Specific Hippocampal Interneurons Shape Consolidation of Recognition Memory

Graphical Abstract

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In Brief

Oliveira da Cruz et al. show that a specific subpopulation of hippocampal CB₁ and D₁ receptor-positive neurons controls late consolidation of recognition memory and associated synaptic plasticity by moderating local inhibitory GABAergic activity in the hippocampus.

Highlights

- CB₁Rs are present in hippocampal D₁R-positive interneurons
- CB₁R/D₁R-positive interneurons control the late phase of recognition memory
- CB₁R/D₁R-positive interneurons control learning-induced facilitation of LTP

Report

Specific Hippocampal Interneurons Shape Consolidation of Recognition Memory

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SUMMARY

A complex array of inhibitory interneurons tightly controls hippocampal activity, but how such diversity specifically affects memory processes is not well understood. We find that a small subclass of type 1 cannabinoid receptor (CB₁R)-expressing hippocampal interneurons determines episodic-like memory consolidation by linking dopamine D₁ receptor (D₁R) signaling to GABAergic transmission. Mice lacking CB₁Rs in D₁-positive cells (D₁-CB₁-KO) display impairment in long-term, but not short-term, novel object recognition memory (NOR). Re-expression of CB₁Rs in hippocampal D₁R-positive cells rescues this NOR deficit. Learning induces an enhancement of *in vivo* hippocampal long-term potentiation (LTP), which is absent in mutant mice. CB₁R-mediated NOR and the associated LTP facilitation involve local control of GABAergic inhibition in a D₁-dependent manner.

This study reveals that hippocampal CB₁R-/D₁R-expressing interneurons control NOR memory, identifying a mechanism linking the diversity of hippocampal interneurons to specific behavioral outcomes.

INTRODUCTION

Formation of episodic memory is a multistep brain process that requires activity of the medial temporal lobe (Squire et al., 2007). The hippocampus in particular participates in long-term storage of recently acquired events. Hippocampal circuits are regulated by a large variety of local inhibitory interneurons that are controlled by neuromodulatory systems ensuring their coordinated function to shape behavioral responses (Klausberger and Somogyi, 2008); the identities and functions of the interneurons are under intense scrutiny (Harris et al., 2018; Pelkey et al., 2017; Parra et al., 1998).

The endocannabinoid system is a brain-modulatory signaling hub formed mainly by type 1 cannabinoid receptors (CB₁Rs), their endogenous ligands (endocannabinoids), and enzymes for their synthesis and degradation. In the hippocampus, CB₁Rs are present in principal neurons and astroglial cells (Busquets-Garcia et al., 2015; Oliveira da Cruz et al., 2016). However, the largest expression of CB₁Rs resides in GABAergic interneurons (Marsicano and Kuner, 2008; Katona and Freund, 2012),

where they modulate local inhibition of hippocampal circuits. Particularly, the largest amount of CB₁Rs is expressed in cholecystokinin (CCK)-positive interneurons, which are characterized by asynchronous neurotransmitter release (Harris et al., 2018; Katona et al., 1999; Marsicano and Lutz, 1999).

Hippocampal CB₁Rs control episodic-like memory processes and synaptic plasticity (Robin et al., 2018; Hebert-Chatelain et al., 2016; Puighermanal et al., 2009). However, the specific locations where these receptors participate in the mechanisms underlying hippocampus-dependent memory are only partially known.

Activity-dependent long-term changes in hippocampal synaptic transmission are considered cellular correlates of memory consolidation (Nicoll, 2017; Whitlock et al., 2006), which involves local dopamine D₁ receptor (D₁R) signaling (Lisman et al., 2011; Yamasaki and Takeuchi, 2017). Exposure to hippocampus-dependent behavioral tasks induces changes in long-term potentiation (LTP) of synaptic transmission that require activation of D₁-like receptors (Frey et al., 1990; Granado et al., 2008; Li et al., 2003; Lemon and Manahan-Vaughan, 2006). A

novel subpopulation of hippocampal CB₁R/CCK-positive interneurons containing D₁R was recently described (Puighermanal et al., 2017; Gangarossa et al., 2012). However, the potential interactions between D₁Rs and CB₁Rs in regulating learning-induced plasticity, activity of hippocampal circuits, and memory processes remain unexplored.

Here we assessed the role of D₁R/CB₁R-positive cells in regulation of episodic-like novel object recognition (NOR) memory. We found that conditional deletion of the CB₁R gene in hippocampal D₁R-positive cells impairs long- but not short-term NOR memory and learning-induced LTP enhancement involving local control of GABAergic transmission. These intriguing results suggest that CB₁R signaling provides a functional link between hippocampal dopaminergic and GABAergic control of synaptic plasticity and memory consolidation.

RESULTS

CB₁Rs in Hippocampal D₁R-Positive Neurons Are Necessary for Consolidation of NOR Memory

Mutant mice bearing a deletion of the CB₁R gene in cells expressing D₁R (D₁-CB₁-knockout [KO] mice; Monory et al., 2007) displayed no phenotype in the short-term version (3 h post-training) of a NOR task (Figures 1A and 1B; Puighermanal et al., 2009; Busquets-Garcia et al., 2011; Robin et al., 2018). Conversely, they showed strong impairment in long-term (24 h) memory compared with their wild-type (WT) littermates (Figure 1C), with no changes in total exploration time (Figures S1A–S1D).

The majority of CB₁Rs in D₁R-positive neurons have been characterized previously in striatonigral circuits (Monory et al., 2007). Considering the involvement of these circuits in NOR memory (Darvas and Palmiter, 2009), we tested the role of striatal CB₁Rs. We infused an adeno-associated virus carrying a Cre-dependent expression of CB₁Rs (pAAV-CAG-DIO-CB₁) into the striatum of D₁-CB₁-KO mice to obtain re-expression (RS) of CB₁Rs in cells where Cre is present (hereafter called D₁R-positive) in this brain region (striatum [STR]-CB₁-RS mice; Figures 1D and 1E), as revealed by immunodetection of a myc-tagged version of CB₁Rs (CB₁R-myc; STAR Methods; Figure 1E). This re-expression was not sufficient to rescue the phenotype of D₁-CB₁-KO mice in long-term NOR (Figures 1F, S1E, and S1F), suggesting that CB₁Rs in striatal D₁R-positive cells do not participate in this type of memory. Anatomical data indicate that a subset of hippocampal neurons contain D₁Rs (Gangarossa et al., 2012), likely co-expressing CB₁R protein (Puighermanal et al., 2017). Thus, we re-express the CB₁R gene in the hippocampus of D₁-CB₁-KO mice to obtain hippocampus (HPC)-CB₁-RS mice (Figures 1D and 1G). This manipulation fully rescued the phenotype of the mutant mice (Figure 1F, S1E, and S1F), indicating that hippocampal CB₁Rs expressed in D₁R-positive cells are required for NOR memory.

We recently reported that deletion of CB₁Rs in hippocampal glial acidic fibrillary protein (GFAP)-positive cells (i.e., mainly astrocytes, GFAP-CB₁-KO mice) also impaired NOR memory (Robin et al., 2018). Indeed, GFAP-CB₁-KO mice were impaired in NOR (Figures S1G–S1I; Robin et al., 2018), but, in contrast to D₁-CB₁-KO mice, this phenotype extended to short-term NOR

memory (Figures S1J–S1L). This difference suggests that CB₁Rs expressed in hippocampal astrocytes or D₁R-positive cells might control distinct phases of NOR memory consolidation.

The primary function of CB₁R activation in neurons is to decrease neurotransmitter release (Castillo et al., 2012; Busquets-Garcia et al., 2017). Accordingly, deletion of CB₁Rs from neurons often results in excessive neurotransmission. Thus, we reasoned that inhibition of hippocampal D₁R-positive neurons during NOR consolidation should be able to rescue the memory impairment of D₁-CB₁-KO mice. Viral vectors carrying Cre-dependent expression of an inhibitory designer receptor exclusively activated by designer drugs (DIO-hM4DGi, Gi-DREADD; Robinson and Adelman, 2015) or control mCherry protein were infused into the hippocampi of D₁-CB₁-KO mice and WT littermates (Figure 1H). Post-training clozapine N-oxide (CNO) injections did not affect the NOR performance of D₁-CB₁-KO and WT mice injected with Gi-DREADD or mCherry, indicating that the drug or its metabolites had no effect per se (Gomez et al., 2017; Figures 1I, S1M, and S1N). Conversely, post-acquisition CNO treatment fully rescued the NOR impairment of D₁-CB₁-KO mice expressing Gi-DREADD (Figures 1I, S1M, and S1N). This strongly suggests that excessive activity of D₁R-positive neurons during the consolidation process is responsible for the memory impairment observed in D₁-CB₁-KO mice.

