Modulation of excitation on parvalbumin interneurons by neuroligin-3 regulates the hippocampal network

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Hippocampal network activity is generated by a complex interplay between excitatory pyramidal cells and inhibitory interneurons. Although much is known about the molecular properties of excitatory synapses on pyramidal cells, comparatively little is known about excitatory synapses on interneurons. Here we show that conditional deletion of the postsynaptic cell adhesion molecule neuroligin-3 in parvalbumin interneurons causes a decrease in NMDA-receptor-mediated postsynaptic currents and an increase in presynaptic glutamate release probability by selectively impairing the inhibition of glutamate release by presynaptic Group III metabotropic glutamate receptors. As a result, the neuroligin-3 deletion altered network activity by reducing gamma oscillations and sharp wave ripples, changes associated with a decrease in extinction of contextual fear memories. These results demonstrate that neuroligin-3 specifies the properties of excitatory synapses on parvalbumin-containing interneurons by a retrograde trans-synaptic mechanism and suggest a molecular pathway whereby neuroligin-3 mutations contribute to neuropsychiatric disorders.

Optimal neural circuit function is critical for effective information processing that mediates cognitive processes and is dependent on the specification of the precise properties of individual synapses. Synapses in the forebrain initially form in excess but are subsequently pruned, strengthened and eliminated in an experience-dependent manner. This process gives rise to functional neuronal ensembles that enable computations critical for precise information processing. In the hippocampus, circuits processing contextual and spatiotemporal information utilize glutamatergic pyramidal cells and various types of GABAergic interneurons, which play critical roles in the information processing underlying learning and memory via, in part, the generation of functionally important neural oscillations1–3. The critical importance of interneurons in optimal circuit performance is emphasized by recent suggestions that a range of prominent neuropsychiatric disorders involve abnormalities in the functioning of GABAergic interneurons4.

Although both excitatory and inhibitory cells in hippocampal ensembles are driven to spike by glutamatergic excitatory synapses, most of our understanding of the molecular specification of synaptic properties comes from study of excitatory synapses on principal glutamatergic cells. In contrast, the molecular architecture underlying excitatory synapses on GABAergic interneurons, which are biophysically and structurally different from excitatory synapses on principal neurons5–7 and which transform incoming excitation into various forms of inhibition, is poorly understood. To more comprehensively elucidate the molecular architecture of excitatory synapses on GABAergic interneurons, here we focus on the role of the postsynaptic cell adhesion protein neuroligin-3 (NL3) in specifying the properties of excitatory synapses on parvalbumin (PV) interneurons in the CA1 region of the hippocampus.

NL3 is of particular interest because of its known genetic association with a variety of neuropsychiatric disorders, notably autism8,9. Moreover, the availability of NL3-conditioned knockout mice, which have been used previously to advance our understanding of its role in circuit function and repetitive behaviors10, facilitates analysis of its cell-type-specific functions. NL3 belongs to the neuroligin family of synaptic cell-adhesion molecules (NL1–4), proteins which are differentially expressed at excitatory and inhibitory synapses9, with NL1 and NL2 localized to excitatory and inhibitory synapses, respectively. Although NL3 is present at both excitatory and inhibitory synapses, its detailed functions at excitatory synapses in general and at excitatory synapses on GABAergic interneurons in particular have yet to be established.

Here we report that genetic deletion of postsynaptic NL3 from PV interneurons in the CA1 region of the hippocampus enhanced presynaptic glutamate release via specific loss of functioning of presynaptic Group III metabotropic glutamate receptors (mGluRs), which normally mediate tonic inhibition of glutamate release on PV interneurons. Combined with a decrease in NMDA receptor (NMDAR)-mediated postsynaptic responses due to the NL3 deletion, this presynaptic modulation resulted in a frequency-dependent change of net circuit function, as evidenced by a decrease in the frequency of network oscillations in the gamma (35–85 Hz) and sharp wave ripple (SWR; 100–200 Hz) ranges. Behavioral assays revealed that the NL3 deletion from hippocampal CA1 PV interneurons specifically impaired contextual fear extinction without altering fear

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conditioning itself. Thus, NL3 conferred specific properties to excitatory synapses on hippocampal CA1 PV GABAergic interneurons that importantly contributed to the role of these interneurons in controlling hippocampal network activity and hippocampal-dependent behavioral functions.

RESULTS

Postsynaptic effects of conditional knockout of NL3 from CA1 PV interneurons

We deleted NL3 specifically from PV interneurons by crossing homozygous female conditional NL3 knockout mice (NL3fl; ref. 10) with male mice that had been engineered to drive Cre recombinase under the parvalbumin promoter (PV-Cre; Jackson Laboratories, stock number 08069). Since NL3 is located on the X chromosome, breeding homozygous loxP-flanked NL3 (NL3fl) female mice to homozygous PV-Cre male mice produced male offspring that carried a PV-specific deletion of NL3 (NL3fl/PV-Cre; Supplementary Fig. 1a). We previously confirmed the deletion of NL3 in NL3fl mice by performing quantitative PCR on NL3fl/Nestin-Cre mice, which showed a complete absence of NL3 while levels of NL1 and NL2 were unaltered10. Histological examination of the forebrain in NL3fl/PV-Cre mice did not reveal any difference in the density or distribution of PV cells in the hippocampal formation (Supplementary Fig. 1b,c) or in the frontal cortex (data not shown), ruling out the possibility of an early disruption of PV cell development.

To examine the electrophysiological consequences of deleting NL3 from PV interneurons in the hippocampus, we stereotactically injected postnatal day (P) 21 PV-Cre and NL3fl/PV-Cre mice with an AAV virus encoding eGFP in a double-inverse opening reading frame (DIO) driven by the human synapsin promoter (AAV-hSyn-DIO-eGFP) (Supplementary Fig. 2a). After 10–14 d, acute hippocampal slices were prepared and whole-cell recordings were made from eGFP-expressing PV cells in the stratum oriens of the hippocampus (Supplementary Fig. 2b). Current-clamp recordings revealed that deletion of NL3 did not detectably alter the basic membrane properties of PV interneurons as assayed by spike frequency in response to a twice-threshold depolarizing pulse (Fig. 1a), resting membrane potential (Fig. 1b) or the magnitude of the afterhyperpolarization potential (Fig. 1c). Neither the amplitude nor the frequency of AMPA receptor (AMPAR)-mediated miniature excitatory postsynaptic currents (mEPSCs) were affected by NL3 deletion from PV interneurons (Fig. 1d,e). There was no change in the amplitude or frequency of GABA	extsubscript{A} receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) (Supplementary Fig. 3).

AMPA EPSCs in PV interneurons display robust inward rectification and fast decay kinetics because a high proportion of the synaptic AMPARs in PV interneurons lack GluA2 subunits11. However, deletion of NL3 from PV cells did not significantly influence the rectification properties or decay kinetics of AMPAR EPSCs ($P = 0.07$; Fig. 1f). Given that synaptic AMPAR function and number seemed unaltered following NL3 deletion from PV interneurons and NMDARs are variably expressed in these cells12, we next assayed NMDAR-mediated synaptic transmission using ratios of NMDAR EPSC to AMPAR EPSC. NL3 deletion caused a $\sim 60\%$ reduction in NMDAR/AMPAR ratios (Fig. 1g) without causing any changes in NMDAR stoichiometry or biophysical properties as assayed by the rise-time and decay-time constants and the current–voltage relationships of pharmacologically isolated NMDAR EPSCs (Fig. 1h–j). Additionally, examining the density of synapses on PV neurons by staining for PSD-95 and the AMPAR subunit GluA4 on the dendrites of PV-Cre and NL3fl/PV-Cre neurons revealed no significant effect of NL3 deletion (Fig. 1k–m), assayed as the number of PSD-95 puncta per dendritic length (Fig. 1l) or as the proportion of PSD-95 puncta containing detectable GluA4 (Fig. 1m). Thus, NL3 deletion in CA1 PV interneurons caused a reduction in postsynaptic NMDAR-mediated synaptic transmission without causing detectable changes in the postsynaptic properties of either AMPAR- or GABA	extsubscript{A}-receptor-mediated synaptic currents, nor did it cause changes in excitatory synapse density.

