Striatal GABAergic and cortical glutamatergic neurons mediate contrasting effects of cannabinoids on cortical network synchrony

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Activation of type 1 cannabinoid receptors (CB1R) decreases GABA and glutamate release in cortical and subcortical regions, with complex outcomes on cortical network activity. To date there have been few attempts to disentangle the region- and cell-specific mechanisms underlying the effects of cannabinoids on cortical network activity in vivo. Here we addressed this issue by combining in vivo electrophysiological recordings with local and systemic pharmacological manipulations in conditional mutant mice lacking CB1R expression in different neuronal populations. First we report that cannabinoids induce hypersynchronous thalamocortical oscillations while decreasing the amplitude of faster cortical oscillations. Then we demonstrate that CB1R at striatonigral synapses (basal ganglia direct pathway) mediate the thalamocortical hypersynchrony, whereas activation of CB1R expressed in cortical glutamatergic neurons decreases cortical synchrony. Finally we show that activation of CB1R in cortical glutamatergic neurons limits the cannabinoid-induced thalamocortical hypersynchrony. By reporting that CB1R activations in cortical and subcortical regions have contrasting effects on cortical synchrony, our study bridges the gap between cellular and in vivo network effects of cannabinoids. Incidentally, the thalamocortical hypersynchrony we report suggests a potential mechanism to explain the sensory “high” experienced during recreational consumption of marijuana.

Cannabis is a family of compounds that activate cannabinoid receptors, which are well known for their psychotropic effects and therapeutic potentials. The type 1 cannabinoid receptor (CB1R) is massively expressed in the brain at the synaptic terminals of excitatory and inhibitory neurons, and its activation by exogenous or endogenous cannabinoids decreases neurotransmitter release (1–3). One largely unanswered question is how the elementary decreases in excitatory and inhibitory synaptic transmissions induced by systemic intake of cannabinoids interact to produce alterations in neuronal network activity in vivo. A number of recent studies combining systemic injections of CB1R agonists and antagonists with electrophysiological recordings in the hippocampus and neocortex of awake or anesthetized rodents have shown that a hallmark of cannabinoids effects on neuronal network activity is a decrease in synchrony (4–9). Specifically, systemic CB1R activation has been shown to decrease (i) the amplitude of the hippocampal theta rhythm (4, 7, 8), (ii) the amplitude of gamma oscillations in the hippocampus (4, 7, 10), entorhinal cortex (8), and prefrontal cortex (7), (iii) the incidence of hippocampal ripples (4, 6, 11), and (iv) spiking correlation in the hippocampus and prefrontal cortex (4, 5, 7, 9).

In contrast to the consistent reports that cannabinoids dampen cortical network oscillations, other findings suggest that they could also increase synchrony. First, CB1R are predominantly expressed by GABAergic forebrain neurons (12), and their activation leads to decreased GABA release (1). Therefore, cannabinoids might be expected to increase network synchrony or generate excessive firing activity, in a similar manner to GABA receptor antagonists, which favor convulsive seizures (13, 14). Second, several studies have reported that cannabinoids are proconvulsant in experimental models of epilepsy (15–17). The concurrent but unbalanced activation of the CB1R at the synaptic terminals of inhibitory and excitatory neurons could be one explanation for these seemingly contradictory observations. Additionally, the high level of CB1R expression in subcortical regions (18–20) leaves open the possibility that at least a part of the cannabinoid-induced alterations in cortical network activity has extracortical origins. To date, the potential cell type- and region-specific impact of CB1R activation on in vivo cortical network activity has received little attention. Here we specifically addressed this issue by comparing the impact of systemic and local injections of the CB1R agonist CP55940 on cortical network oscillations recorded from freely moving mice lacking CB1R expression in distinct neuronal populations. The results reveal the cell- and region-specific mechanisms underlying a dual modulation of cortical synchrony by exogenous cannabinoids and pave the way to a refined understanding of the cognitive alterations associated with marijuana consumption (21, 22).