CB₁Rs in Hippocampal D₁R-Positive Neurons Control Learning-Induced Changes of LTP *In Vivo*

Cellular and molecular mechanisms underlying activity-dependent changes in synaptic plasticity are proposed to underlie long-term memory (Aggleton and Morris, 2018). Previous studies showed that conditional and global deletion of CB₁Rs in neuronal and glial cell populations induces deficits in learning and associated synaptic plasticity (Busquets-Garcia et al., 2017; Robin et al., 2018). To address the role of CB₁Rs in hippocampal D₁R-positive neurons in modulation of synaptic plasticity, we recorded *in-vivo*-evoked field excitatory postsynaptic potentials (fEPSPs) in the hippocampal CA3-CA1 pathway of anesthetized mice. High-frequency stimulation (HFS) induced similar long-lasting LTP of synaptic fEPSPs in D₁-CB₁-KO and WT littermates (Figures 2A and 2B), indicating that hippocampal D₁R/CB₁R-positive neurons are dispensable for expression of LTP in naive animals.

HPC-dependent memory-related processes such as LTP are sensitive to pharmacological and genetic modulation of hippocampal D₁Rs, particularly after learning (Li et al., 2003; Lemon and Manahan-Vaughan, 2006; Takeuchi et al., 2016; Yamasaki and Takeuchi, 2017). Thus, we hypothesized that CB₁Rs in D₁R-positive neurons may modulate learning-dependent hippocampal synaptic plasticity. To explore whether acquisition of the NOR task modulates *in vivo* LTP, we recorded fEPSPs from C57Bl6/NRj mice after a NOR task (Figure 2C). HFS induced stronger LTP in animals exposed to NOR acquisition than in control mice (Figures 2D and 2E), showing that the training modulates hippocampal synaptic plasticity. Strikingly, D₁-CB₁-KO mice lacked this learning-induced enhancement of LTP (Figures 2F and 2G). Thus, physiological activation of CB₁Rs in

Figure 1. Hippocampal CB₁Rs in D₁R-Positive Cells Are Necessary for Late but Not Early Consolidation of NOR

(A) Schematic representation of the NOR memory task.

(B) Short-term (3 h) NOR memory performance of D₁-CB₁-WT mice (n = 10) and D₁-CB₁-KO littermates (n = 7).

(C) Long-term (24 h) memory performance of D₁-CB₁-WT mice (n = 9) and D₁-CB₁-KO littermates (n = 8).

(D) Schematic representation of the experiment using viral re-expression of the CB₁R gene in the striatum (STR) or the hippocampus (HPC) of D₁-CB₁-WT mice and D₁-CB₁-KO littermates.

(E) Representative images of Cre-expressing D₁-CB₁-KO mice injected with CB₁R-myc in the STR using the same procedure as described in (D) (STAR Methods). Scale bar, 2 mm.

(F) NOR memory performance of mice with re-expression of the CB₁R gene in the STR or HPC. Control, n (D₁-CB₁-WT) = 17 and n (D₁-CB₁-KO) = 5; STR-CB₁-RS, n (D₁-CB₁-KO) = 6; HPC-CB₁-RS, n (D₁-CB₁-KO) = 9.

(G) Immunofluorescence of cells expressing CB₁R-myc in the HPC. Scale bar, 500 μm.

(H) Schematic representation of the experiment using viral expression of the Gi-DREADDs or mCherry in the HPC of D₁-CB₁-WT mice and D₁-CB₁-KO littermates. Clozapine N-oxide (CNO; 2 mg/kg) injections take place after the training phase of the NOR task.

(I) NOR memory performance of D₁-CB₁-WT mice injected intra-hippocampally with hM4D(Gi) virus or mCherry (n VEH = 16, n CNO = 21), D₁-CB₁-KO mice injected with mCherry (n VEH = 6, n CNO = 7), and D₁-CB₁-KO mice injected intra-hippocampally with hM4D(Gi) (n VEH = 11, n CNO = 14).

Data, mean ± SEM. *p < 0.05, ***p < 0.001, ****p < 0.0001. ns, not significant. See also Figure S1 and Table S1.

(legend on next page)

hippocampal D₁R-positive neurons is required for learning-dependent facilitation of LTP.

CB₁R in Hippocampal D₁R-Positive Neurons Modulate NOR Memory Consolidation through a GABA-Dependent Mechanism

D₁Rs are expressed in different hippocampal cells, including subsets of GABAergic and glutamatergic neurons (Gangarossa et al., 2012). Considering that CB₁R signaling decreases the activity of hippocampal neurons (Busquets-Garcia et al., 2017; Castillo et al., 2012), we asked whether excessive glutamatergic or GABAergic neurotransmission might underlie the phenotype of D₁-CB₁-KO mice. Thus, we injected non-amnesic doses (Puighermanal et al., 2009) of the NMDA receptor blocker MK-801, the AMPA/kainate receptor antagonist NBQX (Figure S2A), or the GABA_A receptor antagonist bicuculline into D₁-CB₁-KO and WT littermates immediately after NOR training. MK-801 and NBQX did not alter memory performance in WT mice, nor did it rescue the amnesic phenotype of D₁-CB₁-KO littermates (Figures 3A, S2B, and S2C). Conversely, bicuculline completely reversed the memory impairment of D₁-CB₁-KO mice when injected immediately after training or 1 h later without affecting WT littermates' performance (Figures 3A, S2B, and S2C).

These data indicate that excessive GABAergic but not glutamatergic ionotropic receptor activity is involved in the phenotype of D₁-CB₁-KO mice. A large proportion of GABAergic hippocampal interneurons contain CB₁R mRNA, which is expressed at different levels (high CB₁R- and low CB₁R-expressing cells; Marsicano and Lutz, 1999). Conversely, D₁R mRNA is expressed at very low levels in the HPC (<http://mouse.brain-map.org/experiment/show/35>; data not shown), which makes it difficult to accurately quantify its expression above background. Therefore, to pinpoint which CB₁R-positive interneurons in the HPC contain D₁R, we combined fluorescence *in situ* hybridization for CB₁R mRNA in D₁-Cre and D₁-CB₁-KO mice carrying viral Cre-dependent expression of mCherry (STAR Methods; Figure 3B). As described (Marsicano and Lutz, 1999), detectable levels of CB₁R mRNA were present throughout the HPC in pyramidal neurons and in GABAergic interneurons (Figure S2D). The distribution of mCherry-tagged D₁-positive neurons in the dorsal CA1 region of D₁-Cre mice was similar to previous findings (Puighermanal et al., 2017; Gangarossa et al., 2012). Double staining revealed that virtually no high CB₁R-expressing interneurons in the *strata oriens*, *pyramidale*, *radiatum*, or *lacunosum*

moleculare contain D₁Rs (Figures 3C–3F and S2D). Conversely, D₁Rs are present in a small subpopulation of low CB₁R-expressing interneurons along the different hippocampal layers (Figures 3C and 3F). Importantly, this co-expression was virtually abolished in hippocampi of D₁-CB₁-KO mice (Figures 3C, 3D, and 3F).

Altogether, these data indicate that CB₁R-dependent modulation of a small subpopulation of D₁R-positive GABAergic interneurons is required during NOR memory consolidation.

Synaptic Mechanisms Underlying NOR Memory Consolidation and Associated Hippocampal Plasticity

The data collected so far show that reduction of GABAergic signaling prevents the deficits in D₁-CB₁-KO mice of NOR consolidation. Therefore, we tested whether inhibition of GABA_A receptors could rescue the lack of learning-induced LTP enhancement observed in D₁-CB₁-KO mice. Trained mice received bicuculline or vehicle (VEH) before testing LTP induction in hippocampal circuits. In vehicle-treated animals, D₁-CB₁-KO mice showed no training-induced LTP enhancement (Figures 4A–4C). Strikingly, although bicuculline did not affect LTP in WT animals, it rescued the training-induced LTP of D₁-CB₁-KO mice (Figures 4A–4C).

