



HAL
open science

Role of cellular and sub-cellular CB1 receptor in Hippocampal D1-positive cells on Memory Processes

Gianluca Lavanco

► **To cite this version:**

Gianluca Lavanco. Role of cellular and sub-cellular CB1 receptor in Hippocampal D1-positive cells on Memory Processes. Neuroscience. Université de Bordeaux; Università degli studi (Catane, Italie), 2022. English. NNT : 2022BORD0027 . tel-03967566

HAL Id: tel-03967566

<https://theses.hal.science/tel-03967566>

Submitted on 1 Feb 2023

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.

2021

université
de BORDEAUX



UNIVERSITÀ
di CATANIA

DOCTORAL DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE JOINT DEGREE OF DOCTOR OF
PHILOSOPHY

UNIVERSITY OF BORDEAUX, FRANCE

DOCTORAL SCHOOL OF LIFE AND HEALTH SCIENCES -
SPECIALTY NEUROSCIENCE
AND

UNIVERSITY OF CATANIA, ITALY

INTERNATIONAL PHD PROGRAM IN NEUROSCIENCE –
XXXIII CYCLE

**Role of cellular and
sub-cellular CB₁ receptor in
Hippocampal D₁-positive cells on
Memory Processes**

Gianluca Lavanco

Presented and defended publicly on 25 January 2022

Thesis Supervisor: Dr. Giovanni Marsicano (Bordeaux, France)
Thesis Co-Supervisor: Prof. Filippo Drago (Catania, Italy)
Members of the Jury: Prof.ssa Alessia Pascale – University of Pavia – President
Prof.ssa Monica Baiula – University of Bologna - Examiner
Prof. Claudio Bucolo – University of Catania – Examiner
Dr. Guillaume Ferreira – University of Bordeaux – Rapporteur
Dr. Silvia Middei – CNR (Rome) - Rapporteur

THÈSE EN COTUTELLE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

DOCTEUR DE

L'UNIVERSITÉ DE BORDEAUX

École Doctorale Sciences de la Vie et de la Santé - SPÉCIALITÉ NEUROSCIENCES

ET DE

L'UNIVERSITÉ DE CATANE

International PhD Program in Neuroscience – XXXIII Cycle

Par Gianluca LAVANCO

Rôle du récepteur CB₁ cellulaire et subcellulaire dans les
cellules D₁-positives de l'hippocampe sur les processus de mémoire

Sous la direction de Giovanni MARSICANO

et de Filippo DRAGO

Soutenue le 25 Janvier 2022

Membres du jury:

Prof.ssa Alessia Pascale – Université de Pavia - Examineur

Prof.ssa Monica Baiula – Université de Bologna - Examineur

Prof. Claudio Bucolo – Université de Catane – Examineur

Dr. Guillaume Ferreira – Université de Bordeaux – Rapporteur

Dr. Silvia Middei – CNR (Rome) - Rapporteur

This work was supported by a fellowship from the International PhD Program in Neuroscience – XXXIII CYCLE of the University of Catania (Italy) and by a doctoral extension provided by Prof. Filippo Drago of University of Catania.

The work in this thesis has been carried out as a joint-PhD program in the laboratories of:

Dr. Giovanni Marsicano

Neurocentre Magendie – INSERM U1215
University of Bordeaux
146 Rue Leo Saignat
33077 Bordeaux cedex
France

Prof. Filippo Drago

Department of Biomedical and
Biotechnological Sciences School of Medicine
University of Catania
Via S. Sofia 64
95125 Catania
Italy

TABLE OF CONTENTS

ACKNOWLEDGMENTS	6
LIST OF PUBLICATIONS	9
Articles published or in process of publication in peer reviewed scientific journals	9
LIST OF COMMUNICATIONS.....	13
Poster communications	13
Oral communications.....	14
RÉSUMÉ.....	15
ABSTRACT.....	17
LIST OF ABBREVIATIONS.....	19
LIST OF FIGURES	21
Section I – GENERAL INTRODUCTION	22
Part 1 – The endocannabinoid system in the brain	23
I – Introduction	23
II – Cannabinoid receptors	26
III – Localization of CB ₁ receptors in the brain.....	28
IV – Endocannabinoids Synthesis, Transport and Degradation.....	32
V – Pharmacological and Genetic Tools to study the ECS.....	35
VI – Pathological roles of the CB ₁ receptor.....	38
Part 2 – Synaptic plasticity and Memory.....	47
I – How to study Synaptic Plasticity?.....	48
II – Hippocampal Synaptic Plasticity	51
III – Mechanisms of NMDA receptor-dependent LTP.....	53
IV – CB ₁ -mediated Modulation of Synaptic Activity.....	56
Part 3 – Memory	61
I – Long-term potentiation (LTP): A Synaptic Mechanism for Memory	62
II – CB ₁ Receptors and Memory Process.....	64
III – Novel Object Recognition as Tool to investigate Memory.....	66

Part 4 – Dopaminergic System and the ECS	68
I – D ₁ receptors in Hippocampal-dependent Synaptic Plasticity and Memory.....	69
II – D ₁ and CB ₁ receptors – Linking in memory functions.....	72
Part 5 – Subcellular Signaling of CB ₁ receptor: Mitochondrial CB ₁ and Memory.....	74
I – Mitochondrial Functions.....	76
II – Role of Mitochondrial CB ₁ in the Brain.....	78
III – Mitochondrial CB ₁ and Memory.....	80
Section II – RESEARCH OBJECTIVES	83
Section III – RESULTS	88
Part 1 – Specific hippocampal interneurons shape consolidation of recognition memory.....	89
Part 2 – Linking Mitochondrial G-protein Signaling to cannabinoids-induced amnesia: A new Mitochondria-specific chemogenetic Strategy.....	133
Section IV – GENERAL DISCUSSION.....	156
Part 1 – Specific hippocampal interneurons shape consolidation of recognition memory.....	157
Part 2 – Linking Mitochondrial G-protein Signaling to cannabinoids-induced amnesia: A new Mitochondria-specific chemogenetic Strategy.....	160
Section V – REFERENCES	163

ACKNOWLEDGMENTS

It was 4 wonderful years which I will always carry in my heart. I refer not only to the scientific path done and carried out, but also to the people I met and had the pleasure to know.

Each one has contributed to the beauty of these 4 years.

First of all, I would like to thank Giovanni Marsicano for welcoming me in his team and giving me the opportunity to develop my project under his supervision. I met a really great person not only from a professional point of view but also from a human one.

I was very lucky to be part of your team. The way you lead it allows each member of the team to bring out the best in yourself and to collaborate with each other, without neglecting non-work events that strengthen even more the link that keeps this team together as a family.

That's exactly how I felt, in a big family.

Thank you!

A special thanks also goes to Professor Filippo Drago for allowing me to take up this PhD experience. I would also like to thank Professor Salomone, Professor Bucolo and Professor Leggio for the enormous help and willingness shown to me during all these years.

I spent the first year of my PhD training at the BIOMETEC Department of the University of Catania. It was a productive year in scientific terms; I learned new and interesting scientific techniques which encouraged me to want to know more about the field of Neuroscience.

I started my PhD training, accompanied by Seby Torrìsi, with whom a very strong friendship was established in a short time. Thank you Seby for giving me your great help.

I met wonderful people and shared unforgettable moments.

Thanks to Chiara, Francesca, Federica, Oriana, Margherita, Anna, Claudia, Giovanni, Giorgia, Giuliana, Giovanna, Carlo and Lucia.

From the 2th year, my PhD training has continued at the Neurocentre Magendie of Bordeaux. At the beginning it was not easy; first experience abroad, new language, new environment. What made it less stressful and pleasant was meeting Nadia and Giovanni, two Sicilians in Bordeaux, like me. THANK YOU.

Fitting in Marsicano team was very easy thanks to the kindness and helpfulness of each team member. I met so many friends and each of them left me something both professionally and humanly.

A special thanks goes to Josè, who accompanied me throughout the first year in Bordeaux. Your advices and professionalism were a great help to me.

I equally thank all Marsicano's people. Arnau, Bastien, Christina and Ana, with whom I shared the I4 office for 3 years, and Geo, Ula, Abel, Roman, Nuria, Emma, Imane, Ignacio, Francisca, Marjo, Doriane, Paula, Christopher, Edgar, Francis, Astrid, Virginie, Sandrine, Zhe, Alex, Julia, Tommaso, Michela, MariaSole, Hazim. I will take with me wonderful memories of each of you.

A very special thanks goes to Yamuna, Antonio and Gigi. Thank you so much for everything.

I think I come out of this experience professionally improved but mainly with many new friends I can count on in my future carrer.

CIAO BELLI!!

I cannot forget to thank all the administrative and scientific staff of the two Universities for their work and the laboratory animal care staff who facilitated the scientific project.

A special thought goes to the members of the Neuropsychopharmacology Lab at the University of Palermo where my passion for science started. In particular, I thank Prof. Carla Cannizzaro for passing on this passion. I equally thank Prof. Plescia and Anna Brancato for their huge help.

Finally, I thank my family for the support, especially Laura for supporting me in every decision I made and for encouraging me on in the most difficult moments.

A special thank also to my dog Tato who, just thinking about him, made me feel better.

I hope I haven't forgotten anyone, and if I have...THANK YOU VERY MUCH!

LIST OF PUBLICATIONS

Articles published or in process of publication in peer reviewed scientific journals

PUBLISHED

- 1) PLESCIA F., PLESCIA F., RAFFA D., CAVALLARO A., LAVANCO G., MAGGIO B., RAIMONDI M.V., DAIDONE G., BRANCATO A. AND CANNIZZARO C. 2018
The role of (E)-6-chloro-3-(3-methyl-1-phenyl-1H-pyrazol-5-yl)-2-styrylquinazolin-4(3H)-one in the modulation of cannabinoidergic system. A pilot study. *Pharmacological Reports*.
- 2) BRANCATO A., CASTELLI V., CAVALLARO A., LAVANCO G., PLESCIA F. AND CANNIZZARO C. 2018
Pre-conceptional and peri-gestational maternal binge alcohol drinking produces inheritance of mood disturbances and alcohol vulnerability in the adolescent offspring. *Frontiers in Psychiatry*.
- 3) LAVANCO G., CASTELLI V., BRANCATO A., TRINGALI G., PLESCIA F. AND CANNIZZARO C. 2018
The endocannabinoid-alcohol crosstalk: recent advances on a bi-faceted target. *Clinical and Experimental Pharmacology and Physiology*.

- 4) LEGGIO G.M., DI MARCO R., GULISANO W., D'ASCENZO M., TORRISI S.A., GERACI F., LAVANCO G., DAHL K., GIURDANELLA G., CASTORINA A., AITTA-AHO T., ACETO G., BUCOLO C., PUZZO D., GRASSI C., KORPI E.R., DRAGO F., SALOMONE S. 2019
Dopamine D3 receptor-dependent expression of alpha6 GABAA receptor subunit in the nucleus accumbens counteracts alcohol intake. *Pharmacological Research*.
- 5) CASTELLI V., LAVANCO G., BRANCATO A., PLESCIA F. 2020
Targeting the stress system during gestation: is early handling a protective strategy for the offspring? *Frontiers in Behavioral Neuroscience*.
- 6) BRANCATO A., CASTELLI V., LAVANCO G., MARINO R.A.M. AND CANNIZZARO C. 2020
In utero THC exposure confers vulnerability towards cognitive impairments and alcohol drinking in adolescent offspring. Is there a role of Neuropeptide Y? *Journal of Psychopharmacology*.
- 7) OLIVEIRA DA CRUZ J.F., BUSQUETS-GARCIA A., ZHAO Z., VARILH M., LAVANCO G., BELLOCCHIO L., ROBIN L., CANNICH A., JULIO-KALAJIĆ F., LESTÉ-LASSERRE T., MAÎTRE M., DRAGO F., MARSICANO G. AND SORIA-GOMEZ E. 2020
Specific hippocampal interneurons shape consolidation of recognition memory. *Cell Reports*.
- 8) BRANCATO A., CASTELLI V., LAVANCO G. AND CANNIZZARO C. 2020
Environmental enrichment during adolescence mitigates cognitive deficits and alcohol vulnerability due to continuous- and intermittent perinatal alcohol

exposure in adult rats. *Front. Behav. Neurosci.*

- 9) TORRISI S., LAVANCO G., MAUREL O., GULISANO W., LAUDANI S., GRASSO M., BARBAGALLO C., CARACI F., BUCOLO C., RAGUSA M., PAPALETTO F., CAMPOLONGO P., PUZZO D., DRAGO F., SALOMONE S., LEGGIO G.M. 2021

A novel arousal-based individual screening reveals susceptibility and resilience to PTSD-like phenotypes in mice. *Neurobiology of Stress.*

- 10) CASARRUBEA M., PALACINO M., BRANCATO A., LAVANCO G., CANNIZZARO C., CRESCIMANNO G. 2021

Detection of a temporal structure in the rat behavioural response to an aversive stimulation in the emotional object recognition (EOR) task. *Physiology & Behavior.*

- 11) BRANCATO A., CASTELLI V., LAVANCO G., TRINGALI G., MICALE V., KUCHAR M., D'AMICO C., PIZZOLANTI G., FEO S. AND CANNIZZARO C. 2021

Binge-like alcohol exposure in adolescence: behavioural, neuroendocrine and molecular evidence of abnormal neuroplasticity... and return. *Biomedicines.*

IN PREPARATION

Linking mitochondrial G-protein signaling to cannabinoids-induced amnesia: a new mitochondria-specific chemogenetic strategy

GIANLUCA LAVANCO*, ANTONIO C. PAGANO ZOTTOLA*, YAMUNA MARIANI, ASTRID CANNICH, FRANCISCA JULIO-KALAJZIĆ, FILIPPO DRAGO, GIOVANNI MARSICANO#, ETIENNE HEBERT-CHATELAIN#, LUIGI BELLOCCHIO#

*: equal contribution, #: equal supervision

LIST OF COMMUNICATIONS

Poster communications

*2019 – Gordon Research Conferences on Cannabinoid Function in The CNS
(Castelldefels, Spain)*

Deletion of CB1 receptors in D1-positive cells impairs object recognition memory and synaptic plasticity

Oliveira da Cruz J. F., Busquets-Garcia A., Lavanco G., Bellocchio L., Zhao Z., Robin L., Cannich A., Varilh M., Maitre M., Marsicano G., Soria-Gomez E.

2020 – FENS Virtual Forum of Neuroscience

Specific hippocampal interneurons shape consolidation of recognition memory

Lavanco G.; Oliveira da Cruz J. F.; Busquets-Garcia A.; Bellocchio L.; Zhao Z.; Robin L.; Cannich A.; Varilh M.; Lesté-Lasserre T.; Maitre M.; Marsicano G.; Soria-Gomez E.

Oral communications

2019 – 1th International Conference Perinatal Origins of Neuropsychiatric Disorders: from Molecular Mechanisms to Therapeutic Perspectives (Palermo, Italy)

**Binge drinking during adolescence as a vulnerability factor for migraine?
Focus on Calcitonin gene-related peptide.**

Lavanco G., Brancato A., Castelli V., Tringali G. and Cannizzaro C.

Titre : Rôle du récepteur CB₁ cellulaire et subcellulaire dans les cellules D₁-positives de l'hippocampe sur les processus de mémoire

Résumé :

Via la modulation de l'activité neuronale par les récepteurs cannabinoïdes de type 1 (CB₁), le système endocannabinoïde représente un système modulateur cérébral majeur contrôlant les fonctions de la mémoire. D'autre part, plusieurs rapports soulignent le rôle crucial de la signalisation de la dopamine hippocampique dans la régulation des processus liés à la mémoire. De plus, des preuves récentes suggèrent que les cellules hippocampiques exprimant des récepteurs de dopamine possèdent également des récepteurs CB₁.

Le travail présenté dans cette thèse vise à établir un lien fonctionnel entre les récepteurs CB₁ et la signalisation dopaminergique dans la régulation des processus de mémoire liés à l'hippocampe, en mettant l'accent sur les mécanismes cellulaires et sous-cellulaires impliqués.

Dans la première partie de la thèse, nous avons observé qu'une lignée de souris dépourvue de CB₁ dans les cellules des récepteurs dopaminergiques de type 1 (D₁-CB₁-KO) présentait une déficience de la mémoire de reconnaissance des objets nouveaux à long terme (NOR) et, la réexpression virale de CB₁ dans les cellules D₁-positives de l'hippocampe des souris D₁-CB₁-KO a inversé la déficience NOR présente chez ces souris. En outre, nous avons mis en évidence une activation excessive des récepteurs GABA_A de l'hippocampe et une altération de la potentialisation à long terme (LTP) *in vivo* dans la voie CA3-CA1 comme étant les principaux mécanismes cellulaires à l'origine des troubles de la mémoire chez les souris D₁-CB₁-KO. Nous avons ainsi fourni des preuves fonctionnelles de l'implication d'une petite sous-classe d'interneurones hippocampiques exprimant le récepteur cannabinoïde de type 1 (CB₁) dans la modulation de circuits hippocampiques spécifiques dans les processus de mémoire.

La deuxième partie de la thèse s'est concentrée sur la localisation subcellulaire de l'activation des CB₁ dans les cellules D₁ positives. En effet, outre la régulation canonique de l'activité neuronale par

le récepteur CB₁ de la membrane plasmique, des preuves récentes suggèrent l'implication du récepteur CB₁ mitochondrial (mtCB₁) dans la régulation des processus bioénergétiques qui ont un impact sur la transmission synaptique et les effets amnésiques des cannabinoïdes. Nous avons découvert que les récepteurs mtCB₁ dans les neurones hippocampiques D₁-positifs ne sont pas nécessaires pour la régulation physiologique de la formation de la mémoire en soi, mais que leur activation est nécessaire pour les troubles de la mémoire induits par le THC. En recherchant la signalisation intracellulaire et intra-mitochondriale de la protéine G impliquée dans ce processus, nous avons développé une nouvelle stratégie chimiogénétique qui module spécifiquement la signalisation mitochondriale de la protéine G et nous avons observé sa contribution dans l'activité mitochondriale du cerveau et les fonctions cognitives. Nous avons observé sa contribution à l'activité mitochondriale du cerveau et aux fonctions cognitives. L'activation chimiogénétique spécifique de la signalisation mitochondriale de la protéine G entraîne une augmentation de la respiration mitochondriale qui, à son tour, résout l'effet amnésique induit par le THC.

Dans l'ensemble, les résultats de cette thèse indiquent les mécanismes reliant la diversité des récepteurs CB₁ cellulaires et subcellulaires dans les fonctions cérébrales supérieures, y compris l'apprentissage et la mémoire, et fournissent la base pour le développement de stratégies thérapeutiques plus sélectives et précises pour les troubles cognitifs.

Mots clés : [Récepteur CB₁, récepteur mtCB₁, cellules D₁-positives, mémoire]

INSERM U1215 – NEUROCENTRE MAGENDIE

Inserm U1215, Neurocentre Magendie, 146 rue Léo Saignat, 33077 Bordeaux cedex, FRANCE

Title: Role of cellular and sub-cellular CB₁ receptor in Hippocampal D₁-positive cells on Memory Processes

Abstract:

Via modulation of neuronal activity by cannabinoid receptor type-1 (CB₁), the endocannabinoid system represents a major brain modulatory system controlling memory functions. On the other hand, several reports point out a crucial role of hippocampal dopamine signaling in the regulation of memory related processes. Furthermore, recent evidence suggests that hippocampal cells expressing dopamine receptors do also possess CB₁ receptors.

The work presented in this Thesis aims at establishing a functional connection between CB₁ receptor and dopaminergic signaling in the regulation of hippocampal related memory processes with particular emphasis on the cellular and sub-cellular mechanisms involved.

In the first part of the thesis we observed that a mouse line lacking CB₁ in dopamine receptor type-1 cells (D₁-CB₁-KO) displayed impaired long-term novel object recognition memory (NOR) and, interestingly, viral-mediated re-expression of CB₁ in D₁-positive cells in the hippocampus of D₁-CB₁-KO mice reversed the NOR impairment present in these mice. Furthermore, we pointed out excessive hippocampal GABA_A receptor activation and impaired *in vivo* long-term potentiation (LTP) in the CA3-CA1 pathway as the main cellular mechanisms for memory impairment in D₁-CB₁-KO. Thus, we provided functional evidence for the involvement of a small subclass of type-1 cannabinoid receptor (CB₁)-expressing hippocampal interneurons in the modulation of specific hippocampal circuits in memory processes.

The second part of the Thesis focused on subcellular location of CB₁ activation in D₁ positive cells. Indeed, besides the canonical regulation of neuronal activity by plasma membrane CB₁ receptor, recent evidence suggests the involvement of mitochondrial CB₁ receptor (mtCB₁) in the regulation of bioenergetic processes which impacts on synaptic transmission and amnesic effects of cannabinoids. We found that mtCB₁ receptors in hippocampal D₁-positive neurons is not required for physiological regulation of memory formation *per se* but its activation is required for THC-induced memory impairment. Looking for the intracellular and intra-mitochondrial G-protein

signaling involved in these processes, we developed a new chemogenetic strategy which specifically modulates the mitochondrial G-protein signaling and we observed its contribution in brain mitochondrial activity and cognitive functions. Specific chemogenetic activation of mitochondrial G-protein signaling results in increased mitochondrial respiration which in turns rescues THC-induced amnesic effect.

Overall, the results of this Thesis indicate the mechanisms linking the diversity of cellular and subcellular CB₁ receptors in higher brain functions, including learning and memory and provide the basis for the development of more selective and precise therapeutic strategies for cognitive disorders.

Keywords: [CB1 receptor, mtCB1 receptor, D1-positive cells, memory]

INSERM U1215 – NEUROCENTRE MAGENDIE

Inserm U1215, Neurocentre Magendie, 146 rue Léo Saignat, 33077 Bordeaux cedex, FRANCE

LIST OF ABBREVIATIONS

2-AG	arachidonoylglycerol
AC	adenylyl cyclase
ACEA	arachidonyl-2'-chloroethylamine
AEA	arachidonoyl ethanolamide
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	adenosine triphosphate
CaMKII	ca ²⁺ /calmodulin-dependent protein kinase II
cAMP	cyclic adenosine monophosphate
CB ₁	cannabinoid type-1
CB ₂	cannabinoid type-2
CB-LTD	cannabinoid-induced LTD
CCK	cholecystokinin
CNS	central nervous system
CRE	CRE recombinase
CREB	cAMP responsive element binding protein
D ₁	dopamine type-1
D ₂	dopamine type-2
DAG	diacylglycerol
DI	discrimination Index
DREADDs	designer receptor exclusively activated by designer drugs
DS	dopaminergic system
DSE	depolarization induced suppression of excitation
DSI	depolarization induced suppression of inhibition
eCB	endocannabinoid
eCB-LTD	endocannabinoid mediated LTD
ECS	endogenous cannabinoid system
EPSP	excitatory postsynaptic potential
ERK	extracellular signal-regulated kinase
ERT	estrogen receptor
FAAH	fatty acid amid hydrolase
fEPSP	field excitatory post synaptic potentials
GABA	gamma-aminobutyric acid
GFAP	glial fibrillary acidic protein
GPCR	G protein coupled receptor

HPA	hypothalamic–pituitary–adrenal
HFS	high frequency stimulation
I-LTD	inhibitory-LTD
IP3	inositol trisphosphate
ISH	in situ hybridization
JNK	c-Jun N-terminal kinase
KO	knockout
LTD	long-term depression
LTP	long-term potentiation
MAGL	monoacylglycerol Lipase
MAPK	mitogen-activated protein kinases
mGlu	metabotropic glutamate receptor
mRNA	messenger ribonucleic acid
MSN	medium spiny neurons
mtCB ₁	mitochondrial CB1 receptor
NAPE	N-arachidonoyl phosphatidyl ethanol
NMDA	N-methyl-D-aspartate receptor
NORT	novel object recognition task
OXPPOS	oxidative phosphorylation
PKA	protein Kinase A
PLC	phospholipase C
PLD	phospholipase D
PV	parvalbumin
STED	stimulated emission depletion
THC	Δ 9-tetrahydrocannabinol
TRPV-1	transient receptor potential vanilloid 1
VGCC	voltage-gated calcium channel
VTA	ventral tegmental area
WT	wild-type

LIST OF FIGURES

Figure 1 – Distribution of CB ₁ Receptors in the adult mouse brain	28
Figure 2 – In the Hippocampus: Presynaptic Terminal Distribution of CB ₁ Receptors.....	29
Figure 3 – In the Hippocampus: Intracellular and Subcellular Distribution of CB ₁ Receptors.....	30
Figure 4 – Different Pathways involved in the Biosynthesis and Degradation of AEA and 2-AG.....	34
Figure 5 – Generation of a cell-type specific KO mouse	37
Figure 6 – Involvement of the Endocannabinoid System in Different Neuropathologies.....	39
Figure 7 – LTP at Hippocampal CA1 Synapses.....	51
Figure 8 – Molecular Mechanism of Long-Term Potentiation.....	55
Figure 9 – Intracellular CB ₁ Receptor Signaling Pathways.....	57
Figure 10 – Endocannabinoid-Mediated Short- And Long-Term Synaptic Plasticity Mechanisms....	60
Figure 11 – Scheme of the Novel Object Recognition Task.....	67
Figure 12 – View of the CB ₁ receptor functional expression: Classic Vs Current.....	75
Figure 13 – Schematic view of the mtCB ₁ -dependent Signalin pathway	81

SECTION I – GENERAL INTRODUCTION

PART 1 – THE ENDOCANNABINOID SYSTEM

I – Introduction

The Endogenous Cannabinoid System (ECS) is a complex set of circuits coordinating many other systems of our organism, thus representing a regulator of many physiological processes (Pacher et al. 2006). Because of multitude of effects in both humans and animals, the interest in understanding the action mechanisms of *cannabis sativa* (commonly named marijuana or simply cannabis) started. Originary from Asia, cannabis has been used for medical, spiritual, religious or recreational purposes for at least 5,000 years (a Chinese pharmacological treatise attributed to Emperor Shen Nung, dated 2737 BC, contains the first reference to the use of cannabis as medicine). The Aryans smoked cannabis and they may have been to teach the properties of cannabis to both the Indian people and the ancient Assyrians (Curran et al., 2016, Mechoulam et al., 2014).

The date on which cannabis was introduced into Central, Northern and Western Europe is unknown, but it probably dates back at least 500 years before Christ, since an urn containing cannabis leaves and seeds was found in Berlin about 2,500 years ago. Also a few centuries before Christ, before the advent of the Roman Empire, various European peoples such as the Celts and the Picts cultivated and used cannabis. From then on, cannabis cultivation in Europe has been common, if not massive, for centuries. Europeans also knew, of course, the recreational potential of the plant (Mechoulam and Parker, 2013).

The medical use of cannabis was introduced into Europe only around 1840 by a young Irish doctor named William O'Shaughnessy. He worked in India, for the East India Trading Company, where the medical use of cannabis was widespread. In the following decades, cannabis had a short period of popularity in both Europe and the United States. At the peak of its popularity, different medicinal preparations were available, with cannabis as an active ingredient, which were prescribed for various indications, such as menstrual cramps, asthma, coughs, insomnia, childbirth pains, migraines, throat infections and opium withdrawal symptoms.

The use of cannabis as a medicine disappeared at the beginning of the 20th century due to some of its chemical and physical properties, which made impossible the creation of medicinal preparations standardized and therefore reliable. Therefore, its place was replaced by opium-derived medicines such as morphine and codeine.

Scientific research aimed at understanding the mechanisms underlying the effects of cannabis on the brain started in the 20th century. Indeed, in the two decades that followed the identification and synthesis of Δ^9 -tetrahydrocannabinol (THC), the psychoactive molecule of Cannabis, by scientists Mechoulam and his colleague Yoel Gaoni in Israel in 1964 (Gaoni and Mechoulam, 1964), scientists collected a huge *plethora* on notions on the pharmacology, biochemistry and clinical effects of Cannabis.

In 1990, a potent THC-like molecule synthesised by Pfizer (CP55,940) enabled researchers to begin mapping the precise positions of cannabinoid receptors in the brain, following the signals emitted by a radioactive tag bound to this molecule (Herkenham et al. 1990).

In the same year, at a conference of the National Academy of Science's Institute of Medicine, Dr. Lisa Matsuda announced that she and her colleagues at the National Institute of Mental Health (NIMH) had made a fundamental discovery by locating the precise sequence of DNA coding for THC-sensitive receptors in the mouse brain. Dr Matsuda also announced that she had successfully cloned the sensitive receptor to Cannabis, and called it Cannabinoid-type 1 receptor (CB₁) (Matsuda, et al. 1990).

The greatest advances in Neurophysiology of cannabinoids started in the 1990s and was followed by 25 years of scientific evidence, which gave a fairly clear picture of the ECS functionality.

In 1992, a collaboration between researchers brought to light a new neurotransmitter, called the "endocannabinoid" (eCB), a molecule that binds with the same receptors in the brain that are sensitive to THC. They called this substance "Anandamide", (AEA) (Devane et al. 1992).

In 1995 the Mechoulam group discovered, in parallel with another group of Japanese researchers, a second important eCB, 2-arachidonylglycerol, (2-AG) (Sugiura et al. 1995).

This eCB binds not only to CB₁ receptors mainly present in the brain, but also to a second type, called CB₂ receptors, which was identified in 1993 (Munro et al. 1993).

In 1998, Professor Vincenzo di Marzo defined the ECS as "A central regulator capable of modulating and balancing the main activities of organisms such as eating, sleeping, relaxing, protecting and forgetting" (Di Marzo et al. 1998).

In the following sections, I will introduce the ECS also making an excursus of the main and important findings that have highlighted the functional relevance of this system in the pathophysiology of the central nervous system.

The aim of the thesis is investigating how the ECS can modulate some of the most important physiological and adaptive functions of our organism: memory, learning and synaptic plasticity. I will focus my attention on hippocampal circuits and dopaminocetive cells in this brain area.

II – Cannabinoid receptors

The isolation and purification of the components of marijuana, cannabidiol and THC, which took place in the '60 years (Mechoulam et al. 1965; Mechoulam and Gaoni, 1965), led to an explosion of cannabinoid research but, paradoxically, their molecular mechanisms of action were still not fully elucidated. One of the first studies that proved the intracellular effects of cannabinoids and their action through a receptor was that carried out by the pioneer Howlett (Howlett, 1984), using neuroblastoma membrane preparations. He demonstrated the decrease in the accumulation of cyclic AMP (cAMP) stimulated by prostanoids, and therefore an inhibition of adenylate cyclase by THC. Later, Howlett and colleagues demonstrated that this effect required a functional Gi protein (Howlett et al. 1986). In 1988 this group was the first to isolate and characterize a cannabinoid receptor in the rat brain (Devane et al. 1988). The gene encoding this receptor was then identified and cloned by Matsuda et al. (1990) and called cannabinoid receptor type 1 (CB₁). A second receptor was identified in the HL-60 cell line in 1993 and named cannabinoid receptor type 2 (CB₂) (Munro et al. 1993).

CB₁ and CB₂ receptors belong to the 7-domain transmembrane receptor family. Most of the biological effects described for cannabinoids are mediated by the coupling of Gi/Go type G proteins (GPCRs). GPCRs are a diverse family of eukaryote-specific membrane receptors which convert external signals, such as light, peptides, lipids and proteins, into specific cellular responses. Their key role in cellular signaling has made them the central focus of modern drug discovery (Hauser et al. 2017). GPCR domains comprise the extracellular N terminus, seven transmembrane alpha helices (TM), loops connecting the TMs, and an intracellular C terminus. The ligand generally binds through a binding site gap formed by the TM bundle, directly by a pocket formed by the extracellular loops, or a combination of extracellular loop and binding site gap residues. Binding induces a conformational change in the receptor, causing activation of a G-protein which then initiates a specific cellular process (Latorraca et al. 2017; Weis and Kobilka, 2014).

The human CB₁ and CB₂ receptors are closely related GPCRs, exhibiting approximately 44% amino acid similarity overall and 68% homology in the TMs (Munro et al. 1993; Hryhorowicz et al. 2019). It appears that CB₁ can pair to both Gi and Go, while CB₂ is thought to preferentially pair to Go (Glass

and Northup, 1999). This coupling difference could explain the variation in THC's effectiveness in activating these CB₁ and CB₂ receptors: although THC binds with the same affinity to both CB₁ and CB₂ receptors, it activates CB₁ but does not, or only partially, the CB₂ (Bayewitch et al. 1996).

Although CB₁ and CB₂ are well known and characterized, numerous pharmacological studies suggest the existence of additional metabotropic and ionotropic cannabinoid receptors able to respond to the endogenous agonists. Among these, the transient receptor potential vanilloid type 1 (TRPV1) ion channel, which was found to bind some cannabinoid ligands (Caterina et al. 1997), G-protein-coupled receptor 55 (GPR55) and G-protein-coupled receptor 119 (GPR119) as novel potential cannabinoid receptors (Brown, 2007).

GPR55 was cloned in mouse, rat and human (Ryberg et al. 2007). The human GPR55 (hGPR55) gene is located on chromosome 2 and encodes a protein of 319 amino acids, shares only 14% sequence identity with the CB₁ and CB₂ receptors and is mainly expressed in the brain (caudate and putamen) (Ross, 2009). The human GPR119 is encoded by a protein of 335 amino acids and isoforms of this receptor are present in various mammalian species (Fredriksson et al. 2003). Expression profiles of GPR119 mRNA receptor seem to be restricted to the pancreas, foetal liver and gastrointestinal tract in human (Overton et al. 2008).

Because CB₁ receptors are known to mediate the majority of the cannabinoid-induced psychotropic effects, studying the role of CB₁ receptors in brain physiology and pathology is a major topic in cannabinoid research. In next sections, I will thereby concentrate on CB₁ receptor and how its physiology and biology is involved in brain plasticity and behavior and what is its role in the modulation of brain functions.

III – Localization of CB₁ receptors in the brain

The cannabinoid receptor type-1 (CB₁) is considered one of the GPCRs with the highest expression rate in the brain (Busquets-Garcia et al. 2018a). CB₁ receptors are most highly expressed by the axons and presynaptic terminals of neurons in the neocortex, olfactory system, amygdala, striatum, cerebellum, thalamus, substantia nigra, ventral tegmental area (VTA), periaqueductal gray and the spinal cord and hippocampus (Figure 1) (Busquets-Garcia et al. 2016; Hu and Mackie, 2015; Marsicano and Kuner, 2008; Soria-Gomez et al. 2017), the latter, which will be the main region of interest of this thesis.

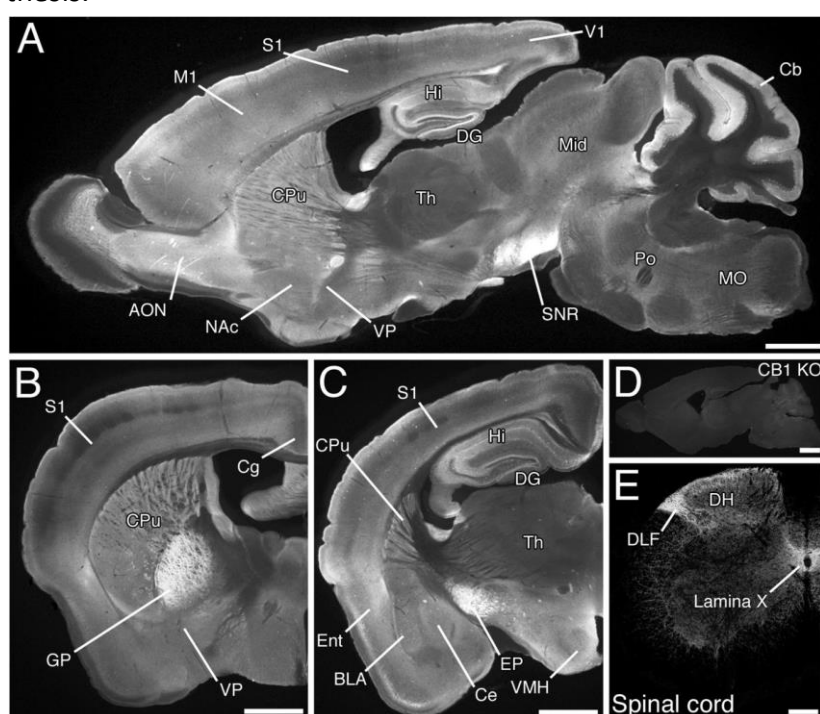


FIGURE 1 – DISTRIBUTION OF CB₁ RECEPTORS IN THE ADULT MOUSE BRAIN

Distribution of CB₁ receptors in the central nervous system of adult mice. A–D: overall distribution in parasagittal (A and D) and coronal (B and C) brain sections of wild-type (A–C) and CB₁-knockout (D) mice immunolabeled with a high-titer polyclonal antibody against the COOH terminus of mouse CB₁ receptor [443–473 amino acid residues, GenBank accession no. NM007726; Fukudome et al., 2004]. CB₁ immunoreactivity is highest along striatal output pathways, including the substantia nigra pars reticulata (SNR), globus pallidus (GP), and entopeduncular nucleus (EP). High levels are also observed in the hippocampus (Hi), dentate gyrus (DG), and cerebral cortex, such as the primary somatosensory cortex (S1), primary motor cortex (M1), primary visual cortex (V1), cingulate cortex (Cg), and entorhinal cortex (Ent). High levels are also noted in the basolateral amygdaloid nucleus (BLA), anterior olfactory nucleus (AON), caudate putamen (CPU), ventromedial hypothalamus (VMH), and cerebellar cortex (Cb). Virtual lack of immunostaining in CB₁-knockout (KO) mice indicates the specificity of the CB₁ immunolabeling. E: CB₁ immunolabeling in the spinal cord. Note that striking CB₁ immunoreactivity is seen in the superficial dorsal horn (DH), dorsolateral funiculus (DLF), and lamina X.

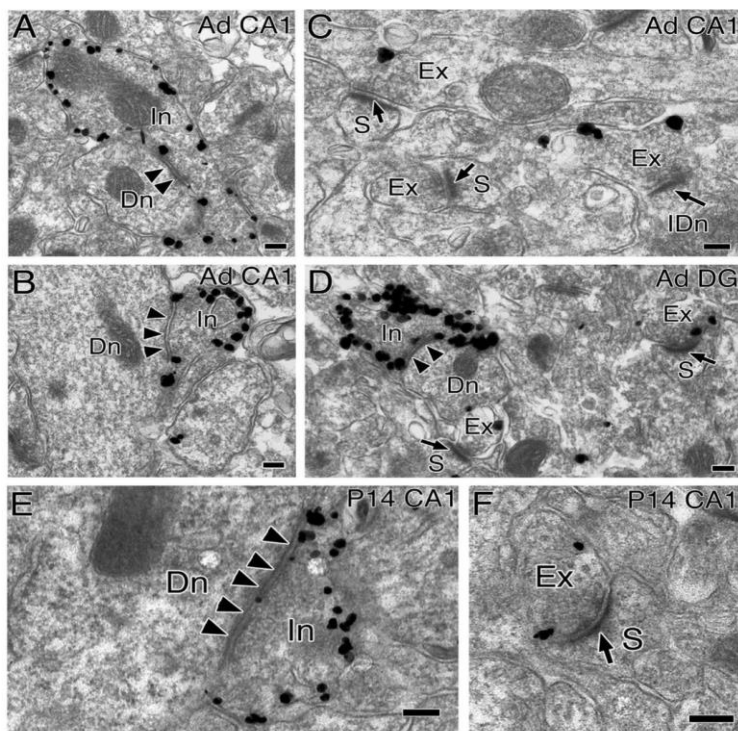
NAc, nucleus accumbens; VP, ventral pallidum; Ce, central amygdaloid nucleus; Th, thalamus; Mid, midbrain; Po, pons; MO, medulla oblongata; Or, stratum oriens; Py, pyramidal cell layer; Ra, stratum radiatum; Lm, lacunosum moleculare layer; Mo, dentate molecular layer; Gr, dentate granular layer; ML, cerebellar molecular layer; PC, Purkinje cell layer; GL, cerebellar granular layer; LI, lamina I; LIIo, outer lamina II; LIII, inner lamina II. Scale bars: 1 mm (A–C, E); 200 μm (D). [(A–E) from Kano et al. 2009].

III.A – CB₁ LOCALIZATION IN DIFFERENT NEURONAL POPULATIONS IN THE HIPPOCAMPUS

The hippocampus is a forebrain structure that participates in cognitive functions such as learning, memory, and sensory integration (Scarante et al. 2017; Galve-Roperh et al. 2013; Mackie, 2005) and is one of the brain regions with the highest expression of CB₁ receptors (Marsicano and Kuner, 2008).

In the hippocampus, the CB₁ receptor is primarily localized in GABAergic neurons, but it can also be found in glutamatergic neurons (Figure 2) (Busquets-Garcia et al. 2018a; Gutierrez-Rodriguez et al. 2018; Hebert-Chatelain et al. 2016; Jimenez-Blasco et al. 2020).

GABAergic inhibitory interneurons are widely distributed throughout the different hippocampal subregions. Although they represent only 10 to 15% of the neuronal population, they provide the



inhibitory input necessary to regulate excitation and facilitate neural oscillation. GABAergic cells are classified by their neuroanatomical characteristics, molecular expression profiles (parvalbumin (PV) or cholecystinin (CCK) expressing cells), developmental origins, or their electrical activity (Pelkey et al. 2017). Notably, most CCK positive interneurons express CB₁ receptors, whereas only a little percentage of PV interneurons do (Katona et al. 1999).

FIGURE 2 – IN THE HIPPOCAMPUS: PRESYNAPTIC TERMINAL DISTRIBUTION OF CB₁ RECEPTORS

Immunoelectron microscopy showing presynaptic localization of CB₁ receptors in the hippocampus. Ultrathin sections were prepared from adult (A–D) or P14 (E, F) mice. A–F: preembedding silver-enhanced immunogold for CB₁ in the stratum radiatum of the CA1 region (A–C, E, F) and in the innermost molecular layer of the dentate gyrus (D). Arrowheads and arrows indicate symmetrical and asymmetrical synapses, respectively. Dn, dendrite; Ex, excitatory terminal; IDn, interneuronal dendrite; In, inhibitory terminal; S, dendritic spine. Scale bar: 100 nm. [(A–F) from Kano et al. 2009].

CB₁ receptors have been described anatomically and functionally in the glutamatergic pyramidal neurons of the CA1 and CA3 regions, although in considerably less amount as compared to GABAergic neurons (Katona et al. 2006; Marsicano and Lutz, 1999; Marsicano et al. 2003).

Besides the classic excitatory/inhibitory transmission regulation by CB₁ receptors, cannabinoid signaling also controls cholinergic and dopaminergic neurotransmitter release (Marsicano et al. 2003; Degroot et al. 2006).

III.B – CB₁ LOCALIZATION IN OTHER CELL TYPES AND INTRACELLULAR ORGANELLES

During the past years, many studies confirmed by both in vitro and in vivo experiments the functional expression of CB₁ receptors in astroglial cells (Figure 3, from Han et al., 2012) (Navarrete and Araque, 2008; Han et al. 2012; Bosier et al. 2013), which can modulate glutamatergic transmission indirectly through astrocyte-dependent mechanisms such as the release of gliotransmitters like glutamate, adenosine and D-serine (Robin et al. 2018; Perea et al. 2009; Navarrete and Araque, 2010).

Although CB₁ receptors are localized primarily at the plasma membrane, more and more evidence suggest the presence of functional intracellular CB₁ receptors such as endosomes (Dudok et al. 2015) and brain mitochondria (mtCB₁) (Figure 3) (Bénard et al. 2012; Hebert-Chatelain et al. 2014; Koch et al. 2015; Hebert-Chatelain et al. 2016).

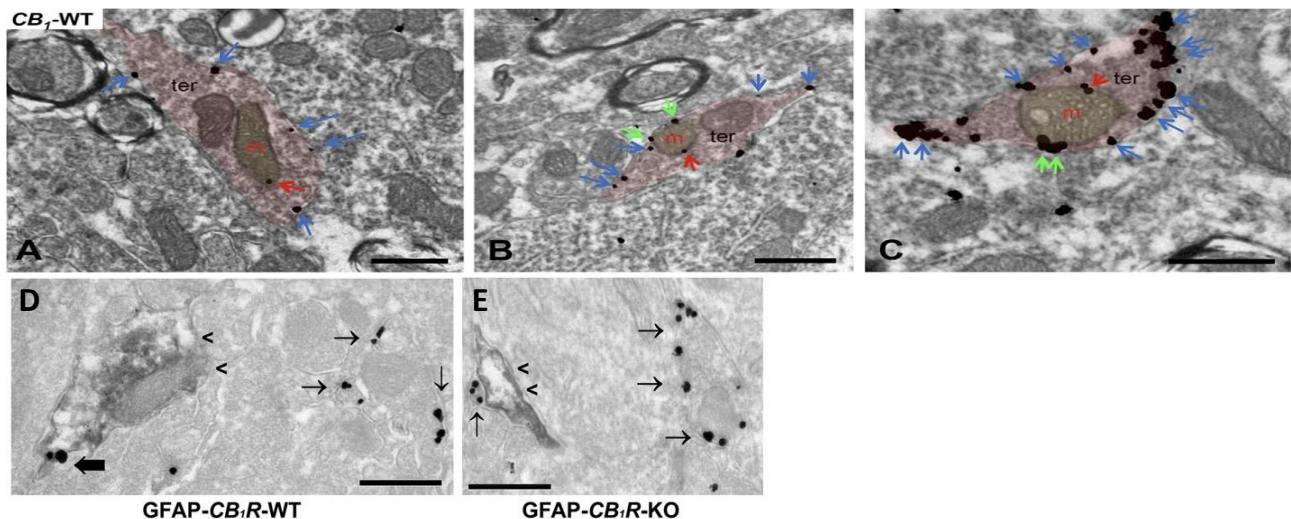


FIGURE 3 – IN THE HIPPOCAMPUS: INTRACELLULAR AND SUBCELLULAR DISTRIBUTION OF CB₁ RECEPTORS

A-C. Immunoelectron detection of CB₁ receptors in neuronal CA1 hippocampal mitochondria in CB₁-WT by a goat anti-CB₁ C-ter31 antibody combined with a pre-embedding silver-intensified immunogold method [(A-C) from Benard et al. 2012]. D-E. Electron microscopic images show a high density of CB₁R immunopositive silver grains (small arrows) in axons/terminals of both tamoxifen-treated GFAP- *CB1R*-WT and GFAP-*CB1R*-KO mice, and a low density of silver grains (large arrow) in DAB-stained astrocytes (arrowheads) of GFAP-*CB1R*-WT mice but not of GFAP-*CB1R*-KO littermates. The scale bar represents 500 nm. [(D,E) from Han et al. 2012]

Mitochondria are a subset of subcellular organelles which play a crucial role in maintaining cellular metabolic and energetic homeostasis. Defects in mitochondrial functions and structure have been associated to different pathologies, including obesity, type-2 diabetes, and neurodegenerative diseases (Bournat and Brown, 2010; Hojlund et al. 2008; Schneeberger et al. 2013; Dietrich et al. 2013). In the central nervous system (CNS), for example in specific neurons of hypothalamus, mitochondria are also important for coordinating energy intake and expenditure regulating diet-induced obesity (Schneeberger et al. 2013; Dietrich et al. 2013).

Several reports showed that different exogenous agonists of CB₁ receptors, such as THC, WIN55,212-2, HU210, or JZL195 (an inhibitor of the two enzymes responsible for the degradation of endocannabinoids) dose-dependently decreased respiration rates of purified brain mitochondria from wild-type (WT) mice, but not from CB₁-KO mice (Bénard et al. 2012) suggesting a direct link between endocannabinoids, CB₁ receptors, and brain cellular bioenergetics, which could constitute a new target for the treatment of biochemical and metabolic disorders.

IV – Endocannabinoids Synthesis, Transport and Degradation

The main endocannabinoids isolated from central and peripheral nervous system tissues are arachidonylethanolamide (also known as anandamide, AEA) (Devane et al. 1992) and 2-arachidonoylglycerol (2-AG) (Figure 4) (Mechoulam et al. 1995). Structurally, endocannabinoids are lipids derived from esters, ethers and amides of long chain polyunsaturated fatty acids (Di Marzo et al. 1994). These lipid compounds are the only known endogenous molecules capable of binding to cannabinoid receptors (CB₁ and CB₂) and mimic the pharmacological and behavioral effects of Δ 9-THC.

Anandamide and 2-AG possess some features that allow them to be classified as full-fledged neurotransmitters. However, a feature that distinguishes endocannabinoids from many other neuromodulators is that they are not synthesized in advance and not stored in vesicles. Classical neurotransmitters are synthesized in the cytoplasm of neurons and stored in synaptic vesicles, from which they are excreted by exocytosis into the synaptic cleft after excitation of the nerve ending by action potentials (Piomelli et al. 2000; Di Marzo et al. 1994). For endogenous cannabinoids, their precursors exist in cell membranes and are cleaved by specific enzymes and released in the synaptic cleft. This form of synthesis is often referred to as "on demand", i.e. when and where necessary.

AEA is the amide formed between arachidonic acid and ethanolamine, from the hydrolysis of N-arachidonoyl-phosphatidylethanolamine. It belongs to the family of the N-acyl-ethanolamines for which the biosynthesis depends on the hydrolysis of the corresponding N-acyl-phosphatidylethanolamines (NAPE) by a phospholipase D-like enzyme (NAPE-PLD) (Di Marzo et al. 1994; Okamoto et al. 2004).

Anandamide levels in the brain are comparable to other neurotransmitters such as dopamine or serotonin. The highest levels correspond to areas of high expression of CB₁ receptors, i.e. the hippocampus, striatum, cerebellum or cortex (Di Marzo et al. 1994; Felder et al. 1996). Anandamide binds preferentially to CB₁ receptors compared to CB₂ receptors (its affinity is four times higher for CB₁).

The enzymatic cascade responsible for the formation of the second messengers inositol (1,4,5)-triphosphate (IP₃) and 1,2-diacylglycerol (DAG), is involved in the biosynthesis of 2-AG. Phospholipase C (PLC) hydrolyses phosphatidylinositol (4,5)-biphosphate into DAG, which in turn is converted into 2-AG by DAG lipase (Stella et al. 1997). Two DAG lipase isoenzymes have been cloned so far, enzymatically characterized and proposed to be responsible for the formation of 2-AG (Sugiura et al. 2006). More recently, several studies provided evidence that DAG lipase α is the major isoform involved in the biosynthesis of 2-AG in the brain, using a DAG lipase knock-out mice model (Gao et al. 2010; Tanimura et al. 2010).

The formation of 2-AG has been shown to be triggered by neuronal activity or the activation of certain receptors (e.g. acetylcholine) (Stella et al. 1997; Mechoulam et al. 1998). After its release, 2-AG can be recaptured by cells via the anandamide transporter (Piomelli et al. 1999) and then hydrolyzed by a monoacylglycerol lipase enzyme (MAGL) or the alpha/beta-Hydrolase 6 (ABDH6) (Marrs et al. 2010). 2-AG equally binds to CB₁ and CB₂ receptors and, in the brain, its levels have been found to be 170 times higher than those of anandamide (Stella et al. 1997).

It is important to remind that classical neurotransmitters are usually inactivated by facilitated re-uptake from neurons and/or astrocytes and subsequent enzymatic degradation. The endocannabinoids, as any other endogenous mediators, need mechanisms for their rapid removal from their molecular targets and subsequent degradation. As lipophilic compounds, they can rapidly and efficiently diffuse through the cell membrane. Once inside the cell, the endocannabinoids are mainly degraded by two enzymes. The fatty acid amide hydrolase (FAAH) catabolises AEA to arachidonic acid and ethanolamine (Cravatt et al. 1996) but it can also degrade 2-AG.

On the other hand, MAGL, was identified as 2-AG hydrolases and localized in both membrane and cytosolic subcellular fractions (Ben-Shabat et al. 1998). This enzyme is highly expressed in the CNS (Dinh et al. 2002), and converts 2-AG into arachidonic acid and glycerol (Ahn et al. 2008). In hippocampal neurons, MAGL is expressed mainly presynaptically in glutamatergic and GABAergic terminals, in contrast to FAAH, which is mainly postsynaptic (Dinh et al. 2002). MAGL is localized in close proximity to CB₁ receptors to ensure a tight regulation of CB₁ receptor activity by 2-AG (Gulyas et al. 2004). At the subcellular level, MAGL have also been functionally and anatomically identified in the mitochondria (Alger and Tang, 2012; Marsicano and Kuner, 2008).

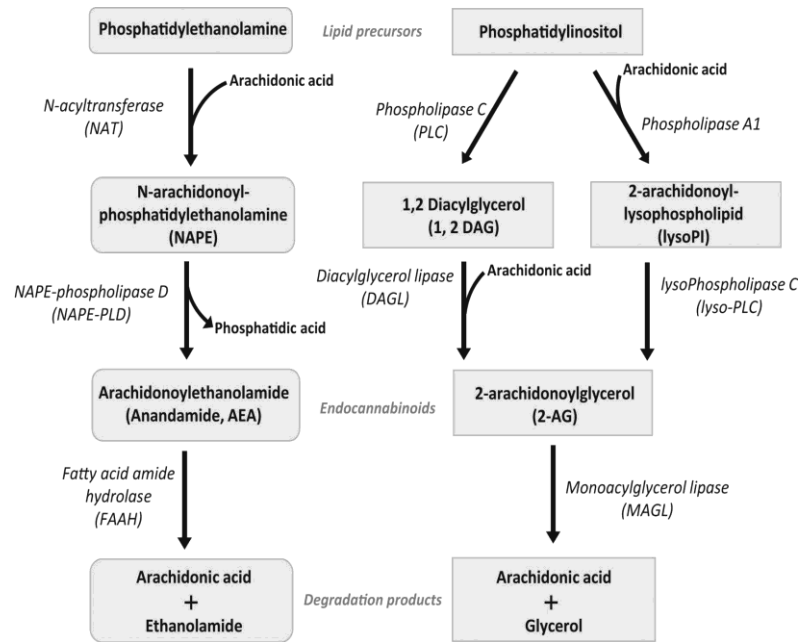


FIGURE 4 – DIFFERENT PATHWAYS INVOLVED IN THE BIOSYNTHESIS AND DEGRADATION OF AEA AND 2-AG
(From Simon and Cota, 2017)

V – Pharmacological and Genetic Tools to study the ECS

Given the wide brain distribution of the CB₁ receptor and the complexity of the endocannabinoid system, it is necessary to describe some of the main pharmacological tools to understand the specific function of CB₁ receptors at cellular, molecular and behavioral levels.

To date, the use of marijuana-derived Δ9-THC and synthetic analogues were key tools in the discovery and characterization of CB₁ receptor.

There are currently different synthetic molecules that can act as complete agonists and partial agonists. Antagonists blocking receptor action and reverse agonists reduce receptor activation below a baseline activity threshold. There are also allosteric modulators which, through binding at the allosteric sites, can modify the function of the CB₁ receptor (Mackie, 2008).

Among the synthetic CB₁ agonists, HU-210 is the most potent synthetic compound. A second group of CB₁ agonists used in pharmacological studies includes analogs of Δ9-THC, such as CP-55,940. A third group of ligands which also exhibit potent agonistic activity at CB₁ receptor includes the aminoalkylindoles, such as WIN-55,212 (Howlett, 2002).

Moreover, ACEA (arachidonoyl-2'-chloroethanolamide), a structural analogue of anandamide, has recently been characterized as a very potent and highly selective CB₁ agonist (Hillard et al. 1999).

Among the synthetic ligands showing antagonistic activity to cannabinoid receptors, SR 141716 (also known as Rimonabant) (Rinaldi-Carmona et al. 1994), AM251 (Gatley et al. 1996; Pertwee et al. 2010) and AM281 (Lan et al. 1999) are specific for CB₁.

Given the huge expression of CB₁ receptors in brain cells (e.g. glutamatergic and GABAergic neurons in the hippocampus) and circuits with apparent functional opposing effects, a valuable tool to study the specific functional role of this receptor at the molecular, cellular and behavioral level was the generation of mouse models which ubiquitously lack CB₁ receptors (Ledent et al. 1999; Marsicano et al. 2002; Zimmer et al. 1999).

However, the constitutive deletion of CB₁ receptor does not allow to study its specific contribution to brain functions (Castillo et al. 2012) but the use of the Cre recombinase (CRE)/loxP technology

allowed us to induce site-specific recombination events and to generate cell type-specific conditional KOs.

Cre recombinase is a 38 kDa protein responsible for intra- and inter-molecular recombination at the loxP recognition sites. LoxP sites are 34-base-pair long recognition sequences generally introduced into the genome by homologous recombination. These sequences do not have impact on the normal animal phenotype. Thus, mice carrying loxP sequences flanking the gene of interest (named floxed mice) are considered as WT animals (Nagy, 2000).

The Cre recombinase catalyzes the site-specific recombination event between two loxP sites (Orban et al. 1992; Sauer and Henderson, 1988), allowing the targeted excision of genes in the genome. In order to achieve the specific deletion of the gene of interest, “floxed” mice (i.e. with the gene of interest flanked by the LoxP sequences) are crossed with a mouse that expresses the Cre recombinase under the control of a promoter specific for the cell-type to be targeted (Nagy, 2000; Orban et al. 1992). The offspring derived from breeding will express the CRE in the cell-type of interest, allowing to modify the genome by excision of the “floxed” gene and generating a cell-type specific KO mouse (Figure 5) (Nagy, 2000).

However, one of the limits of CRE/LoxP system for genetic recombination lies on the tissue- and developmental- specific activity of the promoter used to drive CRE expression (Malatesta et al. 2003). For example, using the glial fibrillary acidic protein (GFAP, a cytoskeleton protein that is commonly used as a marker for astrocytic identification) promoter to drive CRE for the generation of a conditional KO would generate a mouse with recombination in both neurons and astrocytes (Garcia et al. 2004), thus making cell-type specific functional dissection undetectable (Brenner et al. 1994).

One way to bypass this problem is to generate a system that allows time-dependent inducible gene deletion. In the case of astrocytes, to achieve cell-type specific KO, Hirrlinger and colleagues (2006) developed the tamoxifen-inducible CRE-ERT2/loxP system (Hirrlinger et al. 2006). In this model the CRE is fused to a heat mutated ligand binding domain of the estrogen receptor (ERT). The CRE-ERT2 is expressed in the cells that have GFAP but it is only active after treatment with the selective estrogen ligand tamoxifen. Accordingly, this method allows temporal control of the generation of the tissue specific KO (Hirrlinger et al. 2006) diminishing the risk of having genetic recombination in neurons.