Results

Systemic CB1R Activation Induces Thalamocortical High-Voltage Spindles While Decreasing Fast Electrocorticogram Oscillations. To investigate in vivo the cell type-specific impact of systemic CB1R activation on cortical network activity, we recorded neocortical electrocorticograms (ECoG) before and after i.p. injections of the high-affinity CB1R agonist CP55940 (0.3 mg/kg of body weight). ECoG were recorded bilaterally above the somatosensory cortex, in the home cage of two groups of mice: C57BL/6N mice (n = 9) and conditional mutant mice that lack CB1R expression in specific neuronal populations [n = 33, including WT littermates (19, 23, 24)]. All ECoG shown and compared in this study were taken from moving mice lacking CB1R expression in distinct neuronal populations (CP55940 on cortical network oscillations recorded from freely moving mice lacking CB1R expression in distinct neuronal populations [n = 33, including WT littermates (19, 23, 24)]. All ECoG shown and compared in this study were taken from moving mice lacking CB1R expression in distinct neuronal populations (CP55940 on cortical network oscillations recorded from freely moving mice lacking CB1R expression in distinct neuronal populations [n = 33, including WT littermates (19, 23, 24)])

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the ECoG from C57BL/6N mice (Fig. 1). After CP55940 injections the ECoG displayed numerous oscillatory bursts, with a shape, frequency (∼5 Hz), length (∼1–2 s), and amplitude (> 0.5 mV) characteristic of thalamocortical high-voltage spindles (HVS, also referred to as spike-and-wave discharges/seizures) (Fig. 1 A–C; arrowheads in Fig. 1C) (25–27). As expected for this type of oscillation, HVS were sparse during control recordings (27) and, after CP55940 injections, were strictly restricted to periods of immobility (28). A quantitative routine developed to compare HVS before and after CP55940 injections (Experimental Procedures, Fig.

**Fig. 1.** Systemic CB1R activation induces HVS while decreasing the amplitude of faster neocortical oscillations. (**A–C**) Single experiment showing the effects of an i.p. injection of the CB1R agonist CP55940 (Right), or its vehicle alone (Left), on a mouse ECoG recorded during immobility epochs. (**A**) Thirty minutes ECoG (black traces) and HVS incidence (colored rasters). (Scale bars, 1 mV and 5 min.) (**B**) Thirty seconds ECoG taken from rectangles in A. (**Middle**) Time–frequency power spectrograms (1-s sliding windows, steps of 0.2 s). (**Bottom**) ECoG power values in the HVS frequency band (4–6 Hz). Dashed line is HVS detection threshold. Circles identify windows detected as part of an HVS. (**C**) Similar to B, but 10 s ECoG are from dashed rectangles in B. Arrowheads indicate detected HVS. ECoG traces in A–C have the same voltage scale. (**D and E**) HVS comparison in all experiments. Each line represents a single experiment, and different colors represent different animals. CP55940 (CP, 0.3 mg/kg) reliably increases HVS, and this effect was reversed (D) and abolished (E) by injection of the CB1R antagonist AM251 (AM, 3 mg/kg). (**F and G**) Similar to D and E, but the amplitude of ECoG oscillations faster than 12 Hz is compared. Same experiments and color code as in D and E. P values in D–G are from Wilcoxon paired two-sided signed rank test. Bold green traces in D and F show the quantification of the illustrative experiment shown in A–C.
1 B and C, and Fig. S2) showed that, across animals and experiments, HVS incidence and power reliably and strongly increased after CP55940 injections (Fig. 1D). This effect was dependent on CB1R activation because it was completely reversed (Fig. 1D and Fig. S3) and prevented (Fig. 1E) by i.p. injections of the CB1R antagonist AM251 (3 mg/kg). Cannabinoids have been previously shown to decrease the power of neocortical local field potential oscillations over a wide range of frequencies (7, 8). Therefore, we next quantified the effect of CP55940 on ECoG oscillations in a frequency band distinct from the one of the cannabinoid-induced HVS. As expected, CP55940 injections reliably decreased the amplitude of fast (>12 Hz) ECoG oscillations (Fig. 1F) (7, 8), an effect that was also reversed (Fig. 1F and Fig. S3) and blocked (Fig. 1G) by AM251 injections. Taken together these data show that systemic CB1R activation, although decreasing synchrony of fast ECoG oscillations, generated hypersynchronous oscillatory bursts characteristic of thalamocortical HVS.

