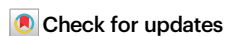


Repetitive neuronal activation regulates cellular maturation state via nuclear reprogramming

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Neural stimulation, such as electroconvulsive therapy (ECT) and repetitive transcranial magnetic stimulation (rTMS), is highly effective clinical intervention for a broad spectrum of psychiatric disorders, including depression and schizophrenia. However, their mechanism of action at the cellular level remains poorly understood. Here, we model ECT with repeated optogenetic neuronal stimulation in the mouse dentate gyrus, and observe ECT-relevant behavioral changes, including decreased depression-like behavior and increased locomotor activity. At the cellular level, we identify dematuration to a long-term stable state, persisting for more than one month, defined by changes in nuclear structure, gene expression patterns resembling the G₂/M phase of the cell cycle, and altered neural coding of navigational information. Moreover, knockout of the G₂/M master regulator Cyclin B attenuates some of behavioral and cellular effects. These findings demonstrate that chronically-repeated brain stimulation triggers plasticity of the cellular state, revealing a form of stimulus-regulated nuclear reprogramming with potential clinical utility.

In the adult mammalian brain, strong synchronous neural activity typically has toxic effects on neuronal function and viability. Cumulative electrical activity of sufficiently high amplitude, frequency, and/or duration interferes with cellular metabolism, flooding cells with excess calcium and resulting in metabolic failure and death^{1,2}. High-intensity neural activity also results in maladaptive neuronal changes, such as dendritic spine loss^{3,4}, abnormal neurite arborization^{4,5}, and toxic excitability⁶. Previous studies have

identified an intermediate state of senescence after overactivation, characterized by the dematuration of adult neurons to an immature-like state, including global de-differentiation of gene-expression patterns, membrane excitability, and neural plasticity^{7–11}. Similar neuronal phenotypes have been observed in genetically engineered mice that exhibit abnormal behaviors mirroring common neuropsychiatric disorders, such as schizophrenia, intellectual disability, and bipolar disorder^{7,12–15}. Our recent study investigated the levels of

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lactate, a surrogate marker of neural excitation, in the brains of mouse models relevant to neuropsychiatric disorders, and found that all mouse models with immature brain phenotypes investigated in this study showed significant increases in lactate levels, suggesting a potential link between cellular hyperexcitability and the immature phenotype¹⁶. Additionally, immature-like gene-expression patterns have been identified in the brain tissues of human patients with neuropsychiatric disorders, such as epilepsy, Alzheimer's disease, and schizophrenia^{11,17–20}, where neural hyperexcitability is a known or suspected etiological contribution.

In marked contrast to the detrimental effects of high-intensity neural activity, there are reported beneficial effects of moderate electrical activity on neural circuit function. Neural activity is essential for brain development, learning, and memory^{21,22}, including enhancements in synaptic weight^{23,24}, neuronal excitability^{25,26}, and gene expression^{27,28}. A clinical application of moderate activity is the use of electrical brain stimulation technologies such as electroconvulsive therapy (ECT), as a class of methods for ameliorating negative symptoms in patients with major depression^{29,30}. ECT in patients promotes neuroplastic changes, such as increased adult neurogenesis in the dentate gyrus and hippocampal volume enlargement^{31–33} with 6–12 repeated stimulations commonly used for long-term positive clinical outcomes^{29,30}. Electroconvulsive stimulation (ECS), the animal equivalent of ECT, also induces molecular and electrophysiological changes, such as enhanced neurogenesis and altered synaptic transmission^{9,14,34,35}. Similar changes occur with chronic administration of fluoxetine, a selective serotonin reuptake inhibitor (SSRI), in mouse models^{35–38}. Repetitive ECS and chronic fluoxetine administration can induce cellular dematuration, suggesting that unspecified cellular plasticity may underpin their antidepressant effects^{9,36,39,40}. Likewise, other noninvasive brain stimulation methods, such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), can induce neuroplastic changes in patients' brains and have promising therapeutic potential^{41,42}. However, despite their common use, the specific cellular mechanisms engaged by these brain stimulation therapies, including clinical ECT, rTMS, and tDCS, remain poorly understood.

In this study, we aimed to elucidate the primary cellular mechanisms by which brain stimulation technologies like ECT cause cellular dematuration. We applied optogenetic stimulation to granule cells of the hippocampal dentate gyrus under a protocol inspired by ECT to produce clinically relevant behavioral effects. This stimulation led to an immature-like gene-expression profile in the brain of mice, and a similar pattern was observed in the post-mortem brains of patients with mood disorders. Our analysis also revealed that repetitive stimulations induce G₂/M cell-cycle re-entry and nuclear structural plasticity with epigenomic and morphonuclear signatures. These findings reveal an immature-like cellular state induced by chronically repeated neuronal activation, which has long-term stability, and a mechanism of activity-dependent nuclear reprogramming, offering mechanistic insight into ECT effects and related applications.

Results

Neuronal activation triggers an immature cellular state in DG granule cells

We challenged neurons in vivo with a stimulation protocol designed to mimic human ECT, in which seizure duration is a critical determinant of therapeutic efficacy and is therefore tightly controlled in clinical settings. Conventional methods, such as seizure-inducing drugs and animal ECS, do not allow precise control over seizure duration, leading to variability in cellular and network-level effects. To overcome this limitation, we used mice expressing channelrhodopsin-2 (ChR2) in granule cells (GCs) of the dentate gyrus (DG) (POMC-Cre::ChR2-EYFP mice) and applied optogenetic stimulation, termed Repetitive Optogenetic Stimulation (REPOPS), enabling precise control over the

frequency and duration of stimulation (Fig. 1a). This optogenetic approach allowed us to establish a precise experimental system to study cellular effects confined to a specific target cell population. We employed a moderate stimulation protocol that did not cause cell death (Supplementary Figs. 1a, b, 2a, b; Supplementary Data 1).

REPOPS was performed either three or ten times consecutively (Fig. 1a). These regimens were selected to contrast a minimal (3-day) protocol with an extended (10-day) regimen, approximating the 6–12 sessions typically required to achieve clinical efficacy in ECT. This design enabled us to assess how the cumulative number of stimulations influences the emergence of stable molecular and behavioral effects. The expression of calbindin (CB), a maturation marker of GCs, was reduced 24 h after REPOPS for three days, but returned to a level that was not significantly different from the pre-stimulus levels within two weeks (Fig. 1b). In contrast, REPOPS for 10 days significantly decreased CB expression at 24 h and two weeks after the last stimulation (Fig. 1b). The decrease in CB was inhibited by the administration of Ca²⁺ blockers (Fig. 2a–c), suggesting that Ca²⁺ influx is crucial for REPOPS-induced dematuration. RNA-seq analysis showed that the number of genes with significant changes in expression ($P < 0.05$, fold change > 1.2 ; vs. No Stim group) decreased over time after 3-day REPOPS, but remained unchanged after 10-day REPOPS (Fig. 1c). Evidently, neuronal activation repeated a few times induces short-term transcriptome changes that spontaneously revert to the original state; however, when neuronal activation is chronically repeated, the changes are sustained for longer periods. To examine whether REPOPS induces an immature-like gene-expression pattern at the transcriptome level, we assessed the similarity between gene-expression patterns of optogenetically stimulated DG and those of normal infant mice (GSE113727⁴¹) by the Running Fisher test⁴³, a rank-based non-parametric algorithm that evaluates the degree of overlap between two gene groups implemented in BaseSpace (Illumina, Cupertino, CA, USA). There was a significant degree of similarity between the datasets (Overlap $P = 3.9 \times 10^{-52}$) with the same directional changes in expression (Fig. 1d and Supplementary Fig. 3). Collectively, these results indicate that REPOPS for 10 consecutive days induces long-lasting immature-like gene-expression patterns in the DG, whereas REPOPS for three days does not.

As REPOPS was designed to model the effects of human ECT, we next asked whether ECT in human patients also induces an immature-like gene-expression pattern. We reanalyzed RNA-seq data from the post-mortem DG of bipolar disorder and major depressive disorder patients with and without ECT (SRP241159⁴⁴). Genes differentially expressed between ECT-treated and non-ECT-treated patients were compared with those changing during typical hippocampal development (0–5 months vs. 20–39 years; GSE25219⁴⁵). This analysis revealed a significant overlap between the two datasets (Overlap $P = 1.2 \times 10^{-8}$), with consistent directional changes (Fig. 1f), suggesting that ECT induces an immature-like gene-expression pattern in the DG of human patients, in line with the pattern observed in REPOPS (see also Supplementary Data 2). Both REPOPS and human ECT-induced long-term alterations in the expression of synapse-related genes: REPOPS significantly downregulated *Gria1*, *Gria2*, *Camk2a*, and *Camk2b*, and upregulated *Arc* (Fig. 1e), while ECT-treated patients exhibited similar alterations in *GRIA1*, *GRIA2*, and *ARC* compared with non-ECT-treated patients (Fig. 1g), suggesting a shift toward an immature synaptic state.

Ca²⁺ influx and subsequent kinase activation are indispensable for inducing a cellular immature-like state

To dissect the signaling pathways underlying REPOPS-dependent dematuration, we developed an integrated optofluidic probe that enables simultaneous drug delivery and optogenetic stimulation within the same brain region through an implanted cannula (Fig. 2a).

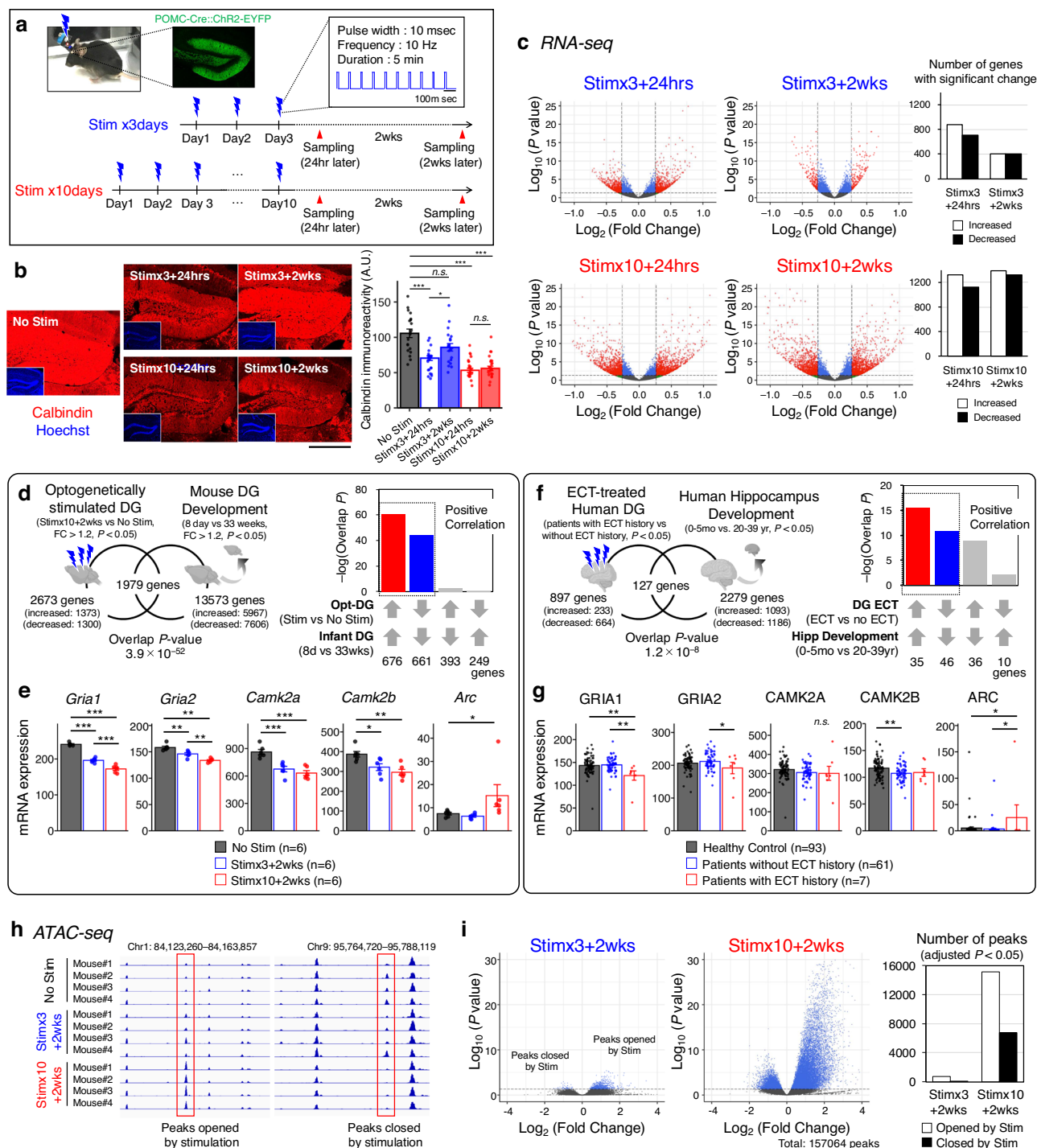


Fig. 1 | REPOPS induces long-term immature-like gene expression and large-scale chromatin remodeling. **a** Experimental design. POMC-Cre::ChR2-EYFP mice express ChR2 in GCs of the DG. Optogenetic stimulation (10 ms pulse, 10 Hz, 5 min/day) was delivered via a wireless LED device for 3 or 10 days. Brains were collected 24 h or 2 weeks after the last stimulation. **b** Calbindin immunoreactivity in dorsal DG. Scale bar, 500 μ m. Bar graph: mean $\pm$ s.e.m. of calbindin immunoreactivity ($n = 20$, 24, 20, 24, 23 sections from 4 mice/group for No Stim, Stim $\times$ 3+24hrs, Stim $\times$ 3+2wks, Stim $\times$ 10+24hrs, Stim $\times$ 10+2wks). One-way ANOVA, $F_{(4, 106)} = 24.9$, $P = 1.5 \times 10^{-14}$, with Bonferroni post hoc (two-sided); *** $P < 0.001$. **c** RNA-seq volcano plots; differential expression by DESeq2 (Wald test, two-sided), each stimulation group vs. No Stim ($n = 6$ mice/group). Red, Benjamini–Hochberg adjusted $P < 0.05$ and fold change > 1.2 . Bar graphs: number of significantly changed genes. **d** Overlap between genes altered by REPOPS (Stim $\times$ 10+2wks vs. No Stim) and during mouse DG development (P8 vs. 33 weeks; GSE113727). Bars: $-\log_{10}(\text{overlap } P\text{-values})$ for up- and downregulated genes (Running Fisher test, one-sided);

Bonferroni-corrected for dataset pairs). Created in BioRender. Murano (2026) <https://BioRender.com/p6ed2mm>. **e** FPKM of representative synapse-related genes; mean $\pm$ s.e.m. ($n = 6$ mice/group). One-way ANOVA with Bonferroni post hoc (two-sided); * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. **f** Same as (d), but for ECT-treated human DG (with vs. without ECT; SRP241159⁴⁵) and human hippocampal development (0–5 mo vs. 20–39 yr; GSE25219⁴⁵). **g** FPKM of representative synapse-related genes in post-mortem DG: healthy controls ($n = 93$), patients without ECT ($n = 61$), patients with ECT ($n = 7$); mean $\pm$ s.e.m. One-way ANOVA with Bonferroni-corrected pairwise t -tests (two-sided); * $P < 0.05$, ** $P < 0.01$. **h** ATAC-seq. IGV tracks of representative gained-open and gained-closed ATAC-seq peaks. **i** Volcano plot of differential chromatin accessibility by DESeq2 (Wald test, two-sided); Stim $\times$ 3+2wks and Stim $\times$ 10+2wks vs. No Stim ($n = 4$ mice/group). Bar graph: number of peaks with significant change (Benjamini–Hochberg adjusted $P < 0.05$). Source data are provided as a Source Data file.

