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1           **Specific hippocampal interneurons shape consolidation of**  
2   **recognition memory**

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24

1 **SUMMARY**

2

3 A complex array of inhibitory interneurons tightly controls hippocampal activity,  
4 but how such diversity specifically impacts on memory processes is scanty  
5 known. We find that a small subclass of type-1 cannabinoid receptor (CB<sub>1</sub>R)-  
6 expressing hippocampal interneurons determines episodic-like memory  
7 consolidation by linking dopamine D<sub>1</sub> receptor (D<sub>1</sub>R) signaling to GABAergic  
8 transmission.

9 Mice lacking CB<sub>1</sub>R in D<sub>1</sub>-positive cells (D<sub>1</sub>-CB<sub>1</sub>-KO) display impairment in long-  
10 term, but not short-term, novel object recognition memory (NOR). Re-  
11 expression of CB<sub>1</sub>R in hippocampal D<sub>1</sub>R-positive cells rescues this NOR deficit.  
12 Learning induces an enhancement of *in vivo* hippocampal long-term  
13 potentiation (LTP), which is absent in mutant mice. The CB<sub>1</sub>R-mediated NOR  
14 and associated LTP facilitation involve the local control of GABAergic inhibition  
15 in a D<sub>1</sub>-dependent manner.

16 This study reveals that hippocampal CB<sub>1</sub>R-/D<sub>1</sub>R-expressing interneurons  
17 control NOR memory, thereby identifying a mechanism linking the diversity of  
18 hippocampal interneurons to specific behavioral outcomes.

19 **KEYWORDS:** CB<sub>1</sub> receptor, novel object recognition memory,  
20 hippocampus, interneurons, dopamine, D<sub>1</sub> receptor, GABA.

21

# 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 (CB<sub>1</sub>R), their endogenous ligands  
13 (endocannabinoids), and the enzymes for their synthesis and degradation. In  
14 the hippocampus, CB<sub>1</sub>R 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 CB<sub>1</sub>R 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 CB<sub>1</sub>R 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 CB<sub>1</sub>R 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 D<sub>1</sub>R 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 D<sub>1</sub>-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 CB<sub>1</sub>R/CCK-positive interneurons  
4 containing D<sub>1</sub>R was recently described (Puighermanal et al., 2017; Gangarossa  
5 et al., 2012). However, the potential interactions between D<sub>1</sub>R and CB<sub>1</sub>R in  
6 regulating learning-induced plasticity, activity of hippocampal circuits, and  
7 memory processes remain unexplored.

8         Here we assessed the role of D<sub>1</sub>R/CB<sub>1</sub>R-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 *CB<sub>1</sub>R* gene in hippocampal  
11 D<sub>1</sub>R-positive cells impairs long- but not short-term NOR memory and learning-  
12 induced LTP enhancement. These intriguing results suggest that CB<sub>1</sub>R  
13 signaling provides a functional link between hippocampal dopaminergic and  
14 GABAergic control of synaptic plasticity and memory consolidation.

15

# 1 RESULTS

## 2 CB<sub>1</sub>R IN HIPPOCAMPAL D<sub>1</sub>R-POSITIVE NEURONS ARE NECESSARY FOR 3 THE CONSOLIDATION OF NOR MEMORY

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

11 The majority of CB<sub>1</sub>R in D<sub>1</sub>R-positive neurons has been previously  
12 characterized in striatonigral circuits (Monory et al., 2007). Considering the  
13 involvement of these circuits in NOR memory (Darvas and Palmiter, 2009), we  
14 tested the role of striatal CB<sub>1</sub>R. We infused an adeno-associated virus carrying  
15 a Cre-dependent expression of CB<sub>1</sub>R (pAAV-CAG-DIO-*CB<sub>1</sub>R*) into the striatum of  
16 D<sub>1</sub>-*CB<sub>1</sub>R*-KO mice to obtain re-expression of CB<sub>1</sub>R in cells where Cre is present  
17 (hereafter called D<sub>1</sub>R-positive) in this brain region (STR-*CB<sub>1</sub>R*-RS mice, **Figure**  
18 **1D and 1E**) as revealed by the immunodetection of a myc-tagged version of  
19 CB<sub>1</sub>R (CB<sub>1</sub>R-myc, see Methods, **Figure 1E**). This re-expression was not  
20 sufficient to rescue the phenotype of D<sub>1</sub>-*CB<sub>1</sub>R*-KO mice in long-term NOR  
21 (**Figure 1F, S1E and S1F**), suggesting that CB<sub>1</sub>R in striatal D<sub>1</sub>R-positive cells  
22 do not participate to this type of memory. Anatomical data indicate that a subset  
23 of hippocampal neurons contain D<sub>1</sub>R (Gangarossa et al., 2012), likely co-  
24 expressing CB<sub>1</sub>R protein (Puighermanal et al., 2017). Thus, we re-express the  
25 *CB<sub>1</sub>R* gene in the hippocampus of D<sub>1</sub>-*CB<sub>1</sub>R*-KO mice to obtain HPC-*CB<sub>1</sub>R*-RS mice  
26 (**Figure 1D and 1G**). This manipulation fully rescued the phenotype of the  
27 mutant mice (**Figure 1F, S1E and S1F**), indicating that hippocampal CB<sub>1</sub>R  
28 expressed in D<sub>1</sub>R-positive cells are required for NOR memory.

29 We recently reported that deletion of CB<sub>1</sub>R in hippocampal glial acidic  
30 fibrillary protein (GFAP)-positive cells (i.e. mainly astrocytes, GFAP-*CB<sub>1</sub>R*-KO  
31 mice) also impaired NOR memory (Robin et al., 2018). Indeed, GFAP-*CB<sub>1</sub>R*-KO  
32 mice were impaired in NOR (**Figure S1G-S1I**; Robin et al., 2018) but, in

1 contrast to D<sub>1</sub>-CB<sub>1</sub>-KO mice, this phenotype extended to short-term NOR  
2 memory (**Figure S1J-S1L**). This difference suggests that CB<sub>1</sub>R expressed in  
3 hippocampal astrocytes or D<sub>1</sub>R-positive cells might control distinct phases of  
4 NOR memory consolidation.

5 The primary function of CB<sub>1</sub>R activation in neurons is to decrease  
6 neurotransmitter release (Castillo et al., 2012, Busquets-Garcia et al., 2017).  
7 Accordingly, the deletion of CB<sub>1</sub>R from neurons often results in excessive  
8 neurotransmission. Thus, we reasoned that inhibition of hippocampal D<sub>1</sub>R-  
9 positive neurons during NOR consolidation should be able to rescue the  
10 memory impairment of D<sub>1</sub>-CB<sub>1</sub>-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 D<sub>1</sub>-CB<sub>1</sub>-KO mice  
14 and WT littermates (**Figure 1H**). Post-training CNO injections did not affect the  
15 NOR performance of D<sub>1</sub>-CB<sub>1</sub>-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 D<sub>1</sub>-CB<sub>1</sub>-KO mice  
19 expressing DREADD-Gi (**Figure 1I, S1M and S1N**). This strongly suggests that  
20 excessive activity of D<sub>1</sub>R-positive neurons during the consolidation process is  
21 responsible for the memory impairment observed in D<sub>1</sub>-CB<sub>1</sub>-KO mice.

