SCIENCE FACULTY

MASTER PROGRAM IN BIOLOGICAL SCIENCES

MENTION NEUROSCIENCE

CB1 RECEPTOR LOCATED IN GABAERGIC NEURONS EXPRESSING SOMATOSTATIN REGULATE SYNAPTIC TRANSMISSION IN THE PREFRONTAL CORTEX

Nicole Sanguinetti González

Thesis to opt for Master Degree in Biological Sciences

Mention Neuroscience

Thesis Director:

Dra. Chiayu Chiu

2019

INDEX

ABSTRACT

Endocannabinoids (eCBs) are potent modulators of synaptic function throughout the central nervous system (CNS). Alterations in the eCB system are observed in several neuropsychiatric disorders, including schizophrenia and attention deficit disorder, prompting the suggestion that eCBs modulate emotional and cognitive processes in associative areas such as the prefrontal cortex (PFC). However, the specific neural circuits and cell types participating in eCB-dependent signaling in the PFC have not been well elucidated. Given the importance of synaptic inhibition in shaping neural activity, we study the potential impact of eCBs at GABAergic synapses from a major population of dendrite-targeting interneurons that express somatostatin (SOM-INs) in the PFC. Using optogenetic tools to selectively activate SOM-INs in acute brain slices, we investigate whether GABA release from SOM-INs are regulated by eCB signaling through activation of its canonical type 1 cannabinoid receptor (CB1R). We found that inhibition onto cortical pyramidal cells evoked by optical stimulation of channelrhodopsin 2 (ChR2)-expressing SOM-INs are depressed by WIN 55,212-2, a potent agonist of CB1Rs, in control mice. Next, we generated mice lacking CB1Rs in SOM-INs (SOM-CB1R KO) and confirmed the absence of WIN-depression at these inhibitory synapses. We further assessed the impact of removing CB1Rs from SOM-INs on inhibitory synaptic transmission. Input-output curves of electrically stimulated inhibitory responses (eIPSCs) in layer 2/3 pyramidal neurons suggest an upregulation of dendritic inhibition in SOM-CB1R KO mice compared to littermate controls. In support of this interpretation, eIPSCs in SOM-CB1R KOs also showed stronger paired pulse depression compared to controls. Moreover, spontaneous

inhibitory activity increased in frequency but not amplitude in SOM-CB1 KOs compared to control mice. Together, these results suggest an important contribution of eCBs to control GABAergic inhibition from SOM-INs. By targeting dendritic regions of pyramidal cells where excitatory synapses congregates, SOM-INs can profoundly modulate synaptic integration to influence principal cell output. Thus, through its effect on inhibition mediated by SOM-INs, the eCB system would be able to fine-tune information flow in the PFC from sources such as the thalamus, hippocampus and amygdala to shape associative cognitive processing.

INTRODUCTION

Communication between neurons is the cellular basis of thinking and movement control. Information processing in normal brain function requires balanced activity between excitatory and inhibitory cells (E-I balance). Disruption of this E-I balance has been linked to a number of neuropsychiatric disorders, including schizophrenia, autism and epilepsy [1][2] , although the underlying mechanisms are unclear.

Endocannabinoids (eCBs), powerful regulators of neural activity throughout the central nervous system (CNS) [3] have been suggested to contribute to maintaining and disrupting E-I balance. Indeed, eCB signaling plays key roles in short-term and long-term plasticity of excitatory and inhibitory synaptic transmission in numerous brain regions [4]. In the prefrontal cortex (PFC), alterations in the eCB system have been associated with neuropsychiatric disorders that reflect PFC dysfunction [5] and preclinical data has demonstrated that eCB signaling is important in regulating stress, emotional processing and cognitive functions [6].

THE ENDOCANNABINOID SYSTEM

The eCB system comprises the eCBs such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG) [7][8], the cannabinoid type 1 and type 2 receptors (CB_1Rs and CB_2Rs), the enzymes responsible for eCB synthesis and metabolism, and transporters that regulate eCB levels in the synaptic cleft [9] eCBs are a group of lipid molecules that get their name from the exogenous cannabinoid Δ9-tetrahydrocannabinol (Δ9-THC), the main psychoactive component in cannabis [3]. They are produced on demand via increased intracellular Ca^{2+} at postsynaptic sites in response to prolonged synaptic activity [4][10].

AEA and 2-AG are the most prominent eCBs [1]. Both are derivatives of arachidonic acid [11],but they differ in their biosynthesis and degradation pathways, as well as affinity for receptor binding [12]. 2-AG is considered a major eCB given that it is found at high concentrations in the brain and is an efficacious eCB ligand, acting as a specific cannabinoid receptor agonist [8][13]. The synthesis of 2-AG occurs through the phosphoslipase (PLC) β- diacylglycerol lipase (DGL) pathway after the activation of Gq/11-coupled receptors such as the muscarinic M1/M3 or group I metabotropic glutamate (mGluR-I) receptors [13][11]. PLCβ hydrolyzes phosphatidylinositol to generate diacylglycerol, which is converted to 2-AG by diacylglycerol lipase α (DGLα) specifically localized in postsynaptic compartments [14][15]. Interestingly, 2-AG production can also be triggered by Ca^{2+} influx via voltage gated Ca^{2+} channels (VGCCs), although the precise mechanisms are unknown. There are two enzymes that metabolizes 2-AG: 1) monoacylglycerol lipase (MGL) that is found more heterogeneously across synapses [16]

and controls the duration and magnitude of 2-AG-mediated synaptic plasticity [17] and 2) serine hydrolase ABHD6 that is located postsynaptically and catabolizes a small fraction of 2-AG [18].

AEA is a nonselective partial agonist of CB_1Rs and CB_2Rs [13] and is therefore a ligand with efficacy lower than 2-AG. The synthesis of AEA occurs after postsynaptic depolarization and intracellular $Ca^{\acute{}2+}$ influx [19] by N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase-D (NAPE-PLD), and the enzyme primarily responsible for its degradation is the fatty acid amide hydrolase (FAAH) [20]. The metabolism of 2-AG and AEA can be decreased with MGL (URB602) and FAAH inhibitors (URB597), respectively. AEA has been shown to contribute to eCB-mediated synaptic transmission in several ways. It is a full agonist of transient receptor potential vanilloid type 1 (TRPV1), a superfamily of non selective cationic channels which participates in eCB-dependent synaptic modulation [21]. AEA-mediated LTD (via a TRPV1-dependent mechanism) has been reported in several studies [22]. The differential recruitment of 2-AG and AEA by various types of presynaptic activity has been described in the extended amygdala [23]. AEA negatively regulates 2-AG metabolism, the effect of which can be mimicked by the activation of TRPV1 [24]. There is also evidence supporting a tonic production of AEA, since chronic blockade of FAAH leads to constant agonism of the eCB system [25].Increasing AEA levels in this way does not cause a decrease in CB1R expression, in contrast to the effect observed with raising 2-AG levels by MAGL antagonism. Hence, AEA and 2-AG is likely more associated with signaling by TRPV1 channels and CB_1Rs, respectively.

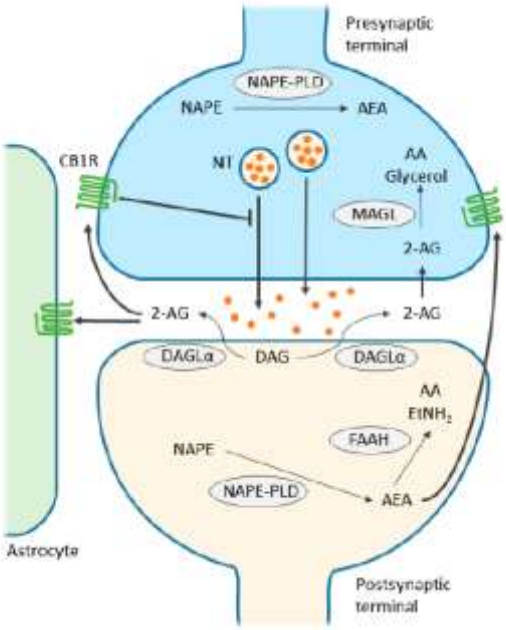

Figure 1.Representative scheme of eCB retrograde signaling mediated synaptic transmission. 2-arachidonolglycerol (2-AG) is biosynthesized from diacylglycerol (DAG) by diacylglycerol lipase-α (DAGLα), and anandamide (AEA) is synthesized from N-acyl-phosphatidylethanolamine (NAPE) by NAPE-specific phospholipase D (NAPE-PLD). (Adapted from Zou, 2018, [11])

THE CANNABINOID RECEPTORS

The CB_1R is expressed predominantly in the CNS, whereas the CB_2R is mostly present peripherally in the immune system [26]. Both are seven transmembrane receptors that are coupled to the pertussis toxin sensitive G protein, Gi/o [9]. Activation of either cannabinoid receptor reduces adenylyl cyclase (AC) activity, thus lowering cAMP levels and protein kinase A (PKA) activity. CB_1Rs are present at presynaptic and axonal compartments, restricting their function to sites of synaptic activity [27][28][29]. The CB_1R binds Δ^9-THC and mediates most of the CNS effects [30]. In addition, they bind synthetic

cannabimimetic compounds such as CP55940, JWH-015, WIN55212-2 and the endogenous compounds AEA and 2-AG [9].

The CB_1R is one of the most highly expressed G protein-coupled receptors in the brain [31] and is widely distributed in many brain areas embodying the cortico-limbic system like prefrontal cortex, hippocampus, cerebellum, striatum and amygdala [32]. In these areas, CB_1Rs are present in many different cell types, and their levels of expression are variable. Notably, cortical GABAergic presynaptic terminals contain prodigious levels of the CB1R protein, whereas cortical glutamatergic neurons have lower levels of these receptors [33].Nevertheless, the high expression of CB1Rs at presynaptic terminals suggest a role of eCBs in the regulation of synaptic efficacy and neuronal activity [12].