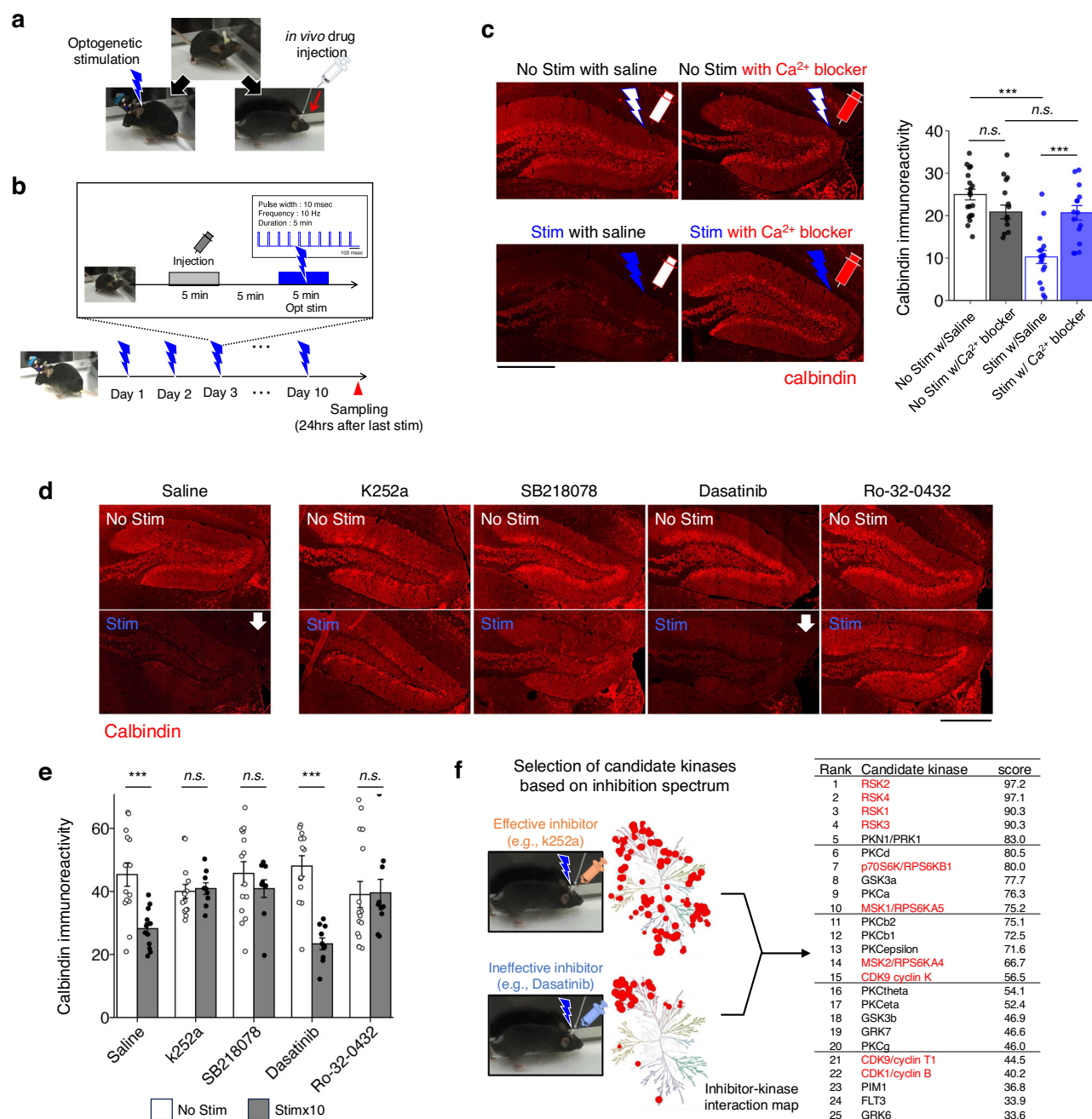


Fig. 2 | Ca^{2+} influx and subsequent kinase activation are indispensable for inducing a cellular immature-like state. **a** Integrated optofluidic probe enabling localized drug injection and optogenetic stimulation through a single cannula implanted in the brain. **b** Experimental design. Intracerebral drug infusion into the DG was followed by optogenetic stimulation (5 min per day) for 10 consecutive days. Brains were sampled 24 h after the final stimulation. **c** Effect of Ca^{2+} blockers on calbindin expression. The Ca^{2+} blockers included NBQX (AMPA-R antagonist), AP5 (NMDA-R antagonist), mibefradil (non-specific blocker of voltage-gated Ca^{2+} channels), and nimodipine (Ca_v1 blocker). Left: representative calbindin immunostaining (red); scale bar, 500 μm . Right: bar graph of calbindin immunoreactivity; mean $\pm$ s.e.m. ($n = 19, 14, 17, 15$ sections from 4 mice for No Stim+NoDrug, No Stim+Drug, Stim+NoDrug, Stim+Drug). One-way ANOVA; $F_{(3, 61)} = 19.17$, $P = 7.13 \times 10^{-9}$, with Bonferroni post hoc (two-sided); $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. **d** Effect of kinase inhibitors on calbindin expression. Four kinase inhibitors with distinct inhibition spectra (K252a, SB218078, Dasatinib, Ro-32-0432) and saline were injected before optogenetic stimulation. Representative calbindin (red) immunostaining images (4 mice per group; quantification in **e**) are shown; scale bar,

500 μm . **e** Calbindin immunoreactivity from **(d)**. Mean $\pm$ s.e.m. (sections per group, No Stim/Stim $\times$ 10: Saline 14/12, K252a 14/9, SB218078 14/10, Dasatinib 13/10, Ro-32-0432 16/10; 4 mice per group). Two-way ANOVA: StimType, $F_{(1, 112)} = 18.34$, $P = 3.9 \times 10^{-5}$; Drug, $F_{(4, 112)} = 1.12$, $P = 0.35$; StimType $\times$ Drug interaction, $F_{(4, 112)} = 5.60$, $P = 3.8 \times 10^{-4}$. Tukey's post hoc (two-sided); $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. **f** Identification of candidate kinases mediating REPOPS-induced dematuration. Left: Kinome dendrogram generated using KinMap (Eid et al.¹³⁵, based on the kinome tree from Cell Signaling Technology, Inc.), available under the Creative Commons Attribution 4.0 International License (CC BY 4.0; <https://creativecommons.org/licenses/by/4.0/>). The KinMap framework was used without modification; symbols overlaid on the dendrogram represent the authors' candidate kinases identified in this study. Kinase inhibition data are from Anastassiadis et al.⁴⁶. Right: candidate kinases identified by comparing inhibition spectra of compounds that blocked (K252a, SB218078, and Ro-32-0432) or did not block (Dasatinib) REPOPS-induced effects. The score is the difference in average % inhibition between the effective and the ineffective inhibitor. Source data are provided as a Source Data file.

Using this device, we administered daily drug infusions followed by 5-min optogenetic stimulation for 10 consecutive days (Fig. 2b).

To test whether Ca^{2+} influx is required for dematuration, REPOPS was performed with a cocktail of Ca^{2+} blockers, including NBQX (AMPA-R antagonist), AP5 (NMDA-R antagonist), mibefradil (non-specific blocker of voltage-gated Ca^{2+} channels), and nimodipine (Ca_v1 blocker). In the saline-treated control group, REPOPS markedly reduced CB expression, whereas CB expression remained unchanged in Ca^{2+} blocker-treated mice (Fig. 2c). These results indicate that Ca^{2+} influx is crucial for neuronal dematuration.

Next, to explore kinases involved in dematuration, we conducted REPOPS with four kinase inhibitors with distinct inhibition spectra (Fig. 2d). K252a, SB218078, and Ro-32-0432 successfully inhibited REPOPS-induced CB reduction, whereas Dasatinib did not (Fig. 2d, e). Based on these results, we screened for candidate kinases potentially mediating dematuration. Using a comprehensive kinase-inhibitor activity dataset⁴⁶, we compared the inhibition spectra of the effective inhibitors with that of the ineffective one (Fig. 2f). Kinases more strongly inhibited by the effective inhibitors (K252a, SB218078, and Ro-32-0432) than by the ineffective inhibitor (Dasatinib) were short-listed as candidates. This analysis identified 57 potential kinases, of which the top 25 are shown in Fig. 2f. These include RSK/MSK, which regulate chromatin remodeling through histone phosphorylation^{47,48}, and CDKs, key regulators of the cell cycle. Together, these findings suggest that Ca^{2+} -dependent signaling pathways and phosphorylation cascades play a pivotal role in REPOPS-induced dematuration.

REPOPS regulates the long-term plasticity of genomic structure

Changes in gene expression are often accompanied by alterations in the chromatin structure of the associated genomic regions⁴⁹. To investigate whether and how neural overactivation alters the chromatin accessibility of neurons, we performed ATAC-seq on optogenetically stimulated DG tissue (Fig. 1h). Volcano plots showing changes in chromatin accessibility are shown in Fig. 1i. We found significant changes in chromatin accessibility in 806 chromatin regions (748 opened and 58 closed) and 21,853 chromatin regions (15,098 opened and 6755 closed) in the Stim×3+2wks and Stim×10+2wks groups, respectively (Fig. 1i). These results indicate that repeated neuronal activation for 10 consecutive days, but not for 3 days, induced widespread and persistent reorganization of three-dimensional genome structure, along with concordant global transcriptome changes (Supplementary Fig. 4 and Supplementary Data 3).

REPOPS initiates long-term anti-depressive behavioral changes in mice

To assess whether repeated neuronal activation could alter anti-depressive behavior, we first performed an open field (OF) test (Fig. 3a, b). Locomotor activity was recorded for 30 min each day, and the dorsal DG was optogenetically stimulated for 5 min (20–25 min after the start) on each stimulation day. As previously reported⁵⁰, mice showed an increase in locomotor activity immediately after stimulation (a1–a3 in Fig. 3a). On the day after the first stimulation, mice in both Stim×3 and Stim×10 groups exhibited a significant increase in distance traveled during the first 15 min compared to the No Stim group ($P=0.0134$, Day 2 in Stim×10 group; $P=8.05 \times 10^{-4}$, Day 9 in Stim×3 group; Fig. 3b). In the Stim×3 group, this increase peaked on Day 9 and returned to control levels within two weeks (blue line in Fig. 3b), whereas in the Stim×10 group, it declined gradually from the last stimulation day but remained significantly elevated two weeks after the last stimulation (Day 24, $P=0.00362$; red line in Fig. 3b). Thus, neuronal activation of the DG repeated for 10 days induced hyperlocomotor activity persisting for at least two weeks, whereas an increase in locomotor activity after neuronal activation for three days was transient. We also conducted 24-h activity monitoring in the home cage (Fig. 3c). The distance traveled by mice in the Stim×10 group

gradually increased after the initial stimulation and was significantly higher than that in the No Stim and Stim×3 groups ($P<0.05$, Days 7–36; Fig. 3c). In the home cage setting, unlike in the open field, locomotor activity did not increase immediately after stimulation, likely reflecting the differences between familiar and novel environments. Mice in the Stim×3 group did not show any significant increase in locomotor activity in the home cage. These results indicate that repeated neuronal activation causes a significant increase in baseline locomotor activity, which persists for more than one month. While it has not been an accepted therapeutic effect, ECT in humans is reported to be accompanied by increased spontaneous motor activity^{51–53}, supporting the relevance of the enhanced activity observed in our model.

Depression-related behavior was assessed with the tail suspension test (Fig. 3d). Two weeks after the last stimulation, mice in the Stim×10 group showed significantly less immobility compared to those in the No Stim ($P=0.0094$) and Stim×3 groups ($P=0.0198$). A similar trend was observed in the forced swim test (Supplementary Fig. 5c), where Stim×10 mice showed reduced immobility two weeks after the last stimulation compared to the No Stim group ($P=0.0304$) and Stim×3 group ($P=0.0770$). These results suggest that repeated neuronal activation mimicking ECT reduces depression-like behavior in mice. We further performed the novelty-suppressed feeding test, a well-established assay for antidepressant-like effects that is less dependent on locomotor activity⁵⁴ (Fig. 3e). In this paradigm, food-deprived mice are placed in a brightly lit open field with a food pellet in the center, and the latency to begin feeding is recorded. Feeding latency differed significantly among the three groups (log-rank Mantel–Cox test: $\chi^2=11.86$, $P=0.0027$). Post hoc pairwise comparisons revealed that feeding latency was significantly shorter in the Stim×10 group than in both the No Stim ($\chi^2=5.09$, $P=0.024$) and Stim×3 groups ($\chi^2=10.20$, $P=0.0014$), whereas no significant difference was detected between the No Stim and Stim×3 groups ($\chi^2=0.25$, $P=0.62$; Fig. 3e). To determine whether this effect was attributable to differences in general activity levels, ANCOVA controlling for locomotor activity confirmed that the main effect of stimulation group on feeding latency remained significant ($F_{(2, 18)}=6.93$, $P=0.0059$). When sex and the interaction term were further entered into the model, there were no significant effects of locomotor activity ($F_{(1, 18)}=0.74$, $P=0.40$), sex ($F_{(1, 18)}=3.44$, $P=0.080$), or their interaction ($F_{(2, 18)}=0.74$, $P=0.49$). These results demonstrate that 10-day, but not 3-day, REPOPS reduces anxiety-related behavior independently of general locomotor activity. We also performed the sucrose preference test. No statistically significant differences were observed among groups in sucrose or water intake or in the percentage of sucrose preference (Supplementary Fig. 5d), suggesting that REPOPS does not alter anhedonia-like behavior. Thus, REPOPS increased locomotor activity and reduced depression-like and anxiety-related behaviors, whereas anhedonia-like behavior remained unchanged. This dissociation may reflect the regional specificity of DG-targeted REPOPS, as the DG and its associated circuits are implicated in exploratory/anxiety-related and approach–avoidance behaviors^{50,55}, whereas hedonic tone is primarily supported by mesolimbic reward circuitry⁵⁶.