CB1R Activation at GABAergic Striatonigral Synapses Increases the Incidence of Thalamocortical HVS. We next set out to understand how CP55940 i.p. injections increase HVS. Although rodent HVS function is still a matter of debate [analog to human how CP55940 i.p. injections increase HVS. Although rodent HVS incidence and power reliably and strongly increased after CP55940 injections (Fig. 1D), this effect was dependent on CB1R activation because it was completely reversed (Fig. 1D and Fig. S3) and prevented (Fig. 1E) by i.p. injections of the CB1R antagonist AM251 (3 mg/kg). Cannabinoids have been previously shown to decrease the power of neocortical local field potential oscillations over a wide range of frequencies (7, 8). Therefore, we next quantified the effect of CP55940 on ECoG oscillations in a frequency band distinct from the one of the cannabinoid-induced HVS. As expected, CP55940 injections reliably decreased the amplitude of fast (>12 Hz) ECoG oscillations (Fig. 1F) (7, 8), an effect that was also reversed (Fig. 1F and Fig. S3) and blocked (Fig. 1G) by AM251 injections. Taken together these data show that systemic CB1R activation, although decreasing synchrony of fast ECoG oscillations, generated hypersynchronous oscillatory bursts characteristic of thalamocortical HVS.

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To test whether these effects shared the same mechanism, we quantified the effect of CP55940 on the amplitude of fast ECoG oscillations in CB1R conditional mutant mice and their WT littermates. The CP55940-induced decrease in fast ECoG oscillations power was intact in D1-CB1R−/− mice (Fig. 4), suggesting that the cannabinoid-induced increase in HVS and decrease in fast ECoG oscillations have distinct mechanisms. In contrast, the decrease in fast ECoG oscillations was significantly reduced in Glu-CB1R−/− and CaMK-CB1R−/− mice compared with WT littermates (Fig. 4). These results show that activation of CB1R expressed by glutamatergic cortical neurons is, at least in part, responsible for the reduced neuronal network synchrony observed in vivo after cannabinoid injections. Additionally, CP55940-induced decrease in fast ECoG oscillations was stronger in GABA-CB1R−/− mice than in WT littermates (Fig. 4), suggesting that activations of CB1R expressed on cortical GABAergic and glutamatergic neurons exert a bidirectional control over fast ECoG oscillations.

A desynchronization function of CB1R expressed by glutamatergic cortical neurons is additionally supported by the observation that the cannabinoid-induced increase in HVS was stronger in Glu-CB1R−/− mice than in WT animals (Fig. 5 A–C). Indeed, CP55940 induced prominent HVS and high-amplitude isolated spike-and-wave complexes in the mutant mice (Fig. 5 D and E). This result suggests that, in WT animals, activation of CB1R expressed by glutamatergic cortical neurons limits the increase in HVS observed after CP55940 injection.

**Discussion**

Here we report that cannabinoids have contrasting effects on neocortical network synchrony, characterized by the appearance of hypersynchronous thalamocortical oscillations and decreased amplitude of fast ECoG oscillations. Taking advantage of conditional mice lacking CB1R expression in specific neuronal populations, we broke down these effects into region- and cell-specific CB1R activation: CB1R at striatonigral inhibitory synapses are responsible for the cannabinoid-induced thalamocortical hyper-synchrony, whereas CB1R activation at cortical excitatory synapses reduces synchrony of fast neocortical oscillations.

![Fig. 4.](image-url) Cannabinoid-induced decrease in fast ECoG oscillations power is reduced in mice lacking CB1R in glutamatergic cortical neurons. (A) Effects of vehicle and CP55940 (CP) injections on the amplitude of fast (>12 Hz) ECoG oscillations in, from left to right, WT, D1-CB1R−/− mice, mice lacking CB1R in glutamatergic cortical neurons (Glu-CB1R−/−), in all principal forebrain neurons (CaMK-CB1R−/−), and in all GABAergic forebrain neurons (GABA-CB1R−/−). (B) Mean changes in HVS and SEM for WT and mutant mice. *P* values are from Wilcoxon rank sum test vs. WT data. Dotted lines represent mean change in the WT group.

