STUDY OF THE NEUROBIOLOGICAL MECHANISMS INVOLVED IN THE DEVELOPMENT OF EATING AND ADDICTIVE DISORDERS

Miriam Gutiérrez Martos

DOCTORAL THESIS UPF / 2017

Thesis directors:

Dr. Miquel Martín
Prof. Rafael Maldonado

Departament de Ciències Experimentals i de la Salut

Universitat Pompeu Fabra Barcelona

A mi familia,
y especialmente al primero que me
llamó doctora aún sin serlo,
mi yayo.

Acknowledgements

Gracias a Rafael Maldonado y Miquel (El Calvo) Martín por guiarme durante esta carrera de obstáculos que ha sido el doctorado. Esta tesis no hubiera sido posible sin la ayuda de vosotros, chicos del 308 y colegas del PRBB, el apoyo incondicional de mi familia, las bocanadas de ánimo indiscriminado de mis queridos amigos y sobretodo sin ti. Gracias por allanarme el terreno y ponerle florecillas.

Abstract

Drug addiction and several eating disorders share striking similarities in their behavioural patterns triggered by the presence of neuronal alterations in the brain reward circuit. In this Thesis, we investigate the neurobiological mechanisms modulating the rewarding effects of a drug of abuse, cocaine, and the progression of some eating disorders. Firstly, we evaluated the effect of three natural isoflavones, daidzin, daidzein and genistein, and a reference compound, disulfiram, in the modulation of the reinforcing and relapsing properties of cocaine in mice. Among them, the natural isoflavone daidzein presents a promising therapeutic effect decreasing cocaine reinforcing effects and relapse that surpasses those of disulfiram. Other rewarding stimuli able to develop abnormal behavioural responses are highly palatable foods such as those rich in sugar and fat. *Ad libitum* exposure to cafeteria diet foments overeating and overweight in mice and leads to neuroplastic modifications in the brain reward system. We have now demonstrated for the first time that microglia reactivity and neuroinflammation are responsible of the neurobiological adaptations observed in this model of free-choice diet that resembles the human food availability environment. Moreover, we also set-up and validated an operant animal model of high palatable food-induced addictive-like behaviour in mice applying the same criteria to diagnose drug addiction in the DSM-5. We have revealed in this model epigenetic changes in the Cnr1 promoter induced by the chronic exposure to palatable food.

Resumen

La adicción a las drogas y algunos trastornos de ingesta comparten importantes parecidos en sus patrones conductuales, provocados por la presencia de alteraciones neuronales en el sistema del refuerzo del cerebro. En esta tesis investigamos los mecanismos neurobiológicos que modulan los efectos recompensantes de una droga, la cocaína, y la progresión de algunos trastornos de ingesta. Primero, evaluamos el efecto de tres isoflavonas naturales, daidzin, daidzein y genistein, y un compuesto de referencia, disulfiram, en la disminución de las propiedades reforzantes y de recaída de la cocaina en ratones. Entre las isoflavonas, daidzein presenta unos efectos terapéuticos prometedores que superan a los del disulfiram reduciendo las propiedades reforzantes y de recaída de la cocaína. Otros estímulos recompensantes capaces de conducir a conductas anómalas son las comidas altamente sabrosas, como las ricas en azúcar y grasas. La exposición *ad libitum* a la dieta de cafetería fomenta la sobreingesta de comida y el sobrepeso en ratones y produce cambios neuroplásticos en el sistema del refuerzo. Hemos demostrado por primera vez que la reactividad microglial y la neuroinflamación son las responsables de estas adaptaciones neurobiológicas observadas en este modelo de libre elección de comida que simula la disponibilidad de comida en humanos. Además, también hemos generado y validado un modelo operante en ratón que conduce a comportamientos adictivos hacia la comida sabrosa usando los criterios de diagnóstico de adicción del DSM-5 y YFAS 2.0. Partiendo de este modelo, hemos desvelado cambios epigenéticos en el promotor de Cnr1 inducidos por la exposición crónica a comida sabrosa.

Abbreviations

2-AG	2-arachidonoylglycerol
AADC	L-amino acid decarbolylase
AEA	N-arachidonylethanolamide
AgRP	agouti-related protein
ALDH	aldehyde dehydrogenase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ARC	arcuate nucleus
ARFID	avoidant/restrictive food intake disorder
BBB	blood brain barrier
BED	binge-eating disorder
BDNF	brain-derived neurotrophic factor
CART	Cocaine- and amphetamine- regulated transcript
CB1R	cannabinoid receptor 1
CB2R	cannabinoid receptor 2
CCK	cholecystokinin
CD	cafeteria diet
CNS	central nervous system
CNTF	ciliaryneurotrophic factor
CRF	corticotropin-releasing factor
CRH	corticotrophin-releasing hormone
CT1	cardiotrophin-1
D1R	dopamine receptor 1
D2R	dopamine receptor 2
DA	dopamine
DBH	dopamine-ß-hydroxylase
DDC	diethyldithiocarbamate
DHA	dorsal hypothalamic area
DMH	dorsomedial nucleus
DOPAC	3,4-dihydroxyphenylacetic acid
DOPAL	3,4-dihydroxyphenylacetaldehyde
DSM	diagnostic and statistical manual of mental disorders
ECS	endocannabinoid system
ER	estrogen receptor
FR	fixed ratio
FTO	fat mass and obesity-associated

GABA	γ-aminobutyric acid
$GABA_A$	GABA ionotropictransmembrane receptors
$GABA_B$	GABA metabotrobic G protein-coupled receptors
GHIH	growth hormone-inhibiting hormone
GLP1	glucagon-like peptide-1
GWAS	genome wide association studies
IFN	interferon
LH	lateral hypothalamus
LIF	leukaemia inhibitory factor
LTD	long-term depression
LTP	long-term potentiation
MBH	mediobasal hypothalamus
MCH	melanin concentrating hormone
MCSF	macrophage colony-stimulating factor
MFB	medial forebrain bundle
mGluR	glutamate G-coupled metabotropic receptors
MHCI	major histocompatibility complex class I
MN	mammillary nuclei
MSH	melanocyte-stimulating hormone
NAc	nucleus accumbens
NMDA	n-methyl-d-aspartate receptors
NPY	neuropeptide Y
NTS	nucleus of the solitary tract
O-DMA	o-desmethylangolensin
OPN	osteopontin
OSM	oncostatin M
PDYN	prodynorphin
PENK	proenkephalin
PFA	perifornical area
PFC	prefrontal cortex
PGE2	prostaglandin E2
POA	preoptic area
POMC	proopiomelanocortin
PP	pancreatic polypeptide
PVN	paraventricular nucleus
PYY	peptide YY
SCN	suprachiasmatic nucleus

SN	substantia nigra pars compacta
SON	supraoptic nucleus
TGF	transforming growth factor
TH	tyrosine hydroxylase
THP	tetrahydropapaveroline
TNF	tumour necrosis factor
VMH	ventromedial nucleus
VTA	ventral tegmental area
YFAS	Yale food addiction scale

Table of Contents

INTRODUCTION

1. Addiction disorder

Addiction is a mental disorder triggered by the compulsive engagement to a pleasurable behaviour (American Psychiatric Association, 2013). Addiction is characterized by compulsive seeking of the substance or behaviour of abuse, loss of control to seek and take the drug even in fully awareness of its associated negative consequences, motivational withdrawal syndrome when the access to the drug is prevented, and high relapsing rate even after long periods of abstinence (American Psychiatric Association, 2013). The onset and escalation to addiction is a long-term process that O'Brien and colleagues summarized in an equation. They predicted that the probability to get addicted was a result of interacting variables involving the drug, the host and the environment. Namely, high drug availability at low price may increase the risk of getting drug addicted if genetic factors (host) or the chance to find alternative pleasures (environment) is low (O'Brien, 2008). Interestingly, only a small percentage of drug consumers, around 10-30%, become addicted, which underlies the relevance of individual and environmental factors (Anthony *et al*, 1994).

Addiction can be diagnosed, classified and graded by several psychiatric manuals such as the Diagnostic and Statistical Manual of Mental Disorders (DSM) from the American Psychiatric Association, or the International Classification of Diseases, from the World Health Organization (Hasin *et al*, 1996).

1.1 Addiction as a Non/Substance Related Disorder

The diagnosis of addiction has evolved in parallel with the expert's definition of the term "addiction" (see Table 1). Early definitions, such as DSM III (1980), were centred on the consequences after prolonged drug consumption ("drug-centred"), such as tolerance and withdrawal (American Psychiatric Association, 1980). In the 90's, the 4th version of the DSM avoided the term "addiction" in support of "Substance Related Disorders" which in turn was divided into "Substance-Abuse", experiencing family or relationship problems motivated by drug consumption, and "Substance-Dependence", aggravating the pattern of drug-taking and experiencing distress from other non-drug-related behaviours. The DSM-IV added 5 additional criteria related to loss of control to the drug-dependence category in a more "individual-centred diagnose". These criteria can be summarized in persistence to use the drug, high motivation to use/obtain the drug and resistance to punishment (American Psychiatric Association, 1994).

In 2013, an integrative approach to the former bivalent classification reached the approval of the scientific community and addiction disorder was termed as "Substance-Related and Addictive Disorders" standing for both Substance and Non-Substance addictions (American Psychiatric Association, 2013). This new definition of addiction was centred in the behavioural effects produced by the drug/behaviour, i.e. "behavioural-centred". The new Non Substance-Related Disorder category permitted the inclusion of a sole non-substance behavioural disorder, Gambling Disorder, in expectancy of controversial potential future inclusions, such as eating addiction, not yet considered within these pathological disorders. Meanwhile, The Yale Food Addiction Scale (YFAS) addressed

DSM-III "drug-centred" (APA, 1980)	DSM-IV "individual-centred" (APA, 1994)	DSM-5 "behavioural-centred" (APA, 2013)
Substance-Abuse 3 criteria	**Substance-Abuse** ≥1 criteria	**Substance-Related and Addictive Disorders** 2-3 criteria: mild; 4-5 criteria: moderate; ≥6 criteria: severe
1. Pattern of pathological use.	1. Recurrent failure to fulfil major role obligations.	1. Recurrent failure to fulfil major role obligations.
2. Impairment in social or occupational functioning due to substance use.	2. Recurrent substance use in physically hazardous situations.	2. Recurrent substance use in physically hazardous situations.
3. Minimal duration of disturbance of at least one month.	3. Recurrent substance-related legal problems.	
	4. Continued substance use despite persistent or recurrent social interpersonal problems.	3. Continued substance use despite persistent or recurrent social interpersonal problems.
Substance-Dependence ≥1 criteria	**Substance-Dependence** ≥3 criteria	
1. Tolerance.	1. Tolerance.	4. Tolerance.
2. Withdrawal.	2. Withdrawal.	5. Withdrawal.
	3. The substance is often taken in larger amounts or over a longer period than intended.	6. The substance is often taken in larger amounts or over a longer period than intended.
	4. Persistent desire or unsuccessful efforts to cut down.	7. Persistent desire or unsuccessful efforts to cut down.
		8. Craving.
	5. Considerable time spent in obtaining the substance or using, or recovering, from its effects.	9. Considerable time spent in obtaining the substance or using, or recovering, from its effects.
	6. Important social, work, or recreational activities given up because of use.	10. Important social, work, or recreational activities given up because of use.
	7. Continued use despite knowledge of problems caused by or aggravated by use.	11. Continued use despite knowledge of problems caused by or aggravated by use.

Table 1. Comparison and classification of the criteria used to diagnose "addiction" in the DSM-III, DSM-IV and DSM-5. Highlighting represent the three criteria related to loss of control used to behaviourally model addiction in rodents: orange highlight indicates those criteria related to (1) persistence to response; blue highlight, (2) motivation; green highlight, (3) resistance to punishment (Deroche-Gamonet *et al*, 2004; Mancino *et al*, 2015). **APA:** American Psychiatric Association; **DSM:** Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1980, 1994, 2013).

this issue in 2009 being the first questionnaire to successfully evaluate addictive-like eating behaviour waiting for the formal inclusion of this disorder in future versions of the DSM, as further detailed in Chapter 9.1 (Gearhardt *et al*, 2009). The DSM-5 also enabled a more accurate diagnostic since it allows the grading of the disorder dependent on the criteria met by the subject from mild to severe. Some authors has adapted this classification to rodent models that will allow a deeper understanding of the neurobiology of the addictive process (Barry *et al*, 2009; Deroche-Gamonet *et al*, 2004; Mancino *et al*, 2015).

2. Neurobiology of addiction

2.1 The brain reward system

Rewards are defined as those objects or goals that trigger a pleasurable and motivational response. Different neurobiological mechanisms are involved in mediating the responses towards pleasurable elementary natural (e.g. eating, drinking, reproduction) and non-natural processes (e.g. gambling or drug-taking) (Schultz, 2010). In this sense, a specific neuronal reward system is involved in the rating and contrast of such rewards. The brain reward system is mainly integrated by a group of dopaminergic neurons that emerge from the midbrain, send connections to the cortex and limbic areas (where motivation, pleasure, learning and decision-making centres belong) that are in charge of managing and establishing conditioned behaviours towards pleasurable stimuli.

2.2 Dopamine

Dopamine (DA) is a neurotransmitter from the family of the monoamines. Among monoamines, DA, norepinephrine (noradrenaline) and epinephrine (adrenaline) are derived from the processing of dietary tyrosine forming the catecholamine subgroup. Tyrosine actively crosses the blood-brain barrier and catecholamines are synthesised in a region-specific way. Dopaminergic neurons are found in the mesencephalon, diencephalon and the olfactory bulb (Arias-Carrión and Pŏppel, 2007; Björklund *et al*, 2007), although most of the neurons remain in the mesencephalon forming the *ventral tegmental area* (VTA) and the *substantia nigra* (SN) *pars compacta*. Noradrenergic neurons form a small subset in the *locus coeruleus*, medulla, brainstem and spinal cord

(Bruinstroop *et al*, 2012; Rinaman, 2011; Swanson and Hartman, 1975) and adrenergic cells in the central nervous system (CNS) are mainly found in the *locus coeruleus*, medulla, brainstem (Mejías-Aponte *et al*, 2009). Tyrosine is processed into DA through the enzymes tyrosine hydroxylase (TH) and L-amino acid decarbolylase (AADC). Noradrenaline and epinephrine are subproducts of DA, which need dopamine-β-hydroxylase (DBH) action (see Figure 1).

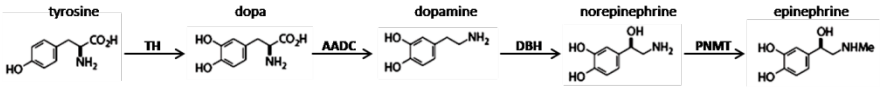

Figure 1. Biosynthetic pathway of catecholamines. TH: tyrosine hydroxylase; **AADC:**L-amino acid decarboxylase; **DBH:** dopamine-β- hydroxylase.

Although the number of dopaminergic neurons in the SN and the VTA of the midbrain is small (around 400,000 and 5,000, respectively (Björklund and Dunnett, 2007), their extensive connections project widely through the brain enabling the modulation of multiple brain functions. Three main brain dopaminergic pathways have been reported to be involved in addictive behaviours (see Figure 2):

a) Mesolimbic pathway: dopaminergic neurons emerging from the VTA project to limbic areas including the ventral *striatum* (*nucleus accumbens*, NAc), a key area involved in motivation and incentive salience, the attribution of a "desirable" tag to a reward. The NAc has been widely described to be involved in the development of addictive behaviours and promotes goal-directed behaviours (Björklund *et al*, 2007). NAc DA levels are generally lower by aversive conditions, such as unavoidable shock, chronic pain, over- or under-eating and withdrawal

from addictive drugs (Parsons and Hurd, 2015). The VTA processes emotionally environmental stimuli as rewarding (motivational salience) by initiating the DA flow onto the NAc (D'Ardenne *et al*, 2008; Phillips *et al*, 2008; Yim and Mogenson, 1980). Indeed, both natural rewards and drugs of abuse increase NAc DA and this neurochemical response contributes to the subjective positive reinforcement (Salamone *et al*, 2005). The VTA contains a mixture of dopaminergic (~65%), gamma-aminobutyric acid (GABA) (~30%), and glutamatergic neurons (~5%) (Margolis et al., 2006; Nair-Roberts et al., 2008; Swanson, 1982; Yamaguchi et al., 2011) that orchestrate reward-seeking behaviour. VTA dopaminergic neurons also innervate other areas of the limbic system including the *amygdala,* the *bed nucleus of the stria terminalis* (BNST) and the *hippocampus*. The *amygdala* is involved in the formation of associative reward- and fear-related memories and the BNST has a prominent role in the withdrawal/negative effects of addiction including anxiety and aversion. Together, they integrate the so called "extended amygdala circuit", processing the aversive effects of withdrawal and self-perceptions of drug-related memories. The *hippocampus* is involved in declarative memory and in contextual associations of drug-related cues (Avery *et al*, 2016; Keleta and Martinez, 2012; Koob, 2009; Koob and Volkow, 2010; Potenza *et al*, 2011). The VTA also sends glutamatergic and GABAergic projections to the NAc to fine-tune reward and aversive events (Qi *et al*, 2016; van Zessen *et al*, 2012). In turn, VTA neurons are also modulated by glutamatergic and/or GABAergic connections coming from stress, fear and memory/conditioned learning areas (Koob and Volkow, 2010). Glutamatergic neurons found in the *habenula, hippocampus*, *amygdala*, lateral *hypothalamus* and *prefrontal cortex* activate the dopaminergic

somas by its numerous projections to the VTA. This glutamatergic inputs mediate the assignment of positive/aversive tags to stimuli (drugs of abuse, a certain behaviour, food, matting and others) according to self-perceptions, memories and contexts coupled to that activity (Geisler and Wise, 2008). In contrast, GABAergic medium spiny neurons found in the NAc and GABAergic interneurons of the VTA modulate dopaminergic activity acting in the VTA on presence of DA picks. This phenomena can occur indirectly, by desinhibiting the GABAergic interneurons in the VTA, or directly by exciting VTA GABAergic neurons (Chiodo and Berger, 1986). Indeed, GABAergic neurotransmission in the VTA is drastically altered by exposure to drugs of abuse, which may result in aberrant activity in DA neurons promoting maladaptive behaviours (Madhavan *et al*, 2010; Taylor *et al*, 2016). Altogether, these connections allow sensory and emotional information conversion into motivational actions. Therefore, this pathway regulates the willingness or motivation to obtain a pleasurable reward.

b) Mesocortical pathway: dopaminergic neurons from the VTA also project to the prefrontal cortex (PFC). The *orbitofrontal cortex* (involved in salience attribution and goal-directed behaviours), the *anterior cingulate cortex* (involved in inhibitory control and awareness), and the *dorsolateral* PFC (involved in higher cognitive operations and decision making) maintain the cognitive control of behaviour (Volkow *et al*, 2007, 2008b). In some conditions, such as during drug addiction, prestablished responses and behaviours tend to dominate all the current activities. In this line, exposure to drug cues can elicit drug seeking. In the event of these cues provided by the environment, the behavioural individual's response towards the attention designated to them, self-control and

decision-making, to balance whether seeking for the drug or not, is mediated by the PFC (Malenka RC, Nestler EJ, 2009; Nestler *et al*, 2009). This pathway is involved in the impulsivity and loss of control found in addiction disorder. The VTA-PFC relationship works as a feed-back loop. The PFC sends glutamatergic excitatory projections onto the VTA acting on dopaminergic and GABAergic inhibitory neurons. These glutamatergic projections from the PFC and VTA GABAergic interneurons directly play an important role in regulating the activity of VTA dopaminergic neurons and, in turn, the extracellular levels of DA within forebrain regions (Carr and Sesack, 2000).

c) Nigrostriatal pathway: dopaminergic neurons located in the SN *pars compacta* project to the *caudate* and *putamen nuclei* (*dorsal striatum*), involved in voluntary movement and goal-directed actions (Björklund *et al*, 2007; Smith and Villalba, 2008). SN increases dopaminergic activity in prediction of rewarding stimuli and in positive reward error when the reward achieved is greater than the expected. DA efflux in the *dorsal striatum* plays a key role in the development of compulsive forms of reward seeking and consumption (Everitt *et al*, 2008; Everitt and Robbins, 2005). This pathway is involved in reward-learning process and a habit formation towards goal-predicted actions (Everitt and Robbins, 2013; Gold *et al*, 2015; Matsumoto and Hikosaka, 2009; Schultz, 2015).

These pathways serve as framework for DA trafficking, the major neurotransmitter involved in reward processing and incentive learning (Volkow *et al*, 2008a; Wise, 2006).

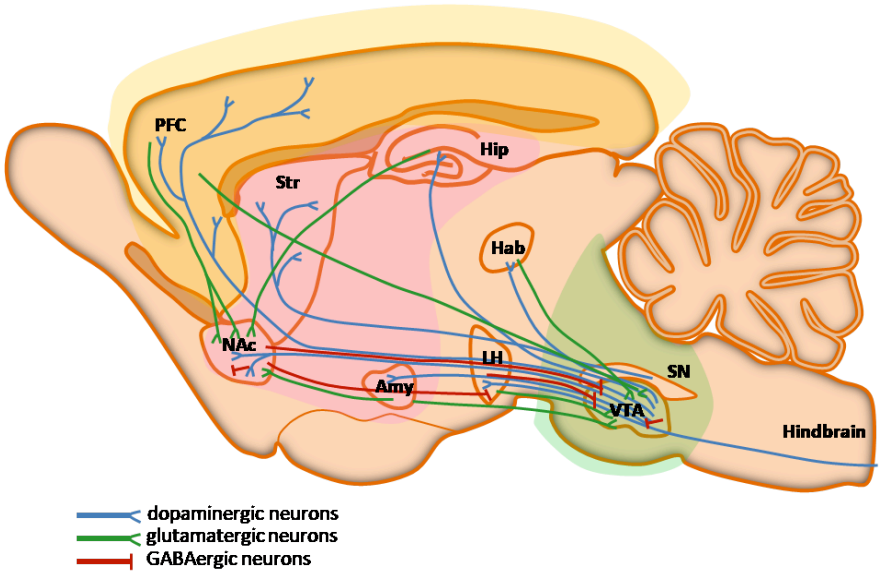

Figure 2. Major dopaminergic, glutamatergic and GABAergic connections in the rodent brain in response to rewarding stimuli. Schematic sagital view of a mouse brain illustrating brain areas involved in reward processing and their dopaminergic projections in blue (Russo and Nestler, 2013). Glutamatergic excitatory projections are represented in green whereas GABAergic inhibitory projections are pictured in red. **PFC**: *prefrontal cortex*; **Str**: *striatum*; **NAc**: *nucleus accumbens*; **Hip**: *hippocampus*; **Hab**: *habenula*; **Amy**: *amygdala*; **LH**: *lateral hypothalamus*; **VTA**: *ventral tegmental area*; **SN**: *substantia nigra pars compacta;* **GABA:** gamma-aminobutyric acid. Cortical areas are highlighted in yellow; limbic areas are highlighted in pink; midbrain areas are highlighted in green.

2.3 Modulation of dopaminergic connections

A precise fine-tuning of dopaminergic activities is orchestrated by other neurotransmitters working together with DA to perform an optimal reward processing considering biological needs (Bardo, 1998; Berridge, 2012; Davis *et al*, 2009; Khanh *et al*, 2014). Two of the most important neurotransmitters regulating dopaminergic activity are GABA and glutamate (Chiodo and Berger, 1986), as seen previously. Changes in GABA and glutamate metabolism play an important role on neuronal excitability.

GABAergic neurons produce and secrete the aminoacid **GABA**, the major inhibitory neurotransmitter in the adult brain (Ganguly *et al*, 2001). GABA targets ionotropic transmembrane receptors ($GABA_A$) or metabotrobic G protein-coupled receptors ($GABA_B$) in order to open ion channels. On their opening, chloride ions are flowed into the cell by $GABA_B$ and/or potassium ions are flowed out the neuron by $GABA_B$, thus changing the transmembranal potential and provoking a hyperpolarization of the neuron (Kuriyama *et al*, 1993). These channels are found both in pre- and post-synaptic neurons and GABA receptor activation results in synapse inhibition. An important GABAergic projection is the one arising from the NAc to the VTA working as feed-back inhibitor of DA release (see Figure 2 for main GABAergic projections in the reward system).

Glutamic acid, also known as **glutamate**, is an aminoacid found in a wide variety of ingested proteins and is considered the major excitatory neurotransmitter (Chiodo and Berger, 1986; Paladini *et al*, 1999). Glutamate is synthesized from glutamine by presynaptic neurons and adjacent glia in the CNS. It can also serve as precursor of GABA under the

action of the enzyme glutamate decarboxylase (Petroff, 2002). Glutamate is found all over the brain and binds to different types of G-coupled metabotropic receptors (mGluR) and three types of ionotropic receptors: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, kainate and N-methyl-D-aspartate (NMDA) receptors. Glutamate binding opens the transmembrane cation channels increasing the permeability of the membrane for sodium and potassium (AMPA and kainate receptors) or calcium (NMDA receptors). In metabotropic receptors, glutamate binds to the extracellular part of the receptor that mediate a change in the intracellular part, where the G protein occurs. G proteins phosphorilate and affect second messengers, such as inositol trisphosphate and diacylglycerol, and ion channels which polarize the neuron (Platt, 2007). The presence of glutamate in the synaptic cleft provokes the hyperpolarization of neurons but not only on a synaptically confined point-to-point transmission manner, but also affecting neighbouring ones in the such called glutamate spillover (Okubo *et al*, 2010). This extrasynaptic glutamate activates extrasynaptic glutamate receptors to regulate fast adjacent synaptic crosstalk, transmission or plasticity among others (DiGregorio *et al*, 2002; Kullmann *et al*, 1996; Urstadt *et al*, 2013). Long-term potentiation, a form of long-lasting enhanced synaptic plasticity, (see chapter 3.1 Synaptic plasticity) takes place in neurons receiving glutamatergic projections (see Figure 2 for main glutamatergic projections in the reward system).

Moreover, the **endocannabinoid system** (ECS) and the opioid system are also key players in DA modulation and the rewarding effects of drugs (Koob and Le Moal, 2008) (see Figure 3). The ECS is comprised of G protein-coupled receptors, small neuromodulatory lipid ligands and

biosynthetic and metabolic enzymes for the synthesis and degradation of the ligands, respectively. The best characterized ligands are N-arachidonylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG), which, due to their lipid nature are not stored in vesicles but synthesized "on demand". Two major types of cannabinoid receptor have been characterized and cloned: cannabinoid receptor 1 (CB1R) and cannabinoid receptor 2 (CB2R). While CB2R is mainly expressed in immune cells (Atwood and Mackie, 2010), CB1R is abundantly expressed in areas involved in reward, addiction and cognitive function (*amygdala,* PFC, *ventral pallidum, caudate putamen*, NAc, VTA, lateral hypothalamus (LH) (Glass *et al*, 1997). CB1R exerts inhibitory effects on glutamatergic and GABAergic signalling indirectly modulating DA release in the NAc and reward processing (Panagis *et al*, 2014). In particular, CB1R regulates approach and avoidance behaviours that mediate reward acquisition by its role in the VTA-NAc DA projection (Melis and Pistis, 2012). Due to their mesolimbic DA modulatory effect, CB1R and endocannabinoids have a prominent influence on the hedonic effects of both natural rewards and drugs of abuse (Maldonado *et al*, 2006).

The **opioid system** consists of three receptors, mu (MOR), delta (DOR), and kappa (KOR), that are activated by endogenous opioid peptides resulting from the precursors proopiomelanocortin (POMC), proenkephalin (PENK), and prodynorphin (PDYN). Opioid receptors are synthetized in response to drug and natural rewards and, together with endogenous opiods, their concentration in the reward system is modulated as addiction is developed (Le Merrer *et al*, 2009). They are involved in nociception and analgesia (Zöllner and Stein, 2007), stress, respiration, gastrointestinal transit, endocrine and immune functions,

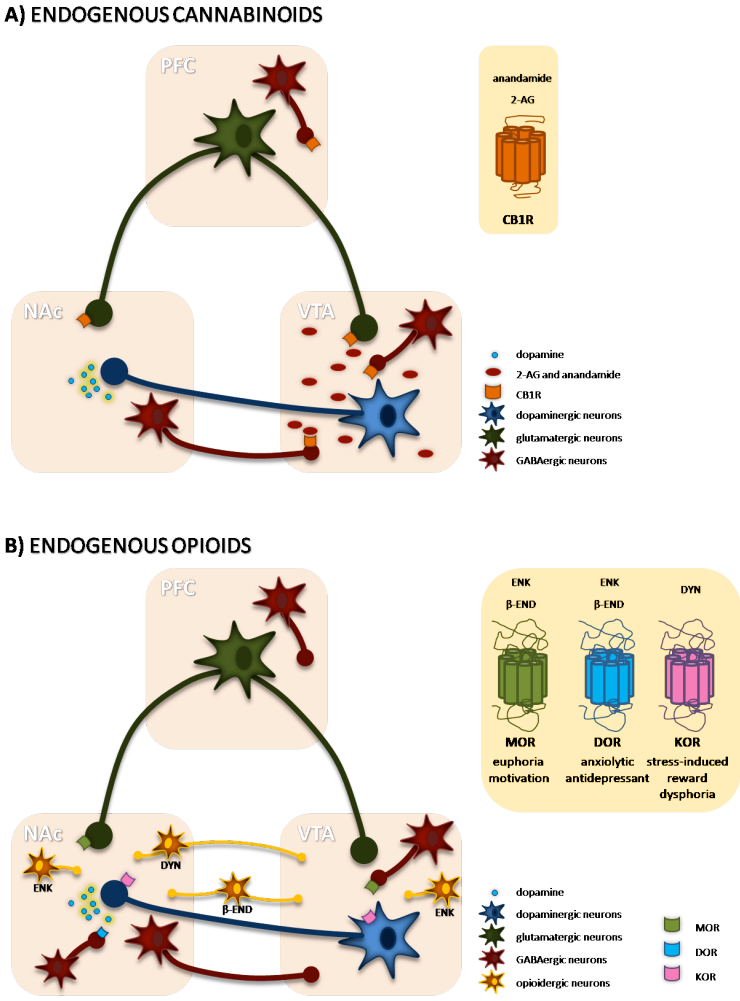

Figure 3. Schematic representation of the possible mechanisms involved in the modulation of the reward circuit by the endocannabinoid and endogenous opioid system. (A) Endocannabinoids are synthetized on demand and act as retrograde messengers on presynaptic CB1R inhibiting both GABAergic and glutamatergic inputs to VTA dopaminergic neurons. **(B)** Endogenous opioids facilitate dopamine release by activating MOR and DOR in the NAc and decreasing VTA GABAergic interneuron inhibition via MOR. Dynorphin/KOR activation acts as a feedback mechanism to counteract the high levels of DA released by drugs of abuse (Charbogne *et al*, 2014; Maldonado *et al*, 2013; Trigo *et al*, 2010). **PFC:** *prefrontal cortex;* **NAc:** *nucleus accumbens*; **VTA:** *ventral tegmental area*; **2-AG:** 2-arachidonoylglycerol; **CB1R:** cannabinoid receptor 1; **GABA:** gamma-aminobutyric acid; **ENK:** enkephalin; **β-END:** β-endorphin; **DYN:** dynorphin; **MOR:** mu opioid receptor; **DOR:** delta opioid receptor; **KOR:** kappa opioid receptor.

among others, and noteworthy they contribute to craving and relapse (Bodnar, 2007). Endogenous opioids, such as enkephalins, are synthesized and released into the NAc shell, considered the hedonic hot-spot, to confer "liking" values to the drug-related context (Peciña and Berridge, 2005; Smith and Berridge, 2007). Endogenous opioids are known to facilitate the release of DA in the NAc by an action either in the VTA or directly in the NAc (Koob, 2009).

In summary, in the event of a relevant reward stimuli, the primary dopaminergic circuits arising from the VTA and the SN *pars compacta* are activated increasing the DA tone in different areas of the brain reward system involved in motivation, goal-directed behaviours, emotions, motor control and memory, among others. This response is mediated by GABA- and glutamate-expressing neurons and fine-tuned by the action of cannabinoids and opiods among others (de Oliveira, Reimer, & Brandão, 2014; Keleta & Martinez, 2012; Pezze& Feldon, 2004; Root, Mejias-Aponte, Qi, & Morales, 2014) (depicted in Figure 2 and 3). Previous studies have demonstrated that these dopaminergic projections to multiple brain areas and its modulators are involved in memory/learning processes (*hippocampus* and *amygdala*), motivation/drive systems (*striatum* and *dorsal cortex*), inhibitory control/executive function (*orbitofrontal* and *anterior cingulate cortex*), stress reactivity (*habenula* and *amygdala*) and aversion/anxiety (BNST) demonstrating that an accurate modulation of the reward processing involves a fine orchestration of many players (Volkow *et al*, 2012).

2.4 Addiction: a chronic brain disease

Drug addiction is a chronic mental disease driven by the pathological changes in brain function produced by repeated pharmacological insults to the reward system. Compulsivity develops through maladaptive learning processes where associations between the rewarding aspects of the drug and drug-related cues take over self-control until limiting drug use becomes unfeasible. Over the course of this progression 3 stages of drug use are differentiated: (1) occasional or social use, (2) repeated and escalated use and (3) loss of control and addiction (see Figure 4). Alterations in neurotransmitter systems and long-term synaptic plasticity are developed through the progression of addiction until they become anchored together with the addictive behaviour (Kalivas and Volkow, 2005; Kelley, 2004; Nestler, 2001).

2.4.1 Occasional or social drug use

A considerable number of individuals take drugs of abuse in a recreational manner. This initial exposure to the drug is mostly motivated by social or environmental factors, such as the socioeconomic status, drug availability or hard life experiences (Kalivas and O'Brien, 2008). Drugs are initially taken in a controlled style and enjoyed due to its hedonic effects. Addictive drugs use the natural neurobiological substrate of natural rewards to exert their action (Volkow *et al*, 2011b). Even though their final goal is to increase DA tone in the mesolimbic system, drugs of abuse are chemically heterogeneous with very distinct molecular targets within the neuronal connections in the reward system. Moreover, an individual drug may have more than one molecular target. Some authors have focused on the mechanisms used by drugs directly

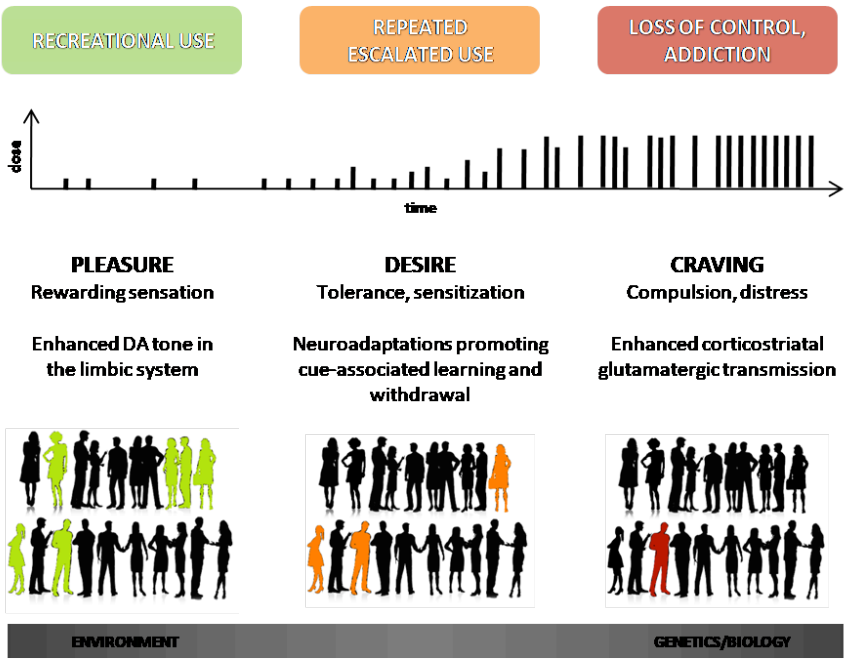

Figure 4. Stages of the addictive process. The development of addiction is a progression through three different phases: (1) Recreational use, in which the drug is taken occasionally in a controlled manner; (2) Escalated use, in which the drug intake intensifies frequency and amount; (3) Addiction, in which the individual cannot control drug intake and drug-related activities become the principal occupation.

responsible for the increase in DA concentration in order to classify drugs of abuse (see Figure 5) (Lüscher and Ungless, 2006).

Class I are drugs that activate to G protein-coupled receptors. This class comprises opioids, cannabinoids and LSD. Class I drugs agonise different types of G protein-coupled receptors (CB1R, serotonergic 5-HT2A receptor, MOR) in VTA GABA inhibitory neurons, which in turn disinhibit VTA dopaminergic neurons. By inhibiting GABA release, they foment DA flow through the dopaminergic NAc axis.

Class II are drugs that interact with ionotropic receptors. This class comprises nicotine, alcohol and benzodiazepines. These drugs act on VTA GABA terminals but also directly modulate dopaminergic neurons in the VTA producing enhanced DA release.

Class III are drugs that block monoamine transporters. This class comprises cocaine, amphetamines and derivatives such as ecstasy (MDMA). These drugs exert their action by blocking (cocaine) or reversing (amphetamine and ecstasy) the reuptake of DA in VTA projecting axons and vesicular monoamine transporters, such as those arriving to the NAc. Moreover, some of these drugs additionally produce synaptic depletion in DA neurons (amphetamine, ecstasy) and direct excitation of dopaminergic neurons (amphetamine) (Di Chiara and Imperato, 1988; Lüscher and Ungless, 2006).

2.4.2 Repeated escalated drug use

Repeated drug use progressively triggers neuroadaptations in the brain reward system of vulnerable individuals that make the drug strongly wanted influencing the way the drug is consumed. Chronic exposure to

the drug increase DA levels in two steps: directly by its consumption and indirectly by related drug-associated memories (cues, drug expectancy) (Phillips *et al*, 2003). Drug users enjoy and form contextual memories tagged to the rewarding "high" sensation produced by the accumulation of accumbal DA and these incentive values are assigned to the drug via

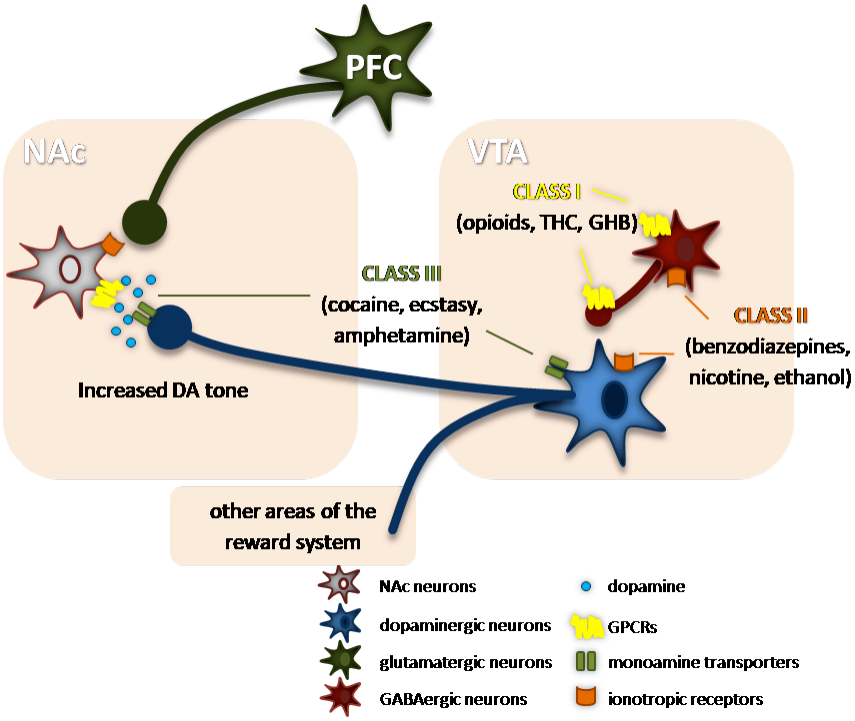

Figure 5. Intrinsic mechanism by which different drugs of abuse modulate dopamine concentrations in the *nucleus accumbens*. Class I drugs inhibit GPCRs in GABA inhibitory interneurons in the ventral tegmental area (VTA), thus desinhibiting DA release in VTA projections. Class II drugs interact with ion channels in two cells populations: inhibiting GABAergic inhibitory neurons in the VTA and directly modulating dopaminergic neurons in the VTA. Class III drugs target monoamine transporters in dopaminergic projections of the VTA blocking the reuptake of DA or stimulating the vesicular release of DA. **GABA:** gamma-aminobutyric acid; **DA:** dopamine; **THC:** tetrahydrocannabinol (marijuana); **GHB:** gamma-hydroxybutyrate ("club drug" or "date rape"); **GPCR**: G-protein-coupled receptors.

glutamatergic stimulation from cortical regions (Bromberg-Martin *et al*, 2010). The PFC and the NAc are two brain areas tightly involved in the transition to addiction. Glutamatergic PFC and dopaminergic NAc interactions are critical in triggering transductional and transcriptional cascades promoting progressive long-term changes in gene expression and neuronal plasticity potentiated by chronic drug intake (Kelley *et al*, 2003). Drug-related cues are associated with the initiation of goal-directed behaviours promoting drug desire and drug seeking (Koob, 2005).

Chronic hyperexcitation of the reward system also foments sensitization to the drug effects mediated by dysregulations in DA receptors levels. Increases in the low-affinity dopamine receptor 1 (D1R) and downregulation of dopamine receptor 2 (D2R), a high-affinity DA receptor, results in less DA caption in the synaptic cleft, less DA bounded in the post-synaptic neuron and less reinforcing sensation (Bertran-Gonzalez *et al*, 2008). Furthermore, the brief absence of the drug induces a negative emotional state in the subject, which includes stress, dysphoria, anxiety and withdrawal symptoms (negative reinforcement) (Koob, 2009; Rothwell *et al*, 2009). This phenomenon occurs due to the decreased DA levels in the *striatum* and the activation of stress pathways involving the *amygdala*. Indeed, the negative affective state that occurs during drug withdrawal is associated with a decrease in mesolimbic DA function, which might lead to compulsive drug seeking to counteract dysphoria (Koob, 2009; Weiss *et al*, 2001). Chronic drug use and acute withdrawal trigger the activation of the stress system which releases adrenocorticotropic hormone, corticosterone and corticotropin-releasing factor (CRF). Microdialysis studies in rats have found that a large increase

in stress-induced CRF release in the *amygdala* is tightly linked with enhanced anxiety and hyperlocomotion (Koob, 2009; Richter *et al*, 1995). The expression of other peptides, such as cholecystokinin (CCK), is also enhanced during chronic drug consumption and withdrawal in the NAc (Beinfeld *et al*, 2002). Indeed, CCK is involved in the behavioural sensitization to psychostimulants and foments DA increases in this area facilitating motivation and attention towards drug-derived activities (Beinfeld *et al*, 2002; Ladurelle *et al*, 1997). Brain-derived neurotrophic factor (BDNF) levels are also increased after cocaine self-administration in the NAc, and this rise in BDNF leads to the temporary increase of glutamatergic AMPA receptors in the NAc (Li and Wolf, 2011). BDNF is also known to be involved in altering dendritic and spine morphology (Russo *et al*, 2009) as well as increased BDNF serum levels have been associated with craving in opiate-dependent patients (Heberlein *et al*, 2011).

Drug withdrawal manifestations have been associated to the presence of a 'Reward Deficit Syndrome'. This syndrome refers to the insufficiency of the usual feelings of satisfaccion resulting from a dysfunction of the reward system. Primarly sensitization of DA receptors, endogenous opioid dysregulation and the action of stress systems mediate this deficiency. Reward Deficiency Syndrome leads to enhance impulsivity and need of drug use as the subject tends to compensate the disruption of DA hedonic effects by escalating drug consumption, increasing regularity of intake and even changing the drug administration route to achieve the usual pleasure (Blum *et al*, 2012; Volkow *et al*, 2011a).

2.4.3 Loss of control and drug addiction

Only 10-30% of individuals with initial contact with the drug develop addiction due to their vulnerability traits (O'Brien, 2008). Dysregulations in corticostriatal glutamatergic neurotransmission altering DA signalling are tightly involved in the transition from recreational drug use to drug addiction. Indeed, reductions of basal glutamate in the NAc core have been described after extended withdrawal in contrast with PFC glutamate release onto the NAc after reinstatement (McFarland *et al*, 2003). After long chronic drug insults, the PFC modulation of incentive salience in the NAc gets disrupted in addicted subjects. Functional alterations in the *orbitofrontal cortex*, the *anterior cingulate cortex* and the *dorsolateral PFC* are involved in salience attribution and goal-directed behaviours, in inhibitory control and awareness, and in higher cognitive operations and decision making, respectively (Volkow *et al*, 2007, 2008b). Altogether, an impaired PFC function leads to compulsive drug taking resulting in distress and completely biased attention to drug-related activities even in the expense of adverse consequences such as legal, economic or familiar problems (Jentsch and Taylor, 1999; Volkow *et al*, 2007).

In the event of the desire to cessate drug-taking, the enhanced incentive value attributed to the drug makes the addicted subject vulnerable to drug cues. The *amygdala* and the *hippocampus* mediate the recall of drug-associated memories sufficient to strongly provoke drug craving in addicted subjects and to precipitate relapse in those former abusers (Hsiang *et al*, 2014).

To sum up, the transition from sporadic drug consumption to drug addiction is a result of long chronic drug use and brain insults that involves the switch from controlled sporadic drug taking to compulsive un-controlled drug use (Deroche-Gamonet *et al*, 2004). Addicted subjects maintain and scalate drug consumption due to a weaken self-control inhibition and in order to compensate the loss of DA and avoid negative withdrawal feelings. The underlying mechanism by which these gradual changes promote the desire and need to take continuous and greater amounts of drug beyond conscious awareness includes dysregulations in neurotransmitter systems and neuronal signalling.