ENDOCANNABINOID SIGNALING IN THE CNS

The eCB system is one of the key regulatory mechanisms in the brain controlling multiple events such as mood, pain perception, learning and memory among others [34][1]. It is also thought to provide a neuroprotective role during traumatic brain injury (TBI) and may be part of the natural brain compensatory repair mechanism during neurodegeneration [35][36][37]. The principal mechanism by which eCBs work in the brain is by regulating synaptic function through retrograde signaling, as observed in short-term and long-term forms of synaptic plasticity [4] that contribute to learning and memory [38] [39] (see Figure 2). eCBs are released from neurons in an activity-dependent manner, act retrogradely on presynaptic CB_1Rs and modulate transmitter release [40]. eCB mobilization can be triggered either by strong neuronal depolarization [41] or by activation of phospholipase Cβ (PLCβ) [42] through Gq-coupled receptors such as group I metabotropic glutamate receptors (mGluR-I) and M1/M3 muscarinic acetylcholine receptors [43] [44]. More recently, it has been revealed that eCBs can act in non-retrograde ways, where the postsynaptically produced eCBs activate postsynaptic CB_1Rs or TRPV1 channels, which are involved in the transduction of remarkably diverse stimuli, including temperature, pH, mechanical and osmotic pressure, taste, xenobiotic substances, and endogenous lipids [45]. Moreover, eCBs can signal via astrocytes by triggering gliotransmission to indirectly modulate presynaptic or postsynaptic function [3].

The eCB system underlies a number of short- and long-term forms of synaptic plasticity at both excitatory and inhibitory synapses in several brain areas [1]. Short-term

depression (STD) is commonly triggered by depolarization of the postsynaptic cell, leading to a short-lived calcium dependent mobilization of eCBs and transient CB1R activation. This process is called depolarization-induced suppression of inhibition (DSI) or excitation (DSE) depending on the inhibitory or excitatory nature of the target synapse [40]. The mechanism involves direct G protein-dependent inhibition of presynaptic Ca+2 influx through VGCCs [46] or facilitation of inward-rectifying K^+ (IRK) channels. eCB-mediated long-term depression (eCB-LTD) is most commonly initiated following repetitive glutamatergic synaptic activity and involves modulation of the presynaptic vesicular release machinery [10]. The predominant mechanism of this form of plasticity requires inhibition of AC and downregulation of the cAMP/PKA pathway via the α i/o limb [10]. CB_1Rs are needed during the induction phase of eCB-LTD that requires combined presynaptic firing with CB1R activation [47]. The expression phase of eCB-LTD involves presynaptic proteins Rab3B/RIM1a [48] or a reduction of VGCCs [49]. The induction of eCB-LTD has been described in several brain regions at both excitatory synapses (E-LTD) and inhibitory synapses (I-LTD)[10]. These forms of plasticity are thought to be the cellular basis of learning and memory.

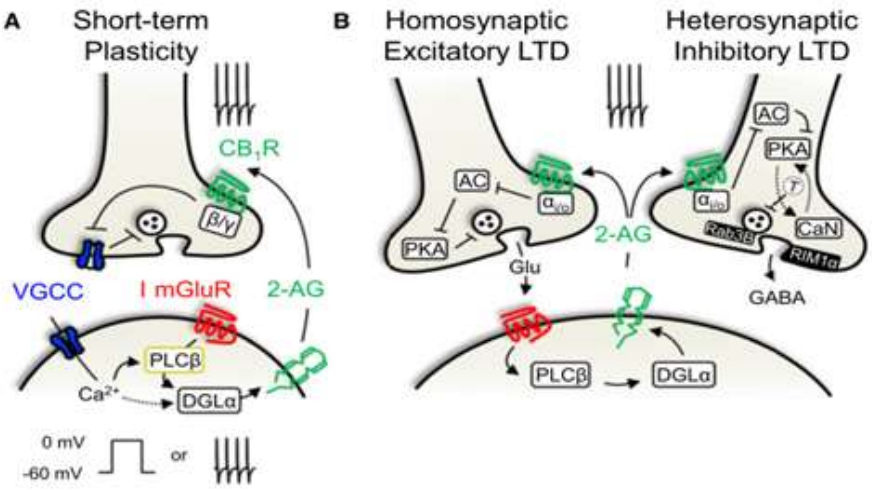

Figure 2.Molecular mechanisms underlying endocannabinoid-mediated short- and long-term synaptic plasticity. (A) Short-term depression. (B) eCB-mediated excitatory long-term depression (E-LTD) and inhibitory LTD (I-LTD). (Adapted from Castillo et al. [3]).

ENDOCANNABINOID AT INHIBITORY SYNAPSES

Studies of synaptic plasticity have largely focused on changes to excitatory glutamatergic connections, including both long-term depression and potentiation. Less is known about the plasticity of inhibitory GABAergic synapses in the mammalian brain where these connections exhibit diverse forms of long-term plasticity. Activation of CB1Rs in neocortex have been shown to suppress inhibitory transmission by reducing GABA release [50][51]. Interestingly, the formation of inhibitory synapses can be directed by excitatory synaptic activity on the same dendrite, a mechanism that requires CB1Rs and postsynaptic activation of DGL, which produces 2-AG [52]. Thus, although CB1Rs are abundantly expressed in the prefrontal cortex (PFC), little is known about the specific neural circuits through which eCBs signal and how eCBs regulate PFC function.

eCB-mediated synaptic plasticity at GABAergic synapses is most well-known for the cholecystokinin-expressing (CCK-) interneurons (INs) in the hippocampus [53] [54] [55] [56].However, recent work suggest three principal groups of INs in the cortex: cells co-expressing either the Ca^{2+} binding protein parvalbumin (PV), the neuropeptide somatostatin (SOM), or the iontotropic serotonin 5HT3A receptor (5HT3AR) [57]. PV-INs account for ~40% of GABAergic neurons and includes fast spiking basket cells and chandelier cells. SOM-INs represent ~30% of GABAergic neurons and includes the Martinotti cells, a set of neurons that target distal dendritic regions. The 5HT3AR group, accounts for ~30% of the total IN population and includes the CCK-INs. Considering that CCK-INs make up a small percent of the GABAergic population in the PFC, the impact of the eCB system on PFC function is unclear. We wondered whether inhibition from GABAergic cells others than CCK-INs are under eCB regulation in the PFC. Because the

majority of GABAergic synapses are made on postsynaptic dendrites [58][59], where they regulate the integration of incoming excitatory synaptic signals, we aimed to investigate the ability of eCBs to modulate synaptic transmission from SOM-INs, a major population of dendrite-targeting interneurons in the prefrontal cortex.

The rich diversity of GABA-producing cell types mediates widely distributed and precisely positioned inhibition. The specific subdomain of GABAergic contact (e.g. dendrite, soma or axon) critically determines the impact of synaptic inhibition in shaping neuronal activity in the neocortex. SOM-INs primarily target distal dendrites of excitatory neurons [60] where they regulate Ca^{2+} signaling, synaptic integration, and dendritic spiking [61]. These GABAergic cells mediate feedback inhibition, being most excited by local cortical pyramidal cells. Immunohistochemical evidence suggests that at least 63% of SOM-INs co-express CB_1Rs in the cortex [50], and in the rat hippocampus, a higher percentage of co-localization between CB_1Rs and somatostatin has been reported [62].Therefore, we hypothesize that SOM-INs may also express CB_1Rs and be another cellular target by which eCBs exert control over synaptic inhibition.

HYPOTHESIS:

"eCBs modulate GABAergic synaptic transmission from SOM-INs in the PFC"

General Aim

Determine the impact of CB1R activation and removal on GABAergic synaptic transmission from SOM interneurons.

Specific aims

1. Assess the effect of an exogenous CB1R agonist on inhibition mediated by SOM-INs onto pyramidal cells.
2. Assess the impact of CB1R deletion in SOM-INs on inhibitory synaptic transmission.

    2.1. Generate mice lacking CB1Rs selectively in SOM-INs.

        2.1.1. Optimize the genotyping protocol to detect DNA sequences for genes encoding for the conditional expression of CB1Rs and for the Cre- Recombinase in second generation offsprings of crosses between the SOM-Cre and the floxed cnr1 (CB1R gene) mice.

        2.1.2. Confirm CB1R deletion in SOM-INs by assessing the impact of an exogenous CB1R agonist on inhibition mediated by SOM-INs onto pyramidal neurons.

    2.2. Determine changes on evoked inhibitory synaptic transmission in mice lacking CB1Rs in SOM-INs.

        2.2.1. Assess changes in the input-output curve of electrically evoked inhibitory responses in pyramidal neurons of SOM-CB1R KO mice.

2.2.2. Evaluate changes in the paired pulse ratio of electrically evoked inhibitory responses in pyramidal neurons of SOM-CB1R KO mice.

2.3.Determine changes in spontaneous activity in mice lacking SOM-CB1R.

METHODS

ANIMALS

All experiments were performed on postnatal day (P) 21-35 wild type and SOM-CB1R knockout mice, from both sex. Mice, born and raised in the animal facility of the Universidad de Valparaiso were maintained at 25ºC under a 12h light/dark cycle with water and food ad libitum. All animal protocols were approved by the bioethics committee of the Universidad de Valparaiso and are in accordance with the bioethics and biosafety regulations of the Chilean Research Council (CONICYT).

