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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 impacts on memory processes is scanty known. 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 CB₁R in D₁-positive cells (D₁-CB₁-KO) display impairment in long-term, but not short-term, novel object recognition memory (NOR). Re-expression of CB₁R 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. The CB₁R-mediated NOR and associated LTP facilitation involve the local control of GABAergic inhibition in a D₁-dependent manner.

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

KEYWORDS: CB₁ receptor, novel object recognition memory, hippocampus, interneurons, dopamine, D₁ receptor, GABA.

1 INTRODUCTION

2
3 The formation of episodic memory is a multistep brain process that
4 requires the activity of the medial temporal lobe (Squire et al., 2007). In
5 particular, the hippocampus participates in the long-term storage of recently
6 acquired events. Hippocampal circuits are regulated by a large variety of local
7 inhibitory interneurons that are controlled by neuromodulatory systems ensuring
8 their coordinated functions to shape behavioral responses (Klausberger and
9 Somogyi, 2008), whose identities and functions are unclear (Harris et al., 2018;
10 Pelkey et al., 2017; Parra et al., 1998).

11 The endocannabinoid system is a brain modulatory signaling hub mainly
12 formed by type-1 cannabinoid receptors (CB₁R), their endogenous ligands
13 (endocannabinoids), and the enzymes for their synthesis and degradation. In
14 the hippocampus, CB₁R are present in principal neurons and astroglial cells
15 (Busquets-Garcia et al., 2015; Oliveira da Cruz et al., 2016). However, the
16 largest expression of CB₁R resides in GABAergic interneurons (Marsicano and
17 Kuner, 2008; Katona and Freund, 2012), where they modulate local inhibition of
18 hippocampal circuits. Particularly, the largest amount of CB₁R is expressed in
19 cholecystokinin (CCK)-positive interneurons, which are characterized by
20 asynchronous neurotransmitter release (Harris et al., 2018; Katona et al., 1999;
21 Marsicano and Lutz, 1999).

22 Hippocampal CB₁R control episodic-like memory processes and synaptic
23 plasticity (Robin et al., 2018; Hebert-Chatelain et al., 2016; Puighermanal et al.,
24 2009). However, the specific locations where these receptors participate in the
25 mechanisms underlying hippocampal-dependent memory are only partially
26 known.

27 Activity-dependent long-term changes in hippocampal synaptic
28 transmission are considered cellular correlates of memory consolidation (Nicoll,
29 2017; Whitlock et al., 2006), which involves local D₁R signaling (Lisman et al.,
30 2011; Yamasaki and Takeuchi, 2017). The exposure to hippocampal-dependent
31 behavioral tasks induces changes in long-term potentiation (LTP) of synaptic

1 transmission that require the activation of D₁-like receptors (Frey et al., 1990;
2 Granado et al., 2008; Li et al., 2003; Lemon and Manahan-Vaughan, 2006). A
3 novel subpopulation of hippocampal CB₁R/CCK-positive interneurons
4 containing D₁R was recently described (Puighermanal et al., 2017; Gangarossa
5 et al., 2012). However, the potential interactions between D₁R and CB₁R in
6 regulating learning-induced plasticity, activity of hippocampal circuits, and
7 memory processes remain unexplored.

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

15

RESULTS

CB₁R IN HIPPOCAMPAL D₁R-POSITIVE NEURONS ARE NECESSARY FOR THE CONSOLIDATION OF NOR MEMORY

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

The majority of CB₁R in D₁R-positive neurons has been previously characterized 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 CB₁R. We infused an adeno-associated virus carrying a Cre-dependent expression of CB₁R (pAAV-CAG-DIO-*CB₁R*) into the striatum of D₁-*CB₁R*-KO mice to obtain re-expression of CB₁R in cells where Cre is present (hereafter called D₁R-positive) in this brain region (STR-*CB₁R*-RS mice, **Figure 1D and 1E**) as revealed by the immunodetection of a myc-tagged version of CB₁R (CB₁R-myc, see Methods, **Figure 1E**). This re-expression was not sufficient to rescue the phenotype of D₁-*CB₁R*-KO mice in long-term NOR (**Figure 1F, S1E and S1F**), suggesting that CB₁R in striatal D₁R-positive cells do not participate to this type of memory. Anatomical data indicate that a subset of hippocampal neurons contain D₁R (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-*CB₁R*-KO mice to obtain HPC-*CB₁R*-RS mice (**Figure 1D and 1G**). This manipulation fully rescued the phenotype of the mutant mice (**Figure 1F, S1E and S1F**), indicating that hippocampal CB₁R expressed in D₁R-positive cells are required for NOR memory.

We recently reported that deletion of CB₁R in hippocampal glial acidic fibrillary protein (GFAP)-positive cells (i.e. mainly astrocytes, GFAP-*CB₁R*-KO mice) also impaired NOR memory (Robin et al., 2018). Indeed, GFAP-*CB₁R*-KO mice were impaired in NOR (**Figure S1G-S1I**; Robin et al., 2018) but, in

1 contrast to D₁-CB₁-KO mice, this phenotype extended to short-term NOR
2 memory (**Figure S1J-S1L**). This difference suggests that CB₁R expressed in
3 hippocampal astrocytes or D₁R-positive cells might control distinct phases of
4 NOR memory consolidation.