Overall, the use of this method allowed the cell-type specific dissection of the CB₁ receptor function in different brain functions and generate several mouse lines in order to go deeper into the knowledge of molecular function of CB₁ receptor in different cell populations.

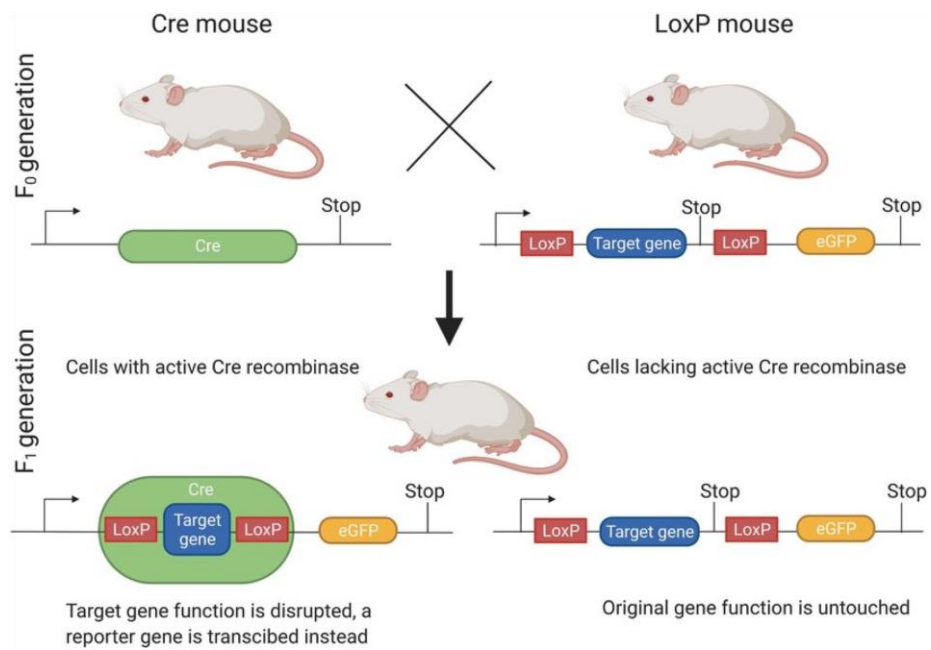


FIGURE 5 – GENERATION OF A CELL-TYPE SPECIFIC KO MOUSE
[from Shi et al. 2021]

VI – Pathological roles of the CB₁ receptor

The first traces of the medicinal use of cannabis derivatives are found in Chinese and Egyptian texts dating back several hundred years BC. Given the widespread distribution of CB₁Rs in the human body and the ubiquity of cannabinoid receptors lends itself to regulate a variety of cellular and physiological processes and, thus, serving essential role in both physiological and pathological conditions (Figure 6) (Reddy et al. 2020).

In recent decades, the endocannabinoid system has attracted considerable attention as a potential therapeutic target in numerous physiological conditions, from regulation of cellular functions, as neuromodulatory, to orchestrating complex metabolic and immune responses. Since the endocannabinoid system is vital for homeostasis of multiple biological processes, several pathological conditions pertaining to cardiovascular, neurological, metabolic and immunological diseases, are often associated with alterations of endocannabinoid system (Haspula and Clark, 2020).

This section describes the roles of cannabinoid receptors in various pathological conditions associated with changes in endocannabinoid tone. This includes some scientific evidence regarding the involvement of CB₁ receptor and the ECS in various aspects of central neural activities and disorders, including energy metabolism and eating disorders, learning and memory, anxiety and depression, pain modulation and addiction, as well as in some of the most important pathological conditions such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and multiple sclerosis which are still being studied (Pacher et al. 2006; Kano et al. 2009; Di Marzo et al. 2015; Iversen, 2003).

I will mention here only some of the best-established examples, to give a general overview of endocannabinoid regulation and function and underscore the significance of understanding and manipulating the endocannabinoid system in a specific pathological condition.

The effects of eCBs on synaptic plasticity and learning and memory will not be mentioned in this section, because the role of CB₁ receptor on hippocampal memory is the main topic of my Thesis and will be discussed in the next sections.

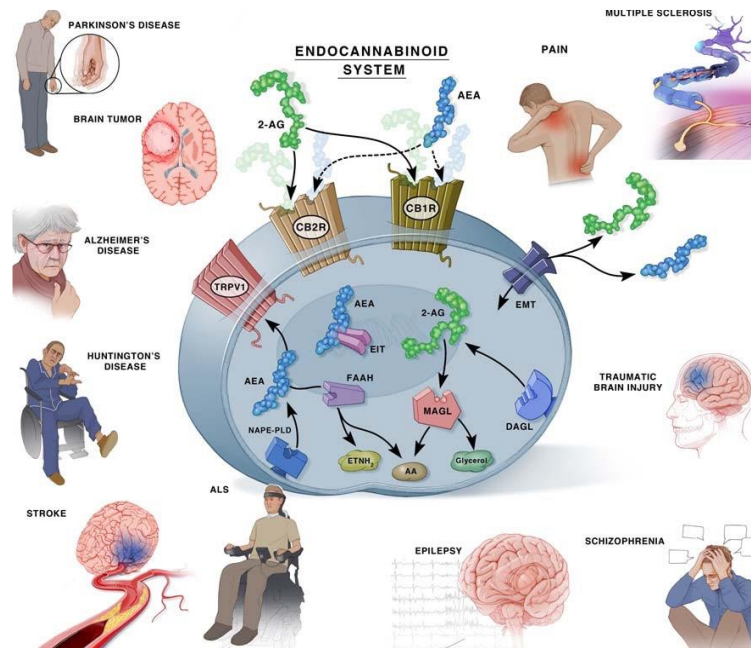


FIGURE 6 – INVOLVEMENT OF THE ENDOCANNABINOID SYSTEM IN DIFFERENT NEUROPATHOLOGIES
[from Reddy et al., 2020]

VI.A – EATING DISORDERS: OBESITY

The activation of CB₁ receptors in the CNS generally induces an increase in the sensitivity to appetitive properties of food and stimulation of food intake beyond satiety. CB₁ receptor agonists increase the intake of palatable food (Brown et al. 1977; Koch and Matthews, 2001; Higgs et al. 2003; Perio et al. 2001), whereas CB₁ receptor blockade reduces it (Arnone et al. 1997; Freedland et al. 2001; Gallate et al. 1999; Simiand et al. 1998). Genetic studies with mice lacking the CB₁ receptor gene (CB₁-KO) display reduced sucrose operant responding and this phenotype appears to depend on the rewarding properties of sucrose (Sanchis-Segura et al. 2004). Different brain regions appear to be the sites where exogenous cannabinoids increase food intake (Mazier et al. 2015). Among these, the hypothalamus is the region to which researchers have pointed the importance of the ECS in the control of food intake. First, because there is abundant expression of CB₁ receptors in the hypothalamic nuclei controlling food intake (Cota et al. 2003a, 2003b; Fernandez-Ruiz et al. 1997; Marsicano and Kuner, 2008; Marsicano and Lutz, 1999; Mazier et al. 2015). Second, because the hypothalamic endocannabinoid levels are strongly modulated by the feeding status of the animals (Di Marzo et al. 2001; Hanus et al. 2003; Kirkham et al. 2002; Mazier et al. 2015). Accordingly, direct infusion of endocannabinoids or synthetic CB₁ agonists into different

hypothalamic nuclei increases food intake (Jamshidi and Taylor, 2001; Mazier et al. 2015; Williams and Kirkham, 1999; Soria-Gomez et al. 2014a, 2014b).

The hypothalamus plays a key role in the context of exostatic functions, because increased food intake can be interpreted as a behavior bound by the change in the incentive property of external stimuli induced by the activation of the lateral hypothalamus (Trojniar and Wise, 1991; Berridge and Valenstein, 1991). On the other hand, the limbic system, has been linked to the food palatability (Jager and Witkamp, 2014; Silvestri and Di Marzo, 2013). Recently the role of the ECS in olfactory circuits and olfactory bulb, where exogenous and endogenous CB₁ receptor agonists increase perception and attractiveness of both odors and food, has been described role (Soria-Gomez et al. 2014a).

Brain CB₁ receptors can also alter satiety, which normally decrease food intake during feeding. For instance, cannabinoid agonists trigger eating in a CB₁ receptor-dependent manner (Williams and Kirkham, 1999) and their activation overcomes satiety prompts, likely by promoting the incentive value of food (Higgs et al. 2003).

CB₁ receptor antagonism reduces body weight and food intake in free-fed animals (Black, 2004; Chambers et al. 2004; Chen et al. 2004; Colombo et al. 1998; McLaughlin et al. 2003) but stronger effects were observed in obesity animals' models (Hildebrandt et al. 2003; Vickers et al. 2003; Zhou and Shearman, 2004). Accordingly, CB₁-KO mice show slight alteration of food intake under basal conditions, but these effects become much more evident under fasting-induced food intake (Bellocchio et al. 2013; Cota et al. 2003a; Di Marzo et al. 2001).

Increased endocannabinoid levels and CB₁ receptor expression are hallmarks of obesity in rodents and humans, which strongly contribute not only to the development, but also to the maintenance of the pathology (Mazier et al. 2015; Silvestri and Di Marzo, 2013). Thus, the exostatic functions of the ECS are regulated by positive feedback mechanisms by which increased ECS activity leads to higher energy accumulation. This is supported by Osei-Hyiaman and colleagues' study (2005) made in conditional mutant mice lacking hepatic CB₁ receptor. In fact, in WT mice, high-fat diet induces a large increase in liver endocannabinoid levels while it is strongly reduced in mutant animals (Osei-Hyiaman et al. 2005).

Thus, we can argue the ECS plays a physiological role in promoting exostatic processes and energy accumulation, contributing to the development of obesity and metabolic disorders when the system

is activated beyond physiological levels.

VI.B – MOOD DISORDERS: ANXIETY AND DEPRESSION

Most of the CB₁ agonists and antagonists can produce both anxiogenic- and anxiolytic-like effects, and antidepressant- and prodepressant-like readouts. These effects are dependent on the animal species, its starting emotional state, the behavioral tests used to investigate anxiety- and depression-like behaviors, and the dose of compounds used (Paton and Pertwee, 1973; Ashton et al. 2005; Ashton et al. 1981; Viveros et al. 2005).

It is possible that the reasons for this lie on the biphasic and bidirectional effects of cannabinoids on anxiety, with low doses having anxiolytic, and high doses having anxiogenic-like effects (Chakrabarti et al. 1998; Berrendero and Maldonado, 2002; Onaivi et al. 1990; Genn et al. 2003; Marco et al. 2004; Rodriguez de Fonseca et al. 1996; Giuliani et al. 2000; Arevalo et al. 2001; Marin et al. 2003; Rey et al. 2012). On the other hand, the CB₁ antagonist SR141716A has been largely reported to induce anxiogenic effects, suggesting that eCB tone contributes to keep anxiety low.

The mechanisms mediating the anxiolytic effects of THC involve CB₁ receptor. For instance, low doses of THC increase the time spent on open arms in elevated plus maze (EPM) in rats, an index of anxiolytic-like effects (Rubino et al. 2007). The response is species-specific because, in mice, THC produces instead a dose-dependent reduction of time spent in the open arm (Patel and Hillard, 2006). These differences could be due to the different responsiveness of GABAergic and glutamatergic neurons to CB₁ activation as well as to different expression/distribution of CB₁ receptors in rats and mice (Haller et al. 2007). Accordingly, in rats, a low dose of THC injected in the prefrontal cortex elicits anxiolytic effects, whereas in the basolateral amygdala it produces an anxiogenic response (Rubino et al. 2008a). High doses usually induce anxiogenic-like responses in rodents (Long et al. 2010; Rubino et al. 2007). Chronic exposure to THC in adolescent rats induces a depressive-like phenotype in adulthood (Realini et al. 2011; Rubino et al. 2008).

CB₁ receptors mediate the extinction of aversive memories as shown by the finding that the pharmacological inhibition or genetic lacking in mice leads to impaired extinction in a fear-conditioning test (Marsicano et al. 2002). Indeed, the administration of THC during extinction learning facilitates extinction by preventing recovery of extinguished fear in rats (Ashton et al. 2005; Grinspoon and Bakalar, 1998).

Several evidence show that the endocannabinoid system may play a role in the aetiology of depression and could represent a new therapeutic target for its treatment. CB₁-KO mice showed altered HPA (hypothalamic–pituitary–adrenal) axis function (Urigen et al. 2004; Cota et al. 2007) and a higher sensitivity to exhibit depressive-like responses in the chronic unpredictable mild stress procedure, which suggests an increased susceptibility to develop an anhedonic state (Martin et al. 2002). These characteristics together with their heightened anxiety (Haller et al. 2002; Urigen et al. 2004) and deficits in extinction of aversive memories (Marsicano et al. 2002) have been proposed to be analogous to certain symptoms of depression (Hill and Gorzalka, 2005).

Several cannabinoid compounds have been evaluated in antidepressant-like behavioral tests such as the forced swimming test (FST) and the tail-suspension test (TST) (McArthur and Borsini, 2006). For example, administration of AM404 (endocannabinoid uptake inhibitor) and HU-210, a potent CB₁ receptor agonist, induced decreases in immobility in FST (indicative of antidepressant activity) This effect was blocked by pretreatment with the selective CB₁ receptor antagonist AM251. In turn, the FAAH inhibitor URB597 exerted potent antidepressant-like actions in the mouse TST and the rat FST, and these effects were prevented or attenuated by rimonabant (Gobbi et al. 2005).

During the last years, there has been a huge interest on the involvement of hippocampal neurogenesis in the pathophysiology and therapy of mood disorders. Preclinical and clinical studies indicate that stress, through glucocorticoids-mediated action, and depression lead to atrophy and loss of neurons in the adult hippocampus. On the other hand, chronic antidepressant treatment up-regulates hippocampal neurogenesis which could counteract the stress-induced damage (Elder et al. 2006; Warner-Schmidt and Duman, 2006).

Furthermore, Jiang and colleagues show that both embryonic and adult rat hippocampal neural stem/progenitor cells are immunoreactive for cannabinoid receptor, indicating a possible eCBs implication in the regulation of neurogenesis. Indeed, a chronic treatment with the potent synthetic cannabinoid HU210 promotes neurogenesis in the hippocampal dentate gyrus of adult rats and exerts anxiolytic- and antidepressant-like effects (Jiang et al. 2005). These evidences strongly suggested that cannabinoid agonists might produce anxiolytic- and antidepressant-like effects by promoting hippocampal neurogenesis (Harkany et al. 2007).

In view of the above, it was speculated that the endocannabinoid system is involved in the action of antidepressant drugs as well as in HPA axis. In favor of this hypothesis, chronic administration of

the antidepressant desipramine resulted in a significant increase in the expression of the cannabinoid CB₁ receptor in both hippocampus and hypothalamus as well as in a reduction in swim stress-induced corticosterone secretion. Moreover, acute treatment with the CB₁ receptor antagonist AM251 before exposure to stress blocked the effects of desipramine on corticosterone secretion (Hill et al. 2006).

VI.C – PAIN

Pain is a “sensation” given by a combination of a subjective experience in psychophysics, an objective sensory neurophysiology, as well as an emotional reaction to distressing stimuli (Rainville, 2002). These multifaced “feelings” make it especially difficult to understand and target therapeutically.

One of the earliest uses of cannabis was treating pain as well as its various facets. As there are several modalities of pain modulation, the endocannabinoid system has shown a particular link to a variety of pain pathways (Woodhams et al. 2015; Mallet et al. 2008; Kinsey et al. 2009; Guindon and Beaulieu 2009; Clapper et al. 2010).

Multiple lines of evidence support the important role of the cannabinoid signaling system in the modulation of pain (Guindon and Hohmann, 2009; Piomelli et al. 2014; Piscitelli and Di Marzo, 2012). Moreover, the role of the ECS in pain has been widely tested in animal models of acute, neuropathic and hyperalgesic pain (Pertwee, 2001; Walker and Huang, 2002; Fox and Bevan, 2005) in immune function and inflammation (Klein, 2005; Klein et al. 1998; Klein et al. 2003; Walter and Stella, 2004) and the antinociceptive power of CB₁ agonists has also been observed in humans (Hamann and di Vadi, 1999).

Cannabinoids exert their antinociceptive effects by complex mechanisms involving effects on the central nervous system (Martin et al. 1993; Hohmann and Herkenham, 1999; Richardson et al. 1997; Richardson et al. 1998; Meng et al. 1998; Strangman et al. 1998; Fox et al. 2001; Soria-Gomez et al. 2021). This is consistent with the massive anatomical localization of CB₁ receptors in brain areas relevant to pain, for example in basal ganglia, peripheral sensory ganglia neurons, dorsal horn of the spinal cord and supraspinal brain areas, such as the periaqueductal grey (PAG), rostral ventromedial medulla (RVM), and cortex (Herkenham et al. 1991; Hohmann and Herkenham, 1998; Hohmann and Herkenham, 1999; Sañudo-Peña et al. 1999). Furthermore, using CB₁ and CB₂

knockout (KO) mice it has become clear that that endocannabinoids produce analgesia mostly via cannabinoid receptor-mediated mechanisms (Kinsey et al. 2010).

Endocannabinoids modulate nociception by lowering sensory excitability and regulating the transmission of nociceptive signals to the CNS. Local injection of AEA is able to control the first phases pain (Calignano et al. 1998) showing a CB₁-dependent action, whereas 2-AG antinociceptive effects seem to require activation of both CB₁ and CB₂ receptors (Guindon and Hohmann, 2009).

These effects seem to occur via both presynaptic CB₁ receptors retrograde activation on GABAergic terminals which disinhibit the antinociceptive excitatory neurons of the PAG, and direct TRPV1-mediated activation of these same neurons (Hu et al. 2014; Maione et al. 2007; Starowicz et al. 2007).

In agreement with the important role of the ECS in the regulation of pain and inflammation, several studies highlight the complex anti-inflammatory effects of cannabinoids in the modulation of cytokine (e.g., TNF- α , IL-12, IL-1, IL-6, and IL-10) and chemokine production (e.g., CCL2, CCL5, CXCL8, and CXCL10), the expression of adhesion molecules, and the migration, proliferation, and apoptosis of inflammatory cells (Hampson et al. 2000; Carrier et al. 2006; Klein et al. 1998; Klein et al. 2003; Walter and Stella, 2004; Klein, 2005).

VI.D – ADDICTION

The endocannabinoid system is certainly the primary site of action for the rewarding and pharmacological responses induced by cannabinoids (Ledent et al. 1999; Lichtman and Martin, 2005; Melis and Pistis, 2014; De Vries and Schoffelmeer, 2005; Fattore et al. 2007; Laviolette and Grace, 2006; Maldonado et al. 2006) and was shown to be important for the rewarding effects of most addictive compounds, including nicotine (Cohen et al. 2002; Melis and Pistis, 2014), ethanol (Wang et al. 2003; Lavanco et al. 2018; Brancato et al. 2020), morphine (Ledent et al. 1999), and cocaine (Maldonado et al. 2006).

The ability to activate the mesolimbic dopaminergic reward pathway and increase dopamine levels in the Nucleus Accumbens (NAc) is the common denominator believed to be responsible for addictive properties of different addictive drugs interacting with distinct receptors (Koob, 1992; Wise, 2004) thus, ECS has been shown to play an overall modulatory effect on the reward circuitry. Indeed, endocannabinoids modulate the activity of the mesolimbic dopaminergic pathway by

multisynaptic strengthening of the release of dopamine in the NAc shell. The dopaminergic neurons of the reward circuit are controlled by excitatory and inhibitory inputs that are modulated by CB₁ cannabinoid receptors. Thus, endocannabinoids can be released following depolarization in the NAc (Robbe et al. 2002) and from dopaminergic neurons in the VTA (Melis et al. 2004; Riegel and Lupica, 2004), and they modulate glutamatergic and GABAergic afferents by acting as retrograde messengers on CB₁ receptors. The presence of CB₁ receptors in other structures related to motivation and reward, such as the basolateral amygdala and the hippocampus, also contributes to this function (Katona et al. 2001).

A further indication that endocannabinoids may be involved in mechanisms of drug reward is the evidence that pharmacological (Cohen et al. 2002) and genetic (Hungund et al. 2003) CB₁ receptor blockade abolishes the increase of extracellular dopamine levels in the NAc shell and the neurochemical and behavioral responses to rewarding as well as addictive substances. Similarly, THC and other agonists have been shown to increase extracellular dopamine levels in the NAc (Chen et al. 1990; Tanda et al. 1999) and boost the activity of dopaminergic neurons in the VTA via activation of CB₁ receptors (Johnson and North, 1992). The latter effect might be due to μ receptor-mediated inhibition of GABA release from the terminals of inhibitory GABAergic interneurons (Cheer et al. 2000). However, cannabinoids also inhibit glutamate release in the VTA, which would have an opposite effect on dopaminergic activity (Melis et al. 2004). Activation of CB₁ receptors on glutamatergic terminals in the NAc was reported to inhibit glutamate release onto GABAergic neurons in the NAc that project to the VTA, which may also result in disinhibition of VTA dopaminergic neurons (Robbe et al. 2001).

THC and related synthetic cannabinoid agonists also fulfill the reward-related behavioral criteria for drugs of abuse: they support conditioned place preference (CPP) (Lepore et al. 1995; Valjent and Maldonado, 2000; Zangen et al. 2006), they are self-administered intravenously or intracerebrally in a CB₁ antagonist-sensitive manner (Martellotta et al. 1998; Ledent et al. 1999; Braida et al. 2001; Zangen et al. 2006), and they reinstate cocaine- or heroine-seeking behavior in rats previously extinguished from self-administration (De Vries et al. 2001).

In a CPP paradigm, nicotine produced a significant rewarding effect in WT mice, but not in CB₁-KO mice (Castane et al. 2002). The CB₁ antagonist SR141716A reduced nicotine self-administration (Cohen et al. 2002) and nicotine-induced CPP (Le Foll and Goldberg, 2004) in rats. In a two-bottle

free-choice paradigm, ethanol preference of WT mice was reduced by SR141716A to the level observed in their CB₁-KO littermates (Wang et al. 2003). Morphine self-administration was abolished in CB₁-KO mice (Ledent et al. 1999).

Effects of CB₁ blockade on rewarding properties of cocaine were different in different paradigms (Maldonado et al. 2006). Cocaine-induced CPP was not modified in CB₁-KO mice. Moreover, cocaine self-administration was neither modified in CB₁-KO mice nor in rats treated with SR141716A. Interestingly, when the effort required to obtain a cocaine infusion was enhanced, however, acquisition of an operant response to self-administrable cocaine was impaired in CB₁-KO mice (Soria et al. 2005). This can be explained as a no-participation in the primary reinforcing effects of psychostimulants by endocannabinoid system, but is important for maintaining psychostimulant-seeking behavior.

These findings suggest that endocannabinoid activation of CB₁ receptors in the mesolimbic reward circuit is part of a “common pathway” of drug reward (De Vries and Schoffelmeer, 2005; Maldonado et al. 2006) supporting the idea that CB₁ antagonists or other drugs interfering with ECs action and release might represent a suitable therapeutic tool to tackle addiction and other drug-related disorders.

PART 2 – SYNAPTIC PLASTICITY AND MEMORY

The circuitry of human brain is composed of a trillion neurons and innumerable synapses, whose connectivity underlies perception, emotion, thought, and behavior (Jorgenson et al. 2015). Several studies have revealed that the complex structure of the nervous system is genetically hard-wired but that neural circuits undergo widespread shaping and remodelling in response to a variety of external or internal stimuli, such as for the storage of information gained through experience (Ho et al. 2011).

This process of experience-dependent changes in synaptic connectivity is called synaptic plasticity, a general mechanism by which stimuli can alter brain neuronal responsiveness and allow changes in the strength and number of synaptic connections between neurons leading to learn new abilities, form new memories and generate new adaptive behaviors (Cheyne and Montgomery, 2020).

In the next sections, I will focus on the hippocampal synaptic plasticity, the most important form of plasticity involved in learning and memory. In addition, I will first attempt to provide a broad overview of the mechanisms of synaptic plasticity emphasize current understanding of the cellular mechanisms and possible functions of phenomena commonly termed long-term potentiation (LTP). Following I will deeply go in the discussion of CB₁ receptor modulation of synaptic transmission and plasticity and the its potential role in the regulation of learning and memory.

I – How to study Synaptic Plasticity?

One of the most versatile approaches to study the role of endocannabinoid system in synaptic plasticity is the electrophysiological investigation of the electrical properties of neuronal networks. Indeed, electrophysiological recordings of synaptic currents have since long been the gold standard for measuring the efficacy of synaptic transmission. Electrophysiology allows the investigation from single cells to populations both in cellular cultures, in vitro slices and in vivo anesthetized, head restrained or freely moving animals. That allows us to understand how neurons communicate and how different cell-types interact, drawing regarding their function and dysfunction in the pathophysiology of the brain.

To investigate neural activity, researchers have developed various types of electrophysiological recording techniques, roughly divided into extracellular and intracellular methods. While extracellular recordings enable us to obtain data on neuronal firing generated by multiple cells surrounding recording electrodes, intracellular recordings allow for the measurement of subthreshold membrane potential dynamics and supra-threshold firing activity at the single-cell level (Noguchi et al. 2021). In vitro electrophysiological techniques in CNS studies exploit ionic conductance of ion-channels and transient modulation of the membrane potential of a neuron. If the membrane potential becomes sufficiently depolarized, an action potential will trigger. Many in vitro electrophysiology techniques have been developed to detect and manipulate ion-channel function and/or action potential generation (Accardi et al. 2016).

The most commonly employed in vitro intracellular electrophysiological technique is the patch-clamp method. In order to attempt to patch-clamp neurons, the researchers press the recording pipette, filled with an artificial intracellular solution onto the cell membrane and tightly seal the membrane with a resistance of $>1 \text{ G}\Omega$ between the pipette and the membrane. This recording mode enables us to capture the dynamics of the membrane currents generated by ions through ion channels on the cell membrane. Once negative pressure is applied and a small hole is made on the cell membrane ("whole-cell"), the net dynamics of currents and voltages ("voltage clamp" and "current clamp" mode) generated through all ion channels expressed on the cell membrane can be measured. Voltage clamp aims at recording the changes in current across the membranes by holding

voltage at a specific value. Current clamp allows the study of the membrane potential when injecting current and give information about the ionic conductance of the membrane (Accardi et al. 2016). One of the principle limitations of both the patch-clamp and impalement techniques is that they are intracellular recording systems and, as such, are limited to assessment of individual neurons. Thus, extracellular recordings have been developed to study neuronal circuit connectivity, physiology and pathology (Spira and Hai, 2013). Extracellular recording techniques insert electrodes into tissue which, depending on the positioning and tip size, measure the extracellular field potential generated by an action potential discharge from either a single neuron or neuronal population. Therefore, extracellular recordings can be used to understand neuronal communication, information encoding, propagation, processing and computation of neuronal circuits (Obien et al. 2014). These recordings can be carried out either in vivo on anesthetized or awake animals, ex vivo on brain slices or in vitro in cultured embryonic neuronal tissue (Legatt et al. 1980; Gray et al. 1995; Juergens et al. 1999; Erickson et al. 2008).

Focusing on the hippocampus, Hahn et al. first achieved in vivo whole-cell recordings from hippocampal pyramidal cells, dentate granule cells and even hippocampal interneurons of unconscious animals (Epsztein et al. 2006; Hahn et al. 2006, 2007, 2012; Leitner et al. 2016). For instance, the study of LTP can be done by recording the ionic extracellular potentials (from a group of neurons) that are artificially induced by a stimulation electrode in another brain region (e.g. Shaffer collateral to CA1 pathway) (Zhang et al. 2014). In this case, what we call potentiation is purely an increase in the extracellular field excitatory post synaptic potentials (fEPSP) that is a correlation of synaptic changes induced by the stimulation (e.g. increase in receptor concentration, among others) (Zhang et al. 2014).

The study of the endocannabinoid system in the brain improved with the development of advanced techniques for example combining in vivo whole-cell recording methods with optic techniques. This combination allowed to optogenetically manipulate specific neural activities on a restricted time scale and capture individual cellular activity based on fluorescence, uncovering additional characteristics of cellular activity in terms of anatomical connections, genetic properties, and collective activity associated with brain function (Kitamura and Häusser, 2011; Mateo et al. 2011; Lien and Scanziani, 2013; Pala and Petersen, 2015; Reinhold et al. 2015; Valeeva et al. 2016; Zucca et al. 2017; Kato et al. 2017; González-Rueda et al. 2018; Noguchi et al. 2021).

To optogenetically manipulate neurons, researchers first genetically expressed channelrhodopsin-2 (ChR2) in neurons projecting to cells in the brain regions where membrane potentials are recorded. It is a protein serving as sensory photoreceptor that is activated/inactivated in response to photostimulation; it functions as light-gated ion channel that allows ion trafficking (i.e., electric current) through the cell membrane (Deubner et al. 2019; Adamantidis et al. 2014; Deisseroth, 2015; Boyden, 2015; Gautier 2014). This technique enabled to demonstrate the feasibility of empirically evaluating synaptic connectivity between specific neurons in vivo (Mateo et al. 2011; Pala and Petersen, 2015; Valeeva et al. 2016; van Welie et al. 2016; Arlt and Häusser, 2020). Currently, a new powerful approach was developed: the combination of electrophysiology with imaging techniques. For example, in vivo optical imaging (including voltage-sensitive dye (VSD) imaging (Petersen et al. 2003), two-photon calcium imaging (Kitamura and Häusser, 2011) or stimulated emission depletion (STED) microscopy was simultaneously performed with whole-cell recording to capture neural activity in a wider area than single whole-cell recording alone and allow the imaging of nanoscopic structures in the brain (Takasaki et al. 2013). These new arrays of techniques have given a fairly broad picture of the endocannabinoid system functionality in different neural circuits, and the advent of new technologies will provide just as much evidence of its contribution to brain physiology.

II – Hippocampal Synaptic Plasticity

A well-studied brain area for investigating synaptic plasticity in the nervous system is the hippocampus (Fig. 7). Critical for memory formation, the anatomy of the hippocampus renders it particularly suitable for electrophysiological investigation (Ho et al. 2011). It is composed of two closely interconnected regions, Ammon's horn and the Dentate Gyrus. Ammon's horn is formed by a layer of principal neurons, the pyramidal neurons, and is subdivided into three regions called CA1, CA2 and CA3. The Dentate Gyrus is formed by a layer of principal neurons called granular cells (Hammond et al. 2015).

The pyramidal cells of CA3 have branched axons. Some of these leave the hippocampus and project to other structures. The other ones are collaterals that form synapses with dendrites of CA1 pyramidal neurons. These collaterals form the Schaffer collateral pathway; and their terminals form asymmetrical synapses with the numerous spines of CA1 dendrites in stratum radiatum and to a lesser extent on the basal dendrites in stratum oriens (Figure 7A) (Hjorth-Simonsen, 1973; Swanson et al. 1978; Laurberg, 1979). These synapses are excitatory and the neurotransmitter is the glutamate.

In the '70 years Bliss and Lomo showed that the Schaffer collateral-commissural projection displays considerable neuronal plasticity (Bliss and Lomo, 1973). Most striking is the LTP produced by brief, high frequency stimulation (HFS) of the pathway (Schwartzkroin and Wester, 1975; Alger and Teyler,

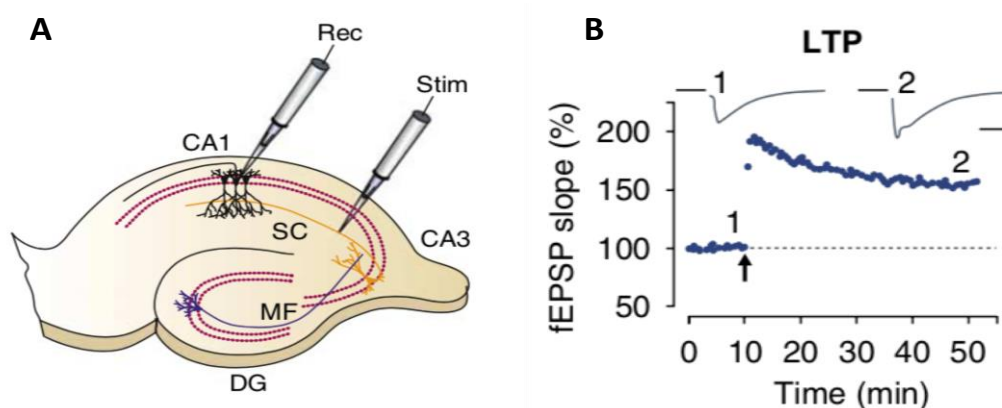


FIGURE 7 – LTP AT HIPPOCAMPAL CA1 SYNAPSES

A. Representation of the CA3-Schaffer collateral to CA1 synaptic pathway in Hippocampus. Axons from the CA3 pyramidal cells (in orange) synapse with the dendrites of the CA1 pyramidal neurons. By electrical stimulation via an electrode in the CA3 axons it is possible to neuronal field or individual responses in the CA1 neurons. **B.** LTP elicited by high-frequency tetanic stimulation. [(A) and (B) from Citri and Malenka, 2008].

1976; Dunwiddie and Lynch, 1978). This potentiation, which in vivo may last for days (Buzsáki, 1980), has been considered as a useful model for memory formation (Cotman et al. 1981).

III – Mechanism of NMDA receptor-dependent LTP

As said previously, it is widely believed that experience of any sort modifies subsequent behavior through activity-dependent, long-lasting modifications of synaptic strength. Experimental support for the existence of such long-lasting, activity-dependent changes in synaptic strength came from Bliss and colleagues (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973) which reported that a brief HFS of hippocampal excitatory synapses, typically referred to as a tetanus, produced a rapid and long-lasting increase in the strength of these synapses that could persist for long time (Bliss and Gardner-Medwin, 1973). Over the last three decades, this phenomenon, named long term potentiation (LTP), has been the object of intense investigation because it is widely believed to be the key to understanding some of the cellular and molecular mechanisms by which memories are shaped (Figure 7B) (Martin et al. 2000; Pastalkova et al. 2006; Whitlock et al. 2006; Citri and Malenka, 2008). It is now clear that hippocampal LTP is only one of several different forms of long-term synaptic plasticity that exist in specific circuits in the mammalian brain.

Others most extensively studied long-term forms of synaptic plasticity observed in the CA1 region of the hippocampus and important in the modulation of neuronal circuits include long-term depression (LTD), spike-timing-dependent plasticity, excitatory postsynaptic potential (EPSP) -spike potentiation and depotentiation (Neves et al. 2008). However, these forms of plasticity won't be discussed in the thesis.

A major advance in the understanding of excitatory synaptic function and hippocampal LTP was the demonstration that two major types of ionotropic glutamate receptors contribute to the postsynaptic response at glutamatergic synapses, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors (Collingridge et al. 1983; Bliss and Collingridge, 1993). Interestingly, Morris and colleagues, by using selective NMDAR antagonists which impaired learning and memory and *in vivo* hippocampal LTP (Morris et al. 1986) provided the first evidence probing the role of LTP and ionotropic glutamatergic transmission in learning and memory (Morris et al. 1986).

LTP can be induced chemically (Stewart et al. 2005), electrically (Bliss and Lomo, 1973; Neves et al. 2008) and optogenetically (Nabavi et al. 2014) both *in vitro* and *in vivo*. These procedures are applied

to mimic neuronal firing able to induce synaptic changes that, depending on the intensity and frequency of the stimulation, potentiate or weaken the synaptic transmission (Nicoll, 2017). LTP is a process that has an early induction phase (early-LTP, lasting around 60 minutes) in which external signals rapidly cause a biochemical cascade that results in a change in synaptic efficacy and a late phase (late-LTP) where these changes are maintained over long periods of time (Figure 8A,B) (Malenka and Bear, 2004). The induction phase requires activation of postsynaptic NMDA receptors by synaptically released glutamate during action potential to the axon terminal. The release of neurotransmitter occurs after the fusion of the vesicles with the membrane from presynaptic terminal (Malenka and Bear, 2004) and binding to α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in the postsynaptic terminals (Figure 8A) (Malinow and Malenka, 2002). The AMPA receptor has a channel that is permeable to Na^+ and K^+ and, when the cell is close to its resting membrane potential, its activation provides most of the inward current that generates the excitatory synaptic response. Consequently, AMPA receptor activation quickly depolarizes the membrane of the spine to prime NMDA receptors (Malinow and Malenka, 2002). In contrast, NMDARs have a high Ca^{2+} permeability relative to AMPA receptors and exhibit a block of its channel at negative membrane potentials by extracellular Mg^{2+} (Mayer et al. 1984; Nowak et al. 1984; Paoletti et al. 2013). However, when the postsynaptic cell is depolarized, the Mg^{2+} block is removed and it results in the entry of Ca^{2+} into the postsynaptic dendrite spine (Nicoll, 2017). The consequent rise in the concentration of intracellular Ca^{2+} is thought to be a necessary and perhaps sufficient for triggering LTP.

Much of the work on NMDAR-dependent LTP has focused on the mechanisms responsible for its initial 30–60min. Nonetheless, the mechanisms that allow LTP to persist for hours, days, or even longer are of great importance. An elevated number of proteins have been suggested to play a key role in translating the calcium signal that is required to trigger LTP into the long-lasting increase in synaptic strength (Malenka and Bear, 2004). Among these, calcium/calmodulin (CaM)-dependent protein kinase II (CaMKII) fulfills these requirements and is a key component of the molecular machinery for LTP. CaMKII undergoes autophosphorylation after the triggering of LTP (Barria et al. 1997), and LTP induction was prevented both in knockout mice lacking a critical CaMKII subunit (Silva et al. 1992), and in knock-in animals in which endogenous CaMKII was replaced with a form lacking the autophosphorylation site (Giese et al. 1998). Furthermore, inhibition of CaMKII activity

by directly loading postsynaptic cells with peptides that impair CaMKII function blocks LTP (Malenka et al. 1989; Malinow et al. 1989), whereas acutely increasing the postsynaptic concentration of active CaMKII increases synaptic strength and occludes LTP (Lledo et al. 1995; Pettit et al. 1994). On the same line, also the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) pathway has also been suggested to be important for LTP, as well as for learning and memory (Sweatt, 2004; Thomas and Huganir, 2004). All these proteins activate key transcription factors that may include cAMP response element-binding protein (CREB) and immediate-early genes such as c-Fos and Zif268/Egr-1 (Thomas and Huganir, 2004). These transcriptional complexes presumably promote expression of effector genes/proteins that are required for maintaining long-lasting LTP. Another compelling possibility for the maintenance mechanism of LTP is the presynaptic structural remodeling of potentiated synapses as well as local dendritic protein synthesis (Figure 8B) (Lüscher and Nicoll, 2000; Reymann and Frey, 2007; Sutton and Schuman, 2006; Zhou et al. 2006). Given the presynaptic localization of CB₁ receptor, in the next section I will discuss about CB₁-dependent modulation of synaptic transmission and its impact on short- and long-term plasticity as well as the role on learning and memory.

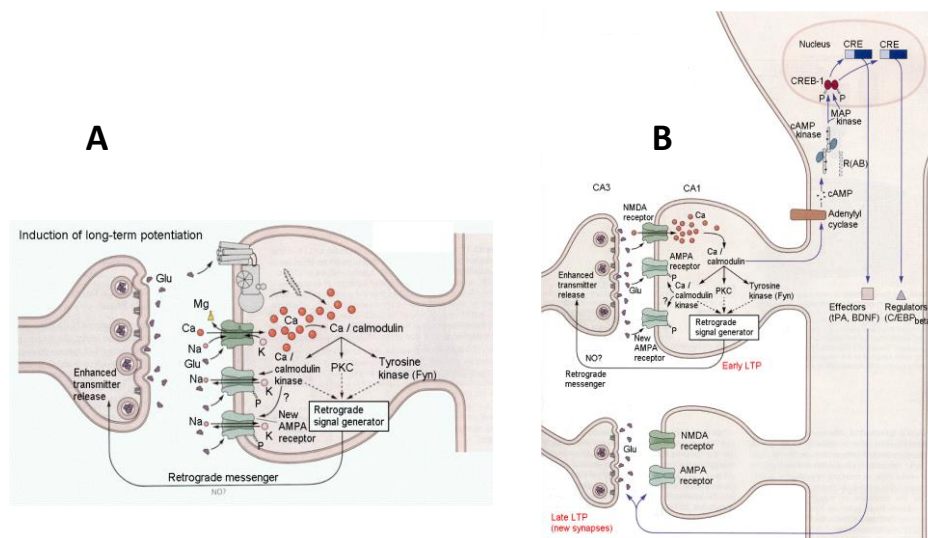


FIGURE 8 – MOLECULAR MECHANISM OF LONG-TERM POTENTIATION

A. Induction of Long-Term Potentiation by high-frequency stimulation. All the events illustrated trigger the Ca²⁺ entry into the cell and activates Ca-dependent kinases leading to the neurotransmitter increase. **B.** Early and late-phase of LTP. [(A) and (B) from Kandel et al. 2000]

IV – CB₁-mediated Modulation of Synaptic Activity

Some forms of plasticity are initiated and maintained by postsynaptic mechanisms, others by presynaptic mechanisms, and still others by retrograde mechanisms (Malinow et al. 2000; Kemp and Bashir, 2001; Tao and Poo, 2001). This latter expression mechanism garnered significant attention because it required the production of a retrograde messenger that was released by postsynaptic cells and acted on presynaptic terminals altering the neurotransmitter release process (Nicoll, 2003). Post-synaptically on-demand production of endocannabinoids (AEA and 2-AG) and release function as such a retrograde signal by activating CB₁ receptors, mainly, in the presynaptic terminals in order to decrease neurotransmitter release (GABA and Glutamate) and are critical to the alteration of synaptic efficacy (Alger, 2002; Wilson and Nicoll, 2002).

IV.A – INTRACELLULAR SIGNALING PATHWAY OF CB₁ RECEPTORS

As discussed previously, CB₁ receptors at the presynaptic terminal are intracellularly primarily coupled with the Gi/o subunits of G proteins (Howlett and Fleming, 1984; Howlett et al. 1986).

The activation of CB₁ receptor acts mainly on three intracellular signaling pathways: adenylate cyclase, the mitogen-activated protein kinase (MAPK) pathway and some ion channels (Figure 9). Through Gi/Gs coupling, the activation of CB₁ or CB₂ receptors simultaneously induces an inhibitory signal and an activating signal depending on the cell type (Howlett and Fleming, 1984; Howlett et al. 1986; Glass and Felder, 1997; Navarrete and Araque, 2008).

Adenylate cyclase is the enzyme responsible for the production of cyclic adenosine monophosphate (cAMP), one of the main intracellular second messengers. The inhibition is reversible, dose-dependent and Gi-mediated (Howlett and Fleming, 1984; Howlett et al. 1986); a decrease in cAMP in the cell causes inhibition of PKA and an increase in tyrosine-phosphorylated proteins such as focal adhesion kinase (FAK) (Derkinderen et al. 1996). It has also been shown that CB₁ can be coupled to the Gs protein, causing an increase in cAMP levels and thus activation of PKA (Glass and Felder, 1997). MAP kinases play a key role in the processes of morphological differentiation and neuronal survival (Fukunaga and Miyamoto, 1998). Several members of the MAP kinase family are abundantly present in the brain and are activated during physiological or pathological events (ischemia,

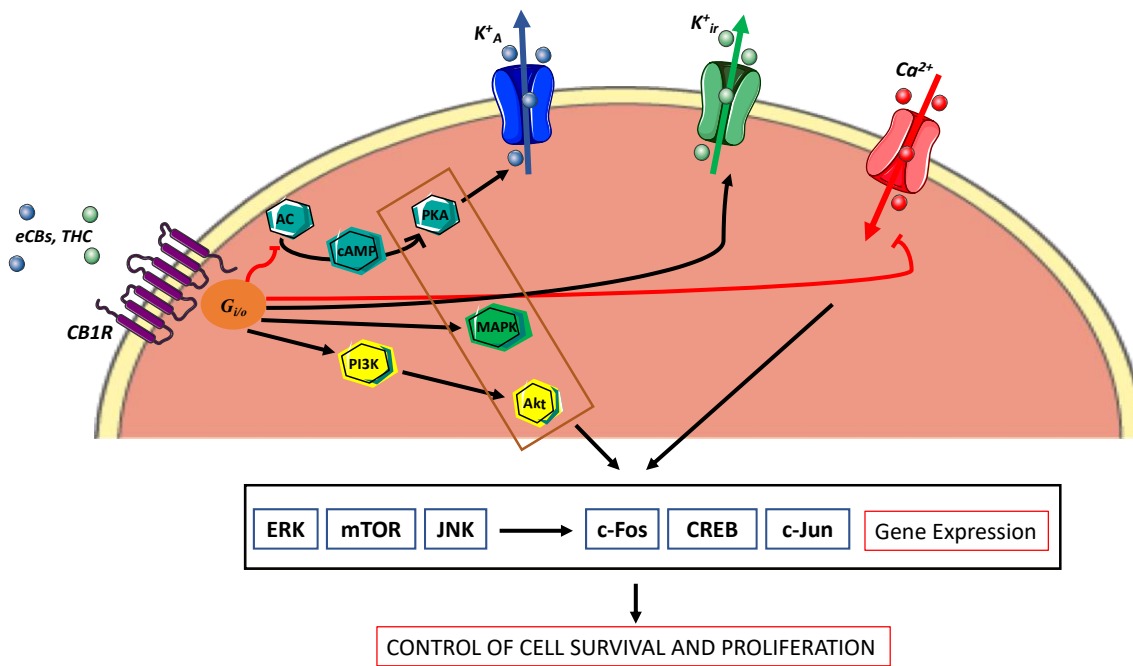


FIGURE 9 – INTRACELLULAR CB1 RECEPTOR SIGNALING PATHWAYS

Stimulation of CB1 receptors lead to multiple cascades of events. A direct modulation of ion channels. Inhibition of Ca²⁺ channel and activation of K⁺ channels. Activation of A-type K⁺ channels can be induced through the inhibition of adenylyl cyclase (AC). Activation of several protein kinases including ERK, JNK and mTOR leads to *de novo* gene expression. [Adapted from Pagotto et al. 2006]

epilepsy). Cannabinoids are able to activate MAP kinase as ERK1/2 (extracellular signal-related protein kinase), involved in the regulation of gene expression and protein synthesis; this effect is dose-dependent and independent inhibition of cAMP production (Bouaboula et al. 1995).

Activation of the CB₁ receptor can also causes inhibition of voltage-sensitive calcium channels (VGCC). These effects are relatively slow, reversible, mediated by a Gi/o type G protein and independent of adenylate cyclase inhibition (Caulfield and Brown, 1992; Mackie and Hille, 1992) and specific for the activation of CB₁ receptor (Ameri, 1999). These calcium channels are preferentially localized at the presynaptic level and are involved in the control of neurotransmitter release.

Cannabinoids cause potassium channel stimulation of incoming rectification (Henry and Chavkin, 1995; Mackie et al. 1995). This effect is mediated by a G-type protein Gi and depends on the state of phosphorylation of the CB₁ receptor by the protein kinase C to the serine of the third intracellular cycle of the receptor (Garcia et al. 1998). Furthermore, activation of CB₁ receptors modulates, in a dose-dependent manner, the voltage sensitivity of rapidly inactivated type A potassium channels

(Deadwyler et al. 1993). This effect is mediated by a Gi-type protein G and depends on the inhibition of adenylate cyclase and the subsequent inhibition of protein kinase A (Hampson et al. 1995; Childers and Deadwyler, 1996).

Other activating signaling pathways are also involved in the binding of cannabinoids to their receptors: Jun (c-Jun N-terminal) kinase (JNK) and p38-kinase, which are involved in the regulation of gene expression and in the processes of cell death apoptosis (Rueda et al. 2000); Akt (or protein kinase B), mainly involved in cell survival, but also in the regulation of glucose metabolism (Gómez del Pulgar et al. 2000). This divergence of signaling pathways could explain why the activation of the endocannabinoid system may exert different biological effects depending on the nature of the tissue/cell type considered.

IV.B – SHORT- AND LONG-TERM FORMS OF ENDOCANNABINOID-MEDIATED PLASTICITY

The first demonstration of retrograde eCB signaling came from the discovery that eCBs mediate forms of short-term synaptic plasticity induced at various types of GABAergic and glutamatergic synapses throughout the brain. They are known as depolarization-induced suppression of inhibition (DSI) (Ohno-Shosaku et al. 2001; Wilson and Nicoll, 2001) and depolarization-induced suppression of excitation (DSE) (Kreitzer and Regehr, 2001). Shortly after it was shown that eCBs also mediate presynaptic forms of long-term depression (eCB-LTD) at both excitatory (Gerdeman et al. 2002; Robbe et al. 2002) and inhibitory synapses (Chevalleyre and Castillo, 2003; Marsicano et al. 2002). Thus, eCBs represent the best characterized retrograde messengers involved in the short- and long-term regulation of synaptic transmission (Wilson and Nicoll, 2002; Kreitzer and Regehr, 2002; Doherty and Dingledine, 2003; Regehr et al. 2009; Heifets and Castillo, 2009; Kano et al. 2009; Castillo et al. 2012).

DSI and DSE

Presynaptic inhibition of transmitter release by endocannabinoids may adopt two different forms of short-term synaptic plasticity, depending on the involvement of GABA or glutamate transmission, respectively: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE) (Figure 10A) (Wilson and Nicoll, 2002; Diana and Marty, 2004).

The contribution of endocannabinoids to these forms of short-term synaptic plasticity remained unknown until the recent work of Wilson and Nicoll: in the hippocampus, inhibition of CB₁ receptors blocks DSI, whereas synthetic CBs lead to DSI occlusion (Wilson and Nicoll, 2002). This phenomenon has been also described in the cerebellum (Diana et al. 2002). Another study with slice preparations demonstrated that DSI could be induced at inhibitory synapses between CCK-positive Schaffer collateral associated interneurons in the stratum radiatum (Ali, 2007), indicating that interneurons can release endocannabinoids. Interestingly, other studies also demonstrated DSI in hippocampal pyramidal neurons can also be triggered by the activation of metabotropic glutamate receptors (mGluRs) (Varma et al. 2001) or muscarinic acetylcholine receptors (Kim et al. 2002), presumably acting on the postsynaptic neuron to stimulate the formation and release of the endocannabinoid. In parallel, the central role of eCBs was also revealed in DSE in the cerebellum where retrograde endoCB signalling modulates glutamatergic excitatory synapses (Kreitzer and Regehr, 2002; Maejima et al. 2001). This mechanism was also observed in the hippocampal pyramidal cells (Ohno-Shosaku et al. 2002).

Hippocampal and cerebellar DSI and DSE as eCB-mediated modulatory events on synaptic transmission were later reported in other brain areas as well, including the amygdala (Zhu and Lovinger, 2005), the neocortex (Bodor et al. 2005; Trettel et al. 2004), the striatum (Uchigashima et al. 2007) and the hypothalamus (Hentges et al. 2005). Taken together, these results gave further proof of the relevant role endocannabinoid-induced DSI or DSE in physiological processes of memory.

eCB-LTD

Other forms of endocannabinoid modulation of synaptic plasticity involve the LTP and LTD (Lu and Mackie, 2016). Mechanistically diverse forms of endocannabinoid-mediated LTD have also been described, which either involve (Kellogg et al. 2009), or don't involve (Chavez et al. 2010) CB₁ receptors. Notably, endocannabinoids can induce both homosynaptic and heterosynaptic LTD (eLTD). Homosynaptic eLTD is LTD at the synapse being stimulated. It is typically evoked by persistent low frequency stimulation and is prominent at glutamatergic synapse in both dorsal and ventral striatum (Gerdeman et al. 2002; Robbe et al. 2002). Heterosynaptic eLTD occurs at synapses adjacent to the stimulated synapses. For example, stimulation of Schaffer collaterals in hippocampal

CA1 leads to a persistent decrease in GABAergic inhibition of CA1 pyramidal neurons (Chevalyere and Castillo, 2003). The mechanism of eLTD at hippocampal inhibitory synapses appears to require inhibition of adenylyl cyclase (AC) and the involvement of the presynaptic proteins, RIM1 α and RAB3B (Chevalyere et al. 2007; Tsetsenis et al. 2011).

As discussed previously, much of the mechanistic work on the transient synaptic effects of endocannabinoids was performed in the hippocampus and cerebellum, but the first evidence regarding a form of LTD that required endocannabinoids, eCB-LTD (Figure 10B), was observed in the glutamatergic synapses onto medium spiny neurons (MNS) in the striatum as well as at synapses between layer V pyramidal neurons (Gerdeman et al. 2002; Robbe et al. 2002; Sjöström et al. 2003). In the hippocampus, in contrast, the endocannabinoid messengers regulate a form of LTD that affects inhibitory GABAergic neurons, but not excitatory ones (Chevalyere and Castillo, 2003).

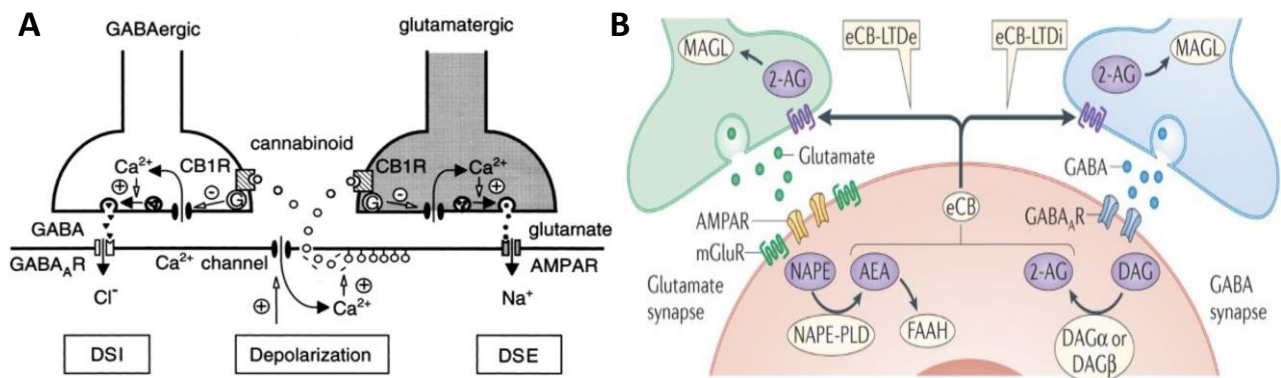


FIGURE 10 – ENDOCANNABINOID-MEDIATED SHORT- AND LONG-TERM SYNAPTIC PLASTICITY MECHANISMS

A. Depolarization of the postsynaptic terminal causes an elevation of intracellular calcium which leads to the production of endocannabinoids that travel to the presynaptic terminal to inhibit neurotransmitters, GABA or Glutamate, release by inhibiting Ca²⁺ channels. **B.** Description of eCB-LTD of both excitatory (glutamatergic) and inhibitory (GABAergic) afferents. [(A) from Kano et al. 2002 and (B) from Parsons and Hurd, 2015].

In conclusion, CB₁ receptors in the brain can modulate several forms of synaptic plasticity in different cell-types in different brain regions and impacting important physiological processes, such as memory which will be discussed in the next Part.

PART 3 – MEMORY

Memory is defined as the faculty of encoding, consolidating, storing, and retrieving information (Squire et al. 2007; Squire, 2009). The term "encoding" refers to the initial process of recognizing and processing newly learned information. The encoding process is influenced by several factors, including emotional-cognitive-motivational factors. The term "consolidation" corresponds to the process of transforming the learned memory. It requires a change in the structure of nerve circuits with the creation of new synaptic connections between neurons. The term "storage" concerns the mechanisms of maintenance of learned information. Finally, the term "retrieval" is fundamental to recall the stored information to the state of consciousness (Squire et al. 2007; Squire, 2009).

Neuroscientists have divided memory systems into two broad categories, declarative and non-declarative. The declarative memory system is the most familiar system of memory. It is the memory system that has a conscious component and it includes the memories of facts and events.

Non-declarative memory, also called implicit memory, includes the types of memory systems that do not have a conscious component but are nevertheless extremely important. They include the memories for skills and habits (e.g., riding a bicycle, driving a car, playing golf or tennis or a piano) and simple forms of associative learning (e.g., Pavlovian conditioning) (Squire and Zola, 1998).

Tulving (1972) distinguished two types of declarative memory: semantic memory and episodic memory. Semantic memory consists of the set of concepts, knowledge and notions that we have acquired in the course of our life experience. Episodic memory allows us to store specific aspects - in spatio-temporal and situational terms- of events.

Due to its complexity, memory formation involves many neuronal circuits within several brain regions. The hippocampus with its adjacent cortical regions (i.e. entorhinal, perirhinal and parahippocampal cortices), constitutes the medial temporal lobe, which is involved in the formation of declarative memory (Eichenbaum, 2017). Thus, it is a key brain region that has been shown to be important for episodic memory, spatial navigation and time perception (Squire et al. 2007; Howard and Eichenbaum, 2015).

I – Long-term potentiation (LTP): A Synaptic Mechanism for Memory

Activity-dependent synaptic plasticity has since long been proposed to represent the subcellular substrate of learning and memory, one of the most important behavioral processes through which we adapt to our environment (Humeau and Choquet, 2019).

The presumptive causal link between synaptic plasticity and memory has been formalized by Morris and colleagues with the “synaptic plasticity and memory hypothesis” (Martin et al. 2000):

“Activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation, and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which that plasticity is observed”.