Alterations of neural coding of navigational information

To investigate whether and how neural overactivation affects circuit function, we performed in vivo Ca^{2+} imaging under freely moving conditions combined with optogenetic stimulation (Fig. 4a, b). On each stimulation day, we recorded DG neuron activity and locomotor activity in the OF during the first 30 min, followed by 5 min of optogenetic stimulation (590–650 nm, 20 ms pulse, 10 Hz; Fig. 4c). The distance traveled during the first 30 min in the Stim×10 group gradually increased and was significantly greater than in the No Stim group two weeks after the last stimulation ($P=0.0358$; Fig. 4d). However, the

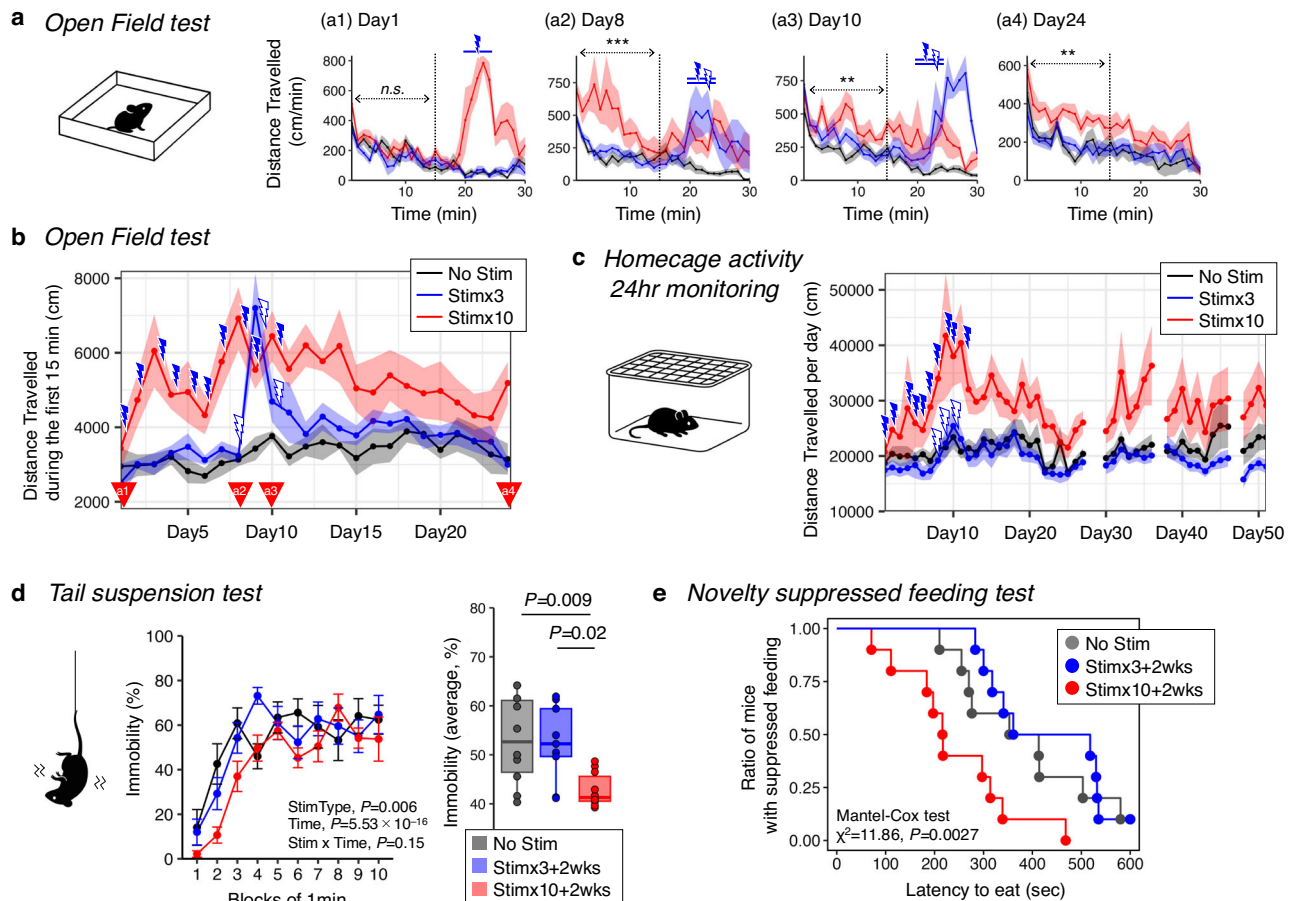


Fig. 3 | REPOPS induces anti-depressive behaviors. **a** Distance traveled in the open field during the first 30 min on Days 1, 8, 10, and 24 (a1–a4; time points marked in **b**) (red arrowheads). Group means (solid lines, $n = 6$ mice/group) $\pm$ s.e.m. (semitransparent area); black, No Stim; blue, Stim $\times$ 3+2wks; red, Stim $\times$ 10+2wks. Optogenetic stimulation: 5 min (20–25 min after the start) on Days 1, 8, 10. Asterisk indicates Stim $\times$ 10+2wks vs. No Stim; One-way ANOVA with Bonferroni post hoc (two-sided); ** $P < 0.01$, *** $P < 0.001$. **b** Total distance traveled in the first 15 min of the open field test from Day 1 to Day 24; mean $\pm$ s.e.m. ($n = 6$ mice/group; same color scheme as **a**). Two-way repeated measures ANOVA revealed significant main effects of Stim type ($P = 2.0 \times 10^{-3}$) and Day, and a significant Stim type $\times$ Day interaction. **c** 24-h locomotor activity in the home cage. Daily average distance; mean $\pm$ s.e.m. ($n = 10, 8, 10$ mice for No Stim, Stim $\times$ 3, Stim $\times$ 10; same color as **a**). Data missing on Days 28, 29, 37, and 47 due to a computer system error. Two-way repeated measures ANOVA: significant main effects of stimulation group

($P = 0.016$) and Day. **d** Immobility (%) in the tail suspension test. Left, immobility per 1-min block; mean $\pm$ s.e.m. ($n = 10, 9, 10$ mice for No Stim, Stim $\times$ 3+2wks, Stim $\times$ 10+2wks). Two-way repeated measures ANOVA: significant main effects of Stimulation group ($P = 0.006$) and Timebin; no significant interaction. Right, box plot of mean immobility over 10 min: center line, median; box bounds, 25th and 75th percentiles; whiskers extend to the most extreme data points within $1.5 \times$ IQR from the box; dots, individual mice (showing minimum and maximum values). One-way ANOVA, $P = 0.0057$, with Tukey's HSD post hoc (two-sided). **e** Novelty-suppressed feeding test. Latency to feed; Kaplan–Meier survival curves ($n = 10$ mice/group). Log-rank Mantel–Cox test (two-sided): all groups, $\chi^2 = 11.86$, $P = 0.0027$. ANCOVA (locomotor activity as covariate): significant effect of stimulation group on feeding latency, $P = 0.0045$. Source data are provided as a Source Data file.

mean Ca^{2+} transient rate did not differ significantly between these groups throughout the experiment (Fig. 4e).

Navigational information, such as position and speed, is encoded in the population activity of DG neurons^{15,57}. To examine how neural overactivation affects this coding, we computed spatial and speed information for individual DG neurons. In the No Stim group, spatial information increased on Days 2–11 and Days 24–26 compared to Day 1 (Cohen's $d = 0.43$ and 0.30), suggesting that OF exposure enhanced spatial coding (Fig. 4f). In contrast, the Stim $\times$ 10 group showed reduced spatial information on Days 2–11 and Days 24–26 compared to the No Stim group ($d = -0.50$ and -0.45 ; Fig. 4f). Speed information remained unchanged in the No Stim group throughout the experiment ($|d| < 0.10$), but increased in the Stim $\times$ 10 group on Days 2–11 and Days 24–26, exceeding that in the No Stim group ($d = 0.46$ and 0.63 ; Fig. 4i). Thus, repeated neuronal activation reduced spatial coding and enhanced speed-related activity in individual DG neurons for over two weeks.

To examine information encoding at the population level, we predicted mouse position and speed from the population activity of DG neurons using machine learning methods. Representative decoding results are shown in Fig. 4g, j. In the No Stim group, position decoding errors decreased on Days 2–11 and Days 24–26 and were significantly smaller than chance (Fig. 4h). In contrast, the Stim $\times$ 10 group showed no significant change in position decoding accuracy, and decoding errors were significantly greater than in the No Stim group on Days 2–11 and Days 24–26 ($P = 0.0133$ and 0.0335 ; Fig. 4h). These findings suggest that position information coding was enhanced by OF exposure but impaired by neural overactivation. Speed decoding accuracy did not change significantly during the experiment in the No Stim group, but was increased by REPOPS in the Stim $\times$ 10 group and was significantly higher than in the No Stim group on Days 2–11 and Days 24–26 ($P = 0.0363$ and 0.0223 ; Fig. 4k). Thus, repeated neuronal activation reshapes information processing in the DG by suppressing spatial map formation and enhancing speed-

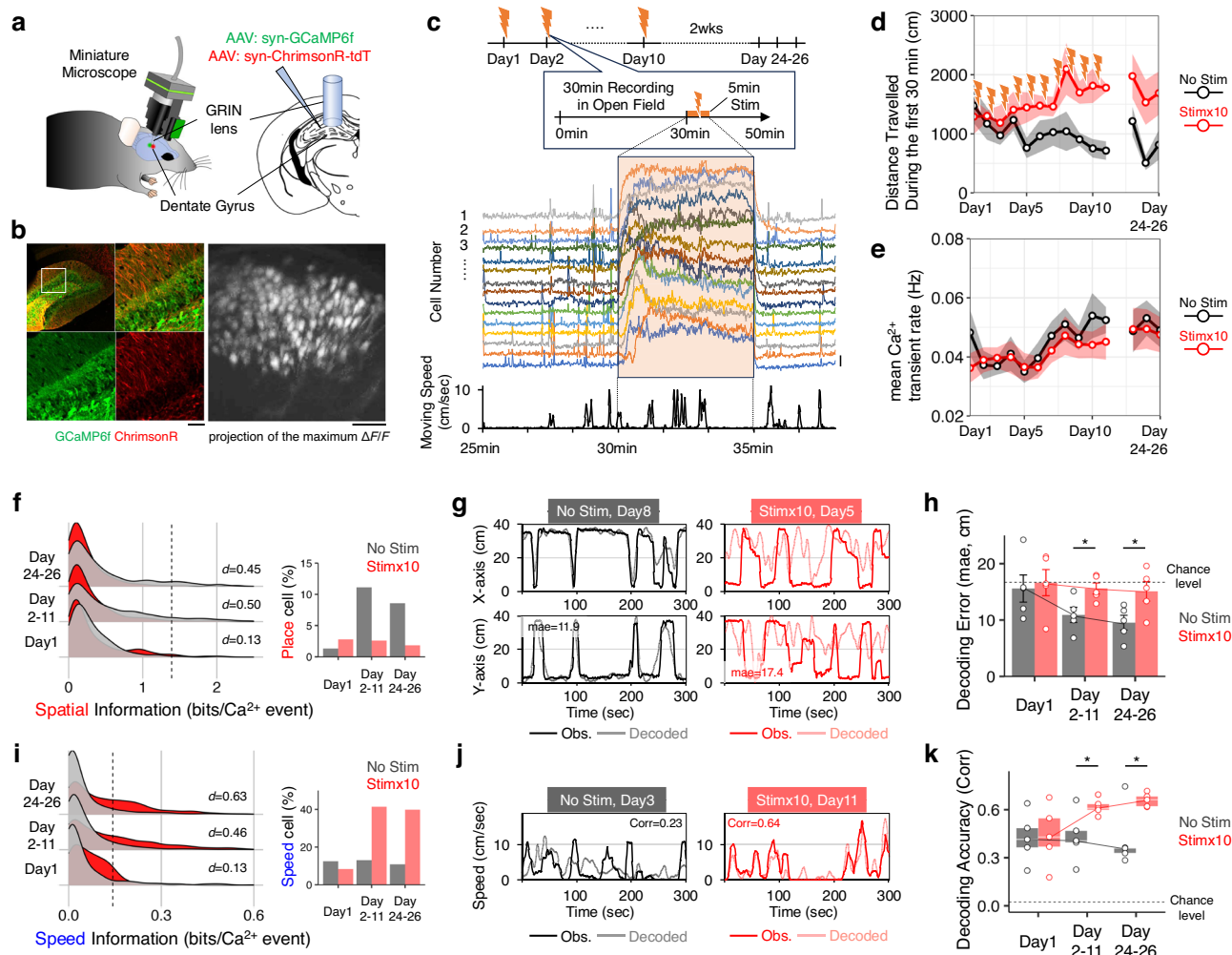


Fig. 4 | REPOPS mediates long-term changes in neural coding of navigational information. **a** Simultaneous in vivo calcium imaging and optogenetic stimulation in freely moving mice using an integrated miniature microscope (nVoke; Inscopix) after AAV-syn-GCaMP6f and AAV-syn-ChrimsonR co-injection and GRIN lens implantation in the DG. **b** Left: GCaMP6f and ChrimsonR expression in the DG. Right: max projection of $\Delta F/F$ from all imaging frames during the first 30 min, showing representative active neurons. Scale bars, 20 μm . **c** Top: experimental design. Each 50-min daily session (10 days) comprised 30-min Ca^{2+} imaging, 5-min optogenetic stimulation, and 15-min post-stimulation recording. No light was given to No Stim mice. Bottom: $\Delta F/F$ traces from 15 representative neurons (25–38 min; scale bar, 10% $\Delta F/F$). **d** Distance traveled during the first 30 min in the open field. Solid lines show group means; semitransparent area indicates $\pm$ s.e.m. ($n = 5$ mice per group; black, No Stim; red, Stim $\times$ 10). **e** Same as (**d**), for the average Ca^{2+} transient rate. **f** Distribution of spatial information for No Stim (gray) and Stim $\times$ 10

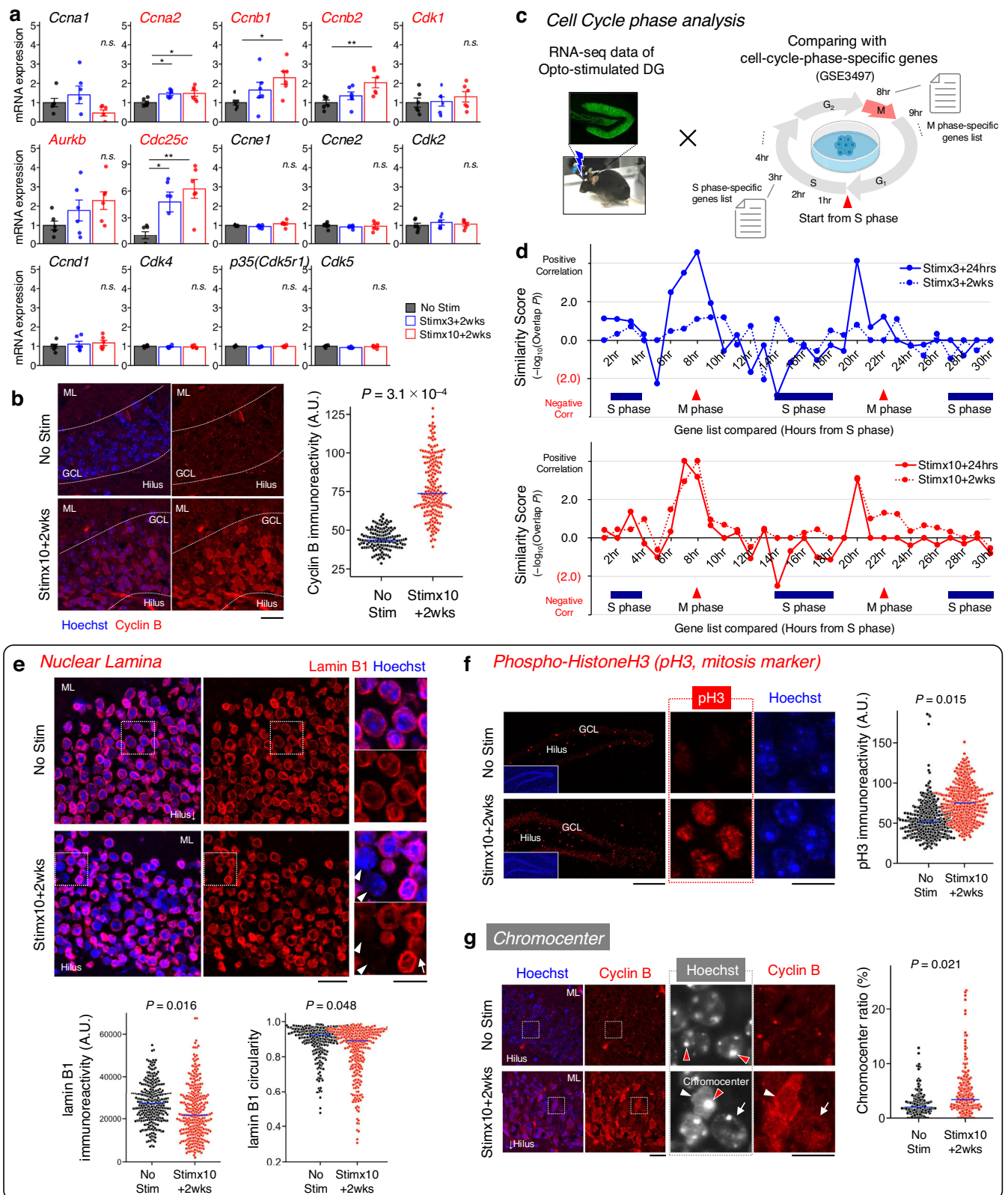
(red). Dotted line: place-cell criterion (top 95% percentile of shuffled distribution; see “Methods” section). Bar graph: proportion of place cells. **g** Representative position decoding; the first 30 min was split into two 15-min halves (training/test). Black/red, observed position; gray/pink, decoded. **h**, Position decoding accuracy (mean absolute error, mae, cm); mean $\pm$ s.e.m. ($n = 5$ mice/group); dots, individual mice. Dotted lines: shuffled control. Two-way repeated measures ANOVA: significant main effect of Stim type ($P = 0.034$); Bonferroni post hoc (two-sided), $*P < 0.05$. **i** Same as (**f**), for speed information and speed cells. **j** Same as (**g**), for speed decoding. **k** Same as (**h**), for speed decoding accuracy. Box plot: center line, median; box bounds, 25th and 75th percentiles; whiskers extend to the most extreme data points within $1.5 \times \text{IQR}$ from the box; dots, individual mice ($n = 5$ mice per group). Two-way repeated measures ANOVA: significant main effect of Day ($P = 0.022$) and Day $\times$ Stim type interaction ($P = 0.039$); Bonferroni post hoc (two-sided), $*P < 0.05$. Source data are provided as a Source Data file.

related activity, with unchanged overall activity levels. This shift may underlie the behavioral changes observed in optogenetically stimulated mice, such as increased locomotor activity and reduced depression-like behavior.

