Loss of function of NCOR1 and NCOR2 impairs memory through a novel GABAergic hypothalamus–CA3 projection

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Nuclear receptor corepressor 1 (NCOR1) and NCOR2 (also known as SMRT) regulate gene expression by activating histone deacetylase 3 through their deacetylase activation domain (DAD). We show that mice with DAD knock-in mutations have memory deficits, reduced anxiety levels, and reduced social interactions. Mice with NCOR1 and NORC2 depletion specifically in GABAergic neurons (NS-V mice) recapitulated the memory deficits and had reduced GABAα2 receptor subunit (GABRA2) expression in lateral hypothalamus GABAergic (LH-GABA) neurons. This was associated with LH-GABA neuron hyperexcitability and impaired hippocampal long-term potentiation, through a monosynaptic LH-GABA to CA3-GABA projection. Optogenetic activation of this projection caused memory deficits, whereas targeted manipulation of LH-GABA or CA3-GABA neuron activity reversed memory deficits in NS-V mice. We describe de novo variants in NCOR1, NCOR2 or HDAC3 in patients with intellectual disability or neurodevelopmental disorders. These findings identify a hypothalamus–hippocampus projection that may link endocrine signals with synaptic plasticity through NCOR-mediated regulation of GABA signaling.

Regulation of gene expression is a key component of intracellular signaling and confers long-lasting effects that are particularly relevant to memory formation. Many endocrine factors use nuclear receptors to regulate gene expression. Nuclear receptors recruit nuclear receptor coreactivators (NCOAs) or nuclear receptor corepressors (NCORs) in a ligand-dependent manner, which alters epigenome modifications such as histone acetylation, remodels chromatin architecture, and regulates gene transcription. NCOR1 and its homolog NCOR2 (also known as SMRT, silencing mediator for retinoid and thyroid hormone receptors) are the fundamental scaffold proteins of the NCOR complex in mammals. NCORs regulate gene expression by recruiting and activating histone deacetylase 3 (HDAC3)1–4. Our previous work has elucidated the metabolic functions of NCORs and HDAC3 in multiple tissues5–11. NCORs have been shown to interact with methylated CpG binding protein 2 (MeCP2), which binds methylated DNA12. In addition, mutations in the human MECP2 gene are known to cause Rett syndrome13, a neurodevelopmental disorder characterized by intellectual disability, developmental regression, autism spectrum disorders, seizures, and acquired microcephaly. Furthermore, it was shown that MeCP2 missense mutations affecting MeCP2–NCOR interactions can cause a Rett syndrome-like phenotype in mice14,15. NCORs can also form a stable protein complex with transducin beta-like 1X-linked receptor 1 (TBL1XR1 or TBLR1)16, another protein that is associated with neurocognitive disorders presenting with variable phenotypes that include autism, intellectual disability, and multiple congenital anomalies in humans (https://www.omim.org/entry/608628). However, whether and how NCORs regulate cognitive functions has not been studied directly.

Results
Disruption of NCOR function by NS-DADM mutations causes cognitive deficits. NCORs regulate gene expression by recruiting and activating HDAC3 (refs. 1,14). The deacetylase activation domain (DAD) on the amino-terminal region of NCORs is responsible for activating HDAC3 enzyme activity (Fig. 1a). HDAC3 can be inactivated both in vitro and in vivo by missense mutations in either DAD or HDAC3 that abolishes the DAD–HDAC3 interaction14,15,17,18. Conversely, HDAC3 is the only HDAC that confers deacetylase activity.

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**Fig. 1** | **NS-DADm mice display cognitive dysfunction, altered anxiety, and social avoidance.**

**a.** The DAD in NCOR mediates transcriptional regulation by nuclear receptors (NR), which counteracts coactivators (NCOA) and histone acetyltransferases (HAT).

**b.** Fluorescence-based HDAC enzyme assay using lysates of mouse brain cortex (CTX), hippocampus (HIP), and hypothalamus (HYP) after immunoprecipitation with α-HDAC3 antibody or normal IgG. Box plot center lines, median; box limits, upper and lower quartiles; whiskers, minimal and maximum values. Two-way ANOVA was used followed by Tukey’s post hoc test; \( n = 4 \) mice per group. Male 4-month-old mice were used. The blot images have been cropped.

**c.** Discrimination index in the NOR test. Data were analyzed by two-tailed unpaired \( t \) test; \( n = 4 \) mice per group. Male 4-month-old mice were used. The experiment was repeated independently once with similar results; \( n = 16 \) male 4-month-old mice.

**d.** Representative heat map and time spent in the target quadrant during MWM test after the hidden platform was removed from the original location (indicated by white crosses). Data were analyzed by two-tailed unpaired \( t \) test; \( n = 13, n_{\text{NS-DADm}} = 14 \) male 4-month-old mice. Data are expressed as mean ± s.e.m. For detailed statistics results, see Supplementary Table 1. * \( P \leq 0.05 \) was considered to be significant.
enzymatic activity to the NCOR complex\textsuperscript{19,20}. Therefore, inactivating HDAC3 renders the NCOR complex deficient in deacetylase activity. We previously constructed an NCOR1/SMRT deacetylase activation domain mutation (NS-DADm) whole-body knock-in mouse model that contains NCOR1-Y478A and NCOR2-Y470A\textsuperscript{16}. NS-DADm mice do not have HDAC3 enzymatic activity but display normal development and appearance\textsuperscript{16}.

In the whole-body knock-in NS-DADm mouse line, HDAC3 enzyme activity was undetectable in all brain regions tested, including cortex, hippocampus, and hypothalamus (Fig. 1b). The interaction between HDAC3 and NCOR or TBLR1 was also reduced in the hypothalamus compared to littermate controls (referred to as wild type), although not completely abolished (Fig. 1c). The HDAC1 enzyme activity remained unaltered in the NS-DADm brain (Supplementary Fig. 1a). NS-DADm mice were born at the Mendelian ratio and had slightly lower body weight than wild-type mice, with normal brain weight, breathing patterns, hindlimb activity, circadian locomotor activity, and gross histological morphology in various brain regions (Supplementary Fig. 1b–i). The novel object recognition (NOR) test revealed a marked deficit in recognition memory in NS-DADm mice (Fig. 1d,e, and Supplementary Fig. 1j–k). This was not confounded by a lack of locomotor activity or more susceptibility to stress during the test, because NS-DADm mice in fact showed hyperactivity and less anxiety compared to control mice in the open field test (Fig. 1f), elevated plus maze test (Fig. 1g,h), and light–dark test (Fig. 1i). The recognition memory deficit was not associated with defective motor learning, because NS-DADm mice had enhanced motor coordination and motor learning in the rotarod test (Fig. 1j), even when compared with body weight-matched control mice (Supplementary Fig. 1d).

Importantly, NS-DADm mice were less interested in social interaction with other animals (Fig. 1k,l) and displayed spatial memory deficits in the Morris water maze (MWM) (Fig. 1m–o). These findings demonstrate that NCOR loss of function causes cognitive deficits, which can be independent of general developmental defects.

Depletion of HDAC3 in the LH impairs memory. To explore which brain regions are susceptible to the loss of HDAC3 enzyme activity in the context of memory deficits, we stereotaxically injected $\text{Hdac}^{\text{loxP/loxP}}$ mice with adeno-associated virus (AAV) expressing Cre recombinase from a neuron-specific promoter using AAV-GFP as a control. Depletion of HDAC3 in the hippocampal CA1 region was shown to enhance memory\textsuperscript{17}, suggesting that CA1 is unlikely to be the region mediating the memory deficit due to the loss of HDAC3 enzyme activity. We started with the hippocampus CA3 region. After injecting AAV-Cre into the CA3, we did not find altered learning behaviors or memory deficits in the NOR or MWM (Supplementary Fig. 2a–i). This suggests that the CA3 region is unlikely to mediate the memory deficit due to the loss of HDAC3 enzyme activity, although we cannot rule out the possibility that the absence of behavioral changes could be due to non-complete HDAC3 depletion (Supplementary Fig. 2c). The lateral hypothalamus (LH) is another brain region involved in motivated behaviors. We found that depletion of HDAC3 at the lateral hypothalamus resulted in a robust memory deficit in both the NOR test and MWM (Supplementary Fig. 2j–r), suggesting that the NCOR/HDAC3 function in the lateral hypothalamus is required for learning and memory. These findings do not exclude the possibility that other brain regions could also be important in the memory deficit in NS-DADm mice, especially considering that NS-DADm mice were deficient in the HDAC3 enzyme activity while HDAC3 regional knockout mice had disrupted HDAC3 protein levels.

Downregulation of $\text{GABA}_A$ receptor subunits in the NS-DADm hypothalamus. To address how NCORs regulate gene expression in the hypothalamus, we performed RNA-seq analysis using total RNAs extracted from the hypothalami of NS-DADm and their wild-type littermates. There were more genes downregulated than upregulated in NS-DADm mice compared to control mice (Fig. 2a). The differentially expressed genes were highly enriched in pathways involved in synapse function and cell junctions (Fig. 2b), with multiple $\text{GABA}_A$ receptor subunits downregulated in NS-DADm mice, compared to wild-type mice (Fig. 2c). The downregulation of the $\text{GABA}_A$ receptors was further confirmed by quantitative PCR with reverse transcription (RT-qPCR) and immunostaining using antibodies against $\text{GABRA2}$ in the NS-DADm hypothalamus, especially in the lateral hypothalamus (Fig. 2d,e, and Supplementary Fig. 3a). HDAC3 chromatin immunoprecipitation followed by deep sequencing (ChiP–seq) revealed several possible HDAC3 binding sites at the genes of $\text{GABA}_A$ receptors. ChiP–qPCR analysis further confirmed that HDAC3 indeed bound to those regions and the binding intensity was lower in NS-DADm mice than in wild-type mice (Fig. 2f). The residual binding in NS-DADm mice was presumably due to the interaction of HDAC3 with the NCORs carboxy-terminal region\textsuperscript{22}. Of note, $\text{GABA}_A$ receptor gene expression remained unaltered in the NS-DADm hippocampus (Supplementary Fig. 3b,c), suggesting that NCOR-mediated transcription regulation is highly region-specific. To address the functional significance of NCOR-mediated regulation of $\text{GABA}$ signaling, we used diazepam, a positive modulator of $\text{GABA}_A$ receptor, at a dosage lower than that commonly shown to cause amnesia\textsuperscript{23}. Diazepam rescued the recognition memory deficit of NS-DADm mice in the NOR test (Fig. 2g), suggesting a causative role of disrupted $\text{GABA}$ signaling in the memory deficit of NS-DADm mice.

