



HAL
open science

CB1 Receptors in the anterior piriform cortex control odor preference memory

Geoffrey Terral, Arnau Busquets-Garcia, Marjorie Varilh, Svein Achicallende, Astrid Cannich, Luigi Bellocchio, Itziar Bonilla-del Río, Federico Massa, Nagore Puente, Edgar Soria-Gomez, et al.

► **To cite this version:**

Geoffrey Terral, Arnau Busquets-Garcia, Marjorie Varilh, Svein Achicallende, Astrid Cannich, et al.. CB1 Receptors in the anterior piriform cortex control odor preference memory. *Current Biology - CB*, 2019, 29 (15), pp.2455-2464. 10.1016/j.cub.2019.06.041 . hal-02618265

HAL Id: hal-02618265

<https://hal.inrae.fr/hal-02618265>

Submitted on 20 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 **CB1 receptors in the anterior piriform cortex** 2 **control odor preference memory**

3 Geoffrey Terral^{1,2}, Arnau Busquets-Garcia^{1,2}, Marjorie Varilh^{1,2}, Svein Achicallende^{3,4},
4 Astrid Cannich^{1,2}, Luigi Bellocchio^{1,2}, Itziar Bonilla-Del Río^{3,4}, Federico Massa^{1,2},
5 Nagore Puente^{3,4}, Edgar Soria-Gomez^{1,2,3,4,5}, Pedro Grandes^{3,4}, Guillaume
6 Ferreira^{2,6,*} & Giovanni Marsicano^{1,2,*}

7 ¹INSERM, U1215 NeuroCentre Magendie, 146 rue Léo Saignat, 33077 Bordeaux
8 cedex, France.

9 ²University of Bordeaux, 146 rue Léo Saignat, 33000 Bordeaux, France.

10 ³Department of Neurosciences, University of the Basque Country UPV/EHU, Barrio
11 sarriena s/n, 48940 Leioa, Spain

12 ⁴Achucarro Basque Center for Neuroscience, Science Park of the UPV/EHU, 48940
13 Leioa, Spain.

14 ⁵IKERBASQUE, Basque Foundation for Science, Maria Diaz de Haro 3, 48013
15 Bilbao, Spain.

16 ⁶INRA, Nutrition and Integrative Neurobiology, UMR 1286, 146 rue Léo Saignat,
17 33076 Bordeaux cedex, France.

18 **Corresponding author:**

19 Giovanni Marsicano, giovanni.marsicano@inserm.fr (Lead Contact)

20 Guillaume Ferreira, guillaume.ferreira@inra.fr

21 *These authors share senior authorship

1 **SUMMARY**

2 The retrieval of odor-related memories shapes animal behavior. The anterior piriform
3 cortex (aPC) is the largest part of the olfactory cortex and it plays important roles in
4 olfactory processing and memory. However, it is still unclear whether specific cellular
5 mechanisms in the aPC control olfactory memory depending on the appetitive or
6 aversive nature of the stimuli involved. Cannabinoid-type 1 (CB1) receptors are
7 present in the aPC (aPC-CB1), but their potential impact on olfactory memory was
8 never explored. Here, we used a combination of behavioral, genetic, anatomical and
9 electrophysiological approaches to characterize the functions of aPC-CB1 receptors
10 in the regulation of appetitive and aversive olfactory memory. Pharmacological
11 blockade or genetic deletion of aPC-CB1 receptors specifically impaired the retrieval
12 of conditioned odor preference (COP). Interestingly, expression of conditioned odor
13 aversion (COA) was unaffected by local CB1 receptor blockade, indicating that the
14 role of aPC endocannabinoid signaling is selective for retrieval of appetitive memory.
15 Anatomical investigations revealed that CB1 receptors are highly expressed on aPC
16 GABAergic interneurons, and *ex vivo* electrophysiological recordings showed that
17 their pharmacological activation reduces miniature inhibitory currents (mIPSCs) onto
18 aPC semilunar (SL), but not pyramidal principal neurons. COP retrieval, but not COA,
19 was associated with a specific CB1 receptor-dependent decrease of mIPSCs in SL
20 cells. Altogether, these data indicate that aPC-CB1 receptor-dependent mechanisms
21 physiologically control the retrieval of olfactory memory depending on odor valence
22 and engaging modulation of local inhibitory transmission.

23 Keywords: anterior piriform cortex; CB1 receptors; conditioned odor preference;
24 conditioned odor aversion; neuroanatomy; miniature inhibitory currents (mIPSCs);
25 semilunar neurons; pyramidal neurons.

1 INTRODUCTION

2 Chemosensory information is crucial for the survival of humans and other
3 animals. For example, a large part of animal behavior including control of emotional
4 states, food intake and social interactions relies on the capacity to perceive odor
5 information and to retrieve its potential meaning based on previous experiences [1,2].
6 Olfactory perception starts when odorant molecules, travelling through orthonasal or
7 retronasal pathways, reach olfactory receptors on sensory neurons located in the
8 olfactory epithelium [3]. These neurons project to the olfactory bulb that in turn
9 transmits the signal to other brain regions, including the anterior piriform cortex (aPC)
10 [4,5], which plays a key role in olfactory processing and memory [6–8]. However, the
11 specific cellular mechanisms governing odor information storage and retrieval in the
12 aPC are still unclear.

13 Cannabinoid type-1 (CB1) receptors together with their endogenous ligands
14 (endocannabinoids) form the core of the so-called endocannabinoid system (ECS) in
15 the brain [9], which is an important modulator of many functions, including learning
16 and memory [10,11]. Activation of presynaptic CB1 receptors is well-known to
17 physiologically control the release of several neurotransmitters in many brain regions
18 [12,13]. CB1 receptors are present in different olfactory structures [14], where they
19 can modulate olfactory processes [15–19]. However, little is known about the specific
20 impact of CB1 receptor signaling in olfactory brain structures on odor-dependent
21 memory functions.

22 Considering that the aPC is an important region for olfactory memory [6–8,20–
23 22], we hypothesized that CB1 receptors in the aPC (aPC-CB1) could modulate odor-
24 related memory processes. Our data show that aPC-CB1 receptors are specifically

1 required for the expression of appetitive, but not aversive, olfactory memory, and they
2 are involved in the direct control of the associated modulation of local inhibitory
3 circuits. Altogether, these results indicate that the physiological activation of CB1
4 receptors in the aPC exerts a fine-tuned regulation of olfactory circuits and
5 functionally discriminates the retrieval of positively- and negatively-motivated
6 olfactory memories.

7 **RESULTS**

8 **CB1 receptors in the aPC are necessary for the retrieval of conditioned odor** 9 **preference**

10 To investigate the potential impact of CB1 receptor signaling in odor-related memory,
11 we set up a behavioral protocol to assess conditioned odor preference (COP) in mice
12 (see STAR Methods, Figures 1A and S1A,B) [23,24]. Using this protocol, mice
13 displayed a reliable preference for the odor-scented solution previously associated to
14 sucrose (C+) as compared to the other one (C-), revealing the formation of COP
15 (Figure S1C), regardless of the odor used as C+ (Figures S1D,E).

16 We next investigated the role of CB1 receptors in the aPC (aPC-CB1) during COP.
17 Specific deletion of the *CB1* gene in the aPC (aPC-*CB1*-KO, see STAR Methods,
18 Figures 1B,C and S1F-I) [25–27] abolished the preference for the C+ solution during
19 test (Figure 1D), without altering sucrose preference upon training (Figure S1J), nor
20 total liquid intake (Figure S1K), and independently from the anatomical extension of
21 the CB1 deletion (Figure S1L). These results indicate that COP requires aPC-CB1
22 receptors. To determine the specific role of CB1 receptor signaling in the different
23 phases of the COP protocol, we acutely injected the CB1 receptor antagonist AM251
24 into the aPC (4 μ g/0.5 μ l per side; Figures S2A-E) prior to each odor-sucrose pairing

1 or before the retrieval test (Figure 1A). Neither consumption during training, nor COP
2 performance were affected by aPC-CB1 blockade before each pairing (AM pairings;
3 Figures 1D, S1J,K and S2F). Conversely, AM251 acutely injected into the aPC prior
4 to the retrieval test abolished COP, without altering total liquid consumption (AM test;
5 Figures 1D, S1J,K and S2F). Acute blockade of aPC-CB1 receptors might
6 permanently impair COP retrieval. On the other hand, longer training might render
7 COP retrieval independent of aPC-CB1 receptors. To simultaneously test for these
8 possibilities, animals previously treated with AM251 or vehicle received 4 additional
9 odor-sucrose pairings (see STAR Methods) and were injected with vehicle ["Veh(AM)
10 test"] or AM251 ["AM(Veh) test"] before the second retrieval test, respectively. In
11 these conditions, vehicle-treated mice displayed clear COP, whereas AM251 blocked
12 this behavior (Figures 1E and S2G). aPC-CB1 receptors might control expression of
13 preference independently of previous learning. However, aPC-CB1 blockade did not
14 impair innate sucrose preference (Figures S3A-C).

15 Altogether, these results indicate that endogenous activation of aPC-CB1 is
16 specifically required during retrieval of COP, without affecting innate responses to
17 attractive stimuli.

18 **CB1 receptors in the aPC are not involved in the retrieval of conditioned odor** 19 **aversion**

20 We next asked whether aPC-CB1 receptors are also involved in the retrieval of
21 conditioned odor aversion (COA) induced by lithium chloride (LiCl) injections (Figure
22 2A, COA-lithium, COA-L) [25,28–30]. Notably, AM251 acutely injected into the aPC
23 did not impair COA-L expression (Figures 2A,B, S2A-F and S3D), suggesting that
24 aPC-CB1 receptors are dispensable for the retrieval of negatively motivated olfactory
25 memory. However, the differential effects of CB1 receptor blockade between COP

1 and COA-L could be due to the different types of associations involved (sensory-
2 sensory *versus* sensory-gastric). Using a sensory-sensory COA protocol, where
3 sucrose was substituted by the aversive taste quinine (COA-Q; Figures 2C and S3E)
4 [31,32], mice treated with vehicle or the CB1 receptor antagonist before the retrieval
5 test displayed the same avoidance towards the conditioned odor (Figures 2D, S2F
6 and S3F).

7 Altogether, these data indicate that aPC-CB1 receptor signaling is necessary for the
8 retrieval of COP but not of COA, suggesting that expression of acquired odor choices
9 rely on different mechanisms depending on the valence of the unconditioned
10 stimulus.

11 **CB1 receptors are highly expressed in GABAergic interneurons in the aPC**

12 As previously reported [14,33], fluorescent immunohistochemistry revealed that CB1
13 receptor protein is highly expressed in layer II of the aPC (Figure 3A), where the aPC
14 principal neurons are mainly localized [34,35]. To detail the cellular distribution of
15 CB1 receptors, we analyzed aPC tissues from conditional mutant mice carrying
16 exclusive expression of the protein in identified specific cell types (Rescue mice)
17 [33,36,37]. A similar pattern of CB1 receptor immunoreactivity was observed across
18 aPC tissues from WT mice and global CB1-Rescue mice (Figure 3A) [33,37], but also
19 from mice with specific re-expression in GABAergic neurons (GABA-CB1 Rescue;
20 Figure 3A) [33,36]. In contrast, the immunoreactivity was extremely low in mice re-
21 expressing the receptor only in cortical glutamatergic neurons (Glu-CB1 Rescue;
22 Figure 3A) [37] and, as expected, was undetectable in mice where CB1 receptor
23 expression is absent (CB1 Stop; Figure 3A) [33,36,37]. To better characterize the
24 expression of CB1 receptors at the synaptic level, we next used immunogold electron
25 microscopy (Figure 3B). As expected, CB1 receptor immunogold particles were

1 specifically present in different amounts at many cellular locations (Figures 3B and
2 S4). Amongst CB1 receptor particles located at terminals (1856 over 3409 total
3 counted in WT; Figures 3B and S4), approximately 88% and 12% were at symmetric
4 (presumably inhibitory) and asymmetric synapses (presumably excitatory),
5 respectively (Figures 3B,C). Moreover, whereas only 23% of excitatory terminals
6 were labeled with CB1 receptor immunogold particles, this percentage was as high
7 as 87% in inhibitory ones (Figures 3B,D). As expected, only background staining was
8 detected in sections from *CB1*-KO mice (18 particles at terminals; Figures 3B,D and
9 S4).

10 To identify the specific topographical distribution of CB1-expressing cells within the
11 aPC, we used double Fluorescent *In Situ* Hybridization (D-FISH) to label the mRNAs
12 of CB1 receptor and of glutamic acid decarboxylase 65KDa (GAD), a marker of
13 GABAergic neurons (Figure 4A). Consistent with previous studies [38], the majority of
14 GAD-expressing cells were observed in deep layer III (Figures 4A,B). CB1 mRNA
15 was also highly expressed in layer III, with scattered positive cells in layer I (Figures
16 4A,C). Accordingly, counting of positive cells revealed that a high proportion of GAD+
17 neurons contained also CB1 mRNA (63%; Figures 4A,D), following the distribution of
18 GAD across layers (Figures 4A,B,E). Similarly to other cortical regions such as the
19 hippocampus [39], CB1 mRNA was expressed at very different levels across CB1-
20 positive aPC cells. Whereas a majority of cells expressed low-to-moderate amounts
21 of the transcript, scattered cells contained very high levels of CB1 mRNA, especially
22 in layers II and III (Figure 4A). Interestingly, virtually 100% of these high CB1-
23 expressing neurons co-expressed GAD mRNA (Figure 4F). Conversely, low CB1-
24 expressing neurons were virtually all identified as GAD-positive in layer I, but this
25 proportion was reduced in layers II and III (Figure 4F).

1 Altogether, these data indicate that the large majority of aPC-CB1 receptor protein is
2 expressed in terminals of local GABAergic neurons, with layer-specific topographical
3 distribution.

