Rapid erasure of hippocampal memory following inhibition of dentate gyrus granule cells

Noelia Madroñal, José M. Delgado-García, Azahara Fernández-Guzmán, Jayanta Chatterjee, Maja Köhn, Camilla Mattucci, Apar Jain, Theodoros Tsetsenis, Anna Illarionova, Valery Grinevich, Cornelius T. Gross & Agnès Gruart

The hippocampus is critical for the acquisition and retrieval of episodic and contextual memories. Lesions of the dentate gyrus, a principal input of the hippocampus, block memory acquisition, but it remains unclear whether this region also plays a role in memory retrieval. Here we combine cell-type specific neural inhibition with electrophysiological measurements of learning-associated plasticity in behaving mice to demonstrate that dentate gyrus granule cells are not required for memory retrieval, but instead have an unexpected role in memory maintenance. Furthermore, we demonstrate the translational potential of our findings by showing that pharmacological activation of an endogenous inhibitory receptor expressed selectively in dentate gyrus granule cells can induce a rapid loss of hippocampal memory. These findings open a new avenue for the targeted erasure of episodic and contextual memories.
The hippocampus is an evolutionarily ancient part of the cortex that makes reciprocal excitatory connections with neocortical association areas and is critical for the acquisition and retrieval of episodic and contextual memories. The hippocampus has been the subject of extensive investigation over the last 50 years as the site of plasticity thought to be critical for memory encoding. Models of hippocampal function propose that sensory information reaching the hippocampus from the entorhinal cortex via dentate gyrus (DG) granule cells is encoded in CA3 auto-association circuits and can in turn be retrieved via Schaffer collateral (SC) projections linking CA3 and CA1 (refs 1–4; Fig. 1a). Learning-associated plasticity in CA3–CA3 auto-associative networks encodes the memory trace, and plasticity in SC connections is necessary for the efficient retrieval of this trace2,5–10. In addition, both CA3 and CA1 regions receive direct, monosynaptic inputs from entorhinal cortex that are thought to convey information about ongoing sensory inputs that could modulate CA3 memory trace acquisition and/or retrieval via SC (refs 11–13; Fig. 1a). In DG granule cells, sensory information is thought to undergo pattern separation into orthogonal cell ensembles before encoding (or reactivating, in the case of retrieval) memories in CA3 (ref. 14). However, how the hippocampus executes both the acquisition and recall of memories stored in CA3 remains a question of debate with some models attributing a role for DG inputs in memory acquisition, but not retrieval2,13–17.

Here we combined a pharmacogenetic approach for the rapid and transient suppression of neural activity with in vivo electrophysiology during trace eye-blink conditioning to examine the contribution of DG to hippocampal learning and plasticity. Our studies demonstrate that activity in DG granule cells is not necessary for the retrieval of hippocampal memory. Unexpectedly, inhibition of DG is associated with a rapid and persistent loss of memory, as revealed by the suppression of both conditioned responding and learning-associated plasticity. Pharmacogenetic inhibition of entorhinal cortex or local delivery of adenosine A1 receptor antagonist into the hippocampus reverses the suppression of learning-associated plasticity, suggesting a role for direct entorhinal-CA1 inputs in promoting memory loss. Similar memory impairment can be induced by DG inhibition during trace fear conditioning, demonstrating its generalization across hippocampus-dependent memory tasks. Finally, we show that activation of an endogenous inhibitory receptor (neuropeptide Y1 receptor) selectively expressed in DG granule cells can similarly induce rapid and persistent memory loss, opening the possibility of the targeted erasure of hippocampal memories.

**Results**

**Rapid pharmacogenetic inhibition of DG granule cells.** Until recently, a major limitation in resolving the mechanism of hippocampal function has been the lack of tools that allow for the rapid, transient, efficient and specific inhibition of selected hippocampal cell-types to directly assess their contribution to memory. Here we applied a pharmacogenetic neuronal inhibition strategy that we had previously used to rapidly suppress the firing of DG granule cells in living mice18,19 to dissect the role of this hippocampal input structure in memory. This pharmacogenetic inhibition system depends on the systemic administration of DG granule cells in living mice18,19 to transgenic mice expressing Htr1a exclusively to transgenic mice expressing Htr1a exclusively

Importantly, the short latency fEPSP component that reflects direct entorhinal inputs to CA3 was unaffected by 8-OH-DPAT treatment20,21 (Supplementary Fig. 1; n = 10, P = 0.477, two-way analysis of variance followed by Holm–Sidak post hoc test). Neither agonist treatment of Htr1aKO control littermates nor vehicle treatment of wild-type mice affected synaptic transmission (Fig. 1c). These results demonstrate that the 8-OH-DPAT/Htr1a pharmacogenetic inhibition system is able to rapidly and selectively suppress neurotransmission via DG granule cells in vivo.