## 22 **CB<sub>1</sub>R IN HIPPOCAMPAL D<sub>1</sub>R-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 CB<sub>1</sub>R 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 CB<sub>1</sub>R in hippocampal D<sub>1</sub>R-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 D<sub>1</sub>-CB<sub>1</sub>-KO and WT

1 littermates (**Figure 2A and 2B**), indicating that hippocampal D<sub>1</sub>R/CB<sub>1</sub>R-positive  
2 neurons are dispensable for the expression of LTP in naïve animals.

3 Hippocampal-dependent memory-related processes such as LTP are  
4 sensitive to pharmacological and genetic modulation of hippocampal D<sub>1</sub>R,  
5 particularly after learning (Li et al., 2003, Lemon and Manahan-Vaughan, 2006,  
6 Takeuchi et al., 2016, Yamasaki and Takeuchi, 2017). Thus, we hypothesized  
7 that CB<sub>1</sub>R in D<sub>1</sub>R-positive neurons may modulate learning-dependent  
8 hippocampal synaptic plasticity. To explore whether acquisition of the NOR task  
9 modulates *in vivo* LTP; we recorded fEPSP from C57Bl6/NRj mice after NOR  
10 task (**Figure 2C**). The HFS induced stronger LTP in animals exposed to NOR  
11 acquisition than in control mice (**Figure 2D and 2E**), showing that the training  
12 modulates hippocampal synaptic plasticity. Strikingly, D<sub>1</sub>-CB<sub>1</sub>-KO mice lacked  
13 this learning-induced enhancement of LTP (**Figure 2F and 2G**). Thus,  
14 physiological activation of CB<sub>1</sub>R in hippocampal D<sub>1</sub>R-positive neurons is  
15 required for learning-dependent facilitation of LTP.

## 16 **CB<sub>1</sub>R IN HIPPOCAMPAL D<sub>1</sub>R-POSITIVE NEURONS MODULATE NOR** 17 **MEMORY CONSOLIDATION THROUGH A GABAERGIC-DEPENDENT** 18 **MECHANISM**

19 D<sub>1</sub>R are expressed in different hippocampal cells, including subsets of  
20 GABAergic and glutamatergic neurons (Gangarossa et al., 2012). Considering  
21 that CB<sub>1</sub>R signaling decreases the activity of both hippocampal neurons  
22 (Busquets-Garcia et al., 2017, Castillo et al., 2012), we asked whether  
23 excessive glutamatergic or GABAergic neurotransmission might underlie the  
24 phenotype of D<sub>1</sub>-CB<sub>1</sub>-KO mice. Thus, we injected non-amnesic doses  
25 (Puighermanal et al., 2009) of the NMDA receptor blocker MK-801, the  
26 AMPA/kainate receptor antagonist NBQX (**Figure S2A**) or of the GABA<sub>A</sub>  
27 receptor antagonist Bicuculline in D<sub>1</sub>-CB<sub>1</sub>-KO and WT littermates immediately  
28 after the NOR training. MK-801 or NBQX did neither alter memory performance  
29 in WT mice nor did it rescue the amnesic phenotype of D<sub>1</sub>-CB<sub>1</sub>-KO littermates  
30 (**Figure 3A, S2B and S2C**). Conversely, Bicuculline completely reversed the  
31 memory impairment of D<sub>1</sub>-CB<sub>1</sub>-KO mice when injected immediately after  
32 training or 1h later, without affecting WT littermates' performance (**Figure 3A,**  
33 **S2B and S2C**).



1           These data indicate that excessive GABAergic, but not glutamatergic,  
2 ionotropic receptor activity is involved in the phenotype of D<sub>1</sub>-CB<sub>1</sub>-KO mice. A  
3 large proportion of GABAergic hippocampal interneurons contain CB<sub>1</sub>R mRNA,  
4 which is expressed at different levels [high CB<sub>1</sub>R- and low CB<sub>1</sub>R-expressing  
5 cells; (Marsicano and Lutz, 1999)]. Conversely, D<sub>1</sub>R mRNA is expressed at very  
6 low levels in the hippocampus ([http://mouse.brain-  
7 map.org/experiment/show/35](http://mouse.brain-map.org/experiment/show/35), data not shown), which makes it difficult to  
8 accurately quantify its expression above background. Therefore, in order to  
9 pinpoint which CB<sub>1</sub>R-positive interneurons in the hippocampus contain D<sub>1</sub>R, we  
10 combined fluorescence *in situ* hybridization for CB<sub>1</sub>R mRNA in D<sub>1</sub>-Cre and D<sub>1</sub>-  
11 CB<sub>1</sub>-KO mice carrying viral Cre-dependent expression of mCherry (see  
12 methods and **Figure 3B**). As described (Marsicano and Lutz, 1999), detectable  
13 levels of CB<sub>1</sub>R mRNA were present throughout the hippocampus both in  
14 pyramidal neurons and in GABAergic interneurons (**Figure S2D**). The  
15 distribution of mCherry-tagged D<sub>1</sub>-positive neurons in the dorsal CA1 region of  
16 D<sub>1</sub>-Cre mice was similar to previous findings (Puighermanal et al., 2017,  
17 Gangarossa et al., 2012). Double staining revealed that virtually no high CB<sub>1</sub>R-  
18 expressing interneurons in *strata oriens*, *pyramidale*, *radiatum* or *lacunosum*  
19 *moleculare* contain D<sub>1</sub>R (**Figure 3C-3F and S2D**). Conversely, D<sub>1</sub>R is present  
20 in a small subpopulation of low CB<sub>1</sub>R-expressing interneurons along the  
21 different hippocampal layers (**Figure 3C and 3F**). Importantly, this co-  
22 expression was virtually abolished in hippocampi of D<sub>1</sub>-CB<sub>1</sub>-KO mice (**Figure**  
23 **3C, 3D and 3F**).

24           Altogether, these data indicate that CB<sub>1</sub>R-dependent modulation of a  
25 small subpopulation of D<sub>1</sub>R-positive GABAergic interneurons is required during  
26 NOR memory consolidation.