4 **CB1 receptors control inhibitory transmission in the aPC**

5 To start addressing the so-far unexplored cannabinoid-dependent control of inhibitory
6 transmission in the aPC, we first determined by *ex vivo* patch-clamp
7 electrophysiological recordings the impact of CB1 receptor activation on GABAergic
8 neurotransmission impinging onto specific populations of principal cells of the aPC
9 (semilunar-like neurons, SL, and pyramidal-like neurons, PNs; Figures S5A-B and
10 see STAR Methods). These cells receive many inputs from the olfactory bulb and
11 other brain regions, project to other olfactory cortical areas [35,40,41] and are
12 extensively innervated by local inhibitory interneurons [38,42,43]. Thus, we reasoned
13 that selective modulation of GABAergic inputs onto SL cells and/or PNs might
14 represent a way through which CB1 receptors rapidly regulate principal cell activity
15 and consequently odor processing. To address this possibility, we recorded miniature
16 inhibitory post-synaptic currents (mIPSCs) representing the global inhibitory inputs of
17 principal cells. These events occur at a frequency of 2.06 ± 0.23 Hz and an amplitude
18 of 76.29 ± 4.41 pA (Vehicle; Figures 5A-C) in SL cells, and at a frequency of $2.50 \pm$
19 0.39 Hz and an amplitude of 64.06 ± 4.27 pA in PNs of naïve animals (Vehicle;
20 Figures 5D-F). Similarly to what observed in the hippocampus [44], the application of
21 the CB1 receptor agonist WIN 55,212-2 (WIN, 5 μ M) significantly reduced the
22 frequency of mIPSCs in SL cells, with only a slight impact, if any, on their amplitude
23 (Figures 5A-C), suggesting a presynaptic inhibitory effect. This decrease was fully
24 reversed by the application of the CB1 receptor antagonist AM251 (4 μ M;
25 WIN+AM251; Figures 5A-C). Conversely and surprisingly, no such effects were

1 observed in PNs (Figures 5D-F), indicating a cell-type specific impact of CB1
2 receptor-dependent control of inhibitory currents in naïve animals.

3 In summary, CB1 receptors are highly expressed in GABAergic interneurons of the
4 aPC, and their activation results in the modulation of inhibitory inputs onto SL
5 principal neurons.

6 **COP retrieval reduces mIPSCs in the aPC *via* presynaptic CB1 receptors**

7 We next hypothesized that retrieval of COP might be associated with aPC-CB1
8 receptor-dependent modulation of principal cells' mIPSCs. A significant reduction of
9 mIPSCs frequency (~26%) was found in SL cells from mice sacrificed during COP
10 retrieval, as compared to a control group exposed to the same number of only water-
11 drinking sessions (Water; Figures 6A,B). SL cells from mice receiving the same
12 number of "training" sessions, but without the presence of sucrose (Sucrose free) or
13 odor (Odor free) did not display any reduction of mIPSCs frequency (Figure 6B).
14 Considering that aPC-CB1 receptors regulate COP but not COA retrieval (Figure 1
15 and Figure 2), we next evaluated the effect of COA-Q retrieval and we found that this
16 condition did not affect mIPSCs frequency (Figure 6B). Notably, no differences in
17 amplitudes were observed across the groups (Figure S6A). Altogether, these results
18 indicate that COP retrieval, but not exposure to odor or sucrose alone or COA
19 retrieval, is associated with a specific reduction of presynaptic inhibitory transmission
20 onto SL neurons in the aPC.

21 As local blockade, the systemic injection of the CB1 receptor antagonist Rimonabant
22 (Rim, 1 mg/kg) impaired COP retrieval (Figures 6C and S6B), independently of total
23 liquid consumption (Figures S6C-F). As expected, systemic injection of vehicle before
24 COP retrieval did not alter the associated reduction of mIPSCs frequency in SL cells

1 (Figures 6D,E; $p>0.8$, as compared to Figure 6B). Conversely, systemic
2 administration of Rim abolished this decrease up to levels undistinguishable from
3 control mice (Figures 6D,E; $p>0.8$, as compared to Figure 6B), with no effect on
4 amplitude (Figure S6G). Notably, mIPSC frequencies, but not amplitudes, of
5 individual animals were inversely correlated with the COP retrieval performance
6 (Figure 6F and data not shown), suggesting that the level of presynaptic inhibition of
7 SL cells is linked to the behavioral retrieval of COP. Next, we examined the impact of
8 COP retrieval on mIPSCs of PNs.

9 Similarly to SL neurons, the frequency of mIPSCs was reduced (~33%) in PNs of
10 animals undergoing COP retrieval, as compared to water control mice (Figures 6G-H)
11 with no change in amplitude (Figure S6H). A slight non-significant increase of
12 mIPSCs amplitude was observed in PNs from mice receiving systemic injection of
13 Rim prior COP retrieval (Figure S6H). However, this treatment was not able to
14 reverse the COP retrieval-associated reduction of mIPSCs frequency in PNs (Figures
15 6G-H). No correlations between COP retrieval and the levels of mIPSC frequencies
16 or amplitudes of PNs in individual mice were observed (Figure 6I and data not
17 shown).

18 Altogether, these results indicate that COP retrieval is associated to a reduction of
19 inhibitory inputs on both SL cells and PNs. However, presynaptic CB1 receptors
20 appear to regulate inhibitory transmission in the aPC in a cell type-specific manner,
21 thereby providing an unforeseen fine-tuned modulation of olfactory memory circuits,
22 likely contributing to appropriate behavioral responses.

23

24

1 **Acute blockade of CB1 receptors affects COP retrieval through GABAergic**
2 **neurons**

3 We then addressed the potential involvement of CB1 receptors expressed in
4 inhibitory neurons (GABAergic CB1) in COP retrieval. Surprisingly, mice lacking *CB1*
5 gene expression from GABAergic neurons (GABA-*CB1*-KO) [27,45] did not display
6 any alteration of COP retrieval as compared to control wild-type littermates (GABA-
7 *CB1*-WT; Figure S6I). This negative result might suggest that CB1 receptors in
8 GABAergic neurons are not necessary for acute COP retrieval. However, GABA-
9 *CB1*-KO mice carry a deletion of CB1 receptors in all GABAergic cells of the whole
10 forebrain, starting from early developmental stages [27,46]. Such diffuse and long-
11 lasting absence of CB1 receptor signaling might induce opposing effects in different
12 brain regions and/or stimulate developmental compensatory phenomena, whose
13 general mechanisms recently started to be elucidated [47,48]. If occurring in GABA-
14 *CB1*-KO mice, these mechanisms might mask the specific acute role of CB1
15 receptors signaling in GABAergic neurons in COP retrieval during adulthood.
16 Considering this possibility, we adopted an alternative strategy to investigate the
17 specific role of CB1 receptors in GABAergic neurons, by testing whether the
18 blockade of COP retrieval by the acute administration of Rim was still effective in
19 GABA-*CB1*-KO mice. A systemic acute injection of the drug blocked COP retrieval in
20 GABA-*CB1*-WT, but it failed to impair this behavior in GABA-*CB1*-KO mice (Figures
21 6J,K and S6J-L), indicating that CB1 receptors in GABAergic neurons are required
22 for the COP retrieval-disrupting effect of the acute pharmacological blockade of CB1
23 receptor signaling.

1 **DISCUSSION**

2 In this study, we functionally characterized the presence and the role of CB1
3 receptors in the aPC. We found that these receptors are specifically involved in the
4 retrieval of appetitive, but not aversive, olfactory memory and in the associated
5 modulation of inhibitory transmission onto specific aPC principal cells. Moreover, our
6 data show that the retrieval impairment of appetitive olfactory memory induced by
7 CB1 antagonism requires CB1 receptors expressed in inhibitory neurons.

8 **aPC-CB1 receptors are necessary for the retrieval of appetitive but not aversive** 9 **olfactory memory**

10 In other brain structures, CB1 receptors have been reported to play crucial roles in
11 different phases of learning and memory processes [10,11]. Our data reveal that the
12 endogenous activation of aPC-CB1 receptors is necessary for COP retrieval, but it is
13 dispensable for its acquisition, thereby enlarging the spectrum of CB1 receptor
14 involvement in different phases of learning and memory. Interestingly, it was recently
15 found that a small enhancement of hippocampal GABAergic inhibition blocked cell
16 firing and memory retrieval but left memory encoding intact [49]. This nicely parallels
17 our results showing that a small, but CB1 receptor- and cell type-dependent reduction
18 in GABAergic inhibition in the aPC, is associated with effective COP memory
19 retrieval. We here propose that CB1 receptors on GABAergic axon terminals might
20 be essential to reduce inhibition onto SL cells, thereby permitting cell firing in the
21 ensemble population to retrieve the COP memory trace. In this context, it is
22 interesting to note that COP retrieval is also associated with a similar reduction of
23 inhibitory drive onto PNs, but in a CB1 receptor-independent manner. This suggests

1 that cell type-specific mechanisms are involved in the coordinated regulation of the
2 activity of distinct aPC principal neurons during retrieval of olfactory memory.

3 Our data show that the retrieval of aversive olfactory memory is independent of aPC-
4 CB1 receptor signaling. These intriguing results might be explained by two
5 possibilities: either COA depends on aPC but it does not involve CB1 receptor
6 signaling, or COA does not depend on aPC. There is currently no clear answer to this
7 question, but some pieces of evidence seem to indicate a certain level of
8 specialization of the aPC for positively motivated olfactory memory. Optogenetic or
9 chemogenetic manipulations of selected neurons in the PC can modulate both
10 aversive and appetitive behavioral responses [50,51]. Despite the fact that specific
11 aPC activity was observed during COA retrieval [52], olfactory cues associated with
12 sucrose activate more aPC neurons than odors associated with quinine [53] and aPC
13 lesions impair appetitive but not aversive odor-related memory [21]. In addition, CB1
14 receptor signaling can mediate aversive olfactory memory in other brain regions. For
15 instance, Laviolette and Grace (2006) showed that CB1 receptors in the medial
16 prefrontal cortex are required for odor-dependent fear conditioning [54], and we
17 recently demonstrated that deletion of the *CB1* gene specifically in medial habenular
18 neurons selectively abolishes COA, but not COP [30]. Moreover, it has been shown
19 that the basolateral nucleus of the amygdala (BLA), which is essential for COA
20 [31,55], is more strongly connected with the posterior PC (pPC) than with the aPC
21 [56], and BLA-pPC interactions are important for aversive odor conditioning [57].
22 Conversely, the aPC is more densely connected than the pPC to the olfactory
23 tubercle, which, by receiving intense dopaminergic inputs, might be specifically
24 involved in the processing of reward-related information [58]. Altogether, this
25 suggests a potential double dissociation in the roles of aPC and pPC in COP and

1 COA, with the aPC being somehow specialized in processing positive acquired
2 values of odors and the pPC more involved in aversive odor memory.

3 **CB1 receptors are highly expressed in aPC GABAergic interneurons and**
4 **regulate local inhibitory neurotransmission**

5 Our immunohistochemical, D-FISH and electron microscopy data show that CB1
6 receptors are found in a high proportion of GABAergic neurons located in the three
7 layers of the aPC and that they are strongly expressed at inhibitory synaptic
8 terminals. Moreover, pharmacological activation of aPC-CB1 receptors decreases
9 miniature inhibitory currents frequency specifically in SL cells. Similarly to the
10 hippocampus [39], cells expressing high levels of CB1 mRNA are exclusively
11 GABAergic interneurons. Recent evidence points to the presence of long-range
12 inhibitory neurons as a novel neuroanatomical and functional feature in cortical areas
13 [59]. These putative long-range inputs to the aPC might contain CB1 receptors, but
14 our viral manipulations exclude their participation in the CB1 receptor-dependent
15 retrieval of COP. Interestingly, depending on the layer, a portion of low CB1-
16 expressing cells do not co-express GAD mRNA and are presumably glutamatergic
17 neurons. Indeed, CB1 receptor protein is abundantly present in the main olfactory
18 bulb at terminals of glutamatergic centrifugal fibers coming from principal neurons of
19 the anterior olfactory nucleus and aPC [18]. Importantly, as these "glutamatergic"
20 CB1 receptors play a key role in the control of olfactory perception and food intake
21 [18], we cannot fully exclude that alterations in olfactory perception might participate
22 in the phenotype of aPC-*CB1*-KO mice. However, intra-aPC pharmacological
23 manipulations indicate that local CB1 receptor signaling is necessary for COP
24 retrieval, but it is dispensable for expression of COA. Therefore, any putative
25 impairment of olfactory perception induced by deletion of the *CB1* gene in projecting

1 glutamatergic neurons of the aPC is unlikely to be responsible for the phenotype of
2 aPC-*CB1*-KO mice. Nevertheless, our electron microscopy immunogold results
3 indicate that a small proportion of CB1 receptors are specifically present at
4 glutamatergic terminals within the aPC. Moreover, a consistent portion of CB1
5 receptors appears to be located outside of terminals. Future studies will investigate
6 the origin and the potential roles of these aPC subpopulations of CB1 receptors,
7 which might still have functional significance.

8 **COP retrieval is associated with CB1 receptor-dependent modulation of**
9 **inhibitory transmission on specific aPC principal cells**

10 An approximate 30% decrease of mIPSCs frequency recorded in both aPC SL cells
11 and PNs was observed in slices from mice undergoing COP retrieval. In PNs, CB1
12 receptor antagonism did not affect COP-dependent frequency decrease. Conversely,
13 the same treatment fully reversed mIPSCs frequency in SL cells up to the same
14 levels of mice exposed to water alone, odor alone, sucrose alone or COA. Together
15 with the fact that mIPSCs amplitude was not affected and that frequency values were
16 inversely correlated with behavioral performance, these results indicate that COP
17 retrieval is likely associated with presynaptic reduction of inhibitory transmission onto
18 aPC SL cells. This idea is reinforced by the fact that the COP retrieval impairment
19 under pharmacological CB1 receptor blockade is absent in mice lacking CB1
20 receptors from forebrain GABAergic neurons.

21 Hence, these results suggest that aPC-CB1 receptors control the behavioral
22 responses induced by appetitive olfactory memory by regulating cell type-specific
23 inhibitory transmission. More generally, they imply a dissociation between the roles of
24 SL cells and PNs in the processing of olfactory information that will be very
25 interesting to study in deeper details.

1 Interestingly, inhibitory circuits within the aPC have been shown to be strongly
2 recruited in olfactory-dependent processes [43,60–62]. For instance, *in vivo* odor
3 exposure widely activates GABAergic interneurons in the aPC [43], potentially
4 participating in the processing of odors and their meaning [63]. In this context, the
5 spatially restricted functions of CB1 receptors (i.e. on SL cells but not on PNs)
6 suggest that CB1 receptor-dependent processes selectively tune the excitability of
7 specific aPC principal neurons during COP retrieval. These processes would, in turn,
8 refine the response to positively conditioned odor stimulations, eventually allowing
9 the precise “funneling” of behavior towards preference responses.