**DG inhibition induces loss of plasticity and memory.** To define the contribution of DG to the retrieval of hippocampal memory, we combined our pharmacogenetic strategy with in vivo electrophysiology during trace eye-blink conditioning (Fig. 2a–c). We selected trace eye-blink conditioning as a learning task, as the hippocampus executes both the acquisition and recall of memories stored in CA3 (ref. 14). However, how the hippocampus executes both the acquisition and recall of memories stored in CA3 remains a question of debate with some models attributing a role for DG inputs in memory acquisition, but not retrieval2,13–17.

Here we combined a pharmacogenetic approach for the rapid and transient suppression of neural activity with in vivo electrophysiology during trace eye-blink conditioning to examine the contribution of DG to hippocampal learning and plasticity. Our studies demonstrate that activity in DG granule cells is not necessary for the retrieval of hippocampal memory. Unexpectedly, inhibition of DG is associated with a rapid and persistent loss of memory, as revealed by the suppression of both conditioned responding and learning-associated plasticity. Pharmacogenetic inhibition of entorhinal cortex or local delivery of adenosine A1 receptor antagonist into the hippocampus reverses the suppression of learning-associated plasticity, suggesting a role for direct entorhinal-CA1 inputs in promoting memory loss. Similar memory impairment can be induced by DG inhibition during trace fear conditioning, demonstrating its generalization across hippocampus-dependent memory tasks. Finally, we show that activation of an endogenous inhibitory receptor (neuropeptide Y1 receptor) selectively expressed in DG granule cells can similarly induce rapid and persistent memory loss, opening the possibility of the targeted erasure of hippocampal memories.

**Figure 1 | Rapid and selective inhibition of DG neurotransmission in vivo.** (a) The hippocampal tri-synaptic circuit receives PP inputs from entorhinal cortex to DG, CA3 and CA1. (b) A stimulating electrode was implanted in the PP and a recording electrode in CA3 pyramidal layer. (c) Strength of CA3 pyramidal layer fEPSPs evoked in anaesthetized mice by electrical stimulation of PP inputs showed fast and slow latency population spike components corresponding to direct PP-CA3 and indirect PP–DG-CA3 inputs, respectively. Systemic administration of the selective Htr1a agonist, 8-OH-DPAT (0.3 mg kg$^{-1}$, subcutaneous), to Htr1aDG (TG) mice caused a rapid and selective decrease in the long-latency component that persisted for several hours. Quantification indicated a significant decrease in DG neurotransmission following agonist treatment of Htr1aDG, but not Htr1aKO (KO) littermates or vehicle treated wild-type mice that reached 80% suppression and persisted for >2 h (mean ± s.e.m.; n = 10; *P < 0.05; two-way analysis of variance followed by Holm–Sidak post hoc test). (d) Representative fEPSPs evoked at CA3 pyramidal layer after stimulation of PP inputs before and after agonist treatment. The fast and the slow latency population spike components are indicated (black arrow, short; grey arrow, long).
paradigm because it is a hippocampus-dependent form of learning in which the neural circuitry supporting conditioned responses is well defined and in which synaptic plasticity correlates are readily identified. 5,22. Htr1aDG and Htr1aKO control littermates that had successfully acquired conditioned behaviour were treated with 8-OH-DPAT (day 8; 0.3 mg kg−1, subcutaneous). Agonist-treated Htr1aDG, but not Htr1aKO mice showed a significant decrease in the per cent of successful conditioned responses on the treatment day (Fig. 2d) suggesting a requirement of neural activity in DG granule cells for retrieval of the conditioned response. Unexpectedly, a significant deficit in conditioned responding persisted on the day following agonist treatment in Htr1aDG mice (day 9; Fig. 2d) and did not reach control levels before day 12. These data point to a persistent effect of transient DG inhibition on memory recall.
To determine whether the persistent loss of memory on transient DG inhibition was associated with a loss of learning-associated plasticity, we monitored synaptic changes in SC during eye-blink conditioning (Fig. 2a–e and Supplementary Fig. 2). SC synaptic plasticity increased in parallel with eye-blink conditioning and reached an asymptotic mean value of 130% on day 8 of conditioning (Fig. 2e). Surprisingly, 8-OH-DPAT treatment of Htr1aKO, but not Htr1aKO littermates on day 8 was associated with a significant depotentiation of SC (Fig. 2e) demonstrating that maintenance of SC synaptic plasticity was dependent on inputs from upstream DG granule cells. Moreover, SC plasticity continued to be significantly reduced in Htr1aKO, but not Htr1aKO mice on the day following agonist treatment (day 9, Fig. 2e) and did not reach control levels before day 12. Similar results were found in an independent experimental group, demonstrating the robust nature of DG inhibition-induced loss of memory and plasticity (Supplementary Fig. 3). No change in conditioned responding or SC plasticity were observed in Htr1aKO mice receiving 8-OH-DPAT or wild-type, Htr1aDG, or Htr1aKO mice receiving saline on day 8 (Supplementary Fig. 3) confirming that the transgene does not interfere with the measured behaviour or plasticity. These observations suggest that the rapid and persistent loss of conditioned responding seen following DG inhibition is caused by a parallel loss of SC plasticity.

