TESI DOCTORAL UPF / 2013

Thomas Guegan

Common neuroplasticity mechanisms underlying drugs and food reward

Common neuroplasticity mechanisms underlying drugs and food reward

Thomas Guegan

Universitat Pompeu Fabra
Barcelona

COMMON NEUROPLASTICITY MECHANISMS UNDERLYING DRUGS AND FOOD REWARD

Thomas Guegan

DOCTORAL THESIS UPF / 2013

Thesis director

Dr. Miquel Martin Sanchez

Pr. Rafael Maldonado López

Departament de Ciències Experimentals i de la Salut

UNIVERSITAT POMPEU FABRA

A mes frères, mon père, ma mère y a ti

Acknowledgements

Agradezco a toda la gente que ha compartido conmigo estos años de tesis, ya que todos ellos han contribuido, bien directa o indirectamente, al desarrollo de este trabajo. Quiero agradecer especialmente a Rafael Maldonado y Miquel Martín por su supervisión y implicación en la realización de esta tesis.

Este trabajo es producto de un esfuerzo continuado, en el cual ha sido fundamental el apoyo científico, técnico y humano recibido. He tenido la suerte de trabajar en un proyecto interesante y tanto la guía de la tesis como el ambiente de trabajo en el laboratorio han sido excelentes. Además, he podido conocer a gente extraordinaria con la que no sólo he compartido el lugar de trabajo, sino también muchos momentos buenos fuera de él. Así mismo, también me gustaría dar las gracias a mi familia y amigos por su apoyo incondicional día a día. Por todos estos motivos, gracias. No lo hubiera conseguido sin vosotros.

Abstract

Drug addiction and some eating disorders present striking similarities in their behavioural symptoms. It has been postulated that excessive consumption of drugs of abuse and palatable food could lead to the development of similar neuronal alterations in the brain reward circuit that may account for the resemblance of these pathologies. In the present thesis, we demonstrate that repeated operant training with palatable food promotes behavioural alterations and structural plasticity changes in the mesocorticolimbic circuit that are reminiscent to those observed with drugs of abuse. Furthermore, we identify the cannabinoid receptor 1 as a common neurobiological substrate underlying these alterations. Finally, we uncover several synaptic proteins commonly implicated in the retrieval of drug and palatable food rewarding memories that may represent part of the common neurobiological basis underlying drug and palatable food craving.

Resumen

La adicción a las drogas de abuso y determinados trastornos alimentarios comparten varios síntomas comportamentales. Algunos estudios han sugerido que el consumo excesivo de drogas y de comida palatable podrían producir alteraciones neuronales similares en el circuito cerebral de recompensa. En esta tesis, hemos demostrado que un aprendizaje operante prolongado con comida palatable provoca la aparición de alteraciones comportamentales y cambios de plasticidad estructurales en el circuito mesocorticolimbico que son reminiscentes de los observados con las drogas de abuso. Así mismo, hemos identificamos al receptor cannabinoide

1 como un sustrato neurobiológico común a estas alteraciones. Finalmente, hemos caracterizado varias proteínas sinápticas implicadas en la reactivacion de la memoria asociada a los efectos placenteros de las drogas y la comida palatable. Nuestras observaciones contribuyen a definir las bases neuronales subyacentes a la necesidad de consumir drogas y comida palatable.

Abbreviations

2-AG: 2-arachidonoylglycerol

AMPA-R: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

BDNF: brain-derived neurotrophic factor

CASP-2: calcium-dependent secretion activator 2

CB1-R: cannabinoid receptor 1

CB2-R: cannabinoid receptor 2

Cdk5: cyclin dependent kinase 5

CK2: caseine kinase 2

CPP: conditioned place preference

CNS: central nervous system

DA: dopamine

DA D1-R: dopamine receptor D1

DA D2-R: dopamine receptor D2

DSM: Diagnostic and Statistical Manual of Mental Disorders

ERK: extracellular signal-regulated kinase

FR: fixed ratio

GABA: gamma-aminobutyric acid

HPC: hippocampus

JNK: c jun N-terminal protein kinase

KO: knockout

LSD: lysergic acid diethylamide

LTD: long-term depression

LTP: long-term potentiation

mGluR: metabotropic glutamate receptor

mPFC: medial prefrontal cortex

mTOR: mammalian target of rapamycin

NAc: nucleus accumbens

NMDA-R: N-methyl-D-aspartate receptor

PFC: prefrontal cortex

PKA: protein kinase A

PR: progressive ratio

THC: Δ9-tetrahydrocannabinol

VTA: ventral tegmental area

WT: wild-type

Tables of figures

Table of Contents

INTRODUCTION

1. DRUG ADDICTION

1.1. Overview

Drug addiction, also known as substance dependence, represents an abnormal behavioural state, in which individuals develop a chronic and uncontrolled consumption toward a natural or synthetic compound, named drug of abuse. Although considered in the past as lack of will power, or a way of self-destruction, it is now clear that drug addiction is a neuropsychiatric disease (O'Brien, 2003). Indeed, growing evidences acknowledge that drug addiction results from different cognitive and psychological dysfunctions, due to the repeated pharmacological insult exerted by drugs of abuse on the brain (Kalivas and Volkow, 2005). Clinically, this brain disease can be diagnosed thanks to several psychiatric manuals, such as the *Diagnostic and Statistical Manual of Mental Disorders* (DSM) of the American Psychiatric Association (DSM-IV; APA, 1994). Thus, according to its last version, the DSM IV, drug consumption is clinically considered pathologic if drug users manifest at least three different symptomatic criteria, among all described, in a same 12-month period (see box 1). Nevertheless, in an attempt to summarize, neuroscientists commonly agreed to defined drug addiction as a chronically relapsing brain disease characterized by three main criteria: (1) loss of control in limiting or stopping drug intake, (2) compulsion to seek and take the drug despite harmful consequences, and (3) emergence of a negative emotional state when access to the drug is prevented (Koob and Volkow, 2010).

1. Tolerance symptoms, as defined by either of the following:
a. A need for increased amounts of the substance to achieve the desired effects
b. Diminished effects with the same amount of the substance
2. Withdrawal, as manifested by either of the following:
a. Appearance of the characteristic withdrawal syndrome
b. The substance is taken to relieve or avoid withdrawal symptoms
3. The substance is taken in larger amounts or over longer periods than intended
4. Persistent desire or unsuccessful efforts to cut down or control substance use
5. A great deal of time spent in activities necessary to obtain or use the substance and recover from its effects
6. Important social, occupational, or recreational activities are given up or reduced because of substance use
7. Substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance

Box 1. Symptomatic criteria that are required by the DSM IV (three or more in the same 12 month period) to define a maladaptive pattern of substance use as drug addiction

In the last decades, drug availability and purity have importantly increased with the improvement of drug processing, and in parallel the rate of drug use. In fact, it is estimated that 230 millions, or 5 % of the entire world's adult population (i.e. people aged 15-64), have used an illicit drug at least once in 2010. Moreover, the legal use of tobacco and alcohol is even more concerning, with an annual prevalence of use of 25% and 42% in the world's adult population, respectively. These high rates of drug use are therefore a critical threat for public health, as chronic drug uses have catastrophic health and social consequences. In fact, drug consumption, including licit and illicit drugs, represents, nowadays, the leading cause of worldwide preventable death (UNODC, World Drug Report 2012).

In this context, few therapeutic options are available and the best strategies still rely on medical prevention and reinforcement of drug control policies. At a clinical level, addiction can be treated by behavioural therapies that provide a psychological support to help to quit the drug, combined with

pharmaco-substitution to alleviate withdrawal syndrome (National institute of drug of abuse, 1999). Nevertheless, actual addiction treatments remain largely unsuccessful. Thus, once become addicted, it is observed that most of individuals (i.e. 80 to 90%) seeking for treatment, actually relapse at least once in their life, if not constantly (Hendershot et al, 2011). Therefore, this high rate of relapse to drug use after detoxification represents a major clinical problem, and understanding the neurobiology of relapse constitutes the primary challenge of drug addiction researches.

1.2. Vulnerability to addiction

Although many people experience with both licit and illicit drugs over their lifetime, not all individuals become addict. Indeed, it is estimated that the percentage of consumers that develop addiction as a function of ever having tried a drug varies from approximately 9% for marijuana to 31% for tobacco (Anthony et al, 1994). Therefore, a question of fundamental relevance concerns the factors that explain who will make the transition from initial use of these substances to the ultimate stage of chronic addiction (Swendsen and Le Moal, 2011).

The factors that can influence vulnerability include sociologic factors. Thus, epidemiology has long shown that the rates for most substance use disorders increase as a function of male sex, younger age, lower education, unmarried status and limited income (Swendsen and Le Moal, 2011). Among these factors, the strongest and most consistent effects are typically seen for age. In particular, adolescence represents the period of greatest vulnerability for this disorder, with an increase of its prevalence of sixfold between the age of 13 and 18 (Merikangas et al, 2010). In this period of

cortical brain development, many important cognitive and emotional functions are still maturing, promoting high vulnerable phenotypes, such as novelty and sensation seeking, risk taking, mood instability or poor inhibitory control (Crews et al, 2007). Importantly, adolescence on-set substance use is a significant predictor of substance use disorders over the lifespan (Grant and Dawson, 2003). For instance, the age at which smoking begins influences the total years of smoking, the number of cigarettes smoked in adulthood, and the likelihood of quitting.

A second factor concerns psychiatric and psychological factors that reflect an individual's own preferences, experiences, or problems. Indeed, a large body of literature has accumulated demonstrating that mental disorders such as depression, anxiety or schizophrenia are associated with substance use disorders, probably in attempt of self-medication (Swendsen and Le Moal, 2011).

Finally, a third factor concerns basic biological and genetic factors that will determine the physiological effects of a given substance and influence its addictive valence, such as genes involved in peripheral drug metabolism or central brain drug targets. Indeed, twin and adoption studies demonstrated that genetic factors could account for approximately 40% of the total variability of the phenotype (Swendsen and Le Moal, 2011).

1.3. Different stages of the addiction process

The occasional but limited use of drugs is clinically distinct from compulsive drug seeking and uncontrolled intake that characterize addiction. In fact, among drug users, three different types of drug consuming behaviour have been defined: (1) occasional, controlled or

social use, (2) abuse or deleterious use and finally (3) compulsive drug intake or drug addiction. These different patterns of drug use suggest that the addiction process is not a unitary process; and that different neuropsychobiological mechanisms may explain distinct drug use patterns. Thus, an important question is to identify when drug use become abuse and addiction. Drug addiction has been conceptualized as the endpoint of a series of transitions: (1) initial drug use, when a drug is voluntarily taken because of its hedonic effects, (2) drug abuse or harmful use, when drugs are chronically consume to avoid abstinence's negative emotional state, and finally (3) drug addiction when drug intake becomes compulsive and uncontrolled (Koob and Nestler, 1997; Koob and Volkow, 2010).

Initially, drug intake may create a sense of well-being and excitement and/or temporarily relieves discomfort. Since these effects may seem ideal, some individuals start repeated drug consumption in order to obtain the positive reinforcement effects. Homeostatic adaptations may occur within cells and circuits stimulated by the drug to counteract this chronic pharmacological insult to the brain. Among these adaptations, one of the most pervasive processes is certainly tolerance, or the progressive decrease of initial drugs' effects (Koob, 2009). Tolerance often develops to the pleasurable effects of drugs, leading individuals to constantly increasing dosage to maintain a stable effect. Indeed, drug addicts often describe their continuing drug use as an attempt to re-experience the initial remembered "high" or "rush" without success (Hyman et al, 2006). Unfortunately, the consequent repeated and increasing pattern of drug intake will in turn exacerbate these previous molecular changes and lead to the development of a more severe homeostatic adaptation, called drug dependence. When unmasked by drug cessation, this adapted state can result in the production

of a strong negative emotional state that includes dysphoria, anxiety and/or irritability, or even "physical" withdrawal symptoms for some drugs like opiates, alcohol and nicotine (Hyman et al, 2006; Kosten and George, 2002). Thus, subjects describe withdrawal changes as a "hunger" or primary need, and the effects of the drug on such a state as "satiation" or gratification of the primary need. This additional source of gratification results in the development of a new pattern in which the drug must be obtained to avoid the aversive consequences of drug abstinence (i.e. negative reinforcement) (Koob and Le Moal, 2008b; Koob, 2009). Thus, drugs become overvalued in comparison to any other goals, and the drug users' life becomes profoundly narrowed to a focus on obtaining and using drugs by almost any means, such as smoking through a tracheotomy tube after a laryngectomy for cancer.

Drug-induced pleasure or the desire to avoid withdrawal symptoms do not explain compulsive use. Indeed, the hedonic effects of drugs often habituate "rapidely", whereas compulsion to take drug can be very persistent (Hyman and Malenka, 2001). Similarly, if the discomfort associated with withdrawal would be the major obstacle to recovery, close supervision of addicted individuals for several weeks until withdrawal symptoms diminished would yield a cure — even for drugs such as alcohol, barbiturates and opiates, which produce the most serious withdrawal syndromes (O'Brien et al, 1998; Hyman and Malenka, 2001). In fact, relapse to drug use has been observed even years or decades after drug abstinence, and long after any withdrawal symptoms subsisted. Moreover, for psychostimulants such as cocaine and amphetamines, physical withdrawal symptoms are absent, and emotional withdrawal symptoms are variable. Finally, it is worth recalling

that withdrawal symptoms from non-addictive compounds can also be severe at times (Hyman and Malenka, 2001).

In summary, pleasure, tolerance, dependence and withdrawal are clinically significant, but do not explain the transition from voluntary to uncontrolled and compulsive drug taking nor the persistent cravings and high risk of relapse to drug use observed in "ex-addicts". Thus, although much of the initial study of the neurobiology of drug addiction focused on the acute impact of drugs of abuse on the brain, the focus now is shifting to understand the long-term neuroadaptive changes underlying these persistent changes in behaviour that characterize addiction.

1.4. Animal models to evaluate drug addiction

Much of the recent progress in understanding the mechanisms of the neurobiology of addiction has derived from a variety of increasingly sophisticated animal models. Although no animal model of addiction totally emulates the human condition, especially in its psychological complexity, critical features of the process of drug addiction can be reliably measured in animal studies. In particular, the use of such approaches have permitted to characterize the neurobiological substrates underlying the rewarding effects of drugs of abuse, the aversive aspects of drug withdrawal, as well as some long-lasting behavioural alterations associated to repeated drug exposure. Examples of models able to measure these specific features include the intracranial electric self-stimulation techniques, drug discrimination tasks, place conditioning methods, self-administration paradigm and the locomotor sensitization model, among others (Sanchís-Segura and Spanagel, 2006).

1.4.1. Intracranial self-stimulation

Early work using the intracranial electric self-stimulation paradigm was fundamental for the identification of the brain reward circuitry (Olds and Milner, 1954). Although reward self-stimulation involves widespread brain circuits, the most sensitive sites involve the trajectory of the medial forebrain bundle that connects the ventral tegmental area to the basal forebrain (Olds and Milner, 1954). In the intracranial electric self-stimulation model, animals previously implanted with intracranial electrodes into reward-related brain areas are trained to maintain an operant behavior to obtain an electric pulse through these electrodes (Figure 1). During these sessions, the threshold of the minimal current needed to promote intracranial self-stimulation is estimated. Typically, rewarding stimuli such as drugs of abuse decrease the self-stimulation threshold, whereas aversive drugs or stimuli, such as drug withdrawal, elevate the threshold for self-stimulation (Markou et al, 1993). Thus, intracranial electric self-stimulation methods are useful to investigate drug reward and withdrawal.

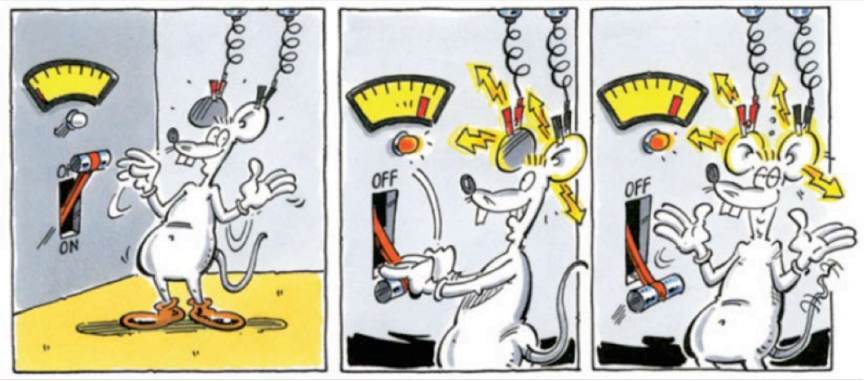


Figure 1. Intracranial self-stimulation paradigm (adapted from Sanchís-Segura and Spanagel, 2006).

1.4.2. Drug discrimination

The drug discrimination is a behavioral procedure in which animals must recognize a particular drug state and choose a correct response to receive a reinforcer. The standard protocol generally consists on daily short training sessions during 10 to 15 minutes over weeks, in which animals are placed in an operant two-lever chamber after receiving at alternative days a drug or vehicle injection. Thus, animals are taught to respond on one lever (e.g., right-side lever) when a dose of training drug is administered before a training session and on the alternative lever (e.g., left-side lever) when vehicle is given; thereby correct responses are reinforced by delivery of food pellet (Figure 2). Therefore, interoceptive cue state produced by the drug controls the behavior as a discriminative stimulus or cue that informs the animal to make the appropriate response in order to gain reinforcement. Many different psychoactive drugs have been shown to serve as discriminative stimuli in this paradigm (Young, 2009).

Finally, once trained, animals perform several test sessions, in which they receive the injection of a novel drug, they are subsequently replace in the operant chamber, and they must then choose between the two reinforced levers. Hence, lever choice during test sessions can then be used as an indication of whether the novel drug has interoceptive effects similar to the training drug, or whether a potential therapeutic, such as receptor antagonist, alters the discriminative effects of the training drug. Thus, drug discrimination procedures have been mainly used to classify and characterize the pharmacological effects of drugs of abuse, and have permitted to elucidate some neurochemical mechanisms involved in the discriminative stimulus properties of a drug (Solinas et al, 2006a).

Additionally, although many discriminative effects of drugs are not related directly to their addictive's abilities, it is important to note that certain interoceptive effects of drugs of abuse might play major roles in the development and maintenance of drug abuse, and contribute to drug craving in humans (Solinas et al, 2006a; Gray and Critchley, 2007). Therefore, the study of the neurobiological substrates underlying drug discrimination might also be useful to investigate the neurobiology of drug addiction.

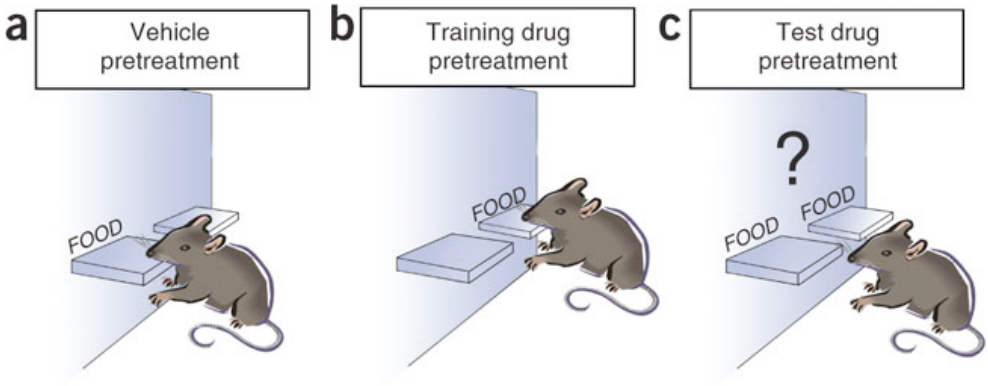


Figure 2. Drug discrimination (Solinas et al, 2006a).

1.4.3. Conditioned place preference

In the conditioned place preference (CPP) model, animals are exposed to an apparatus generally consisting of two initially neutral environments that can differ in terms of a number of stimulus modalities, including colour, texture, odour and lighting. Animals are exposed to one environment following drug pre-treatment, whereas the other environment is paired with vehicle pre-treatment. After a number of conditioning sessions, the animals, in a drug-free state, are allowed free access to the apparatus and

the preference/avoidance for one of the two environments is assessed (Figure 3). Results are usually expressed with a score calculated as the difference of the time spent in the drug-paired compartment during the post-conditioning and the pre-conditioning phase (Bardo and Bevins, 2000).

According to the principles of classical conditioning, if a drug has reinforcing properties, the animal should prefer the previously drug-paired environment. Consistent with this hypothesis, various drugs of abuse, including opiates, nicotine and cocaine, typically induce CPP. Although drug consumption in humans can induce conditioned approach to specific drug-related stimuli, CPP is not intended to model any particular feature of human behaviour. This paradigm mainly represents an indirect measure of the rewarding effects of a drug by evaluating the response of the animal towards the conditioned stimulus (Sanchís-Segura and Spanagel, 2006).

Two variables with different implications are usually analyzed using this paradigm: the acquisition and the expression of CPP. The acquisition phase has been proposed to be mainly related to learning and memory mechanisms, whereas the expression phase would be more linked to incentive motivation, memory recall or sign tracking. The CPP procedure has proven to be a useful and inexpensive means for assessing rewarding properties in a fairly quick manner, since animals require no surgery, minimal training and can be tested in a drug-free state (Tzschentke, 2007).

Finally, during the past decade, drug reinstatement procedures have been developed using the CPP model, suggesting that this paradigm might also serve in some particular circumstances for modelling relapse in human addicts. Indeed, CPP apparatus comprise very discriminative contextual

cues that can promote potent conditioned association. Thus, re-exposure to this paradigm after prevented its access for certain amount of time could in some way model human drug craving situation, often triggered by re-exposure to drug associated-cues, that represent a major cause of relapse (Aguilar et al, 2009).

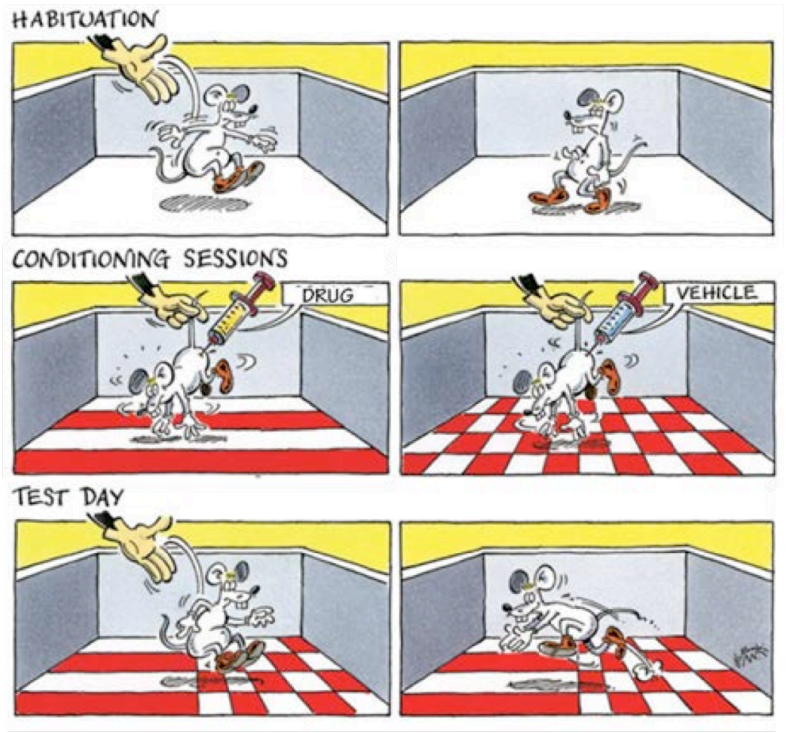


Figure 3. Conditioned place preference paradigm (adapted from Sanchís-Segura and Spanagel, 2006).

1.4.4. Self-administration paradigm

The self-administration paradigm is a more "motivational" approach than the CPP paradigm, since animals have to work to obtain the drug. Drug self-administration has both reliability and predictive validity for the study of

addiction. Consequently, this technique is widely used in preclinical research to directly evaluate the primary reinforcing effects of drugs of abuse. Most drugs of abuse are readily self-administered by experimental animals, although, there are a few exceptions (e.g. LSD). In general, drugs that are self-administered are those that have high abuse potential. Additionally, the neural chemistry and the anatomical substrates underlying drug self-administration are assumed to be similar in these animal models and in human addicts. Therefore, these procedures seem to be adequate models to identify common neural mechanisms and search for useful strategies for the treatment of drug addiction (Sanchís-Segura and Spanagel, 2006).

In drug self-administration procedures, the reinforcer can be delivered by different routes of administration. Accordingly, intravenous, intraventricular, intracranial, intragastric delivery of drugs and also oral (alcohol or food) sustain operant behaviour. Typically, the operant chamber consists of 2 manipulandi that transmit the operant response, and devices that deliver the reinforcer. The manipulandi are defined as "active" or "inactive" and typically consist of levers or nose-pokes. The response in the active manipulandum is associated with the delivery of the reinforcer whereas activation of the "inactive" manipulandum lacks any programmed consequences. Additionally, the active manipulandum can be paired with other stimuli (e.g. light or tone), which improves learning of the operant behaviour (Figure 4). The most common schedules of reinforcement used in these studies are the fixed-ratio and the progressive-ratio programs. Under a fixed- ratio schedule, the drug is delivered each time a preselected number of responses are completed. After each reinforcer delivery, a time-out period (usually from 10 to 30 sec) occurs in which operant responses

are not rewarded, neither in active nor in the inactive manipulandum. This specific period aimed to avoid any potential drug overdoses and can also serve to evaluate impulsive-like behaviours (Diergaarde et al, 2009). In contrast, under a progressive-ratio schedule, the required ratio to deliver the reinforcer increases following an arithmetic progression, usually from 1 up to 5000. When using the latter schedule, the "breaking-point" value is commonly measured, which refers to the highest response rate accomplished to obtain a single reinforcer. Thus, the "breaking-point" is considered to be a measure of the motivation of the animal to obtain the reward. The analysis of the patterns of self-administration using both the fixed-ratio and the progressive-ratio (PR) schedules of reinforcement provide valuable information about the different factors that determine reinforcer consumption (Collins et al, 1984).

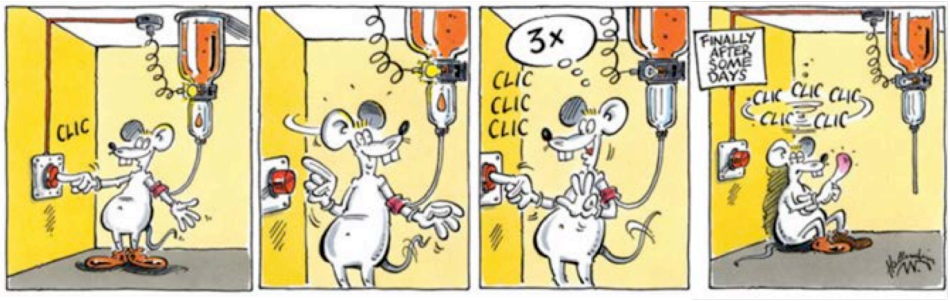


Figure 4. Intravenous drug self-administration (adapted from Sanchís-Segura and Spanagel, 2006).

1.4.5. Locomotor sensitization

In drug addiction, the term sensitization refers to the increase of drug initial effects in response to repeated consumption of the drug. This phenomenon is thought to be an opposite process of drug tolerance, and a

reliable mechanism underling the increasing motivation of addicts to consume drug, despite the development of a progressive indulgence to their pleasurable effects (Robinson and Berridge, 2000). This phenomenon is mainly assessed with respect to locomotor activity in experimental animals. Thus, the ability of addictive drugs to increase locomotion after an acute administration is progressively enhanced, when drug exposure is repeated (Figure 4). This so-called psychomotor sensitization is a very robust phenomenon that has been observed across several species, and elicited by almost all prototypical drugs abused by humans (Robinson and Berridge, 2000).

From a procedural perspective, psychomotor sensitization generally relies on a repeated drug intermittent treatment and route of administration with a fast onset of drug effect (e.g. intraperitoneal, intravenous and subcutaneous injections). Moreover, sensitization is stronger, when the dose is higher or when escalating doses are administered. Some drugs such as psychostimulants, and morphine can even trigger a sensitized response after a single pre-exposure if the dose used was high enough. Finally, although sensitization is in essence a non-associative learning process, the context plays a major role in the development and expression of locomotor sensitization. Thus, a higher degree of sensitization is usually observed when drug injections are administered in a context different from that of the home cage (Sanchís-Segura and Spanagel, 2006; Phillips et al, 2011).

Different parameters can be evaluated in this model: the initial drug effect with the first injection of the drug, the induction of sensitization during the chronic treatment and the expression of sensitization, usually measured after a period of drug withdrawal, by the injection of a challenge dose.

Typically, animals display a progressive slight enhancement of drug locomotor effects during the induction phase, and a disproportionate hyperlocomotion with the challenge dose (Leith and Kuczenski, 1982).

Although drug sensitization in humans remains controversial, psychomotor sensitization in animals is considered an attractive model of human drug craving and relapse, since it relies on the major events that establish their appearance including chronic drug intoxication, drug free period and re-exposure to the drug or its associated context. In addition, sensitized locomotor responses can persist for at least several months. Therefore, psychomotor sensitization is hypothesized to provide a behavioural measure that reflects underlying neural adaptations to repeated drug exposure and "incubation" period during abstinence (Robinson and Berridge, 2000; Sax and Strakowski, 2001).

Sensitized animals exhibit facilitated acquisition of drug self-administration, CPP, an enhanced motivation to obtain drug (i.e. increase breaking point on PR), as well as enhanced drug-seeking behaviour in priming-induced reinstatement (Vezina 2004; Shaham and Hope, 2005). Thus, psychomotor sensitization has been proposed as a possible model to mimic the transition from a regular pattern of voluntary drug intake to compulsive drug-seeking and -taking behaviour; thereby an appealing paradigm to study the long-lasting neuronal alterations that mediate persistent behavioural abnormalities that characterized addiction (Robinson and Berridge, 2000).

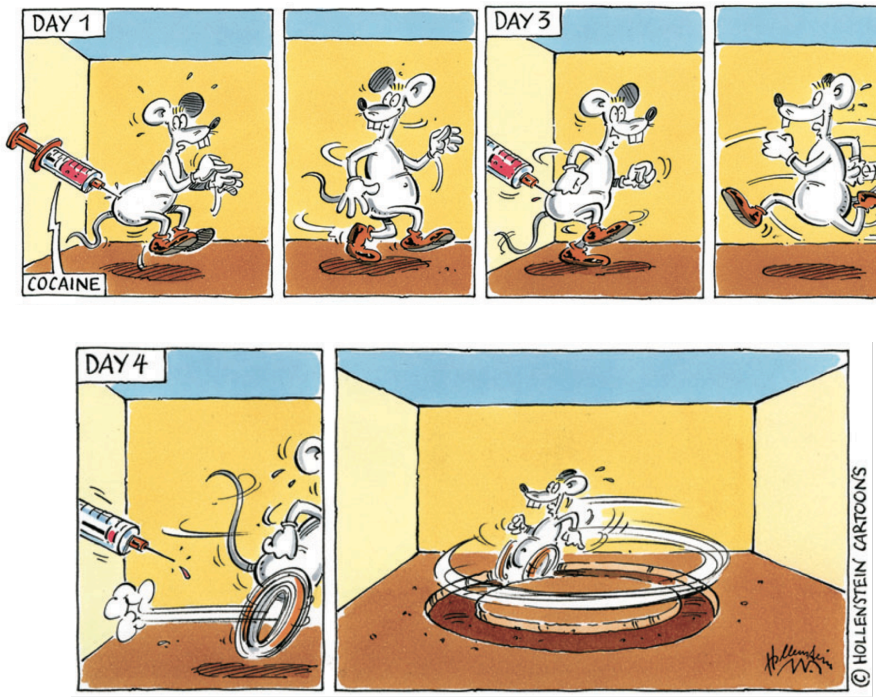


Figure 5. Locomotor sensitization (adapted from Sanchís-Segura and Spanagel, 2006).

1.5. Neurobiological substrates of addiction

Many different drugs, with distinct pharmacological properties, are known to potentially lead to addiction. However, despite distinct primary brain target and initial physiological effects, all drugs of abuse have in common the capability to activate a same neuronal circuit called the mesocorticolimbic system (Camí and Farré M 2003).

This circuit is based on neuronal dopaminergic pathways, that originate principally from the ventral tegmental area (VTA) in the mid-brain, and

project most notably to several limbic structures, such as the amygdala, the hippocampus (HPC), the nucleus accumbens (NAc), but also to cortical areas in the forebrain, in particular the prefrontal cortex (PFC) (Figure 6) (Morales and Pickel, 2012). The mesocorticolimbic circuit is often refereed as the brain reward system. Indeed, initial work have shown that rats can spend 92% of their time pressing a lever to electrically stimulating different parts of this circuit, ignoring even thirst and hunger (Olds and Milner, 1954). This effect was initially attributed to the activation of the medial forebrain bundle, through which dopamine (DA) neurons course from cell bodies in the midbrain to limbic and cortical targets. Thus, as pharmalogical activation or blockade of DA transmission, respectively enhances or attenuates the rewarding effects of medial forebrain bundle stimulation, the dopaminergic system has been considered a major neurobiological substrate for reward processing (Wise, 2008). Consequently, the common ability of drugs of abuse to enhace DA release in the mesocorticolimbic circuit (see section 1.5.1.1) has been proposed as an underlying mechanism mediating drugs reinforcing effects (Di Chiara and Bassareo, 2007). However, DA transmission does not fully account for the reinforcing effects of all drugs of abuse. Other neurotransmitter/ neuromodulator systems such as opioid peptides, glutamate and endocannabinoids (see chapter 4), among others, play also key roles in the rewarding effects of addictive drugs (Koob and Le Moal, 2008b). Drug-induced alterations in these neurotransmitter system disrupt the neuronal functions of the brain structures that composed the mesocorticolimbic system, and ultimately produce cognitive and emotional dysfunctions that characterize the addictive disorder (Koob and Volkow, 2010).

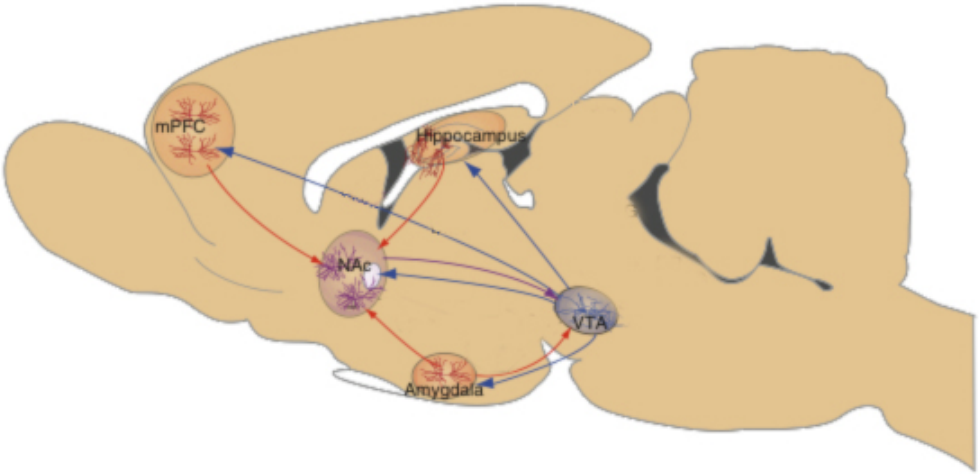


Figure 6. Mesocorticolimbic circuit. Simplified schema of the mesocorticolimbic system circuitry in rodent brain highlighting the major dopaminergic projections (blue lines) arising from VTA and innervating the principal limbic (NAc, HPC and amygdala) and cortical (medial prefrontal cortex (mPFC)) components of this circuit. The red lines represent excitatory glutamatergic afferents to NAc from mPFC, amygdala and hippocampus, and glutamatergic innervation of VTA by amygdala. GABAergic afferents (purple) are inhibitory circuits and include connections from NAc to VTA (adapted from Russo et al, 2012).

In the present section, some of the major neurotransmitter systems that mediate the addictive properties of drugs of abuse are described, as well as the main functional alterations triggered by chronic drug consumption in some components of the mesocorticolimbic system, such as the NAc, the mPFC and the HPC.

1.5.1. Main neurotransmitter systems involved in the addictive properties of drugs of abuse

1.5.1.1. The dopaminergic system

DA neurons in the brain are localized in nine distinctive cell groups, but the major part resides in the ventral part of the mid-brain, in particular in the substancia negra pars compacta and VTA. From this latter, emerge the mesolimbic and mesocortical dopaminergic pathways that together form the basis of the brain reward circuit (Björklund and Dunnett, 2007; Arias-Carrión et al, 2010).

DA's action is mediated by two subfamily of DA receptors: the D1-like (D1 and D5) and the D2-like (D2, D3 and D4) receptors. DA acting on D1-like and D2-like receptors can have either an excitatory or inhibitory modulatory effect on neurotransmission (Tritsch and Sabatini, 2012).

One function attributed to the DA system in the mesocorticolimbic circuit is to promote reward-related learning. This proposal is supported by evidences showing that DA neurons activity correlates with the achievement of rewarding stimuli, and DA release in this circuit (i.e. NAc shell) can vary in response to the incentive value of the received stimulus. Thus, DA release in NAc shell is stimulated by unfamiliar appetitive tastes and unaffected or even decreased by aversive ones (Bassareo et al, 2002). In addition, repetead experiences to a given stimulus lead that DA neurons activation is shifted from the presentation of the reward to environmental cues that predict its appearance. Therefore, it has been postulated that DA activity provides a predictor-error signal for the learning of stimulus–reward associations. Thus, phasic DA release is enhanced when conditioned cues lead to the achievement of rewards that are better than

expected, whereas tonic DA release is paused if reward is withheld after the presentation of its predictive associated stimuli (Schultz, 2002). Importantly, blockade of DA D1 and/or D2-R activity in specific locations of the mesocorticolimbic circuit, such as the NAc shell and core, prevents acquisition of conditioned learning tasks (Fenu et al, 2001; Di Ciano et al, 2001). Thus, DA seems to serve mainly two physiological functions: (1) to facilitate initial learning of adaptive responding to important stimuli, and (2) to cue the retrieval of the information needed to execute the adaptive behavioural response when environmental circumstances predict that reward is imminent (Di Chiara and Bassareo, 2007; Glimcher, 2011).

From a psychological perspective, it is thought that the mesocorticolimbic dopaminergic system may serve to assign motivational value to biologically relevant stimuli, and thus to direct the behaviour toward goals that are normally beneficial and that promote survival of the individual (e.g. food and water intake) or the species (e.g. reproductive behaviour). Such survival-relevant natural goals act as "rewards," i.e., they are pursued with the anticipation that their consumption will produce desired outcomes. Thus, behaviours with rewarding goals tend to persist strongly to a conclusion and increase over time (Kelley and Berridge, 2002).

Although addictive drugs have no intrinsic ability to serve a biological need and do not represent a beneficial goal, they all share the common feature to "artificially" enhance DA activity and release in the mesocorticolimbic circuit. Thus, addictive drugs can strongly shape behaviour by mimicking the effects of natural rewards. This process may be dependent on the drug itself, and addictive drugs can be distinguished into three main groups according to the way that they activate DA neurons in the mesocorticolimbic system (Figure 7) (Lüscher and Ungless, 2006).

Class I: Drugs that bind to G protein–coupled receptors. This group includes the opioids (e.g. morphine, heroin, through mu opioid receptors), cannabinoids (e.g. Δ9-tetrahydrocannabinol (THC), through cannabinoid receptor 1 (CB1-R)), and gamma-hydroxy butyrate (GHB, through $GABA_B$ receptors). These drugs, acting on G protein–coupled receptors that are of the $G_{i/o}$ family, inhibit principally gamma-aminobutyric acid (GABA) neurons in the VTA. As GABA neurons act as local inhibitory interneurons in the VTA, their inhibition leads to a net disinhibition of DA neurons and increase DA release.

Class II: Drugs that interact with ionotropic receptors or ion channels. This group includes nicotine (acetylcholine receptors), alcohol ($GABA_A$ and others receptors), and benzodiazepines ($GABA_A$ receptors). These drugs have a complex mechanism of action that has not been entirely elucidated, but in general have a combined effect: inhibit GABA neurons and also directly modulate DA neurons activity in the VTA leading to enhanced release of DA.

Class III: Drugs that target monoamine transporters. This group comprises cocaine, amphetamines, and methylenedioxymetamphetamine (MDMA, ecstasy). Whereas all other drugs directly act on VTA neurons, these drugs block the re-uptake of DA, or stimulate non-vesicular release of DA in their projecting axons, causing an accumulation of extracellular DA in target structures such as the NAc.

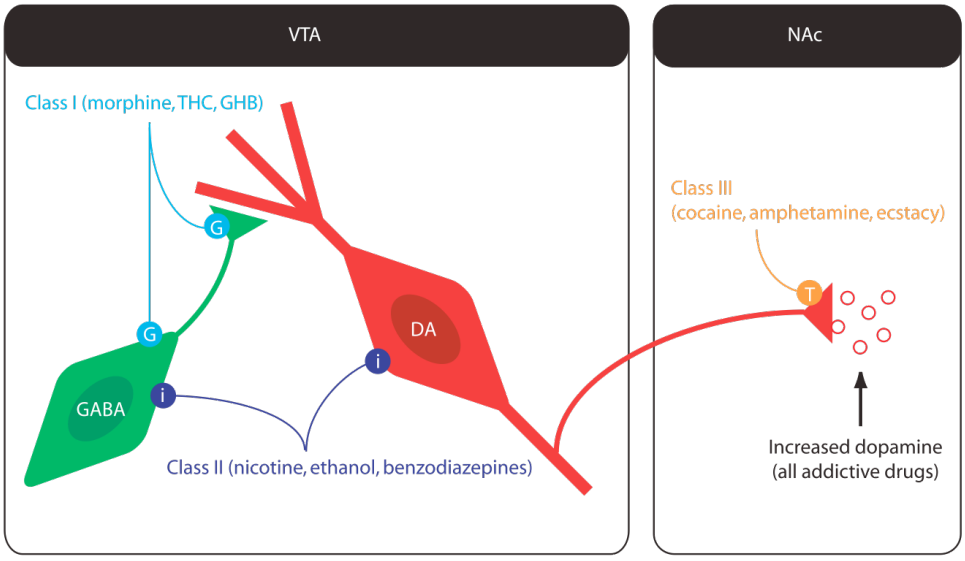


Figure 7. The dominant targets involved in increasing DA for the major types of addictive drugs: G: Gi/o-coupled receptors; i: ionotropic receptors/ion channels; T: monoamine transporters (Lüscher and Ungless, 2006).

Given the likelihood that addictive drugs exceed natural stimuli in the reliability, quantity, and persistence of increased synaptic DA levels, a predicted consequence of the DA function hypotheses would be that brain repeatedly get the signal that drugs are "better than expected", leading to a profound overlearning of the motivational significance of cues that predict its delivery (Hyman, 2005).

Indeed, the main prototypical drugs consumed by humans such as alcohol, nicotine, opioids, cannabinoids and psychostimulants, all produce and maintain operant self-administration behaviours in experimental animals (Panlilio and Goldberg, 2007). Moreover, some drugs (e.g. psychostimulants) also promote the acquisition of even more complex

behaviour, such as drug seeking under second order schedule or Pavlovian instrumental transfer that required potent association between conditioning stimuli and the reward (Everitt and Robbins, 2005). Finally, conditional stimuli associated with consumption of all drugs of abuse can be so powerful that they can reinstate drug-seeking behaviours even after active behavioural extinction or prolonged protracted abstinence during several months (Marchant and Shaham, 2013).

There is considerable evidence for a dysregulation of the DAergic system following repeated drug intake, and the changes observed persist at least during the early phases of abstinence (Volkow et al, 2009). Thus, human imaging studies have demonstrated a reduction in the availability of DA D2-R and alterations of the DA transporter (DAT) density in subjects addicted to different drugs including cocaine, alcohol, methamphetamine, heroin and nicotine (Volkow et al, 2009).

All together, these findings strongly suggest that DA plays an important role in the reinforcing effects of drugs of abuse, which has lead to the DA hypothesis of addiction, and to the proposal that alterations of DA system triggered by abuse consumption of drugs could be one of the main factors leading to the emergence addictive behaviours (Diana, 2011).

1.5.1.2. The endogenous opioid system

The endogenous opioid system is composed by three major subtypes of seven-transmembrane, G protein-coupled opioid receptors, mu, delta, and kappa which are stimulated by three different families of endogenous opioid peptides: derivates of pre-pro-opiomelanocortin, pre-enkephalin, and pre-dynorphin; or by exogenous opiates, such as morphine and heroin.

The endogenous opioid system is largely distributed through the central nervous system (CNS) and peripheral tissues, which confer to this system a key role in the control of several physiological responses including nociception, emotional behaviour, learning and memory and regulation of reward circuits, among others (Benarroch, 2012).

The different components of the endogenous opioid system are highly expressed in brain areas of the mesocorticolimbic circuit, such as the VTA, NAc, PFC, and extended amygdala (Le Merrer et al, 2009). One important function of the endogenous opioid system inside this circuit is the control of hedonic processes. In this view, microinjection of mu receptor agonists in the NAc amplifies positive affective orofacial reactions to natural reward, such as sucrose, and increase food consumption in rodents (Peciña, 2008; Kelley et al, 2002). In contrast, injection of a kappa receptor agonist into the NAc decreased the consumption of pre-fed palatable foods (Peciña, 2008; Woolley et al, 2007). Thus, opioid peptides released in the NAc during consumption of palatable foods can produce opposite effects on flavor preference depending on the opioid receptors activated. The endogenous opioid system in the mesocorticolimbic circuit may contribute to assign an emotional valence to an aimed or goal. In general, systemic mu and, to a lesser extent, delta agonists produce positive reinforcement, whereas kappa agonists induce aversive effects (Le Merrer et al, 2009).

The endogenous opioid system has been considered a key substrate for the development of addictive behaviours (Trigo et al, 2010). The first compelling evidence is that drugs that directly target the endogenous opioid system, such as heroin or morphine, are extremely addictive. Moreover, exposure to many different drugs, including alcohol, nicotine, psychostimulants and cannabinoids, modifies the activity of the

endogenous opioid system in the mesocorticolimbic system. In addition, pharmalogical or genetic blockade of mu and delta opioid receptors decreased the reinforcing properties of these drugs in different behavioural paradigms (self-administration and CPP) (Trigo et al, 2010). On the other hand, the endogenous opioid system through kappa signalling mediates aversive effects of drugs such as anxiety and dysphoria, and even regulates several manifestations of the withdrawal syndrome (Trigo et al, 2010). Finally, repeated exposure to all prototypical drugs produces long-term adaptive changes in the endogenous opioid system, particularly an increase in dynorphin/kappa receptor signalling in different brain areas of the mesocorticolimbic system, which participates in the aversive component of the withdrawal syndrome (Mathieu-Kia and Besson, 1998; Di Benedetto et al., 2006).

In summary, while DA system appears to be preferentially involved in motivational and/or learning aspects of drug reinforcement, the endogenous opioid system seems to mediate the emotional hedonic/aversive response to drugs of abuse. Importantly, the endogenous opioid system participates in many aspects of the addictive process, including the hedonic effects that drive initial drug use and the subsequent negative reinforcement associated with drug abuse.

1.5.1.3. The glutamatergic system

Glutamate, together with GABA, represent the major neurotransmitter systems in the CNS. Glutamate is the main excitatory neurotransmitter, while GABA is the principal inhibitory one. The glutamatergic system is ubiquitously present throughout the brain, and many cortical and limbic

structures of the mesocorticolimbic system spread out their neural activity through glutamatergic efferents (Tzschentke and Schmidt, 2003).

Several evidences demonstrate the crucial role played by the glutamatergic system in drug addiction (Kalivas, 2009). In particular, glutamatergic signalling in the mesocorticolimbic system, via its ionotropic (AMPA, NMDA and kainate receptors) and metabotropic ($mGluR_{1-8}$) receptors, has been considered an essential substrate for neuronal plasticity, learning and memory mechanisms involved in different aspects of the addictive process, such as reinforcement learning, drug sensitization and craving/relapse (Tzschentke and Schmidt, 2003).

The involvement of glutamate transmission in reinforcement learning is crucial, and glutamate blockade in different part of the mesocorticolimbic system prevents the acquisition of operant self-administration behaviour and/or of Pavlovian conditioning (CPP) of both natural rewards and drugs of abuse (Schenk et al., 1993; Del Pozo et al., 1996; Kelley et al., 1997; Kotlińska and Biała, 2000). These effects may rely on the capacity of glutamatergic transmission to activate dopaminergic cells in the VTA and DA release in the NAc (Karreman et al., 1996). Thus, co-infusion of low doses of the DA D1-R and glutamate receptor antagonist in the NAc strongly potentiates the impairment in the acquisition of instrumental learning that each drug produced when administered alone, suggesting that a glutamate–DA interaction is a key mechanism in the acquisition of appetitive instrumental learning (Smith-Roe and Kelley, 2000).

The requirement of glutamatergic transmission in drug sensitization highlights its involvement in driving contextual information and neuroplastic mechanisms (Vanderschuren and Kalivas, 2000). Thus,

pharmalogical blockade of glutamate transmission in the VTA and NAc can prevent the induction and expression, respectively, of context-dependent behavioural sensitization to differents drugs of abuse (Vanderschuren and Kalivas, 2000). Interestingly, injection of an AMPA-R agonist in the NAc of animals re-exposed to the context where they received chronic injection of cocaine is sufficient to induce psychomotor sensitization, without injecting a challenge cocaine dose (Bell and Kalivas, 1996). As this behavioural model is thought to emulate some long-lasting addicts' behavioural abnormalities, the glutamatergic system has been considered a key substrate for the development of long-term neuronal adaptations that promote the occurrence of addiction (Robinson and Berridge, 2000; Kalivas, 2009)

The glutamatergic system is also a key substrate for drug relapse (Knackstedt and Kalivas, 2009). Relapse in human addicts is mainly induced by three different factors: a punctual and even low consumption of the drug, a stressful situation, or the re-exposure to environmental cues previously associated with drug intake such as places or people with whom addicts used to take drugs. Such stimuli activate an "addiction memory" that has developed during the period of active drug-taking and that is very stable, perhaps permanent (Stewart, 2008). Imaging studies in human addicts have shown that the presentation of drug-related cues that typically promote drug relapse were associated with increased activity of several glutamatergic structures, such as the amygdala and the PFC. Moreover, pharmalogical inactivation of AMPA-Rs in these same structures in rodents prevents cocaine reinstatement induced by both drug-priming or cues (Tzschentke and Schmidt, 2003). This predominant role of glutamate in cocaine relapse is further confirmed by the fact that AMPA infusions in the

NAc, alone, reinstates previous extinguished cocaine-seeking behaviour in rodents (Cornish and Kalivas, 2000). Thus, the glutamatergic system represents an interesting neurobiological target to prevent relapse.

In summary, behavioural dysfunctions in addictive disorders have been mainly related to drug-induced altertations in the dopaminergic, opioid and glutamatergic system in the mesocorticolimbic circuit. However, other neurochemical systems, such as the endogenous cannabinoid system (see chapter 4), also play an important role in the addictive process. A remaining critical issue not yet fully clarified is to understand how these alterations lead to persistently compromise the physiological functions of the different cerebral areas of the brain reward circuit, and ultimately to the development of addiction.

1.5.2. Main brain structures involved in the development of addictive-like behaviours

The different brain structures that composed the brain reward circuit receive important dopaminergic innervations that are modulated by the endogenous opioid and cannabinoid system, and most of these structures spread out their neural activity through glutamatergic efferents (Koob and Volkow, 2010). This section describes some components of the mesocorticolimbic circuit in which alterations of these neurotransmitter systems promoted by the different drugs of abuse lead to the long-lasting cognitive and psychological impairments that characterize addiction.

1.5.2.1. The nucleus accumbens

The NAc is a subcortical forebrain structure located in the septal region that forms, together with the olfactory tubercule, the ventral part of the striatum complex. The NAc is considered an essential integrative brain area where neural activity from the limbic system and the basal ganglia/motor system can interact, providing the interface between emotion and action (Day and Carelli, 2007).

The vast majority (90–95%) of neurons in the striatum complex, including the NAc, are GABA-containing medium spiny neurons that have been divided in two subpopulations: those that co-express DA D1-like receptors and the endogenous opioid peptide dynorphin, and those that co-express DA D2- like receptors and the endogenous opioid peptide enkephalin (Meredith, 1999).

The NAc receives extensive excitatory afferents from the thalamus, cerebral cortex including the mPFC, and multiple limbic associated areas such as the HPC and amygdala. These excitatory inputs often synapse onto the dendritic spines of medium spiny neurons that also receive synapses from DA afferents from the VTA. The so-called triad of elements: spine, glutamate synapse, and DA synapse, creates the potential for DA to modulate discretely specific sources of glutamate transmission in the NAc, thus gating the activation of medium spiny neurons (Sesack and Grace, 2010). It has been shown that DA D1 and D2-R oppositely modulate these excitatory inputs. Thus, DA D1-R stimulation potentiates glutamatergic drive, while DA acting on D2-R potently inhibits medium spiny neurons, preventing the glutamate stimulation (West and Grace, 2002). As DA neurons in the VTA respond to both rewarding and aversive stimuli (Lammel et al., 2011), the integration of these excitatory (glutamate, DA on

D1-R) and modulatory-inhibitory (DA on D2-R) inputs in the medium spiny neurons provides to the NAc the ability to regulate motor actions in a most adaptive manner (Horvitz et al., 2002). Thus, cortical activation of D1-expressing medium spiny neurons in the NAc would lead ultimately to disinhibition of appropriate action plans that facilitate reward acquisition; while cortical activation of the D2-expressing medium spiny neurons related circuit is likely to inhibit motor plans that are maladaptive, either for obtaining reward or for avoiding punishment (Sesack and Grace, 2010).

The functional activity of the NAc can be segregated anatomically into two subregions: the NAc shell and the NAc core. The NAc core represents the central portion directly beneath and continuous with the dorsal part of the striatum and surrounding the anterior commissure, whereas, the NAc shell occupies the most ventral and medial portions of the NAc. The NAc core and shell differ in their precise cellular morphology, neurochemistry, projection patterns, and functions (Meredith et al., 2008). The NAc shell is considered a transitional zone between the striatum and emotional limbic structures included in the extended amygdala, and may be preferentially involved in mediating the assignment of hedonic and motivational value to environmental stimuli (Meredith et al., 2008). Indeed, the modulation by DA of responses to novel rewarding stimuli occurs within the shell region. Hence, the unconditioned DA release to new rewarding stimuli is significantly greater in the shell than in the core (Di Chiara et al., 2004; Aragona et al., 2008). Moreover, opioid signalling mediates principally the pleasurable effects of rewarding stimuli in the shell, into specific regions named “hedonic hotspot” (Peciña et al., 2005). Finally, inhibition of the NAc shell, but not the NAc core, enhances feeding responses (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997). In contrast, the NAc core is

considered a functional extension of the dorsal striatum and may be particularly important to regulate goal-directed exploratory activity and to promote association of rewarding stimuli with contextual cues and behavioural actions (Meredith et al., 2008). Indeed, infusions of an NMDA-R antagonist in the core produces decrements in locomotor activity, rearing, and novel object exploration that were much more pronounced than similar infusions into the shell (Maldonado-Irizarry and Kelley, 1994). In addition, conditioned stimuli that trigger motivated behaviours, such as cue-light promoted lever-pressing, specifically enhance DA release in the NAc core, but not in the shell (Ito et al., 2000); and lesion of the NAc core, but not of the shell, prevents the motivational consequences of outcome devaluation, which tests an animal's ability to respond appropriately to a recent change in the motivational significance of a stimulus (Corbit et al. 2001).

The NAc also mediates the rewarding properties of drugs of abuse, in agreement with its crucial role in coordinating behavioural responses towards natural reward achievement. Indeed, operant responses are maintained in rodents by self-administration into the NAc of different drugs of abuse, such as amphetamine, cocaine and morphine. Conversely, lesions of the NAc reduce self-administration of psychostimulants and opiates (Feltenstein and See, 2008).

In addition, chronic consumption of drugs of abuse profoundly alters NAc's functionality. Indeed, several studies showed that neurons in the NAc are still highly activated by drug associated stimuli even 1 month after repeated cocaine self-administration, an effect that was not observed with natural rewards (Ghitza et al., 2003; Jones et al., 2008). Importantly, this activation was significantly greater following one month compared to one

day of abstinence, correlating with the progressive enhancement of cocaine-seeking behaviour observed during the same period of time, a phenomenon referred as "drug craving incubation" (Hollander and Carelli, 2007). Finally, human addicts with prolonged history of drug use present functional alterations in the NAc, such as reduced DA D2-R binding, which has been viewed as a reward-deficiency state that leads drug users to increase drug-taking in an attempt to achieve the same level of reward (Volkow et al., 2009).

1.5.2.2. The hippocampus

The HPC is an important component of the limbic system localized in the temporal lobe that plays a crucial role in the control of emotion and memory. In particular, the hippocampal formation plays a prominent role in the storage, consolidation and retrieval of a specific form of memory, named declarative memory. Declarative memory processes are involved in defining our idendity and anchor us to past events, places, and experiences. Thus, the HPC is essential to form spatial and contextual memory, to move in space or to associate specific places with emotional events (i.e. contextual conditioning) (Tulving and Markowitsch, 1998). In addition, the HPC is also a target structure of the mesocorticolimbic dopaminergic pathways that integrate the brain reward circuit. Thus, hippocampal functions seem determinant to remember the location of natural rewards or to associate a place, or specific stimulus with the occurrence of rewarding or aversive outcomes.

DA signalling plays an important modulatory role in the HPC on memory consolidation. Indeed, injection of D1-R agonists or antagonists in rodents, respectively, enhances or decreases retention of memory (Bernabeu et al.,

1997; Morris et al., 2003), and polymorphism of COMT gene, which codes the enzyme that metabolizes DA, is associated with improved episodic memory in humans (de Frias et al., 2004). Importantly, this functional interaction is reciprocal, and the HPC, through the ventral subiculum, also modulates the activity of DA neurons in the VTA. Indeed, the ventral subiculum send many excitatory projections to the NAc, and specific stimulation of these neural inputs is sufficient to produce an increase in DA release in the mesocorticolimbic system (Taepavarapruk et al., 2000).

The hippocampal influence on the NAc/VTA neurons is thought to serve two important functions implicated in reward-directed behaviours (Lisman and Grace, 2005). First, excitatory afferents from the ventral subiculum of HPC signal to the NAc the novelty aspects of a stimulus, and promote approach behaviour toward the salient stimuli leading to the potential achievement of new source of reward. Second, the subsequent activation of VTA DA neurons triggered by these excitatory inputs will in turn increase DA release in target structures of the mesocorticolimbic system, including the HPC, which enhances the entry of behaviourally relevant information into long-term memory. This will be essential to shape in a most efficient manner future goal-directed behaviours.

It has been shown that the HPC represents an important neurobiological substrate for the acquisition, consolidation and expression of drug-related memories. Thus, lesions or functional alterations of specific parts of the HPC prevent cocaine-induced CPP, disrupt consolidation of an established morphine-induced-CPP and reduce alcohol-maintained responding in an operant task (Meyers et al., 2003; Milekic et al., 2006; June et al., 2001).

Moreover, chronic drug consumption has deleterious effects on hippocampal functions, leading to strong maladaptive contextual associations. Indeed, DA signalling in the HPC promotes rewarding relevant information to come into long-term memory, and drug-induced "abnormal" DA release can potentially consolidates drugs-related memories for a lifetime. Thus, drug-associated stimuli that evoke memories of the drug induce potent craving or desire of drug consumption, an effect that can persist for years or decades after abstinence (Weiss, 2005). Both, presentation of drug associated stimuli or drug craving evoked by autobiographical scripts in addicts strongly enhance neural activity in the HPC (Kilts et al., 2001; Hermann et al., 2006). Similarly, natural reward-predictive cues can guide efficiently goal directed-behaviours, whereas drugs-related cues can dominate the behaviour. Indeed, exposure to drug associated stimuli is a major cause of relapse in humans addicts, and the HPC seems a crucial structure mediating this process. Thus, lesions of the HPC prevent reinstatement of drug seeking behaviour by drug-associated context (Fuchs et al., 2005), while its stimulation can cause reinstatement of drug taking behaviour (Vorel et al., 2001). Finally, conditioned associations disappear in "normal" individuals when environmental cues no longer predict reward achievement, while in addicts drug conditioned associations tend to subsist. Indeed, human addicts often relapse when re-exposed to drug-associated cues, despite being "tolerant" to drug hedonic effects and had suffered harmful outcomes due to drug consumption, such as cancers derived of smoking or alcoholic cirrhoses. This unflexible learning-reponse has been hypothezised to reflect, in part, a dysfunction of hippocampal declarative memory processes and pathological involvement of striatal procedural memories processes that form "aberrant" habit learning (Kalivas and O'Brien, 2008; Everitt et al., 2008).

1.5.2.3. The prefrontal cortex

The PFC represents the anterior part of the frontal lobes of brain that lyes in front of the motor and premotor areas. This brain region has been implicated in a variety of cognitive and executive functions, such as attention, working memory, inhibitory responses control and decision-making. In particular, these functions allow to differentiate among conflicting thoughts, such as determining good and bad, to predict action's outcomes, or to suppress urges. The PFC serves to represent information that is not currently in the environment to intelligently guide thought, action, and emotion in accordance with internal goals (Miller et al., 2002).

The PFC is highly interconnected with multiple brain areas, including extensive connections with other cortical areas that include the HPC, and subcortical regions, such as the NAc and VTA. The main output of the PFC is composed of excitatory glutamatergic pyramidal neurons that can either directly activate its target structures, or inhibiting them via activation of local inhibitory GABAergic interneurons. Reciprocally, these structures influence neural activity in the PFC, and DA afferents from the mesocortical pathway emerging in the VTA greatly contribute to regulate PFC's functions (Tzschentke, 2000). Phasic DA signalling in the PFC provides a gating signal that "synchronises" PFC neural activity in processing information relative to a salient stimulus. Thereby, the PFC permits to elaborate behavioural plans, to limit impulsive actions, and to evaluate benefit/cost ratio to select the most appropriate behavioural strategies to obtain the desired stimuli. (Seamans and Yang, 2004; Dalley et al., 2004)

A significant aspect of addiction is the pathological narrowing of goal selection to those that are drug related. Indeed, phasic DA release provides

a gating signal in the PFC and addictive drugs produce a potent and highly distorted signal that disrupts normal DA-related learning in the PFC. The upshot of such a scenario would be a biased representation of the world, powerfully overweighted toward drug-related cues and away from other choices, thus contributing to the loss of control over drug use that characterizes addiction (Hyman, 2005).

In this line, accumulating evidences show the contribution of the PFC, especially its medial part, in the pathological rewarding and reinforcing effects of drugs of abuse (Tzschentke, 2000). Thus, administration of differents drugs of abuse, such as cocaine and heroin, enhances mPFC neural activity (Breiter et al., 1997; Chang et al., 1998), and lesions of the mPFC facilitate cocaine self-administration (Weissenborn et al., 1997). This latter effect has been considered to reflect a loss of behavioural inhibition that enhances new and potentially risky behaviour. In agreement, lesions of the mPFC in rodents increase impulsive-like responses (Pezze et al., 2009), which facilitate drug self-administration acquisition (Dalley et al., 2007). Conversely, human abstinent drugs addicts present hypo-activity of several regions of PFC, which has been related to impaired impulse control and decision making (Volkow et al., 1992; Jentsch and Taylor, 1999; Rogers et al., 1999). Thus, compulsive drug taking has been interpreted as an aberrant behaviour resulting from poorly modulated decisions due to the inability to learn from the negative consequences of drug use (George and Koob, 2010).

Moreover, dysfunction of the PFC has been related to drug craving and relapse. Indeed, imaging studies show that drug abusers present a hyperactivation of some parts of the PFC in response to drug related stimuli when compared compared with non-abusers ones, which correlates with

subjective feelings of drug craving (Goldstein and Volkow, 2002). In addition, reinstatement to drug-seeking is accompanied by increased neuronal activation in the dorsal mPFC in rodents (Koya et al., 2006; Zavala et al., 2008), and mPFC inactivation reduces reinstament of both cocaine and heroin seeking behaviour (McFarland and Kalivas, 2001; Rogers et al., 2008). Interestingly, the PFC seems to play a bidirectional role in drug relapse. Indeed, inactivation of the prelimbic part of the mPFC decrease cue-, context- and stress-induced reinstatement of cocaine-seeking (Capriles et al., 2003; McLaughlin and See, 2003), while inactivation of the infralimbic part enhances spontaneous recovery of cocaine-seeking (Peters et al., 2008). These results suggest that the prelimbic circuitry excites drug-seeking in the presence of reinstating cues, whereas the infralimbic circuitry has an inhibitory influence on spontaneous recovery of drug-seeking.

In summary, converging evidence suggests that chronic substance abuse is associated with frontal and executive impairments. These dysfunctions seem to contribute to the enhanced drive to take drug and the emergence of drug craving and relapse observed in drug addicts. A better understanding of these dysregulation in the mPFC may provide insight into the mechanisms underlying the development of drug addiction.

1.6. Theories of addiction

Animal's studies have permitted to reveal several crucial neurobiological mechanisms that participate in the development of drug addiction in vulnerable individuals. However, the whole picture of the physiopathology of this disease is still far from being achieved. Thus, many neuroscientists

have postulated several models of addiction to blend the multidimensional aspects of the pathology that results from complex interactions of biological, psychological and social-environmental processes. Among the most influential theories, we can highlight the allostatic model of addiction, the habit learning theory and the incentive sensitization hypothesis. All these theories emphasise the critical role that DA plays in the development of addiction and propose distinct neurobiological mechanisms to explain the appearance of compulsive behaviours, one of the main features of addictive disorders.

1.6.1.The allostatic model of addiction (Koob and Le Moal, 2008a)

In this theory, addiction is considered as the combined desregulation of the brain reward system and its opponent, the "anti-reward" circuit or stress system. This theory proposes that the development of compulsive behaviour would result from two successive brain homeostatic processes generated in these two neuronal systems to counteract chronic brain insults by drugs: the opponent and the allostasis process.

The opponent process represents a negative feedback mechanism or opponent loop to countervail excessive hedonic stimulation by chronic exposure to drugs. Indeed, affective and emotional states are control by two opposing mechanisms: the "a-process" that tends to up-regulate mood in aversive situations, and the "b-process" that down-regulates mood in excessive euphoric states, both to maintain brain reward homeostasis. Thus, acute drug consumption strongly activates an artificial "a-process", which constitutes the initial rewarding effect of the drug, and will consequently trigger the delayed appearance of the "b-process" to reduce all departures from hedonic neutrality. At initial stage of addiction, the a-

process is always greater than b-process, leading to the positive reinforcement. In contrast, chronic drug use is associated with tolerance to a-process while b-process gets larger with repeated exposure (Figure 8). This results in small pleasurable effects when drug is taking after repeated exposure and important negative emotional state when access to the drug is prevented. Therefore, drug is then taking mainly to avoid or alleviate dysphoric effect (Solomon and Corbit, 1974). This mechanism referred as the "dark side" of addiction has been proposed to explain tolerance, withdrawal, and the aversive craving state observed during abstinence. However, this simple break in reward homeostasis cannot account for other key characteristics of addiction, such as the persistent compulsion to take drug even after years of abstinence. Thus, this theory proposes the involvement of a second subsequent mechanism, the allostasis process.

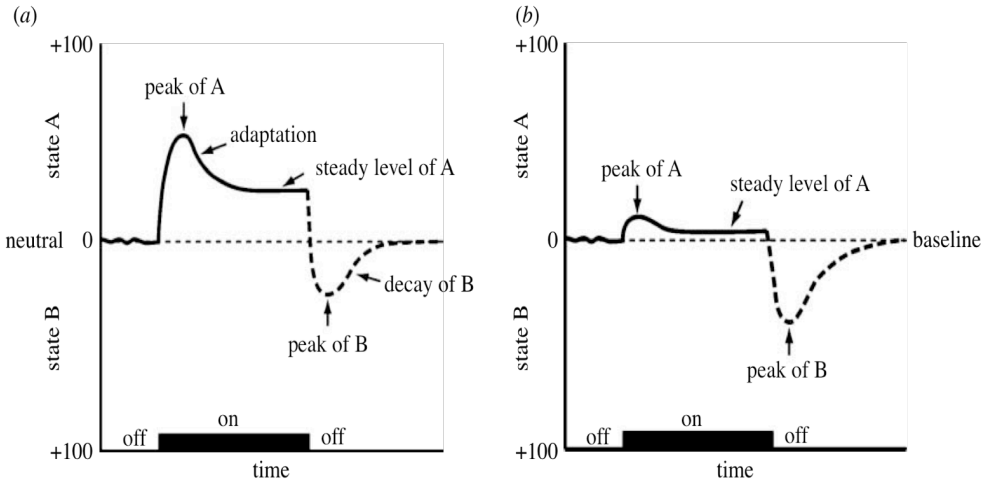


Figure 8. Opponent process theory of affective dynamics relevant to addiction. (a) The standard pattern of affective dynamics produced by a relatively novel unconditioned stimulus (first few stimulations). (b) The standard pattern of affective dynamics produced by a familiar, frequently repeated unconditioned stimulus (after many stimulations) (Koob and Le Moal, 2008).

The allostasis ("stability through change") process: this concept proposes that chronic drug exposure produces an alteration of the whole regulatory system rather than temporary variation in a- and b- process. Thus, the continuous over-activation of the reward circuit and the consequent continuous increase in b-process result in a chronic deviation of the regulatory system from its normal operating level. The system fails to return within the normal homeostatic range, which leads to a readjustment of all parameters towards a new abnormal homeostatic set point. If the threats to the system continue to produce disequilibrium, the process of allostasis continues to down-regulate the homeostatic set point, and the organism must mobilize enormous amounts of energy to maintain apparent stability at a now pathological set point (Figure 9).