10 In conclusion, this study provides a first characterization of the functional role of CB1
11 receptor signaling in aPC circuitry and related behaviors, thereby contributing to a
12 better understanding of how the aPC participates in specific memory functions.

13 **ACKNOWLEDGMENTS**

14 We thank all the personal of the Animal Facility of the NeuroCentre Magendie for
15 mouse care and genotyping. We also thank Gabriel Lepousez, Antoine Nissant,
16 Pierre-Marie Lledo and all the members of Marsicano’s lab for useful discussions.
17 This work was supported by INSERM (to G.M.), INRA (to G.F.), Fondation pour la
18 Recherche Médicale (DRM20101220445 to G.M. and FDT20170436845 to G.T.),
19 French State/Agence Nationale de la Recherche (LABEX BRAIN ANR-10-LABX-43
20 to G.M., G.F. and A.B.G., ANR-10-IDEX-03-02 to A.B.-G, NeuroNutriSens ANR-13-
21 BSV4- 0006-02 to G.M., Orups ANR-16-CE37-0010 to G.M. and G.F., CaCoVi ANR-
22 18-CE16-0001 to G.M., MitObesity ANR-18-CE14-0029 to G.M.), EU-FP7
23 (PAINCAGE, HEALTH-603191 to G.M.; FP7-PEOPLE-2013-IEF-623638 to A.B.-G.),
24 European Research Council (Endofood, ERC–2010–StG–260515, CannaPreg, ERC-

1 2014-PoC-640923 and MiCaBra, ERC-2017-AdG-786467, to G.M.), Human Frontiers
2 Science Program (to G.M.), Region Aquitaine (to G.M.), Fyssen Foundation (to E.S.-
3 G.). MINECO/FEDER, UE (SAF2015-65034-R to PG), The Basque Government
4 (BCG IT764-13 to PG), Red de Trastornos Adictivos, Instituto de Salud Carlos III
5 (ISC-III) and European Regional Development Funds-European Union (ERDF-EU)
6 (RD16/0017/0012 to P.G.).

7 **AUTHOR CONTRIBUTIONS**

8 G.T., G.F., and G.M. designed research; G.T., M.J., S.A., A.C., L.B., N.P., E.S-G.,
9 performed research; G.T., A.B-G., F.M., P.G., G.F. and G.M. supervised research;
10 G.T.,S.A. and I.B-D.R analyzed data; G.T., G.F., and G.M. wrote the manuscript. All
11 authors edited and approved the manuscript.

12 **DECLARATION OF INTERESTS**

13 The authors declare no competing interests.

14 **MAIN-TEXT FIGURE LEGENDS**

15 **Figure 1. CB1 receptors in the aPC are necessary for retrieval of odor**
16 **preference**

17 **(A)** Schematic protocol used for conditioned odor preference (COP). During
18 conditioning, the two odor-scented solutions (O1 and O2) are associated to the
19 absence or presence of sucrose, becoming neutral (C-) or conditioned (C+) stimuli,
20 respectively. Blue arrows, aPC infusions before pairings; red arrow, aPC infusion
21 before test for pharmacological experiments. **(B)** Representative images of
22 Fluorescent *In Situ* Hybridization against CB1 mRNA (red), showing the virally
23 induced deletion of CB1 receptors in the aPC of *CB1*-Flox mice locally injected with

1 AAV-GFP or AAV-CRE. Scale bar, 200 μ m. **(C)** Quantification of fluorescence
2 intensity of CB1 mRNA in the insular cortex, the aPC and the lateral olfactory
3 tubercle from sections where maximal deletion of CB1 expression was observed in
4 aPC-*CB1*-KO and from equally located sections in control mice (n=7-10). **(D)**
5 Consumption of C+ and C- odor-scented solutions in control mice (Control, n=36),
6 mice carrying deletion of aPC-CB1 receptors (aPC-*CB1*-KO, n=10), mice receiving
7 aPC infusions of the CB1 receptor antagonist AM251 (4 μ g/0.5 μ l per side) before
8 each odor-sucrose pairing (AM pairings, n=8), or before the COP retrieval test (AM
9 test, n=12). **(E)** Consumption of C+ and C- solutions during a second COP retrieval
10 test performed after retraining (see STAR Methods). Mice previously infused with AM
11 were infused with vehicle before the second test ["Veh(AM) test", n=12] and those
12 previously infused with vehicle were now infused with AM ["AM(Veh) test", n=13]. **,
13 p<0.01, ***, p<0.001; ****, p<0.0001; ns, not significant. For statistical details, see
14 Tables S1-S3. For supplemental information, see Figures S1 and S2.

1 **Figure 2. CB1 receptors in the aPC are not involved in the retrieval of odor**
2 **aversion**

3 **(A)** Schematic representation of the protocol used for LiCl-induced conditioned odor
4 aversion (COA-L). **(B)** Consumption of the odor-scented solutions (C+ and C-) during
5 test of COA-L in mice receiving aPC infusions of the CB1 receptor antagonist AM251
6 (4µg/0.5µl per side, n=11) or vehicle (n=13). **(C)** Schematic representation of the
7 protocol used for quinine-induced conditioned odor aversion (COA-Q). **(D)**
8 Consumption of the odor-scented solution during test of COA-Q in mice receiving
9 aPC infusions of AM251 (n=12) or vehicle (n=11). C+, odor-scented solutions
10 previously paired with LiCl injections **(B)** or quinine **(D)**. C-, odor-scented solutions
11 paired with saline injections **(B)** or water **(D)**. Red arrows, time of intra-aPC infusions.
12 ****, $p < 0.0001$ general solution effect. For statistical details, see Tables S1-S3. For
13 supplemental information, see Figures S2 and S3.

1 **Figure 3. CB1 receptors are highly expressed in GABAergic interneurons in the**
2 **aPC**

3 **(A)** Representative coronal brain sections showing immunostaining of CB1 receptors
4 in the aPC of wild-type (WT), CB1 Rescue, GABA-CB1 Rescue, Glu-CB1 Rescue
5 and CB1 Stop mice. Dotted lines delimitate the different cortical layers (I, II and III).
6 Scale bars, 100 μ m. **(B)** Electron microscopy micrographs of immunogold staining for
7 CB1 receptors in the aPC of CB1 wild-type (*CB1*-WT) and knockout mice (*CB1*-KO).
8 Black arrowheads, synapses; sp, spines; den, dendrites; red areas, presumably
9 inhibitory terminals and preterminals; green areas, presumably excitatory terminals;
10 purple areas, mitochondria; red arrows, CB1 receptors on inhibitory terminals and
11 pre-terminals; green arrows, CB1 receptors on excitatory terminals; purple arrows,
12 CB1 receptors on mitochondria. Scale bars, 1 μ m. **(C)** Proportion of CB1 receptor
13 immunoparticles on inhibitory and excitatory terminals over total CB1 labeling on
14 terminals (100 %). **(D)** Percentage of CB1 receptor-labeled inhibitory and excitatory
15 terminals in *CB1*-WT and *CB1*-KO. ****, $p < 0.0001$. For statistical details, see Tables
16 S1-S3. For supplemental information, see Figure S4.

1 **Figure 4. Topographic distribution of CB1 receptor-positive GABAergic**
2 **interneurons in the aPC**

3 **(A)** Representative images showing double Fluorescent *In Situ* Hybridization (D-
4 FISH) of GAD 65kDa mRNA (GAD, red) and CB1 mRNA (green) in the aPC. Lower
5 panels, higher magnifications of the boxed in the top panels. Yellow arrows, GAD-
6 positive cells that do not express CB1; blue arrows, GAD-positive cells containing low
7 levels of CB1 mRNA; white arrows, GAD-positive cells containing high levels of CB1
8 mRNA. Lines in top panels delimitate the different cortical layers (I, II and III). Scale
9 bar, 100 μ m (top) and 50 μ m (bottom). **(B-E)** Pie-charts representing percentage
10 distribution of **(B)** cells expressing GAD mRNA in different layers, **(C)** cells
11 expressing CB1 mRNA in different layers, **(D)** GAD-positive cells expressing or not
12 CB1 mRNA, and **(E)** cells expressing both GAD and CB1 mRNAs in different layers.
13 Total numbers of counted cells are below each chart (n=118 sections from 4
14 animals). **(F)** Percentages of GAD mRNA expression in total high- (green) and low-
15 CB1 expressing cells (light green) in the different layers of the aPC.

1 **Figure 5. CB1 receptors control inhibitory transmission in SL cells of the aPC**

2 **(A)** Representative traces of miniature inhibitory postsynaptic currents (mIPSCs)

3 recorded in aPC semilunar-like neurons (SL) under different sequential treatments:

4 Vehicle; WIN, CB1 receptor agonist WIN55,212-2 (5 μ M); WIN + AM251, WIN

5 together with the CB1 receptor antagonist AM251 (4 μ M). **(B, C)** Quantifications of

6 mIPSCs frequency **(B)** and amplitude **(C)** recorded in SL cells under Vehicle, WIN

7 and WIN+AM251 treatments (n=10 cells from 4 mice). **(D)** Representative traces of

8 mIPSCs recorded in aPC pyramidal-like neurons (PNs) under the same sequential

9 treatments as in **(A-C)**. **(E, F)** Quantifications of mIPSCs frequency **(E)** and amplitude

10 **(F)** recorded in PNs under the different treatments (n=9 cells from 5 mice). *, p<0.05;

11 ns, not significant (vs Vehicle). For statistical details, see Table S1. For supplemental

12 information, see Figure S5.

1 **Figure 6. Involvement of GABAergic transmission in the CB1 receptor-**
2 **dependent control of COP retrieval**

3 **(A)** Representative traces of mIPSCs recorded in SL neurons in the aPC, from mice
4 sacrificed during control water consumption (Water) or COP retrieval test (COP). **(B)**
5 Quantifications of mIPSCs frequency in SL neurons of mice sacrificed during water
6 consumption (Water, n=44 cells from 10 animals), COP retrieval test (COP, n=30
7 cells from 8 animals), exposure to odor-scented solutions without sucrose (Sucrose
8 free, n=17 cells from 4 animals), exposure to sucrose solution without odors (Odor
9 free, n= 27 cells from 4 animals), or COA retrieval test (COA, n= 24 cells from 4
10 animals). **(C)** Consumption of C+ and C- during COP retrieval test after
11 administration of Vehicle or the CB1 receptor antagonist Rimonabant (IP, Rim, 1
12 mg/kg). **(D-F)** Effect of IP injections of Vehicle or Rim on mIPSCs of SL cells from
13 mice sacrificed during COP retrieval test. **(D)** Representative traces. **(E)**
14 Quantification of mIPSCs frequency (Vehicle, n=30 cells from 5 animals; Rim, n=32
15 cells from 5 animals). **(F)** Correlation between the average of mIPSC frequencies in
16 SL neurons of individual animals and their COP retrieval performances expressed as
17 Preference index (COP vehicle, n=5; COP Rim, n=5). **(G-I)** Effect of IP injections of
18 Vehicle or Rim on mIPSCs of PNs in mice sacrificed during water consumption
19 (Water vehicle) or COP retrieval test (COP vehicle and COP Rim). **(G)**
20 Representative traces. **(H)** Quantification of mIPSCs frequency (Water vehicle, n=21
21 from 3 animals; COP vehicle, n=26 from 4 animals; COP Rim, n=27 from 5 animals).
22 **(I)** Correlation between the average of mIPSC frequencies in PNs from individual
23 animals and their COP retrieval performances, expressed as Preference index (COP
24 vehicle, n=4; COP Rim, n=5). **(J-K)** Consumption of the C+ and C- odor-scented
25 solutions in **(J)** GABA-CB1-WT (n=31) and in **(K)** GABA-CB1-KO mice (n=23)

1 receiving IP injections of either vehicle or Rim before a COP test (see STAR
2 Methods). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant. For statistical
3 details, see Tables S1, S3. For supplemental information, see Figures S5 and S6.

1 **STAR METHODS**

2 **KEY RESOURCES TABLE**

3 **LEAD CONTACT AND MATERIALS AVAILABILITY**

4 This study did not generate new unique reagents. Further information and requests
5 for resources and reagents should be directed to and will be fulfilled by the Lead
6 Contact, Giovanni Marsicano (giovanni.marsicano@inserm.fr).

7 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

8 All experimental procedures were approved by the local Committee on Animal Health
9 and Care of Bordeaux and the French Ministry of Agriculture and Forestry
10 (authorization number A33063098) and Committee of Ethics for Animal Welfare of
11 the University of the Basque Country (CEEA/408/2015/Grandes Moreno, CEIAB/
12 213/2015/Grandes Moreno). Two to three months-old naive male *CB1-flox* [mice
13 carrying the “floxed” *CB1* gene (*CB1* f/f)] were used [26,27,64]. Rescue, stop and
14 knockout lines were generated as described [33,36,37,64]. Briefly, Stop-*CB1* mouse
15 line was produced by silencing the endogenous *CB1* gene with a *loxP*-flanked stop
16 cassette in the 5' UTR of the *CB1* receptor start codon. To rescue the expression of
17 the *CB1* receptor, Stop-*CB1* line was crossed with a Cre-deleter mouse line.
18 Conditional rescue mice were obtained by crossing Stop-*CB1* mice with *Dlx5/6*-CRE
19 mice (gene expressed in differentiating GABAergic neurons) allowing the expression
20 of *CB1* in GABAergic neurons, named as “GABA-*CB1* rescue”, and with *Nex*-CRE
21 mice (gene expressed in cortical glutamatergic neurons) allowing the expression of
22 *CB1* in cortical glutamatergic neurons, named as “Glu-*CB1* rescue”. Total *CB1*
23 receptor knockout (*CB1*-KO) mice and conditional knockout animals lacking *CB1*
24 receptor in forebrain GABAergic *Dlx5/6* positive neurons (GABA-*CB1*-KO) were

1 obtained as described [27,45,64]. All behavioral experiments were performed during
2 the light phase (from 9am to 1pm) and animals were kept in individual cages under a
3 12h light/dark cycle (lights on 7 am) and were maintained under standard conditions
4 with food and water *ad libitum* prior undergoing behavioral procedures. At least three
5 animals from each genotype or experimental group were used for
6 immunohistochemistry, fluorescent *in situ* hybridization and electrophysiology
7 recordings.