Finally, we examined whether the loss of memory and plasticity induced by DG inhibition was persistent. Mice were exposed from day 1 to 8 to tone–shock (conditioned stimulus-unconditioned stimulus, CS-US) pairs and pretreated on day 8 with 8-OH-DPAT to induce DG inhibition. As before, Htr1aDG, but not Htr1aKO mice showed a significant deficit in conditioned responding (Fig. 2f) and SC plasticity (Fig. 2g) on day 8. To test the persistence of these deficits, mice were left in their home cages on days 9–13 and then exposed to paired CS–US presentations from day 14 to 18. Remarkably, both conditioned responding and SC plasticity remained suppressed in Htr1aDG mice when compared with Htr1aKO littermates on day 14 in a manner indistinguishable from day 8 (Fig. 2f,g). From day 14 to 18 conditioned responding and SC plasticity in Htr1aDG mice rose gradually to control levels as a result of learning associated with paired CS–US presentation. These data demonstrate that the rapid loss of memory and plasticity induced by DG inhibition is persistent and that subsequent relearning of the CS–US association follows the same time course as initial learning. Memory loss depends on paired CS–US exposure. To determine whether DG inhibition was sufficient to induce loss of plasticity and memory, we tested mice exposed from day 1 to 7 to tone–shock pairs, but left in the home cage on day 8. Under these conditions DG inhibition on day 8 was not associated with a significant alteration in conditioned responding or SC plasticity (Fig. 3a,b) demonstrating that DG inhibition by itself does not induce synaptic depotentiation or memory loss and is not associated with a general deficit in performance or SC plasticity. Next, we tested whether DG inhibition coupled to tone only exposure on day 8 would induce memory loss. Under these conditions DG inhibition was not associated with a significant alteration in conditioned responding or SC plasticity (Fig. 3c,d). This finding suggests that presentation of both CS and US are necessary during DG inhibition to induce synaptic depotentiation and memory loss. It also shows that DG inhibition does not induce memory loss by enhancing extinction, a form of memory loss and synaptic depotentiation induced by exposure to tone presentation2 (Supplementary Fig. 3).

Finally, we examined whether associative pairing of CS and US during DG inhibition was necessary for memory loss. DG inhibition on day 8 was coupled with exposure to explicitly unpaired CS and US presentations, a pseudoconditioning protocol that we have previously shown does not support associative learning2. Under these conditions DG inhibition was not able to induce loss of memory or plasticity (Fig. 3e,f) demonstrating a requirement for the associative, paired presentation of CS and US in the memory loss mechanism. Importantly, spectral power of CA1 recordings were unaffected by DG inhibition (Supplementary Fig. 4) ruling out an indirect effect of altered rhythmic activity on SC plasticity. Together, these findings argue that blockade of neural activity in DG unmasks a depotentiation mechanism that rapidly reverses SC plasticity during associative presentation of tone and shock and that causes a persistent impairment in hippocampal memory retrieval.