Presynaptic effects of conditional knockout of NL3 from CA1 PV interneurons

As a further test for effects of the NL3 knockout in PV interneurons on basal synaptic transmission, we examined the input–output relationship of AMPAR EPSCs at incremental stimulus intensities and, unexpectedly, observed a significant increase in EPSC amplitudes in NL3fl/PV-Cre cells as a function of stimulus strength (Fig. 2a). We then measured paired-pulse ratios (PPRs) at multiple interstimulus intervals, a measure that inversely correlates with presynaptic release probability13, and found that postsynaptic deletion of NL3 from PV interneurons caused a clear decrease in PPRs (Fig. 2b), indicative of an increase in glutamate release probability. At central synapses, different presynaptic machinery may mediate spontaneous release in the absence of action potentials (i.e., mEPSCs recorded...

Figure 1 NL3 deletion reduces NMDA-EPSCs in hippocampal PV interneurons. (a) Firing properties of CA1 PV interneurons in wild-type PV-Cre and NL3fl/PV-Cre mice. Left: sample traces in response to twice-threshold current injection. Right: spike frequency as a function of a spike number is unchanged by NL3 deletion (frequency at spikes 4–8: PV-Cre, 111 ± 10 Hz, n = 6 cells; NL3fl/PV-Cre, 115 ± 10 Hz, n = 6 cells). (b) Resting membrane potential of PV interneurons is unchanged by NL3 deletion (PV-Cre, −64 ± 2 mV, n = 6 cells; NL3fl/PV-Cre, −67 ± 3 mV, n = 6 cells). (c) Amplitude of fast afterhyperpolarization potential is unchanged by NL3 deletion (PV-Cre, 18 ± 1 mV, n = 4 cells; NL3fl/PV-Cre, 15 ± 2 mV, n = 4 cells). (d,e) Top: sample traces showing mEPSCs. Bottom: cumulative distribution plots for AMPAR mEPSC (d) amplitudes and (e) frequency showing no change due to NL3 deletion ($P > 0.05$, Kolmogorov-Smirnov test for both amplitude and frequency). Insets show average mEPSC amplitudes (d; PV-Cre, 21 ± 2 pA, n = 9 cells; NL3fl/PV-Cre, 19 ± 2 pA, n = 8 cells; $P > 0.05$) and mEPSC frequency (e; PV-Cre, 14 ± 2 Hz, n = 9 cells; NL3fl/PV-Cre, 17 ± 3 Hz, n = 8 cells; $P > 0.05$). (f) Top: sample average traces showing evoked AMPAR EPSCs at −60 and +40 mV. Bottom: rectification ratio (left), measured as a ratio of peak AMPAR current at +40 mV and −60 mV, is unchanged by NL3 deletion (PV-Cre, 0.13 ± 0.01, n = 19 cells; NL3fl/PV-Cre, 0.09 ± 0.02, n = 11 cells; $P > 0.05$). AMPAR EPSC decay kinetics (right) fitted with a single exponential fit are unchanged by NL3 deletion (PV-Cre, 2.3 ± 0.2, n = 12 cells; NL3fl/PV-Cre, 2.0 ± 0.2, n = 11 cells; $P > 0.05$). (g) Left: representative traces of AMPAR EPSCs recorded at −60 mV and dual component EPSC at +40 mV. Right: NMDAR/AMPAR ratio is reduced by NL3 deletion (PV-Cre, 0.18 ± 0.1, n = 12 cells; NL3fl/PV-Cre, 0.07 ± 0.1, n = 12 cells; $P < 0.05$). (h) NMDAR EPSC rise time is unchanged by NL3 deletion (NL3fl/PV-Cre, 1.37 ± 0.10 ms, n = 8 cells; PV-Cre, 1.64 ± 0.15 ms, n = 10 cells; $P = 0.17$). (i) Weighted decay time constants for NMDAR EPSCs are unchanged by NL3 deletion (NL3fl/PV-Cre, 31.0 ± 1.8 ms, n = 20 cells; PV-Cre, 36.2 ± 2.1 ms, n = 22 cells; $P > 0.05$). (j) Current–voltage (I−V) relationship of NMDAR EPSCs is unchanged by NL3 deletion. (k) Representative images from dendrites (30 μm) of hippocampal (left) PV-Cre and (right) NL3fl/PV-Cre neurons infected with AAV-DIO-GFP and immunolabeled for GFP (top), GluA4 and PSD-95 (middle) and merged images (bottom). Online figure (Fig. 1f) quantifies the number of PSD-95 puncta per 30 μm of the dendrite (PV-Cre $2.2 ± 1.5$, n = 9; NL3fl/PV-Cre, $6.5 ± 1.9$, n = 8; $P > 0.1$). (m) Bar graph showing the colocalization of GluA4 and PSD-95 puncta (colocalization coefficient in PV-Cre, $0.46 ± 0.02$, n = 9; in NL3fl/PV-Cre, $0.42 ± 0.02$, n = 8; $P = 0.21$). Data are presented as mean ± s.e.m.
in the presence of tetrodotoxin) versus action-potential-dependent evoked and spontaneous EPSCs. Given that we did not see a change in mEPSC properties in these cells (Fig. 1d,e), we examined whether spontaneous EPSCs (sEPSCs) were altered in NL3fl/PV-Cre cells. Deletion of postsynaptic NL3 caused an increase in the frequency of sEPSCs without any significant change in their amplitude (Fig. 2c,d), findings consistent with the observed decrease in PPRs. To test whether these changes in input–output relationship, PPRs and sEPSCs did indeed indicate a change in presynaptic release probability, we examined the rate of block of pharmacologically isolated NMDAR EPSCs using MK-801, a measure that directly correlates with release probability. Consistent with the change in PPRs and sEPSC frequency caused by NL3 deletion, blockade of NMDAR EPSCs by MK-801 was accelerated in NL3fl/PV-Cre cells (Fig. 2e,f). Thus, three different electrophysiological measures of evoked presynaptic release probability were all consistent with the hypothesis that NL3 deletion from PV interneurons increased the release probability at excitatory synapses on these neurons.