![Fig. 5.](image-url) CB1R expressed by glutamatergic cortical neurons limit the cannabinoid-induced increase in HVS. (A and B) Effects of vehicle and CP55940 (CP) injections on HVS incidence and power in WT (A) and Glu-CB1R−/− (B) mice. (C) Comparison of the mean changes in HVS for these mice. (D) Single experiment in which ECoG (black) was recorded from a Glu-CB1R−/− mouse after vehicle (Upper) and CP55940 (Lower) i.p. injections. Colored rasters show detected HVS. (Scale bar, 5 min.) (E) Upper trace is taken from the rectangle in D. Arrowheads show two HVS. The two lower traces are taken from the upper trace (1, 2) and show, respectively, a strong HVS and isolated spike-and-wave seizure-like discharges. (Scale bars, 1 s and 0.75 mV.)
We found that in immobile mice, systemic CB1R activation generated highly synchronous oscillatory bursts of the ECoG with a frequency, duration, and amplitude characteristic of thalamocortical HVS (25, 26, 40). We further showed that this hypersynchronous effect was mediated by CB1R located in the SNr on striatal GABAergic synaptic terminals. Finally, intra-SNr injections of GABA-A agonist and antagonist, respectively, reversed and mimicked the effects of CP55940 on HVS. Altogether, our results are best explained by the following mechanism: systemic CB1R activation induces thalamocortical HVS by decreasing GABA release at striatonigral synapses (Fig. 6). This hypothesis is supported by previous studies on CB1R synaptic physiology in the basal ganglia and the known role of the SNr in controlling thalamocortical oscillations. First, the highest level of CB1R immunoreactivity in rodent is found in the SNr (18). In this nucleus, CB1R is localized on axons and terminals of striatal neurons (20), and its activation potently inhibits striatonigral GABAergic transmission (20, 39). Second, the basal ganglia are known to modulate thalamocortical oscillations (28, 36). Specifically, hyperpolarization of thalamocortical relay cells is a condition sine qua non for the emergence of thalamocortical oscillations (13, 31, 34), and the giant inhibitory synapses between GABAergic neurons of the SNr and thalamocortical relay cells have been proposed to contribute to this hyperpolarization (37). In agreement with an oscillation-promoting role of nigrothalamic cells, pharmacological excitation or inhibition of SNr neurons increased or decreased HVS incidence, respectively (38, 41). Finally, dopamine depletion or blockade of dopamine receptors in the striatum, which decreased the activity of direct pathway striatal neurons (42), also enhanced the incidence of HVS (28, 43). Altogether these studies suggest that the cannabinoid-induced increase in HVS we report is best explained by the disinhibition of nigrothalamic GABAergic neurons (Fig. 6).

In mice lacking CB1R on glutamatergic cortical neurons (Glu-CB1R−/−), the cannabinoid-induced increase in HVS was stronger than in their WT littermates, and CP55940 generated a seizure-like pattern of activity. This result suggests that in WT mice, striatonigral and cortical CB1R compete and modulate thalamocortical oscillations in opposite directions. The partial counterbalancing effect of “cortical” CB1R could be due to a decrease in release of glutamate in the neocortex, in the thalamus (from corticothalamic excitatory neurons), or in both areas. This is in agreement with a role of cortical input on the thalamic reticular neurons to control thalamocortical HVS oscillations (34, 44). A role for CB1R expressed by cortical glutamatergic neurons in reducing network synchrony is further supported by the observation that cannabinoids-induced decrease in fast neocortical oscillations was reduced in Glu- and CaMK-CB1R−/− mice. This function is in line with studies showing that “glutamatergic cortical” CB1R provide protection against kainic acid-induced seizures (23, 24) and with recent works suggesting that cannabinoids decrease hippocampal γ and ripple oscillations by decreasing excitatory transmission (6, 11). CB1R expressed by brain astroglial cells have been recently shown to participate in the control of glutamatergic synaptic transmission and plasticity (45–47). Our results suggest that the effects of CP55940 on fast ECoG oscillations are mostly independent from astroglial CB1R: in fact, at odds with astroglial CB1R-dependent long-term depression of excitatory synaptic transmission (47), CP55940 effects were reversed by injections of a CB1R antagonist and were significantly reduced in Glu-CB1R−/− mice. Still, at this stage, we cannot entirely exclude that astroglial CB1R contribute to the decrease in fast ECoG oscillations, and future investigation will address directly this possibility.