Long-term forms of synaptic plasticity were discovered in the 1970s (Bliss and Lomo, 1970), and have since been extensively studied at various synapse subtypes, with the objective of understanding whether and how they mediate the modifications in brain activity that underlie behavioral adaptation. An enduring form of synaptic plasticity called LTP is believed to be involved in declarative memory. Indeed, LTP was first described in the hippocampus, a structure well established to be critically important for declarative memory (Squire et al. 2004). Much research over the last 20 years aimed at demonstrating a role for hippocampal LTP in encoding new memories (Martin et al. 2000; Morris, 2007). Animal studies revealed that controlled lesions, pharmacological inactivation or molecular knockouts limited to the hippocampus result in either a failure to learn or a loss of spatial memory (Tsien et al. 1996; Martin et al. 2005; Morris et al. 1986; Pastalkova et al. 2006). Electrophysiological recordings and molecular imaging studies in animals, as well as MRI imaging studies in humans, provide correlative evidence that episodic or episodic-like learning and memory involves changes in hippocampal activity (Berger et al. 1983; Vazdarjanova and Guzowski, 2004; Guzowski et al. 2001; Gabrieli et al. 1997; Maguire, 2001; Henke et al. 1997).

However, linking LTP and memory has always been a difficult task. The first effort to link the two

phenomena was mainly supported by the observation that in vivo NMDA receptors antagonist (which prevent LTP manifestation) induced memory impairments in behavioral tests (Martin et al. 2005; Morris et al. 1986). In addition, Giese and colleagues, generated a knockin mouse containing the mutation of CaMKII. This prevented autophosphorylation and thereby blocked CaM-independent persistent activity of the enzyme. In this mouse, NMDA receptor function was normal, but LTP was absent and learning and memory were strongly impaired (Giese et al. 1998).

Furthermore, during an inhibitory avoidance task, LTP recorded in vivo in a subset of hippocampal CA1 pyramidal cells demonstrated that the patterns of activity generated during a real learning task were sufficient to elicit LTP (Whitlock et al. 2006). Perhaps even more convincing was the demonstration that in vivo infusion of a PKM-delta inhibitor into the hippocampus abolished the maintenance of LTP and simultaneously the storage of a long-lasting spatial memory (Pastalkova et al. 2006). These findings strongly suggest that maintained LTP was required for the engram that stored the spatial information.

Several recent studies have detected LTP-like synaptic changes in the hippocampus in vivo during certain behaviors (e.g. following learning) (Gruart et al. 2006; Whitlock et al. 2006). For instance, learning has been shown to control synapse formation and elimination (Yang et al. 2009) and, on the other hand, ablation of synapses that were formed during the acquisition phase of a certain behavior decreases the performance of that specific behavior (Hayashi-Takagi et al. 2015), according to the synaptic theory of memory storage.

Those concepts have been reinforced by the emergence of sophisticated genetic techniques, optogenetics (Govindarajan et al. 2006; Kim et al. 2017) and chemogenetics (Armbruster et al. 2007; Gomez et al. 2017) that have allowed investigators to manipulate given genes/cell populations and observe their contribution to both memory and synaptic plasticity (Silva, 2003), demonstrating that certain memories can be stored in engrams which can eventually be manipulated and eventually artificially generated (Nabavi et al. 2014; Ramirez et al. 2013).

II – CB₁ Receptors and Memory Process

The high level of expression of CB₁ receptors in the hippocampus, in conjunction with the ability to affect short- and long-term changes in synaptic strengthening, had drawn attention to processes of memory and learning (Scarante et al. 2017; Busquets-Garcia et al. 2015; Hampson and Deadwyler, 1999; Marsicano and Kuner, 2008; Soria-Gomez et al. 2017). It's known since long time that cannabinoids induce learning and memory impairments in both rodents and humans by CB₁ receptors in the brain (Broyd et al. 2016). In the hippocampus, the CB₁ receptor is primarily localized in GABAergic neurons, but it can also be found in glutamatergic neurons and astrocytes as well as in subcellular compartments (Busquets-Garcia et al. 2018a; Gutierrez-Rodriguez et al. 2018; Hebert-Chatelain et al. 2016; Jimenez-Blasco et al. 2020).

In the last decade, the role of the endocannabinoid system, mainly the CB₁ receptor, in memory processes was studied pharmacologically and genetically through its distinct action in different cell types shaping our current understanding of this relevant and complex system. For instance, Puighermanal and colleagues have demonstrated that the suppression in GABA transmission in the hippocampus is the primary cause of the long-term amnesic effects produced by cannabinoids, as assessed by Novel Object Recognition Task (NOR, which I will discuss in detail in the next paragraph) (Puighermanal et al. 2009). Moreover, they showed that the rescue of this effect was possible by local injection of an NMDA receptors antagonist suggesting that an excess of excitation, most likely due to suppression of GABAergic inhibition, was the substrate for the observed impairment in this memory process induced by cannabinoids. Interestingly, Han and colleagues, by analyzing the acute effect of THC in short-term working memory, confirmed what had previously been suggested by Puighermanal and colleagues, namely that, the glutamate excess can also be responsible for short-term memory impairment (Han et al. 2012).

Conversely, there is evidence that physiological activation of CB₁ receptors in GABAergic neurons is essential for certain types of memory (Busquets-Garcia et al. 2015; Busquets-Garcia et al. 2018b; Oliveira da Cruz et al. 2020). For instance, the inhibition produced by endocannabinoid signaling in hippocampal GABAergic neurons is necessary for complex learning processes such as incidental associations (Busquets-Garcia et al. 2018b). Mice lacking CB₁ receptors from GABAergic cells show

deficits in mediated aversion while maintaining normal expression of direct learning (Busquets-Garcia et al. 2018b). As previously discussed, eCBs modulate synaptic function primarily through their effects on presynaptically expressed CB₁ receptors in both GABAergic and glutamatergic terminals (Castillo et al. 2012; Kano et al. 2009). In the hippocampus, eCBs exert a profound effect on inhibition by reducing GABA release in a transient (Wilson and Nicoll, 2002) or long-lasting manner by triggering long-term depression (LTD) of inhibition (iLTD) (Chevalleyre and Castillo, 2003). Thus, the excitatory-inhibitory balance can be altered and by this means contribute to associative learning (Letzkus et al. 2015). By reducing inhibition, eCBs facilitate the induction of excitatory-LTP at Schaffer collateral (SC) to CA1 pyramidal cell synapse (SC-CA1) (Carlson et al. 2002; Chevalleyre and Castillo, 2004). Changes in the LTP could play an important role in the formation memory (Xu et al. 2014).

Relevant to neuronal plasticity, is the ability of astrocytes to communicate with neurons through mobilization of calcium and neurotransmitters (Fellin, 2009). Endocannabinoids released from neurons could then activate distant neurons by activating the astroglial CB₁R, thereby impacting synaptic plasticity (Navarrete and Araque, 2010; Gómez-Gonzalo et al. 2015) and memory formation (Han et al. 2012). Activation of CB₁ receptors in astrocytes, by regulating synaptic function, may have important consequences on animal behavior. Taking advantage of transgenic mice lacking CB₁ selectively in astrocytes, it has been reported that CB₁ activation in hippocampal astrocytes likely mediates the impairment of spatial working memory and LTD at CA3-CA1 synapses induced by THC (Han et al. 2012). Furthermore, the activation CB₁ receptor through astrocyte-dependent mechanisms can modulate glutamatergic transmission triggering the release of gliotransmitters like glutamate and D-serine and thereby potentiates synaptic transmission (Robin et al. 2018; Perea et al. 2009; Navarrete and Araque, 2010). The biochemical pathway leading to production of D-serine, co-agonist of the NMDAR, takes place primarily in astrocytes (Murtas et al. 2020). Concerning the role of the gliotransmitter D-Serine in memory process, Robin and colleagues have showed recognition memory is dependent on astroglial CB₁ receptor modulation of astrocytic D-Serine production (Robin et al. 2018). Overall, it is known that CB₁ receptors in specific cell populations can modulate several aspects of brain physiology, and regulation of memory processes gives an exquisite example. Thus, dissecting the endogenous and exogenous function of CB₁ receptor will provide important insights on how CB₁ receptor is involved in the pathophysiology of brain function.

III – Novel Object Recognition (NOR) as Tool to investigate Memory

Novel object recognition (NOR) memory task is a commonly used experimental behavioral task aimed at studying different phases of learning and memory in rodents (Ennaceur and Delacour, 1988; Ennaceur, 2010).

The test relies on as few as three sessions: one habituation session, one training session, and one test session. Training simply involves exploration of two identical objects, while the test session involves replacing one of the previously explored objects with a novel object (Figure 11). Because rodents have an innate preference for novelty, a rodent that remembers the familiar object will spend more time exploring the novel object (Berlyne, 1950; Ennaceur and Delacour, 1988; Ennaceur, 2010). Thus, the increased exploration of the novel object is interpreted as indirect evidence that animals acquired a memory of the familiar object.

Finally, the NOR task can be easily modified to interfere with different phases of learning and memory (i.e., acquisition, consolidation, or recall), to assess different types of memory (e.g., spatial memory), or to assess different retention intervals (i.e., short-term vs long-term memory). NOR task is also considered a reliable model to test hippocampal and temporal lobe function, as lesions within these brain regions abolish recognition memory (Winters et al. 2008; Broadbent et al. 2010) and several components of novelty versus familiarity preference are encoded in regions such as the hippocampus (De Lima et al. 2006; Puighermanal et al. 2009, 2013).

The NOR task has been replicated using a variety of environmental designs: in a large open field (Bevins and Besheer, 2006) or in a Y-shaped maze (Winters et al. 2004). In order to overcome some limitations of the open field and Y-maze settings, such as the pro-anxiogenic effects of the environment (Hale et al. 2008) and the short length of the corridors (Winters et al. 2004) respectively, for the aim of this thesis, we used a NOR version in an L- or V-maze (Oliveira da Cruz et al. 2020b) because it has been shown to allow the study of hippocampal-dependent object recognition memory and because it has several technical advantages compared to behavioral tests for memory functions (Puighermanal et al. 2009, 2013; Busquets-Garcia et al. 2011, 2013, 2017,

2018c; Robin et al. 2018; Aso et al. 2012; Hebert-Chatelain et al. 2016; Aloisi et al. 2017).

This version of the maze maximizes the exploration time of the objects relative to the surrounding context and increases the accuracy of the test. The versatility of the NOR task provides a platform for innumerable research applications: pharmacologic and pharmacogenetic studies with agents to either disrupt or enhance memory (Urban and Roth, 2015); varying the time of drug administration before or after training, or prior to testing can lead to disrupted or enhanced memory (Lueptow et al. 2015; Rutten et al. 2007; Prickaerts et al. 2002; Bertaina-Anglade et al. 2006); studies with the optogenetic technology to look at the neural activation/inhibition that contributes to the different phases of learning and memory. The NOR task is also appropriate for assessing differences in transgenic animals or in neurodegenerative models or in aging studies (Li et al. 2008; Frick and Gresack, 2003; Grayson et al. 2015; Tuscher et al. 2015; Balderas et al. 2013; Akkerman et al. 2014). In conclusion, it provides high reproducibility and low variability in the study of memory processes.

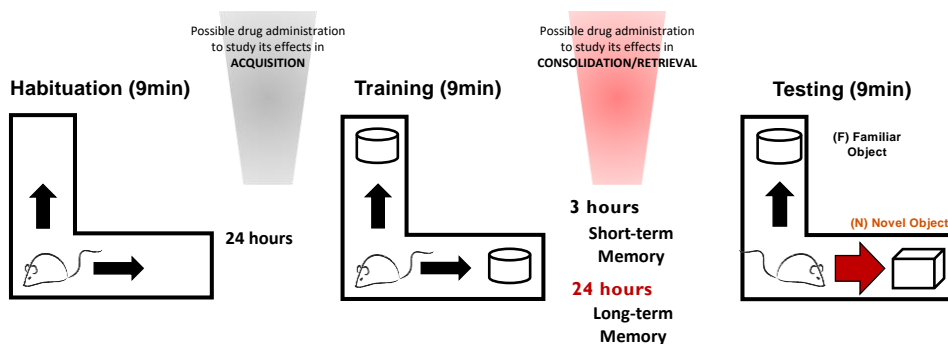


FIGURE 11 – SCHEME OF THE NOVEL OBJECT RECOGNITION TASK

The novel object recognition task consists in 3 sequential daily trials of 9 minutes each. During the habituation session (day 1, Habituation), mice are placed in the center of the maze and allowed to freely explore the arms in the absence of any objects. The acquisition session (day 2, Training) consisted in placing the mice again in the corner of the maze in the presence of two identical objects positioned at the extremities of each arm and left to freely explore the maze and the objects. The test session (day 3, Testing) consisted in replacing one of the previously explored objects with a novel object.

PART 4 – DOPAMINERGIC SYSTEM and the ECS

The dopaminergic system (DS) is involved in a multitude of brain functions: it regulates learning and memory, motivation, mood, movement, and neuroendocrine integration (Undieh, 2010). Dopamine (DA), mainly produced by the VTA, is released after novel (Ljungberg et al. 1992), salient sensory (Ungless, 2004), aversive (Bromberg-Martin et al. 2010), or reinforcement-relevant (reward) stimuli (Schultz et al. 1993). For many decades, the DS role in multiple disorders and brain disease has been intensely studied (Goto and Grace, 2007; Lodge and Grace, 2011; Del Campo et al. 2011; Kumar and Patel, 2007; Jürgensen et al. 2011). The different physiological actions of DA are mediated by two distinct groups of G protein-coupled receptor subtypes. D₁-like receptor subtypes (D₁/D₅) couple to the G_s protein that activate AC pathway. The other receptor subtypes belong to the D₂-like subfamily (D₂/D₃/D₄) and are G_i protein-coupled receptors that inhibit AC pathway and activate K⁺ channels (Missale et al. 1998; Vallone et al. 2000).

D₁-like receptors show high concentration in the prefrontal cortex, whereas the concentration of D₂-like receptors is elevated in the caudate nucleus, putamen, and nucleus accumbens (Missale et al. 1998; Jaber et al. 1996). Their opposite effect at the molecular level (Verhoeff, 1999; Piccini, 2004) and the expression in different cell populations makes the study of DS relatively difficult (Nagatomo et al. 2017; Romanelli et al. 2010) and, moreover, its complexity lies in its interactions with other modulatory systems, such as the ECS. Indeed, DS and ECS shares an “*intimate relationship*” through which they cooperate in the modulation of several physiological processes.

In this chapter, I will focus on the D₁ receptors, because it has been shown to have an important role in the modulation of learning and memory and synaptic plasticity (Hansen and Manahan-Vaughan, 2014) and I will also go deeper inside crosstalk between CB₁ receptor and D₁ receptor, principal topic in this thesis.

I – D₁ receptors in Hippocampal-dependent Synaptic Plasticity and Memory

DA is highly relevant for the modulation of hippocampus-dependent synaptic plasticity and memory (Lisman and Grace, 2005; Lisman et al. 2011) mostly because of the relevant role of D₁ receptors in such processes (Hansen and Manahan-Vaughan, 2014; Huang and Kandel, 1995; Lemon and Manahan-Vaughan, 2006; Bethus et al. 2010; Clausen et al. 2011; da Silva et al. 2012).

Recently, several studies from the Valjent lab provided evidence regarding the existence of D₁-positive cells in hippocampal sub-regions (Gangarossa et al. 2012; Puighermanal et al. 2017). Among these, D₁-positive cells have been showed in the subiculum, in the granule cells of the dentate gyrus (Fremeau et al. 1991), in glutamatergic pyramidal cells in the CA1-CA3 (Bergson et al. 1995) and very recently immunohistochemical investigations have revealed D₁ receptors to multiple types of inhibitory GABAergic interneurons of CA3/CA1 fields and in stratum radiatum in the mouse hippocampus (Gangarossa et al. 2012; Puighermanal et al. 2017), indicating that DA acting on these interneurons may influence information processing in the hippocampal circuit.

As previously mentioned activity-dependent alterations in synaptic strength encode new information in the brain, with LTP/LTD phenomena as the underlying mechanism of hippocampal learning and the encoding of different elements of a memory representation (Bliss and Collingridge, 1993; Bear, 1996; Kemp and Manahan-Vaughan, 2008). In this scenario, some studies showed that LTP and LTD are under the control of D₁/D₅ receptors, suggesting that these receptors contribute in different forms of synaptic plasticity to memory representations (Huang and Kandel, 1995; Lemon and Manahan-Vaughan, 2006). In particular, D₁/D₅ receptors appear to regulate both early-LTP and late-LTP in the CA1 region of the hippocampus by altering the excitability in the hippocampus (Ito and Schuman, 2007; Hamilton et al. 2010) and therefore influence the thresholds for the induction of synaptic plasticity or memory encoding.

This effect could be due to the engaging of signal cascades triggered by D₁ activation. Indeed, this receptor signaling is coupled to adenylyl cyclase (AC) (Undiehl, 2010) which catalyzes the conversion of adenosine triphosphate to the intracellular second messenger cAMP, which in turns activate

protein kinase A (PKA) activity (Vallone et al. 2000; Undieh, 2010). A target of PKA phosphorylation is the DA and cAMP-regulated 32-kDa phosphoprotein (DARPP-32) expressed in the DG of the hippocampus (Undieh, 2010), whose activation results in the potentiation of NMDA receptor function (Cepeda and Levine, 2006). DA-mediated PKA activation also regulates Ca²⁺ currents (Drolet et al. 1997) and stimulation of nuclear transcription factors such as calcium-response element-binding (CREB) proteins (Undieh, 2010). All this intracellular pathway triggered by D₁ receptors activation are well known supporters of long-term synaptic plasticity in the hippocampus (Barco et al. 2002). Furthermore, D₁/D₅ receptors regulate the NMDA receptor directly (Cepeda et al. 1998; Stramiello and Wagner, 2008; Varela et al. 2009) and could affect: a) LTP and LTD induction thresholds (Cummings et al. 1996); b) signaling cascades activated by the D₁/D₅ receptors that lead to the activation of CREB and protein synthesis (Smith et al. 2005; Moncada et al. 2011; Sarantis et al. 2012). Moreover, both *in vitro* early- (Otmakhova and Lisman, 1996) and late-LTP (Frey et al. 1991; Huang and Kandel, 1995) are inhibited by D₁/D₅ blockade (Swanson-Park et al. 1999), whereas agonists of those receptors lead to enhanced early-LTP (Otmakhova and Lisman, 1996), as well as *in vivo* HFS-induced LTP in freely behaving rats (Lemon and Manahan-Vaughan, 2006).

DA is a key component in enabling processing of novel information in the hippocampus. Novelty exploration induces DA release, triggering an up-regulation of the immediate early gene *Arc* in the CA1 region (Guzowski et al. 1999) and the expression of *Zif268* in the DG (Gangarossa et al. 2011). This suggests that DA, via D₁/D₅ receptors, stimulates transcriptional processes leading to consolidate long-term plasticity and memory (Moncada et al. 2011). Learning-facilitated early-LTP and late-LTP by exploration of novel empty space can be prevented by D₁/D₅ receptor blockade in CA1 synapses and the pharmacological activation of D₁/D₅ receptors mimics the spatial novelty-induced facilitation of LTP (Li et al. 2003). Consistent with this observation, local intra-hippocampal injection of D₁/D₅ receptors antagonists blocks late-LTP and prevents place novelty memory (Wang et al. 2010) and optogenetic stimulation of locus coeruleus to dorsal hippocampus terminals induced a D₁-like dependent facilitation of LTP (Takeuchi et al. 2016).

The aforementioned findings suggest that a very tight link exists between the regulation of synaptic plasticity by D₁/D₅ receptors and their role in hippocampus-dependent learning such as spatial and episodic memory. In particular, what drives changes in DA levels in the hippocampus and the relative contribution of D₁/D₅ receptors to synaptic plasticity and memory formation is the response to

novelty.

Hippocampal-mediated long-term episodic-like memory acquisition requires the activation of D₁/D₅ receptors whereas short-term memory does not involve these receptors (Bethus et al. 2010).

In addition, D₁ agonist treatment in rats enhances hippocampus-dependent spatial memory (Bach et al. 1999; da Silva et al. 2012) whereas D₁/D₅ receptor blockade impairs short- and long-term spatial memory (Clausen et al. 2011; da Silva et al. 2012). On the same line, D₁/D₅ receptor activation is required during memory encoding to generate a persistent memory trace in the hippocampus (O'Carroll et al. 2006). All these pharmacological observations were further confirmed by studies in transgenic mice providing further evidence for the essential role of the D₁ receptor for spatial learning (Granado et al. 2008; Xing et al. 2010) and for the encoding of novel environments and hippocampal representations of plasticity (Tran et al. 2008).

Another interesting aspect of dopaminergic signaling in hippocampal memory is that the processing of novelty by the hippocampus may be supported by structures other than the VTA. The hippocampus receives inputs not only from VTA (Lisman and Grace, 2005; Rossato et al. 2009) but also from the substantia nigra pars compacta (Beckstead et al. 1979) and indirectly from NAC (Floresco et al. 2001; Legault and Wise, 2001). In novelty-related context, all these dopaminergic nuclei represent an important source of DA for the hippocampus, which this brain region integrates and encodes this information into a memory engram (Lisman and Grace, 2005). Recently, it has been shown that also the locus coeruleus (LC) noradrenergic fibers have been shown to directly release DA in the CA1 region (Smith and Greene, 2012) thus regulating synaptic plasticity and learning processes mediated by hippocampal D₁ receptors (Kempadoo et al. 2016; Takeuchi et al. 2016; Matta et al. 2017).

Thus, it has become clear that hippocampal neurons express D₁/D₅ receptors and DA, by activating these receptors, play a pivotal role in the enablement of hippocampal information encoding and storage.

II – D₁ and CB₁ receptors: Linking in memory functions

In addition to complex functions in the intracellular signaling, changes in neurotransmitter release and synaptic plasticity, newly genetic and pharmacological studies have demonstrated the importance of CB₁ receptors on the modulation of the dopaminergic system and its participation on the modulation of behavioral responses (Bloomfield et al. 2016; Fernandez-Ruiz et al. 2010). CB₁Rs and the endocannabinoid ligands AEA and 2-AG are abundant in dopaminergic pathways, where they act as a retrograde feedback system on presynaptic glutamatergic and GABA nerve terminals, modulating dopaminergic transmission (Herkenham et al. 1991). *In situ* hybridization (ISH) analysis have showed CB₁ receptor mRNA co-expression with D₁ receptors in several brain regions (Hermann et al. 2002). Among these, functional interaction between the CB₁ and the D₁ receptors have been widely studied in the striatum (Martin et al. 2008) and in the prefrontal cortex (Scheggia et al. 2017), given the high expression of CB₁ and D₁ receptors in this brain area (Paspalas et al. 2005; Marsicano and Kuner, 2008; Freeze et al. 2013). Accordingly, cognitive impairment, which includes executive dysfunction (Hooker and Jones, 1987), working memory impairments (Ranganathan and D'Souza, 2006) and amotivation (McGlothlin, 1968) induced by cannabis use are susceptible to mesocortical dopaminergic manipulation (Goldman-Rakic, 1996), and to prefrontal D₁ receptor blockade (Sawaguchi and Goldman-Rakic, 1991).

Furthermore, mice lacking the CB₁ gene in D₁R+ cells (D₁-CB₁-KO) do not display cannabinoid-induced catalepsy (Monory et al. 2006). Consequently, a recent intriguing article showed that CB₁ receptors in D₁-positive striatonigral neurons are the substrate of both adverse cataleptic and clinically relevant antinociceptive cannabinoid effects (Soria-Gomez et al. 2021).

Interestingly, Terzian and colleagues provided a well clear demonstration of a physiological cross-talk between the cannabinoid CB₁ receptors and the dopamine D₁ receptors examining the anxiety-like profile of conditional CB₁ mutant mice lacking CB₁Rs exclusively in neurons containing D₁Rs receptors (D₁-CB₁-KO mice) by using different tests (Terzian et al. 2011). The authors brought to light the specific role of CB₁ receptors in D₁-positive cells in modulating emotional states under conditions of stress-induced conflict/frustration (grooming behavior) or inescapable situations (social interactions). D₁-CB₁-KO mice showed a behavior reminiscent of a mild anhedonia-like state

(commonly associated with depression) even if this constatation does not support a depressive-like behavioral phenotype, as also suggested by the observation that KO mice performed as WT in the forced swim test. Finally, in the fear-related memory conditioning task, D₁-CB₁-KO mice showed significantly increased auditory-cued and contextual fear responses, which is not surprising when considering the important role of DA receptors in fear adaptation processes (El-Ghundi et al. 2001; de la Mora et al. 2010) and that of CB₁ receptors in fear alleviation (Marsicano et al. 2002; Kamprath et al. 2006).

Although the contribution of dopamine and cannabinoid neurotransmission in brain circuits regulating motivational and emotional neural processing has been well characterized by both animal and clinical studies (LeDoux, 2000; Laviolette and Grace, 2006) the mechanisms by which both D₁ and CB₁ receptor collaborate in mediating their effect in the hippocampus, more specifically in learning and memory, remain largely unknown. Furthermore, although the precise expression of hippocampal cells expressing D₁Rs was already characterized, suggesting that various classes of GABAergic interneurons expressed D₁R (Gangarossa et al. 2012; Wheeler et al. 2015) there is currently no anatomical evidence demonstrating the presence of CB₁ receptors in hippocampal D₁-positive cells.

The study of the potential interaction between D₁ and CB₁ receptor in the hippocampus, and its role in modulating learning and memory could provide a new mechanism for the role of the ECs in cognitive processes and memory-related disorders.

PART 5 – SUBCELLULAR SIGNALING OF CB₁ RECEPTOR:

MITOCHONDRIAL CB₁ AND MEMORY

As discussed previously, CB₁ receptors are G-proteins coupled receptors (GPCRs). GPCRs are classically seen as presynaptic plasma membrane proteins in order to convert extracellular signals into intracellular responses (Freund and Hajos, 2003). Until recently CB₁-dependent effects have been exclusively attributed to plasma membrane CB₁ (pmCB₁) and its possible presence at intracellular level was disputed mainly for the aspecificity of the anti-CB₁ immunolabelling (Campbell, 2001; Athanasiou et al. 2007). However, the idea that GPCRs targeted by water-soluble ligands were believed to be functional only at plasma membranes has been challenged over the years by some studies, showing that different types of GPCRs can also be located intracellularly, including endosomes, nuclei and mitochondria (Figure 12) (Irannejad et al. 2013, 2017; Jong et al. 2014; Tsvetanova et al. 2015; Jalink and Moolenaar, 2010; Belous et al. 2004; Irannejad and von Zastrow, 2014; Rozenfeld and Devi, 2008; *reviewed in* Busquets Garcia et al. 2016).

As we know, the CB₁ receptor is primarily a lipid receptor and there is now evidence that both eCBs and exogenous cannabinoids reach their binding sites on CB₁ and CB₂Rs via the lipid bilayer (Hurst et al. 2010, 2019; Reggio, 2010; Kimura et al. 2009) and that is also confirmed from a recent analysis of the crystal structure of the CB₁ receptor protein (Hua et al. 2016, 2017; Shao et al. 2016) which suggests that these compounds can easily move either via active or passive mechanisms within cellular membranes (Voelker, 1991). Interestingly, lipid eCBs are produced and degraded within both the plasma membrane and organelle membranes inside cells (Gulyas et al. 2004; Morozov et al. 2004). For example, the FAAH enzyme, which degrades the AEA, and MAGL, the degrading enzyme of 2-AG, are present in intracellular purified brain mitochondria (Morozov et al. 2004; et al. 2007) modulating mitochondrial energetic activity (Benard et al. 2012).

In this context, in 2012, an elegant study from our laboratory established the specific localization of CB₁ receptors within CA1 hippocampal mitochondrial membranes by using electron microscopy and immunohistochemical labelling. Approximately 30% of WT hippocampal mitochondria were observed to contain CB₁ receptors labelling and named mtCB₁, whereas only 3% of mitochondria

showed non-specific immunoreactivity in CB₁-KO mice (Benard et al. 2012). Altogether, these observations revealed that the constituent pieces of the ECS are present inside cells and set the stage for more recent studies demonstrating a functional role for intracellular CB₁ receptor signaling in brain functions.

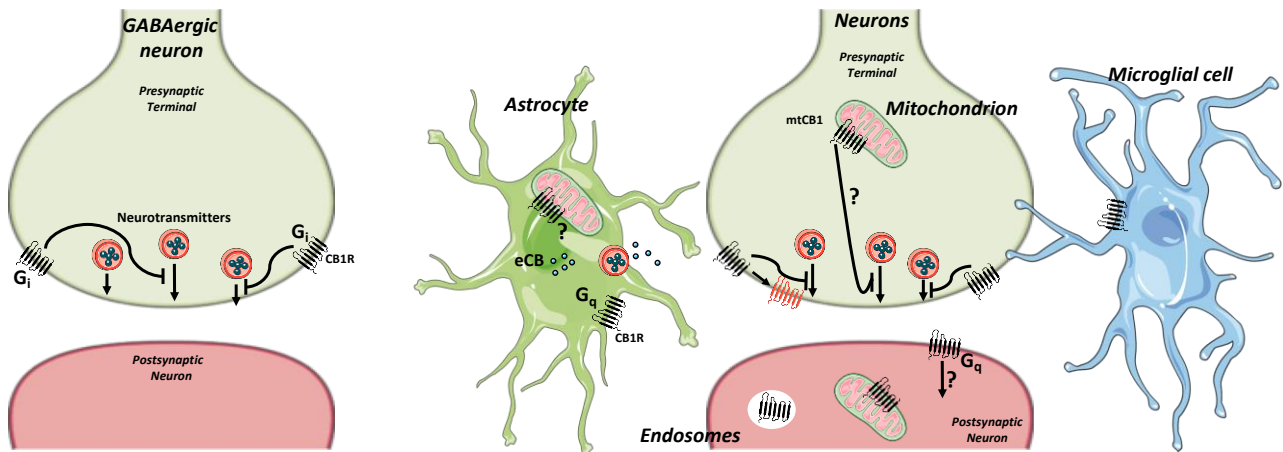


FIGURE 12 – VIEW OF THE CB₁ RECEPTOR FUNCTIONAL EXPRESSION: CLASSIC Vs CURRENT

On the left panel the classic distribution of CB₁ receptor in the brain where it was thought to be exclusively localized in GABAergic neurons. On the right panel, the current view where it was showed to be expressed in different neuronal and glial cells, astrocytes and microglia. Moreover it was found intracellularly, in the mitochondria and endosomes [Adapted from Busquets Garcia et al. 2016]

I – Mitochondria Functions

According to the first electron microscopy studies (Palade, 1953), mitochondria are organelles resembling to bacteria, composed by a highly specialized double membrane and containing their own DNA (Taanman, 1999). The outer (OM) and inner membrane (IM) have differential properties and functions and create two different mitochondrial compartments: the internal lumen called the matrix, and a much narrow intermembrane space (Alberts et al. 2002). The OM contains large lipid channels which render it permeable to high molecular weight molecules, including adenosine triphosphate (ATP) (Shoshan-Barmatz and Gincel, 2003). Conversely, the IM is largely impermeable, but it contains a variety of carrier proteins that makes it selectively permeable (Alberts et al. 2002). Mitochondria are organelles essential for the survival of our cells and perform various functions. The best known is the production of ATP during oxidative phosphorylation (OXPHOS). That takes place thanks to the succession of a series of redox reactions mediated by a set of four enzyme complexes, which form the respiratory chain, located on the IM of the mitochondrion (Stroud and Ryan, 2013; Brown, 2004). Through this mitochondrial process, the brain, which is one of the most energy demanding organs of the mammalian body (Erecinska and Silver, 2001; Rolfe and Brown, 1997), generates its own high energetic support, which is used to maintain ion flow, neuronal excitability and regulation of synaptic activity (Attwell et al. 2010; Attwell and Laughlin, 2001). Beyond their central role in energy metabolism, brain mitochondria participate to several other important physiological processes including Ca^{2+} homeostasis, production of reactive oxygen species (ROS), synthesis and metabolism of neurotransmitters and other signaling molecules, and apoptosis (Turrens, 2003; Ruggiero et al. 2021; Bock and Tait, 2020).

Thus, it is not surprising that mitochondrial malfunctions have been associated to different pathologies affecting the CNS amongst the others (Manji et al. 2012; McInnes, 2013). For example, the insulin-resistance is associated with mitochondrial dysfunctions in myocytes, hepatocytes, adipocytes, and islets b-cells and neurons (Hojlund et al. 2008; Sivitz and Yorek, 2010). In the arcuate nucleus of the hypothalamus, a brain region involved in energy homeostasis, diet-induced obesity tremendously impacts on mitochondrial activity (Schneeberger et al. 2013; Dietrich et al. 2013). Furthermore, recent evidence indicates that events associated with cellular energy homeostasis can

impact synaptic and cognitive function (Suzuki et al. 2011; Wu et al. 2004) highlighting mitochondria as a node for neurotransmission, synaptic plasticity, network activity and behavioral processes (Benard et al. 2012; Kann and Kovacs, 2007; Li-Byarlay et al. 2014; Sun et al. 2013). Therefore, the study of brain mitochondrial activity and regulation is getting an important step to fully understand brain functioning in general and the onset and progression of several neurodegenerative diseases. Interestingly, the study of different types of G proteins and associated-mitochondria signaling pathways are nowadays on the rise (Kuyznierewicz and Thomson, 2002; Lyssand and Bajjalieh, 2007; Andreeva et al. 2008; Zhang et al. 2010; Beninca et al. 2014) rendering the demonstration that CB₁ receptors are functionally present on brain mitochondrial membranes (Benard et al. 2012; Hebert-Chatelain et al. 2014, 2016; Koch et al. 2015; Jimenez-Blasco et al. 2020; Soria-Gomez et al. 2021) part of a novel field of research.

However, the specific roles of G proteins in the mitochondria and the mechanisms regulating their functions are poorly explored so far and understanding of that will be part of my Thesis.

II – Role of Mitochondrial CB₁ in the Brain

Already in the Seventies, different studies reported the impact of exogenous cannabinoids on mitochondrial processes, including reduction of complex I or V activities of the respiratory chain and changes in mitochondrial structure (Bartova and Birmingham, 1976; Bino et al. 1972; Chari-Bitron and Bino, 1971; Mahoney and Harris, 1972; Schurr and Livne, 1975). With the identification of CB₁ receptors as typical plasma membrane GPCRs, these effects were explained and ascribed to indirect signaling of CB₁ receptor-dependent activation (Campbell, 2001; Athanasiou et al. 2007) or to unspecific alterations of mitochondrial membrane by lipid molecules (Bartova and Birmingham, 1976; Howlett et al. 2002).

One of the first pieces of evidence for the presence of G protein-dependent intracellular cannabinoid signaling came from the observation that cannabinoids can activate CB₁ receptors localized in endosomes and lysosomes (Thibault et al. 2013; Rozenfeld and Devi, 2008). However, the most complete evidence for intracellular functional cannabinoid signaling relies on the study lead by Benard and colleagues on mitochondria (Benard et al. 2012).

This study and subsequently also the one by Hébert-Chatelain (2016), Koch and colleagues (2015) have been demonstrated the importance of mitochondrial CB₁ receptors coupled to the Gi/o protein in physiology and pathophysiology, using experiments with mice lacking CB₁ receptor.

Through electron microscopy of hippocampal CA1 neurons from WT and CB₁-KO mice the authors established that approximately 15.5% of the total amount of CB₁ receptors is located in mitochondria at the OM and only 30% of the mitochondria analysed show CB₁ immunolabelling. On the contrary, only 3% of CA1 mitochondria in CB₁-KO mice shows nonspecific labeling (Benard et al. 2012).

A similar mitochondrial localization also was shown in mouse hypothalamic pro-opiomelanocortin (POMC) neurons in which the activation of CB₁ receptors negatively regulates leptin-induced reactive oxygen species formation (Palomba et al. 2015) and increases coupled mitochondria respiration that associates with the generation of reactive oxygen species (Koch et al. 2015). Recent results also indicate the presence of mtCB₁ receptors in the periphery, such as in spermatozoa (Aquila et al. 2010) or skeletal and cardiac striated muscle cells (Arrabal et al. 2015; Mendizabal-

Zubiaga et al. 2016) and in astrocytes (Gutiérrez-Rodríguez et al. 2018).

Moreover, Benard and colleagues has shown that activation of mtCB₁, by using different exogenous agonists of CB₁ receptors, negatively regulates the respiratory function of mitochondria decreasing in parallel the activity of complex I of the electron transfer chain (Benard et al. 2012). Indeed, in mitochondria isolated from WT mouse brains, exogenous application of CB₁ agonists decreases mitochondrial oxygen consumption, whereas no change is observed in mitochondria from CB₁-KO mouse brains (Benard et al. 2012). Furthermore, DSI which was previously attributed to presynaptic plasma membrane CB₁ receptors has been shown to be partially dependent on mtCB₁ receptor activation (Benard et al. 2012; Soria-Gomez et al. 2021).

These results suggested a direct link between endocannabinoids, CB₁ receptors, and brain cellular energy homeostasis. Interestingly, brain mitochondrial functions alterations have been recently causally associated to anxiety-related responses in the nucleus accumbens (Hollis et al. 2015), suggesting that brain energetic processes can impact behavior. For instance, a recent elegant study linked astroglial mtCB₁ receptor activation to a disruption of glucose metabolism and lactate production (Jimenez-Blasco et al. 2020) providing the mechanistic link between metabolic changes and cannabinoid-induced social impairment in mice.

Another interesting study revealed, by immunogold electron microscopy, the existence of mtCB₁ in striatonigral terminals (Soria-Gomez et al. 2021), the output of a well known neuronal circuits in which the activation of CB₁ receptors impairs motor control (Giuffrida and Seillier, 2012; et al. 2007; Freeze et al. 2013). This study further investigated the subcellular-specific CB₁ receptor signaling within striatonigral circuits on behavioral control (Soria-Gomez et al. 2021) and found that the activation of mtCB₁ receptors in striatonigral terminals is responsible for the effects of cannabinoids on cellular respiration, synaptic transmission, and behavioral catalepsy (Soria-Gomez et al. 2021). In detail, the authors elegantly showed that cannabinoid-induced catalepsy involves inhibition of striatonigral mitochondrial soluble AC activity and intra-mitochondrial PKA-dependent cellular respiration (Soria-Gomez et al. 2021).

These results establish a link between mitochondrial activity, synaptic transmission, and motor control, thus by providing further evidence of the importance of mitochondrial functions for the regulation of behavior and the therapeutic potential of selective subcellular manipulations of CB₁ receptors.

III – Mitochondrial CB₁ and Memory

The impact of mitochondrial function or dysfunction in the brain relating to long-term pathological conditions is well known (Cheng et al. 2010; Mattson et al. 2008; Picard, 2015; Raefsky and Mattson, 2017). Conversely, very little is known about the direct impact of physiological regulation of mitochondrial activity on cognitive functions, mainly considering the fact that mitochondrial activity controls a series of processes absolutely necessary for normal synaptic transmission, such as the production of ATP (Rangaraju et al. 2014), the Ca²⁺ homeostasis (Brini et al. 2014), or the generation of reactive oxygen species (Accardi et al. 2014). Thus, it is very likely that slight alterations of these mitochondrial functions through mtCB₁ receptors modulations could have an impact on memory functions and behavior.

With the discovery of the subcellular signaling of CB₁ receptors in the brain (Benard et al. 2012) the molecular mechanisms resulting from the activation of the Gi/0 protein of mtCB₁ receptors and the physiological and behavioral effects mediated by mtCB₁ receptors started to be deciphered.

In 2016 our lab, by using pharmacological and genetic approaches, characterized the intra-mitochondrial signaling pathway triggered by mtCB₁ receptor activation and its behavioral consequences in a hippocampus-dependent novel object recognition task (Hebert-Chatelain et al. 2016). We showed that mtCB₁ receptors signal through intra-mitochondrial G α i protein activation and consequent inhibition of the activity of the soluble form of adenylate cyclase (sAC) leading to lower amount of intramitochondrial cAMP. This results in a decreased protein kinase A (PKA) activity in the mitochondria and consequently decreases the phosphorylation of proteins involved in OXPHOS, in particular the NDUFS2, specific subunit of complex I of respiratory chain (Busquets-Garcia et al. 2018a; Hebert-Chatelain et al. 2014a, 2014b), leading finally to a reduction of ATP production (Figure 13). Interestingly, manipulation of sAC activity, intra-mitochondrial PKA signalling or the phosphorylation of the Complex I subunit NDUFS2 completely abolished both blocked the bioenergetic and amnesic effects of cannabinoids (Hebert-Chatelain et al. 2016) further confirming the role of this intra-mitochondrial pathway. Thus, the G α i-coupled mtCB₁ receptors regulate memory functions via modulation of mitochondrial bioenergy processes (Hebert-Chatelain et al. 2016).

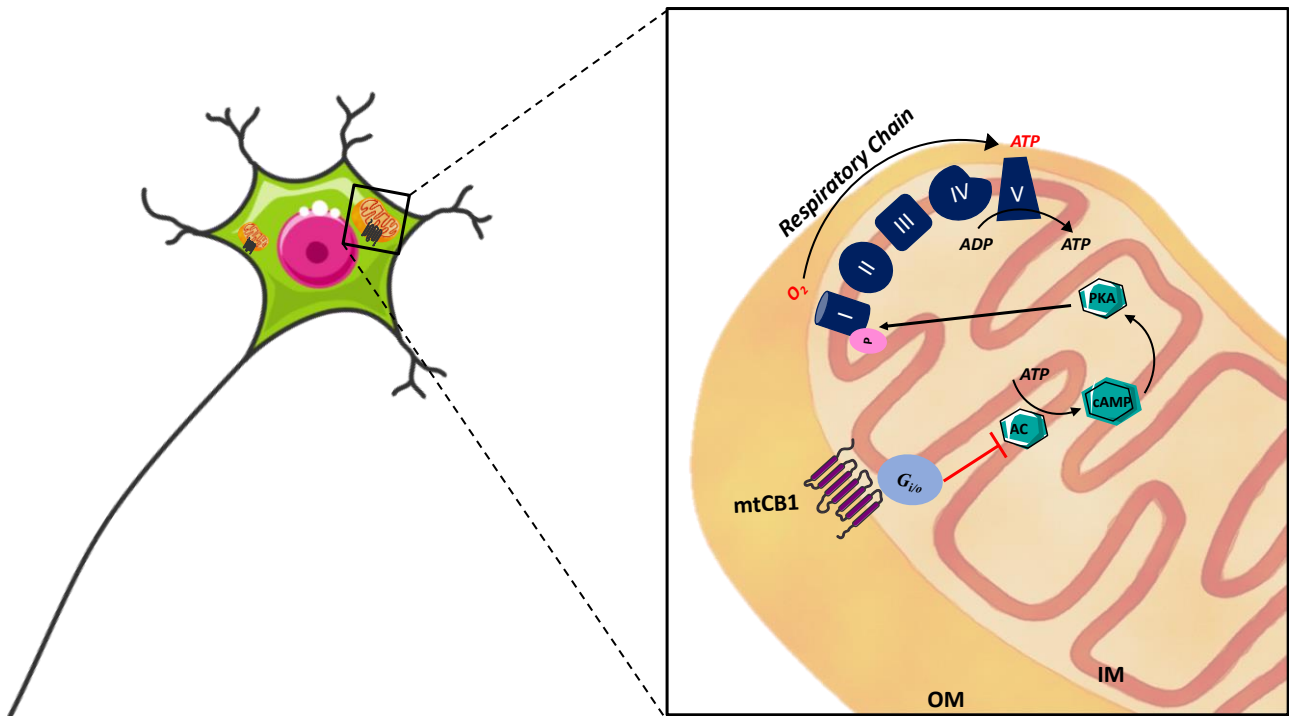


FIGURE 13 – SCHEMATIC VIEW OF THE mtCB₁-DEPENDENT SIGNALING PATHWAY

mtCB₁ receptors in the brain regulate the respiratory chain and the mitochondrial functions (eg, ATP production). mtCB₁ receptors mediate its effects involving intra-mitochondrial Gi/o protein signaling, mitochondrial cAMP synthesis that is catalyzed by adenyl cyclase (sAC), and the decrease of intra-mitochondrial PKA activity which reduces phosphorylation of respiratory chain complex I, decreasing mitochondrial respiration and affecting important mitochondria bioenergetic processes. [Adapted from Busquets Garcia et al. 2018]

It is well noted that acute cannabinoid intoxication induces amnesia in humans and animals (Broyd et al. 2016; Marsicano and Lafenêtre, 2009), and the activation of type-1 cannabinoid receptors present at brain mitochondria membranes (mtCB₁) can directly alter mitochondrial energetic activity (Bénard et al. 2012; Hebert-Chatelain et al. 2014a; Koch et al. 2015). By directly linking mitochondrial activity to memory formation, Hebert-Chatelain and colleagues reveal that bioenergetic processes are primary regulators of cognitive functions (Hebert-Chatelain et al. 2016). Interestingly, mtCB₁ receptors seem not to be required for normal long-term memory formation as the absence of mtCB₁ receptors did not impair memory functions in a novel object recognition task, but it accounts for the acute cannabinoid-induced memory impairment as well as the cannabinoid-induced reduction of mitochondrial mobility and synaptic transmission (Hebert-Chatelain et al. 2016). Indeed, although eCB-mediated plasticity was not fully investigated in this study, the activation of the mtCB₁ receptor in hippocampal neurons interrupts excitatory synaptic transmission in the CA1-CA3 circuit, resulting in an impairment of long-term memory (Hebert-

Chatelain et al. 2016).

As in neurons also in astrocytes CB₁ receptors are involved in memory processes and CB₁ receptors in hippocampal astroglial mitochondria have recently been described (Gutierrez-Rodriguez et al. 2018). However, their implications on astroglial-dependent memory functions are ongoing studied by our lab. The most auspicious aspect about the study by Hebert-chatelain and colleagues is the demonstration that a mutant version of the CB₁ protein lacking the first 22 amino acids in the N-terminal part of the CB₁ receptor (called DN22-CB₁) is no longer physically and functionally addressed in the mitochondria, but it maintains its functionality at plasma membranes (Hebert-Chatelain et al. 2016). Thus, recently our lab generated a new transgenic mouse model, a knockin mutant mouse line, called DN22-CB₁-KI (Pagano Zottola et al. 2020; Soria-Gomez et al. 2021), in which CB₁ receptor gene is replaced by the coding sequence of the DN22-CB₁ protein, thereby, opening the door to understanding the functionality of the mtCB₁ receptor (McKeon and Mathur, 2021).

SECTION II – RESEARCH OBJECTIVES

Physiological or pharmacological modulation of the endocannabinoid system has been shown to impact the synaptic plasticity and transmission and learning and memory via CB₁ receptor-dependent control of different cell types (i.e. GABAergic, glutamatergic neurons, astrocytes) within the hippocampus (Busquets-Garcia et al., 2017, Soria-Gomez et al., 2017, Robin et al. 2018). This multimodal impact of CB₁ receptors on recognition memory raises the question about the differential cell type-, temporal- and spatial-specific contributions of the endocannabinoid system to memory processes. For instance, CB₁ receptors have been identified in a subclass of hippocampal CCK-positive interneurons which contain dopamine D₁ receptors, thus potentially representing a novel subpopulation of CB₁ expressing hippocampal interneurons (Puighermanal et al., 2017, Gangarossa et al., 2012) with a likely impact in hippocampal mediated functions. As previously mentioned, the consolidation of episodic like memory has been shown to require functional D₁ receptors in the hippocampus (Lisman et al., 2011, Yamasaki and Takeuchi, 2017), where activity-dependent long-term changes in synaptic transmission are considered cellular correlates of learning and memory (Nicoll, 2017, Whitlock et al., 2006). Furthermore, the activation of D_{1/5} receptors mediates LTP induced by the exposure of the animal to hippocampal-dependent behavioral tasks (Frey et al., 1990, Granado et al., 2008, Li et al., 2003, Lemon and Manahan-Vaughan, 2006). Given the neuromodulatory role of the ECS, this Thesis aims at studying the role of CB₁ receptors in hippocampal D₁-positive cells at biochemical, electrophysiological and behavioral levels. The results will provide novel insights regarding the modulation of memory and learning functions, as well as new mechanisms for the role of the ECs in cognitive processes and memory-related disorders.

The first objective of the thesis is to identify the cellular and molecular mechanisms by which CB₁ receptors in D₁-positive cells contribute to the physiological modulation of learning and memory.

For this purpose, we used a combination of genetics (constitutive and conditional mutagenesis of CB₁ receptors in mice) and chemogenetics coupled with behavioral, pharmacological and *in vivo* electrophysiological approaches.

During this work we addressed several questions regarding: 1) the role of CB₁ receptors in hippocampal D₁⁺ cells in the modulation of long-term memory formation, 2) the role of CB₁ receptors in D₁⁺ cells in the training-induced modulation of hippocampal plasticity, 3) how CB₁

receptors in D₁+ cells modulate LTP and memory.

To address these questions, we used mice lacking CB₁ receptors in D₁+ cells in the hippocampus and we further complemented our study with the use of pharmacological and pharmacogenetics approaches to dissect the cellular and molecular mechanism involved in modulation of memory functions. This first part of this thesis is resumed in the manuscript:

(Published)

Specific hippocampal interneurons shape consolidation of recognition memory

Jose F. Oliveira da Cruz^{*}, Arnau Busquets-Garcia^{*}, Zhe Zhao, Marjorie Varilh, **Gianluca Lavanco**, Luigi Bellocchio, Laurie Robin, Astrid Cannich, Francisca Julio-Kalajzić Thierry Lesté-Lasserre, Marlène Maître, Filippo Drago, Giovanni Marsicano[#], and Edgar Soria-Gomez[#]

^{*}: equal contribution, [#]: equal supervision

My main contribution to this work was to set up and perform in vivo electrophysiology in anesthetized mice and to analyze the data acquired. In addition, I performed experiments in novel object recognition task and participated to the analysis of the behavioral data. I also performed hippocampal extractions from the mouse brain that were subsequently used for amino acid quantification.

In the Seventies, a number of reports demonstrated that cannabinoid drugs interfered with mitochondrial functions (Bartova and Birmingham, 1976; Bino et al, 1972; Chari-Bitron and Bino, 1971; Mahoney and Harris, 1972; Schurr and Livne, 1975). There is now compelling evidence that CB₁ receptors are present in mitochondrial membranes of different tissues (eg, brain, spermatozoa, and skeletal muscles) (Aquila et al, 2010; Benard et al, 2012; Hebert-Chatelain et al, 2014; Koch et al, 2015; Mendizabal-Zubiaga et al, 2016). The study by Hebert-Chatelain and colleagues (2016) showed the behavioral relevance of mtCB₁ receptors indicating that the inhibition or genetic exclusion of CB₁ receptors from hippocampal mitochondria prevents the cannabinoid-induced reduction of mitochondrial mobility and synaptic transmission and bioenergetic and amnesic effects of cannabinoids.

Thus, acute cannabinoid-induced memory impairment in mice requires activation of hippocampal mtCB₁ receptors. The discovery of this subcellular pool of CB₁ receptors has paved the way to a novel field of research dealing with linking GPCR mitochondrial signaling, cannabinoids and their impact on behavior. These observations fit with the idea that, by regulating innumerable cellular processes beyond ATP production, mitochondria exert a plethora of functions that are particularly crucial for one of the most energy-avid organs of the body, such as the brain (Mattson et al, 2008). However, the direct biochemical and behavioral impact of intra-mitochondrial receptor GPCR signaling modulation is far from being completely understood and further studies are required to clarify this and other issues linked to the discovery of mtCB₁ receptors. Thus, it's crucial undertaking efforts directed at identifying and understanding both the specific effects of cannabinoids on mitochondrial functions and the potential impact that GPCR–mitochondrial signaling might have on ECS function.

The second objective of the thesis is extending our knowledge on mitochondrial G-protein activity in order to better understand the intra-mitochondrial signaling and its effects on brain functions.

For this purpose, we developed a new mitochondria-specific chemogenetic tool based on DREADDs (designer receptor exclusively activated by designer drugs) technology and we combined it with *in vitro* and *in vivo* approaches. For *in vitro* studies, we used immunoblotting, immunofluorescence and oxigraphy techniques in order to reveal the specific subcellular target location and signaling

mechanism of our tool. Moreover, we were able to show its ability to regulate the intra-mitochondrial functions such as cellular respiration and membrane potential.

For *in vivo* studies, we combined this new tool with genetic and pharmacological approaches in order to investigate how the mitochondrial G-protein signaling modulation can impact bioenergetic processes and memory. The results obtained are presented in a manuscript that is currently in preparation:

Linking Mitochondrial G-protein Signaling to cannabinoids-induced amnesia: A new Mitochondria-specific chemogenetic Strategy

Gianluca Lavanco^{*}, Antonio C. Pagano Zottola^{*}, Yamuna Mariani, Astrid Cannich, Francisca Julio-Kalajzić, Filippo Drago, Giovanni Marsicano[#], Etienne Hebert-Chatelain[#] and Luigi Bellocchio[#]

^{*}: equal contribution, [#]: equal supervision

My main contribution to this work was to perform and analyze data collected from *in vivo* studies. I also performed experiments in novel object recognition task and participated to the analysis of the behavioral data. I participated in the writing of the manuscript.

SECTION III – RESULTS

PART 1 – SPECIFIC HIPPOCAMPAL INTERNEURONS SHAPE CONSOLIDATION OF RECOGNITION MEMORY

Specific Hippocampal Interneurons Shape Consolidation of Recognition Memory

Jose F. Oliveira da Cruz,^{1,2,3,9} Arnau Busquets-Garcia,^{1,2,4,9} Zhe Zhao,¹ Marjorie Varilh,^{1,2} Gianluca Lavanco,^{1,2,5} Luigi Bellocchio,^{1,2} Laurie Robin,¹ Astrid Cannich,^{1,2} Francisca Julio-Kalajzić,^{1,2} Thierry Lesté-Lasserre,^{1,2} Marlène Maître,^{1,2} Filippo Drago,⁴ Giovanni Marsicano,^{1,2,10,11,*} and Edgar Soria-Gómez^{1,2,6,7,8,10,*}

¹ INSERM U1215, NeuroCentre Magendie, Bordeaux 33300, France

² University of Bordeaux, Bordeaux 33300, France

³ New York University, Center for Neural Science, New York, NY 10003, USA

⁴ Integrative Pharmacology and System Neuroscience, IMIM-Hospital del Mar Medical Research Institute, Barcelona 08003, Spain

⁵ Department of Biomedical and Biotechnological Sciences, Section of Pharmacology, University of Catania, Catania 95124, Italy

⁶ Ikerbasque-Basque Foundation for Science, Bilbao 48013, Spain

⁷ Department of Neuroscience, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU) Leioa 48940, Spain

⁸ Achucarro Basque Center for Neuroscience, Leioa 48940, Spain

⁹ These authors contributed equally

¹⁰ Senior authors

¹¹ Lead Contact

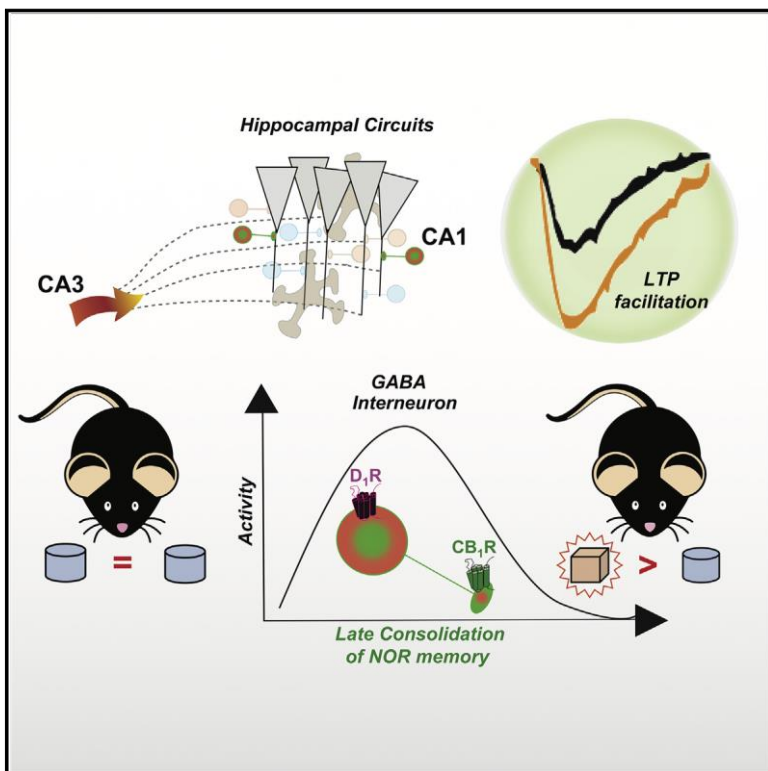
*Correspondence: giovanni.marsicano@inserm.fr (G.M.), edgarjesus.soria@ehu.eus (E.S.-G.)

SUMMARY

A complex array of inhibitory interneurons tightly controls hippocampal activity, but how such diversity specifically affects memory processes is not well understood. We find that a small subclass of type 1 cannabinoid receptor (CB₁R)-expressing hippocampal interneurons determines episodic-like memory consolidation by linking dopamine D₁ receptor (D₁R) signaling to GABAergic transmission. Mice lacking CB₁Rs in D₁-positive cells (D₁-CB₁-KO) display impairment in long-term, but not short-term, novel object recognition memory (NOR). Re-expression of CB₁Rs in hippocampal D₁R-positive cells rescues this NOR deficit. Learning induces an enhancement of *in vivo* hippocampal long-term potentiation (LTP), which is absent in mutant mice. CB₁R-mediated NOR and the associated LTP facilitation involve local control of GABAergic inhibition in a D₁-dependent manner.

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

Graphical Abstract



INTRODUCTION

Formation of episodic memory is a multistep brain process that requires activity of the medial temporal lobe (Squire et al., 2007). The hippocampus in particular participates in long-term storage of recently acquired events. Hippocampal circuits are regulated by a large variety of local inhibitory interneurons that are controlled by neuromodulatory systems ensuring their coordinated function to shape behavioral responses (Klausberger and Somogyi, 2008); the identities and functions of the interneurons are under intense scrutiny (Harris et al., 2018; Pelkey et al., 2017; Parra et al., 1998). The endocannabinoid system is a brain-modulatory signaling hub formed mainly by type 1 cannabinoid receptors (CB₁Rs), their endogenous ligands (endocannabinoids), and enzymes for their synthesis and degradation. In the hippocampus, CB₁Rs are present in principal neurons and astroglial cells (Busquets-Garcia et al., 2015; Oliveira da Cruz et al., 2016). However, the largest expression of CB₁Rs resides in GABAergic interneurons (Marsicano and Kuner, 2008; Katona and Freund, 2012),

where they modulate local inhibition of hippocampal circuits. Particularly, the largest amount of CB₁Rs is expressed in cholecystokinin (CCK)-positive interneurons, which are characterized by asynchronous neurotransmitter release (Harris et al., 2018; Katona et al., 1999; Marsicano and Lutz, 1999).