3. Neuronal plasticity

Life experiences, learning at school, a stressful event, or even taking a psychoactive substance, impact the brain by modifying the activity and organization of neural networks. A major mechanism by which the neural activity generated by an experience modifies brain functions is via modifications of synaptic transmission. Neuronal plasticity is the capacity of the neural reaction generated by a life experience to modify a neural network and therefore modify subsequent reactions such as thoughts, feelings, and behaviours (Citri and Malenka, 2008). It refers to the capacity of the brain to integrate transient experiences into persistent memories. For this purpose, neuronal circuits modulate their activity, structure and connections by modifying existing synapses or forming new ones (Malenka, 2003).

According to their nature, two main types of close associated neuronal plasticity mechanisms can be described: changes in the strength of the synaptic connections contribute to synaptic plasticity while morphological remodelling changes of spines and the number of synaptic contacts between neurons leads to structural plasticity.

3.1 Synaptic plasticity

This term is applied to describe the ability of the synapse to strength or weaken its activity during a certain period of time. Neurons maintain voltage gradients through their membranes by allowing or actively enabling the flow of ions, such as sodium, potassium, chloride and calcium. A sufficient voltage change in their membrane generates an action potential, the main signalling mechanism to activate synaptic

transmission at axon terminals. Action potentials travel along the axons to postsynaptic dendrites and activate postsynaptic neurons on its arrival (Rutecki, 1992). Synaptic transmission modulation can by classified according to its durability and its effect:

a) Short-term plasticity comprises rapid and reversible changes that happen in urge of adaptation. It includes several rapid synaptic processes that happen in milliseconds to minutes to modulate synaptic efficacy in an activity-dependent manner (Deng and Klyachko, 2011).

b) Long-term plasticity comprises long-lasting modifications that range from hours to well-established long-lasting behaviours (Martin *et al*, 2000).

- Long-term potentiation (LTP) is suggested to strengthen the efficacy of synaptic transmission, which could remain from hours to weeks, by a mechanism that classically initiates by calcium influx through NMDA receptors in order to increase the number and function of AMPA receptors (Malenka and Bear, 2004).
- Long-term depression (LTD) is suggested to weaken the efficacy of synaptic transmission. The mechanism is mostly mediated by NMDA receptors producing a decrease in the function and number of AMPA receptors (Collingridge *et al*, 2010).

3.2 Structural plasticity

Structural plasticity is the capability of brain circuits to reorganize and form or destroy synaptic connections. New connectivity patterns can also anchor different behaviours. Neuronal restructuring is tightly linked to synaptic plasticity. Thus, LTP/D can affect the size of somas, arborization of dendrites and the morphology and density of synaptic contacts.

Dendritic spines are small protuberances found in dendrites where excitatory glutamatergic synapses occur. They account for the cellular machinery disposed in a becoming shape so as to enable efficient or deficient synapses (Shepherd, 1996). Both LTP and LTD trigger changes at the molecular level involving cytoskeletal proteins. Indeed, cytoskeletal elements (i.e. actin filaments, scaffold proteins, adhesion molecules) are abundant in dendritic spines (Kasai *et al*, 2003). Among them, actin filaments and adhesion molecules are fundamental for synaptogenesis and spine motility, and to stabilize the synapse, respectively (as broadly reviewed by (Kasai *et al*, 2003). Synaptic plasticity also affects spine morphology. Indeed, LTP is associated with spine thicken, growth and new formation, whereas LTD promotes spine constriction and synapse depletion (Bastrikova *et al*, 2008).

Spines undergo morphological changes related to the maturation of excitatory synapses (Bourne and Harris, 2008). Indeed, they can be classified according to these morphological particularities in four different subtypes (see Figure 6):

a) Stubby are small spines with no neck. They are considered an immature-like type as they are transiting spines to be converted into other or just disappear (Kasai *et al*, 2002).

b) Mushroom are thick large spines with wide head and a defined neck. They are more prone to contain organelles (i.e. polyribosomes, smooth endoplasmatic reticulum) and high post-synaptic density (i.e. more post-synaptic receptors). These large mature-like spines contain more AMPA receptors and are more sensitive to glutamate stimulation (Nimchinsky *et al*, 2002; Takumi *et al*, 1999).

c) Thin spines represent new stable formation protrusions with early synaptic machinery. They are characterized by an elongated neck and a small bulbous head (Bourne and Harris, 2007).

d) Filopodia are precursors of dendritic spines mainly found during early neurodevelopment. The role of these long filaments absent of head may be establishing surrounding contacts with nearby axons (Garcia-Lopez *et al*, 2010).

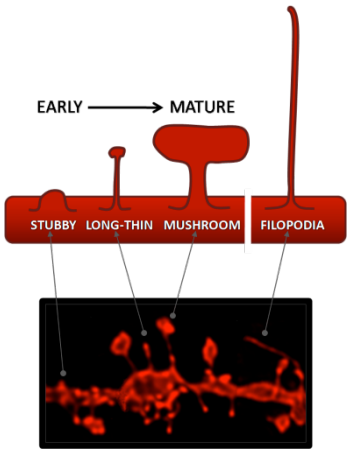

Figure 6: Morphology of spine subtypes and their schematic representation. Stubby spines are early tiny spines with no neck. Long-thin immature spines have an elongated shape with small bulbous head. Mushroom are mature spines with a huge wide head provided with synaptic machinery. Filopodia are long ephemera filaments that create linkages with nearby structures.

3.3 Reward-induced alterations in neuronal plasticity in the mesocorticolimbic system

Drugs of abuse are stimuli that can modify neuronal plasticity in a progressive long-lasting manner. It is suggested that drug-induced long-lasting modifications in the reward system relay as a neurobiological substrate of drug addiction and related behavioural alterations enabling the consolidation of new drug-related behaviours evoking the loss of control that characterize addiction (Kasanetz *et al*, 2010; Kauer and Malenka, 2007). In contrast with non addicts, addicted subjects present alterations in their different neuronal networks in a way that drug-derived behaviours got anchored as neuronal plasticity modifications. In this sense, increasing consensus suggests that addiction to drugs of abuse usurps learning and memory mechanisms normally related to natural rewards, ultimately producing long-lasting neuroadaptations that may underlie drug seeking and relapse. Neuronal plasticity modifications driven by drug exposure have been described to be primary triggered by multiple drugs in the VTA (Faleiro *et al*, 2004; Mansvelder and McGehee, 2000) and NAc (Li and Kauer, 2004; Thomas *et al*, 2001) and then all through the reward system in areas including the PFC (Otani *et al*, 2015), the *dorsal striatum* (Gerdeman *et al*, 2003), the *amygdala* (Bissière *et al*, 2003) and the *hippocampus* (Thompson *et al*, 2002). This progressive detrimental changes alter the function of the system and contribute to the persistent modifications and behavioural disruption associated with drug use and addiction (Robinson and Kolb, 2004).

Previous studies have shown that both LTP at glutamatergic synapses of dopaminergic VTA neurons and increases in the AMPA/NMDA receptor ratio can be triggered by a single dose of several drugs of abuse (Mameli

et al, 2011; Niehaus *et al*, 2010; Ungless *et al*, 2001). These affirmations indicate that neuronal plasticity in the VTA may be the first modification of the reward system in drug exposure. Further impairments in neuronal plasticity are triggered by subsequent repeated drug seeking. In contrast with evidence showing that consumption of natural rewards (standard food and water) or even passive administration of drugs, such as cocaine, do not produce persistent LTP in the VTA, drug-seeking behaviour is able to trigger LTP in the VTA. Thus, an active drug-seeking would be determinant to develop modifications in VTA functioning (Chen *et al*, 2008). In this line, subsequent neuroplasticity changes in the reward system will develop in a drug use-dependent manner (see Figure 7).

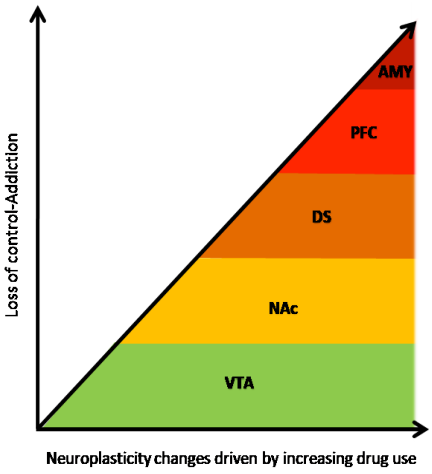

Figure 7. Schematic drawing describing the sequential and cumulative effects in neuroplasticity changes hypothesized to contribute to addiction. Acute administration of drugs of abuse primary affects VTA excitability. Repeated excitation of mesolimbic dopaminergic neurons induced by drugs of abuse posteriory affect the NAc, highly sensitive to modifications in DA tone. Progression of the addictive phenotype will then involve the dorsal striatum. Finally, compulsion will develop by drug-induced glutamatergic signalling modifications in the PFC and the amygdala (Koob and Volkow, 2010). **VTA:** *ventral tegmental area*; **NAc**: *nucleus accumbens*; **DS***: dorsal striatum*; **PFC:** *prefrontal cortex*; **AMY:** *amygdala*.

Activation of dopaminergic VTA neurons generates an increased mesolimbic DA tone. A constant and repeated overload of DA in the NAc contributes to an increased activation and maladaptation of this area. In this line, preclinical studies have demonstrated that after a prolonged period of cocaine self-administration, the ability to generate LTD in the NAc core and shell impaired. Even more, the disability remained during two weeks after cessation of cocaine self-administration (Martin *et al*, 2006; Russo *et al*, 2010). These observations indicate the prominent role of NAc neuronal plasticity in drug addiction by early stages of conditioning and pathological drug consumption. On repeated escalated drug consumption, the *dorsal striatum* also contributes to the transition from occasional to compulsive use. Plasticity modifications in the *dorsal striatum* have been implicated in habit-formation and automaticity that trigger drug-seeking seen in drug consumers (Koob and Volkow, 2010). Subsequently, habits transform into compulsive seeking of drug-evoked actions and progressively into loss of control towards drug-seeking and –taking. This behavioural modification is paired with neuronal plasticity adaptations occurring in the PFC. Specially, glutamatergic PFC connections to the NAc are key to anchor compulsive behaviours that define drug addiction. Impairments in the corticolimbic glutamatergic connections anchor the addictive behaviour characterized by an impaired executive control towards drug-related activities. Therefore, the engagement of plasticity modifications in PFC connections to the NAc may be critical to induce loss of control. Finally, co-ocurring neuronal plasticity modifications inducing poor decision-making (PFC) and gain of function in the brain stress systems (extended *amygdala* circuit) may contribute to an enhanced incentive salience for drugs motivated by the aversive effects of non-drug episodes. In turn, this increased salience

towards drug-related actions to compensate withdrawal symptoms would foment distress towards other actions or natural reinforcers (Koob and Volkow, 2010). The progression of these neuronal plasticity modifications in vulnerable individuals anchor disrupted behaviours conducing to loss of control towards drug seeking and consumption that remain in a long-lasting manner. Noteworthy, these changes in neuronal architecture and activity remain even after discontinuation of drug use for the majority of the drugs. Preclinical studies using the cocaine self-administration paradigm indicate that after cessation of cocaine seeking and intake 'addicted' animals expressed persistently impairment of LTD ability while 'non-addicted' animals slowly recovered this skill (Schramm-Sapyta *et al*, 2006). Therefore, these maladaptations in neuronal connections also limit the new formation of natural structural plasticity modifications promoted by other later experiences. This observations indicate the stability and power of these changes once they are acquired (Kolb *et al*, 2003).

Noteworthy, even the progression of the plasticity modifications induced by drugs of abuse engage the different brain areas as previously described, the nature of the changes underwent by neurons depend on the drug type. Thus, while psychostimulants and nicotine increase AMPA receptor availability and spine densities in the NAc, VTA and PFC, opiates are known to decrease spine densities in the same reward-related areas (Robinson and Kolb, 2004).

4. Neuroglial cells

Neuroglial cells are tightly involved in neuronal function. Neuroglial cells are implicated in neuronal connectivity, synaptic efficiency, neuronal adaptations to experiences and stimuli and, thus, are of striking importance in the modulation of neuronal restructuration driven by some mental disorders such as the addictive process.

Neuroglial cells, usually known as glial cells, constitute a voluminous fraction of the mammalian nervous system, representing between 33 and 66% of total brain mass (Herculano-Houzel, 2014). First identified in the 19th century (by Ramon y Cajal, among others), their initial suggested function was to serve as glue for nerve cells, which was reflected in their name ("glia" is the ancient Greek word for "glue"). Since then, many different roles have been proposed and shown for glia cells, although their full properties remain unveiled (reviewed in Jäkel & Dimou, 2017). There are several types of glial cells, which can be divided in four groups depending on their morphology and functions:

1) Astrocytes: They are the most abundant population of glial cells in the brain (Kettenmann and Ransom, 2005). Their major functions are the regulation of water and ion homeostasis, modulation of blood brain barrier (BBB) permeability and control of tripartite synapses (Kimelberg and Nedergaard, 2010).

2) Oligodendrocytes: They are the myelin-producing cells and, therefore, the responsible of axon myelination allowing the fast signal conduction. Nevertheless, there are different patterns of myelination between axons,

which may reflect different oligodendrocyte functions depending on the signals coming from neurons (de Hoz and Simons, 2015).

3) NG2-glia: Although they are the progenitors of mature oligodendrocytes, they are considered as an independent glial population due to their particular characteristics. Their main function is generating mature oligodendrocytes (Simon *et al*, 2011). However, they also form functional synapses with neurons in different parts of the brain (Sun *et al*, 2016).

4) Microglia: They represent the immune and phagocytic cells of the nervous system and will be extensively discussed in the next chapter since the role of microglia cells on the neurobiological alterations promoted by repeated reward exposure has been investigated in this Thesis.

4.1 Microglial cells

Microglial cells are a type of glial cells found in the brain and spinal cord. They function as immune cells in the CNS and can be functionally compared to the resident tissue macrophages of other organs (Saijo and Glass, 2011). They have an hematopoietic origin from macrophages arising from the yolk sac and migrating to the CNS on early embryogenesis (Alliot *et al*, 1999). These immune effectors represent between a 0.5-16.6% of the brain cell population in human and around a 10% in rodents (Lawson *et al*, 1990; Mittelbronn *et al*, 2001). Microglial cells are architected in a net-like style along the brain parenchyma. The net varies their thickness anatomically and between species: e.g. their density ranges between 5% in mouse *corpus callosum* to 12% in mouse

SN and are found to be more abundant in grey than in white brain matter (Lawson *et al*, 1990; Zhang and Sejnowski, 2000). In humans, their concentration may vary by up to one order of magnitude between brain areas and are more abundant in white than in grey matter (Mittelbronn *et al*, 2001).

4.1.1 Morphology

Every single microglial cell is settled as a sentinel guarding its own territory in a non-overlapped manner. These particular cells exert visible changes in their morphology, switching from resting to motile behaviour and varying the expression of cell adhesion molecules, cytoeskeletal organization and antigen presentation molecules according to the changes in their surroundings. Microglial activation can be classified according to their morphology and/or the expression of cell surface antigens (see Figure 8). Indeed, their conformation range from a normal brain "resting state" to an amoeboid-like "activated state" as reviewed by Boche et al, (2013).

a) Resting/ramified state

In this quiescent conformation, microglia cells present a tiny soma with highly ramified and elongated branches that sweep the nearby tissue (Benarroch, 2013; Kettenmann *et al*, 2011). In the quiescent state microglia monitors either astrocytes or neurons in the search of perturbations. Contrary to the "resting" assumption, two photon microscopy revealed that microglial cells present high motility even in surveillance conditions on a healthy brain (Nimmerjahn *et al*, 2005).

Microglial cells maintain an active sensoring by elongating or retracting their branches from 1 to 3 µm/min without translocating the soma.

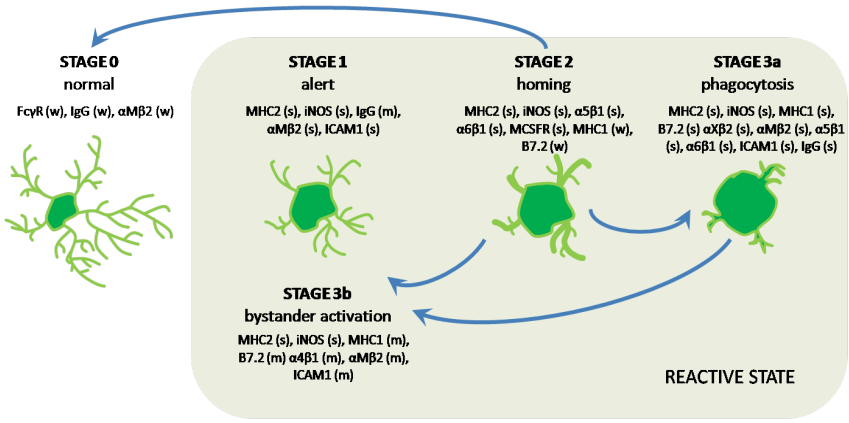

Figure 8: Different stages of microglial activation from a morphological and molecular point of view. Neuronal injury activates microglia cells, which shapes from a tiny soma and highly ramified conformation (stage 0) to a more corpulent and less ramified state (stage 1). Alert microglia detects and attachs to damaged cells (stage 2). With no further stimulation, homing microglia returns to resting state. However, with additional cell death or trauma, microglial cells turn into phagocytes (stage 3a) and activate neighbouring microglia (stage 3b). Activation of microglial cells also happens undergoing infection or autoimmune response. Activated microglia share common membrane antigens being MHC2 and iNOS. Each state exerts specific membrane markers Raivich et al., 1999. **(w):** weak; **(m):** moderate; **(s):** strong immunoreactivity. **FcγR:** Fc gamma receptor; **IgG:** immunoglobulin G; **αβ:** integrin; **iNOS:** Inducible nitric oxide synthase; **ICAM1:** intercellular adhesion molecule 1; **MCSFR:** macrophage colony-stimulating factor receptor; **MHC:** major histocompatibility complex.

b) Activated/amoeboid state

Infections, trauma, ischemia and neurodegeneration cause microglia to undergo activation. Microglial cells shape to a motile amoeboid-like conformation where their branches become retracted and simplified, their soma expanded and its inner machinery starts working as a macrophage. Active microglial functions comprise migration to the injured site, phagocytocis, proliferation, T-cell antigen presentation and release of pro-inflammatory or anti-inflammatory cytokines and chemokines to attract nearby cells (Kettenmann *et al*, 2011; Ransohoff and Perry, 2009).

4.2 Functions

Multiple functions are attributed to the microglial cells that can be detrimental or beneficial for the surrounding cells. These functions are related to brain development, homeostasis and modulation of immune responses and inflammation: CNS development, regulation of stem cell and tumour proliferation, removal of cell debris, matrix remodelling, demyelination, recognition of pathogens and antibodies, phagocytosis, antigen presentation, cytotoxicity, among others (as reviewed in Boche, Perry, & Nicoll, 2013). Noteworthy, microglial cells are key players in synaptic remodelling in healthy and diseased brain. This function called pruning is of notable importance during brain development in order to refine neuronal circuitries in an activity-dependent manner, but it also remains in adulthood (see Figure 9)(Graeber, 2010).

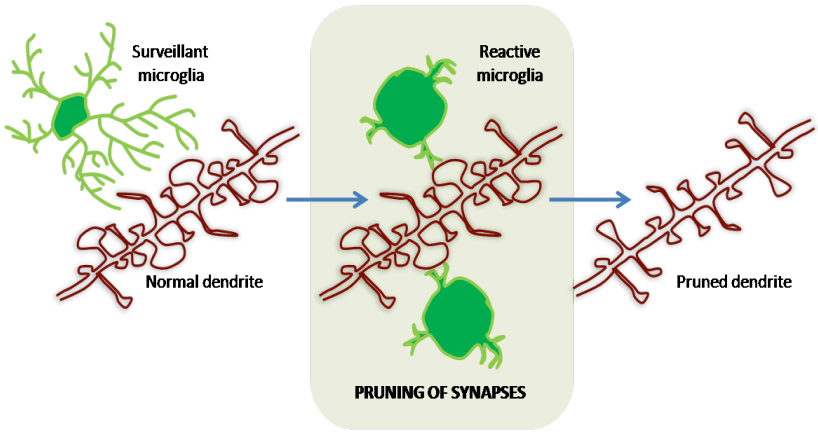

Figure 9. Pruning synapses by microglial cells. Dendritic spines are postsynaptic components found in dendrites which contain the synaptic machinery needed for synaptic transmission. Activated microglia is in charge of removing spines for circuitry remodelling. Excessive or unnecessary pruning of synapses is linked with neurodegenerative processes.

5. Cytokines

Cytokines are small proteins secreted by different types of immunological cells (monocytes, macrophages and lymphocytes, as well as microglia and astrocytes) and key modulators of inflammation. They have a specific effect on the interactions and communications between cells, orchestrating the immunological response. Accordingly, depending on their site of action, cytokines are involved in autocrine signalling (on the same cells secreting them), paracrine signalling (on nearby cells), or endocrine signalling (on distant cells) (Zhang and An, 2007). Cytokine is a general name for a group of over 300 proteins, including lymphokines (cytokines secreted by lymphocytes), monokines (cytokines generated in monocytes), chemokines (cytokines with chemotactic activities), interleukins (cytokines produced in leukocytes and acting on other leukocytes), interferons (IFNs), tumour necrosis factors (i.e. TNFα) and growth factors (TGF) such as TGFβ (Turner *et al*, 2014). Thus, cytokines involve a great variety of proteins with pleiotropic effects in a complex, and sometimes contradictory, network of interactions. For example, the same cytokine can produce proliferation in one cell type and, in combination with another cytokine, cycle arrest in another (Turner M et al, 2014). However, it is possible to classify cytokines depending on the nature of the immune response in pro-inflammatory (i.e. IL-1, TNFα or IFN-I), anti-inflammatory (i.e. IL-10 and IL-12) or both depending on the circumstances (i.e. IL-6 or TGFβ) (Turner *et al*, 2014; Zhang and An, 2007).

5.1 Pro-inflammatory cytokines

Pro-inflammatory cytokines, produced mainly by activated macrophages, are involved in the up-regulation of inflammatory responses. The major families of this class of cytokines are IL-1 (IL-1α, IL-1β, IL-1ra, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Ra, IL-37 and IL-1Hy2), IL-6 (IL-6, IL-11, IL-31, ciliary neurotrophic factor -CNTF-, cardiotrophin-1 -CT1-, leukaemia inhibitory factor -LIF-, osteopontin -OPN-, and oncostatin M -OSM-), TNFα (TNFα, TNFβ, BAFF, and APRIL), IL-17 (IL-17A-F and IL-25, also known as IL-17E), Type I IFN (IFNα, IFNβ, IFNω, IFNκ, Limitin), Type II IFN (IFNγ), and Type III IFN (IFNλ1 (IL-29), IFNλ2 (IL-28A), and IFNλ3 (IL-28B)). Many of them have important roles in the CNS, either in brain homeostasis or in pathological situations. Thus, IL-1β is expressed in nociceptive neurons and its expression is increased after trauma in microglia and astrocytes in the CNS causing hyperalgesia and augmented production of substance P and prostaglandin E2 (PGE2) in glial cells (Zhang and An, 2007). In addition, the IL-6 family of cytokines has been implicated in a multitude of functions within the CNS, such as differentiation of neurons, astrocytes and oligodendrocytes (Turner *et al*, 2014).

5.2 Anti-inflammatory cytokines

The anti-inflammatory cytokines are a class of immunoregulatory proteins that control the pro-inflammatory cytokine response to regulate the human immune action. Major anti-inflammatory cytokines include IL-1 receptor antagonist, IL-4, IL-10 family (IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, IL-29), IL-11, and IL-12 family (IL-12, IL-23, IL-27, IL-35and IL-13) (Turner *et al*, 2014; Zhang and An, 2007). In addition, Leukemia inhibitory

factor (LIF), IFNα, IL-6 and transforming growth factor (TGF)-β can be classified as either anti-inflammatory or pro-inflammatory cytokines, depending on the circumstances. Also, specific cytokine receptors for IL-1, TNFα, and IL-18 work as inhibitors for pro-inflammatory cytokines (Zhang and An, 2007).

Among anti-inflammatory citokines, IL-10 is a potent anti-inflammatory one which suppresses the expression of several pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1. Moreover, IL-10 can up-regulate anti-cytokines and down-regulate pro-inflammatory cytokine receptors. It controls the production and action of pro-inflammatory cytokines at multiple levels.

Other anti-inflammatory cytokines are the 5 members of TGF-β family (TGF-β1 to -β5). TGF-β represses cytokine synthesis by blocking the activity of macrophages and Th1 lymphocytes; counteracts IL-1, IL-2, IL-6, and TNF action; and induces IL-1ra 6. Interestingly, its expression is induced after axotomy and may be involved in a negative-feedback loop to control glial activation (Kiefer *et al*, 1993).

5.3 Cytokines in neuroinflammation

The BBB restricts leukocyte migration but cytokines can cross this barrier through leakages or by BBB transporter (Kim et al, 2016). Moreover, endothelial cells of the BBB can secrete cytokines, being a major source of CNS cytokines (Verma *et al*, 2006). Nevertheless, the main regulators of cytokines and inflammatory processes within the brain are microglia cells (Fenn *et al*, 2014).

Neuroinflammation accounts for many different pathological conditions, ranging from morphological alterations in glial cells to tissue destruction by blood immune invading cells. It can be initiated after a brain injury, infection, toxic contact, or autoimmunity. Then, microglia secrete pro-inflammatory cytokines, chemokines and reactive oxidants to protect and restore the injured region of the CNS. However, prolonged inflammatory responses can increase the tissue damage that together with cytokine networks dysregulation may result in pathological situations. For instance, the CNS can be the target of inflammatory response by invading immune cells in several disorders such as encephalitides and inflammatory demyelinating diseases. On the other hand, some pathologies such as Alzheimer disease or Parkinson disease can also trigger inflammation through glia cells (Fenn *et al*, 2014).

It is known that cytokine expression in the brain shows a pyramidal graded production depending on the severity of the suffered trauma as pictured in Figure 10. In normal brain (grade 0), there is already a basal expression of two cytokines: macrophage colony-stimulating factor (MCSF) and TGFβ1. The MCSF is a pro-inflammatory cytokine, member of the colony stimulating factors family in charge of mitotic division of macrophage-like cells (including microglia) (Kloss *et al*, 1997), fomenting the proliferation of microglial cells. It also has been described as a non-redundant pathogenic factor in the progression of autoimmune CNS inflammation (Becher et al, 2016). In contrast, TGFβ1 acts as anti-inflammatory cytokine (as described before) and it is up-regulated to tune the strong immunological process (Koefer *et al*, 1995). With increased pathology, more cytokines are progressively recruited to the injured zone. On indirect trauma (grade 1), such as experimental cut of

axons (axotomy), ischemia, autoinmune disease or obesity, several studies have reported the production of the almost ubiquitous pro-inflammatory interleukin 6 (IL-6) acting on neurons and astrocytes (Raivich *et al*, 1996). After cell death (grade 2), interleukin 1β (IL-1β) and tumour necrosis factor α (TNFα) are recruited to the damaged site. IL-1β is a key pro-inflammatory cytokine emerging from activated microglia and strongly upregulated in severe brain injury regulating citotoxicity (Wang *et al*, 1997). TNFα is also a pro-inflammatory cytokine acting on neurons, astrocytes and activating microglia, which is strongly produced after severe brain pathologies such as severe trauma, ischemia or dementias (Bruce *et al*, 1996; Seilhean *et al*, 1997; Uno *et al*, 1997). In presence of strong brain injury (grade 3) also IFNγ is recruited. IFNγ is a key regulator in the T-cell mediated inflammation. Apart from trigger the production of cytotoxic oxygen radicals, it induces phagocytosis and activate microglial cells by presenting the antigen in the MHC2 (Suzumura *et al*, 1987).

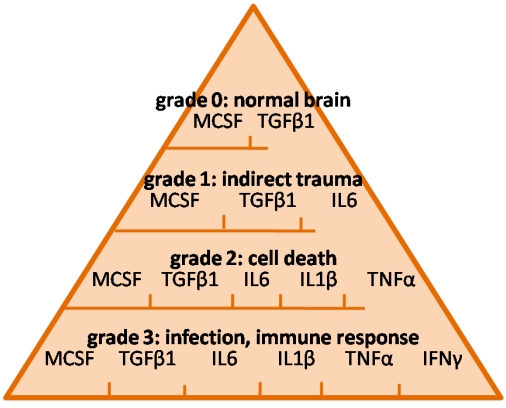

Figure 10. Microglial cytokine expression pyramid. The recruitment of cytokines in the normal and damaged brain depends on the strength of the leisure. With increased pathology, more cytokines are recruited to the damaged area. Adapted from (Raivich *et al*, 1999). **MCSF:** macrophage colony-stimulating factor; **TGFβ1:** transforming growth factor beta 1; **IL-6:** interleukin 6; **IL1β:** interleukin 1 beta; **TNFα:** tumour necrosis factor alpha; **IFNγ:** interferon gamma.

6. Microglia, cytokines and synaptic plasticity

In the **healthy brain**, microglia removes unwanted spines and synapses contributing to the maturation of neuronal circuits (Wu *et al*, 2015). Two-photon data showed microglia activities associated with dendritic spine remodelling in an experience-dependent manner (Graeber, 2010). However, inappropriate engulfment of synapses causes excessive loss of running or new-born synapses as in neurodegenerative diseases such as Alzheimer's Disease (Hong *et al*, 2016). In the absence of inflammation, microglia activities and secretion of immune-related signalling molecules control the homeostasis of the brain maintaining the balance between neurogenesis and neuronal cell death. Some proteins also intervene in the modification of synaptic connections in addition to their immunological roles. These include pro-inflammatory cytokines (e.g., TNFα, IL-6), proteins of the innate immune system (e.g., complement C1q and C3, pentraxins, Dscam), members of the major histocompatibility complex class I (MHCI) family, and MHCI-binding immunoreceptors and their components (e.g., PIRB, Ly49, DAP12, CD3zeta) (Boulanger, 2009).

In the **diseased brain**, inflammatory factors and microglia modify synaptic connections and synaptic plasticity in a pathological manner (Wu *et al*, 2015). In this line, dietary obesity has been shown to dysregulate the neuron-microglia relationship leading to plasticity deficits due to excessive pruning and enhanced inflammation. Studies in diet-induced obesity in mice have vastly shown overacting microglia and inflammation in the *hypothalamus*, one of the most important areas of the brain controlling feeding behaviour (Erion *et al*, 2014; Kälin *et al*, 2015; Thaler *et al*, 2012; Valdearcos *et al*, 2015). Moreover, microglial

activation has also been described in hippocampal neurons. Unlike neurodegenerative diseases, high fat diet-induced microglia excessive pruning in *hippocampus* is restablished after time (Hao *et al*, 2016).

In this line, cytokines has also been described to be dysregulated in obesity. General obesity has been associated with significantly elevated levels of IL-5, IL-10, IL-12, IL-13, IFN-γ and TNF-α while central obesity was related to significantly elevated IL-5, IL-10, IL-12, IL-13 and IFN-γ-levels. Moreover, sedentarism and physical activity significantly correlate with the increase and decrease of these levels (Schmidt *et al*, 2015).

Microglial activities are also directly modulated by drugs of abuse by targeting their receptors including DA receptors, opioid receptors, morphine-coupled toll-like receptor 4, CB1R and CB2R (Cabral and Marciano-Cabral, 2005; Racz *et al*, 2008; Wang *et al*, 2012; Zou *et al*, 2011). In addition, the action of drugs of abuse in the brain can also indirectly activate microglia by a mechanism independent of the former receptors (Lee *et al*, 2009). In this line, glial cells have also been involved in the process of drug addiction by interfering in drug-evoked synaptic plasticity rearrangements, such as the loss of function of cortical circuits in the development of drug addiction (Smith *et al*, 2015). Indeed, it has been postulated that new-born neurons may block consolidated drug-associative memories or enhance an extinction prone learning. Drugs of abuse foment the activation of microglia to provoke the reduction of neurogenesis in order to establish a more robust long-lasting memory of drug seeking and drug taking (Mandyam and Koob, 2012). Moreover, preclinical studies revealed that repeated cocaine injection in mice activated microglia. Furthermore, microglia rather than astrocytes was

pointed to be the major source of chronic cocaine-induced TNF-α in the NAc (Lewitus *et al*, 2016). Even more, evaluations in cocaine withdrawal indicated that microglia activity decreased during this drug-free periods (Ferrarelli, 2016).

Thus, unravel the role of non-neuronal cells such as microglial cells must be a jackpot on the treatment of addictive disorders and disrupted maladaptive behaviours.

7. Cocaine addiction

Cocaine is a tropane ester alkaloid found in leaves of the *Erythroxylum* coca plant, a bush that grows in the region of the Andes Mountain in South America (Karch, 2006). Cocaine is widely consumed drug, particularly in the young adult population, in different format presentations alone or in combination with other drugs. Cocaine consumers are in high risk of developing co-ocurring conditions and often develop addiction (Center for Behavioral Health Statistics, 2014).

7.1 Epidemiology

Cocaine is used by an estimated 18.2 million people worldwide especially by individuals aged from 15 to 64 (World Drug Report, 2016). However, most cocaine use is by urban men from 15 to 35 years old. Indeed, approximately 13.3% of young adults in the USA reported at least one use of cocaine, with an estimated 600,000 new users per year (SAaMHSA, 2010). Its use is more prevalent in North, Central and South America followed by Western and Central Europe (European Monitoring Centre for Drugs and Drug Addiction, 2015).

Cocaine consumption has considerable health and economic impact. This drug causes potentially fatal cerebral haemorrhage and cardiovascular events such as arrhythmias, myocardial infarction resulting in US$581 million in direct health-care costs annually (Caulkins *et al*, 2002). Its use is associated with abusing from other legal and illegal substances, such as tobacco and alcohol. Many cocaine users simultaneously use other substances, such as opiates, to enhance the "high" or to ameliorate the cocaine-related adverse effects of

intoxication or withdrawal, such as alcohol, cannabis, or benzodiazepines. Importantly, alcohol and cocaine abuse is usually co-ocurrent. Indeed, an 85% of people diagnosed with cocaine addiction is also diagnosed with alcoholism (Flannery *et al*, 2004). Also, cocaine consumers have doubled probability of developing depressive or anxiety disorders (Zöllner and Stein, 2007). Chronic cocaine use is associated with cognitive impairment in visuo-motor performance, attention, verbal memory, and risk-reward decision-making including suicidal ideation and attempts (Friedman *et al*, 2004; Kandel *et al*, 2001). Moreover, repeated cocaine consumption may develop cocaine addiction in one out of six individuals (Anthony *et al*, 1994). The interval between first cocaine use and diagnosis of dependence in those cocaine users who become addicted has been reported to be 4–12 months (Ridenour *et al*, 2006). Cocaine addiction is characterized by a compulsive need to seek and take the drug, a loss of control over the amount of drug consumed and by periods of attempted abstinence followed by relapse. Indeed, approximately 69% of cocaine addicts completing cocaine addiction treatment programs and 80% of addicts completing long-term residential cocaine treatment relapse to their cocaine habit within 1 year (Institute for Health Policy, 2001).

7.2 Pharmacology of cocaine

Illegal cocaine can be bought in two different **presentations** of the same molecule that trigger the same pharmacological action when reaching the targeted organs. Cocaine base ("crack") melts at 98ºC and can be smoked but not injected as it is insoluble in water. Cocaine salt has a higher melting point (195ºC) that impedes being smoked before its pyrolytic destruction. This variant is normally injected or snorted through the nose (Hatsukami and Fischman, 1996). The onset of action and duration of cocaine **effects** depends on the route of administration being intravenous and smoked within seconds and lasting 15-30 min; intranasal in 20-30 min and remaining for 1 h and gastrointestinal up to 90 min and staying for 3 h.

Cocaine **absorption** happens in the mucous membranes of the nose and mouth and in the genitourinary, gastrointestinal and respiratory tracts. In addition, contact with the skin or second-hand cocaine smoke inhalation are passive absorption routes that can even cause important negative effects in infants (Cone *et al*, 1995; Kavanagh *et al*, 1992; Mott *et al*, 1994). Subsequently, cocaine arrives to a vast collection of organs including the brain, heart, kidney, adrenal glands, and liver. Cocaine is mainly metabolized by carboxyesterases in the liver by hydrolysis of its ester bonds to benzoylecgonine and in brain, liver, lung by butyrylcholinesterases to ecgonine methylester and finally eliminated in the orine (Cone, 1995; Warner and Norman, 2000).

Importantly, cocaine is used together with alcohol (Gossop *et al*, 2006), which leads to the formation of a new compound, **cocaethylene** exerting less potent pharmacological actions than those of cocaine but with

longer half-life (Baker *et al*, 2007; Center for Behavioral Health Statistics, 2014; Pennings *et al*, 2002). Cocaethylene induces more intense feelings of 'high' beyond those perceived with either drug alone, less intense feelings of alcohol-induced inebriation and tempering of discomfort when coming down from cocaine 'high'. However, combining alcohol and cocaine provokes magnified deleterious effects on heart rate and toxicity that are greater than those obtained adding the effects from each drug consumed alone. Moreover, its combined consumption increases blood cocaine levels up to a 30% more than taking solely cocaine (Baker *et al*, 2007; Herbst *et al*, 2011; Pennings *et al*, 2002). Therefore, cocaine and alcohol co-occurrence may have more severe and longer lasting toxic effects than taking cocaine or alcohol alone.

7.3 Neurobiological mechanism

Cocaine exerts its action in the mesolimbic system enhancing monoamine activity. It blocks monoamine reuptake by acting on presynaptic monoamine transporters in the *striatum*. DA tone increases in the *dorsal* and *ventral striatum*, mediating the psychomotor stimulant effects of cocaine and contributing to its conditioned reinforcing effects, respectively (see Figure 11) (Camí and Farré, 2003; Suto *et al*, 2009; Suto and Wise, 2011). Chronic cocaine use can result in tolerance (decreased drug response) or sensitization (increased drug response). The escalation of cocaine abuse depends on the tolerance and sensitization of each subject to the effects of the drug over time. Both tolerance and sensitization to cocaine are produced by the neurochemical effects of cocaine, but these changes will depend on the amount and frequency of cocaine use (Calipari ES et al, 2013). Tolerance is the most described neuroadaptation present in cocaine addicts and results from frequent,

high-dose, or long-term exposure (O'Brien *et al*, 2006). According to the DSM-5, tolerance is defined as either 1) a need for markedly increased amounts of the substance to achieve intoxication or desired effect, or 2) markedly diminished effect with continued use of the same amount (American Psychiatric Association, 2013). Tolerance to cocaine's DA enhanced effects is possibly due to a decreased ability of cocaine to inhibit DA transporters, as shown by several studies using self-administration sessions (Mateo et al, 2005; Ferris et al, 2011; Calipari et al, 2012). On the other hand, low-dose, intermittent exposure may potentiate sensitization to DA binding in postsynaptic D1R and D2R in the *striatum* which can be long-lasting (reviewed in Vanderschuren & Kalivas, 2000). Indeed, neurochemical sensitization to cocaine-induced extracellular DA increases is associated not only to mesolimbic adaptations concerning alterations in the regulation of DA release and reuptake (Kalivas and Duffy, 1990, 1993; Parsons and Justice, 1993; Addy et al, 2010) but also changes in glutamate, GABA and serotonin release in the system (Vanderschuren and Kalivas, 2000; Steketee, 2005; Filip et al, 2006; Neumaier et al, 2002; Filip et al, 2010). These pharmacological neurobiological actions are responsible for the development of maladaptive modifications in neuronal excitability and connections leading in some subjects to the loss of control that characterize drug addiction (Dackis and O'Brien, 2001).

.

7.4 Therapeutic approaches

Treatment of cocaine addiction, including both cocaine dependence and cocaine relapse, requires a pharmacologic approach to be combined with psychological therapies to soothe withdrawal symptoms and re-educate established drug behavioural patterns (Potenza *et al*, 2011). However, to date, no effective treatment is known.

Psychosocial/Behavioural therapies developed to treat cocaine addiction include strategies to help patients clarify their ambivalence about cocaine use (Motivational Enhancement Therapy/Motivational Interviewing), cope more effectively without cocaine consume (Cognitive

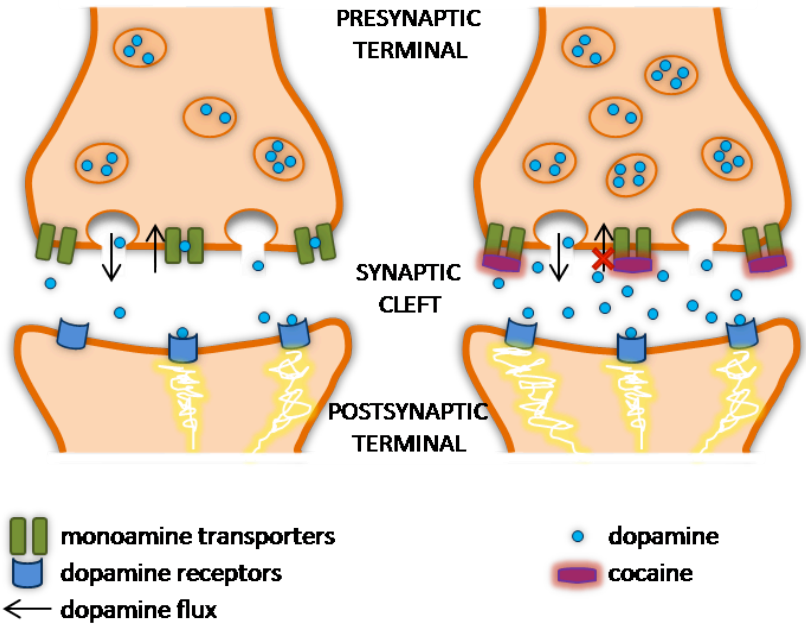

Figure 11. Synapse on normal conditions compared with a synapse under cocaine exposure. Cocaine enhances monoamine activity by stimulating dopamine release in the dorsal and ventral striatum and acting on monoamine transporters inhibiting monoamine reuptake.

Behavioural Therapy), think differently about their consume behaviour (Cognitive Therapy), use techniques to avoid triggers for cocaine consume), change the reinforcing environmental stimuli (Contingency Management and Community Reinforcement Approach), and increase detachment from thoughts that may lead to cocaine use (meditation/mindfulness-based therapies) (reviewed in Penberthy, Ait-Daoud, Vaughan, & Fanning, 2010). Nevertheless, all these therapies are often used in combination with pharmacological approaches to treat cocaine dependence and relapse. Numerous drugs have been tested being the most used antidepressants, mood stabilizers, dopaminergic agonists, stimulants, and precursors of neurotransmitters (Sánchez-Hervás, 2016). A brief description of the most used pharmacotherapies for cocaine addiction follows.

7.4.1 Topiramate

Topiramate is a sulphamate fructopyranose derivate that reduces the rewarding effects associated with cocaine use (Penberthy *et al*, 2010). Topiramate inhibits mesocorticolimbic DA release in the VTA projecting to the NAc through two actions. It facilitates GABA activity and inhibits glutamatergic kainate and AMPA receptors decreasing glutamate function after drug consumption. Moreover, topiramate inhibits noradrenergic neurons, which are involved in the development of withdrawal symptoms. Even more, it is a carbonic anhydrase inhibitor, an enzyme involved in the convulsant effects suffered in withdrawal. Thus, topiramate may act in reducing cocaine rewarding effects and moderating cocaine withdrawal (Rugino, 2007). Topiramate therapeutic concentrations range from 25 mg to 300 mg. However, doses higher than

100 mg increase adverse reactions such as dizziness, paraesthesia or psychomotor slowing (Penberthy *et al*, 2010).

7.4.2 Ondansetron

Ondansetron is a serotonin (5-HT3) antagonist that decreases DA release, especially on corticomesolimbic regions (Jasinki *et al*, 1990). Ondansetron treatment is associated with a decrease in cocaine-induced withdrawal symptoms and cue-associated relapse (Penberthy *et al*, 2010).

7.4.3 Baclofen

Baclofen is a $GABA_B$ receptor agonist thought to modulate cocaine-induced DA release in the NAc. Baclofen was shown to decrease cocaine self-administration, reinstatement, and cocaine seeking behaviours in rats (Roberts, 2005). In humans, it reduces cocaine use only in a subgroup of patients with the highest cocaine use (Penberthy *et al*, 2010) (Ling *et al*, 1998). In a human laboratory study, Baclofen 60 mg/d decreased cocaine self-administration in non-treatment seeking cocaine dependent volunteers who were non-opioid dependent (Haney, 2006). However, although this data was replicated (reviewed in Vocci and Elkashef, 2005), several divergent studies suggested that Baclofen does not eliminate all subjective effects of cocaine (Haney *et al*. 2006; Kahn et al. 2009) or of cocaine-associated cues (Young *et al*. 2014). Importantly, a recent study in rodents demonstrated that baclofen selectively decreased reinstatement of cocaine self-administration in a dose-dependent manner, providing further support for the potential anticraving effect of Baclofen in the treatment of cocaine-seeking (Froger-Colléaux, 2016).

Other GABAergic modulators with therapeutic potential for cocaine addiction treatment are gabapentin (GABAergic agonist), vigabatrin (inhibits GABA transaminase) and tiagabine (blocks the presynaptic reuptake of GABA) (Penberthy *et al*, 2010).