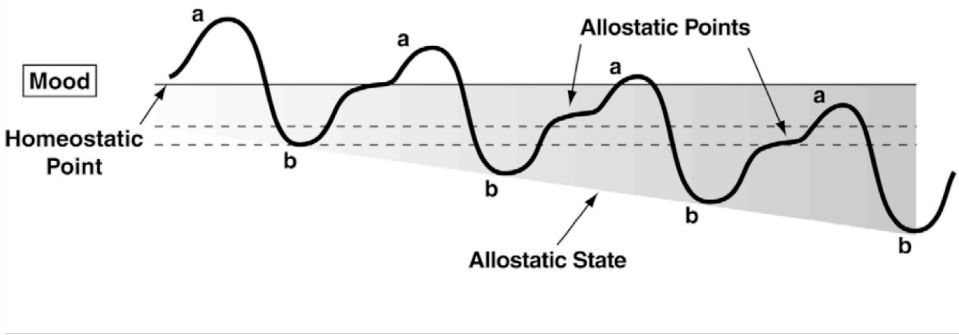


Figure 9. Allostatic process in drug addiction. Diagram illustrating the progressive dysregulation of the brain reward homeostatic set point due to repeated frequent drug use. Note that the apparent b-process never returns to the original homeostatic level before drug-taking begins again, thus does not balance the activational process (a-process) but instead "down-regulated" the mood regulatory system over the time. Consequently, drug takings do not anymore elevate mood but allieviate a depressed state, and abstinence periods unmasked a basal aversive affective state (adapted from, Koob and Le Moal., 2001).

The system is at the limit of its capability, and a small challenge can lead to breakdown. When the organism has reached a state of dysregulation so severe that it cannot recover by mobilizing its own resources, allostasis has reached the point tha is considered illness. This is the beginning of spiralling distress and the addiction cycle, in which addicts are trapped, constantly repeating and passing through three different period: preoccupation-anticipation, binge-intoxication, and withdrawal-negative affect (Figure 10) (Koob and Le Moal, 2008b).

In summary, this theory states that after repeated periods of drugs binge/intoxification, a transition is made from positive reinforcement driving the motivated behaviour to negative reinforcement and automaticity driving the motivated behaviour. Consequently, drug seeking and taking behaviours are initially produced by impulsive acts that are characterized by a sense of tension or arousal before executing the behaviour, and pleasure, gratification, or relief at the time of committing the act. At later stages, drug seeking is a consequence of a compulsive act, which is reflected by anxiety and stress before committing the behaviour and relief from the stress by performing the compulsive act. Therefore, these behavioural alterations will lead to intense states of preoccupation (drug craving) if the compulsive act is not performed, such as during drug abstinence, which anticipates inevitable relapses to drug consumption. This model has leaded researches in understanding the long-term alterations in the "anti-reward" system in addiction, to explain the compulsion to seek and take drugs despite harmful consequences.

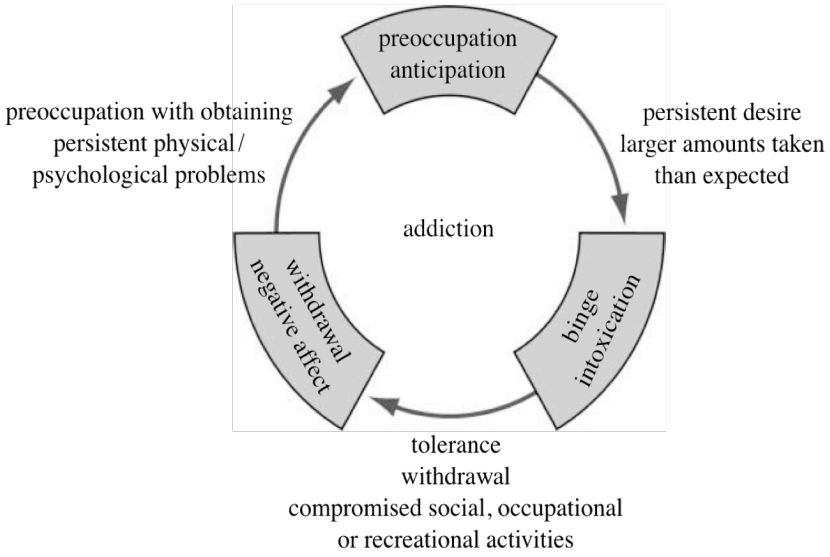


Figure 10. Diagram describing the addiction cycle: preoccupation/anticipation ("craving"), binge/intoxication and withdrawal/negative affect; with the different criteria for substance dependence incorporated from the DSM-IV (Koob and Le Moal, 2008).

1.6.2. The habit learning theory of addiction (Everitt et al., 2008)

In this theory, addiction is considered as a pathology of learning and memory processes in the brain. In particular, the development of compulsive behaviours is viewed as a progressive alteration in one specific memory system that underline procedural learning resulting in inflexible drug seeking habits. Therefore, the autors states that the abundant DA release induced by drugs would produce abnormal neuroadaptations in the striato-nigro-striatal circuitry, accelerating and more effectively consolidating drug seeking as a habitual, well-learned behaviour (Figure 11).

This theory is based on several experimental evidences. First, DA release shift from the ventral to dorsal part of the striatum during adaptive procedural learning. Similarly, drug-induced DA release is more important in the NAc shell during initial drug exposures, then more in the core when drug conditioned stimuli "consciously" drive seeking behaviour, and finally predominant in the dorsal striatum after prolonged history of drug consumption when drug seeking become habitual and inflexible. Importantly, this process is faster with drugs than natural reward (Ito et al., 2000 and 2002; Dickinson et al., 2002). In addition, ventral/dorsal disconnection of the striatal loop specifically decreases cocaine seeking at later stage when it had become automatic, but not at early stage when it still respond to response-outcome mechanisms (Belin and Everitt, 2008). In a similar way, chronic cocaine self-administration provokes neuro-adaptations in D2/3 DA receptors in the dorsal striatum, while at earlier stages of training these adaptations were largely restricted to the ventral NAc region (Porrino et al., 2004). Finally, the dorsal striatum plays an important role in drug relapse at late stages of the human addiction cycle. Indeed, presentation of drug cues to human cocaine addicts induces both drug craving and dorsal striatum activation (Garavan et al., 2000), and specific pharmacological inhibition of dorsal striatum, but not NAc shell or core, prevents in rodents the reinstatement of cocaine seeking after protracted withdrawal (See et al., 2007).

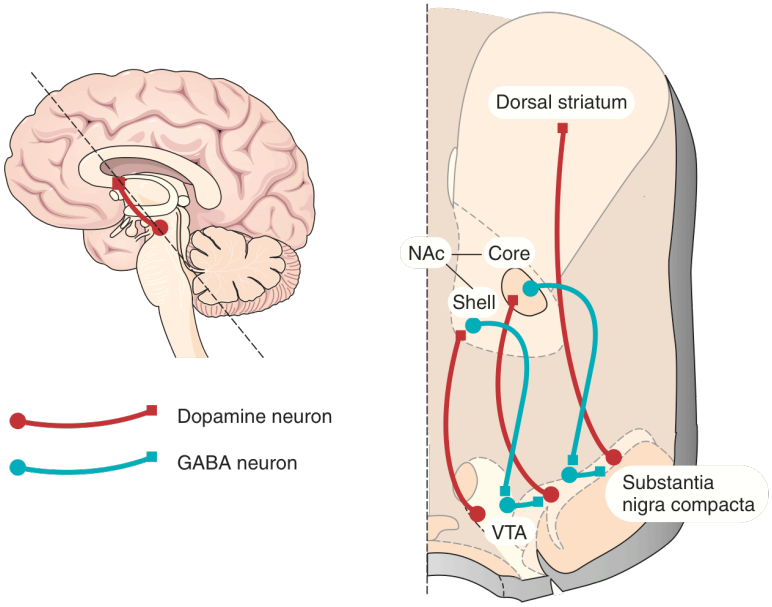


Figure 11. Striato-nigro-striatal circuitry involved in the formation of drug-seeking habit. DA neurons of the medial VTA project to the NAc shell. GABAergic neurons from the NAc shell then back-project onto an interneuron in the VTA, which in turn connects to DA neurons of the lateral VTA. These cells then send their axons to the NAc core, from which cells project to the substantia nigra pars compacta. The nigra cells finally innervate the dorsal striatum. Drugs gradually activate this spiraling connection and animals develop a drug seeking habit once the dorsal striatum is recruited (adapted from Lüscher and Bellone, 2008).

Taken together, these results indicate the progressive evolution of control over drug seeking from ventral to dorsal striatum supporting the habit theory of addition. However, this process occurs in all animals exposed to drugs and may not account, by itself, for the development of addiction. Indeed, it is well known that not all individuals that take drug become addict. Therefore, this theory proposes that this neurobiological alterations lead to compulsive drug taking behaviour only in high impulsive individuals. Indeed, impulsivity is associated with enhanced habit learning formation, determines the predisposition to escalate cocaine self-

administration, increases propensity to relapse to drug seeking after abstinence, and promotes the switch from controlled to compulsive cocaine taking (Dalley et al., 2007; Belin et al., 2008; Broos et al., 2012; Hogarth et al., 2012). Interestingly, impulsive animals showed intrinsically a markedly reduction in D2/3 dopamine receptor availability in the ventral striatum that might explain their greater and/or faster engagement in these addictive-like behaviour (Dalley et al., 2007).

This theory conceptualized addictive disorders as a transition from declarative to automatic behaviours. Thus, initial social drug use is compared with the acquisition of new memories or learning new "adaptive" behavioural responses to "important" stimuli. At this stage, relapse to drug use is considered as a regulated response resulting from a conscious decision process, such as the retrieval of declaratives memories. On the contrary, drug abuse and addiction are considered equivalent to habit learning, and compulsive and late relapses as the retrieval of procedural memories, not verbalized and guiding unconsciously the execution of behavioural responses. This process can be adaptive and positive in physiological conditions, permitting well-learned behaviours to proceed most efficiently without ongoing decision-making, such as riding a bike. However, these well learning behaviours become in addicts their sword of Damocles since additional executive function alterations in these individuals disrupt their capacity to stop automatic behaviours.

This theory has greatly contributed to investigate the alterations in the neurobiological mechanisms of learning and memory during addiction. In particular, this theory have leaded researches on determining the underpinnings of drug-induced altered forms of synaptic plasticity in this

striato-nigro-striatal circuitry that may account for drug's ability to promote abberant habit learning and compulsive drug seeking and taking in vulnerable individuals.

1.6.3. The incentive sensitization hypothesis (Robinson and Berridge, 2008)

This theory postulates that compulsive drug use do not result from a pathologic negative reinforcement nor from aberrant habit learnings, but rather from the senstization of the motivational processes of incentive salience ("wanting"). Indeed, negative withdrawal states may well contribute to drug abuse, but only while they last, for a relative short period of time. Instead, addiction typically persists long after withdrawal syndrome dissipates. On the contrary, psychomotor sensitization and related changes in the brain can persist during very long period after drug use (Robinson and Berridge, 2008), providing a more valid mechanism to explain why addicts continue to want drugs and relapse even after long periods of abstinence. In addition, habits learning alterations would be only a partial part of the addictive process. Indeed, tying shoes or brushing teeth are strong habits that do not lead to compulsive behaviours, even after being performed more than 10.000 times. Conversely, compulsive drug-seeking displayed by drug addicts can be quite "flexible" since addicts can be engaged in actions and routes that they have never performed before, if required to get the drug. Thus, additional alterations in motivational processes may explain the development of compulsive drug seeking and taking. This theory proposes that "liking", defined as hedonic feelings, and "wanting", defined as motivation, are dissociated processes inside the brain reward circuit. Repeated drug use sensitizes only motivational neural

systems that assign incentive salience to drugs and drug-related cues, but not neural hedonic systems that mediate pleasurable effects of drugs. This process may account for the continious enhancement of motivation to take the drug in addicts, despite that they progressively develop tolerance to the drug's hedonic effects. With further drug consumption, this dissociation between "wanting" and "liking" progressively increases, producing a bias of attentional processing towards drug-associated stimuli and pathological motivation for drugs. Incentive sensitization culminates in the core symptoms of addiction when combined with impaired executive control over behaviour.

The importance of sensitization-related changes in the development of drug addiction is supported by evidences showing that sensitizing drug treatments facilitate the emergence of addiction-like behaviours in animals. Thus, an amphetamine treatment regimen that produces psychomotor sensitization accelerates the subsequent escalation of cocaine intake in self-administration paradigms, and increases the motivation to work for drugs as revealed by the enhancement of "break point" on a PR schedule (Ferrario et al., 2007). It has been postulated that incentive sensitization can also spill over in animals or humans to other targets, such as food, sex or gambling. Thus, chronic treatment with dopaminergic medications in some patient populations, such as Parkinson disease, can lead to a "DA dysregulation syndrome" that is manifested not only by compulsive drug use but also sometimes by pathological gambling, hypersexuality or food bingeing (Robinson and Berridge, 2008). Finally, another central element of this theory is the persistent characteristic of these behavioural changes. Indeed, psychomotor sensitization lasts for several months in animals (Robinson and Berridge, 2008), and repeated intermittent administration

of amphetamine causes sensitization of DA release in humans, even when a drug challenge is given a year later (Boileau et al. 2006). Therefore, this theory postulates that repeated exposure to potentially addictive drugs can produce progressive "sensitization" of DA release in susceptible individuals, leading under particular circumstances to the development of persistent morphological changes in brain cells and circuits that mediate motivation. This theory has permitted to open new lines of research in the drug addiction field, such as evaluating the role of drug-induced structural plasticity in the development and persistent of the behavioural alterations that characterized addiction.

All these theories have greatly contributed to lead research toward the discovery of common neurobiological mechanisms across the different types of substance dependence and to explain how chronic abuse of different drugs can lead to the development of a same brain pathology. Recently, it has been proposed that the concept of addiction should be enlarged to new addictive-like disorders, such as pathological gambling, hypersexuality or even some eating disorders (Holden, 2001; Blumenthal and Gold, 2010). This is an important debate to hold because it can bring new therapeutic perspectives for either drug addiction or these supposed behavioural manifestation of addictive-like disorders.

2. FEEDING BEHAVIOUR AND EATING DISORDERS

2.1. Overview

Eating behaviour is a complex response mediated by a large variety of physiological system. Indeed, the expression of appetite reflects the interaction of three distinct and co-ordinated domains: (1) the psychological experiences that include sensations of hunger and satiety, cravings and hedonics triggering eating behaviour; (2) peripheral metabolic events arising as a consequence of nutrient absorption, utilisation and storage; (3) the central neural processes by the CNS that translate all neurotransmitter and metabolic signals into neurochemical brain activity triggering the subsequent act of eating behaviour (Harrold et al., 2012).

A key issue when considering the regulation of eating behaviour is the relationships between the homeostatic and hedonic expression of appetite (Berthoud, 2011). The homeostatic control of appetite is mediated by a biological need to maintain the body's energy stores, which is achieved by increasing the motivation to eat following depletion of the stores. Negative feedback signals are generated once this need is met to finish the period of eating (Schwartz et al., 2000). The hedonic control of appetite is mediated by reward. Reward pathways are activated by palatable food and maintain a drive to eat over satiety, stimulating overconsumption, based on sensory pleasure and reward rather than biological need (Kenny, 2011). In evolutionary terms, this hedonic control was advantageous in environments where food sources were scarce and/or unreliable. Indeed, it provides crucial information about the beneficial properties of food and ensures that palatable food is eaten when available, enabling energy to be

stored in the body for future use. However, this hedonic control has become a dangerous liability in societies like ours, where food is plentiful and ubiquitous. Indeed, many individuals exposed to this environment overconsume palatable foods and many of them become obese (Volkow and Wise, 2005; Swinburn et al., 2009). Thus, it is estimated that nowadays approximately 400 million adults are obese and 1.6 billion are overweight worldwide (WHO, 2006). Moreover, obesity-related diseases, such as type II diabetes, hypertension, and liver disease, have replaced smoking as the leading cause of preventable death in adults, reducing life expectancy by an estimated 6 to 7 years (Haslam et al., 2005; Jia et al., 2010). The health and science communities have responded to this epidemic by working to discover the physiological and psychological underpinnings of excessive food consumption.

Previous researches mainly focused on identifying the metabolic feedback signals and neural systems that could be altered in obese people (Duca and Covasa, 2012). However, several scientists have recently proposed that obesity should not be considered only as a metabolic disease and suggested that some forms of obesity also represent a psychological condition, in which individuals overconsume palatable food due to an excessive hedonic drive, to make them feel better and/or relieve stress. It has been even suggested that some individuals could exhibit an addiction-like behaviour towards food, and parallels have been drawn to drug addiction (Volkow and Wise, 2005).

A better understanding of how these hedonic pathways interact with the metabolic regulation of food intake and in particular by which mechanisms they can override this homeostatic regulation is an important scientific

issue to better define the physiopathology of eating disorders and their potential relationships to addiction.

2.2. Homeostatic regulation of food intake

Consuming sufficient amount of food to maintain adequate energy stores is mandatory for survival in all animal species. Therefore, food intake, energy expenditure and body weight are highly regulated by an active process termed energy homeostasis (Morton et al., 2006). Controlling energy homeostasis demands extensive coordination from the CNS to adjust tightly the balance between energy intake and expenditure. It requires reciprocal communication between peripheral organs that provide information about the nutrient status and energy stores of the body, and the brain that informs about the availability of food in the external environment (Berthoud, 2007). One key brain structure in orchestrating these informations is the hypothalamus. The hypothalamus represents the main hub for sensing nutrients' availability and for generating an integrated behavioural adaptive response to deviations from energetic status. This brain structure is connected to the periphery via the median eminence and to cortico-striatal areas involved in the control of motivated behaviour (Minokoshi et al., 2004; Berthoud, 2007). The hypothalamus integrates hormonal (insulin, leptin, ghrelin and others), nutrient signals and neural inputs from different peripheral organs (mainly stomach, liver and adipose tissue) through the arcuate nucleus and its connection with the brain stem. These signals provide crucial information concerning the amount and the nutritional composition of the food consumed or the level of available energy (carbohydrates, lipids and glycogen), and the hypothalamus will

activate either an orexigenic/anabolic or anorexigenic/catabolic process in response to these inputs (Figure 12) (Schwartz et al., 2000).

- Orexigenic/anabolic pathway: in response to a low energetic state, the release of orexigenic peripheral signals, such as ghrelin, stimulates neuropeptide Y and agouti-related protein expressing neurons within the arcuate nucleus, and the subsequent release of orexigenic neurotransmitters, such as melanin-concentrating hormone and hypocretins by the lateral hypothalamus. These neuronal responses lead to the sensation of hunger and the motivation to seek food that initiate a feeding episode.

- Anorexigenic/catabolic pathway: secretion of anorectic peripheral signals, such as insulin and leptin, stimulates the activity of pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript expressing neurons within the arcuate nucleus. This activation promotes the release of anorexigenic neurotransmitters, such as corticotropin-releasing hormone, thyrotropin-releasing hormone and oxytocin, leading to increase metabolic rate and promote satiety, which finish the episode of eating.

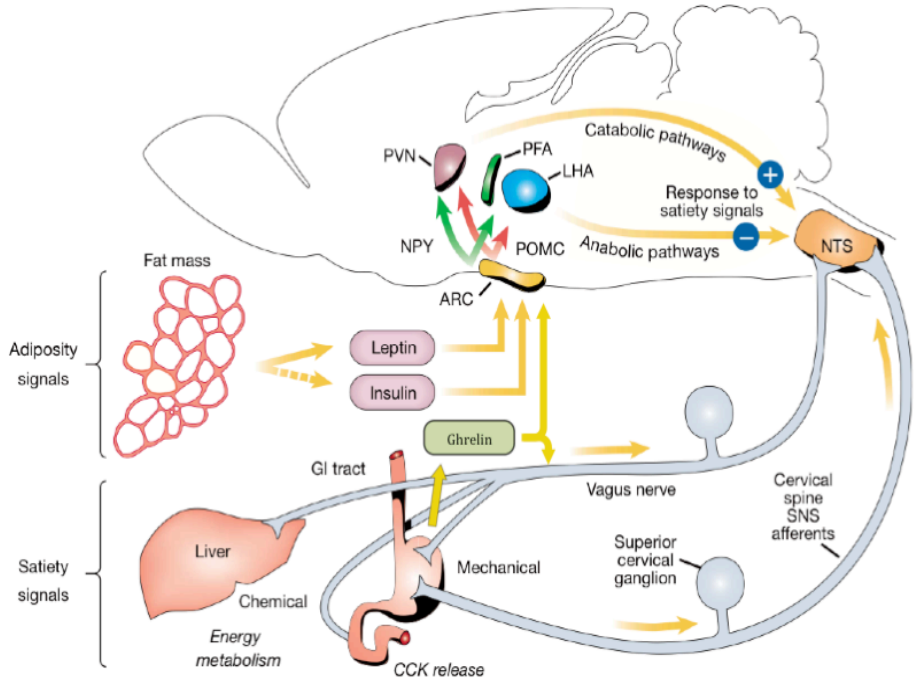


Figure 12. Homeostatic control of food intake. Simplified scheme of the interaction between peripheral hormonal signals and central autonomic circuits regulating appetite and meal size. See explanation in the text (adapted from Schwartz et al., 2000).

The activation of one of these two pathways provides to the brain the necessary homeostatic information to select the appropriate behavioural actions related to food intake.

In summary, the homeostatic system controls eating behaviour at virtually all the different stages: physiological needs (energy absorption, assimilation and storage), psychological drive (hunger and satiety), and behaviour (modulates eating motor action). Alterations in specific components of this system lead to severe metabolic and/or eating disorders. Indeed, insulin, leptin and ghrelin dysfunctions have been

associated in humans to type I and II diabetes, obesity and anorexia nervosa (Pratley and Weyer, 2001; Feng et al., 2012; Zhang et al., 2012). Conversely, genetic deletion of leptin leads to hyperphagia and obesity in rodents (Zhang et al., 1994), while ghrelin-receptor knockout and male ghrelin-deficient mice show resistance to diet-induced obesity when supplied with a high-fat diet early in life (Cummings, 2006).

The initial clinical strategies for the treatment of these disorders were to intend to compensate these metabolic deficiencies by exogenous administration of leptin or insulin (Andrews et al., 1984; Heymsfield et al., 1999). However, most of obese individuals already present resistance to both hormones in the hypothalamus (Williams, 2012) limiting the validity of these treatments. Thus, obesity is often associated with a progressive increase in circulating leptin that do not prevent over-consumption of palatable foods. Therefore, the homeostatic system, including leptin, has not evolved as a signal to prevent obesity. In contrast, other mechanisms should have been strengthened across the ages to actively dampen the anorectic effects of supra-normal leptin level, as it may have conferred a disadvantage in a restrictive environment (Zheng and Berthoud, 2007). Hence, it has been proposed that the high sensitivity of hedonic system to food reward may account for the development of obesity in some individuals. In this context, an emerging issue has been to better understand the role of the brain reward circuit in food intake and eating disorders (Davis et al., 2007).

2.3. Hedonic control of food intake

Apart from being a biological necessity, eating can be a source of joy and we often eat when not metabolically hungry and in spite of large fat reserves. Indeed, reward related brain structures play an important role in the regulation of food intake, and certain types of food, such as high-energy dense and sweet foods elicit strong rewarding effects and greatly influence eating behaviour. Thus, food palatability constitutes a major predictor of food choice and intake, stimulates appetite even in absence of metabolic needs, and thus promotes overeating. Clearly this ''power of food'' is thought to undermine weight regulation and to contribute to the development of eating disorders (Zheng and Berthoud, 2007).

The motivation to eat or stop eating is clearly more complex than a "simple" homeostatic system that responds to metabolic and satiety signals from the periphery. Indeed, sensory perception, mood, memory of food past experiences, social factors, time of day, convenience and cost are a few of the multiple variables that are not metabolically determinant but affect meal-to-meal energy intake (Schwartz et al., 2000). These observations clearly suggest the participation of cognitive and emotional related brain structures in the control of food intake. Indeed, the hypothalamus is highly connected to crucial brain areas involved in hedonic pleasure, emotion and memory, such as the NAc, amygdala and the HPC, or inhibitory control and decision-taking such the PFC; and these structures are strongly activated in response to the presentation of food stimuli (Kelley et al., 2005; Volkow et al., 2012). Thus, the control of food intake critically results from a tight intra-neural communication between the homeostatic and the hedonic system, or between the hypothalamic/peripheral metabolic circuit and the mesocorticolimbic system. This interaction is well illustrated by the facts

that satisfying a metabolic need can be rewarding and that in absence of homeostatic drive to eat, food consumption loses much of its allure.

Emerging evidence now supports the idea that metabolic signals can also directly act on the brain reward circuit adding a potent emotional and motivational meaning to the energetic feelings of hunger and satiety to more efficiently ensure metabolic needs to be fulfilled. First, insulin and leptin can directly influence food sensory perception by acting on taste lingual or olfactory mucosal cells (Shigemura et al., 2004; Julliard et al., 2007; Ketterer et al., 2010). Moreover, receptors for insulin, leptin, ghrelin or orexin are all expressed in different areas of the mesocorticolimbic system. It has been shown that anorexigenic peptides decrease the sensitivity of the brain reward system to the hedonic effects of food, whereas orexigenic peptides increase this sensitivity. Thus, leptin inhibits firing of DA neurons in the VTA and decreases DA release in the NAc, and administration of ghrelin enhances DA release, sucrose preference and intake (Zheng and Berthoud, 2007).

On the other hand, signals generated by cortico-limbic structures processing sensory, cognitive, and reward information can influence hypothalamic processes relevant for energy balance regulation, in a similar way than hypothalamic energy regulatory signals can modulate the activity of cortico-limbic structures (Figure 13).

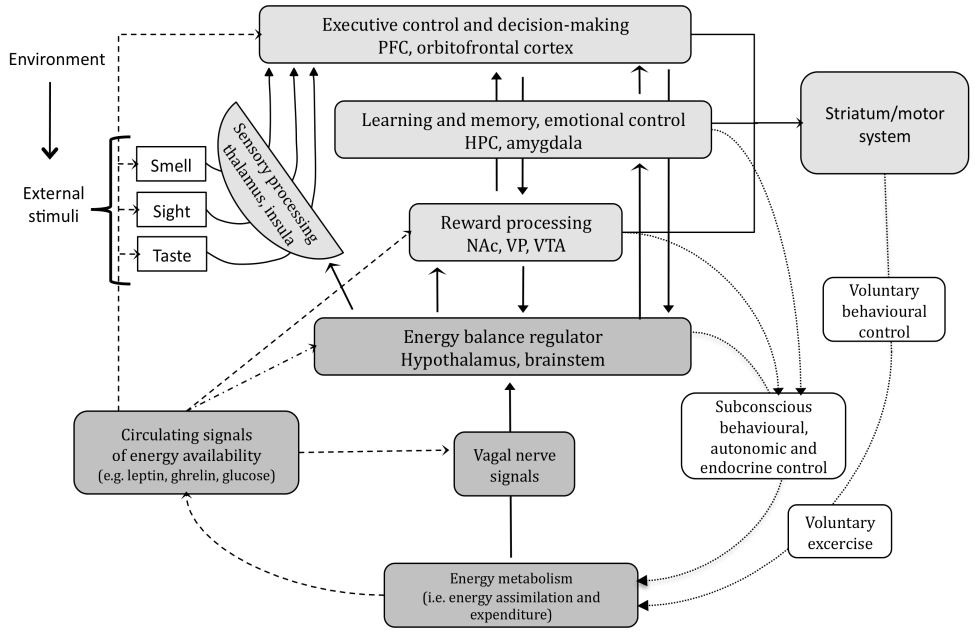


Figure 13. Interaction of the homeostatic and hedonic system in the control of food intake. Schematic diagram showing neural systems and flow of informations involved in the control of food intake and regulation of energy balance. The traditional regulatory circuitry using neural and hormonal feedback from the internal milieu acting on hypothalamus and brainstem is shown on the bottom (dark grey boxes). Sensory and cortico-limbic brain areas used for processing information from the environment are shown in the upper half (light gray boxes). The broken lines with open arrows on the left indicate modulation of sensory, cognitive, and reward processes by circulating signals of fuel availability, such as leptin, ghrelin, and glucose. The full lines/open arrows indicate modulation by nutritionally relevant neural signals such as taste and visceral sensory information, as well as signals originating from the hypothalamus. Full lines/closed arrows represent neural interconnections, and dotted lines/full arrows represent conscious and subconscious behavioral., autonomic, and endocrine output/effector pathways. (adapted from Zheng and Berthoud, 2007).

One important non-homeostatic signal that influences food intake is DA transmission. Indeed, rodents lacking DA greatly loose motivation to work for food and can even die of starvation (Szczypka et al., 2001). In addition, the hunger-stimulating hormone, ghrelin, fails to enhance motivation to

work for palatable food when DA D1-R activity is blocked (Overduin et al., 2012). DA also controls opposite feeding responses, as enhanced DA signalling has been associated with decrease food intake by its inhibitory effects on the hypothalamus (Yang et al., 1997). In general, DA signalling in the mesocorticolimbic system permits to coordinate many aspects of an animal's attempts to obtain food, including increased arousal, motivation, and conditioned learning (Kelley et al., 2005). Thus, dysregulation of the DA system is likely to promote altered motivation to eat food and disrupt energy homeostasis.

The endogenous opioid system in the mesocorticolimbic circuit is also an important brain neurotransmitter that mediates feelings of pleasure derived from food consumption. Indeed, as mentioned before, administration of mu opioid receptor agonists in the NAc enhances hedonic taste reactions to palatable food and promotes voracious appetite in rodents, even in satiated animals (Peciña and Berridge, 2005). In addition, the endogenous opioid system can directly affect the activity of different satiety signals in the hypothalamus, such as oxytocin and alpha-melanocyte stimulating hormone (Olszewski et al., 2004). Therefore, the activation of the endogenous opioid system by palatable foods may represent another mechanism by which the hedonic system can override the homeostatic control of food intake.

Finally, other key structures of the mesocorticolimbic circuit different from the NAc have shown to tightly control eating behaviour. In particular, the prominent projections from PFC, amygdala and HPC to the hypothalamus are thought to play an important role in cognitive suppression of metabolic satiation signals (Berthoud, 2002). Thus, a neutral stimulus paired repeatedly with food acquires the ability to trigger consumption of that

food even in sated animals by increasing the palatability of the paired food (Weingarten, 1983). This cue-potentiated feeding is thought to be a form of craving rather than a non-specific increase in appetite and is dependent of intact projections from the amygdala and PFC to the lateral hypothalamus (Petrovich et al., 2002 and 2007). Moreover, it has been reported that food sated rats with hippocampal lesions show increased appetitive behavior relative to intact controls, and are impaired in using interoceptive cues arising from short- and long-term food deprivation as discriminative stimuli. These results suggest that the behavioural inhibition by energy state signals depends on the HPC (Davidson et al., 2007). Similarly, patients with fronto-temporal dementia demonstrate increased drive to eat suggesting that loss of cortical control can disinhibit circuits that promote food intake (Ikeda et al., 2002). Lastly, it has been even suggested that prefrontal cognitive processes, such as economic choice and decision-making, may also control ingestive behaviour in humans. Indeed several lines of evidence suggest that damage to the right PFC can cause a passion for eating and a specific preference for fine and expensive foods, which has been called the "gourmand syndrome" (Regard and Landis, 1997; Uher and Treasure, 2005). Conversely, alteration of PFC activity in rodents causes hyperphagia and enhances preference for sweet palatable foods (Mena et al., 2011).

In summary, increasing evidences underline the important contribution of the mesocorticolimbic circuit in the control of eating behaviour. Therefore, dysregulations of the brain reward circuit are likely to contribute to the physiopathology of some eating disorders. In this sense, several scientists postulate that the pattern of food intake observed in some obese people is reminiscent of the compulsive drug taking seen in addicts, and claim that

some types of obesity should be considered as a form of "food addiction" (Volkow and Wise, 2005; Blumenthal and Gold, 2010). This is an important debate to hold and resolve because of the potential role of this addictive-like behaviour in the obesity epidemic.

2.4. The concept of food addiction

The relationships between eating disorders and drug addiction are controversial. Indeed, while some scientists prone that both pathologies present strinking similarities (Volkow and Wise, 2005; Blumenthal and Gold, 2010), others point out the lack of convicing evidences for "food addiction" in humans, mainly based in the absence of clinical symptomps such food dependence or withdrawal (Ziauddeen and Fletcher, 2013). To date, addiction is exclusively diagnosed in individuals that present a pathological consumption of drugs of abuse (DSM-IV; APA, 1994). However, the forthcoming DSM V suggests replacing the previous category "Substance-Related Disorders" with "Addiction and Related Disorders", thus for the first time allowing the diagnosis of behavioural addictions (Holden, 2001; draft DSM V, APA). At the present moment, this new category pretends to include only a single entity: gambling addiction. Nevertheless, this theoretical advance will permit to promote new researches on determining whether other excessive engagements in adaptative behaviours, such as hypersexuality and overating, can present addictive-like features.

The controversy of considering some eating disorders as addictive-like behaviours is exemplified by the lack of an accepted definition of food addiction. In response to this issue, it has been recently created the Yale

food addiction scale that translates the substance dependence diagnostic criteria outlined in the DSM IV to eating behaviour (Gearhardt et al., 2009). Thus, according to the Yale food addiction scale, the excessive consumption of food in obese and the pathological drug intake in addicts present striking similarities. Indeed, obese individuals often eat more than they intended and make frequent unsuccessful efforts to control overeating. In addition, obesity can reduce an individual's ability to participate in a full range of social, occupational, and recreational activities. Finally, many individuals continue to overeat despite knowledge that overeating causes obesity and may contribute to, or complicate serious health problems. Not all overweight or obese persons would meet these clinical criteria for food addiction, just as not all drug abusers are considered addicts. Therefore, interindividual differences in reactions to food and vulnerability phenotypes may certainly account for the transition from excessive to compulsive food intake, similarly to those that facilitate the shift from drug abuse to addiction. Indeed, several common behavioural traits have been described between overeating and substances disorders. First, obese persons have more likely high novelty seeking and lower self-directedness scores in specific psychological tests than normal weight individuals. Interestingly, a negative correlation is observed between high novelty scores and the successfulness at losing weight (Sullivan et al., 2007). Similar behavioural traits have been associated to drug addiction, and addicts with high novelty scores are more likely to be dependent on two or more substances (Le Bon et al., 2004; Conway et al., 2003). In addition, overweight and obese individuals often have high scores on a personality measure of impulsivity, and positive correlations are observed between these impulsivity scores and the amount of meal supplement consumed (Galanti et al., 2007). Finally, this high impulsivity in obese people has also

been correlated with poor decision-making on the Iowa gambling task. Indeed, obese individuals perform more poorly on the Iowa glambling task than normal weight peers and similarly to drug addicts. In particular, obese and drug addicts people show a preference for high and immediate reward despite an increased chance of suffering higher future losses (Davis et al., 2004; Bechara and Damasio 2002).

Similarities in neural substrates and neurochemical profile between drug addicts and obese individuals have also been recently revealed. These findings have been determinant to respond to many legitimate doubts concerning resemblances between these two pathologies and further support the concept of "food addiction". One example of major discordance is that food intake, unlike drug intake, is a physiological process that relies on more complex neuronal circuitry, with important contribution of the hypothalamic/metabolic system. Thus, many scientists claim that eating disorders cannot be reduced as "simple" alterations of the reward system, such as in the case of drug addiction. In contradiction to this proposal, accumulative evidences demonstrate the participation of the hypothalamus in drug addiction. Indeed, DA neurons are also present in the hypothalamus where they represent one of the four major brain dopaminergic patways: the tuberoinfundibular pathway. Although this pathway is not directly involved in rewarding processes, drugs of abuse can alter DA neurons activity in the hypothalamus, and thereby its functions (Gudelsky et al., 1986; Baumann and Rothman, 1993). In particular, drugs induce alterations in the hypothalamic-pituitary-adrenal axis and responses to stress have shown to play an important role in drug addiction (Armario, 2010; Goeders, 2003). Moreover, opioid, GABA and acetylcholine receptors are expressed in the hypothalamus, highlighting the direct influence of

morphine, alcohol and nicotine on its functioning (Zhou et al., 2006; Volgin, 2008; Pasumarthi and Fadel, 2010). Importantly, one of the highest rates of intracranial electric self-stimulation, a reward-related behaviour, is obtained in the lateral hypothalamus, and this response can be modulated by opiates and several other drugs of abuse (Singh et al., 1996; Ornstein and Huston, 1977; Bauco and Wise, 1997). In addition, mice can self-administer some drugs of abuse such as opiates directly into the lateral hypothalamus (Cazala et al., 1987). Finally, it has been recently reported that the orexin system plays a crucial role in the lateral hypothalamus in different aspects of drug addictive processes, including reward/reinforcement, withdrawal and relapse (Plaza-Zabala et al., 2012).

Therefore, the hypothalamus is now well recognized to be an important component of the reward system and thus a key substrate for addictive disorders. From this perspective, some eating disorders that involve alterations of the hypothalamic circuit could present some addictive-like characteristics based on this physiological role of the hypothalamus. Indeed, growing evidences show that several metabolic hormones or peptides that are altered in obese patients, such as ghrelin, leptin, insulin, orexins and melanocortin, modulate the rewarding properties of drugs of abuse and modify DA activity (Davis et al., 2010; Dickson et al., 2011; Chung et al., 2011). Conversely, alterations in energetic status, such as food deprivation and obesity, increases and attenuates respectively responses to drug reward (Carr, 2002; Blendy et al., 2005). Importantly, an inverse relationship has been reported in humans between body mass index and recent illicit drug use (Blüml et al., 2012), as well as an association between obesity and a lower risk for substance use disorders (Simon et al., 2006). Taken together, these results strongly suggest the possibility that food and

drugs may be competing for overlapping reward mechanisms including those generated through the hypothalamus.

Reticent scientists often point out that food-induced DA release in the mesocorticolimbic system, in particular in the NAc shell, is lower than the one induced by drugs of abuse, and unlike this latter undergoes habituation (Pothos et al., 1991; Wise et al., 1995; Bassareo and Chiara, 1997). Therefore, overactivation of the DA system by food seems unlikely to explain the development of eating disorders. In contrast to this hypothesis, recent data have demonstrated that the increased DA release in the NAc shell triggered by palatabale food does not habituate and can still be elicited several weeks after the first exposure in animals that develop binge-like eating behaviour (Rada et al., 2005). Thus, prolonged overconsumption of these types of food could give rise to dysfunctions of the dopaminergic system in susceptible individuals, as it occurs with drugs of abuse. In support to this proposal, it has been shown that obese individuals present a decrease in striatal DA D2-R availability similar to the one reported in drug addicts (Wang et al., 2001). Importantly, a negative correlation between individual's body mass and the availability of D2-R in the striatum has been observed (Wang et al., 2001). Conversely, a recent study shows that rodents chronically exposed to a high palatable diet develop an obese phenotype, a decreased D2-R striatal expression and compulsive palatable food intake (Johnson and Kenny, 2010). Moreover, these striatal DA D2-R dysfunctions have been associated with decreased metabolic activity in the PFC in obese individuals, similarly to drug addicts (Volkow and Fowler, 2000). This decreased activity in the PFC is likely to contribute to poor self-control, impulsivity and high compulsivity, both in drug addicts and obese patients. Therefore, DA D2-R alterations in the

mesocorticolimbic circuit have been considered a potential common neuroadaptation among addictive-like behaviours that could represent both a vulnerability factor and/or adverse consequence of overconsumption of drugs or palatable food (Volkow and Wise, 2005).

Moreover, the concept of food addiction is debated due to the absence of convincing physiological markers of food dependence, such as a withdrawal syndrome. However, it has been recently reported that rodents previously exposed to diets rich in sugar and then withdrawn to less palatable food or injected with naloxone display a withdrawal-like syndrome similar to those observed in drug-dependent animals, including specific somatic signs, anxiety, aggressiveness and depressive-like behaviour (Colantuoni et al., 2002; Avena et al., 2008). These findings suggest that overconsumption of palatable food can trigger alterations in the endogenous opioid system, as this food withdrawal syndrome was also precipitated by an opioid antagonist. In agreement, prolonged intermittent access to sugar was associated with decrease gene expression of preproenkephalin in the NAc and caudate-putamen (Spangler et al., 2004).

Finally, it is often doubted whether food can induce feelings of craving, and if relapse to food consumption exist. However, it is now well-known that the three main factors that induce craving and relapse in human addicts, reinforcer-related cues, stress and re-exposure to the reinforcer, also reinstate food seeking behaviour in animal models of relapse (Nair et al., 2009). In addition, food-seeking responses are enhanced during the time course of palatable food “abstinence”, a phenomenon refered as incubation of food craving that is reminiscent from the one observed with drugs of abuse (Avena et al., 2005 and 2008). Finally, imaging studies show that the

expression and inhibition of food and drug craving modulate similar subregions in the PFC in humans (Volkow et al., 2012).

In summary, whether or not certain types of eating disorders should be defined as an addiction, accumulating evidences acknowledge that similar brain dysfunctions could mediate both disorders. Therefore, translating our extensive knowledge on the neurobiology of drug addiction into the study of eating disorders might permit to better understand the physiopathology that underlines abnormal food consumption. A recent line of research in drug addiction is the characterisation of the neuronal plasticity mechanisms underlying the development of the disease (Kalivas and O'Brien, 2008). Therefore, it will be interesting to know whether similar neuroplastic alterations could be involved in eating disorders that present addictive-like characteristic.

3. NEURONAL PLASTICITY

3.1. Overview

Neuronal plasticity refers to the intrinsic ability of the neurons to modify their morphology or functioning in response to external factors, resulting in synaptic activity modifications or brain neural circuitry changes. This plasticity is not exclusively restricted to the period of brain development and it is now well recognised that the brain remains plastic even into adulthood (Lillard and Erisir, 2011). Indeed, adult neuroplasticity has been described to contribute to the recovery process that follows brain injuries (Johansson, 2000). Moreover, it is thought that the functioning of some cognitive processes, such as learning and memory, could rely on neuroplasticity mechanisms (Martin et al., 2000a; Lamprecht and LeDoux, 2004). Thus, the concept of adult neuronal plasticity has replaced the formerly-held position that the brain is a physiologically static organ with amitotic cells, uncapacity of self-renewal and hard-wired circuitry, and provides a reliable explanation for the brain's ability to improve motor function and learning despite a limited cerebral space.

One important feature of adult neuronal plasticity is to adapt neuronal activity and brain functioning in response to constant variations in internal physiology and external environment, a phenomenon called activity-dependent plasticity (Malenka and Bear, 2004; Holtmaat and Svoboda, 2009). Thus, adult neuroplasticity is considered a crucial process for individuals to adjust their own behaviour in response to experience. This neuronal process can also have negative physiological consequences in some particular circumstances, such as in the phenomenon of phantom limbs (Ramachandran et al., 1998). Drug addiction and addictive-like

disorders have been suggested to also represent deemed examples of "negative plasticity", in which neuronal adaptations result in highly maladaptive behaviours (Kauer and Malenka, 2007). This maladaptive neuronal plasticity in addiction includes homeostatic adaptations triggered by repeated exposure to drugs, such as drug tolerance and dependence. Indeed, these adaptations aimed to counteract chronic brain insult induced by repeated drugs use, but their undesirable counterpart is the subsequent increase of drug consumption. (Hyman and Malenka, 2001; Hyman et al., 2006). Morevoer, drug addiction is associated with the emergence of other forms of maladaptive neuronal plasticity that are more long-lasting than drug tolerance and dependence and may underline the development of persistent behavioural alterations that characterize the pathology, such as drug craving and relapse (Hyman and Malenka, 2001; Hyman et al., 2006). To better understand addiction, it would be thus essential to know how the effects of a drug during an initial exposure lead progressively to stable molecular and cellular changes after repeated exposure. This will be determinant in order to explain: (1) how repeated episodes of DA release consolidate drug-taking behaviour into compulsive use, (2) how drug-related cues become dominant in the control of behaviour, (3) how risk of relapse from a drug-free state can persist for years.

Substantial progress has been made in understanding the molecular and cellular mechanisms of tolerance, dependence and withdrawal. However, less it is known about the neurobiological substrates of these long-lived behavioural abnormalities. It is postulated that drugs of abuse “hijack” plasticity mechanisms involved in learning and memory processes in circuits responsible for reinforcement and reward. In this way, addiction has been concieved as powerful pathological form of learning and memory

(Hyman et al., 2006). Thus, the transient neuroplasticity triggered by initial social drugs use has been compared to the necessary changes that are antecedent to acquiring a new motivated-behaviour, whereas the stable neuroplasticity that mediates compulsive drug use is thought to correspond to the stable information that is retrieved to guide the execution of well-learned automatic behaviour (Everitt et al., 2008). Evolution from transient to stable neuroplasticity is thought to mediate the shift from regulated to compulsive relapse: the transition from the conscious decision to take drugs to automatic and inconscious relapsing behaviours triggered by drug conditioned cues and stressors (Figure 14) (Kalivas and O'Brien, 2008).

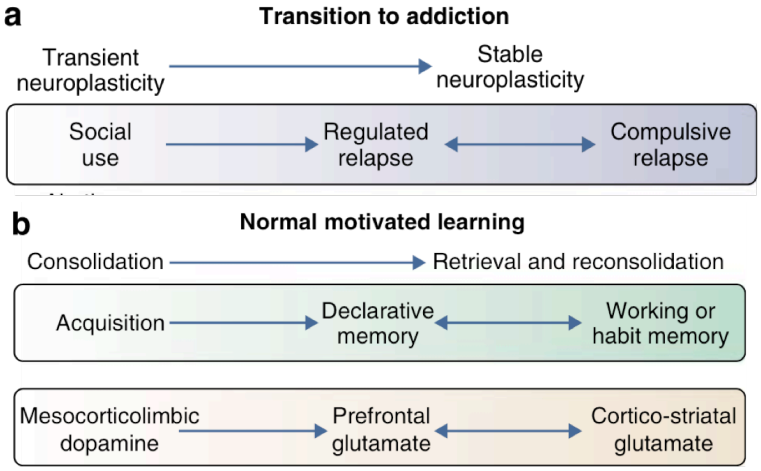


Figure 14. Neuroplasticity changes through the addiction process. Illustration of the relationship between neuroplasticity, motivated learning, brain circuitry, and the stages of addiction. (a) The phases of addiction from the development of addiction (social use) to vulnerability to relapse (transitioning from regulated to compulsive). (b) Mapping normal motivated learning processes and the relevant dopaminergic and glutamatergic circuitry onto the stages of addiction. (Kalivas and O'Brien, 2008).

These neuroplastic changes are thought to alter transitorily DA synaptic transmission in key areas of the mesocorticolimbic system such as the VTA, the NAc and PFC at initial drugs use, and thereby strongly reinforcing drug-taking behaviours. In later stages, the neuroplastic changes are thought to be more drastic, leading to long-lasting impairments of synaptic transmission and a stable reorganization of the mesocorticolimbic synaptic connectivity. Indeed, accumulative evidences have shown that chronic exposure to many drugs abused by humans lead to the emergence of enduring forms of synaptic and structural plasticity in the brain reward circuit (Lüscher and Malenka, 2011; Robinson and Kolb, 2004).

3.2. Synaptic plasticity

Synaptic plasticity describes the ability of individual synapses to alter their strength of transmission over the time in response to either use or disuse of transmission over synaptic pathways (Feldman, 2009). Synaptic transmission relies on several elements that include the pre-synaptic release of neurotransmitters, post-synaptic receptors and transporters and enzymes that terminate the transmission. Thus, synapses can be considered as filters with distinctive properties, and synaptic transmission appears to vary importanly depending on the recent history of activity of the synapse. Activity-dependent changes in synaptic transmission arise from a large number of mechanisms, and synaptic plasticity can be divided into three broad categories: (1) short-term plasticity, that occurs over milliseconds to minutes, allows a rapid and reversible modulation of synaptic transmission strengh and has been viewed as a dynamic filter that optimizes information processing (Zucker and Regehr, 2002; Deng and Klyachko, 2011) (2) long-term plasticity, involving changes that last for

hours or longer, is thought to underpin learning and memory (Martin et al., 2000a) (3) and homeostatic plasticity of both synapses and neurons allows neural circuits to maintain appropriate levels of excitability and connectivity despite continuous occurrence of short- and long-term plasticity. This metaplasticity represents an essential negative feedback to maintain proper neuronal network function over the time (Pérez-Otaño and Ehlers, 2005).

Long-term form of synaptic plasticity is probably the most studied type of neuronal plasticity, and alterations of synaptic plasticity processes have been linked to several pathologies, including drug addiction (Lüscher and Isaac, 2009; Kauer and Malenka, 2007). Long-term synaptic plasticity encompasses both a long-lasting strengthen or weaken in the efficacy of synaptic transmission, respectively referred as long-term potentiation (LTP) and long-term depression (LTD). This form of neuronal plasticity usually depends on the pattern of pre and postsynaptic firing. Thereby, most forms of long-term synaptic plasticity appear to be an associative form of plasticity, reflecting the Hebbian theory: “any two cells or systems of cells that are repeatedly active at the same time will tend to become “associated”, so that activity in one facilitates activity in the other". The theory is often summarized as "*Cells that fire together, wire together*" (Hebb, 1961). Thus, long-term changes in the transmission properties of synapses are thought to provide a physiological substrate for associative learning.

LTP and LTD have been found to occur in different types of neurons that release various neurotransmitters. However, the most common neurotransmitter involved in long-term forms of synaptic plasticity is glutamate. Long-term plasticity at glutamatergic synapses is mediated by

different neuronal mechanisms, and one of the most studied required NMDA-R activity (Figure 15). Thus, depending on the nature of pre-synaptic signals, activation of NMDA-R leads to opposite mechanisms (Malenka and Bear, 2004):

- High frequency pre-synaptic stimuli lead typically to strong post-synaptic depolarization, complete displacing of the magnesium ions that block NMDA ions channels and a large entry of calcium in post-synaptic neurons. This important raise in intracellular calcium leads to the activation of specific protein kinases that will phosphorylate synaptic AMPA-Rs, improving their cation conduction, and will also promote insertion of new AMPA-Rs in the synapse, thereby potentiating the synapse and inducing LTP.

- At the contrary, low frequency pre-synaptic stimuli lead to weaker depolarization, only partially displace the magnesium ions, resulting in less calcium entering the post-synaptic neuron. This lower intracellular calcium concentration will instead activate particular protein phosphatases leading to AMPA-Rs dephosphorylation, decreasing their conductance and promoting their removal from the post synaptic membrane, thus resulting in weaken synaptic transmission and LTD induction.

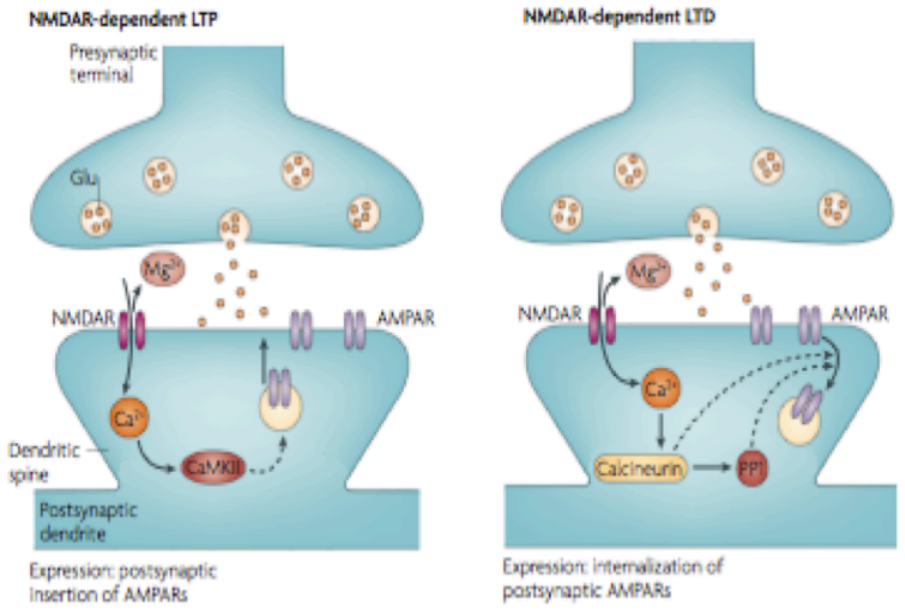


Figure 15. NMDA-R dependent long-term forms of synaptic plasticity. Schematic diagrams of the induction and expression of NMDA-R dependent LTP and LTD observed in the rodent brain (see description in the text) (adapted from Kauer and Malenka, 2007).

The induction of these strengthening and weakening synaptic mechanisms can modulate synaptic transmission up to one hour (Malenka and Bear, 2004). However, the activation of additional synaptic mechanisms that required the synthesis of new protein and/or gene expression leads to stabilize these initial synaptic changes, and allows LTP and LTD to last hours, days, or even weeks (Malenka and Bear, 2004). Therefore, long-term synaptic plasticity has been hypothesized as neurobiological substrate for long-lasting memory storage (Martin et al., 2000a).

LTP and LTD are expressed at excitatory synapses throughout the brain and have both been described at corticostriatal connections, where they

might underline motor-skill learning, cognitive performance and reward mechanisms (Calabresi et al., 2007). Importantly, both forms of synaptic plasticity in the striatum strongly depend on the activation of DA receptors. Indeed, striatal LTP and LTD are impaired after pharmacological manipulation or genetic disruption of the dopaminergic system (Centonze et al., 2001). Thus, the role of the dopaminergic system in gating long-term synaptic plasticity may account for the ability of DA to promote memory consolidation (Wittmann et al., 2005). Hence, a simple model to link learning and addiction predicts that addictive drugs might induce abnormal long-term synaptic plasticity in the reward system by excessively enhancing DA release in this circuit, which would result in the formation of persistent drug-related memories. In support to this proposal, accumulative evidences have demonstrated that most of addictive drugs, such as cocaine, morphine, nicotine, ethanol and amphetamines, induce long-term synaptic plasticity in neurons of the brain's reward system (Saal et al., 2003, Lüscher and Malenka, 2011). Such drug-evoked plasticity has been reported in the VTA, NAc and PFC, and seems to contribute to all the different phases of the addiction process (Figure 16) (Lüscher and Malenka, 2011).

Drug-induced synaptic plasticity may participate in the intital effects of drugs of abuse. Indeed, a single exposure to several drugs of abuse, but not non-addictive compounds, triggers LTP of AMPA receptor-mediated currents at excitatory synapses onto DA neurons in the VTA (Ungless et al., 2001; Saal et al., 2003; Niehaus et al., 2010). In addition, the initial rewarding and reinforcing properties of many drugs of abuse evaluated in the CPP and self-administration paradigms are impaired by NMDA-R blockade, a treatment that prevents LTP and LTD in many brain regions

(Mameli and Lüscher, 2011; Malenka and Bear, 2004). Futhermore, drug-induced synaptic plasticity mediates the long-lasting changes promoted by chronic consumption of drugs of abuse. Thus, chronic administration of cocaine provokes synaptic plasticity changes in many components of the brain reward circuit (Borgland et al., 2004; Thomas et al., 2001; Huang et al., 2007), and some of these alterations can persist up to several weeks or months after prolonged cocaine self-administration (Martin et al., 2006; Chen et al., 2008). In addition, reversal of cocaine-evoked synaptic plasticity in the NAc has shown to abolish cocaine locomotor sensitization, a model of drug-induced long-lasting behavioural changes (Pascoli et al, 2011). Finally, synaptic plasticity changes triggered during drug abstinence have been proposed to be a neurobiological substrate for drug craving. Indeed, incubation of cocaine craving correlates with a progressive increase in GluA2-lacking AMPARs in the NAc, which has been interpreted as a transition from LTD-like state at early withdrawal stage to LTP-like state with prolonged abstinence (Conrad et al., 2008). In addition, selective inhibition of the GluA2-lacking AMPARs in the NAc reduces cue-induced cocaine seeking in mice, which is generally accepted as a model for relapse in humans (Conrad et al., 2008).

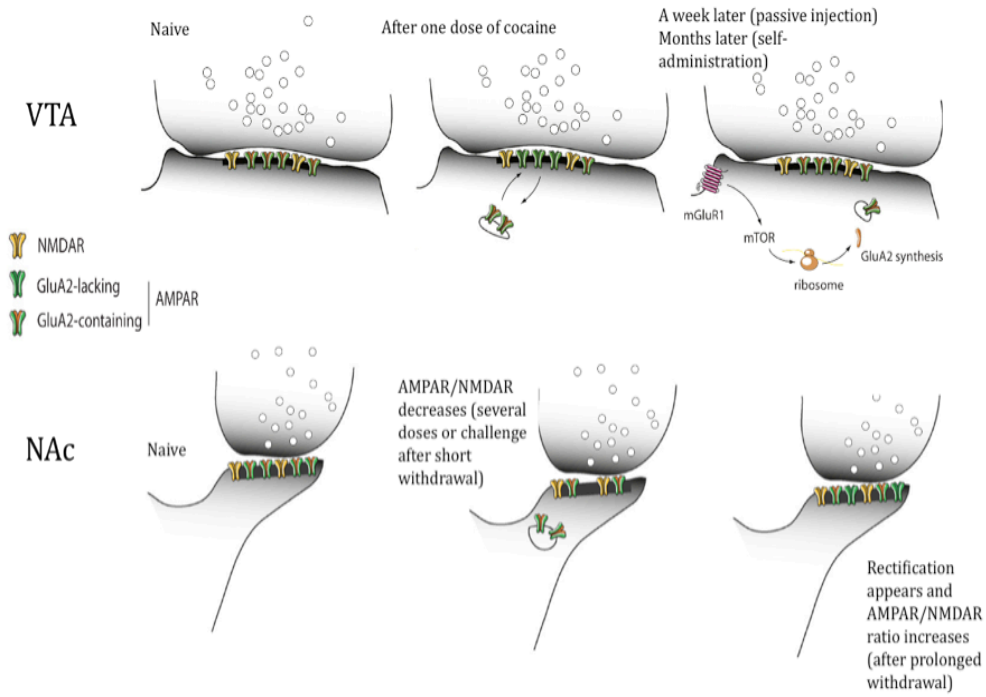


Figure 16. Drug-evoked synaptic plastic changes in the mesocorticolimbic circuit. Schematic diagrams illustrating the alterations of synaptic transmission in the VTA and NAc induced by single or repeated injections of cocaine, as well as those observed during cocaine withdrawal. In naïve animals, synapses in the VTA and NAc contain NMDARs, and AMPARs that all contain the GluA2 subunit. After one dose of cocaine, synaptic plastic changes occur in the VTA, in particular AMPARs are exchanged for GluA2-lacking ones through mechanisms involving endo- and exocytosis, leading to potentiate synaptic transmission. In the NAc, synaptic plastic changes are observed after repeated injection of cocaine and some AMPARs are endocytosed leading to synapse depression. Finally, during cocaine withdrawal, potentiated synaptic transmission in the VTA persist up to one week or several months depending on the nature of the past cocaine treatment (passive injection vs. self administration) before returning to a basal level through de novo synthesis of GluA2 containning AMPARs. In the NAc, a progressive increase in synaptic GluA2-lacking AMPARs is observed during cocaine abstinence, leading to potentiation of synapses (adapted from Lüscher and Malenka, 2011).

From these findings, drug-induced synaptic plasticity in the mesocorticolimbic system has been considered an important neurobiological substrate for the development of different classes of addictive disorders. Nevertheless, it is also clear that these synaptic adaptations do not predict that addiction will follow. Indeed, a recent study shows that while all animals develop impaired LTD in the NAc following prolonged cocaine self-administration, only a modest proportion of rats develop behaviours analogous to human addicts. Interestingly, after 2 weeks of drug withdrawal the ability to generate LTD slowly recovered in ''non-addicted'' animals, while ''addicted'' animals expressed persistently impaired LTD (Kasanetz et al., 2010). Therefore, additional neuronal plasticity changes certainly occur in some drug users that contribute to the persistence of synaptic plasticity alterations and the development of addictive-like behaviours. In this perspective, it has been recently proposed that the ability of drugs of abuse to induce structural plasticity changes in the brain reward circuit may represent one of these additional mechanisms by which drugs of abuse alter neuronal physiology and drug users behaviour in a long-lasting manner (Robinson and Kolb, 2004).

3.3. Structural plasticity

Structural plasticity represents a physical reorganization of synaptic connectivity and neuronal circuitry. This morphological adaptation includes changes in the size of cell bodies, dendritic three arborizations or spine number and shape. Therefore, unlike synaptic plasticity, which alters locally the activity of specific pre-exiting synapses, structural plasticity can potentially affect a neuronal network in its entirety, removing or creating new connectivity pattern between neurons, thus substantially impacting

cerebral functions (Chklovskii et al., 2004). Like synaptic plasticity, morphological plastic changes can be triggered by activity-dependent mechanisms and represent a neuronal substrate underlying experience-induced behavioural changes (Butz et al., 2009). In fact, both forms of neuronal plasticity seem strinkingly associated. Thus, stimulation protocols that induce LTD have shown to also promote spines shrinkage or synapse elimination (Zhou et al., 2004; Bastrikova et al., 2008), whereas induction of LTP have been associated with enlargement of pre-existing spines or even with the formation of new ones (Figure 17) (Matsuzaki et al., 2004; Engert and Bonhoeffer, 1999).

At a molecular level, it is believed that the maintenance of LTP and LTD processes can initiate changes in signalling pathways, and in the synthesis of proteins that modulate the organization of cytoskeletal proteins, such as actin, and transynaptic adhesion molecules including cadherins, thus altering synaptic spine structure and stability (Kasai et al., 2003). Indeed, induction of LTP has been associated with an increased concentration of actin filaments (F-actin) at the synapse through the polymerization of globular actin monomer (G-actin), which lead to the enlargement of dendritic spines; while induction of LTD provokes convertion of F-actin into G-action and the destabilization of the cytoskeleton of dendritic spines (Segal, 2005). Upon stabilization, imaging studies show that the spine lifespan can strongly increase, and a new created spine can even "survive" for months (Trachtenberg et al., 2002). Therefore, it is believed that stabilization of a "transient" form of activity-dependent neuronal plasticity could occur through morphological changes, in particular at the dendritic spines level.

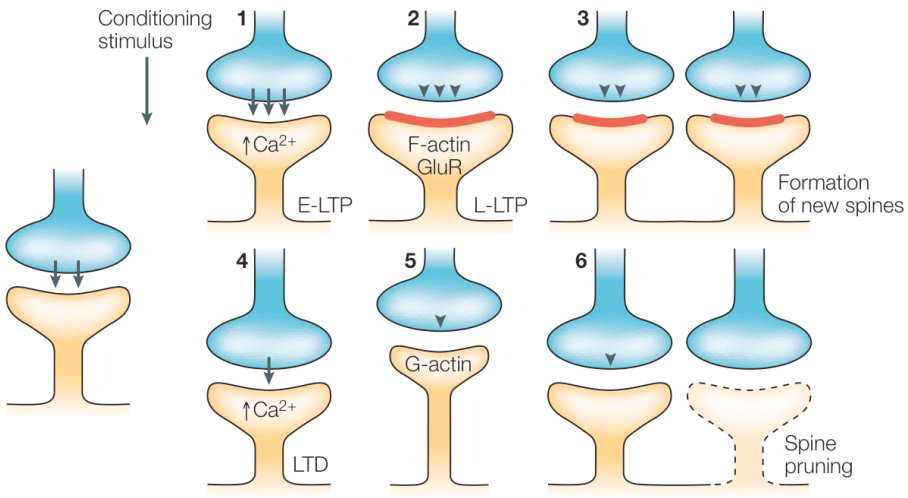


Figure 17. Long-term synaptic plasticity promotes structural plastic changes. Schematic depiction of changes in dendritic spine morphology associated with LTP and LTD. (1-3) Stimulation patterns that induce LTP lead to increase neuroransmitter release, intracellular calcium (Ca2+); cause spine enlargement, which involves an influx of glutamate receptors (GluR) and an accumulation of F-actin. Finally new spines can be formed on the conversion to the late phase of LTP (L-LTP). (4-6) By contrast, when the stimulus pattern leads to LTD there is a decrease of neurotransmitter release, conversion of F-actin into G-actin, spine shrinkage and eventually spine pruning. E-LTP, early-phase LTP. (adapted from Segal, 2005).

3.3.1. Dendritic spines

Spines are tiny specialized membranous protrusions, generally arising from the dendritic arm, that represent the main structural element by which many neurons establish synaptic connections with surrounding axons (Shepherd, 1996). Potentially, a higher number of dendritic spines reflects a greater possibility of making synaptic contacts. Indeed, pyramidal cell spines are virtually never seen without a presynaptic element (Mates and Lund, 1983). Therefore, structural plasticity and dendritic spines density changes are more likely than synaptic plasticity to impact neuronal

physiology and network functions since activation of neurons cannot be triggered only by the activity on one individual synapse, but rather the sum of all their synaptic inputs.

Dendritic spines are composed by two distinct structural elements: a bulbous head connected typically to the dendritic shaft by a narrow neck. The geometric constriction of the spine neck permits to isolate physically and functionally the synaptic elements from the rest of the neuronal cell. In particular, the spine neck is thought to function as resistive barriers limiting synaptic currents and signaling molecules exchanges between spine head and parent dendrite. Upon synaptic activation, this compartmentalization allows membrane potential, second messengers and activated enzymes to activate synapse-specific regulatory mechanisms on a reduced time scale, thus providing to the synapse the ability of fast plastic responses (Nimchinsky et al., 2005). The spine head contains all the essential elements that permit proper synaptic functions: (1) the post-synaptic density that includes neurotransmitter receptors, cytoskeletal and adaptor proteins, mediates reception and transition of synaptic inputs, (2) polyribosmoses that might provide to the synapse the intrinsic capacity of protein synthesis, thus giving rise to a localized and rapid protein production that could be critical for activity-dependent plasticity, (3) and the smooth endoplasmic reticulum and/or the spine apparatus that allow post-translational protein processing, receptor spatial delivery and regulation of intracellular calcium levels (Figure 18) (Nimchinsky et al., 2005).

One of the most striking characteristics of dendritic spines is their morphological diversity. In fact, an infinite number of spine shape and size can be observed, describing a continuum from short and squat to long and

bulbous. However, spines are often classified into five subtypes (Figure 16) (Harris et al., 1989). This morphological heterogeneity underlines important anatomic and functional differences between spines. Thus, the different spines subtypes have been further classified into two anatomo-functional categories: the small and immature spines, and the large and mature ones (Kasai et al., 2003).

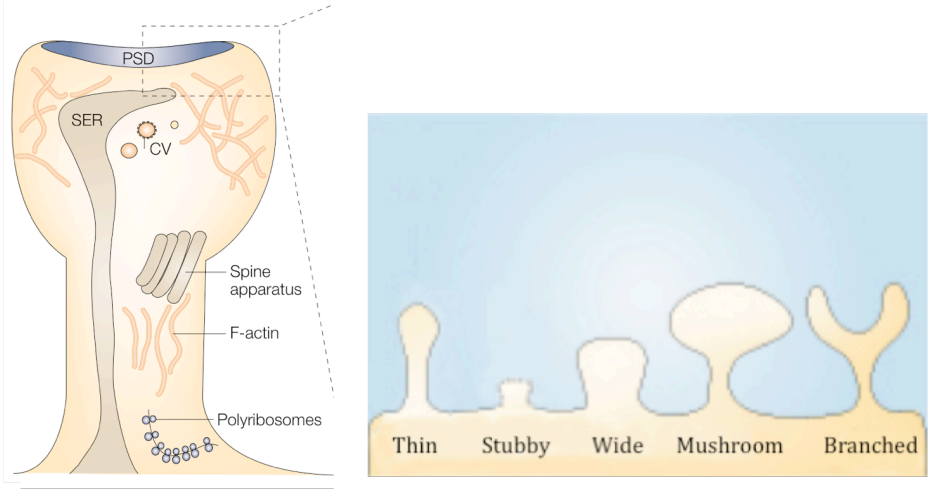


Figure 18. Structure and morphology of dendritic spines. Illustration of the principal components (left panel) and morphological shapes (right panel) of dendritic spines. SER: smooth endoplasmic reticulum (adapted from Hering and Sheng, 2001).