5 The primary function of CB₁R activation in neurons is to decrease
6 neurotransmitter release (Castillo et al., 2012, Busquets-Garcia et al., 2017).
7 Accordingly, the deletion of CB₁R from neurons often results in excessive
8 neurotransmission. Thus, we reasoned that inhibition of hippocampal D₁R-
9 positive neurons during NOR consolidation should be able to rescue the
10 memory impairment of D₁-CB₁-KO mice. Viral vectors carrying Cre-dependent
11 expression of an inhibitory Designer Receptor Exclusively Activated by
12 Designer Drugs (DIO-hM4DGi, DREADD-Gi; Robinson and Adelman, 2015) or
13 control mCherry protein were infused into the hippocampi of D₁-CB₁-KO mice
14 and WT littermates (**Figure 1H**). Post-training CNO injections did not affect the
15 NOR performance of D₁-CB₁-KO and WT mice injected with either DREADD-Gi
16 or mCherry, indicating that the drug or its metabolites had no effect *per se*
17 [(Gomez et al., 2017), **Figure 1I, S1M and S1N**]. Conversely, post-acquisition
18 CNO treatment fully rescued the NOR impairment of D₁-CB₁-KO mice
19 expressing DREADD-Gi (**Figure 1I, S1M and S1N**). This strongly suggests that
20 excessive activity of D₁R-positive neurons during the consolidation process is
21 responsible for the memory impairment observed in D₁-CB₁-KO mice.

22 **CB₁R IN HIPPOCAMPAL D₁R-POSITIVE NEURONS CONTROL LEARNING-** 23 **INDUCED CHANGES OF LTP IN VIVO**

24 Cellular and molecular mechanisms underlying activity-dependent
25 changes in synaptic plasticity are proposed to underlie long-term memory
26 (Aggleton and Morris, 2018). Previous studies showed that conditional and
27 global deletion of CB₁R in neuronal and glial cell populations induces deficits in
28 learning and associated synaptic plasticity (Busquets-Garcia et al., 2017; Robin
29 et al., 2018). To address the role of CB₁R in hippocampal D₁R-positive neurons
30 in the modulation of synaptic plasticity, we recorded *in vivo* evoked field
31 excitatory postsynaptic potentials (fEPSPs) in the hippocampal CA3-CA1
32 pathway of anesthetized mice. High Frequency Stimulation (HFS) induced
33 similar long-lasting LTP of synaptic fEPSPs in both D₁-CB₁-KO and WT

littermates (**Figure 2A and 2B**), indicating that hippocampal D₁R/CB₁R-positive neurons are dispensable for the expression of LTP in naïve animals.

Hippocampal-dependent memory-related processes such as LTP are sensitive to pharmacological and genetic modulation of hippocampal D₁R, particularly after learning (Li et al., 2003, Lemon and Manahan-Vaughan, 2006, Takeuchi et al., 2016, Yamasaki and Takeuchi, 2017). Thus, we hypothesized that CB₁R 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 fEPSP from C57Bl6/NRj mice after NOR task (**Figure 2C**). The HFS induced stronger LTP in animals exposed to NOR acquisition than in control mice (**Figure 2D and 2E**), showing that the training modulates hippocampal synaptic plasticity. Strikingly, D₁-CB₁-KO mice lacked this learning-induced enhancement of LTP (**Figure 2F and 2G**). Thus, physiological activation of CB₁R in hippocampal D₁R-positive neurons is required for learning-dependent facilitation of LTP.

CB₁R IN HIPPOCAMPAL D₁R-POSITIVE NEURONS MODULATE NOR MEMORY CONSOLIDATION THROUGH A GABAERGIC-DEPENDENT MECHANISM

D₁R 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 both 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 of the GABA_A receptor antagonist Bicuculline in D₁-CB₁-KO and WT littermates immediately after the NOR training. MK-801 or NBQX did neither alter memory performance in WT mice nor did it rescue the amnesic phenotype of D₁-CB₁-KO littermates (**Figure 3A, S2B and S2C**). Conversely, Bicuculline completely reversed the memory impairment of D₁-CB₁-KO mice when injected immediately after training or 1h later, without affecting WT littermates' performance (**Figure 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 hippocampus (<http://mouse.brain-map.org/experiment/show/35>, data not shown), which makes it difficult to accurately quantify its expression above background. Therefore, in order to pinpoint which CB₁R-positive interneurons in the hippocampus 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 (see methods and **Figure 3B**). As described (Marsicano and Lutz, 1999), detectable levels of CB₁R mRNA were present throughout the hippocampus both 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 *strata oriens*, *pyramidale*, *radiatum* or *lacunosum moleculare* contain D₁R (**Figure 3C-3F and S2D**). Conversely, D₁R is present in a small subpopulation of low CB₁R-expressing interneurons along the different hippocampal layers (**Figure 3C and 3F**). Importantly, this co-expression was virtually abolished in hippocampi of D₁-CB₁-KO mice (**Figure 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 of D₁-CB₁-KO mice in 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 before testing LTP induction in hippocampal

1 circuits. In vehicle-treated animals, D₁-CB₁-KO mice showed no training-
2 induced LTP enhancement (**Figure 4A-4C**). Strikingly, whereas Bicuculline did
3 not affect LTP in WT animals, it rescued the training-induced LTP of D₁-CB₁-KO
4 mice (**Figure 4A-4C**).

5 Recent data suggest that hippocampal D₁R-like receptors participate in
6 memory formation, but little is known concerning the cell types involved (Lisman
7 et al., 2011, Yamasaki and Takeuchi, 2017). Our data indicate that CB₁R-
8 dependent control of GABAergic transmission from a low number of
9 hippocampal interneurons expressing D₁R is required to guarantee late
10 consolidation of NOR memory. Therefore, it is possible that endocannabinoid
11 actions are secondary to an activation of D₁R in these cells. To address this
12 issue, we first reasoned that partial inhibition of D₁R should "replace" the lack of
13 CB₁R-dependent control of neurotransmission in D₁-CB₁-KO mice. Thus, we
14 administered a sub-effective dose of the D_{1/5}R antagonist SCH-23390 (**Figure**
15 **S3A-S3C**) to D₁-CB₁-KO mice and WT littermates after NOR acquisition and we
16 analyzed the training-induced enhancement of *in vivo* LTP. This treatment
17 slightly reduced the late phase of LTP in WT animals (**Figure 4A-4C**). However,
18 the antagonist abolished the differences between D₁-CB₁-KO mice and WT
19 littermates (**Figure 4A-4C**), indicating that reducing D₁R activity counteracts the
20 absence of CB₁R in the mutants. If LTP is mechanistically linked to NOR
21 consolidation, the same treatment should rescue the memory impairment of D₁-
22 CB₁-KO mice. The administration of SCH-23390 did not alter the behavior of
23 WT mice (**Figure 4D, S3D and S3E**) but, strikingly, it fully rescued the memory
24 impairment of D₁-CB₁-KO littermates (**Figure 4D, S3D and S3E**).