Loss of plasticity depends on entorhinal cortex inputs. In vitro electrophysiological experiments with hippocampal slices have shown that stimulation of PP inputs to CA1 is able to reverse long-term potentiation induced at SC synapses22,27 suggesting that learning-associated activity in PP inputs to CA1 might be involved in the DG inhibition-induced SC depotentiation. To test this hypothesis we repeated our trace eye-blink conditioning experiments in mice in which activity in entorhinal cortex neurons could be selectively inhibited. Htr1aDG mice were bilaterally infected in the entorhinal cortex with adenosine-doped virus (AAV) expressing Venus fluorescent protein and the inhibitory mH4D DREADD receptor24 under a neuron-specific promoter25 (AAV-hSyn::Venus-2A-hM4D; Fig. 4a). Selective expression of Venus reporter in entorhinal cortex neurons and their efferents to DG, CA3 and CA1 was confirmed by immunofluorescence (Fig. 4b). Control electrophysiology experiments in anaesthetized wild-type mice similarly infected with AAV-hSyn::Venus-2A-hM4D and systemically injected with the selective hM4D agonist clozapine-N-oxide (CNO (ref. 24)) demonstrated a significant suppression of PP–CA1 neurotransmission, as revealed by a ~50% reduction of fEPSPs evoked in CA1 by electrical stimulation of the olfactory bulb–entorhinal cortex pathway26 (Fig. 4c and Supplementary Fig. 5), although we cannot exclude a contribution of volume conduction from PP–DG inputs to the measured evoked responses. SC synaptic plasticity was monitored in infected Htr1aDG mice during trace eye-blink conditioning (Fig. 4d). On day 8 of training, mice were pretreated with either vehicle or CNO to suppress PP-CAL inputs and administered 8-OH-DPAT to induce SC depotentiation (Fig. 4d). As expected, learning-induced SC plasticity was strongly depotentiated in vehicle-pretreated animals receiving 8-OH-DPAT (Fig. 4e). However, depotentiation was significantly attenuated in CNO-pretreated mice (Fig. 4e). These data support a role for entorhinal cortex inputs to the hippocampus in promoting the depotentiation of SC synaptic plasticity induced by DG inhibition.

Loss of plasticity involves local adenosine signalling. Hippocampal slice experiments demonstrated that PP stimulation-induced SC depotentiation could be blocked by treatment with an antagonist of adenosine A1 receptors, suggesting that local release of adenosine in response to PP-CAL stimulation was necessary to induce long-term depression at SC synapses22,27. To test whether a similar mechanism might underlie the depotentiation of SC synapses seen during DG inhibition in vivo, we repeated our earlier experiment with local, bilateral administration of the adenosine A1 receptor antagonist 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) into dorsal CA1 on day 8 (Fig. 4f,g and Supplementary Fig. 6). As expected, Htr1aDG mice treated with vehicle showed a marked depotentiation of SC plasticity following DG inhibition on day 8 and this effect was significantly...
two-way analysis of variance followed by Holm–Sidak post hoc test). On day 8, failed to cause a decrease in (d) conditioned responding or (f) fEPSP responses in either Htr1aKO mice (mean ± s.e.m.; n = 9–10; two-way analysis of variance followed by Holm–Sidak post hoc test).

Figure 3 | Memory loss depends on paired CS-US presentations. Htr1aDG or Htr1aKO mice were exposed (a,b) to tone–shock pairs on days 1–7 and 9–10, but left in the home cage on day 8, (c,d) to paired tone–shock presentations on days 1–7 and 9–10, but received tone only presentations on day 8, or (e,f) to tone–shock pairs on days 1–7 and 9–10, but received unpaired tone and shock presentations on day 8. Under these conditions treatment with 8-OH-DPAT on day 8 failed to cause a decrease in (a,c,e) conditioned responding or (b,d,f) fEPSP responses in either Htr1aDG or Htr1aKO mice (mean ± s.e.m.; n = 9–10; two-way analysis of variance followed by Holm–Sidak post hoc test).