Recent studies demonstrate that genetic deletion of NL3 from CA1 pyramidal cells or their presynaptic partners neurexins can influence...
Figure 2 Deletion of postsynaptic NL3 causes an increase in glutamate release probability at synapses on PV interneurons. (a) Representative traces (top) and plot (bottom) show increased input–output relationship of AMPAR EPSCs in cells lacking NL3 for incremental stimulation intensities (in V) 2.5, 5.7, 6, 10, 12.5, 15, 17.5 and 20 (PV-Cre, 12.3 ± 7.1, 49.8 ± 21.6, 114.9 ± 34.7, 181.1 ± 56.5, 241.3 ± 68.8, 252.4 ± 78.5, 224.6 ± 62.1 and 215.1 ± 56.4 pA, respectively, n = 7 cells; NL3fl/PV-Cre, 4.9 ± 2.0, 74.5 ± 12.5, 189.7 ± 32.5, 319.8 ± 55.8, 446.8 ± 59.7, 563.4 ± 77.6, 576.5 ± 75.3 and 521.2 ± 72.7 pA, respectively, n = 7 cells; P = 0.33, 0.34, 0.14, 0.10, 0.02, 0.01, 0.003 and 0.006 for each stimulus intensity). (b) Representative average traces (top) and plot (bottom) show reduced PPRs at interstimulus intervals of 20, 50, 100 and 200 ms in cells lacking NL3 (PV-Cre, 2.5 ± 0.1, 2.2 ± 0.1, 1.6 ± 0.1 and 1.4 ± 0.1, n = 11 cells; NL3fl/PV-Cre, 1.8 ± 0.1, 1.6 ± 0.1, 1.4 ± 0.1 and 1.1 ± 0.1, n = 12 cells; P < 0.01, 0.01, 0.05 and 0.05 for each interstimulus interval). (c,d) Top: sample traces showing spontaneous EPSCs. Bottom: cumulative distribution plot showing unchanged sEPSC amplitude and increased sEPSC frequency as a result of NL3 deletion (P = 0.3, Mann-Whitney test for amplitude; P = 0.03, Mann-Whitney test for frequency). Insets show average sEPSC amplitudes (0.1, 1.4, 2.5, 20, 5, 7.5, 10, 12.5, 15, 17.5 and 20; PV-Cre, 12.3 ± 7.1, 49.8 ± 21.6, 114.9 ± 34.7, 181.1 ± 56.5, 241.3 ± 68.8, 252.4 ± 78.5, 224.6 ± 62.1 and 215.1 ± 56.4 pA; respectively, n = 7 cells; NL3fl/PV-Cre, 4.9 ± 2.0, 74.5 ± 12.5, 189.7 ± 32.5, 319.8 ± 55.8, 446.8 ± 59.7, 563.4 ± 77.6, 576.5 ± 75.3 and 521.2 ± 72.7 pA; respectively, n = 7 cells; P = 0.33, 0.34, 0.14, 0.10, 0.02, 0.01, 0.003 and 0.006 for each stimulus intensity). (e) Top: Representative traces (top) for 1st, 10th, 50th and 120th NMDAR EPSC at +40 mV following 10 min wash-in of 40 µM MK-801 block (PV-Cre, 36.4 ± 7.2 pA; NL3fl/PV-Cre, 120.3 ± 27.2 pA). Bottom: summary bar graph with mean decay time constant (τw) of MK-801 block in PV-Cre and NL3fl/PV-Cre cells. Bottom: summary bar graph with mean decay time constant of MK-801 block (PV-Cre, τw = 215 ± 25 ms, n = 6 cells; NL3fl/PV-Cre, 112 ± 19 ms, n = 5 cells; *P < 0.05, t-test). Error bars represent s.e.m.

the tonic release of endocannabinoids (eCBs) and the consequent activity of presynaptic CB1 cannabinoid receptors, thereby influencing transmitter release. To determine whether a similar mechanism occurs at excitatory synapses on PV interneurons, we applied the CB1 receptor agonist WIN 55,212-2. Application of WIN 55,212-2 elicited comparable reductions in EPSCs and increases in the PPRs of presynaptic CB1 cannabinoid receptors, thereby influencing the tonic release of endocannabinoids (eCBs) and the consequent activity of presynaptic CB1 cannabinoid receptors, thereby influencing transmitter release. To determine whether a similar mechanism occurs at excitatory synapses on PV interneurons, we applied the CB1 receptor agonist WIN 55,212-2. Application of WIN 55,212-2 elicited comparable reductions in EPSCs and increases in the PPRs of presynaptic CB1 cannabinoid receptors, thereby influencing the tonic release of endocannabinoids (eCBs) and the consequent activity of presynaptic CB1 cannabinoid receptors, thereby influencing transmitter release. To determine whether a similar mechanism occurs at excitatory synapses on PV interneurons, we applied the CB1 receptor agonist WIN 55,212-2. Application of WIN 55,212-2 elicited comparable reductions in EPSCs and increases in the PPRs of presynaptic CB1 cannabinoid receptors, thereby influencing the tonic release of endocannabinoids (eCBs) and the consequent activity of presynaptic CB1 cannabinoid receptors, thereby influencing transmitter release.
Figure 3  Increase in glutamate release caused by NL3 deletion at synapses on PV interneurons is due to loss of Group III mGluR-mediated presynaptic inhibition. (a–d) Depression of excitatory synaptic transmission in PV interneurons by the CB1 receptor agonist WIN 55,212-2 (5 µM) is not affected by NL3 deletion. (a) Time course of normalized AMPAR EPSCs during application of WIN 55,212-2 and (b) sample EPSCs in response to paired-pulse stimulation before and after WIN 55,212-2 application in PV-Cre (before, black; after, blue) and NL3/PV-Cre (before, orange; after, blue) neurons. Summary plot (c) of normalized EPSC changes due to WIN 55,212-2 and (d) normalized PPR changes in individual cells (PV-Cre, 0.68 ± 0.05, 1.3 ± 0.1, n = 5; NL3/PV-Cre, 0.66 ± 0.07, 1.2 ± 0.1, n = 7). (e–h) As in a–d but for effect of CB1-receptor antagonist AM251 (10 µM). (f) Representative EPSCs before and after AM251 for PV-Cre cell (before, black; after, blue) and NL3/PV-Cre (before, orange; after, blue). Individual normalized (g) EPSC and (h) PPR after AM251 application (PV-Cre, 1.0 ± 0.1, 0.9 ± 0.1, n = 5; NL3/PV-Cre, 1.05 ± 0.08, 1.07 ± 0.1, n = 5; P > 0.1). (i–l) As in a–d but for effect of Group III mGluR agonist L-AP4 (10 µM). (j) Representative EPSCs before and after L-AP4 for PV-Cre cell (before, black; after, blue) and NL3/PV-Cre (before, orange; after, blue). Individual normalized EPSC (k) and PPR (l) changes caused by L-AP4 (PV-Cre, 0.7 ± 0.1, 1.3 ± 0.1, n = 6; NL3/PV-Cre, 1.1 ± 0.1, 1.0 ± 0.05, n = 8; *P < 0.001 for EPSC amplitude; *P = 0.04 for PPR). (m–p) As in i–l but for application of Group III mGluR antagonist LY341495 (1 µM) showing changes in EPSC and PPR in PV-Cre cells (1.7 ± 0.2, 0.9 ± 0.03, n = 11) and NL3/PV-Cre cells (1.0 ± 0.1, 1.1 ± 0.1, n = 8; *P < 0.01 for EPSC amplitude; *P = 0.04 for PPR). Data are represented as mean ± s.e.m.
**Figure 4** Changes in synaptic responses to repetitive stimulation as a result of NL3 deletion from PV interneurons. (a,b) Normalized EPSC amplitudes in response to stimulation trains applied at increasing frequencies. Response in PV-Cre and NL3fl/PV-Cre cells for (a) 2 Hz, 5 Hz, 10 Hz and 20 Hz stimulation and for (b) 30 Hz, 50 Hz, 100 Hz and 200 Hz stimulation (PV-Cre, n = 10 cells; NL3fl/PV-Cre, n = 12 cells; *P < 0.05, post hoc Bonferroni test). (c,d) The probability of generating spikes in response to prolonged stimulus trains in PV-Cre and NL3fl/PV-Cre cells for (e) 5 Hz and (d) 100 Hz trains. Representative traces (left) and summary graphs (right) are shown. Spikes were reliably evoked at 5 Hz in both PV-Cre (n = 8 cells) and NL3fl/PV-Cre cells (n = 5; P > 0.99, Bonferroni test) but showed increased attenuation as the train progresses at 100 Hz in NL3fl/PV-Cre (*P = 0.0554, 0.0481, 0.0175, 0.0413, 0.0137, 0.0074, 0.0498 and 0.0074, respectively; Bonferroni test). (e) Schematic of experimental setup for recording synaptic integration in CA1 pyramidal neurons in the absence of GABA A receptor blockers. Stimulating electrode was placed in stratum oriens to evoke monosynaptic EPSPs and disynaptic inhibitory postsynaptic potentials (IPSPs). PN, pyramidal neuron; SC, schaffer collateral; CA3, cornus ammonis. (f) Representative traces of synaptic potentials in CA1 pyramidal cells from PV-Cre and NL3fl/PV-Cre held at −60 mV during 50 Hz stimulation. (g) 2 Hz, 5 Hz, 10 Hz and 20 Hz stimulus number (PV-Cre, n = 8 cells; *P < 0.05, t-test). (h) Bar graph showing the slope of the EPSP summation as a function of stimulus number and the corresponding fitted curve with 95% confidence limits (*P < 0.05, Bonferroni test). (i) Bar graph showing normalized EPSP amplitude after the tenth stimulation (PV-Cre, 3.7 ± 0.5; n = 9 cells; NL3fl/PV-Cre, 5.8 ± 0.8, n = 8 cells; *P < 0.05, t-test). Error bars represent s.e.m.