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

1 DISCUSSION

2 The present study reveals that a specific subpopulation of hippocampal
3 D₁R/CB₁R-positive neurons controls late consolidation of NOR memory and
4 associated synaptic plasticity by moderating local inhibitory GABAergic activity
5 in the hippocampus. Specifically, CB₁R expressed in D₁R-positive interneurons
6 participate in learning-induced facilitation of *in vivo* LTP and are required for
7 consolidation of NOR memory. Moreover, CB₁R in D₁R-positive neurons are
8 necessary for physiological D₁R-dependent modulation of memory processes,
9 suggesting that cannabinoid signaling is part of a complex modulatory circuit
10 regulated by dopamine transmission in the hippocampus. By determining
11 cellular and behavioral functions of a specific CB₁R-expressing interneuron
12 subpopulation, these data uncover an unforeseen role of CB₁R in the D₁R-
13 dependent control of long-term memory.

14 The endocannabinoid system regulates episodic-like recognition memory
15 processes *via* CB₁R-dependent control of different cell types within the
16 hippocampus (Busquets-Garcia et al., 2017; Soria-Gomez et al., 2017;
17 Busquets-Garcia et al., 2016; Puighermanal et al., 2009; Robin et al., 2018). In
18 the present study we observed that the transition from short- to long-term
19 memory processes is controlled by a functional interaction between D₁R and
20 CB₁R receptors in a specific subpopulation of hippocampal interneurons. In
21 contrast, CB₁R receptor deletion from all body cells or in all forebrain
22 GABAergic neurons does not reproduce the phenotype of D₁-CB₁-KO mice
23 (Puighermanal et al., 2009; Hebert-Chatelain et al., 2016). These apparently
24 contrasting observations can be explained by different possibilities. Long-term
25 deletion of the *CB₁R* gene starting from early developmental stages both in
26 *CB₁-KO* and *GABA-CB₁-KO* mice might induce compensatory mechanisms (El-
27 Brolosy et al., 2019; El-Brolosy and Stainier, 2017) masking the functional role
28 of the CB₁R in NOR memory. An alternative or complementary explanation
29 might point to the presence of different subpopulations of brain cells expressing
30 CB₁R and exerting opposite effects on memory processes. For instance,
31 endocannabinoid signaling might promote or inhibit memory formation when
32 acting at D₁R-positive cells or at other neuronal subpopulations, respectively.
33 We have previously shown that astroglial CB₁R are necessary for consolidation

1 of NOR memory by allowing D-Serine availability at glutamatergic synapses
2 (Robin et al., 2018). We cannot fully exclude that deletion of CB₁R in D₁R-
3 positive cells does not involve also astrocytes (Nagatomo et al., 2017).
4 However, no conclusive anatomical evidence so far has been presented for the
5 expression of D₁R in hippocampal astrocytes [(Chai et al., 2017; Zhang et al.,
6 2014), but see (Jennings et al., 2017) for D_{1/5}R pharmacological experiments].
7 Moreover, our current and past results suggest that endocannabinoid control of
8 astrocytes is likely involved in the initial phases of memory formation, whereas
9 the CB₁R-dependent inhibition of D₁R-positive hippocampal interneurons
10 determines later phases of NOR memory consolidation. The time-course effects
11 of pharmacological treatments indicate that D-serine can rescue memory
12 performance of GFAP-CB₁-KO mice only when administered immediately after
13 learning (Robin et al., 2018). This idea is reinforced by the fact that these
14 mutants do not express *in vivo* LTP even in basal "home-cage" conditions
15 (Robin et al., 2018), whereas D₁-CB₁-KO mice only lack the specific facilitation
16 of LTP induced by learning. Altogether, these observations allow speculating
17 that at least two distinct temporal windows exist in the CB₁R-dependent control
18 of NOR. First, astroglial CB₁R are necessary for the plastic processes to initiate
19 the memory. Later, the endocannabinoid-dependent regulation of D₁R-positive
20 interneurons is required to maintain the memory trace for longer periods.

21 Hippocampal D₁R were previously shown to be mainly on GABAergic
22 interneurons, but lower levels were also detected on glutamatergic neurons
23 (Gangarossa et al., 2012; Puighermanal et al., 2017; [http://celltypes.brain-
24 map.org/rnaseq/mouse_ctx-hip_smart-seq](http://celltypes.brain-map.org/rnaseq/mouse_ctx-hip_smart-seq)). Our data show that the D₁-Cre
25 mouse line used in the present study (Lemberger et al., 2007) induces
26 recombination in a small sub-fraction of hippocampal interneurons containing
27 low levels of CB₁R mRNA, but also in pyramidal neurons and mossy cells.
28 Therefore, we cannot fully exclude that other cell types than hippocampal
29 interneurons might participate in the D₁R/CB₁R-dependent control of memory
30 consolidation. However, our data show that partial blockade of GABA_A
31 receptors, but not of AMPA/Kainate or NMDA glutamatergic ones, reverse the
32 memory impairment of D₁-CB₁-KO mice. Therefore, our findings strongly
33 suggest that CB₁R-control of GABA release from D₁R-positive interneurons

1 regulates late consolidation of NOR memory. However, recent data using
2 emerging technologies suggest that hippocampal cells are more diverse and
3 functionally segregated than previously thought (Harris et al., 2018; Soltesz and
4 Losonczy, 2018). By identifying specific markers, future studies will extend our
5 genetic and pharmacological evidence that a specific subpopulation of
6 D₁R/CB₁R-positive hippocampal interneurons regulates consolidation of NOR
7 memory.