REPOPS-driven long-term increases in the expression of cell-cycle-related genes

We next explored potential molecular markers associated with REPOPS-induced neuronal alterations by conducting gene ontology analysis of RNA-seq data from optogenetically stimulated DG. Notably, gene categories significantly altered in the Stim $\times$ 10+2wks group—but not in the Stim $\times$ 3+2wks group—included cell-cycle-related terms, such as “cell division” and “mitotic cell-cycle process” (Supplementary Fig. 7). Expression levels of

representative cell-cycle-related genes are shown in Fig. 5a. Genes related to the G₂/M phase, such as *Ccna2*, *Ccnb1*, *Ccnb2*, and *Cdc25c*, were significantly increased in Stim $\times$ 10+2wks compared to the No Stim group, whereas genes related to other phases, such as *Ccne1*, *Ccne2*, *Cdk2* (G₁-S phase), and *Ccnd1*, *Cdk4* (G₁ phase), showed no significant changes (Fig. 5a). We found that cyclin B, an essential regulator for the transition from G₂ to M phase, was expressed in most DG neurons in Stim $\times$ 10+2wks, while it was barely detectable in the No Stim group (Fig. 5b). It was broadly expressed throughout the granule cell layer and was not restricted to the subgranular zone, where newborn neurons are abundant, indicating that the majority of cyclin B expression reflects induction in mature neurons, rather than enhanced neurogenesis (see also Supplementary Fig. 8).



To assess whether gene-expression patterns induced by REPOPS resembled specific cell-cycle phases, we used a publicly available microarray dataset of HeLa cells that captures gene-expression pattern dynamics throughout the cell cycle (Fig. 5c; GSE3497³⁸). In this dataset, genes characteristic of each phase were identified through microarray analysis of HeLa cells sampled at multiple time points across the cell cycle. We compared these cell-cycle phase-specific gene lists (1–30 h

from S phase) with RNA-seq data from optogenetically stimulated DG (Fig. 5c) and computed a similarity score for each time point, defined as the $-\log_{10}$ of the overlap P -value (Fig. 5d). In Stimx3+24hrs and Stimx10+24hrs, similarity score peaks were observed at 7, 8, and 20 h from S phase (scores >3.0; Fig. 5d), corresponding approximately to the G₂/M phase. In Stimx3+2wks, these peaks declined (scores <2.0), whereas in the Stimx10+2wks group, they remained elevated (scores

Fig. 5 | REPOPS confers G₂/M phase-like gene-expression patterns and nuclear structure plasticity. **a** mRNA expression of representative cell-cycle-related genes. FPKM normalized to No Stim; mean $\pm$ s.e.m. ($n = 6$ mice/group). One-way ANOVA with Bonferroni post hoc (two-sided); * $P < 0.05$, ** $P < 0.01$. **b** Cyclin B immunostaining (red) in the DG. Scale bar, 10 μ m. Beeswarm plot: cyclin B immunoreactivity; blue bars, median ($n = 147$ No Stim, 202 Stim $\times$ 10+2wks neurons from 3 mice/group). Linear mixed-effects model (LMM, two-sided), mouse as random effect; $P = 3.1 \times 10^{-4}$. **c** Cell-cycle phase analysis. To infer the cell-cycle-like status of DG neurons after REPOPS, RNA-seq data from optogenetically stimulated DG (Fig. 1c) were compared with reference gene-expression profiles for each cell-cycle phase (HeLa cells released from S-phase arrest, sampled at 1-h intervals; Whitfield et al.; GSE3497)⁵⁸. Created in BioRender. Murano (2026) <https://BioRender.com/p6ed2mm>. **d** Similarity scores ($-\log_{10}$ of overlap P -value, Running Fisher test, one-sided) between stimulated DG transcriptomes and cell-cycle reference profiles. Positive/negative scores indicate concordant/opposite changes. S and M phase

positions on the x-axis are estimated from the HeLa cell data (GSE3497)⁵⁸. **e** Lamin B1 immunostaining (red) in the DG; enlarged views at right. Arrows/arrowheads: neurons with partial/complete lamin B1 disruption. Scale bars: 20 μ m (left), 10 μ m (right). Beeswarm plots: immunoreactivity and circularity ($n = 289$ No Stim, 320 Stim $\times$ 10+2wks from 3 mice/group); blue bars, medians. LMM (two-sided): immunoreactivity, $P = 0.016$; circularity, $P = 0.048$. **f** Phospho-histone H3 (pH3; red) immunostaining in the DG. Scale bars: 200 μ m (left), 10 μ m (right). Beeswarm plot of pH3 signal ($n = 415$ and 459 neurons from 3 mice/group). LMM (two-sided): $P = 0.015$. **g** Hoechst (blue/white pseudo-color) and cyclin B (red) immunostaining; enlarged views at right. Red arrowheads, chromocenters; white arrowheads/arrows, neurons with large/small chromocenters and high/low cyclin B. Scale bars: 100 μ m (left), 10 μ m (right). Beeswarm plot of chromocenter ratio (%) per neuron ($n = 147$ and 202 neurons from 3 mice/group). LMM (two-sided); $P = 0.021$. Source data are provided as a Source Data file.

>3.0; Fig. 5d). Although neurons are considered post-mitotic and do not undergo cell-cycle progression, these findings suggest that repeated neuronal activation reinstates G₂/M phase-like transcriptional programs in mature neurons that persist for over two weeks.

Long-term G₂/M phase-like epigenomic plasticity in nuclear structure

We investigated whether REPOPS can induce changes in nuclear structure, focusing on three major events that occur during the G₂/M phase: (i) nuclear lamina disruption, (ii) an increase in the phosphorylation of histone H3 (pH3), and (iii) chromatin condensation.

(i) The nuclear lamina is a dense fibrillar network lining the inner nuclear membrane and providing mechanical support for chromatin organization, which is disrupted during mitosis due to nuclear division^{59,60}. In the No Stim group, most neurons exhibited normal, round-shaped lamin B1, the main component of the nuclear lamina (Fig. 5e and Supplementary Fig. 9). In contrast, neurons in the Stim $\times$ 10+2wks group showed varying degrees of reduced expression and/or disrupted lamin B1 structure, indicating that 10 days of REPOPS-induced nuclear lamina disruption (Fig. 5e).

(ii) Increased phosphorylated histone H3 (pH3) is another characteristic change during mitosis, which is a known “mitosis marker.” In the No Stim group, most neurons showed minimal immunoreactivity of pH3, whereas the Stim $\times$ 10+2wks group exhibited a significant increase in pH3 immunoreactivity (Fig. 5f), suggesting that repeated neuronal activation induces mitosis-like epigenetic modifications of chromatin in mature neurons.

(iii) The chromocenter is an aggregation of heterochromatin domains, which can be visualized as concentrated dot-like signals in the nucleus by DNA counterstaining (indicated by red arrowheads in Fig. 5g). It is considered a central site for chromatin condensation during mitosis^{61,62}. The chromocenter ratio—defined as the ratio of total chromocenter area to the nucleus size—was significantly increased by REPOPS, indicating an enlargement of heterochromatin domains (Fig. 5g).

Thus, REPOPS induces G₂/M phase-like nuclear states, characterized by nuclear lamina disruption, increased pH3, and chromatin condensation. These changes largely overlapped and were most prominent in the granule cell layer, suggesting that they occur predominantly in granule cells (see also Supplementary Fig. 10). This G₂/M-like nuclear state aligns with the idea that REPOPS-induced dematuration is likely mediated by RSK/MSK, which regulate chromatin remodeling through histone phosphorylation, and by CDKs, which are key regulators of the cell cycle (Fig. 2f).

Cyclin B mediates REPOPS-induced changes in nuclear structure, cellular immaturity, and behavior

To investigate the molecular mechanisms underlying G₂/M phase-like nuclear alterations, we knocked out the expression of cyclin B—an

essential gene for the G₂/M phase transition—by injecting a mixture of AAV9-SpCas9 and AAV9-sgRNA encoding sequences targeting *Ccnb1* and *Ccnb2* (CyclinB-KO) into the DG (Fig. 6a, b). AAV9-sgRNA encoding scrambled sequences (Scram-KO) was used as a control. The REPOPS-induced expression of cyclin B, observed in neurons in the granule cell layer, was effectively eliminated in the CyclinB-KO group (Supplementary Fig. 11).

Lamin B1 expression was significantly reduced by REPOPS in both Scram-KO and CyclinB-KO groups, but the degree of reduction was significantly smaller in the CyclinB-KO group (Fig. 6c). Similarly, REPOPS significantly increased pH3 signals in both groups, but the degree of increase was significantly reduced in the CyclinB-KO group (Fig. 6d). The chromocenter ratio was significantly increased by REPOPS in the Scram-KO group, but not in the CyclinB-KO group (Fig. 6e). Calbindin expression was significantly decreased in both groups, but the reduction was significantly smaller in the CyclinB-KO group compared to the Scram-KO group (Fig. 6f). In the open field test, REPOPS significantly increased locomotor activity in the Scram-KO group from the day after the first stimulation, which remained significantly greater than that of the No Stim group two weeks after the last stimulation ($P = 0.0231$, Day 24; Fig. 6g). In contrast, in the CyclinB-KO group, locomotor activity showed a transient increase but gradually returned to baseline levels by Day 24 ($P > 0.99$; Fig. 6g). Collectively, these findings demonstrate that cyclin B is a critical molecular mediator linking repeated neuronal activation to G₂/M phase-like nuclear remodeling, neuronal immaturity, and prolonged hyperlocomotor activity in mice.

Δ FosB is upregulated by Cyclin B/Cdk1 activity and mediates persistent genomic remodeling

We next explored mechanisms underlying long-term genomic changes by performing motif enrichment analysis⁶³ of ATAC-seq data (Fig. 1h, i). We found that open chromatin peaks showed the highest similarity to ChIP-seq profiles of AP-1 transcription factors, dimers composed of Fos and Jun family proteins, such as Fra1, Atf3, Batf, and JunB (Supplementary Fig. 12a), suggesting that AP-1 mediates long-term genomic remodeling. We focused on Δ FosB, a truncated FosB isoform, as a candidate driver of these long-term changes due to its long half-life (~208 h⁶⁴). We analyzed its expression time course following REPOPS. After 3-day REPOPS, Δ FosB was transiently increased at 24 h but returned to baseline at 14 and 40 days (Fig. 7a). In contrast, after 10-day REPOPS, Δ FosB was elevated at 24 h and remained high at 14 and 40 days (Fig. 7a), suggesting that chronic repetition of activation induces long-lasting elevation in Δ FosB. This sustained elevation likely reflects increased baseline expression of FosB rather than residual REPOPS-induced Δ FosB (Fig. 7a, Supplementary Fig. 12b). Δ FosB levels negatively correlated with calbindin expression (Fig. 7b, Supplementary Fig. 13), which is consistent with previous reports¹⁸, suggesting that prolonged Δ FosB upregulation