8 **METHOD DETAILS**

9 **Behavioral procedures**

10 *Conditioned Odor Preference (COP)*

11 Mice were water deprived during the whole protocol. During three consecutive days,
12 animals had 1-hour access to two bottles of water. Over the following 4 days, animals
13 received simultaneously (1-hour access) one bottle with an odor-scented solution,
14 either banana (isoamyl acetate, 0.05%) or almond (benzaldehyde, 0.01%) diluted in
15 water and one bottle with a different odor-scented solution (either almond or banana)
16 mixed with the sweet taste sucrose (0.15M, 5%). Concentrations of banana (0.05%)
17 and almond (0.01%) solutions were chosen to be equally consumed when diluted in
18 water and prior to any associations with other stimuli [25,28,29]. Moreover, these
19 almond- and banana-scented solutions were chosen to specifically served as odor
20 cue based on previous studies indicating anosmic animals were unable to reliably
21 detect almond- or banana-scented water (at higher concentrations than the ones
22 used here), whereas they performed as well as control for taste detection [23,24].
23 This provides evidence that these aqueous banana and almond compounds did not
24 confer any behaviorally detected gustatory sensation to the drinking solution.

1 During COP training, the odor-scented solution present in water was named odor 1
2 (O1) and the other odor-scented solution associated with sucrose was named odor
3 2+Sucrose (O2+Sucrose). Half of the mice received banana-sucrose and the other
4 half almond-sucrose. No differences were observed between either conditions in all
5 the experiments performed. The position of the bottles was changed every day. After
6 this training, a preference test was performed using a 1-hour two bottles choice: each
7 bottle was presented with an odor-alone solution (almond versus banana diluted in
8 water without sucrose). Subjects showing COP will drink more liquid in the bottle with
9 the odor previously associated with sucrose (C+) than in the other bottle (C-).

10 In order to test the impact of CB1 receptor blockade in some experiments (Figures
11 1E and 6J,K), mice injected with either the CB1 receptor antagonist (either AM251 in
12 aPC or Rimonabant IP) or vehicle prior to the first COP test received 4 additional
13 odor-sucrose pairings and were injected with the other treatment (either vehicle or
14 CB1 receptor antagonist) before a second COP test.

15 *Sucrose Preference*

16 All subjects underwent 3 days habituation to water followed by 3 days with two
17 bottles containing either water or sucrose. Finally, we evaluated the effect of aPC
18 injection of AM251, or its vehicle, on their preference for sucrose over water.

19 *Conditioned Odor Aversion (COA)*

20 COA induced by gastric malaise

21 COA using gastric malaise was adapted from previous studies [25,28–30]. Mice
22 followed the same habituation phase as described above. The conditioning phase
23 consisted in 4 days. On days 4 and 6 the subjects received 1-hour access to
24 odorized water (banana or almond) followed by an injection of Saline immediately

1 after the session (O1 + sal.). On Days 5 and 7 subjects received 1-hour access to the
2 other odor (almond or banana) that they did not receive on Days 4 and 6, followed by
3 an injection of lithium chloride (LiCl, 0.3 M, 1% b.w.; Sigma-Aldrich; O2 + LiCl)
4 immediately after the session. The different odors were counterbalanced between
5 each group. After this conditioning, the subjects were given a recovery day during
6 which they received water dispensed in two bottles during 1 hour. The following day,
7 a preference test was performed using a 1-hour two bottles choice: each bottle was
8 presented with an odor (almond versus banana). During the test, subjects showing
9 COA will drink less liquid in the bottle with the odor previously associated with LiCl
10 (C+) than in the other bottle (C-).

11 COA induced by quinine

12 The COA with quinine followed the same procedure as the COP by replacing the
13 sucrose by 0.1mM of quinine (Sigma-Aldrich). During the test, subjects showing COA
14 will drink less liquid in the bottle with the odor previously associated with quinine (C+)
15 than in the other bottle (C-). An index of preference or aversion was calculated for
16 COP or COA experiments as the following:

$$17 \quad \frac{\textit{Liquid intake of (C +)} - \textit{liquid intake of (C -)}}{\textit{Total liquid intake}}$$

18 **Surgery**

19 Mice were anesthetized by IP injection of a mixture of ketamine (100mg/kg, Imalgene
20 500®) and xylazine (10mg/kg, Rompun®) or with isoflurane. Then, animals were
21 placed into a stereotaxic apparatus (Model 900, Kopf instruments, CA, USA) with a
22 mouse adaptor and lateral ear bars. For local deletion of CB1 receptors [25,27] in the
23 aPC, CB1 flox mice were injected with an AAV-cag-CRE or its control AAV-cag-GFP

1 (mixed serotype AAV1/AAV2, 10^{10} Vg/ml) into the aPC (250 μ l per side, 125 μ l/min)
2 with the following coordinates according to Paxinos and Franklin's mouse brain atlas
3 [65]: AP +1.6, L \pm 2.5, DV -4.8. For each animal receiving AAV-cag-CRE, CB1
4 deletion was verified by Fluorescent *In Situ* Hybridization against CB1 mRNA. To
5 control that recombination did not involve the posterior PC or the anterior olfactory
6 nucleus, data were obtained by averaging CB1 mRNA fluorescence intensity from 2/4
7 slices for each level in the antero-posterior axis (Figures S1H,I). In order to check
8 region specificity across the medio-lateral axis, data were obtained from brain regions
9 (insular cortex, aPC and lateral olfactory tubercle) bilaterally in the section for each
10 mouse where maximal deletion of CB1 mRNA was observed (Figure 1C).
11 Corresponding sections were quantified in both antero-posterior and medio-lateral
12 axes in control mice injected with AAV-cag-GFP.

13 For local pharmacology experiments, mice were bilaterally implanted with 3.5mm
14 stainless steel guide cannulae (Bilaney, UK) targeting the aPC with the following
15 coordinates [65]: AP +1.6, L \pm 2.5, DV -4.5. Guide cannulae were secured in place
16 with dental cement. Mice were allowed to recover for 2 weeks in individual cages
17 before the beginning of the experiments.

18 The placement of aPC cannulae was determined by injection of 2% pontamine sky
19 blue solution (0.5 μ l per side).

20 **Drugs**

21 For *in vitro* patch-clamp experiment, WIN 55,212-2 (5 μ M) (Tocris Bioscience) and
22 AM251 (4 μ M) (Tocris Bioscience) were prepared in Dimethyl Sulfoxide (DMSO) and
23 applied for 10min.

1 For behavioral experiments, AM251 was dissolved in a mixture of 10% Cremophor-
2 EL, 10% DMSO and 80% saline (NaCl 0.9%). AM251 (4 μ g/0.5 μ l per side) or its
3 vehicle was injected bilaterally in the aPC using silicone tubing connected to a
4 peristaltic pump (PHD 22/2000 Syringe Pump Infusion, Harvard Apparatus,
5 Massachusetts, USA, flow rate: 0.5 μ l/min). Rimonabant (Cayman Chemical) was
6 dissolved in a mixture of 1.25% Tween20, 1.25% DMSO and 97.5% saline (NaCl
7 0.9%). Rimonabant (1 mg/kg) or its vehicle was injected intraperitoneally (IP) in a
8 volume of 10 ml/kg.

9 Mice injected with AM251 (4 μ g/0.5 μ l per side) or Rimonabant (1mg/kg) were left in
10 their home cage 10 min or 30min before bottles presentation, respectively. In order to
11 habituate animals to receive aPC infusion and systemic injection, animals were
12 injected with a saline solution (NaCl 0.9%) in the same manner during the two
13 previous days. Mice receiving local aPC infusion were kept awake and maintained by
14 the tail during the injection.

15 **Immunohistochemistry**

16 Mice were anesthetized with pentobarbital (Exagon, 400 mg/kg body weight),
17 transcardially perfused with phosphate-buffered solution (PBS 0.1M, pH 7.4) before
18 being fixed with 4% formaldehyde prepared at 4°C. Serial coronal sections were cut
19 at 40 μ m and collected in PBS at room temperature (RT). Sections were
20 permeabilized in a blocking solution of 10% donkey serum, 0.3% Triton X-100 and
21 0.02% sodium azide in PBS for 1 hour at RT. Free-floating sections were incubated
22 with a goat polyclonal antibody against C-terminal sequence of the mouse CB1
23 receptor (1:2000, Frontier Science Co.) for 48h at 4°C. After several washes, slices
24 were incubated for 2 hours with a secondary anti-goat antibody conjugated to Alexa
25 488 (1:500, Fisher Scientific) and then washed in PBS at RT. Finally, sections were

1 incubated with DAPI (1:20 000, Fisher Scientific) for 5 minutes before being washed,
2 mounted and coverslipped. The fluorescence was visualized with an epifluorescence
3 Leica DM6000 microscope.

4 **Immunocytochemistry for electron microscopy**

5 For detailed methodological procedure see [66]. Coronal anterior Piriform Cortex
6 (aPC) vibrosections were cut at 50 μm and collected in 0.1 M phosphate buffer (pH
7 7.4) at RT. Sections were preincubated in a blocking solution of 10% BSA, 0.1%
8 sodium azide, and 0.02% saponin prepared in 1X Tris-HCl-buffered saline, pH 7.4,
9 for 30 minutes at RT. A pre-embedding silver-intensified immunogold method was
10 used for localization of the CB1 receptor protein. Briefly, aPC sections were
11 incubated with the primary goat polyclonal anti-CB₁ receptor antibody (2 $\mu\text{g}/\text{ml}$
12 Frontier Sciences Institute; goat polyclonal) in 10% BSA/Tris-HCl-buffered saline
13 containing 0.1% sodium azide and 0.004% saponin on a shaker for 48h at 4°C. After
14 several washes in 1% BSA/Tris-HCl-buffered saline, tissue sections were incubated
15 with a secondary 1.4-nm gold-labeled rabbit anti-goat Immunoglobulin G (Fab
16 fragment; 1:100; Nanoprobes Inc.) in 1% BSA/Tris-HCl-buffered saline with 0.004%
17 saponin on a shaker for 4 hours at RT. Sections were washed in 1% BSA/ Tris-HCl-
18 buffered saline overnight at 4°C and postfixed in 1% glutaraldehyde in Tris-HCl-
19 buffered saline for 10 minutes at RT. After several washes in double-distilled water,
20 gold particles were silver intensified with an HQ Silver kit (Nanoprobes Inc.) for
21 approximately 12 minutes in the dark and then washed in double-distilled water first,
22 and in a 0.1M phosphate buffer, pH 7.4 later. Stained sections were osmicated (1%
23 osmium tetroxide, in 0.1 M phosphate buffer, pH 7.4, 20 minutes), dehydrated in
24 graded alcohols to propylene oxide, and plastic-embedded in Epon resin 812.
25 Ultrathin sections of 50 nm were collected on nickel mesh grids, stained with 2.5%

1 lead citrate for 20 minutes, and examined in a JEOL JEM 1400 Plus electron
2 microscope. Tissue preparations were photographed by using a digital camera
3 coupled to the electron microscope. Adjustments in contrast and brightness were
4 made to the figures in Adobe Photoshop (Adobe Systems, San Jose, CA).

5 **Fluorescent *in situ* hybridization**

6 The procedure was performed as described [18,39]. Briefly, mice were sacrificed by
7 cervical dislocation. Their brains were extracted, frozen on dry ice and stored at
8 -80°C until sectioning in a cryostat (14 μm , Microm HM 500M, Microm Microtech).
9 Fluorescein (FITC)-labeled riboprobes against mouse CB1 receptor and digoxigenin
10 (DIG)-labeled riboprobes against mouse GAD65 were prepared as described [39].
11 After hybridization overnight at 60°C with the mixture of probes, the slides were
12 washed with different stringency wash buffers at 65°C . Then, the slides were blocked
13 with a blocking buffer prepared according to the manufacturer's protocol. Anti-DIG or
14 anti-FITC antibodies conjugated to horseradish peroxidase (HRP) (Roche; 1 :2000)
15 were applied 2 hours at RT or overnight at 4°C to detect respectively GAD65-DIG or
16 CB1-FITC probes. Probes hybridization was revealed by a tyramide signal
17 amplification (TSA) reaction using Cyanine 3-labeled tyramide (Perkin Elmer; 1:100
18 for 10 minutes) to detect GAD65 signal or FITC-conjugated tyramide (Perkin Elmer;
19 1:80 for 12 minutes) to amplify the signal of CB1. The slides were incubated in 4',6-
20 diamidino-2-phenylindole (DAPI; 1:20 000; FISHER Scientific) before being washed,
21 coverslipped and visualized with an epifluorescence Leica DM6000 microscope.

22 **Electrophysiology**

23 All the animals were sacrificed by dislocation during the light phase (9am to 12am).
24 The brains were quickly removed and immersed in ice-cold oxygenated cutting

1 solution containing in mM: 180 Sucrose, 26 NaHCO₃, 12 MgCl₂, 11 Glucose, 2.5 KCl,
2 1.25 NaH₂PO₄, 0.2 CaCl₂, oxygenated with 95% O₂/5% CO₂ ≈ 300mOsm. Coronal
3 aPC slices (300µm thick) were obtained using a vibratome (VT1200S, Leica) and
4 transferred for 30min into a 34°C bath of oxygenated ACSF containing in mM: 123
5 NaCl, 26 NaHCO₃, 11 Glucose, 2.5 KCl, 2.5 CaCl₂, 1.3 MgCl₂, 1.25 NaH₂PO₄ ≈ 305
6 mOsm. After a minimum of 30min recovery at RT (22-25°C), slices were transferred
7 to a recording chamber in ACSF at 32°C. Recordings were performed using a
8 Multiclamp 700B amplifier (Molecular devices) in principal glutamatergic neurons
9 clamped with glass pipettes (3-5 MΩ) filled with an internal solution containing in mM:
10 130 KCl, 10 HEPES, 1 EGTA, 2 MgCl₂, 0.3 CaCl₂, 7 Phosphocreatin, 3 Mg-ATP, 0.3
11 Na-GTP; pH=7.2; 290mOsm. These cells were identified based on their morphology
12 and somatic location using a contrast microscope (axio examiner.A1, Zeiss) and
13 through electrical properties by measuring their resting potential and their excitability
14 in current-clamp mode after 300ms steps of current injections from -50 to 300pA with
15 steps of 25pA [34,35,51]. Neurons with large apical dendrites, soma located in the
16 upper half of layer II, resting potential of around -70mV, input resistance of around
17 200-300 MΩ and displaying regular spiking were considered as semilunar-like cells
18 (SL, see Figures S5A-B). Instead, neurons with large basal dendrites, soma located
19 in the lower half of layer II and upper part of layer III, resting potential around -75mV,
20 input resistance of around 100-150 MΩ and showing initial burst firing were classified
21 as pyramidal-like neurons (PN, see Figures S5A-B). Given the complex layered
22 structure of the aPC, miniature inhibitory post-synaptic currents (mIPSCs), known to
23 be regulated by CB1 receptors [44], were specifically chosen to avoid restricting the
24 study of inhibitory inputs coming from a specific layer where the stimulating electrode
25 would have been placed. mIPSCs were obtained in voltage clamp mode in presence

1 of NMDA and AMPA/Kainate receptor antagonists (50 μ M D-APV and 10 μ M NBQX)
2 and of the voltage-gated sodium channels blocker, tetrodotoxin (1 μ M TTX). Vehicle
3 (DMSO) was applied before starting the recording and CB1 agonist (WIN 5 μ M) and
4 antagonist (AM251 4 μ M) were applied for 10min successively. For experiments
5 performed after behavior, animals underwent the two bottles choice test for 15min
6 and were sacrificed 5min later. mIPSCs were collected in the same manner as for
7 naïve animals, for 5 min in presence of vehicle (DMSO).

