NOVEL INSIGHTS IN NICOTINE ADDICTION: FOCUS ON COGNITIVE FUNCTION

Rocio Saravia Santos

DOCTORAL THESIS UPF / 2018

Thesis directors:

Dr. Fernando Berrendero Díaz

Pr. Rafael Maldonado López

Departament de Ciències Experimentals i de la Salut

Universitat Pompeu Fabra Barcelona

*"**It is our choices, that show what we truly are, far more than our abilities**"*

Harry Potter and the Chamber of secrets

JK Rowling

ABSTRACT

Cigarette smoking continues to be leading cause of preventable cause of death worldwide. Cognitive modulation by nicotine seems to be a key factor in nicotine addiction. Several studies indicate that initial nicotine intake has a positive effect on cognition, which may contribute to the development of nicotine dependence. Conversely, when chronic nicotine treatment ceases, cognitive functioning is altered. The orexin and the endocannabinoid system have been reported to play a crucial role in different stages of nicotine addiction and in learning and memory processes. Our results show that orexin receptors influence the pro-cognitive effects of acute nicotine treatment, whereas the endocannabinoid system acting through CB1R modulates the cognitive deficits associated with nicotine withdrawal. In addition, our work reveals an inflammatory process associated with the cognitive deficits of early nicotine abstinence. Given that the presence of cognitive alterations is associated with increased smoking relapse risk, our results identify CB1R and anti-inflammatory drugs as new potential therapeutic strategies for nicotine dependence.

RESUMEN

El consumo de cigarrillos es una de las principales causas de muerte prevenible en el mundo. Los efectos de la nicotina sobre la memoria parecen parece ser un factor clave en la adicción a la nicotina. Diversos estudios indican que el consumo inicial de nicotina tiene un efecto positivo sobre la cognición, lo que puede contribuir al desarrollo de la dependencia de la nicotina. Por el contrario, cuando el consumo de nicotina cesa, se altera el funcionamiento cognitivo. Las orexinas y el sistema endocannabinoide desempeñan un papel crucial en las diferentes etapas de la adicción a la nicotina y en los procesos de aprendizaje y memoria. Nuestros resultados demuestran que los receptores de orexina la mejora de memoria inducido por un el tratamiento agudo de nicotina, mientras que el sistema endocannabinoide, actuando a través de los receptores CB1 modula los déficits cognitivos asociados con la abstinencia de nicotina. Además, hemos revelado que un proceso inflamatorio está asociado al desarrollo de los déficits cognitivos de la abstinencia a nicotina. Dado que la presencia de alteraciones cognitivas se asocia con un mayor riesgo de recaída en el hábito de fumar, nuestros resultados identifican a los receptores CB1 y fármacos antiinflamatorios como potenciales nuevas estrategias terapéuticas para la dependencia de la nicotina.

ABREVIATTIONS

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid

AEA: N-arachidonoylethanol-amide

2-AG: 2-arachidonoylglycerol

BNST: bed nucleus of the stria terminalis

CB1R: cannabinoid receptor 1

CB2R: cannabinoid receptor 2

CBD: cannabidiol

CNS: central nervous system

CPP: conditioned place preference

CRF: corticotrophin-releasing factor

DG: dentate gyrus

GABA: γ-aminobutyric acid

HPC: hippocampus

LTP: long-term potentiation

NAc: nucleus accumbens

nAChR: nicotinic acetylcholine receptor

NAc: nucleus accumbens

NMDA: N-methyl-D-aspartate

mGlu receptor: metabotropic glutamate receptor

OXR1: orexin receptor-1

ORX2: orexin receptor-2

PFC: prefrontal cortex

PKA protein kinase A

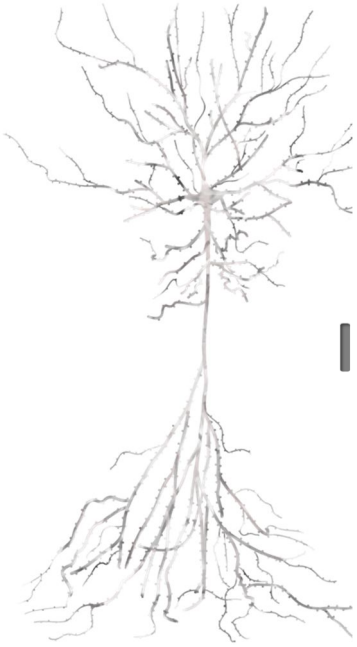
PKC: protein kinase C

PVN: paraventricular nucleus of the hypothalamus

SVG: subgranular zone

VTA: ventral tegmental area

INDEX



INTRODUCTION

1. Nicotine

1.1 Tobacco use: a public health concern

Tobacco consumption remains one of the leading causes of preventable disease and death worldwide, resulting in approximately 7 million premature deaths per year. This mortality is mainly due to lung cancer, coronary heart disease, respiratory and chronic obstructive pulmonary disease, which represent an important impact on health care expenses (US surgeon report, 2014). Despite public awareness of the harmful effects of tobacco use, it is estimated that 22.5% (32% men, 7% women) of global adult population are current smokers (Gowing *et al*, 2015).

Tobacco is mainly consumed in the form of cigarettes, which are conceived as the most effective form to deliver nicotine to the organism. Nicotine is the main psychoactive component of tobacco and responsible for the addictive and cognitive properties of tobacco. Aside from nicotine, tobacco smoke contains more than 4000 compounds with many been reported as irritant, carcinogenic and toxic contributing to the development of smoking-related diseases. In an effort to avoid the toxic molecules present in tobacco smoke, some smokers have shifted to the use of smokeless tobacco (e-cigarettes). Since this alternative is perceived as "healthier" than traditional cigarettes its use has rapidly increased and now also represents a major public health concern (Etter and Eissenberg, 2015; Pulvers *et al*, 2016).

Quitting smoking at any age has substantial health benefits, which encourage near 50% of adult smokers to make a quit attempt every year. However, 80% of smokers who try to quit on their own relapse within the first month of abstinence with only a 3% of them remaining abstinent at six months (Benowitz, 2010; Control Center for Disease, 2016). These data

illustrate the powerful force of tobacco addiction and the chronic nature of the disorder.

1.2 Nicotine: neuropharmacology

Nicotine accounts for the 95% of the alkaloids present in the leaves of *Nicotiana tabacum* plant where it acts as a natural insecticide (Schmeltz and Hoffmann, 1977). Nicotine is a tertiary amine consisting of a pyridine and a pyrrolidine ring and is predominantly found as the (S)-nicotine isomer. The (R)-nicotine only represents a 1–0.6% of total nicotine and is less active pharmacologically than (S)-nicotine (Armstrong *et al*, 1998)(Figure 1).

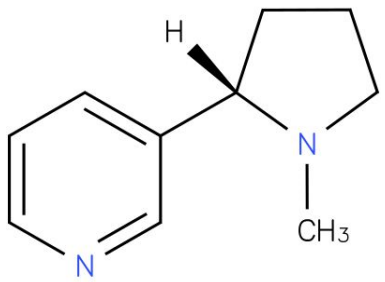


Figure 1. Chemical structure of nicotine [3-(1-methyl-2-pyrrolidinyl)pyridine].

1.2.1 Pharmacokinetics and metabolism

Nicotine is a weak base (pKa of 8.0), whose ability to cross membranes depends on the environmental pH. During tobacco burning, nicotine is transported in small smoke particles into the lungs. Tobacco smoke is an acidic environment (pH 5.5–6.0) where nicotine is ionized and can no longer cross membranes easily. When tobacco smoke arrives to alveoli and small airways, the surrounding fluids (pH 7.4) unionize nicotine so it can be rapidly absorbed and transferred to the bloodstream (Hukkanen *et*

al, 2005). Following a puff, nicotine can reach the brain within 10-20 seconds. This short time gap between smoke inhalation and perception of the effects of nicotine makes smoking the most reinforcing form of nicotine administration facilitating the development of addiction (Hukkanen *et al*, 2005; Perkins *et al*, 2016).

Other forms of nicotine administration, such as nicotine replacement therapy, increase gradually nicotine blood levels, resulting in lower nicotine levels in the brain and poor abuse liability (Flowers, 2016). Smokeless tobacco (e-cigarettes) was first advertised as a tool to reduce nicotine dependence given its reduced nicotine delivery. However, recent studies have shown that e-cigarettes can produce nicotine plasma levels similar to those observed with conventional cigarettes smokers (Dawkins and Corcoran, 2014; Etter and Bullen, 2011; Pulvers *et al*, 2016). Since the amount of nicotine obtained from e-cigarettes may determine its addictiveness, it is not surprising that users, especially young ones, can develop dependence on e-cigarettes (Etter and Eissenberg, 2015).

Nicotine is metabolized primarily by the liver enzyme CYP2A6 (Hukkanen *et al*, 2005). In humans, approximately 70% of nicotine is converted to cotinine. Many animal species, including mice, rabbits, and monkeys, metabolize nicotine primarily to cotinine as in humans (Matta *et al*, 2007). Genetic variants affecting CYP2A6 function are associated with cigarette use (Benowitz, 2009), vulnerability to develop nicotine addiction (Audrain-McGovern *et al*, 2007; Chenoweth *et al*, 2016) and responsiveness to smoking cessation pharmacotherapy (Lerman *et al*, 2015), suggesting that nicotine metabolism contributes to nicotine addiction.

The plasma half-life (t1/2) of nicotine in human averages about 2 hours (Hukkanen *et al*, 2005). Nevertheless, rodents display faster nicotine metabolism and are less sensitive to the effects of nicotine (t1/2 rat: 45

minutes; t1/2 mouse: 6-7 minutes). Thus, it is important to adjust doses in order to obtain a response comparable to humans, when using a murine model of nicotine addiction (Matta *et al*, 2007).

1.2.2 Mechanism of action: nicotinic acetylcholine receptors

Nicotine exerts its physiological and psychological effects through binding and activation of nicotinic acetylcholine receptors (nAChRs). These receptors are widely distributed through the central nervous system (CNS) (Figure 2), being expressed by neurons, microglia and astrocytes (Egea *et al*, 2015; Maurer and Williams, 2017). nAChRs are also abundantly expressed in the peripheral nervous system (Gotti *et al*, 2006) and in non-neuronal cells including keratinocytes, endothelial cells, digestive, respiratory, and immune cells (Albuquerque *et al*, 2009; Kawashima *et al*, 2015).

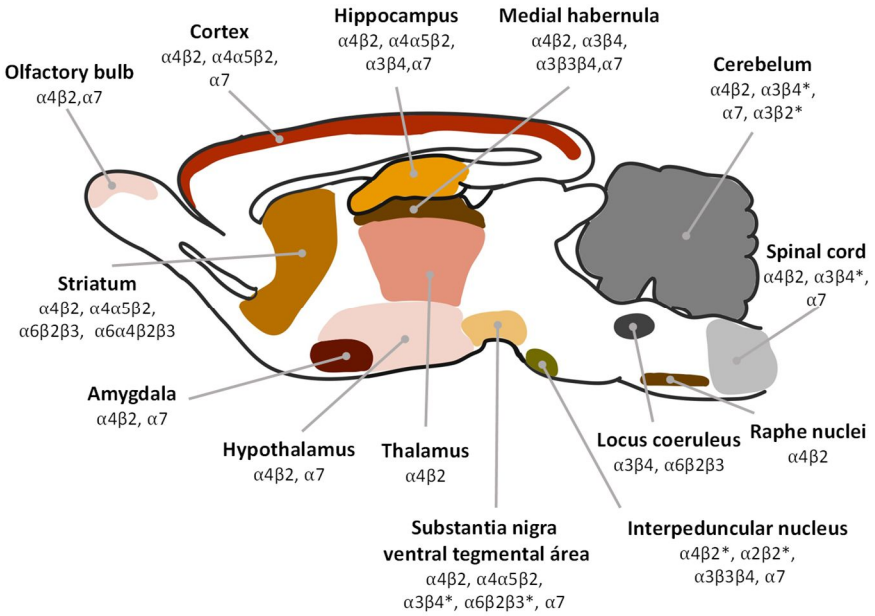


Figure 2 Distribution of nicotinic acetylcholine receptors in the rodent central nervous system (Adapted from Gotti *et al,* 2006).

Nicotinic receptors are ligand-gated ion channels (Changeux, 1990) made of a combination of five alpha (α2–α7, α9, and α10) and beta subunits (β2–β4) arranged around a central permeable pore. The alpha subunits can conform homo-oligomeric and hetero-oligomeric receptors because these subunits contain the binding site for the ligand. In contrast, beta subunits only arrange hetero-oligomeric receptors and exhibit 2 ligands binding sites at the interface between α and β subunits (Le Novere *et al*, 2002) (Figure 3). The different combination of subunits accounts for the diverse structure and function of nAChRs.

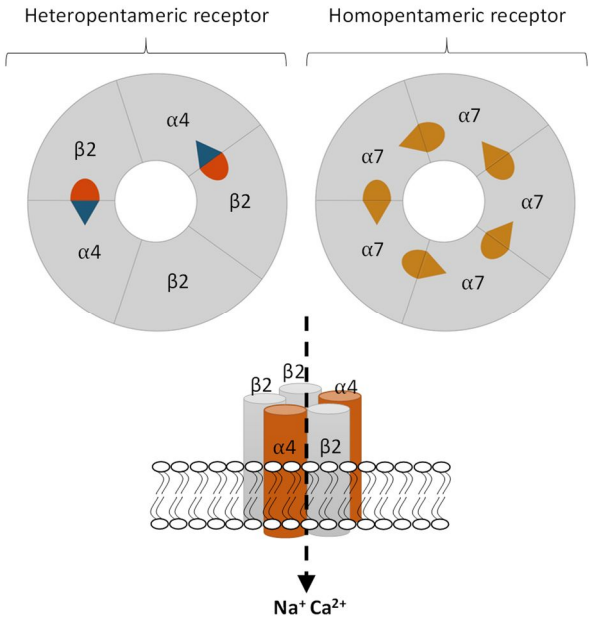


Figure 3. Schematic representation of nicotinic acetylcholine receptors (nAChRs). Nicotinic receptors consist of five transmembrane subunits arranged around a water-filled cation-permeable pore. Nicotinic extracellular domain carries the acetylcholine/nicotine binding sites at the boundary between subunits. The most common nAChRs in the brain are hetero-oligomeric α4β2 nAChRs and homo-oligomeric α7 nAChRs (Adapted from Zoli et al, 2015).

Upon binding of the endogenous neurotransmitter acetylcholine, the open conformation of the nAChRs stabilizes and allow the influx of small cations, such as Na+, K+, and Ca^{2+} (Albuquerque *et al*, 2009; Dani, 2015). This open conformation lasts several milliseconds before it changes to a close-resting state (responsive to agonist) or a close-desensitized state (unresponsive to agonists). Interestingly, nAChRs in the close-resting state have 20 times lower affinity for agonists than nAChRs in the close-desensitized state. In physiological conditions, brief and high concentration of acetylcholine at the synapse induces opening of the nAChRs' pores followed by a rapid recovery from desensitization. However, prolonged exposure to low concentrations of nicotine, as occur during tobacco use, induces activation accompanied by a significant desensitization (Picciotto *et al*, 2008). Since regular smokers maintain levels of circulating nicotine over the course of the day, nAChRs remain longer in a desensitized state promoting an increase in the number of high-affinity receptors (De Biasi and Dani, 2011; Fasoli *et al*, 2016). Long-term forms of nAChRs inactivation could explain several aspects of nicotine addiction. Thus, tolerance and withdrawal might be a consequence of the slow recovery of nAChRs into functional states from different levels of desensitization and inactivation (De Biasi and Dani, 2011; Giniatullin *et al*, 2005; Picciotto *et al*, 2008). In agreement, a human study showed that after 2 days of smoking abstinence, participants' cravings were reduced only when nAChRs were again nearly saturated (Brody *et al*, 2006).Depending on the localization, the gating of the receptor promotes different responses. Thus, activation of nicotinic receptors in presynaptic terminals triggers the direct release of neurotransmitters, including acetylcholine, glutamate, GABA, dopamine, norepinephrine, and serotonin. Postsynaptic nAChRs activation allows the influx of cations

promoting the depolarization of the neuron and, in the case of Ca2+, also induces Ca2+ depending signaling cascades (Albuquerque *et al*, 2009; Dani, 2015).

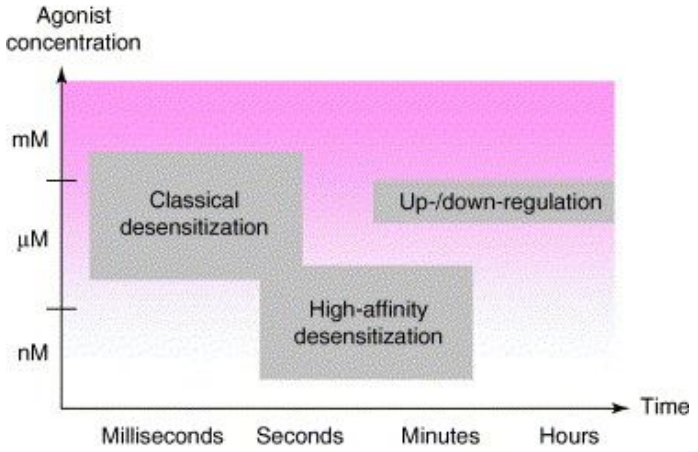


Figure 4. **Nicotinic acetylcholine receptor desensitization depends on agonist concentration and time exposure**. Classical desensitization induced by relatively high (micromolar to millimolar) agonist concentrations proceeds from the open receptor state in milliseconds (e.g. for α7 nAChRs) or seconds (e.g. for α4β2 nAChRs). High-affinity desensitization induced by low agonist concentrations proceeds from the agonist-bound closed state before channel opening (i.e. without receptor activation) with slow kinetics (seconds to minutes). The upregulation or downregulation of nAChRs is usually observed with long-lasting application of agonist (Giniatullin et al, 2005).

In the mammalian brain, the heteromeric $\alpha4\beta2^{*}$ receptor (asterisk indicates that other subunits may be present in this receptor) and the α7 homomeric receptor are mostly expressed (Dani, 2015; Zoli *et al*, 2015). The $\alpha4\beta2^{*}$ receptor is considered the principal mediator of nicotine dependence given its high affinity for nicotine and slow desensitization. Conversely, the α7 receptor exhibits lower affinity to nicotine with a fast activation which explains its involvement in rapid synaptic transmission, long-term potentiation (LTP) and learning (Dani, 2015; Picciotto and Kenny, 2013). Other nAChRs subtypes have a more restricted distribution

in the brain, but they might also represent the most abundant receptor subtype in a specific brain area.

1.2.3 Role of nAChRs subunits in nicotine addiction

Investigating the nAChRs at which nicotine acts may provide valuable insights to better understand the neurobiology of nicotine addiction and facilitate the development of novel therapeutic strategies. However, it is difficult to attribute any particular behavioral effect of nicotine to a specific nAChRs subtype has been difficult, due to the lack of selective antagonist and agonist for all nAChRs subtypes (Zoli *et al*, 2015). The development of genetically modified animal models with knockout (KO), knockin or selective expression of nAChRs subunits has enabled the investigation of the role of specific nAChRs in nicotine addiction.

1.2.3.1 α4β2 nAChRs*

The α4β2*-containing nAChRs are the most abundant in the CNS, with several evidence supporting the role of this nAChR subtype in the reinforcement and withdrawal of nicotine (McLaughlin *et al*, 2015; Stoker and Markou, 2013). In the ventral tegmental area (VTA), α4β2* nAChRs are located in GABAergic presynaptic terminals, where they regulate the release of the inhibitory neurotransmitter (Mansvelder *et al*, 2002). However, nicotine binding rapidly desensitizes these nicotinic receptors (Mansvelder *et al*, 2002), disrupting inhibitory GABAergic transmission in the VTA might contribute, at least in part, to the reinforcing properties of nicotine.

Early studies reported reduced nicotine reward in β2 (KO), using the intravenous nicotine self-administration (Picciotto *et al*, 1998) and the conditioning place preference (CPP) (Walters *et al*, 2006). These observations could be the result of nicotine's inability to stimulate the

mesocorticolimbic dopaminergic system in β2 KO mice, since a reduced dopamine release and responsiveness of dopaminergic neurons has been observed in the absence of β2 subunits (Maskos *et al*, 2005; Stoker and Markou, 2013). Furthermore, lentiviral re-expression of β2 in the VTA reinstated nicotine self-administration behavior (Maskos *et al*, 2005) suggesting that β2 subunit is essential for the reinforcing effects of nicotine. Nicotine withdrawal affective signs also seem to be dependent on β2* nAChRs (Hamouda *et al*, 2018). Indeed, anxiety-like behavior associated to nicotine withdrawal were reduced in β2 KO mice (Jackson *et al*, 2008; McLaughlin *et al*, 2015). Additionally, β2 KO mice did not exhibit the learning deficits in fear conditioning associated with nicotine withdrawal (Portugal *et al*, 2008). In contrast, β2* nAChRs do not participate in the expression of the somatic signs of nicotine withdrawal (De Biasi and Salas, 2008; McLaughlin *et al*, 2015). These findings indicate that nAChRs containing the β2 subunit are critical for the development of nicotine dependence and expression of withdrawal signs upon cessation of nicotine administration.

Similar to mice lacking β2 KO mice, α4 KO mice failed to enhance dopamine levels in the mesocorticolimbic system after nicotine exposure and to acquire nicotine self-administration (Pons *et al*, 2008). Interestingly, mice with a single point mutation in the α4 gene self-administered nicotine at lower doses than their wildtype counterparts and exhibited conditioned place preference also at very low nicotine doses (Wilking and Stitzel, 2015). Altogether, these findings suggest a modulatory role for the α4 subunit in nicotine reinforcement.

1.2.3.2 α7 nAChRs*

Similar to the α4β2 nAChRs, homomeric α7 receptors exhibit a widespread expression through the brain and a significantly lower affinity for nicotine. In the VTA, α7 nAChRs regulate presynaptic glutamate release onto dopaminergic neurons (Feduccia *et al*, 2012; Zoli *et al*, 2015). The effects of α7* nAChRs on nicotine reinforcement appear to be subtler than those of α4β2 nAChRs. Importantly, α7 nAChRs rapidly recover from nicotine-induced desensitization (Subramaniyan and Dani, 2015). This rapid recovery suggests that α7* nAChRs, unlike α4β2* nAChRs, may remain sensitive to fluctuations in nicotine levels during continuous nicotine exposure and might consequently be important in the maintenance of nicotine dependence (Stoker and Markou, 2013). Nevertheless, contradictory results have been obtained when evaluating the role of the α7 subunit in the reinforcing properties of nicotine. Thus, even though pharmacological blockage of the α7 subunits decreased nicotine self-administration in rats (Walters *et al*, 2006), genetic deletion of the α7 subunit unaltered nicotine self-administration (Brunzell and McIntosh, 2012). In addition, a mouse strain that had lower α7 mRNA expression tended to have a greater sensitivity for the rewarding properties of nicotine (Harenza *et al*, 2014). This observation was confirmed by the increased nicotine reward revealed in the CPP paradigm. The different observations between all of these studies might be explained by the differences in the experimental approaches (pharmacological vs genetic), the use of different animal models (rats vs mice) and the genetic differences of mice strains. Therefore, the precise role of α7* nAChRs in nicotine reinforcement remains unclear.

Withdrawal signs seem to be independent of α7* nAChRs. Thus, both wildtype and α7 KO mice exhibited similar somatic signs of nicotine

withdrawal (Stoker *et al*, 2012). In addition, the learning deficits in fear conditioning associated with nicotine withdrawal were unaffected in the α7 KO mice (Kutlu and Gould, 2016a; Portugal *et al*, 2008).

1.2.3.3 α5 nAChRs*

Human genetic studies have highlighted the polymorphic nature of the CHRNA5- CHRNA3-CHRNB4 genomic cluster, encoding subunits α5, α3, and β4, respectively, and its implication in smoking behaviors, dependence risk and lung cancer (Bierut *et al*, 2008; Bierut and Tyndale, 2018). Polymorphisms in the CHRNA5 gene have been associated with a reduced function of α5* receptors (Bierut, 2011). This alteration has been described to increase the risk of tobacco dependence by 30%, which can be doubled if the individual carries 2 risks alleles instead of a single allele (Saccone *et al*, 2009). In addition, the α5 subunit gene variant is a major risk factor for lung cancer and chronic obstructive pulmonary disease in smokers (Bierut, 2011). The α5 subunit seems to be determinant to the sensitivity and aversion to nicotine (Antolin-fontes *et al*, 2015; Frahm *et al*, 2011). Thus, mice lacking the α5 subunit self-administered high doses of nicotine that are otherwise aversive in wildtype mice (Fowler and Kenny, 2014). Interestingly, re-expression α5 nAChR subunits in the habernulo-interpeduncular pathway in KO mice restored similar nicotine intake levels than in wildtype mice (Antolin-fontes *et al*, 2015). Therefore, it seems that deletion of the α5 subunit increases the reinforcing effects of high doses of nicotine perhaps by attenuating the adverse effects associated with high nicotine concentrations. During nicotine withdrawal, somatic signs were decreased in α5 KO compared with wildtype mice (Jackson *et al*, 2008; Stoker and Markou, 2013). In contrast, α5* nAChRs

do not appear to mediate affective symptoms of nicotine abstinence (Jackson *et al*, 2015; Salas *et al*, 2009).

1.2.3.4 β4 nAChRs*

Genome-wide association studies have revealed that variants in CHRNB4's coding region reduce the risk for nicotine dependence (Haller *et al*, 2012), and variants in CHRNB4's regulatory domain decrease the age of onset for tobacco intake (Haller *et al*, 2012; Saccone *et al*, 2009). Notably, mouse studies have shown that the lack of β4* nAChR reduces the reinforcing properties of nicotine (Harrington *et al*, 2016), whereas overexpression of this subunit induced a strong aversion to nicotine (Frahm *et al*, 2011). These divergent data highlight the balance of positive and aversive signaling mechanisms associated with nicotine intake. During nicotine withdrawal, β4 KO mice displayed decreased somatic signs compared with wildtype mice (Stoker *et al*, 2012). Overall, these results suggest that the β4 subunit is involved in many aspects of nicotine dependence.

1.2.3.5 α6 nAChRs*

Genetic variation on the CHRNA6-CHRNAB3 gene cluster, encoding the α6 and β3 subunits respectively, has been reported to increase vulnerability to tobacco smoking (Bierut, 2011; Thorgeirsson *et al*, 2010). In the VTA, α6* nAChRs appear to regulate GABA release onto dopaminergic neurons (Yang *et al*, 2011). Notably, the presence of α6 in nAChRs containing α4β2 subunits seem to maintain the activation produced by nicotine, since it slows the rate of desensitization (Liu *et al*, 2012). Mice lacking the α6 subunit failed to acquire nicotine-self administration suggesting a role of this subunit in the reinforcing effects of nicotine. Furthermore, the α6 subunit is also involved in the affective component of nicotine abstinence

(McLaughlin *et al*, 2015). Thus, pharmacological blockage of the α6β2* prevents nicotine withdrawal-induced anxiety-like effects (Jackson *et al*, 2009). In agreement one of the currently used treatments for smoking cessation, varenicline, acts as a partial agonist at α6β2* nAChRs (Bordia *et al*, 2012).

1.3 Nicotine addiction: a complex brain disease

Similarly to other addictive processes, nicotine addiction is a chronically relapsing brain disease characterized by compulsive tobacco use, loss of control over tobacco consumption despite its negative consequences, the appearance of withdrawal symptoms upon cessation of tobacco smoking, and relapse even after long periods of abstinence (adapted from Koob and Le Moal, 2008).

For many years, addiction was considered a personal "lifestyle choice" instead of a brain disorder. Although initial drug use is certainly a voluntary behavior, multiple preclinical and clinical studies have evidence that addiction to drugs, including tobacco addiction, is based on pathological changes in brain function produced by the repeated pharmacological insult to specific brain circuits. In this regard, continuous drug exposure has been reported to affect the expression of genes involved in neuroplasticity through epigenetic and possibly RNA modifications, ultimately altering intracellular signaling cascades and neuronal circuits (Volkow and Morales, 2015). Hence, repeated stimulation of motivational circuitries by addictive drugs leads to maladaptive changes that progressively redirect the behavioral strategies, originally driven in response to biological stimuli, towards drug-seeking and drug-taking (Kalivas and O'Brien, 2008). Addiction can be conceptualized as a three-stage, recurring cycle of binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (craving), that worsens over time and involves neuroplastic changes in the brain reward, stress, and executive function systems (Koob and Volkow, 2016). One of the main challenges in the field of addiction is identifying the neuroadaptative changes that contribute to the progression from experimental use to addiction.

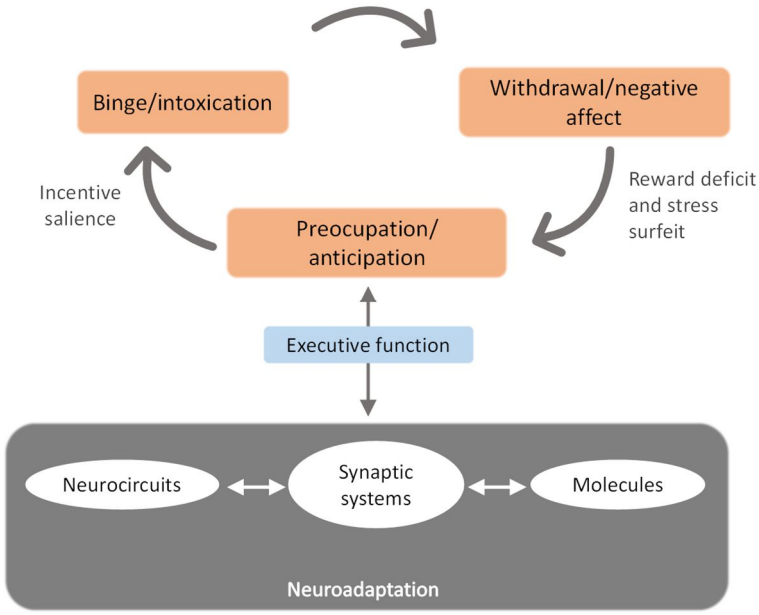


Figure 5. Conceptual representation of the addiction process. Addiction can be conceptualized as a three-stage, recurring cycle—binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (craving)—that worsens over time and involves neuroplastic changes in the brain reward, stress, and executive function systems Derived from a confluence of information from social psychology of human self-regulation failure, psychiatry, and brain imaging, these three stages provide a heuristic framework for the study of the neurobiology of addiction (Adapted from Volkow and Koob, 2016).

Experiencing with drugs, such as nicotine, does not imply becoming an addict. Indeed, the actual percentage of consumers that transit from recreational use to addiction ranges from approximately 9% for marijuana to 31% for tobacco (Anthony *et al*, 1994). Thus, addiction is a complex disease influenced by a variety of environmental and genetic factors and the mechanisms underlying the vulnerability or resilience are still not well understood (Addy and Picciotto, 2013). In tobacco addiction, the initial use of cigarettes is strongly influenced by environmental factors, such as availability and accessibility to tobacco, whereas genetic factors play more important role in the transition from regular use to the development of addiction (Vink *et al*, 2005).

The 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association [APA], 2013) describes the clues for the diagnosis of substance-related and addictive disorders. According to this, substance-related disorders are divided into two groups: (1) substance use disorders, referring to changes produced in brain circuitry that can persist beyond detoxification, leading to cognitive, behavioral and psychological symptoms directly related to the substance use, and (2) substance-induced disorders, including intoxication, withdrawal and mental disorders induced by substances or medications. Therefore, the DSM-5 has combined the substance abuse and substance dependence categories, previously separated in the 4th edition, into a single substance use disorder. Indeed, the nicotine dependence diagnosis present in the DMS-IV has been replaced for the diagnose of tobacco use disorder, defined as “a problematic pattern of tobacco use leading to clinically significant impairment or distress, as manifested by at least 2 of the 11 symptoms listen in Table 1 in a 12-month period.

Table 1. DMS-5 criteria for substance use disorders.

A. Impaired control
1. Substance is often taken in larger amounts or over longer period than was intended.
2. There is a persistence desire or unsuccessful efforts to cut down or control substance use.
3. A large amount of time is spent in activities necessary to obtain the substance, use the substance, or recover from it effects.
4. Development of craving, or a strong desire or urge to use the substance.
B. Social impairment
5. Recurrent substance use resulting in a failure to fulfill major role obligations.
6. Continued substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance.
7. Important social, occupational, or recreational activities are given up or reduced because of substance use.
C. Risky use of substance
8. Recurrent substance use in situations in which it is physically hazardous
9. Substance use is continued despite knowledge of having a psychological problem that is likely to have been caused by the substance.
D. Pharmacological criteria
10. Tolerance, as defined by either: a need for markedly increased amounts of substance to achieve intoxication or desired effect or markedly diminished effect with continued use of the same amount of substance.
11. Withdrawal, as manifested by either: the characteristic withdrawal syndrome for the substance or the substance is taken to relieve or avoid withdrawal symptoms.

1.3.1 Acute effects: reward

In humans, nicotine obtained from tobacco smoking produces a moderate pleasure, reduction in stress and anxiety, increased arousal, improve concentration, reaction and performance of certain tasks (Benowitz, 2010; Henningfield *et al*, 1985). These behavioral effects seem to be primary sources of reinforcement by nicotine and motivations for smoking (Hall *et al*, 2015a; Pulvers *et al*, 2014). However, the strong addictive profile of tobacco contrasts with the relatively weak primary reinforcing effect of nicotine revealed in both clinical and preclinical studies (Caggiula *et al*, 2001; Chaudhri *et al*, 2006). Indeed, complex interactions between environmental cues and nicotine also play a critical role in promoting and maintaining nicotine seeking (Bani *et al*, 2014; Garcia-Rivas and Deroche-Gamonet, 2018; Stoker and Markou, 2015).

Over the last 30 years, advances in the knowledge of the neurobiological mechanisms underlying the psychopharmacological effects of nicotine have been possible by the use of preclinical models. In agreement with the findings revealed in humans, nicotine administration has reinforcing and cognitive effects in non-human primates (Le Foll *et al*, 2007), dogs (Risner and Goldberg, 1983), rats (Caille *et al*, 2012) and mice (Martín-García *et al*, 2009; Stoker and Markou, 2013).

Nicotine, as other drugs of abuse, induces its rewarding effects by enhancing the activity of the mesocorticolimbic reward system. In physiological conditions, the reward system is entitled of promoting learning of goal-directed behaviors, generate positive emotions, and subsequently stimulate the repetition of those learned behaviors (Schultz, 2010). Many of the behaviors stimulated by the reward system are implied in survival, which might explain why the reward mechanisms are preserved across species, being especially complex in the case of humans

(O'Doherty *et al*, 2001).

The mesocorticolimbic system is composed of the VTA, which contains the cell bodies of dopaminergic neurons, and the terminal areas, where dopamine is released, in the nucleus accumbens (NAc), amygdala, and frontal and limbic cortices, (Kelley, 2004; Wise, 2004) (Figure 6). This system interacts with diverse brain regions including the amygdala, the bed nucleus of the stria terminalis (BNST), the hypothalamus and the hippocampus (HPC) that provide information about external context and about internal emotional and physiological states (Nestler, 2005). Hence, alterations in Nac projections, as occurs during chronic drug intake, contribute to addiction by promoting reward-directed behavior (Hyman and Malenka, 2001; Kauer, 2004).

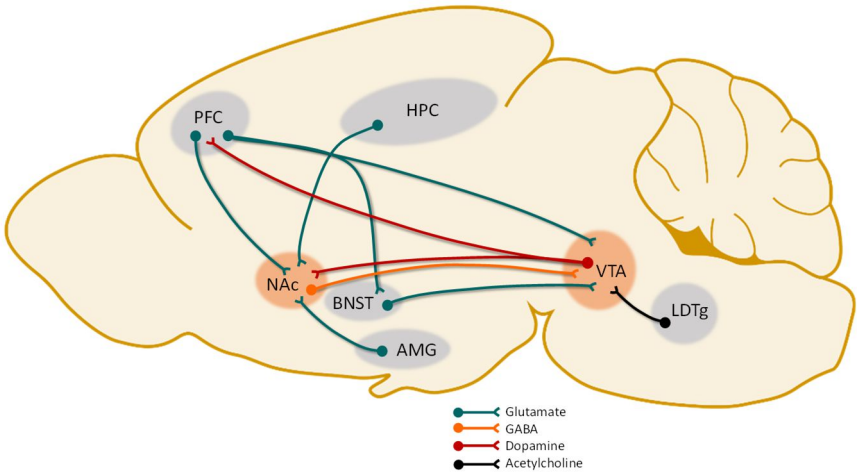


Figure 6. Mesocorticolimbic dopamine system. Simplified illustration of the circuitry of the mesolimbic dopamine system in rodent brain highlighting the major inputs to the nucleus accumbens (NAc) and ventral tegmental area (VTA). The release of dopamine from VTA neurons increases in response to administration of all drugs of abuse. AMG, amygdala; BNST, bed nucleus of the stria terminalis; LDTg, laterodorsal tegmental nucleus; PFC, prefrontal cortex; HPC, hippocampus.

Dopamine release has been described to facilitate reward-induced learning and promote recurrent drug-taking (Cardinal and Everitt, 2004; Russo *et al*, 2012; Wise, 2004). Different excitatory and inhibitory inputs directly regulate the activity of the dopamine neurons in the VTA (Markou, 2008). As an excitatory signaling, glutamate release from different projection sites, including the NAc and the prefrontal cortex (PFC), promotes the depolarization of the VTA dopamine neurons and the consequent release of dopamine into the NAc. Conversely, inhibitory GABA signaling from interneurons within the VTA and projections from the NAc inhibits dopamine neurons in the VTA. The activity of dopaminergic neurons in the VTA is not only modulated by glutamate and GABA, but also by acetylcholine. Indeed, direct stimuli of cholinergic neurotransmission has been shown to evoke dopamine release into the NAc (Cachope *et al*, 2012; Threlfell *et al*, 2012) and inhibition of cholinergic interneurons disrupt drug reinforcing effects (Witten *et al*, 2010). Acetylcholine promotes dopamine release through nAChRs expressed in dopaminergic cell bodies and indirectly through glutamatergic and GABAergic terminals within the VTA.

Similar to other drugs of abuse, nicotine exerts its rewarding effects by acting on the mesocorticolimbic pathway. Nicotine uses the nAChRs that in physiological conditions respond to acetylcholine (Korpi *et al*, 2015). Animal studies reveal that nicotine enhances dopamine neurotransmission in mesolimbic areas through direct stimulation of nAChRs within the VTA. Thus, intra-VTA infusion of nAChR antagonists reduced nicotine-elicited dopaminergic outflow in the NAc, while infusion of the same antagonists in the NAc did not have any effect (Nisell *et al*, 1994). In agreement, nAChRs located in the VTA, but not in the NAc, modulate nicotine self-administration in rats (Corrigall *et al*, 1994).