Small spines are less likely than larger ones to contain smooth endoplasmic reticulum and spine apparatus, have few or no polyribosomes. In comparison, most of larger spines contain smooth endoplasmic reticulum, spines apparatus, polyribosomes and have larger post-synaptic densities than small ones (Nimchinsky et al., 2005). As the size of post-synaptic density correlates with the number of post-synaptic receptor, larger spines have shown to contain more AMPA-R and thus to be more sensitive to

glutamate stimulation than small spines (Takumi et al., 1999). In addition, larger post-synaptic densities have been also associated with increased number of presynaptic vesicles, and thus greater neurotransmitter release (Schikorski and Stevens, 1997). Taking together, these evidences suggest that larger spines are preferential sites for strong synaptic transmissions. In fact, the likelihood of large spine to have smooth endoplasmic reticulum and spine apparatus also supports an increased synaptic strength, as calcium concentration is a crucial event to amplify synaptic signals. At the contrary, small spines contain few AMPA-R but, unlike larger ones, do express NMDA-R. Therefore, small spines have been considered as "silent" spines, less sensitive to glutamate, and preferential sites for the induction of NMDA-R dependent synaptic plasticity (Isaac et al., 1995). The propensity of small spines to undergo synaptic plasticity confers to these types of spines a high motility and a low stability. Indeed, small spines can "easily" be subjected to synaptic plasticity induced morphological changes, but can also be readily eliminated (Kasai et al., 2003). By contrast, large spines lack NMDA-R and have greater F-actin/G-actin ratio, render them less subject to activity-induced calcuim influx and, at the same time, less structurally sensitive to this types of environmental changes. In addition, large spines possess more adhesion molecules, such N-cadherin, which promotes their physical anchoring to the pre-synaptic compartment. Therefore, large spines hold a decreased motility and consequently display an impressive structural and functional stability (Kasai et al., 2003). In this view, large spines are ascribed to mature spines that are unlikely to experience synaptic plasticity, but ensure a high synaptic transmission and can survive for more than a month or even for a year (Nimchinsky et al., 2005; Trachtenberg, et al., 2002; Grutzendler et al., 2002). The differences in function and stability between types of spines have further suggested

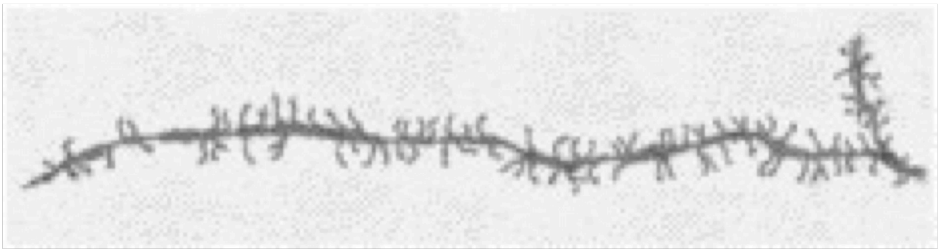
that small and large spines may play distinct roles in learning and memory. Indeed, large spines are stable and maintain preexisting strong connections, and thus are considered a structural basis for long-term memory. At the contrary, small spines are highly plastic and can be generated during activity-dependent processes, providing an inexhaustible source of new synapses and memory (Kasai et al., 2003).

3.3.2. Drug-induced structural plasticity

Accumulative evidences acknowledge that chronic administration of most drug of abuse, including amphetamine, cocaine, nicotine and morphine induces structural plasticity changes in differents areas of the brain reward circuit (Robinson and Kolb, 2004; Russo et al., 2010). Thus, repeated exposure to psychostimulants and nicotine consistently increases dendritic spine density in the NAc, VTA and mPFC (Figure 19) (Robinson and Kolb, 2004). In sharp contrast, chronic exposure to opiates decreases the number of dendritic spines in the NAc, mPFC and HPC, and also decreases the soma size of VTA dopaminergic neurons (Sklair-Tavron et al, 1996; Robinson et al., 2002). These differences between drugs on structural plasticity are not understood, and highlight that drug-induced structural plasticity could depend on different brain primary pharmacologic effects of each drug. Nevertheless, it is possible that structural plasticity changes that are different in nature could lead to similar alterations of brain functioning and behaviour, since the impact of drug-induced structural plasticity on neuronal network activity is still largely unknown. In addition to the density, drugs also alter the morphological shapes of dendritic spines. For instance, amphetamine or cocaine approximately doubles the proportion of spines with multiple heads (branched spines) (Robinson and Kolb, 2004).

Although little is known about branched spines, it is hypothessized that branched spines are formed by ‘‘splitting’’ an existing presynaptic bouton to form two new synapses, and may represent a radical alteration in synaptic organization (Sorra et al., 1998).

Drug naive rat



Cocaine self-administering rat

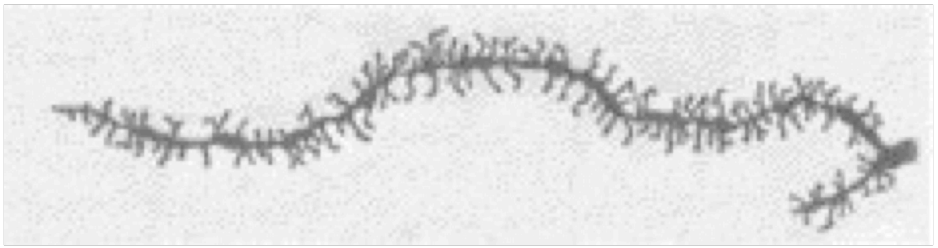


Figure 19. Ilustration of cocaine-induced increase in dendritic spine density. Digital images of Golgi-stained medium spiny neuron terminal tips in the NAc core from a drug naïve rat (up panel) and one that self-administered cocaine (bottow panel) (Ferrario et al., 2005).

Drug-induced structural plasticity in different part of the mesocorticolimbic circuit has been found as little as 1 or 3 days after the last daily injections (Sarti et al., 2007; Kolb et al., 2003), but importantly, these alterations can still be observed several months after the discontinuation of drug treatment. These findings suggest that drugs of abuse can produce a persistent reorganization of patterns of synaptic connectivity in the mesocorticolimbic system (Robinson and Kolb, 2004).

Correlative evidences suggest that certain morphological changes triggered by drugs of abuse could be important mediators of addictive behaviours. Thus, morphine alters dendritic spines density in different parts of the brain reward circuit to a greater extent in animals self-administering the drug than in those receiving passively the drug, suggesting that volition can be important for key aspects of this plasticity (Robinson et al., 2002). Moreover, some drug related structural changes, such as cocaine increases spine density in the NAc core, appear to be tightly correlated with the induction of behavioural sensitization (Li et al., 2004a). Conversely, exposure to high doses of amphetamine that are well known to induce stronger behavioural sensitization induces greater increase in spine density in the NAc core than small doses (Kolb et al., 2003). Interestingly, a cocaine treatment regimen that produces only relatively transient behavioural sensitization also produces an increase in spine density in the NAc core that is similarly transient (Robinson and Kolb, 2004). These evidences suggest that drug-induced behavioural and structural plasticity are tight interrelated events that appear and disappear concomitantly. Consequently, these evidences highlight that drug-induced spines density changes may be a reversible phenomenon depending on the treatment regimen. Finally, it has been reported that rats given long-access to intravenous cocaine over weeks, dramatically escalate their drug intake and show a significant greater increase in spine density in the NAc core than animals presenting a stable pattern of intake with short access (Ferrario et al., 2005). It seems that the extend of drug-induced structural changes could correlate with the magnitude of the "addictive state" since animals that escalate their drug intake also present higher motivation to seek for the drug and greater propensity to "relapse" than animals that do

not escalate their drug consumption (Paterson and Markou 2003; Ahmed and Cador, 2006),

In summary, most of the prototypical drugs abused by humans induces structural plasticity changes in the brain reward circuit. These morphological changes vary in parallel to drug-induced behavioural changes and their extent seem to correlate with the magnitude of the "addictive state". Certain morphological changes appear to be very persistent and participate in drug-induced enduring behavioural changes, such as locomotor sensitization. Thus, drug-induced structural plasticity in the mesocorticolimbic system seems to be a common neuronal alteration underlying the development and the persistence of the behavioural abnormalities associated to differents types of addiction. A better knowledge of its underlying cellular mechanisms may be important to improve our understanding of addiction and to develop new pharmacotherapies for drug addiction and related addictive behaviours, such as compulsive overeating. One endogenous system that may be potentially involved in the structural changes induced by drugs of abuse and other rewarding stimuli, such as food, is the endogenous cannabinoid system.

4. THE ENDOGENOUS CANNABINOID SYSTEM

4.1. Overview

The endogenous cannabinoid system is a ubiquitous lipid signalling found in most mammalian organs and tissues, with an especially high expression in the CNS. The endogenous cannabinoid system is composed of the cannabinoid receptors, their endogenous ligands (i.e. endocannabinoids) that include anandamide and 2-arachidonoylglycerol (2-AG), and the enzymes involved in the synthesis and degradation of these endocannabinoids (Pazos et al., 2005; Mechoulam and Parker, 2013). To date, two subtypes of cannabinoid receptors have been well characterized and cloned: CB1 and CB2. Both receptors are G-protein-coupled receptors with seven-transmembrane domains. There are considerable differences regarding their body distribution and function. CB2-Rs are poorly expressed in the CNS and are mainly located in the cells of the immune system. The functional role of CB2Rs in the CNS has not been yet clarified, although it has been shown that they can modulate neuroinflammatory responses upon microglial activation (Atwood and Mackie, 2010). In contrast, CB1-Rs are highly expressed in the CNS and to a lesser extent in some peripheral organs such as the liver, smooth muscle and gastrointestinal tract among others (Pertwee et al., 2010). The ubiquitous presence of CB1-R in the brain (Figure 20) correlates with its role as a modulator of multiple physiological processes. Thus, the presence of CB1Rs in the basal ganglia and cerebellar circuits is responsible of the modulation of motor behaviour (Rodríguez de Fonseca et al., 1998). CB1-Rs are also found in the thalamus, periaqueductal grey, amygdala and HPC, where they play an important role in the control of pain, emotional responses and

memory (Manzanares et al., 2006; Wotjak, 2005; Puighermanal et al., 2009). Important for the frame of this thesis, CB1-Rs are also expressed in the hypothalamus and in several components of the mesocorticolimbic circuit, including the NAc, VTA and mPFC, where they play a crucial regulatory role in energy homeostasis, reward processes and motivated behaviour (Osei-Hyiaman et al., 2006; Solinas et al., 2008). The orphan G-protein coupled receptor GPR55 has been recently classified as another member of the cannabinoid family, potentially explaining some of the physiological effects that are non-CB1-R/CB2-R mediated (Sharir and Abood, 2010).

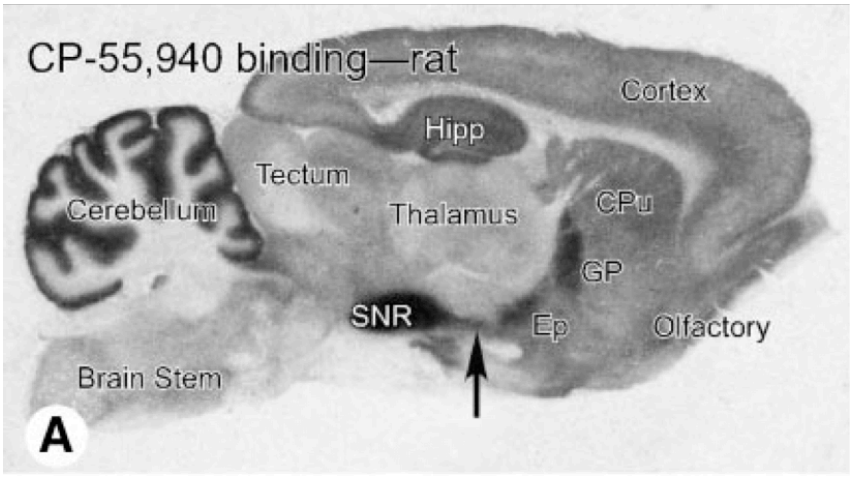


Figure 20. CB1-R localization in the rat brain. Autoradiographic images show CB1-R localization in rat brain marked by the tritiated ligand CP-55,940. High levels of CB1-R are visible in the globus pallidus (GP), entopeduncular nucleus (Ep), substantia nigra pars reticulata (SNR), cerebellum, hippocampus (Hipp), cortex, and caudate putamen (CPu). Low binding is seen in the brain stem and thalamus (adapted from Freund et al., 2003).

Dysregulation of the endogenous cannabinoid system has been linked to both drug addiction and some eating disorders (Maldonado et al., 2006; Marco et al., 2012). Therefore, a better understanding of the mechanisms by which the endogenous cannabinoid system mediates food and drug intake, reward and seeking behaviour may be of relevance for the treatment of these two pathologies.

4.2. The endogenous cannabinoid system in drug addiction

One compelling evidence supporting the role of the endogenous cannabinoid system in brain reward processes is the wide spread abuse of drugs that directly act on this system, such as the derivates of the plant Cannabis sativa (UNODC, World Drug Report 2012). The main psychoactive ingredient of cannabis, delta-9-tetrahydrocannabinol (THC), is an exogenous agonist of CB1-R that produces subjective feelings of "high", well-being and euphoria in humans (Green et al., 2003). Despite the clinical evidence for abuse liability of cannabis, rewarding and reinforcing effects of THC or other cannabinoid agonists have been difficult to demonstrate in animals. However, several recent findings have now demonstrated that exogenous cannabinoid agonists (e.g. WIN 55,212-2 and THC) and even the endogenous anandamine can initiate and sustain self-administration behaviour and/or elicit CPP in monkeys and rodents (Tanda et al., 2000; Fattore et al., 2001; Justinova et al., 2005; Valjent and Maldonado, 2000). In addition, rats can directly self-administer THC into different parts of the mesocorticolimbic system, such as the VTA and NAc, highlighting the particular importance of the endogenous cannabinoid system in this circuit in mediating cannabinoid reinforcing effects (Zangen et al., 2006).

The role of the endogenous cannabinoid system in mediating rewarding and reinforcing processes is not limited to cannabinoid drugs, and accumulative evidences acknowledge the involvement of this system in those related to natural reward (Sanchis-Segura et al., 2004) and differents classes of drugs of abuse (Maldonado et al., 2006). In addition, the endogenous cannabinoid system is implicated in several phases of the addictive process and thereby this system has been considered an important common neuronal substrate of drug addiction (Maldonado et al., 2006). Thus, this system is directly involved in the primary rewarding and reinforcing effects of many drugs of abuse, including THC, nicotine, alcohol and opioids (Maldonado et al., 2006). Indeed, genetic deletion of CB1-R greatly impaired acquisition of CPP and self-administration maintained by each of these drugs (Maldonado et al., 2006). Moreover, self-administration of heroin and alcohol directly modify endocannabinoid levels in the NAc shell (Caillé et al., 2007). The ability of the endogenous cannabinoid system to regulate DA transmission in the mesocorticolimbic circuit (Gardner, 2005), and in particular the phasic DA release commonly triggered by drugs of abuse (Cheer et al., 2007), may represent one mechanism by which the endogenous cannabinoid system participates in drug reinforcement. Thus, the endogenous cannabinoid system seems to be a crucial substrate mediating the positive reinforcement that is involved in the initial step of the addictive process.

The endogenous cannabinoid system is also involved in the motivation to seek the drug. Indeed, genetic and pharmacological blockade of CB1-R reduce the breaking point on a PR schedule task, a measure of motivation, when rodents are trained to self-administer psychostimulants and opioids and might also be the case for other drugs of abuse (Solinas et al., 2003;

Soria et al., 2005). Thus, the endogenous cannabinoid system could have an important role in mediating the pathological motivation for drug revealed in addicts.

The endogenous cannabinoid system participates in the aversive effects of the withdrawal syndrome of several drugs (Maldonado et al., 2006). Thus, THC decreases somatic and motivational manifestations of nicotine withdrawal in mice (Balerio et al., 2004); while genetic deletion of CB1-R increases alcohol withdrawal (Naassila et al., 2004). Therefore, the endogenous cannabinoid system may represent an important system underlying drug negative reinforcement and drug dependence.

Finally, the endogenous cannabinoid system plays a major role in relapse to drug consumption. Indeed, pharmacologic inactivation of CB1-R strongly reduces both drug priming and cues induced reinstament to drug-seeking behaviour in mice, a common effect observed with almost all prototypical drugs of abuse (De Vries and Schoffelmeer, 2005). Thus, the endogenous cannabinoid system could represent a crucial neurobiological substrate underlying the long-term behavioural abnormalities that characterize addiction, such as persistent relapse.

According to these evidences, many scientists point out the endogenous cannabinoid system and in particular CB1-R as a major target for the development of new pharmacological agents for the treatment of many different types of drug abuse and dependence (Maldonado et al., 2006; Parolaro et al., 2007). Preclinicals trials have already shown promising results for smoking cessation with the use of the CB1-R antagonist rimonabant (Fernandez and Allison, 2004). However, these clinical trials have also revealed that rimonabant produces severe psychiatric side

effects, such as anxiety and depression (Moreira and Crippa JA, 2009). Therefore, a better understanding of the cellular mechanisms by which the endogenous cannabinoid system and CB1-R mediate drug induced persitent behavioural alterations could help to more specifically target determinant processes involved in pathological drug intake, without altering other brain functions, and thus limiting the side effects.

4.3. The endogenous cannabinoid system in the control of eating behaviour

The ability of Cannabis sativa to promote eating has been documented for many centuries and drug users often report to experience voracious appetites, a general enhanced enjoyment of food and cravings for sweets (Foltin et al., 1988). In experimental animals, hyperphagia induced by peripheral administration of THC is so powerful that some scientists compare its effect to those induced by central injection of neuropeptide Y (Harrold and Williams, 2003). These findings highlight the crucial physiological role of the endogenous cannabinoid system in the control of appetite and the hedonic evaluation of food. Thus, one of the currently licensed uses of cannabinoid drugs in the USA is the stimulation of appetite in patients suffering cancer or AIDS (Plasse et al., 1991; Haney et al., 2005).

The endogenous cannabinoid system is strategically positioned to influence virtually every key point of the regulatory network that controls eating behaviour, either at central level regulating appetite or at the periphery contributing to energy metabolism (Figure 21). In the brain, the endogenous cannabinoid system regulates food intake mainly at two functional levels: the hypothalamus, where it participates in the control of

energy homeostatis, and the mesocorticolimbic system, in which it modulates hedonic responses to palatable food (Di Marzo and Matias, 2005). In the periphery, the endogenous cannabinoid system expressed in the liver, adipose tissue, muscles and pancreas regulates fat metabolism and energy expenditure (Di Marzo and Matias, 2005).

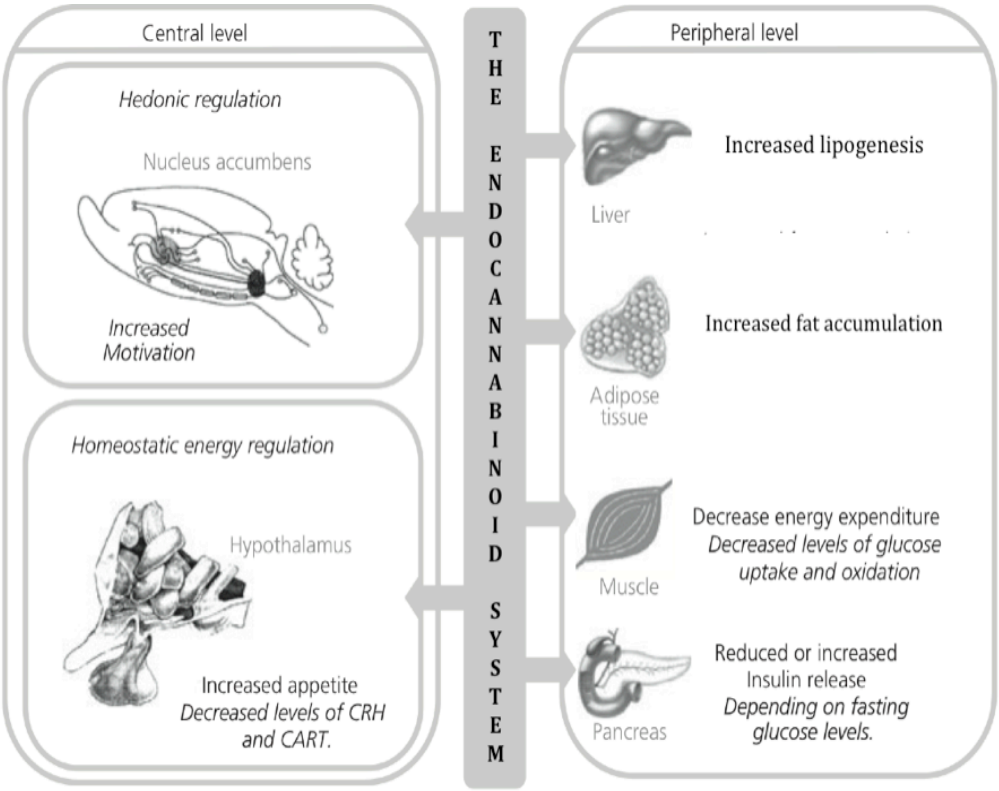


Figure 21. The endogenous cannabinoid system in food intake and energy balance. Schematic diagram showing the location and function of the endogenous cannabinoid system in different central brain structures and peripheral organs involved in the control of food intake and energy metabolism (adapted from Matias et al., 2008).

4.3.1.The endogenous cannabinoid system in the homeostatic control of food intake

CB1-Rs are expressed at relatively low levels in the hypothalamus (Breivogel and Childers, 1998), but their activation in this region is sufficient to produce profound effects on food intake. Thus, infusion of anadamide into some hypothalamic nuclei strongly promotes food intake through CB1-R activation, even in satiated rats (Jamshidi and Taylor, 2001). This observation suggests that tonic cannabinoid release may be crucial to the normal regulation of feeding. Indeed, hypothalamic 2-AG levels increase during acute fasting, decline as the animals are refed, and return to normal values in satiated animals (Kirkham et al., 2002). The hyperphagic properties of cannabinoids could be due to a direct regulation of orexigenic peptides in the hypothalamus, as CB1-R is expressed in melanin concentring hormone and orexins producing neurons in the lateral hypothalamus (Cota et al., 2003). In fact, CB1-R activation strongly enhances the orexin-A-stimulated intracellular pathway, while CB1-R antagonists inhibit the stimulation of food intake induced by melanocortin receptor MCR4 antagonists (Hilairet et al., 2003; Verty et al., 2004). In addition, CB1-R is expressed in cocaine and amphetamine regulated transcript (CART) expressing neurons and corticotropin-releasing hormone (CRH) producing neurons and the endogenous cannabinoid system may also promote appetite by decreasing satiety signals (Cota et al., 2003). Functional interactions between the endogenous cannabinoid system and the satiety system is supported by the observation that defect in leptin signaling is associated with elevated hypothalamic levels of endocannabinoids (Di Marzo et al., 2001). In addition, the endogenous cannabinoid system presented in different peripheral organs plays a key

role in energy expenditure and storage. Thus, activation of CB1-R in the adipose tissue increases expression of proteins involved in fatty acid synthesis, leading to enhance lipid levels and fat accumulation (Matias et al., 2008). In addition, CB1-R participates in adipogenesis and controls the number and size of adipocytes independently from the amount of food ingested (Matias et al., 2008). Conversely, CB1-R KO mice present lower amounts of fat mass (Cota et al., 2003). In the liver, some evidences suggest that activity of CB1-R enhances lipogenesis and CB1-R KO mice do not develop hepatic steatosis (fatty liver) after several weeks of a high-fat diet (Matias et al., 2008). Furthermore, the endogenous cannabinoid system decreases energy expenditure in part due to its inhibitory effects on glucose utilisation by the skeletal muscle (Matias et al., 2008). CB1-R expressed in the pancreas regulates insulin release and subsequently control blood glucose levels (Matias et al., 2008). All these metabolic processes strongly influence the behavioural expression of appetite. Thus, central and peripheral CB1-Rs act in a coordinated fashion to regulate energy homeostasis and eating behaviour.

4.3.2.The endogenous cannabinoid system in the hedonic control of food intake

Marijuana increases appetite and especially enhances the desire to consume palatable food (Foltin et al., 1988). Conversely, several animal studies show that peripheral administration of rimonabant reduces the intake of palatable foods whereas THC increases to a greater extent the consumption of sweet foods than less palatable ones (Simiand et al., 1998; Koch and Matthews, 2001). Thus, an important mechanism by which the

endogenous cannabinoid system regulates food intake seems to rely on the ability of endocannabinoids to modulate hedonic responses to food.

One mechanism by which the endogenous cannabinoid system modulates food palatability is the regulation of gustatory perception. In fact, CB1Rs are present in taste buds, and their activation enhances neural responses to sweet foods (Yoshida et al., 2010). In addition, CB1-Rs are present in the parabrachial nucleus, a hinbrain area that integrates taste information, where local administration of 2-AG increases intake of palatable fat-rich diet, but not standard chow, revealing the key role of the endogenous cannabinoid system in gating neurotransmission inherent to fat and sweet taste (DiPatrizio and Simansky, 2008).

In addition, the endogenous cannabinoid system regulates the hedonic properties of palatable food through its activity in the mesocorticolimbic system. Indeed, endocannabinoid hedonic hotspots have been described in the NAc shell, and microinfusions of anadamide into these regions increase positive "liking" responses to sucrose and enhance the intake of sweet solutions (Mahler et al., 2007; Shinohara et al., 2009). Morevover, the endogenous cannabinoid system in the mesocorticolimbic system mediates the motivation to obtain palatable food. Indeed, blockade of CB1-R activity decreases palatable food induced DA release in the NAc shell, suggesting that the endogenous cannabinoid system regulates the incentive value of the palatable food (Melis et al., 2007). Conversely, inactivation of CB1-R decreases operant responses in a PR task maintained by palatable food by altering the activity of NAc neurons that mediate the generation of this motivated behaviour (Hernandez and Cheer, 2012).

In summary, the endogenous cannabinoid system participates in the homeostatic, pleasurable and motivational aspects of food intake. The extended role of the endogenous cannabinoid system in the control of eating behaviour underscores that alterations of this system may lead to the development of eating disorders.

4.3.2. The endogenous cannabinoid system in eating disorders

Growing evidences suggest that obesity and some eating disorders are associated with a pathological overactivation of the endogenous cannabinoid system. Thus, a significant elevation of anandamide's plasma levels has been detected in anorexics, binge-eaters and some obese patients (Monteleone et al., 2005). Conversely, genetic deletion of CB1-R has shown to prevent high fat diet-induced hyperphagia and obesity in mice, while CB1 antagonism reduces food intake, body weight and improves metabolic profiles in obese mice (Ravinet Trillou et al., 2004; Nogueiras et al., 2008). In addition, prolonged consumption of palatable food, a key characteristic of obesogenic diets, produces important alterations in the endogenous cannabinoid system's functionality in rodents, such as a downregulation of CB1-R expression in severals regions of the mesocorticolimbic circuit, including the NAc and the HPC (Harrold et al., 2002). Surprisingly, these alterations were not observed in the hypothalamus (Harrold et al., 2002). These evidences suggest that an overactivation of the endogenous cannabinoid system in the mesocorticolimbic system could represent an important event in mediating dysregulation of the hedonic control of food intake, leading to overconsume palatable food. On the other hand, several studies have reported association between genetic polymorphisms for different components of the endogenous cannabinoid system and the

propensity to develop overweight and obesity (Sipe et al., 2005; Jaeger et al., 2008).

In this context, pharmacological manipulation of the endogenous cannabinoid system could be an appropriate therapeutical strategy for obesity management. In fact, clinical trials with the CB1 antagonists rimonabant and taranabant have already demonstrating efficacy at reducing food intake as well as obesity and metabolic alterations (Van Gaal, 2005; Hagmann, 2008). Consequently, rimonabant has even been prescribed to treat obesity in more than 40 countries around the world from 2006 to 2008. However, the European Medicines Agency recommended the suspension of the marketing authorization for rimonabant in 2008 due to the presence of undesired central side-effects including depression and suicidal ideation (Moreira and Crippa JA, 2009). Therefore, it will be interesting to uncover the specific neurobiological mechanisms through which the endogenous cannabinoid system contributes to the development of eating disorders to develop new pharmacotherapies that entail less side effects. One appealing neurophysiological process regulated by the endogenous cannabinoid system in the brain that may account for the involvement of this system in drug addiction and presumably in some eating disorders is neuronal plasticity (Sidhpura and Parsons, 2011).

4.4. The endogenous cannabinoid system in neuronal plasticity.

The relevance of the endogenous cannabinoid system in neuronal plasticity is highlighted by the crucial role of CB1-R and endocannabinoids in regulating neurogenesis, axonal navigation and synapse formation during

brain development (Fernández-Ruiz et al., 2000; Diaz-Alonso et al., 2012). Importantly, the endogenous cannabinoid system remains a crucial substrate of neuronal plasticity above this period, and it is even considered as a major modulator of synaptic function and plasticity throughout the adult brain (Castillo et al., 2012).

In the CNS, the endogenous cannabinoid system behaves as a neuromodulator, and thereby modulates the release of many different excitatory and inhibitory neurotransmitters (Wilson and Nicoll, 2002). This particular synaptic function of the endogenous cannabinoid system results from the atypical cellular organization of its components. Indeed, CB1Rs are mainly confined at the presynaptic terminals in the CNS, while endocannabinoids are synthetized and released from the post-synaptic compartement. Therefore, following postsynaptic neuronal activation, endocannabinoids synthetized "on demand" from cleavage of membrane lipids, travel retrogradely across the synapse to bind presynaptic CB1Rs, thereby suppressing the release of the neurotransmitter that caused their activation (Wilson and Nicoll, 2002). This neuromodularoty activity provides to the endogenous cannabinoid system the ability to guarantee a fine adjustment of information processed in the brain, a function reminiscent with synaptic plasticity. In fact, it is now well known that the endogenous cannabinoid system participates in different forms of synaptic plasticity at both excitatory and inhibitory synapses and in a short- and long-term manner (Castillo et al., 2012).

Retrograde signalling is the principal mode by which endocannabinoids mediate synaptic plasticity. At short-term, endocannabinnoids promote mainly two different forms of plasticity that typically persist for a minute or less: (1) depolarization-induced suppression of inhibition (DSI) that

dampens presynaptic GABA release, and (2) depolarization-induced suppression of excitation (DSE) that dampens presynaptic glutamate release. In addition, endocannabinnoids are also involved in more persistent forms of synaptic plasticity, including LTP and LTD. Interestingly, endocannabinnoid-mediated LTD can target the original afferents responsible for its appearance (homosynaptic plasticity) or afferents in close proximity (heterosynaptic plasticity) (Chevaleyre et al., 2006). In the same target cell, the difference between endocannabinnoid-mediated LTD and endocannabinnoid-mediated DSI/DSE relies on the duration of CB1R activity, which engages distinct signaling events in the neuron leading to a short or long suppression of neurotransmitter release (Figure 22).

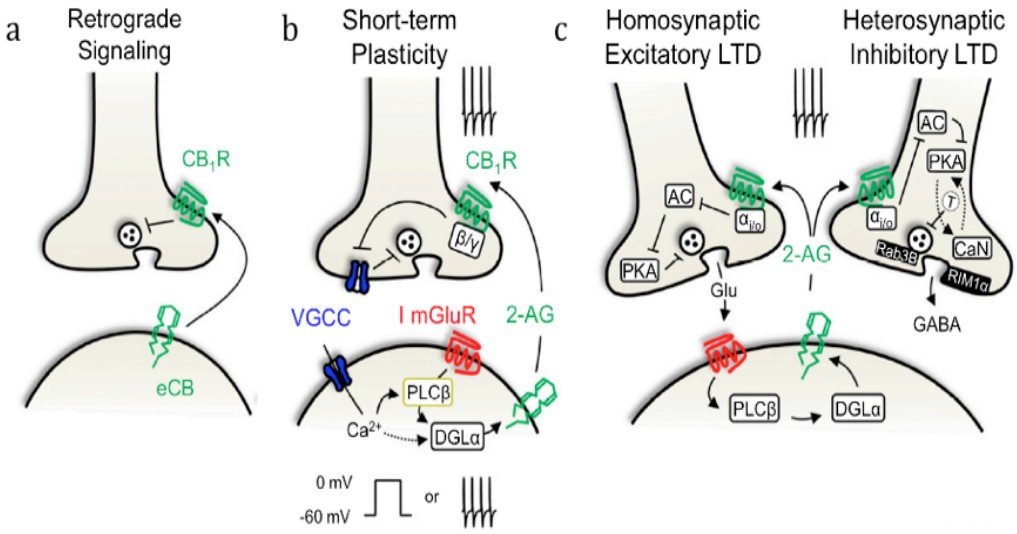


Figure 22. CB1-R in synaptic transmission and plasticity. Schematic diagrams illustrating the synaptic retrograde endocannabinoid signaling that lead to decrease neurotransmitter release (a), endocannabinoid mediated short-term (b) and long-term (c) synaptic depression. (b) Postsynaptic activity triggers calcium (Ca2+) influx via voltage-gated Ca2+ channels (VGCCs). Ca2+ promotes diacylglycerol lipase (DGL alpha)-mediated endocannabinoid (2-AG) production.

Presynaptic activity can also lead to endocannabinnoid mobilization by activating postsynaptic group I metabotropic glutamate receptors (I mGluRs). 2-AG travels retrogradely and targets presynaptic CB1Rs, and the beta gamma subunits probably couple to presynaptic VGCCs to reduce neurotransmitter release. (c) Glutamate (Glu) release triggered by presynaptic stimulation activates postsynaptic mGluRs and promotes the production of 2-AG, through phospholipase-C beta (PLCb) and DGL alpha dependent mechanism. 2-AG homosynaptically targets CB1Rs localized to excitatory terminals and heterosynaptically engages CB1Rs at inhibitory terminals. A protein G alpha i/o-dependent reduction in adenylyl cyclase (AC) and protein kinase A (PKA) activity durably suppresses neurotransmitter release (adapted from Castillo et al., 2012).

Importanlty, both endocannabinoid-mediated short-term and long-term plasticity has been observed in several brain areas relevant to drug reward and addiction including the VTA, NAc, PFC, amygdala and HPC; and different drugs of abuse alter endocannabinoid-mediated neuronal plasticity (Sidhpura and Parsons, 2011). Thus, sub-chronic (7 days) administration and even a single exposure to THC can abolish endocannabinoid-mediated LTD at excitatory and/or inhibitory synapse in the NAc (Hoffman et al., 2003; Mato et al., 2004). Importantly, alterations of endocannabinoid-mediated synaptic plasticity is not restricted to THC and has been also observed with other drugs of abuse, such as cocaine and alcohol, following acute or chronic exposure, and occurring in NAc, VTA, HPC and dorsal striatum (Fourgeaud et al., 2004; Pan et al., 2008; Clarke and Adermark, 2010). Drug-induced disruption of endocannabinoid-mediated synaptic plasticity has been proposed to heightened behavioural and neurochemical responses to drug exposure, decreased extinction of drug-related memories and increased cue-induced drug craving, among others (Sidhpura and Parsons, 2011). Accordingly, therapeutic approaches aimed at restoring normal endocannabinoid signaling may have clinical benefit for the treatment of addiction.

On the other hand, recent evidences suggest that unhealthy eating habits can alter endocannabinoid-mediated synaptic plasticity in the mesocorticolimbic circuit. Thus, deficiency in n-3 polyunsaturated fatty acids can impair endocannabinoid-mediated LTD in both the PFC and NAc, which is associated with defects in mood and emotional behaviour (Lafourcade et al., 2011). Of importance, obesigenic diets often contain low levels of this essential fatty acid (Simopoulos, 2009), suggesting that alterations of endocannabinoid-mediated LTD may possibly occurs in individuals overconsuming such unheathly diet. Moreover, diet-induced obesity in mice increases endocannabinoid-mediated DSI and LTD at inhibitory synapses in the HPC (Massa et al., 2010). Together, these evidences suggest that alterations of endocannabinoid-mediated synaptic plasticity in particular areas of the brain reward circuit could participate in the genesis of eating disorders.

In general, a better understanding of intracellular mechanisms by which the endogenous cannabinoid system mediates the altered neuronal plasticity in the mesocorticolimbic system triggered by excessive consumption of drug and palatable food may be useful to develop new pharmacological tools for the treatment of drug addiction and eating disorders.

OBJECTIVES

Objective 1

To assess whether long-term exposure to palatable food in an operant task can trigger the emergence of structural plasticity changes in the mesocorticolimbic circuit. Additionally, we were interested to define the cellular mechanisms underlying these neuroadaptations by studying the specific role of CB1-R and the ERK signaling pathway.

Article #1

Operant behaviour to obtain palatable food modifies neuronal plasticity in the brain reward circuit

Thomas Guegan, Laura Cutando, Eduard Ayuso, Emanuela Santini, Gilberto Fisone, Fatima Bosch, Albert Martinez, Emmanuel Valjent, Rafael Maldonado*, Miquel Martin*

European Neuropsychopharmacology (2013)

* Co-corresponding authors

Article #2

Operant behavior to obtain palatable food modifies ERK activity in the brain reward circuit

Thomas Guegan, Laura Cutando, Giuseppe Gangarossa, Emanuela Santini, Gilberto Fisone, Fatima Bosch, Albert Martinez, Emmanuel Valjent, Rafael Maldonado*, Miquel Martin*

European Neuropsychopharmacology (2013).

* Co-corresponding authors.

Objective 2

To evaluate the participation of the endogenous cannabinoid system in drug-induced structural plasticity in the mesocorticolimbic circuit. In particular, to determine whether the dendritic spines rearragement associated to the development of behavioural sensitization to morphine was dependent of CB1-R activity.

Title: Studying the role of CB1-R in morphine-induced behavioural and structural plasticity.

Thomas Guegan, Joan Pau Cerbià Costa, Rafael Maldonado and Miquel Martin.

Objective 3

To uncover the common synaptic signaling events mediating the retrieval of drug of abuse and palatable food related rewarding memories; as an approach to study the neurobiological substrates of drug and palatable food craving.

Title: Characterization of the common synaptic signalling events that mediate drug of abuse and palatable food reward memories

Thomas Guegan, Joan Pau Cerbià Costa, Rafael Maldonado and Miquel Martin

RESULTS

OBJECTIVE 1

Identification of the neuroplastic mechanisms underlying food reward and seeking behaviour

Articles 1 and 2

ARTICLE 1

Operant behaviour to obtain palatable food modifies neuronal plasticity in the brain reward circuit

Thomas Guegan, Laura Cutando, Eduard Ayuso, Emanuela Santini, Gilberto Fisone, Fatima Bosch, Albert Martinez, Emmanuel Valjent, Rafael Maldonado*, Miquel Martin*

European Neuropsychopharmacology. 2013; 23(2):146-59.

*Co-corresponding authors

Supplementary methods

Ballistic Labeling with the Fluorescent Dye DiI

Immediately after the last training session, mice were deeply anesthetized by intraperitoneal injection (0.2 ml/10 g body weight) of a mixture of ketamine (100 mg/kg) and xylazine (20 mg/kg) prior to rapid intracardiac perfusion, delivered with a peristaltic pump at 20 ml/min, with 10 ml of Na2HPO4/NaH2PO4/NaCl buffer (PBS) 0.1 m, pH 7.5, and followed by perfusion with 40 ml of 4 % paraformaldehyde (PFA) in PBS 0.1 M, pH 7.5. Brains were quickly removed from the skull and postfixed in 4 % PFA for 10 min. Brain coronal sections (100 μm) containing the NAc (Paxinos and Franklin, 2001) (from bregma 1.54 mm to bregma 1.10 mm) or the medial prefrontal cortex (mPFC) (from bregma 1.98 mm to bregma 1.70 mm) were obtained by using a vibratome (Leica VT 1000 S, Nussloch, Germany) and kept in PBS 0.1 M until they were processed for fluorescent labeling. Brain slices were labeled by ballistic delivery of fluorescent dye DiI (Molecular Probes, Eugene, OR, USA) using a gene gun apparatus (Helios Gene Gun System, Bio-Rad, Deutschland) as described previously (Grutzendel et al., 2003) and postfixed with PFA for 4 hours at room temperature to further preserve structures and to allow the diffusion of the dye DiI. Sections were placed on microscope gelatine-coated slides and coverslipped with mounting medium (Mowiol). Then, images were acquired with confocal microscope (Zeiss LSM 510, Germany) with an oil immersion lens (40x) to analyze dendritic spine density and structure.

Acute bilateral intraNAc microinjection of rimonabant

CB_1+/+ were trained to lever-press for standard, highly caloric or highly palatable isocaloric pellets as described above. After reaching a stable response and pellet consumption on FR5 for a minimum of 8 days (10 +/- 2 days), mice underwent stereotaxic surgery and guide cannuli (11 mm long, 26 gauge) were bilaterally implanted into the NAc. For surgery, mice were first anaesthetized with a ketamine/xylazine mixture (100 mg/kg ketamine and 20 mg/kg xylazine on 0.2 ml/10 g of body weight) and subsequently mounted in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). The coordinates (AP: +1.35 mm; ML: +/- 0.95mm ; DV: -4.4mm) were taken from bregma and the skull surface according to the stereotaxic atlas (Paxinos and Franklin, 2001). After implantation, cannuli were fixed to the skull with dental cement. Mice were left undisturbed for recovering during 3 days after surgery and were then trained again in the operant boxes for each type of food on FR5. After reaching a stable response for a minimum of 4 days, the effects of a bilateral microinjection into the NAc of the selective **CB_1-R** antagonist rimonabant (1, 2 and 4 μg/site) (gently provided by Sanofi, France) or vehicle (saline 20% /DMSO 80%) on operant behavior were tested. Rimonabant and vehicle were administered in a volume of 0.5 μl per side, delivered at a rate of 0.1 μl/10 sec and kept undisturbed in the cannula for an extra 60 sec to prevent backflow. Each animal received all different treatments by using a Latin square design. After each treatment, mice underwent training on the operant boxes to recover basal lever-pressing responding on FR5 before receiving a new dose of the tested compound or vehicle (currently after 2-3 days). At the end of the experiment, mice were sacrificed and their brain removed to verify the final

site of the injection. Only data from mice with both final sites of injection correctly placed into the NAc were considered.

Design and Construction of AAV Vectors and Viral production and purification

To generate shRNA expressing vectors, the U6 promoter, the hairpin sequence of interest and terminator sequences were subcloned into the multicloning site of pAAV9-MCS (stratagene). An AAV9 expressing a scrambled shRNA sequence was used as control for the knock-down studies. To knock-down murine CB_1-R expression, we selected two different hairpin sequences (TRCN0000027886 and TRCN0000027943) from the RNAi consortium database (www.broadinstitute.org/rnai/public) targeting murine Cnr1 gene (CB_1). -shRNACB_1a (hairpin sequence from TRCN0000027886): 5'-CCGG-CACCGCAAAGATAGTCCCAAT-CTCGAG-ATTGGGACTATCTTTGCGGTG-TTTTT-3' -shRNACB_1b (hairpin sequence from TRCN0000027943): 5'-CCGG-TCAGACATCTTCCCACTCATT-CTCGAG-AATGAGTGGGAAGATGTCTGA-TTTT-3'.

Vectors were generated by triple transfection of HEK293 cells according to standard methods. Cells were cultured in 10 roller bottles (850 cm2, flat from Corning, New York, NY, USA) in DMEM 10% FBS to 80% confluence and co-transfected by calcium phosphate method with a plasmid carrying the expression cassette flanked by the AAV2 viral ITRs (described above), a helper plasmid carrying the AAV rep2 and cap gene from serotype 9, and a plasmid carrying the adenovirus helper functions (all plasmids kindly provided by K.A. High, Children's Hospital of Philadelphia). We selected

adeno-associated virus serotype 9 (AAV9) to infect into the NAc to ensure a good transduction efficiency and larger distribution of the vector upon stereotaxic injection, as previously reported (Cearley et al., 2008). AAV9 directly injected by stereotaxic delivery in the brain tissue only transduces neurons, as previously reported (Cearley et al., 2008). This selective transduction on neurons, but not on glial cells, was also revealed in this study after AAV9 locally microinjection in restricted brain areas, as shown in Fig. 6. AAVs were purified with an optimized method based on a PEG precipitation step and two consecutives cesium chloride (CsCl) gradients (Ayuso et al., 2010). This second generation CsCl-based protocol dramatically reduce empty AAV capsids and DNA and protein impurities from the viral stock thus increasing AAV purity that ultimately results in higher transduction in vivo. Purified AAV vectors were dialyzed against PBS, filtered and stored at -80ºC. Titers of viral genomes were determined by quantitative PCR assay following the protocol described for the AAV2 Reference Standard Material using linearized plasmid DNA as standard curve.

Bilateral intraNAc administration of AAV-scrambled or AAV-shCB$_1$

CB$_1$+/+ underwent stereotaxic surgery and received a bilateral administration of AAV9-scrambled (7.4 x 1012 vector genomes/ml) or AAV9-sh to knock-down the CB$_1$-R (AAV9-shCB$_1$). For knocking down CB$_1$-R, we produced two AAV9 vectors carrying different shRNA sequences against CB$_1$-R and a 1:1 mixture of the two vectors was injected bilaterally (AAV9-shRNACB$_1$-a: 1.3 x 1013 vector genomes/ml and AAV9-shRNACB$_1$-b: 6.5 x 1012 vector genomes/ml) into the NAc. The use of a combination of two AAV vectors ensures the achievement of an appropriate reduction of

CB_1-R expression since the AAV vectors are able to enter the cells at a high multiplicity of infection, as previously reported in other studies successfully using the combination of two AAV vectors (Mas et al., 2006; Tafuro et al., 2009). For surgery, mice were first anaesthetized with a ketamine/xylazine mixture (100 mg/kg ketamine and 20 mg/kg xylazine on 0.2 ml/10 g of body weight) and mounted in a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA). AAV9 preparations were bilaterally infused into the NAc by using a glass pipette in a volume of 0.7 μl/site and delivered at a constant rate of 0.15 μl/min using a microinfusion pump. The glass pipette was left for an extra 5-10 min inside the NAc to prevent backflow. Mice were then left undisturbed for 15 days. Then, animals were trained to lever-press for highly palatable isocaloric pellets as described above.

For tropism studies, we used an AAV9 vector expressing GFP cDNA (7.7 x 1012 vector genomes/ml) under the control of the hybrid cytomegalovirus enhancer/chicken beta-actin constitutive promoter (CAG) and also contained the WPRE regulatory element.

Tissue preparation and immunofluorescence analysis

To verify the specificity of the AAV9 infecting neurons but not glial cells, we performed an immunoflurorescence study, as previously described (Bertran-Gonzalez et al., 2008). Fifteen days after the administration of the AAV9, mice were deeply anesthetized by intraperitoneal injection (0.2 ml/10 g body weight) of a mixture of ketamine (100 mg/kg) and xylazine (20 mg/kg) prior to rapid intracardiac perfusion with 120 ml of 4 % PFA in 0.1 M PBS, pH 7.5, delivered with a peristaltic pump at 30 ml/min for 4 min. Brains were post-fixed overnight in the same solution and stored at 4°C.

Thirty µm-thick sections were cut with a vibratome (Leica, VT 1000 S, Nussloch, Germany) and stored at -20°C in a solution containing 30 % (vol/vol) ethylene glycol, 30 % (vol/vol) glycerol and 0.1 M sodium phosphate buffer, until they were processed for immunofluorescence. Brain regions were identified using a mouse brain atlas (Paxinos and Franklin, 2001) as described above, and sections equivalent to the following bregma coordinates were taken (mm): 1.32 for NAc. Free-floating sections were rinsed in Tris-buffered saline (TBS; 0.25 M Tris and 0.5 M NaCl, pH 7.5), three times for 10 min each. After 20 min incubation in 0.2 % Triton X-100 in TBS, sections were rinsed three times in TBS again. Finally, they were incubated overnight at 4°C with the different primary antibodies (NeuN, 1:1000, Chemicon; GFAP, 1:800, DAKO; Iba-1, 1:500, WAKO; for GFP no antibody was used, we evaluated directly the intrinsic fluorescence of the GFP). Following incubation with the primary antibodies, sections were rinsed three times for 10 min in TBS and incubated for 45 min with mice Cy3-coupled (for NeuN positive cells, 1:400, Jackson Lab) or rabbit Alexa-fluor 647 (for GFAP or Iba-1 positive cells) secondary antibodies. Sections were rinsed for 10 min twice in TBS and twice in Tris-buffered (TB, 0.25 M Tris) before mounting in 1, 4 – diazabicyclo-[2. 2. 2]-octane (DABCO, Sigma).

For immunofluorescence analysis, double-labeled images from the region of interest were obtained bilaterally using sequential laser scanning confocal microscopy (Zeiss LSM 510, Germany). Analysis was performed by a blind observer by evaluating Cy3- or infra-red Alexa-fluor 647 immunoreactive cells immunofluorescence for each marker analyzed.

Immunoblot analysis

The efficiency of the viral infections was verified by immunoblot analysis. NAc (positive control) and hippocampus (Hc) (negative control) of mice treated with AAV-scrambled or AAV-shCB_1 were extracted. Frozen Hc and NAc tissues were processed, as previously reported (Puighermanal et al., 2009). For immunoblotting, antibodies to **CB_1-R** (1:700) from Frontier-Science were used. Blots containing equal amounts of Hc or NAc protein samples to compare in each specific experiment were cut horizontally. Bound primary antibodies were detected with horseradish peroxidase–conjugated antibodies to rabbit or mouse antibodies (Pierce, diluted 1:5,000) and visualized by enhanced chemiluminescence detection (West-Femto-SuperSignal, Pierce). The optical density of the immunoreactive bands was quantified after acquisition on a ChemiDoc XRS System (Bio-Rad) controlled by The Quantity One software v4.6.3 (Bio-Rad). Representative immunoblots were cropped with Adobe Photoshop 7.0 for display. For quantitative purposes, the optical density values of the specific antibodies were normalized to the detection of specific antibodies to GAPDH values in the same sample and expressed as a percentage of control treatment.

Supplementary figures

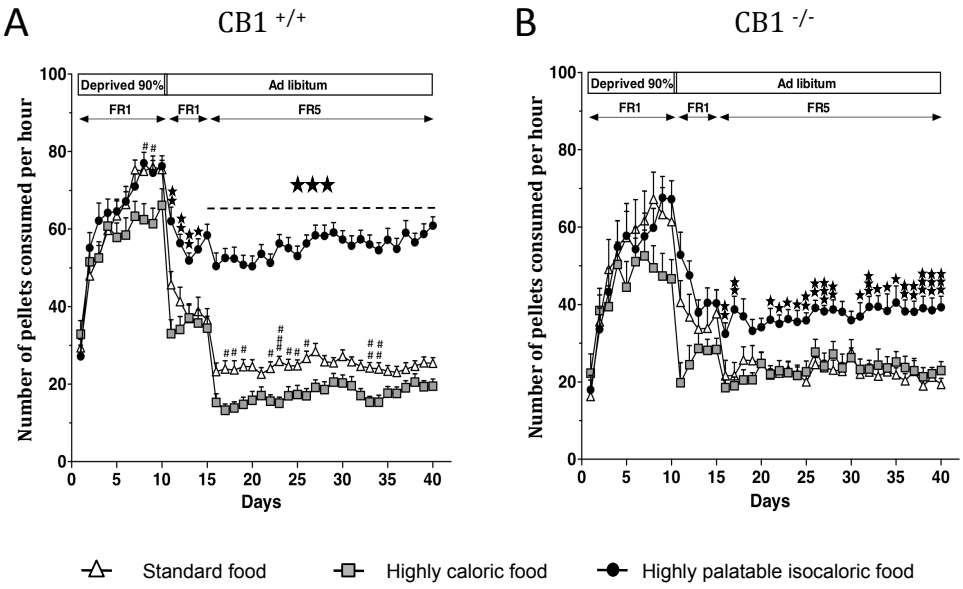


Figure 23: Comparison of the reinforcing properties of standard, highly caloric and highly palatable isocaloric food in CB1-/- and CB1+/+ mice. (**A**) Daily consumption of standard (white triangle, n=36), highly caloric (grey square, n=29) and highly palatable isocaloric (black circle, n=37) pellets in CB1+/+ mice. (**B**) Daily consumption of standard (white triangle, n=14), highly caloric (grey square, n=13) and highly palatable isocaloric (black circle, n=17) pellets in CB1-/- mice. Data are expressed as mean ± s.e.m. ★P < 0.05, ★★P<0.01, ★★★P < 0.001 (highly palatable pellets vs. standard); # P < 0.05, ## P<0.01, ### P < 0.001 (high caloric pellets vs. standard).

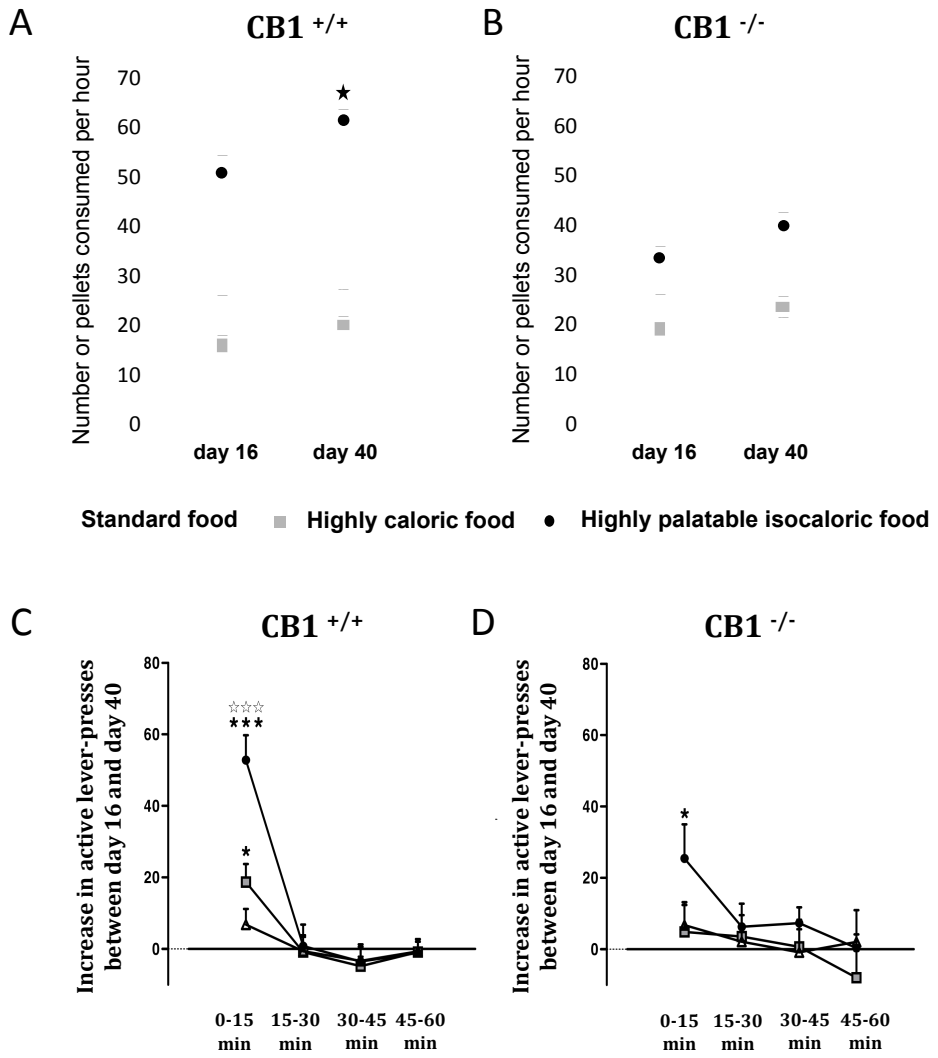


Figure 24: **Repeated operant training with highly palatable isocaloric pellets promotes progressive escalation of food intake and the development of a binge-like eating behaviour, through a CB1-R dependent mechanism. (A)** and **(B)** amount of standard, highly caloric and highly palatable isocaloric pellets consumed during the first (i.e. day 16) and last (i.e. day 40) day of operant training in FR5 in CB1+/+ (n=29–37 mice per group) and CB1-/- (n=13–17 mice per group) mice. This graph illustrates that CB1+/+, but not CB1-/- mice, working for palatable pellets, but not for other types of food, display a significant progressive increase of their pellets intake across the operant sessions. **(C)** and **(D)** Time course (15 min intervals) of the variation of reinforced responses between the first

(i.e. day 16) and last (i.e. day 40) day of operant training in FR5 in CB1+/+ (n=29–37 mice per group) and CB1-/- (n=13–17 mice per group) mice trained for each kind of food. The vertical axes represent the difference between active-lever pressing recorded during "active periods" (i.e. not included lever-pressing during time-out periods) at day 16 and those recorded at day 40. These graph show that the increased pellets intake observed in CB1+/+ mice trained with highly palatable pellets at day 40 is due to an enhancement of their reinforced responses during the first 15 min of the last FR5 session. No significant variation of reinforced responses were detected in the rest of the last session (i.e. from 15 to 60min) in these mice. Increased responses at the initiation of the last session are also observed in CB1+/+ and CB1-/- mice that worked for highly caloric and highly palatable food, respectively. However, in contrast to CB1+/+ mice trained highly palatable food, these increased responses are not significantly different from those displayed by standard-trained mice at the same time interval. Data are expressed as mean ± s.e.m. ★ $P < 0.05$, ★★ $P < 0.001$ (day 16 vs. day 40); ☆☆☆ $P < 0.001$ (comparison vs. standard).

ARTICLE 2

Operant behavior to obtain palatable food modifies ERK activity in the brain reward circuit

Thomas Guegan, Laura Cutando, Giuseppe Gangarossa, Emanuela Santini, Gilberto Fisone, Fatima Bosch, Albert Martinez, Emmanuel Valjent, Rafael Maldonado*, Miquel Martin*

European Neuropsychopharmacology. 2013; 23(3): 240-52.

* Co-corresponding authors

OBJECTIVE 2

Studying the role of CB1-R in morphine-induced behavioural and structural plasticity

Thomas Guegan, Joan Pau Cerbià Costa, Rafael Maldonado and Miquel Martin

Role of CB1-R in morphine-induced locomotor sensitization

Drug addiction is marked by long-lasting behavioural changes that can even persist for a lifetime in certain patients. Therefore, understanding by which mechanisms chronic exposure to drugs of abuse can lead to the emergence of stable neuroadaptations is a primary challenge for the treatment of the disease. Drugs of abuse cause alterations in the synaptic plasticity mechanisms underlying learning and memory processes in the brain reward circuit, leading to maladaptive behavioural learning such as compulsive drug-intake, and ingrained drug memories revealed by recurrent drug craving (Hyman and Malenka, 2001; Kalivas and O'Brien, 2008; Lüscher and Malenka, 2011). The persistence of these behavioural alterations in addicts is thought to be, in part, the consequence of drug-induced structural plasticity changes in the mesocorticolimbic system (Robinson and Kolb, 2004). Indeed, synaptic morphological changes can enhance the stability of synaptic connexions, and promote the emergence of enduring forms of synaptic plasticity (Grutzendler et al., 2002; Kasai et al., 2003; O'Donnell et al., 2011; Robert et al., 2010). In addition, drug-induced structural plasticity in the mesocorticolimbic system can persist for at least several months after cessation of drug treatment in rodents (Robinson and Kolb, 2004). Although these structural changes have been well documented, little is known regarding the underlying molecular mechanisms and whether structural plasticity drive addictive behaviors or whether they reflect homeostatic compensations to the drug (Russo et al., 2010).

Our previous results demonstrate that CB1-R mediates the structural plasticity changes that underline food reward and seeking-behaviour

alterations (Guegan et al., 2013). On the other hand, the endogenous cannabinoid system through CB1-R is an important mediator of drug reward and drug-induced long-lasting effects (Maldonado et al., 2006). Interestingly, one study has shown that CB1-R is involved in cocaine-induced structural plasticity in the motor cortex (Ballesteros-Yáñez et al., 2007a). Therefore, we sought that CB1-R could represent a common neurobiological substrate for the development of distinct pathologic consummatory behaviour by contributing to the emergence of morphological synaptic alterations in the brain reward circuit.

We have used the locomotor sensitization paradigm that has been considered a reliable animal model of drug-induced long-lasting behavioural changes (Robinson and Berridge, 2008) to evaluate the role of CB1-R in drug-induced structural plasticity. Indeed, sensitization treatments lead to an hypersentivity to drug stimulating effects that can last for several months (Robinson and Berridge, 2008). Moreover, sensitized animals rapidly escalate their drug intake in self-administration paradigms, display enhanced motivation to work for drug and high propensity to reinstate drug seeking after priming injection in the NAc (Ferrario et al., 2007; Suto et al., 2004). Interestingly, chronic drug treatments that induce locomotor sensitization also promote the development of morphological synaptic changes in different parts of the mesocorticolimbic system (Robinson and Kolb, 2004). In addition, some of these structural changes tightly correlate with the expression of locomotor sensitization (Li et al., 2004a). Thus, this paradigm may represent a valuable animal model to investigate the neurobiological basis of drug-induced structural plasticity changes and their implication in the long lasting behavioural alterations that characterize addiction.

In this study, we evaluated the involvement of CB1-R in the structural plasticity changes associated with morphine sensitization. Opioid drugs have extremely high abuse potential in humans and opiate addiction is associated with elevated rates of relapse after abstinence (Veilleux et al., 2010). Previous studies have demonstrated the important role played by the endogenous cannabinoid system in the rewarding effects and long-term alterations triggered by opioids drugs (Robledo et al., 2005). Interestingly, CB1-R is an important mediator of morphine induced locomotor sensitization (Martin et al., 2000b). Therefore, we hypothesized that the endogenous cannabinoid system, via CB1-R, could mediate the long-lasting behavioural changes induced by morphine, such as locomotor sensitization, by contributing to the development structural plasticity changes in the mesocorticolimbic system.

Methods

Animals

This experiment was carried out in male CB1-R knockout mice and wild-type littermates with a C57BL6/J genetic background. The generation of mice lacking CB1 receptors was described previously (Zimmer et al., 1999). The mice were aged of 8 to 12 weeks at the beginning of the experiment, and were housed 4/5 per cage in a temperature-controlled room (21 ± 1 °C) with a 12 h light: dark cycle (light on between 8:00 and 20:00h). Food and water were available ad libitum. Mice were acclimated to handling and received subcutaneous saline injection for one week before the beginning

of experiments. All animal procedures met the guidelines of the European Communities directive 86/609/EEC regulating animal research and were approved by the Local Ethical Committee.

Drugs

Morphine was provided by the Ministerio de Sanidad y Consumo (Spain), and dissolved in saline (0.9%). Morphine was administered subcutaneously at the dose of 15 mg/kg in the locomotor sensitization study.

Locomotor sensitization

The sensitization to the locomotor responses induced by chronic morphine treatment was evaluated using locomotor activity boxes (9 x 20 x 11 cm; Imetronic, France). The boxes contained 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 (i.e. rearing). Mice were individually placed in the boxes and their general locomotor activity was recorded during 15 min in a low luminosity environment (20 ± 25lux). All the locomotor activity tests were performed between 09.30 and 10.30. In order to habituate the animals to the test environment and to obtain a stable baseline, basal locomotor activity was measured 10 min after a subcutaneous (s.c.) injection of saline on days 1, 2 and 3. On day 4, chronic treatment with morphine (15 mg/kg, s.c.) began for a period of 15 days. The animals received two injections of the drug or saline per day. Morning injections were performed between 9 and 10 h, and evening injections between 18 and 19 h. The acute locomotor effects of morphine were evaluated 10 min after morphine (15 mg/kg, s.c.) or saline injection on day

4. During chronic drug treatment, the locomotion was recorded 10 min after the morning injection every 3 days (days 7, 10, 13, 16 and 19). After finishing chronic drug administration on day 19, the animals did not receive any treatment from day 20 to day 25. On day 26, animals received a challenge injection of morphine (15 mg/kg, s.c.) or saline, and locomotor activity was measured again, to evaluate the maintenance of morphine induced locomotor sensitization (Figure 25). Mice were divided in 4 different experimental conditions for each genotype at this time point (i.e. day 26), to better assess the impact of different morphine treatment on structural plastic changes:

1. Sal/sal group: mice chronically treated with saline and challenged at day 26 with saline injection that represent the control group.

2. Sal/mor group: mice chronically treated with saline and challenged at day 26 with morphine injection, which allows to evaluate the effects of an acute morphine injection on structural plasticity.

3. Mor/sal group: mice chronically treated with morphine and challenged at day 26 with saline injection, that permit to assess the long-term morphological changes associated with a morphine chronic treatment.

4. Mor/mor group: mice chronically treated with morphine and challenged at day 26 with morphine injection, which allows to determine specifically the structural plasticity changes involved in the late expression or maintenance of morphine induced behavioural sensitization.

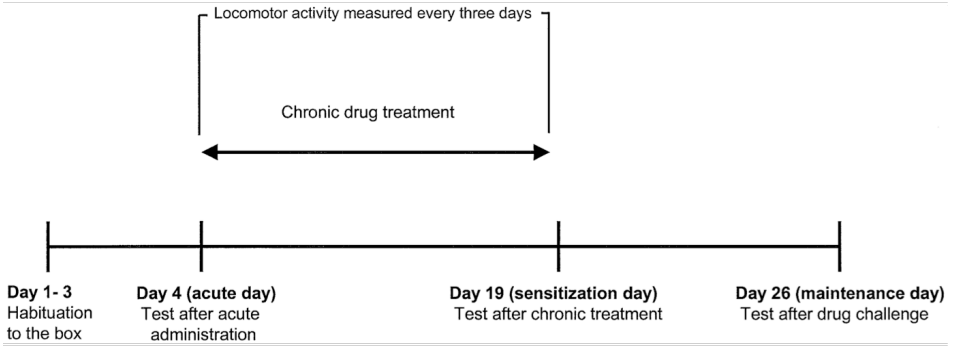


Figure 25: Schematic representation of the protocol used to evaluate the sensitization to locomotor effects of morphine.

Structural plasticity analysis

At day 26, thirty minutes after the challenge injection of morphine or saline (10 min post treatment injection, 15 min locomotor activity recording, and 5 additional min in activity boxes), mice were deeply anesthetized by intraperitoneal injection (0.2 ml/10 g body weight) of a mixture of ketamine (100 mg/kg) and xylazine (20 mg/kg) prior to rapid intracardiac perfusion, delivered with a peristaltic pump at 20 ml/min, with 10 ml of Na2HPO4/NaH2PO4/NaCl buffer (PBS) 0.1 M, pH 7.5, and followed by perfusion with 40 ml of 4 % paraformaldehyde (PFA) in PBS 0.1 M, pH 7.5. Brains were quickly removed from the skull and postfixed in 4 % PFA for 10 min. Brain coronal sections (100 µm) containing the NAc (Paxinos and Franklin, 2001) (from bregma 1.54 mm to bregma 1.10 mm) or the medial prefrontal cortex (mPFC) (from bregma 1.98 mm to bregma 1.70 mm) were obtained by using a vibratome (Leica VT 1000 S, Nussloch, Germany) and kept in PBS 0.1 M until they were processed for fluorescent labeling. Brain

slices were labeled by ballistic delivery of fluorescent dye DiI (Molecular Probes, Eugene, OR, USA) using a gene gun apparatus (Helios Gene Gun System, Bio-Rad, Deutschland) as described previously (Grutzendel et al., 2003) and postfixed with PFA for 4 h at room temperature to further preserve structures and to allow the diffusion of the dye DiI. Sections were placed on microscope gelatine-coated slides and coverslipped with mounting medium (Mowiol). Then, images were acquired with confocal microscope (Leica TCS SP5 II CW-STED, Germany) with a glycerol immersion lens (63x), and an additional 3x objective zoom, to analyze dendritic spine density and structure. Individual medium spiny neurons in the NAc and pyramidal neurons from the mPFC were chosen for spine analysis based on several criteria, as described previously (Lee et al., 2006): (i) there was minimal or no overlap with other labelled cells to ensure that processes from different cells would not be confused, (ii) at least three primary dendrites needed to be visible for cells to be used for analysis and (iii) distal dendrites (from secondary dendrites to terminal dendrites) were examined. Dendrites from medium spiny neurons in the core and shell of the NAc (from bregma 1.54 to bregma 1.10) and basilar dendrites of pyramidal neurons taken predominantly from the prelimbic and infralimbic areas of the mPFC (from bregma 1.98 to bregma 1.70) were analyzed. To calculate spine density, a length of dendrite (at least 20 μm long) was traced by using a confocal microscope (Leica TCS SP5 II CW-STED, Germany) with a glycerol immersion lens (63x), and an additional 3x objective zoom. All images of dendrites were taken at different z levels (0.25 μm depth intervals) to examine the morphology of dendritic spines. All measurements were made using IMAGE J analysis software. Protrusions from dendrites were classified into five types based on their morphology:

class 1 protrusions, also called stubby protuberances were 0.5 µm in length, lacked a large spine head, and did not appear to have a neck; class 2, or mushroom-shaped spines were between 0.5 and 1.25 µm in length and were characterized by a short neck and large spine head; class 3, or thin spines ranged between 1.25 and 3.0 µm and had elongated spine necks with small heads; class 4, or wide spine were between 0.5 and 1.25 µm in length and were characterized by a large neck and a large spine head; and class 5 or branched spine ranged between 1.25 and 3.0 µm and had elongated spine necks with two or more spine heads. Quantification of dendritic spine densities was performed in blind conditions.

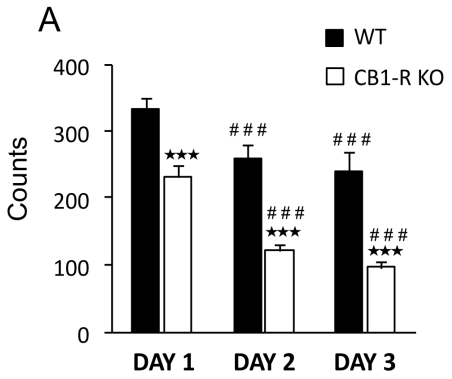
Statistical analysis

Behavioural data were analysed by using a three-way or two-way ANOVA with genotype (between subjects) and/or treatment (between subjects) and day (within subjects) as factors of variations (Table 2). Post hoc comparisons were performed by using the Fischer F-test when appropriate. Morphological data were analysed by using a two-way ANOVA with genotype and treatment as factors of variation (between subjects) (Table 3). Post hoc comparisons were performed by using the Fischer F-test when appropriate. Student's t-tests were achieved when assessing two-group comparisons. Statistical tests were performed with Statistica© (StatSoft, Tulsa, OK, USA).

Results

Genetic deletion of CB1-R alters basal locomotor activity and acute locomotor effects of morphine

CB1-R KO mice presented a lower basal locomotor activity than WT mice during the habituation phase since the first day (Figure 26 A). This result is in accordance with previous studies showing that genetic deletion of CB1-R in C57BL6/J mice promotes a hypolocomotor phenotype (Zimmer et al., 1999). Our experimental measures not only reveal a general alteration of motor behaviour in CB1-R KO mice, but also highlight the involvement of CB1-R in the process of habituation. Indeed, after three days of exposure to the activity boxes, CB1-R KO mice displayed a decrease of their basal locomotor activity of about 63%, while this decrease was only of 27% in WT mice, suggesting that the lack of CB1-R accelerates the habituation (Figure 26 A and C). Although of scientific interest, these behavioural characteristics represent for our experimental measures a potential bias that must be taken into consideration to accurately interpret the experimental data of morphine induced hyperlocomotion.