Hippocampal CB₁Rs control episodic-like memory processes and synaptic plasticity (Robin et al., 2018; Hebert-Chatelain et al., 2016; Puighermanal et al., 2009). However, the specific locations where these receptors participate in the mechanisms underlying hippocampus-dependent memory are only partially known.

Activity-dependent long-term changes in hippocampal synaptic transmission are considered cellular correlates of memory consolidation (Nicoll, 2017; Whitlock et al., 2006), which involves local dopamine D₁ receptor (D₁R) signaling (Lisman et al., 2011; Yamasaki and Takeuchi, 2017). Exposure to hippocampus-dependent behavioral tasks induces changes in long-term potentiation (LTP) of synaptic transmission that require activation of D₁-like receptors (Frey et al., 1990; Granada et al., 2008; Li et al., 2003; Lemon and Manahan-Vaughan, 2006). A novel subpopulation of hippocampal CB₁R/CCK-positive interneurons containing D₁R was recently described (Puighermanal et al., 2017; Gangarossa et al., 2012). However, the potential interactions between D₁Rs and CB₁Rs in regulating

learning-induced plasticity, activity of hippocampal circuits, and memory processes remain unexplored.

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

RESULTS

CB₁Rs in Hippocampal D₁R-Positive Neurons Are Necessary for Consolidation of NOR Memory

Mutant mice bearing a deletion of the CB₁R gene in cells expressing D₁R (D₁-CB₁-KO mice; Monory et al., 2007) displayed no phenotype in the short-term version (3 h post-training) of a NOR task (Figures 1A and 1B; Puighermanal et al., 2009; Busquets-Garcia et al., 2011; Robin et al., 2018). Conversely, they showed strong impairment in long-term (24 h) memory compared with their wild-type (WT) littermates (Figure 1C), with no changes in total exploration time (Figures S1A–S1D).

The majority of CB₁Rs in D₁R-positive neurons have been characterized previously in striatonigral circuits (Monory et al., 2007). Considering the involvement of these circuits in NOR memory (Darvas and Palmiter, 2009), we tested the role of striatal CB₁Rs. We infused an adeno-associated virus carrying a Cre-dependent expression of CB₁Rs (pAAV-CAG-DIO-CB₁) into the striatum of D₁-CB₁-KO mice to obtain re-expression (RS) of CB₁Rs in cells where Cre is present (hereafter called D₁R-positive) in this brain region (striatum [STR]-CB₁-RS mice; Figures 1D and 1E), as revealed by immunodetection of a myc-tagged version of CB₁Rs (CB₁R-myc; STAR Methods; Figure 1E). This re-expression was not sufficient to rescue the phenotype of D₁-CB₁-KO mice in long-term NOR (Figures 1F, S1E, and S1F), suggesting that CB₁Rs in striatal D₁R-positive cells do not participate in this type of memory. Anatomical data indicate that a subset of hippocampal neurons contain D₁Rs (Gangarossa et al., 2012), likely co-expressing CB₁R protein (Puighermanal et al., 2017). Thus, we re-express the CB₁R gene in the hippocampus of D₁-CB₁-KO mice to obtain hippocampus (HPC)-CB₁-RS

mice (Figures 1D and 1G). This manipulation fully rescued the phenotype of the mutant mice (Figure 1F, S1E, and S1F), indicating that hippocampal CB₁Rs expressed in D₁R-positive cells are required for NOR memory.

We recently reported that deletion of CB₁Rs in hippocampal glial acidic fibrillary protein (GFAP)-positive cells (i.e., mainly astrocytes, GFAP-CB₁-KO mice) also impaired NOR memory (Robin et al., 2018). Indeed, GFAP-CB₁-KO mice were impaired in NOR (Figures S1G–S1I; Robin et al., 2018), but, in contrast to D₁-CB₁-KO mice, this phenotype extended to short-term NOR memory (Figures S1J–S1L). This difference suggests that CB₁Rs expressed in hippocampal astrocytes or D₁R-positive cells might control distinct phases of NOR memory consolidation.

The primary function of CB₁R activation in neurons is to decrease neurotransmitter release (Castillo et al., 2012; Busquets-Garcia et al., 2017). Accordingly, deletion of CB₁Rs from neurons often results in excessive neurotransmission. Thus, we reasoned that inhibition of hippocampal D₁R-positive neurons during NOR consolidation should be able to rescue the memory impairment of D₁-CB₁-KO mice. Viral vectors carrying Cre-dependent expression of an inhibitory designer receptor exclusively activated by designer drugs (DIO-hM4DGi, Gi-DREADD; Robinson and Adelman, 2015) or control mCherry protein were infused into the hippocampi of D₁-CB₁-KO mice and WT littermates (Figure 1H). Post-training clozapine N-oxide (CNO) injections did not affect the NOR performance of D₁-CB₁-KO and WT mice injected with Gi-DREADD or mCherry, indicating that the drug or its metabolites had no effect per se (Gomez et al., 2017; Figures 1I, S1M, and S1N). Conversely, post-acquisition CNO treatment fully rescued the NOR impairment of D₁-CB₁-KO mice expressing Gi-DREADD (Figures 1I, S1M, and S1N). This strongly suggests that excessive activity of D₁R-positive neurons during the consolidation process is responsible for the memory impairment observed in D₁-CB₁-KO mice.

CB₁Rs in Hippocampal D₁R-Positive Neurons Control Learning-Induced Changes of LTP In Vivo

Cellular and molecular mechanisms underlying activity-dependent changes in synaptic plasticity are proposed to underlie long-term memory (Aggleton and Morris, 2018). Previous studies showed that conditional and global deletion of CB₁Rs in neuronal and glial cell populations induces deficits in learning and associated synaptic plasticity (Busquets-Garcia et al., 2017; Robin et al., 2018). To address the role of CB₁Rs in hippocampal D₁R-positive neurons in modulation of synaptic plasticity, we recorded in-vivo-evoked field excitatory postsynaptic potentials (fEPSPs) in the hippocampal CA3-CA1 pathway of anesthetized mice. HFS induced similar long-lasting LTP of synaptic fEPSPs in

D₁-CB₁-KO and WT littermates (Figures 2A and 2B), indicating that hippocampal D₁R/CB₁R-positive neurons are dispensable for expression of LTP in naive animals.

HPC-dependent memory-related processes such as LTP are sensitive to pharmacological and genetic modulation of hippocampal D₁Rs, particularly after learning (Li et al., 2003; Lemon and Manahan-Vaughan, 2006; Takeuchi et al., 2016; Yamasaki and Takeuchi, 2017). Thus, we hypothesized that CB₁Rs in D₁R-positive neurons may modulate learning-dependent hippocampal synaptic plasticity. To explore whether acquisition of the NOR task modulates in vivo LTP, we recorded fEPSPs from C57Bl6/NRj mice after a NOR task (Figure 2C). HFS induced stronger LTP in animals exposed to NOR acquisition than in control mice (Figures 2D and 2E), showing that the training modulates hippocampal synaptic plasticity. Strikingly, D₁-CB₁-KO mice lacked this learning-induced enhancement of LTP (Figures 2F and 2G). Thus, physiological activation of CB₁Rs in hippocampal D₁R-positive neurons is required for learning-dependent facilitation of LTP.

CB₁R in Hippocampal D₁R-Positive Neurons Modulate NOR Memory Consolidation through a GABA-Dependent Mechanism

D₁Rs are expressed in different hippocampal cells, including subsets of GABAergic and glutamatergic neurons (Gangarossa et al., 2012). Considering that CB₁R signaling decreases the activity of hippocampal neurons (Busquets-Garcia et al., 2017; Castillo et al., 2012), we asked whether excessive glutamatergic or GABAergic neurotransmission might underlie the phenotype of D₁-CB₁-KO mice. Thus, we injected non-amnesic doses (Puighermanal et al., 2009) of the NMDA receptor blocker MK-801, the AMPA/kainate receptor antagonist NBQX (Figure S2A), or the GABA_A receptor antagonist bicuculline into D₁-CB₁-KO and WT littermates immediately after NOR training. MK-801 and NBQX did not alter memory performance in WT mice, nor did it rescue the amnesic phenotype of D₁-CB₁-KO littermates (Figures 3A, S2B, and S2C). Conversely, bicuculline completely reversed the memory impairment of D₁-CB₁-KO mice when injected immediately after training or 1 h later without affecting WT littermates' performance (Figures 3A, S2B, and S2C).

These data indicate that excessive GABAergic but not glutamatergic ionotropic receptor activity is involved in the phenotype of D₁-CB₁-KO mice. A large proportion of GABAergic hippocampal interneurons contain CB₁R mRNA, which is expressed at different levels (high CB₁R- and low CB₁R-

expressing cells; Marsicano and Lutz, 1999). Conversely, D₁R mRNA is expressed at very low levels in the HPC (<http://mouse.brain-map.org/experiment/show/35>; data not shown), which makes it difficult to accurately quantify its expression above background. Therefore, to pinpoint which CB₁R-positive interneurons in the HPC contain D₁R, we combined fluorescence ISH for CB₁R mRNA in D₁-Cre and D₁-CB₁-KO mice carrying viral Cre-dependent expression of mCherry (STAR Methods; Figure 3B). As described (Marsicano and Lutz, 1999), detectable levels of CB₁R mRNA were present throughout the HPC in pyramidal neurons and in GABAergic interneurons (Figure S2D). The distribution of mCherry-tagged D₁-positive neurons in the dorsal CA1 region of D₁-Cre mice was similar to previous findings (Puighermanal et al., 2017; Gangarossa et al., 2012). Double staining revealed that virtually no high CB₁R-expressing interneurons in the *strata oriens*, *pyramidale*, *radiatum*, or *lacunosum moleculare* contain D₁Rs (Figures 3C–3F and S2D). Conversely, D₁Rs are present in a small subpopulation of low CB₁R-expressing interneurons along the different hippocampal layers (Figures 3C and 3F). Importantly, this co-expression was virtually abolished in hippocampi of D₁-CB₁-KO mice (Figures 3C, 3D, and 3F).

Altogether, these data indicate that CB₁R-dependent modulation of a small subpopulation of D₁R-positive GABAergic interneurons is required during NOR memory consolidation.

Synaptic Mechanisms Underlying NOR Memory Consolidation and Associated Hippocampal Plasticity

The data collected so far show that reduction of GABAergic signaling prevents the deficits in D₁-CB₁-KO mice of NOR consolidation. Therefore, we tested whether inhibition of GABA_A receptors could rescue the lack of learning-induced LTP enhancement observed in D₁-CB₁-KO mice. Trained mice received bicuculline or vehicle (VEH) before testing LTP induction in hippocampal circuits. In vehicle-treated animals, D₁-CB₁-KO mice showed no training-induced LTP enhancement (Figures 4A–4C). Strikingly, although bicuculline did not affect LTP in WT animals, it rescued the training-induced LTP of D₁-CB₁-KO mice (Figures 4A–4C).

Recent data suggest that hippocampal D₁R-like receptors participate in memory formation, but little is known concerning the cell types involved (Lisman et al., 2011; Yamasaki and Takeuchi, 2017). Our data indicate that CB₁R-dependent control of GABAergic transmission from a low number of

hippocampal interneurons expressing D₁R is required to guarantee late consolidation of NOR memory. Therefore, it is possible that endocannabinoid actions are secondary to activation of D₁Rs in these cells. To address this issue, we first reasoned that partial inhibition of D₁Rs should “replace” the lack of CB₁R-dependent control of neurotransmission in D₁-CB₁-KO mice. Thus, we administered a sub-effective dose of the D_{1/5}R antagonist SCH-23390 (Figures S3A–S3C) to D₁-CB₁-KO mice and WT littermates after NOR acquisition and analyzed the training-induced enhancement of *in vivo* LTP. This treatment slightly reduced the late phase of LTP in WT animals (Figures 4A–4C). However, the antagonist abolished the differences between D₁-CB₁-KO mice and WT littermates (Figures 4A–4C), indicating that reducing D₁R activity counteracts the absence of CB₁Rs in the mutants. If LTP is mechanistically linked to NOR consolidation, then the same treatment should rescue the memory impairment of D₁-CB₁-KO mice. Administration of SCH-23390 did not alter the behavior of WT mice (Figures 4D, S3D, and S3E), but, strikingly, it fully rescued the memory impairment of D₁-CB₁-KO littermates (Figures 4D, S3D, and S3E).

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

DISCUSSION

The present study reveals that a specific subpopulation of hippocampal D₁R/CB₁R-positive neurons controls late consolidation of NOR memory and associated synaptic plasticity by moderating local inhibitory GABAergic activity in the HPC. Specifically, CB₁Rs expressed in D₁R-positive interneurons participate in learning-induced facilitation of *in vivo* LTP and are required for consolidation of NOR memory. Moreover, CB₁Rs in D₁R-positive neurons are necessary for physiological D₁R-dependent modulation of memory processes, suggesting that cannabinoid signaling is part of a complex modulatory circuit regulated by dopamine transmission in the HPC. By determining cellular and behavioral functions of a specific CB₁R-expressing interneuron subpopulation, these data uncover an unforeseen role of CB₁Rs in the D₁R-dependent control of long-term memory.

The endocannabinoid system regulates episodic-like recognition memory processes via CB₁R-dependent control of different cell types in the HPC (Busquets-Garcia et al., 2017; Soria-Gomez et

al., 2017; Busquets Garcia et al., 2016; Puighermanal et al., 2009; Robin et al., 2018). In the present study, we observed that the transition from short- to long-term memory processes is controlled by a functional interaction between D₁Rs and CB₁Rs in a specific subpopulation of hippocampal interneurons. In contrast, CB₁R deletion from all body cells or in all forebrain GABAergic neurons does not reproduce the phenotype of D₁-CB₁-KO mice (Puighermanal et al., 2009; Hebert-Chatelain et al., 2016). These apparently contrasting observations can be explained by different possibilities. Long-term deletion of the CB₁R gene starting from early developmental stages in CB₁-KO and GABA-CB₁-KO mice might induce compensatory mechanisms (El-Brolosy et al., 2019; El-Brolosy and Stainier, 2017), masking the functional role of the CB₁R in NOR memory. An alternative or complementary explanation might point to the presence of different subpopulations of brain cells expressing CB₁Rs and exerting opposite effects on memory processes. For instance, endocannabinoid signaling might promote or inhibit memory formation when acting at D₁R-positive cells or at other neuronal subpopulations, respectively. We have shown previously that astroglial CB₁Rs are necessary for consolidation of NOR memory by allowing D-serine availability at glutamatergic synapses (Robin et al., 2018). We cannot fully exclude that deletion of CB₁Rs in D₁R-positive cells does not also involve astrocytes (Nagatomo et al., 2017). However, so far, no conclusive anatomical evidence has been presented for expression of D₁Rs in hippocampal astrocytes (Chai et al., 2017; Zhang et al., 2014; but see Jennings et al., 2017 for D_{1/5}R pharmacological experiments). Moreover, our current and past results suggest that endocannabinoid control of astrocytes is likely involved in the initial phases of memory formation, whereas CB₁R-dependent inhibition of D₁R-positive hippocampal interneurons determines later phases of NOR memory consolidation. The time-course effects of pharmacological treatments indicate that D-serine can rescue memory performance of GFAP-CB₁-KO mice only when administered immediately after learning (Robin et al., 2018). This idea is reinforced by the fact that these mutants do not express in vivo LTP even under basal “home cage” conditions (Robin et al., 2018), whereas D₁-CB₁-KO mice only lack the specific facilitation of LTP induced by learning. Altogether, these observations allow speculation that at least two distinct temporal windows exist in CB₁R-dependent control of NOR. First, astroglial CB₁R are necessary for the plastic processes to initiate the memory. Later, endocannabinoid-dependent regulation of D₁R-positive interneurons is required to maintain the memory trace for longer periods.

Hippocampal D₁R have been shown previously to be mainly on GABAergic interneurons, but lower levels were also detected on glutamatergic neurons (Gangarossa et al., 2012; Puighermanal et al., 2017; http://celltypes.brain-map.org/rnaseq/mouse_ctx-hip_smart-seq). Our data show that the D₁-Cre mouse line used in the present study (Lemberger et al., 2007) induces recombination in a small sub-fraction of hippocampal interneurons containing low levels of CB₁R mRNA but also in pyramidal neurons and mossy cells. Therefore, we cannot fully exclude that cell types other than hippocampal interneurons might participate in D₁R/CB₁R-dependent control of memory consolidation. However, our data show that partial blockade of GABA_A receptors, but not of AMPA/kainate or NMDA glutamatergic ones, reverse the memory impairment of D₁-CB₁-KO mice. Therefore, our findings strongly suggest that CB₁R control of GABA release from D₁R-positive interneurons regulates late consolidation of NOR memory. However, recent data using emerging technologies suggest that hippocampal cells are more diverse and functionally segregated than previously thought (Harris et al., 2018; Soltesz and Losonczy, 2018). By identifying specific markers, future studies will extend our genetic and pharmacological evidence that a specific subpopulation of D₁R/CB₁R-positive hippocampal interneurons regulates consolidation of NOR memory.

LTP at the CA3-CA1 pathway is a potential molecular and cellular mechanism underlying behavioral expression of episodic-like memory processes (Morris, 2013). Interestingly, although deletion of CB₁Rs from D₁R-positive cells impairs NOR memory, the same manipulation does not impair *in vivo* LTP of hippocampal synaptic transmission in naive animals. In agreement with previous evidence under other experimental conditions (Li et al., 2003; Lemon and Manahan-Vaughan, 2006), WT mice exposed to the NOR learning task display facilitation of *in vivo* LTP compared with animals exposed to the same environment without any learning. Importantly, this facilitation is absent in D₁-CB₁-KO mice, suggesting that endocannabinoid control of D₁R-positive hippocampal interneurons is recruited only after learning. The facilitation might be due to “real” stronger synaptic transmission after learning or a decrease in baseline synaptic activity (Lisman, 2017), which might be occluded in D₁-CB₁-KO mice. The fact that partial blockade of GABA_A receptors in trained WT mice does not alter LTP facilitation suggests that this phenomenon is due to a genuine increase in LTP. In addition, our data indicate that reducing GABAergic transmission in D₁R-positive neurons is required for this form of learning-induced synaptic plasticity. These results reinforce the idea that, to reveal relevant mechanisms, investigations of synaptic plasticity associated with memory processes should include

not only naive animals but also behaviorally challenged ones (Lisman et al., 2011).

D₁R activity in the HPC is necessary for long-term memory, synaptic plasticity, and network dynamics (Lisman et al., 2011; Yamasaki and Takeuchi, 2017; Kaufman et al., 2020; Bethus et al., 2010). Consistently, our results show that high doses of the D_{1/5}R antagonist SCH-23390 impair memory performance in the NOR task. In addition, our data suggest that D₁R/CB₁R-positive hippocampal interneurons are one of the targets of dopaminergic control of learning and memory processes. Interestingly, it has been shown that parvalbumin (PV)-expressing interneurons require D₁R activity for late phases of memory consolidation through coordinated control of the activity of hippocampal pyramidal neurons (Karunakaran et al., 2016). Particularly, the authors describe that this D₁R activity modulates hippocampal network oscillations (i.e., sharp-wave ripples), which is a proposed correlate for synaptic plasticity and memory consolidation (Buzsáki, 2015). In addition, previous studies have shown that PV/CB₁R-negative and CCK/CB₁R-positive interneurons have complementary roles in ensuring such high oscillatory ripple events with consequent capacity to modulate synaptic plasticity (Klausberger et al., 2005; Buzsáki, 2015). Therefore, we speculate that the subpopulation of D₁R/CB₁R-positive interneurons described in our work could play a complementary role in maintaining a proper excitation/inhibition balance in the hippocampal network activity required for memory consolidation.

Although complete elucidation of the complex microcircuitry requires further characterization, our findings support the hypothesis that D₁R/CB₁R-positive hippocampal interneurons belong to a broader circuit participating in dopaminergic control of memory (Yamasaki and Takeuchi, 2017). Our data are compatible with a scenario where D₁R activation during the learning/consolidation process potentiates GABAergic transmission. However, this D₁R-dependent increase in inhibition is kept within adequate limits by activation of CB₁Rs, allowing proper flow of information. In this sense, in the absence of CB₁R-dependent control of D₁R/CB₁R-positive interneurons (i.e., D₁-CB₁-KO mice), partial inhibition of D₁-like or GABA_A receptors rescues the phenotype. In other words, although activation of D₁Rs in interneurons seems to be necessary for the memory process, their abnormally high activity (e.g. in the absence of CB₁Rs) impairs such functions. In this context, an interesting question relates to the functional link between endogenous activation of D₁Rs and CB₁Rs. Our results allow speculation about two potential scenarios based on autocrine or paracrine modes of action of endocannabinoid signaling (Busquets-Garcia et al., 2017). (1) General D₁R-dependent

dopaminergic signaling in the HPC might activate pyramidal neurons (Roggenhofer et al., 2013; Shivarama Shetty et al., 2016) targeted by D₁R/CB₁R-positive interneurons. This depolarization of pyramidal neurons would, in turn, induce canonical endocannabinoid-dependent retrograde inhibition of GABAergic release (Castillo et al., 2012), moderating, among others, activation of D₁R/CB₁R-positive interneurons. (2) Following D₁R activation and consequent interneuron depolarization (Anastasiades et al., 2019; Gorelova et al., 2002), endocannabinoids might be mobilized locally and act in an autocrine manner to decrease the membrane potential and thereby moderate the activity of the neuron (Bacci et al., 2004). These two possibilities are not mutually exclusive, and they might reflect the effect of the mechanisms described on general network activity and/or on specific plastic cellular processes, respectively. Future studies will investigate these intriguing scenarios using adapted experimental approaches.

Altogether, these data reveal that functionally distinct cell types are present in the general population of hippocampal GABAergic interneurons expressing CB₁Rs. In particular, D₁R/CB₁R-positive interneurons provide specific behavioral and hippocampal synaptic mechanisms sustaining the fine-tuned regulation of memory processes. The close interaction of CB₁Rs and D₁Rs in modulating recognition memory might provide novel therapeutic frame-works for treatment of cognitive diseases characterized by alterations of endocannabinoid or dopaminergic systems or both.

ACKNOWLEDGMENTS

We thank Delphine Gonzales, Nathalie Aubailly, and the personnel of the Animal Facilities of the NeuroCentre Magendie for mouse care. We thank the Biochemistry Platform of Bordeaux NeuroCampus and the members of Marsicano's lab for useful discussions. This work was funded by INSERM; the European Research Council (Endofood ERC-2010-StG-260515, CannaPreg ERC-2014-PoC-640923, and MiCaBra ERC-2017-AdG-786467 to G.M.); Fondation pour la Recherche Medicale (FRM; DRM20101220445 to G.M. and DT20160435664 to J.F.O.d.C.); the Human Frontiers Science Program, Region Aquitaine, Agence Nationale de la Recherche (ANR; NeuroNutriSens ANR-13-BSV4-0006, ORUPS ANR-16-CE37-0010-01, and CaCoVi ANR-18-CE16- 0001-02 to G.M.); BRAIN ANR-10-LABX-0043 (to G.M.); NIH/NIDA (1R21DA037678-01); the European Regional Development Fund;

the European Union Horizon 2020 Research and Innovation Program (grant agreement 686009); French State/Agence Nationale de la Recherche/IdEx (ANR-10- IDEX-03-02), Eu-Fp7 (FP7-PEOPLE-2013-IEF-623638), and MINECO/AEI (RYC-2017-21776) (to A.B.-G.); FRM (ARF20140129235 to L.B.); and Ikerbasque (The Basque Foundation for Science) and MINECO (Ministerio de Economía y Competitividad) PGC2018-093990-A-I00 (MICIU/AEI/FEDER, UE) (to E.S.-G.).

AUTHOR CONTRIBUTIONS

J.F.O.d.C., A.B.-G., G.M., and E.S.-G. conceived and supervised the whole project. J.F.O.d.C. performed and analyzed in vivo electrophysiology and behavioral experiments. A.B.-G. and E.S.-G. performed and analyzed behavioral experiments. L.B. and A.C. contributed to experiments using viral vectors. L.R. and G.L. contributed to behavioral experiments. M.V., F.J.-K., T.L.-L., and M.M. performed cytochemical experiments. Z.Z. and M.V. helped with analysis of the data. J.F.O.d.C., A.B.-G., G.M., and E.S.-G. wrote the manuscript. F.D. contributed to writing. All authors edited and approved the manuscript.

Oliveira da Cruz et al. Figure 1

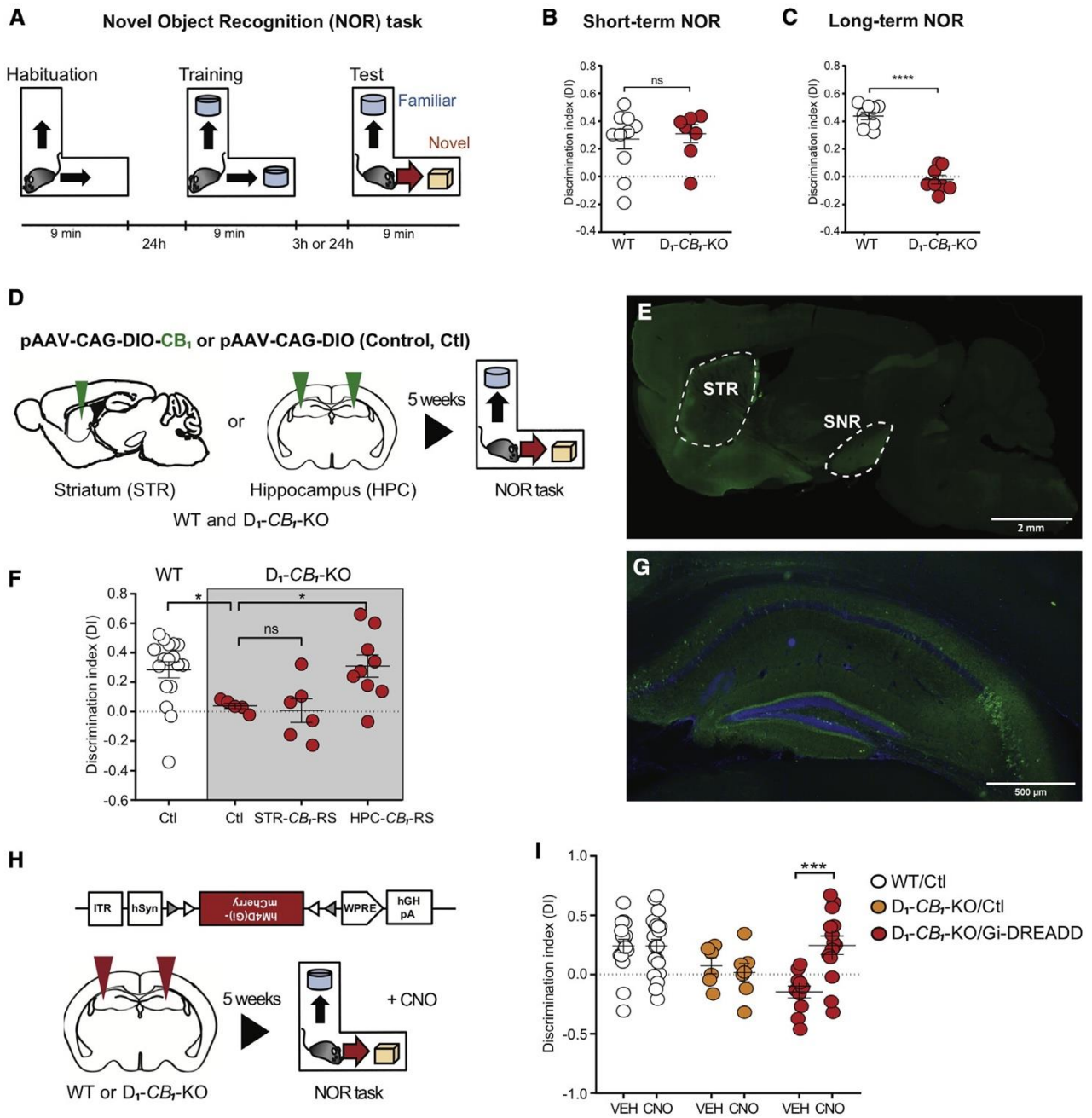


Figure 1. Hippocampal CB₁Rs in D₁R-Positive Cells Are Necessary for Late but Not Early Consolidation of NOR (A) Schematic representation of the NOR memory task. (B) Short-term (3 h) NOR memory performance of D₁-CB₁-WT mice (n = 10) and D₁-CB₁-KO littermates (n = 7). (C) Long-term NOR (24 h) memory performance of D₁-CB₁-WT mice (n = 9) and D₁-CB₁-KO littermates (n = 8). (D) Schematic representation of the experiment using viral re-expression of the CB₁R gene in the striatum (STR) or the hippocampus (HPC) of D₁-CB₁-WT mice and D₁-CB₁-KO littermates. (E) Representative images of Cre-expressing D₁-CB₁-KO mice injected with CB₁R-myc in the STR using the same procedure as described in (D) (STAR Methods). Scale bar, 2 mm. (F) NOR memory performance of mice with re-expression of the CB₁R gene in the STR or HPC. Control, n (D₁-CB₁-WT) = 17 and n (D₁-CB₁-KO) = 5; STR-CB₁-RS, n (D₁-CB₁-KO) = 6; HPC-CB₁-RS, n (D₁-CB₁-KO) = 9. (G) Immunofluorescence of cells expressing CB₁R-myc in the HPC. Scale bar, 500 mm. (H) Schematic representation of the experiment using viral expression of the Gi-DREADDs or mCherry in the HPC of D₁-CB₁-WT mice and D₁-CB₁-KO littermates. Clozapine N-oxide (CNO; 2 mg/kg) injections take place after the training phase of the NOR task. (I) NOR memory performance of D₁-CB₁-WT mice injected intra-hippocampally with hM4D(Gi) virus or mCherry (n VEH = 16, n CNO = 21), D₁-CB₁-KO mice injected with mCherry (n VEH = 6, n CNO = 7), and D₁-CB₁-KO mice injected intra-hippocampally with hM4D(Gi) (n VEH = 11, n CNO = 14). Data, mean ± SEM. *p < 0.05, ***p < 0.001, ****p < 0.0001. ns, not significant. See also Figure S1 and Table S1.

Oliveira da Cruz et al. Figure 2

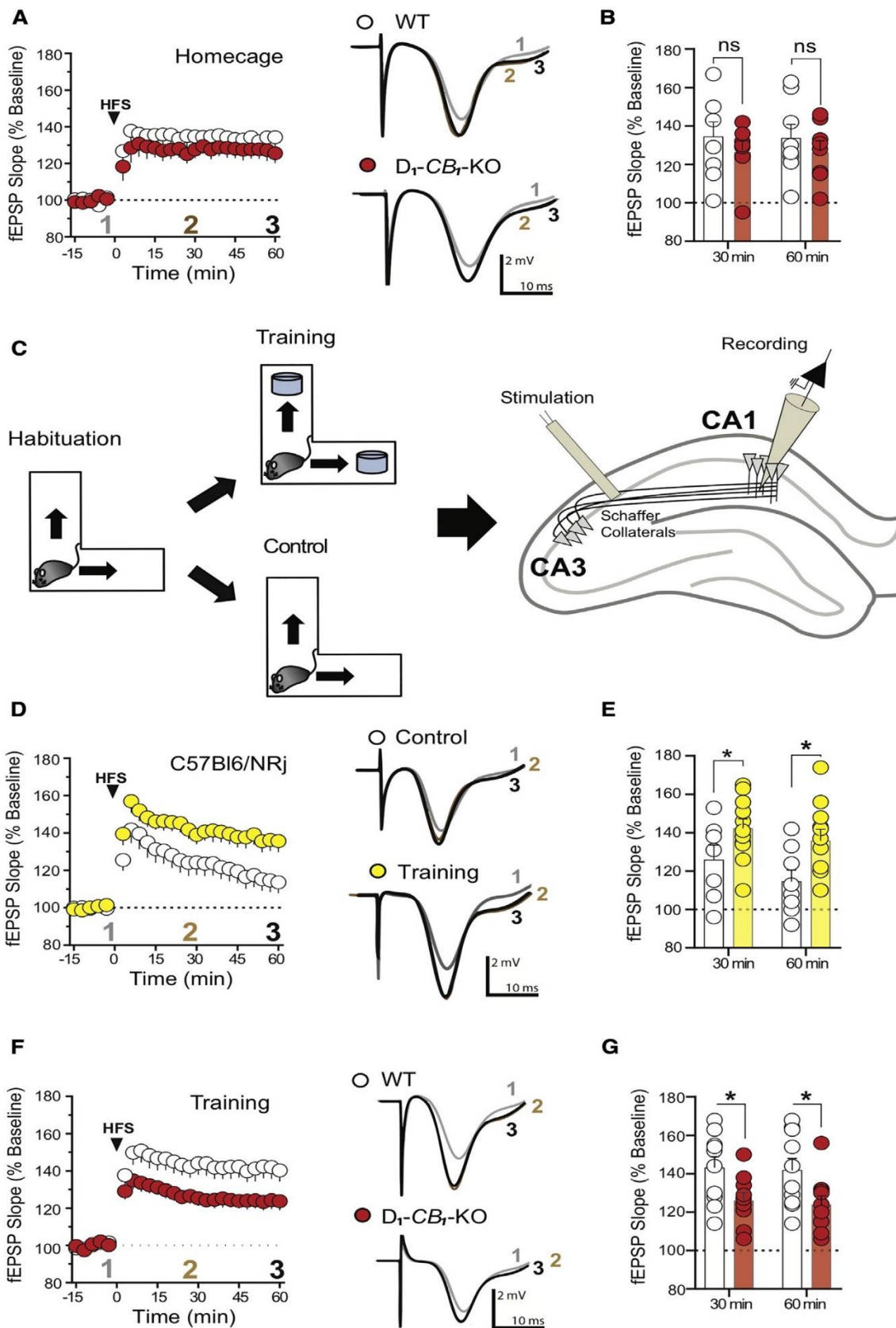


Figure 2. Learning-Induced Facilitation of In Vivo Hippocampal LTP Requires CB₁Rs at D₁R-Positive Neurons (A and B) HFS in the dorsal hippocampal CA3 Schaffer collateral pathway induces in vivo LTP in the dorsal CA1 stratum radiatum. (A) Summary plots of recorded evoked fEPSPs in anesthetized D₁-CB₁-WT (n = 8) and D₁-CB₁-KO (n = 8) mice. (B) Bar histograms of normalized fEPSPs from (A), representing 30 and 60 min after HFS. (C) Schematic representation of the experimental setup (STAR Methods). (D and E) Learning modulates in vivo LTP. (D) Summary plots of recorded evoked fEPSPs from mice exposed to control (n = 8) and NOR training (n = 11) conditions. (E) Bar histograms of normalized of evoked fEPSPs from (D), representing 30 and 60 min after HFS. (F and G) Learning-induced modulation of in vivo LTP is impaired in D₁-CB₁-KO mice. (F) Summary plots of recorded fEPSPs in anesthetized D₁-CB₁-WT (n = 10) and D₁-CB₁-KO (n = 10) mice. (G) Bar histograms of normalized of evoked fEPSPs from (F), representing 30 and 60 min after HFS. Traces on the right side of the summary plots represent 150 superimposed evoked fEPSPs before HFS (1, gray) and 30 min (2, brown) and 60 min (3, black) after HFS. Data, mean ± SEM. *p < 0.05. See also Table S1.

Oliveira da Cruz et al. Figure 3

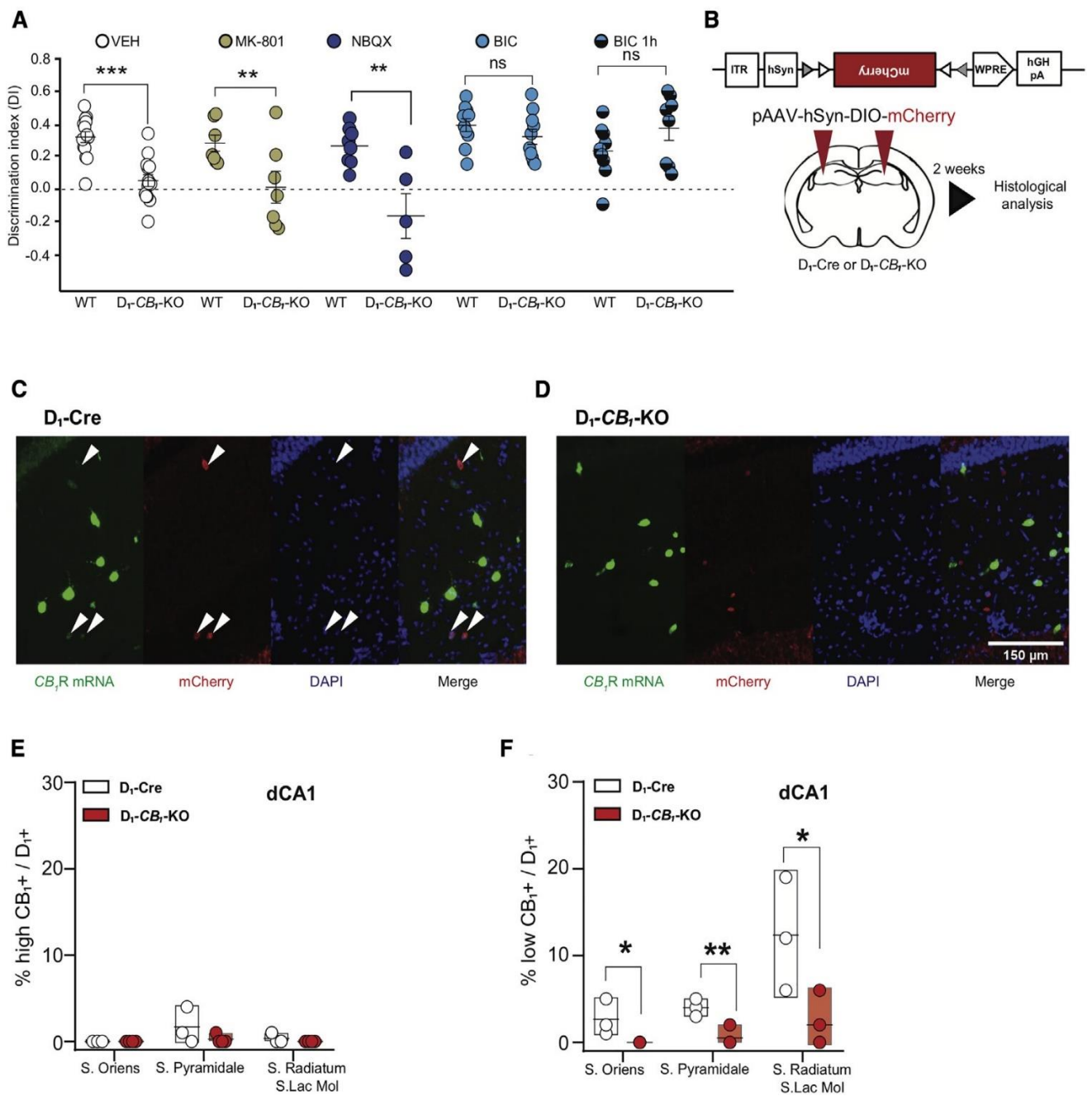


Figure 3. Hippocampal CB₁R/D₁R-Positive Interneurons Modulate Synaptic GABAergic Transmission (A) NOR memory performance of mutant mice administered vehicle (n D₁-CB₁-WT = 14, n D₁-CB₁-KO = 14), MK-801 (0.1 mg/kg, intraperitoneally [i.p.]; n D₁-CB₁-WT = 7, n D₁-CB₁-KO = 7), NBQX (5 mg/kg, i.p.; n D₁-CB₁-WT = 8, n D₁-CB₁-KO = 5), or bicuculline immediately after (n D₁-CB₁-WT = 10, n D₁-CB₁-KO = 10) or 1 h after the training phase (n D₁-CB₁-WT = 10, n D₁-CB₁-KO = 8). (B) Schematic representation of the experimental procedure to detect CB₁R mRNA in D₁R-positive cells. (C and D) Representative images of CB₁R mRNA (green) and mCherry protein (red) labeling in the hippocampal CA1 region of D₁-Cre (C) and D₁-CB₁-KO (D) mice. White arrows indicate colocalization of CB₁R-positive and D₁R-positive cell bodies. Scale bar, 150 μm. (E and F) Layer-specific distribution of the percentage of cell bodies expressing high (E) and low amounts (F) of CB₁Rs, which colocalize with mCherry-positive (i.e., D₁R-positive) in D₁-Cre (n = 3) and D₁-CB₁-KO (n = 3). Data, mean ± SEM. *p < 0.05, **p < 0.01. See also Figure S2 and Table S1.

Oliveira da Cruz et al. Figure 4

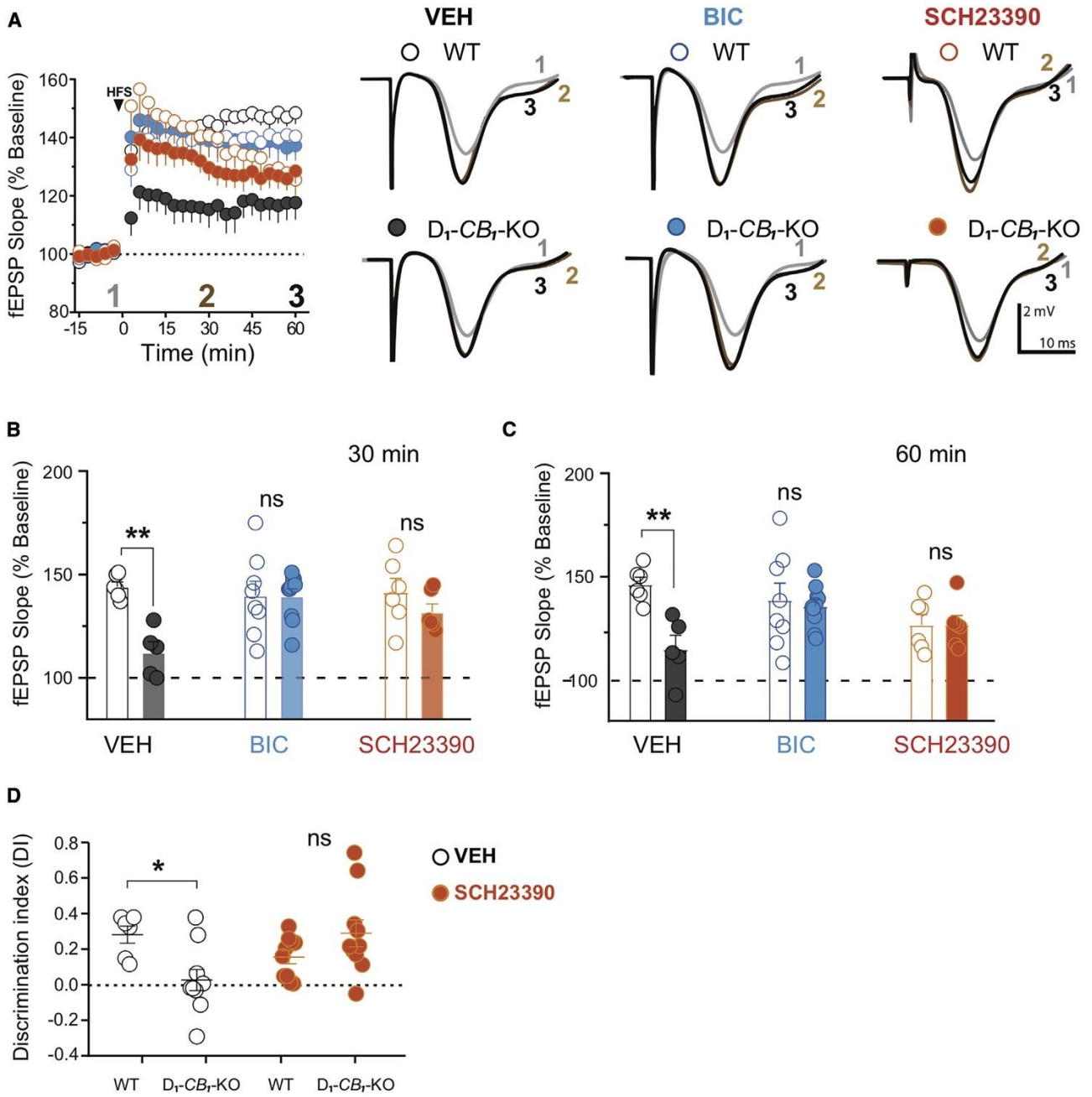


Figure 4. Cellular Mechanisms Linking D₁R Signaling with GABAergic Activity during Learning-Induced Facilitation of In Vivo LTP and Memory Consolidation (A) Effects of the GABA_A receptor antagonist bicuculline and the D_{1/5}R antagonist SCH-23390 on learning-induced modulation of in vivo LTP in D₁-CB₁-WT and D₁-CB₁-KO mice. Shown are summary plots of recorded evoked fEPSPs in vehicle (n D₁-CB₁-WT = 6, n D₁-CB₁-KO = 8), bicuculline (0.5 mg/kg, i.p.; n D₁-CB₁-WT = 9, n D₁-CB₁-KO = 11), and SCH-23390 (0.3 mg/kg, i.p.; n D₁-CB₁-WT = 6, n D₁-CB₁-KO = 6). (B and C) Bar histograms of (A), representing normalized fEPSPs from 30 (B) and 60 (C) min after HFS. (D) Memory performance D₁-CB₁-WT and D₁-CB₁-KO mice after being injected with vehicle (n D₁-CB₁-WT = 6, n D₁-CB₁-KO = 10) or SCH-23390 (0.3 mg/kg, i.p.; n D₁-CB₁-WT = 10, n D₁-CB₁-KO = 10). Traces on the right side of the summary plot (A) represent 150 superimposed evoked fEPSPs before HFS (1, gray) and 30 min (2, brown) and 60 min (3, black) after HFS. Data, mean ± SEM. *p < 0.05, **p < 0.01. See also Figure S3 and Table S1.

REFERENCES

Aggleton, J.P., and Morris, R.G.M. (2018). Memory: Looking back and looking forward. *Brain Neurosci. Adv.* 2, 2398212818794830.

Anastasiades, P.G., Boada, C., and Carter, A.G. (2019). Cell-Type-Specific D1 Dopamine Receptor Modulation of Projection Neurons and Interneurons in the Prefrontal Cortex. *Cereb. Cortex* 29, 3224–3242.

Atasoy, D., Aponte, Y., Su, H.H., and Sternson, S.M. (2008). A FLEX switch targets Channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. *J. Neurosci.* 28, 7025–7030.

Bacci, A., Huguenard, J.R., and Prince, D.A. (2004). Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature* 431, 312–316.

Bethus, I., Tse, D., and Morris, R.G. (2010). Dopamine and memory: modulation of the persistence of memory for novel hippocampal NMDA receptor-dependent paired associates. *J. Neurosci.* 30, 1610–1618.

Busquets-Garcia, A., Puighermanal, E., Pastor, A., de la Torre, R., Maldonado, R., and Ozaita, A. (2011). Differential role of anandamide and 2-arachidonoyl-glycerol in memory and anxiety-like responses. *Biol. Psychiatry* 70, 479–486.

Busquets-Garcia, A., Gomis-Gonzalez, M., Guegan, T., Agustín-Pavón, C., Pastor, A., Mato, S., Pérez-Samartín, A., Matute, C., de la Torre, R., Dierssen, M., et al. (2013). Targeting the endocannabinoid system in the treatment of fragile X syndrome. *Nat. Med.* 19, 603–607.

Busquets-Garcia, A., Desprez, T., Metna-Laurent, M., Bellocchio, L., Marsicano, G., and Soria-Gomez, E. (2015). Dissecting the cannabinergic control of behavior: The where matters. *BioEssays* 37, 1215–1225.

Busquets Garcia, A., Soria-Gomez, E., Bellocchio, L., and Marsicano, G. (2016). Cannabinoid receptor type-1: breaking the dogmas. *F1000Res.* 5, 5.

Busquets-Garcia, A., Bains, J., and Marsicano, G. (2017). CB1 Receptors Signaling in the Brain: Extracting Specificity from Ubiquity. *Neuropsychopharmacology* 43, 4–20.

Buzsáki, G. (2015). Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus* 25, 1073–1188.

Castillo, P.E., Younts, T.J., Chavez, A.E., and Hashimoto, Y. (2012). Endocannabinoid signaling and synaptic function. *Neuron* 76, 70–81.

Chai, H., Diaz-Castro, B., Shigetomi, E., Monte, E., O'Carroll, J.C., Yu, X., Cohn, W., Rajendran, P.S., Vondriska, T.M., Whitelegge, J.P., et al. (2017). Neural Circuit-Specialized Astrocytes: Transcriptomic, Proteomic, Morphological, and Functional Evidence. *Neuron* 95, 531–549.e9.

Darvas, M., and Palmiter, R.D. (2009). Restriction of dopamine signaling to the dorsolateral striatum is sufficient for many cognitive behaviors. *Proc. Natl. Acad. Sci. USA* 106, 14664–14669.

El-Brolosy, M.A., and Stainier, D.Y.R. (2017). Genetic compensation: A phenomenon in search of mechanisms. *PLoS Genet.* 13, e1006780.

El-Brolosy, M.A., Kontarakis, Z., Rossi, A., Kuenne, C., Guenther, S., Fukuda, N., Kikhi, K., Boezio, G.L.M., Takacs, C.M., Lai, S.L., et al. (2019). Genetic compensation triggered by mutant mRNA degradation. *Nature* 568, 193–197.

Frey, U., Schroeder, H., and Matthies, H. (1990). Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. *Brain Res.* 522, 69–75.

Gangarossa, G., Longueville, S., De Bundel, D., Perroy, J., Hervé, D., Girault, J.A., and Valjent, E. (2012). Characterization of dopamine D1 and D2 receptor-expressing neurons in the mouse hippocampus. *Hippocampus* 22, 2199–2207.

Gomez, J.L., Bonaventura, J., Lesniak, W., Mathews, W.B., Sysa-Shah, P., Rodriguez, L.A., Ellis, R.J., Richie, C.T., Harvey, B.K., Dannals, R.F., et al. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science* 357, 503–507.

Gorelova, N., Seamans, J.K., and Yang, C.R. (2002). Mechanisms of dopamine activation of fast-spiking interneurons that exert inhibition in rat prefrontal cortex. *J. Neurophysiol.* 88, 3150–3166.

Granado, N., Ortiz, O., Suarez, L.M., Martín, E.D., Cenã, V., Solís, J.M., and Moratalla, R. (2008). D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-Induced arc and zif268 expression in the hippocampus. *Cereb. Cortex* 18, 1–12.

Harris, K.D., Hochgerner, H., Skene, N.G., Magno, L., Katona, L., Bengtsson Gonzales, C., Somogyi, P., Kessaris, N., Linnarsson, S., and Hjerling-Leffler, J. (2018). Classes and continua of hippocampal CA1 inhibitory neurons revealed by single-cell transcriptomics. *PLoS Biol.* 16, e2006387.

Hebert-Chatelain, E., Desprez, T., Serrat, R., Bellocchio, L., Soria-Gomez, E., Busquets-Garcia, A., Pagano Zottola, A.C., Delamarre, A., Cannich, A., Vincent, P., et al. (2016). A cannabinoid link between mitochondria and memory. *Nature* 539, 555–559.

Jennings, A., Tyurikova, O., Bard, L., Zheng, K., Semyanov, A., Henneberger, C., and Rusakov, D.A. (2017). Dopamine elevates and lowers astroglial Ca²⁺ through distinct pathways depending on local synaptic circuitry. *Glia* 65, 447–459.

Karunakaran, S., Chowdhury, A., Donato, F., Quairiaux, C., Michel, C.M., and Caroni, P. (2016). PV plasticity sustained through D1/5 dopamine signaling required for long-term memory consolidation. *Nat. Neurosci.* 19, 454–464.

Katona, I., and Freund, T.F. (2012). Multiple functions of endocannabinoid signaling in the brain. *Annu. Rev. Neurosci.* 35, 529–558.

Katona, I., Sperlagh, B., Sik, A., Káfalvi, A., Vizi, E.S., Mackie, K., and Freund, T.F. (1999). Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J. Neurosci.* 19, 4544–4558.

Kaufman, A.M., Geiller, T., and Losonczy, A. (2020). A Role for the Locus Coeruleus in Hippocampal CA1 Place Cell Reorganization during Spatial Reward Learning. *Neuron* 105, 1018–1026.e4.

Klausberger, T., and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Science* 321, 53–57.

Klausberger, T., Marton, L.F., O’Neill, J., Huck, J.H., Dalezios, Y., Fuentealba, P., Suen, W.Y., Papp, E., Kaneko, T., Watanabe, M., et al. (2005). Complementary roles of cholecystinin- and parvalbumin-expressing GABAergic neurons in hippocampal network oscillations. *J. Neurosci.* 25, 9782–9793.

Lemberger, T., Parlato, R., Dasselze, D., Westphal, M., Casanova, E., Turiault, M., Tronche, F., Schiffmann, S.N., and Schuetz, G. (2007). Expression of Cre recombinase in dopaminergic neurons. *BMC Neurosci.* 8, 4.

Lemon, N., and Manahan-Vaughan, D. (2006). Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *J. Neurosci.* 26, 7723–7729.

Li, S., Cullen, W.K., Anwyl, R., and Rowan, M.J. (2003). Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. *Nat. Neurosci.* 6, 526–531.

Lisman, J. (2017). Criteria for identifying the molecular basis of the engram (CaMKII, PKMzeta). *Mol. Brain* 10, 55.

Lisman, J., Grace, A.A., and Duzel, E. (2011). A neoHebbian framework for episodic memory; role of dopamine-dependent late LTP. *Trends Neurosci.* 34, 536–547.

Marsicano, G., and Kuner, R. (2008). Anatomical distribution of receptors, ligands and enzymes in the brain and the spinal cord: circuitries and neurochemistry. In *Cannabinoids and the brain*, A. Kofalvi, ed. (Springer).

Marsicano, G., and Lutz, B. (1999). Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur. J. Neurosci.* 11, 4213–4225.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutiérrez, S.O., van der Stelt, M., et al. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302, 84–88.

Monory, K., Blaudzun, H., Massa, F., Kaiser, N., Lemberger, T., Schuëtz, G., Wotjak, C.T., Lutz, B., and Marsicano, G. (2007). Genetic dissection of behavioural and autonomic effects of Delta(9)-tetrahydrocannabinol in mice. *PLoS Biol.* 5, e269.

Morris, R.G. (2013). NMDA receptors and memory encoding. *Neuropharmacology* 74, 32–40.

Nagatomo, K., Suga, S., Saitoh, M., Kogawa, M., Kobayashi, K., Yamamoto, Y., and Yamada, K. (2017). Dopamine D1 Receptor Immunoreactivity on Fine Processes of GFAP-Positive Astrocytes in the Substantia Nigra Pars Reticulata of Adult Mouse. *Front. Neuroanat.* 11, 3.

Nicoll, R.A. (2017). A Brief History of Long-Term Potentiation. *Neuron* 93, 281–290.

Oliveira da Cruz, J.F., Robin, L.M., Drago, F., Marsicano, G., and Metna-Laurent, M. (2016). Astroglial type-1 cannabinoid receptor (CB1): A new player in the tripartite synapse. *Neuroscience* 323, 35–42.

Parra, P., Gulyás, A.I., and Miles, R. (1998). How many subtypes of inhibitory cells in the hippocampus? *Neuron* 20, 983–993.

Pelkey, K.A., Chittajallu, R., Craig, M.T., Tricoire, L., Wester, J.C., and McBain, C.J. (2017). Hippocampal GABAergic Inhibitory Interneurons. *Physiol. Rev.* 97, 1619–1747.

Puighermanal, E., Marsicano, G., Busquets-Garcia, A., Lutz, B., Maldonado, R., and Ozaita, A. (2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nat. Neurosci.* 12, 1152–1158.

Puighermanal, E., Busquets-Garcia, A., Gomis-Gonzalez, M., Marsicano, G., Maldonado, R., and Ozaita, A. (2013). Dissociation of the pharmacological effects of THC by mTOR blockade. *Neuropsychopharmacology* 38, 1334–1343.

Puighermanal, E., Cutando, L., Boubaker-Vitre, J., Honoré, E., Longueville, S., Hervé, D., and Valjent, E. (2017). Anatomical and molecular characterization of dopamine D1 receptor-expressing neurons of the mouse CA1 dorsal hippocampus. *Brain Struct. Funct.* 222, 1897–1911.

Robin, L.M., Oliveira da Cruz, J.F., Langlais, V.C., Martin-Fernandez, M., Metna-Laurent, M., Busquets-Garcia, A., Bellocchio, L., Soria-Gomez, E., Papouin, T., Varilh, M., et al. (2018). Astroglial CB1 Receptors Determine Synaptic D-Serine Availability to Enable Recognition Memory. *Neuron* 98,

935–944.e5.

Robinson, S., and Adelman, J.S. (2015). A Method for Remotely Silencing Neural Activity in Rodents During Discrete Phases of Learning. *J. Vis. Exp.* (100), e52859.

Roggenhofer, E., Fidzinski, P., Shor, O., and Behr, J. (2013). Reduced threshold for induction of LTP by activation of dopamine D1/D5 receptors at hippocampal CA1-subiculum synapses. *PLoS ONE* 8, e62520.

Shivarama Shetty, M., Gopinadhan, S., and Sajikumar, S. (2016). Dopamine D1/D5 receptor signaling regulates synaptic cooperation and competition in hippocampal CA1 pyramidal neurons via sustained ERK1/2 activation. *Hippocampus* 26, 137–150.

Soltész, I., and Losonczy, A. (2018). CA1 pyramidal cell diversity enabling parallel information processing in the hippocampus. *Nat. Neurosci.* 21, 484–493.

Soria-Gomez, E., Metna, M., Bellocchio, L., Busquets-Garcia, A., and Marsicano, G. (2017). The endocannabinoid system in the control of behavior. In *Handbook of Neurobehavioral Genetics and Phenotyping*, V. Tucci, ed. (John Wiley & Sons), pp. 323–355.