Recent data suggest that hippocampal D₁R-like receptors participate in memory formation, but little is known concerning the cell types involved (Lisman et al., 2011; Yamasaki and Takeuchi, 2017). Our data indicate that CB₁R-dependent control of GABAergic transmission from a low number of hippocampal interneurons expressing D₁R is required to guarantee late consolidation of NOR memory. Therefore, it is possible that endocannabinoid actions are secondary to activation of D₁Rs in these cells. To address this issue, we first reasoned that partial inhibition of D₁Rs should “replace” the lack of CB₁R-dependent control of neurotransmission in D₁-CB₁-KO mice. Thus, we administered a sub-effective dose of the D_{1/5}R antagonist SCH-23390 (Figures S3A–S3C) to D₁-CB₁-KO mice and WT littermates after NOR acquisition and analyzed the training-induced enhancement of *in vivo* LTP. This treatment slightly reduced the late phase of LTP in WT animals (Figures 4A–4C). However, the antagonist abolished the differences between D₁-CB₁-KO mice and WT littermates (Figures 4A–4C), indicating that reducing D₁R activity counteracts the absence of CB₁Rs in the mutants. If LTP is mechanistically linked to NOR consolidation, then the same treatment should rescue the memory impairment of

Figure 2. Learning-Induced Facilitation of *In Vivo* Hippocampal LTP Requires CB₁Rs at D₁R-Positive Neurons

(A and B) HFS in the dorsal hippocampal CA3 Schaffer collateral pathway induces *in vivo* LTP in the dorsal CA1 *stratum radiatum*.

(A) Summary plots of recorded evoked fEPSPs in anesthetized D₁-CB₁-WT (n = 8) and D₁-CB₁-KO (n = 8) mice.

(B) Bar histograms of normalized fEPSPs from (A), representing 30 and 60 min after HFS.

(C) Schematic representation of the experimental setup (STAR Methods).

(D and E) Learning modulates *in vivo* LTP.

(D) Summary plots of recorded evoked fEPSPs from mice exposed to control (n = 8) and NOR training (n = 11) conditions.

(E) Bar histograms of normalized evoked fEPSPs from (D), representing 30 and 60 min after HFS.

(F and G) Learning-induced modulation of *in vivo* LTP is impaired in D₁-CB₁-KO mice.

(F) Summary plots of recorded fEPSPs in anesthetized D₁-CB₁-WT (n = 10) and D₁-CB₁-KO (n = 10) mice.

(G) Bar histograms of normalized evoked fEPSPs from (F), representing 30 and 60 min after HFS.

Traces on the right side of the summary plots represent 150 superimposed evoked fEPSPs before HFS (1, gray) and 30 min (2, brown) and 60 min (3, black) after HFS. Data, mean ± SEM. *p < 0.05. See also Table S1.

Figure 3. Hippocampal CB1R/D1R-Positive Interneurons Modulate Synaptic GABAergic Transmission

(A) NOR memory performance of mutant mice administered vehicle (n D1-CB1-WT = 14, n D1-CB1-KO = 14), MK-801 (0.1 mg/kg, intraperitoneally [i.p.]; n D1-CB1-WT = 7, n D1-CB1-KO = 7), NBQX (5 mg/kg, i.p.; n D1-CB1-WT = 8, n D1-CB1-KO = 5), or bicuculline immediately after (n D1-CB1-WT = 10, n D1-CB1-KO = 10) or 1 h after the training phase (n D1-CB1-WT = 10, n D1-CB1-KO = 8).

(B) Schematic representation of the experimental procedure to detect CB1R mRNA in D1R-positive cells.

(C and D) Representative images of CB1R mRNA (green) and mCherry protein (red) labeling in the hippocampal CA1 region of D1-Cre (C) and D1-CB1-KO (D) mice. White arrows indicate colocalization of CB1R-positive and D1R-positive cell bodies. Scale bar, 150 μ m.

(E and F) Layer-specific distribution of the percentage of cell bodies expressing high (E) and low amounts (F) of CB1Rs, which colocalize with mCherry-positive (i.e., D1R-positive) in D1-Cre (n = 3) and D1-CB1-KO (n = 3).

Data, mean $\pm$ SEM. *p < 0.05, **p < 0.01. See also Figure S2 and Table S1.

D1-CB1-KO mice. Administration of SCH-23390 did not alter the behavior of WT mice (Figures 4D, S3D, and S3E), but, strikingly, it fully rescued the memory impairment of D1-CB1-KO littermates (Figures 4D, S3D, and S3E).

Altogether, these results indicate that endocannabinoid-dependent regulation of hippocampal D1R-positive interneurons is a necessary step in dopaminergic control of NOR memory consolidation and associated synaptic plasticity.

DISCUSSION

The present study reveals that a specific subpopulation of hippocampal D1R/CB1R-positive neurons controls late consolidation of NOR memory and associated synaptic plasticity by moderating local inhibitory GABAergic activity in the HPC. Specifically, CB1Rs expressed in D1R-positive interneurons participate in learning-induced facilitation of *in vivo* LTP and are required for

Figure 4. Cellular Mechanisms Linking D₁R Signaling with GABAergic Activity during Learning-Induced Facilitation of *In Vivo* LTP and Memory Consolidation

(A) Effects of the GABA_A receptor antagonist bicuculline and the D_{1/5}R antagonist SCH-23390 on learning-induced modulation of *in vivo* LTP in D₁-CB₁-WT and D₁-CB₁-KO mice. Shown are summary plots of recorded evoked fEPSPs in vehicle (n D₁-CB₁-WT = 6, n D₁-CB₁-KO = 8), bicuculline (0.5 mg/kg, i.p.; n D₁-CB₁-WT = 9, n D₁-CB₁-KO = 11), and SCH-23390 (0.3 mg/kg, i.p.; n D₁-CB₁-WT = 6, n D₁-CB₁-KO = 6).

(B and C) Bar histograms of (A), representing normalized fEPSPs from 30 (B) and 60 (C) min after HFS.

(D) Memory performance D₁-CB₁-WT and D₁-CB₁-KO mice after being injected with vehicle (n D₁-CB₁-WT = 6, n D₁-CB₁-KO = 10) or SCH-23390 (0.3 mg/kg, i.p.; n D₁-CB₁-WT = 10, n D₁-CB₁-KO = 10).

Traces on the right side of the summary plot (A) represent 150 superimposed evoked fEPSPs before HFS (1, gray) and 30 min (2, brown) and 60 min (3, black) after HFS. Data, mean $\pm$ SEM. *p < 0.05, **p < 0.01.

See also Figure S3 and Table S1.

consolidation of NOR memory. Moreover, CB₁R_s in D₁R-positive neurons are necessary for physiological D₁R-dependent modulation of memory processes, suggesting that cannabinoid signaling is part of a complex modulatory circuit regulated by dopamine transmission in the HPC. By determining cellular and behavioral functions of a specific CB₁R-expressing interneuron subpopulation, these data uncover an unforeseen role of CB₁R_s in the D₁R-dependent control of long-term memory.

The endocannabinoid system regulates episodic-like recognition memory processes via CB₁R-dependent control of different cell types in the HPC (Busquets-Garcia et al., 2017; Soria-Gomez et al., 2017; Busquets Garcia et al., 2016; Puighermanal et al., 2009; Robin et al., 2018). In the present study, we observed that the transition from short- to long-term memory processes is controlled by a functional interaction between D₁R_s and CB₁R_s in a specific subpopulation of hippocampal interneurons. In contrast, CB₁R deletion from all body cells or in all forebrain GABAergic neurons does not reproduce the phenotype of D₁-CB₁-KO mice (Puighermanal et al., 2009; Hebert-Chatelain et al., 2016). These apparently contrasting observations can be explained by different possibilities. Long-term deletion of the CB₁R gene starting from early developmental stages in CB₁-KO and GABA-CB₁-KO mice might induce compensatory mechanisms (El-Brolosy et al., 2019; El-Brolosy and Stainier, 2017), masking the functional role of the CB₁R in NOR memory. An alternative or complementary explanation might point to the presence of different subpopulations of brain cells expressing CB₁R_s and exerting opposite effects on memory processes. For instance, endocannabinoid signaling might promote or inhibit memory formation when acting at D₁R-positive cells or at other neuronal subpopulations, respectively. We have shown previously that astroglial CB₁R_s are necessary for consolidation of NOR memory by allowing D-serine availability at glutamatergic synapses (Robin et al., 2018). We cannot fully exclude that deletion of CB₁R_s in D₁R-positive cells does not also involve astrocytes (Nagatomo et al., 2017). However, so far, no conclusive anatomical evidence has been presented for expression of D₁R_s in hippocampal astrocytes (Chai et al., 2017; Zhang et al., 2014; but see Jennings et al., 2017 for D_{1/5}R pharmacological experiments). Moreover, our current and past results suggest that endocannabinoid control of astrocytes is likely involved in the initial phases of memory formation, whereas CB₁R-dependent inhibition of D₁R-positive hippocampal interneurons determines later phases of NOR memory consolidation. The time-course effects of pharmacological treatments indicate that D-serine can rescue memory performance of GFAP-CB₁-KO mice only when administered immediately after learning (Robin et al., 2018). This idea is reinforced by the fact that these mutants do not express *in vivo* LTP even under basal “home cage” conditions (Robin et al., 2018), whereas D₁-CB₁-KO mice only lack the specific facilitation of LTP induced by learning. Altogether, these observations allow speculation that at least two distinct temporal windows exist in CB₁R-dependent control of NOR. First, astroglial CB₁R are necessary for the plastic processes to initiate the memory. Later, endocannabinoid-dependent regulation of D₁R-positive interneurons is required to maintain the memory trace for longer periods.