8 **QUANTIFICATION AND STATISTICAL ANALYSIS**

9 **Behavioral data**

10 For all the experiments, data are presented as absolute liquid intake. Considering the
11 variability of liquid consumption during the two first days of pairings (likely due to the
12 random choice of a bottle at day 1 and the confusion that might appear because of
13 the inverted position of the bottles at day 2), only the last two days of the learning
14 phase showing a reliable preference/aversion behavior were presented for each
15 experiments.

16 **Numerical evaluation for electron microscopy**

17 Semi-quantitative analysis of CB1 receptor presence in excitatory or inhibitory
18 terminals was done in aPC layers I and II of *CB1*-WT and *CB1*-KO according to our
19 published procedure [66]. Total analyzed area was more than 2200 μ m² per genotype
20 (n=3).

21 **Numerical evaluation for FISH**

22 Cells expressing mRNAs were quantified in the three layers of the aPC. Because
23 CB1 mRNA level is variable, CB1 positive cells were classified according to the level

1 of transcript visualized by the intensity of fluorescence [39]. “High-CB1” cells were
2 considered to be round-shaped and intense staining covering the entire nucleus
3 whereas “Low-CB1” cells were defined with discontinuous shape and lowest intensity
4 of fluorescence allowing the discrimination of grains of staining. Numerical evaluation
5 of the double FISH was performed manually in 118 sections from 4 animals, by
6 evaluating the coexpression of CB1-positive cells with GAD 65 marker.

7 **Electrophysiology**

8 Electrophysiological data were filtered at 4kHz by a Digidata 1440A (Molecular
9 devices) and they were collected during the last 5min of recording in each condition.
10 Electrical properties were analyzed with Clampfit and mIPSCs were analyzed using
11 Axograph software.

12 **Statistics**

13 Electrophysiological and behavioral data were analyzed with Prism Software
14 (GraphPad). Repeated or unpaired statistical analyses were obtained with Student’s
15 t-test, ANOVA (one-way or two way), mixed effects analysis and linear regression to
16 compare two or multiple groups and for correlation where appropriate. When ANOVA
17 provided significant main factor effects or significant interactions, Dunnett or Sidak
18 post-hoc analyses were performed as appropriate. Statistical details are presented in
19 tables S1, S2 and S3. Significance was set at $p < 0.05$ and data are expressed as
20 mean \pm SEM.

21 **DATA AND CODE AVAILABILITY**

22 This study did not generate datasets/code. Further data information are available
23 upon request by contacting the Lead Contact, Giovanni Marsicano

1 (giovanni.marsicano@inserm.fr).

2

1 REFERENCES

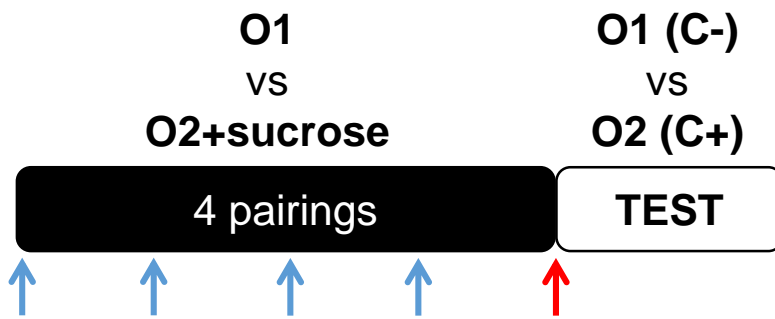
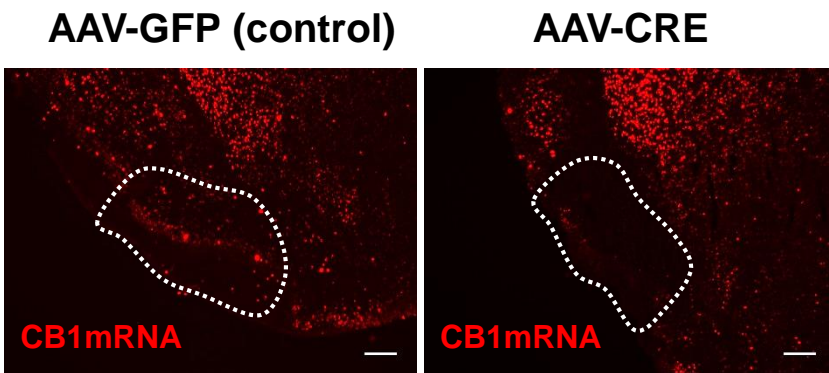
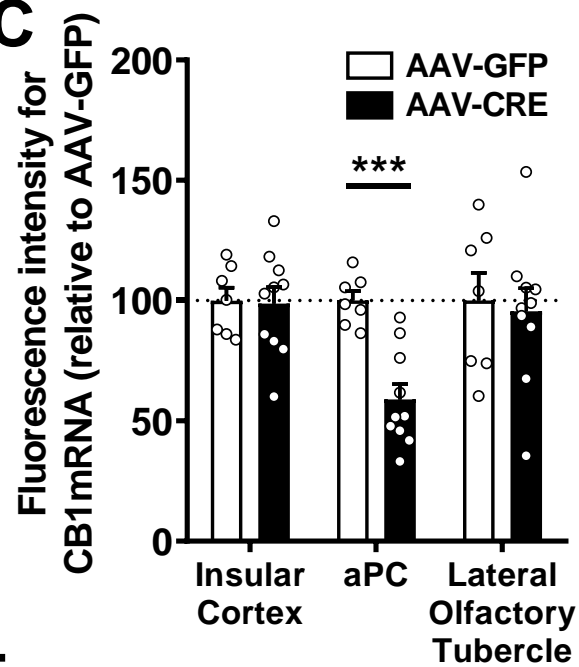
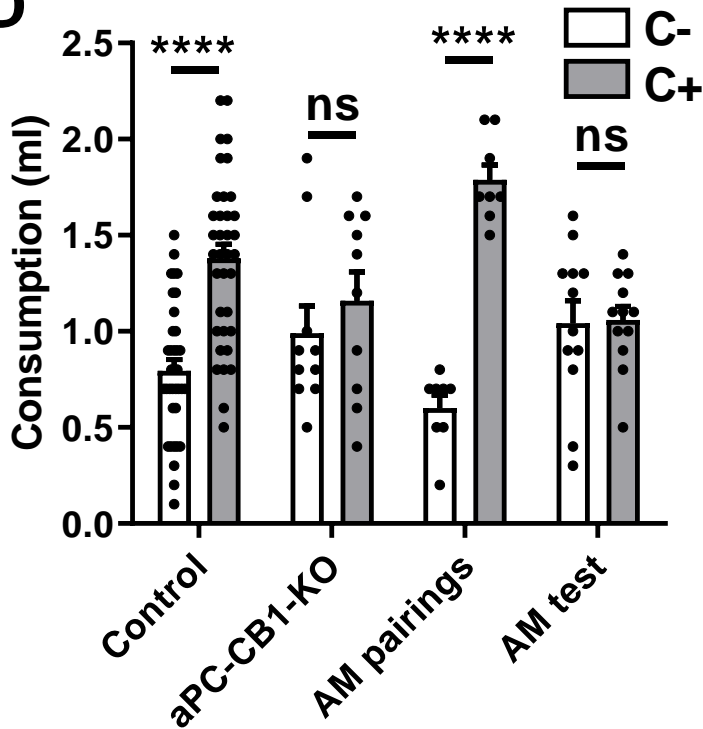
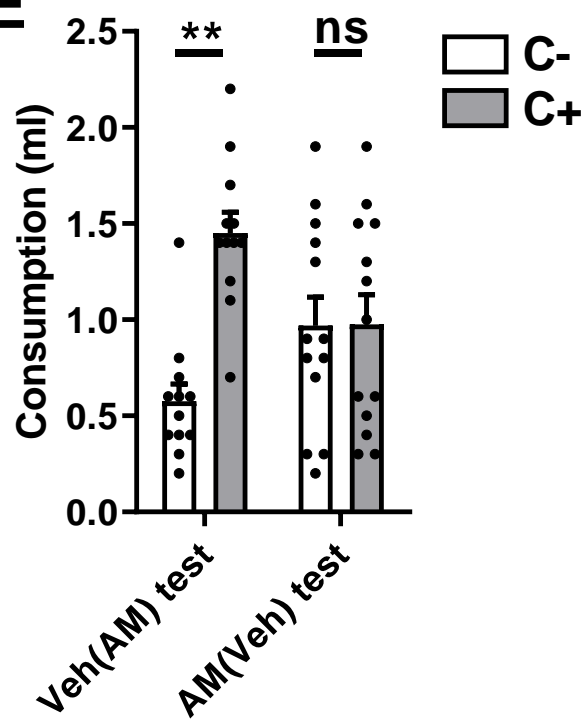
- 2 1. Smeets, M.A.M., and Dijksterhuis, G.B. (2014). Smelly primes – when olfactory primes
3 do or do not work. *Front. Psychol.* *5*.
- 4 2. Sullivan, R.M., Wilson, D.A., Ravel, N., and Mouly, A.-M. (2015). Olfactory memory
5 networks: from emotional learning to social behaviors. *Front. Behav. Neurosci.* *9*.
- 6 3. Buck, L., and Axel, R. (1991). A Novel Multigene Family May Encode Odorant
7 Receptors: A Molecular Basis for Odor Recognition. *Cell* *65*, 175–187.
- 8 4. Ghosh, S., Larson, S.D., Hefzi, H., Marnoy, Z., Cutforth, T., Dokka, K., and Baldwin, K.K.
9 (2011). Sensory maps in the olfactory cortex defined by long-range viral tracing of single
10 neurons. *Nature* *472*, 217–220.
- 11 5. Sosulski, D.L., Bloom, M.L., Cutforth, T., Axel, R., and Datta, S.R. (2011). Distinct
12 representations of olfactory information in different cortical centres. *Nature* *472*, 213–216.
- 13 6. Bekkers, J.M., and Suzuki, N. (2013). Neurons and circuits for odor processing in the
14 piriform cortex. *Trends Neurosci.* *36*, 429–438.
- 15 7. Neville, and Haberly (2004). Olfactory cortex. In *The Synaptic Organization of the Brain*
16 (Gordon M. Shepherd), pp. 415–454.
- 17 8. Wilson, and Sullivan, R.M. (2011). Cortical Processing of Odor Objects. *Neuron* *72*, 506–
18 519.
- 19 9. Piomelli, D. (2003). The molecular logic of endocannabinoid signalling. *Nat. Rev.*
20 *Neurosci.* *4*, 873–884.
- 21 10. Drumond, A., Madeira, N., and Fonseca, R. (2017). Endocannabinoid signaling and
22 memory dynamics: A synaptic perspective. *Neurobiol. Learn. Mem.* *138*, 62–77.
- 23 11. Marsicano, G., and Lafenêtre, P. (2009). Roles of the endocannabinoid system in
24 learning and memory. *Curr. Top. Behav. Neurosci.* *1*, 201–230.
- 25 12. Araque, A., Castillo, P.E., Manzoni, O.J., and Tonini, R. (2017). Synaptic functions of
26 endocannabinoid signaling in health and disease. *Neuropharmacology* *124*, 13–24.
- 27 13. Kano, M., Ohno-Shosaku, T., Hashimoto-dani, Y., Uchigashima, M., and Watanabe, M.
28 (2009). Endocannabinoid-Mediated Control of Synaptic Transmission. *Physiol. Rev.* *89*,
29 309–380.
- 30 14. Marsicano, G., and Kuner, R. (2008). Anatomical Distribution of Receptors, Ligands and
31 Enzymes in the Brain and in the Spinal Cord: Circuitries and Neurochemistry. In
32 *Cannabinoids and the Brain*, A. Köfalvi, ed. (Boston, MA: Springer US), pp. 161–201.
- 33 15. Ghosh, Reuveni, I., Zidan, S., Lamprecht, R., and Barkai, E. (2018). Learning-induced
34 modulation of the effect of endocannabinoids on inhibitory synaptic transmission. *J.*
35 *Neurophysiol.* *119*, 752–760.
- 36 16. Pouille, F., and Schoppa, N.E. (2018). Cannabinoid Receptors Modulate Excitation of an
37 Olfactory Bulb Local Circuit by Cortical Feedback. *Front. Cell. Neurosci.* *12*.