GABA neuron-specific depletion of NCORs impairs memory. We found that NCOR1 was ubiquitously expressed across different brain regions, including those that are highly populated with $\text{GABA}$ergic neurons (Supplementary Fig. 3d). To address the function of NCORs specifically in $\text{GABA}$ergic neurons, we crossedbred $\text{NCOR1}^{\text{loxP/loxP}}/\text{NCOR2}^{\text{loxP/loxP}}$ mice with Vgat-Cre mice\textsuperscript{24} that express Cre recombinase only in $\text{GABA}$ergic neurons. The $\text{NCOR1}^{\text{loxP/loxP}}/\text{NCOR2}^{\text{loxP/loxP}}/\text{Vgat-Cre (NS-V)}$ mice were born at the Mendelian ratio and showed no obvious abnormality compared to littermate $\text{NCOR1}^{\text{loxP/loxP}}/\text{NCOR2}^{\text{loxP/loxP}}$ or Vgat-Cre controls (referred to as wild type). In situ hybridization confirmed depletion of NCOR1 in $\text{GABA}$ergic neurons in multiple brain regions (Supplementary Fig. 3e–f). Unlike NS-DADm mice, NS-V mice maintained normal locomotor activity and anxiety levels in the open field test and elevated plus maze test (Fig. 3a,b). However, NS-V mice displayed memory and learning deficits in both the NOR test (Fig. 3c,d) and the MWM (Fig. 3e–g) to a similar degree to the whole-body knock-in NS-DADm mice, demonstrating that NCOR loss of function specifically in $\text{GABA}$ergic neurons is sufficient to cause cognitive deficits. These data do not necessarily suggest that NCOR dysfunction in $\text{GABA}$ergic neurons fully accounts for the memory deficit in NS-DADm mice, considering that NCORs have functions other than activation of HDAC3 enzyme activity.

NCORs regulate $\text{GABA}_A$ receptor expression in LH$\text{GABA}$ neurons. Does the $\text{GABA}_A$ receptor downregulation occur in $\text{GABA}$ergic neurons? NS-V mice and control Vgat-Cre mice were stereotaxically injected at the LH with AAV expressing a Flag-tagged 60 S ribosomal protein L10a (Rpl10a) using the flip-excision (FLEX) system\textsuperscript{24} to ensure Cre-dependent expression of Rpl10a-Flag (Fig. 3h,i). Subsequent immunoprecipitation with anti-Flag antibodies enriched ribosome-bound mRNAs that were only expressed in LH$\text{GABA}$ neurons (Fig. 3h). The expression of $\text{GABA}_A$ receptors was downregulated in LH$\text{GABA}$ neurons upon NCOR depletion (Fig. 3i), which confirmed that NCORs regulate expression of $\text{GABA}_A$ receptors in LH$\text{GABA}$ neurons.
NCORs regulate GABA<sub>α</sub> receptor gene expression in the hypothalamus. How do NCORs regulate firing activities of GABAergic neurons? After crossbreeding NS-V mice or Vgat-Cre mice (referred to as wild-type control) with Rosa26-tdTomato mice that express the red fluorescent protein tdTomato in a Cre-dependent manner<sup>25</sup>, we used electrophysiology patch clamp to survey tdTomato-labeled GABAergic neurons in different brain regions. LH was the region used for synaptic analysis of GABAergic neurons. LH GABA neurons was higher in the absence of NCORs compared to control littermates, as identified by RNA-seq analysis. Three biological replicates were performed, after controls were analyzed by two-way ANOVA followed by post hoc LSD test; n<sub>saline</sub> = 6, n<sub>Diazepam</sub> = 6 female 4-month-old mice. Data were analyzed by two-tailed unpaired t test; n<sub>WT</sub> = 6, n<sub>NS-DADm</sub> = 8 male 4-month-old mice. Data were expressed as mean ± s.e.m. For detailed statistics results, see Supplementary Table 1. *P < 0.05 was considered significant.

NCORs depletion causes LH<sub>GABA</sub> hyperexcitability. The concurrence of the LH<sub>GABA</sub> hyperexcitability and the apparent CA3<sub>GABA</sub> hyperpolarization prompted us to check the neural connection between LH<sub>GABA</sub> and CA3<sub>GABA</sub> using wheat germ agglutinin (WGA)-mediated anterograde tracing<sup>26</sup>. WGA travels across the axon terminals and labels downstream neurons. Vgat-Cre/tdTomato mice were stereotaxically injected at LH with adenovirus expressing channelrhodopsin-2 (ChR2) to produce a WGA-GFP fusion protein in a Cre-dependent manner (Ad-iN/CaMKIIα-WGA-GFP) (Fig. 5a). Robust co-labeling of WGA-GFP (green) and tdTomato (red) at CA3<sub>GABA</sub> projection, we used channelrhodopsin-2 (ChR2)-assisted circuit mapping (CRACM). ChR2 is an excitatory light-gated ion channel that can be activated by blue light, allowing for selective neuronal excitation in vivo. In the presence of WGA-GFP, the ChR2-expressing LH<sub>GABA</sub> neurons remained unchanged (Fig. 4g), suggesting a postsynaptic mechanism for the hyperexcitability in LH<sub>GABA</sub> neurons. This could be explained by the lower GABA<sub>α</sub> receptor expression in these neurons.
channel. Its expression was restricted in LH\textsubscript{GABA} neurons by injecting an AAV vector expressing ChR2 in a Cre-dependent manner (AAV.FLEX.hChR2-EYFP) into the LH of Vgat-Cre mice (Fig. 5d). Ad-in/WGA-GFP was co-injected to label CA3\textsubscript{GABA} neurons that receive LH\textsubscript{GABA} projections. Upon excitation of LH\textsubscript{GABA} neurons with blue light on the CA3 region, evoked IPSCs (eIPSCs) were detected within 10 ms in GFP-labeled CA3\textsubscript{GABA} neurons (Fig. 5e–g). The eIPSCs were insensitive to potassium channel blocker 4-amino-pyridine (4-AP) and sodium channel blocker tetrodotoxin (TTX), but could be blocked by GABA{\textsubscript{A}} receptor antagonist bicuculline (Bic) (Fig. 5e), suggesting a monosynaptic GABA input from LH to CA3. There were similar findings in NS-V mice (Supplementary Fig. 4h), further confirming the monosynaptic projections from LH to CA3. Taken together, the data suggest that the hyperexcitability of LH\textsubscript{GABA} neurons in NS-V mice could inhibit CA3\textsubscript{GABA} neurons through monosynaptic GABA input.

The LH\textsubscript{GABA} to CA3\textsubscript{GABA} projection regulates memory and learning behaviors. Could the altered excitability in LH\textsubscript{GABA} and CA3\textsubscript{GABA} neurons explain the cognitive deficit in NS-V mice? DREADD (designer receptor exclusively activated by designer drugs) uses engineered G-protein coupled receptors (GPCRs) to activate or silence specific neurons in response to clozapine-N-oxide (CNO)\textsuperscript{14}. We first used an activating DREADD receptor hM3Dq to address the role of CA3\textsubscript{GABA} in the memory deficit of the NS-V mice. NS-V mice and control Vgat-Cre mice were injected at the CA3 with AAV.FLEX.hM3Dq-mCherry or a control virus, AAV.FLEX.
mCherry (Fig. 5h). All mice were subjected to behavior tests after administration of CNO to control for potential direct pharmacological effects of CNO itself. In the NOR test and MWM, NS-V mice showed improved spatial memory and recognition memory after the DREADD-mediated activation of CA3 neural activities (Fig. 5i–j). To specifically activate the LH GABA neurons in NS-V mice we used an optogenetic approach by expressing ChR2 in LH GABA neurons in Vgat-Cre mice and shedding blue light at CA3 (Fig. 5k). Such stimulation during the NOR test reduced the discrimination index (Fig. 5l) without affecting the total exploration time (Supplementary Fig. 5e). This finding demonstrates that the targeted activation of the LH GABA–CA3 circuit is sufficient to cause memory deficits.

We next addressed whether the hyperexcitability of LH GABA neurons is necessary for the memory deficits in NS-V mice. We used hM4Di, a silencing DREADD. AAV.FLEX.hM4Di-mCherry was injected into the LH of the NS-V mice and the control Vgat-Cre mice (Fig. 5m–p). DREADD-mediated suppression of LH GABA neurons in NS-V mice rescued their spatial memory (Fig. 5m,n) and recognition memory (Fig. 5o) without affecting explorative or locomotor activity (Supplementary Fig. 5f–g). To further address whether the LH GABA–CA3 projection per se mediates this effect, we used an enhanced natronomonas halorhodopsin (eNpHR), a suppressing optogenetic receptor. NS-V mice and control Vgat-Cre mice were injected at the LH with AAV.FLEX.eNpHR-EYFP or AAV.FLEX.EYFP, and implanted with an optical fiber at CA3 (Fig. 5g). The NS-V mice showed improved recognition memory in the NOR test when all four groups of mice received photosuppression (Fig. 5r, Supplementary Fig. 5h). The NS-V mice showed improved recognition memory in the NOR test when all four groups of mice received photosuppression (Fig. 5r, Supplementary Fig. 5h).
of the normal range. These data demonstrate that LH\textsuperscript{GABA} hyperexcitability contributes to the memory deficits in NS-V mice.