Nicotine enhances dopamine release into the NAc by primarily acting on α4β2* and α7 homomeric nAChRs within the VTA. Nicotine acts in the VTA through the preferential activation of α4β2* nAChRs that have high affinity for nicotine and are mostly expressed in the body of dopaminergic neurons and in GABAergic terminals (Zoli *et al*, 2015). Thus, nicotine acting on these receptors induces the depolarization of dopaminergic neurons and the release of GABA from GABAergic terminals (Dani, 2015). Nicotine binds, to a lesser degree, to the lower-affinity α7 nAChRs highly expressed in glutamatergic terminals (Dani, 2015). Nicotine binding to these receptors induces the release of glutamate to dopaminergic neurons, promoting the activation of these neurons (Dani, 2015; Yan *et al*, 2018). Therefore, nicotine in the VTA triggers both inhibitory and excitatory signaling (Figure 7). Interestingly, low concentration of nicotine achieved through smoking desensitizes rapidly the nAChRs located in GABAergic and dopaminergic neurons, whereas higher nicotine doses are required to desensitize nAChRs in glutamatergic neurons. This difference in desensitization rates translates to a diminished inhibitory tone while the excitation persists leading to depolarization of dopaminergic neurons and an overall increase of dopamine transmission from the VTA to the NAc (Dani, 2015; Markou, 2008; Yan *et al*, 2018). Other brain regions are involved in nicotine reinforcing effects, aside from the mesocorticolimbic pathway. Nicotine administration enhances dopaminergic transmission in the BNST (Carboni *et al*, 2000). In addition, α5 nAChRs in the habenulo-interpenduncular pathway are key to control nicotine intake (Fowler *et al*, 2011). This pathway projecting to and from the VTA acts as an opposite mechanism to the reward system and transmits inhibitory signals that limit intake of noxious substances. Hence, knockdown of α5 nAChRs signaling decreases the stimulatory effects of nicotine and enhances nicotine

intake. Finally, smokers with insula damage, a cortical region involved in processing interoceptive information of emotional and motivational states, are more likely to quit smoking compared to those without insula injury (Naqvi *et al*, 2007).

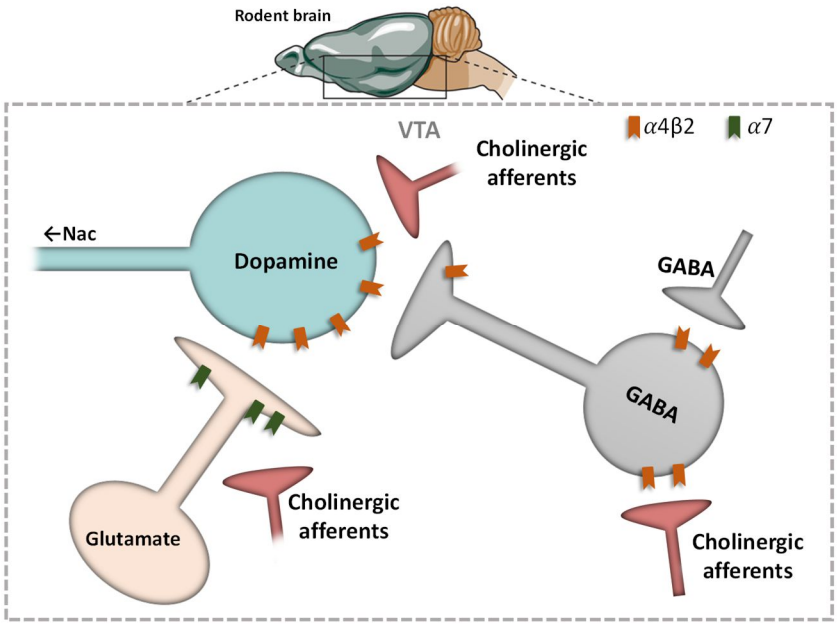


Figure 7. Nicotine modulation of dopaminergic transmission in the VTA. Schematic representation of the neurotransmission interactions in the VTA involved in the rewarding effects of nicotine and contribute to nicotine dependence. Dopaminergic neurons receive inputs from glutamatergic, GABAergic and cholinergic neurons. Nicotine elicits the release of GABA and glutamate towards dopaminergic neurons, thus promoting both inhibitory and excitatory signaling. Notably, α4β2* nAChRs located in GABAergic terminals desensitized rapidly with the consequent decrease of the inhibitory input. α7 nAChRs located in glutamatergic terminals remain active longer generating an overall excitation of dopaminergic neurons and increase dopamine release from the VTA to the NAc (adapted from Markou, 2008).

1.3.2 Chronic effects: tolerance and sensitization

All drugs of abuse, when administered acutely, decrease brain stimulation reward thresholds (i.e increasing reward; Kornetsky and Esposito, 1979). In contrast, when the drug is chronically administered the reward thresholds increase (i.e decrease reward; Koob and Volkow, 2010), this phenomenon is termed tolerance. With continued nicotine exposure, smokers develop tolerance to some nicotine effects with the consequent need of progressively higher doses of nicotine to obtain the same effects (Wang and Sun, 2005). It has been proposed that desensitization and up-regulation of nAChRs are behind the phenomenon of nicotine tolerance and dependence. Following nicotine binding, nAChRs activate and rapidly enter in a desensitize (inactive) state. The rate of desensitization and recovery varies among receptor subtypes, expressing neuron and brain areas. During chronic nicotine exposure, smokers exhibit low plasmatic levels of nicotine responsible for maintaining most nAChRs in a desensitized state. Moreover, chronic nicotine usage enhances substantially the time that nAChRs need to recover from the desensitized state, contributing to a generalized nAChRs inactive state in the brain (Dani, 2015; Picciotto *et al*, 2008). Indeed, a brain image study showed that cigarette smoking in amounts used by typical daily smokers maintains near-complete saturation, and thus desensitization, of brain nAChRs (Brody *et al*, 2006, 2014).

Long-term exposure to an agonist produces excessive receptor activation that is homeostatically compensated by down-regulation of the receptor (Dani, 2015). Paradoxically, chronic nicotine exposure increases nicotine-binding sites in the brain, a phenomenon termed up-regulation of nAChRs (Cosgrove *et al*, 2015; Zoli *et al*, 2015). Up-regulation of nAChRs observed in humans, mice and rats, may be a response to the prolonged

desensitization of nAChRs (Picciotto *et al*, 2008). This up-regulation only occurs in nicotinic, but not muscarinic, nAChRs and differs among receptor subtypes and brain regions (Gaimarri *et al*, 2007; Zoli *et al*, 2015). The interplay between the increased nAChRs expression and the low desensitization recovery rate might explain why smokers report that they receive the most pleasurable effect from the first cigarette of the day (Benowitz, 2010; Russell, 1989).

1.3.3 Acute nicotine withdrawal: physical, affective and cognitive symptoms

Tobacco-dependent subjects seem to maintain nicotine consumption to avoid or alleviate the distressing withdrawal symptoms rather than to obtain the positive reinforcing effects of nicotine (Koob *et al*, 2013; Koob and Le Moal, 2008b). Indeed, nicotine withdrawal syndrome is considered as a major cause of relapse into smoking (Le Foll and Goldberg, 2009). Thus, the severity and the duration of withdrawal symptoms has been suggested to predict relapse in abstinent smokers (Allen *et al*, 2008; Ashare and Schmidt, 2014; Killen and Fortmann, 1997; Rukstalis *et al*, 2005; Zhou *et al*, 2009).

Smoking cessation produces a wide range of undesirable effects that can be classified as somatic, affective and cognitive withdrawal symptoms (Hughes, 2007a; Hughes and Hatsukami, 1986). Somatic or "physical" signs of withdrawal include bradycardia, gastrointestinal discomfort, fatigue, insomnia, and restlessness. The affective withdrawal symptoms include depressed mood, irritability, severe craving for nicotine, anxiety and decreased arousal. Cognitive deficits associated with nicotine withdrawal include impairments in attention, working memory and episodic memory (Hall *et al*, 2015a; Hughes, 2007a; Wesnes *et al*, 2013). In general, all withdrawal signs onset approximately at 4h after the last tobacco

consumption, peak within the first few days of abstinence and could still be observed for weeks (Benowitz, 1992; Hughes, 2007a). Detailed information regarding the cognitive impairments associated with nicotine withdrawal and the neurobiological mechanism involved will be provided in section 1.4.3 of this thesis.

Many of the abstinent symptoms observed in humans can be recapitulated in rodent models and withdrawal (McLaughlin *et al*, 2015). In this regard, withdrawal signs can be studied by observing the frequencies of certain stereotypes or by evaluating changes in behavior during abstinence. As in humans, behavioral manifestations of nicotine withdrawal in rodents can be classified also as somatic, affective and cognitive. The somatic signs in rodents include teeth chattering, palpebral ptosis, tremor, wet dog shakes, changes in locomotor activity, and other behavioral consequences (Castañé *et al*, 2002; Jackson *et al*, 2018). The affective manifestations of nicotine withdrawal in rodents consist of increased anxiety-like behavior, aversive effects, and reward deficits (Jackson *et al*, 2008; Johnson *et al*, 2008; Stoker *et al*, 2012). Finally, the cognitive deficits associated with nicotine withdrawal have been often studied using hippocampal-dependent memory tasks in rodents (Kutlu *et al*, 2016; Yildirim *et al*, 2015). In mice, spontaneous and precipitated withdrawal from nicotine results in memory impairment in the contextual fear conditioning (Hall *et al*, 2015a; Leach *et al*, 2013) and object recognition test (Borkar *et al*, 2017). Similar to humans, attentional deficits have been observed in rodents during nicotine withdrawal. Thus, rats withdrawn from nicotine exhibit attention deficits in the operant signal detection paradigm (Shoaib and Bizarro, 2005). In addition, nicotine withdrawal results in cognitive flexibility alterations in mice and the

consequent inability to execute new learning strategies (Parikh *et al*, 2016).

1.3.4 Long-term abstinence: relapse to nicotine consumption

Former smokers remain vulnerable to relapse to tobacco consumption, even after years of abstinence. One significant factor thought to be important in relapse to drug taking is exposure to environmental stimuli previously associated with drug intake (Franklin *et al*, 2007; Liu, 2016). Smoking may be particularly effective in establishing the incentive properties of nicotine-associated environmental stimuli (cues), such as the smell and taste of cigarettes or contexts within which smoking occurs. Stress plays an important role in relapse to smoking. Indeed, external stressors are important triggers of relapse, and nicotine withdrawal itself produces a "stress-like state" of negative affect (Hughes, 2007b; Wardle *et al*, 2011). Furthermore, relapse to tobacco can also be triggered by a single smoked cigarette (re-exposure) (Liu, 2016). The use of animal models has been determinant to advance in the study of the mechanisms underlying nicotine relapse. Notably, reinstatement models of relapse in animals have shown that nicotine-seeking can be triggered by nicotine-associated conditioned cues, stressors, and re-exposure to nicotine (Feltenstein *et al*, 2012; Liu, 2016; Martín-García *et al*, 2009; Nygard *et al*, 2016), which are the same events that trigger resumption of smoking behavior in humans.

Animal studies have shown that relapse to nicotine-seeking could be mediated by the orexin system. Thus, blockade of the orexin receptor 1 (OXR1) attenuates cue-induced reinstatement of nicotine-seeking in mice (Plaza-Zabala *et al*, 2013). In addition, nicotine relapse also involves the corticotropin-releasing factor (CRF). Blockade of CRF1 receptors

prevented stress-induced reinstatement of nicotine-seeking behavior in rats (Bruijnzeel, 2017; Bruijnzeel *et al*, 2009). Moreover, blockade of CRF1 receptors also prevents stress-induced reinstatement of nicotine-seeking in the mouse (Plaza-Zabala *et al*, 2010). The endogenous cannabinoid system also has a crucial role in the reinstatement of nicotine-seeking behavior. Antagonism of cannabinoid receptor 1 (CB1) blocks the reinstatement of previously extinguished nicotine-seeking behavior in rats (Cohen *et al*, 2005; Gamaleddin *et al*, 2015). Interestingly, all these mentioned mechanisms also have a role in other effects of nicotine, including cognitive modulation, that will be further discussed in this thesis. Reinstatement of nicotine-seeking behavior has also been found to be sensitive to antagonists for noradrenergic (Forget *et al*, 2010) and β-adrenergic receptors (Chiamulera *et al*, 2010), and metabotropic and ionotropic glutamate receptors (Dravolina *et al*, 2007; Gipson *et al*, 2013).

1.4 Cognitive effects of nicotine

Nicotine addiction involves maladaptive changes that go beyond the reward system (Le Foll and Goldberg, 2005). The existing overlap between the neural substrates of reward and learning and memory processes could explain the participation of cognitive processes in nicotine addiction (Kelley, 2004; Volkow and Baler, 2014). Thus, nicotine addiction involves maladaptive learning and memories that contribute to the development of drug-taking behaviors. Human and animal research have shown that initial nicotine intake facilitates the association of nicotine reinforcing effects with specific context and cues (Garcia-Rivas and Deroche-Gamonet, 2018; Thewissen *et al*, 2005). These associative memories grow stronger, with extended nicotine use, and conduct to drug-seeking behaviors. In addition, cognitive inflexibility and cognitive deficits that emerge during nicotine abstinence might both contribute to the maintenance and relapse to nicotine (Kutlu and Gould, 2016b). Therefore, cognitive modulation by nicotine seems to be a key factor in nicotine addiction.

This section focusses on the effects that nicotine exerts on cognition with a particular emphasis in the effects of acute, chronic, and nicotine withdrawal. While acute nicotine improves learning and promotes consumption, cognitive deficits that arise during nicotine withdrawal might influence relapse into tobacco consumption. A better knowledge of the neurobiological mechanisms underlying the acute and chronic effects of nicotine might provide novel insights into nicotine addiction, which represents the main objective of this thesis.

1.4.1 Acute nicotine effects on cognition

Human and animal research reveal that nicotine given acutely can improve memory performance (Gould and Leach, 2014). Indeed, growing evidence supports that the pro-cognitive effects of nicotine during initial use might facilitate the development of drug-context associations, contributing to repeated nicotine use and the development of nicotine addiction (Hall *et al*, 2015a; Kenney *et al*, 2011; Stoker and Markou, 2015).

1.4.1.1 Human studies

Several human studies have shown that nicotine has cognitive enhancing effects, when given acutely (Newhouse *et al*, 2012; Valentine and Sofuoglu, 2017; Zandonai *et al*, 2018). However, the first studies that evaluated the effect of nicotine on cognition failed to distinguish between the improvements due to the acute administration of nicotine and the normalization of the cognitive deficits of nicotine withdrawal. (Hall *et al*, 2015a; Kutlu *et al*, 2016). Thus, many studies from the 1970s to the 1990s that claimed that nicotine improved memory were, in fact, demonstrating the reversion of withdrawal-induced cognitive deficits in tobacco-dependent smokers (Heishman *et al*, 1994). Taking this premise into account, the studies supporting the pro-cognitive effects of nicotine presented in this section only correspond to research performed in nonsmokers or in smokers that no longer exhibit withdrawal-cognitive deficits. Thus, human studies have shown that acute nicotine administration improves performance in a variety of attention tasks, short-term episodic memory and working memory (Hall *et al*, 2015a; Heishman *et al*, 2010; Valentine and Sofuoglu, 2017). In nonsmoker adults, transdermal nicotine treatment significantly improves attention when evaluated in a continuous performance test (Barr *et al*, 2008; Newhouse

et al, 2012; Poltavski and Petros, 2006). Thus, nicotine-treated subjects presented an enhancement in both the ability to maintain focus in the task (sustained attention) and the ability to discriminate a relevant from a competing stimulus (selective attention). In addition, acute nicotine treatment also improved spatial attention and oriented attention (File *et al*, 2001; Foulds *et al*, 1996; Hahn *et al*, 2007; Heishman and Henningfield, 2000; Rusted and Alvares, 2008; Thiel *et al*, 2005; Vossel *et al*, 2008). Indeed, it has been suggested that nicotine improves learning by involving aspects of cognitive function as well as other related processes, such as attention. Indeed, nicotine improved working memory only when maintaing attention was key to perform the task correctly (McClernon *et al*, 2003).

Besides the cognitive effects of nicotine at the beginning of tobacco dependence, nicotine and other agonists might be of potential therapeutic interest in a population with pre-existing cognitive deficits. Thus, some clinical studies have reported that nicotine can ameliorate cognitive impairments associated with Alzheimer disease (Lombardo and Maskos, 2015), schizophrenia (D'Souza and Markou, 2012) and attention deficits/hyperactivity disorders (ADHD) (van Amsterdam *et al*, 2018), at lower doses than those observed during smoking. Thus, a single dose of transdermal nicotine improved attentional performance in schizophrenia patients by ameliorating deficits in response inhibition (Barr et al, 2008). Similarly, treatment with the α7 nAChRs agonist 3-[(2,4-dimethoxy)benzylidene]anabaseine significantly improves neurocognition, as measured by a battery for assessment of neuropsychological status, suggesting that nAChRs could represent a target to enhance cognition in schizophrenia (Olincy et al, 2006). Additionally, nicotine improved attention in patients with ADHD (van

Amsterdam *et al*, 2018; Kutlu *et al*, 2015). The positive impact that nicotine has on these populations may explain the high comorbidity observed between smoking and the mentioned CNS disorders. This suggests that individuals with pre-existing cognitive deficits might smoke as an attempt to self-medicate (Garcia-Rivas and Deroche-Gamonet, 2018; Kumari and Postma, 2005; Valentine and Sofuoglu, 2017).

1.4.1.2 Animal studies

In agreement with human studies, many animal studies indicate that acute nicotine administration enhances learning and memory. In this regard, animal models represent a useful tool to elucidate the mechanisms underlying the pro-cognitive effect of nicotine. The most used approaches to evaluate the effects of nicotine on memory in animals are paradigms based on hippocampal-dependent learning, such as the object recognition and fear conditioning tests (Gould and Leach, 2014).

In the fear conditioning paradigm, a training session can involve two different types of learning: a tone-shock association (cued conditioning) that is hippocampal-independent and amygdala-dependent, and a context-shock association (contextual conditioning) that is hippocampal- and amygdala-dependent (Fanselow and Dong, 2010; Logue *et al*, 1997) (Figure 8). Multiple fear conditioning experiments have shown that acute nicotine treatment enhances contextual, but not cue conditioning (Davis *et al*, 2005; Davis and Gould, 2006; Gould and Leach, 2014; Gould and Wehner, 1999; Gulick and Gould, 2008; Portugal *et al*, 2012b). These behavioral effects were present even 1 week after nicotine treatment (Wilkinson and Gould, 2013). Nicotine, as other drugs of abuse, presents an inherent biphasic nature, which translates

into opposing effects depending on the used dose. Interestingly, the pro-cognitive effects are observed at low nicotine doses, similar to those achieved by smokers (Davis *et al*, 2005). It has been suggested that nicotine enhances contextual memory by acting mainly in the HPC, since differential brain areas are recruited in the contextual and the cue version of the fear task. Indeed, nicotine infusion into the dorsal, but not ventral, HPC enhances contextual fear conditioning (Davis *et al*, 2007; Kenney *et al*, 2012b). Conversely, direct administration of nicotine into the PFC or the thalamus, two areas well connected to the HPC and with a high density of nAChRs, did not affect contextual fear

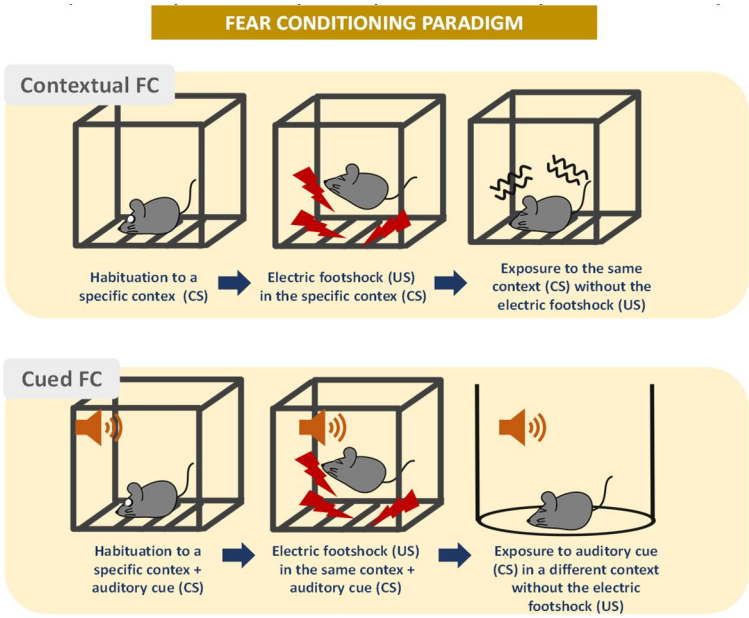


Figure 8. Schematic representation of fear conditioning and extinction in rodents. In this paradigm, a particularly neutral conditioned stimulus (CS), usually a chamber in contextual fear conditioning or a tone in cued fear conditioning, is presented together with an aversive unconditioned stimulus (US), typically an electrical footshock, resulting in an US–CS association. New exposure to the CS in absence of the US evokes an evaluable conditioned response: the freezing behavior, a natural response in rodents experiencing fear. FC, fear conditioning.

In the spatial object recognition, animals are presented with two identical objects to explore in a maze or arena. 24 hours after this training session, one of the objects (familiar) is replaced by a new one (new). A discrimination index is calculated with time that animals spend exploring the familiar and the new object. High discrimination indexes reflect good memory performance while low discrimination indexes suggest memory deficits (Figure 9). Using this test, nicotine exhibited a tendency to improve memory performance in mice (Kenney *et al*, 2011). Notably, this tendency became significant when the complexity of the task was increased by leaving a gap of 48h between the training session and the test (Kenney *et al*, 2011). Thus, these results suggest that nicotine's ability to improve learning might be associated with the complexity of the task.

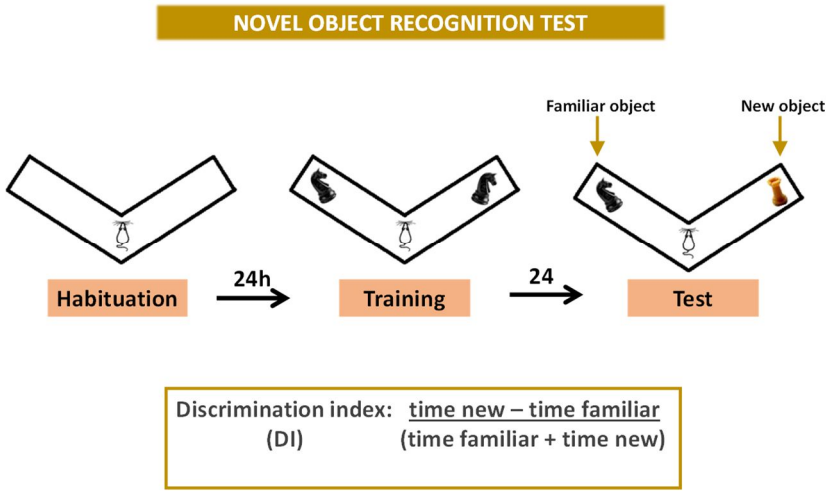


Figure 9. Schematic representation of the novel object recognition test. This test is useful to study short-term and long-term memory and evaluates the rodents' ability to recognize a novel object in the environment. This test is based on the natural preference for novel objects displayed by rodents. The task procedure consists of three phases: habituation, training, and test phase. In the habituation phase, each animal is allowed freely exploring the maze in the absence of objects for 9 minutes. During the familiarization phase, a single animal is placed in the maze containing two identical sample objects for 9 minutes. During the test phase, one of the familiar objects is replaced by a new one and the animal is returned to the maze. The time that the animal spends exploring both objects is recorded to calculate a discrimination index. Normal rodents spend more time exploring the novel object than the familiar one resulting in a high discrimination index. Low discrimination indexes suggest memory deficits.

1.4.1.3 Neurobiological mechanisms

The HPC seems to play a crucial role in the positive impact of acute nicotine on cognitive tasks. Thus, several studies have focused on the HPC to examine the neurobiological mechanisms involved in nicotine pro-cognitive effects. nAChRs are widely abundant in the HPC. The main nAChRs present in the HPC are the α4β2* and the α7 (Yakel, 2012; Zoli *et al*, 2015). α4β2* are more abundant in GABAergic hippocampal

interneurons, whereas α7 nAChRs are predominantly expressed in pyramidal cells (Ji and Dani, 2000; Kenney *et al*, 2012b). Early studies suggested that the α7 receptors are the dominant nAChRs involved in hippocampus-dependent learning. α7 nAChRs have a relevant role in diseases with cognitive impairment, such as schizophrenia and Alzheimer disease (Brunzell and McIntosh, 2012; Lombardo and Maskos, 2015). Animal studies have suggested that α7 nAChRs do not have a predominant role in the enhancement of learning induced by nicotine in spite of the cognitive effects produced by the activation of α7 nAChRs by endogenously released acetylcholine (Kenney and Gould, 2008). Thus, antagonism of the α7 nAChRs did not disrupt the nicotine improvement of learning (Davis *et al*, 2007; Davis and Gould, 2006). Conversely, antagonism of the α4β2* blocked the effects of nicotine on contextual learning, suggesting that this receptor subtype is involved in nicotine effects on memory (Davis *et al*, 2007; Davis and Gould, 2006). Given the localization of α4β2*, it seems that nicotine improves learning by acting on hippocampal interneurons rather than directly at hippocampal pyramidal cells (Jia *et al*, 2010). In agreement, electrophysiology experiments have shown that nicotine facilitates LTP in CA1 by acting on α4β2* receptors located in hippocampal interneurons (Jia *et al*, 2010), but not through α7* receptors (Nakauchi and Sumikawa, 2012). These data indicate that nicotine (exogenous) and acetylcholine (endogenous) modulate learning and memory by acting on different nAChRs suggesting a dissociation between normal neural communication and drug-induced alterations.

The molecular mechanisms involved in the effects of nicotine on learning and memory have not been yet fully clarified. It is feasible that cellular processes downstream from nAChRs must interact with cell signaling

cascades involved in learning and memory to enhance hippocampus-dependent learning (Kutlu and Gould, 2016a). On the other hand, NMDA receptors in the HPC are critical to learning and memory (Khakpai *et al*, 2016; Place *et al*, 2012). Interestingly, multiple studies have suggested that the depolarization triggered by nAChRs could facilitate activation of NMDA receptors (Gould and Leach, 2014; López-Hidalgo *et al*, 2012). In this sense, currents mediated by postsynaptic nAChRs could contribute to the depolarization needed to remove the blockade of NMDA receptors exerted by the presence of magnesium (Ji *et al*, 2001). The interaction between NMDA and nAChRs could explain that nicotine administration reversed the deficits in contextual fear learning produced by two different NMDA antagonists (André *et al*, 2011). nAChRs and NMDA receptor activation will lead to a greater internal release of Ca^{2+} and the consequent activation of several cell signaling cascades. PKA and ERK are plausible kinases to be modulated by nicotine given their involvement in hippocampal-dependent learning (Abel and Nguyen, 2008). In agreement, the decrease of ERK and PKA activity blocked the enhancing effect of nicotine on contextual fear (Raybuck and Gould, 2007). Interestingly, nicotine administration modulates other components of the PKA-ERK cascade. Several studies have demonstrated a crucial role of CREB in the formation of long-termed memories in a wide range of animal models (Kida and Serita, 2014). Indeed, gain or loss of CREB function improves and impairs, respectively, the formation of memories (Kida and Serita, 2014). Notably, nicotine paired with learning leads to an increase in expression of Jnk1 in the HPC (Kenney *et al*, 2010) and was associated with increased CREB phosphorylation at the Jnk1 promoter region in the HPC (Kenney *et al*, 2012a) (Figure 10).

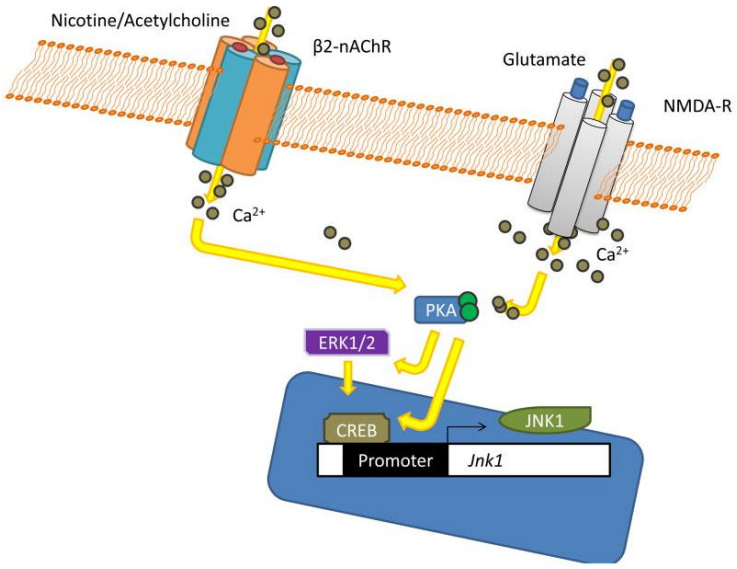


Figure 10. Cell signaling cascade involved in the acute effect of nicotine on hippocampus-dependent learning. Activation of nAChRs will lead to an increase in intracellular calcium that could provide the necessary depolarization to allow NMDA-receptor mediated calcium influx. Calcium leads to the activation of PKA and ERK, which in turn activate CREB and consequently stimulates *Jnk1*. Activation of JNK kinase seems critical for nicotine to enhance learning (Gould and Leach, 2014).

Alternatively, nicotine may activate other pathways to modulate learning considering that the increase in intracellular calcium, triggered by activation of nAChRS, is common to many signaling cascades involved in cognitive processes (Berridge, 2014). Activation of other pathways would probably act in parallel with those usually activated to enhance learning. One of the objectives of this thesis is to identify other mechanisms involved in the effects that nicotine exerts on learning, given their contribution to the development of nicotine dependence.

1.4.2 Chronic nicotine effects on cognition

Nicotine intake improves hippocampus-dependent learning, although this effect disappears with sustained nicotine consumption. Indeed, contextual and cued fear conditioning in mice remained unaltered after a chronic treatment with nicotine (Davis *et al*, 2005). In addition, similar results have been observed in other types of hippocampus-dependent learning. Thus, chronic treatment with nicotine did not affect memory in the object recognition task in mice (Kenney *et al*, 2011). In agreement, other cognitive processes related to executive functions remained unaltered when mice were chronically treated with nicotine (Cole *et al*, 2015; Ortega *et al*, 2013). These studies suggest that chronic nicotine treatment may develop tolerance to the pro-cognitive effect, which may be related to differences in activation and desensitization states of nAChRs (Picciotto and Mineur, 2014).

1.4.3 Effects of withdrawal from chronic nicotine on cognition

In contrast to the positive cognitive effects of acute nicotine, nicotine withdrawal alters a variety of cognitive processes in humans, including impairments in attention, working memory and episodic memory (Hall *et al*, 2015a; Hughes, 2007a; Wesnes *et al*, 2013). During the last decade, increasing attention has focused on cognitive impairments that emerge during smoking abstinence, since these impairments seem to play a critical role in relapse in tobacco consumption (Ashare and Schmidt, 2014).

1.4.3.1 Human studies

Difficulty in concentrating has often been reported by abstinent smokers (Hughes, 2007a). These deficits arise as soon as 30 minutes after smoking cessation (Hendricks *et al*, 2006), last for days and are observed as more

omission errors and slower reaction times in sustained attention tasks, compared to non-abstinent smokers (Harrison *et al*, 2009). Several human studies describe that abstinence from smoking also affects working memory performance (Hall *et al*, 2015a). Following overnight abstinence, smokers had less accuracy and exhibited slower reaction times when performing working memory tasks (Jacobsen *et al*, 2005, 2007; Loughead *et al*, 2010; Patterson *et al*, 2010). Alterations in working memory became more visible when the difficulty of the task was increased (Jacobsen *et al*, 2007; Loughead *et al*, 2009, 2010). There is also strong evidence that deficits in episodic memory could be a primary trait of nicotine withdrawal during tobacco abstinence. Thus, smokers following a 24 hours period of abstinence exhibited diminished episodic memory when evaluated in a recognition memory task, compared to their performance when smoking normally (Merritt *et al*, 2010; Wesnes *et al*, 2013). Interestingly, a pilot study reported that abstinent male smokers showed reduced performance in the recognition test compared to their female counterparts, suggesting substantial sex differences in the cognitive effects of tobacco abstinence (Merritt *et al*, 2012).

These cognitive deficits that appear within the first few days of tobacco cessation are gaining importance as a core dependence phenotype of nicotine withdrawal and a target for medication development efforts (Ashare and Schmidt, 2014). Indeed, cognitive deficits observed during nicotine withdrawal could directly promote short-term relapse to tobacco smoking (Culhane *et al*, 2008; Miglin *et al*, 2017; Patterson *et al*, 2010). These cognitive symptoms during withdrawal can be alleviated by nicotine re-exposure (Myers *et al*, 2008; Soar *et al*, 2008), suggesting that smokers might relapse to tobacco use to recover from the cognitive impairments observed during nicotine abstinence. In addition, current pharmacological

agents used for smoking cessation, such as varenicline and bupropion, enhance mood and cognitive function during early nicotine abstinence (Ashare and McKee, 2012; Loughead *et al*, 2010; Patterson *et al*, 2009). The cognitive-enhancing effects of these compounds seem to be an important part of their efficacy as smoking cessation agents.

1.4.3.2 Animal studies

Many of the abstinent symptoms observed in humans, including the cognitive alterations, can be recapitulated in rodent models of nicotine withdrawal (McLaughlin *et al*, 2015). Among preclinical models, the use of osmotic minipumps is a well-stablished model to study the effects of chronic nicotine exposure (Hamouda *et al*, 2018; Jackson *et al*, 2018; Matta *et al*, 2007). Minipumps only require a minimal surgical procedure to be implanted and produce a constant level of circulating nicotine in the animal (Damaj, 2003; Jackson *et al*, 2018). Plasma nicotine levels obtained using minipumps with appropriate dosage could resemble those reported in smokers i.e plasma levels between 0.06–0.31 μM of humans consuming an average of 17 cigarettes a day (Benowitz and Peyton, 1984; Davis *et al*, 2005; Turner *et al*, 2014a). Withdrawal symptoms can be assessed after the sudden discontinuation of nicotine treatment (spontaneous withdrawal) or administration of nAChRs antagonists, such as mecamylamine (De Biasi and Salas, 2008). One advantage of the use of mecamylamine is that induction of nicotine withdrawal can be timed to behavioral assessment.

Similar to the observed in humans, mouse experiments have consistently shown that withdrawal from nicotine results in hippocampus-dependent cognitive deficits (Gould and Leach, 2014; Hall *et al*, 2015a; Leach *et al*, 2013; Parikh *et al*, 2016). Thus, impairments in fear conditioning have

been reported following spontaneous (Davis *et al*, 2005; Portugal *et al*, 2012a; Wilkinson and Gould, 2013) and precipitated (Raybuck and Gould, 2009) nicotine withdrawal. Spontaneous nicotine withdrawal has also been reported to alter spatial object-recognition (Kenney *et al*, 2011). Furthermore, mecamylamine-precipitated nicotine withdrawal has been associated with alterations in cognitive flexibility. Thus, withdrawn mice were unable to execute a new learning strategy, manifested as an increase in learning errors in an operant strategy switching task (Parikh *et al*, 2016). These data suggest that cognitive difficulties, inability to learn adaptive coping strategies and the frustration that can result from them could influence relapse. Therefore, treating these changes in cognition could facilitate abstinence and prevent relapse. Effective therapeutic development requires a deeper understanding of the neurobiological basis for these symptoms, which represents an objective of this thesis.

1.4.3.3 Neurobiological mechanisms

Experiments in mice suggest a selectivity of nicotine withdrawal to alter hippocampus-dependent learning (Gould and Leach, 2014). Indeed, withdrawal from chronic infusion of nicotine into the dorsal HPC disrupted learning, whereas withdrawal from chronic infusion into the cortical area above the HPC or the thalamic region directly below the HPC did not disrupt learning (Davis and Gould, 2009). In humans, abstinent smokers recover from the withdrawal-cognitive deficits over time (Hughes, 2007a), which suggests that the underlying changes in brain function should also improve in a similar time frame. It seems that nicotine withdrawal could affect cognition by modulating nAChRs, as occurs with the positive effects that acute nicotine has on learning. In mice, nicotine withdrawal deficits in hippocampus-dependent learning lasted 4 days and

by the 5th day, learning was similar to controls (Gould *et al*, 2012). Interestingly, upregulation of the high-affinity nAChRs (β2*) in the HPC was paralleled with the duration of cognitive deficits. This pattern of upregulation was exclusive to the HPC as it was not observed in cortex or cerebellum (Gould *et al*, 2012). In addition, withdrawal deficits were absent in mice lacking the β2 subunit supporting its role in the cognitive deficits associated with nicotine withdrawal (Portugal *et al*, 2008; Yildirim *et al*, 2015). Therefore, changes in high-affinity nAChR upregulation in the HPC seem to be an important contributing factor to the development of cognitive deficits during nicotine withdrawal.

Several studies have implicated other receptors and mechanisms different of nAChRs in the cognitive symptoms of nicotine withdrawal. Thus, a variation on the cannabinoid receptor 1 gene (CNR1) seems to moderate cognitive disruption during nicotine withdrawal in humans (Evans *et al*, 2016). This study found that homozygotes for a major allele of CNR1, associated with an increased expression of this gene, exhibited greater nicotine withdrawal-related cognitive disruption (Evans *et al*, 2016). Moreover, cocaine-and amphetamine-regulated transcript peptide (CART), a neuropeptide known for its pro-cognitive properties, seems to be involved in memory impairment induced by nicotine withdrawal. Early nicotine withdrawal resulted in drastic reduction in CART immunoreactivity that correlated to memory impairment (Borkar *et al*, 2017). Furthermore, nicotine withdrawal deficits might be associated with changes in noradrenergic function. Thus, the norepinephrine reuptake inhibitor atomoxetine restored cognitive function during nicotine withdrawal (Davis and Gould, 2007).

These findings provide some initial steps to understand the mechanisms involved in the cognitive deficits associated with nicotine withdrawal.

However, more research in this field is necessary to understand how nicotine withdrawal affects cognitive function and facilitate the development of more specific therapeutic targets for nicotine addiction.

In summary, several studies indicate that acute or initial nicotine intake has a positive effect on cognition which may contribute to the development of nicotine dependence. However, this pro-cognitive effect disappears during chronic nicotine treatment, indicating the development of tolerance to this effect. Interestingly, when chronic nicotine treatment ceases, cognitive functioning is altered, especially those that rely on hippocampal function. Therefore, chronic nicotine usage seems to induce neuroadaptive changes in the brain resulting in tolerance to the pro-cognitive effects of acute nicotine and then deficits in learning are uncovered when nicotine exposure ends (Figure 11).