## 27 **SYNAPTIC MECHANISMS UNDERLYING NOR MEMORY CONSOLIDATION** 28 **AND ASSOCIATED HIPPOCAMPAL PLASTICITY**

29           The data collected so far show that reduction of GABAergic signaling  
30 prevents the deficits of D<sub>1</sub>-CB<sub>1</sub>-KO mice in NOR consolidation. Therefore, we  
31 tested whether inhibition of GABA<sub>A</sub> receptors could rescue the lack of learning-  
32 induced LTP enhancement observed in D<sub>1</sub>-CB<sub>1</sub>-KO mice. Trained mice  
33 received Bicuculline or vehicle before testing LTP induction in hippocampal

1 circuits. In vehicle-treated animals, D<sub>1</sub>-CB<sub>1</sub>-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 D<sub>1</sub>-CB<sub>1</sub>-KO  
4 mice (**Figure 4A-4C**).

5         Recent data suggest that hippocampal D<sub>1</sub>R-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 CB<sub>1</sub>R-  
8 dependent control of GABAergic transmission from a low number of  
9 hippocampal interneurons expressing D<sub>1</sub>R is required to guarantee late  
10 consolidation of NOR memory. Therefore, it is possible that endocannabinoid  
11 actions are secondary to an activation of D<sub>1</sub>R in these cells. To address this  
12 issue, we first reasoned that partial inhibition of D<sub>1</sub>R should "replace" the lack of  
13 CB<sub>1</sub>R-dependent control of neurotransmission in D<sub>1</sub>-CB<sub>1</sub>-KO mice. Thus, we  
14 administered a sub-effective dose of the D<sub>1/5</sub>R antagonist SCH-23390 (**Figure**  
15 **S3A-S3C**) to D<sub>1</sub>-CB<sub>1</sub>-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 D<sub>1</sub>-CB<sub>1</sub>-KO mice and WT  
19 littermates (**Figure 4A-4C**), indicating that reducing D<sub>1</sub>R activity counteracts the  
20 absence of CB<sub>1</sub>R in the mutants. If LTP is mechanistically linked to NOR  
21 consolidation, the same treatment should rescue the memory impairment of D<sub>1</sub>-  
22 CB<sub>1</sub>-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 D<sub>1</sub>-CB<sub>1</sub>-KO littermates (**Figure 4D, S3D and S3E**).

25         Altogether, these results indicate that endocannabinoid-dependent  
26 regulation of hippocampal D<sub>1</sub>R-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 D<sub>1</sub>R/CB<sub>1</sub>R-positive neurons controls late consolidation of NOR memory and  
4 associated synaptic plasticity by moderating local inhibitory GABAergic activity  
5 in the hippocampus. Specifically, CB<sub>1</sub>R expressed in D<sub>1</sub>R-positive interneurons  
6 participate in learning-induced facilitation of *in vivo* LTP and are required for  
7 consolidation of NOR memory. Moreover, CB<sub>1</sub>R in D<sub>1</sub>R-positive neurons are  
8 necessary for physiological D<sub>1</sub>R-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 CB<sub>1</sub>R-expressing interneuron  
12 subpopulation, these data uncover an unforeseen role of CB<sub>1</sub>R in the D<sub>1</sub>R-  
13 dependent control of long-term memory.

14       The endocannabinoid system regulates episodic-like recognition memory  
15 processes *via* CB<sub>1</sub>R-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 D<sub>1</sub>R and  
20 CB<sub>1</sub>R receptors in a specific subpopulation of hippocampal interneurons. In  
21 contrast, CB<sub>1</sub>R receptor deletion from all body cells or in all forebrain  
22 GABAergic neurons does not reproduce the phenotype of D<sub>1</sub>-CB<sub>1</sub>-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 *CB<sub>1</sub>R* gene starting from early developmental stages both in  
26 *CB<sub>1</sub>-KO* and *GABA-CB<sub>1</sub>-KO* mice might induce compensatory mechanisms (El-  
27 Brolosy et al., 2019; El-Brolosy and Stainier, 2017) masking the functional role  
28 of the CB<sub>1</sub>R in NOR memory. An alternative or complementary explanation  
29 might point to the presence of different subpopulations of brain cells expressing  
30 CB<sub>1</sub>R and exerting opposite effects on memory processes. For instance,  
31 endocannabinoid signaling might promote or inhibit memory formation when  
32 acting at D<sub>1</sub>R-positive cells or at other neuronal subpopulations, respectively.  
33 We have previously shown that astroglial CB<sub>1</sub>R 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 CB<sub>1</sub>R in D<sub>1</sub>R-  
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 D<sub>1</sub>R in hippocampal astrocytes [(Chai et al., 2017; Zhang et al.,  
6 2014), but see (Jennings et al., 2017) for D<sub>1/5</sub>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 CB<sub>1</sub>R-dependent inhibition of D<sub>1</sub>R-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-CB<sub>1</sub>-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 D<sub>1</sub>-CB<sub>1</sub>-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 CB<sub>1</sub>R-dependent control  
18 of NOR. First, astroglial CB<sub>1</sub>R are necessary for the plastic processes to initiate  
19 the memory. Later, the endocannabinoid-dependent regulation of D<sub>1</sub>R-positive  
20 interneurons is required to maintain the memory trace for longer periods.

21 Hippocampal D<sub>1</sub>R 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 D<sub>1</sub>-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 CB<sub>1</sub>R 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 D<sub>1</sub>R/CB<sub>1</sub>R-dependent control of memory  
30 consolidation. However, our data show that partial blockade of GABA<sub>A</sub>  
31 receptors, but not of AMPA/Kainate or NMDA glutamatergic ones, reverse the  
32 memory impairment of D<sub>1</sub>-CB<sub>1</sub>-KO mice. Therefore, our findings strongly  
33 suggest that CB<sub>1</sub>R-control of GABA release from D<sub>1</sub>R-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 D<sub>1</sub>R/CB<sub>1</sub>R-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 CB<sub>1</sub>R from  
11 D<sub>1</sub>R-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 D<sub>1</sub>-CB<sub>1</sub>-KO mice, suggesting that the endocannabinoid  
18 control of D<sub>1</sub>R-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 D<sub>1</sub>-CB<sub>1</sub>-KO mice. The fact that partial blockade of GABA<sub>A</sub>  
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 D<sub>1</sub>R-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 D<sub>1</sub>R 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<sub>1/5</sub>R antagonist SCH23390 impair memory  
33 performance in the NOR task. In addition, our data suggest that D<sub>1</sub>R/CB<sub>1</sub>R-

1 positive hippocampal interneurons are one of the targets of the dopaminergic  
2 control of learning and memory processes. Interestingly, it has been shown that  
3 parvalbumin (PV)-expressing interneurons require D<sub>1</sub>R activity for late phases  
4 of memory consolidation through the coordinate control of the activity of  
5 hippocampal pyramidal neurons (Karunakaran et al., 2016). Particularly, the  
6 authors describe that this D<sub>1</sub>R activity modulates hippocampal network  
7 oscillations (i.e. sharp-wave ripples), which is a proposed correlate for synaptic  
8 plasticity and memory consolidation (Buzsaki, 2015). In addition, previous  
9 studies have shown that PV/CB<sub>1</sub>R-negative and CCK/CB<sub>1</sub>R-positive  
10 interneurons have complementary roles in ensuring such high oscillatory ripple  
11 events with consequent capacity to modulate synaptic plasticity (Klausberger et  
12 al., 2005; Buzsaki, 2015). Therefore, we speculate that the subpopulation of  
13 D<sub>1</sub>R/CB<sub>1</sub>R-positive interneurons described in our work could play a  
14 complementary role in maintaining proper excitation/inhibition balance in the  
15 hippocampal network activity required for memory consolidation.