In addition to chemogenetic and optogenetic rescue, we sought to treat the cognitive defects in NS-V mice by pharmaceutical manipulation of LH neurons. We found that the recognition memory deficit in the NS-V mice was ameliorated by infusion of SL651498, a selective positive modulator of GABA\textsubscript{A} receptors\cite{10}, specifically into the LH via an implanted cannula (Fig. 5u, and Supplementary Fig. 5j-k). SL651498 did not change total travel distance or exploration time (Supplementary Fig. S1). These data demonstrate an indispensable role of LH\textsuperscript{GABA} neurons in regulating memory formation, which underlies the cognitive deficit observed in the NS-V mice.

**LH\textsuperscript{GABA} hyperexcitability impairs hippocampal synaptic plasticity.** Synaptic plasticity in the hippocampus is considered an important cellular mechanism for memory formation. Long-term potential (LTP) in hippocampal interneurons is a form of synaptic plasticity involved in memory\textsuperscript{13,14}. We found that hippocampal CA\textsubscript{3}GABA neurons in NS-V mice displayed impaired LTP when induced by high-frequency stimulation (HFS) at the dentate gyrus (Fig. 6a). We used DREADD to address whether the hyperexcitability of LH\textsuperscript{GABA} neurons has a role in impaired LTP formation in CA\textsubscript{3}GABA neurons. AAV.FLEX.hM4Di was co-injected with Ad-iN/WGA-GFP into the LH of NS-V mice and control Vgat-Cre mice (Fig. 6b–j). Administration of CNO silenced the difference in LH\textsuperscript{GABA} resting membrane potential between wild-type and NS-V mice to a comparable level (Fig. 6d). CNO also abolished the difference in LH\textsuperscript{GABA} expressing CA\textsubscript{3}GABA neurons (Fig. 6d), validating efficient suppression of LH\textsuperscript{GABA} neurons. Remarkably, CNO completely rescued LTP at LH\textsuperscript{GABA}-innervated CA\textsubscript{3}GABA neurons (co-labeled with WGA-GFP and tdTomato) (Fig. 6e,f). These data demonstrate that LH\textsuperscript{GABA} hyperexcitability contributes to the impaired hippocampal synaptic plasticity in NS-V mice. We then used optogenetics to address the role of the LH\textsuperscript{GABA-CA3GABA} projection. NS-V mice and control Vgat-Cre mice were injected at the LH with AAV.FLEX.eNhPr-EYFP or AAV.FLEX.EYFP, and LTP was measured after shedding yellow light on CA3 in brain slices (Fig. 6g). Such photosuppression rescued LTP at LH\textsuperscript{GABA}-innervated CA\textsubscript{3}GABA neurons (Fig. 6h,i). These data demonstrate that LH\textsuperscript{GABA} hyperexcitability impaired hippocampal synaptic plasticity through the LH\textsuperscript{GABA-CA3GABA} projection.

The changes in AMPA receptors (AMPA\textsubscript{R}s, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR\textsubscript{R}s) and NMDA receptors (NMDAR\textsubscript{R}s, N-methyl-D-aspartate receptors) constitute a molecular basis of synaptic plasticity. Using WGA-GFP and tdTomato to label LH\textsuperscript{GABA}-innervated CA\textsubscript{3}GABA neurons, we recorded AMPAR- and NMDAR-dependent spontaneous excitatory postsynaptic current (sEPSC) as a surrogate for learning-induced synaptic plasticity in vivo in this specific neuron population. Mice were left untrained (naïve) or trained for 3 days to memorize the location of a hidden platform in the MWM, and were then allowed to rest for a day before recording. Training increased the amplitude of AMPAR-dependent sEPSCs in LH\textsuperscript{GABA}-innervated CA\textsubscript{3}GABA neurons in wild-type mice (Fig. 6j,k), which led to an elevated AMPAR/NMDAR ratio (Supplementary Fig. 6a). Such learning-induced changes were not observed in NS-V mice (Fig. 6j,k). Training slightly increased the expression of the GABAR\textsubscript{B2} in LH\textsuperscript{GABA} neurons (Supplementary Fig. 6b–d). We did not observe obvious changes in firing activities of CA3- innervating LH\textsuperscript{GABA} neurons (labeled with retrograde beads) in response to MWM training (Supplementary Fig. 6e–h), although a small increase in the mIPSCs amplitude was observed (Supplementary Fig. 6e). This suggests that learning-induced CA\textsubscript{3} plasticity in wild-type mice is not directly driven by a possible suppression of LH\textsuperscript{GABA} neurons. Instead, LH\textsuperscript{GABA} neurons may have a permissive role in learning-induced CA\textsubscript{3} plasticity.

To directly address the role of LH\textsuperscript{GABA} in learning-induced plasticity in NS-V mice in vivo, we injected AAV.FLEX.hM4Di-mCherry...
or AAV.FLEX.mCherry into the LH of the NS-V mice (Fig. 6l). Upon recovery, both groups of mice were trained in the MWM after CNO administration in each training session, followed by the recording of AMPAR- and NMDAR-dependent sEPSCs. Such chemogenetic suppression of LHGABA neural activity in NS-V mice largely rescued training-induced increase in AMPAR-dependent sEPSCs (Fig. 6m,n, and Supplementary Fig. 6i). Chemogenetic suppression of LHGABA did not affect AMPAR-dependent sEPSCs in naive untrained NS-V mice (Fig. 6o), which suggests that the learning experience and the normal LHGABA neural activity are both required for CA3 plasticity. These results demonstrate that LHGABA hyperexcitability contributes to the impaired synaptic plasticity in NS-V mice in vivo. Collectively, the data obtained from the animal models delineate a circuitry pathway through which NCORs regulate neurocognition (Fig. 6p).

**Genetic variants of the NCOR complex in human neurocognitive disorders.** Estimates from exome sequencing data from more than 60,000 apparently healthy individuals suggest that the observed numbers of loss-of-function variants for NCOR1 (n = 8), NCOR2 (n = 6) and HDAC3 (n = 3) are significantly smaller than the expected numbers (NCOR1, n = 87; NCOR2, n = 65; HDAC3, n = 20), with the probability of loss-of-function intolerance (pLI) scores of 1, 1, and 0.91, respectively, strongly suggesting that NCOR1, NCOR2, or HDAC3 are intolerant to loss-of-function variants in humans (a pLI score of >0.9 is considered to reflect intolerance34).
Fig. 6 | The LHGABA to CA3GABA projection regulates hippocampal synaptic plasticity in NS-V mice. a, LTP formation in CA3GABA neurons upon HFS at DG in brain slices. Data were analyzed by one-way repeated ANOVA; nWT = 7 mice, nNS-V = 5 mice. Male 3-month-old mice were used. b, Injection scheme for DREADD. c, Spontaneous firing frequency of LHGABA neurons with or without CNO. Data were analyzed by two-way ANOVA; nWT_Saline = 11 neurons/2 mice, nNS-V_Saline = 9 neurons/2 mice, nWT_CNO = 11 neurons/2 mice, nNS-V_CNO = 9 neurons/2 mice. Male 3-month-old mice were used. d, Resting membrane potential of LHGABA neurons in response to CNO. Data were analyzed by two-way ANOVA; nWT_Saline = 11 neurons/2 mice, nNS-V_Saline = 9 neurons/2 mice, nWT_CNO = 11 neurons/2 mice, nNS-V_CNO = 9 neurons/2 mice. Male 3-month-old mice were used. e,f, LTP formation in LHGABA-innervated CA3GABA neurons without or in the presence of CNO. Data were analyzed by two-way repeated ANOVA; nWT_Saline = 6 mice, nNS-V_Saline = 6 mice, nWT_CNO = 6 mice, nNS-V_CNO = 7 mice. Male 3-month-old mice were used. g, Scheme of AAV injection and laser probe implant for optogenetic manipulation. h, LTP formation with yellow light on in LHGABA-innervated CA3GABA neurons in brain slices from EYFP-infected mice. i, LTP formation with yellow light on in LHGABA-innervated CA3GABA neurons in brain slices from eNpHR-infected mice. Data were analyzed by two-way repeated ANOVA; nWT_Saline = 6 mice, nNS-V_Saline = 6 mice, nWT_CNO = 6 mice, nNS-V_CNO = 7 mice. Male 3-month-old mice were used. j,k, Representative traces and the amplitude of AMPAR-dependent sEPSC in LHGABA-innervated CA3GABA neurons in mice either trained during the MWM or left untrained (naive). Data were analyzed by two-way ANOVA; nWT_Train = 10 neurons/2 mice, nNS-V_Train = 13 neurons/2 mice, nWT_Train = 10 neurons/2 mice, nNS-V_Train = 14 neurons/2 mice. Male 3-month-old mice were used. l, Scheme of intracranial injection of AAV.FLEX.mCherry or AAV.FLEX.hM4Di in the LH of NS-V mice. m, Injected NS-V mice were trained in the MWM for 3 days with CNO administration (1 mg kg⁻¹, i.p.) before each training session. The amplitude of AMPAR-dependent sEPSC in the LHGABA-innervated CA3GABA neurons was measured in brain slices at the fifth day. Data were analyzed by two-way ANOVA; nWT_Train = 17 neurons/3 mice, nNS-V_Train = 18 neurons/3 mice. Male 3-month-old mice were used. n, Representative traces of AMPAR sEPSC. o, Measurement of AMPAR-dependent sEPSC in naive NS-V mice without MWM training. Data were analyzed by two-way ANOVA; nWT_Train = 20 neurons/3 mice, nNS-V_Train = 22 neurons/3 mice. Male 3-month-old mice were used. p, A model of LHGABA to CA3GABA projection regulating cognitive function. Data are expressed as mean ± s.e.m. For detailed statistics results, see Supplementary Table 1. *P ≤ 0.05 was considered significant. Independently, the electrophysiological data was re-analyzed using the linear mixed-effects models (Supplementary Table 2).
**Fig. 7** | Genetic variants involving NCORs and HDAC3 in human with neurocognitive disorders.  