C

			SENSITIZATION	
	HAB.	ACUTE	INDUCTION	EXPRESSION
WT	-27,3%	114,2%	49,9%	197,3%
CB1-R KO	-63,1%	579,9%	-30,5%	55,5%

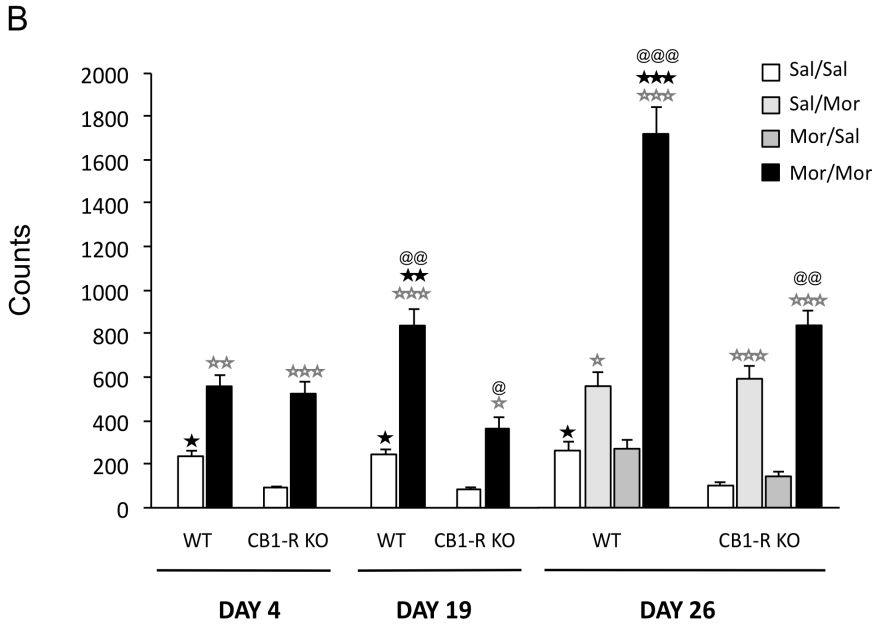


Figure 26: Genetic deletion of CB1-R alters basal locomotor activity, acute locomotor-stimulating effects of morphine and the development and expression of locomotor sensitization to morphine. (A) Basal locomotor activity of WT (black columns; n=60) and CB1-R KO (white columns; n=59) mice during the three days of habituation. **(B)** Locomotor-stimulating effects of morphine (15mg/kg, s.c.) in WT and CB1-R KO mice. Day 4 represents the acute effect of morphine, day 19 represents the sensitization effect at the end of the chronic treatment (induction phase), and the maintenance or late expression of sensitization is shown on day 26. From day 4 to 19, mice of each genotype were treated, twice daily, with morphine (black columns; WT, n=31; CB1-R, n=28) or saline (white columns; WT, n=29; CB1-R, n=31). At day 26, after 1 week without treatment, mice chronically treated with saline received either an injection of

saline (white columns; WT, n=16; CB1-R KO, n=17), or morphine (light grey columns; WT, n=13; CB1-R KO, n=14); similarly, mice chronically treated with morphine received either an injection of saline (dark grey columns; WT, n=12; CB1-R KO, n=12) or morphine (black columns; WT, n=19; CB1-R KO, n=16). **(C)** Table showing the percentage of increase or decrease of locomotor activity in WT and CB1-R mice, triggered by the habituation procedure (HAB: comparision day 3 vs. day 1), an acute injection of morphine (ACUTE: comparison day 4 vs. day 3), a morphine injection after chronic treatment (INDUCTION: comparison day 19 vs. day 4), and challenge injection of morphine (EXPRESSION: comparision day 26 vs. day 4). Data are expressed as mean of the total number photocells beam crossed during the 15 min session, ± s.e.m. ★ P< 0.05, ★★ P<0.01, ★★★ P < 0.001 (WT vs. CB1-R KO, Fisher F-test); ### P < 0.001 (comparison vs. day 1, Fisher F-test); ☆ P<0.05, ☆☆ P<0.01, ☆☆☆ P < 0.001 (morphine- treated vs. saline-treated mice, Fisher F-test); @ P < 0.05, @@ P<0.01, @@@ P < 0.001 (comparison vs. day 4, Fisher F-test).

Morphine acute effects were evaluated at day 4. Both genotypes displayed a significant enhancement of their locomotor activity in response to this first morphine injection, when compared to their saline treated counterparts. No statistical differences in the absolute locomotor values were observed between morphine-treated mice of both genotypes (Figure 26B). However, it is important to note that the increment of locomotor activity induced by acute injection of morphine was 5 times higher in CB1-R KO than in WT mice in proportion to their respective basal activity established at day 3 (Figure 26C). Therefore, our results suggest that CB1-R plays an important modulatory role in the acute locomotor effects of morphine, as its absence leads to a disproportionate enhancement of acute morphine-induced hyperlocomotion.

The lack of CB1-R prevents the induction locomotor sensitization to morphine, and promotes the development of a tolerance-like process to morphine-induced hyperlocomotion

Following repeated intermittent administration of morphine, WT mice displayed sensitized responses to the locomotor effects of morphine. Indeed, at day 19, morphine (15mg/kg) produced a higher hyperlocomotor effect in WT mice than initially (day 4) (Figure 26B). In sharp contrast, this drug regimen failed to further increase the acute hyperlocomotor effect of morphine in CB1-KO mice, and even leaded to the development of the opposite behavioural outcome, i.e., decrease of morphine's initial effect (Figure 26B). The locomotor sensitization starts as soon as four days after the initiation of morphine chronic treatment (day 7), and the lack of CB1-R disrupts the induction of this phenomenon. Thus, while WT mice displayed an enhancement of 55,7% of their initial locomotor response to morphine at day 7 , CB1-KO mice showed similar locomotor responses between day 4 and day 7 (Figure 27A and B). These sensitized responses remained stable all over the chronic treatment in WT mice, whereas in CB1-R KO mice repeated exposure to morphine produced a progressive decrease of the acute hyperlocomotor effects of this drug, which reached statistical significance from day 10 to day 19 (Figure 26 A and B). Therefore, the genetic deletion of CB1-R prevents the development of locomotor sensitization and promotes the appearance of tolerance-like process to morphine hyperlocomotor effects during chronic administration. This process seems specific to morphine effects since locomotor activity of saline treated CB1-R KO mice remained stable during the same period (Figure 27B).

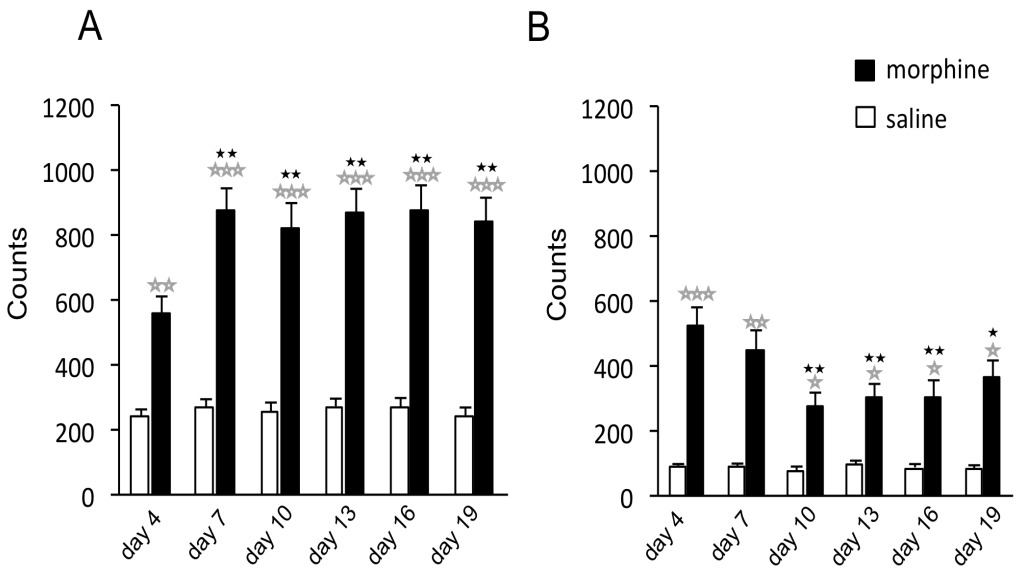


Figure 27: Lack of CB1-R prevents morphine induced locomotor sensitization and promotes tolerance to morphine hyperlocomotor effects during the chronic treatment. Locomotor activity of saline-treated (white columns; WT, n=29; CB1-R, n=31) and morphine-treated (black columns; WT, n=31; CB1-R, n=28) WT **(A)** and CB1-R KO **(B)** mice during the chronic treatment (from day 4 to 19). Data are expressed as mean of the total number photocells beam crossed during the 15 min session, ± s.e.m. ★ $P < 0.05$, ★★ $P < 0.01$ (comparison vs day 4, Fisher F-test). ☆ $P<0.05$ ☆☆ $P < 0.01$, ☆☆☆ $P < 0.001$ (morphine vs saline, Fisher F-test).

Deletion of CB1-R reduces the expression of morphine sensitization after chronic treatment disruption

After 7 days of cessation of the morphine chronic treatment, mice were challenged with a similar dose of morphine (15mg/kg) to evaluate the maintenance of behavioural sensitization (day 26). Our results show that once established, sensitized locomotor responses to morphine can be

maintained for at least one week in WT mice after chronic treatment disruption (Figure 26B). Thus, morphine sensitization may rely on enduring forms of neuronal plasticity changes. Moreover, we also observed that the sensitized locomotor responses to morphine were significantly more important at this time point than during the chronic treatment (increased by 98,4% day 26 vs day 19)(Figure 26B), highlighting the emergence of additional neuronal adaptations after chronic morphine treatment disruption.

On the other hand, tolerance to the hyperlocomotor effects of morphine was no longer revealed in CB1-R KO mice that received a challenge dose of morphine after one week of chronic treatment cessation (Figure 26B). Thus, the neuronal adaptations mediating tolerance to the hyperlocomotor effects of morphine are not as persistent than those underlying sensitization. Interestingly, the ability of morphine to enhance locomotor activity in CB1-R KO mice was not only restored after chronic morphine treatment disruption, but this hyperlocomotion was also sensitized at this time point. Indeed, we observed that the challenge injection of morphine at day 26 produces significantly higher hyperlocomotion than acute morphine injection at day 4 in CB1-R KO mice (Figure 26B and C). This result suggests that chronic morphine treatment may also trigger the generation sensitization-related plastic changes in CB1-R KO mice after the disruption of the chronic treatment. However, it is important to note that the sensitized responses induced by challenge injection of morphine were significantly smaller in CB1-R KO mice than in WT mice (Figure 25C).

At day 26, mice from both genotypes were divided in four experimental groups to better assess the impact of different morphine treatments on

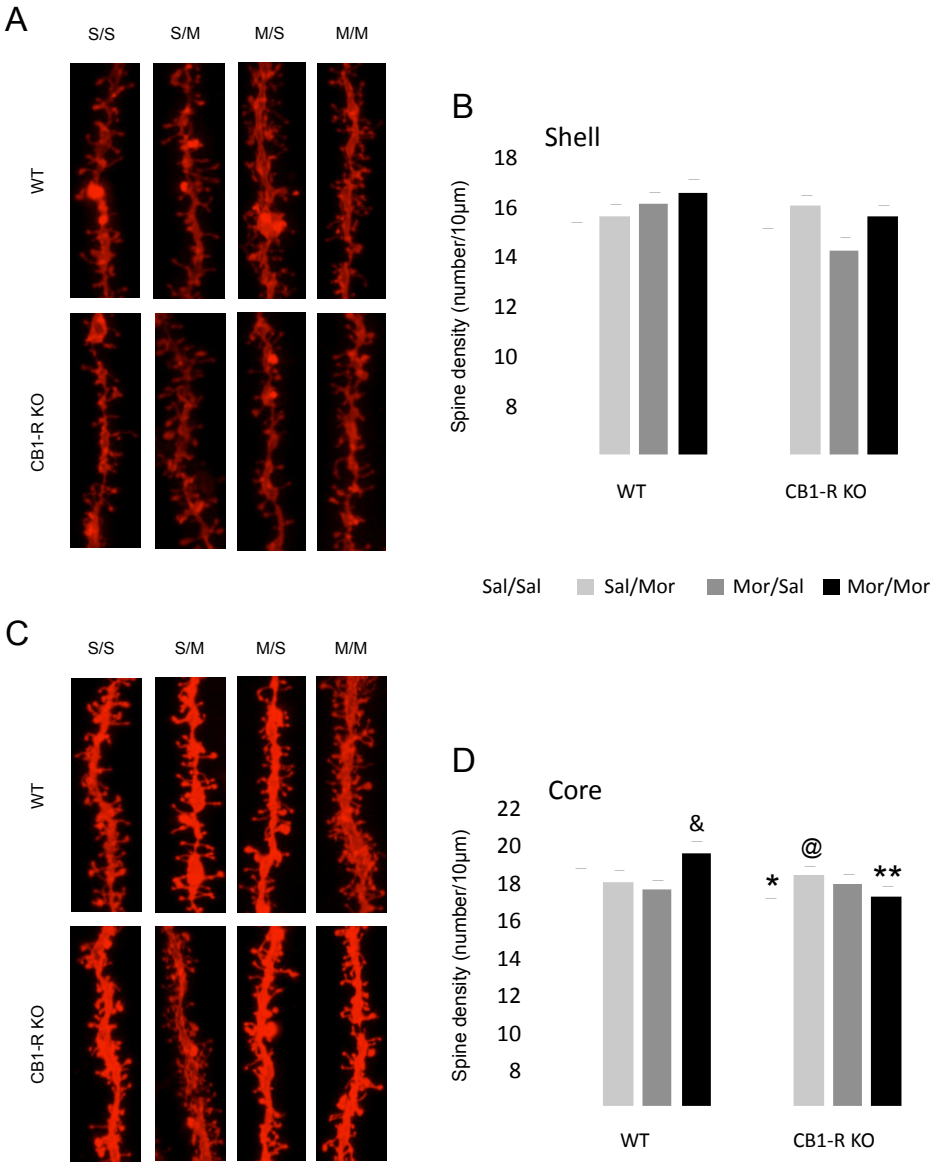
structural plasticity changes. Thus, WT and CB1-R KO mice chronically treated with saline received either a challenge injection of saline (sal/sal group) or morphine (sal/mor group) at day 26 to evaluate the impact of an acute exposure to morphine on structural plasticity. Similarly, mice from both genotypes chronically treated with morphine received either a challenge injection of saline (mor/sal group) or morphine (mor/mor group) at day 26, to distinguish the structural changes specifically involved in the late expression of morphine sensitization from those associated to the disruption of the chronic morphine treatment. Mice from both genotypes receiving an acute injection of morphine at day 26 (sal/mor group) behaved similarly than their respective counterparts receiving this acute injection at day 4. In addition, both CB1-R KO and WT mice chronically treated with morphine that received a challenge injection of saline at day 26 did not show any significant hyperlocomotor effects in response to the re-exposure to the drug associated context (Figure 26C).

Genetic deletion of CB1-R produces basal morphological impairments and "exacerbates" the structural plasticity changes induced by acute morphine in the mesocorticolimbic circuit

Thirty minutes after the administration of the challenge dose of morphine or saline, mice were sacrificed and medium spiny neurons from the NAc shell and core, and pyramidal neurons from the mPFC were stained with a ballistic delivery of a DiI colorant to perform the structural analysis. The comparison of dendritic spines density in mice never exposed to morphine (sal/sal groups) reveals that the deletion of CB1-R alters basal neuronal morphology in the NAc core. Indeed, saline/saline-treated CB1-R KO mice

present a decreased total spine density in the NAc core when compared to saline/saline-treated WT mice (Figure 28). This basal morphological alteration may be the consequence of an impairment of brain developmental processes due to the absence of CB1-R. Indeed, CB1-R plays an important role in neural progenitor proliferation, axonal navigation and synapse formation during embryonic neuronal development (Fernández-Ruiz et al., 2000; Diaz-Alonso et al., 2012). In addition, several studies also reported alterations in neuronal morphology in different brain areas in CB1-R KO mice (Ballesteros-Yanez et al., 2007a; Hill et al., 2011). This structural alteration could account for basal behavioural impairments observed in these mutant mice, such as their basal hypoactivity (present study; Zimmer et al., 1999).

Furthermore, our morphological analysis reveals that an acute injection of morphine do not produce any significant change in the total number of dendritic spines in any of the three studied areas in WT mice. In contrast, the same acute administration of morphine significantly increases gobal dendritic spines density in the NAc shell and core in CB1-R KO mice (Figure 28).


A
S/S
S/M
M/S
M/M
WT
CB1-R KO
B
Shell
Spine density (number/10µm)
18
16
14
12
10
8
WT
CB1-R KO
Sal/Sal
Sal/Mor
Mor/Sal
Mor/Mor
C
S/S
S/M
M/S
M/M
WT
CB1-R KO
D
Core
Spine density (number/10µm)
22
20
18
16
14
12
10
8
&
@
*
**
WT
CB1-R KO

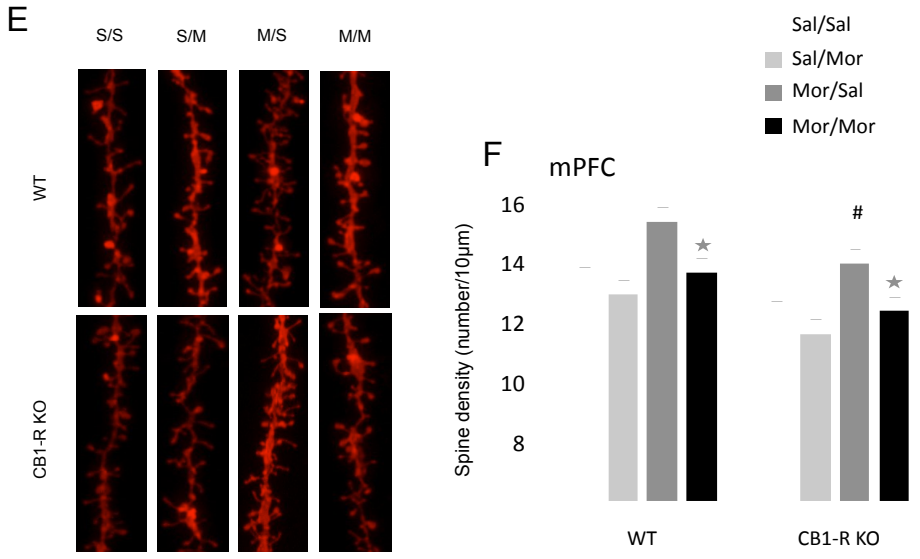


Figure 28: Structural plasticity changes induced by morphine in the mesocorticolimbic circuit are dependent on the activity of CB1-R. Illustration of DiI-labeled dendrites of medium spiny neurons in the NAc shell **(A)**, NAc core **(C)** and DiI-labeled basilar dendrites of pyramidal neurons in the mPFC **(E)** of WT and CB1-R KO mice chronically treated with saline and receiving at day 26 an injection of saline (S/S and Sal/Sal) or morphine (S/M and Sal/Mor); and of WT and CB1-R KO mice chronically treated with morphine and receiving at day 26 an injection of saline (M/S and Mor/Sal) or morphine (M/M and Mor/Mor). Quantification of total dendritic spines density (number/10 µm) in neurons from the NAc shell **(B)**, core **(D)** and mPFC **(F)** in the different experimental groups of WT and CB1-R KO mice. Data represents the average of 42-52 dendrites per experimental group; a total of 6-8 dendrites per animal (n=7-8 per experimental group) and brain area were analyzed; and no more than 2 dendrites were evaluated from the same neuron. Data are expressed as mean ± s.e.m. & $P < 0.05$ (comparision vs. Mor/Sal, Fisher F-test); @ $P < 0.05$ (comparision vs. Sal/Sal, Fisher F-test); ★ $P < 0.05$, ★★ $P < 0.01$ (WT vs. CB1-R KO mice, Fisher F-test); ☆ $P < 0.05$, ☆☆ $P < 0.01$ (comparison vs. Sal/Sal, Student's t-test); ★ $P < 0.05$ (comparision vs. Mor/Sal, Student's t-test); # $P < 0.05$ (WT vs. CB1-R KO mice, Student's t-test).

As dendritic spines vary morphologically in length and shape and consequently in their biophysical characteristics, stability and physiological functions (see introduction), we evaluated the particular types of spines (see methods) that result specifically involved in morphine-induced structural plasticity changes. This analysis reveals that acute morphine increases specifically the dendritic density of mushroom-type in the NAc shell and stubby-types in the NAc core in CB1-R KO mice (Table 1). Interestingly, although global changes in dendritic spines density were not revealed in WT mice after a single injection of morphine, this detailed analysis shows that acute morphine administration increases the proportion of stubby-types spines in the NAc shell and mushroom-types in the NAc core (Figure 29A and B).

In summary, our results show that acute morphine exposure produces structural plasticity changes in the mesocorticolimbic circuit in WT mice by modifying the morphology of pre-existing spines in the NAc core and shell. In addition, our results suggest that acute morphine-induced structural changes in the mesocoticolimbic circuit could be dependent of the activity of the endogenous cannabinoid system, as the lack of CB1-R "exacerbates" these neuronal adaptations. Indeed, acute morphine exposure leads to increase global spines density in the NAc core and shell in CB1-R KO mice, but not in WT mice.

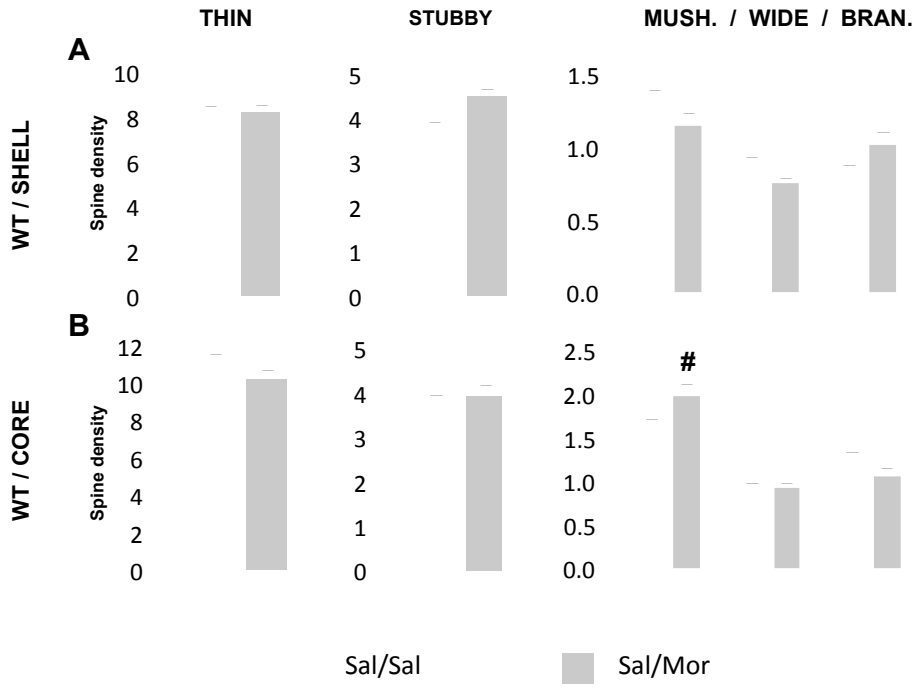


Figure 29: Acute morphine induces spines morphology changes in the NAc shell and core in WT mice . Quantification of the density (number/10 µm) of the different types of spines in the NAc shell (**A**) NAc core (**B**) in WT mice. Acute morphine increases the density of stubby-types spines in the NAc shell (**A**) and mushroom-types in the NAc core (**B**) in WT mice, without changing the global spines density in these regions (Figure 28B and D). Data represents the average of 42-52 dendrites per experimental group; a total of 6-8 dendrites per animal (n=7-8 per experimental group) and brain area were analyzed; and no more than 2 dendrites were evaluated from the same neuron. Data are expressed as mean ± s.e.m. ☆☆ $P < 0.01$ (comparison vs. Sal/Sal, Student's t-test); # $P < 0.05$ (comparison vs. Sal/Sal, Fisher F-test). MUSH.: mushroom, BRAN.: branched.

The lack of CB1-R differentially alters the structural plasticity changes induced by chronic morphine treatment and morphine re-exposure in the NAc and mPFC

After one week of abstinence from a chronic morphine treatment (mor/sal group), we did not observe any significant structural change in the NAc shell and core in WT mice, neither in the global dendritic spines density nor in the density of the differents sub-types of spines (Figure 28 and Table 1). Similarly, no major stuctural plasticity changes were found in the NAc core and shell in morphine/saline-treated CB1-R KO mice, altought an increased proportion of stubby-types spines was revealed in the NAc core in these animals (Figure 28 and Table 1). Globally, these results suggest that the morphological changes induced by acute morphine exposure in the NAc core and shell are not persistent and disappear rapidly after the disruption of the treatment. Nevertheless, a tendency to enhance global dendritic spine density in the NAc shell was revealed in morphine/saline-treated WT mice when compared with saline/saline-treated WT mice (P=0.097, Student's t-test) (Figure 28). These results could reflect that the disruption of chronic morphine treatment might be associated with the progressive waning of structural changes established during the morphine chronic treatment in the NAc shell. This hypothetical process may be absent or have a shorter duration in CB1-R KO mice.

In contrast, we observed that the disruption of chronic morphine treatment (mor/sal group) was associated with a significant increase of dendritic spines density in the mPFC in CB1-R KO and WT mice (Figure 28). In both genotypes, these changes in the mPFC were mainly due to an increase of the density of thin-types spines (Table 1). These results suggest that CB1-R

is not involved in the generation of these changes. However, dendritic spines density in morphine/saline-treated WT mice were significantly higher than in the equivalent CB1-R KO mice group in this brain area (Figure 28). Thus, CB1-R is certainly not necessary for the formation of these changes, although its genetic deletion appears to reduce their magnitude.

Finally, the morphological analysis in morphine/morphine-treated groups revealed that the injection of a challenge dose of morphine one week after the disruption of the chronic treatment significantly increases total dendritic spines density in the NAc shell and core in WT mice, when compared with saline/saline treated WT mice and morphine/saline-treated WT mice, respectively. In the NAc core, these changes were also significant when compared to the morphine/morphine-treated CB1-R KO mice (Figure 28). The structural changes observed in WT mice were mainly due to an increase in stubby and branched types spines density in the NAc shell, while in the NAc core these changes rely on an increase of thin spines (Table 1). In contrast, similar challenge injection did not produce significant global spines density changes in CB1-R KO in both sub-regions of the NAc (Figure 28) although leaded to enhance the proportion mushroom-types spines in the NAc shell and mushroom and stubby-types spines in the NAc core (Figure 30). On the other hand, our results show that a challenge dose of morphine decreases the prior enhancement of total spines density in mPFC that has been revealed after the disruption of the chronic morphine treatment in both genotypes (Figure 28). These results further indicate that structural changes in the mPFC associated to chronic morphine treatment and re-exposure are in part independent of CB1-R activity.

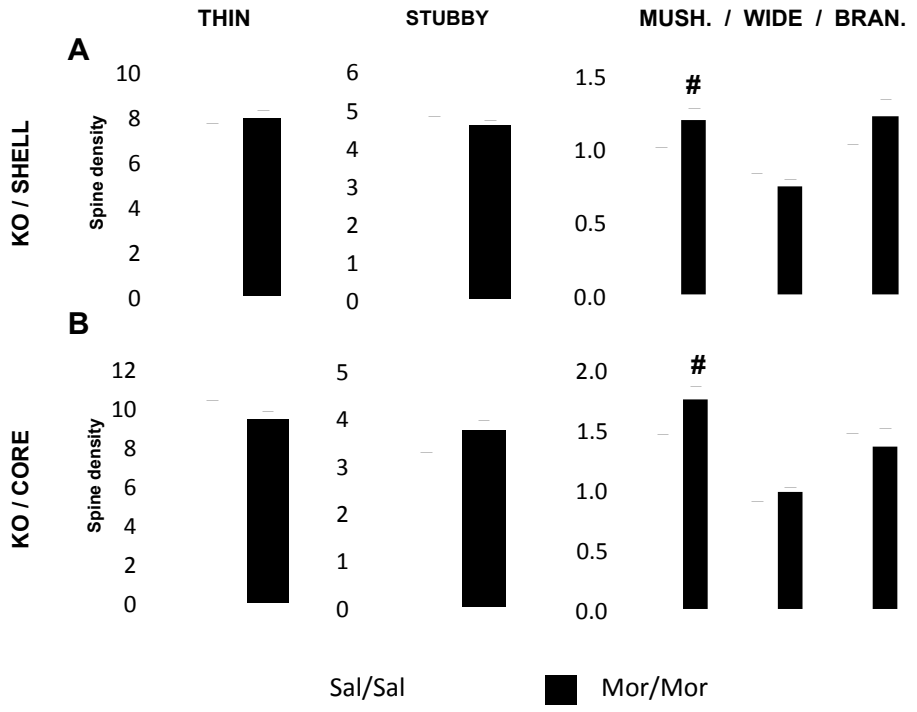


Figure 30: Re-exposure to morphine after disruption of the chronic treatment induces spines morphology changes in the NAc shell and core in CB1-R KO mice. Quantification of the density (number/10 µm) of the different types of spines in the NAc shell (**A**) NAc core (**B**) in CB1-R KO mice. Challenge injection of morphine increases the density of mushroom-types spines in the NAc shell (**A**) and stubby-types and mushroom-types spines in the NAc core (**B**) in CB1-R KO mice, without altering the total spines density in these area (Figure 28B and D). Data represents the average of 42-52 dendrites per experimental group; a total of 6-8 dendrites per animal (n=7-8 per experimental group) and brain area were analyzed; and no more than 2 dendrites were evaluated from the same neuron. Data are expressed as mean ± s.e.m. ☆ $P < 0.05$, (comparison vs. Sal/Sal, Student's t-test); # $P < 0.05$ (comparison vs. Sal/Sal, Fisher F-test). MUSH.: mushroom, BRAN.: branched.

In summary, our study demonstrates that morphine-induced structural plasticity in the NAc and mPFC is a dynamic phenomenon that evolves during the time course of the sensitization process. Indeed, our data in WT mice show that a single morphine administration triggers spines morphology changes in the NAc core and shell, which can potentially participate in the acute hyperlocomotor effects of morphine. In addition, structural plasticity changes were no longer observed in the NAc core after the disruption of chronic morphine treatment, whereas increased spines density in the mPFC was revealed at this time point, which can potentially support the long-term maintenance of morphine sensitization in WT mice. Finally, re-exposure to morphine one week after the cessation of the chronic treatment produces in WT mice further spines density changes in all the three brain areas studied. This challenge dose of morphine after chronic treatment produces higher hyperlocomotor effects and more extended structural changes in the mesocorticolimbic system than the first acute injection. The enhancement of morphine induced structural plasticity after chronic morphine treatment could represent a neuronal mechanism underlying morphine sensitization. Finally, our results highlight the involvement of CB1-R in morphine-induced structural plasticity in the NAc core and shell and in lesser extent in the mPFC, which can possibly account for the altered acute hyperlocomotor effects of morphine and impaired morphine-induced locomotor sensitization observed in CB1-R KO mice.

		THIN	STUBBY	MUSHROOM	WIDE	BRANCHED
mPFC						
WT	S/S	6,36 ± 0,33	3,90 ± 0,20	1,37 ± 0,10	0,73 ± 0,05	0,90 ± 0,07
	S/M	6,29 ± 0,30	3,86 ± 0,20	1,31 ± 0,10	0,67 ± 0,07	0,73 ± 0,06
	M/S	**7,93 ± 0,27** ###	4,29 ± 0,19	1,42 ± 0,09	0,69 ± 0,05	1,02 ± 0,09
	M/M	6,87 ± 0,32 %	3,61 ± 0,16	1,37 ± 0,10	0,73 ± 0,05	1,05 ± 0,09
CB1-R	S/S	5,61 ± 0,29	3,70 ± 0,15	1,34 ± 0,09	0,77 ± 0,05	0,84 ± 0,08
KO	S/M	5,44 ± 0,31	3,69 ± 0,13	1,01 ± 0,07 @	0,53 ± 0,04	0,90 ± 0,09
	M/S	**7,05 ± 0,33** ##; @	3,83 ± 0,14	1,25 ± 0,10	0,73 ± 0,06	1,05 ± 0,11
	M/M	6,00 ± 0,30 %	3,58 ± 0,16	1,27 ± 0,08	0,68 ± 0,07	0,82 ± 0,08
NAc shell						
WT	S/S	8,11 ± 0,34	3,78 ± 0,12	1,32 ± 0,07	0,87 ± 0,06	0,81 ± 0,06
	S/M	8,16 ± 0,34	**4,46 ± 0,18** ##	1,14 ± 0,09	0,74 ± 0,04	1,01 ± 0,09
	M/S	8,86 ± 0,41	4,03 ± 0,19	1,37 ± 0,10	0,82 ± 0,06	0,91 ± 0,08
	M/M	8,73 ± 0,39	**4,45 ± 0,17** ##	1,24 ± 0,08	0,89 ± 0,06	**1,16 ± 0,10** ##
CB1-R	S/S	7,28 ± 0,38	4,63 ± 0,15 @@@	0,92 ± 0,08 §§§	0,77 ± 0,06	0,94 ± 0,09
KO	S/M	8,02 ± 0,30	4,57 ± 0,17	**1,28 ± 0,09** $$	0,91 ± 0,08	1,14 ± 0,12
	M/S	7,31 ± 0,38 @@	4,23 ± 0,17	0,88 ± 0,06 §§§	0,74 ± 0,06 §	0,99 ± 0,11
	M/M	7,84 ± 0,37	4,52 ± 0,16	**1,18 ± 0,08** $	0,73 ± 0,06	1,21 ± 0,12
NAc core						
WT	S/S	11,15 ± 0,36	3,35 ± 0,16	1,59 ± 0,11	0,91 ± 0,07	1,21 ± 0,12
	S/M	10,09 ± 0,55	3,91 ± 0,25	**1,97 ± 0,15** $	0,92 ± 0,05	1,05 ± 0,10
	M/S	10.17 ± 0.48	3.77 ± 0.29	1.60 ± 0.12	0.80 ± 0,07	1.15 ± 0,11
	M/M	**11,82 ± 0,55** €€	3,84 ± 0,20	1,64 ± 0,10	0,91 ± 0,06	1,26 ± 0,15
CB1-R	S/S	9,81 ± 0,51 §	3,07 ± 0,18	1,36 ± 0,10	0,83 ± 0,07	1,32 ± 0,15
KO	S/M	10,91 ± 0,44	**3,88 ± 0,21** ##	1,38 ± 0,09 §§§	0,85 ± 0,05	1,22 ± 0,17
	M/S	10,28 ± 0,39	**3,83 ± 0,23** ##	1,53 ± 0,12	0,83 ± 0,08	1,34 ± 0,14
	M/M	9,37 ± 0,40 §§§	**3,72 ± 0,22** #	**1,74 ± 0,12** $	0,96 ± 0,05	1,34 ± 0,16

Table 1: Dendritic density of thin, stubby, mushroom, wide and branched types spines in WT and CB1-R KO mice. Data are expressed as mean (± s.e.m) of the density (number/10 µm) of each spine sub-types measured in n= 42 to 52 dendrites per experimental group (i.e. 7 to 8 animals per group, 6 to 8 dendrites analyzed per animals and brain area, no more than 2 dendrites were evaluated from the same neuron). $ P < 0.05, $$ P < 0.01 (comparison vs. S/S, Fisher F-test); § P < 0.05, §§§ P < 0.001 (WT vs. CB1-R KO, Fisher F-test); €€ P < 0.01 (comparison vs. M/S, Fisher F-test); ## P < 0.01, ### P< 0.001 (comparison vs. S/S, Student's t-test); @ P < 0.05, @@ P < 0.01, @@@ P< 0.001 (WT vs. CB1-R KO, Student's t-test); % P < 0.05 (comparison vs. M/S, Student's t-test). S/S: saline/saline group; S/M: saline/morphina group; M/S: morphine/saline group; M/M: morphine/morphine group.

	Two-way ANOVA		Three-way ANOVA			
	Habituation (Figure 26A)		Sensitization (Figure 26B)		Chronic treament (Figure 27A and B)	
	Total movements	P-value	Total movements	P-value	Total movements	P-value
Genotype (G)	F(1,94) = 79.5318	P < 0.001	F(1,111) = 40.3113	P < 0.001	F(1,115) = 59.8966	P < 0.001
Day (D)	F(2,232) = 107.0572	P < 0.001	F(2,222) = 34.8736	P < 0.001	F(5,575) = 3.3810	P < 0.01
Treatment (T)	-	-	F(3,111) = 67.0171	P < 0.001	F(1,115) = 112.2351	P < 0.001
G X D	F(2,232) = 4.8896	P < 0.01	F(2,222) = 12.6161	P < 0.001	F(5,575) = 16.6058	P < 0.001
G X T	-	-	F(3,111) = 9.7889	P < 0.001	F(1,115) = 11.5672	P < 0.001
T X D	-	-	F(6,222) = 12.6161	P < 0.001	F(5,575) = 2.2221	n.s.
G X T X D	-	-	F(6,222) = 9.8936	P < 0.001	F(5,575) = 13.8514	P < 0.001

Table 2: Statistical analysis of habituation and locomotor effects of morphine in WT and CB1-R KO mice.

	Two-way ANOVA					
	Spine density					
	Genotype	P-value	Treatment	P-value	Interaction	P-value
Shell						
Total	F(1,349) = 3.628	n.s.	F(3,349) = 2.668	P < 0.05	F(3,349) = 1.678	n.s.
Thin	F(1,349) = 10.548	P < 0.01	F(3,349) = 0.948	n.s.	F(3,349) = 1.153	n.s.
Stubby	F(1,349) = 6.986	P < 0.01	F(3,349) = 2.764	P < 0.05	F(3,349) =2 .540	n.s.
Mushroom	F(1,349) = 11.249	P < 0.001	F(3,349) = 0.773	n.s.	F(3,349) = 6.049	P < 0.001
Wide	F(1,349) = 1.020	n.s.	F(3,349) = 0.202	n.s.	F(3,349) = 2.942	P < 0.05
Branched	F(1,349) = 1.82898	n.s.	F(3,349) = 4.0580	P < 0.01	F(3,349) = 0.0839	n.s.
Core						
Total	F(1,363) = 4.588	P < 0.05	F(3,363)=1.204	n.s.	F(3,363) =2.839	P < 0.05
Thin	F(1,363) = 4.479	P < 0.05	F(3,363)=0.276	n.s.	F(3,363) = 5.182	P < 0.01
Stubby	F(1,363) = 0.525	n.s.	F(3,363)=4.584	P < 0.01	F(3,363) = 0.181	n.s.
Mushroom	F(1,363) = 6.032	P < 0.05	F(3,363) = 1.620	n.s.	F(3,363) = 3.283	P < 0.05
Wide	F(1,363) =0.063	n.s.	F(3,363) = 1.203	n.s.	F(3,363) = 0.608	n.s.
Branched	F(1,363) = 1.5338	n.s.	F(3,363) = 0.4647	n.s.	F(3,363) = 0.0233	n.s.
mPFC						
Total	F(1,365) = 13.571	P < 0.001	F(3,365) = 8.954	P < 0.001	F(3,365) = 0.062	n.s.
Thin	F(1,365) = 14.753	P < 0.001	F(3,365) = 11.162	P < 0.001	F(3,365) = 0.018	n.s.
Stubby	F(1,365) = 3.228	n.s.	F(3,365) =2.522	n.s.	F(3,365) = 0.549	n.s.
Mushroom	F(1,365) = 5.125	P < 0.05	F(3,365) = 1.732	n.s.	F(3,365) = 0.739	n.s.
Wide	F(1,365) = 0.509	n.s.	F(3,365) = 2.617	n.s.	F(3,365) = 1.209	n.s.
Branched	F(1,365) = 0.1104	n.s.	F(3,365) = 2.3171	n.s.	F(3,365) = 1.9605	n.s.

Table 3: Statiscal analysis of the effects of morphine on dendritic spines density in the NAc shell, core and mPFC in WT and CB1-R KO mice.

OBJECTIVE 3

Characterization of the common synaptic signalling events that mediate drug of abuse and palatable food reward memories

Thomas Guegan, Joan Pau Cerbià Costa, Rafael Maldonado and Miquel Martin

Drug addiction is charactherized by recurrent episodes of drug relapse that can even occur after long periods of drug abstinence (Stewart, 2008). This behavioural abnormality is therefore a major problem to obtain the recovery of drug addicts, and understanding its underlying neurobiological mechanisms represents a primary challenge for the treatment of addiction.

Relapse to drug consumption is often triggered in human addicts by a re-exposition to drug-associated environmental stimuli, such as drug paraphernalia (e.g. syringes, crack pipes) and place or people, where or with whom they used to take drugs (Carter BL, Tiffany 1999). These drug-related cues may lead to "re-activate" drug's memories and produce intense feeling of drug craving in humans (Grant et al., 1996). This unconscious drive to consume drugs is thought to be a determinant factor that challenges addicts' own willpower in controlling their drug consumption or the possible maintenance of abstinence. In animals, such drug associated cues lead to reinstate drug-seeking behaviour even after devaluation of their drug predictive value or prolonged protracted drug withdrawal (Weiss, 2010). On the other hand, growing evidences suggest the existence of some form of "food addiction", and obese humans often self-reported feelings of food craving that contribute to their excessive food intake and unsuccessful diet control (Wilson, 2010). In animals, these food-associated cues promote reinstatement of food-seeking behaviour, an effect that is potentiated along dieting period from a previous extend access to palatable food (Avena et al, 2008).

Recent research on this topic has identified several brain structures implicated in drug and food craving and relapse (Weiss, 2005; Nair et al., 2009). Among them, the HPC and the NAc have shown to play a prominent

role in these processes (Weiss, 2005; Nair et al., 2009). Thus, presentation of drug associated stimuli or drug craving evoked by autobiographical scripts in human addicts strongly enhance neural activity in the HPC (Kilts et al., 2001; Hermann et al., 2006). In addition, lesion of the HPC in experimental animals prevents reinstatement of drug seeking behaviour by drug-associated context (Fuchs et al., 2005), while its stimulation reinstates drug-taking behaviour (Vorel et al., 2001). However, the role of HPC in food relapse in animal models has not been yet investigated, although imaging studies in humans show that mental representation of highly desirable food that induces food craving also activates the HPC (Pelchat et al., 2004). On the other, the NAc is an important brain structure in medating incubation of drug craving, a process that correlates with enhancement of drug-seeking behaviour (Hollander and Carelli, 2007). Pharmalogical modifications of NAc activity prevent cue- and priming-induced reinstament of cocaine seeking behaviour (Cornish and Kalivas, 2000; Fuchs et al., 2004). Similarly, pharmacological inactivation of the NAc core attenuates cue-induced reinstatement of food seeking behaviour (Floresco et al., 2008) and context-induced reinstatement of sucrose seeking is associated with c-Fos induction in the NAc shell (Hamlin et al., 2006). A better understanding of the cellular mechanisms that mediate drug and palatable food craving in the HPC and NAc can represent an important advance for the development of anti-relapse pharmacotherapies.

Pavlovian learning mechanisms seem to be critically involved in both drug of abuse and food craving. The formation of long-term memories and stimulus-cued retrieval of memories are thought to be mediated by synaptic plasticity processes (Berke and Hyman, 2000). A recent study has demonstrated that plasticity changes at synaptic level in the NAc represent

an important neurobiological substrate for cocaine craving (Conrad et al., 2008). It will be therefore interesting to know whether similar synaptic plasticity changes in reward related brain areas, such as the NAc and HPC, could mediate craving to different classes of drugs of abuse and natural rewards, such as palatable food; which may account for the common relapsing feature that characterizes drug addiction and some eating disorders.

The CPP paradigm is a useful animal model to study drug or food-related memories, in which few conditioning sessions elicit potent drug or food-related contextual associations that seem to be long-lasting. In addition, CPP behaviour is considered a motivated approach to a drug- or food paired environment with the purpose to re-experience a pleasurable feeling (Tzschentke, 2007). Therefore, the CPP paradigm in mice may represent an interesting behavioural model to study the molecular underpinnings of drug and food craving.

In our experiments, we used an adapted version of the classic CPP model in mice to evaluate the synaptic mechanisms that underline the retrieval of drug and palatable food reward-related memories. For this purpose, we performed a quantitative protein and phospho-protein profiling in synaptoneurosomes purified from the HPC and NAC of mice re-exposed to the drug or palatable food conditioned compartment. This approach intend to model drug and palatable food craving-like situations.

Methods

Animals

Male CD1 mice aged of 8 weeks at the beginning of the experiment were used. Mice were housed four per cage in a temperature-controlled room (21 ± 1 °C) with a 12 h light : dark cycle (light on between 8:00 and 20:00h). Food (i.e. regular chow) and water were available ad libitum, excepted when indicated. All mice were acclimated to handling and received subcutaneous and intraperitoneal saline injection for 1 week before experiments. All animal procedures met the guidelines of the European Communities directive 86/609/EEC regulating animal research and were approved by the Local Ethical Committee.

Chemicals and injection procedure

Morphine and cocaine were provided by the Ministerio de Sanidad y Consumo (Spain). Both drugs were dissolved in saline (0.9%). In the CPP paradigm, morphine was administered subcutaneously (s.c.) at the dose of 5 mg/kg, and cocaine was administered intraperitoneally (i.p.) at the dose of 20 mg/kg.

Palatable food preparation

The palatable food used for the CPP paradigm was manufactured as previously described (Heyne et al. 2009). It is composed by a mix of equal amounts of Bounty®, Snickers®, Mars® and Milka® chocolate prepared as homogenous food pellets (65% energy from carbohydrates, 6.5% from protein and 23% from fat with in total 4.846 kcal/g).

Drug conditioned place preference

The rewarding effects of cocaine and morphine were evaluated by using the CPP paradigm as previously reported (Martin et al, 2000b; Valjent et al, 2006b). The place preference apparatus consisted of two different compartments (15x15x15cm) separated by a central neutral area. The place preference protocol was performed on four different phases:

Preconditioning phase (day1): mice were placed in the middle of the neutral area and allowed to explore both compartments. The time spent in each compartment was measured during 18 min in order to pair one compartment to drug administration and the other one to saline during the conditioning phase. The treatments were counterbalanced between compartments to use an unbiased procedure.

Conditioning phase (days 2 to 7): animals were treated for 6 consecutive days with alternate injections of drug (morphine 5 mg/kg, s.c. or cocaine 20 mg/kg, i.p.) or saline. Mice were confined in the corresponding compartment immediately after injection by using guillotine doors matching walls for 20 min. Drugs were administered on days 1, 3 and 5, and vehicle on days 2, 4 and 6. Control animals received vehicle every day.

First testing phase (day 8, Test 1): this phase was conducted exactly as the preconditioning phase, i.e. free access to both compartments. The time spent in each compartment was measured for 18 min. A score value was calculated for each mouse as the difference between time spent in the drug-paired compartment during the post-conditioning and pre-conditioning phases.

Second testing phase (day 10, Test 2): the analysis of the individual scores on the first CPP test (Test 1) revealed an important heterogeneity in the performance of mice conditioned to cocaine, morphine or palatable food (see results). This variability may interfere with our proteomic analysis. Therefore, we distinguished in each experimental group two different sub-populations according to a median division: the good performers and the bad performers. The good performers group contained animals with CPP scores above the median of the whole group, while the bad performers contained all the animals with scores equal or below the median. This segregation allows to differentiate mice that have acquired the CPP task (good performers) from those that did not (bad performers). Good and bad performers groups were divided in two homogenous sub-groups according to the CPP score on Test 1, and assigned to different experimental conditions in the next step. Indeed, mice were assigned to each sub-group maintaining a similar mean CPP score on Test 1 in both sub-groups. After one day of no disturbance (day 9), good and bad performers sub-groups were assigned to a second CPP test (Test 2) or a "re-exposure" session at day 10. "Re-exposure" sessions consisted to confine (as the conditioning phase) mice to the drug-associated compartment during 20 min and were used as a model of craving-like situations, while Test 2 conditions were strictly similar to those of Test 1 and were performed to evaluate the maintenance or not of drug conditioned responses at this time point. Immediately after both conditions ("re-exposure" or Test 2), mice were sacrificed, and the HPC and NAc were extracted. HPC and NAc from mice that underwent the "re-exposure" session were processed to isolate synaptoneurosomes. HPC and NAc of mice evaluated on the CPP Test 2

were immediately frozen for latter analysis.

Palatable food conditioned place preference procedure

The rewarding effects of palatable food were evaluated by using a CPP protocol adapted from a previous study (Perello et al, 2009). Briefly, one week before starting the CPP experiment, mice were placed under a restricted access to laboratory chow to maintain their body weights to 90% of the average body weights of the ad libitum fed mice. Caloric-restricted mice were provided ad libitum access to laboratory chow in their home cages between 12:00 PM and 4:00 PM, and extra laboratory chow pellets were eventually given at the end of the day (from 8:00 PM) to adjust weight loss. This food regimen was maintained until the end of the conditioning phase. After conditioning, mice were given full ad libitum access to laboratory chow. Additionally, during the week that preceded the start of the CPP experiment mice were exposed in their home cage to the palatable diet (1 g per animals/day) to avoid neophobia during the conditioning. Food CPP experiments were started after the initial adjustment week and were performed just before the 4 h period of food availability. The palatable food CPP experiment was performed exactly as the CPP with drugs, expected during the conditioning phase. Indeed, mice were conditioned during 12 days. On even days, mice were confined to one conditioning chamber in the presence of 2 g of palatable food pellet for 30 min; while on odd days, mice were confined to the other conditioning chamber in the absence of food for 30 min. Test 1 was conducted at day 14, and Test 2 and re-exposure session (20min) at day 16; all performed in mice fed ad libitum since day 13, after last the conditioning session. Similarly to the drug CPP study, mice conditioned to palatable food were

divided in good and bad performers according to a median division (see drug conditioned place preference methods). Subsequently, good and bad performers were further divided in two homogenized sub-groups that present a similar mean of CPP scores on Test 1. Sub-groups of each sub-population were assigned either to the "re-exposure" session or Test 2. Immediately after both conditions ("re-exposure" or Test 2), mice were sacrificed, and the HPC and NAc were extracted. HPC and NAc from mice that underwent the "re-exposure" session were processed to isolate synaptoneurosomes. HPC and NAc of mice evaluated on the CPP Test 2 were immediately frozen for latter analysis.

Statistical analysis

CPP scores were analysed by using a one-way ANOVA test between groups (cocaine, morphine, palatable food and control group). Post hoc comparisons following significant main effects of treatment by one-way ANOVA were performed by using the Fischer F-test. Statistical tests were performed with Statistica 6.0© (StatSoft, Tulsa, OK, USA).

Purification of synaptoneurosomes

Synaptoneurosomes from the HPC and the NAc of mice conditioned to cocaine, morphine, palatable food or saline and assigned to the "re-exposure" conditions were purificated as previously reported (Villasana et al, 2006). Briefly, immediately after the re-exposure session, brains were removed and HPC and NAc were isolated and homogenized, at 4 °C, in 30 volumes of synaptoneurosome buffer (10 mM HEPES, 1 mM EDTA, 2 mM EGTA, 0.5 mM DTT, 10 µg/ml leupeptin, and 50 µg/ml soybean trypsin inhibitor, pH 7.0). From this step, the homogenates were kept ice-cold at all

times to minimize proteolysis throughout the isolation procedure. The homogenates were then further diluted with the same volume of synaptoneurosome buffer and briefly and gently sonicated. The samples were loaded into a 60 ml syringe and filtered twice through three layers of a pre-wetted 100 µm pore nylon filter held in a 13 mm diameter filter holder. The resulting filtrates were then loaded into a 5 ml syringe and filtered through a pre-wetted 5 µm pore hydrophilic filter held in a 13 mm diameter filter holder. The resulting filtrates were centrifuged at 1000 × *g* for 10 min. The pellets obtained corresponded to the synaptoneurosome fraction. Isolated synaptoneurosomes were resuspended with the synaptoneurosome buffer. Resuspended synaptoneurosome fractions extracted from 4 animals of the same experimental group and sub-groups (i.e. good or bad performers) were pooled to ensure sufficient protein concentration for the proteomic analysis.

Proteomic analysis

Sample preparation: Samples were resuspended in 1 ml of resuspension buffer (4% SDS, 0.1M DTT) and lysed by heating at 95°C for 3 min and sonication. Lysates were centrifuged (4ºC, 10 min, 16.000 *g*), supernatants were collected, and protein content was determined using the BCA Protein Quantification Kit (Thermo Fisher Scientific). Samples were reduced with dithiothreitol (10 µM, 30min, 56°C), alkylated in the dark with iodoacetamide (5 mM, 30 min, 25 ºC) and digested with 2 mg LysC (Wako, cat # 129-02541) overnight at 37ºC and then with 2 mg of trypsin (Promega, cat # V5113) for 8 h at 37˚C following Wiśniewski fasp procedure (Wiśniewski et al, 2009). After digestion, peptides were eluted

and cleaned up on a homemade Empore C18 column (3M, St. Paul, MN, USA) (Rappsilber et al, 2007).

Samples were analyzed using a LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) coupled to an EasyLC (Thermo Fisher Scientific (Proxeon), Odense, Denmark). Peptides were loaded directly onto the analytical column at 1.5-2 µl / min using a wash-volume of 4 to 5 times injection volume and were separated by reversed-phase chromatography using a 12-cm column with an inner diameter of 75 µm, packed with 5 µm C18 particles (Nikkyo Technos Co., Ltd. Japan). Chromatographic gradients started at 97% buffer A and 3% buffer B with a flow rate of 300 nl/min, and gradually increased to 93% buffer A and 7% buffer B in 1 min, and to 65% buffer A / 35% buffer B in 120 min. After each analysis, the column was washed for 10 min with 10% buffer A (0.1% formic acid in water) / 90% buffer B (0.1% formic acid in acetonitrile).

The mass spectrometer was operated in positive ionization mode with nanospray voltage set at 2.2 kV and source temperature at 250 °C. Ultramark 1621 for the Fourier transform mass analyzer was used for external calibration prior the analyses. Moreover, an internal calibration was also performed using the background polysiloxane ion signal at 445.1200 m/z (mass/charge). The instrument was operated in data dependent acquisition mode and full mass spectrometry scans with 1 micro scans at resolution of 60.000 were used over a mass range of m/z 250-2000 with detection in the Orbitrap. Auto gain control was set to $1E^6$, dynamic exclusion (60 seconds) and charge state filtering disqualifying singly charged peptides was activated. In each cycle of DDA analysis, following each survey scan the top ten most intense ions with multiple charged ions above a threshold ion count of 5000 were selected for

fragmentation at normalized collision energy of 35%. Fragment ion spectra produced via collision-induced dissociation were acquired in the Ion Trap, auto gain control was set to $5e^4$, isolation window of 2.0 m/z, activation time of 0.1 ms and maximum injection time of 100 ms was used. All data were acquired with Xcalibur software v2.2.

Data analysis**:** Proteome Discoverer software suite (v1.3.0.339, Thermo Fisher Scientific) and the Mascot search engine (v2.3, Matrix Science (Perkins et al, 1999) were used for peptide identification and quantification. The data were searched against an in-house generated database containing all proteins corresponding to Mouse in the Swissprot database plus the most common contaminants previously described (Bunkenborg et al, 2010). A precursor ion mass tolerance of 7 ppm at the mass spectrometry 1 level was used, and up to three miscleavages for trypsin were allowed. The fragment ion mass tolerance was set to 0.5 Da. Oxidation of methionine, protein acetylation at the N-terminal, phosphorilation at serine, threonine and tyrosine were defined as variable modification; whereas carbamidomethylation on cysteines was set as a fix modification. False discovery rate in peptide identification was evaluated by using a decoy database and it was set to a maximum of 5 %.

All calculations and comparisons were processed using R v2.15 (Gentleman et al, 2004). Median normalization between- and within-sample was used at peptide level. The normalized areas were subsequently log2 transformed. An in-house R script was adopted in order to quantify proteins. This script makes use of Top3 idea that quantify a protein using the three most abundant peptides of the proteins. A distribution of protein abundances of each group vs control was then compared with the relative

distribution of control vs control. For each protein, a Z-score was calculated and a p-value was computed using a two-sided t-test and adjusted (Benjamini and Hochberg, 1995). A value of 0.05 was used as cutoff.

Finally, each phospho-peptide fold-change was compared with the fold-change of the relative protein, and only those that show a change of 1.5 times were taken into consideration for following analysis.

Results

Interindividual variability in sensitivity to rewarding conditioned stimuli

After the conditioning phase, all mice underwent a first CPP test (Test 1) to evaluate their preference for the different compartments of the CPP apparatus. According to this preference, a CPP score (see methods) was calculated for each animal, which permits to assign an affective value to the stimuli experienced during conditioning phase. As previously described (Martin et al, 2000b; Valjent et al, 2006b; Perello et al, 2009), we observed that mice conditioned with cocaine, morphine and palatable food expressed a significant preference for the drug or palatable food associated compartement during the CPP test, when compared to the saline group (Figure 31), indicating that these three stimuli produce rewarding effects in mice.

Although the three experimental cohorts developed significant CPP behaviour, our data indicate the existence of an important interindividual variability in the conditioning responses to cocaine (standard deviation= 179.4), morphine (standard deviation= 152.9) and palatable food (standard

deviation= 163.3) during the Test 1. Indeed, the analysis of individual CPP scores shows a high heterogeneity in the performance of mice on Test 1 in the three experimental groups (Figure 32).

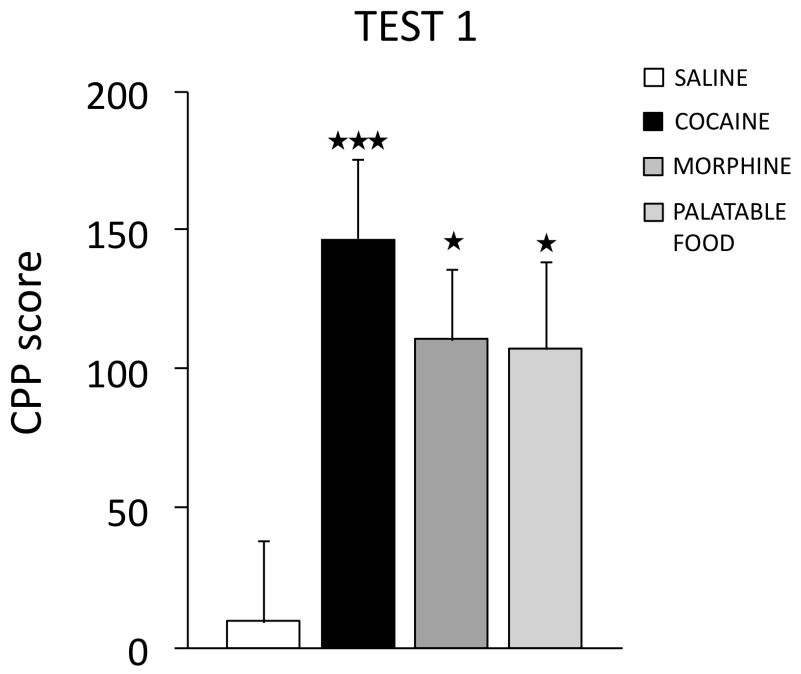


Figure 31: CPP induced by cocaine, morphine and palatable food. Rewarding effects of cocaine (20mg/kg, i.p.), morphine (5mg/kg, s.c.) and palatable food (2g of palatable pellets) evaluated in the CPP paradigm in CD1 mice, the day after the conditioning phase (i.e. TEST1). The vertical axis represents the place preference score, calculated as the time spent in the conditioned compartment on the testing day minus the time spent in the same compartment on the preconditioning day. Data are expressed as mean ± s.e.m. White columns represent saline control animals (n=37), black columns represent cocaine treated animals (n=40), dark grey columns represent morphine treated animals (n=36), and light grey columns represent palatable food conditioned animals (n=27). ★ $P < 0.05$, ★★★ $P < 0.001$ (comparison vs. saline group, Fisher F-test).

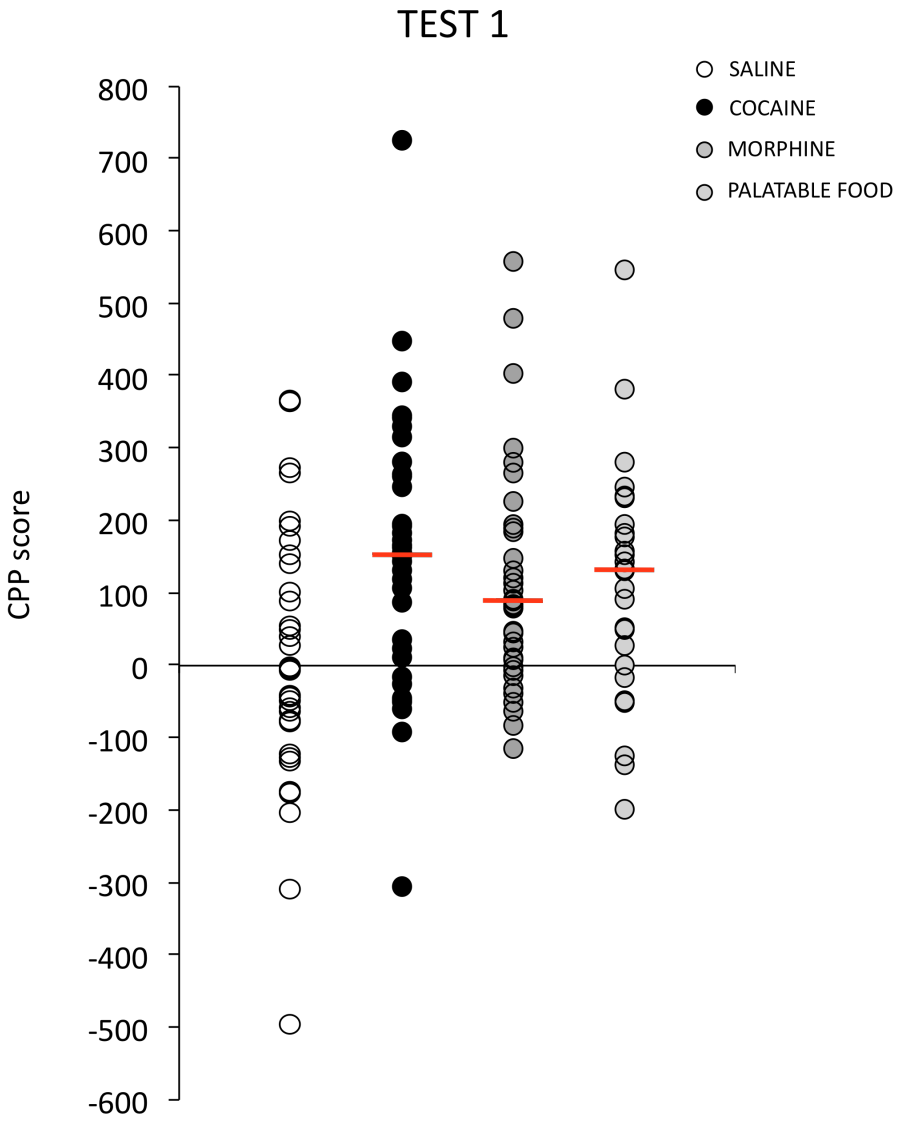


Figure 32: Individual CPP scores analysis reveals an important interindividual heterogeneity of performances on TEST 1. Graphic illustrating individual performances on CPP TEST 1 of mice conditioned with saline (white circles), cocaine (black circles), morphine (dark grey circles) and palatable food (light grey circles). Red lines represent the median value of the performances of the cohort, which has been used to delimitate good (i.e. all animals which CPP score was above the median) and bad (i.e. all animals which CPP score was equal or inferior to the median) performers on TEST 1 in each experimental group.

Therefore, it was important to take into consideration this interindividual variability to accurately design our proteomic analysis. Based on their CPP scores on Test 1, we distinguished good and bad performer mice according to a median division (see methods) (Figure 32). This segregation may allow to differentiate mice that have established strong rewarding stimuli/conditional cues associations (good performers) from those that were presumably less sensitive to the rewarding effects of cocaine, morphine or palatable food and did not acquire the CPP task (bad performers). Thus, good and bad performers of each experimental condition were divided in two homogenous sub-groups to perform either a "re-exposition" session or a second CPP test (Test 2) at day 10 (see methods). Subsequently, we performed our proteomic analysis in synaptoneurosomes extracted from good performer sub-groups that have been re-exposed to the drug or palatable food conditioned compartment in order to accurately identify the synaptic mechanisms mediating drug and palatable food reward memories. Synaptoneurosomes extracted from bad performer sub-groups re-exposed to these same stimuli were frozen, and will be use as negative control group for future validation of the protein candidates. Finally, the second CPP test conducted in good performer sub-groups permitted to ensure that this sub-population still displayed significant CPP behaviour at this time point.

The analysis of our behavioural data dissociated in good and bad performers revealed that only the good performer sub-population expressed a strong CPP behaviour to cocaine, morphine and palatable food during the Test 1, which is maintained two days later in the Test 2 (Figure 33A and B). In contrast, bad performers did not develop any significant preference to the cocaine, morphine or palatable food conditioned

compartment at both time points (Figure 33C and D). These results support the hypothesis that our CPP paradigm conditions could permit to distinguish interindividual variability in mice sensitivity to rewarding stimuli and their propensity to elaborate long-lasting conditioned memories. Finally, the maintenance of cocaine-, morphine- or palatable food-induced CPP in good performers during the Test 2 demonstrates that "re-exposure" sessions with similar performers would trigger the retrieval of drug or palatable food reward memories, which support the use of these sub-populations for the proteomic analysis.

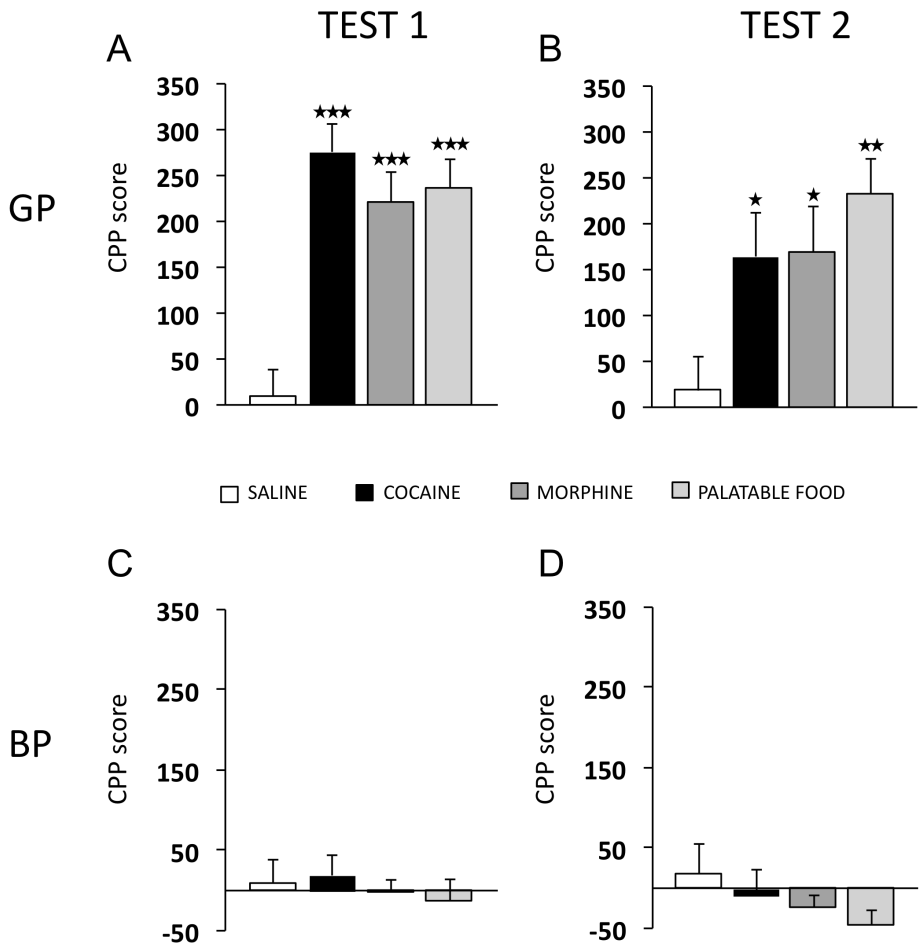


Figure 33: Cocaine, morphine and palatable food promote the establishment of durable CPP behaviour in good performers, but not in bad performers. (**A**) and (**B**) CPP scores display by good performers (GP) conditioned to cocaine (black columns; TEST1, n=20; TEST2, n=12), morphine (dark grey columns; TEST1, n=18; TEST2, n=10) and palatable food (light grey columns; TEST1, n=13; TEST2, n=9), during the TEST 1 (**A**) and the TEST 2 (**B**). (**C**) and (**D**) CPP scores display by bad performers (BP) conditioned to cocaine (black columns; TEST1, n=20; TEST2, n=12), morphine (dark grey columns; TEST1, n=18; TEST2, n=10) and palatable food (light grey columns; TEST1, n=14; TEST2, n=10), during the TEST 1 (**C**) and the TEST 2 (**D**). Data are expressed as mean ± s.e.m. ★ $P < 0.05$, ★★ $P < 0.01$, ★★★ $P < 0.001$ (comparison vs. saline group, Fisher F-test).

Characterisation of the synaptic molecular processes underlying the retrieval of drug and palatable food reward-related memories

Synaptic plasticity has emerged as an important mechanism in the neuroadaptations believed to underlie drug craving and relapse (Kauer and Malenka, 2007; Bowers et al., 2010). Therefore, we restricted our screen to protein and phospho-protein changes within a synaptoneurosome fraction that contains both the pre- and post-synaptic compartments.