16 While the complete elucidation of the complex microcircuitry requires  
17 further characterization, our findings support the hypothesis that D<sub>1</sub>R/CB<sub>1</sub>R-  
18 positive hippocampal interneurons belong to a broader circuit participating in  
19 the dopaminergic control of memory (Yamasaki and Takeuchi, 2017). Our data  
20 are compatible with a scenario where D<sub>1</sub>R activation during the  
21 learning/consolidation process potentiates GABAergic transmission. However,  
22 this D<sub>1</sub>R-dependent increase of inhibition is kept within adequate limits by the  
23 activation of CB<sub>1</sub>R, thereby allowing a proper flow of information. In this sense,  
24 in the absence of CB<sub>1</sub>R-dependent control of D<sub>1</sub>R/CB<sub>1</sub>R-positive interneurons  
25 (i.e. D<sub>1</sub>-CB<sub>1</sub>-KO mice), a partial inhibition of either D<sub>1</sub>-like or GABA<sub>A</sub> receptors  
26 rescues the phenotype. In other words, whereas activation of D<sub>1</sub>R in  
27 interneurons seems to be necessary for the memory process, their abnormally  
28 high activity (e.g. in the absence of CB<sub>1</sub>R) impairs such functions. In this  
29 context, an interesting question relates to the functional link between the  
30 endogenous activation of D<sub>1</sub>R and CB<sub>1</sub>R. Our results allow speculating about  
31 two potential scenarios, based on autocrine or paracrine modes of action of  
32 endocannabinoid signaling (Busquets-García et al., 2017): (i) General D<sub>1</sub>R-  
33 dependent dopaminergic signaling in the hippocampus might activate pyramidal

1 neurons (Roggenhofs et al., 2013; Shivarama-Shetty et al., 2018) targeted by  
2 D<sub>1</sub>R/CB<sub>1</sub>R-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 D<sub>1</sub>R/CB<sub>1</sub>R-positive interneurons. (ii) Following  
6 D<sub>1</sub>R 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 CB<sub>1</sub>R. In particular, D<sub>1</sub>R/CB<sub>1</sub>R-positive interneurons provide specific behavioral  
17 and hippocampal synaptic mechanisms sustaining the fine-tuned regulation of  
18 memory processes. The close interaction of CB<sub>1</sub>R and D<sub>1</sub>R 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.

29



1 **DECLARATION OF INTERESTS**

2 The authors declare no competing interests.

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**FIGURE 1. HIPPOCAMPAL CB<sub>1</sub>R IN D<sub>1</sub>R-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 D<sub>1</sub>-CB<sub>1</sub>-WT mice (n = 10) and D<sub>1</sub>-CB<sub>1</sub>-KO littermates (n = 7).

(C) Long-term NOR (24 hours) memory performance of D<sub>1</sub>-CB<sub>1</sub>-WT mice (n = 9) and D<sub>1</sub>-CB<sub>1</sub>-KO littermates (n = 8).

(D) Schematic representation of the experiment using viral re-expression of the CB<sub>1</sub>R gene in the striatum (STR) or the hippocampus (HPC) of D<sub>1</sub>-CB<sub>1</sub>-WT mice and D<sub>1</sub>-CB<sub>1</sub>-KO littermates.

(E) Representative images of Cre-expressing D<sub>1</sub>-CB<sub>1</sub>-KO mice injected with CB<sub>1</sub>R-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 CB<sub>1</sub>R gene in striatum (STR) or hippocampus (HPC). Control [n(D<sub>1</sub>-CB<sub>1</sub>-WT)=17 and n(D<sub>1</sub>-CB<sub>1</sub>-KO)=5], STR-CB<sub>1</sub>-RS [n(D<sub>1</sub>-CB<sub>1</sub>-KO)=6] or HPC-CB<sub>1</sub>-RS [n(D<sub>1</sub>-CB<sub>1</sub>-KO)=9].

(G) Immunofluorescence of cells expressing CB<sub>1</sub>R-myc in the hippocampus. Scale bar = 500 μm.

(H) Schematic representation of the experiment using viral expression of the Gi-DREADDs or mCherry in the hippocampus of D<sub>1</sub>-CB<sub>1</sub>-WT mice and D<sub>1</sub>-CB<sub>1</sub>-KO littermates. Clozapine N-oxide (CNO, 2mg/kg) injections take place after the Training phase of the NOR task.

(I) NOR memory performance of D<sub>1</sub>-CB<sub>1</sub>-WT mice intra-hippocampally injected with hM4D(Gi) virus or mCherry [n(VEH)=16 and n(CNO)=21], D<sub>1</sub>-CB<sub>1</sub>-KO mice injected with mCherry [n(VEH)=6 and n(CNO)=7] and D<sub>1</sub>-CB<sub>1</sub>-KO mice intra-hippocampally injected with hM4D(Gi) [n(VEH)=11 and 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**.

1 **FIGURE 2. LEARNING-INDUCED FACILITATION OF *IN VIVO* HIPPOCAMPAL**  
2 **LTP REQUIRES CB<sub>1</sub>R AT D<sub>1</sub>R-POSITIVE NEURONS**

3 (A and B) HFS in the dorsal hippocampal CA3 Shaffer-Collateral pathway  
4 induces an *in vivo* LTP in dorsal CA1 *stratum radiatum*. (A) Summary plots of  
5 recorded evoked fEPSP in anesthetized D<sub>1</sub>-CB<sub>1</sub>-WT (n=8) and D<sub>1</sub>-CB<sub>1</sub>-KO  
6 (n=8) mice. (B) Bar histograms of normalized fEPSP from (A) representing 30  
7 and 60 minutes after HFS.

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

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

13 (F and G) Learning-induced modulation of *in vivo* LTP is impaired in D<sub>1</sub>-CB<sub>1</sub>-KO  
14 mice. (F) Summary plots of recorded fEPSP in anesthetized D<sub>1</sub>-CB<sub>1</sub>-WT (n=10)  
15 and D<sub>1</sub>-CB<sub>1</sub>-KO (n=10) mice. (G) Bar histograms of normalized of evoked  
16 fEPSP from (F) representing 30 and 60 minutes after HFS.