**a.** Patients carrying copy number variants (CNVs) or SNVs in NCOR1, NCOR2, or HDAC3. Genomic coordinates are shown in hg19. DDD_SNV, single nucleotide variants retrieved from the Deciphering Developmental Disorders (DDD) website (United Kingdom); kb, kilobase.  

**b–d.** Schematic representations for the deletions and point mutations affecting NCOR1, NCOR2, or HDAC3, respectively, observed in patients with neurodevelopmental disorders. The locations of deletions are depicted in red, and the point mutations in pink.  

**e.** Western blot of HEK-293 cells transfected with plasmids expressing WT HDAC3 with or without mutant L266S. The experiment was repeated independently once with similar results. Data were analyzed by two-tailed unpaired t test; n = 4 biological independent samples for each group.  

**f.** Fluorescence-based HDAC enzyme assay after anti-HDAC3 immunoprecipitates from cell lysates overexpressing the indicated HDAC3 proteins. Box plot center lines, median; box limits, upper and lower quartiles; whiskers, minimal and maximum values. Data were analyzed by two-tailed unpaired t test; n = 4 biological independent samples for each group. The blot images have been cropped.  

**g.** Western blot of HEK-293 cells transfected with plasmids expressing WT HDAC3, WT NCOR1, with or without the NCOR1 deletion mutant (Del). The experiment was repeated independently once with similar results. Data were analyzed by two-tailed unpaired t test; n = 3 biological independent samples for each group. The blot images have been cropped.  

**h.** Chromatin immunoprecipitation (ChIP) with anti-HDAC3 antibodies followed by qPCR using primers targeting promoters of the indicated genes ARNTL and CDKN1A. RPLPO serves as a negative control. Data are expressed as mean ± s.e.m. For detailed statistics results, see Supplementary Table 1. *P ≤ 0.05 was considered significant.
Importantly, we identified subjects carrying deleterious mutations in NCOR1, NCOR2 or HDAC3 with neurocognitive disorders from the DECIPHER database, including one individual with a pathogenic deletion, one individual with a pathogenic single-nucleotide variant (SNV), and two individuals with probable pathogenic SNVs (Fig. 7a). For NCOR1, a 152 kb heterozygous de novo deletion was found in patient 251746, a boy with severe global developmental delay and no language expression. He developed refractory epilepsy before the age of 4 years, severe intellectual disability, poor motor coordination, stereotyped hand and head movements (jacking capitis), and auto-aggressive attitude (Fig. 7a,b). The deletion removes the last 25 exons of NCOR1, which is predicted to result in deletion of the truncated allele through nonsense-mediated decay. The deletion also removes three other genes, ADORA2B, TTC19, and ZSWIM7. However, heterozygous loss-of-function variants in any of these three genes are tolerated in healthy individuals from the Exome Aggregation Consortium database (ExAC), with pLI scores of 0.09 and 0 for ZSWIM7 and ADORA2B, respectively. Defects in TTC19 cause mitochondrial complex III deficiency, nuclear type 2 (MIM: 615157), an autosomal recessive disorder that cannot explain the phenotypes in this patient with a heterozygous deletion. No other known pathogenic deletion was found in this patient. Therefore, we consider that NCOR1 is likely to be the contributing gene in patient 251746. A de novo heterozygous c.2182+2T>G variant of NCOR1 was identified in an 8-year-old boy with global developmental delay, moderate learning difficulties, autism spectrum disorder, epilepsy, absence and drop attacks, tip-toe walking, and double incontinence (Fig. 7a,b). This SNV affects the canonical splice donor site of NCOR1 and has not been observed in the control cohort in the ExAC. It is therefore classified as a potential pathogenic variant.

Heterozygous de novo missense variants in NCOR2 [c.6887G>A (p.R2296Q)] and HDAC3 [c.797T>C (p.L2665S)] were observed in two patients (Fig. 7c,d). The NCOR2 variant was found in a 17-year-old female with denticogenesis imperfecta, obesity, severe learning difficulties, and coordination problems. The HDAC3 variant was found in a 12-year-old girl who displayed learning difficulties, focus problems, and coordination problems. The HDAC3 variant is predicted to result in a frameshift in the coding sequence with the loss of the active site and catalytic tunnel and is likely to be important for the substrate to access the active site. Overexpression of wild-type HDAC3 forms one wall of the catalytic tunnel and is likely to be important for the substrate to access the active site. Overexpression of wild-type HDAC3 itself did not increase the total HDAC3 deacetylase activity, in keeping with the previous finding that endogenous NCOR is a limiting factor for HDAC3 enzyme activity. HDAC3-L2665S overexpression reduced the enzyme activity below the baseline, suggesting a dominant negative effect. The de novo deletion variant in NCOR1 in patient 251746 results in a C-terminal deleted NCOR1 protein (Del) with amino acids 1–666. Cells co-overexpressing NCOR1 wild-type and Del at a 1:1 molar ratio showed significantly reduced chromatin occupancy of HDAC3 compared to the control cells coexpressing two copies of wild-type NCOR1 (Fig. 7g.h). These results suggest that the human genetic variants could cause loss of function for the NCOR–HDAC3 complex in the heterozygous scenario. The causative role of these mutations in cognitive behaviors needs to be further addressed in vivo using new genetic mouse models. In summary, NCOR1, NCOR2, and HDAC3 are promising Mendelian disease genes whose loss of function in humans could contribute to intellectual disability, neurodevelopmental defects, or multiple congenital anomalies.

Discussion

Our results delineate a molecular and circuitry pathway through which NCORs regulate neurocognition (Fig. 6p). NCOR loss of function causes downregulation of GABA_A receptor expression in LH_GABA neurons, leading to hyperexcitability of these inhibitory neurons. Such an excitation/inhibition imbalance in the hypothalamus inhibits LTP formation in the hippocampus, probably through monosynaptic LH_GABA-CA3_GABA projections, which underlies memory deficits caused by NCOR loss of function. This model is supported by the following observations: (1) deletion of NCORs specifically in GABAergic neurons in NS-V mice recapitulated memory deficits observed in the NS-DADm mice and impaired LTP formation in the hippocampal CA3 region; (2) electrophysiology in NS-V mice identified hyperexcitability in LH_GABA neurons; (3) LH_GABA neurons send monosynaptic projects to CA3_GABA neurons; (4) transcriptomic profiling identified GABA_A receptor subunits as the molecular targets of NCORs in the hypothalamus, and down-regulation of GABA_A receptor in LH_GABA neurons was further confirmed by immunostaining and ribosomal profiling; (5) targeted activation of the LH_GABA-CA3_GABA projection is sufficient to cause memory deficits; (6) learning behaviors facilitate synaptic plasticity in LH_GABA, innervated CA3_GABA neurons in an NCOR-dependent manner; (7) targeted manipulation of LH_GABA neurons or the LH_GABA-CA3_GABA projection rescued LTP in hippocampal CA3 and cognitive deficits in NS-V mice; (8) targeted manipulation of CA3_GABA neurons rescued cognitive deficits in NS-V mice; and (9) positive modulators of GABA_A receptor administrated in the LH rescued cognitive deficits in NS-V mice. Collectively, these findings delineate a molecular and circuitry mechanism through which NCORs regulate synaptic plasticity and cognitive function.

Our study established a pivotal role of LH_GABA neurons in memory and hippocampal synaptic plasticity. LH glutamatergic neurons expressing orexin were previously implicated in regulating memory formation. LH_GABA neurons were less studied in the context of learning and memory. In addition to the direct monosynaptic LH_GABA-CA3_GABA projection, it is possible that LH_GABA neurons could affect hippocampal synaptic plasticity indirectly through projections to other brain regions. LH_GABA neurons have been shown to regulate feeding. However, we did not observe abnormal eating behaviors in NS-V mice or NS-DADm mice. It is possible that loss of NCORs in other brain regions compensates for orexigenic effects of LH_GABA activation. It is also possible that alteration of LH_GABA excitability in NS-V or NS-DADm mice is not sufficient to change consummatory behaviors, unlike high-frequency optogenetic stimulation or genetic ablation of the LH_GABA neurons.

It remains an open question whether LH_GABA hyperexcitability can fully explain the seemingly hypexcitability of CA3_GABA neurons in the NS-V mice. The frequency of IPSCs and EPSCs generally reflects presynaptic inputs, whereas the amplitude is often attributable to postsynaptic mechanisms. However, synapses are complex and this general principle may not be applicable in all situations. CA3_GABA neurons are likely to receive multiple inputs that are integrated in a complex manner that is yet to be fully defined. It is possible that the dysfunction of NCORs in other regions of the brain can also contribute to the cognitive deficits.