8 LTP at the CA3-CA1 pathway represents a potential molecular and
9 cellular mechanism underlying the behavioral expression of episodic-like
10 memory processes (Morris, 2013). Interestingly, whereas deletion of CB₁R from
11 D₁R-positive cells impairs NOR memory, the same manipulation does not
12 impair *in vivo* LTP of hippocampal synaptic transmission in naïve animals. In
13 agreement with previous evidence in other experimental conditions (Li et al.,
14 2003; Lemon and Manahan-Vaughan, 2006), WT mice exposed to the NOR
15 learning task display a facilitation of *in vivo* LTP as compared to animals
16 exposed to the same environment without any learning. Importantly, this
17 facilitation is absent in D₁-CB₁-KO mice, suggesting that the endocannabinoid
18 control of D₁R-positive hippocampal interneurons is recruited only after learning.
19 The facilitation might be due to a "real" stronger synaptic transmission after
20 learning or to a decrease of baseline synaptic activity (Lisman, 2017), which
21 might be occluded in D₁-CB₁-KO mice. The fact that partial blockade of GABA_A
22 receptors in trained WT mice does not alter the LTP facilitation, suggests that
23 this phenomenon is due to a genuine increase of LTP. In addition, our data
24 indicate that reducing GABAergic transmission in D₁R-positive neurons is
25 required for this form of learning-induced synaptic plasticity. These results
26 reinforce the idea that, in order to reveal relevant mechanisms, investigations
27 on synaptic plasticity associated to memory processes should include not only
28 naïve animals, but also behaviorally challenged ones (Lisman et al., 2011).

29 D₁R activity in the hippocampus is necessary for long-term memory,
30 synaptic plasticity and network dynamics (Lisman et al., 2011, Yamasaki and
31 Takeuchi, 2017; Kaufman et al., 2020; Bethus et al., 2010). Consistently, our
32 results show that high-doses of the D_{1/5}R antagonist SCH23390 impair memory
33 performance in the NOR task. In addition, our data suggest that D₁R/CB₁R-

positive hippocampal interneurons are one of the targets of the 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 the coordinate 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 (Buzsaki, 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; Buzsaki, 2015). Therefore, we speculate that the subpopulation of D₁R/CB₁R-positive interneurons described in our work could play a complementary role in maintaining proper excitation/inhibition balance in the hippocampal network activity required for memory consolidation.

While the 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 the 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 of inhibition is kept within adequate limits by the activation of CB₁R, thereby allowing a 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), a partial inhibition of either D₁-like or GABA_A receptors rescues the phenotype. In other words, whereas activation of D₁R in interneurons seems to be necessary for the memory process, their abnormally high activity (e.g. in the absence of CB₁R) impairs such functions. In this context, an interesting question relates to the functional link between the endogenous activation of D₁R and CB₁R. Our results allow speculating about two potential scenarios, based on autocrine or paracrine modes of action of endocannabinoid signaling (Busquets-García et al., 2017): (i) General D₁R-dependent dopaminergic signaling in the hippocampus might activate pyramidal

1 neurons (Roggenhofe et al., 2013; Shivarama-Shetty et al., 2018) targeted by
2 D₁R/CB₁R-positive interneurons. This depolarization of pyramidal neurons
3 would, in turn, induce the canonical endocannabinoid-dependent retrograde
4 inhibition of GABAergic release (Castillo et al., 2012), thereby moderating,
5 amongst others, the activation of D₁R/CB₁R-positive interneurons. (ii) Following
6 D₁R activation and consequent interneuron depolarization (Anastasiades et al.,
7 2019; Gorelova et al., 2002), endocannabinoids might be mobilized locally and
8 act in an autocrine manner to decrease the membrane potential and thereby
9 moderate the activity of the neuron (Bacci et al., 2004). These two possibilities
10 are not mutually exclusive and they might reflect the impact of the mechanisms
11 described on general network activity and/or on specific plastic cellular
12 processes, respectively. Future studies will investigate these intriguing
13 scenarios using adapted experimental approaches.

14 Altogether, these data reveal that functionally distinct cell types are present in
15 the general population of hippocampal GABAergic interneurons expressing
16 CB₁R. In particular, D₁R/CB₁R-positive interneurons provide specific behavioral
17 and hippocampal synaptic mechanisms sustaining the fine-tuned regulation of
18 memory processes. The close interaction of CB₁R and D₁R in modulating
19 recognition memory might provide novel therapeutic frameworks for the
20 treatment of cognitive diseases characterized by alterations of both or either
21 endocannabinoid and dopaminergic systems.

22

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20 **AUTHOR CONTRIBUTIONS**

21 JFOdC, AB-G, GM and ES-G conceived and supervised the whole
22 project. JFOdC performed and analyzed *in vivo* electrophysiology and
23 behavioral experiments. AB-G and ES-G performed and analyzed behavioral
24 experiments. LB and AC contributed to experiments using viral vectors. LR and
25 GL contributed to behavioral experiments. MV, FJ-K, TLL and MM performed
26 cytochemical experiments. ZZ, MV helped with the analysis of the data. JFOdC,
27 AB-G, GM and ES-G wrote the manuscript. FD contributed to the writing. All
28 authors edited and approved the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

**FIGURE 1. HIPPOCAMPAL CB₁R IN D₁R-POSITIVE CELLS ARE
NECESSARY FOR LATE, BUT NOT EARLY, CONSOLIDATION OF NOR**

(A) Schematic representation of NOR Memory task.