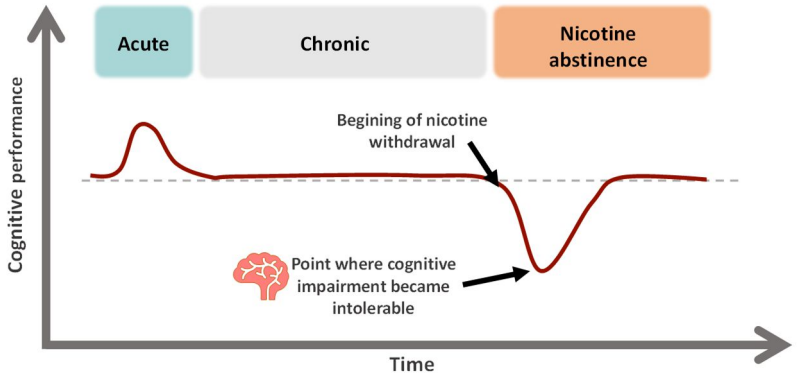


Figure 11. Theoretical model of the cognitive effects of smoking and withdrawal-induced relapse. Initiation of nicotine use produces cognitive benefits which dissipate over time with chronic nicotine use. Cognitive performance declines upon nicotine abstinence. Cognition recovers over time (red line), however altered cognition for some individuals drops to intolerable levels which might induce relapse (blue line). Developing a treatment that could alleviate the withdrawal-induced deficits in cognition would substantially enhance the likelihood of successfully quitting (green line) (Adapted from Hall *et al,* 2015).

1.5 Therapeutic strategies for nicotine addiction

Nicotine addiction is difficult to treat given its complex nature of chronic and relapsing disorder. The majority of attempts to quit smoking in the EU continue to be without any professional cessation assistance (Filippidis *et al*, 2018), even though reports estimate that near 80% of smokers who try to quit on their own relapse within the first month of abstinence (Benowitz, 2009). During the last years, the use of established aids such as pharmacotherapy has become less popular, while the use of e-cigarettes as a potential cessation method has grown (Filippidis *et al*, 2018; Prochaska and Benowitz, 2016). The most promising approaches for achieving long-term abstinence after smoking cessation include a combination of behavioral approaches along with pharmacotherapies, probably because counseling improves medication adherence (Polosa and Benowitz, 2011). However, the outcome of nicotine abstinence, even with combined therapy, is still modest. Interestingly, the ability to maintain smoking abstinence during the first week of a quit attempt is a strong predictor of success at end of treatment and at 6 months (Ashare *et al*, 2013). Thus, the early withdrawal period is a vulnerable time for most smokers and represents a critical window to evaluate novel smoking cessation treatments.

Present pharmacotherapies for tobacco addiction include nicotine replacement therapy, bupropion and varenicline. Generally, all these medications, if used properly, increase significantly the quitting rates compared to placebo treatments. Clinical trials have revealed that varenicline has a greater efficacy than bupropion and nicotine replacement therapy (abstinence rates from 9-52 weeks: 23% for varenicline, 15% for bupropion, and 10% for nicotine replacement therapy, approximately) (Prochaska and Benowitz, 2016).

1.5.1 Nicotine replacement therapy

Nicotine medications act on nAChRs to mimic or replace the effects of nicotine from tobacco. Nicotine replacement therapy provides nicotine to counteract the physical dependence that arise during abstinence without exposure to toxic combustion products (Benowitz, 2009). However, it does not completely prevent withdrawal symptoms since nicotine replacement methods provide lower and slower rising plasma nicotine concentrations compared to cigarettes smoking. Nicotine replacement therapy also provides positive reinforcing by increasing arousal and exerting a stress-relieving effect, aside from ameliorating withdrawal signs (Prochaska and Benowitz, 2016). The degree of reinforcement is related to the rapidity of absorption and the peak nicotine level achieved in arterial blood. Thus, reinforcement better achieved with rapid-delivery formulations such as nicotine nasal spray and, to a lesser extent, nicotine gum or inhaler. By using these products, smokers can dose themselves with nicotine when they have the urge to smoke cigarettes. On the other hand, nicotine patches deliver nicotine gradually and produce sustained nicotine levels throughout the day, thus not providing much positive reinforcement. Combination of nicotine patches (slow release) with nicotine gum, inhaler, or nasal spray (rapid release) are more effective than the use of single nicotine replacement products (Prochaska and Benowitz, 2015).

1.5.2 Varenicline

Varenicline is a partial agonist of the α4β2 receptor, which mediates dopamine release and is thought to be the major receptor involved in nicotine addiction (Mihalak *et al*, 2006). Varenicline activates the α4β2 nAChRs with a maximal effect at around 50% that of nicotine. This action relieves nicotine withdrawal symptoms, including craving, and at the same

time blocks effects of nicotine from tobacco use on the receptor, thereby decreasing the rewarding effects of cigarettes that are smoked. Preclinical studies have revealed that varenicline blocks the rewarding properties of nicotine using the CPP in mice (Bagdas *et al*, 2018) and rats (Biala *et al*, 2010). In addition, high doses of varenicline induced place aversion in mice (Bagdas *et al*, 2018). Smokers under varenicline treatment often reduce the number of cigarettes smoked per day even before their target quit day (Ebbert *et al*, 2010). Interestingly, human studies have demonstrated that varenicline is effective in alleviating cognitive withdrawal symptoms (Ashare and Schmidt, 2014; Loughead *et al*, 2010; Patterson *et al*, 2009). Given the varenicline's site of action, these results support the hypothesis that nicotine withdrawal deficits may be related to hypersensitivity of nAChRs.

Major side effects of varenicline are nausea, vomiting, and insomnia (Fiore and Jaén, 2008). Shortly after varenicline was released the market, multiple studies reported an association between varenicline use and psychiatric adverse events, such as depression, psychosis, and suicide (Prochaska and Benowitz, 2016). Due to these psychiatric effects, the FDA placed a *Box Warning* on varenicline, and recommended caution when prescribing the drug (Drug Safety and Availability - FDA, 2016). Recently, and after evaluating large clinical trials, the FDA has determined that the risk of serious side effects on mood, behavior, or thinking with the use of varenicline is lower than previously suspected and removed the *Boxed Warning* (Drug Safety and Availability - FDA, 2016).

1.5.3 Bupropion

Bupropion hydrochloride was initially developed and marketed as an antidepressant (Stahl *et al*, 2004). It was the unexpected observation of

spontaneous smoking cessation among veterans treated with bupropion for depression that led to the exploration of bupropion as a smoking cessation medication (Benowitz, 2009). Bupropion blocks dopamine and, to a lesser extent, norepinephrine reuptake and has some weak nAChR antagonist effect (Carroll *et al*, 2014). Thus, bupropion increased brain levels of dopamine and norepinephrine, simulating the effects of nicotine on these neurotransmitters. In non-withdrawn rats, bupropion decreased the reward thresholds indicating an increase in reward, measured by intracranial self-stimulation (Cryan *et al*, 2003). Interestingly, bupropion also normalized the elevated brain reward thresholds and reduced the somatic signs of withdrawal in rats (Cryan *et al*, 2003) . In agreement, a human study revealed a reduced responding for a reward unrelated to smoking during smoking abstinence (Perkins *et al*, 2013b). This effect of withdrawal was reversed by bupropion treatment suggesting the reinforcement enhancing effect of this compound. Furthermore, it has been suggested that bupropion's mechanisms for aiding smoking cessation could be the attenuation of mild cognitive effects that occur early in nicotine withdrawal (Perkins *et al*, 2013).

1.5.4 Electronic nicotine delivery systems

Electronic nicotine delivery systems (ENDS; e-cigarettes, e-hookah, vape pens) are battery-powered devices that generate an aerosol for inhalation, typically containing nicotine. Although ENDS are pictured as a "safer" alternative to traditional cigarettes for smokers unable or unwilling to quit, their innocuity is still on debate. Recent investigations have indicated that END's users can achieve nicotine blood concentration similar to that observed in cigarettes smokers, suggesting that ENDs could be potentially addictive (Pulvers *et al*, 2016). Alarmingly, an increase in the use of these

devices has been observed among youth. These could be partially explained because ENDS are sold in child-friendly flavorings (e.g., cotton candy, gummy bear, Froot Loops®, Oreo, Skittles) and in low-cost single units. With only a few years of surveillance data, it is uncertain whether ENDS use in adolescence could be a doorway to nicotine addiction, later conventional tobacco use, and other drugs of abuse (i.e., vaping cannabis) (Prochaska and Benowitz, 2016).

2. Neuroinflammation

For many years, neuroinflammation has been described as a complex response that the CNS exerts to manage pathogens, toxins, trauma and neurodegeneration. However, recent publications indicate that the organized actions of glial cells, neurons, inflammatory cytokines and chemokines that constitute neuroinflammation are not only provoked by pathological conditions, but they can also be induced by alterations in neuronal activity (Xanthos and Sandkühler, 2014). In this regard, neuroinflammation has been linked to depression (Blank and Prinz, 2013; Wohleb *et al*, 2016), chronic exposure to stress (Delpech *et al*, 2015), epilepsy (Vezzani *et al*, 2011), sleep alteration (Zhu *et al*, 2012), and deficits in learning and memory formation (Hein and O'Banion, 2011; McKim *et al*, 2016).

2.1 Cellular and molecular mechanisms of inflammatory processes

2.1.1 Overview of microglial cells

Microglial cells are the immune effector cells in the CNS considered as key cellular mediators of neuroinflammatory processes in the CNS. Microglia comprise cells approximately 10% of the total cell brain population in rodents and between 0.5% to 16.6% in humans (Lawson *et al*, 1990; Mittelbronn *et al*, 2001). They are considered the resident macrophages of the brain and represent 5–20% of total glial cells in rodents (Ginhoux *et al*, 2013). Microglial cells distributed differently along the brain parenchyma and their density varies depending on the brain region, going from 5% in corpus callosum to 12% in substantia nigra (Lawson *et al*, 1990)

and being more abundant in gray matter compared to white matter (Kofler and Wiley, 2011).

The ability to visualize microglia without the need for immunolabeling and the development of novel two-photon *in vivo* imaging methodologies provided unprecedented sights into the role of these cells in vivo. Traditionally, microglia in the normal CNS was considered as functionally inert and hence described as "quiescent" or "resting". However, it is now clear that microglia are highly active and plastic in physiological conditions, changing the previous notion of "resting" microglial phenotype in normal adult CNS to the concept of surveillance phenotype (Davalos *et al*, 2005; Kettenmann *et al*, 2011; Nimmerjahn *et al*, 2005). In this state, microglia morphology is characterized by a small soma and long and highly dynamic ramifications through which monitors neural function and contact synapses (Kettenmann *et al*, 2011). In normal conditions, microglia plays a functionally dynamic role in synaptic plasticity, possibly through the release of cytokines and growth factors. Ramified microglial cells also contribute to structural plasticity through the elimination of synapses via phagocytic mechanisms, which is necessary for normal cognition (Kettenmann *et al*, 2013). Upon injury or alteration in the CNS, microglia cells shift from a ramified surveilling state to an activated phagocytic one, characterized by an ameboid morphology with a large soma and retraction of its ramification (Kitamura *et al*, 1978; Stence *et al*, 2001). This morphological change is generally accompanied by changes in the expression of pro-inflammatory and anti-inflammatory molecules (Blank and Prinz, 2013; Delpech *et al*, 2015) (Figure 12).

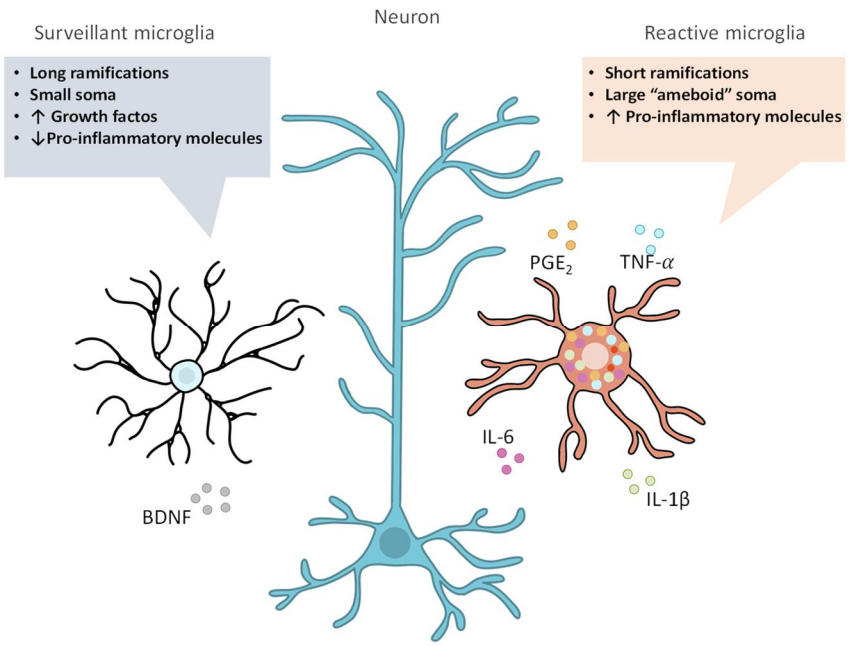


Figure 12. Schematic representation of the different morphology of microglial cells. Upon a harmful stimulus, microglia cells shift from the state to a reactive state. Significant morphological accompany both states IL, interleukin; PGE2, prostaglandin E2 ; TNF, tumour necrosis factor.

2.1.2 Cytokines

Cytokines are small proteins or glycoproteins released by different types of cells such as microglia, neurons and astrocytes within the CNS. Cytokines are key modulators of inflammation but also have primary roles in neuronal plasticity, neurogenesis and learning and memory in physiological conditions (Hanisch, 2002; Makhija and Karunakaran, 2013; Turner *et al*, 2014b). The term cytokine comprises more than 300 peptides, where interleukins (ILs), interferons (IFNs) and tumor necrosis factors (TNFs) represent the three most important families. In addition, cytokines can be classified based on the nature of the immune response

as pro-inflammatory (i.e IL-1, TNFα or IFN-γ) or anti-inflammatory (i.e IL-10 and IL-12) (Turner *et al*, 2014b) (Figure 13).

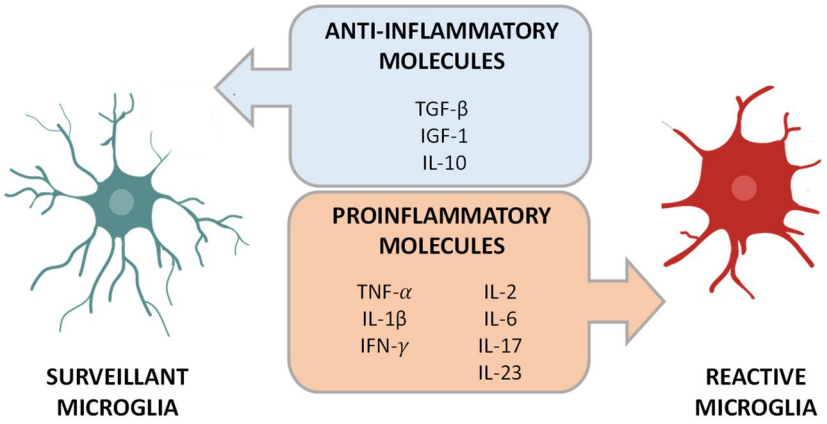


Figure 13. Proinflamamtory and anti-inflammatory molecules.
IL, interleukins; IF, interferon; IFG, insulin-like growth factor; TGF, tumor growth factor; TNF, tumor necrosis factor.

2.2 Neuroinflammation and tobacco use

A potential link between smoking and inflammation has been widely established. Although most of these studies have focused on the relationships between inflammatory markers and increased cardiovascular risk (Lo Sasso *et al*, 2016; Wannamethee *et al*, 2005), there are some studies that have evaluated the role of inflammatory molecules in other aspects of nicotine dependence. Thus, a preclinical study found that precipitation of nicotine withdrawal results in an upregulation of CX3CR1, a key component of a signaling pathway associated with the induction of proinflammatory cytokines in microglial cells (Ding *et al*, 2015). Additionally, a pilot human study found that an inflammatory response, observed as increased levels of c-reactive protein, was associated with the affective signs of nicotine withdrawal in a sub-group

of abstinent smokers (Corwin and Klein, 2003). Interestingly, enhanced levels of c-reactive protein in humans have also been associated with neurocognitive decline following surgery (Ramlawi *et al*, 2006) and in older adults (Bettcher *et al*, 2012).

2.3 Neuroinflammation and cognitive impairment

Many disorders of the CNS that course with cognitive deficits may have an inflammatory component underneath (Blank and Prinz, 2013). Thus, changes in microglial morphology have been directly associated with deficits in cognition in a mice model of schizophrenia (Gomes *et al*, 2015). In line with this study, an increase in microglia reactivity has been found to be underlying the cognitive deficits following an intermittent alcohol exposure (Zhao *et al*, 2013) and chronic stress exposure (McKim *et al*, 2016). Interestingly, microglial reactiveness has been associated with an increase in the expression pro-inflammatory genes, such as IL-1β and TNFα, in models with memory alterations (Blank and Prinz, 2013; Hanisch, 2002; Streit *et al*, 1999, 2004). Notably, these two cytokines have a central role in learning and memory processes in both healthy (Kohman and Rhodes, 2013; Moraes *et al*, 2015; Santello and Volterra, 2012) and pathological brain (Cacci *et al*, 2005; Cho *et al*, 2015; Kitazawa *et al*, 2011). A recent study revealed that microglial-derived IL-1β seems to be critical for normal hippocampus-dependent learning and memory (Williamson *et al*, 2011). Indeed, expression of IL-1β increases in response to normal learning (Ross *et al*, 2003; Schneider *et al*, 1998). Moreover, mice lacking IL-1β or its receptor exhibited impairments in fear memory (Goshen *et al*, 2007) and spatial learning (Hein *et al*, 2010). In contrast, high levels of IL-1β impair contextual and spatial memory (Barrientos *et al*, 2002, 2009;

Hein *et al*, 2010; Spulber *et al*, 2009). It has been suggested that IL-1β could mediate memory processes by affecting LTP in the HPC. Indeed, It is known that IL-1β gene expression is increased after LTP induction *ex vivo,* but also *in vivo* in freely moving rats (Balschun *et al*, 2003; Schneider *et al*, 1998). This enhancement of IL-1β expression seems to play a role not only during induction, but also during LTP maintenance (Loscher *et al*, 2003; Schmid *et al*, 2009). These data suggest that physiological levels of IL-1β are necessary for normal memory, whereas concentrations that are either too low or too high impair memory.

An increase in the levels of TNFα also contributes to cognitive impairment (Belarbi *et al*, 2012). Similar to IL-1β, physiological levels of TNFα can be beneficial but an exaggerated production of this cytokine is detrimental to the induction of LTP in both its early and late phases (Butler *et al*, 2004). TNFα also plays a clear role in a form of long-term plasticity called synaptic scaling, a plasticity mechanism able to adjust the strengths in synapses through increased AMPA receptors expression in response to an episode of strong cell activation. This leads to neuronal network stabilization. Indeed, it has been shown that TNFα is necessary for increased surface AMPA receptors and synaptic strength after chronic blockade of neuronal activity (Stellwagen and Malenka, 2006).

Inflammatory cytokines including IL-1β, TNFα, and IFNγ are also key modulators of neurogenesis (Borsini *et al*, 2015). Neurogenesis is a complex neurobiological process by which new neurons are generated from neural stem cells (Boldrini *et al*, 2018; Imayoshi *et al*, 2009). Current data have estimated that approximately 700 new neurons are added to the adult human HPC daily, suggesting that neurogenesis has a critical role in mediating human brain functions, such as memory formation and learning (Deng *et al*, 2010; Spalding *et al*, 2013). In rodents, 2 neurogenic

niches have been described: (1) the subventricular zone of the lateral ventricles and (2) the subgranular zone of the dentate gyrus (DG) in the HPC. Several studies support the involvement of adult hippocampal neurogenesis in memory and learning (Aimone *et al*, 2014; Deng *et al*, 2010; Saxe *et al*, 2006; Zhao *et al*, 2008). These studies have revealed that new granule cell neurons have higher levels of excitability and plasticity and are thought to play an important role in forming memories (Ge *et al*, 2007), spatial learning (Deng *et al*, 2010), pattern separation (Sahay et al., 2011), cognitive flexibility, and the association between old and new memories (Jessberger and Gage, 2014; Kohman and Rhodes, 2013).

Under physiological conditions, surveilling microglia cells play a supporting role in neurogenesis (Ekdahl *et al*, 2009; Hanisch and Kettenmann, 2007; Ziv and Schwartz, 2008). This, exposure of rats to environmental enrichment induced not only increased neurogenesis, but also a significant increase in the number of hippocampal microglia (Ziv *et al*, 2006). Conversely, reactive microglia and secretion of cytokines have been suggested to impaired neurogenesis in an inflammatory environment (Chesnokova *et al*, 2016; Monje *et al*, 2003).

In summary, strong evidence supports the role of microglia, cytokines and neurogenesis as key players of neuroinflammation-induced cognitive dysfunction. However, further research is needed to better understand the role of neuroinflammation in memory processes. Identifying the key molecules involved in these processes could lead to the development of new tools to prevent the cognitive dysfunction associated with nicotine withdrawal.

3. The endocannabinoid system

The endogenous cannabinoid system is one of the most important physiological systems involved in establishing and maintaining human health. Endocannabinoids and their receptors are found throughout the body: in the brain, connective tissues, glands, and immune cells.

3.1 Overview of the endocannabinoid system

Albeit the extensive consumption of cannabis derivatives over thousands of years, it was not until the 1980s when it was unraveled that cannabinoid compounds exert their biological effects through the activation of specific endogenous receptors, instead of by altering the cellular membrane permeability as it was formerly believed (Devane *et al*, 1988; Herkenham *et al*, 1990; Howlett and Fleming, 1984; Matsuda *et al*, 1990). This discovery was followed by the identification of the endogenous ligands of cannabinoid receptors, which were referred to as endocannabinoids (Devane *et al*, 1992; Mechoulam *et al*, 1995). Hence, the endocannabinoid system consists of endocannabinoids, their GPCR-family receptors and the enzymatic machinery that synthesizes and degrades endocannabinoids. Therefore, exogenous cannabinoids act through the hijacking of the endocannabinoid system, which represents the major site of action of Δ^9-tetrahidrocannabinol. (THC) and other cannabinoids. The biological effects induced by these compounds are directly linked to the neuroanatomical distribution and physiological role of this endogenous system. Three characteristics distinguish the endocannabinoids from other neurotransmitters systems: (1) the endocannabinoid system has a retrograde signaling, (2) endocannabinoids are synthesized on demand since they cannot be stored in vesicles due to its lipophilic nature and (3) activation of cannabinoid receptors modulates excitability of the neurons

inhibiting the release of both excitatory and inhibitory neurotransmitters. This system is involved in a wide variety of biological functions, including brain development, control of energy expenditure, motivation, pain perception, reward, cognition and stress, among others (Chen, 2015; Mechoulam and Parker, 2013).

3.1.1 Cannabinoid receptors

Endocannabinoids and external cannabinoids exert their pharmacological actions through the activation of at least two distinct cannabinoid receptors: CB1R and CB2R. CB1R was the first cloned and characterized cannabinoid receptor (Matsuda *et al*, 1990), abundantly expressed throughout the CNS (Herkenham *et al*, 1990, 1991). Shortly after, CB2R was identified and initially considered as a peripheral receptor, since it was first found in the spleen (Munro *et al*, 1993). Both belong to the GPCR family and are mainly coupled to Gi/o protein. Diverse studies also point to the existence of other receptors that bind cannabinoid ligands, such as G protein-coupled receptor 55 (GPR55) (Pertwee, 2009), the sphingosine-1-phosphate lipid receptors GPR3, GPR6 and GPR12 (Kostenis, 2004; Yin *et al*, 2009), the peroxisome proliferator-activated receptor (PPAR) (O'Sullivan, 2009), or the transient receptor potential cation channel subfamily V member 1 (TRPV1) (De Petrocellis and Di Marzo, 2010).

3.1.1.1 Cannabinoid receptor type 1

CB1R is the most abundant GPCRs receptor in the CNS and constitutes the main cannabinoid receptor involved in the psychoactive effects of THC and other cannabinoid ligands. Its distribution has been well characterized both in rodents (Herkenham *et al*, 1991; Tsou *et al*, 1998) and humans (Lee *et al*, 2018; Terry *et al*, 2010; Westlake *et al*, 1994). The highest density of CB1R has been observed in the basal ganglia, cerebellum, and HPC. These

receptors have also been found in cortex, amygdala, thalamus and hypothalamus, among other brain regions (Herkenham *et al*, 1991). The high CB1R levels in the sensory and motor regions are consistent with the important role of CB1R receptors in motivation and cognition. CB1R is also expressed in peripheral tissues, including the retina, gonads, peripheral neurons, adipocytes, heart, lung, liver, adrenal gland, and immune and vascular system (Pertwee *et al*, 2010). At the cellular level, CB1R expression is mainly restricted to presynaptic terminals, where they modulate the release of multiple excitatory and inhibitory neurotransmitters, usually by promoting the inhibition of their release (Wilson and Nicoll, 2002) (Figure 14).

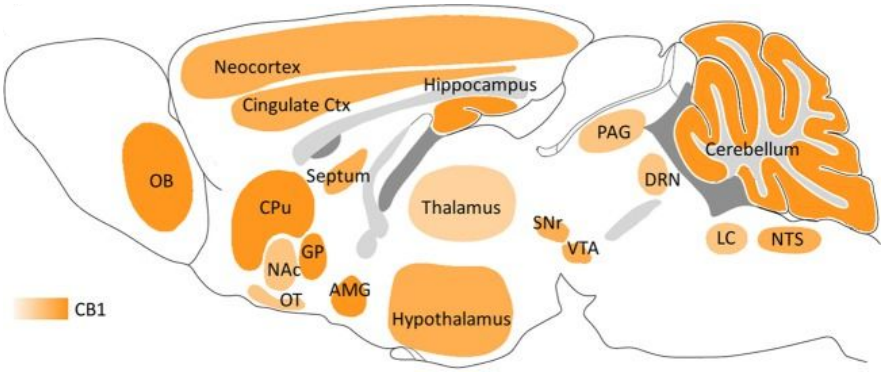


Figure 14. Schematic representation of the main areas expressing CB1 in the mouse brain. AMG, amygdala; CPu, caudate putamen; Ctx, cortex; DRN, dorsal raphe nucleus; GP, globus pallidus; LC, locus coeruleus; NAc, nucleus accumbens; NTS, nucleus of the solitary tract; OB, olfactory bulb; OT, olfactory tubercle; PAG, periaqueductal gray; SNr, substantia nigra pars reticulata; VTA, ventral tegmental area (Flores *et al,* 2013).

3.1.1.2 Cannabinoid receptor type 2

CB2R is primarily located in the immune system including the spleen, thymus and immune cells, and are deeply involved in inflammatory processes (Walter and Stella, 2004). Originally, it was assumed that CB2R was absent in CNS neurons (Munro *et al*, 1993). However, further research suggests that CB2R is expressed by some neurons, particularly under certain pathological conditions (Atwood and Mackie, 2010; Van Sickle *et al*, 2005; Viscomi *et al*, 2009). In addition, CB2R may be upregulated under neuroinflammatory conditions in certain cell populations within the brain, such as microglial cells (Cabral *et al*, 2008; Carlisle *et al*, 2002; Stella, 2013). Interestingly, recent studies suggest that besides their role in neuroinflammation, CB2R also controls the rewarding properties of diverse addictive drugs, such as cocaine (Aracil-Fernández *et al*, 2012; Xi *et al*, 2011), alcohol (Ortega-Álvaro *et al*, 2015) and nicotine (Navarrete *et al*, 2013).

3.1.2 Endocannabinoids

The discovery of the cannabinoid receptors prompted the research to identify the endogenous molecules that stimulate these receptors. Two molecules, known as endocannabinoids, were isolated, one from the brain, N-arachidonoylethanol-amide (anandamide or AEA) and a second from peripheral tissue, 2-arachidonoyl glycerol (2-AG) (Devane *et al*, 1992; Mechoulam *et al*, 1995).

Anandamide behaves as a partial agonist at both CB1R and CB2R, although presents lower affinity for CB2R, and binds also to the TRPV1 receptor (Cristino *et al*, 2008). 2-AG concentration in the brain is much higher than anandamide and acts as a full agonist for both CB1R and CB2R with higher potency than anandamide (Reggio, 2010). Beside these molecules, other

putative endocannabinoids have also been identified, such as 2-arachidonylglycerolether (Hanus *et al*, 2001) and O-arachidonoylethanolamine, among others (Porter *et al*, 2002) (Figure 15). Despite the ability of these additional endogenous lipids to bind to cannabinoid receptors, their functional relevance remains to be elucidated.

Unlike most of the neurotransmitters, anandamide and 2-AG are not stored in presynaptic vesicles, but rather synthesized and released on demand in the postsynaptic terminals in an activity-dependent manner (Di Marzo *et al*, 2005). Once released from the postsynaptic neurons, endocannabinoids travel retrogradely across synapses and activate CB1R on presynaptic terminals, acting as fast retrograde synaptic messengers to produce a transient decrease of the release of other neurotransmitters (Ohno-Shosaku *et al*, 2001; Wilson and Nicoll, 2002). Given its fast-modulatory effects, endocannabinoid tone is finely controlled by balancing its biosynthesis and degradation.

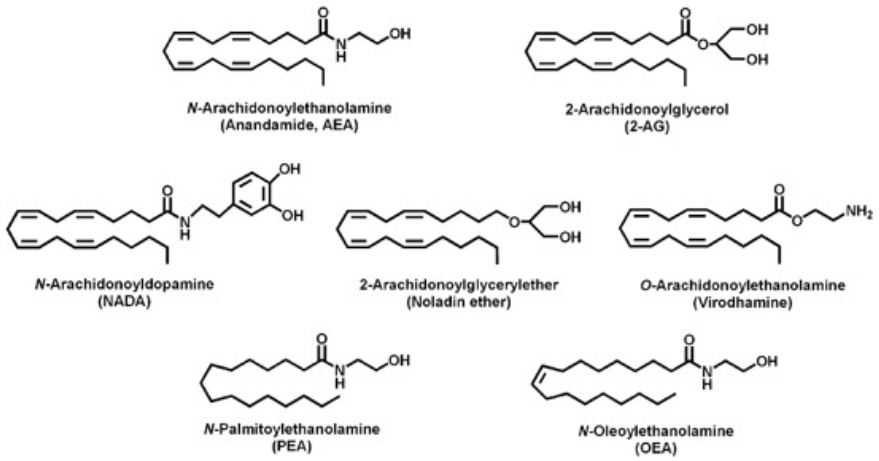


Figure 15. Endocannabinoids structure

3.1.3 Synthesis and degradation of endocannabinoids

Anandamide is principally synthetized as a consequence of the hydrolysis of its phospholipid precursor N-arachidonoyl-phosphatidylethanolamine by the action of a specific phospholipase D (Di Marzo *et al*, 2005). 2-AG results from the hydrolysis of diacylglycerol by a diacylglycerol lipase (DAGL) (Di Marzo et al, 2005). Endocannabinoids are removed from the synaptic cleft and taken up by the cell following their release and upon activation of their molecular targets. After their reuptake in the cell, endocannabinoids are degraded by the effect of specific hydrolases. Anandamide is hydrolyzed to arachidonic acid and ethanolamine by fatty acid amide hydrolase (FAAH) (Cravatt *et al*, 1996), while 2-AG is mainly hydrolyzed by the monoacylglycerol lipase (MAGL) to arachidonic acid and glycerol (Dinh *et al*, 2002a, 2002b). Both are intracellular enzymes, but FAAH is primarily expressed in the soma and dendrites of postsynaptic neurons (Egertová *et al*, 2003), whereas MAGL is expressed in presynaptic terminals (Gulyas *et al*, 2004) (Figure 16).

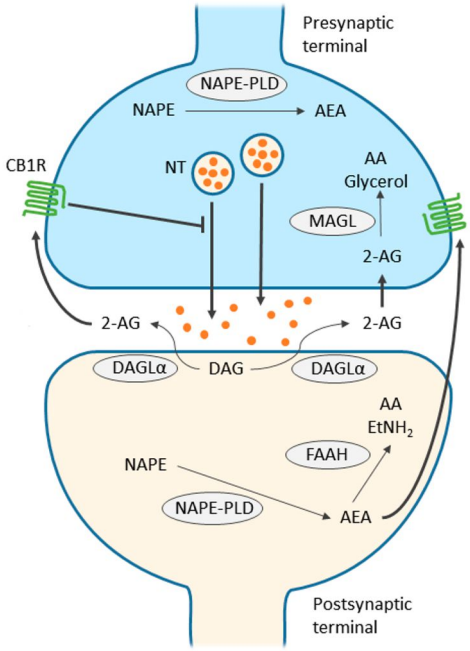


Figure 16. Simplified scheme representing the synthesis and degradation of two main endocannabinoids. 2-arachidonolglycerol (2-AG) is biosynthesized from diacylglycerol (DAG) by diacylglycerol lipase-α (DAGLα), and anandamide (AEA) is synthesized from N-acyl-phosphatidylethanolamine (NAPE) by NAPE-specific phospholipase D (NAPE-PLD). As lipids, endocannabinoids, mainly 2-AG, readily cross the membrane and travel in a retrograde fashion to activate CB1Rs located in the presynaptic terminals. 2-AG in the synaptic cleft is taken up into the presynaptic terminals, via a yet unclear mechanism, and degraded to arachidonic acid (AA) and glycerol by monoacylglycerol lipase (MAGL). On the other hand, AEA, synthesized in postsynaptic terminal, Although endocannabinoid retrograde signaling is mainly mediated by 2-AG, AEA can activate presynaptic CB1Rs as well. Fatty acid amide hydrolase (FAAH) is primarily found in postsynaptic terminals and is responsible for degrading AEA to AA and ethanolamine ($EtNH_2$). Although NAPE-PLD is expressed in presynaptic terminals in several brain regions, it is not clear yet whether AEA is responsible for anterograde signaling in the endocannabinoid system. Alternative routes exist for the metabolism of endocannabinoids, depending on the brain region and physiological conditions (Zhou and kumar, 2018)

3.1.4 Cannabinoid receptor signaling

Stimulation of cannabinoid receptors produces a wide variety of effects through the activation of diverse signal transduction pathways (Bosier *et al*, 2010). Both CB1R and CB2R exert their reported biological effects by activating heterotrimeric Gi/o type G proteins. Through coupling to Gi/o, CB1R activation mediates the inhibition of adenylyl cyclase, with subsequent reduction in cAMP levels and protein kinase A activity (Howlett, 2005). In addition, CB1R coupling to Gβγi/o can stimulate the phosphorylation and activation of various members of the MAPK family, including ERK1/2, p38 and c-Jun N-terminal kinase (Bouaboula *et al*, 1995; Howlett, 2005). CB1R also modulates the activity of several ion channels, including the activation of the inward-rectifying K^+ channels, and the inhibition of N-type and P/Q-type Ca2+ channels, triggering the repolarization of the plasmatic membrane and impeding neurotransmitter release (Bosier *et al*, 2010). The lipid composition of the cellular membrane in the surroundings of the receptor, and particularly cholesterol content, seems to be critical for the regulation of signal transduction pathways triggered upon CB1R stimulation (Maccarrone,

2010) (Figure 17).

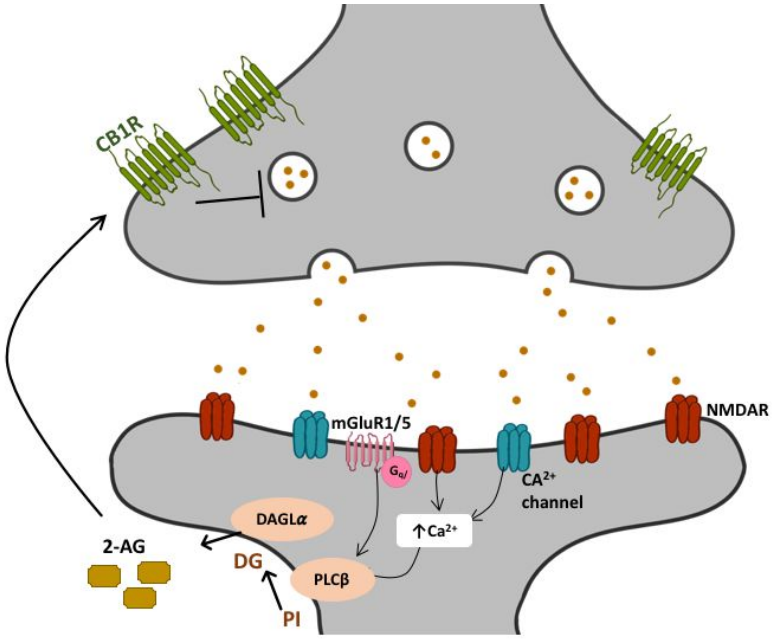


Figure 17. Endocannabinoid-mediated retrograde inhibition of neurotransmitter release in glutamatergic transmission. Endocannabinoids are synthesized and released on demand in the postsynaptic terminals in an activity-dependent manner after postsynaptic Ca2+ elevations. Once released from the postsynaptic neurons, endocannabinoids act retrogradely on the CB1R located at the presynaptic terminals to produce transient decrease of neurotransmitter release. N-Methyl-D-aspartate receptor (NMDAR); phosphatidylinositol (PI); diacylglycerol (DG); 1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta (PLCβ) (Adapted from Kano, 2014).

3.1.5 Exogenous cannabinoids: focus on cannabidiol

Over 100 compounds, called phytocannabinoids, have been isolated from *Cannabis Sativa* extracts. Among them, the two major active constituents are Δ^9-tetrahydrocannabinol (THC) (Gaoni and Mechoulam, 1964) and cannabidiol (CBD) (Mechoulam and Shvo, 1963). THC is the main psychoactive component of the plant and mediates the rewarding properties of cannabis (Huestis *et al*, 2001). In contrast, CBD does not have reinforcing effects nor abuse potential (Katsidoni *et al*, 2013; Parker *et al*, 2004).

Although CBD was isolated more than 50 years ago, the interest in this phytocannabinoid experimented a dramatic increase in recent years. Several studies have positioned CBD as a potential therapeutic strategy for the treatment of several neuropsychiatric disorders (Campos *et al*, 2012, 2016; Fernández-Ruiz *et al*, 2013). In this sense, CBD has been proposed to exert neuroprotective effects in neurodegenerative conditions, including Alzheimer's (Martín-Moreno *et al*, 2011) and Parkinson's disease (Garcia-Arencibia et al., 2007), epilepsy and multiple sclerosis (Leo *et al*, 2016). Furthermore, CBD has been described as an anti-inflammatory (Ben-Shabat *et al*, 2006; Esposito *et al*, 2011; Mecha *et al*, 2013; Napimoga *et al*, 2009), and immunomodulatory (Kozela *et al*, 2010; Malfait *et al*, 2000) compound. Indeed, CBD has been shown to decrease the production of inflammatory cytokines, the activation of microglial cells (Alvarez *et al*, 2008; Kozela *et al*, 2011; Napimoga *et al*, 2009) and to improve cell proliferation and neurogenesis in the HPC (Mori *et al*, 2016; Schiavon *et al*, 2016). In agreement, a recent investigation reported that CBD protects against memory impairments and hippocampal cell loss in a mouse model of artery occlusion (Schiavon *et al*, 2014)

The pharmacological actions of CBD in the modulation of the addictive properties of different drugs of abuse have also been studied. In this sense, most of the effects of CBD have been investigated in the context of opiate drugs. In morphine-dependent rats, CBD reduces withdrawal signs (Bhargava, 1976; Chesher and Jackson, 1985; Hine *et al*, 1975a, 1975b). CBD does not reduce heroin self-administration or heroin-primed reinstatement of drug seeking in an animal model of relapse, however CBD has been reported to be effective in diminishing heroin cue-primed drug seeking and normalizes heroin-induced impairments in accumbal AMPA and CB1R levels (Ren *et al*, 2009). In addition to these preclinical studies, a human study demonstrated that CBD does not alter the subjective effects of fentanyl, but attenuates heroin cue-induced drug craving and anxiety (Hurd *et al*, 2015). These results indicate that CBD effectively reduces opioid-paired cue reactivity but has little effect on the acute reinforcing properties of opioids. However, additional work shows that the reward-facilitating effects of morphine on intracranial self-stimulation are decreased by systemic CBD administration (Katsidoni *et al*, 2013). Interestingly, CBD could be a potential therapeutic agent to treat nicotine dependence. Preliminary findings from a pilot study in humans revealed that CBD reduced cigarette smoking significantly compared to placebo in smokers trying to quit (Morgan *et al*, 2013). In addition, CBD treatment ameliorated the salience and pleasantness of cigarette cues after overnight abstinence in dependent smokers (Hindocha *et al*, 2018).