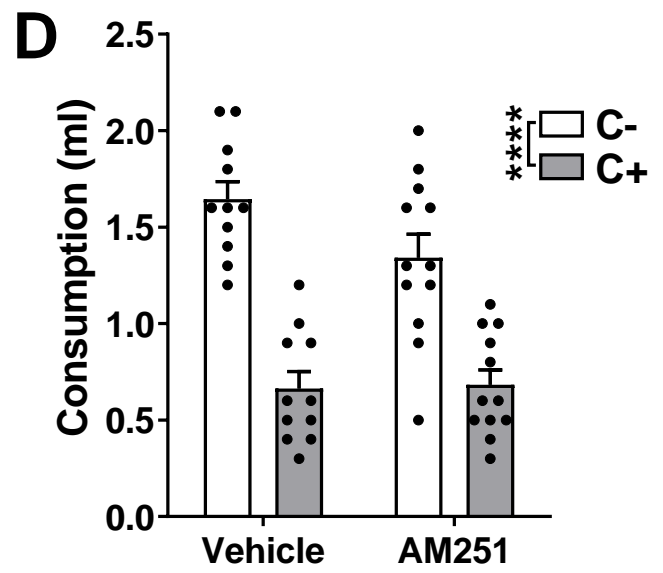
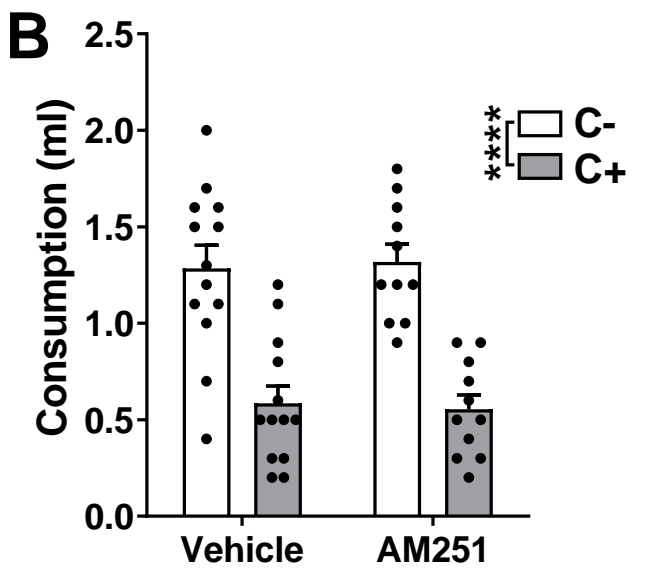
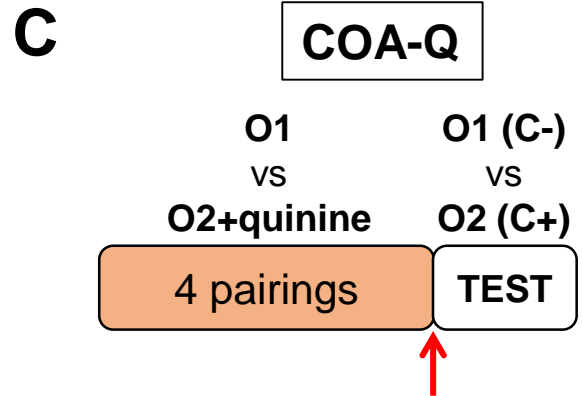
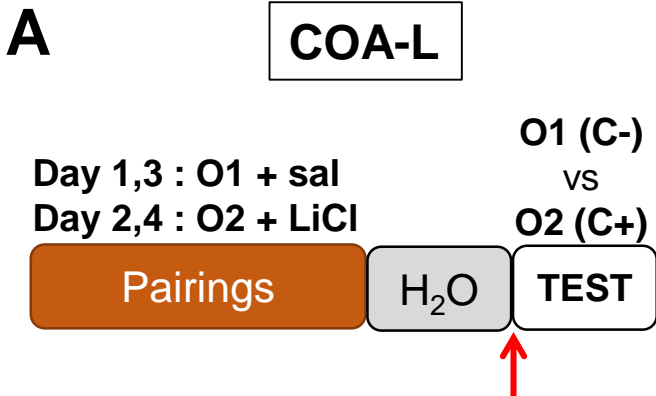
- 1 17. Soria-Gómez, E., Bellocchio, L., and Marsicano, G. (2014). New insights on food intake
2 control by olfactory processes: The emerging role of the endocannabinoid system. *Mol.*
3 *Cell. Endocrinol.* *397*, 59–66.
- 4 18. Soria-Gómez, E., Bellocchio, L., Reguero, L., Lepousez, G., Martin, C., Bendahmane,
5 M., Ruehle, S., Remmers, F., Desprez, T., Matias, I., *et al.* (2014). The endocannabinoid
6 system controls food intake via olfactory processes. *Nat. Neurosci.* *17*, 407–415.
- 7 19. Wang, Z.-J., Sun, L., and Heinbockel, T. (2012). Cannabinoid receptor-mediated
8 regulation of neuronal activity and signaling in glomeruli of the main olfactory bulb. *J.*
9 *Neurosci. Off. J. Soc. Neurosci.* *32*, 8475–8479.
- 10 20. Barnes, Hofacer, Zaman, Rennaker, R.L., and Wilson, D.A. (2008). Olfactory perceptual
11 stability and discrimination. *Nat. Neurosci.* *11*, 1378–1380.
- 12 21. Mediavilla, C., Martin-Signes, M., and Risco, S. (2016). Role of anterior piriform cortex in
13 the acquisition of conditioned flavour preference. *Sci. Rep.* *6*, 33365.
- 14 22. Schoenbaum, G., and Eichenbaum, H. (1995). Information coding in the rodent prefrontal
15 cortex. I. Single-neuron activity in orbitofrontal cortex compared with that in pyriform
16 cortex. *J. Neurophysiol.* *74*, 733–750.
- 17 23. Rusiniak, K.W., Hankins, W.G., Garcia, J., and Brett, L.P. (1979). Flavor-illness
18 aversions: potentiation of odor by taste in rats. *Behav. Neural Biol.* *25*, 1–17.
- 19 24. Slotnick, B.M., Bell, G.A., Panhuber, H., and Laing, D.G. (1997). Detection and
20 discrimination of propionic acid after removal of its 2-DG identified major focus in the
21 olfactory bulb: a psychophysical analysis. *Brain Res.* *762*, 89–96.
- 22 25. Busquets-Garcia, Oliveira da Cruz, J.F., Terral, G., Zottola, A.C.P., Soria-Gómez, E.,
23 Contini, A., Martin, H., Redon, B., Varilh, M., Ioannidou, C., *et al.* (2018). Hippocampal
24 CB1 Receptors Control Incidental Associations. *Neuron* *99*, 1247–1259.
- 25 26. Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad,
26 S.C., Cascio, M.G., Gutiérrez, S.O., van der Stelt, M., *et al.* (2003). CB1 cannabinoid
27 receptors and on-demand defense against excitotoxicity. *Science* *302*, 84–88.
- 28 27. Monory, Massa, F., Egertová, M., Eder, M., Blaudzun, H., Westenbroek, R., Kelsch, W.,
29 Jacob, W., Marsch, R., Ekker, M., *et al.* (2006). The Endocannabinoid System Controls
30 Key Epileptogenic Circuits in the Hippocampus. *Neuron* *51*, 455–466.
- 31 28. Busquets-Garcia, Soria-Gómez, E., Ferreira, G., and Marsicano, G. (2017).
32 Representation-mediated Aversion as a Model to Study Psychotic-like States in Mice.
33 *Bio-Protoc.* *7*.
- 34 29. Busquets-Garcia, Soria-Gómez, E., Redon, B., Mackenbach, Y., Vallée, M., Chaouloff,
35 F., Varilh, M., Ferreira, G., Piazza, P.-V., and Marsicano, G. (2017). Pregnenolone blocks
36 cannabinoid-induced acute psychotic-like states in mice. *Mol. Psychiatry* *22*, 1594–1603.
- 37 30. Soria-Gómez, E., Busquets-Garcia, A., Hu, F., Mehidi, A., Cannich, A., Roux, L., Louit, I.,
38 Alonso, L., Wiesner, T., Georges, F., *et al.* (2015). Habenular CB1 Receptors Control the
39 Expression of Aversive Memories. *Neuron* *88*, 306–313.
- 40 31. Sevelinges, Y., Desgranges, B., and Ferreira, G. (2009). The basolateral amygdala is
41 necessary for the encoding and the expression of odor memory. *Learn. Mem.* *16*, 235–
42 242.

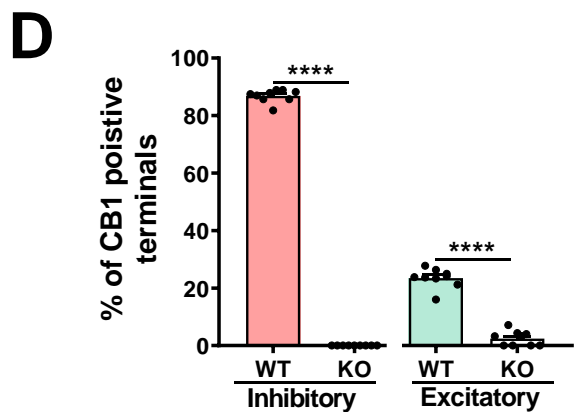
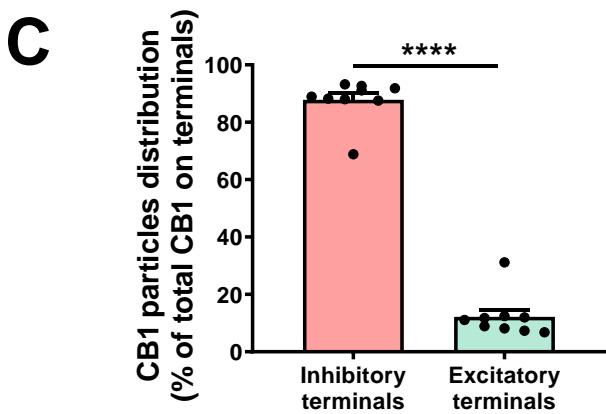
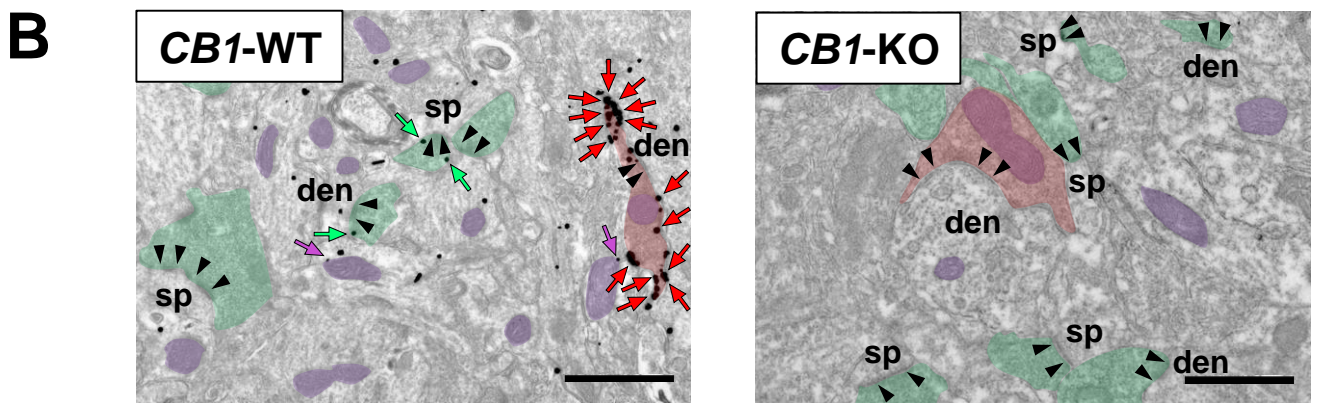
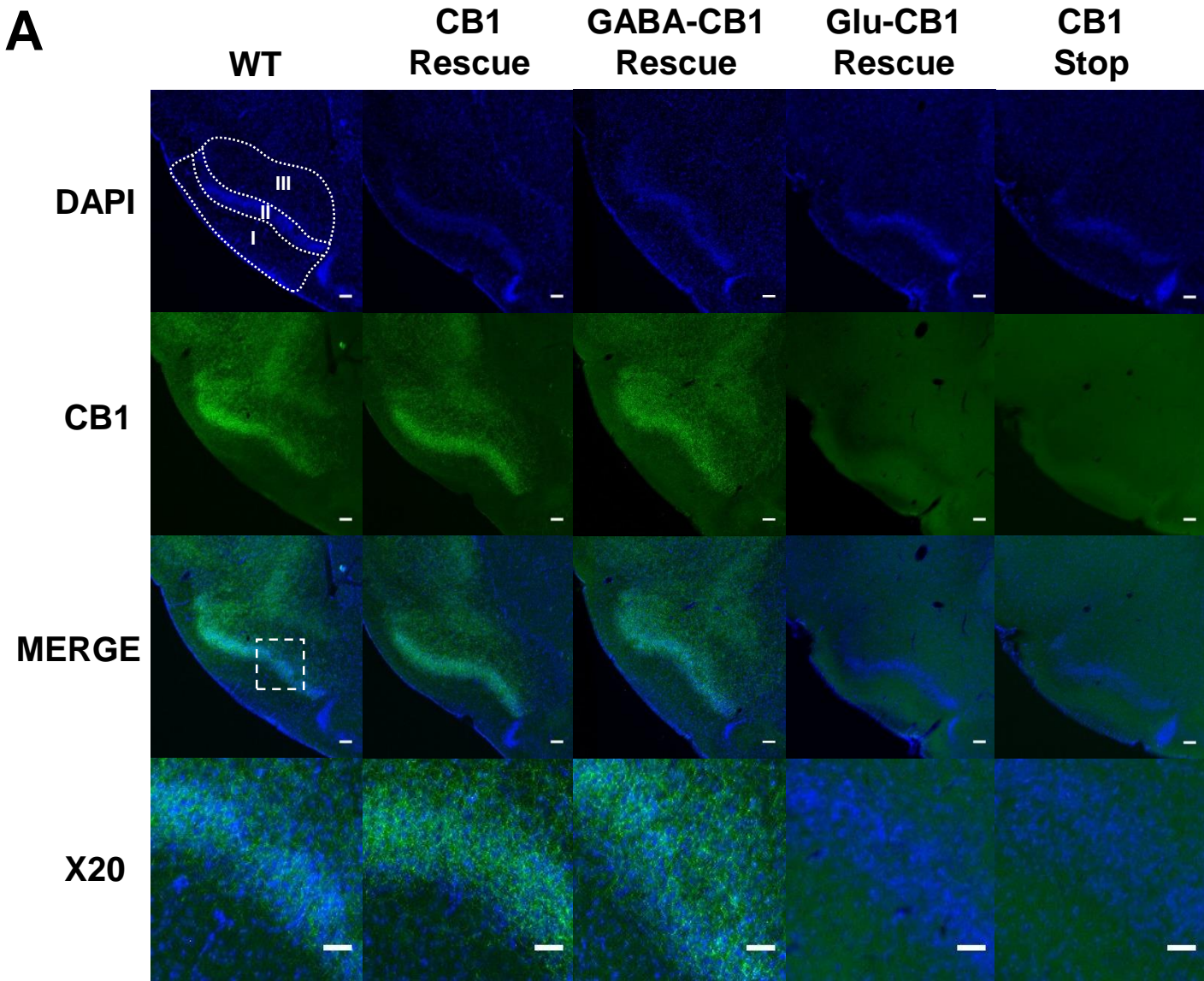
- 1 32. Yasoshima, Y., Morimoto, T., and Yamamoto, T. (2000). Different disruptive effects on
2 the acquisition and expression of conditioned taste aversion by blockades of amygdalar
3 ionotropic and metabotropic glutamatergic receptor subtypes in rats. *Brain Res.* 869, 15–
4 24.
- 5 33. Gutiérrez-Rodríguez, A., Puente, N., Elezgarai, I., Ruehle, S., Lutz, B., Reguero, L.,
6 Gerrikagoitia, I., Marsicano, G., and Grandes, P. (2017). Anatomical characterization of
7 the cannabinoid CB1 receptor in cell-type-specific mutant mouse rescue models. *J.*
8 *Comp. Neurol.* 525, 302–318.
- 9 34. Suzuki, and Bekkers, J.M. (2006). Neural Coding by Two Classes of Principal Cells in the
10 Mouse Piriform Cortex. *J. Neurosci.* 26, 11938–11947.
- 11 35. Suzuki, N., and Bekkers, J.M. (2011). Two Layers of Synaptic Processing by Principal
12 Neurons in Piriform Cortex. *J. Neurosci.* 31, 2156–2166.
- 13 36. Remmers, F., Lange, M.D., Hamann, M., Ruehle, S., Pape, H.-C., and Lutz, B. (2017).
14 Addressing sufficiency of the CB1 receptor for endocannabinoid-mediated functions
15 through conditional genetic rescue in forebrain GABAergic neurons. *Brain Struct. Funct.*
16 222, 3431–3452.
- 17 37. Ruehle, S., Remmers, F., Romo-Parra, H., Massa, F., Wickert, M., Wortge, S., Haring,
18 M., Kaiser, N., Marsicano, G., Pape, H.-C., *et al.* (2013). Cannabinoid CB1 Receptor in
19 Dorsal Telencephalic Glutamatergic Neurons: Distinctive Sufficiency for Hippocampus-
20 Dependent and Amygdala-Dependent Synaptic and Behavioral Functions. *J. Neurosci.*
21 33, 10264–10277.
- 22 38. Suzuki, and Bekkers, J.M. (2010). Inhibitory neurons in the anterior piriform cortex of the
23 mouse: Classification using molecular markers. *J. Comp. Neurol.* 518, 1670–1687.
- 24 39. Marsicano, and Lutz, B. (1999). Expression of the cannabinoid receptor CB1 in distinct
25 neuronal subpopulations in the adult mouse forebrain. *Eur. J. Neurosci.* 11, 4213–4225.
- 26 40. Mazo, C., Grimaud, J., Shima, Y., Murthy, V.N., and Lau, C.G. (2017). Distinct projection
27 patterns of different classes of layer 2 principal neurons in the olfactory cortex. *Sci. Rep.*
28 7.
- 29 41. Haberly, and Price, J.L. (1978). Association and commissural fiber systems of the
30 olfactory cortex of the rat. I. Systems originating in the piriform cortex and adjacent
31 areas. *J. Comp. Neurol.* 178, 711–740.
- 32 42. Suzuki, and Bekkers, J.M. (2012). Microcircuits Mediating Feedforward and Feedback
33 Synaptic Inhibition in the Piriform Cortex. *J. Neurosci.* 32, 919–931.
- 34 43. Poo, C., and Isaacson, J.S. (2009). Odor Representations in Olfactory Cortex: “Sparse”
35 Coding, Global Inhibition, and Oscillations. *Neuron* 62, 850–861.
- 36 44. Wilson, and Nicoll, R.A. (2001). Endogenous cannabinoids mediate retrograde signalling
37 at hippocampal synapses. *Nature* 410, 588–592.
- 38 45. Bellocchio, L., Lafenêtre, P., Cannich, A., Cota, D., Puente, N., Grandes, P., Chaouloff,
39 F., Piazza, P.V., and Marsicano, G. (2010). Bimodal control of stimulated food intake by
40 the endocannabinoid system. *Nat. Neurosci.* 13, 281–283.
- 41 46. Zerucha, T., Stühmer, T., Hatch, G., Park, B.K., Long, Q., Yu, G., Gambarotta, A.,
42 Schultz, J.R., Rubenstein, J.L.R., and Ekker, M. (2000). A Highly Conserved Enhancer in