7.4.4 Naltrexone

Naltrexone is a MOR antagonist used for the treatment of alcoholism and heroin addiction (standard dose, 50 mg/d). Opioid antagonists have been shown in preclinical studies to decrease the reinforcing properties of cocaine (Bain and Kornetsky, 1986) and the rate of cocaine self-administration during acquisition (De Vry *et al*, 1989; Ramsey *et al*, 1991) and maintenance (Corrigal and Coen, 1991). Thus, naltrexone is used in combination with relapse prevention therapies to treat cocaine addiction (Schmitz *et al*, 2001) although other studies have reported that naltrexone does not reduce cocaine self-administration (Ettenberg, Pettit, Bloom, & Koob, 1982; Mello et al., 1995; Walsh, Sullivan, Preston, Garner and Bigelow, 1996; Winger, Skjoldager and Woods, 1992).

7.4.5 Bupropion

Bupropion, like cocaine, inhibits DA reuptake by occupying DA transporter therefore increasing DA concentration in the synaptic cleft (Stahl *et al*, 2004). It is mainly used as a treatment for depression and nicotine dependence (Kline, 2004; Richmond and Zwar, 2003). Although a clinical trial of bupropion for cocaine dependence showed no differences between the medication and placebo, an exploratory analysis of the study suggested some effect of bupropion on those participants with higher levels of depression (Margolin *et al*, 1995).

7.4.6 Modafinil

Modafinil is a functional stimulant used for the treatment of narcolepsy and idiopathic hypersomnia specially in kids with attention-deficit/hyperactivity disorder (Rugino, 2007). Although its mechanism of action is not clear, one possibility is that it occupies the DA and norepinephrine transporters thus triggering the stimulant-like effects (Madras *et al*, 2006). Moreover, it seems to increase glutamate release and decrease GABA secretion (Ballon and Feifel, 2006). First studies in humans showed a reduction in cocaine use in patients treated with modafinil compared to placebo (Dackis *et al*, 2005).

7.4.7 Glutamate modulators

Drugs that interact with glutamate systems have some therapeutic potential for cocaine addiction treatment. Among others, the NMDA receptor modulators memantine (blocks the NMDA receptor channel) and acamprosate (a synthetic compound structurally similar to glutamate) have been tested in cocaine addiction (Eden E et al, 2009) (Penberthy *et al*, 2010). One study in rats has shown that memantine attenuates the acquisition or maintenance of intravenous self-administration of cocaine (Hyytiä, 1999) as well as its rewarding effects (Kotlińska, 2000; Maldonado, 2007). However, it was also shown that memantine failed to block the reinstatement of cocaine-seeking behavior (Bespalov, 2000). Acamprosate inhibits conditioned place preference to cocaine (McGeehan, 2003) and attenuates both drug and cue-induced reinstatement of cocaine-seeking behavior (Bowers MS, 2007).

7.4.8 Disulfiram

Disulfiram (Antabuse, Odyssey Pharmaceuticals) is broadly used drug for the treatment of alcoholism. It is an inhibitor of aldehyde dehydrogenase (ALDH), an enzyme necessary to convert acetaldehyde to acetate in alcohol metabolism. Acetaldehyde is a toxic metabolite and its accumulation in the liver provokes discomfort and ailment in the subject. Hence, alcoholic patients develop an aversive conditioning towards alcohol ingestion under the treatment of disulfiram (Fuller *et al*, 1986). Studies performed with ALDH-1 & ALDH-2 inhibitors showed promising results on reducing cocaine craving and consumption (Koppaka *et al*, 2012; Yao *et al*, 2010). Disulfiram has been described to be effective decreasing cocaine use in humans by two distinct mechanisms (see Figure 12) (Pani *et al*, 2010a; Suh *et al*, 2006a). The biphasic action of disulfiram in cocaine addiction may consist on blocking cocaine-driven DA and norephinephrine overaccumulation in the synaptic cleft, thus, preventing the elevated rewarding properties of cocaine and mood disorders on withdrawal. Disulfiram blocks ALDH-2, which catalyzes the reaction from 3,4-dihydroxyphenylacetaldehyde (DOPAL) to 3,4-dihydroxyphenylacetic acid (DOPAC) in the DA biosynthetic pathway. DOPAL condenses with DA to produce tetrahydropapaveroline (THP), which inhibits TH, the rate limiting enzyme in DA biosynthesis blunting the rewarding effects of cocaine and cocaine-associated cues (Yao *et al*, 2010).

In addition, disulfiram can also decrease norepinephrine abundance by potently chelating copper, an ion needed for DBH activity, the enzyme converting DA to norepinephrine. The modulation of the excess in norephinephrine triggered by cocaine prevents future drug exposure and

stress on withdrawal interfering with the ability of environmental stimuli to trigger relapse (Schroeder *et al*, 2010).

Although the benefits of the treatment with disulfiram, its efficacy on cocaine addiction is moderate. Indeed, recent clinical findings provide a limited support for its efficacy on reducing cocaine use which need further attention (Carroll *et al*, 2016a; Schottenfeld *et al*, 2014). In addition, disulfiram has been associated with numerous cardiovascualr and toxic side-effects including cardiac arrest, prolonged QRS and QT-interval, atrioventricular block, increased blood pressure and liver toxicity (Jerónimo *et al*, 2009). On the other hand, cocaine has been broadly described to induce severe cardiovascular effects including hypertension, arrhythmia and heart failure that get potentiated on the co-ocurring consumption with alcohol (Havakuk *et al*, 2017; Herbst *et al*, 2011; Riezzo *et al*, 2012). Furthermore, cocaine consumers have a high comorbidity with ethanol intake that notably reaches the 85% of combined diagnosed abuse (Gossop *et al*, 2006). Clinical studies with heavy drinkers cocaine consumers have demonstrated that disulfiram effects on cocaine use were only evident among those abstaining from alcohol (Carroll *et al*, 2004a). The elevated co-occurrence of cocaine and alcohol abuse and the associated aggravated cardiovascular risk of dual consumers and disulfiram treatment, incapacitates the treatment of cocaine abuse with disulfiram (Filip *et al*, 2005). Thus, finding new drugs for the treatment of cocaine addiction is urgently needed.

7.4.9 Isoflavones

Isoflavones are bioactive natural compounds synthesized by plants. They are also known as phytoestrogens due to their resemblance to

mammalian estrogens and their mild estrogenic activity (Preedy, 2013). Numerous health and relief properties have been attributed to isoflavones in chronic diseases such as menopausal symptoms (Taku *et al*, 2012), breast cancer (Loibl, Lintermans, Dieudonné, & Neven, 2011; Magee, Mcglynn, & Rowland, 2004), prostate cancer (Ganry, 2005), incidence of cardiovascular disease (Merz-Demlow *et al*, 2000), osteoporosis (Ma *et al*, 2008), obesity and diabetes (Velasquez and Bhathena, 2007; Zimmermann *et al*, 2012), cognitive functions

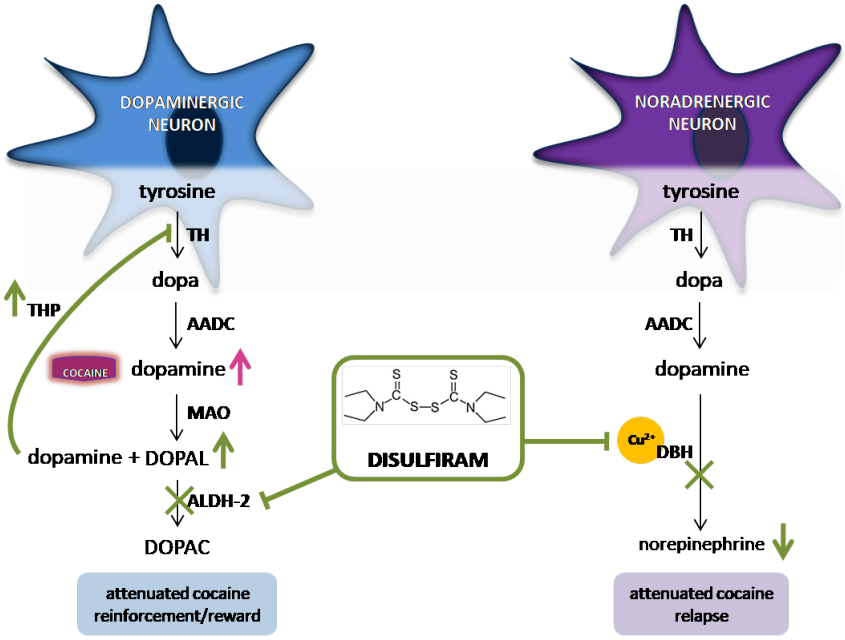

Figure 12. Metabolic interference of disulfiram in alcoholism and in cocaine addiction. The biphasic role of disulfiram in cocaine addiction: blocking cocaine-driven dopamine and norephinephrine overaccumulation in the synaptic cleft (Weinshenker, 2010). **TH:** tyrosine hydroxylase; **dopa:** l-3,4-dihydroxyphenylalanine; **AADC:** L-amino acid decarboxylase; **MAO:** monoamine oxidase; **THP:** tetrahydropapaveroline; **DOPAL:** 3,4-dihydroxyphenylacetaldehyde; **DOPAC:** 3,4-dihydroxyphenylacetic acid; **DBH:** dopamine-β-hydroxylase; **ALDH-2:** aldehyde dehydrogenase 2.

(Henderson *et al*, 2000) and prevention of viral infections (Andres *et al*, 2009).

Isoflavones are mostly biosynthesized in legumes and a Chinese root named Kudzu but are specially abundant in soybeans and their related processed foods such as tofu (Mazur *et al*, 1998). The amounts of the different isoflavones vary greatly among crops and the region where they are cultivated ranging from 360 μg/g in Eastern Canada to 4.6 mg/g in Southern Ontario being the most abundant isoflavones in the products **genistein** and **daidzein** (Seguin *et al*, 2004). Another common isoflavone is **daidzin**, the natural analogue of the synthetic compound CVT-10216, a potent inhibitor of ALDH-2 activity and the glycoside conjugated version of daidzein (Keung *et al*, 1997; Keung and Vallee, 1998; Overstreet *et al*, 2009). Daidzein and genistein share many features with 17β-estradiol including the aromatic ring with a hydroxyl group (Song, 1998; Zhu et al, 2006). Indeed, they can act as selective estrogen receptor (ER) modulators, presenting both estrogenic and antiestrogenic effects depending on the targeted tissue (Setchell, 2001; Lecomte, 2017). Moreover, due to their chemical isoflavan bone structure, daidzin, daidzein, and genistein, are good candidates to mimic CVT-10216 modulation of drug rewarding effects and relapse (see Figure 13 for compared molecular characteristics). Nevertheless, there are several differences on the activity between these isoflavones. Genistein is a strong ERβ agonist (Kuiper *et al*, 1997, 1998), daidzein is known to bind to ER and DA receptors and daidzin may acts on both serotonergic and DA receptors (Bare *et al*, 1995; Keung and Vallee, 1998; Schottenfeld *et al*, 2014; Zaheer and Humayoun Akhtar, 2017), although some studies

propose that it may indirectly inhibit serotonin and DA metabolism by accumulating their metabolization products (Keung and Vallee, 1998).

For these reasons, the treatment of cocaine addiction could be another possible beneficial health property of natural isoflavones that needs to be investigated.

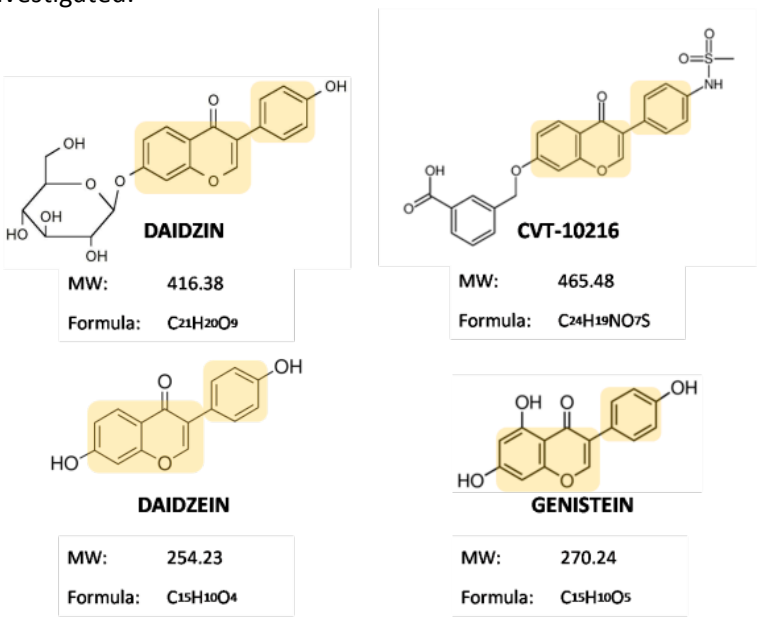

Figure 13. Molecular structure and molecular data of synthetic and natural compounds potentially involved in the modulation of addictions. CVT-10216 is a potent ALDH-2 inhibitor synthesized from the crystallized structure of daidzin binding ALDH-2. Daidzin, daizein and genistein are natural isoflavones with similar chemical structure known to contribute in the modulation of ALDH activity and potentially in the rewarding and relapsing properties of drugs of abuse. **MW:** molecular weight (g/mol). Orange highlight indicates the shared isoflavan frame of these compounds.

8. Neurobiological mechanisms of food intake

8.1 Natural rewards

Natural stimuli such as eating, drinking or sex, are intrinsically reinforcing *per se* and are well-known to activate the reward system (Kelley, 2004). Palatable foods rich in sugar, carbohydrates, fat or even salt are massively craved not only to satiate hunger, but also for pleasure (Alonso-Alonso *et al*, 2015). This concern implies two mechanisms of regulation of food intake: "homeostatic feeding" or need to eat to fulfil biological needs, and "non-homeostatic, allostatic" or hedonic feeding related to the pleasure to eat. The homeostatic feeding involves the brain monitoring of available energy stocks via integrating peripheral signals mostly inducing satiate states. The role of allostatic or hedonic feeding was originally to trigger food consumption in advance of periods where food was not available. This regulation is able to surpass energy requirements and favour reward perception in order to foment the accumulation of energy. However, in our developed societies, where scarcity of food is not an issue, allostatic feeding potentiates overeating and weight gain (Pandit *et al*, 2011). In order to maintain energy stores and weight balanced, both regulatory circuits must work together for the common aim of maintaining a stable weight and nutritional status adapted to the individual environment.

8.2 Homeostatic regulation of food intake

The different organs of the body act together to maintain an appropriate store of energy. Peripheral organs act as body's energy sensors for nutrient status and energy deposits sending information to the brain, which integrates these inputs with the external environmental availability of food and reacts accordingly (Berthoud, 2006, 2007).

8.2.1 Central regulation of homeostatic food intake

The central regulation of homeostatic feeding is an organized circuit where inputs coming from the periphery are integrated mainly by the *hypothalamus* and outputs are sent through the vagal and spinal nerves (Palkovits, 2003).

The ***hypothalamus*** is a small brain area in the *diencephalon* that regulates multiple homeostatic functions comprising endocrine, autonomic and behavioural responses. Indeed, the *hypothalamus* controls the release of hypophysal hormones, regulates body temperature, feeding and eating behaviour, sexual behaviour and reproduction, controls circadian rhythm and mediates emotional responses. Among these roles, the *hypothalamus* is the main brain area that receives feeding inputs and orchestrates homeostatic feeding behaviour (Williams and Elmquist, 2012).

The *hypothalamus* is conformed of a large number of nuclei interconnected by axonal projections that can be grouped into 3 regions according their prominence in the ventral surface (see Figure 14): the supraoptic or anterior region, the tuberal or middle region, and the

mammillary or posterior region. Due to their relevance the following nuclei are to mention:

- In the **anterior region**, two important hormone-secreting nuclei can be found: the *supraoptic* (SON) and the *paraventricular* (PVN) *hypothalamic nuclei*. In addition, the *suprachiasmatic nucleus* (SCN) remains here in charge of the day-night body synchronization, and the preoptic area (POA).

- The **tuberal or medial region** contains the LH and the medial part where the *dorsomedial nucleus* (DMH), dorsal hypothalamic area (DHA), *ventromedial nucleus* (VMH), the *arcuate nucleus* (ARC) also known as (infundibular or periventricular) are found. Feeding behaviour and fear processing are functions attributed to this medial part. Hormones coming from the gastrointestinal tract, pancreas, liver, guts, nutrients and neuronal inputs play a crucial role in the regulation of these hypothalamic nuclei functions.

- The **mammillary or posterior region** of the *hypothalamus* consists of the *perifornical area* (PFA) and *mammillary nuclei* (MN). The former is involved in thermoregulation, whereas the later has been associated with memory.

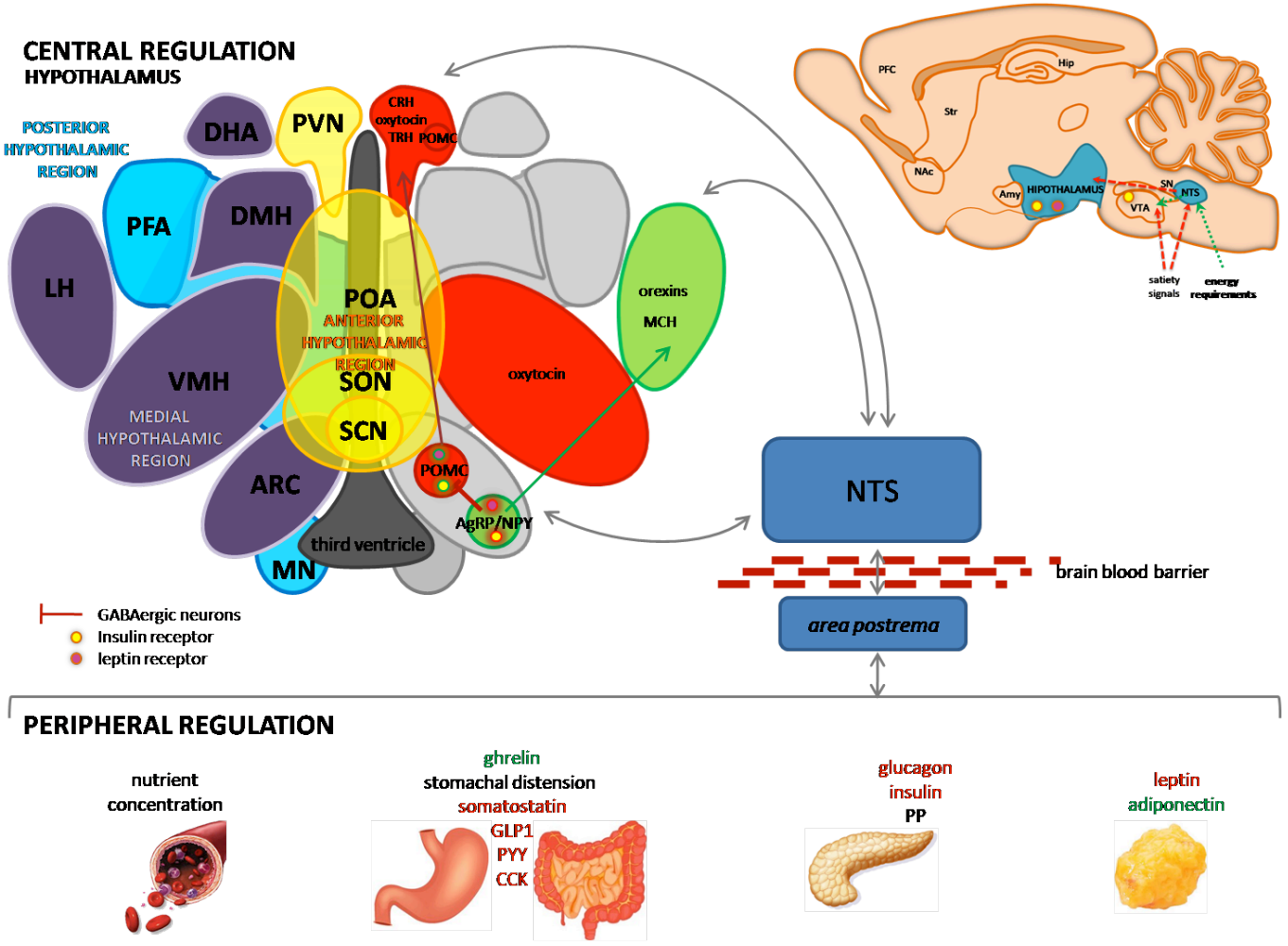
CENTRAL REGULATION
HYPOTHALAMUS
POSTERIOR HYPOTHALAMIC REGION
DHA
PVN
CRH
oxytocin
TRH
POMC
PFA
DMH
LH
POA
ANTERIOR HYPOTHALAMIC REGION
SON
SCN
oxytocin
orexins
MCH
VMH
MEDIAL HYPOTHALAMIC REGION
ARC
POMC
AgRP/NPY
third ventricle
MN
GABAergic neurons
Insulin receptor
leptin receptor
PFC
Hip
Str
NAc
Amy
HIPOTHALAMUS
SN
NTS
VTA
satiety signals
energy requirements
NTS
brain blood barrier
area postrema
PERIPHERAL REGULATION
nutrient concentration
ghrelin
stomachal distension
somatostatin
GLP1
PYY
CCK
glucagon
insulin
PP
leptin
adiponectin

Figure 14. Homeostatic food intake regulatory systems. The sagittal view of the mouse brain highlights the orientative situation of the key structures on the homeostatic regulation of food intake and their regulatory inputs. The coronal diagram presents the distribution of hypothalamic nuclei **(left)** and a simplification of the key regulatory transcripts and inputs in the hypothalamic nuclei involved in food intake and weight regulation **(right)**. The central system is connected with the peripheral elements by a constant loop of inputs/outputs through the *nucleus of the solitary tract* (NTS) and directly by hormone receptors. The peripheral regulation of food intake comes from effectors in the gastrointestinal tract, pancreas, adipose tissue and blood vessels. Red font or background indicates inhibition of food intake; green font or background indicates promotion of food intake. **PFC**: *prefrontal cortex*; **Str**: *striatum*; **NAc**: *nucleus accumbens*; **Hip**: *hippocampus*; **Hab**: *habenula*; **Amy**: *amygdala*; **VTA**: *ventral tegmental area*; **SN**: *substantia nigra pars compacta;* **NTS:** *nucleus of the solitary tract;* **CRH:** corticotrophin releasing hormone; **TRH,** thyrotropin-releasing hormone; **MCH:** melanin concentrating hormone; **NPY:** neuropeptide-Y; **POMC:** pro-opiomelanocortin; **AgRP:** agouti-related peptide; **GABA:** gamma aminobutyric acid; **PVN:** *paraventricular hypothalamic nucleus;* **DHA:** dorsal hypothalamic area; **PFA:** *perifornical area;* **LH:** *lateral hypothalamus;* **VMH:** *ventromedial nucleus;* **DMN:** *dorsomedial nucleus;* **ARC:** *arcuate nucleus;* **SCN:** *suprachiasmatic nucleus;* **SON:** *supraoptic nucleus;* **POA:** *preoptic area;* **MN:** *mammillary nucleus* **GLP1:** glucagon-like peptide 1; **PYY:** peptide YY; **CCK:** cholecystokinin; nuclei in yellow belong to the *anterior hypothalamic region;* nuclei in purple belong to the *medial hypothalamic region;* nuclei in blue belong to the *posterior hypothalamic region.*

Several hypothalamic nuclei in the medial hypothalamic region, the *nucleus of the solitary tract* (NTS) and reward-related areas have a fundamental role in the regulation of food intake:

- The **LH** works as a hunger centre promoting feeding behaviour. Two peptides driving feeding are expressed only in these LH neurons: melanin concentrating hormones (MCH) and orexins (Broberger *et al*, 1998; Elias *et al*, 1998). Orexin neurons respond to decreases in physiological glucose levels in the LH (Oomura and Yoshimatsu, 1984). These two peptides are found to be overexpressed in deprivation (Cai *et al*, 1999; Ludwig *et al*, 2001). Orexins and MCHs are not coexpressed in the same neuron although they share similar expression patterns. Neurons synthesizing each of them are interlinked in the LH and widely project together to several brain areas including the cortex (Marcus *et al*, 2001). In addition, receptors of both peptides are similarly distributed in the brain in sympathetic and parasympathetic areas in charge of chewing, swallowing, or promoting salivation, gastric motility and secretion of pancreatic hormones (Ahrén, 2000; Marcus *et al*, 2001; Saito *et al*, 2001). Noteworthy, one of the few sites where MCH and orexin neurons differ is the NAc. Only MCH neurons project and activate the NAc enabling a direct link from homeostatic feeding to the hedonic rewarding components of food intake (Bittencourt *et al*, 1992; Marcus *et al*, 2001; Peyron *et al*, 1998; Saito *et al*, 2001).

- The **VMH** plays important roles in the regulation of behavioural, endocrine, and autonomic functions, and is also known to be involved in producing both instinctive (feeding and sexual) and emotional (flight, defence and aggressive) behaviours (King, 2006; Narita *et al*, 2016). For our concern, it is involved in the regulation of feeding working as a

satiety centre. Oxytocin receptors are densely found within the VMH reducing feeding and increasing energy expenditure (Noble *et al*, 2014). Moreover, estrogens have been described to act in this nucleus modulating oxytocin receptors, which means that they can also influence in feeding behaviour indirectly (Narita *et al*, 2016).

- The **ARC** contains mixed populations of orexigenic and anorexigenic neurons and is targeted by satiety hormones from the gastrointestinal tract. Thus, the function of this area totally depends on the general nutritional status (Elias *et al* 1998).

- Receptors of hormones from the gastrointestinal tract, such as insulin and leptin (food intake inhibitory hormones) can be widely found both in peripheral tissues and the CNS. However, the medial hypothalamic region and specially the ARC, recruits an important concentration of these receptors (Bellinger and Bernardis, 2002). This peripheral signalling activates the brain orexigenic (anabolic) and anorexigenic (catabolic) machinery in response to peripheral energy homeostasis (Schwartz et al 2000).

- Orexigenic neurons express neuropeptide Y (NPY) and agouti-related protein (AgRP). ARC NPY/AgRP neurons fire in response to low energy availability and are inhibited by insulin and leptin. When activated, they induce the release of peripheral orexigenic signals and MCH and potentiate the orexin secretion of the LH. Orexigenic neurons from the ARC stimulate food intake enhancing the sensation of hunger and the motivation to seek food.

- Anorexigenic neurons are found in the ARC and the PVN. These neurons express pro-opiomelanocortin (POMC), the precursor of melanocyte peptides stimulating hormone, α-, β- and γ-melanocyte-stimulating hormone (MSH), and cocaine- and amphetamine- regulated transcript (CART). ARC POMC neurons reduce food intake when activated. They are stimulated by insulin and leptin and inhibited by GABAergic projections from NPY/AgRP neurons (Saper *et al*, 2002). ARC POMC neurons release the anorexigenic peptides corticotrophin-releasing hormone (CRH), thyrotropin-releasing hormone and oxytocin, which induce satiety and increase metabolic rate finishing the feeding episode.

These two orexigenic and anorexigenic neurons under the control of satiety signals project to nearby medial hypothalamic areas, such as the PVN (to stop eating) and the LH (to potentiate eating).

- In addition to the *hypothalamus,* the reward system also collaborates in the homeostatic regulation of food intake. In the event of hunger, the reward system is activated in order to promote a feeding episode by increasing the motivation to obtain food (Lutter and Nestler, 2009). When energy stores are full, the reward-related area, **VTA**, is also involved in promoting satiation. Indeed, insulin receptors are expressed in the somas of dopaminergic VTA neurons. Insulin acts in the VTA to suppress feeding by a mechanism that involve increased reuptake of DA via DA transporters. Insulin-mediated decrease of DA actively decreases salience of food on satiation (Mebel *et al*, 2012).

Outputs from the peripheral tissues arrive to the **NTS** in the hindbrain. The NTS transfers body energy necessities to the reward system and the *hypothalamus* in order to orchestrate feeding necessities. Among the peptides reaching the NTS, the enterogastric peptide, CCK, promotes satiation by activating gastrointestinal vagal afferents that synapse in the NTS (Campos *et al*, 2012). Although the NTS is protected by the brain blood barrier, peptides from the periphery manage to inform the NTS indirectly by sensing to the *area postrema* (Broadwell and Brightman, 1976). Neurons from the *area postrema* remain outside the brain blood barrier just above the NTS and present these circulating intestinal signals to the NTS (Herbert *et al*, 1990). Thus, there is a constant feedback loop from the peripheral and central systems in the regulation of food intake.

8.2.2 Peripheral regulation of food intake

Several peripheral signals and circulating hormones are released from the gastrointestinal tract, pancreas, liver, muscles, and adipose tissue to the CNS providing information on the status of energy consumption. Multiple peptides participate in this regulation including ghrelin, glucagon-like peptide-1 (GLP1) peptide YY (PYY), CCK, insulin, glucagon, pancreatic polypeptide (PP), leptin and adiponectin, among others (Saper *et al*, 2002).

Enterogastric modulation of food intake

Gastric satiaty signals arise primarily from mechanical distension, whereas those from the rest of the gastrointestinal tract mainly derive from the effects of food composition or metabolism of nutrients (Powley and Phillips, 2004). Evidence shows that while the pylorus stomachal exit

remains open and food volume drained out the stomach, animals could constantly eating unnecessary voluminous meals (Davis and Smith, 1990). The stomach wall comprise neural sensors of tension (Berthoud and Powley, 1992), strength (Phillips and Powley, 2000), and volume (Ritter, 2004) whose output are sent to the brain by vagal and spinal sensory nerves (Ritter, 2004; Schwartz *et al*, 1999). Enteroendocrine cells are also stimulated by food and nutrients or intracellular metabolism. Gut cells are polarized and disposed with several taste receptors whose coupling with tastant molecules increase the secretion of gut peptides (Dyer *et al*, 2005; Wu *et al*, 2002). Secreted intestinal peptides activate the vagal-, enteric-, and spinal-afferent nerves and/or enter blood vessels (Reimann and Gribble, 2002). The enterogastric secreted peptides comprise ghrelin, GLP1, PYY, CCK and somatostatin among others.

Ghrelin is a peptide of stomachal-duodenal origin whose levels increase in response to negative energy balance. Its main role is stimulating food intake and energy storage to compensate the energy imbalance both at short- and long-term manner (Ritter, 2004). Ghrelin surges in advance of regularly scheduled meals and meal anticipation. It seems to have a role in meal initiation to prepare the body machinery for food intake and dispose current nutrient disposition (Drazen *et al*, 2006). In accordance, ghrelin circulating levels are high before meals and lower after ingestion of nutrients. Ghrelin levels decrease faster with carbohydrates than proteins, which in turn are more effective on descending ghrelin levels than lipids (Cummings *et al*, 2005). At the CNS, ghrelin receptors are found in energy homeostasis areas, such as the ARC NPY/AGRP neurons but also in the reward system. Thus, ghrelin influences coordinately not

only homeostatic, but also hedonic mechanisms of food intake (Date *et al*, 2002).

GLP1 and PYY are hormones secreted by intestinal L cells in the distal intestine, which favour meal termination. These cells express sweet and bitter taste receptors (Rozengurt *et al*, 2006). GLP1 secretion is affected by cellular glucose uptake and metabolism and lipid concentration. GLP1 acts in the stomach inhibiting acid secretion and gastric emptying. In the pancreas, it regulates islets' cell differentiation increasing pancreatic β cell populations modulating insulin release (Edvell and Lindström, 1999). In addition, it suppresses glucagon release and enhances insulin sensitivity (Miki *et al*, 2005). These multiple actions reduce appetite and food intake (Verdich *et al*, 2001).

PYY is secreted by L cells to the circulation in a proportional manner to the caloric load ingested and meal composition (Chaudhri *et al*, 2006). PYY acts in the stomach by lowering its emptying process and also nutrient absorption. PYY interferes in the digestive process of meal making it more effective and inhibiting the necessity to eat further (Chaudhri *et al*, 2006).

CCK is another satiety factor released by the upper gut to suppress appetite. It is produced in the small bowel, especially in the duodenum and jejunum mucosa by I cells. CCK secretion is stimulated by the presence of nutrients, mainly aminoacids and fatty acids in the duodenum. CCK flow foments meal termination by coupling brain receptors. Additionally, it also inhibits food intake by reaching the CNS via neurons in the *area postrema* (Chaudhri *et al*, 2006).

Pancreatic modulation of food intake

Several pancreatic hormones synthesized in the islets of Langerhans serve as feeding signals of opposed nature in the regulation of food intake and energy metabolism.

Insulin and glucagon are counterparts in the regulation of glucose metabolism. Insulin is the pancreatic peptide produced by β cells. During meals, insulin is released in the blood and targets the liver to calm down glucose production and stimulate glucose uptake by peripheral tissues, thus, reducing circulating meal-derived glucose concentration and avoiding glucose picks. Apart from its role in glucose metabolism, insulin also circulates in the bloodstream in proportion to fat deposits (Bagdade *et al*, 1967). It serves as a sensor and messenger of body fat content and gives an inhibitory intake feedback by acting in the mediobasal *hypothalamus* (MBH) (Gerozissis, 2004). Insulin receptors can be broadly found in the ARC together with anorexigenic POMC and orexigenic NPY/AgRP expressing neurons. Insulin achieves its role in the reduction of feeding by inhibiting NPY/AgRP peptide production in the ARC while enhancing POMC expression (Plum *et al*, 2006).

Glucagon a peptide produced by α cells that has the opposite role of insulin in the regulation of glucose blood levels (Kieffer and Francis Habener, 1999). Contrary to insulin, glucagon stimulates hepatic gluconeogenesis and glycogenolysis to obtain fuel during fast energy requirements and to prevent falling of blood glucemia levels. In addition, glucagon is also secreted during meals into the *vagus nerve* to signal for meal termination (Geary, 1990).

PP is synthesized in γ cells in a proportional manner to the calories ingested. This hormone exerts a dual action. It foments meal termination when acting at the peripheral level and foments food intake in the CNS. Termination of meal is caused by its direct action in the stomach by lowering the rate of stomach emptying during the eating episode. However, it also modulates gastrointestinal function by stimulating receptor in the dorsal vagal complex mainly in the *area postrema* and NTS (Whitcomb *et al*, 1997). It is known that activation of these receptors produce a slight increase in food ingestion, although the mechanism to trigger this anorexigenic effect remains to be clarified (Katsuura *et al*, 2002).

Somastotatin (growth hormone-inhibiting hormone, GHIH) is an inhibitory hormone produced in the pancreatic islets in δ cells, the stomachal pylorus and the duodenum and in the brain. Somatostatin exerts an inhibitory action on numerous physiological functions, acting as a classical endocrine hormone or a local (paracrine) regulator. In the pancreas, it inhibits the secretion of other hormones such as insulin, glucagon and CCK. In the gastrointestinal tract, it acts locally to reduce gastric acid secretion, gastrointestinal motility and to inhibit the secretion of gastrointestinal hormones, including gastrin and secretin (Boron and Boulpaep, 2012). Somatostatin is also produced by neurons of the VMH to reach other brain areas such as the ARC and the NTS to inhibit the effects of growth hormone.

Adipose tissue modulation of food intake

The adipose tissue is not only a depot for lipids but also an important endocrine organ involved in the integration of endocrine, metabolic and inflammatory signals for the control of energy homeostasis. Leptin, adiponectin and other hormones and cytokines are secreted by the adipose tissue (Chandran *et al*, 2003).

Leptin is an hormone secreted from the adipose tissue that induces satiety perception, inhibits food intake and increases metabolism rate to reduce excessive energy stores (Lutter and Nestler, 2009). Leptin and the afore mentioned ghrelin are a sort of opposite role hormones with similar CNS targets. Leptin concentration in blood decreases proportionally to body fat content. In fed conditions, blood leptin increases whereas it falls in deprivation (Ahima and Flier, 2000; Frederich *et al*, 1995a, 1995b; Maffei *et al*, 1995). Accordingly, studies administering leptin showed inhibition of food intake, weight loss and enhanced energy consumption, while decreased levels in leptin were coupled with weight gain and obesity (Friedman and Halaas, 1998). Indeed, the leptin deficient ob/ob mice are broadly used as models of diabetes and obesity (Drel *et al*, 2006).

Adiponectin is a peptide secreted by the white adipose tissue. Its concentration is high in serum and inversely correlated with body fat percentage (Ukkola and Santaniemi, 2002). Adiponectin regulates glucose and fatty acid metabolism in insulin-sensitive tissues. In addition, this adipocytokine is a mediator of insulin action. Indeed, low circulating adiponectin levels have been related to directly contribute to induce

resistance to insulin in mouse models and type 2 diabetic humans (Yamauchi *et al*, 2001). In the CNS, it induces feeding stimulation through activation of its receptors in the ARC (Kubota *et al*, 2007).

8.3 Hedonic regulation of food intake

The *hypothalamus* is also vastly connected to **the reward system**. The reward system is responsible for the hedonic control of food intake and it can be activated with food cues (Kelley *et al*, 2005; Volkow *et al*, 2013a). Indeed, the environment, rewarding stimuli and emotional factors are implicated in food intake and food palatability plays a prominent role in the regulation of feeding even in periods of energy abundance (Berthoud, 2006).

Some brain areas in the reward system are strikingly controlling hedonic eating. The VTA, NAc, *amygdala*, PFC and *hippocampus* are paticipants in the control of feeding under DA, opioid and ECs modulatory actions. These areas are involved in incentive learning and pleasure and confer effort towards obtaining food rewards, integrating information about energy stores and visceral sensory information with food availability in the environment (Dagher, 2009).

The **NAc** is in charge of the motivation towards food consumption and the pleasure associated to its consumption (Wang *et al*, 2009). Projections from the NAc and the VTA to the LH mediate hedonic feeding (Castro *et al*, 2015). The release of DA from the VTA to the NAc and *dorsal striatum* enhances the drive to obtain a particular food but seems not responsible for the hedonic component inherent to a palatable food. As described in Chapter 2.3, endogenous opioids and ECs reaching these

areas mediate the hedonic value of food (and drug) rewards (Cota *et al*, 2006; Maldonado *et al*, 2006). 'Liking', which refers to the hedonic value or palatability associated with a food, often shows overlapping neuronal circuits and brain areas with 'wanting', which refers to the desire that potentiates goal-directed actions to obtain that food (motivation). The DA striatal system is predominantly (although not exclusively) implicated in 'wanting' while the opioid and cannabinoid systems are predominantly (although not exclusively) implicated in food 'liking'. Concerning the anatomy of the NAc, the core subterritory has been described to be more reactive to cue-driven motivation (DA conditioning) while the NAc shell has been postulated to be more engaged with the hedonic component of rewards influencing in palatability. Indeed, the NAc shell has been called the 'hedonic hot-spot' for some authors referring to the high densities of opioid and EC receptors that are recruited there (Castro and Berridge, 2014). Liking (mediated by opioids) and wanting (mediated by DA) are separate mechanisms that work together to modulate eating behaviour. Both the hedonic component of rewards and reward-associated conditioning may be processed in concert by the NAc. Sometimes motivation to obtain food and food palatability can also work in a more independent manner. Indeed, cues for palatable food could still evoke excessive desire and consumption, in expenses of hedonic drive. Cues can provoke hyperreactivity in mesocorticolimbic DA-glutamate mechanisms involved in incentive salience as well as the related CRF stress circuits that potentiate these mechanisms. Therefore, cues such as sight, smell, or imagination of food could trigger a urge to eat, even though the subject would not experiment eating that specific food as extremely pleasurable (Berridge *et al*, 2010). Also, DA conditioning can modify behaviour in an opposite manner. Tasty foods may become

unwanted after an aversive conditioning such as coupling their ingestion with nausea or food poisoning (Finlayson *et al*, 2008). On the other hand, food can become excessively hedonic by pathological overactivation of the opioid or ECs hedonic hot-spots in the NAc shell. The enhanced activation of this substrate would magnify the hedonic impact of foods triggering the subsequent wanting responses contributing to binge eating or overeating. In contrast, suppression or dysfunction of this hedonic mechanism is described in anorexia (Kaye et al., 2009). These hedonic and motivation systems can be downregulated by satiety influences (insulin, leptin). However, satiety signals could not strongly stop the drive but tone down its intensity. This phenomenon can be exemplified in the decision of taking a succulent dessert even after been full with a plenteous meal (Blum *et al*, 2012).

Apart from the NAc, the projections from the *cortex*, *amygdala* and *hippocampus* to the *hypothalamus* have also an important role in modulating homeostatic metabolic processing (Berthoud, 2002). Perception of food reward begins with information generated by oral taste receptor cells transmitted to the NTS by afferent sensory fibres. In turn, the NTS sends the taste information to other brain areas in the hindbrain, midbrain (i.e. the VTA) and forebrain (e.g. NAc, *dorsal striatum*, thalamus and cortex). These areas assign a reward value to food according to the subject preferred textures or taste. Within the ***cortex***, the *insular cortex* and the *orbitofrontal cortex* have a notable role in the processing of palatability. Primary taste neurons allocated in the *insular cortex* higher process taste information from the NTS inputs and project to secondary taste neurons. These cells are found in the *orbitofrontal cortex* and integrate taste and smell information with the

relevant gustatory, olfactory, visual, sensory and somatosensory inputs (Rolls, 2012). These later cells output concerning taste stimuli is hunger-dependent and decreases during the eating episode, meaning that these cells are able to integrate taste information with satiety signals. Thus, the *orbitofrontal cortex* has an important role in reward related feeding (Rolls, 2012). According to this, reduced firing of secondary taste neurons in the *orbitofrontal cortex* decreases the reward value of foods contributing to meal termination by projections to the striatum and *amygdala*, two areas in charge of assigning a motivational value to rewards (Kringelbach *et al*, 2003). Indeed, studies in rodents with impaired PFC function showed increased preference for sweet palatable foods and hyperphagia (Mena *et al*, 2011).

The ***amygdala*** accounts of two different regions: the basolateral amygdala and the central nucleus of the amygdala. The connections between the basolateral *amygdala* and the forebrain potentiate cue-related feeding and have a role in the assessment of food palatability (Petrovich *et al*, 2007). Moreover, the central nucleus of the *amygdala* is reciprocally connected to the NAc controlling opioid-mediated eating (Kim *et al*, 2004). In addition, the *amygdala* is close-related to the BNST in the so called "extended *amygdala* circuit" that can potentiate stress-induced hyperphagia (Sharma *et al*, 2013).

The ***hippocampus*** is also involved in feeding behaviour through its processing of memories. Hippocampal-derived processes include remembering whether one ate, remembering conditioning associations, remembering where food is located, self-identifying the own state of hunger and remembering how to relieve these states (Volkow *et al*,

2011a). Indeed, hippocampal lesions in rodents have been described to impair their ability to discriminate between the state of hunger and satiety. In the same line, rats with impaired hippocampal function showed a significant increase in hedonic feeding (Davidson *et al*, 2007). In humans, brain-imaging studies have reported activation of the hippocampus with food craving, in hunger, in the event of trying new food and in response to food-conditioned cues (Haase *et al*, 2009). In addition, imaging studies in human have shown that in obese but not in lean individuals, the hippocampus gets hyperactivated in response to food stimuli indicating the implication of this area also in obesity (Bragulat *et al*, 2010).

Metabolic signals also act modulating reward circuits by adding a motivational component to hunger and satiety (Palmiter, 2007). Anorexigenic peptides decrease the hedonic component of food, while orexigenic peptides increase it (Zheng *et al*, 2007). Satiety signals indicating full stores of fat (leptin) or glucose (insulin) are recently known to contribute in the regulation of motivation towards food intake through modelling DA release in the mesolimbic system (Khanh *et al*, 2014). Preclinical studies show that leptin decreases DA release in the NAc and feeding behaviour (Krügel *et al*, 2003). In addition, leptin receptor activation in the VTA inhibits the firing of VTA neurons (Hommel *et al*, 2006). The same action in VTA neurons inhibiting DA release has been recently described for insulin, which appears to be co-expressed with TH, the rate limiting enzyme for DA synthesis (see chapter 2.2) (Figlewicz *et al*, 1994). Ghrelin receptors are also expressed in TH-positive VTA neurons. Ghrelin increases the excitatory rate of VTA dopaminergic neurons through glutamatergic inputs (Abizaid *et al*, 2006).

The **LH** is the integrative node from all these signals. Indeed, the LH receives homeostatic inputs about energy stores from the ARC and NTS. Also, it is targeted by satiety hormones and metabolic signals and it receives hedonic inputs from the other reward-related areas including the *ventral striatum* and the *cortex*. This area can attenuate the response to homeostasis concerning energy stores and meal termination in favour of reward-related signals to modify the amount of food consumed in a meal (see Figure 15).

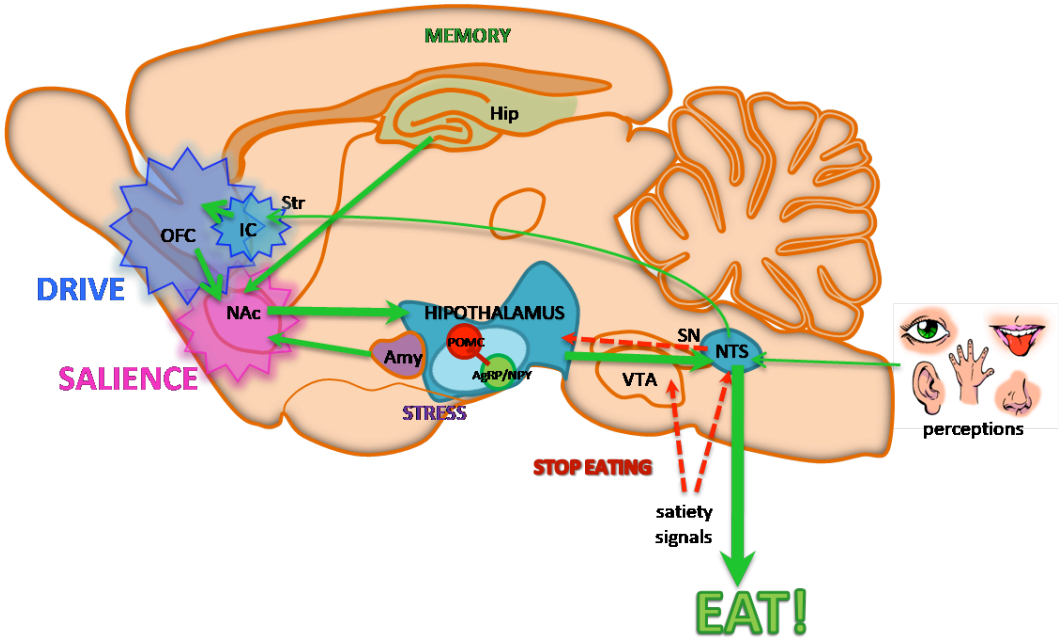

Figure 15. Schematic sagital view of a mouse brain modelling the hedonic overcome in the regulation of food intake. Hedonic mechanisms involving the brain reward system easily overcome the influence of homeostatic signals in the presence of food palatability, food cues and stress. **IC**: *insular cortex*; **OFC:** *obitofrontal cortex*; **Str**: *striatum*; **NAc**: *nucleus accumbens*; **Hip**: *hippocampus*;; **Amy**: *amygdala*; **NTS**: *nucleus of the solitary tract;* **POMC:** pro-opiomelanocortin; **AgRP:** agouti-related peptide; **VTA**: *ventral tegmental area*; **SN**: *substantia nigra*. Red arrows stand for inhibitory effects; green arrow stand for excitatory effects.