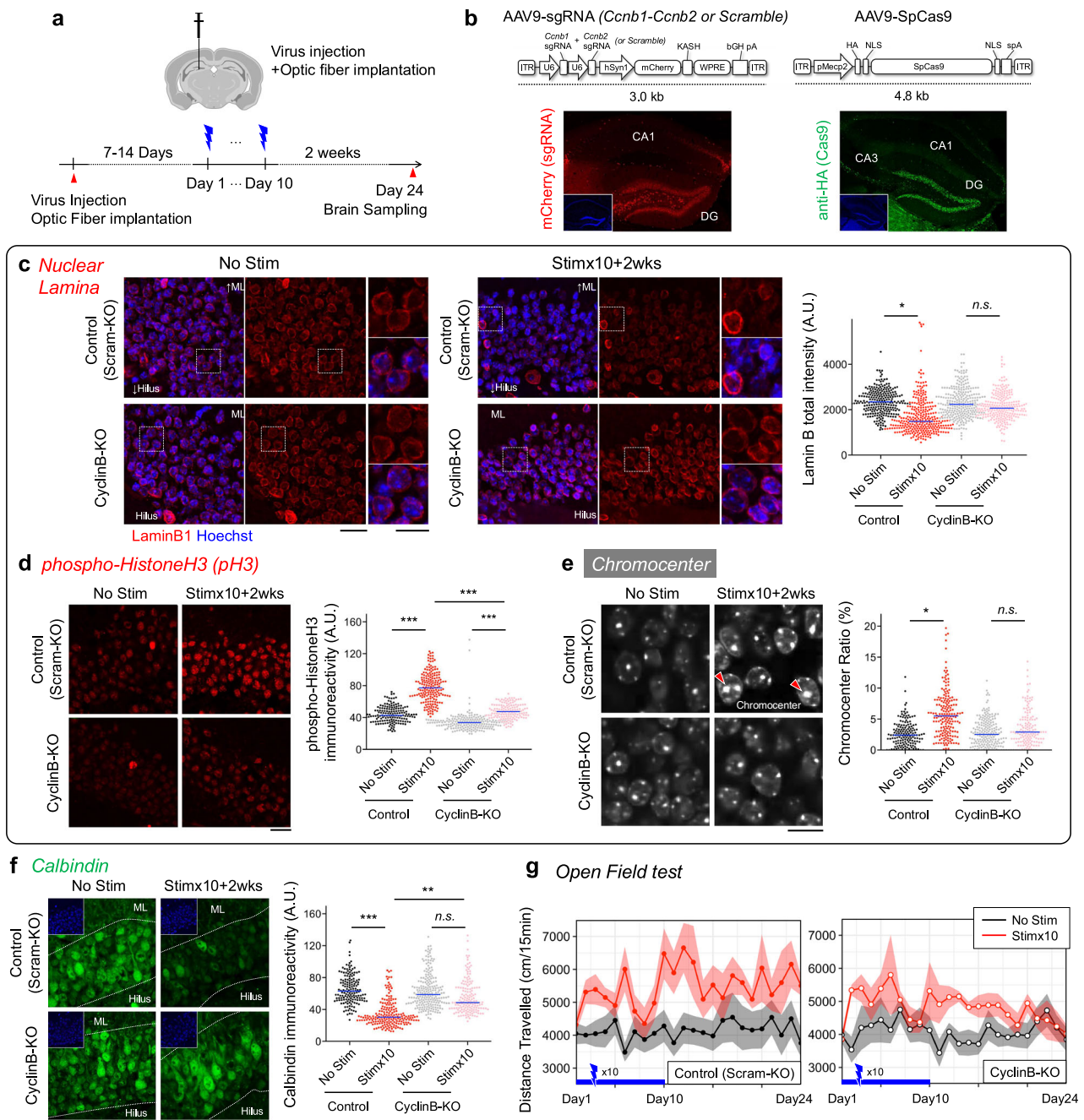
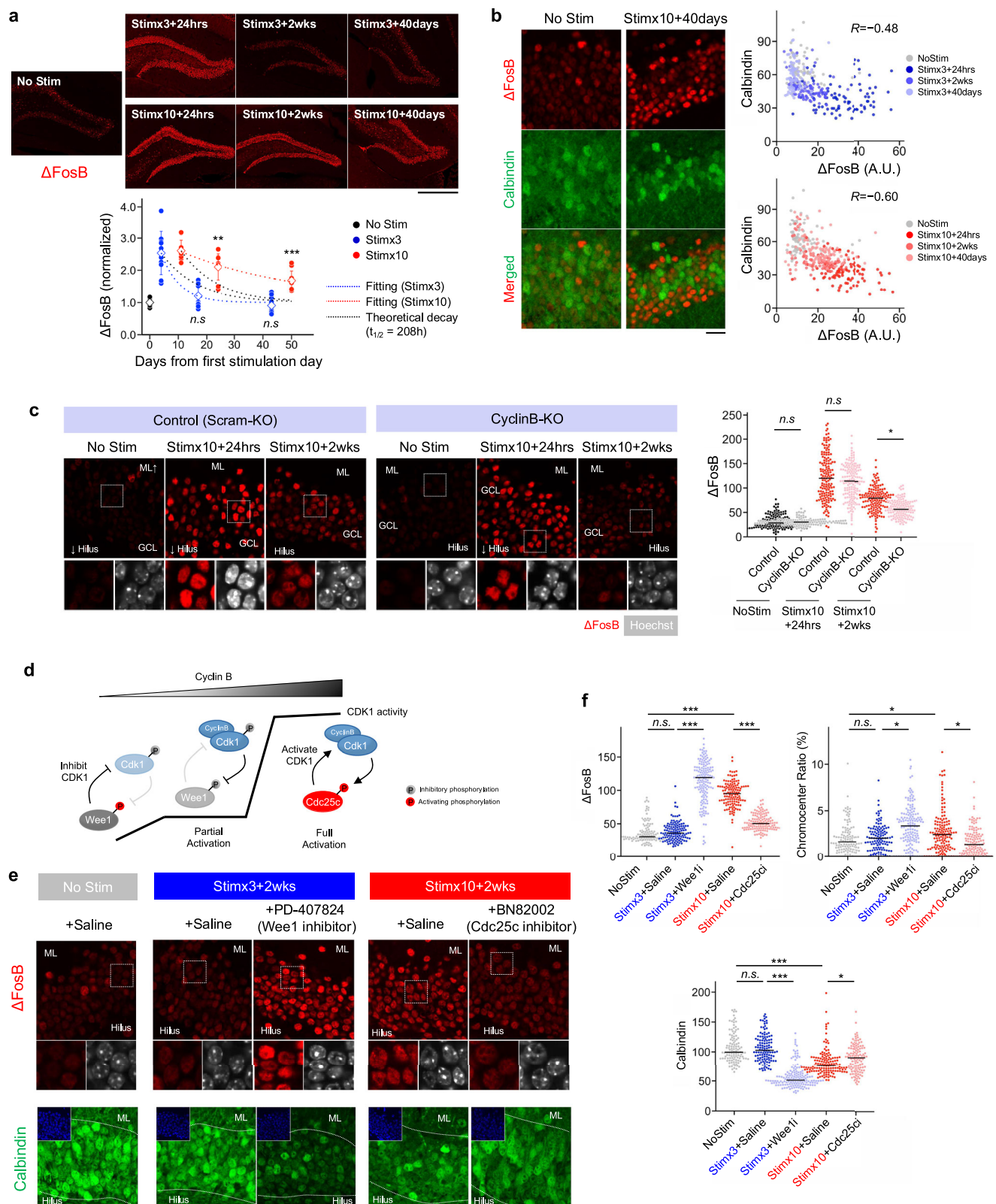


Fig. 6 | Cyclin B deletion reverses multiple REPOPS-induced phenotypes.

a Experimental design. AAV9 vectors encoding Cas9 and sgRNAs targeting *Ccnb1* and *Ccnb2* (CyclinB-KO) or a *Scramble* sequence (Scram-KO) were co-injected into the DG, followed by optoelectric stimulation. Created in BioRender. Murano (2026) <https://BioRender.com/p6ed2mm>. **b** Co-expression of sgRNAs and Cas9 in the DG. sgRNAs were co-expressed with mCherry, and Cas9 was tagged with HA. Most granule cell-layer neurons co-expressed mCherry and HA. **c** Lamin B1 immunostaining in the DG (converted to red for visibility; originally stained with Alexa647). Upper and lower panels show control (Scramble-KO) and *Ccnb1-Ccnb2*-KO (CyclinB-KO) mice, respectively. Scale bars: 20 μ m (left), 10 μ m (right). Beeswarm plot shows lamin B1 immunoreactivity in individual neurons; red bars, median. $n = 245, 301, 271$, and 236 neurons from 4 mice each for Scram-KO No Stim, Scram-KO Stim $\times$ 10+2wks, CyclinB-KO No Stim, and CyclinB-KO Stim $\times$ 10+2wks, respectively. Linear mixed-effects model (LMM, two-sided), mouse as random effect; $P = 0.039$.

Tukey's post hoc, $*P < 0.05$. **d** pH3 immunostaining in the DG. Scale bar, 20 μ m. Same four-group analysis as (c) ($n = 158, 183, 199, 143$ neurons from 3 mice each). LMM (two-sided), $P = 1.2 \times 10^{-7}$; Tukey's post hoc, $***P < 0.001$. **e** Chromocenter visualized by Hoechst staining (white, pseudo-color). Scale bar, 10 μ m. $n = 158, 183, 199, 143$ (groups as in c). LMM (two-sided), $P = 0.035$; Tukey's post hoc, $*P < 0.05$. **f** Calbindin staining in the DG. Scale bar, 20 μ m. Same four-group analysis as (c) ($n = 157, 197, 217, 158$ neurons from 3 mice each). LMM (two-sided), $P = 1.3 \times 10^{-4}$; Tukey's post hoc, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. **g** Open field test: total distance traveled during the first 15 min from Day 1 to Day 24. Left: Scramble-KO; Right: CyclinB-KO. Black and red lines represent No Stim and Stim $\times$ 10+2wks groups, respectively. Solid lines, group means ($n = 7$ mice/group); semitransparent areas, $\pm$ s.e.m. Two-way repeated measures ANOVA: significant main effects of Group ($P = 0.015$) and Day ($P = 0.020$); no significant interaction. Source data are provided as a Source Data file.



contributes to pseudo-immaturity of DG neurons (see also Supplementary Fig. 14).

To examine whether cell-cycle re-entry is involved in this sustained Δ FosB upregulation, we analyzed its expression in CyclinB-KO mice after REPOPS. At 24 h after 10-day REPOPS, Δ FosB was similarly increased in both CyclinB-KO and Scram-KO groups, indicating that cyclin B is dispensable for acute induction (Fig. 7c). In contrast, at two

weeks, Δ FosB was significantly lower in CyclinB-KO than in Scram-KO, suggesting that cyclin B expression is necessary for the sustained elevation of Δ FosB. To further assess the role of Cyclin B/Cdk1, we performed REPOPS while continuously perfusing its modulators—PD-407824 (a Wee1 inhibitor) or BN82002 (a Cdc25c inhibitor)—via an optofluidic neural probe (Fig. 2a) and measured Δ FosB expression (Fig. 7d). Two weeks after 3-day REPOPS, Δ FosB was not elevated, but

Fig. 7 | Cyclin B/Cdk1 activity mediates long-term Δ FosB expression and pseudo-immaturity of neurons. **a** Top, Δ FosB immunostaining in the DG. Scale bar, 500 μ m. Bottom, time course of Δ FosB expression normalized to No Stim mean. Day 0, first stimulation day. Black dashed line: theoretical decay of Δ FosB (half-life = 208 h); blue and red curves represent exponential fits for the Stim $\times$ 3 and Stim $\times$ 10 groups, respectively. $n = 10, 11, 10, 11, 9$, and 10 slices per group for No Stim, Stim $\times$ 3+1day, Stim $\times$ 3+14days, Stim $\times$ 3+40days, Stim $\times$ 10+1day, Stim $\times$ 10+14-days, and Stim $\times$ 10+40days, respectively. One-sample t -tests (one-sided, testing for elevation above the theoretical decay curve) at each time point; $^{**}P < 0.01$, $^{***}P < 0.001$. **b** Immunostaining of Δ FosB and calbindin in the DG (same data in **a**). Only No Stim and Stim $\times$ 10 + 40 days are shown. Scale bar, 20 μ m. Right, scatter plot of Δ FosB and calbindin immunoreactivity. **c** Δ FosB immunostaining in the DG of Scram-KO and CyclinB-KO mice. Bottom: enlarged views of white boxes; grayscale, Hoechst. Scale bar, 20 μ m. Beeswarm plot of Δ FosB immunoreactivity. $n = 138, 128$,

155, 164, 148, 132 cells from 4 mice each (groups left to right). black bars, median. LMM (two-sided), $P = 5.1 \times 10^{-11}$; Tukey's HSD post hoc pairwise comparisons, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. **d** Schematic of Cyclin B/Cdk1 activation. Cdk1 is activated by accumulating cyclin B and is further regulated by inhibitory Wee1 phosphorylation and activating Cdc25c dephosphorylation. **e** Top, Δ FosB immunostaining in the DG. Middle, enlarged view of the boxed region. Grayscale indicates Hoechst staining. Bottom, calbindin immunostaining. Scale bar, 20 μ m. **f** Beeswarm plots showing Δ FosB, calbindin, and chromocenter ratio in DG neurons of mice treated with PD-407824 (Wee1 inhibitor) or BN82002 (Cdc25c inhibitor). Black bars, median. n (groups, left to right): No Stim, Stim $\times$ 3+Saline, Stim $\times$ 3+Wee1, Stim $\times$ 10+Saline, Stim $\times$ 10+Cdc25ci: Δ FosB, 116, 128, 151, 129, 130 neurons; calbindin and chromocenter ratio, 113, 120, 155, 147, 125 neurons; 4 mice per group. LMM (two-sided), mouse as random effect, with Tukey's post hoc; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Source data are provided as a Source Data file.

PD-407824 infusion significantly increased its expression (Fig. 7e). Conversely, after 10-day REPOPS, Δ FosB remained elevated for two weeks, but this increase was suppressed by BN82002 (Fig. 7e). Calbindin showed a complementary pattern: it remained unchanged two weeks after 3-day REPOPS but decreased with PD-407824; whereas it was reduced after 10-day REPOPS but reversed by BN82002 (Fig. 7e). These findings indicate that Cyclin B/Cdk1 activity is essential for sustained elevation of Δ FosB and the associated reduction in calbindin. Thus, repeated neuronal activation and cell-cycle re-entry induce long-lasting and stable changes in activity-dependent transcription factors, global genomic architecture, and cellular maturity.

Discussion

In this study, we tested the hypothesis that brain stimulation methods, such as electroconvulsive therapy (ECT) and repetitive transcranial magnetic stimulation (rTMS), may achieve beneficial clinical effects by reprogramming cellular state into an immature but stable configuration. We developed an optogenetic stimulation, REPetitive OPtogenetic Stimulation (REPOPS), which reproducibly induces dematuration as indexed by global changes in the cellular transcriptome and 3D genome structure. Most of these changes were transient when REPOPS was repeated for three consecutive days, but could persist for more than one month after 10 consecutive days of activation, consistent with the 6–12 stimulus repeat regimen for ECT. RNA-seq data of human DG also showed that ECT induces immature-like gene-expression patterns in patients, resembling the effects by REPOPS. Underlining the clinically relevant behavioral effects of REPOPS, our stimulation protocol induces cell-cycle re-entry resembling the G₂/M phase-like nuclear structure. These changes were reversed by deletion of Cyclin B, a master regulator of the G₂/M phase transition. We further found that activation of Cyclin B/Cdk1 stabilizes Δ FosB expression, thereby promoting sustained reorganization of genome structure. Our results establish a candidate cellular mechanism underpinning the beneficial clinical effects of ECT and offer a cell-based perspective for the development of brain stimulation therapies.

Our findings reveal that optogenetic stimulation of hippocampal granule cells induces a G₂/M phase-like transcriptional signature and nuclear structural plasticity, which collectively define an immature-like cell state. There was increased expression of G₂/M phase-related genes such as Cyclin A2, Cyclin B1, Cyclin B2, and Cdc25c, and changes in nuclear structure, including nuclear lamina disruption, an increase in pH3, and chromocenter enlargement. In the literature, cell-cycle re-entry has been implicated in several neuropsychiatric and neurological disorders. For example, aberrant expression of cell-cycle-related genes, such as Cyclin A, Cyclin B, and Cdk1, has been reported in Alzheimer's disease^{65–70} and temporal lobe epilepsy⁷¹, as well as in animal models of risk events for these diseases, such as epileptic seizures^{72,73}, ischemia⁷⁴, and encephalitis⁷⁵. Nuclear alterations related

to the cell cycle, such as nuclear lamina disruption and increased pH3, are observed in the brains of patients and animal models of Alzheimer's disease^{76–78} and epileptic seizures⁷⁹. Recent large-scale GWAS studies identified several M-phase-related genes (e.g., *CCNB2*, *CDC25C*, *CENPM*, *CENPT*, *MAD1L1*, and *STAG1*) near the risk loci for schizophrenia^{80,81}. While these diseases have diverse etiologies, neural hyperexcitability and cell-cycle-like processes may be common contributors to their pathophysiology^{82–85}. This study demonstrates a causal link between chronic neural activation and G₂/M phase-like transcriptional and nuclear signatures. The cell-cycle re-entry observed in various diseases is typically associated with apoptosis^{86,87}; however, our REPOPS method did not induce significant cell death (Supplementary Fig. 2). REPOPS-triggered nuclear structural dynamics, but they were less pronounced than those in dividing cells during the late M phase^{87,88} and there were no fully compacted chromosomes (Fig. 5e, g), suggesting partial activation of G₂/M-associated programs rather than true mitotic progression. We do not assert that this immature-like cellular state is a perfect regression to developmental immaturity but rather an analog state of immaturity, a state that is phenotypically immature relative to the mature state. Another key finding of this study is the involvement of Δ FosB in neuronal dematuration. Δ FosB has previously been reported to be highly expressed in the striatum of addiction models^{89,90}, as well as in the DG of patients with epileptic seizures and Alzheimer's disease^{18,91}. Owing to its long half-life (~208 h)⁶⁴, Δ FosB is widely believed to mediate long-lasting cellular changes. However, given this half-life, stimulus-induced Δ FosB would be expected to decay to ~4% of its peak level within 40 days. Therefore, the marked increase observed in the Stim $\times$ 10+40day group (Fig. 7a) is unlikely to reflect residual Δ FosB from prior stimulation, but rather a sustained elevation of its basal expression (Supplementary Fig. 12b). This persistent upregulation of Δ FosB likely contributes to long-lasting 3D genome reorganization and neuronal dematuration. We also suggest that REPOPS triggers repeated calcium influx that initiates dematuration and cell-cycle re-entry (Fig. 2a–c). Activation of calcium signaling is essential for cell-cycle progression^{92,93}; therefore, REPOPS-stimulated neurons may transduce a specific pattern of calcium signaling that causes cell-cycle re-entry. Further studies will clarify this signaling pattern, and we predict that variations of REPOPS methodology may enable fine-scale external control of cell dematuration in vivo.