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

(C) Long-term NOR (24 hours) 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) (see methods). Scale bar = 2mm.

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

(G) Immunofluorescence of cells expressing CB₁R-myc in the hippocampus. Scale bar = 500 μ m.

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

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

Data, mean \pm SEM. *p < 0.05, ***p < 0.001, ****p < 0.0001, ns=not significant.

See also **Figure S1** and **Table S1**.

FIGURE 2. LEARNING-INDUCED FACILITATION OF *IN VIVO* HIPPOCAMPAL LTP REQUIRES CB₁R AT D₁R-POSITIVE NEURONS

(A and B) HFS in the dorsal hippocampal CA3 Shaffer-Collateral pathway induces an *in vivo* LTP in dorsal CA1 *stratum radiatum*. (A) Summary plots of recorded evoked fEPSP in anesthetized D₁-CB₁-WT (n=8) and D₁-CB₁-KO (n=8) mice. (B) Bar histograms of normalized fEPSP from (A) representing 30 and 60 minutes after HFS.

(C) Schematic representation of the experimental setup (see methods).

(D and E) Learning modulates *in vivo* LTP. (D) Summary plots of recorded evoked fEPSP from mice expose to Control (n=8) and NOR Training (n=11) conditions. (E) Bar histograms of normalized of evoked fEPSP from (D) representing 30 and 60 minutes after HFS.

(F and G) Learning-induced modulation of *in vivo* LTP is impaired in D₁-CB₁-KO mice. (F) Summary plots of recorded fEPSP in anesthetized D₁-CB₁-WT (n=10) and D₁-CB₁-KO (n=10) mice. (G) Bar histograms of normalized of evoked fEPSP from (F) representing 30 and 60 minutes after HFS.

Traces on the right side of the summary plots represent 150 superimposed evoked fEPSP before HFS (1, grey), 30 minutes (2, brown) and 60 minutes (3, black) after HFS.

Data, mean \pm SEM. *p < 0.05, ns=not significant.

See also **Table S1**.

FIGURE 3. HIPPOCAMPAL CB₁R/D₁R-POSITIVE INTERNEURONS

MODULATE SYNAPTIC GABAERGIC TRANSMISSION.

(A) NOR memory performance of mutant mice administered with: vehicle [n(D₁-CB₁-WT)=14 and n(D₁-CB₁-KO)=14], MK-801 [0.1 mg/kg, IP; n(D₁-CB₁-WT)=7 and n(D₁-CB₁-KO)=7], NBQX [5 mg/kg, IP; n(D₁-CB₁-WT)=8 and n(D₁-CB₁-KO)=5], or Bicuculline immediately after [n(D₁-CB₁-WT)=10 and n(D₁-CB₁-KO)=10] or 1 hour after the training phase [n(D₁-CB₁-WT)=10 and 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 (in green) and mCherry protein (in red) labeling in the hippocampal CA1 region of D₁-Cre (C) and D₁-CB₁-KO (D) mice. White arrows indicate colocalization between CB₁R-positive and D₁R-positive cell bodies. Scale bar = 150 μm.

(E and F) Floating bars indicating the layer specific distribution of the % of cell bodies expressing high (E) and low amounts (F) of CB₁R, which colocalize with mCherry-positive (i.e. D₁R-positive) in D₁-Cre (n=3) and D₁-CB₁-KO (n=3).

Data, mean ± SEM. *p < 0.05, **p < 0.01, ns=not significant.

See also **Figure S2** and **Table S1**.

FIGURE 4. CELLULAR MECHANISMS LINKING D₁R-SIGNALLING WITH GABAERGIC ACTIVITY DURING LEARNING-INDUCED FACILITATION OF *IN VIVO* LTP AND MEMORY CONSOLIDATION

(A) Effects of the GABA_A receptor antagonist Bicuculline, 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. Summary plots of recorded evoked fEPSP in Vehicle [n(D₁-CB₁-WT)=6 and n(D₁-CB₁-KO)=8], Bicuculline [0.5 mg/kg; IP, n(D₁-CB₁-WT)=9 and n(D₁-CB₁-KO)=11] and SCH 23390[0.3 mg/kg; IP, n(D₁-CB₁-WT)=6 and n(D₁-CB₁-KO)=6].

(B and C) Bar histograms of (A) representing normalized fEPSP from 30 (B) and 60 (C) minutes after HFS.

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

Traces on the right side of the summary plot (A) represent 150 superimposed evoked fEPSP before HFS (1, grey), 30 minutes (2, brown) and 60 minutes (3, black) after HFS.

Data, mean ± SEM. *p < 0.05, **p < 0.01, ns=not significant.

See also **Figure S3** and **Table S1**.

STAR METHODS

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.

1 EXPERIMENTAL MODEL AND SUBJECT DETAILS

2 ANIMAL MODEL

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

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

METHOD DETAILS

DRUG PREPARATION AND ADMINISTRATION

Bicuculline, MK-801, NBQX and SCH23390 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., 2013; Busquets-Garcia et al., 2011; Hebert-Chatelain et al., 2016; Puighermanal et al., 2013; Puighermanal et al., 2009; Robin et al., 2018).