9. The addictive dimensionality of obesity

Obesity has reached epidemic magnitudes in developed societies becoming a public health problem. Overeating, in turn, is a major cause of overweight and obesity contributing to the high rate of prevalence of these conditions in the first world (Bott, 2014). The broad and increasing availability of highly palatable energy dense foods but especially those rich in sugar and fat (Lenoir *et al*, 2007), are a crucial factor in the decision to eat even when body energy stores are supplied. Indeed, palatable foods and its associated cues can trigger this non-homeostatic overfeeding where hedonic mechanisms easily override homeostatic signals, sometimes in a compulsive uncontrolled manner (Lerma-Cabrera *et al*, 2015). For instance, an apparently tasty cake can be desired to be eaten after an overwhelming lunch even having previously refused to take dessert. In this line, this chapter highlights the shared framework between overeating and addictive behaviours that can be responsible of the uncontrolled hedonic eating that leads to overweight and obesity.

9.1 The shared neurobiological and behavioural framework

Palatable foods and drugs activate the same **reward-learning regions** in the reward system (Geiger *et al*, 2009; Small *et al*, 2003). Their mechanism, however, remains different. While drugs of abuse increase DA tone in the NAc by their pharmacological effect, palatable foods rise DA levels in two steps: fast sensory inputs due to the pleasure associated to palatability itself, and low postingestive inputs due to nutrient concentration in blood and brain (Koob and Volkow, 2010). After long periods of chronic exposure to certain foods, some vulnerable individuals may develop an enhanced saliency. This could lead to maladaptations in

the reward system processing that can be associated with compulsive actions towards food seeking and taking mimicking addictive behaviours (Pelchat, 2009).

Maladaptations in the reward system by drugs of abuse include **rewiring of neuronal connections,** especially glutamatergic corticolimbic ones. Neuronal connections are restructured after chronic drug insults in reward-related areas fomenting the anchorage of compulsive behaviours that define addiction (Kalivas and O'Brien, 2008). Past research maintained that only drugs, but not food, produced changes in synaptic plasticity in the VTA (Chen *et al*, 2008). However, recent data on the field demonstrated that palatable foods are also capable of rearranging neuronal connections in the NAc in operant self-administration paradigms in mice (Guegan *et al*, 2013). Moreover, other studies have focussed on the impairments on hippocampal-dependent memory induced by high-fat diets backed up with less LTD in hippocampal neurons (Boitard *et al*, 2014; Krishna *et al*, 2015). Recently, research has also focussed on the role of plasticity in the hypothalamus. Some studies examined the role of plasticity changes in glutamatergic neurons activating POMC and inhibiting AgRP neurons of the ARC in fasting conditions (Nuzzaci *et al*, 2015). Others have revised the plasticity changes regulating ARC activity associated to exercise, insulin or caloric restriction (Mainardi *et al*, 2013). Moreover, some authors have described the stress-induced impairment of the capacity of PVN synapses to undergo plasticity rearrangements (Bains *et al*, 2015). However, little is known about neuronal rearrangements in the mesolimbic system in other environmental food intake conditions or paradigms.

From the behavioural point of view, **escalation** to higher and recurrent doses is observed in drug users, but also with pathological food consumption (Ahmed *et al*, 2002). As described preclinically, rats on a high-fat diet showed constant increase in food consumption in binge-eating episodes where the involvement of the DA mesolimbic system was described. This pattern of escalation in food consumption resembles the incipient loss of control towards drug consumption shown by drug abusers (Valdivia *et al*, 2015).

Moreover, **tolerance** is developed after chronic drug exposure leading to the development of addiction. Accordingly, reward tolerance for a specific food can be also described after long periods of ingestion. Blunted striatal response for ice-creams was shown in fMRI studies in regular ice-cream eaters humans versus sporadically consumers. Moreover, reward related areas were activated differently when regular ice-cream eaters were offered candies or other energy-dense foods (Burger and Stice, 2012). In the same line, D2R downregulations and D1R upregulations have been described in drug abusers and obese subjects (Johnson and Kenny, 2010; Stice *et al*, 2010; Volkow *et al*, 2008b). Obese subjects have less striatal D2R availability in comparison with lean subjects (Stice *et al*, 2013). Decreases in striatal D2R have been linked to impaired reward processing (NAc) and drug/overeating-related habit-forming (*dorsal striatum*) (Geiger *et al*, 2009; de Weijer *et al*, 2011). Indeed, obese individuals have similar D2R-associated neurological activity in the *striatum* than the one described for drug abusers as revealed by clinical functional magnetic resonance imaging (fMRI) studies. Preclinical studies also show reduced D2R availability, less DA flow and turnover and reduced response in reward-related regions

towards food and drug intake in overfed animals in comparison with controls (Geiger *et al*, 2009; Johnson and Kenny, 2010). Thus, overeating-induced obesity and drug addiction may share prolonged decrease in striatal D2R availability and reactivity implicating DA binding in reward-related areas in the development of these two conditions (Volkow *et al*, 2006).

Quitting or **cutting down** the intake of drugs is difficult for drug abusers partially because drug cues stimulate the reward system and precipitate relapse in former drug abusers (Kosten *et al*, 2006). For overeating subjects, quitting or cutting down the amount of palatable food share these same characteristics due to food **cues** and conditioning (Stoeckel *et al*, 2008). Indeed, an important factor to obesity is overeating motivated by food cues such as sounds, smells or sights associated to palatable foods (Stice *et al*, 2013). Studies in young adults have shown an elevated incentive salience manifested in some individuals as hyper-responsivity towards food-cues. These subjects were accordingly predicted to gain weight in an easier manner (Demos *et al*, 2012). Hyper-responsivity towards food-cues does not couple with initial subject vulnerability to overeating, but with an enhanced associative learning capacity after a period of overeating (Burger and Stice, 2014). However, the potential to trigger relapse after long periods of abstinence remains different for drugs of abuse and food. Preclinical studies demonstrate that stress can induce reinstatement in drug-deprived animals more effectively than in palatable food-deprived ones indicating that stress is more tightly involved in drug than in food relapse (Kearns *et al*, 2011). In a similar way, drug-related cues seem more powerful to precipitate relapse than food cues (Kearns *et al*, 2011) and its efficiency depends more on the

nutrition status than on stress (Rudenga *et al*, 2013; Stockburger *et al*, 2009).

Distress towards other activities is found in drug addicts, who regularly increase the time spent in drug-related activities. Hunger provokes a similar effect. Thus, food craving interferes with competing cognitive demands shifting cognitive resources to getting food (Kemps *et al*, 2008).

Taken together, drug addiction and hedonic overeating can be defined as disorders where the incentive salience of a specific reward is exacerbated accompanied with an enhanced DA tone and restructuration of reward-prone neuronal wiring. From a behavioural point of view, escalation on consumption, tolerance and distress from other activities are present in chronic drug consumers and recurrent overeaters. These maladaptations manifest the mental counterpart involved in overeating-induced obesity and indicate that some forms of obesity can be triggered by food addictive-like behaviours neurobiological and behaviourally resembling addiction (Volkow and Wise, 2005).

9.2 Eating disorders

Due to the similarities between hedonic overeating and drug addiction highlighted in the previous chapter, some authors defend that "food or eating addiction" should be included in future versions of the DSM (Volkow *et al*, 2013a; Volkow and O'Brien, 2007). The current DSM-5 states that "Feeding and eating disorders are characterized by a persistent disturbance of eating or eating-related behaviour that results in the altered consumption or absorption of food and that significantly

impairs physical health or psychosocial functioning" and comprise the following cases:

a) Pica: It is characterized by the eating of substances that are considered non-food and non-nutritive, such as paper, hair, paint or chalk (Delaney *et al*, 2015).

b) Rumination disorder: This disorder is described as repetitive regurgitation of previously swallowed and potentially partially digested food. The regurgitation is voluntary and does not involve physical or somatic reasons such as reflux (Delaney *et al*, 2015).

c) Avoidant/Restrictive food intake disorder (ARFID): It is manifested as persistent difficulty to meet appropriate nutritional and/or energy needs despite a subjective lack of body image or weight apprehensions. Importantly to be diagnosed, ARFID cannot be explained by lack of available food or by an associated culturally sanctioned practice, like religious fasting or intentional dieting (Zimmerman and Fisher, 2017).

d) Anorexia nervosa: This disease is a psychiatric condition defined by severe loss-of-weight and secondary problems associated with malnutrition. Main characteristics are food intake restriction, odd eating habits or rituals due to an obsessive fear of gaining weight and an unrealistic perception of low body weight (Treasure J et al, 2015).

e) Bulimia nervosa: It is characterized by recurrent binge eating followed by compensatory behaviours, such as purging, fasting, and excessive exercise (American Psychiatric Association, 2013).

f) Binge-eating disorder (BED): This disorder is characterized by recurrent episodes of binge eating accompanied with the subjective sense of loss of control over food consumption. These episodes are not followed by compensatory behaviours, such as self-induced vomiting, excessive use of laxatives/diuretics, fasting, or excessive physical exercise (American Psychiatric Association, 2013).

g) Other specified feeding or eating disorder: This category includes those eating disorders causing social and occupational impairment that do not fulfil DSM-5 criteria for anorexia nervosa, bulimia nervosa or BED (American Psychiatric Association, 2013).

h) Unspecified feeding or eating disorder: According to the DSM-5 criteria this category applies to those behaviours causing clinically significant distress/impairment of functioning, but do not meet the full criteria of any of the feeding or eating disorder criteria previously stated (American Psychiatric Association, 2013).

Similar to eating disorders, some forms of addictive-like eating that lead to overweight are conducted by excessive motivation and loss-of-control towards food consumption. Standard procedures to treat overweight/obesity involve healthy lifestyle changes to diminish food intake and increase exercise. However, even they can be effective in normalizing body weight, they are difficult to sustain by the patients (Volkow and O'Brien, 2007). This phenomenon highlights the mental and altered behavioural dimension of hedonic eating-induced overweight (Volkow and Wise, 2005).

Despite the partial overlap between the recognised eating disorders and food addiction-induced overeating, they differentiate in some aspects that make us require a new different taxonomy. Also, food addicted individuals present a diminished control over food consumption (the more they eat, the lesser control they have), but in the case of some eating disorders (for example, ARFID) subject's control is not impaired (Zimmerman and Fisher, 2017). Moreover, subjects diagnosed with other eating disorders usually show guilt feelings after food intake (Ziauddeen and Fletcher, 2013) followed by compensatory behaviours such as vomiting or fast (American Psychiatric Association, 2013). Food-addicted subjects, however, do not present these behaviours after eating.

Thus, the concept of "food addiction" or "eating addiction" still remains controversial, although parallels and discrepancies between addictions and eating disorders may support the inclusion as a new different mental disorder. However, criticism comes out when considering food, a natural vital necessity, as addictive. To fill this diagnostic requirement, some authors developed the Yale Food Addiction Scale (YFAS). The YFAS is the first psychometrical questionnaire to assess addictive-like eating behaviour, originally based on the diagnostic criteria for substance-use disorders stated in the DSM-IV Text Revision (Gearhardt *et al*, 2009). Indeed, elevated scores on the YFAS's have been associated with higher rates of obesity and more severe pathological eating, such as BED (Gearhardt *et al*, 2013). With the launch of the 5Th edition of the DSM, the YFAS 2.0 was developed to maintain consistency with the current diagnostic of addiction (Gearhardt *et al*, 2016). Accordingly, while the original YFAS only assessed food dependence (not abuse), YFAS 2.0 determines food addiction based on the DSM-5, which, as described

before, combines the dependence and abuse criteria from DSM-IV. The YFAS 2.0 consists of a series of 35 self-reported items that address the subject's eating habits over the previous 12 months. Each question from the YFAS 2.0 is related to a DSM-5 Substance-Related and Addictive Disorders criterion (Gearhardt *et al*, 2016). Importantly, both original YFAS and YFAS 2.0 were similarly associated with higher BMI, binge eating and weight cycling. However, only the YFAS 2.0 food addiction threshold, but not the original YFAS, was associated with obesity. Moreover, compared to the original YFAS, the YFAS 2.0 is more strongly associated with indicators of excess food consumption (such as binge eating and obesity) (Gearhardt *et al*, 2016). Altogether, the YFAS 2.0 is a good psychometrically measure in consonance with the current diagnostic understanding of addiction (based on DSM-5) to evaluate the potential role of an addictive-like process in eating disorders (Gearhardt *et al*, 2016).

9.3 Clinical implications of hedonic eating: obesity

Overeating produced by "food addiction" is a major cause for overweight and obesity (Ferrario, 2017). Nevertheless, it is important to state that eating addiction not always leads to overweight and obesity and not all obese subjects are food addicted (Lerma-Cabrera *et al*, 2015; Meule *et al*, 2014).

Overweight and obesity are considered a 21st century epidemic and their prevalence is expected to increase in coming years, and so its adverse-associated health, social and economic consequences (González-Muniesa *et al*, 2017). Obesity prevalence has doubled since 1980 in more than 70 countries, being 39% of world's adult population obese in 2014 and some

countries extremely overweight (see Figure 16) (WHO, 2014). Combined prevalence of obesity and overweight is similar between both genders being >36% in 2013 (Ng *et al*, 2014). However, although adult women are found to be more obese than adult men (14.9% and 10.8%, respectively), the risk of being overweight is higher in adult men (NCD Risk Factor Collaboration (NCD-RisC), 2016; Ng *et al*, 2014). This pandemic and the associated comorbidities accounts for a global health cost of approximately US$2 trillion a year (Dobbs *et al*, 2014).

The Body Mass Index (BMI), although it is imprecise, is the most common tool used to detect obesity and overweight. It does not distinguish muscle weight from fatness and does not relate with body fat distribution, but it estimates adiposity and it is strongly associated with obesity-related morbidity. Therefore, it can be complemented by measuring the waist circumference in order to discriminate between subcutaneous or visceral obesity (James *et al*, 2001a). The BMI is calculated as the weight of the subject expressed in kilograms divided by the square height in meters. Common classification by the WHO defines normal weight as 18.5 – 24.9 kg/m^2, overweight as 25 – 29.9 kg/m^2, obesity as >30 kg/m^2, and >40 kg/m^2 is considered extreme obesity (González-Muniesa *et al*, 2017).

Obesity is a multifactorial metabolic disorder defined as abnormal or increased fat deposition and accumulation of adipose tissue with negative health consequences, usually co-occurring with mild-chronic systemic inflammation (James *et al*, 2001b; Sellayah *et al*, 2014; Williams *et al*, 2015). The onset of obesity and overweight involves the interaction

of genetic, environmental, neural, hormonal, nutritional and psychosocial factors that affect body weight and fat deposition (Bray and Champagne, 2005). By definition, obesity and overweight come from the imbalance between caloric intake and energy expenditure which results in fat deposition (Sellayah *et al*, 2014). Thus, overeating driven by food addiction, food availability, alterations in dietary patterns, preference of energy-dense fat and sweet foods accompanied with a too sedentary

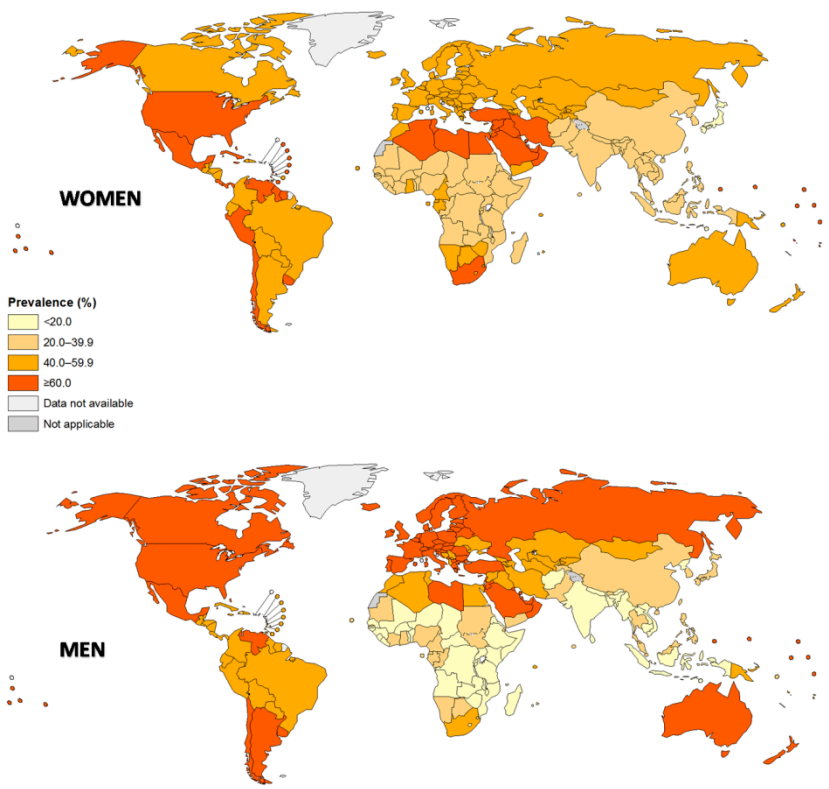

Figure 16. Prevalence of overweight in 2014. Body Mass Index>25kg/m^2 prevalence in each country is pictured for women and men older than 18 years old according to the colour scale (Adapted from WHO, 2014).

lifestyle contributes to the high prevalence of obesity and overweight (Martinez, 2000; Rolls, 2011; Young and Nestle, 2002). As mentioned in previous chapters, last studies strongly demonstrate that the **rewarding properties** of highly palatable food (hedonic regulation) can override the homeostatic pathway of food intake, leading to progressive increase in BMI and, hence, obesity (Lutter and Nestler, 2009).

Interaction of **genetic and environmental factors** contributes to the heterogeneity of individuals with obesity, which complicates the understanding of the disease (González-Muniesa *et al*, 2017). Around 70% of inter-individual variation in BMI could be due to the genetic differences between subjects and the identification of genes implicated in the susceptibility to obesity will provide new tools and pharmacological targets to treat these disorders (Elks *et al*, 2012). During the last years, several Genome Wide Association Studies (GWAS) identified >300 genetic loci for obesity traits. Importantly, the fat mass and obesity-associated (FTO) genetic locus was discovered, which showed high significant association with obesity risk in young and adults (Frayling *et al*, 2007; Scuteri *et al*, 2007). First studies on the biological significance of this association showed that the FTO locus might regulate the expression of RPGRIP1L (Retinitis Pigmentosa GTPase Regulator-Interacting Protein-1 Like) or IRX3-IRX5 (Iroquois homeobox 3 - Iroquois homeobox 5) to modulate body weight by affecting appetite, satiety, thermogenesis and adipocyte browning (Milagro *et al*, 2016; Yang *et al*, 2012). In addition, studies on monogenic obesity in mice have identified several genes that play essential roles in the appetite control, food intake and energy homeostasis. Among others, genes affecting body weight through CNS pathways, such as genes encoding the hormone leptin and

its receptor, the melanocortin 4 receptor and the POMC (Bott, 2014; Myers and Leibel, 2015). Interestingly, mutations in these human orthologous genes cause monogenic obesity (van der Klaauw and Farooqi, 2015). Accordingly with the role of the reward system in obesity, variations of genes involved in DA neurotransmission, like the D2R Taq I A1 allele, and the gene encoding for CB1R have also been associated with obesity and overeating (Schleinitz *et al*, 2010). Polimorphisms of genes involved in adipocyte metabolism, such as fatty acid binding protein 2, insulin-induced gene 2, Niemann-pick disease type C1 protein could also have a role in the development of overweight and obesity (Hofker and Wijmenga, 2009; Shabana and Hasnain, 2016). Moreover, polymorphisms in the glucocorticoid receptor have been associated to fat accumulation, mainly in the central abdomen, and obesity (Kezia J, 2016). Other interesting candidates include genes encoding for adrenergic receptors, insulin and insulin receptors, insulin-like growth factor, glucose transport proteins, CCK and apolipoproteins (Bell GC, 2005; Klöckener T, 2011; Wang W, 2017), among others.

Another cause for the development of obesity and overweight is **stress**. This **environmental factor** plays an important role since individuals suffering stress are more predisposed to consume high palatable food to find comfort as highly palatable food presents powerful reinforcing properties that trigger the reward system, which alleviates the feelings of anxiety and discomfort promoting overeating (Pecoraro *et al*, 2004). Other factors are the so called “obesogenic” food environments, which are believed to promote unhealthy food choices increasing energy intake, such as easy access to fast food restaurants, takeaway shops, convenience store that will not likely sell healthy food and thereby

contributing to poor food choices. Moreover, these obesogenic food environments are associated with sedentariness and low physical activity (Giskes *et al*, 2011).

Finally, several **psychosocial factors** and some **psychological pathologies**, such as depression or social discrimination, also contribute to the obesity prevalence. Indeed, the number of depressive episodes positively associates with the risk of developing obesity, suggesting mood as the main trigger of emotional eating (Björntorp *et al*, 2001). Moreover, obesity has often been described as a major social prejudice with a significant social tendency to stigmatize overweight individuals (Muennig, 2008), and evidences of bidirectional association between depression and obesity have also been described (Marmorstein *et al*, 2014).

Altogether, the multiple aetiology of overweight and obesity, makes these disorders difficult to treat nowadays. Further research in the field is needed to prevent and treat effectively this pathology. New animal models taking into account a more realistic approach of an individual life are required to serve as tools to better study the intricacies of these pathologies.

OBJECTIVES

General objective

The general objective of this thesis is to deepen into the knowledge of cocaine and food related addictive behaviours taking into consideration their numerous neurobiological and behavioural parallelisms.

Specific objectives

1. To evaluate the efficacy in decreasing the addictive properties of cocain of three natural isoflavones (daidzin, daidzein and genistein) and a synthetic drug (disulfiram) able to regulate ALDH activity (Article 1).

2. To investigate the neurobiological and functional modifications in the mesocorticolimbic reward system induced by overeating-induced overweight (Article 2).

3. To generate a valid mouse model to study eating addictive-like behaviour (Article 3).

RESULTS

ARTICLE 1

DAIDZEIN MODULATES COCAINE-REINFORCING EFFECTS AND CUE-INDUCED COCAINE RELAPSE

Miriam Gutiérrez-Martos*, Miquel Martin*, Roberto Cabrera, Rafael Maldonado, Magí Farré, Rafael de la Torre

Scientific Reports (2017) under review

DAIDZEIN MODULATES COCAINE-REINFORCING EFFECTS AND CUE-INDUCED COCAINE RELAPSE

Miriam Gutiérrez-Martos[2#], Miquel Martin[2#], Roberto Cabrera[2], Rafael Maldonado[1,2,&], Magi Farre[1,3,&]*, Rafael de la Torre[1,4,5,&]

1. Integrative Pharmacology and Systems Neuroscience Research Group, Hospital del Mar Medical Research Institute (IMIM), Parc de Recerca Biomedica de Barcelona (PRBB), C/Dr. Aiguader 88, 08003, Barcelona, Spain
2. Laboratory of Neuropharmacology, Universitat Pompeu Fabra, Parc de Recerca Biomedica de Barcelona (PRBB), C/ Dr. Aiguader 88, 08003, Barcelona, Spain
3. Universitat Autònoma de Barcelona (UDIMAS-UAB), C/Dr. Aiguader 88, 08003, Barcelona, Spain
4. Universitat Pompeu Fabra (CEXS-UPF), C/Dr. Aiguader 88, 08003, Barcelona, Spain
5. CIBER Fisiopatologia de la Obesidad y la Nutrición (CIBERobn) Choupana s/n | 15706 Santiago de Compostela, Spain

#. These authors equally contributed to this study

&. These authors equally supervised this study

*Current address: Farmacología Clínica. Hospital Universitari Germans Trias i Pujol - HUGTiP- IGTP Universitat Autònoma de Barcelona - UAB Carretera de Canyet, s/n. 08916 Badalona.

ABSTRACT

Cocaine addiction is a chronic relapsing disease that nowadays lacks of an effective treatment. Previous studies and clinical evidences have shown that compounds acting on aldehyde- dehydrogenase (ALDH) activities such as disulfiram, can modulate cocaine addiction. Isoflavones are natural compounds present in different plants and vegetables like soybeans. Several isoflavones can regulate ALDH activities, and therefore may potentially modulate cocaine responses related to its addictive properties. This study describes the effects of three natural isoflavones, daidzin, daidzein and genistein, on the modulation of the cocaine reinforcing effects and on cue-induced relapse in an operant mouse model of cocaine self- administration. Chronic treatment with daidzein or genistein decreased operant responding to obtain cocaine intravenous infusions. On the other hand, daidzein and daidzin, but not genistein, were effective modifying cue-induced cocaine relapse. Complementary studies revealed that daidzein effects on cocaine reinforcement were mediated through regulatory actions on dopamine type-II/III receptors activities. Therefore, our results suggest that these natural compounds alone or in combination can be of interest as a potential therapeutic approach for cocaine addiction. Further clinical studies are required in order to ascertain their potential therapeutic use.

INTRODUCTION

Cocaine addiction is a chronic relapsing disorder characterized by compulsive drug seeking and use. Cocaine addicts present a high rate of relapse even after prolonged periods of abstinence. This drug exerts its rewarding effects by inhibiting the monoamine transporters in presynaptic neurons in the brain reward system, producing an increase in the concentrations of dopamine (DA) and other monoamines in the synaptic cleft in the mesocorticolimbic system[1]. These pharmacological actions are directly involved in the development of cocaine addiction[2].

Nowadays, no effective treatment for cocaine addiction and relapse has been developed. However, studies performed with inhibitors of aldehyde dehydrogenase type 1 and 2 enzymes (ALDH-1 & ALDH-2), involved in monoamine metabolism[3.4], indicate that these compounds might decrease cocaine craving and consumption[5]. Clinical reports with disulfiram, an inhibitor of ALDH-1 and 2 activities used for the treatment of alcoholism, have shown to be effective to decrease cocaine use in humans[6,7]. More recently, the compound CVT-10216, a synthetic analogue of the natural isoflavone daidzin, has also been described as a potent inhibitor of the ALDH-2 activities[8] that can regulate cocaine rewarding effects and cocaine relapse in rodents[9]. Previous studies have pointed out the relevance of ALDH activities on DA metabolism as a possible mechanism of action modulating cocaine consumption. In this sense, the blockade of ALDH activities by CVT-10216 inhibits the conversion of the DA metabolite DOPAL to DOPAC, leading to the accumulation of DOPAL in dopaminergic neurons. This excess of DOPAL combines with DA to

form tetrahydropapaveroline, a compound that inhibits tyrosine hydroxylase (TH) activity, the enzyme responsible of DA synthesis. TH inhibition leads to a decrease in DA synthesis and release, which can attenuate cocaine rewarding effects and cocaine seeking and relapse[10]. In agreement, CVT-10216 reduces DA release into the nucleus accumbens[11], a crucial brain area of the mesocorticolimbic system involved in cocaine reinforcement, and decreases cocaine self-administration and cocaine seeking behavior in rats[9].

Natural isoflavones obtained from the plant *Radix puerariae*, like daidzin, are also potent inhibitors of ALDH activities[12]. These compounds can decrease DA metabolism by inhibiting ALDH-2 activities[13], suggesting that natural isoflavones might be of potential interest for the treatment of cocaine addiction.

In our study, we have evaluated the effects of three different natural isoflavones, daidzin, daidzein and genistein in cocaine reinforcing effects and cocaine seeking behavior using an operant mouse model of cocaine self-administration. We have also performed complementary studies to elucidate the possible mechanisms of action of these isoflavones on the modulation of cocaine reinforcement. Results obtained with these natural compounds have been compared with those produced by a reference ALDH inhibitor, disulfiram.

RESULTS

Daidzin, daidzein, genistein and disulfiram effects on locomotor activity

This experiment was carried out in order to choose the appropriate dose of each isoflavone to be tested in cocaine self-administration studies with no intrinsic locomotor effects. Based on previous studies[14,15], we evaluated the effects of daidzein and genistein at the doses of 100, 200 and 400 mg/kg, i.p.; and daidzin and disulfiram at the doses of 75, 150 and 300 mg/kg, i.p. in locomotor activity boxes 60 min after injection. Only daidzin and genistein produced hypolocomotor effects at the highest doses ($F(3,30)=6.7093$, $p<0.001$; $p<0.01$ vehicle vs daidzin 150 and 300 mg/kg) (Fig. 1B); ($F(3,25)=4.809$, $p<0.01$; $p<0.01$ vehicle vs genistein 200 mg/kg and $p<0.05$ 400 mg/kg) (Fig. 1C). Daidzein and disulfiram did not produce substantial locomotor changes at any of the doses, although the highest ones tended to reduce locomotor activity ($F(3,30)=3.8821$, n.s and $F(3,28)=2,369$, n.s, respectively) (Fig. 1A and 1D). Based on these results, we chose the dose of 100 mg/kg for daidzein and genistein, and the dose of 75 mg/kg for daidzin and disulfiram to be used at the operant cocaine self-administration paradigm.

Daidzein produces its hypolocomotor effects through a mechanism that requires estrogen and dopaminergic receptor activities

Based on a preliminary study performed in the laboratory that revealed the potential therapeutic effects of daidzein modulating cocaine reward, we conducted a more in depth investigation on

Figure 1

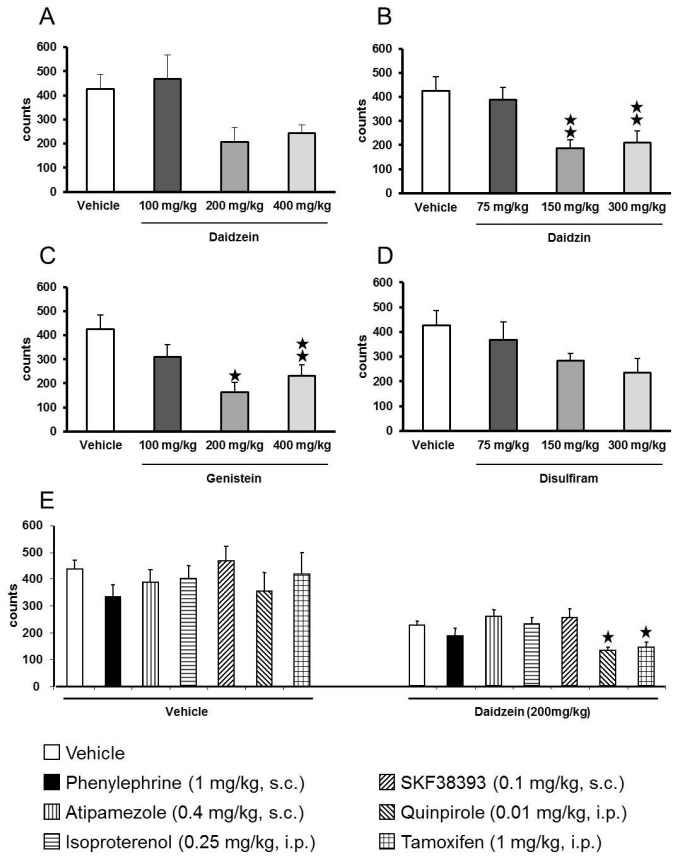

FIGURE 1: Locomotor effects induced after acute isoflavone or disulfiram administration. Involvement of dopamine type-II/III and estrogen-receptor systems in the locomotor effects induced by daidzein. Total locomotor activity 60 min after acute administration (i.p.) of different doses of **(A)** daidzein (n=8-10), **(B)** daidzin (n=8-10), **(C)** genistein (n=8-10) or **(D)** disulfiram (n=8-10). Effects of acute administration (30 min before locomotion was evaluated) of different agonist or antagonist for the adrenergic, dopaminergic or estrogen receptor systems (n=9-15 per group) in the locomotor activity induced by **(E)** acute vehicle (60 min before locomotion) or daidzein (200 mg/kg, 60 min before locomotion) administration. A total of 9-15 mice were included per experimental group. Results are expressed as average +SEM, ★★$p<0.01$, ★ $p<0.05$ vs vehicle or vs daidzein-vehicle treated mice, Fisher's multiple comparison test.

the mechanism involved in this isoflavone-induced locomotor effect. We specifically evaluated the involvement of the estrogen, noradrenergic and dopaminergic systems on daidzein-pharmacological effects. We performed an additional locomotor study injecting this isoflavone at the first dose producing clear hypolocomotor effects (200 mg/kg, i.p.) or vehicle in combination (30 min later) with the following agonists and antagonists of its potential pharmacological targets (Fig. 1E): estrogen receptor (tamoxifen, 1 mg/kg, i.p.), noradrenergic receptor (phenylephrine, 1 mg/kg, s.c.; atipamezole, 0.4 mg/kg, s.c.; isoproterenol, 0.25 mg/kg, i.p.) and dopaminergic receptor (SKF38393, 0.1 mg/kg, s.c.; quinpirole, 0.01 mg/kg, i.p.). Locomotion was evaluated 30 min after the administration of these last compounds and for a period of 60 min. Results show that the estrogen-, adrenergic- or dopaminergic- modulators did not produce intrinsic locomotor effects in control mice pretreated with vehicle at the doses used in this experiment ($F(6,69)=0,6962$, n.s.) (Fig. 1E). Daidzein at the dose of 200 mg/kg, i.p. produced significant hypolocomotor effects when compared with vehicle treated mice (Student t-test (3.994, 22), $p<0.05$). Further statistical analysis demonstrated that the treatment with type- II/III dopaminergic receptor agonist quinpirole (0.01 mg/kg) and with estrogen receptor agonist tamoxifen (1 mg/kg) (Fig. 1E) modified daidzein-induced hypolocomotion ($F(6,60)=4.460$, $p<0.001$; Fisher post-hoc analysis: daidzein-vehicle vs daidzein-quinpirole ($p<0.01$) and daidzein-vehicle vs daidzein-tamoxifen ($p<0.05$)). These results suggest that daidzein exerts its locomotor effects by regulating dopamine type-II/III and estrogen activities.

Chronic treatment with daidzein and genistein, but not with daidzin or disulfiram, modulates cocaine reinforcement

Mice were trained in operant boxes to actively nose-poke to obtain i.v. infusions of cocaine (0.5 mg/kg/infusion) in order to evaluate the effects of daidzein, daidzin, genistein and disulfiram on cocaine reinforcement. After reaching the stability criteria, vehicle, daidzein (100 mg/kg, i.p.), daidzin (75 mg/kg, i.p.), genistein (100 mg/kg, i.p.) or disulfiram (75 mg/kg, i.p.) were administered for 5 consecutive days 60 min before the exposure to the self- administration task. Mice were divided into the different experimental groups homogenously, based on their previous baseline operant responding levels.

When the effects of chronic daidzein (100 mg/kg, i.p.) were analyzed, three-way ANOVA (hole x day x treatment) revealed a significant interaction between the hole and day ($F(1,21)=6.2413$, $p<0.05$) with no interaction between the 3 factors ($F(4,84)=0.3398$, n.s.). Posterior one-way ANOVA (hole x treatment) on each day of treatment with the isoflavone demonstrated a significant difference when compared with vehicle-treated mice on day 1 ($F(1,21)=5.2515$, $p<0.05$), day 2 ($F(1,21)=5.7031$, $p<0.05$) and day 3 ($F(1,21)=7.9934$, $p<0.05$). On day 4, one-way ANOVA revealed an effect of the hole ($F(1,21)=20.6743$, $p<0.01$). On day 5, the effect of the hole ($F(1,21)=24.9150$, $p<0.01$) and treatment ($F(1,21)=5.1155$, $p<0.05$), but not of the interaction ($F(1,21)=3.8767$, n.s.) were observed. Posterior Fisher post-hoc analysis revealed a significant difference in the active nose poke responding in between animals treated with vehicle and daidzein on day 1 ($p<0.01$), day 2 ($p<0.01$),

day 3 ($p<0.01$), day 4 ($p<0.05$) and day 5 ($p<0.01$).

Chronic daidzin (75 mg/kg, i.p.) effects were also analyzed by applying a three-way ANOVA (hole x day x treatment), revealing an interaction between the 3 factors ($F(4,80)=2.6037$, $p<0.05$). Posterior Fisher post-hoc analysis demonstrated no significant difference in the active responding in between mice treated with vehicle and daidzin.

When the effects of chronic genistein (100 mg/kg, i.p.) were analyzed, three-way ANOVA (hole x day x treatment) revealed a significant interaction between the hole and treatment ($F(1,20)=6.3023$, $p<0.05$) and in between the hole and the day ($F(4,80)=2.5184$, $p<0.05$) with no interaction between the 3 factors ($F(4,80)=1.640598$, n.s.). Posterior one-way ANOVA (hole x treatment) performed on each day of treatment with the isoflavone demonstrated a significant difference when compared with vehicle mice on day 2 ($F(1,20)=4.9997$, $p<0.05$), day 3 ($F(1,20)=4.4436$, $p<0.05$), day 4 ($F(1,20)=7.0883$, $p<0.05$) and day 5 ($F(1,20)=7.3437$, $p<0.05$). Posterior Fisher post-hoc analysis revealed a significant difference in the active nose poke responding in between animals treated with vehicle and daidzein on day 2 ($p<0.01$), day 3 ($p<0.01$), day 4 ($p<0.01$) and day 5 ($p<0.05$).

Chronic disulfiram (75 mg/kg, i.p.) effects were also analyzed by applying a three-way ANOVA (hole x day x treatment) revealing no significant effect of the interaction of all 3 factors ($F(4,76)=0.5803$, n.s.) neither of the hole x treatment ($F(1,19)=1.9470$, n.s.), day x treatment ($F(4,76)=0.6196$, n.s.) or hole x day ($F(4,76)=0.5803$, n.s.).

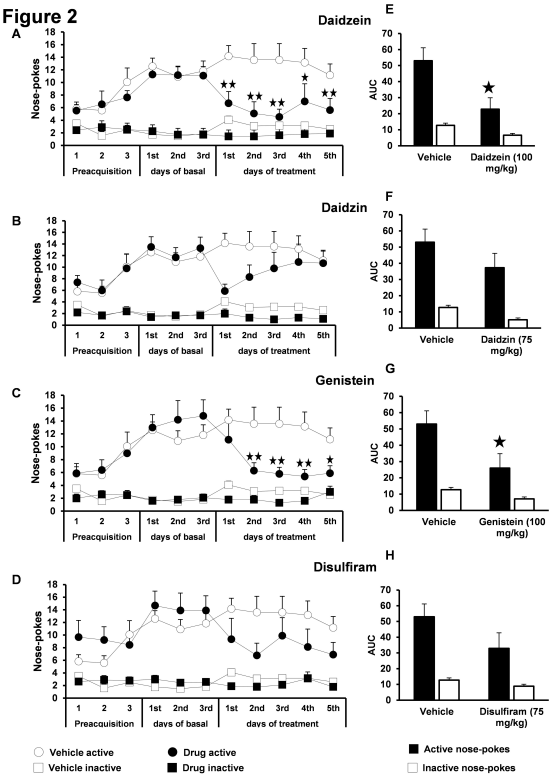

FIGURE 2: Chronic treatment with isoflavones decreases operant responding for cocaine.

(A to D) Effects of the chronic treatment with vehicle (n=12) and daidzein (100 mg/kg/day, i.p., n=11) (A), daidzin (75 mg/kg/day, i.p., n=10) **(B)**, genistein (100 mg/kg/day, i.p., n=10) **(C)** and disulfiram (75 mg/kg/day, i.p., n=9) **(D)** on cocaine self-administration. **(E to H)** Area under the curve of the 5 days of chronic treatment with vehicle (n=12) and daidzein (100 mg/kg/day, i.p., n=11) **(E)**, daidzin (75 mg/kg/day, i.p., n=10) **(F)**, genistein (100 mg/kg/day, i.p., n=10) **(G)** and disulfiram (75 mg/kg/day, i.p., n=9) **(H)** on cocaine self-administration. Animals received the injection of the vehicle, isoflavone or disulfiram for 5 consecutive days 1 h before the beginning of the operant training session. Data is represented as the mean number of nose-pokes in the active (circles) and inactive (square) hole to obtain cocaine (0.5 mg/kg/infusion) during the pre-acquisition and acquisition-training, and following the chronic treatment for 5 days with vehicle, isoflavone or disulfiram. Data are expressed as mean +SEM. ★ $p<0.05$ vs vehicle treated mice, Fisher's multiple comparison test.

The AUC of cocaine self-administration responses was also calculated for each compound and compared with the control vehicle treated animals. Results obtained demonstrated that daidzein significantly decreased cocaine self-administration responses ($F(1,42)=4.717$, $p<0.05$). Posterior post-hoc analysis revealed a significant difference in the active nose-poke response between vehicle and daidzein treatment (Fisher's F-test; $p<0.05$) (Fig. 2E). Interestingly, two-way ANOVA also revealed a significant effect of genistein modifying cocaine self- administration responses ($F(1,40)=4.878$, $p<0.05$, Fisher's F-test analysis $p<0.05$ vehicle vs genistein effects in the active hole; Fig. 2G). Modifications in the AUC after daidzin and disulfiram treatment were also calculated. No significant effects in the two-way ANOVA interaction were observed after daidzin ($F(1,40)=0.444$, n.s.; Fig. 2F) or disulfiram administration ($F(1,38)=1.586$, n.s.; Fig. 2H). These results suggest that isoflavone effects on drug reinforcement are heterogeneous and point to different mechanisms of action involved on the regulatory actions produced by each isoflavone.

Involvement of the dopamine receptor on daidzein-induced modulatory effects in cocaine self-administration

Based on the mechanistic locomotion study (Fig.1), we evaluated the involvement of the estrogen and dopamine type-II/III receptors in the modulatory effects induced by daidzein on drug reinforcement. Daidzein was chosen because it showed effective modulatory actions on both, the maintenance of cocaine self-administration (Fig. 2) and cue-induced cocaine seeking (Fig. 4).

Figure 3

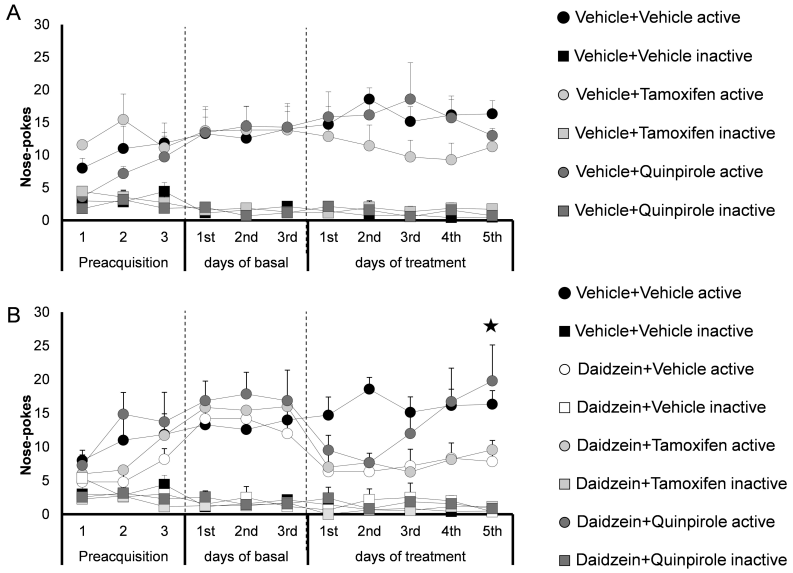

FIGURE 3: Involvement of the dopamine type-II/III receptor on daidzein-induced modulatory actions in cocaine self-administration paradigm. Effects of the exposure to vehicle, quinpirole (0.01 mg/kg, i.p.) or tamoxifen (1 mg/kg, i.p.) in the operant responding to obtain cocaine in mice previously treated with **(A)** vehicle (i.p., n=7-10) or **(B)** daidzein (100 mg/kg/day, i.p., n=8- 9). Data is represented as the mean number of nose-pokes in the active (circles) and inactive (square) hole to obtain cocaine (0.5 mg/kg/infusion) during the pre-acquisition and acquisition-training, and following the chronic treatment for 5 days with vehicle or daidzein (60 min before the beginning of the training session) and subsequent administration of vehicle, quinpirole or tamoxifen (30 min before the beginning of the training session). Results are expressed as average +SEM.★ $p<0.05$ vs daidzein-vehicle treated animals on the same day, Fisher's multiple comparison test.

Estrogen receptor agonist tamoxifen (1 mg/kg, i.p.), dopamine type-II/III agonist quinpirole (0.01 mg/kg, i.p.) or vehicle were administered 30 min after daidzein (100 mg/kg, i.p.) during 5 consecutive days. Thirty min after the last injection, animals were exposed to the self- administration task. Chronic exposure to quinpirole (0.01 mg/kg, i.p.) or tamoxifen (1 mg/kg, i.p.) for 5 consecutive days produced no significant effects in control animals pre-exposed to vehicle ($F(10,110)=0.6868$, n.s.) (Fig. 3A).