Figs. 4f and 5e–h) was not altered by the NL3 deletion. Thus, postsynaptic deletion of NL3 from PV interneurons specifically impaired the presynaptic inhibition of glutamate release by Group III mGluRs but had no effect on the presynaptic modulation mediated by CB1 receptors, GABA B receptors or A1 adenosine receptors.

**Network effects of the NL3 deletion from CA1 PV interneurons**

To begin to elucidate the network effects of the NL3 deletion from PV interneurons, we examined the effects of repetitive activation of excitatory synapses at a wide range of frequencies (20 stimuli at 2–200 Hz), including those that occur during various forms of hippocampal oscillatory activity, such as in theta, gamma and SWR frequencies1,2,21–25. To avoid inducing postsynaptic plasticity during these trains, we added the calcium chelator BAPTA (10 mM) to the pipette solution. At moderate frequencies (2–10 Hz), the facilitation of EPSCs during the train was modest in PV-Cre interneurons and not substantially affected by the NL3 deletion (Fig. 4a and Supplementary Fig. 6a). At 20–50 Hz, an impairment of the train-induced facilitation by NL3 deletion became more apparent, while at...
100 Hz and 200 Hz, the differences between PV-Cre and NL3fl/PV-Cre cells were pronounced (Fig. 4a,b and Supplementary Fig. 6a,b). Furthermore, during repetitive activation of excitatory synapses in current-clamp configuration, spikes were reliably generated in both PV-Cre and NL3fl/PV-Cre cells at a low frequency (5 Hz; Fig. 4c), but spiking was substantially attenuated at a high frequency (100 Hz) in PV cells lacking NL3 (Fig. 4d). This frequency-dependent attenuation in information transfer (i.e., EPSC amplitudes and spiking probability) in NL3fl/PV-Cre interneurons suggests that these cells were now functioning as low-pass filters, in that they reliably integrated information (i.e., EPSCs) at lower frequencies but not at higher frequencies (Supplementary Fig. 6c).

The marked reduction in facilitation of EPSCs and spiking during high-frequency stimulation in PV interneurons lacking NL3 would be expected to have an effect on the net inhibition generated in CA1 pyramidal cells during high frequency trains. To test this hypothesis, we made current-clamp recordings from CA1 pyramidal cells in the absence of GABA receptor antagonists during a 50-Hz train (Fig. 4e). In wild-type slices, by the fourth stimulus inhibitory postsynaptic potentials (IPSPs) seemed to shunt excitatory postsynaptic potential...
(EPSP) summation, giving rise to a plateau effect during which EPSP amplitude stabilized at approximately fourfold its initial amplitude (Fig. 4f–i). In NL3\(^{fl}\)/PV-Cre slices, EPSPs in CA1 pyramidal cells summated in a supralinear manner during the early phase of the stimulus train, as indicated by an increase in the slope of the synaptic summation and the much larger EPSP summation (approximately six-fold).

### Figure 6
Deletion of NL3 from CA1 PV interneurons compromises contextual fear extinction. (a) Schematic representation of fear conditioning and extinction protocols. (b) Left: schematic representation of DIO-hM4Di-mCherry construct. Right: bar graph shows percent of time freezing for control PV-Cre (all of which received injections of AAV-expressing Cre-dependent eGFP) and PV-Cre mice expressing hM4Di in hippocampal PV cells treated with CNO (10 mg/kg). Baseline (BL) habituation to context (PV-Cre mice, 0.7 ± 0.4%, \( n = 12 \); hM4Di expressing mice, 1.0 ± 0.3%, \( n = 8 \)), average percent of time freezing for fear conditioning bouts (FC) 1–3 (PV-Cre mice, 31.3 ± 5.5%; hM4Di injected mice 33.8 ± 5.8%) and average percent of time freezing during Days 2–5 of extinction (Ext2–5; PV-Cre mice, 14.0 ± 6.2%; hM4Di injected mice, 29.5 ± 6.2%, *\( P = 0.01 \), Bonferroni test). Middle and right graphs show time course of percent of time freezing during BL, FC1–3 and 4 d of fear extinction (Ext2–5). (c) Left: schematic representation of AAV-DIO-eGFP construct injected into NL3\(^{fl}\)/PV-Cre and PV-Cre mouse hippocampus. Right: graphs same as in b; data for control PV-Cre mice are the same as shown in b. Bar graph: BL NL3\(^{fl}\)/PV-Cre, 0.6 ± 0.2%, \( n = 16 \); FC1–3 NL3\(^{fl}\)/PV-Cre, 34.6 ± 3.4%; Ext2–5 NL3\(^{fl}\)/PV-Cre, 27.9 ± 3.1; *\( P = 0.002 \), Bonferroni test. (d) Left: schematic representation of AAV-DIO-NL3-t2a-Venus construct injected into NL3\(^{fl}\)/PV-Cre mouse hippocampus. Graphs same as in b; data for control PV-Cre mice are the same as shown in b. Bar graph: BL NL3\(^{fl}\)/PV-Cre rescue, 0.9 ± 0.2%, \( n = 8 \) mice; FC1–3 NL3\(^{fl}\)/PV-Cre rescue, 29.5 ± 5.2%; Ext2-5 NL3\(^{fl}\)/PV-Cre rescue, 17.2 ± 3.5%; *\( P < 0.05 \), Bonferroni test. Data are represented as mean ± s.e.m.
during the latter half of the train (Fig. 4h,i), presumably due to a decrease in PV-interneuron-mediated inhibition at this frequency.