Hippocampal D₁R have been shown previously to be mainly on GABAergic interneurons, but lower levels were also detected on glutamatergic neurons (Gangarossa et al., 2012; Puighermanal et al., 2017; http://celltypes.brain-map.org/maseq/mouse_ctx-hip_smart-seq). Our data show that the D₁-Cre mouse line used in the present study (Lemberger et al., 2007) induces recombination in a small sub-fraction of hippocampal interneurons containing low levels of CB₁R mRNA but also in pyramidal neurons and mossy cells. Therefore, we cannot fully exclude that cell types other than hippocampal interneurons might participate in D₁R/CB₁R-dependent control of memory consolidation. However, our data show that partial blockade of GABA_A receptors, but not of AMPA/kainate or NMDA glutamatergic ones, reverse the memory impairment of D₁-CB₁-KO mice. Therefore, our findings strongly suggest that CB₁R control of GABA release from D₁R-positive interneurons regulates late consolidation of NOR memory. However, recent data using emerging technologies suggest that hippocampal cells are more diverse and functionally segregated than previously thought (Harris et al., 2018; Soltesz and Losonczy, 2018). By identifying specific markers, future studies will extend our genetic and pharmacological evidence that a specific subpopulation of D₁R/CB₁R-positive hippocampal interneurons regulates consolidation of NOR memory.

LTP at the CA3-CA1 pathway is a potential molecular and cellular mechanism underlying behavioral expression of episodic-like memory processes (Morris, 2013). Interestingly, although deletion of CB₁R_s from D₁R-positive cells impairs NOR memory, the same manipulation does not impair *in vivo* LTP of hippocampal synaptic transmission in naive animals. In agreement with previous evidence under other experimental conditions (Li et al., 2003; Lemon and Manahan-Vaughan, 2006), WT mice exposed to the NOR learning task display facilitation of *in vivo* LTP compared with animals exposed to the same environment without any learning. Importantly, this facilitation is absent in D₁-CB₁-KO mice, suggesting that endocannabinoid control of D₁R-positive hippocampal interneurons is recruited only after learning. The facilitation might be due to “real” stronger synaptic transmission after learning or a decrease in baseline synaptic activity (Lisman, 2017), which might be occluded in D₁-CB₁-KO mice. The fact that partial blockade of GABA_A receptors in trained WT mice does not alter LTP facilitation suggests that this phenomenon is due to a genuine increase in LTP. In addition, our data indicate that reducing GABAergic transmission in D₁R-positive neurons is required for this form of learning-induced synaptic plasticity. These results reinforce the idea that, to reveal relevant mechanisms, investigations of synaptic plasticity associated with memory processes should include not only naive animals but also behaviorally challenged ones (Lisman et al., 2011).

D₁R activity in the HPC is necessary for long-term memory, synaptic plasticity, and network dynamics (Lisman et al., 2011; Yamasaki and Takeuchi, 2017; Kaufman et al., 2020; Bethus et al., 2010). Consistently, our results show that high doses of the D_{1/5}R antagonist SCH-23390 impair memory performance in the NOR task. In addition, our data suggest that D₁R/CB₁R-positive hippocampal interneurons are one of the targets of dopaminergic control of learning and memory processes. Interestingly, it

has been shown that parvalbumin (PV)-expressing interneurons require D₁R activity for late phases of memory consolidation through coordinated control of the activity of hippocampal pyramidal neurons (Karunakaran et al., 2016). Particularly, the authors describe that this D₁R activity modulates hippocampal network oscillations (i.e., sharp-wave ripples), which is a proposed correlate for synaptic plasticity and memory consolidation (Buzsáki, 2015). In addition, previous studies have shown that PV/CB₁R-negative and CCK/CB₁R-positive interneurons have complementary roles in ensuring such high oscillatory ripple events with consequent capacity to modulate synaptic plasticity (Klausberger et al., 2005; Buzsáki, 2015). Therefore, we speculate that the subpopulation of D₁R/CB₁R-positive interneurons described in our work could play a complementary role in maintaining a proper excitation/inhibition balance in the hippocampal network activity required for memory consolidation.

Although complete elucidation of the complex microcircuitry requires further characterization, our findings support the hypothesis that D₁R/CB₁R-positive hippocampal interneurons belong to a broader circuit participating in dopaminergic control of memory (Yamasaki and Takeuchi, 2017). Our data are compatible with a scenario where D₁R activation during the learning/consolidation process potentiates GABAergic transmission. However, this D₁R-dependent increase in inhibition is kept within adequate limits by activation of CB₁Rs, allowing proper flow of information. In this sense, in the absence of CB₁R-dependent control of D₁R/CB₁R-positive interneurons (i.e., D₁-CB₁-KO mice), partial inhibition of D₁-like or GABA_A receptors rescues the phenotype. In other words, although activation of D₁Rs in interneurons seems to be necessary for the memory process, their abnormally high activity (e.g. in the absence of CB₁Rs) impairs such functions. In this context, an interesting question relates to the functional link between endogenous activation of D₁Rs and CB₁Rs. Our results allow speculation about two potential scenarios based on autocrine or paracrine modes of action of endocannabinoid signaling (Busquets-Garcia et al., 2017). (1) General D₁R-dependent dopaminergic signaling in the HPC might activate pyramidal neurons (Roggenhofer et al., 2013; Shivarama Shetty et al., 2016) targeted by D₁R/CB₁R-positive interneurons. This depolarization of pyramidal neurons would, in turn, induce canonical endocannabinoid-dependent retrograde inhibition of GABAergic release (Castillo et al., 2012), moderating, among others, activation of D₁R/CB₁R-positive interneurons. (2) Following D₁R activation and consequent interneuron depolarization (Anastasiades et al., 2019; Gorelova et al., 2002), endocannabinoids might be mobilized locally and act in an autocrine manner to decrease the membrane potential and thereby moderate the activity of the neuron (Bacci et al., 2004). These two possibilities are not mutually exclusive, and they might reflect the effect of the mechanisms described on general network activity and/or on specific plastic cellular processes, respectively. Future studies will investigate these intriguing scenarios using adapted experimental approaches.

Altogether, these data reveal that functionally distinct cell types are present in the general population of hippocampal GABAergic interneurons expressing CB₁Rs. In particular, D₁R/CB₁R-positive interneurons provide specific behavioral and hippocampal synaptic mechanisms sustaining the fine-tuned regulation of memory

processes. The close interaction of CB₁Rs and D₁Rs in modulating recognition memory might provide novel therapeutic frameworks for treatment of cognitive diseases characterized by alterations of endocannabinoid or dopaminergic systems or both.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.celrep.2020.108046>.