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

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

21 See also **Table S1**.

22

1 **FIGURE 3. HIPPOCAMPAL CB<sub>1</sub>R/D<sub>1</sub>R-POSITIVE INTERNEURONS**  
2 **MODULATE SYNAPTIC GABAERGIC TRANSMISSION.**

3 (A) NOR memory performance of mutant mice administered with: vehicle [n(D<sub>1</sub>-  
4 CB<sub>1</sub>-WT)=14 and n(D<sub>1</sub>-CB<sub>1</sub>-KO)=14], MK-801 [0.1 mg/kg, IP; n(D<sub>1</sub>-CB<sub>1</sub>-WT)=7  
5 and n(D<sub>1</sub>-CB<sub>1</sub>-KO)=7], NBQX [5 mg/kg, IP; n(D<sub>1</sub>-CB<sub>1</sub>-WT)=8 and n(D<sub>1</sub>-CB<sub>1</sub>-  
6 KO)=5], or Bicuculline immediately after [n(D<sub>1</sub>-CB<sub>1</sub>-WT)=10 and n(D<sub>1</sub>-CB<sub>1</sub>-  
7 KO)=10] or 1 hour after the training phase [n(D<sub>1</sub>-CB<sub>1</sub>-WT)=10 and n(D<sub>1</sub>-CB<sub>1</sub>-  
8 KO)=8].

9 (B) Schematic representation of the experimental procedure to detect CB<sub>1</sub>R  
10 mRNA in D<sub>1</sub>R-positive cells.

11 (C and D) Representative images of CB<sub>1</sub>R mRNA (in green) and mCherry  
12 protein (in red) labeling in the hippocampal CA1 region of D<sub>1</sub>-Cre (C) and D<sub>1</sub>-  
13 CB<sub>1</sub>-KO (D) mice. White arrows indicate colocalization between CB<sub>1</sub>R-positive  
14 and D<sub>1</sub>R-positive cell bodies. Scale bar = 150 μm.

15 (E and F) Floating bars indicating the layer specific distribution of the % of cell  
16 bodies expressing high (E) and low amounts (F) of CB<sub>1</sub>R, which colocalize with  
17 mCherry-positive (i.e. D<sub>1</sub>R-positive) in D<sub>1</sub>-Cre (n=3) and D<sub>1</sub>-CB<sub>1</sub>-KO (n=3).

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

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

1 **FIGURE 4. CELLULAR MECHANISMS LINKING D<sub>1</sub>R-SIGNALLING WITH**  
2 **GABAERGIC ACTIVITY DURING LEARNING-INDUCED FACILITATION OF *IN***  
3 ***VIVO* LTP AND MEMORY CONSOLIDATION**

4 (A) Effects of the GABA<sub>A</sub> receptor antagonist Bicuculline, the D<sub>1/5</sub>R antagonist  
5 SCH 23390 on learning-induced modulation of *in vivo* LTP in D<sub>1</sub>-CB<sub>1</sub>-WT and  
6 D<sub>1</sub>-CB<sub>1</sub>-KO mice. Summary plots of recorded evoked fEPSP in Vehicle [n(D<sub>1</sub>-  
7 CB<sub>1</sub>-WT)=6 and n(D<sub>1</sub>-CB<sub>1</sub>-KO)=8], Bicuculline [0.5 mg/kg; IP, n(D<sub>1</sub>-CB<sub>1</sub>-WT)=9  
8 and n(D<sub>1</sub>-CB<sub>1</sub>-KO)=11] and SCH 23390[0.3 mg/kg; IP, n(D<sub>1</sub>-CB<sub>1</sub>-WT)=6 and  
9 n(D<sub>1</sub>-CB<sub>1</sub>-KO)=6].

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

12 (D) Memory performance D<sub>1</sub>-CB<sub>1</sub>-WT and D<sub>1</sub>-CB<sub>1</sub>-KO mice after being injected  
13 with vehicle [n(D<sub>1</sub>-CB<sub>1</sub>-WT)=6 and n(D<sub>1</sub>-CB<sub>1</sub>-KO)=10] or SCH 23390 [0.3 mg/kg;  
14 IP, n(D<sub>1</sub>-CB<sub>1</sub>-WT)=10 and n(D<sub>1</sub>-CB<sub>1</sub>-KO)=10]

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

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

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

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1 **STAR METHODS**

2 **RESOURCE AVAILABILITY**

3

4 **LEAD CONTACT**

5

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

9

10 **MATERIALS AVAILABILITY**

11

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

15

16 **DATA AND CODE AVAILABILITY**

17

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

20

# 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  $D_1$ - $CB_1$ -KO mice were generated as previously described (Monory et al., 2007;  
8 Terzian et al., 2011). Briefly,  $CB_1$  floxed mice (Marsicano et al., 2003) were  
9 crossed with  $D_1$ -Cre line (Lemberger et al., 2007), in which the Cre  
10 recombinase was placed under the control of the  $D_1$  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  $D_1$ - $CB_1$ -KO mice with homozygous  $CB_1$ -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  $D_1$ - $CB_1$ -KO and WT littermates were used. 8-14  
20 weeks old male C57BL/6Rj mice purchased from Janvier (France). 8-12 weeks-  
21 old  $D_1$ -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.

29

## 1 **METHOD DETAILS**

2

### 3 **DRUG PREPARATION AND ADMINISTRATION**

4 Bicuculline, MK-801, NBQX and SCH23390 were purchased from Merck  
5 (formerly Sigma-Aldrich, France) and were dissolved to their final concentration  
6 in physiological saline (NaCl 0.9%). The exogenous DREADD ligand clozapine-  
7 N-oxide (CNO, 2 mg/kg) was purchased from Tocris Bioscience (Bristol, UK)  
8 and dissolved in saline after gently mixing with a vortex. All drugs were injected  
9 intraperitoneally in a volume of 10 ml/kg. Vehicle in all the conditions was  
10 composed of physiological saline (NaCl 0.9%) injections.

### 11 **NOVEL OBJECT RECOGNITION MEMORY**

12 We used the novel object recognition (NOR) memory task in an L-maze  
13 (Busquets-Garcia et al., 2013; Busquets-Garcia et al., 2011; Hebert-Chatelain  
14 et al., 2016; Puighermanal et al., 2013; Puighermanal et al., 2009; Robin et al.,  
15 2018).