To date, the mechanisms through which CBD exerts its effects remain to be elucidated. Multiple targets have been proposed to mediate the pharmacological effects of CBD. Early studies indicated that CBD was a CB1R inverse agonist similar to rimonabant (Pertwee, 2008; Thomas *et al*, 2009). However, it has been demonstrated that CBD has negligible activity

on CB1R and CB2R (Mechoulam *et al*, 2002), but may interfere with the endocannabinoid system and directly or indirectly stimulate FAHH (Bisogno *et al*, 2001; De Petrocellis *et al*, 2011), 5-hydroxytryptamine 1A (5-HT1A) receptors (Gomes *et al*, 2011; Zanelati *et al*, 2010), adenosine receptors, TRPV1, and nuclear receptors of the peroxisome proliferator-activated receptor family (Esposito *et al*, 2011; Fernández-Ruiz *et al*, 2013). Although these results have shed a light on how CBD acts to modulate behavior, further research is needed to fully elucidate CBD's mechanism of action.

3.2 Main physiological functions of the endocannabinoid system

The extensive distribution of the endocannabinoid system in the CNS and numerous peripheral tissues correlates with its role as a modulator of multiple physiological functions. The presence of CB1R in the basal ganglia and cerebellum has been related to fine control of motor coordination and cerebellar learning performance (Fernández-Ruiz and Gonzáles, 2005; Kishimoto and Kano, 2006). The endocannabinoid system also controls nociception under diverse sorts of acute and chronic pain conditions (Maldonado *et al*, 2016; La Porta *et al*, 2014). Notably, this neuromodulatory system ensures an appropriate reaction to stressful events, regulating anxiety and fear responses (Lutz *et al*, 2015). It has also been associated with the modulation of emotions and motivation (Mechoulam and Parker, 2013), reward processing and addiction (Maldonado *et al*, 2006; Parsons and Hurd, 2015). CB1R expression in the HPC has been widely investigated because of the deleterious effects of cannabis on learning and memory (Kano *et al*, 2009; Puighermanal *et al*, 2009). In this regard, agonism of CB1R has been shown to alter hippocampal oscillatory activity by depressing synaptic activity and

plasticity at CA3-CA1 synapses (Hampson and Deadwyler, 2000). In addition, this neuromodulatory system regulates synapse formation and remodeling (Harkany *et al*, 2008; Kano *et al*, 2009), and diverse processes involved in neuronal development, including neuronal survival, differentiation, proliferation and migration (Galve-Roperh *et al*, 2013; Harkany *et al*, 2008; Rueda *et al*, 2002). Acting at the peripheral level, the endocannabinoid system modulates the immune and cardiovascular systems, controls gastrointestinal motility and metabolism, and regulates the function of the liver, the adipose tissue and the reproductive system, among others (Grotenhermen and Müller-Vahl, 2003; Watkins and Kim, 2014).

3.3 Involvement of the endocannabinoid system in nicotine addiction

Several studies support the crucial role of the endocannabinoid system in nicotine addiction. Animals pretreatment with the selective CB1R antagonist rimonabant (SR141716A) did not exhibit nicotine-enhanced dopamine extracellular levels in the NAc (Cheer *et al*, 2007; Cohen *et al*, 2002) and the BNST (Cheer *et al*, 2007). Consistent with this, nicotine-induced CPP was absent in rats and mice pretreated with rimonabant (Le Foll and Goldberg, 2004; Merritt *et al*, 2008), and in mice lacking CB1R (Castañé *et al*, 2002; Merritt *et al*, 2008). Furthermore, CB1R antagonists reduce nicotine self-administration in rats (Cohen *et al*, 2002; Shoaib, 2008), suggesting that signaling through CB1R is necessary for the rewarding and reinforcing properties of nicotine. The endocannabinoid system also plays a role in the reinstatement of nicotine-seeking. Rimonabant attenuated cue- (Cohen *et al*, 2005; De Vries and

Schoffelmeer, 2005), and context-induced (Diergaarde *et al*, 2008) nicotine-seeking in rats.

Besides CB1R, the role of CB2R in the rewarding, reinforcing and physical effects of nicotine has also been addressed (Gamaleddin *et al*, 2012; Navarrete *et al*, 2013). Interestingly, pharmacological and genetical blockade of CB2R abolished nicotine-induced CPP and reduced nicotine self-administered. In addition, somatic signs of nicotine withdrawal were significantly decreased in CB2KO mice. In contrast, another study reported that genetic deletion of CB2 receptors does not alter the expression of anxiogenic-like withdrawal responses and somatic withdrawal signs (Ignatowska-Jankowska *et al*, 2013). These contradictory results could be due to differences in the genetic background (CD-1 vs C57BL/6), which would emphasize the significant influence of genetics on nicotine withdrawal behaviors. Therefore, it is not clear the role of CB2R in the rewarding, reinforcing, and motivational effects of nicotine.

Given the role of CB1R in nicotine reinforcement and nicotine-seeking behavior, CB1R was pictured as a possible target to treat nicotine addiction. Additionally, rimonabant was proposed as potential drug to protect successful quitters from significant post-cessation weight gain, since rimonabant was initially developed as a potential treatment for obesity (Cahill and Mh, 2012). Rimonabant at the dose of 20 mg was licensed as an aid for weight control in the European Union in June 2006, but was not licensed for smoking cessation (Acomplia, 2006). Phase III trials were conducted to test the use of rimonabant for long-term smoking cessation with the avoidance of significant weight gain. The STRATUS program (STudies with Rimonabant And Tobacco USe), engaged two cessation trials (STRATUS-EU and STRATUS-US), and one relapse prevention trial (STRATUS-WW) (Cinciripini *et al*, 2006)

Results from STRATUS-EU and STRATUS-US indicated that 20 mg rimonabant treatment increased the chances of quitting approximately by 1.5-fold (Cahill and Ussher, 2011; Le Foll and Goldberg, 2009; Steinberg and Foulds, 2007). The STRATUS-WW trial reported that who had quit on the 20 mg regimen were more likely to remain abstinent. In addition, it was reported that adding a nicotine patch to rimonabant was well tolerated and increased smoking cessation rates over rimonabant alone (Rigotti *et al*, 2009). Concerns on the safety of rimonabant were raised by reports of 1-year treatment in overweight and obese patients. Rimonabant significantly increased the risk of suicide attempts or ideation (Moreira and Crippa, 2009). In addition, it was revealed that many patients abandoned rimonabant treatment due to anxiety and depression (Moreira and Crippa, 2009). Due to these psychiatric side effects, the European Medicines Agency (EMEA) recommended the suspension of the marketing authorization for rimonabant on October 23th, 2008.

Contradictory results have been found regarding the participation of the endocannabinoid system in nicotine withdrawal. Somatic signs of withdrawal remained unaltered in KO mice for CB1R (Castañé *et al*, 2002; Merritt *et al*, 2008). However, an amelioration of physical signs has been observed in mice pretreated with rimonabant (Merritt *et al*, 2008). Few studies have assessed the role of endocannabinoids in withdrawal signs. Mice treated with a high dose of URB597, a selective FAAH inhibitor, significantly enhanced spontaneous nicotine somatic withdrawal signs (Merritt *et al*. 2008). In contrast, URB597 did not modify the withdrawal somatic signs in rats, although it was effective in reducing withdrawal-induced anxiety (Cippitelli *et al*. 2011). The discrepancy between these two studies could suggest possible species differences in the regulation of nicotine withdrawal mechanisms by FAAH inhibition between rats and

mice (Muldoon *et al*, 2013). Furthermore, inhibition of MAGL by administration of JZL184, dose-dependently reduced somatic and aversive withdrawal signs, an effect blocked by rimonabant, indicating a CB1 receptor-dependent mechanism (Muldoon *et al*, 2015).

Although the role of the endocannabinoid system in the somatic and affective symptoms of withdrawal has relatively been studied, the involvement of this system in other aspects of withdrawal, such as cognitive symptoms, have not been explored yet and constitutes one of the main objectives of the present thesis.

4. The orexin/hypocretin system

Two decades ago, the orexin/hypocretin system was discovered simultaneously by two independent groups through different scientific approaches in the US (de Lecea *et al*, 1998) and Japan (Sakurai *et al*, 1998). De Lecea's group identified a hypothalamic mRNA species that encoded for a polypeptide precursor that after cleavage form two peptide transmitters (de Lecea *et al*, 1998). One of these peptides was shown to be strongly neuroexcitatory in neuronal cultures. These peptides were named hypocretin-1 and hypocretin-2 ("hypo" for hypothalamus, "cretin" for the sequence resemblance to the hormone secretin). At the same time, the research group of Sakurai identified two peptide transmitters that activated two orphan receptors (Sakurai *et al*, 1998). Molecular cloning studies showed that these peptides derive from a common precursor peptide, and were able to stimulate food intake in rats upon intracerebroventricular infusion (Sakurai *et al*, 1998). The peptides were termed orexin-A and orexin-B, from *orexis*, the Greek word for appetite) and the orphan receptors became the OX1R and OX2R orexin receptors. Both sets of names are still in use, with hypocretin-1 being equivalent to orexin A and hypocretin-2 to Orexin-B.

4.1 Overview of the orexin system

4.1.1 Orexin peptides

Orexin-A and orexin-B are 33- and 28-amino acid peptides, respectively, result from the proteolysis of a common precursor, prepro-orexin (131 amino acids) (de Lecea *et al*, 1998; Sakurai *et al*, 1998). The gene encoding prepro-orexin is located on chromosome 17 in humans (Sakurai *et al*, 1999). The sequence of orexin-A is identical in rats, mice, pigs, dogs, and humans, whereas orexin-B differs only in one or two amino acids between these species (Wong *et al*, 2011). The strong preservation of the orexin system across vertebrate evolution reveals its functional relevance. Diverse post-translational modifications take place in order to obtain the mature functional orexin peptides. Orexin-A and orexin-B share 46% of their sequence (Sakurai *et al*, 1998), and their overall 3D structures are quite similar, which explains their ability to bind the same receptors (Kim *et al*, 2004; Lee *et al*, 1999). However, orexin A appears to be more stable and lipophilic than orexin B. In the CNS, orexin peptides act as neuromodulators. Hence, they are stored in secretory vesicles, transferred through the axon to the neuronal terminals and released in a Ca^{2-}-dependent manner (de Lecea *et al*, 1998).

4.1.2 Orexin receptors

Two GPCR receptors respond to orexin stimulation: OXR1 (425 amino acids) and OXR2 (444 amino acids). These receptors are also known as hypocretin receptor 1 (Hcrtr-1) and hypocretin receptor 2 (Hcrtr-2), respectively (de Lecea *et al*, 1998; Sakurai *et al*, 1998). Orexin receptors, similar to orexin peptides, are highly conserved across mammalian species and there is an overall 64% identity between them in humans (Sakurai *et al*, 1998). Studies on heterologous expression systems have shown that orexin receptors differ in their ligand binding affinities. Thus, OX2R presents a rather equal affinity for both orexin peptides, while OX1R shows a 10- to 100-fold higher affinity for orexin-A than orexin-B (Ammoun *et al*, 2003; Sakurai *et al*, 1998) (Figure 18).

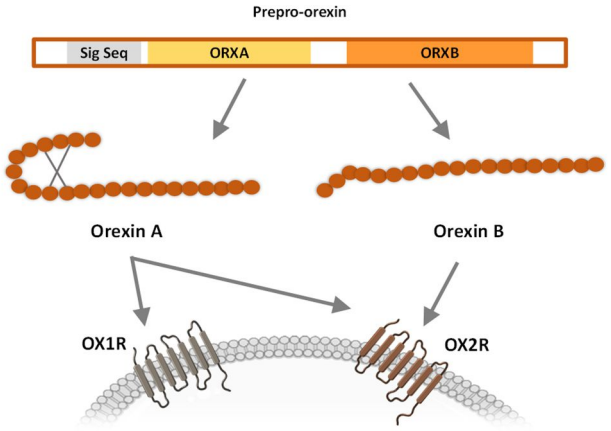


Figure 18. Orexin peptides and their receptors. Orexin-A and orexin-B are cleaved from their precursor prepro-orexin (Adapted from Sakurai 2014).

4.1.3 Orexin receptors signaling

The signaling pathways activated by orexin receptor stimulation have been extensively investigated in transfected heterologous cell systems. However, these studies provide limited information about the particular signaling pathways taking place in native receptor-expressing neurons, from which available data are still limited (Kukkonen, 2017). The main signal transduction mechanism accepted for orexins indicates that OX1R couples to Gq proteins and that OX2R couples to Gq and Gi/o family members. In neurons, the most frequent response after agonist binding to orexin receptors is an enhancement of intracellular Ca2+ concentrations, explaining the commonly reported neuroexcitatory nature of orexin peptides on the brain (Eriksson *et al*, 2001; van den Pol *et al*, 1998). Upon ligand binding, orexin receptor promotes the activation of Gq proteins, which induces the stimulation of phospholipase C (PLC) and subsequent production of the second messengers diacylglycerol and inositol trisphosphate (IP3) from membrane phospholipids. In turn, this triggers the activation of protein kinase C (PKC), which phosphorylates and modulates effector ion channels leading to Ca2+ entrance (Kohlmeier *et al*, 2004; Uramura *et al*, 2001; Xia *et al*, 2009), as well as further IP3-mediated entry via store-operated Ca2+ channels (Kukkonen and Akerman, 2001; Larsson *et al*, 2005). Therefore, activation of orexin receptors commonly translates into an increase in action potential frequency (Figure 19).

As previously mentioned, the activation of PLC upon OX1R stimulation leads to the production of diacylglycerol and concomitant activation of PKC. Among other effectors, PKC phosphorylates extracellular signal-regulated kinase (ERK) and p38 kinase, both in recombinant cells (Ammoun *et al*, 2006; Tang *et al*, 2008) and neurons (Gorojankina *et al*,

2007; Selbach *et al*, 2010). These kinases are two well-known members of the mitogen-activated protein kinase (MAPK) pathway, which is involved in several cellular processes, including synaptic plasticity, cell survival and proliferation (Selbach *et al*, 2010; Thornton and Rincon, 2009).

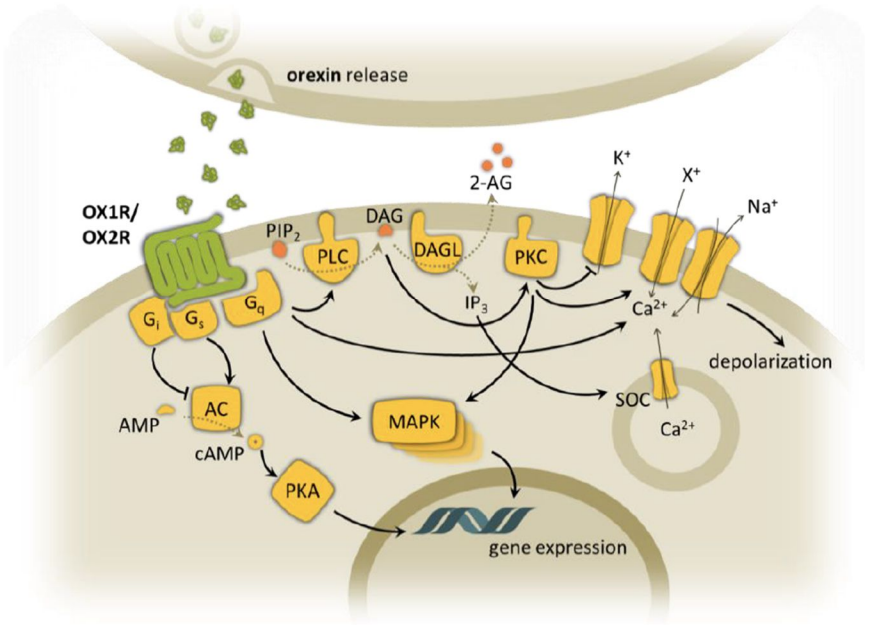


Figure 19. Main cellular signalling pathways activated upon orexin receptor stimulation. Orexin receptor stimulation is associated with Gq-dependent activation of the PLC/PKC pathway and diverse MAPK cascades, as well as membrane depolarization through modulation of cation channels. Gs and Gi protein stimulation has also been observed, leading to increase or decrease of AC activity, respectively. 2-AG, 2-arachidonoylglycerol; AC, adenylyl cyclase; DAGL, diacylglycerol lipase; IP3, inositol trisphosphate; MAPK, diverse members of the mitogen-activated protein kinase cascade; PIP2, phosphatidylinositol bisphosphate; PKA and PKC, protein kinases A and C; PLC, phospholipase C; SOC, store-operated Ca2+ channels.

4.2 Orexin main physiological functions in the CNS

Orexin-expressing neurons represent a small population exclusively located in the lateral hypothalamus (LH), the perifornical area (PFA), and the dorsomedial hypothalamus (DMH). Although limited in number and localization, they have extensive projections throughout the brain (Peyron *et al*, 1998). The widespread projections of the orexin system reflect the variety of physiological functions of orexin peptides.

Soon after the identification of orexins, two groups demonstrated an association between orexin deficiency and narcolepsy (Chemelli *et al*, 1999; Lin *et al*, 1999; Peyron *et al*, 2000). Thus, regulation of sleep/wakefulness is one of the best-understood roles of the orexin system. Indeed, suvorexant, a dual OXR antagonist, has been approved by the FDA for the treatment of insomnia (Herring *et al*, 2014; Michelson *et al*, 2014).

Orexins were also initially reported to be regulators of feeding behavior based on their capacity to elicit food intake when centrally administered to rats (Sakurai *et al*, 1998). Thus, pharmacological and genetical blockade of OX1R attenuates food consumption (Hara *et al*, 2001; Haynes *et al*, 2000; Yamada *et al*, 2000).

The role of the orexin system in stress responses has been well-established on the basis of three kinds of evidences. First, orexinergic neurons mainly located in the PFA/DMH are activated by different stressors, including immobilization, footshock, cold exposure, and food deprivation (Berridge *et al*, 2010; Johnson *et al*, 2012). Second, some stress-induced responses, such as stress-induced analgesia (Xie *et al*, 2008), footshock-induced reinstatement of cocaine seeking (Boutrel *et al*, 2005) as well as stress-induced ACTH and cardiovascular responses (Chang *et al*, 2007; Kayaba *et al*, 2003; Samson and Taylor, 2001) induce activation of the orexin system.

Finally, direct modulation of orexin system by the intracerebroventricular administration of orexin-A induces anxiety-like effects in several behavioral models of anxiety (Suzuki *et al*, 2005). The existence of reciprocal interactions between orexin and CRF neurons (Winsky-Sommerer, 2004) suggests that the orexin system is an important component of the pathways contributing to the physiological CRF-mediated behaviors that occur in response to stressful situations (Giardino and de Lecea, 2014). Dysregulation of stress responses could lead to the development of different anxiety disorders, which might be influenced by the activity of the orexin system (Flores *et al*, 2015; Johnson *et al*, 2010).

Increasing evidence suggests the involvement of orexins in higher brain functions. However, the possible mechanisms of action by which orexin could promote learning and memory, are still poorly understood. Alterations in orexin regulation of hippocampal cholinergic activity have been linked to age-related dysfunctions in arousal, learning, and memory (Stanley and Fadel, 2012). In addition, a pilot human study showed that during a hippocampal-dependent social task, participants exhibited an increase in orexin-A (Blouin *et al*, 2013). Other studies also suggest the contribution of the orexin system to spatial and nonsocial learning and memory. In this regard, intracerebroventricular injection of orexin-A improved memory in both an active and passive avoidance paradigm (Jaeger *et al*, 2002). Furthermore, antagonism of OX1R in the CA1 or DG has been shown to impaired acquisition, consolidation and retrieval in the Morris water maze (Akbari *et al*, 2007; Akbari *et al*, 2006) and passive avoidance tasks (Akbari *et al*, 2008). However, the exact role of orexin in learning and memory is still unclear.

Multiple evidence supports a role for orexins in the reinforcing properties of different drugs of abuse (Plaza-Zabala *et al*, 2012b). The dopamine

neurons in the VTA might be a crucial site of action for orexins to mediate these effects (Aston-Jones *et al*, 2009). Accordingly, direct injections of orexin-A in the VTA increase dopamine levels in the NAc (España *et al*, 2010, 2011; Narita *et al*, 2006). Although most of the research on the involvement of orexins in drug addiction has focused on elucidating the function of OX1R (Khoo and Brown, 2014; Plaza-Zabala *et al*, 2012b), recent studies point to a role for OX2R in reward regulation. Thus, antagonism of OX2R reduces heroin (Schmeichel *et al*, 2015) and ethanol self-administration (Brown *et al*, 2013), as well as the cue-induced reinstatement of nicotine-seeking behavior (Uslaner *et al*, 2014).

4.3 Involvement of the orexin system in nicotine addiction

Several reports suggest that orexin transmission may regulate the addictive properties of nicotine. Orexins could participate in the attention-enhancing effects of nicotine (Lambe *et al*, 2005), which suggests that the orexin system may contribute to nicotine addiction through the modification of nicotine-cognitive effects. Indeed, orexins and nicotine have been found to activate the same thalamocortical synapses in PFC with the consequent improvement of attention in rats (Lambe *et al*, 2005). Interestingly, narcoleptic patients usually report cognitive deficits. A high percentage of this population smoke tobacco regularly, and it has been hypothesised that smoking could be a way of self-medication to improve cognition (Peřinová *et al*, 2016).

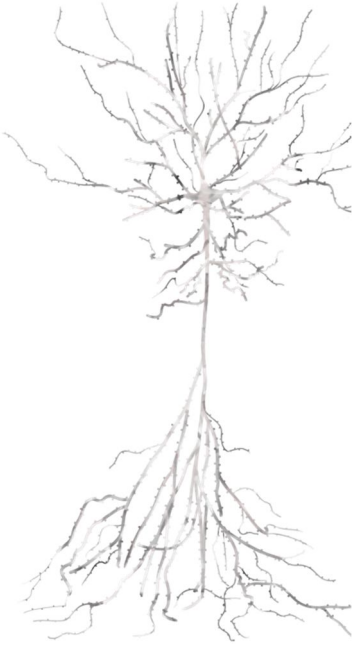
Nicotine has also been shown to alter anxiety-like behavior when given acutely by a mechanism involving orexin signaling (Plaza-Zabala *et al*, 2010). Thus, the anxiogenic-like effect produced by acute administration of nicotine was abolished by pretreatment with the OX1R antagonist SB334867 and in orexin KO mice. In addition, acute nicotine injection

increased c-Fos expression in orexin neurons (Pasumarthi *et al*, 2006). Furthermore, intravenous nicotine self-administration modified orexin-A mRNA levels in the arcuate nucleus and the rostral lateral areas of the hypothalamus in rats (LeSage *et al*, 2010). Similarly, non-contingent chronic nicotine administration regulated prepro-orexin and orexin receptor mRNA levels in the rat hypothalamus (Kane *et al*, 2000). At the behavioral level, pretreatment with the OX1R antagonist SB334867 or dual orexin antagonist almorexant decreased intravenous nicotine self-administration in rats under a fixed-ratio 5 schedule of reinforcement (Hollander *et al*, 2008; LeSage *et al*, 2010).

Moreover, the OX1R antagonist SB334867 decreased the number of nicotine rewards earned under a progressive-ratio schedule of reinforcement (Hollander *et al*, 2008), suggesting that orexins acting on OX1R regulate nicotine reinforcement and the motivation to seek the drug. Stroke-associated damage to the insular cortex in human smokers results in spontaneous cessation of the smoking habit and a low urge to smoke (Naqvi *et al*, 2007). Interestingly, intra-insular infusion of SB334867 decreased nicotine intake in rats in a self-administration paradigm (Hollander *et al*, 2008), suggesting that insular OX1R transmission is crucial for the reinforcing effects of nicotine. In addition, the orexin system seems to have an important role in the somatic manifestations of mecamylamine-precipitated nicotine withdrawal (Plaza-Zabala *et al*, 2012a). Thus, somatic nicotine withdrawal signs were attenuated in mice lacking the prepro-orexin gene or treated with the OX1R antagonist SB334867, but not with the OX2R antagonist TCSOX229 prior mecamylamine administration. Notably, direct administration of SB334867 into the paraventricular nucleus of the hypothalamus (PVN) decreased somatic signs of abstinence (Plaza-Zabala *et al*, 2012a),

suggesting that orexin signaling acting on OX1R in this brain area is critically involved in the modulation of nicotine withdrawal. Given the association of withdrawal signs and relapse (Killen and Fortmann, 1997; Rukstalis *et al*, 2005; Zhou *et al*, 2009), its not surprising that the orexin system might have a role in relapse to tobacco consumption. Preclinical research has shown that intracerebroventricular administration of orexin-A reinstates previously extinguished nicotine-seeking behavior, and this effect was abolished by the OX1R antagonist SB334867 (Plaza-Zabala *et al*, 2010). In addition, blockade of OX1R, but not blockade of OX2R, attenuated cue-induced reinstatement of nicotine-seeking (Plaza-Zabala *et al*, 2013), suggesting that reinstatement of nicotine seeking is mediated via OX1R.

These findings support the implication of orexins in nicotine addiction and highlight the importance of understanding the role of the orexin system in the addictive properties of nicotine.



OBJECTIVES

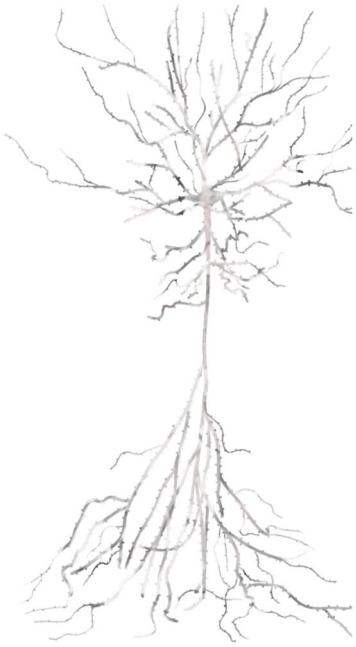
General objective

Extensive data suggest that the effects that nicotine exerts on memory are relevant for the initiation and maintenance of nicotine dependence. Therefore, the main goal of this thesis is to investigate the neurobiological mechanisms mediating the cognitive deficits associated with nicotine withdrawal and the pro-cognitive effect of acute nicotine administration.

Specific objectives

1. To set up a rodent model to evaluate the cognitive deficits that arise during nicotine withdrawal (Article 1).
2. To evaluate the participation of the endocannabinoid system in the cognitive deficits of nicotine withdrawal (Article 1).
3. To study the participation of inflammatory processes in the memory deficits of nicotine withdrawal (Article 2).
4. To evaluate the potential therapeutic use of anti-inflammatory compounds to prevent the cognitive deficits associated with nicotine abstinence (Article 2)

1. To evaluate the role of the orexin system in the improvement of memory induced by acute nicotine administration (Supplementary results).



RESULTS

ARTICLE 1

CB1 Cannabinoid Receptors Mediate Cognitive Deficits and Structural Plasticity Changes During Nicotine Withdrawal

Rocio Saravia, África Flores, Ainhoa Plaza-Zabala, Arnau Busquets-García, Antoni Pastor, Rafael de la Torre, Vincenzo Di Marzo, Giovanni Marsicano, Andrés Ozaita, Rafael Maldonado and Fernando Berrendero

Biol. Psychiatry **(2017) 81 (7): 625-634**

Saravia R, Flores Á, Plaza-Zabala A, Busquets-Garcia A, Pastor A, de la Torre R, et al. CB1 Cannabinoid Receptors Mediate Cognitive Deficits and Structural Plasticity Changes During Nicotine Withdrawal. Biol Psychiatry. 2017 Apr 1;81(7):625–34. DOI: 10.1016/j.biopsych.2016.07.007

ARTICLE 2

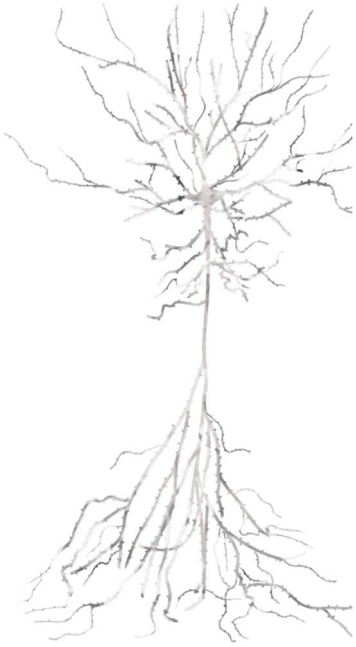
Anti-inflammatory agents for smoking cessation? Focus on cognitive deficits associated with nicotine withdrawal

Rocio Saravia, Marc Ten-Blanco, María T. Grande, Rafael Maldonado and Fernando Berrendero

Brain Behavior and Immunity (under second revision)

Saravia R, Ten-Blanco M, Grande MT, Maldonado R, Berrendero F. Anti-inflammatory agents for smoking cessation? Focus on cognitive deficits associated with nicotine withdrawal in male mice. Brain Behav Immun. 2019 Jan 1;75:228–39. DOI: 10.1016/j.bbi.2018.11.003

Results



DISCUSSION

Tobacco smoking represents the main leading cause of preventable death worldwide (World Health Organization, 2017). Among the 4000 components present in tobacco, nicotine is the main psychoactive compound and responsible for the addictive properties of tobacco. Several studies suggest that the effects that nicotine exerts on cognition are crucial to the development and maintenance of tobacco addiction (Hall *et al*, 2015a; Kutlu and Gould, 2016b). In this sense, acute or initial nicotine intake has a positive effect on cognition, which may contribute to the development of nicotine dependence (Gould and Leach, 2014). Conversely, nicotine abstinence impacts negatively on cognitive functioning (Ashare *et al*, 2014). Interestingly, these cognitive impairments associated with nicotine abstinence ameliorate after nicotine re-exposure, suggesting that relapse might occur to reverse the cognitive alterations (Hall *et al*, 2015a; Myers *et al*, 2008). During the last decade, the cognitive deficits associated with nicotine abstinence have gained attention as a core dependence phenotype and a predictor for relapse into tobacco consumption (Ashare *et al*, 2014; Loughead *et al*, 2015). Although the mechanisms that mediate nicotine withdrawal-induced cognitive impairments are not clear, different studies have suggested a role for CB1R (Evans *et al*, 2016), neuropeptide CART (Borkar *et al*, 2017), α4β2 nAChRs (Yildirim *et al*, 2015) and noradrenaline (Davis and Gould, 2007) in these deficits. The endocannabinoid system has been widely reported as a key modulator of learning and memory processes (Marsicano and Lutz, 2006; Mechoulam and Parker, 2013). This system to regulates the reinforcing properties of different drugs of abuse including nicotine (Cahill and Mh, 2012; Gamaleddin *et al*, 2015). However, the potential participation of the endocannabinoid system in the neurobiological mechanisms underlying

the cognitive deficits associated with nicotine withdrawal remains to be elucidated.

On the other hand, there are well-established links between inflammation and tobacco smoking (Corwin and Klein, 2003; Lo Sasso *et al*, 2016; Wannamethee *et al*, 2005). Thus, higher levels of inflammatory markers have been associated with the presence of some smoking-withdrawal signs (Corwin and Klein, 2003) and with an increased cardiovascular risk in current smokers (Wannamethee *et al*, 2005). In addition, growing evidence implicates neuroinflammatory processes in the development of cognitive impairments (McKim *et al*, 2016; Wohleb *et al*, 2016; Zhao *et al*, 2013). However, the possible role of neuroinflammation and the consequent use of anti-inflammatory agents to treat the cognitive deficits associated with nicotine abstinence has not been addressed yet.

This thesis has mainly focused on the neuronal substrates of the cognitive effects of nicotine. Using mainly pharmacological and genetical approaches, we investigated: (1) The possible participation of the endocannabinoid system in the cognitive deficits associated with nicotine abstinence, (2) the involvement of an inflammatory phenotype in nicotine-withdrawal cognitive impairments and (3) the implication of the orexin system in the pro-cognitive effect of acute nicotine administration.

The results described in this thesis indicate that CB1R located in a specific hippocampal neural population is associated with the cognitive deficits of nicotine withdrawal. Moreover, our results suggest that anti-inflammatory agents could be a new therapeutical tool to modulate the cognitive alterations observed during abstinence. In addition, we revealed results that orexin signaling could be mediating the memory-enhancing effect of acute nicotine administration.

Role of the endocannabinoid system in the memory deficits associated with nicotine abstinence

Despite the well-known harmful consequences of tobacco smoking, 80% of individuals trying to quit relapse within the first month of abstinence (Prochaska and Benowitz, 2016). This high relapse rate reflects the limited efficacy of current therapies and the complex nature of nicotine addiction. During a quit attempt, smokers experiment a range of undesirable signs of nicotine abstinence, which seem to play a critical role in relapsing into tobacco smoking (Ashare *et al*, 2014; Hughes, 2007a; Jackson *et al*, 2015). These signs can be classified as somatic, affective and cognitive, and have been associated with relapse into tobacco consumption (Ashare *et al*, 2014; Garcia-Rivas and Deroche-Gamonet, 2018; Hughes, 2007a). Nowadays, these cognitive deficits can be recapitulated in different animal models. In rodents, withdrawal from chronic nicotine administration has been shown to produce cognitive impairments in hippocampal-dependent tasks (Hall *et al*, 2015a). In this study, we evaluated the role of the endocannabinoid system in the memory deficits associated with nicotine withdrawal. To assess these memory deficits, we used the novel object recognition test and precipitate withdrawal with an injection of mecamylamine during the training phase of the task. This allowed to test the effect of nicotine withdrawal in the consolidation of memory. Precipitation of withdrawal in nicotine-dependent mice resulted in a decrease in the discrimination index in the object recognition test. The reduced discrimination index was only observed in nicotine withdrawn mice and was not due to alteration in locomotor activity since both experimental groups (saline and nicotine) exhibited similar exploration times. In agreement with our results, precipitation of nicotine withdrawal has been described to alter memory performance in other types of

hippocampal-dependent tasks, such as the spatial object-recognition and contextual fear conditioning during spontaneous and precipitated nicotine withdrawal (Gould *et al*, 2012; Kenney *et al*, 2011; Raybuck and Gould, 2009; Yildirim *et al*, 2015). Consistent with other studies, chronic treatment with nicotine did not alter the discrimination index, indicating that the deficit in memory performance was only due to precipitation of nicotine withdrawal (Article 1).

Signaling through CB1R has been widely reported to regulate learning and memory processes (Kruk-Slomka *et al*, 2017). Several studies have demonstrated that activation of CB1R by exogenous cannabinoids significantly alters learning and memory in various animal models, including the object recognition task (Lupica *et al*, 2017; Marsicano and Lafenêtre, 2009; Schneider *et al*, 2008). In our study, pharmacological blockade of CB1R with rimonabant prior precipitation of nicotine withdrawal prevented the memory impairment observed during nicotine abstinence (Article 1). A similar result was obtained in the KO mice for CB1R, suggesting that CB1R has a crucial role in the development of the cognitive deficits associated with nicotine withdrawal (Article 1). Expression of CB1R differs among brain areas and different cell types, within the brain. In the HPC, CB1R is mainly located in GABAergic interneurons (Kawamura *et al*, 2006). CB1R are also present in glutamatergic terminals, but with a lower density than in GABAergic terminals (Steindel *et al*, 2013). Growing evidence indicates that cellular and molecular effects could be differentially mediated by CB1R depending on their cell-type localization (i.e. glutamatergic or GABAergic neurons) (Busquets-Garcia *et al*, 2015). In this regard, THC has been shown to act as a full agonist of CB1R located in GABAergic terminals, whereas it acts as a partial agonist of the glutamatergic ones (Laaris *et al*, 2010). The

differential implications for hippocampal functioning that could result from acting on CB1R at glutamatergic or GABAergic terminals prompted us to examine which CB1R-expressing neurons were involved in the memory impairment associated with nicotine withdrawal. Our results show that CB1R expressed in GABAergic neurons mediate the memory impairment associated with nicotine abstinence (Article 1). Consistent with this, targeted deletion of CB1R from GABAergic neurons has been shown to prevent the disruption of hippocampal-dependent behavior by THC, whereas deletion of this receptor from glutamatergic neurons was ineffective in abolishing other pharmacological effects of THC, such as memory impairment (Puighermanal *et al*, 2009). In agreement, mice displaying a reduced GABA transmission exhibited memory deficits when evaluated in the object recognition task (Zhu and Lovinger, 2007). In addition, CB1R on GABAergic neurons seems to play a crucial role in protecting against age-related cognitive decline (Albayram *et al*, 2011). These data, together with our results suggest that CB1R expressed in GABAergic neurons is crucial for proper learning and memory processes.

In physiological conditions, activation of CB1R is always triggered by the previous release of endocannabinoids. We found that 2-AG was the endocannabinoid responsible for the cognitive deficits associated with nicotine withdrawal (Article 1). A significant increase in 2-AG, but not AEA, was observed 10 minutes after precipitation of nicotine withdrawal. 2-AG tone depends on the balance between its synthesis by DAGLs, and its degradation by the hydrolytic enzyme MAGL (Di Marzo *et al*, 2005). Our immunoblot studies showed that the increase of 2-AG was the result of a decrease in its degradation since reduced protein levels of MAGL were observed in nicotine withdrawn mice (Article 1). Interestingly, reduction of 2-AG synthesis triggered by the inhibitor of DAGL O7460 restored

memory performance in nicotine abstinent mice. Conversely, inhibition of 2-AG degradation by administration of the inhibitor of MAGL ZL184 did not modify the memory impairment associated with nicotine withdrawal. Indeed, JZL184 administration at the highest dose (20 mg/kg) induced a cognitive impairment by itself (Article 1). In agreement, other studies have reported that blockade of 2-AG degradation impairs hippocampal-dependent learning and memory in rodents (Griebel *et al*, 2015; Wise *et al*, 2012). Our results suggest that precipitation of nicotine withdrawal selectively boosts 2-AG signaling, that through activation of hippocampal CB1R in GABAergic neurons, contribute to impair memory performance.

