Role of the type-1 cannabinoid receptor in the control of water intake

Zhe Zhao

THÈSE PRÉSENTÉE

POUR OBTENIR LE GRADE DE

DOCTEUR DE L'UNIVERSITÉ DE BORDEAUX

École doctorale des Sciences de la Vie et de la Santé

Spécialité: Neurosciences

Zhe ZHAO

LE ROLE DU RECEPTEUR DES CANNABINOIDES DE TYPE 1 DANS LA CONSOMMATION D'EAU

Sous la direction de Dr. Giovanni MARSICANO
Co-supervision du Dr. Arnau Busquets-Garcia et du Dr. Luigi Bellocchio

Soutenue publiquement le 9 décembre 2019

Membres du jury :

M. FERREIRA, Guillaume Dr. *Université de Bordeaux, France*	**Président**
M. WOTJAK, Carsten Dr. *Max Planck Institute of Psychiatry Munich, Allemagne*	**Rapporteur**
M. GUZMAN, Manuel Pr. *Universidad Complutense de Madrid, Espagne*	**Rapporteur**
Mme LLORENS-CORTES, Catherine Dr. *Collège de France, France*	**Examinateur**
M. FRICK, Andreas Dr. *Université de Bordeaux, France*	**Examinateur**
M. MARSICANO, Giovanni Dr. *Université de Bordeaux, France*	**Directeur de thèse**

THESIS FOR THE

DOCTORATE OF THE UNIVERSITY OF BORDEAUX

Doctoral school of Life Science and Health

Specialty: Neurosciences

Zhe ZHAO

ROLE OF THE TYPE-1 CANNABINOID RECEPTOR IN THE CONTROL OF WATER INTAKE

Under the supervision of Dr. Giovanni MARSICANO
Co-supervision of Dr. Arnau Busquets-Garcia and Dr. Luigi Bellocchio

Defended in public on December 9th, 2019

Jury members:

M. FERREIRA, Guillaume Dr. *University of Bordeaux, France*	**President**
M. WOTJAK, Carsten Dr. *Max Planck Institute of Psychiatry Munich, Germany*	**Reviewer**
M. GUZMAN, Manuel Pr. *Complutense University of Madrid, Spain*	**Reviewer**
Mme LLORENS-CORTES, Catherine Dr. *Collège de France, France*	**Examiner**
M. FRICK, Andreas Dr. *University of Bordeaux, France*	**Examiner**
M. MARSICANO, Giovanni Dr. *University of Bordeaux, France*	**Thesis supervisor**

Unité de recherche

Physiopathologie de la plasticité neuronale - NeuroCentre Magendie U 1215

Addresse du laboratoire:

NeuroCentre Magendie

INSERM U1215

Groupe “Endocannabinoïdes et Neuroadaptation”

146, rue Léo Saignat

33077 Bordeaux Cedex

Each towering giant of a tree used to be a tiny bud.

A terrace nine-storey high came from baskets of soil piled.

A journey of a thousand miles begins with a single step.

Laozi

Tao Te Ching

INDEX

Abstract

L'apport hydrique est crucial pour le maintien homéostatique des fluides corporels et pour la survie de l'animal. Des processus cérébraux complexes déclenchent la soif, qui survient suite à une perte de volume sanguin (déshydratation extracellulaire) ou une augmentation de l'osmolalité sanguine (déshydratation intracellulaire), afin d'apporter de l'eau pour l'équilibre des fluides. Cependant, les mécanismes centraux favorisant la prise hydrique sont encore peu caractérisés. Les récepteurs aux cannabinoïdes de type 1 (CB_1) sont exprimés de manière large et abondante dans le système nerveux central où ils modulent une variété de fonctions telles que la mémoire, l'anxiété et les comportements alimentaires. Cependant, le rôle des récepteurs CB_1 dans le contrôle de l'apport hydrique est encore matière à débat du fait de résultats contradictoires d'expériences d'activation ou de blocage pharmacologique de ces récepteurs sur les comportements de consommation d'eau.

Mon travail de thèse s'est concentré sur le rôle des récepteurs CB_1 dans le contrôle de l'apport hydrique. Par l'utilisation d'approches génétiques, pharmacologiques, comportementales et de traçage neuronal, j'ai examiné l'implication des récepteurs CB_1 dans le contrôle de l'apport hydrique induit par différentes conditions physiologiques de déshydratation extra- et intracellulaire. Les résultats montrent que la signalisation du récepteur CB_1 est requise pour promouvoir l'apport hydrique. En particulier, la délétion globale du récepteur CB_1 ne change pas l'osmolalité plasmatique ni la composition corporelle en eau, mais elle diminue l'apport hydrique induit par une privation d'eau, une administration systémique ou intracérébroventriculaire (ICV) de chlorure de sodium ou une injection ICV de l'hormone peptidergique angiotensine II. Dans le but de mieux décrire les mécanismes neuronaux de cette fonction, j'ai découvert que la présence des récepteurs CB_1 dans les neurones glutamatergiques corticaux, en particulier ceux présents dans le cortex cingulaire antérieur (CCA) favorisent le comportement de consommation d'eau. En effet, les récepteurs CB_1 sont exprimés abondamment dans les terminaisons axonales des neurones glutamatergiques du CCA projetant sur l'amygdale basolatérale (ABL) et une réexpression sélective des récepteurs CB_1 dans ce circuit est suffisante pour garantir un comportement de consommation d'eau normal chez la souris.

Dans l'ensemble, ces données révèlent que les récepteurs CB_1 sont nécessaires pour promouvoir l'apport hydrique, et que leur présence dans le circuit CCA-ABL est suffisant pour le contrôle descendant des comportements de consommation d'eau.

De plus, j'ai également montré que les récepteurs CB_1 contrôlaient l'apport hydrique dans différentes conditions à d'autres niveaux comme au niveau du cortex insulaire, des cellules cholinergiques et des mitochondries.

En résumé, mon travail de thèse a analysé le rôle des récepteurs CB_1 dans des populations cellulaires / circuits neuronaux distincts dans le contrôle de l'apport hydrique. Ces résultats apportent de plus amples preuves permettant la compréhension des fonctions du système endocannabinoïde et de la régulation cérébrale de la soif.

Mot clés : soif, récepteur CB_1, apport hydrique, cerveau, type cellulaire, cortex cingulaire antérieur, cortex insulaire, amygdale basolatérale, circuit neuronal.

Abstract

Water intake is crucial for maintaining body fluid homeostasis and animals' survival. Complex brain processes trigger thirst, which arises upon losing blood volume (i.e. extracellular dehydration) or increasing blood osmolality (i.e. intracellular dehydration), to replenish water for fluid balance. Although the brain plays a key role in modulating these processes, the central mechanisms regulating water intake are not fully understood. Type-1 cannabinoid receptors (CB_1) are widely and abundantly expressed in the central nervous system where they modulate a variety of functions, such as memory, anxiety and feeding behavior. However, the role of CB_1 receptors in the control of water intake is still a matter of debate, since pharmacological activation or blockade of CB_1 receptors produced contradictory results in drinking behavior experiments.

My thesis work focuses on the role of CB_1 receptors in the control of water intake. By using genetic, pharmacological, anatomical, imaging, and behavioral approaches, I examined the involvement of CB_1 receptors in the control of water intake induced by different physiological conditions of extracellular or intracellular dehydration. The results showed that CB_1 receptor signaling is required to promote water intake. In particular, global deletion of CB_1 receptors does not change plasma osmolality and body water composition, but it decreases water intake induced by water deprivation, systemic or intracerebroventricular (ICV) administration of sodium chloride, or ICV injection of the peptide hormone angiotensin II. In the attempt to better detail the neuronal mechanisms of this function, I discovered that the presence of CB_1 receptors in cortical glutamatergic neurons, particularly the ones located in the anterior cingulate cortex (ACC) glutamatergic neurons promote drinking behavior. CB_1 receptors are abundantly expressed in axon terminal of ACC glutamatergic neurons projecting to the basolateral amygdala (BLA) and selective expression of CB_1 receptors in this circuit is sufficient to guarantee proper drinking behavior in mice. Altogether, these data reveal that CB_1 receptors are necessary to promote water intake, and that their presence in the ACC-BLA circuit is sufficient for the top-down control of drinking behavior.

Furthermore, I also provided evidence that CB_1 controls water intake in different conditions at other levels, e.g. insular cortex, cholinergic cells, and mitochondria.

Abstract (English)

In summary, my thesis work analyzed the role of CB_1 receptors in distinct cell populations/neuronal circuits for the control of water intake. These results will help further understanding the functions of the ECS and the brain regulation of thirst.

Keywords: thirst, CB_1 receptor, water intake, brain, cell type, the anterior cingulate cortex, the insular cortex, the basolateral amygdala, neural circuit.

Rôle des récepteurs CB_1 dans le contrôle de l'apport hydrique

La soif est une perception fondamentale qui pousse les animaux à ingérer de l'eau pour maintenir l'homéostasie des fluides corporels et qui survient lors de la déshydratation du corps. Les substrats physiologiques qui sous-tendent ce processus et sa régulation ne sont que partiellement connus. Les récepteurs CB_1 sont abondamment présents dans le cerveau et régulent une grande variété de fonctions telles que l'apprentissage, la mémoire, l'alimentation et la balance énergétique (Busquets-Garcia et al 2018, Kano et al 2009, Piazza et al 2017). Le rôle des récepteurs CB_1 dans le contrôle du comportement de consommation d'eau fait encore l'objet d'un débat, puisque les manipulations pharmacologiques des récepteurs ont donné des résultats contradictoires pour différentes espèces, conditions et doses (Abel 1975, Drewnowski & Grinker 1978, Higgs et al 2003, Ruginsk et al 2015, Verty et al 2004). Nous avons émis l'hypothèse que les récepteurs CB_1 dans des endroits distincts joueraient des rôles différents dans le contrôle du comportement de consommation d'eau. Dans ce travail de thèse, j'ai contribué à élucider le rôle des récepteurs CB_1 dans des types cellulaires spécifiques, des structures cérébrales, des circuits neuronaux et des organites subcellulaires dans la régulation de l'apport hydrique.

1) Rôle des récepteurs CB_1 dans le contrôle de l'apport hydrique.

J'ai constaté que la délétion globale des récepteurs CB_1 diminuait la consommation d'eau en réponse à une privation de 24 heures, à l'administration de chlorure de sodium (NaCl) hypertonique et à l'administration d'angiotensine II (Ang II). Afin de s'assurer que ces effets ne sont pas dus à l'absence constitutive des récepteurs CB_1, j'ai bloqué de manière systémique les récepteurs CB_1 par le rimonabant, ce qui a entraîné une réduction de l'apport en eau chez la souris contrôle, comparable à celle observée chez la souris mutante pour les récepteurs CB_1 (*CB_1*-KO). Parallèlement, la réexpression globale des récepteurs CB_1 chez les souris stop-*CB_1* a entraîné une augmentation du comportement de consommation d'eau par rapport aux souris contrôles. Les données révèlent que les récepteurs CB_1 favorisent l'absorption d'eau provoquée par la privation d'eau sur 24 heures, le NaCl hypertonique et l'Ang II chez la souris.

2) Rôle des récepteurs CB_1 dans des types cellulaires spécifiques dans le contrôle de l'apport hydrique.

Les neurones excitateurs et inhibiteurs de la *lamina terminalis* jouent des rôles opposés dans le contrôle de la prise d'eau. (Abbott et al 2016, Betley et al 2015, Nation et al 2016, Oka et al 2015, Zimmerman et al 2016). Par conséquent, nous avons émis l'hypothèse que les récepteurs CB_1 des neurones glutamatergiques (Glu) corticaux et des neurones GABAergiques (GABA) du cerveau antérieur exerceraient différentes fonctions dans la régulation du comportement de consommation. Étonnamment, nous n'avons observé aucune différence dans la consommation d'eau des souris mutantes Glu-*CB_1*-KO et GABA-*CB_1*-KO par rapport à leurs congénères contrôles.

Les astrocytes exercent de nombreuses fonctions dans le cerveau, comme l'apport de nutriments aux tissus nerveux, le maintien de l'équilibre ionique extracellulaire et la modulation de la transmission synaptique (Han et al 2013, Piet et al 2004, Santello et al 2019). Nous avons utilisé des souris mutantes pour le récepteur CB_1 sur ces astrocytes (GFAP-*CB_1*-KO) et constaté que la suppression spécifique des récepteurs CB_1 dans les astrocytes ne présentait pas de différences significatives par rapport aux congénères contrôles.

Le récepteur de la dopamine D_1 est l'un des récepteurs dopaminergiques les plus abondants du système nerveux central et contribue à la modulation de la motivation et de la récompense (Hasbi et al 2011). Nous nous sommes donc demandé si le récepteur CB_1 des cellules D_1 positives pouvait contrôler la consommation d'eau. Pour répondre à cette question, nous avons utilisé des souris D_1-*CB_1*-KO. Cependant, les souris D_1-*CB_1*-KO n'ont pas montré de différence significative dans la consommation d'eau par rapport à leurs congénères contrôles, ce qui exclut un rôle possible du récepteur CB_1 dans les cellules dopaminoceptives dans le contrôle du comportement de consommation.

Fait intéressant, nous avons constaté que la suppression des récepteurs CB_1 dans les cellules cholinergiques favorisait la consommation d'eau de façon sélective en réponse à l'application centrale de NaCl hypertonique, mais non à la privation d'eau sur 24 heures, à l'administration systémique hypertonique de NaCl ou à l'application de l'Ang

II, indiquant que les récepteurs CB_1 des neurones cholinergiques pourraient participer au contrôle des apports en eau résultant de changements d'osmolalité centrale.

Du fait qu'aucune sous-population cérébrale de récepteurs CB_1 ne semble jouer un rôle nécessaire dans l'absorption d'eau, j'ai décidé d'examiner si l'une d'elles pourrait jouer un rôle suffisant dans ce processus. Dans ce but, nous avons appliqué une stratégie de réexpression génétique. J'ai d'abord utilisé des souris portant la réexpression de CB_1 exclusivement dans les neurones glutamatergiques corticaux (Glu-*CB_1*-RS) ou dans les neurones GABAergiques du cerveau antérieur (GABA-*CB_1*-RS). Fait intéressant, les souris Glu-*CB_1*-RS ont une consommation d'eau supérieure aux souris Stop-*CB_1*, mais les souris GABA-*CB_1*-RS n'ont montré aucune différence significative par rapport aux souris contrôles. Ces résultats indiquent que les récepteurs CB_1 des neurones glutamatergiques corticaux jouent un rôle suffisant dans le contrôle de la consommation d'eau.

3) Rôle des récepteurs CB_1 dans des structures cérébrales spécifiques dans le contrôle de l'apport hydrique.

On sait que le cortex insulaire (CI) et le cortex cingulaire antérieur (CCA) sont impliqués dans le contrôle du comportement de consommation (Denton et al 2009, Gizowski & Bourque 2018, Ichiki et al 2019, Robinson & Mishkin 1968, Zimmerman et al 2017). Considérant les résultats obtenus avec des souris Glu-*CB_1*-RS, je me suis demandé si la modulation par les récepteurs CB_1 de l'absorption d'eau pourrait impliquer le contrôle de la transmission glutamatergique des neurones situés dans ces zones du cerveau. Ainsi, j'ai ré-exprimé les récepteurs CB_1 dans le CI chez des souris Stop-*CB_1* par injection de virus adéno-associés(VAA) exprimant la Cre recombinase (enzyme nécessaire à la réexpression génétique). La réexpression des récepteurs CB_1 dans le CI ou le CI postérieur n'a pas eu d'influence sur la consommation d'eau comparativement aux souris Stop-*CB_1*. Inversement, lorsque nous avons injecté le VAA exprimant de la Cre recombinase par l'intermédiaire du promoteur CaMKIIα dans le CCA pour obtenir la réexpression des récepteurs CB_1 dans les neurones glutamatergiques du CCA (CCA-CaMKIIα-*CB_1*-RS), cette manipulation a permis d'augmenter la quantité d'eau absorbée par les souris contrôles Stop-*CB_1*. Ainsi, les récepteurs CB_1 dans les neurones

glutamatergiques du CCA mais pas dans ceux du CI semblent être nécessaires pour promouvoir le comportement de consommation.

La stimulation optogénétique du CI influence le comportement de consommation (Schiff et al 2018, Wang et al 2018). Puisque les récepteurs CB_1 sont principalement des récepteurs couplés à la protéine Gi, la stimulation des récepteurs exerce une inhibition de la neurotransmission. Inversement, la suppression des récepteurs CB_1 facilite la libération des neurotransmetteurs, ce qui récapitule en quelque sorte la stimulation optogénétique. Par conséquent, nous avons supprimé les récepteurs CB_1 du CI par injection de VAA exprimant la Cre recombinase dans le CI (CI-*CB_1*-KO). Nous avons observé que la suppression des récepteurs CB_1 n'avait pas d'incidence sur la consommation quotidienne d'eau et sur le poids corporel, ni sur la consommation d'eau et d'aliments suite à une privation de 24 heures. Il est intéressant de noter que les souris CI-*CB_1*-KO présentaient un comportement de consommation d'eau significativement inhibé suite à l'administration systémique de NaCl hypertonique et à l'administration centrale d'Ang II, mais non pas suite à l'administration centrale de NaCl hypertonique. Par conséquent, les récepteurs CB_1 dans le cortex insulaire sont impliqués dans la promotion de l'absorption d'eau à deux niveaux différents, comme la réponse aux changements d'osmolalité périphérique et de la signalisation de l’Ang II au niveau cérébral.

4) Rôle des récepteurs CB_1 dans des circuits cérébraux spécifiques dans le contrôle de l’apport hydrique.

Les récepteurs CB_1 sont principalement des récepteurs présynaptiques dans les neurones. Par conséquent, je me suis demandé quelles terminaisons des projections du CCA pourraient être la zone où s’exerce le contrôle endocannabinoïde de la prise d'eau. En examinant l'expression des récepteurs CB_1 dans l'ensemble du cerveau, j'ai identifié que les récepteurs CB_1 des terminaisons axonales provenant du CCA sont situés dans le Claustrum (CL), l'amygdale basolatérale (ABL), le cortex ectorhinal et le cortex périrhinal. Parmi ces régions du cerveau, l’ABL a été montré comme régularisant la consommation d'eau (Kim et al 2016, Kim et al 2017). Par conséquent, nous avons caractérisé le rôle des récepteurs CB_1 dans le circuit CCA-ABL sur le comportement de

consommation. Nous avons ré-exprimé les récepteurs CB_1 spécifiquement dans le circuit CCA-ABL (CCA-ABL-*CB_1*-RS) en combinant l'injection de virus rétrograde exprimant une flipase dans l'ABL avec l'injection de VAA exprimant une Cre recombinase dépendante de la flipase dans le CCA des souris Stop-*CB_1*. Les souris CCA-ABL-*CB_1*-RS ont remarquablement bu plus d'eau que les souris Stop-*CB_1*. Par conséquent, les récepteurs CB_1 dans le circuit CCA-ABL sont essentiels pour faciliter la prise d'eau.

5) Rôle des récepteurs CB_1 mitochondriaux dans le contrôle de l'apport hydrique.

Les mitochondries sont essentielles à la production d'énergie dans les cellules. Des études récentes ont montré que les récepteurs CB_1 sont fonctionnellement associés aux membranes mitochondriales (Benard et al 2012). Les fonctions mitochondriales pourraient également moduler les phénomènes liés à la consommation d'eau, comme les effets de l'Ang II dans le cerveau (Zimmerman et al 2002). Ainsi, la stimulation des récepteurs CB_1 mitochondriaux dans les neurones liés à la soif pourrait avoir un impact sur le comportement de consommation. J'ai utilisé des souris DN22-*CB_1*-KI, chez lesquelles les récepteurs CB_1 mitochondriaux sont absents, mais les récepteurs CB_1 plasmatiques sont toujours présents, et j'ai constaté que les souris DN22-*CB_1*-KI diminuent significativement leur consommation quotidienne d'eau par rapport à leurs congénères contrôles. De plus, les souris DN22-*CB_1*-KI ont remarquablement bu moins d'eau que les souris contrôles en réponse à l'administration systémique de NaCl hypertonique, mais pas après 24 heures de privation d'eau (bien qu'une forte tendance à boire moins d'eau soit observée). Ainsi, ces résultats révèlent que les récepteurs CB_1 mitochondriaux pourraient jouer un rôle nécessaire dans la régulation de la prise d'eau, et les mécanismes sous-jacents méritent d'être étudiés.

En conclusion, les récepteurs CB_1 centraux de différentes régions sont essentiels au contrôle du comportement de consommation. Ce contrôle a lieu sur les neurones glutamatergiques corticaux, situés dans le cortex cingulaire antérieur projetant sur l'amygdale basolatérale, révélant un nouveau circuit neuronal pour le contrôle descendant de l'apport en eau par le système endocannabinoïde.

Abbreviations

2-AG	2-arachidonoylglycerol
5-HT	5-hydroxytryptamine
Abh4	α/β-hydrolase 4
Abh6	α/β-hydrolase 6
AC	Adenylyl cyclase
ACC	Anterior cingulate cortex
ACh	Acetylcholine
AEA	Anandamide
AKT	Protein kinase B
Ang I	Angiotensin I
Ang II	Angiotensin II
ANP	Atrial natriuretic peptide
AON	Anterior olfactory nucleus
AT1	Ang II receptor type 1
ATP	Adenosine triphosphate
BLA	Basolateral amygdala
BNST	Bed nucleus of the stria terminalis
Ca^{2+}	Calcium
CaMK II	Ca^{2+}/calmoduin dependent kinase II
cAMP	Cyclic adenosine monophosphate
Cb	Cerebellar cortex
CB_1	Type-1-cannabinoid receptor
CB_2	Type-2 cannabinoid receptor
CBD	Cannabidiol
CeA	Central amygdala
CeL	Lateral nucleus of the central amygdala
CeM	Medial nucleus of the central amygdala
CNR1	CB_1 gene
CNS	Central nervous system
COX	Cyclooxygenase
Cpu	Caudate putamen

D1	Type-1 dopamine receptor
D2	Type-2 dopamine receptor
DAG	Diacylglycerol
DG	Dentate gyrus
DGL	Diacylglycerol lipase
DN22	Deletion of 22 amino acids at the beginning of N-terminal
DNA	Deoxyribonucleic acid
DREADD	Designer Receptors Exclusively Activated by Designer Drugs
DSE	Depolarization-induced suppression of excitation
DSI	Depolarization-induced suppression of inhibition
EBC	Eyeblink conditioning
eCBs	Endogenous cannabinoids
ECS	Endocannabinoid system
EMT	Endocannabinoid membrane transporter
EPSC	Excitatory postsynaptic currents
ERK	Extracellular signal-regulated kinase
ETC	Electron transport chain
ETV-1	ETS translocation variant 1
FAAH	Fatty acid amide hydrolase
FADH2	Flavin adenine dinucleotide
FCCP	4-(trifluoromethoxyl)s-phenyl-hydrazone
FLAT	FAAH-like protein complex
GABA	γ-aminobutyric acid
GAD65	Glutamic acid decarboxylase 65
GDP	Guanosine diphosphate
GFAP	Glial fibrillary acidic protein
GFP	Green fluorescent protein
GLP-1	Glucagon-like peptide 1
GP	Globus pallidus
GPCR	G protein-coupled receptor
GTP	Guanosine triphosphate
H1	Type-1 histamine receptor
H2	Type-2 histamine receptor
HETE-G	Hydroxyeicosatetraenoyl-glycerol

HFS	High-frequency stimulation
Hi	Hippocampus
HPETEA	Hydroxyperoxyeicosatetraenoylethanolamide
HPETE-G	Hydroxyperoxyeicosatetraenoyl-glycerol
IC	Insular cortex
ICV	Intracerebroventricular
IP	Intraperitoneal
IPSC	Inhibitory postsynaptic currents
K^+	Potassium
KCl	Potassium chloride
KI	Knockin
KO	Knockout
LA	Lateral amygdala
LH	Lateral hypothalamus
LOX	Lipoxygenase
LT	Lamina terminalis
LTD	Long-term depression
M1	Primary motor cortex
MAFP	Methylarachidonoylfluorophosphonate
MAGL	Monoacylglycerol lipase
MAPK	Mitogen-activated protein kinase
MeA	Medial amygdala
MGL	Monoacyl-glycerol Lipase
Mid	Midbrain
MnPO	Median preoptic nucleus
MO	Medulla oblongata
mRNA	Messenger RNA
mtCB_1	Mitochondrial CB_1 receptors
NAAA	N-acylethanolamine acid amide hydrolase
NAc	Nucleus accumbens
NaCl	Sodium chloride
NAM	N-arachidonoylmaleimide
NAPE	N-acylphosphatidylethanolamine
NArPE	N-arachidonoylphosphatidyl-ethanolamine

nNOS	Nitric oxide synthase 1
NTS	Nucleus of the solitary tract
OVLT	Organum vasculosum of the lamina terminalis
PAG	Periaqueductal gray
PBN	Parabrachial nucleus
PE	Protein tyrosine phosphatase
PEG	Polyethylene glycol
PG	Prostaglandin
PI	Inositol phospholipids
PI3K	Phosphatidylinositol 3-kinase
PKA	Protein kinase A
PLA1	Phospholipase A1
PLC	Phospholipase C
PLD	Phospholipase D
Po	Pons
PTPN22	Protein tyrosine phosphatase 22
PVH	Paraventricular nucleus of the hypothalamus
RS	Rescue
RVM	Rostral ventromedial medulla
S1	Primary somatosensory cortex
sAC	Soluble adenylyl cyclase
SC	Subcutaneous
SCN	Suprachiasmatic nucleus
SFO	Subfornical organ
SNR	Substantia nigra pars reticulata
SON	Supraoptic nucleus
STD	Short-term depression
STN	Subthalamic nucleus
Th	Thalamus
THC	Δ^9-tetrahydrocannabinol
V1	Primary visual cortex
VGCC	Voltage-gated Ca^{2+} channel
VP	Ventral pallidum

CHAPTER I

General introduction

Introduction

Thirst is a feeling mainly arising from body dehydration that is paramount for driving water intake to maintain body fluid balance. Both the periphery and the central nervous system are involved in the control of thirst and drinking behavior. Previous studies revealed that peripheral hormones stimulate brain regions that regulate water intake. However, the cellular mechanisms and neural networks underlying thirst are largely unknown. Whereas many studies addressed the role of subcortical brain region (especially the hypothalamus) in thirst and water intake, cortical circuits are starting to emerge as important elements in the top-down control of drinking behavior. However, their nature and the mechanisms underlying are scantly known. Therefore, more studies on the mechanisms controlling drinking behavior in the brain are required for a better understanding of this vital function.

Type-1 cannabinoid receptor (CB_1) is a G protein-coupled receptor, widely and abundantly expressed in the central nervous system and in the periphery. CB_1 receptors are able to be activated by Δ^9-tetrahydrocannabinol, the main psychoactive component of *cannabis sativa*, by synthetic ligands, and by endogenous cannabinoids, including 2-arachidonoyglycerol (2-AG) and anandamide (AEA). CB_1 receptors are located in different cell types, including different types of neuronal and non-neuronal cells (e.g. astrocyte), and in different brain structures, including the olfactory bulb, striatum, hippocampus, and neocortex. CB_1 receptors modulate a large variety of functions, such as feeding behavior, energy balance, learning and memory, analgesia, or reward, through different mechanisms. However, it is still not clear how CB_1 receptors participate in the control of thirst and drinking behavior.

Pharmacological manipulation of CB_1 receptors in water intake experiments produced contradictory results, depending on species, conditions, and dosages of drugs. Since CB_1 receptors are distributed in different cell types and brain regions, I hypothesized that the functions of CB_1 receptors in the control of water intake depend on their locations.

In this chapter, I will review the studies on physiological substrates of thirst and the endocannabinoid system. In addition, the relationships between thirst and the endocannabinoid system will be introduced.

I.1 Thirst

Water represents a fundamental element for life because it is the medium in which most of the biochemical reactions occur (Jequier & Constant 2010, Popkin et al 2010). In living organisms, water is necessarily supplied from the outer world and it is eliminated by respiration, secretion by the kidney, and transpiration from the skin. Importantly, a sophisticated system is necessary to sense water loss and elicit drinking behavior to maintain body water balance.

Thirst is a subjective perception that provides an urge for humans and animals to drink potable fluids, which is crucial for maintaining body fluid homeostasis. Cannon and Rullier mentioned that researchers have investigated the mechanisms of thirst since the 18th century, but they did not show references (Cannon 1918, Rullier 1821). The questions are as follows: Which are the brain regions or peripheral organs controlling thirst in the body? What are the factors inducing thirst? How do animals and humans act to maintain body fluid balance in response to thirst factors? Early studies pointed out that the brain plays an important role in the control of water intake, especially the hypothalamus (Fitzsimons 1972, Fitzsimons 1976). Thanks to the advances of new technologies in neuroscience, recent studies revealed the central mechanisms underlying thirst at cellular and neural circuitry levels (Gizowski & Bourque 2018, Ichiki et al 2019, Zimmerman et al 2017). Despite these important advances, however, the central mechanisms regulating thirst and drinking behavior are far from being fully understood.

Here, I will review early and recent studies regarding the central and peripheral mechanisms controlling water intake and the main tools used to study thirst and drinking behavior.

I.1.1 Body water composition and balance

Being the solvent for all body fluids, water is one of the most important elements in living organisms, providing the environment for most biochemical reactions, and playing critical roles to maintain physiological functions (Jequier & Constant 2010, Popkin et al 2010). Water is eliminated through the expired air, the secretion of the kidney and

transpiration from the skin. Thus, organisms need to replenish enough water from the outer world to keep their body water balance.

I.1.1.1 Body water composition and functions

On average, 60% of the bodyweight is comprised of water in adult men, and 55% in adult women. Among the total body water, two-thirds of it locates in the intracellular space, and one-third of it locates in the extracellular space. The extracellular water contains interstitial and plasma water, 75% of the extracellular water is in the interstitial space and 25% of it locates in the plasma (Farag et al 2017, Popkin et al 2010). Body water plays a wide variety of roles in physiological functions. For example, water is the solvent for the transportation of nutrients to the whole body, a medium where most of the biochemical reactions and metabolic processes occur, a lubricant for joints, it is a medium for the secretion in the kidney and the skin, and participates in the control of body temperature (Jequier & Constant 2010, Popkin et al 2010).

I.1.1.2 Maintenance of water balance

Mammals are continuously losing water from their bodies and they need a system to keep their body water balance. In this sense, mammals have evolved the sensation of water imbalance in order to promote actions to recover the balance. For example, water balance can be recovered by ingesting fluids, by enhancing the reabsorption in the kidneys or by reducing water secretion when needed (Farag et al 2017, Sparks et al 2014).

In mammals and humans, thirst arises when the body is losing water to a threshold, and causes drinking behavior to replenish body water volume. In addition, the renin-angiotensin system works on re-absorption of water through the renal and stimulation of water intake by angiotensin II (Ang II). When there is a decrease in body water volume, the kidneys sense the loss of water and releases renin. Then plasma renin carries out the conversion of angiotensinogen, which is released by the liver, to angiotensin I (Ang I). Subsequently, the angiotensin-converting enzyme converts the Ang I to Ang II, which causes the thirst by stimulating the Ang II receptor type 1 (AT1) in the brain. Simultaneously, Ang II induces the blood vessel narrows and increases blood

pressure. In addition, Ang II stimulates the adrenal gland to secrete aldosterone, which causes the renal tubules to reabsorb water and sodium (Farag et al 2017, Sparks et al 2014). Therefore, Ang II is a very important hormone eliciting water intake and enhancing reabsorption of water from blood to maintain body water balance.

I.1.1.3 Physiological and pathological consequences of dehydration

Humans are usually euhydrated as thirst drives to drink water to compensate for the fluid deficiency. However, sometimes, we lose water in our bodies that is not compensated with fluid intake, leading to dehydration. In addition, in aged people or in some diseases such as metabolic disorders, there is a higher threshold to elicit thirst than in healthy persons, causing frequent dehydration states. In general, it seems that dehydration is often overlooked by medical practitioners, even though studies show the beneficial and harmful effects in euhydration and dehydration respectively (Jequier & Constant 2010, Popkin et al 2010).

Physical performance

Dehydration effects have been well described in athletes (Maughan et al 2007a, Murray 2007, Sawka & Noakes 2007). Under mild levels of dehydration, athletes experience decrements in performance related to a reduction of endurance, increased fatigue, altered thermoregulatory capability, reduced motivation, and increased perceived effort (Cheuvront et al 2003, Montain & Coyle 1992, Paik et al 2009). In addition, an exercise in a hot condition with inadequate water replacement is associated with hyperthermia, reduced cardiac output, a decrease in blood pressure, and reduced blood flow to the muscles (Maughan et al 2007b). Importantly, rehydration can reverse all these effects (Paik et al 2009)

Cognitive performance

Dehydration is assumed to affect cognition. Studies showed that mild dehydration alters a set of important aspects of cognitive functions such as concentration, alertness and short-term memory in children, young adults and elderly adults (Bar-Or et al 1980, Cian et al 2001, Cian et al 2000, D'Anci K et al 2009, Gopinathan et al 1988, Suhr et al 2004). Furthermore, mild to moderate levels of dehydration impairs performance on

tasks such as short-term memory, perceptual discrimination, arithmetic ability, visuomotor tracking, and psychomotor skills (Bar-Or et al 1980, Cian et al 2001, Cian et al 2000, D'Anci K et al 2009, Gopinathan et al 1988).

Delirium

Dehydration is a risk factor for delirium, particularly the one present in dementia (Culp et al 2004, Lawlor 2002, Voyer et al 2009). A recent study showed that dehydration is one of several predisposing factors in observed confusion in a long-term care resident (Voyer et al 2009).

Gastrointestinal function

The proximal small intestine absorbs fluids with a capacity of up to 15 L/day (Ritz & Berrut 2005). A number of reasons cause constipation, which is characterized by slow gastrointestinal transit and difficulty in passing stool. Inadequate fluid intake is touted as a common culprit in constipation, and an increase in water intake is a usually recommended treatment against constipation (Arnaud 2003). In addition, diarrhea, which represents a large loss of body water and electrolytes through the gastrointestinal tract, is one of the highest causes of death in children resulting in approximately 1.5-2.5 million deaths per year (Kosek et al 2003). However, the appropriate oral rehydration therapy prevents the high rate of mortality (Atia & Buchman 2009).

Headache

Dehydration can lead to the development of headaches (Shirreffs et al 2004). Some observational studies showed that water deprivation can serve as a trigger for migraines and also prolong migraines (Blau 2005, Blau et al 2004). In individuals suffering from dehydration-induced headaches, water intake alleviates the headache within 30 min to 3 hours (Blau et al 2004). On the other hand, a recent study showed correlates between headache and increase of water intake. In this case, patients with different types of headache including migraine and tension headache were either assigned to a placebo condition or an increase in water consumption condition. The participants that increased water intake did not decrease the number of headache

episodes, but reduced the headache intensity and duration of headache (Spigt et al 2005), providing evidence that a reduction of headache by increasing water intake.

Chronic diseases

There are increasing pieces of evidence indicating that mild dehydration promotes the development of various chronic diseases. Manz and Wentz summarized the studies regarding dehydration and chronic diseases (Manz & Wentz 2005). It has been shown that good hydration reduces the risk of urolithiasis, the incidence of constipation, asthma, hypertonic dehydration in the infants, and hyperglycemia in diabetic ketoacidosis. In addition, good hydration is associated with a reduction in urinary tract infections, hypertension, fatal coronary heart disease, venous thromboembolism, and stroke, but all these effects need to be confirmed by clinical trials. Overall, good hydration contributes to a number of beneficial effects without any harmful effects.

I.1.2 Early studies on thirst

The earliest studies of thirst were performed in the 18th century, and cited in the later studies (Cannon 1918, Rullier 1821). The questions these investigators addressed included what factors trigger thirst and where the origin(s) of thirst is (are). It is clear that dehydration causes thirst. In addition to dehydration, other factors also induce thirst and drinking behavior, such as prandial drinking and circadian drinking. Regarding the origin(s) of thirst in animals, there were three hypotheses. First, thirst might be a general sensation, because water is almost everywhere in the body. When our bodies need to replenish water, thirst arises without external stimuli. Second, thirst is caused by mouth and pharynx, because dryness in these organs is the first sign of dehydration. However, the dryness is sufficient, but not necessary for the generation of thirst. Third, thirst perception is generated by the brain. The following studies pointed out the brain sensing internal thirst stimuli as a key factor in eliciting drinking behavior. Furthermore, peripheral organs send thirst hormonal signals to the brain, which are processed by the brain to generate thirst and drive drinking behavior (Fitzsimons 1972, Fuller 1984). In this section, I will focus on reviewing these questions and the related early studies, mainly on factors inducing thirst, origin(s) of thirst, hormonal stimuli for water intake, and pharmacological approaches to study fluid balance (**Table 1,2**).

I.1.2.1 Drinking behaviors triggered by distinct factors

Thirst is a subjective perception and drives drinking behavior. Because thirst promotes drinking, investigators considered that drinking represents thirst to a certain extent in animals (Watts 2001). Here, I will describe different kinds of drinking elicited by different factors (Gizowski & Bourque 2018, Leib et al 2016, Stricker & Sved 2000).

Dehydration-elicited drinking

Thirst is a sensation accompanying dehydration urging mammals to drink that can soon become very aversive, insistent and torturous (Cannon 1918). The feeling will be relieved after drinking water. This kind of drinking is the most common in animals and humans (Gizowski & Bourque 2018, Leib et al 2016, Stricker & Sved 2000).

Associated drinking

Associated drinking is a kind of cue-responsive behavior. It is not really due to thirst. Sometimes mammals anticipate dehydration and drink water prior to dehydration in response to the thirst signs. For example, dryness of the mouth. Because dehydration produces dryness of mouth, this state is associated with dehydration. When we feel the dryness of the mouth, we feel that we should drink water. This kind of drinking is called associated drinking (Fitzsimons 1972).

Prandial drinking

Prandial drinking is caused by eating in humans (Bellisle & Le Magnen 1981, Phillips et al 1984) and rodents (Epstein et al 1964, Fitzsimons & Le Magnen 1969, Kissileff 1969a, Kissileff 1969b). It may be due to three reasons. The first reason is dehydration. When we eat dry food, we take body water to digest food, which leads to dehydration. The second reason is that eating food leads to dry mouth, which is a sufficient trigger of thirst. The third reason is that drinking water facilitates eating food smoothly. Therefore, prandial drinking is caused by complex reasons.

Circadian drinking

Most drinking occurs during active daytime in humans and nighttime in rodents (Zucker 1971). Some persons drink water before going to sleep at night. This behavior is likely linked to the fact that we need water before sleep at night to avoid dehydration (Gizowski et al 2016). This kind of water consumption is also not really due to dehydration but a kind of anticipated thirst. A recent study showed that the circadian regulation of drinking also exists in rodents (Gizowski et al 2016).

Drinking beverages and wines

Some other kinds of drinking behaviors are not caused by dehydration or thirst. For example, when we drink beverages and wines because of the good tastes of the beverage or the rewarding properties of alcohol. In addition, when we are at a party, we drink beers and wines for social purposes, but not for supplementing fluid (Fitzsimons 1972, Watts 2001).

I.1.2.2 Origins of thirst

What are the physiological substrates of thirst? Using several tools, including lesions, electrical stimulations, and pharmacological approaches applied to different types of drinking behavioral assays, investigators have discovered many central and peripheral regions contributing to the regulation of drinking behavior (Cannon 1918, Fitzsimons 1972). In this section, I will review early studies on investigating the origins of thirst in the central nervous system and periphery.

The peripheral organs are involved in the control of drinking behavior

Mouth and pharynx

The fact that dryness of mouth and pharynx promotes drinking water induced investigators hypothesizing that these places might be at the origin of thirst. To address this possibility, they first asked whether moist mouth and pharynx can attenuate thirst without restoring body water content. In 1856, Bernard showed that a horse with an esophageal fistula or a dog with a gastric fistula was unable to relieve thirst by drinking water as the swallowed water escaped through the fistula (Bernard 1856). In addition, investigators observed that blocking the transmission of dryness to the brain by severing the glossopharyngeal nerve did not abolish thirst (Longet 1868). Furthermore, the injection of water into the veil is enough to relieve thirst (Magendie 1823), indicating the dryness of mouth and pharynx are not necessary to stimulate thirst and water intake.

Why do we feel the dryness of mouth and pharynx when our bodies are dehydrated? Dryness of mouth is one of the first signs of dehydration, but it can be induced also by emotions (e.g. anxiety or fear) and certain activities (e.g. intense speaking) without concurrent body dehydration (Guggenheimer & Moore 2003, Porter et al 2004). The dryness of the mouth is due to the buccal glands (Edgar et al 2012). In mammals, there are three pairs of salivary glands, the parotid, sub-maxillary, and sub-lingual. The action of these organs is to secrete a fluid that consists of almost 100% water. When water is lacking in the body, these glands fail to provide enough liquid to moisten the mouth and throat, thereby triggering dryness. Interestingly, Epstein et al. found that salivarectomized rats drink more water than the normal ones when they eat (Epstein et al 1964).

Altogether, these observations indicate that whereas dryness of the mouth, tongue, and pharynx is associated with thirst and it is often sufficient to trigger drinking, this phenomenon is not necessary for the generation of thirst.

Plasma osmolality

Mammals are able to sense the increase of serum pressure and loss of blood volume (Mayer 1900, Wettendorff 1901), leading to mechanisms promoting drinking behavior. Later studies showed artificial changes of plasma osmolality by administering hypertonic sodium Chloride (NaCl) solutions either intraperitoneally or intracerebrally induces drinking behavior (Arden 1934, Janssen 1936, Leschke 1918). However, further mechanisms were not clear. Since the treatment increases plasma osmolality as well as the Na^+ and Cl^- concentrations, it was still not clear whether drinking was a result of high osmolality or a direct effect of these ions. In addition, it was not known which part of the body senses ion concentrations or high osmolality to trigger drinking behavior. In the section of I.1.2.4 and table 3, some related studies show that the hypothalamus is a key region to promote drinking in response to hypertonic NaCl.

Kidneys

Kidneys are very important for the control of the volume of various body fluid compartments, fluid osmolality, various electrolyte concentrations and the removal of toxins (Fountain & Lappin 2019). These organs have been proposed as a sensor of the osmolality and blood pressure, and to produce different elements to counteract any unbalance detected. For example, the renin-angiotensin cascade is activated by kidneys for restoring body water balance, regulation of blood pressure, and systemic vascular resistance (Fountain & Lappin 2019). The Renin/Ang II cascade described above starts from the kidneys. Besides stimulating drinking, Ang II also induces the contraction of blood vessels, increases blood pressure and stimulates the adrenal gland to secrete aldosterone, which causes the reabsorption of water and sodium by the renal tubules (Farag et al 2017, Sparks et al 2014). Therefore, the kidney-derived Renin/Ang II system can elicit water intake and reabsorption of water from the blood to maintain the body water balance.

The brain is involved in the control of drinking behavior

The subfornical organ

The subfornical organ (SFO) is a brain region belonging to the group of circumventricular areas. It is located on the ventral surface of the fornix near the interconnection between the lateral and the third ventricles. Routtenberg and Simpson showed that the local administration of the cholinergic agonist carbachol stimulation in the SFO promotes drinking behavior (Routtenberg & Simpson 1971, Simpson & Routtenberg 1972). Furthermore, the local injection of Ang II into the SFO elicits drinking behavior in water-satiated rats (Simpson & Routtenberg 1973). In contrast, lesions of the SFO in rats block stimulated water intake by application of Ang II to the basal telencephalon (Simpson & Routtenberg 1973). Altogether, these results indicate that the SFO is an important brain area in the control of drinking behavior.

The paraventricular nucleus and the supraoptic nucleus of the hypothalamus

The paraventricular nucleus of the hypothalamus (PVH), located in the ventral diencephalon adjacent to the third ventricle, is a highly conserved brain region present in species from zebrafish to humans (Qin et al 2018). The supraoptic nucleus (SON) is located at the base of the brain, adjacent to the optic chiasm (Leng et al 1982). Verney et al. found that increases in osmolality cause the release of the antidiuretic hormone, called vasopressin (Verney 1947), which is synthesized in the paraventricular nucleus and the supraoptic nucleus of the hypothalamus (Lederis 1961, Swanson & Sawchenko 1983). In the posterior pituitary, vasopressin is released from vesicles into the circulation in response to extracellular hypertonicity and it stimulates the kidney to promote water reabsorption (Farag et al 2017, Sparks et al 2014).

The ventromedial hypothalamus

The ventromedial nucleus of the hypothalamus (VMH) is a nucleus of the hypothalamus. The descriptions of hypothalamic obesity by Hetherington and Ranson (Hetherington & Ranson 1942) and of hyperphagia by Brobeck et al. (Brobeck et al 1943) after damage to both ventromedial nuclei were later supported by Anand and Brobeck's paper, "Hypothalamic control of food intake in rats and cats" (Anand & Brobeck 1951). In this seminal paper, aphagia (the inability or refusal to swallow) after

lesions in the lateral hypothalamus were reported, suggesting that the lateral hypothalamus is an active feeding center and that the ventromedial regions have the function of stopping the animal from eating when the necessary amount of food has been ingested. However, these authors did not analyze water intake in their experiments. Stevenson and his colleagues (Stevenson 1949, Stevenson et al 1950) reported that lesions in or near the ventromedial hypothalamic nuclei in rats caused a decrease in the proportion of water to food taken by the animal, a relative failure to excrete a water load, and increases in urinary concentration and in serum sodium. The animals became chronically dehydrated owing to an inadequate intake of water.

The anterior and medial hypothalamus

The anterior hypothalamus is a nucleus of the hypothalamus, and located in the anterior part of the hypothalamus. Witt et al. in 1952 described the complete absence of thirst in the dog after extensive electrocoagulation lesions of the anterior hypothalamus, extending dorsally and laterally, a phenotype accompanied by the development of diabetes insipidus (Witt et al 1952). Andersson and McCann, with more restricted lesions in the medial hypothalamus between the fornix and mamillothalamic tracts anterior to the mamillary region and not involving the supraoptic region, were able to produce adipsia (the inappropriately decreased or absent feelings of thirst) without diabetes insipidus in the dog (Andersson & McCann 1956). In both experiments, it should be noted that the dogs continued to feed normally.

The lateral hypothalamus

The lateral hypothalamus is a brain nucleus located in the hypothalamus of the brain in mammals. Lesions in the lateral hypothalamus of rats were shown to cause adipsia by Teitelbaum and Stellar, but this might have been secondary to aphagia or to general debility (Teitelbaum & Stellar 1954). Montemurro and Stevenson, however, demonstrated that it is possible to produce adipsia without aphagia in the rat with lesions in the dorsal and lateral regions of the lateral hypothalamic area opposite the plane between the ventromedial nucleus and the premamillary nucleus (Montemurro & Stevenson 1957).

Interestingly, later experiments showed that water-deprived rats choose to self-stimulate the lateral hypothalamus rather than press a lever for water. It may be that the self-stimulation is the partial activation of a drive system of the visceral brain (Mogenson & Stevenson 1966). This evidence appears to favor two separate drive systems, one subserving drive for water, and the other subserving drive for other reinforcements. The concurrent drinking and self-stimulation results from the activation of both systems. Mogenson confirmed this hypothesis by using amphetamine, which depresses drinking, but it enhances the rate of self-stimulation (Mogenson 1968).

Hypothalamic damage-caused aphagia and adipsia in rats were well studied by Teitelbaum and Epstein. Lesions of the lateral part of the lateral hypothalamus, invading the internal capsules and optic tracts, extending to the subthalamus and the base of the brain caused permanent impairment of thirst, without, however, affecting prandial drinking (Teitelbaum & Epstein 1962).

Greer et al. showed a striking increase in water intake by stimulation of the lateral edge of the dorsomedial nucleus in the lateral hypothalamus (Greer 1955). In addition, Mogenson and Stevenson found a similar phenotype by self-stimulation of the lateral hypothalamus (Mogenson & Stevenson 1966). Thus, it seems that the neural mechanisms of thirst-induced behavior have a relationship with the reward system. However, neither water deprivation nor water loading affects the rates of self-stimulation, but food deprivation increases and excess feeding decreases the rate of self-stimulation (Mogenson 1969). This may indicate the possibility that the thirst drive is much stronger than the hunger drive.

The medial forebrain bundle

Since lesions in the lateral hypothalamus cause adipsia and aphagia, Morrison et al. supposed that the medial forebrain bundle may be as important as the lateral area for the control of food and water intake (Morrison et al 1958). In the late 1950s, Nauta carefully characterized the medial forebrain bundle in detail (Nauta 1958). This structure is a major projection system that traverses the entire lateral hypothalamic area. The medial components originate in the septal area, where they receive connections from the hippocampal formation and the amygdala. The lateral components come directly

from the amygdaloid-hippocampal system. Medial and lateral components pass through the lateral preoptic and lateral hypothalamic regions, receiving fresh fibers and shedding others, to leave the diencephalon and enter the midbrain. The medial components terminate in the limbic midbrain area, in the periaqueductal gray matter, and in the ventral tegmentum, while the lateral components merge with the reticular formation in the central and lateral tegmental regions. The lateral components contain fibers of lateral hypothalamic origin, many of which relay on the mesencephalic reticular formation to end finally on the bulbar motor nuclei which are important for the basic ingestive reflexes. The bundle also contains an extensive system of ascending fibers coming from the mesencephalic-septum and hypothalamic-septum. The mesencephalic projections contain a substantial contribution from the mamillary peduncles that bypass the mamillary bodies and end in the lateral hypothalamus, lateral preoptic region, and medial septal nucleus. Another major connection between the limbic midbrain and the medial hypothalamus is the periventricular system and the dorsal longitudinal fasciculus of Schiitz, which contain descending and ascending fibers running between the medial hypothalamus and periaqueductal gray matter. Some ascending fibers also end in the intralaminar thalamic nuclei (Nauta 1958).

The aphagia and adipsia due to lesions in the lateral hypothalamus area can be attributable to damage to cells or to the fiber tract passing through it. The lateral hypothalamic area serves as the “bed nucleus” of the medial forebrain bundle, it is, therefore, difficult to separate them by classical lesions and/or electrical stimulation. However, later studies showed that bilateral cuts either anterior or posterior to the lateral hypothalamus without lesions of the lateral hypothalamus itself, lead to aphagia and adipsia, a similar effect caused by lateral hypothalamus damage (LBERT et al 1970). Thus, the medial forebrain bundle participates in the control of ingestive behaviors.

The pallidofugal fibers

The pallidofugal fibers are nerve projections that conduct impulses from the globus pallidus across the internal capsule to the thalamus and nearby areas (Parent & Parent 2004). Interruption of pallidofugal fibers, including the pallido-hypothalamic connections,

causes complete and permanent aphagia and adipsia (Morgane 1961a, Morgane 1961b, Morgane 1964), pointing to the essential role of the globus pallidus in the control of feeding and drinking. Morgane et al. made the typical midlateral lesions of pallidofugal fibers, which led to “motivational inertia” and prevented the rats from lever pressing for food or water, but the lesions did not prevent free feeding and drinking. In addition, lesions of the far-lateral pallidofugal fibers produced the same deficit in ingestive behavior. Both lesions decreased motivation to eat and drink without changing motor functions.

The septal area

The septal area is a brain structure in the lower, posterior part of the medial surface of the frontal lobe, and refers to the nearby septum pellucidum (St-Amant 2019). Lesions of the septal area in rats increased daily water intake and thirst drive measured in operant conditions (Harvey & Hunt 1965). The animals with septal injury were approaching water and food even though this behavior was punished, indicating a deficit of passive response avoidance. In addition, the bilateral lesions of the septum in rats have been reported to increase the intake of 1.5% saline and water (Négro-Vilar et al 1967). Morita et al. reported that the effect of hyperdipsia in the septal lesioned rats was blocked by the lesions of the subfornical organ (SFO) (Morita et al 1980). These results point to an antagonistic relationship between the septum and SFO: the septum being an inhibitory center, the SFO being an excitatory center for drinking behavior.

The amygdala

The amygdala is one of two almond-shaped clusters of nuclei located deep and medially within the temporal lobes of the brain in complex vertebrates, including humans (Science 2004). Lesions of the amygdala in rats and cats led to aphagia (Anand 1961). It is not clear which part of the amygdala is involved and if water intake was affected by the lesions. Later Grossman et al. showed that lesions of the anterior part of the ventral amygdala caused an increase in food intake and a decrease in water intake. Lesions of the posterior part of the ventral amygdala caused a gradual rise in food intake and water intake (Grossman & Grossman 1963). In addition, lesions of the ventromedial amygdala in rats caused a decrease in water intake without affecting food

intake. However, Fonberg et al. showed that limited lesions of the dorsomedial portion of the amygdaloid complex in dogs caused aphagia and adipsia (Fonberg 1966, Fonberg 1969a, Fonberg 1969b, Fonberg & Sychowa 1968).

Besides the lesion approach in the amygdala, Grossman et al. investigated the feeding and drinking behavior after stimulation of the amygdala. Electrical stimulation of the anteroventral amygdala increased drinking, but decreased feeding, whereas posteroventral stimulation inhibited both behaviors (Grossman & Grossman 1963).

The tegmentum

The tegmentum is the ventral part of the midbrain, and located between the ventricular system and distinctive basal or ventral structures at each level (Reinoso-Suarez et al 1994). In the midbrain, bilateral lesions of the tegmentum or of the periaqueductal gray matter caused hyperphagia, but no effects on the drinking behavior in monkeys (Ruch et al 1942, Skultety 1958, Skultety 1966, Skultety & Gary 1962). However, later studies showed the opposite effect: Parker and Feldman found that lesions of the ventral tegmental area at the level of the red nucleus caused aphagia and adipsia in rats (Parker & Feldman 1967). Gold also reported aphagia and adipsia after tegmentum lesions (Gold 1967). Like the effects of the lateral hypothalamic lesions, Ungerstedt claimed that destruction of nigrostriatal fibers might be the cause of the aphagia and adipsia induced by tegmental lesions (Ungerstedt 1971).

The subcommissural organ

The subcommissural organ is an ependymal structure in the roof of the cerebral aqueduct, ventral to the posterior commissure. This area, located outside of the blood-brain barrier, can respond rapidly to changes in the composition of body fluids and it is considered as an important neural and endocrine organ involved in thirst (Rodriguez et al 1998). However, the function of the subcommissural in thirst has been disputed (Crow 1967, Gilbert 1957).

Lesions of a brain region	Species	Drinking	Feeding	Reference
Septal area	Rat	-		Harvey & Hunt 1965, Morita et al 1980
Amygdala	Rat and cat	-	-	Anand 1961
Anterior ventral amygdala	Rat	-	+	Grossman & Grossman 1963
Posterior ventral amygdala	Rat	+	+	Grossman & Grossman 1963
Ventromedial amygdala	Rat	-	NS	Fonberg 1969b
Dosalmedial amygdala	Dog	-	-	Fonberg 1966, Fonberg 1969a, Fonberg & Sychowa 1968
Anterior hypothalamus	Dog	-		Witt et al 1952, Andersson & McCann 1956
Ventromedial hypothalamus	Rat	-	+	Stevenson 1949, Stevenson et al 1950
Lateral hypothalamus	Rat	-	-	Teitelbaum & Stellar 1954
Medial forebrain bundle	Rat	-	-	Lbert et al 1970
Tegmentum	Monkey	NS	+	Ruch et al 1942, Skultety 1958, Skultety 1966, Skultety & Gary 1962
Tegmentum	Rat	-	-	Gold 1967.
Pallidofugal fibers	Rat	-	-	Morgane 1961a, Morgane 1961b, Morgane 1964
Nigrostriatal system	Rat	-	-	Ungerstedt 1971

Table 1. Early studies of lesions on drinking and feeding behaviors. “+” and “-” represent an increase and a decrease respectively, “NS” is the abbreviation of “no significant difference”.

The cerebral cortex

The cerebral cortex is the outer layer of the neural tissue of the cerebrum of the brain in humans and other mammals (McKinley 2011). The induction of spreading cortical depression on both cerebral hemispheres with 25% KCl leads to adipsia, but the

unilateral spreading cortical depression has no effect on drinking behavior. In addition, the bilateral depression in the cerebral cortex also leads to aphagia. This may be due to a general depression of the subcortical behavioral system (Levitt & Krikstone 1968). However, in the rat, unilateral decortication was reported to increase food intake (Covian et al 1954) and selective cortical ablations cause changes in food preferences, but have no effect on the water intake.

Electrical stimulation	**Species**	**Drinking**	**Feeding**	**Reference**
Anterior ventral amygdala	Rat	+	-	Grossman & Grossman 1963
Posterior ventral amygdala	Rat	-	-	Grossman & Grossman 1963
Area between the fornix and anterior commissure	Goat	+		Andersson & McCann 1955
Area between the anterior columns of the fornix and the mamillothalamic tract	Goat	+		Andersson & McCann 1955
Supraoptic nucleus	Goat	NS		Andersson & McCann 1955
Paraventricular nucleus	Goat	NS		Andersson & McCann 1955
Dorsomedial nucleus in the lateral hypothalamus	Rat	+		Greer 1955

Table 2. Early studies of electrical stimulations on drinking and feeding behaviors. "+" and "-" represent an increase and a decrease respectively, "NS" is the abbreviation of "no significant difference".

The perifornical region

The perifornical region is a brain structure located in the hypothalamus (Tortorella et al 2013). Andersson and his colleagues found that the injection of 0.1-0.2ml of 2%

NaCl into the perifornical region leads to drinking immediately in goats (Andersson 1967). In addition, Andersson et al. found that electrical stimulation of the area between the anterior columns of the fornix and the mamillothalamic tract, extending from dorsal to ventral hypothalamus, causes drinking and antidiuresis. Similar effects were obtained by stimulating the area between the fornix and anterior commissure, lateral to the paraventricular nucleus. Moreover, the authors found that the stimulation of the paraventricular nucleus and the supraoptic nucleus leads to antidiuresis, but it has no effect on water intake (Andersson et al 1960, Andersson & McCann 1955).

In conclusion, lesion and electrical stimulation approaches in the brain of different species provided valuable information, even though sometimes contradictory results about the brain systems subserving drinking behavior. However, these approaches present several disadvantages. For example, animal behaviors upon electrical stimulation are difficult to compare with untreated animals. Chemical stimulation is more specific and causes fewer side effects than lesions and electrical stimulation. Therefore, chemical stimulation may be a good approach to study drinking behavior and therapy.

I.1.2.3 Hormonal control of water intake

Fluid balance is pivotal for life and its control in response to changes in water osmolality and contents in the body is tightly regulated. Different parts of the body release hormones regulating water intake and reabsorption in order to keep the fluid balance. In addition, dehydration promotes the production of dipsogenic hormones to enhance drinking behavior, whereas excess content of water promotes anti-dipsogenic hormones' production and release (Leib et al 2016). Here, I will briefly review the hormones that participate in the control of water intake (**Figure 1**).