There are several possible specific behavioral implications of our findings. First, the reported dual pro- and antioscillatory functions of CB1R expressed in, respectively, subcortical and cortical regions might be related to the complex control of network excitability exerted by CB1R in physiopathological conditions, such as during epileptiform seizures (15–17, 23, 48). Additionally, we believe that the increase in thalamocortical HVS after CB1R activation in the SNr offers a perspective to understand the psychoactive effects associated with marijuana consumption. In this context, it is important to emphasize that thalamocortical HVS have been proposed to constitute a brain state that facilitates detection of weak sensory stimulation (29, 49). More generally, the activity of the thalamocortical system controls vigilance states and gates the perception of sensory stimulation (50). The recreational consumption of marijuana is well known to produce a “high” characterized by an altered consciousness and an intensification of sensory perceptions (51). Strikingly, in both human and rodent brains, the highest expression of CB1R is found in the SNr on striatonigral synapses (18, 20, 52, 53). Therefore, an exciting hypothesis for future investigation is that the sensory/behavioral “high” experienced during marijuana consumption is due to an aberrant thalamocortical synchrony via massive CB1R activation in the SNr.

Experimental Procedures

Experimental procedures are described in SI Experimental Procedures. This section describes the conditional CB1R mutant mice used, electrophysiological recording methods, local and systemic pharmacological injections, and data analysis. All animal procedures were conducted in accordance with standard ethical guidelines (European Communities Directive 86/609-EEC) and were approved by the local ethical committee (Comité d’Expérimentation Animal, Universitat de Barcelona, Ref 520008).

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Animals. In vivo chronic electrocorticogram (ECoG) recordings were performed in three groups of animals: (i) 9 C57BL/6N (Charles River), (ii) conditional type 1 cannabinoid receptor (CB1R) mutant mice (4 Glu-CB1R<sup>−/−</sup>, 5 GABA-CB1R<sup>−/−</sup>, 4 CaMK-CB1R<sup>−/−</sup>, and 7 D1-CB1R<sup>−/−</sup>), and (iii) 13 WT littermates (1–3). All mice (male and female) were aged 4–7 mo. Regarding ECoG baseline activity and CP55940 effects on ECoG, no differences between sexes were observed. Similarly, no differences were observed between WT littermates of the four different mutant lines. Therefore, all WT animals were pooled into a single group for statistical comparison.

Surgical Procedures. All surgical procedures were performed in accordance with standard ethical guidelines (European Communities Directive 86/609/EEC) and were approved by the local ethics committee (Comitè d’Ex- perimentació Animal, Universitat de Barcelona, Ref 520/08).

Supporting Information

SI Experimental Procedures

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Animals. In vivo chronic electrocorticogram (ECoG) recordings were performed in three groups of animals: (i) 9 C57BL/6N (Charles River), (ii) conditional type 1 cannabinoid receptor (CB1R) mutant mice (4 Glu-CB1R<sup>−/−</sup>, 5 GABA-CB1R<sup>−/−</sup>, 4 CaMK-CB1R<sup>−/−</sup>, and 7 D1-CB1R<sup>−/−</sup>), and (iii) 13 WT littermates (1–3). All mice (male and female) were aged 4–7 mo. Regarding ECoG baseline activity and CP55940 effects on ECoG, no differences between sexes were observed. Similarly, no differences were observed between WT littermates of the four different mutant lines. Therefore, all WT animals were pooled into a single group for statistical comparison.
Power Spectrum Analysis. Spectral analyzes were made using a multitaper approach as implemented in the Chronux toolbox (http://chronux.org/). ECoG was digitally high-pass filtered at 1 Hz. Frequency-domain analysis was restricted to data recorded during immobility periods. Power spectrum density functions were computed with nine tapers and averaged over 10-s–long epochs.