ECT is well documented as an effective treatment for psychiatric disorders such as severe depression and anxiety. The current results demonstrate that cellular dematuration triggered by chronically repeated stimulation can produce positive behavioral phenotypes, including decreased depression-like symptoms and increased motor activity, similar to the clinical ECT effects. An important question is how these behaviors are enabled by neuronal stimulation at the neural circuit level. REPOPS increased the number of speed cells and the speed coding accuracy in the DG (Fig. 4i–k). Prior studies indicate that activation of DG neurons can elicit robust exploration of the

environment⁵⁰, and our observation of increased speed-related activity in the DG may account for the increased locomotor activity observed in the open field (Fig. 3a, b) and home cage (Fig. 3c). Activation of DG neurons is also known to suppress depression-like behaviors^{94,95}, therefore, increased speed-related activities of DG neurons may explain the link between chronically repeated stimulation and the reduction in depression-like behavior observed in the tail suspension and forced swim tests (Fig. 3d and Supplementary Fig. 5c). In contrast to these beneficial effects, a potential negative side effect of ECT is memory loss, including retrograde and anterograde amnesia^{96,97}. Indeed, we observed a deficit in remote memory, or the ability to recall information from the neocortex into the hippocampal circuit (Supplementary Fig. 5a and Supplementary Result 1). REPOPS caused long-term (>2 weeks) alterations in synapse-related genes, including *Gria1*, *Gria2*, *Camk2a*, *Camk2b*, and *Arc* (Fig. 1e). Reanalysis of RNA-seq data from human patients with and without ECT also revealed significant changes in the expression of *GRIA1*, *GRIA2*, and *ARC* (Fig. 1g). These genes play pivotal roles in synaptic and homeostatic plasticity^{98–100} and their decline may be related to memory impairment due to neural hyperexcitation. Long-term down-regulation in the expression of synapse-related genes may interfere with the development of place cells and spatial information coding in the DG (Fig. 4f–h) and CA1^{15,101–103}. Such REPOPS-dependent deficit in neural plasticity may share a common mechanism with ECT-induced amnesia. Thus, REPOPS alters neural coding, leading to both decreased depressive-like behaviors and memory deficits. Further research will address the stimulus conditions that preserve beneficial clinical therapeutic efficacy without impairing memory or learning.

Our findings also provide a perspective on the mechanism of action of antidepressant therapy. In this study, we found that REPOPS-triggered cellular and behavioral effects share similarities to previously reported behavioral effects and neuronal dematuration induced by ECS or selective serotonin reuptake inhibitors (SSRIs)^{9,10,36,39}. Chronic SSRI treatment may reactivate the plastic state in the adult brain, comparable to that observed during development^{14,40}. For example, in the adult visual cortex, chronic fluoxetine reopens a critical period of plasticity for the recovery of visual function^{104,105}, and, in the hippocampus, increased neural circuit plasticity can promote fear memory extinction^{14,106}. SSRIs may regulate neural plasticity by increasing the environmental susceptibility of neural circuits, thereby reducing depression via exposure to favorable environments^{107,108}. This form of meta-plasticity^{109,110}, or the induction of changes in neural plasticity itself, can also manifest in opposite forms by neural hyperactivity. A recent study showed that recurrent seizures cause a long-term reduction in neuronal excitability, indicating decreased neural plasticity¹¹¹. In the current study, REPOPS for 10 days inhibited spatial map formation by exposure to the environment for more than two weeks (Fig. 4f–h), suggesting an altered meta-plasticity for spatial coding. Thus, neural overactivation and dematuration can alter the meta-plasticity of neural circuits, which may underlie the effects of antidepressant treatments such as ECT and rTMS, or the development of neuropsychiatric disorders involving neural hyperexcitability in their etiology. A key insight from the present work is that the immature cellular state appears to be bistable; that is, it can serve as a reversible therapeutic intermediate, as observed in ECT, or consolidate into an irreversible pathological lesion, as seen in disease models. This bistable nature likely depends on the intensity and repetition of neural hyperactivity. Therapeutic paradigms such as ECT or REPOPS involve brief, intermittent, and well-regulated stimulation^{29,112}. In contrast, pathological conditions such as epilepsy and Alzheimer's disease are characterized by uncontrolled and chronic hyperexcitability¹¹³. Timing is also critical: whether the system is in a stable recovery phase after transient hyperactivity or remains in a state of recurrent excitation profoundly influences the outcome. In our paradigm, behavioral testing

conducted approximately two weeks after the final stimulation session likely reflects a stabilized phase of circuit reorganization, whereas patients are often in a state of persistent hyperexcitability, in which neuronal homeostasis is disrupted¹¹⁴.

In summary, we propose a working model for the mechanism of brain stimulation therapies in the treatment of psychiatric disorders. This class of brain diseases may represent neuronal populations trapped in various energy minima of metabolic and/or synaptic inflexibility, such as the loss of plasticity by the pathological closure of neural circuits to environmental stimulation, as discussed above. In this model, repetitive brain stimulation methods like ECT or rTMS trigger patterned calcium influx and signaling to drive cellular dematuration that temporarily resets cellular metabolic and synaptic homeostasis to restore normal neural circuit flexibility and beneficial clinical outcomes like anti-depressive behavior. This immature-like state can persist for more than one month, and excessive neuronal activation may eventually reinforce rather than reset metabolic or synaptic dysfunction in a vicious cycle associated with chronic psychiatric disorders associated with hyperactivity. Therefore, our model suggests that the immature-like cell state may serve as a bistable switch for cellular health at moderate levels of electrical brain stimulation, or disease progression at higher levels. We believe that these findings open a window to new methods and protocols for artificial neural stimulation that may enable cell reprogramming for brain health.

Methods

Animals

All experimental procedures complied with relevant ethical regulations and were approved by the Institutional Animal Care and Use Committee of Fujita Health University (Approval No. APU22016, APU25150), based on the Law for the Humane Treatment and Management of Animals and the Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain. All experiments were conducted during the light period. Every effort was made to minimize the number of animals used. The omics experiments (RNA-seq and ATAC-seq) used male mice only to minimize potential estrogenic-cycle effects on transcriptome and chromatin accessibility; this is acknowledged as a study limitation. A subset of behavioral and histological experiments described in the Supplementary Information also included female mice in addition to males, and sex was included as a covariate in ANCOVA analyses where indicated (e.g., Fig. 3e and Supplementary Result 3); no significant main effects of sex were detected. Sex-disaggregated values for individual experiments are provided in the Source Data file.

ROSA26-CAG-stop^{fllox}-ChR2(H134R)-EYFP (Strain#012569; RRI-D:IMSR JAX:012569) and *POMC-Cre* (Strain #010714; RRI-D:IMSR JAX:010714), both maintained on a C57BL/6J background, were obtained from Jackson Laboratories (Bar Harbor, Maine). These mouse lines were crossed to generate *POMC-Cre::ChR2-EYFP* mice, which express ChR2-EYFP selectively in the granule cells (GCs) of the dentate gyrus (DG) (Fig. 1a). Three or four mice were housed in one cage and switched to individual housing 1–2 weeks prior to the experiment. They were housed with a 12 h light/dark cycle (lights on at 7:00 a.m., off at 7:00 p.m.) with access to food and water *ad libitum*. The room temperature was maintained at 23 ± 2 °C with relative humidity of 50 ± 10%.

For RNA-seq and ATAC-seq experiments, male mice aged 3–4 months were used. For other experiments, approximately equal numbers of male and female mice aged 6–12 months were used. Age-matched control groups were included in each experiment. Our previous study¹¹⁵ reported minimal behavioral differences between 2- and 11-month-old mice, indicating that the use of 3–4 month-old and 6–12 month-old mice in the present study is unlikely to confound the interpretation of the results. For the omics analyses, six mice per group were used for RNA-seq and four per group for ATAC-seq, which is

consistent with standard practice in transcriptomic and epigenomic studies^{27,28}. For behavioral experiments, 7–16 mice per group were used. Post hoc power analyses ($\alpha = 0.05$) based on the observed effect sizes indicated that the achieved statistical powers ($1 - \beta$) exceeded 0.80 in most tests, confirming that the sample sizes were sufficient to detect the main effects.

Stereotactic surgery

Optic fiber implantation. For optogenetic stimulation, a blue-light optic fiber was implanted into the dorsal DG of adult POMC-Cre::Chr2-EYFP mice using conventional methods¹¹⁶. Briefly, adult mice were anesthetized with 1.5–3% isoflurane at an oxygen flow rate of 1 L/min. The head fur was shaved, and the incision site was sterilized with 70% ethanol before the surgical procedure. The mice were mounted on a stereotaxic device (Narishige, Tokyo, Japan). After the scalp was incised and pulled aside, a 1.0 mm diameter craniotomy was created with a surgical drill on the skull above the implantation site. Using the stereotaxic device, a 250 μ m diameter optic fiber (470 nm, fiber diameter 250 μ m; TeleC-B-3mm-250 μ m, BioResearch Center, Aichi, Japan) was precisely inserted into the target site (2.0 mm posterior to bregma (AP), 1.5 mm lateral to midline (ML), and 1.5 mm ventral to bregma (DV)) and fixed to the skull with dental cement. After implantation, the scalp was sutured and treated with povidone-iodine. To avoid the effects of surgery on mouse behavior, a recovery period of 5–10 days after surgery was provided when performing behavioral experiments.

Stereotactic injection of AAV9. Anesthetization and craniotomy were performed as described above. A glass-capillary pipette (25 μ m inner-diameter tip) filled with virus suspended in mineral oil was inserted into the target site (2.0 mm posterior to bregma (AP), 1.5 mm lateral to midline (ML)) through the surgical drill on the skull. For the genome-editing experiment (Fig. 6), a mixture of adeno-associated viruses (AAVs) carrying Cas9 and sgRNA was injected into the DG. A 200 nL volume of a 1:3 mixture of AAV9-U6-sgRNA (*Ccnb1-Ccnb2* or *Scramble*)-hSyn1-mCherry (2.0×10^{13} vector genomes vg/mL) and AAV9-pMecp2-HA-SpCas9 (2.0×10^{13} vg/mL) was injected at 200 nL/min using a microinjection pump (Nanoliter 2010, WPI, FL) at three depths (1.5, 2.0, and 2.5 mm). After each injection, the glass pipette was held in place for 1–2 min before retraction to prevent leakage. The optic fiber was implanted in the same site immediately after injection. For the Δ FosB inhibition experiment (Supplementary Fig. 14), an AAV expressing Δ JunD (a dominant-negative inhibitor of AP-1 transcription factors), provided by A.J.R. and previously described in Eagle et al.¹¹⁷, was injected using the same procedure.

For SpCas9 target selection and sgRNA design, 20-nt target sequences preceding a 5'-NGG PAM motif were selected. To minimize off-target effects, the CRISPR design tool (<https://crispr.dbcls.jp/>) was used. sgRNAs targeting *Ccnb1*, *Ccnb2* and *Scramble* control were designed as follows: *Ccnb1*: 5'-GCTAC AGCTG GTCGG TGTA CCG-3', *Ccnb2*: 5'-GGCGC TGCTC CGACG CCCGA CCG-3', *Scramble*: 5'-GCACT ACCAG AGCTA ACTCA CCG -3'. The backbone plasmid for sgRNA (PX552; pAAV-U6sgRNA(Sap1)_hSyn-GFP-KASH-bGH) was obtained from Addgene. *Ccnb*-sgRNA (tandemly connecting *Ccnb1* and *Ccnb2*; Fig. 6b) and *Scramble*-sgRNA nucleotides were synthesized and cloned into AAV backbone plasmids (Vigene Biosciences, USA). To avoid fluorescent overlap with Chr2-EYFP, the GFP cassette was replaced with mCherry (Fig. 6b). The plasmid for SpCas9 (PX551; pAAV-pMecp2-SpCas9-spA) was also obtained from Addgene. Concentrated AAV vectors were produced from these plasmids with support from the Gunma University Initiative for Advanced Research Viral Vector Core.

Implantation of the injection cannula. In the experiment that combined optogenetic stimulation and drug injection (Fig. 2 and Fig. 7d–f), a guide cannula (24-gauge, 11 mm length, C316G, Plastics One) was

implanted into the dorsal DG using the same procedure as that used for optic fiber implantation.

Optogenetic stimulation and drug infusion

For optogenetic stimulation under freely moving conditions, we employed a wireless LED illuminator, Teleopto (BioResearch Center, Nagoya, Japan). A 2.0 g infrared receiver (TeleR-2-P, BioResearch Center) was attached to an optic fiber implanted in the mouse head, whereas in the control group, a dummy receiver was used as a substitute. This receiver was signaled by an infrared generator (Tele-Emitter-C, BioResearch Center) to light up the optical fiber. The light intensity varied depending on the custom-made fiber, typically within a range of 3–5 mW. We set the stimulation protocol to 10 ms pulses at a frequency of 10 Hz, utilizing a pulse generator (STOmK-2, BioResearch Center). These photostimulation intensities are sufficiently weak to avoid tissue damage¹¹⁸.

For optogenetic stimulation with an intracerebral drug infusion (Fig. 2 and Fig. 7d–f), we employed injection cannulas (31-gauge, 11 mm length, C316I, Plastics One) and customized optic fibers designed to fit the implanted guide cannula (Bio Research Center). For acute injection (Fig. 2), the injection cannula was attached to the implanted guide cannula, and 2 μ L of the drugs was injected over approximately 5 min. The Ca^{2+} blockers consisted of 100 mM NBQX (ab120046, Abcam), 100 mM AP5 (ab120003, Abcam), 100 mM mibefradil (ab120343, Abcam), and 100 mM nimodipine (ab120138, Abcam) diluted in saline (Fig. 2c). Kinase inhibitors, K252a (sc-200517, Santa Cruz), SB218078 (sc-203692, Santa Cruz), Dasatinib (sc-218081, Santa Cruz), and Ro-32-0432 (sc-3549, Santa Cruz), were used at the same concentration (1.0 mM). Five minutes after the injection, the injection cannula was gently detached, and a customized optic fiber was inserted through the guide cannula. For chronic drug infusion (Fig. 7d–f), an osmotic pump (Alzet model 1004) was implanted subcutaneously and connected to a guide cannula. The pump was filled with PD-407824 (sc-203669, Santa Cruz) or BN82002 (#217691, Calbiochem) and delivered the drug at a constant rate. For the effective infusion of drugs into brain tissues in vivo, the concentration of the solution was set to 100–1000 times higher than that used for bath applications in acute slice cultures according to previous studies¹¹⁹. Stimulation was performed in the same manner as described above. All procedures were conducted in an awake state.

RNA-sequencing library construction, sequencing, mapping, and data analysis

RNA-seq was performed with six biological replicates per group ($n = 6$). All animals were male mice and exhibited high RNA integrity numbers (RINs); thus, sex and RIN were not included as covariates in the differential analysis. The dentate gyrus was rapidly dissected from the adult mouse hippocampus under a dissection microscope¹²⁰. Briefly, the brain was rapidly removed and placed in ice-cold PBS. A coronal cut was made at the midbrain level, and the cerebral hemispheres were separated. The hippocampus was carefully isolated from each hemisphere, and the dentate gyrus was microdissected from the hippocampal formation under a stereomicroscope using fine forceps, avoiding contamination from CA1, CA3, or other adjacent regions. Previous studies have shown that such preparations are highly enriched with mature dentate granule neurons (~90% NeuN⁺ neurons^{27,121}; see also Supplementary Fig. 15a, d). Total RNA from dissected tissue samples was extracted using NucleoSpin RNA (Macherey-Nagel, Duren, Germany), according to the manufacturer's instructions. The RNA quality was assessed with an Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA, United States). Sequencing libraries were prepared using the TruSeq Stranded mRNA Sample Prep Kit (Illumina, San Diego, CA, USA) and sequenced as 100-bp paired-end reads on the Illumina HiSeq 2500. Sequenced data were mapped onto the *Mus musculus* genome (GRCm38/mm10) using TopHat (v2.0.14).