We detected approximately 3.000 different proteins throughout the distinct synaptoneurosomal samples of both studied cerebral areas. Our statistical analysis permitted to isolate between 80 and 208 proteins in each experimental condition and brain area, which expression level was significantly different from the one measured in the saline control group; as well as 30 to 59 proteins differently phosphorylated in each experimental condition and brain area, when compared to the saline control group (Table 4).

	HPC		NAc	
Total number of detected protein	2973		3202	
Versus Saline	Protein	Phospho-protein	Protein	Phospho-protein
COCAINE	81	37	73	48
MORPHINE	80	39	118	59
PALATABLE FOOD	81	30	208	42

Table 4: Table showing the total number of peptides detected in our synaptoneurosomes samples, as well as the total number of proteins that were significantly more or less expressed (i.e. protein) or phosphorylated (i.e. phospho-protein) in mice re-exposed to cocaine, morphine and palatable food conditioned compartement compared to the saline control group.

From the protein candidate list of each experimental group, we conducted a bioinformatic analysis using the Ingenuity system software (Mountain View, CA; http://www.ingenuity.com) to uncover the major biological functions and signalling pathways involved in the retrieval of reward-related memories triggered by cocaine, morphine and palatable food conditioned cues. The biological functions and signalling pathways that were commonly regulated in our three experimental conditions are represented in tables 5 and 6. Thus, re-exposure to cocaine, morphine and palatable food conditioned contextual cues commonly modulate the expression level and/or phosphorylation state of proteins described to be involved in contextual conditioning, emotional behaviour, neurotransmission, synaptic plasticity or neuronal structure (axonal and dendritic growth, microtubule dynamics and polymerisation of filaments). In addition, the retrieval of drug and palatable food reward memories rely on the activity of well-known signalling pathways involved in reward processing, synaptic plasticity and addictive disorders, such as the cAMP response element-binding protein (CREB) and the protein kinase A (PKA) signalling cascades (Beninger et al, 1998; Waltereit et al, 2003; Benito et al, 2010; Carlezon et al, 2005). On the other hand, we observed an involvement of the P2Y purigenic receptor and α-adrenergic signaling pathways, both known to participate in the modulation of neurotransmission in the CNS (Bücheler et al, 2002; Burnstock, 2006), as well as the Rho family GTPases and RhoGDI signalling that are closely related to the reorganisation of the actin cytoskeleton (Ingenuity target explorer, http:// www.targetexplorer.ingenuity.com). Finally, this bioinformatic analysis also suggests the involvement of the semaphorin signalling pathway that has been described as an important regulator of

cell morphology, axon guidance and maturation of dendritic spines (Pasterkamp et al, 2009).

HPC :

	COCAINE		MORPHINE		PALATABLE FOOD	
	# Molecules	p-Value	# Molecules	p-Value	# Molecules	p-Value
behavior	23	1,05E-07	13	2,00E-02	19	1,38E-05
microtubule dynamics	16	6,60E-06	12	1,99E-03	15	1,68E-05
formation of cellular protrusions	14	4,79E-06	10	2,27E-03	14	2,86E-06
morphology of nervous system	13	8,55E-03	13	1,19E-02	17	7,16E-05
Huntington's Disease	8	7,83E-05	5	1,54E-02	8	5,77E-05
contextual conditioning	6	1,66E-05	3	1,89E-02	4	1,81E-03
growth of axons	5	5,75E-04	4	5,41E-03	6	4,57E-05
plasticity of synapse	3	1,06E-02	3	1,19E-02	3	9,46E-03
synaptic depression	3	2,31E-02	3	2,56E-02	8	1,15E-07
excitatory postsynaptic potential	6	6,14E-05	3	3,43E-02	7	3,98E-06
assembly of intercellular junctions	4	9,31E-03	5	1,68E-03	7	1,37E-05

NAc :

	COCAINE		MORPHINE		PALATABLE FOOD	
	# Molecules	p-Value	# Molecules	p-Value	# Molecules	p-Value
behavior	22	1,15E-06	21	8,17E-04	37	1,47E-07
microtubule dynamics	19	1,29E-07	24	2,99E-08	36	7,29E-12
morphology of nervous system	17	2,35E-04	23	4,24E-05	28	2,37E-04
formation of cellular protrusions	16	3,19E-07	21	1,84E-08	33	2,78E-13
conditioning	11	6,29E-08	10	1,47E-05	15	8,75E-08
growth of axons	7	6,94E-06	8	7,39E-06	10	2,38E-06
long-term potentiation	7	9,19E-04	10	7,07E-05	18	4,40E-09
polymerization of filaments	6	7,80E-07	4	1,11E-03	6	5,54E-05
emotional behavior	5	2,02E-02	7	6,84E-03	11	4,68E-04
exocytosis	4	1,33E-03	8	3,56E-07	7	6,28E-05
synaptic depression	3	2,63E-02	8	3,38E-06	11	9,49E-08
neurotransmission	9	1,70E-04	13	5,03E-06	17	1,04E-06
contextual conditioning	7	1,57E-06	5	1,28E-03	8	2,53E-05
excitatory postsynaptic potential	5	7,59E-04	6	5,41E-04	6	4,02E-03
long-term potentiation of synapse	5	3,08E-03	9	1,09E-05	10	4,32E-05
dendritic growth/branching	4	1,65E-03	5	7,50E-04	10	9,66E-08
endocytosis	4	7,79E-03	4	2,49E-02	5	2,44E-02

Table 5: Principal biological functions mediated by the proteins that are differently expressed and/or phosphorylated in mice re-exposed to cocaine, morphine or palatable food conditioned CPP compartement, compared to the saline control group. Analysis performed with the total list of proteins differentially expressed and/or phosphorylated compared to the saline control group for each experimental conditions, through the Ingenuity system software (Mountain View, CA; http://www.ingenuity.com).

HPC :

	COCAINE		MORPHINE		PALATABLE FOOD	
	Ratio	-log(p-value)	Ratio	-log(p-value)	Ratio	-log(p-value)
α-Adrenergic Signaling	4,60E-02	2,64E+00	4,60E-02	2,57E+00	5,75E-02	3,72E+00
G Beta Gamma Signaling	4,17E-02	2,58E+00	4,17E-02	2,52E+00	4,17E-02	2,65E+00
CREB Signaling in Neurons	2,79E-02	2,29E+00	2,79E-02	2,22E+00	3,91E-02	4,03E+00
Androgen Signaling	3,39E-02	2,26E+00	4,24E-02	3,06E+00	5,08E-02	4,22E+00
P2Y Purigenic Receptor Signaling Pathway	3,39E-02	2,12E+00	3,39E-02	2,06E+00	3,39E-02	2,18E+00
Melatonin Signaling	4,55E-02	2,01E+00	4,55E-02	1,96E+00	6,06E-02	3,08E+00
Corticotropin Releasing Hormone Signaling	2,63E-02	1,46E+00	2,63E-02	1,41E+00	3,51E-02	2,31E+00
nNOS Signaling in Neurons	4,35E-02	1,42E+00	6,52E-02	2,40E+00	8,70E-02	3,68E+00
Synaptic Long Term Potentiation	2,59E-02	1,37E+00	2,59E-02	1,33E+00	5,17E-02	4,00E+00

NAc :

	COCAINE		MORPHINE		PALATABLE FOOD	
	Ratio	-log(p-value)	Ratio	-log(p-value)	Ratio	-log(p-value)
Huntington's Disease Signaling	3,20E-02	3,14E+00	2,74E-02	1,72E+00	5,02E-02	3,52E+00
Semaphorin Signaling in Neurons	7,84E-02	3,35E+00	7,84E-02	2,78E+00	1,57E-01	6,13E+00
Protein Kinase A Signaling	2,47E-02	2,99E+00	2,19E-02	1,54E+00	4,11E-02	3,49E+00
Synaptic Long Term Depression	3,55E-02	2,58E+00	2,84E-02	1,34E+00	6,38E-02	3,75E+00
CXCR4 Signaling	3,29E-02	2,42E+00	3,29E-02	1,80E+00	7,89E-02	5,73E+00
Signaling by Rho Family GTPases	2,51E-02	2,29E+00	2,51E-02	1,60E+00	6,28E-02	5,90E+00
CREB Signaling in Neurons	2,79E-02	2,20E+00	3,91E-02	2,86E+00	6,15E-02	4,44E+00
Synaptic Long Term Potentiation	3,45E-02	2,04E+00	4,31E-02	2,22E+00	6,03E-02	2,78E+00
Melatonin Signaling	4,55E-02	1,95E+00	4,55E-02	1,55E+00	6,06E-02	1,79E+00
G Beta Gamma Signaling	3,12E-02	1,64E+00	5,21E-02	2,78E+00	8,33E-02	4,41E+00
RhoGDI Signaling	2,19E-02	1,51E+00	2,73E-02	1,58E+00	5,46E-02	3,70E+00
Androgen Signaling	3,39E-02	2,18E+00	5,08E-02	3,18E+00	6,78E-02	3,76E+00
P2Y Purigenic Receptor Signaling Pathway	3,39E-02	2,04E+00	3,39E-02	1,54E+00	7,63E-02	4,26E+00
α-Adrenergic Signaling	3,45E-02	1,68E+00	4,60E-02	2,02E+00	1,03E-01	5,44E+00
Gαs Signaling	2,75E-02	1,42E+00	4,59E-02	2,40E+00	1,01E-01	6,38E+00

Table 6: Principal signalling pathways implicating the proteins that are differently expressed and/or phosphorylated in mice re-exposed to cocaine, morphine or palatable food conditioned CPP compartement, compared to the saline control group. Analysis performed with the total list of proteins differentially expressed and/or phosphorylated compared to the saline control group for each experimental conditions, through the Ingenuity system software (Mountain View, CA; http://www.ingenuity.com).

Close examination of the protein candidate lists derived from cocaine, morphine and palatable food "re-exposed" groups revealed that few changes in synaptic protein expression and phosphorylation detected in our study were common to the three rewarding conditioned stimuli in both studied brain areas (Figure 34).

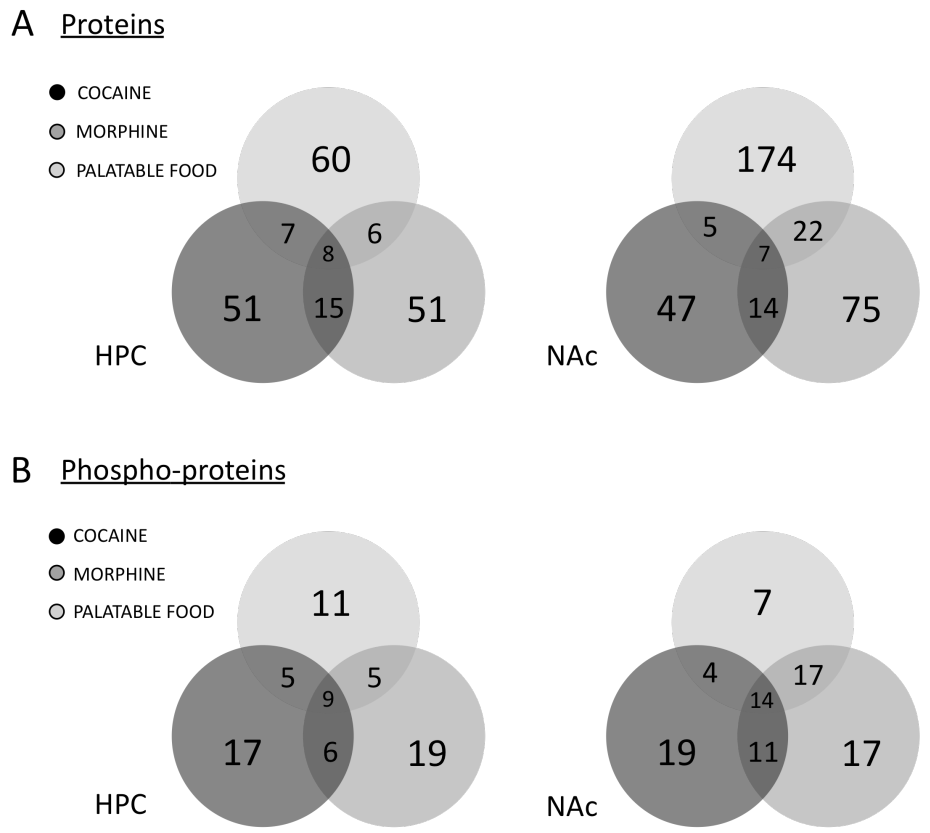


Figure 34: **A small proportion of synaptic proteins changes is common to cocaine, morphine and palatable food re-exposure groups.** (**A**) and (**B**) Venn diagrams illustrating the number of common and specific synaptic proteins changes in the HPC and NAc between mice re-exposed to cocaine, morphine or palatable food conditioned compartement. Comparison of the proteins that are significantly more or less expressed (**A**) or phosphorylated (**B**) compared to the saline group.

Thus, the re-activation of cocaine, morphine and palatable food reward memories may mainly rely on the activity of distinct subset of synaptic molecular mechanism defined by the nature of the unconditional reinforcer. Nevertheless, we had observed that these synaptic mechanisms lead to the activation of several similar signalling pathways and biological processes (table 5 and 6), and these few common proteomic changes triggered by re-exposure to cocaine, morphine and palatable food conditioned cues could represent important synaptic events in which different mechanisms converge to produce a similar cognitive process, such a retrieval of hedonic memories, and a common behavioural response represented by a motivated approach to the conditonned place.

These proteins and phospho-proteins changes common to our three experimental groups are represented in tables 7,8 and 9. Proteins that were differentially regulated by cocaine, morphine and palatable food conditioned cues have not been included in these lists. We therefore performed a bibliographic analysis to identify the major functions attributed to these selected proteins (table 12 and 13). We observed that a majority of proteomic changes common to our three experimental conditions in both brain areas are related to proteins involved in the regulation of the cytoskeleton or neuronal morphology. Thus, we detected changes in the expression of tubulin beta-2A and striatin-4, and in the phosphorylation state of plakophilin-4, MARCKS-related protein in the HPC. In the NAc, these changes include an increased expression of the spectrin alpha chain, and changes in the phosphorylation state of dihydropyrimidinase-related protein 1 and 3, tubulin polymerization-promoting protein and alpha-adducin. Another important type of common proteomic change detected in our study concerns protein associated to

mitochondrial functions, such as the 6.8 kDa mitochondrial proteolipid, reticulon-4-interacting protein 1, inorganic pyrophosphatase 2, isoform 2 of nitrilase homolog 1, calcium-binding mitochondrial carrier protein SCaMC-3 in the HPC, and cytochrome b-c1 complex subunit 10 and mitochondrial import receptor subunit TOM22 homolog in the NAc. Finally, several proteins implicated in neurotransmission were similarly regulated by re-exposure to drug of abuse and palatable food conditioned cues. Hence, we observed a decreased expression of the isoform 5 of calcium-dependent secretion activator 2 (CASP-2) in the HPC, and a decreased expression of Rab3 GTPase-activating protein non-catalytic subunit and metabotropic glutamate receptor 7 (mGluR 7) in the NAc, as well as changes in the phosphorylation state of syntaxin-1B in this same brain area.

On the other hand, we intended to identify the protein kinases and phosphatases that mediate the phosphorylation changes observed in our study (table 12 and 13). Few evidences have linked kinases or phosphatases activity with the specific amino acid residues in which phosphorylation changes were detected in our experiment. Nevertheless, it has been reported that phosphorylation of MARCKS-related protein at threonine 148, detected in the HPC, is dependent on c-jun N-terminal protein kinase (JNK) (Björkblom et al, 2012). In addition, two phosphorylation changes detected in the NAc occur at phosphorylation sites that are specific substrates of PKA: serine 112 in cAMP-dependent protein kinase type II-beta regulatory subunit (Budillon et al, 1995) and serine 31 in tubulin polymerization-promoting protein (Hlavanda E, 2007). In addition, changes in the activity of cyclin dependent kinase 5 (cdk5) and caseine kinase 2 (CK2) is suggested in this brain area by changes in phosphorylation state of dihydropyrimidinase-related protein 1 at

threonine 509 and prostaglandin E synthase 3 at serine 113, respectively (Yamashita et al., 2007; Kobayashi et al., 2004). These observations suggest that JNK in the HPC and PKA, cdk5 and CK2 in the NAc may contribute to the retrieval/reconsolidation of drug and palatable food reward-related memories.

Finally, our analysis also highlights several proteomic changes that were specifically triggered by re-exposure to cocaine and morphine condtionned cues, but not by palatable food related cues (tables 10 and 11). In table 14 and 15 are summarized the main functions attributed to these proteins. These proteomic changes include modifications of the expression level or phosphorylation states of proteins involved in the regulation of cytoskeletal dynamics and neuronal morphology, such as formin 2, beta-actin-like protein 2, microtuble associated protein 2 and spectrin beta chain 1 in the HPC, as well as stathmin, microtuble associated protein 2 and neuronal membrane glycoprotein M6-a in the NAc. In addition, re-exposure to cocaine and morphine condtionned cues alters the expression level or phosphorylation state of mitochondrial proteins including oxidation resistance protein 1 in the HPC and hydroxyacyl-coenzyme A dehydrogenase in the NAc, as well as proteins of the endoplasmic reticulum involved in protein trafficking, such as the protein transport protein Sec31A and protein transport protein Sec61 subunit beta in the NAc. Finally, our analysis indicates that the retrieval of cocaine and morphine reward memories commonly rely on glutamatergic and dopaminergic signaling in the HPC and NAc, respectively. Indeed, we detected a decreased expression of the isoform Glt-1B of excitatory amino acid transporter 2 and a change in the phosphorylation state of proline-rich transmembrane protein 2 in the HPC, while we observed a decreased phosphorylation of

the dopamine- and cAMP-regulated neuronal phosphoprotein (DARPP-32) at serine 10 in the NAc.

In summary, our experiment provides some new insight in the characterization of a hypothetical common neurobiological mechanism mediating the retrieval of drug and palatable food reward memories. In addition, our results highlight several synaptic proteins that were differentially regulated by re-exposure to drug and palatable conditonned cues. Our study may help future work to define the neurobiological substrates of drug and palatable food craving and to better define the similarities and differences between drug addiction and some eating disorders.

HPC

	PROTEIN NAMES	NUMBER OF PEPTIDES	MEAN DETECTED VALUE				p-value vs. saline		
			SALINE	COCAINE	MORPHINE	PF	COCAINE	MORPHINE	PF
Down-regulated	**Peroxisomal acyl-coenzyme A oxidase 1**	6	28,552	23,338	19,796	24,221	1,58E-10	0,00E+00	1,07E-07
	Isoform 2 of Protein FAM114A2	2	24,366	22,029	21,313	22,687	4,11E-03	1,78E-04	3,91E-02
	6.8 kDa mitochondrial proteolipid	2	27,821	26,207	25,326	25,794	4,72E-02	2,18E-03	1,28E-02
	Isoform 2 of Striatin-4	7	23,255	21,910	21,517	21,529	9,82E-02	3,28E-02	3,39E-02
	Isoform 2 of ELKS/Rab6-interacting/CAST family member 1	5	23,398	22,025	21,790	21,761	9,11E-02	4,80E-02	4,41E-02
	Isoform 5 of Calcium-dependent secretion activator 2	3	24,758	22,094	23,376	22,983	1,07E-03	8,91E-02	2,91E-02
	Reticulon-4-interacting protein 1, mitochondrial	2	23,742	22,333	22,300	21,944	8,30E-02	7,62E-02	2,71E-02
Up-regulated	**COP9 signalosome complex subunit 2**	8	21,400	23,650	24,254	23,513	5,89E-03	4,75E-04	9,70E-03
	Inorganic pyrophosphatase 2, mitochondrial	6	22,701	24,628	24,621	24,514	1,84E-02	1,88E-02	2,66E-02
	Nucleoside diphosphate kinase 3	3	20,369	22,509	22,464	22,915	8,81E-03	1,03E-02	1,83E-03
	ELAV-like protein 1	3	18,192	21,192	22,639	20,731	2,41E-04	5,13E-08	1,89E-03
	Isoform 2 of Nitrilase homolog 1	3	20,391	24,706	22,816	22,372	1,26E-07	3,00E-03	1,54E-02
	Calcium-binding mitochondrial carrier protein SCaMC-3	7	20,966	22,797	23,535	22,439	2,51E-02	1,66E-03	7,17E-02
	Tubulin beta-2A chain	2	27,808	30,618	29,178	29,983	5,82E-04	9,42E-02	7,79E-03

Table 7: Proteins commonly down- and up-regulated by re-exposure to cocaine, morphine and palatable food conditioned contextual cues, in the HPC. Dark red and green filled boxes highlight significant protein expression changes (p-value< 0,05). Light red and green filled boxes highlight protein expression changes nearly significant (p-value< 0,1). PF: palatable food.

NAc

	PROTEIN NAMES	NUMBER OF PEPTIDES	MEAN DETECTED VALUE				p-value vs. saline		
			SALINE	COCAINE	MORPHINE	PF	COCAINE	MORPHINE	PF
Down-regulated	**Rab3 GTPase-activating protein non-catalytic subunit**	5	22,186	18,600	18,714	20,614	1,98E-05	3,49E-05	4,50E-02
	Metabotropic glutamate receptor 7	3	25,787	21,550	22,642	22,537	5,79E-07	1,62E-04	1,00E-04
	Protein C17orf37 homolog	2	23,491	21,623	21,703	21,088	1,94E-02	2,46E-02	3,30E-03
	E3 ubiquitin-protein ligase RNF181	2	27,901	26,322	26,269	26,129	4,41E-02	3,83E-02	2,58E-02
	Aminopeptidase B	10	23,738	21,585	21,824	22,228	7,86E-03	1,69E-02	5,29E-02
Up-regulated	**Peroxisomal acyl-coenzyme A oxidase 1**	4	19,292	21,429	21,261	21,395	3,06E-02	4,85E-02	3,37E-02
	Secretory carrier-associated membrane protein 5	2	22,740	25,865	24,934	25,720	1,06E-03	2,60E-02	1,87E-03
	Spectrin alpha chain, brain	2	22,689	25,209	25,141	24,413	9,52E-03	1,19E-02	8,94E-02

Table 8: Proteins commonly down- and up-regulated by re-exposure to cocaine, morphine and palatable food conditioned contextual cues, in the NAc. Dark red and green filled boxes highlight significant protein expression changes (p-value< 0,05). Light red and green filled boxes highlight protein expression changes nearly significant (p-value< 0,1). PF: palatable food.

HPC

			normalized fold change vs. saline		
	Protein name	Phosphorylation sites	Cocaine	Morphine	PF
Less phosphorylated	Isoform 2 of Plakophilin-4	S775	1,652	0,790	0,596
	MARCKS-related protein	T148	0,735	0,548	1,014
More phosphorylated	Hepatocyte cell adhesion molecule	T378 and S386	1,280	1,012	1,045
	Secretogranin-2	S270	0,524	1,165	1,579

NAC

			normalized fold change vs. saline		
	Protein name	Phosphorylation sites	Cocaine	Morphine	PF
Less phosphorylated	Mitochondrial import receptor subunit TOM22 homolog	S 15	0,996	1,562	0,558
	Dihydropyrimidinase-related protein 1	M498 (oxydation) T509	0,809	1,503	0,996
	Dihydropyrimidinase-related protein 3	M498 (oxydation) T509	0,754	0,862	0,912
	cAMP-dependent protein kinase type II-beta regulatory subunit	S112 and C114 (carbamidomethyl)	0,640	1,158	2,867
	Tubulin polymerization-promoting protein	S31	0,539	1,346	3,573
	Prostaglandin E synthase 3	S113	1,104	2,276	0,569
	Syntaxin-1B	S14	0,665	1,384	5,172
More phosphorylated	Alpha-adducin	S355 and T358	0,757	0,871	0,828

Table 9: Proteins commonly more or less phosphorylated by re-exposure to cocaine, morphine and palatable food conditioned contextual cues, in the HPC and NAc.. PF: palatable food.

HPC

	PROTEIN NAMES	NUMBER OF PEPTIDES	MEAN DETECTED VALUE				p-value vs. saline		
			SALINE	COCAINE	MORPHINE	PF	COCAINE	MORPHINE	PF
Down-regulated	**Multifunctional protein ADE2**	9	23,149	21,458	20,902	23,678	3,77E-02	5,78E-03	5,20E-01
	Exportin-1	9	24,108	21,809	22,204	24,031	4,75E-03	1,94E-02	9,21E-01
	Proton myo-inositol cotransporter	5	23,441	20,683	18,697	23,188	7,08E-04	5,85E-09	7,53E-01
	Transportin-3	4	22,593	19,780	20,922	22,474	5,51E-04	3,99E-02	8,79E-01
	Formin-2	3	21,228	19,252	19,099	22,142	1,52E-02	8,93E-03	2,65E-01
	Surfeit locus protein 4	3	24,496	21,840	21,222	23,227	1,11E-03	5,84E-05	1,19E-01
	Protein FAM136A	2	22,777	20,898	20,835	23,037	2,10E-02	1,70E-02	7,54E-01
	Isoform Glt-1B of Excitatory amino acid transporter 2	1	23,225	20,666	21,202	23,957	1,67E-03	1,29E-02	3,72E-01
	UTP--glucose-1-phosphate uridylyltransferase	1	23,228	21,585	19,533	23,250	4,35E-02	5,76E-06	9,83E-01
Up-regulated	**Carnitine O-acetyltransferase**	5	20,513	23,231	22,267	19,781	8,77E-04	3,19E-02	3,66E-01
	Beta-actin-like protein 2	2	22,982	25,075	24,985	24,115	1,05E-02	1,43E-02	1,67E-01

NAc

	PROTEIN NAMES	NUMBER OF PEPTIDES	MEAN DETECTED VALUE				p-value vs. saline		
			SALINE	COCAINE	MORPHINE	PF	COCAINE	MORPHINE	PF
Down-regulated	**Protein transport protein Sec31A**	7	23,428	20,959	21,889	23,039	2,60E-03	4,92E-02	5,00E-01
	Emerin	6	22,936	20,594	20,964	22,335	4,11E-03	1,41E-02	3,61E-01
	Cell cycle exit and neuronal differentiation protein 1	3	25,018	22,598	23,225	25,472	3,10E-03	2,43E-02	7,85E-01
	Protein transport protein Sec61 subunit beta	3	23,085	21,219	21,465	22,439	1,96E-02	3,95E-02	3,35E-01

Table 10: Proteins specifically down- and up-regulated by re-exposure to cocaine and morphine conditioned contextual cues, but not palatable food related ones, in the HPC and NAc. PF: palatable food

HPC

	Protein name	Phosphorylation sites	normalized fold change vs. saline		
			Cocaine	Morphine	PF
Less phosphorylated	SH3-containing GRB2-like protein 3-interacting protein 1	S287	1,580	2,538	N.S.
	Tumor protein p63-regulated gene 1-like protein	T34	0,917	1,068	N.S.
	Oxidation resistance protein 1	S346	0,669	1,249	N.S.
	Proline-rich transmembrane protein 2	S244 and S250	0,620	0,537	N.S.
More phosphorylated	Microtubule-associated protein 2	S1612	1,039	1,479	N.S.
	Spectrin beta chain, brain 1	S2102	0,700	1,965	N.S.

NAc

	Protein name	Phosphorylation sites	normalized fold change vs. saline		
			Cocaine	Morphine	PF
Less phosphorylated	Isoform 2 of Protein phosphatase 1 regulatory subunit 1B	S10	1,291	1,428	N.S.
	Stathmin	S38	1,148	0,971	N.S.
	Neuronal membrane glycoprotein M6-a	S267	0,946	2,441	N.S.
	Heat shock protein HSP 90-alpha	S263	0,897	1,208	N.S.
	14-3-3 protein zeta/delta	S207	0,577	0,747	N.S.
	Microtubule-associated protein 2	T1160	0,573	1,413	N.S.
More phosphorylated	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial	S11	1,261	0,614	N.S.
	DnaJ homolog subfamily C member 5	S10	0,920	1,512	N.S.

Table 11: Proteins specifically more or less phosphorylated by re-exposure to cocaine and morphine conditioned contextual cues, but not palatable food related ones, in the HPC and NAc. PF: palatable food; N.S.: non-significant (normalized fold changes inferior to 0.5).

Table 12: Principal functions attributed to the proteins which expression or phosphorylation state in the HPC is similarly modulated by re-exposure to cocaine, morphine and palatable food conditioned CPP compartement, when compared to the saline control group.

Peroxisomal acyl-coenzyme A oxidase 1:
Subcellular location: peroxisome.
Function: metabolic function: lipid metabolism, peroxisomal fatty acid beta-oxidation. The first enzyme of the fatty acid beta-oxidation pathway, which catalyzes the desaturation of acyl-CoAs to 2-trans-enoyl-CoAs. Acts on prostagladin (Farioli-Vecchioli et al., 2001).
Isoform 2 of Protein FAM114A2:
Subcellular location: no data.
Function: no data.
6.8 kDa mitochondrial proteolipid:
Subcellular location: mitochondria.
Function: component of an ATP synthase complex.
Isoform 2 of Striatin-4:
Subcellular location: cytoplasm and membranes, enriched in dendritic spines.
Function: binds calmodulin in a calcium dependent manner. May function as scaffolding or signalling protein. Participates in estrogen receptor non-genomic cellular signaling (Lu et al., 2004). Interacts with caveolin 1, a protein involved in endocytosis and nitric oxide signalling (Gaillard et al., 2001). Regulates dendritic growth (Bartoli et al., 1999). Regulates protein phosphatases 2A activity (Gordon et al., 2011). Participates in actin organization (Breitman et al., 2008).
Isoform 2 of ELKS/Rab6-interacting/CAST family member 1:
Subcellular location: Presynaptic membrane. Recruited on Golgi membrane by RAB6A in a GTP-dependent manner.
Function: regulatory subunit of the IKK complex, a regulator of NF-κB pathway (Ducut Sigala et al., 2004). May be involved in the organization of the cytomatrix at the nerve terminals active zone, which regulates neurotransmitter release (Wagh et al., 2006). May be involved in Rab-6 regulated endosomes to Golgi transport (Wang et al., 2002).

Isoform 5 of Calcium-dependent secretion activator 2:
Subcellular location: vesicle and peripheral membrane. Strongly enriched in synaptic fractions. **Function**: calcium-binding protein involved in exocytosis of vesicles filled with neurotransmitters and neuropeptides, regulates fast phasic transmitter release (Jockusch et al., 2007). Regulates neurotrophin release, such as NT3 and BDNF (Sadakata et al., 2007). Interacts with the DA-D2R, and regulation monoamine uptake and storage (Binda et al., 2005). Splicing of its gene is associated with autism and lower intelligence quotient (Hattori et al., 2012; Sadakata et al., 2007).
Reticulon 4-interacting protein 1:
Subcellular location: mitochondria. **Function**: interacts with reticulon 4 (NOGO), a inhibitor of neurite outgrowth (Hu et al., 2002)
COP9 signalosome complex subunit 2:
Subcellular location: cytoplasm and nucleus. **Function**: essential component of the COP9 signalosome complex, which is an important regulator of the ubiquitin proteasome system.
Inorganic pyrophosphatase 2, mitochondrial:
Subcellular location: mitochondria. **Function**: involved in lipid metabolism and calcium absorption. Hydrolyses pyrophosphate, an important molecule for intracellular signalling.
Nucleoside diphosphate kinase 3:
Subcellular location: probably located in mitochondria. **Function**: major role in the synthesis of nucleoside triphosphates other than ATP. Nucleoside diphosphate kinases regulate a variety of cellular activities, including proliferation, development, and differentiation (Otero et al., 2000), participates in dynamin-dependent synaptic vesicle recycling (Krishnan et al., 2001).

ELAV-like protein 1:
Subcellular location: cytoplasm and nucleus. **Function**: Increases mRNA stability and/or rate of translation (e.g. GAP-43 or neuromodulin, a protein involved in neuronal growth cones and LTP). Involves in spatial learning (Quattrone et al., 2001). In kidney cells, stabilize mRNA of Gls-l, the major glutamate-synthesizing enzyme in neurons (Ibrahim et al., 2008).
Isoform 2 of Nitrilase homolog 1:
Subcellular location: mitochondria. **Function**: participates in metabolic process of nitrogen compound. Plays a role in cell growth and apoptosis (loss of expression promotes cell growth). Has apparently no omega-amidase activity such as nitrilase homolog 2.
Calcium-binding mitochondrial carrier protein SCaMC-3:
Subcellular location: mitochondria. **Function**: Transmembrane transport. Participates in the increase in oxidative phosphorylation, accumulation of adenine nucleotide and increase calcium retention capacity in liver mitochondria in response to glucagon (Amigo et al., 2013).
Tubulin beta-2A chain:
Subcellular location: cytoplasm. **Function**: tubulin beta forms dimers with tubulin alpha, which regulate microtubules gathering and stability. Its expression can be modulated by 17beta-estradiol, known to improve spatial and non-spatial memory (Pechenino et al., 2009). Increased expression of tubulin beta in chronic cocaine users (Tannu et al., 2007).
Isoform 2 of Plakophilin-4:
Subcellular location: cytoplasm **Phosphorylation site/kinase:** serine 775/not described. **Function**: plays a role as a regulator of Rho activity in cytoskeleton reorganization during cytokinesis (Wolf et al., 2006). Plays a role in stabilizing cadherin-mediated adhesion (Hatzfeld, 2007).

MARCKS-related protein:

Subcellular location: cytoplasm and plasma membrane.

Phosphorylation site/kinase: threonine 148/ c Jun N-terminal protein kinase (Björkblom et al., 2012).

Function: controls cell movement by regulating actin cytoskeleton homeostasis. Involved in coupling the protein kinase C and calmodulin signal transduction systems. Phosphorylation at T148 enables MARCKS-related protein to bundle and stabilize F-actin, increase filopodium numbers and dynamics, and retard migration in neurons. Conversely, when MARCKS-related protein phosphorylation is inhibited, actin mobility increases and filopodium formation is compromised whereas lamellipodium formation is enhanced, as is cell migration (Björkblom et al., 2012).

Hepatocyte cell adhesion molecule:

Subcellular location: cytoplasm and plasma membrane.

Phosphorylation site/kinase: threonine 378 and serine386/ not described.

Function: involved in regulating cell motility and adhesion via its association with actin cytoskeleton (Moh et al., 2009).

Secretogranin-2:

Subcellular location: secretory granule and secreted in the extracellular space.

Phosphorylation site/kinase: serine 270/not described.

Function: a neuroendocrine secretory granule protein, which may be the precursor for other biologically active peptides. Involved in the packaging or sorting of peptide hormones and neuropeptides into secretory vesicles (Entrez Gene: SCG2). Participates in the liberation of neutrophins and interleukin 6, which are involved in synaptic plasticity (Möller et al., 2006).

Table 13: Principal functions attributed to the proteins which expression or phosphorylation state in the NAc is similarly modulated by re-exposure to cocaine, morphine and palatable food conditioned CPP compartement, when compared to the saline control group.

Rab3 GTPase-activating protein non-catalytic subunit:
Subcellular location: cytoplasm, enriched in synaptic fractions. **Function**: regulatory subunit of a GTPase activating protein that has specificity for Rab3 subfamily, proteins involved in exocytosis of neurotransmitters and hormones. May participate in neuronal proliferation, migration and differentiation before synapse formation. Regulates synaptic transmission and plasticity through the inactivation of Rab3 (Sakane et al., 2006), and participates synaptic homeostasis (neurotransmitter receptor abundance, ion channel density, and presynaptic neurotransmitter release)(Müller et al., 2011).
Metabotropic glutamate receptor 7:
Subcellular location: plasma membrane. **Function**: receptor for glutamate. Regulates NMDAR trafficking and function through cofilin/actin signaling (Gu et al 2012). Regulates the expression and extinction of conditioned fear in mice (Morawska et, 2012), reinstatement of ethanol-induced CPP (Bahi 2012a) and metamphetamine seeking behaviours (Schwendt et al., 2012).
Protein C17orf37 homolog:
Subcellular location: cytoplasm. **Function**: plays a role in regulation of apoptosis. Participates in filopodia formation in migrating cells (Dasgupta et al., 2011).
E3 ubiquitin-protein ligase RNF181:
Subcellular location: not well-known. **Function**: involved in protein ubiquitination, a post-translational modification that regulates cellular degradation of proteins, protein trafficking and signaling networks. Interacts with the ERK/MAPK signaling (Wang et al., 2011).

Aminopeptidase B:
Subcellular location: Golgi apparatus, plasma membrane and secreted in the extracellular space. **Function**: Exopeptidase that selectively removes arginine and/or lysine residues from the N-terminus of several peptide substrates including Leu-enkephalin, Met-enkephalin and somatostatin-14.
Peroxisomal acyl-coenzyme A oxidase 1:
Subcellular location: peroxisome. **Function**: see table 10.
Secretory carrier-associated membrane protein 5:
Subcellular location: mainly localizes in Golgi apparatus membrane. Upon calcium-triggered exocytosis, it translocates to the cell membrane. Highly enriched in synaptic vesicles. **Function**: required for the calcium-dependent exocytosis of signal peptide-containing cytokines, such as CCL5. Probably acts in cooperation with the SNARE machinery. High expression after the second postnatal week, a period of intense synaptogenesis (Fernández-Chacón et al., 2000). Associated with autism disorders (Castermans et al., 2010).
Spectrin alpha chain, brain:
Subcellular location: cytoplasm. **Function**: protein of the cytoskeleton. It is involved in secretion, interacts with calmodulin in a calcium-dependent manner and may be candidate for the calcium-dependent movement of the cytoskeleton at the membrane. Involved in axonal and neurites outgrowth (Ramser et al., 2010). Cocaine and morphine exposure alters its integrity and expression (Lepsch et al., 2009; Prokai et al., 2005).

Mitochondrial import receptor subunit TOM22 homolog:
Subcellular location: mitochondria.
Phosphorylation site/kinase: serine 15/not described.
Function: Central receptor component of the translocase of the outer membrane of mitochondria responsible for the recognition and translocation of cytosolically synthesized mitochondrial preproteins. The N-terminal domain (residues 1-62) is important for binding to the unfolded mature imported proteins. Caseine kinase 2 phosphorylates TOM 22 at several sites, and plays an important stimulatory role in the biogenesis of the TOM complex (Schmidt et al., 2011).
Dihydropyrimidinase-related protein 1:
Subcellular location: cytoplasm.
Phosphorylation site/kinase: threonine 509/ cyclin-dependent kinase 5 (Yamashita et al., 2007).
Function: Necessary for signaling by class 3 semaphorins and subsequent remodeling of the cytoskeleton. Plays a role in axon guidance, invasive growth and cell migration. This phosphorylation modulates the role of Dihydropyrimidinase-related protein 1 in Semaphorin3A-induced spine development (Yamashita et al., 2007).
Dihydropyrimidinase-related protein 3:
Subcellular location: cytoplasm.
Phosphorylation site/kinase: threonine 509/not described
Function: regulates dendrite arborization and spine morphology in the hippocampus and is required for LTP formation (Quach et al., 2008).
cAMP-dependent protein kinase type II-beta regulatory subunit:
Subcellular location: cytoplasm.
Phosphorylation site/kinase: serine 112/ protein kinase A (autophosphorylation) (Budillon et al., 1995).
Function: regulatory subunit of protein kinase A. Type II regulatory chains mediate the association of protein kinase A to the plasma membrane by binding to anchoring proteins, including the microtubule-associated protein 2 kinase. Involved in conditioned taste aversion (Koh et al., 2003). Involved in ethanol induced reward effects, locomotor activity and sensitization (Thiele et al., 2000; Fee et al., 2006). Described as a transcription factor capable of interacting physically and functionally with cAMP response element in the DNA (Srivastava et al., 1998). Its deletion decreases body weight and increases energy expenditure in leptin-deficient ob/ob mice (Newhall et al., 2005). Involved in synaptic plasticity (Yang et al., 2009).

Tubulin polymerization-promoting protein

Subcellular location: cytoplasm and nucleus.

Phosphorylation site/kinase: serine 31/ protein kinase A (Hlavanda E, 2007).

Function: may play a role in the polymerization of tubulin into microtubules, microtubule bundling and the stabilization of existing microtubules, thus maintaining the integrity of the microtubule network. May play a role in mitotic spindle assembly and nuclear envelope breakdown (uniprot, http://www.uniprot.org).

Prostaglandin E synthase 3

Subcellular location: cytoplasm and nucleus.

Phosphorylation site/kinase: serine 113/ caseine kinase 2 (Kobayashi et al., 2004).

Function: protein chaperone, also known as p23, which is required for proper functioning of the glucocorticoid and other steroid receptors (Lovgren et al., 2007). Binds to the progesterone receptor and interact with heat shock protein 90. Regulates estrogen receptor alpha induced gene expression (Oxelmark et al., 2006). Its phosphorylation at serine 133 is important for its activation and association with heat shock protein 90 (Kobayashi et al., 2004).

Syntaxin-1B

Subcellular location: membranes.

Phosphorylation site/kinase: serine 14/ not described, however syntaxin 1A is phosphorylated at serine 14 by caseine kinase 2 (Gil et al., 2011).

Function: potentially involved in the docking of synaptic vesicles at presynaptic active zones (Gerber et al., 2008). Involved in spatial learning, synaptic and trans-synaptic plasticity (Davis et al., 1998 and 2000). Increased expression of its mRNA in NAc shell in amphetamine sensitizated rats (Bhardwaj et al., 2006).

Table 14: Principal functions attributed to the proteins which expression or phosphorylation state in the HPC is similarly modulated by re-exposure to cocaine and morphine conditioned CPP compartement, when compared to the saline control group.

Multifunctional protein ADE2:
Subcellular location: not well-described. **Function**: involved in de novo purine biosynthesis pathways (essential for RNA and DNA synthesis, regulate energy metabolism, protein synthesis and function, enzyme activity, coenzyme function, and cell signaling) (Duval et al., 2013). May be involved in cell proliferation during ocular development, trought ATP signalling pathways (Ng et al., 2009).
Exportin-1:
Subcellular location: cytoplasm and nucleus. **Function**: mediates the nuclear export of cellular proteins and of RNAs (Stade et al., 1997). Participates in the cytoplasmic localisation of Neurogenin 3, a proneural gene, that regulates dendritogenesis and synaptogenesis in mouse hippocampal neurons (Simon-Areces et al., 2013). May be involved in the nuclear export of NUFIP1, a FMRP interactors involved in the regulation of local protein synthesis near synapses (Bardoni et al., 2003).
Proton myo-inositol cotransporter:
Subcellular location: membrane. **Function**: H+-myo-inositol cotransporter. May be a regulator of phosphoinositide signalling, which is involved in the development of growth cones, synaptic activity and mood disorders (Uldry et al., 2004; Di Daniel et al., 2009).
Transportin-3:
Subcellular location: cytoplasm and nucleus. **Function**: Nuclear import receptor for serine/arginine-rich proteins, which are essential precursor-mRNA splicing factors.
Formin-2:
Subcellular location: cytoplasm. **Function**: actin cytoskeleton organization (Goode et al., 2007). Formins play a role in synaptic growth (Pawson et al., 2008).

Surfeit locus protein 4:
Subcellular location: membrane of endoplasmic reticulum and Golgi. **Function**: Plays a role in the maintenance of the architecture of the endoplasmic reticulum-Golgi (Mitrovic et al., 2008).
Protein FAM136A:
Subcellular location: mitochondria. **Function**: not described.
Isoform Glt-1B of Excitatory amino acid transporter 2:
Subcellular location: membrane . **Function**: transports L-glutamate and also L- and D-aspartate. Essential for terminating the postsynaptic action of glutamate by rapidly removing released glutamate from the synaptic cleft. Up regulated by chronic stress (Reagan et al., 2004).
UTP-glucose-1-phosphate uridylyltransferase:
Subcellular location: cytoplasm. **Function**: UDP-glucose metabolic process, glycogenesis. It synthesizes UDP-glucose from glucose-1-phosphate and UTP. UDP-glucose has been described as a ligand for the purinergic receptor P2Y14, which promotes cytoskeleton rearrangement via Rho/Rho kinase activation (Sesma et al., 2012).
Carnitine O-acetyltransferase:
Subcellular location: endoplasmic reticulum, membrane, mitochondria and peroxisome. **Function**: Fatty acid and lipid metabolism and transport. May be involved in the transport of acetyl-CoA into mitochondria. Involved in carnitine metabolism. Acetyl-L-carnitine ameliorates spatial memory deficits associated to alzheimer disease, has anti-depressive like effects, increases DA outflow and phosphorylation of DARPP-32 (Thr34) in the NAc, and stimulates production of nerve growth factor (Jiang et al., 2011;Di Cesare Mannelli et al., 2011; Scheggi et al., 2004, Taglialatelae et al., 1994).
Beta-actin-like protein 2:
Subcellular location: cytoplasm (cytoskeleton) **Function**: cell motility. β-actin exists as a globular actin (G-actin) or filamentous actin (F-actin). Involved in cell growth (Chang et al., 2011).

SH3-containing GRB2-like protein 3-interacting protein 1:
Subcellular location: membrane. **Phosphorylation site/kinase:** serine 287/ not described. **Function**: may be implicated in clathrin-mediated endocytosis. Recruit proteins essential to the formation of functional clathrin-coated pits (Li et al., 2011). May also bind tubulin. May play a role in the regulation of energy homeostasis. Up-regulated in the hypothalamus of obese mice (Trevaskis et al 2005).
Tumor protein p63-regulated gene 1-like protein:
Subcellular location: presynaptic nerve terminals (synaptic vesicles). **Phosphorylation site/kinase:** threonine 34/ not described. **Function**: not described.
Oxidation resistance protein 1:
Subcellular location: mitochondria. **Phosphorylation site/kinase:** serine 346/ not described. **Function**: may be involved in protection from oxidative damage, such as neurodegeneration (Oliver et al., 2011).
Proline-rich transmembrane protein 2:
Subcellular location: membrane, cell junction at the synapse. **Phosphorylation site/kinase:** serine 244 and 250/ not described. **Function**: Component of the outer core of AMPAR complex, participates in the control of the gating and pharmacology of the AMPAR complex (Schwenk et al 2012). Interacts with SNAP-25, co-localised with synapsin-1 and may play a role in neurotransmitter release; mutation of its gene cause paroxysmal kinesigenic dyskinesia (Heron et al., 2013).

Microtubule-associated protein 2 :
Subcellular location: cytoplasm (cytoskeleton) **Phosphorylation site/kinase:** serine 1612/ not described **Function**: may stabilize microtubules against depolymerization. Involved in the localisation of type II PKA in dendritic shaft, which is important for the regulation of synaptic strength and long-term potentiation (Zhong et al., 2009). Considered as neurosteroid receptor, and mediates neurosteroid-enhanced neurite outgrowth of nerve growth (Fontaine-Lenoir et al., 2006).
Spectrin beta chain, brain 1:
Subcellular location: cytoplasm (cytoskeleton). **Phosphorylation site/kinase:** serine 2101/ not described. **Function**: involved in calcium-dependent movement of the cytoskeleton at the membrane. Presynaptic spectrin (alpha and beta) is an essential scaffold that is required to maintain synapse stability (Pielage et al., 2005). beta-Spectrin is required for the localization of alpha-Spectrin and ankyrin to the postsynaptic membrane, in the absence of postsynaptic α- or β-Spectrin, active zone size is increased, which correlate with an increase in quantal size; spectrin are required for the organization of ion channels and cell adhesion molecules and participates in the clustering of postsynaptic neurotransmitter receptors (Pielage et al., 2006).

Table 15: Principal functions attributed to the proteins which expression or phosphorylation state in the NAc is similarly modulated by re-exposure to cocaine and morphine conditioned CPP compartement, when compared to the saline control group.

Protein transport protein Sec31A:
Subcellular location: cystoplasm, cytoplasmic vesicles, endoplasmic reticulum and membrane. **Function**: component of the coat protein complex II that promotes the formation of transport vesicles from the endoplasmic reticulum.
Emerin:
Subcellular location: nucleus membrane. **Function**: stabilizes and promotes the formation of a nuclear actin cortical network. Stimulates actin polymerization. Inhibits beta-catenin activity by preventing its accumulation in the nucleus, through a CRM1-dependent export pathway. Facilitates repressive chromatin formation at the nuclear periphery by increasing the catalytic activity of HDAC3 (Demmerle et al., 2012).
Cell cycle exit and neuronal differentiation protein 1:
Subcellular location: membrane **Function**: Co-ordinates cell cycle exit and differentiation of neuronal progenitors (Politis et al., 2008). Although highly expressed in mature neurons, its function in adult brain is unknown, but it might have a neuroprotective role (Boutou et al., 2000).
Protein transport protein Sec61 subunit beta:
Subcellular location: endoplasmic reticulum membrane. **Function**: necessary for protein translocation in the endoplasmic reticulum. Participates in the epidermal growth factor receptor trafficking to the nucleus, functions to insert secretory and transmembrane proteins into the endoplastic reticulum during protein synthesis and traffics toxins from the cell surface to the endoplasmic reticulum and to the cytosol (intoxication process) (Liao et al., 2007)

Isoform 2 of Protein phosphatase 1 regulatory subunit 1B (DARPP-32):
Subcellular location: cytoplasma and nucleus. **Phosphorylation site/kinase:** serine 10/ not described. **Function**: Inhibitor of protein-phosphatase 1. DARPP-32 in the brain is highly enriched in DA neurons and is important mediator of DA signalling. Depending on its phosphorylation state, it can acts as an amplifier of PKA-mediated signaling or inhibitor PKA activity. It participates also in glutamate and serotonin signalling. It plays an important role in the induction of both LTD and LTP. It mediates the short- and some long-term effects of several drugs of abuse. The acquisition of cocaine and ethanol self-administration and CPP is attenuated in DARPP-32 KO mice. DARPP-32 KO mice show increased locomotor sensitization to cocaine (Nairn et al., 2004).
Stathmin:
Subcellular location: cytoplasm (cytoskeleton). **Phosphorylation site/kinase:** serine 38/ Cdk5 (Hayashi et al., 2006). **Function**: Stathmin is a neuronal growth-associated protein that has been implicated in neural development, plasticity, learning, degeneration and aging (Mori and Morii 2002). It is involved in the regulation of the microtubule filament system by destabilizing microtubules. Involved in the maintenance of synaptic connexions (Graf et al., 2011). Involved in the control of the learned and innate fear (Shumyatsky et al., 2005). Serine 38 is predominantly phosphorylated by Cdk5, which may causes weakened stathmin-tubulin binding, and thus increases the concentration of tubulin available in the cytoplasm for microtubule assembly (Hayashi et al., 2006).
Neuronal membrane glycoprotein M6-a:
Subcellular location: membrane, in particular of dendrtitic spines and axons. **Phosphorylation site/kinase:** serine 267/ protein kinase C (Huang et al., 2011). **Function**: involved in neuronal differentiation. Plays a role in neuronal plasticity and is involved in neurite and filopodia outgrowth, filopodia motility and probably synapse formation; it is a stress-responsive gene in the hippocampal formation (Alfonso et al., 2005). May be involved in regulation of endocytosis and intracellular trafficking of G-protein-coupled receptors; enhances internalization and recycling of mu-type opioid receptor (Liang et al., 2008). Phosphorylation at serine 267 regulates filopodium motility (Brocco, 2010)

Heat shock protein HSP 90-alpha:

Subcellular location: cytoplasm

Phosphorylation site/kinase: serine 263/ CK2 (Lees-Miller SP et al., 1998)

Function: Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction, such as the glucocorticoid receptor or the epidermal growth factor receptor. In the endometrium, its expression is stimulated by oestradiol and inhibited by progesterone (Tang et al., 2005). Participated in nitric oxide biosynthetic process and protein import into mitochondrial outer membrane. Involved in synaptic function by controlling neurotransmitter release and, independently, by mediating the continuous cycling of synaptic AMPA receptors (Gerges et al., 2004). Increased expression in cocaine withdrawn rats (García-Fuster et al., 2012).

14-3-3 protein zeta/delta:

Subcellular location: cytoplasm.

Phosphorylation site/kinase: serine 207/ not described.

Function: Adapter protein implicated in the regulation of several signaling pathways. Modulation of its expression during the RISE phenomenon (repetitive LTP-induced synaptic enhancement) (Kawaai, 2010). May be involved in THC-induced neuroprotection (Chen et al., 2007). 14-3-3ζ deficiency is associated with behavioural, cognitive and neurodevelopmental (altered glutamatergic synapse formation) anomalies similar to those seen in neuropsychiatric disorders such as schizophrenia, autism spectrum disorder and bipolar disorder (Cheah et al., 2012). Involved in cAMP-induced BDNF transcription (Neasta et al., 2012). Conditioned cue/context-mediated cocaine memory is associated with accumbal 14-3-3ζ protein downregulation (Kao et al., 2011). Involved in escalation and inflexibility of alcohol intake (Lesscher et al., 2012). Its expression is altered by morphine administration (Morón et al., 2007).

Microtubule-associated protein 2:

Subcellular location: cytoplasm (cytoskeleton).

Phosphorylation site/kinase: threonine 1160/ not described.

Function: see table 12.

Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial:
Subcellular location: mitochondria. **Phosphorylation site/kinase:** serine 11/ not described. **Function**: lipid metabolism. Plays an essential role in the mitochondrial beta-oxidation of short chain fatty acids. In the brain, participates in androgen metabolism: catalyze the oxidation of 17β-estradiol and 5α-androstane-3α,17β-diol and convert them to dihydrotestosterone (He et al., 2000).
DnaJ homolog subfamily C member 5:
Subcellular location: membrane and synaptic vesicles. **Phosphorylation site/kinase:** serine 10/ PKA (Evans et al., 2001). **Function**: may be involved in calcium-dependent neurotransmitter release at nerve endings (Buchner et al., 1997), could be essential for synaptic function maintenance and refolding of synaptic proteins (Fernández-Chacón et al., 2004; Tobaben et al 2001). Phosphorylation at serine 10 modulates the activity of the exocytotic machinery (Evans et al., 2001).

DISCUSSION

A. ROLE OF CB1-R IN THE NEUROPLASTIC CHANGES ASSOCIATED TO PALATABLE FOOD REWARD AND SEEKING BEHAVIOUR

Growing evidences suggest that some eating disorders should be considered as a form of "food addiction". In particular, some scientists have proposed that the pattern of food intake observed in certain obese individuals present striking similarities with the uncontrolled and compulsive drug intake seen in drug-addicts (Volkow et al., 2008). Like drugs of abuse, certain types of food, particularly those enriched in carbohydrates and lipids, can trigger an excessive release of DA in mesocorticolimbic system mainly when consumed in a binge-like manner (Avena et al., 2009). It is proposed that excessive consumption of these types of food could progressively alter the functionality of the brain reward circuit, such as drugs of abuse, generating a pathologic motivational state towards hedonic foods that could facilitate compulsive eating (Avena et al., 2009; Johnson and Kenny, 2010). Some forms of obesity can be viewed as a chronic condition with repetitive periods of protracted abstinence (restriction of seductive foods) and periods of relapse (compulsive eating) (Volkow and Wise, 2005). In drug addiction, the persistence of behavioural alterations (drug relapse after years of abstinence) may be due, at least in part, to drug-induced structural changes in the mesocorticolimbic circuit (Robinson and Kolb, 2004). Although several studies have investigated the underpinnings of "food addiction" (Blumenthal and Gold, 2010), few to date have studied the involvement of structural plasticity changes in food reward and eating behaviour alterations (Crombag et al., 2005).

In the first part of this thesis, we have evaluated whether prolonged operant training to obtain hedonic foods can alter the morphological

structure of the neurons of the mesocorticolimbic circuit. In particular, we evaluated the specific involvement of different characteristics of obesogenic diets in promoting behavioural and neurophysiological alterations: the palatability by using isocaloric chocolate-flavoured pellets and the caloric content by using high fat pellets. Different studies have demonstrated the role of the endogenous cannabinoid system in the mesocorticolimbic system in controlling food hedonic value and consumption (Harrold and Williams, 2003). In addition, some evidences have highlighted the implication of this system in promoting structural plasticity changes involved in drug-seeking behaviour (Ballesteros-Yáñez et al., 2007a). Thus, we sought that the endogenous cannabinoid system could also be involved in the development of palatable food-induced behavioural alterations by a mechanism that implies structural plasticity changes in the brain reward pathways.

Our results show that palatability represents a key factor that influences motivation to consume and seek food. Indeed, despite satiety and the increased effort required to obtain food (fixed ratio 5 (FR5)), mice trained with the palatable diet displayed high levels of operant responding, consuming as much as two-fold more pellets than mice trained with standard diet. These mice trained with palatable food were able to lever-pressing at least 300 times to get one single pellet in the PR task. In contrast, such strong reinforcing effects were not observed with high fat pellets, and mice trained with this type of food importantly reduced their pellets intake when fed ad libitum and tested in a FR5 schedule (-77% compared to the intake during the training under food deprivation). In addition, these mice presented similar motivation to obtain the reinforcer in the PR test than mice trained with the standard diet. These results

suggest that in absence of pleasurable taste, fatty diets fail to produce enough rewarding effects to override satiety signals in rodents.

In support to our results, a previous study has shown that palatable pellets improve performance on operant training when compared with a standard diet, a response that was not observed with high fat pellets (Barbano et al., 2009). However, another study suggests that the low reinforcing properties of high fat food in sated animals tested in operant task do not reflect their lack of rewarding effects but rather their strong ability to elicit satiety signals (Ward et al., 2007). Indeed, these data reveal that mice trained with high fat food exhibit higher operant reponses than mice trained with sweet food in operant tasks that do not involve food consumption, such as extinction and cue reinstatement sessions (Ward et al., 2007). Thus, the authors state that high fat foods could produce high rewarding effects, even greater than sweet foods under these experimental conditions. In addition, fat enriched foods promote CPP acquisition in animals (Imaizumi et al., 2000), and fat foods are largely preferred than low-energy-density ones in humans (Drewnowski and Almiron-Roig, 2010).

Our results support the proposal that the high rewarding effects of palatable food can override satiety signals, and palatability may represent a key factor leading to excessive food intake (Lutter and Nestler, 2009). In addition, our results suggest that the ability of fat-enriched food to promote overeating may be less prominent than palatable one, without additional pleasurable taste and/or the emergence of metabolic alterations, although the rewarding effects of this type of food has been well documented (Imaizumi et al., 2000; Drewnowski and Almiron-Roig, 2010).

On the other hand, our results confirm that the endogenous cannabinoid system plays an important role in both the homeostatic and hedonic control of food intake (Harrold and Williams, 2003). Indeed, CB1-R KO mice consumed all kinds of diet in a less amount than WT mice when food-deprived, suggesting a general decrease of appetite under these experimental conditions. This effect was not due to learning impairments because both WT and CB1-R KO mice similarly acquired the task. In agreement, previous studies have demonstrated that administration of a CB1 antagonist in food-deprived animals decreases operant responses and intake of both palatable and non-palatable foods (Freedland et al., 2000; Pério et al., 2001). These results are in accordance with the well-described role of endocannabinoids in the regulation of appetite and the modulation of orexigenic signals (Kirkham et al., 2002, Di Marzo and Matias, 2005). In addition, our results show that CB1-R mediates the rewarding and reinforcing effects of palatable food. Indeed, sated CB1-R KO mice consumed significantly less palatable pellets than sated WT mice, and in contrast to WT mice, exhibited similar motivation for this type of food than for a standard diet. These effects were not due to compensatory mechanisms related to the genetic deletion of CB1-R, as pharmalogical and viral inactivation of CB1-R in the NAc in WT mice also leaded to decrease palatable food consumption in the FR schedule, and breaking point in a PR test. In agreement, several studies have shown that inactivation of CB1-R reduces operant responses in both FR and PR schedule, as well as the intake of palatable food in animals fed ad libitum, while administration of CB1-R agonists produces opposite effects (Simiand et al., 1998; Higgs et al., 2003; Ward et al., 2005). In addition, our pharmacological and viral approaches highlight the prominent role of the endogenous cannabinoid

system in the NAc in mediating these responses. Accordingly, previous studies have shown that the endogenous cannabinoid system is a critical regulator of accumbal activity during motivational responses towards palatable food achievement (Hernandez et al., 2012), and infusion of a CB1-R agonist in the NAc increases palatable food consumption (Shinohara et al 2009). This later effect has been related to the presence of endocannabinoid hotspots in the NAc shell that would mediate food sensory pleasure or palatability perception (Mahler et al., 2007).

Furthermore, we observed that sated CB1-R KO mice consumed significantly more high fat pellets than sated WT mice, suggesting that CB1-R KO mice may be less sensitive to the satiety effects derived from the consumption of this diet and/or more sensitive to its rewarding properties. We also observed that CB1-R KO mice seem more motivated to work for high fat foods than WT mice in a PR task. However, it is important to note that breaking points reached by CB1-R KO mice trained with high fat and standard pellets were not statistically different. These findings are somewhat surprising considering that several studies have shown that pharmalogical inactivation of CB1-R decreases operant responses and intake of fat foods (McLaughlin et al., 2003; Randall et al., 2010). In addition, endocannabinoid signalling in the small intestine through CB1-R serves as an orosensory positive feedback mechanism that facilitates fat intake (DiPatrizio et al., 2011). Nevertheless, another study found that genetic deletion of CB1-R-KO does not decrease operant responses for high fat food (Ward et al., 2005). Thus, data in CB1-R deficient mice suggest that the role of the endogenous cannabinoid system in fat intake could be more complex than just modulating the rewarding properties of fat foods. Indeed, the endogenous cannabinoid system has shown to be also involved in

adipocytes fat storage and lipogenesis, two processes that can influence appetite (Cota et al., 2003; Trayhurn et al., 2006). Thus, it is possible that a constant alteration of these processes in CB1-R KO mice leads to enhance incentive value and/or appetite for fat foods in these mice, in order to promote fat nutrients supply and compensate their impaired storage as well as the decreased body mass of these mutants mice (Cota et al., 2003). Hence, CB1-R KO mice could present both a decreased sensitivity to the hedonic effect of fat food together with an enhanced motivation to eat this type of food to fulfil a metabolic necessity. Thus, such opposite responses could lead to either no apparent changes in fat intake in CB1-R KO mice due to the compensation of both mechanisms (Ward et al., 2005), or even an increased in fat consumption and motivation (our results) depending on the ratio of hedonic effects/caloric content of the fat diet used. Although further experiments will be necessary to validate this hypothesis, our results together with the previous studies suggest that the endogenous cannabinoid system plays an important role in mediating both palatability-induced rewarding effects and the perception of the caloric value of food. These findings provide further evidences that endogenous cannabinoid system could be an important neurobiological substrate underlying the development of eating disorders.

A more detailed analysis of our experimental data revealed that long-term exposure to palatable food, but not standard or high fat diet, leads to progressive alterations of food-seeking behaviour through a CB1-R dependent mechanism. Thus, we observed that mice repeatedly trained with palatable food progressively increased their operant responses and food intake throughout the experimental sequence (+ 20% between day 16 and day 40, figure 24A). This behaviour suggests that chronic exposure to

palatable food may lead to an enhancement of its hedonic properties and/or the desire to obtain this kind of food. In support to this hypothesis, other studies have reported similar alterations of eating behaviour after long-term intermittent exposure to a palatable beverage (Colantuoni et al., 2001). Indeed, rats exposed to this diet regimen dramatically increase their glucose intake along the time, and display enhanced motivation to obtain this beverage after abstinence, an effect interpreted as the expression of palatable food craving (Avena et al., 2005). As it occurs with drugs of abuse, this enhanced motivation has shown to incubate or grow with the length of the abstinence (Avena et al., 2008; Grimm et al. 2005). Together, these findings suggest the gradual emergence of long-term changes in the neural circuitry underlying motivation as a result of palatable food self-administration, which seem to be maintained or even enhanced during dieting period.

Interestingly, similar responses have been described in animals allowed to self-administer drugs of abuse during prolonged intermittent period using extended access protocols (Ahmed and Koob, 1998). This phenomenon, named escalation of drug intake, has been interpreted either as reflecting the development of tolerance to the drug's hedonic effects or the development of sensitization to the drug's reinforcing effects and/or incentive salience of the drug-associated stimuli (Zernig et al., 2007). Animals that escalate their drug intake present an enhanced motivation to seek the drug in a PR schedule compared to animals that do not escalate their drug consumption (Paterson and Markou, 2003). One important feature with escalation of drug intake in long-access model is that animals develop a prominent binge-pattern of drug self-administration revealed by discontinuous and repetitive episodes of large drug intake. Under these

conditions, animals display one large binge at the onset of the drug-availability period, and most drug is self-administered during the first 10 min of the session (Ahmed and Koob, 1998). Similarly, rats feed intermittently with sugar escalate their sugar intake and consume a large amount of this food during the first hour of daily access, which can be defined as a binge (Colantuoni et al., 2001). In accordance, we also observed the development of a binge-like eating pattern in mice trained with palatable pellets (Figure 24C). Indeed, the enhancement of food intake observed in these mice was mainly due to an increase of their operant responses during the first 15 min of the last 1 h session. Together, these evidences suggest that prolonged exposure to palatable food can cause the emergence of sensitization or tolerance to the incentive effects or pleasurable properties of palatable food, respectively. This process may possibly lead some vulnerable individuals to consume hedonic foods in an excessive and uncontrolled manner.

Furthermore, we observed that long-term operant access to palatable pellets appears to also alter inhibitory control processes in WT mice. Indeed, mice trained with palatable food that have well acquired the task and its contingencies progressively developed an unadapted behaviour reflected by a high number of unreinforced “active” lever presses during the time-out period. In comparison, these responses were dramatically decreased in sated mice trained with a standard or high-fat food during the whole experiment. Therefore, repeated palatable food exposure might lead mice to experience strong urges before consuming the desirable food challenging their capacity to wait even for a short period of time since the time-out was only 10 sec. These unadapted responses were significanlty increased within the training, suggesting a progressive loss of control over

palatable food-seeking behaviour. In accordance to our results, a previous work also described the emergence of such unadapted behaviour with extend period of operant training with palatable food (Ghitza et al., 2006). Interestingly, this peculiar behaviour has been correlated with elevated impulsivity (Diergaarde et al., 2009). Similar increase in unreinforced responses has been observed in rats that develop compulsive drug-intake (Deroche-Gamonet et al., 2004). Together, these data suggest that prolonged access to palatable food can promote the development impulsive-like behaviours and potentially lead to compulsive food intake. In agreement, another study shows that rodents chronically exposed to a high palatable diet develop an obese phenotype and compulsive palatable food intake (Johnson and Kenny, 2010).

We further show that these alterations of food-seeking behaviour triggered by palatable food are mediated by the endogenous cannabinoid system. Indeed, CB1-R KO mice did not escalate their food consumption, nor developed a pronounced binge-eating pattern nor impulsive-like responses when trained with palatable food (figure 24B and D), although they were sensitive to its rewarding properties and largely prefered this type of food to a standard diet (figure 23B). In support to our results, the pharmalogical blockade of CB1-R reduces the enhanced motivation for cocaine developed by rats that have previously escalated their cocaine intake (Orio et al., 2009). In addition, several studies have suggested that the endogenous cannabinoid system could be involved in binge eating disorders, and pharmalogical blockade of CB1-R reduces binge-like eating behaviour in animals (Scherma et al., 2012; Parylak et al., 2012). Finally, the administration of CB1-R agonists and antagonists has shown to enhance and decrease, respectively, certain forms of impulsive behaviours,

highlighting the role of the endogenous cannabinoid system in impulsivity and inhibitory control (McDonald et al., 2003; Pattij et al., 2007). Therefore, the endogenous cannabinoid system may represent an important neurobiological substrate for the neuroadaptations triggered by excessive palatable food consumption that mediate the development of eating behaviour alterations.

In a next step, we have evaluated whether these neuroadaptations include structural plasticity changes in the mesocorticolimbic system and the possible intracellular mechanisms involved. Our results show that the alterations in food-seeking behaviour triggered by prolonged consumption of palatable food are associated with dendritic spines density changes in specific areas of the mesocorticolimbic circuit. We also provide evidences that the endogenous cannabinoid system through CB1-R activity and probably ERK signalling pathway mediates these morphological adaptations. Indeed, we observed that repeated operant training with palatable food, but not other kinds of food, increases spine density in the NAc shell and in a lesser extent in the mPFC. Interestingly, similar structural changes induced by chronic exposure to psychostimulants or nicotine have been hypothesized to participate in the development of addictive behaviour (Robinson and Kolb, 2004; Russo et al., 2010). Our correlative analyses reveal a significant inter-dependence between the dendritic spine density in the NAc shell and mPFC and the amount of pellets consumed or the number of unreinforced lever presses during the time-out period. These results suggest that synaptic structural adaptations may contribute to the development of food-seeking behaviour alterations.

The NAc shell and the mPFC are two important brain areas involved in

reward processes, and their functional activities are altered in addictive disorders (Koob and Volkow, 2010). In parallel, both regions are also crucial for regulating the hedonic aspects of food, and dysfunctions of these areas have been related to some eating disorders (Kelley and Berridge, 2002, Volkow et al., 2008). Thus, the NAc shell encodes pleasurable sensation derived from food consumption, and contributes to the motivational aspect of food seeking behaviour (Baldo and Kelley, 2007). Pharmacological alterations of NAc activity can induce voracious appetite, especially for palatable food, even in sated animals (Baldo and Kelley, 2007). Therefore, the structural plasticity changes triggered by repeated operant training with palatable food in the NAc shell may possibly underline the development of a sensitization process to pleasurable and/or motivational effects of palatable food. On the other hand, the PFC plays a crucial role in decision-making and inhibitory processes, and seems to be an important structure in mediating food choice and intake (Dalley et al., 2004; Rolls, 2011). Alterations of neurochemical activity in the mPFC promote hyperphagia in mice, mainly for sweet food (Mena et al., 2011). In addition, dysfunctions of mPFC have been associated with behavioural inflexibility, perseverative and impulsive actions (Ragozzino et al., 1999; Passetti et al., 2002; Dalley et al., 2002). Thus, palatable food induced structural plasticity changes in the mPFC may possibly contribute to the emergence of an enhanced sensitivity to hedonic value of palatable food and impulsive-like responses toward this type of food.