Generalization of memory loss phenomenon. To examine whether DG inhibition-induced memory loss is a general feature of hippocampal learning, we examined the role of DG granule cells in trace fear conditioning, a widely used hippocampus-dependent memory task. Htr1aDG and control Htr1aKO littermate mice were subjected to trace fear conditioning by placing them into a chamber (context A) and exposing them to a series of paired tone and foot shock stimuli separated by 20 s (Fig. 5a; six tone–shock pairs; tone: 20 s, 3 kHz; shock: 2 s, 0.4 mA). One day later the mice were placed into a novel context (context B) and freezing behaviour during the presentation of the tone was assessed as a measure of fear conditioning recall (recall 1; Fig. 5a). Immediately thereafter, mice were removed from context B and placed back into the same context with an exposed grid floor and subjected to either a second training session (six tone–shock pairs; tone: 20 s, 3 kHz; shock: 2 s, 0.4 mA) or to a series of identical tones without shock (tone only). Mice were pretreated on the second day with 8-OH-DPAT to induce DG inhibition during both recall and subsequent learning. Finally, 1 day later, mice were placed into a third context (context C) to test their recall in the absence of DG inhibition (recall 2; Fig. 5a). Although treatment of Htr1aDG mice with 8-OH-DPAT did not affect trace fear conditioning recall (recall 1), it was associated with a significant impairment of recall on the day following treatment (recall 2, Fig. 5b). Importantly, similar treatment of Htr1aDG mice exposed only to tones on the second day (Fig. 5c) was not associated with deficient recall, consistent with tone–shock association being required for memory loss. Htr1aKO littermates treated with 8-OH-DPAT did not show impaired recall on either day (Fig. 5b,c) confirming a requirement for DG inhibition in the fear conditioning deficit.
Figure 4 | Loss of plasticity depends on entorhinal cortex inputs and local adenosine signalling. (a–c) Mice locally infected with an (a) AAV expressing the Venus fluorescent protein and HA-tagged hM4D DREADD receptor (HA-hM4D) for pharmacogenetic neural inhibition under control of the Synapsin (Syn) gene promoter showed expression in (b) entorhinal cortex (EC) and PP projections to hippocampus (DG, CA3 and CA1). (c) Systemic administration of the selective hM4D agonist CNO (3 mg kg$^{-1}$, intraperitoneal) to wild-type mice caused a significant decrease in fEPSP recorded in CA1 following stimulation of olfactory bulb-entorhinal cortex projections in animals infected with AAV-hM4D, but not in non-infected (control) animals. Significant suppression of fEPSPs was observed 1 h after agonist treatment and persisted for at least 2 h (mean ± s.e.m.; n = 4–7; *P < 0.05; two-way analysis of variance followed by Holm–Sidak post hoc test). (d,e) Htr1a$^{DG}$ (Tg) mice (d) expressing hM4D in the entorhinal cortex and its projections to hippocampus (red arrows) and implanted with stimulating and recording electrodes in the SC and CA1 regions, respectively, were (e) subjected to trace eye-blink conditioning with tone–shock presentations on days 1–12. Systemic pre-treatment with the hM4D agonist CNO (3 mg kg$^{-1}$, intraperitoneal) on day 8 reversed the loss of SC plasticity induced by 8-OH-DPAT treatment on that day (mean ± s.e.m.; n = 12; *P < 0.05; two-way analysis of variance followed by Holm–Sidak post hoc test). (f,g) Htr1a$^{DG}$ mice (f) implanted with stimulating and recording electrodes in the SC and CA1 regions, respectively, and bilateral cannulae aimed at dorsal CA1 were (g) subjected to trace eye-blink conditioning as above. Pre-treatment on day 8 with the adenosine A1 receptor antagonist, DPCPX (82 nmol) caused a significant reversal of the SC depotentiation induced by 8-OH-DPAT treatment on that day when compared with vehicle pretreated mice. Notably, reversal of DG inhibition-induced plasticity by DPCPX persisted through day 12 (mean ± s.e.m.; n = 7; †P < 0.05; two-way analysis of variance followed by Holm–Sidak post hoc test).