On the other hand, three-way ANOVA (hole x day x treatment) revealed a significant effect of the treatment with quinpirole or tamoxifen in animals pre-exposed to daidzein (100 mg/kg, i.p.) ($F(12,92)=1.8896$, $p<0.05$). Subsequent Fisher post-hoc analysis demonstrated that quinpirole, but not tamoxifen, progressively abolished daidzein effects on the maintenance of cocaine self-administration (Fisher's post-hoc, $p<0.05$ quinpirole vs vehicle on the 5^{th} day of treatment) (Fig. 3B). Our results suggest that daidzein modifies cocaine self-administration behavior by a mechanism that requires dopamine type-II/III receptor activities.

Daidzein, daidzin and disulfiram, but not genistein, decreased cue-induced cocaine seeking behavior and relapse

We exposed a new set of mice to an extinction protocol after reaching cocaine self- administration acquisition criteria. Once mice acquired the extinction criteria the effects of an acute injection of daidzein (100 mg/kg, i.p.), daidzin (75 mg/kg, i.p.), genistein (100 mg/kg, i.p.) or disulfiram (75 mg/kg, i.p.) in cue-induced cocaine seeking behavior were evaluated by using a Latin square

experimental design. All compounds were administered 60 min before starting the training session.

Student t-Test revealed that responding in the active hole was not modified when evaluated in two different days after pre-treatment with vehicle (Fig. 4A).

Figure 4

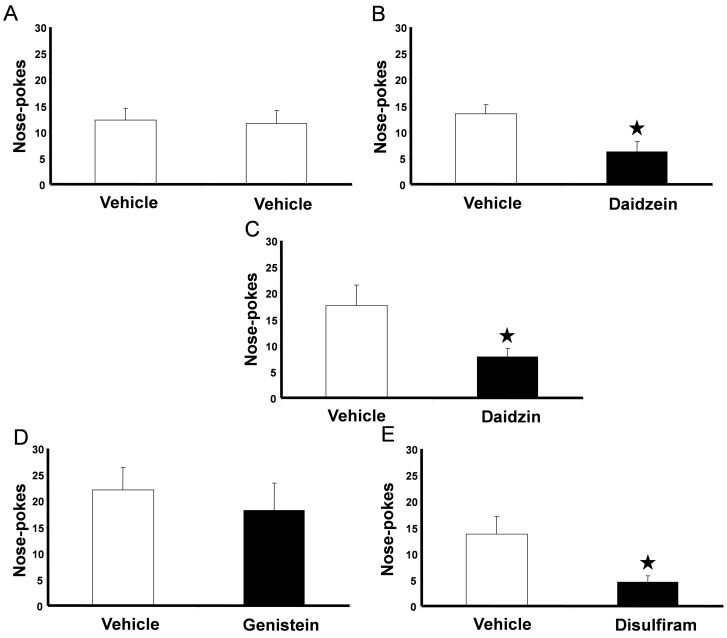

FIGURE 4: Acute administration of daidzein, daidzin and disulfiram, but not genistein, decreases cue-induced cocaine seeking behavior and relapse. Comparison of the effects of the administration of vehicle vs **(A)** vehicle (n=9), **(B)** daidzein (100 mg/kg, i.p., n=9), **(C)** daidzin (75 mg/kg, i.p., n=9), **(D)** genistein (100 mg/kg, i.p., n=9) or **(E)** disulfiram (75 mg/kg, i.p., n=8) in the active nose-poke responding after exposure to cue-induced cocaine seeking behaviour paradigm. All compounds were administered acutely 1 h before the beginning of the session. Results are expressed as average +SEM. ★$p<0.05$ vs vehicle treated animals, Student's t- test.

Daidzein (100 mg/kg, i.p.) effects on cocaine relapse were also studied. Student's t-test revealed that this compound decreased cue-induced responding when compared with vehicle administration ($t<0.05$) (Fig. 4B). Moreover, daidzin (75 mg/kg, i.p.) pre-exposure was also effective to decrease cocaine relapse (Student's t-test; $t<0.05$). Similar results were also observed in mice pretreated with disulfiram (75 mg/kg, i.p.), showing a significant decreased in the active nose-pokes responses when compared with vehicle pre-treatment ($t<0.05$) (Fig. 4E). However, genistein (100 mg/kg, i.p.) did not modify the behavior in cue-induced seeking behavior paradigm, leading to the same active responding as when mice received vehicle injections (Student's t-test, n.s.) (Fig. 4D).

Involvement of dopamine receptor on daidzein, but not daidzin, modulatory effects in cue- induced cocaine seeking behavior

Because of the similar chemical structure share by daidzin and daidzein, we studied the role of estrogen and dopamine type-II/III receptors modulating the effects of both isoflavones in cue- induced cocaine relapse.

After extinction of cocaine self-administration, animals received vehicle, daidzein or daidzin and 30 min later tamoxifen, quinpirole or vehicle. Thirty min after the last injection, animals were exposed to cue-induce relapse. Both tamoxifen and quinpirole did not modify cocaine- seeking behavior in mice treated with vehicle (one-way ANOVA $F(2,30)=0.0639$, n.s.) (Fig.5A). Daidzein (100 mg/kg, i.p.) decreased the number of active responses after cue-induced cocaine relapse when compared with vehicle-treated animals (Fig. 5B). One-

way ANOVA revealed a significant effect of quinpirole and/or tamoxifen modulating daidzein actions on cue-induced cocaine relapse ($F(1,26)=3.9221$, $p<0.05$). Subsequent post-hoc analysis demonstrated that dopamine type-II/III receptor agonist quinpirole (Fisher's F-test post-hoc analysis, $p<0.05$), but not estrogen receptor agonist tamoxifen (Fisher's F-test post-hoc analysis, n.s.) significantly abolished the pharmacological effect of daidzein in the operant responses when compared with vehicle treatment 30 min after daidzein injection ($F(2,26)=3.9221$, $p<0.05$) (Fig. 5B).

In addition, acute administration of daidzin also decreased cue-induced cocaine seeking behavior when compared to vehicle treated mice. In contrast with daidzein, administration of tamoxifen or quinpirole did not modify the effects of daidzin on operant responding ($F(2,25)=1.0989$, n.s.) (Fig. 5C).

These data suggest that daidzein effects on cocaine seeking behavior and relapse are mediated by mechanism that involves dopamine type-II/III receptor activities. Moreover, our data suggest that isoflavones, daidzein and daidzin, act through different mechanisms of action regulating cocaine seeking responses.

Figure 5

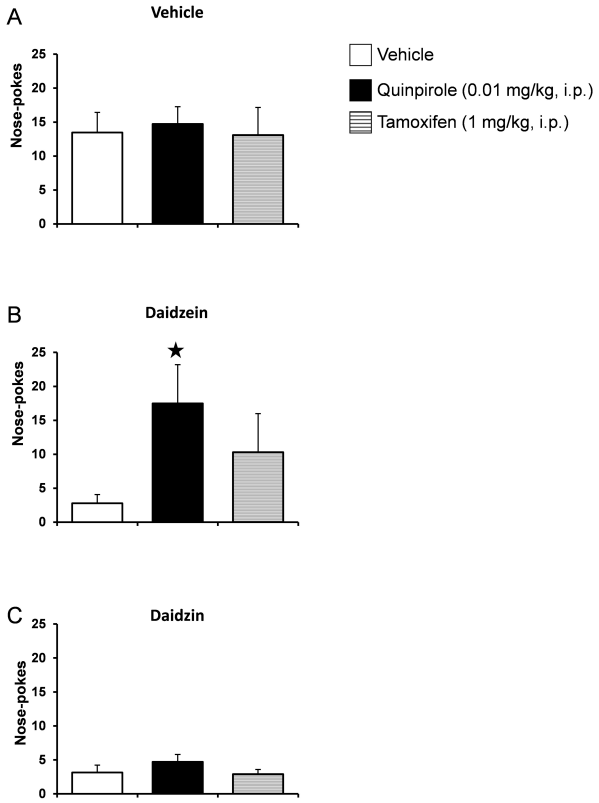

FIGURE 5: Involvement of the estrogen and dopamine type-II-receptor systems on daidzein-, but not daidzin-, effects in cue-induced cocaine seeking behavior and relapse. Comparison of the effects of an acute administration of vehicle or a sub-effective dose of quinpirole (0.01 mg/kg, i.p.) or tamoxifen (1 mg/kg, i.p.) on cue-induced cocaine seeking behavior and relapse in mice acutely treated with **(A)** vehicle, **(B)** daidzein (100 mg/kg, i.p.) or **(C)** daidzin (75 mg/kg, i.p.). Animals were injected with vehicle, daidzein or daidzin and thirty minutes later with tamoxifen, quinpirole or vehicle. Thirty minutes after the last injection animals were exposed to cue-induced cocaine seeking behavior paradigm. Results are expressed as average +SEM. ★$p<0.05$ vs daidzein-vehicle treated animals, Fisher's multiple comparison test.

DISCUSSION

In this study, we have demonstrated that the isoflavones daidzein, daidzin and genistein are natural compounds that can modulate the reinforcing effects of cocaine and cocaine seeking behavior in an operant self-administration paradigm in mice.

We compared the effects of these isoflavones with those produced by disulfiram, a classical drug known to inhibit ALDH activities and alcohol intake[16,17] that has been evaluated for the treatment of cocaine addiction in humans[7] (however see also[18]).

Our study has revealed that daidzein is the most promising compound able to modulate cocaine reinforcing effects and cocaine seeking behavior in this operant paradigm. This isoflavone produced its modulatory actions on cocaine self-administration (Fig. 2B) and cue- induced relapse (Fig. 4B) by a mechanism that involved dopamine type-II/III receptor, but not estrogen receptor, activities.

Daidzein-modulatory actions on cocaine self-administration (Fig. 2B) and cue-induced relapse (Fig. 4B) were modified by the pretreatment with the dopamine type-II/III receptor agonist quinpirole but not the estrogen receptor agonist tamoxifen (Fig. 3B, 5B), although both were shown to alter hypolocomotor effects produced by high doses of daidzein (200 mg/kg, i.p.) (Fig. 1). Our observations suggest that daidzein modulates cocaine addiction by modifying DA transmission. In agreement, previous studies have hypothesized that other ALDH inhibitors can regulate cocaine consumption and relapse by a mechanism that also involves a

decrease in DA synthesis and release[9,11]. Based on our results, we suggest that the alterations in DA transmission induced by daidzein might preferentially modify dopamine type-II/III functionality and subsequently modulate cocaine addictive properties.

In sharp contrast with daidzein, disulfiram exposure did not modify cocaine self-administration (Fig. 2E). This is in agreement with previous preclinical studies in rats also describing no effect of acute disulfiram administration using the operant cocaine self-administration approach[14]. Importantly, when disulfiram is administered *in vivo* it becomes metabolized into different compounds that present a wide array of pharmacological targets and actions. It has been shown that disulfiram metabolites can modulate different neurotransmitter systems crucially involved in cocaine reward and addiction, such as the dopaminergic, noradrenergic and glutamatergic systems[19]. The lack of effects of disulfiram on cocaine reward might be a reflection of the vast spectrum of pharmacological actions produced by this compound and its metabolites, with some of them triggering opposite effects on cocaine rewarding actions. Further research is required to clarify this topic.

Interestingly, clinical studies have demonstrated that disulfiram was effective decreasing cocaine addictive response in cocaine addicts (even if recently, some studies are questioning this statement[18]). Discrepancies between clinical observations and the preclinical studies in rats and mice could be explained by previous studies describing that disulfiram inhibit cocaine- metabolizing enzymes and increase peak plasma cocaine levels in human addicts[20,21], an effect

that has never been observed in rodents[19]. These differences between species might account for the differential effect of disulfiram modulating cocaine consumption between human and rodents, even if recent studies might have also questioned the effectiveness of this compound in human addicts[18]. More research is required in order to clarify the role of disulfiram modulating the rewarding effects of cocaine.

On the other hand, our results revealed that disulfiram was effective decreasing cue-induced drug seeking behavior (Fig. 4E) in agreement with previous preclinical and clinical observations describing the inhibitory properties of this compound on cocaine craving and relapse[14.6]. Different mechanisms of action have been proposed to explain this effect: Disulfiram might decrease cue-induced cocaine seeking behavior by altering DA release after the inhibition of ALDH activities, as described above. However, a different mechanism has also been suggested[22,19]. Thus, disulfiram is metabolized producing a compound called diethyldithiocarbamate, which acts as a copper chelator that inhibits dopamine *β*-hydroxylase (DBH), the enzyme responsible for the conversion of DA to noradrenaline (NE) in the final step of NE biosynthesis. Therefore, disulfiram can reduce NE and increase DA release and DA- metabolites, an effect reported in different brain areas in rodents[23,24,25] and humans[26]. Consequently, the modifications in NE and DA neurotransmission after the inhibition of DBH activities by disulfiram has been hypothesized as a possible mechanism involved in the modulatory effects of this compound in cue-induced cocaine seeking behavior and relapse. A recent study suggests that the suppressant effects of DBH inhibitors on cocaine

reinstatement is mediated by an increase in extracellular DA levels leading to a hyper-stimulation of dopamine type-I receptors in the rat medium prefrontal cortex, a critical area involved in cocaine seeking behavior[27].

Even if no statistical significance was observed, chronic administration of daidzin also tends to decrease cocaine self-administration after the 1st and 2nd administrations of the compound. However, tolerance developed after prolonged treatment (Fig. 2C). Furthermore, the acute administration of daidzin was also effective decreasing cocaine seeking behavior in the cue- induced cocaine relapse paradigm (Fig. 4C). Previous studies performed with this isoflavone and with synthetic analogues such as CVT-10216 suggest that daidzin might decrease cocaine effects by a mechanism that involves modulatory actions in ALDH-2 and DA activities[9,11,15]. In contrast, our results show that the blockade of dopamine type-II/III receptors did not modify daidzin modulatory actions (Fig. 5C). Moreover, daidzin acting through ALDH-2 can also affect the metabolism of 5-HT[13], and this mechanism may also be important for the modulation of cocaine addictive properties. In this sense, previous studies have shown that 5-HT influences cocaine reinforcement, seeking behavior and relapse by a mechanism that requires interactions with DA and/or glutamate neurotransmission[28].

Genistein was also effective decreasing cocaine-induced operant responding (Fig. 2E). In contrast with daidzein and daidzin, this isoflavone did not modify the active nose-poke response in the cue-induced cocaine relapse paradigm (Fig. 4D). Genistein is a potent agonist of the β-estrogen receptors[29], and previous studies have

shown an involvement of these receptors in modulating cocaine-rewarding effects by a mechanism not fully understood. Thus, blocking the brain estrogen receptors completely inhibits the development and expression of cocaine-induced locomotor sensitization in rats[30]. Based on our results, we hypothesize that the chronic treatment with genistein may lead to a desensitization in the estrogen signaling, an effect that develops after the 2nd day of treatment with this isoflavone. This alteration in estrogen receptor functionality might lead to a decrease in the rewarding effects of cocaine. On the other hand, genistein is also a potent inhibitor of TH activities and previous studies have shown that genistein can increase DA release from mouse striatal slices by modifying the activity of this enzyme[31]. Chronic treatment with genistein might lead to alterations in TH activity that can change DA release in different areas of the mesocorticolimbic system and decrease cocaine-rewarding effects. Moreover, genistein can also modulate different intracellular signaling pathways, including the nuclear factor-kappa β (NF-kβ)[32,33] which is shown to be increased in the nucleus accumbens after cocaine administration. This increase in neuronal NF-kβ signaling pathways has been implicated in the long-term effects of cocaine that lead to the development of cocaine addiction[34]. Based on these results, we can suggest that genistein interferes in cocaine rewarding effects by inhibiting the activity of NF-kβ and consequently decreases the level of responding in the self-administration paradigm. Interestingly, cocaine-induced NF-kβ expression occurred after the chronic, but not the acute treatment with this psychostimulant[34]. This observation also fits well with our results where no effect of genistein was observed after its acute

administration modulating cocaine reward and cue-induced cocaine relapse.

We can conclude that natural isoflavones, but specially daidzein, can be useful for the treatment against cocaine consumption and cocaine relapse. Moreover, because of the safety of these natural products[35,36], the translationality of the observations made in this report to clinics in humans is feasible and promising. Each isoflavone produces its effects on cocaine addiction modulating different neurotransmitter, receptor systems and intracellular pathways so further research is required in order to unravel each specific mechanism. Moreover, under a clinical therapeutic perspective, it will be important to take into consideration whether treatment to patients should be performed with soybean extracts or with specific isoflavones.

METHODS

Animals

Male CD-1 mice from 20-22 g at the beginning of the experiments were used. Mice were maintained in a temperature (21±1 ºC) and humidity (65±10%) controlled room with a normal light-dark cycle (lights on from 08:00 to 20:00 h) except for the self-administration studies where a reversed light-dark cycle was used during the whole experimental sequence (lights off from 08:00 to 20:00 h) was used. Animals were housed 4 per cage, except in the self-administration studies where mice were single-housed after the surgery. Behavioral tests and animal care were conducted in accordance with the standard ethical guidelines (European Commission Directive 86/609 EC) and approved by the local ethical committee (CEEA-PRBB). All behavioral studies were performed in blind conditions.

Drugs

Cocaine hydrochloride was obtained from the Spanish Agency of Medicines and Medical Devices (AEMPS, Madrid, Spain), dissolved in sterile saline and administered at 0.5 mg/kg (intravenously, i.v.). All natural isoflavones (daidzin, daidzein and genistein) were purchased from LC Laboratories (Woburn, MA) or PhytoLab (Vestenbergsgreuth, Germany), dissolved in 50 µl of Tween 80/0.5 % carboxymethylcellulose (CMC) in sterile saline and administered at different doses by intraperitoneal (i.p.) route. Disulfiram was purchased from TOCRIS (Bio- Techne, Minneapolis, MN), dissolved

in 50 µl of Tween 80/0.5 % CMC in sterile saline and administered at different doses by i.p. route. Phenylephrine (1 mg/kg, subcutaneous (s.c.)), atipamezole (0.4 mg/kg, s.c.), isoproterenol (0.25 mg/kg, i.p.), SKF38393 (0.1 mg/kg, s.c.), quinpirole (0.01 mg/kg, i.p.) and tamoxifen (1 mg/kg, i.p.) were purchased from TOCRIS and dissolved in 50 µl of Tween 80/0.5% CMC in sterile saline.

Locomotor activity

Locomotion was evaluated in locomotor activity boxes (9 × 20 × 11 cm; Imetronic, France) containing a line of photocells 2 cm above the floor to measure horizontal movements, and another line located 6 cm above the floor to measure vertical activity (rearing). Mice were individually placed in the boxes and the total activity was recorded during 60 min in a low luminosity environment (20–25 lux). Total locomotor activity was evaluated as the sum of the horizontal and vertical movements. All the locomotor activity tests were performed between 09:30 and 16:30 h. Isoflavones and disulfiram were administered 60 min before locomotor activity measurements. Phenylephrine, atipamezole, isoproterenol, SKF38393, quinpirole and tamoxifen were administered 30 min before locomotor activity was evaluated. All locomotor activity studies were performed with no previous habituation to the activity boxes.

Cocaine self-administration procedure

Apparatus

Self-administration training and testing occurred in operant chambers (Model ENV-307A-CT, Med-Associates, Georgia, VT, USA)

equipped with two holes, one selected as the active hole for delivering the reinforcer and the other as the inactive hole. Acquisition of drug self- administration was performed using a fixed ratio 1 (FR1) schedule of reinforcement such that one nose-poke in the active hole resulted in one cocaine infusion, while nose-poking in the inactive hole had no programmed consequences. A stimulus light, located above the active hole, was paired contingently with the delivery of the reinforcer. Infusions were delivered in a volume of 23.5 µl over 2 sec. Cocaine was infused via a syringe that was mounted on a microinfusion pump (PHM-100A, Med-Associates, Georgia, VT, USA) and connected, via Tygon tubing (0.96 mm o.d., Portex Fine Bore Polythene Tubing, Portex Limited, Kent, England) to a single channel liquid swivel (375/25, Instech Laboratories, Plymouth Meeting, PA, USA), and to the mouse i.v. catheters. The swivel was mounted on a counter-balanced arm above the operant chamber.

Surgery

Mice were anesthetized with a ketamine/xylazine mixture (0.1 ml/10 g body weight, i.p.) and then implanted with indwelling i.v. silastic catheters in the right jugular vein, as previously described[37]. After surgery, animals were individually housed and allowed 4 days for recovery before starting the operant training. The patency of the catheters was evaluated at the end of the operant cocaine self-administration experimental sequence, and whenever drug self-administration behavior appeared to deviate more than 50% from the one previously observed, by infusing 0.1 ml of thiopental (5 mg/ml) through the catheter. If prominent signs of anesthesia were

not apparent within 3 sec of the infusion, the animal was removed from the experiment.

Procedure

Animals were trained to nose-poke under a FR1 schedule of reinforcement to receive cocaine (0.5 mg/kg/infusion). Self-administration session started with a priming infusion of the drug, lasted for 60 min and was conducted 7 days a week. After each session, mice were returned to their home-cages. The number of reinforcers was limited to 50 infusions per session. Each infusion was followed by a 15 sec time-out period during which an active nose-poke had no consequence. Stable acquisition of self-administration behavior was achieved when the 3 following conditions were met: (i) < 20% deviation from the mean of the total number of reinforcers earned in 3 consecutive sessions (80% stability), (ii) at least 75% responding on the active hole, and (iii) a minimum of 4 reinforcers earned per session. When stability criteria were achieved, the effects of daidzin (75 mg/kg, i.p.), daidzein (100 mg/kg, i.p.), genistein (100 mg/kg, i.p.), disulfiram (75 mg/kg, i.p.) and vehicle were evaluated on cocaine self- administration for 5 consecutive days. These compounds were administered 60 min before starting the self-administration session on each day. Phenylephrine, atipamezole, isoproterenol, SKF38393, quinpirole or tamoxifen were injected 30 min after vehicle or isoflavone administration during 5 consecutive days to evaluate the involvement of adrenergic, dopaminergic or estrogen receptors on isoflavone effects on cocaine self-administration.

In a different set of mice, after cocaine self-administration training, animals underwent to an extinction process. During this phase, nose-poking into the reinforced poke resulted in the activation of the pump to maintain the usual experimental environment, but mice did not receive the infusion of the drug nor the conditioned stimulus (light). Mice were exposed to 1 h daily extinction sessions 6 days a week until extinction criterion was reached. The extinction criterion was achieved when mice made a mean number of active responses in three consecutive extinction sessions of less than 30% of the responses performed during the last day of the cocaine-training period. A small percentage of animals never reached the extinction criteria after the exposure to the extinction protocol for a month and a half (less than around 10%) and were excluded from the experiment.

After achieving the extinction criterion, mice were exposed to cue (stimulus light)-induced reinstatement of cocaine seeking behavior. Cue-induced seeking behavior experimental conditions were the same as for acquisition training sessions except that the pump was turned off to make cocaine not available. Animals were re-exposed to several sessions of cue-induced relapse using a Latin-square design. Each consecutive relapse session was preceded by an extinction period and not performed until mice reached the extinction criterion again. This methodological approach allowed evaluating in the same animal the effects of vehicle and one isoflavone or disulfiram on cue-induced relapse. The doses used and period of administration of these compounds were the same as when tested during cocaine self-administration (75 or 100 mg/kg, i.p., 60 min

before testing), but the animals received only an acute injection. We also followed a Latin-square design to investigate the involvement of the adrenergic, dopaminergic or estrogen receptors modulating isoflavone effects on cue-induced cocaine relapse. These compounds were injected 30 min after vehicle or daidzein administration.

Statistical analysis

Locomotor activity results were analyzed for each compound evaluated by using one-way ANOVA followed by Dunnett's multiple comparison tests when required. The same statistical approach was applied when the effects of estrogen, dopaminergic and adrenergic systems on daidzein-induced hypolocomotor effects were studied.

The effects of the isoflavones or disulfiram on cocaine self-administration were evaluated for each compound by applying a three-way ANOVA of repeated measures with day and manipulandum (active/inactive) as within-subjects and treatment as between-subjects factors. Subsequent Fisher's F-tests were performed when required. The area under the curve (AUC) was also calculated for each compound in the acquisition of cocaine self-administration and was compared with the control vehicle-treated mice using a two-way ANOVA (treatment and manipulandum as factors) followed by Fisher's F-test when required.

Tamoxifen and quinpirole modulatory effects on cocaine self-administration were evaluated separately for control vehicle- and daidzein-treated mice by using a three-way ANOVA of repeated measures with day and manipulandum (active/inactive) as within-

subjects and treatment as between-subjects factors. Subsequent Fisher's F-tests were performed when required.

Isoflavone or disulfiram effects on the active nose-poke responses after cue-induced cocaine relapse were analyzed by applying a Student's t-Test.

Finally, the effects of quinpirole and tamoxifen modulating the active nose-poke responses on cue-induced cocaine relapse were analyzed separately for vehicle-, daidzein- or daidzin-treated animals by applying a one-way ANOVA, followed by Fisher's post-hoc analysis tests when required.

All data were analyzed using STATISTICA software. A result was considered significant if $p<0.05$. All the results are expressed as mean + SEM.

REFERENCES

1. Camí J, Farré M (2003). Drug addiction. *N Engl J Med*.**349**:975-986.

2. Dackis CA, O'Brien CP (2001). Cocaine dependence: a disease of the brain's reward centers. *J Subst Abuse Treat.***21:**111-117.

3. Kopin IJ (1994). Monoamine oxidase and catecholamine metabolism. *J Neural Transm Suppl.***41:**57-67.

4. Erwin VG, Deitrich RA (1966). Brain aldehyde dehydrogenase. Localization, purification, and properties. *J Biol Chem.***241:**3533-3539.

5. Koppaka V, Thompson DC, Chen Y, Ellermann M, Nicolaou KC, Juvonen RO et al (2012). Aldehyde dehydrogenase inhibitors: a comprehensive review of the pharmacology, mechanism of action, substrate specificity, and clinical application. *Pharmacol Rev.***64:**520-239.

6. Suh JJ, Pettinati HM, Kampman KM, O'Brien CP (2006). The status of disulfiram: a half of a century later. *J Clin Psychopharmacol.***26:**290-302.

7. Pani PP, Trogu E, Vacca R, Amato L, Vecchi S, Davoli M (2010). Disulfiram for the treatment of cocaine dependence. *Cochrane Database Syst Rev.* 1:CD007024.

8. Overstreet DH, Knapp DJ, Breese GR, Diamond I (2009). A selective ALDH-2 inhibitor reduces anxiety in rats. *Pharmacol Biochem Behav.***94:**255-261.

9. Yao L, Fan P, Arolfo M, Jiang Z, Olive MF, Zablocki J et al (2010). Inhibition of aldehyde dehydrogenase-2 suppresses cocaine seeking by generating THP, a cocaine use-dependent inhibitor of dopamine synthesis. *Nat Med.***16:**1024-1028.

10. Diamond I, Yao L (2015) From Ancient Chinese Medicine to a Novel Approach to Treat Cocaine Addiction. *CNS Neurol Disord Drug Targets*. **14**:716-26.

11. Arolfo MP, Overstreet DH, Yao L, Fan P, Lawrence AJ, Tao G et al (2009). Suppression of heavy drinking and alcohol seeking by a selective ALDH-2 inhibitor. *Alcohol Clin Exp Res.***33:**1935-1944.

12. Keung WM, Klyosov AA, Vallee BL (1997). Daidzin inhibits mitochondrial aldehyde dehydrogenase and suppresses ethanol intake of Syrian golden hamsters. *Proc Natl Acad SciU SA.***94:**1675-1679.

13. Keung WM, Vallee BL (1998). Daidzin and its antidipsotropic analogs

inhibit serotonin and dopamine metabolism in isolated mitochondria.*Proc Natl Acad Sci U S A.***95:**2198-2203.

14. Schroeder JP, Cooper DA, Schank JR, Lyle MA, Gaval-Cruz M, Ogbonmwan YE et al (2010). Disulfiram attenuates drug-primed reinstatement of cocaine seeking via inhibition of dopamine β-hydroxylase. *Neuropsychopharmacology.***35:**2440-2449.

15. Keung WM, Vallee BL (1993). Daidzin and daidzein suppress free-choice ethanol intake by Syrian golden hamsters. *Proc Natl Acad Sci U S A.***90:**10008-10012.

16. Hald J, Jacobsen E (1948). A drug sensitizing the organism to ethyl alcohol. *Lancet.* **2:**1001- 1004.

17. Johansson B(1992). A review of the pharmacokinetics and pharmacodynamics of disulfiram and its metabolites. *Acta Psychiatr Scand Suppl.***369:**15-26.

18. Carroll KM, Nich C, Petry NM, Eagan DA, Shi JM, Ball SA (2016) A randomized factorial trial of disulfiram and contingency management to enhance cognitive behavioral therapy for cocaine dependence. *Drug Alcohol Depend*. **160**:135-142.

19. Gaval-Cruz M, Weinshenker D (2009). Mechanisms of disulfiram-induced cocaine abstinence: antabuse and cocaine relapse. *Mol Interv.***9:**175-187.

20. McCance-Katz E F, Kosten T R, Jatlow P (1998). Disulfiram effects on acute cocaine administration. *Drug and alcohol dependence* 52:27-39.

21. Baker FC, Kahan TL, Trinder J & Colrain I. M (2007). Sleep quality and the sleep electroencephalogram in women with severe premenstrual syndrome. *Sleep***30**, 1283–91.

22. Weinshenker D, Schroeder JP (2007). There and back again: a tale of norepinephrine and drug addiction. *Neuropsychopharmacology.***32:**1433-1451.

23. Goldstein M, Nakajima K (1966). The effects of disulfiram on the repletion of brain catecholamine stores. *Life Sci.***5:**1133-1138.

24. Bourdélat-Parks BN, Anderson GM, Donaldson ZR, Weiss JM, Bonsall RW, Emery MS et al (2005). Effects of dopamine beta-hydroxylase genotype and disulfiram inhibition on catecholamine homeostasis in mice. *Psychopharmacology (Berl).* **183:**72-80.

25. Devoto P, Flore G, Saba P, Cadeddu R, Gessa GL (2012). Disulfiram stimulates dopamine release from noradrenergic terminals and potentiates cocaine-induced dopamine release in the prefrontal cortex. *Psychopharmacology (Berl).***219:**1153-1164.

26. Paradisi R, Grossi G, Pintore A, Venturoli S, Porcu E, Capelli M et al (1991). Evidence for a pathological reduction in brain dopamine metabolism in idiopathic hyperprolactinemia. *Acta Endocrinol (Copenh).***125:**246-252.

27. Devoto P, Fattore L, Antinori S, Saba P, Frau R, Fratta W et al (2014). Elevated dopamine in the medial prefrontal cortex suppresses cocaine seeking via D1 receptor overstimulation. *Addict Biol.***21**:61-71.

28. Howell LL, Cunningham KA (2015). Serotonin 5-HT2 receptor interactions with dopamine function: implications for therapeutics in cocaine use disorder. *Pharmacol Rev.***67:**176-197.

29. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT et al (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* **139**, 4252–63.

30. Segarra AC, Torres-Díaz YM, Silva RD, Puig-Ramos A, Menéndez-Delmestre R, Rivera- Bermúdez JG et al (2014). Estrogen receptors mediate estradiol's effect on sensitization and CPP to cocaine in female rats: role of contextual cues. *Horm Behav*. **65:**77-87.

31. Bare DJ, Ghetti B, Richter JA (1995). The tyrosine kinase inhibitor genistein increases endogenous dopamine release from normal and weaver mutant mouse striatal slices. *J Neurochem.***65:**2096-2104.

32. Kazi A, Daniel KG, Smith DM, Kumar NB, Dou QP (2003). Inhibition of the proteasome activity, a novel mechanism associated with the tumor cell apoptosis-inducing ability of genistein. *Biochem Pharmacol.***66:**965-976.

33. Nagaraju GP, Zafar SF, El-Rayes BF (2013). Pleiotropic effects of genistein in metabolic, inflammatory, and malignant diseases. *Nutr Rev.***71:**562-572.

34. Ang E, Chen J, Zagouras P, Magna H, Holland J, Schaeffer E et al (2001). Induction of nuclear factor-kappaB in nucleus accumbens by chronic cocaine administration. *J Neurochem.***79:**221- 224.

35. Munro IC, Harwood M, Hlywka JJ, Stephen AM, Doull J, Flamm WG, Adlercreutz H (2003). Soy isoflavones: a safety review. *Nutr Rev*. **61**:1-33.

36. Choi MS, Rhee KC (2006). Production and processing of soybeans and

nutrition and safety of isoflavone and other soy products for human health. *J Med Food*. **9**:1-10.

37. Trigo JM, Panayi F, Soria G, Maldonado R, Robledo P (2006). A reliable model of intravenous MDMA self-administration in naïve mice. *Psychopharmacology* (Berl*).***184:**212-220.

AUTHOR CONTRIBUTION

RT, MF and RF elaborated the study design. MM and MG-M collected the experimental data. RG and MG-M performed the surgical procedures. MG-M analysed the data. MM, MG-M, RT and RM drafted the article. RT, MF, RF, MM and MG-M contributed to data interpretation. All authors critically reviewed the content and approved the final version for publication.

ADDITIONAL INFORMATION

We acknowledge the financial support of the Spanish "Instituto de Salud Carlos III" (#RD12/0028/0023 to R.M. and #CIBEROBN to R. dl T.), Spanish "Ministerio de Sanidad y Política Social-Investigación Clínica Independiente" (#EC10-166 to M.F.), the Catalan Government (#2014-SGR-1547 and #TECCIT2014 and 2015-NeuroPhar to R.M., and #2014- SGR-680 and #TECCIT2014 and 2015-BAPP to R dl T.). M.M. was a "Beatriu de Pinós" fellow (#2010-BP-B00221). Partial support of FEDER funds is also acknowledged.

None of the authors reported biomedical financial interests or potential conflicts of interest.

ARTICLE 2

CAFETERIA DIET INDUCES NEUROPLASTIC MODIFICATIONS IN THE NUCLEUS ACCUMBENS MEDIATED BY MICROGLIA ACTIVATION

Miriam Gutiérrez-Martos, Benoit Girard, Sueli Mendonça-Netto, Julie Perroy, Emmanuel Valjent, Rafael Maldonado, Miquel Martin

Addiction Biology. 2017 Sep 5. [Epub ahead of print]

doi: 10.1111/adb.12541.

PMID: 28872733

http://onlinelibrary.wiley.com/wol1/doi/10.1111/adb.12541/full

ARTICLE 3

EPIGENETIC AND PROTEOMIC EXPRESSION CHANGES PROMOTED BY EATING ADDICTIVE-LIKE BEHAVIOR

Samantha Mancino*, Aurelijus Burokas*, Javier Gutiérrez-Cuesta*,
Miriam Gutiérrez-Martos, Elena Martín-García, Mariangela Pucci,
Anastasia Falconi, Claudio D'Addario, Mauro Maccarrone,
Rafael Maldonado

Neuropsychopharmacology. 2015 Nov;40(12):2788-800.
doi: 10.1038/npp.2015.129. Epub 2015 May 6.
PMID: 25944409
https://www.nature.com/npp/journal/v40/n12/full/npp2015129a.html

DISCUSSION

Overview

Drugs of abuse and palatable food intake stimulate the brain reward system, that constitutes the neurobiological substrate involved in addictive disorders and the management of the hedonic component of eating (Ikemoto, 2007). In this thesis we present a compendium of several studies aiming to better understand the mechanisms involved in the development of addictive-like behaviours, specially focusing on the effects induced by a psychostimulant drug (cocaine) and the alterations in the mesocorticolimbic system involved in food-induced behavioural impairment and addictive-like behaviour. This doctoral thesis is divided in 3 original research studies involving several scopes:

- First (objective 1, Article 1), we evaluate the efficacy in decreasing cocaine reinforcement and cue-induced relapse of several natural compounds and a synthetic drug able to regulate ALDH activities.

- Second (objective 2, Article 2), we focus on the intrinsic neurobiological and functional changes associated with overeating-induced overweight in the reward system. We use a translational free-choice feeding animal model to mimic the human obesogenic environment, tightly linked with the development of overweight and obesity.

- Finally (objective 3, Article 3), we validate a new animal model of high palatable food-induced addictive-like behaviour. This model adapts the DSM-5 criteria to diagnose drug addiction to the self-administration paradigm enabling us to separate extreme populations of palatable food addictive-vulnerable and -resistant individuals.

A. Isoflavones and cocaine addiction

Cocaine addiction is a chronic brain disease which affects 15-17% of cocaine consumers (American Psychiatric Association, 2013). Unfortunately, nowadays cocaine addiction lacks of an effective treatment and most cocaine addict subjects relapse even after long periods of abstinence. Each drug of abuse has unique and individual characteristics that are important in developing improved and specific treatments, although all drugs share a common final objective of increasing dopaminergic tone (Edens *et al*, 2010). In this line, although a certain medication may be useful in the treatment of a specific drug of abuse, the same approach may not have the desired effect in cocaine addiction. In this line, the withdrawal symptoms of nicotine and cocaine can respectively precipitate relapse in addicted subjects. Nicotine abstinence can be accompanied with nicotine replacement therapies or nicotinic acetylcholine receptor antagonists such as mecamylamine that successfully increases the efficiency of cessating nicotine use (Edens *et al*, 2010). However, the treatment of cocaine withdrawal with mecamylamine only results in reduced cocaine craving but no differences in self-reported abstinence rates (Reid et al. 1999; Reid et al. 2005a). In addition, some antidepressants have demonstrated efficacy in the treatment of nicotine dependence. However, antidepressants, such as bupropion, have also been used to treat cocaine with only some success in combination with psychosocial treatments or for patients with comorbid psychiatric disorders (Levin et al. 2002). GABA modulators, such as baclofen or gabapentin, have been used to treat the withdrawal symptoms of alcohol cessation and alcohol craving in alcoholism (Edens *et al*, 2010; Furieri and Nakamura-Palacios, 2007). In contrast, the action

of these treatments in cocaine addiction was limited and gabapentin was even paired to serious side-effects including visual field deficits (Angehagen et al. 2003). Even though these GABA modulators worked for alcoholism treatment, they failed to decrease the subjective effects of cocaine and only slightly decreased cocaine taking (Haney et al. 2006; Rotheram-Fuller et al. 2007; Bisaga et al. 2006; Gonzalez et al. 2007; Hart et al. 2007; Hart et al. 2004). Disulfiram is a potent ALDH inhibitor broadly commercialized for the treatment of alcoholism (Fuller *et al*, 1986). Moreover, it has also shown promising results on reducing cocaine craving and consumption (Gaval-Cruz and Weinshenker, 2009). Apart from the recent disagreement in this later affirmation (Caroll et al., 2016), this drug has associated side-effects including cardiac arrest, increased blood pressure and liver toxicity (Jerónimo *et al*, 2009). Cocaine's cardiovascular effects have been widely described including hypertension, arrhythmia and heart failure that strikingly aggravate when cocaine is consumed with alcohol (Havakuk *et al*, 2017; Herbst *et al*, 2011; Riezzo *et al*, 2012). The elevated comorbidity of cocaine and alcohol abuse (85%) and the associated cardiovascular risk of consumers and disulfiram treatment incapacitate the treatment of cocaine abuse with disulfiram (Filip *et al*, 2005). However, due to the beneficial effects of disulfiram in treating cocaine addictive properties, other compounds with ALDH inhibitory action have been considered as potential candidates to treat cocaine addiction (Keung *et al*, 1997; Yao *et al*, 2010). CVT-10216 is a synthetic inhibitor of ALDH-2 that was synthesized based on the X-ray co-crystal structure of the natural isoflavone daidzin binding the ALDH receptor. Moreover, CVT-10216 has been described to effectively modulate cocaine reinforcing effects and DA release in the NAc (Chen *et al*, 2014; Yao *et al*, 2010). Based on these affirmations, we

considered daidzin, CVT-10216's natural analogue, and two isoflavan derivates, daidzein (the hydrolysed derivate of daidzin) and genistein (a hydroxide isoflavan derivate and the most present isoflavone in soybeans), as ALDH inhibitors and possible natural therapies with to treat the rewarding and relapsing effects of cocaine in rodents (Yao *et al*, 2010). These compounds are naturally found in soybeans and Kudzu root, so they can be regarded as safe profiled compounds, broad nature available (and associated low cost) and, therefore, potentially of easy incorporation to the market. So, proving the efficacy of natural isoflavones in decreasing cocaine addictive properties have great interest for the safety, easy and cheap development of treatments for cocaine addiction.

Cocaine possesses high affinity for the transporters of DA, serotonin (5-HT) and noradrenaline and blocks the reuptake of the above-mentioned monoamines (Filip *et al*, 2005). In addition, estrogens have been described to enhance DA release in the *dorsoventral striatum* and interfere in DA-mediated behaviours (Becker, 1999). Although the three natural isoflavones share similar chemical structures, each isoflavone was chosen for the study due to their different affinities for distinct types of receptors and their ALDH inhibitory properties (Keung *et al*, 1997; Yao *et al*, 2010). Daidzein has been described to bind to dopaminergic receptors; daidzin, to serotonergic and dopaminergic; and genistein, to ERs (Bare *et al*, 1995; Keung and Vallee, 1998; Schottenfeld *et al*, 2014; Zaheer and Humayoun Akhtar, 2017). Thus, the effect of daidzin, daidzein and genistein in decreasing the reinforcing effects of cocaine and cue-induced cocaine relapse was assessed in comparison with

disulfiram, the reference ALDH inhibitor used for the treatment of cocaine addiction.

Cocaine-addictive effects and isoflavones

The action of daidzin, daidzein, genistein and disulfiram decreasing cocaine reinforcing properties was tested using the cocaine self-administration paradigm in mice and the potential to attenuate cue-induced relapse assessed with the same operant self-administration protocol followed by an extinction phase and a cue-induced relapse session. Daidzein and genistein, but not the reference compound disulfiram, decreased the rewarding properties of the drug. Acute daidzin administration attenuated the rewarding effects of cocaine, although this effect was present only after the first injection and not maintained over the following days. Our results in cue-induced cocaine relapse showed that both daidzin and daidzein, but not genistein, could diminish this cocaine property effectively. In addition, the reference compound disulfiram strongly decreased cocaine seeking responses.

These results demonstrate that daidzein is the only compound that effectively modulates both the reinforcing effects of cocaine and cue-induced relapse with a better performing than the reference compound disulfiram. Although the three isoflavones share a similar backbone isoflavan structure, they have different effects in the modulation of cocaine addictive properties. These differences could be due to (1) distinct metabolization of the compounds, or (2) the compounds interfering in discrete neurobiological mechanisms.

Metabolization of the compounds: Daidzein and genistein are simpler molecules in comparison with daidzin. Daidzin chemical structure integrates a glycoside group that makes this compound more complex and less readily bioavailable in the body. Indeed, glycoside-conjugated isoflavones, such as genistin or daidzin, require a first glycosilation step that removes the glycoside conjugated group to become bioavailable. Daidzein and genistein are the compounds resulting from this metabolization of daidzin and genistin, respectively. We can hypothesize that this metabolization step required for daidzin but not daidzein must enable daidzein to be effective before urine excretion. In this line, an aglycon structure (or even a simpler structure) could have a role inducing a prolonged behavioural modification in cocaine seeking that is not shown by daidzin (Heinonen *et al*, 2003). This hypothesis could explain why daidzin is an efficient modulator of the addictive properties of cocaine in acute administrations (same as in the cue-induced relapse experiment), while daidzein can exert its modulatory actions in both acute and chronic administrations.

Possible neurobiological mechanisms of isoflavones: Several neurobiological mechanisms may be involved in the reinforcing and relapsing effects of cocaine (Dackis and O'Brien, 2001; Volkow *et al*, 2012). The mechanisms by which the reference ALDH inhibitor, disulfiram modifies the addictive properties of cocaine is not well defined. However, the most accepted hypothesis concerning the role of disulfiram in preventing cue-induced relapse is its indirect inhibition of DBH, the subsequent blockade of norepinephrine synthesis and so, the normalization of the DA tone in cocaine addicts (Gaval-Cruz and Weinshenker, 2009). This hypothesis supports that upon absorption, disulfiram is immediately

reduced to diethyldithiocarbamate (DDC) when it reacts with thiol groups (Johnston, 1953), a potent copper chelator compound (Törrönen and Marselos, 1978). DDC can thereby affect the activity of copper-dependent enzymes, such as DBH (Frigon *et al*, 1978), which is the enzyme that determines the ratio of conversion of DA to norepinephrine in noradrenergic neurons (Goldstein *et al*, 1964; Musacchio *et al*, 1966). DBH inhibition leads to decreased norepinephrine synthesis in the *locus coeruleus* and brainstem and norepinephrine release in the midbrain. Dopaminergic neurons require noradrenergic modulation for normal firing and neurotransmitter release (Ventura *et al*, 2003, 2007). Thus, DA release is decreased by the lack of action of norephinephrine and a compensatory upregulation of high-affinity state DA receptors follows, resulting in behavioural hypersensitivity to psychostimulants. Chronic cocaine users are described to be hypodopaminergic and a current treatment is normalizing the dopaminegic tone (Grabowski *et al*, 2004). Therefore, disulfiram action in DBH may promote DA agonist effects and facilitate cocaine abstinence by "normalizing" DA levels in addicts. This hypothesis related to the inhibition of DBH and blockade of norepinephrine synthesis is the most sustained mechanism by which disulfiram could modulate cocaine abstinence. However, the different nature and metabolization of natural isoflavones and disulfiram may suggest that this mechanism may not be the one reproduced by isoflavones to exert their action treating cocaine addictive properties. Actually, some studies have demonstrated that CVT-10216, daidzin's synthetic analogue, inhibits ALDH-2 and suppresses cocaine seeking by generating THP, a cocaine use-dependent inhibitor of DA synthesis that prevents DOPAC formation (Yao *et al*, 2010). However, other studies on ethanol intake suggest that daidzin and its analogs have an

antidipsotropic action by indirectly inhibiting serotonin and DA metabolism by acting on serotonin and DA metabolization products, respectively. Indeed, daidzin potently inhibits the formation of 5-hydroxyindole-3-acetic acid (5-HIAA), the major break up product of serotonin, and DOPAC, the final metabolite of DA, from their respective amines, which accumulate in the presence of daidzin (Keung and Vallee, 1998). Therefore, we can suggest that the modulation of cue-induced reinstatement by daidzin could be also mediated by interfering in serotonin metabolism.