Our results show that CB1-R KO mice repeatedly trained with palatable food do not present any structural plasticity change neither in the NAc shell nor in the mPFC. Therefore, the endogenous cannabinoid system may play

a role in mediating the neuroadaptations triggered by excessive and/or prolonged palatable food intake. In agreement, viral inactivation of CB1-R activity in the NAc of WT mice also prevents the emergence of palatable food induced structural changes in the NAc shell and mPFC, ruling out possible compensatory mechanisms in mutant mice that could interfere with the generation of these morphological changes. Therefore, our results extend the role of CB1-R in drug-induced structural plasticity changes (Ballesteros-Yáñez et al., 2007a) to those triggered by palatable food, and suggest that CB1-R dependent morphological alterations in the mesocorticolimbic system could represent a common neuroadaptation involved in the development of different pathological consumatory behaviours.

The present results are of revelance to further support the hypothesis that drug and natural reward, such as palatable food, can influence similar neurobiological mechanisms and that excessive consumption of palatable food, like drugs of abuse, can potentially alter mechanisms involved in reward processing.

Our data also highlight some important differences between drug- and palatable food-induced structural plasticity. First, we observed that in contrast to drugs of abuse (Robinson and Kolb, 2004) long-term exposure to palatable food in an operant paradigm do not significantly alter dendritic spines density in the NAc core. This result suggests that prolonged drug and palatable food consumption may have a different impact on NAc core functionality. Thus, recent studies have shown that drug and palatable food conditioned stimuli differentially affect the activity of the dopaminergic system in the NAc core (Bassareo et al., 2011), and drug-reinforced

learning, but not palatable food learning, rely on acetylcholine neurotransmission in the NAc core (Crespo et al., 2008). Distinct alterations of these systems by drugs and palatable food may have different consequences on activity-dependent structural changes since both DA and acetylcholine have been linked to synaptic plasticity (Jay, 2003; Rasmusson, 2000). In addition, functions attributed to the NAc core could subserve more directly behavioural responses to drugs than to palatable food. Thus, the NAc core seems to play a prominent role in response-reinforcement learning, by promoting the conditioned association of drug and natural reward-related stimuli (Kelley, 1999; Parkinson et al., 1999; Di Ciano and Everitt, 2001). This particular function of the NAc core is important for the elaboration of habit learning, a process that is thought to be impaired in addiction (Everitt and Robbins, 2005). Thus, drug-induced structural alterations in the NAc core could possibly contribute to the emergence of pathological habit behaviours, and the absence of these changes with palatable food may possibly reflect the lower ability of food to promote such habit learning (Everitt and Robbins, 2005). However, it will be interesting to know whether more extended palatable-food operant training could promote spines density changes in the NAc core. Furthermore, drug-induced structural changes in the NAc core have been tightly correlated with the expression of cocaine sensitization (Robinson and Kolb, 2004). This phenomenon seems to be dependent on additional neuronal adaptations that occur during the time course of drug abstinence (Vanderschuren and Kalivas, 2000**)**. Therefore, it will be also of interest to evaluate whether a similar sensitization-like procedure with palatable food that includes prolonged intermittent exposure, absence of exposure and subsequent re-expousre to a palatable diet, can produce structural

plasticity changes in the NAc core. Although we did not observe changes in total spines density in the NAc core after repeated palatable food operant training, we found that this training leads to increase the proportion of thin-types spines in this brain area. These spines morphology changes in the NAc core could also participate the food-seeking behaviour alterations triggered by palatable food.

Secondly, palatable food-induced structural plasticity changes in the mesocorticolimbic circuit appear to be a longer time-dependent process than drug-induced structural plasticity changes. Indeed, a short operant training with palatable food do not alter dendritic spines density in the three studied brain areas. In contrast, drug-induced structural plasticity can be revealed after one single injection of the drug (Sarti et al., 2007; Kolb et al., 2003), or after short training in drug self-administration (Ballesteros-Yáñez et al., 2007b). This difference could reflect distinct capability of drugs and palatable food to modify neuronal activity in the mesocorticolimbic circuit. In contrast to drugs, DA release triggered by palatable food in the NAc shell rapidly wanes with further exposure (Di chiara and Bassareo, 2007), unless repeatedly consumed in an binge-like manner (Rada et al., 2005). In addition, mice display higher motivation to seek for cocaine than for palatable food after a short operant training (Cantin et al., 2010), suggesting that the ability of drugs to alter motivational circuitry following short exposure is greater than palatable food. However, the motor-stimulating effect of cocaine can have possibly biased the experimental measures in this previous study (Cantin et al., 2010). Alternatively, it is possible that food restriction have unmasked an effect of palatable food on structural plasticity during our short training

since it can promote synaptic and structural plasticity changes (Fontán-Lozano et al., 2007; Spolidoro et al., 2011)

Palatable food-induced structural changes in the mesocorticolimbic circuit seem to be strictly associated with learning processes. Indeed, mice that did not learn an operant behaviour and received passively palatable food during a same extended period did not present any structural change in the mesocorticolimbic system in spite of consuming the same amount of pellets. Although volition and learning processes seem also important factors that determine the extend of drug-induced structural changes, considerable data have shown that these structural changes also occur in animals that passively received the drug, even in their home cage (Robinson and Kolb, 2004). These differences certainly rely on the particular pharmacological properties of drugs of abuse, which can durably alter synaptic plastic processes in a learning independent manner (Lüscher and Malenka, 2011).

A previous study did not observe structural changes in the NAc and mPFC after operant training with palatable food (Crombag et al., 2005). A possible explanation for this discrepancy could be the different operant procedure used in the two studies. Indeed, the morphological analysis of the previous study was performed in rats trained during 14 to 20 days in an operant task maintained by sucrose, while our analysis revealed structural changes after 41 days of palatable food operant training but not following a short training. In addition, the previous analysis was conducted one month after the last sucrose self-administration session, while our analysis was performed just after the last session. Therefore, another possible

explanation for this discrepancy could be the transitory nature of the plasticity changes promoted by palatable food.

Finally, we evaluated whether the ERK signalling pathway could be involved in the behavioural and morphological alterations induced by palatable food. We observed that WT mice trained with palatable food present an enhanced phosphorylation of ERK in the NAc shell and mPFC, when compared with standard food-trained WT mice. In contrast, no changes in p-ERK were detected in the NAc core. This specific pattern of ERK activation correlates with the total spines density changes suggesting that ERK signalling may mediate palatable food-induced structural plasticity. In agreement, WT mice that performed a palatable food short-training or received passively palatable food did not develop morphological alterations and did not present a hyperphosphorylation of ERK in any studied area. Prolonged high fat training was also associated with a strong activation of ERK in the NAc shell and mPFC in WT mice. Thus, ERK activation may be necessary but not essential for the development of palatable food-induced structural changes. Instead, increased ERK activation in response to palatable and high fat food suggests that ERK signalling in the mesocorticolimbic system may have a prominent role in promoting learning processes towards goals with high rewarding value. Indeed, high fat foods can produce strong rewarding effects under some experimental conditions (Ward et al., 2007). In support to this hypothesis, psychoactive substances that do not sustain self-administration behaviour in rodents neither activate ERK in the NAc nor the mPFC, while drugs of abuse does (Valjent et al., 2004).

The absence of a direct correlation between ERK activation and the structural changes do not necessarily exclude its participation in the initiation of these morphological alterations. In fact, our results show that two ERK down-stream targets, the ribosomal protein S6 (rpS6) and the histone 3 (H3) (Roux et al., 2007; Brami-Cherrier et al., 2005), are selectively hyperphosphorylated (serine 235/236 and serine 10, respectively) in WT mice trained with palatable food, mainly in brain areas in which structural changes are observed. Double immunolabeling of p-ERK/p-H3 and p-ERK/p-rS6 demonstrate an almost complete co-localization of these two phosphorylated targets and ERK phosphorylation in the NAc. In addition, phosphorylations of rpS6 and p-H3 are dependent on operant learning processes and CB1-R activity, as it is the case for the behavioural and morphological alterations. Together these results support the implication of ERK signalling pathway in palatable food induced structural and behavioural changes through a CB1-R dependent mechanism.

In agreement, several studies have reported the involvement of ERK signalling in the regulation of dendritic structural plasticity in vitro (Goldin et al., 2003; Wu et al., 2001). In addition, ERK plays a key role in synaptic plasticity and long-lasting behavioural changes triggered by drugs of abuse (Thomas and Huganir, 2004; Girault et al., 2007). Drug-induced ERK activation in different parts of the mesocorticolimbic circuit seems to be dependent on CB1-R (Corbille et al., 2007; Pan et al., 2011). Together, these results suggest that ERK signalling in the mesocorticolimbic circuit may represent a common neurobiological substrate for the generation of maladaptive responses in the brain reward system induced by both drugs of abuse and natural rewards.

Histone 3 is a nuclear DNA-binding protein involved in the relaxation/condensation of chromatin and its phoshorylation at serine 10 has been linked to the activation of gene transcription (Nowak and Corces, 2004), and long-term forms of synaptic plasticity (Nestler, 2001). Phosphorylation of H3 at serine 10 through a DA dependent mechanism has been proposed as a common intracellular process by which drugs of abuse and food modulate striatal activity, included the NAc shell, and promote reward-seeking behaviour (Stipanovich et al., 2008). Interestingly, disruption of this process prevents locomotor sensitization induced by drugs (Stipanovich et al., 2008). Therefore, these studies and our results suggest that alteration of H3 dependent nucleosomal responses in the NAc shell may represent a common neuronal adaptations involved in drug and palatable food induced behavioural changes.

Further studies will be necessary to identify the specific genes activated by ERK-dependent phosphorylation of H3 that contribute to palatable food-induced behavioural and structural changes. Previous studies have shown that several downstream effectors of the transcriptional regulator ΔFosB, which is regulated by ERK, could mediate drug-induced dendritic remodeling in the NAc (Russo et al., 2010). In this sense, the cdk5-MEF2 module and the transcription factor NFkB have shown to regulate cocaine-induced structural plasticity in the NAc (Russo et al., 2010; Pulipparacharuvil et al., 2008). Interestingly, a recent study has described the involvement of ΔFosB in regulating responses to natural rewards (Wallace et al., 2008). Thus, overexpression of ΔFosB in the NAc increases sucrose intake and promotes sexual behavior in rats, and it is involved in the development of cross-sensitization between different types of rewards (Wallace et al., 2008). The authors suggest the possibility that ΔFosB

induction in the NAc may mediate not only key aspects of drug addiction, but also the development of "natural addictions" involving compulsive consumption of natural rewards (Wallace et al., 2008). It will be therefore interesting to evaluate the possible involvement of ΔFosB and its downstream effectors in our experimental model to clarify whether drug and palatable food induced structural plasticity can be mediated by similar mechanisms.

In the present study, ERK activation was associated by concomitant phosphorylation of rpS6. This protein is a component of the small 40S ribosomal subunit involved in mRNA decoding (Ruvinsky and Meyuhas, 2006) and its phosphorylation at Ser235/236 promotes protein synthesis by facilitating rpS6 recruitment to the 5'cap complex during the formation of the pre-initiation translation complex (Roux et al., 2007). Activation of rpS6 at dendritic level has been considered to support local protein translation, and can promote neural plasticity processes without implication of transcriptional mechanisms (Kelleher et al., 2004a). ERK-dependent phosphorylation of rpS6 at the synaptodendritic level is important for the establishment of late-LTP and memory consolidation (Kelleher et al., 2004b). In addition, the activation of rpS6 could mediate BDNF-induced dendritic spine remodeling (Lai et al., 2012), and BDNF-induced protein synthesis may be dependent on ERK activity (Takei et al., 2001). Therefore, the increased rpS6 phosphorylation observed in WT mice trained with palatable food may represent an enhancement of ERK dependent protein synthesis that possibly contribute to the morphological rearrangements observed in our study. Further studies will be necessary to determine the specific proteins that mediate these plastic events, and

whether structural plasticity changes induced by drugs and palatable food dependent on similar proteomic changes.

Our findings cannot rule out the possible involvement of other intracellular signalling systems in palatable food operant training-induced structural plasticity changes in the mesocorticolimbic system. Indeed, the two downstream proteins of ERK analyzed in this study, p-H3 and p-rS6, are also modulated by other intracellular pathways. Thus, the Akt/mTOR signaling pathway is a direct regulator of the activity of prS6 and participates in the modulation of H3 (Dobashi et al., 2011). This signalling cascade is involved in neuroplasticity, including spines formation (Hoeffer and Klann, 2010; Li et al., 2010a), and in the responses related to CB1-R activation (Puighermanal et al., 2009). Similarly, PKA signalling promotes phosphorylation of rpS6 and H3 at the same residues than ERK (DeManno et al., 1999; Valjent et al., 2011). In addition, PKA participates in CB1-R signalling, and plays an important role in synaptic plasticity and addictive-like behaviours (Childers et al., 1996; Wolf et al., 2003; Kauer and Malenka, 2007; Self, 2004). Further studies will be required to evaluate the possible participation of mTOR and PKA signalling pathways in the behavioural and neuroplastic changes promoted by palatable food. These studies would help to understand how repeated palatable food training promotes spines morphology changes and phosphorylation of rpS6 in the NAc core independently of ERK activation, and thus clarify the implication of the NAc core in food-seeking behaviour alterations.

In conclusion, our data show that repeated operant training to obtain palatable food is associated with the progressive development of seeking behaviour changes, which present similarities with those observed after

long-term exposure to drugs of abuse. Furthermore, we provide evidences that structural alterations in the neurons of the NAc shell and mPFC could mediate the generation of these behavioural alterations. Similar morphological changes have been observed in these brain structures after exposure to drugs of abuse, which are thought to play an important role in addictive-like behaviours (Robinson and Kolb, 2004; Russo et al., 2010). Finally, our results suggest that activation of several components of the ERK signalling pathway through a CB1-R dependent mechanism could represent an important intracellular event mediating palatable food-induced structural and behavioural alterations. These neural changes induced by palatable food could represent a neurobiological substrate for the development of some forms of obesity and eating disorders. This study provides some advances in the understanding of the common links between eating disorders and drug addiction, and highlights that the endogenous cannabinoid system and its downstream effectors may represent potential therapeutic targets to treat different addictive-like disorders.

B. ROLE OF CB1-R IN THE BEHAVIOURAL AND NEURONAL PLASTICITY ASSOCIATED TO MORPHINE-INDUCED LOCOMOTOR SENSITIZATION

Addictive disorders have been considered as a mental disease related to learning and memory systems, in particular those that subserve reward processing (Hyman, 2005). At the cellular level, learning and memory are thought to rely on plasticity phenomena that can durably modify synaptic transmission facilitating either the encryption or the consolidation of information (Martin et al., 2000a). It has been proposed that the ability of drugs of abuse to create altered forms of neuronal plasticity in the brain reward circuit could represent a neurobiological substrate for the development of unadapted behavioural learning, such as compulsive drug-intake and persistent drugs memories including drug craving (Hyman and Malenka, 2001; Kalivas and O'Brien, 2008 Lüscher and Malenka, 2011).

Drug-induced structural plasticity in the mesocorticolimbic circuit has been proposed as a possible mechanism to explain the ability of drugs of abuse to durably alter behaviour (Robinson and Kolb, 2004). Indeed, synaptic morphological changes can greatly enhance the stability of synaptic connexions, and can promote the emergence of enduring forms of synaptic plasticity (Grutzendler et al., 2002; Kasai et al., 2003; O'Donnell et al., 2011; Robert et al., 2010). In addition, drug-induced structural plasticity in the mesocorticolimbic system can persist for at least several months after cessation of drug treatment in rodents (Robinson and Kolb, 2004). A better knowledge of the cellular mechanisms underlying drug-induced structural plasticity may be useful to clarify the long-lasting alterations associated to addiction.

In the second part of the present thesis, we have investigated the possibility that the endogenous cannabinoid system could be a neurobiological substrate for drug-induced structural plasticity in the brain reward circuit. Indeed, the endogenous cannabinoid system is a key regulator of synaptic plasticity in this circuit (Sidhpura and Parsons, 2011), a phenomenon that can lead to structural changes (Matsuzaki et al., 2004; Zhou et al., 2004), and an important modulatory system of drug reward and drug-induced long-lasting effects (Maldonado et al., 2006). In addition, one study has shown that cocaine-induced structural changes in the motor cortex are dependent on CB1-R activity (Ballesteros-Yáñez et al., 2007a), and we previously demonstrated the relevance of this receptor in mediating the morphorlogical changes triggered by palatable food in mesocorticolimbic circuit (Guegan et al., 2013). In particular, we have evaluated the role of the endogenous cannabinoid system in mediating structural changes associated with the development of morphine locomotor sensitization, an animal model of drug-induced enduring behavioural changes (Robinson and Berridge, 2000). This opioid drug has been selected considering the particular involvement of the endogenous cannabinoid system in its rewarding effects (Robledo et al., 2008).

By using CB1-R KO mice, we show that the activity of the endogenous cannabinoid system is a determinant neuronal event that facilitates the development of morphine-induced locomotor sensitization. In addition, we provide evidences that disruption of CB1-R activity alters the ability of morphine to induce morphological changes in the mesocorticolimbic circuit, which may possibly account for the impairment of locomotor sensitization to morphine observed in CB1-R KO mice.

The detailed analysis of our behavioural results reveals that CB1-R plays an important role in all the different phases of the morphine sensitization process. Thus, genetic deletion of CB1-R enhances acute hyperlocomotor effects of morphine, prevents sensitization and facilitates tolerance to the stimulating effects of morphine during the chronic treatment, as well as reduces the expression of morphine sensitization after abstinence.

Role of CB1-R in acute locomotor effects of morphine

Our results show that the enhancement of locomotion induced by acute injection morphine is substantially greater in CB1-R KO than in WT mice, suggesting that the endogenous cannabinoid system could have an inhibitory modulatory role in the initial hyperlocomotor effects of this drug. In agreement, it has been reported that administration of a CB1-R agonist (i.e. HU-210) decreases acute hyperlocomotor effects of morphine, while the administration of a CB1-R antagonist (i.e. SR141716) enhances this effect (Hagues et al., 2007).

Locomotor-stimulating effects of drugs of abuse, included morphine, are thought to be the consequence of drug-induced enhancement of DA release in the striatum (Chiara and Imperato, 1998; Joyce et al., 1983), and CB1-R has shown to modulate DA transmission in this brain area (De Fonseca F et al 2001, Fitzgerald et al., 2012). Therefore, it is possible that the inactivation of CB1-R leads to an enhancement of morphine-induced DA release, and consequently of morphine-induced hyperlocomotor effects. However, such explanation seems unlikely since several studies have shown that morphine-induced DA release in the NAc and striatum was either reduced or unchanged in CB1-R KO mice or in WT mice treated with

a CB1-R antagonist (Mascia et al., 1999; Caillé et al., 2006; Melis et al., 2000). Alternatively, it could be possible that the inactivation of CB1-R enhances the efficacy of DA signalling in the striatum. Thus, CB1-R is highly presents in D1- and D2-expressing striatal neurons (Martín et al., 2007), and a tight functional interaction between CB1-R and DA D2-R has been reported (Kearn et al., 2005). Interestingly, the activation of DA-D2-R increases the release of anandamide in the striatum, and pharmalogical blockade of CB1-R enhances the hyperlocomotor effects of the DA-D2-R agonist quinpirole (Giuffrida et al., 1999). This effect could be due to the absence of anandamide-induced negative feedback control on DA signalling in the striatum (Giuffrida et al., 1999). Some studies suggest that the acute hyperlocomotor effects of morphine are dependent on DA-D2-R activity (Magnus-Ellenbroek et al., 1993; Manzanedo et al., 1999). Therefore, it will be interesting to evaluate whether a similar mechanism can account for the enhanced hyperlocomotor effects of morphine in CB1-R KO mice.

On the other hand, the lack of CB1-R could also enhance the acute stimulating effects of morphine by a DA-independent mechanism since acute morphine injection still produces a small hyperlocomotion in DA-deficient mice (Hnasko et al., 2005). Thus, acute morphine increases enkephalin release in the ventral pallidum, a region that mediates morphine stimulanting effects (Olive et al., 1998; Kalivas et al., 1991; Johnson et al., 2000), and enkephalin increases locomotion in part by DA-independent mechanisms (Kalivas et al., 1983). Interestingly, CB1-R KO mice present an increased expression of enkephalin in the striatum, a region that send important enkephalinergic afferents to the ventral pallidum (Steiner et al., 1999; Olive et al., 1998). Thus, the enhanced hyperlocomotor effects of morphine in CB1-R KO could also result from an

increased morphine-induced enkephalin release and/or enkephalin effects in these animals.

Finally, the endogenous cannabinoid system and the endogenous opioid system have shown to be functionally inter-connected. Both systems mediate similar physiological processes, CB1-R and mu opioid receptor are co–expressed in several brain structures including motor areas, share similar intracellular pathways and can even form heterodimers (Robledo et al., 2008; Vigano et al., 2005; Pickel et al., 2004; Rodriguez et al., 2001; Hojo et al., 2008). Therefore, the deletion of CB1-R could alter the functionality of the endogenous opioid system, and consequently mice sensitivity to the acute hyperlocomotor effects of morphine. In agreement, several studies have reported that CB1-R KO mice present an altered expression and increased activity of kappa and delta opioid receptor in the CNS (Urigüen et al., 2005; La Porta C et al., 2013). Interestingly, when CB1-R and mu opioid receptor are co-expressed in the same cell, the stimulation of one receptor can attenuate the signal transduction of the other receptor (Rios et al. 2006). Similarly, basal activity of CB1-R also attenuates signalling of mu opioid receptor (Canals and Milligan, 2008). This effect is thought to reflect a mechanism named sequestration of G proteins (Vasques and Lewis 1999, Nie and Lewis 2001). Therefore, the deletion of CB1-R could enhance the availability of G-proteins for mu opioid receptor signalling, and consequently increases morphine effects, such as hyperlocomotion.

Further experiments will be necessary to verify if the lack of CB1-R potentiates morphine-induced endogenous opioid or DA signalling in the striatum or other locomotor related regions. This will be important to understand the apparent enhanced hyperlocomotor effects of morphine

observed in CB1-R KO mice. Indeed, this increase was relative to the basal hypoactivity of these mice in this particular genetic background (C57B6/J) (the present study; Li et al., 2009a; Zimmer et al., 1999), but was not an absolute increase. In contrast, it has been shown that genetic deletion of CB1-R in a different strain of mice (CD1) leads to enhance basal locomotor activity (Ledent et al., 1999), and does not alter the acute hyperlocomotor effects of morphine (Martin et al., 2000b).

Genetic deletion of CB1-R prevents sensitization facilitating tolerance to the stimulating effects of morphine during the chronic treatment

Our results show that the genetic deletion of CB1-R prevents the induction of locomotor sensitization to morphine during the chronic treatment, in agreement with a previous study (Martin et al., 2000b). This lack of sensitized responses in CB1-KO mice points out the possible involvement of CB1-R in the plasticity mechanisms mediating the process of behavioural sensitization. In agreement, pharmalogical and genetic blockade of CB1-R has shown to disrupt the induction of sensitization to the locomotor effects of other drugs of abuse, such as cocaine and amphetamine (Corbillé et al., 2007). Thus, the endogenous cannabinoid system could represent a common neurobiological substrate of drug-induced long-lasting behavioural changes. One intracellular mechanism underlying the involvement of the endogenous cannabinoid system in these alterations could be the activation of the ERK signalling pathway. Indeed, the ERK cascade is an important signal transduction of CB1-R activity in the brain (Turu and Hunyady, 2010). Most drugs of abuse trigger the activation of ERK in the mesocorticolimbic system (Valjent et al., 2004) and this

activation is considered an important substrate of drug-induced neuronal plasticity (Girault et al., 2007). In the case of psychostimulants, the induction of locomotor sensitization is mediated by ERK signalling through a CB1-R dependent mechanism (Valjent et al., 2006a; Corbillé et al., 2007). It will be therefore interesting to evaluate the involvement of ERK in morphine sensitization and whether the inability of morphine to activate ERK in the mesocorticolimbic system in CB1-R KO mice can account for the absence of morphine sensitization in these animals.

Our data further reveal that the lack of CB1-R promotes the development of tolerance to the hyperlocomotor effects of morphine after chronic administration. Conversely, other studies have shown that administration of different CB1-R agonists reduces the development of tolerance to the analgesic properties of morphine (Cichewicz and Welch, 2003; Fischer et al., 2010).

At the cellular level, one proposed mechanism mediating tolerance to opioid effects relies on desensitization of opioids receptors, which disrupts receptor-G-protein coupling, dampens signal transduction processes, and reduces the efficacy of opioid agonists to produce their pharmacological effects (Williams et al., 2001). Mechanisms such as protein kinase C-dependent phosphorylation of mu opioid receptor, binding of beta-arrestin-2 to mu opioid receptor, or changes in adenylate cyclase 7 signal tranduction system have been described to participate in morphine tolerance (Bailey et al., 2006; Bohn et al., 2000, Yoshimura et al., 2000). Interestingly, protein kinase C, beta-arrestin-2 and adenylate cyclase 7 are all involved in CB1-dependent signaling processes (Garcia et al., 1998; Jin et al., 1999; Rhee et al., 1998), and cross-tolerance and densitization between

CB1-R and mu opioid receptor has been reported (Garzon et al., 2009). Thus, disruption of CB1-R activity may possibly alter some of these mechanisms, facilitating mu opioid receptor desensitization and accelerating the development of tolerance to morphine hyperlocomotor effects. In agreement, overexpression of adenylate cyclase 7 enhances acute analgesic effects of morphine and accelerates the emergence of tolerance to these effects (Yoshimura et al., 2000). Although, the overexpression of adenylate cyclase 7 also leads to enhance morphine locomotor sensitization (Yoshimura et al., 2000), changes in other common signaling molecules between the endogenous cannabinoid system and the endogenous opioid system, in CB1-R KO mice, could potentially produce similar reciprocal responses between acute and chronic effects of morphine on locomotion.

The emergence of a tolerance process in CB1-R KO mice raises the possibility that the endogenous cannabinoid system does not specifically participate in the neuroplastic mechanisms that mediate morphine sensitization. Indeed, the lack of morphine sensitization in CB1-R KO mice could be due to the reduced efficacy of morphine to engage the cellular processes that trigger the neuroadaptations associated to morphine sensitization, because of a potential accelerated desensitization of mu opioid receptor in these animals after repeated morphine administration. Nevertheless, sensitized locomotor responses were observed as soon as the fourth day of morphine treatment in WT mice, a time point where no tolerant responses were revealed in CB1-R KO mice. Therefore, the lack of sensitized responses in CB1-R KO mice after four day of morphine treatment is unlikely to be directly due to the tolerance of drug effects. Consequently, CB1-R could participate in both morphine tolerance and sensitization. Hence, CB1-R activation could facilitate plasticity

mechanisms associated with morphine sensitization, while its inactivation might promote those related to morphine tolerance. Accordingly, a recent study has reported that sensitization and tolerance to the hyperlocomotor effects of morphine could share common neurobiological substrates. Indeed, delta opioid receptor-KO mice present both enhanced sensitization and decreased tolerance to the locomotor stimulating effects of morphine (Chefer et al., 2009). Interestingly, CB1-R KO mice present an increased activity of delta opioid receptor signaling in the caudate putamen (Urigüen et al., 2005). Therefore, the interaction between CB1-R and delta opioid receptor signaling in the striatum could contribute to establish the balance between the induction of morphine sensitization and tolerance, in response to repeated injection of morphine. Hence, activation of CB1-R could promote sensitization process while its inactivation might impair this process and concomitantly facilitate delta opioid receptor-dependent morphine tolerance process.

Lack of CB1-R reduces late expression of morphine sensitization

We observed that sensitized responses to morphine were still induced in WT mice one week after the disruption of the chronic morphine treatment; confirming that drug sensitization is a long-lasting process that may rely on enduring form of drug-induced neuronal plasticity (Babbini et al., 1975; Robinson and Berridge, 2008). At this time point, tolerant-like responses were no longer observed in CB1-R KO mice, highlighting the shorter-duration of this process. In accordance, another study has shown that tolerance to the stimulating effects of morphine disappears rapidly during drug abstinence (Timár et al 2005). On the other hand, our results suggest

that further neuroadaptations may occur after the disruption of morphine chronic treatment since WT mice displayed greater sensitized responses after drug challenge than during chronic treatment. Accordingly, hyperlocomotor effects of morphine in CB1-R KO mice were not only restored after cessation of chronic morphine treatment, but also sensitized. This latter result suggests that while CB1-R KO mice do not express locomotor sensitization to morphine during the chronic treatment, this drug regimen may nevertheless trigger sensitization-related plastic processes after cessation of drug administration. However, it is important to note that sensitized locomotor responses induced by a challenge dose of morphine were significantly lower in CB1-R KO mice than in WT mice. Together, these findings suggest that CB1-R could play a more prominent role in the neuroadaptations underlying the induction of morphine sensitization during the chronic treatment than those implicated in the late expression of sensitization after a drug-free period. In agreement, pharmacological inactivation of CB1-R blocks cocaine-induced locomotor sensitization when injected during the induction, but not during the expression phase (Corbillé et al., 2007).

Morphine promotes structural plasticity changes through a CB1-R dependent mechanism

We next performed a morphological analysis of the neurons of the NAc shell and core, and mPFC of mice receiving distinct morphine treatments (see methods) to evaluate the involvement of structural plasticity changes in the different phases of morphine sensitization. Our study reveals that morphine-induced structural plasticity is a highly dynamic phenomenon

that continually evolves during the time course of the sensitization process. In our conditions, morphological changes are observed as soon as after the first morphine exposure. Some of these plasticity changes disappear after the disruption of the chronic treatment, while others are maintained or induced during this drug-free period. In addition, our data suggest that the endogenous cannabinoid system could represent a major neurobiological substrate in mediating morphine-induced structural plasticity, as this process is importantly altered in CB1-R KO mice.

We first show that a single administration of morphine rapidly (i.e. 30 min) triggers some structural adaptations in the NAc shell and core of WT mice, such as changes in spine morphology. Drug-induced structural plasticity has been mainly studied following chronic drug regimens (Robinson and Kolb, 2004), and thus initially considered as a form of neuronal plasticity that required repeated drug exposure. Nevertheless, early studies already demonstrated that even a single exposure to some drugs of abuse, such as cocaine and amphetamine, could also produce structural changes in specific areas of the mesocorticolimbic circuit (Sarti et al., 2007; Kolb et al., 2003). However, these changes were revealed 1 or 3 days after drug administration (Sarti et al., 2007; Kolb et al., 2003). This rapid induction is quite surprising considering that structural plasticity has been originally thought to be dependent on gene transcription and de novo protein synthesis, processes that are generally not observed within this short time window (Miyamoto, 2006). However, in vitro studies have shown that structural plasticity can be a rapid dynamic process, and dendritic spines morphology changes and growth have been observed in a time scale of seconds or minutes (Kwon and Sabatini, 2011). In addition, a similar fast onset is considered to occur in vivo since induction of LTP in slice culture

promotes spines enlargement or creation of new ones within 30 min or less (Trommald et al 1996; Tanaka et al., 2008; Bourne and Harris, 2011; Wosiski-Khun et al., 2012). It is now clear that dendrites contain all required elements to support locally de novo protein synthesis (polyribosomes and local mRNA) and posttranslational modification (endoplasmic reticulum and Golgi elements), which can permit rapid production of new functional proteins (Kelleher el, 2004), and structural changes mediated by rapid translational mechanisms have been reported (Tanaka et al., 2008).

Our results suggest that acute morphine-induced rapid structural changes can be mediated by CB1-R. Indeed, these changes appear exacerbated in CB1-R KO mice. Thus, acute morphine produces a global increase of spines density in the NAc shell and core in CB1-R KO mice, whereas the same treatment in WT mice only changes the morphology of pre-existing spines in these same areas. Hence, the "greater magnitude" of the morphological changes observed in CB1-R KO mice may represent an additional evidence for a potential enhanced effect of acute morphine in these animals.

The endogenous cannabinoid system, via CB1-R activation, has shown to mediate different forms of synaptic plasticity in the NAc and mPFC (Sidhpura and Parsons, 2011), and synaptic plasticity can promote the emergence of synaptic structural changes (Matsuzaki et al., 2004; Zhou et al., 2004). Interestingly, a single exposure to cocaine or THC can abolish endocannabinoid-mediated LTD in the NAc (Fourgeaud et al., 2004; Mato et al., 2004). Endocannabinoid-mediated LTD in the NAc has been mainly described at excitatory synapses, and is thought to participate in a negative feedback loop reducing the strength of excitatory synapses during

sustained cortical activity (Fourgeaud et al., 2004; Sidhpura and Parsons, 2011). Thus, drug-induced impairement of this form of plasticity could lead to heightened glutamatergic signaling in this brain region (Sidhpura and Parsons, 2011). Consequently, this process could lead to the generation structural plasticity changes since it has been shown that exogenous infusion of glutamate promotes the rapid growth of dendritic spines (Richards et al., 2005; Kwon and Sabatini, 2011). Therefore, it will be interesting to evaluate whether acute morphine exposure similarly alters endocannabinoid-mediated LTD in the NAc to better understand the underlying mechanism of acute morphine-induced structural plasticity.

The presence of "more important" structural changes in the mesocorticolimbic system of CB1-R KO after acute morphine could be due to the basal disruption of endocannabinoid-mediated LTD, which may possibly enhances the sensitivity of accumbal synapses to undergo activity-dependent changes in glutamatergic transmission. In support to this proposal, several studies have shown that high-frequency stimulation produces stronger LTP at excitatory synapses in the HPC and basolateral amygdala in CB1-R KO than in WT mice (Bohme et al., 2000; Marsicano et al., 2002; Jacob et al., 2002).

Mechanisms implicating BDNF, ERK or mTOR signaling participate in local dendritic translation, and have been proposed to support rapid induction of synaptic and/or structural plasticity changes (Besse and Ephrussi, 2008; Swanger et al., 2011; Harvey et al 2008; Li et al., 2010a). Moreover, morphine can trigger activation of mTOR pathway (Polakiewicz et al 1998), enhance BDNF expression by microgial cells (Takayama et al., 2005, Matsushita et al., 2013), and activate ERK in the NAc (Valjent et al., 2004).

Interestingly, mTOR, ERK and BDNF also participate in CB1-R signaling (Puighermanal et al., 2010; Corbillé et al., 2007; De Chiara et al 2010). Therefore, morphine could activate one or several of these mechanisms through a CB1-R dependent mechanism stimulating the rapid translational machinery in dendritic spines, and leading to rapid structural plasticity changes in the NAc.

Alternatively, the rapid development of synaptic and structural plasticity can be independent on gene expression and de novo protein synthesis (Wosiski-Khun et al., 2012). Indeed, other mechanisms such as rapid actin restructuration (Okamoto et al., 2004; Kramar et al., 2006) or membrane trafficking from recycling endosomes (Park et al., 2006) have also been proposed to account for fast structural plastic events. Interestingly, acute morphine administration increases the expression of the metalloproteinase-9 (Liu et al., 2012), an extracellular protease that promote rapid consolidation-like process of LTP and rapid structural synaptic remodeling, through the modulation of actin polymerization in dendritic spines (Huntley, 2012). Of importance, activation of metalloproteinase-9 can even drive structural synaptic remodeling in the absence of LTP-inducing stimuli (Huntley, 2012). In a similar way, acute morphine administration increases expression of the tissue plasminogen activator in the NAc (Nagai et al., 2004), a protease of the extracellular matrix that is rapidly released during LTP and can also mediate rapid structural plasticty changes (Lochner et al., 2006; Oray et al., 2004). Interestingly, tissue plasminogen activator KO mice display reduced acute morphine hyperlocomotion (Nagai et al., 2004). A direct involvement of CB1-R in rapid actin reorganization has not been yet investigated, although one study reports that CB1-R activation can modulate metalloproteinase-9

activity (Mukhopadhyay et al., 2011). Therefore, these mechanisms could also account for the fast induction of structural changes by a single morphine exposure.

Further studies will be necessary to determine the functional consequences of these structural changes induced by acute morphine administration. Nevertheless, our results indicate that these CB1-R-dependent changes are rapidly induced suggesting that morphological spines plasticity in the NAc core and shell could participate in the acute hyperlocomotor effects of morphine. In agreement, CB1-R KO mice that are more sensitive to these hyperlocomotor effects also present structural changes of "greater magnitude" in these brain regions. Furthermore, the induction of morphine sensitization is known to be dependent on the development of neuroadaptations in the VTA, but also in other regions of the mesocorticolimbic system including the NAc (Cunningham et al., 1997; Vanderschuren and Kalivas, 2000). Thus, acute morphine-induced spines rearrangement in the NAc core and shell could represent one these neuroadaptations, and account for the sensitized locomotor responses induced by subsequent morphine injection in WT mice. It would be interesting to know whether the stability of these structural changes is different between WT and CB1-R KO mice. Indeed, it is possible that these changes persist during the chronic morphine treatment in WT mice promoting locomotor sensitization, whereas in CB1-R KO mice they could rapidly wane preventing the development of sensitization.

Role of CB1-R in the structural changes associated to chronic morphine treatment and re-exposure

Structural changes induced by chronic morphine in different areas of the mesocorticolimbic circuit, including the NAc and mPFC, have shown to persist at least up to one month (Robinson et al., 1999 and 2002). These changes could therefore contribute to the long-lasting maintenance of behavioural sensitization to morphine (Robinson and Kolb, 2004). Surprisingly, we did not observe any significant structural change in the NAc shell and core in WT mice 7 days after the cessation of the chronic morphine treatment in our experimental conditions. Differences in the technique used to stain neurons (Golgi-Cox vs. DiI labelling) that can influence the result of the quantification (Shen et al., 2009), as well as in the animal model (rats vs. mice) and the experimental protocol (self-adiministration and non-contigent repeated injection vs. context-associated behavioural sensitization) could explain these discrepancies. Additionally, the time point at which the morphological analysis was performed (one month vs. one week after the last morphine injection) may also account for the differences between both studies. In fact, studies made at different time points after chronic cocaine administration suggest that drug-induced synaptic functional changes in NAc can be a highly time-dependent process (Russo et al., 2010). Thus, while early cocaine withdrawal times seem associated with increased synaptic depression and silent synapses that often engaged immature spines (Thomas et al., 2001; Huang et al., 2009), prolonged abstinences have been related to synaptic potentiation and a progressive increase in post-synaptic expression of AMPA-R that are characteristics of large spines (Boudreau et al., 2007; Conrad at al 2008). Interestingly, these latter changes have been related to the incubation of

cocaine craving (Conrad at al 2008). These findings indicate that abstinence from chronic cocaine exposure is associated with a transition from immature to large spines, thus an evolution of the structural plasticity changes triggered by drug during abstinence. A similar process could explain the tendency of an increased spines density in the NAc shell that we observed after one week of morphine abstinence, while previous studies found a decreased spines density in this brain area after one month of morphine abstinence (Robinson et al., 1999 and 2002). Together, these data suggest that morphine induced structural plasticity in the NAc could be a dynamic process that evolves after disruption of the chronic treatment, which might potentially underline an incubation of locomotor sensitization (Vanderschuren et al., 1999; Robinson and Berridge, 2008).

On the other hand, we observed that WT mice chronically treated with morphine present an increased spines density in the mPFC one week after the end of this drug treatment. The persistence of these structural changes in the mPFC across morphine abstinence may possibly support the long-term maintenance of the sensitization process. However, this interpretation is speculative since we did not evaluate whether these morphological changes were present during the chronic treatment or were triggered during morphine abstinence. Previous studies have shown that abstinence from a chronic morphine treatment is associated with decreased spines density in the mPFC (Robinson et al., 1999 and 2002). As described above, these discrepancies may be explained by several differences in the experimental procedures, or could reflect an evolution of these changes during drug abstinence. In this view, one study has observed an increase of dendritic spines density in the mPFC just after 15 days of morphine self-administration (Ballesteros-Yáñez et al., 2007b). Together, these results

suggest that repeated morphine administration is associated with increased spines density in the mPFC (Ballesteros-Yáñez et al., 2007b) that is maintained during early withdrawal (our results) and then reverses at later stage of abstinence (Robinson et al., 1999 and 2002). Similarly to the NAc shell, this evolution in morphine-induced structural plasticity changes in the mPFC after the disruption of chronic morphine treatment could also underscore an incubation of morphine sensitization.

Furthermore, we observed that re-exposure to morphine after one week of drug-free period produces structural changes in all the three studied areas in WT mice. Indeed, this challenge injection increases spines density in the NAc shell and core and reverts the structural changes triggered by the disruption of chronic morphine treatment in the mPFC. Similarly to the acute morphine administration, this challenge injection induces rapid structural plasticity changes (30 min). However, the extent of these changes is substantially more important since acute morphine only modifies spines morphology in the NAc shell and core. These results highlight that chronic morphine exposure and subsequent abstinence increase the propensity of the mesocorticolimbic circuit to undergo structural plasticity changes following morphine injection. Morphine sensitization is thought to rely on the facilitation of morphine-induced DA release in the mesocorticolimbic system after repeated exposure to this drug (Acquas et al., 1992; Spanagel et al., 1993). Accordingly, we can hypothesize that the enhancement of morphine-induced structural plasticity in the NAc shell, core and mPFC, after chronic morphine treatment and abstinence, could also represent an underlying mechanism of morphine sensitization. Conversely, a similar process has been described with cocaine (Shen et al., 2009). Indeed, both acute cocaine administration

and challenge injection of cocaine after abstinence from a chronic treatment produce a rapid increase in spine density in the NAc core. However, the acute cocaine-related structural changes have a slower induction phase and are qualitatively different from those induced by a challenge injection of cocaine (Shen et al., 2009).

Finally, our results suggest that different neurobiological mechanisms could mediate the structural plasticity changes in the NAc and mPFC induced by chronic morphine treatment and morphine re-exposure. Indeed, both CB1-R KO and WT mice present an increase in dendritic spines density in the mPFC after one week of abstinence from chronic morphine treatment, and subsequent challenge injection similarly decreases spines density in this region in both genotypes. Therefore, these structural changes in the mPFC seem to be largely independent of CB1-R activity and could possibly account for the development of sensitized locomotor responses to a challenge dose of morphine in CB1-R KO mice. In contrast, challenge injection of morphine after the withdrawal of the chronic treatment produces significant changes in total spines density in the NAc shell and core in WT mice, but not in CB1-R KO mice. Thus, the structural plasticity changes triggered by this challenge injection of morphine in the NAc core and shell seem to be mediated by CB1-R. The absence of these neuroadaptations in CB1-R KO could account for the reduced expression of morphine sensitization observed in these mutants following morphine re-exposure.

The underlying cellular mechanisms of drug-induced structural plasticity and the contribution of these structural changes in the development of addictive behaviours are still poorly understood (Russo et al., 2010). Our

results suggest that structural changes can contribute to the initial effects of morphine as well as the long-term maintenance of the behavioural alterations triggered by repeated exposure to this drug. In addition, our data reveal that morphine-induced structural plasticity can be a highly dynamic phenomenon in contrast to what it was initially thought (Robinson and Kolb, 2004). A better knowledge of the functional consequences of the evolution of drug-induced structural plasticity may be important to understand the distinct behavioural alterations that characterized the different periods of the addiction cycle. Finally, our results highlight the relevance of the endogenous cannabinoid system in morphine-induced structural plasticity changes, which may help to lead future research in determining the CB1-R downstream effectors that mediate these alterations.

C. CHARACTERIZATION OF THE SYNAPTIC PROTEOMIC CHANGES THAT UNDERLINE THE RETRIEVAL OF DRUG AND PALATABLE FOOD REWARD MEMORIES

The persistence of drug craving and relapse after abstinence is a major behavioural change associated to addiction that makes difficult the treatment of the disease (Weiss, 2005). Similarly, some obese patients report to experience food craving, especially for palatable foods, and their recurrent failure in maintaining an appropriate diet has been viewed as a form of food relapse (Volkow and Wise, 2005; Wilson, 2010). Our previous results have provided new evidences supporting the hypothesis that drugs of abuse and palatable food can share common neurobiological substrates and that similar neuronal adaptations may participate in the development of addictive-like behaviours and food-seeking behaviour alterations (Volkow et al., 2012; Avena et al., 2009). In the last part of the present thesis, we were interested to study the neurobiological basis of drug of abuse and palatable food craving and uncover a possible common neuronal mechanism between both phenomena.

Craving and relapse are often triggered in drug addicts by re-exposure to drug associated environmental stimuli, such as drug paraphernalia (e.g. syringes, crack pipes) and place or people, where or with whom they used to take drugs (Carter and Tiffany, 1999). Similarly, palatable food smell, sight and TV commercial strongly challenge obese willpower to absent from medically advised forbidden foods (Fortuna, 2012). Critical for craving and relapse is the process of associative learning, whereby environmental stimuli repeatedly paired with drug or palatable food intake

acquire incentive motivational value. Consequently, re-exposure to these conditioned environmental stimuli evokes expectation of drug or palatable food availability as well as hedonic memories derived from past experiences that can lead drug addicts and obese people to experience strong craving and to relapse (Everitt et al., 2001). The CPP paradigm represents an appropriate model to evaluate this process, and specific CPP protocols have been designed to mimic some features of craving and relapse (Martin-Fardon and Weiss, 2013). The formation of reward-related memories in the CPP paradigm includes the acquisition during the conditioning phase, in which associations reward/context are progressively consolidate into long-term memories, and the retrieval during CPP tests, in which previously learned associations are rendered labile and must be reconsolidated to be converted in stable and persistent memory traces (Tronson and Taylor 2007). Previous studies have shown that pharmalogical manipulations during this reconsolidation period can alter the maintenance of contextual drug memories (Valjent et al., 2006b; Milton and Everitt, 2012). A better understanding of the neurobiological mechanisms mediating drug and palatable food conditioned cues memory reconsolidation may be useful for the development of novel therapeutic strategies to limit craving and relapse.

Synaptic plasticity represents a neuronal substrate of learning and memory (Martin et al., 2000) and some evidences have directly linked plasticity changes in accumbal synapses with the development of drug craving (Conrad et al., 2008). Thus, we have focused our study on determining the common synaptic mechanisms underlying the retrieval/reconsolidation of drug and palatable food conditioned cues evoked reward-related memories. For this purpose, we used an adapted form of the CPP paradigm

that has been previously used to evaluate drug memories reconsolidation (Valjent et al., 2006b), and performed a quantitative protein and phospho-protein analysis of synaptoneurosomes extracted from the NAc and the HPC of mice re-exposed to the CPP compartment where they have previously experienced cocaine, morphine or palatable food reward.

As previously described (Martin et al., 2000b; Valjent et al., 2006b; Perello et al., 2009), our results show that cocaine, morphine and palatable food promote the acquisition of a CPP paradigm, confirming that all these three stimuli produce rewarding effects in mice. However, our data also indicates that CD1 mice present intrinsic variability in their performances on this task. Indeed, while each experimental cohort expresses globally a significant place preference for the cocaine, morphine or palatable food conditioned compartment in the Test 1, a substantial number of mice from these different groups displays a CPP score similar or inferior to the average CPP score recorded in the saline conditioned group at this time point. Different factors may have prevented the acquisition of the CPP task in those latter mice including anxiogenic effects or low discriminative properties of the CPP apparatus. Indeed, these factors could impair the establishment of accurate conditioned associations in mice that are more vulnerable to stress or have poor learning abilities. Alternatively, mice's interindividual differences in reward sensitivity could possibly account for the variability of CPP performances observed in our study. In this regard, several studies have demonstrated that rodents are intrinsically different in face of drug effects. Thus, high-responding rats to novelty display a greater propensity to acquire psychostimulant self-administration (Piazza et al., 1989), a higher cocaine intake whatever the dose tested (Piazza et al., 2000) and are more vulnerable than low-responding rats to the induction

of behavioural sensitization produced by repeated injections of amphetamine (Hooks et al., 1992). The hypothesis that drugs have an increased efficacy in high-responding rats is supported by the enhanced propensity for cocaine-induced DA release in the NAc in these animals (Hooks et al. 1991). In addition, high impulsive rats show greater propensity to develop compulsive drug taking in the face of aversive consequences than low impulsive ones (Belin et al., 2008). Interindividual differences in drug responses observed in experimental animals are indeed reminiscent of the concept of vulnerability to drug addiction in humans. In fact, not all drug users become addicts and the predisposition to drug addiction has been associated with several distinct personality traits, including sensation seeking, anxiety, and impulsivity (Franques et al., 2000; Lejuez et al., 2008; Ersche et al., 2010).

This variability of CPP performance on Test 1 is an important experimental factor that we took in consideration to design our proteomic analysis. Thus, we distinguished good and bad performers on CPP Test 1 according to the median CPP score of each experimental group in agreement with previous studies that discriminate high and low responders to novelty (Piazza et al. 1989). We sought that this segregation could allow to differentiate mice that did not acquire the CPP task from those that had established stable reward/context association. In agreement, our data reveal that good performers conditioned to cocaine, morphine or palatable food express strong CPP behaviour on Test 1 that is maintained two days later on Test 2. In contrast, bad performers do not exhibit such conditioned responses neither on Test 1 nor on Test 2. Further studies will be necessary to better define the phenotypical differences between good and bad performers that can account for their distinct performances in the CPP test.

Thus, the comparison of their performances on drug and palatable food operant task could clarify whether these two sub-groups present intrinsic differences in reward sensitivity.

Taking advantage of our CPP model, we performed our proteomic and phosho-proteomic screening analysis in synaptoneurosomes extracted from good performers, to unravel more accurately the synaptic mechanisms mediating drug and palatable food conditioned cues-evoked hedonics memories. This analysis identified several proteins, kinases and intracellular pathways involved in these responses. We have mainly focused our interest in the synaptic processes that were similarly regulated by re-exposure to cocaine, morphine and palatable food conditioned cues to intend to define a potential common synaptic mechanism underlying drug and palatable food craving. Our analysis shows that re-exposure to drug and palatable food conditioned stimuli were associated with common changes in the expression of proteins involved in neurotransmission. In particular, we identify a down-regulation of CAPS-2 in the HPC, and down-regulation of the non-catalytic subunit of Rab3 GAP and mGluR7 in the NAc. CAPS-2 is an important component of the synaptic vesicles priming machinery that supports phasic calcium triggered transmitter release. The regulation of this process controls the strength and plasticity of synaptic transmission (Jockusch et al, 2007). Therefore, the down-regulation of CASP-2 in the HPC suggests a decrease of the release of synaptic vesicles and probably a diminished synaptic transmission strength in this brain area. Similarly, Rab3 GAP is regulator of Rab3A activity, a small G protein involved in trafficking cycle of synaptic vesicles at nerve terminals that plays an important role in neurotransmitter release (Sakane et al, 2006). Deletion of Rab3 GAP catalytic subunit decreases expression of its non-

catalytic subunit and inhibits calcuim-dependent glutamate release (Sakane et al, 2006). Thus, the down-regulation of Rab3 GAP non-catalytic subunit may also suggest a decreased synaptic transmission in the NAc. Conversely, presynaptic mGluR7 activation depresses synaptic transmission at a variety of central synapses (Losonczy et al., 2003, O'Connor et al., 1999; Perroy et al., 2002a), and leads to a rapid internalization of mGluR7 (Pelkey et al, 2007). Therefore, the decreased expression of mGluR 7 detected in our experiment could result from this internalization process, which may further support a decreased synaptic transmission in the NAc following re-exposure to drug and palatable food conditioned cues. Changes in regulatory mechanisms occurring in the presynaptic compartment, such as synaptic vesicle availability and release are considered to be key processes in mediating short-term and long-term forms of synaptic plasticity (Fioravante and Regehr WG 2011, Zakharenko et al, 2002). Thus, we can speculate that the induction of short-term depression or LTD in the HPC and NAC could represent a common neuronal event underlying the re-activation of reward-related memories associated to both drug of abuse and palatable food.

Alternatively, the changes cited above may also reflect an enhanced synaptic transmission. Indeed, high-frequency stimulation can result in synaptic vesicle depletion, and their reappearance can be time-demanding (Heuser and Reese, 1973). A similar process could explain the decreased expression of CASP-2 and Rab3 GAP observed in our study after the CPP re-exposure session. In addition, internalization of mGluR7 can facilitate the emergence of LTP at some synapses (Pelkey et al., 2005). Further studies will be necessary to accurately determinate the consequences of these

changes on synaptic transmission. A previous study has shown that re-exposure to cocaine and its associated context can trigger LTD in the NAc shell, in a behavioural sensitization paradigm (Thomas et al., 2001; Brebner et al., 2005). In contrast, re-exposure to discrete stimuli (light/tone) associated to cocaine self-administration has been associated with the emergence of synaptic potentiation in the NAc core (Gipson et al., 2013). Thus, the re-activation of drug memories can be mediated by different synaptic plastic processes depending on the past drug regimen, modality of the conditioned stimuli and/or the brain region. Our analysis was performed in the whole NAc and it will be of interest to determine whether the proteomic changes observed in our study differ between shell and core.

A role of CASP-2 or Rab3 GAP in drug and palatable food reward has not been investigated yet. In contrast, several studies have reported that mGlur7 plays an important role in the development of addictive-like behaviours. Indeed, systemic administration of a mGluR7 agonist reduces intravenous cocaine self-administration and decreases ethanol consumption in rodents (Li et al., 2009b; Bahi et al., 2012b). The activity of mGluR7 in the NAc seems of particular importance since local infusion of a mGluR7 agonist in this region is sufficient to inhibit cocaine self-administration (Li et al., 2009b), while knockdown of mGluR7 expression in the NAc enhances ethanol-induced CPP and alcohol consumption (Bahi, 2012b). Importantly, activation of mGluR7 in the NAc inhibits priming injection induced reinstatement of cocaine-seeking behaviour (Li et al., 2010b) suggesting that decreased activity of mGluR7 in the NAc could be a neurobiological substrate of drug relapse. In accordance, we show that re-exposure to cocaine and morphine conditioned contextual cues, an event that also promotes reinstatement of drug-seeking behaviour, decreases the

expression of mGluR7 in the NAc and thus probably its activity in this brain region. Our data also reveal that a similar neuronal mechanism in the NAc is triggered by re-exposure to palatable food conditioned contextual cues. Together these findings indicate that decreased activity of mGluR7 in the NAc could be a common neuronal process underlying the re-activation of hedonic memories and presumably a neuronal substrate for drug and palatable food craving and relapse.

On the other hand, we observed that re-exposure to both drug and palatable food conditioned stimuli modify the expression level of proteins of the cytoskeleton. Thus, we detected an increased expression of tubulin beta 2A in the HPC and alpha-spectrin in the NAc. Tubulin beta 2A is one of the main beta-tubulin isotype in the brain, which forms part of the neuronal microtubules network. Alpha-spectrins interact with beta-spectrin subunits to form the spectrin skeleton. Microtubules and spectrins are expressed in both the pre- and post-synaptic compartments participating in the transport of vesicles, organelles, transmitter receptor and other structural components of synapses. In addition, both proteins interact with the actin cytoskeleton to regulate synapses morphology (Gu and Zheng, 2009; Goodman and Zagon, 1986). It has been shown that the loss of presynaptic alpha-spectrin leads to presynaptic retraction and synapse elimination (Pielage et al., 2005). Consequently, presynaptic spectrins are thought to be important for synapse stability (Pielage et al., 2005). Hence, an increased expression of alpha spectrin may promote the consolidation and persistence of synaptic connections. On the other hand, microtubules are rarely observed in dendritic spines, but strong tetanic stimulation used to induce LTP causes an entry of microtubules into dendritic spines in the HPC (Mitsuyama et al., 2008) and this entry has

been related to dendritic spines growth and enlargement (Jaworski et al., 2009). Thus, our proteomic analysis suggests that the retrieval of drug and palatable food conditioned memories could be associated with the development of synaptic structural changes in the NAc and HPC. Changes such as enhanced stability of synapses and enlargement of dendritic spines are reminiscent of the morphologic changes associated to LTP (De Roo et al., 2008; Harris et al., 2003). This observation further supports that re-exposure to drug and palatable food conditioned cues could trigger the potentiation of synaptic transmission in the NAc and HPC. In agreement, cue-induced reinstatement of cocaine-seeking behaviour has been associated with rapid enlargement of dendritic spines and synaptic potentiation in the NAc core (Gipson et al., 2013). However, similar changes were not observed with re-exposure to cues predicting sucrose in this study (Gipson et al., 2013).

In addition, our analysis highlights changes in the phosphorylation state of several proteins involved in the regulation of cytoskeleton dynamics, which further support the emergence of structural changes after re-exposure to drug and palatable food conditioned cues. Thus, we observed a decreased JNK-dependent phosphorylation of MARCKS-related protein in the HPC. This phosphorylation enables MARCKS-related protein to bundle and stabilize F-actin, while inhibition of this phosphorylation increases actin motility and formation of cellular projection (Björkblom et al., 2012). In the NAc, we observed a decreased cdk-5 dependent phosphorylation of dihydropyrimidinase-related protein 1 (DRP-1), a protein involved in semaphorin 3A induced spine growth (Yamashita et al., 2007). This phosphorylation decreases DRP-1 binding affinity to tubulin, thereby enabling spines rearrangement (Yamashita et al., 2007). The removal of

this phosphorylation that strengthens DRP-1/tubulin association should possibly stabilize spines structures. Moreover, we detected a decreased PKA dependent-phosphorylation of tubulin polymerization-promoting protein (TPPP) in the same brain area, a protein involved in the polymerization of tubulin into microtubules and stabilization of existing microtubules (Hlavanda et al., 2007). The functional consequence of this phosphorylation is not known, but could possibly modulate the function of TPPP in regulating microtubules polymerization/stabilization and thereby spines morphology and/or stability. In addition, we also observed an increased phosphorylation of alpha adducin in the NAc. Adducin controls actin polymerization by capping the fast-growing ends of actin filaments and promotes the interaction of actin with the cytoskeletal protein spectrin. The regulation of adducin activity alters both the stability and the morphology of synapses (Stevens and Littleton, 2011). Although we do not know the consequences of the phosphorylation detected in our study, it has been shown that once alpha adducing is phosphorylated, it is removed from the barbed end of the actin filament allowing the actin filament to either polymerize or depolymerize and permitting spines rearrangement or creation (Matsuoka et al. 1998; Hotulainen et al., 2009). Interestingly, phosphorylation of alpha adducin in the NAc has been described to occur in response to cocaine exposure (Lavaur et al., 2009). In addition, other studies show that beta-adducin KO mice that present changes in expression and phosphorylation levels of alpha-adducin also present basal alterations in spines morphology in the NAc and enhanced cocaine-induced structural plasticity in this region (Porro et al 2009, Jung et al 2013). Additional experiments would be necessary to evaluate the nature of the possible

structural changes triggered by re-exposure to drugs of abuse and palatable food conditioned cues.

One possible mechanism underlying these synaptic and structural plasticity related changes triggered by re-exposure to drug and palatable food conditioned cues may be the involvement of BDNF signalling, at least in the HPC. Indeed, CAPS-2 is also associated to secretory vesicles and mediates the depolarization-dependent release of neurotrophins such as BDNF (Sadakata et al., 2004). Interestingly, the expression of amphetamine-induced CPP has been linked to an increase expression of TrkB receptors in the HPC, which are activated by BDNF (Shen et al., 2006). BDNF promotes the induction of LTP, contributes to memory consolidation, triggers rapid spines morphological changes and participates in those mediated by drugs of abuse (Mizuno et al., 2000; Monteggia et al., 2004; Russo et al., 2009; Swanger et al., 2011). Thus, changes in the expression of CAPS-2 detected in our study could reflect that the retrieval of drug and palatable food related memories commonly rely on the activity of BDNF in the HPC. BDNF-induced synaptic plasticity may be in part due to the ability of BDNF to enhance mitochondrial energy production (Mattson et al., 2008). Interestingly, we also observed in our analysis that re-exposure to drug and palatable food conditioned stimuli is associated with changes in protein involved in mitochondrial ATP metabolism in the HPC. Indeed, we detected changes in the expression of the 6.8 kDa mitochondrial proteolipid, a direct component of the ATP synthase complex (Meyer et al., 2007), and in the expression of the calcium-binding mitochondrial carrier protein SCaMC-3, which acts as a ATP-Mg/Pi exchanger in the mitochondria (Bassi et al., 2005). ATP generated by mitochondria plays an essential role in several synaptic processes including neurotransmitter accumulation and release

and trafficking of post-synaptic receptors. Therefore, mitochondria are considered important modulators of synaptic functional plasticity (Manji et al., 2012). The changes in mitochondrial functions observed here may represent another evidence for the involvement of synaptic plasticity processes in mediating retrieval of drug and palatable food reward memories. Most of the observed changes in mitochondrial proteins were related to an increased expression of these proteins in the HPC, suggesting an enhanced number of mitochondria at the synapse. Delivery of mitochondria into dendritic protrusions promotes morphological plasticity and increased dendritic mitochondrial content enhances the number and plasticity of spines and synapses (Li et al., 2004b). Thus, a possible increased synaptic content of mitochondria following drug and palatable food cues re-exposure can also account for the emergence synaptic structural changes.

Our analysis suggests a possible involvement of neurosteroids in the mnemonic processes related to drug and palatable food reward. Indeed, we detected changes in the expression of the protein striatin-4 in the HPC, a protein described as a mediator of the rapid and non-genomic cellular effects of estrogen, among other functions (Lu et al., 2004). In addition, we observed changes in the phosphorylation state of prostanglin synthase E2 in the NAc. This CK2-dependent phosphorylation modulates the association of prostagladin synthase E2 with heat shock protein 90 (Kobayashi et al, 2004), and this protein complex regulates glucocorticoid receptor dependent transcriptional activities (Lovgren et al., 2007). Interestingly, both estrogens and glucocorticoids promote synaptic and structural plasticity (Srivastava et al., 2011; Krugers et al., 2010). In addition, estrogens can induce the formation of novel dendritic spines within 30 min

through a rapid and non-genomic modulation of protein translation in dendritic spines (Srivastava et al., 2011). This process can possibly account for the modulation of cytoskeletal proteins observed in our study, after short re-exposure (20min) to drug and palatable food conditioned context. Of importance, several studies linked both estrogens and glucocorticoids activity with the acquisition of drug-induced CPP. Thus, infusion of a glucocorticoid receptor antagonist into the HPC or NAc prevents morphine-induced CPP (Dong et al., 2006), whereas estrogens enhance cocaine-induced CPP and promote reinstatement of cocaine-seeking behaviour (Segarra et al., 2009; Larson et al., 2005). In agreement, our results suggest that estrogens and glucocorticoids signalling could be common neurobiological mechanisms underlying the re-activation of drug and palatable food rewarding memories.

On the other hand, our analysis identified several protein kinases including JNK, PKA, Cdk5 and CK2 that might contribute to the neuroplasticity changes triggered by re-exposure to drug and palatable food conditioned cues. However, a decreased phosphorylation in the protein substrates of these kinases was detected in our study. Thus, it would be of interest to also determine which phosphatases could have mediated these changes, although our bibliographic research did not permit to obtain such information. In our study, the protein substrates of JNK, PKA and cdk5 that present phosphorylation changes are proteins involved in the control of cytoskeleton dynamic. Therefore, these kinases may contribute the retrieval of drug and palatable food reward memories by mediating the hypothetical structural changes underlying these processes. In support to this hypothesis, the activity cdk5 has been implicated in cocaine-induced structural plasticity (Norrholm et al., 2003) and in the expression of

cocaine-induced CPP (Li et al., 2010c). Similarly, PKA activity is required to stabilize the enlargement of spines that accompanies the induction of LTP (Yang et al., 2008), and is important for the consolidation of stimulus-drug reward associations (Cervo et al., 1997). PKA signalling in the NAc mediates incentive value elicited by both food and drug of abuse (Beninger and Gerdjikov, 2006). In contrast, previous data show that JNK signalling does not play an important role in the acquisition of psychostimulants-induced CPP (Gerdjikov et al., 2004; Chen and Xu, 2010), and no changes in the activity of this signalling pathway are observed during reinstatement of cocaine-induced CPP (Chen and Xu, 2010). We found a decrease JNK-dependent phosphorylation of MARCKS-related protein in the HPC after re-exposure to cocaine, morphine and palatable food conditioned CPP compartment. It will be therefore of interest to identify the phosphatase potentially responsible for this decreased phosphorylation. However, it is not excluded that decreased activity of JNK could account for this decreased phosphorylation in the HPC, as the activity of JNK during the acquisition and/or reinstatement of psychostimulants-induced CPP has not been evaluated in this region (Gerdjikov et al., 2004; Chen and Xu, 2010). In the case of CK2, few evidences linked its activity with drug reward. Nevertheless, it has been recently reported that CK2 may be an important component of the striatal signalling pathways by which food and drugs of abuse regulate nucleosomal response, and thereby promote motivated behaviours (Stipanovich et al., 2008). In agreement, our results confirm that CK2 signalling in the NAc could be a common neuronal substrate for drugs and palatable food effects.

Finally, our analysis highlights several synaptic protein changes in the NAc and HPC that were specifically triggered by cocaine and morphine

conditioned cues, but not by palatable food related ones. A better knowledge of the function of these proteins in the physiological activity of hippocampal and accumbal synapses could help to characterize the differences between the re-activation of drug and palatable food reward memories. Indeed, while accumulating evidences support the hypothesis that drug of abuse and palatable food craving and relapse share similar neurobiological substrates (Nair et al., 2009), other data highlight differences in the neuronal mechanisms underlying these responses. Thus, cocaine-associated contextual stimuli elicit reinstatement for one year, whereas the same stimuli paired with a palatable sweet solution remain effective for only three months (Ciccocioppo et al., 2004). In addition, systemic administration of a group II metabotropic glutamate receptor agonist is more efficient at reducing contextual cues induced reinstatement of cocaine-seeking behaviour than contextual cues induced reinstatement of palatable food-seeking (Baptista et al., 2004).

In summary, our study identified several synaptic proteins which expression and/or phosphorylation state is commonly modulated by re-exposure to cocaine, morphine and palatable food conditioned contextual cues. These experimental conditions may mimic some features of drug and palatable food craving (Martin-Fardon and Weiss, 2013) and our data may be of relevance to define a hypothetical common neurobiological mechanism underlying these processes. Our results highlight the importance of several presynaptic proteins involved in neurotransmssion, and components of cytoskeleton and its regulatory proteins that control synaptic morphology in the retrieval/reconsolidation of drug and palatable food reward memories, suggesting a possible involvement of synaptic and structural plasticity changes in these processes. In addition, our results

suggest that these neuroplastic changes in the HPC and NAc could be mediated by BDNF signalling, mitochondrial proteins activity, neurosteroids such as estrogens and glucocorticoids, as well as protein kinases such as JNK, PKA, cdk5 and CK2. To further validate our protein candidates, it will be interesting to evaluate whether re-exposure to cocaine, morphine and palatable conditioned cues fail to induce similar synaptic changes in animals that did not previously acquire the CPP task (i.e. bad performers). Additionally, the evaluation of these synaptic changes in other animal models of craving/relapse, such as cue-induced reinstatement of drug or palatable food operant seeking behaviour may be crucial to support our results. Finally, our data also revealed several synaptic proteins changes in the NAc and HPC that differentially participate in the retrieval of drug and palatable food reward memories. In general, our screening analysis may help future studies to better understand the neurobiology of drug of abuse and palatable food craving, defining their similarities and differences.

CONCLUSIONS

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

1. Palatability, but not caloric content, is a determinant factor that enhances food motivation and intake over satiety. Prolonged operant training with palatable food, but not with high caloric diet, promotes food-seeking behaviour changes that are reminiscent of the behavioural alterations observed with extended drug self-administration.

2. Food-seeking behaviour changes triggered by palatable food are associated with the emergence of structural plasticity changes in the NAc shell and mPFC. Similar neuronal alterations have been related to the development of addictive-like behaviour.

3. The endogenous cannabinoid system, through CB1-R, mediates both palatability-induced rewarding effects and the perception of the caloric value of food. Genetic deletion and local inactivation of CB1-R in the NAc prevent palatable food-induced structural plasticity and food-seeking behaviour changes.

4. The CB1-R dependent activation of ERK signalling pathway in the mesocorticolimbic system participates in the neuronal and behavioural alterations triggered by palatable food.

5. The endogenous cannabinoid system, through CB1-R, is an important neurobiological substrate for the development of behavioural sensitization induced by morphine. CB1-R modulates both the acute hyperlocomotor effects of morphine and the

induction and expression of morphine sensitization triggered by chronic morphine treatment.

6. Both acute and chronic morphine treatment promote structural plasticity changes in different parts of the mesocorticolimbic system through a CB1-R dependent mechanism. The rapid induction of these structural changes in the mesocorticolimbic system suggests their direct involvement in the hyperlocomotor effects of morphine. The magnification of these changes after chronic treatment could represent an underlying mechanism of morphine locomotor sensitization.

7. Swiss albino mice present interindividual differences in reward sensitivity and propensity to elaborate long-lasting conditioned memories that were revealed in our CPP paradigm.

8. In the NAc and HPC, we identify several presynaptic proteins involved in neurotransmission and cytoskeleton regulation as common neurobiological substrates mediating the retrieval of cocaine, morphine and palatable food reward memories. Our proteomic analysis further suggests the involvement of BDNF, estrogens and glucocorticoids signalling, as well as JNK, PKA, cdk5 and CK2 in the retrieval of drug and palatable food reward memories.

9. CB1-R dependent structural plasticity changes in the mesocorticolimbic system could represent a common neurobiological substrate for drug addiction and some eating disorders. The characterization of the CB1-R downstream effectors

mediating these neuronal alterations could facilitate the design of new therapeutic approaches for both pathologies that minimize the side effects of previous CB1-R targeting treatments.