Memory loss can be induced by Npy1r activation. Finally, we asked whether we could induce memory loss by manipulating neural activity in DG granule cells without the need for transgenic pharmacogenetic inhibition technology. Such an approach might offer a promising method for the targeted reversal of unwanted memories in other species, including humans. A search of the Allen Brain Atlas gene expression database identified a somato-dendritic inhibitory G-protein coupled receptor, Npy1r, expressed selectively in DG granule cells. Although effective small molecule agonists are not presently available for this receptor, at least one peptidic agonist, [Pro$^{30}$, Nle$^{31}$, Bpa$^{32}$, Leu$^{34}$]NPY(28–36), has been reported to selectively activate Npy1r (ref. 29). Control experiments in anaesthetized wild-type mice showed that administration of [Pro$^{30}$, Nle$^{31}$, Bpa$^{32}$, Leu$^{34}$]NPY(28–36), but not vehicle into the cerebral ventricles rapidly and significantly suppressed neurotransmission via DG mossy fibres in vivo as revealed by a ~60% reduction of fEPSPs evoked in CA3 by electrical stimulation of PP inputs (Supplementary Fig. 7). These results show that endogenous Npy1r expressed on DG granule cells can be targeted by systemic agonists to induce DG inhibition in wild-type animals. Next, we repeated our trace fear conditioning experiments to determine whether the Npy1r agonist could induce memory loss. Similar to data obtained with transgenic mice treated with 8-OH-DPAT, wild-type mice receiving the Npy1r agonist, but not those receiving vehicle showed a significant decrease in conditioned...
Freezing on the second recall session (recall 2; Fig. 5d). No deficit in conditioned responding was observed on the first recall day (recall 1) during drug treatment, nor on either day in mice exposed to only tones on the first recall day (tone only; Fig. 5e).

These data show that activation of an endogenous inhibitory receptor expressed selectively in DG granule cells can be used to induce learning-associated memory loss, and suggest an avenue for the translation of these findings to other organisms.

Discussion

In the present study we examined the contribution of DG granule cells to learning and recall and its associated synaptic plasticity in animals that had previously acquired a hippocampal memory. We found that transient pharmacogenetic inhibition of DG granule cells did not impair conditioned responding to CS presentation nor alter SC synaptic plasticity demonstrating that DG is not required for memory recall (Fig. 3c,d). However, when DG inhibition occurred during paired presentation of CS and US, we observed a rapid loss of SC synaptic plasticity and conditioned responding to CS (Fig. 2d,e and Supplementary Fig. 3). Strikingly, the synaptic plasticity and behavioural impairment persisted in the absence of further stimulus presentation and later relearning occurred at a rate indistinguishable from initial learning, suggesting a loss of the memory trace (Fig. 2f-g).

One possible explanation for the memory loss seen on DG inhibition is that presentation of paired CS–US has a dual effect on CA1 plasticity, on the one hand strengthening SC synapses via a DG-dependent mechanism (indirect inputs to CA1 through the tri-synaptic circuit) and on the other hand weakening SC synapses in a non-DG-dependent manner (direct PP-CA1 inputs). This explanation is consistent with several studies in the literature reporting mechanistic and functional differences between the direct and the indirect inputs to CA1 (refs 12,13,30–32). Furthermore, earlier in vitro12,23 and in vivo33 electrophysiology studies found that stimulation of PP-CA1 inputs to the hippocampus could depotentiate synaptic plasticity that had been previously acquired at SC synapses suggesting that the direct PP pathway might promote depotentiation during hippocampal learning. To test this possibility, we used dual, orthogonal pharmacogenetic inhibition of DG and entorhinal cortex to show that the memory loss phenomenon we observed depended on PP inputs (Fig. 4e). Furthermore, one of the earlier studies23 had shown that PP stimulation-induced SC depotentiation could be inhibited by blockade of adenosine A1 receptors, but not several other receptors, and we found that bilateral administration of DPCPX to the CA1 region of the hippocampus blocked synaptic depotentiation in our model (Fig. 4g).