As previously mentioned, CB1R is more densely expressed in GABAergic than in glutamatergic neurons in the HPC (Kawamura *et al*, 2006; Steindel *et al*, 2013). Thus, activation of CB1R primary suppresses GABA release, resulting in an increase of excitatory signaling (Katona and Freund, 2012). Following THC exposure, this excitatory input seems to modulate memory by promoting mTOR signaling (Graber *et al*, 2013; Puighermanal *et al*, 2009). In the brain, the mTOR pathway regulates many physiological functions, including neurogenesis, synaptic plasticity, information storage and cognition (Bockaert and Marin, 2015). Given its broad implication in many brain functions, it is not surprising that proteins within the mTOR signaling cascade are implicated in diseases associated with cognitive deficits, such as Down syndrome and Fragile X syndrome (Bockaert and Marin, 2015; Costa-Mattioli and Monteggia, 2013). In this sense, several reports indicate that the mTOR pathway regulates synaptic plasticity and memory by controlling protein synthesis (Bockaert and Marin, 2015; Hoeffer and Klann, 2010). Interestingly, administration of the mTOR inhibitor temsirolimus prevented the memory impairment observed during nicotine abstinence (Article 1). Likewise, treatment with the protein

synthesis inhibitor anisomycin also blocked the nicotine withdrawal-cognitive deficits (Article 1). Thus, mTOR activation and excessive protein synthesis might underlie the behavioral deficit induced by nicotine withdrawal. In this regard, animal studies have revealed an excessive increase in protein synthesis in other conditions characterized by memory impairment such as the fragile X syndrome (Bolduc *et al*, 2008) or the administration of amnesic doses of THC (Puighermanal *et al*, 2009).

In abstinent smokers, somatic, affective and cognitive withdrawal signs, tend to dissipate with time (Hall *et al*, 2015a; Hughes, 2007a). In our model, we observed that memory impairment was still present 4 days after precipitation of withdrawal, with mice recovering from these cognitive impairments by the 8th day of abstinence (Article 1). Consisting with our results, a previous study showed a similar duration of these memory impairments in the hippocampal-dependent contextual fear conditioning test in C57BL/6J mice (Gould *et al*, 2012). The fact that withdrawal memory deficits had a duration of days suggests the existence of changes in synaptic plasticity during nicotine withdrawal. It is widely accepted that memories are stored as changes in the "strength" of synaptic connections between neurons (Bosch and Hayashi, 2012). Synaptic spines exhibit a wide range of size and shape, with several studies indicating a positive correlation between the spine head volume, the postsynaptic density area, the number of AMPA-type glutamate receptors, and synaptic "strength" (Bannerman *et al*, 2014; Bosch and Hayashi, 2012; Matsuzaki *et al*, 2001). Thus, it seems that spine structure is tightly coupled to synaptic functions. Interestingly, morphological evaluation of pyramidal neurons in the CA1 of the HPC revealed a decrease of mushroom (mature) spines 4 days after precipitation of nicotine abstinence when the cognitive deficits were still present (Article 1) (Figure

20).

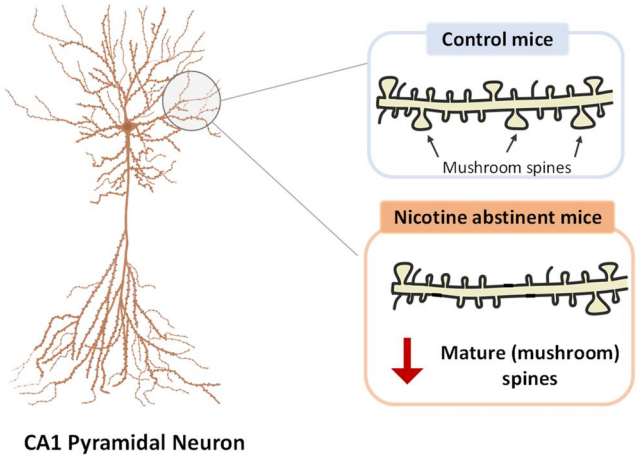


Figure 20. Schematic representation of the structural plasticity changes observed during nicotine abstinence in CA1 pyramidal neurons.

This type of dendritic spine has been widely associated with long-term memory or "memory storage" (Kasai *et al*, 2010; Segal, 2017). Spines are extremely plastic with their density and shape being modifiable by several factors, including stress (Maras and Baram, 2012). The stressful condition of nicotine withdrawal could be responsible for the reduced density of mature spines, probably leading to synaptic dysfunction and cognitive deficits. Nonetheless, the mechanisms described in our study should not be extended to other types of stressful situations such as acute corticosterone administration. Indeed, the injection of compounds affecting the endocannabinoid system such as the mGluR5 antagonist MTEP and O7460, before an acute amnesic dose of corticosterone did not prevent memory impairment in the object-recognition test (Article 1). As previously mentioned, glutamate receptors are strongly linked to synaptic strength, and are abundant in mushroom spines, but are sparsely distributed in thin and filopodia spines of CA1 hippocampal pyramidal

neurons (Matsuzaki *et al*, 2001). Consistent with this, we found a decrease of GluR2 receptors in nicotine abstinent mice that could be related to the reduced number of mature spines observed in nicotine-abstinent mice (Article 1). In line with our study, cortical neuroplasticity alterations have been reported during early withdrawal in abstinent smokers (Grundey *et al*, 2012). It seems that altered neuroplasticity in the PFC may be responsible for disrupting the motivation necessary to remain abstinence or decreasing the ability to sustain the cognitive control necessary to maintain abstinence in front of smoking cravings (Ashare *et al*, 2014). However, no modifications of structural plasticity were observed in pyramidal neurons of PFC under our experimental conditions (Article 1).

The reduced density of mushroom-type spines in the CA1 pyramidal neurons observed in nicotine abstinent mice was normalized by a subchronic treatment with rimonabant and in GABA-CB1R KO mice. In agreement, a similar treatment with rimonabant has been shown to reverse cognitive and hippocampal dendritic spine deficits in a model of fragile X syndrome (Busquets-Garcia *et al*, 2013). Although reduced dendritic spines of pyramidal neurons in the CA1 in GABA-CB1 KO has been reported, we did not observe any modification in the average of spine density under basal conditions (Monory *et al*, 2015). The different methodology used to quantify spines (apical versus apical and basal dendrites) could explain these contradictory results. Taken together, our data suggest that CB1R activation in GABAergic neurons is determinant to modulate the morphology of hippocampal spines resulting in the cognitive deficits observed during nicotine abstinence. Several studies the role of GABAergic-CB1R in other behaviors aside from learning and memory processes (Albayram *et al*, 2016), such as food intake (Bellocchio *et al*, 2010), exercise-related behaviors (Fuss *et al*, 2015) and drug addiction

(Martín-García *et al*, 2016; Talani and Lovinger, 2015)

In order to understand the role of 2-AG during nicotine abstinence, we evaluated the participation of this endocannabinoid in other aspects of nicotine withdrawal. During nicotine abstinence, 2-AG seems to have bimodal effects. In this regard, modulation of 2-AG levels induced opposite effects on the somatic signs and memory impairment revealed during withdrawal. The MAGL inhibitor JZL184 reduced the severity of nicotine physical dependence, whereas the inhibition of DAGLs by O7460 exacerbated somatic signs of withdrawal (Article 1). In agreement with our results, a recent study reported that inhibition of MAGL reduced the expression of somatic signs of withdrawal (Muldoon *et al*, 2015). These data suggest that on the one hand increased levels of 2-AG might be triggered to alleviate somatic signs but on the other hand these higher levels of 2-AG have detrimental effects on memory during nicotine withdrawal. Since these cognitive deficits last longer, targeting them could be more relevant for avoiding early nicotine relapse than physical symptoms. Furthermore, several studies have shown an increase in 2-AG during stress exposure, suggesting that 2-AG could be released to counteract the negative effects of stress (Morena *et al*, 2016).

Our results suggest that different neurobiological mechanisms mediate the somatic and the cognitive aspects of nicotine abstinence. Thus, the mTOR inhibitor temsirolimus and the inhibitor of mGluR5 MTEP blocked memory impairment without affecting somatic signs. In agreement, no changes in the physical severity of nicotine abstinence were observed in a previous study using the mGluR5 antagonist MTEP (Liechti *et al*, 2007). These results reveal the crucial involvement of CB1R located in GABAergic cells in the cognitive impairment and neuronal plasticity changes in the HPC occurring during nicotine withdrawal (Figure 21). This subpopulation

of CB1R could be targeted to prevent smoking relapse by increasing cognitive performance during early nicotine abstinence.

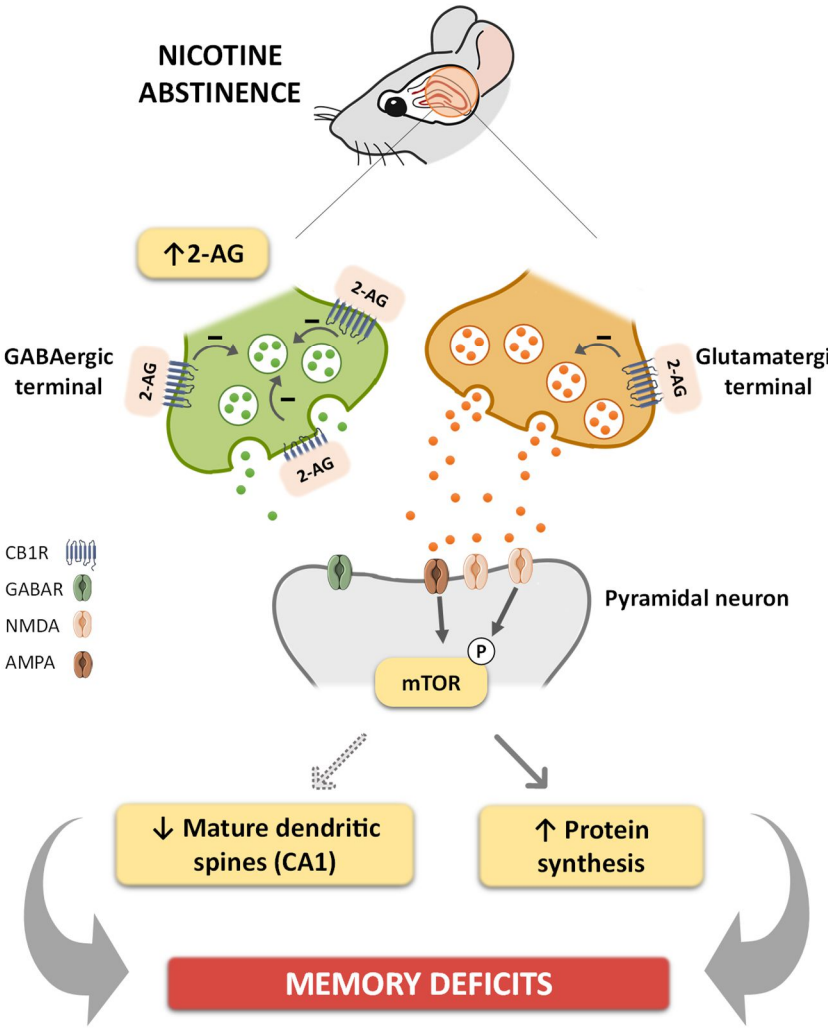


Figure 21. The endocannabinoid system mediates the cognitive deficits associated with nicotine withdrawal. Upon precipitation of nicotine withdrawal, brain levels of 2-AG increase probably to counteract the physical signs of withdrawal. However, high levels of 2-AG triggered by nicotine abstinence impact negatively on memory. 2-AG acting mainly on CB1R located in GABAergic terminals leads to a disbalance between excitatory and inhibitory neural transmission. As a consequence, the mTOR pathway is activated, which in turn induces an aberrant increase in protein synthesis and alter structural plasticity. Structural plasticity is affected as a reduced density of mature spines is observed in pyramidal neurons of the CA1 region of the hippocampus during nicotine abstinence. These changes in structural plasticity could explain the long-lasting effects of nicotine withdrawal on cognition.

Participation of inflammatory processes in the cognitive deficits associated with nicotine withdrawal

Unrevealing the mechanisms involved in the cognitive deficits observed during nicotine abstinence are determinant to identify new therapeutical targets and, therefore, develop more efficient smoking cessation treatments. Since cognitive deficits seem relevant for smoking relapse, pharmacotherapies should emphasize on increasing cognitive performance during nicotine withdrawal. In this sense, repurposing available pharmacotherapies that enhance cognition could be a good strategy (Ashare *et al*, 2014). Several reports have associated the development of cognitive impairments with inflammatory processes (Allison and Ditor, 2014; McKim *et al*, 2016). Additionally, there are well-stablished links between inflammation and tobacco smoking, although these studies have mainly focused on the relationship between inflammatory markers and increased cardiovascular risk (Lo Sasso *et al*, 2016; Wannamethee *et al*, 2005). However, the possible role of neuroinflammation in the cognitive deficits associated with early nicotine withdrawal remains to be elucidated.

As observed in our first study (Article 1), precipitation of nicotine withdrawal resulted in a memory impairment in nicotine-treated mice when memory was evaluated on the 4th day of withdrawal (Article 2). In agreement, several studies have shown similar cognitive deficits in other hippocampal-dependent tasks in rodents (Raybuck and Gould, 2009; Wilkinson and Gould, 2013; Yildirim *et al*, 2015). In many CNS disorders, the presence of cognitive deficits has been associated with inflammatory responses (McKim *et al*, 2016; Wohleb *et al*, 2016; Zhao *et al*, 2013). Microglia cells play crucial roles in normal development, plasticity, and maintenance of neural circuits (Wake et al, 2013). Upon disturbance of

brain homeostasis, microglial cells move from a surveillance state, indicated by a ramified morphology with small soma, to a reactive state characterized by retracted ramifications and larger amoeboid soma (Kohman and Rhodes, 2013). Interestingly, a significant increase in the perimeter of microglial soma was observed in the CA1 and DG regions of the HPC in nicotine abstinent mice (Article 2). This increase in the soma perimeter was also present in PFC, but not in the dorsal striatum of abstinent mice, suggesting that the change in microglial morphology is restricted to areas relevant to cognitive processing (Article 2). Indeed, we observed a negative correlation between the perimeter of the soma and the performance of mice in the object-recognition task in the DG of the HPC and in the PFC (Article 2). The stressful condition of nicotine withdrawal could be responsible for this microglial activation since ample evidence demonstrates how stress exposure can induce changes in microglia structure and function leading to cognitive deficits (Tay *et al*, 2017; Yirmiya *et al*, 2015). Thus, corticotropin-releasing factor has been shown to be involved in the dysphoria and anxiety-like behavior observed during nicotine abstinence (Bruijnzeel, 2017), while blockade of corticosterone effects abolishes acute and chronic stress-induced microglial proliferation and activation (Frank *et al*, 2012; de Pablos *et al*, 2014). Other factors aside from stress also modulate microglial response. Given the sensitive nature of microglial cells, these cells can be activated in response to the slightest CNS insult (Salter and Stevens, 2017). In this regard, it has been reported that glutamatergic neurotransmission increases the activity of microglial cells, while GABAergic neurotransmission decreases it (Fontainhas *et al*, 2011). As suggested in our first study, precipitation of nicotine abstinence could result in an overall increase of glutamatergic response in the HPC mediated through

CB1R (Article 1). Therefore, it is plausible that this unbalance between excitatory and inhibitory signaling could contribute to the morphological change observed in microglial cells. Furthermore, substantial evidence supports the role of microglia in synaptic and structural plasticity, where microglia interaction with neuronal components can determine the fate of dendritic synapses in the adult brain (Delpech *et al*, 2015; Morris *et al*, 2013). Indeed, a recent publication demonstrated that microglial cells directly remodel dendritic spines shape, turning mature into immature spines (Weinhard *et al*, 2018). Therefore, an aberrant spine remodeling driven by activated microglia could be contributing to the reported reduced density of mature spines during nicotine abstinence (Article 1).

It is still controversial whether the sole change of microglial morphology could indicate a particular response state (Salter and Stevens, 2017). Therefore, aside from evaluating the morphology of microglial cells, it is essential to evaluate their molecular expression profiles. In addition to microglial activation, we found an increased mRNA expression of several inflammatory markers such as IL1β, TNFα, and IFNγ in both the HPC and the PFC during nicotine withdrawal (Article 2). Overexpression of pro-inflammatory cytokines in the CNS has been associated with several neuropsychiatric disorders including depression, Alzheimer's disease and Parkinson's disease (Borsini *et al*, 2015). Indeed, changes in cytokine levels have a profound impact on hippocampal dependent-memory systems and have been associated with synaptic plasticity processes (Patterson, 2015). This cumulative evidence has demonstrated that IL1β inhibits hippocampal-dependent learning (Jones and Lynch, 2015). In addition, it has been suggested that increased levels of TNFα, produced by microglia in the HPC, seem to be underlying stress-induced memory impairments (Ohgidani *et al*, 2016). Remarkably, an elevation of plasmatic levels of

TNFα and IFNγ was also observed 4 days after nicotine withdrawal precipitation (Article 2), pointing these cytokines as possible biomarkers of the cognitive deficits present during tobacco abstinence. Indeed, cytokine levels in plasma were normalized by the 8th day of withdrawal when mice have completely recovered from memory impairment under our experimental conditions (Article 1).

Inflammatory cytokines including IL1β, TNFα, and IFNγ are also key modulators of neurogenesis (Borsini *et al*, 2015), a process with a key role in mediating human brain functions including memory formation and cognition (Kohman and Rhodes, 2013). Interestingly, cognitive deficits of nicotine withdrawal were associated with reduced expression of cell proliferation and young neuron markers in the subgranular zone of the HPC. Therefore, these results suggest that inflammation-induced deficits in cognitive performance during nicotine withdrawal could be related to the reductions in hippocampal neurogenesis (Article 2).

Current pharmacotherapies for smoking cessation have been associated with psychiatric adverse events (Hughes, 2016; Prochaska and Benowitz, 2016). This has increased the demand to develop new treatments for smoking cessation. Indeed, many smokers would try to quit smoking if effective, inexpensive and less sided-effects approaches were available (Volkow, 2018). During the last decade, the interest in the non-psychoactive cannabinoid CBD has grown exponentially. CBD has been featured as an anti-inflammatory and neuroprotective compound (Burstein, 2015; Fernández-Ruiz *et al*, 2013), with potential benefits for the treatment of motivational disorders such as drug addiction, anxiety and depression (Shoval *et al*, 2016; Zlebnik and Cheer, 2016). In our study, subchronic treatment with CBD prevented the memory impairment in the object-recognition task and the activation of microglia in the HPC and the

PFC observed 4 days after nicotine withdrawal (Article 2). Consisting with this, CBD has been shown to improve object-recognition memory in preclinical models that course with cognitive impairment, including schizophrenia (Gomes *et al*, 2015), Alzheimer's disease (Cheng *et al*, 2014a), brain ischemia (Pazos *et al*, 2013) and cerebral malaria (Campos *et al*, 2015). Furthermore, treatment with CBD also normalized the increased expression of IL1β and TNFα in the HPC and the PFC, respectively, observed during nicotine withdrawal (Article 2). These results suggest that normalizing the levels of proinflammatory cytokines might be a mechanism by which CBS restores cognitive function, although studies supporting this idea are sparse and controversial (Osborne *et al*, 2017). Nonetheless, some studies propose a possible involvement of TNFα in the mechanisms underlying the ability of CBD to improve cognition (Barichello *et al*, 2012; Cheng *et al*, 2014b). Indeed, chronic administration of CBD reduced mRNA levels of TNFα in the PFC which was associated with the prevention of memory impairments in a rodent model of meningitis (Barichello *et al*, 2012). Moreover, CBD treatment promoted cell proliferation during nicotine withdrawal in the subgranular zone of the hippocampal DG. Accordingly, an increase of hippocampal neurogenesis following CBD administration was observed in rodent models of Alzheimer's disease (Esposito *et al*, 2011) and chronic stress (Campos *et al*, 2013). As a whole, these data indicate that CBD might improve cognitive performance during nicotine withdrawal through the modulation of inflammation and cell proliferation. Interestingly, preliminary findings in humans show that CBD treatment reduced cigarette consumption (Morgan et al, 2013), and pleasantness of cigarette cues after overnight abstinence (Hindocha et al, 2018a) in tobacco smokers. However, a recent study has shown that acute administration of

a single dose of CBD did not improve memory performance in tasks previously shown to be impaired during cigarette abstinence (Hindocha et al, 2018b). CBD's lack of effect in this study could be due to the dose used, considering the bell-shaped dose-response effects widely reported for this compound (Zuardi *et al*, 2017). Indeed, low, but not high, doses of cannabidiol have been found to be effective in alleviating memory deficits in a rat model of Parkinson's disease and tardive dyskinesia (Peres *et al*, 2016).

In light of the relationships between the inflammatory profile and the cognitive impairments observed during nicotine abstinence, we evaluated whether the improvement of memory performance by CBD could be generalized to other anti-inflammatory agents. Interestingly, administration of the NSAID indomethacin prevented the development of the cognitive deficits associated with nicotine withdrawal and microglia activation in the HPC (Article 2). In agreement, indomethacin has been found to rescue the memory deficits induced by stress (Emad *et al*, 2017; Perveen *et al*, 2018), intermittent ethanol intoxication (Pascual *et al*, 2007) and in a mouse model of Alzheimer's disease (Balducci *et al*, 2017), but not in a model of social defeat stress (Duque *et al*, 2017). Indomethacin is a broad spectrum NSAID that inhibits cyclooxygenase (Cox)-1 and -2 activity, although hippocampal mRNA expression of Cox-1 and -2 was not modified under our experimental conditions (Article 2). Further experiments evaluating the activity of COX-1 and COX-2 should be performed. Recently, other mechanisms have been described to be involved in the memory rescuing effect of indomethacin. Thus, indomethacin might rescue memory impairments by acting on acetylcholinesterase (Emad *et al*, 2017), an enzyme tightly related to formation and encoding of memories (Hasselmo, 2006). In addition, increasing serotonin and dopamine

transmission in the brain seems to be another mechanism by which indomethacin prevented the memory impairment induced by stress exposure (Perveen *et al*, 2018). Given the stressful condition of nicotine withdrawal, indomethacin could act through these mechanisms to rescue memory impairment during nicotine withdrawal.

In summary, our work reveals for the first time an inflammatory process associated with the cognitive deficits that characterize early nicotine abstinence (Figure 22). Moreover, these findings underline the efficacy of anti-inflammatory agents to improve cognitive deficits during nicotine withdrawal. Given that the presence of cognitive alterations is associated with increased smoking relapse risk, our results identify anti-inflammatory drugs as new potential therapeutic strategies for nicotine dependence.

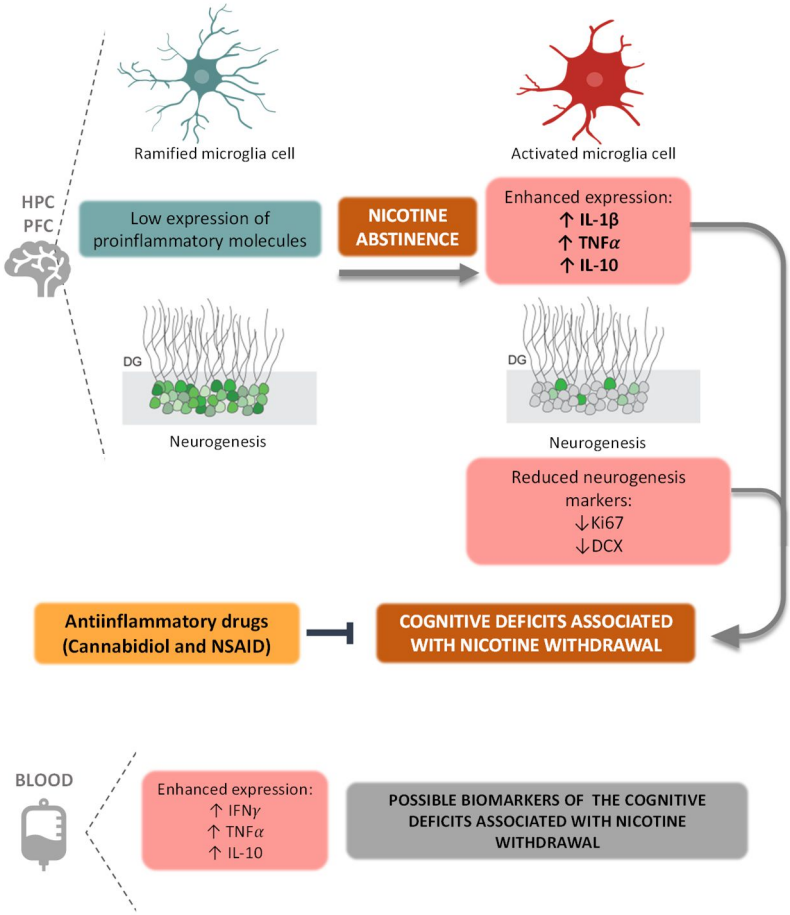


Figure 22. Neuroinflammation underlies the cognitive deficits associated with nicotine withdrawal. In brain areas related with cognitive processing, such as prefrontal cortex and hippocampus, microglia cells move from its surveilling state to a more reactive state characterized by short ramification and large soma when nicotine withdrawal is precipitated. Microglia activation is accompanied with increased expression of proinflammatory cytokines such as IL1-β and TNFα. Proinflammatory cytokines also affect the proliferation and differentiation of new neurons within the subgranular zone of the hippocampus. Neuroinflammation and altered neurogenesis contribute to the development of the cognitive deficits associated with nicotine withdrawal. Anti-inflammatory compounds, such as cannabidiol and the NSAID indomethacin reduced microglial reactivity, normalize neurogenesis and prevent memory deficits during nicotine abstinence. The increase of inflammatory cytokines was also observed in blood samples only when cognitive deficits were present. Therefore, these molecules could serve as possible peripheral biomarkers of the cognitive deficits associated with nicotine withdrawal.

Involvement of orexin signaling in the improvement of memory induced by acute nicotine administration

Human and animal studies have demonstrated that nicotine can enhance cognition (Hall *et al*, 2015a; Kutlu and Gould, 2016a), which might contribute to initial tobacco use and smoking dependence (Pulvers *et al*, 2014). Moreover, cognitive processes that rely on hippocampal function have been suggested to be more sensitive to the effects of acute nicotine administration (Kutlu and Gould, 2016a). Several reports suggest a potential role of the orexin system in cognition, learning and memory (Chen *et al*, 2017; Li *et al*, 2014). In addition, this system is also involved in behaviors associated with nicotine dependence, such as reinstatement of nicotine-seeking behavior (Bruijnzeel, 2017; Plaza-Zabala *et al*, 2013). In this study, we evaluated the possible role of the orexin signaling in effects that acute nicotine exerts on memory. To assess memory, we used the novel object recognition test. Conventionally, the test session in the object recognition task is performed 24 hours after the training session (Antunes and Biala, 2012). However, under our experimental conditions, this protocol was not sensitive enough to observe a memory improvement. Therefore, to properly evaluate an enhancement of memory, we set a gap of 48 hours between training and test. With this modification of the novel object recognition test, control mice exhibited low discrimination indexes and the effect of nicotine on memory was revealed (Supplementary results). A similar modification to the task has been previously used to evaluate the pro-cognitive effect of nicotine in the spatial version of the object recognition (Kenney *et al*, 2011). Increasing the time gap between training and test made the task more challenging and converted the tendency to improve memory (24 hours) in a significant enhancement of memory (48h)(Kenney *et al*, 2011).

Nicotine was administered at different doses (0.25, 0.50, 0.75 and 1 mg/kg) but only the dose of 0.75 mg/kg resulted in a significant increase in the discrimination index in the object recognition test (Supplementary results). This difference in the discrimination index was not due to altered locomotor activity since all groups exhibited similar exploration times. Stress can have detrimental or enhancing effects of memory depending on the type and duration of the stress stimuli (Maras and Baram, 2012). Indeed, some studies have shown that acute stressful experiences that occur shortly after learning (during memory consolidation) can improve memory performance (Glienke and Piefke, 2016; McCullough *et al*, 2015). Interestingly, a similar dose to the one used in our study (0.8 mg/kg of nicotine) has been reported to have anxiogenic-like effects, to activate orexin neurons and to activate the PVN, a stress-related brain region, in an orexigenic-dependent manner (Plaza-Zabala *et al*, 2010). Thus, it is possible that nicotine might enhance memory by acting as a mild stressor and involving orexin signaling. In our study, pharmacological and genetical blockade of the OX1R prevented the memory improvement induced by nicotine administration (Supplementary results). Consistent with our results, several studies support the role of OX1R in learning and memory in a variety of hippocampal-dependent tasks (Akbari *et al*, 2006, 2008; Flores *et al*, 2014; Yang *et al*, 2013; Zhao *et al*, 2014). Notably, antagonism of the OX2R also abolished the pro-cognitive effect of nicotine (Supplementary results). Most of the studies point to orexin-A as the orexin peptide involved in learning and memory (Akbari *et al*, 2007; Chen *et al*, 2017; Zhao *et al*, 2013, 2014). Since orexin-A binds with equal affinity to OX1R and OX2R, this peptide could be the one mediating the effects of nicotine on memory. However, the participation of OXB should not be discarded as this peptide has been also shown to improve cognition

(Lambe *et al*, 2005). Indeed, deletion of the prepro-orexin gene, that codifies for both orexin-A and orexin-B, prevented the enhancement of memory induced by nicotine (Supplementary results).

In summary, our data reveal that orexin signaling through OX1R and OX2R modulates the pro-cognitive effect of nicotine. However, further research will be needed to fully understand the mechanism by which the orexins system regulated the memory-enhancing effect of nicotine. Considering the existence of a reciprocal link between the CRF system and the orexin neurons (Tsujino and Sakurai, 2013) and that several studies have demonstrated role of CRF on cognition (Gafford *et al*, 2014; Hupalo and Berridge, 2016), the involvement of CRF signaling in the improvement of memory induced by nicotine should be explored.

The current thesis has identified new neurobiological mechanisms involved in the cognitive effects of nicotine. We have identified new possible therapeutic targets to treat the cognitive deficits that arise during nicotine withdrawal (Article 1 and 2). These cognitive deficits have been proposed to be a hallmark of nicotine withdrawal and to predict smoking relapse. We have shown that signaling through the CB1R located in GABAergic neurons is crucial for the cognitive impairments associated with nicotine withdrawal (Article 1). Moreover, we found that structural plasticity changes in CA1 pyramidal neurons in the HPC underlie the cognitive deficits of nicotine abstinence (Article 1). Furthermore, we have revealed for the first time that inflammatory processes are associated with the cognitive deficits that characterize early nicotine abstinence (Article 2). Given the role of microglia in synaptic plasticity, it is hypothesized that the increased microglial reactivity observed during nicotine abstinence (Article 2) could be mediating the alteration of structural plasticity that underlies the cognitive deficits of nicotine withdrawal (Article 1) (Figure 23). Therefore, blockade of inflammatory processes might be an interesting therapeutic strategy to ameliorate the cognitive deficits of nicotine withdrawal. Indeed, we propose the study of the use of anti-inflammatory compounds as potential therapeutic strategy for nicotine dependence. In our pilot investigation, we have demonstrated that the NSAID indomethacin is effective in normalizing memory performance during nicotine withdrawal. However, the use of indomethacin has been associated with a number of side effects including gastrointestinal erosions, and renal and hepatic insufficiency (Süleyman *et al*, 2007). Therefore, the use of other NSAIDs to with similar therapeutic but less side effects could be probably of better interest for such a purpose.

In addition, we studied the mechanisms involved in the pro-cognitive effect of acute nicotine administration, since this effect has been hypothesized to contribute to initial nicotine intake. Our results suggest that orexin signaling through both OX1R and OX2R participates in the improvement of memory induced by acute treatment with nicotine (Supplementary results). The improvement of memory induced by acute nicotine administration might be crucial to develope dependence in a population with preexisting cognitive deficits that find in smoking a way for alleviating their cognitive deficits.

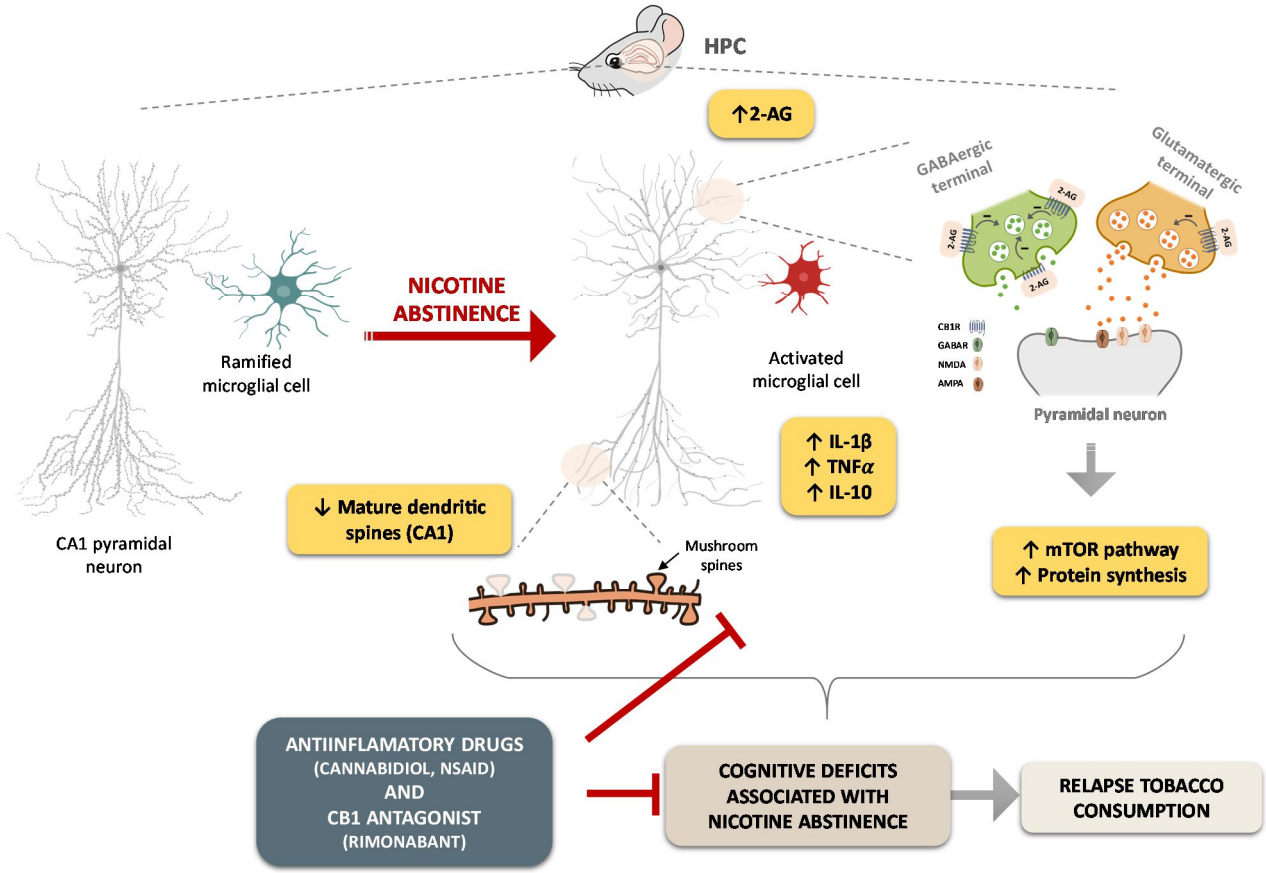
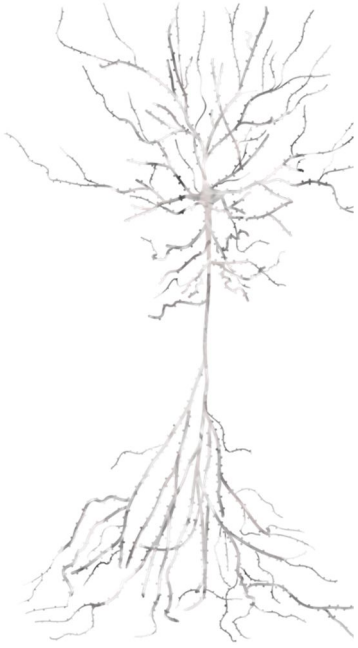

HPC
↑2-AG
GABAergic terminal
Glutamatergic terminal
2-AG
CB1R
GABAR
NMDA
AMPA
Pyramidal neuron
NICOTINE ABSTINENCE
Ramified microglial cell
Activated microglial cell
↑ IL-1β
↑ TNFα
↑ IL-10
↓ Mature dendritic spines (CA1)
CA1 pyramidal neuron
Mushroom spines
↑ mTOR pathway
↑ Protein synthesis
ANTIINFLAMATORY DRUGS (CANNABIDIOL, NSAID) AND CB1 ANTAGONIST (RIMONABANT)
COGNITIVE DEFICITS ASSOCIATED WITH NICOTINE ABSTINENCE
RELAPSE TOBACCO CONSUMPTION

Figure 23. Schematic representation of the main mechanisms involved in the cognitive deficits associated with nicotine withdrawal assessed in this thesis. Precipitation of nicotine withdrawal results in an increase in 2-AG levels, that through activation of the CB1R in GABAergic neurons might generate an imbalance between excitatory and inhibitory signaling. The resulting increase in excitatory transmission could be responsible for the shift of microglia to a more reactive or "inflammatory" phenotype. Microglial activation also contributes to the inflammatory environment by increasing the release of proinflammatory molecules such as IL1-β and TNFα. On the other hand, activation of CB1R could increase activity in the mTOR pathway and produces an aberrant increase in protein synthesis, which could impact negatively on structural plasticity, contributing to the cognitive impairment observed during nicotine withdrawal. Indeed, a decrease in the density of mature spines was observed in CA1 pyramidal neurons of the hippocampus in abstinent mice. Given the role of microglia in synaptic and structural plasticity, an aberrant spine remodelling driven by activated microglia could be contributing to the reported reduced density of mature spines during nicotine abstinence. Restorage of normal microglia phenotype by anti-inflammatory drugs prevented memory impairment of nicotine withdrawal. Therefore, the use of anti-inflammatory agents to normalize cognition during early abstinence could represent a new therapeutic approach to treat nicotine dependence since cognitive deficits are related to increased risk of relapse to tobacco consumption in humans.



CONCLUSIONS

The findings revealed in the present thesis allow to draw the following conclusions:

1. The endocannabinoid system plays a key role in the cognitive deficits that arise during nicotine withdrawal. CB1R located in GABAergic neurons modulates the structural plasticity alterations and the cognitive deficits associated with nicotine withdrawal.

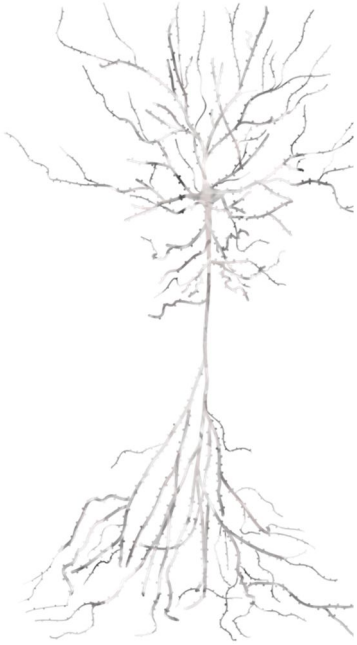
2. 2-AG seems to play divergent functional effects during nicotine abstinence. Thus, modulation of 2-AG levels induced opposite effects on the somatic signs and memory impairment revealed during withdrawal.