Vasopressin

Vasopressin is an antidiuretic hormone first discovered in 1913 (Farini 1913, Vongraven 1913). It is synthesized by neurons in the paraventricular nucleus of the hypothalamus and the supraoptic nucleus. It is directly released to the posterior pituitary through the axons and stored there. When the osmolality of the extracellular fluid is increased, vasopressin is secreted to the circulation provoking water retention by the

kidney and raising blood pressure by constricting arterioles (Nielsen et al 1995, Robertson et al 1976). After drinking water, vasopressin levels are decreased within minutes (Geelen et al 1984). On the other hand, some studies showed that vasopressin also participates in the regulation of drinking. Early studies showed a direct anti-dipsogenic effect in dogs (Bellows 1939, Holmes & Gregersen 1950). However, later studies reported a dipsogenic effect of vasopressin (Adolph & Barker 1953) or no effect on drinking behavior (De wied 1966). The contradictory results may be due to doses of vasopressin and timing. Therefore, vasopressin regulates water excretion by kidneys, but its role in the control of water intake is still not very clear.

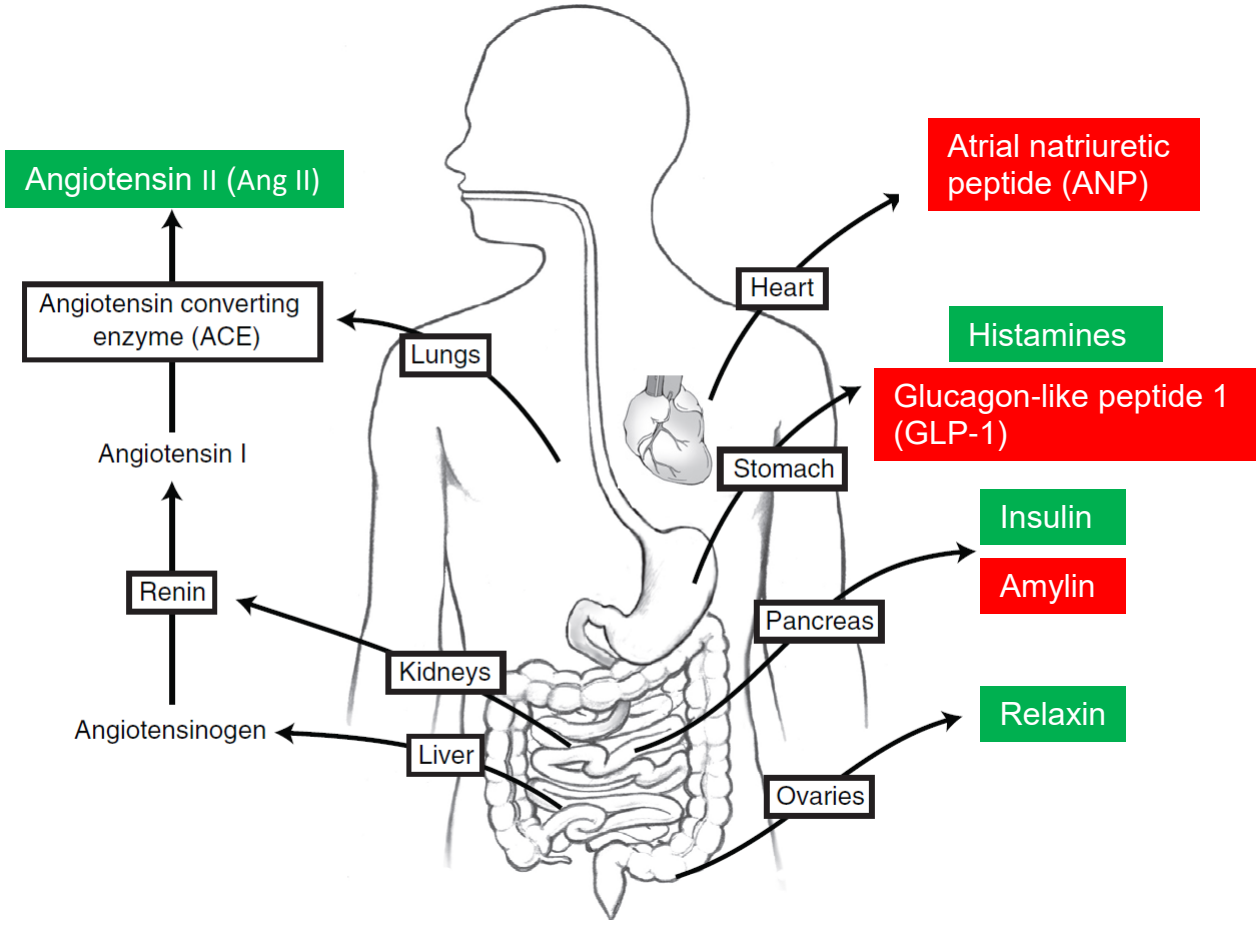


Figure 1. Hormones control water intake. Hormones in green backgound promotes drinking, and in red brckgound suppress drinking (modified from Leib et al 2016).

Insulin

Insulin is a peptide hormone produced by beta cells of the pancreas and was discovered at the beginning of 1920s (Banting & Best 1922, Banting et al 1922). It is the main anabolic hormone of the body and promotes absorption of glucose, from the blood to the liver, fat and skeletal muscle cells (Stryer 1995, Voet & Voet 2011). In early studies, insulin was shown to enhance the secretion of vasopressin (Verney 1947), and intravenous injections of insulin promoted water intake in rats (Novin 1964, Spitz 1975). In addition, intraperitoneal injection of insulin increased plasma volume and decreased plasma osmolality in rats (Waldbillig & Bartness 1981) and, in humans, intravenous administration of insulin significantly increased water and food intake. Meanwhile, the authors recorded thirst and hunger degrees reported by insulin-injected humans, and found that the persons feel thirst prior to hunger (Vijande et al 1990). Thus, these studies clearly indicate that insulin is a dipsogenic hormone.

Amylin

Amylin is a 37-residue peptide hormone first reported in 1987 (Cooper et al 1987). It is co-secreted with insulin from pancreatic beta cells. The ratio of insulin to amylin in the blood is approximately 20:1 (Kruger et al 1999). Amylin plays a key role in the down-regulation of glycemia and inhibition of ingestive behavior (Chance et al 1992, Lutz et al 1994, Morley et al 1997). Investigators studied the effects of local amylin application on drinking and feeding, and found that the injection of amylin into the subfornical organ inhibits drinking behavior (Riediger et al 1999). In addition, recent studies showed that administration of amylin into the nucleus accumbens or the ventral tegmental area reduces both water and food intake (Baldo & Kelley 2001, Mietlicki-Baase et al 2017). Therefore, amylin is now considered to be an anti-dipsogenic hormone.

Thirst in Hyperparathyroidism

Hyperparathyroidism is a condition in which the parathyroid glands produce too much parathyroid hormone and the calcium level in the blood becomes elevated (Albright 1948, Beard et al 1989). One early study showed that polydipsia and polyuria are the first and most prominent symptoms of hyperparathyroidism (Heesen et al 1976).

Angiotensin II

Angiotensin II (Ang II) is a peptide hormone first isolated in the late 1930s (Page 1939, Page & Helmer 1940). It plays a key role in eliciting water intake and reabsorbing water from the blood to maintain body water balance, causing vasoconstriction and increasing blood pressure (Scroop & Lowe 1968). Intravenous injection of Ang II promotes water intake in rats (Fitzsimons & Simons 1969). This might be due to three causes. *1).* The sensitivity of vascular stretch receptors to preexisting hypovolemia might be increased (Fitzsimons 1966b, Scroop & Lowe 1968); *2).* Plasma volume may fall further because of increased capillary permeability (Asscher & Anson 1963, Haefeli & Peters 1971); *3).* Drinking behavior elicited by Ang II may be due to the direct target on the brain (Fitzsimons 1966a). Later studies support the third possibility, and indicate that the Ang II stimulates thirst-related brain areas in order to promote water intake. Indeed, intraventricular injection of Ang II produces a dipsogenic effect (Severs et al 1970) and causes release of vasopressin (Iovino & Steardo 1984, Knepel et al 1982). In addition, local injections of Ang II into the septum, the medial preoptic nucleus, or the anterior hypothalamic area causes water intake in water-replete rats (Epstein et al 1970).

Relaxin

Relaxin is a protein hormone of about 6kDa first reported in 1926, which is mainly produced by the corpus luteum of the ovary and the breast during pregnancy in females, and in semen in the male (Bani 1997, Hisaw 1926). A strong feeling of thirst frequently occurs premenstrually, during pregnancy and lactation, which is partially due to an increase in relaxin secretion (Lindheimer et al 1989). Investigators showed that central administration of relaxin promotes drinking behavior in the male and female rats (Summerlee & Robertson 1995, Thornton & Fitzsimons 1995).

Histamine

Histamine is an organic nitrogenous compound involved in local immune responses and other functions, and was first described in 1910 (Dale & Laidlaw 1910). They also regulate physiological functions of the gut and the uterus, and they act as neurotransmitters in the brain and the spinal cord (Marieb 2001). There are two main

receptor types, H_1 and H_2. H_1 receptors exert the vascular and inflammatory effects, H_2 receptors control gastric acid secretion and intestinal motility (Panula et al 2015).

Systemic administration of histamine causes an increase in water intake in rats (Leibowitz 1973). H_1- and H_2-receptor antagonists have additive inhibitory effects on histamine-induced water intake (Leibowitz 1979)(Leibowitz, 1979). Furthermore, local injection of histamine into the lateral hypothalamus (Gerald & Maickel 1972), the preoptic area, the lateral or anteroventral hypothalamus increases water intake in thirsty and sated rats (Leibowitz 1973). However, the administration of histamine into the ventromedial hypothalamus and mammillary bodies did not produce any dipsogenic effect (Myers & Sharpe 1968). Therefore, histamine participates to regulate drinking behavior as a dipsogenic agent.

Atrial natriuretic peptide

Atrial natriuretic peptide (ANP) is a hormone secreted from the cardiac atria, and was first reported in 1981 (de Bold et al 1981, Macchia 1987). The main function of ANP is to reduce the expanded extracellular fluid by increasing renal sodium excretion and decreasing thirst (Burrell et al 1992, Ehrlich & Fitts 1990, Tarjan et al 1988, Tian et al 1992, Unger et al 1989, Weisinger et al 1992). Central administration of ANP inhibits water intake in rabbits, sheep, and rats (Tarjan et al 1988, Tian et al 1992, Weisinger et al 1992). Furthermore, local stimulation of the SFO by ANP suppresses drinking behavior (Ehrlich & Fitts 1990). Thus, ANP plays a negative role in the control of water intake.

Glucagon-like peptide 1

Glucagon-like peptide 1 (GLP-1) is a 30 or 31 amino acid long peptide hormone first identified in the early 1980s (Lund et al 1982). It is produced and secreted by intestinal enteroendocrine L-cells and certain neurons within the nucleus of the solitary tract. Its well-known functions are inhibiting food and water intake (McKay et al 2014, McKay et al 2011, Navarro et al 1996). Injection of GLP-1 into the third cerebral ventricle inhibits water intake in rats (McKay & Daniels 2013). GLP-1 is another hormone that suppresses drinking behavior.

I.1.2.4 Pharmacology of water balance

Body water balance is crucial to health and diseases (Roumelioti et al 2018). This is largely achieved by the regulation of drinking behavior. In animals and humans, a large variety of disparate substances contribute to a stimulatory or an inhibitory effect on drinking. This may be due to a direct action on neurons controlling water intake, or changing water or electrolyte balance causing secondary regulation of drinking (Fuller 1984, Roumelioti et al 2018). In this section, I will review pharmacological approaches to water balance, with particular emphasis on the study of central effects (**Table 3**).

Electrolytes

Systemic application of hypertonic NaCl elicits drinking behavior (Arden 1934, Janssen 1936, Leschke 1918). Andersson et al. found that drinking is elicited by the injection of hypertonic NaCl (2-3%) into the medial hypothalamus of the goat, in the vicinity of the third ventricle, or into the ventricle (Andersson 1953, Andersson & McCann 1955). However, it was not clear whether this effect is due to changes in osmotic pressure or to specific effects of the ions. In following studies, Andersson et al. investigated this issue (Andersson et al 1967). First, they observed that the injection of 0.1ml of 0.85M NaCl into the third ventricle triggered drinking in water-replete goats. Similar amounts of 0.85M NH_4Cl, 1.7M d-glucose or 1.7M urea injected into the third ventricle did not elicit drinking. The results indicate that sodium may be the key component to trigger drinking, not the chloride and osmotic pressure (Andersson et al 1967). In addition, the same amount of 0.85 M $NaHCO_3$ was much less effective in causing drinking and 0.85M CH_3COONa failed to cause drinking. Therefore, it is likely that Na^+ and Cl^- coming from other sources than NaCl do not have a strong effect on eliciting drinking.

Cholinergic system and drinking

The cholinergic system comprises of acetylcholine (ACh) and acetylcholine receptors, including nicotinic and muscarinic ACh receptors. As a neurotransmitter, ACh is released by nerve cells to send signals to other cells, like neurons, muscle cells, and gland cells (Everitt & Robbins 1997, Houser et al 1985). The cholinergic system is distributed in the central and peripheral nervous system and exerts a variety of functions

(Sarter & Parikh 2005). Here, mechanisms of the cholinergic system in the control of drinking are reviewed.

Looking back to 1939, Pickford reported that the intravenous injection of acetylcholine inhibits water diuresis in dogs (Pickford 1939). Later studies showed that the injection of acetylcholine into the supraoptic nucleus causes antidiuresis (Pickford 1947). In rats, Grossman found that injection of acetylcholine into the lateral hypothalamus elicits drinking, whereas epinephrine and especially norepinephrine causes feeding (Grossman 1960, Grossman 1962a). In addition, muscarine also enhances drinking through the stimulation of the ACh receptor in the hypothalamus, but nicotine does not have the same effect (Stein & Seifter 1962). Therefore, stimulation of muscarinic acetylcholine receptors in the lateral hypothalamus enhances drinking in rats. Atropine is a competitive antagonist of muscarinic acetylcholine receptors, including the types M1, M2, M3, M4, and M5. Grossman reported that the systemic administration of atropine sulfate blocks drinking induced by acetylcholine administration (Grossman 1962b). Furthermore, the systemic or intracranial injection of atropine blocks the drinking induced by water deprivation, subcutaneous hypertonic saline injection, and schedule-induced polydipsia (De wied 1966, Grossman 1962b). In contrast, blockade of the peripheral cholinergic system by the methylatropine nitrate, which can not cross the blood-brain barrier, is much less effective in blocking drinking (Miller 1965), suggesting that the central cholinergic system plays a key role here.

Fisher and Coury studied elicited drinking by the stimulation of cholinergic receptors in the limbic system in rats (Fisher & Coury 1962, Fisher & Coury 1964). Carbachol is an agonist of the acetylcholine receptor, that is not hydrolyzed by cholinesterase. Injection of carbachol into most parts of the limbic forebrain and midbrain elicits drinking in water-replete rats. The most responsive brain areas include the dorsomedial hippocampus, the septal region, the area of the diagonal band, and the reuniens nucleus in the thalamus. Some other areas are also responsive to the carbachol, including the perifornical area, the preoptic nuclei, the lateral hypothalamic-medial forebrain bundle region, the mammillary-interpeduncular region, the anterior thalamic nuclei, and the cingulate gyrus. Some areas like the ventromedial region of the hypothalamus, the paraventricular nucleus, the frontal cortex, the caudate nucleus, the

pyriform and the entorhinal cortices, the posterior and lateral hippocampus and the amygdala do not respond to carbachol (Fisher & Coury 1962, Fisher & Coury 1964). These results indicate the central cholinergic system plays a key role in the control of drinking.

However, it seems that the cholinergic system in the control of drinking is surprisingly dependent on the species. For example, cholinergic stimulation of the lateral hypothalamus in cats causes rage, aggression, and sleep, but not drinking (Myers & Sharpe 1968). To complicate things even more, the stimulation of the cholinergic system causes opposite effects in rats and monkeys. Injection of carbachol, acetylcholine, or eserine (a reversible cholinesterase inhibitor) into different parts of the diencephalon blocked all ingestive behavior in the hunger or thirsty monkey, and atropine reversed these effects (Sharpe & Myers 1969). Thereby it is possible that the effects of the cholinergic system on drinking are dependent on the species and doses, and the further mechanisms of the cholinergic drinking need to be studied.

Drug	**Species**	**Location/injection**	**Drinking**	**Feeding**	**Reference**
NaCl	Goat	Perifornical region	+		Andersson 1967
NaCl	Goat	Medial hypothalamus	+		Andersson 1953
Acetylcholine	Dog	Intravenous injection	+		Pickford 1939
Acetylcholine	Rat	Lateral hypothalamus	+		Grossman 1960, Grossman 1962a
Acetylcholine	Cat	Lateral hypothalamus	NS		Myers & Sharpe 1968
Acetylcholine	Monkey	Diencephalon	-	-	Myers & Sharpe 1968
Muscarine	Rat	Hypothalamus	+		Stein & Seifter 1962
Nicotine	Rat	Hypothalamus	NS		Stein & Seifter 1962
Epinephrine	Rat	Lateral hypothalamus	NS	+	Grossman 1960, Grossman 1962a

Norepinephrine	Rat	Lateral hypothalamus	NS	+	Grossman 1960, Grossman 1962a
Isoprenaline	Rat	Subcutaneous injection	+		Lehr et al 1967, Zamboni & Siro-Brigiani 1966
Isoprenaline	Rat	Hippocampus	+		Grossman 1964, Mountford 1969)
Isoprenaline	Rat	Ventral hippocampus	+		Grossman 1964, Mountford 1969)
Metaraminol	Rat	Subcutaneous injection	NS		Lehr et al 1967, Zamboni & Siro-Brigiani 1966
Carbachol	Rat	Subfornical organ	+		(Routtenberg & Simpson 1971, Simpson & Routtenberg 1972
Carbachol	Rat	Limbic forebrain and midbrain	+		Fisher & Coury 1962, Fisher & Coury 1964
5-HT	Rat	Subcutaneous injection	+		Goldman et al 1968
Indoleamine 5-HT	Monkey	Diencephalon	+	+	Sharpe & Myers 1969
Amphetamine	Dog	Intramuscular injection	-		Andersson & Larsson 1956
Dopamine	Rat	Intracranial injection	+		Poat et al 1980
Haloperidol	Rat	Intracranial injection	-		Poat et al 1980
Spiroperidol	Rat	Intracranial injection	-		Poat et al 1980

Table 3. Pharmacological approaches to drinking behavior. “+” and “-” represent an increase and a decrease respectively, “NS” is the abbreviation of “no significant difference”.

Adrenergic systems and drinking

Adrenergic system is a group of organs and nerves in which epinephrine (adrenaline) and/or norepinephrine (noradrenaline) act as neurotransmitters

(Lymperopoulos et al 2013). As mentioned above, Grossman found that local injection of epinephrine, and especially norepinephrine into the lateral hypothalamus causes eating in satiated rats, but had no effect on drinking (Grossman 1960, Grossman 1962a). In later studies, investigators found that injection of adrenaline into the lateral hypothalamus can either decrease or increase water intake in rats (Grossman 1962a, Miller 1965, Myers 1964), suggesting that there may be two mechanisms leading to opposite effects. In view of these contradicting results, investigators studied α- and β-adrenergic agonists in the control of drinking. Subcutaneous injection of the β-adrenergic agonist isoprenaline enhances drinking in rats (Lehr et al 1967, Zamboni & Siro-Brigiani 1966). In contrast, the α-adrenergic agonist, metaraminol, has no effect on water intake, whereas the β-adrenergic blocker propranolol blocks drinking induced by β-adrenergic agonist isoprenaline. Peskar et al. found that systemic administration of the β-adrenergic agonist nylidrine or isoxsuprine and α-adrenergic blockers phentolamine or phenoxybenzamine stimulate drinking and simultaneous antidiuresis in a β-adrenergic receptor-dependent manner (Peskar et al 1970). Furthermore, the injection of the β-adrenergic agonist isoprenaline into the rat hippocampus, but not into the septum, causes significant drinking (Grossman 1964, Mountford 1969). Thus, these studies indicate that α-adrenergic signaling likely acts a satiety system, whereas β-adrenergic signaling promotes thirst.

5-Hydroxytryptamine and drinking

5-hydroxytryptamine (5-HT, serotonin) is a monoamine neurotransmitter, and has a variety of neural functions, such as modulating cognition, reward, learning, memory, and numerous physiological processes (Mohammad-Zadeh et al 2008, Young 2007). 5-HT acts through metabotropic and ionotropic receptors. Subcutaneous injection of 5-HT caused drinking and decrease of urine excretion in rats (Goldman et al 1968). In monkeys, the indoleamine 5-HT applied in the diencephalon caused drinking and eating (Sharpe & Myers 1969). However, Booth et al. found that no or little drinking is elicited by the injection of 5-HT into the anterior thalamus. Goldman and Lehr found the inhibition of eating by the injection of 5-HT into the region of the fornix, but no effect on drinking (Goldman & Lehr 1971). Therefore, the further central mechanisms of 5-HT induced drinking are still not clear.

Inhibition of drinking by amphetamine

Amphetamine is a central nervous system stimulant (Heal et al 2013). It is used for treating attention-deficit hyperactivity disorders, narcolepsy, and obesity through enhancing the signaling of monoamines by inhibition of the monoamine transporters (Lee et al 2018). Amphetamine decreases drinking (Andersson & Larsson 1956), but the mechanisms involved are not clear. It may be due to the alteration of monoamine levels, but further studies are required to explain the involvement of monoamines in the depression of drinking by amphetamine. Another study showed that inhibition of drinking by amphetamine is eliminated in the prefrontal lobotomized rats (Epstein 1959). These results indicate that the prefrontal lobe may be an important target of amphetamine for suppressing drinking.

Dopamine

Dopamine is a neurotransmitter and a hormone, and plays important roles in the brain and body (Volkow et al 2017). Destruction of the monoaminergic system by intracerebroventricular applications of 6-Hydroxydopamine (6-OHDA, a neurotoxic synthetic organic compound used to selectively destroy dopaminergic and noradrenergic neurons in the brain) inhibit Ang II-induced water intake (Fitzsimons & Setler 1971, Sumners et al 1981). Furthermore, intracranial injection of the dopamine receptor antagonists haloperidol or spiroperidol blocks Ang II-induced water intake (Fitzsimons & Setler 1975). On the other hand, intracranial injection of dopamine causes drinking behavior in water-replete rats (Poat et al 1980) and it increases food-associated water intake in monkeys (Myers & Sharpe 1968). Therefore, dopamine is indispensable in the control of water intake.

I.1.3 Latest advances in central mechanisms of thirst

A set of recent studies identified several brain areas regulating drinking behavior through specific neural circuits. The lamina terminalis is considered as the main area sensing thirst signals (Gizowski & Bourque 2018, Ichiki et al 2019, Leib et al 2016, Zimmerman et al 2017). In addition, the parabrachial nucleus (PBN) and the nucleus of the solitary tract in the hindbrain are involved in the control of water intake (Ryan et al 2017, Vivas et al 2014). Furthermore, investigators started dissecting the specific circuits which play key roles in thirst. Here, recent advances in our understanding of thirst are reviewed, with particular attention to specific brain regions, cell types, and brain circuits.

I.1.3.1 Brain areas controlling thirst.

As mentioned above, early studies on thirst used lesions, electrostimulation, and pharmacological treatments in specific brain areas. Many brain structures were identified and considered as key nodes in the control of thirst and drinking behavior, such as the lateral hypothalamus, the septum, the subfornical organ, the supraoptic nucleus, and the paraventricular nucleus of the hypothalamus. However, the cellular and molecular mechanisms were poorly understood. Thanks to recently developed viral approaches, transgenic mouse lines, optogenetics, and Designer Receptors Exclusively Activated by Designer Drugs (DREADD)-based chemogenetics, we can now directly manipulate neural activity in specific brain structures and cell types. This provides possibilities to better understand the neural mechanisms of thirst and drinking behavior (**Figure 2**).

The lamina terminalis

The lamina terminalis (LT) is a thin sheet of gray matter and pia mater that attaches to the upper surface of the chiasm and stretches upward to fill the interval between the optic chiasm and the rostrum of the corpus callosum (Plant & Zeleznik 2015). It is composed of the subfornical organ (SFO), the organum vasculosum of the lamina terminalis (OVLT), and the median preoptic nucleus (MnPO). Among the three brain areas, SFO and OVLT locate outside of the blood-brain barrier, and directly sense

thirst signals, such as Ang II and blood osmolality (Ichiki et al 2019, Zimmerman et al 2017). Recent studies have genetically identified specific neural populations involved in the control of drinking behavior in the LT. Optogenetic stimulation of glutamatergic neurons in the SFO elicits voracious water intake (Betley et al 2015, Nation et al 2016, Oka et al 2015, Zimmerman et al 2016). These glutamatergic neurons are characterized by the expression of the transcription factor ETV-1 (Oka et al 2015), nitric oxide synthase 1 (nNOS) (Betley et al 2015, Nation et al 2016, Oka et al 2015, Zimmerman et al 2016), and Ca^{2+}/calmoduin-dependent kinase II (Nation et al 2016, Oka et al 2015). Conversely, activation of GABAergic neurons in the SFO suppresses water intake in the thirsty mice (Oka et al 2015). In the MnPO, optogenetic stimulation of excitatory neurons expressing nNOS or adenylate cyclase-activating polypeptide 1 promotes drinking (Augustine et al 2018, Leib et al 2017). In the OVLT, optogenetic stimulation of nNOS- or angiotensin 1A receptor-expressing cells promote drinking behavior (Leib et al 2017). In contrast, activation of the inhibitory neurons in the MnPO/OVLT suppresses water intake (Leib et al 2017). Therefore, it appears that stimulation of excitatory neurons of the LT enhances thirst, whereas activation of inhibitory neurons suppresses thirst.

Supraoptic nucleus and Paraventricular nucleus of the hypothalamus

Vasopressin neurons in the supraoptic nucleus (SON) and the paraventricular nucleus of the hypothalamus (PVH) are able to sense changes of plasma osmolality, subsequently, they secrete vasopressin to the posterior pituitary gland, which releases vasopressin into the circulation (Bourque 2008). A recent study showed that SON vasopressin neurons anticipate changes in plasma osmolality (Mandelblat-Cerf et al 2017). Drinking water decreases plasma osmolality, whereas eating food has an opposing effect. Water deprivation increases the activity of SON vasopressin neurons. When water is available, the neural activity decreases within seconds. Conversely, eating food increases neural activity of these neurons within seconds (Mandelblat-Cerf et al 2017). The data indicates that SON vasopressin neurons anticipate drinking-elicited hypo-osmolality and feeding-elicited hyper-osmolality.

Parkyong Song et al. showed that the PVH contributes to the control of fluid homeostasis through the activity of the FGF21 receptor. FGF21 is a hormone synthesized by the liver and released in response to a wide variety of nutritional stresses like starvation, ketogenic diet, and alcohol (Song et al 2018). Systemic administration of FGF21 induced water intake by stimulation of the receptor in PVH cells (Song et al 2018). Therefore, the PVH is a key brain area in the control of drinking water.

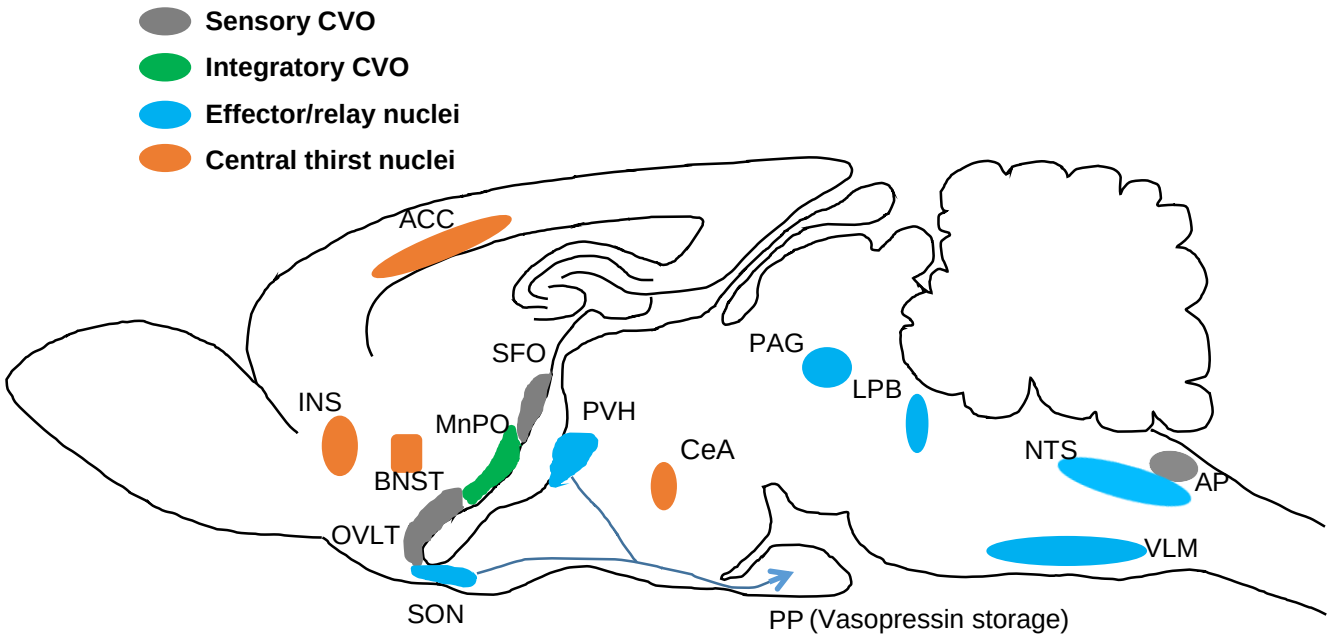


Figure 2. Brain areas are involved in the control of water intake (adapted from Leib et al 2016).

Anterior cingulate cortex (ACC)
Area postrema (AP)
Basolateral amygdala (BLA)
Bed nucleus of stria terminalis (BNST)
Circumventricular organ (CVO)
Insular cortex (IC)
Lateral parabrachial nucleus (LPB)
Median preoptic nucleus (MnPO)
Nucleus of solitary tract (NTS)
Paraventricular nucleus of hypothalamus (PVH)
Periaqueductal gray (PAG)
Posterior pituitary gland (PP)
Subfornical organ (SFO)
Supraoptic nucleus (SON)
Vascular organ of lamina terminalis (OVLT)
Ventrolateral medulla (VLM)

The suprachiasmatic nucleus

The suprachiasmatic nucleus (SCN) is a tiny region in the hypothalamus, located above the optic chiasm, and is involved in the control of circadian rhythms (Patton & Hastings 2018). Gizowski et al. found that the vasopressin-expressing neurons in the SCN mediate drinking before sleeping, which may be due to anticipation of potential dehydration during sleep [see above I.1.2.1 (Gizowski et al 2016)].

The lateral hypothalamus

The lateral hypothalamus (LH) also controls drinking and feeding behaviors (Bernardis & Bellinger 1996). Kurt et al. recently showed that activation of neurotensin-expressing neurons in the LH enhances water intake, but not food intake (Kurt et al 2019).

The bed nucleus of the stria terminalis

The bed nucleus of the stria terminalis (BNST) is a heterogeneous and complex limbic forebrain structure, which surrounds the stria terminalis (Dong et al 2001). It is considered as an important relay connecting limbic forebrain structures to the hypothalamus and brainstem regions. It plays a key role in the control of automatic, neuroendocrine and behavioral responses (Crestani et al 2013). Regarding drinking behavior, researchers discovered that local injections of hypertonic sucrose into the BNST increase water intake (Peck & Blass 1975). This reveals that the BNST is also functioning as an osmosensor. In addition, intraventricular infusion of angiotensin II increases the expression of c-Fos protein in this brain region (Herbert et al 1992), indicating that the BNST is activated during thirst conditions. Thus, the BNST is a key brain area that regulate drinking.

The hindbrain

The nucleus of solitary tract (NTS) is a series of purely sensory nuclei (clusters of nerve cell bodies) forming a vertical column of grey matter embedded in the medulla oblongata of the hindbrain (Haines 2004). It receives peripheral inputs including visceral and baroreceptor signals and it participates in the control of appetitive behaviors (Hyde & Miselis 1984, Ohman & Johnson 1989, Vivas et al 2014, Zheng et al 2010). Animals

lesioned in the NTS and adjacent area postrema significantly drink more water than the controls after the intracerebroventricular application of angiotensin II (Ohman & Johnson 1989). Recently, Ryan et al. showed that activation of NTS neurons suppresses drinking, feeding, and salt appetite. In addition, stimulation of cholecystokinin-expressing neurons in the NTS suppresses water intake as well as salt appetite. The parabrachial nuclei (PBN) are a group of nuclei in the dorsolateral pons that surrounds the superior cerebellar peduncle as it enters the brainstem from the cerebellum (Haines 2004). The PBN receives visceral afferent information from the NTS, and relays satiety signal to higher brain regions (Ryan et al 2017). Lesions of the PBN lead to an increase in water intake elicited by the intracerebroventricular injection of angiotensin II (Ohman & Johnson 1989). Ryan et al. found that activation of calcitonin-gene-related peptide-expressing neurons in the PBN suppresses water, food, and salt intake (Ryan et al 2017). In addition, these authors showed that activation of oxytocin receptor-expressing neurons in the PBN suppresses water and salt intake, but not feeding behavior (Ryan et al 2017). This reveals that the NTS and PBN are relays for satiety signal controlling ingestive behaviors, likely with selected cell populations involved in the regulation of specific intakes.

Thalamus

The thalamus is a large mass of gray matter in the dorsal part of the diencephalon (Herrero et al 2002), and is a very important relay of sensory signals, including the ones related to thirst (Herrero et al 2002, Sherman 2017). The hypothesis is that the midline nuclei of the thalamus are in charge of transmitting thirst signal from the lamina terminalis, the NTS, and the PBN to the ACC and the IC (Gizowski & Bourque 2018, Zimmerman et al 2017). In human studies, the medial nuclei of the thalamus are activated by the systemic infusion of hypertonic saline (Denton et al 1999a) and exercise-induced dehydration (Farrell et al 2011). Similar to human studies, rat medial thalamic neurons that project to the ACC and IC increase the expression of c-Fos protein in response to systemic administration of hypertonic saline. These studies indicate that the thalamo-ACC/IC circuit is engaged in thirst perception.

The amygdala

Amygdala is an almond-shaped group of nuclei in the temporal lobe, present in all mammals. It consists of the lateral amygdala (LA), the basolateral amygdala (BLA), the central amygdala (CeA), and the medial amygdala (MeA). The LA, BLA, and MeA are cortex-like structures, whereas the CeA is a striatum-like structure (Cassell et al 1999, Sah et al 2003). The amygdala is essential for affective and appetitive behaviors, decision-making, and memory (Seymour & Dolan 2008). Recent studies identified genetically distinct, spatially separated population of neurons in the BLA and CeA, and found that these different populations have opposing impact on the processing of positive and negative valences of stimuli (Kim et al 2016, Kim et al 2017). Activation of Protein Phosphatase 1 Regulatory Inhibitor Subunit 1B (Ppp1r1b)-positive cells in the posterior BLA leads to increased nose pokes to get water (Kim et al 2016). Similarly, stimulation of the lateral nucleus of the CeA (CeL) and the medial nucleus of the CeA (CeM) enhances nose pokes for drinking water. Whereas inhibition of somatostatin (Sst)-positive neurons in the CeL decreases drinking behavior as well as inhibition of the CeL neurons co-expressing hormone (Crh), neurotensin (Nts), and tachykinin 2 (Tac2). Conversely, inhibition of the protein kinase C-δ (Prkcd)-positive cells in the CeL increases drinking behavior (Kim et al 2017). Thus, the BLA and CeA are crucial for drinking behavior, and genetically distinct neuronal populations control water intake in different ways.

The anterior cingulate cortex and the insular cortex

Experiments in rhesus monkeys showed the first evidence that the prefrontal cortex may be an area for conscious perception of thirst in mammals. Robinson and Mishkin showed that the electrical stimulation of the anterior cingulate cortex (ACC) in awake rhesus monkeys provokes water intake (Robinson & Mishkin 1968). The ACC is known to be associated with affective motivation, and thirst is considered as a primordial emotion (Denton et al 2009). Therefore, the ACC may be involved in the regulation of thirst motivation.

In studies of human brain imaging using PET and functional MRI (fMRI), the ACC and the insular cortex (IC) are consistently activated by the systemic infusion of hypertonic saline (Denton et al 1999a, Denton et al 1999b, Farrell et al 2006) and

exercise-induced dehydration (Farrell et al 2011, Saker et al 2014). In addition, the ACC and IC are immediately deactivated after ingestion of water (Becker et al 2017, Egan et al 2003, Farrell et al 2011, Saker et al 2014, Saker et al 2016). In rats, c-Fos protein expression is increased in the ACC by systemic hypertonic saline administration (Ma et al 2019). Hypovolaemic thirst induced by injection of the diuretic furosemide enhances the c-Fos protein expression in the rat IC (Pastuskovas et al 2003). It appears that thirst increases the neural activity in the ACC and the IC similar to humans. Recent studies in mice showed that activation of the posterior IC inhibits water licking, whereas activation of anterior IC promotes water licking (Kim et al 2017, Wang et al 2018). Thus, the function of the IC in the control of water is known. The role the ACC in thirst is still not very clear.

I.1.3.2 Neural circuits underlying thirst

A neural circuit is defined as a population of neurons connecting with another population of neurons by synapses to carry out a specific function when activated (Purves 2011). Classical and contemporary studies have unveiled neural populations underlying the regulation of thirst. However, because the tools to study them were limited, the identities of specific neural circuits controlling thirst and the underlying mechanisms have started to be investigted only recently. Thanks to the advances of genetic manipulations and the use of chemogenetics and optogenetics approaches, investigators can now study functions of neural connections. Here, the recent work studying neural circuits underlying thirst is reviewed.

As mentioned above, the SFO is a key brain area sensing a thirst signal to regulate water intake. Activation of the SFO glutamatergic neurons projecting to the MnPO or the OVLT elicits water intake, whereas inhibition of the SFO glutamatergic neurons projecting to OVLT suppresses water intake (Oka et al 2015).

Photostimulation of the SCN vasopressin neurons projecting to the OVLT increases water intake, whereas photoinhibition of the projections suppresses water intake (Gizowski et al 2016, Matsuda et al 2017).

Activation of the PVH oxytocin neurons projecting to the PBN attenuated water intake induced by dehydration and hypertonic saline. Conversely, inactivation of the projections increases water intake (Ryan et al 2017).

In conclusion, some neural circuits underlying thirst have been recently discovered in the hypothalamus and hindbrain. However, the projections to and from higher brain structures and the top-down control of drinking behavior are mostly unknown.

I.1.4 Factors eliciting thirst and drinking behavior

Thirst is a primordial emotion driving animals and humans to drink water for maintaining body water homeostasis. It is caused by loss of body water through breathing, urine, sweating, and feces. In animals and humans, there are many factors inducing water intake, e.g. intracellular and extracellular dehydration, circadian clock, and high temperature, food intake. I will briefly describe the different factors.

I.1.4.1 Intracellular dehydration (An increase in plasma osmolality)

Intracellular dehydration is defined as losing water from the cells and it is due to an increase in blood plasma osmolality. This happens when fluid intake is insufficient or when the blood content is unbalanced and it is associated with poor health (Jequier & Constant 2010, Popkin et al 2010). In the brain, the SFO, OVLT, PVH, and SON are crucial to the sensation of plasma osmolality (Leib et al 2017, Prager-Khoutorsky & Bourque 2010).

I.1.4.2 Extracellular dehydration (A decrease in blood volume)

Extracellular dehydration is water-loss from the circulating fluids without change of plasma osmolality and due to excess blood loss, vomiting, or diarrhea. The consequent effect is hypotension because of hypovolemia (Jequier & Constant 2010, Popkin et al 2010). This kind of dehydration promotes the production of Ang II, but does not change plasma osmolality (Fitzsimons 1966b, Fitzsimons 1980).

I.1.4.3 Circadian clock

Humans and rodents display a surge of water intake before sleep (Gizowski et al 2016). Even if not dehydrated, they are still driven to drink water, to anticipate dehydration during sleep. Therefore, the circadian clock mediates anticipatory thirst. Gizowski et al. studied the neural basis of the circadian clock-driven thirst. They found that the SCN releases vasopressin to promote water intake prior to sleep (Gizowski et al 2016).

I.1.4.4 Feeding

Feeding elicits thirst within seconds, although plasma osmolality did not change yet. Drinking induced by feeding is known as prandial thirst (Bellisle & Le Magnen 1981, Epstein et al 1964, Fitzsimons & Le Magnen 1969, Kissileff 1969a, Kissileff 1969b, Phillips et al 1984). Conversely, thirst suppresses feeding before water is available, a mechanism known as dehydration anorexia (Zimmerman et al 2017, Zimmerman et al 2016). The neural bases of prandial thirst were studied by the optical recording of neural activity. SFO glutamatergic neurons, driving water intake, are activated by eating. In addition, eating also rapidly activates SON vasopressin neurons to release vasopressin for enhancing water retention (Mandelblat-Cerf et al 2017, Zimmerman et al 2016). Thus, prandial thirst can be seen as a kind of anticipatory thirst.

I.1.4.5 Body temperature

Exposure to high temperatures promotes body water loss by an increase of sweating, panting, and saliva spreading in endotherms (Tan & Knight 2018). Prolonged exposure can lead to thermal dehydration, which is intracellular dehydration, and hyperthermia (Barney & Folkerts 1995). Besides thermal dehydration, hyperthermia also plays a key role in the promotion of water intake (Budgell 1970a, Budgell 1970b). Previous studies showed that the hypothalamus responds to locally thermal stimuli and regulates water intake. Cooling of the hypothalamus reduces water intake in sheep. Whereas heating the same region increases drinking (Strauss et al 2015). This suggests that the body temperature controls water intake independent of body water volume and osmolality.

I.1.5 Animal models for studying thirst and drinking behavior

Thirst is an instinctive drive for ingestion of water, and a primordial emotion. It is generated by stimulation of dehydration signals, temperature, and anticipatory factors. It is an aversive feeling that promotes drinking behavior. The neural pathway involved can be described as follows: thirst signals activate sensory neurons, which transmit information to higher brain structures that generate thirst. The brain processes thirst and other perceptions, then it takes the decision to drink. This process looks very simple, but the underlying mechanisms are complicated. So far, there is no experimental assay for quantifying thirst directly in animals. Therefore, investigators considered drinking behavior a close representation of thirst (Greenleaf 1992, Saker et al 2014). Here, I describe a set of animal models for studying drinking behavior and for extrapolating information about the mechanisms of thirst.

I.1.5.1 Water deprivation

Water is discharging by expired air, secretion of the kidney, and sweat from the skin in animals and humans. Water-loss causes thirst and drinking behavior. Therefore, the simplest way to elicit thirst is water deprivation. Normally animals are deprived for less than 24 hours, which leads to both intracellular and extracellular dehydration, and water intake is monitored for 2 hours after re-exposure to drinking bottles (Gizowski & Bourque 2018). Therefore, we need other approaches to study intracellular and extracellular dehydration respectively.

I.1.5.2 Osmolality challenge (intracellular dehydration)

Systemic intraperitoneal or subcutaneous injection of the hypertonic NaCl triggers water intake eliciting a state of intracellular dehydration. In addition, injection of the hypertonic NaCl into the brain elicits drinking behavior, such as the lateral ventricle and the subfornical organ (Johnson et al 2003).

I.1.5.3 Extracellular dehydration (angiotensin II and polyethylene glycol, PEG)

To mimic extracellular dehydration, subcutaneous injection of 25-30% PEG with (10ml/kg body weight) is applied and water intake is monitored. The administration induces a hypovolemia effect due to losing blood volume but not changing blood osmolality (Johnson et al 2003). In addition, hypovolemia results in extracellular dehydration and promotes Ang II production (Fitzsimons 1966b). Thus application of Ang II is another approach to mimic extracellular dehydration (Fitzsimons 1980). Intracerebroventricular (ICV) injection of angiotensin II rapidly induces water intake. However, the subcutaneous or intraperitoneal injection of angiotensin II does not elicit water intake (Johnson et al 2003).

1.1.5.4 Thermal dehydration and hyperthermia

Another way of stimulating water intake is to expose animals to 40 ℃ for 4 hours, causing thermal dehydration and hyperthermia (Barney & Folkerts 1995). Therefore, other approaches need to be applied to make either thermal dehydration or hyperthermia. For thermal dehydration, first, animals are exposed to 40 ℃ for 4 hours, which lead to dehydration and high body temperature in these animals. Then, to avoid the effect of high temperature, the animals are staying at 25 ℃ for half an hour for recovering body temperature to normal value, then water is available by animals and drinking is monitored (Barney & Folkerts 1995). For hyperthermia, cold or hot electrodes are placed in a specific brain area, then water intake is monitored (Strauss et al 2015).

1.1.5.5 Alcohol and Ketogenic diet

Parkyong Song et al. showed that the administration of alcohol (3.5 g/kg/day) by oral gavage increases daily water intake in mice. In addition, they also showed that mice having a ketogenic diet drink more water than the mice having a standard diet (Song et al 2018).

1.1.5.6 Hypotension

The beta-adrenergic agonist isoproterenol (isoproterenol hydrochloride, Sigma) produces hypotension in animals and humans, and is a powerful thirst eliciting agent, which also induces a substantial release of renin (Johnson et al 2003, Rettig et al 1981). Isoproterenol is injected subcutaneously at a dose of 15-200 μg/kg. Immediately after the injection, the animals are given access to water in their cages and water intake is measured (Johnson et al 2003).

Supplementary information for the section of I.1 Thirst.

In this section, I learned many good ideas from some review papers, which explained detailed information about thirst (Fitzsimons 1972, Fuller 1984, Gizowski & Bourque 2018, Ichiki et al 2019, Leib et al 2016, Popkin et al 2010, Zimmerman et al 2017). I thank the authors for providing valuable reviews in specific fields of thirst, including brain structures, neural populations, and neural circuits for the control of thirst, and helping a lot in the writing of this section.

I.2 The endocannabinoid system

The endocannabinoid system (ECS) is a biological system in animals and humans. It comprises endocannabinoids and cannabinoid receptors (Pagotto et al 2006, Viveros et al 2005). In the 1990s and early 2000s, type-1 and type-2 cannabinoid receptors (CB_1 and CB_2) in rodents and humans were identified (Brown et al 2002, Chakrabarti et al 1995, Gerard et al 1990, Griffin et al 2000, Matsuda et al 1990, Munro et al 1993, Shire et al 1996). Meanwhile, in the 1990s, the endocannabinoids anandamide and 2-arachidonoylglycerol were isolated (Devane et al 1992, Mechoulam et al 1995, Sugiura et al 1995). The cannabinoid receptors are stimulated by endocannabinoids, phytocannabinoids isolated from *cannabis sativa*, and synthetic cannabinoids (Kano et al 2009). *Cannabis sativa* has been used as a medicine for thousands of years, although the ECS was discovered in the last decade of the 20th century (Touw 1981). Δ^9-tetrahydrocannabinol (THC) is the most important psychoactive component in the *cannabis sativa*, and was first isolated in the 1940s and then better characterized in the 1960s (Adams 1940, Gaoni & Mechoulam 1964, Petrzilka & Sikemeier 1967). A plethora of studies showed that the ECS exerts a wide variety of functions both in the central nervous system and in peripheral organs (Busquets-Garcia et al 2018a, Kano et al 2009, Piazza et al 2017). Here, I will briefly introduce the ECS and its components.

I.2.1 *Cannabis* and its uses in humans

Cannabis is a genus of flowering plants. It was widely cultivated and used in the world as a medicine and a recreational drug (Bridgeman & Abazia 2017). The earliest trace of *cannabis* use as a drug was found in 2,700 before the Christian era in China. In parallel, the recreational and medical effects were well known in India (Touw 1981). The description of *cannabis* in Western medicine occurred in the middle of the 19th century (Touw 1981) (**Figure 3**). However, the psychoactive components of *cannabis* were unknown until the middle of the 20th century (Adams 1940, Gaoni & Mechoulam 1964, Petrzilka & Sikemeier 1967). In the last decade of the 20th century, cannabinoid receptors and endocannabinoids were identified successively (Kano et al 2009). At

present, more attention is being paid to *cannabis* medical uses due to its diverse beneficial functions (Araujo et al 2019, Basavarajappa et al 2017, Soltesz et al 2015).

I.2.1.1 *Cannabis'* uses in China

Cannabis was cultivated for fibers since 4000 B.C. in China (Li & Lin 1974). *Cannabis* fruits were cooked as food around 206 B.C. to 220 A.D. during the Han dynasty (Li & Lin 1974). In addition, ancient Chinese have used *cannabis* as medicine, which was recorded in the world's oldest pharmacopoeia, the *pen-ts'ao ching*, in the first century of this Era. At that time, c*annabis* was applied in the treatment of rheumatic pain, intestinal constipation, disorders of the female reproductive system, malaria, and others (Touw 1981). Hua T'o (A.D. 110 - 207) a very famous doctor who developed Chinese surgery, used *cannabis* powder combined with wine to anesthetize patients during surgeries (Li & Lin 1974).

The Chinese physicians mainly used the seeds of *cannabis* for medical purposes including laxative, eczema, psoriasis, atherosclerosis, osteoporosis, rheumatoid arthritis, and some inflammatory diseases (Leson & Pless 2002, Touw 1981). The psychoactive effect of *cannabis* was first reported in the *pen-ts'ao ching*, however, the ancient Chinese rarely used it as a hallucinogen. In modern China, investigators have attempted to clarify the complicated nomenclature regarding *cannabis* in traditional Chinese medicine because of confused interpretation of the plants, such as *mafen*, *mahua*, and *mabo*, and different parts of the plants, including seeds, leaves, and flowers (Brand & Zhao 2017).

I.2.1.2 *Cannabis*' uses in India

Cannabis was widely used as a medicine, a recreational drug, and a religious supply in ancient India around 1000 B.C. (Mikuriya 1969). It was applied in a broad variety of diseases, and mainly as analgesic (neuralgia, headache, and toothache), anticonvulsant (epilepsy, tetanus, and rabies), hypnotic, tranquilizer (anxiety, mania, and hysteria), anesthetic, anti-inflammatory (rheumatism and other inflammatory diseases), antibiotic (topical use on skin infections, erysipelas, and tuberculosis), anti-parasite (internal and external worms), anti-spasmodic (colic, diarrhea), digestive,

appetite stimulant, diuretic, aphrodisiac or anaphrodisiac, anti-tussive, and expectorant (bronchitis, asthma) (Aldrich 1997, Mikuriya 1969, Touw 1981).

The psychoactive effects of *cannabis* were well-known in India. The products were different and classified three types depending on the efficacy. The weakest type, called *Bhang*, consists of dry leaves without flowers; a stronger one, called *Ganja*, comprises the flowers; the strongest one is called *Charas*, and made of the resin that covers flowers (Touw 1981). Currently, we know that cannabinoids are mainly located in the female plant's flowers, and in a smaller amount in the leaves. Solitary resin glands mostly form at the tips of the trichome stalks, and contain a considerable amount of cannabinoids (Paris & Nahas 1984).

The broad use of *cannabis* in India was due to the association with religion, which assigned sacred virtues to the plant. The *Atharva Veda* (an ancient Hindu scripture) mentioned *cannabis* as one of five sacred plants, referring to it as a *source of happiness, donator of joy and bringer of freedom*. Therefore, numerous Indian religions have used *cannabis* in rituals (Touw 1981).

I.2.1.3 *Cannabis*' uses in the Western world

European physicians started using *cannabis* as a medicine since the beginning of the 19th century (Zuardi 2006). Years later, the effective introduction of *cannabis* has been recorded in the works of Willian B. O'Shaughnessy, an Irish physician, and of Jacques-Joseph Moreau, a French psychiatrist in the midst 19th century. O'Shaughnessy described various successful human therapeutic uses of plant extracts, such as rheumatism, convulsions, muscular spasms of tetanus, and rabies (Fankhauser 2002, Mikuriya 1969). Moreau, a French physician, reported the use of different *cannabis* preparations systemically describing the acute effects on mental abilities. He also considered *cannabis* as a powerful and unique approach to the investigation of mental illness (Moreau 1845).

More than 100 studies about *cannabis* were published in Europe and the United States until the end of the 19th century (Grinspoon 1971). The medical use of *cannabis* reached a climax in the late 19th century and early 20th century, and was summarized by

the *Sajous's Analytic Cyclopedia of Practical Medicine* (1924). *Cannabis* was mainly applied as sedative, hypnotic, analgesic, and appetite stimulant (Aldrich 1997). However, these effects were difficult to replicate due to the extreme heterogeneity of plant preparations (Fankhauser 2002). Therefore, legal restrictions reduced the medical use of *cannabis* (Grinspoon & Bakalar 1993, Mikuriya 1969) but the recreational use of *cannabis* rapidly spreads since the 1960s in the western world, and became an important social activity.

Investigators have identified the components of *cannabis* between the 1940s and the 60s, then discovered the cannabinoid receptors and endogenous cannabinoids in the 1990s. With a better understanding of the cannabinoid system, the medical use of cannabinoids is promising to treat kinds of diseases including chronic pain, epilepsy, psychoses, and multiple sclerosis (Carlini 2004, Mechoulam et al 2002, Zuardi et al 2002).

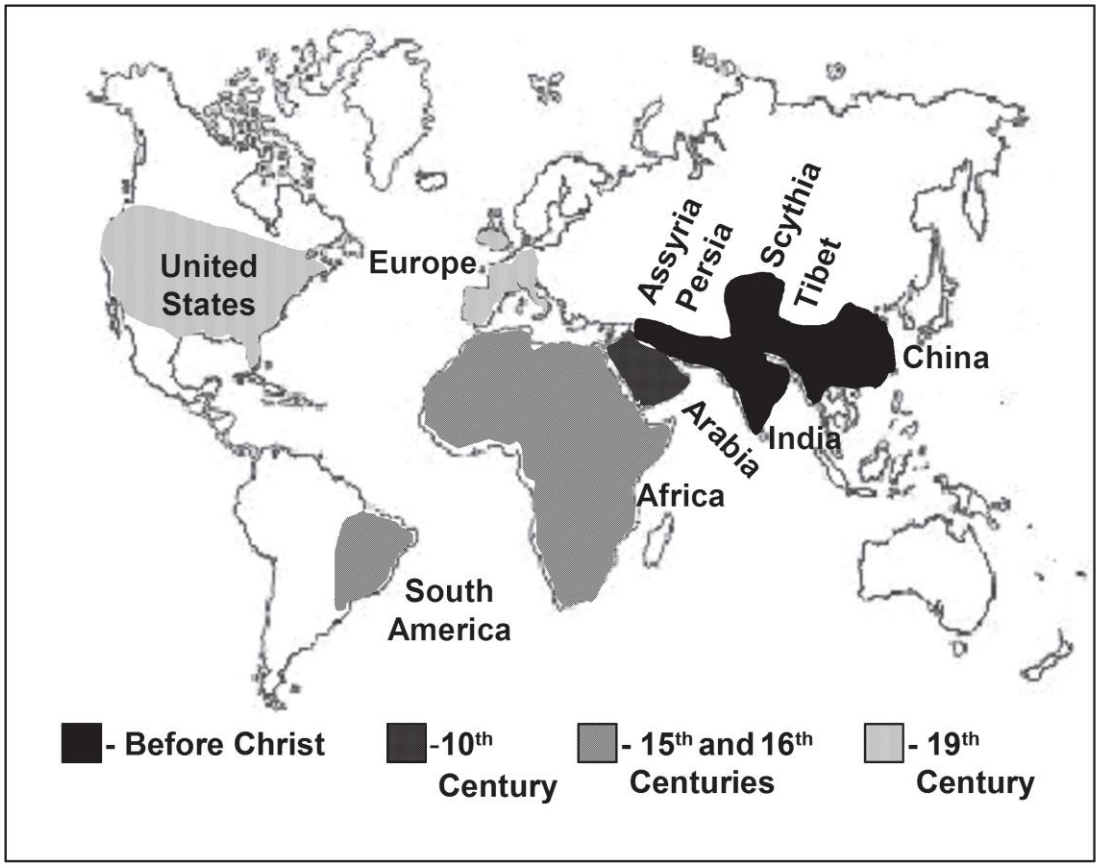


Figure 3. Age of the beginning of *cannabis* use as a medicine (this figure is from Zuardi 2006).

I.2.2 Cannabinoids and cannabinoid receptors

Cannabinoids consist of phytocannabinoids (from *cannabis sativa)*, endocannabinoids (from animal bodies), and synthetic cannabinoids. Here, I focus on the cannabinoids which exist in nature, such as phytocannabinoids and endocannabinoids (synthetic cannabinoid agonist and antagonist will be introduced in the section of I.2.4.1.), and their binding properties at cannabinoid receptors CB_1 and CB_2. Cannabinoid receptors are G-protein coupled receptors, which can signal through different pathways ($G_{i/O}$ G_q and G_s proteins for CB_1 receptors; $G_{i/O}$ protein for CB_2 receptors) (Bouaboula et al 1999, Demuth & Molleman 2006, Howlett et al 2002, Lauckner et al 2005, Pertwee 1997, Sugiura et al 1997).

I.2.2.1 Phytocannabinoids and endogenous cannabinoids

Phytocannabinoids

Phytocannabinoids are present in *cannabis sativa* and other species. In *cannabis sativa*, at least 113 different cannabinoids have been isolated, among them, the most studied ones are Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) (Aizpurua-Olaizola et al 2016) (**Figure 4**). Other plant species such as *Echinacea purpurea*, *Echinacea angustifolia*, *Acmella oleracea*, *Helichrysum umbraculigerum*, and *Radula marginata* have been reported to produce cannabinoids (Woelkart et al 2008). The lipophilic alkamides (alkylamides) isolated from various *Echinacea* species presented affinities to CB_2 receptors (Bauer & Remiger 1989, Raduner et al 2006). Yangonin group found a series of alkaloids with a significant affinity to CB_1 receptors in the Kava plant (Ligresti et al 2012). Black truffles also contain anandamide (Pacioni et al 2015). Furthermore, perrottetinene, a moderately psychoactive cannabinoid, was isolated from different *Radula* varieties (Chicca et al 2018).

Δ^9-tetrahydrocannabinol

Δ^9-tetrahydrocannabinol (Δ^9-THC) is the principal psychoactive component in *cannabis sativa*, and has been purified in the middle of the last century (Adams 1940, Gaoni & Mechoulam 1964, Petrzilka & Sikemeier 1967). It can activate type-1 and type-2 cannabinoid receptors, and can affect many brain functions, such as learning and

memory, analgesia, depression, and appetite (Kano et al 2009). Systemic administration of Δ^9-THC impairs learning and memory in different species (Davies et al 2002, Lichtman et al 2002). Moreover, Δ^9-THC has been proposed to treat nausea and anorexia (Pagotto et al 2006). The pharmacology of cannabinoids is complex and it often follows biphasic dose-response curves. For instance, investigators found that a low dose of Δ^9-THC produces an increase in appetite, but a high dose of Δ^9-THC generates hypophagia in mice (Bellocchio et al 2010, Soria-Gomez et al 2014). Therefore, the application of Δ^9-THC produces a diversity of effects, ranging from beneficial to adverse, depending on factors that are just starting to be elucidated (Busquets-Garcia et al 2015).