The task took place in a L-shaped maze made of dark grey 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; Puighermanal 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 homoeothermic 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 micropositioner (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

1 response. *In vivo* recordings of evoked field excitatory postsynaptic potentials
2 (fEPSPs) were amplified 1000 times and filtered (low-pass at 1Hz and high-
3 pass 3000Hz) by a DAGAN 2400A amplifier (DAGAN Corporation, MN, USA).
4 fEPSPs were digitized and collected on-line using a laboratory interface and
5 software (CED 1401, SPIKE 2; Cambridge Electronic Design, Cambridge, UK).
6 Test pulses were generated through an Isolated Constant Current Stimulator
7 (DS3, Digitimer, Hertfordshire, UK) triggered by the SPIKE 2 output sequencer
8 via CED 1401 and collected every 2 s at a 10 kHz sampling frequency and then
9 averaged every 180 s. Test pulse intensities were typically between 40-250 μ A
10 with a duration of 50 ms. Basal stimulation intensity was adjusted to 30%–50%
11 of the current intensity that evoked a maximum field response. All responses
12 were expressed as percent from the average responses recorded during the 15
13 min before high frequency stimulation (HFS). HFS was induced by applying 3
14 trains of 100 Hz (1 s each), separated by 20 s interval. fEPSP were then
15 recorded for a period of 60 min. C57BL6/NRj mice underwent this *in vivo*
16 electrophysiology procedure after the training phase of NOR task. Also, where
17 specified, D₁-CB₁-KO and D₁-CB₁-WT received an injection of Bicuculine (0.5
18 mg/kg, intraperitoneal) or SCH 23390 (0.3 mg/kg, intraperitoneal) or vehicle
19 immediately after undergoing training in NORT and before being subjected to
20 the *in vivo* electrophysiology procedure. At the end the experiment, the position
21 of the electrodes was marked (recording area: iontophoretic infusion of the
22 recording solution during 180 s at 20mA; stimulation area: continuous current
23 discharge over 20 s at +20mA) and histological verification was performed ex
24 vivo.

25

1 SURGERY AND VIRAL ADMINISTRATION

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

1 inactivated by incubating the free-floating sections with 3% H₂O₂ in PBS-DEPC
2 for 30 minutes. All endogenous biotin, biotin receptors, and avidin binding sites
3 present in the tissue were blocked by using the Avidin/Biotin Blocking Kit
4 (Vector Labs, USA). Then, the slices were incubated overnight at RT with a
5 rabbit polyclonal primary antibody against DsRed (1:1000, Takara Bio) diluted in
6 a blocking solution (0.3% Triton X-100 diluted in PBS-DEPC). The following
7 day, after several washes, the sections were incubated with a secondary
8 antibody goat anti-rabbit conjugated to a horseradish peroxidase (HRP) (1:500,
9 Cell Signaling Technology) during 2 hours at RT followed by TSA Biotin System
10 (Biotin TSA 1:100, PerkinElmer) for 10 minutes at RT. After several washes, the
11 slices were fixed with 4% of formaldehyde (Sigma Aldrich) for 10 minutes and
12 blocked with 0.2M HCl for 20 minutes at RT. Then, the section were acetylated
13 in 0.1 M Triethanolamine, 0.25% Acetic Anhydride for 10 minutes. This step
14 was performed to reduce non-specific probe binding. Sections were hybridized
15 overnight at 60°C with Digoxigenin (DIG)-labeled riboprobe against mouse CB₁
16 receptor (1:1000, prepared as described in (Marsicano and Lutz, 1999). After
17 hybridization, the slices were washed with different stringency wash buffers at
18 65°C. Then, the sections were incubated with 3% of H₂O₂ for 30 minutes at RT
19 and blocked 1 hour with NEN blocking buffer prepared according to the
20 manufacturer's protocol (PerkinElmer). Anti-DIG antibody conjugated to HRP
21 (1:2000, Roche) was applied for 2 hours at RT. The signal of CB₁ receptor
22 hybridization was revealed by a TSA reaction using fluorescein isothiocyanate
23 (FITC)-labeled tyramide (1:80 for 12 minutes, Perkin Elmer). After several
24 washes, the free-floating slices were incubated overnight at 4°C with
25 Streptavidin-Texas Red (1:400, PerkinElmer). Finally, the slices were incubated
26 with DAPI (1:20000; Fisher Scientific) diluted in PBS, following by several
27 washes, to finally be mounted, cover slipped and imaged with an
28 epifluorescence Leica DM 6000 microscope (Leica, Germany).

1 QUANTIFICATION AND STATISTICAL ANALYSIS

2 DATA COLLECTION

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

8 FLUORESCENCE QUANTIFICATIONS

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

17 STATISTICAL ANALYSES

18 Data were expressed as mean \pm SEM or single data points and were analyzed
19 with Prism 6.0 (Graphpad Software), using two-tails *t*-test (paired, unpaired) or
20 one-way ANOVA (Dunnett’s), two-way ANOVA (sidak’s). Sample sizes and p-
21 values can be found in figure legends and Table S1.