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AUTHOR CONTRIBUTIONS

J.F.O.d.C., A.B.-G., G.M., and E.S.-G. conceived and supervised the whole project. J.F.O.d.C. performed and analyzed *in vivo* electrophysiology and

behavioral experiments. A.B.-G. and E.S.-G. performed and analyzed behavioral experiments. L.B. and A.C. contributed to experiments using viral vectors. L.R. and G.L. contributed to behavioral experiments. M.V., F.J.-K., T.L.-L., and M.M. performed cytochemical experiments. Z.Z. and M.V. helped with analysis of the data. J.F.O.d.C., A.B.-G., G.M., and E.S.-G. wrote the manuscript. F.D. contributed to writing. All authors edited and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit antibody against the C-myc epitope tag	BioLegend	Cat# 906301; RRID:AB_2565064
Goat anti-rabbit antibody Alexa Fluor 488	Fisher Scientific	Cat# A-11008; RRID:AB_143165
4',6-diamidino-2-phenylindole	Fisher Scientific	Cat# D3571; RRID:AB_2307445
Rabbit polyclonal antibody against DsRed	Takara Bio	Cat# 632496; RRID:AB_10013483
Secondary antibody goat anti-rabbit conjugated to a horseradish peroxidase	Cell signaling	Cat#7074S; RRID:AB_2099233
Digoxigenin (DIG)-labeled riboprobe against mouse CB ₁	Marsicano and Lutz, 1999	N/A
Anti-DIG antibody conjugated to HRP	Roche	Cat#11207733910; RRID:AB_514500
Bacterial and Virus Strains		
rAAV-CAG-DIO	Lead contact lab	rAAV-30
AAV-CAG-DIO-CB ₁	Lead contact lab	rAAV-37
AAV-CAG-DIO-CB ₁ -myc	Lead contact lab	rAAV-21
hSyn-DIO-hM4D(Gi)-mCherry	Addgene	44361-AAV8
pAAV-hSyn-DIO-mCherry	Addgene	50459-AAV8
Chemicals, Peptides, and Recombinant Proteins		
2-methylbutane	Sigma-Aldrich	M32631-1L
TSA plus fluorescein system	Perkin Elmer	NEL741001KT
Streptavidin-Texas Red	Perkin Elmer	NEL721001EA
Normal donkey serum	Merck	S30-100ML
Sheep Serum	Sigma Aldrich	S3772-10ML
Formaldehyde 4%	Sigma Aldrich	HT501128-4L
Blocking reagent (to prepare NEN)	Perkin Elmer	FP1012
TSA Biotin Systems	Perkin Elmer	NEL700A001KT
SSC 20X	Sigma	93017-10L-F
Fluoromount-GSlide Mounting Medium	Electron microscopy sciences	17984-25
Isoflurane	Virbac	Vnr137317
Bicuculine	Sigma-Aldrich	14343-50MG
SCH 23390	Sigma-Aldrich	D054-10MG
MK-801	Sigma-Aldrich	77086-22-7
clozapine-N-oxide CNO	Tocris	4936
Critical Commercial Assays		
Avidin/Biotin Blocking Kit	Vector Labs	SP-2001
Experimental Models: Organisms/Strains		
D ₁ -CB ₁ -WT and D ₁ -CB ₁ -KO	Lead contact lab	N/A
C57BL/6N	Janvier Labs	C57BL/6NRj
D ₁ -Cre	Lead contact lab	Tg(Drd1a-cre)AGsc/KndIJ,
CB ₁ ^{flox/flox}	Lead contact lab	Cnr1tm1.2Ltz, MGI:3045419
GFAP-CB ₁ -WT and GFAP-CB ₁ -KO	Lead contact lab	N/A
Software and Algorithms		
Prism	Graphpad Software	V6.0
CED 1401 Spike2	Cambridge Electronic Design	V6.18
ImageJ	NIH	V1.52

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Giovanni Marsicano (giovanni.marsicano@inserm.fr).

Materials Availability

Mouse lines generated and used in the current study are available from the lead contact upon request. We are glad to share the mouse lines with reasonable compensation by requestor for its processing and shipping.

Data and Code Availability

The data supporting the current study have not been deposited in a public repository but are available from the lead contact on request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal Model

All experimental procedures were approved by the ethical committee of the French Ministry of Higher Education, Research and Innovation (authorization APAFIS#18111). Maximal efforts were made to reduce the suffering of the animals. Male mice were used in this study.

D₁-CB₁-KO mice were generated as previously described (Monory et al., 2007; Terzian et al., 2011). Briefly, CB₁ floxed mice (Marsicano et al., 2003) were crossed with D₁-Cre line (Lemberger et al., 2007), in which the Cre recombinase was placed under the control of the D₁ gene (Drd1a) regulatory sequences using transgenesis with modified bacterial artificial chromosomes. The pattern of Cre expression recapitulated the expression pattern of the endogenous Drd1a (Lemberger et al., 2007). Breeding was performed by mating male Cre-positive D₁-CB₁-KO mice with homozygous CB₁-flox female mice deriving from a separate colony. In order to detect possible germline or ectopic recombination events, genotyping of tail samples from pups (PD10) was performed by genomic PCR using primers suited to identify WT, “floxed” and “recombined” bands. No germline or ectopic recombination was detected. Eight to 14 weeks-old naive male D₁-CB₁-KO and WT littermates were used. 8–14 weeks old male C57BL/6NRj mice purchased from Janvier (France). 8–12 weeks-old D₁-Cre mice breed in the animal facilities of the U1215 we also used. Animals were housed collectively under standard conditions of temperature and humidity in a day/night cycle of 12/12 hours (light on at 7 am). Animals that underwent surgery were kept in individual cages after the procedures to avoid conflict with their littermates. Food and water were provided *ad libitum*. All the experiments were performed during the light phase. Behavioral experiments were performed from 9 am to 3 pm. Electrophysiology experiments were performed from 8 am to 7 pm.

METHOD DETAILS

Drug preparation and administration

Bicuculline, MK-801, NBQX and SCH-23390 were purchased from Merck (formerly Sigma-Aldrich, France) and were dissolved to their final concentration in physiological saline (NaCl 0.9%). The exogenous DREADD ligand clozapine-N-oxide (CNO, 2 mg/kg) was purchased from Tocris Bioscience (Bristol, UK) and dissolved in saline after gently mixing with a vortex. All drugs were injected intraperitoneally in a volume of 10 ml/kg. Vehicle in all the conditions was composed of physiological saline (NaCl 0.9%) injections.

Novel object recognition memory

We used the novel object recognition (NOR) memory task in an L-maze (Busquets-Garcia et al., 2011, 2013; Hebert-Chatelain et al., 2016; Puighearnan et al., 2009, 2013; Robin et al., 2018).

The task took place in a L-shaped maze made of dark gray polyvinyl chloride made by two identical perpendicular arms (35 cm and 30 cm long respectively for external and internal L walls, 4.5cm wide and 15 cm high walls) placed on a white background (Busquets-Garcia et al., 2011; Puighearnan et al., 2009). The task occurred in a room adjacent to the animal house with a light intensity fixed at 50 lux. The maze was overhung by a video camera allowing the detection and offline scoring of animal's behavior. The task consisted in 3 sequential daily trials of 9 minutes each. During the habituation phase (day 1), mice were placed in the center of the maze and allowed to freely explore the arms in the absence of any objects. The training phase (day 2) consisted in placing the mice again in the corner of the maze in the presence of two identical objects positioned at the extremities of each arm and left to freely explore the maze and the objects. The testing phase occurred 24 hours later (day 3): one of the familiar objects was replaced by a novel object different in its shape, color and texture and mice were left to explore both objects. The position of the novel object and the associations of novel and familiar were randomized. All objects were previously tested to avoid biased preference. Memory performance was assessed by the discrimination index (DI). The DI was calculated as the difference between the time spent exploring the novel (TN) and the familiar object (TF) divided by the total exploration time (TN+TF): $DI = [TN - TF] / [TN + TF]$. Memory was also evaluated by directly comparing the exploration time of novel and familiar objects, respectively. Object exploration was defined as the orientation

of the nose to the object at less than 2 cm. Experienced investigators evaluating the exploration were blind of treatment and/or genotype of the animals. Pharmacological treatments were immediately administered after the training phase.