3. Administration of an inhibitor of the mTOR, an inhibitor of mGluR5, and cannabidiol administration blocked memory impairment without affecting somatic signs of nicotine withdrawal. These results suggest that different neurobiological mechanisms mediate somatic signs and cognitive deficits during nicotine withdrawal.

4. Precipitation of nicotine abstinence results in an increase of microglial reactivity and proinflammatory molecules in brain areas related with cognition, and a decrease in neurogenesis in the HPC. These data suggest that modulation of inflammation is a potential novel target for alleviating cognitive deficits of nicotine abstinence.

5. Subchronic treatment with cannabidiol and the NSAID indomethacin prevented the memory impairment observed during nicotine withdrawal through the modulation of microglial reactivity. Since the presence of cognitive alterations are associated with increased risk of smoking relapse, our results point to anti-inflammatory drugs as new potential therapeutic strategies for nicotine dependence.

6. The orexin system participates in the enhancement of the memory triggered by acute nicotine treatment. Signaling through both OX1R and OX2R contribute to the pro-cognitive effect of nicotine.



REFERENCES

References

Abel T, Nguyen P V. (2008). Regulation of hippocampus-dependent memory by cyclic AMP-dependent protein kinase. *Prog Brain Res* **169**: 97–115.

Acomplia S (2006). *Sanofi-Aventis. Acomplia 20mg Summary of Product Characteristics. http://emc.medicines.org.uk/emc/assets/c/html/displaydoc.asp?documentid=18283*. .

Addy NA, Picciotto MR (2013). Nicotine, Striatum, and Reward. *Biol Psychiatry* **73**: 205–206.

Aimone JB, Li Y, Lee SW, Clemenson GD, Deng W, Gage FH (2014). Regulation and function of adult neurogenesis: from genes to cognition. *Physiol Rev* **94**: 991–1026.

Akbari E, Motamedi F, Naghdi N, Noorbakhshnia M (2008). The effect of antagonization of orexin 1 receptors in CA1 and dentate gyrus regions on memory processing in passive avoidance task. *Behav Brain Res* **187**: 172–177.

Akbari E, Naghdi N, Motamedi F (2006). Functional inactivation of orexin 1 receptors in CA1 region impairs acquisition, consolidation and retrieval in Morris water maze task. *Behav Brain Res* **173**: 47–52.

Akbari E, Naghdi N, Motamedi F (2007). The selective orexin 1 receptor antagonist SB-334867-A impairs acquisition and consolidation but not retrieval of spatial memory in Morris water maze. *Peptides* **28**: 650–656.

Albayram O, Alferink J, Pitsch J, Piyanova A, Neitzert K, Poppensieker K, *et al* (2011). Role of CB1 cannabinoid receptors on GABAergic neurons in brain aging. *Proc Natl Acad Sci U S A* **108**: 11256–61.

Albayram Ö, Passlick S, Bilkei-Gorzo A, Zimmer A, Steinhäuser C (2016). Physiological impact of CB1 receptor expression by hippocampal GABAergic interneurons. *Pflügers Arch - Eur J Physiol* **468**: 727–737.

Albuquerque EX, Pereira EF, Alkondon M, Rogers SW (2009). Mammalian Nicotinic Acetylcholine Receptors: From Structure Function. *Physiol Rev* **89**: 73–120.

Allen S, Bade T, Hatsukami D, Center B (2008). Craving, withdrawal, and smoking urges on days immediately prior to smoking relapse. *Nicotine Tob Res* **10**: 35–45.

Allison DJ, Ditor DS (2014). The common inflammatory etiology of depression and cognitive impairment: a therapeutic target. *J Neuroinflammation* **11**: 151.

Alvarez FJ, Lafuente H, Carmen Rey-Santano M, Mielgo VE, Gastiasoro E, Rueda M, *et al* (2008). Neuroprotective Effects of the Nonpsychoactive Cannabinoid Cannabidiol in Hypoxic-Ischemic Newborn Piglets. *Pediatr Res* **64**: 653–658.

Ammoun S, Holmqvist T, Shariatmadari R, Oonk HB, Detheux M, Parmentier M, *et*

al (2003). Distinct Recognition of OX1 and OX2 Receptors by Orexin Peptides. *J Pharmacol Exp Ther* **305**: 507–514.

Ammoun S, Johansson L, Ekholm ME, Holmqvist T, Danis AS, Korhonen L, *et al* (2006). OX_1 Orexin Receptors Activate Extracellular Signal-Regulated Kinase in Chinese Hamster Ovary Cells via Multiple Mechanisms: The Role of Ca^{2+} Influx in OX_1 Receptor Signaling. *Mol Endocrinol* **20**: 80–99.

Amsterdam J van, Velde B van der, Schulte M, Brink W van den (2018). Causal Factors of Increased Smoking in ADHD: A Systematic Review. *Subst Use Misuse* **53**: 432–445.

André JM, Leach PT, Gould TJ (2011). Nicotine ameliorates NMDA receptor antagonist-induced deficits in contextual fear conditioning through high-affinity nicotinic acetylcholine receptors in the hippocampus. *Neuropharmacology* **60**: 617–25.

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

Antolin-fontes B, Ables JL, Görlich A, Ibañez-Tallon I (2015). The Habenulo-Interpeduncular pathway in nicotine aversion and withdrawal. *Neuropharmacology* **96**: 213–222.

Antunes M, Biala G (2012). The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process* **13**: 93–110.

Aracil-Fernández A, Trigo JM, García-Gutiérrez MS, Ortega-Álvaro A, Ternianov A, Navarro D, *et al* (2012). Decreased cocaine motor sensitization and self-administration in mice overexpressing cannabinoid CB_2 receptors. *Neuropsychopharmacology* **37**: 1749–63.

Armstrong DW, Wang X, Ercal N (1998). Enantiomeric composition of nicotine in smokeless tobacco, medicinal products, and commercial reagents. *Chirality* **10**: .

Ashare RL, Falcone M, Lerman C (2014). Cognitive function during nicotine withdrawal: Implications for nicotine dependence treatment. *Neuropharmacology* **76 Pt B**: 581–91.

Ashare RL, McKee SA (2012). Effects of varenicline and bupropion on cognitive processes among nicotine-deprived smokers. *Exp Clin Psychopharmacol* **20**: 63–70.

Ashare RL, Schmidt HD (2014). Optimizing treatments for nicotine dependence by increasing cognitive performance during withdrawal. *Expert Opin Drug Discov* 1–16doi:10.1517/17460441.2014.908180.

Ashare RL, Wileyto EP, Perkins KA, Schnoll RA (2013). The First 7 Days of a Quit Attempt Predicts Relapse. *J Addict Med* **7**: 249–254.

Aston-Jones G, Smith RJ, Moorman DE, Richardson KA (2009). Role of lateral hypothalamic orexin neurons in reward processing and addiction. *Neuropharmacology* **56 Suppl 1**: 112–21.

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

Audrain-McGovern J, Koudsi NA, Rodriguez D, Wileyto EP, Shields PG, Tyndale RF (2007). The Role of CYP2A6 in the Emergence of Nicotine Dependence in Adolescents. *Pediatrics* **119**: e264–e274.

Bagdas D, Alkhlaif Y, Jackson A, Carroll FI, Ditre JW, Damaj MI (2018). New insights on the effects of varenicline on nicotine reward, withdrawal and hyperalgesia in mice. *Neuropharmacology* **138**: 72–79.

Balducci C, Frasca A, Zotti M, Vitola P La, Mhillaj E, Grigoli E, *et al* (2017). Toll-like receptor 4-dependent glial cell activation mediates the impairment in memory establishment induced by β-amyloid oligomers in an acute mouse model of Alzheimer's disease. *Brain Behav Immun* **60**: 188–197.

Balschun D, Randolf A, Pitossi F, Schneider H, Rey A Del, Besedovsky HO (2003). Hippocampal interleukin-1 beta gene expression during long-term potentiation decays with age. *Ann N Y Acad Sci* **992**: 1–8.

Bani M, Andorn A, Heidbreder C (2014). Pharmacologically, are smokers the same as non-smokers? *Curr Opin Pharmacol* **14**: 42–49.

Bannerman DM, Sprengel R, Sanderson DJ, McHugh SB, Rawlins JNP, Monyer H, *et al* (2014). Hippocampal synaptic plasticity, spatial memory and anxiety. *Nat Rev Neurosci* **15**: 181–92.

Barichello T, Ceretta RA, Generoso JS, Moreira AP, Simões LR, Comim CM, *et al* (2012). Cannabidiol reduces host immune response and prevents cognitive impairments in Wistar rats submitted to pneumococcal meningitis. *Eur J Pharmacol* **697**: 158–164.

Barr RS, Culhane MA, Jubelt LE, Mufti RS, Dyer MA, Weiss AP, *et al* (2008). The Effects of Transdermal Nicotine on Cognition in Nonsmokers with Schizophrenia and Nonpsychiatric Controls. *Neuropsychopharmacology* **33**: 480–490.

Barrientos RM, Frank MG, Hein AM, Higgins EA, Watkins LR, Rudy JW, *et al* (2009). Time course of hippocampal IL-1 beta and memory consolidation impairments in aging rats following peripheral infection. *Brain Behav Immun* **23**: 46–54.

Barrientos RM, Higgins EA, Sprunger DB, Watkins LR, Rudy JW, Maier SF (2002). Memory for context is impaired by a post context exposure injection of interleukin-1 beta into dorsal hippocampus. *Behav Brain Res* **134**: 291–298.

Belarbi K, Jopson T, Tweedie D, Arellano C, Luo W, Greig NH, *et al* (2012). TNF-α protein synthesis inhibitor restores neuronal function and reverses

cognitive deficits induced by chronic neuroinflammation. *J Neuroinflammation* **9**: 518.

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

Ben-Shabat S, Hanuš LO, Katzavian G, Gallily R (2006). New Cannabidiol Derivatives: Synthesis, Binding to Cannabinoid Receptor, and Evaluation of Their Antiinflammatory Activity. *J Med Chem* **49**: 1113–1117.

Benowitz NL (1992). Cigarette smoking and nicotine addiction. *Med Clin North Am* **76**: 415–437.

Benowitz NL (2009). Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. *Annu Rev Pharmacol Toxicol* **49**: 57–71.

Benowitz NL (2010). Nicotine Addiction. *N Engl J Med* **362**: 2295–2303.

Benowitz NL, Peyton J (1984). Daily intake of nicotine during cigarette smoking. *Clin Pharmacol Ther* **35**: 499–504.

Berridge CW, España RA, Vittoz NM (2010). Hypocretin/orexin in arousal and stress. *Brain Res* **1314**: 91–102.

Berridge MJ (2014). Calcium regulation of neural rhythms, memory and Alzheimer's disease. *J Physiol* **592**: 281–293.

Bettcher BM, Wilheim R, Rigby T, Green R, Miller JW, Racine CA, *et al* (2012). C-Reactive Protein is Related to Memory and Medial Temporal Brain Volume in Older Adults. *Brain Behav Immun* **26**: 103–108.

Bhargava HN (1976). Effect of some cannabinoids on naloxone-precipitated abstinence in morphine-dependent mice. *Psychopharmacology (Berl)* **49**: 267–70.

Biala G, Staniak N, Budzynska B (2010). Effects of varenicline and mecamylamine on the acquisition, expression, and reinstatement of nicotine-conditioned place preference by drug priming in rats. *Naunyn Schmiedebergs Arch Pharmacol* **381**: 361–370.

Biasi M De, Dani JA (2011). Reward, addiction, withdrawal to nicotine. *Annu Rev Neurosci* **34**: 105–30.

Biasi M De, Salas R (2008). Influence of Neuronal Nicotinic Receptors over Nicotine Addiction and Withdrawal. *Exp Biol Med* **233**: 917–929.

Bierut LJ (2011). Genetic Vulnerability and Susceptibility to Substance Dependence. *Neuron* **69**: 618–627.

Bierut LJ, Stitzel JA, Wang JC, Hinrichs AL, Grucza RA, Xuei X, *et al* (2008). Variants in Nicotinic Receptors and Risk for Nicotine Dependence. *Am J Psychiatry* **165**: 1163–1171.

Bierut LJ, Tyndale RF (2018). Preparing the Way: Exploiting Genomic Medicine to Stop Smoking. *Trends Mol Med* **24**: 187–196.

Bisogno T, Hanus L, Petrocellis L De, Tchilibon S, Ponde DE, Brandi I, *et al* (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* **134**: 845–52.

Blank T, Prinz M (2013). Microglia as modulators of cognition and neuropsychiatric disorders. *Glia* **61**: 62–70.

Block ML, Zecca L, Hong J-S (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci* **8**: 57–69.

Blouin AM, Fried I, Wilson CL, Staba RJ, Behnke EJ, Lam HA, *et al* (2013). Human hypocretin and melanin-concentrating hormone levels are linked to emotion and social interaction. *Nat Commun* **4**: 1547.

Bockaert J, Marin P (2015). mTOR in Brain Physiology and Pathologies. *Physiol Rev* **95**: 1157–87.

Boldrini M, Fulmore CA, Tartt AN, Simeon LR, Pavlova I, Poposka V, *et al* (2018). Human Hippocampal Neurogenesis Persists throughout Aging. *Cell Stem Cell* **22**: 589–599.e5.

Bolduc F V, Bell K, Cox H, Broadie KS, Tully T (2008). Excess protein synthesis in Drosophila Fragile X mutants impairs long-term memory. *Nat Neurosci* **11**: 1143–1145.

Bordia T, Hrachova M, Chin M, McIntosh JM, Quik M (2012). Varenicline Is a Potent Partial Agonist at 6 2* Nicotinic Acetylcholine Receptors in Rat and Monkey Striatum. *J Pharmacol Exp Ther* **342**: 327–334.

Borkar CD, Sagarkar S, Sakharkar AJ, Subhedar NK, Kokare DM (2017). Neuropeptide CART prevents memory loss attributed to withdrawal of nicotine following chronic treatment in mice. *Addict Biol* doi:10.1111/adb.12579.

Borsini A, Zunszain PA, Thuret S, Pariante CM (2015). The role of inflammatory cytokines as key modulators of neurogenesis. *Trends Neurosci* **38**: 145–157.

Bosch M, Hayashi Y (2012). Structural plasticity of dendritic spines. *Curr Opin Neurobiol* **22**: 383–388.

Bosier B, Muccioli GG, Hermans E, Lambert DM (2010). Functionally selective cannabinoid receptor signalling: Therapeutic implications and opportunities. *Biochem Pharmacol* **80**: 1–12.

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

Boutrel B, Kenny PJ, Specio SE, Martin-Fardon R, Markou A, Koob GF, *et al* (2005). Role for hypocretin in mediating stress-induced reinstatement of cocaine-seeking behavior. *Proc Natl Acad Sci* **102**: 19168–19173.

Brody AL, Mandelkern MA, London ED, Olmstead RE, Farahi J, Scheibal D, *et al* (2006). Cigarette Smoking Saturates Brain α4β2 Nicotinic Acetylcholine Receptors. *Arch Gen Psychiatry* **63**: 907.

Brody AL, Mukhin AG, Mamoun MS, Luu T, Neary M, Liang L, *et al* (2014). Brain Nicotinic Acetylcholine Receptor Availability and Response to Smoking Cessation Treatment. *JAMA Psychiatry* **71**: 797.

Brown RM, Khoo SY-S, Lawrence AJ (2013). Central orexin (hypocretin) 2 receptor antagonism reduces ethanol self-administration, but not cue-conditioned ethanol-seeking, in ethanol-preferring rats. *Int J Neuropsychopharmacol* **16**: 2067–79.

Bruijnzeel AW (2017). Neuropeptide systems and new treatments for nicotine addiction. *Psychopharmacology (Berl)* **234**: 1419–1437.

Bruijnzeel AW, Prado M, Isaac S (2009). Corticotropin-Releasing Factor-1 Receptor Activation Mediates Nicotine Withdrawal-Induced Deficit in Brain Reward Function and Stress-Induced Relapse. *Biol Psychiatry* **66**: 110–117.

Brunzell DH, McIntosh JM (2012). Alpha7 Nicotinic Acetylcholine Receptors Modulate Motivation to Self-Administer Nicotine: Implications for Smoking and Schizophrenia. *Neuropsychopharmacology* **37**: 1134–1143.

Burstein S (2015). Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. *Bioorg Med Chem* **23**: 1377–85.

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

Busquets-Garcia A, Gomis-González M, Guegan T, Agustín-Pavón C, Pastor A, Mato S, *et al* (2013). Targeting the endocannabinoid system in the treatment of fragile X syndrome. *Nat Med* **19**: 603–607.

Butler M., O'Connor J., Moynagh P. (2004). Dissection of tumor-necrosis factor-α inhibition of long-term potentiation (LTP) reveals a p38 mitogen-activated protein kinase-dependent mechanism which maps to early—but not late—phase LTP. *Neuroscience* **124**: 319–326.

Cabral GA, Raborn ES, Griffin L, Dennis J, Marciano-Cabral F (2008). CB 2 receptors in the brain: Role in central immune function. *Br J Pharmacol* **153**: 240–251.

Cacci E, Claasen J-H, Kokaia Z (2005). Microglia-derived tumor necrosis factor-α exaggerates death of newborn hippocampal progenitor cells in vitro. *J Neurosci Res* **80**: 789–797.

Cachope R, Mateo Y, Mathur BN, Irving J, Wang H-L, Morales M, *et al* (2012). Selective Activation of Cholinergic Interneurons Enhances Accumbal Phasic

Dopamine Release: Setting the Tone for Reward Processing. *Cell Rep* **2**: 33–41.

Caggiula AR, Donny EC, White AR, Chaudhri N, Booth S, Gharib MA, *et al* (2001). Cue dependency of nicotine self-administration and smoking. *Pharmacol Biochem Behav* **70**: 515–530.

Cahill K, Mh U (2012). Cannabinoid type 1 receptor antagonists for smoking cessation (Review). .

Cahill K, Ussher MH (2011). Cannabinoid type 1 receptor antagonists for smoking cessation. *Cochrane database Syst Rev* CD005353doi:10.1002/14651858.CD005353.pub4.

Caille S, Clemens K, Stinus L, Cador M (2012). Modelling nicotine addiction in rats. **829**: .

Campos AC, Brant F, Miranda AS, Machado FS, Teixeira AL (2015). Cannabidiol increases survival and promotes rescue of cognitive function in a murine model of cerebral malaria. *Neuroscience* **289**: 166–80.

Campos AC, Fogaça M V., Sonego AB, Guimarães FS (2016). Cannabidiol, neuroprotection and neuropsychiatric disorders. *Pharmacol Res* **112**: 119–127.

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

Campos AC, Ortega Z, Palazuelos J, Fogaça M V., Aguiar DC, Díaz-Alonso J, *et al* (2013). The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. *Int J Neuropsychopharmacol* **16**: 1407–1419.

Carboni E, Bortone L, Giua C, Chiara G Di (2000). Dissociation of physical abstinence signs from changes in extracellular dopamine in the nucleus accumbens and in the prefrontal cortex of nicotine dependent rats. *Drug Alcohol Depend* **58**: 93–102.

Cardinal RN, Everitt BJ (2004). Neural and psychological mechanisms underlying appetitive learning: links to drug addiction. *Curr Opin Neurobiol* **14**: 156–162.

Carlisle SJ, Marciano-Cabral F, Staab A, Ludwick C, Cabral GA (2002). Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. *Int Immunopharmacol* **2**: 69–82.

Carroll FI, Blough BE, Mascarella SW, Navarro HA, Lukas RJ, Damaj MI (2014). Bupropion and Bupropion Analogs as Treatments for CNS Disorders. *Adv Pharmacol* **69**: 177–216.

Castañé A, Valjent E, Ledent C, Parmentier M, Maldonado R, Valverde O (2002). Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. *Neuropharmacology* **43**: 857–867.

Chang H, Saito T, Ohiwa N, Tateoka M, Deocaris CC, Fujikawa T, *et al* (2007). Inhibitory effects of an orexin-2 receptor antagonist on orexin A- and stress-induced ACTH responses in conscious rats. *Neurosci Res* **57**: 462–466.

Changeux J-P (1990). The TiPS lecture the nicotinic acetylcholine receptor: an allosteric protein prototype of ligand-gated ion channels. *Trends Pharmacol Sci* **11**: 485–492.

Chaudhri N, Caggiula AR, Donny EC, Palmatier MI, Liu X, Sved AF (2006). Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement. *Psychopharmacology (Berl)* **184**: 353–366.

Cheer JF, Wassum KM, Sombers LA, Heien MLA V, Ariansen JL, Aragona BJ, *et al* (2007). Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. *J Neurosci* **27**: 791–5.

Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, *et al* (1999). Narcolepsy in orexin Knockout MiceMolecular Genetics of Sleep Regulation. *Cell* **98**: 437–451.

Chen C (2015). Homeostatic regulation of brain functions by endocannabinoid signaling. *Neural Regen Res* **10**: 691–692.

Chen X-Y, Chen L, Du Y-F (2017). Orexin-A increases the firing activity of hippocampal CA1 neurons through orexin-1 receptors. *J Neurosci Res* **95**: 1415–1426.

Cheng D, Low JK, Logge W, Garner B, Karl T (2014a). Chronic cannabidiol treatment improves social and object recognition in double transgenic APPswe/PS1ΔE9 mice. *Psychopharmacology (Berl)* **231**: 3009–3017.

Cheng D, Spiro AS, Jenner AM, Garner B, Karl T (2014b). Long-term cannabidiol treatment prevents the development of social recognition memory deficits in Alzheimer's disease transgenic mice. *J Alzheimers Dis* **42**: 1383–96.

Chenoweth MJ, Sylvestre M-P, Contreras G, Novalen M, O'Loughlin J, Tyndale RF (2016). Variation in CYP2A6 and tobacco dependence throughout adolescence and in young adult smokers. *Drug Alcohol Depend* **158**: 139–146.

Chesher GB, Jackson DM (1985). The quasi-morphine withdrawal syndrome: effect of cannabinol, cannabidiol and tetrahydrocannabinol. *Pharmacol Biochem Behav* **23**: 13–5.

Chesnokova V, Pechnick RN, Wawrowsky K (2016). Chronic peripheral inflammation, hippocampal neurogenesis, and behavior. *Brain Behav Immun* **58**: 1–8.

Chiamulera C, Tedesco V, Zangrandi L, Giuliano C, Fumagalli G (2010). Propranolol transiently inhibits reinstatement of nicotine-seeking behaviour in rats. *J Psychopharmacol* **24**: 389–395.

Cho S-H, Chen JA, Sayed F, Ward ME, Gao F, Nguyen TA, *et al* (2015). SIRT1 Deficiency in Microglia Contributes to Cognitive Decline in Aging and Neurodegeneration via Epigenetic Regulation of IL-1. *J Neurosci* **35**: 807–818.

Cinciripini P, Aubin H-J, Dale L, Niaura R, Anthenelli R, Robinson J (2006). *STRATUS META 2006. Pooled analysis of three short-term, randomised, double-blind, placebo-controlled trials with rimonabant 20 mg/d in smoking cessation [PI-TS-01]. Society for Research on Nicotine and Tobacco 8th European Conference, September 2006, Kusa*. .

Cippitelli A, Astarita G, Duranti A, Caprioli G, Ubaldi M, Stopponi S, *et al* (2011). Endocannabinoid Regulation of Acute and Protracted Nicotine Withdrawal: Effect of FAAH Inhibition. *PLoS One* **6**: e28142.

Cohen C, Kodas E, Griebel G (2005). CB1 receptor antagonists for the treatment of nicotine addiction. *Pharmacol Biochem Behav* **81**: 387–95.

Cohen C, Perrault G, Voltz C, Steinberg R, Soubrié P (2002). SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behav Pharmacol* **13**: 451–63.

Cole RD, Poole RL, Guzman DM, Gould TJ, Parikh V (2015). Contributions of β2 subunit-containing nAChRs to chronic nicotine-induced alterations in cognitive flexibility in mice. *Psychopharmacology (Berl)* **232**: 1207–1217.

Control Center for Disease (2016). Health effects of cigarette smoking-fact sheet. .

Corrigall WA, Coen KM, Adamson KL (1994). Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. *Brain Res* **653**: 278–284.

Corwin EJ, Klein LC (2003). C-reactive protein and depressed mood in a sub-group of smokers during nicotine abstinence. *Hum Psychopharmacol Clin Exp* **18**: 329–337.

Cosgrove KP, Esterlis I, Sandiego C, Petrulli R, Morris ED (2015). Imaging Tobacco Smoking with PET and SPECT. 1–17doi:10.1007/978-3-319-13482-6_1.

Costa-Mattioli M, Monteggia LM (2013). mTOR complexes in neurodevelopmental and neuropsychiatric disorders. *Nat Neurosci* **16**: 1537–43.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**: 83–87.

Cristino L, Starowicz K, Petrocellis L De, Morishita J, Ueda N, Guglielmotti V, *et al* (2008). Immunohistochemical localization of anabolic and catabolic enzymes for anandamide and other putative endovanilloids in the hippocampus and cerebellar cortex of the mouse brain. *Neuroscience* **151**: 955–968.

Cryan JF, Bruijnzeel AW, Skjei KL, Markou A (2003). Bupropion enhances brain reward function and reverses the affective and somatic aspects of nicotine withdrawal in the rat. *Psychopharmacology (Berl)* **168**: 347–358.

Culhane MA, Schoenfeld DA, Barr RS, Cather C, Deckersbach T, Freudenreich O, *et al* (2008). Predictors of early abstinence in smokers with schizophrenia. *J Clin Psychiatry* **69**: 1743–50.

D'Souza MS, Markou A (2012). Schizophrenia and tobacco smoking comorbidity: nAChR agonists in the treatment of schizophrenia-associated cognitive deficits. *Neuropharmacology* **62**: 1564–73.

Damaj MI (2003). Characterization of Spontaneous and Precipitated Nicotine Withdrawal in the Mouse. *J Pharmacol Exp Ther* **307**: 526–534.

Dani JA (2015). Neuronal Nicotinic Acetylcholine Receptor Structure and Function and Response to Nicotine. *Int Rev Neurobiol* **124**: 3–19.

Davalos D, Grutzendler J, Yang G, Kim J V, Zuo Y, Jung S, *et al* (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci* **8**: 752–758.

Davis JA, Gould TJ (2006). The effects of DHBE and MLA on nicotine-induced enhancement of contextual fear conditioning in C57BL/6 mice. *Psychopharmacology (Berl)* **184**: 345–352.

Davis JA, Gould TJ (2007). Atomoxetine Reverses Nicotine Withdrawal-Associated Deficits in Contextual Fear Conditioning. *Neuropsychopharmacology* **32**: 2011–2019.

Davis JA, Gould TJ (2009). Hippocampal nAChRs mediate nicotine withdrawal-related learning deficits. *Eur Neuropsychopharmacol* **19**: 551–61.

Davis JA, James JR, Siegel SJ, Gould TJ (2005). Withdrawal from Chronic Nicotine Administration Impairs Contextual Fear Conditioning in C57BL/6 Mice. *J Neurosci* **25**: 8708–8713.

Davis JA, Kenney JW, Gould TJ (2007). Hippocampal 4 2 Nicotinic Acetylcholine Receptor Involvement in the Enhancing Effect of Acute Nicotine on Contextual Fear Conditioning. *J Neurosci* **27**: 10870–10877.

Dawkins L, Corcoran O (2014). Acute electronic cigarette use: nicotine delivery and subjective effects in regular users. *Psychopharmacology (Berl)* **231**: 401–407.

Delpech J-C, Madore C, Nadjar A, Joffre C, Wohleb ES, Layé S (2015). Microglia in neuronal plasticity: Influence of stress. *Neuropharmacology* **96**: 19–28.

Deng W, Aimone JB, Gage FH (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nat Rev Neurosci* **11**: 339–350.

Department of Health U, Services H (2014). The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office. at <https://www.surgeongeneral.gov/library/reports/50-years-of-progress/full-report.pdf>.

Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* **34**: .

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

Diergaarde L, Vries W de, Raasø H, Schoffelmeer ANM, Vries TJ De (2008). Contextual renewal of nicotine seeking in rats and its suppression by the cannabinoid-1 receptor antagonist Rimonabant (SR141716A). *Neuropharmacology* **55**: 712–716.

Ding Y, Shi W, Xie G, Yu A, Wang Q, Zhang Z (2015). CX3CR1 Mediates Nicotine Withdrawal-Induced Hyperalgesia via Microglial P38 MAPK Signaling. *Neurochem Res* **40**: 2252–2261.

Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, *et al* (2002a). Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci* **99**: 10819–10824.

Dinh TP, Freund TF, Piomelli D (2002b). A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem Phys Lipids* **121**: 149–58.

Dravolina OA, Zakharova ES, Shekunova E V., Zvartau EE, Danysz W, Bespalov AY (2007). mGlu1 receptor blockade attenuates cue- and nicotine-induced reinstatement of extinguished nicotine self-administration behavior in rats. *Neuropharmacology* **52**: 263–269.

Drug Safety and Availability - FDA (Center for Drug Evaluation and Research: 2016). *Drug Safety Communication: FDA revises description of mental health side effects of the stop-smoking medicines Chantix (varenicline) and Zyban (bupropion) to reflect clinical trial findings*. at <https://www.fda.gov/Drugs/DrugSafety/ucm532221.htm>.

Duque A, Vinader-Caerols C, Monleón S (2017). Indomethacin counteracts the effects of chronic social defeat stress on emotional but not recognition memory in mice. *PLoS One* **12**: e0173182.

Ebbert JO, Wyatt KD, Hays JT, Klee EW, Hurt RD (2010). Varenicline for smoking

cessation: efficacy, safety, and treatment recommendations. *Patient Prefer Adherence* **4**: 355–62.

Egea J, Buendia I, Parada E, Navarro E, León R, Lopez MG (2015). Anti-inflammatory role of microglial alpha7 nAChRs and its role in neuroprotection. *Biochem Pharmacol* **97**: 463–472.

Egertová M, Cravatt BF, Elphick MR (2003). Comparative analysis of fatty acid amide hydrolase and cb(1) cannabinoid receptor expression in the mouse brain: evidence of a widespread role for fatty acid amide hydrolase in regulation of endocannabinoid signaling. *Neuroscience* **119**: 481–96.

Ekdahl CT, Kokaia Z, Lindvall O (2009). Brain inflammation and adult neurogenesis: The dual role of microglia. *Neuroscience* **158**: 1021–1029.

Emad S, Qadeer S, Sadaf S, Batool Z, Haider S, Perveen T (2017). Attenuation of stress induced memory deficits by nonsteroidal anti-inflammatory drugs (NSAIDs) in rats: Role of antioxidant enzymes. *Pharmacol Reports* **69**: 300–305.

Ennaceur A (2010). One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behav Brain Res* **215**: 244–254.

Eriksson KS, Sergeeva O, Brown RE, Haas HL (2001). Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. *J Neurosci* **21**: 9273–9.

España RA, Melchior JR, Roberts DCS, Jones SR (2011). Hypocretin 1/orexin A in the ventral tegmental area enhances dopamine responses to cocaine and promotes cocaine self-administration. *Psychopharmacology (Berl)* **214**: 415–26.

España RA, Oleson EB, Locke JL, Brookshire BR, Roberts DCS, Jones SR (2010). The hypocretin-orexin system regulates cocaine self-administration via actions on the mesolimbic dopamine system. *Eur J Neurosci* **31**: 336–48.

Esposito G, Scuderi C, Valenza M, Togna GI, Latina V, Filippis D De, *et al* (2011). Cannabidiol reduces Aβ-induced neuroinflammation and promotes hippocampal neurogenesis through PPARγ involvement. *PLoS One* **6**: e28668.

Etter J-F, Bullen C (2011). Electronic cigarette: users profile, utilization, satisfaction and perceived efficacy. *Addiction* **106**: 2017–2028.

Etter J-F, Eissenberg T (2015). Dependence levels in users of electronic cigarettes, nicotine gums and tobacco cigarettes. *Drug Alcohol Depend* **147**: 68–75.

Evans DE, Sutton SK, Jentink KG, Lin H-Y, Park JY, Drobes DJ (2016). Cannabinoid receptor 1 (CNR1) gene variant moderates neural index of cognitive disruption during nicotine withdrawal. *Genes, Brain Behav* **15**: 621–626.

Fanselow MS, Dong H-W (2010). Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? *Neuron* **65**: 7–19.

Fasoli F, Moretti M, Zoli M, Pistillo F, Crespi A, Clementi F, *et al* (2016). In vivo chronic nicotine exposure differentially and reversibly affects upregulation and stoichiometry of α4β2 nicotinic receptors in cortex and thalamus. *Neuropharmacology* **108**: 324–331.

Feduccia AA, Chatterjee S, Bartlett SE (2012). Neuronal nicotinic acetylcholine receptors: neuroplastic changes underlying alcohol and nicotine addictions. *Front Mol Neurosci* **5**: 83.

Feltenstein MW, Ghee SM, See RE (2012). Nicotine self-administration and reinstatement of nicotine-seeking in male and female rats. *Drug Alcohol Depend* **121**: 240–6.

Fernández-Ruiz J, Gonzáles S (2005). Cannabinoid control of motor function at the basal ganglia. *Handb Exp Pharmacol* 479–507at <http://www.ncbi.nlm.nih.gov/pubmed/16596785>.

Fernández-Ruiz J, Sagredo O, Pazos MR, García C, Pertwee R, Mechoulam R, *et al* (2013). Cannabidiol for neurodegenerative disorders: important new clinical applications for this phytocannabinoid? *Br J Clin Pharmacol* **75**: 323–33.

File SE, Fluck E, Leahy A (2001). Nicotine has calming effects on stress-induced mood changes in females, but enhances aggressive mood in males. *Int J Neuropsychopharmacol* **4**: .

Filippidis FT, Laverty AA, Mons U, Jimenez-Ruiz C, Vardavas CI (2018). Changes in smoking cessation assistance in the European Union between 2012 and 2017: pharmacotherapy versus counselling versus e-cigarettes. *Tob Control* tobaccocontrol-2017-054117doi:10.1136/tobaccocontrol-2017-054117.

Fiore MC, Jaén CR (2008). A Clinical Blueprint to Accelerate the Elimination of Tobacco Use. *JAMA* **299**: 2083.

Flores Á, Saravia R, Maldonado R, Berrendero F (2015). Orexins and fear: implications for the treatment of anxiety disorders. *Trends Neurosci* **38**: 550–559.

Flores Á, Valls-Comamala V, Costa G, Saravia R, Maldonado R, Berrendero F (2014). The Hypocretin/Orexin System Mediates the Extinction of Fear Memories. *Neuropsychopharmacology* **39**: 2732–2741.

Flowers L (2016). Nicotine Replacement Therapy. *Am J Psychiatry Resid J* **11**: 4–7.

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

Foll B Le, Goldberg SR (2005). Control of the reinforcing effects of nicotine by associated environmental stimuli in animals and humans. *Trends Pharmacol Sci* **26**: 287–293.

Foll B Le, Goldberg SR (2009). Effects of nicotine in experimental animals and humans: An update on addictive properties. *Handb Exp Pharmacol* **192**:

335–367.

Foll B Le, Wertheim C, Goldberg SR (2007). High Reinforcing Efficacy of Nicotine in Non-Human Primates. *PLoS One* **2**: e230.

Fontainhas AM, Wang M, Liang KJ, Chen S, Mettu P, Damani M, *et al* (2011). Microglial Morphology and Dynamic Behavior Is Regulated by Ionotropic Glutamatergic and GABAergic Neurotransmission. *PLoS One* **6**: e15973.

Forget B, Wertheim C, Mascia P, Pushparaj A, Goldberg SR, Foll B Le (2010). Noradrenergic α1 Receptors as a Novel Target for the Treatment of Nicotine Addiction. *Neuropsychopharmacology* **35**: 1751–1760.

Foulds J, Stapleton J, Swettenham J, Bell N, McSorley K, Russell MA (1996). Cognitive performance effects of subcutaneous nicotine in smokers and never-smokers. *Psychopharmacology (Berl)* **127**: 31–8.

Fowler CD, Kenny PJ (2014). Nicotine aversion: Neurobiological mechanisms and relevance to tobacco dependence vulnerability. *Neuropharmacology* **76**: 533–544.

Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ (2011). Habenular α5 nicotinic receptor subunit signalling controls nicotine intake. *Nature* **471**: 597–601.

Frahm S, Ślimak MA, Ferrarese L, Santos-Torres J, Antolin-Fontes B, Auer S, *et al* (2011). Aversion to Nicotine Is Regulated by the Balanced Activity of β4 and α5 Nicotinic Receptor Subunits in the Medial Habenula. *Neuron* **70**: 522–535.

Frank MG, Thompson BM, Watkins LR, Maier SF (2012). Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. *Brain Behav Immun* **26**: 337–345.

Franklin TR, wang Z, Wang J, Sciortino N, Harper D, Li Y, *et al* (2007). Limbic Activation to Cigarette Smoking Cues Independent of Nicotine Withdrawal: A Perfusion fMRI Study. *Neuropsychopharmacology* **32**: 2301–2309.

Fuss J, Steinle J, Bindila L, Auer MK, Kirchherr H, Lutz B, *et al* (2015). A runner's high depends on cannabinoid receptors in mice. *Proc Natl Acad Sci* **112**: 13105–13108.

Gafford G, Jasnow AM, Ressler KJ (2014). Grin1 Receptor Deletion within CRF Neurons Enhances Fear Memory. *PLoS One* **9**: e111009.

Gaimarri A, Moretti M, Riganti L, Zanardi A, Clementi F, Gotti C (2007). Regulation of neuronal nicotinic receptor traffic and expression. *Brain Res Rev* **55**: 134–143.

Galve-Roperh I, Chiurchiù V, Díaz-Alonso J, Bari M, Guzmán M, Maccarrone M (2013). Cannabinoid receptor signaling in progenitor/stem cell proliferation and differentiation. *Prog Lipid Res* **52**: 633–650.

Gamaleddin I, Wertheim C, Zhu AZX, Coen KM, Vemuri K, Makryannis A, *et al*

(2012). Cannabinoid receptor stimulation increases motivation for nicotine and nicotine seeking. *Addict Biol* **17**: 47–61.

Gamaleddin IH, Trigo JM, Gueye AB, Zvonok A, Makriyannis A, Goldberg SR, *et al* (2015). Role of the endogenous cannabinoid system in nicotine addiction: novel insights. *Front psychiatry* **6**: 41.

Gaoni Y, Mechoulam R (1964). Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *J Am Chem Soc* **86**: 1646–1647.

Garcia-Rivas V, Deroche-Gamonet V (2018). Not all smokers appear to seek nicotine for the same reasons: Implications for preclinical research in nicotine dependence. *Addict Biol* doi:10.1111/adb.12607.

Ge S, Yang C-H, Hsu K-S, Ming G-L, Song H (2007). A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron* **54**: 559–66.

Giardino WJ, Lecea L de (2014). Hypocretin (orexin) neuromodulation of stress and reward pathways. *Curr Opin Neurobiol* **29**: 103–8.

Ginhoux F, Lim S, Hoeffel G, Low D, Huber T (2013). Origin and differentiation of microglia. *Front Cell Neurosci* **7**: 45.

Giniatullin R, Nistri A, Yakel JL (2005). Desensitization of nicotinic ACh receptors: Shaping cholinergic signaling. *Trends Neurosci* **28**: 371–378.