GENOTYPING PROTOCOL

Founder lines for the conditional CB1R mice were a kind gift from Dr. Eric Delpire (Vanderbilt University, Tennessee USA). They were genotyped by PCR with primers for floxed cnr1 (Forward: 5′-TGGCTCCTGTCTGCAAGTATAGG-3′; Rev: 5′-ACTCAAAATGTCCATGTCTTATAACCAG-3′, 400 bp amplicon). PCR conditions were 0.4 µM primer and cycle: 95 °C 3 min; [95 °C 15 s, 62 °C 15s, 72 °C 15 s] × 30 cycles; 72 °C 1 min. Homozygous conditional CB1R female mice were bred with heterozygous SOM-Cre male mice (JAX 005359, Jackson Laboratories, Bar Harbor, ME, USA) to generate mice in which the CB1R was deleted from INs and their control littermates. We developed a double PCR assay for CRE and somatostatin as internal control (IC). CRE primers were CRE-forward:5′-CGGTCGATGCAACGAGTGATG-3 ′; CRE-reverse:5′-AGCCTGTTTTGCACGTTCACC-3′, amplicon bp. IC primers were IC- forward: 5′-CTGGAAGACATTCACATCCTG-3′; IC-reverse:5′-TATGGCAGCTGTTCCCAATAG-3′, amplicon 200-465 bp. Double PCR used 0.4 µM primer and cycle: 95 °C 3 min; [95 °C

15 s, 62 °C 15 s, 72 °C 15 s] × 30 cycles; 72 °C 1 min. Amplicons were run on 2% agarose electrophoresis in TBE buffer (pH 8.4).

SLICE PREPARATION

Experiments were performed in acute prefrontal cortical slices (300 μm thick) taken from male and female mice (P26-P40). For the experiments in specific aim 1, we crossed conditional CB1R mice with SOM-Cre mice to generate transgenic mice lacking CB1R expression in all SOM-INs. Controls were littermates in which Cre is not expressed. For slice preparation, the animals were anesthetized with isoflurane, decapitated and their brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 127 NaCl, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$ and 20 glucose (pH=7,4) bubbled with 95% O_2 and 5% CO_2. Coronal slices were cut in ice-cold external solution containing (in mM): 110 choline, 25 $NaHCO_3$, 1.25 NaH_2PO_4, 2.5 KCl, 7 $MgCl_2$, 0.5 $CaCl_2$, 20 glucose, 11.6 sodium ascorbate and 3.1 sodium pyruvate, bubbled with 95% O_2 and 5% CO_2 and transferred to ACSF. After an incubation period of 10 min at 34°C, the slices were maintained at 20–22 °C for at least 30 min before use.

ELECTROPHYSIOLOGY AND ANALYSIS

Experiments were conducted at room temperature (20–22°C), in a submersion-type recording chamber. Whole-cell patch-clamp recordings were obtained from layer 2/3 pyramidal cells (200-300 μm from the pial surface). For voltage-clamp recordings, cells were held at +10 mV to obtain IPSCs, and glass electrodes (2.8–3.2 MΩ) were filled with internal solution containing (in mM): 130 CsGluconate, 10 HEPES, 4 MgCl2, 4 Na2ATP, 0.4 NaGTP and 10 sodium creatine phosphate, adjusted to pH 7.3 with CsOH. Recording were made from visually identified pyramidal-shaped somata of principal neurons in the PFC. In specific aim 2.1, IPSCs were evoked by optogenetic means. The magnitude of the WIN effect was calculated as the percentage change between baseline (averaged responses for 5 minutes before drug application) and 25–30 minutes after the start of drug application to the bath. In specific aim 2.2, IPSCs were evoked by means of a theta-stimulating electrode positioned within layer 1 of the PFC. The paired-pulse ratio (PPR), was defined as the ratio of the amplitude of the second IPSC over the amplitude of the first IPSC. To obtain the amplitude of the second IPSC, the first IPSC waveform was first subtracted from the paired-pulse responses. Electrophysiological recordings were made using a Multiclamp 700B amplifier, filtered at 4 kHz, and digitized at 10 kHz. Data was analyzed in custom-written Igor Pro software. Statistics were performed using $p < 0.05$ in Graph Pad Prism.

CHR2 EXPRESSION AND PHOTO ACTIVATION

To stimulate specific interneurons in aims 1 and 2.1, recombinant adeno-associated virus containing a plasmid coding for the conditional expression of a ChR2-EYFP fusion protein (AAV-DIO-Ef1a-ChR2-EYFP; UNC Vector Core, USA) was injected into the prefrontal cortex of SOM-Cre mice at P18-22. Mice were sacrificed 2-3 weeks post-injection for slice preparation. To focally activate ChR2-positive fibers, we overfilled the back aperture of the microscope objective (60x, 1.0 NA) with collimated blue light from a 455-nm LED light source (3.2 mW power at sample).

RESULTS

1. Assess the effect of an exogenous CB1R agonist on inhibition mediated by SOM-INs onto pyramidal cells.

To examine whether CB1Rs regulate inhibitory synaptic transmission from SOM-INs in the PFC, we assessed the effect of an exogenous CB1R agonist (5uM WIN) on inhibitory responses evoked by optogenetic means onto pyramidal cells. To this end, we first injected stereotaxically a viral vector carrying a CRE dependent construct coding for channelrhodopsin2 (ChR2), a light-activated cationic channel, in mice that express the Cre-recombinase (Cre) in SOM-INs. After two weeks of incubation, cell-type specific expression of ChR2 enables optical stimulation of genetically targeted SOM-INs. The animals that express ChR2 were confirmed by the presence of positive EYFP cell bodies and processes in PFC slices (Figure 3A). Pyramidal cells in layer 2/3 were clamped at +10 mV to register IPSCs mediated by SOM-INs (SOM-IPSCs) that were generated with blue light stimulation. We found that bath application of WIN depressed SOM-IPSCs to 56.5 ± 14.49 % (n=6, p=0.0159) (Figure 3C-D).

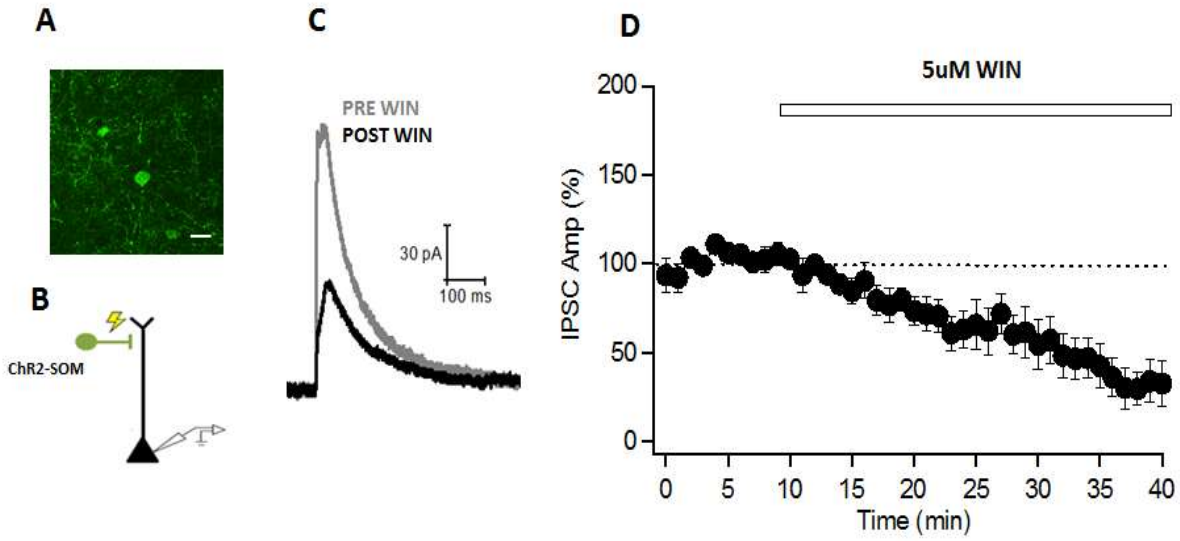

Figure 3.A: Representative image show a PFC slice with SOM-INs expressing positive EYFP cell bodies. Image was obtained by Confocal Microscope of fixed slice. Objective 60X, scale bar 25um. B: Schematic of proposed experimental design.C:Representative traces of inhibitory response on pyramidal cells using ChR2 stimulation before and after WIN application in SOM-INs.D: WIN effect on the IPSC amplitude mediate by SOM-INs onto pyramidal cells over time (n=6 cells, 5 mice). The amplitude is normalized to 10 min of baseline for each single experiment. Bars indicate the standard error.

2. Assess the impact of CB1 deletion in SOM-INs in inhibitory transmission.

2.1. Generate mice lacking CB1Rs selectively in SOM-INs

2.1.1. Optimize the genotyping protocol to detect DNA sequences for genes encoding for the conditional expression of CB1Rs and for the Cre- Recombinase.

To confirm that the WIN-mediated depression on synaptic transmission is dependent on CB1R activation, we generated transgenic mice lacking CB1R expression specifically in SOM-INs (SOM-Cre x floxed CB1). We first optimized a genotyping protocol that includes DNA extraction, polymerase chain reaction (PCR) and gel electrophoresis to identify mice in which both alleles of cnr1, the gene encoding for CB1R, are floxed (Figure 4B, blue arrow) with at least one copy of the Cre recombinase. These animals will constitute the experimental group (Figure 4A, red arrow) and those that do not have Cre recombinase expression will serve as the control group.