Given that PV interneurons control the excitation of principal cells and thus gate network activity, we next examined the effects of NL3 deletion from PV interneurons on hippocampal circuit function by making in vivo recordings from freely behaving PV-Cre and NL3fl/PV-Cre mice implanted with a linear four-channel recording electrode in the CA1 region (Supplementary Fig. 7a). The power spectra of the local field potentials (LFPs) in the theta oscillation range (3–12 Hz) were not significantly altered in NL3fl/PV-Cre mice (Fig. 5a–c), with only a left shift in the peak theta frequency in NL3fl/PV-Cre mice (peak theta frequency: 6.0 ± 0.6 Hz in PV-Cre mice; 4.0 ± 0.5 in NL3fl/PV-Cre mice). In contrast, LFP power spectra at higher frequency oscillations including gamma (35–85 Hz) were reduced by ~60% in NL3fl/PV-Cre mice (Fig. 5c). This resulted in a ~70% reduction in the gamma-to-theta ratio in NL3fl/PV-Cre mice (Supplementary Fig. 7b).

We also examined the power spectra, amplitude and frequency of individual SWRs during immobility. Similarly to gamma oscillations, the power spectra of the isolated SWRs substantially decreased in NL3fl/PV-Cre mice (Fig. 5d–f) due to a ~70% decrease in the amplitude of the SWRs (Fig. 5g and Supplementary Fig. 7c) and a ~50% decrease in the length of the SWRs (Fig. 5h). However, the rate of SWRs was not significantly affected (Fig. 5i).

We also examined SWRs in acute hippocampal slices, a preparation in which acute hippocampal oscillatory activity cannot be influenced by changes in afferent activity to the hippocampus due to the NL3 deletion from PV interneurons in brain regions other than the hippocampus. Application of a high-potassium solution reliably induced SWRs25 (Fig. 5j), which exhibited qualitatively similar effects of deletion of NL3 from PV interneurons compared to the SWRs recorded in vivo (Fig. 5k–o). Specifically, slices from NL3fl/PV-Cre mice exhibited lower power in the 100–200-Hz range (Fig. 5k,l), a decrease in SWR length (Fig. 5n) and a decrease in SWR rate (Fig. 5o), but they did not exhibit a detectable change in SWR amplitude (Fig. 5m).

Thus, deletion of NL3 from PV neurons caused a decrease in hippocampal gamma oscillations and SWRs, patterns of activity that have been implicated in various phases of hippocampal-dependent learning and memory3,23,24.

Behavioral effects of the NL3 deletion from hippocampal PV interneurons

Since the synaptic changes caused by the NL3 deletion in PV interneurons modulated hippocampal circuit function in a manner that might be expected to influence hippocampal-dependent memory functions, we investigated whether PV interneurons are critical for hippocampal dependent learning and memory processes, as previously suggested13,26. In an initial set of experiments, we expressed in these neurons hM4Di, an inhibitory designer receptor exclusively activated by a designer drug (DREADD), using stereotoxic injection of an appropriate Cre-dependent adeno-associated virus (AAV-hSyn-DIO-hM4Di-mCherry) into the hippocampus of PV-Cre mice. Mice were exposed to a hippocampal-dependent contextual fear conditioning task, during which mice learned to associate a neutral context with an aversive foot shock, and the learned fear was measured by freezing behavior22 (Fig. 6a). Experimental mice were injected with the hM4Di agonist clozapine-N-oxide (CNO) twice a day on both conditioning day 1 and extinction days 2–5. Control PV-Cre mice expressing GFP and hM4Di-expressing mice showed virtually identical freezing behavior during both the baseline habituation session and the three conditioning sessions. However, hM4Di-expressing mice displayed much less extinction of freezing when the footshock no longer occurred (Fig. 6b). Thus, inhibition of CA1 PV interneurons did not influence the acquisition of conditioned fear but significantly impaired its extinction.

To investigate the role of NL3 in PV interneurons in mediating this behavior, we tested NL3fl/PV-Cre mice using this protocol. The behavioral results were essentially identical to those observed when PV interneurons were inhibited by DREADD. The NL3 knockout in PV interneurons had no effect on learning to associate the context with the footshock as assessed by freezing behavior, but the retention of fear on day 2 was higher in NL3fl/PV-Cre mice and the extinction of context-induced freezing was significantly reduced (Fig. 6c). To determine if loss of NL3 specifically in CA1 PV interneurons was responsible for the impairment in extinction, we expressed full-length NL3 in the hippocampal PV interneurons of NL3fl/PV-Cre mice by injecting an AAV encoding NL3 in a double-inverse open reading frame (AAV-hSyn-DIO-NL3-t2a-Venus) 3–4 weeks before the behavioral experiments. Histological examination revealed a ~90% colocalization for NL3-Venus and PV in hippocampal PV interneurons through the dorsal–ventral axis (Supplementary Fig. 8a–c). This hippocampal PV interneuron-specific rescue manipulation reversed contextual fear extinction in NL3fl/PV-Cre mice while having no effect on conditioned freezing (Fig. 6d).

To test whether the rescue of the extinction deficit due to NL3 deletion from PV neurons was associated with rescue of the synaptic changes caused by this genetic manipulation, we made recordings from NL3fl/PV-Cre cells expressing the full-length NL3. These cells exhibited NMDAR/AMPAR ratios (Supplementary Fig. 9a) and PPRs (Supplementary Fig. 9b) that were very similar to those recorded from control PV neurons, as well as normal reductions in EPSC amplitudes in response to application of L-AP4 (Supplementary Fig. 9c–e). Thus, the major synaptic changes caused by NL3 deletion from PV neurons were rescued by expression of NL3 in young adult hippocampus.

To examine whether other forms of hippocampal-dependent learning might be disrupted in NL3fl/PV-Cre mice, we subjected these mice to a reward alternation task in a T maze. In this assay, both PV-Cre and NL3fl/PV-Cre mice performed at chance on day 1 and learned the task at similar rates during days 2–7 (Supplementary Fig. 10a). The NL3fl/PV-Cre mice also did not exhibit any atypical motor behaviors as assayed by total distance traveled (Supplementary Fig. 10b), low motion bouts (Supplementary Fig. 10c) or stereotypy (Supplementary Fig. 10d), all measured on a force-plate actometer.28 Thus, deletion of NL3 from PV interneurons in the hippocampus specifically affected extinction of conditioned fear, likely due to its complex modification of excitatory synaptic drive, which in turn influenced hippocampal network activity.

DISCUSSION

Using a cell-restricted conditional knockout approach, we have defined a critical role for the postsynaptic cell-adhesion molecule NL3 in controlling the properties of excitatory synapses on PV interneurons in the CA1 region of the hippocampus. Moreover, we demonstrated that changes in synaptic excitation to hippocampal PV interneurons modified hippocampal circuit properties, specifically the frequencies of gamma oscillations and SWRs, and, presumably as a consequence of these changes in network properties, modified the extinction of conditioned fear. Given that NL3 deletions and point mutations are genetically associated with autism spectrum disorders8,9, it is plausible to suggest that the abnormalities described here contribute to some of the cognitive symptoms manifested by these prominent disorders. In contrast, distinct cell-type-specific and circuit-specific changes due
to the NL3 deletion cause abnormalities in other behavioral domains, such as repetitive motor routines\textsuperscript{13}, thus emphasizing the daunting complexity of defining even how a ‘simple’ single genetic manipulation influences circuit function and behavior.