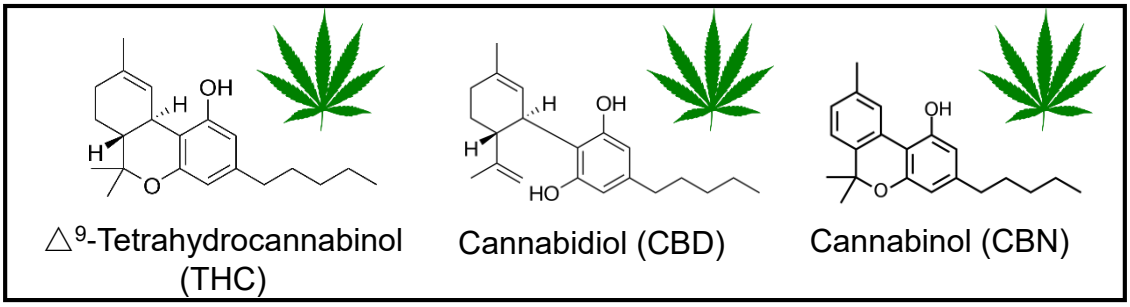


Figure 4. Structural formulas of phytocannabinoids (the structures are first identified by Adams 1940, Gaoni & Mechoulam 1964).

Cannabidiol

Cannabidiol (CBD) was isolated in 1940 and it accounts for up to 40% of the plant's extract (Adams 1940, Campos et al 2012). CBD is not an agonist of the cannabinoid receptors, and does not have psychoactive effects as described for Δ^9-THC (Iseger & Bossong 2015, Pisanti et al 2017). Interestingly, CBD may change the effect of Δ^9-THC on the body when both are present (Aizpurua-Olaizola et al 2016, Boggs et al 2018, Campos et al 2012, Pisanti et al 2017). Up to now, the mechanisms of the CBD's biological effects are still not clear (Boggs et al 2018, Pisanti et al 2017).

Recently CBD has been proposed as medical intervention in epilepsy, especially refractory epilepsy in children. Indeed, this compound is able to reduce seizure frequency and improving quality of life in combination with conventional drugs

(Stockings et al 2018). In the United States, Epidiolex, a cannabidiol preparation, was approved by the Food and Drug Administration in 2018 for treatment of epilepsy associated with Lennox-Gastaut syndrome or Dravet syndrome in patients 2 years of age and older (Letter 2018).

Cannabinol

Cannabinol (CBN) is a mildly psychoactive cannabinoid first isolated in 1940 and found only in small amounts in *Cannabis* (Adams 1940, Karniol et al 1975). It mostly occurs in aged *Cannabis* (Andre et al 2016). CBN is a metabolite of the tetrahydrocannabinolic acid (THCA). If *cannabis* is exposed to air or ultraviolet for a long time, THCA will be converted to cannabinolic acid (CBNA). Then CBNA is degraded to CBN. CBN acts as a partial agonist of CB_1 receptors but has a higher affinity of CB_2 receptors. However, CBN has lower affinities to cannabinoid receptors as compared to Δ^9-THC (Huestis 2005, Mahadevan et al 2000, Petitet et al 1998).

Endogenous cannabinoids

The natural endogenous ligands of cannabinoid receptors are collectively called “endocannabinoids”. Endocannabinoids are derived (amides, esters, and ethers) from long-chain polyunsaturated fatty acid (PUFA), mainly arachidonic acid (AA). They are able to bind and activate cannabinoid receptors (Di Marzo 2004). Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are best-characterized endocannabinoids, others include N-Dihomo-γ-linolenoyl ethanolamine and N-oleoyl dopamine (OLDA) (Pertwee 2005), 2-arachidonoylglycerol ether (Noladin ether, 2-AGE) (Hanus 2001), *O*-arachidonoylethanolamine (virodhamine) (Porter 2002), *N*-arachidonoyldopamine (NADA) (Huang 2002) or the allosteric endogenous inhibitor pregnenolone (Vallee et al. 2014; Piazza et al. 2017). Here, I will mainly describe AEA and 2-AG.

Anandamide

Anandamide (AEA), from the Sanskrit word *ananda*, which means *bliss*, is the first identified endogenous cannabinoid (Devane et al 1992, Hanus 2007). It is the ethanolamide of arachidonic acid and acts as a partial agonist at cannabinoid receptors (Pertwee et al 2010). However, AEA was found to interact with other receptors,

including the transient receptor potential vanilloid 1 (TRPV1) (Zygmunt et al 1999) and the peroxisome proliferator-activated receptor (PPAR) family (O'Sullivan 2007). In agreement, some effects of AEA are independent of cannabinoid receptors (Breivogel et al 2001, Monory et al 2002).

There are many complex pathways of AEA biosynthesis (Di Marzo et al 2005, Ligresti et al 2005), but they share the first step, consisting in the transfer of an acyl chain from the *sn*-1 position of a glycerophospholipid to the amino group of the hydroxyethy moiety of phophatidylethanolamine (PE), which is catalyzed by a calcium-dependent *N*-acyltransferase (NAT). Then, there are at least 4 ways to produce AEA. First, the generated *N*-acylphosphatidylethanolamine (NAPE) is hydrolyzed to AEA and phophatidic acid through a reaction catalyzed by a phosphodiesterase of the phospholipase D-type (NAPE-PLD). Second, AEA is formed from *N*-acyl-lysophosphatidylethanolamine by a lysophospholipase-D-enzyme (lyso-PLD) (Sun et al 2004). Third, the generation of glycerophospho-arachidonoylethanolamide (GpAEA) occurs by α/β-hydrolase 4 (Abh4) acting on either NAPE or lyso-NAPE, then the GpAEA is subsequently converted to AEA by a phosphodiesterase (Simon & Cravatt 2006). Fourth, NAPE can also be hydrolyzed to phosphatidylethanolamine (pAEA) by phospholipase C. Then pAEA is dephosphorylated by phosphatases to AEA (Liu et al 2006) (**Figure 5**).

AEA can be degraded by hydrolytic and oxidative pathways. It is mainly hydrolyzed by fatty acid amide hydrolase (FAAH) (Bracey et al 2002, Cravatt et al 1996, Giang & Cravatt 1997), which was first cloned in 1996 (Cravatt et al 1996). FAAH is an integral membrane protein widely distributed in various tissues of rats (Cravatt et al 1996, Desarnaud et al 1995, Katayama et al 1997), mouse (Sun et al 2005), and human (Giang & Cravatt 1997). In the brain (Egertova et al 1998), intense expression of FAAH was detected in the cerebellum, hippocampus, and neocortex, which are enriched with CB_1 receptors. In addition, also N-acylethanolamine acid amide hydrolase (NAAA) is involved in AEA hydrolysis (Tsuboi et al 2005, Ueda et al 2010). This enzyme is a cysteine hydrolase belonging to the *N*-terminal nucleophile hydrolase superfamily and is present in cellular lysosomes or the Golgi apparatus. NAAA is highly expressed in a

number of blood cell lines (Sun et al 2005, Tsuboi et al 2004, Ueda et al 1999). In humans, NAAA mRNA is abundantly distributed in prostate followed by leukocytes,

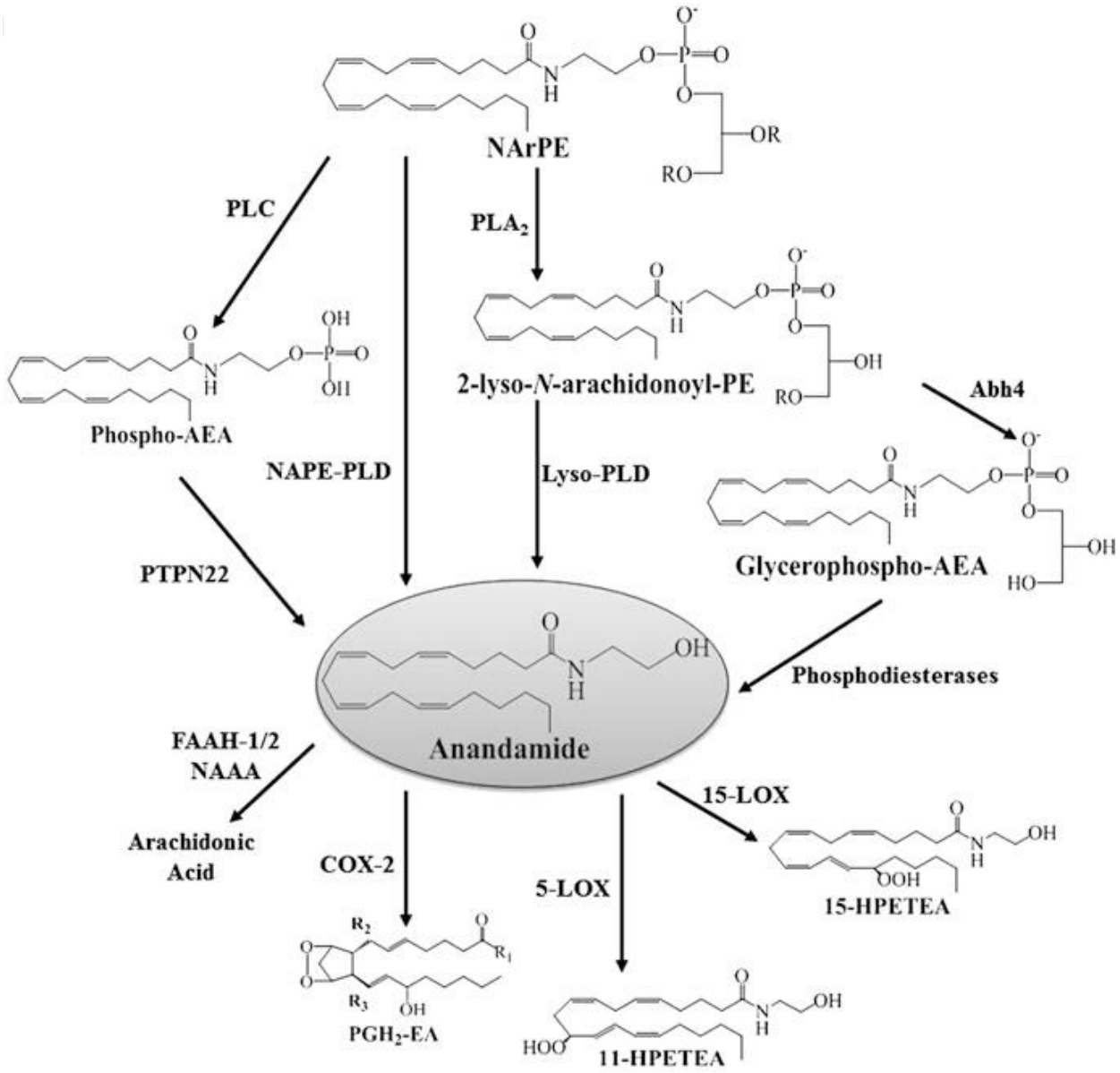


Figure. 5 Schematic representation of anandamide biosynthesis and degradation. NArPE (N-arachidonoylphosphatidyl-ethanolamine), PLC (phospholipase C), PTPN22 (protein tyrosine phosphatase), PLA2 (phospholipase A2), PE (phosphatidyl-ethanolamine), PLD (phospholipase D), Abh4 (α/β-hydrolase 4), PG (prostaglandin), HPETEA (hydroxyperoxyeicosatetraenoylethanolamide), LOX (lipoxygenase), COX (cyclooxygenase), FAAH (fatty acid amide hydrolase), NAAA (N-acylethanolamine-hydrolysing acid amidase), R_1 (ethanolamine) (this figure is from Cascio & Marini 2015).

2-Arachidonoylglycerol

2-Arachidonoylglycerol (2-AG) is an arachidonate ester of glycerol and able to stimulate both CB_1 and CB_2 receptors with similar potency and efficacy (Mechoulam et al 1995, Sugiura et al 1995) as well as γ-aminobutyric acid (GABA) receptors (Sigel et al 2011).

The biosynthetic pathway of 2-AG consists of the hydrolysis of membrane phospholipids, catalyzed by phospholipase C (PLC), which produces 1,2-diacylglycerol (DAG), eventually converted into 2-AG by diacylglycerol lipase (DGL) (Bisogno et al 2003). The inositol phospholipids (PI) are also catalyzed by the phospholipase A_1, which produces the lysophospatidyl-inositol (lyso PI), which can be converted into 2-AG by the lyso PI-PLC (**Figure 6**).

2-AG is mainly hydrolyzed by Monoacyl-glycerol Lipase (MGL) (Dinh et al 2002). MGL is a serine hydrolase responsible for about 85% of the 2-AG hydrolyzing activity of the mouse brain (Blankman et al 2007). It is distributed in a number of tissues in rats (Karlsson et al 1997). MGL mRNA mainly presents in the adrenal gland, heart, adipose tissue, kidney, ovary, testis, spleen, lung, liver, skeletal muscle and brain (Dinh et al 2002). In the brain, MGL is abundantly present in the hippocampus, the cortex, the thalamus, and the cerebellum, where CB_1 receptors are also abundantly expressed. In addition, MGL is mainly distributed on the presynaptic membrane and co-localizes with CB_1 receptors in the axon terminals (Savinainen et al 2012). In addition, 2-AG is also catalyzed by two integral membrane proteins, α/β-hydrolase domain containing protein-6 (ABHD6) and -12 (ABHD12) (**Figure 6**). ABHD6 is mainly distributed at the sites of 2-AG generation, including postsynaptic dendrites of principal glutamatergic neurons as well as some GABAergic interneurons (Savinainen et al 2012). ABHD12 is intensively expressed in microglia, macrophages, and osteoclasts (Fiskerstrand et al 2010).

Transport and uptake of endocannabinoids

Endocannabinoids are released in extracellular space, where they activate CB_1 receptors present on the surface of presynaptic nerve terminals (Piomelli 2014). So far, it is not clear how endocannabinoids are transported in extracellular space and

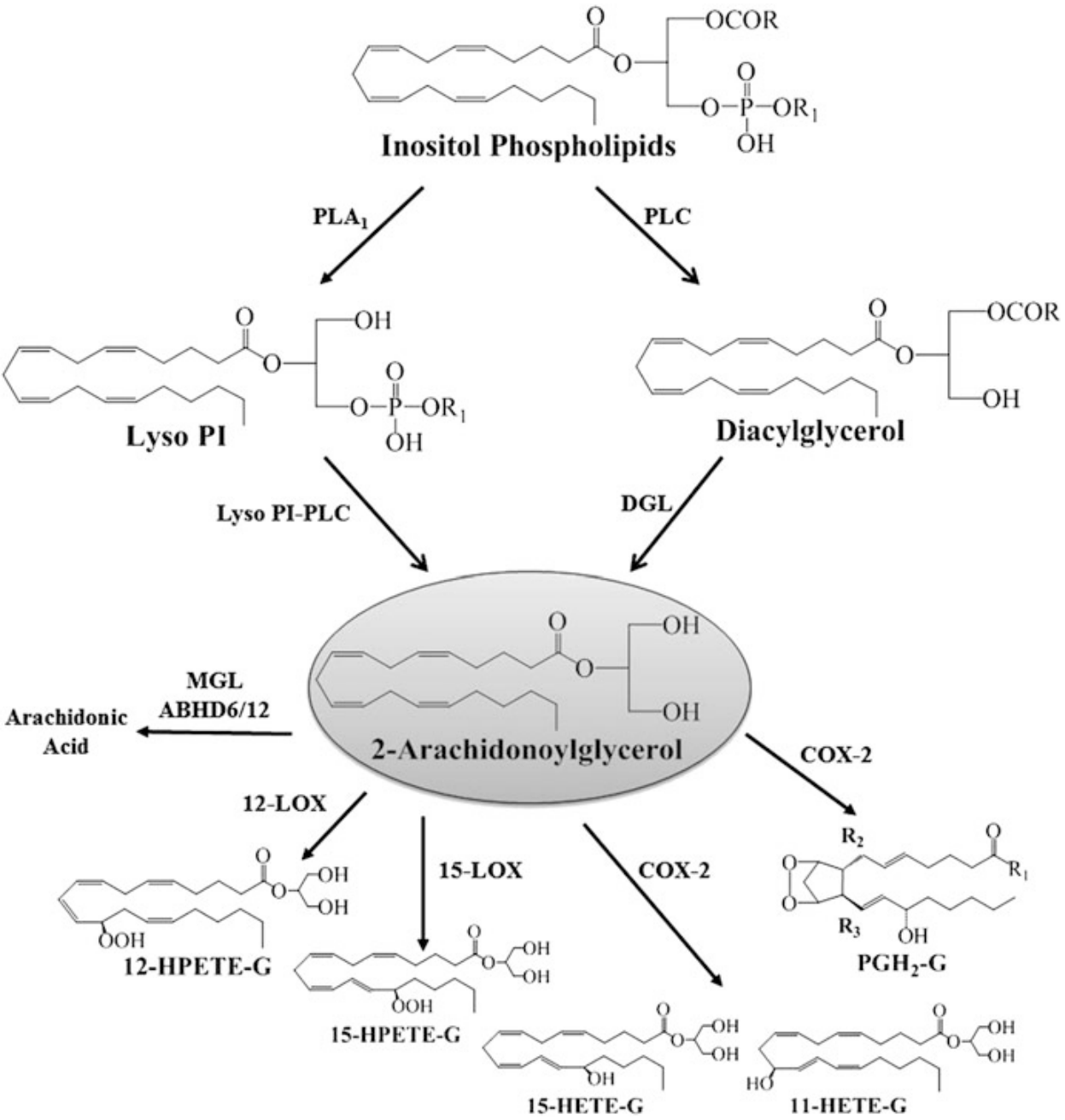


Figure. 6 Schematic representation of 2-arachidonoylglycerol biosynthesis and degradation. PLC (phospholipase C), PLA1 (phospholipase A1), PI (phospatidyl-inositol), DGL (diacylglycerol lipase), HETE-G (hydroxyeicosatetraenoyl-glycerol), HPETE-G (hydroxyperoxyeicosatetraenoyl-glycerol), LOX (lipoxygenase), COX (cyclooxygenase), MGL (monoacylglycerol lipase), ABHD (α/β-hydrolase domain), R1 (glycerol) (this figure is from Cascio & Marini 2015).

how their uptake works. Briefly, AEA is a lipophilic molecule and it could easily diffuse through the cell membrane. However, the diffusion would cease when the equilibrium in the AEA gradient between the extracellular and intracellular environment is reached. The other way of transport is through the endocannabinoid membrane transporter (EMT). But the existence of EMT is only supported by indirect evidence. Recent studies have shown that carrier proteins facilitate diffusion of AEA through the plasma membranes, such as the fatty acid-binding proteins FABP5 and FABP7 (Kaczocha et al 2009), the heat shock protein 70 (Hsp 70), albumin (Oddi et al 2009), and the FAAH-like anandamide transporter (FLAT) (Fu et al 2011).

I.2.2.2 Cannabinoid receptors

Cannabinoid receptors belong to a class of membrane receptors in G protein-coupled receptor superfamily (Graham et al 2009, Howlett 2002) and mainly include CB_1 and CB_2 receptors. CB_1 receptors are abundantly distributed in distinct cell types in the central nervous system and in the periphery (Busquets-Garcia et al 2018a, Kano et al 2009, Piazza et al 2017). CB_2 receptors are mainly expressed on T cells of the immune system, macrophages, and B cells, but they are also present in the brain (Cabral & Griffin-Thomas 2009). When cannabinoid receptors are activated by exogenous and endogenous ligands, they trigger multiple intracellular signal transduction pathways. Here, I will describe the structures and signaling pathways of cannabinoid receptors.

CB_1 receptor

In 1990, a 473-amino acid G protein-coupled receptor encoded by a brain cDNA clone was identified as a cannabinoid receptor in rats (Matsuda et al 1990) and named CB_1. Soon afterward, CB_1 receptor was cloned in humans and in mice as a protein consisting of 472 amino acids (Gerard et al 1990) and of 473 amino acids (Chakrabarti et al 1995), respectively. Across the CB_1 receptors of these 3 different species, there is a 97-99% amino acid sequence identity. CB_1 receptor is encoded by the gene *CNR1*, which is located on human chromosome 6 (Elphick & Egertova 2001, NCBI-Gene 2009). *CNR1* orthologs exist and have been identified in most mammals. In addition, two splice variants of the human CB_1 receptor have been reported (Ryberg et al 2005,

Shire et al 1995). The variants are short-length receptors and are expressed at very low levels in various tissues. Ligand binding properties are altered in the variants compared to the full-length receptor (Ryberg et al 2005). By using site-directed mutagenesis, binding sites of cannabinoids were shown to be embedded in the transmembrane helices of the CB_1 receptor (Song & Bonner 1996). Moreover, NMR experiments showed that lipid cannabinoid ligands diffuse laterally within one membrane leaflet, and interact with a hydrophobic groove formed by helices 3 and 6 of the CB_1 receptor (Makriyannis et al 2005, Tian et al 2005).

CB_1 receptor is supposed to exist as a homodimer in vivo (Wager-Miller et al 2002) and also as a heterodimer (Mackie 2005). The extent of CB_1 receptor dimerization was proposed to be modulated by cannabinoids (Mackie 2005). It was also reported that cannabinoid stimulation promotes the formation of CB_1 receptor/D_2 receptor heterodimer which alters the CB_1 signaling (Marcellino et al 2008). Another potential heterodimer is formed by CB_1 receptor and orexin 1 receptor (OX1R) (Hilairet et al 2003). Indeed activation of CB_1 receptor enhances the OX1R signaling and co-expression CB_1 receptor and OX1R results in a heteromeric complex *in vitro* (Ellis et al 2006).

CB_1 Signaling

Cannabinoids binding to CB_1 receptors cause activation of multiple intracellular signaling pathways. Several studies have pointed out the mechanisms of CB_1 signaling (Demuth & Molleman 2006, Diaz-Laviada & Ruiz-Llorente 2005, Howlett 2004, Howlett & Mukhopadhyay 2000, McAllister & Glass 2002, Mukhopadhyay et al 2002). Activation of CB_1 receptor primarily stimulates the $G_{i/o}$ transduction pathway resulting in increased conversion of GTP to GDP and a decreased cAMP production (Pertwee 1997). Consistently, blockade of $G_{i/o}$ protein by pertussis toxin (PTX) eliminates the CB_1-mediated inhibition of adenylyl cyclase and cAMP. However, some studies have also shown that CB_1 receptor is also coupled to G_s in order to increase cAMP production (Demuth & Molleman 2006, Howlett et al 2002) **(Figure 7)**. Furthermore, via Gq coupling, activation of CB_1 receptor can evoke transient Ca^{2+} elevations in a phospholipase C (PLC)-dependent manner (Lauckner et al 2005, Sugiura et al 1997). Activation of CB_1 receptor regulates several ion channels and enzymes. Administration

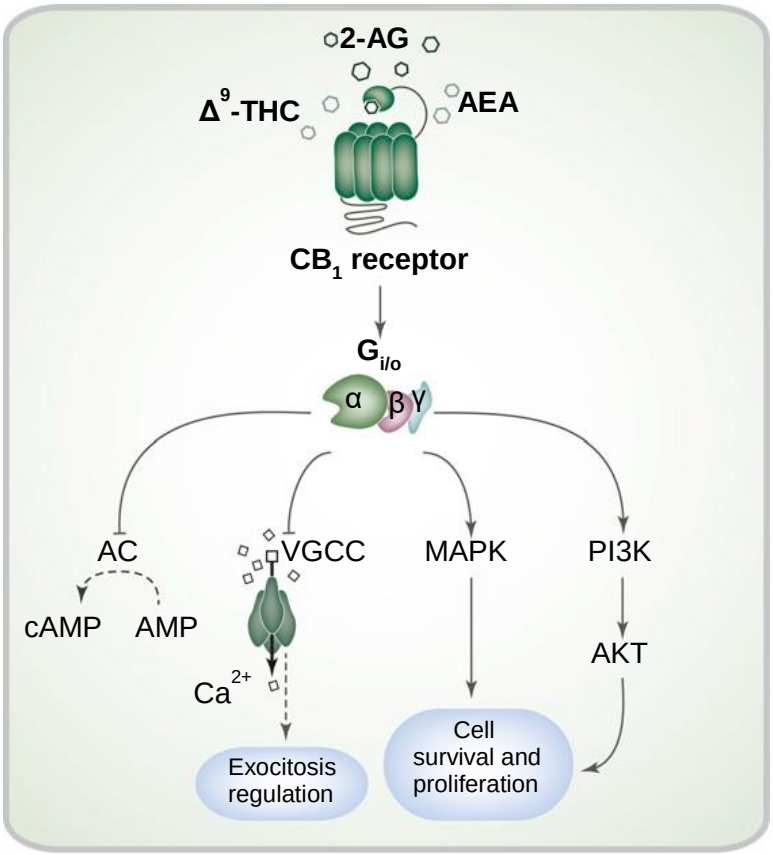


Figure 7. Cell signaling pathways triggered by CB_1 receptor's activation. Activation of the CB_1 receptor by the endocannabinoids 2-AG and AEA can initiate a series of intracellular events mediated by the Gi/o protein. CB_1 receptor activation can inhibit adenylate cyclase activity and therefore modulate cAMP production. CB_1 receptor activation also promotes closure of the voltage-gated Ca^{2+} channel, which can reduce Ca^{2+} entry into the presynaptic neurons and interfere with synaptic vesicle exocytosis and neurotransmission. Furthermore, CB_1 receptor stimulation can induce both MAPK and AKT phosphorylation and activation, which regulate proliferation and cell survival. AC, adenylyl cyclase; VGCC, voltage-gated Ca^{2+} channel (this figure is adapted from Olma et al 2016).

of cannabinoids activates A-type (Hampson et al 1995), and inwardly rectifying K^+ channels (Mackie et al 1995), and inhibits N- and P/Q Ca^{2+} channels (Twitchell et al

1997) and D- and M-type K^+ channels (Mu et al 1999, Schweitzer 2000). The enzymes influenced by CB_1 activation include focal adhesion kinase (FAK) (Derkinderen et al 1996), mitogen-activated protein kinase (MAPK) (Sanchez et al 1998), phosphatidylinositol 3-kinase (PI3K) (Bouaboula et al 1995), cAMP-activated protein kinase (PKA) (Mackie et al 1995).

CB_2 receptor

In 1993, a human cDNA clone encoding another type of cannabinoid receptor was identified and named CB_2 (Munro et al 1993). The human CB_2 receptor is a 360 amino acids G protein-coupled receptor, sharing a 44% amino acid sequence identity with the human CB_1 receptor. Soon afterward, *CB_2* genes were cloned in mice (Shire et al 1996) and rats (Brown et al 2002, Griffin et al 2000). The mouse CB_2 receptor consists of 347 amino acids and has 82% amino acid sequence identity with the human CB_2 receptor. The rat CB_2 receptor is complicated since its gene may be polymorphic, encoding a protein of 360 or 410 amino acids (Brown et al 2002, Griffin et al 2000). CB_2 receptors are mainly expressed in macrophages and other immune cells (Munro et al 1993).

I.2.3 CB_1 receptors in the central nervous system

CB_1 receptors are abundantly distributed in the central nervous system and in distinct cell types (Busquets-Garcia et al 2018a, Piazza et al 2017). The main neuronal effect of the activation of CB_1 receptor is a presynaptic reduction of neurotransmitter release (Kano et al 2009). Here I will briefly discuss the distribution and synaptic functions of CB_1 receptor.

I.2.3.1 The distribution of CB_1 receptors

CB_1 receptors are widely and intensively expressed in the brain. In early studies, the distribution of cannabinoid receptors was examined in different brain structures and spinal cord by using the radiolabeled synthetic cannabinoid [^{3}H]CP55,940 (Herkenham et al 1991, Herkenham et al 1990, Mailleux & Vanderhaeghen 1992). Ligand binding sites are widely distributed in different brain areas at various levels. Brain structures with high levels of [^{3}H]CP55,940 binding include the innermost layers of the olfactory bulb, the dentate molecular layer of the hippocampus, the lateral part of the striatum, the globus pallidus, the entopeduncular nucleus, the substantia nigra pars reticulata, and the cerebellar molecular layer. Moderate levels of the binding were observed in the cerebral cortex, the septum, the amygdala, the hypothalamus, the lateral subnucleus of interpeduncular nucleus, the parabrachial nucleus, the nucleus of the solitary tract, and the spinal dorsal horn. Low levels of ligand binding were noted in the thalamus, other nuclei in the brain stem, and spinal ventral horn. Kano and his colleagues found similar distributions of CB_1 receptors (Kano et al 2009) (**Figure 8**).

A number of studies of CB_1 mRNA expression have shown characteristic features of the distribution. CB_1 mRNA was found in many brain areas (Mailleux & Vanderhaeghen 1992, Matsuda et al 1993). For example, CB_1 mRNA expression is observed in the cerebral cortex, the striatum, the hippocampus, the amygdala, the thalamus, the hypothalamus, the cerebellum, and the brainstem.

CB_1 receptors are mainly located on the presynaptic membrane (Kawamura et al 2006, Matyas et al 2006, Nyiri et al 2005). Therefore, CB_1 proteins are distributed in the axonal terminals of downstream targets. For example, medium spiny neurons are

projecting neurons in the striatum, and their CB_1 receptor immunoreactivity is detected in the target regions, e.g. the globus pallidus and substantia nigra pars reticulata rather than the striatum (Matyas et al 2006). Furthermore, by using electron microscopy, it was shown that CB_1 receptors are expressed mainly at the presynaptic membrane (Kawamura et al 2006, Nyiri et al 2005).

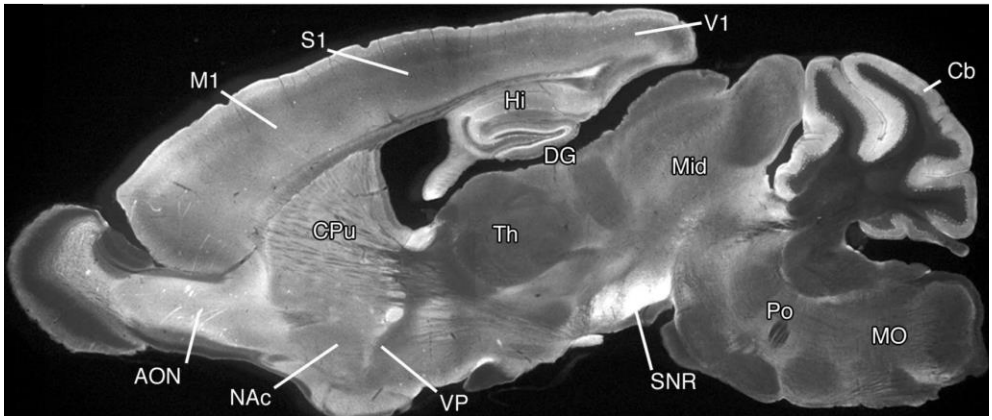
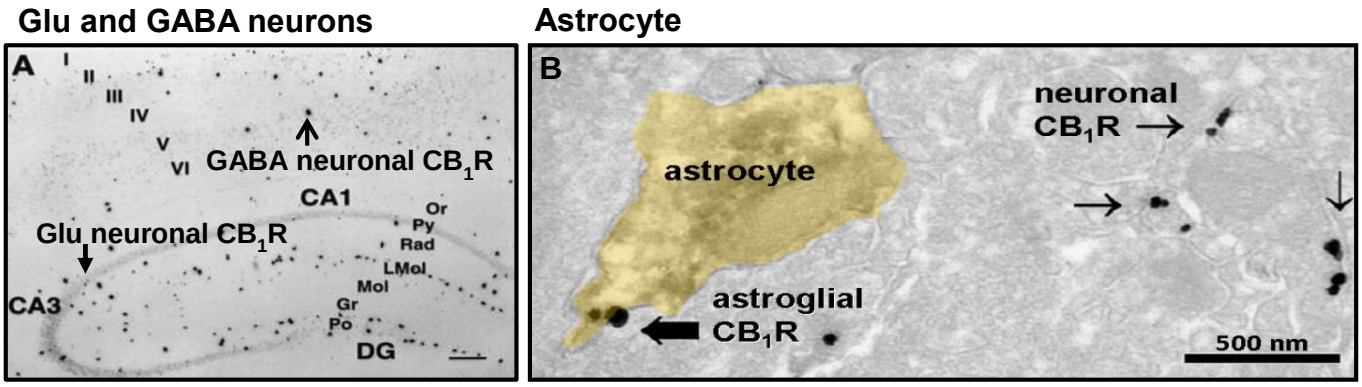


Figure 8. Distribution of CB_1 receptors in the central nervous system of adult mice.
CB_1 immunoreactivity is highest along striatal output pathways, including the substantia nigra pars reticulata (SNR), globus pallidus (GP). High levels are also observed in the hippocampus (Hi), dentate gyrus (DG), and cerebral cortex, such as the primary somatosensory cortex (S1), primary motor cortex (M1), and primary visual cortex (V1). High levels are also noted in anterior olfactory nucleus (AON), caudate putamen (CPu), nucleus accumbens (NAc), and cerebellar cortex (Cb). Low levels are observed in the ventral pallidum (VP), thalamus (Th), midbrain (Mid), pons (Po), and medulla oblongata (MO) (this figure is from Kano 2009).

Mitochondria are subcellular organelles essential for generating most of the cell's supply of adenosine triphosphate (ATP), which is used as a source of chemical energy. Recent studies have shown that CB_1 receptors are physically associated with mitochondrial membranes. Stimulation of CB_1 receptors regulates mitochondrial energetics, endocannabinoid-dependent short-term synaptic plasticity in the hippocampus, and memory processing (Benard et al 2012, Hebert-Chatelain et al 2016).

I.2.3.2 CB_1 receptors located in distinct cell types

CB_1 receptors are located in distinct cell types, including glutamatergic and GABAergic neurons, and astrocytes (Han et al 2012, Marsicano & Lutz 1999) (**Figure 9A**). The expression of CB_1 receptors has been shown in neurons by in situ hybridization and immunostaining. However, astrocytic CB_1 receptors are only observed by immunogold electron microscopy due to the low quantity (Han et al 2012) (**Figure 9B**). In cortical neurons, CB_1 mRNA is expressed at higher levels in GABAergic neurons than in glutamatergic neurons (Marsicano & Lutz 1999). In addition, inhibitory synapses have higher levels of CB_1 receptors than excitatory ones (Kano et al 2009).



Marsicano and Lutz et al 1999 Han et al 2012

Figure 9. CB_1 receptors are located in distinct cell types.

I.2.3.3 CB_1 receptors in the control of synaptic transmission

Endocannabinoid-mediated inhibition of neurotransmission

Activation of CB_1 receptors is generally known to inhibit neurotransmitter release as shown by using electrophysiological and biochemical techniques (Schlicker & Kathmann 2001). Many neurotransmitters have been reported to be under the control of CB_1 receptors, such as glutamate (Levenes et al 1998), GABA (Szabo et al 1998), glycine (Jennings et al 2001), acetylcholine (Gifford & Ashby 1996), norepinephrine (Ishac et al 1996), dopamine (Cadogan et al 1997), serotonin (Nakazi et al 2000), and CCK (Beinfeld & Connolly 2001).

Regarding the mechanisms, the inhibition of voltage-gated Ca^{2+} channels has been proposed to mediate the suppression of GABA release in the hippocampus

(Hoffman & Lupica 2000) and glutamate release at corticostriatal synapses (Huang et al 2001). The possible involvement of K^+ channels has also been reported for the suppression of glutamate release at cerebellar synapses (Daniel & Crepel 2001, Daniel et al 2004) and in the nucleus accumbens (Robbe et al 2001). In addition, the inhibition of Ca^{2+} influx has also been identified as a potential mechanism for the presynaptic suppression of inhibitory and excitatory transmission in the cerebellum (Takahashi & Linden 2000, Yamasaki et al 2006). Therefore, the mechanisms underlying the suppression of transmitter release by CB_1 are through voltage-gated Ca^{2+} channels or K^+ channels in different brain structures and synapses.

Endocannabinoid-mediated retrograde signaling

Endocannabinoid-mediated retrograde signaling was first reported in 2001 (Kreitzer & Regehr 2001, Maejima et al 2001, Ohno-Shosaku et al 2001, Wilson & Nicoll 2001). These and later studies have highlighted that endocannabinoid-mediated retrograde signaling happens at various synapses in the central nervous system (Kano et al 2009). Endocannabinoids are mobilized from postsynaptic neurons either phasically in an activity-dependent manner or tonically under basal conditions. In turn, mobilized endocannabinoids activate presynaptic CB_1 receptors, then transmitter release is suppressed either transiently (endocannabinoid-mediated short-term depression, eCB-STD) or persistently (endocannabinoid-mediated long-term depression, eCB-LTD).

Endocannabinoid-mediated short-term depression

Depolarization-induced suppression of GABAergic inhibitory inputs was originally discovered in the cerebellum (Llano et al 1991). They recorded the spontaneous IPSCs from Purkinye cells in cerebellar slices and found that the IPSCs were transiently suppressed following depolarizing voltage pulses applied postsynaptically. Pilter and Alger found a similar phenomenon in the hippocampus (Pitler & Alger 1992). However, the nature of the retrograde signal remained elusive for almost a decade, when different groups simultaneously and independently showed that such depolarization-induced suppression of inhibition (DSI) was completely blocked by the CB_1 antagonist SR141716A, AM251, or AM281, but not by the mGluR antagonist MCPG (Ohno-

Shosaku et al 2001, Wilson & Nicoll 2001). On the other hand, Kreitzer and Regehr discovered depolarization-induced suppression of excitation (DSE) in the cerebellar Prukinye cells (Kreitzer & Regehr 2001). They observed that excitatory transmission to Purkinye cells was transiently suppressed by postsynaptic depolarization, and this DSE was blocked by the CB_1 antagonist AM251, but not by the antagonists for mGluRs, $GABA_B$, and adenosine A_1 receptors. Overall, these data revealed that both DSI and DSE are mediated by the endocannabinoid system, which is to date the best characterized mediator of retrograde synaptic regulation.

Endocannabinoid-mediated long-term depression

It is known that high-frequency stimulation (HFS) induces long-term depression (LTD) at excitatory synapses in the striatum (Calabresi et al 1992, Gerdeman et al 2002). LTD is an activity-dependent reduction in the efficacy of neuronal synapses lasting hours or longer following a long patterned stimulus (Massey & Bashir 2007). Many studies have shown that endocannabinoid signaling mediates LTD induced by stimulation of synaptic inputs in several brain structures (Kano et al 2009). In the dorsal striatum, HFS of the corticostriatal glutamatergic inputs to medium spiny neurons induces LTD, which requires postsynaptic Ca^{2+} elevation (Calabresi et al 1994) and is accompanied by a decrease in the probability of glutamate release (Choi & Lovinger 1997a, Choi & Lovinger 1997b). These studies indicate that retrograde synaptic signaling is involved in the process. Gerdeman and his colleagues found that the LTD induced by stimulation of corticostriatal synapse was blocked by the CB_1 antagonist SR141716A or in the *CB_1*-KO mice (Gerdeman et al 2002, Ronesi et al 2004). Kreitzer and Malenka confirmed the CB_1 dependence of striatal LTD soon after with the same induction protocol (Kreitzer & Malenka 2005).

In summary, CB_1 receptors are widely located in different brain regions and cell types. The endocannabinoid system participates in the short- and long-term control of neural transmission. Therefore, the endocannabinoid system is one of the most interesting and essential neural modulators in the central nervous system.

I.2.3.4 Endogenous allosteric modulation of CB_1 receptors

In addition to endocannabinoid stimulation of cannabinoid receptors, there are endogenous allosteric modulators mediating functions of the receptors in animals (Piazza et al 2017). The endogenous anti-inflammatory lipid lipoxin A4 has been shown to increase the affinity of AEA and CB_1 receptors in the brain as an allosteric enhancer (Pamplona et al 2012). Therefore, lipoxin A4 is enhancing the activation of cannabinoid receptors. On the other hand, pregnenolone, a neurosteroid, was identified as an allosteric signal-specific inhibitor of CB_1 receptors, and is able to protect the brain from excessive cannabinoid intoxication (Vallee et al 2014). Pregnenolone exerts full inhibition of the CB_1-mediated activation of the ERK pathway and inhibition of mitochondrial functions, but it does not alter the decrease of cAMP by stimulation of CB_1 receptors (Vallee et al 2014). A recent study showed that pregnenolone blocks a wide spectrum of THC-induced endophenotypes typically associated with psychotic-like states, including impairments in cognitive functions, somatosensory gating, social interaction and perceptual delusion (Busquets-Garcia et al 2017). Thus, pregnenolone could be a potential drug treating diseases caused by excessive activation of CB_1 receptors.

I.2.4 Tools for studying the endocannabinoid system

The endocannabinoid system (ECS) comprises endocannabinoids and cannabinoid receptors. Current tools for studying the ECS include different pharmacological and genetic approaches. They are applied to either manipulate endocannabinoid levels or cannabinoid receptors' activity. In addition, the ECS participates in the regulation of neural transmission, making electrophysiological approaches suitable tools for understanding its functions. Recently a newly developed cannabinoid sensor provides an effective tool for monitoring dynamic cannabinoid levels *in vivo* (poster in a conference). Here, I mainly review the tools for studying the ECS in the central nervous system.

I.2.4.1 Cannabinoid agonists and antagonists

Δ^9-THC and endocannabinoids (2-AG and AEA) exist in nature and have been introduced in the section of I.2.2.1. Here, I focus on the synthetic CB_1 agonists and antagonists. Activation of CB_1 receptors has both beneficial effects, such as antidepression, analgesia, and neuroprotection, and negative effects, including impairment of learning and memory and of locomotor functions (Di Marzo et al 2001, Kano et al 2009, Piazza et al 2017, van der Stelt & Di Marzo 2005). Therefore, it is difficult to develop the natural CB_1 agonists, including Δ^9-THC, 2-AG, and AEA, as drugs due to the complex consequences of their use. However, thanks to the synthetic CB_1 agonists and antagonists, investigators can have more options to manipulate the endocannabinoid system and better understand its functions.

Synthetic selective CB_1 agonists are widely used, including the ACEA and O-1812. The WIN55,212-2 and HU-210 are also popular in the studies as synthetic CB_1/CB_2 agonists. The selective CB_1 antagonists contain SR141716 (Rimonabant), LY-320135, AM 251 and AM281 (Cota et al 2006, Merck) (**Figure 10**).

I.2.4.2 Inhibitors of endocannabinoid degradation

The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are metabolized by both hydrolytic enzymes, including the fatty acid amide hydrolase

(FAAH) and monoacylglycerol lipase (MGL). Here, I will describe the effects of selective inhibitors of the hydrolytic enzymes as well as the potential clinical significance.

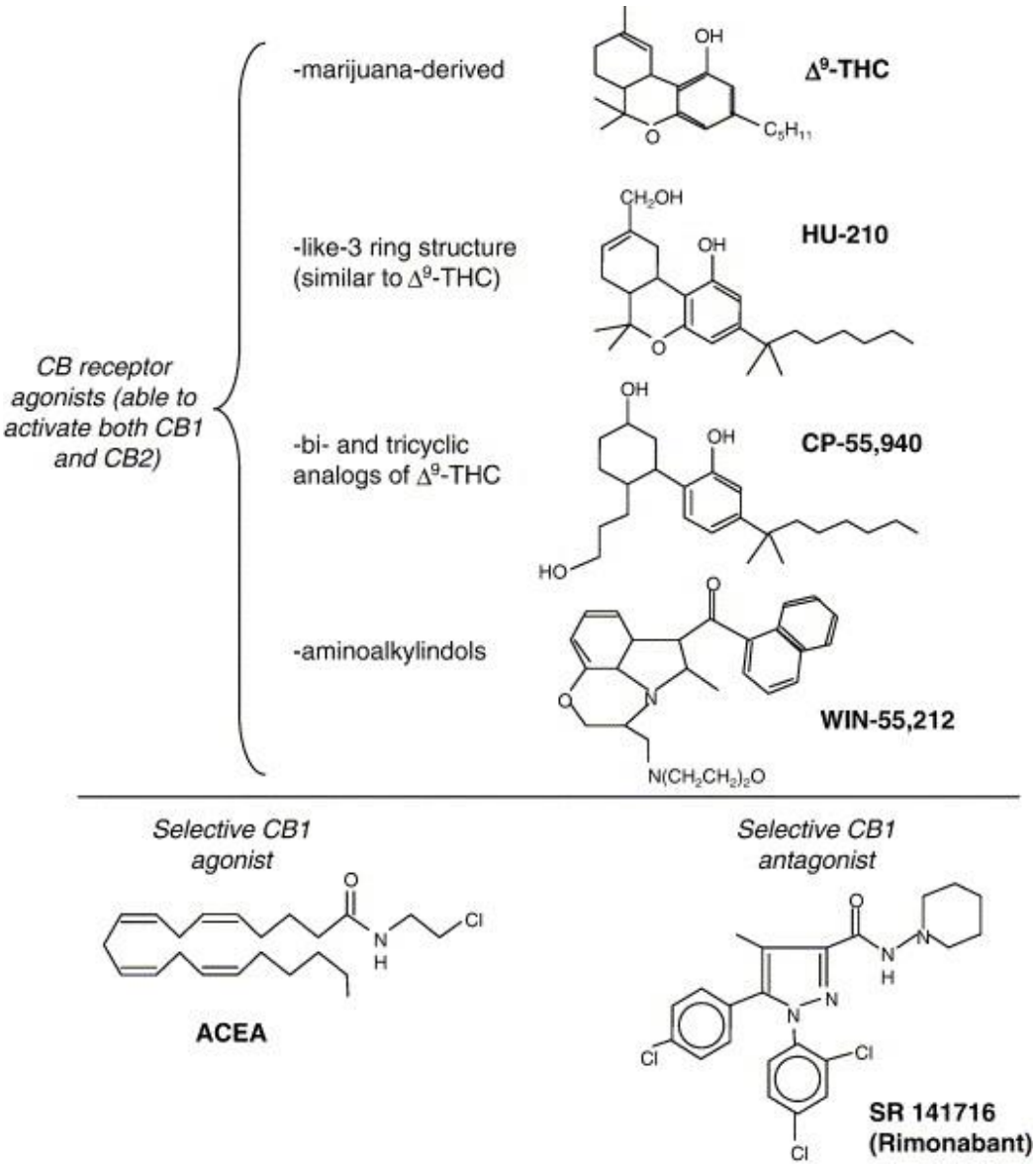


Figure 10. Structral formulas of cannabinoid agonists and antagonist (this figure is from Cota et al 2016).

Fatty acid amide hydrolase (FAAH)

FAAH is a hydrolytic enzyme for the AEA. Several FAAH inhibitors have been produced in the last years. Amongst them, URB597, OL135, O-1887, URB532, AM374, *N*-arachidonoylglycine and *N*-arachidonoyl serotonin, JNJ1661010, and CAY10401,

AM3506 and AM5206, ST4070, PF3845 and PF04457845 (Pertwee 2014). The inhibitors of FAAH produce increases in the levels of AEA but not the 2-AG (Ahn et al 2009, Kathuria et al 2003) (Ahn et al 2011, Ignatowska-Jankowska et al 2014, Long et al 2009a, Long et al 2009b). Selective FAAH inhibitor produced a modest degree of analgesia in the hotplate test. However, the side effects of Δ^9-THC on motor behavior and memory performance did not appear after FAAH inhibition (Ahn et al 2011, Chobanian et al 2014, Justinova et al 2008, Karbarz et al 2009, Kathuria et al 2003, Solinas et al 2007, Stewart & McMahon 2011). In humans, PF04457845, a FAAH inhibitor, increased the levels of AEA, but did not show any effects on cognitive functions, such as spatial memory, problem-solving, psychomotor function, attention and learning (Li et al 2011).

Monoacylglycerol lipase (MGL)

MGL is a hydrolytic enzyme of 2-AG. JZL184 is a selective inhibitor of MGL, and produced antinociception in the hotplate test as well as a decrease in locomotor activity (Long et al 2009b). Up to now, several MGL inhibitors have been reported, for example, methylarachidonoylfluorophosphonate (MAFP) (Saario et al 2004, Savinainen et al 2012, Savinainen et al 2010), N-arachidonoylmaleimide (NAM) (Blankman et al 2007, Saario et al 2005, Savinainen et al 2012), URB602, JZL184, OMDM169 (Petrosino & Di Marzo 2010). Among them, MAFP is not selective since it inhibits most metabolic serine hydrolases. NAM is selective but it inhibits MGL partially (85%). URB602 and JZL184 are non-competitive/irreversible inhibitors. OMDM169 is a reversible inhibitor. The more selective inhibitor of MGL KML29 only produced thermal antinociception without other effects which could be induced by the Δ^9-THC (Ignatowska-Jankowska et al 2014).

I.2.4.3 Inhibitors of endocannabinoid transport

FABP5 is a fatty acid-binding protein encoded by the *FABP5* gene (Madsen et al 1992). It is able to carry endocannabinoids across cellular membranes for their degradation (Kaczocha et al 2009). *FABP*-knockout mice showed higher levels of AEA in the brain than wild-type mice (Yu et al 2014). The systemic application of SBFI-26, which is an inhibitor of FABP5, produced an increase of AEA in the brain, but not 2-AG.

The application also reduced thermal nociception and oedema following intraplantar administration of γ-carrageenan. These effects were blocked by the CB_1 and CB_2 antagonists rimonabant and SR144528, respectively (Berger et al 2012). In addition, several synthetic compounds are able to inhibit the cellular uptake of AEA, such as AM404, VDM11, UCM707, OMDM1, OMDM2, and LY2183211 (Pertwee 2014). These compounds, by likely increasing levels of endocannabinoids in the extracellular space, have shown promising pharmacological properties for the treatment of cancer, pain, multiple sclerosis, Parkinson's disease, Huntington disease, anxiety, nicotine addiction, and obsessive-compulsive disorders (Pertwee 2014).

I.2.4.4 Genetic manipulation of the endocannabinoid system (ECS)

The ECS has been widely studied in different mutant mice, including the deletions of cannabinoid receptors and metabolic enzymes, and the re-expression of CB_1 receptors (Busquets-Garcia et al 2015). By using the approach of conditional deletion or re-expression of CB_1 receptors in combination with a viral expression system, we can study the role of CB_1 receptors in specific cell types, brain structures, and neural circuits. Here, I will introduce the genetic tools used so far for studying the ECS.

Global deletion of CB_1 receptors

CB_1 receptors are encoded by the *CNR1* gene. Several labs have generated mouse strains with a globally disrupted *CNR1* gene. The first reported *CB_1*-knockout (*CB_1*-KO) was in 1999 by Ledent and his colleagues (Ledent et al 1999). In their targeting strategy, they replaced the first 233 codons of the gene with a PGK-neo (phosphoglycerokinase promoter driving aminoglycoside phosphotransferase expression) cassette in embryonic stem cells from a 129 mouse strain. Then the mutant mice were crossed with CD1 mice, resulting in a genetically heterogeneous mouse stock with a *CNR1* deletion. Soon afterward, another mutant mouse strain was generated by disruption of the CB_1 coding region after amino acid 32 with a neo cassette (Steiner et al 1999, Zimmer et al 1999). These mutant mice are here called *CB_1*-KO^{Zim}, and were backcrossed to C57BL/6J animals for more than ten generations, resulting in mutation in an almost pure congenic C57BL/6J background (Steiner et al

1999, Zimmer et al 1999). Another CB_1 mutant mouse line was reported in 2002. It is replaced parts of 5' untranslated region plus most of the CB_1 open reading frame with a neo cassette, the mutant is here named *CB₁*-KO^{Ojm} (Robbe et al 2002) (**Table 4**).

Lutz and his colleagues have described a global CNR1 mutation on a C57BL/6N background, which is called here *CB₁*-KO^{Ltz} (Marsicano et al 2002). This mutant was derived from a floxed allele for the conditional deletion of *CB₁*, and contains the deletion of the whole *CB₁* open reading frame. Years later, a second floxed line was generated in the laboratory of Pierre Chambon for Sanofi Aventis (Vianna et al 2012). The floxed mutant mice were crossed with the ubiquitous Cre mouse line, and generated global deletion of *CB₁* on a mixed C57BL/6 X 129 mice background. The mutants are here named *CB₁*-KO^{Pch}. Deltagen produced *CB₁*-KO^{Dgen} on a pure 129/SvJ genetic background (Ibrahim et al 2003, Pan et al 2008a, Pan et al 2008b) (**Table 4**).

In summary, the CB_1 mutant lines have provided tools for better understanding the functions of the endocannabinoid system. Most studies showed that cannabinoid effects in the central nervous system were absent in different *CB₁*-KO lines (Han et al 2012, Marsicano et al 2003, Marsicano et al 2002, Monory et al 2007, Monory et al 2006) However, some reports showed that few central cannabinoid effects are still present in *CB₁*-KO mice, indicating that other mechanisms exist (Zimmer et al 1999). Therefore, the mutant lines are very important tools for the study of the ECS.

Mouse line	**Genetic background**	**Reference**
CB₁-KO^{Map}	*Unique CD1 derived*	Ledent et al 1999
CB₁-KO^{Zim}	C57BL/6J	Steiner et al 1999, Zimmer et al 1999
CB₁-KO^{Ojm}	C67BL/6	Robbe et al 2002
CB₁-KO^{Ltz}	C57BL/6N	Marsicano et al 2002
CB₁-KO^{Dgen}	129/SvJ	Ibrahim et al 2003, Pan et al 2008a, Pan et al 2008b
CB₁-KO^{Pch}	C57BL/6 X 129	Vianna et al 2012

Table 4. *CB₁*-KO mouse lines.

Conditional deletion of CB_1 receptors

CB_1 receptors are widely present in different brain structures and cell types (Han et al 2012, Kano et al 2009, Marsicano & Lutz 1999), but their specific roles are poorly known. Thus, some years ago, the research directions focus on the dissection of CB_1 receptors in precise brain areas, cell populations, and neural circuits (Bellocchio et al 2010, Busquets-Garcia et al 2018b, Monory et al 2007, Monory et al 2006, Soria-Gomez et al 2014). The conditional deletion of CB_1 receptors makes the studies possible by applying viral expression of Cre or crossing Cre mouse lines in/with *CB_1*-Flox mice.

As mentioned before, there are two *CB_1*-Flox^{Ltz} and *CB_1*-Flox^{Pch} lines (Marsicano et al 2003, Vianna et al 2012). Two loxP sites were located upstream and downstream of the *CB_1* coding exon in both mouse lines. The difference between the two lines is the genetic background. *CB_1*-Flox^{Ltz} was from C57BL/6N, the *CB_1*-Flox^{Pch} was from the C57BL/6 X 129. The *CB_1*-Flox^{Ltz} has been crossed with many Cre-expressing mouse lines to delete *CB_1* in different cell populations, such as principal forebrain neurons (CamKII-*CB_1*-KO^{Ltz}), forebrain GABAergic neurons (Dlx5/6-*CB_1*-KO^{Ltz}), cortical glutamatergic neurons (Nex-*CB_1*-KO^{Ltz}), dopamine D_1 receptor-positive neurons (D_1-*CB_1*-KO^{Ltz}), astrocytes (GFAP-*CB_1*-KO^{Ltz}), serotonergic neurons (TPH-*CB_1*-KO^{Ltz}), acetylcholinergic neurons (ChAT-*CB_1*-KO^{Ltz}), epinephrine/norepinephrine neurons (DBH-*CB_1*-KO^{Ltz}), PVH neurons (Sim-*CB_1*-KO^{Ltz}), VMH neurons (SF1-*CB_1*-KO^{Ltz}), and others (Balthasar et al 2005, Bingham et al 2006, Busquets-Garcia et al 2016, Han et al 2012, Monory et al 2007, Monory et al 2006, Weber et al 2009) (**Table 5**).

Regarding *CB_1*-KO in the specific brain structures or neural circuits, Cre-expressing adeno-associated virus (AAV) was injected into specific sites by using a stereotaxic injection system in *CB_1*-Flox mice. Injection of the virus into the hippocampus, hypothalamus, anterior olfactory nucleus, or anterior piriform cortex of *CB_1*-Flox resulted in a profound reduction of CB_1 mRNA expression (Monory et al 2006, Soria-Gomez et al 2014). Furthermore, a double viral approach was applied for deletion of *CB_1* in a specific neural circuit, injection of a retrograde virus expressing flippase into the innervated site

in conjugation with injection of flipase dependent Cre-expressing AAV into the area with soma (Gremel et al 2016).

Mouse line	**Cre line used**	**Location of CB1 receptors**	**Reference**
CamKII-*CB$_1$*-KO^{Ltz}	CamKIIa-BAC-iCre	Forebrain projection neurons	Monory et al 2006, Monory et al 2007
Dlx5/6-*CB$_1$*-KO^{Ltz}	Dlx5/6-Cre	Forebrain GABAergic neurons	Monory et al 2006, Monory et al 2007
Nex-*CB$_1$*-KO^{Ltz}	Nex-Cre	Cortical glutamatergic neurons	Monory et al 2006, Monory et al 2007
D$_1$-*CB$_1$*-KO^{Ltz}	D1R-YAC-Cre	Dpoamine D1 receptor positive neurons	Monory et al 2007
PVH-*CB$_1$*-KO^{Ltz}	Sim1-BAC-Cre	PVH, PH nuclei, scattered in the amygdala, BNST, PAG, kidney	Balthasar et al 2005
VMH-*CB$_1$*-KO^{Ltz}	SF1-Cre	VMH hypothalamus, pituitary and gonads	Bingham et al 2006
TPH-*CB$_1$*-KO^{Ltz}	TPH2-CreERT2	5-HT neurons of all raphe nuclei	Weber et al 2009
ChAT-*CB$_1$*-KO^{Ltz}	ChAT-Cre	Cholinergic neuron in the nervous system	Unpublished from our lab
DBH-*CB$_1$*-KO^{Ltz}	DBH-Cre	epinephrine/norepinephrine neurons	Busquets-Garcia et al 2016
GFAP-*CB$_1$*-KO^{Ltz}	GFAP-Cre-ERT2	Astrocytes	Han et al 2012
Stop-*CB$_1$*		No expression of CB1 receptors	Ruehle et al 2013
Rescue-*CB$_1$*	CMV-Cre	Widely experssed in all tissues	Ruehle et al 2013
Nex-*CB$_1$*-RS	Nex-Cre	Cortical glutamatergic neurons	Ruehle et al 2013
GABA-*CB$_1$*-RS	Dlx5/6-Cre	Forebrain GABAergic neurons	Remmers et al 2017

Table 5. Conditinal deletion or re-expression of CB_1 receptors.

Conditional re-expression of CB_1 receptors

Lutz and colleagues have developed an approach for CB_1 re-expression in Stop-*CB_1* mice, which have no expression of the receptor. Stop-*CB_1* mice contain a transcriptional Stop cassette flanked by loxP sequences inserted into the 5' untranslated region of the *CNR1* gene, just upstream of the CB_1 open reading frame (Ruehle et al 2013). Therefore, Stop-*CB_1* represents a global knockout background, but can be used for re-expression by Cre-expressing strategy (**Figure 11**). Lutz's lab produced and published the Nex-*CB_1*-RS, GABA-*CB_1*-RS, and global *CB_1*-RS mice (Remmers et al 2017, Ruehle et al 2013).