Squire, L.R., Zola-Morgan, J., and Zola-Morgan, J. (2007). Recognition memory and the medial temporal lobe: a new perspective. *Nat. Rev. Neurosci.* 8, 872–883.

Takeuchi, T., Duszkiewicz, A.J., Sonneborn, A., Spooner, P.A., Yamasaki, M., Watanabe, M., Smith, C.C., Fernandez, G., Deisseroth, K., Greene, R.W., and Morris, R.G. (2016). Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature* 537, 357–362.

Terral, G., Busquets-García, A., Varilh, M., Achicallende, S., Cannich, A., Bellocchio, L., Bonilla-Del Río, I., Massa, F., Puente, N., Soria-Gomez, E., et al. (2019). CB1 receptors in the anterior piriform cortex control odor preference memory. *Curr. Biol.* 29, 2455–2464.e5.

Terzian, A.L., Drago, F., Wotjak, C.T., and Micale, V. (2011). The Dopamine and Cannabinoid Interaction in the Modulation of Emotions and Cognition: Assessing the Role of Cannabinoid CB1 Receptor in Neurons Expressing Dopamine D1 Receptors. *Front. Behav. Neurosci.* 5, 49.

Whitlock, J.R., Heynen, A.J., Shuler, M.G., and Bear, M.F. (2006). Learning induces long-term potentiation in the hippocampus. *Science* 313, 1093–1097.

Yamasaki, M., and Takeuchi, T. (2017). Locus Coeruleus and Dopamine-Dependent Memory Consolidation. *Neural Plast.* 2017, 8602690.

Zhang, Y., Chen, K., Sloan, S.A., Bennett, M.L., Scholze, A.R., O’Keefe, S., Phatnani, H.P., Guarnieri, P., Caneda, C., Ruderisch, N., et al. (2014). An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* 34, 11929–11947.

Supplemental Material for

Specific Hippocampal Interneurons Shape Consolidation of Recognition Memory

Jose F. Oliveira da Cruz,^{1,2,3,9} Arnau Busquets-Garcia,^{1,2,4,9} Zhe Zhao,¹ Marjorie Varilh,^{1,2} Gianluca Lavanco,^{1,2,5} Luigi Bellocchio,^{1,2} Laurie Robin,¹ Astrid Cannich,^{1,2} Francisca Julio-Kalajzić,^{1,2} Thierry Lesté-Lasserre,^{1,2} Marlène Maître,^{1,2} Filippo Drago,⁴ Giovanni Marsicano,^{1,2,10,11, *} and Edgar Soria-Gómez^{1,2,6,7,8,10, *}

¹ INSERM U1215, NeuroCentre Magendie, Bordeaux 33300, France

² University of Bordeaux, Bordeaux 33300, France

³ New York University, Center for Neural Science, New York, NY 10003, USA

⁴ Integrative Pharmacology and System Neuroscience, IMIM-Hospital del Mar Medical Research Institute, Barcelona 08003, Spain

⁵ Department of Biomedical and Biotechnological Sciences, Section of Pharmacology, University of Catania, Catania 95124, Italy

⁶ Ikerbasque-Basque Foundation for Science, Bilbao 48013, Spain

⁷ Department of Neuroscience, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU) Leioa 48940, Spain

⁸ Achucarro Basque Center for Neuroscience, Leioa 48940, Spain

Content:

Gianluca Lavanco – Doctoral Thesis – University of Bordeaux and University of Catania

Material and Methods

Figures S1-S3 (relative to main figures)

Table S1

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Animal Model

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

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

METHOD DETAILS

Drug preparation and administration

Bicuculline, MK-801, NBQX and SCH-23390 were purchased from Merck (formerly Sigma-Aldrich, France) and were dissolved to their final concentration in physiological saline (NaCl 0.9%). The exogenous DREADD ligand clozapine-N-oxide (CNO, 2 mg/kg) was purchased from Tocris Bioscience (Bristol, UK) and dissolved in saline after gently mixing with a vortex. All drugs were injected intraperitoneally in a volume of 10 ml/kg. Vehicle in all the conditions was composed of physiological saline (NaCl 0.9%) injections.

Novel object recognition memory

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

The task took place in a L-shaped maze made of dark gray polyvinyl chloride made by two identical perpendicular arms (35 cm and 30 cm long respectively for external and internal L walls, 4.5cm wide and 15 cm high walls) placed on a white background (Busquets-Garcia et al., 2011; Puighermanal et al., 2009). The task occurred in a room adjacent to the animal house with a light intensity fixed at 50 lux. The maze was overhung by a video camera allowing the detection and offline scoring of animal's behavior. The task consisted in 3 sequential daily trials of 9 minutes each. During the habituation phase (day 1), mice were placed in the center of the maze and allowed to freely explore the arms in the absence of any objects. The training phase (day 2) consisted in placing the mice again in the corner of the maze in the presence of two identical objects positioned at the extremities of each arm and left to freely explore the maze and the objects. The testing phase occurred 24 hours later (day 3): one of the familiar objects was replaced by a novel object different in its shape, color and texture and mice were left to explore both objects. The position of the novel object and the associations of novel and familiar were randomized. All objects were previously tested to avoid biased preference. Memory performance was assessed by the discrimination index (DI). The DI was calculated as the difference between the time spent exploring the novel (TN) and the familiar object (TF) divided by the total exploration time (TN+TF): $DI = [TN-TF]/[TN+TF]$. Memory was also evaluated by directly comparing the exploration time of novel and familiar objects, respectively. Object exploration was defined as the orientation of the nose to the object at less than 2 cm. Experienced investigators evaluating the exploration were blind of treatment and/or genotype of the animals. Pharmacological treatments were immediately administered after the training phase.

In vivo electrophysiology in anesthetized mice

Experiments were performed as described in Robin et al. (2018). Mice were anesthetized in a box containing 5% Isoflurane (Virbac, France) before being placed in a stereotaxic frame (Model 900, Kopf instruments, CA, USA) in which 1.0% to 1.5% of Isoflurane was continuously supplied via an anesthetic mask during the whole duration of the experiment. The body temperature was maintained at $\pm 36.5^{\circ}\text{C}$ using a homeothermic system (model 50-7087-F, Harvard Apparatus, MA, USA) and the state of anesthesia was assessed by mild tail pinch. Before surgery, 100 mL of the local anesthetic lurocaine (vetoquinol, France) was injected in the scalp region. Surgical procedure started with a longitudinal incision of 1.5 cm in length aimed to expose Bregma and Lambda. After ensuring the correct alignment of the head, two holes were drilled in the skull for electrode placement. Glass recording electrodes were inserted in the CA1 stratum radiatum, and a concentric stimulating bipolar electrode (Model CBARC50, FHC, ME, USA) placed in the CA3 region. Coordinates were as follows: CA1 stratum radiatum: A/P 1.5, M/L 1.0, DV 1.20; CA3: A/P 2.2, M/L 2.8, D/V 1.3 (20° insertion angle). The recording electrode (tip diameter = 1–2 mm, 2–4 MU) was filled with a 2% pontamine sky blue solution in 0.5M sodium acetate. At first the recording electrode was placed by hand until it reached the surface of the brain and then to the final depth using a hydraulic micro-positioner (Model 2650, KOPF instruments, CA, USA). The stimulation electrode was placed in the correct area using a standard manipulator. Both electrodes were adjusted to find the area with maximum response. In vivo recordings of evoked field excitatory postsynaptic potentials (fEPSPs) were amplified 1000 times and filtered (low-pass at 1Hz and high-pass 3000Hz) by a DAGAN 2400A amplifier (DAGAN Corporation, MN, USA). fEPSPs were digitized and collected on-line using a laboratory interface and software (CED 1401, SPIKE 2; Cambridge Electronic Design, Cambridge, UK). Test pulses were generated through an Isolated Constant Current Stimulator (DS3, Digitimer, Hertfordshire, UK) triggered by the SPIKE 2 output sequencer via CED 1401 and collected every 2 s at a 10 kHz sampling frequency and then averaged every 180 s. Test pulse intensities were typically between 40–250 mA with a duration of 50 ms. Basal stimulation intensity was adjusted to 30%–50% of the current intensity that evoked a maximum field response. All responses were expressed as

percent from the average responses recorded during the 15 min before HFS. HFS was induced by applying 3 trains of 100 Hz (1 s each), separated by 20 s interval. fEPSP were then recorded for a period of 60 min. C57BL6/NRj mice underwent this in vivo electrophysiology procedure after the training phase of NOR task. Also, where specified, D₁-CB₁-KO and D₁-CB₁-WT received an injection of Bicuculine (0.5 mg/kg, intraperitoneal) or SCH 23390 (0.3 mg/kg, intraperitoneal) or vehicle immediately after undergoing training in NORT and before being subjected to the in vivo electrophysiology procedure. At the end the experiment, the position of the electrodes was marked (recording area: iontophoretic infusion of the recording solution during 180 s at 20mA; stimulation area: continuous current discharge over 20 s at +20mA) and histological verification was performed ex vivo.

Surgery and viral administration

Mice were anesthetized in a box containing 5% Isoflurane (Virbac, France) before being placed in a stereotaxic frame (Model 900, Kopf instruments, CA, USA) in which 1.0% to 1.5% of Isoflurane was continuously supplied via an anesthetic mask during the whole duration of the experiment. For viral intra-HPC AAV delivery, mice were submitted to stereotaxic surgery (as above) and AAV vectors were injected with the help of a microsyringe (0.25 mL Hamilton syringe with a 30-gauge beveled needle) attached to a pump (UMP3-1, World Precision Instruments, FL, USA). Where specified, D₁-CB₁-WT and D₁-CB₁-KO mice were injected directly into the hippocampus (HPC) or striatum (STR) (0.5 ml per injection site at a rate of 0.5 ml per min), with the following coordinates: HPC, AP -1.8; ML ± 1; DV -2.0 and -1.5; Striatum: AP -1.34; ML ± 2.8; DV -1.84. Following virus delivery, the syringe was left in place for 1 minute before being slowly withdrawn from the brain. CB₁ floxed mice were injected with rAAV-CAG-DIO (empty control vector), AAV-CAG-DIO-CB₁ or AAV-CAG-DIO-CB₁-myc to induce re-expression of the CB₁ receptor gene in hippocampal or striatal D₁-positive cells. To generate the aforementioned rAAVs, mouse CB₁ receptor coding sequence (either native or fused to myc-tag at the C term) was cloned in rAAV-CAG-DIO vector using standard molecular cloning technology. The coding sequence was cloned inverted in orientation to allow Cre-dependent expression of CB₁ receptors (Atasoy et al., 2008). In another experiment, and using the same procedure as described as above, D₁-CB₁-WT and D₁-CB₁-KO mice were injected intra hippocampally (AP -1.8; ML ± 1; DV -2.0 and -1.5), with pAAV-hSyn-DIO-hM4D(Gi)-mCherry or pAAV-hSyn-DIO-

mCherry (addgene, USA). For anatomical experiments and using the same procedure as above, D₁-Cre and D₁-CB₁-KO were injected intra hippocampally with pAAV-hSyn-DIO-mCherry. In this specific experiment, expression was allowed to take place for 2 weeks. For the remaining experiments, animals were used around 4-5 weeks after local infusions. Mice were weighed daily and individuals that failed to regain the pre-surgery body weight were excluded from the following experiments.

Immunohistochemistry on free-floating sections

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

Combined Fluorescent in situ hybridization (FISH)/ Immunohistochemistry (IHC) on free-floating frozen sections

Mice were anesthetized with pentobarbital (Exagon, Axience SAS, 400 mg/kg body weight), transcardially perfused with PBS (0.1M, pH 7.4) before being fixed with 4% formaldehyde (Sigma-Aldrich). The brains were extracted and incubated overnight at 4°C in the same fixative, then embedded with sucrose 30% for 3 days and finally frozen in 2-methylbutane (Sigma-Aldrich) at -

80°C. Free-floating frozen coronal sections were cut out with a cryostat (30 mm, Microm HM 500M Microm Microtech) and collected in an anti-freeze solution and conserved at -20°C.

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

QUANTIFICATION AND STATISTICAL ANALYSIS

Data collection

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

Fluorescence quantifications

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

Statistical analyses

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

Figure S1

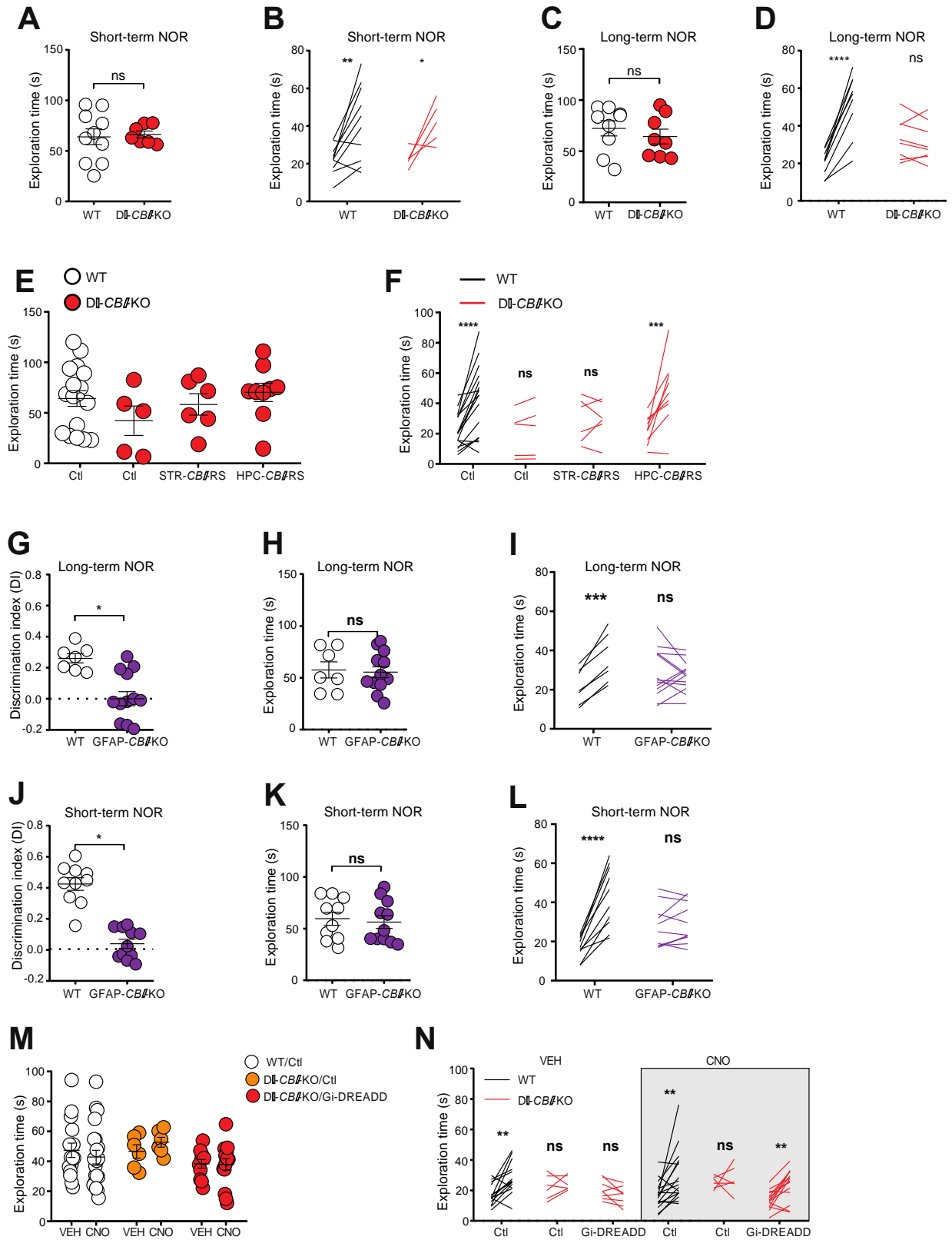


FIGURE S1. RELATED TO FIGURE 1.

(A) Total exploration time, and (B) exploration time of the familiar versus the novel object of D₁-CB₁-KO mice and WT littermates in the Short-term NOR task. (C) Total exploration time, and (D) exploration time of the familiar versus the novel object of D₁-CB₁-KO mice and WT littermates in the Long-term NOR task. (E) Total exploration time, and (F) exploration time of the familiar versus the novel object of STR-CB₁-RS, HPC-CB₁-RS and control mice in the Long-term NOR task. (G) Memory performance, (H) total exploration, and (I) exploration time of the familiar versus the novel object of D₁-CB₁-KO mice and WT littermates in the Long-term NOR task. (J) Memory performance, (K) total exploration, and (L) exploration time of the familiar versus the novel object of D₁-CB₁-KO mice and WT littermates in the Short-term NOR task. (M) Total exploration time, and (N) exploration time of the familiar versus the novel object of D₁-CB₁-KO and WT littermates intra-hippocampally injected with hM4D(Gi) virus or mCherry, with VEH or CNO in the Long-term NOR task. See also Table S1.

Figure S2

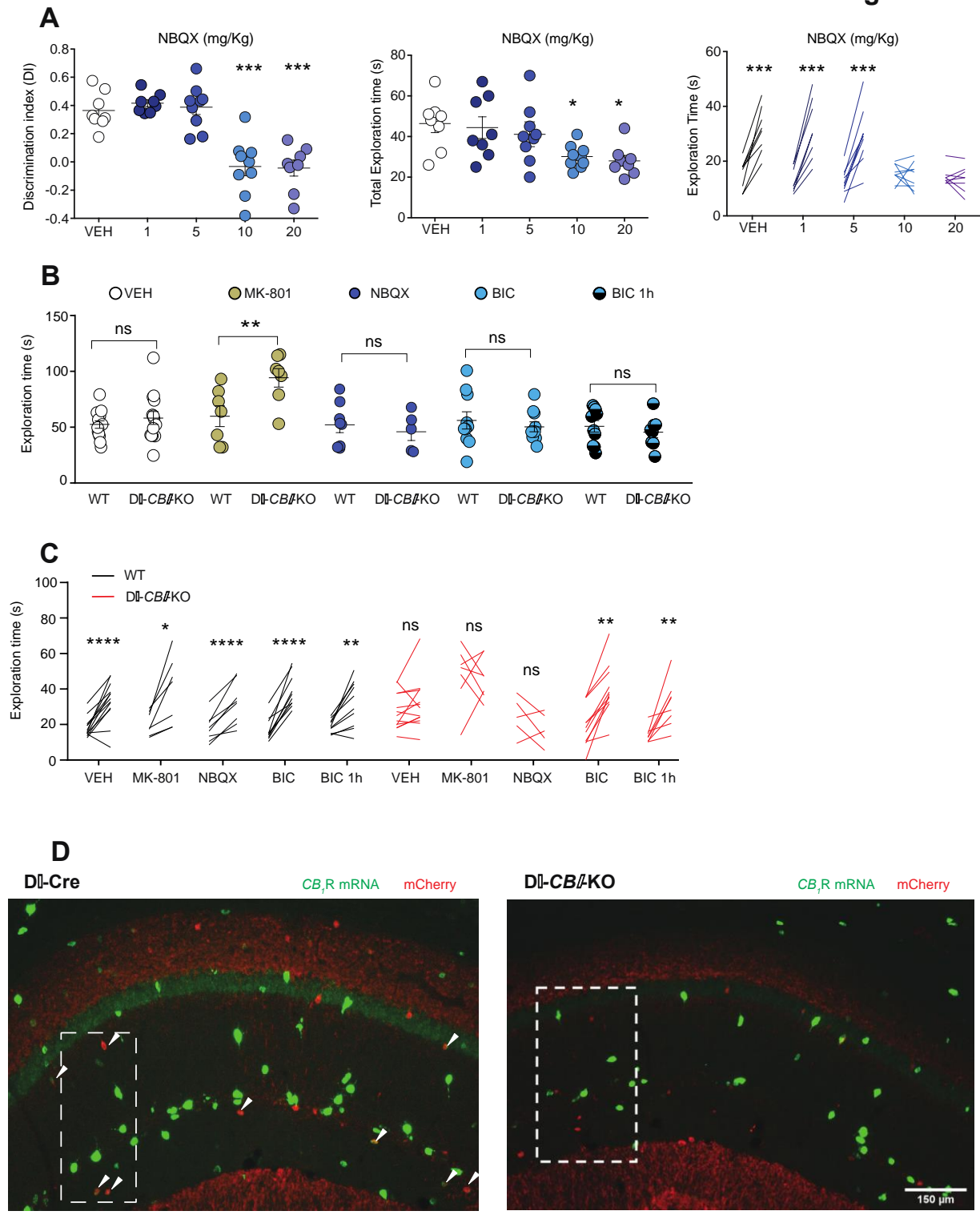


FIGURE S2. RELATED TO FIGURE 3

(A) Memory performance, total exploration time, and exploration time of the familiar versus the novel object of C57BL6/NRj mice injected systemically with different doses of NBQX (in mg/kg, IP). (B) Total exploration, and (C) exploration time of the familiar versus the novel object time of D₁-CB₁-KO mice and WT littermates with systemic injections of VEH, MK-801, NBQX and Bicuculine (BIC). (D) Representative micrographs of the dorsal hippocampus of D₁-Cre and D₁-CB₁-KO mice showing the co-expression of CB₁R mRNA and mCherry (D₁R-positive cells). The dotted white square is the area showed in main Figure 3C. Scale bar = 150 μm. See also Table S1.

Figure S3

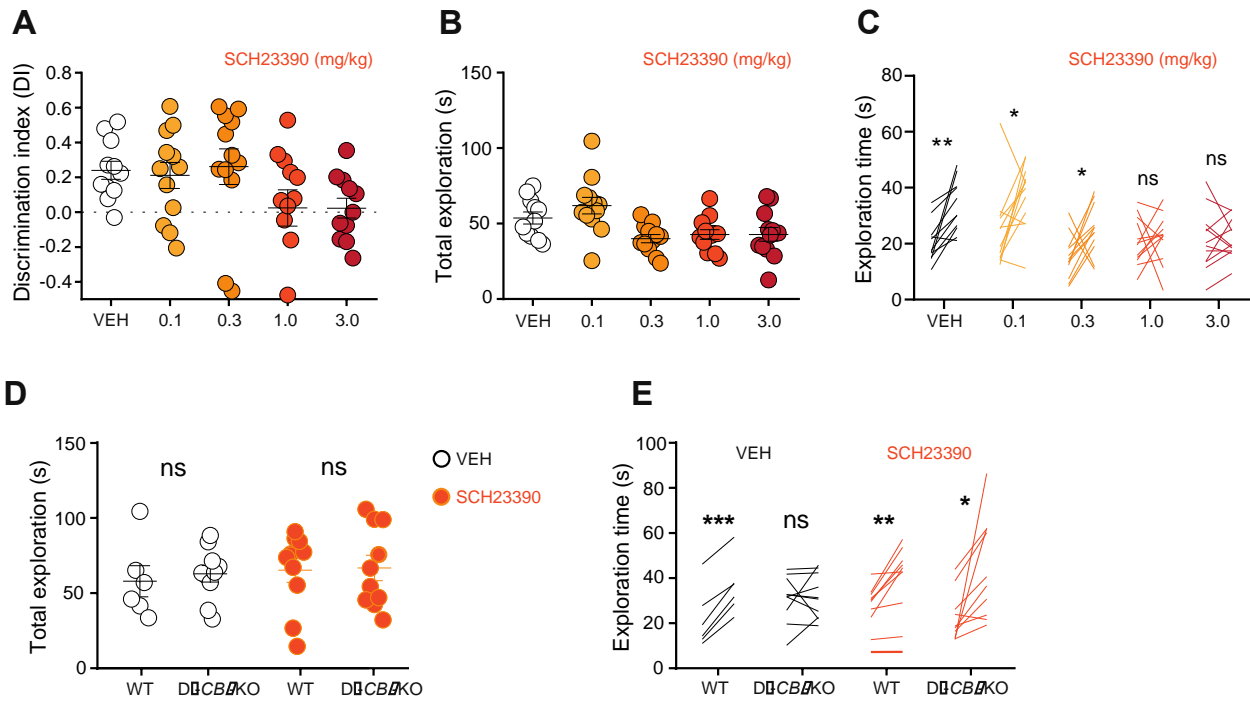


FIGURE S3. RELATED TO FIGURE 4

(A) Memory performance, (B) Total exploration time, and (C) Exploration time of the familiar versus the novel object of C57BL6/NRj mice injected with different doses of SCH23390 (in mg/kg, IP). (D) Total exploration, and (E) exploration time of the familiar versus the novel object time of D₁-CB₁-KO mice and WT littermates with systemic injections of SCH23390 (0.3 mg/kg, IP). See also Table S1.

Table S1

Figure	Panel	Conditions	"n"	Analysis (post-hoc test reported)	Factors analyzed	F-ratios	P values
1	B	D1-CB1-WT vs D1-CB1-KO	10, 7	Unpaired t-test	-	-	0,708
	C	D1-CB1-WT vs D1-CB1-KO	9, 8	Unpaired t-test	-	-	< 0,0001
	E	D1-CB1-WT(Control) vs D1-CB1-KO (Control)	17, 5	Unpaired t-test	-	-	0,0271
	E	D1-CB1-KO: (Control) vs (R-CB1-STR) vs (R-CB1-HPC)	5, 6, 9	One-way ANOVA (Dunnet)	-	-	0,0133
	I	" VEH vs CNO": "Control", D1-CB1-KO-DREADD-Gi", " D1-CB1-KO-mCherry"	(16, 14) (11, 14) (6, 7)	2-WAY ANOVA (Sidak)	Treatment vs Genotype	Interaction F (2, 62) = 5,107 Genotype F (2, 62) = 7,266 Treatment F (1, 62) = 4,587	0,0088 0,0015 0,0362
2	B	D1-CB1-WT vs D1-CB1-KO - 30 min	8, 8	Unpaired t-test	-	-	0,9444
	B	D1-CB1-WT vs D1-CB1-KO - 60 min	8, 8	Unpaired t-test	-	-	0,4546
	E	D1-CB1-WT vs D1-CB1-KO - 30 min	8, 11	Unpaired t-test	-	-	0,0455
	E	D1-CB1-WT vs D1-CB1-KO - 60 min	8, 11	Unpaired t-test	-	-	0,0253
	G	D1-CB1-WT vs D1-CB1-KO - 30 min	10, 10	Unpaired t-test	-	-	0,0266
G	D1-CB1-WT vs D1-CB1-KO - 60 min	10, 10	Unpaired t-test	-	-	0,0306	
3	A	" D1-CB1-WT vs D1-CB1-KO": "VEH" / "MK-801" / "NBQX" / "BIC" / "BIC 1h"	(14, 14) (7, 7) (8, 5) (10, 10) (10, 8)	2-WAY ANOVA (Sidak)	Treatment vs Genotype	Interaction F (4, 83) = 2,811 Treatment F (4, 83) = 6,787 Genotype F (1, 83) = 10,17	0,0003 0,0001 0,0021
	F	S Oriens: D1-CRE vs D1-CB1-KO	(3, 4)	Unpaired t-test	-	-	0,0453
	F	S Pyramidale: D1-CRE vs D1-CB1-KO	(3, 4)	Unpaired t-test	-	-	0,0059
	F	S Radiatum / L. Moleculare: D1-CRE vs D1-CB1-KO	(3, 4)	Unpaired t-test	-	-	0,0337
4	B	" D1-CB1-WT vs D1-CB1-KO": "VEH" / "Bicuculline" / "SCH 23390" at 30 min	(6, 5)	2-WAY ANOVA (Sidak)	Genotype vs Treatment	Interaction F (2, 35) = 4,670	0,0159
			(8, 10)			Genotype F (1, 35) = 10,30	0,0028
			(6, 6)			Treatment F (2, 35) = 2,492	0,0973
	C	" D1-CB1-WT vs D1-CB1-KO": "VEH" / "Bicuculline" / "SCH 23390" at 60 min	(6, 5)	2-WAY ANOVA (Sidak)	Genotype vs Treatment	Interaction F (2, 35) = 4,232	0,0226
			(8, 10)			Treatment F (1, 35) = 6,108	0,0185
			(6, 6)			Genotype F (2, 35) = 2,014	0,1487
	D	" D1-CB1-WT vs D1-CB1-KO": "VEH"/"SCH 23390"	(6, 10)		Genotype vs Treatment	Interaction F (1, 32) = 10,15	0,0032
			(10, 10)			Treatment F (1, 32) = 0,9844	0,3285
						Genotype F (1, 32) = 1,236	0,2746

TABLE S1. STATISTICAL ANALYSIS RELATED TO MAIN AND SUPPLEMENTARY FIGURES

PART 2 – LINKING MITOCHONDRIAL G-PROTEIN
SIGNALING TO CANNABINOIDS-INDUCED AMNESIA: A NEW
MITOCHONDRIA-SPECIFIC CHEMOGENETIC STRATEGY

Linking Mitochondrial G-protein Signaling to cannabinoids-induced amnesia: A new Mitochondria- specific chemogenetic Strategy

Gianluca Lavanco^{1,2,3 *}, Antonio C. Pagano Zottola^{1,2 *}, Yamuna Mariani^{1,2},
Astrid Cannich^{1,2}, Francisca Julio-Kalajzić^{1,2}, Filippo Drago³, Giovanni
Marsicano^{1,2, #}, Etienne Hebert-Chatelain[#] and Luigi Bellocchio^{1,2 #}

¹ INSERM U1215, NeuroCentre Magendie, Bordeaux, France | ² University of
Bordeaux, Bordeaux, France, ³ Dept. of Biomedical and Biotechnological Sciences,
Section of Pharmacology, University of Catania, Catania, Italy

*: share first authorship

#: share senior authorship

Until recently physiological and behavioral CB₁R-dependent effects had been exclusively attributed to canonical activation of plasma membrane CB₁Rs (Campbell, 2001; Athanasiou et al. 2007). With the identification of intracellular mitochondrial CB₁Rs in different tissues, in particular in the brain (Aquila et al, 2010; Benard et al, 2012; Hebert-Chatelain et al, 2014; Koch et al, 2015; Mendizabal-Zubiaga et al, 2016), this idea has been progressively changing.

Considering the brain as a high demander of energy support (Erecinska and Silver, 2001; Rolfe and Brown, 1997), the study of the impact of mitochondrial CB₁ receptor and its G-protein intra-mitochondrial signaling on cellular energy metabolism is becoming a highly interesting research field. So far, studies on the role of mitochondria on brain functions have led to understand their involvement in neuronal excitability and regulation of synaptic activity, beyond their central role in the ATP production, Ca²⁺ homeostasis, production of ROS, synthesis and metabolism of neurotransmitters and apoptosis (Turens, 2003; Ruggiero et al. 2021; Bock and Tait, 2019). However, the consequences of brain mitochondrial modulation via factivation of the intra-mitochondrial G-protein signaling and its physiological and behavioral effects are far from being completely understood.

In the last years, some studies have shown that slight alterations of mitochondrial functions through mtCB₁ receptors modulations could have an impact on energy metabolism and memory functions and behavior (Benard et al. 2012; Hebert-Chatelain et al. 2016; Jimenez-Blasco et al. 2020; Soria-Gomez et al. 2021). Indeed, the particular activation of this subcellular pool of the receptor results in mitochondrial inhibition and decrease of synaptic transmission, providing the physiological bases for cannabinoid-induced behavioral alterations such as memory and motor impairment (Hebert-Chatelain et al. 2016; Soria-Gomez et al. 2021).

Thus, our work aims at investigating how the mitochondrial G-protein signaling may represent a regulator of cellular functions and thus analyzing the effects of its modulation. Indeed, we aim at understanding how playing with mitochondrial G-protein signaling can affect the bioenergetic processes and behavior, in particular focusing on hippocampal neurons and the amnesic effects of cannabinoids.

RESULTS

As shown in Oliveira da Cruz et al. (2020), mutant mice bearing a deletion of the CB₁R gene in cells expressing D₁R (D₁-CB₁-knockout [KO] mice) displayed an impaired phenotype in long-term memory (24h) in NOR which was shown to be hippocampus-dependent.

To understand whether and how mtCB₁ receptor in D₁-positive cells can contribute to memory impairment we infused a viral vector leading to the Cre-dependent re-expression of the CB₁ receptor (AAV-CAG-Flexx-CB₁) or the mutant form DN22-CB₁ (which is excluded from mitochondria without losing functionality [Hebert-Chatelain et al. 2016], AAV-CAG-Flexx-DN22-CB₁) or control (AAV-CAG-Flexx) in the hippocampus of mutant mice lacking CB₁ receptors in D₁-positive cells (D₁-CB₁-KO mice) in order to obtain a re-expression of total CB₁Rs or pmCB₁ respectively, exclusively in cells where Cre is present (hereafter called D₁R-positive) in this brain region (**Fig. 1A,B**). As expected, D₁-CB₁-KO mice injected with AAV-CAG-Flexx showed strong memory impairment in long-term memory performance as compared to their wild-type littermates (**Fig. 1C**), with no changes in total exploration time (**Fig. 1D**). Re-expression of CB₁R in D₁-CB₁-KO mice was able to rescue the memory performance of the mutant mice (**Fig. 1C**) and strikingly, also the infusion of AAV-CAG-Flexx-DN22-CB₁ in the hippocampus of D₁-CB₁-KO mice fully rescued the NOR memory impairment (**Fig. 1C**), indicating that mtCB₁ receptors in D₁-positive cells is not required for normal long-term memory formation as well as the absence of mtCB₁ receptors in the same cells did not impair memory functions in NOR task (**Fig. 1C**).

As previously showed not only from our team, THC treatment after training induced strong memory impairment in the NOR test via CB₁ receptor. Rescue of CB₁ receptor in D₁-CB₁-KO mice resulted in amnesia when mice were treated with THC (**Fig. 1C**), as also shown for control wild type mice (**Fig. 1C**). Interestingly, rescue of DN22-CB₁ was not able to produce amnesia after THC treatment despite recovery of normal memory performance (**Fig. 1C**).

Therefore, our results showed that appropriate memory performance in the NOR require CB₁ receptors in D₁R-positive hippocampal neurons. Amongst these receptors the one associated to mitochondria-are the ones responsible for THC induced memory impairment, highlighting the difference between endogenous system activation and pharmacological stimulation.

The primary function of CB₁R activation in mitochondria by exogenous cannabinoids is to decrease

mitochondrial oxygen consumption with negative effects on cellular respiration, synaptic transmission, and behavior (Benard et al. 2012; Hebert-Chatelain et al. 2016; Soria-Gomez et al. 2021). Furthermore, we showed that the activation of the $G_{i\alpha}$ protein of $mtCB_1$ receptors is relevant for amnesic effects of cannabinoids presumably through inhibition of sAC and intra-mitochondrial PKA signaling (Hebert-Chatelain et al. 2016).

Thus, we reasoned that the activation of mitochondrial Gs-protein signalling, possibly by increasing mitochondrial respiration with a mechanism that counteract $mtCB_1$ activation, should be able to rescue the THC-induced memory impairment during NOR consolidation in D_1R -positive neurons.

For this purpose, we took advantage of the DREADDs technology (Robinson and Adelman, 2015) and developed a new mitochondria-specific Gs-coupled designer receptor. Indeed, we created a mito-DREADD by fusing the Gs-coupled designer receptor exclusively activated by designer drugs (DREADD-Gs receptor; CNO, clozapine N-oxide, ligand) to a mitochondria leader sequence (MLS) (MLS-DREADD) (**Fig. 2A**). In this way we aimed at obtaining a mutant Gs coupled receptor which can be targeted to mitochondria, expressed in given cell types and, when activated by a specific ligand (CNO), triggering Gs signaling in mitochondria.

Immunodetection of HA-tag and mitochondrial protein TOM20, in HEK293 cells expressing control vector, native DREADD (used as control) or MLS-DREADD, by confocal microscopy revealed that DREADD spreads throughout the cell, whereas MLS-DREADD-HA is specifically addressed to mitochondria (**Fig. 2B,C**) indicating the successful targeting of DREADD to this intracellular compartment.

One useful readout of Gs activation at plasma membrane level is the phosphorylation of extracellular signal regulated kinases (ERKs) (Goldsmith and Dhanasekaran, 2007). The activation of Gs-protein by CNO was able to strongly activate ERK phosphorylation in DREADD-transfected cells. Interestingly MLS-DREADD-transfected cells did not increase the phosphorylation of ERK upon CNO treatment, further suggesting the intracellular (mitochondrial) targeting of MLS-DREADD. (**Fig. 2D,E**).

The functional impact of DREADD and MLS-DREADD on mitochondrial activity was studied by mitochondrial respiration assays. MLS-DREADD activation was able to increase mitochondrial oxygen consumption rate both whole HEK293 cells and isolated mitochondrias from the same cell type. This effect was strongly specific to mitochondrial Gs activation since not present in control or

DREADD-Gs transfected cells (**Fig. 3A,B**). Similarly, when MLS-DREADD-Gs and DREADD-Gs were virally expressed in mouse hippocampi the mitochondrial respiration was increased by CNO only in hippocampal homogenates from MLS-DREADD-Gs injected mice (**Fig.3D**).

Moreover, the mitochondrial membrane potential, an important parameter of OXPHOS status, was increased by CNO administration in HEK293 cells expressing MLS-DREADD (and not native DREADD-Gs) suggesting that a higher activation of OXPHOS can be induced via specific modification of mitochondrial G-proteins activity (**Fig. 3C**) together with increased mitochondrial activity.

To understand how the modulation of the mitochondrial signaling can be specifically involved in cannabinoids-induced memory impairment, we expressed DREADD-Gs or MLS-DREADD-Gs in the hippocampal D₁-positive cells, by using CRE-dependent viral vector in D₁-Cre mice. Post-training clozapine N-oxide (CNO) injections did not affect the NOR performance of C57Bl/6N and does not rescue the amnesic effect induced by THC, indicating that the drug or its metabolites have no effect per se (Gomez et al., 2017; **Fig. 4A-C**). Similarly, post-training CNO treatment does not rescue the NOR impairment induced by THC of D₁-Cre mice expressing DREADD-Gs (**Fig. 5A-D**), indicating that plasma membrane Gs activation in D₁-positive neurons is not sufficient for memory rescue. Conversely, post-training CNO treatment fully rescue the NOR impairment induced by THC in D₁-Cre mice expressing MLS-DREADD-Gs, revealing that the specific activation of mitochondrial signaling in D₁-positive neurons is necessary for rescue of THC-induced amnesia during the consolidation process in NOR (**Fig. 6A-D**).

Altogether, the results obtained *in vivo* by using MLS-DREADD, confirm an impairment of mitochondrial activity in D₁-positive hippocampal neurons as the leading mechanism for THC-induced amnesia, further strengthening the link between brain bio-energetic status and behavioral processes.

AUTHOR CONTRIBUTIONS

GL performed and analyzed *in vivo* characterization and behavioral experiments and participated to the manuscript writing. ACPZ performed and analyzed *in vitro* characterization experiments and participated to the manuscript writing. GM, LB and EH-C conceived and supervised the whole project and wrote the manuscript. All authors edited and approved the manuscript.

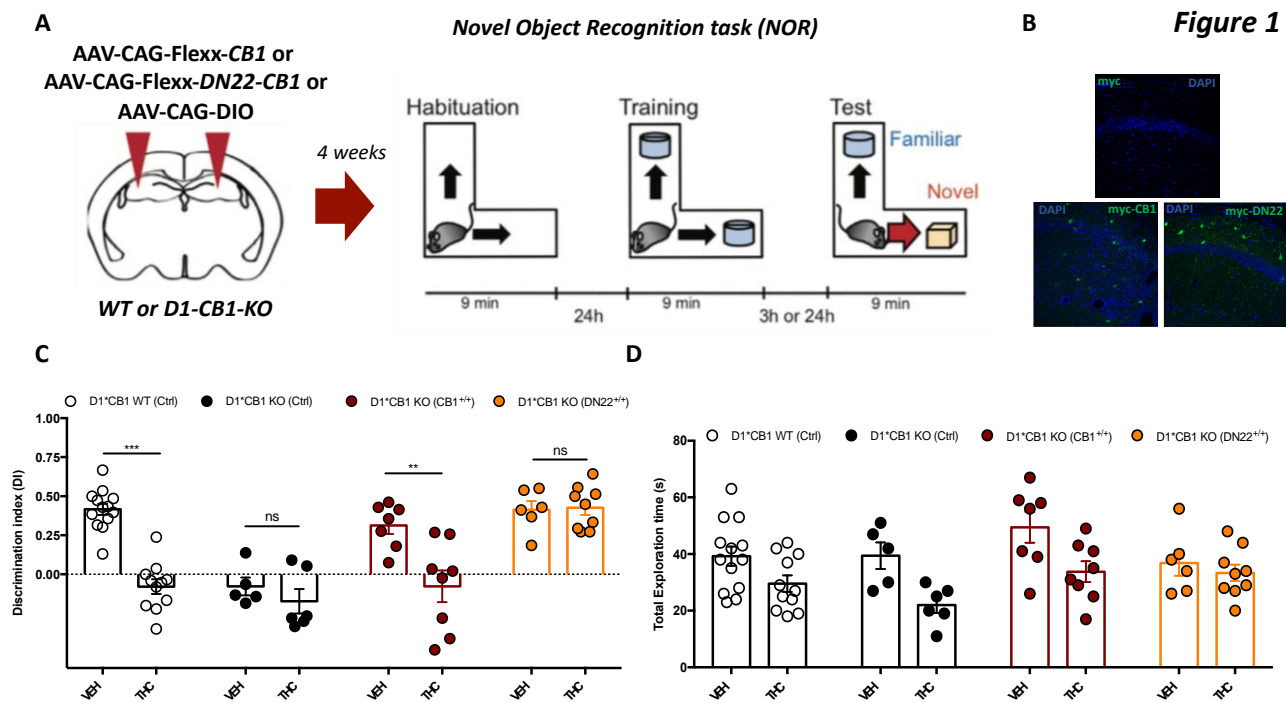


FIGURE 1. HIPPOCAMPAL mtCB₁RS IN D1R-POSITIVE CELLS ARE REQUIRED FOR THC-INDUCED MEMORY IMPAIRMENT OF NOR

(A) Schematic representation of the NOR memory task. Schematic representation of the experiment using viral re-expression of the CB₁R or DN22-CB₁ in the hippocampus of D₁-CB₁-WT mice and D₁-CB₁-KO littermates. VEH or THC (5 mg/kg) injections take place after the training phase of the NOR task. (B) Immunofluorescence for myc-tag in order to detect CB₁ or DN22-CB₁. (C) NOR memory performance of D₁-CB₁-WT (n VEH = 13, n THC = 11) and D₁-CB₁-KO mice injected intra-hippocampally with control virus (n VEH = 5, n THC = 6), virus re-expressing CB₁Rs (n VEH = 7, n THC = 8), virus re-expressing DN22-CB₁ (n VEH = 6, n THC = 9). (D) Total object exploration during the testing phase for all experimental groups. Data, mean ± SEM. *p < 0.05, ***p < 0.001, ****p < 0.0001. ns, not significant.

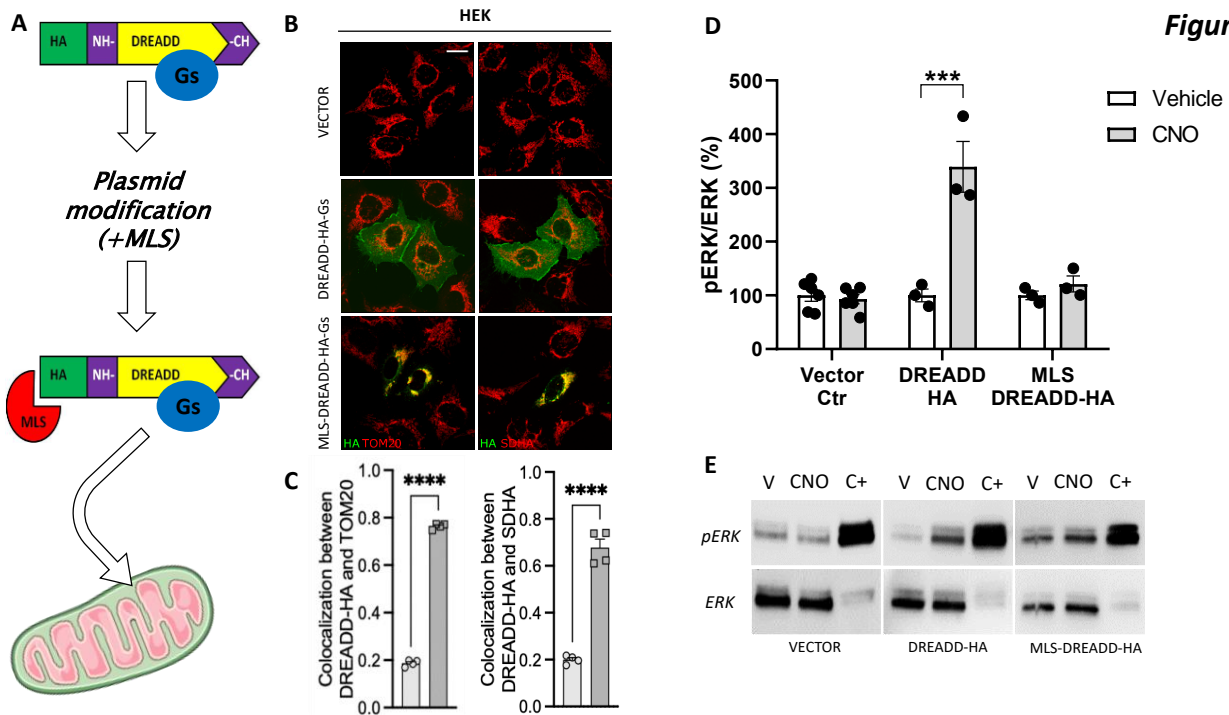


FIGURE 2. CREATION AND CHARACTERIZATION OF MITOCHONDRIA LEADER SEQUENCE DREADD-HA (MLS-DREADD-HA). (A) Cloning strategy to express DREADD-HA at mitochondria. (B) Representative immunofluorescence of HEK cells transfected with empty vector or DREADD-HA or MLS-DREADD-HA expressing plasmid. Green, HA tag staining; red, mitochondrial staining TOM20 or SDHA, succinate dehydrogenase A. (C) Quantification of (MLS-)DREADD-HA expressing plasmids and TOM20 or SDHA colocalization. (D) Effect of CNO (50 μ M) on ERK phosphorylation in HEK293 cells transfected with empty vector, DREADD-HA or MLS-DREADD-HA-expressing plasmids. FBS was used as positive control (C+). (E) Representative immunoblot showing the effect of CNO (50 μ M) on pERK and ERK levels in HEK293 cells transfected with with plasmids expressing empty vector, DREADD-Gs or MLS-DREADD-Gs.

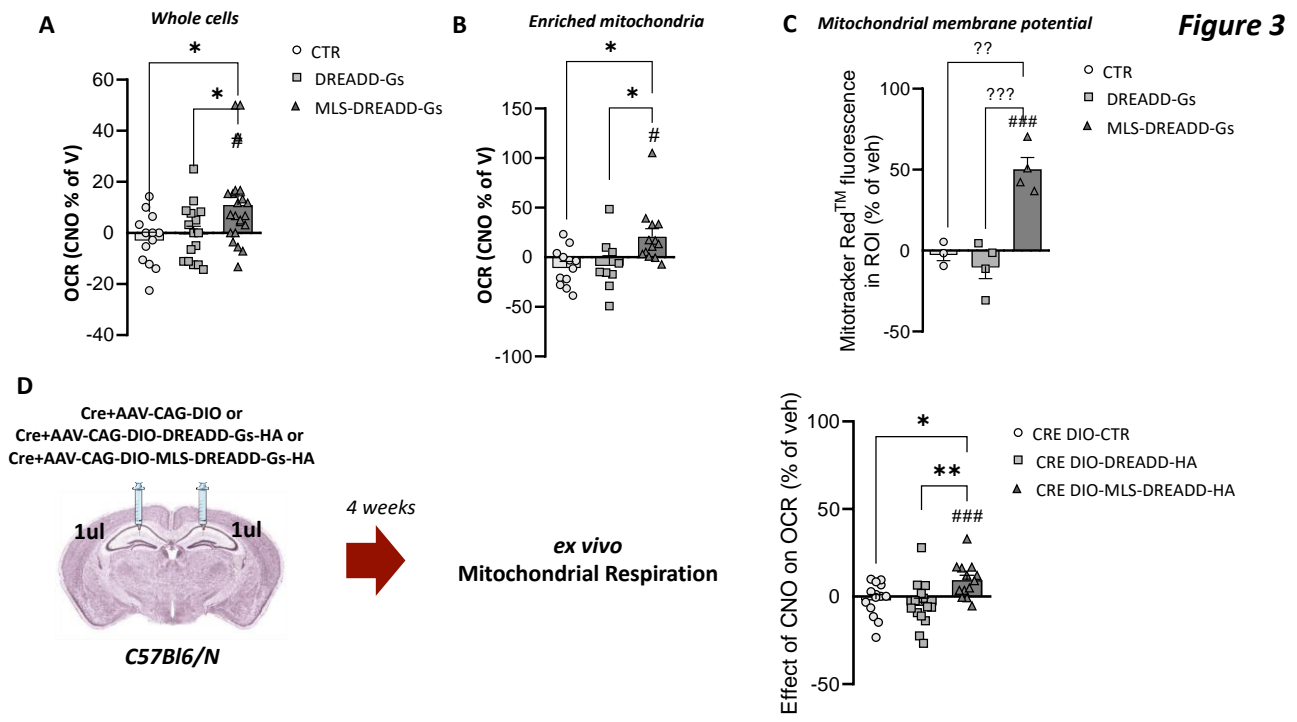


FIGURE 3. THE ACTIVATION OF THE MITOCHONDRIAL G-PROTEIN SIGNALING via MLS-DREADD-HA INCREASES MITOCHONDRIAL RESPIRATION AND MEMBRANE POTENTIAL. (A-B) Effect of CNO (50 μ M) on oxygen consumption rate (OCR) in HEK293 whole cells (A) or enriched mitochondrial fraction (B) transfected with control (Ctr) or DREADD-HA or MLS-DREADD-HA plasmid. (C) MitoTracker Red™ fluorescence in HEK293 cells expressing empty vector (CTR), DREADD-HA or MLS-DREADD-HA. (D) The effect of CNO (50 μ M) on mitochondrial respiration of homogenized hippocampi from C57Bl/6N mice that received intrahippocampal injection of AAV-Cre and Cre-dependent AAV-DIO-DREADD-HA or AAV-MLS-DREADD-HA or AAV-DIO-CTRL.

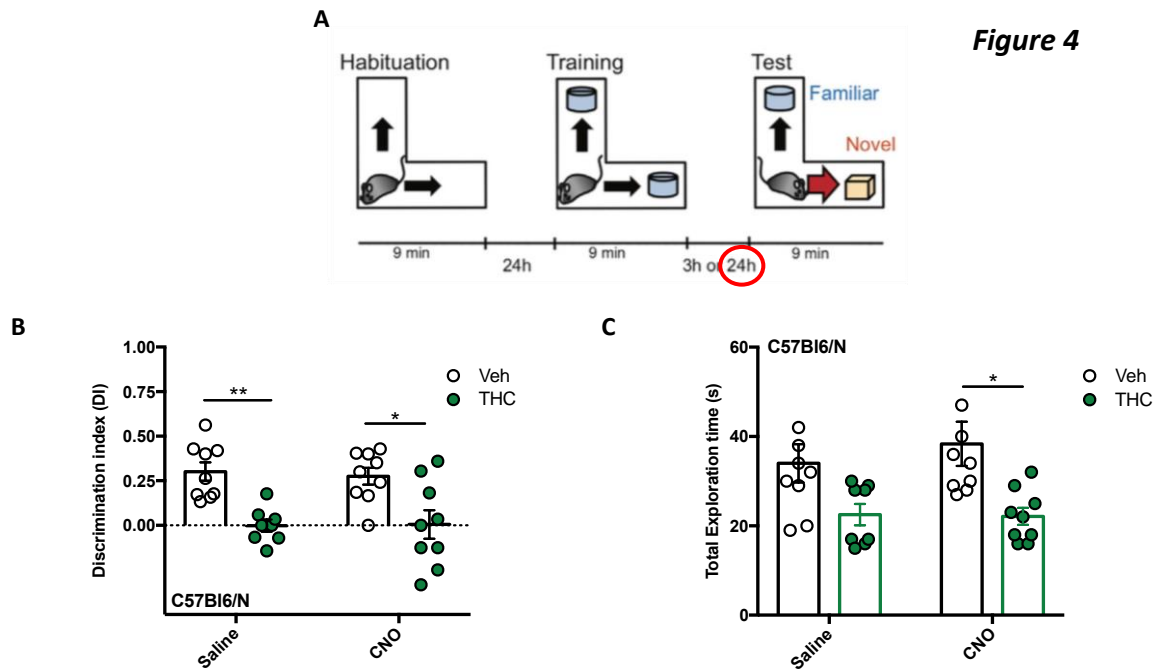


FIGURE 4. NO ALTERATIONS ON MEMORY PERFORMANCE AND THC-INDUCED AMENSIC EFFECT BY CLOZAPINE N-OXIDE (CNO)

(A) Schematic representation of the NOR memory task with treatment after training. (B) NOR memory performance of C57Bl/6N mice. Clozapine N-oxide (CNO; 5 mg/kg) injections take place after the training phase of the NOR task and THC (5 mg/kg) 15 minutes after CNO injection. (B) NOR memory performance of C57Bl/6N mice (n VEH/VEH = 9, n VEH/THC = 8, n CNO/VEH = 9, n CNO/THC = 9). (C) Total object exploration during the testing phase for all treatment groups.

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

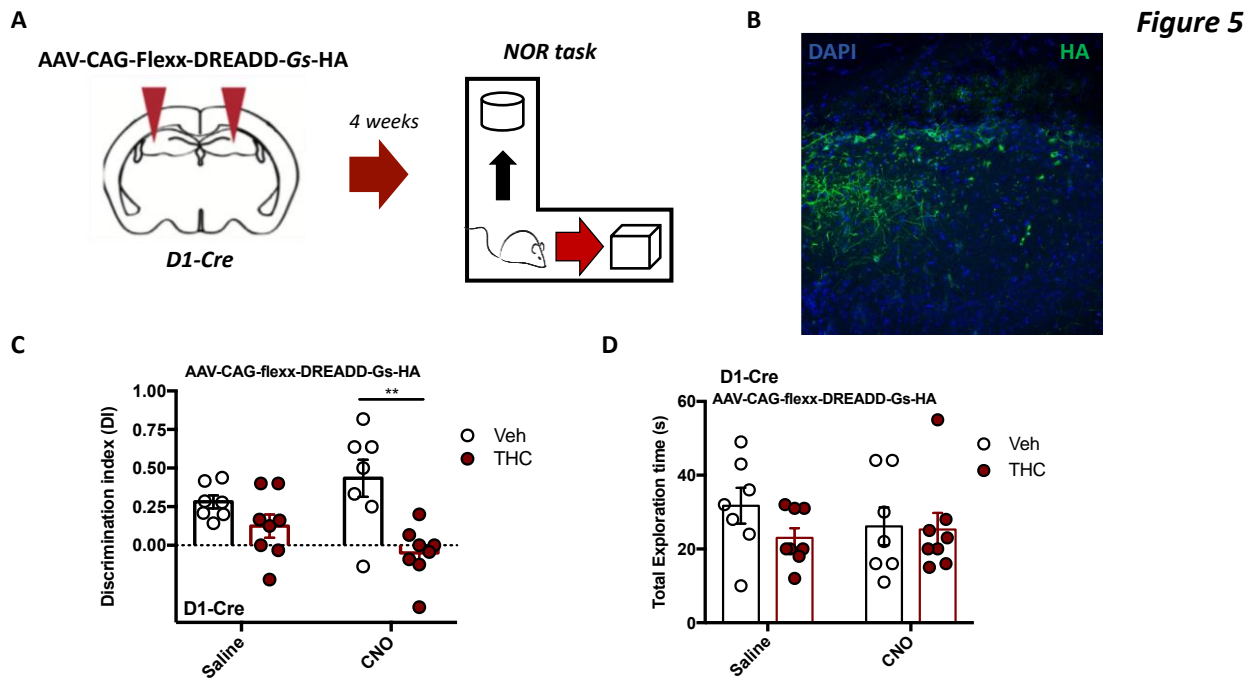


Figure 5

FIGURE 5. THE ACTIVATION OF G_s PROTEIN IN D₁-POSITIVE NEURONS IS NOT ABLE TO RESCUE THE THC-INDUCED AMNESIC EFFECT

(A) Schematic representation of the experiment using viral expression of the DREADD-Gs in the hippocampus of D₁-Cre mice. Clozapine N-oxide (CNO; 5 mg/kg) injections take place after the training phase of the NOR task and THC (5 mg/kg) 15 minutes after CNO injection. (B) Immunofluorescence for HA-tag in order to detect DREADD-Gs. (C) NOR memory performance of D₁-Cre mice injected intra-hippocampally with DREADD-Gs virus (n VEH/VEH = 8, n VEH/THC = 8, n CNO/VEH = 7, n CNO/THC = 8). (D) Total object exploration during the testing phase for all treatment groups. Data, mean ± SEM. *p < 0.05, ***p < 0.001, ****p < 0.0001. ns, not significant.