REFERENCES

Acquas E, Di Chiara G (1992)Depression of mesolimbic dopamine transmission and sensitization to morphine during opiate abstinence. J Neurochem. 58(5):1620-5.

Aguilar MA, Rodríguez-Arias M, Miñarro J (2009) Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. Brain Res Rev 59:253-277.

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science. 282(5387):298-300

Ahmed SH, Cador M (2006) Dissociation of psychomotor sensitization from compulsive cocaine consumption. Neuropsychopharmacology. 31(3):563-71.

Alfonso J, Fernández ME, Cooper B, Flugge G, Frasch AC (2005) The stress-regulated protein M6a is a key modulator for neurite outgrowth and filopodium/spine formation. Proc Natl Acad Sci U S A. 102(47):17196-201.

American Psychiatric Association (APA): DSM-5. www.dsm5.org /ProposedRevisions /Pages/Substance-RelatedDisorders.aspx.

Amigo I, Traba J, González-Barroso MM, Rueda CB, Fernández M, Rial E, Sánchez A, Satrústegui J, Del Arco A (2013) Glucagon regulation of oxidative phosphorylation requires an increase in matrix adenine nucleotide content through Ca2+ activation of the mitochondrial ATP-Mg/Pi carrier SCaMC-3.J Biol Chem. 288(11):7791-802.

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. Ex Clin Psychopharmacol 2:244-268.

Andrews WJ, Vasquez B, Nagulesparan M, Klimes I, Foley J, Unger R, Reaven GM (1984) Insulin therapy in obese, non-insulin-dependent diabetes induces improvements in insulin action and secretion that are maintained for two weeks after insulin withdrawal. Diabetes. 33(7):634-42.

Aragona BJ, Cleaveland NA, Stuber GD, Day JJ, Carelli RM, Wightman RM (2008) Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is attributable to a direct increase in phasic dopamine release events. J Neurosci. 28(35):8821-31.

Arias-Carrión O, Stamelou M, Murillo-Rodríguez E, Menéndez-González M, Pöppel E (2010) Dopaminergic reward system: a short integrative review. Int Arch Med. 3:24.

Armario A (2010) Activation of the hypothalamic-pituitary-adrenal axis by addictive drugs: different pathways, common outcome.Trends Pharmacol Sci. 31(7):318-25.

Atwood BK, Mackie K (2010) CB2: a cannabinoid receptor with an identity crisis. Br J Pharmacol. 160(3):467-79.

Avena NM, Long KA, Hoebel BG (2005) Sugar-dependent rats show enhanced responding for sugar after abstinence: evidence of a sugar deprivation effect. Physiol Behav. 84(3):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(1):20-39.

Avena NM, Rada P, Hoebel BG (2009) Sugar and fat bingeing have notable differences in addictive-like behavior. J Nutr. 139(3):623-8.

Ayuso E, Mingozzi F, Montane J, Leon X, Anguela XM, Haurigot V, Edmonson SA, Africa L, Zhou S, High KA, Bosch F, Wright JF (2010) High AAV vector purity results in serotype- and tissue-independent enhancement of transduction efficiency. Gene Ther. 17(4):503-10.

Babbini M, Gaiardi M, Bartoletti M (1975) Persistence of chronic morphine effects upon activity in rats 8 months after ceasing the treatment. Neuropharmacology. 14(8):611-4.

Bahi A, Fizia K, Dietz M, Gasparini F, Flor PJ (2012) Pharmacological modulation of mGluR7 with AMN082 and MMPIP exerts specific influences on alcohol consumption and preference in rats. Addict Biol. 17(2):235-47.

Bahi A (2012a) The selective metabotropic glutamate receptor 7 allosteric agonist AMN082 prevents reinstatement of extinguished ethanol-induced conditioned place preference in mice. Pharmacol Biochem Behav. 101(2):193-200.

Bahi A (2012b) Viral-Mediated Knockdown of mGluR7 in the Nucleus Accumbens Mediates Excessive Alcohol Drinking and Increased Ethanol-Elicited Conditioned Place Preference in Rats. Neuropsychopharmacology. doi: 10.1038/npp.2012.122.

Bailey CP, Smith FL, Kelly E, Dewey WL, Henderson G (2006) How important is protein kinase C in mu-opioid receptor desensitization and morphine tolerance? Trends Pharmacol Sci. 27(11):558-65.

Baldo BA, Kelley AE (2007) Discrete neurochemical coding of distinguishable motivational processes: insights from nucleus accumbens control of feeding. Psychopharmacology (Berl). 191(3):439-59.

Balerio GN, Aso E, Berrendero F, Murtra P, Maldonado R (2004) Delta9-tetrahydrocannabinol decreases somatic and motivational manifestations of nicotine withdrawal in mice. Eur J Neurosci. 20(10):2737-48.

Ballesteros-Yáñez I, Valverde O, Ledent C, Maldonado R, DeFelipe J (2007a) Chronic cocaine treatment alters dendritic arborization in the adult motor cortex through a CB1 cannabinoid receptor-dependent mechanism. Neuroscience. 146(4):1536-45.

Ballesteros-Yáñez I, Ambrosio E, Benavides-Piccione R, Pérez J, Torres I, Miguéns M, García-Lecumberri C, DeFelipe J (2007b) The effects of morphine self-administration on cortical pyramidal cell structure in addiction-prone lewis rats. Cereb Cortex. 17(1):238-49.

Baptista MA, Martin-Fardon R, Weiss F (2004) Preferential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on conditioned reinstatement versus primary reinforcement: comparison between cocaine and a potent conventional reinforcer. J Neurosci. 24(20):4723-7.

Barbano MF, Castañé A, Martín-García E, Maldonado R (2009) Delta-9-tetrahydrocannabinol enhances food reinforcement in a mouse operant conflict test. Psychopharmacology (Berl). 205(3):475-87.

Bardo MT, Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology (Berl) 153:31-43.

Bartoli M, Ternaux JP, Forni C, Portalier P, Salin P, Amalric M, Monneron A (1999) Down-regulation of striatin, a neuronal calmodulin-binding protein, impairs rat locomotor activity. J Neurobiol. 40(2):234-43.

Bardoni B, Willemsen R, Weiler IJ, Schenck A, Severijnen LA, Hindelang C, Lalli E, Mandel JL (2003) NUFIP1 (nuclear FMRP interacting protein 1) is a nucleocytoplasmic shuttling protein associated with active synaptoneurosomes. Exp Cell Res. 289(1):95-107.

Bassareo V, Di Chiara G (1997) Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. J Neurosci. 17(2):851-61.

Bassareo V, De Luca MA, Di Chiara G (2002) Differential expression of motivational stimulus properties by dopamine in nucleus accumbens shell versus core and prefrontal cortex. J Neurosci . 22:4709-4719.

Bassareo V, Musio P, Di Chiara G (2011) Reciprocal responsiveness of nucleus accumbens shell and core dopamine to food- and drug-conditioned stimuli. Psychopharmacology (Berl). 214(3):687-97.

Bassi MT, Manzoni M, Bresciani R, Pizzo MT, Della Monica A, Barlati S, Monti E, Borsani G (2005) Cellular expression and alternative splicing of SLC25A23, a member of the mitochondrial Ca2+-dependent solute carrier gene family. Gene. 345(2):173-82.

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(8):3123-7.

Bauco P, Wise RA (1997) Synergistic effects of cocaine with lateral hypothalamic brain stimulation reward: lack of tolerance or sensitization. J Pharmacol Exp Ther. 283(3):1160-7.

Baumann MH, Rothman RB (1993) Effects of acute and chronic cocaine on the activity of tuberoinfundibular dopamine neurons in the rat. Brain Res. 608(1):175-9.

Bechara A, Damasio H (2002) Decision-making and addiction (part I): impaired activation of somatic states in substance dependent individuals when pondering decisions with negative future consequences. Neuropsychologia. 2002;40:1675–1689.

Bell K, Kalivas PW (1996) Context-specific cross-sensitization between systemic cocaine and intra-accumbens AMPA infusion in the rat. Psychopharmacology (Berl). 127(4):377-83.

Belin D, Everitt BJ (2008) Cocaine seeking habits depend upon dopamine-dependent serial connectivity linking the ventral with the dorsal striatum. Neuron. 57(3):432-41.

Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ (2008) High impulsivity predicts the switch to compulsive cocaine-taking. Science. 320(5881):1352-5.

Benarroch EE (2012) Endogenous opioid systems: current concepts and clinical correlations. Neurology. 79(8):807-14.

Beninger RJ, Miller R (1998) Dopamine D1-like receptors and reward-related incentive learning. Neurosci Biobehav Rev. 1998 22(2):335-45

Beninger RJ, Gerdjikov T (2006) The role of signaling molecules in reward-related incentive learning. Neurotox Res. 2004;6(1):91-104.

Benito E, Barco A (2010) CREB's control of intrinsic and synaptic plasticity: implications for CREB-dependent memory models. Trends Neurosci. 33(5):230-40.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society. Series B (Methodological), 289--300.

Berke JD, Hyman SE (2000) Addiction, dopamine, and the molecular mechanisms of memory. Neuron. 25(3):515-32.

Bernabeu R, Bevilaqua L, Ardenghi P, Bromberg E, Schmitz P, Bianchin M, Izquierdo I, Medina JH (1997) Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. Proc Natl Acad Sci U S A. 94(13):7041-6.

Berthoud HR (2002) Multiple neural systems controlling food intake and body weight. Neurosci Biobehav Rev. 26(4):393-428.

Berthoud HR (2007) Interactions between the "cognitive" and "metabolic" brain in the control of food intake. Physiol Behav. 91(5):486-98.

Berthoud HR (2011) Metabolic and hedonic drives in the neural control of appetite: who is the boss? Curr Opin Neurobiol. 21(6):888-96.

Besse F, Ephrussi A (2008)Translational control of localized mRNAs: restricting protein synthesis in space and time.Nat Rev Mol Cell Biol. 9(12):971-80.

Bhardwaj SK, Cassidy CM, Srivastava LK (2006) Changes in syntaxin-1B mRNA in the nucleus accumbens of amphetamine-sensitized rats. Int J Neuropsychopharmacol. 9(6):751-9.

Binda AV, Kabbani N, Levenson R (2005) Regulation of dense core vesicle release from PC12 cells by interaction between the D2 dopamine receptor and calcium-dependent activator protein for secretion (CAPS). Biochem Pharmacol. 69(10):1451-61.

Björkblom B, Padzik A, Mohammad H, Westerlund N, Komulainen E, Hollos P, Parviainen L, Papageorgiou AC, Iljin K, Kallioniemi O, Kallajoki M, Courtney MJ, Mågård M, James P, Coffey ET (2012) c-Jun N-terminal kinase phosphorylation of MARCKSL1 determines actin stability and migration in neurons and in cancer cells. Mol Cell Biol. 32(17):3513-26.

Björklund A, Dunnett SB (2007) Dopamine neuron systems in the brain: an update. Trends Neurosci. 30(5):194-202.

Blendy JA, Strasser A, Walters CL, Perkins KA, Patterson F, Berkowitz R, Lerman C (2005) Reduced nicotine reward in obesity: cross-comparison in human and mouse. Psychopharmacology (Berl). 180(2):306-15.

Blumenthal DM, Gold MS (2010) Neurobiology of food addiction Curr Opin Clin Nutr Metab Care13(4):359-65.

Blüml V, Kapusta N, Vyssoki B, Kogoj D, Walter H, Lesch OM (2012) Relationship between substance use and body mass index in young males. Am J Addict. 21(1):72-7.

Bohme GA, Laville M, Ledent C, Parmentier M, Imperato A (2000) Enhanced long-term potentiation in mice lacking cannabinoid CB1 receptors. Neuroscience. 95(1):5-7.

Bohn LM, Gainetdinov RR, Lin FT, Lefkowitz RJ, Caron MG (2000) Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. Nature. 2000 408(6813):720-3.

Boileau I, Dagher A, Leyton M, Gunn RN, Baker GB, Diksic M, Benkelfat C (2006) Modeling sensitization to stimulants in humans: an [11C]raclopride/positron emission tomography study in healthy men. Arch Gen Psychiatry. 63(12):1386-95.

Borgland SL, Malenka RC, Bonci A, (2004) Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area: electrophysiological and behavioral correlates in individual rats. J. Neurosci. 24, 7482e7490.

Boudreau AC, Reimers JM, Milovanovic M, Wolf ME (2007) Cell surface AMPA receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. J Neurosci. 27(39):10621-35.

Bourne JN, Harris KM (2011) Coordination of size and number of excitatory and inhibitory synapses results in a balanced structural plasticity along mature hippocampal CA1 dendrites during LTP. Hippocampus. 21(4):354-73.

Boutou E, Hurel C, Matsas R (2000) Early expression of the BM88 antigen during neuronal differentiation of P19 embryonal carcinoma cells. Int J Dev Neurosci. 18(2-3):321-8.

Brami-Cherrier K, Valjent E, Hervé D, Darragh J, Corvol JC, Pages C, Arthur SJ, Girault JA, Caboche J (2005) Parsing molecular and behavioral effects of cocaine in mitogen- and stress-activated protein kinase-1-deficient mice. J Neurosci. 25(49):11444-54.

Brebner K, Wong TP, Liu L, Liu Y, Campsall P, Gray S, Phelps L, Phillips AG, Wang YT (2005) Nucleus accumbens long-term depression and the expression of behavioral sensitization. Science. 310(5752):1340-3.

Breitman M, Zilberberg A, Caspi M, Rosin-Arbesfeld R (2008) The armadillo repeat domain of the APC tumor suppressor protein interacts with Striatin family members. Biochim Biophys Acta. 1783(10):1792-802.

Breiter HC, Gollub RL, Weisskoff RM, Kennedy DN, Makris N, Berke JD, Goodman JM, Kantor HL, Gastfriend DR, Riorden JP, Mathew RT, Rosen BR, Hyman SE (1997) Acute effects of cocaine on human brain activity and emotion. Neuron. 19(3):591-611.

Breivogel CS, Childers SR (1998) The functional neuroanatomy of brain cannabinoid receptors. Neurobiol Dis. 5(6 Pt B):417-31.

Brocco MA, Fernández ME, Frasch AC (2010) Filopodial protrusions induced by glycoprotein M6a exhibit high motility and aids synapse formation. Eur J Neurosci. 31(2):195-202.

Broos N, Diergaarde L, Schoffelmeer AN, Pattij T, De Vries TJ (2012) Trait impulsive choice predicts resistance to extinction and propensity to relapse to cocaine seeking: a bidirectional investigation. Neuropsychopharmacology. 37(6):1377-86.

Bücheler MM, Hadamek K, Hein L (2002) Two alpha(2)-adrenergic receptor subtypes, alpha(2A) and alpha(2C), inhibit transmitter release in the brain of gene-targeted mice. Neuroscience.;109(4):819-26.

Buchner E, Gundersen CB (1997) The DnaJ-like cysteine string protein and exocytotic neurotransmitter release. Trends Neurosci. 20(5):223-7.

Budillon A, Cereseto A, Kondrashin A, Nesterova M, Merlo G, Clair T, Cho-Chung YS (1995) Point mutation of the autophosphorylation site or in the nuclear location signal causes protein kinase A RII beta regulatory subunit to lose its ability to revert transformed fibroblasts. Proc Natl Acad Sci U S A. 1995 Nov 7;92(23):10634-8.

Bunkenborg J, García GE, Paz MI, Andersen JS, Molina H (2010) The minotaur proteome: avoiding cross-species identifications deriving from bovine serum in cell culture models. Proteomics.10(16):3040.

Burnstock G (2006) Purinergic signalling. Br J Pharmacol. 147 Suppl 1:S172-81.

Butz M, Wörgötter F, van Ooyen A (2009) Activity-dependent structural plasticity. Brain Res Rev. 60(2):287-305.

Caillé S, Parsons LH (2006) Cannabinoid modulation of opiate reinforcement through the ventral striatopallidal pathway. Neuropsychopharmacology. 31(4):804-13.

Caillé S, Alvarez-Jaimes L, Polis I, Stouffer DG, Parsons LH (2007) Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. J Neurosci. 27(14):3695-702.

Camí J, Farré M (2003) Drug addiction. N Engl J Med. 2003 349(10):975-86.

Calabresi P, Picconi B, Tozzi A, Di Filippo M (2007) Dopamine-mediated regulation of corticostriatal synaptic plasticity. Trends Neurosci30(5):211-9. Epub 2007 Mar 23.

Canals M, Milligan G (2008) Constitutive activity of the cannabinoid CB1 receptor regulates the function of co-expressed Mu opioid receptors.J Biol Chem. 283(17):11424-34.

Cantin L, Lenoir M, Augier E, Vanhille N, Dubreucq S, Serre F, Vouillac C, Ahmed SH (2010) Cocaine is low on the value ladder of rats: possible evidence for resilience to addiction. PLoS One. 5(7):e11592.

Capriles N, Rodaros D, Sorge RE, Stewart J (2003) A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl). 168(1-2):66-74.

Carlezon WA Jr, Duman RS, Nestler EJ (2005) The many faces of CREB. Trends Neurosci. 28(8):436-45.

Carr KD (2002) Augmentation of drug reward by chronic food restriction: behavioral evidence and underlying mechanisms. Physiol Behav. 76(3):353-64.

Carter BL, Tiffany ST (1999) Meta-analysis of cue-reactivity in addiction research. Addiction. 94:327-340.

Castermans D, Volders K, Crepel A, Backx L, De Vos R, Freson K, Meulemans S, Vermeesch JR, Schrander-Stumpel CT, De Rijk P, Del-Favero J, Van Geet C, Van De Ven WJ, Steyaert JG, Devriendt K, Creemers JW (2010) SCAMP5, NBEA and AMISYN: three candidate genes for autism involved in secretion of large dense-core vesicles. Hum Mol Genet. 2010 19(7):1368-78.

Castillo PE, Younts TJ, Chávez AE, Hashimotodani Y (2012) Endocannabinoid signaling and synaptic function. Neuron. 76(1):70-81.

Cazala P, Darracq C, Saint-Marc M (1987) Self-administration of morphine into the lateral hypothalamus in the mouse. Brain Res. 416(2):283-8.

Cearley CN, Vandenberghe LH, Parente MK, Carnish ER, Wilson JM, Wolfe JH (2008) Expanded repertoire of AAV vector serotypes mediate unique patterns of transduction in mouse brain. Mol Ther. 16(10):1710-8.

Centonze D, Picconi B, Gubellini P, Bernardi G, Calabresi P (2001) Dopaminergic control of synaptic plasticity in the dorsal striatum. Eur J Neurosci. 13(6):1071-7.

Cervo L, Mukherjee S, Bertaglia A, Samanin R (1997) Protein kinases A and C are involved in the mechanisms underlying consolidation of cocaine place conditioning. Brain Res. 1997 Nov 14;775(1-2):30-6.

Chang JY, Janak PH, Woodward DJ (1998) Comparison of mesocorticolimbic neuronal responses during cocaine and heroin self-administration in freely moving rats. J Neurosci. 18(8):3098-115.

Cheah PS, Ramshaw HS, Thomas PQ, Toyo-Oka K, Xu X, Martin S, Coyle P, Guthridge MA, Stomski F, van den Buuse M, Wynshaw-Boris A, Lopez AF, Schwarz QP (2012) Neurodevelopmental and neuropsychiatric behaviour defects arise from 14-3-3ζ deficiency. Mol Psychiatry. 17(4):451-66.

Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ, Phillips PE, Wightman RM (2007) Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. J Neurosci. 27(4):791-5.

Chefer VI, Shippenberg TS (2009) Augmentation of morphine-induced sensitization but reduction in morphine tolerance and reward in delta-opioid receptor knockout mice. Neuropsychopharmacology. 34(4):887-98.

Chen J, Lee CT, Errico SL, Becker KG, Freed WJ (2007) Increases in expression of 14-3-3 eta and 14-3-3 zeta transcripts during neuroprotection induced by delta9-tetrahydrocannabinol in AF5 cells. J Neurosci Res. 2007 Jun;85(8):1724-33.

Chen BT, Bowers MS, Martin M, Hopf FW, Guillory AM, Carelli RM, Chou JK, Bonci A (2008) Cocaine but not natural reward self-administration nor passive cocaine infusion produces persistent LTP in the VTA. Neuron. 59(2):288-97.

Chen L, Xu M (2010) Dopamine D1 and D3 receptors are differentially involved in cue-elicited cocaine seeking. J Neurochem. 114(2):530-41.

Chevaleyre V, Takahashi KA, Castillo PE (2006) Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci. 29:37-76.

Childers SR, Deadwyler SA (1996) Role of cyclic AMP in the actions of cannabinoid receptors. Biochem Pharmacol. 52(6):819-27.

Chklovskii DB, Mel BW, Svoboda K (2004) Cortical rewiring and information storage. Nature. 431(7010):782-8.

Chang KW, Chou A, Lee CC, Yeh C, Lai MW, Yeh TS, Chen TC, Liang KH, Yeh CT (2011) Overexpression of kappa-actin alters growth properties of hepatoma cells and predicts poor postoperative prognosis. Anticancer Res. 31(6):2037-44.

Chung S, Hopf FW, Nagasaki H, Li CY, Belluzzi JD, Bonci A, Civelli O (2009) The melanin-concentrating hormone system modulates cocaine reward. Proc Natl Acad Sci U S A. 106(16):6772-7.

Ciccocioppo R, Martin-Fardon R, Weiss F (2004) Stimuli associated with a single cocaine experience elicit long-lasting cocaine-seeking. Nat Neurosci. 7(5):495-6.

Cichewicz DL, Welch SP (2003) Modulation of oral morphine antinociceptive tolerance and naloxone-precipitated withdrawal signs by oral Delta 9-tetrahydrocannabinol.J Pharmacol Exp Ther. 305(3):812-7.

Clarke RB, Adermark L (2010) Acute ethanol treatment prevents endocannabinoid-mediated long-lasting disinhibition of striatal output. Neuropharmacology. 58(4-5):799-805.

Colantuoni C, Schwenker J, McCarthy J, Rada P, Ladenheim B, Cadet JL, Schwartz GJ, Moran TH, Hoebel BG (2001) Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. Neuroreport 12, 3549–3552.

Colantuoni C, Rada P, McCarthy J, Patten C, Avena NM, Chadeayne A, Hoebel BG (2002) Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. Obes Res. 10(6):478-88.

Collins RJ, Weeks JR, Cooper MM, Good PI, Russell RR (1984) Prediction of abuse liability of drugs using IV self-administration by rats. Psychopharmacology (Berl) 82:6-13.

Conrad KL, Tseng KY, Uejima JL, Reimers JM, Heng LJ, Shaham Y, Marinelli M, Wolf ME (2008) Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. Nature. 454(7200):118-21.

Conway KP, Kane RJ, Ball SA, Poling JC, Rounsaville BJ (2003) Personality, substance of choice, and polysubstance involvement among substance dependent patients. Drug Alcohol Depend. 71:65–75.

Corbillé AG, Valjent E, Marsicano G, Ledent C, Lutz B, Hervé D, Girault JA (2007) Role of cannabinoid type 1 receptors in locomotor activity and striatal signaling in response to psychostimulants. J Neurosci. 27(26):6937-47.

Corbit LH, Muir JL, Balleine BW (2001) The role of the nucleus accumbens in instrumental conditioning: Evidence of a functional dissociation between accumbens core and shell. J Neurosci. 2001 May 1;21(9):3251-60.

Cornish JL, Kalivas PW (2000) Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. J Neurosci. 20(15):RC89.

Cota D, Marsicano G, Tschöp M, Grübler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thöne-Reineke C, Ortmann S, Tomassoni F, Cervino C, Nisoli E, Linthorst AC, Pasquali R, Lutz B, Stalla GK, Pagotto U (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest. 112(3):423-31.

Crespo JA, Stöckl P, Zorn K, Saria A, Zernig G (2008) Nucleus accumbens core acetylcholine is preferentially activated during acquisition of drug- vs food-reinforced behavior. Neuropsychopharmacology. 33(13):3213-20.

Crews F, He J, Hodge C (2007) Adolescent cortical development: a critical period of vulnerability for addiction. Pharmacol Biochem Behav. 86(2):189-99.

Crombag HS, Gorny G, Li Y, Kolb B, Robinson, TE (2005) Opposite effects of amphetamine self-administration experience on dendritic spines in the medial and orbital prefrontal cortex. Cereb. Cortex. 15, 341–348

Cummings DE (2006) Ghrelin and the short- and long-term regulation of appetite and body weight. Physiol Behav. 89(1):71-84.

Cunningham ST, Finn M, Kelley AE (1997) Sensitization of the locomotor response to psychostimulants after repeated opiate exposure: role of the nucleus accumbens. Neuropsychopharmacology 16:147–155

Dalley JW, Theobald DE, Eagle DM, Passetti F, Robbins TW (2002) Deficits in impulse control associated with tonically-elevated serotonergic function in rat prefrontal cortex. Neuropsychopharmacology. 26(6):716-28.

Dalley JW, Cardinal RN, Robbins TW (2004) Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. Neurosci Biobehav Rev. 28(7):771-84.

Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Lääne K, Peña Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW (2007) Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. Science. 315(5816):1267-70.

Dasgupta S, Cushman I, Kpetemey M, Casey PJ, Vishwanatha JK (2011) Prenylated c17orf37 induces filopodia formation to promote cell migration and metastasis. J Biol Chem. 286(29):25935-46.

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(6):613-6.

Davis S, Rodger J, Stéphan A, Hicks A, Mallet J, Laroche S (1998) Increase in syntaxin 1B mRNA in hippocampal and cortical circuits during spatial learning reflects a mechanism of trans-synaptic plasticity involved in establishing a memory trace. Learn Mem. 5(4-5):375-90.

Davis S, Salin H, Helme-Guizon A, Dumas S, Stéphan A, Corbex M, Mallet J, Laroche S (2000) Dysfunctional regulation of alphaCaMKII and syntaxin 1B transcription after induction of LTP in the aged rat. Eur J Neurosci. 2000 Sep;12(9):3276-82.

Davis C, Levitan RD, Muglia P, Bewell C, Kennedy JL (2004) Decision-making deficits and overeating: a risk model for obesity. Obes Res. 2004;12:929–935.

Davis C, Patte K, Levitan R, Reid C, Tweed S, Curtis C (2007) From motivation to behaviour: a model of reward sensitivity, overeating, and food preferences in the risk profile for obesity. 48(1):12-9. Epub 2006 Jul 26.

Davis JF, Choi DL, Benoit SC (2010) Insulin, leptin and reward. Trends Endocrinol Metab. 21(2):68-74.

Day JJ, Carelli RM (2007) The nucleus accumbens and Pavlovian reward learning. Neuroscientist. 13(2):148-59.

De Chiara V, Angelucci F, Rossi S, Musella A, Cavasinni F, Cantarella C, Mataluni G, Sacchetti L, Napolitano F, Castelli M, Caltagirone C, Bernardi G, Maccarrone M, Usiello A, Centonze D (2010) Brain-derived neurotrophic factor controls cannabinoid CB1 receptor function in the striatum. J Neurosci. 30(24):8127-37.

de Frias CM, Annerbrink K, Westberg L, Eriksson E, Adolfsson R, Nilsson LG (2004) COMT gene polymorphism is associated with declarative memory in adulthood and old age. Behav Genet. 2004 Sep;34(5):533-9.

Del Pozo E, Barrios M, Baeyens JM (1996) The NMDA receptor antagonist dizocilpine (MK-801) stereoselectively inhibits morphine-induced place preference conditioning in mice. Psychopharmacology (Berl). 125(3):209-13.

DeManno DA, Cottom JE, Kline MP, Peters CA, Maizels ET, Hunzicker-Dunn M (1999) Follicle-stimulating hormone promotes histone H3 phosphorylation on serine-10. Mol Endocrinol. 13(1):91-105.

Demmerle J, Koch AJ, Holaska JM (2012) The nuclear envelope protein emerin binds directly to histone deacetylase 3 (HDAC3) and activates HDAC3 activity. J Biol Chem. 287(26):22080-8.

Deng PY, Klyachko VA (2011)The diverse functions of short-term plasticity components in synaptic computations. Commun Integr Biol. 4(5):543-8.

Deroche-Gamonet V, Belin D, Piazza PV (2004) Evidence for addiction-like behavior in the rat. Science. 305(5686):1014-7.

De Roo M, Klauser P, Muller D (2008) LTP promotes a selective long-term stabilization and clustering of dendritic spines. PLoS Biol. 6(9):e219.

De Vries TJ, Schoffelmeer AN (2005) Cannabinoid CB1 receptors control conditioned drug seeking. Trends Pharmacol Sci. 26(8):420-6.

Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (1994) Washington, DC: American Psychiatric Association.

Diana M (2011) The dopamine hypothesis of drug addiction and its potential therapeutic value. Front Psychiatry. 2:64.

Díaz-Alonso J, Guzmán M, Galve-Roperh I (2012) Endocannabinoids via CB_1 receptors act as neurogenic niche cues during cortical development. Philos Trans R Soc Lond B Biol Sci. 367(1607):3229-41.

Di Benedetto M, D'addario C, Candeletti S, Romualdi P (2006) Chronic and acute effects of 3,4-methylenedioxy-N-methylamphetamine ('Ecstasy') administration on the dynorphinergic system in the rat brain. Neuroscience. 137(1):187-96.

Di Cesare Mannelli L, Vivoli E, Salvicchi A, Schiavone N, Koverech A, Messano M, Nicolai R, Benatti P, Bartolini A, Ghelardini C (2011) Antidepressant-like effect of artemin in mice: a mechanism for acetyl-L-carnitine activity on depression. Psychopharmacology (Berl). 218(2):347-56

Di Chiara G, 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(14):5274-8.

Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D (2004) Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology. 47 Suppl 1:227-41.

Di Chiara G, Bassareo V (2007) Reward system and addiction: what dopamine does and doesn't do. Curr Opin Pharmacol. 7(1):69-76.

Di Ciano P, Everitt BJ (2001) Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. Neuropsychopharmacology. 25:341–360.

Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BJ (2001) Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. J Neurosci. 21(23):9471-7.

Dickinson A, Wood N, Smith JW (2002) Alcohol seeking by rats: action or habit? Q J Exp Psychol B. 2002 Oct;55(4):331-48.

Dickson SL, Egecioglu E, Landgren S, Skibicka KP, Engel JA, Jerlhag E (2011) The role of the central ghrelin system in reward from food and chemical drugs. Mol Cell Endocrinol. 340(1):80-7.

Di Daniel E, Kew JN, Maycox PR (2009) Investigation of the H(+)-myo-inositol transporter (HMIT) as a neuronal regulator of phosphoinositide signalling. Biochem Soc Trans. 37(Pt 5):1139-43.

Diergaarde L, Pattij T, Nawijn L, Schoffelmeer AN, De Vries TJ (2009) Trait impulsivity predicts escalation of sucrose seeking and hypersensitivity to sucrose-associated stimuli. Behav Neurosci. 123(4):794-803.

Di Marzo V, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T, Kunos G (2001) Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature. 410(6830):822-5.

Di Marzo V, Matias I (2005) Endocannabinoid control of food intake and energy balance. Nat Neurosci. 8(5):585-9.

DiPatrizio NV, Simansky KJ (2008) Activating parabrachial cannabinoid CB1 receptors selectively stimulates feeding of palatable foods in rats. J Neurosci. 28(39):9702-9.

DiPatrizio NV, Astarita G, Schwartz G, Li X, Piomelli D (2011) Endocannabinoid signal in the gut controls dietary fat intake. Proc Natl Acad Sci U S A. 108(31):12904-8.

Dobashi Y, Suzuki S, Kimura M, Matsubara H, Tsubochi H, Imoto I, Ooi A (2011) Paradigm of kinase-driven pathway downstream of epidermal growth factor receptor/Akt in human lung carcinomas. Hum. Pathol. 42, 214–426.

Dong Z, Han H, Wang M, Xu L, Hao W, Cao J (2006) Morphine conditioned place preference depends on glucocorticoid receptors in both hippocampus and nucleus accumbens. Hippocampus. 16(10):809-13.

Drewnowski A, Almiron-Roig E (2010) Human Perceptions and Preferences for Fat-Rich Foods. Fat Detection: Taste, Texture, and Post Ingestive Effects. Boca Raton (FL): CRC Press; 2010. Chapter 11.
Frontiers in Neuroscience.

Duca FA, Covasa M (2012) Current and emerging concepts on the role of peripheral signals in the control of food intake and development of obesity. 108(5):778-93.

Ducut Sigala JL, Bottero V, Young DB, Shevchenko A, Mercurio F, Verma IM (2004) Activation of transcription factor NF-kappaB requires ELKS, an IkappaB kinase regulatory subunit. Science. 304(5679):1963-7.

Duval N, Luhrs K, Wilkinson TG 2nd, Baresova V, Skopova V, Kmoch S, Vacano GN, Zikanova M, Patterson D. (2013) Genetic and metabolomic analysis of AdeD and AdeI mutants of de novo purine biosynthesis: cellular models of de novo purine biosynthesis deficiency disorders. Mol Genet Metab. 108(3):178-89.

Engert F, Bonhoeffer T (1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. Nature. 399(6731):66-70.

Evans GJ, Wilkinson MC, Graham ME, Turner KM, Chamberlain LH, Burgoyne RD, Morgan A (2001) Phosphorylation of cysteine string protein by protein kinase A. Implications for the modulation of exocytosis. J Biol Chem. 276(51):47877-85.

Ersche KD, Turton AJ, Pradhan S, Bullmore ET, Robbins TW (2010) Drug addiction endophenotypes: Impulsive versus sensation-seeking personality traits. Biol Psychiatry.

Everitt BJ, Dickinson A, Robbins TW (2001) The neuropsychological basis of addictive behaviour. Brain Res Brain Res Rev. 36(2-3):129-38.

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci. 8(11):1481-9.

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(1507):3125-35.

Farioli-Vecchioli S, Moreno S, Cerù MP (2001) Immunocytochemical localization of acyl-CoA oxidase in the rat central nervous system. J Neurocytol. 30(1):21-33.

Fattore L, Cossu G, Martellotta CM, Fratta W (2001) Intravenous self-administration of the cannabinoid CB1 receptor agonist WIN 55,212-2 in rats. Psychopharmacology (Berl). 156:410–6.

Fee JR, Knapp DJ, Sparta DR, Breese GR, Picker MJ, Thiele TE (2006) Involvement of protein kinase A in ethanol-induced locomotor activity and sensitization. Neuroscience. 140(1):21-31.

Feldman DE (2009) Synaptic mechanisms for plasticity in neocortex. Annu Rev Neurosci. 32:33-55.

Feltenstein MW, See RE (2008) The neurocircuitry of addiction: an overview. Br J Pharmacol. 2008 May;154(2):261-74.

Feng H, Zheng L, Feng Z, Zhao Y, Zhang N (2012) The role of leptin in obesity and the potential for leptin replacement therapy. Endocrine.

Fenu S, Bassareo V, Di Chiara G (2001) A role for dopamine D1 receptors of the nucleus accumbens shell in conditioned taste aversion learning. J Neurosci. 2001 Sep 1;21(17):6897-904.

Fernandez JR, Allison DB (2004) Rimonabant Sanofi- Synthelabo. Curr. Opin. Investig. Drugs 5, 430–435.

Fernández-Chacón R, Südhof TC (2000) Novel SCAMPs lacking NPF repeats: ubiquitous and synaptic vesicle-specific forms implicate SCAMPs in multiple membrane-trafficking functions. J Neurosci. 20(21):7941-50.

Fernández-Chacón R, Wölfel M, Nishimune H, Tabares L, Schmitz F, Castellano-Muñoz M, Rosenmund C, Montesinos ML, Sanes JR, Schneggenburger R, Südhof TC (2004) The synaptic vesicle protein CSP alpha prevents presynaptic degeneration. Neuron. 42(2):237-51.

Fernández-Ruiz J, Berrendero F, Hernández ML, Ramos JA (2000) The endogenous cannabinoid system and brain development. Trends Neurosci. 23(1):14-20.

Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE (2005) Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry. 58(9):751-9.

Ferrario CR, Robinson TE (2007) Amphetamine pretreatment accelerates the subsequent escalation of cocaine self-administration behavior. Eur Neuropsychopharmacol. 17(5):352-7.

Fioravante D, Regehr WG (2011) Short-term forms of presynaptic plasticity. Curr Opin Neurobiol 21:269–274.

Fischer BD, Ward SJ, Henry FE, Dykstra LA (2010) Attenuation of morphine antinociceptive tolerance by a CB(1) receptor agonist and an NMDA receptor antagonist: Interactive effects. 58(2):544-50.

Fitzgerald ML, Shobin E, Pickel VM (2012) Cannabinoid modulation of the dopaminergic circuitry: implications for limbic and striatal output. Prog Neuropsychopharmacol Biol Psychiatry. 38(1):21-9.

Floresco SB, McLaughlin RJ, Haluk DM (2008) Opposing roles for the nucleus accumbens core and shell in cue-induced reinstatement of food-seeking behavior. Neuroscience. 154:877–884.

Foltin RW, Fischman MW, Byrne MF (1988) Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. Appetite. 1988 Aug;11(1):1-14.

Fontán-Lozano A, Sáez-Cassanelli JL, Inda MC, de los Santos-Arteaga M, Sierra-Domínguez SA, López-Lluch G, Delgado-García JM, Carrión AM (2007) Caloric restriction increases learning consolidation and facilitates synaptic plasticity through mechanisms dependent on NR2B subunits of the NMDA receptor. J Neurosci. 27(38):10185-95.

Fontaine-Lenoir V, Chambraud B, Fellous A, David S, Duchossoy Y, Baulieu EE, Robel P (2006) Microtubule-associated protein 2 (MAP2) is a neurosteroid receptor. Proc Natl Acad Sci U S A. 103(12):4711-6.

Fortuna JL (2012) The obesity epidemic and food addiction: clinical similarities to drug dependence. J Psychoactive Drugs. 44(1):56-63.

Fourgeaud L, Mato S, Bouchet D, Hémar A, Worley PF, Manzoni OJ (2004) A single in vivo exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. J Neurosci. 24(31):6939-45.

Franques P, Auriacombe M, Tignol J (2000) Addiction and personality. Encephale 26: 68–78 (in French)

Freedland CS, Poston JS, Porrino LJ (2000) Effects of SR141716A, a central cannabinoid receptor antagonist, on food-maintained responding. Pharmacol Biochem Behav. 2000 Oct;67(2):265-70.

Freund TF, Katona I, Piomelli D (2003) Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 83, 1017-1066.

Fuchs RA, Evans KA, Parker MC, See RE (2004) Differential involvement of the core and shell subregions of the nucleus accumbens in conditioned cue-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl). 176(3-4):459-65.

Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, See RE (2005) The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology. 30(2):296-309.

Gaillard S, Bartoli M, Castets F, Monneron A (2001) Striatin, a calmodulin-dependent scaffolding protein, directly binds caveolin-1. FEBS Lett. 508(1):49-52.

Galanti K, Gluck ME, Geliebter A (2007) Test meal intake in obese binge eaters in relation to impulsivity and compulsivity. Int J Eat Disord. 40:727–732.

Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA (2000) Cue-induced cocaine craving:

neuroanatomical specificity for drug users and drug stimuli. Am J Psychiatry. 157(11):1789-98

Garcia DE, Brown S, Hille B, Mackie K (1998) Protein kinase C disrupts cannabinoid actions by phosphorylation of the CB1 cannabinoid receptor. J Neurosci. 18(8):2834-41.

García-Fuster MJ, Flagel SB, Mahmood ST, Watson SJ, Akil H (2012) Cocaine withdrawal causes delayed dysregulation of stress genes in the hippocampus. PLoS One. 7(7):e42092.

Gardner EL (2005) Endocannabinoid signaling system and brain reward: emphasis on dopamine. Pharmacol Biochem Behav. 81(2):263-84.

Garzón J, de la Torre-Madrid E, Rodríguez-Muñoz M, Vicente-Sánchez A, Sánchez-Blázquez P (2009) Gz mediates the long-lasting desensitization of brain CB1 receptors and is essential for cross-tolerance with morphine. 5:11.

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JYH, Zhang J (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5, R80.

Gearhardt AN, Corbin WR, Brownell KD (2009) Preliminary validation of the Yale Food Addiction Scale. Appetite52(2):430-6.

George O, Koob GF (2010) Individual differences in prefrontal cortex function and the transition from drug use to drug dependence. Neurosci Biobehav Rev. 35(2):232-47.

Gerber SH, Rah JC, Min SW, Liu X, de Wit H, Dulubova I, Meyer AC, Rizo J, Arancillo M, Hammer RE, Verhage M, Rosenmund C, Südhof TC (2009) Conformational switch of syntaxin-1 controls synaptic vesicle fusion. Science. 321(5895):1507-10.

Gerdjikov TV, Ross GM, Beninger RJ (2004) Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats. Behav Neurosci. 118(4):740-50.

Gerges NZ, Tran IC, Backos DS, Harrell JM, Chinkers M, Pratt WB, Esteban JA (2004) Independent functions of hsp90 in neurotransmitter release and in the continuous synaptic cycling of AMPA receptors. J Neurosci. 2004 May 19;24(20):4758-66.

Ghitza UE, Fabbricatore AT, Prokopenko V, Pawlak AP, West MO (2003) Persistent cue-evoked activity of accumbens neurons after prolonged abstinence from self-administered cocaine. J Neurosci. 23(19):7239-45.

Ghitza UE, Gray SM, Epstein DH, Rice KC, Shaham Y (2006) The anxiogenic drug yohimbine reinstates palatable food seeking in a rat relapse model: a role of CRF1 receptors. Neuropsychopharmacology. 31(10):2188-96.

Gil C, Falqués A, Sarró E, Cubi R, Blasi J, Aguilera J, Itarte E (2011) Protein kinase CK2 associates to lipid rafts and its pharmacological inhibition enhances neurotransmitter release. FEBS Lett. 585(2):414-20.

Gipson CD, Kupchik YM, Shen H, Reissner KJ, Thomas CA, Kalivas PW (2013) Relapse induced by cues predicting cocaine depends on rapid, transient synaptic potentiation. Neuron. 77(5):867-72.

Girault JA, Valjent E, Caboche J, Hervé D (2007) ERK2: a logical AND gate critical for drug-induced plasticity? Curr Opin Pharmacol. 7(1):77-85.

Giuffrida A, Parsons LH, Kerr TM, Rodríguez de Fonseca F, Navarro M, Piomelli D (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci. 2(4):358-63.

Glimcher PW (2011) Understanding dopamine and reinforcement learning: the dopamine reward prediction error hypothesis. Proc Natl Acad Sci U S A. 108 Suppl 3:15647-54.

Goeders NE (2003) The impact of stress on addiction. Eur Neuropsychopharmacol. 2003 Dec;13(6):435-41.

Goldin M, Segal M (2003) Protein kinase C and ERK involvement in dendritic spine plasticity in cultured rodent hippocampal neurons. Eur J Neurosci. 17(12):2529-39.

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry159(10):1642-52.

Goode BL, Eck MJ (2007) Mechanism and function of formins in the control of actin assembly. Annu Rev Biochem. 76:593-627.

Goeders NE (2003)The impact of stress on addiction.Eur Neuropsychopharmacol. 13(6):435-41.

Goodman SR and Zagon IS (1986) The neural cell spectrin skeleton: a review. Am J Physiol Cell Physiol 250:C347-C360.

Gordon J, Hwang J, Carrier KJ, Jones CA, Kern QL, Moreno CS, Karas RH, Pallas DC (2011) Protein phosphatase 2a (PP2A) binds within the oligomerization domain of striatin and regulates the phosphorylation and activation of the mammalian Ste20-Like kinase Mst3. BMC Biochem. 2011 Oct 10;12:54. doi: 10.1186/1471-2091-12-54.

Graf ER, Heerssen HM, Wright CM, Davis GW, DiAntonio A (2011) Stathmin is required for stability of the Drosophila neuromuscular junction. J Neurosci. 31(42):15026-34.

Grant S, London ED, Newlin DB, Villemagne VL, Liu X, Contoreggi C, Phillips RL, Kimes AS, Margolin A (1996) Activation of memory circuits during cue-elicited cocaine craving. Proc Natl Acad Sci U S A. 1996 Oct 15;93(21):12040-5.

Grant BF, Dawson DA (1998) Age of onset of drug use and its association with DSM-IV drug abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. J .Subst. Abuse 10: 163–173.

Gray MA, Critchley HD (2007) Interoceptive basis to craving. Neuron. 54(2):183-6.

Green B, Kavanagh D, Young R (2003) Being stoned: a review of self-reported cannabis effects. Drug Alcohol Rev. 22(4):453-60.

Grimm JW, Fyall AM, Osincup DP (2005) Incubation of sucrose craving: effects of reduced training and sucrose pre-loading. Physiology & Behavior 84, 73–79.

Grutzendler J, Kasthuri N, Gan WB (2002) Long-term dendritic spine stability in the adult cortex. Nature. 420(6917):812-6.

Grutzendler J, Tsai J, Gan WB (2003) Rapid labeling of neuronal populations by ballistic delivery of fluorescent dyes. Methods. 2003 May;30(1):79-85.

Gu J and Zheng JQ (2009) Microtubules in Dendritic Spine Development and Plasticity. The Open Neuroscience Journal. 3, 128-133.

Gu Z, Liu W, Wei J, Yan Z (2012) Regulation of N-methyl-D-aspartic acid (NMDA) receptors by metabotropic glutamate receptor 7. J Biol Chem. 287(13):10265-75.

Gudelsky GA, Passaro E, Meltzer HY (1986) Delayed activation of tuberoinfundibular dopamine neurons and suppression of prolactin secretion in the rat after morphine administration. J Pharmacol Exp Ther. 236(3):641-5.

Guegan T, Cutando L, Ayuso E, Santini E, Fisone G, Bosch F, Martinez A, Valjent E, Maldonado R, Martin M (2013) Operant behavior to obtain palatable food modifies neuronal plasticity in the brain reward circuit. Eur Neuropsychopharmacol. 23(2):146-59.

Hagmann WK (2008) The discovery of taranabant, a selective cannabinoid-1 receptor inverse agonist for the treatment of obesity. Arch Pharm (Weinheim). 341(7):405-11.

Hagues G, Costentin J, Duterte-Boucher D (2007) Modulation of morphine and alcohol motor stimulant effects by cannabinoid receptors ligands. Behav Brain Res. 178(2):274-82.

Hamlin AS, Blatchford KE, McNally GP (2006) Renewal of an extinguished instrumental response: neural correlates and the role of D1 dopamine receptors. Neuroscience. 143:25–38.

Haney M, Rabkin J, Gunderson E, Foltin RW (2005) Dronabinol and marijuana in HIV+ marijuana smokers: Acute effects on caloric intake and mood. Psychopharmacology (Berlin). 181, 170–178.

Harris KM, Jensen FE, Tsao BH (1989) Ultrastructure, development and plasticity of dendritic spine synapses in area CA1 of the rat hippocampus: extending our vision with serial electron microscopy and three-dimensional analyses. The Hippocampus . pp. 33–52.

Harris KM, Fiala JC, Ostroff L (2003) Structural changes at dendritic spine synapses during long-term potentiation. Philos Trans R Soc Lond B Biol Sci. 358(1432):745-8.

Harrold JA, Elliott JC, King PJ, Widdowson PS, Williams G (2002) Down-regulation of cannabinoid-1 (CB-1) receptors in specific extrahypothalamic regions of rats with dietary obesity: a role for endogenous cannabinoids in driving appetite for palatable food? Brain Res. 952(2):232-8.

Harrold JA, Williams G (2003) The cannabinoid system: a role in both the homeostatic and hedonic control of eating? Br J Nutr. 90(4):729-34.

Harrold JA, Dovey TM, Blundell JE, Halford JC (2012) CNS regulation of appetite. Neuropharmacology. 63(1):3-17.

Harvey CD, Yasuda R, Zhong H, Svoboda K (2008) The spread of Ras activity triggered by activation of a single dendritic spine. Science. 321(5885):136-40.

Haslam DW, James WP (2005): Obesity. Lancet 366:1197–1209.

Hattori K, Tanaka H, Yamamoto N, Teraishi T, Hori H, Kinoshita Y, Matsuo J, Kawamoto Y, Kunugi H (2012) Biol Psychol. (1):117-22. Blood CADPS2ΔExon3 expression is associated with intelligence and memory in healthy adults.

Hatzfeld M (2007) Plakophilins: Multifunctional proteins or just regulators of desmosomal adhesion? Biochim Biophys Acta. 1773(1):69-77.

Hayashi K, Pan Y, Shu H, Ohshima T, Kansy JW, White CL 3rd, Tamminga CA, Sobel A, Curmi PA, Mikoshiba K, Bibb JA (2006) Phosphorylation of the tubulin-binding protein, stathmin, by Cdk5 and MAP kinases in the brain. J Neurochem. 99(1):237-50.

He XY, Merz G, Yang YZ, Pullakart R, Mehta P, Schulz H, Yang SY (2000) Function of human brain short chain L-3-hydroxyacyl coenzyme A dehydrogenase in androgen metabolism. Biochim Biophys Acta. 1484 (2-3):267-77.

Hebb DO, (1961) "Distinctive features of learning in the higher animal". In J. F. Delafresnaye (Ed.). Brain Mechanisms and Learning. London: Oxford University Press.

Hendershot CS, Witkiewitz K, George WH, Marlatt GA (2011) Relapse prevention for addictive behaviors. Subst Abuse Treat Prev Policy 19;6:17.

Hering H, Sheng M (2001)Dendritic spines: structure, dynamics and regulation. Nat Rev Neurosci. 2(12):880-8.

Hermann D, Smolka MN, Wrase J, Klein S, Nikitopoulos J, Georgi A, Braus DF, Flor H, Mann K, Heinz A (2006) Blockade of cue-induced brain activation of abstinent alcoholics by a single administration of amisulpride as measured with fMRI. Alcohol Clin Exp Res. 30(8):1349-54.

Hernandez G, Cheer JF (2012) Effect of CB1 receptor blockade on food-reinforced responding and associated nucleus accumbens neuronal activity in rats. J Neurosci. 32(33):11467-77.

Heron SE, Dibbens LM (2013) Role of PRRT2 in common paroxysmal neurological disorders: a gene with remarkable pleiotropy. J Med Genet. 50(3):133-9.

Heuser, J. E. & Reese, T. S (1973) Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. J. Cell Biol. 57, 315–344.

Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M (1999) Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA. 282(16):1568-75.

Heyne A, Kiesselbach C, Sahún I, McDonald J, Gaiffi M, Dierssen M, Wolffgramm J (2009) An animal model of compulsive food-taking behaviour. Addict Biol. 14(4):373-83.

Higgs S, Williams CM, Kirkham TC (2003) Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after delta(9)-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. Psychopharmacology (Berl). 165(4):370-7.

Hilairet S, Bouaboula M, Carrière D, Le Fur G, Casellas P (2003) Hypersensitization of the Orexin 1 receptor by the CB1 receptor: evidence for cross-talk blocked by the specific CB1 antagonist, SR141716. 278(26):23731-7.

Hill MN, Hillard CJ, McEwen BS (2011) Alterations in corticolimbic dendritic morphology and emotional behavior in cannabinoid CB1 receptor-deficient mice parallel the effects of chronic stress. Cereb Cortex. 21(9):2056-64.

Hlavanda E, Klement E, Kókai E, Kovács J, Vincze O, Tökési N, Orosz F, Medzihradszky KF, Dombrádi V, Ovádi J (2007) Phosphorylation blocks the

activity of tubulin polymerization-promoting protein (TPPP): identification of sites targeted by different kinases. J Biol Chem. 282(40):29531-9.

Hnasko TS, Sotak BN, Palmiter RD (2005)Morphine reward in dopamine-deficient mice. Nature. 438(7069):854-7

Hogarth L, Chase HW, Baess K (2012) Impaired goal-directed behavioural control in human impulsivity. Q J Exp Psychol (Hove). 65(2):305-16.

Hoeffer CA, Klann E (2010) mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci. 33(2):67-75.

Hoffman AF, Oz M, Caulder T, Lupica CR (2003) Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. J Neurosci. 23(12):4815-20.

Hojo M, Sudo Y, Ando Y, Minami K, Takada M, Matsubara T, Kanaide M, Taniyama K, Sumikawa K, Uezono Y (2008) mu-Opioid receptor forms a functional heterodimer with cannabinoid CB1 receptor: electrophysiological and FRET assay analysis.J Pharmacol Sci. 108(3):308-19.

Hollander JA, Carelli RM (2007) Cocaine-associated stimuli increase cocaine seeking and activate accumbens core neurons after abstinence. J Neurosci. 27(13):3535-9.

Holtmaat A, Svoboda K (2009) Experience-dependent structural synaptic plasticity in the mammalian brain. Nat Rev Neurosci. 10(9):647-58.

Holden C (2001) 'Behavioral' addictions: do they exist? Science. 294(5544):980-2.

Hooks MS, Jones GH, Liem BJ, Justice JB (1992) Sensitization and individual differences to IP amphetamine, cocaine, or caffeine following repeated intracranial amphetamine infusions. Pharmacol Biochem Behav 43: 815–823.

Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB (1991) Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse. 9: 121–128.

Horvitz JC (2002) Dopamine gating of glutamatergic sensorimotor and incentive motivational input signals to the striatum. Behav Brain Res. 137(1-2):65-74.

Hotulainen P, Llano O, Smirnov S, Tanhuanpää K, Faix J, Rivera C, Lappalainen P (2009) Defining mechanisms of actin polymerization and depolymerization during dendritic spine morphogenesis. J Cell Biol. 185(2):323-39.

Hu WH, Hausmann ON, Yan MS, Walters WM, Wong PK, Bethea JR (2002) Identification and characterization of a novel Nogo-interacting mitochondrial protein (NIMP). J Neurochem. 81(1):36-45.

Huang CC, Lin HJ, Hsu KS (2007) Repeated cocaine administration promotes long-term potentiation induction in rat medial prefrontal cortex. Cereb Cortex. 17(8):1877-88.

Huang YH, Lin Y, Mu P, Lee BR, Brown TE, Wayman G, Marie H, Liu W, Yan Z, Sorg BA, Schlüter OM, Zukin RS, Dong Y (2009)In vivo cocaine experience generates silent synapses. Neuron. 63(1):40-7.

Huang KY, Chen GD, Cheng CH, Liao KY, Hung CC, Chang GD, Hwang PP, Lin SY, Tsai MC, Khoo KH, Lee MT, Huang CJ (2011) Phosphorylation of the zebrafish M6Ab at serine 263 contributes to filopodium formation in PC12 cells and neurite outgrowth in zebrafish embryos. PLoS One. 6(10):e26461.

Huntley GW (2012) Synaptic circuit remodelling by matrix metalloproteinases in health and disease. Nat Rev Neurosci. 13(11):743-57.

Hyman SE, Malenka RC (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. Nat Rev Neurosci. 2(10):695-703.

Hyman SE (2005) Addiction: a disease of learning and memory. Am J Psychiatry. 162(8):1414-22.

Hyman SE, Malenka RC, Nestler EJ (2006) Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci. 29:565-98.

Ibrahim H, Lee YJ, Curthoys NP (2008) Renal response to metabolic acidosis: role of mRNA stabilization. Kidney Int. 73(1):11-8.

Ikeda M, Brown J, Holland AJ, Fukuhara R, Hodges JR (2002)Changes in appetite, food preference, and eating habits in frontotemporal dementia and Alzheimer's disease.J Neurol Neurosurg Psychiatry. 73(4):371-6.

Imaizumi M, Takeda M, Fushiki T (2000) Effects of oil intake in the conditioned place preference test in mice. Brain Res. 870(1-2):150-6.

Isaac JT, Nicoll RA, Malenka RC (1995) Evidence for silent synapses: implications for the expression of LTP. Neuron. 15(2):427-34.

Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ (2000) Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. J Neurosci. 20(19):7489-95.

Ito R, Dalley JW, Robbins TW, Everitt BJ (2002) Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. J Neurosci. 22(14):6247-53.

Jacob W, Marsch R, Marsicano G, Lutz B, Wotjak CT (2012) Cannabinoid CB1 receptor deficiency increases contextual fear memory under highly aversive conditions and long-term potentiation in vivo. Neurobiol Learn Mem. 98(1):47-55.

Jaeger JP, Mattevi VS, Callegari-Jacques SM, Hutz MH (2008) Cannabinoid type-1 receptor gene polymorphisms are associated with central obesity in a Southern Brazilian population. Dis Markers. 25(1):67-74.

Jamshidi N, Taylor DA (2001) Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. Br J Pharmacol. 134(6):1151-4.

Jaworski J, Kapitein LC, Gouveia SM, Dortland BR, Wulf PS, Grigoriev I, Camera P, Spangler SA, Di Stefano P, Demmers J, Krugers H, Defilippi P, Akhmanova A, Hoogenraad CC (2009) Dynamic microtubules regulate dendritic spine morphology and synaptic plasticity. Neuron. 61(1):85-100.

Jay TM (2003) Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. Prog Neurobiol. 69(6):375-90.

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(4):373-90.

Jia H, Lubetkin EI (2010) Trends in quality-adjusted life-years lost contributed by smoking and obesity. Am J Prev Med 38:138–144.

Jiang X, Tian Q, Wang Y, Zhou XW, Xie JZ, Wang JZ, Zhu LQ (2011) Acetyl-L-carnitine ameliorates spatial memory deficits induced by inhibition of phosphoinositol-3 kinase and protein kinase C. J Neurochem. 118(5):864-78.

Jin W, Brown S, Roche JP, Hsieh C, Celver JP, Kovoor A, Chavkin C, Mackie K (1999) Distinct domains of the CB1 cannabinoid receptor mediate desensitization and internalization. J Neurosci. 1999 19(10):3773-80.

Jockusch WJ, Speidel D, Sigler A, Sørensen JB, Varoqueaux F, Rhee JS, Brose N (2007) CAPS-1 and CAPS-2 are essential synaptic vesicle priming proteins. Cell. 131(4):796-808.

Johansson BB (2000) Brain plasticity and stroke rehabilitation. The Willis lecture. Stroke. 31(1):223-30.

Johnson PI, Napier TC (2000) Ventral pallidal injections of a mu antagonist block the development of behavioral sensitization to systemic morphine.Synapse. 2000 Oct;38(1):61-70.

Johnson PM, Kenny PJ (2010) Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nat Neurosci. 13(5):635-41.

Jones JL, Wheeler RA, Carelli RM (2008) Behavioral responding and nucleus accumbens cell firing are unaltered following periods of abstinence from sucrose. Synapse. 62(3):219-28.

Joyce EM, Stinus L, Iversen SD (1983) Effect of injections of 6-OHDA into either nucleus accumbens septi or frontal cortex on spontaneous and drug-induced activity. Neuropharmacology. 22(9):1141-5.

Julliard AK, Chaput MA, Apelbaum A, Aimé P, Mahfouz M, Duchamp-Viret P (2007) Changes in rat olfactory detection performance induced by orexin and leptin mimicking fasting and satiation. Behav Brain Res. 183(2):123-9.

June HL, Harvey SC, Foster KL, McKay PF, Cummings R, Garcia M, Mason D, Grey C, McCane S, Williams LS, Johnson TB, He X, Rock S, Cook JM (2001) GABA(A) receptors containing (alpha)5 subunits in the CA1 and CA3 hippocampal fields regulate ethanol-motivated behaviors: an extended ethanol reward circuitry. J Neurosci. 21(6):2166-77.

Jung Y, Mulholland PJ, Wiseman SL, Judson Chandler L, Picciotto MR (2013) Constitutive knockout of the membrane cytoskeleton protein beta adducin decreases mushroom spine density in the nucleus accumbens but does not prevent spine remodeling in response to cocaine. 37(1):1-9.

Justinova Z, Solinas M, Tanda G, Redhi GH, Goldberg SR (2005) The endogenous cannabinoid anandamide and its synthetic analog R(+)-methanandamide are intravenously self-administered by squirrel monkeys. J Neurosci. 25:5645–50.

Kalivas PW, Widerlöv E, Stanley D, Breese G, Prange AJ Jr (1983) Enkephalin action on the mesolimbic system: a dopamine-dependent and a dopamine-

independent increase in locomotor activity.J Pharmacol Exp Ther. 227(1):229-37.

Kalivas PW, Klitenick MA, Hagler H, Austin MC (1991) GABAergic and enkephalinergic regulation of locomotion in the ventral pallidum: involvement of the mesolimbic dopamine system.Adv Exp Med Biol. 1991;295:315-26.

Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403-1413.

Kalivas PW, O'Brien C (2008) Drug addiction as a pathology of staged neuroplasticity. Neuropsychopharmacology. 33(1):166-80.

Kalivas PW, Lalumiere RT, Knackstedt L, Shen H (2009) Glutamate transmission in addiction. Neuropharmacology56 Suppl 1:169-73.

Kalivas PW (2009) The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci. 10(8):561-72.

Kao GS, Chuang JY, Cherng CF, Yu L (2011) Accumbal 14-3-3ζ protein downregulation is associated with cocaine-conditioned memory. Neurosignals. 19(4):175-88.

Karreman M, Westerink BH, Moghaddam B (1996) Excitatory amino acid receptors in the ventral tegmental area regulate dopamine release in the ventral striatum. J Neurochem. 1996 Aug;67(2):601-7.

Kasai H, Matsuzaki M, Noguchi J, Yasumatsu N, Nakahara H (2003) Structure-stability-function relationships of dendritic spines. Trends Neurosci. 26(7):360-8.

Kasanetz F, Deroche-Gamonet V, Berson N, Balado E, Lafourcade M, Manzoni O, Piazza PV (2010) Transition to addiction is associated with a persistent impairment in synaptic plasticity. Science. 328(5986):1709-12.

Kauer JA, Malenka RC (2007) Synaptic plasticity and addiction. Nat Rev Neurosci. 8(11):844-58.

Kawaai K, Tominaga-Yoshino K, Urakubo T, Taniguchi N, Kondoh Y, Tashiro H, Ogura A, Tashiro T (2010) Analysis of gene expression changes associated with long-lasting synaptic enhancement in hippocampal slice cultures after repetitive exposures to glutamate. J Neurosci Res. 88(13):2911-22.

Kearn CS, Blake-Palmer K, Daniel E, Mackie K, Glass M (2005) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? Mol Pharmacol. 67(5):1697-704.

Kelleher RJ 3rd, Govindarajan A, Tonegawa S (2004a) Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron. 44(1):59-73.

Kelleher RJ 3rd, Govindarajan A, Jung HY, Kang H, Tonegawa S (2004) Translational control by MAPK signaling in long-term synaptic plasticity and memory. Cell. 116(3):467-79.

Kelley AE, Smith-Roe SL, Holahan MR (1997) Response-reinforcement learning is dependent on N-methyl-D-aspartate receptor activation in the nucleus accumbens core. Proc Natl Acad Sci U S A. 94(22):12174-9.

Kelley AE (1999) Functional specificity of ventral striatal compartments in appetitive behaviors. Ann N Y Acad Sci. 877:71-90.

Kelley AE, Berridge KC (2002) The neuroscience of natural rewards: relevance to addictive drugs. J. Neurosci. 22 (9), 3306–3311.

Kelley AE, Bakshi VP, Haber SN, Steininger TL, Will MJ, Zhang M (2002) Opioid modulation of taste hedonics within the ventral striatum Physiol Behav 76: 365–377.

Kelley AE, Baldo BA, Pratt WE, Will MJ (2005) Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. Physiol Behav. 86(5):773-95.

Kenny PJ (2011) Reward mechanisms in obesity: new insights and future directions. Neuron. 69(4):664-79.

Ketterer C, Heni M, Thamer C, Herzberg-Schafer SA, Haring HU Fritsche A (2010) Acute, short-term hyperinsulinemia increases olfactory threshold in healthy subjects. Int J Obes (Lond). 35(8):1135-8.

Kilts CD, Schweitzer JB, Quinn CK, Gross RE, Faber TL, Muhammad F, Ely TD, Hoffman JM, Drexler KP (2001) Neural activity related to drug craving in cocaine addiction. Arch Gen Psychiatry. 58(4):334-41.

Kirkham TC, Williams CM, Fezza F, Di Marzo V (2002) Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. Br J Pharmacol. 136(4):550-7.

Knackstedt LA, Kalivas PW (2009) Glutamate and reinstatement. Curr Opin Pharmacol. 9(1):59-64.

Kobayashi T, Nakatani Y, Tanioka T, Tsujimoto M, Nakajo S, Nakaya K, Murakami M, Kudo I (2004) Regulation of cytosolic prostaglandin E synthase by phosphorylation. Biochem J. 2004 Jul 1;381(Pt 1):59-69.

Koch JE, Matthews SM (2001) Delta9-tetrahydrocannabinol stimulates palatable food intake in Lewis rats: effects of peripheral and central administration. Nutr Neurosci. 4: 179–187.

Koh MT, Clarke SN, Spray KJ, Thiele TE, Bernstein IL (2003) Conditioned taste aversion memory and c-Fos induction are disrupted in RIIbeta-protein kinase A mutant mice. Behav Brain Res. 143(1):57-63.

Kolb B, Gorny G, Li Y, Samaha AN, 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. USA 100, 10523–

10528.

Koob GF, Nestler EJ (1997) The neurobiology of drug addiction. J Neuropsychiatry Clin Neurosci. 9(3):482-97.

Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology. 24(2):97-129.

Koob GF, Le Moal M (2008a) Neurobiological mechanisms for opponent motivational processes in addiction. Philos Trans R Soc Lond B Biol Sci. 363(1507):3113-23.

Koob GF, Le Moal M (2008b) Addiction and the brain antireward system. Annu Rev Psychol. 2008;59:29-53.

Koob GF (2009) Dynamics of neuronal circuits in addiction: reward, antireward, and emotional memory. Pharmacopsychiatry. 42 Suppl 1:S32-41.

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35(1):217-38.

Kosten TR, George TP (2002) The neurobiology of opioid dependence: implications for treatment. Sci Pract Perspect. 1(1):13-20.

Kotlińska J, Biała G (2000) Memantine and ACPC affect conditioned place preference induced by cocaine in rats. Pol J Pharmacol. 2000 May-Jun;52(3):179-85.

Kramár EA, Lin B, Rex CS, Gall CM, Lynch G (2006) Integrin-driven actin polymerization consolidates long-term potentiation. Proc Natl Acad Sci U S A. 103(14):5579-84.

Krishnan KS, Rikhy R, Rao S, Shivalkar M, Mosko M, Narayanan R, Etter P, Estes PS, Ramaswami M (2001) Nucleoside diphosphate kinase, a source of GTP, is required for dynamin-dependent synaptic vesicle recycling. Neuron. 2001 Apr;30(1):197-210.

Krugers HJ, Hoogenraad CC, Groc L (2010) Stress hormones and AMPA receptor trafficking in synaptic plasticity and memory. Nat Rev Neurosci. 11(10):675-81.

Kwon HB, Sabatini BL (2011) Glutamate induces de novo growth of functional spines in developing cortex. Nature. 2011 474(7349):100-4.

Koya E, Spijker S, Voorn P, Binnekade R, Schmidt ED, Schoffelmeer AN, De Vries TJ, Smit AB (2006) Enhanced cortical and accumbal molecular reactivity associated with conditioned heroin, but not sucrose-seeking behaviour. J Neurochem. 98(3):905-15.

Lafourcade M, Larrieu T, Mato S, Duffaud A, Sepers M, Matias I, De Smedt-Peyrusse V, Labrousse VF, Bretillon L, Matute C, Rodríguez-Puertas R, Layé S, Manzoni OJ (2011) Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions. Nat Neurosci. 14(3):345-50.

Lai KO, Wong AS, Cheung MC, Xu P, Liang Z, Lok KC, Xie H, Palko ME, Yung WH, Tessarollo L, Cheung ZH, Ip NY (2012) TrkB phosphorylation by Cdk5 is required for activity-dependent structural plasticity and spatial memory. Nat Neurosci. 15(11):1506-15.

Liao HJ, Carpenter G (2007) Role of the Sec61 translocon in EGF receptor trafficking to the nucleus and gene expression. Mol Biol Cell. 18(3):1064-72.

Liang YJ, Wu DF, Stumm R, Höllt V, Koch T (2008) Membrane glycoprotein M6A promotes mu-opioid receptor endocytosis and facilitates receptor sorting into the recycling pathway. Cell Res. 18(7):768-79.

Lammel S, Ion DI, Roeper J, Malenka RC (2011) Projection-specific modulation of dopamine neuron synapses by aversive and rewarding stimuli. Neuron. 70(5):855-62.

Lamprecht R, LeDoux J (2004) Structural plasticity and memory. Nat Rev Neurosci. 5(1):45-54.

Larson EB, Roth ME, Anker JJ, Carroll ME (2005) Effect of short- vs. long-term estrogen on reinstatement of cocaine-seeking behavior in female rats. Pharmacol Biochem Behav. 82(1):98-108.

Lavaur J, Mineur YS, Picciotto MR (2009) The membrane cytoskeletal protein adducin is phosphorylated by protein kinase C in D1 neurons of the nucleus accumbens and dorsal striatum following cocaine administration. J Neurochem. 111(5):1129-37.

Le Bon O, Basiaux P, Streel E, Tecco J, Hanak C, Hansenne M, Ansseau M, Pelc I, Verbanck P, Dupont S (2004) Personality profile and drug of choice; a multivariate analysis using Cloninger's TCI on heroin addicts, alcoholics, and a random population group. Drug Alcohol Depend. 73(2):175-82.

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, Böhme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, Parmentier M (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science. 283(5400):401-4

Lee KW, Kim Y, Kim AM, Helmin K, Nairn AC, Greengard P (2006) Cocaine-induced dendritic spine formation in D1 and D2 dopamine receptor-containing medium spiny neurons in nucleus accumbens. Proc Natl Acad Sci U S A. 103(9):3399-404.

Lees-Miller SP, Anderson CW (1989) Two human 90-kDa heat shock proteins are phosphorylated in vivo at conserved serines that are phosphorylated in vitro by casein kinase II. J Biol Chem. 264(5):2431-7.