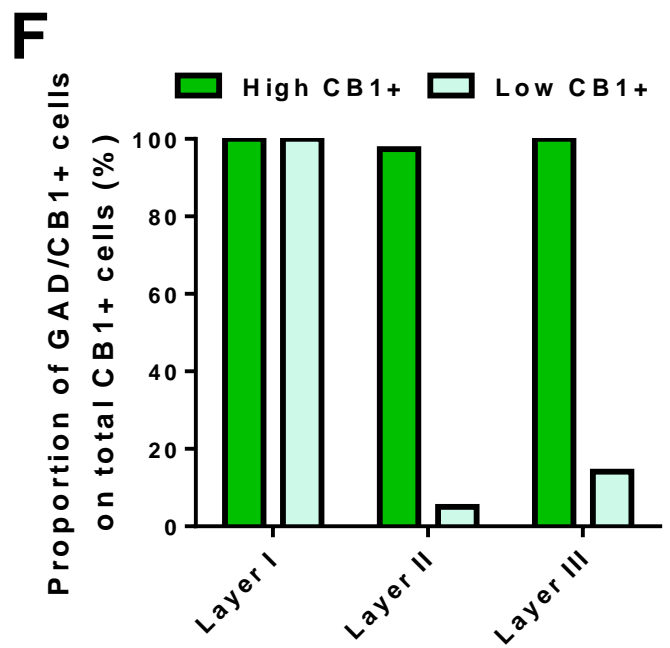
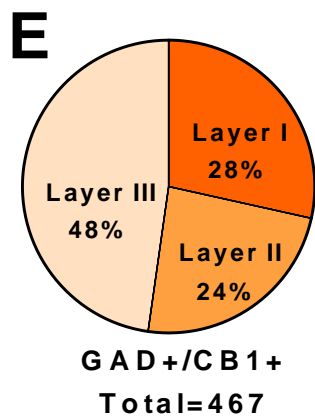
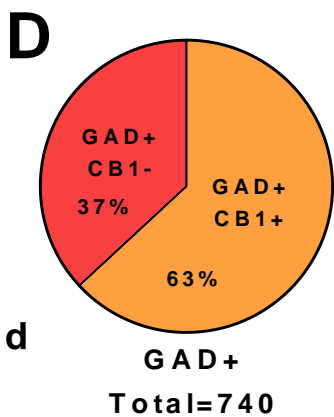
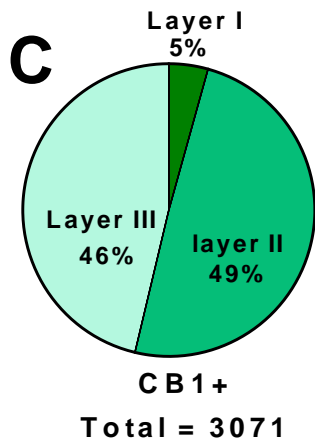
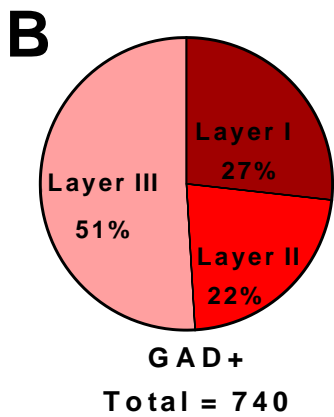
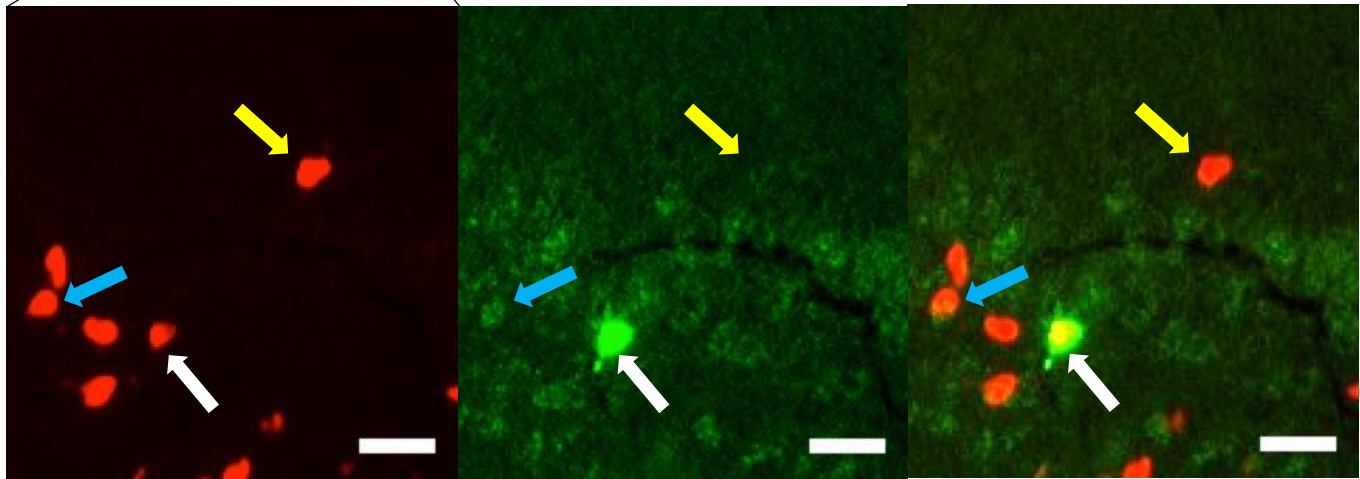
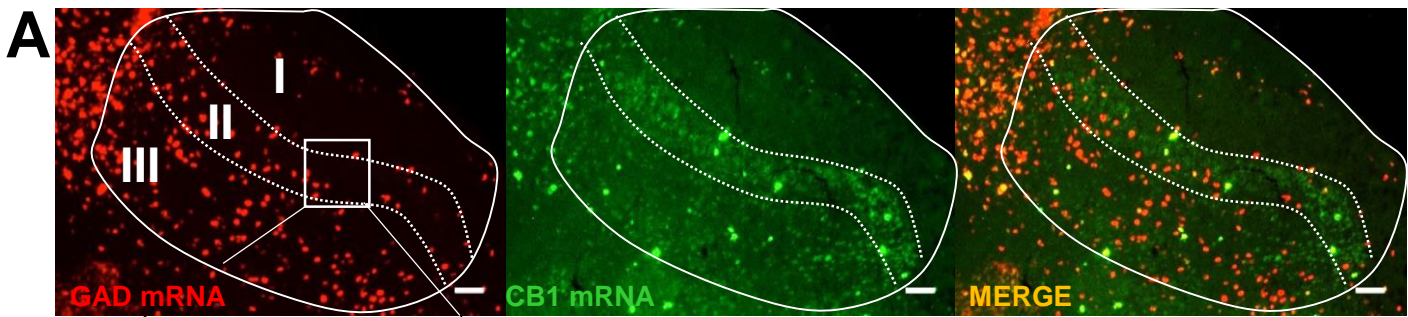
- 1 the *Dlx5/Dlx6* Intergenic Region is the Site of Cross-Regulatory Interactions between *Dlx*
2 Genes in the Embryonic Forebrain. *J. Neurosci.* *20*, 709–721.
- 3 47. El-Brolosy, M.A., and Stainier, D.Y.R. (2017). Genetic compensation: A phenomenon in
4 search of mechanisms. *PLOS Genet.* *13*, e1006780.
- 5 48. El-Brolosy, M.A., Kontarakis, Z., Rossi, A., Kuenne, C., Günther, S., Fukuda, N., Kikhi,
6 K., Boezio, G.L.M., Takacs, C.M., Lai, S.-L., *et al.* (2019). Genetic compensation
7 triggered by mutant mRNA degradation. *Nature* *568*, 193–197.
- 8 49. Rossato, J.I., Moreno, A., Genzel, L., Yamasaki, M., Takeuchi, T., Canals, S., and
9 Morris, R.G.M. (2018). Silent Learning. *Curr. Biol.* *28*, 3508-3515.e5.
- 10 50. Choi, G.B., Stettler, D.D., Kallman, B.R., Bhaskar, S.T., Fleischmann, A., and Axel, R.
11 (2011). Driving Opposing Behaviors with Ensembles of Piriform Neurons. *Cell* *146*,
12 1004–1015.
- 13 51. Meissner-Bernard, C., Dembitskaya, Y., Venance, L., and Fleischmann, A. (2019).
14 Encoding of Odor Fear Memories in the Mouse Olfactory Cortex. *Curr. Biol.* *29*, 367-
15 380.e4.
- 16 52. Chapuis, J., Garcia, S., Messaoudi, B., Thevenet, M., Ferreira, G., Gervais, R., and
17 Ravel, N. (2009). The Way an Odor Is Experienced during Aversive Conditioning
18 Determines the Extent of the Network Recruited during Retrieval: A Multisite
19 Electrophysiological Study in Rats. *J. Neurosci.* *29*, 10287–10298.
- 20 53. Roesch, M.R., Stalnaker, T.A., and Schoenbaum, G. (2007). Associative Encoding in
21 Anterior Piriform Cortex versus Orbitofrontal Cortex during Odor Discrimination and
22 Reversal Learning. *Cereb. Cortex* *17*, 643–652.
- 23 54. Laviolette, S.R., and Grace, A.A. (2006). Cannabinoids Potentiate Emotional Learning
24 Plasticity in Neurons of the Medial Prefrontal Cortex through Basolateral Amygdala
25 Inputs. *J. Neurosci.* *26*, 6458–6468.
- 26 55. Miranda, M.I., Ferry, B., and Ferreira, G. (2007). Basolateral amygdala noradrenergic
27 activity is involved in the acquisition of conditioned odor aversion in the rat. *Neurobiol.*
28 *Learn. Mem.* *88*, 260–263.
- 29 56. Luna, V.M., and Morozov, A. (2012). Input-specific excitation of olfactory cortex
30 microcircuits. *Front. Neural Circuits* *6*.
- 31 57. Hegoburu, C., Parrot, S., Ferreira, G., and Mouly, A.-M. (2014). Differential involvement
32 of amygdala and cortical NMDA receptors activation upon encoding in odor fear memory.
33 *Learn. Mem.* *21*, 651–655.
- 34 58. Wesson, D.W., and Wilson, D.A. (2011). Sniffing out the contributions of the olfactory
35 tubercle to the sense of smell: Hedonics, sensory integration, and more? *Neurosci.*
36 *Biobehav. Rev.* *35*, 655–668.
- 37 59. Caputi, A., Melzer, S., Michael, M., and Monyer, H. (2013). The long and short of
38 GABAergic neurons. *Curr. Opin. Neurobiol.* *23*, 179–186.
- 39 60. Franks, K.M., Russo, M.J., Sosulski, D.L., Mulligan, A.A., Siegelbaum, S.A., and Axel, R.
40 (2011). Recurrent Circuitry Dynamically Shapes the Activation of Piriform Cortex. *Neuron*
41 *72*, 49–56.