***In vivo* electrophysiology in anesthetized mice**

Experiments were performed as described in [Robin et al. \(2018\)](#). Mice were anesthetized in a box containing 5% Isoflurane (Virbac, France) before being placed in a stereotaxic frame (Model 900, Kopf instruments, CA, USA) in which 1.0% to 1.5% of Isoflurane was continuously supplied via an anesthetic mask during the whole duration of the experiment. The body temperature was maintained at $\pm 36.5^{\circ}\text{C}$ using a homeothermic system (model 50-7087-F, Harvard Apparatus, MA, USA) and the state of anesthesia was assessed by mild tail pinch. Before surgery, 100 mL of the local anesthetic lurocaine (vetoquinol, France) was injected in the scalp region. Surgical procedure started with a longitudinal incision of 1.5 cm in length aimed to expose Bregma and Lambda. After ensuring the correct alignment of the head, two holes were drilled in the skull for electrode placement. Glass recording electrodes were inserted in the CA1 stratum radiatum, and a concentric stimulating bipolar electrode (Model CBARC50, FHC, ME, USA) placed in the CA3 region. Coordinates were as follows: CA1 stratum radiatum: A/P 1.5, M/L 1.0, DV 1.20; CA3: A/P 2.2, M/L 2.8, D/V 1.3 (20 insertion angle). The recording electrode (tip diameter = 1–2 mm, 2–4 M Ω) was filled with a 2% pontamine sky blue solution in 0.5M sodium acetate. At first the recording electrode was placed by hand until it reached the surface of the brain and then to the final depth using a hydraulic micro-positioner (Model 2650, KOPF instruments, CA, USA). The stimulation electrode was placed in the correct area using a standard manipulator. Both electrodes were adjusted to find the area with maximum response. *In vivo* recordings of evoked field excitatory postsynaptic potentials (fEPSPs) were amplified 1000 times and filtered (low-pass at 1Hz and high-pass 3000Hz) by a DAGAN 2400A amplifier (DAGAN Corporation, MN, USA). fEPSPs were digitized and collected on-line using a laboratory interface and software (CED 1401, SPIKE 2; Cambridge Electronic Design, Cambridge, UK). Test pulses were generated through an Isolated Constant Current Stimulator (DS3, Digitimer, Hertfordshire, UK) triggered by the SPIKE 2 output sequencer via CED 1401 and collected every 2 s at a 10 kHz sampling frequency and then averaged every 180 s. Test pulse intensities were typically between 40–250 μA with a duration of 50 ms. Basal stimulation intensity was adjusted to 30%–50% of the current intensity that evoked a maximum field response. All responses were expressed as percent from the average responses recorded during the 15 min before high frequency stimulation (HFS). HFS was induced by applying 3 trains of 100 Hz (1 s each), separated by 20 s interval. fEPSP were then recorded for a period of 60 min. C57BL6/NRj mice underwent this *in vivo* electrophysiology procedure after the training phase of NOR task. Also, where specified, D₁-CB₁-KO and D₁-CB₁-WT received an injection of Bicuculine (0.5 mg/kg, intraperitoneal) or SCH 23390 (0.3 mg/kg, intraperitoneal) or vehicle immediately after undergoing training in NOR and before being subjected to the *in vivo* electrophysiology procedure. At the end the experiment, the position of the electrodes was marked (recording area: iontophoretic infusion of the recording solution during 180 s at 20mA; stimulation area: continuous current discharge over 20 s at +20mA) and histological verification was performed *ex vivo*.

Surgery and viral administration

Mice were anesthetized in a box containing 5% Isoflurane (Virbac, France) before being placed in a stereotaxic frame (Model 900, Kopf instruments, CA, USA) in which 1.0% to 1.5% of Isoflurane was continuously supplied via an anesthetic mask during the whole duration of the experiment. For viral intra-HPC AAV delivery, mice were submitted to stereotaxic surgery (as above) and AAV vectors were injected with the help of a microsyringe (0.25 mL Hamilton syringe with a 30-gauge beveled needle) attached to a pump (UMP3-1, World Precision Instruments, FL, USA). Where specified, D₁-CB₁-WT and D₁-CB₁-KO mice were injected directly into the hippocampus (HPC) or striatum (STR) (0.5 μL per injection site at a rate of 0.5 μL per min), with the following coordinates: HPC, AP -1.8 ; ML ± 1 ; DV -2.0 and -1.5 ; Striatum: AP -1.34 ; ML ± 2.8 ; DV -1.84 . Following virus delivery, the syringe was left in place for 1 minute before being slowly withdrawn from the brain. CB₁ *floxed* mice were injected with rAAV-CAG-DIO (empty control vector), AAV-CAG-DIO-CB₁, or AAV-CAG-DIO-CB₁-myc to induce re-expression of the CB₁ receptor gene in hippocampal or striatal D₁-positive cells. To generate the aforementioned rAAVs, mouse CB₁ receptor coding sequence (either native or fused to myc-tag at the C term) was cloned in rAAV-CAG-DIO vector using standard molecular cloning technology. The coding sequence was cloned inverted in orientation to allow Cre-dependent expression of CB₁ receptors ([Atasoy et al., 2008](#)). In another experiment, and using the same procedure as described as above, D₁-CB₁-WT and D₁-CB₁-KO mice were injected intra hippocampally (AP -1.8 ; ML ± 1 ; DV -2.0 and -1.5), with pAAV-hSyn-DIO-hM4D(Gi)-mCherry or pAAV-hSyn-DIO-mCherry (addgene, USA). For anatomical experiments and using the same procedure as above, D₁-Cre and D₁-CB₁-KO were injected intra hippocampally with pAAV-hSyn-DIO-mCherry. In this specific experiment, expression was allowed to take place for 2 weeks. For the remaining experiments, animals were used around 4–5 weeks after local infusions. Mice were weighed daily and individuals that failed to regain the pre-surgery body weight were excluded from the following experiments.

Immunohistochemistry on free-floating sections

Mice were anesthetized with pentobarbital (Exagon, Axience SAS, 400 mg/kg body weight), transcardially perfused with phosphate-buffered solution (PBS 0.1M, pH 7.4) before being fixed with 4% formaldehyde (Sigma-Aldrich). The brains were extracted and incubated overnight at 4°C in the same fixative, then embedded with sucrose 30% for 3 days and finally frozen in 2-methylbutane (Sigma-Aldrich) at -80°C . Free-floating frozen coronal sections (40 μm) were cut out with a cryostat (Microm HM 500M Microm Microtech), collected in an antifreeze solution and conserved at -20°C . Sections were permeabilized in a blocking solution

(in PBS: 10% donkey serum, 0.3% Triton X-100) for 1 hour at room temperature (RT). Then, sections were incubated with a rabbit primary antibody against the C-myc epitope tag (1:1000, BioLegend) overnight at 4°C. After several washes with PBS, slices were incubated for 2 hours with a secondary antibody goat anti-rabbit Alexa Fluor 488 (1:500, Fisher Scientific) and then washed in PBS at RT. Finally, sections were incubated with 4',6-diamidino-2-phenylindole (DAPI 1:20000, Fisher Scientific) diluted in PBS for 5 minutes to visualize cell nuclei and then were washed, mounted and coverslipped. All the antibodies were diluted in blocking solution. The sections were imaged with a slides scanner Hamamatsu Nanozoomer 2.0 HT.

Combined Fluorescent *in situ* hybridization (FISH)/ Immunohistochemistry (IHC) on free-floating frozen sections

Mice were anesthetized with pentobarbital (Exagon, Axience SAS, 400 mg/kg body weight), transcardially perfused with PBS (0.1M, pH 7.4) before being fixed with 4% formaldehyde (Sigma-Aldrich). The brains were extracted and incubated overnight at 4°C in the same fixative, then embedded with sucrose 30% for 3 days and finally frozen in 2-methylbutane (Sigma-Aldrich) at –80°C. Free-floating frozen coronal sections were cut out with a cryostat (30 µm, Microm HM 500M Microm Microtech) and collected in an anti-freeze solution and conserved at –20°C.

Section were washed several times with PBS with diethyl pyrocarbonate (PBS-DEPC) to wash out the antifreeze solution. The endogenous peroxidases were inactivated by incubating the free-floating sections with 3% H₂O₂ in PBS-DEPC for 30 minutes. All endogenous biotin, biotin receptors, and avidin binding sites present in the tissue were blocked by using the Avidin/Biotin Blocking Kit (Vector Labs, USA). Then, the slices were incubated overnight at RT with a rabbit polyclonal primary antibody against DsRed (1:1000, Takara Bio) diluted in a blocking solution (0.3% Triton X-100 diluted in PBS-DEPC). The following day, after several washes, the sections were incubated with a secondary antibody goat anti-rabbit conjugated to a horseradish peroxidase (HRP) (1:500, Cell Signaling Technology) during 2 hours at RT followed by TSA Biotin System (Biotin TSA 1:100, PerkinElmer) for 10 minutes at RT. After several washes, the slices were fixed with 4% of formaldehyde (Sigma Aldrich) for 10 minutes and blocked with 0.2M HCl for 20 minutes at RT. Then, the section were acetylated in 0.1 M Triethanolamine, 0.25% Acetic Anhydride for 10 minutes. This step was performed to reduce non-specific probe binding. Sections were hybridized overnight at 60°C with Digoxigenin (DIG)-labeled riboprobe against mouse CB₁ receptor (1:1000, prepared as described in [Marsicano and Lutz, 1999](#)). After hybridization, the slices were washed with different stringency wash buffers at 65°C. Then, the sections were incubated with 3% of H₂O₂ for 30 minutes at RT and blocked 1 hour with NEN blocking buffer prepared according to the manufacturer's protocol (PerkinElmer). Anti-DIG antibody conjugated to HRP (1:2000, Roche) was applied for 2 hours at RT. The signal of CB₁ receptor hybridization was revealed by a TSA reaction using fluorescein isothiocyanate (FITC)-labeled tyramide (1:80 for 12 minutes, Perkin Elmer). After several washes, the free-floating slices were incubated overnight at 4°C with Streptavidin-Texas Red (1:400, PerkinElmer). Finally, the slices were incubated with DAPI (1:20000; Fisher Scientific) diluted in PBS, following by several washes, to finally be mounted, coverslipped and imaged with an epifluorescence Leica DM 6000 microscope (Leica, Germany).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data collection