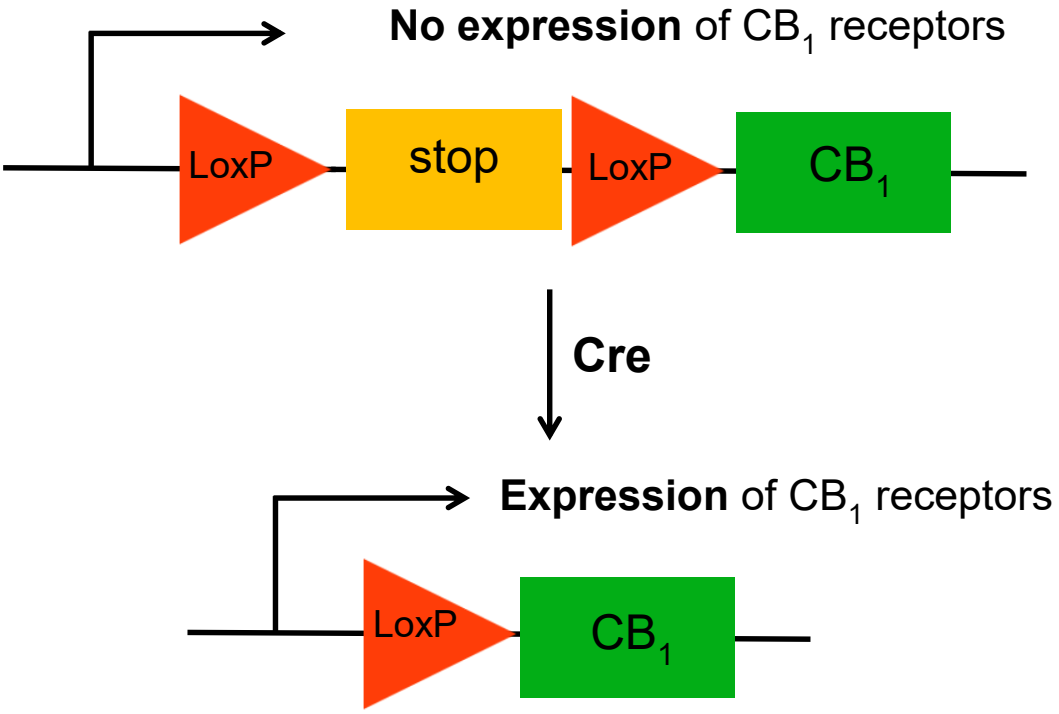


Figure 11. Schematic presentation of re-expression of CB_1 receptors in Stop-*CB_1* mice.

The strategy of CB_1 re-expression is particularly useful for studying the cannabinoid function in specific brain regions. For instance, an AAV1/2 virus expressing Cre recombinase under a chicken β-actin promoter was recently injected into the anterior olfactory nucleus of Stop-*CB_1* mice, thereby locally restoring the expression of CB_1 receptors (Soria-Gomez et al 2014). In addition, Metna-Laurent developed the AAV1/2 expressing CB_1 receptors, and injection of the virus into the amygdala restored the normal switch between different fear-coping strategies (Metna-Laurent et al 2012).

Furthermore, Hebert-Chatelain and his colleagues established a viral tool specifically expressing CB_1 receptors in plasma membrane but not mitochondrial membranes. They found that deletion of the first 22 amino acids in N-terminal of CB_1 receptor (DN22-CB_1) significantly decreases the presence of CB_1 receptor in mitochondria, displaying normal presence and function in the plasma membrane (Hebert-Chatelain et al 2016).

Deletions of biosynthetic enzymes (NAPE-PLD, DAGLs)

N-Acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) catalyzes the hydrolytic cleavage of *N*-acyl phosphatidylethanolamines (NAPES) to generate *N*-acylethanolamines, including AEA (Petersen & Hansen 1999, Ueda et al 2001). Palmiter and his colleagues generated the *Napepld*-KO mice (Liu et al 2008). However, the AEA levels in the *Napepld*-KO mice were comparable to the ones observed in wild-type mice (Leung et al 2006, Liu et al 2008), or only showed a slight reduction (Tsuboi et al 2011). The result was quite surprising and indicated the existence of an alternative AEA biosynthetic pathways.

2-Arachidonoylglycerol (2-AG) is generated by hydrolysis of diacylglycerol through an sn-1 specific diacylglycerol lipase (DGL), which consists of DGLα and DGLβ encoded by Dglα and Dglβ (Bisogno et al 2003). Doherty and his colleagues have generated the *Dglα*-Flox and *Dglα*-KO mice (Gao et al 2010, Tanimura et al 2010). In addition, Gao and Yoshino have independently generated *Dglβ*-KO mice (Gao et al 2010). *Dglα*-KO mice displayed a remarkable reduction in 2-AG levels, whereas no changes were observed in *Dglβ*-KO (Gao et al 2010). However, *Dglα*-KO X *Dglβ*-KO further decreased the 2-AG levels compared with *Dglα*-KO mice (Yoshino et al 2011). Furthermore, the retrograde synaptic suppression was absent in the *Dglα*-KO mice but not in the *Dglβ*-KO mice (Yoshino et al 2011) (**Table 6**).

Deletions of metabolic enzymes (FAAH, MGL)

Fatty acid amid hydrolase (FAAH) is the main metabolic enzyme of AEA. *Faah*-KO mice have been generated by Cravatt lab (Cravatt et al 2001). The first coding exon of this gene and approximately 2 kb of upstream sequences was replaced by a neo selection cassette. These mutant mice display a profound reduction in the degradation

of AEA and other related compounds, with AEA levels much higher than in wild-type mice (Cravatt et al 2001, Ortega-Gutierrez et al 2004).

Mouse line	**Genetic background**	**Reference**
Napepld-KO	C57BL/6 X 129SV	Liu et al 2008
Dglα-KO	C67BL/6	Gao et al 2010
Dglβ-KO	C57BL/6	Gao et al 2010
Daglα-GT (Gene trap)	Not reported	Yoshino et al 2011
Daglβ-GT (Gene trap)	Not reported	Yoshino et al 2011
Dglα-GT X *Dglβ*-GT	Not reported	Yoshino et al 2011
Faah-KO	129SvJ X C57BL/6	Cravatt et al 2001
Mgll-GT (Gene trap)	Not reported	Schlosburg et al 2010
Mgll-Flox	C57BL/6N	Uchigashima et al 2011
Mgll-KO	C57BL/6N	Uchigashima et al 2011

Table 6. Genetic manipulation of endogeneous cannabinoids.

Monoacylglycerol lipase (MGL) is the main degrading enzyme of 2-AG (Dinh et al 2002). *Mgl* mutant mice have been generated by a gene trapping approach, inducing a tenfold elevation of brain 2-AG (Schlosburg et al 2010). In addition, Uchigashima and his colleagues have generated *Mgl*-Flox mice, which were crossed with ubiquitous Cre expressing mouse line to produce global *Mgl*-KO mice (Uchigashima et al 2011). The *Mgl*-Flox mice were used to delete *Mgll* in specific cerebellar granule cells (GC-*Mgl*-KO) for examining cannabinoid effects on synaptic plasticity in the cerebellum. Investigators found that GC-*Mgl*-KO mice, compared to wild type mice, display a significantly longer DSE at the climbing fiber to purkinje cell synapses (Tanimura et al 2012) (**Table 6**).

In conclusion, pharmacological and genetic approaches provide tremendous tools for dissecting cannabinoid functions, and have markedly enhanced our understanding of the endocannabinoid system in physiological process and pathophysiological mechanisms. However, both of the tools have their disadvantages: Pharmacological approaches may have off-targets (Rudmann 2013), whereas genetic manipulations can suffer from compensatory mechanisms, which may change the expression of other genes (Monory et al 2007). Therefore, investigators should be aware of that, and the combinatory pharmacological and genetic methods can be an optional approach to get more trustable phenotypes than only using one of the two.

I.2.5 Central CB_1 receptors contribute to a variety of functions

Central CB_1 receptors play important roles in a wide variety of functions, such as learning and memory, anxiety, depression, reward processing, feeding behavior, pain, and neuroprotection (Busquets-Garcia et al 2018a, Kano et al 2009, Piazza et al 2017, Zou & Kumar 2018). Here, I briefly introduce some behavioral effects of manipulation of CB_1 receptors, the brain structures involved and the potential therapeutic significance of these processes.

I.2.5.1 Learning and memory

It is well known that the administration of Δ^9-THC produces memory impairment in humans (Makela et al 2006, Pope et al 1997). A number of studies have shown that exogenous cannabinoids impair learning and memory in different species as assessed using various behavioral paradigms (Davies et al 2002, Lichtman et al 2002). In cannabinoid-treated animals, certain aspects of memory are impaired, while others are mostly normal. Here, I will mainly limit my discussion to specific behavior paradigms, such as Morris water maze, fear conditioning, and eyeblink conditioning.

Spatial memory

The Morris water maze is one of the most widely used spatial learning paradigms, and well known to be sensitive to disruptions of hippocampal functions (D'Hooge & De Deyn 2001, Morris et al 1982). Here, the animals need to navigate in a water pool to locate a hidden platform by learning its position relative to visual cues. In a standard version, the hidden platform remains in a fixed location between trials. In a working memory version, the location of the platform is changed before each session (Vorhees & Williams 2006). Studies have shown that systemic administration of synthetic cannabinoid agonists impairs both the fixed hidden platform task (Ferrari et al 1999) and the working memory version (Varvel et al 2001). In addition, the doses of Δ^9-THC producing the impairment of performance are much lower in the working memory version than in the fixed platform version (Varvel et al 2001). These data suggest that working memory is more sensitive to the application of synthetic cannabinoid agonists than Δ^9-THC.

Several studies have shown the effects of CB_1 blockade and CB_1 deletion on spatial memory in the Morris water maze paradigm. In *CB_1*-knockout mice and wild-type mice treated CB_1 antagonist SR141716, the performance in the fixed hidden platform task was intact (Varvel et al 2005, Varvel & Lichtman 2002). However, when the platform was moved to a new place after the mice acquired the task, the *CB_1*-knockout mice had significant differences in behavior as compared to wild type mice. In these conditions, the *CB_1*-knockout mice continued to return to the previous platform location and had a prominent deficit in learning the new location. These results revealed that *CB_1*-knockout mice are impaired in reversal learning, indicating a likely lack of ability to extinguish previously acquired information (Varvel & Lichtman 2002). Soon afterwards, the same group found that blockade of CB_1 receptor by CB_1 antagonist has the same phenotype as the *CB_1*-knockout mice in the Morris water maze paradigm (Varvel et al 2005).

CB_1 receptors in the hippocampus have also been shown to contribute to the extinction of the spatial memory (Robinson et al 2008), in accordance with the well-known involvement of this brain region. Altogether, these findings reveal that CB_1 receptors are involved in the modulation of spatial learning and memory. Therefore, in human patients, it should be cautious to apply therapies based on cannabinoid agonists or antagonists, since amnesia may represent important side effects.

Aversive memory

Fear conditioning is a behavioral paradigm in which organisms learn to predict aversive events by associated neutral cues (Maren 2001). In this paradigm, an aversive stimulus (unconditioned stimulus, US, e.g. an electrical foot shock) is coupled to *a priori* neural stimulus such as a specific context or a discrete sensory cue (e.g. a tone, a smell, a light, etc.). Animals express fear behavior (e.g. freezing or others) in response to the neutral stimulus (conditioned stimulus, CS) after the conditioning, which indicates the acquisition of fear memory. Animals extinguish the fear response to the stimulus gradually if they are not receiving the foot shock repeatedly. This process is called the extinction of aversive memory (LeDoux 2000, Pavlov 1927, Rescorla 1996).

Systemic application of the synthetic cannabinoid agonist WIN55,212-2 in animals resulted in an impaired acquisition of fear memory in the contextual but not auditory cued fear conditioning (Pamplona & Takahashi 2006). In addition, systemic administration of WIN55,212-2 facilitated the extinction of fear memory at a low dose (0.25 mg/kg), but disrupted it at a higher dose (2.5 mg/kg) (Pamplona et al 2006). On the other hand, blockade or deletion of CB_1 receptors has consistently produced an impairment of fear response extinction (Chhatwal et al 2005, Marsicano et al 2002, Suzuki et al 2004). During extinction, animals are thought to actively learn that the CS is not anymore predictive US ("associative safety learning"), and/or gradually and passively habituate to the repeated CS (Kong et al 2014). Interestingly, CB_1 receptors were shown to play an important role in the habituation process but to be less prominent in the associative safety learning in auditory fear conditioning in mice (Kamprath et al 2006). In addition, both passive fear responses (freezing) and active behaviors (escape attempts and risk assessment) exist in classical fear conditioning (Gozzi et al 2010). Metna and his colleagues found that deletion of CB_1 receptors in cortical glutamatergic neurons enhances freezing, whereas deletion of CB_1 receptors in forebrain GABAergic neurons inhibits freezing and promotes active coping, suggesting a bimodal control of fear-coping strategies by CB_1 receptors (Metna-Laurent et al 2012).

It is known that contextual conditioning depends on the hippocampus and cued auditory conditioning requires the basolateral amygdala (BLA) (Anagnostaras et al 2001). Therefore, the hippocampus is likely involved in the impairment in the acquisition of fear memory by activation of CB_1 receptors. The acquisition of fear memory is reported to be controlled by the basolateral amygdala, so CB_1 receptors in the BLA are proposed to play a key role here (Anglada-Figueroa & Quirk 2005). In summary, these studies indicate that activation of CB_1 receptors has potential therapeutic significance for the treatments of psychiatric disorders related to retrieval and extinction of fear memories, such as panic disorders, phobias, and posttraumatic stress disorder (PTSD).

Eyeblink conditioning

Eyeblink conditioning (EBC) is a form of classical conditioning that has been extensively used to study the neural basis of learning and memory (Green & Woodruff-

Pak 2000). This paradigm consists of pairing an auditory or visual stimulus with a mild puff of air or a mild shock to the cornea (the transparent front part of the eye) eliciting an eyeblink (Green & Woodruff-Pak 2000). There are two classical types of EBC, delay and trace paradigms, which are thought to reflect cerebellum-dependent discrete motor learning and hippocampus-dependent associative learning, respectively (Kishimoto & Kano 2006, Weiss et al 1999). In the delay paradigm, a brief periorbital electrical shock (unconditional stimulus, US) is applied during a tone with longer duration (conditioned stimulus, CS), the two stimuli terminating simultaneously (Kishimoto & Kano 2006, Weiss et al 1999). In the trace paradigm, the US occurs 500-750 ms after the termination of the CS (Kishimoto & Kano 2006, Weiss et al 1999). *CB_1*-knockout mice are severely impaired in the delay paradigm, but not in the trace version (Kishimoto & Kano 2006). In addition, systemic blockade of CB_1 receptors 20 minutes before the daily training caused severe impairment in acquisition but not extinction of the delay eyeblink conditioning (Kishimoto & Kano 2006), indicating that CB_1 receptors likely play a key role in cerebellum-dependent motor learning.

I.2.5.2 Anxiety

Whereas fear is the response to actual and determined threats, anxiety is induced by the presence of potential and undetermined dangers (American Psychiatric Association 2013). In humans, anxiety is an emotion characterized by an unpleasant state of inner turmoil, often accompanied by nervous behaviors such as pacing back and forth, somatic complaints, and rumination (Seligman et al 2000). Short-term anxiety produces appropriate responses, such as escape. However long-term and persistent anxiety can lead to psychiatric diseases including depression (American Psychiatric Association 2013). Anxious states are controlled by a complex system consisting of inhibitory and excitatory mechanisms. Anxiety-related behaviors are assessed in rodents by several paradigms, such as the elevated plus-maze, the light-dark box, vocalizations, social interaction, and others (Buccafusco 2009). There are many studies showing links between cannabinoids and anxiety (Haring et al 2015, Viveros et al 2005).

Previous studies showed complex and sometimes contradictory effects by the application of cannabinoid agonists (Lutz et al 2015). However, it is now clear that low

doses of cannabinoids produce anxiolytic effects, whereas higher doses generate anxiogenic effects (Viveros et al 2005). Interestingly, these biphasic effects seem to be due to the respective activation of CB_1 receptors in cortical glutamatergic neurons or in forebrain GABAergic ones, respectively (Rey et al 2012). Blockade of CB_1 receptors by SR141716 produced anxiogenic effects in elevated plus-maze and vocalization tests (Arevalo et al 2001, McGregor et al 1996, Navarro et al 1997). Similarly, *CB_1*-knockout mice displayed anxious behaviors in elevated plus-maze, light-dark box, and social interaction tests (Haller et al 2002, Martin et al 2002, Uriguen et al 2004). Moreover, mice lacking the CB_1 receptor in serotonergic neurons are more anxious than control littermates in novelty suppressed food intake and forced swimming tests (Haring et al 2015). Therefore, these studies reveal that endocannabinoids likely exert anxiolytic functions. According to this hypothesis, pharmacological blockade of FAAH by URB597 and URB532 led to an increase of anandamide levels, and produced anxiolytic effects in a CB_1-dependent manner (Kathuria et al 2003). Thus, strategies acting on inhibition of endocannabinoid degradation might have therapeutic relevance to anxiety-related disorders.

I.2.5.3 Depression

Depression is a mental disorder characterized by at least two weeks of low moods, such as prolonged feelings of sadness and loss of interest in daily activities (NIMH 2016, World Health Organization 2018). It is often accompanied by low self-esteem, loss of interest in a normally enjoyable activity, low energy, and pain without a clear cause (NIMH 2016) and it is a high-risk factor of suicide (Nestler et al 2002). There are no definitive ways to assess a depressive state in rodents. However, because antidepressant drugs are able to modify them, tests of the forced swim, tail suspension, sucrose preference and others are often used as surrogates to define “depression-like” states in experimental animals (Belovicova et al 2017, Cryan & Mombereau 2004, Porsolt et al 1977, Willner et al 1987). Previous studies have shown the relationship between the endocannabinoid system and depressive-like behaviors (Vinod & Hungund 2006, Witkin et al 2005).

In the rats, systemic stimulation of CB_1 receptors by HU210 (5-25 µg/kg) showed an antidepressant-like activity in the forced swim test (FST). Similarly, enhancing CB_1 signaling by the administration of the anandamide-transporter inhibitor AM404 induced an antidepressant-like effect. However, blockade of CB_1 receptors did not induce significant effects on immobility in forced swimming test (Hill & Gorzalka 2005). In humans, the CB_1 antagonist SR141716A (rimonabant) was reported to increase the incidence of depression and suicidal thoughts (Christensen et al 2007, Mitchell & Morris 2007). However, in rats and mice, systemic treatments of rimonabant produced antidepressant-like effects (Griebel et al 2005, Steiner et al 2008). Therefore, there is still no unequivocal evidence elucidating relations between CB_1 receptors and depression.

I.2.5.4 Addiction

Addiction is a disorder characterized by compulsive engagement in rewarding stimuli despite adverse consequences (Volkow et al 2016). Many compounds lead to addiction, such as alcohol, nicotine, opioids, psychostimulants, and cannabinoids. There is a well-established relationship between the endocannabinoid system and addiction (De Vries & Schoffelmeer 2005, Fattore et al 2007, Laviolette & Grace 2006, Maldonado et al 2006). In *CB_1*-knockout mice, the rewarding effects of nicotine (0.5 mg/kg SC) disappeared in a conditioned place preference paradigm (Castane et al 2002) and, accordingly, administration of the CB_1 antagonist SR141713A reduced nicotine self-administration at 0.3-1 mg/kg (Cohen et al 2002) and nicotine-induced conditioned place preference at 1-3 mg/kg (Le Foll & Goldberg 2004) in rats. In ethanol preference test, both the pharmacological blockade of CB_1 receptors and their genetic deletion produced a reduction of ethanol preference (Wang et al 2003). Moreover, morphine self-administration was blocked in the *CB_1*-knockout mice (Ledent et al 1999). Overall, this evidence reveals that blockade of CB_1 receptors suppresses the rewarding effects of many addictive compounds.

I.2.5.5 Neuroprotection

Cannabinoids have been shown to exert neuroprotection in different kinds of in vitro and in vivo models of neurodegeneration (van der Stelt & Di Marzo 2005). Application of the cannabinoid agonist WIN55,212-2 (0.1-1 mg/kg) decreased hippocampal neuronal loss or infarct volume in the transient global or permanent focal cerebral ischemia of adult rats (Nagayama et al 1999). In the neonatal hypoxic-ischemic encephalopathy model of rats, administration of WIN55,212-2 (0.1 mg/kg) after the hypoxia-ischemia procedure decreased the final necrotic area (Fernandez-Lopez et al 2007). In closed head injury mouse models, the application of 2-AG (0.1-10 mg/kg) decreased brain edema, infarct volume, and hippocampal death and improved clinical recovery (Panikashvili et al 2001). On the other hand, in *CB_1*-knockout mice, the spontaneous recovery from behavioral deficits after closed head injury was extremely slow compared to wild type littermates (Panikashvili et al 2005). Kainic acid-induced excitotoxicity was also elevated in the *CB_1*-knockout mice (Marsicano et al 2003). Specifically, CB_1 receptors in the gulatamatertic hippocampal neurons provided substantial endogenous protection against kainic acid-induced seizures (Monory et al 2006). In an animal model of Huntington disease, chronic stimulation of cannabinoid receptors prevented motor impairments (Pietropaolo et al 2015). Furthermore, CB_1 receptors in the coticostriatal gulatamatergic neurons were identified as an indispensable player against excitotoxic damage and Huntington disease-like neurodegeneration (Chiarlone et al 2014). A recent study showed that astroglial monoacylglycerol lipase controls mutant huntingtin-induced damage of striatal neurons, and 2-AG exerts a protective function here (Ruiz-Calvo et al 2019). Taken together, these studies indicate that CB_1 receptors play an important role in neuroprotection.

I.2.5.6 Pain

Pain is a distressing feeling often caused by intense or damaging stimuli. Whereas it is an important factor to avoid dangers, excessive and persistent pain can lead to important debilitating states (Bonica 1979, Chapman et al 1979). The analgesic and antinociceptive effects of cannabinoids have been described in several models (Cravatt & Lichtman 2004, Hohmann & Suplita 2006, Jhaveri et al 2007, Walker et al 2002). Indeed, systemic application of cannabinoids predominantly suppresses pain through

CB_1 receptors in the model of acute noxious stimuli, inflammatory pain, and nerve injury (Calignano et al 1998, Hohmann & Suplita 2006, Richardson et al 1998, Strangman et al 1998). Local injections of cannabinoid agonists to different brain structures have identified the sites of cannabinoid-induced antinociception, including the dorsolateral periaqueductal gray (PAG), the dorsal raphe nucleus, the rostral ventromedial medulla (RVM), and amygdala (Hohmann & Suplita 2006). Conversely, the CB_1 antagonist SR141716A produced hyperalgesia in the Formalin (Calignano et al 1998, Strangman et al 1998) and hot plate tests (Richardson et al 1998). Thus, the data shows that enhancement of CB_1 signaling causes an analgesic effect, whereas blockade of CB_1 signaling promotes pain sensitivity.

Manipulation of endocannabinoid levels is another approach to the inhibition of pain. The inhibition of cannabinoid degradation is proposed to produce analgesia by increasing the levels of endocannabinoid levels (Manzanares et al 2006). In this *scenario,* FAAH inhibitor URB597 has been shown to remarkably decrease pain without toxicity in preclinical safety studies with rats and monkeys (Saario & Laitinen 2007). Therefore, endocannabinoids have potential clinical use in analgesia.

I.2.5.7 Feeding behavior

It is well known that hyperphagia is one of the most notable effects of *cannabis* in humans. In animals, a low dose of cannabinoids increases food intake in a CB_1-dependent manner (Pagotto et al 2006). Conversely, systemic application of CB_1 antagonists reduced food intake in normal and obese animals (Pagotto et al 2006). Therefore, the studies show that CB_1 receptors are involved in the control of food intake.

In the brain, endocannabinoid levels were shown an increase in the limbic forebrain and the hypothalamus after 24-hour food deprivation in rats (Kirkham et al 2002), and injection of 2-AG into the nucleus accumbens promotes feeding behavior (Kirkham et al 2002). Leptin is a hormone suppressing food intake, which decreases endocannabinoid levels in the hypothalamus (Di Marzo et al 2001). In addition, application of anandamide into the ventromedial hypothalamus, as well as in the

nucleus accumbens produced hyperphagia in a CB_1-dependent manner (Jamshidi & Taylor 2001, Soria-Gomez et al 2007). Furthermore, Bellocchio and colleagues discovered a bimodal control of food intake by the ECS. Whereas deletion of CB_1 receptors in cortical glutamatergic neurons decreases food intake, lack of CB_1 receptors in forebrain GABAergic neurons increases food intake in food-deprived mice (Bellocchio et al 2010).

Preclinical studies in animal models and clinical trials have confirmed that the CB_1 receptor is a promising target for treating appetitive disorders and obesity. Δ^9-THC or its synthetic analog has been proposed for antinausea and treatment of anorexia (Pagotto et al 2006). The CB_1 antagonist SR141716A (rimonabant) has a clear hypophagic effect. Although it has serious psychiatric side effects, it is still worth to study this agent for decreasing its side effects. A recent study reported a novel peripherally restricted cannabinoid receptor antagonist, AM6545, which remarkably reduced food intake in rodents (Cluny et al 2010). Therefore, AM6545 may be a potential anti-obesity drug avoiding psychiatric side effects.

Supplementary information for the section of I.2 The endocannabinoid system.

In this section, some review papers and a textbook (Endocannabinoids), which systemically concluded knowledge of the endocannabinoid system (Busquets-Garcia et al 2018a, Cascio & Marini 2015, Kano et al 2009, Olmo et al 2016, Piazza et al 2017, Touw 1981, Zimmer 2015, Zuardi 2006), provided helpful information in designing the structure of this section. I am so grateful to the contribution in the field of the endocannabinoid system from the authors.

I.3 The relationship between thirst and the endocannabinoid system

Thirst is determined by internal states, such as plasma osmolality, hormones and nervous signals (Gizowski & Bourque 2018, Ichiki et al 2019, Leib et al 2016, Zimmerman et al 2017). The brain generates the perception of thirst, and successively drives drinking behavior in response to thirst signals (Gizowski & Bourque 2018, Leib et al 2016). Type-1 cannabinoid receptor (CB_1) is abundantly expressed and widely distributed in the central nervous system. Many brain regions involved in the control of drinking behavior have an intense expression of CB_1 mRNA (**Figure 12,13**). In addition, CB_1 receptors participate in the control of other ingestive behaviors, such as food intake (Bellocchio et al 2010, Di Marzo et al 2001, Soria-Gomez et al 2014a, Soria-Gomez et al 2014b, Soria-Gomez et al 2007). Indeed, the pharmacological stimulation or inactivation of CB_1 receptors influences drinking behavior (Abel 1975, Higgs et al 2003, Ruginsk et al 2015, Verty et al 2004b), providing however inconsistent results, likely due to the use of different doses of drugs and in distinct species. Altogether, these observations point out that CB_1 receptors may play a crucial role in the control of water intake.

I.3.1 CB_1 mRNA is located in the brain areas regulating water intake

CB_1 mRNA is widely distributed in the central nervous system, including brain structures participating in the control of water intake. Here, I will describe the distribution of CB_1 mRNA in these brain structures. Considering that the CB_1 protein is mainly expressed at the presynaptic membrane in neurons (Kawamura et al 2006, Matyas et al 2006, Nyiri et al 2005), the detection of mRNA at somatic level allows the identification of the cellular location and identity of the cells expressing the receptor.

The subfornical organ (SFO)

The SFO is one of brain areas in the lamina terminalis, situated on the ventral surface of fornix and in the wall of the third ventricle, and it is located outside of the blood-brain barrier (Kaur & Ling 2017). As I mentioned in the sections of I.1.2.2 and

I.1.3, the SFO is an essential brain structure in the control of drinking behavior. Oka and colleagues reported that optogenetic stimulation of glutamatergic neurons of the SFO promotes drinking behavior, but the same treatment in GABAergic neurons suppresses water intake (Oka et al 2015). As shown in **Figure 12**, there is a strong expression of CB_1 mRNA in the SFO. Altogether, these observations point out that CB_1 receptors in the SFO cells may contribute to the regulation of drinking behavior.

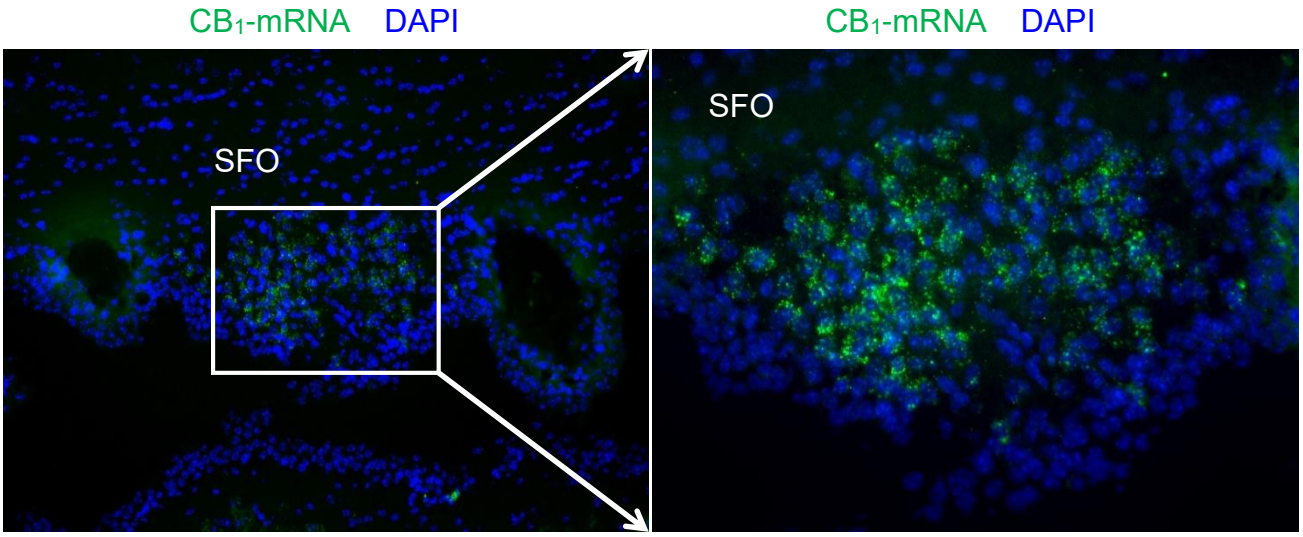


Figure 12. CB_1 mRNA is locating in the SFO. The green signals are CB_1 mRNA, the blue signals are DAPI.

The paraventricular nucleus of the hypothalamus (PVH)

The PVH is a brain structure producing the antidiuretic hormone, which is also called vasopressin (Lederis 1961, Swanson & Sawchenko 1983). An early study showed that a 1-2% increase in blood osmolality causes the release of vasopressin (Verney 1947). A recent study reported that ketogenic diet- or alcohol-induced water intake is dependent on the signaling of fibroblast growth factor 21 (FGF21) in the PVH (Song et al 2018). Therefore, the PVH plays an important role in the control of water retention and drinking behavior for the regulation of body water homeostasis. In this area, there is a strong expression of CB_1 mRNA (**Figure 13**), suggesting that CB_1 receptors in the PVH cells may regulate water retention and drinking behavior.

The lateral hypothalamus

The lateral hypothalamus (LH) is a crucial brain area for the control of ingestive behavior, including both drinking and feeding behaviors. Lesions of the LH led to adipsia and aphagia (Teitelbaum & Stellar 1954). In addition, electrical and pharmacological stimulations of the LH have shown an influence on both drinking and feeding behavior (Bernardis & Bellinger 1996). A recent study showed that activation of neurotensin-expressing neurons in the LH promotes water intake, but not food intake (Kurt et al 2019). These results indicate that the LH is an essential brain structure for the regulation of drinking behavior. By doing fluorescent in situ hybridization, we found that a strong expression of CB_1 mRNA is present in the LH (**Figure 13**), suggesting that CB_1 receptors in LH neurons may also contribute to the control of water intake.

The amygdala

Early studies using lesion and stimulation approaches have shown that the amygdala is crucial for drinking behavior (Anand 1961, Fonberg 1966, Fonberg 1968, Fonberg 1969a, Fonberg 1969b, Fonberg & Sychowa 1968, Grossman & Grossman 1963). Recently, Kim and his colleagues reported that the basolateral amygdala (BLA) and the central amygdala (CeA) control drinking behavior (Kim et al 2016, Kim et al 2017). In addition, different cell types in the amygdala exert different roles in the regulation of water intake. For example, activation of protein phosphatase 1 regulatory subunit 1B (Ppp1r1b)-positive cells expressed in the posterior BLA led to increased nose pokes to get water (Kim et al 2016). Conversely, inhibition of somatostatin (Sst)-positive neurons in the CeA decreases drinking behavior as well as inhibition of neurons co-expressing corticotropin-releasing hormone (Crh), neurotensin (Nts), and tachykinin 2 (Tac2). However, inhibition of the protein kinase C-δ (Prkcd)-positive neurons in the CeA increases drinking behavior (Kim et al 2017). Altogether, these data reveal that neurochemically distinct neuronal populations in the amygdala control water intake in different ways. Both BLA and CeA contain the expression of CB_1 mRNA at different levels (**Figure 13**), indicating that CB_1 receptors in the neurons of the amygdala may control drinking behavior in different manners depending on the cell types, for example,

deletion of CB_1 receptors Ppp1r1b-positive cells or Prkcd-positive may increase or decrease drinking, respectively.

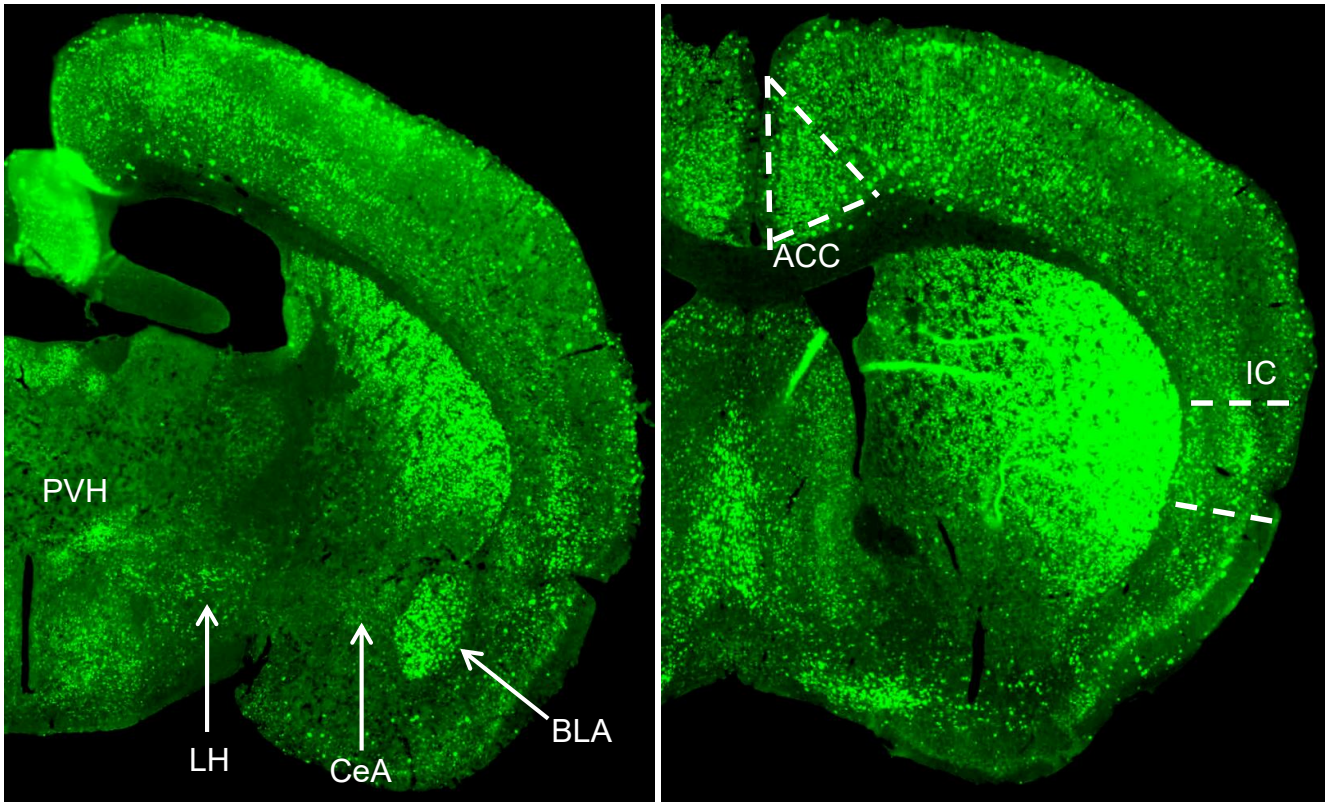


Figure 13. CB_1 mRNA in brain sections. The green signals are CB_1 mRNA.

The anterior cingulate cortex (ACC) and the insular cortex (IC)

Several studies showed that the ACC and the IC are involved in the control of thirst and drinking (Denton et al 2009, Gizowski & Bourque 2018, Ichiki et al 2019, Robinson & Mishkin 1968, Zimmerman et al 2017). By using fMRI, investigators found that the activity of the ACC and IC is correlated to the subjective feeling of thirst in humans (Denton et al 1999a, Denton et al 1999b). Recent studies showed activation of the posterior IC inhibits licking water, whereas activation of anterior IC promotes this behavior in mice (Schiff et al 2018, Wang et al 2018). In rats, the ACC c-fos protein expression is increased by systemic hypertonic saline administration (Ma et al 2019). These results indicate that the ACC and IC are involved in the control of drinking

behavior. CB_1 mRNA is present in both principal neurons and interneurons of the ACC and the IC (**Figure 13**). Therefore, CB_1 receptors in different cell types of these cortical regions may play different roles in the control of drinking behavior.

In summary, many brain regions regulating drinking behavior display expression of CB_1 mRNA, but their role in the control of water intake is mostly unknown. Here, we hypothesize that CB_1 receptors in these brain regions play an important role in the regulation of water intake.

I.3.2 CB_1 receptors are crucial to the control of feeding behavior

Ingestive behavior encompasses feeding and drinking. It is well known that CB_1 receptors contribute to the control of feeding behavior (Bellocchio et al 2010, Di Marzo et al 2001, Soria-Gomez et al 2014a, Soria-Gomez et al 2014b, Soria-Gomez et al 2007, Pagotto et al 2006). As drinking behavior is another ingestive behavior, we supposed that CB_1 receptors may play a role in the regulation of drinking behavior.

Regarding feeding behavior, Δ^9-tetrahydrocannabinol (Δ^9-THC) stimulates food intake with a low dose (1mg/kg). However, a high dose of Δ^9-THC exerts inhibition of food intake (Bellocchio et al 2010, Soria-Gomez et al 2014a). Administration of endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG) increased food intake in a dose-dependent manner. The orexigenic effects induced by AEA and 2-AG were lower efficient than the application of Δ^9-THC (Williams & Kirkham 2002). This indicates that exogenous AEA and 2-AG may be an amplification of endocannabinoid signaling in regulating normal, episodic patterns of meal-taking in rats (Kirkham & Williams 2001, Williams & Kirkham 2002).

Global deletion or blockade of CB_1 receptors inhibits feeding behavior (Bellocchio et al 2010). In addition, the deletion of CB_1 receptors in cortical glutamatergic neurons also decreases food intake. Conversely, the deletion of CB_1 receptors in forebrain GABAergic neurons increases food intake (Bellocchio et al 2010). These results showed that CB_1 receptors control food intake in a bimodal. Furthermore, by using a stop-*CB_1* mouse line, which is similar to the *CB_1*-KO mouse line until the presence of

Cre recombinase, re-expression of CB_1 receptors in the cortical glutamatergic neurons rescued the reduction in food intake of these mice (Soria-Gomez et al 2014a).

CB_1 receptors and endocannabinoids are present in the limbic forebrain (Bisogno et al 1999). In the striatum, CB_1 receptors co-localize with dopamine D1 and D2 receptors (Hermann et al 2002). Injection of AEA or FAAH inhibitor into the nucleus accumbens increased food intake by enhancing endocannabinoid signaling (Soria-Gomez et al 2007). Furthermore, dopamine D1 receptor antagonists reduced the food intake elicited by Δ^9-THC (Verty et al 2004b). These results suggest that the orexigenic effect of Δ^9-THC might partially depend on the enhancement of dopamine signaling.

Hunger promotes olfactory detection to facilitate seeking and ingesting food (Albrecht et al 2009, Cameron et al 2012, Goetzl & Stone 1947, Julliard et al 2007, Tong et al 2011). The anterior olfactory nucleus (AON) and the anterior piriform cortex (APC) send projections to and receive inputs from the olfactory bulb (Bisogno et al 1999, Boyd et al 2012, Davis & Macrides 1981, Markopoulos et al 2012, Shepherd 1972). A recent study showed that the deletion of CB_1 receptors in the AON and APC decreases food intake, whereas re-expression of CB_1 receptors in some brain structures promotes food intake in stop-*CB1* mice (Soria-Gomez et al 2014a). These data suggest that CB_1 receptors of the AON and APC play sufficient and necessary roles in the control of food intake.

The paraventricular nucleus (PVN) of the hypothalamus is a center for the control of food intake (Fenselau et al 2017, Krashes et al 2014). Blockade of ECS in the PVN by CB_1 receptor antagonist AM251 increased food intake in fasted rats. In addition, inactivation of CB_1 receptors in the PVN facilitated ghrelin-induced food intake (Soria-Gomez et al 2014b). The data suggest that endocannabinoid signaling in the PVN plays an inhibitory role in the regulation of feeding behavior.

In conclusion, CB_1 receptors contribute to the control of feeding behavior in different manners depending on their distributions in brain regions and cell types. However, it is totally unknown how CB_1 receptors control drinking behavior. Here, we would like to study cannabinoid-dependent drinking behavior by using similar approaches like the ones used to investigate feeding behavior.

I.3.3 Pharmacological manipulation of CB_1 receptors influences drinking behavior

The effect of *Cannabis* and *Cannabis* extracts on water intake was equivocal in early studies (Abel 1975). Chronic administration of Δ^9-THC decreased daily water intake in both of the Zuker obese (fa/fa) and lean (Fa/-) rats (Drewnowski & Grinker 1978). Interestingly, stimulation of CB_1 receptors by Δ^9-THC, 2-AG, or AEA promotes the consumption of sucrose liquid in adult male hooded Lister rats, whereas blockade of CB_1 receptors by SR141716 decreased the consumption of sucrose liquid (Higgs et al 2003). These data indicate that enhancement of cannabinoid signaling facilitates palatability and promotes sucrose drinking. However, injection of the CB_1 receptors antagonist SR141716 into lateral ventricle did not influence water intake in male Wistar rats (Verty et al 2004a). Systemic stimulation of CB_1 receptors by ACEA (10mg/kg) promoted water intake in water-deprived male Wistar rats, whereas blockade of CB_1 receptors by AM251 (3mg/kg) inhibits drinking behavior (Ruginsk et al 2015). These data indicate that CB_1 receptor modulates water deprivation-induced homeostatic responses. However, there is still no unequivocal studies showing the role of CB_1 receptors in the control of water intake and the underlying mechanisms are unknown.

In summary, the given evidence suggests that CB_1 receptors participate in the control of water intake. Previous pharmacological studies provide contradictory results, but it is still clear that CB_1 receptors control drinking behavior in distinct ways depending on the species and the dosage of drugs. Thanks to the new genetic approaches, we can use diverse conditional CB_1 receptor mutant mice to study the role of CB_1 receptor in precise locations (Busquets-Garcia et al 2018, Hebert-Chatelain et al 2016, Metna-Laurent et al 2012, Soria-Gomez et al 2014a). Therefore, it is now feasible to dissect the role of CB_1 receptors in different cell types, brain structures, and neural circuits in the control of water intake.

CHAPTER II

Objectives

Water intake is one of the basic instinctive behaviors of all living beings. Thirst drives drinking behavior to maintain proper body water homeostasis. Inadequate water intake leads to dehydration, which causes nausea, trembling, and may have serious consequences, such as seizure and death (Gizowski & Bourque 2018, Ichiki et al 2019, Leib et al 2016, Zimmerman et al 2017). The endocannabinoid system (ECS) modulates a variety of higher brain functions, such as learning, memory, and anxiety, but is also involved in the regulation of basic physiological processes, such as food intake and energy balance (Bellocchio et al 2010, Di Marzo et al 2001, Pagotto et al 2006, Soria-Gomez et al 2014a, Soria-Gomez et al 2014b, Soria-Gomez et al 2007). However, the role of CB_1 receptors in the control of thirst processes and subsequent drinking behavior has not been clearly elucidated yet. Given the ECS modulation of ingestive behaviors, such as feeding, one may postulate that drinking behavior is regulated by the ECS as well. In addition, CB_1 receptors and endocannabinoids are present in many brain structures involved in water intake (**Figure 12,13**). Furthermore, few reports suggest that pharmacological manipulation of CB_1 receptors impact water intake, although results are contradictory according to species, conditions, and drug/dosage used. Therefore, I hypothesize that the control of water intake by CB_1 receptors may be multimodal depending on their locations in the brain. **In this Thesis work, I aimed at investigating how CB_1 receptors in specific cell types, brain structures, and neural circuits regulate drinking behavior.**

As described in the following chapters, this question was addressed through different and complementary experimental approaches.

First, I established different approaches to eliciting drinking behavior, including water deprivation, systemic and intracerebroventricular injections of hypertonic sodium chloride (inducing intracellular dehydration), and systemic and intracerebroventricular injections of angiotensin II (inducing extracellular dehydration) dehydration (Fitzsimons 1966, Fitzsimons 1980). To study the

general role of the ECS in these processes I applied the aforementioned experimental conditions to mice globally lacking CB_1 receptors (*CB_1*-KO mice compared to their WT littermates).

Second, I studied the role of CB_1 receptors in specific cell types in the control of water intake. CB_1 receptors are mainly G_i protein-coupled receptors and their activation at presynaptic level suppresses neurotransmitter release. Therefore, CB_1 receptors might be required to exert different roles in different cell types, depending on their neurochemical nature. To untangle the potential necessary role of CB_1 receptor signaling in different cell types in the control of drinking behavior, I tested how the conditional deletion of CB_1 receptors in different cell populations impact dehydration-induced drinking. To this aim, I used Glu-*CB_1*-KO, GABA-*CB_1*-KO, GFAP-*CB_1*-KO, D_1-*CB_1*-KO, and ChAT-*CB_1*-KO mice compared to their WT littermates (**Table 7**). In addition, to establish whether CB_1 receptors play a sufficient role in the control of water intake in specific cell types, I studied the drinking behavior of conditional re-expression of CB_1 receptors over a knockout background, using *CB_1*-RS, Glu-*CB_1*-RS, and GABA-*CB_1*-RS mice (**Table 7**). Furthermore, to study the role of recently discovered mitochondrial associated CB_1 receptors in the control of water intake, we also used a novel mouse line lacking mitochondrial CB_1 receptors, called DN22-*CB_1* mice (**Table 7**).

Third, I addressed the role of CB_1 receptors in specific brain structures, and their targeted projections for the control of water intake, using specific viral approaches to delete or re-express CB_1 receptors in specific neural circuits. *CB_1*-Flox mice were locally injected with viral vectors expressing Cre recombinase in order to achieve deletion of the gene. Conversely, the same viral injections were made in stop-*CB_1* mice, in which the expression of the receptor is impaired in normal conditions and triggered by Cre recombinase. To address specific circuits more precisely, I also used injections of retrograde viral vectors in combination with the injection of AAV(**Table 7**).

In summary, by using genetic, pharmacologic, and behavioral approaches, this Thesis work started dissecting the role of CB_1 receptors in distinct cell populations/neuronal circuits for the control of water intake.

Lines	**Cre line used**	**Cre expression pattern or CB_1 deletion pattern**	**References**
CB_1-KO		All cells of the body.	Marsicano et al., 2002
Glu-*CB_1*-KO	NEX-Cre	Cortical glutamatergic neurons, including hippocampus, neocortex, archicortex and amygdala.	Goebbels et al., 2006 Monory et al., 2006 Monory et al., 2007
GABA-*CB_1*-KO	Dlx5/6-Cre	GABAergic neurons of the forebrain.	Monory et al., 2006 Monory et al., 2007
GFAP-*CB_1*-KO	GFAP-Cre-ERT2	Astrocytes	Han et al 2012
D1-*CB_1*-KO	D1R-YAC-Cre	Striatum, nucleus accumbens, olfactory tubercles, weak in cortex and thalamus.	Lemberger et al., 2007 Monory et al., 2007
ChAT-*CB_1*-KO	ChAT-Cre	Cholinergic neurons in the nervous system.	Unpublished from our lab
CB_1-RS	CMV-Cre	Widespread expression in all tissues.	Ruehle et al., 2013
Glu-*CB_1*-RS	NEX-Cre	Cortical glutamatergic neurons including hippocampus, neocortex, archicortex and amygdala.	Ruehle et al., 2013
GABA-*CB_1*-RS	Dlx5/6-Cre	GABAergic neurons of the forebrain.	Remmers et al., 2017
DN22-*CB_1*-KI	AAV-CaMKIIα Cre in the ACC	All cells of the body.	
ACC-CaMKIIα-*CB_1*-RS	AAV-CaMKIIα Cre in the ACC	The glutamatergic neurons in the anterior cingulate cortex.	Ruehle et al., 2013
IC-*CB_1*-RS	AAV-Cre in theIC	The cells in the insular cortex.	Ruehle et al., 2013
ACC-BLA-*CB_1*-RS	AAV-FRT-Cre in the ACC, AAV2-retro-FLPo in the BLA	BLA projecting neurons inthe anterior cingulate cortex.	Ruehle et al., 2013

Table 7. CB_1 receptor-mutant mouse lines.

CHAPTER III

Results

III.1 Top-down control of water intake by the endocannabinoid system: Role of CB_1 receptors in the anterior cingulate cortex neurons projecting to the basolateral amygdala

Thirst is a fundamental perception driving drinking behavior for maintenance of body water homeostasis. However, the neural mechanisms underlying thirst are still not fully understood. In particular, some recent evidence points out neocortical regions as key players for the top-down control of water intake. The endocannabinoid system (ECS) regulates a variety of brain functions, from feeding behavior and energy balance to learning, memory, and anxiety. CB_1 receptors are abundantly present in the central nervous system, including in neocortical areas. In this project, I aimed at investigating the role of CB_1 receptors in the control of drinking behavior. Since CB_1 receptors are located in different cell types, brain regions, and neural circuits, some of which regulate water intake, a potential differential role of CB_1 receptor-mediated control water intake might depend on the receptors' distribution. Notably, the global deletion of CB_1 receptors decreases water intake elicited by water deprivation, hypotonic sodium chloride, and angiotensin II. Furthermore, specific expression of CB_1 receptors in cortical glutamatergic neurons, but not in the forebrain GABAergic neurons, is sufficient to promote water intake.

As the anterior cingulate cortex is involved in the regulation of thirst, we re-expressed CB_1 receptors in ACC glutamatergic neurons, and found that this manipulation rescues water intake. Interestingly, we identified that presynaptic CB_1 receptors are intensely expressed in projections from ACC to the basolateral amygdala (BLA). The specific re-expression of CB_1 in the ACC-BLA circuit of mice lacking the receptors in all cells of the body rescues drinking behavior.

Altogether, these data reveal that CB_1 receptors are crucial to water intake, and we deciphered a novel circuit for the top-down control of drinking behavior.

This work is going to be submitted soon, and the manuscript is as below.

Top-down control of water intake by the endocannabinoid system

Zhe Zhao[1,2], Edgar Soria-Gómez[1,2,3], Marjorie Varilh[1,2], Francisca Julio-Kalajzić[1,2], Astrid Cannich[1,2], Adriana Castiglione[1,2], Léonie Vanhoutte[1,2], Alexia Duveau[1,2], Philippe Zizzari[1,2], Anna Beyeler[1,2], Daniela Cota[1,2], Luigi Bellocchio[1,2,*], Arnau Busquets-Garcia[1,2,4,*], Giovanni Marsicano[1,2,*]

[1] INSERM, NeuroCentre Magendie, Physiopathologie de la Plasticité Neuronale U1215, F33077 Bordeaux, France.

[2] University of Bordeaux, NeuroCentre Magendie, Physiopathologie de la Plasticité Neuronale U1215, F33077 Bordeaux, France.

[3] IKERBASQUE Basque Foundation for Science, University of the Basque Country UPV/EHU, Achucarro Basque Center for Neuroscience.

[4] Integrative Pharmacology and Systems Neuroscience, IMIM-Hospital del Mar Medical Research Institute, Barcelona, Spain.

[*] Senior author

Correspondence should be addressed to:
Giovanni Marsicano DVM, PhD
NeuroCentre Magendie,
INSERM U1215 Université Bordeaux
Group Endocannabinoids and Neuroadaptation
146 rue Léo-Saignat 33077 Bordeaux Cedex- France
Tel: +33 (0)5 5757 3756 Fax: +33 (0)5 5757 3669
giovanni.marsicano@inserm.fr

Abstract

Water intake is regulated by neocortical top-down circuits, but their identity and the cellular mechanisms involved are scantly known. Here, we show that endogenous activation of type-1 cannabinoid receptors (CB_1) promotes water intake and that endocannabinoid modulation of excitatory projections from the anterior cingulate cortex to the basolateral amygdala is sufficient to guarantee physiological drinking. These data reveal a new circuit involved in the homeostatic control of water intake.

Water intake is crucial for maintaining body fluid homeostasis and animals' survival (Gizowski & Bourque 2018). Complex brain processes trigger thirst and drinking behavior, which arise upon losing blood volume (i.e. extracellular dehydration) or increasing blood osmolality (i.e. intracellular dehydration) (Gizowski & Bourque 2018, Johnson et al 2003). However, the central mechanisms promoting water intake are still poorly understood. In the brain, the anterior wall of the third ventricle formed by the subfornical organ (SFO), the median preoptic nucleus, and the organum vasculosum of the lamina terminalis (OVLT) constitutes the primary structure sensing thirst signals and promoting water intake (Abbott et al 2016, Oka et al 2015). These subcortical regions are connected with the neocortex (Gizowski & Bourque 2018). In particular, insular and anterior cingulate cortices (IC and ACC, respectively) have been shown to receive indirect projections from the SFO and OVLT in rats (Hollis et al 2008), and water consumption after dehydration decreases ACC activity in humans (Saker et al 2018). Furthermore, recent evidence shows that stimulation of the anterior part of IC promotes drinking behavior, whereas stimulation of the posterior part exerts the opposite effect (Wang et al 2018). These studies highlight the importance of cortical regions in the regulation of water intake (Gizowski & Bourque 2018, Hollis et al 2008, Saker et al 2018, Wang et al 2018).

Type-1 cannabinoid receptors (CB_1) are widely and abundantly expressed in the central nervous system where they modulate a variety of functions, including feeding behavior (Bellocchio et al 2010, Busquets-Garcia et al 2018a, Piazza et al 2017). However, the role of CB_1 receptors in the control of water intake is still a matter of

debate, since pharmacological activation or blockade of CB_1 receptors produced contradictory results in drinking behavior experiments (Abel 1975, Ruginsk et al 2015). In this study, we identified a novel and specific cortical circuit where CB_1 receptors modulate water intake.

To examine the role of CB_1 receptors in the control of water intake, we first tested *CB_1* knockout mice (*CB_1*-KO) (Marsicano et al 2002) under different experimental conditions. No significant difference was observed between *CB_1* wild-type (*CB_1*-WT) and *CB_1*-KO littermates in daily water intake (**Supplementary Fig. 1a**). However, *CB_1*-KO mice drunk less than WT littermates after 24-hour water deprivation (**Fig. 1a, Supplementary Fig. 1b**), without any change in food intake (**Supplementary Fig. 1c**), indicating that CB_1 receptors participate in water deprivation-induced drinking behavior. Water deprivation triggers both intracellular and extracellular dehydration that can lead to water intake through different pathways (Gizowski & Bourque 2018). To discriminate the impact of CB_1 receptor signaling on either of these mechanisms, we first applied systemic (intraperitoneal, IP) or intracerebroventricular (ICV) injections of sodium chloride (NaCl), which are known to induce water intake by mimicking intracellular dehydration (Gizowski & Bourque 2018). As compared to wild-type littermates, mice lacking CB_1 receptors displayed a lower water intake induced by both IP or ICV NaCl administration (**Fig. 1b, c, Supplementary Fig. 1d**). Extracellular dehydration promotes the production of angiotensin II (ANG), which can induce drinking behavior and salt appetite (Gizowski & Bourque 2018). Thus, to mimic this condition, mice received ICV injections of ANG. Notably, the ANG-induced

water intake was blunted in *CB1*-KO mice **(Fig. 1d)**, indicating that endocannabinoid signaling controls drinking behavior induced by both intracellular and extracellular dehydration mechanisms. Importantly, the acute systemic pharmacological blockade of CB_1 receptors decreased drinking under water deprivation and NaCl injections (**Fig. 1e, f**), indicating that endocannabinoid signaling is required at the moment of drinking and that the phenotype of *CB1*-KO mice is not due to the long-lasting deletion of the gene (Marsicano et al 2002). Concomitantly with the abundant brain expression, CB_1 receptors are also present in peripheral organs (Piazza et al 2017), suggesting that peripheral control of body water levels or blood osmolality might underlie the endocannabinoid-dependent regulation of water intake. However, measurements of body water composition and blood osmolality did not reveal any difference between *CB1*-KO mice and *CB1*-WT littermates (**Supplementary Fig. 1e, f**). Altogether, these results indicate that endogenous activation of CB_1 receptors contributes to drinking behavior induced by both intracellular and extracellular dehydration conditions, likely through central mechanisms.

CB_1 receptors are expressed in many different brain regions and in distinct cell types (Busquets-Garcia et al 2015, Busquets-Garcia et al 2018b, Piazza et al 2017). To identify the specific cell-type involved in CB_1 receptor-dependent control of water intake, we used conditional mutant mice carrying deletion of the *CB1* gene in specific cell types, such as cortical glutamatergic neurons (Glu-*CB1*-KO) (Marsicano et al 2003, Monory et al 2006), forebrain GABAergic neurons (GABA-*CB1*-KO) (Marsicano et al 2003, Monory et al 2006), glial fibrillary acidic protein-positive cells (mainly

astrocytes, GFAP-*CB$_1$*-KO) (Han et al 2012, Marsicano et al 2003) and dopamine receptor D$_1$-positive cells (D$_1$-*CB$_1$*-KO) (Marsicano et al 2003, Monory et al 2007). All these cell types have been implicated in the control of water intake (Abbott et al 2016, Gizowski & Bourque 2018, Oka et al 2015). Surprisingly, however, none of these mutant lines displayed significant phenotypes in drinking behavior induced by water deprivation or NaCl injections (**Supplementary Fig. 2a-h**).