Leith NJ, Kuczenski R (1982) Two dissociable components of behavioral sensitization following repeated amphetamine administration. Psychopharmacology (Berl) 76:310–315.

Lejuez CW, Zvolensky MJ, Daughters SB, Bornovalova MA, Paulson A, Tull MT, Ettinger K, Otto MW (2008) Anxiety sensitivity: A unique predictor of dropout

among inner-city heroin and crack/cocaine users in residential substance use treatment. Behav Res Ther 46: 811–818.

Le Merrer J, Becker JA, Befort K, Kieffer BL (2009) Reward processing by the opioid system in the brain. Physiol Rev. 89(4):1379-412.

Lepsch LB, Munhoz CD, Kawamoto EM, Yshii LM, Lima LS, Curi-Boaventura MF, Salgado TM, Curi R, Planeta CS, Scavone C (2009) Cocaine induces cell death and activates the transcription nuclear factor kappa-B in PC12 cells. Mol Brain. 2:3.

Lesscher HM, Houthuijzen JM, Groot Koerkamp MJ, Holstege FC, Vanderschuren LJ (2012) Amygdala 14-3-3ζ as a novel modulator of escalating alcohol intake in mice. PLoS One. 7(5):e37999.

Li Y, Acerbo MJ, Robinson TE (2004a) The induction of behavioural sensitization is associated with cocaine-induced structural plasticity in the core (but not shell) of the nucleus accumbens. Eur J Neurosci. 20(6):1647-54.

Li Z, Okamoto K, Hayashi Y, Sheng M (2004b) The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. Cell. 119(6):873-87.

Li X, Hoffman AF, Peng XQ, Lupica CR, Gardner EL, Xi ZX (2009a) Attenuation of basal and cocaine-enhanced locomotion and nucleus accumbens dopamine in cannabinoid CB1-receptor-knockout mice. Psychopharmacology (Berl). 204(1):1-11.

Li X, Li J, Peng XQ, Spiller K, Gardner EL, Xi ZX (2009b) Metabotropic glutamate receptor 7 modulates the rewarding effects of cocaine in rats: involvement of a ventral pallidal GABAergic mechanism. Neuropsychopharmacology. 34(7):1783-96

Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, Li XY, Aghajanian G, Duman RS (2010a) mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science. 329(5994):959-64.

Li X, Li J, Gardner EL, Xi ZX (2010b) Activation of mGluR7s inhibits cocaine-induced reinstatement of drug-seeking behavior by a nucleus accumbens glutamate-mGluR2/3 mechanism in rats. J Neurochem 114: 1368–1380.

Li FQ, Xue YX, Wang JS, Fang Q, Li YQ, Zhu WL, He YY, Liu JF, Xue LF, Shaham Y, Lu L (2010c) Basolateral amygdala cdk5 activity mediates consolidation and reconsolidation of memories for cocaine cues. J Neurosci. 30(31):10351-9.

Li HD, Liu WX, Michalak M (2011) Enhanced clathrin-dependent endocytosis in the absence of calnexin. PLoS One. 6(7):e21678.

Lillard SA, Erisir A (2011) Old dogs learning new tricks: Neuroplasticity beyond the juvenile period. Developmental Review. Volume 31, issue 4, pages 207-239.

Lisman JE, Grace AA (2005) The hippocampal-VTA loop: controlling the entry of information into long-term memory. Neuron. 46(5):703-13.

Liu YC, Berta T, Liu T, Tan PH, Ji RR (2012) Acute morphine induces matrix metalloproteinase-9 up-regulation in primary sensory neurons to mask opioid-induced analgesia in mice. Mol Pain. 8:19.

Lochner JE, Honigman LS, Grant WF, Gessford SK, Hansen AB, Silverman MA, Scalettar BA (2006) Activity-dependent release of tissue plasminogen activator from the dendritic spines of hippocampal neurons revealed by live-cell imaging. J Neurobiol. (6):564-77.

Losonczy, A., Somogyi, P., and Nusser, Z. (2003). Reduction of excitatory postsynaptic responses by persistently active metabotropic glutamate receptors in the hippocampus. J. Neurophysiol. 89, 1910–1919.

Lovgren AK, Kovarova M, Koller BH (2007) cPGES/p23 is required for glucocorticoid receptor function and embryonic growth but not prostaglandin E2 synthesis. Mol Cell Biol. 27(12):4416-30.

Lu Q, Pallas DC, Surks HK, Baur WE, Mendelsohn ME, Karas RH (2004) Striatin assembles a membrane signaling complex necessary for rapid, nongenomic activation of endothelial NO synthase by estrogen receptor alpha. Proc Natl Acad Sci U S A. 101(49):17126-31

Lüscher C, Ungless MA (2006) The mechanistic classification of addictive drugs. PLoS Med. 3(11):e437.

Lüscher C, Isaac JT (2009) The synapse: center stage for many brain diseases. J Physiol. 587(Pt 4):727-9.

Lüscher C, Bellone C (2008) Cocaine-evoked synaptic plasticity: a key to addiction? Nat Neurosci. 11(7):737-8.

Lüscher C, Malenka RC (2011) Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. Neuron. 69(4):650-63.

Lutter M, Nestler EJ (2009) Homeostatic and hedonic signals interact in the regulation of food intake. J Nutr. 139(3):629-32.

Magnus-Ellenbroek B, Havemann-Reinecke U (1993) Morphine-induced hyperactivity in rats--a rebound effect? Naunyn Schmiedebergs Arch Pharmacol. 347(6):635-42.

Mahler SV, Smith KS, Berridge KC (2007) Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. Neuropsychopharmacology. 32(11):2267-78.

Malenka RC, Bear MF (2004) LTP and LTD: an embarrassment of riches. Neuron. 44(1):5-21.

Maldonado R, Valverde O, Berrendero F (2006) Involvement of the endocannabinoid system in drug addiction. Trends Neurosci. 29(4):225-32.

Maldonado-Irizarry CS, Kelley AE (1994) Differential behavioral effects following microinjection of an NMDA antagonist into nucleus accumbens subregions. Psychopharmacology (Berl). 116(1):65-72.

Maldonado-Irizarry CS, Swanson CJ, Kelley AE (1995) Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. J Neurosci. 15(10):6779-88.

Mameli and Lüscher C (2011) Synaptic plasticity and addiction: learning mechanisms gone awry. Neuropharmacology. 61(7):1052-9.

Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G (2012) Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci. 13(5):293-307

Manzanares J, Julian M, Carrascosa A (2006) Role of the cannabinoid system in pain control and therapeutic implications for the management of acute and chronic pain episodes. Curr Neuropharmacol. 4(3):239-57.

Manzanedo C, Aguilar MA, Miñarro J (1999) The effects of dopamine D2 and D3 antagonists on spontaneous motor activity and morphine-induced hyperactivity in male mice. Psychopharmacology (Berl). 143(1):82-8.

Marchant NJ, Li X, Shaham Y (2013) Recent developments in animal models of drug relapse. Curr Opin Neurobiol. pii: S0959-4388(13)00019-6.

Marco EM, Romero-Zerbo SY, Viveros MP, Bermudez-Silva FJ (2012) The role of the endocannabinoid system in eating disorders: pharmacological implications. Behav Pharmacol. 23(5-6):526-36.

Markou A, Weiss F, Gold LH, Caine SB, Schulteis G, Koob GF (1993) Animal models of drug craving. Psychopharmacology (Berl) 112:163-182.

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgänsberger W, Di Marzo V, Lutz B (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature. 2002 Aug 1;418(6897):530-4.

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

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O (2000b) Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice. Eur J Neurosci. 12(11):4038-46.

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(7):868-9.

Martín AB, Fernandez-Espejo E, Ferrer B, Gorriti MA, Bilbao A, Navarro M, Rodriguez de Fonseca F, Moratalla R (2008) Expression and function of CB1 receptor in the rat striatum: localization and effects on D1 and D2 dopamine receptor-mediated motor behaviors. Neuropsychopharmacology. 33(7):1667-79.

Martin-Fardon R, Weiss F (2013) Modeling relapse in animals. Curr Top Behav Neurosci.13:403-32.

Mas A, Montané J, Anguela XM, Muñoz S, Douar AM, Riu E, Otaegui P, Bosch F (2006) Reversal of type 1 diabetes by engineering a glucose sensor in skeletal muscle.Diabetes. 2006 Jun;55(6):1546-53.

Mascia MS, Obinu MC, Ledent C, Parmentier M, Böhme GA, Imperato A, Fratta W (1999) Lack of morphine-induced dopamine release in the nucleus accumbens of cannabinoid CB(1) receptor knockout mice. Eur J Pharmacol. 383(3):R1-2.

Massa F, Mancini G, Schmidt H, Steindel F, Mackie K, Angioni C, Oliet SH, Geisslinger G, Lutz B (2010) Alterations in the hippocampal endocannabinoid system in diet-induced obese mice. J Neurosci. 30(18):6273-81.

Mates SL, Lund JS (1983) Spine formation and maturation of type 1 synapses on spiny stellate neurons in primate visual cortex. J Comp Neurol.221(1):91-7.

Matias I, Cristino L, Di Marzo V (2008) Endocannabinoids: some like it fat (and sweet too). J Neuroendocrinol. 20 Suppl 1:100-9.

Mathieu-Kia AM, Besson MJ (1998) Repeated administration of cocaine, nicotine and ethanol: effects on preprodynorphin, preprotachykinin A and preproenkephalin mRNA expression in the dorsal and the ventral striatum of the rat. Brain Res Mol Brain Res. 54(1):141-51.

Mato S, Chevaleyre V, Robbe D, Pazos A, Castillo PE, Manzoni OJ (2004) A single in-vivo exposure to delta 9THC blocks endocannabinoid-mediated synaptic plasticity. Nat Neurosci. 7(6):585-6.

Matsuoka Y, Li X, Bennett V (1998) Adducin is an in vivo substrate for protein kinase C: phosphorylation in the MARCKS-related domain inhibits activity in

promoting spectrin-actin complexes and occurs in many cells, including dendritic spines of neurons. The Journal of cell biology. 142:485–497

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H (2004) Structural basis of long-term potentiation in single dendritic spines. Nature. 429(6993):761-6.

Mattson MP, Gleichmann M, Cheng A (2008) Mitochondria in neuroplasticity and neurological disorders. Neuron. 60(5):748-66.

McDonald J, Schleifer L, Richards JB, de Wit H (2003) Effects of THC on behavioral measures of impulsivity in humans. Neuropsychopharmacology. 28(7):1356-65.
McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci. 21(21):8655-63.

McLaughlin J, See RE (2003) Selective inactivation of the dorsomedial prefrontal cortex and the basolateral amygdala attenuates conditioned-cued reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology (Berl). 168(1-2):57-65.

McLaughlin PJ, Winston K, Swezey L, Wisniecki A, Aberman J, Tardif DJ, Betz AJ, Ishiwari K, Makriyannis A, Salamone JD (2003) The cannabinoid CB1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. Behav Pharmacol. 14(8):583-8.

Mechoulam R, Parker LA (2013) The endocannabinoid system and the brain. Annu Rev Psychol. 64:21-47.

Melis M, Gessa GL, Diana M (2000) Different mechanisms for dopaminergic excitation induced by opiates and cannabinoids in the rat midbrain. Prog Neuropsychopharmacol Biol Psychiatry. 24(6):993-1006.

Melis T, Succu S, Sanna F, Boi A, Argiolas A, Melis MR (2007) The cannabinoid antagonist SR 141716A (Rimonabant) reduces the increase of extra-cellular dopamine release in the rat nucleus accumbens induced by a novel high palatable food. Neurosci Lett. 419(3):231-5.

Mena JD, Sadeghian K, Baldo BA (2011) Induction of hyperphagia and carbohydrate intake by μ-opioid receptor stimulation in circumscribed regions of frontal cortex. J Neurosci. 31(9):3249-60.

Meredith GE (1999) The synaptic framework for chemical signaling in nucleus accumbens. Ann N Y Acad Sci. 877:140-56.

Meredith GE, Baldo BA, Andrezjewski ME, Kelley AE (2008) The structural basis for mapping behavior onto the ventral striatum and its subdivisions. Brain Struct Funct. 213(1-2):17-27.

Merikangas KR, He JP, Burstein M, Swanson SA, Avenevoli S, Cui L, Benjet C, Georgiades K, Swendsen J (2010) Lifetime prevalence of mental disorders in U.S. adolescents: results from the National Comorbidity Survey Replication--Adolescent Supplement (NCS-A). J Am Acad Child Adolesc Psychiatry. 49(10):980-9.

Meyers RA, Zavala AR, Neisewander JL (2003) Dorsal, but not ventral, hippocampal lesions disrupt cocaine place conditioning. Neuroreport. 14(16):2127-31.

Meyer B, Wittig I, Trifilieff E, Karas M, Schägger H (2007) Identification of two proteins associated with mammalian ATP synthase. Mol Cell Proteomics. 6(10):1690-9.

Milekic MH, Brown SD, Castellini C, Alberini CM (2006) Persistent disruption of an established morphine conditioned place preference. J Neurosci. 26(11):3010-20.

Miller EK, Freedman DJ, Wallis JD (2002) The prefrontal cortex: categories, concepts and cognition. Philos Trans R Soc Lond B Biol Sci. 2002 Aug 29;357(1424):1123-36.

Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foufelle F, Ferré P, Birnbaum MJ, Stuck BJ, Kahn BB (2004) AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature. 428(6982):569-74.

Mitrovic S, Ben-Tekaya H, Koegler E, Gruenberg J, Hauri HP (2008) The cargo receptors Surf4, endoplasmic reticulum-Golgi intermediate compartment (ERGIC)-53, and p25 are required to maintain the architecture of ERGIC and Golgi. Mol Biol Cell. 19(5):1976-90.

Mitsuyama F, Niimi G, Kato K, Hirosawa K, Mikoshiba K, Okuya M, Karagiozov K, Kato Y, Kanno T, Sanoe H, Koide T (2008) Redistribution of microtubules in dendrites of hippocampal CA1 neurons after tetanic stimulation during long-term potentiation. Ital J Anat Embryol. 113(1):17-27.

Miyamoto E (2006) Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. J Pharmacol Sci. 100(5):433-42.

Mizuno M, Yamada K, Olariu A, Nawa H, Nabeshima T (2006) Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. J Neurosci. 20(18):7116-21.

Moh MC, Tian Q, Zhang T, Lee LH, Shen S (2009) The immunoglobulin-like cell adhesion molecule hepaCAM modulates cell adhesion and motility through direct interaction with the actin cytoskeleton. J Cell Physiol. 219(2):382-91.

Möller JC, Krüttgen A, Burmester R, Weis J, Oertel WH, Shooter EM (2006) Release of interleukin-6 via the regulated secretory pathway in PC12 cells. Neurosci Lett. 400(1-2):75-9.

Monteggia LM, Barrot M, Powell CM, Berton O, Galanis V, Gemelli T, Meuth S, Nagy A, Greene RW, Nestler EJ (2004) Essential role of brain-derived neurotrophic factor in adult hippocampal function. Proc Natl Acad Sci U S A. 101(29):10827-32.

Monteleone P, Matias I, Martiadis V, De Petrocellis L, Maj M, Di Marzo V (2005) Blood levels of the endocannabinoid anandamide are increased in anorexia nervosa and in binge-eating disorder, but not in bulimia nervosa. Neuropsychopharmacology.30(6):1216-21.

Morales M, Pickel VM (2012) Insights to drug addiction derived from ultrastructural views of the mesocorticolimbic system. Ann N Y Acad Sci. 1248:71-88.

Morawska MM, Fendt M (2012) The effects of muscimol and AMN082 injections into the medial prefrontal cortex on the expression and extinction of conditioned fear in mice. J Exp Biol. 215(Pt 8):1394-8.

Mori N. and Morii H. (2002) SCG10-related neuronal growth-associated proteins in neural development, plasticity, degeneration, and aging. J. Neurosci. Res. 70, 264–273.

Morón JA, Abul-Husn NS, Rozenfeld R, Dolios G, Wang R, Devi LA (2007) Morphine administration alters the profile of hippocampal postsynaptic density-associated proteins: a proteomics study focusing on endocytic proteins. Mol Cell Proteomics. 6(1):29-42.

Morris RG, Moser EI, Riedel G, Martin SJ, Sandin J, Day M, O'Carroll C (2003) Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. Philos Trans R Soc Lond B Biol Sci.

358(1432):773-86.

Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW (2006) Central nervous system control of food intake and body weight. Nature. 443(7109):289-95.

Mukhopadhyay S and Sheng H (2011) Targetting CB1 Cannabinoid Receptor for Neuroprotetion in Spinal Cord Injury. The FASEB Journal. 2011;25:lb422.

Müller M, Pym EC, Tong A, Davis GW (2011) Rab3-GAP controls the progression of synaptic homeostasis at a late stage of vesicle release. Neuron. 69(4):749-62.

Naassila M, Pierrefiche O, Ledent C, Daoust M (2004) Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. Neuropharmacology. 46(2):243-53.

Nagai T, Yamada K, Yoshimura M, Ishikawa K, Miyamoto Y, Hashimoto K, Noda Y, Nitta A, Nabeshima T (2004) The tissue plasminogen activator-plasmin system participates in the rewarding effect of morphine by regulating dopamine release. Proc Natl Acad Sci U S A. 101(10):3650-5.

Nair SG, Adams-Deutsch T, Epstein DH, Shaham Y (2009) The neuropharmacology of relapse to food seeking: methodology, main findings, and comparison with relapse to drug seeking. Prog Neurobiol. 89(1):18-45.

Nairn AC, Svenningsson P, Nishi A, Fisone G, Girault JA, Greengard P (2004) The role of DARPP-32 in the actions of drugs of abuse. Neuropharmacology. 47 Suppl 1:14-23.

National institute of drug of abuse (1999) Principles of Drug Addiction Treatment: A Research-Based Guide (Third Edition) Revised: December 2012, NIH Pub Number: 12-4180.

Neasta J, Kiely PA, He DY, Adams DR, O'Connor R, Ron D (2012) Direct interaction between scaffolding proteins RACK1 and 14-3-3ζ regulates brain-derived neurotrophic factor (BDNF) transcription. J Biol Chem. 287(1):322-36.

Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci. 2001 Feb;2(2):119-28.

Newhall KJ, Cummings DE, Nolan MA, McKnight GS (2005) Deletion of the RIIbeta-subunit of protein kinase A decreases body weight and increases energy expenditure in the obese, leptin-deficient ob/ob mouse. Mol Endocrinol. 19(4):982-91.

Ng A, Uribe RA, Yieh L, Nuckels R, Gross JM (2009) Zebrafish mutations in gart and paics identify crucial roles for de novo purine synthesis in vertebrate pigmentation and ocular development. Development. 136(15):2601-11.

Nie J, Lewis DL (2001) Structural domains of the CB1 cannabinoid receptor that contribute to constitutive activity and G-protein sequestration. J Neurosci. 21(22):8758-64.

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(1):108-17.

Nimchinsky EA, Sabatini BL, Svoboda K (2002) Structure and function of dendritic spines. Annu Rev Physiol. 2002;64:313-53.

Nogueiras R, Veyrat-Durebex C, Suchanek PM, Klein M, Tschöp J, Caldwell C, Woods SC, Wittmann G, Watanabe M, Liposits Z, Fekete C, Reizes O, Rohner-Jeanrenaud F, Tschöp MH (2008) Peripheral, but not central, CB1 antagonism provides food intake-independent metabolic benefits in diet-induced obese rats. Diabetes. 57(11):2977-91.

Norrholm SD, Bibb JA, Nestler EJ, Ouimet CC, Taylor JR, Greengard P (2003) Cocaine-induced proliferation of dendritic spines in nucleus accumbens is

dependent on the activity of cyclin-dependent kinase-5. Neuroscience.116:19–22

Nowak SJ, Corces VG (2004) Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. Trends Genet. 2004 Apr;20(4):214-20.

O'Brien CP, Childress AR, Ehrman R, Robbins SJ (1998) Conditioning factors in drug abuse: can they explain compulsion? *J. Psychopharmacol.* 12, 15–22.

O'Brien CP (2003) Research advances in the understanding and treatment of addiction. Am J Addict 12 (Suppl 2):S36-47.

O'Connor V, El Far O, Bofill-Cardona E, Nanoff C, Freissmuth M, Karschin A, Airas JM, Betz H, Boehm S (1999). Calmodulin dependence of presynaptic metabotropic glutamate receptor signaling. Science 286, 1180–1184.

O'Donnell C, Nolan MF, van Rossum MC (2011)Dendritic spine dynamics regulate the long-term stability of synaptic plasticity.J Neurosci. 31(45):16142-56.

Okamoto K, Nagai T, Miyawaki A, Hayashi Y (2004) Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity.Nat Neurosci. 7(10):1104-12.

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J Comp Physiol Psychol 47:419-427.

Olive MF, Maidment NT (1998) Opioid regulation of pallidal enkephalin release: bimodal effects of locally administered mu and delta opioid agonists in freely moving rats.J Pharmacol Exp Ther. 285(3):1310-6.

Oliver PL, Finelli MJ, Edwards B, Bitoun E, Butts DL, Becker EB, Cheeseman MT, Davies B, Davies KE (2011) Oxr1 is essential for protection against oxidative stress-induced neurodegeneration. PLoS Genet. 7(10):e1002338.

Olszewski PK, Levine AS (2004) Minireview: Characterization of influence of central nociceptin/orphanin FQ on consummatory behavior. Endocrinology. 145(6):2627-32.

Oray S, Majewska A, Sur M (2004) Dendritic spine dynamics are regulated by monocular deprivation and extracellular matrix degradation. Neuron. 44(6):1021-30.

Orio L, Edwards S, George O, Parsons LH, Koob GF (2009) A role for the endocannabinoid system in the increased motivation for cocaine in extended-access conditions. J Neurosci. 29(15):4846-57.

Ornstein K, Huston JP (1977) Interaction between morphine and reinforcing lateral hypothalamic stimulation. Psychopharmacology (Berl). 54(3):227-35.

Osei-Hyiaman D, Harvey-White J, Bátkai S, Kunos G (2006) The role of the endocannabinoid system in the control of energy homeostasis. Int J Obes (Lond). 30 Suppl 1:S33-8.

Otero AS (2000) NM23/nucleoside diphosphate kinase and signal transduction.J Bioenerg Biomembr. 32(3):269-75.

Overduin J, Figlewicz DP, Bennett-Jay J, Kittleson S, Cummings DE (2012) Ghrelin increases the motivation to eat, but does not alter food palatability. Am J Physiol Regul Integr Comp Physiol. (3):R259-69.

Oxelmark E, Roth JM, Brooks PC, Braunstein SE, Schneider RJ, Garabedian MJ (2006) The cochaperone p23 differentially regulates estrogen receptor target genes and promotes tumor cell adhesion and invasion.Mol Cell Biol. 26(14):5205-13.

Pan B, Hillard CJ, Liu QS (2008) Endocannabinoid signaling mediates cocaine-induced inhibitory synaptic plasticity in midbrain dopamine neurons. J Neurosci. 28(6):1385-97.

Pan B, Zhong P, Sun D, Liu QS (2011) Extracellular signal-regulated kinase signaling in the ventral tegmental area mediates cocaine-induced synaptic plasticity and rewarding effects. J Neurosci. 31(31):11244-55.

Panlilio LV, Goldberg SR (2007) Self-administration of drugs in animals and humans as a model and an investigative tool. Addiction. 102(12):1863-70.

Park M, Salgado JM, Ostroff L, Helton TD, Robinson CG, Harris KM, Ehlers MD (2006) Plasticity-induced growth of dendritic spines by exocytic trafficking from recycling endosomes. Neuron. 52(5):817-30.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. J Neurosci. 19:2401–2411.

Parolaro D, Vigano D, Realini N, Rubino T (2007) Role of endocannabinoids in regulating drug dependence. Neuropsychiatr Dis Treat. 3(6):711-21.

Parylak SL, Cottone P, Sabino V, Rice KC, Zorrilla EP (2012) Effects of CB1 and CRF1 receptor antagonists on binge-like eating in rats with limited access to a sweet fat diet: lack of withdrawal-like responses. Physiol Behav. 107(2):231-42.

Pascoli V, Turiault M, Lüscher C (2011) Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour. Nature. 481(7379):71-5.

Passetti F, Chudasama Y, Robbins TW (2002) The frontal cortex of the rat and visual attentional performance: dissociable functions of distinct medial prefrontal subregions. Cereb Cortex. 2002 Dec;12(12):1254-68.

Pasterkamp RJ, Giger RJ (2009) Semaphorin function in neural plasticity and disease. Curr Opin Neurobiol. 19(3):263-74.

Pasumarthi RK, Fadel J (2010) Stimulation of lateral hypothalamic glutamate and acetylcholine efflux by nicotine: implications for mechanisms of nicotine-induced activation of orexin neurons. J Neurochem. 113(4):1023-35.

Paterson NE, Markou A (2003) Increased motivation for self-administered cocaine after escalated cocaine intake. Neuroreport. 14(17):2229-32.

Pattij T, Janssen MC, Schepers I, González-Cuevas G, de Vries TJ, Schoffelmeer AN (2007) Effects of the cannabinoid CB1 receptor antagonist rimonabant on distinct measures of impulsive behavior in rats. Psychopharmacology (Berl). 193(1):85-96.

Pawson C, Eaton BA, Davis GW (2008) Formin-dependent synaptic growth: evidence that Dlar signals via Diaphanous to modulate synaptic actin and dynamic pioneer microtubules. J Neurosci. 28(44):11111-23.

Pazos MR, Núñez E, Benito C, Tolón RM, Romero J (2005) Functional neuroanatomy of the endocannabinoid system. Pharmacol Biochem Behav. 81(2):239-47.

Pechenino AS, Frick KM (2008) The effects of acute 17beta-estradiol treatment on gene expression in the young female mouse hippocampus. Neurobiol Learn Mem. 91(3):315-22.

Peciña S, Berridge KC (2005) Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? J Neurosci. 25(50):11777-86.

Peciña S (2008) Opioid reward 'liking' and 'wanting' in the nucleus accumbens. Physiol Behav. 94(5):675-80.

Pelchat ML, Johnson A, Chan R, Valdez J, Ragland JD (2004) Images of desire: food-craving activation during fMRI. Neuroimage. 23(4):1486-93.

Pelkey KA, Lavezzari G, Racca C, Roche KW, McBain CJ (2005) mGluR7 is a metaplastic switch controlling bidirectional plasticity of feedforward inhibition. Neuron. 46(1):89-102.

Pelkey KA, Yuan X, Lavezzari G, Roche KW, McBain CJ (2007) mGluR7 undergoes rapid internalization in response to activation by the allosteric agonist AMN082. Neuropharmacology. 52(1):108-17.

Pérez-Otaño I, Ehlers MD (2005) Homeostatic plasticity and NMDA receptor trafficking. Trends Neurosci. 28(5):229-38.

Pério A, Barnouin MC, Poncelet M, Soubrié P (2001) Activity of SR141716 on post-reinforcement pauses in operant responding for sucrose reward in rats. Behav Pharmacol. 2001 Dec;12(8):641-5.

Perello M, Sakata I, Birnbaum S, Chuang JC, Osborne-Lawrence S, Rovinsky SA, Woloszyn J, Yanagisawa M, Lutter M, Zigman JM (2010) Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. Biol Psychiatry. 67(9):880-6.

Perkins DN, Pappin DJ, Creasy DM, Cottrell JS (1999) Probability-based protein identification by searching sequence databases using mass spectrometry data. Electrophoresis 20, 3551-67.

Perroy J, El Far O, Bertaso F, Pin JP, Betz H, Bockaert J, Fagni L (2002) PICK1 is required for the control of synaptic transmission by the metabotropic glutamate receptor 7. EMBO J. 21, 2990–2999

Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA (2010) International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB_1 and CB_2. Pharmacol Rev. 62(4):588-631.

Peters J, LaLumiere RT, Kalivas PW (2008) Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. J Neurosci. 28(23):6046-53.

Petrovich GD, Setlow B, Holland PC, Gallagher M (2002) Amygdalohypothalamic circuit allows learned cues to override satiety and promote eating. J Neurosci. ;22:8748–8753.

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.

Pezze MA, Dalley JW, Robbins TW (2009) Remediation of attentional dysfunction in rats with lesions of the medial prefrontal cortex by intra-accumbens administration of the dopamine D(2/3) receptor antagonist sulpiride. Psychopharmacology (Berl). 202(1-3):307-13.

Phillips JT, Pastor R, Scibelli AC, Reed C, Tarragón E (2011) Behavioral Sensitization to Addictive Drugs: Clinical Relevance and Methodological Aspects. Animal Models of Behavioral Analysis. Neuromethods Volume 50, 2011, pp 267-305.

Piazza PV, Deminiere JM, Le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science 245: 1511–1513.

Piazza PV, Deroche-Gamonet V, Rouge-Pont F, Le Moal M (2000) Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. J Neurosci 20: 4226–4232.

Pickel VM, Chan J, Kash TL, Rodríguez JJ, MacKie K (2004) Compartment-specific localization of cannabinoid 1 (CB1) and mu-opioid receptors in rat nucleus accumbens. Neuroscience. 127(1):101-12.

Pielage J, Fetter RD, Davis GW (2005) Presynaptic spectrin is essential for synapse stabilization. Curr Biol. 15(10):918-28.

Pielage J, Fetter RD, Davis GW (2006) A postsynaptic spectrin scaffold defines active zone size, spacing, and efficacy at the Drosophila neuromuscular junction. J Cell Biol. 175(3):491-503.

Plasse TF, Gorter RW, Krasnow SH, Lane M, Shepard KV, Wadleigh RG (1991) Recent clinical experience with dronabinol. Pharmacological Biochemistry Behaviour. 40, 695–700.

Plaza-Zabala A, Maldonado R, Berrendero F (2012) The hypocretin/orexin system: implications for drug reward and relapse. Mol Neurobiol. 45(3):424-39.

Polakiewicz RD, Schieferl SM, Gingras AC, Sonenberg N, Comb MJ (1998) mu-Opioid receptor activates signaling pathways implicated in cell survival and translational control. J Biol Chem. 273(36):23534-41.

Politis PK, Thomaidou D, Matsas R (2008) Coordination of cell cycle exit and differentiation of neuronal progenitors. Cell Cycle. 7(6):691-7.

Porrino LJ, Daunais JB, Smith HR, Nader MA (2004) The expanding effects of cocaine: studies in a nonhuman primate model of cocaine self-administration. Neurosci Biobehav Rev. 2004 Jan;27(8):813-20.

Porro F, Rosato-Siri M, Leone E, Costessi L, Iaconcig A, Tongiorgi E, Muro AF (2009) beta-adducin (Add2) KO mice show synaptic plasticity, motor coordination and behavioral deficits accompanied by changes in the expression and phosphorylation levels of the alpha- and gamma-adducin subunits. Genes Brain Behav. 9(1):84-96.

Pothos E, Rada P, Mark GP, Hoebel BG (1991) Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment. Brain Res. 566(1-2):348-50.

Pratley RE, Weyer C (2001) The role of impaired early insulin secretion in the pathogenesis of Type II diabetes mellitus. Diabetologia. 2001 Aug;44(8):929-45.

Prokai L, Zharikova AD, Stevens SM Jr (2005) Effect of chronic morphine exposure on the synaptic plasma-membrane subproteome of rats: a quantitative protein profiling study based on isotope-coded affinity tags and liquid chromatography/mass spectrometry. J Mass Spectrom. 40(2):169-75.

Puighermanal E, Marsicano G, Busquets-Garcia A, Lutz B, Maldonado R, Ozaita A (2009) Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. Nat Neurosci. 12(9):1152-8.

Pulipparacharuvil S, Renthal W, Hale CF, Taniguchi M, Xiao G, Kumar A, Russo SJ, Sikder D, Dewey CM, Davis MM, Greengard P, Nairn AC, Nestler EJ, Cowan CW (2008) Cocaine regulates MEF2 to control synaptic and behavioral plasticity. Neuron 59, 621–633.

Quach TT, Massicotte G, Belin MF, Honnorat J, Glasper ER, Devries AC, Jakeman LB, Baudry M, Duchemin AM, Kolattukudy PE (2008) CRMP3 is required for hippocampal CA1 dendritic organization and plasticity. FASEB J. 22(2):401-9.

Quattrone A, Pascale A, Nogues X, Zhao W, Gusev P, Pacini A, Alkon DL (2001) Posttranscriptional regulation of gene expression in learning by the neuronal ELAV-like mRNA-stabilizing proteins. Proc Natl Acad Sci U S A. 2001 Sep 25;98(20):11668-73.

Rada P, Avena NM, Hoebel BG (2005) Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. Neuroscience 134, 737–744.

Ragozzino ME, Detrick S, Kesner RP (1999) Involvement of the prelimbic-infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. J Neurosci. 19(11):4585-94.

Ramachandran VS, Hirstein W (1998) The perception of phantom limbs. The D. O. Hebb lecture. Brain. 121 (Pt 9):1603-30.

Ramser EM, Buck F, Schachner M, Tilling T (2010) Binding of alphaII spectrin to 14-3-3beta is involved in NCAM-dependent neurite outgrowth. Mol Cell Neurosci. 45(1):66-74.

Randall PA, Vemuri VK, Segovia KN, Torres EF, Hosmer S, Nunes EJ, Santerre JL, Makriyannis A, Salamone JD (2010) The novel cannabinoid CB1 antagonist AM6545 suppresses food intake and food-reinforced behavior. Pharmacol Biochem Behav. 97(1):179-84.

Rappsilber J, Mann M, Ishihama Y (2007) Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. Nat Protoc 2, 1896-906.

Ravinet Trillou C, Delgorge C, Menet C, Arnone M, Soubrié P (2004) CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. Int J Obes Relat Metab Disord. 28(4):640-8.

Rasmusson DD (2000) The role of acetylcholine in cortical synaptic plasticity. Behav Brain Res. 2000 Nov; 115(2):205-18.

Reagan LP, Rosell DR, Wood GE, Spedding M, Muñoz C, Rothstein J, McEwen BS (2004) Chronic restraint stress up-regulates GLT-1 mRNA and protein expression in the rat hippocampus: reversal by tianeptine. Proc Natl Acad Sci U S A. 101(7):2179-84.

Regard M, Landis T (1997) "Gourmand syndrome": eating passion associated with right anterior lesions. Neurology.48:1185–90.

Rhee MH, Bayewitch M, Avidor-Reiss T, Levy R, Vogel Z (1998) Cannabinoid receptor activation differentially regulates the various adenylyl cyclase isozymes. J Neurochem. 71(4):1525-34.

Richards DA, Mateos JM, Hugel S, de Paola V, Caroni P, Gähwiler BH, McKinney RA (2005) Glutamate induces the rapid formation of spine head protrusions in hippocampal slice cultures. Proc Natl Acad Sci U S A. 102(17):6166-71.

Rios C, Gomes I, Devi LA (2006) mu opioid and CB1 cannabinoid receptor interactions: reciprocal inhibition of receptor signaling and neuritogenesis. Br J Pharmacol. 148(4):387-95.

Roberts TF, Tschida KA, Klein ME, Mooney R (2010) Rapid spine stabilization and synaptic enhancement at the onset of behavioural learning. Nature. 463(7283):948-52.

Robinson TE, Kolb B (1999) Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. Synapse. 33(2):160-2.

Robinson TE, Berridge KC (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction.95, S91–117.

Robinson TE, Gorny G, Savage VR, Kolb B (2002) Widespread but regionally specific effects of experimenter- versus self-administered morphine on dendritic spines in the nucleus accumbens, hippocampus, and neocortex of adult rats. Synapse. 46(4):271-9.

Robinson TE, Kolb B (2004) Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology. 47 Suppl 1:33-46.

Robinson TE, Berridge KC (2008) Review. The incentive sensitization theory of addiction: some current issues. Philos Trans R Soc Lond B Biol Sci. 363(1507):3137-46.

Robledo P, Berrendero F, Ozaita A, Maldonado R (2008)Advances in the field of cannabinoid--opioid cross-talk. Addict Biol. 13(2):213-24.

Rodríguez de Fonseca F, Del Arco I, Martín-Calderón JL, Gorriti MA, Navarro M (1998) Role of the endogenous cannabinoid system in the regulation of motor activity. Neurobiol Dis. 5(6 Pt B):483-501.

Rodríguez De Fonseca F, Gorriti MA, Bilbao A, Escuredo L, García-Segura LM, Piomelli D, Navarro M (2001) Role of the endogenous cannabinoid system as a modulator of dopamine transmission: implications for Parkinson's disease and schizophrenia. Neurotox Res. 3(1):23-35.

Rodriguez JJ, Mackie K, Pickel VM (2001) Ultrastructural localization of the CB1 cannabinoid receptor in mu-opioid receptor patches of the rat Caudate putamen nucleus. J Neurosci.21(3):823-33.

Rogers RD, Everitt BJ, Baldacchino A, Blackshaw AJ, Swainson R, Wynne K, Baker NB, Hunter J, Carthy T, Booker E, London M, Deakin JF, Sahakian BJ, Robbins TW (1999) Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: evidence for monoaminergic mechanisms. Neuropsychopharmacology. 20(4):322-39.

Rogers JL, Ghee S, See RE (2008) The neural circuitry underlying reinstatement of heroin-seeking behavior in an animal model of relapse. Neuroscience. 151(2):579-88.

Rolls ET (2011) Taste, olfactory and food texture reward processing in the brain and obesity. Int J Obes (Lond). 35(4):550-61.

Roux PP, Shahbazian D, Vu H, Holz MK, Cohen MS, Taunton J, Sonenberg N, Blenis J (2007) RAS/ERK signaling promotes site-specific ribosomal protein S6 phosphorylation via RSK and stimulates cap-dependent translation. J Biol Chem. 282(19):14056-64.

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, 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(6):267-76.

Russo SJ, Murrough JW, Han MH, Charney DS, Nestler EJ (2012) Neurobiology of resilience. Nat Neurosci. 15(11):1475-84.

Ruvinsky I, Meyuhas O (2006) Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. Trends Biochem Sci. 31(6):342-8.

Saal D, Dong Y, Bonci A, Malenka RC (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron. 37(4):577-82.

Sadakata T, Mizoguchi A, Sato Y, Katoh-Semba R, Fukuda M, Mikoshiba K, Furuichi T (2004) The secretory granule-associated protein CAPS2 regulates neurotrophin release and cell survival. J Neurosci. 24(1):43-52.

Sakane A, Manabe S, Ishizaki H, Tanaka-Okamoto M, Kiyokage E, Toida K, Yoshida T, Miyoshi J, Kamiya H, Takai Y, Sasaki T (2006) Rab3 GTPase-activating protein regulates synaptic transmission and plasticity through the inactivation of Rab3. Proc Natl Acad Sci U S A. 103(26):10029-34.

Sanchis-Segura C, Cline BH, Marsicano G, Lutz B, Spanagel R (2004) Reduced sensitivity to reward in CB1 knockout mice. Psychopharmacology (Berl). 176(2):223-32.

Sanchis-Segura C, Spanagel R (2006) Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. Addict Biol 11:2-38.

Sarti F, Borgland SL, Kharazia VN, Bonci A (2007) Acute cocaine exposure alters spine density and long-term potentiation in the ventral tegmental area.Eur J Neurosci. 26(3):749-56.

Sax KW, Strakowski SM (2001) Behavioural sensitization in humans. J Addict Dis 20:55–65.

Scheggi S, Rauggi R, Nanni G, Tagliamonte A, Gambarana C (2004) Repeated acetyl-l-carnitine administration increases phospho-Thr34 DARPP-32 levels and antagonizes cocaine-induced increase in Cdk5 and phospho-Thr75 DARPP-32 levels in rat striatum. Eur J Neurosci. 19(6):1609-20.

Schenk S, Valdez A, Worley CM, and McNamara C (1993) Blockade of the acquisition of cocaine self-administration by the NMDA antagonist MK-801 (dizocilpine). Behav Pharmacol 4: 652-659.

Scherma M, Fattore L, Satta V, Businco F, Pigliacampo B, Goldberg S, Dessy C, Fratta W Fadda P (2012) Pharmacological modulation of the endocannabinoid signalling alters binge-type eating behaviour in female rats. Br J Pharmacol.

Schikorski T, Stevens CF (1997) Quantitative ultrastructural analysis of hippocampal excitatory synapses. J Neurosci. 1997 Aug 1;17(15):5858-67.

Schmidt O, Harbauer AB, Rao S, Eyrich B, Zahedi RP, Stojanovski D, Schönfisch B, Guiard B, Sickmann A, Pfanner N, Meisinger C (2011) Regulation of mitochondrial protein import by cytosolic kinases. Cell. 144(2):227-39.

Schultz W (2002) Getting formal with dopamine and reward. Neuron. 36(2):241-63.

Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature. 404(6778):661-71.

Schwendt M, Reichel CM, See RE (2012) Extinction-dependent alterations in corticostriatal mGluR2/3 and mGluR7 receptors following chronic methamphetamine self-administration in rats. PLoS One. 7(3):e34299.

Schwenk J, Harmel N, Brechet A, Zolles G, Berkefeld H, Müller CS, Bildl W, Baehrens D, Hüber B, Kulik A, Klöcker N, Schulte U, Fakler B (2012) High-resolution proteomics unravel architecture and molecular diversity of native AMPA receptor complexes. Neuron. 74(4):621-33.

Seamans JK, Yang CR (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol. 74(1):1-58.

See RE, Elliott JC, Feltenstein MW (2007) The role of dorsal vs ventral striatal pathways in cocaine-seeking behavior after prolonged abstinence in rats. Psychopharmacology (Berl). 194(3):321-31.

Segal M (2005) Dendritic spines and long-term plasticity. Nat Rev Neurosci. 6(4):277-84.

Segarra AC, Agosto-Rivera JL, Febo M, Lugo-Escobar N, Menéndez-Delmestre R, Puig-Ramos A, Torres-Diaz YM (2010) Estradiol: a key biological substrate mediating the response to cocaine in female rats. Horm Behav. 58(1):33-43.

Self DW (2004) Regulation of drug-taking and -seeking behaviors by neuroadaptations in the mesolimbic dopamine system. Neuropharmacology. 2004;47 Suppl 1:242-55.

Sesack SR, Grace AA (2010) Cortico-Basal Ganglia reward network: microcircuitry. Neuropsychopharmacology. 35(1):27-47.

Sesma JI, Kreda SM, Steinckwich-Besancon N, Dang H, García-Mata R, Harden TK, Lazarowski ER (2012) The UDP-sugar-sensing P2Y(14) receptor promotes Rho-mediated signaling and chemotaxis in human neutrophils. Am J Physiol Cell Physiol. 303(5):C490-8.

Shaham Y, Hope BT (2005) The role of neuroadaptations in relapse to drug-seeking. Nat Neurosci 8:1437–1439.

Sharir H, Abood ME (2010) Pharmacological characterization of GPR55, a putative cannabinoid receptor. Pharmacol Ther. **126**, 301- 313.

Shen F, Meredith GE, Napier TC (2006) Amphetamine-induced place preference and conditioned motor sensitization requires activation of tyrosine kinase receptors in the hippocampus. J Neurosci. 26(43):11041-51.

Shen HW, Toda S, Moussawi K, Bouknight A, Zahm DS, Kalivas PW (2009) Altered dendritic spine plasticity in cocaine-withdrawn rats. J Neurosci. 29(9):2876-84.

Shepherd GM (1996) The dendritic spine: a multifunctional integrative unit. J Neurophysiol. 75(6):2197-210.

Shigemura N, Ohta R, Kusakabe Y, Miura H, Hino A, Koyano K, Nakashima K, Ninomiya Y (2004) Leptin modulates behavioral responses to sweet

substances by influencing peripheral taste structures. Endocrinology. 145:839-847.

Shinohara Y, Inui T, Yamamoto T, Shimura T (2009) Cannabinoid in the nucleus accumbens enhances the intake of palatable solution. Neuroreport.20(15):1382-5.

Shumyatsky GP, Malleret G, Shin RM, Takizawa S, Tully K, Tsvetkov E, Zakharenko SS, Joseph J, Vronskaya S, Yin D, Schubart UK, Kandel ER, Bolshakov VY (2005) stathmin, a gene enriched in the amygdala, controls both learned and innate fear. Cell. 123(4):697-709.

Sidhpura N, Parsons LH (2011) Endocannabinoid-mediated synaptic plasticity and addiction-related behavior. Neuropharmacology. 61(7):1070-87.

Simiand J, Keane M, Keane PE, Soubrie P (1998) SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. Behav Pharmacol 9: 179–181.

Simon GE, Von Korff M, Saunders K, Miglioretti DL, Crane PK, van Belle G, Kessler RC (2006) Association between obesity and psychiatric disorders in the US adult population. Arch Gen Psychiatry. 63(7):824-30.

Simon-Areces J, Acaz-Fonseca E, Ruiz-Palmero I, Garcia-Segura LM, Arevalo MA (2013) A CRM1-mediated nuclear export signal is essential for cytoplasmic localization of neurogenin 3 in neurons. PLoS One. 8(1):e55237.

Simopoulos AP (2009) Evolutionary aspects of the dietary omega-6:omega-3 fatty acid ratio: medical implications. World Rev Nutr Diet. 100:1-21.

Singh J, Desiraju T, Raju TR (1996) Comparison of intracranial self-stimulation evoked from lateral hypothalamus and ventral tegmentum: analysis based on stimulation parameters and behavioural response characteristics. Brain Res Bull. 41(6):399-408.

Sipe JC, Waalen J, Gerber A, Beutler E (2005) Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). Int J Obes (Lond). 29(7):755-9.

Sklair-Tavron L, Shi WX, Lane SB, Harris HW, Bunney BS, Nestler EJ (1996) Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons. Proc Natl Acad Sci U S A. 93(20):11202-7.

Smith-Roe SL and Kelley AE (2000). Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. J. Neurosci. 20:7737 -7742.

Solinas M, Panlilio LV, Antoniou K, Pappas LA, Goldberg SR (2003) The cannabinoid CB1 antagonist N-piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl) -4-methylpyrazole-3-carboxamide (SR-141716A) differentially alters the reinforcing effects of heroin under continuous reinforcement, fixed ratio, and progressive ratio schedules of drug self-administration in rats. J Pharmacol Exp Ther. 306(1):93-102.

Solinas M, Panlilio LV, Justinova Z, Yasar S, Goldberg SR (2006a) Using drug-discrimination techniques to study the abuse-related effects of psychoactive drugs in rats. Nat Protoc. 1(3):1194-206.

Solinas M, Goldberg SR, Piomelli D (2008) The endocannabinoid system in brain reward processes. Br J Pharmacol. 154(2):369-83.

Solomon RL, Corbit JD (1974) An opponent-process theory of motivation. I. Temporal dynamics of affect. Psychol Rev. 1974 Mar;81(2):119-45.

Soria G, Mendizábal V, Touriño C, Robledo P, Ledent C, Parmentier M, Maldonado R, Valverde O (2005) Lack of CB1 cannabinoid receptor impairs cocaine self-administration. Neuropsychopharmacology. 30(9):1670-80.

Sorra KE, Fiala JC, Harris KM (1998) Critical assessment of the involvement of perforations, spinules, and spine branching in hippocampal synapse formation. J.Comp.Neurol. 398,225–240.

Spanagel R, Almeida OF, Shippenberg TS (1993) Long lasting changes in morphine-induced mesolimbic dopamine release after chronic morphine exposure. Synapse. 14(3):243-5.

Spangler R, Wittkowski KM, Goddard NL, Avena NM, Hoebel BG, Leibowitz SF (2004) Opiate-like effects of sugar on gene expression in reward areas of the rat brain. Brain Res Mol Brain Res. 124(2):134-42.

Spolidoro M, Baroncelli L, Putignano E, Maya-Vetencourt JF, Viegi A, Maffei L (2011) Food restriction enhances visual cortex plasticity in adulthood. Nat Commun. 2011;2:320.

Srivastava RK, Lee YN, Noguchi K, Park YG, Ellis MJ, Jeong JS, Kim SN, Cho-Chung YS (1998) The RIIbeta regulatory subunit of protein kinase A binds to cAMP response element: an alternative cAMP signaling pathway. Proc Natl Acad Sci U S A. 95(12):6687-92.

Srivastava DP, Waters EM, Mermelstein PG, Kramár EA, Shors TJ, Liu F (2011) Rapid estrogen signaling in the brain: implications for the fine-tuning of neuronal circuitry. J Neurosci. 31(45):16056-63.

Stade K, Ford CS, Guthrie C, Weis K (1997) Exportin 1 (Crm1p) is an essential nuclear export factor. Cell. 90(6):1041-50.

Steiner H, Bonner TI, Zimmer AM, Kitai ST, Zimmer A (1999) Altered gene expression in striatal projection neurons in CB1 cannabinoid receptor knockout mice. Proc Natl Acad Sci U S A. 96(10):5786-90.

Stevens RJ, Littleton JT (2011) Synaptic growth: dancing with adducin. Curr Biol. 21(10):R402-5

Stewart J (2008) Review. Psychological and neural mechanisms of relapse. Philos Trans R Soc Lond B Biol Sci. 363(1507):3147-58.

Stipanovich A, Valjent E, Matamales M, Nishi A, Ahn JH, Maroteaux M, Bertran-Gonzalez J, Brami-Cherrier K, Enslen H, Corbillé AG, Filhol O, Nairn AC,

Greengard P, Hervé D, Girault JA (2008) A phosphatase cascade by which rewarding stimuli control nucleosomal response. Nature. 453(7197):879-84.

Stratford TR, Kelley AE (1997) GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. J Neurosci. 17(11):4434-40.

Sullivan S, Cloninger CR, Przybeck TR, Klein S (2007) Personality characteristics in obesity and relationship with successful weight loss. Int J Obes. 31:669–674.

Suto N, Tanabe LM, Austin JD, Creekmore E, Pham CT, Vezina P (2004) Previous exposure to psychostimulants enhances the reinstatement of cocaine seeking by nucleus accumbens AMPA. Neuropsychopharmacology. 29:2149–2159.

Swanger SA, Yao X, Gross C, Bassell GJ (2011) Automated 4D analysis of dendritic spine morphology: applications to stimulus-induced spine remodeling and pharmacological rescue in a disease model. Mol Brain. 4:38.

Swendsen J, Le Moal M (2011) Individual vulnerability to addiction. Ann N Y Acad Sci. 1216:73-85.

Swinburn B, Sacks G, Ravussin E (2009) Increased food energy supply is more than sufficient to explain the US epidemic of obesity. Am J Clin Nutr. 90:1453–1456.

Szczypka MS, Kwok K, Brot MD, Marck BT, Matsumoto AM, Donahue BA, Palmiter RD (2001) Dopamine production in the caudate putamen restores feeding in dopamine-deficient mice. Neuron. (3):819-28.

Taepavarapruk P, Floresco SB, Phillips AG (2000) Hyperlocomotion and increased dopamine efflux in the rat nucleus accumbens evoked by electrical stimulation of the ventral subiculum: role of ionotropic glutamate and dopamine D1 receptors. Psychopharmacology (Berl). 151(2-3):242-51.

Tafuro S, Ayuso E, Zacchigna S, Zentilin L, Moimas S, Dore F, Giacca M (2009) Inducible adeno-associated virus vectors promote functional angiogenesis in adult organisms via regulated vascular endothelial growth factor expression. Cardiovasc Res. 83(4):663-71.

Taglialatela G, Navarra D, Cruciani R, Ramacci MT, Alemà GS, Angelucci L (1994) Acetyl-L-carnitine treatment increases nerve growth factor levels and choline acetyltransferase activity in the central nervous system of aged rats. Exp Gerontol. 29(1):55-66.

Takayama N, Ueda H (2005) Morphine-induced chemotaxis and brain-derived neurotrophic factor expression in microglia. J Neurosci. 25(2):430-5.

Takei N, Kawamura M, Hara K, Yonezawa K, Nawa H (2001) Brain-derived neurotrophic factor enhances neuronal translation by activating multiple initiation processes: comparison with the effects of insulin. J Biol Chem. 276(46):42818-25.

Takumi Y, Ramírez-León V, Laake P, Rinvik E, Ottersen OP (1999) Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. Nat Neurosci. 1999 Jul;2(7):618-24.

Tanaka J, Horiike Y, Matsuzaki M, Miyazaki T, Ellis-Davies GC, Kasai H (2008) Protein synthesis and neurotrophin-dependent structural plasticity of single dendritic spines. Science. 319(5870):1683-7.

Tanda G, Munzar P, Goldberg SR (2000) Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat Neurosci. 3:1073–4.

Tang PZ, Gannon MJ, Andrew A, Miller D (1995) Evidence for oestrogenic regulation of heat shock protein expression in human endometrium and steroid-responsive cell lines. Eur J Endocrinol. 133(5):598-605.

Tannu N, Mash DC, Hemby SE (2007) Cytosolic proteomic alterations in the nucleus accumbens of cocaine overdose victims. Mol Psychiatry. 12(1):55-73.

Thiele TE, Willis B, Stadler J, Reynolds JG, Bernstein IL, McKnight GS (2000) High ethanol consumption and low sensitivity to ethanol-induced sedation in protein kinase A-mutant mice. J Neurosci. 20(10):RC75.

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.

Thomas GM, Huganir RL (2004) MAPK cascade signalling and synaptic plasticity. Nat Rev Neurosci. 5(3):173-83.

Timár J, Gyarmati Z, Fürst Z (2005) The development of tolerance to locomotor effects of morphine and the effect of various opioid receptor antagonists in rats chronically treated with morphine. Brain Res Bull. 64(5):417-24.

Tobaben S, Thakur P, Fernández-Chacón R, Südhof TC, Rettig J, Stahl B (2001) A trimeric protein complex functions as a synaptic chaperone machine. Neuron. 31(6):987-99.

Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K (2002). Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature. 420(6917):788-94.

Trayhurn P, Bing C (2006) Appetite and energy balance signals from adipocytes. Philos Trans R Soc Lond B Biol Sci. 361(1471):1237-49.

Trevaskis J, Walder K, Foletta V, Kerr-Bayles L, McMillan J, Cooper A, Lee S, Bolton K, Prior M, Fahey R, Whitecross K, Morton GJ, Schwartz MW, Collier GR (2005) Src homology 3-domain growth factor receptor-bound 2-like (endophilin) interacting protein 1, a novel neuronal protein that regulates energy balance. Endocrinology. 146(9):3757-64.

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(3):183-94.

Tritsch NX, Sabatini BL (2012) Dopaminergic modulation of synaptic transmission in cortex and striatum. Neuron. 76(1):33-50.

Trommald M, Hulleberg G, Andersen P (1996) Long-term potentiation is associated with new excitatory spine synapses on rat dentate granule cells. Learn Mem. 3(2-3):218-28.

Tronson NC, Taylor JR 2007. Molecular mechanisms of memory reconsolidation. Nat Rev Neurosci 8: 262–275.

Tulving E, Markowitsch HJ (1998) Episodic and declarative memory: role of the hippocampus. Hippocampus. 8(3):198-204.

Turu G, Hunyady L (2010) Signal transduction of the CB1 cannabinoid receptor.J Mol Endocrinol. 44(2):75-85.

Tzschentke TM (2000) The medial prefrontal cortex as a part of the brain reward system. Amino Acids. 19(1):211-9.

Tzschentke TM, Schmidt WJ (2003) Glutamatergic mechanisms in addiction. Mol Psychiatry. (4):373-82.

Tzschentke TM (2007) Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 12:227- 462.

Uher R, Treasure J (2005) Brain lesions and eating disorders. J. Neurol. Neurosurg. Psychiatry. 76:852–57.

Uldry M, Steiner P, Zurich MG, Béguin P, Hirling H, Dolci W, Thorens B (2004) Regulated exocytosis of an H+/myo-inositol symporter at synapses and growth cones. EMBO J. 23(3):531-40.

Ungless MA, Whistler JL, Malenka RC, Bonci A (2001) Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. Nature. 411(6837):583-7.

UNODC, World Drug Report (2012) United Nations publication, Sales No. E.12.XI.1.

Urigüen L, Berrendero F, Ledent C, Maldonado R, Manzanares J (2012) Kappa- and delta-opioid receptor functional activities are increased in the caudate putamen of cannabinoid CB1 receptor knockout mice. 2005 22(8):2106-10

Valjent E, Maldonado R (2000) A behavioural model to reveal place preference to delta 9-tetrahydrocannabinol in mice. Psychopharmacology (Berl). 147:436–8.

Valjent E, Pagès C, Hervé D, Girault JA, Caboche J (2004) Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain.Eur J Neurosci. 19(7):1826-36.

Valjent E, Corvol JC, Trzaskos JM, Girault JA, Hervé D (2006a) Role of the ERK pathway in psychostimulant-induced locomotor sensitization. BMC Neurosci. 2;7:20.

Valjent E, Corbille AG, Bertran-Gonzalez J, Herve D, Girault JA (2006b) Inhibition of ERK pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. Proc Natl Acad Sci 103: 2932–2937.

Valjent E, Bertran-Gonzalez J, Bowling H, Lopez S, Santini E, Matamales M, Bonito-Oliva A, Hervé D, Hoeffer C, Klann E, Girault JA, Fisone G (2011) Haloperidol regulates the state of phosphorylation of ribosomal protein S6 via activation of PKA and phosphorylation of DARPP-32. Neuropsychopharmacology. 36(12):2561-70.

Vanderschuren LJ, Schmidt ED, De Vries TJ, Van Moorsel CA, Tilders FJ, Schoffelmeer AN (1999) A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. J Neurosci. 19:9579–9586.

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(2-3):99-120.

Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rössner S; RIO-Europe Study Group (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study Lancet. 365(9468):1389-97.

Vásquez C, Lewis DL (1999)The CB1 cannabinoid receptor can sequester G-proteins, making them unavailable to couple to other receptors. J Neurosci. 19(21):9271-80.

Veilleux JC, Colvin PJ, Anderson J, York C, Heinz AJ (2010) A review of opioid dependence treatment: pharmacological and psychosocial interventions to treat opioid addiction. Clin Psychol Rev. 30(2):155-66.

Verty AN, McFarlane JR, McGregor IS, Mallet PE (2004) Evidence for an interaction between CB1 cannabinoid and melanocortin MCR-4 receptors in regulating food intake. Endocrinology. 145:3224–3231.

Vezina P (2004) Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. Neurosci Biobehav Rev 27:827–839.

Viganò D, Rubino T, Parolaro D (2005) Molecular and cellular basis of cannabinoid and opioid interactions.Pharmacol Biochem Behav. 81(2):360-8.

Villasana LE, Klann E, Tejada-Simon MV (2006) Rapid isolation of synaptoneurosomes and postsynaptic densities from adult mouse hippocampus. J Neurosci Methods. 158(1):30-6.

Volgin DV (2008) Perinatal alcohol exposure leads to prolonged upregulation of hypothalamic GABA A receptors and increases behavioral sensitivity to gaboxadol. Neurosci Lett. 439(2):182-6.

Volkow ND, Hitzemann R, Wang GJ, Fowler JS, Wolf AP, Dewey SL, Handlesman L (1992) Long-term frontal brain metabolic changes in cocaine abusers. Synapse. 11(3):184-90.

Volkow ND, Fowler JS (2000) Addiction, a disease of compulsion and drive: involvement of the orbitofrontal cortex. Cereb Cortex. 10(3):318-25.

Volkow ND, Wise RA (2005) How can drug addiction help us understand obesity? Nat Neurosci. 8(5):555-60.

Volkow ND, Wang GJ, Fowler JS, Telang F (2008) Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. Philos Trans R Soc Lond B Biol Sci. 363(1507):3191-200.

Volkow ND, Fowler JS, Wang GJ, Baler R, Telang F (2009) Imaging dopamine's role in drug abuse and addiction. Neuropharmacology. 56 Suppl 1:3-8.

Volkow ND, Wang GJ, Fowler JS, Tomasi D, Baler R (2012) Food and drug reward: overlapping circuits in human obesity and addiction. Curr Top Behav Neurosci. 11:1-24.

Vorel SR, Liu X, Hayes RJ, Spector JA, Gardner EL (2001) Relapse to cocaine-seeking after hippocampal theta burst stimulation. Science. 292(5519):1175-8.

Wagh DA, Rasse TM, Asan E, Hofbauer A, Schwenkert I, Dürrbeck H, Buchner S, Dabauvalle MC, Schmidt M, Qin G, Wichmann C, Kittel R, Sigrist SJ, Buchner E (2006) Bruchpilot, a protein with homology to ELKS/CAST, is required for structural integrity and function of synaptic active zones in Drosophila. Neuron. 49(6):833-44.

Wallace DL, Vialou V, Rios L, Carle-Florence TL, Chakra-varty S, Kumar A, Graham DL, Green TA, Kirk A, Iñiguez SD, Perrotti LI, Barrot M, DiLeone RJ,

Nestler EJ, Bolanos-Guzman CA (2008) The influence of DeltaFosB in the nucleus accumbens on natural reward-related behavior. J. Neurosci. 28, 10272–10277.

Waltereit R, Weller M (2003) Signaling from cAMP/PKA to MAPK and synaptic plasticity. Mol Neurobiol. 2003 27(1):99-106.

Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N, Fowler JS (2001) Brain dopamine and obesity. Lancet. 357(9253):354-7.

Wang Y, Liu X, Biederer T, Südhof TC (2002) A family of RIM-binding proteins regulated by alternative splicing: Implications for the genesis of synaptic active zones. Proc Natl Acad Sci U S A. 99(22):14464-9.

Wang S, Huang X, Li Y, Lao H, Zhang Y, Dong H, Xu W, Li JL, Li M (2011) RN181 suppresses hepatocellular carcinoma growth by inhibition of the ERK/MAPK pathway. Hepatology. 53(6):1932-42.

Ward SJ, Dykstra LA (2005) The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940). Behav Pharmacol. 16(5-6):381-8.

Ward SJ, Walker EA, Dykstra LA (2007) Effect of cannabinoid CB1 receptor antagonist SR141716A and CB1 receptor knockout on cue-induced reinstatement of Ensure and corn-oil seeking in mice. Neuropsychopharmacology. 32(12):2592-600.

Wee S, Mandyam CD, Lekic DM, Koob GF (2008) Alpha 1-noradrenergic system role in increased motivation for cocaine intake in rats with prolonged access. Eur Neuropsychopharmacol. 18(4):303-11.

Weingarten HP (1983) Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. Science. 220:431-433.

Weiss F (2005) Neurobiology of craving, conditioned reward and relapse. Curr Opin Pharmacol. 5(1):9-19.

Weissenborn R, Robbins TW, Everitt BJ (1997) Effects of medial prefrontal or anterior cingulate cortex lesions on responding for cocaine under fixed-ratio and second-order schedules of reinforcement in rats. Psychopharmacology (Berl). 134(3):242-57.

West AR, Grace AA (2002) Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining in vivo intracellular recordings and reverse microdialysis. J Neurosci. 22(1):294-304.

WHO. Obesity and overweight fact sheet. (2006). Sep, http://www.who.int/dietphysicalactivity/publications/facts/obesity/en/ Volume, DOI.

Williams JT, Christie MJ, Manzoni O (2001) Cellular and synaptic adaptations mediating opioid dependence. Physiol Rev. 81(1):299-343.

Williams LM (2012) Hypothalamic dysfunction in obesity. Proc Nutr Soc. 71(4):521-33.

Wilson RI, Nicoll RA (2002) Endocannabinoid signaling in the brain. Science. 2002 Apr 26;296(5568):678-82.

Wilson GT (2010) Eating disorders, obesity and addiction. Eur Eat Disord Rev. 18(5):341-51

Wise RA, Newton P, Leeb K, Burnette B, Pocock D, Justice JB Jr (1995) Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. Psychopharmacology (Berl). 120(1):10-20.

Wise RA (2008) Dopamine and reward: the anhedonia hypothesis 30 years on. Neurotox Res. 14(2-3):169-83.

Wiśniewski J, Zougman A, Nagaraj N, Mann M (2009) Universal sample preparation method for proteome analysis. Nature Methods 6, 359-362.

Wittmann BC, Schott BH, Guderian S, Frey JU, Heinze HJ, Düzel E. (2005) Reward-related FMRI activation of dopaminergic midbrain is associated with enhanced hippocampus-dependent long-term memory formation. Neuron. 45(3):459-67.

Wolf ME, Mangiavacchi S, Sun X (2003) Mechanisms by which dopamine receptors may influence synaptic plasticity. Ann N Y Acad Sci. 1003:241-9.

Wolf A, Keil R, Götzl O, Mun A, Schwarze K, Lederer M, Hüttelmaier S, Hatzfeld M. (2006) The armadillo protein p0071 regulates Rho signalling during cytokinesis. Nat Cell Biol. 8(12):1432-40.

Wosiski-Kuhn M, Stranahan AM (2012) Transient increases in dendritic spine density contribute to dentate gyrus long-term potentiation.Synapse. 66(7):661-4.

Wotjak CT (2005) Role of endogenous cannabinoids in cognition and emotionality. Mini Rev Med Chem. 5(7):659-70.

Woolley JD, Lee BS, Kim B, Fields HL (2007) Opposing effects of intra-nucleus accumbens mu and kappa opioid agonists on sensory specific satiety. Neuroscience. 146(4):1445-52. Epub 2007 Apr 19.

Wu GY, Deisseroth K, Tsien RW (2001) Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology. Nature Neurosci. 4(2):151-8.

Yamashita N, Morita A, Uchida Y, Nakamura F, Usui H, Ohshima T, Taniguchi M, Honnorat J, Thomasset N, Takei K, Takahashi T, Kolattukudy P, Goshima Y (2007) Regulation of spine development by semaphorin3A through cyclin-dependent kinase 5 phosphorylation of collapsin response mediator protein 1. J Neurosci. 27(46):12546-54.

Yang ZJ, Meguid MM, Chai JK, Chen C, Oler A (1997) Bilateral hypothalamic dopamine infusion in male Zucker rat suppresses feeding due to reduced meal size. Pharmacol Biochem Behav. 58(3):631-5.

Yang Y, Wang XB, Frerking M, Zhou Q (2008) Spine expansion and stabilization associated with long-term potentiation. J Neurosci. 28(22):5740-51.

Yang Y, Takeuchi K, Rodenas-Ruano A, Takayasu Y, Bennett MV, Zukin RS (2009)Developmental switch in requirement for PKA RIIbeta in NMDA-receptor-dependent synaptic plasticity at Schaffer collateral to CA1 pyramidal cell synapses. Neuropharmacology. 56(1):56-65.

Yoshida R, Ohkuri T, Jyotaki M, Yasuo T, Horio N, Yasumatsu K, Sanematsu K, Shigemura N, Yamamoto T, Margolskee RF, Ninomiya Y (2010) Endocannabinoids selectively enhance sweet taste. Proc Natl Acad Sci U S A. 107(2):935-9.

Yoshimura M, Wu PH, Hoffman PL, Tabakoff B (2000) Overexpression of type 7 adenylyl cyclase in the mouse brain enhances acute and chronic actions of morphine.Mol Pharmacol. 58(5):1011-6.

Young R (2009) Drug discrimination. Source Methods of Behavior Analysis in Neuroscience. 2nd edition. Boca Raton, CRC Press. Chapter 3.

Zakharenko SS, Zablow L, Siegelbaum SA (2002) Altered presynaptic vesicle release and cycling during mGluR-dependent LTD. Neuron. 35(6):1099-110.

Zangen A, Solinas M, Ikemoto S, Goldberg SR, Wise RA (2006) Two brain sites for cannabinoid reward. J Neurosci. 26:4901–7.

Zavala AR, Osredkar T, Joyce JN, Neisewander JL (2008) Upregulation of Arc mRNA expression in the prefrontal cortex following cue-induced reinstatement of extinguished cocaine-seeking behavior. Synapse. 62(6):421-31.

Zernig G, Ahmed SH, Cardinal RN, Morgan D, Acquas E, Foltin RW, Vezina P, Negus SS, Crespo JA, Stöckl P, Grubinger P, Madlung E, Haring C, Kurz M, Saria

A (2007) Explaining the escalation of drug use in substance dependence: models and appropriate animal laboratory tests. Pharmacology. 80(2-3):65-119.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature. 372(6505):425-32.

Zhang L, Yagi M, Herzog H (2012) The role of NPY and ghrelin in anorexia nervosa. Curr Pharm Des18(31):4766-78.

Zheng H, Berthoud HR (2007) Eating for pleasure or calories. Curr Opin Pharmacol. 7(6):607-12.

Zhong H, Sia GM, Sato TR, Gray NW, Mao T, Khuchua Z, Huganir RL, Svoboda K (2009) Subcellular dynamics of type II PKA in neurons. Neuron. 62(3):363-74.

Zhou Q, Homma KJ, Poo MM (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron. 2004 Dec 2;44(5):749-57.

Zhou Y, Bendor J, Hofmann L, Randesi M, Ho A, Kreek MJ (2006) Mu opioid receptor and orexin/hypocretin mRNA levels in the lateral hypothalamus and striatum are enhanced by morphine withdrawal. J Endocrinol. 191(1):137-45.

Ziauddeen H, Fletcher PC (2013)Is food addiction a valid and useful concept? Obes Rev. 14(1):19-28.

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI (1999) Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci U S A. 96(10):5780-5.

Zucker RS, Regehr WG (2002) Short-term synaptic plasticity. Annu Rev Physiol. 64:355-405.

ANNEX

ARTICLE 3

Operant self-administration of a sigma ligand improves nociceptive and emotional manifestations of neuropathic pain.

Bura AS, Guegan T, Zamanillo D, Vela JM, Maldonado R.

European Journal of Pain. 2013; 17(6):832-43.

ARTICLE 4

Targeting the endocannabinoid system in the treatment of fragile X syndrome.

Busquets-Garcia A, Gomis-González M, Guegan T, Agustín-Pavón C, Pastor A, Mato S, Pérez-Samartín A, Matute C, de la Torre R, Dierssen M, Maldonado R, Ozaita A.

Nature Medicine 2013; 19(5): 603-7.