No statistical methods were used to pre-determine sample sizes, but they are similar to those reported in previous publications. All data collection and/or analysis were performed blind to the conditions of the experimenter except for the *in vivo* electrophysiological experiments. All mice were assigned randomly to the different experimental conditions.

Fluorescence quantifications

Cells expressing mRNAs were quantified in the different layers (*stratum oriens*, *stratum pyramidale*, *stratum radiatum* and *stratum lacunosum moleculare*) of the dorsal hippocampus. CB₁ receptor positive cells were classified according to the level of transcript visualized by the intensity of fluorescence ([Marsicano and Lutz, 1999](#); [Terral et al., 2019](#)). “High-CB₁” cells were considered to be round-shaped and intense staining covering the entire nucleus whereas “Low-CB₁” cells were defined with discontinuous shape and lowest intensity of fluorescence allowing the discrimination of grains of staining.

Statistical analyses

Data were expressed as mean ± SEM or single data points and were analyzed with Prism 6.0 (Graphpad Software), using two-tails t test (paired, unpaired) or one-way ANOVA (Dunnett's), two-way ANOVA (sidak's). Sample sizes and p values can be found in figure legends and [Table S1](#).

Supplemental Information

Specific Hippocampal Interneurons Shape

Consolidation of Recognition Memory

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Figure S1

FIGURE S1. RELATED TO FIGURE 1.

(A) Total exploration time, and (B) exploration time of the familiar versus the novel object of D_1 - CB_1 -KO mice and WT littermates in the Short-term NOR task. (C) Total exploration time, and (D) exploration time of the familiar versus the novel object of D_1 - CB_1 -KO mice and WT littermates in the Long-term NOR task. (E) Total exploration time, and (F) exploration time of the familiar versus the novel object of STR- CB_1 -RS, HPC- CB_1 -RS and control mice in the Long-term NOR task. (G) Memory performance, (H) total exploration, and (I) exploration time of the familiar versus the novel object of GFAP- CB_1 -KO mice and WT littermates in the Long-term NOR task. (J) Memory performance, (K) total exploration, and (L) exploration time of the familiar versus the novel object of GFAP- CB_1 -KO mice and WT littermates in the Short-term NOR task. (M) Total exploration time, and (N) exploration time of the familiar versus the novel object of D_1 - CB_1 -KO and WT littermates intra-hippocampally injected with hM4D(Gi) virus or mCherry, with VEH or CNO in the Long-term NOR task.

See also **Table S1**.

Figure S2

FIGURE S2. RELATED TO FIGURE 3

(A) Memory performance, total exploration time, and exploration time of the familiar versus the novel object of C57BL6/NRj mice injected systemically with different doses of NBQX (in mg/kg, IP). (B) Total exploration, and (C) exploration time of the familiar versus the novel object time of D₁-CB₁-KO mice and WT littermates with systemic injections of VEH, MK-801, NBQX and Bicuculine (BIC). (D) Representative micrographs of the dorsal hippocampus of D₁-Cre and D₁-CB₁-KO mice showing the co-expression of CB₁R mRNA and mCherry (D₁R-positive cells). The dotted white square is the area showed in main Figure 3C. Scale bar = 150 μ m.

See also **Table S1**.

Figure S3

FIGURE S3. RELATED TO FIGURE 4

(A) Memory performance, (B) Total exploration time, and (C) Exploration time of the familiar versus the novel object of C57BL6/NRj mice injected with different doses of SCH23390 (in mg/kg, IP). (D) Total exploration, and (E) exploration time of the familiar versus the novel object time of D_1 - CB_1 -KO mice and WT littermates with systemic injections of SCH23390 (0.3 mg/kg, IP).

See also **Table S1**.

Table S1

Figure	Panel	Conditions	"n"	Analysis (post-hoc test reported)	Factors analyzed	F-ratios	P values
1	B	D1-CB1-WT vs D1-CB1-KO	10, 7	Unpaired t-test	-	-	0,708
	C	D1-CB1-WT vs D1-CB1-KO	9, 8	Unpaired t-test	-	-	< 0,0001
	E	D1-CB1-WT(Control) vs D1-CB1-KO (Control)	17, 5	Unpaired t-test	-	-	0,0271
	E	D1-CB1-KO: (Control) vs (R-CB1-STR) vs (R-CB1-HPC)	5, 6, 9	One-way ANOVA (Dunnet)	-	-	0,0133
	I	" VEH vs CNO": "Control", D1-CB1-KO-DREADD-Gi", " D1-CB1-KO-mCherry"	(16, 14) (11, 14) (6, 7)	2-WAY ANOVA (Sidak)	Treatment vs Genotype	Interaction F (2, 62) = 5,107 Genotype F (2, 62) = 7,266 Treatment F (1, 62) = 4,587	0,0088 0,0015 0,0362
2	B	D1-CB1-WT vs D1-CB1-KO - 30 min	8, 8	Unpaired t-test	-	-	0,9444
		D1-CB1-WT vs D1-CB1-KO - 60 min	8, 8	Unpaired t-test	-	-	0,4546
	E	D1-CB1-WT vs D1-CB1-KO - 30 min	8, 11	Unpaired t-test	-	-	0,0455
		D1-CB1-WT vs D1-CB1-KO - 60 min	8, 11	Unpaired t-test	-	-	0,0253
	G	D1-CB1-WT vs D1-CB1-KO - 30 min	10, 10	Unpaired t-test	-	-	0,0266
		D1-CB1-WT vs D1-CB1-KO - 60 min	10, 10	Unpaired t-test	-	-	0,0306
3	A	" D1-CB1-WT vs D1-CB1-KO": "VEH" / "MK-801" / "NBQX" / "BIC" / "BIC 1h"	(14, 14)	2-WAY ANOVA (Sidak)	Treatment vs Genotype	Interaction F (4, 83) = 2,811	0,0003
			(7, 7)			Treatment F (4, 83) = 6,787	0,0001
			(8, 5)			Genotype F (1, 83) = 10,17	0,0021
			(10, 10) (10, 8)				
	F	S Oriens: D1-CRE vs D1-CB1-KO	(3, 4)	Unpaired t-test	-	-	0,0453
		S Pyramidale: D1-CRE vs D1-CB1-KO	(3, 4)	Unpaired t-test	-	-	0,0059
		S Radiatum / L Moleculare: D1-CRE vs D1-CB1-KO	(3, 4)	Unpaired t-test	-	-	0,0337
4	B	" D1-CB1-WT vs D1-CB1-KO": "VEH" / "Bicuculline" / "SCH 23390" at 30 min	(6, 5)	2-WAY ANOVA (Sidak)	Genotype vs Treatment	Interaction F (2, 35) = 4,670	0,0159
			(8, 10)			Genotype F (1, 35) = 10,30	0,0028
			(6, 6)			Treatment F (2, 35) = 2,492	0,0973
	C	" D1-CB1-WT vs D1-CB1-KO": "VEH" / "Bicuculline" / "SCH 23390" at 60 min	(6, 5)	2-WAY ANOVA (Sidak)	Genotype vs Treatment	Interaction F (2, 35) = 4,232	0,0226
			(8, 10)			Treatment F (1, 35) = 6,108	0,0185
			(6, 6)			Genotype F (2, 35) = 2,014	0,1487
	D	" D1-CB1-WT vs D1-CB1-KO": "VEH"/"SCH 23390"	(6, 10)		Genotype vs Treatment	Interaction F (1, 32) = 10,15	0,0032
			(10, 10)			Treatment F (1, 32) = 0,9844	0,3285
						Genotype F (1, 32) = 1,236	0,2746

Table S1 (Cont.)