Our electrophysiological characterization of genetically labeled CA1 PV interneurons revealed two unexpected changes at excitatory synapses due to the NL3 deletion: a decrease in NMDAR-mediated synaptic responses and an increase in presynaptic release probability, both of which could be rescued by selectively replacing NL3 in NL3\textsuperscript{fl}/PV-Cre cells. While in other cell types in hippocampus, striatum and amygdala, NL1 seems to be required to maintain normal NMDAR-mediated synaptic transmission\textsuperscript{29–31}, our findings suggest that NL3 fulfills this role in CA1 PV interneurons and that NL1 does not compensate for the loss of NL3.

The mechanism by which the NL3 deletion enhances glutamate release is more surprising. In CA1 pyramidal cells, the NL3 deletion enhances GABA release at a subset of inhibitory synapses due to disruption of tonic endocannabinoid signaling\textsuperscript{16}. In marked contrast, we demonstrated here that tonic inhibition of glutamate release at excitatory synapses on CA1 PV interneurons was not mediated by endocannabinoids but instead by Group III mGluRs and that this mGluR\textsubscript{1} mediated inhibition was eliminated by NL3 deletion. This presynaptic consequence of the postsynaptic NL3 deletion was specific, in that the effects of other prominent presynaptic neurotransmitters were unaffected. Furthermore, mIPSCs were unaffected by NL3 deletion from CA1 PV interneurons, in marked contrast to the robust decrease in mIPSCs caused by NL3 deletion from nucleus accumbens area D1 medium spiny neurons\textsuperscript{10}. Together these results suggest that postsynaptic NL3 in hippocampal PV neurons is required for the normal function and/or recruitment of presynaptic Group III mGluRs, which are selectively enriched at synaptic terminals on hippocampal interneurons\textsuperscript{20,32–38}.

The autoreceptor activity of group III mGluRs seems to be critical for maintaining sustained release of glutamate at stimulation frequencies above ~50 Hz, as shown by our finding that, following the NL3 deletion, EPSCs and synchronously evoked spikes showed marked attenuation with successive stimuli (Fig. 4b,d). As a consequence of this decrease in excitatory drive onto CA1 PV interneurons at these frequencies, postsynaptic potentials in CA1 pyramidal neurons exhibited supralinear summation (Fig. 4e–i). Because normal PV interneuron function is required for the generation of oscillatory network activity including gamma oscillations and SWRs\textsuperscript{2,39,40}, we suggest that the observed decrease in gamma oscillations and SWRs (Fig. 5) due to the deletion of NL3 in these neurons was the result of a net decrease in PV interneuron activity at high frequencies and the consequent reduction in circuit-level inhibition.

SWRs play a critical role in hippocampal-dependent learning and consolidation of recently formed memories\textsuperscript{3,23,24}. Although the decrease in SWRs due to the deletion of NL3 from PV interneurons did not influence the development of contextual fear conditioning or the learning of a spatial reward alternation task, extinction of conditioned fear was markedly impaired. Notably, by performing cell-type-restricted expression of inhibitory DREADDs and local Cre-dependent NL3 rescue experiments, we demonstrated that this impairment was in fact due to dysfunctions specifically in hippocampal PV interneurons. Although we did not include CNO- or vehicle-treated mice as controls for the DREADD experiments, the lack of change in baseline freezing and fear conditioning itself after CNO injections in the DREADD mice suggests that any stress caused by the injection did not account for the observed impairment of fear extinction. Deleting the requisite NMDAR subunit GluN1 from PV interneurons causes more pronounced effects than NL3 deletion in various learning and memory assays\textsuperscript{3,41}. However, in this previous work, experiments were not performed to directly test whether these deficits were solely due to deficits in hippocampal PV interneuron function.

In summary, we have shown that deletion of NL3 from CA1 PV interneurons causes changes at excitatory synapses unlike those observed at any of the distinct synapses previously studied. These findings emphasize that the function of individual synaptic proteins often depends on the specific molecular architecture in which they are embedded. This appears to be particularly the case for the neurexin–neurexin family of synaptic cell-adhesion proteins\textsuperscript{8,10,16,17,42,43}. Furthermore, combined with previous work on neurelin\textsuperscript{10,29,44–47}, our findings emphasize the challenges and importance of defining the specific synapses and circuits responsible for the behavioral abnormalities caused by manipulations of proteins that are genetically associated with brain disorders.

**METHODS**

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

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**AUTHOR CONTRIBUTIONS**

J.S.P., T.C.S. and R.C.M. conceived the project, designed the experiments, and wrote and edited the manuscript. H.W. performed the in vivo electrophysiology experiments with input from C.H.H., and D.G. performed the immunohistochemistry experiments.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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12. Nyiri, G., Stephenson, F.A., Freund, T.F. & Somogyi, P. Large variability in synaptic NMDA receptor density on interneurons and a comparison with pyramidal-cell spines in the rat hippocampus. Neuroscience 119, 347–363 (2003).

13. Zucker, R.S. & Regehr, W.G. Short-term synaptic plasticity. Annu. Rev. Physiol. 64, 355–405 (2002).

14. Kavalali, E.T. The mechanisms and functions of spontaneous neurotransmitter release. Nat. Rev. Neurosci. 16, 5–16 (2015).

15. Hessler, N.A., Shirke, A.M. & Malinow, R. The probability of transmitter release at a mammalian central synapse. Nature 366, 569–572 (1993).

16. Földy, C., Malenka, R.C. & Südhof, T.C. Autism-associated neuroligin-3 mutations commonly disrupt tonic endocannabinoid signaling. Neuron 78, 498–509 (2013).

17. Anderson, G.R. et al. β-Neurexins control neural circuits by regulating synaptic endocannabinoid signaling. Cell 162, 593–606 (2015).

18. Gibson, H.E., Edwards, J.G., Pago, R.S., Van Hook, M.J. & Kauer, J.A. TRPV1 channels mediate long-term depression at synapses on hippocampal interneurons. Neuron 57, 746–759 (2008).

19. Thompson, S.M., Capogna, M. & Scanziani, M. Presynaptic inhibition in the hippocampus. Trends Neurosci. 16, 222–227 (1993).

20. Shigemoto, R. et al. Target-cell-specific concentration of a metabotropic glutamate receptor in the presynaptic active zone. Nature 381, 523–525 (1996).

21. Allen, K. & Monyer, H. Interneuron control of hippocampal oscillations. Curr. Opin. Neurobiol. 31, 81–87 (2015).

22. Stark, E. et al. Pyramidal cell-interneuron interactions underlie hippocampal ripple oscillations. Neuron 83, 467–480 (2014).

23. Jadhav, S.P., Kemere, C., German, P.W. & Frank, L.M. Awake hippocampal sharp wave ripples support spatial memory. Science 336, 1454–1458 (2012).

24. Girardeau, G., Benchenane, K., Wiener, S.I., Buzsáki, G. & Zugaro, M.B. Selective suppression of hippocampal ripples impairs spatial memory. Nat. Neurosci. 12, 1222–1223 (2009).

25. Dzhala, V.I. & Staley, K.J. Mechanisms of fast ripples in the hippocampus. J. Neurosci. 24, 8896–8906 (2004).

26. Donato, F., Rompani, S.B. & Caroni, P. Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. Nature 504, 272–276 (2013).

27. Xu, W. et al. Distinct neuronal coding schemes in memory revealed by selective erasure of fast synchronous synaptic transmission. Neuron 73, 990–1001 (2012).