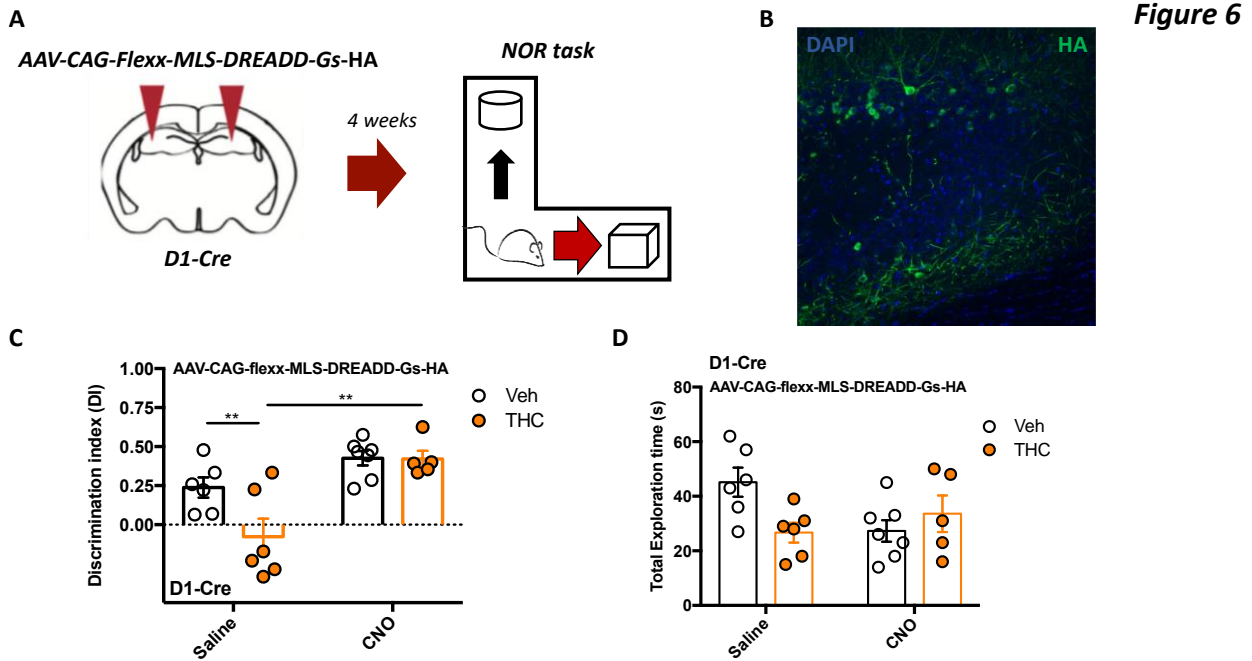


Figure 6

FIGURE 6. THE SPECIFIC ACTIVATION OF G_s PROTEIN IN MITOCHONDRIA OF D₁-POSITIVE NEURONS IS ABLE TO RESCUE THE THC-INDUCED AMNESIC EFFECT

(A) Schematic representation of the experiment using viral expression of the MLS-DREADD-Gs in the hippocampus of D₁-Cre mice. Clozapine N-oxide (CNO; 5 mg/kg) injections take place after the training phase of the NOR task and THC (5 mg/kg) 15 minutes after CNO injection. (B) Immunofluorescence for HA-tag in order to detect MLS-DREADD-Gs. (C) NOR memory performance of D₁-Cre mice injected intra-hippocampally with MLS-DREADD-Gs virus (n VEH/VEH = 6, n VEH/THC = 6, n CNO/VEH = 7, n CNO/THC = 5). (D) Total object exploration during the testing phase for all treatment groups. Data, mean ± SEM. *p < 0.05, ***p < 0.001, ****p < 0.0001. ns, not significant.

REFERENCES

Aquila S, Guido C, Santoro A, et al. (2010) Rimonabant (SR141716) induces metabolism and acquisition of fertilizing ability in human sperm. *Br J Pharmacol.*; 159(4): 831–41.

Athanasiou, A., Clarke, A.B., Turner, A.E., Kumaran, N.M., Vakilpour, S., Smith, P.A., Bagiokou, D., Bradshaw, T.D., Westwell, A.D., Fang, L., et al. (2007). Cannabinoid receptor agonists are mitochondrial inhibitors: a unified hypothesis of how cannabinoids modulate mitochondrial function and induce cell death. *Biochemical and biophysical research communications* 364, 131-137.

Benard, G., Massa, F., Puente, N., Lourenco, J., Bellocchio, L., Soria-Gomez, E., Matias, I., Delamarre, A., Metna-Laurent, M., Cannich, A., et al. (2012). Mitochondrial CB(1) receptors regulate neuronal energy metabolism. *Nature neuroscience* 15, 558- 564.

Bock FJ, Tait SWG. (2020) Mitochondria as multifaceted regulators of cell death. *Nat Rev Mol Cell Biol.*; 21(2):85-100. doi: 10.1038/s41580-019-0173-8. Epub 2019 Oct 21. PMID: 31636403.

Busquets-Garcia, A., Gomis-Gonzalez, M., Guegan, T., Agustín-Pavón, C., Pastor, A., Mato, S., Pérez-Samartín, A., Matute, C., de la Torre, R., Dierssen, M., et al. (2013). Targeting the endocannabinoid system in the treatment of fragile X syndrome. *Nat. Med.* 19, 603–607.

Busquets-Garcia, A., Puighermanal, E., Pastor, A., de la Torre, R., Maldonado, R., and Ozaita, A. (2011). Differential role of anandamide and 2-arachidonoyl-glycerol in memory and anxiety-like responses. *Biol. Psychiatry* 70, 479–486.

Campbell, V.A. (2001). Tetrahydrocannabinol-induced apoptosis of cultured cortical neurones is associated with cytochrome c release and caspase-3 activation. *Neuropharmacology* 40, 702-709.

Di Benedetto G, Scalzotto E, Mongillo M, Pozzan T. (2013). Mitochondrial Ca²⁺ uptake induces cyclic AMP generation in the matrix and modulates organelle ATP levels. *Cell Metab.* 2013 Jun 4;17(6):965-975. doi: 10.1016/j.cmet.2013.05.003. PMID: 23747252.

Erecinska, M., and Silver, I.A. (2001). Tissue oxygen tension and brain sensitivity to hypoxia. *Respiration physiology* 128, 263-276.

Goldsmith, Z., Dhanasekaran, D. G. (2007). Protein regulation of MAPK networks. *Oncogene* 26, 3122–3142.

Gomez, J.L., Bonaventura, J., Lesniak, W., Mathews, W.B., Sysa-Shah, P., Rodriguez, L.A., Ellis, R.J., Richie, C.T., Harvey, B.K., Dannals, R.F., et al. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science* 357, 503–507.

Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., Koehl, M., Abrous, D. N., Mendizabal-Zubiaga, J., Grandes, P., Liu, Q., Bai, G., Wang, W., Xiong, L., Ren, W., Marsicano, G. & Zhang, X. 2012. Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell*, 148, 1039-50.

Hebert-Chatelain E, Desprez T, Serrat R, Bellocchio L, Soria-Gomez E, Busquets-Garcia A, Pagano Zottola AC, Delamarre A, Cannich A, Vincent P et al. (2016) A cannabinoid link between mitochondria and memory. *Nature* 539, 555–559.

Jimenez-Blasco D, Busquets-Garcia A, Hebert-Chatelain E, Serrat R, Vicente-Gutierrez C, Ioannidou C, Gomez-Sotres P, Lopez-Fabuel I, Resch-Beusher M, Resel E et al. (2020) Glucose metabolism links astroglial mitochondria to cannabinoid effects. *Nature* 583, 603–608.

Koch M, Varela L, Kim JG, et al. (2015) Hypothalamic POMC neurons promote cannabinoid-induced feeding. *Nature.*; 519(7541): 45–50

Lemberger, T., Parlato, R., Dassel, D., Westphal, M., Casanova, E., Turiault, M., Tronche, F., Schiffmann, S.N., and Schütz, G. (2007). Expression of Cre recombinase in dopaminergic neurons. *BMC Neurosci.* 8, 4.

Makrecka-Kuka, M., Krumschnabel, G., and Gnaiger, E. (2015). High-Resolution Respirometry for Simultaneous Measurement of Oxygen and Hydrogen Peroxide Fluxes in Permeabilized Cells, Tissue Homogenate and Isolated Mitochondria. *Biomolecules* 5, 1319–1338.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutiérrez, S.O., van der Stelt, M., et al. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302, 84–88.

Mendizabal-Zubiaga J, Melser S, Bénard G, Ramos A, Reguero L, Arrabal S, Elezgarai I, Gerrikagoitia I, Suarez J, Rodríguez De Fonseca F, Puente N, Marsicano G, Grandes P. (2016) Cannabinoid CB1 Receptors Are Localized in Striated Muscle Mitochondria and Regulate Mitochondrial Respiration. *Front Physiol.*; 7:476. doi: 10.3389/fphys.2016.00476. PMID: 27826249; PMCID: PMC5078489.

Monory, K., Blaudzun, H., Massa, F., Kaiser, N., Lemberger, T., Schütz, G., Wotjak, C.T., Lutz, B., and Marsicano, G. (2007). Genetic dissection of behavioural and autonomic effects of Delta(9)-tetrahydrocannabinol in mice. *PLoS Biol.* 5, e269.

Oliveira da Cruz JF, Busquets-Garcia A, Zhao Z, Varilh M, Lavanco G, Bellocchio L, Robin L, Cannich A, Julio-Kalajzić F, Lesté-Lasserre T, Maître M, Drago F, Marsicano G, Soria-Gómez E. (2020) Specific Hippocampal Interneurons Shape Consolidation of Recognition Memory. *Cell Rep.*; 32(7):108046. doi: 10.1016/j.celrep.2020.108046. PMID: 32814049; PMCID: PMC7443618.

Oliveira da Cruz, J. F., Gomis-Gonzalez, M., Maldonado, R., Marsicano, G., Ozaita, A. and Busquets-Garcia, A. (2020). An Alternative Maze to Assess Novel Object Recognition in Mice. *Bio-protocol* 10(12): e3651. DOI: 10.21769/BioProtoc.3651.

Puighermanal, E., Busquets-Garcia, A., Gomis-Gonzalez, M., Marsicano, G., Maldonado, R., and Ozaita, A. (2013). Dissociation of the pharmacological effects of THC by mTOR blockade. *Neuropsychopharmacology* 38, 1334–1343.

Puighermanal, E., Marsicano, G., Busquets-Garcia, A., Lutz, B., Maldonado, R., and Ozaita, A. (2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nat. Neurosci.* 12, 1152–1158.

Robin, L.M., Oliveira da Cruz, J.F., Langlais, V.C., Martin-Fernandez, M., Metna-Laurent, M., Busquets-Garcia, A., Bellocchio, L., Soria-Gomez, E., Papouin, T., Varilh, M., et al. (2018). Astroglial CB1 Receptors Determine Synaptic D-Serine Availability to Enable Recognition Memory. *Neuron* 98, 935–944.e5.

Robinson, S., and Adelman, J.S. (2015). A Method for Remotely Silencing Neural Activity in Rodents During Discrete Phases of Learning. *J. Vis. Exp.* (100), e52859.

Rolfe, D.F., and Brown, G.C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiological reviews* 77, 731-758.

Ruggiero A, Katsenelson M, Slutsky I. (2021) Mitochondria: new players in homeostatic regulation of firing rate set points. *Trends Neurosci.*; 44(8):605-618. doi: 10.1016/j.tins.2021.03.002. Epub 2021 Apr 14. PMID: 33865626.

Soria-Gomez E, Pagano Zottola AC, Mariani Y, Desprez T, Barresi M, Bonilla-Del Río I, Muguruza C, Le Bon-Jego M, Julio-Kalajzić F, Flynn R et al. (2021) Subcellular specificity of cannabinoid effects in striatonigral circuits. *Neuron*; 109(9):1513-1526.e11. doi: 10.1016/j.neuron.2021.03.007. Epub 2021 Mar 25. PMID: 33770505.

Terzian, A.L., Drago, F., Wotjak, C.T., and Micale, V. (2011). The Dopamine and Cannabinoid Interaction in the Modulation of Emotions and Cognition: Assessing the Role of Cannabinoid CB1 Receptor in Neurons Expressing Dopamine D1 Receptors. *Front. Behav. Neurosci.* 5, 49.

Turrens, J.F. (2003). Mitochondrial formation of reactive oxygen species. *The Journal of physiology* 552, 335-344.

Vallée, M., Vitiello, S., Bellocchio, L., Hébert-Chatelain, E., Monlezun, S., Martin-Garcia, E., Kasanetz, F., Baillie, G.L., Panin, F., Cathala, A., et al. (2014). Pregnenolone can protect the brain from cannabis intoxication. *Science* 343, 94–98.

Wise, L. E., Thorpe, A. J., & Lichtman, A. H. (2009). Hippocampal CB(1) receptors mediate the memory impairing effects of Delta(9)-tetrahydrocannabinol. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology*, 34(9), 2072–2080. <https://doi.org/10.1038/npp.2009.31>.

SUPPLEMENTARY METHODS

Mice

All experimental procedures were approved by the ethical committee of the French Ministry of Higher Education, Research and Innovation. Maximal efforts were made to reduce the suffering of the animals. Male mice were used in this study.

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

Plasmids

pcDNA-DREADD-Gs (fused to HA-tag) was kindly provided by Brian Roth (UNC). pcDNA-MLS-DREADD-Gs (fused to HA-tag) was obtained by cloning the coding sequence of DREADD-Gs in frame with 4× repetition of the mitochondrial leader sequence (aa. 1-30) of human COX-VIII (Di Benedetto et al. 2013). PcDNA based vectors were used for HEK293T expression as well as donor inserts for AAV generation (see after). HA tag was kept in both constructs for in vitro and in vivo immune-detection.

Cell lines

Certified HEK293T (ATCC, CRL-3216TM, lot 62729596) cells were cultured in DMEM Glutamax, supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 mg/mL streptomycin (except for Oxygen consumption measurements on cells), nonessential amino acids (0.1 mM of Glycine, L-Alanine, L-Asparagine, L-Aspartic acid, L-Glutamic Acid, L-Proline, and L-Serine), 10 mM HEPES (Thermo Fisher Scientific, Waltham, MA) and maintained at 37C in the presence of 5% CO₂. 24H after the seeding, cells were transfected with control plasmid or (MLS-) DREADD-HA-Gs plasmid using polyethylenimine (Polysciences, Warrington, PA).

Drugs

THC was obtained from THC Pharm GmbH (Frankfurt, Germany). For in vivo administration, THC (5 mg/Kg) was prepared freshly before the experiments and was dissolved in a mixture of 5% ethanol, 4% cremophor and saline. Corresponding vehicle solutions were used in control experiments. The exogenous DREADD ligand clozapine-N-oxide (CNO, 5 mg/kg) was purchased from Tocris Bioscience (Bristol, UK) and dissolved in saline after gently mixing with a vortex. For in vitro experiment, CNO was dissolved in DMSO. All drugs were injected intraperitoneally in a volume of 10 ml/kg. Vehicle in all the conditions was composed of physiological saline (NaCl 0.9%) injections. Doses and concentrations of the different drugs were chosen on the basis of previous published data or preliminary experiments.

Viral vectors

To generate AAV-DIO-CB1 and AAV-DIO-DN22, AAV-Flexx-MLS-DREADD and AAV-Flexx-DREADD the coding sequences for the 4 proteins (Hebert-Chatelain et al., 2016) were subcloned in pAAV-CAG-flex plasmid (kindly gifted by Matthias Klugmann, UNSW, Australia) by using standard molecular cloning techniques. The same pAAV-CAG-flex plasmid was used as empty control (AAV-DIO-ctr). AAVs were generated by PEI transfection of HEK293T cells and purified by iodixanol-gradient ultracentrifugation as previously described (Hebert-Chatelain et al., 2016).

Virus titers were between 10¹⁰ and 10¹¹, expressed as genomic copies (GC) x ml.

Oxygen consumption measurements on tissue homogenates and enriched mitochondria

Hippocampus extracts: Preparation of hippocampus extracts was done as previously described (Hebert-Chatelain et al., 2016) with some modification. Briefly, C57Bl/6N mice that received intrahippocampal injection of AAV-Cre and Cre-dependent AAV-DIO-DREADD-HA or AAV-MLS-DREADD-HA or AAV-DIO-CTRL were sacrificed by cervical dislocation, the mouse brain was extracted and the hippocampus was rapidly dissected. Hippocampus was homogenized in 450 μ L of Mito5 buffer without taurine supplementation (Makrecka-Kuka et al., 2015) using a Politron homogenizer (11.000 rpm 3-5 s). After brief centrifugation at 4 C the supernatant was treated with saponin at a final concentration of 12.5 mg/ml.

Mitochondrial enrichment from HEK cells: HEK cells expressing DREADD-GS or MLS-DREADD-Gs or transfected with control plasmid were collected in cold Isolation buffer (70mM Sucrose, 210mM Mannitol, 1mM EDTA, 50mM Tris base, pH 7.4, supplemented with protease inhibitor (Sigma-aldrich, France)) and lysed by passing the cell suspension through a 25G needle. After a first centrifugation step at 1000g (5 min, 4C), the supernatant was collected and a second centrifugation at 6900g was carried out for 10 min at 4C. The pellet containing the mitochondrial fraction was resuspended in fresh isolation buffer and used for mitochondrial respiration

Respiration analyses were carried out using a 2K Oroboros device (Makrecka-Kuka et al., 2015). 30 μ L of sample were put in each chamber and complex I-dependent respiration was triggered by adding malate (2mM), pyruvate (5mM) and glutamate (10 mM) (MPG) (Makrecka-Kuka et al., 2015). Vehicle or CNO were added at final concentration of 50 μ M and 12 minutes after 6.25 μ M ADP was injected. Each measure of oxygen consumption rate (OCR) was expressed as ratio of ADP/MPG stimulation and the effect of CNO was calculated as percentage of vehicle conditions. Only samples for which the ratio of ADP/MPG in the vehicle was equal or superior to 1.5 were retained for the analyses.

Oxygen consumption measurements on cells

HEK cells were transfected with control plasmid or expressing DREADDs-Gs or MLS-DREADDs-Gs. Each cell suspension was divided in two and transferred into Oroboros chambers (Makrecka-Kuka et al., 2015) Routine respiration in culture medium was recorded until a stable signal was reached. CNO (50 μ M) or Vehicle were added directly into the chamber after 20 minutes, Rotenone (0.5 μ M)

and Antimycin A (2.5 μ M) were injected allowing to correct the mitochondrial respiration for residual oxygen consumption (Makrecka-Kuka et al., 2015).

Oxygen consumption rate (OCR) after CNO or Veh treatment was normalized on the values recorded before the injection. CNO effect on OCS was expressed as % of vehicle condition.

ERK phosphorylation assays

After the transfection with empty vector (CTRL), DREADD-HA or MLS-DREADD-HA plasmid, HEK cells were allowed to recover for 48 h. Cells were then starved overnight in serum-free DMEM before treatment with CNO (50 μ M) or vehicle or FBS (10% of final volume) for 30 min. The medium was rapidly aspirated and the samples were snap-frozen in liquid nitrogen and stored at -80°C before preparation for western blotting.

Protein extraction and western immunoblotting

For ERK-phosphorylation assays, lysis buffer (1 mM EGTA, 50 mM Tris pH 7.5, 0.1% triton X-100, 30 mM 2-mercaptoethanol) supplemented with protease and phosphatase inhibitors (Sigma-aldrich, France) was added, cells were collected by scraping and pelleted by centrifugation at 12,500g (4°C) for 5 min to remove cell debris. Protein concentrations were measured using the Roti-Nanoquant protein quantification assay, following manufacturer's instruction (Carl Roth, Karlsruhe, Germany), mixed with Laemmli buffer and kept at -20°C .

For western blotting, the proteins were separated on Tris-glycine 10% acrylamide gels and transferred to PVDF membranes. Membranes were soaked in 5% BSA in TBS-T (20 mM Tris-HCl pH 7.6, 150 mM NaCl, 0.05% Tween 20). Antibodies against pERK (phospho-p44/42 MAPK, #4370, Cell Signaling Technology, Danvers, MA) and ERK (p44/42 MAPK, #9102, Cell Signaling Technology Danvers, MA, USA) were used. Bound primary antibodies were detected with HRP-linked antibodies (Cell Signaling Technology, Danvers, MA, USA). HRP signal was revealed with Clarity Western ECL Substrate using ChemiDoc MP System (Bio-Rad, Hercules, CA, USA). Image processing was performed in ImageLab software. pERK levels were normalized to the amount of total ERK. CNO effect was expressed as percentage of vehicle effect.

Mitochondrial membrane potential

Mitochondrial membrane potential was evaluated using Mitotracker Red™ (Life Technologies, CA, USA). Briefly, cells were incubated with 150 nM Mitotracker Red™ during 30 min at 37 °C in 5% CO₂ and 95% humidity.

After incubation with probes, cells were rinsed and imaging was performed. Mitotracker Red™ fluorescence were examined using the EVOS FL Auto 2 imaging system (Thermo Fisher Scientific, MA, USA) with a ×40 objective (LPLAN 40×, 0.65NA, EVOS). For each independent experiment, total fluorescence intensity was quantified for 50 cells using ImageJ (NIH, MD, USA).

Novel object-recognition memory task

We used the novel object recognition (NOR) memory task in an L-maze (Busquets-Garcia et al., 2011, 2013; Hebert-Chatelain et al., 2016; Puighermanal et al., 2009, 2013; Robin et al., 2018) as described in detail in Oliveira da Cruz et al. (2020).

The task consisted in 3 sequential daily trials of 9 minutes each. During the habituation phase (day 1), mice were placed in the center of the maze and allowed to freely explore the arms in the absence of any objects. The training phase (day 2) consisted in placing the mice again in the corner of the maze in the presence of two identical objects positioned at the extremities of each arm and left to freely explore the maze and the objects. The testing phase occurred 24 hours later (day 3): one of the familiar objects was replaced by a novel object different in its shape, color and texture and mice were left to explore both objects. The position of the novel object and the associations of novel and familiar were randomized. All objects were previously tested to avoid biased preference. Memory performance was assessed by the discrimination index (DI). The DI was calculated as the difference between the time spent exploring the novel (TN) and the familiar object (TF) divided by the total exploration time (TN+TF): $DI = [TN-TF]/[TN+TF]$. Memory was also evaluated by directly comparing the exploration time of novel and familiar objects, respectively. Object exploration was defined as the orientation of the nose to the object at less than 2 cm.

Pharmacological treatments were immediately administered after the training phase.

Surgery and virus administration

Mice were anesthetized in a box containing 5% Isoflurane (Virbac, France) before being placed in a stereotaxic frame (Model 900, Kopf instruments, CA, USA) in which 1.5% to 2.0% of Isoflurane was continuously supplied via an anesthetic mask during the whole duration of the experiment. For viral intra-hippocampal AAV delivery, mice were submitted to stereotaxic surgery and AAV vectors were injected with the help of a microsyringe (0.25 mL Hamilton syringe with a 30-gauge beveled needle) attached to a pump (UMP3-1, World Precision Instruments, FL, USA). Where specified, D_1 -CB₁-WT and D_1 -CB₁-KO mice were injected directly into the hippocampus (HPC) (0.5 μ l per injection site at a rate of 0.5 μ l per min), with the following coordinates: HPC, AP -1.8; ML \pm 1; DV -2.0 and -1.5. Following virus delivery, the syringe was left in place for 1 minute before being slowly withdrawn from the brain. D_1 -CB₁-WT mice were injected with rAAV-CAG-DIO (empty control vector) and D_1 -CB₁-KO with rAAV-CAG-DIO (empty control vector), AAV-CAG-DIO-CB1 or AAV-CAG-DIO-DN22-CB1-myc to induce re-expression of the CB₁ receptor gene or DN22-CB₁ respectively in hippocampal D_1 -positive cells. The aforementioned rAAVs were generated as described in Hebert-Chatelain et al. (2016). In another experiment, and using the same procedure as described as above, D_1 -Cre mice were injected intra hippocampally (AP -1.8; ML \pm 1; DV -2.0 and -1.5), with pAAV-CAG-flexx-Gs-HA or pAAV-CAG-flexx-4MLS-Gs-HA (1 μ l per injection site at a rate of 0.5 μ l per min). For these experiments, animals were used around 4-5 weeks after local infusions. Mice were weighed daily and individuals that failed to regain the pre-surgery body weight were excluded from the following experiments. Mice that underwent behavioral experiments were fixed by transcardial perfusion of 4% pfa and their brain were processed for immunofluorescence (Hebert-Chatelain et al., 2016), with primary antibodies directed against myc-tag (#2276; Cell Signaling Technology), to detect CB1 or DN22-CB1 rescue or against HA-tag (#26183, Thermo Fisher Scientific, France) to confirm overexpression of DREADD-Gs-HA and MLS-DREADD-Gs-HA. Mice that did not fulfill histological positive criteria were excluded from the study.

Statistical analyses

Data were expressed as mean \pm SEM or single data points and were analyzed with Prism 8.0 (Graphpad Software), using t-test (unpaired), one-way ANOVA (post-hoc dunnett's test) or 2-way ANOVA (post-hoc Bonferroni's test).

SECTION IV — GENERAL DISCUSSION

PART 1 – SPECIFIC HIPPOCAMPAL INTERNEURONS SHAPE CONSOLIDATION OF RECOGNITION MEMORY

It is well known that the endocannabinoid system is present in different cell types in the brain where it poses an active role in the modulation of synaptic plasticity and in learning and memory processes. There is evidence that memory formation does not rely solely on hippocampal function but it could require also other components directly or indirectly. Among these, it is known that striatal D₁-positive MSNs express functional CB₁ receptors (Monory et al., 2007) and the dopaminergic cells in the striatum can indirectly modulate the hippocampal activity (Goldfarb et al. 2016; Sales-Carbonell et al. 2013; Tort et al. 2008) but there are not currently known direct connections between striatal structures and the hippocampus which can prove the involvement in memory formation.

Simultaneously, it is known that D₁Rs are expressed mainly on GABAergic interneurons in the hippocampus (Gangarossa et al., 2012; Puighermanal et al., 2017), in which CB₁ receptors are mainly expressed in GABAergic cells (Marsicano and Lutz, 1999, Marsicano and Kuner, 2008).

In line with this evidence, the first aim of this thesis was to investigate the physiological role of CB₁ receptors in hippocampal D₁+positive cells in the modulation of learning and memory processes.

We showed that specific deletion of CB₁ receptors from D₁-positive cells impairs long-term, but not short-term, object recognition memory formation. Although it is quite well clear the role of dopamine in the modulation of consolidation and storage of new memories (Lisman et al., 2011), the mechanism of this modulation remains poorly explored.

Given the higher expression of CB₁R in the striatum, our results show that the local re-expression of CB₁ receptors in the hippocampus but not the striatum of D₁-CB₁-KO mice is able to rescue the memory impairment displayed by these animals. Thus, we provide evidence that hippocampal CB₁ receptors in D₁-positive cells are necessary and sufficient for the consolidation of object recognition memory. Since the endocannabinoid/dopamine crosstalk has been shown to be important in aversive memory (in particular mice lacking CB₁ receptors in D₁-positive cells have impaired extinction of freezing in fear conditioning tasks (Micale et al., 2017)) and for spatial memory formation (Xing et al., 2010; Riedel and Davies, 2005), we can speculate that CB₁ receptors in D₁-

positive cells might be also involved in this episodic-like memory. Supporting this idea, LTP phenomenon is widely accepted as a cellular model of memory consolidation and dopamine, via D₁ receptors, is highly relevant for the modulation of hippocampus-dependent synaptic plasticity and memory (Lisman and Grace, 2005; Lisman et al. 2011; Hansen and Manahan-Vaughan, 2014; Huang and Kandel, 1995; Lemon and Manahan-Vaughan, 2006; Bethus et al. 2010; Clausen et al. 2011; da Silva et al. 2012). Accordingly, LTP at the CA3-CA1 schaffer collater pathway is a potential molecular and cellular mechanism underlying behavioral expression of episodic-like memory processes (Morris, 2013). Our results showed that deletion of CB₁ from D₁-positive cells in homecage conditions do not alter in vivo LTP. However, when the animals are exposed to the objects in the training phase of NORT, LTP is impaired in D₁-CB₁-KO mice as compared with their WT littermates. These results demonstrate, in accordance with the literature, that the presentation of a novel stimulus (learning) induces hippocampal dopamine release that modulates consolidation of memory and enhances the LTP by acting on D₁ receptors in the hippocampus (Kempadoo et al., 2016, Takeuchi et al., 2016) which cannot be done in the absence of CB₁ receptors in the D₁-positive cells, suggesting that endocannabinoid control of D₁R-positive hippocampal interneurons is recruited only after learning and is responsible for the consolidation of memory and LTP enhancement. Unfortunately, we cannot fully exclude that cell types other than hippocampal interneurons might participate in D₁R/CB₁R-dependent control of memory consolidation. Indeed, our data show that the D₁-Cre mouse line used in the present study (Lemberger et al. 2007) induces recombination in a small sub-fraction of hippocampal interneurons containing low levels of CB₁R mRNA but also in pyramidal neurons and mossy cells. Thus, it could represent most likely a subclass within CB₁-expressing GABAergic interneurons.

However, our data show that partial blockade of GABA_A receptors, but not of AMPA/kainate or NMDA glutamatergic ones, reverse the memory impairment of D₁-CB₁-KO mice. Therefore, our findings indicate that excessive GABAergic activity is involved in the phenotype of D₁-CB₁-KO mice and CB₁R control of GABA release from D₁R-positive interneurons regulates the consolidation of NOR memory. Moreover, partial blockade of GABA_A receptors in trained WT mice does not alter LTP facilitation suggests that this phenomenon is due to a genuine increase in LTP. In addition, our data indicate that reducing GABAergic transmission in D₁R-positive neurons is required for this form of learning-induced synaptic plasticity.

D₁R activity in the HPC is necessary for long-term memory and synaptic plasticity (Lisman et al., 2011; Yamasaki and Takeuchi, 2017; Kaufman et al. 2020; Bethus et al., 2010). Consistently, our results show that high doses of the D_{1/5}R antagonist SCH-23390 impair memory performance in the NOR task. Although activation of D₁Rs in interneurons seems to be necessary for the memory process, their abnormally high activity (e.g. in the absence of CB₁Rs) impairs such functions.

Thus, our results show that partial reducing D₁R activity counteracts the absence of CB₁Rs in D₁-CB₁-KO mice by abolishing the differences in LTP enhancement and rescuing the NOR memory consolidation impairment. Altogether, our results suggest an important role of this small subpopulation of D₁R/CB₁R-positive interneurons in maintaining a proper excitation/inhibition balance in the hippocampal network activity required for memory consolidation.

Taking advantage of a study from our team, the astroglial CB₁Rs are also necessary for consolidation of NOR does memory by allowing D-serine availability at glutamatergic synapse (Robin et al., 2018). Thus, we cannot fully exclude that deletion of CB₁Rs in D₁R-positive cells does not also involve astrocytes. In this study, D-serine rescues memory performance of GFAP-CB₁-KO mice only when administered immediately after training. Moreover, GFAP-CB₁-KO mice do not express in vivo LTP even in basal “home cage” conditions (Robin et al., 2018). Conversely, D₁-CB₁-KO mice only lack the specific facilitation of LTP induced by learning. Comparing the current and past results, we could speculate that at least two distinct temporal windows exist and CB₁R in hippocampal astrocytes is likely involved in the starting memory formation, whereas CB₁R in D₁R-positive hippocampal interneurons is required in the maintaining the memory for longer periods.

In conclusion, although it is very complex elucidating the neuro-biological and physiological mechanism of hippocampal D₁R/CB₁R microcircuit, our data provide a novel link between CB₁ receptor-mediated activity and D₁-positive cells in learning-dependent facilitation of hippocampal LTP and in the consolidation of object recognition memory, which might provide novel therapeutic strategies for treatment of cognitive diseases.

PART 2 – LINKING MITOCHONDRIAL G-PROTEIN SIGNALING TO CANNABINOIDS-INDUCED AMNESIA: A NEW MITOCHONDRIA-SPECIFIC CHEMOGENETIC STRATEGY

Our brain consumes up to 80% of body energy in order to maintain a series of active bioenergetic processes. Our brain cellular activity depends on the high energetic support provided by mitochondria, the cell organelles which process the energy sources and transform them into ATP (MacAskill and Kittler, 2010; Mattson et al., 2008; Rangaraju et al., 2014). In this regard, memory formation and storage are among the most important brain functions which requires an intense consume of energy (Watts et al. 2018). Moreover, events associated with perturbation in cellular energy homeostasis can dramatically impact on synaptic and cognitive function (Suzuki et al. 2011; Wu et al. 2004). In this *scenario*, mitochondrial alterations (e.g. interruptions of the ATP production) have been showed to alter memory processes (Lin et al. 2013; Roubertoux et al. 2003).

Cannabinoid drugs and CB₁R activation have a multitude of therapeutic potentials (Pacher et al. 2006) but the amnesic side effect is amongst the well known side effects of these compounds (Broyd et al. 2016; Marsicano and Lafenêtre, 2009). With the discovery of the functional presence of GPCRs on mitochondrial membranes (Beninca et al., 2014) and the mitochondrial CB₁ receptors in the brain (Benard et al. 2012), the hypothesis that the effects induced by exogenous cannabinoids on mitochondrial functions are due to an indirect signaling of plasma membrane CB₁ receptor-dependent activation (Campbell, 2001; Athanasiou et al. 2007) is now remote and, the molecular signaling pathways mechanisms resulting from the activation of the mtCB₁ G-protein and the physiological and behavioral effects mediated by mtCB₁ receptors started to emerge (Hebert-Chatelain et al. 2014, 2016; Koch et al. 2015; Gutiérrez-Rodríguez et al. 2018; Jimenez-Blasco et al. 2020; Soria-Gomez et al. 2021).

Accordingly, direct bioenergetic effects of cannabinoids play a central role on brain mitochondrial activity, memory and motor regulation. The activation of intra-mitochondrial G α i proteins by exogenous CB₁ receptor agonists decreases the respiratory function of mitochondria, in particular the oxygen consumption and ATP production, through a sAC- and PKA- dependent mechanism.

Overall, this chain of events leads to a decrease of brain mitochondrial activity, which is responsible for cannabinoids-induced amnesia (Hebert-Chatelain et al. 2016). Conversely, mice lacking hippocampal mtCB₁R lose this effect of cannabinoids during NOR consolidation task, indicating that the mtCB₁ activation is required for cannabinoid-induced amnesia via the modulation of bioenergetics processes.

The first part of thesis demonstrates that a small subpopulation of hippocampal D₁R/CB₁R interneurons is necessary for NOR memory consolidation (Oliveira da Cruz et al. 2020a). Taking advantage of these findings, we supposed that mtCB₁Rs in hippocampal D₁-positive interneurons can play a key role in THC-induced memory impairment during NOR consolidation. Accordingly, our data show that THC impairs the NOR performance of D₁-CB₁-WT mice without altering total exploration and confirm the impaired NOR memory phenotype of D₁-CB₁-KO mice. The amnesic effect of THC is fully rescued in D₁-CB₁-KO re-expressing CB₁ but not in re-expressing DN22-CB₁ ones (mutant version of CB₁ receptor not targeted to mitochondria, [Hebert-Chatelain et al. 2016]) suggesting that mtCB₁ in hippocampal D₁-positive neurons is not necessary for memory formation per se but its activation is involved in memory consolidation impairment induced by THC during NOR task.

Thus, the second challenge of this thesis was to investigate how the modulation of mitochondrial G-protein signaling can affect the mitochondrial bioenergetic processes. To this aim, we adopted a novel chemogenetic tool. We developed a new adeno-associated virus expressing a Cre-dependent Gs-coupled DREADD specifically targeted to mitochondrial membrane. To this aim, we first fused a DREADD-Gs construct to a mitochondrial leading sequence (MLS) in order to induce the specific mitochondrial localization of the designer receptor mutant construct (MLS-DREADD-Gs).

MLS-DREADD-Gs was unable to activate the ERK phosphorylation, but its activation by CNO resulted in an increased mitochondrial respiration and membrane potential, representing the ideal tool to manipulate mitochondrial Gs activity and counteract the inhibitory effects of CB₁ activation on mitochondrial respiration.

Thus, given the relevance of mitochondrial G-protein signaling and bioenergetic processes in behavioral cognitive aspects, we evaluated how in vivo MLS-DREADD-Gs activation can interfere with the effects of cannabinoids on memory consolidation.

The activation of DREADD-Gs in D₁-positive hippocampal cells during NOR memory consolidation did not block the THC amnesic effect, suggesting that the excessive cytoplasmic Gs-cAMP-PKA signaling in hippocampal D₁-positive neurons is not required for this effect. Conversely, over-activation mitochondrial respiration by MLS-DREADD-Gs in hippocampal D₁ neurons inhibited the amnesic effect of THC, indicating the direct control of mitochondrial activity as the mechanism for cannabinoid-induced memory impairment.

In summary, this study extends our knowledge on direct link between the impact of cannabinoids on brain mitochondrial signaling and some of their most important intracellular and behavioral effects, such as cellular respiration and amnesia respectively. In particular, it introduces an innovative genetic tool able to modify the intra-mitochondrial GPCRs signaling and regulate mitochondrial bioenergetics processes and relevant brain functions as memory. The importance of mitochondrial-dependent effects opens new hopes for the development of innovative therapeutical strategies against several brain disorders which can specifically take advantage of the functional and energetic potentials of these intracellular organelles, possibly avoiding/limiting undesired side effects.

SECTION V — REFERENCES

ACCARDI MV, DANIELS BA, BROWN PM, FRITSCHY JM, TYAGARAJAN SK, BOWIE D. 2014. Mitochondrial reactive oxygen species regulate the strength of inhibitory GABA- mediated synaptic transmission. *Nat Commun* 5: 3168.

ACCARDI, M. V., PUGSLEY, M. K., FORSTER, R., TRONCY, E., HUANG, H. & AUTHIER, S. 2016. The emerging role of in vitro electrophysiological methods in CNS safety pharmacology. *Journal of Pharmacological and Toxicological Methods*, 81, 47-59.

ADAMANTIDIS, A.R., ZHANG, F., DE LECEA, L., DEISSEROTH, K. 2014. Optogenetics: Opsins and Optical Interfaces in Neuroscience. *Cold Spring Harb. Protoc.* pdb-top083329.

AHN, K., MCKINNEY, M. K. & CRAVATT, B. F. 2008. Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chem Rev*, 108, 1687-707.

AKKERMAN, S., BLOKLAND, A., & PRICKAERTS, J. 2014. Mind the gap: delayed manifestation of long-term object memory improvement by phosphodiesterase inhibitors. *Neurobiol. Learn. Mem.* 109, 139-43.

ALBERTS, B.J., A.; LEWIS, J; RAFF, M.; ROBERTS, K.; WALTER, P. 2002. *Molecular Biology of the cell*, 4th edition edn (New York: Garland Science).

ALGER BE, TEYLER TJ. 1976. Long-term and short-term plasticity in the CA1, CA3, and dentate regions of the rat hippocampal slice. *Brain Res.* 110(3):463-80. doi: 10.1016/0006-8993(76)90858-1.

ALGER BE. 2002. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. *Prog Neurobiol.* 68:247–286.

ALGER, B.E. & TANG, A. H. 2012. Do cannabinoids reduce brain power? *Nat Neurosci*, 15, 499-501.

ALI AB. 2007. Presynaptic inhibition of GABAA receptor-mediated unitary IPSPs by cannabinoid receptors at synapses between CCK-positive interneurons in rat hippocampus. *J Neurophysiol* 98: 861– 869.

ALOISI, E., LE CORF, K., DUPUIS, J., ZHANG, P., GINGER, M., LABROUSSE, V., SPATUZZA, M., GEORG HABERL, M., COSTA, L., SHIGEMOTO, R., TAPPE-THEODOR, A., DRAGO, F., VINCENZO PIAZZA, P., MULLE, C., GROG, L., CIRANNA, L., CATANIA, M. V. AND FRICK, A. 2017. Altered surface mGluR5 dynamics provoke synaptic NMDAR dysfunction and cognitive defects in Fmr1 knockout mice. *Nat Commun* 8(1): 1103.

AMERI A. 1999. The effects of cannabinoids on the brain. *Prog Neurobiol.* 58(4):315-48. doi: 10.1016/s0301-0082(98)00087-2.

ANDREEVA, A.V., KUTUZOV, M.A., AND VOYNO-YASENETSKAYA, T.A. 2008. G alpha12 is targeted to the mitochondria and affects mitochondrial morphology and motility. *FASEB journal.* 22, 2821-2831.

AQUILA S, GUIDO C, SANTORO A, et al. 2010. Rimonabant (SR141716) induces metabolism and acquisition of fertilizing ability in human sperm. *Br J Pharmacol.* 159(4): 831–41.

AREVALO C., DE MIGUEL R., AND HERNANDEZ-TRISTAN R. 2001. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. *Pharmacology Biochemistry and Behavior*, vol. 70, no. 1, pp. 123–131.

ARLT, C., HÄUSSER, M. 2020. Microcircuit Rules Governing Impact of Single Interneurons on Purkinje Cell Output In Vivo. *Cell Rep.* 30, 3020–3035.e3

ARMBRUSTER, B.N., LI, X., PAUSCH, M.H., HERLITZE, S. & ROTH, B.L. 2007. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proc Natl Acad Sci U S A*, 104, 5163-8.

ARNONE, M., MARUANI, J., CHAPERON, F., THIEBOT, M.H., PONCELET, M., SOUBRIE, P., AND LE FUR, G. 1997. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology (Berl.)* 132, 104–106.

ARRABAL S, LUCENA MA, CANDUELA MJ, et al. 2015. Pharmacological Blockade of Cannabinoid CB1 Receptors in Diet-Induced Obesity Regulates Mitochondrial Dihydrolipoamide Dehydrogenase in Muscle. *PLoS One.* 10(12): e0145244.

ASHTON CH, MOORE PB, GALLAGHER P, YOUNG AH. 2005. Cannabinoids in bipolar affective disorder: a review and discussion of their therapeutic potential. *J Psychopharmacol.* 19:293–300.

ASHTON H, GOLDING J, MARSH VR, MILLMAN JE, THOMPSON JW. 1981. The seed and the soil: effect of dosage, personality and starting state on the response to Δ^9 tetrahydrocannabinol in man. *Br J Clin Pharmacol.* 12:705–720.

ASO, E., PALOMER, E., JUVES, S., MALDONADO, R., MUNOZ, F. J. AND FERRER, I. 2012. CB1 agonist ACEA protects neurons and reduces the cognitive impairment of AbetaPP/PS1 mice. *J Alzheimers Dis* 30(2): 439-459.

ATHANASIOU, A., CLARKE, A.B., TURNER, A.E., KUMARAN, N.M., VAKILPOUR, S., SMITH, P.A., BAGIOKOU, D., BRADSHAW, T.D., WESTWELL, A.D., FANG, L., et al. 2007. Cannabinoid receptor agonists are mitochondrial inhibitors: a unified hypothesis of how cannabinoids modulate mitochondrial function and induce cell death. *Biochemical and biophysical research communications* 364, 131-137.

ATTWELL, D., AND LAUGHLIN, S.B. 2001. An energy budget for signaling in the grey matter of the brain. *Journal of cerebral blood flow and metabolism*. 21, 1133-1145.

ATTWELL, D., BUCHAN, A.M., CHARPAK, S., LAURITZEN, M., MACVICAR, B.A., AND NEWMAN, E.A. 2010. Glial and neuronal control of brain blood flow. *Nature* 468, 232-243.

BACH ME, BARAD M, SON H, ZHUO M, LU YF, SHIH R, MANSUY I, HAWKINS RD, KANDEL ER. 1999. Age-related defects in spatial memory are correlated with defects in the late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signaling pathway. *Proc Natl Acad Sci U S A*. 96:5280–5285.

BALDERAS, I., MORENO-CASTILLA, P., & BERMUDEZ-RATTONI, F. 2013. Dopamine D1 receptor activity modulates object recognition memory consolidation in the perirhinal cortex but not in the hippocampus. *Hippocampus*. 23 (10), 873-878.

BARCO A, ALARCON JM, KANDEL ER. 2002. Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. *Cell*. 108(5):689-703. doi: 10.1016/s0092-8674(02)00657-8.

BARRIA A, MULLER D, DERKACH V, GRIFFITH LC, SODERLING TR. 1997. Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science*. 276(5321):2042-5. doi: 10.1126/science.276.5321.2042.

BARTOVA A, BIRMINGHAM MK. 1976. Effect of delta9-tetrahydrocannabinol on mitochondrial NADH-oxidase activity. *J Biol Chem*. 251(16): 5002–6.

BAYEWITCH M, RHEE MH, AVIDOR-REISS T, BREUER A, MECHOULAM R, VOGEL Z. 1996. (-) - Delta9-tetrahydrocannabinol antagonizes the peripheral cannabinoid receptor-mediated inhibition of adenylyl cyclase. *J Biol Chem*, 271: 9902-9905.

BEAR MF. 1996. A synaptic basis for memory storage in the cerebral cortex. *Proc Natl Acad Sci U S A*. 93:13453–13459.

BECKSTEAD RM, DOMESICK VB, NAUTA WJ. 1979. Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res.* 175:191–217.

BELLOCCHIO, L., LAFENETRE, P., CANNICH, A., COTA, D., PUENTE, N., GRANDES, P., CHAOULOFF, F., PIAZZA, P.V., AND MARSICANO, G. 2010. Bimodal control of stimulated food intake by the endocannabinoid system. *Nat. Neurosci.* 13, 281–283.

BELLOCCHIO L, SORIA-GÓMEZ E, QUARTA C, METNA-LAURENT M, CARDINAL P, BINDER E, CANNICH A, DELAMARRE A, HÄRING M, MARTÍN-FONTECHA M, VEGA D, LESTE-LASSERRE T, BARTSCH D, MONORY K, LUTZ B, CHAOULOFF F, PAGOTTO U, GUZMAN M, COTA D, MARSICANO G. 2013. Activation of the sympathetic nervous system mediates hypophagic and anxiety-like effects of CB₁ receptor blockade. *Proc Natl Acad Sci U S A.* 110(12):4786-91. doi: 10.1073/pnas.1218573110.

BELOUS, A., WAKATA, A., KNOX, C.D., NICOUD, I.B., PIERCE, J., ANDERSON, C.D., PINSON, C.W., AND CHARI, R.S. 2004. Mitochondrial P2Y-Like receptors link cytosolic adenosine nucleotides to mitochondrial calcium uptake. *Journal of cellular biochemistry* 92, 1062-1073.

BEN-SHABAT S, FRIDE E, SHESKIN T, TAMIRI T, RHEE MH, VOGEL Z, BISOGNO T, DE PETROCELLIS L, DI MARZO V, MECHOULAM R. 1998. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol.* 353(1):23-31. doi: 10.1016/s0014-2999(98)00392-6. PMID: 9721036.

BÉNARD G, MASSA F, PUENTE N, et al. 2012. Mitochondrial CB receptors regulate neuronal energy metabolism. *Nat Neurosci.* 15(4): 558–64.

BENINCA, C., PLANAGUMA, J., DE FREITAS SHUCK, A., ACIN-PEREZ, R., MUNOZ, J.P., DE ALMEIDA, M.M., BROWN, J.H., MURPHY, A.N., ZORZANO, A., ENRIQUEZ, J.A., et al. 2014. A new non-canonical pathway of Galpha(q) protein regulating mitochondrial dynamics and bioenergetics. *Cellular signalling* 26, 1135-1146.

BERGER, T.W., RINALDI, P.C., WEISZ, D.J. & THOMPSON, R.F. 1983. Single-unit analysis of different hippocampal cell types during classical conditioning of rabbit nictitating membrane response. *J. Neurophysiol.* 50, 1197–1219.

BERGSON C, MRZLJAK L, SMILEY JF, PAPPY M, LEVENSON R, GOLDMAN-RAKIC PS. 1995. Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J Neurosci.* 15:7821–7836.

BERLYNE, D. 1950. Novelty and curiosity as determinants of exploratory behavior. *Br. J. Psychol.* 41 (1-2), 68-80.

BERRENDERO F, MALDONADO R. 2002. Involvement of the opioid system in the anxiolytic-like effects induced by Δ^9 -tetrahydrocannabinol. *Psychopharmacology*; 163: 111-7.

BERRIDGE, K.C., AND VALENSTEIN, E.S. 1991. What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? *Behav. Neurosci.* 105, 3–14.

BERTAINA-ANGLADE, V., ENJUANES, E., MORILLON, D., & DRIEU LA ROCHELLE, C. 2006. The object recognition task in rats and mice: A simple and rapid model in safety pharmacology to detect amnesic properties of a new chemical entity. *J. Pharmacol. Toxicol. Methods.* 54 (2), 99-105.

BETHUS I, TSE D, MORRIS RG. 2010. Dopamine and memory: modulation of the persistence of memory for novel hippocampal NMDA receptor-dependent paired associates. *J Neurosci.* 30: 1610–1618.

BEVINS, R. A. AND BESHEER, J. 2006. Object recognition in rats and mice: a one-trial non- matching-to-sample learning task to study 'recognition memory'. *Nat Protoc* 1(3): 1306-1311.

BINO, T., CHARI-BITRON, A., AND SHAHAR, A. 1972. Biochemical effects and morphological changes in rat liver mitochondria exposed to Δ^9 -tetrahydrocannabinol. *Biochimica et biophysica acta* 288, 195-202.

BLACK, S.C. 2004. Cannabinoid receptor antagonists and obesity. *Curr. Opin. Investig. Drugs* 5, 389–394.

BLANKMAN JL, SIMON GM, CRAVATT BF. 2007. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol* 14: 1347–1356.

BLISS TV, GARDNER-MEDWIN AR. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaesthetized rabbit following stimulation of the perforant path. *J Physiol.* 232(2):357-74. doi: 10.1113/jphysiol.1973.sp010274.

BLISS TV, LOMO T. 1970. Plasticity in a monosynaptic cortical pathway. *J Physiol.* 207(2):61P.

BLISS TV, LOMO T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* 232: 331–356.

BLISS, T.V. & COLLINGRIDGE, G.L. 1993. A Synaptic Model of Memory: Long-Term Potentiation in The Hippocampus. *Nature*, 361, 31-9.

BLOOMFIELD, M. A., ASHOK, A. H., VOLKOW, N. D. & HOWES, O. D. 2016. The effects of Delta9-tetrahydrocannabinol on the dopamine system. *Nature*, 539, 369-377.

BOCK FJ, TAIT SWG. 2020. Mitochondria as multifaceted regulators of cell death. *Nat Rev Mol Cell Biol.* 21(2):85-100. doi: 10.1038/s41580-019-0173-8.

BODOR, A.L., KATONA, I., NYIRI, G., MACKIE, K., LEDENT, C., HAJOS, N. & FREUND, T.F. 2005. Endocannabinoid signaling in rat somatosensory cortex: laminar differences and involvement of specific interneuron types. *J Neurosci*, 25, 6845-56.

BOSIER B, BELLOCCHIO L, METNA-LAURENT M, SORIA-GOMEZ E, MATIAS I, HEBERT-CHATELAIN E, CANNICH A, MAITRE M, LESTE-LASSERRE T, CARDINAL P, MENDIZABAL-ZUBIAGA J, CANDUELA MJ, REGUERO L, HERMANS E, GRANDES P, COTA D, MARSICANO G. 2013. Astroglial CB1 cannabinoid receptors regulate leptin signaling in mouse brain astrocytes. *Mol Metab* 2:393–404.

BOUABOULA M, POINOT-CHAZEL C, BOURRIÉ B, CANAT X, CALANDRA B, RINALDI-CARMONA M, LE FUR G, CASELLAS P. 1995. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J.* 312 (Pt 2)(Pt 2):637-41. doi: 10.1042/bj3120637.

BOURNAT, J.C., BROWN, C.W., 2010. Mitochondrial dysfunction in obesity. *Current Opinion in Endocrinology Diabetes and Obesity* 17:446e452.

BOYDEN, E.S. 2015. Optogenetics and the future of neuroscience. *Nat. Neurosci.* 18,1200–1201.

BRAIDA D, POZZI M, PAROLARO D, SALA M. 2001. Intracerebral self-administration of the cannabinoid receptor agonist CP 55,940 in the rat: interaction with the opioid system. *Neuroscience* 413:227–234.

BRANCATO A, CASTELLI V, LAVANCO G, MARINO RAM, CANNIZZARO C. 2020. In utero Δ^9 -tetrahydrocannabinol exposure confers vulnerability towards cognitive impairments and alcohol drinking in the adolescent offspring: Is there a role for neuropeptide Y? *J Psychopharmacol.* 34(6):663-679. doi: 10.1177/0269881120916135. PMID: 32338122.

BRENNER, M., KISSEBERTH, W. C., SU, Y., BESNARD, F. & MESSING, A. 1994. GFAP promoter directs astrocyte-specific expression in transgenic mice. *J Neurosci*, 14, 1030-7.

BRINI M, CALI T, OTTOLINI D, CARAFOLI E. 2014. Neuronal calcium signaling: function and dysfunction. *Cell Mol Life Sci* 71: 2787–2814.

BROADBENT, N. J., GASKIN, S., SQUIRE, L. R. AND CLARK, R. E. 2010. Object recognition memory and the rodent hippocampus. *Learn Mem* 17(1): 5-11.

BROMBERG-MARTIN ES, MATSUMOTO M, HIKOSAKA O. 2010. Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron*. 68:815–834.

BROWN AJ. 2007. Novel cannabinoid receptors. *Br J Pharmacol*. 152(5):567-75. doi: 10.1038/sj.bjp.0707481. PMID: 17906678; PMCID: PMC2190013.

BROWN, A.M. 2004. Brain glycogen re-awakened. *Journal of neurochemistry* 89, 537-552.

BROWN, J.E., KASSOUNY, M., AND CROSS, J.K. 1977. Kinetic studies of food intake and sucrose solution preference by rats treated with low doses of delta9-tetrahydrocannabinol. *Behav. Biol.* 20, 104–110.

BROYD, S. J., VAN HELL, H. H., BEALE, C., YÜCEL, M. & SOLOWIJ, N. 2016. Acute and chronic effects of cannabinoids on human cognition—a systematic review. *Biol. Psychiatry* 79, 557–567.

BUSQUETS GARCIA, A., SORIA-GOMEZ, E., BELLOCCHIO, L., AND MARSICANO, G. 2016. Cannabinoid receptor type-1: breaking the dogmas. *F1000Research* 5.

BUSQUETS-GARCIA A, BAINS J & MARSICANO G. 2018a. CB1 receptor signaling in the brain: extracting specificity from ubiquity. *Neuropsychopharmacology* 43, 4–20.

BUSQUETS-GARCIA A, DESPREZ T, METNA-LAURENT M, BELLOCCHIO L, MARSICANO G & SORIA-GOMEZ E. 2015. Dissecting the cannabinergic control of behavior: the where matters. *BioEssays* 37, 1215–1225.

BUSQUETS-GARCIA A, OLIVEIRA DA CRUZ JF, TERRAL G, ZOTTOLA ACP, SORIA-GOMEZ E, CONTINI A, MARTIN H, REDON B, VARILH M, IOANNIDOU C et al. 2018b. Hippocampal CB1 receptors control incidental associations. *Neuron* 99, 1247–1259.e7.

BUSQUETS-GARCIA, A., GOMIS-GONZALEZ, M., GUEGAN, T., AGUSTIN-PAVON, C., PASTOR, A., MATO, S., PEREZ-SAMARTIN, A., MATUTE, C., DE LA TORRE, R., DIERSSEN, M., MALDONADO, R. & OZAITA, A. 2013. Targeting the Endocannabinoid System in The Treatment of Fragile X Syndrome. *Nature Medicine*, 19, 603-7.

BUSQUETS-GARCIA, A., GOMIS-GONZALEZ, M., SALGADO-MENDIALDUA, V., GALERA-LOPEZ, L., PUIGHERMANAL, E., MARTIN-GARCIA, E., MALDONADO, R. & OZAITA, A. 2017. Hippocampal Protein Kinase C Signaling Mediates the Short-Term Memory Impairment Induced by Delta9-Tetrahydrocannabinol. *Neuropsychopharmacology*.

BUSQUETS-GARCIA, A., GOMIS-GONZALEZ, M., SALGADO-MENDIALDUA, V., GALERA-LOPEZ, L., PUIGHERMANAL, E., MARTIN-GARCIA, E., MALDONADO, R. AND OZAITA, A. 2018c. Hippocampal protein kinase C signaling mediates the short-term memory impairment induced by delta9-tetrahydrocannabinol. *Neuropsychopharmacology* 43(5): 1021-1031.

BUSQUETS-GARCIA, A., PUIGHERMANAL, E., PASTOR, A., DE LA TORRE, R., MALDONADO, R. & OZAITA, A. 2011. Differential Role of Anandamide and 2-Arachidonoylglycerol in Memory and Anxiety-Like Responses. *Biological Psychiatry*, 70, 479-86.

BUZSÁKI G. 1980. Long-term potentiation of the commissural path-CA1 pyramidal cell synapse in the hippocampus of the freely moving rat. *Neurosci Lett*. 19(3):293-6. doi: 10.1016/0304-3940(80)90276-1.

CALIGNANO A, LA RANA G, GIUFFRIDA A, PIOMELLI D. 1998. Control of pain initiation by endogenous cannabinoids. *Nature* 394: 277–281.

CAMPBELL, V.A. 2001. Tetrahydrocannabinol-induced apoptosis of cultured cortical neurones is associated with cytochrome c release and caspase-3 activation. *Neuropharmacology* 40, 702-709.

CARLSON, G., WANG, Y., AND ALGER, B. E. 2002. Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nat. Neurosci.* 5, 723–724. doi: 10.1038/nn879.

CARRIER EJ, AUCHAMPACH JA, HILLARD CJ. 2006. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc Natl Acad Sci USA*. 103:7895–7900.

CASTANE A, VALJENT E, LEDENT C, PARMENTIER M, MALDONADO R, VALVERDE O. 2002. Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. *Neuropharmacology* 43: 857–867.

CASTILLO PE, YOUNTS TJ, CHÁVEZ AE, HASHIMOTODANI Y. 2012. Endocannabinoid signaling and synaptic function. *Neuron*. 76(1):70-81. doi: 10.1016/j.neuron.2012.09.020.

CATERINA, M. J., SCHUMACHER, M. A., TOMINAGA, M., ROSEN, T. A., LEVINE, J. D. & JULIUS, D. 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*, 389, 816-24.

CAULFIELD MP, BROWN DA. 1992. Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. *Br J Pharmacol*. 106(2):231-2. doi: 10.1111/j.1476-5381.1992.tb14321.x.

CEPEDA C, COLWELL CS, ITRI JN, CHANDLER SH, LEVINE MS. 1998. Dopaminergic modulation of NMDA-induced whole cell currents in neostriatal neurons in slices: contribution of calcium conductances. *J Neurophysiol*. 79:82–94.

CEPEDA C, LEVINE MS. 2006. Where do you think you are going? The NMDA-D1 receptor trap. *Sci STKE*. 333:20.

CHAKRABARTI A, EKUTA JE, ONAIVI ES. 1998. Neurobehavioral effects of anandamide and cannabinoid receptor gene expression in mice. *Brain Res Bull*; 45: 67-74.