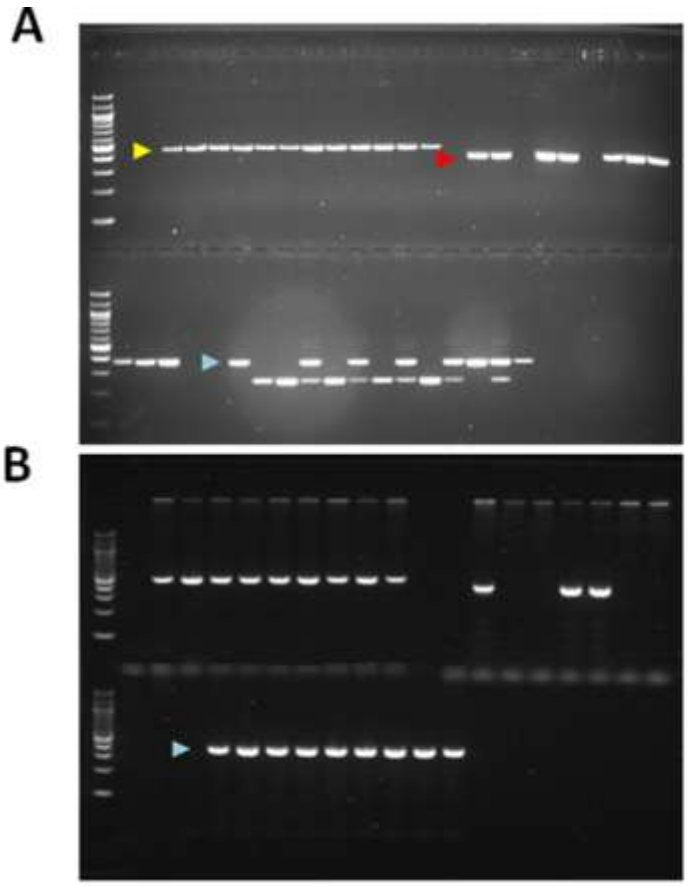

Figure 4.Electrophoresis gel with amplicons for SOM, Cre and CB1. A, genotyping from SOM-Cre crossed with co-Cb1. Amplicon for SOM (yellow arrow), Cre (Red arrow), and double floxed, wild type and heterozygotes for CB1 (blue arrow) are shown. B, Amplicons for crosses of SOM-Cre floxed Cb1 x SOM-Cre floxed Cb1.

2.1.2. Confirm CB1R deletion in SOM-INs by assessing the impact of an exogenous CB1R agonist on inhibition mediated by SOM-INs onto pyramidal neurons.

We further investigated whether the loss of CB_1Rs in SOM-INs exerted an impact on their inhibition. To verify complete CB_1R deletion in SOM-INs, we evaluated the effect of WIN 55,212-2 on inhibitory responses onto pyramidal cells mediated by SOM-CB_1 KO by optogenetic means. To this end, we injected stereotaxically channelrhodopsin 2 in mice that express the Cre-recombinase (Cre) in SOM-CB_1 KO mice. After two weeks, cell-type specific expression of ChR2 enables optical stimulation of genetically targeted SOM-CB_1 KO. The animals that express ChR2 were confirmed by the presence of positive EYFP cell bodies and processes in PFC slices. Like control mice, pyramidal cells in layer 2/3 were clamped at +10 mV to register IPSCs mediated by SOM-CB_1 KO (SOM-IPSCs) that were generated with blue light stimulation in KO mice. We found that WIN application did not depress SOM-IPSCs in SOM-CB_1 KO mice (n=6, p=1.0000, paired t-test; Figure 5). WIN effect was significantly different between control mice and KO mice (p =0.0159, Mann Whitney test) (Figure 6).

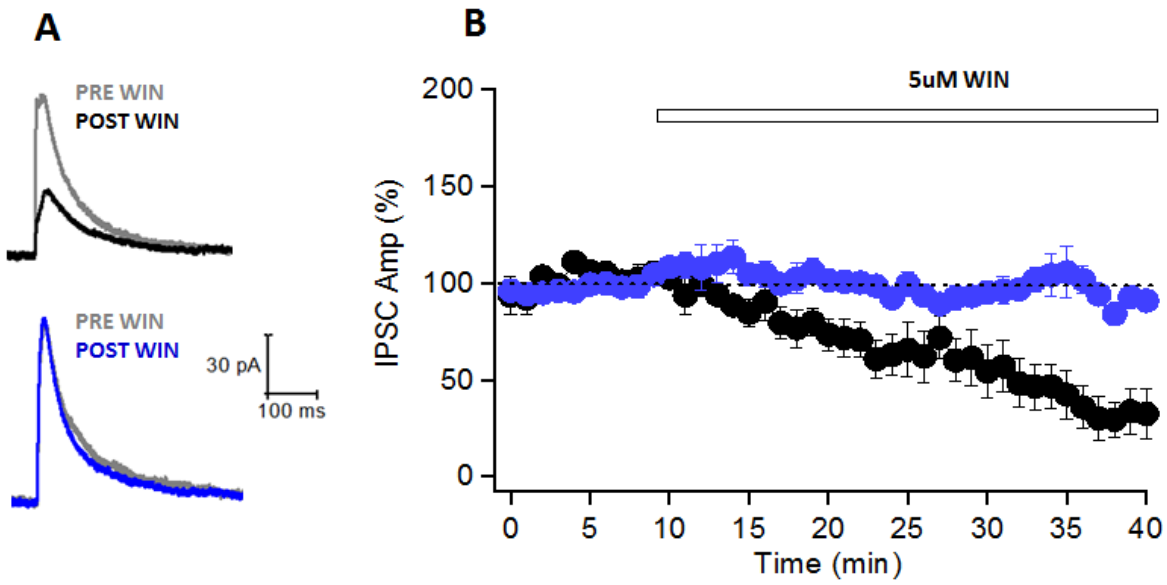

Figure 5.A: Representative traces of inhibitory response on pyramidal cells using ChR2 stimulation before and after WIN application in SOM-Cre (Top) and mice lacking CB_1R in SOM-INs (Bottom). B: WIN 55,212-2 effect on the IPSC amplitude mediate by SOM-INs onto pyramidal cells over time in SOM-CB_1 KO (n=6) and Control mice (n=6).The amplitude is normalized to 10 minutes of baseline for each single experiment. Bars indicate the standard error.

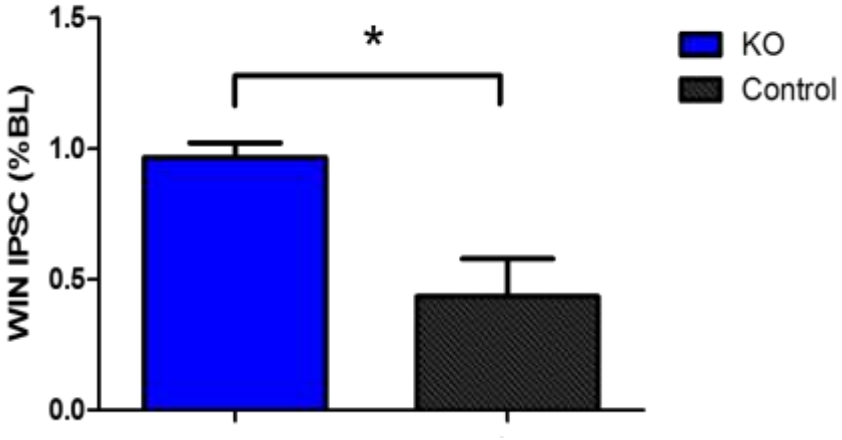

Figure 6.WIN 55,212-2 effect on the IPSC amplitude evoked with blue light stimulation over time in SOM-CB_1 KO (n=6) and Control mice (n=6). The amplitude was normalized to baseline. WIN 55,212-2 effect in control mice was significant after 30min of WIN application compared with KO mice, as tested by Mann Whitney test (*$P < 0.05$).

2.2. Determine changes on evoked inhibitory synaptic transmission in mice lacking CB1Rs in SOM-INs.

To assess changes in inhibitory transmission, we next set out to construct input-output (I-O) curves for evoked IPSCs in SOM-CB_1 KO and control mice. Because the control group does not express Cre-recombinase, we cannot compare IPSCs mediated by SOM-INs by optogenetic means. Instead, we electrically stimulated inhibitory synapses by placement of a theta-stimulating electrode in layer 1 of the PFC to focally recruit dendritic inhibition. These experiments were performed and analyzed in a blind manner. We found that the I-O curve for evoked IPSCs was different between the SOM-CB_1 KO mice (n=10) and controls (n=10) (Figure 7B). First, evoked IPSCs in the SOM-CB_1 KOs showed higher maximal amplitudes compared to controls and significant differences were found at 40V, 50V, 60V, 70V and 80V ($p < 0.0001$, two way ANOVA). We also determined the voltage at half maximal response in controls and KO mice and found no difference (Figure 8A). Thus, inhibitory synapses responded more strongly to the same range of electrical stimulation intensities in SOM-CB_1R KO mice compared to controls.

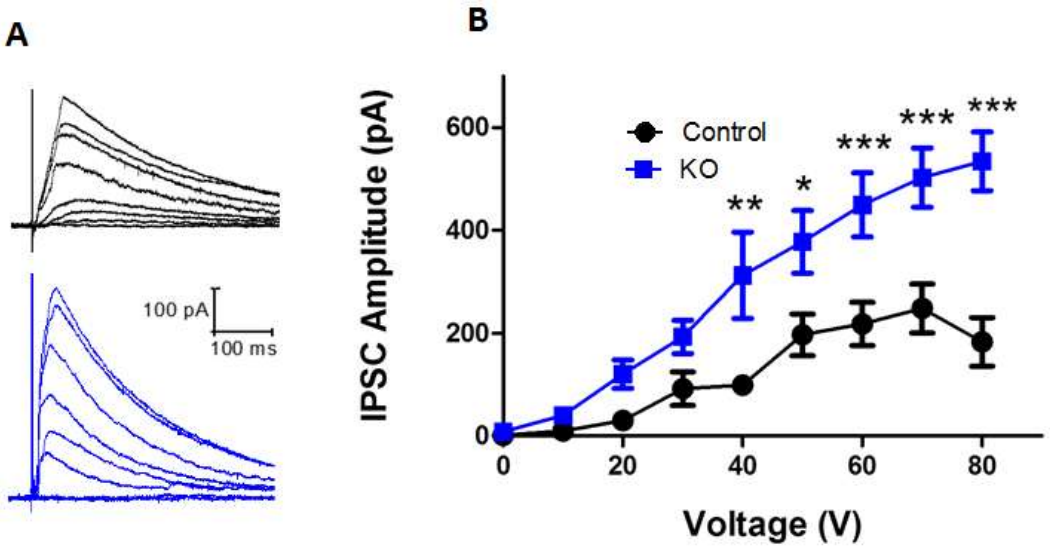

Figure 7. A: Representative traces of electrical inhibitory response in KO (Blue) and Control mice (Black). B:Inhibitory response evoked by electrical stimulation at different voltages. Input-Ouput curve represent summary plots of IPSCs responses to electrical stimulation between Control (n=10) and KO (n=10) mice. Significant difference were found at 40V, 50V, 60V, 70V and 80 V tested by two way ANNOVA (*$p<0.05$;**$p<0.01$;***$p<0.001$). Bars indicate the standard error.