We found that several human patients with genetic variants involving NCORs or HDAC3 had growth abnormalities in multiple systems. This is consistent with previous findings that NCORs and HDAC3 are essential for development. However, growth abnormalities were not observed in NS-DADm mice or NS-V mice, except slightly lower body weight in both mouse lines compared to...
their lissencephalic controls. This suggests that the abnormal growth in various systems in human patients is attributable to HDAC3-independent functions of NCoRs in cells that are distinct from GABAergic neurons. This further suggests that the function of NCoRs in neurocognition can be separated from developmental regulation. Our previous study demonstrated normal development and survival of NS-DADm mice despite lack of HDAC3 enzymatic activity. Therefore, the developmental function of HDAC3 does not require its enzymatic activity, in line with its known deacetylase-independent function. Our circuit mapping study revealed intact LH\GABA\-CA3\GABA projections in NS-V mice, demonstrating normal anatomy and development of GABAergic neurons in those regions. The memory deficit in NS-DADm mice and NS-V mice can be rescued by small molecule modulators or chemogenetic manipulation in adult mice, indicating that the cognitive dysfunction is not caused by an irreversible anomaly originated from early development. One caveat is that the rescue experiments in the current study were performed acutely, so the long-term reversibility of cognitive deficits remains an open question.

Several previous studies have demonstrated that HDAC3 can have different or opposite roles in neurocognition. Depletion of HDAC3 in hippocampus CA1 regions facilitated the formation of context memory, while depletion of HDAC3 in forebrain excitatory neurons caused memory deficits in multiple neurobehavioral tests. Transcripтомic changes were also context-dependent. Our RNA-seq showed that expression of Fos, Nr4a1, and Nr4a2 were not altered than upregulated in the hypothalamus. The molecular basis of such regulation remains elusive in the hypothalamus and further studies are required to delineate the relationship between NCoRs and MeCP2 in various brain regions. As far as neuronal activity is concerned, both MeCP2-KO mice and NS-V mice showed neuronal hyperexcitability at certain brain regions. These findings support excitation/inhibition imbalance as an important factor contributing to cognitive dysfunction and pinpoint inhibitory GABA signaling as a possible therapeutic target. We attempted treatment in adult NS-DADm and NS-V mice with small-molecule modulators or chemogenetic manipulation and successfully reversed the cognitive and memory deficits, which provides strategic insights for the treatment of NCoR-related neurocognitive disorders in human patients.

NCoRs are fundamental players in the action of many endocrine factors. Hypothalamic is the central nexus of neuroendocrine regulation that governs many basic biological processes through hormones. Our findings thus establish hypothalamus–hippocampus communication that potentially links endocrine signals with synaptic plasticity through NCoR-mediated regulation of GABA signaling.

**Accession codes.** RNA-seq and ChIP–seq data are available in the GEO (accession code GSE92452).

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41593-018-0311-1.

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46. Author contributions Z.S. conceived the study, W.Z., Y.H., X.H., Q.W., Q.T., P.L., Y.X., and Z.S. designed the experiments. W.Z. and C.W. performed and analyzed the studies involving behavior tests, injection surgery, histology analysis, ChIP, and gene expression analysis. Y.H. performed and analyzed the electrophysiology recordings. Y.K., S.H., G.D., and Y.G. performed and analyzed western blot, molecular cloning, immunoprecipitation, and HDAC assays. I.D., E.S., M.O., J.E.V.M., and the DDD research team provided the human sequencing data resource. A.U.R. and P.L. analyzed the human sequencing data. H.K.Y., Y.W., B.P., and Z.L. analyzed the ChIP-seq and RNA-seq data. W.Z. and H.L. analyzed the electrophysiology data. W.Z., Y.H., X.H., Q.W., Q.T., P.L., X.Y., and Z.S. interpreted the data. Y.X. and Z.S. secured the funding. Z.S. wrote the manuscript with input from the other authors.

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Methods

Animals. NS-DADm mice were generated by crossing N-DADM mice with S-DADM mice. \( ^{4} \) HDAC3\textsuperscript{loxP/loxP} mice were generated as described previously. \( ^{5} \) Vgat-Cre mice \( ^{6} \) and Rosa26-tdTomato mice \( ^{7} \) were obtained from The Jackson Laboratory. The NCOR\textsuperscript{fl/fl}NOR\textsuperscript{fl/fl} mice were generated by PHENOMIN, Institut Clinique de la Souris (ICS), CNRS, INSERM, University of Strasbourg, France (http://www.phenomin.fr/). \( ^{8} \) NCOR\textsuperscript{fl/fl}Vgat-Cre (NS-V) mice were generated through crossbreeding. \( ^{9} \) tDTomato-labeled NS-V mice \( ^{10} \) were generated for circuit mapping and electrophysiology studies. All mice were from a C57BL/6 genetic background. Male mice aged 2–6 months were used for all experiments unless otherwise noted. Female mice were also used for some of the tests and no sexual dimorphism was observed for the phenotypes of NS-DADm mice or NS-V mice. For NS-DADm mice, wild-type littermates were used as the control and referred to as wild type. For the initial characterization of NS-V mice, NCOR\textsuperscript{fl/fl}Vgat-Cre mice were used as the control and referred to as wild type. All tests were repeated at least two times. Five mice were housed in each cage in a 12–12 light–dark (7 am to 7 pm) facility with free access to water and food. After surgery, mice were housed singly in the same facility. All animals were grouped according to their sex, age, and genotype. Mice with the same sex, age, and genotypes were randomized into different surgery groups or treatment groups. All the animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Baylor College of Medicine.

Behavioral tests. All the behavior tests were carried out between 12pm and 6pm in a dim light environment (300 lumens) unless stated otherwise. Mice were given a 2-week interval to recover from the first behavioral test before the next behavioral test. Mice were not subjected to more than four behavioral tests.

Novel object recognition test. For the NOR test, the objects used were built from Lego and were provided by the Neurobehavioral Core at Baylor College of Medicine. We used a small (22 cm x 44 cm) arena to facilitate object exploration and reduce the time needed to habituate mice to the arena. The arena was surrounded on three sides by a white screen to limit spatial information and prevent spatial biases. During the training session, two identical objects were placed at right and left sides of the arena. During the first day, mice were placed in the center of the arena and allowed to explore freely for 5 min. Mice were returned to their home cages. Then 24 h later, one of the objects was replaced by a novel object with different color and shape, and mice were allowed to freely explore the whole arena for 5 min. Animal behavior during the training and test session was tracked by a top camera and analyzed by ANY-maze software. Sniffing, touching (\( > 1 \) s), and staring the objects were judged as exploration behaviors. \( ^{11} \)

Elevated plus maze. For the elevated plus maze test, we used a plus-shaped platform that was elevated to 40 cm above the floor. Two opposite arms of the maze were walled (15 cm high), whereas the other two arms were open with a 5 mm high ridge to prevent falling. Each arm was 8 cm wide, 25 cm long. The test lasted for 10 min and was started by placing a mouse in the center part of the maze facing one of the two open arms under a bright environment (900 lumens). An overhead camera and the ANY-maze software program were used to track the position of the mouse in the elevated plus maze. The number of visits to the open arms and the time spent on the open arms were used as measures for anxiety. \( ^{12} \)

Open-field arena test. The open-field arena test was performed using the Versamass animal activity monitor equipped with infrared photo beams as horizontal \( X \)-Y sensors and/or \( Z \) sensors. Mice were placed in the center of the open-field arena (40 cm x 40 cm x 30 cm) and allowed to explore for 60 min. The locomotor activity and location of the mice were scored automatically by VersaMax software. The percentage of time spent in the center area measures anxiety levels.

Light–dark test. The light–dark test was performed in a box (42 cm x 20 cm x 25 cm) that contained a dark area (one-third of the box) and light area (two-thirds of the box) connected by a small opening to allow mice to move from one area to the other. The test lasted for 10 min and started by placing a mouse in the bright area. The activity and location of the mouse was scored automatically by VersaMax software. The number of transitions between dark and light zones and the time spent in the light and dark area were the index for anxiety. \( ^{13} \)

Morris water maze. The MWM was performed as described previously \( ^{14} \), with modifications. During the training session, a transparent rescue platform was submerged under the painted water (0.5 cm–1 cm) and was placed in a fixed position in the pool. On the first day of training, mice were first allowed to stand on the platform for 10 s. After that, mice were gently placed into the water facing the wall of the pool and allowed to freely explore the whole maze for 1 min. Mice were then guided to the rescue platform if they did not find it. Mice were allowed to take a rest on the platform for 10 s, and then re-trained from a different start position. After the four training trials, mice were injected with a paper towel and returned to home cages. Twenty-four hours later, mice were trained again following the same procedure without the initial habituation session. Mice were trained for five consecutive days. At the end of the fourth trial on day 5, mice were returned to home cages for a rest. One hour later, mice were put into the water maze for 1 min, where the platform had been removed. Mouse behaviors were videotaped and analyzed by the Noldus EthoVision XT. The MWM was virtually divided into four quadrants. The rescue platform was located in the target quadrant. Escape latency was defined as the time spent before finding the platform. Escape latency during the 5-day training sessions served as an independent measurement of spatial learning and memory.

Rotarod test. The rotarod test was performed using an accelerating rotarod (Ugo Basile) \( ^{15} \), with minor modifications. Mice were placed on a rotating drum, which was accelerated from 4 to 40 rpm over a 5 min period, without the accommodation training described in ref. \( ^{16} \). Time spent walking on top of the rod before falling off the rod or hanging on and riding completely around the rod was recorded. The rod was only 20 cm above the platform, so mice were always falling off the rod. Mice were given two trials per day for four consecutive days, with a maximum time of 5 min per trial and a 30–60 min inter-trial rest interval.