Our data lead us to propose a novel function for PP-CA1 inputs to the hippocampus. During CS–US presentation, but not during presentation of unpaired CS–US or CS alone, information arriving via this pathway actively promotes depotentiation of SC synapses, while information arriving via the DG pathway opposes this depotentiation. Thus, in an animal that has successfully acquired a hippocampal-dependent memory, and in which the direct and indirect pathways are intact, SC synaptic strength is stable and memories can be retrieved. However, when the DG pathway is blocked, as we have done artificially in our study, depotentiation is favoured and memory is lost (see scheme, Fig. 6). The precise function of PP-dependent SC depotentiation remains unclear at this point, but we speculate that it may play a role in weakening previously acquired associations to facilitate the encoding of new memories. Existing data show that selective blockade of synaptic activity in entorhinal cortex neurons projecting to CA1 impairs the acquisition of trace fear conditioning34 and support our hypothesis of a positive role for this pathway in learning13,30,32,33. Moreover, our DPCPX experiments suggest that blockade of the depotentiation mechanism promotes SC synaptic plasticity during CS–US presentation in otherwise intact animals (Fig. 4g). However,
field potentials does not allow us to rule out that changes in synaptic plasticity at non-SC inputs underlie our plasticity effects. Experiments using targeted optogenetic stimulation of CA3 efferents could be used to more selectively measure SC synaptic strength. Fourth, our observation that SC depotentiation and memory loss occurred only during paired, but not unpaired CS–US presentation (Fig. 2d,e) suggests that the memory loss phenomenon we describe is distinct from other well-described avenues for memory degradation, including enhancement of extinction\cite{33} and blockade of reconsolidation\cite{44}.

Finally, our findings demonstrating generalization of DG inhibition-induced memory loss across tasks coupled with our identification of an endogenous pharmacological target that can induce similar memory loss raise the possibility that the novel memory mechanism we have uncovered may be useful for erasing unwanted memories in a clinical setting.

**Methods**

**Animals.** Transgenic mice (genetic background: B6.CBA;129S6/SvEVTac) were littermates derived from breeding Htr1a\(^{-/-}\), Htr1a\(^{-/-}\)Nrip2+/+, and Htr1a\(^{+/+}\)/Htr1a\(^{+/+}\) mice\(^{18}\) (called respectively DG\(^{+/-}\), DG\(^{-/-}\) and CA3\(^{+/+}\), respectively following protocols approved by the Italian Ministry of Health and shipped to Pablo Olavide University for experimental testing following experimental protocols approved by the University Ethics Committee and the Spanish Ministry of Health and in accordance with the guidelines of the European Union (2010/63/UE) and Spanish regulations (BOE 34:1370-421, 2013). Wild-type animals used for trace fear conditioning (genetic background: C57BL/6J) were derived from an internal EMBL breeding colony and tested following an experimental protocol approved by the Italian Ministry of Health. Experiments were carried out with male and female littermates that were 3–5 months old. Animals were group housed, switched to individual cages following surgery, and kept on a 12 h light/dark cycle (lights on at 8:00) with constant ambient temperature (21.5 \(\pm\) 1 \(^\circ\)C) and humidity (55 \(\pm\) 8\%). Food and water were available ad libitum. All mice that completed experimental protocols in the absence of deteriorated fEPSPs, electroencephalogram and/or electromyographic recordings were included. Additional animals were used in preliminary studies to select the appropriate drug doses and test the stability of recording and stimulating systems.

**Surgery.** Mice were anaesthetized with 0.8–1.5% isoflurane, supplied from a calibrated Fluotec 5 vaporizer (Fluotec-Ohmeda, Tewksbury, MA) at a flow rate of 1–21 min\(^{-1}\) oxygen (AstraZeneca, Madrid, Spain) and delivered by a mouse anaesthesia mask (David Kopf Instruments, Tujunga, CA). For eye blink conditioning animals were implanted with bipolar recording electrodes in the orbicularis oculi muscle and stimulating electrodes on the ipsilateral supraorbital nerve (Fig. 2a). These electrodes were made from 50 \(\mu\)m, Teflon-coated, annealed stainless steel wire (A-M Systems, Carlsborg, WA). For measurement of SC synaptic efficacy animals were implanted with stimulating electrodes in the right olfactory bulb (4.0 mm anterior to bregma, 2.0 mm lateral, and 1–1.5 mm from the brain surface) and with a recording electrode in the right CA1 stratum radiatum (2.2 mm posterior to bregma, 1.2 mm lateral and 1–1.5 mm from the brain surface; Figs 2a,c and 4d,f and Supplementary Fig. 3a). Hippocampal electrodes were made from 50 \(\mu\)m, Teflon-coated, tungsten wire (Advent Research, Eynsham, UK). All the implanted wires were soldered to two four-pin sockets (RS Amidata, Madrid, Spain) and fixed to the skull with dental cement\(^{46}\). Recording electrodes were aimed at the apical dendrites of pyramidal CA1 cells\(^{5,46}\) and their final location was adjusted by the profile of fEPSPs evoked by SC stimulation during surgery. For experiments to determine the activity of DG granule cells, animals were implanted with stimulating electrodes in the perforant pathway (2.2 mm lateral to bregma) and with a recording electrode in the right CA1 stratum radiatum (2.2 mm posterior to bregma, 1.2 mm lateral and 1–1.5 mm from the brain surface; Figs 2a,c and 4d,f and Supplementary Fig. 3a). Hippocampal electrodes were made from 50 \(\mu\)m, Teflon-coated, tungsten wire (Advent Research, Eynsham, UK). 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and delivered via unilateral guide cannula to the right lateral ventricle (20 nmol per mouse; injection volume: 2 μl; flow rate: 1 μl/min) 20 min before testing. The selective 5-HT1A receptor agonist 8-OH-DPAT (1 mg/kg, s.c.) was dissolved in saline (0.9% NaCl) and systemically injected (3 mg/kg, i.p.) 60 min before testing.