28. Fowler, S.C. et al. A force-plate actometer for quantitating rodent behaviors: illustrative data on locomotion, rotation, spatial patterning, stereotypies, and tremor. J. Neurosci. Methods 107, 107–124 (2001).

29. Chubykin, A.A. et al. Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2. Neuron 54, 919–931 (2007).

30. Blundell, J. et al. Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. J. Neurosci. 30, 2115–2129 (2010).

31. Jung, S.Y. et al. Input-specific synaptic plasticity in the amygdala is regulated by neuroligin-1 via postsynaptic NMDA receptors. Proc. Natl. Acad. Sci. USA 107, 4710–4715 (2010).

32. Losonczy, A., Somogyi, P. & Nusser, Z. Reduction of excitatory postsynaptic responses by persistently active metabotropic glutamate receptors in the hippocampus. J. Neurophysiol. 89, 1910–1919 (2003).

33. Scanziani, M., Gähwiler, B.H. & Charpak, S. Target cell-specific modulation of transmitter release at terminals from a single axon. Proc. Natl. Acad. Sci. USA 95, 12034–12039 (1998).

34. Losonczy, A., Zhang, L., Shigemoto, R., Somogyi, P. & Nusser, Z. Cell type dependence and variability in the short-term plasticity of EPSPs in identified mouse hippocampal interneurons. J. Physiol. (Lond.) 562, 543–550 (2004).

35. Pellegrino, L.A., Tertulliani, R., Pellegrino, M. & Van der Zee, S.E. A force-plate actometer for quantitating rodent behaviors: illustrative data on locomotion, rotation, spatial patterning, stereotypies, and tremor. J. Neurosci. Methods 196, 74–84 (2010).

36. Blundell, J. et al. Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. J. Neurosci. 30, 1228–1240 (2010).

37. Tabuchi, K. et al. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. J. Neurosci. 30, 10765–10776 (2010).

38. Steinbach, L. et al. Target-cell-specific concentration of a metabotropic glutamate receptor in the presynaptic active zone. Nature 381, 523–525 (1996).

39. Schlingloff, D., Käll, S., Freund, T.F., Hájos, N. & Gulyás, A.I. Mechanisms of long-term depression at hippocampal synapses. Curr. Opin. Neurobiol. 21, 222–227 (2011).

40. Foro, F., Alleva, J., Pasino, F. & Di Cuzzio, M. Selective suppression of hippocampal ripples impairs spatial memory. Nat. Neurosci. 12, 1222–1223 (2009).

41. Dzhala, V.I. & Staley, K.J. Mechanisms of fast ripples in the hippocampus. J. Neurosci. 24, 8896–8906 (2004).

42. Donato, F., Rompani, S.B. & Caroni, P. Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. Nature 504, 272–276 (2013).

43. Xu, W. et al. Distinct neuronal coding schemes in memory revealed by selective erasure of fast synchronous synaptic transmission. Neuron 73, 990–1001 (2012).

44. Fowler, S.C. et al. A force-plate actometer for quantitating rodent behaviors: illustrative data on locomotion, rotation, spatial patterning, stereotypies, and tremor. J. Neurosci. Methods 107, 107–124 (2001).

45. Chubykin, A.A. et al. Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2. Neuron 54, 919–931 (2007).

46. Blundell, J. et al. Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. J. Neurosci. 30, 2115–2129 (2010).

47. Jung, S.Y. et al. Input-specific synaptic plasticity in the amygdala is regulated by neuroligin-1 via postsynaptic NMDA receptors. Proc. Natl. Acad. Sci. USA 107, 4710–4715 (2010).
Slice electrophysiology. Ten to 15 d following the injection of AAV virus, animals were anesthetized with halothane and the brains rapidly removed and placed in ice-cold, high-sucrose cutting solution containing (in mM): 228 sucrose, 26 NaHCO3, 11 glucose, 2.5 KCl, 1 NaH2PO4, 7 MgSO4 and 0.5 CaCl2. We cut 300-µm sections on a Leica vibratome in the high sucrose cutting solution and immediately transferred them to an incubation chamber containing artificial cerebrospinal fluid (ACSF) containing (in mM) 135 CsMeSO4, 8 NaCl, 10 HEPES, 0.25 EGTA, 2 Mg2ATP, 0.3 Na3GTP, 0.1 spermine and 7 phosphocreatine (pH 7.25–7.3; osmolarity 294–298). Data was collected with a MultiClamp 700B amplifier (Axon Instruments), digitized at 8 kHz using an ITC-18 A/D converter (Instrutech Corporation) and filtered at 4 kHz. Data were acquired and analyzed using Axograh-X (Axograh).

PV interneurons were identified by the presence of GFP on an upright microscope (Olympus BX51WI, Olympus Optical) and visualized by infrared differential interference contrast. mEPSCs were recorded at −60 mV in the presence of 100 µM picrotoxin, 50 µM AP-5 and 0.1 mM TTX. For mIPSCs, picrotoxin was replaced by 20 µM NBQX. A theta glass pipette filled with ACSF was used as a bipolar stimulation electrode and was placed in the CA1 oriens alveus layer to evoke EPSCs in PV cells. Cells were held at −60 or −70 mV to record AMPAR EPSCs while stimulating afferent inputs at 0.1 Hz. NMDAR EPSCs were evoked by injecting square-step current injections (500 ms) into cells held at their resting membrane potentials. For pharmacology experiments, paired-pulse AMPAR EPSCs were evoked with an ISI of 20 ms at +40 mV. Drugs were applied following a 10 min baseline. For current-clamp recordings, CsMeSO4 in the internal solution was replaced with KMeSO4. Spike frequency adaptation was measured for spikes 1–8 at rheobase. The afterhyperpolarization potential was measured as the peak hyperpolarization after the first spike in the train at rheobase. To evoke spiking in PV cells in current-clamp mode, a theta glass stimulator was placed in the oriens layer and stimulation intensity was increased to the minimal necessary level to produce spiking in two consecutive stimulations given 50 ms apart. The same stimulation intensity was used during the prolonged stimulation trains. CA1 pyramidal cells (closer to oriens) were held at −60 mV in current-clamp mode for EPSP–IPSP integration experiments, and postsynaptic potentials were evoked by a theta glass electrode placed in the CA1 oriens alveus layer.

Sharp wave ripple oscillations (SWRs) were recorded from 450-µm coronal sections of the hippocampus using a 4–5 ΜΩ pipette filled with aCSF. To induce SWRs, the slices were perfused with modified aCSF containing 8.5 mM potassium at a flow rate of 10 ml/min at 32–34 °C (ref. 25). To detect SWRs, local field potentials were bandpass filtered at 100–400 Hz and smoothened with a six-point boxcar filter. Individual events were detected using an amplitude threshold algorithm that detected peaks higher than 6 s.d. of the noise of the eventless LFP. The minimum separation between events was set to 50 ms, and 200 ms of the oscillatory event were captured. The captured events were visually examined to discard false positives that had fewer than two peaks crossing the set threshold.

Statistics. All data are presented as means ± s.e.m. Statistical significance was calculated between the two genotypes using unpaired two-tailed t-tests (*P < 0.05). For LFP recordings, power spectrum data are presented as means ± 95% confidence level. Data distribution was assumed to be normal, but this was not formally tested.