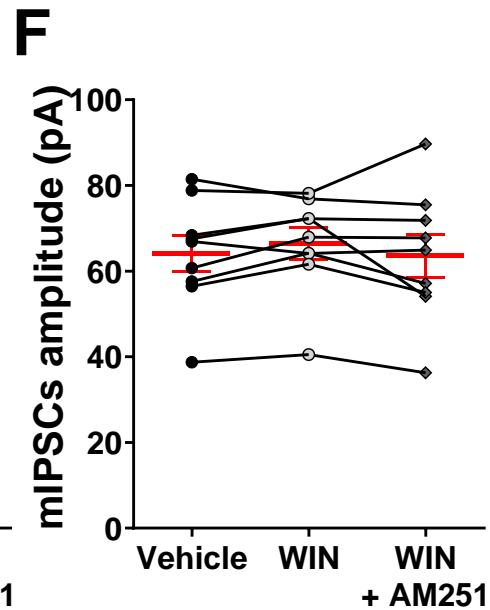
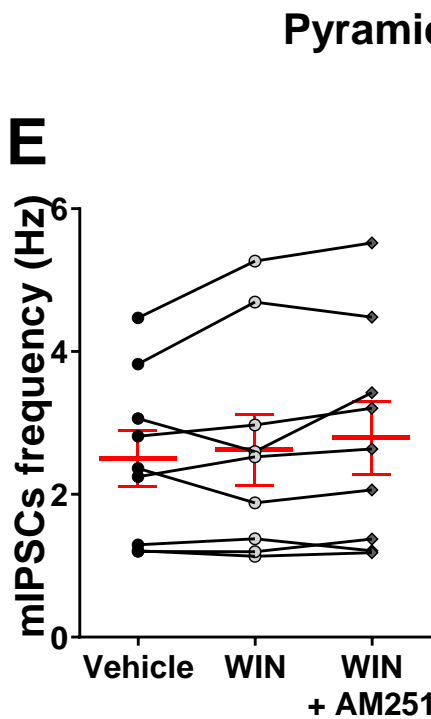
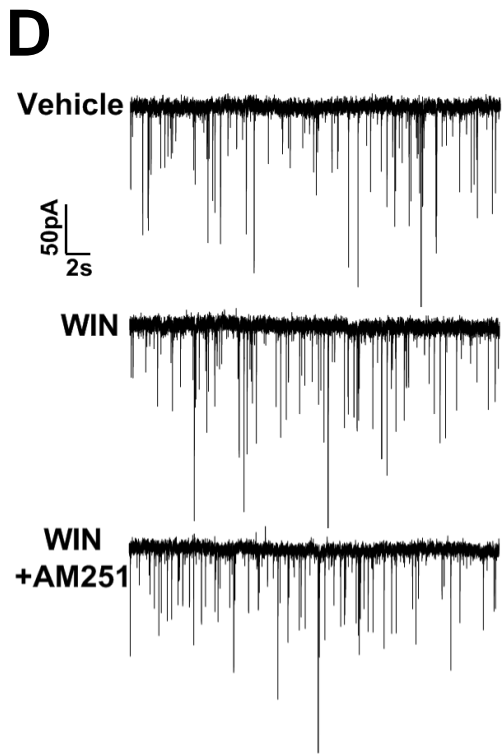
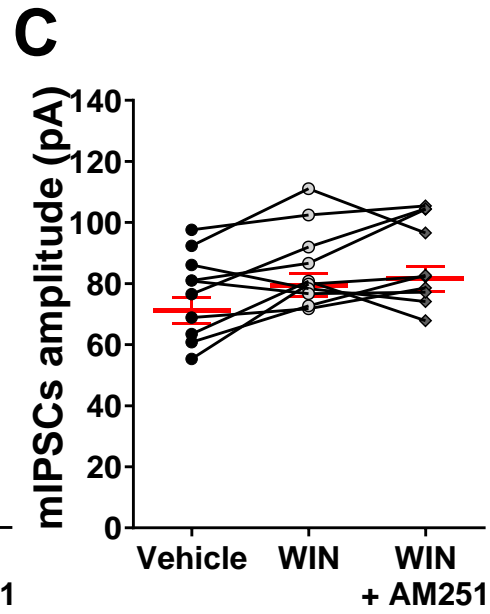
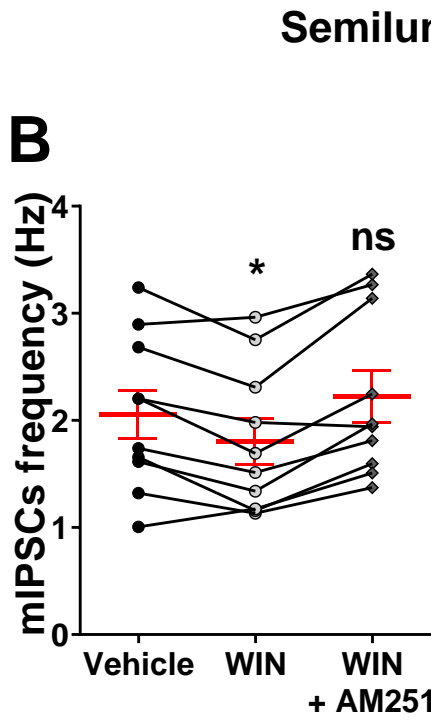
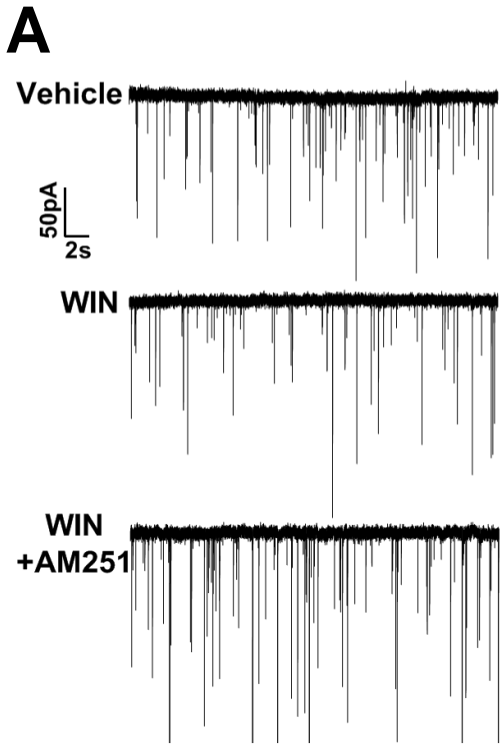
- 1 61. Reuveni, I., Lin, L., and Barkai, E. (2018). Complex-learning Induced Modifications in
2 Synaptic Inhibition: Mechanisms and Functional Significance. *Neuroscience* 381, 105–
3 114.
- 4 62. Zhan, C., and Luo, M. (2010). Diverse Patterns of Odor Representation by Neurons in
5 the Anterior Piriform Cortex of Awake Mice. *J. Neurosci.* 30, 16662–16672.
- 6 63. Sturgill, J.F., and Isaacson, J.S. (2015). Somatostatin cells regulate sensory response
7 fidelity via subtractive inhibition in olfactory cortex. *Nat. Neurosci.* 18, 531–535.
- 8 64. Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G.,
9 Hermann, H., Tang, J., Hofmann, C., Zieglgänsberger, W., *et al.* (2002). The
10 endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418,
11 530–534.
- 12 65. Paxinos, G., and Franklin, K.B.J. (2005). *The Mouse Brain in Stereotaxic Coordinates* 4th
13 Edition. (Academic Press).
- 14 66. Puente, N., Bonilla-Del Rio, I., Achicallende, S., Nahirney, P., and Grandes, P. (2019).
15 High-resolution Immunoelectron Microscopy Techniques for Revealing Distinct
16 Subcellular Type 1 Cannabinoid Receptor Domains in Brain. *Bio-Protoc.* 92 E3145.
- 17

A**B****C****D****E**

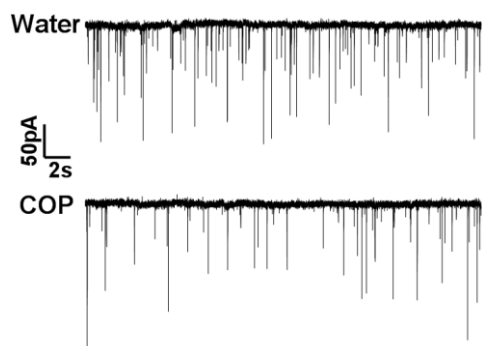




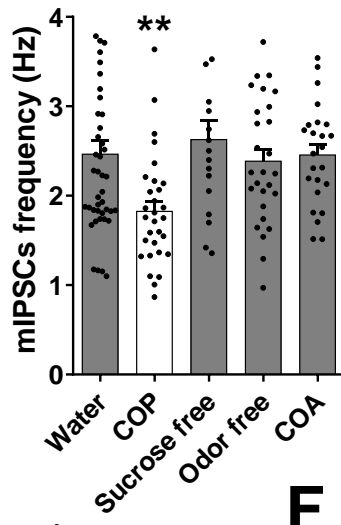




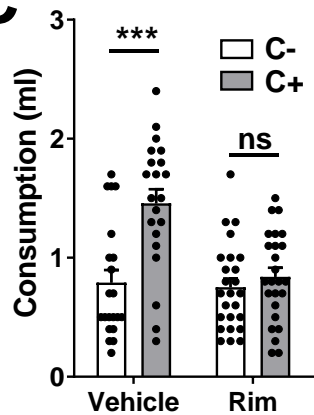
A Semilunar-like neurons



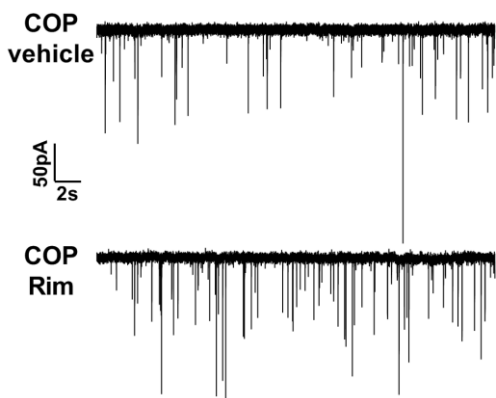
B



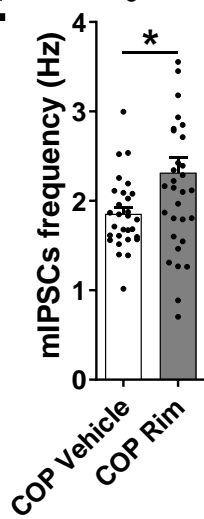
C



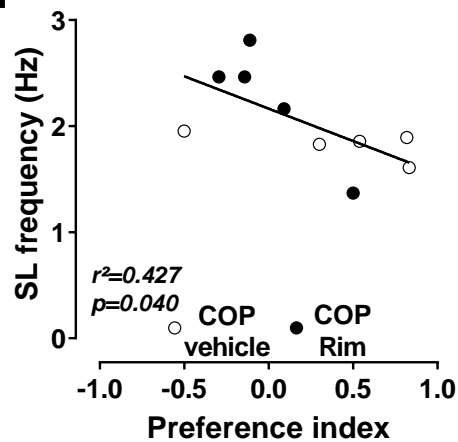
D Semilunar-like neurons



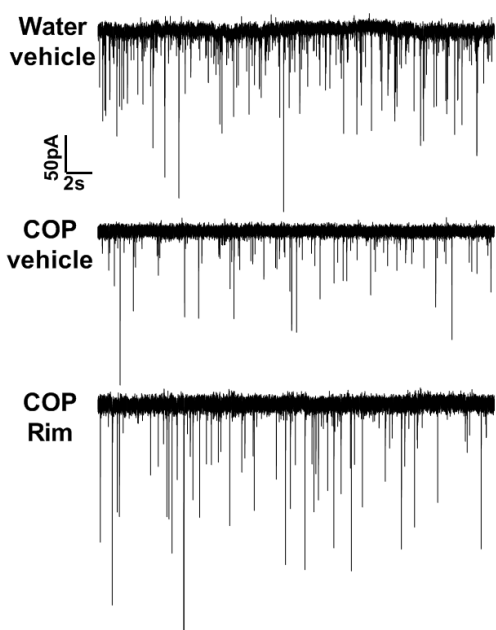
E



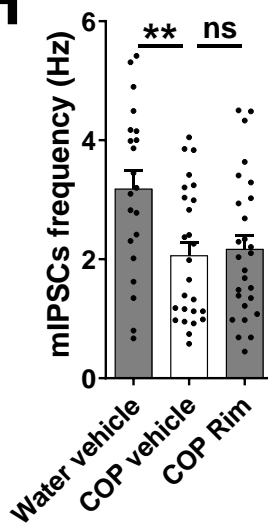
F



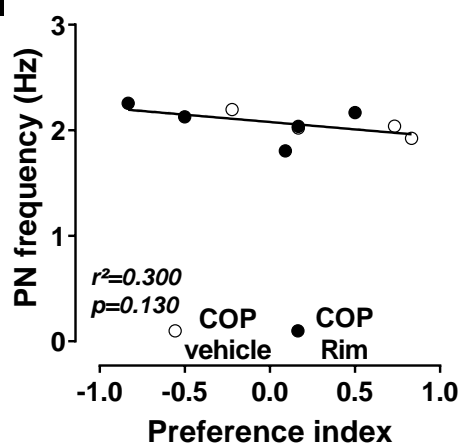
G Pyramidal-like neurons



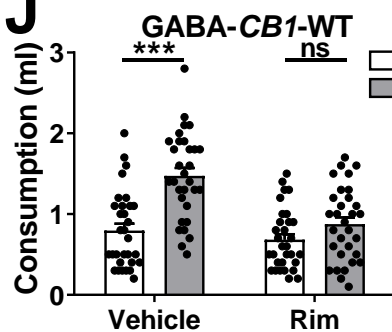
H



I



J



K