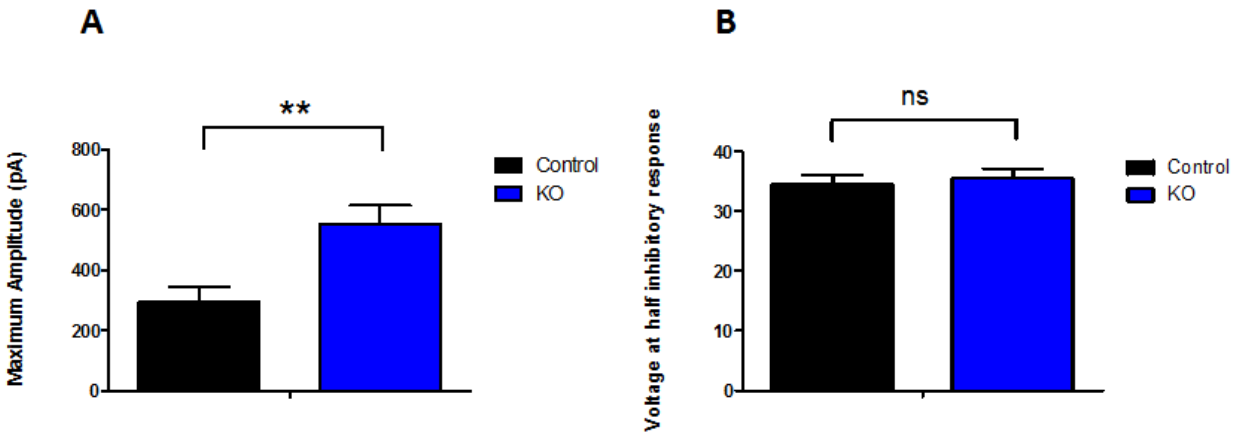

Figure 8.A: Maximum amplitude of evoked inhibitory response in Control (n=10) and KO mice (n=10). Inhibitory responses at higher peak values can be triggered in SOM-CB1R KO mice compared to controls (P=0.0052, Mann Whitney test). B: Voltage necessary to obtain half maximal inhibitory response in Control (n=10) and KO (n=10) mice. No significant difference was found (p = 0.3999, Mann Whitney test). Bars indicate the standard error.

2.2.2. Evaluate changes in the paired pulse ratio of electrically evoked inhibitory responses in pyramidal neurons of SOM-CB1R KO mice.

Because CB1Rs are expected to act presynaptically, we next set out to determine whether the probability of GABA release is altered in SOM-CB_1R KO mice. We first recorded inhibitory responses evoked by light stimulation at an inter-stimulus interval of 100 ms to calculated the paired pulse ratio (PPR) before and after WIN application. We found no difference between experimental groups (p= 0.0865, Wilcoxon test) (Figure 9B).

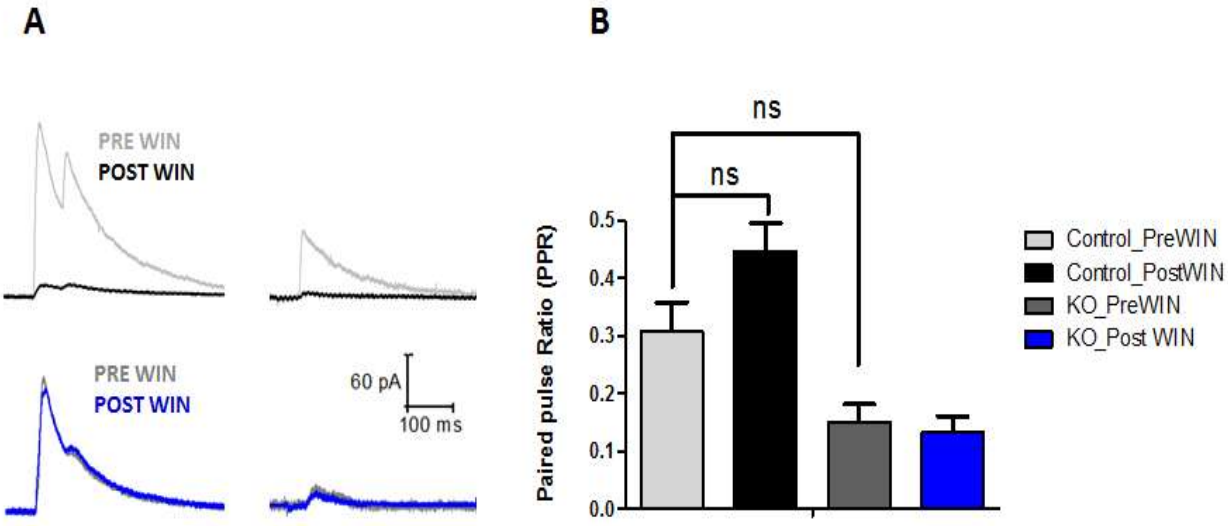

Figure 9.A: Representative traces of light evoked inhibitory responses at 100ms of inter-stimulus interval in SOM-INs (Top; left) and KO mice (Bottom; left), and representative traces for subtraction of second pulse in control to calculated PPR (Top; right) and KO mice (Bottom; right). B: Paired pulse ratio (PPR) in Control (n=3) and KO (n=3) mice before and after WIN application. No significant difference between experimental groups, as tested by Wilcoxon signed rank test (Control PreWIN v/s Control PostWIN) and Mann Whitney test (Control pre WIN v/s KO Pre WIN). Bars indicate the standard error.

Because CB1Rs are expected to act presynaptically, we next set out to determine whether the probability of GABA release is altered in SOM-CB1R KO mice. We analyzed the Paired Pulse Ratio (PPR) at variable intervals, collected with the electrical evoked stimulation intensity at half maximum (40). Evoked IPSCs tended to show stronger paired pulse depression at all inter-stimulus intervals in SOM-C1R KO mice, however we found significant difference only at 30 ms, tested by two-way ANOVA (Figure 10).

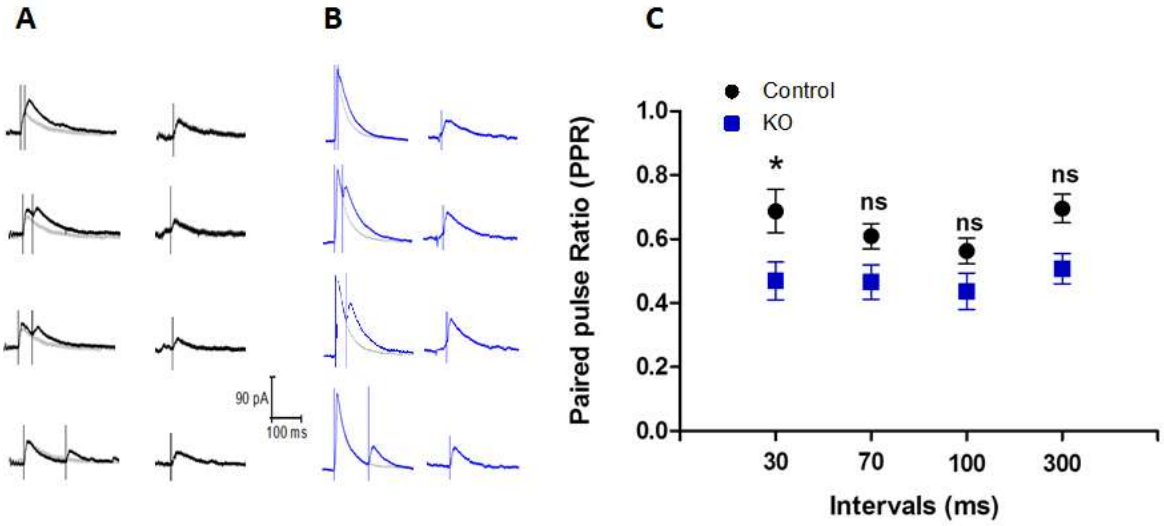

Figure 10.A: Representative traces of evoked inhibitory responses at 30ms, 70ms, 100ms and 300ms (left) and subtraction of the single pulse response (gray) to obtain isolated second pulse traces (right) in Control (Black traces) and B, KO mice (Blue traces). C: Paired pulse ratio for IPSCs between Control and KO mice collected at half max intensity (40V). Significant difference was found at the 30 ms inter-stimulus interval (30 ms: p= 0.0115; 70ms: p= 0.0630; 100 ms: p= 0.1903; 300 ms: p= 0.0630, two-way ANOVA). Bars indicate standard error.

Aim 2.3.Determine changes of spontaneous activity in mice lacking SOM-CB1R.