High-Voltage Spindles Comparison. Comparison of high-voltage spindles (HVS) incidence and power between control and drug conditions was made from ECoG (25–30 min long) recorded during periods of immobility (i.e., periods of immobility, interleaved by behavioral activity, were concatenated until they amounted to 25–30 min). The rationale for comparing HVS over such relatively short periods was to avoid as much as possible the inclusion of slow-wave sleep in our control recordings. During immobility, the incidence of HVS in mice was extremely low in control condition but strongly and reliably increased after CP55940 systemic injection (6). Noticeably, across experiments and animals, we found variability in the mean frequency of the HVS appearing after CP55940 injections (range, 4–8 Hz). The first step of our HVS quantification consisted therefore in determining the cannabinoind-induced HVS frequency band. To avoid ECoG power at low frequencies (<3 Hz) contaminating the estimation of the HVS frequency band (owing to the presence 1/f pink noise in this type of signal), the ECoG obtained after CP55940 injection was detrended (locdetrend function in Chronux toolbox, 300-ms–long moving windows). The average power spectrum density function of the detrended ECoG was computed as described earlier. The HVS frequency band was defined as a 2-Hz–wide window centered around the peak of the power spectrum in the drug condition (Fig. S2A, I). Using a distinct method (7) to reduce 1/f pink noise in the ECoG resulted in similar results. Note that ECoG detrending was only done to define the HVS frequency band. CP55940 globally reduced the amplitude of the ECoG fluctuations (Fig. 1). To avoid this effect affecting HVS detection, the ECoGs to compare were standardized. Time–frequency spectrograms of the standardized ECoGs were computed (1–s–long windows, advanced in steps of 0.2 s; examples in Fig. 1C and Fig. S2B). For each window, the power in the HVS frequency band was calculated (Fig. S2 A, 2). An arbitrary threshold for HVS detection was then defined as the mean plus 2 SDs of the HVS frequency band power values obtained in all of the windows (vehicle + CP55940, Fig. S2 A, 3). HVS were defined as the times when four or more consecutive windows had power values in the HVS frequency band superior to the threshold (Fig. 1 B and C and Fig. S2 A, 4 and B). Finally, the time–frequency spectrograms of the ECoGs (nonstandardized) were computed as above. In the pharmacological conditions to compare (e.g., vehicle vs. CP55940), we computed the sum of the ECoG power in the HVS frequency band from the windows detected as part of an HVS (Fig. S2 A, 5). These sums provide a quantification of HVS incidence and power during a single experiment. It is very important to note that the threshold is a relative value that depends on the intraexperiment variability of the ECoG power in the HVS frequency band. For instance, a strong increase in HVS after CP55940 injection would bias the threshold toward a high value. Consequently, the number of detected HVS after vehicle injection would be low. Conversely, if a CP55940 injection had little effect on HVS (as in the D1-CB1R−/− mice), the threshold would be low and more HVS would be detected in the control recording (this is the reason why baseline HVS incidence is higher in Fig. 3C than in Fig. 3A). Using this “relative” method to compare HVS in a given pharmacological condition (e.g., vehicle) between different animals or experiments is therefore meaningless. Only change in HVS power and incidence (calculated as the ratio “HVS in CP55940”/“HVS in vehicle”) were compared across animals.

Statistics. To statistically assess electrophysiological differences between pharmacological conditions (e.g., vehicle vs. CP55940) in a given group of animals (e.g., WT mice) we used the Wilcoxon paired signed rank test (nonparametric test). To statistically assess differences in CP55940 effect between two groups of animals (e.g., WT vs. Glu-CB1R−/− mice) we used the Wilcoxon rank sum test (nonparametric test).

Fig. S1. Separation of immobility from mobility periods. (A and B) Illustrative experiment during which ECoG (black) and vertical head acceleration (gray) were recorded simultaneously. Orange and green lines show detected periods of activity and immobility, respectively. (A) Full recording; (B) 10-s epoch taken from A, at the time indicated by the dash lines. (C) Power spectrum density function of the ECoG during immobility and activity (same color code as in A and B). Note that the strong increase in ECoG power above 30 Hz during behavioral activity is partially accounted by contamination (via passive volume conduction) of the ECoG with electromyographic signals (mainly showing artifacts).