Gipson CD, Reissner KJ, Kupchik YM, Smith ACW, Stankeviciute N, Hensley-Simon ME, *et al* (2013). Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. *Proc Natl Acad Sci* **110**: 9124–9129.

Glienke K, Piefke M (2016). Acute social stress before the planning phase improves memory performance in a complex real life-related prospective memory task. *Neurobiol Learn Mem* **133**: 171–181.

Gomes F V., Llorente R, Bel EA Del, Viveros M-P, López-Gallardo M, Guimarães FS (2015). Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol. *Schizophr Res* **164**: 155–163.

Gomes F V., Resstel LBM, Guimarães FS (2011). The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. *Psychopharmacology (Berl)* **213**: 465–473.

Gorojankina T, Grébert D, Salesse R, Tanfin Z, Caillol M (2007). Study of orexins signal transduction pathways in rat olfactory mucosa and in olfactory sensory neurons-derived cell line Odora: Multiple orexin signalling pathways. *Regul Pept* **141**: 73–85.

Goshen I, Kreisel T, Ounallah-Saad H, Renbaum P, Zalzstein Y, Ben-Hur T, *et al* (2007). A dual role for interleukin-1 in hippocampal-dependent memory processes. *Psychoneuroendocrinology* **32**: 1106–1115.

Gotti C, Zoli M, Clementi F (2006). Brain nicotinic acetylcholine receptors: native

subtypes and their relevance. *Trends Pharmacol Sci* **27**: 482–91.

Gould TJ, Leach PT (2014). Cellular, molecular, and genetic substrates underlying the impact of nicotine on learning. *Neurobiol Learn Mem* doi:10.1016/j.nlm.2013.08.004.

Gould TJ, Portugal GS, André JM, Tadman MP, Marks MJ, Kenney JW, *et al* (2012). The duration of nicotine withdrawal-associated deficits in contextual fear conditioning parallels changes in hippocampal high affinity nicotinic acetylcholine receptor upregulation. *Neuropharmacology* **62**: 2118–25.

Gould TJ, Wehner JM (1999). Nicotine enhancement of contextual fear conditioning. *Behav Brain Res* **102**: 31–9.

Gowing LR, Ali RL, Allsop S, Marsden J, Turf EE, West R, *et al* (2015). Global statistics on addictive behaviours: 2014 status report. *Addiction* **110**: 904–919.

Graber TE, McCamphill PK, Sossin WS (2013). A recollection of mTOR signaling in learning and memory. *Learn Mem* **20**: 518–30.

Griebel G, Pichat P, Beeské S, Leroy T, Redon N, Jacquet A, *et al* (2015). Selective blockade of the hydrolysis of the endocannabinoid 2-arachidonoylglycerol impairs learning and memory performance while producing antinociceptive activity in rodents. *Sci Rep* **5**: 7642.

Grotenhermen F, Müller-Vahl K (2003). IACM 2nd Conference on Cannabinoids in Medicine. *Expert Opin Pharmacother* **4**: 2367–2371.

Grundey J, Thirugnanasambandam N, Kaminsky K, Drees A, Skwirba AC, Lang N, *et al* (2012). Neuroplasticity in cigarette smokers is altered under withdrawal and partially restituted by nicotine exposition. *J Neurosci* **32**: 4156–62.

Gulick D, Gould TJ (2008). Interactive effects of ethanol and nicotine on learning in C57BL/6J mice depend on both dose and duration of treatment. *Psychopharmacology (Berl)* **196**: 483–495.

Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, *et al* (2004). Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur J Neurosci* **20**: 441–458.

Hahn B, Ross TJ, Yang Y, Kim I, Huestis MA, Stein EA (2007). Nicotine enhances visuospatial attention by deactivating areas of the resting brain default network. *J Neurosci* **27**: 3477–3489.

Hall FS, Der-Avakian A, Gould TJ, Markou A, Shoaib M, Young JW (2015a). Negative affective states and cognitive impairments in nicotine dependence. *Neurosci Biobehav Rev* **58**: 168–185.

Hall SM, Shi Y, Humfleet GL, Muñoz RF, Reus VI, Prochaska JJ (2015b). Smoking cessation abstinence goal in treatment-seeking smokers. *Addict Behav* **42**:

148–153.

Haller G, Druley T, Vallania FL, Mitra RD, Li P, Akk G, *et al* (2012). Rare missense variants in CHRNB4 are associated with reduced risk of nicotine dependence. *Hum Mol Genet* **21**: 647–655.

Hamouda AK, Jackson A, Bagdas D, Imad Damaj M (2018). Reversal of Nicotine Withdrawal Signs Through Positive Allosteric Modulation of α4β2 Nicotinic Acetylcholine Receptors in Male Mice. *Nicotine Tob Res* **20**: 903–907.

Hampson RE, Deadwyler SA (2000). Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. *J Neurosci* **20**: 8932–42.

Hanisch U-K (2002). Microglia as a source and target of cytokines. *Glia* **40**: 140–155.

Hanisch U-K, Kettenmann H (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci* **10**: 1387–1394.

Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, *et al* (2001). 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci U S A* **98**: 3662–5.

Harenza JL, Muldoon PP, Biasi M De, Damaj MI, Miles MF (2014). Genetic variation within the Chrna7 gene modulates nicotine reward-like phenotypes in mice. *Genes, Brain Behav* **13**: 213–225.

Harkany T, Keimpema E, Barabás K, Mulder J (2008). Endocannabinoid functions controlling neuronal specification during brain development. *Mol Cell Endocrinol* **286**: S84–S90.

Harrington L, Viñals X, Herrera-Solís A, Flores A, Morel C, Tolu S, *et al* (2016). Role of β4* Nicotinic Acetylcholine Receptors in the Habenulo–Interpeduncular Pathway in Nicotine Reinforcement in Mice. *Neuropsychopharmacology* **41**: 1790–1802.

Harrison ELR, Coppola S, McKee SA (2009). Nicotine deprivation and trait impulsivity affect smokers' performance on cognitive tasks of inhibition and attention. *Exp Clin Psychopharmacol* **17**: 91–98.

Hasselmo ME (2006). The role of acetylcholine in learning and memory. *Curr Opin Neurobiol* **16**: 710–715.

Hein AM, O'Banion MK (2011). Neuroinflammation and memory : the role of prostaglandins. *Mol Neurobiol* **40**: 15–32.

Hein AM, Stasko MR, Matousek SB, Scott-McKean JJ, Maier SF, Olschowka JA, *et al* (2010). Sustained hippocampal IL-1β overexpression impairs contextual and spatial memory in transgenic mice. *Brain Behav Immun* **24**: 243–253.

Heishman SJ, Henningfield JE (2000). Tolerance to repeated nicotine administration on performance, subjective, and physiological responses in

nonsmokers. *Psychopharmacology (Berl)* **152**: 321–33.

Heishman SJ, Kleykamp BA, Singleton EG (2010). Meta-analysis of the acute effects of nicotine and smoking on human performance. *Psychopharmacology (Berl)* **210**: 453–469.

Heishman SJ, Taylor RC, Henningfield JE (1994). Nicotine and Smoking: A Review of Effects on Human Performance. **2**: 345–395.

Hendricks PS, Ditre JW, Drobes DJ, Brandon TH (2006). The early time course of smoking withdrawal effects. *Psychopharmacology (Berl)* **187**: 385–396.

Henningfield JE, Miyasato K, Jasinski DR (1985). Abuse liability and pharmacodynamic characteristics of intravenous and inhaled nicotine. *J Pharmacol Exp Ther* **234**: 1–12.

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

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

Herring WJ, Connor KM, Ivgy-May N, Snyder E, Liu K, Snavely DB, *et al* (2014). Suvorexant in Patients with Insomnia: Results from Two 3-Month Randomized Controlled Clinical Trials. *Biol Psychiatry* doi:10.1016/j.biopsych.2014.10.003.

Hindocha C, Freeman TP, Grabski M, Stroud JB, Crudgington H, Davies AC, *et al* (2018). Cannabidiol reverses attentional bias to cigarette cues in a human experimental model of tobacco withdrawal. *Addiction* **113**: 1696–1705.

Hine B, Torrelio M, Gershon S (1975a). Interactions between cannabidiol and Δ9-THC during abstinence in morphine-dependent rats. *Life Sci* **17**: 851–857.

Hine B, Torrelio M, Gershon S (1975b). Differential effect of cannabinol and cannabidiol on THC-induced responses during abstinence in morphine-dependent rats. *Res Commun Chem Pathol Pharmacol* **12**: 185–8.

Hoeffer CA, Klann E (2010). mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends Neurosci* **33**: 67–75.

Hollander JA, Lu Q, Cameron MD, Kamenecka TM, Kenny PJ (2008). Insular hypocretin transmission regulates nicotine reward. *Proc Natl Acad Sci* **105**: 19480–19485.

Howlett AC (2005). Cannabinoid receptor signaling. *Handb Exp Pharmacol* 53–79at <http://www.ncbi.nlm.nih.gov/pubmed/16596771>.

Howlett AC, Fleming RM (1984). Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Mol Pharmacol* **26**: 532–8.

Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, *et al* (2001). Blockade of Effects of Smoked Marijuana by the CB1-Selective Cannabinoid Receptor Antagonist SR141716. *Arch Gen Psychiatry* **58**: 322.

Hughes JR (2007a). Effects of abstinence from tobacco: Valid symptoms and time course. *Nicotine Tob Res* **9**: 315–327.

Hughes JR (2007b). Effects of abstinence from tobacco: Etiology, animal models, epidemiology, and significance: A subjective review. *Nicotine Tob Res* **9**: 329–339.

Hughes JR (2016). Varenicline as a Cause of Suicidal Outcomes. *Nicotine Tob Res* **18**: ntu275.

Hughes JR, Hatsukami D (1986). Signs and Symptoms of Tobacco Withdrawal. *Arch Gen Psychiatry* **43**: 289.

Hukkanen J, Jacob P, Benowitz NL (2005). Metabolism and disposition kinetics of nicotine. *Pharmacol Rev* **57**: 79–115.

Hupalo S, Berridge CW (2016). Working Memory Impairing Actions of Corticotropin-Releasing Factor (CRF) Neurotransmission in the Prefrontal Cortex. *Neuropsychopharmacology* **41**: 2733–40.

Hurd YL, Yoon M, Manini AF, Hernandez S, Olmedo R, Ostman M, *et al* (2015). Early Phase in the Development of Cannabidiol as a Treatment for Addiction: Opioid Relapse Takes Initial Center Stage. *Neurotherapeutics* **12**: 807–15.

Hyman SE, Malenka RC (2001). Addiction and the brain: The neurobiology of compulsion and its persistence. *Nat Rev Neurosci* **2**: 695–703.

Ignatowska-Jankowska BM, Muldoon PP, Lichtman AH, Damaj MI (2013). The cannabinoid CB2 receptor is necessary for nicotine-conditioned place preference, but not other behavioral effects of nicotine in mice. *Psychopharmacology (Berl)* **229**: 591–601.

Imayoshi I, Sakamoto M, Ohtsuka T, Kageyama R (2009). Continuous neurogenesis in the adult brain. *Dev Growth Differ* **51**: 379–386.

Jackson A, Papke RL, Damaj MI (2018). Pharmacological modulation of the α7 nicotinic acetylcholine receptor in a mouse model of mecamylamine-precipitated nicotine withdrawal. *Psychopharmacology (Berl)* 1–9doi:10.1007/s00213-018-4879-7.

Jackson KJ, Martin BR, Changeux JP, Damaj MI (2008). Differential role of nicotinic acetylcholine receptor subunits in physical and affective nicotine withdrawal signs. *J Pharmacol Exp Ther* **325**: 302–12.

Jackson KJ, McIntosh JM, Brunzell DH, Sanjakdar SS, Damaj MI (2009). The Role of 6-Containing Nicotinic Acetylcholine Receptors in Nicotine Reward and Withdrawal. *J Pharmacol Exp Ther* **331**: 547–554.

Jackson KJ, Muldoon PP, Biasi M De, Damaj MI (2015). New mechanisms and perspectives in nicotine withdrawal. *Neuropharmacology* **96**: 223–234.

Jacobsen LK, Krystal JH, Mencl WE, Westerveld M, Frost SJ, Pugh KR (2005). Effects of smoking and smoking abstinence on cognition in adolescent tobacco smokers. *Biol Psychiatry* **57**: 56–66.

Jacobsen LK, Mencl WE, Constable RT, Westerveld M, Pugh KR (2007). Impact of smoking abstinence on working memory neurocircuitry in adolescent daily tobacco smokers. *Psychopharmacology (Berl)* **193**: 557–566.

Jaeger LB, Farr SA, Banks WA, Morley JE (2002). Effects of orexin-A on memory processing. *Peptides* **23**: 1683–8.

Jessberger S, Gage FH (2014). Adult neurogenesis: bridging the gap between mice and humans. *Trends Cell Biol* **24**: 558–563.

Ji D, Dani JA (2000). Inhibition and Disinhibition of Pyramidal Neurons by Activation of Nicotinic Receptors on Hippocampal Interneurons. *J Neurophysiol* **83**: 2682–2690.

Ji D, Lape R, Dani JA (2001). Timing and Location of Nicotinic Activity Enhances or Depresses Hippocampal Synaptic Plasticity. *Neuron* **31**: 131–141.

Jia Y, Yamazaki Y, Nakauchi S, Ito KI, Sumikawa K (2010). Nicotine facilitates long-term potentiation induction in oriens-lacunosum moleculare cells via Ca2+ entry through non-α7 nicotinic acetylcholine receptors. *Eur J Neurosci* **31**: 463–476.

Johnson PL, Molosh A, Fitz SD, Truitt WA, Shekhar A (2012). Orexin, stress, and anxiety/panic states. *Prog Brain Res* **198**: 133–61.

Johnson PL, Truitt W, Fitz SD, Minick PE, Dietrich A, Sanghani S, *et al* (2010). A key role for orexin in panic anxiety. *Nat Med* **16**: 111–115.

Johnson PM, Hollander JA, Kenny PJ (2008). Decreased brain reward function during nicotine withdrawal in C57BL6 mice: Evidence from intracranial self-stimulation (ICSS) studies. *Pharmacol Biochem Behav* **90**: 409–415.

Jones RS, Lynch MA (2015). How dependent is synaptic plasticity on microglial phenotype? *Neuropharmacology* **96**: 3–10.

Kalivas PW, O'Brien C (2008). Drug Addiction as a Pathology of Staged Neuroplasticity. *Neuropsychopharmacology* **33**: 166–180.

Kane JK, Parker SL, Matta SG, Fu Y, Sharp BM, Li MD (2000). Nicotine Up-Regulates Expression of Orexin and Its Receptors in Rat Brain [1]. *Endocrinology* **141**: 3623–3629.

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

Kasai H, Fukuda M, Watanabe S, Hayashi-Takagi A, Noguchi J (2010). Structural

dynamics of dendritic spines in memory and cognition. *Trends Neurosci* **33**: 121–9.

Katona I, Freund TF (2012). Multiple functions of endocannabinoid signaling in the brain. *Annu Rev Neurosci* **35**: 529–58.

Katsidoni V, Anagnostou I, Panagis G (2013). Cannabidiol inhibits the reward-facilitating effect of morphine: involvement of 5-HT $_{1A}$ receptors in the dorsal raphe nucleus. *Addict Biol* **18**: 286–296.

Kauer JA (2004). Learning Mechanisms in Addiction: Synaptic Plasticity in the Ventral Tegmental Area as a Result of Exposure to Drugs of Abuse. *Annu Rev Physiol* **66**: 447–475.

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

Kawashima K, Fujii T, Moriwaki Y, Misawa H, Horiguchi K (2015). Non-neuronal cholinergic system in regulation of immune function with a focus on α7 nAChRs. *Int Immunopharmacol* **29**: 127–134.

Kayaba Y, Nakamura A, Kasuya Y, Ohuchi T, Yanagisawa M, Komuro I, *et al* (2003). Attenuated defense response and low basal blood pressure in orexin knockout mice. *Am J Physiol Integr Comp Physiol* **285**: R581–R593.

Kelley AE (2004). Memory and Addiction: Shared Neural Circuitry and Molecular Mechanisms. *Neuron* **44**: 161–179.

Kenney JW, Adoff MD, Wilkinson DS, Gould TJ (2011). The effects of acute, chronic, and withdrawal from chronic nicotine on novel and spatial object recognition in male C57BL/6J mice. *Psychopharmacology (Berl)* **217**: 353–365.

Kenney JW, Florian C, Portugal GS, Abel T, Gould TJ (2010). Involvement of Hippocampal Jun-N Terminal Kinase Pathway in the Enhancement of Learning and Memory by Nicotine. *Neuropsychopharmacology* **35**: 483–492.

Kenney JW, Gould TJ (2008). Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. *Mol Neurobiol* **38**: 101–21.

Kenney JW, Poole RL, Adoff MD, Logue SF, Gould TJ (2012a). Learning and Nicotine Interact to Increase CREB Phosphorylation at the jnk1 Promoter in the Hippocampus. *PLoS One* **7**: e39939.

Kenney JW, Raybuck JD, Gould TJ (2012b). Nicotinic receptors in the dorsal and ventral hippocampus differentially modulate contextual fear conditioning. *Hippocampus* **22**: 1681–90.

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

Kettenmann H, Kirchhoff F, Verkhratsky A (2013). Microglia: new roles for the synaptic stripper. *Neuron* **77**: 10–8.

Khakpai F, Nasehi M, Zarrindast M-R (2016). The role of NMDA receptors of the medial septum and dorsal hippocampus on memory acquisition. *Pharmacol Biochem Behav* **143**: 18–25.

Khoo SY-S, Brown RM (2014). Orexin/hypocretin based pharmacotherapies for the treatment of addiction: DORA or SORA? *CNS Drugs* **28**: 713–30.

Kida S, Serita T (2014). Functional roles of CREB as a positive regulator in the formation and enhancement of memory. *Brain Res Bull* **105**: 17–24.

Killen JD, Fortmann SP (1997). Craving is associated with smoking relapse: Findings from three prospective studies. *Exp Clin Psychopharmacol* **5**: 137–142.

Kim H-Y, Hong E, Kim J-I, Lee W (2004). Solution structure of human orexin-A: regulator of appetite and wakefulness. *J Biochem Mol Biol* **37**: 565–73.

Kishimoto Y, Kano M (2006). Endogenous Cannabinoid Signaling through the CB1 Receptor Is Essential for Cerebellum-Dependent Discrete Motor Learning. *J Neurosci* **26**: 8829–8837.

Kitamura T, Tsuchihashi Y, Fujita S (1978). Initial response of silver-impregnated ?resting microglia? to stab wounding in rabbit hippocampus. *Acta Neuropathol* **44**: 31–39.

Kitazawa M, Cheng D, Tsukamoto MR, Koike MA, Wes PD, Vasilevko V, *et al* (2011). Blocking IL-1 Signaling Rescues Cognition, Attenuates Tau Pathology, and Restores Neuronal -Catenin Pathway Function in an Alzheimer's Disease Model. *J Immunol* **187**: 6539–6549.

Kofler J, Wiley CA (2011). Microglia. *Toxicol Pathol* **39**: 103–114.

Kohlmeier KA, Inoue T, Leonard CS (2004). Hypocretin/Orexin Peptide Signaling in the Ascending Arousal System: Elevation of Intracellular Calcium in the Mouse Dorsal Raphe and Laterodorsal Tegmentum. *J Neurophysiol* **92**: 221–235.

Kohman RA, Rhodes JS (2013). Neurogenesis, inflammation and behavior. *Brain Behav Immun* **27**: 22–32.

Koob GF, Gilpin NW, Boutrel B (2013). Addiction is a reward deficit and stress surfeit disorder. *Front Psychiatry* **4**: 1–18.

Koob GF, Moal M Le (2008a). Review. Neurobiological mechanisms for opponent motivational processes in addiction. *Philos Trans R Soc Lond B Biol Sci* **363**: 3113–23.

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

Koob GF, Volkow ND (2010). Neurocircuitry of addiction. *Neuropsychopharmacology* **35**: 217–38.

Koob GF, Volkow ND (2016). Neurobiology of addiction: a neurocircuitry analysis. *The Lancet Psychiatry* **3**: 760–773.

Kornetsky C, Esposito RU (1979). Euphorigenic drugs: effects on the reward pathways of the brain. *Fed Proc* **38**: 2473–6.

Korpi ER, Hollander B den, Farooq U, Vashchinkina E, Rajkumar R, Nutt DJ, *et al* (2015). Mechanisms of Action and Persistent Neuroplasticity by Drugs of Abuse. *Pharmacol Rev* **67**: 872–1004.

Kostenis E (2004). Novel clusters of receptors for sphingosine-1-phosphate, sphingosylphosphorylcholine, and (lyso)-phosphatidic acid: New receptors for ?Old? ligands. *J Cell Biochem* **92**: 923–936.

Kozela E, Lev N, Kaushansky N, Eilam R, Rimmerman N, Levy R, *et al* (2011). Cannabidiol inhibits pathogenic T cells, decreases spinal microglial activation and ameliorates multiple sclerosis-like disease in C57BL/6 mice. *Br J Pharmacol* **163**: 1507–1519.

Kozela E, Pietr M, Juknat A, Rimmerman N, Levy R, Vogel Z (2010). Cannabinoids Delta(9)-tetrahydrocannabinol and cannabidiol differentially inhibit the lipopolysaccharide-activated NF-kappaB and interferon-beta/STAT proinflammatory pathways in BV-2 microglial cells. *J Biol Chem* **285**: 1616–26.

Kruk-Slomka M, Dzik A, Budzynska B, Biala G (2017). Endocannabinoid System: the Direct and Indirect Involvement in the Memory and Learning Processes—a Short Review. *Mol Neurobiol* **54**: 8332–8347.

Kukkonen JP (2017). Orexin/Hypocretin signaling. *Curr Top Behav Neurosci* **33**: 17–50.

Kukkonen JP, Akerman KE (2001). Orexin receptors couple to Ca2+ channels different from store-operated Ca2+ channels. *Neuroreport* **12**: 2017–20.

Kumari V, Postma P (2005). Nicotine use in schizophrenia: The self medication hypotheses. *Neurosci Biobehav Rev* **29**: 1021–1034.

Kutlu MG, Gould TJ (2016a). Nicotinic modulation of hippocampal cell signaling and associated effects on learning and memory. *Physiol Behav* **155**: 162–171.

Kutlu MG, Gould TJ (2016b). Effects of drugs of abuse on hippocampal plasticity and hippocampus-dependent learning and memory: contributions to development and maintenance of addiction. *Learn Mem* **23**: 515–33.

Kutlu MG, Holliday E, Gould TJ (2016). High-affinity α4β2 nicotinic receptors mediate the impairing effects of acute nicotine on contextual fear extinction. *Neurobiol Learn Mem* **128**: 17–22.

Kutlu MG, Parikh V, Gould TJ (2015). Nicotine Addiction and Psychiatric Disorders. *Int Rev Neurobiol* **124**: 171–208.

Laaris N, Good CH, Lupica CR (2010). Δ9-tetrahydrocannabinol is a full agonist at CB1 receptors on GABA neuron axon terminals in the hippocampus. *Neuropharmacology* **59**: 121–127.

Lambe EK, Olausson P, Horst NK, Taylor JR, Aghajanian GK (2005). Hypocretin and nicotine excite the same thalamocortical synapses in prefrontal cortex: correlation with improved attention in rat. *J Neurosci* **25**: 5225–9.

Larsson KP, Peltonen HM, Bart G, Louhivuori LM, Penttonen A, Antikainen M, *et al* (2005). Orexin-A-induced Ca ^{2} + Entry. *J Biol Chem* **280**: 1771–1781.

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

Leach PT, Cordero KA, Gould TJ (2013). The effects of acute nicotine, chronic nicotine, and withdrawal from chronic nicotine on performance of a cued appetitive response. *Behav Neurosci* **127**: 303–10.

Lecea L de, Kilduff TS, Peyron C, Gao X-B, Foye PE, Danielson PE, *et al* (1998). The hypocretins: Hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci* **95**: 322–327.

Lee H-W, Park S-H, Weng M, Wang H-T, Huang WC, Lepor H, *et al* (2018). E-cigarette smoke damages DNA and reduces repair activity in mouse lung, heart, and bladder as well as in human lung and bladder cells. *Proc Natl Acad Sci* 201718185doi:10.1073/PNAS.1718185115.

Lee JH, Bang E, Chae KJ, Kim JY, Lee DW, Lee W (1999). Solution structure of a new hypothalamic neuropeptide, human hypocretin-2/orexin-B. *Eur J Biochem* **266**: 831–9.

Leo A, Russo E, Elia M (2016). Cannabidiol and epilepsy: Rationale and therapeutic potential. *Pharmacol Res* **107**: 85–92.

Lerman C, Schnoll RA, Hawk LW, Cinciripini P, George TP, Wileyto EP, *et al* (2015). Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: A randomised, double-blind placebo-controlled trial. *Lancet Respir Med* **3**: 131–138.

LeSage MG, Perry JL, Kotz CM, Shelley D, Corrigall WA (2010). Nicotine self-administration in the rat: effects of hypocretin antagonists and changes in hypocretin mRNA. *Psychopharmacology (Berl)* **209**: 203–12.

Li J, Hu Z, Lecea L de (2014). The hypocretins/orexins: integrators of multiple physiological functions. *Br J Pharmacol* **171**: 332–350.

Liechti ME, Lhuillier L, Kaupmann K, Markou A (2007). Metabotropic glutamate 2/3 receptors in the ventral tegmental area and the nucleus accumbens shell are involved in behaviors relating to nicotine dependence. *J Neurosci* **27**: 9077–85.

Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, *et al* (1999). The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* **98**: 365–76.

Liu L, Zhao-Shea R, McIntosh JM, Gardner PD, Tapper AR (2012). Nicotine persistently activates ventral tegmental area dopaminergic neurons via nicotinic acetylcholine receptors containing α4 and α6 subunits. *Mol Pharmacol* **81**: 541–8.

Liu X (2016). Characterization of Cue-Induced Reinstatement of Nicotine-Seeking Behavior in Smoking Relapse: Use of Animal Models. *Neuropathol Drug Addict Subst Misuse* 237–245doi:10.1016/B978-0-12-800213-1.00022-5.

Logue SF, Paylor R, Wehner JM (1997). Hippocampal lesions cause learning deficits in inbred mice in the Morris water maze and conditioned-fear task. *Behav Neurosci* **111**: 104–113.

Lombardo S, Maskos U (2015). Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. *Neuropharmacology* **96**: 255–262.

López-Hidalgo M, Salgado-Puga K, Alvarado-Martínez R, Medina AC, Prado-Alcalá R a, García-Colunga J (2012). Nicotine uses neuron-glia communication to enhance hippocampal synaptic transmission and long-term memory. *PLoS One* **7**: e49998.

Loscher CE, Mills KHG, Lynch MA (2003). Interleukin-1 receptor antagonist exerts agonist activity in the hippocampus independent of the interleukin-1 type I receptor. *J Neuroimmunol* **137**: 117–124.

Loughead J, Ray R, Wileyto EP, Ruparel K, Sanborn P, Siegel S, *et al* (2010). Effects of the alpha4beta2 partial agonist varenicline on brain activity and working memory in abstinent smokers. *Biol Psychiatry* **67**: 715–21.

Loughead J, Wileyto EP, Ruparel K, Falcone M, Hopson R, Gur R, *et al* (2015). Working memory-related neural activity predicts future smoking relapse. *Neuropsychopharmacology* **40**: 1311–20.

Loughead J, Wileyto EP, Valdez JN, Sanborn P, Tang K, Strasser AA, *et al* (2009). Effect of abstinence challenge on brain function and cognition in smokers differs by COMT genotype. *Mol Psychiatry* **14**: 820–826.

Lupica CR, Hu Y, Devinsky O, Hoffman AF (2017). Cannabinoids as hippocampal network administrators. *Neuropharmacology* **124**: 25–37.

Lutz B, Marsicano G, Maldonado R, Hillard CJ (2015). The endocannabinoid system in guarding against fear, anxiety and stress. *Nat Rev Neurosci* **16**: 705–718.

Makhija K, Karunakaran S (2013). The role of inflammatory cytokines on the aetiopathogenesis of depression. *Aust New Zeal J Psychiatry* **47**: 828–839.

Maldonado R, Baños JE, Cabañero D (2016). The endocannabinoid system and neuropathic pain. *Pain* **157**: S23–S32.

Maldonado R, Valverde O, Berrendero F (2006). Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci* **29**: 225–32.

Malfait AM, Gallily R, Sumariwalla PF, Malik AS, Andreakos E, Mechoulam R, *et al* (2000). The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. *Proc Natl Acad Sci U S A* **97**: 9561–6.

Mansvelder HD, Keath JR, McGehee DS (2002). Synaptic Mechanisms Underlie Nicotine-Induced Excitability of Brain Reward Areas. *Neuron* **33**: 905–919.

Maras PM, Baram TZ (2012). Sculpting the hippocampus from within: stress, spines, and CRH. *Trends Neurosci* **35**: 315–24.

Markou A (2008). Neurobiology of nicotine dependence. *Philos Trans R Soc Lond B Biol Sci* **363**: 3159–68.

Marsicano G, Lafenêtre P (2009). Roles of the Endocannabinoid System in Learning and Memory. *Curr Top Behav Neurosci* **1**: 201–230.

Marsicano G, Lutz B (2006). Neuromodulatory functions of the endocannabinoid system. *J Endocrinol Invest* **29**: 27–46.

Martín-García E, Barbano MF, Galeote L, Maldonado R (2009). New operant model of nicotine-seeking behaviour in mice. *Int J Neuropsychopharmacol* **12**: 343.

Martín-García E, Bourgoin L, Cathala A, Kasanetz F, Mondesir M, Gutiérrez-Rodriguez A, *et al* (2016). Differential Control of Cocaine Self-Administration by GABAergic and Glutamatergic CB1 Cannabinoid Receptors. *Neuropsychopharmacology* **41**: 2192–205.

Martín-Moreno AM, Reigada D, Ramírez BG, Mechoulam R, Innamorato N, Cuadrado A, *et al* (2011). Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. *Mol Pharmacol* **79**: 964–73.

Marzo V Di, Petrocellis L De, Bisogno T (2005). The biosynthesis, fate and pharmacological properties of endocannabinoids. *Handb Exp Pharmacol* 147–85at <http://www.ncbi.nlm.nih.gov/pubmed/16596774>.

Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux J-P, *et al* (2005). Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. *Nature* **436**: 103–107.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**: 561–564.

Matsuzaki M, Ellis-Davies GCR, Nemoto T, Miyashita Y, Iino M, Kasai H (2001). Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nat Neurosci* **4**: 1086–1092.

Matta SG, Balfour DJ, Benowitz NL, Boyd RT, Buccafusco JJ, Caggiula AR, *et al* (2007). Guidelines on nicotine dose selection for in vivo research. *Psychopharmacology (Berl)* **190**: 269–319.

Maurer S V., Williams CL (2017). The Cholinergic System Modulates Memory and Hippocampal Plasticity via Its Interactions with Non-Neuronal Cells. *Front Immunol* **8**: 1489.

McClain JA, Morris SA, Deeny MA, Marshall SA, Hayes DM, Kiser ZM, *et al* (2011). Adolescent binge alcohol exposure induces long-lasting partial activation of microglia. *Brain Behav Immun* **25**: S120–S128.

McClernon FJ, Gilbert DG, Radtke R (2003). Effects of transdermal nicotine on lateralized identification and memory interference. *Hum Psychopharmacol Clin Exp* **18**: 339–343.

McCullough AM, Ritchey M, Ranganath C, Yonelinas A (2015). Differential effects of stress-induced cortisol responses on recollection and familiarity-based recognition memory. *Neurobiol Learn Mem* **123**: 1–10.

McKim DB, Niraula A, Tarr AJ, Wohleb ES, Sheridan JF, Godbout JP (2016). Neuroinflammatory Dynamics Underlie Memory Impairments after Repeated Social Defeat. *J Neurosci* **36**: 2590–2604.

McLaughlin I, Dani JA, Biasi M De (2015). Nicotine Withdrawal. 99–123doi:10.1007/978-3-319-13482-6_4.

Mecha M, Feliú A, Iñigo PM, Mestre L, Carrillo-Salinas FJ, Guaza C (2013). Cannabidiol provides long-lasting protection against the deleterious effects of inflammation in a viral model of multiple sclerosis: A role for A2A receptors. *Neurobiol Dis* **59**: 141–150.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, *et al* (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* **50**: 83–90.

Mechoulam R, Parker LA (2013). The Endocannabinoid System and the Brain. *Annu Rev Psychol* **64**: 21–47.

Mechoulam R, Parker LA, Gallily R (2002). Cannabidiol: an overview of some pharmacological aspects. *J Clin Pharmacol* **42**: 11S–19S.

Mechoulam R, Shvo Y (1963). Hashish—I : The structure of Cannabidiol. *Tetrahedron* **19**: 2073–2078.

Merritt LL, Martin BR, Walters C, Lichtman AH, Damaj MI (2008). The endogenous cannabinoid system modulates nicotine reward and dependence. *J Pharmacol Exp Ther* **326**: 483–92.

Merritt PS, Cobb AR, Cook GI (2012). Sex differences in the cognitive effects of tobacco abstinence: A pilot study. *Exp Clin Psychopharmacol* **20**: 258–263.

Merritt PS, Cobb AR, Moissinac L, Hirshman E (2010). Evidence That Episodic

Memory Impairment During Tobacco Abstinence Is Independent of Attentional Mechanisms. *J Gen Psychol* **137**: 331–342.

Michelson D, Snyder E, Paradis E, Chengan-Liu M, Snavely DB, Hutzelmann J, *et al* (2014). Safety and efficacy of suvorexant during 1-year treatment of insomnia with subsequent abrupt treatment discontinuation: a phase 3 randomised, double-blind, placebo-controlled trial. *Lancet Neurol* **13**: 461–71.

Miglin R, Kable JW, Bowers ME, Ashare RL (2017). Withdrawal-related changes in delay discounting predict short-term smoking abstinence. *Nicotine Tob Res* **19**: 694–702.

Mihalak KB, Carroll FI, Luetje CW (2006). Varenicline Is a Partial Agonist at 4beta2 and a Full Agonist at 7 Neuronal Nicotinic Receptors. *Mol Pharmacol* **70**: 801–805.

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

Monje ML, Toda H, Palmer TD (2003). Inflammatory Blockade Restores Adult Hippocampal Neurogenesis. *Science (80-)* **302**: 1760–1765.

Monory K, Polack M, Remus A, Lutz B, Korte M (2015). Cannabinoid CB1 receptor calibrates excitatory synaptic balance in the mouse hippocampus. *J Neurosci* **35**: 3842–50.

Moraes CA, Santos G, Spohr TCL de S e, D'Avila JC, Lima FRS, Benjamim CF, *et al* (2015). Activated Microglia-Induced Deficits in Excitatory Synapses Through IL-1β: Implications for Cognitive Impairment in Sepsis. *Mol Neurobiol* **52**: 653–663.

Moreira FA, Crippa JAS (2009). The psychiatric side-effects of rimonabant. *Rev Bras Psiquiatr* **31**: 145–153.

Morena M, Patel S, Bains JS, Hill MN (2016). Neurobiological Interactions Between Stress and the Endocannabinoid System. *Neuropsychopharmacology* **41**: 80–102.

Morgan CJA, Das RK, Joye A, Curran HV, Kamboj SK (2013). Cannabidiol reduces cigarette consumption in tobacco smokers: preliminary findings. *Addict Behav* **38**: 2433–6.

Mori MA, Meyer E, Soares LM, Milani H, Guimarães FS, Oliveira RMW de (2016). Cannabidiol reduces neuroinflammation and promotes neuroplasticity and functional recovery after brain ischemia. *Prog Neuro-Psychopharmacology Biol Psychiatry* doi:10.1016/j.pnpbp.2016.11.005.

Morris GP, Clark IA, Zinn R, Vissel B (2013). Microglia: A new frontier for synaptic plasticity, learning and memory, and neurodegenerative disease research. *Neurobiol Learn Mem* **105**: 40–53.

Muldoon PP, Chen J, Harenza JL, Abdullah RA, Sim-Selley LJ, Cravatt BF, *et al* (2015). Inhibition of monoacylglycerol lipase reduces nicotine withdrawal. *Br J Pharmacol* **172**: 869–882.

Muldoon PP, Lichtman AH, Parsons LH, Damaj MI (2013). The role of fatty acid amide hydrolase inhibition in nicotine reward and dependence. *Life Sci* **92**: .

Munro S, Thomas KL, Abu-Shaar M (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365**: 61–65.

Myers CS, Taylor RC, Moolchan ET, Heishman SJ (2008). Dose-Related Enhancement of Mood and Cognition in Smokers Administered Nicotine Nasal Spray. *Neuropsychopharmacology* **33**: 588–598.

Nakauchi S, Sumikawa K (2012). Endogenously released ACh and exogenous nicotine differentially facilitate long-term potentiation induction in the hippocampal CA1 region of mice. *Eur J Neurosci* **35**: 1381–1395.

Napimoga MH, Benatti BB, Lima FO, Alves PM, Campos AC, Pena-dos-Santos DR, *et al* (2009). Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. *Int Immunopharmacol* **9**: 216–222.

Naqvi NH, Rudrauf D, Damasio H, Bechara A (2007). Damage to the insula disrupts addiction to cigarette smoking. *Science* **315**: 531–4.

Narita M, Nagumo Y, Hashimoto S, Narita M, Khotib J, Miyatake M, *et al* (2006). Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. *J Neurosci* **26**: 398–405.

Navarrete F, Rodríguez-Arias M, Martín-García E, Navarro D, García-Gutiérrez MS, Aguilar M a, *et al* (2013). Role of CB2 Cannabinoid Receptors in the Rewarding, Reinforcing, and Physical Effects of Nicotine. *Neuropsychopharmacology* **38**: 2515–24.

Nestler EJ (2005). Is there a common molecular pathway for addiction? *Nat Neurosci* **8**: 1445–1449.

Newhouse P, Kellar K, Aisen P, White H, Wesnes K, Coderre E, *et al* (2012). Nicotine treatment of mild cognitive impairment: a 6-month double-blind pilot clinical trial. *Neurology* **78**: 91–101.

Nimmerjahn A, Kirchhoff F, Helmchen F (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* **308**: 1314–8.

Nisell M, Nomikos GG, Svensson TH (1994). Infusion of Nicotine in the Ventral Tegmental Area or the Nucleus Accumbens of the Rat Differentially Affects Accumbal Dopamine Release. *Pharmacol Toxicol* **75**: 348–352.

Novere N Le, Grutter T, Changeux J-P (2002). Models of the extracellular domain of the nicotinic receptors and of agonist- and Ca2+-binding sites. *Proc Natl*

Acad Sci **99**: 3210–3215.

Nygard SK, Hourguettes NJ, Sobczak GG, Carlezon WA, Bruchas MR (2016). Stress-Induced Reinstatement of Nicotine Preference Requires Dynorphin/Kappa Opioid Activity in the Basolateral Amygdala. *J Neurosci* **36**: 9937–48.

O'Doherty J, Kringelbach ML, Rolls ET, Hornak J, Andrews C (2001). Abstract reward and punishment representations in the human orbitofrontal cortex. *Nat Neurosci* **4**: 95–102.