Enhancement of GABAergic synaptic transmision may also be reflected in spontaneous inhibitory events. We recorded and analyzed spontaneous IPSCs to determine whether the frequency, amplitude and decay kinetics were altered. We found an increase in sIPSC frequency in SOM-CB1R KO mice compared with controls (n=10, p= 0.0274, Mann-Whitney). However, the sIPSC amplitude and decay were not different between KOs and controls (Figure 11). Thus, consistent with the PPR analysis, the probability of GABA release may be enhanced when CB1Rs are absent from axon terminals of SOM-INs. This idea remains to be confirmed in future experiments such as with recordings of miniature IPSCs.

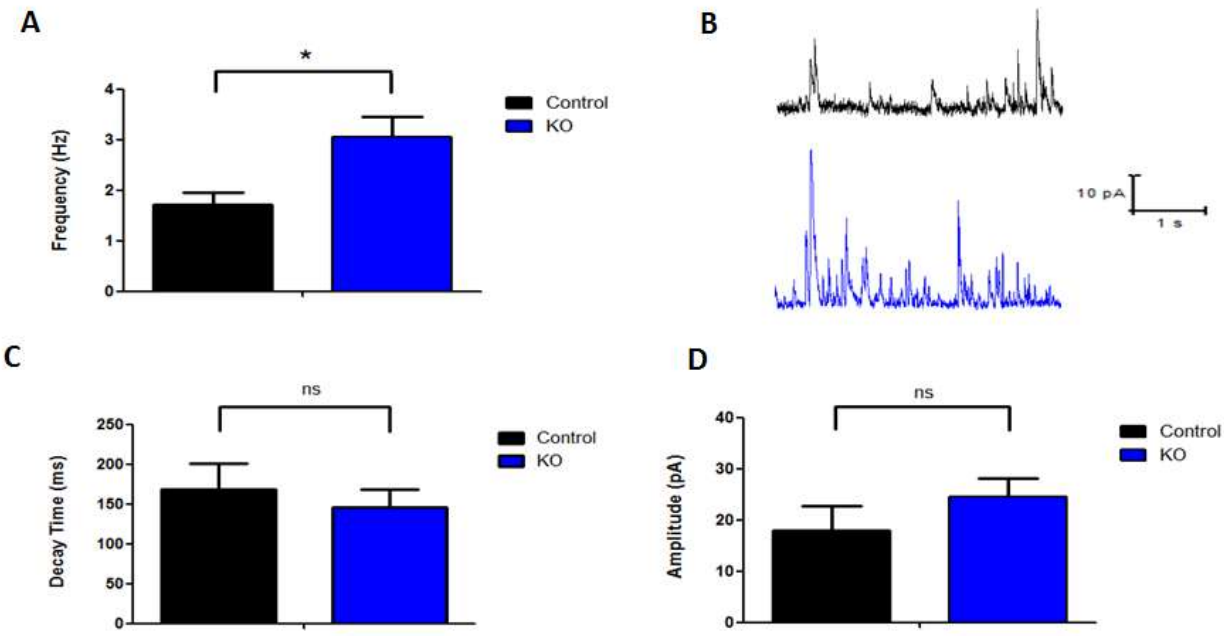

Figure 11.Spontaneous Activity of Inhibitory response in Control (n=5) and KO (n=10) mice. A: IPSCs frequency (Hz) of spontaneous IPSCs, an increase of frequency were found in KO mice compare control (*p= 0.0274). B: Representative traces of spontaneous activity in Control (Black) and KO (Blue) mice. C: Amplitude (pA) and D: Decay Time (ms) of spontaneous IPSCs, no significant difference was found between experimental groups, tested by Mann Whitney test.

DISCUSSION

eCBs have a critical role in regulating neural activity in the brain through the control of both inhibitory and excitatory neurotransmission. Although the CB_1R is expressed widely in the nervous system, the pattern is not uniform. In cortex and hippocampus, strong receptor expression is seen in CCK-INs, whereas principal neurons and PV-INs show low to no expression, respectively [1]. Retrograde endocannabinoid signaling can powerfully suppress GABA release from presynaptic terminals and thereby disinhibit pyramidal neurons [33] [11]. Some evidence in subcortical regions suggests that SOM-IPSCs, but not PV-IPSCs, are sensitive to CB_1R activation [61] SOM-INs in the neocortex targets the dendrites of postsynaptic glutamatergic pyramidal neurons (PNs), forming contacts on both dendritic shafts and spines [63]. Through their inhibitory contact on dendritic arbors, SOM-INs regulate Ca^{2+} signaling, synaptic integration, and dendritic excitability. Dendritic inhibitory synapses are essential for dendritic computation and can change the integration of excitatory synaptic inputs [61][64].

We found that CB_1R activation by WIN depressed IPSCs mediated by SOM-INs (Figure 3D). The depression of IPSC amplitude was 56.5 ± 14.49 % of baseline after 30 minutes of WIN application. It is worth mentioning that we also analyzed PPR in these experiments but only saw a trend for PPR to be higher after WIN application in controls (Figure 7B). The low number of experiments (n=3 cells) may be a culprit. More importantly, we confirmed that the depression of IPSCs is mediated by CB_1Rs in SOM-INs by testing the effect of WIN in mice lacking CB_1Rs specifically in SOM-INs. |Additionally, the lack of WIN-depression also demonstrates that CB_1Rs are efficiently removed from SOM-INs in our transgenic crosses.

By removing CB_1Rs selectively from SOM-INs, we were also able to assess the impact of this loss on inhibitory synaptic transmission. Since eCBs typically act by reducing GABA release, we would expect that CB_1R deletion to increase the probability of GABA release mediated by SOM-INs. This is supported by our finding that peak eIPSC amplitudes are higher in the SOM-CB_1R KO mice.

We are aware that electrical stimulation will recruit GABAergic synapses from different sources. Thus, to enhance the contribution of inhibitory transmission from SOM-INs, we used theta-stimulating electrodes that focally excites nearby neuronal processes and placed them in layer 1. We found that the putative dendritic inhibition evoked in this way was stronger in the SOM-CB_1R KO mice compared to controls, with significant differences found at stimulation intensities at 40V, 50, 60V, 70V and 80V in the I-O curve (Figure 7B). Moreover, no changes were found at the voltage required to evoke the half maximal inhibitory response (Figure 8A), suggesting that inhibitory synapses in both KO and control mice are activated by the same range of stimulation intensities.

Considering that mice lacking C1BRs in SOM-INs have stronger inhibitory responses, we would expect this effect to be accompanied by an increase in GABA release mediated by SOM-INs. Indeed, we found a significant difference in the PPR at 30 ms of inter-stimulus interval between experimental groups, but not at other inter-stimulus intervals (70ms, 100ms and 300ms). However, there is a trend to show more depression by PPR in KO mice, where the first pulse was higher compared to second that decrease the ratio. When two stimuli are delivered within a short interval, the response to the second stimulus can be either enhanced or depressed relative to the response to the first stimulus [65][66]. Paired-pulse depression is observed at synapses with short inter-stimulus

intervals, where many synapses exhibit paired-pulse facilitation at longer inter-stimulus intervals [67][68], that could partially explain why we were not able to find significance at longer inter-stimulus intervals. Since facilitation is considered to indicate a low initial probability of release (PPR>1) and depression a high initial probability of release (PPR<1), we observed that KO mice have more GABA release and is consistent with high inhibition observed KO mice through I-O curve. Paired-pulse depression is thought to be due to vesicle depletion that could result from inactivation of voltage-dependent sodium or calcium channels or from a transient depletion of the readily-releasable pool of vesicles docked at the presynaptic terminal [68]. Thus, although PPR is commonly used to determine whether an effect like the one we observed has presynaptic mechanisms, it is not the most definitive or sensitive way.

Another factor that contribute to the lack of significance at longer inter-stimulus intervals is that we may not be monitoring IPSCs solely mediated by SOM-INs by electrically stimulating in layer 1. It is formally possible that the enhancement of inhibition mediated by SOM-INs may induce a compensatory decrease of inhibition mediated by PV-INs. In future experiments, it would be necessary to isolate pharmacologically the IPSC response from PV-INs and SOM-INs. PV-INs exclusively depend on P/Q-type Ca^{2+} channels for GABA release, while SOM-INs uses both P/Q-type and N-type channel types to mediate GABAergic transmission [64]. For a next step, we can take advantage of this selectivity by including the P/Q-type Ca^{+2} channel blocker Agatoxin TK to block GABA release from PV-INs [69] to substantiate our findings.

Interestingly, we found a significance increase in spontaneous activity in SOM-CB1R KOs compared to Controls, as well as a trend towards an increase and a decrease in

sIPSC amplitude and decay time, respectively. These results highlight the importance of CB_1Rs at SOM-INs where a dys-regulation of the eCB system may contribute to develop several neuropsychiatric diseases that are characterized by inhibitory hyperactivity. A recent study has shown that optically silencing SOM-expressing interneurons resulted in a greater decrease in the frequency of spontaneous IPSCs in striatal spiny projection neurons (SPNs) in a Huntington disease (HD) model, suggesting that SOM-expressing interneurons are the main contributors to the overall increased GABA synaptic activity in HD SPNs [70].

Information processing requires temporally and spatially coordinated synaptic communication, which constitutes a balance between excitatory and inhibitory (E/I ratio) cells and is implicated in neuropsychiatic disorders such as schizophrenia. Our result shows that CB_1R deletion increases GABA inhibition mediated by SOM-INs. Consequently, the excitatory transmission may also change to maintain homeostasis (E-I balance) or if not, a decrease in the E/I ratio would be expected. Disinhibition can robustly shift the E-I balance in a network and by this means can contribute to associative learning [71][54]. It is important to assess the impact on excitatory transmission by the removal of CB_1Rs on SOM-INs as a next step. Such experiments will be required to more fully understand the physiological consequences of CB_1R removal from SOM-INs.