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Figure 1

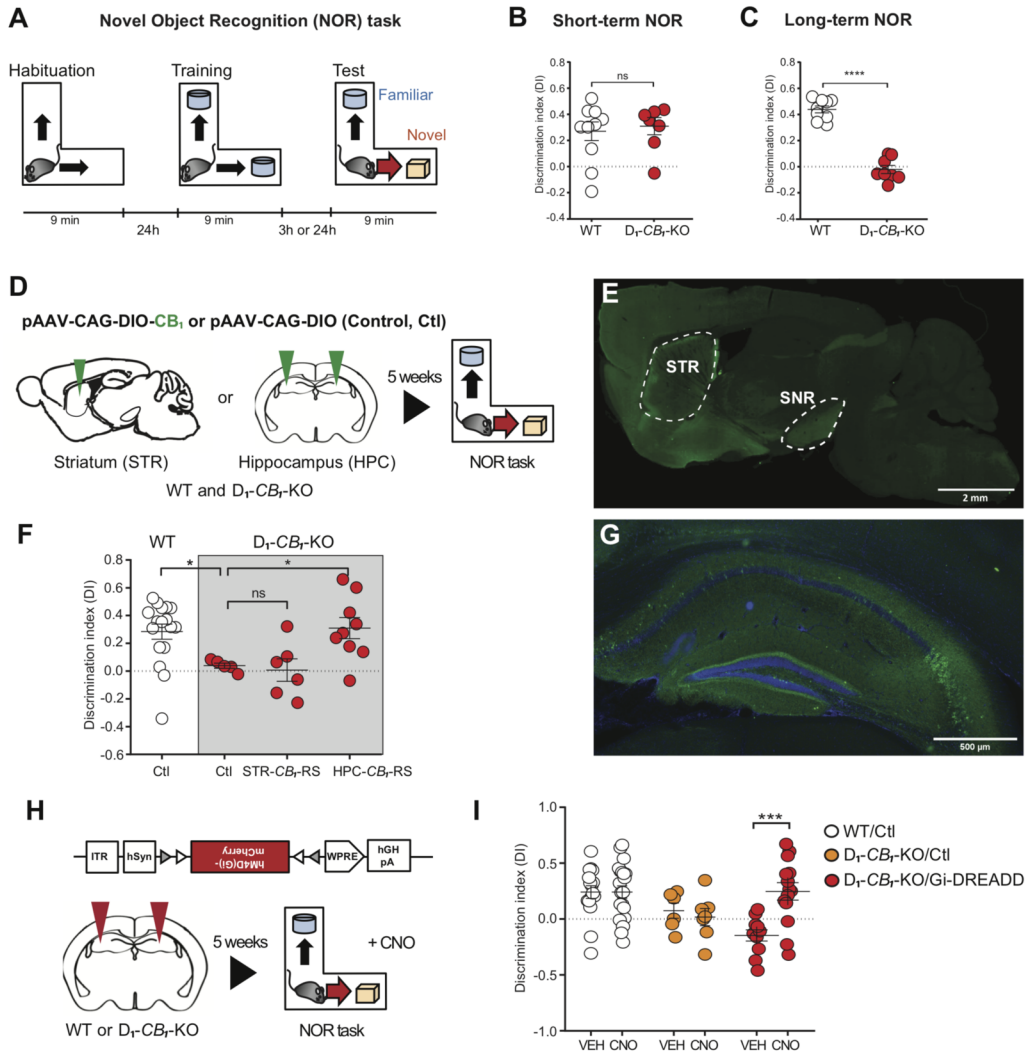


Figure 2

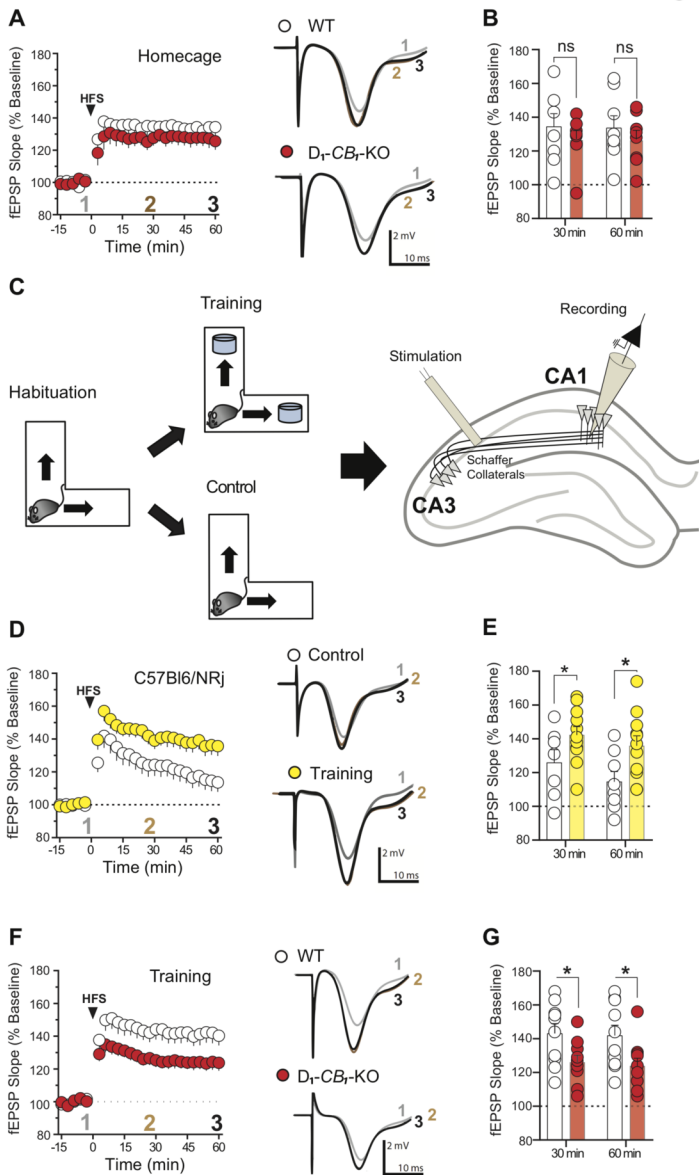