Social interaction test. The social interaction test was performed in a three-chamber (Crawley) apparatus (60.8 x 40.5 x 23 cm) that consisted of three chambers (left, center, and right) of equal size with 10 x 5 cm openings between the chambers, slightly narrower than those in the published report \( ^{17} \). Mice were exposed to a novel peer in the test chamber for 10 min. The first test measured baseline activity in the apparatus and the second test measured sociability of the test mouse given a choice of exploring a chamber containing another mouse under a mesh pencil cup or a chamber containing a novel object under a mesh pencil cup. A camera and the ANY-maze software program were used to track the position of the mouse in the three-chamber box, while the experimenters scores the approaches to the object or partner mouse using a wireless keyboard. Partner mice (sex-, age- and weight-matched) were purchased from the Center for Comparative Medicine (CCM) at Baylor College of Medicine and habituated to the mesh pencil cups in the apparatus for 1 h per day for 2 days before the day of testing. Partner mice were used up to three times, with one test per day.

Virus, stereotaxic injection, DREADD, and optogenetics. AAVCAG.FLEX. Rpl10a-GFP-Flag vector was constructed based on the pAAV-CAG-FLEX-GFP plasmid (Addgene 28304, from Edward Boyden), and was pseudotyped with AAV9 scFv vector. During virus production, AAVS5.FLEX.mCherry was generated in S-DADm mice. \( ^{18} \) AAVS5.FLEX.mCherry was obtained from Bryan Roth through the Vector Core at the University of Northern California at Chapel Hill. AAVS5.FLEX.mCherry was obtained from Addgene (43631-AAV5-3). \( ^{19} \) AAV2.EF1a.FLEX. hChR2(H134R)-EYFP and AAV2.EF1a.FLEX.ChR2(H134R)-EYFP were from Karl Deisseroth at Stanford University through the Vector Core at the University of North Carolina. AAV8.TR.eGFP and AAV8.hSyn.GFP were generated by the Vector Core at University of Northern California at Chapel Hill. Ad.IN.GFP was provided by Martin Meyer at the University of Michigan. \( ^{20} \) AAV.FIR1.FLEX. synaptophysin-EYFP.WPRE.GHPA, serotype D/I, was from Ben Arenkiel at BCM for synaptophysin tracing. All virus was titrated at around 10 \( ^{10} \) GC ml\( ^{-1} \). Mice were anesthetized with ketamine and xylazine (100 mg kg\(^{-1}\) : 10 mg kg\(^{-1}\)), and the head were fixed on the surgery platform by a stereotaxic system (Stoeling). For virus injection, a blur hole was drilled on each side of the skull (0.1 mm posterior and 1.2 mm lateral to the bregma, depth 5.48 mm for LH; 1.98 mm posterior and 2.0 mm lateral to the bregma, depth 2.25 mm for CA3), and mice were injected with 150 nl of each virus on each side of the skull, unless stated otherwise. \( ^{21} \) Ad.IN.WGA-GFP or AAV.EF1a.synaptophysin-EYFP.WPRE.GHPA was injected with 100 nl unilaterally. For intracerebroventricular injection, a mouse was implanted with a guide cannula (0.8 mm posterior and 1.6 mm lateral to the bregma, depth 2.2 mm for intracerebroventricular injections). One or two weeks after the surgery, diazepam (0.5 mg per mouse per 1 g) or Sal51498 (1 mg per 2 g per mouse) was injected through the guide cannula in the freely moving mice before the tests. DREADDS engineered GPCRs to activate or silence specific neurons in response to CNO. \( ^{22} \) Mice were intraperitoneally injected with 3 mg kg\(^{-1}\) CNO shortly before each session of neurobehavioral tests. For optogenetics, two photo fibers (Thorlabs, CFML12U-20) were implanted above the CA3 regions (2.18 mm posterior and 2.2 mm lateral to the bregma) before the injection of the virus. Mice were housed singly after the surgeries and were allowed to recover for 1 or 2 weeks before test. To examine the mPSCs and mEPSCs at CA3-inverting LH\( ^{40} \) neurons, 50 ml green Retrobeads IX (Lunaflour) were injected into the CA3 (2.18 mm posterior and 2.2 mm lateral to the bregma, depth 2.5 mm for CA3). \( ^{23} \) Mice were allowed to recover in their home cages for 1 week before being placed and testing. For optogenetic activation in CA3 neurons, 10 ml 473 nm blue light pulses with 3 s on and 2 s off were delivered from a 473 nm blue solid state laser (MBL-III-473, Changshun New Industries Optoelectronics).
and wild-type control mice. Mice aged 6–12 weeks were deeply anesthetized with 100 μg/mouse U-74382F and 50 μg/mouse medetomidine (Phoenix Pharmaceutical), injected intraperitoneally, and then surgically prepared. For electrophysiology, animals were placed on a warm plate, and the head was fixed with the Stereotaxic Instruments (David Kopf Instruments). For imaging experiments, animals were deeply anesthetized with 100 mg/mouse ketamine and 10 mg/mouse xylazine, injected intraperitoneally, and then surgically prepared. The animals were placed in a temperature-controlled stereotaxic frame (Brown Instrument) and then fixed under anesthetics. Mice were maintained at 37 °C and then allowed to equilibrate for at least 10 min before recording. Mice were perfused transcardiacally using a mixture of 0.9% NaCl and 0.01% heparin. Sample brains were removed and post-fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS). Three series of sagittal sections were cut at a thickness of 30 μm using a Leica CM1850 cryostat slicer. The sections were immersed in 30% sucrose, and embedded in the Tissue-Tek compound (Sakura Finetek). Coronal sections corresponding to Bregma 0.0 mm were prepared on the Leica CM1850 cryostat slicer. The sections were then washed three times in PBS at room temperature (25 °C) and incubated for 1 hr with the following solution: 0.5% Triton X-100, 0.2% sodium deoxycholate, 2% nonylphenol, and 1% BSA. The sections were then incubated with mouse anti-GFAP (dilution 1:4000), rabbit anti-NCOR1 (dilution 1:500), and mouse anti-β-III-tubulin (dilution 1:4000) overnight at 4 °C. Then, the slides were washed three times with PBS, and incubated with secondary antibodies (1:4000) at room temperature for 2 hr. The sections were then washed three times with PBS and mounted on glass slides using the(Vector). The thickness of the collected sections was about 100 μm. Immunostaining was performed on 100-μm-thick frozen coronal sections of the time of analysis, the correct total membrane potential was monitored during the experiment. Three brain slices containing the hippocampus (CA3, CA1, and dentate gyrus (DG) regions) were visualized using epifluorescence and infrared differential interference contrast (IR-DIC) imaging on an upright microscope (Eclipse FN1, Nikon). DRAPE was stained with a mixture of MPM-2 and DAPI (Thermo Fisher). For the samples stained with MPM-2, the coverslip was removed before imaging. Images were acquired with an Axioimager M1 fluorescence microscope (Zeiss) equipped with a ColorView 107 digital camera (Prima Vision) and the Axiovision software (Zeiss). The immunostained coronal sections were processed using ImageJ (National Institutes of Health, Bethesda, MD) for image analysis and quantification of the data. For the analysis of the optical density of the immunostained sections, the ImageJ region of interest tool was used to draw regions of interest around the different cell types. The mean optical density of the drawn regions was then calculated and used for the analysis. The sections were then washed three times with PBS and mounted on glass slides using the Fluoromount-G mounting medium (Southern Biotech).
dissolved in DMso in a stock solution of 60 mg ml⁻¹ and then diluted to a final concentration of 30 mg ml⁻¹. The gramicidin-free pipette solution was backfilled into the pipette first and the gramicidin-containing pipette solution was then added before the experiment. After 10 to 15 min of cell-attached formation (pipette tip attached tightly to cell membrane), series resistance (R) decreased and stabilized at around 10 to 50 MΩ. the resting membrane potential was then recorded in the current clamp model.

Channelrhodopsin-2-assisted circuit mapping. To determine the LH projection to CA3, we used the circuit diagram, AAAV.EF1α.FLEX.CBCh-1H34β-EYFP and Ad.dI.PWP.WGA were co-injected into the LH region of Vgat-Cre mice. Evoked IPSCs of the mCherry and GFP-positive CA3 neurons (holding potential at ~70 mV) in brain slices were recorded in response to 473 nm blue light to validate the functional neural circuits from LH to CA3 neurons. TTX (1 μM) and 4-AP (100 μM) were added to the ACSF to confirm the response is monosynaptic responses.

In vivo optogenetic behavior assays. To reduce the interference from the optic cord, mice injected with AAV.FLEX.ChR2-EYFP, AAV.FLEX.eNhHR3.0-EYFP or AAV.FLEX.eYFP were acclimated by attachment to the optic cord in an open arena containing two identical objects, with the light on for three consecutive days. On each day the mice were trained and acclimated to the environment for 5 min. During the training session, mice were chronically stimulated with trains of blue light (5–7 mW, 15 ms pulses, 20 Hz, 30 s on and 2 s off) or yellow light (5–7 mW, 15 ms pulses, 6000 pulse sequence). Twenty-four hours after the third training, mice were re-exposed to a similar environment and the same light stimulus with one familiar object and one novel object. The animal behavior was recorded and analyzed by an experienced technician.