Another important factor is to determine which eCBs are involved in regulating on inhibitory transmission from SOM-INs in the PFC, given that AEA and 2-AG are the most relevant eCBs that regulate synaptic function [3]. Our data suggest an important role of eCB signalling to fine-tune dendritic inhibitory synapses. Deregulation of this system could have profound consequences for information processing and cognitive functions. It is important to elucidate the molecular mechanisms by which eCBs mediate these responses.

It is known that deletion or pharmacological blockade of CB_1Rs on GABA interneurons disrupt eCB-mediated plasticity in many brain regions [72] and genetic deletion of CB_1R from GABAergic neurons (GABA/CB1-/-) leads to behavioral changes such as enhanced interest to social stimuli [73] and enhanced neuroinflammation in ageing [72] as well as hyperphagia [74] and developmental deficits [75]. Pharmacological studies reveal that the cannabinoid system regulates the development of the GABAergic system. For example, the exposure to the CB_1R agonist WIN 55,212-2 (WIN) during adolescence impairs brain maturation and prenatal WIN treatment alters migration of GABAergic neurons in the cortex [76][77]. Exogenous activation of CB_1Rs on GABAergic interneurons disrupts hippocampal-dependent learning in vivo [78] and inhibits LTD of excitatory synapses in the amygdala in vitro [79]. In this sense, CB_1Rs at GABAergic synapses play a role in modulating synaptic plasticity and may underlie certain learning and memory deficits.

BIBLIOGRAFY

[1] M. Kano, T. Ohno-Shosaku, Y. Hashimotodani, M. Uchigashima, and M. Watanabe, "Endocannabinoid-Mediated Control of Synaptic Transmission," *Physiol Rev*, vol. 89, no. 1, pp. 309–80, 2009.

[2] V. Di Marzo, N. Stella, and A. Zimmer, "Endocannabinoid signalling and the deteriorating brain," *Nat. Publ. Gr.*, vol. 16, no. 1, pp. 30–42, 2015.

[3] P. Castillo, T. Younts, A. Chávez, and Y. Hashimotodani, "Endocannabinoid Signaling and Synaptic Function," *Neuron*, vol. 76, no. 1, pp. 70–81, 2012.

[4] V. Chevaleyre, K. A. Takahashi, and P. E. Castillo, "Endocannabinoid-Mediated Synaptic Plasticity in the CNS," *Annu Rev Neurosci*, vol. 29, pp. 37–76, 2006.

[5] R. A. Sewell, M. Ranganathan, and D. C. D. Souza, "Cannabinoids and psychosis," *Int. Rev. Psychiatry*, vol. 21, no. April, pp. 152–162, 2009.

[6] C. J. Hillard, K. M. Weinlander, and K. L. Stuhr, "Contributions of Endocannabinoid Signaling to Psychiatric Disorders in Humans: Genetic and Biochemical Evidence," *Neurocience*, vol. 204, pp. 207–229, 2012.

[7] W. A. Devane *et al.*, "Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor," *Science (80-.).*, vol. 258, no. 5090, pp. 1946–9, 1992.

[8] R. et al Mechoulam, "Identification of An Endogenous 2-Monoglyceride, Present In Canine Gut, That Binds To Cannabinoid Receptors," *Biochem. Pharmacol.*, vol. 50, no. 1, pp. 83–90, 1995.

[9] A. C. Howlett *et al.*, "Classification of Cannabinoid Receptors," *Pharmacol Rev*, vol. 54, no. 2, pp. 161–202, 2002.

[10] B. D. Heifets and P. E. Castillo, "Endocannabinoid Signaling and Long-term Synaptic Plasticity," *Annu Rev Physiol*, no. 71, pp. 283–306, 2009.

[11] S. Zou and U. Kumar, "Cannabinoid Receptors and the Endocannabinoid System : Signaling and Function in the Central Nervous System," 2018.

[12] S. M. Augustin and D. M. Lovinger, "Functional relevance of endocannabinoid-dependent synaptic plasticity in the CNS," 2018.

[13] T. Sugiura, Y. Kobayashi, S. Oka, and K. Waku, "Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance," *Prostaglandins Leukot Essent Fat. Acids*, vol. 66, no. 2–3, pp. 173–

192, 2002.

[14] I. Katona *et al.*, “Molecular Composition of the Endocannabinoid System at Glutamatergic Synapses,” *J. Neurosci.*, vol. 26, no. 21, pp. 5628–5637, 2006.

[15] M. Lafourcade, I. Elezgarai, S. Mato, Y. Bakiri, P. Grandes, and O. J. Manzoni, “Molecular Components and Functions of the Endocannabinoid System in Mouse Prefrontal Cortex,” *PLoS One*, vol. 2, no. 8, p. e709, 2007.

[16] A. Tanimura, M. Uchigashima, M. Yamazaki, N. Uesaka, T. Mikuni, and M. Abe, “Synapse type-independent degradation of the endocannabinoid 2-arachidonoylglycerol after retrograde synaptic suppression,” *PNAS*, vol. 109, no. 30, pp. 12195–12200, 2012.

[17] Y. Hashimotodani, T. Ohno-shosaku, and M. Kano, “Presynaptic Monoacylglycerol Lipase Activity Determines Basal Endocannabinoid Tone and Terminates Retrograde Endocannabinoid Signaling in the Hippocampus,” *J. Neurol.*, vol. 27, no. 5, pp. 1211–1219, 2007.

[18] W. R. Marrs *et al.*, “The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors,” *Nat. Neurosci.*, vol. 13, no. 8, pp. 951–957, 2010.

[19] V. Di Marzo, “Endocannabinoid signaling in the brain: biosynthetic mechanisms in the limelight,” *Nat. Neurosci.*, vol. 14, no. 1, pp. 9–15, 2011.

[20] A. I. Gulyas *et al.*, “Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus , cerebellum and amygdala,” *Eur. J. Neurosci.*, vol. 20, no. 2, pp. 441–458, 2004.

[21] A. E. Chávez, C. Q. Chiu, and P. E. Castillo, “TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus,” *Nat. Publ. Gr.*, vol. 13, no. 12, pp. 1511–1518, 2010.

[22] A. Khlaifia, H. Farah, F. Gackiere, and F. Tell, “Anandamide , Cannabinoid Type 1 Receptor , and NMDA Receptor Activation Mediate Non-Hebbian Presynaptically Expressed Long-Term Depression at the First Central Synapse for Visceral Afferent Fibers,” vol. 33, no. 31, pp. 12627–12637, 2013.

[23] N. Puente *et al.*, “Polymodal activation of the endocannabinoid system in the extended amygdala,” *Nat. Neurosci.*, vol. 14, no. 12, pp. 1542–1547, 2011.

[24] M. Maccarrone *et al.*, “Anandamide inhibits metabolism and physiological actions of 2-arachidonoylglycerol in the striatum,” vol. 11, no. 2, pp. 152–159, 2008.

[25] J. E. Schlosburg *et al.*, “Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system,” *Nat. Publ. Gr.*, vol. 13, no. 9, pp. 1113–1119, 2010.

[26] S. Munro, K. Thomas, and M. Abu-Shaar, “Molecular characterization of a peripheral receptor for cannabinoids,” *Nature*, vol. 365, no. 6441, pp. 61–5, 1993.

[27] K. Mackie, “Distribution of Cannabinoid Receptors in the Central and Peripheral Nervous System,” pp. 299–325, 2005.

[28] L. Bodor, K. Mackie, C. Ledent, and N. Ha, “Endocannabinoid Signaling in Rat Somatosensory Cortex : Laminar Differences and Involvement of Specific Interneuron Types,” *J. Neurosci.*, vol. 25, no. 29, pp. 6845–6856, 2005.

[29] T. Hashimoto, H. H. Bazmi, K. Mirnics, Q. Wu, A. R. Sampson, and D. A. Lewis, “Conserved Regional Patterns of GABA-Related Transcript Expression in the Neocortex of Subjects With Schizophrenia,” *Am J Psychiatry*, vol. 165, no. 4, pp. 479–489, 2008.

[30] L. Matsuda, S. Lolait, M. Brownstein, A. Young, and T. Bonner, “Structure of a cannabinoid receptor and functional expression of the cloned cDNA,” *Nature*, vol. 346, no. 6284, pp. 561–564, 1990.

[31] M. Herkenham *et al.*, “Cannabinoid receptor localization in brain,” *Proc Natl Acad Sci U S A*, vol. 87, no. 5, pp. 1932–1936, 1990.

[32] I. Katona *et al.*, “Distribution of CB1 Cannabinoid Receptors in the Amygdala and their Role in the Control of GABAergic Transmission,” *J. Neurosci.*, vol. 21, no. 23, pp. 9506–9518, 2001.

[33] A. Busquets-garcia, J. Bains, and G. Marsicano, “CB 1 Receptor Signaling in the Brain : Extracting Specificity from Ubiquity,” vol. 43, no. 1, pp. 4–20, 2017.

[34] G. Marsicano and B. Lutz, “Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain,” vol. 11, no. August, pp. 4213–4225, 1999.

[35] G. Pryce *et al.*, “Cannabinoids inhibit neurodegeneration in models of multiple sclerosis,” 2003.

[36] A. Gowran, “Alzheimer ’ s disease ; taking the edge off with cannabinoids ?,” no. September, pp. 655–662, 2007.

[37] A. Bilkei-gorzo, “The endocannabinoid system in normal and pathological brain ageing,” pp. 3326–3341, 2012.

[38] G. Marsicano, C. T. Wotjak, and S. C. Azad, "The endogenous cannabinoid system controls extinction of aversive memories," *Nature*, vol. 418, pp. 530–534, 2002.