In vivo electrophysiology. Linear multielectrode arrays containing four channels (75 µm spacing between channels; Microprobes, Gaithersburg, MD) were stereotactically implanted in the CA1 region of the hippocampus in P6–P90 mice (n = 6 PV-Cre; n = 6 NL3f/PV-Cre). In brief, mice were anesthetized with ketamine/xylazine and placed in a stereotaxic frame. Craniotomies were made to allow a multielectrode array to be implanted in the left hippocampus (2 mm posterior to the bregma, 1.7 mm lateral to the midline and 2.1 mm below the brain surface for the deepest contact). Skull screws in the frontal cortex served as reference. After 1 week of recovery and acclimation, each mouse was tethered to a neural recording device (Alphalab SnR, Alpha Omega, Nazareth, Israel) and put in an open-field box for 1 h, during which hippocampal signals were recorded continuously at 22 kHz together with synchronized behavioral data (Ethovision, Noldus, Wageningen, Netherlands). To extract information in the local field potential range, signals were downsampled offline to 1.375 Hz, and segments corresponding to locomotion and paradoxical sleep were extracted. A short-time Fourier transform was used to approximate the power spectrum in the time–frequency domain (window of 1 s, 50% overlap). Signal processing was performed using the Matlab software package and custom scripts. Data for theta and gamma oscillations is presented as mean ± s.e.m. SWRs were detected using Axograh-X analysis software. LFPs during immobility were normalized, bandpass filtered at 100–400 Hz and smoothened with a six-point boxcar filter.
Individual events were detected using an amplitude threshold algorithm that detected peaks higher than 6 s.d. of the noise of the eventless LFP. The minimum separation between the highest peaks in two events was set to 100 ms, and 500 ms of the oscillatory event were captured. The captured events were visually examined to discard false positives that had fewer than two peaks crossing a threshold of 4 s.d. of the eventless noise.

**Statistics.** Power spectrum data are presented as mean ± s.e.m. Statistical significance was calculated between the two genotypes using unpaired two-tailed t-tests (*P < 0.05). Data distribution was assumed to be normal, but this was not formally tested.

**Behavior: fear conditioning and extinction.** Three to four weeks following the injection of AAV virus, 3–4-month-old mice were handled daily for 3 d before training. On day 1 of training, individual mice were placed in a sound-attenuated fear-conditioning chamber cleaned with 10% ethanol. After a 2 min exploration period, 3 context-footshock pairings were delivered, separated by 1 min intervals. The mice remained in the training chamber for another 30 s before being returned to home cages. From day 2 until day 5, mice were reintroduced to the fear-conditioning chamber for 5 min twice a day, with a gap of 2–3 h between sessions. The behavior of the mice was recorded with FreezeFrame software and analyzed with FreezeView software (Colburn Instruments, Holliston, MA). Motionless bouts lasting more than 1 s were considered as freezing. Freezing was analyzed for 30 s periods. Baseline freezing was calculated as the percent of time freezing for the first 30 s after the mice were first introduced to the chamber on day 1. Fear learning was calculated as the percent of time freezing during the 30 s following context–footshock pairings. Fear retention during extinction on days 2–5 was calculated as percent of time freezing for the first 30 s when the mice were first reintroduced into the fear-conditioning chamber. Percent of times freezing were averaged for the two sessions for each day during extinction. Mice previously injected with AAV-syn-hM4Di-mCherry were injected with 10 mg/kg clozapine-N-oxide (CNO) 1 h before the start of the behavioral experiments and 12 h thereafter for all 5 d of the behavioral procedure. Animal experiments were conducted following protocols approved by Administrative Panel on Laboratory Animal Care at Stanford University.

**Reward alternation task.** We used 10–12-week-old PV-Cre and NL3/+/PV-Cre mice that were food restricted until they reached 85% of their body weight. During the first 2 d of food restriction, they were fed only the chocolate pellets that they would subsequently receive in the task, and they were moved to a 50–50 mixture of dry food and chocolate pellets until the end of the experimental procedure. Mice were handled for 3 d before habituation, and during habituation mice were allowed to explore all three arms of the T-maze. Food pellets were placed in the reward cups in the two arms of the maze and were constantly replaced during the 10-min session as they were consumed. After two habituation sessions over 2 d, mice were given 10 trials per d. Each trial consisted of two runs, a sample run and a choice run. In the sample run, a reward was placed in the reward cup at the end of an arm and mice were forced to run into this arm by blocking the other arm. Over the 10 trials, the sample arm was randomly assigned to left or right to avoid biases. At the end of the trial, mice were allowed to consume the reward and were gently placed back into the start box, and the arms were wiped down with 10% ethyl alcohol while the mouse was trapped in the start box. The previously blocked door was opened so that both arms of the T-maze were open for the choice run and mice were rewarded for choosing the opposite arm from the sample run. If a mouse chose the same arm as the sample run, it was blocked in the arm for 10 s as a punishment, before being returned to the start box. Ten consecutive trials were performed in each session for 6 d. Mice were scored for the percent of correct choices made in each session.

**Force-plate actometer assays.** Mice were tested for locomotor activity and stereotypical behaviors on a custom made force-plate actometer as described23. In brief, mice were placed individually in the actometer for 20-min recording sessions. The distance traveled by the mice was calculated as the sum of distances between coordinates of the location of the center of force (COF) recorded every 0.5 s over the 20-min recording session. A low-mobility bout was defined as the mouse’s COF remaining within a 15-mm radius for 5 s. Stereotypy score was defined as the product of the score of head mobility and the score of lack of foot mobility during the same time interval.

**Behavioral data analysis and statistics.** All data were collected and analyzed blind to the genotype of mice or the virus injected. Mice were randomized into groups consisting of a mixture of control and knockout and DREADD–containing mice, or control and knockout or rescue, or control and rescue and DREADD mice. Data are presented as mean ± s.e.m. Repeated measures ANOVAs with α = 0.05 were used to analyze the data, and statistical interference was further verified with Bonferroni tests. For force-plate actometer assays, data between the two genotypes were compared using two-tailed Student’s t-tests with statistical significance set to P < 0.05. Data distribution was assumed to be normal, but this was not formally tested. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications48.

**Immunohistochemistry.** PV-Cre and NL3/+/PV-Cre mice injected with AAV virus were anesthetized with sodium pentobarbital (150 mg/kg) and perfused transcardially with 50 ml of 4% paraformaldehyde (in 0.1 M PB, pH 7.4) 45–60 d after virus injection. Brains were then removed and postfixed overnight in the same fixative at 4 °C and then washed in 0.1 M PBS, after which 50-µm thick coronal sections containing the hippocampus and the frontal cortex were cut using a vibratome (Leica VT1000S). Sections were serially washed four times in PBS and blocked using a blocking buffer (PBS + 0.1% Triton X-100 + 2% bovine serum albumin + 2% goat serum) for 30 min. Antibodies to parvalbumin (1:1,000; Sigma-Aldrich, P3088), GluA4 (1:100; Abcam, ab13552-50) and GFP (1:1,000; Abcam, ab13970) were made and used to detect parvalbumin, GluA4, and GFP, respectively. Images were processed and analyzed using ImageJ. To quantify the percent of hippocampal parvalbumin neurons infected with AAV-DIO-NL3-GFP we used 5–6 labeled sections from each hemisphere along the rostrocaudal axis of the hippocampus. Cells bodies labeled with parvalbumin and GFP antibodies throughout the hippocampus were visually analyzed using ImageJ, and the numbers of colabeled cells per section were averaged.

**Data availability statement.** The data that support the findings of this study are available from the corresponding author upon request. A **Supplementary Methods Checklist** is available.

48. Celn, R.L. & Huganir, R.L. Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science* **330**, 1108–1112 (2010).