CHAMBERS, A.P., SHARKEY, K.A., AND KOOPMANS, H.S. 2004. Cannabinoid (CB) 1 receptor antagonist, AM 251, causes a sustained reduction of daily food intake in the rat. *Physiol. Behav*. 82, 863–869.

CHARI-BITRON, A., AND BINO, T. 1971. Effect of 1-tetrahydrocannabinol on ATPase activity of rat liver mitochondria. *Biochemical pharmacology* 20, 473-475.

CHAVEZ AE, CHIU CQ, CASTILLO PE. 2010. TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. *Nat Neurosci*. 13:1511–1518.

CHEER JF, MARSDEN CA, KENDALL DA, MASON R. 2000. Lack of response suppression follows repeated ventral tegmental cannabinoid administration: an in vitro electrophysiological study. *Neuroscience* 99:661–667.

CHEN JP, PAREDES W, LI J, SMITH D, LOWINSON J, GARDNER EL. 1990. Δ^9 -Tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely moving rats, as measured by intracerebral microdialysis. *Psychopharmacology* 102:156–162.

CHEN, R.Z., HUANG, R.R., SHEN, C.P., MACNEIL, D.J., AND FONG, T.M. 2004. Synergistic effects of cannabinoid inverse agonist AM251 and opioid antagonist nalmefene on food intake in mice. *Brain Res*. 999, 227–230.

- CHENG A, HOU Y, MATTSON MP. 2010. Mitochondria and neuroplasticity. *ASN Neuro* 2: e00045.
- CHEVALEYRE V, CASTILLO PE. 2003. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron*. 38:461–472.
- CHEVALEYRE V, CASTILLO PE. 2004. Endocannabinoid-mediated metaplasticity in the hippocampus. *Neuron*. 43(6):871-81. doi: 10.1016/j.neuron.2004.08.036.
- CHEVALEYRE V, HEIFETS BD, KAESER PS, SUDHOF TC, CASTILLO PE. 2007. Endocannabinoid-mediated long-term plasticity requires cAMP/PKA signaling and RIM1alpha. *Neuron*. 54:801–812.
- CHEYNE JE, MONTGOMERY JM. 2020. The cellular and molecular basis of in vivo synaptic plasticity in rodents. *Am J Physiol Cell Physiol*. 318(6):C1264-C1283. doi: 10.1152/ajpcell.00416.2019.
- CHILDERS SR, DEADWYLER SA. 1996. Role of cyclic AMP in the actions of cannabinoid receptors. *Biochem Pharmacol*. 52(6):819-27. doi: 10.1016/0006-2952(96)00419-4.
- CITRI, A., MALENKA, R. 2008. Synaptic Plasticity: Multiple Forms, Functions, and Mechanisms. *Neuropsychopharmacol* 33, 18–41.
- CLAPPER JR, MORENO-SANZ G, RUSSO R, GUIJARRO A, VACONDIO F, DURANTI A, et al. 2010. Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nat Neurosci*. 13(10):1265–70. <https://doi.org/10.1038/nn.2632>.
- CLAUSEN B, SCHACHTMAN TR, MARK LT, REINHOLDT M, CHRISTOFFERSEN GR. 2011. Impairments of exploration and memory after systemic or prelimbic D1-receptor antagonism in rats. *Behav Brain Res*. 223:241–254.
- COHEN C, PERRAULT G, VOLTZ C, STEINBERG R, SOUBRIE P. 2002. SR141716, a central cannabinoid [CB(1)] receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behav Pharmacol* 13: 451– 463.
- COLLINGRIDGE, G.L., KEHL, S.J. & MCLENNAN, H. 1983. Excitatory Amino Acids in Synaptic Transmission in The Schaffer Collateral-Commissural Pathway of The Rat Hippocampus. *The Journal of Physiology*, 334, 33-46.
- COLOMBO, G., AGABIO, R., DIAZ, G., LOBINA, C., REALI, R., AND GESSA, G.L. 1998. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. *Life Sci*. 63, PL113–PL117.

COTA, D., MARSICANO, G., LUTZ, B., VICENNATI, V., STALLA, G.K., PASQUALI, R., AND PAGOTTO, U. 2003a. Endogenous cannabinoid system as a modulator of food intake. *Int. J. Obes. Relat. Metab. Disord.* 27, 289–301.

COTA, D., MARSICANO, G., TSCHOP, M., GRUEBLER, Y., FLACHSKAMM, C., SCHUBERT, M., AUER, D., YASSOURIDIS, A., THONE-REINEKE, C., ORTMANN, S., et al. 2003b. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J. Clin. Invest.* 112, 423–431.

COTA D, STEINER MA, MARSICANO G, CERVINO C, HERMAN JP, GRÜBLER Y, STALLA J, PASQUALI R, LUTZ B, STALLA GK, PAGOTTO U. 2007. Requirement of cannabinoid receptor type 1 for the basal modulation of hypothalamic-pituitary-adrenal axis function. *Endocrinology.* 148(4):1574-81. doi: 10.1210/en.2005-1649.

COTMAN CW, FOSTER A, LANTHORN T. 1981. An overview of glutamate as a neurotransmitter. *Adv Biochem Psychopharmacol.* 27:1-27.

CRAVATT, B. F., GIANG, D. K., MAYFIELD, S. P., BOGER, D. L., LERNER, R. A. & GILULA, N. B. 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature,* 384, 83-7.

CUMMINGS JA, MULKEY RM, NICOLL RA, MALENKA RC. 1996. Ca²⁺ signaling requirements for long-term depression in the hippocampus. *Neuron.* 16:825–833.

CURRAN, H. V., FREEMAN, T. P., MOKRYSZ, C., LEWIS, D. A., MORGAN, C. J. & PARSONS, L. H. 2016. Keep off the grass? Cannabis, cognition and addiction. *Nat Rev Neurosci,* 17, 293-306.

DA SILVA WC, KÖHLER CC, RADISKE A, CAMMAROTA M. 2012. D1/D5 dopamine receptors modulate spatial memory formation. *Neurobiol Learn Mem.* 97:271–275.

DE LA MORA MP, GALLEGOS-CARI A, ARIZMENDI-GARCÍA Y, MARCELLINO D, FUXE K. 2010. Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: Structural and functional analysis. *Prog Neurobiol.* 90(2):198-216. doi: 10.1016/j.pneurobio.2009.10.010.

DE LIMA, M. N., LUFT, T., ROESLER, R. & SCHRODER, N. 2006. Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. *Neurosci Lett,* 405, 142-6.

DE VRIES TJ, SCHOFFELMEER AN. 2005. Cannabinoid CB1 receptors control conditioned drug seeking. *Trends Pharmacol Sci* 26: 420–426.

DE VRIES TJ, SHAHAM Y, HOMBERG JR, CROMBAG H, SCHUURMAN K, DIEBEN J, VANDER-SCHUREN LJ, SCHOFFELMEER AN. 2001. A cannabinoid mechanism of relapse to cocaine seeking. *Nat Med* 7:1151– 1154.

DEADWYLER SA, HAMPSON RE, BENNETT BA, EDWARDS TA, MU J, PACHECO MA, WARD SJ, CHILDERS SR. 1993. Cannabinoids modulate potassium current in cultured hippocampal neurons. *Recept Channels*. 1(2):121-34.

DEGROOT A, KÖFALVI A, WADE MR, DAVIS RJ, RODRIGUES RJ, REBOLA N, CUNHA RA & NOMIKOS GG. 2006. CB1 receptor antagonism increases hippocampal acetylcholine release: site and mechanism of action. *Mol Pharmacol* 70, 1236–1245.

DEISSEROTH, K. 2015. Optogenetics: 10 years of microbial opsins in neuroscience. *Nat. Neurosci.* 18,1213–1225.

DEL CAMPO N, CHAMBERLAIN SR, SAHAKIAN BJ, ROBBINS TW. 2011. The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. *Biol Psychiatry*. 69:145–157.

DERKINDEREN P, TOUTANT M, BURGAYA F, LE BERT M, SICILIANO JC, DE FRANCISCIS V, GELMAN M, GIRAULT JA. 1996. Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science*. 273(5282):1719-22. doi: 10.1126/science.273.5282.1719.

DEUBNER, J., COULON, P., DIESTER, I. 2019. Optogenetic approaches to study the mammalian brain. *Curr. Opin. Struct. Biol.* 57, 157–163.

DEVANE, WA, et al. 1988. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol*. 34 (5): p. 605-13.

DEVANE, WILLIAM A., et al. 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*. 258.5090: 1946-1949.

DI MARZO V, MELCK D, BISOGNO T, DE PETROCELLIS L. 1998. Endocannabinoids: Endogenous cannabinoid receptor ligands with neuromodulatory action. *Neuroscience Trends*. 21(12):521-8).

DI MARZO, V., FONTANA, A., CADAS, H., SCHINELLI, S., CIMINO, G., SCHWARTZ, J.-C. & PIOMELLI, D. 1994. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature*, 372, 686-691.

DI MARZO, V., GOPARAJU, S.K., WANG, L., LIU, J., BATKAI, S., JARAI, Z., FEZZA, F., MIURA, G.I., PALMITER, R.D., SUGIURA, T., AND KUNOS, G. 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 410, 822–825.

DI MARZO, V., STELLA, N., ZIMMER, A. 2015. Endocannabinoid signalling and the deteriorating brain. *Nat. Rev. Neurosci.* 16, 30–42.

DIANA MA, LEVENES C, MACKIE K, MARTY A. 2002. Short-term retrograde inhibition of GABAergic synaptic currents in rat Purkinje cells is mediated by endogenous cannabinoids. *J Neurosci.* 22(1):200-208. doi:10.1523/JNEUROSCI.22-01-00200.2002.

DIANA MA, MARTY A. 2004. Endocannabinoid-mediated short-term synaptic plasticity: depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE). *Br J Pharmacol.* 142(1):9-19. doi: 10.1038/sj.bjp.0705726.

DIETRICH, M.O., LIU, Z.W., HORVATH, T.L., 2013. Mitochondrial dynamics controlled by mitofusins regulate *Agpr* neuronal activity and diet-induced obesity. *Cell* 155:188e199.

DINH, T. P., CARPENTER, D., LESLIE, F. M., FREUND, T. F., KATONA, I., SENSI, S. L., KATHURIA, S. & PIOMELLI, D. 2002. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A*, 99, 10819-24.

DOHERTY J, DINGLELINE R. 2003. Functional interactions between cannabinoid and metabotropic glutamate receptors in the central nervous system. *Curr Opin Pharmacol.* 3 : 46-53.

DROLET P, BILODEAU L, CHORVATOVA A, LAFLAMME L, GALLO-PAYET N, PAYET MD. 1997. Inhibition of the T-type Ca²⁺ current by the dopamine D1 receptor in rat adrenal glomerulosa cells: requirement of the combined action of the G betagamma protein subunit and cyclic adenosine 3',5'-monophosphate. *Mol Endocrinol.* 11:503–514.

DUDOK B, BARNA L, LEDRI M, et al. 2015. Cell-specific STORM super-resolution imaging reveals nanoscale organization of cannabinoid signaling. *Nat Neurosci.* 18(1): 75–86.

DUNWIDDIE T, LYNCH G. 1978. Long-term potentiation and depression of synaptic responses in the rat hippocampus: localization and frequency dependency. *J Physiol.* 276:353-67. doi: 10.1113/jphysiol.1978.sp012239.

EICHENBAUM, H. 2017. Memory: Organization and Control. *Annu Rev Psychol*, 68, 19-45.

EL-GHUNDI, M., O'DOWD, B. F. & GEORGE, S. R. 2001. Prolonged fear responses in mice lacking dopamine D1 receptor. *Brain Research*, 892, 86-93.

ELDER G. A., DE GASPERI R., AND GAMA SOSA M. A. 2006. Research update: neurogenesis in adult brain and neuropsychiatric disorders. *Mount Sinai Journal of Medicine*, vol. 73, no. 7, pp. 931–940.

ENNACEUR, A. 2010. One-trial object recognition in rats and mice: methodological and theoretical issues. *Behav Brain Res* 215(2): 244-254.

ENNACEUR, A. AND DELACOUR, J. 1988. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav Brain Res* 31(1): 47-59.

EPSZTEIN, J., MILH, M., ID BIHI, R., JORQUERA, I., BEN-ARI, Y., REPRESA, A., CRÉPEL, V. 2006. Ongoing Epileptiform Activity in the Post- Ischemic Hippocampus Is Associated with a Permanent Shift of the Excitatory-Inhibitory Synaptic Balance in CA3 Pyramidal Neurons. *J. Neurosci.* 26, 7082–7092.

ERECINSKA, M., AND SILVER, I.A. 2001. Tissue oxygen tension and brain sensitivity to hypoxia. *Respiration physiology* 128, 263-276.

ERICKSON J, TOOKER A, TAI YC, PINE J. 2008. Caged neuron MEA: a system for long-term investigation of cultured neural network connectivity. *J Neurosci Methods* 175:1–16.

FATTORE L, SPANO MS, DEIANA S, MELIS V, COSSU G, FADDA P, FRATTA W. 2007. An endocannabinoid mechanism in relapse to drug seeking: a review of animal studies and clinical perspectives. *Brain Res Rev* 53: 1–16.

FELDER CC, NIELSEN A, BRILEY EM, PALKOVITS M, PRILLER J et al. 1996. Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. *FEBS Lett*, 393 : 231-235.

FELLIN, T. 2009. Communication between neurons and astrocytes: Relevance to the modulation of synaptic and network activity. *J. Neurochem.* 108, 533–544, doi:10.1111/j.1471-4159.2008.05830.x.

FERNANDEZ-RUIZ, J., HERNANDEZ, M. & RAMOS, J.A. 2010. Cannabinoid-dopamine interaction in the pathophysiology and treatment of CNS disorders. *CNS Neurosci. Ther.* 16, e72–e91.

FERNANDEZ-RUIZ, J.J., MUNOZ, R.M., ROMERO, J., VILLANUA, M.A., MAKRIYANNIS, A., AND RAMOS, J.A. 1997. Time course of the effects of different cannabimimetics on prolactin and gonadotrophin secretion: evidence for the presence of CB1 receptors in hypothalamic structures and their involvement in the effects of cannabimimetics. *Biochem. Pharmacol.* 53, 1919–1927.

FLORESCO SB, TODD CL, GRACE AA. 2001. Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. *J Neurosci.* 21:4915–4922.

FOX A, BEVAN S. 2005. Therapeutic potential of cannabinoid receptor agonists as analgesic agents. *Expert Opin Investig Drugs.* 14:695–703.

FOX A, KESINGLAND A, GENTRY C, MCNAIR K, PATEL S, URBAN L, JAMES I. 2001. The role of central and peripheral cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. *Pain.* 92:91–100.

FREDRIKSSON R, HÖGLUND PJ, GLORIAM DEI, LAGERSTROM MC, SCHIÖTH HB. 2003. Seven evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close relatives. *FEBS Lett.* 554:381–388.

FREEDLAND, C.S., SHARPE, A.L., SAMSON, H.H., AND PORRINO, L.J. 2001. Effects of SR141716A on ethanol and sucrose self-administration. *Alcohol. Clin. Exp. Res.* 25, 277–282.

FREEZE, B.S., KRAVITZ, A.V., HAMMACK, N., BERKE, J.D., AND KREITZER, A.C. 2013. Control of basal ganglia output by direct and indirect pathway projection neurons. *J. Neurosci.* 33, 18531–18539.

FREMEAU RT JR, DUNCAN GE, FORNARETTO MG, DEARRY A, GINGRICH JA, BREESE GR, CARON MG. 1991. Localization of D1 dopamine receptor mRNA in brain supports a role in cognitive, affective, and neuroendocrine aspects of dopaminergic neurotransmission. *Proc Natl Acad Sci U S A.* 88:3772–3776.

FREUND, T.F., AND HAJOS, N. 2003. Excitement reduces inhibition via endocannabinoids. *Neuron* 38, 362-365.

FREY U, MATTHIES H, REYMANN KG, MATTHIES H. 1991. The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region in vitro. *Neurosci Lett.* 129:111–114.

FRICK, K.M., & GRESACK, J.E. 2003. Sex Differences in the Behavioral Response to Spatial and Object Novelty in Adult C57BL/6 Mice. *Behav. Neurosci.* 117 (6), 1283-1291.

FUKUDOME Y, OHNO-SHOSAKU T, MATSUI M, OMORI Y, FUKAYA M, TSUBOKAWA H, TAKETO MM, FUKUNAGA K, MIYAMOTO E. 1998. Role of MAP kinase in neurons. *Mol Neurobiol.* 16(1):79-95. doi: 10.1007/BF02740604.

GABRIELI, J.D., BREWER, J.B., DESMOND, J.E. & GLOVER, G.H. 1997. Separate neural bases of two fundamental memory processes in the human medial temporal lobe. *Science.* 276, 264–266.

GALLATE, J.E., SAHAROV, T., MALLET, P.E., AND MCGREGOR, I.S. 1999. Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur. J. Pharmacol.* 370, 233–240.

GALVE-ROPERH I, CHIURCHIÙ V, DÍAZ-ALONSO J, BARI M, GUZMÁN M, MACCARRONE M. 2013. Cannabinoid receptor signaling in progenitor/stem cell proliferation and differentiation. *Prog Lipid Res.* 52(4):633-50. doi: 10.1016/j.plipres.2013.05.004. PMID: 24076098.

GANGAROSSA G, DI BENEDETTO M, O'SULLIVAN GJ, DUNLEAVY M, ALCACER C, BONITO-OLIVA A, HENSHALL DC, WADDINGTON JL, VALJENT E, FISONE G. 2011. Convulsant doses of dopamine D1 receptor agonist result in Erk-dependent increases in Zif268 and Arc/Arg3.1 expression in mouse dentate gyrus. *PLoS One.* 3:e19415.

GANGAROSSA, G., LONGUEVILLE, S., DE BUNDEL, D., PERROY, J., HERVE, D., GIRAULT, J.A. & VALJENT, E. 2012. Characterization of dopamine D1 and D2 receptor-expressing neurons in the mouse hippocampus. *Hippocampus*, 22, 2199-207.

GAO Y, VASILYEV DV, GONCALVES MB, HOWELL FV, HOBBS C, REISENBERG M, SHEN R, ZHANG MY, STRASSLE BW, LU P, MARK L, PIESLA MJ, DENG K, KOURANOVA EV, RING RH, WHITESIDE GT, BATES B, WALSH FS, WILLIAMS G, PANGALOS MN, SAMAD TA, DOHERTY P. 2010. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *J Neurosci.* 30(6):2017-24. doi: 10.1523/JNEUROSCI.5693-09.2010.

GAONI AND MECOULAM. 1964. Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *Journal of the American Chemical Society*. 86 (8), 1646-1647. DOI: 10.1021/ja01062a046.

GARCIA DE, BROWN S, HILLE B, MACKIE K. 1998. Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor. *J Neurosci*. 18(8):2834-41. doi: 10.1523/JNEUROSCI.18-08-02834.1998.

GARCIA, A. D., DOAN, N. B., IMURA, T., BUSH, T. G. & SOFRONIEW, M. V. 2004. GFAP-expressing progenitors are the principal source of constitutive neurogenesis in adult mouse forebrain. *Nat Neurosci*, 7, 1233-41.

GARDNER EL, PAREDES W, SMITH D, DONNER A, MILLING C, COHEN D, MORRISON D. 1988. Facilitation of brain stimulation reward by Δ^9 -tetrahydrocannabinol. *Psychopharmacology (Berl)* 96:142–144.

GATLEY SJ, GIFFORD AN, VOLKOW ND, LAN R, MAKRIYANNIS A. 1996. ¹²³I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors. *Eur J Pharmacol*. 307(3):331-8. doi: 10.1016/0014-2999(96)00279-8. PMID: 8836622.

GAUTIER, A., GAURON, C., VOLOVITCH, M., BENSIMON, D., JULLIEN, L., VRIZ, S. 2014. How to control proteins with light in living systems. *Nat. Chem. Biol.* 10, 533–541.

GENN R. F., TUCCI S., MARCO E.-M., VIVEROS M.-P., AND FILE S. E. 2003. Anxiolytic and anxiogenic effects of the cannabinoid agonist CP 55,940 in animal tests of anxiety. *Journal of Psychopharmacology*, vol. 17, p. A27.

GERDEMAN GL, RONESI J, LOVINGER DM. 2002. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci*. 5:446–451.

GIESE KP, FEDOROV NB, FILIPKOWSKI RK, SILVA AJ. 1998. Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science*. 279(5352):870-3. doi: 10.1126/science.279.5352.870.

GIUFFRIDA, A., AND SEILLIER, A. 2012. New insights on endocannabinoid transmission in psychomotor disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 38, 51–58.

GIULIANI D., FERRARI F., AND OTTANI A. 2000. The cannabinoid agonist HU 210 modifies rat behavioural responses to novelty and stress. *Pharmacological Research*, vol. 41, no. 1, pp. 47– 53.

GLASS M, NORTHUP JK. 1999. Agonist selective regulation of G proteins by cannabinoid CB (1) and CB (2) receptors. *Mol Pharmacol*, 56: 1362-1369.

GLASS, M., FELDER, C.C. 1997. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J Neurosci*, 17, 5327-33.

GOBBI G., BAMBICO F.R, MANGIERI R., ET AL. 2005. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 51, pp. 18620–18625.

GOLDFARB, E.V., CHUN, M.M. & PHELPS, E.A. 2016. Memory-Guided Attention: Independent Contributions of the Hippocampus and Striatum. *Neuron*, 89, 317-24.

GOLDMAN-RAKIC, P.S. 1996. Regional and cellular fractionation of working memory. *Proc. Natl Acad. Sci. USA* 93, 13473–13480.

GÓMEZ DEL PULGAR T, VELASCO G, GUZMÁN M. 2000. The CB1 cannabinoid receptor is coupled to the activation of protein kinase B/Akt. *Biochem J.* 347(Pt 2):369-73. doi: 10.1042/0264-6021:3470369.

GÓMEZ-GONZALO M., NAVARRETE M., PEREA G., COVELO A., MARTÍN-FERNÁNDEZ M. SHIGEMOTO R., LUJÁN R., ARAQUE A. 2015. Endocannabinoids induce lateral long-term potentiation of transmitter release by stimulation of gliotransmission. *Cereb. Cortex* 25, 3699–3712, doi:10.1093/cercor/bhu231.

GOMEZ, J. L., BONAVENTURA, J., LESNIAK, W., MATHEWS, W. B., SYSA-SHAH, P., RODRIGUEZ, L. A., ELLIS, R. J., RICHIE, C. T., HARVEY, B. K., DANNALS, R. F., POMPER, M. G., BONCI, A. & MICHAELIDES, M. 2017. Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science*, 357, 503-507.

GONZÁLEZ-RUEDA, A., PEDROSA, V., FEORD, R.C., CLOPATH, C., PAULSEN, O. 2018. Activity-Dependent Down scaling of Subthreshold Synaptic Inputs during Slow-Wave-Sleep-like Activity In Vivo. *Neuron* 97, 1244–1252.e5.

GOTO Y, GRACE AA. 2007. The dopamine system and the pathophysiology of schizophrenia: a basic science perspective. *Int Rev Neurobiol.* 78:41–68.

GOVINDARAJAN, A., KELLEHER, R. J. & TONEGAWA, S. 2006. A clustered plasticity model of long-term memory engrams. *Nat Rev Neurosci*, 7, 575-83.

GRANADO N, ORTIZ O, SUÁREZ LM, MARTÍN ED, CEÑA V, SOLÍS JM, MORATALLA R. 2008. D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-induced arc and zif268 expression in the hippocampus. *Cereb Cortex.* 18:1–12.

GRAY CM, MALDONADO PE, WILSON M, MCNAUGHTON B. 1995. Tetrodes markedly improve the reliability and yield of multiple single-unit isolation from multi-unit recordings in cat striate cortex. *J Neurosci Methods* 63:43–54.

GRAYSON, B., LEGER, M., PIERCY, C., ADAMSON, L., HARTE, M., & NEILL, J.C. 2015. Assessment of disease-related cognitive impairments using the novel object recognition (NOR) task in rodents. *Behav. Brain Res.* 285, 176-193.

GRINSPOON L, BAKALAR JB. 1998. The use of cannabis as a mood stabilizer in bipolar disorder: Anecdotal evidence and the need for clinical research. *J Psychoactive Drugs* 30: 171– 177.

GRUART, A., MUNOZ, M.D. & DELGADO-GARCIA, J.M. 2006. Involvement of the CA3-CA1 synapse in the acquisition of associative learning in behaving mice. *J. Neurosci.* 26, 1077–1087.

GUINDON J, BEAULIEU P. 2009. The role of the endogenous cannabinoid system in peripheral analgesia. *Curr Mol Pharmacol.* 2(1): 134–9.

GUINDON J, HOHMANN AG. 2009. The endocannabinoid system and pain. *CNS Neurol Disord Drug Targets* 8: 403– 421.

GULYAS, A. I., CRAVATT, B. F., BRACEY, M. H., DINH, T. P., PIOMELLI, D., BOSCIA, F. & FREUND, T. F. 2004. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur J Neurosci*, 20, 441-58.

GUTIERREZ-RODRIGUEZ A, BONILLA-DEL RIO I, PUENTE N, GOMEZ-URQUIJO SM, FONTAINE CJ, EGANA-HUGUET J, ELEZGARAI I, RUEHLE S, LUTZ B, ROBIN LM et al. 2018. Localization of the cannabinoid type-1 receptor in subcellular astrocyte compartments of mutant mouse hippocampus. *Glia* 66, 1417–1431.

GUZOWSKI, J.F., SETLOW, B., WAGNER, E.K. & MCGAUGH, J.L. 2001. Experience-dependent gene expression in the rat hippocampus after spatial learning: a comparison of the immediate-early genes Arc, c-fos, and zif-268. *J. Neurosci.* 21, 5089–5098.

GUZOWSKI JF, MCNAUGHTON BL, BARNES CA, WORLEY PF. 1999. Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles. *Nat Neurosci.* 2:1120–1124.

HAHN, T.T.G., MCFARLAND, J.M., BERBERICH, S., SAKMANN, B., MEHTA, M.R. 2012. Spontaneous persistent activity in entorhinal cortex modulates cortico-hippocampal interaction in vivo. *Nat. Neurosci.* 15, 1531–1538.

HAHN, T.T.G., SAKMANN, B., MEHTA, M.R. 2006. Phase-locking of hippocampal interneurons' membrane potential to neocortical up-down states. *Nat. Neurosci.* 9, 1359–1361.

HAHN, T.T.G., SAKMANN, B., MEHTA, M.R. 2007. Differential responses of hippocampal subfields to cortical up-down states. *Proc. Natl. Acad. Sci. USA* 104, 5169–5174.

HALE, M.W., HAY-SCHMIDT, A., MIKKELSEN, J.D., POULSEN, B., SHEKHAR, A. AND LOWRY, C.A. 2008. Exposure to an open-field arena increases c-Fos expression in a distributed anxiety-related system projecting to the basolateral amygdaloid complex. *Neuroscience* 155(3): 659-672.

HALLER J, MATYAS F, SOPRONI K, VARGA B, BARSY B, NEMETH B, MIKICS E, FREUND TF, HAJOS N. 2007. Correlated species differences in the effects of cannabinoid ligands on anxiety and on GABAergic and glutamatergic synaptic transmission. *Eur J Neurosci* 25: 2445–2456.

HALLER J., BAKOS N., SZIRMAY M., LEDENT C., AND FREUND T.F. 2002. The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *European Journal of Neuroscience*, vol. 16, no. 7, pp. 1395–1398.

HAMANN W, DI VADI PP. 1999. Analgesic effect of the cannabinoid analogue nabilone is not mediated by opioid receptors. *Lancet.* 353:560.

HAMILTON TJ, WHEATLEY BM, SINCLAIR DB, BACHMANN M, LARKUM ME, COLMERS WF. 2010. Dopamine modulates synaptic plasticity in dendrites of rat and human dentate granule cells. *Proc Natl Acad Sci U S A.* 107:18185–18190.

HAMMOND C, GOAILLARD JM, DEBANNE D, GAIARSA JL. 2015. Synaptic plasticity. In book: Cellular and Molecular Neurophysiology: Fourth Edition. 361-389. DOI: 10.1016/B978-0-12-397032-9.00018-2.

HAMPSON AJ, GRIMALDI M, LOLIC M, WINK D, ROSENTHAL R, AXELROD J. 2000. Neuroprotective antioxidants from marijuana. *Ann NY Acad Sci* 899: 274–282.

HAMPSON RE & DEADWYLER SA. 1999. Cannabinoids, hippocampal function and memory. *Life Sci* 65, 715– 723.

HAMPSON RE, EVANS GJ, MU J, ZHUANG SY, KING VC, CHILDERS SR, DEADWYLER SA. 1995. Role of cyclic AMP dependent protein kinase in cannabinoid receptor modulation of potassium "A-current" in cultured rat hippocampal neurons. *Life Sci.* 56(23-24):2081-8. doi: 10.1016/0024-3205(95)00192-9.

HAN J, KESNER P, METNA-LAURENT M, DUAN T, XU L, GEORGES F, KOEHL M, ABROUS DN, MENDIZABAL-ZUBIAGA J, GRANDES P, LIU Q, BAI G, WANG W, XIONG L, REN W, MARSICANO G, ZHANG X. 2012. Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell* 148:1039–1050.

HANSEN, N. & MANAHAN-VAUGHAN, D. 2014. Dopamine D1/D5 receptors mediate informational saliency that promotes persistent hippocampal long-term plasticity. *Cereb Cortex*, 24, 845-58.

HANUS, L., AVRAHAM, Y., BEN-SHUSHAN, D., ZOLOTAREV, O., BERRY, E.M., AND MECHOULAM, R. 2003. Short-term fasting and prolonged semistarvation have opposite effects on 2-AG levels in mouse brain. *Brain Res.* 983, 144–151.

HARKANY T, GUZMÁN M, GALVE-ROPERH I, BERGHUIS P, DEVI LA, MACKIE K. 2007. The emerging functions of endocannabinoid signaling during CNS development. *Trends Pharmacol Sci.* 28(2):83-92. doi: 10.1016/j.tips.2006.12.004.

HASPULA, D., & CLARK, M. A. 2020. Cannabinoid Receptors: An Update on Cell Signaling, Pathophysiological Roles and Therapeutic Opportunities in Neurological, Cardiovascular, and Inflammatory Diseases. *International journal of molecular sciences*, 21(20), 7693. <https://doi.org/10.3390/ijms21207693>.

HAUSER, AS, ATTWOOD, MM, RASK-ANDERSEN, M., SCHIÖTH, HB, AND GLORIAM, DE. 2017. Trends in GPCR drug discovery: new agents, targets and indications. *Nat. Rev. Drug Discov.* 16, 829–842.

HAYASHI-TAKAGI, A., YAGISHITA, S., NAKAMURA, M., SHIRAI, F., WU, Y. I., LOSHBAUGH, A. L., KUHLMAN, B., HAHN, K. M. & KASAI, H. 2015. Labelling and optical erasure of synaptic memory traces in the motor cortex. *Nature*, 525, 333-8.

HEBERT-CHATELAIN E, DESPREZ T, SERRAT R, BELLOCCHIO L, SORIA-GOMEZ E, BUSQUETS-GARCIA A, PAGANO ZOTTOLA AC, DELAMARRE A, CANNICH A, VINCENT P et al. 2016. A cannabinoid link between mitochondria and memory. *Nature* 539, 555–559.

HEBERT-CHATELAIN E, REGUERO L, PUENTE N, et al. 2014a. Cannabinoid control of brain bioenergetics: Exploring the subcellular localization of the CB1 receptor. *Mol Metab*. 3(4): 495–504.

HEBERT-CHATELAIN E, REGUERO L, PUENTE N, LUTZ B, CHAOULOFF F, ROSSIGNOL R, PIAZZA P-V, BENARD G, GRANDES P & MARSICANO G. 2014b. Studying mitochondrial CB1 receptors: yes we can. *Mol Metab* 3, 339.

HEIFETS BD, CASTILLO PE. 2009. Endocannabinoid signaling and long-term synaptic plasticity. *Annu Rev Physiol*. 71:283–306.

HENKE, K., BUCK, A., WEBER, B. & WIESER, H. G. 1997. Human hippocampus establishes associations in memory. *Hippocampus*. 7, 249–256.

HENRY DJ, CHAVKIN C. 1995. Activation of inwardly rectifying potassium channels (GIRK1) by co-expressed rat brain cannabinoid receptors in *Xenopus* oocytes. *Neurosci Lett*. 186(2-3):91-4. doi: 10.1016/0304-3940(95)11289-9.

HENTGES, S.T., LOW, M.J. & WILLIAMS, J.T. 2005. Differential regulation of synaptic inputs by constitutively released endocannabinoids and exogenous cannabinoids. *J Neurosci*, 25, 9746-51.

HERKENHAM M, GROEN BG, LYNN AB, DE COSTA BR, RICHFIELD EK. 1991. Neuronal localization of cannabinoid receptors and second messengers in mutant mouse cerebellum. *Brain Res*. 552:301–310.

HERKENHAM, M., LYNN, A.B., DECOSTA, B.R. & RICHFIELD, E.K. 1991. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res*. 547, 267–274.

HERKENHAM, MILES, et al. 1990. Cannabinoid receptor localization in brain. *Proceedings of the national Academy of sciences*. 87.5: 1932-1936.

HERMANN, H., MARSICANO, G. & LUTZ, B. 2002. Coexpression of the cannabinoid receptor type 1 with dopamine and serotonin receptors in distinct neuronal subpopulations of the adult mouse forebrain. *Neuroscience*, 109, 451-60.

HIGGS, S., WILLIAMS, C.M., AND KIRKHAM, T.C. 2003. Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after delta(9)-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. *Psychopharmacology (Berl.)* 165, 370–377.

HILDEBRANDT, A.L., KELLY-SULLIVAN, D.M., AND BLACK, S.C. 2003. Antiobesity effects of chronic cannabinoid CB1 receptor antagonist treatment in diet- induced obese mice. *Eur. J. Pharmacol.* 462, 125–132.

HILL M.N. AND GORZALKA B.B. 2005. Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? *Behavioural Pharmacology*, vol. 16, no. 5- 6, pp. 333–352.

HILL M.N., HO W.-S.V., SINOPOLI K.J., VIAU V., HILLARD C.J., AND GORZALKA B.B. 2006. Involvement of the endocannabinoid system in the ability of long-term tricyclic antidepressant treatment to suppress stress-induced activation of the hypothalamic-pituitary-adrenal axis. *Neuropsychopharmacology*, vol. 31, no. 12, pp. 2591–2599.

HILLARD CJ, MANNA S, GREENBERG MJ, DICAMELLI R, ROSS RA, STEVENSON LA, MURPHY V, PERTWEE RG, CAMPBELL WB. 1999. Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). *J Pharmacol Exp Ther.* 289(3):1427-33.

HIRRLINGER, P. G., SCHELLER, A., BRAUN, C., HIRRLINGER, J. & KIRCHHOFF, F. 2006. Temporal control of gene recombination in astrocytes by transgenic expression of the tamoxifen-inducible DNA recombinase variant CreERT2. *Glia*, 54, 11-20.

HJORTH-SIMONSEN A. 1973. Some intrinsic connections of the hippocampus in the rat: an experimental analysis. *J Comp Neurol.* 147(2):145-61. doi: 10.1002/cne.901470202.

HO VM, LEE JA, MARTIN KC. 2011. The cell biology of synaptic plasticity. *Science* 334(6056):623-8. doi: 10.1126/science.1209236.

HOHMANN AG, HERKENHAM M. 1998. Regulation of cannabinoid and mu opioid receptors in rat lumbar spinal cord following neonatal capsaicin treatment. *Neurosci Lett.* 252:13–16.

HOHMANN AG, HERKENHAM M. 1999. Cannabinoid receptors undergo axonal flow in sensory nerves. *Neuroscience*. 92:1171–1175.

HOJLUND, K., MOGENSEN, M., SAHLIN, K., BECK-NIELSEN, H., 2008. Mitochondrial dysfunction in type 2 diabetes and obesity. *Endocrinology and Metabolism Clinics of North America* 37:713e731 x.

HOLLIS F, VAN DER KOOIJ MA, ZANOLETTI O, et al. 2015. Mitochondrial function in the brain links anxiety with social subordination. *Proc Natl Acad Sci U S A*. 112(50): 15486–91.

HOOKER, W.D. & JONES, R.T. 1987. Increased susceptibility to memory intrusions and the Stroop interference effect during acute marijuana intoxication. *Psychopharmacology (Berl.)* 91, 20–24.

HOWARD, M. W. & EICHENBAUM, H. 2015. Time and space in the hippocampus. *Brain Res*, 1621, 345-54.

HOWLETT AC, BARTH F, BONNER TI, et al. 2002. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev*. 54(2): 161–202.

HOWLETT AC. 2002. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat*. 68-69:619-31. doi: 10.1016/s0090-6980(02)00060-6. PMID: 12432948.

HOWLETT, A. C. & FLEMING, R. M. 1984. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Molecular pharmacology*, 26, 532-8.

HOWLETT, A. C., QUALY, J. M. & KHACHATRIAN, L. L. 1986. Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol Pharmacol*, 29, 307-13.

HOWLETT, AC. 1984. Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. *Life Sci*. 35 (17)): p. 1803-10.

HRYHOROWICZ, S., KACZMAREK-RYS, M., ANDRZEJEWSKA, A., STASZAK, K., HRYHOROWICZ, M., KORCZ, A., AND SLOMSKI, R. 2019. Allosteric modulation of cannabinoid receptor 1- current challenges and future opportunities. *Int. J. Mol. Sci*. 20, 1–19.

HU SSJ, HO YC, CHIOU LC. 2014. No more pain upon G(q)-protein-coupled receptor activation: role of endocannabinoids. *Eur J Neurosci* 39: 467– 484.

HU, S. S.-J. & MACKIE, K. 2015. Distribution of the Endocannabinoid System in the Central Nervous System. In: PERTWEE, R. G. (ed.) *Endocannabinoids*. Cham: Springer International Publishing;

HUA T, VEMURI K, NIKAS SP, LAPRAIRIE RB, WU Y, QU L et al. 2017. Crystal structures of agonist-bound human cannabinoid receptor CB(1). *Nature* 547: 468–471.

HUA T, VEMURI K, PU M, QU L, HAN GW, WU Y et al. 2016. Crystal structure of the human cannabinoid receptor CB1. *Cell* 167: 750–762 e714.

HUANG YY, KANDEL ER. 1995. D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. *Proc Natl Acad Sci U S A.* 92:2446–2450.

HUMEAU Y, CHOQUET D. 2019. The next generation of approaches to investigate the link between synaptic plasticity and learning. *Nat Neurosci.* 2019 Oct;22(10):1536-1543. doi: 10.1038/s41593-019-0480-6.

HUNGUND BL, SZAKALL I, ADAM A, BASAVARAJAPPA BS, VADASZ C. 2003. Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. *J Neurochem* 84: 698 –704.

HURST, D. P., GARAI, S., KULKARNI, P. M., SCHAFFER, P. C., REGGIO, P. H., & THAKUR, G. A. 2019. Identification of CB1 Receptor Allosteric Sites Using Force-Biased MMC Simulated Annealing and Validation by Structure-Activity Relationship Studies. *ACS medicinal chemistry letters*, 10(8), 1216–1221.

HURST, D. P., GROSSFIELD, A., LYNCH, D. L., FELLER, S., ROMO, T. D., GAWRISCH, K., PITMAN, M. C., & REGGIO, P. H. 2010. A lipid pathway for ligand binding is necessary for a cannabinoid G protein-coupled receptor. *The Journal of biological chemistry*, 285(23),17954–17964.

IRANNEJAD, R., AND VON ZASTROW, M. 2014. GPCR signaling along the endocytic pathway. *Current opinion in cell biology* 27, 109-116.

IRANNEJAD, R., PESSINO, V., MIKA, D. et al. 2017. Functional selectivity of GPCR-directed drug action through location bias. *Nat Chem Biol* 13, 799–806.

IRANNEJAD, R., TOMSHINE, J.C., TOMSHINE, J.R., CHEVALIER, M., MAHONEY, J.P., STEYAERT, J., RASMUSSEN, S.G., SUNAHARA, R.K., EL-SAMAD, H., HUANG, B., et al. 2013. Conformational biosensors reveal GPCR signalling from endosomes. *Nature* 495, 534-538.

ITO HT, SCHUMAN EM. 2007. Frequency-dependent gating of synaptic transmission and plasticity by dopamine. *Front Neural Circuits.* 1:1.

IVERSEN, L. 2003. Cannabis and the brain. *Brain*. 126, 1252–1270.

JABER M, ROBINSON SW, MISSALE C, CARON MG. 1996. Dopamine receptors and brain function. *Neuropharmacology*. 35(11):1503–1519. doi: 10.1016/S0028-3908(96)00100-1.

JAGER, G., AND WITKAMP, R.F. 2014. The endocannabinoid system and appetite: relevance for food reward. *Nutr. Res. Rev.* 27, 172–185.

JALINK K, MOOLENAAR WH. 2010. G protein-coupled receptors: the inside story. *Bioessays*. 32(1):13-6. doi: 10.1002/bies.200900153.

JAMSHIDI, N., AND TAYLOR, D.A. 2001. Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. *Br. J. Pharmacol.* 134, 1151–1154.

JIANG W., ZHANG Y., XIAO L., et al. 2005. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. *Journal of Clinical Investigation*, vol. 115, no. 11, pp. 3104–3116.

JIMENEZ-BLASCO D, BUSQUETS-GARCIA A, HEBERT- CHATELAIN E, SERRAT R, VICENTE-GUTIERREZ C, IOANNIDOU C, GOMEZ-SOTRES P, LOPEZ-FABUEL I, RESCH-BEUSHER M, RESEL E et al. 2020. Glucose metabolism links astroglial mitochondria to cannabinoid effects. *Nature* 583, 603–608.

JOHNSON SW, NORTH RA. 1992. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neurosci* 12:483–488.

JONG, Y.J., SERGIN, I., PURGERT, C.A., AND O'MALLEY, K.L. 2014. Location-dependent signaling of the group 1 metabotropic glutamate receptor mGlu5. *Molecular pharmacology* 86, 774-785.

JORGENSON, L. A., NEWSOME, W. T., ANDERSON, D. J., BARGMANN, C. I., BROWN, E. N., DEISSEROTH, K., DONOGHUE, J. P., HUDSON, K. L., LING, G. S., MACLEISH, P. R., MARDER, E., NORMANN, R. A., SANES, J. R., SCHNITZER, M. J., SEJNOWSKI, T. J., TANK, D. W., TSIEN, R. Y., UGURBIL, K., & WINGFIELD, J. C. 2015. The BRAIN Initiative: developing technology to catalyse neuroscience discovery. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 370(1668), 20140164.

JUERGENS E, GUETTLER A, ECKHORN R. 1999. Visual stimulation elicits locked and induced gamma oscillations in monkey intracortical- and EEG-potentials, but not in human EEG. *Exp Brain Res* 129:247–259.

JÜRGENSEN S, ANTONIO LL, MUSSI GE, BRITO-MOREIRA J, BOMFIM TR, DE FELICE FG, GARRIDO-SANABRIA ER, CAVALHEIRO ÉA, FERREIRA ST. 2011. Activation of D1/D5 dopamine receptors protects neurons from synapse dysfunction induced by amyloid-beta oligomers. *J Biol Chem.* 286:3270–3276.

KAMPRATH K, MARSICANO G, TANG J, MONORY K, BISOGNO T, DI MARZO V, LUTZ B, WOTJAK CT. 2006. Cannabinoid CB1 receptor mediates fear extinction via habituation-like processes. *J Neurosci.* 26(25):6677-86. doi: 10.1523/JNEUROSCI.0153-06.2006.

KANDEL, ER, SCHWARTZ JH AND JESSELL TM. 2000. *Principles of Neural Science.* New York: McGraw-Hill.

KANN, O., AND KOVACS, R. 2007. Mitochondria and neuronal activity. *American journal of physiology Cell physiology* 292, C641-657.

KANO M, OHNO-SHOSAKU T, HASHIMOTODANI Y, UCHIGASHIMA M, WATANABE M. 2009. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev.* 89:309–380.

KANO, M., OHNO-SHOSAKU, T. & MAEJIMA, T. 2002. Retrograde signaling at central synapses via endogenous cannabinoids. *Mol Psychiatry* 7, 234–235.

KATO, H.K., ASINOF, S.K., ISAACSON, J.S. 2017. Network-Level Control of Frequency Tuning in Auditory Cortex. *Neuron* 95, 412–423.e4.

KATONA I, SPERLÁGH B, SÍK A, KÁFALVI A, VIZI ES, MACKIE K & FREUND TF. 1999. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* 19, 4544–4558.

KATONA, I. et al. 2001. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. *J. Neurosci.* 21, 9506–9518.

KATONA, I., URBAN, G. M., WALLACE, M., LEDENT, C., JUNG, K. M., PIOMELLI, D., MACKIE, K. & FREUND, T. F. 2006. Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci*, 26, 5628-37.

KAUFMAN, A.M., GEILLER, T., AND LOSONCZY, A. 2020. A Role for the Locus Coeruleus in Hippocampal CA1 Place Cell Reorganization during Spatial Reward Learning. *Neuron* 105, 1018–1026.e4.

KELLOGG R, MACKIE K, STRAIKER A. 2009. Cannabinoid CB1 receptor-dependent long-term depression in autaptic excitatory neurons. *J Neurophysiol.* 102:1160–1171.

KEMP A, MANAHAN-VAUGHAN D. 2008. The hippocampal CA1 region and dentate gyrus differentiate between environmental and spatial feature encoding through long-term depression. *Cereb Cortex.* 18: 968–977.

KEMP N, BASHIR ZI. 2001. Long-term depression: a cascade of induction and expression mechanisms. *Prog Neurobiol.* 65(4):339-65. doi: 10.1016/s0301-0082(01)00013-2.

KEMPADOO, K. A., MOSHAROV, E. V., CHOI, S. J., SULZER, D. & KANDEL, E. R. 2016. Dopamine release from the locus coeruleus to the dorsal hippocampus promotes spatial learning and memory. *Proc Natl Acad Sci U S A*, 113, 14835-14840.

KIM J, ISOKAWA M, LEDENT C, ALGER BE. 2002. Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. *J Neurosci* 22: 10182–10191.

KIM, C. K., ADHIKARI, A. & DEISSEROTH, K. 2017. Integration of optogenetics with complementary methodologies in systems neuroscience. *Nat Rev Neurosci*, 18, 222-235.

KIMURA T, CHENG K, RICE KC, GAWRISCH K. 2009. Location, structure, and dynamics of the synthetic cannabinoid ligand CP-55,940 in lipid bilayers. *Biophys J* 96: 4916–4924.

KINSEY SG, LONG JZ, CRAVATT BF, LICHTMAN AH. 2010. Fatty acid amide hydrolase and monoacylglycerol lipase inhibitors produce anti-allodynic effects in mice through distinct cannabinoid receptor mechanisms. *J Pain* 11: 1420 –1428.

KINSEY SG, LONG JZ, O'NEAL ST, ABDULLAH RA, POKLIS JL, BOGER DL, et al. 2009. Blockade of endocannabinoid-degrading enzymes attenuates neuropathic pain. *J Pharmacol Exp Ther.* 330(3):902– 10. <https://doi.org/10.1124/jpet.109.155465>.

KIRKHAM, T.C., WILLIAMS, C.M., FEZZA, F., AND DI MARZO, V. 2002. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br. J. Pharmacol.* 136, 550–557.

KITAMURA, K., HÄUSSER, M. 2011. Dendritic Calcium Signaling Triggered by Spontaneous and Sensory-Evoked Climbing Fiber Input to Cerebellar Purkinje Cells In Vivo. *J. Neurosci.* 31, 10847–10858.

KLEIN TW, FRIEDMAN H, SPECTER SC. 1998. Marijuana, immunity and infection. *J Neuroimmunol.* 83:102– 115.

KLEIN TW, NEWTON C, LARSEN K, LU L, PERKINS I, NONG L, FRIEDMAN H. 2003. The cannabinoid system and immune modulation. *J Leukoc Biol.* 74:486–496.

KLEIN TW. 2005. Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat Rev Immunol.* 5:400– 411.

KOCH, J.E., AND MATTHEWS, S.M. 2001. Delta9-tetrahydrocannabinol stimulates palatable food intake in Lewis rats: effects of peripheral and central administration. *Nutr. Neurosci.* 4, 179–187.

KOCH, M., VARELA, L., KIM, J.G., KIM, J.D., HERNANDEZ-NUNO, F., SIMONDS, S.E., CASTORENA, C.M., VIANNA, C.R., ELMQUIST, J.K., MOROZOV, Y.M., et al. 2015. Hypothalamic POMC neurons promote cannabinoid-induced feeding. *Nature* 519, 45- 50.

KOOB GF. 1992. Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci.* 13:177–184.

KREITZER AC, REGEHR WG. 2001. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron.* 29:717–727.

KREITZER AC, REGEHR WG. 2002. Retrograde signaling by endocannabinoids. *Curr Opin Neurobiol* 12 : 324-30.

KUMAR U, PATEL SC. 2007. Immunohistochemical localization of dopamine receptor subtypes (D1R-D5R) in Alzheimer's disease brain. *Brain Res.* 1131:187–196.

KUYZNIEREWICZ, I., AND THOMSON, M. 2002. GTP-binding proteins G(salpha), G(ialpha), and Ran identified in mitochondria of human placenta. *Cell biology international* 26, 99-108.

LAN R, GATLEY J, LU Q, FAN P, FERNANDO SR, VOLKOW ND, PERTWEE R, MAKRIYANNIS A. 1999. Design and synthesis of the CB1 selective cannabinoid antagonist AM281: a potential human SPECT ligand. *AAPS PharmSci.* 1(2):E4. doi: 10.1208/ps010204. PMID: 11741201; PMCID: PMC2761119.

LATORRACA, NR, VENKATAKRISHNAN, AJ, AND DROR, RO. 2017. GPCR dynamics: structures in motion. *Chem. Rev.* 117, 139–155.

LAURBERG S. 1979. Commissural and intrinsic connections of the rat hippocampus. *J Comp Neurol.* 184(4):685-708. doi: 10.1002/cne.901840405.

LAVANCO G, CASTELLI V, BRANCATO A, TRINGALI G, PLESCIA F, CANNIZZARO C. 2018. The endocannabinoid-alcohol crosstalk: Recent advances on a bi-faceted target. *Clin Exp Pharmacol Physiol.* doi: 10.1111/1440-1681.12967. PMID: 29770478.

LAVIOLETTE SR, GRACE AA. 2006. The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. *Cell Mol Life Sci* 63: 1597–1613.

LAVIOLETTE SR, GRACE AA. 2006. The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. *Cell Mol Life Sci.* 63(14):1597-613. doi: 10.1007/s00018-006-6027-5.

LE FOLL B, GOLDBERG SR. 2004. Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences. *Neuroreport* 15: 2139 –2143.

LEDENT C, VALVERDE O, COSSU C, PETITET F, AUBERT LF, BESLOT F, BOHME GA, IMPERATO A, PEDRAZZINI T, ROQUES BP, VASSART G, FRATTA W, PARMENTIER M. 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* 283: 401– 404.

LE DOUX JE. 2000. Emotion circuits in the brain. *Annu Rev Neurosci.* 23:155-84. doi: 10.1146/annurev.neuro.23.1.155.

LEGATT AD, AREZZO J, VAUGHAN HG JR. 1980. Averaged multiple unit activity as an estimate of phasic changes in local neuronal activity: effects of volume-conducted potentials. *J Neurosci Methods* 2:203–217.

LEGAULT M, WISE RA. 2001. Novelty-evoked elevations of nucleus accumbens dopamine: dependence on impulse flow from the ventral subiculum and glutamatergic neurotransmission in the ventral tegmental area. *Eur J Neurosci.* 13:819–828.

- LEITNER, F.C., MELZER, S., LÜTCKE, H., PINNA, R., SEEBURG, P.H., HELMCHEN, F., MONYER, H. 2016. Spatially segregated feedforward and feedback neurons support differential odor processing in the lateral entorhinal cortex. *Nat. Neurosci.* 19, 935–944.
- LEMBERGER, T., PARLATO, R., DASSESSE, D., WESTPHAL, M., CASANOVA, E., TURIAULT, M., TRONCHE, F., SCHIFFMANN, S.N., AND SCHUETZ, G. 2007. Expression of Cre recombinase in dopaminergic neurons. *BMC Neurosci.* 8, 4.
- LEMON N, MANAHAN-VAUGHAN D. 2006. Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *J Neurosci.* 26:7723–7729.
- LEPORE M, VOREL SR, LOWINSON J, GARDNER EL. 1995. Conditioned place preference induced by Δ^9 -tetrahydrocannabinol: comparison with cocaine, morphine and food reward. *Life Sci* 56:2073–2080.
- LETZKUS JJ, WOLFF SB, LÜTHI A. 2015. Disinhibition, a Circuit Mechanism for Associative Learning and Memory. *Neuron.* 88(2):264-76. doi: 10.1016/j.neuron.2015.09.024.
- LI S, CULLEN WK, ANWYL R, ROWAN MJ. 2003. Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. *Nat Neurosci.* 6:526–531
- LI S., WANG, C., WANG, W., DONG, H., HOU, P., & TANG, Y. 2008. Chronic mild stress impairs cognition in mice: From brain homeostasis to behavior. *Life Sci.* 82 (17), 934-942.
- LI-BYARLAY, H., RITTSCHOF, C.C., MASSEY, J.H., PITTENDRIGH, B.R., AND ROBINSON, G.E. 2014. Socially responsive effects of brain oxidative metabolism on aggression. *Proceedings of the National Academy of Sciences of the United States of America* 111, 12533-12537.
- LICHTMAN, A.H. AND MARTIN, B.R. 2005. Cannabinoid tolerance and dependence. In *Cannabinoids* (Pertwee, R., ed.), pp. 691–717, Springer-Verlag.
- LIEN, A.D. & SCANZIANI, M. 2013. Tuned thalamic excitation is amplified by visual cortical circuits. *Nat. Neurosci.* 16,1315–1323.
- LIN, A.L. et al. 2013. Decreased in vitro mitochondrial function is associated with enhanced brain metabolism, blood flow, and memory in Surf1-deficient mice. *J. Cereb. Blood Flow Metab.* 33, 1605–1611

LISMAN, J., GRACE, A.A. & DUZEL, E. 2011. A neoHebbian framework for episodic memory; role of dopamine-dependent late LTP. *Trends Neurosci*, 34, 536-47.

LISMAN, J.E. & GRACE, A.A. 2005. The hippocampal-VTA loop: controlling the entry of information into long-term memory. *Neuron*, 46, 703-13.

LJUNGBERG T, APICELLA P, SCHULTZ W. 1992. Responses of monkey dopamine neurons during learning of behavioral reactions. *J Neurophysiol*. 67:145–163.

LLEDO PM, HJELMSTAD GO, MUKHERJI S, SODERLING TR, MALENKA RC, NICOLL RA. 1995. Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *Proc Natl Acad Sci U S A*. 92(24):11175-9. doi: 10.1073/pnas.92.24.11175.

LODGE DJ, GRACE AA. 2011. Hippocampal dysregulation of dopamine system function and the pathophysiology of schizophrenia. *Trends Pharmacol Sci*. 32:507–513.

LONG LE, CHESWORTH R, HUANG XF, MCGREGOR IS, ARNOLD JC, KARL T. 2010. A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. *Int J Neuropsychopharmacol* 13: 861– 876.

LU HC, MACKIE K. 2016. An Introduction to the Endogenous Cannabinoid System. *Biol Psychiatry*. 79(7):516-525. doi:10.1016/j.biopsych.2015.07.028.

LUEPTOW, L.M., ZHANG, C.-G., & O'DONNELL, J.M. 2015. Cyclic GMP-mediated memory enhancement in the object recognition test by inhibitors of phosphodiesterase-2 in mice. *Psychopharmacology (Berl)*.

LÜSCHER C, NICOLL RA, MALENKA RC, MULLER D. 2000. Synaptic plasticity and dynamic modulation of the postsynaptic membrane. *Nat Neurosci*. 3(6):545-50. doi: 10.1038/75714.

LYSSAND, J.S., AND BAJJALIEH, S.M. 2007. The heterotrimeric [corrected] G protein subunit G alpha i is present on mitochondria. *FEBS letters* 581, 5765-5768.

MACASKILL, A.F., AND KITTLER, J.T. 2010. Control of mitochondrial transport and localization in neurons. *Trends in cell biology* 20, 102-112.

MACKIE K, HILLE B. 1992. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci U S A*. 89(9):3825-9. doi: 10.1073/pnas.89.9.3825.

MACKIE K, LAI Y, WESTENBROEK R, MITCHELL R. 1995. Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J Neurosci.* 15(10):6552-61. doi: 10.1523/JNEUROSCI.15-10-06552.1995.

MACKIE K. 2005. Cannabinoid receptor homo- and heterodimerization. *Life Sci.* 77(14):1667-73. doi: 10.1016/j.lfs.2005.05.011. PMID: 15978631.

MACKIE, K. 2008. Cannabinoid receptors: where they are and what they do. *J Neuroendocrinol*, 20

MAEJIMA T., HASHIMOTO K., YOSHIDA T., AIBA A., KANO M. 2001. Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron.* 31:463–475.

MAGUIRE, E.A. 2001. Neuroimaging, memory and the human hippocampus. *Rev. Neurol. (Paris)* 157, 791–794.

MAHONEY JM, HARRIS RA. 1972. Effect of D9-tetrahydrocannabinol on mitochondrial processes. *Biochem Pharmacol.* 21(9):1217-26. doi: 10.1016/0006-2952(72)90283-3.

MAIONE S, DE PETROCELLIS L, DE NOVELLIS V, SCHIANO MORIELLO A, PETROSINO S, PALAZZO E, ROSSI FS, WOODWARD DF, DI MARZO V. 2007. Analgesic actions of N-arachidonoyl-serotonin, a fatty acid amide hydrolase inhibitor with antagonistic activity at vanilloid TRPV1 receptors. *Br J Pharmacol* 150: 766 –781.