Other isoflavones may exert its regulatory properties using different mechanisms than the above mentioned. Indeed, genistein is the major natural phytoestrogen present in soybeans and has been attributed weak estrogenic properties and ER-mediated actions (McClain *et al*, 2007). It binds ERs with 7-8 times higher affinity for ERβ than ERα (Kuiper *et al*, 1997, 1998) which defines genistein as a potent ERβ agonist. Estrogens are known to rapidly and directly act on the *dorsal striatum* and the NAc, via a G-protein–coupled external membrane receptor, to enhance DA release and DA-mediated behaviours (Becker, 1999). Estrogens can also induce a rapid change in neuronal excitability by acting on G-protein–coupled membrane receptors located in GABAergic neurons and DA terminals (Becker, 1999). The combined effect of these two actions results in enhanced stimulated DA release through modulation of terminal excitability. Moreover, nuclear ERβ has also been associated with mediating cocaine self-administration in rats (Larson and Carroll, 2007). Therefore, an enhanced release of DA promoted by genistein may be the explanation for the decrease in the number of cocaine infusions needed to obtain the same DA tone in the reward system. ERβ is

majoritary expressed in neural and immune cells and other studies has described that selective activation of this receptor increases cocaine-induced reinstatement responding. This suggests that ERβ agonists, such as genistein, can influence the propensity for reinstatement of extinguished cocaine-seeking behaviour (Larson and Carroll, 2007). All these observations point to ERβ agonist effect of genistein as a possible mechanism to mediate cue-induced relapse of cocaine. Altogether, the potential mechanism of action of disulfiram and the natural isoflavones daidzin and genistein afore suggested could involve dopaminergic, noradrenergic, estrogenic and serotonergic actions.

Daidzein was the compound with better results decreasing the addictive properties of cocaine. In order to discern the specific mechanism by which daidzein exerts its regulatory actions decreasing cocaine reinforcement and relapse, we performed a locomotion study where we injected a hypolocomotor dose of daidzein (200 mg/kg) in combination with ER, D1, D2/3R and adrenergic receptor antagonists and agonists. The dose of 200 mg/kg was previously found to decrease locomotion in our animals. The D2/3R antagonist quinpirole and the ER antagonist tamoxifen significantly affected daidzein-induced hypolocomotion suggesting the involvement of two different mechanisms in the effects of daidzein. When D2/D3 and ERs antagonists were tested in the cocaine self-administration paradigm, we observed that the specific action of daidzein in the rewarding properties of cocaine and cue-induced relapse was mediated only by dopaminergic D2/3R activities. Only the D2/3R antagonist quinpirole, but not the ER antagonist tamoxifen, effectively blocked the effects of daidzein in both cocaine self-administration and cue-induced relapse tests. Several observations may explain this

phenomenon. Firstly, daidzein has been given 17β-estradiol properties at a lower scale (Zhu *et al*, 2006) and 17β-estradiol binds equally to membrane and nuclear ERs, which can be ERα or ERβ (Kuiper *et al*, 1997). As previously mentioned, membrane ERs can modulate GABAergic synaptic plasticity and DA release in the *dorsoventral striatum* altering the increased DA tone induced by cocaine (Becker, 1999). In addition, some authors have also described the involvement of the nuclear receptor ERβ, (but not ERα) mediating estrogen's effect of cocaine self-administration (Larson and Carroll, 2007), and the role of ERα (but not ERβ) in mediating locomotor activity in mice. These observations indicate that both processes may be regulated in an independent manner by ERα and ERβ (Ogawa *et al*, 2003). The low 17β-estradiol properties attributed to daidzein together with these facts and the different dose of daidzein used in our locomotion and cocaine experiments may explain our contrasted observations. Daidzein could slightly bind ERs when administered at a high dose (200 mg/kg), such as the one used in the locomotor to produce hyperlocomotor effects. However, low doses of daidzein, such as the one used in our self-administration paradigm (100 mg/kg) would not sufficiently interact with ERs to affect cocaine reinforcing effects. Thus, we can suggest that daidzein at the dose of 100 mg/kg may exert its action blunting cocaine reinforcing effects and cue-induced relapse by a major mechanism involving D2/D3Rs activity.

The use of daidzein for the treatment of cocaine addiction in clinical studies must be taken carefully due to its metabolization in humans. Daidzein, when ingested, is metabolized in the human gut to several diverse compounds: equol, O-desmethylangolensin (O-dma), dihydrodaidzein and cis-4-OH-equol (Heinonen *et al*, 2003). Among

daidzein metabolites, equol and O-dma are considered the end products of the metabolism of daidzein. Equol has some structural features similar to the endogenous hormone estradiol, conferring daidzein its estrogenic properties (Axelson *et al*, 1982). However, equol can only be synthesized by the microbiota of one third of the population (Axelson *et al*, 1982; Rowland *et al*, 2000). Due to the uneven metabolization in the human population and the intraperitoneal route used in our self-administration studies, a complete translationality for this study must further include equol experimentation for the treatment of cocaine addiction or a different administration route in humans with the entire molecule. Further in-depth studies concerning equol modulation of cocaine addictive effects will be required to further clarify the potential interest of this natural compound for the use in clinical studies.

Altogether, our results compare the effectiveness of three natural isoflavones with disulfiram, the commercialized ALDH inhibitor to treat alcoholism and a promising compound in cocaine abuse therapies with cardiopathic side-effects (Pani *et al*, 2010b). Our data striking and robustly demonstrate that natural isoflavones, and specially daidzein, are far more efficient in modulating the additive properties of cocaine in a mouse model of cocaine self-administration (see Figure 17). In contrast with the first studies showing the potential of disulfiram in treating cocaine abuse (Pani *et al*, 2010b; Suh *et al*, 2006b), some others have also reported discrepancies using food and cocaine operant self-administration approaches (Schroeder *et al*, 2010) but also in clinical studies with human addicts (Carroll *et al*, 2004b, 2016b). Therefore, new therapies such as daidzein, a natural compound that significantly decreases both the rewarding properties and cue-induced relapse of

cocaine in the self-administration paradigm in mice, are of striking clinical relevance. Further studies with its ingested active metabolite, equol, could be of clinical interest to ensure a controlled metabolization of the isoflavone independently of the microbiota of the subject.

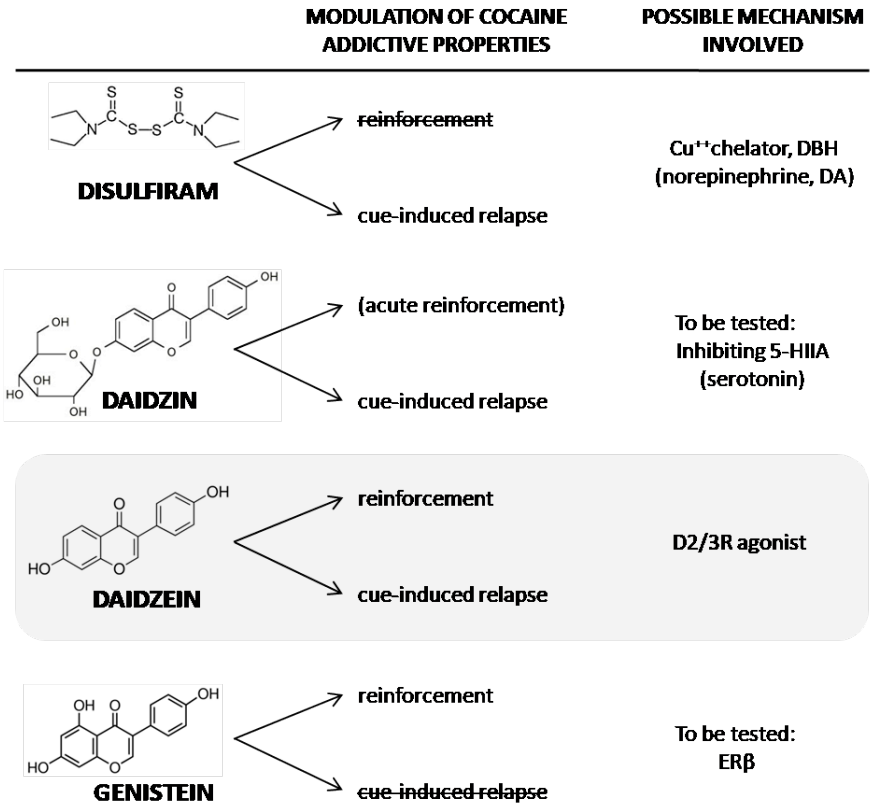

Figure 17. Summary of the actions of natural isoflavones in the addictive properties of cocaine compared with the ALDH inhibitor disulfiram. Cu^{++}: copper; **DBH:** dopamine-β-hydroxylase; **5-HHIA:** 5-hydroxyindoleacetic acid; **D2/3R:** dopamine 2 or 3 receptor; **ERβ:** estrogen receptor β. Crossed font represents no significative effect.

B. Food palatability and eating disorders

Feeding is a vital necessity mainly orchestrated by the *hypothalamus* and the reward mesocorticolimbic system. While the *hypothalamus* receives peripheral body inputs considering energy stores and nutrient disposition, the reward system confers hedonic values to foods and motivation to initiate feeding episodes (Berthoud, 2006, 2007). In some individuals, the hedonic component of foods may overcome energy necessities leading to overeating that can contribute to the development of overweight and obesity (Corwin and Grigson, 2009; Hebebrand *et al*, 2014). Actually, growing evidence indicates that some eating disorders could be considered as a form of "eating addiction". Indeed, persons with high scores in the YFAS 2.0 have a great probability to be diagnosed with other co-ocurring eating disorders described in the DSM-5 and obesity. Noteworthy, studies using the YFAS scale have found prevalence rates of food addiction ranging 5-10% of normoweight individuals and 15-25% in obese subjects (Gearhardt *et al*, 2013; Meule *et al*, 2014) and 56,8% of comorbidity with food addiction in people diagnosed with BED (Gearhardt *et al*, 2009).

Foods rich in carbohydrates and fat, like chocolate, are highly palatable can potentiate addictive-like behaviours towards eating. Indeed, palatable foods trigger opioid and EC expression in the reward system and an enhanced DA tone evoking a pleasant palatability-related feeling (Davis *et al*, 2009; Robinson *et al*, 2015). Prolonged excessive exposure to palatable foods can enhance the incentive value attributed to these foods and modify the pattern of food intake in a compulsive manner (Volkow *et al*, 2008a). Actually, this compulsive intake presents striking similarities with the behaviours observed in addictive disorders and has

associated underlying impairments in the normal functionality of the mesocorticolimbic system similar to the changes observed in drug addicts. This deleterious functionality of the reward system can lead to a pathological motivational state that could end up in a crystallized disruption of eating behaviour (Avena *et al*, 2009; Johnson and Kenny, 2010). Indeed, some forms of overweight and obesity can be seen as a chronic state with repetitive compulsive hedonic eating involving similar behavioural and neurobiological adaptations with drug addiction (Volkow *et al*, 2013b). Our studies shed some light into these maladaptations by assessing the neurobiological modifications in the mesocorticolimbic reward system and behavioural alterations driven by chronic exposure to palatable food.

Modelling hedonic eating

In order to evaluate the neurobiological mechanisms governing the behavioural impairments produced by palatable foods in humans, we evaluated hedonic eating using two complementary studies. First, we tried to mimic the human obesogenic scenario exposing our animals to regular and highly palatable and caloric food (namely cafeteria diet, CD) in a free-choice manner (Article 2). Then, we model food addictive-like behaviour by chronically training mice to self-administer sweet palatable cocoa-flavoured pellets. This long chronic training was able to induce compulsive behaviour towards palatable food in our animals (Article 3).

In our first study (Article 2) we consider the free will to choose between palatable and normal food as basis. In developed countries, food is readily accessible at any moment. Food can be easily and broadly bought on chose between healthy and hypercaloric choices that share room

within supermarkets. Moreover, not all foods has the same palatability for mice. Chocolate, peanut butter, cookies, saccharine, sucrose or fructose are palatable foods that are preferred for mice among other plain flavours such as their standard food (Ackroff *et al*, 2007). The CD used in this first assay was a combination of chocolate bars broadly craved by humans that has also been demonstrated to be highly palatable and trigger compulsive eating in rodents (Heyne *et al*, 2009). CD had a prominent odour and soft texture and was periodically renewed to ensure these organoleptic characteristics were maintained. Moreover, CD was presented to mice a week in advance of the beginning of the data acquisition in order to avoid neophobia. CD and standard food choice was presented in the comfort of the mouse home cage. This way, the free will in food choices seen in the human environment was translated into the animal microenvironment accommodated in its own cage with no potential stress associated (Czyzyk *et al*, 2010). Moreover, we evaluated whether the pattern of CD intake was involved in triggering behavioural and neurobiological changes. Therefore, CD was offered to a second group of mice in a restrictive intermittent schedule of 24 h every 7 days inducing BED. Patterns of intermittent access to palatable foods have been described to lead to emotional and somatic signs of withdrawal when the food is no longer available (Mattes *et al*, 2005; Parylak *et al*, 2011) and episodes of huge consumption of food in discrete periods of time may follow the deprivation. BED represents this definition (American Psychiatric Association, 2013). Although BED is more frequently in overweight and obese subjects, it can also occur in normal weight individuals in a similar trend as food addiction (Hebebrand *et al*, 2014). Comparing these two exposures to CD will enable to

understand the intrinsic neurobiological changes driven by overeating highly palatable and highly caloric CD to the onset of obesity.

Overeating due to transitions from a non pathological food intake to a more compulsive pattern are behaviours described in overweight and obese patients with high YFAS 2.0 scores (Gearhardt *et al*, 2013; Meule *et al*, 2014). In order to better understand the neurobiological mechanisms by which palatable foods can become craved in an addictive manner, a reliable animal model for the study of eating addiction-like behaviour found in some obese subjects was required (Corwin and Grigson, 2009; Hebebrand *et al*, 2014). Some animal models have already been used to represent some eating behaviours such as diet-induced obesity and specially BED (Ackroff *et al*, 2007). However, some individuals present addictive like behaviours towards food that have not been model yet. Indeed, our model considers the numerous underpinnings that long chronic palatable food intake shares with the transition from sporadic drug consumption to drug addiction to model food addictive-like behaviours. In a similar manner, an overwhelming number of models can be found to study specific behaviours related to substance abuse. Drug abuse models using a self-administration training can evaluate drug-induced behaviours such as drug-seeking, motivation, tolerance, withdrawal, escalation in drug intake or relapse (Georgiou *et al*, 2015; Martín-García *et al*, 2009; Soria *et al*, 2005). However, these models do not comprise the sequential dimensionality of the transition from drug use to addiction. In this line, some authors were able to reveal evidence of the full long process of addiction in rats by exposing them to a chronic training of operant cocaine self-administration (Deroche-Gamonet *et al*, 2004). This addiction model raised from merging and adapting DSM-IV criteria to diagnose addiction to three feasible rat behavioural tests using

the self-administration paradigm (Deroche-Gamonet *et al*, 2004). Self-administration procedures are able to perfectly quantify free willing individual responses and enable to assess different progressive behavioural changes related to the development of an addictive-like behaviour in a quantitative manner. Our food addictive-like behaviour model uses the cocaine addiction model validated in rat as starting point and adapts the DSM-5 and YFAS criteria to evaluate the progression from palatable food consumption to addictive-like behaviour towards palatable food seeking and eating (Deroche-Gamonet *et al*, 2004). In this model, mice were exposed to long daily operant conditioning sessions maintained by isocaloric highly palatable sweet cocoa-flavoured pellets or standard food pellets. The pressing of the active lever was coupled with the dispensing of a food pellet and a cue light. The use of an additional inactive lever was required in order to reveal that the animals are actively seeking for a pellet. In addition, pellet delivery-associated cues were added to the model as cues are crucial to induce both drug and food seeking also in humans (Berthoud, 2007; Oginsky *et al*, 2016). A first 5-day period of fixed ratio (FR) 1, where one active lever-pressing was coupled with the delivery of the pellet and the associated cue, was required to facilitate the learning of the task for the animals. The subsequent more challenging FR5 period, where five active lever-presses are required to obtain a pellet, represents the active seeking will of the animal to obtain the food. As addiction is a long chronic process, a long-lasting daily training under the FR5 self-administration paradigm was required in order to reveal the development of food-induced addiction-like phenotype (Piazza and Deroche-Gamonet, 2013). Moreover, we assessed the progression of these behavioural adaptations by exposing the animals to three special tests at two different time points. The three

tests correspond to three addiction criteria and were performed at an early stage (at day 5 of FR5 training) and at a later stage (day 105 of FR5) during the FR5 training period. The early stage would represent an occasional controlled eating where animals enjoy the hedonic effects of eating palatable (or standard) pellets. The late stage would assess whether a long chronic exposure to these foods had provoked a behavioural disruption as seen in overweight and obese subjects (Volkow *et al*, 2013b). At these time points, the food self-administration paradigm enables us to evaluate three criteria that summarize addictive behaviour (see Table 1 Chapter 1.1 in the Introduction) (American Psychiatric Association, 2013; Gearhardt *et al*, 2016): (1) persistence of food-seeking during a period of non-availability of food, (2) motivation for food, and (3) perseverance of food-seeking even aversive consequences. Persistence of food seeking (criteria 1) was evaluated in a 10 min pellet-free period in the middle of the 1 h daily session. The house light was on during this 10 min period indicating the unavailability of pellets. Motivation to food seeking (criteria 2) was assessed using a progressive ratio schedule. In this test, the number of active lever-presses to obtain a pellet sequentially increased and when reached (breaking point) the count reinitiates in 0. Therefore, the higher the breaking point, the more motivation exerted an animal to obtain a single pellet. Finally, an electric shock was coupled with the pellet delivery in order to check the perseverance in food seeking associated to aversive consequences (criteria 3). Making a comparison with human population, some subjects with addictive-like behaviour towards food are found to manifest high motivation (e.g. taking long distances to get a certain snack when all regular shops are closed) and impulsivity (e.g. hitting the vending machine if the chocolate bar does not fall) and continued eating in the

event of paired bad consequences (e.g. eating junk hypercaloric food being on cardiovascular risk due to morbid obesity) (Gearhardt *et al*, 2016). For each test, animals were ranked according to their lever-presses and those performing on or over the 75% greater responses were consider to have achieved the criteria for that test. Animals scoring two o three criteria were consider the 'Addict' or addiction-vulnerable group while those with no criteria achieved represented the `No addict' or addiction-resistant.

Chronic exposure to palatable food

In the experimental conditions of Article 3, only 8 days of training lever-pressing for cocoa flavoured pellets were required to learn the task by 100% of the mice, in contrast with the only 66% of the animals trained to lever-press for standard food pellets that reached the acquisition criteria by a more prolonged period of time (20 sessions). The training sessions required to learn the self-administration task for an animal with no cognitive or locomotion impairments may give an idea of how rewarding a stimulus can be. In the same line, a striking difference of almost 2-folds was found in the amount of pellets received in one daily session by animals pressing for standard versus cocoa-flavoured pellets from the beginning of the more challenging FR5 phase. The easier acquisition of the task and the greater number of active lever-pressings in comparison with the standard pellets-trained group indicate that cocoa-flavoured pellets were highly palatable and rewarding for mice. Importantly, our experimental conditions did not consider deprivation of food or fasting conditions in order to facilitate the training process and sweet cocoa-flavoured pellets were isocaloric than standard chow ones. Thus, the

effects seen on responding were exclusively driven by cocoa palatability but neither animal nutritional status nor pellet fat content were implied. Indeed, no significant changes in body weight were observed between animals lever-pressing for either palatable or standard pellets.

In the case of the animals chronically fed with highly caloric CD (Article 2), the prevalence of hedonic mechanisms over caloric necessities was the main cause leading to the development of overweight (Murray *et al*, 2014; Volkow *et al*, 2008a). Those animals chronically exposed to highly caloric CD overeat and significantly increase their weight by only 4 weeks of exposure to CD. In this study, the group of animals that had CD available only 24h every 7 days presented CD-induced BED the first hours of CD presence. However, they did not present alterations in body weight when compared with control mice. These data suggest that only a maintained diet of energy-dense food may interfere in weight regulation. Indeed, studies in rats with a similar intermittent sweet-fat food exposure, that also produced BED, demonstrated that after the bingeing episode, when the binging food was removed, rats reduced standard food consumption and lose some weight until the next sweet-fat food exposure (Berner *et al*, 2008). This compensatory behaviour could explain the slight loss of weight of our binging animals (although not statistically relevant), exposed only once a week to CD, during the experiment and is in agreement with the observations indicating that BED does not need to be paired with overweight or obesity (Hebebrand *et al*, 2014). This phenomenon indicates that palatable food availability is also a key factor to gain weight in a similar manner than human obesogenic environments are, where highly palatable and hypercaloric food can be broadly found and trigger overeating (Giskes *et al*, 2011).

The compendium of the results obtained with these two different approaches demonstrates the predominant reinforcing effect of food palatability and that this palatability can trigger enhanced food-seeking and overconsumption of palatable food even in satiation states.

Reward system maladaptations

Indeed, only animals lever-pressing for palatable pellets (Article 3) were found to score in two or more addiction criteria at the late period of training, while none of the animals trained to lever-press for standard food pellets reach any criteria. Some authors have also found escalation in other palatable foods consumption and seeking to be related with high impulsivity and hypersensitivity to palatable food-associated cues that may be in agreement with our observations seen comparing the early and late responses in the three tests (Diergaarde *et al*, 2009). Moreover, this comparing leads us to affirm that compulsive behaviour requires of chronic exposure to palatable pellets to develop. Indeed, a greater number of animals reaching addictive-like criteria were observed in the latest tests (7.4% got two criteria; 14.8%, three criteria) in comparison with the early period (14.8% got two criteria, 3.7%, three criteria) suggesting that neurobiological adaptations that resemble those driven long-chronic exposure to drugs of abuse may have happened at the level of the reward system so that hedonic mechanisms and loss of control over food intake governed behaviour (Piazza and Deroche-Gamonet, 2013). Behavioural disruption and loss of control that defines addiction is experienced by 10-30% of initial occasional alcohol, tobacco and psychostimulants consumers in general terms (Anthony *et al*, 1994). We observed that 22.2% of mice developed an addictive-like phenotype in

the late phase in agreement with this numbers and those accounted in the cocaine addiction model in rats from which our model is inspired (Deroche-Gamonet *et al*, 2004). Food addiction resistant individuals are found in the human population and are also represented in this model. Some animals consumed high amounts of cocoa-flavoured pellets in the daily self-administration basis but did not reach high numbers in the addictive criteria tests. In parallel, some human subjects consume palatable food (or drugs) in a regular basis but did not develop addiction. This means that these subjects, represented by our 'No addict' animals, enjoy hedonic eating in a controlled manner which may be in expenses of addictive neurobiological modifications responsible of compulsive behaviours (Reboussin and Anthony, 2006; Tossmann *et al*, 2001). These variability among subjects can prevent and lead some vulnerable individuals to develop compulsive behaviours towards palatable foods, same as for drugs (Everitt *et al*, 2008; Piazza and Deroche-Gamonet, 2013). Interindividual variations were also observed in these two models in terms of preferences and genetic component. Although animals exposed to CD preferred this food rather than their standard food, individual food preference was found constant but preferences varied among individuals (Article 2). In the same line, few animals trained to lever-press for cocoa flavoured pellets had to be excluded from the study as cocoa pellets were not reinforcing enough for them to acquire the self-administration behaviour (Article 3). Palatability is a matter of subjective taste, preferences and incentive value attribution to food and, in a similar manner as not all foods are similarly palatable for all humans, these models were also conditioned to this fact (Garzaro *et al*, 2010).

Interindividual genetic variations can be also involved in facilitating addictive behaviours. Indeed, chronic exposure to drugs of abuse has been described to produce epigenetic modifications favouring addiction which, in turn, can become inherited contributing to the individual vulnerability to develop addictive behaviours (Schroeder *et al*, 2008). Some authors have described the involvement of the ECS, and specially the CB1R, mediating the reinforcing properties of drugs and natural rewards (D'Addario et al, 2014). Therefore, we used the two distinct populations obtained with the food addiction-like behaviour model to assess if epigenetic modifications in the Cnr1 gene promoter, the gene codifying the CB1R, were present in Addict mice. This way, we compared the methylation of the Cnr1 promoter of those animals lever-pressing for cocoa-flavoured pellets that had achieved two or three criteria in the late phase tests (a 22% of the total) with the methylation of the Cnr1 promoter in those animals that did not reach any criteria ('No addict) (Article 3). Long-term operant training to obtain this highly palatable food produced adaptative changes at the epigenetic level in the reward system of 'Addict' animals when compared to those categorized as food addiction resistant. Our results showed only decreased *Cnr1* gene promoter methylation in the PFC of animals showing food addictive-like behaviour coupled with a significant increase in the protein levels of the gene product, CB1R, in the same area. As previously mentioned, the maladaptive changes in the glutamatergic corticostriatal connexion are tightly linked with the final steps of addiction where compulsion towards drug-seeking and taken govern behaviour (Kelley *et al*, 2003; Koob and Volkow, 2010). Indeed, the PFC is the brain area regulating decision-making and emotions and has also been related with enhanced impulsivity and compulsive food intake disorders (Tomasi and Volkow,

2013). In addition, CB1R has a crucial role in the reinforcing and motivational properties of highly palatable foods (Maccioni et al, 2008). It can decrease glutamatergic excitatory and GABAergic inhibitory synaptic inputs in several brain regions, thus, acting as a regulatory mechanism to modulate synaptic transmission (D'Addario et al, 2014). To proof this hypothesis, we carried out a second set of experiments using the food addictive-like behaviour model and *knocked-out* animals with total deletion of CB1R and WT animals with pharmacological blockade of CB1R by rimonabant intraperitoneal injection. The exposure of these animals to the long daily self-administration training with cocoa-flavoured pellets revealed that CB1R expression was tightly involved in the reinforcing properties of palatable food and that its pharmacological blockade or deletion can prevent the development of loss of control towards palatable food. We can suggest that CB1R epigenetic regulation in PFC and the subsequent translation in increased CB1R protein level could modulate (1) the primary glutamatergic neuronal output of this region to the VTA and NAc, as described by other studies (Steketee, 2003) or (2) PFC GABAergic interneurons that modulate the previous glutamatergic outputs (see Figure 18). Indeed, PFC CB1R has a more powerful effect in glutamatergic cells than in GABAergic ones, even though is less abundant in the first population (Steindel *et al*, 2013). We can suggest that a decreased methylation in the *Cnr1* gene promoter in the PFC may trigger food intake indirectly due to an upregulation of CB1R in the PFC, which in turn will inhibit glutamatergic PFC neurons. This action could decrease PFC glutamate release onto the VTA producing less VTA excitation. Finally, it will result in less GABAergic inhibition of VTA ending up in enhanced VTA DA release in the brain reward circuit (Lupica *et al*, 2004; Melis *et al*, 2004). Also, inhibition of PFC glutamatergic cells

will end up in less glutamate delivery onto the NAc. Furthermore, with less glutamatergic excitation of this area, less GABAergic inputs to the VTA will be activated. Therefore, less inhibition of VTA DA release will result. Even the feasibility of this hypothesis, little is known about the mechanism of CB1R in glutamatergic neurons in addiction.

On the other hand, we can hypothesize that CB1R overexpression can occur in PFC GABAergic interneurons, instead. Cortical GABAergic interneurons in turn would produce less GABA release followed by an increase in the activation of the PFC principal glutamatergic neurons. This increased excitatory transmission towards the VTA could indirectly stimulate the dopaminergic VTA neurons firing rate with the consequent release of DA in the NAc, as reported by cocaine studies (Almodóvar-Fabregas *et al*, 2002; Geisler and Wise, 2008). In parallel, glutamatergic projections from the PFC stimulate the GABAergic neurons in the NAc to compensate the excessive DA release. So, as the combination of both actions will define the final behaviour (Maldonado and Berrendero, 2010), we can suggest that the first hypothesis is more feasible to represent our observations in long-chronic seeking of palatable food. Further studies with antagonists injected directly in the PFC in glutamatergic and GABAergic CB1R mutants will be required to finally ascertain whether the mechanism by which CB1R modulates the reinforcing and addictive properties of palatable food are due to direct effects in the glutamatergic and GABAergic populations and specifics of the PFC region. Therefore, the implication of CB1R activity in food palatability is a fact. Concerning our results, CB1R seems to play a role in the motivation, impulsivity and craving triggered by chronic exposure to palatable food in the self-administration paradigm.

In this line, some authors have described the requirement of CB1R to induce neuroplastic changes in neurons of the NAc shell and PFC after exposure to a shorter (41 days) operant training to obtain highly palatable isocaloric food that were associated to changes in food-seeking behaviour (Guegan *et al*, 2013). Noteworthy, structural plasticity modifications in the reward system induced by palatable food have only been studied by few authors. Research on neuronal plasticity has mainly focused in the involvement of areas of the brain close related with homeostatic control of feeding, such as the *hypothalamus.* There, ghrelin and leptin signals were described to trigger increased spine densities in glutamatergic neurons resulting in a greater activation of AgRP neurons in fasting conditions (Nuzzaci *et al*, 2015). Indeed, a lesson learned from drug addiction indicates that the hedonic component of drugs can trigger compulsive seeking behaviours by inducing neuronal maladaptations in

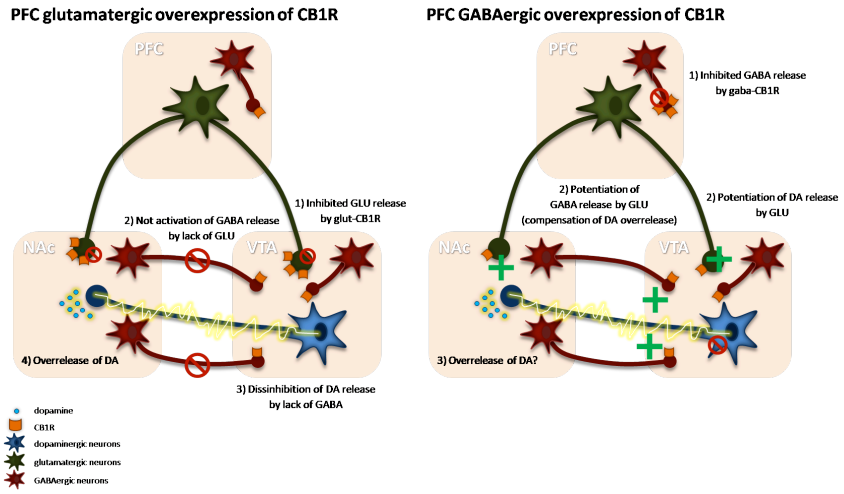

Figure 17. Schematic representation of the two potential mechanisms by which CB1R overexpression in glutamatergic (left) or GABAergic (right) terminals in the PFC could mediate eating addictive-like behaviour. CB1R: cannabinoid receptor 1; **DA:** dopamine; **glut:** glutamatergic terminal; **GLU:** glutamate; **gaba:** GABAergic terminal; **GABA:** gamma-aminobutyric acid;

the reward system, especially in the corticostrial connections (Smith *et al*, 2016). Therefore, we studied the structural plasticity modifications in the PFC, *ventral* (NAc core and NAc shell) and *dorsal striatum* of mice chronically exposed to CD (Article 2). Our approach considers a more translational exposure to palatable food than those studies exposing animals to self-administration schedules (Guegan *et al*, 2013). Indeed, the novelty of our study resides on the obesogenic environment that we recreate by the free-choice manner that CD is presented in animals' home cage and the caloric compound of CD. In accordance with the results obtained by the former study (Guegan *et al*, 2013), our observations indicate an important alteration in total structural plasticity in the NAc shell but also specific alterations in mature spine densities in both the NAc shell and core. However, we declare no structural plasticity modification either in the PFC or in the *dorsal striatum* of hedonic eating-induced overweight animals chronically exposed to CD diet that could be due to the presence of overweight in our animals in contrast with those fed with isocaloric palatable pellets (Guegan *et al*, 2013). In agreement with our observations, some studies have indicated the enhanced reactivity of the VTA and NAc over the *dorsal striatum* and PFC concerning the reward system alterations produced by other reinforcing stimuli such as drugs of abuse (Koob and Volkow, 2010). Furthermore, the NAc is the crucial brain area involved in motivation and its two anatomical and functional differentiated territories (NAc core and the NAc shell) slightly differ in their functionality (Chaudhri *et al*, 2010). Interestingly, our results are in accordance with previous studies reporting a more prominent role of the NAc shell in food consumption than the core. Thus, studies with high-frequency electrostimulation indicate that only NAc shell but not core stimulation resulted in

decreased high-fat or standard food consumption, suggesting a more important role of the shell in feeding behaviour, in agreement with our observations (Halpern *et al*, 2013; Zhang *et al*, 2015). Medium spiny neurons projecting from the NAc shell are known to provide direct inhibition to the LH GABAergic neurons providing a rapid control over feeding (O'Connor *et al*, 2015). Furthermore, the NAc shell congregates high densities of opioid receptors that mediate food gustatory hedonic impact, together with great concentration of CB1Rs that are known to mediate the pleasurable feeling towards palatable food (Guegan *et al*, 2013). Stimulation of these opioid populations enhance hedonic reactions towards food conferring higher hedonic feeding modulator properties to the NAc shell in comparison with the NAc core (Castro and Berridge, 2014).

In order to evaluate whether these alterations in structural plasticity in the NAc observed in our mice with a free-choice diet could have lead to possible functional modifications in the reward system, we exposed our animals to amphetamine-induced hyperlocomotor effects. Noteworthy, only animals fed with *ad libitum* free-choice of CD but not those with restricted access or not access to this diet exert an anomalous decreased locomotor response after the first injection of amphetamine. This observation may indicate a modification in the normal functionality of the mesolimbic system as a consequence of the alterations in neuronal plasticity triggered by long-term exposure to CD. In agreement, previous studies also showed aberrant functionality of the brain reward system in overweight or obese rodents, revealed by the development of abnormal conditioned place preference or behavioral sensitization after psychostimulant injection (Morales *et al*, 2012; Robinson *et al*, 2015).

Therefore, chronic long-term CD exposure was able to induce changes in structural plasticity in the NAc that were linked to an anomalous function of reward processing. As previously described in the Chapter 6 of the Introduction, microglia activities and neuroinflammation are tightly involved in the natural modifying of structural plasticity and also are an important player in the deleterious neuronal rearrangements part of the maladaptations induced in reward processing that anchor disrupted behaviours such as those seen on drug addicts (Graeber, 2010). Even though some studies have described obesity-derived detrimental inflammatory activities present in brain areas such as the *hippocampus* (Bocarsly *et al*, 2015; Davidson *et al*, 2007; Erion *et al*, 2014) and the *hypothalamus* (Valdearcos *et al*, 2014), the role of neuroinflammation in the reward system during the onset of obesity is also still unknown. Therefore, we hypothesized that neuroinflammation and especially microglia activity could mediate the neuronal plasticity adaptations triggered by overeating previously described in the NAc. In order to ascertain this hypothesis, we studied microglia reactivity and neuroinflammatory markers in the NAc in overweight animals exposed to a free-choice between CD and standard food. After 7 weeks of *ad libitum* exposure to CD, overweight mice presented morphologically reactive microglia in the NAc shell and NAc core but not in the PFC or the *dorsal striatum*. Interestingly, no modifications were observed neither in those mice with restricted access to CD nor in those only eating standard food. The presence of increased neuroinflammatory markers in the NAc, but not in the PFC or the *dorsal striatum,* went in agreement with our results in microglia reactivity. Two pro-inflammatory factors, IL1β and IFNγ, were found to be upregulated in the NAc only in animals chronically exposed to CD. IL1β is a mid-early inflammation expressed pro-

inflammatory cytokine (Wang *et al*, 1997). IFNγ is a T-cell regulator in the event of neuroinflammation known to activate microglial cells (Suzumura *et al*, 1987). Both IL1β and IFNγ have been described to have a negative impact on synaptic plasticity contributing to inhibit LTP (Maher *et al*, 2006).

Apart from CNS neuroinflammation, low-grade peripheral inflammation has also been widely described in obesity (Esser *et al*, 2014; Monteiro *et al*, 2010; Schmidt *et al*, 2015). Surprisingly, several authors have highlighted the importance of food composition and microbiota in the low-grade inflammation present in obesity. In this line, saturated fatty acids consumption (such as those present in chocolate bars) has been linked to a pro-inflammatory obesity-related gene expression profile, while consumption of monounsaturated fatty acid-rich diet caused the opposite (van Dijk *et al*, 2009). Total fat intake is also known to be linked with subclinical inflammatory responses in obese patients (Aeberli *et al*, 2008). Fat deposits are integrated by adipocytes among others. Adipocytes behave like inmune cells and are capable of synthesize and release pro-inflammatory cytokines, such as IL1 and, TNFα. In addition, macrophages are also found in the adipose tissue and have been described to reinforce the inflammatory reaction of adipocytes (Schäffler *et al*, 2006, 2007). Food composition has been also shown to affect gut microbiota. Some recent studies have demonstrated that high-fat feeding increases gut permeability and provokes metabolic endotoxemia mediated by gut microbiota (Monteiro *et al*, 2010). Therefore, we can suggest that foods rich in fat, such as CD, may exert direct pro-inflammatory effects, long-term low-grade inflammatory effects related to the overload of the adipose tissue, and indirectly mediate

endotoxemia and inflammation via gut microbiota. These findings concerning the inflammation in peripheral tissues driven by high-fat food would be in line with the neuroinflammation that we describe in animals chronically fed with CD. Moreover, based on previous studies showing microglia involvement in adult neurodegeneration (Ekdahl, 2012) and our results, we can hypothesize that exposure to long chronic energy-dense CD-driven microglia activation and neuroinflammation in the NAc, could have modified synaptic remodelling. Microglia reactivity could have increased NAc shell adult dendritic spine pruning and prevented mature mushroom-shaped spines formation in the NAc shell and core. To ascertain this hypothesis, we decided to systemically inhibit microglia with a chronic daily intraperitoneal injection of minocycline. Minocycline was injected after week 4 of CD exposure, an experimental time point where *ad libitum* CD-exposed animals were already significantly overweighed. Microglia morphology and neuroinflammatory factors in minocycline injected mice indicated that minocycline injection had effectively decreased neuroinflammation in the NAc. Outstandingly, microglia inhibition normalized the anomalies described in neuronal plasticity densities in those animals exposed to a free-choice between CD and standard food, with the subsequent normalization in the reaction in amphetamine-induced hyperlocomotion. Moreover, microglia inhibition did not modify any of these items in those animals only fed with standard food or in those with intermittent exposure to CD. Our results demonstrated the striking involvement of microglia in regulating the physical, neurobiological and behavioural impairments driven by long access to highly palatable and energy-dense food driven overweight. Moreover, chronic minocycline treatment was paired with a significant decrease in food intake and body weight gain in mice exposed to CD

when compared with those animals exposed to standard diet. Importantly, microglia inhibition did not alter food preferences in free-choice eating mice. Instead, minocycline treatment did not modify food intake and body weight in animals with restricted exposure to CD, an effect that can be explained as a result of the absence of alterations in neuroinflammation in the reward system of these animals. Moreover, inhibition of microglia also reversed those structural plasticity modifications in the NAc and, indeed, enabled the new formation of immature thin-shaped early spines only in *ad libitum* fed CD animals.

Further studies considering the specific microglial inhibition in the NAc must be carried on following the same experimental approach in order to pull apart the possible systemic effect of minocycline in the physical, neurobiological and behavioural modifications here described. Due to our experimental approach, we cannot conclude whether microglia inhibition by minocycline affected structural plasticity and weight; or, minocycline affected weight gain and subsequently regulated structural plasticity and neuroinflammation. Despite this limitation in our study, the identification of this neuroinflammatory process in the reward system driven by microglia activity highlights the relevance of the reward circuitry, and especially the NAc, in the development of behavioural alterations coupled to overeating and overweight. Therefore, we can suggest that targeting these neuroinflammatory responses is of striking interest for the development of future obesity treatments.

Concluding remarks

Altogether, the dissertations include in this Thesis indicate that palatable food and cocaine are highly reinforcing rewards. Indeed, palatable food and cocaine seeking are governed by hedonic mechanisms that prevail over homeostatic regulation. Even more, chronic long-term availability and intake of palatable foods or drugs can trigger adaptations in the reward system that can foment the overconsumption of these substances. Particularly, these modifications occur in the cortico-ventrostriatal axis that regulates decision-making and motivation. The furhter in depth study of natural isoflavones, CB1Rs and microglia populations modulating the neurobiological, functional and/or behavioural adaptations in the brain reward system produced by cocaine and palatable food may be of potential interest to treat cocaine addiction and stop the obesity epidemic.

CONCLUSIONS

The results obtained in the present thesis allow to draw the following conclusions:

1) Natural isoflavones are able to decrease the reinforcing properties of cocaine and cue-induced relapse in a cocaine self-administration paradigm in mice in a more efficiently manner than the reference commercialized ALDH inhibitor disulfiram.

2) Daidzein is a promising natural compound to treat the addictive properties of cocaine in clinical research, able to decrease the reinforcing properties of cocaine and cue-induced relapse in a cocaine self-administration paradigm in mice via D2/3R mechanisms.

3) Long *ad libitum* free-choice exposure to highly palatable energy-dense CD provokes overeating and overweight coupled with neuroinflammation, decreased neuronal spine densities and abnormal amphetamine-induced hyperlocomotion response in mice.

4) Microglia reactivity mediates the detrimental effects in the reward system caused by long *ad libitum* free-choice exposure to highly palatable energy-dense CD.

5) The rewarding properties of palatable food permits the study of eating addictive-like behaviour in mice by identifying two extreme addictive phenotypes using the DSM-5 substance use

disorder and YFAS 2.0 food addiction criteria adapted to a long-term operant palatable food self-administration paradigm.

6) A decreased *Cnr1* gene promoter methylation in the PFC represents an important adaptive mechanism for addictive-like behaviour towards palatable food vulnerability.

7) CB1R mediates the reinforcing properties of palatable food and its inhibition prevents compulsive palatable food-seeking in a long-term operant palatable food self-administration paradigm.

REFERENCES

Abizaid A, Liu Z-W, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, *et al* (2006). Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest* **116**: 3229–39.

Ackroff K, Bonacchi K, Yeh-Min Y, Magee M, Jonathan VG, Sclafani A (2007). Obesity by choice revisited: Effects of food availability, flavor variety and nutrient composition on energy intake. *Physiol Behav* **92**: 468–478.

Aeberli I, Beljean N, Lehmann R, l'Allemand D, Spinas GA, Zimmermann MB (2008). The increase of fatty acid-binding protein aP2 in overweight and obese children: interactions with dietary fat and impact on measures of subclinical inflammation. *Int J Obes* **32**: 1513–1520.

Ahima RS, Flier JS (2000). Leptin. *Annu Rev Physiol* **62**: 413–437.

Ahmed SH, Kenny PJ, Koob GF, Markou A (2002). Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. *Nat Neurosci* **5**: 625–6.

Ahrén B (2000). Autonomic regulation of islet hormone secretion - Implications for health and disease. *Diabetologia* **43**: 393–410.

Alliot F, Godin I, Pessac B (1999). Microglia derive from progenitors, originating from the yolk sac, and which proliferate in the brain. *Brain Res Dev Brain Res* **117**: 145–52.

Almodóvar-Fabregas LJ, Segarra O, Colón N, Dones JG, Mercado M, Mejías-Aponte CA, *et al* (2002). Effects of cocaine administration on VTA cell activity in response to prefrontal cortex stimulation. *Ann N Y Acad Sci* **965**: 157–71.

Alonso-Alonso M, Woods SC, Pelchat M, Grigson PS, Stice E, Farooqi S, *et al* (2015). Food reward system: current perspectives and future research needs. *Nutr Rev* **73**: 296–307.

American Psychiatric Association (Washington, DC, USA, 1980). *Diagnostic and Statistical Manual of Mental Disorders (DSM-III)*. .

American Psychiatric Association (Washington, DC. USA, 1994). *Diagnostic and Statistical MAnual of Mental Disorders (DSM-IV)*. .

American Psychiatric Association (Washington, DC, USA, 2013). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5)*. .

Andres A, Donovan SM, Kuhlenschmidt MS (2009). Soy isoflavones and virus infections. *J Nutr Biochem* **20**: 563–569.

Anthony JC, Warner LA, Kessler RC (1994). Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: Basic findings from the National Comorbidity Survey. *Exp Clin Psychopharmacol* **2**: 244–268.

Arias-Carrión O, Pöppel E (2007). Dopamine, learning, and reward-seeking behavior. *Acta Neurobiol Exp (Wars)* **67**: 481–8.

Atwood BK, Mackie K (2010). CB2: a cannabinoid receptor with an identity crisis. *Br J Pharmacol* **160**: 467–479.

Avena NM (2010). The study of food addiction using animal models of binge eating. *Appetite* **55**: 734–7.

Avena NM, Long K a, Hoebel BG (2005). Sugar-dependent rats show enhanced responding for sugar after abstinence: evidence of a sugar deprivation effect. *Physiol Behav* **84**: 359–62.

Avena NM, Rada P, Hoebel BG (2008). Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci Biobehav Rev* **32**: 20–39.