It is particularly puzzling how global, but not cell type-specific, CB$_1$ deletion can impact water intake. This may be due to the redundancy of CB$_1$ receptor-dependent pathways controlling a function as vital as water intake. In this context, despite the general necessary role of the endocannabinoid system in controlling drinking behavior, this redundancy would decrease the specific *necessity* of selected subpopulations of CB$_1$ receptors. This, however, does not exclude that CB$_1$ receptor-dependent control of specific cell populations might play *sufficient* roles in controlling stimulated water intake. To address this possibility, we adopted a rescue approach and we used mice carrying specific and exclusive re-expression of the CB$_1$ protein in specific cell types (Stop-*CB$_1$* mice approach) (Remmers et al 2017, Ruehle et al 2013). A “floxed-stop” cassette prevents the expression of CB$_1$ receptors in the stop-*CB$_1$* mutant line, similarly as in global *CB$_1$*-KO mice. Viral or transgenic expression of the Cre recombinase, however, induces the re-expression of the CB$_1$ receptors in particular brain regions and/or cell types over a “knockout-like” background (Remmers et al 2017, Ruehle et al 2013).

First, we verified that Stop-*CB$_1$* mice displayed the same impaired water intake as

CB_1-KO mice and that global re-expression of the CB_1 protein is able to fully rescue water intake under deprivation and NaCl injections (*CB_1*-RS for CB_1 "rescued"; **Fig. 1g,h**) (Remmers et al 2017, Ruehle et al 2013). Re-expression of CB_1 protein in GABAergic neurons (GABA-*CB_1*-RS mice) (Remmers et al 2017, Ruehle et al 2013), which include the large majority of brain CB_1 receptors (Busquets-Garcia et al 2018a, Busquets-Garcia et al 2015, Piazza et al 2017), did not rescue drinking behavior either after water deprivation or IP NaCl injection (**Supplementary Fig. 2i, j**). Interestingly, however, re-expression of CB_1 receptors in cortical glutamatergic neurons (Glu-*CB_1*-RS) (Ruehle et al 2013), which represents a minority of the receptor in the brain (Busquets-Garcia et al 2018a, Busquets-Garcia et al 2015, Piazza et al 2017), significantly rescued water intake induced by water deprivation, by systemic or central injection of NaCl, or by ICV ANG administration (**Fig. 1i-l**). These data indicate that the presence of CB_1 receptors in cortical glutamatergic neurons is sufficient to promote water intake induced by different conditions.

Amongst other neocortical areas, the insular cortex (IC) has been directly shown to regulate water intake (Schiff et al 2018, Wang et al 2018). Therefore, we tested whether specific re-expression of CB_1 receptors in this brain region might rescue the impairment of water intake observed in Stop-*CB_1* mice. Multiple local injections of an adeno-associated virus expressing Cre recombinase (AAV-Cre) into the IC of Stop-*CB_1* mice resulted in a consistent *CB_1* re-expression in both anterior and posterior portions of this brain region (IC-*CB_1*-RS; **Supplementary Fig. 3a-e**). However, this manipulation did not rescue the water intake associated with lack of

CB_1 receptor protein (**Supplementary Fig. 3f,g**). Recent evidence points to the idea that the anterior and posterior parts of the IC play opposite roles in the control of drinking behavior[6]. In particular, activation of neurons located in the anterior IC (aIC) increases water intake, whereas the same manipulation of the posterior IC (pIC) exerts the opposite effect (Wang et al 2018). Considering that activation of CB_1 receptors generally reduces neuronal activity (Busquets-Garcia et al 2018a), we reasoned that endocannabinoid control of the pIC leads to decreased neuronal activity and promotes drinking behavior. To test this possibility, we re-expressed the CB_1 protein exclusively in the pIC of Stop-*CB_1* mice (pIC-*CB_1*-RS, **Supplementary Fig. 4a,b and 3e**), where the lack of the receptor should logically induce a reduction of drinking. However, also this partial re-expression did not rescue the phenotype of Stop-*CB_1* mice (**Supplementary Fig. 4c,d**), strongly suggesting that CB_1 receptors in this brain region do not play a major role in water intake.

Recent studies suggest that the anterior cingulate cortex (ACC) might participate in the regulation of water intake (Gizowski & Bourque 2018, Hollis et al 2008, Saker et al 2018). Using a similar approach as above, we generated ACC-*CB_1*-RS mice, in which the CB_1 protein is re-expressed only in ACC principal neurons (**Fig. 2a-c**). Notably, ACC-*CB_1*-RS mice displayed significantly higher water intake than Stop-*CB_1* littermates (ACC-*CB_1*-SS) both after water deprivation and IP NaCl injection (**Fig. 2d,e**), indicating that the presence of CB_1 receptors in principal neurons of the ACC is sufficient to promote drinking behavior induced by water deprivation and NaCl treatment.

As the ACC is a heterogeneous structure targeting multiple downstream regions, we next aimed at identifying which CB_1-positive projections from ACC are responsible for the stimulation of drinking behavior. First, we mapped the ACC neuronal projections interested by our local viral treatments. The injection of an AAV-CaMKIIα-GFP virus into the ACC revealed that principal neurons of this neocortical region project to many brain areas, including the basolateral amygdala (BLA), the claustrum (Cl), the medial caudate putamen, the lateral habenula (**Supplementary Fig. 5 and video 1**). In order to analyze the expression of presynaptic CB_1 receptors in these ACC projections, we evaluated the distribution of the CB_1 protein in ACC-*CB_1*-RS mice. Interestingly, CB_1 receptors were mainly present in the claustrum, the BLA, as well as in the ectorhinal and perirhinal cortices (**Fig. 2f-h, Supplementary video 2**). Recent evidence actually indicates that BLA is involved in the control of drinking behavior (Kim et al 2017, Wang et al 2018). We therefore asked whether CB_1 receptors expressed in ACC to BLA projections (ACC-BLA) are sufficient to promote water intake (**Fig. 3a**). To obtain selective rescue of the CB_1 protein in ACC-BLA terminals, we used a retrograde viral approach in the Stop-*CB_1* mice. The injection of a retrograde AAV (rAAV2-retro) expressing flippases coupled to blue fluorescent protein (rAAV2-retro-FLIPo-EBFP) into the BLA of Stop-*CB_1* mice was associated with the simultaneous infusion of another AAV carrying a FLIPo-dependent expression of Cre recombinase (AAV-FRT-iCre) into the ACC (**Fig. 3b**). These combinatorial viral manipulations resulted in a strong re-expression of CB_1 protein in ACC-BLA projecting neurons of Stop-*CB_1* mice

(ACC-BLA-*CB_1*-RS mice; **Fig. 3c-e**). Strikingly, after water deprivation and IP NaCl injection, ACC-BLA-*CB_1*-RS mice consumed significantly more water than control mice (**Fig. 3f,g**), revealing the key role of CB_1 receptor-dependent control of drinking in this specific brain circuit.

This study reveals an unforeseen circuit mechanism for top-down control of a fundamental life function such as water intake. Specifically, general CB_1 receptor activity is necessary to promote water intake, and its control of ACC principal neurons impinging onto BLA is sufficient to promote drinking behavior. These data highlight the complexity of brain control of water intake and underline the importance of top-down regulatory circuits in these processes.

Author contributions

Z.Z. and G.M. conceived the project. Z.Z. and G.M. designed the experiments and analyzed data with the input of E.S., L.B., and A.B. Z.Z. performed the experiments and collected data. Z.Z., L.B., and G.M. wrote the manuscript. M.V. and F.J. performed immunohistochemistry experiments. A.C., A.C., L.V., A.D., and P.Z. assisted in performing experiments. A.B. and D.C. discussed the study. All authors read and edited the manuscript.

Acknowledgments

We thank the animal facility and the genotyping platform of the NeuroCentre Magendie (INSERM U1215 Unit) for providing assistance in the animal breeding,

maintenance and genotyping. We also thank Drs. Aude Panatier and Stéphane Oliet of NeuroCentre Magendie for providing the Osmometer. We thank the Bordeaux Imaging Center, a service unit of the CNRS-INSERM and Bordeaux University, member of the national infrastructure France BioImaging supported by the French National Research Agency (ANR-10-INBS-04), for providing the confocal microscope (Leica TCS SP8), the slide scanner (Nanozoomer 2.0HT, Hamamatsu Photonics France), and Imaris software (Imaris, Oxford instrument, UK), the help of Sébastien Marais is acknowledged. HHMI Janelie farm research campus is acknowledged for providing the rAAV2-retro helper. We thank the Viral Vector Facility (VVF) of Neuroscience Center Zurich (ZNZ) for providing the rAAV2-retro-FLIPo-EBFP and the rAAV2-retro-EBFP viral vectors. We also thank Dr. Karl Deisseroth from Stanford University, Stanford, CA for providing the plasmid of AAV-CaMKIIα-GFP. This work is supported by the China Scholarship Council (to Z.Z.), INSERM (to G.M., D.C., A.B.), Nouvelle Aquitaine Region (to D.C., G.M.), European Research Council (Endofood, ERC–2010–StG–260515 and CannaPreg, ERC-2014-PoC-640923, MiCaBra, ERC-2017-AdG-786467, to G.M.), Fondation pour la Recherche Medicale (FRM, DRM20101220445, to G.M.), 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.) and BRAIN ANR-10-LABX-0043, to G.M.

Figure 1

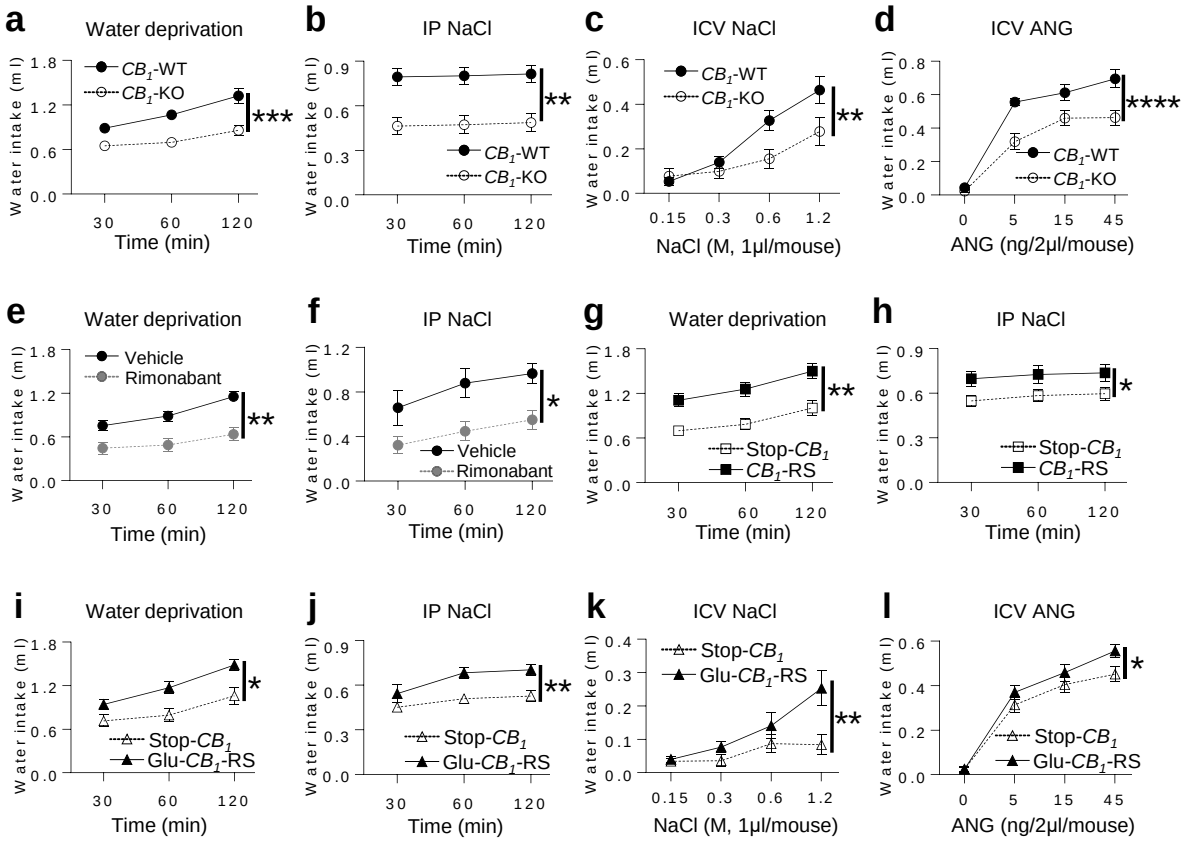

a
Water deprivation
Water intake (ml)
CB1-WT
CB1-KO
***
Time (min)
b
IP NaCl
**
c
ICV NaCl
**
NaCl (M, 1µl/mouse)
d
ICV ANG
****
ANG (ng/2µl/mouse)
e
Water deprivation
Vehicle
Rimonabant
**
f
IP NaCl
*
g
Water deprivation
**
Stop-CB1
CB1-RS
h
IP NaCl
*
i
Water deprivation
*
Glu-CB1-RS
j
IP NaCl
**
k
ICV NaCl
**
l
ICV ANG
*

Figure 1. Global deletion of CB_1 decreases water intake induced by different dehydrations, whereas re-expression of *CB_1* in cortical glutamatergic neurons is sufficient to promote water intake. **a-d,** Cumulative water intake of *CB_1*-WT (Black circles) and *CB_1*-KO (Open circles) mice after 24-hour water deprivation (*CB_1*-WT n=10, *CB_1*-KO n=8), IP 1M NaCl, 10ml/kg body weight (*CB_1*-WT n=10, *CB_1*-KO n=8), ICV NaCl (*CB_1*-WT n=13, *CB_1*-KO n=10), and ICV ANG (*CB_1*-WT n=11, *CB_1*-KO n=13). **e-f,** Cumulative water intake induced by 24-hour water deprivation (Vehicle n=9, Rimonabant n=10) or IP 1.5M NaCl, 10ml/kg body weight (Vehicle n=6, Rimonabant n=7) after systemic blockade of CB_1 receptors (Rimonabant, 3mg/kg, gray circles; Vehicle, black circles). **g-h,** Cumulative water intake induced by 24-hour water deprivation (Stop-*CB_1* n=9, *CB_1*-RS n=12), IP 1M NaCl, 10ml/kg body weight (Stop-*CB_1* n=9, *CB_1*-RS n=11) in Stop-*CB_1* (Open squares) and *CB_1*-RS (Black squares) mice. **i-l,** Cumulative water intake induced by 24-hour water deprivation (Stop-*CB_1* n=11, Glu- *CB_1*-RS n=11), IP 1M NaCl, 10ml/kg body weight (Stop-*CB_1* n=11, Glu-*CB_1*-RS n=11), ICV NaCl (Stop-*CB_1* n=13, Glu-*CB_1*-RS n=11), and ICV ANG (Stop-*CB_1* n=15, Glu-*CB_1*-RS n=13) in Stop-*CB_1* (Open triangles) and Glu-*CB_1*-RS (Black triangles) mice. All data are showed as mean ± s.e.m, and were statistically analyzed by the two-way repeated measures ANOVA, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

Figure 2

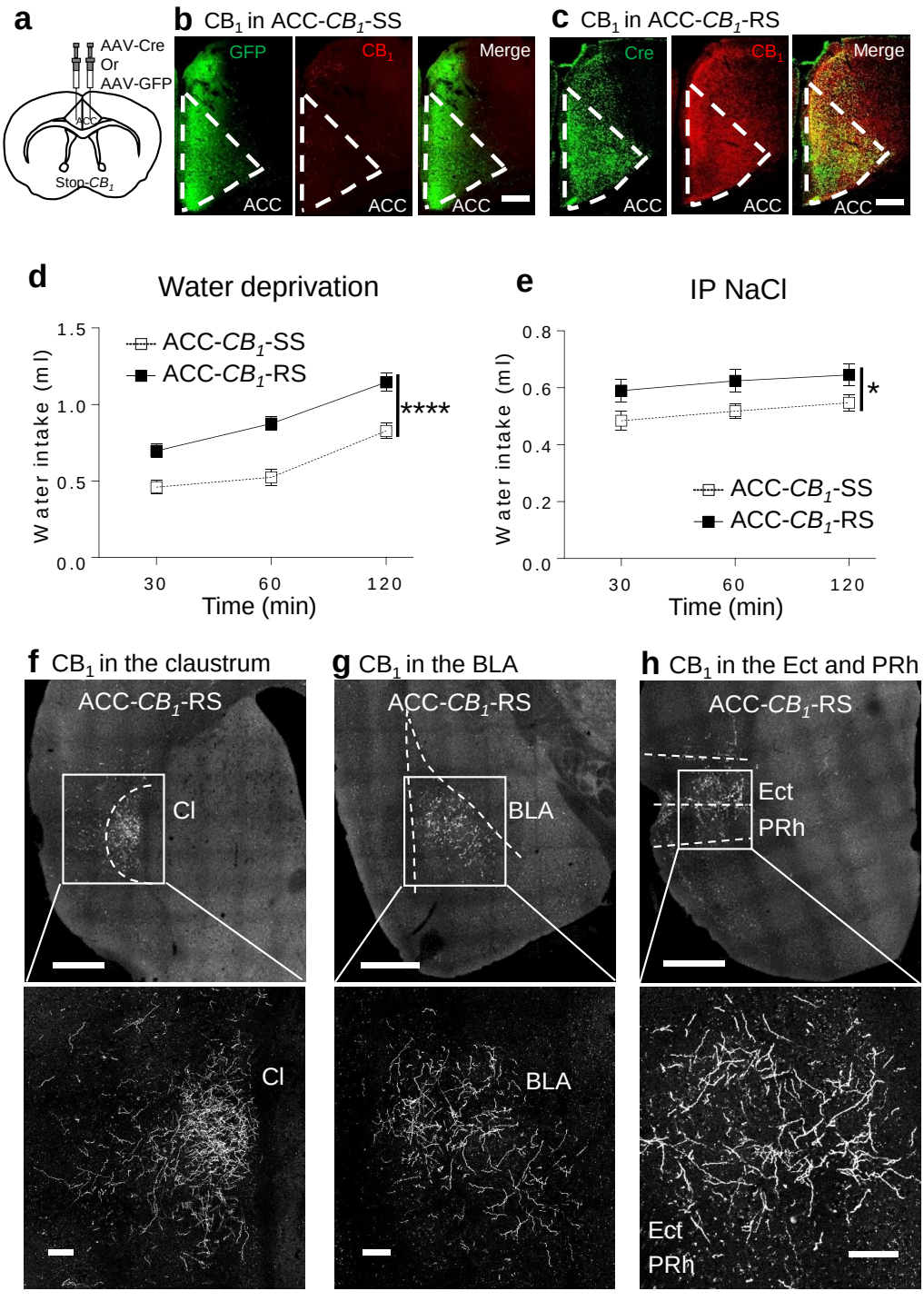

a
AAV-Cre
Or
AAV-GFP
ACC
Stop-CB1
b CB1 in ACC-CB1-SS
GFP
CB1
Merge
ACC
c CB1 in ACC-CB1-RS
Cre
CB1
Merge
ACC
d
Water deprivation
Water intake (ml)
ACC-CB1-SS
ACC-CB1-RS
****
Time (min)
e
IP NaCl
Water intake (ml)
*
ACC-CB1-SS
ACC-CB1-RS
Time (min)
f CB1 in the claustrum
ACC-CB1-RS
Cl
g CB1 in the BLA
ACC-CB1-RS
BLA
h CB1 in the Ect and PRh
ACC-CB1-RS
Ect
PRh
Ect
PRh

Figure 2. Re-expression of CB_1 in the ACC is sufficient to promote water intake. **a**, Schematic representation of CB_1 rescue approach in the ACC of Stop-*CB1* mice. **b-c**, CB_1 (red) immunostaining in the ACC of ACC-*CB1*-SS and ACC-*CB1*-RS, respectively. Scale bar, 200 µm. **d-e**, Cumulative water intake of ACC-*CB1*-SS (Open squares) and ACC-*CB1*-RS (Black squares) mice after 24-hour water deprivation (ACC-*CB1*-SS n=17, ACC-*CB1*-RS n=20) or IP 1M NaCl, 10ml/kg body weight (ACC-*CB1*-SS n=18, ACC-*CB1*-RS n=20). **f-h**, Presynaptic CB_1 receptors located in the CI, BLA and Ect/PRh in a ACC-*CB1*-RS mouse. Scale bar, 500 µm and 100 (Amplified images) µm. All data are showed as mean ± s.e.m, and were statistically analyzed by the two-way repeated measures ANOVA, $*P < 0.05$, $****P < 0.0001$.

Figure 3

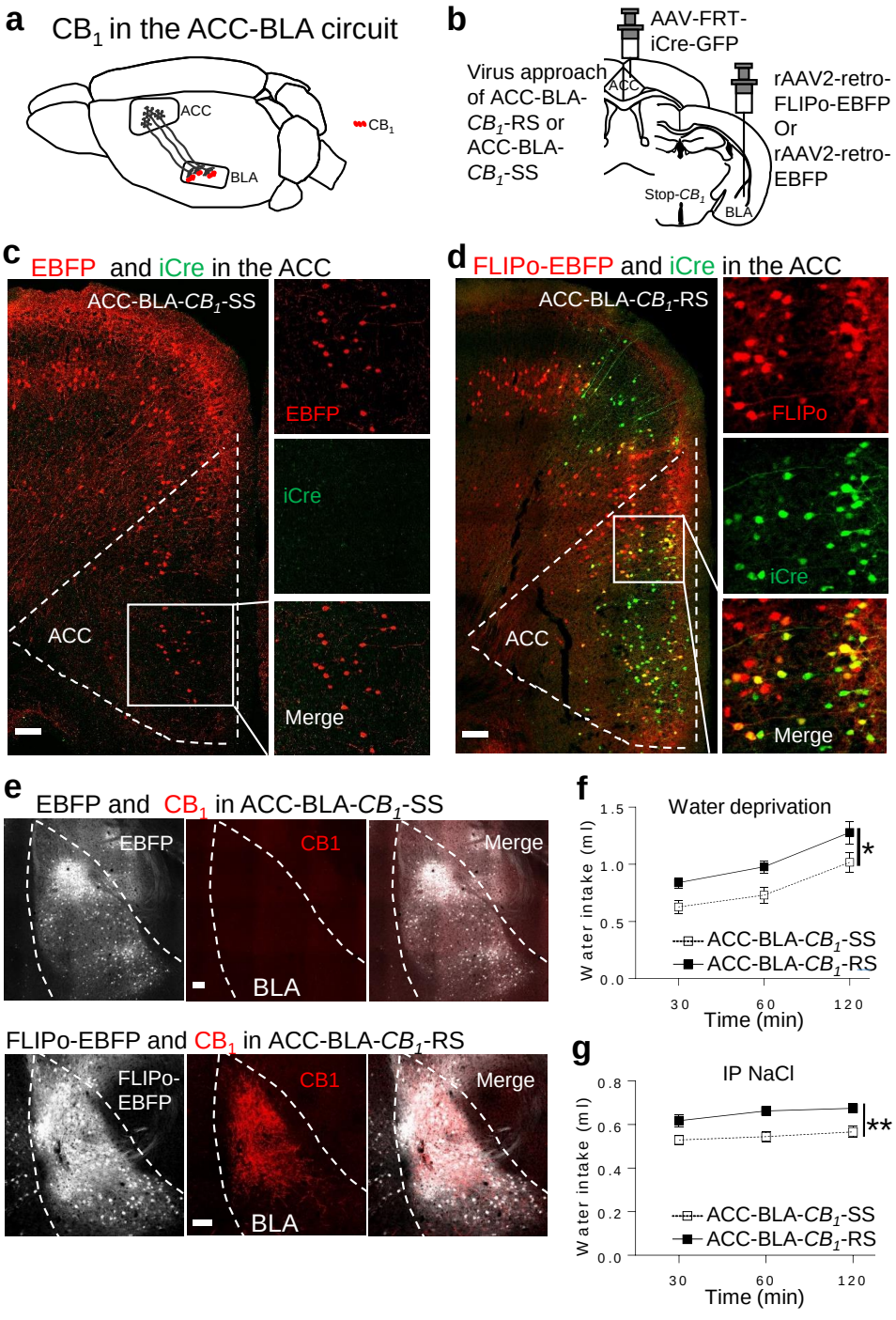

a
CB1 in the ACC-BLA circuit
ACC
BLA
CB1
b
AAV-FRT-iCre-GFP
Virus approach of ACC-BLA-CB1-RS or ACC-BLA-CB1-SS
rAAV2-retro-FLIPo-EBFP Or rAAV2-retro-EBFP
Stop-CB1
c
EBFP and iCre in the ACC
ACC-BLA-CB1-SS
EBFP
iCre
Merge
ACC
d
FLIPo-EBFP and iCre in the ACC
ACC-BLA-CB1-RS
FLIPo
iCre
Merge
ACC
e
EBFP and CB1 in ACC-BLA-CB1-SS
EBFP
CB1
Merge
BLA
FLIPo-EBFP and CB1 in ACC-BLA-CB1-RS
FLIPo-EBFP
CB1
Merge
BLA
f
Water deprivation
Water intake (ml)
1.5
1.0
0.5
0.0
*
ACC-BLA-CB1-SS
ACC-BLA-CB1-RS
30
60
120
Time (min)
g
IP NaCl
Water intake (ml)
0.8
0.6
0.4
0.2
0.0
**
ACC-BLA-CB1-SS
ACC-BLA-CB1-RS
30
60
120
Time (min)

Figure 3. CB_1 receptors located in ACC-BLA is sufficient to promote water intake. **a,** Schematic representation of CB_1 receptors located in the ACC-BLA circuit. **b,** Viral approach to specifically rescue *CB_1* in the ACC-BLA circuit. **c,** EBFP (pseudo red) and iCre-GFP (green) in ACC sections of ACC-BLA-*CB_1*-SS. Scale bar, 100 μm. **d,** FLIPo-EBFP (pseudo red) and iCre-GFP (green) in ACC section of ACC-BLA-*CB_1*-RS. Scale bar, 100 μm. **e-f,** CB_1 (red) immunostaining in BLA section of ACC-BLA-*CB_1*-SS and ACC-BLA-*CB_1*-RS. Scale bar, 100 μm. **g-h,** Cumulative water intake of ACC-BLA-*CB_1*-SS (Open squares) and ACC-BLA-*CB_1*-RS (Black squares) mice after 24-hour water deprivation (ACC-BLA-*CB_1*-SS n=10) and 1M IP NaCl, 10ml/kg body weight (ACC-BLA-*CB_1*-SS n=12). All data are shown as mean ± s.e.m, and were statistically analyzed by the two-way repeated-measures ANOVA, $*P < 0.05$, $**P < 0.01$.

Supplementary information for: Top-down control of water intake by the endocannabinoid system

Zhe Zhao[1,2], Edgar Soria-Gómez[1,2,3], Marjorie Varilh[1,2], Francisca Julio-Kalajzić[1,2], Astrid Cannich[1,2], Adriana Castiglione[1,2], Léonie Vanhoutte[1,2], Alexia Duveau[1,2], Philippe Zizzari[1,2], Anna Beyeler[1,2], Daniela Cota[1,2], Luigi Bellocchio[1,2,*], Arnau Busquets-Garcia[1,2,4,*], Giovanni Marsicano[1,2,*]

[1] INSERM, NeuroCentre Magendie, Physiopathologie de la Plasticité Neuronale U1215, F33077 Bordeaux, France.

[2] University of Bordeaux, NeuroCentre Magendie, Physiopathologie de la Plasticité Neuronale U1215, F33077 Bordeaux, France.

[3] IKERBASQUE Basque Foundation for Science, University of the Basque Country UPV/EHU, Achucarro Basque Center for Neuroscience.

[4] Integrative Pharmacology and Systems Neuroscience, IMIM-Hospital del Mar Medical Research Institute, Barcelona, Spain.

[*] Senior author

Correspondence should be addressed to:
Giovanni Marsicano DVM, PhD
NeuroCentre Magendie,
INSERM U1215 Université Bordeaux
Group Endocannabinoids and Neuroadaptation
146 rue Léo-Saignat 33077 Bordeaux Cedex- France
Tel: +33 (0)5 5757 3756 Fax: +33 (0)5 5757 3669
giovanni.marsicano@inserm.fr

Contents:

Supplementary online methods

Mice. All experiments were approved by the Committee on Animal Health and Care of INSERM and the French Ministry of Agriculture and Forestry. The authorizing number from the ethical committee is 15493. Maximal efforts were made to reduce the suffering and the number of used mice. All behavioral experiments were performed during the light phase and animals were kept in individual cages under standard conditions in a day/night cycle of 12/12 hours (lights on at 7 am). Male wild-type C57BL/6n mice purchased from Janvier (France) were used for the pharmacological experiments. All the used mutant mice were generated and identified in previous studies, e.g. global CB_1 knockout (*CB_1*-KO) mice (Marsicano et al 2002), deletion of CB_1 receptors is specific in cortical glutamatergic Nex positive neurons (Glu-*CB_1*-KO) (Marsicano et al 2003, Monory et al 2006), forebrain GABAergic Dlx5/6 positive neurons (GABA-*CB_1*-KO) (Marsicano et al 2003, Monory et al 2007), astrocytes (GFAP-*CB_1*-KO) (Han et al 2012, Marsicano et al 2003), and dopamine receptor type 1 positive neurons (D_1-*CB_1*-KO) (Marsicano et al 2003, Monory et al 2007). The stop-*CB_1* mice (lack of CB_1) (Remmers et al 2017, Ruehle et

al 2013), global re-expression of CB_1 receptors (*CB_1*-RS) (Remmers et al 2017, Ruehle et al 2013), re-expression of CB_1 receptors is specific in forebrain GABAergic Dlx5/6 positive neurons (GABA-*CB_1*-RS) (Remmers et al 2017) and cortical glutamatergic Nex positive neurons (Glu-*CB_1*-RS) (Ruehle et al 2013). The mice used in this study were 7-10 weeks old at the beginning of the experiments.

Water intake assays. Water intake was observed at 30, 60 and 120 minutes after 24-hour water deprivation and intraperitoneal (IP) injection of 1M sodium chloride (NaCl, VWRV0241) with 10ml/kg body weight. In the pharmacological experiments, Rimonabant (3mg/kg, 9000484, Cayman Chemical Company US) and vehicle (4% ethanol, 4% Cremophor, 92% saline) were injected half an hour prior to the water intake test of the water deprivation or IP injection of 1.5 M NaCl with 10ml/kg body weight. For the mice of ICV injection, water intake was observed at 30 minutes after intracerebroventricular (ICV) injection of Angiotensin II (ANG, Bachem, H-1705.0025) and NaCl. We started to test water intake 7 days after the ICV cannula implantation. ICV injection was once a day in each mouse. In the progressive ANG dose-response experiments, we did ICV injections of saline, 5 ng, 15 ng, and 45 ng ANG (2µl/mouse) in different days. Then, we start the ICV NaCl injection 3 days after the last ICV ANG injection, ICV injection was once a day in each mouse. In the progressive NaCl dose-response experiments, we did ICV NaCl injections of 0.15M, 0.3M, 0.6M, and 1.2M NaCl (1µl/mouse) in the different days. In order to make sure that mice were drinking normally before the treatments, each mouse was observed the daily water intake during the experiments.

Body water composition analysis. The basal body water composition test was performed in mice by using a mouse-specific nuclear magnetic resonance whole body composition analyzer (EchoMRITM-900, EchoMedical Systems, Houston, TX). Mice were placed in a specific chamber without strong movements, each readout was done within 1 minute. Mice were put back to home cages after the test.

Plasma osmolality analysis. Plasma osmolality was tested by Osmometer 3320 (Advanced Instruments, France). Facial veil blood collection was applied in this experiment. Blood was collected and put in the Micro tube 1.3 ml K3E (SARSTEDT, 41.1395.005), then blood samples remained in room temperature for 30 minutes. By using a refrigerated centrifuge (VWR Micro Star 17R), blood samples were centrifuged with 4000 rpm for 15 minutes at 4℃. Following centrifugation, the plasma was immediately transferred to a clean eppendorf tube and put on the ice for the osmolality test.

Surgery and viral administration. Mice were anesthetized by isoflurane (5% induction, then, 2% during the surgery) and placed on a stereotaxic apparatus (Model 900, KOPF instruments, CA, USA) with a mouse adaptor and lateral ear bars. For viral vectors delivery, AAV vectors were loaded in a glass pipette and fused by a pump (UMP3-1, World Precision Instruments, FL, USA). AAV-GFP (Hybrid AAV1/2, 5 x 10E10 vg/ml), AAV-Cre-GFP (Hybrid AAV1/2, 4.5 x 10E10 vg/ml) were injected into the insula (IC) (200nl/side, 100nl/min). The coordinate of anterior IC injection is AP +1.2mm, ML ± 3.0mm, DV 3.5mm, and the coordinate of posterior IC injection is AP -0.3mm, ML ± 3.7mm, DV 4.0mm. AAV-CaMKIIα-GFP (Hybrid AAV1/2, >1 x 10E10

vg/ml) or AAV-CaMKIIα-Cre-HA (Hybrid AAV1/2, >1 x 10E10 vg/ml. The plasmids were provided by Karl Deisseroth, Stanford University, Stanford, CA) were injected into the anterior cingulate cortex (ACC) (200nl/side, 100nl/min). The coordinate of ACC injection is AP +0.6mm, ML ± 0.3mm, DV 2.0mm, For the ACC-BLA-*CB_1*-RS or ACC-BLA-*CB_1*-SS mice, the AAV-FRT-iCre-GFP (Addgene #24593, ZNZ VVF v245, 6.3 x 10E12 vg/ml) was injected into ACC with the coordinates mentioned above in both group mice (200nl/side, 100nl/min). The rAAV2-retro-FLIPo-EBFP (Addgene #60663, ZNZ VVF v151, 6.4 x 10E12 vg/ml) or rAAV2-retro-EBFP (ZNZ VVF v140, 4.1 x 10E12 vg/ml) were injected into the BLA with the coordinates AP -1.6mm, ML ±3.3mm, DV 4.9 mm (150nl/side, 100nl/min). AAV-FRT-iCre-GFP, rAAV2-retro-FLIPo-EBFP, and rAAV2-retro-EBFP were produced by the Viral Vector Facility (VVF) of the Neuroscience Center Zurich (ZNZ). The re-expression of CB_1 receptors was verified by the immunohistochemistry in all the mice used in the behavioral experiments. Above coordinates were according to the mouse brain in stereotaxic coordinates by Paxinos and Franklin, 2001 (Second edition).

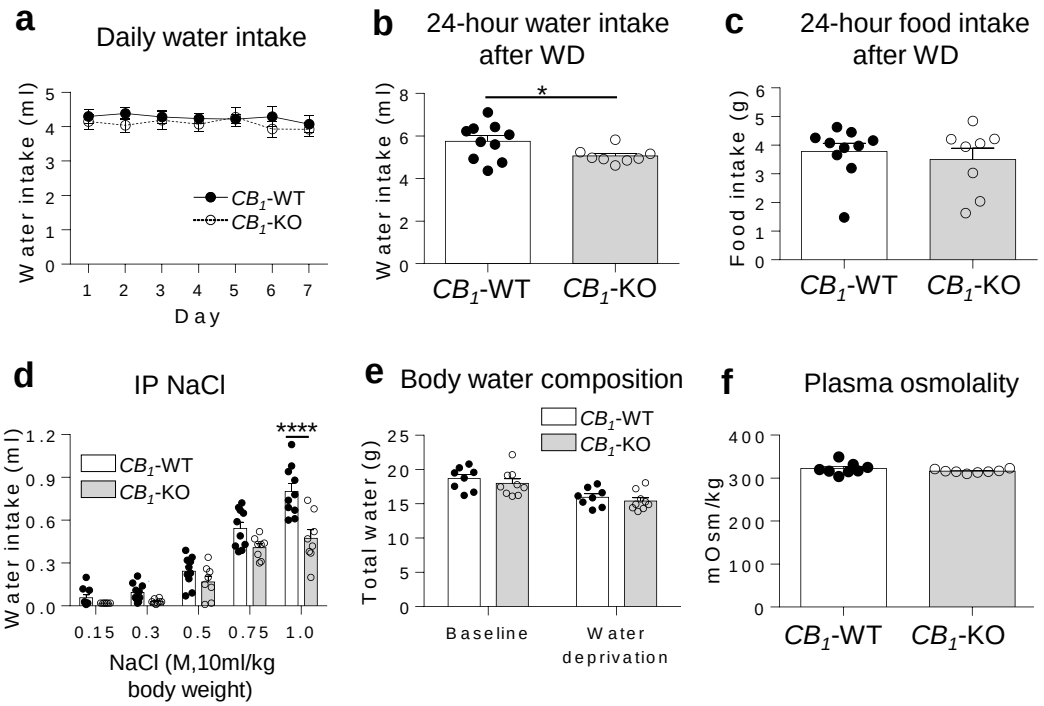
Immunohistochemistry and imaging. After the behavioral experiment, mice were anesthetized with pentobarbital (Exagon, 400 mg/kg body weight), transcardially perfused first with the phosphate-buffered solution (PBS, 0.1M, pH 7.4), then fixed by 4% formaldehyde. After brain extraction, serial brain coronal sections were cut at 40 μm and collected in PBS at room temperature (RT). Sections were permeabilized in a blocking solution of 4% donkey serum, 0.3% Triton X-100 and 0.02% sodium azide prepared in PBS for 1 hour at RT. For the CB_1 immunohistochemistry, free-floating

sections were incubated with goat CB_1 receptors polyclonal primary antibodies (CB_1-Go-Af450-1; 1:2000, Frontier Science Co. ShinKO-nishi, Ishikari, Hokkaido, Japan) for 48 hours at 4℃. The antibody was prepared in the blocking solution. After three washes, the sections were incubated with a secondary antibody anti-goat Alexa Fluor 555 (A21432, 1:500, Fisher Scientific) for 2 hours at RT and then washed in PBS at RT. For the HA immunohistochemistry, it is similar with the CB_1. Sections were incubated in anti-HA tag monoclonal antibody (1:1000, Fisher Scientific, 2-2.2.14) for 18 hours at 4℃ and in secondary antibody anti-mouse Alexa Fluor 488 (A21202, 1:500, Fisher Scientific) for 2 hours at RT. All sections were mounted, dried and cover slipped. The sections were analyzed with a Nanozoomer microscope (Hamamatsu, Japan) and Leica SP8 confocal microscope (Leica, Germany). Images were analyzed by Image J (NIH). For the mouse brain reconstruction, images were collected by Nanazoomer, Z-stack images were made by Image J, and the 3D reconstruction and videos were made by Imaris software (Imaris, Oxford instrument, UK).

Statistics. Data handling and statistical analysis were performed using Microsoft Excel and GraphPad Prism 6 software. For the dose-response experiments of ICV NaCl and ICV ANG, and body water composition data were statistically analyzed by two-way analysis of variance (ANOVA). For the water intake test with several time points, data were statistically analyzed by the two-way repeated measures ANOVA. The data of IP NaCl dose response and plasma osmolality were statistically analyzed by two-tailed Student's t-test. P values of ≤0.05 were considered statistically

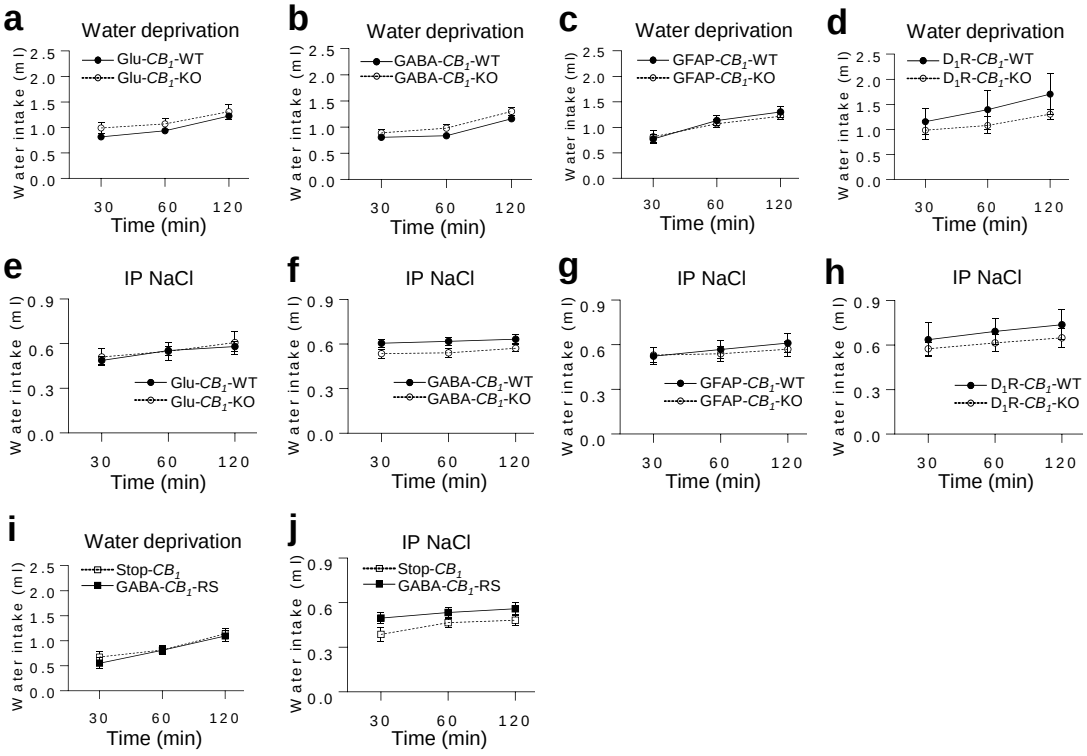
significant at a confidence interval of 95%. For detailed statistical analysis, see statistical tables (Supplementary tables 1-2).

Supplementary Figure 1



Supplementary figure 1. Decrease of stimulated water intake in *CB_1*-KO mice is independent of food intake, body water composition, and plasma osmolality. **a**, Daily water intake of *CB_1*-WT (Black circles, n=9) and *CB_1*-KO (Open circles, n=8). **b**, Water intake in 24 hours after 24-hour water deprivation in *CB_1*-WT (White, n=10) and *CB_1*-KO (Gray, n=8) mice. **c**, Food intake in 24 hours after 24-hour water deprivation in *CB_1*-WT (White, n=10) and *CB_1*-KO (Gray, n=8) mice. **d**, Water intake in 1 hour after IP NaCl, 10ml/kg body weight at different doses in *CB_1*-WT (White, n=10) and *CB_1*-KO (Gray, n=8) mice. **e**, Body water composition test in *CB_1*-WT (White, n=8) and *CB_1*-KO (Gray, n=9) mice. **f**, Blood plasma osmolality test in *CB_1*-WT (White, n=8) and *CB_1*-KO (Gray, n=8) mice. All data are shown as mean ± s.e.m. Data of IP NaCl dose-response and 24-hour water intake after water deprivation were statistically analyzed by two-tailed Student's t-test. *$P < 0.05$, **$P < 0.01$.

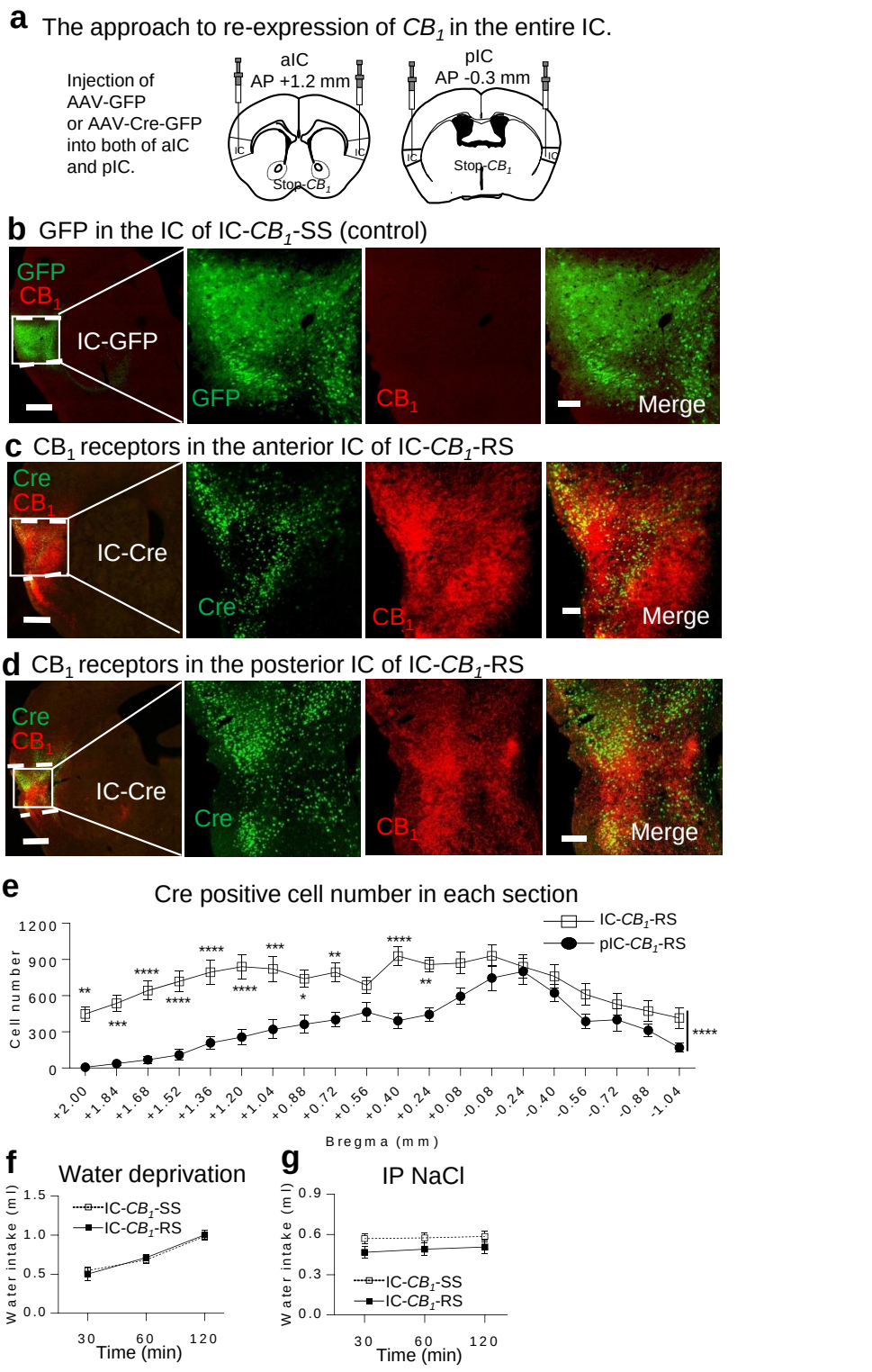
Supplementary Figure 2



Supplementary figure 2. Deletion or re-expression of CB_1 receptors in specific cell types did not affect water intake.

a-d, Cumulative water intake after 24-hour water deprivation in Glu-*CB_1*-WT (Black circles, n=18) and Glu-*CB_1*-KO (Open circles, n=11), GABA-*CB_1*-WT (Black circles, n=6) and GABA-*CB_1*-KO (Open circles, n=10), GFAP-*CB_1*-WT (Black circles, n=7) and GFAP-*CB_1*-KO(Open circles, n=11), D_1-*CB_1*-WT (Black circles, n=5) and D_1-*CB_1*-KO(Open circles, n=7). **e-h**, Cumulative water intake after IP 1M NaCl, 10ml/kg body weight in Glu-*CB_1*-WT (Black circles, n=18) and Glu-*CB_1*-KO (Open circles, n=11), GABA-*CB_1*-WT (Black circles, n=6) and GABA-*CB_1*-KO (Open circles, n=10), GFAP-*CB_1*-WT (Black circles, n=7) and GFAP-*CB_1*-KO (Open circles, n=11), D_1-*CB_1*-WT (Black circles, n=5) and D_1-*CB_1*-KO(Open circles, n=7). **i**, Cumulative water intake after 24-hour water deprivation in stop-*CB_1* (Open squares, n=8) and GABA-*CB_1*-RS (Black squares, n=8). **j**, Cumulative water intake after IP NaCl, 10ml/kg body weight in stop-*CB_1* (Open squares, n=10) and GABA-*CB_1*-RS (Black squares, n=8). All data are shown as mean ± s.e.m, and were statistically analyzed by the two-way repeated measurements ANOVA.

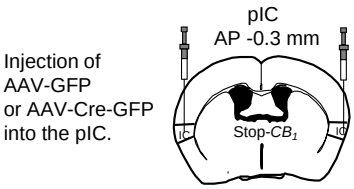
Supplementary Figure 3



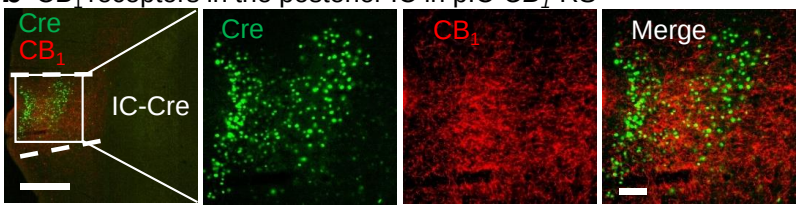
Supplementary figure 3. Re-expression of CB_1 in the entire IC did not affect stimulated water intake. **a**, Schematic representation of CB_1 rescue approach in the entire IC of Stop-*CB_1* mice. **b-d**, CB_1 (red) immunostaining in the IC of IC-*CB_1*-SS and IC-*CB_1*-RS, respectively. Scale bar, 500μm and 100 (Amplified images) μm. **e**, Cre positive cell number in sequential brain sections of the IC-*CB_1*-RS (Open squares, n=9) and pIC-*CB_1*-RS (Black circles, n=8; the pIC data in the supplementary Figure 4). **f-g**, Cumulative water intake of IC-*CB_1*-SS (Open squares) and IC-*CB_1*-RS (Black squares) mice after 24-hour water deprivation (IC-*CB_1*-SS n=8, IC-*CB_1*-RS n=8) or IP 1M NaCl, 10ml/kg body weight (IC-*CB_1*-SS n=9, IC-*CB_1*-RS n=9). All data are showed as mean ± s.e.m. The data of Cre positive cell number were statistically analyzed by the two-way ANOVA. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ ****$P < 0.0001$.

Supplementary Figure 4

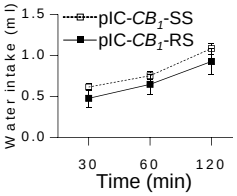
a The approach to re-expression of CB_1 in the pIC.



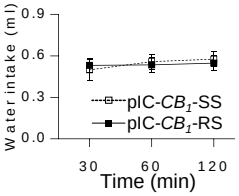
b CB_1 receptors in the posterior IC in pIC-*CB_1*-RS



c Water deprivation

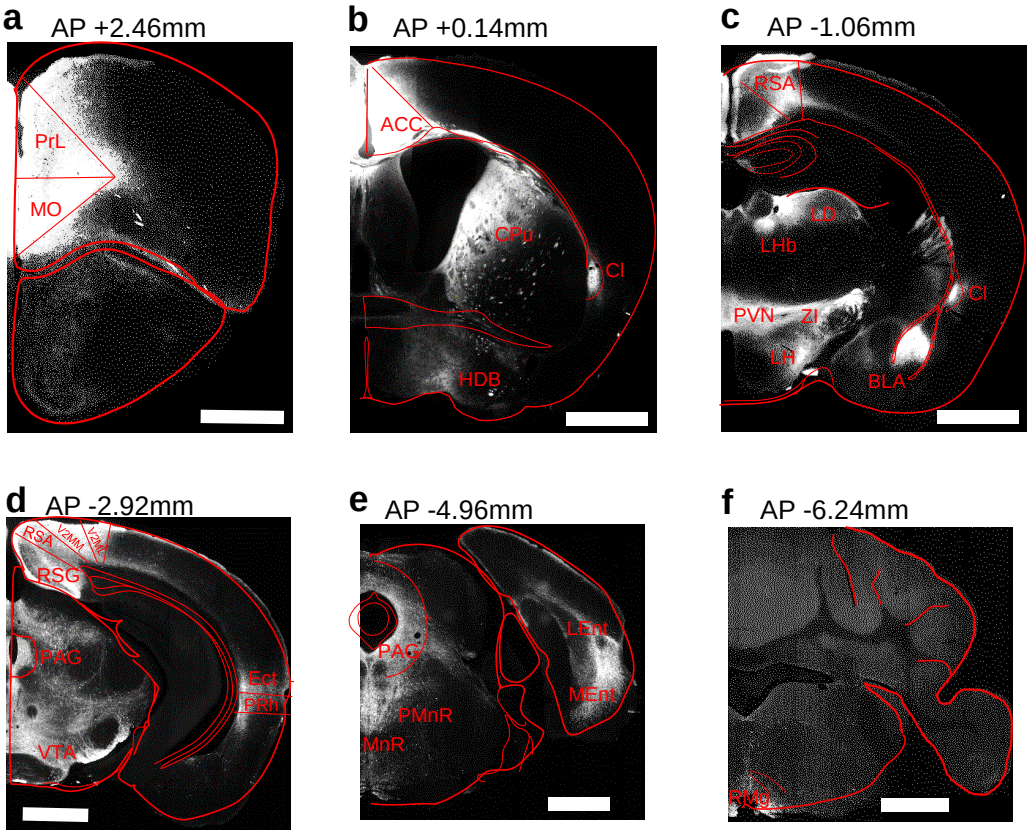


d IP NaCl



Supplementary figure 4. Re-expression of CB_1 in the posterior IC did not affect stimulated water intake. **a**, Schematic representation of CB_1 rescue approach in the posterior IC of Stop-*CB1* mice. **b**, CB_1 (red) immunostaining in the IC of pIC-*CB1*-RS. Scale bar, 500μm and 100 (Amplified images) μm. **c-d**, Cumulative water intake of pIC-*CB1*-SS (Open squares) and pIC-*CB1*-RS (Black squares) mice after 24-hour water deprivation (pIC-*CB1*-SS n=9, pIC-*CB1*-RS n=7) or IP 1M NaCl, 10ml/kg body weight (pIC-*CB1*-SS n=9, pIC-*CB1*-RS n=8). All data are showed as mean ± s.e.m, and were statistically analyzed by the two-way repeated measurements ANOVA.

Supplementary Figure 5



Supplementary figure 5. Brain-wide ACC neural projections revealed by injection of AAV-CaMKIIα-GFP into the ACC. **a,** Brain section at AP+2.46mm, PrL (Prelimbic cortex), MO (Medial obital cortex). **b,** Brain section at AP+0.14mm, CPu (Caudate putamen), CI (Claustrum), HDB (nucleus of the horizontal limb of the diagonal band). **c,** Brain section at AP-1.06mm, RSA (retrosplenial agranular cortex), LD (laterodorsal thalamic nucleus), LHb (Lateral habenula), PVN (paraventricular hypothalamic nucleus), ZI (zona incerta), LH (lateral hypothalamic area), BLA (basolateral amygdala). **d,** Brain section at AP-2.92mm, RSA (retrosplenial agranular cortex), RSG (retrosplenial granular cortex), V2MM (secondary visual cortex, mediomedial area), V2ML(secondary visual cortex, mediolateral area), PAG (periaqueductal gray), VTA (ventral tegmental area), Ect (ectorhinal cortex), PRh (perirhinal cortex). **e,** Brain section at AP-4.96mm, PAG (periaqueductal gray), MnR (median raphe nucleus), PMnR (paramedian raphe nucleus), LEnt (lateral entorhinal cortex), MEnt (medial entorhinal cortex). **f,** Brain section at AP-6.24mm, RMg (raphe magnus nucleus). Scale bar of a-f, 1 mm.

Supplementary table 1

Figure	Experiment, sample, size (n)	Analysis (post-hoc test)	Factors analyzed	F-ratios	P values
1a	Water deprivation *CB1*-WT (10) *CB1*-KO (8)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 32) = 2.254	P = 0.1213
				Time F (2, 32) = 18.59	P < 0.0001
				Genotype F (1, 16) = 19.49	P = 0.0004
1b	IP 1M NaCl 10ml/kg *CB1*-WT (10) *CB1*-KO (8)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 32) = 0.7755	P = 0.4689
				Time F (2, 32) = 111.1	P < 0.0001
				Genotype F (1, 16) = 16.02	P = 0.0010
1c	ICV NaCl *CB1*-WT (13) *CB1*-KO (10)	Two-way ANOVA	Genotype and dose	Interaction F (3, 84) = 2.776	P = 0.0463
				Dose F (3, 84) = 19.55	P < 0.0001
				Genotype F (1, 84) = 9.189	P = 0.0032
1d	ICV ANG *CB1*-WT (11) *CB1*-KO (13)	Two-way ANOVA	Genotype and dose	Interaction F (3, 88) = 3.292	P = 0.0243
				Dose F (3, 88) = 79.76	P < 0.0001
				Genotype F (1, 88) = 33.11	P < 0.0001
1e	Water deprivation C57BL/6 Vehicle (9) C57BL/6 Rimonabant 3mg/kg (10)	Two-way repeated mesures ANOVA	Drug and time	Interaction F (2, 34) = 6.461	P = 0.0042
				Time F (2, 34) = 53.81	P < 0.0001
				Drug F (1, 17) = 14.97	P = 0.0012
1f	IP 1.5M NaCl 10ml/kg C57BL/6 Vehicle (6) C57BL/6 Rimonabant 3mg/kg (7)	Two-way repeated mesures ANOVA	Drug and time	Interaction F (2, 22) = 0.9549	P = 0.4002
				Time F (2, 22) = 27.89	P < 0.0001
				Genotype F (1, 11) = 7.492	P = 0.0193
1g	Water deprivation Stop-*CB1*(9) *CB1*-RS (12)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 40) = 1.343	P = 0.2726
				Time F (2, 40) = 80.70	P < 0.0001
				Genotype F (1, 20) = 13.66	P = 0.0014
1h	IP 1M NaCl 10ml/kg Stop-*CB1*(10) *CB1*-RS (11)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 38) = 0.1556	P = 0.8564
				Time F (2, 38) = 14.88	P < 0.0001
				Genotype F (1, 19) = 4.395	P = 0.0497
1i	Water deprivation Glu-*CB1*-SS (11) Glu-*CB1*-RS (11)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 40) = 5.614	P = 0.0071
				Time F (2, 40) = 103.9	P < 0.0001
				Genotype F (1, 20) = 7.918	P = 0.0107
1j	IP 1M NaCl 10ml/kg Glu-*CB1*-SS (11) Glu-*CB1*-RS (11)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 40) = 2.098	P = 0.1360
				Time F (2, 40) = 14.26	P < 0.0001
				Genotype F (1, 20) = 9.155	P = 0.0067

1k	ICV NaCl Glu-CB_1-SS (13) Glu-CB_1-RS (11)	Two-way ANOVA	Genotype and dose	Interaction F (3, 84) = 3.132	P = 0.0298
				Dose F (3, 84) = 8.909	P < 0.0001
				Genotype F (1, 84) = 11.41	P = 0.0011
1l	ICV ANG Glu-CB_1-SS (15) Glu-CB_1-RS (13)	Two-way ANOVA	Genotype and dose	Interaction F (3, 104) = 1.026	P = 0.3845
				Dose F (3, 104) = 100.8	P < 0.0001
				Genotype F (1, 104) = 6.506	P = 0.0122
2d	Water deprivation ACC-CB_1-SS (17) ACC-CB_1-RS (20)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 70) = 3.598	P = 0.0325
				Time F (2, 70) = 182.5	P < 0.0001
				Genotype F (1, 35) = 20.47	P < 0.0001
2e	IP 1M NaCl 10ml/kg ACC-CB_1-SS (18) ACC-CB_1-RS (20)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 72) = 0.1684	P = 0.8454
				Time F (2, 72) = 26.92	P < 0.0001
				Genotype F (1, 36) = 4.432	P = 0.0423
3f	Water deprivation ACC-BLA-CB_1-SS (10) ACC-BLA-CB_1-RS (12)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 40) = 0.2143	P = 0.8080
				Time F (2, 40) = 59.32	P < 0.0001
				Genotype F (1, 20) = 7.133	P = 0.0147
3g	IP 1M NaCl 10ml/kg ACC-BLA-CB_1-SS (10) ACC-BLA-CB_1-RS (12)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 40) = 1.556	P = 0.2235
				Time F (2, 40) = 16.58	P < 0.0001
				Genotype F (1, 20) = 10.28	P = 0.0044

Supplementary table 1. Statistical details related to figures 1-3.