MALATESTA, P., HACK, M. A., HARTFUSS, E., KETTENMANN, H., KLINKERT, W., KIRCHHOFF, F. & GOTZ, M. 2003. Neuronal or glial progeny: regional differences in radial glia fate. *Neuron*, 37, 751-64.

MALDONADO R, VALVERDE O, BERRENDERO F. 2006. Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci* 29: 225–232.

MALENKA RC, KAUER JA, PERKEL DJ, MAUK MD, KELLY PT, NICOLL RA, WAXHAM MN. 1989. An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. *Nature.* 340(6234):554-7. doi: 10.1038/340554a0.

MALENKA, R. C. & BEAR, M. F. 2004. LTP and LTD: an embarrassment of riches. *Neuron*, 44, 5-21.

MALINOW R, MAINEN ZF, HAYASHI Y. 2000. LTP mechanisms: from silence to four-lane traffic. *Curr Opin Neurobiol.* 10(3):352-7. doi: 10.1016/s0959-4388(00)00099-4.

MALINOW R, SCHULMAN H, TSIEN RW. 1989. Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science*. 245(4920):862-6. doi: 10.1126/science.2549638.

MALINOW, R. & MALENKA, R. C. 2002. AMPA Receptor Trafficking and Synaptic Plasticity. *Annual Review of Neuroscience*, 25, 103-126.

MALLET C, DAULHAC L, BONNEFONT J, LEDENT C, ETIENNE M, CHAPUY E, et al. 2008. Endocannabinoid and serotonergic systems are needed for acetaminophen-induced analgesia. *Pain*. 139(1):190–200. <https://doi.org/10.1016/j.pain.2008.03.030>.

MANJI, H., KATO, T., DI PROSPERO, N.A., NESS, S., BEAL, M.F., KRAMS, M., AND CHEN, G. 2012. Impaired mitochondrial function in psychiatric disorders. *Nature reviews Neuroscience* 13, 293-307.

MARCO E.-M., PEREZ-ALVAREZ L., BORCEL E., et al. 2004. Involvement of 5-HT1A receptors in behavioural effects of the cannabinoid receptor agonist CP 55,940 in male rats. *Behavioural Pharmacology*, vol. 15, no. 1, pp. 21–27.

MARIN S., MARCO E.-M., BISCAIA M., et al. 2003. Involvement of the κ -opioid receptor in the anxiogenic-like effect of CP 55,940 in male rats. *Pharmacology Biochemistry and Behavior*, vol. 74, no. 3, pp. 649–656.

MARRS WR, BLANKMAN JL, HORNE EA, THOMAZEAU A, LIN YH, COY J, BODOR AL, MUCCIOLI GG, HU SS, WOODRUFF G, FUNG S, LAFOURCADE M, ALEXANDER JP, LONG JZ, LI W, XU C, MÖLLER T, MACKIE K, MANZONI OJ, CRAVATT BF, STELLA N. 2010. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci*. 13(8):951-7. doi: 10.1038/nn.2601.

MARSICANO G, WOTJAK CT, AZAD SC, BISOGNO T, RAMMES G, CASCIO MG, HERMANN H, TANG J, HOFMANN C, ZIEGLGANSBERGER W, DI MARZO V, LUTZ B. 2002. The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418: 530 –534.

MARSICANO, G. & KUNER, R. 2008. Anatomical distribution of receptors, ligands and enzymes in the brain and the spinal cord: circuitries and neurochemistry. In: KOFALVI, A. (ed.) *Cannabinoids and the brain*. New York: Springer.

MARSICANO, G. & LAFENÊTRE, P. 2009. Roles of the endocannabinoid system in learning and memory. *Curr. Top. Behav. Neurosci*. 1, 201–230.

MARSICANO, G., AND LUTZ, B. 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur. J. Neurosci.* 11, 4213–4225.

MARSICANO, G., GOODENOUGH, S., MONORY, K., HERMANN, H., EDER, M., CANNICH, A., AZAD, S. C., CASCIO, M. G., GUTIERREZ, S. O., VAN DER STELT, M., LOPEZ-RODRIGUEZ, M. L., CASANOVA, E., SCHUTZ, G., ZIEGLGANSBERGER, W., DI MARZO, V., BEHL, C. & LUTZ, B. 2003. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*, 302, 84-8.

MARTELLOTTA MC, COSSU G, FATTORE L, GESSA GL, FRATTA G. 1998. Self-administration of the cannabinoid receptor agonist WIN 55,212-2 in drug-naïve rats. *Neuroscience* 85:327–330.

MARTIN M., LEDENT C., PARMENTIER M., MALDONADO R., AND VALVERDE O. 2002. Involvement of CB1 cannabinoid receptors in emotional behavior. *Psychopharmacology*, vol. 159, no. 4, pp. 379–387.

MARTIN SJ, GRIMWOOD PD, MORRIS RG. 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci.* 23:649-711. doi: 10.1146/annurev.neuro.23.1.649.

MARTIN WJ, LAI NK, PATRICK SL, TSOU K, WALKER JM. 1993. Antinociceptive actions of WIN 55,212-2 following intraventricular administration in rats. *Brain Res.* 629:300–304.

MARTIN, A. B., FERNANDEZ-ESPEJO, E., FERRER, B., GORRITI, M. A., BILBAO, A., NAVARRO, M., RODRIGUEZ DE FONSECA, F. & MORATALLA, R. 2008. Expression and function of CB1 receptor in the rat striatum: localization and effects on D1 and D2 dopamine receptor-mediated motor behaviors. *Neuropsychopharmacology*, 33, 1667-79.

MARTIN, S.J., DE HOZ, L. & MORRIS, R.G. 2005. Retrograde amnesia: neither partial nor complete hippocampal lesions in rats result in preferential sparing of remote spatial memory, even after reminding. *Neuropsychologia.* 43, 609–624.

MASON B.L. AND PARIANTE C.M. 2006. The effects of antidepressants on the hypothalamic-pituitary-adrenal axis. *Drug News & Perspectives*, vol. 19, no. 10, pp. 603–608.

MATEO, C. AVERMANN, M. GENTET, L.J. ZHANG, F. DEISSEROTH, K. PETERSEN, C.C.H. 2011. In Vivo Optogenetic Stimulation of Neocortical Excitatory Neurons Drives Brain-State-Dependent Inhibition. *Curr. Biol.* 21, 1593–1602.

MATSUDA, LISA A., et al. 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature.* 346.6284: 561.

MATTA, R., TIESSEN, A. N. & CHOLERIS, E. 2017. The Role of Dorsal Hippocampal Dopamine D1-Type Receptors in Social Learning, Social Interactions, and Food Intake in Male and Female Mice. *Neuropsychopharmacology*.

MATTSON MP, GLEICHMANN M, CHENG A. 2008. Mitochondria in neuroplasticity and neurological disorders. *Neuron* 60: 748–766.

MAYER ML, WESTBROOK GL, GUTHRIE PB. 1984. Voltage-dependent block by Mg²⁺ of NMDA responses in spinal cord neurones. *Nature*. 309(5965):261-3. doi: 10.1038/309261a0.

MAZIER, W., SAUCISSE, N., GATTA-CHERIFI, B., AND COTA, D. 2015. The endocannabinoid system: pivotal orchestrator of obesity and metabolic disease. *Trends Endocrinol. Metab.* 26, 524–537.

MCARTHUR R.AND BORSINI F. 2006. Animal models of depression in drug discovery: a historical perspective. *Pharmacology Biochemistry and Behavior*, vol. 84, no. 3, pp. 436–452.

MCGLOTHLIN, W. H.W.L. 1968. The marihuana problem: an overview. *Am. J. Psychiatry* 125, 126–134.

MCINNES, J. 2013. Mitochondrial-associated metabolic disorders: foundations, pathologies and recent progress. *Nutrition & metabolism* 10, 63.

MCKEON PN, MATHUR BN. 2021. On location for cannabinoid control of multimodal behavior. *Neuron*. 109(9):1416-1418. doi: 10.1016/j.neuron.2021.04.013.

MCLAUGHLIN, P.J., WINSTON, K., SWEZEY, L., WISNIECKI, A., ABERMAN, J., TARDIF, D.J., BETZ, A.J., ISHIWARI, K., MAKRIYANNIS, A., AND SALAMONE, J.D. 2003. The cannabinoid CB1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. *Behav. Pharmacol.* 14, 583–588.

MECHOULAM R, BEN-SHABAT S, HANUS L, LIGUMSKY M, KAMINSKI NE et al. 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol*, 50 : 83-90.

MECHOULAM R, FRIDE E, BEN-SHABAT S, MEIRI U, HOROWITZ M. 1998. Carbachol, an acetylcholine receptor agonist, enhances production in rat aorta of 2-arachidonoyl glycerol, a hypotensive endocannabinoid. *Eur J Pharmacol*, 362 : R1-R3.

MECHOULAM, R. & PARKER, L. A. 2013. The endocannabinoid system and the brain. *Annu Rev Psychol*, 64, 21-47.

MECHOULAM, R. AND Y. GAONI, HASHISH. IV. 1965. The isolation and structure of cannabinolic cannabidiolic and cannabigerolic acids. *Tetrahedron*. 21 (5): p. 1223-9

MECHOULAM, R. AND Y. GAONI. 1965. A Total Synthesis of Δ^1 -Tetrahydrocannabinol, the Active Constituent of Hashish. *J Am Chem Soc*. 87: p. 3273- 5.

MECHOULAM, R., HANUS, L. O., PERTWEE, R. & HOWLETT, A. C. 2014. Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nat Rev Neurosci*, 15, 757-64.

MELIS M, PISTIS M, PERRA S, MUNTONI AL, PILLOLLA G, GESSA GL. 2004. Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in ventral tegmental area dopamine neurons through activation of CB1 receptors. *J Neurosci* 24:53–62.

MELIS M, PISTIS M. 2014. Targeting the interaction between fatty acid ethanolamides and nicotinic receptors: therapeutic perspectives. *Pharmacol Res* 86: 42– 49.

MENDIZABAL-ZUBIAGA J, MELSER S, BÉNARD G, RAMOS A, REGUERO L, ARRABAL S, ELEZGARAI I, GERRIKAGOITIA I, SUAREZ J, RODRÍGUEZ DE FONSECA F, PUENTE N, MARSICANO G, GRANDES P. 2016. Cannabinoid CB1 Receptors Are Localized in Striated Muscle Mitochondria and Regulate Mitochondrial Respiration. *Front Physiol*. 7:476. doi: 10.3389/fphys.2016.00476.

MENG ID, MANNING BH, MARTIN WJ, FIELDS HL. 1998. An analgesia circuit activated by cannabinoids. *Nature (Lond)*. 395:381–383.

MISSALE C, NASH SR, ROBINSON SW, JABER M, CARON MG. 1998. Dopamine receptors: from structure to function. *Physiological reviews*. 78(1):189–225.

MONCADA D, BALLARINI F, MARTINEZ MC, FREY JU, VIOLA H. 2011. Identification of transmitter systems and learning tag molecules involved in behavioral tagging during memory formation. *Proc Natl Acad Sci U S A*. 108:12931–12936.

MONORY, K., BLAUDZUN, H., MASSA, F., KAISER, N., LEMBERGER, T., SCHUÖTZ, G., WOTJAK, C.T., LUTZ, B., AND MARSICANO, G. 2007. Genetic dissection of behavioural and autonomic effects of Δ^9 -tetrahydrocannabinol in mice. *PLoS Biol*. 5, e269.

MONORY, K., MASSA, F., EGERTOVA, M., EDER, M., BLAUDZUN, H., WESTENBROEK, R., KELSCH, W., JACOB, W., MARSCH, R., EKKER, M., LONG, J., RUBENSTEIN, J. L., GOEBBELS, S., NAVE, K. A., DURING, M., KLUGMANN, M., WOLFEL, B., DODT, H. U., ZIEGLGANSBERGER, W., WOTJAK, C. T., MACKIE, K., ELPHICK, M. R., MARSICANO, G. & LUTZ, B. 2006. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron*, 51, 455-66.

MOROZOV YM, BEN-ARI Y, FREUND TF. 2004. The spatial and temporal pattern of fatty acid amide hydrolase expression in rat hippocampus during postnatal development. *Eur J Neurosci* 20: 459–466.

MORRIS, R. G., ANDERSON, E., LYNCH, G. S. & BAUDRY, M. 1986. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature*, 319, 774-6.

MORRIS, R.G.M. 2007. in *The Hippocampus Book* (eds Andersen, P., Morris, R. G. M., Amaral, D. G., Bliss, T. V. P. & O'Keefe, J.) 581–714 (Oxford Univ. Press, New York).

MORRIS, R.G. 2013. NMDA receptors and memory encoding. *Neuropharmacology* 74, 32–40.

MUNRO, S., THOMAS, K. L. & ABU-SHAAR, M. 1993. Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, 365, 61-5.

MURTAS G, MARCONE GL, SACCHI S & POLLEGIONI L. 2020. L-serine synthesis via the phosphorylated pathway in humans. *Cell Mol Life Sci* 77, 5131–5148.

NABAVI, S., FOX, R., PROULX, C. D., LIN, J. Y., TSIEN, R. Y. & MALINOW, R. 2014. Engineering a memory with LTD and LTP. *Nature*, 511, 348-352.

NAGATOMO, K., SUGA, S., SAITOH, M., KOGAWA, M., KOBAYASHI, K., YAMAMOTO, Y. & YAMADA, K. 2017. Dopamine D1 Receptor Immunoreactivity on Fine Processes of GFAP-Positive Astrocytes in the Substantia Nigra Pars Reticulata of Adult Mouse. *Front Neuroanat*, 11, 3.

NAGY, A. 2000. Cre recombinase: the universal reagent for genome tailoring. *Genesis*, 26, 99-109.

NAVARRETE M, ARAQUE A. 2008. Endocannabinoids mediate neuron-astrocyte communication. *Neuron* 57:883–893.

NAVARRETE M, ARAQUE A. 2010. Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. *Neuron* 68:113–126.

NEVES, G., COOKE, S.F. & BLISS, T.V. 2008. Synaptic Plasticity, Memory and The Hippocampus: A Neural Network Approach to Causality. *Nat Rev Neurosci*, 9, 65-75.

NICOLL, R.A. 2003. Expression mechanisms underlying long-term potentiation: a postsynaptic view. *Philos Trans R Soc Lond B Biol Sci.* 358:721–726.

NICOLL, R.A. 2017. A Brief History of Long-Term Potentiation. *Neuron*, 93, 281-290.

NOGUCHI A, IKEGAYA Y, MATSUMOTO N. 2021. In Vivo Whole-Cell Patch-Clamp Methods: Recent Technical Progress and Future Perspectives. *Sensors (Basel)*. 21(4):1448. doi: 10.3390/s21041448.

NOWAK L, BREGESTOVSKI P, ASCHER P, HERBET A, PROCHIANTZ A. 1984. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature*. 307(5950):462-5. doi: 10.1038/307462a0.

O'CARROLL CM, MARTIN SJ, SANDIN J, FRENGUELLI B, MORRIS RG. 2006. Dopaminergic modulation of the persistence of one-trial hippocampus-dependent memory. *Learn Mem.* 13:760–769.

OBIEN, M. E., DELIGKARIS, K., BULLMANN, T., BAKKUM, D. J., & FREY, U. 2014. Revealing neuronal function through microelectrode array recordings. *Frontiers in Neuroscience*, 8, 423.

OHNO-SHOSAKU T, MAEJIMA T, KANO M. 2001. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron*. 29:729–738.

OHNO-SHOSAKU T., TSUBOKAWA H., MIZUSHIMA I., YONEDA N., ZIMMER A., KANO M. 2002. Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. *J. Neurosci.* 22:3864–3872.

OKAMOTO Y, MORISHITA J, TSUBOI K, TONAI T, UEDA N. 2004. Molecular characterization of a phospholipase D generating anandamide and its congeners. *J Biol Chem*. 279(7):5298-305. doi: 10.1074/jbc.M306642200. PMID: 14634025.

OLIVEIRA DA CRUZ JF, BUSQUETS-GARCIA A, ZHAO Z, VARILH M, LAVANCO G, BELLOCCHIO L, ROBIN L, CANNICH A, JULIO-KALAJZIC F, LESTE-LASSERRE T et al. 2020a. Specific hippocampal interneurons shape consolidation of recognition memory. *Cell Rep* 32, 108046.

OLIVEIRA DA CRUZ, J.F., GOMIS-GONZALEZ, M., MALDONADO, R., MARSICANO, G., OZAITA, A. AND BUSQUETS-GARCIA, A. 2020b. An Alternative Maze to Assess Novel Object Recognition in Mice. *Bio-protocol* 10(12): e3651. DOI: 10.21769/BioProtoc.3651.

ONAVI E.S., GREEN M.R., AND MARTIN B.R. 1990. Pharmacological characterization of cannabinoids in the elevated plus maze. *Journal of Pharmacology and Experimental Therapeutics*, vol. 253, no. 3, pp. 1002–1009.

ORBAN, P. C., CHUI, D. & MARTH, J. D. 1992. Tissue- and site-specific DNA recombination in transgenic mice. *Proc Natl Acad Sci U S A*, 89, 6861-5.

OSEI-HYIAMAN, D., DEPETRILLO, M., PACHER, P., LIU, J., RADAIEVA, S., BATKAI, S., HARVEY-WHITE, J., MACKIE, K., OFFERTALER, L., WANG, L., AND KUNOS, G. 2005. Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *J. Clin. Invest.* 115, 1298–1305.

OTMAKHOVA NA, LISMAN JE. 1996. D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. *J Neurosci.* 16:7478–7486.

OVERTON HA, FYFE MC, REYNET C. 2008. GPR119, a novel G protein-coupled receptor target for the treatment of type 2 diabetes and obesity. *Br J Pharmacol.* 153 Suppl 1(Suppl 1):S76-81. doi: 10.1038/sj.bjp.0707529. PMID: 18037923; PMCID: PMC2268073.

PACHER, P., BATKAI, S., & KUNOS, G. 2006. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacological reviews*, 58(3), 389–462. <https://doi.org/10.1124/pr.58.3.2>

PAGANO ZOTTOLA, A.C., SORIA-GOMEZ, E., BONILLA-DEL-RÍO, I., MUGURUZA, C., TERRAL, G., ROBIN, L.M., DA CRUZ, J.F.O., REDON, B., LESTÉ-LASSERRE, T., TOLENTINO-CORTES, T., et al. 2020. A new mutant mouse model lacking mitochondrial-associated CB1 receptor. *bioRxiv*. 2003.2030.009472.

PAGOTTO, U., MARSICANO, G., COTA, D., LUTZ, B., AND PASQUALI, R. 2006. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocrine reviews* 27, 73-100.

PALA, A. & PETERSEN, C.C.H. 2015. In Vivo Measurement of Cell-Type-Specific Synaptic Connectivity and Synaptic Transmission in Layer 2/3 Mouse Barrel Cortex. *Neuron* 85, 68–75.

PALADE, G.E. 1953. An electron microscope study of the mitochondrial structure. *The journal of histochemistry and cytochemistry* 1, 188-211.

PALOMBA L, SILVESTRI C, IMPERATORE R, MORELLO G, PISCITELLI F, MARTELLA A, CRISTINO L, DI MARZO V. 2015. Negative Regulation of Leptin-induced Reactive Oxygen Species (ROS) Formation by Cannabinoid CB1 Receptor Activation in Hypothalamic Neurons. *J Biol Chem.* 290(22):13669-77. doi: 10.1074/jbc.M115.646885.

PAOLETTI, P., BELLONE, C. & ZHOU, Q. 2013. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nat Rev Neurosci*, 14, 383-400.

PARSONS, L., HURD, Y. 2015. Endocannabinoid signalling in reward and addiction. *Nat Rev Neurosci* 16, 579–594.

PASPALAS, C. D. & GOLDMAN-RAKIC, P. S. 2005. Presynaptic D1 dopamine receptors in primate prefrontal cortex: target-specific expression in the glutamatergic synapse. *J Neurosci*, 25, 1260-7

PASTALKOVA E, SERRANO P, PINKHASOVA D, WALLACE E, FENTON AA, SACKTOR TC. 2006. Storage of spatial information by the maintenance mechanism of LTP. *Science* 313: 1141–1144.

PATEL S, HILLARD CJ. 2006. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. *J Pharmacol Exp Ther* 318: 304 –311.

PATON, WDM. AND PERTWEE, RG. 1973. The actions of cannabis in man. In: Nahas, GG.; Paton, WDM., editors. *Marijuana: Chemistry, Pharmacology, Metabolism and Clinical Effects*. Pergamon Press; Oxford: 1973. p. 735-738.

PELKEY KA, CHITTAJALLU R, CRAIG MT, TRICOIRE L, WESTER JC & MCBAIN CJ. 2017. Hippocampal gabaergic inhibitory interneurons. *Physiol Rev* 97, 1619–1747.

PEREA G, NAVARRETE M & ARAQUE A. 2009. Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci* 32, 421–431.

PERIO, A., BARNOUIN, M.C., PONCELET, M., AND SOUBRIE, P. 2001. Activity of SR141716 on post-reinforcement pauses in operant responding for sucrose reward in rats. *Behav. Pharmacol.* 12, 641–645.

PERTWEE RG. 2001. Cannabinoid receptors and pain. *Prog Neurobiol.* 63:569-611.

PERTWEE, R. G., HOWLETT, A. C., ABOOD, M. E., ALEXANDER, S. P., DI MARZO, V., ELPHICK, M. R., GREASLEY, P. J., HANSEN, H. S., KUNOS, G., MACKIE, K., MECHOULAM, R., & ROSS, R. A. 2010. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB₁ and CB₂. *Pharmacological reviews*, 62(4), 588–631.

PETERSEN, C.C.H., GRINVALD, A., SAKMANN, B. 2003. Spatiotemporal Dynamics of Sensory Responses in Layer 2/3 of Rat Barrel Cortex Measured In Vivo by Voltage-Sensitive Dye Imaging Combined with Whole-Cell Voltage Recordings and Neuron Reconstructions. *J. Neurosci.* 23, 1298–1309.

PETTIT DL, PERLMAN S, MALINOW R. 1994. Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons. *Science.* 266(5192):1881-5. doi: 10.1126/science.7997883.

PICARD M. 2015. Mitochondrial synapses: intracellular communication and signal integration. *Trends Neurosci* 38: 468–474.

PICCINI P. 2004. Neurodegenerative movement disorders: the contribution of functional imaging. *Current opinion in neurology.* 17(4):459–466. doi: 10.1097/01.wco.0000137538.84115.3c.

PIOMELLI D, BELTRAMO M, GLASNAPP S, LIN SY, GOUTOPOULOS A et al. 1999. Structural determinants for recognition and translocation by the anandamide transporter. *Proc Natl Acad Sci USA*, 96: 5802-5807.

PIOMELLI D, GIUFFRIDA A, CALIGNANO A, RODRIGUEZ DE FONSECA F. 2000. The endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol Sci*, 21 : 218-224.

PIOMELLI D, HOHMANN AG, SEYBOLD V, HAMMOCK BD. 2014. A lipid gate for the peripheral control of pain. *J Neurosci* 34: 15184 –15191.

PISCITELLI F, DI MARZO V. 2012. “Redundancy” of endocannabinoid inactivation: new challenges and opportunities for pain control. *ACS Chem Neurosci* 3: 356 –363.

PRICKAERTS, J., DE VENDE, J., HONIG, W., STEINBUSCH, H.W.M., & BLOKLAND, A. 2002. cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. *Eur. J. Pharmacol.* 436 (1-2), 83-87.

PUENTE, N., RIO, I.B., ACHICALLENDE, S., NAHIRNEY, P.C., AND GRANDES, P. 2019. High-resolution Immunoelectron Microscopy Techniques for Revealing Distinct Subcellular Type 1 Cannabinoid Receptor Domains in Brain. *Bio Protoc.* 9, e3145.

PUIGHERMANAL E, MARSICANO G, BUSQUETS-GARCIA A, LUTZ B, MALDONADO R & OZAITA A. 2009. Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nat Neurosci* 12, 1152– 1158.

PUIGHERMANAL, E., BUSQUETS-GARCIA, A., GOMIS-GONZALEZ, M., MARSICANO, G., MALDONADO, R. & OZAITA, A. 2013. Dissociation of the pharmacological effects of THC by mTOR blockade. *Neuropsychopharmacology*, 38, 1334-43.

PUIGHERMANAL, E., CUTANDO, L., BOUBAKER-VITRE, J., HONORE, E., LONGUEVILLE, S., HERVE, D. & VALJENT, E. 2017. Anatomical and molecular characterization of dopamine D1 receptor-expressing neurons of the mouse CA1 dorsal hippocampus. *Brain Struct Funct*, 222, 1897-1911.

RAEFSKY SM, MATTSON MP. 2017. Adaptive responses of neuronal mitochondria to bioenergetic challenges: roles in neuroplasticity and disease resistance. *Free Radic Biol Med* 102: 203–216.

RAINVILLE P. 2002. Brain mechanisms of pain affect and pain modulation. *Curr Opin Neurobiol.* 12(2):195–204

RAMIREZ, S., LIU, X., LIN, P. A., SUH, J., PIGNATELLI, M., REDONDO, R. L., RYAN, T. J. & TONEGAWA, S. 2013. Creating a false memory in the hippocampus. *Science*, 341, 387-91.

RANGANATHAN, M. & D'SOUZA, D.C. 2006. The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology (Berl.)* 188, 425–444.

RANGARAJU V, CALLOWAY N, RYAN TA. 2014. Activity-driven local ATP synthesis is required for synaptic function. *Cell* 156: 825–835.

REALINI N, VIGANO D, GUIDALI C, ZAMBERLETTI E, RUBINO T, PAROLARO D. 2011. Chronic URB597 treatment at adulthood reverted most depressive-like symptoms induced by adolescent exposure to THC in female rats. *Neuropharmacology* 60: 235–243

REDDY V, GROGAN D, AHLUWALIA M, SALLES ÉL, AHLUWALIA P, KHODADADI H, ALVERSON K, NGUYEN A, RAJU SP, GAUR P, BRAUN M, VALE FL, COSTIGLIOLA V, DHANDAPANI K, BABAN B, VAIBHAV K. 2020. Targeting the endocannabinoid system: a predictive, preventive, and

personalized medicine-directed approach to the management of brain pathologies. *EPMA J.* 11(2):217-250. doi: 10.1007/s13167-020-00203-4.

REGEHR WG, CAREY MR, BEST AR. 2009. Activity-dependent regulation of synapses by retrograde messengers. *Neuron.* 63:154–170.

REGGIO PH. 2010. Endocannabinoid binding to the cannabinoid receptors: what is known and what remains unknown. *Curr Med Chem.* 17(14):1468-86. doi: 10.2174/092986710790980005.

REINHOLD, K., LIEN, A.D., SCANZIANI, M. 2015. Distinct recurrent versus afferent dynamics in cortical visual processing. *Nat.Neurosci.* 18, 1789–1797.

REY AA, PURRIO M, VIVEROS MP, LUTZ B. 2012. Biphasic effects of cannabinoids in anxiety responses: CB1 and GABA(B) receptors in the balance of GABAergic and glutamatergic neurotransmission. *Neuropsychopharmacology.* 37(12):2624-34. doi: 10.1038/npp.2012.123.

REYMANN KG, FREY JU. 2007. The late maintenance of hippocampal LTP: requirements, phases, 'synaptic tagging', 'late-associativity' and implications. *Neuropharmacology.* 52(1):24-40. doi: 10.1016/j.neuropharm.2006.07.026.

RICHARDSON JD, AANONSEN L, HARGREAVES KM. 1997. SR141716A, a cannabinoid receptor antagonist, produces hyperalgesia in untreated mice. *Eur J Pharmacol.* 319:R3–R4.

RICHARDSON JD, AANONSEN L, HARGREAVES KM. 1998. Antihyperalgesic effects of spinal cannabinoids. *Eur J Pharmacol.* 345:145–153.

RIEDEL, G. & DAVIES, S. N. 2005. Cannabinoid Function in Learning, Memory and Plasticity. In: PERTWEE, R. G. (ed.) *Cannabinoids.* Berlin, Heidelberg: Springer Berlin Heidelberg.

RIEGEL, A.C. AND LUPICA, C.R. 2004. Independent presynaptic and postsynaptic mechanisms regulate endocannabinoid signaling at multiple synapses in the ventral tegmental area. *J. Neurosci.* 24, 11070–11078.

RINALDI-CARMONA M, BARTH F, HÉAULME M, SHIRE D, CALANDRA B, CONGY C, MARTINEZ S, MARUANI J, NÉLIAT G, CAPUT D, et al. 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* 350(2-3):240-4. doi: 10.1016/0014-5793(94)00773-x. PMID: 8070571.

ROBBE D, ALONSO G, DUCHAMP F, BOCKAERT J, MANZONI OJ. 2001. Localization and mechanisms of action of cannabinoid receptors at the glutamatergic synapses of the mouse nucleus accumbens. *J Neurosci* 21:109–116.

ROBBE D, KOPF M, REMAURY A, BOCKAERT J, MANZONI OJ. 2002. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc Natl Acad Sci U S A*. 99:8384–8388.

ROBIN LM, OLIVEIRA DA CRUZ JF, LANGLAIS VC, MARTIN- FERNANDEZ M, METNA-LAURENT M, BUSQUETS-GARCIA A, BELLOCCHIO L, SORIA-GOMEZ E, PAPOUIN T, VARILH M et al. 2018. Astroglial CB1 receptors determine synaptic D-serine availability to enable recognition memory. *Neuron* 98, 935–944.e5.

RODRIGUEZ DE FONSECA F., RUBIO P., MENZAGHI F., et al. 1996. Corticotropin-releasing factor (CRF) antagonist [D-Phe¹²,Nle^{21,38},CαMeLeu³⁷]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *Journal of Pharmacology and Experimental Therapeutics*, vol. 276, no. 1, pp. 56–64.

ROLFE, D.F., AND BROWN, G.C. 1997. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiological reviews* 77, 731-758.

ROMANELLI, R. J., WILLIAMS, J. T. & NEVE, K. A. 2010. Dopamine Receptor Signaling: Intracellular Pathways to Behavior. In: NEVE, K. A. (ed.) *The Dopamine Receptors*. Totowa, NJ: Humana Press.

ROSS RA. 2009. The enigmatic pharmacology of GPR55. *Trends Pharmacol Sci*. 30(3):156-63. doi: 10.1016/j.tips.2008.12.004. PMID: 19233486.

ROSSATO JI, BEVILAQUA LR, IZQUIERDO I, MEDINA JH, CAMMAROTA M. 2009. Dopamine controls persistence of long-term memory storage. *Science*. 325:1017–1020.

ROUBERTOUX, P.L. et al. 2003. Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice. *Nat. Genet.* 35, 65–69.

ROZENFELD, R., AND DEVI, L.A. 2008. Regulation of CB1 cannabinoid receptor trafficking by the adaptor protein AP-3. *FASEB journal*. 22, 2311-2322.

ROZENFELD, R., AND DEVI, L.A. 2008. Regulation of CB1 cannabinoid receptor trafficking by the adaptor protein AP-3. *FASEB J*. 22, 2311–2322.

RUBINO T, SALA M, VIGANO D, BRAIDA D, CASTIGLIONI C, LIMONTA V, GUIDALI C, REALINI N, PAROLARO D. 2007. Cellular mechanisms underlying the anxiolytic effect of low doses of peripheral Delta9-tetrahydrocannabinol in rats. *Neuropsychopharmacology* 32: 2036 – 2045.

RUBINO T, VIGANO D, REALINI N, GUIDALI C, BRAIDA D, CAPURRO V, CASTIGLIONI C, CHERUBINO F, ROMUALDI P, CANDELETTI S, SALA M, PAROLARO D. 2008. Chronic Delta(9)-tetrahydrocannabinol during adolescence provokes sex-dependent changes in the emotional profile in adult rats: behavioral and biochemical correlates. *Neuropsychopharmacology* 33: 2760 –2771.

RUEDA D, GALVE-ROPERH I, HARO A, GUZMÁN M. 2000. The CB(1) cannabinoid receptor is coupled to the activation of c-Jun N-terminal kinase. *Mol Pharmacol.* 58(4):814-20. doi: 10.1124/mol.58.4.814.

RUGGIERO A, KATSENELSON M, SLUTSKY I. 2021. Mitochondria: new players in homeostatic regulation of firing rate set points. *Trends Neurosci.* 44(8):605-618. doi: 10.1016/j.tins.2021.03.002.

RUTTEN, K., et al. 2007. Time-dependent involvement of cAMP and cGMP in consolidation of object memory: studies using selective phosphodiesterase type 2, 4 and 5 inhibitors. *Eur. J. Pharmacol.* 558 (1-3), 107-12.

RYBERG E, LARSSON N, SJÖGREN S, HJORTH S, HERMANSSON NO, LEONOVA J, ELEBRING T, NILSSON K, DRMOTA T, GREASLEY PJ. 2007. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol.* 152(7):1092-101. doi: 10.1038/sj.bjp.0707460.

SALES-CARBONELL, C., RUEDA-OROZCO, P. E., SORIA-GOMEZ, E., BUZSAKI, G., MARSICANO, G. & ROBBE, D. 2013. Striatal GABAergic and cortical glutamatergic neurons mediate contrasting effects of cannabinoids on cortical network synchrony. *Proc Natl Acad Sci U S A*, 110, 719-24.

SANCHIS-SEGURA, C., CLINE, B.H., MARSICANO, G., LUTZ, B., AND SPANAGEL, R. 2004. Reduced sensitivity to reward in CB1 knockout mice. *Psychopharmacology (Berl.)* 176, 223–232.

SAÑUDO-PEÑA MC, STRANGMAN NM, MACKIE K, WALKER JM, TSOU K. 1999. CB1 receptor localization in spinal cord and roots, dorsal root ganglion, and peripheral nerve. *Acta Pharmacol Sin.* 12:1115– 1120.

SARANTIS K, ANTONIOU K, MATSOKIS N, ANGELATOU F. 2012. Exposure to novel environment is characterized by an interaction of D1/NMDA receptors underlined by phosphorylation of the NMDA and AMPA receptor subunits and activation of ERK1/2 signaling, leading to epigenetic changes and gene expression in rat hippocampus. *Neurochem Int.* 60:57–67.

SAUER, B. & HENDERSON, N. 1988. Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. *Proc Natl Acad Sci U S A*, 85, 5166-70.

SAWAGUCHI, T. & GOLDMAN-RAKIC, P.S. 1991. D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* 251, 947–950.

SCARANTE, F. F., VILA-VERDE, C., DETONI, V. L., FERREIRA-JUNIOR, N. C., GUIMARÃES, F. S., & CAMPOS, A. C. 2017. Cannabinoid Modulation of the Stressed Hippocampus. *Frontiers in molecular neuroscience*, 10, 411. <https://doi.org/10.3389/fnmol.2017.00411,20,23>

SCHEGGIA, D., ZAMBERLETTI, E., REALINI, N., MEREU, M., CONTARINI, G., FERRETTI, V., MANAGO, F., MARGIANI, G., BRUNORO, R., RUBINO, T., DE LUCA, M. A., PIOMELLI, D., PAROLARO, D. & PAPALETTO, F. 2017. Remote memories are enhanced by COMT activity through dysregulation of the endocannabinoid system in the prefrontal cortex. *Mol Psychiatry*.

SCHNEEBERGER, M., DIETRICH, M.O., SEBASTIAN, D., IMBERNON, M., CASTANO, C., GARCIA, A., et al., 2013. Mitofusin 2 in POMC neurons connects ER stress with leptin resistance and energy imbalance. *Cell* 155:172e187.

SCHULTZ W, APICELLA P, LJUNGBERG T. 1993. Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J Neurosci*. 13:900–913.

SCHURR A, LIVNE A. 1975. Proceedings: Differential inhibition of mitochondrial monoamine oxidase from brain by hashish components. *Isr J Med Sci*. 11(11):1188.

SCHWARTZKROIN PA, WESTER K. 1975. Long-lasting facilitation of a synaptic potential following tetanization in the in vitro hippocampal slice. *Brain Res*. 89(1):107-19. doi: 10.1016/0006-8993(75)90138-9.

SHAO Z, YIN J, CHAPMAN K, GRZEMSKA M, CLARK L, WANG J et al. 2016. High-resolution crystal structure of the human CB1 cannabinoid receptor. *Nature* doi:10.1038/nature20613.

SHI, H., CHEER, K., SIMANAINEN, U., LESMANA, B., MA, D., HEW, J. J., PARUNGAO, R. J., LI, Z., COOPER, M. S., HANDELSMAN, D. J., MAITZ, P. K., & WANG, Y. 2021. The contradictory role of androgens in cutaneous and major burn wound healing. *Burns & trauma*, 9, tkaa046.

SHOSHAN-BARMATZ, V., AND GINCEL, D. 2003. The voltage-dependent anion channel: characterization, modulation, and role in mitochondrial function in cell life and death. *Cell biochemistry and biophysics* 39, 279-292.

SILVA AJ, STEVENS CF, TONEGAWA S, WANG Y. 1992. Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. *Science*. 257(5067):201-6. doi: 10.1126/science.1378648.

SILVA AJ. 2003. Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. *J Neurobiol*. 54(1):224-37. doi: 10.1002/neu.10169. PMID: 12486706.

SILVESTRI, C., AND DI MARZO, V. 2013. The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. *Cell Metab*. 17, 475–490.

SIMIAND, J., KEANE, M., KEANE, P.E., AND SOUBRIE, P. 1998. SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. *Behav. Pharmacol*. 9, 179–181.

SIMON V, COTA D. 2017. Mechanisms in Endocrinology: Endocannabinoids and metabolism: past, present and future. *Eur J Endocrinol*. 176(6):R309-R324. doi: 10.1530/EJE-16-1044. PMID: 28246151.

SIVITZ, W.I., YOREK, M.A., 2010. Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. *Antioxidants & Redox Signaling* 12:537e577.

SJÖSTRÖM PJ, TURRIGIANO GG, NELSON SB. 2003. Neocortical LTD via coincident activation of presynaptic NMDA and cannabinoid receptors. *Neuron*. 39(4):641-54. doi: 10.1016/s0896-6273(03)00476-8.

SMITH CC, GREENE RW. 2012. CNS dopamine transmission mediated by noradrenergic innervation. *J Neurosci*. 32:6072–6080.

SMITH WB, STARCK SR, ROBERTS RW, SCHUMAN EM. 2005. Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. *Neuron*. 45:765–779.

SORIA G, MENDIZABAL V, TOURINO C, ROBLEDO P, LEDENT C, PARMENTIER M, MALDONADO R, VALVERDE O. 2005. Lack of CB1 cannabinoid receptor impairs cocaine self-administration. *Neuropsychopharmacology* 30: 1670–1680.

SORIA-GOMEZ E, PAGANO ZOTTOLA AC, MARIANI Y, DESPREZ T, BARRESI M, BONILLA-DEL RÍO I, MUGURUZA C, LE BON-JEGO M, JULIO-KALAJZIĆ F, FLYNN R, TERRAL G, et al. 2021. Subcellular specificity of cannabinoid effects in striatonigral circuits. *Neuron*. 109(9):1513-1526.e11. doi: 10.1016/j.neuron.2021.03.007.

SORIA-GOMEZ, E., BELLOCCHIO, L., REGUERO, L., LEPOUSEZ, G., MARTIN, C., BENDAHMANE, M., RUEHLE, S., REMMERS, F., DESPREZ, T., MATIAS, I., et al. 2014a. The endocannabinoid system controls food intake via olfactory processes. *Nat. Neurosci.* 17, 407–415.

SORIA-GOMEZ, E., MASSA, F., BELLOCCHIO, L., RUEDA-OROZCO, P.E., CIOFI, P., COTA, D., LIET, S.H., PROSPERO-GARCIA, O., AND MARSICANO, G. 2014b. Cannabinoid type-1 receptors in the paraventricular nucleus of the hypothalamus inhibit stimulated food intake. *Neuroscience* 263, 46–53.

SORIA-GOMEZ, E., METNA, M., BELLOCCHIO, L., BUSQUETS-GARCIA, A. & MARSICANO, G. 2017. *Handbook of Neurobehavioral Genetics and Phenotyping*. 323-355.

SPIRA, M. E., & HAI, A. 2013. Multi-electrode array technologies for neuroscience and cardiology. *Nature Nanotechnology*, 8, 83–94.

SQUIRE LR, STARK CE, CLARK RE. 2004. The medial temporal lobe. *Annu Rev Neurosci.* 27:279-306. doi: 10.1146/annurev.neuro.27.070203.144130.

SQUIRE LR, WIXTED JT, CLARK RE. 2007. Recognition memory and the medial temporal lobe: a new perspective. *Nat Rev Neurosci.* 8(11):872-83. doi: 10.1038/nrn2154.

SQUIRE LR, ZOLA SM. 1998. Episodic memory, semantic memory, and amnesia. *Hippocampus.* 8(3):205-11. doi: 10.1002/(SICI)1098-1063(1998)8:3<205:AID-HIPO3>3.0.CO;2-I.

SQUIRE LR. 2009. Memory and brain systems: 1969-2009. *J Neurosci.* 29(41):12711-6. doi: 10.1523/JNEUROSCI.3575-09.2009.

STAROWICZ K, MAIONE S, CRISTINO L, PALAZZO E, MARABESE I, ROSSI F, DE NOVELLIS V, DI MARZO V. 2007. Tonic endovanilloid facilitation of glutamate release in brainstem descending antinociceptive pathways. *J Neurosci* 27: 13739 –13749.

STELLA N, SCHWEITZER P, PIOMELLI D. 1997. A second endogenous cannabinoid that modulates long-term potentiation. *Nature*, 388 : 773-778.

STEWART, M. G., MEDVEDEV, N. I., POPOV, V. I., SCHOEPFER, R., DAVIES, H. A., MURPHY, K., DALLERAC, G. M., KRAEV, I. V. & RODRIGUEZ, J. J. 2005. Chemically induced long-term potentiation increases the number of perforated and complex postsynaptic densities but does not alter dendritic spine volume in CA1 of adult mouse hippocampal slices. *Eur J Neurosci*, 21, 3368-78.

STRAMIELLO M, WAGNER JJ. 2008. D1/D5 receptor-mediated enhancement of LTP requires PKA, Src family kinases, and NR2B-containing NMDARs. *Neuropharmacology*. 55:871–877.

STRANGMAN NM, PATRICK SL, HOHMANN AG, TSOU K, WALKER JM. 1998. Evidence for a role of endogenous cannabinoids in the modulation of acute and tonic pain sensitivity. *Brain Res*. 813:323–328.

STROUD, D.A., AND RYAN, M.T. 2013. Mitochondria: organization of respiratory chain complexes becomes cristae-lized. *Current biology: CB* 23, R969-971.

SUGIURA T, KISHIMOTO S, OKA S, GOKOH M. 2006. Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Prog Lipid Res*. 45(5):405-46. doi: 10.1016/j.plipres.2006.03.003. PMID: 16678907.

SUGIURA, T., KONDO, S., SUKAGAWA, A., NAKANE, S., SHINODA, A., ITOH, K., YAMASHITA, A., WAKU, K., 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun*. 215, 89–97.

SUN, T., QIAO, H., PAN, P.Y., CHEN, Y., AND SHENG, Z.H. 2013. Motile axonal mitochondria contribute to the variability of presynaptic strength. *Cell reports* 4, 413- 419.

SUTTON MA, SCHUMAN EM. 2006. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell*. 127(1):49-58. doi: 10.1016/j.cell.2006.09.014.

SUZUKI, A., STERN, S.A., BOZDAGI, O., HUNTLEY, G.W., WALKER, R.H., MAGISTRETTI, P.J., AND ALBERINI, C.M. 2011. Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810-823.

SWANSON LW, WYSS JM, COWAN WM. 1978. An autoradiographic study of the organization of intrahippocampal association pathways in the rat. *J Comp Neurol*. 181(4):681-715. doi: 10.1002/cne.901810402.

SWANSON-PARK JL, COUSSENS CM, MASON-PARKER SE, RAYMOND CR, HARGREAVES EL, DRAGUNOW M, COHEN AS, ABRAHAM WC. 1999. A double dissociation within the hippocampus of dopamine D1/D5 receptor and beta-adrenergic receptor contributions to the persistence of long-term potentiation. *Neuroscience*. 92:485–497.

SWEATT JD. 2004. Hippocampal function in cognition. *Psychopharmacology (Berl)*. 174(1):99-110. doi: 10.1007/s00213-004-1795-9.

TAANMAN, J.W. 1999. The mitochondrial genome: structure, transcription, translation and replication. *Biochimica et biophysica acta* 1410, 103-123.

TAKASAKI, KEVIN T., DING, JUN B. & SABATINI, BERNARDO L. 2013. Live-Cell Super-resolution Imaging by Pulsed STED Two-Photon Excitation Microscopy. *Biophysical Journal*, 104, 770-777.

TAKEUCHI, T., DUSZKIEWICZ, A. J., SONNEBORN, A., SPOONER, P. A., YAMASAKI, M., WATANABE, M., SMITH, C. C., FERNANDEZ, G., DEISSEROTH, K., GREENE, R. W. & MORRIS, R. G. 2016. Locus coeruleus and dopaminergic consolidation of everyday memory. *Nature*, 537, 357-362.

TANDA G, LODDO P, DI CHIARA G. 1999. Dependence of mesolimbic dopamine transmission on delta9- tetrahydrocannabinol. *Eur J Pharmacol* 376:23–26.

TANIMURA A, YAMAZAKI M, HASHIMOTODANI Y, UCHIGASHIMA M, KAWATA S, ABE M, KITA Y, HASHIMOTO K, SHIMIZU T, WATANABE M, SAKIMURA K, KANO M. 2010. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. *Neuron*. 65(3):320-7. doi: 10.1016/j.neuron.2010.01.021.

TAO HW, POO M. 2001. Retrograde signaling at central synapses. *Proc Natl Acad Sci U S A*. 98(20):11009-15. doi: 10.1073/pnas.191351698.

TERZIAN, A.L., DRAGO, F., WOTJAK, C.T. & MICALE, V. 2011. The Dopamine and Cannabinoid Interaction in the Modulation of Emotions and Cognition: Assessing the Role of Cannabinoid CB1 Receptor in Neurons Expressing Dopamine D1 Receptors. *Front Behav Neurosci*, 5, 49.

THIBAUT K, CARREL D, BONNARD D, et al. 2013. Activation-dependent subcellular distribution patterns of CB1 cannabinoid receptors in the rat forebrain. *Cereb Cortex*. 23(11): 2581–91.

THOMAS GM, HUGANIR RL. 2004. MAPK cascade signalling and synaptic plasticity. *Nat Rev Neurosci*. 5(3):173-83. doi: 10.1038/nrn1346.

- TORT, A. B. L., KRAMER, M. A., THORN, C., GIBSON, D. J., KUBOTA, Y., GRAYBIEL, A. M. & KOPELL, N. J. 2008. Dynamic cross-frequency couplings of local field potential oscillations in rat striatum and hippocampus during performance of a T-maze task. *Proceedings of the National Academy of Sciences*, 105, 20517-20522.
- TRAN AH, UWANO T, KIMURA T, HORI E, KATSUKI M, NISHIJO H, ONO T. 2008. Dopamine D1 receptor modulates hippocampal representation plasticity to spatial novelty. *J Neurosci*. 28:13390–13400.
- TRETTEL, J., FORTIN, D.A. & LEVINE, E.S. 2004. Endocannabinoid signalling selectively targets perisomatic inhibitory inputs to pyramidal neurones in juvenile mouse neocortex. *J Physiol*, 556, 95-107.
- TROJNAR, W., AND WISE, R.A. 1991. Facilitory effect of delta 9-tetrahydrocannabinol on hypothalamically induced feeding. *Psychopharmacology (Berl.)* 103, 172–176.
- TSETSENIS T, YOUNTS TJ, CHIU CQ, KAESER PS, CASTILLO PE, SUDHOF TC. 2011. Rab3B protein is required for long-term depression of hippocampal inhibitory synapses and for normal reversal learning. *Proc Natl Acad Sci U S A*. 108:14300–14305.
- TSIEN, J. Z., HUERTA, P. T. & TONEGAWA, S. 1996. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. *Cell*. 87, 1327–1338.
- TSVETANOVA NG, IRANNEJAD R, VON ZASTROW M. 2015. G protein-coupled receptor (GPCR) signaling via heterotrimeric G proteins from endosomes. *J Biol Chem*. 290(11):6689-96. doi: 10.1074/jbc.R114.617951.
- TULVING E. 1972. Episodic and semantic memory. In E. Tulving & W. Donaldson, *Organization of memory*. Academic Press.
- TURRENS, J.F. 2003. Mitochondrial formation of reactive oxygen species. *The Journal of physiology* 552, 335-344.
- TUSCHER, J.J., FORTRESS, A.M., KIM, J., & FRICK, K.M. 2015. Regulation of object recognition and object placement by ovarian sex steroid hormones. *Behav. Brain Res*. 285, 140-157.
- UCHIGASHIMA, M., NARUSHIMA, M., FUKAYA, M., KATONA, I., KANO, M. & WATANABE, M. 2007. Subcellular arrangement of molecules for 2-arachidonoyl-glycerol-mediated retrograde signaling and its physiological contribution to synaptic modulation in the striatum. *J Neurosci*, 27, 3663-76.

UNDIEH A.S. 2010. Pharmacology of signaling induced by dopamine D(1)-like receptor activation. *Pharmacology & therapeutics*, 128(1), 37–60.

UNGLESS MA. 2004. Dopamine: the salient issue. *Trends Neurosci.* 27:702–706.

URBAN, D.J. & ROTH, B.L. 2015. DREADDs (designer receptors exclusively activated by designer drugs): chemogenetic tools with therapeutic utility. *Annu Rev Pharmacol Toxicol*, 55, 399-417.

URIGUEN L., PEREZ-RIAL S., LEDENT C., PALOMO T., AND MANZANARES J. 2004. Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. *Neuropharmacology*, vol. 46, no. 7, pp. 966–973.

VALEEVA, G., TRESSARD, T., MUKHTAROV, M., BAUDE, A., KHAZIPOV, R. 2016. An Optogenetic Approach for Investigation of Excitatory and Inhibitory Network GABA Actions in Mice Expressing Channelrhodopsin-2 in GABAergic Neurons. *J. Neurosci.* 36, 5961–5973.

VALJENT E, MALDONADO R. 2000. A behavioural model to reveal place preference to Δ^9 -tetrahydrocannabinol in mice. *Psychopharmacology* 147:436–438.

VALLONE D, PICETTI R, BORRELLI E. 2000. Structure and function of dopamine receptors. *Neuroscience and biobehavioral reviews.* (1):125–132. doi: 10.1016/S0149-7634(99)00063-9.

VAN WELIE, I., ROTH, A., HO, S.S.N., KOMAI, S., HÄUSSER, M. 2016. Conditional Spike Transmission Mediated by Electrical Coupling Ensures Millisecond Precision-Correlated Activity among Interneurons In Vivo. *Neuron* 90, 810–823.

VARELA JA, HIRSCH SJ, CHAPMAN D, LEVERICH LS, GREENE RW. 2009. D1/ D5 modulation of synaptic NMDA receptor currents. *J Neurosci.* 29:3109–3119.

VARMA N, CARLSON GC, LEDENT C, ALGER BE. 2001. Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J Neurosci* 21: RC188.

VAZDARJANOVA, A. & GUZOWSKI, J.F. 2004. Differences in hippocampal neuronal population responses to modifications of an environmental context: evidence for distinct, yet complementary, functions of CA3 and CA1 ensembles. *J. Neurosci.* 24, 6489–6496.

VERHOEFF NP. 1999. Radiotracer imaging of dopaminergic transmission in neuropsychiatric disorders. *Psychopharmacology.* 147(3):217–249. doi: 10.1007/s002130051163.

VICKERS, S.P., WEBSTER, L.J., WYATT, A., DOURISH, C.T., AND KENNETT, G.A. 2003. Preferential effects of the cannabinoid CB1 receptor antagonist, SR 141716, on food intake and body weight gain of obese (fa/fa) compared to lean Zucker rats. *Psychopharmacology (Berl.)* 167, 103–111.

VIVEROS MP, MARCO EM, FILE SE. 2005. Endocannabinoid system and stress and anxiety responses. *Pharmacol Biochem Behav.* 81:331–342.

VOELKER DR. 1991. Organelle biogenesis and intracellular lipid transport in eukaryotes. *Microbiol Rev.* 55(4):543-60. doi: 10.1128/mr.55.4.543-560.1991.

WALKER JM, HUANG SM. 2002. Cannabinoid analgesia. *Pharmacol Ther.* 95: 127-35.

WALTER L, STELLA N. 2004. Cannabinoids and neuroinflammation. *Br J Pharmacol.* 141:775–785.

WANG L, LIU H, HARVEY-WHITE J, ZIMMER A, KUNOS G. 2003. Endocannabinoid signaling via cannabinoid receptor 1 is involved in ethanol preference and its age-dependent decline in mice. *Proc Natl Acad Sci USA* 100: 1393–1398.

WANG SH, REDONDO RL, MORRIS RG. 2010. Relevance of synaptic tagging and capture to the persistence of long-term potentiation and everyday spatial memory. *Proc Natl Acad Sci U S A.* 107:19537–19542.

WARNER-SCHMIDT J. L. AND DUMAN R. S. 2006. Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus*, vol. 16, no. 3, pp. 239–249.

WATANABE M, MANABE T, KANO M. 2004. 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* 19: 2682–2692.

WATTS, M. E., POCOCK, R., & CLAUDIANOS, C. 2018. Brain Energy and Oxygen Metabolism: Emerging Role in Normal Function and Disease. *Frontiers in molecular neuroscience*, 11, 216.

WEIS, WI, AND KOBILKA, BK. 2014. The molecular basis of G protein – coupled receptor activation. *Annu. Rev. Biochem.* 87, 897– 919.

WHEELER DW, WHITE CM, REES CL, KOMENDANTOV AO, HAMILTON DJ, ASCOLI GA. 2015. Hippocampome.org: a knowledge base of neuron types in the rodent hippocampus. *Elife* 4. doi:10.7554/eLife.09960.

WHITLOCK JR, HEYNEN AJ, SHULER MG, BEAR MF. 2006. Learning induces long-term potentiation in the hippocampus. *Science* 313: 1093–1097.

WILLIAMS, C.M., AND KIRKHAM, T.C. 1999. Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. *Psychopharmacology (Berl.)* 143, 315–317.

WILSON RI, NICOLL RA. 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature*. 410:588–592.

WILSON RI, NICOLL RA. 2002. Endocannabinoid signaling in the brain. *Science*. 296(5568):678-82. doi: 10.1126/science.1063545.

WINTERS, B. D., FORWOOD, S. E., COWELL, R. A., SAKSIDA, L. M. AND BUSSEY, T. J. 2004. Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. *J Neurosci* 24(26): 5901-5908.

WINTERS, B. D., SAKSIDA, L. M. AND BUSSEY, T. J. 2008. Object recognition memory: neurobiological mechanisms of encoding, consolidation and retrieval. *Neurosci Biobehav Rev* 32(5): 1055-1070.

WISE RA. 2004. Dopamine, learning and motivation. *Nat Rev Neurosci* 5:483–494.

WOODHAMS SG, SAGAR DR, BURSTON JJ, CHAPMAN V. 2015. The role of the endocannabinoid system in pain. *Handb Exp Pharmacol*. 227:119–43. https://doi.org/10.1007/978-3-662-46450-2_7.

WU, A., YING, Z., AND GOMEZ-PINILLA, F. 2004. The interplay between oxidative stress and brain-derived neurotrophic factor modulates the outcome of a saturated fat diet on synaptic plasticity and cognition. *The European journal of neuroscience* 19, 1699-1707.

XING B, KONG H, MENG X, WEI SG, XU M, LI SB. 2010. Dopamine D1 but not D3 receptor is critical for spatial learning and related signaling in the hippocampus. *Neuroscience*. 169:1511–1519.

XU J, ANTION MD, NOMURA T, KRANIOTIS S, ZHU Y, CONTRACTOR A. 2014. Hippocampal metaplasticity is required for the formation of temporal associative memories. *J Neurosci*. 34(50):16762-73. doi: 10.1523/JNEUROSCI.2869-13.2014.

YAMASAKI, M., AND TAKEUCHI, T. 2017. Locus Coeruleus and Dopamine-Dependent Memory Consolidation. *Neural Plast*. 8602690.

YANG, G., PAN, F. & GAN, W.B. 2009. Stably maintained dendritic spines are associated with lifelong memories. *Nature*, 462, 920-4.

ZANGEN A, SOLINAS M, IKEMOTO S, GOLDBERG SR, WISE RA. 2006. Two brain sites for cannabinoid reward. *J Neurosci* 26:4901–4907.

ZHANG, J., LIU, W., LIU, J., XIAO, W., LIU, L., JIANG, C., SUN, X., LIU, P., ZHU, Y., ZHANG, C., et al. 2010. G-protein beta2 subunit interacts with mitofusin 1 to regulate mitochondrial fusion. *Nature communications* 1, 101.

ZHANG, J., XIA, J. & XIONG, H. 2014. Techniques for Extracellular Recordings. In: XIONG, H. & GENDELMAN, H. E. (eds.) *Current Laboratory Methods in Neuroscience Research*. New York, NY: Springer New York.

ZHOU Y, WU H, LI S, CHEN Q, CHENG XW, ZHENG J et al. 2006. Requirement of TORC1 for Late-Phase Long-Term Potentiation in the Hippocampus. *PLoS ONE* 1: E16.

ZHOU, D., AND SHEARMAN, L.P. 2004. Voluntary exercise augments acute effects of CB1-receptor inverse agonist on body weight loss in obese and lean mice. *Pharmacol. Biochem. Behav.* 77, 117–125.

ZHU, P.J. & LOVINGER, D.M. 2005. Retrograde endocannabinoid signaling in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. *J Neurosci*, 25, 6199-207.

ZIMMER, A., ZIMMER, A. M., HOHMANN, A. G., HERKENHAM, M. & BONNER, T. I. 1999. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 5780-5.

ZUCCA, S., GRIGUOLI, M., MALÉZIEUX, M., GROSJEAN, N., CARTA, M., MULLE, C. 2017. Control of Spike Transfer at Hippocampal Mossy Fiber Synapses In Vivo by GABA A and GABA B Receptor-Mediated Inhibition. *J. Neurosci.* 37, 587–598.