Ribosomal profiling, RT-qPCR, ChIP-qPCR, RNA-seq, and ChIP–seq. Ribosomal profiling, RT-qPCR, ChIP-qPCR, RNA-seq, and ChIP–seq were as described previously10. In brief, brain tissues were ground by TE buffer followed by RNA degradation by RNase inhibitor (Ambion) with a Downs homogenizer on ice. A one-ninth sample of 10% NP-40 and 1% Saponin were added and mixed, centrifuged at 20,000 g at 4 °C. The soluble fraction of RNA was saved, and cDNA was generated by using bar-coded M2 affinity gel (Sigma-Aldrich) for 30 min in the cold room (4 °C) in an end-over-end shaker. After washing the gel beads three times with washing buffer (20 mM HEPEs-KOH (pH 7.4), 5 mM MgCl2, 350 mM KCl, 1% NP-40, 0.5 mM DTT, and 100 μg ml⁻¹ CHX), we recovered enriched mRNA with phenol–chloroform extraction and ethanol precipitation with glycogen. The RT-qPCR analysis was performed using the reverse transcription kit (Life Technologies) and SYBR green master mixture (Life Technologies) to determine the fold enrichment.

RNA-seq was performed using total RNA extracted from the hypothalami in NS-DA Dm mice and their littermate wild-type control mice (n = 3). ChIP and ChIP–seq were as described previously. In brief, brain tissues were ground in liquid nitrogen and linked in 1% formalin for 20 min at room temperature. Whole-cell extracts were sonicated followed by immunoprecipitation with antibodies for HDAC3 (Abcam, ab7030) and Protein A Sepharose CL-4B (GE, 17-0780-01). After the immunoprecipitation, beads were washed by ChIP dilution buffer (50 mM HEPEs (pH 7.5), 155 mM NaCl, 1.1% Triton-X-100, 0.1% NaDeoxycholate, 1 mM EDTA) three times followed by a wash with ChIP wash buffer (50 mM Tris- HCl (pH 8.0), 10 mM EDTA, 0.5% NaDeoxycholate, 250 mM LiCl, and 0.5% NP-40). Sepharose beads were further washed once with TE buffer (Promega, E260A). RNA were enriched with phenol–chloroform extraction and dissolved in 20 μl TE buffer followed by RNA degradation by RNase Roche (Roche, 11119915001) for 30 min at 37 °C. Four HDAC3 ChIP reactions were performed with each reaction containing samples from three mice. The ChIP reactions were then pooled to be sequenced as one sample, together with the pooled total input DNA as the control. The precipitation DNA was then pooled and amplified according to the manufacturer’s guide of Illumina (Illumina TrueSeq ChIP Library Preparation Kit), followed by 50 bp single-end deep sequencing on Illumina Genome Analyzer Ix. For ChIP-qPCR analysis, primers were as follows: GABRA2 forward GATATGAGAAGATGGAAGATG, reverse GGTTCTGTGGGGAGT, distance to TSS is approximately 160bp; GABRA4 forward CAAACCGACACTACACTGT, reverse TGATGCCCGAGTGCAGGT, distance to TSS is around 1.5 kb; GABRD forward CTGGCTGGAGCCAAACAGCA, reverse TAAGACCTATGTTTGGACAA, distance to TSS is around 1.5 kb; GABRB1 forward AGGAGGAGCATACCTCTGG, reverse AGGAGGAGCATACCTCTGG, distance to TSS is around 270 bp. ChIP-qPCR was performed on four independent ChIP reactions with each reaction containing samples from three mice. A non-binding region near the gene Arhg was selected as the negative control (forward primer GAGTTGCGTGTGGAAACAGAG, reverse primer TCTTGGTCTCCTGGCTGAAAA).

Analysis of behavioral trials. For all behavioral studies, including those results from circuitry-targeted chemogenetic and optogenetic experiments, Student’s two-tailed paired t-test, one-way ANOVA, two-way ANOVA, and repeated ANOVA were used to analyze data, as appropriate. All intracranially injected mice were analyzed post mortem, and misinjected mice were excluded from final data analysis. Mice that did not recover to their pre-surgery body weight were also excluded. Sample sizes were based on literature and were not determined by statistical methods. All data meet the assumptions of the specific statistical tests. Data collection and analysis were not completely blind to the conditions of the experiments. Behavioral experiments were carried out by experimenters who knew the drug treatment information but not the genotype or surgery information. During the early stage of the experiment, some duration from video slices was blind to both genotype and treatment information. The statistical analysis and data plotting was then carried out by experimenters who knew both genotype and treatment information. All data were individually plotted (Prism 7, GraphPad). The exact N (number of animals), F, T, and P values are reported in the figure legends and Supplementary Table 1. P < 0.05 was considered to be significant.

Analysis of ex vivo electrophysiological data. Multiple single neurons were recorded from 2–4 mice in each group and were pooled for statistical analysis. We performed recording in pairs of mice, with one mouse from each group per day. We used two-way ANOVA to compare between two groups. The genotype or treatment serves as one variable, and the recording day served as another independent variable to take into account the animal-to-animal or day-to-day variability. We also analyzed the data using the linear mixed-effects (LME) models with the packages “lme4” and “lmerTest” in the statistical package R version 3.5.1 (https://www.gbif.org/tool/81287/r-language-and-environment-for-statistical-computing). The comparison between the experimental groups was made using the individual effects while the possible correlation due to the ANOVA was not considered. The initial analysis was considered to be significant.

Analysis of immunostaining, in situ hybridization, and anterograde tracing. To evaluate the expression of GABRA2 in NS-DA Dm mice and their wild-type littermates, coronal brain sections including the lateral hypothalamic structures were analyzed. The average optical density was measured by ImageJ software. The background was manually measured in the area near the analyzed GABARA2+ cells. The final results were calculated as OD = OD^GABARA2− OD^GABARA2^ background. Student’s two-tailed t-test was used for analysis, as appropriate. P < 0.05 was considered to be significant.

To evaluate the expression of NCOR1 in GABAerger neurons, GABAergic neurons were manually identified according to the expression of GAD1 and the optical density of NCOR1 in the GABAergic neurons was measured by ImageJ software. The final results were calculated as OD = OD^GABAergic− OD^GABAergic^ background. Student’s two-tailed t-test was used for analysis, as appropriate. P < 0.05 was considered to be significant.

To identify the projection of LH, Ad.dI.PWP.WGA was injected into one side of the lateral hypothalammus of tdTomato+/−/Vgat-Cre mice. The number of GFP+ or tdTomato+ cells in CA3 region was characterized. The number of co-localized GFP+ and tdTomato+ cells was also characterized. The number of GFP+ or glutaamate+ cells in CA3 was characterized. The number of co-localized GFP+ and glutamate+ cells was also characterized. Student’s two-tailed t-test was used for analysis, as appropriate. P < 0.05 was considered to be significant.

To confirm the projection of LH to CA3, we used the circuit diagram (https://www.gbif.org/tool/81287/r-language-and-environment-for-statistical-computing). The comparison between the experimental groups was made using the individual effects while the possible correlation due to the ANOVA was not considered.

Analysis of ChIp–seq and RNA-seq data. ChIP–seq reads were aligned to the Mus musculus genome (mm10) using Bowtie2 v2.1.0 with the following parameters: -D 20 -R 3 -N 0 -L 20 -i S,1,0.50. The average mappability across all the samples was approximately 96%. SAMtools V0.1.19 and BEDtools v2.17.0 were used to convert SAM files to bigWig format using bamCompare (with–normalizeTo1x) module from deepTools. Normalized bigwig files were visualized using Integrative Genome Browser (IGV). HDAC3 peaks were called using using SICER v1.1 with a window size of 200 nt, a gap of 300 nt and a false discovery rate (FDR) of ≤0.05. Called peaks were annotated and plotted using Chipseeker. For RNA-seq, raw sequence reads from each biological replicate of total RNA were first aligned to the UCSC mm10 genome with TopHat v2.1.0 using default parameters. Then, HTSeq was used to obtain read counts from the aligned reads. Finally, DESeq2 (v1.8.2) was used to normalized the read counts and perform differential gene analysis. Significantly differentially expressed genes were identified based on 5% FDR threshold.

Patient data. Data from the Exome Aggregation Consortium (ExAC) were used to estimate the intolerance of heterozygous loss-of-function variants in the apparently healthy human population. Data regarding the observed and the expected numbers for each variant were used to estimate the intolerance.
of loss-of-function variants and the probability of being loss-of-function intolerant (pLI) for each gene were obtained from http://exac.broadinstitute.org/ and calculated based on previous methods. In brief, the higher the pLI score (ranging from 0 to 1) is, the more likely it is that the gene is intolerant to heterozygous loss-of-function variants in humans. A pLI score higher than 0.9 is defined as being loss-of-function intolerant.

Patient data were obtained from the DECIPHER database (https://decipher.sanger.ac.uk/). For copy number variants, only small deletions affecting NCOR1, NCOZ2, or HADC3 were selected for analysis; large deletions that encompass additional disease genes for which heterozygous deletions are known or can potentially be associated with Mendelian disorders are excluded. Potential deleterious effects of the variants on protein functions were assessed by both the SIFT and the PolyPhen2 prediction programs. The patients selected do not have additional known pathogenic deletions. For single-nucleotide variants, only de novo changes are included. Single-nucleotide variants that have been observed in apparently healthy individuals from the ExAC database are excluded. The patients selected are not known to carry additional pathogenic variants that could complicate their clinical presentations. Pathogenicities (pathogenic or likely pathogenic significance) of the variants from the patients are classified according to the guidelines from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. The study has UK Research Ethics Committee approval (10/H3003/83, granted by the Cambridge South Research Ethics Committee, and GEN/284/12 granted by the Republic of Ireland Research Ethics Committee).