Supplementary table 2

Supplementary figure	Experiment, sample, size (n)	Analysis (post-hoc test)	Factors analyzed	F-ratios	P values
1a	Daily water intake *CB1*-WT (9) *CB1*-KO (8)	Two-way ANOVA	Genotype and day	Interaction F (6, 105) = 0.2138	P = 0.9717
				Days F (6, 105) = 0.3389	P = 0.9149
				Genotype F (1, 105) = 2.150	P = 0.1455
1b	Water intake in a day after water deprivation *CB1*-WT (10) *CB1*-KO (8)	Unpaired *t-test*			P = 0.0500
1c	Food intake in a day after water deprivation *CB1*-WT (10) *CB1*-KO (8)	Unpaired *t-test*			P = 0.5666
1d	IP NaCl dose response *CB1*-WT (10) *CB1*-KO (8)	Two-way ANOVA		Interaction F (4, 80) = 5.196	P = 0.0009
				Dose F (4, 80) = 102.6	P < 0.0001
				Genotype F (1, 80) = 30.26	P < 0.0001
1e	Body water composition *CB1*-WT (8) *CB1*-KO (9)	Two-way ANOVA		Interaction F (1, 30) = 0.01329	P = 0.9090
				Days F (1, 30) = 23.10	P < 0.0001
				Genotype F (1, 30) = 1.242	P = 0.2739
1f	Plasma osmolality *CB1*-WT (8) *CB1*-KO (8)	Unpaired t-test			P = 0.2496
2a	Water deprivation Glu-*CB1*-WT (18) Glu-*CB1*-KO (11)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 54) = 1.410	P = 0.2529
				Time F (2, 54) = 109.5	P < 0.0001
				Genotype F (1, 27) = 1.673	P = 0.2068
2b	Water deprivation GABA-*CB1*-WT (6) GABA-*CB1*-KO (10)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 28) = 0.7316	P = 0.4901
				Time F (2, 28) = 119.7	P < 0.0001
				Genotype F (1, 14) = 2.026	P = 0.1766
2c	Water deprivation GFAP-*CB1*-WT (7) GFAP-*CB1*-KO (11)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 32) = 0.8915	P = 0.4200
				Time F (2, 32) = 46.29	P < 0.0001
				Genotype F (1, 16) = 0.07548	P = 0.7870
2d	Water	Two-way	Genotype and	Interaction F (2, 20) = 0.6907	P = 0.5128

	deprivation D1R-*CB1*-WT (5) D1R-*CB1*-KO (7)	repeated mesures ANOVA	time	Time F (2, 20) = 9.770 Genotype F (1, 10) = 0.7693	P = 0.0011 P = 0.4010
2e	IP 1M NaCl 10ml/kg Glu-CB1-WT (18) Glu-CB1-KO (11)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 54) = 0.4799 Time F (2, 54) = 13.36 Genotype F (1, 27) = 0.06145	P = 0.6215 P < 0.0001 P = 0.8061
2f	IP 1M NaCl 10ml/kg GABA-*CB1*-WT (6) GABA-*CB1*-KO (10)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 28) = 0.6378 Time F (2, 28) = 10.47 Genotype F (1, 14) = 2.738	P = 0.5360 P = 0.0004 P = 0.1202
2g	IP 1M NaCl 10ml/kg GFAP-*CB1*-WT (7) GFAP-*CB1*-KO (11)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 32) = 3.281 Time F (2, 32) = 22.50 Genotype F (1, 16) = 0.06804	P = 0.0506 P < 0.0001 P = 0.7975
2h	IP 1M NaCl 10ml/kg D1R-*CB1*-WT (5) D1R-*CB1*-KO (7)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 20) = 0.1803 Time F (2, 20) = 7.256 Genotype F (1, 10) = 0.5358	P = 0.8364 P = 0.0043 P = 0.4810
2i	Water deprivation Stop-*CB1* (8) GABA-*CB1*-RS (8)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 28) = 0.8169 Time F (2, 28) = 56.11 Genotype F (1, 14) = 0.2042	P = 0.4520 P < 0.0001 P = 0.6583
2j	IP 1M NaCl 10ml/kg Stop-*CB1* (10) GABA-*CB1*-RS (8)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 32) = 1.099 Time F (2, 32) = 15.22 Genotype F (1, 16) = 2.626	P = 0.3454 P < 0.0001 P = 0.1246
3e	Cre positive cell number IC-*CB1*-RS (9) pIC-*CB1*-RS (8)	Two-way ANOV	Brain area	Interaction F (19, 300) = 2.726 Bregma F (19, 300) = 9.849 Brain area F (1, 300) = 211.7	P = 0.0002 P < 0.0001 P < 0.0001
3f	Water deprivation IC-*CB1*-SS (8) IC-*CB1*-RS (8)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 28) = 0.5276 Time F (2, 28) = 73.13 Genotype F (1, 14) = 4.499e-015	P = 0.5958 P < 0.0001 P > 0.9999
3g	IP 1M NaCl 10ml/kg IC-*CB1*-SS (9) IC-*CB1*-RS (9)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 32) = 2.137 Time F (2, 32) = 13.32 Genotype F (1, 16) = 2.095	P = 0.1346 P < 0.0001 P = 0.1671
4c	Water deprivation pIC-*CB1*-SS (9) pIC-*CB1*-RS (7)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 28) = 0.2249 Time F (2, 28) = 70.61 Genotype F (1, 14) = 1.216	P = 0.8000 P < 0.0001 P = 0.2888

4d	IP 1M NaCl 10ml/kg pIC-*CB*$_1$-SS (9) pIC-*CB*$_1$-RS (8)	Two-way repeated mesures ANOVA	Genotype and time	Interaction F (2, 45) = 0.1708	P = 0.8435
				Time F (2, 45) = 0.3459	P = 0.7095
				Genotype F (1, 45) = 0.02060	P = 0.8865

Supplementary table 2. Statistical details related to supplementary figures 1-4.

Supplementary video 1. Whole-brain mapping of ACC neural projections by injection of AAV-CaMKIIα-GFP into ACC.

Supplementary video 2. Whole-brain mapping of CB_1 receptors' distribution in ACC-*CB*$_1$-RS mice.

III.2 Insular cortex, cholinergic cells, and mitochondria: other potential localizations where CB_1 receptors might modulate water intake

Besides the circuit described in the previous chapter, I also focused my attention on another brain region, the insular cortex, which contains high levels of CB_1 mRNA and is involved in drinking behavior. The results showed that CB_1 receptor in this area is selectively required to promote water intake following changes in peripheral osmolarity and angiotensin II signaling.

Then, I asked whether CB_1 receptors present in cholinergic cells play a role in the control of water intake. Interestingly, the data obtained with conditional mutant mice lacking CB_1 receptor expression from cholinergic neurons, revealed that this subpopulation of receptors are selectively required to promote drinking only when the osmolality changes are induced at central levels.

Last but not least, I analyzed the role of a recently discovered subcellular pool of CB_1 receptors located at mitochondrial membranes ($mtCB_1$). Surprisingly, genetic exclusion of CB_1 from mitochondria reveals that the overall control of water intake by the ECS might partly involve this subcellular localization.

Overall, these sets of results further highlight the complexity of ECS signaling in the regulation of thirst and drinking behavior and will be part of future studies addressing the mechanistic details.

III.2.1 Control of water intake by the insular CB_1 receptors

The insular cortex (IC) is a portion of the cerebral neocortex. It is folded deep within the lateral sulcus in human and primate brains. It is divided into two parts, the anterior IC, which is part of the gustatory cortex and responsible for the perception of tastes, and the posterior IC, which is involved in recognition, learning and memory (Gogolla 2017). The IC plays diverse roles including the control of emotions, body homeostasis, perception, motor control, self-awareness, cognition, and visceral functions (Gogolla 2017). Recently the IC has been identified as a crucial area for the control of drinking behavior. Optogenetic stimulation of the anterior IC promotes licking water, whereas optogenetic stimulation of the posterior IC suppresses licking water (Schiff et al 2018, Wang et al 2018). These results indicate that the two parts of IC likely play opposite roles in the control of drinking behavior.

The G protein-coupled cannabinoid CB_1 receptors are mainly located on the presynaptic membrane. CB_1 mRNA is highly expressed in the IC, including both the anterior and posterior portions. Pharmacological stimulation of CB_1 receptors within this brain area increased the hedonic valence of tastes (Kobilo et al 2007), suggesting that CB_1 receptors in the IC may modulate ingestive behaviors, including feeding and drinking. Since CB_1 receptors are essential for the regulation of drinking behavior, I investigated the role of IC CB_1 receptors in the control of water intake.

To study the role of the IC CB_1 receptors in the control of water intake, I used a viral approach to remove the *CB_1* gene in the IC by injection of an adeno-associated virus expressing the Cre recombinase into the IC of *CB_1*-Flox mice to generate IC-*CB_1*-KO mutants (**Figure 12 A**). Combined fluorescent in situ hybridization (FISH)/immunohistochemistry (IHC) to respectively reveal CB_1 mRNA and Cre recombinase, showed that the IC *CB_1* gene was deleted in the IC-*CB_1*-KO mice, but not in the control mice (**Figure 12 B-D**). I first checked body weight and daily water intake of IC-*CB_1*-KO compared to their WT controls. *CB_1* deletion in this region did not impact these parameters (**Figure 13**), thereby excluding a role of endocannabinoid signaling in the IC for the control of drinking in basal conditions. Then, I tested the mutant mice in the different behavioral paradigms to generate thirst and elicit water intake, including

24-hour water deprivation, systemic administration of sodium chloride (NaCl), and intracerebroventricular (ICV) administration of NaCl or angiotensin II (Ang II).

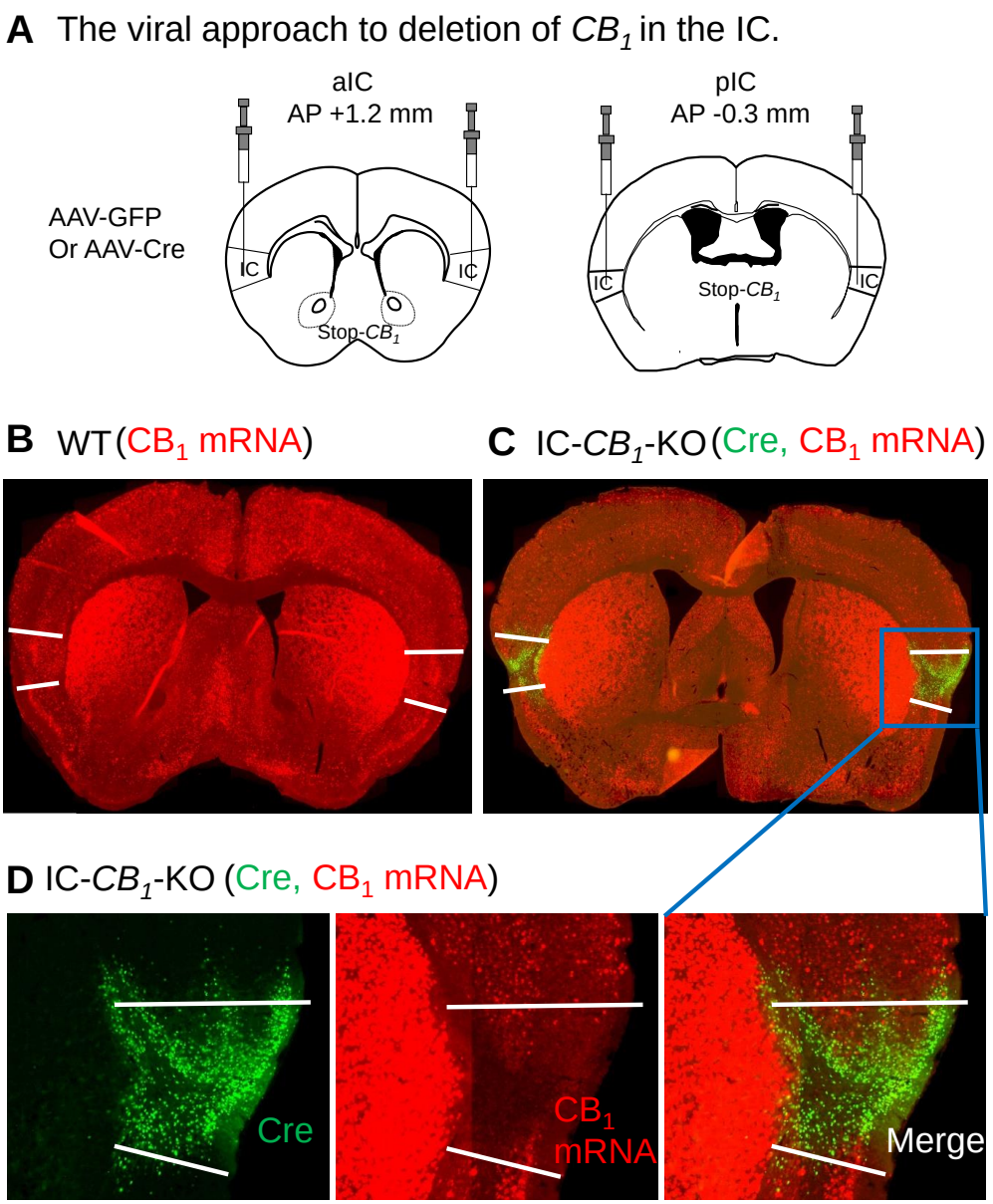


Figure 12. The viral approach to deletion of the IC CB_1 receptors. **A.** diagrammatic presentation of the viral approach. **B.** CB_1 mRNA in the IC of the control mouse. **C** and **D.** Deletion of CB_1 mRNA in the IC.

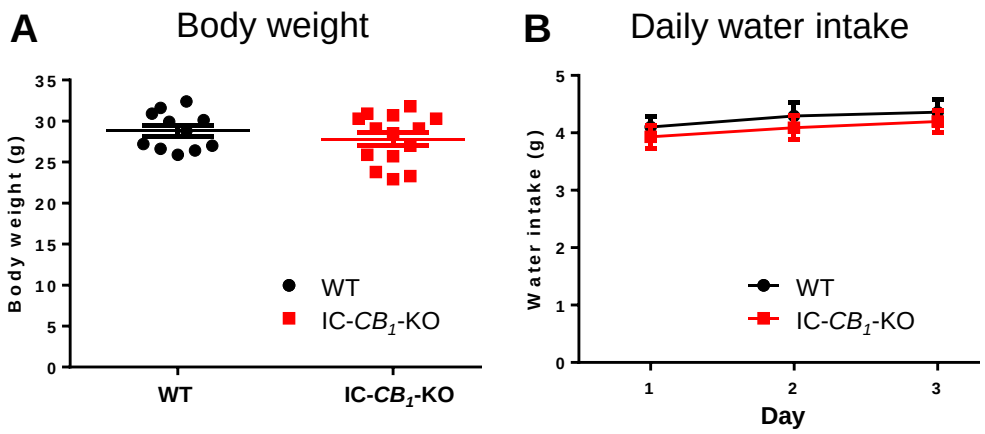


Figure 13. Body weight and daily water intake in the IC-*CB*$_1$-KO and the control mice. **A.** Body weight at the beginning of light phase (WT n=11, IC-*CB*$_1$-KO n=14). **B.** Daily water intake is detected in the first 3 days before the dehydration treatment (WT n=11, IC-*CB*$_1$-KO n=14).

As expected, water deprivation led to a decrease in body weight and increased water consumption (**Figure 14 A,B**). Surprisingly, IC-*CB*$_1$-KO mice did not show any differences in water or food intake (**Figure 14 B-E**), suggesting that CB_1 receptor in this brain area is dispensable for dehydration-induced drinking or eating. Long-term water restriction produces many complicated changes in the body, such as the induction of both intracellular and extracellular dehydration, and associated physiological alterations in blood osmolality, hormone and neurotransmitter levels, and mental state. Therefore, it is still possible that IC CB_1 receptors might differentially and/or oppositely impact on these diverse processes. To start addressing these issues, I decided to use other approaches to induce drinking, such as the systemic or central injection of hypertonic NaCl in order to induce specific intracellular dehydration and associated drinking behavior or the ICV infusion of Ang II, able to stimulate drinking in the absence of any dehydration.

The systemic administration of hypertonic NaCl produced a lower water intake in IC-*CB*$_1$-KO mice than in control mice, without any difference in food intake (**Figure 15 A,B**). However, in the condition of ICV hypertonic NaCl injection, IC-*CB*$_1$-KO mice did not show a significant difference in water intake comparing to the control mice (**Figure**

15 C). Furthermore, IC-*CB*$_1$-KO mice drank less water than control mice after the ICV injection of Ang II (**Figure 15 D**).

Thus CB1 receptor in the insular cortex is involved in promoting water intake at two different levels, such as response to peripheral (but not central) osmolality changes and brain angiotensin signaling.

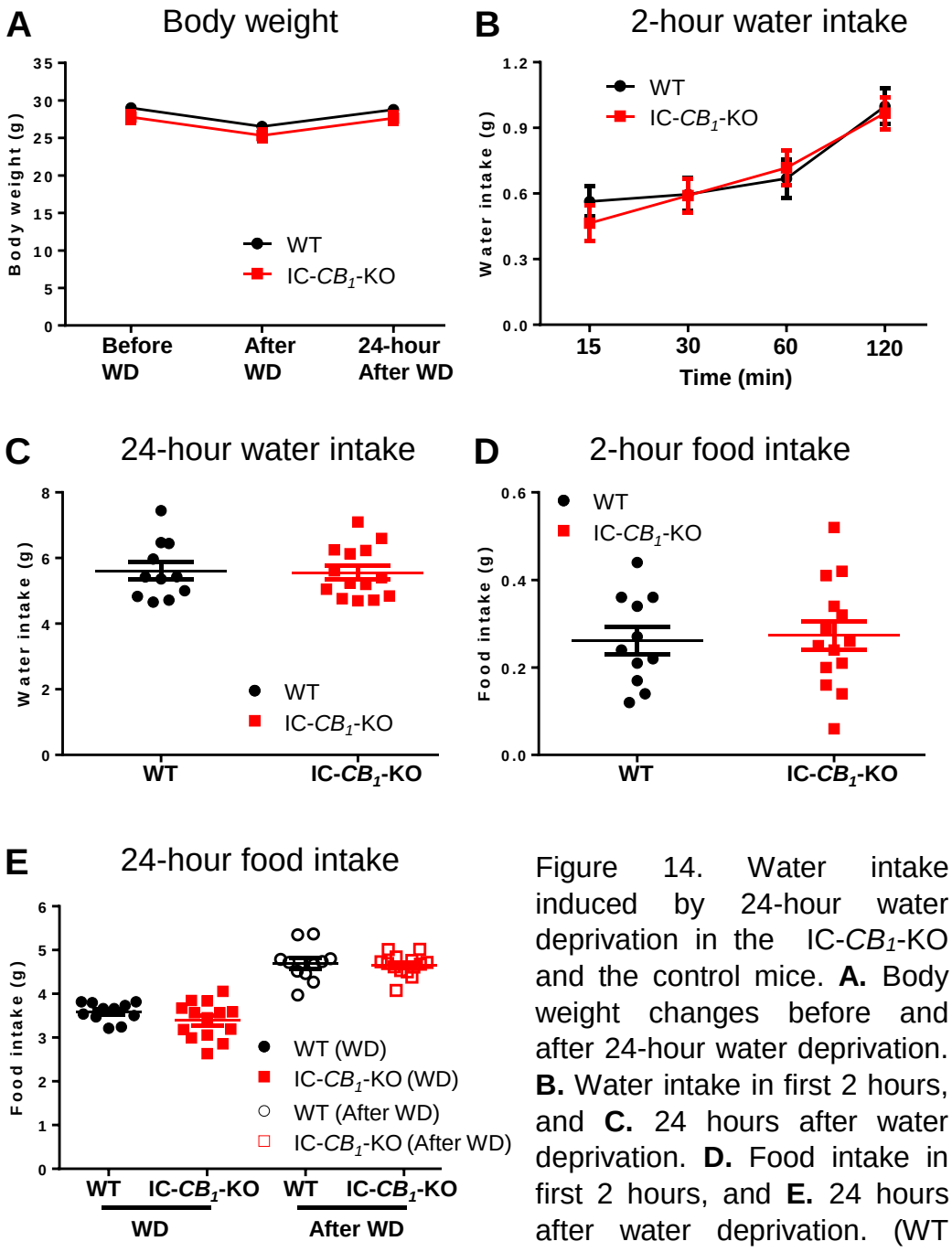


Figure 14. Water intake induced by 24-hour water deprivation in the IC-*CB*$_1$-KO and the control mice. **A.** Body weight changes before and after 24-hour water deprivation. **B.** Water intake in first 2 hours, and **C.** 24 hours after water deprivation. **D.** Food intake in first 2 hours, and **E.** 24 hours after water deprivation. (WT n=11, IC-*CB*$_1$-KO n=14)

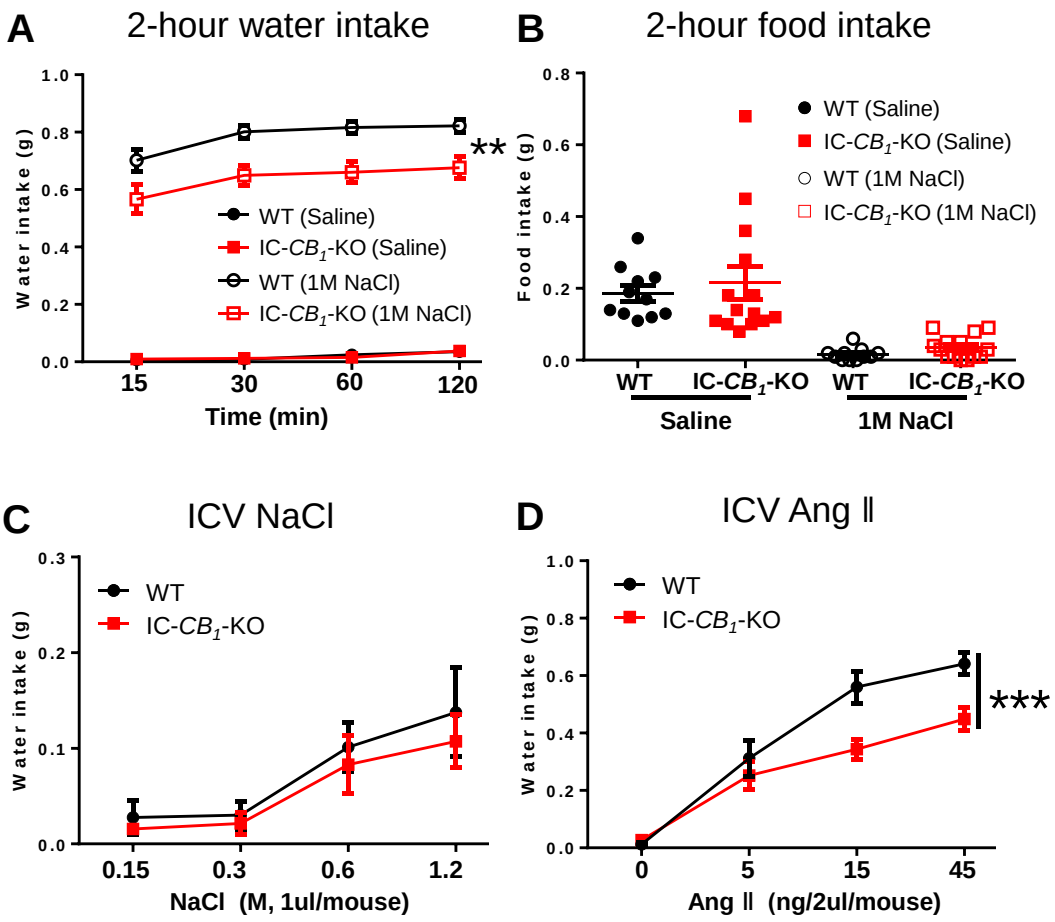


Figure 15. Water intake induced by hypertonic NaCl and Ang II in the IC-*CB*$_1$-KO and the control mice. **A** and **B.** Physiological saline and 1M NaCl (10 ml/kg) induced water and food intake (WT n=11, IC-*CB*$_1$-KO n=14). **C.** ICV NaCl injection-induced water intake (WT n=8, IC-*CB*$_1$-KO n=7). **D.** ICV Ang II injection-induced water intake (WT n=6, IC-*CB*$_1$-KO n=10). (** presents p<0.01, *** presents p<0.001, the data were analized by two way ANOVA)

Methods

Please see the methods in III.1.

Combined fluorescent in situ hybridization (FISH)/immunohistochemistry (IHC) on free-floating frozen sections.

Mice were sacrificed by cervical dislocation. Their brains were extracted, frozen on dry ice and stored at 80 ℃ until sectioning in a cryostat (14 mm, MicromHM500M,

Microm Microtech). Digoxigenin (DIG)-labeled riboprobes against mouse CB_1 receptor. After hybridization overnight at 60℃ with the mixture of probes, the slides were washed with different stringency wash buffers at 65℃. Then, the slides were blocked with a blocking buffer prepared according to the manufacturer's protocol. Anti-DIG antibody conjugated to horseradish peroxidase (HRP) (Roche; 1:2000) were applied 2 hours at RT or overnight at 4℃ to detect or CB_1-DIG probe. Probe hybridization was revealed by a tyramide signal amplification (TSA) reaction using Cyanine 3-labeled tyramide (Perkin Elmer; 1:80 for 12 minutes) to amplify the signal of CB_1. After FISH for detection of CB_1 signal, immunohistochemistry (IHC) was applied to reveal green fluorescent protein (GFP) signal. Anti-green fluorescent protein (GFP) antibody was used here. After FISH and IHC, the slides were incubated in 4',6-diamidino-2-phenylindole (DAPI; 1:20 000; FISHER Scientific) before being washed, coverslipped and visualized with an epifluorescence Nanozoomer microscope.

III.2.2 CB_1 receptors in cholinergic cells for the control of water intake

The cholinergic system comprises the neurotransmitter acetylcholine (ACh) and acetylcholine receptors. It is distributed in the central and peripheral nervous system and modulates a variety of functions, including cognition, addiction, learning and memory, muscle contraction, etc (Everitt & Robbins 1997, Houser et al 1985, Sarter & Parikh 2005). However, how the cholinergic system control water intake is still a matter of debate (Routtenberg & Simpson 1971, Simpson & Routtenberg 1972, Stein & Seifter 1962, Myers & Sharpe 1968). CB_1 receptors are mainly presynaptic receptors and exert an inhibition in neurotransmission in glutamatergic and GABAergic terminals, as well as many other neurotransmitters, including cholinergic neurons (Gifford & Ashby 1996).

Here, I focused on the role of CB_1 receptors in cholinergic cells for the control of water intake. I used ChAT-*CB_1*-KO mice carrying a specific deletion of CB_1 receptors in cholinergic neurons previously developed and characterized in the lab (unpublished). ChAT-*CB_1*-KO mice did not show significant differences in body weight and daily water intake as compared to their WT littermates (**Figure 16**), suggesting that drinking behavior in basal conditions does not require cholinergic CB_1 receptors. I then tried the previously mentioned different approaches to generate thirst and eliciting water intake, including 24-hour water deprivation, systemic sodium chloride (NaCl) application, and intracerebroventricular (ICV) administration of NaCl or angiotensin II (Ang II). ChAT-*CB_1*-KO and control mice consumed the same amount of food and water after 24h water deprivation (**Figure 17**) as well as after systemic hypertonic NaCl treatment (**Figure 18 A,B**). Interestingly, when thirst was induced by ICV hypertonic NaCl injection, ChAT-*CB_1*-KO mice significantly drunk more water than the control mice (**Figure 18 C**). This phenotype was not observed when dinking was induced by ICV injection of Ang II (**Figure 18 D**).

Altogether, these data reveal that the CB_1 receptors in cholinergic cells play a specific role in the control of water intake elicited by central hypertonic NaCl application, differentially from insular cortex neurons where the same receptor promotes drinking in response to peripheral osmolality changes and angiotensin signaling.

Methods

Please see the methods in III.1.

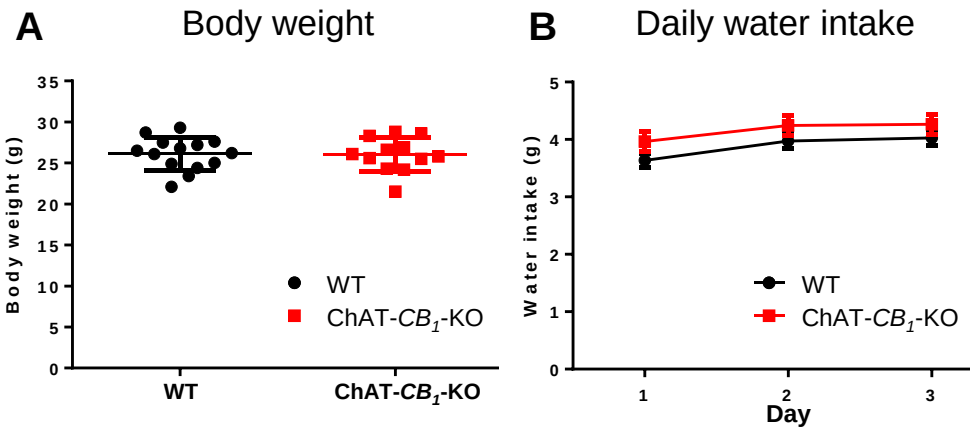


Figure 16. Body weight and daily water intake in the ChAT-*CB*$_1$-KO and the control mice. **A.** Body weight at the beginning of light phase (WT n=14, ChAT-*CB*$_1$-KO n=12). **B.** Daily water intake is detected in the first 3 days before the dehydration treatment (WT n=14, ChAT-*CB*$_1$-KO n=12).

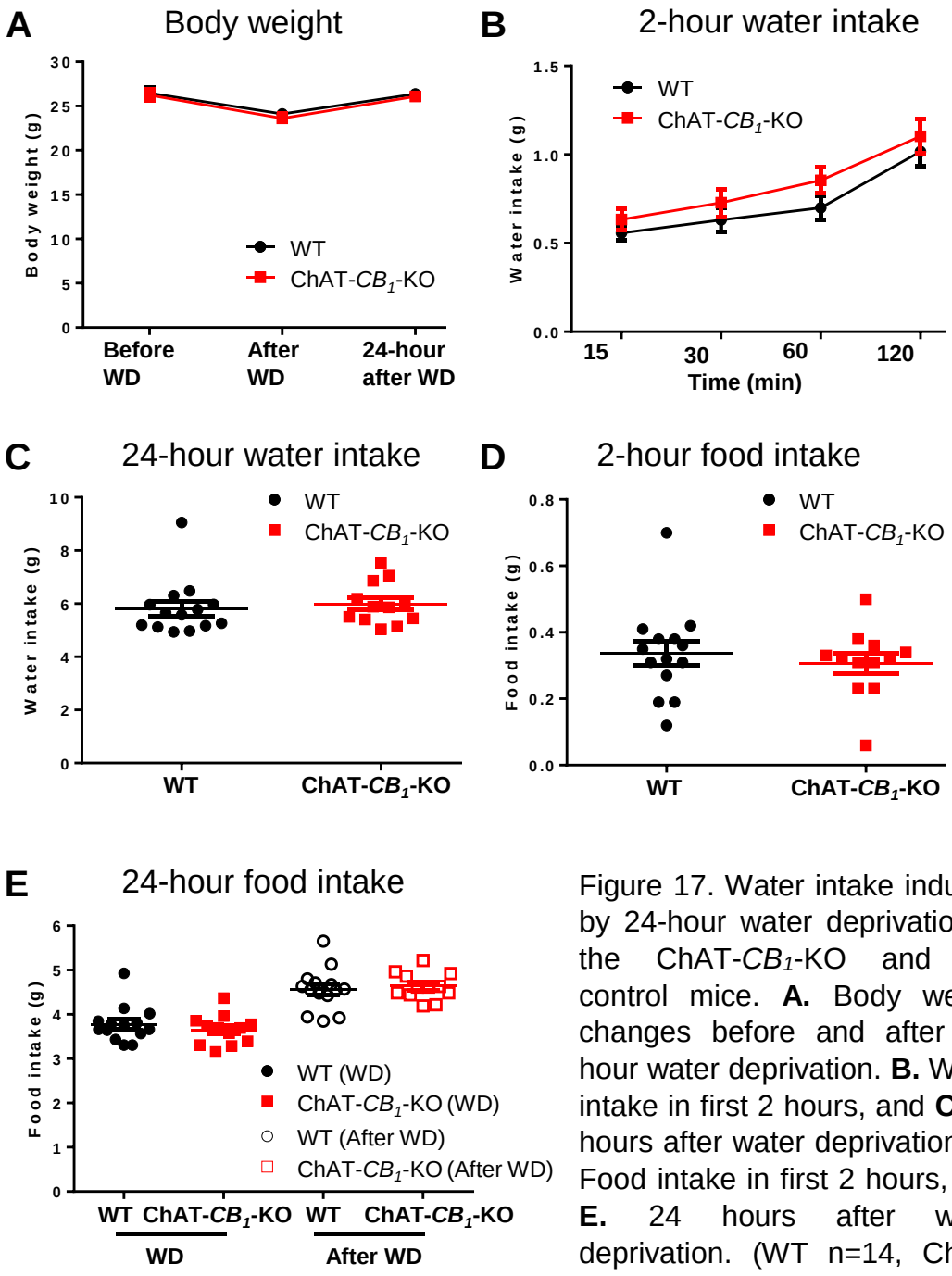


Figure 17. Water intake induced by 24-hour water deprivation in the ChAT-*CB1*-KO and the control mice. **A.** Body weight changes before and after 24-hour water deprivation. **B.** Water intake in first 2 hours, and **C.** 24 hours after water deprivation. **D.** Food intake in first 2 hours, and **E.** 24 hours after water deprivation. (WT n=14, ChAT-*CB1*-KO n=12)

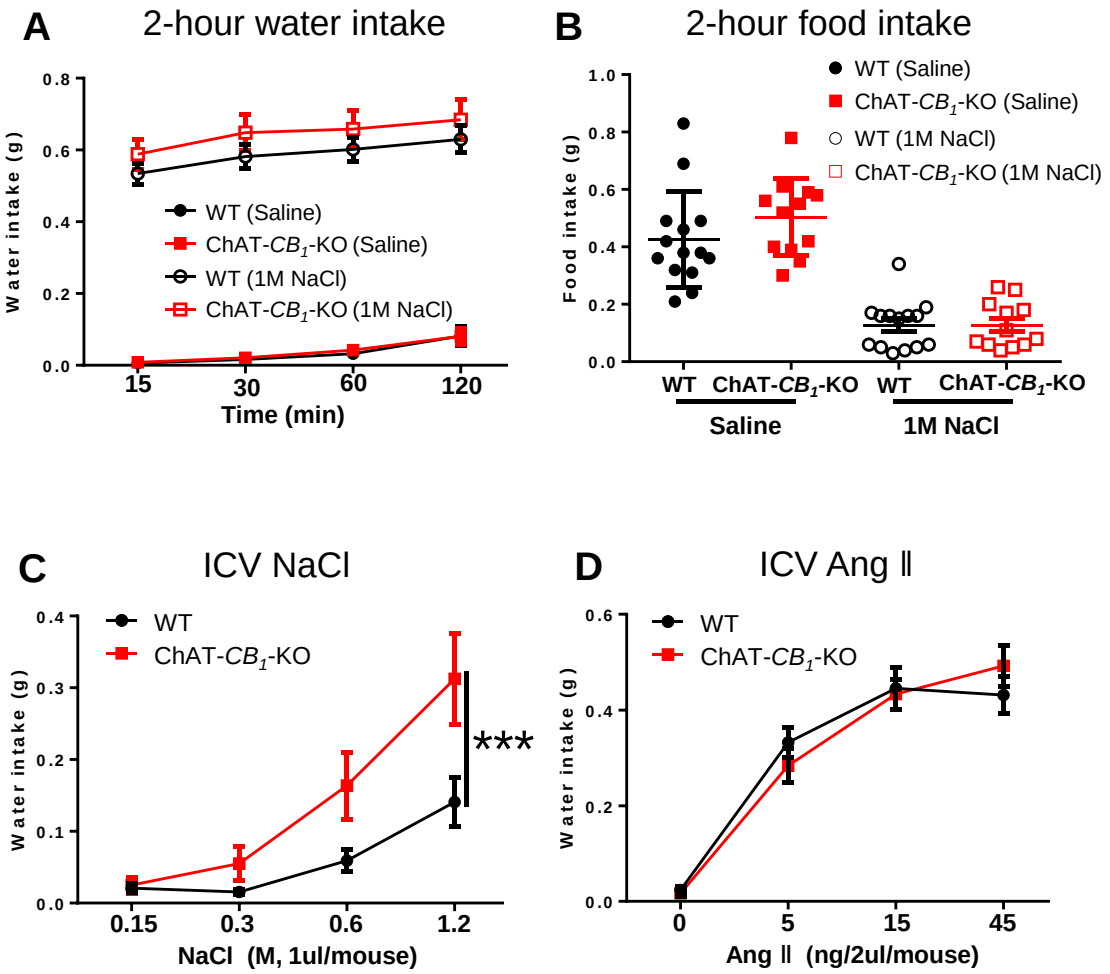


Figure 18. Water intake induced by hypertonic NaCl and Ang II in the ChAT-*CB*$_1$-KO and the control mice. **A** and **B.** Physiological saline and 1M NaCl (10 ml/kg) induced water and food intake (WT n=14, ChAT-*CB*$_1$-KO n=12). **C.** ICV NaCl injection-induced water intake (WT n=11, ChAT-*CB*$_1$-KO n=8). **D.** ICV Ang II injection-induced water intake (WT n=16, ChAT-*CB*$_1$-KO n=13). (*** presents $p<0.001$, the data were analized by two way ANOVA)

III.2.3 Mitochondrial CB_1 receptors for the control of water intake

Besides many other functions, mitochondria are essential organelles for generating most of the cell's supply of adenosine triphosphate (ATP), which is used as a source of chemical energy (Campbell et al 2006). Water is known to be a product resulting from oxygen consumption by these organelles and mitochondrial diseases are characterized by excessive thirst among other clinical signs (Johannsen & Ravussin 2009). However little is known about a direct role of brain mitochondrial metabolism on drinking behavior. Recent studies have shown that CB_1 receptors are closely associated with mitochondrial membranes ($mtCB_1$) (Benard et al 2012). Stimulation of CB_1 receptors regulates mitochondrial energetics, endocannabinoid-dependent short-term synaptic plasticity in the hippocampus, and memory (Benard et al 2012, Hebert-Chatelain et al 2016). Since CB_1 receptors are required to promote water intake, I hypothesized that $mtCB_1$ might also contribute to this process.

To study the role of $mtCB_1$ receptors in the control of water, I used DN22-*CB_1* knock-in mice (DN22-*CB_1*-KI), generated and previously characterized in the lab (unpublished). This mouse strain carries a mutant form of CB_1 receptor (deletion of the first 22 amino acids, DN22-CB_1), which is still present and functional at the plasma membrane, but lacks mitochondrial localization and related effects (Hebert-Chatelain et al 2016). First, I checked body weight and daily water intake. DN22-*CB_1*-KI mice did not show significant differences in body weight as compared to the control mice but, interestingly, DN22-*CB_1* mice drunk less water in basal conditions (**Figure 19**). I then tried the different approaches to generate thirst and elicit water intake, including 24-hour water deprivation and systemic hypertonic sodium chloride (NaCl) application. After water deprivation, DN22-*CB_1*-KI mice showed a strong tendency to drink less water than their WT littermates (although not statistically significant) (**Figure 20**). Strikingly, when thirst was induced by systemic hypertonic NaCl administration, DN22-*CB_1*-KI mice drunk less water when compared to their WT littermates (**Figure 21**).

Altogether, these results point that the water intake promoted by the endocannabinoid system in dehydrated condition, might have also a direct mitochondrial component which will be worth to further investigate.

Methods

Please see the methods in III.1.

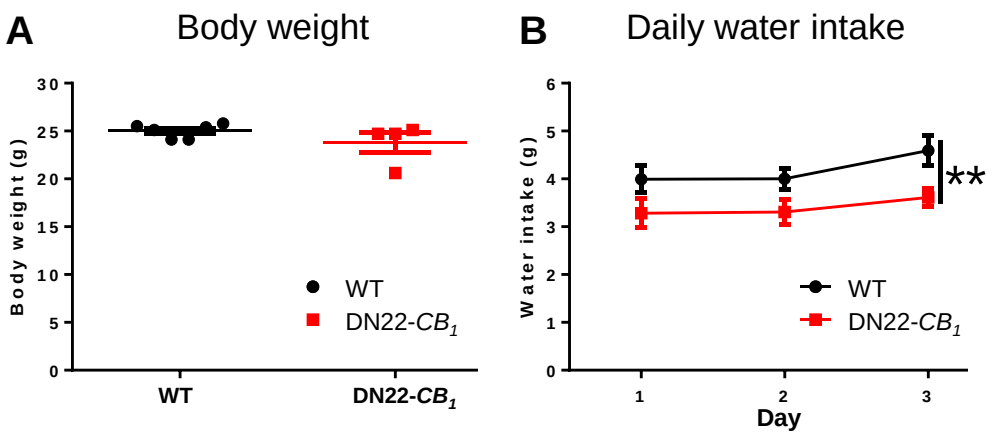


Figure 19. Body weight and daily water intake in the DN22-*CB*$_1$ and the control mice. **A.** Body weight at the beginning of light phase (WT n=6, DN22-*CB*$_1$=4). **B.** Daily water intake is detected in the first 3 days before the dehydration treatment(WT n=6, DN22-*CB*$_1$=4). (** presents $p<0.01$, the data were analized by two way ANOVA)

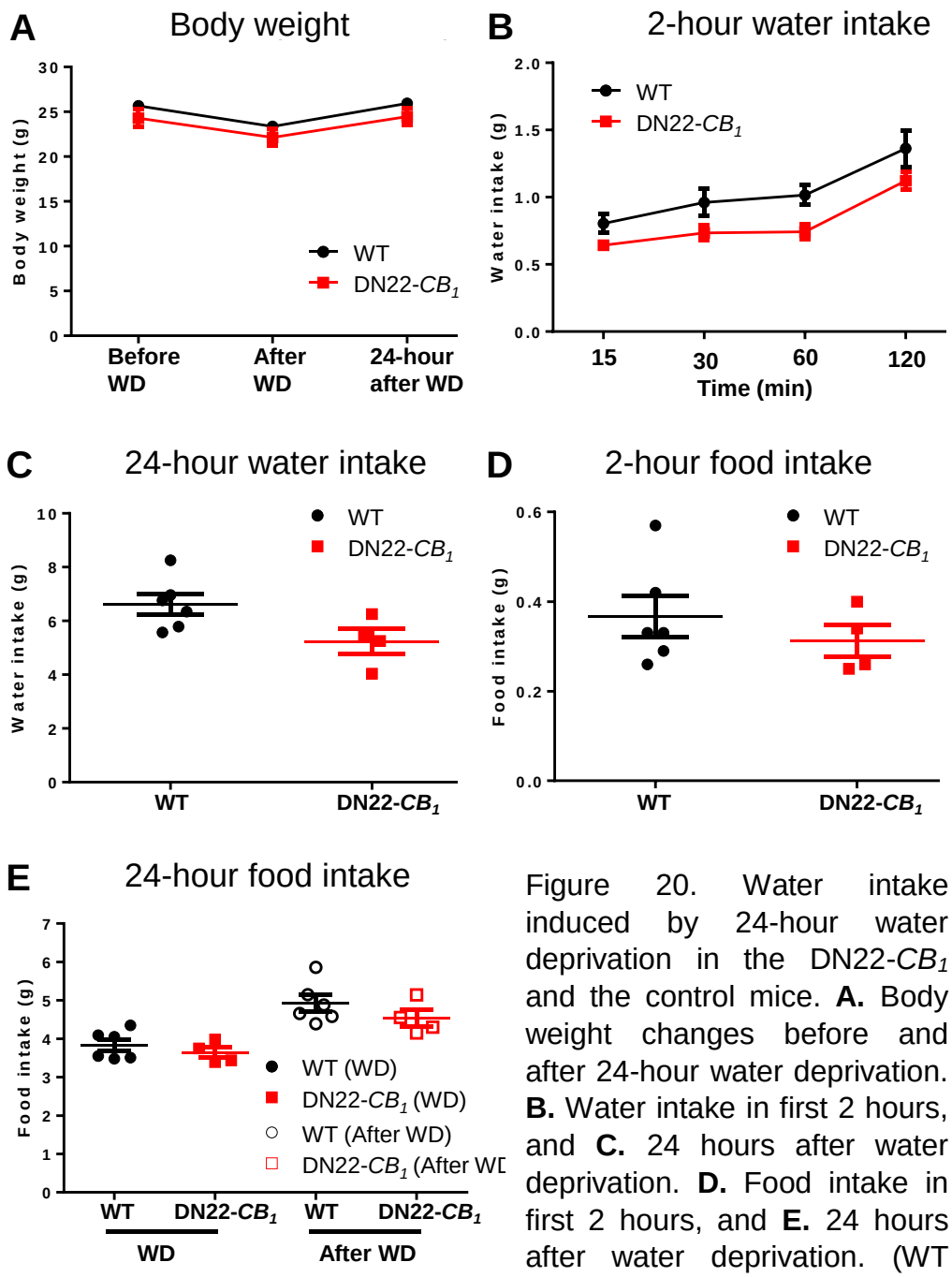


Figure 20. Water intake induced by 24-hour water deprivation in the DN22-*CB*$_1$ and the control mice. **A.** Body weight changes before and after 24-hour water deprivation. **B.** Water intake in first 2 hours, and **C.** 24 hours after water deprivation. **D.** Food intake in first 2 hours, and **E.** 24 hours after water deprivation. (WT n=6, DN22-*CB*$_1$=4)

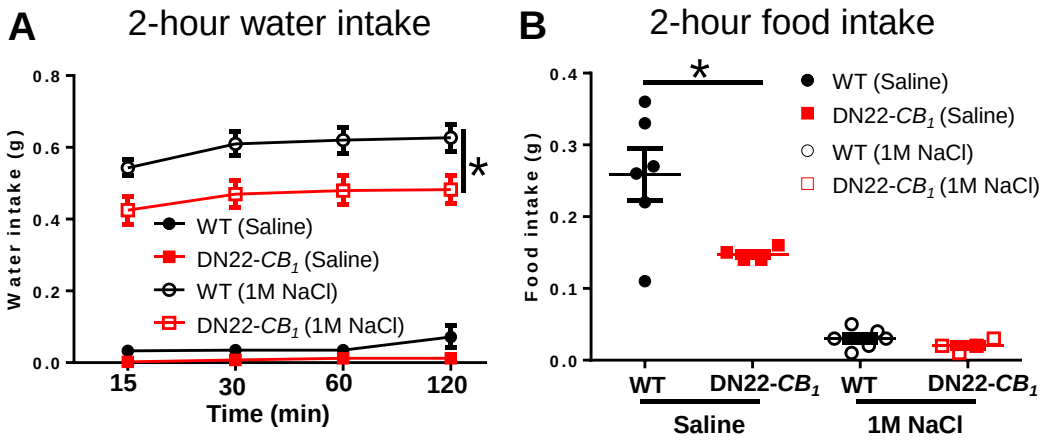


Figure 21. Water intake induced by hypertonic NaCl and Ang II in the DN22-*CB*$_1$ and the control mice. **A** and **B.** Physiological saline and 1M NaCl (10 ml/kg) induced water and food intake. (WT n=6, DN22-*CB*$_1$=4. Statistical analysis of Figure 21 A and B were applied two way ANOVA, ∗presents $p<0.05$.)

CHAPTER IV

Discussion

Thirst is a fundamental perception driving animals arising upon body dehydration to ingest water and maintain body fluid homeostasis. The physiological substrates underlying this process and its regulation are only partially known. Early studies pointed out that the brain receives interoceptive signals from the periphery, such as an increase in osmolality and dehydration-related hormones, to regulate drinking behavior. After decades of work on thirst in the brain, a hierarchical neural system involved in the control of thirst and drinking is appearing. It is comprised of sensory nuclei, effector/relay nuclei, and cortical nuclei. The sensory and effector/relay nuclei have been extensively investigated, but the cortical nuclei are not well characterized (Gizowski & Bourque 2018, Ichiki et al 2019, Zimmerman et al 2017).

CB_1 receptors are abundantly present in the brain, and regulate a wide variety of functions, such as learning, memory, feeding and energy balance (Busquets-Garcia et al 2018, Kano et al 2009, Piazza et al 2017). The role of CB_1 receptors in the control of drinking behavior is still a matter of debate, since pharmacological manipulations of the receptors have provided contradictory results in different species, conditions, and dosage of drugs (Abel 1975, Drewnowski & Grinker 1978, Higgs et al 2003, Ruginsk et al 2015, Verty et al 2004). However, it is clear that the endocannabinoid system (ECS) contributes to the regulation of water intake. As CB_1 receptors are distributed in different cell types and brain structures, we hypothesized that CB_1 receptors in distinct locations play different roles in the control of drinking behavior. In this Thesis work, I have contributed elucidating the roles of CB_1 receptors in specific cell types, brain structures, neural circuits, and subcellular organelles in the regulation of water intake.

IV.1 Role of CB_1 receptors in the control of water intake

Neural mechanisms underlying CB_1 receptor-dependent control of water intake were mostly unknown. Many brain regions eliciting drinking behavior have an intense expression of CB_1 receptors. Furthermore, pharmacological stimulation or inactivation of CB_1 receptors influence drinking behavior, but the results were inconsistent because of the application of different doses of drugs in distinct species and under different experimental conditions (Abel 1975, Drewnowski & Grinker 1978, Higgs et al 2003, Ruginsk et al 2015, Verty et al 2004). In this project, I found that the global deletion of

CB_1 receptors decreases water intake in response to 24-hour water deprivation, hypertonic sodium chloride (NaCl), and angiotensin II (Ang II) administrations. In order to make sure that these effects are not due to the long-term lack of CB_1 receptors, I systemically blocked CB_1 receptors by rimonabant, which produced a reduction in water intake in WT mice, comparable to the one observed in *CB_1*-KO mice. In parallel, global re-expression of CB_1 receptors in stop-*CB_1* mice produced an increase in drinking behavior as compared to the control mice. The data reveals that CB_1 receptors promote water intake elicited by 24-hour water deprivation, hypertonic NaCl, and Ang II in mice.

IV.2 Role of CB_1 receptors in specific cell types in the control of water intake

Excitatory and inhibitory neurons of the *lamina terminalis* play opposite roles in the control of water intake. Optogenetic stimulation of glutamatergic neurons in the SFO and OVLT/MnPO elicits voracious water intake (Abbott et al 2016, Betley et al 2015, Nation et al 2016, Oka et al 2015, Zimmerman et al 2016), whereas activation of GABAergic neurons in the SFO and MnPO suppresses water intake in thirsty mice (Abbott et al 2016, Oka et al 2015). In addition, the deletion of CB_1 receptors in the cortical glutamatergic neurons decreased food intake, whereas the deletion of CB_1 receptors in the forebrain GABAergic neurons resulted in a hyperphagic phenotype (Bellocchio et al 2010). Therefore, we hypothesized that the CB_1 receptors in the cortical glutamatergic and forebrain GABAergic neurons exert different functions in the regulation of drinking behavior. Surprisingly, we did not see any differences in water consumption in Glu-*CB_1*-KO and GABA-*CB_1*-KO mice as compared to their WT littermates.

Astrocytes exert many functions in the brain, such as the provision of nutrients to nervous tissue, maintenance of extracellular ion balance, and modulation of synaptic transmission (Han et al 2013, Piet et al 2004, Santello et al 2019). Astrocytes influence neuronal activity through the so-called tripartite synapse, which is composed of pre- and post-synaptic neuronal components and the surrounding astrocyte processes. Shimizu and his colleagues found that glial Na_x channels control lactate signaling to neurons for brain Na^+ sensing (Shimizu et al 2007). Furthermore, a recent study showed that local injection of a reversible glial inhibitor into the lateral ventricle significantly decreases salt

appetite in rats, but not water intake (Flor et al 2018). Optogenetic stimulation of astrocytes in the SFO did not impact water intake (Oka et al 2015). Therefore, these results reveal that astrocytes play an important role in the control of salt appetite, but not water intake. Since astrocytes regulate synaptic transmission by the tripartite synapse, we still hypothesized that astrocytes might play a role in the control of drinking behavior in specific conditions, and astrocytic CB_1 receptors are involved in the process. Here, we used GFAP-*CB_1*-KO mice, and found that specific deletion of CB_1 receptors in astrocyte did not show significant differences compared to the control mice.

Dopamine plays important roles in the brain as a neurotransmitter, such as reward, motivation and drinking behavior (Volkow et al 2017). Destruction of the monoaminergic system by intracerebroventricular 6-OHDA inhibits angiotensin II-induced water intake (Fitzsimons & Setler 1971, Sumners et al 1981), whereas intracranial injection of dopamine causes drinking behavior in rats (Poat et al 1980). Dopamine D_1 receptor is the most abundant dopamine receptor in the central nervous system (Paul et al 1992) and contributes to the modulation of motivation and reward (Robbins & Everitt 1996). Thus we asked whether CB_1 receptor in D_1 positive cells might control water intake. To answer this question we used D_1-*CB_1*-KO mice. However, D_1-*CB_1*-KO mice did not show any significant difference in water intake compared to their WT littermates ruling out a possible role of CB_1 receptor in dopaminoceptive cells in the control of drinking behavior.

The cholinergic system comprises of the neurotransmitter acetylcholine (ACh) and acetylcholine receptors. It is distributed in the central and peripheral nervous system and exerts a variety of functions, such as modulating cognition, addiction, learning and memory, muscle contraction, etc. However, how cholinergic neurons control water intake is still a matter of debate. Previous studies have shown contradictory results in water consumption by manipulations of the cholinergic system of different species. Local injection of muscarine into the lateral hypothalamus or carbachol into the subfornical organ promotes drinking behavior in rats (Routtenberg & Simpson 1971, Simpson & Routtenberg 1972, Stein & Seifter 1962). However, injection of carbachol, acetylcholine, or eserine (a reversible cholinesterase inhibitor) into different parts of the

diencephalon blocks all ingestive behavior in hungry or thirsty monkeys; in cats, there are no effects on drinking behavior after the stimulation of the acetylcholine receptors (Myers & Sharpe 1968). Therefore, it is still not clear how the cholinergic system regulates drinking behavior. CB_1 receptor was reported to regulate acetylcholine release in the brain (Gifford & Ashby 1996). Interestingly, we found that deletion of CB_1 receptors in cholinergic cells promotes drinking selectively in response to central application of hypertonic NaCl, but not 24-hour water deprivation, systemic hypertonic NaCl administration and application of Ang II, indicating that CB_1 receptors in cholinergic neurons may be involved in the control of water intake elicited by central osmolality changes.

Altogether, these results indicate that global CB_1 receptors exert a positive control of water intake but none of the different subpopulations analyzed are involved. In other words, whereas CB_1 receptors are generally necessary to promote water intake, we were not able to find a specific subpopulation of receptors responsible alone for this general function This suggests that the general control of a vital function, such as thirst, by CB_1 receptors is likely exerted through redundant pathways and mechanisms, which can compensate each other. However, CB_1 receptors are expressed in many more cell types and brain regions. Therefore, the present data cannot exclude that a selected subgroup of CB_1 receptors in the brain might be necessary alone to provide correct drinking behavior. Moreover, also peripheral mechanisms regulated by endocannabinoid signaling might be involved.

If no brain subpopulation of CB_1 receptors seems to play a specific necessary role in water intake, I decided to investigate whether any of them might exert a sufficient role in the process, because both necessary and sufficient roles are essential to the control of body fluid balance. To this aim, we applied a rescue strategy. Stop-*CB_1* mice do not express CB_1 receptors unless in the presence of Cre recombinase and displayed a reduced water intake comparable to the one observed in full *CB_1*-KO mice. When crossed with specific Cre-expressing lines or when injected with Cre-expressing viruses, these mice carry specific CB_1 receptors expression exclusively in given cell types or brain regions (Remmers et al 2017, Ruehle et al 2013, Soria-Gomez et al 2014). I first

used mice carrying re-expression of CB_1 exclusively in cortical glutamatergic (Glu-*CB_1*-RS) or in forebrain GABAergic neurons (GABA-*CB_1*-RS). Interestingly, Glu-*CB_1*-RS mice drunk more water than stop-*CB_1* littermates, but GABA-*CB_1*-RS mice did not show any significant difference compared to the control mice. These results indicate that CB_1 receptors in cortical glutamatergic neurons play a sufficient role in the control of water intake.

IV.3 Role of CB_1 receptors in specific brain areas in the control of water intake

It is known that the insular cortex (IC) and the anterior cingulate cortex (ACC) are involved in the control of drinking behavior (Denton et al 2009, Gizowski & Bourque 2018, Ichiki et al 2019, Robinson & Mishkin 1968, Zimmerman et al 2017). The IC is divided into two parts, the anterior IC, which is part of the gustatory cortex and responsible for the perception of tastes, and the posterior IC, which is involved in recognition, learning and memory (Gogolla 2017). It plays diverse roles including the regulation of emotions, body homeostasis, perception, motor control, self-awareness, cognition, and visceral control (Gogolla 2017). Recent studies have shown that optogenetic stimulation of the anterior IC in mice promotes the licking of water, whereas optogenetic stimulation of the posterior IC suppresses this behavior (Schiff et al 2018, Wang et al 2018). These results indicate that the two parts of IC play opposite roles in the control of drinking behavior. In addition, pharmacological stimulation of CB_1 receptors in the IC produced a hedonic taste valance (Kobilo et al 2007), suggesting that CB_1 receptors in the IC may modulate ingestive behavior, including feeding and drinking.

The anterior cingulate cortex (ACC) is a medial part of the frontal cortex, located above the corpus callosum. It is involved in the process of cognitive and emotional information, such as attention allocation, reward anticipation, decision-making, ethics and morality, and emotion (Bush et al 2002, Decety & Jackson 2004, Jackson et al 2006, Pardo et al 1990, Sevinc et al 2017). Previous studies have also shown that the ACC participates in the control of thirst consciousness (McKinley et al 2006, Saker et al 2018). A recent study reported that the treatment of hypertonic NaCl increases neural

activity in the ACC (Ma et al 2019). Therefore, these studies suggested that ACC may be an essential brain area for the control of drinking behavior.