Fig. S2. Method to compare the incidence and power of HVS after vehicle and CP55940 injections. (A, 1) Same-length ECoGs, recorded during immobility epochs, are compared after systemic injections of vehicle and CP55940. Power spectra of the detrended ECoGs are computed, and HVS frequency band is determined. (A, 2) Time-frequency spectograms are computed from the standardized ECoG, and the time-varying power in the HVS frequency band is reported. (A, 3) Threshold detection is set as the mean plus 2 SDs of all the HVS band power values (vehicle + CP55940). (A, 4) HVS are defined as at least four consecutive windows with power values above threshold. (A, 5) The sum of the ECoG power (in the HVS band) from the windows detected as part of an HVS is computed for each pharmacological condition and compared. (B) Examples of detected HVS. (Top) Traces are ECoG epochs taken from A, at times indicated by stars. (Middle) Time–frequency spectograms. White dotted lines show HVS frequency band for this experiment. (Bottom) ECoG power in the HVS frequency band. Dashed lines represent the threshold and circled points the values detected as part of a HVS.
CP55940 effects on HVS and fast ECoG oscillations are still present 2 h 45 min after injection and are reversed by injection of a CB1 antagonist. (A) HVS were compared in 30-min-long epochs (during immobility) starting 15 min after vehicle and both 15 min and 2 h 30 min after CP55940 injections (0.3 mg/kg, \( n = 6 \) experiments in six mice). (B) Same experiments as in A, but the amplitudes of fast ECoG oscillations (>12 Hz) were compared. \( P \) values in A and B are from Wilcoxon paired two-sided signed rank test. (C) Average ratio between late and immediate effects of CP55940 on HVS. Comparison is made between experiments in which only CP55940 was injected (left bar, same data as in A) and experiments in which an i.p. injection of AM251 was made 1 h after CP55940 injection (right bar, same data as in Fig. 1D). In both conditions late effects were quantified starting 2 h 30 min after the CP55940 injection. \( P \) value is from the Wilcoxon ranksum test. (D) Similar comparison as in C, but for the amplitude of fast (>12 Hz) ECoG oscillations. Left bar is calculated from data in B, whereas right bar is from the same data as in Fig. 1F.

Effect of CP55940 on HVS is abolished in mice lacking CB1R expression in forebrain GABAergic neurons (GABA-CB1R\(^{-/-}\)) and forebrain principal neurons (CaMK-CB1R\(^{-/-}\)). (A) HVS comparison in all experiments. Each line represents a single experiment, and different colors represent different animals. CP55940 (0.3 mg/kg) reliably increases HVS in WT animals. Such effect is absent in GABA-CB1R\(^{-/-}\) mice and small in CaMK-CB1R\(^{-/-}\) mice. \( P \) values are from Wilcoxon paired two-sided signed rank test. (B) Mean HVS change (CP55940/vehicle) + SEM for all experiments. \( P \) values are from Wilcoxon rank sum test vs. WT data. Note that in GABA-CB1R\(^{-/-}\) and CaMK-CB1R\(^{-/-}\) mice, CB1R expression is absent from D1-positive striatonigral neurons.
Fig. S5. Relative ECoG oscillatory content in control condition recorded from WT and CB1R conditional mutant mice. (A, Left) ECoG power spectrum density function of a representative WT mouse, in control condition. Three frequency bands (1–4, 4–10, 10–20 Hz) were arbitrarily defined. ECoG was high-pass filtered above 1 Hz. The three histograms show the ECoG relative power in the three bands during baseline or vehicle recordings for all experiments displayed in Figs. 2, 4, 5, and Fig. S4. P values are from Wilcoxon rank sum test vs. WT and were not graphically reported if above 0.05. (B) Same data as in A, except that the 1/f power relationship was removed from the ECoG. In this case, the relative power in the lowest frequency band is meaningless and is not shown. Note that there was no significant difference between WT and CB1 conditional mutant mice in the 10- to 20-Hz band.