Fragments per kilobase per million (FPKM) values were computed by Genedata Profiler Genome (v10.1.12). Genes with low expression (FPKM < 0.1) were excluded from the analysis to avoid an excessively high FDR due to low expression levels. Differential expression analyses were performed using DESeq2. The R scripts used for these analyses are publicly available on GitHub (<https://github.com/tmurano/REpeatedOPtogeneticStimulation>)¹²².

ATAC-sequencing library construction, sequencing, mapping, and data analysis

ATAC-seq was performed using $n = 4$ biological replicates per group. The dentate gyrus of adult mice was dissected in the same manner as that used for RNA-seq. The dentate gyrus was slowly frozen in a freezing box (Cat. No. 5100-0001; Mr. Frosty Freezing Container, Thermo Fisher Scientific). Flash-frozen tissue was sent to Active Motif to perform the ATAC-seq assay. The tissue was manually dissociated, isolated nuclei were quantified using a hemocytometer, and 100,000 nuclei were tagmented as previously described¹²³, with some modifications based on Corces et al.¹²⁴ using the enzyme and buffer provided in the Nextera Library Prep Kit (Illumina). Tagmented DNA was purified using the MinElute PCR purification kit (Qiagen), amplified with 10 cycles of PCR, and purified using Agencourt AMPure SPRI beads (Beckman Coulter). The resulting material was quantified using the KAPA Library Quantification Kit for Illumina platforms (KAPA Biosystems) and sequenced using paired-end 42 bp sequencing on a NextSeq 500 sequencer (Illumina).

Reads were aligned to the mm10 genome using BWA (mem mode, default parameters). Duplicate reads were removed, and only reads mapping as matched pairs and uniquely mapped reads (mapping quality ≥ 1) were used for further analysis. Alignments were extended in silico at their 3' ends to a length of 200 bp and assigned to 32-nt bins along the genome. The resulting histograms (genomic signal maps) were stored in the bigWig files. Peaks were identified using the MACS 2.1.0 algorithm at a cutoff of P -value 1.0×10^{-7} , without the control file, and with the -nomodel option. Peaks on the ENCODE blacklist of known false ChIP-Seq peaks were excluded. Intervals identified by MACS2 were merged by taking the union of overlapping peaks across all samples, ensuring consistent quantification among replicates. Read counts within merged regions were obtained using Subread, and differential accessibility was analyzed using DESeq2 (adjusted $P < 0.05$). Quality control metrics confirmed robust data quality: FRiP scores ranged from 37 to 51%, exceeding the standard 10% benchmark. Aggregate accessibility profiles around promoters (TSS ± 5 kb) showed clear enrichment in all samples, confirming high signal-to-noise ratios (Supplementary Fig. 16). Significantly up- and downregulated regions were exported as BED files, and 200-bp windows centered on each peak midpoint were analyzed for motif enrichment using HOMER. Both known and de novo motifs were identified after repeat masking to minimize false positives. Correlation analysis further confirmed high reproducibility across biological samples. Statistical analyses were performed using an in-house R script, unless otherwise specified.

Immunohistochemistry

Immunostaining of a brain sample with PFA fixation. The mice were deeply anesthetized with isoflurane and transcardially perfused with PBS, followed by 4% PFA in PBS. Brains were dissected, immersed overnight in 4% PFA in PBS, and cryoprotected by incubation in 20% sucrose in PBS for three days at 4 °C. After cryoprotection, the brains were mounted in Tissue-Tek optimal cutting temperature compound (Miles, Elkhart, IN) and frozen in liquid nitrogen. Brain samples were cut into 10- μ m-thick coronal sections using a microtome (CM1850; Leica Microsystems, Wetzlar, Germany). The sections were stained

with the following antibodies: Calbindin (1:1000, rabbit, polyclonal, Synaptic Systems Cat# 214 002, RRID:AB_2068199), Cyclin B (1:500, mouse, monoclonal, Thermo Fisher Scientific Cat# MA1-155, RRID:AB_2536863), phospho-Histone H3 (1:500, rabbit, polyclonal, Millipore Cat# 06-570, RRID:AB_310177), HA-tag (1:500, rabbit, polyclonal, Cell Signaling Technology Cat# 3724, RRID:AB_1549585), Δ FosB (1:1000, rabbit, monoclonal, Cell Signaling Technology Cat# 14695, RRID:AB_2798577), Calbindin (1:500, goat, polyclonal, Frontier Institute Cat# MSFR100410, RRID:AB_2571569), NeuN (1:500, mouse, monoclonal, Millipore, Cat# MAB377X, RRID:AB_2149209), GFAP (1:500, rabbit, polyclonal, Sigma-Aldrich, Cat# G9269, RRID:AB_477035), Iba1 (1:500, rabbit, polyclonal, FUJIFILM, Cat# 019-19741, RRID:AB_839504), JunD (1:500, rabbit, polyclonal, Abcam, Cat# ab28837, RRID:AB_2130167), and tri-methyl-Histone H3 (Lys9me3) (1:500, mouse, monoclonal, FUJIFILM Cat# MAB10308, RRID:AB_2887767). The Δ FosB antibody (Cell Signaling Technology, Cat# 14695, clone D3S8R, RRID:AB_2798577) specifically recognizes the Δ FosB isoform (35–37 kDa) without cross-reactivity to the full-length FosB (46–48 kDa), as verified by both manufacturer validation and independent reports¹²⁵. Before the first antibody reaction, antigen retrieval was performed in 120 °C autoclave for 5 min in 0.01 M sodium citrate buffer (S1699, Target Retrieval Solution, Dako). An autoclave at 120 °C is essential, especially for detecting weak signals of nuclear antigens (e.g., pH3 and Cyclin B). This procedure also eliminates EYFP/mCherry fluorescence, thus preventing overlap with secondary antibody staining with Alexa 488/594. After antigen retrieval, sections were cooled at room temperature for 2 h. Sections were pre-incubated for 1 h at room temperature in 5% skim milk in PBST for blocking, and then incubated overnight at 4 °C in PBS containing the primary antibodies. The next day, secondary antibody reactions were performed for 1 h at room temperature using Alexa Fluor 488-, Alexa Fluor 594-, and Alexa Fluor 647-conjugated secondary antibodies (Invitrogen). Nuclear staining was performed using Hoechst 33258 (Polysciences, Warrington, PA).

Immunostaining of a brain sample with acetone fixation. For Lamin B1 staining (1:200, mouse, monoclonal, Thermo Fisher Scientific Cat# MA1-06103, RRID:AB_2281281), a method omitting PFA perfusion was employed^{126,127}. In this protocol, brains were perfused with PBS alone, without PFA. Immediately after PBS perfusion, the dissected brains were mounted in Tissue-Tek compound and frozen in liquid nitrogen. Brain samples were cut into 10- μ m-thick coronal sections using a microtome. Before the first antibody staining, the brain sections were fixed with ice-cold acetone. Antigen retrieval before the blocking step was omitted, and subsequent procedures were performed in the same manner as for immunostaining of the fixed brain samples.

TUNEL staining. In Situ Cell Death Detection Kit TMR red (Roche, 12156792910) was used for the detection of apoptotic cells. Brain sections were rinsed in PBS, permeabilized with 0.1% Tween for 30 min, and incubated with TUNEL reaction reagent (TdT Enzyme and Labeling Safe Buffer) and Hoechst for 60 min at room temperature. Embryo brain tissue was used as the positive control. The localization of TUNEL to the nucleus allows for accurate counting of dead or dying cells.

Image acquisition and analysis. Confocal microscopy (LSM 700; Zeiss) was used to obtain images of stained sections. For image analysis, the regions of interest (ROIs) were manually delineated using ZEN (Zeiss) and ImageJ software.

Behavioral tests

Open field test. The open field test was performed in an open field apparatus (40 cm $\times$ 40 cm $\times$ 30 cm; width, depth, and height, respectively; O'Hara, Japan) made of opaque white plastic, with the center

area illuminated to 100 lux by an LED light attached above the ceiling. Before each behavioral experiment, the open field apparatus was cleaned with weakly acidified hypochlorous water (super-hypochlorous water; Shimizu Laboratory Supplies, Kyoto, Japan) to prevent bias due to olfactory cues. All behavioral experiments were conducted in a soundproof room. During each recording session, mouse movements were captured using a CCD camera above the open field arena. Mouse behavior was monitored using a computer screen located outside the room to minimize artifacts caused by the experimenter's presence. Mouse behavior was recorded at a sampling rate of 10 Hz. Mouse images were automatically processed using the ImageJ plugin (Image OF, freely available on the Mouse Phenotype Database website: <http://www.mouse-phenotype.org/software.html>) to obtain the location-time sequence for each mouse. From the traces of mouse movements, we calculated the total distance traveled by the mice during the session¹¹⁵.

Locomotor activity monitoring in a home cage. Locomotor activity monitoring was performed on mice that had been operated on at least one month before. The system automatically analyzed the locomotor activity of the mice in their home cage¹²⁸. The system contains a home cage (29 × 18 × 12 cm) and a filtered cage top, separated by a 13-cm-high metal stand containing an infrared video camera, which is attached to the top of the stand. Each mouse was individually housed in each home cage, and their locomotor activity was monitored 24 h a day. Outputs from the video cameras were fed into a computer, and images from each cage were captured at a rate of one frame per second. The distance traveled was calculated automatically using the ImageJ plugin¹¹⁵.

Tail suspension test. The tail suspension test was performed to assess depression-related behavior in mice. Each mouse was suspended 30 cm above the floor by the tail in a white plastic chamber (44 cm height × 49 cm length × 32 cm width; O'Hara, Japan) with a video camera mounted on the wall (O'Hara, Japan). The behavior was recorded for 10 min. Images were captured at two frames per second using a video camera and transferred to a computer. The area (pixels) within which the mouse moved was measured for each pair of successive frames. When the area was below a certain threshold, the mouse behavior was judged as immobile. When the area equaled or exceeded the threshold, the mouse was considered moving. The optimal threshold for this judgment was determined by adjusting it to the amount of immobility measured by a trained human observer. Immobility lasting for <2 s was not included in the analysis. Data acquisition and analysis were performed automatically using the ImageJ plugin software¹¹⁵.

Porsolt forced swim test. The Porsolt forced swim test was performed to assess depression-related behavior. A Plexiglas cylinder (20 cm height × 10 cm diameter) was placed in a test chamber (49 cm height × 44 cm length × 32 cm width; O'Hara, Japan). A video camera was mounted on the ceiling of the test chamber and positioned directly above the cylinder. The mice were placed into a cylinder, which was filled with water (approximately 23 °C) to a height of 7.5 cm.

The immobility time was recorded for 10 min. Images were captured at two frames per second through the video camera. The immobility status of each mouse was determined in the same manner as for the tail suspension test. Data acquisition and analysis were performed using the ImageJ plugin¹¹⁵.

Novelty-suppressed feeding test (NSFT). Mice were individually housed in plastic cages (250 × 182 × 139 mm) with fresh paper-chip bedding. After 24-h food deprivation with ad libitum access to water, each mouse was placed in a brightly lit (400 lux) novel open field (square acrylic arena, 40 × 40 cm) containing a single familiar chow

pellet at the center. Latency to feed was recorded as the time from placement in the arena until the mouse grasped and bit the pellet with its forepaws while sitting on its haunches. A cutoff time of 600 s was applied to non-eaters. Immediately after the test, mice were returned to their home cage, and percent body-weight loss during deprivation was calculated to control for appetite and motivational differences. The procedure followed established protocols⁵⁴.

Sucrose preference test (SPT). Mice were individually housed in plastic cages (250 × 182 × 139 mm) and given two bottles of filtered tap water for acclimation. On the following day, one bottle was replaced with 1% (w/v) sucrose solution while the other contained water. Fluid intake was measured by weighing the bottles at approximately 24-h intervals for 2 consecutive days, with left-right positions alternated daily to prevent side bias. Sucrose preference (%) was calculated as $100 \times [\text{mean sucrose intake} / (\text{mean sucrose} + \text{mean water intake})]$ across the 2-day testing period.

Contextual and cued fear-conditioning tests. Fear conditioning tests were conducted to assess contextual memory (Supplementary Fig. 5a). In the conditioning session, each mouse was placed in an acrylic chamber consisting of white (side) and transparent (front, rear, and top) walls (33 × 25 × 28 cm) with a stainless-steel grid floor (0.2 cm diameter, spaced 0.5 cm apart; O'Hara, Japan). The mice were allowed to explore the chamber freely for 120 s, and 55 dB white noise then served as the conditioned stimulus (CS) for 30 s. During the last 2 s of the CS presentation, a mild foot shock (0.3 mA, 2 s) was delivered as the unconditioned stimulus (US). The mice were subjected to two more CS-US pairings with a 2-min interstimulus interval. Approximately 24 h or one month after conditioning, a context test was conducted for 300 s. In the context test, mice were placed in the same chamber in which they had been conditioned. After the context test, a cued test with an altered context was performed for 360 s. In the cued test, the mice were placed in a triangular chamber (33 × 29 × 32 cm) made of white plastic walls and floor, which was located in a different sound-attenuating room and allowed to explore the triangular chamber for 180 s. Then, the CS was presented during the last 180 s of the cued test. In each session, the percentage of freezing and distance traveled was calculated automatically using the ImageJ plugin¹¹⁵.

Three-chamber social interaction test. The testing apparatus consisted of a rectangular three-chambered box and lid with a video camera (O'Hara, Japan; Supplementary Fig. 5b). The dividing walls of the chamber were made of transparent plastic, with small square openings (5 × 3 cm) allowing access to each chamber (20 × 40 × 47 cm). A small round wire cage (9 cm in diameter, 11 cm in height, with vertical bars 0.5 cm apart) was located in the corner of the chambers. The test mice were first placed in the middle chamber and allowed to explore the entire test chamber for 10 min. Immediately after the 10-min period, the test mice were placed in a clean holding cage, and a male C57BL/6J mouse (stranger mouse) with no prior contact with the test mice was enclosed in one of the wire cages. The test mice were returned to the middle chamber and allowed to explore for 10 min. The time spent in each chamber and the time spent around each cage were automatically measured using ImageCSI software¹¹⁵.

Corticosterone treatment. Corticosterone (95%, FUJIFILM Wako Pure Chemical Co., Japan) was dissolved in ethanol (99.5%, FUJIFILM Wako Pure Chemical Co., Japan) at 10 mg/mL and then diluted with filtered drinking water to a final concentration of 0.1 mg/mL corticosterone in 1% ethanol. Mice had ad libitum access to bottles containing either the CORT or vehicle solution. The solutions were replaced every 2–4 days. Oral administration continued throughout the experiment except during the sucrose preference test.

in vivo Ca^{2+} imaging with optogenetic stimulation

Viral constructs. Purified and concentrated adeno-associated virus serotype 5 coding GCaMP6f under the synapsin promoter (AAV5-syn-GCaMP6f; #100837-AAV5) and adeno-associated virus serotype 5 coding for ChrimsonR-tdTomato under the synapsin promoter (AAV5-syn-ChrimsonR-tdT) were obtained from Addgene and UNC Vector Core Facility.