Figure 3

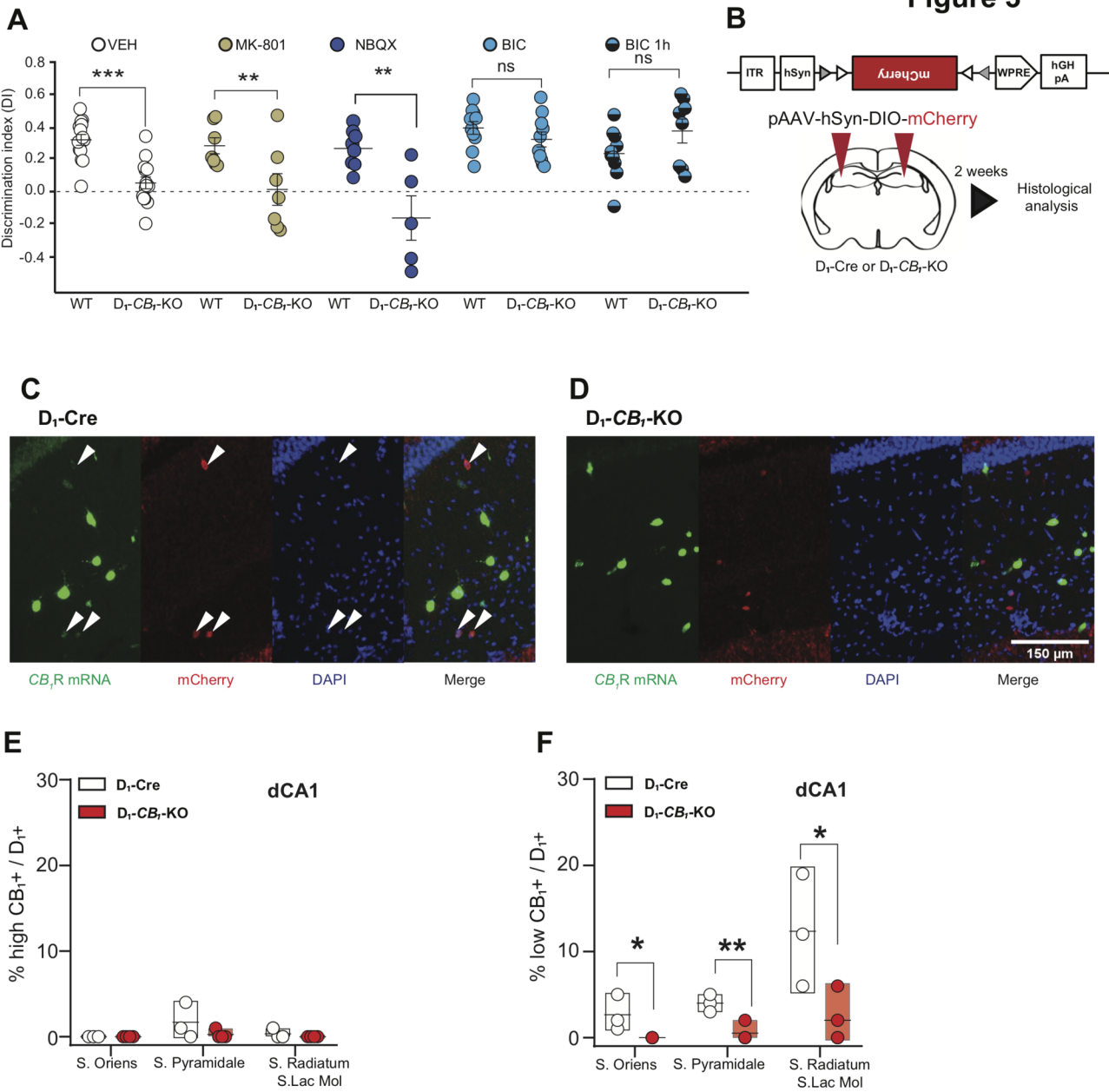
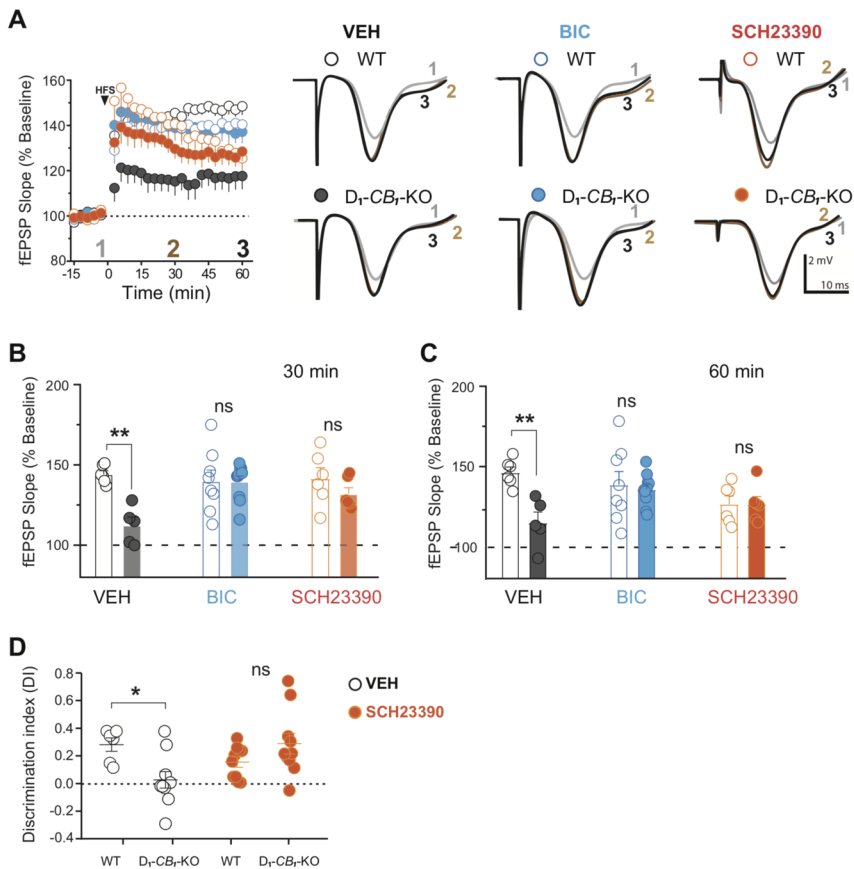


Figure 4

Hippocampal Circuits