Avena NM, Rada P, Hoebel BG (2009). Sugar and Fat Bingeing Have Notable Differences in Addictive-like Behavior. *J Nutr* **139**: 623–628.

Avery SN, Clauss JA, Blackford JU (2016). The Human BNST: Functional Role in Anxiety and Addiction. *Neuropsychopharmacology* **41**: 126–41.

Axelson M, Kirk DN, Farrant RD, Cooley G, Lawson AM, Setchell KD (1982). The identification of the weak oestrogen equol [7-hydroxy-3-(4'-hydroxyphenyl)chroman] in human urine. *Biochem J* **201**: 353–7.

Bagdade JD, Bierman EL, Porte D (1967). The Significance of Basal Insulin Levels in the Evaluation of the Insulin Response to Glucose in Diabetic and Nondiabetic Subjects*. *J Clin Invest* **46**: 1549–1557.

Bains JS, Cusulin JIW, Inoue W (2015). Stress-related synaptic plasticity in the hypothalamus. *Nat Rev Neurosci* **16**: 377–388.

Bake T, Morgan DG a, Mercer JG (2014). Feeding and metabolic consequences of scheduled consumption of large, binge-type meals of high fat diet in the Sprague-Dawley rat. *Physiol Behav* **128**: 70–9.

Baker J, Jatlow P, Pade P, Ramakrishnan V, McCance-Katz EF (2007). Acute cocaine responses following cocaethylene infusion. *Am J Drug Alcohol Abuse* **33**: 619–25.

Ballon JS, Feifel D (2006). A Systematic Review of Modafinil: Potential Clinical Uses and Mechanisms of Action. *J Clin PsychiatryJ Clin Psychiatry* **67467**: 554–566.

Bardo MT (1998). Neuropharmacological Mechanisms of Drug Reward: Beyond Dopamine in the Nucleus Accumbens. *Crit Rev Neurobiol* **12**: 37–68.

Bare DJ, Ghetti B, Richter JA (1995). The tyrosine kinase inhibitor genistein increases endogenous dopamine release from normal and weaver mutant mouse striatal slices. *J Neurochem* **65**: 2096–104.

Barry D, Clarke M, Petry NM (2009). Obesity and its relationship to addictions: is overeating a form of addictive behavior? *Am J Addict* **18**: 439–51.

Bastrikova N, Gardner GA, Reece JM, Jeromin A, Dudek SM (2008). Synapse elimination accompanies functional plasticity in hippocampal neurons. *Proc Natl Acad Sci U S A* **105**: 3123–7.

Becker JB (1999). Gender Differences in Dopaminergic Function in Striatum and Nucleus Accumbens. *Pharmacol Biochem Behav* **64**: 803–812.

Beinfeld MC, Connolly KJ, Pierce RC (2002). Cocaine treatment increases extracellular cholecystokinin (CCK) in the nucleus accumbens shell of awake, freely moving rats, an effect that is enhanced in rats that are behaviorally sensitized to cocaine. *J Neurochem* **81**: 1021–7.

Bellinger LL, Bernardis LL (2002). The dorsomedial hypothalamic nucleus and its role in ingestive behavior and body weight regulation: lessons learned from lesioning studies. *Physiol Behav* **76**: 431–42.

Benarroch EE (2013). Microglia: Multiple roles in surveillance, circuit shaping, and response to injury. *Neurology* **81**: 1079–88.

Benelam B (2009). Satiation, satiety and their effects on eating behaviour. *Nutr Bull* **34**: 126–173.

Berner LA, Avena NM, Hoebel BG (2008). Bingeing, Self-restriction, and Increased Body Weight in Rats With Limited Access to a Sweet-fat Diet. *Obesity* **16**: 1998–2002.

Berridge KC (2012). From prediction error to incentive salience: mesolimbic computation of reward motivation. *Eur J Neurosci* **35**: 1124–43.

Berridge KC, Ho C-Y, Richard JM, DiFeliceantonio AG (2010). The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. *Brain Res* **1350**: 43–64.

Berthoud H-R (2002). Multiple neural systems controlling food intake and body weight. *Neurosci Biobehav Rev* **26**: 393–428.

Berthoud H-R (2006). Homeostatic and Non-homeostatic Pathways Involved in the Control of Food Intake and Energy Balance. *Obesity* **14**: 197S–200S.

Berthoud H-R (2007). Interactions between the "cognitive" and "metabolic" brain in the control of food intake. *Physiol Behav* **91**: 486–498.

Berthoud H-R, Powley TL (1992). Vagal afferent innervation of the rat fundic stomach: Morphological characterization of the gastric tension receptor. *J Comp Neurol* **319**: 261–276.

Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamales M, Herve D, Valjent E, *et al* (2008). Opposing Patterns of Signaling Activation in Dopamine D1 and D2 Receptor-Expressing Striatal Neurons in Response to Cocaine and Haloperidol. *J Neurosci* **28**: 5671–5685.

Bissière S, Humeau Y, Lüthi A (2003). Dopamine gates LTP induction in lateral amygdala by suppressing feedforward inhibition. *Nat Neurosci* **6**: 587–92.

Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon J-L, *et al* (1992). The melanin-concentrating hormone system of the rat brain: An immuno- and hybridization histochemical characterization. *J Comp Neurol* **319**: 218–245.

Björklund A, Dunnett SB (2007). Dopamine neuron systems in the brain: an update. *Trends Neurosci* **30**: 194–202.

Björklund A, Dunnett SB, Falck B, al. E, Carlsson A, al. E, *et al* (2007). Dopamine neuron systems in the brain: an update. **30**: 194–202.

Björntorp P, Rössner S, Uddén J (2001). "Consolatory eating" is not a myth. Stress-induced increased cortisol levels result in leptin-resistant obesity. *Lakartidningen* **98**: 5458–61.

Blum K, Gardner E, Oscar-Berman M, Gold M (2012). "Liking" and "wanting" linked to Reward Deficiency Syndrome (RDS): hypothesizing differential responsivity in brain reward circuitry. *Curr Pharm Des* **18**: 113–8.

Blundell JE, Macdiarmid JI (1997). Fat as a Risk Factor for Overconsumption: Satiation, Satiety, and Patterns of Eating. *J Am Diet Assoc* **97**: S63–S69.

Bocarsly ME, Fasolino M, Kane GA, Lamarca EA, Kirschen GW, Karatsoreos IN, *et al* (2015). Obesity diminishes synaptic markers, alters microglial morphology, and impairs cognitive function. *Proc Natl Acad Sci U S A* **112**: 15731–6.

Boche D, Perry VH, Nicoll JAR (2013). Review: Activation patterns of microglia and their identification in the human brain. *Neuropathol Appl Neurobiol* **39**: 3–18.

Bodnar RJ (2007). Endogenous opiates and behavior: 2006. *Peptides* **28**: 2435–513.

Boitard C, Cavaroc A, Sauvant J, Aubert A, Castanon N, Layé S, *et al* (2014). Impairment of hippocampal-dependent memory induced by juvenile high-fat diet intake is associated with enhanced hippocampal inflammation in rats. *Brain Behav Immun* **40**: 9–17.

Boron WF, Boulpaep EL (Saunders Elsevier: 2012). *Medical physiology : a cellular and molecular approach*. .

Bott R (2014). *Obesity Epidemiology: Methods and Applications*. *Igarss 2014* doi:10.1007/s13398-014-0173-7.2.

Bouchard C (CRC Press: 1994). *The Genetics of obesity*. at <https://www.crcpress.com/The-Genetics-of-Obesity/Bouchard/p/book/9780849348808>.

Boulanger LM (2009). Immune Proteins in Brain Development and Synaptic Plasticity. *Neuron* **64**: 93–109.

Bourne J, Harris KM (2007). Do thin spines learn to be mushroom spines that remember? *Curr Opin Neurobiol* **17**: 381–6.

Bourne JN, Harris KM (2008). Balancing structure and function at hippocampal dendritic spines. *Annu Rev Neurosci* **31**: 47–67.

Bragulat V, Dzemidzic M, Bruno C, Cox CA, Talavage T, Considine R V., *et al* (2010). Food-Related Odor Probes of Brain Reward Circuits During Hunger: A Pilot fMRI Study. *Obesity* **18**: 1566–1571.

Bray GA, Champagne CM (2005). Beyond Energy Balance: There Is More to Obesity than Kilocalories. *J Am Diet Assoc* **105**: 17–23.

Broadwell RD, Brightman MW (1976). Entry of peroxidase into neurons of the central and peripheral nervous systems from extracerebral and cerebral blood. *J Comp Neurol* **166**: 257–283.

Broberger C, Lecea L De, Sutcliffe JG, Hökfelt T (1998). Hypocretin/orexin- and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems. *J Comp Neurol* **402**: 460–74.

Bromberg-Martin ES, Matsumoto M, Hikosaka O (2010). Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron* **68**: 815–34.

Bruce AJ, Boling W, Kindy MS, Peschon J, Kraemer PJ, Carpenter MK, *et al* (1996). Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. *Nat Med* **2**: 788–94.

Bruinstroop E, Cano G, Vanderhorst VGJM, Cavalcante JC, Wirth J, Sena-Esteves M, *et al* (2012). Spinal projections of the A5, A6 (locus coeruleus), and A7 noradrenergic cell groups in rats. *J Comp Neurol* **520**: 1985–2001.

Bura SA, Burokas A, Martín-García E, Maldonado R (2010). Effects of chronic nicotine on food intake and anxiety-like behaviour in CB(1) knockout mice. *Eur Neuropsychopharmacol* **20**: 369–78.

Burger KS, Stice E (2012). Frequent ice cream consumption is associated with reduced striatal response to receipt of an ice cream-based milkshake. *Am J Clin Nutr* **95**: 810–7.

Burger KS, Stice E (2014). Greater striatopallidal adaptive coding during cue?reward learning and food reward habituation predict future weight gain. *Neuroimage* **99**: 122–128.

Cabral GA, Marciano-Cabral F (2005). Cannabinoid receptors in microglia of the central nervous system: immune functional relevance. *J Leukoc Biol* **78**: 1192–7.

Cai XJ, Widdowson PS, Harrold J, Wilson S, Buckingham RE, Arch JR, *et al* (1999). Hypothalamic orexin expression: modulation by blood glucose and feeding. *Diabetes* **48**: 2132–7.

Camí J, Farré M (2003). Drug addiction. *N Engl J Med* **349**: 975–86.

Campos CA, Wright JS, Czaja K, Ritter RC (2012). CCK-Induced Reduction of Food Intake and Hindbrain MAPK Signaling Are Mediated by NMDA Receptor

Activation. *Endocrinology* **153**: 2633–2646.

Carr DB, Sesack SR (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J Neurosci* **20**: 3864–73.

Carroll KM, Fenton LR, Ball SA, Nich C, Frankforter TL, Shi J, *et al* (2004a). Efficacy of disulfiram and cognitive behavior therapy in cocaine-dependent outpatients: a randomized placebo-controlled trial. *Arch Gen Psychiatry* **61**: 264–72.

Carroll KM, Fenton LR, Ball SA, Nich C, Frankforter TL, Shi J, *et al* (2004b). Efficacy of Disulfiram and Cognitive Behavior Therapy in Cocaine-DependentOutpatients. *Arch Gen Psychiatry* **61**: 264.

Carroll KM, Nich C, Petry NM, Eagan DA, Shi JM, Ball SA (2016a). A randomized factorial trial of disulfiram and contingency management to enhance cognitive behavioral therapy for cocaine dependence. *Drug Alcohol Depend* **160**: 135–42.

Carroll KM, Nich C, Petry NM, Eagan DA, Shi JM, Ball SA (2016b). A randomized factorial trial of disulfiram and contingency management to enhance cognitive behavioral therapy for cocaine dependence. *Drug Alcohol Depend* **160**: 135–142.

Castro DC, Berridge KC (2014). Opioid Hedonic Hotspot in Nucleus Accumbens Shell: Mu, Delta, and Kappa Maps for Enhancement of Sweetness "Liking" and "Wanting" *J Neurosci* **34**: 4239–4250.

Castro DC, Cole SL, Berridge KC (2015). Lateral hypothalamus, nucleus accumbens, and ventral pallidum roles in eating and hunger: interactions between homeostatic and reward circuitry. *Front Syst Neurosci* **9**: 90.

Center for Behavioral Health Statistics S (2014). *Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings*. at <https://www.samhsa.gov/data/sites/default/files/NSDUHresultsPDFWHTML2013/Web/NSDUHresults2013.pdf>.

Chandran M, Phillips SA, Ciaraldi T, Henry RR (2003). Adiponectin: More Than Just Another Fat Cell Hormone? *Diabetes Care* **26**: .

Charbogne P, Kieffer BL, Befort K (2014). 15 years of genetic approaches in vivo for addiction research: Opioid receptor and peptide gene knockout in mouse models of drug abuse. *Neuropharmacology* **76 Pt B**: 204–17.

Chaudhri N, Sahuque LL, Schairer WW, Janak PH (2010). Separable roles of the nucleus accumbens core and shell in context- and cue-induced alcohol-seeking. *Neuropsychopharmacology* **35**: 783–91.

Chaudhri O, Small C, Bloom S (2006). Gastrointestinal hormones regulating appetite. *Philos Trans R Soc Lond B Biol Sci* **361**: 1187–209.

Chen BT, Bowers MS, Martin M, Hopf FW, Guillory AM, Carelli RM, *et al* (2008). Cocaine but Not Natural Reward Self-Administration nor Passive Cocaine Infusion Produces Persistent LTP in the VTA. *Neuron* **59**: 288–297.

Chen C-H, Ferreira JCB, Gross ER, Mochly-Rosen D (2014). Targeting aldehyde dehydrogenase 2: new therapeutic opportunities. *Physiol Rev* **94**: 1–34.

Chiara G Di, Imperato A (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A* **85**: 5274–8.

Chiodo LA, Berger TW (1986). Interactions between dopamine and amino acid-induced excitation and inhibition in the striatum. *Brain Res* **375**: 198–203.

Citri A, Malenka RC (2008). Synaptic Plasticity: Multiple Forms, Functions, and Mechanisms. *Neuropsychopharmacology* **33**: 18–41.

Collingridge GL, Peineau S, Howland JG, Wang YT (2010). Long-term depression in the CNS. *Nat Rev Neurosci* **11**: 459–473.

Cone EJ (1995). Pharmacokinetics and pharmacodynamics of cocaine. *J Anal Toxicol* **19**: 459–78.

Cone EJ, Yousefnejad D, Hillsgrove MJ, Holicky B, Darwin WD (1995). Passive inhalation of cocaine. *J Anal Toxicol* **19**: 399–411.

Corwin RL, Buda-Levin A (2004). Behavioral models of binge-type eating. *Physiol Behav* **82**: 123–30.

Corwin RL, Grigson PS (2009). Symposium overview--Food addiction: fact or fiction? *J Nutr* **139**: 617–9.

Cota D, Tschöp MH, Horvath TL, Levine AS (2006). Cannabinoids, opioids and eating behavior: The molecular face of hedonism? *Brain Res Rev* **51**: 85–107.

Cottone P, Sabino V, Steardo L, Zorrilla EP (2008). Opioid-dependent anticipatory negative contrast and binge-like eating in rats with limited access to highly preferred food. *Neuropsychopharmacology* **33**: 524–35.

Cottone P, Wang X, Park JW, Valenza M, Blasio A, Kwak J, *et al* (2012). Antagonism of sigma-1 receptors blocks compulsive-like eating. *Neuropsychopharmacology* **37**: 2593–604.

Cummings DE, Foster-Schubert KE, Overduin J (2005). Ghrelin and energy balance: focus on current controversies. *Curr Drug Targets* **6**: 153–69.

Czyzyk TA, Sahr AE, Statnick MA (2010). A model of binge-like eating behavior in mice that does not require food deprivation or stress. *Obesity (Silver Spring)* **18**: 1710–7.

D'Ardenne K, McClure SM, Nystrom LE, Cohen JD (2008). Responses Reflecting Dopaminergic Signals in the Human Ventral Tegmental Area. *Science (80-)* **319**: 1264–1267.

Dackis CA, Kampman KM, Lynch KG, Pettinati HM, O'Brien CP (2005). A Double-Blind, Placebo-Controlled Trial of Modafinil for Cocaine Dependence. *Neuropsychopharmacology* **30**: 205–211.

Dackis CA, O'Brien CP (2001). Cocaine dependence: a disease of the brain's reward centers. *J Subst Abuse Treat* **21**: 111–7.

Dagher A (2009). The neurobiology of appetite: hunger as addiction. *Int J Obes* **33**: S30–S33.

Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, *et al* (2002). The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* **123**: 1120–8.

Davidson TL, Kanoski SE, Schier LA, Clegg DJ, Benoit SC (2007). A potential role for the hippocampus in energy intake and body weight regulation. *Curr Opin Pharmacol* **7**: 613–6.

Davis CA, Levitan RD, Reid C, Carter JC, Kaplan AS, Patte KA, *et al* (2009). Dopamine for "wanting" and opioids for "liking": a comparison of obese adults with and without binge eating. *Obesity (Silver Spring)* **17**: 1220–5.

Davis JD, Smith GP (1990). Learning to sham feed: behavioral adjustments to loss of physiological postingestional stimuli. *Am J Physiol* **259**: R1228-35.

Delaney CB, Eddy KT, Hartmann AS, Becker AE, Murray HB, Thomas JJ (2015). Pica and rumination behavior among individuals seeking treatment for eating disorders or obesity. *Int J Eat Disord* **48**: 238–48.

Demos KE, Heatherton TF, Kelley WM (2012). Individual Differences in Nucleus Accumbens Activity to Food and Sexual Images Predict Weight Gain and Sexual Behavior. *J Neurosci* **32**: 5549–5552.

Deng P-Y, Klyachko VA (2011). The diverse functions of short-term plasticity components in synaptic computations. *Commun Integr Biol* **4**: 543–8.

Deroche-Gamonet V, Belin D, Piazza PV (2004). Evidence for addiction-like behavior in the rat. *Science* **305**: 1014–7.

Diergaarde L, Pattij T, Nawijn L, Schoffelmeer ANM, Vries TJ De (2009). Trait Impulsivity Predicts Escalation of Sucrose Seeking and Hypersensitivity to Sucrose-Associated Stimuli. *Biol Psychol* **123**: 794–803.

DiGregorio DA, Nusser Z, Silver RA (2002). Spillover of glutamate onto synaptic AMPA receptors enhances fast transmission at a cerebellar synapse. *Neuron* **35**: 521–33.

Dijk SJ van, Feskens EJ, Bos MB, Hoelen DW, Heijligenberg R, Bromhaar MG, *et al* (2009). A saturated fatty acid-rich diet induces an obesity-linked proinflammatory gene expression profile in adipose tissue of subjects at risk of metabolic syndrome. *Am J Clin Nutr* **90**: 1656–1664.

Dobbs R, Sawers C, Thompson F, Manyika J, Woetzel J, Child P, *et al* (2014). *How the world could better fight obesity | McKinsey & Company*. at <http://www.mckinsey.com/industries/healthcare-systems-and-services/our-insights/how-the-world-could-better-fight-obesity>.

Drazen DL, Vahl TP, D'Alessio DA, Seeley RJ, Woods SC (2006). Effects of a Fixed Meal Pattern on Ghrelin Secretion: Evidence for a Learned Response Independent of Nutrient Status. *Endocrinology* **147**: 23–30.

Drel VR, Mashtalir N, Ilnytska O, Shin J, Li F, Lyzogubov V V., *et al* (2006). The Leptin-Deficient (ob/ob) Mouse: A New Animal Model of Peripheral Neuropathy of Type 2 Diabetes and Obesity. *Diabetes* **55**: 3335–3343.

Dyer J, Salmon KSH, Zibrik L, Shirazi-Beechey SP (2005). Expression of sweet taste receptors of the T1R family in the intestinal tract and enteroendocrine cells. *Biochem Soc Trans* **33**: 302–305.

Edens E, Massa A, Petrakis I (2010). Novel Pharmacological Approaches to Drug Abuse Treatment. *Curr Top Behav Neurosci* **3**: 343–386.

Edvell A, Lindström P (1999). Initiation of increased pancreatic islet growth in young normoglycemic mice (Umeå +/?). *Endocrinology* **140**: .

Ekdahl CT (2012). Microglial activation - tuning and pruning adult neurogenesis. *Front Pharmacol* **3**: 41.

Elias CF, Saper CB, Maratos-Flier E, Tritos NA, Lee C, Kelly J, *et al* (1998). Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area. *J Comp Neurol* **402**: 442–59.

Elks CE, Hoed M den, Zhao JH, Sharp SJ, Wareham NJ, Loos RJF, *et al* (2012). Variability in the heritability of body mass index: a systematic review and meta-regression. *Front Endocrinol (Lausanne)* **3**: 29.

Erion JR, Wosiski-Kuhn M, Dey A, Hao S, Davis CL, Pollock NK, *et al* (2014). Obesity elicits interleukin 1-mediated deficits in hippocampal synaptic plasticity. *J Neurosci* **34**: 2618–31.

Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N (2014). Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clin Pract* **105**: 141–150.

Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, Robbins TW (2008). Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos Trans R Soc Lond B Biol Sci* **363**: 3125–35.

Everitt BJ, Robbins TW (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nat Neurosci* **8**: 1481–1489.

Everitt BJ, Robbins TW (2013). From the ventral to the dorsal striatum: Devolving views of their roles in drug addiction. *Neurosci Biobehav Rev* **37**: 1946–1954.

Faleiro LJ, Jones S, Kauer JA (2004). Rapid synaptic plasticity of glutamatergic synapses on dopamine neurons in the ventral tegmental area in response to acute amphetamine injection. *Neuropsychopharmacology* **29**: 2115–25.

Fenn AM, Gensel JC, Huang Y, Popovich PG, Lifshitz J, Godbout JP (2014).

Immune activation promotes depression 1 month after diffuse brain injury: a role for primed microglia. *Biol Psychiatry* **76**: 575–84.

Ferrarelli LK (2016). Microglia combat addiction. *Sci Signal* **9**: ec110-ec110.

Ferrario CR (2017). Food Addiction and Obesity. *Neuropsychopharmacology* **42**: 361–361.

Figlewicz DP, Szot P, Chavez M, Woods SC, Veith RC (1994). Intraventricular insulin increases dopamine transporter mRNA in rat VTA/substantia nigra. *Brain Res* **644**: 331–4.

Filip M, Frankowska M, Zaniewska M, Gołda A, Przegaliński E (2005). The serotonergic system and its role in cocaine addiction. *Pharmacol Rep* **57**: 685–700.

Finlayson G, King N, Blundell J (2008). The role of implicit wanting in relation to explicit liking and wanting for food: implications for appetite control. *Appetite* **50**: 120–7.

Flannery BA, Morgenstern J, McKay J, Wechsberg WM, Litten RZ (2004). Co-Occurring Alcohol and Cocaine Dependence: Recent Findings From Clinical and Field Studies. *Alcohol Clin Exp Res* **28**: 976–981.

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, *et al* (2007). A Common Variant in the FTO Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. *Science (80-)* **316**: 889–894.

Frederich RC, Hamann A, Anderson S, Löllmann B, Lowell BB, Flier JS (1995a). Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nat Med* **1**: 1311–4.

Frederich RC, Löllmann B, Hamann A, Napolitano-Rosen A, Kahn BB, Lowell BB, *et al* (1995b). Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. *J Clin Invest* **96**: 1658–1663.

Friedman AS, Terras A, Zhu W, McCallum J (2004). Depression, Negative Self-Image, and Suicidal Attempts as Effects of Substance Use and Substance Dependence. *J Addict Dis* **23**: 55–71.

Friedman JM, Halaas JL (1998). Leptin and the regulation of body weight in mammals. *Nature* **395**: 763–70.

Frigon RP, Converse JL, Stone RA (1978). Plasma dopamine beta-hydroxylase species dependence and in the vitro influence of NEM, coppor, and PH. *Biochem Med* **19**: 1–15.

Fuller RK, Branchey L, Brightwell DR, Derman RM, Emrick CD, Iber FL, *et al* (1986). Disulfiram treatment of alcoholism. A Veterans Administration cooperative study. *JAMA* **256**: 1449–55.

Furieri FA, Nakamura-Palacios EM (2007). Gabapentin reduces alcohol consumption and craving: a randomized, double-blind, placebo-controlled trial. *J*

Clin Psychiatry **68**: 1691–700.

Ganguly K, Schinder AF, Wong ST, Poo M (2001). GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell* **105**: 521–32.

Ganry O (2005). Phytoestrogens and prostate cancer risk. *Prev Med (Baltim)* **41**: 1–6.

Garcia-Lopez P, Garcia-Marin V, Martinez-Murillo R, Freire M (2010). Cajal's achievements in the field of the development of dendritic arbors. *Int J Dev Biol* **54**: 1405–1417.

Garzaro M, Raimondo L, Nadalin J, Pecorari G, Giordano C Subjective assessment of palatability, digestibility and emotions in healthy volunteers after ingestion of an iced dessert: preliminary report. *J Biol Regul Homeost Agents* **24**: 391–5.

Gaval-Cruz M, Weinshenker D (2009). Mechanisms of disulfiram-induced cocaine abstinence: antabuse and cocaine relapse. *Mol Interv* **9**: 175–87.

Gearhardt AN, Corbin WR, Brownell KD (2009). Preliminary validation of the Yale Food Addiction Scale. *Appetite* **52**: 430–436.

Gearhardt AN, Corbin WR, Brownell KD (2016). Development of the Yale Food Addiction Scale Version 2.0. *Psychol Addict Behav* **30**: 113–121.

Gearhardt AN, White MA, Masheb RM, Grilo CM (2013). An examination of food addiction in a racially diverse sample of obese patients with binge eating disorder in primary care settings. *Compr Psychiatry* **54**: 500–5.

Geary N (1990). Pancreatic glucagon signals postprandial satiety. *Neurosci Biobehav Rev* **14**: 323–38.

Geiger BMM, Haburcak M, Avena NMM, Moyer MCC, Hoebel BGG, Pothos ENN (2009). Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. *Neuroscience* **159**: 1193–1199.

Geisler S, Wise RA (2008). Functional implications of glutamatergic projections to the ventral tegmental area. *Rev Neurosci* **19**: 227–44.

Georgiou P, Zanos P, Ehteramyan M, Hourani S, Kitchen I, Maldonado R, *et al* (2015). Differential regulation of mGlu $_5$ R and MOPr by priming- and cue-induced reinstatement of cocaine-seeking behaviour in mice. *Addict Biol* **20**: 902–912.

Gerdeman GL, Partridge JG, Lupica CR, Lovinger DM (2003). It could be habit forming: drugs of abuse and striatal synaptic plasticity. *Trends Neurosci* **26**: 184–92.

Gerozissis K (2004). Brain insulin and feeding: a bi-directional communication. *Eur J Pharmacol* **490**: 59–70.

Gerstein DE, Woodward-Lopez G, Evans AE, Kelsey K, Drewnowski A (2004). Clarifying concepts about macronutrients' effects on satiation and satiety. *J Am*

Diet Assoc **104**: 1151–3.

Giskes K, Lenthe F van, Avendano-Pabon M, Brug J (2011). A systematic review of environmental factors and obesogenic dietary intakes among adults: are we getting closer to understanding obesogenic environments? *Obes Rev* **12**: e95–e106.

Glass M, Dragunow M, Faull RL (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77**: 299–318.

Gold MS, Badgaiyan RD, Blum K (2015). A Shared Molecular and Genetic Basis for Food and Drug Addiction: Overcoming Hypodopaminergic Trait/State by Incorporating Dopamine Agonistic Therapy in Psychiatry. *Psychiatr Clin North Am* **38**: 419–62.

GOLDSTEIN M, ANAGNOSTE B, LAUBER E, MCKEREGHAM MR (1964). Inhibition of dopamine-beta-hydroxylase by disulfiram. *Life Sci* **3**: 763–7.

González-Muniesa P, Mártinez-González M-A, Hu FB, Després J-P, Matsuzawa Y, Loos RJF, *et al* (2017). Obesity. *Nat Rev Dis Prim* **3**: 17034.

Gossop M, Manning V, Ridge G (2006). Concurrent use of alcohol and cocaine: differences in patterns of use and problems among users of crack cocaine and cocaine powder. *Alcohol Alcohol* **41**: 121–125.

Grabowski J, Shearer J, Merrill J, Negus SS (2004). Agonist-like, replacement pharmacotherapy for stimulant abuse and dependence. *Addict Behav* **29**: 1439–1464.

Graeber MB (2010). Changing Face of Microglia. *Science (80-)* **330**: 783–788.

Guegan T, Cutando L, Ayuso E, Santini E, Fisone G, Bosch F, *et al* (2013). Operant behavior to obtain palatable food modifies neuronal plasticity in the brain reward circuit. *Eur Neuropsychopharmacol* **23**: 146–59.

Haase L, Cerf-Ducastel B, Murphy C (2009). Cortical activation in response to pure taste stimuli during the physiological states of hunger and satiety. *Neuroimage* **44**: 1008–1021.

Halpern CH, Tekriwal A, Santollo J, Keating JG, Wolf JA, Daniels D, *et al* (2013). Amelioration of binge eating by nucleus accumbens shell deep brain stimulation in mice involves D2 receptor modulation. *J Neurosci* **33**: 7122–9.

Hansen MJ, Schiöth HB, Morris MJ (2005). Feeding responses to a melanocortin agonist and antagonist in obesity induced by a palatable high-fat diet. *Brain Res* **1039**: 137–45.

Hao S, Dey A, Yu X, Stranahan AM (2016). Dietary obesity reversibly induces synaptic stripping by microglia and impairs hippocampal plasticity. *Brain Behav Immun* **51**: 230–239.

Hasin DS, Trautman KD, Miele GM, Samet S, Smith M, Endicott J (1996).

Psychiatric Research Interview for Substance and Mental Disorders (PRISM): reliability for substance abusers. *Am J Psychiatry* **153**: 1195–1201.

Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong E a, Gill S, *et al* (2012). Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab* **15**: 848–60.

Hatsukami DK, Fischman MW (1996). Crack cocaine and cocaine hydrochloride. Are the differences myth or reality? *JAMA* **276**: 1580–8.

Havakuk O, Rezkalla SH, Kloner RA (2017). The Cardiovascular Effects of Cocaine. *J Am Coll Cardiol* **70**: 101–113.

Hebebrand J, Albayrak Ö, Adan R, Antel J, Dieguez C, Jong J de, *et al* (2014). "Eating addiction", rather than "food addiction", better captures addictive-like eating behavior. *Neurosci Biobehav Rev* **47**: 295–306.

Heberlein A, Dürsteler-MacFarland KM, Lenz B, Frieling H, Grösch M, Bönsch D, *et al* (2011). Serum levels of BDNF are associated with craving in opiate-dependent patients. *J Psychopharmacol* **25**: 1480–1484.

Heinonen S-M, Hoikkala A, Wähälä K, Adlercreutz H (2003). Metabolism of the soy isoflavones daidzein, genistein and glycitein in human subjects. *J Steroid Biochem Mol Biol* **87**: 285–299.

Henderson VW, Paganini-Hill A, Miller BL, Elble RJ, Reyes PF, Shoupe D, *et al* (2000). Estrogen for Alzheimer's disease in women: randomized, double-blind, placebo-controlled trial. *Neurology* **54**: 295–301.

Herbert H, Moga MM, Saper CB (1990). Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat. *J Comp Neurol* **293**: 540–580.

Herbst ED, Harris DS, Everhart ET, Mendelson J, Jacob P, Jones RT (2011). Cocaethylene formation following ethanol and cocaine administration by different routes. *Exp Clin Psychopharmacol* **19**: 95–104.

Herculano-Houzel S (2014). The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. *Glia* **62**: 1377–91.

Heyne A, Kiesselbach C, Sahún I, McDonald J, Gaiffi M, Dierssen M, *et al* (2009). An animal model of compulsive food-taking behaviour. *Addict Biol* **14**: 373–383.

Hill JO (1998). Environmental Contributions to the Obesity Epidemic. *Science (80-)* **280**: 1371–1374.

Hofker M, Wijmenga C (2009). A supersized list of obesity genes. *Nat Genet* **41**: 139–140.

Hommel JD, Trinko R, Sears RM, Georgescu D, Liu Z-W, Gao X-B, *et al* (2006). Leptin Receptor Signaling in Midbrain Dopamine Neurons Regulates Feeding. *Neuron* **51**: 801–810.

Hong S, Dissing-Olesen L, Stevens B (2016). New insights on the role of microglia in synaptic pruning in health and disease. *Curr Opin Neurobiol* **36**: 128–134.

Hoz L de, Simons M (2015). The emerging functions of oligodendrocytes in regulating neuronal network behaviour. *Bioessays* **37**: 60–9.

Hsiang H-LL, Epp JR, Oever MC van den, Yan C, Rashid AJ, Insel N, *et al* (2014). Manipulating a "cocaine engram" in mice. *J Neurosci* **34**: 14115–27.

Ikemoto S (2007). Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex. *Brain Res Rev* **56**: 27–78.

Jäkel S, Dimou L (2017). Glial Cells and Their Function in the Adult Brain: A Journey through the History of Their Ablation. *Front Cell Neurosci* **11**: 24.

James PT, Leach R, Kalamara E, Shayeghi M (2001a). The worldwide obesity epidemic. *Obes Res* **9 Suppl 4**: 228S–233S.

James PT, Leach R, Kalamara E, Shayeghi M (2001b). The Worldwide Obesity Epidemic. *Obes Res* **9**: 228S–233S.

Jentsch JD, Taylor JR (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl)* **146**: 373–90.

Jerónimo A, Meira C, Amaro A, Campello GC, Granja C (2009). Cardiogenic shock caused by disulfiram. *Arq Bras Cardiol* **92**: e43–e45.

Johnson PM, Kenny PJ (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci* **13**: 635–641.

JOHNSTON CD (1953). The in vitro reaction between tetraethylthiuram disulfide (antabuse) and glutathione. *Arch Biochem Biophys* **44**: 249–51.

Kälin S, Heppner FL, Bechmann I, Prinz M, Tschöp MH, Yi C-X (2015). Hypothalamic innate immune reaction in obesity. *Nat Rev Endocrinol* **11**: 339–51.

Kalivas PW, O'Brien C (2008). Drug addiction as a pathology of staged neuroplasticity. *Neuropsychopharmacology* **33**: 166–180.

Kalivas PW, Volkow ND (2005). The Neural Basis of Addiction: A Pathology of Motivation and Choice. *Am J Psychiatry* **162**: 1403–1413.

Kandel DB, Huang FY, Davies M (2001). Comorbidity between patterns of substance use dependence and psychiatric syndromes. *Drug Alcohol Depend* **64**: 233–41.

Karch SB (CRC/Taylor & Francis: 2006). *A brief history of cocaine : from Inca monarchs to Cali cartels : 500 years of cocaine dealing*. .

Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N (2002). Dendritic spine structures and functions. *Nihon Shinkei Seishin Yakurigaku Zasshi* **22**: 159–64.

Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003). Structure–stability–function relationships of dendritic spines. *Trends Neurosci* **26**: 360–368.

Kasanetz F, Deroche-Gamonet V, Berson N, Balado E, Lafourcade M, Manzoni O, *et al* (2010). Transition to Addiction Is Associated with a Persistent Impairment in Synaptic Plasticity. *Science (80-)* **328**: .

Katsuura G, Asakawa A, Inui A (2002). Roles of pancreatic polypeptide in regulation of food intake. *Peptides* **23**: 323–9.

Kauer JA, Malenka RC (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci* **8**: 844–858.

Kavanagh KT, Maijub AG, Brown JR (1992). Passive exposure to cocaine in medical personnel and its effect on urine drug screening tests. *Otolaryngol Head Neck Surg* **107**: 363–6.

Kearns DN, Gomez-Serrano MA, Tunstall BJ (2011). A review of preclinical research demonstrating that drug and non-drug reinforcers differentially affect behavior. *Curr Drug Abuse Rev* **4**: 261–9.

Keleta YB, Martinez JL (2012). Brain Circuits of Methamphetamine Place Reinforcement Learning: The Role of the Hippocampus-VTA Loop. *Brain Behav* **2**: 128–141.

Kelley AE (2004). Memory and Addiction. *Neuron* **44**: 161–179.

Kelley AE, Andrzejewski ME, Baldwin AE, Hernandez PJ, Pratt WE (2003). Glutamate-mediated plasticity in corticostriatal networks: role in adaptive motor learning. *Ann N Y Acad Sci* **1003**: 159–68.

Kelley AE, Baldo BA, Pratt WE (2005). A proposed hypothalamic-thalamic-striatal axis for the integration of energy balance, arousal, and food reward. *J Comp Neurol* **493**: 72–85.

Kemps E, Tiggemann M, Grigg M (2008). Food cravings consume limited cognitive resources. *J Exp Psychol Appl* **14**: 247–254.

Kettenmann H, Hanisch U-K, Noda M, Verkhratsky A (2011). Physiology of Microglia. *Physiol Rev* **91**: 461–553.

Kettenmann H, Ransom BR (2005). *Neuroglia*. .

Keung WM, Klyosov AA, Vallee BL (1997). Daidzin inhibits mitochondrial aldehyde dehydrogenase and suppresses ethanol intake of Syrian golden hamsters. *Proc Natl Acad Sci U S A* **94**: 1675–9.

Keung WM, Vallee BL (1998). Daidzin and its antidipsotropic analogs inhibit serotonin and dopamine metabolism in isolated mitochondria. *Proc Natl Acad Sci U S A* **95**: 2198–203.

Khanh D V., Choi Y-H, Moh SH, Kinyua AW, Kim KW (2014). Leptin and insulin signaling in dopaminergic neurons: relationship between energy balance and reward system. *Front Psychol* **5**: 846.

Khisti RT, Wolstenholme J, Shelton KL, Miles MF (2006). Characterization of the ethanol-deprivation effect in substrains of C57BL/6 mice. *Alcohol* **40**: 119–26.

Kiefer R, Gold R, Gehrmann J, Lindholm D, Wekerle H, Kreutzberg GW (1993). Transforming growth factor beta expression in reactive spinal cord microglia and meningeal inflammatory cells during experimental allergic neuritis. *J Neurosci Res* **36**: 391–398.

Kieffer TJ, Francis Habener J (1999). The Glucagon-Like Peptides. *Endocr Rev* **20**: 876–913.

Kim E-M, Quinn JG, Levine AS, O'Hare E (2004). A bi-directional ?-opioid?opioid connection between the nucleus of the accumbens shell and the central nucleus of the amygdala in the rat. *Brain Res* **1029**: 135–139.

Kimelberg HK, Nedergaard M (2010). Functions of astrocytes and their potential as therapeutic targets. *Neurotherapeutics* **7**: 338–53.

King BM (2006). The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. *Physiol Behav* **87**: 221–244.

Kloss CU, Kreutzberg GW, Raivich G (1997). Proliferation of ramified microglia on an astrocyte monolayer: characterization of stimulatory and inhibitory cytokines. *J Neurosci Res* **49**: 248–54.

Koefer R, Streit WJ, Toyka K V., Kreutzberg GW, Hartung H-P (1995). Transforming growth factor-β1: A lesion-associated cytokine of the nervous system. *Int J Dev Neurosci* **13**: 331–339.

Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, *et al* (2007). High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab* **6**: 414–21.

Kolb B, Gorny G, Li Y, Samaha A-N, Robinson TE (2003). Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens. *Proc Natl Acad Sci* **100**: 10523–10528.

Koob GF (2005). The neurocircuitry of addiction: Implications for treatment. *Clin Neurosci Res* **5**: 89–101.

Koob GF (2009). Brain stress systems in the amygdala and addiction. *Brain Res* **1293**: 61–75.

Koob GF, Moal M Le (2008). Addiction and the Brain Antireward System. *Annu Rev Psychol* **59**: 29–53.

Koob GF, Volkow ND (2010). Neurocircuitry of Addiction. *Neuropsychopharmacology* **35**: 217–238.

Koppaka V, Thompson DC, Chen Y, Ellermann M, Nicolaou KC, Juvonen RO, *et al* (2012). Aldehyde Dehydrogenase Inhibitors: a Comprehensive Review of the Pharmacology, Mechanism of Action, Substrate Specificity, and Clinical

Application. *Pharmacol Rev* **64**: 520–539.

Kosten TR, Scanley BE, Tucker KA, Oliveto A, Prince C, Sinha R, *et al* (2006). Cue-Induced Brain Activity Changes and Relapse in Cocaine-Dependent Patients. *Neuropsychopharmacology* **31**: 644–650.

Kringelbach ML, O'Doherty J, Rolls ET, Andrews C (2003). Activation of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness. *Cereb Cortex* **13**: 1064–71.

Krishna S, Keralapurath MM, Lin Z, Wagner JJ, La Serre CB de, Harn DA, *et al* (2015). Neurochemical and electrophysiological deficits in the ventral hippocampus and selective behavioral alterations caused by high-fat diet in female C57BL/6 mice. *Neuroscience* **297**: 170–181.

Krügel U, Schraft T, Kittner H, Kiess W, Illes P (2003). Basal and feeding-evoked dopamine release in the rat nucleus accumbens is depressed by leptin. *Eur J Pharmacol* **482**: 185–7.

Kubota N, Yano W, Kubota T, Yamauchi T, Itoh S, Kumagai H, *et al* (2007). Adiponectin Stimulates AMP-Activated Protein Kinase in the Hypothalamus and Increases Food Intake. *Cell Metab* **6**: 55–68.

Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, Saag PT van der, *et al* (1998). Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* **139**: 4252–63.

Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Häggblad J, Nilsson S, *et al* (1997). Comparison of the Ligand Binding Specificity and Transcript Tissue Distribution of Estrogen Receptors α and β. *Endocrinology* **138**: 863–870.

Kullmann DM, Erdemli G, Asztély F (1996). LTP of AMPA and NMDA receptor-mediated signals: evidence for presynaptic expression and extrasynaptic glutamate spill-over. *Neuron* **17**: 461–74.

Kuriyama K, Hirouchi M, Nakayasu H (1993). Structure and function of cerebral GABAA and GABAB receptors. *Neurosci Res* **17**: 91–9.

Ladurelle N, Keller G, Blommaert A, Roques BP, Daugé V (1997). The CCK-B agonist, BC264, increases dopamine in the nucleus accumbens and facilitates motivation and attention after intraperitoneal injection in rats. *Eur J Neurosci* **9**: 1804–14.

Larson EB, Carroll ME (2007). Estrogen Receptor β, but not α, Mediates Estrogen's Effect on Cocaine-Induced Reinstatement of Extinguished Cocaine-Seeking Behavior in Ovariectomized Female Rats. *Neuropsychopharmacology* **32**: 1334–1345.

Lawson LJ, Perry VH, Dri P, Gordon S (1990). Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* **39**: 151–70.

Lee C-T, Lehrmann E, Hayashi T, Amable R, Tsai S-Y, Chen J, *et al* (2009). Gene

expression profiling reveals distinct cocaine-responsive genes in human fetal CNS cell types. *J Addict Med* **3**: 218–26.

Lenoir M, Serre F, Cantin L, Ahmed SH (2007). Intense sweetness surpasses cocaine reward. *PLoS One* **2**: e698.

Lerma-Cabrera JM, Carvajal F, Lopez-Legarrea P (2015). Food addiction as a new piece of the obesity framework. *Nutr J* **15**: 5.

Lewitus GMM, Konefal SCC, Greenhalgh ADD, Pribiag H, Augereau K, Stellwagen D (2016). Microglial TNF-α Suppresses Cocaine-Induced Plasticity and Behavioral Sensitization. *Neuron* **90**: 483–491.

Li X, Wolf ME (2011). Brain-derived neurotrophic factor rapidly increases AMPA receptor surface expression in rat nucleus accumbens. *Eur J Neurosci* **34**: 190–198.

Li Y, Kauer JA (2004). Repeated exposure to amphetamine disrupts dopaminergic modulation of excitatory synaptic plasticity and neurotransmission in nucleus accumbens. *Synapse* **51**: 1–10.

Loibl S, Lintermans A, Dieudonné AS, Neven P (2011). Management of menopausal symptoms in breast cancer patients. *Maturitas* **68**: 148–154.

Ludwig DS, Tritos NA, Mastaitis JW, Kulkarni R, Kokkotou E, Elmquist J, *et al* (2001). Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J Clin Invest* **107**: 379–86.

Lupica CR, Riegel AC, Hoffman AF (2004). Marijuana and cannabinoid regulation of brain reward circuits. *Br J Pharmacol* **143**: 227–34.

Lüscher C, Ungless MA (2006). The Mechanistic Classification of Addictive Drugs. *PLoS Med* **3**: e437.

Lutter M, Nestler EJ (2009). Homeostatic and Hedonic Signals Interact in the Regulation of Food Intake. *J Nutr* **139**: 629–632.

Ma D-F, Qin L-Q, Wang P-Y, Katoh R (2008). Soy isoflavone intake inhibits bone resorption and stimulates bone formation in menopausal women: meta-analysis of randomized controlled trials. *Eur J Clin Nutr* **62**: 155–161.

Madhavan A, Bonci A, Whistler JL (2010). Opioid-Induced GABA potentiation after chronic morphine attenuates the rewarding effects of opioids in the ventral tegmental area. *J Neurosci* **30**: 14029–35.

Madras BK, Xie Z, Lin Z, Jassen A, Panas H, Lynch L, *et al* (2006). Modafinil Occupies Dopamine and Norepinephrine Transporters in Vivo and Modulates the Transporters and Trace Amine Activity in Vitro. *Massachusetts Gen Hosp* **319**: 561–569.

Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, *et al* (1995). Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* **1**: 1155–61.

MAGEE P, MCGLYNN H, ROWLAND I (2004). Differential effects of isoflavones and lignans on invasiveness of MDA-MB-231 breast cancer cells in vitro. *Cancer Lett* **208**: 35–41.