[39] Y. Kishimoto and M. Kano, "Endogenous Cannabinoid Signaling through the CB 1 Receptor Is Essential for Cerebellum-Dependent Discrete Motor Learning," *J. Neurosci.*, vol. 26, no. 34, pp. 8829–8837, 2006.

[40] B. E. Alger, *Retrograde signaling in the regulation of synaptic transmission : focus on endocannabinoids*, vol. 68. 2002.

[41] T. Ohno-shosaku, H. Tsubokawa, I. Mizushima, N. Yoneda, and A. Zimmer, "Presynaptic Cannabinoid Sensitivity Is a Major Determinant of Depolarization-Induced Retrograde Suppression at Hippocampal Synapses," *J. Neurosci.*, vol. 22, no. 10, pp. 3864–3872, 2002.

[42] Y. Hashimotodani *et al.*, "Phospholipase Cβ Serves as a Coincidence Detector through Its Ca2+ Dependency for Triggering Retrograde Endocannabinoid Signal," *Neuron*, vol. 45, no. 2, pp. 257–268, 2005.

[43] T. Maejima, K. Hashimoto, T. Yoshida, A. Aiba, and M. Kano, "Presynaptic Inhibition Caused by Retrograde Signal from Metabotropic Glutamate to Cannabinoid Receptors," *Neuron*, vol. 31, pp. 463–475, 2001.

[44] Y. Fukudome *et al.*, "Two distinct classes of muscarinic action on hippocampal inhibitory synapses : M2-mediated direct suppression and M1/M3-mediated indirect suppression through endocannabinoid signalling," *Eur. J. Neurosci.*, vol. 19, no. 10, pp. 2682–92, 2004.

[45] K. Venkatachalam and C. Montell, "TRP Channels," 2007.

[46] S. P. Brown, S. D. Brenowitz, and W. G. Regehr, "Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids," *Nat. Neurosci.*, vol. 6, no. 10, pp. 1048–1057, 2003.

[47] B. D. Heifets, V. Chevaleyre, and P. E. Castillo, "Interneuron activity controls endocannabinoid- mediated presynaptic plasticity through calcineurin," *PNAS*, vol. 105, no. 29, pp. 10250–10255, 2008.

[48] V. Chevaleyre, B. D. Heifets, P. S. Kaeser, and T. C. Su, "Endocannabinoid-Mediated Long-Term Plasticity Requires cAMP / PKA Signaling and RIM1 a," *Neuron*, vol. 54, no. 5, pp. 801–812, 2007.

[49] S. Mato, M. Lafourcade, D. Robbe, Y. Bakiri, and O. J. Manzoni, "Role of the cyclic-AMP/PKA cascade and of P/Q-type Ca+2 channels in endocannabinoid-mediated long-term depression in the nucleus accumbens," *Neuropharmacology*, vol.

54, no. 2008, pp. 87–94, 2007.

[50] E. L. Hill *et al.*, "Functional CB1 Receptors Are Broadly Expressed in Neocortical GABAergic and Glutamatergic Neurons," *J Neurophysiol*, vol. 97, no. 4, pp. 2580–2589, 2007.

[51] M. Galarreta, F. Erdelyi, G. Szabo, and S. Hestrin, "Cannabinoid Sensitivity and Synaptic Properties of 2 GABAergic Networks in the Neocortex," *Cereb Cortex*, vol. 18, no. 10, pp. 2296–2305, 2008.

[52] H. Y. Hu, D. L. H. Kruijssen, P. Frias, C. C. Hoogenraad, and C. J. Wierenga, "Endocannabinoid Signaling Mediates Local Dendritic Coordination between Excitatory and Inhibitory Synapses Report Endocannabinoid Signaling Mediates Local Dendritic Coordination between Excitatory and Inhibitory Synapses," pp. 666–675, 2019.

[53] K. Tsou and K. Mackie, "Cannabinoid CB1 Receptors Are Localized Primarily On Cholecystokinin-Containing Gabaergic Interneurons In The Rat Hippocampal Formation," *Neurocience*, vol. 93, no. 3, pp. 969–975, 1999.

[54] A. Araque, P. E. Castillo, O. J. Manzoni, and R. Tonini, "disease," pp. 13–24, 2018.

[55] A. Kepecs and G. Fishell, "Interneuron cell types are fit to function," *Nature*, vol. 505, no. 7483, pp. 318–26, 2014.

[56] A. Neu, M. V Jones, and I. Soltesz, "Presynaptic , Activity-Dependent Modulation of Cannabinoid Type 1 Receptor-Mediated Inhibition of GABA Release," vol. 26, no. 5, pp. 1465–1469, 2006.

[57] B. Rudy, G. Fishell, S. Lee, and J. Hjerling-Leffler, "Three Groups of Interneurons Account for Nearly 100% of Neocortical GABAergic Neurons," *dev neurobiol.*, vol. 71, no. 1, pp. 45–61, 2011.

[58] A. Bacci, J. R. Huguenard, and D. A. Prince, "Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids," *Nature*, vol. 431, pp. 312–316, 2004.

[59] I. Katona *et al.*, "Gabaergic interneurons are the targets of cannabinoid actions in the human hippocampus," *Neuroscience*, vol. 100, no. 4, pp. 797–804, 2000.

[60] L. M. Mcgarry, A. M. Packer, E. Fino, V. Nikolenko, T. Sippy, and R. Yuste, "Quantitative classification of somatostatin-positive neocortical interneurons identifi es three interneuron subtypes," *Front Neural Circuits*, vol. 4, no. 12, pp. 1–19, 2010.

[61] Chiu, G. Lur, T. Morse, N. Carnevale, G. Ellis-Davies, and M. Higley,

"Compartmentalization of GABAergic Inhibition by Dendritic Spines," *Science (80-.).*, vol. 340, no. 6133, pp. 759–62, 2013.

[62] S. Zou and U. Kumar, "Colocalization of cannabinoid receptor 1 with somatostatin and neuronal nitric oxide synthase in rat brain hippocampus," *Brain Res.*, pp. 1–13, 2015.

[63] M. J. Higley, "HHS Public Access," vol. 15, no. 9, pp. 567–572, 2015.

[64] C. Q. Chiu *et al.*, "Input-specific NMDAR-dependent potentiation of dendritic GABAergic inhibition," 2017.

[65] B. Y. B. Katz and R. Miledi, "THE ROLE OF CALCIUM IN NEUROMUSCULAR," pp. 481–492, 1968.

[66] R. S. Zucker and W. G. Regehr, "S HORT -T ERM S YNAPTIC P LASTICITY," pp. 355–405, 2002.

[67] R. C. Malenka and M. F. Bear, "LTP and LTD : An Embarrassment of Riches," *Neuron*, vol. 44, no. 1, pp. 5–21, 2004.

[68] A. Citri and R. C. Malenka, "Synaptic Plasticity : Multiple Forms , Functions , and Mechanisms," pp. 18–41, 2008.

[69] I. K. and B. Rudy, "Perisomatic GABA Release and Thalamocortical Integration onto Neocortical Excitatory Cells Are Regulated by Neuromodulators," *Neuron*, vol. 58, no. 6, pp. 911–924, 2008.

[70] S. M. Holley, L. Galvan, T. Kamdjou, A. Dong, M. S. Levine, and C. Cepeda, "Major Contribution of Interneurons and Cannabinoid Receptors to Increased GABA Synaptic Activity in the Striatum of Huntington ' s Disease Mice," vol. 11, no. May, pp. 1–19, 2019.

[71] J. J. Letzkus, S. B. E. Wolff, and A. Lu, "Review Disinhibition , a Circuit Mechanism for Associative Learning and Memory," 2015.

[72] Ö. Albayram, S. Passlick, A. Bilkei-gorzo, A. Zimmer, and C. Steinhäuser, "Physiological impact of CB1 receptor expression by hippocampal GABAergic interneurons," *Pflugers Arch*, vol. 468, no. 4, pp. 727–37, 2015.

[73] A. Terzian, V. Micale, and C. Wotjak, "Cannabinoid receptor type 1 receptors on GABAergic vs . glutamatergic neurons differentially gate sex-dependent social interest in mice," *Eur. J. Neurosci.*, vol. 40, pp. 2293–2298, 2014.

[74] L. Bellocchio *et al.*, "Bimodal control of stimulated food intake by the endo

-cannabinoid system," *Nat Neurosci*, vol. 13, no. 3, pp. 281–283, 2010.

[75] J. Díaz-alonso, M. Guzmán, and I. Galve-roperh, "Endocannabinoids via CB1 receptors act as neurogenic niche cues during cortical development," *Philos Trans R Soc L. B Biol Sci*, vol. 367, no. 1607, pp. 3229–3241, 2012.

[76] D. K. Cass, E. Flores-Barrera, D. R. Thomases, W. F. Vital, A. Caballero, and K. Y. Tseng, "CB1 cannabinoid receptor stimulation during adolescence impairs the maturation of GABA function in the adult rat prefrontal cortex," *Mol Psychiatry*, vol. 19, no. 5, pp. 536–543, 2014.

[77] T. Saez, M. Aronne, L. Caltana, and A. Brusco, "Prenatal exposure to the CB1 and CB2 cannabinoid receptor agonist WIN 55,212-2 alters migration of early-born glutamatergic neurons and GABAergic interneurons in the cerebral cortex," *J. Neurochem.*, vol. 129, no. 4, pp. 637–648, 2014.

[78] E. Puighermanal, G. Marsicano, A. Busquets-garcia, B. Lutz, and R. Maldonado, "Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling," *Nat. Neurosci.*, vol. 12, no. 9, 2009.

[79] S. C. Azad, J. Kurz, G. Marsicano, B. Lutz, W. Zieglgänsberger, and G. Rammes, "Activation of CB1 specifically located on GABAergic interneurons inhibits LTD in the lateral amygdala," *Learn Mem*, vol. 15, no. 3, pp. 143–152, 2008.