16 The task took place in a L-shaped maze made of dark grey polyvinyl chloride  
17 made by two identical perpendicular arms (35 cm and 30 cm long respectively  
18 for external and internal L walls, 4.5cm wide and 15 cm high walls) placed on a  
19 white background (Busquets-Garcia et al., 2011; Puighermanal et al., 2009).  
20 The task occurred in a room adjacent to the animal house with a light intensity  
21 fixed at 50 lux. The maze was overhung by a video camera allowing the  
22 detection and offline scoring of animal's behavior. The task consisted in 3  
23 sequential daily trials of 9 minutes each. During the habituation phase (day 1),  
24 mice were placed in the center of the maze and allowed to freely explore the  
25 arms in the absence of any objects. The training phase (day 2) consisted in  
26 placing the mice again in the corner of the maze in the presence of two identical  
27 objects positioned at the extremities of each arm and left to freely explore the  
28 maze and the objects. The testing phase occurred 24 hours later (day 3): one of  
29 the familiar objects was replaced by a novel object different in its shape, color  
30 and texture and mice were left to explore both objects. The position of the novel  
31 object and the associations of novel and familiar were randomized. All objects



1 were previously tested to avoid biased preference. Memory performance was  
2 assessed by the discrimination index (DI). The DI was calculated as the  
3 difference between the time spent exploring the novel (TN) and the familiar  
4 object (TF) divided by the total exploration time (TN+TF):  $DI = [TN - TF] / [TN + TF]$ .  
5 Memory was also evaluated by directly comparing the exploration time of novel  
6 and familiar objects, respectively. Object exploration was defined as the  
7 orientation of the nose to the object at less than 2 cm. Experienced  
8 investigators evaluating the exploration were blind of treatment and/or genotype  
9 of the animals. Pharmacological treatments were immediately administered  
10 after the training phase.

### 11 ***IN VIVO* ELECTROPHYSIOLOGY IN ANESTHETIZED MICE**

12 Experiments were performed as described in (Robin et al., 2018). Mice were  
13 anesthetized in a box containing 5% Isoflurane (Virbac, France) before being  
14 placed in a stereotaxic frame (Model 900, Kopf instruments, CA, USA) in which  
15 1.0% to 1.5% of Isoflurane was continuously supplied via an anesthetic mask  
16 during the whole duration of the experiment. The body temperature was  
17 maintained at  $\pm 36.5^{\circ}\text{C}$  using a homoeothermic system (model 50-7087-F,  
18 Harvard Apparatus, MA, USA) and the state of anesthesia was assessed by  
19 mild tail pinch. Before surgery, 100 ml of the local anesthetic lurocaine  
20 (vetoquinol, France) was injected in the scalp region. Surgical procedure started  
21 with a longitudinal incision of 1.5 cm in length aimed to expose Bregma and  
22 Lambda. After ensuring the correct alignment of the head, two holes were  
23 drilled in the skull for electrode placement. Glass recording electrodes were  
24 inserted in the CA1 stratum radiatum, and a concentric stimulating bipolar  
25 electrode (Model CBARC50, FHC, ME, USA) placed in the CA3 region.  
26 Coordinates were as follows: CA1 stratum radiatum: A/P 1.5, M/L 1.0, DV 1.20;  
27 CA3: A/P 2.2, M/L 2.8, D/V 1.3 (20 insertion angle). The recording electrode (tip  
28 diameter = 1–2 mm, 2-4 M $\Omega$ ) was filled with a 2% pontamine sky blue solution  
29 in 0.5M sodium acetate. At first the recording electrode was placed by hand  
30 until it reached the surface of the brain and then to the final depth using a  
31 hydraulic micropositioner (Model 2650, KOPF instruments, CA, USA). The  
32 stimulation electrode was placed in the correct area using a standard  
33 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  $\mu$ 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, D<sub>1</sub>-CB<sub>1</sub>-KO and D<sub>1</sub>-CB<sub>1</sub>-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, D<sub>1</sub>-CB<sub>1</sub>-WT and D<sub>1</sub>-CB<sub>1</sub>-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. CB<sub>1</sub> *floxed* mice were injected with rAAV-CAG-  
15 DIO (empty control vector), AAV-CAG-DIO-CB<sub>1</sub> or AAV-CAG-DIO-CB<sub>1</sub>-myc to  
16 induce re-expression of the CB<sub>1</sub> receptor gene in hippocampal or striatal D<sub>1</sub>-  
17 positive cells. To generate the aforementioned rAAVs, mouse CB<sub>1</sub> 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 CB<sub>1</sub> receptors (Atasoy et al., 2008). In another experiment, and  
22 using the same procedure as described as above, D<sub>1</sub>-CB<sub>1</sub>-WT and D<sub>1</sub>-CB<sub>1</sub>-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, D<sub>1</sub>-  
26 Cre and D<sub>1</sub>-CB<sub>1</sub>-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.

32

1 **IMMUNOHISTOCHEMISTRY ON FREE-FLOATING SECTIONS.**

2 Mice were anesthetized with pentobarbital (Exagon, Axience SAS, 400 mg/kg  
3 body weight), transcardially perfused with phosphate-buffered solution (PBS  
4 0.1M, pH 7.4) before being fixed with 4% formaldehyde (Sigma-Aldrich). The  
5 The brains were extracted and incubated overnight at 4°C in the same fixative,  
6 then embedded with sucrose 30% for 3 days and finally frozen in 2-  
7 methylbutane (Sigma-Aldrich) at -80°C. Free-floating frozen coronal sections  
8 (40 µm) were cut out with a cryostat (Microm HM 500M Microm Microtech),  
9 collected collected in an antifreeze solution and conserved at -20°C. Sections  
10 were permeabilized in a blocking solution (in PBS: 10% donkey serum, 0.3%  
11 Triton X-100) for 1 hour at room temperature (RT). Then, sections were  
12 incubated with a rabbit primary antibody against the C-myc epitope tag (1:1000,  
13 BioLegend) overnight at 4°C. After several washes with PBS, slices were  
14 incubated for 2 hours with a secondary antibody goat anti-rabbit Alexa Fluor  
15 488 (1:500, Fisher Scientific) and then washed in PBS at RT. Finally, sections  
16 were incubated with 4',6-diamidino-2-phenylindole (DAPI 1:20000, Fisher  
17 Scientific) diluted in PBS for 5 minutes to visualize cell nuclei and then were  
18 washed, mounted and coverslipped. All the antibodies were diluted in blocking  
19 solution. The sections were imagedimaged with a slides scanner Hamamatsu  
20 Nanozoomer 2.0 HT.

21 **COMBINED FLUORESCENT *IN SITU* HYBRIDIZATION (FISH)/**  
22 **IMMUNOHISTOCHEMISTRY (IHC) ON FREE-FLOATING FROZEN SECTIONS**

23 Mice were anesthetized with pentobarbital (Exagon, Axience SAS, 400 mg/kg  
24 body weight), transcardially perfused with PBS (0.1M, pH 7.4) before being  
25 fixed with 4% formaldehyde (Sigma-Aldrich). The brains were extracted and  
26 incubated overnight at 4°C in the same fixative, then embedded with sucrose  
27 30% for 3 days and finally frozen in 2-methylbutane (Sigma-Aldrich) at -80°C.  
28 Free-floating frozen coronal sections were cut out with a cryostat (30 µm,  
29 Microm HM 500M Microm Microtech) and collected in an antifreeze solution and  
30 conserved at -20°C.