Considering the results obtained with Glu-CB_1-RS mice, I wondered whether the modulation of water intake by CB_1 receptors might involve the control of glutamatergic transmission of neurons located in these brain areas. Thus, I re-expressed CB_1 receptors in the IC in stop-*CB_1* mice by injection of adeno-associated virus expressing Cre recombinase. Re-expression of CB_1 receptors in the IC or the posterior IC did not influence water intake as compared to the stop-*CB_1* mice. Conversely, when we injected AAV expressing Cre recombinase driven by CaMKIIα promotor into the ACC to get re-expression of CB_1 receptors in the ACC glutamatergic neurons (ACC-CaMKIIα-*CB_1*-RS), this manipulation was able to increase water intake respect to stop-*CB_1* mice controls. Thus, CB_1 receptors in the ACC glutamatergic neurons but not in the IC ones seems to be necessary to promote drinking behavior.

As mentioned above, optogenetic stimulation of the IC influences drinking behavior. Since CB_1 receptors are mainly Gi protein-coupled receptors, stimulation of the receptors exerts inhibition of neurotransmission. Conversely, deletion of CB_1 receptors facilitates neurotransmitter release, which will somehow recapitulate optogenetic stimulation. Therefore, we deleted the IC CB_1 receptors by injection of AAV expressing Cre recombinase into the IC (IC-*CB_1*-KO). We observed that deletion of CB_1 receptors does not impact on daily water intake and body weight as well as 24-hour water deprivation-induced water and food intake. Interestingly, the IC-*CB_1*-KO mice displayed a significantly suppressed drinking behavior elicited by systemic hypertonic NaCl application and central administration of Ang II, but not central application of hypertonic NaCl. Therefore, CB_1 receptors in the insular cortex are involved in promoting water intake at two different levels, such as response to peripheral osmolality changes and brain Ang II signaling.

IV.4 Role of CB_1 receptors in specific brain circuits in the control of water intake

CB_1 receptors are mainly presynaptic receptors in neurons. Therefore, I wondered which ACC projection terminals might be the location for the endocannabinoid control of

water intake. By examining the expression of CB_1 receptors in the whole brain, I identified that CB_1 receptors in axonal terminals originating from the ACC are located in the Claustrum (CL), the basolateral amygdala (BLA), the ectorhinal cortex and the perirhinal cortex. Among these brain areas, the BLA has been reported to regulate water intake (Kim et al 2016, Kim et al 2017). Therefore, we characterized the role of CB_1 receptors in the ACC-BLA circuit on drinking behavior. We re-expressed CB_1 receptors specifically in the ACC-BLA circuit (ACC-BLA-*CB_1*-RS) by combining injection of retrograde virus expressing flipase into the BLA with the injection of AVV expressing flipase-dependent Cre recombinase into the ACC of stop-*CB_1* mice. ACC-BLA-*CB_1*-RS mice remarkably drunk more water than the stop-*CB_1* mice. Hence, CB_1 receptors in the ACC-BLA circuit is crucial for facilitating water intake.

IV.5 Role of mitochondrial CB_1 receptors in the control of water intake

Mitochondria are essential for producing energy supplies in cells. Recent studies have shown that CB_1 receptors are functionally associated with mitochondrial membranes (Benard et al 2012). Activation of CB_1 receptors regulates mitochondrial energetics, endocannabinoid-dependent short-term synaptic plasticity in the hippocampus, and memory (Benard et al 2012, Hebert-Chatelain et al 2016). Mitochondrial functions might also modulate drinking-related phenomena, such as the effects of Ang II in the brain (Zimmerman et al 2002). Thus, stimulation of mitochondrial CB_1 receptors in the thirst-related neurons might impact drinking behavior. I used DN22-*CB_1*-KI mice, in which mitochondrial CB_1 receptors are absent but plasma CB_1 receptors are still present, and found that DN22-*CB_1*-KI mice significantly decrease daily water intake compared to the WT littermates. Furthermore, DN22-*CB_1*-KI remarkably drunk less water than the control mice in response to the systemic hypertonic NaCl application, but not upon 24-hour water deprivation (although a strong tendency to drink less water was present). Thus, these results reveal that mitochondrial CB_1 receptors might play a necessary role in the regulation of water intake, and further mechanisms are worth to investigate.

In conclusion, central CB_1 receptors in different locations are essential for the control of drinking behavior. This control takes place on cortical glutamatergic neurons,

located in the anterior cingulate cortex projecting to the basolateral amygdala, revealing a novel neuronal circuit for the top-down control of water intake by the endocannabinoid system.

IV.6 Perspectives

I have shown CB_1 receptors in the ACC glutamatergic neurons and particularly in the ACC-BLA circuit play a sufficient role to promote drinking behavior. Since the ACC also contributes to the regulation of cognition and emotion (Bush et al 2002, Decety & Jackson 2004, Jackson et al 2006, Pardo et al 1990, Sevinc et al 2017), the control of water intake may involve cognitive and emotional components. For future studies, we would like to study whether thirst regulates learning and memory, anxiety, and neural mechanisms underlying these regulations. In addition, it will also be very interesting to understand which brain area projecting to the ACC for the transfer of interoceptive signals. As thalamus is a key relay between the cortical and subcortical areas, the first approach may be focusing on the thalamus to the ACC for the control of drinking behavior.

This work has also provided significant insight into therapeutics of adipsia and aphagia. Elderly persons and Alzheimer's disease patients tend to loose their perception of thirst, leading to inadequate water intake (Hahr 2015, Hahr 2019, Popkin et al 2010). Simultaneously, these people have lower expression of CB_1 receptors than normal people (Hodges & Ashpole 2019). Therefore, there is a correlation between the decrease in perception of thirst and the decrease in the expression of CB_1 receptors. For future studies, it will be worth to try stimulation or overexpression of CB_1 receptors in the animal model of aging or Alzheimer's disease for restoring proper water intake. Furthermore, I have shown that an increase in plasma osmolality by systemic injection of hypertonic NaCl suppresses food intake. This provides an insight into inadequate water intake-induced anorexia in certain types of patients. Thus, I hypothesize that decreasing expression of CB_1 receptors leads to inadequate water intake, which causes an increase in plasma osmolality, subsequently anorexia occurs.

Regarding the ChAT-*CB_1*-KO mice, it is very interesting that they selectively displayed an increase in water intake in response to the central application of hypertonic

NaCl, suggesting that CB_1 receptors in cholinergic neurons control water intake. Cholinergic neurons are largely located in the medial septum, the medial habenula, and other brain areas (Li et al 2018). It is known that the lesion of medial septum promotes drinking, indicating that the elimination of acetylcholine signaling increases water intake, which is inconsistent with the phenotype of ChAT-*CB_1*-KO mice. The medial habenula is expressing phosphodiesterase 2A, which is activated by atrial natriuretic peptide (ANP) and successively inhibits neurotransmission (Hu et al 2012). Since ANP is a peptide inhibiting water intake, it is possible that inhibition of the neural transmission by the ANP stimulation decreases water intake, whereas the deletion of CB_1 receptors in the medial habenula leads to an increase in neurotransmission, and promotes drinking behavior.

REFERENCES

Abbott SB, Machado NL, Geerling JC, Saper CB. 2016. Reciprocal Control of Drinking Behavior by Median Preoptic Neurons in Mice. *J Neurosci* 36: 8228-37

Abel EL. 1975. Cannabis: effects on hunger and thirst. *Behav Biol* 15: 255-81

Adams R. 1940. Marihuana. *Science* 92: 115-9

Adolph EF, Barker JP. 1953. Thirst tests in dogs and modifications of thirst with experimental lesions of the neurohypophysis. *Am J Physiol* 173: 233-45

Ahn K, Johnson DS, Mileni M, Beidler D, Long JZ, et al. 2009. Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. *Chem Biol* 16: 411-20

Ahn K, Smith SE, Liimatta MB, Beidler D, Sadagopan N, et al. 2011. Mechanistic and pharmacological characterization of PF-04457845: a highly potent and selective fatty acid amide hydrolase inhibitor that reduces inflammatory and noninflammatory pain. *J Pharmacol Exp Ther* 338: 114-24

Aizpurua-Olaizola O, Soydaner U, Ozturk E, Schibano D, Simsir Y, et al. 2016. Evolution of the Cannabinoid and Terpene Content during the Growth of Cannabis sativa Plants from Different Chemotypes. *J Nat Prod* 79: 324-31

Albrecht J, Schreder T, Kleemann AM, Schopf V, Kopietz R, et al. 2009. Olfactory detection thresholds and pleasantness of a food-related and a non-food odour in hunger and satiety. *Rhinology* 47: 160-5

Albright F. 1948. A page out of the history of hyperparathyroidism. *J Clin Endocrinol Metab* 8: 637-57

Aldrich M. 1997. *History of therapeutic cannabis. In: Mathre ML, eds. Cannabis in medical practice*. pp. 35-55. Jefferson, NC: Mc Farland.

American Psychiatric Association. 2013. *Diagnostic and Statistical Manual of Mental Disorders (Fifth ed.)*. Arlington, VA: American Psychiatric Publishing.

Anagnostaras SG, Gale GD, Fanselow MS. 2001. Hippocampus and contextual fear conditioning: recent controversies and advances. *Hippocampus* 11: 8-17

Anand BK. 1961. Nervous regulation of food intake. *Physiol Rev* 41: 677-708

Anand BK, Brobeck JR. 1951. Hypothalamic control of food intake in rats and cats. *Yale J Biol Med* 24: 123-40

Andersson B. 1953. The effect of injections of hypertonic NaCl-solutions into different parts of the hypothalamus of goats. *Acta Physiol Scand* 28: 188-201

Andersson B. 1967. The thirst mechanism as a link in the regulation of the "milieu intérieur." In: Les conceptes de Claudz Bernaud sur le milieu inlérieur. *Paris: Masson*: 13-26

Andersson B, Jobin M, Olsson K. 1967. A study of thirst and other effects of an increased sodium concentration in the 3rd brain ventricle. *Acta Physiol Scand* 69: 29-36

Andersson B, Larsson S. 1956. Water and food intake and the inhibitory effect of amphetamine on drinking and eating before and after prefrontal lobotomy in dogs. *Acta Physiol Scand* 38: 22-30

Andersson B, Larsson S, Persson N. 1960. Some characteristics of the hypothalamic "drinking centre" in the goat as shown by the use of permanent electrodes. *Acta Physiol Scand* 50: 140-52

Andersson B, McCann SM. 1955. A further study of polydipsia evoked by hypothalamic stimulation in the goat. *Acta Physiol Scand* 33: 333-46

Andersson B, McCann SM. 1956. The effect of hypothalamic lesions on the water intake of the dog. *Acta Physiol Scand* 35: 312-20

Andre CM, Hausman JF, Guerriero G. 2016. Cannabis sativa: The Plant of the Thousand and One Molecules. *Front Plant Sci* 7: 19

Anglada-Figueroa D, Quirk GJ. 2005. Lesions of the basal amygdala block expression of conditioned fear but not extinction. *J Neurosci* 25: 9680-5

Araujo DJ, Tjoa K, Saijo K. 2019. The Endocannabinoid System as a Window Into Microglial Biology and Its Relationship to Autism. *Front Cell Neurosci* 13: 424

Arden F. 1934. Experimental observations upon thirst and on potassium overdosage. *Australian J Exptl Biol Med Sci* 12: 121-22

Arevalo C, de Miguel R, Hernandez-Tristan R. 2001. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. *Pharmacol Biochem Behav* 70: 123-31

Arnaud MJ. 2003. Mild dehydration: a risk factor of constipation? *Eur J Clin Nutr* 57 Suppl 2: S88-95

Asscher AW, Anson SG. 1963. A vascular permeability factor of renal origin. *Nature* 198: 1097-8

Atia AN, Buchman AL. 2009. Oral rehydration solutions in non-cholera diarrhea: a review. *Am J Gastroenterol* 104: 2596-604; quiz 605

Augustine V, Gokce SK, Lee S, Wang B, Davidson TJ, et al. 2018. Hierarchical neural architecture underlying thirst regulation. *Nature* 555: 204-09

Baldo BA, Kelley AE. 2001. Amylin infusion into rat nucleus accumbens potently depresses motor activity and ingestive behavior. *Am J Physiol Regul Integr Comp Physiol* 281: R1232-42

Balthasar N, Dalgaard LT, Lee CE, Yu J, Funahashi H, et al. 2005. Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell* 123: 493-505

Bani D. 1997. Relaxin: a pleiotropic hormone. *Gen Pharmacol* 28: 13-22

Banting FG, Best CH. 1922. The internal secretion of the pancreas. *The journal of laboratory and clinical medicine.* 7: 251-66

Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. 1922. Pancreatic Extracts in the Treatment of Diabetes Mellitus. *Can Med Assoc J* 12: 141-6

Bar-Or O, Dotan R, Inbar O, Rotshtein A, Zonder H. 1980. Voluntary hypohydration in 10- to 12-year-old boys. *J Appl Physiol Respir Environ Exerc Physiol* 48: 104-8

Barney CC, Folkerts MM. 1995. Thermal dehydration-induced thirst in rats: role of body temperature. *Am J Physiol* 269: R557-64

Basavarajappa BS, Shivakumar M, Joshi V, Subbanna S. 2017. Endocannabinoid system in neurodegenerative disorders. *J Neurochem* 142: 624-48

Bauer R, Remiger P. 1989. TLC and HPLC Analysis of Alkamides in Echinacea Drugs1,2. *Planta Med* 55: 367-71

Beard CM, Heath H, 3rd, O'Fallon WM, Anderson JA, Earle JD, Melton LJ, 3rd. 1989. Therapeutic radiation and hyperparathyroidism. A case-control study in Rochester, Minn. *Arch Intern Med* 149: 1887-90

Becker CA, Flaisch T, Renner B, Schupp HT. 2017. From Thirst to Satiety: The Anterior Mid-Cingulate Cortex and Right Posterior Insula Indicate Dynamic Changes in Incentive Value. *Front Hum Neurosci* 11: 234

Beinfeld MC, Connolly K. 2001. Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release,

a possible mechanism contributing to the spatial memory defects produced by cannabinoids. *Neurosci Lett* 301: 69-71

Bellisle F, Le Magnen J. 1981. The structure of meals in humans: eating and drinking patterns in lean and obese subjects. *Physiol Behav* 27: 649-58

Bellocchio L, Lafenetre P, Cannich A, Cota D, Puente N, et al. 2010. Bimodal control of stimulated food intake by the endocannabinoid system. *Nat Neurosci* 13: 281-3

Bellows RT. 1939. Time factors in water drinking in dogs. *Am J Physiol* 125: 87-97

Belovicova K, Bogi E, Csatlosova K, Dubovicky M. 2017. Animal tests for anxiety-like and depression-like behavior in rats. *Interdiscip Toxicol* 10: 40-43

Benard G, Massa F, Puente N, Lourenco J, Bellocchio L, et al. 2012. Mitochondrial CB(1) receptors regulate neuronal energy metabolism. *Nat Neurosci* 15: 558-64

Berger WT, Ralph BP, Kaczocha M, Sun J, Balius TE, et al. 2012. Targeting fatty acid binding protein (FABP) anandamide transporters - a novel strategy for development of anti-inflammatory and anti-nociceptive drugs. *PLoS One* 7: e50968

Bernard C. 1856. Leçons de l'hysiologie expérimentale appliquée à la Medicine faites au Collége de France. *Paris:Baillière* 2: 50-51

Bernardis LL, Bellinger LL. 1996. The lateral hypothalamic area revisited: ingestive behavior. *Neurosci Biobehav Rev* 20: 189-287

Betley JN, Xu S, Cao ZFH, Gong R, Magnus CJ, et al. 2015. Neurons for hunger and thirst transmit a negative-valence teaching signal. *Nature* 521: 180-85

Bingham NC, Verma-Kurvari S, Parada LF, Parker KL. 2006. Development of a steroidogenic factor 1/Cre transgenic mouse line. *Genesis* 44: 419-24

Bisogno T, Berrendero F, Ambrosino G, Cebeira M, Ramos JA, et al. 1999. Brain regional distribution of endocannabinoids: implications for their biosynthesis and biological function. *Biochem Biophys Res Commun* 256: 377-80

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, et al. 2003. Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J Cell Biol* 163: 463-8

Blankman JL, Simon GM, Cravatt BF. 2007. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol* 14: 1347-56

Blau JN. 2005. Water deprivation: a new migraine precipitant. *Headache* 45: 757-9

Blau JN, Kell CA, Sperling JM. 2004. Water-deprivation headache: a new headache with two variants. *Headache* 44: 79-83

Boggs DL, Nguyen JD, Morgenson D, Taffe MA, Ranganathan M. 2018. Clinical and Preclinical Evidence for Functional Interactions of Cannabidiol and Delta(9)-Tetrahydrocannabinol. *Neuropsychopharmacology* 43: 142-54

Bonica JJ. 1979. The relation of injury to pain. *Pain* 7: 203-7

Bouaboula M, Desnoyer N, Carayon P, Combes T, Casellas P. 1999. Gi protein modulation induced by a selective inverse agonist for the peripheral cannabinoid receptor CB2: implication for intracellular signalization cross-regulation. *Mol Pharmacol* 55: 473-80

Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, et al. 1995. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J* 312 (Pt 2): 637-41

Bourque CW. 2008. Central mechanisms of osmosensation and systemic osmoregulation. *Nat Rev Neurosci* 9: 519-31

Boyd AM, Sturgill JF, Poo C, Isaacson JS. 2012. Cortical feedback control of olfactory bulb circuits. *Neuron* 76: 1161-74

Bracey MH, Hanson MA, Masuda KR, Stevens RC, Cravatt BF. 2002. Structural adaptations in a membrane enzyme that terminates endocannabinoid signaling. *Science* 298: 1793-6

Brand EJ, Zhao Z. 2017. Cannabis in Chinese Medicine: Are Some Traditional Indications Referenced in Ancient Literature Related to Cannabinoids? *Front Pharmacol* 8: 108

Breivogel CS, Griffin G, Di Marzo V, Martin BR. 2001. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol Pharmacol* 60: 155-63

Bridgeman MB, Abazia DT. 2017. Medicinal Cannabis: History, Pharmacology, And Implications for the Acute Care Setting. *P T* 42: 180-88

Brobeck JR, Tepperman J, Long CN. 1943. Experimental Hypothalamic Hyperphagia in the Albino Rat. *Yale J Biol Med* 15: 831-53

Brown SM, Wager-Miller J, Mackie K. 2002. Cloning and molecular characterization of the rat CB2 cannabinoid receptor. *Biochim Biophys Acta* 1576: 255-64

Buccafusco JJ. 2009. *Methods of Behavior Analysis in Neuroscience, 2nd edition*.

Budgell P. 1970a. The effect of changes in ambient temperature on water intake and evaporative water loss. *Psychonomic Sci* 20: 275-76

Budgell P. 1970b. Modulation of drinking by ambient temperature changes. *Anim Behav* 18: 753-7

Burrell LM, Palmer JM, Baylis PH. 1992. Atrial natriuretic peptide inhibits fluid intake in hyperosmolar subjects. *Clin Sci (Lond)* 83: 35-9

Bush G, Vogt BA, Holmes J, Dale AM, Greve D, et al. 2002. Dorsal anterior cingulate cortex: a role in reward-based decision making. *Proc Natl Acad Sci U S A* 99: 523-8

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: 1215-25

Busquets-Garcia A, Gomis-Gonzalez M, Srivastava RK, Cutando L, Ortega-Alvaro A, et al. 2016. Peripheral and central CB1 cannabinoid receptors control stress-induced impairment of memory consolidation. *Proc Natl Acad Sci U S A* 113: 9904-9

Busquets-Garcia A, Oliveira da Cruz JF, Terral G, Pagano Zottola AC, Soria-Gomez E, et al. 2018b. Hippocampal CB1 Receptors Control Incidental Associations. *Neuron* 99: 1247-59 e7

Busquets-Garcia A, Soria-Gomez E, Redon B, Mackenbach Y, Vallee M, et al. 2017. Pregnenolone blocks cannabinoid-induced acute psychotic-like states in mice. *Mol Psychiatry* 22: 1594-603

Cabral GA, Griffin-Thomas L. 2009. Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation. *Expert Rev Mol Med* 11: e3

Cadogan AK, Alexander SP, Boyd EA, Kendall DA. 1997. Influence of cannabinoids on electrically evoked dopamine release and cyclic AMP generation in the rat striatum. *J Neurochem* 69: 1131-7

Calabresi P, Maj R, Pisani A, Mercuri NB, Bernardi G. 1992. Long-term synaptic depression in the striatum: physiological and pharmacological characterization. *J Neurosci* 12: 4224-33

Calabresi P, Pisani A, Mercuri NB, Bernardi G. 1994. Post-receptor mechanisms underlying striatal long-term depression. *J Neurosci* 14: 4871-81

Calignano A, La Rana G, Giuffrida A, Piomelli D. 1998. Control of pain initiation by endogenous cannabinoids. *Nature* 394: 277-81

Cameron JD, Goldfield GS, Doucet E. 2012. Fasting for 24 h improves nasal chemosensory performance and food palatability in a related manner. *Appetite* 58: 978-81

Campos AC, Moreira FA, Gomes FV, Del Bel EA, Guimaraes FS. 2012. Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philos Trans R Soc Lond B Biol Sci* 367: 3364-78

Cannon BWM. 1918. The physiological basis of thirst. *Croonian lecture*

Carlini EA. 2004. The good and the bad effects of (-) trans-delta-9-tetrahydrocannabinol (Delta 9-THC) on humans. *Toxicon* 44: 461-7

Cassell MD, Freedman LJ, Shi C. 1999. The intrinsic organization of the central extended amygdala. *Ann N Y Acad Sci* 877: 217-41

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: 857-67

Chakrabarti A, Onaivi ES, Chaudhuri G. 1995. Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. *DNA Seq* 5: 385-8

Chance WT, Balasubramaniam A, Chen X, Fischer JE. 1992. Tests of adipsia and conditioned taste aversion following the intrahypothalamic injection of amylin. *Peptides* 13: 961-4

Chapman CR, Sola AE, Bonica JJ. 1979. Illness behavior and depression compared in pain center and private practice patients. *Pain* 6: 1-7

Cheuvront SN, Carter R, 3rd, Sawka MN. 2003. Fluid balance and endurance exercise performance. *Curr Sports Med Rep* 2: 202-8

Chhatwal JP, Davis M, Maguschak KA, Ressler KJ. 2005. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology* 30: 516-24

Chiarlone A, Bellocchio L, Blazquez C, Resel E, Soria-Gomez E, et al. 2014. A restricted population of CB1 cannabinoid receptors with neuroprotective activity. *Proc Natl Acad Sci U S A* 111: 8257-62

Chicca A, Schafroth MA, Reynoso-Moreno I, Erni R, Petrucci V, et al. 2018. Uncovering the psychoactivity of a cannabinoid from liverworts associated with a legal high. *Sci Adv* 4: eaat2166

Chobanian HR, Guo Y, Liu P, Chioda MD, Fung S, et al. 2014. Discovery of MK-4409, a Novel Oxazole FAAH Inhibitor for the Treatment of Inflammatory and Neuropathic Pain. *ACS Med Chem Lett* 5: 717-21

Choi S, Lovinger DM. 1997a. Decreased frequency but not amplitude of quantal synaptic responses associated with expression of corticostriatal long-term depression. *J Neurosci* 17: 8613-20

Choi S, Lovinger DM. 1997b. Decreased probability of neurotransmitter release underlies striatal long-term depression and postnatal development of corticostriatal synapses. *Proc Natl Acad Sci U S A* 94: 2665-70

Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. 2007. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *Lancet* 370: 1706-13

Cian C, Barraud PA, Melin B, Raphel C. 2001. Effects of fluid ingestion on cognitive function after heat stress or exercise-induced dehydration. *Int J Psychophysiol* 42: 243-51

Cian C, Koulmann PA, Barraud PA, Raphel C, Jimenez C, Melin B. 2000. Influence of variations of body hydration on cognitive performance. *J Psychophysiol* 14: 29-36

Cluny NL, Vemuri VK, Chambers AP, Limebeer CL, Bedard H, et al. 2010. A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents. *Br J Pharmacol* 161: 629-42

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: 451-63

Cooper GJ, Willis AC, Clark A, Turner RC, Sim RB, Reid KB. 1987. Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients. *Proc Natl Acad Sci U S A* 84: 8628-32

Cota D, Tschop MH, Horvath TL, Levine AS. 2006. Cannabinoids, opioids and eating behavior: the molecular face of hedonism? *Brain Res Rev* 51: 85-107

Covian MR, Hartmann LF, Granel JA. 1954. Hyperphagia par hémidécortication chez le rat blanc. *Compt Rend Soc Biol* 148: 909-10

Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, et al. 2001. Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci U S A* 98: 9371-6

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. 1996. Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384: 83-7

Cravatt BF, Lichtman AH. 2004. The endogenous cannabinoid system and its role in nociceptive behavior. *J Neurobiol* 61: 149-60

Crestani CC, Alves FH, Gomes FV, Resstel LB, Correa FM, Herman JP. 2013. Mechanisms in the bed nucleus of the stria terminalis involved in control of autonomic and neuroendocrine functions: a review. *Curr Neuropharmacol* 11: 141-59

Crow LT. 1967. Subcommissural organ, lateral hypothalamus, and dorsal longitudinal fasciculus in water and salt metabolism. *The anatomical record*

Cryan JF, Mombereau C. 2004. In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry* 9: 326-57

Culp KR, Wakefield B, Dyck MJ, Cacchione PZ, DeCrane S, Decker S. 2004. Bioelectrical impedance analysis and other hydration parameters as risk factors for delirium in rural nursing home residents. *J Gerontol A Biol Sci Med Sci* 59: 813-7

D'Anci K E, Vibhakar A, Kanter JH, Mahoney CR, Taylor HA. 2009. Voluntary dehydration and cognitive performance in trained college athletes. *Percept Mot Skills* 109: 251-69

D'Hooge R, De Deyn PP. 2001. Applications of the Morris water maze in the study of learning and memory. *Brain Res Brain Res Rev* 36: 60-90

Dale HH, Laidlaw PP. 1910. The physiological action of beta-iminazolylethylamine. *J Physiol* 41: 318-44

Daniel H, Crepel F. 2001. Control of Ca(2+) influx by cannabinoid and metabotropic glutamate receptors in rat cerebellar cortex requires K(+) channels. *J Physiol* 537: 793-800

Daniel H, Rancillac A, Crepel F. 2004. Mechanisms underlying cannabinoid inhibition of presynaptic Ca2+ influx at parallel fibre synapses of the rat cerebellum. *J Physiol* 557: 159-74

Davies SN, Pertwee RG, Riedel G. 2002. Functions of cannabinoid receptors in the hippocampus. *Neuropharmacology* 42: 993-1007

Davis BJ, Macrides F. 1981. The organization of centrifugal projections from the anterior olfactory nucleus, ventral hippocampal rudiment, and piriform cortex to the main olfactory bulb in the hamster: an autoradiographic study. *J Comp Neurol* 203: 475-93

de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. 1981. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci* 28: 89-94

De Vries TJ, Schoffelmeer AN. 2005. Cannabinoid CB1 receptors control conditioned drug seeking. *Trends Pharmacol Sci* 26: 420-6

De wied D. 1966. Effect of autonomic blocking agents and structurally related substances on the "salt arousal of drinking.". *Physiol Behavior* 1: 193-97

Decety J, Jackson PL. 2004. The functional architecture of human empathy. *Behav Cogn Neurosci Rev* 3: 71-100

Demuth DG, Molleman A. 2006. Cannabinoid signalling. *Life Sci* 78: 549-63

Denton D, Shade R, Zamarippa F, Egan G, Blair-West J, et al. 1999a. Correlation of regional cerebral blood flow and change of plasma sodium concentration during genesis and satiation of thirst. *Proc Natl Acad Sci U S A* 96: 2532-7

Denton D, Shade R, Zamarippa F, Egan G, Blair-West J, et al. 1999b. Neuroimaging of genesis and satiation of thirst and an interoceptor-driven theory of origins of primary consciousness. *Proc Natl Acad Sci U S A* 96: 5304-9

Denton DA, McKinley MJ, Farrell M, Egan GF. 2009. The role of primordial emotions in the evolutionary origin of consciousness. *Conscious Cogn* 18: 500-14

Derkinderen P, Toutant M, Burgaya F, Le Bert M, Siciliano JC, et al. 1996. Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science* 273: 1719-22

Desarnaud F, Cadas H, Piomelli D. 1995. Anandamide amidohydrolase activity in rat brain microsomes. Identification and partial characterization. *J Biol Chem* 270: 6030-5

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, et al. 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946-9

Di Marzo V, De Petrocellis L, Bisogno T. 2005. The biosynthesis, fate and pharmacological properties of endocannabinoids. *Handb Exp Pharmacol*: 147-85

Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, et al. 2001. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 410: 822-5

Diaz-Laviada I, Ruiz-Llorente L. 2005. Signal transduction activated by cannabinoid receptors. *Mini Rev Med Chem* 5: 619-30

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, et al. 2002. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A* 99: 10819-24

Dong HW, Petrovich GD, Swanson LW. 2001. Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res Brain Res Rev* 38: 192-246

Drewnowski A, Grinker JA. 1978. Food and water intake, meal patterns and activity of obese and lean Zucker rats following chronic and acute treatment with delta9-tetrahydrocannabinol. *Pharmacol Biochem Behav* 9: 619-30

Edgar M, Dawes C, O'Mullane D. 2012. *Saliva and oral health*. Stephen Hancocks Limited.

Egan G, Silk T, Zamarripa F, Williams J, Federico P, et al. 2003. Neural correlates of the emergence of consciousness of thirst. *Proc Natl Acad Sci U S A* 100: 15241-6

Egertova M, Giang DK, Cravatt BF, Elphick MR. 1998. A new perspective on cannabinoid signalling: complementary localization of fatty acid amide hydrolase and the CB1 receptor in rat brain. *Proc Biol Sci* 265: 2081-5

Ehrlich KJ, Fitts DA. 1990. Atrial natriuretic peptide in the subfornical organ reduces drinking induced by angiotensin or in response to water deprivation. *Behav Neurosci* 104: 365-72

Ellis J, Pediani JD, Canals M, Milasta S, Milligan G. 2006. Orexin-1 receptor-cannabinoid CB1 receptor heterodimerization results in both ligand-dependent and -independent coordinated alterations of receptor localization and function. *J Biol Chem* 281: 38812-24

Elphick MR, Egertova M. 2001. The neurobiology and evolution of cannabinoid signalling. *Philos Trans R Soc Lond B Biol Sci* 356: 381-408

Epstein AN. 1959. Suppression of eating and drinking by amphetamine and other drugs in normal and hyperphagic rats. *J Comp Physiol Psychol* 52: 37-45

Epstein AN, Fitzsimons JT, Rolls BJ. 1970. Drinking induced by injection of angiotensin into the brain of the rat. *J Physiol* 210: 457-74

Epstein AN, Spector D, Samman A, Goldblum C. 1964. Exaggerated Prandial Drinking in the Rat without Salivary Glands. *Nature* 201: 1342-3

Everitt BJ, Robbins TW. 1997. Central cholinergic systems and cognition. *Annu Rev Psychol* 48: 649-84

Fankhauser M. 2002. *History of cannabis in Western Medicine. In: Grotenhermen F, Russo E, eds. Cannabis and Cannabinoids.* pp. 37-51. New York: The Haworth Integrative Healing Press.

Farag E, Sessler DI, Ebrahim Z, Kurz A, Morgan J, et al. 2017. The renin angiotensin system and the brain: New developments. *J Clin Neurosci* 46: 1-8

Farini F. 1913. Diabete insipido ed opoterapia. *Gazz Osped Clin* 34: 1135-39

Farrell MJ, Bowala TK, Gavrilescu M, Phillips PA, McKinley MJ, et al. 2011. Cortical activation and lamina terminalis functional connectivity during thirst and drinking in humans. *Am J Physiol Regul Integr Comp Physiol* 301: R623-31

Farrell MJ, Egan GF, Zamarripa F, Shade R, Blair-West J, et al. 2006. Unique, common, and interacting cortical correlates of thirst and pain. *Proc Natl Acad Sci U S A* 103: 2416-21

Fattore L, Spano MS, Deiana S, Melis V, Cossu G, et al. 2007. An endocannabinoid mechanism in relapse to drug seeking: a review of animal studies and clinical perspectives. *Brain Res Rev* 53: 1-16

Fenselau H, Campbell JN, Verstegen AM, Madara JC, Xu J, et al. 2017. A rapidly acting glutamatergic ARC-->PVH satiety circuit postsynaptically regulated by alpha-MSH. *Nat Neurosci* 20: 42-51

Fernandez-Lopez D, Pazos MR, Tolon RM, Moro MA, Romero J, et al. 2007. The cannabinoid agonist WIN55212 reduces brain damage in an in vivo model of hypoxic-ischemic encephalopathy in newborn rats. *Pediatr Res* 62: 255-60

Ferrari F, Ottani A, Vivoli R, Giuliani D. 1999. Learning impairment produced in rats by the cannabinoid agonist HU 210 in a water-maze task. *Pharmacol Biochem Behav* 64: 555-61

Fisher AE, Coury JN. 1962. Cholinergic tracing of a central neural circuit underlying the thirst drive. *Science* 138: 691-3

Fisher AE, Coury JN. 1964. *Cholinergic tracing of neural pathways mediating the thirst drive. In: Thirst: Prog. First Intern. Symp. Thirst in the regulation of Body Water.* pp. 515-539. Oxford: Pergamon.

Fiskerstrand T, H'Mida-Ben Brahim D, Johansson S, M'Zahem A, Haukanes BI, et al. 2010. Mutations in ABHD12 cause the neurodegenerative disease PHARC: An inborn error of endocannabinoid metabolism. *Am J Hum Genet* 87: 410-7

Fitzsimons JT. 1966a. The hypothalamus and drinking. *Br Med Bull* 22: 232-7

Fitzsimons JT. 1966b. Hypovolaemic drinking and renin. *J. Physiol. Exptl. Pathol.* 186: 130-31

Fitzsimons JT. 1972. Thirst. *Physiol Rev* 52: 468-561

Fitzsimons JT. 1976. The physiological basis of thirst. *Kidney Int* 10: 3-11

Fitzsimons JT. 1980. The eighth J. A. F. Stevenson memorial lecture. Angiotensin II in the control of hypovolaemic thirst and sodium appetite. *Can J Physiol Pharmacol* 58: 441-4

Fitzsimons JT, Setler PE. 1971. Catecholaminergic mechanisms in angiotensin-induced drinking. *J Physiol* 218 Suppl: 43P-44P

Fitzsimons JT, Setler PE. 1975. The relative importance of central nervous catecholaminergic and cholinergic mechanisms in drinking in response to antiotensin and other thirst stimuli. *J Physiol* 250: 613-31

Fitzsimons JT, Simons BJ. 1969. The effect on drinking in the rat of intravenous infusion of angiotensin, given alone or in combination with other stimuli of thirst. *J Physiol* 203: 45-57

Fitzsimons TJ, Le Magnen J. 1969. Eating as a regulatory control of drinking in the rat. *J Comp Physiol Psychol* 67: 273-83

Flor AFL, de Brito Alves JL, Franca-Silva MS, Balarini CM, Elias LLK, et al. 2018. Glial Cells Are Involved in ANG-II-Induced Vasopressin Release and Sodium Intake in Awake Rats. *Front Physiol* 9: 430

Fonberg E. 1966. Aphagia, produced by destruction of the dorsomedial amygdala in dogs. *Bull Acad Pol Sci Biol* 14: 719-22

Fonberg E. 1968. The role of the amygdaloid nucleus in animal behaviour. *Prog Brain Res* 22: 273-81

Fonberg E. 1969a. Effects of small dorsomedial amygdala lesions on food intake and acquisition of instrumental alimentary reactions in dogs. *Physiol Behavior* 4: 739-43

Fonberg E. 1969b. The role of the hypothalamus and amygdala in food intake, alimentary motivation and emotional reactions. *Acta Biol Exp (Warsz)* 29: 335-58

Fonberg E, Sychowa B. 1968. Effects of partial lesions of the amygdala in dogs. I. Aphagia. *Acta Biol Exp (Warsz)* 28: 35-46

Fountain JH, Lappin SL. 2019. *Physiology, Renin Angiotensin System*.

Fu J, Bottegoni G, Sasso O, Bertorelli R, Rocchia W, et al. 2011. A catalytically silent FAAH-1 variant drives anandamide transport in neurons. *Nat Neurosci* 15: 64-9

Fuller LM. 1984. The pharmacology of drinking behavior. *Pharmacol Ther* 24: 179-206

Gao Y, Vasilyev DV, Goncalves MB, Howell FV, Hobbs C, et al. 2010. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. *J Neurosci* 30: 2017-24

Gaoni Y, Mechoulam R. 1964. Isolation, structure and partial synthesis of an active constituent of hashish. *J. Am..Chem. Soc.* 86: 1646-47

Geelen G, Keil LC, Kravik SE, Wade CE, Thrasher TN, et al. 1984. Inhibition of plasma vasopressin after drinking in dehydrated humans. *Am J Physiol* 247: R968-71

Gerald MC, Maickel RP. 1972. Studies on the possible role of brain histamine in behaviour. *Br J Pharmacol* 44: 462-71

Gerard C, Mollereau C, Vassart G, Parmentier M. 1990. Nucleotide sequence of a human cannabinoid receptor cDNA. *Nucleic Acids Res* 18: 7142

Gerdeman GL, Ronesi J, Lovinger DM. 2002. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci* 5: 446-51

Giang DK, Cravatt BF. 1997. Molecular characterization of human and mouse fatty acid amide hydrolases. *Proc Natl Acad Sci U S A* 94: 2238-42

Gifford AN, Ashby CR, Jr. 1996. Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. *J Pharmacol Exp Ther* 277: 1431-6

Gilbert GJ. 1957. The subcommissural organ: a regulator of thirst. *Am J Physiol* 191: 243-7

Gizowski C, Bourque CW. 2018. The neural basis of homeostatic and anticipatory thirst. *Nat Rev Nephrol* 14: 11-25

Gizowski C, Zaelzer C, Bourque CW. 2016. Clock-driven vasopressin neurotransmission mediates anticipatory thirst prior to sleep. *Nature* 537: 685-8

Goetzl FR, Stone F. 1947. Diurnal variations in acuity of olfaction and food intake. *Gastroenterology* 9: 444-53

Gogolla N. 2017. The insular cortex. *Curr Biol* 27: R580-R86

Gold RM. 1967. Aphagia and adipsia following unilateral and bilaterally asymmetrical lesions in rats. *Physiol Behavior* 2: 211-20

Goldman W, Krukowski M, Lehr D. 1968. Prevention of serotonin (5-HT) induced water conservation by beta-adrenergic blockade. *Federation Proc* 27: 539

Goldman W, Lehr D. 1971. Suppression of eating and enhancement of drinking induced by serotonin (5-HT) and abolition of these effects by β-adrenergic blockade. *Federation Proc* 30: 503

Gopinathan PM, Pichan G, Sharma VM. 1988. Role of dehydration in heat stress-induced variations in mental performance. *Arch Environ Health* 43: 15-7

Gozzi A, Jain A, Giovannelli A, Bertollini C, Crestan V, et al. 2010. A neural switch for active and passive fear. *Neuron* 67: 656-66

Graham ES, Ashton JC, Glass M. 2009. Cannabinoid Receptors: A brief history and what not. *Front Biosci (Landmark Ed)* 14: 944-57

Green JT, Woodruff-Pak DS. 2000. Eyeblink classical conditioning: hippocampal formation is for neutral stimulus associations as cerebellum is for association-response. *Psychol Bull* 126: 138-58

Greenleaf JE. 1992. Problem: thirst, drinking behavior, and involuntary dehydration. *Med Sci Sports Exerc* 24: 645-56

Greer MA. 1955. Suggestive evidence of a primary drinking center in hypothalamus of the rat. *Proc Soc Exp Biol Med* 89: 59-62

Griebel G, Stemmelin J, Scatton B. 2005. Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. *Biol Psychiatry* 57: 261-7

Griffin G, Tao Q, Abood ME. 2000. Cloning and pharmacological characterization of the rat CB(2) cannabinoid receptor. *J Pharmacol Exp Ther* 292: 886-94

Grinspoon L. 1971. *Marihuana reconsidered.*: Cambridge, MA: Harvard University Press.

Grinspoon L, Bakalar JB. 1993. *Marihuana: the forbidden medicine*. New Haven: Yale University Press.

Grossman SP. 1960. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. *Science* 132: 301-2

Grossman SP. 1962a. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. *Am J Physiol* 202: 872-82

Grossman SP. 1962b. Effects of adrenergic and cholinergic blocking agents on hypothalamic mechanisms. *Am J Physiol* 202: 1230-36

Grossman SP. 1964. *Some neurochemical aspects of the central regulation of thirst. In: Thirst: Prog. First Intern. Symp. Thirst in the regulation of Body Water.* pp. 487-511. Oxford: Pergamon.

Grossman SP, Grossman L. 1963. Food and Water Intake Following Lesions or Electrical Stimulation of the Amygdala. *Am J Physiol* 205: 761-5

Guggenheimer J, Moore PA. 2003. Xerostomia: etiology, recognition and treatment. *J Am Dent Assoc* 134: 61-9; quiz 118-9

Haefeli L, Peters G. 1971. Induction of hypovolaemia by thirst-inducing doses of renin or angiotensin II. *Br J Pharmacol* 42: 25-30

Hahr JY. 2015. Physiology of the Alzheimer's disease. *Med Hypotheses* 85: 944-6

Hahr JY. 2019. Physiology of aging. *Med Hypotheses* 123: 83-85

Haines DE. 2004. Neuroanatomy: An Atlas of Structures, Sections, and Systems: Lippincott Williams & Wilkins, 2004

Haller J, Bakos N, Szirmay M, Ledent C, Freund TF. 2002. The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur J Neurosci* 16: 1395-8

Hampson RE, Evans GJ, Mu J, Zhuang SY, King VC, et al. 1995. Role of cyclic AMP dependent protein kinase in cannabinoid receptor modulation of potassium "A-current" in cultured rat hippocampal neurons. *Life Sci* 56: 2081-8

Han J, Kesner P, Metna-Laurent M, Duan T, Xu L, et al. 2012. Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. *Cell* 148: 1039-50

Han X, Chen M, Wang F, Windrem M, Wang S, et al. 2013. Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice. *Cell Stem Cell* 12: 342-53

Hanus LO. 2007. Discovery and isolation of anandamide and other endocannabinoids. *Chem Biodivers* 4: 1828-41

Haring M, Enk V, Aparisi Rey A, Loch S, Ruiz de Azua I, et al. 2015. Cannabinoid type-1 receptor signaling in central serotonergic neurons regulates anxiety-like behavior and sociability. *Front Behav Neurosci* 9: 235

Harvey JA, Hunt HF. 1965. Effects of septal lesions on thirst in rats as indicated by water consumption and operant responding for water reward. *j Comp Physiol Psychol* 59: 49-56

Heal DJ, Smith SL, Gosden J, Nutt DJ. 2013. Amphetamine, past and present--a pharmacological and clinical perspective. *J Psychopharmacol* 27: 479-96

Hebert-Chatelain E, Desprez T, Serrat R, Bellocchio L, Soria-Gomez E, et al. 2016. A cannabinoid link between mitochondria and memory. *Nature* 539: 555-59

Heesen D, Stausberg A, Winkelmann W. 1976. [Polydipsia and polyuria as clinical leading symptoms of a hypercalcemia syndrome in primary hyperparathyroidism]. *Med Welt* 27: 1964-7

Herbert J, Forsling ML, Howes SR, Stacey PM, Shiers HM. 1992. Regional expression of c-fos antigen in the basal forebrain following intraventricular infusions of angiotensin and its modulation by drinking either water or saline. *Neuroscience* 51: 867-82

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. 1991. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* 11: 563-83

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, et al. 1990. Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A* 87: 1932-6

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

Herrero MT, Barcia C, Navarro JM. 2002. Functional anatomy of thalamus and basal ganglia. *Childs Nerv Syst* 18: 386-404

Hetherington AW, Ranson SW. 1942. The spontaneous activity and food intake of rats with hypothalamic lesion. *Am J Physiol* 136: 609-17

Higgs S, Williams CM, Kirkham TC. 2003. Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after delta(9)-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. *Psychopharmacology (Berl)* 165: 370-7

Hilairet S, Bouaboula M, Carriere D, Le Fur G, Casellas P. 2003. Hypersensitization of the Orexin 1 receptor by the CB1 receptor: evidence for cross-talk blocked by the specific CB1 antagonist, SR141716. *J Biol Chem* 278: 23731-7

Hill MN, Gorzalka BB. 2005. Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test. *Eur Neuropsychopharmacol* 15: 593-9

Hisaw FL. 1926. Experimental relaxation of the pubic ligament of guinea pig. *Proc. Soc. Exp. Biol. Med.* 23: 661-63

Hodges EL, Ashpole NM. 2019. Aging circadian rhythms and cannabinoids. *Neurobiol Aging* 79: 110-18

Hoffman AF, Lupica CR. 2000. Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *J Neurosci* 20: 2470-9

Hohmann AG, Suplita RL, 2nd. 2006. Endocannabinoid mechanisms of pain modulation. *AAPS J* 8: E693-708

Hollis, J.H., McKinley, M.J., D'Souza, M., Kampe, J. & Oldfield, B.J. 2008.The trajectory of sensory pathways from the lamina terminalis to the insular and cingulate cortex: a neuroanatomical framework for the generation of thirst. *Am J Physiol Regul Integr Comp Physiol* 294, R1390-1401.

Holmes JH, Gregersen MI. 1950. Role of sodium and chloride in thirst. *Am J Physiol* 162: 338-47

Houser CR, Crawford GD, Salvaterra PM, Vaughn JE. 1985. Immunocytochemical localization of choline acetyltransferase in rat cerebral cortex: a study of cholinergic neurons and synapses. *J Comp Neurol* 234: 17-34

Howlett AC. 2002. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat* 68-69: 619-31

Howlett AC. 2004. Efficacy in CB1 receptor-mediated signal transduction. *Br J Pharmacol* 142: 1209-18

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, et al. 2002. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 54: 161-202

Howlett AC, Mukhopadhyay S. 2000. Cellular signal transduction by anandamide and 2-arachidonoylglycerol. *Chem Phys Lipids* 108: 53-70

Hu F, Ren J, Zhang JE, Zhong W, Luo M. 2012. Natriuretic peptides block synaptic transmission by activating phosphodiesterase 2A and reducing presynaptic PKA activity. *Proc Natl Acad Sci U S A* 109: 17681-6

Huang CC, Lo SW, Hsu KS. 2001. Presynaptic mechanisms underlying cannabinoid inhibition of excitatory synaptic transmission in rat striatal neurons. *J Physiol* 532: 731-48

Huestis MA. 2005. Pharmacokinetics and metabolism of the plant cannabinoids, delta9-tetrahydrocannabinol, cannabidiol and cannabinol. *Handb Exp Pharmacol*: 657-90

Hyde TM, Miselis RR. 1984. Area postrema and adjacent nucleus of the solitary tract in water and sodium balance. *Am J Physiol* 247: R173-82

Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, et al. 2003. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci U S A* 100: 10529-33

Ichiki T, Augustine V, Oka Y. 2019. Neural populations for maintaining body fluid balance. *Curr Opin Neurobiol* 57: 134-40

Ignatowska-Jankowska BM, Ghosh S, Crowe MS, Kinsey SG, Niphakis MJ, et al. 2014. In vivo characterization of the highly selective monoacylglycerol lipase inhibitor KML29: antinociceptive activity without cannabimimetic side effects. *Br J Pharmacol* 171: 1392-407

Iovino M, Steardo L. 1984. Vasopressin release to central and peripheral angiotensin II in rats with lesions of the subfornical organ. *Brain Res* 322: 365-8

Iseger TA, Bossong MG. 2015. A systematic review of the antipsychotic properties of cannabidiol in humans. *Schizophr Res* 162: 153-61

Ishac EJ, Jiang L, Lake KD, Varga K, Abood ME, Kunos G. 1996. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. *Br J Pharmacol* 118: 2023-8

Jackson PL, Brunet E, Meltzoff AN, Decety J. 2006. Empathy examined through the neural mechanisms involved in imagining how I feel versus how you feel pain. *Neuropsychologia* 44: 752-61

Jamshidi N, Taylor DA. 2001. Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. *Br J Pharmacol* 134: 1151-4

Janssen S. 1936. Pharmakologische beeinflussung des durstes. *Arch Exptl Fathol Pharmakol* 181: 126-27

Jennings EA, Vaughan CW, Christie MJ. 2001. Cannabinoid actions on rat superficial medullary dorsal horn neurons in vitro. *J Physiol* 534: 805-12

Jequier E, Constant F. 2010. Water as an essential nutrient: the physiological basis of hydration. *Eur J Clin Nutr* 64: 115-23

Jhaveri MD, Richardson D, Chapman V. 2007. Endocannabinoid metabolism and uptake: novel targets for neuropathic and inflammatory pain. *Br J Pharmacol* 152: 624-32

Johnson RF, Beltz TG, Thunhorst RL, Johnson AK. 2003. Investigations on the physiological controls of water and saline intake in C57BL/6 mice. *Am J Physiol Regul Integr Comp Physiol* 285: R394-403

Julliard AK, Chaput MA, Apelbaum A, Aime P, Mahfouz M, Duchamp-Viret P. 2007. Changes in rat olfactory detection performance induced by orexin and leptin mimicking fasting and satiation. *Behav Brain Res* 183: 123-9

Justinova Z, Mangieri RA, Bortolato M, Chefer SI, Mukhin AG, et al. 2008. Fatty acid amide hydrolase inhibition heightens anandamide signaling without producing reinforcing effects in primates. *Biol Psychiatry* 64: 930-7

Kaczocha M, Glaser ST, Deutsch DG. 2009. Identification of intracellular carriers for the endocannabinoid anandamide. *Proc Natl Acad Sci U S A* 106: 6375-80

Kamprath K, Marsicano G, Tang J, Monory K, Bisogno T, et al. 2006. Cannabinoid CB1 receptor mediates fear extinction via habituation-like processes. *J Neurosci* 26: 6677-86

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. 2009. Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev* 89: 309-80

Karbarz MJ, Luo L, Chang L, Tham CS, Palmer JA, et al. 2009. Biochemical and biological properties of 4-(3-phenyl-[1,2,4] thiadiazol-5-yl)-piperazine-1-carboxylic acid phenylamide, a mechanism-based inhibitor of fatty acid amide hydrolase. *Anesth Analg* 108: 316-29

Karlsson M, Contreras JA, Hellman U, Tornqvist H, Holm C. 1997. cDNA cloning, tissue distribution, and identification of the catalytic triad of monoglyceride lipase. Evolutionary relationship to esterases, lysophospholipases, and haloperoxidases. *J Biol Chem* 272: 27218-23

Karniol IG, Shirakawa I, Takahashi RN, Knobel E, Musty RE. 1975. Effects of delta9-tetrahydrocannabinol and cannabinol in man. *Pharmacology* 13: 502-12

Katayama K, Ueda N, Kurahashi Y, Suzuki H, Yamamoto S, Kato I. 1997. Distribution of anandamide amidohydrolase in rat tissues with special reference to small intestine. *Biochim Biophys Acta* 1347: 212-8

Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, et al. 2003. Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med* 9: 76-81

Kaur C, Ling EA. 2017. The circumventricular organs. *Histol Histopathol* 32: 879-92

Kawamura Y, Fukaya M, Maejima T, Yoshida T, Miura E, et al. 2006. The CB1 cannabinoid receptor is the major cannabinoid receptor at excitatory presynaptic sites in the hippocampus and cerebellum. *J Neurosci* 26: 2991-3001

Kim J, Pignatelli M, Xu S, Itohara S, Tonegawa S. 2016. Antagonistic negative and positive neurons of the basolateral amygdala. *Nat Neurosci* 19: 1636-46

Kim J, Zhang X, Muralidhar S, LeBlanc SA, Tonegawa S. 2017. Basolateral to Central Amygdala Neural Circuits for Appetitive Behaviors. *Neuron* 93: 1464-79 e5

Kirkham TC, Williams CM. 2001. Synergistic efects of opioid and cannabinoid antagonists on food intake. *Psychopharmacology (Berl)* 153: 267-70

Kirkham TC, Williams CM, Fezza F, 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: 550-7

Kishimoto Y, Kano M. 2006. Endogenous cannabinoid signaling through the CB1 receptor is essential for cerebellum-dependent discrete motor learning. *J Neurosci* 26: 8829-37

Kissileff HR. 1969a. Food-associated drinking in the rat. *J Comp Physiol Psychol* 67: 284-300

Kissileff HR. 1969b. Oropharyngeal control of prandial drinking. *J Comp Physiol Psychol* 67: 309-19

Knepel W, Nutto D, Meyer DK. 1982. Effect of transection of subfornical organ efferent projections on vasopressin release induced by angiotensin or isoprenaline in the rat. *Brain Res* 248: 180-4

Kobilo T, Hazvi S, Dudai Y. 2007. Role of cortical cannabinoid CB1 receptor in conditioned taste aversion memory. *Eur J Neurosci* 25: 3417-21

Kong E, Monje FJ, Hirsch J, Pollak DD. 2014. Learning not to fear: neural correlates of learned safety. *Neuropsychopharmacology* 39: 515-27

Kosek M, Bern C, Guerrant RL. 2003. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull World Health Organ* 81: 197-204

Krashes MJ, Shah BP, Madara JC, Olson DP, Strochlic DE, et al. 2014. An excitatory paraventricular nucleus to AgRP neuron circuit that drives hunger. *Nature* 507: 238-42

Kreitzer AC, Malenka RC. 2005. Dopamine modulation of state-dependent endocannabinoid release and long-term depression in the striatum. *J Neurosci* 25: 10537-45

Kreitzer AC, Regehr WG. 2001. Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29: 717-27

Kruger DF, Gatcomb PM, Owen SK. 1999. Clinical implications of amylin and amylin deficiency. *Diabetes Educ* 25: 389-97; quiz 98

Kurt G, Woodworth HL, Fowler S, Bugescu R, Leinninger GM. 2019. Activation of lateral hypothalamic area neurotensin-expressing neurons promotes drinking. *Neuropharmacology* 154: 13-21

Lauckner JE, Hille B, Mackie K. 2005. The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB1 receptor coupling to Gq/11 G proteins. *Proc Natl Acad Sci U S A* 102: 19144-9

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: 1597-613

Lawlor PG. 2002. Delirium and dehydration: some fluid for thought? *Support Care Cancer* 10: 445-54

LBERT DJ, STORLIEN LH, WOOD DJ, EHMAN GK. 1970. Further evidence for a complex system controlling feeding behavior. *Physiol. Behavior* 5: 1075-7082

Le Foll B, Goldberg SR. 2004. Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences. *Neuroreport* 15: 2139-43

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, et al. 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* 283: 401-4

Lederis K. 1961. Vasopressin and oxytocin in the mammalian hypothalamus. *Gen Comp Endocrinol* 1: 80-9

LeDoux JE. 2000. Emotion circuits in the brain. *Annu Rev Neurosci* 23: 155-84

Lee NK, Jenner L, Harney A, Cameron J. 2018. Pharmacotherapy for amphetamine dependence: A systematic review. *Drug Alcohol Depend* 191: 309-37

Lehr D, Mallow J, Krukowski M. 1967. Copious drinking and simultaneous inhibition of urine flow elicited by beta-adrenergic stimulation and contrary effect of alpha-adrenergic stimulation. *J Pharmacol Exp Ther* 158: 150-63

Leib DE, Zimmerman CA, Knight ZA. 2016. Thirst. *Curr Biol* 26: R1260-R65

Leib DE, Zimmerman CA, Poormoghaddam A, Huey EL, Ahn JS, et al. 2017. The Forebrain Thirst Circuit Drives Drinking through Negative Reinforcement. *Neuron* 96: 1272-81 e4

Leibowitz SF. 1973. Histamine: a stimulatory effect on drinking behavior in the rat. *Brain Res* 63: 440-4

Leibowitz SF. 1979. *Histamine receptors*. pp. 219-253. Spectrum, New York.

Leng G, Mason WT, Dyer RG. 1982. The supraoptic nucleus as an osmoreceptor. *Neuroendocrinology* 34: 75-82

Leschke E. 1918. Ueber die Durstempfindung. *Arch Psychiat Nervenkrank* 59: 775-81

Leson G, Pless P. 2002. *Hemp seed and hemp oil. In: Grotenhermen, F., Russo, E., eds. Cannabis and cannabinoids.* pp. 411-25. New York: The Haworth Integrative Healing Press.