Viral delivery of Ca^{2+} sensor and opsin. For delivery of a fluorescent Ca^{2+} sensor and red-shifted opsin into dDG neurons, AAV5-syn-GCaMP6f and AAV5-syn-ChrimsonR-tdT were injected into the dorsal DG in adult mice (left hemisphere; 2.0 mm posterior to bregma [AP], 1.0–1.45 mm lateral to midline [ML], and 2.0 mm ventral to bregma [DV]). Briefly, adult mice (>8 weeks old) were anesthetized with 1.5–3% isoflurane at an oxygen flow rate of 1 L/min. The head fur was shaved, and the incision site was sterilized with 70% ethanol prior to the surgical procedure. Mice were mounted on a stereotaxic device (catalog no. 51730D, Stoelting), and heat pad (catalog no. BWT-100A, BRC) was placed underneath each mouse to maintain body temperature at 37 °C. After the scalp was incised and pulled aside, a 2-mm-diameter craniotomy was created with a surgical drill on the skull above the injection site. Through a glass-capillary injection pipette (25- μm inner-diameter tip), a mixture of 300 nl of AAV5-Syn-GCaMP6f-WPRE-SV40 (titer 4.3×10^{12} GC/mL) and AAV5-syn-ChrimsonR-tdT (titer 1.53×10^{12} GC/mL) was injected using a microinjection pump (Nanoliter 2010, WPI). After the injection, the scalp was sutured and treated with povidone-iodine. The expression of GCaMP6f is driven by the Syn promoter and is expected to be induced not only in GCs but also in other cell types. We previously reported that the majority (approximately 90%) of neurons expressing GCaMP in the DG are GCs¹⁵.

GRIN lens implantation. At least two weeks after viral injection, a gradient refractive index (GRIN) lens (Inscopix catalog no. 1050-004623, 1 mm diameter) was implanted at the same position where the virus was injected. The mouse was mounted on a stereotaxic device, and the scalp was removed. After exposing the skull and removing the overlying connective tissue, we made a cranial hole slightly larger than the diameter of the GRIN lens. To secure an entry path for the GRIN lens, the cortex and white matter were aspirated using a 26-gauge blunt needle attached to a vacuum pump; to prevent aspiration of the GCs, the depth of the needle tip was set at 1.6 mm from the bregma, and the tissue below was carefully aspirated. Saline was continuously applied during aspiration to prevent the tissue from drying. During implantation, the lenses were held in place and slowly lowered using a ProView Implant kit (nVoke, Inscopix, Palo Alto, California, USA) attached to the stereotaxic arm. The GRIN lens connected to the manual stereotaxic arm was slowly lowered (5–10 $\mu\text{m/s}$) until the field of view was in focus, referring to the fluorescence intensity of the GCaMP. After temporarily immobilizing the lens with ultraviolet-curable resin (Primefil, Tokuyama Dental), it was fixed to the exposed skull using dental cement (Super-Bond C&B, Sun Medical). The exposed tip of the GRIN lens was covered with dental silicone (Dent Silicone-V, Shofu). After lens implantation, analgesic and anti-inflammatory agents (Flunixin, 2 mg/kg; Fujita) and antibiotics (Tribrissen, 0.12 mL/kg; Kyoritsu Seiyaku) were injected intraperitoneally.

Baseplate attachment. At least two weeks after GRIN lens implantation, the baseplate of a miniature microendoscope (nVoke, Inscopix) was attached to the GRIN lens using a previously described method¹⁵. Briefly, the mice were anesthetized and mounted onto a stereotaxic device, and a baseplate attached to the miniature microscope was placed on the GRIN lens using a gripper (nVoke accessory, Inscopix). The optimal location was determined by monitoring the fluorescent images of GCaMP-expressing neurons (where the largest number of neurons was in focus), and the baseplate was fixed with ultraviolet-

curable resin and dental cement at this position. In cases where we failed to identify neurons at this stage (usually due to the failure of GRIN lens implantation), the baseplate was not attached, and such mice were excluded from the experiment. After the baseplate attachment, a baseplate cover was placed on the baseplate until Ca^{2+} imaging was performed.

Ca^{2+} imaging and optogenetic stimulation in freely moving mice. Ca^{2+} imaging of the DG neurons was performed while the mice traveled freely in the OF. Prior to Ca^{2+} imaging, the mice were lightly anesthetized, and a miniature microscope (nVoke, Inscopix) was mounted onto the baseplate of the mice. The mice were then placed back in their home cages and transferred to a soundproof behavioral experiment room. At least 30 min after recovery from anesthesia, the mice were subjected to OF. The Ca^{2+} signals of their DG neurons were obtained at a sampling rate of 10 Hz with a 1440×1080 -pixel resolution. Recordings per day totaled 50 min, consisting of before (0–30 min), during (30–35 min), and after (35–50 min) optogenetic stimulation; no stimulation was introduced in the control group. These 50-min recordings were performed daily throughout the experimental period, and the first 30 min recordings were used for data analysis. The experimental period consisted of before stimulation (Day 1), during stimulation (Days 2–11), and two weeks after stimulation (Days 24–26). We applied EX-LED (filtered with a 435–460 nm excitation filter) for the excitation of GCaMP6f fluorescence (0.4 mW/mm² at the bottom of the GRIN lens) and OG-LED (filtered with a 590–650 nm excitation filter) for the excitation of ChrimsonR (10 mW/mm² at the bottom of the GRIN lens). A previous study reported that EX-LED at 2.0 mW/mm² resulted in small current changes of approximately 10 pA via activation of ChrimsonR under slice preparation (approximately 130 pA by OG-LED irradiation)¹²⁹. Given that the EX-LED used in this study is much lower than these conditions (0.4 mW/mm²) and that under in vivo imaging conditions, the EX-LEDs are scattered in the brain tissue, and the current changes are much smaller than in slice preparation¹²⁹, unintended activation of ChrimsonR by EX-LED would be negligible.

Detection of Ca^{2+} transients. To extract the activity patterns of individual DG neurons from the obtained fluorescent images, we used Inscopix Data Processing Software (IDPS 1.8.0). Briefly, the data files of the raw sequential fluorescent images obtained by nVoke (Inscopix) were imported to the IDPS, and movie trimming, data preprocessing, spatial filtering, and motion correction were applied. The instantaneous fluorescence of the motion-corrected images was then normalized by its average fluorescence over the entire recording period, producing fluorescence-change ratio ($\Delta F/F$) images. Individual DG neurons were identified using the automated principal component analysis (PCA) and independent component analysis (ICA) segmentation of activity traces. For PCA/ICA segmentation, default parameters, whose values were confirmed to work well across most of the neuronal activity patterns in the cortex and CA1 of the hippocampus, were used (number of independent components, 120; number of principal components, 150; ICA max iterations, 100; ICA random seed, 0; ICA convergence threshold, 0.00001; block size, 1000; ICA unmixing dimension, spatial; ICA temporal weights, 0.00; and arbitrary units within the IDPS). Finally, the timing of DG neuron activation was identified by an event-detection algorithm available in IDPS (event threshold factor, four median absolute deviations; event shortest decay time, 0.2 s), and the temporal pattern of activity was expressed as a time series of binarized signals for further analysis.

Information coding in individual neurons

Definitions of the position and speed of mice. (i) Position: The OF arena (which had an area of 40 cm $\times$ 40 cm) was represented as a 200 $\times$ 200 pixel grid. The position of the mouse was determined from

the centroid of its shadow on the CCD camera, above the OF. We then assigned a label corresponding to the discrete location of the mouse (e.g., [10, 100]) to each time bin (≈ 0.1 s).

(ii) Speed: From the distance traveled between 1 s before and after a given time point, we calculated the speed of the mouse at that moment and assigned this speed (cm/s) to each time bin.

Statistical analysis of spatial and speed information. To quantify the tuning specificities of neurons with respect to the position and speed of the mouse, we measured their specificity in terms of the information rate of cell activity, and defined them as (i) spatial and (ii) speed information^{15,130}. The Ca^{2+} event rate in Ca^{2+} imaging was considerably lower than that in electrophysiological recordings (average, approximately 0.05 Hz). If the discretization of position and speed is too fine for the number of events in the recorded cells, a proper null distribution cannot be obtained when creating shuffled data for that cell. Therefore, we set the resolution of the discretization of the position and speed to be lower than those that are commonly used.

(i) We used a 2×2 square grid to measure spatial information and computed the amount of Shannon information conveyed by a single Ca^{2+} transient about the animal's position. The spatial information I (bits per Ca^{2+} transient) of a cell was calculated as the mutual information score between the occurrence of a single Ca^{2+} transient in the cell and the animal's behavioral state of position using the following formula:

$$I_{(\text{bits}/\text{Ca}^{2+} \text{ transient})} = \sum_{i=1}^N p_i \frac{r_i}{r} \log_2 \frac{r_i}{r}$$

where i is the bin number corresponding to the physical parameter (in this case, spatial position in the OF; $i = 1-4$ from the 2×2 square grid), N is the total number of bins, p is the probability that the mouse occupied bin i , r_i is the mean transient rate at bin i , and r is the overall mean transient rate.

(ii) To measure speed information, we applied the same formula to the speed after discretizing it to a binary state: Run (speed > 1 cm/s) and Stop (speed < 1 cm/s).

Definitions of place cells and speed cells. To evaluate the statistical significance of the information encoded in individual neurons, we employed the random permutation method as previously outlined¹⁵. The calcium event data were divided into 1000 segments along the time axis and randomly shuffled to generate permuted calcium event data. This method disrupts the temporal structures of neural activity and the temporal correlations between neural activity and behavioral variables. Using this randomly permuted data, we derived a null distribution for the position and speed information of neurons. Neurons with original information content exceeding the top 95% and 99% of these null distributions were classified as place or speed cells, respectively, indicating cells with significant spatial or speed information^{131,132}.

Decoding position and speed in the open field

To determine how OF behavioral parameters are encoded in the population activities of DG neurons, we trained decoders using machine learning methods to predict the position and speed of mice from the population Ca^{2+} activity. We assigned labels to the discretized behavioral parameters of the mouse (position (cm) and speed (cm/s)) and the binary values of the Ca^{2+} signal (0 or 1) of all neurons in each time bin. We then divided the Ca^{2+} imaging data and behavioral data of the first 30 min (before 5-min of optogenetic stimulation each day) into the first 15 min and last 15 min halves, which were designated as training and test data, respectively. For each pair of behavioral parameters (position and speed) and the value of the Ca^{2+} signal in the training data, we trained the decoders using a machine learning

method (the code was obtained and modified from Glaser et al.¹³³). The decoding accuracy of the position was reported as the mean absolute error of the distance between the predicted and actual positions. In the case of speed, decoding accuracy was reported as the correlation between the predicted and actual instantaneous speeds. This is because speed, unlike position, is not limited to a certain range, and the mean absolute error of speed depends on the average locomotion speed of each mouse, which is inappropriate for the evaluation of the decoding error. If the number of neurons recorded in each session was less than 10, the session was excluded from the decoding analysis.

Stimulated Emission Depletion Microscopy (STED) observation

Brain slices were double-immunostained with anti-tri-methyl-Histone H3 (Lys9me3) antibody (1:500, mouse, monoclonal, MAB10308, FUJIFILM) and anti-phospho-Histone H3 antibody (1:500, rabbit, polyclonal #06-570, Cell Signaling) as the primary antibodies, followed by Alexa Fluor 488- and Alexa Fluor 555- conjugated antibodies (Molecular Probes) as the secondary antibodies. STED images were acquired using a TCS SP8 STED 3X system (Leica Microsystems, Wetzlar, Germany) with 2ch HyDSMD detectors¹³⁴. A white laser (for excitation) and a 660 nm laser with a donut beam (for stimulated emission depletion) were used for STED imaging. For the detection of signals of tri-methyl-Histone H3 (Lys9me3) and phospho-Histone H3, we used excitation wavelengths of 488 and 561 nm, respectively. Raw STED images (pixel size: 20 nm) were recorded with a $93\times$ glycerol immersion objective (Leica HC PL APO 93 $\times$ /1.30 GLYC motC STED W) and deconvoluted using the Huygens software (SVI, Hilversum, Netherlands). Image analysis was performed with a custom-developed code using the image processing toolbox in MATLAB (MathWorks, R2022a).

Data collection, preprocessing, and meta-analysis

All microarray and RNA-seq data used for meta-analysis in this study were obtained from the BaseSpace Correlation Engine (<https://japan.uscc.informatics.illumina.com/c/nextbio.nb>; Illumina, Cupertino, CA), a publicly available database containing over 100,000 microarray and RNA-seq datasets. The datasets registered in BaseSpace underwent several preprocessing, quality control, and organization stages. Quality control ensures the integrity of samples and datasets. Genes with a P -value < 0.05 (without correction for multiple testing) were included in the differentially expressed gene datasets. This sensitivity threshold is typically the lowest used with commercial microarray platforms and the default criterion in BaseSpace analyses⁴³. Correction for multiple testing was omitted to minimize false negatives at this stage. Expression values were used to calculate fold changes and P -values between the two conditions (infants–adults and treated–untreated). To determine fold changes, we divided the expression values of the probes/genes in the test datasets by those in the control datasets. If the fold change was < 1.0 , these values were converted to a negative reciprocal or $-1/(\text{fold change})$. Genes with a t -test P -value < 0.05 were imported into the BaseSpace Correlation Engine according to the instructions provided by the manufacturer. The rank order of these genes was determined based on their absolute fold changes. All meta-statistical analyses comparing two datasets were performed in BaseSpace, and the similarities between any two datasets were evaluated as overlap P -values using the Running Fisher algorithm⁴⁴.

Statistical analyses

Mice of the same age and sex were raised under identical conditions and were randomly allocated to different experimental groups. Complete blinding during data collection was not always feasible because experimenters had to deliver group-specific optogenetic stimulation. To minimize bias, behavioral and imaging analyses were performed using automated, predefined pipelines (ImageJ plugins; Inscopix Data Processing Software; Huygens; MATLAB), and group identifiers were concealed during manual histological quantification whenever

feasible. The sample sizes were determined based on those reported in similar publications^{27,28}. Welch's *t*-test was used to compare group means. One-way or two-way ANOVA was applied for comparisons involving more than two groups, followed by Bonferroni's or Tukey's HSD post hoc tests, as indicated in the corresponding figure legend. When pooling the data of neurons from multiple mice per group, a linear mixed-effects model (LMM) was applied, treating mouse ID as a random effect to account for within-animal correlations. All results of the statistical analyses of behavioral tests are provided in the Source Data file.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The RNA-seq and ATAC-seq data generated in this study have been deposited in the Gene Expression Omnibus (GEO) under accession codes [GSE227200](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE227200) and [GSE227201](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE227201), respectively. The post-mortem human dentate gyrus RNA-seq data reanalyzed in this study are available in the Sequence Read Archive under accession [SRP241159](https://www.ncbi.nlm.nih.gov/sra/acc.cgi?acc=SRP241159). The de-identified ECT history metadata linked to the SRP241159 samples were provided by Astellas Pharma Inc. and are not available for external redistribution. The raw in vivo Ca^{2+} imaging data have been deposited in the Systems Science of Biological Dynamics (SSBD) repository at [RIKEN](https://www.riken.jp/en/brain/brain-science/brain-science-repository). The confocal image data underlying the histological quantifications are available at figshare [<https://doi.org/10.6084/m9.figshare.28853303>]. Source data are provided with this paper.

Code availability

The custom code used for RNA-seq, ATAC-seq, and Ca^{2+} imaging analyses in this study is publicly available on GitHub [<https://github.com/tmurano/REPeatedOPtoGeneticStimulation>] and is archived on Zenodo with a citable DOI.

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Author contributions

T. Murano, K. Takao, and T. Miyakawa designed the experiments and analyses. T. Murano performed RNA-seq, ATAC-seq, histological analysis, in vivo genome-editing experiments, and data analyses. T. Murano and K. Takao performed the behavioral experiments. T. Murano and Y. Takamiya performed the Ca^{2+} imaging experiments. M. Namihira and K. Katoh performed the STED microscopy observations. K. Tajinda and M. Matsumoto provided RNA-seq data of post-mortem DG of human patients. A. J. Robison provided the AAV- Δ JunD reagent and scientific input. T. Murano prepared all the figures and wrote the manuscript. H. Hagihara and T. Miyakawa supervised all aspects of the study. All authors reviewed the manuscript.

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Competing interests

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Additional information

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