31 Section were washed several times with PBS with diethyl pyrocarbonate (PBS-  
32 DEPC) to wash out the antifreeze solution. The endogenous peroxidases were

1 inactivated by incubating the free-floating sections with 3% H<sub>2</sub>O<sub>2</sub> 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 CB<sub>1</sub>  
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 H<sub>2</sub>O<sub>2</sub> 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 CB<sub>1</sub> 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).

29

30

31

# 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. CB<sub>1</sub> 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-CB<sub>1</sub>” cells were considered to be round-  
14 shaped and intense staining covering the entire nucleus whereas “Low-CB<sub>1</sub>”  
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 ± 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.

22

23

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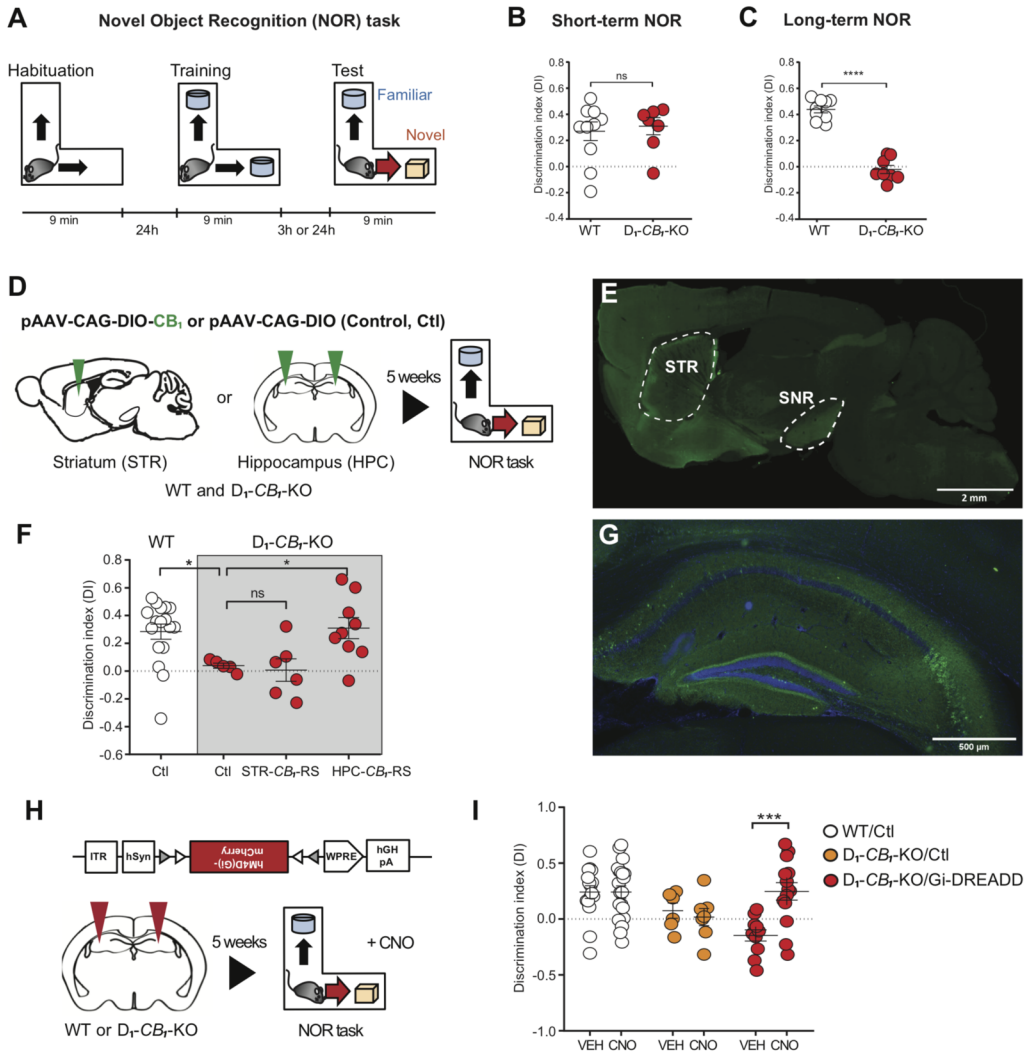
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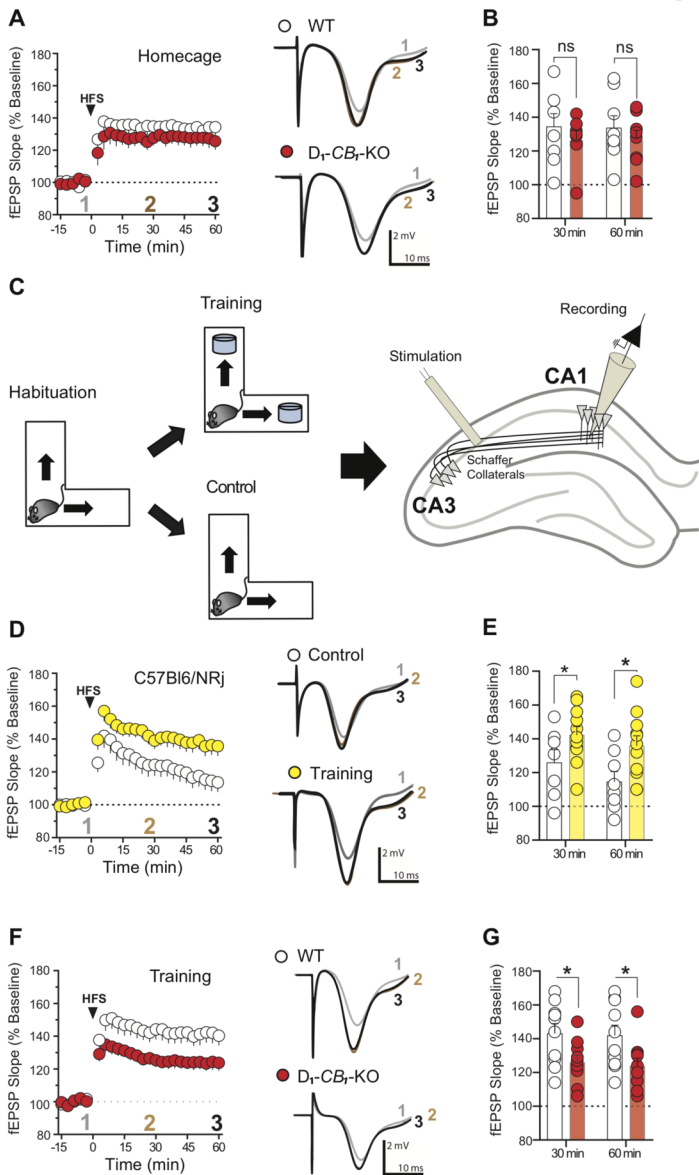
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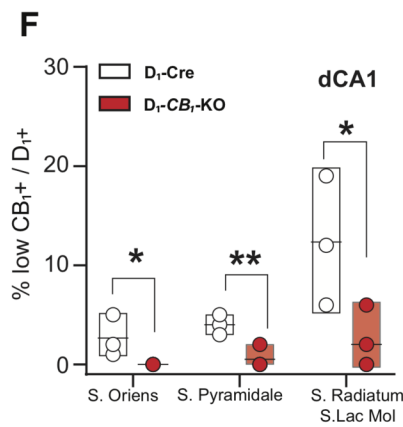
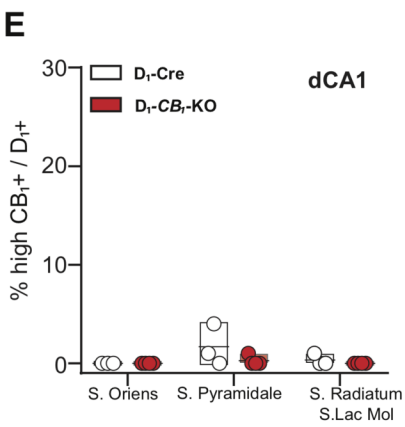
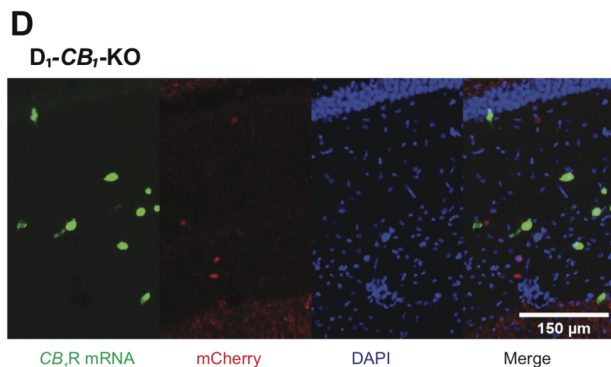
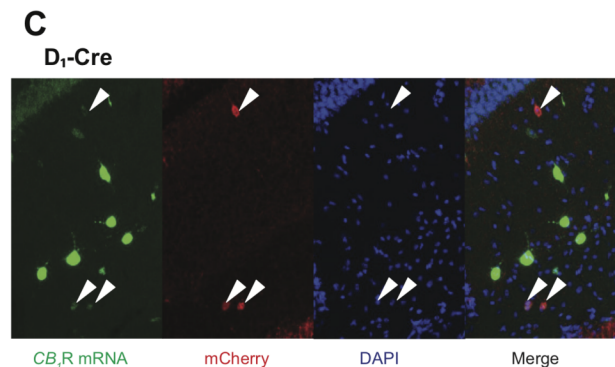
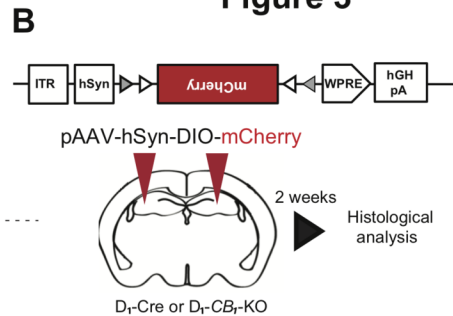
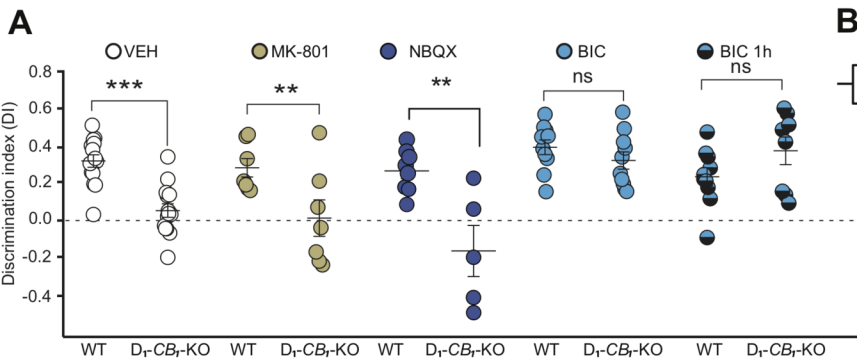
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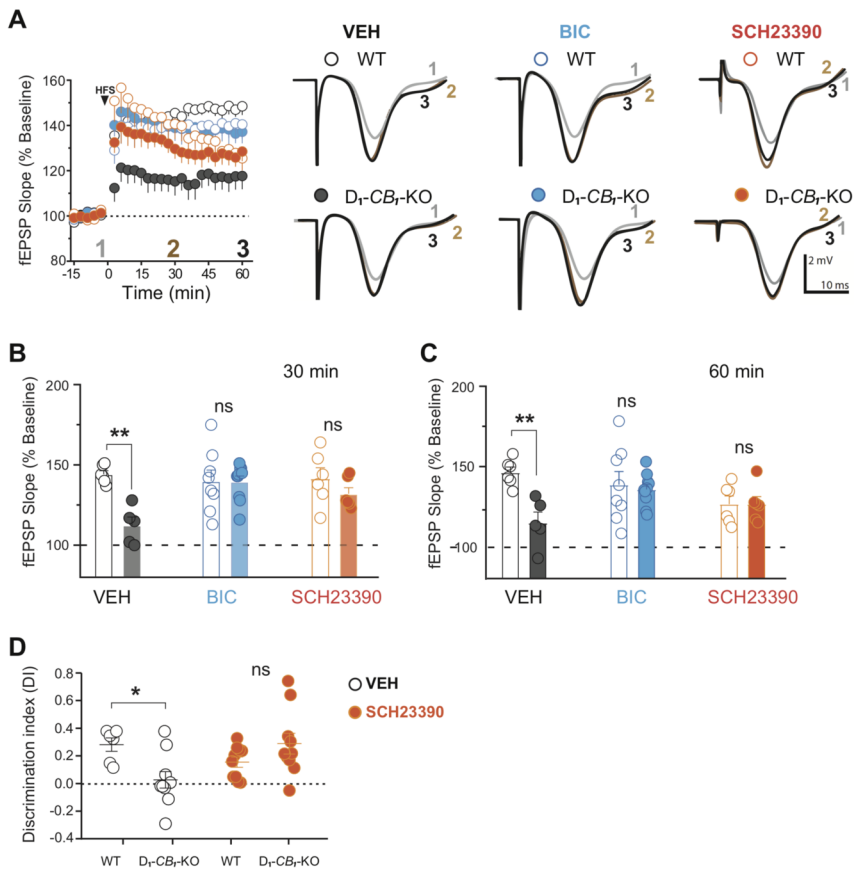
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**Figure 2**









### Hippocampal Circuits