Statistical analysis. Data analysis was performed using SPSS (v21.0, IBM), or packages “lme4” and “lmerTest” (statistical package R v3.5.1). For statistical significance, two-tailed matched t-test, or one-way repeated ANOVA was used for experiments with two groups. Experiments with more than two groups were tested by one-way ANOVA, two-way ANOVA, or two-way repeated ANOVA followed by Tukey’s post hoc or Fisher’s LSD post hoc tests for multiple comparisons. We also analysed the ex vivo electrophysiological data using linear mixed-effects models, due to the limited number of mice. RNA-seq data were analyzed by DESeq2 (v1.8.2) with a 5% FDR significance threshold. All experiments were performed at least twice using independent biological samples or distinct cohorts of mice, except the RNA-seq and ChIP-seq studies, which were performed once. No statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications. All data were presented as means ± s.e.m.

Key reagents list. A list of key reagents are summarized in the Supplementary Table 3.

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

Data availability. The data that support the findings of this study are available from the corresponding author upon request. RNA-seq and ChIP-seq data are available in GEO (GSE92452).

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Reporting Summary

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- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
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- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection | Ethovision XT (Noldus), Any-Maze Behavior Tracking Software (Stoelting), Digidata1440A (Axon Instruments), VersaMax (Autoscan Instruments), SAMtools V0.1.19 , and BEDtools v2.17.0, SICER v1.1, and Tophat v2.1.0 |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data analysis  | SPSS (V.21.0, IBM), GraphPad Prism 7 (Graphpad), packages “lme4” and “lmerTest” (statistical package R version 3.5.1.), Ethovision XT (Noldus), Any-Maze Behavior Tracking Software (Stoelting), ImageJ (NIH), Mini Analysis Program (Synaptosoft Inc.), pClamp 10.3 software (Axon Instruments), SICER v1.1, DESeq2 (version 1.8.2) |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

RNA-seq and ChIP-seq data are available in GEO (GSE92452).

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample size were based on literature and were not determined by statistical methods. |
| Data exclusions | Animals were excluded and euthanized before behavior tests if they showed distress, infection, bleeding, or anorexia due to surgery. Animals were excluded after behavior tests if they showed mis-injection at postmortem examination. |
| Replication | All the experiments were repeated at least once, except that the ChIP sequencing and RNA sequencing were only done once. All attempts at replication were successful. |
| Randomization | All animals were grouped according to their sex, age, genotype, w/wo surgery, and w/wo drug/virus. |
| Blinding | Data collection and analysis were not performed completely blind to the conditions of the experiments. Behavioral experiments were done by experimentalists who know the drug treatment information but do not know the genotype or surgery information. During the early stage of analysis such as counting the time duration from video clips, experimentalists were blind to both genotype and treatment information. The statistical analysis and data plotting was done by experimentalists who knew both genotype and treatment information. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ Unique biological materials |
| ☒ Antibodies |
| ☒ Eukaryotic cell lines |
| ☒ Palaeontology |
| ☒ Animals and other organisms |
| ☒ Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ ChIP-seq |
| ☒ Flow cytometry |
| ☒ MRI-based neuroimaging |

Antibodies

| Antibodies used |
|-----------------|
| Anti-GABA-A Receptor alpha2 (Catlog #224104, Synaptic Systems), dilution 1:100 |
| Anti-HDAC3 (AbCam, AB7030), IHC 1:100, WB 1:2000; IP 6μg per IP. |
| Anti-HDAC1 (AbCam, AB7028), IP 4μg per IP. |
| Anti-NCOR1 (Bethyl Laboratories, A301-146A), dilution 1:1000. |
| Anti-TBLR1 (IMGENEX, IMG591), dilution 1:1000. |
| Anti-GFP (D5.1) (Cell Signaling, 2956), IHC 1:100. |
| Anti Glutamate antibody, (Sigma, G6642), IHC 1:100. |
| Alexa Fluor 488 goat anti guinea pig IgG (H+L) (Life Tech, A11073), dilution 1:1000. |
| Alexa Fluor 488 goat anti rabbit IgG (H+L) (Life Tech, A11034), dilution 1:1000. |
**Validation**

- Alexa Fluor 546 goat anti rabbit IgG(H+L) (Life Tech, A11010), dilution 1:1000.
- Alexa Fluor 647 goat anti rabbit IgG(H+L) (A32733, Life Tech), dilution 1:1000.
- Anti-GABA-A Receptor alpha2 (Catlog #224104, Synaptic Systems). IHC on mouse; https://www.sysy.com/products/gaba-a-facts-224104.php; PMID: 24554721.
- Anti-HDAC3 (AbCam, AB7030). IHC, ChIP and WB on mouse; https://www.abcam.com/hdac3-antibody-chip-grade-ab7030-references.html#top-600; PMID: 22647876.
- Anti-HDAC1 (AbCam, AB7028). IP on mouse; https://www.abcam.com/hdac1-antibody-chip-grade-ab7028-references.html#top-310; PMID: 28115699.
- Anti-NCO1 (Bethyl Laboratories, A301-146A). WB on mouse; PMID: 24268577.
- Anti-TBLR1 (IMGENEX, IMG591). WB on mouse; PMID: 26098212.
- Anti-Glu (DS.1) (Cell Signaling , 2956). IHC on mouse; https://www.citeab.com/antibodies/124367-2956-gfp-d5-1-xp-rabbit-mab?utm_campaign=Widget+All+Citations&utm_medium=Widget&utm_source=Cell+Signaling+Technology; PMID: 29158396.

**Eukaryotic cell lines**

- **Cell line source(s)**: HEK293T (ATCC).
- **Authentication**: Morphology check by microscope and fluorescent Hoechst staining.
- **Mycoplasma contamination**: Negative for mycoplasma contamination.
- **Commonly misidentified lines** (See ICLAC register): Not in ICLAC.

**Animals and other organisms**

- **Laboratory animals**:
  - NS-DADm mice were generated from crossing N-DADm and S-DADm mice. Vgat-Cre mice and Rosa26-tdTomato mice were obtained from JAX. Hdad3loxP/loxP mice were published and can be obtained from JAX. The NCO1/2loxP mice were provided by PHENOMIN, Institut Clinique de la Souris (ICS), CNRS, INSERM, University of Strasbourg, France (http://www.phenomin.fr/). NCO1/2loxP/loxP mice were generated through crossbreeding. tdTomato/loxP mice were used as control and referred to as WT. For the initial characterization of NS-V mice, NCO1/2loxP/loxP mice were further generated for circuit mapping and electrophysiology studies. All mice were C57BL/6 genetic background. Male mice at the age of 2-6 months were used for all experiments except otherwise noted. Female mice (2-6 months old) were used for some of the tests and no sexual dimorphism was observed for the phenotypes of NS-DADm mice or NS-V mice. For NS-DADm mice, wild-type littermates were used as control and referred to as WT. For electrophysiology studies, ribosomal profiling, circuit mapping, and chemogenetics experiments involving NS-V mice, Vgat-Cre mice or tdTomato/Vgat-Cre mice served as control and referred to as WT. All tests were repeated at least 2 times. 5 mice were housed in each cage in a 12-12 light-dark (7am - 7pm) facility with free access to water and food. After surgery, mice were housed singly in the same facility. All the animal care and use procedures followed the guidelines of the Institutional Animal Care and Use Committee in Baylor College of Medicine.

**ChIP-seq**

- **Data deposition**:
  - Confirm that both raw and final processed data have been deposited in a public database such as GEO.
  - Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

**Data access links**

- **May remain private before publication**.
- **Files in database submission**:
  - Raw files: Run1_ZN5264_GCCAATGT_L001_R1_001.fastq.gz, Run2_ZN5264_GCCAATGT_L001_R1_001.fastq.gz, Run2_ZN5264_GCCAATGT_L002_R1_001.fastq.gz, Run1_ZN5624_CTTGTACT_L001_R1_001.fastq.gz, Run2_ZN5624_CTTGTACT_L002_R1_001.fastq.gz.
  - Processed files: HDAC3.IP.bw, HDAC3.IN.bw.

**Genome browser session**

- **UCSC**.
  - https://genome.ucsc.edu/cgi-bin/hgTracks?db=mm10&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr2%3A112129023-112135205&hsid=683266175_dno3IAgp21spw81Dd6canmCY4GW7.
# Methodology

| Replicates | One ChIP sample and one input sample were created and sequenced after pooling 4 independent ChIP samples. |
|------------|----------------------------------------------------------------------------------------------------------|
| Sequencing depth | IP sample: Single end, 50bp, total reads: 234629536, uniquely aligned reads: 159488867  
Input sample: Single end, 50bp, total reads: 157256519, uniquely aligned reads: 97771305 |
| Antibodies | Anti-HDAC3 (Abcam, ab7030) |
| Peak calling parameters | HDAC3 peaks were called using SICER v1.1 by extending reads to 200bp in the 5’ to 3’ direction. A window size of 200nt, gap of 300nt and an FDR of 0.05 were set. HDAC3 ChIP samples were ratio normalized to their respective inputs and converted to bigwig format using the bamCompare (with --normalizeTo1x) module from deepTools. |
| Data quality | Read quality using fastqc: average phred score is greater than 34. ChIP-seq reads were aligned to the mus musculus genome (mm10) using Bowtie2 V2.1.0 with following parameters: -D 20 -R 3 -N 0 -L 20 -i S,1,0.50. The average mappability across all the samples was ~96%. HDAC3 ChIP samples were ratio normalized to their respective inputs and converted to bigwig format using the bamCompare (with --normalizeTo1x) module from deepTools. |
| Software | Bowtie2 V2.1.0, SAMtools V0.1.19, BEDtools v2.17.0, deepTools, SICER v1.1, Integrative Genome Browser (IGV) and ChIPseeker. |