Maher FO, Clarke RM, Kelly A, Nally RE, Lynch MA (2006). Interaction between interferon ? and insulin-like growth factor-1 in hippocampus impacts on the ability of rats to sustain long-term potentiation. *J Neurochem* **96**: 1560–1571.

Mainardi M, Pizzorusso T, Maffei M (2013). Environment, leptin sensitivity, and hypothalamic plasticity. *Neural Plast* **2013**: 438072.

Maldonado R, Berrendero F (2010). Endogenous cannabinoid and opioid systems and their role in nicotine addiction. *Curr Drug Targets* **11**: 440–9.

Maldonado R, Robledo P, Berrendero F (2013). Endocannabinoid system and drug addiction: new insights from mutant mice approaches. *Curr Opin Neurobiol* **23**: 480–6.

Maldonado R, Valverde O, Berrendero F (2006). Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci* **29**: 225–232.

Malenka RC (2003). Synaptic plasticity and AMPA receptor trafficking. *Ann N Y Acad Sci* **1003**: 1–11.

Malenka RC, Bear MF (2004). LTP and LTD. *Neuron* **44**: 5–21.

Malenka RC, Nestler EJ HS (2009). Chapter 13: Higher Cognitive Function and Behavioral Control. *Mol Neuropharmacol A Found Clin Neurosci* 313–321.

Mameli M, Bellone C, Brown MTC, Lüscher C (2011). Cocaine inverts rules for synaptic plasticity of glutamate transmission in the ventral tegmental area. *Nat Neurosci* **14**: 414–416.

Mancino S, Burokas A, Gutiérrez-Cuesta J, Gutiérrez-Martos M, Martín-García E, Pucci M, *et al* (2015). Epigenetic and Proteomic Expression Changes Promoted by Eating Addictive-Like Behavior. *Neuropsychopharmacology* doi:10.1038/npp.2015.129.

Mandyam CD, Koob GF (2012). The addicted brain craves new neurons: putative role for adult-born progenitors in promoting recovery. *Trends Neurosci* **35**: 250–60.

Mansvelder HD, McGehee DS (2000). Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* **27**: 349–57.

Marcus JN, Aschkenasi CJ, Lee CE, Chemelli RM, Saper CB, Yanagisawa M, *et al* (2001). Differential expression of orexin receptors 1 and 2 in the rat brain. *J Comp Neurol* **435**: 6–25.

Marmorstein NR, Iacono WG, Legrand L (2014). Obesity and depression in adolescence and beyond: reciprocal risks. *Int J Obes* **38**: 906–911.

Martín-García E, Barbano MF, Galeote L, Maldonado R (2009). New operant model of nicotine-seeking behaviour in mice. *Int J Neuropsychopharmacol* **12**:

343–356.

Martín-García E, Burokas A, Martín M, Berrendero F, Rubí B, Kiesselbach C, *et al* (2010). Central and peripheral consequences of the chronic blockade of CB1 cannabinoid receptor with rimonabant or taranabant. *J Neurochem* **112**: 1338–13351.

Martin M, Chen BT, Hopf FW, Bowers MS, Bonci A (2006). Cocaine self-administration selectively abolishes LTD in the core of the nucleus accumbens. *Nat Neurosci* **9**: 868–869.

Martin SJ, Grimwood PD, Morris RG (2000). Synaptic plasticity and memory: an evaluation of the hypothesis. *Annu Rev Neurosci* **23**: 649–711.

Martinez JA (2000). Body-weight regulation: causes of obesity. *Proc Nutr Soc* **59**: 337–45.

Matsumoto M, Hikosaka O (2009). Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature* **459**: 837–841.

Mattes RD, Hollis J, Hayes D, Stunkard AJ (2005). Appetite: measurement and manipulation misgivings. *J Am Diet Assoc* **105**: S87-97.

Mazur WM, Duke JA, Wähälä K, Rasku S, Adlercreutz H, Morton M, *et al* (1998). Isoflavonoids and Lignans in Legumes: Nutritional and Health Aspects in Humans. 2 R01 CA56289-04, and analytical work by the EU research contract FAIR-CT95-0894. *J Nutr Biochem* **9**: 193–200.

McClain RM, Wolz E, Davidovich A, Edwards J, Bausch J (2007). Reproductive safety studies with genistein in rats. *Food Chem Toxicol* **45**: 1319–1332.

McFarland K, Lapish CC, Kalivas PW (2003). Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci* **23**: 3531–7.

Mebel DM, Wong JCY, Dong YJ, Borgland SL (2012). Insulin in the ventral tegmental area reduces hedonic feeding and suppresses dopamine concentration via increased reuptake. *Eur J Neurosci* **36**: 2336–2346.

Mejías-Aponte CA, Drouin C, Aston-Jones G (2009). Adrenergic and noradrenergic innervation of the midbrain ventral tegmental area and retrorubral field: prominent inputs from medullary homeostatic centers. *J Neurosci* **29**: 3613–26.

Melis M, Pistis M (2012). Hub and switches: endocannabinoid signalling in midbrain dopamine neurons. *Philos Trans R Soc B Biol Sci* **367**: 3276–3285.

Melis M, Pistis M, Perra S, Muntoni AL, Pillolla G, Gessa GL (2004). Endocannabinoids Mediate Presynaptic Inhibition of Glutamatergic Transmission in Rat Ventral Tegmental Area Dopamine Neurons through Activation of CB1 Receptors. *J Neurosci* **24**: 53–62.

Mena JD, Sadeghian K, Baldo BA (2011). Induction of Hyperphagia and

Carbohydrate Intake by -Opioid Receptor Stimulation in Circumscribed Regions of Frontal Cortex. *J Neurosci* **31**: 3249–3260.

Merrer J Le, Becker JAJ, Befort K, Kieffer BL (2009). Reward processing by the opioid system in the brain. *Physiol Rev* **89**: 1379–412.

Merz-Demlow BE, Duncan AM, Wangen KE, Xu X, Carr TP, Phipps WR, *et al* (2000). Soy isoflavones improve plasma lipids in normocholesterolemic, premenopausal women. *Am J Clin Nutr* **71**: 1462–9.

Meule A, Heckel D, Jurowich CF, Vögele C, Kübler A (2014). Correlates of food addiction in obese individuals seeking bariatric surgery. *Clin Obes* **4**: n/a-n/a.

Miki T, Minami K, Shinozaki H, Matsumura K, Saraya A, Ikeda H, *et al* (2005). Distinct effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 on insulin secretion and gut motility. *Diabetes* **54**: 1056–63.

Milagro FI, Moreno-Aliaga MJ, Martinez JA (2016). FTO Obesity Variant and Adipocyte Browning in Humans. *N Engl J Med* **374**: 190–1.

Mittelbronn M, Dietz K, Schluesener HJ, Meyermann R (2001). Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. *Acta Neuropathol* **101**: 249–55.

Monteiro R, Azevedo I, Azevedo I (2010). Chronic inflammation in obesity and the metabolic syndrome. *Mediators Inflamm* **2010**: .

Morales L, Olmo N Del, Valladolid-Acebes I, Fole A, Cano V, Merino B, *et al* (2012). Shift of circadian feeding pattern by high-fat diets is coincident with reward deficits in obese mice. *PLoS One* **7**: e36139.

Morton GJ, Meek TH, Schwartz MW (2014). Neurobiology of food intake in health and disease. *Nat Rev Neurosci* **15**: 367–378.

Mott SH, Packer RJ, Soldin SJ (1994). Neurologic manifestations of cocaine exposure in childhood. *Pediatrics* **93**: 557–60.

Muennig P (2008). The body politic: the relationship between stigma and obesity-associated disease. *BMC Public Health* **8**: 128.

Murray S, Tulloch A, Gold MS, Avena NM (2014). Hormonal and neural mechanisms of food reward, eating behaviour and obesity. *Nat Rev Endocrinol* **10**: 540–52.

Musacchio JM, Goldstein M, Anagnoste B, Poch G, Kopin IJ (1966). Inhibition of dopamine-beta-hydroxylase by disulfiram in vivo. *J Pharmacol Exp Ther* **152**: 56–61.

Myers MG, Leibel R (2015). Lessons from Rodent Models of Obesity. *NCBI Bookshelf A Serv Natl Libr Med Natl Institutes Heal* 1–32at <http://www.ncbi.nlm.nih.gov/pubmed/25905346>.

Narita K, Murata T, Matsuoka S (2016). The ventromedial hypothalamus oxytocin induces locomotor behavior regulated by estrogen. *Physiol Behav* **164**:

107–112.

Naukkarinen J, Surakka I, Pietiläinen KH, Rissanen A, Salomaa V, Ripatti S, *et al* (2010). Use of genome-wide expression data to mine the "Gray Zone" of GWA studies leads to novel candidate obesity genes. *PLoS Genet* **6**: e1000976.

NCD Risk Factor Collaboration (NCD-RisC) (2016). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *Lancet* **387**: 1377–1396.

Nefti W, Chaumontet C, Fromentin G, Tome D, Darcel N (2009). A high-fat diet attenuates the central response to within-meal satiation signals and modifies the receptor expression of vagal afferents in mice. *AJP Regul Integr Comp Physiol* **296**: R1681–R1686.

Nestler EJ (2001). Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci* **2**: 119–128.

Nestler EJ, Hyman SE, Holtzman DM, Malenka RC (McGraw-Hill Medical: New York, 2009). *Molecular Neuropharmacology: A Foundation for Clinical Neuroscience|Neurology Collection | McGraw-Hill Medical*. at <http://neurology.mhmedical.com>.

Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, *et al* (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **384**: 766–781.

Nicklas TA, Baranowski T, Cullen KW, Berenson G (2001). Eating patterns, dietary quality and obesity. *J Am Coll Nutr* **20**: 599–608.

Niehaus JL, Murali M, Kauer JA (2010). Drugs of abuse and stress impair LTP at inhibitory synapses in the ventral tegmental area. *Eur J Neurosci* **32**: 108–17.

Nimchinsky EA, Sabatini BL, Svoboda K (2002). Structure and function of dendritic spines. *Annu Rev Physiol* **64**: 313–353.

Nimmerjahn A, Kirchhoff F, Helmchen F (2005). Resting Microglial Cells Are Highly Dynamic Surveillants of Brain Parenchyma in Vivo. *Science (80-)* **308**: 1314–1318.

Noble EE, Billington CJ, Kotz CM, Wang C (2014). Oxytocin in the ventromedial hypothalamic nucleus reduces feeding and acutely increases energy expenditure. *Am J Physiol - Regul Integr Comp Physiol* **307**: R737–R745.

Nuzzaci D, Laderrière A, Lemoine A, Nédélec E, Pénicaud L, Rigault C, *et al* (2015). Plasticity of the Melanocortin System: Determinants and Possible Consequences on Food Intake. *Front Endocrinol (Lausanne)* **6**: 143.

O'Brien CP (2008). Review. Evidence-based treatments of addiction. *Philos Trans R Soc Lond B Biol Sci* **363**: 3277–86.

O'Brien CP, Volkow N, Li T-K (2006). What's in a Word? Addiction Versus Dependence in DSM-V. *Am J Psychiatry* **163**: 764–765.

O'Connor EC, Kremer Y, Lefort S, Harada M, Pascoli V, Rohner C, *et al* (2015). Accumbal D1R Neurons Projecting to Lateral Hypothalamus Authorize Feeding. *Neuron* **88**: 553–64.

Ogawa S, Chan J, Gustafsson J-Å, Korach KS, Pfaff DW (2003). Estrogen Increases Locomotor Activity in Mice through Estrogen Receptor α: Specificity for the Type of Activity. *Endocrinology* **144**: 230–239.

Oginsky MF, Goforth PB, Nobile CW, Lopez-Santiago L, Ferrario CR (2016). Eating "Junk-Food" Produces Rapid and Long-Lasting Increases in NAc CP-AMPA Receptors; Implications for Enhanced Cue-Induced Motivation and Food Addiction. *Neuropsychopharmacology* **41**: .

Okubo Y, Sekiya H, Namiki S, Sakamoto H, Iinuma S, Yamasaki M, *et al* (2010). Imaging extrasynaptic glutamate dynamics in the brain. *Proc Natl Acad Sci U S A* **107**: 6526–31.

Oliveira AR de, Reimer AE, Brandão ML (2014). Mineralocorticoid receptors in the ventral tegmental area regulate dopamine efflux in the basolateral amygdala during the expression of conditioned fear. *Psychoneuroendocrinology* **43**: 114–125.

Oomura Y, Yoshimatsu H (1984). Neural network of glucose monitoring system. *J Auton Nerv Syst* **10**: 359–72.

Oswald KD, Murdaugh DL, King VL, Boggiano MM (2011). Motivation for palatable food despite consequences in an animal model of binge eating. *Int J Eat Disord* **44**: 203–11.

Otani S, Bai J, Blot K (2015). Dopaminergic modulation of synaptic plasticity in rat prefrontal neurons. *Neurosci Bull* **31**: 183–190.

Overstreet DH, Knapp DJ, Breese GR, Diamond I (2009). A selective ALDH-2 inhibitor reduces anxiety in rats. *Pharmacol Biochem Behav* **94**: 255–261.

Paladini C., Celada P, Tepper J. (1999). Striatal, pallidal, and pars reticulata evoked inhibition of nigrostriatal dopaminergic neurons is mediated by GABAA receptors in vivo. *Neuroscience* **89**: 799–812.

Palkovits M (2003). Hypothalamic regulation of food intake. *Ideggyogy Sz* **56**: 288–302.

Palmiter RD (2007). Is dopamine a physiologically relevant mediator of feeding behavior? *Trends Neurosci* **30**: 375–381.

Panagis G, Mackey B, Vlachou S (2014). Cannabinoid Regulation of Brain Reward Processing with an Emphasis on the Role of CB1 Receptors: A Step Back into the Future. *Front Psychiatry* **5**: 92.

Pandit R, Jong JW de, Vanderschuren LJMJ (2011). Neurobiology of overeating

and obesity: The role of melanocortins and beyond. *Eur J Pharmacol* **660**: 28–42.

Pani PP, Trogu E, Vacca R, Amato L, Vecchi S, Davoli M (2010a). Disulfiram for the treatment of cocaine dependence. *Cochrane Database Syst Rev* CD007024doi:10.1002/14651858.CD007024.pub2.

Pani PP, Trogu E, Vacca R, Amato L, Vecchi S, Davoli M (2010b). Disulfiram for the treatment of cocaine dependence. *Cochrane Database Syst Rev* doi:10.1002/14651858.CD007024.pub2.

Parsons LH, Hurd YL (2015). Endocannabinoid signalling in reward and addiction. *Nat Rev Neurosci* **16**: 579–94.

Parylak SL, Koob GF, Zorrilla EP (2011). The dark side of food addiction. *Physiol Behav* **104**: 149–56.

Peciña S, Berridge KC (2005). Hedonic Hot Spot in Nucleus Accumbens Shell: Where Do μ-Opioids Cause Increased Hedonic Impact of Sweetness? *J Neurosci* **25**: .

Pecoraro N, Reyes F, Gomez F, Bhargava A, Dallman MF (2004). Chronic Stress Promotes Palatable Feeding, which Reduces Signs of Stress: Feedforward and Feedback Effects of Chronic Stress. *Endocrinology* **145**: 3754–3762.

Pelchat ML (2009). Food Addiction in Humans. *J Nutr* **139**: 620–622.

Penberthy JK, Ait-Daoud N, Vaughan M, Fanning T (2010). Review of treatment for cocaine dependence. *Curr Drug Abuse Rev* **3**: 49–62.

Pendergast JS, Branecky KL, Yang W, Ellacott KLJ, Niswender KD, Yamazaki S (2013). High-fat diet acutely affects circadian organisation and eating behavior. *Eur J Neurosci* **37**: 1350–6.

Pennings EJM, Leccese AP, Wolff FA de (2002). Effects of concurrent use of alcohol and cocaine. *Addiction* **97**: 773–83.

Petroff OAC (2002). Book Review: GABA and Glutamate in the Human Brain. *Neurosci* **8**: 562–573.

Petrovich GD, Ross CA, Holland PC, Gallagher M (2007). Medial Prefrontal Cortex Is Necessary for an Appetitive Contextual Conditioned Stimulus to Promote Eating in Sated Rats. *J Neurosci* **27**: 6436–6441.

Peyron C, Tighe DK, Pol AN van den, Lecea L de, Heller HC, Sutcliffe JG, *et al* (1998). Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* **18**: 9996–10015.

PEZZE M, FELDON J (2004). Mesolimbic dopaminergic pathways in fear conditioning. *Prog Neurobiol* **74**: 301–320.

Phillips AG, Vacca G, Ahn S (2008). A top-down perspective on dopamine, motivation and memory. *Pharmacol Biochem Behav* **90**: 236–249.

Phillips PEM, Stuber GD, Heien MLA V., Wightman RM, Carelli RM (2003).

Subsecond dopamine release promotes cocaine seeking. *Nature* **422**: 614–618.

Phillips RJ, Powley TL (2000). Tension and stretch receptors in gastrointestinal smooth muscle: re-evaluating vagal mechanoreceptor electrophysiology. *Brain Res Brain Res Rev* **34**: 1–26.

Piazza PV, Deroche-Gamonet V (2013). A multistep general theory of transition to addiction. *Psychopharmacology (Berl)* **229**: 387–413.

Platt SR (2007). The role of glutamate in central nervous system health and disease – A review. *Vet J* **173**: 278–286.

Plum L, Belgardt BF, Brüning JC (2006). Central insulin action in energy and glucose homeostasis. *J Clin Invest* **116**: 1761–1766.

Potenza MN, Sofuoglu M, Carroll KM, Rounsaville BJ (2011). Neuroscience of behavioral and pharmacological treatments for addictions. *Neuron* **69**: 695–712.

Powley TL, Phillips RJ (2004). Gastric satiation is volumetric, intestinal satiation is nutritive. *Physiol Behav* **82**: 69–74.

Preedy VR (Royal Society of Chemistry: Cambridge, UK, 2013). *Isoflavones : chemistry, analysis, function and effects*. .

Qi J, Zhang S, Wang H-L, Barker DJ, Miranda-Barrientos J, Morales M (2016). VTA glutamatergic inputs to nucleus accumbens drive aversion by acting on GABAergic interneurons. *Nat Neurosci* **19**: 725–33.

R Core Team (2015). R: A Language and Environment for Statistical Computing. .

Racz I, Nadal X, Alferink J, Banos JE, Rehnelt J, Martin M, *et al* (2008). Interferon-Is a Critical Modulator of CB2 Cannabinoid Receptor Signaling during Neuropathic Pain. *J Neurosci* **28**: 12136–12145.

Raivich G, Bluethmann H, Kreutzberg GW (1996). Signaling molecules and neuroglial activation in the injured central nervous system. *Keio J Med* **45**: 239–47.

Raivich G, Bohatschek M, Kloss CU, Werner A, Jones LL, Kreutzberg GW (1999). Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function. *Brain Res Brain Res Rev* **30**: 77–105.

Ransohoff RM, Perry VH (2009). Microglial Physiology: Unique Stimuli, Specialized Responses. *Annu Rev Immunol* **27**: 119–145.

Reboussin BA, Anthony JC (2006). Is there Epidemiological Evidence to Support the Idea that a Cocaine Dependence Syndrome Emerges Soon after Onset of Cocaine Use? *Neuropsychopharmacology* **31**: 2055–2064.

Reimann F, Gribble FM (2002). Glucose-sensing in glucagon-like peptide-1-secreting cells. *Diabetes* **51**: 2757–63.

Richter RM, Pich EM, Koob GF, Weiss F (1995). Sensitization of cocaine-

stimulated increase in extracellular levels of corticotropin-releasing factor from the rat amygdala after repeated administration as determined by intracranial microdialysis. *Neurosci Lett* **187**: 169–72.

Riezzo I, Fiore C, Carlo D De, Pascale N, Neri M, Turillazzi E, *et al* (2012). Side effects of cocaine abuse: multiorgan toxicity and pathological consequences. *Curr Med Chem* **19**: 5624–46.

Rinaman L (2011). Hindbrain noradrenergic A2 neurons: diverse roles in autonomic, endocrine, cognitive, and behavioral functions. *Am J Physiol Regul Integr Comp Physiol* **300**: R222-35.

Ritter RC (2004). Gastrointestinal mechanisms of satiation for food. *Physiol Behav* **81**: 249–73.

Roberts DCS (2005). Preclinical evidence for GABAB agonists as a pharmacotherapy for cocaine addiction. *Physiol Behav* **86**: 18–20.

Robinson MJF, Fischer AM, Ahuja A, Lesser EN, Maniates H (2015). Roles of "Wanting" and "Liking" in Motivating Behavior: Gambling, Food, and Drug Addictions. *Curr Top Behav Neurosci* doi:10.1007/7854_2015_387.

Robinson TE, Kolb B (2004). Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology* **47 Suppl 1**: 33–46.

Rolls ET (2011). Taste, olfactory and food texture reward processing in the brain and obesity. *Int J Obes* **35**: 550–561.

Rolls ET (2012). Taste, olfactory and food texture reward processing in the brain and the control of appetite. *Proc Nutr Soc* **71**: 488–501.

Root DH, Mejias-Aponte CA, Qi J, Morales M (2014). Role of Glutamatergic Projections from Ventral Tegmental Area to Lateral Habenula in Aversive Conditioning. *J Neurosci* **34**: 13906–13910.

Rothwell PE, Thomas MJ, Gewirtz JC (2009). Distinct profiles of anxiety and dysphoria during spontaneous withdrawal from acute morphine exposure. *Neuropsychopharmacology* **34**: 2285–95.

Rowland IR, Wiseman H, Sanders TAB, Adlercreutz H, Bowey EA (2000). Interindividual Variation in Metabolism of Soy Isoflavones and Lignans: Influence of Habitual Diet on Equol Production by the Gut Microflora. *Nutr Cancer* **36**: 27–32.

Rozengurt N, Wu SV, Chen MC, Huang C, Sternini C, Rozengurt E (2006). Colocalization of the alpha-subunit of gustducin with PYY and GLP-1 in L cells of human colon. *Am J Physiol Gastrointest Liver Physiol* **291**: G792-802.

Rudenga KJ, Sinha R, Small DM (2013). Acute stress potentiates brain response to milkshake as a function of body weight and chronic stress. *Int J Obes (Lond)* **37**: 309–16.

Rugino T (2007). A review of modafinil film-coated tablets for attention-

deficit/hyperactivity disorder in children and adolescents. *Neuropsychiatr Dis Treat* **3**: 293–301.

Russo SJ, Dietz DM, Dumitriu D, Morrison JH, Malenka RC, Nestler EJ (2010). The addicted synapse: mechanisms of synaptic and structural plasticity in nucleus accumbens. *Trends Neurosci* **33**: 267–276.

Russo SJ, Mazei-Robison MS, Ables JL, Nestler EJ (2009). Neurotrophic factors and structural plasticity in addiction. *Neuropharmacology* **56 Suppl 1**: 73–82.

Russo SJ, Nestler EJ (2013). The brain reward circuitry in mood disorders. *Nat Rev Neurosci* **14**: 609–25.

Rutecki PA (1992). Neuronal excitability: voltage-dependent currents and synaptic transmission. *J Clin Neurophysiol* **9**: 195–211.

Saijo K, Glass CK (2011). Microglial cell origin and phenotypes in health and disease. *Nat Rev Immunol* **11**: 775–787.

Saito Y, Cheng M, Leslie FM, Civelli O (2001). Expression of the melanin-concentrating hormone (MCH) receptor mRNA in the rat brain. *J Comp Neurol* **435**: 26–40.

Salamone JD, Correa M, Mingote SM, Weber SM (2005). Beyond the reward hypothesis: alternative functions of nucleus accumbens dopamine. *Curr Opin Pharmacol* **5**: 34–41.

Sampey BP, Vanhoose AM, Winfield HM, Freemerman AJ, Muehlbauer MJ, Fueger PT, *et al* (2009). Cafeteria Diet Is a Robust Model of Human Metabolic Syndrome With Liver and Adipose Inflammation : Comparison to High-Fat Diet. *Obesity* **19**: 1109–1117.

Sánchez-Hervás E (2016). Cocaine addiction: treatments and future perspectives. *Trends Psychiatry Psychother* **38**: 242–243.

Saper CBCB, Chou TC, Elmquist JKJK, Barone M, Leopold L, Friedman JM, *et al* (2002). The need to feed: homeostatic and hedonic control of eating. *Neuron* **36**: 199–211.

Schäffler A, Müller-Ladner U, Schölmerich J, Büchler C (2006). Role of Adipose Tissue as an Inflammatory Organ in Human Diseases. *Endocr Rev* **27**: 449–467.

Schäffler A, Schölmerich J, Salzberger B (2007). Adipose tissue as an immunological organ: Toll-like receptors, C1q/TNFs and CTRPs. *Trends Immunol* **28**: 393–399.

Schleinitz D, Carmienke S, Böttcher Y, Tönjes A, Berndt J, Klöting N, *et al* (2010). Role of genetic variation in the cannabinoid type 1 receptor gene (*CNR1*) in the pathophysiology of human obesity. *Pharmacogenomics* **11**: 693–702.

Schmidt FM, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T, *et al* (2015). Inflammatory cytokines in general and central obesity and modulating effects of physical activity. *PLoS One* **10**: e0121971.

Schottenfeld RS, Chawarski MC, Cubells JF, George TP, Lappalainen J, Kosten TR (2014). Randomized clinical trial of disulfiram for cocaine dependence or abuse during buprenorphine treatment. *Drug Alcohol Depend* **136**: 36–42.

Schramm-Sapyta NL, Olsen CM, Winder DG (2006). Cocaine Self-Administration Reduces Excitatory Responses in the Mouse Nucleus Accumbens Shell. *Neuropsychopharmacology* **31**: 1444–1451.

Schröder H, Marrugat J, Vila J, Covas MI, Elosua R (2004). Adherence to the traditional mediterranean diet is inversely associated with body mass index and obesity in a spanish population. *J Nutr* **134**: 3355–61.

Schroeder FA, Penta KL, Matevossian A, Jones SR, Konradi C, Tapper AR, *et al* (2008). Drug-induced activation of dopamine D(1) receptor signaling and inhibition of class I/II histone deacetylase induce chromatin remodeling in reward circuitry and modulate cocaine-related behaviors. *Neuropsychopharmacology* **33**: 2981–92.

Schroeder JP, Cooper DA, Schank JR, Lyle MA, Gaval-Cruz M, Ogbonmwan YE, *et al* (2010). Disulfiram Attenuates Drug-Primed Reinstatement of Cocaine Seeking via Inhibition of Dopamine β-Hydroxylase. *Neuropsychopharmacology* **35**: 2440–2449.

Schultz W (2010). Subjective neuronal coding of reward: temporal value discounting and risk. *Eur J Neurosci* **31**: 2124–2135.

Schultz W (2015). Neuronal reward and decision signals: from theories to data. *Physiol Rev* **95**: 853–951.

Schwartz GJ, Salorio CF, Skoglund C, Moran TH (1999). Gut vagal afferent lesions increase meal size but do not block gastric preload-induced feeding suppression. *Am J Physiol* **276**: R1623-9.

Scuteri A, Sanna S, Chen W-M, Uda M, Albai G, Strait J, *et al* (2007). Genome-Wide Association Scan Shows Genetic Variants in the FTO Gene Are Associated with Obesity-Related Traits. *PLoS Genet* **3**: e115.

Seguin P, Zheng W, Smith DL, Deng W (2004). Isoflavone content of soybean cultivars grown in eastern Canada. *J Sci Food Agric* **84**: 1327–1332.

Seilhean D, Kobayashi K, He Y, Uchihara T, Rosenblum O, Katlama C, *et al* (1997). Tumor necrosis factor-alpha, microglia and astrocytes in AIDS dementia complex. *Acta Neuropathol* **93**: 508–17.

Sellayah D, Cagampang FR, Cox RD (2014). On the Evolutionary Origins of Obesity: A New Hypothesis. *Endocrinology* **155**: 1573–1588.

Shabana, Hasnain S (2016). Obesity, More than a “Cosmetic” Problem. Current Knowledge and Future Prospects of Human Obesity Genetics. *Biochem Genet* **54**: 1–28.

Sharma S, Fernandes MF, Fulton S (2013). Adaptations in brain reward circuitry

underlie palatable food cravings and anxiety induced by high-fat diet withdrawal. *Int J Obes (Lond)* **37**: 1183–91.

Shepherd GM (1996). The dendritic spine: a multifunctional integrative unit. *J Neurophysiol* **75**: 2197–210.

Simon C, Götz M, Dimou L (2011). Progenitors in the adult cerebral cortex: Cell cycle properties and regulation by physiological stimuli and injury. *Glia* **59**: 869–881.

Small DM, Jones-Gotman M, Dagher A (2003). Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. *Neuroimage* **19**: 1709–15.

Smith ACW, Scofield MD, Kalivas PW (2015). The tetrapartite synapse: Extracellular matrix remodeling contributes to corticoaccumbens plasticity underlying drug addiction. *Brain Res* **1628**: 29–39.

Smith D V, Rigney AE, Delgado MR (2016). Distinct Reward Properties are Encoded via Corticostriatal Interactions. *Sci Rep* **6**: 20093.

Smith KS, Berridge KC (2007). Opioid Limbic Circuit for Reward: Interaction between Hedonic Hotspots of Nucleus Accumbens and Ventral Pallidum. *J Neurosci* **27**: .

Smith Y, Villalba R (2008). Striatal and extrastriatal dopamine in the basal ganglia: An overview of its anatomical organization in normal and Parkinsonian brains. *Mov Disord* **23**: S534–S547.

Soria G, Mendizábal V, Touriño C, Robledo P, Ledent C, Parmentier M, *et al* (2005). Lack of CB1 Cannabinoid Receptor Impairs Cocaine Self-Administration. *Neuropsychopharmacology* **30**: 1670–1680.

Steindel F, Lerner R, Häring M, Ruehle S, Marsicano G, Lutz B, *et al* (2013). Neuron-type specific cannabinoid-mediated G protein signalling in mouse hippocampus. *J Neurochem* **124**: 795–807.

Steketee JD (2003). Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Res Brain Res Rev* **41**: 203–28.

Stice E, Figlewicz DP, Gosnell BA, Levine AS, Pratt WE (2013). The contribution of brain reward circuits to the obesity epidemic. *Neurosci Biobehav Rev* **37**: 2047–2058.

Stice E, Yokum S, Blum K, Bohon C (2010). Weight gain is associated with reduced striatal response to palatable food. *J Neurosci* **30**: 13105–9.

Stockburger J, Schmälzle R, Flaisch T, Bublatzky F, Schupp HT (2009). The impact of hunger on food cue processing: An event-related brain potential study. *Neuroimage* **47**: 1819–1829.

Stoeckel LE, Weller RE, Cook EW, Twieg DB, Knowlton RC, Cox JE (2008).

Widespread reward-system activation in obese women in response to pictures of high-calorie foods. *Neuroimage* **41**: 636–47.

Suh JJ, Pettinati HM, Kampman KM, O'Brien CP (2006a). The Status of Disulfiram. *J Clin Psychopharmacol* **26**: 290–302.

Suh JJ, Pettinati HM, Kampman KM, O'Brien CP (2006b). The status of disulfiram: a half of a century later. *J Clin Psychopharmacol* **26**: 290–302.

Sun W, Matthews EA, Nicolas V, Schoch S, Dietrich D (2016). NG2 glial cells integrate synaptic input in global and dendritic calcium signals. *Elife* **5**: .

Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro a E, Opara EC, *et al* (1995). Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. *Metabolism* **44**: 645–51.

Suto N, Ecke LE, Wise RA (2009). Control of within-binge cocaine-seeking by dopamine and glutamate in the core of nucleus accumbens. *Psychopharmacology (Berl)* **205**: 431–439.

Suto N, Wise RA (2011). Satiating Effects of Cocaine Are Controlled by Dopamine Actions in the Nucleus Accumbens Core. *J Neurosci* **31**: 17917–17922.

Suzumura A, Mezitis SG, Gonatas NK, Silberberg DH (1987). MHC antigen expression on bulk isolated macrophage-microglia from newborn mouse brain: induction of Ia antigen expression by gamma-interferon. *J Neuroimmunol* **15**: 263–78.

Swanson LW, Hartman BK (1975). The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-B-hydroxylase as a marker. *J Comp Neurol* **163**: 467–505.

Taku K, Melby MK, Kronenberg F, Kurzer MS, Messina M (2012). Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity. *Menopause J North Am Menopause Soc* **19**: 776–790.

Takumi Y, Matsubara A, Rinvik E, Ottersen OP (1999). The arrangement of glutamate receptors in excitatory synapses. *Ann N Y Acad Sci* **868**: 474–82.

Taylor AMW, Castonguay A, Ghogha A, Vayssiere P, Pradhan AAA, Xue L, *et al* (2016). Neuroimmune Regulation of GABAergic Neurons Within the Ventral Tegmental Area During Withdrawal from Chronic Morphine. *Neuropsychopharmacology* **41**: 949–59.

Teegarden SL, Bale TL (2007). Decreases in dietary preference produce increased emotionality and risk for dietary relapse. *Biol Psychiatry* **61**: 1021–9.

Thaler JP, Yi C-X, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, *et al* (2012). Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest* **122**: 153–62.

Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001). Long-term depression in the

nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat Neurosci* **4**: 1217–1223.

Thompson AM, Gosnell BA, Wagner JJ (2002). Enhancement of long-term potentiation in the rat hippocampus following cocaine exposure. *Neuropharmacology* **42**: 1039–42.

Tomasi D, Volkow ND (2013). Striatocortical pathway dysfunction in addiction and obesity: differences and similarities. *Crit Rev Biochem Mol Biol* **48**: 1–19.

Törrönen R, Marselos M (1978). Changes in the Hepatic Copper Content after Treatment with Foreign Compounds. 247–249doi:10.1007/978-3-642-66896-8_46.

Tossmann P, Boldt S, Tensil MD (2001). The use of drugs within the techno party scene in European metropolitan cities. *Eur Addict Res* **7**: 2–23.

Trigo JM, Martin-García E, Berrendero F, Robledo P, Maldonado R (2010). The endogenous opioid system: a common substrate in drug addiction. *Drug Alcohol Depend* **108**: 183–94.

Turner MD, Nedjai B, Hurst T, Pennington DJ (2014). Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochim Biophys Acta - Mol Cell Res* **1843**: 2563–2582.

Ukkola O, Santaniemi M (2002). Adiponectin: a link between excess adiposity and associated comorbidities? *J Mol Med* **80**: 696–702.

Ungless MA, Whistler JL, Malenka RC, Bonci A (2001). Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature* **411**: 583–587.

Uno H, Matsuyama T, Akita H, Nishimura H, Sugita M (1997). Induction of tumor necrosis factor-alpha in the mouse hippocampus following transient forebrain ischemia. *J Cereb Blood Flow Metab* **17**: 491–9.

Urstadt KR, Kally P, Zaidi SF, Stanley BG (2013). Ipsilateral feeding-specific circuits between the nucleus accumbens shell and the lateral hypothalamus: regulation by glutamate and GABA receptor subtypes. *Neuropharmacology* **67**: 176–82.

Valdearcos M, Robblee MMM, Benjamin DII, Nomura DKK, Xu AWW, Koliwad SKK (2014). Microglia Dictate the Impact of Saturated Fat Consumption on Hypothalamic Inflammation and Neuronal Function. *Cell Rep* **9**: 2124–2139.

Valdearcos M, Xu AW, Koliwad SK (2015). Hypothalamic inflammation in the control of metabolic function. *Annu Rev Physiol* **77**: 131–60.

Valdivia S, Cornejo MP, Reynaldo M, Francesco PN De, Perello M (2015). Escalation in high fat intake in a binge eating model differentially engages dopamine neurons of the ventral tegmental area and requires ghrelin signaling. *Psychoneuroendocrinology* **60**: 206–216.

van der Klaauw AA, Farooqi IS (2015). The Hunger Genes: Pathways to Obesity.

Cell **161**: 119–132.

Vanderschuren LJ, Kalivas PW (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology (Berl)* **151**: 99–120.

Velasquez MT, Bhathena SJ (2007). Role of dietary soy protein in obesity. *Int J Med Sci* **4**: 72–82.

Ventura R, Cabib S, Alcaro A, Orsini C, Puglisi-Allegra S (2003). Norepinephrine in the prefrontal cortex is critical for amphetamine-induced reward and mesoaccumbens dopamine release. *J Neurosci* **23**: 1879–85.

Ventura R, Morrone C, Puglisi-Allegra S (2007). Prefrontal/accumbal catecholamine system determines motivational salience attribution to both reward- and aversion-related stimuli. *Proc Natl Acad Sci* **104**: 5181–5186.

Verdich C, Toubro S, Buemann B, Lysgård Madsen J, Juul Holst J, Astrup A (2001). The role of postprandial releases of insulin and incretin hormones in meal-induced satiety--effect of obesity and weight reduction. *Int J Obes Relat Metab Disord* **25**: 1206–14.

Verma S, Nakaoke R, Dohgu S, William AB (2006). Release of cytokines by brain endothelial cells: A polarized response to lipopolysaccharide. *Brain Behav Immun* **20**: 449–455.

Volkow ND, O'Brien CP (2007). Issues for DSM-V: should obesity be included as a brain disorder? *Am J Psychiatry* **164**: 708–10.

Volkow ND, Wang G-J, Baler RD (2011a). Reward, dopamine and the control of food intake: implications for obesity. *Trends Cogn Sci* **15**: 37–46.

Volkow ND, Wang G-J, Begleiter H, Porjesz B, Fowler JS, Telang F, *et al* (2006). High levels of dopamine D2 receptors in unaffected members of alcoholic families: possible protective factors. *Arch Gen Psychiatry* **63**: 999–1008.

Volkow ND, Wang G-J, Fowler JS, Telang F (2008a). Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. *Philos Trans R Soc Lond B Biol Sci* **363**: 3191–200.

Volkow ND, Wang G-J, Fowler JS, Tomasi D (2012). Addiction Circuitry in the Human Brain. *Annu Rev Pharmacol Toxicol* **52**: 321–36.

Volkow ND, Wang G-J, Telang F, Fowler JS, Logan J, Jayne M, *et al* (2007). Profound Decreases in Dopamine Release in Striatum in Detoxified Alcoholics: Possible Orbitofrontal Involvement. *J Neurosci* **27**: 12700–12706.

Volkow ND, Wang G-J, Telang F, Fowler JS, Thanos PK, Logan J, *et al* (2008b). Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. *Neuroimage* **42**: 1537–43.

Volkow ND, Wang G-J, Tomasi D, Baler RD (2013a). The addictive dimensionality

of obesity. *Biol Psychiatry* **73**: 811–8.

Volkow ND, Wang G-J, Tomasi D, Baler RD (2013b). Obesity and addiction: neurobiological overlaps. *Obes Rev* **14**: 2–18.

Volkow ND, Wang GJ, Fowler JS, Tomasi D, Baler R (2011b). Food and Drug Reward: Overlapping Circuits in Human Obesity and Addiction. *Curr Top Behav Neurosci* **11**: 1–24.

Volkow ND, Wise RA (2005). How can drug addiction help us understand obesity? *Nat Neurosci* **8**: 555–60.

Wang G-J, Volkow ND, Thanos PK, Fowler JS (2009). Imaging of Brain Dopamine Pathways. *J Addict Med* **3**: 8–18.

Wang X, Barone FC, Aiyar N V, Feuerstein GZ (1997). Interleukin-1 receptor and receptor antagonist gene expression after focal stroke in rats. *Stroke* **28**: 155-61–2.

Wang X, Loram LC, Ramos K, Jesus AJ de, Thomas J, Cheng K, *et al* (2012). Morphine activates neuroinflammation in a manner parallel to endotoxin. *Proc Natl Acad Sci U S A* **109**: 6325–30.

Warner A, Norman AB (2000). Mechanisms of cocaine hydrolysis and metabolism in vitro and in vivo: a clarification. *Ther Drug Monit* **22**: 266–70.

Weijer BA de, Giessen E van de, Amelsvoort TA van, Boot E, Braak B, Janssen IM, *et al* (2011). Lower striatal dopamine D2/3 receptor availability in obese compared with non-obese subjects. *EJNMMI Res* **1**: 37.

Weinshenker D (2010). Cocaine sobers up. *Nat Med* **16**: 969–970.

Weiss F, Ciccocioppo R, Parsons LH, Katner S, Liu X, Zorrilla EP, *et al* (2001). Compulsive drug-seeking behavior and relapse: Neuroadaptation, stress, and conditioning factors. *Biol basis cocaine Addict* **937**: 1–26.

Whitcomb DC, Puccio AM, Vigna SR, Taylor IL, Hoffman GE (1997). Distribution of pancreatic polypeptide receptors in the rat brain. *Brain Res* **760**: 137–49.

WHO (2013). The world health report 2013. *World Heal Organ Press* 146doi:10.1126/scitranslmed.3006971.

Williams EP, Mesidor M, Winters K, Dubbert PM, Wyatt SB (2015). Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem. *Curr Obes Rep* **4**: 363–370.

Williams KW, Elmquist JK (2012). From neuroanatomy to behavior: central integration of peripheral signals regulating feeding behavior. *Nat Neurosci* **15**: 1350–1355.

Willyard C (2014). Heritability: The family roots of obesity. *Nature* **508**: S58–S60.

Wise RA (2006). Role of brain dopamine in food reward and reinforcement. *Philos Trans R Soc Lond B Biol Sci* **361**: 1149–58.

Withrow D, Alter D a (2011). The economic burden of obesity worldwide: a systematic review of the direct costs of obesity. *Obes Rev* **12**: 131–41.

Wu S V., Rozengurt N, Yang M, Young SH, Sinnett-Smith J, Rozengurt E (2002). Expression of bitter taste receptors of the T2R family in the gastrointestinal tract and enteroendocrine STC-1 cells. *Proc Natl Acad Sci* **99**: 2392–2397.

Wu Y, Dissing-Olesen L, MacVicar BA, Stevens B (2015). Microglia: Dynamic Mediators of Synapse Development and Plasticity. *Trends Immunol* **36**: 605–613.

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, *et al* (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* **7**: 941–946.

Yang J, Loos RJF, Powell JE, Medland SE, Speliotes EK, Chasman DI, *et al* (2012). FTO genotype is associated with phenotypic variability of body mass index. *Nature* **490**: 267–272.

Yanovski S (2003). Sugar and Fat: Cravings and Aversions. *J Nutr* **133**: 835S–837.

Yao L, Fan P, Arolfo M, Jiang Z, Olive MF, Zablocki J, *et al* (2010). Inhibition of aldehyde dehydrogenase-2 suppresses cocaine seeking by generating THP, a cocaine use–dependent inhibitor of dopamine synthesis. *Nat Med* **16**: 1024–1028.

Yim CY, Mogenson GJ (1980). Electrophysiological studies of neurons in the ventral tegmental area of Tsai. *Brain Res* **181**: 301–13.

Young LR, Nestle M (2002). The contribution of expanding portion sizes to the US obesity epidemic. *Am J Public Health* **92**: 246–9.

Zaheer K, Humayoun Akhtar M (2017). An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. *Crit Rev Food Sci Nutr* **57**: 1280–1293.

Zessen R van, Phillips JL, Budygin EA, Stuber GD (2012). Activation of VTA GABA neurons disrupts reward consumption. *Neuron* **73**: 1184–94.

Zhang C, Wei N-L, Wang Y, Wang X, Zhang J-G, Zhang K (2015). Deep brain stimulation of the nucleus accumbens shell induces anti-obesity effects in obese rats with alteration of dopamine neurotransmission. *Neurosci Lett* **589**: 1–6.

Zhang J-M, An J (2007). Cytokines, inflammation, and pain. *Int Anesthesiol Clin* **45**: 27–37.

Zhang K, Sejnowski TJ (2000). A universal scaling law between gray matter and white matter of cerebral cortex. *Proc Natl Acad Sci* **97**: 5621–5626.

Zheng H, Patterson LM, Berthoud H-R (2007). Orexin signaling in the ventral tegmental area is required for high-fat appetite induced by opioid stimulation of the nucleus accumbens. *J Neurosci* **27**: 11075–82.

Zhu BT, Han G-Z, Shim J-Y, Wen Y, Jiang X-R (2006). Quantitative Structure-

Activity Relationship of Various Endogenous Estrogen Metabolites for Human Estrogen Receptor α and β Subtypes: Insights into the Structural Determinants Favoring a Differential Subtype Binding. *Endocrinology* **147**: 4132–4150.

Ziauddeen H, Fletcher PC (2013). Is food addiction a valid and useful concept? *Obes Rev* **14**: 19–28.

Zimmerman J, Fisher M (2017). Avoidant/Restrictive Food Intake Disorder (ARFID). *Curr Probl Pediatr Adolesc Health Care* **47**: 95–103.

Zimmermann C, Cederroth CR, Bourgoin L, Foti M, Nef S (2012). Prevention of Diabetes in *db/db* Mice by Dietary Soy Is Independent of Isoflavone Levels. *Endocrinology* **153**: 5200–5211.

Zöllner C, Stein C (2007). Opioids. *Handb Exp Pharmacol* 31–63at <http://www.ncbi.nlm.nih.gov/pubmed/17087119>.

Zou S, Fitting S, Hahn Y-K, Welch SP, El-Hage N, Hauser KF, *et al* (2011). Morphine potentiates neurodegenerative effects of HIV-1 Tat through actions at -opioid receptor-expressing glia. *Brain* **134**: 3616–3631.

ANNEX

ARTICLE 4

TIME-COURSE AND DYNAMICS OF OBESITY-RELATED BEHAVIORAL CHANGES INDUCED BY ENERGY-DENSE FOODS IN MICE

Jose Espinosa-Carrasco[*], Aurelijus Burokas[*], Marta Fructuoso, Ionas Erb, Elena Martín-García, **Miriam Gutiérrez-Martos**, Cedric Notredame, Rafael Maldonado, Mara Dierssen

Addict Biol. 2018 Mar 1;23(2):531–43. DOI: 10.1111/adb.12595