**Histology.** At the end of the recording sessions mice were deeply anesthetized (sodium pentobarbital, 50 mg·kg⁻¹) and perfused transcardially with saline and 4% paraformaldehyde. Brains were dissected, postfixed overnight at 4 °C and cryoprotected in 30% sucrose in PBS. Sections were obtained on a microtome (Leica, Wetzlar, Germany) at 50 μm. Selected sections including the dorsal hippocampus were mounted on gelatinized glass slides and stained using the NiSSL technique with 0.1% toluidine blue to determine the location of stimulations producing electrodes and cannula unilaterally implanted in dorsal CA1. To determine the location of cannulae implanted into the lateral ventricle mice were infused with 2 μl of 2% cresyl violet dye. Correcting euthanasia, intact brains were rapidly removed from the skull and flash frozen in isopentane. Brain slices were prepared at 30 μm and mounted on glass slides. Correct guide cannula placement was judged by dye staining of the ventricular border. To determine the location of viral infection animals were transcardially perfused (4% paraformaldehyde, 0.1 M phosphate buffer and pH 7.4) and brains and left overnight in fixative. Coronal sections (40 μm) were cut on a vibratome (Leica Microsystems). All sections were imaged for Venus and 4,6-diamidino-2-phenylindole fluorescence with a motorized wide-field microscope (Leica Microsystems). No entorhinal cortex-infected animals were excluded from the analysis.

**Data collection and analysis.** Electromyographic, extracellular hippocampal activity and 1-volt rectangular pulses corresponding to tone and shock presentations were stored digitally on a computer via an analogue/digital converter (CED 1401 Plus, Cambridge, England) at a sampling frequency of 11–22 kHz and with an amplitude resolution of 12 bits. Data were analysed off-line for quantification of conditioned responses and fEPSP slopes with the help of commercial (Spike 2 and SIGAVG from CED) and custom⁴⁸,⁴⁹ programs. The slope of evoked fEPSPs was computed as the first derivative (V s⁻¹ of fEPSP recordings (V)). For this, five successive fEPSPs were averaged, and the mean value of the slope during the rise time period (that is, between the initial and final 10% of the fEPSP) was determined. Computed results were processed for statistical analysis using the Sigma Stat software package (SSl, San Jose, CA). Power spectra of hippocampal field activity were computed using fast Fourier transform with a Hanning window and normalized relative power and averaged for each session for each group. Average power spectra were analysed and compared using the wide-band model, considering theta, beta and gamma bands described previously⁵¹. Acquired data were analysed using two-way analysis of variance, with days as repeated measure followed by Holm–Sidak post hoc testing, or t-test.

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Author contributions

N.M. and A.G. designed, carried out and analysed all experiments, A.F.-G. helped carry out experiments, A.I. and V.G. packaged the viral vector, C.M. helped with histology experiments, J.C. and M.K. synthesized the peptidic Npy1r agonist and A.J. and T.T. bred and genotyped mice for the experiments. C.T.G, A.G. and J.-M.-D.G. conceived of, helped design, and supervised the project and together with N.M. wrote the manuscript.

Additional information

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