Letter， M. 2018. Cannabidiol (Epidiolex) for epilepsy. *Med Lett Drugs Ther* 60: 182-84

Leung D, Saghatelian A, Simon GM, Cravatt BF. 2006. Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry* 45: 4720-6

Levenes C, Daniel H, Soubrie P, Crepel F. 1998. Cannabinoids decrease excitatory synaptic transmission and impair long-term depression in rat cerebellar Purkinje cells. *J Physiol* 510 (Pt 3): 867-79

Levitt RA, Krikstone BJ. 1968. Cortical spreading depression and thirst. *Physiol Behavior* 3: 421-23

Li GL, Winter H, Arends R, Jay GW, Le V, et al. 2011. Assessment of the pharmacology and tolerability of PF-04457845, an irreversible inhibitor of fatty acid amide hydrolase-1, in healthy subjects. *Br. J. Clin. Pharmacol.* 75: 706-16

Li HL, Lin H. 1974. An archaeological and historical account of cannabis in China. *Econ. Bot.* 28: 437-47

Li X, Yu B, Sun Q, Zhang Y, Ren M, et al. 2018. Generation of a whole-brain atlas for the cholinergic system and mesoscopic projectome analysis of basal forebrain cholinergic neurons. *Proc Natl Acad Sci U S A* 115: 415-20

Lichtman AH, Varvel SA, Martin BR. 2002. Endocannabinoids in cognition and dependence. *Prostaglandins Leukot Essent Fatty Acids* 66: 269-85

Ligresti A, Cascio MG, Di Marzo V. 2005. Endocannabinoid metabolic pathways and enzymes. *Curr Drug Targets CNS Neurol Disord* 4: 615-23

Ligresti A, Villano R, Allara M, Ujvary I, Di Marzo V. 2012. Kavalactones and the endocannabinoid system: the plant-derived yangonin is a novel CB(1) receptor ligand. *Pharmacol Res* 66: 163-9

Lindheimer MD, Barron WM, Davison JM. 1989. Osmoregulation of thirst and vasopressin release in pregnancy. *Am J Physiol* 257: F159-69

Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, et al. 2008. Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology* 54: 1-7

Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, et al. 2006. A biosynthetic pathway for anandamide. *Proc Natl Acad Sci U S A* 103: 13345-50

Llano I, Leresche N, Marty A. 1991. Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. *Neuron* 6: 565-74

Long JZ, Li W, Booker L, Burston JJ, Kinsey SG, et al. 2009a. Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat Chem Biol* 5: 37-44

Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, et al. 2009b. Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. *Proc Natl Acad Sci U S A* 106: 20270-5

Longet. 1868. Traité de Physiologie. *Pqris* 1: 35

Lund PK, Goodman RH, Dee PC, Habener JF. 1982. Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. *Proc Natl Acad Sci U S A* 79: 345-9

Lutz B, Marsicano G, Maldonado R, Hillard CJ. 2015. The endocannabinoid system in guarding against fear, anxiety and stress. *Nat Rev Neurosci* 16: 705-18

Lutz TA, Del Prete E, Scharrer E. 1994. Reduction of food intake in rats by intraperitoneal injection of low doses of amylin. *Physiol Behav* 55: 891-5

Lymperopoulos A, Rengo G, Koch WJ. 2013. Adrenergic nervous system in heart failure: pathophysiology and therapy. *Circ Res* 113: 739-53

Ma L, Zhang Y, Yue L, Zhang X, Cui S, et al. 2019. Anterior cingulate cortex modulates the affective-motivative dimension of hyperosmolality-induced thirst. *J Physiol*

Macchia DD. 1987. Atrial natriuretic factor: a hormone secreted by the heart. *Pharm Weekbl Sci* 9: 305-14

Mackie K. 2005. Cannabinoid receptor homo- and heterodimerization. *Life Sci* 77: 1667-73

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: 6552-61

Madsen P, Rasmussen HH, Leffers H, Honore B, Celis JE. 1992. Molecular cloning and expression of a novel keratinocyte protein (psoriasis-associated fatty acid-binding protein [PA-FABP]) that is highly up-regulated in psoriatic skin and that shares similarity to fatty acid-binding proteins. *J Invest Dermatol* 99: 299-305

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: 463-75

Magendie F. 1823. Historie d'un hydrophobe, traité à l'Hôtel-Dieu de Paris, au moyen de l'injection de l'eau dans les veines. *J Physiol Exptl Pathol* 3: 382-92

Mahadevan A, Siegel C, Martin BR, Abood ME, Beletskaya I, Razdan RK. 2000. Novel cannabinol probes for CB1 and CB2 cannabinoid receptors. *J Med Chem* 43: 3778-85

Mailleux P, Vanderhaeghen JJ. 1992. Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. *Neuroscience* 48: 655-68

Makela P, Wakeley J, Gijsman H, Robson PJ, Bhagwagar Z, Rogers RD. 2006. Low doses of delta-9 tetrahydrocannabinol (THC) have divergent effects on short-term spatial memory in young, healthy adults. *Neuropsychopharmacology* 31: 462-70

Makriyannis A, Tian X, Guo J. 2005. How lipophilic cannabinergic ligands reach their receptor sites. *Prostaglandins Other Lipid Mediat* 77: 210-8

Maldonado R, Valverde O, Berrendero F. 2006. Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci* 29: 225-32

Mandelblat-Cerf Y, Kim A, Burgess CR, Subramanian S, Tannous BA, et al. 2017. Bidirectional Anticipation of Future Osmotic Challenges by Vasopressin Neurons. *Neuron* 93: 57-65

Manz F, Wentz A. 2005. The importance of good hydration for the prevention of chronic diseases. *Nutr Rev* 63: S2-5

Manzanares J, Julian M, Carrascosa A. 2006. Role of the cannabinoid system in pain control and therapeutic implications for the management of acute and chronic pain episodes. *Curr Neuropharmacol* 4: 239-57

Marcellino D, Carriba P, Filip M, Borgkvist A, Frankowska M, et al. 2008. Antagonistic cannabinoid CB1/dopamine D2 receptor interactions in striatal CB1/D2 heteromers. A combined neurochemical and behavioral analysis. *Neuropharmacology* 54: 815-23

Maren S. 2001. Neurobiology of Pavlovian fear conditioning. *Annu Rev Neurosci* 24: 897-931

Marieb E. 2001. *Human anatomy & physiology.* pp. 414. San Francisco: Benjamin Cummings.

Markopoulos F, Rokni D, Gire DH, Murthy VN. 2012. Functional properties of cortical feedback projections to the olfactory bulb. *Neuron* 76: 1175-88

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, et al. 2003. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302: 84-8

Marsicano G, Lutz B. 1999. Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *Eur J Neurosci* 11: 4213-25

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, et al. 2002. The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418: 530-4

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. 2002. Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl)* 159: 379-87

Massey PV, Bashir ZI. 2007. Long-term depression: multiple forms and implications for brain function. *Trends Neurosci* 30: 176-84

Matsuda LA, Bonner TI, Lolait SJ. 1993. Localization of cannabinoid receptor mRNA in rat brain. *J Comp Neurol* 327: 535-50

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 346: 561-4

Matsuda T, Hiyama TY, Niimura F, Matsusaka T, Fukamizu A, et al. 2017. Distinct neural mechanisms for the control of thirst and salt appetite in the subfornical organ. *Nat Neurosci* 20: 230-41

Matyas F, Yanovsky Y, Mackie K, Kelsch W, Misgeld U, Freund TF. 2006. Subcellular localization of type 1 cannabinoid receptors in the rat basal ganglia. *Neuroscience* 137: 337-61

Maughan RJ, Shirreffs SM, Watson P. 2007a. Exercise, heat, hydration and the brain. *J Am Coll Nutr* 26: 604S-12S

Maughan RJ, Watson P, Shirreffs SM. 2007b. Heat and cold : what does the environment do to the marathon runner? *Sports Med* 37: 396-9

Mayer A. 1900. Variations de la tension osmotique du sang chez les animarux privés de liquides. *Compt Rend Soc Biol* 52: 153-55

McAllister SD, Glass M. 2002. CB(1) and CB(2) receptor-mediated signalling: a focus on endocannabinoids. *Prostaglandins Leukot Essent Fatty Acids* 66: 161-71

McGregor IS, Dastur FN, McLellan RA, Brown RE. 1996. Cannabinoid modulation of rat pup ultrasonic vocalizations. *Eur J Pharmacol* 313: 43-9

McKay NJ, Daniels D. 2013. Glucagon-like peptide-1 receptor agonist administration suppresses both water and saline intake in rats. *J Neuroendocrinol* 25: 929-38

McKay NJ, Galante DL, Daniels D. 2014. Endogenous glucagon-like peptide-1 reduces drinking behavior and is differentially engaged by water and food intakes in rats. *J Neurosci* 34: 16417-23

McKay NJ, Kanoski SE, Hayes MR, Daniels D. 2011. Glucagon-like peptide-1 receptor agonists suppress water intake independent of effects on food intake. *Am J Physiol Regul Integr Comp Physiol* 301: R1755-64

McKinley MJ, Denton DA, Oldfield BJ, De Oliveira LB, Mathai ML. 2006. Water intake and the neural correlates of the consciousness of thirst. *Semin Nephrol* 26: 249-57

McKinley MP. 2011. *Human Anatomy, 3rd Edition* McGraw-Hill; 3rd edition (January 5, 2011).

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, Parker LA, Gallily R. 2002. Cannabidiol: an overview of some pharmacological aspects. *J Clin Pharmacol* 42: 11S-19S

Merck S-A. Cannabinoid receptor.

Metna-Laurent M, Soria-Gomez E, Verrier D, Conforzi M, Jego P, et al. 2012. Bimodal control of fear-coping strategies by CB(1) cannabinoid receptors. *J Neurosci* 32: 7109-18

Mietlicki-Baase EG, McGrath LE, Koch-Laskowski K, Krawczyk J, Reiner DJ, et al. 2017. Amylin receptor activation in the ventral tegmental area reduces motivated ingestive behavior. *Neuropharmacology* 123: 67-79

Mikuriya TH. 1969. Marijuana in medicine: past, present and future. *Calif Med* 110: 34-40

Miller NE. 1965. Chemical Coding of Behavior in the Brain. *Science* 148: 328-38

Mitchell PB, Morris MJ. 2007. Depression and anxiety with rimonabant. *Lancet* 370: 1671-2

Mogenson G, J,. 1968. Effects of amphetamne on self-stimulation and induced drinking. *Physiol Behavior* 3: 133-36

Mogenson G, J,. 1969. Water deprivation and excessive water intake during self-stimulation. *Physiol Behavior* 4: 393-97

Mogenson G, J,, Stevenson JAF. 1966. Drinking and self-stimulation with electrical stimulation of the lateral hypothalamus. *Physiol Behavior* 1: 251-54

Mohammad-Zadeh LF, Moses L, Gwaltney-Brant SM. 2008. Serotonin: a review. *J Vet Pharmacol Ther* 31: 187-99

Monory K, Blaudzun H, Massa F, Kaiser N, Lemberger T, et al. 2007. Genetic dissection of behavioural and autonomic effects of Delta(9)-tetrahydrocannabinol in mice. *PLoS Biol* 5: e269

Monory K, Massa F, Egertova M, Eder M, Blaudzun H, et al. 2006. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* 51: 455-66

Monory K, Tzavara ET, Leximе J, Ledent C, Parmentier M, et al. 2002. Novel, not adenylyl cyclase-coupled cannabinoid binding site in cerebellum of mice. *Biochem Biophys Res Commun* 292: 231-5

Montain SJ, Coyle EF. 1992. Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *J Appl Physiol (1985)* 73: 1340-50

Montemurro DG, Stevenson JA. 1957. Adipsia produced by hypothalamic lesions in the rat. *Can J Biochem Physiol* 35: 31-7

Moreau JJ. 1845. *Du Hachisch et de l'Alienation Mentale: Etudes Psychologiques.*: Paris: Librarie de Fortin Mason.

Morgane PJ. 1961a. Alterations in feeding and drinking behavior of rats with lesions in globi pallidi. *Am J Physiol* 201: 420-8

Morgane PJ. 1961b. Distinct "feeding" and "hunger motivating" systems in the lateral hypothalamus of the rat. *Science* 133: 887-8

Morgane PJ. 1964. *Limbic-hypothalamic-midbrain interaction in thirst and thirst motivated behavior. In: Thirst: Prog. First Intern. Symp. Thirst in the regulation of Body Water.* pp. 429-453. Oxford: Pergamon.

Morita N, Saad WA, Camargo LA, Silva Neto CR, Antunes-Rodrigues J, Covian MR. 1980. Interaction between the septal area and the subfornical organ in the control of water intake induced by thirst-eliciting procedures. *Physiol Behav* 24: 243-9

Morley JE, Suarez MD, Mattamal M, Flood JF. 1997. Amylin and food intake in mice: effects on motivation to eat and mechanism of action. *Pharmacol Biochem Behav* 56: 123-9

Morris RG, Garrud P, Rawlins JN, O'Keefe J. 1982. Place navigation impaired in rats with hippocampal lesions. *Nature* 297: 681-3

Morrison SD, Barrnett RJ, Mayer J. 1958. Localization of lesions in the lateral hypothalamus of rats with induced adipsia and aphagia. *Am J Physiol* 193: 230-4

Mountford D. 1969. Alterations in drinking following isoproterenol stimulation of hippocampus. *Physiolgist* 12: 309

Mu J, Zhuang SY, Kirby MT, Hampson RE, Deadwyler SA. 1999. Cannabinoid receptors differentially modulate potassium A and D currents in hippocampal neurons in culture. *J Pharmacol Exp Ther* 291: 893-902

Mukhopadhyay S, Shim JY, Assi AA, Norford D, Howlett AC. 2002. CB(1) cannabinoid receptor-G protein association: a possible mechanism for differential signaling. *Chem Phys Lipids* 121: 91-109

Munro S, Thomas KL, Abu-Shaar M. 1993. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365: 61-5

Murray B. 2007. Hydration and physical performance. *J Am Coll Nutr* 26: 542S-48S

Myers RD. 1964. *Modification of drinking patterns by choronic intracranial chemical infusion. In: Thirst: Prog. First Intern. Symp. Thirst in the regulation of Body Water.* pp. 533-549. Oxford: Pergamon.

Myers RD, Sharpe LG. 1968. Chemical activation of ingestive and other hypothalamic regulatory mechanisms. *Physiol Behavior* 3: 987-95

Nagayama T, Sinor AD, Simon RP, Chen J, Graham SH, et al. 1999. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J Neurosci* 19: 2987-95

Nakazi M, Bauer U, Nickel T, Kathmann M, Schlicker E. 2000. Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB1 receptors. *Naunyn Schmiedebergs Arch Pharmacol* 361: 19-24

Nation HL, Nicoleau M, Kinsman BJ, Browning KN, Stocker SD. 2016. DREADD-induced activation of subfornical organ neurons stimulates thirst and salt appetite. *J Neurophysiol* 115: 3123-9

Nauta WJ. 1958. Hippocampal projections and related neural pathways to the midbrain in the cat. *Brain* 81: 319-40

Navarro M, Hernandez E, Munoz RM, del Arco I, Villanua MA, et al. 1997. Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. *Neuroreport* 8: 491-6

Navarro M, Rodriquez de Fonseca F, Alvarez E, Chowen JA, Zueco JA, et al. 1996. Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. *J Neurochem* 67: 1982-91

NCBI-Gene. 2009. CNR1 cannabinoid receptor 1 [Homo sapiens (human)]. NCBI Gene

Négro-Vilar A, Gentil GG, Covian MR. 1967. Alterations in sodium chloride and water intake after septal lesions in rat. *Physiol Behavior* 2: 167-70

Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. 2002. Neurobiology of depression. *Neuron* 34: 13-25

Nielsen S, Chou CL, Marples D, Christensen EI, Kishore BK, Knepper MA. 1995. Vasopressin increases water permeability of kidney collecting duct by inducing translocation of aquaporin-CD water channels to plasma membrane. *Proc Natl Acad Sci U S A* 92: 1013-7

NIMH. 2016. Depression. NIMH

Novin D. 1964. *The effects of insulin on water intake in the rat. In: Thirst: Prog. First Intern. Symp. Thirst in the regulation of Body Water.* pp. 177-182. Oxford: Pergamon.

Nyiri G, Cserep C, Szabadits E, Mackie K, Freund TF. 2005. CB1 cannabinoid receptors are enriched in the perisynaptic annulus and on preterminal segments of hippocampal GABAergic axons. *Neuroscience* 136: 811-22

O'Sullivan SE. 2007. Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Br J Pharmacol* 152: 576-82

Oddi S, Fezza F, Pasquariello N, D'Agostino A, Catanzaro G, et al. 2009. Molecular identification of albumin and Hsp70 as cytosolic anandamide-binding proteins. *Chem Biol* 16: 624-32

Ohman LE, Johnson AK. 1989. Brain stem mechanisms and the inhibition of angiotensin-induced drinking. *Am J Physiol* 256: R264-9

Ohno-Shosaku T, Maejima T, Kano M. 2001. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. *Neuron* 29: 729-38

Oka Y, Ye M, Zuker CS. 2015. Thirst driving and suppressing signals encoded by distinct neural populations in the brain. *Nature* 520: 349-52

Ortega-Gutierrez S, Hawkins EG, Viso A, Lopez-Rodriguez ML, Cravatt BF. 2004. Comparison of anandamide transport in FAAH wild-type and knockout neurons: evidence for contributions by both FAAH and the CB1 receptor to anandamide uptake. *Biochemistry* 43: 8184-90

Pacioni G, Rapino C, Zarivi O, Falconi A, Leonardi M, et al. 2015. Truffles contain endocannabinoid metabolic enzymes and anandamide. *Phytochemistry* 110: 104-10

Page IH. 1939. On the Nature of the Pressor Action of Renin. *J Exp Med* 70: 521-42

Page IH, Helmer OM. 1940. A Crystalline Pressor Substance (Angiotonin) Resulting from the Reaction between Renin and Renin-Activator. *J Exp Med* 71: 29-42

Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R. 2006. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev* 27: 73-100

Paik IY, Jeong MH, Jin HE, Kim YI, Suh AR, et al. 2009. Fluid replacement following dehydration reduces oxidative stress during recovery. *Biochem Biophys Res Commun* 383: 103-7

Pamplona FA, Ferreira J, Menezes de Lima O, Jr., Duarte FS, Bento AF, et al. 2012. Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. *Proc Natl Acad Sci U S A* 109: 21134-9

Pamplona FA, Prediger RD, Pandolfo P, Takahashi RN. 2006. The cannabinoid receptor agonist WIN 55,212-2 facilitates the extinction of contextual fear memory and spatial memory in rats. *Psychopharmacology (Berl)* 188: 641-9

Pamplona FA, Takahashi RN. 2006. WIN 55212-2 impairs contextual fear conditioning through the activation of CB1 cannabinoid receptors. *Neurosci Lett* 397: 88-92

Pan B, Hillard CJ, Liu QS. 2008a. D2 dopamine receptor activation facilitates endocannabinoid-mediated long-term synaptic depression of GABAergic synaptic transmission in midbrain dopamine neurons via cAMP-protein kinase A signaling. *J Neurosci* 28: 14018-30

Pan B, Hillard CJ, Liu QS. 2008b. Endocannabinoid signaling mediates cocaine-induced inhibitory synaptic plasticity in midbrain dopamine neurons. *J Neurosci* 28: 1385-97

Panikashvili D, Mechoulam R, Beni SM, Alexandrovich A, Shohami E. 2005. CB1 cannabinoid receptors are involved in neuroprotection via NF-kappa B inhibition. *J Cereb Blood Flow Metab* 25: 477-84

Panikashvili D, Simeonidou C, Ben-Shabat S, Hanus L, Breuer A, et al. 2001. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature* 413: 527-31

Panula P, Chazot PL, Cowart M, Gutzmer R, Leurs R, et al. 2015. International Union of Basic and Clinical Pharmacology. XCVIII. Histamine Receptors. *Pharmacol Rev* 67: 601-55

Pardo JV, Pardo PJ, Janer KW, Raichle ME. 1990. The anterior cingulate cortex mediates processing selection in the Stroop attentional conflict paradigm. *Proc Natl Acad Sci U S A* 87: 256-9

Parent M, Parent A. 2004. The pallidofugal motor fiber system in primates. *Parkinsonism Relat Disord* 10: 203-11

Paris M, Nahas GG. 1984. *Botany: the unstabilizad species. In: Nahas GG, editor. Marihuana in science and medicine.* pp. 3-36. New York: Raven Press.

Parker SW, Feldman SM. 1967. Effect of mesencephalic lesions on feeding behavior in rats. *Exp Neurol* 17: 313-26

Pastuskovas CV, Cassell MD, Johnson AK, Thunhorst RL. 2003. Increased cellular activity in rat insular cortex after water and salt ingestion induced by fluid depletion. *Am J Physiol Regul Integr Comp Physiol* 284: R1119-25

Patton AP, Hastings MH. 2018. The suprachiasmatic nucleus. *Curr Biol* 28: R816-R22

Paul ML, Graybiel AM, David JC, Robertson HA. 1992. D1-like and D2-like dopamine receptors synergistically activate rotation and c-fos expression in the dopamine-depleted striatum in a rat model of Parkinson's disease. *J Neurosci* 12: 3729-42

Pavlov PI. 1927. *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. Oxford University Press, London.

Peck JW, Blass EM. 1975. Localization of thirst and antidiuretic osmoreceptors by intracranial injections in rats. *Am J Physiol* 228: 1501-09

Pertwee RG. 1997. Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther* 74: 129-80

Pertwee RG. 2014. Elevating endocannabinoid levels: pharmacological strategies and potential therapeutic applications. *Proc Nutr Soc* 73: 96-105

Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, et al. 2010. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). *Pharmacol Rev* 62: 588-631

Peskar B, Leodolter S, Hertting G. 1970. [Effect of various blood pressure-lowering drugs on water uptake and output in rats]. *Naunyn Schmiedebergs Arch Pharmakol* 265: 335-46

Petersen G, Hansen HS. 1999. N-acylphosphatidylethanolamine-hydrolysing phospholipase D lacks the ability to transphosphatidylate. *FEBS Lett* 455: 41-4

Petitet F, Jeantaud B, Reibaud M, Imperato A, Dubroeucq MC. 1998. Complex pharmacology of natural cannabinoids: evidence for partial agonist activity of delta9-tetrahydrocannabinol and antagonist activity of cannabidiol on rat brain cannabinoid receptors. *Life Sci* 63: PL1-6

Petrosino S, Di Marzo V. 2010. FAAH and MAGL inhibitors: therapeutic opportunities from regulating endocannabinoid levels. *Curr Opin Investig Drugs* 11: 51-62

Petrzilka T, Sikemeier C. 1967. [Conversion from (-)-delta-6,1-3,4-trans-tetrahydrocannabinol in (-)-delta-1,2-3,4-trans-tetrahydrocannabinol]. *Helv Chim Acta* 50: 2111-3

Phillips PA, Rolls BJ, Ledingham JG, Morton JJ. 1984. Body fluid changes, thirst and drinking in man during free access to water. *Physiol Behav* 33: 357-63

Piazza PV, Cota D, Marsicano G. 2017. The CB1 Receptor as the Cornerstone of Exostasis. *Neuron* 93: 1252-74

Pickford M. 1939. The inhibitory effect of acetylcholine on water diuresis in the dog, and its pituitary transmission. *J Physiol* 95: 226-38

Pickford M. 1947. The action of acetylcholine in the supraoptic nucleus of the chloralosed dog. *J Physiol* 106: 264-70

Piet R, Vargova L, Sykova E, Poulain DA, Oliet SH. 2004. Physiological contribution of the astrocytic environment of neurons to intersynaptic crosstalk. *Proc Natl Acad Sci U S A* 101: 2151-5

Pietropaolo S, Bellocchio L, Ruiz-Calvo A, Cabanas M, Du Z, et al. 2015. Chronic cannabinoid receptor stimulation selectively prevents motor impairments in a mouse model of Huntington's disease. *Neuropharmacology* 89: 368-74

Piomelli D. 2014. More surprises lying ahead. The endocannabinoids keep us guessing. *Neuropharmacology* 76 Pt B: 228-34

Pisanti S, Malfitano AM, Ciaglia E, Lamberti A, Ranieri R, et al. 2017. Cannabidiol: State of the art and new challenges for therapeutic applications. *Pharmacol Ther* 175: 133-50

Pitler TA, Alger BE. 1992. Postsynaptic spike firing reduces synaptic GABAA responses in hippocampal pyramidal cells. *J Neurosci* 12: 4122-32

Plant TM, Zeleznik AJ. 2015. *Knobil and Neill's Physiology of Reproduction*.

Poat JA, Sumners C, Woodruff GN. 1980. The effects of centrally administered dopamine and 2-amino-6,7-dihydroxyl-1,2,3,4-tetrahydronaphthalene. *Br J Pharmacol* 70: 151-52

Pope HG, Jr., Jacobs A, Mialet JP, Yurgelun-Todd D, Gruber S. 1997. Evidence for a sex-specific residual effect of cannabis on visuospatial memory. *Psychother Psychosom* 66: 179-84

Popkin BM, D'Anci KE, Rosenberg IH. 2010. Water, hydration, and health. *Nutr Rev* 68: 439-58

Porsolt RD, Le Pichon M, Jalfre M. 1977. Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266: 730-2

Porter SR, Scully C, Hegarty AM. 2004. An update of the etiology and management of xerostomia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 97: 28-46

Prager-Khoutorsky M, Bourque CW. 2010. Osmosensation in vasopressin neurons: changing actin density to optimize function. *Trends Neurosci* 33: 76-83

Purves D. 2011. *Neuroscience (5th ed.)*. pp. 507. Sunderland, Mass.: Sinauer.

Qin C, Li J, Tang K. 2018. The Paraventricular Nucleus of the Hypothalamus: Development, Function, and Human Diseases. *Endocrinology* 159: 3458-72

Raduner S, Majewska A, Chen JZ, Xie XQ, Hamon J, et al. 2006. Alkylamides from Echinacea are a new class of cannabinomimetics. Cannabinoid type 2 receptor-dependent and -independent immunomodulatory effects. *J Biol Chem* 281: 14192-206

Reinoso-Suarez F, De Andres I, Rodrigo-Angulo ML, Rodriguez-Veiga E. 1994. Location and anatomical connections of a paradoxical sleep induction site in the cat ventral pontine tegmentum. *Eur J Neurosci* 6: 1829-36

Remmers F, Lange MD, Hamann M, Ruehle S, Pape HC, Lutz B. 2017. Addressing sufficiency of the CB1 receptor for endocannabinoid-mediated functions through conditional genetic rescue in forebrain GABAergic neurons. *Brain Struct Funct* 222: 3431-52

Rescorla RA. 1996. Preservation of Pavlovian Associations through Extinction. *The Quarterly Journal of Experimental Psychology Section B-Comparative and Physiological Psychology*: 245-58

Rettig R, Ganten D, Johnson AK. 1981. Isoproterenol-induced thirst: renal and extrarenal mechanisms. *Am J Physiol* 241: R152-7

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: 2624-34

Richardson JD, Aanonsen L, Hargreaves KM. 1998. Hypoactivity of the spinal cannabinoid system results in NMDA-dependent hyperalgesia. *J Neurosci* 18: 451-7

Riediger T, Rauch M, Schmid HA. 1999. Actions of amylin on subfornical organ neurons and on drinking behavior in rats. *Am J Physiol* 276: R514-21

Ritz P, Berrut G. 2005. The importance of good hydration for day-to-day health. *Nutr Rev* 63: S6-13

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: 109-16

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: 8384-8

Robbins TW, Everitt BJ. 1996. Neurobehavioural mechanisms of reward and motivation. *Curr Opin Neurobiol* 6: 228-36

Robertson GL, Shelton RL, Athar S. 1976. The osmoregulation of vasopressin. *Kidney Int* 10: 25-37

Robinson BW, Mishkin M. 1968. Alimentary responses to forebrain stimulation in monkeys. *Exp Brain Res* 4: 330-66

Robinson L, McKillop-Smith S, Ross NL, Pertwee RG, Hampson RE, et al. 2008. Hippocampal endocannabinoids inhibit spatial learning and limit spatial memory in rats. *Psychopharmacology (Berl)* 198: 551-63

Rodriguez EM, Rodriguez S, Hein S. 1998. The subcommissural organ. *Microsc Res Tech* 41: 98-123

Ronesi J, Gerdeman GL, Lovinger DM. 2004. Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. *J Neurosci* 24: 1673-9

Roumelioti ME, Glew RH, Khitan ZJ, Rondon-Berrios H, Argyropoulos CP, et al. 2018. Fluid balance concepts in medicine: Principles and practice. *World J Nephrol* 7: 1-28

Routtenberg A, Simpson JB. 1971. Carbachol-induced drinking at ventricular and subfornical organ sites of application. *Life Sci I* 10: 481-90

Ruch T, Patton H, Brobeck J. 1942. Hperphagia and adiposity in relation to disturbances of taste. *Federation Proc* 1: 76

Rudmann DG. 2013. On-target and off-target-based toxicologic effects. *Toxicol Pathol* 41: 310-4

Ruehle S, Remmers F, Romo-Parra H, Massa F, Wickert M, et al. 2013. Cannabinoid CB1 receptor in dorsal telencephalic glutamatergic neurons: distinctive sufficiency for hippocampus-dependent and amygdala-dependent synaptic and behavioral functions. *J Neurosci* 33: 10264-77

Ruginsk SG, Vechiato FM, Uchoa ET, Elias LL, Antunes-Rodrigues J. 2015. Type 1 cannabinoid receptor modulates water deprivation-induced

homeostatic responses. *Am J Physiol Regul Integr Comp Physiol* 309: R1358-68

Ruiz-Calvo A, Bajo-Graneras R, Maroto IB, Zian D, Grabner GF, et al. 2019. Astroglial monoacylglycerol lipase controls mutant huntingtin-induced damage of striatal neurons. *Neuropharmacology* 150: 134-44

Rullier. 1821. Dictionnqire des science médicales par une société de médecins et de chirurgiens. *Paris: Panckoucke* 51: 488-90

Ryan PJ, Ross SI, Campos CA, Derkach VA, Palmiter RD. 2017. Oxytocin-receptor-expressing neurons in the parabrachial nucleus regulate fluid intake. *Nat Neurosci* 20: 1722-33

Ryberg E, Vu HK, Larsson N, Groblewski T, Hjorth S, et al. 2005. Identification and characterisation of a novel splice variant of the human CB1 receptor. *FEBS Lett* 579: 259-64

Saario SM, Laitinen JT. 2007. Therapeutic potential of endocannabinoid-hydrolysing enzyme inhibitors. *Basic Clin Pharmacol Toxicol* 101: 287-93

Saario SM, Salo OM, Nevalainen T, Poso A, Laitinen JT, et al. 2005. Characterization of the sulfhydryl-sensitive site in the enzyme responsible for hydrolysis of 2-arachidonoyl-glycerol in rat cerebellar membranes. *Chem Biol* 12: 649-56

Saario SM, Savinainen JR, Laitinen JT, Jarvinen T, Niemi R. 2004. Monoglyceride lipase-like enzymatic activity is responsible for hydrolysis of 2-arachidonoylglycerol in rat cerebellar membranes. *Biochem Pharmacol* 67: 1381-7

Sah P, Faber ES, Lopez De Armentia M, Power J. 2003. The amygdaloid complex: anatomy and physiology. *Physiol Rev* 83: 803-34

Saker P, Farrell MJ, Adib FR, Egan GF, McKinley MJ, Denton DA. 2014. Regional brain responses associated with drinking water during thirst and after its satiation. *Proc Natl Acad Sci U S A* 111: 5379-84

Saker P, Farrell MJ, Egan GF, McKinley MJ, Denton DA. 2016. Overdrinking, swallowing inhibition, and regional brain responses prior to swallowing. *Proc Natl Acad Sci U S A* 113: 12274-79

Saker P, Farrell MJ, Egan GF, McKinley MJ, Denton DA. 2018. Influence of anterior midcingulate cortex on drinking behavior during thirst and following satiation. *Proc Natl Acad Sci U S A* 115: 786-91

Sanchez C, Galve-Roperh I, Rueda D, Guzman M. 1998. Involvement of sphingomyelin hydrolysis and the mitogen-activated protein kinase cascade in the Delta9-tetrahydrocannabinol-induced stimulation of glucose metabolism in primary astrocytes. *Mol Pharmacol* 54: 834-43

Santello M, Toni N, Volterra A. 2019. Astrocyte function from information processing to cognition and cognitive impairment. *Nat Neurosci* 22: 154-66

Sarter M, Parikh V. 2005. Choline transporters, cholinergic transmission and cognition. *Nat Rev Neurosci* 6: 48-56

Savinainen JR, Saario SM, Laitinen JT. 2012. The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. *Acta Physiol (Oxf)* 204: 267-76

Savinainen JR, Yoshino M, Minkkila A, Nevalainen T, Laitinen JT. 2010. Characterization of binding properties of monoglyceride lipase inhibitors by a versatile fluorescence-based technique. *Anal Biochem* 399: 132-4

Sawka MN, Noakes TD. 2007. Does dehydration impair exercise performance? *Med Sci Sports Exerc* 39: 1209-17

Schiff HC, Bouhuis AL, Yu K, Penzo MA, Li H, et al. 2018. An Insula-Central Amygdala Circuit for Guiding Tastant-Reinforced Choice Behavior. *J Neurosci* 38: 1418-29

Schlicker E, Kathmann M. 2001. Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci* 22: 565-72

Schlosburg JE, Blankman JL, Long JZ, Nomura DK, Pan B, et al. 2010. Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. *Nat Neurosci* 13: 1113-9

Schweitzer P. 2000. Cannabinoids decrease the K(+) M-current in hippocampal CA1 neurons. *J Neurosci* 20: 51-8

Scroop GC, Lowe RD. 1968. Central pressor effect of angiotensin mediated by the parasympathetic nervous system. *Nature* 220: 1331-2

Seligman ME, Walker EF, Rosenhan DL. 2000. *Abnormal psychology (4th ed.)*. New York: W.W. Norton & Company.

Severs WB, Summy-Long J, Taylor JS, Connor JD. 1970. A central effect of angiotensin: release of pituitary pressor material. *J Pharmacol Exp Ther* 174: 27-34

Sevinc G, Gurvit H, Spreng RN. 2017. Salience network engagement with the detection of morally laden information. *Soc Cogn Affect Neurosci* 12: 1118-27

Seymour B, Dolan R. 2008. Emotion, decision making, and the amygdala. *Neuron* 58: 662-71

Sharpe LG, Myers RD. 1969. Feeding and drinking following stimulation of the diencephalon of the monkey with amines and other substances. *Exp Brain Res* 8: 295-310

Shepherd GM. 1972. Synaptic organization of the mammalian olfactory bulb. *Physiol Rev* 52: 864-917

Sherman SM. 2017. Functioning of Circuits Connecting Thalamus and Cortex. *Compr Physiol* 7: 713-39

Shimizu H, Watanabe E, Hiyama TY, Nagakura A, Fujikawa A, et al. 2007. Glial Nax channels control lactate signaling to neurons for brain [Na+] sensing. *Neuron* 54: 59-72

Shire D, Calandra B, Rinaldi-Carmona M, Oustric D, Pessegue B, et al. 1996. Molecular cloning, expression and function of the murine CB2 peripheral cannabinoid receptor. *Biochim Biophys Acta* 1307: 132-6

Shire D, Carillon C, Kaghad M, Calandra B, Rinaldi-Carmona M, et al. 1995. An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. *J Biol Chem* 270: 3726-31

Shirreffs SM, Merson SJ, Fraser SM, Archer DT. 2004. The effects of fluid restriction on hydration status and subjective feelings in man. *Br J Nutr* 91: 951-8

Sigel E, Baur R, Racz I, Marazzi J, Smart TG, et al. 2011. The major central endocannabinoid directly acts at GABA(A) receptors. *Proc Natl Acad Sci U S A* 108: 18150-5

Simon GM, Cravatt BF. 2006. Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. *J Biol Chem* 281: 26465-72

Simpson JB, Routtenberg A. 1972. The subfornical organ and carbachol-induced drinking. *Brain Res* 45: 135-52

Simpson JB, Routtenberg A. 1973. Subfornical organ: site of drinking elicitation by angiotensin II. *Science* 181: 1172-5

Skultety FM. 1958. The behavioral effects of destructive lesions of the periaqueductal gray matter in adult cats. *J Comp Neurol* 110: 337-65

Skultety FM. 1966. Changes in caloric intake following brain stem lesions. I. Preoperative observations. *Arch Neurol* 14: 428-37

Skultety FM, Gary TM. 1962. Experimental hyperphagia in cats following destructive mid-brain lesions. *Neurology* 12: 394-401

Solinas M, Tanda G, Justinova Z, Wertheim CE, Yasar S, et al. 2007. The endogenous cannabinoid anandamide produces delta-9-tetrahydrocannabinol-like discriminative and neurochemical effects that are enhanced by inhibition of fatty acid amide hydrolase but not by inhibition of anandamide transport. *J Pharmacol Exp Ther* 321: 370-80

Soltesz I, Alger BE, Kano M, Lee SH, Lovinger DM, et al. 2015. Weeding out bad waves: towards selective cannabinoid circuit control in epilepsy. *Nat Rev Neurosci* 16: 264-77

Song P, Zechner C, Hernandez G, Canovas J, Xie Y, et al. 2018. The Hormone FGF21 Stimulates Water Drinking in Response to Ketogenic Diet and Alcohol. *Cell Metab* 27: 1338-47 e4

Song ZH, Bonner TI. 1996. A lysine residue of the cannabinoid receptor is critical for receptor recognition by several agonists but not WIN55212-2. *Mol Pharmacol* 49: 891-6

Soria-Gomez E, Bellocchio L, Reguero L, Lepousez G, Martin C, et al. 2014a. The endocannabinoid system controls food intake via olfactory processes. *Nat Neurosci* 17: 407-15

Soria-Gomez E, Massa F, Bellocchio L, Rueda-Orozco PE, Ciofi P, et al. 2014b. Cannabinoid type-1 receptors in the paraventricular nucleus of the hypothalamus inhibit stimulated food intake. *Neuroscience* 263: 46-53

Soria-Gomez E, Matias I, Rueda-Orozco PE, Cisneros M, Petrosino S, et al. 2007. Pharmacological enhancement of the endocannabinoid system in the nucleus accumbens shell stimulates food intake and increases c-Fos expression in the hypothalamus. *Br J Pharmacol* 151: 1109-16

Sparks MA, Crowley SD, Gurley SB, Mirotsou M, Coffman TM. 2014. Classical Renin-Angiotensin system in kidney physiology. *Compr Physiol* 4: 1201-28

Spigt MG, Kuijper EC, Schayck CP, Troost J, Knipschild PG, et al. 2005. Increasing the daily water intake for the prophylactic treatment of headache: a pilot trial. *Eur J Neurol* 12: 715-8

Spitz R. 1975. Induction of drinking by insulin in the rat. *Eur J Pharmacol* 31: 110-4

St-Amant M. 2019. Septal nuclei Radiology Reference Article Radiopaedia.org

Stein L, Seifter J. 1962. Muscarinic synapses in the hypothalamus. *Am J Physiol* 202: 751-6

Steiner H, Bonner TI, Zimmer AM, Kitai ST, Zimmer A. 1999. Altered gene expression in striatal projection neurons in CB1 cannabinoid receptor knockout mice. *Proc Natl Acad Sci U S A* 96: 5786-90

Steiner MA, Marsicano G, Nestler EJ, Holsboer F, Lutz B, Wotjak CT. 2008. Antidepressant-like behavioral effects of impaired cannabinoid receptor type 1 signaling coincide with exaggerated corticosterone secretion in mice. *Psychoneuroendocrinology* 33: 54-67

Stevenson JA. 1949. Effects of hypothalamic lesions on water and energy metabolism in the rat. *Recen Frogr Hormone Res* 4: 363-94

Stevenson JA, Welt LG, Orloff J. 1950. Abnormalities of water and electrolyte metabolism in rats with hypothalamic lesions. *Am J Physiol* 161: 35-9

Stewart JL, McMahon LR. 2011. The fatty acid amide hydrolase inhibitor URB 597: interactions with anandamide in rhesus monkeys. *Br J Pharmacol* 164: 655-66

Stockings E, Zagic D, Campbell G, Weier M, Hall WD, et al. 2018. Evidence for cannabis and cannabinoids for epilepsy: a systematic review of controlled and observational evidence. *J Neurol Neurosurg Psychiatry* 89: 741-53

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: 323-8

Strauss WM, Hetem RS, Mitchell D, Maloney SK, Meyer LC, Fuller A. 2015. Selective brain cooling reduces water turnover in dehydrated sheep. *PLoS One* 10: e0115514

Stricker EM, Sved AF. 2000. Thirst. *Nutrition* 16: 821-6

Stryer L. 1995. *Biochemistry*. pp. 773-774. New York: W.H.

Sugiura T, Kodaka T, Kondo S, Nakane S, Kondo H, et al. 1997. Is the cannabinoid CB1 receptor a 2-arachidonoylglycerol receptor? Structural requirements for triggering a Ca2+ transient in NG108-15 cells. *J. Biochem.* 122: 890-5

Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, et al. 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 215: 89-97

Suhr JA, Hall J, Patterson SM, Niinisto RT. 2004. The relation of hydration status to cognitive performance in healthy older adults. *Int J Psychophysiol* 53: 121-5

Summerlee AJ, Robertson GF. 1995. Central administration of porcine relaxin stimulates drinking behaviour in rats: an effect mediated by central angiotensin II. *Endocrine* 3: 377-81

Sumners C, Woodruff GN, Poat JA. 1981. Effects of specific dopamine lesions and dopamine receptor sensitivity on angiotensin II- and carbachol-induced thirst in rats. *Psychopharmacology (Berl)* 73: 180-3

Sun YX, Tsuboi K, Okamoto Y, Tonai T, Murakami M, et al. 2004. Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. *Biochem J* 380: 749-56

Sun YX, Tsuboi K, Zhao LY, Okamoto Y, Lambert DM, Ueda N. 2005. Involvement of N-acylethanolamine-hydrolyzing acid amidase in the degradation of anandamide and other N-acylethanolamines in macrophages. *Biochim Biophys Acta* 1736: 211-20

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S. 2004. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J Neurosci* 24: 4787-95

Swanson LW, Sawchenko PE. 1983. Hypothalamic integration: organization of the paraventricular and supraoptic nuclei. *Annu Rev Neurosci* 6: 269-324

Szabo B, Dorner L, Pfreundtner C, Norenberg W, Starke K. 1998. Inhibition of GABAergic inhibitory postsynaptic currents by cannabinoids in rat corpus striatum. *Neuroscience* 85: 395-403

Takahashi KA, Linden DJ. 2000. Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. *J Neurophysiol* 83: 1167-80

Tan CL, Knight ZA. 2018. Regulation of Body Temperature by the Nervous System. *Neuron* 98: 31-48

Tanimura A, Uchigashima M, Yamazaki M, Uesaka N, Mikuni T, et al. 2012. Synapse type-independent degradation of the endocannabinoid 2-

arachidonoylglycerol after retrograde synaptic suppression. *Proc Natl Acad Sci U S A* 109: 12195-200

Tanimura A, Yamazaki M, Hashimotodani Y, Uchigashima M, Kawata S, et al. 2010. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. *Neuron* 65: 320-7

Tarjan E, Denton DA, Weisinger RS. 1988. Atrial natriuretic peptide inhibits water and sodium intake in rabbits. *Regul Pept* 23: 63-75

Teitelbaum P, Epstein AN. 1962. The lateral hypothalamic syndrome: recovery of feeding and drinking after lateral hypothalamic lesions. *Psychol Rev* 69: 74-90

Teitelbaum P, Stellar E. 1954. Recovery from the failure to eat produced by hypothalamic lesions. *Science* 120: 894-5

Thornton SM, Fitzsimons JT. 1995. The effects of centrally administered porcine relaxin on drinking behaviour in male and female rats. *J Neuroendocrinol* 7: 165-9

Tian DR, Zhang DM, Ma LY. 1992. [Effect of atrial natriuretic peptide on angiotensin II--and water deprivation-induced drinking behavior in rats]. *Sheng Li Xue Bao* 44: 186-91

Tian X, Guo J, Yao F, Yang DP, Makriyannis A. 2005. The conformation, location, and dynamic properties of the endocannabinoid ligand anandamide in a membrane bilayer. *J Biol Chem* 280: 29788-95

Tong J, Mannea E, Aime P, Pfluger PT, Yi CX, et al. 2011. Ghrelin enhances olfactory sensitivity and exploratory sniffing in rodents and humans. *J Neurosci* 31: 5841-6

Tortorella S, Rodrigo-Angulo ML, Nunez A, Garzon M. 2013. Synaptic interactions between perifornical lateral hypothalamic area, locus coeruleus nucleus and the oral pontine reticular nucleus are implicated in the stage succession during sleep-wakefulness cycle. *Front Neurosci* 7: 216

Touw M. 1981. The religious and medicinal uses of Cannabis in China, India and Tibet. *J Psychoactive Drugs* 13: 23-34

Tsuboi K, Hilligsmann C, Vandevoorde S, Lambert DM, Ueda N. 2004. N-cyclohexanecarbonylpentadecylamine: a selective inhibitor of the acid amidase hydrolysing N-acylethanolamines, as a tool to distinguish acid amidase from fatty acid amide hydrolase. *Biochem J* 379: 99-106

Tsuboi K, Okamoto Y, Ikematsu N, Inoue M, Shimizu Y, et al. 2011. Enzymatic formation of N-acylethanolamines from N-acylethanolamine plasmalogen through N-acylphosphatidylethanolamine-hydrolyzing phospholipase D-dependent and -independent pathways. *Biochim Biophys Acta* 1811: 565-77

Tsuboi K, Sun YX, Okamoto Y, Araki N, Tonai T, Ueda N. 2005. Molecular characterization of N-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. *J Biol Chem* 280: 11082-92

Twitchell W, Brown S, Mackie K. 1997. Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *J Neurophysiol* 78: 43-50

Uchigashima M, Yamazaki M, Yamasaki M, Tanimura A, Sakimura K, et al. 2011. Molecular and morphological configuration for 2-arachidonoylglycerol-mediated retrograde signaling at mossy cell-granule cell synapses in the dentate gyrus. *J Neurosci* 31: 7700-14

Ueda N, Liu Q, Yamanaka K. 2001. Marked activation of the N-acylphosphatidylethanolamine-hydrolyzing phosphodiesterase by divalent cations. *Biochim Biophys Acta* 1532: 121-7

Ueda N, Tsuboi K, Uyama T. 2010. N-acylethanolamine metabolism with special reference to N-acylethanolamine-hydrolyzing acid amidase (NAAA). *Prog Lipid Res* 49: 299-315

Ueda N, Yamanaka K, Terasawa Y, Yamamoto S. 1999. An acid amidase hydrolyzing anandamide as an endogenous ligand for cannabinoid receptors. *FEBS Lett* 454: 267-70

Unger T, Gohlke P, Kotrba M, Rettig R, Rohmeiss P. 1989. Angiotensin II and atrial natriuretic peptide in the brain: effects on volume and Na+ balance. *Resuscitation* 18: 309-19

Ungerstedt U. 1971. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiol Scand Suppl* 367: 95-122

UniversityOfIdahoCollegeOfScience. 2004. Amygdala. web.archive.org

Uriguen L, Perez-Rial S, Ledent C, Palomo T, Manzanares J. 2004. Impaired action of anxiolytic drugs in mice deficient in cannabinoid CB1 receptors. *Neuropharmacology* 46: 966-73

Vallee M, Vitiello S, Bellocchio L, Hebert-Chatelain E, Monlezun S, et al. 2014. Pregnenolone can protect the brain from cannabis intoxication. *Science* 343: 94-8

van der Stelt M, Di Marzo V. 2005. Cannabinoid receptors and their role in neuroprotection. *Neuromolecular Med* 7: 37-50

Varvel SA, Anum EA, Lichtman AH. 2005. Disruption of CB(1) receptor signaling impairs extinction of spatial memory in mice. *Psychopharmacology (Berl)* 179: 863-72

Varvel SA, Hamm RJ, Martin BR, Lichtman AH. 2001. Differential effects of delta 9-THC on spatial reference and working memory in mice. *Psychopharmacology (Berl)* 157: 142-50

Varvel SA, Lichtman AH. 2002. Evaluation of CB1 receptor knockout mice in the Morris water maze. *J Pharmacol Exp Ther* 301: 915-24

Verney EB. 1947. The antidiuretic hormone and the factors which determine its release. *Proc R Soc Lond B Biol Sci* 135: 25-106

Verty AN, McFarlane JR, McGregor IS, Mallet PE. 2004a. Evidence for an interaction between CB1 cannabinoid and oxytocin receptors in food and water intake. *Neuropharmacology* 47: 593-603

Verty AN, McGregor IS, Mallet PE. 2004b. The dopamine receptor antagonist SCH 23390 attenuates feeding induced by Delta9-tetrahydrocannabinol. *Brain Res* 1020: 188-95

Vianna CR, Donato J, Jr., Rossi J, Scott M, Economides K, et al. 2012. Cannabinoid receptor 1 in the vagus nerve is dispensable for body weight homeostasis but required for normal gastrointestinal motility. *J Neurosci* 32: 10331-7

Vijande M, Lopez-Sela P, Brime JI, Bernardo R, Diaz F, et al. 1990. Insulin stimulation of water intake in humans. *Appetite* 15: 81-7

Vinod KY, Hungund BL. 2006. Role of the endocannabinoid system in depression and suicide. *Trends Pharmacol Sci* 27: 539-45

Vivas L, Godino A, Dalmasso C, Caeiro XE, Macchione AF, Cambiasso MJ. 2014. Neurochemical Circuits Subserving Fluid Balance and Baroreflex: A Role for Serotonin, Oxytocin, and Gonadal Steroids In *Neurobiology of Body Fluid Homeostasis: Transduction and Integration*, ed. LA De Luca, Jr., JV Menani, AK Johnson. Boca Raton (FL)

Viveros MP, Marco EM, File SE. 2005. Endocannabinoid system and stress and anxiety responses. *Pharmacol Biochem Behav* 81: 331-42

Voet D, Voet JG. 2011. *Biochemistry* New York: Wiley.

Volkow ND, Koob GF, McLellan AT. 2016. Neurobiologic Advances from the Brain Disease Model of Addiction. *N Engl J Med* 374: 363-71

Volkow ND, Wise RA, Baler R. 2017. The dopamine motive system: implications for drug and food addiction. *Nat Rev Neurosci* 18: 741-52

Vongraven D. 1913. Die nierenwirkung von hypophysenextrakten meschen. *Berl Klin Wochenscgr* 50: 2083-86

Vorhees CV, Williams MT. 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc* 1: 848-58

Voyer P, Richard S, Doucet L, Carmichael PH. 2009. Predisposing factors associated with delirium among demented long-term care residents. *Clin Nurs Res* 18: 153-71

Wager-Miller J, Westenbroek R, Mackie K. 2002. Dimerization of G protein-coupled receptors: CB1 cannabinoid receptors as an example. *Chem Phys Lipids* 121: 83-9

Waldbillig RJ, Bartness TJ. 1981. Insulin-induced drinking: an analysis of hydrational variables. *Physiol Behav* 26: 787-93

Walker JM, Krey JF, Chu CJ, Huang SM. 2002. Endocannabinoids and related fatty acid derivatives in pain modulation. *Chem Phys Lipids* 121: 159-72

Wang L, Gillis-Smith S, Peng Y, Zhang J, Chen X, et al. 2018. The coding of valence and identity in the mammalian taste system. *Nature* 558: 127-31

Wang L, Liu J, 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 U S A* 100: 1393-8

Watts AG. 2001. Thirst and Drinking. *International Encyclopedia of the Social & Behavioral Sciences*: 15676-81

Weber T, Bohm G, Hermann E, Schutz G, Schonig K, Bartsch D. 2009. Inducible gene manipulations in serotonergic neurons. *Front Mol Neurosci* 2: 24

Weisinger RS, Blair-West JR, Denton DA, Tarjan E. 1992. Central administration of atrial natriuretic peptide suppresses sodium and water intake of sheep. *Brain Res* 579: 113-8

Weiss C, Bouwmeester H, Power JM, Disterhoft JF. 1999. Hippocampal lesions prevent trace eyeblink conditioning in the freely moving rat. *Behav Brain Res* 99: 123-32

Wettendorff H. 1901. Modifications du sang sous l'influence de la privation d'eau. Contribution à l'étude de la soif. *Trav Lab Physiol Inst Solvay* 4: 353-484

Williams CM, Kirkham TC. 2002. Observational analysis of feeding induced by Delta9-THC and anandamide. *Physiol Behav* 76: 241-50

Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. 1987. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl)* 93: 358-64

Wilson RI, Nicoll RA. 2001. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 410: 588-92

Witkin JM, Tzavara ET, Davis RJ, Li X, Nomikos GG. 2005. A therapeutic role for cannabinoid CB1 receptor antagonists in major depressive disorders. *Trends Pharmacol Sci* 26: 609-17

Witt DM, Keller AD, Batsel HL, Lynch JR. 1952. Absence of thirst and resultant syndrome associated with anterior hypothalamectomy in the dog. *Am J Physiol* 171: 780

Woelkart K, Salo-Ahen OM, Bauer R. 2008. CB receptor ligands from plants. *Curr Top Med Chem* 8: 173-86

World Health Organization. 2018. Depression.

Yamasaki M, Hashimoto K, Kano M. 2006. Miniature synaptic events elicited by presynaptic Ca2+ rise are selectively suppressed by cannabinoid receptor activation in cerebellar Purkinje cells. *J Neurosci* 26: 86-95

Yoshino H, Miyamae T, Hansen G, Zambrowicz B, Flynn M, et al. 2011. Postsynaptic diacylglycerol lipase mediates retrograde endocannabinoid suppression of inhibition in mouse prefrontal cortex. *J Physiol* 589: 4857-84

Young SN. 2007. How to increase serotonin in the human brain without drugs. *J Psychiatry Neurosci* 32: 394-9

Yu S, Levi L, Casadesus G, Kunos G, Noy N. 2014. Fatty acid-binding protein 5 (FABP5) regulates cognitive function both by decreasing anandamide levels and by activating the nuclear receptor peroxisome proliferator-activated receptor beta/delta (PPARbeta/delta) in the brain. *J Biol Chem* 289: 12748-58

Zamboni P, Siro-Brigiani G. 1966. [Effect of catecholamines on thirst and diuresis in the rat]. *Boll Soc Ital Biol Sper* 42: 1657-9

Zheng H, Patterson LM, Rhodes CJ, Louis GW, Skibicka KP, et al. 2010. A potential role for hypothalamomedullary POMC projections in leptin-induced suppression of food intake. *Am J Physiol Regul Integr Comp Physiol* 298: R720-8

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. 1999. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci U S A* 96: 5780-5

Zimmerman CA, Leib DE, Knight ZA. 2017. Neural circuits underlying thirst and fluid homeostasis. *Nat Rev Neurosci* 18: 459-69

Zimmerman CA, Lin YC, Leib DE, Guo L, Huey EL, et al. 2016. Thirst neurons anticipate the homeostatic consequences of eating and drinking. *Nature* 537: 680-84

Zimmerman MC, Lazartigues E, Lang JA, Sinnayah P, Ahmad IM, et al. 2002. Superoxide mediates the actions of angiotensin II in the central nervous system. *Circ Res* 91: 1038-45

Zou S, Kumar U. 2018. Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System. *Int J Mol Sci* 19

Zuardi AW. 2006. History of cannabis as a medicine: a review. *Braz J Psychiatry* 28: 153-7

Zuardi AW, Guimarães FS, Guimarães VM, Del Benl EA. 2002. *Cannabidiol: possible therapeutic application. In: Grotenhermen F, Russo E, eds. Cannabis and cannabinoids: pharmacology, toxicology and therapeutic potential.* pp. 359-69. New York: The Haword Interactive Healing Press.

Zucker I. 1971. Light-dark rhythms in rat eating and drinking behavior. *Physiol Behav* 6: 115-26

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, et al. 1999. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 400: 452-7

ANNEXES

Annexe I List of publications

Zhao Z., Soria-Gómez E., Varilh M., Julio-Kalajzic F., Cannich A., Castiglione A., Vanhoutte L., Duveau A., Zizzari F., Beyeler A., Cota D., Bellocchio L., Busquets-Garcia A., Marsicano G.

Top-down control of water intake by the endocannabinoid system.

BioRxiv. doi: https://doi.org/10.1101/729970, 2019.

Martin-Fernandez M., Jamison S., Robin L., **Zhao Z.**, Martin D., Aguilar J., Benneyworth M., Marsicano G., Araque A.

Synapse-specific astrocyte gating of amygdala-related behavior.

Nature Neuroscience, 2017 (IF:17.839)

Zhao Z., Wang L., Gao W., Hu F., Zhang J., Ren Y., Lin R., Feng Q., Cheng M., Ju D., Chi Q., Wang D., Song S., Luo M., Zhan C.

A central catecholaminergic circuit controls blood glucose levels during stress.

Neuron, Volume 95, Issue 1, p138–152.e5, 5 July 2017 (IF: 14.024, **Featured article**)

Wang D., He X., **Zhao Z.**, Feng Q., Lin R., Sun Y., Ding T., Xu F., Luo M., Zhan C.

Whole-brain mapping of the direct inputs and axonal projections of POMC and AgRP neurons.

Frontiers in Neuroanatomy 9:40, 2015 (IF: 3.26)

Annexe II Oral presentations

Cannabinoids, the relevance to central nervous system and pain. Modena, Italy, January 20th, 2018.

Role of the cortical cannabinoid receptor type 1 in the control of water intake. Xi'an, China, September 29th, 2018.

Top-down control of water intake by the endocannabinoid system. 17th synapse day meeting, Bordeaux, France. September 17th, 2019

Top-down control of water intake by the endocannabinoid system. 8th symposium of NeuroCentre Magendie, Bordeaux, France. September 26th, 2019

Annexe III Conferences attended

2018

Society for neuroscience 48th annual meeting, November 3-7 2018, San Diego, CA

New therapeutic strategies for chronic pain: from Botulinum Toxin to Medical Cannabis, January 20th, 2018, Modena, Italy **(Oral presentation)**

2017

Frontier in Neurophotonics, October 15-18, 2017, Bordeaux, France

4th Bordeaux neurocampus conference, September 27-29, 2017, Bordeaux, France

Super-resolution in photonic microscopy, May 30-June 1, 2017, Bordeaux, France

NeuroFrance, May 17-19, 2017, Bordeaux, France

2016

3rd Bordeaux neurocampus conference, September 28-30, 2016, Bordeaux, France

The Catania international school in neuroscience “Cannabinoid receptors: their role in physiology and pathology”, Noto, Italy, July 11-15 2016 (Travel grant award)

Annexe IV Acknowledgments

First of all, I would like to express my sincere gratitude to my supervisor Dr. Giovanni Marsicnao for offering me the opportunity of Ph.D. studies sponsored by the China Scholarship Council and the continuous support, patience, and instructions during the 4-year study and writing the thesis. I could not have imagined that I have been improving so much in considering and solving scientific questions due to his abundant supervision.

Besides my official supervisor Giovanni, I deeply thank other supervisors, Drs. Edgar Soria-Gomez, Arnau Busquets-Garcia, and Luigi Bellocchio. I thank Edgar for the supervision in the first half part of my Ph.D. study, and thank Arnau and Luigi for the supervision of the second half part and correction of my thesis.

I am very grateful to Marjorie Varilh. During the 4 years, she helped in behavioral experiments, anatomy, and kind suggestions for the project. Besides the help in scientific work, she also contributes to administrative work and kinds of orders. In addition, she personally helped in my life in Bordeaux, such as lots of translations from French to English. In short, she is really a very nice colleague and friend,

I very much appreciate the help of Astrid Cannich. She provided all kinds of viral vectors and probes for fluorescent in situ hybridization. She helped a lot in giving suggestions for my thesis project and presentations. In addition, she corrected part of my Ph.D. thesis and made lots of helpful comments.

I thank Francisca Julio-Kalajzic very much for anatomical experiments, thank Virginie Morales very much for administrative work, and thank Bastien Redon very much for the translation of thesis abstract and brief results from English to French. I also really appreciate the help of every colleague in our group.

I am very grateful to Dr. Daniela Cota for her helpful discussion and comments on my project and paper. I thank Philippe Zizzari very much for showing the body composition test and blood collection. I also thank everyone from the Cota group for help in my thesis study.

I thank Dr. Aude Panatier from Oliet group very much for the help in showing the osmolality test. I also thank Dr. Anna Beyeler very much for discussions and comments on my thesis project and paper.

I am very grateful to everyone from the Animal Facility and Genotyping Center of NeuroCentre Magendie for providing assistance in the animal breeding, maintenance and genotyping. I thank everyone from the Bordeaux Imaging Center, particularly Sébastien Marais, for proving microscopes and the help in image analysis.

I greatly appreciate every member of my thesis defense committee for receiving the invitation and revising my thesis.

I have been living in Bordeaux for 4 years. I would like to thank NeuroCentre Magendie, Bordeaux Neurocampus and Bordeaux University for creating a so nice condition for every researcher. I really gain amazing knowledge and broaden my vision, which is not only in neuroscience but also in science and life. I was so lucky that I came to Bordeaux, a super nice city, for studying and living. I really thank France, a very nice

country, very much for helping me and my family. I thank everyone, who I met in Bordeaux, for showing different personalities and sharing different cultures and minds, which made us experience so exciting life here.

Finally, I would like to thank my family and friends for supporting and helping my studies and life in Bordeaux. I thank my wife, Lisi Zhang, for coming to Bordeaux with me 4 years ago, and spending both a happy and a tough time together here. We are so lucky having two kids, Zoé and Léo, and very happy they are growing up and always bring surprises.

En bref, je veux dire: je t'aime, Bordeaux, France!

Zhe