O'Sullivan SE (2009). Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Br J Pharmacol* **152**: 576–582.

Ohgidani M, Kato TA, Sagata N, Hayakawa K, Shimokawa N, Sato-Kasai M, *et al* (2016). TNF-α from hippocampal microglia induces working memory deficits by acute stress in mice. *Brain Behav Immun* **55**: 17–24.

Ohno-Shosaku T, Maejima T, Kano M (2001). Endogenous Cannabinoids Mediate Retrograde Signals from Depolarized Postsynaptic Neurons to Presynaptic Terminals. *Neuron* **29**: 729–738.

Olincy A, Harris JG, Johnson LL, Pender V, Kongs S, Allensworth D, *et al* (2006). Proof-of-Concept Trial of an α7 Nicotinic Agonist in Schizophrenia. *Arch Gen Psychiatry* **63**: 630.

Ortega-Álvaro A, Ternianov A, Aracil-Fernández A, Navarrete F, García-Gutiérrez MS, Manzanares J (2015). Role of cannabinoid CB_2 receptor in the reinforcing actions of ethanol. *Addict Biol* **20**: 43–55.

Ortega LA, Tracy BA, Gould TJ, Parikh V (2013). Effects of chronic low- and high-dose nicotine on cognitive flexibility in C57BL/6J mice. *Behav Brain Res* **238**: 134–145.

Osborne AL, Solowij N, Weston-Green K (2017). A systematic review of the effect of cannabidiol on cognitive function: Relevance to schizophrenia. *Neurosci Biobehav Rev* **72**: 310–324.

Pablos RM de, Herrera AJ, Espinosa-Oliva AM, Sarmiento M, Muñoz MF, Machado A, *et al* (2014). Chronic stress enhances microglia activation and exacerbates death of nigral dopaminergic neurons under conditions of inflammation. *J Neuroinflammation* **11**: 34.

Parikh V, Cole RD, Patel PJ, Poole RL, Gould TJ (2016). Cognitive control deficits during mecamylamine-precipitated withdrawal in mice: Possible links to frontostriatal BDNF imbalance. *Neurobiol Learn Mem* **128**: 110–116.

Parker LA, Burton P, Sorge RE, Yakiwchuk C, Mechoulam R (2004). Effect of low doses of ?9-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. *Psychopharmacology (Berl)* **175**: 360–366.

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

Pascual M, Blanco AM, Cauli O, Miñarro J, Guerri C (2007). Intermittent ethanol exposure induces inflammatory brain damage and causes long-term behavioural alterations in adolescent rats. *Eur J Neurosci* **25**: 541–550.

Pasumarthi RK, Reznikov LR, Fadel J (2006). Activation of orexin neurons by acute nicotine. *Eur J Pharmacol* **535**: 172–176.

Patterson F, Jepson C, Loughead J, Perkins K, Strasser A a, Siegel S, *et al* (2010). Working memory deficits predict short-term smoking resumption following brief abstinence. *Drug Alcohol Depend* **106**: 61–4.

Patterson F, Jepson C, Strasser AA, Loughead J, Perkins KA, Gur RC, *et al* (2009). Varenicline improves mood and cognition during smoking abstinence. *Biol Psychiatry* **65**: 144–9.

Patterson SL (2015). Immune dysregulation and cognitive vulnerability in the aging brain: Interactions of microglia, IL-1β, BDNF and synaptic plasticity. *Neuropharmacology* **96**: 11–18.

Pazos MR, Mohammed N, Lafuente H, Santos M, Martínez-Pinilla E, Moreno E, *et al* (2013). Mechanisms of cannabidiol neuroprotection in hypoxic–ischemic newborn pigs: Role of 5HT1A and CB2 receptors. *Neuropharmacology* **71**: 282–291.

Peres FF, Levin R, Suiama MA, Diana MC, Gouvêa DA, Almeida V, *et al* (2016). Cannabidiol Prevents Motor and Cognitive Impairments Induced by Reserpine in Rats. *Front Pharmacol* **7**: 343.

Peřinová P, Feketeová E, Kemlink D, Kovalská P, Chlebušová K, Nepožitek J, *et al* (2016). Smoking Prevalence and Its Clinical Correlations in Patients with Narcolepsy-cataplexy. *Prague Med Rep* **117**: 81–89.

Perkins KA, Karelitz JL, Jao NC, Gur RC, Lerman C (2013). Effects of bupropion on cognitive performance during initial tobacco abstinence. *Drug Alcohol Depend* **133**: 283–286.

Perkins KA, Kunkle N, Michael VC, Karelitz JL, Donny EC (2016). Assessing Discrimination of Nicotine in Humans Via Cigarette Smoking. *Nicotine Tob Res* **18**: 1830–6.

Perry DC, Xiao Y, Nguyen HN, Musachio JL, Dávila-García MI, Kellar KJ (2002). Measuring nicotinic receptors with characteristics of α4β2, α3β2 and α3β4 subtypes in rat tissues by autoradiography. *J Neurochem* **82**: 468–481.

Pertwee RG (2008). The diverse CB_1 and CB_2 receptor pharmacology of three plant cannabinoids: Δ^9-tetrahydrocannabinol, cannabidiol and Δ^9-tetrahydrocannabivarin. *Br J Pharmacol* **153**: 199–215.

Pertwee RG (2009). GPR55: a new member of the cannabinoid receptor clan? *Br J Pharmacol* **152**: 984–986.

Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Marzo V Di, Elphick MR, *et al* (2010). International Union of Basic and Clinical Pharmacology. LXXIX.

Cannabinoid Receptors and Their Ligands: Beyond CB1 and CB2. *Pharmacol Rev* **62**: 588–631.

Perveen T, Emad S, Haider S, Sadaf S, Qadeer S, Batool Z, *et al* (2018). Role of Cyclooxygenase Inhibitors in Diminution of Dissimilar Stress-induced Depressive Behavior and Memory Impairment in Rats. *Neuroscience* **370**: 121–129.

Petrocellis L De, Ligresti A, Moriello AS, Allarà M, Bisogno T, Petrosino S, *et al* (2011). Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. *Br J Pharmacol* **163**: 1479–1494.

Petrocellis L De, Marzo V Di (2010). Non-CB1, Non-CB2 Receptors for Endocannabinoids, Plant Cannabinoids, and Synthetic Cannabimimetics: Focus on G-protein-coupled Receptors and Transient Receptor Potential Channels. *J Neuroimmune Pharmacol* **5**: 103–121.

Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, *et al* (2000). A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* **6**: 991–7.

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

Picciotto MR, Addy NA, Mineur YS, Brunzell DH (2008). It is not "either/or": Activation and desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. *Prog Neurobiol* **84**: 329–342.

Picciotto MR, Kenny PJ (2013). Molecular mechanisms underlying behaviors related to nicotine addiction. *Cold Spring Harb Perspect Med* **3**: a012112.

Picciotto MR, Mineur YS (2014). Molecules and circuits involved in nicotine addiction: The many faces of smoking. *Neuropharmacology* **76**: 545–553.

Picciotto MR, Zoli M, Rimondini R, Léna C, Marubio LM, Pich EM, *et al* (1998). Acetylcholine receptors containing the β2 subunit are involved in the reinforcing properties of nicotine. *Nature* **391**: 173–177.

Place R, Lykken C, Beer Z, Suh J, McHugh TJ, Tonegawa S, *et al* (2012). NMDA signaling in CA1 mediates selectively the spatial component of episodic memory. *Learn Mem* **19**: 164–169.

Plaza-Zabala A, Flores Á, Maldonado R, Berrendero F (2012a). Hypocretin/orexin signaling in the hypothalamic paraventricular nucleus is essential for the expression of nicotine withdrawal. *Biol Psychiatry* **71**: 214–23.

Plaza-Zabala A, Flores Á, Martín-García E, Saravia R, Maldonado R, Berrendero F (2013). A role for hypocretin/orexin receptor-1 in cue-induced reinstatement of nicotine-seeking behavior. *Neuropsychopharmacology* **38**:

1724–36.

Plaza-Zabala A, Maldonado R, Berrendero F (2012b). The hypocretin/orexin system: Implications for drug reward and relapse. *Mol Neurobiol* **45**: 424–439.

Plaza-Zabala A, Martín-García E, Lecea L de, Maldonado R, Berrendero F (2010). Hypocretins regulate the anxiogenic-like effects of nicotine and induce reinstatement of nicotine-seeking behavior. *J Neurosci* **30**: 2300–10.

Pol AN van den, Gao XB, Obrietan K, Kilduff TS, Belousov AB (1998). Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. *J Neurosci* **18**: 7962–71.

Polosa R, Benowitz NL (2011). Treatment of nicotine addiction: present therapeutic options and pipeline developments. *Trends Pharmacol Sci* **32**: 281–9.

Poltavski DV, Petros T (2006). Effects of transdermal nicotine on attention in adult non-smokers with and without attentional deficits. *Physiol Behav* **87**: 614–624.

Pons S, Fattore L, Cossu G, Tolu S, Porcu E, McIntosh JM, *et al* (2008). Crucial Role of 4 and 6 Nicotinic Acetylcholine Receptor Subunits from Ventral Tegmental Area in Systemic Nicotine Self-Administration. *J Neurosci* **28**: 12318–12327.

Porta C La, Bura SA, Negrete R, Maldonado R (2014). Involvement of the endocannabinoid system in osteoarthritis pain. *Eur J Neurosci* **39**: 485–500.

Porter AC, Sauer J-M, Knierman MD, Becker GW, Berna MJ, Bao J, *et al* (2002). Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther* **301**: 1020–4.

Portugal GS, Kenney JW, Gould TJ (2008). Beta2 subunit containing acetylcholine receptors mediate nicotine withdrawal deficits in the acquisition of contextual fear conditioning. *Neurobiol Learn Mem* **89**: 106–13.

Portugal GS, Wilkinson DS, Kenney JW, Sullivan C, Gould TJ (2012a). Strain-dependent effects of acute, chronic, and withdrawal from chronic nicotine on fear conditioning. *Behav Genet* **42**: 133–50.

Portugal GS, Wilkinson DS, Turner JR, Blendy JA, Gould TJ (2012b). Developmental effects of acute, chronic, and withdrawal from chronic nicotine on fear conditioning. *Neurobiol Learn Mem* **97**: 482–94.

Prochaska JJ, Benowitz NL (2015). Smoking cessation and the cardiovascular patient. *Curr Opin Cardiol* **30**: 506–11.

Prochaska JJ, Benowitz NL (2016). The Past, Present, and Future of Nicotine Addiction Therapy. *Annu Rev Med* **67**: 467–86.

Puighermanal E, Marsicano G, Busquets-Garcia A, Lutz B, Maldonado R, Ozaita A

(2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nat Neurosci* **12**: 1152–8.

Pulvers K, Emami AS, Nollen NL, Romero DR, Strong DR, Benowitz NL, *et al* (2016). Tobacco Consumption and Toxicant Exposure of Cigarette Smokers Using Electronic Cigarettes. *Nicotine Tob Res* **20**: ntw333.

Pulvers K, Scheuermann TS, Emami AS, Basora B, Luo X, Khariwala SS, *et al* (2014). Reasons for smoking among tri-ethnic daily and nondaily smokers. *Nicotine Tob Res* **16**: 1567–76.

Ramlawi B, Rudolph JL, Mieno S, Feng J, Boodhwani M, Khabbaz K, *et al* (2006). C-Reactive protein and inflammatory response associated to neurocognitive decline following cardiac surgery. *Surgery* **140**: 221–6.

Raybuck JD, Gould TJ (2007). Extracellular signal-regulated kinase 1/2 involvement in the enhancement of contextual fear conditioning by nicotine. *Behav Neurosci* **121**: 1119–24.

Raybuck JD, Gould TJ (2009). Nicotine withdrawal-induced deficits in trace fear conditioning in C57BL/6 mice--a role for high-affinity beta2 subunit-containing nicotinic acetylcholine receptors. *Eur J Neurosci* **29**: 377–87.

Reggio P (2010). Endocannabinoid Binding to the Cannabinoid Receptors: What Is Known and What Remains Unknown. *Curr Med Chem* **17**: 1468–1486.

Ren Y, Whittard J, Higuera-Matas A, Morris C V, Hurd YL (2009). Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. *J Neurosci* **29**: 14764–9.

Rigotti N a, Gonzales D, Dale LC, Lawrence D, Chang Y (2009). A randomized controlled trial of adding the nicotine patch to rimonabant for smoking cessation: efficacy, safety and weight gain. *Addiction* **104**: 266–76.

Risner ME, Goldberg SR (1983). A comparison of nicotine and cocaine self-administration in the dog: fixed-ratio and progressive-ratio schedules of intravenous drug infusion. *J Pharmacol Exp Ther* **224**: 319–26.

Ross FM, Allan SM, Rothwell NJ, Verkhratsky A (2003). A dual role for interleukin-1 in LTP in mouse hippocampal slices. *J Neuroimmunol* **144**: 61–7.

Rueda D, Navarro B, Martínez-Serrano A, Guzmán M, Galve-Roperh I (2002). The Endocannabinoid Anandamide Inhibits Neuronal Progenitor Cell Differentiation through Attenuation of the Rap1/B-Raf/ERK Pathway. *J Biol Chem* **277**: 46645–46650.

Rukstalis M, Jepson C, Patterson F, Lerman C (2005). Increases in hyperactive-impulsive symptoms predict relapse among smokers in nicotine replacement therapy. *J Subst Abuse Treat* **28**: 297–304.

Russell MAH (1989). Subjective and behavioural effects of nicotine in humans: some sources of individual variation. *Prog Brain Res* **79**: 289–302.

Russo P, Cardinale A, Margaritora S, Cesario A (2012). Nicotinic receptor and tobacco-related cancer. *Life Sci* **91**: 1087–1092.

Rusted JM, Alvares T (2008). Nicotine effects on retrieval-induced forgetting are not attributable to changes in arousal. *Psychopharmacology (Berl)* **196**: 83–92.

Saccone NL, Wang JC, Breslau N, Johnson EO, Hatsukami D, Saccone SF, *et al* (2009). The CHRNA5-CHRNA3-CHRNB4 nicotinic receptor subunit gene cluster affects risk for nicotine dependence in African-Americans and in European-Americans. *Cancer Res* **69**: 6848–56.

Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, *et al* (1998). Orexins and Orexin Receptors: A Family of Hypothalamic Neuropeptides and G Protein-Coupled Receptors that Regulate Feeding Behavior. *Cell* **92**: 573–585.

Sakurai T, Moriguchi T, Furuya K, Kajiwara N, Nakamura T, Yanagisawa M, *et al* (1999). Structure and function of human prepro-orexin gene. *J Biol Chem* **274**: 17771–6.

Salas R, Sturm R, Boulter J, Biasi M De (2009). Nicotinic receptors in the habenulo-interpeduncular system are necessary for nicotine withdrawal in mice. *J Neurosci* **29**: 3014–8.

Salter MW, Stevens B (2017). Microglia emerge as central players in brain disease. *Nat Med* **23**: 1018–1027.

Samson WK, Taylor MM (2001). Hypocretin/orexin suppresses corticotroph responsiveness in vitro. *Am J Physiol Integr Comp Physiol* **281**: R1140–R1145.

Santello M, Volterra A (2012). TNFα in synaptic function: switching gears. *Trends Neurosci* **35**: 638–647.

Saravia R, Flores Á, Plaza-Zabala A, Busquets-Garcia A, Pastor A, la Torre R de, *et al* (2017). CB1Cannabinoid Receptors Mediate Cognitive Deficits and Structural Plasticity Changes During Nicotine Withdrawal. *Biol Psychiatry* **81**: 625–634.

Sasso G Lo, Schlage WK, Boué S, Veljkovic E, Peitsch MC, Hoeng J (2016). The Apoe−/− mouse model: a suitable model to study cardiovascular and respiratory diseases in the context of cigarette smoke exposure and harm reduction. *J Transl Med* **14**: 146.

Saxe MD, Battaglia F, Wang J-W, Malleret G, David DJ, Monckton JE, *et al* (2006). Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc Natl Acad Sci* **103**: 17501–17506.

Schiavon AP, Bonato JM, Milani H, Guimarães FS, Weffort de Oliveira RM (2016). Influence of single and repeated cannabidiol administration on emotional

behavior and markers of cell proliferation and neurogenesis in non-stressed mice. *Prog Neuro-Psychopharmacology Biol Psychiatry* **64**: 27–34.

Schiavon AP, Soares LM, Bonato JM, Milani H, Guimarães FS, Weffort de Oliveira RM (2014). Protective Effects of Cannabidiol Against Hippocampal Cell Death and Cognitive Impairment Induced by Bilateral Common Carotid Artery Occlusion in Mice. *Neurotox Res* **26**: 307–316.

Schmeichel BE, Barbier E, Misra KK, Contet C, Schlosburg JE, Grigoriadis D, *et al* (2015). Hypocretin Receptor 2 Antagonism Dose-Dependently Reduces Escalated Heroin Self-Administration in Rats. *Neuropsychopharmacology* **40**: 1123–1129.

Schmeltz I, Hoffmann D (1977). Nitrogen-Containing Compounds in Tobacco and Tobacco Smoke. *Chem Rev* **77**: .

Schmid AW, Lynch MA, Herron CE (2009). The effects of IL-1 receptor antagonist on beta amyloid mediated depression of LTP in the rat CA1 in vivo. *Hippocampus* **19**: 670–676.

Schneider H, Pitossi F, Balschun D, Wagner A, Rey A del, Besedovsky HO (1998). A neuromodulatory role of interleukin-1beta in the hippocampus. *Proc Natl Acad Sci U S A* **95**: 7778–83.

Schneider M, Schömig E, Leweke FM (2008). Acute and chronic cannabinoid treatment differentially affects recognition memory and social behavior in pubertal and adult rats. *Addict Biol* **13**: 345–357.

Schultz W (2010). Dopamine signals for reward value and risk: basic and recent data. *Behav Brain Funct* **6**: 24.

Segal M (2017). Dendritic spines: Morphological building blocks of memory. *Neurobiol Learn Mem* **138**: 3–9.

Selbach O, Bohla C, Barbara A, Doreulee N, Eriksson KS, Sergeeva OA, *et al* (2010). Orexins/hypocretins control bistability of hippocampal long-term synaptic plasticity through co-activation of multiple kinases. *Acta Physiol* **198**: 277–285.

Shoaib M (2008). The cannabinoid antagonist AM251 attenuates nicotine self-administration and nicotine-seeking behaviour in rats. *Neuropharmacology* **54**: 438–444.

Shoaib M, Bizarro L (2005). Deficits in a sustained attention task following nicotine withdrawal in rats. *Psychopharmacology (Berl)* **178**: 211–222.

Shoval G, Shbiro L, Hershkovitz L, Hazut N, Zalsman G, Mechoulam R, *et al* (2016). Prohedonic Effect of Cannabidiol in a Rat Model of Depression. *Neuropsychobiology* **73**: 123–9.

Sickle MD Van, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, *et al* (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310**: 329–32.

Soar K, Dawkins L, Begum H, Parrott AC (2008). The effects of cigarette smoking and abstinence on auditory verbal learning. *Hum Psychopharmacol Clin Exp* **23**: 621–627.

Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, *et al* (2013). Dynamics of Hippocampal Neurogenesis in Adult Humans. *Cell* **153**: 1219–1227.

Spulber S, Mateos L, Oprica M, Cedazo-Minguez A, Bartfai T, Winblad B, *et al* (2009). Impaired long term memory consolidation in transgenic mice overexpressing the human soluble form of IL-1ra in the brain. *J Neuroimmunol* **208**: 46–53.

Stahl SM, Pradko JF, Haight BR, Modell JG, Rockett CB, Learned-Coughlin S (2004). A Review of the Neuropharmacology of Bupropion, a Dual Norepinephrine and Dopamine Reuptake Inhibitor. *Prim Care Companion J Clin Psychiatry* **6**: 159–166.

Stanley EM, Fadel J (2012). Aging-related deficits in orexin/hypocretin modulation of the septohippocampal cholinergic system. *Synapse* **66**: 445–52.

Steinberg MB, Foulds J (2007). Rimonabant for treating tobacco dependence. *Vasc Health Risk Manag* **3**: 307–11.

Steindel F, Lerner R, Häring M, Ruehle S, Marsicano G, Lutz B, *et al* (2013). Neuron-type specific cannabinoid-mediated G protein signalling in mouse hippocampus. *J Neurochem* **124**: 795–807.

Stella N (2013). Cannabinoid and cannabinoid-like receptors in microglia, astrocytes and astrocytomas. **58**: 1017–1030.

Stellwagen D, Malenka RC (2006). Synaptic scaling mediated by glial TNF-α. *Nature* **440**: 1054–1059.

Stence N, Waite M, Dailey ME (2001). Dynamics of microglial activation: A confocal time-lapse analysis in hippocampal slices. *Glia* **33**: 256–266.

Stoker AK, Markou A (2013). Unraveling the neurobiology of nicotine dependence using genetically engineered mice. *Curr Opin Neurobiol* **23**: 493–499.

Stoker AK, Markou A (2015). Neurobiological Bases of Cue- and Nicotine-induced Reinstatement of Nicotine Seeking: Implications for the Development of Smoking Cessation Medications. 125–154doi:10.1007/978-3-319-13482-6_5.

Stoker AK, Olivier B, Markou A (2012). Role of α7- and β4-containing nicotinic acetylcholine receptors in the affective and somatic aspects of nicotine withdrawal: studies in knockout mice. *Behav Genet* **42**: 423–36.

Streit WJ, Mrak RE, Griffin WST (2004). Microglia and neuroinflammation: a pathological perspective. *J Neuroinflammation* **1**: 14.

Streit WJ, Walter SA, Pennell NA (1999). Reactive microgliosis. *Prog Neurobiol* **57**:

563–581.

Subramaniyan M, Dani JA (2015). Dopaminergic and cholinergic learning mechanisms in nicotine addiction. *Ann N Y Acad Sci* **1349**: 46–63.

Süleyman H, Demircan B, Karagöz Y (2007). *Anti-inflammatory and side effects of cyclooxygenase inhibitors*. at <http://www.if-pan.krakow.pl/pjp/pdf/2007/3_247.pdf>.

Suzuki M, Beuckmann CT, Shikata K, Ogura H, Sawai T (2005). Orexin-A (hypocretin-1) is possibly involved in generation of anxiety-like behavior. *Brain Res* **1044**: 116–21.

Talani G, Lovinger DM (2015). Interactions between ethanol and the endocannabinoid system at GABAergic synapses on basolateral amygdala principal neurons. *Alcohol* **49**: 781–794.

Tang J, Chen J, Ramanjaneya M, Punn A, Conner AC, Randeva HS (2008). The signalling profile of recombinant human orexin-2 receptor. *Cell Signal* **20**: 1651–1661.

Tay TL, Savage JC, Hui CW, Bisht K, Tremblay M-È (2017). Microglia across the lifespan: from origin to function in brain development, plasticity and cognition. *J Physiol* **595**: 1929–1945.

Terry GE, Hirvonen J, Liow J-S, Zoghbi SS, Gladding R, Tauscher JT, *et al* (2010). Imaging and quantitation of cannabinoid CB1 receptors in human and monkey brains using (18)F-labeled inverse agonist radioligands. *J Nucl Med* **51**: 112–20.

Thewissen R, Hout M van den, Havermans RC, Jansen A (2005). Context-dependency of cue-elicited urge to smoke. *Addiction* **100**: 387–396.

Thiel CM, Zilles K, Fink GR (2005). Nicotine Modulates Reorienting of Visuospatial Attention and Neural Activity in Human Parietal Cortex. *Neuropsychopharmacology* **30**: 810–820.

Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG (2009). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br J Pharmacol* **150**: 613–623.

Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F, *et al* (2010). Sequence variants at CHRNB3–CHRNA6 and CYP2A6 affect smoking behavior. *Nat Genet* **42**: 448–453.

Thornton TM, Rincon M (2009). Non-classical p38 map kinase functions: cell cycle checkpoints and survival. *Int J Biol Sci* **5**: 44–51.

Threlfell S, Lalic T, Platt NJ, Jennings KA, Deisseroth K, Cragg SJ (2012). Striatal Dopamine Release Is Triggered by Synchronized Activity in Cholinergic Interneurons. *Neuron* **75**: 58–64.

Tian S, Pan S, You Y (2015). Nicotine enhances the reconsolidation of novel object

recognition memory in rats. *Pharmacol Biochem Behav* **129**: 14–8.

Tsou K, Brown S, Sañudo-Peña M., Mackie K, Walker J. (1998). Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* **83**: 393–411.

Tsujino N, Sakurai T (2013). Role of orexin in modulating arousal, feeding, and motivation. *Front Behav Neurosci* **7**: 28.

Turner JR, Ray R, Lee B, Everett L, Xiang J, Jepson C, *et al* (2014a). Evidence from mouse and man for a role of neuregulin 3 in nicotine dependence. *Mol Psychiatry* **19**: 801–10.

Turner MD, Nedjai B, Hurst T, Pennington DJ (2014b). Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochim Biophys Acta - Mol Cell Res* **1843**: 2563–2582.

Uramura K, Funahashi H, Muroya S, Shioda S, Takigawa M, Yada T (2001). Orexin-a activates phospholipase C- and protein kinase C-mediated Ca2+ signaling in dopamine neurons of the ventral tegmental area. *Neuroreport* **12**: 1885–9.

Uslaner JM, Winrow CJ, Gotter AL, Roecker AJ, Coleman PJ, Hutson PH, *et al* (2014). Selective orexin 2 receptor antagonism blocks cue-induced reinstatement, but not nicotine self-administration or nicotine-induced reinstatement. *Behav Brain Res* **269**: 61–5.

Valentine G, Sofuoglu M (2017). Cognitive Effects of Nicotine: Recent Progress. *Curr Neuropharmacol* **15**: .

Vezzani A, French J, Bartfai T, Baram TZ (2011). The role of inflammation in epilepsy. *Nat Rev Neurol* **7**: 31–40.

Vink JM, Willemsen G, Boomsma DI (2005). Heritability of Smoking Initiation and Nicotine Dependence. *Behav Genet* **35**: 397–406.

Viscomi MT, Oddi S, Latini L, Pasquariello N, Florenzano F, Bernardi G, *et al* (2009). Selective CB2 Receptor Agonism Protects Central Neurons from Remote Axotomy-Induced Apoptosis through the PI3K/Akt Pathway. *J Neurosci* **29**: 4564–4570.

Volkow N (2018). Toward precision medicine in addiction treatment. *Am J Addict* **27**: 35–36.

Volkow ND, Baler RD (2014). Addiction science: Uncovering neurobiological complexity. *Neuropharmacology* **76**: 235–249.

Volkow ND, Morales M (2015). The Brain on Drugs: From Reward to Addiction. *Cell* **162**: 712–725.

Vossel S, Thiel CM, Fink GR (2008). Behavioral and Neural Effects of Nicotine on Visuospatial Attentional Reorienting in Non-Smoking Subjects. *Neuropsychopharmacology* **33**: 731–738.

Vries TJ De, Schoffelmeer ANM (2005). Cannabinoid CB1 receptors control conditioned drug seeking. *Trends Pharmacol Sci* **26**: 420–6.

Walter L, Stella N (2004). Cannabinoids and neuroinflammation. *Br J Pharmacol* **141**: 775–85.

Walters CL, Brown S, Changeux J-P, Martin B, Damaj MI (2006). The β2 but not α7 subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. *Psychopharmacology (Berl)* **184**: 339–344.

Wang H, Sun X (2005). Desensitized nicotinic receptors in brain. *Brain Res Brain Res Rev* **48**: 420–37.

Wannamethee SG, Lowe GDO, Shaper AG, Rumley A, Lennon L, Whincup PH (2005). Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease. *Eur Heart J* **26**: 1765–73.

Wardle MC, Munafò MR, Wit H de (2011). Effect of social stress during acute nicotine abstinence. *Psychopharmacology (Berl)* **218**: 39–48.

Watkins BA, Kim J (2014). The endocannabinoid system: directing eating behavior and macronutrient metabolism. *Front Psychol* **5**: 1506.

Weinhard L, Bartolomei G di, Bolasco G, Machado P, Schieber NL, Neniskyte U, *et al* (2018). Microglia remodel synapses by presynaptic trogocytosis and spine head filopodia induction. *Nat Commun* **9**: 1228.

Wesnes KA, Edgar CJ, Kezic I, Salih HM, Boer P de (2013). Effects of nicotine withdrawal on cognition in a clinical trial setting. *Psychopharmacology (Berl)* **229**: 133–140.

Westlake TM, Howlett AC, Bonner TI, Matsuda LA, Herkenham M (1994). Cannabinoid receptor binding and messenger RNA expression in human brain: An in vitro receptor autoradiography and in situ hybridization histochemistry study of normal aged and Alzheimer's brains. *Neuroscience* **63**: 637–652.

Wilking JA, Stitzel JA (2015). Natural genetic variability of the neuronal nicotinic acetylcholine receptor subunit genes in mice: Consequences and confounds. *Neuropharmacology* **96**: 205–212.

Wilkinson DS, Gould TJ (2013). Withdrawal from chronic nicotine and subsequent sensitivity to nicotine challenge on contextual learning. *Behav Brain Res* **250**: 58–61.

Williamson LL, Sholar PW, Mistry RS, Smith SH, Bilbo SD (2011). Microglia and memory: modulation by early-life infection. *J Neurosci* **31**: 15511–21.

Wilson RI, Nicoll RA (2002). Endocannabinoid Signaling in the Brain. *Science (80-)* **296**: 678–682.

Winsky-Sommerer R (2004). Interaction between the Corticotropin-Releasing Factor System and Hypocretins (Orexins): A Novel Circuit Mediating Stress Response. *J Neurosci* **24**: 11439–11448.

Wise LE, Long KA, Abdullah RA, Long JZ, Cravatt BF, Lichtman AH (2012). Dual Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase Blockade Produces THC-Like Morris Water Maze Deficits in Mice. doi:10.1021/cn200130s.

Wise RA (2004). Dopamine, learning and motivation. *Nat Rev Neurosci* **5**: 483–494.

Witten IB, Lin S-C, Brodsky M, Prakash R, Diester I, Anikeeva P, *et al* (2010). Cholinergic interneurons control local circuit activity and cocaine conditioning. *Science* **330**: 1677–81.

Wohleb ES, Franklin T, Iwata M, Duman RS (2016). Integrating neuroimmune systems in the neurobiology of depression. *Nat Publ Gr* **17**: .

Wong KKY, Ng SYL, Lee LTO, Ng HKH, Chow BKC (2011). Orexins and their receptors from fish to mammals: A comparative approach. *Gen Comp Endocrinol* **171**: 124–130.

World Health Organization (WHO) (2017). *WHO report on the global tobacco epidemic. Monitoring tobacco use and prevention policies*. .

Xanthos DN, Sandkühler J (2014). Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. *Nat Rev Neurosci* **15**: 43–53.

Xi Z-X, Peng X-Q, Li X, Song R, Zhang H-Y, Liu Q-R, *et al* (2011). Brain cannabinoid CB2 receptors modulate cocaine's actions in mice. *Nat Neurosci* **14**: 1160–1166.

Xia JX, Fan SY, Yan J, Chen F, Li Y, Yu ZP, *et al* (2009). Orexin A-induced extracellular calcium influx in prefrontal cortex neurons involves L-type calcium channels. *J Physiol Biochem* **65**: 125–136.

Xie X, Wisor JP, Hara J, Crowder TL, LeWinter R, Khroyan T V., *et al* (2008). Hypocretin/orexin and nociceptin/orphanin FQ coordinately regulate analgesia in a mouse model of stress-induced analgesia. *J Clin Invest* **118**: 2471–81.

Yakel JL (2012). Nicotinic ACh receptors in the hippocampus: Role in excitability and plasticity. *Nicotine Tob Res* **14**: 1249–1257.

Yan Y, Peng C, Arvin MC, Mcintosh JM, Lavis LD, Drenan Correspondence RM (2018). Nicotinic Cholinergic Receptors in VTA Glutamate Neurons Modulate Excitatory Transmission. doi:10.1016/j.celrep.2018.04.062.

Yang K, Buhlman L, Khan GM, Nichols RA, Jin G, McIntosh JM, *et al* (2011). Functional Nicotinic Acetylcholine Receptors Containing 6 Subunits Are on GABAergic Neuronal Boutons Adherent to Ventral Tegmental Area Dopamine Neurons. *J Neurosci* **31**: 2537–2548.

Yang L, Zou B, Xiong X, Pascual C, Xie J, Malik A, *et al* (2013). Hypocretin/orexin neurons contribute to hippocampus-dependent social memory and synaptic plasticity in mice. *J Neurosci* **33**: 5275–84.

Yildirim E, Connor DA, Gould TJ (2015). ABT-089, but not ABT-107, ameliorates nicotine withdrawal-induced cognitive deficits in C57BL6/J mice. *Behav Pharmacol* **26**: 241–8.

Yin H, Chu A, Li W, Wang B, Shelton F, Otero F, *et al* (2009). Lipid G protein-coupled receptor ligand identification using beta-arrestin PathHunter assay. *J Biol Chem* **284**: 12328–38.

Yirmiya R, Rimmerman N, Reshef R (2015). Depression as a Microglial Disease. *Trends Neurosci* **38**: 637–658.

Zandonai T, Chiamulera C, Mancabelli A, Falconieri D, Diana M (2018). A Preliminary Investigation on Smokeless Tobacco Use and Its Cognitive Effects Among Athletes. *Front Pharmacol* **9**: 216.

Zanelati T V, Biojone C, Moreira FA, Guimarães FS, Joca SRL (2010). Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. *Br J Pharmacol* **159**: 122–8.

Zhao C, Deng W, Gage FH (2008). Mechanisms and Functional Implications of Adult Neurogenesis. *Cell* **132**: 645–660.

Zhao X, Zhang R xue, Tang S, Ren Y yan, Yang W xia, Liu X min, *et al* (2014). Orexin-A-induced ERK1/2 activation reverses impaired spatial learning and memory in pentylenetetrazol-kindled rats via OX1R-mediated hippocampal neurogenesis. *Peptides* **54**: 140–147.

Zhao Y-N, Wang F, Fan Y-X, Ping G-F, Yang J-Y, Wu C-F (2013). Activated microglia are implicated in cognitive deficits, neuronal death, and successful recovery following intermittent ethanol exposure. *Behav Brain Res* **236**: 270–282.

Zhou X, Nonnemaker J, Sherrill B, Gilsenan AW, Coste F, West R (2009). Attempts to quit smoking and relapse: Factors associated with success or failure from the ATTEMPT cohort study. *Addict Behav* **34**: 365–373.

Zhu B, Dong Y, Xu Z, Gompf HS, Ward SAP, Xue Z, *et al* (2012). Sleep disturbance induces neuroinflammation and impairment of learning and memory. *Neurobiol Dis* **48**: 348–55.

Zhu PJ, Lovinger DM (2007). Persistent Synaptic Activity Produces Long-Lasting Enhancement of Endocannabinoid Modulation and Alters Long-Term Synaptic Plasticity. *J Neurophysiol* **97**: 4386–4389.

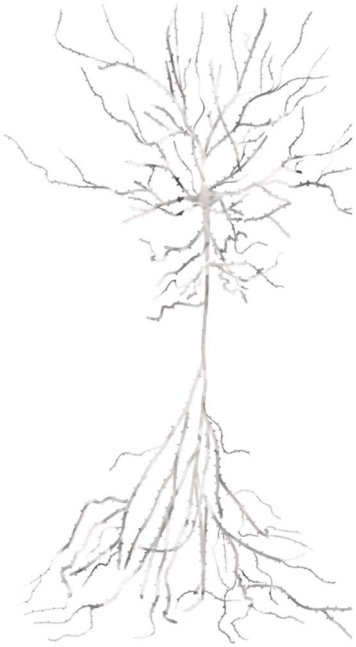
Ziv Y, Ron N, Butovsky O, Landa G, Sudai E, Greenberg N, *et al* (2006). Immune cells contribute to the maintenance of neurogenesis and spatial learning abilities in adulthood. *Nat Neurosci* **9**: 268–275.

Ziv Y, Schwartz M (2008). Immune-based regulation of adult neurogenesis: Implications for learning and memory. *Brain Behav Immun* **22**: 167–176.

Zlebnik NE, Cheer JF (2016). Beyond the CB1 Receptor: Is Cannabidiol the Answer for Disorders of Motivation? doi:10.1146/annurev-neuro-070815-014038.

Zoli M, Pistillo F, Gotti C (2015). Diversity of native nicotinic receptor subtypes in mammalian brain. *Neuropharmacology* **96**: 302–311.

Zuardi AW, Rodrigues NP, Silva AL, Bernardo SA, Hallak JEC, Guimarães FS, *et al* (2017). Inverted U-Shaped Dose-Response Curve of the Anxiolytic Effect of Cannabidiol during Public Speaking in Real Life. *Front Pharmacol* **8**: 259.



ANNEX

ARTICLE 3

A Role for Hypocretin/Orexin Receptor-1 in Cue-Induced Reinstatement of Nicotine-Seeking Behavior

Ainhoa Plaza-Zabala, África Flores, Elena Martín-García, **Rocío Saravia**, Rafael Maldonado, and Fernando Berrendero

Neuropsychopharmacology (2013) **38**:1724–1736

Plaza-Zabala A, Flores Á, Martín-García E, Saravia R, Maldonado R, Berrendero F. A role for hypocretin/ orexin receptor-1 in cue-induced reinstatement of nicotine-seeking behavior. Neuropsychopharmacology. 2013 Aug;38(9):1724–36. DOI: 10.1038/npp.2013.72

ARTICLE 4

The Hypocretin/Orexin System Mediates the Extinction of Fear Memories

África Flores, Victòria Valls-Comamala, Giulia Costa, **Rocío Saravia**, Rafael Maldonado and Fernando Berrendero

Neuropsychopharmacology (2014) **39**:2732–2741

Flores Á, Valls-Comamala V, Costa G, Saravia R, Maldonado R, Berrendero F. The hypocretin/orexin system mediates the extinction of fear memories. Neuropsychopharmacology. 2014;39(12):2732–41. DOI: 10.1038/npp.2014.146

ARTICLE 5

Orexins and fear: implications for the treatment of anxiety disorders

África Flores, **Rocío Saravia**, Rafael Maldonado and Fernando Berrendero

Trends Neurosci. (2015) 38 (9): 550-559

Flores Á, Saravia R, Maldonado R, Berrendero F. Orexins and fear: Implications for the treatment of anxiety disorders. Trends Neurosci. 2015 Sep;38(9):550-9. DOI: 10.1016/j.tins.2015.06.005.

ARTICLE 6

Concomitant THC and stress adolescent exposure induces impaired fear extinction and related neurobiological changes in adulthood

Rocio Saravia, Marc Ten-Blanco, Marina Julià-Hernández, Humberto Gagliano, Raül Andero, Antonio Armario, Rafael Maldonado and Fernando Berrendero

Neuropharmacology (under second revision)

Saravia R, Ten-Blanco M, Julià-Hernández M, Gagliano H, Andero R, Armario A, et al. Concomitant THC and stress adolescent exposure induces impaired fear extinction and related neurobiological changes in adulthood. Neuropharmacology. 2019 Jan 1;144:345–57. DOI: 10.1016/j.neuropharm.2018.11.016