Figure	Panel	Conditions	"n"	Analysis (post-hoc test reported)	Factors analyzed	F-ratios	P values
S1	A	D1-CB1-WT vs D1-CB1-KO	10, 7	Unpaired t-test	-	-	0,8161
	B	D1-CB1-WT: Familiar vs Novel	10	Paired t-test	-	-	0,0072
	B	D1-CB1-KO: Familiar vs Novel	5	Paired t-test	-	-	0,0421
	C	D1-CB1-WT vs D1-CB1-KO	9, 8	Unpaired t-test	-	-	0,4572
	D	D1-CB1-WT: Before vs After	9	Paired t-test	-	-	<0,0001
	D	D1-CB1-KO: Before vs After	8	Paired t-test	-	-	0,4821
	E	D1-CB1-KO: (Control) vs (R-CB1-STR) vs (R-CB1-HPC)	5, 6, 9	One-way ANOVA (Dunnet)	-	-	0,2335
	F	D1-CB1-WT(Ctl): Familiar vs Novel	17	Paired t-test	-	-	<0,0001
	F	D1-CB1-KO(Ctl): Familiar vs Novel	5	Paired t-test	-	-	0,2137
	F	D1-CB1-KO(STR-CB ₁ -RS): Familiar vs Novel	6	Paired t-test	-	-	0,8794
	F	D1-CB1-KO(HPC-CB ₁ -RS): Familiar vs Novel	9	Paired t-test	-	-	0,0064
	G	GFAP-CB1-WT vs GFAP-CB1-KO	7, 13	Unpaired t-test	-	-	0,001
	H	GFAP-CB1-WT vs GFAP-CB1-KO	7, 13	Unpaired t-test	-	-	0,8118
	I	GFAP-CB1-WT: Familiar vs Novel	7	Paired t-test	-	-	0,0004
	I	GFAP-CB1-KO: Familiar vs Novel	13	Paired t-test	-	-	0,6032
	J	GFAP-CB1-WT vs GFAP-CB1-KO	10, 11	Unpaired t-test	-	-	<0,0001
	K	GFAP-CB1-WT vs GFAP-CB1-KO	10, 11	Unpaired t-test	-	-	0,7143
	L	GFAP-CB1-WT: Familiar vs Novel	10	Paired t-test	-	-	<0,0001
	L	GFAP-CB1-KO: Familiar vs Novel	11	Paired t-test	-	-	0,2716
	M	D1-CB1-WT(Ctl): VEH vs CNO	16, 21	Unpaired t-test	-	-	0,5043
	M	D1-CB1-KO(Ctl): VEH vs CNO	6, 6	Unpaired t-test	-	-	0,2957
	M	D1-CB1-KO(Gi-DREADD): VEH vs CNO	11, 14	Unpaired t-test	-	-	0,9387
	N	D1-CB1-WT(Ctl): Vehicle: Familiar vs Novel	16	Paired t-test	-	-	0,0017
	N	D1-CB1-KO(Ctl): Vehicle: Familiar vs Novel	6	Paired t-test	-	-	0,461
	N	D1-CB1-KO(Gi-DREADD): Vehicle: Familiar vs Novel	11	Paired t-test	-	-	0,0133
	N	D1-CB1-WT(Ctl): CNO: Familiar vs Novel	21	Paired t-test	-	-	0,0057
	N	D1-CB1-KO(Ctl): CNO: Familiar vs Novel	6	Paired t-test	-	-	0,701
	N	D1-CB1-KO(Gi-DREADD): CNO: Familiar vs Novel	14	Paired t-test	-	-	0,001
S2	A	DI: VEH, NBQX (1, 5, 10, 20 mg/kg)	8-9	1-WAY ANOVA		Treatment: F (4, 37) = 20,12	P<0.0001
		Total Explor: VEH, NBQX (1, 5, 10, 20 mg/kg)	8-9	1-WAY ANOVA		Treatment: F (4, 37) = 4,3	P=0,0058
		Vehicle	8	Paired t-test		-	P=0,003
		NBQX 1 mg/kg	8	Paired t-test		-	P=0,008
		NBQX 5 mg/kg	9	Paired t-test		-	P=0,008
		NBQX 10 mg/kg	9	Paired t-test		-	P=0,8822
		NBQX 20 mg/kg	8	Paired t-test		-	P=0,9999
	B	" D1-CB1-WT vs D1-CB1-KO": "VEH" / "Mk-801" / "NBQX" / "Bicuculline" / "Bicuculline" 1h	14, 14	2-WAY ANOVA (Sidak)	Genotype vs Treatment	Interaction: F (4, 83) = 3,058	P=0,0363
			7, 7			Genotype: F (1, 83) = 5,457	P=0,0223
			8, 5			Treatment: F (4, 83) = 6,508	P=0,0005
			10, 10			-	-
			10, 8			-	-
	C	D1-CB1-WT: Vehicle: Familiar vs Novel	15	Paired t-test	-	-	<0,0001
	C	D1-CB1-WT: Mk-801: Familiar vs Novel	7	Paired t-test	-	-	0,0129
	C	D1-CB1-WT: Bicuculline: Familiar vs Novel	10	Paired t-test	-	-	<0,0001
	C	D1-CB1-WT: Bicuculline 1h: Familiar vs Novel	10	Paired t-test	-	-	0,0033
	C	D1-CB1-KO: Vehicle: Familiar vs Novel	14	Paired t-test	-	-	0,1869
	C	D1-CB1-KO: MK-801: Familiar vs Novel	7	Paired t-test	-	-	0,8454
	C	D1-CB1-KO: Vehicle: Familiar vs Novel	8	Paired t-test	-	-	0,0087
	C	D1-CB1-KO: NBQX: Familiar vs Novel	5	Paired t-test	-	-	0,1
	C	D1-CB1-KO: Bicuculline: Familiar vs Novel	10	Paired t-test	-	-	0,0034
	C	D1-CB1-KO: Bicuculline 1h: Familiar vs Novel	8	Paired t-test	-	-	0,0081
	C	Vehicle	11	Paired t-test	-	-	0,0018
	C	SCH 23390 0.1 mg/kg	12	Paired t-test	-	-	0,0259
	C	SCH 23390 0.3 mg/kg	12	Paired t-test	-	-	0,0295
	C	SCH 23390 1.0 mg/kg	12	Paired t-test	-	-	0,768
	C	SCH 23390 3.0 mg/kg	12	Paired t-test	-	-	0,8451
	D	" D1-CB1-WT vs D1-CB1-KO": "VEH" / "SCH23390"	6, 10	2-WAY ANOVA (Tukey)	Genotype vs Treatment	Interaction: F (1, 32) = 0,04417	P=0,8349
			10, 10			Genotype: F (1, 32) = 0,4676	P=0,4990
						Treatment: F (1, 32) = 0,1547	P=0,6967
	E	D1-CB1-WT: Vehicle: Familiar vs Novel	6	Paired t-test	-	-	0,0002
	E	D1-CB1-KO: Vehicle: Familiar vs Novel	10	Paired t-test	-	-	0,7749
	E	D1-CB1-WT: SCH23390: Familiar vs Novel	10	Paired t-test	-	-	0,0038
	E	D1-CB1-KO: SCH23390: Familiar vs Novel	10	Paired t-test	-	-	0,0143
S3	D	" D1-CB1-WT vs D1-CB1-KO": "VEH" / "SCH23390"	6, 10	2-WAY ANOVA (Tukey)	Genotype vs Treatment	Interaction: F (1, 32) = 0,04417	P=0,8349
			10, 10			Genotype: F (1, 32) = 0,4676	P=0,4990
						Treatment: F (1, 32) = 0,1547	P=0,6967
	E	D1-CB1-WT: Vehicle: Familiar vs Novel	6	Paired t-test	-	-	0,0002
	E	D1-CB1-KO: Vehicle: Familiar vs Novel	10	Paired t-test	-	-	0,7749
	E	D1-CB1-WT: SCH23390: Familiar vs Novel	10	Paired t-test	-	-	0,0038
	E	D1-CB1-KO: SCH23390: Familiar vs Novel	10	Paired t-test	-	-	0,0143

**TABLE S1. STATISTICAL ANALYSIS RELATED TO MAIN AND
SUPPLEMENTARY FIGURES**