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Abdallah Zayed

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THESE

Presented at the

SORBONNE UNIVERSITE

**École doctorale : CERVEAU, COGNITION, COMPORTEMENT
Spécialité : Neuroscience**

Presented by

Abdallah Zayed, MD

To obtain the degree of

DOCTEUR de L'UNIVERSITE SORBONNE

ROLE OF CHROMATIN REMODELERS IN DOPAMINOCEPTIVE NEURONS

Date: 26th MARCH 2019

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To Eman and Elein

To my parents

To my brothers

Abbreviation

ACTH: Adrenocorticotrophic hormone

AVP: Arginine vasopressin

BAF: Brg1 Associated Factors

BAP: Brahma Associated Protein

BDNF: Brain-derived neurotrophic factor

BLA: Basolateral amygdala

Brg1: Brahma-related Gene 1

BST: Bed Nucleus of the Stria Terminalis

CaMKII: Calcium/calmodulin-dependent protein kinase II

CBP: (CREB)-binding protein

CeA: Central Amygdala

CpG: Cytosine (C) linked by a phosphate bond to the base guanine (G)

CPP: Conditioned Place Preference

CREB: Cyclic AMP response element protein

CRH: Corticotropin releasing hormones

CSDS: Chronic social defeat stress.

DBD: DNA-binding domain

DST: Dexamethasone suppression test

ERK: Extracellular signal-regulated kinases

EPSCs: Excitatory postsynaptic currents

esBAF: Embryonic stem Brg1 Associated Factors

GC: Glucocorticoid

GR: Glucocorticoid receptor

GRE: Glucocorticoid responsive element

HDAC: Histone deacetylase

HATs: Histone acetyltransferases

hBrm: Human Brahma

HPA: hypothalamo–pituitary–adrenocortical

LBD: Ligand binding domain

LTD: Long Term Depression

LTP: Long Term Potentiation

MMTV: Mouse mammary tumor virus

MSNs: Medium spiny neurons

NAc: nucleus accumbens

nBAF: Neuronal Brg1 Associated Factors

npBAF: Neuronal progenitor Brg1 Associated Factors

mPFC: Medial prefrontal cortex

PKA: Protein kinase A

SN: Substantia nigra

SWI: Mating type switching

PBAF: Polybromo-associated BAF

PFC: Prefrontal cortex

PVN: Paraventricular nucleus

VTA: Ventral Tegmental Area

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Abstract

Glucocorticoid (GC) hormone release is a key physiological response to stress exposure enabling the organism to cope with environmental challenges. Beneficial when working, a dysfunction of this adaptive response is associated to multiples pathologies including psychiatric disorders. GC act through the binding to their receptor, glucocorticoid receptor (GR), a ubiquitously expressed transcription factor. It has been shown that GR gene inactivation in dopaminergic neurons (GR^{D1Cre} mice) reduces dopamine neurons activity, decreases responses to cocaine and blocks social aversion induced by repeated social defeat.

GR can control genes expression through different mechanisms. Among others, it can interact with SWI/SNF chromatin remodeling complexes. These complexes include either Brahma (Brm) or Brahma-related gene 1 (Brg1) as an ATP catalytic core subunit. Chromatin remodeling complexes can move the DNA along the nucleosomes thereby opening the chromatin and favoring gene transcription. To examine the role of SWI/SNF complexes in stress-related behaviors, we used two different mouse models: the Brm constitutive mutant mice (Brm^{-/-}) and the Brg1^{D1Cre} mice in which Brg1 gene is inactivated only in dopaminergic cells, as was the case for the above-mentioned GR gene mutant model.

We found that both mouse models showed a complete resilience to repeated social defeat as they exhibited similar social interactions as unstressed animals. Moreover, similarly to the phenotype of GR^{D1Cre} mice, both Brg1^{D1Cre} and Brm^{-/-} mice showed decreased responses to cocaine in behavioral sensitization and/or conditioned place preference paradigms. Social aversion induced by repeated social defeats correlates with a long-term increase of dopamine neurons firing in the ventral tegmental area (VTA). Whilst this increase is blocked in GR mutants, Brg1^{D1Cre} mice on the contrary showed a normal increase of dopamine neuron firing after social defeat despite their behavioral resilience. We therefore examined cell-signaling and immediate early genes induction in the mutated brain areas (including dorsal striatum, nucleus accumbens (NAc) and cortex) of our models and showed that while ERK signaling pathway is normally induced by an acute defeat or an acute cocaine injection, the phosphorylation of histone H3 (H3S10P) and the induction of *c-Fos* and *Egr1* genes expression are reduced in the dorsal striatum and the NAc. Altogether, these results lead to further evidences for an involvement of chromatin remodelers in stress-related behaviors. The inactivation of Brm and /or Brg1 might result in a lack of molecular plasticity leading to a blockade of maladaptive behaviors.

Part 1: Introduction

Chapter 1: Stress Response

I. Stress and the general syndrome of adaptation

Biologists have always been impressed by the ability of living beings to maintain their own stability and to adapt to external world. In the late XIXth century, Claude Bernard introduced the idea of the “inner world” stating that organs of the body work together to maintain a constant internal environment or “milieu intérieur”. In the beginning of XXth century, based on the work of Claude Bernard, Walter B. Cannon coined the concept of homeostasis to describe the maintenance within acceptable ranges of several physiological variables, enabling the organism to maintain its balance despite environmental challenges.

Cannon assumed that the brain coordinates with body systems, with the aim of maintaining a set of goal values for key internal variables. The body temperature is kept at 37,0 °C, the serum sodium level at 140 mEq/L, the blood glucose level at 90 mg/dL, and so forth. Any disturbances of the homeostasis either internal or external, by causing large enough deviations from the target values, lead to the arousal of the sympatho-adrenal system enabling what he called the “fight or flight” response. This observation brought attention to the role of the adrenal gland medulla and its release of adrenaline. In the 1930's, Hans Selye built on Cannon's work and described for the first time the hypothalamo-pituitary-adrenal (HPA) axis, introducing the role of adrenal cortex and steroid hormones in the observations made by Cannon (Selye, 1936). Both of them used the notion of “stress” and “strain”, an analogy with physics in which stress is a force that tends to deform a solid by compression, elongation, or torsion. The deformation produced by the force is strain. At relatively low levels of stress, deformation is elastic and may be considered analogous to homeostatic mechanisms, but when excessive physical forces are exerted, the strain might turn permanent. This notion was popularized by both researchers review articles and in “large audience” books during the 1930s', although only the term « stress » remained in current

language, often used instead of strain and the notion of « stressors » became popular to describe forces.

Selye viewed all forms of stress as leading to a stereotyped pathological response pattern, including enlargement of the adrenal glands, shrinkage of the thymus gland (associated with atrophy of the lymph nodes and inhibition of inflammatory or immune responses), and ulcers or bleeding in the stomach or gastrointestinal tract. Based on these observations, he later framed the General Adaptation Syndrome theory to describe a non-specific response of the body to any demand placed upon it (Figure 1).

The General Adaptation Syndrome theory relies on three steps. The alarm reaction is the immediate reaction to a stressor described by Cannon and triggering the fight or flight response. It involves different sets of brain areas (the cortex, the limbic system, the hypothalamus) and the autonomous nervous system. If the stressor lasts, the organism enters in the phase of resistance during which the body aims at controlling the physiological changes triggered during the alarm phase. This step involves the HPA axis and helps the organism to cope with the stressor. However, if the stressor can't be erased, the energy coast of this homeostasis maintenance can become too high and lead to mental and physical pathologies and eventually to death.

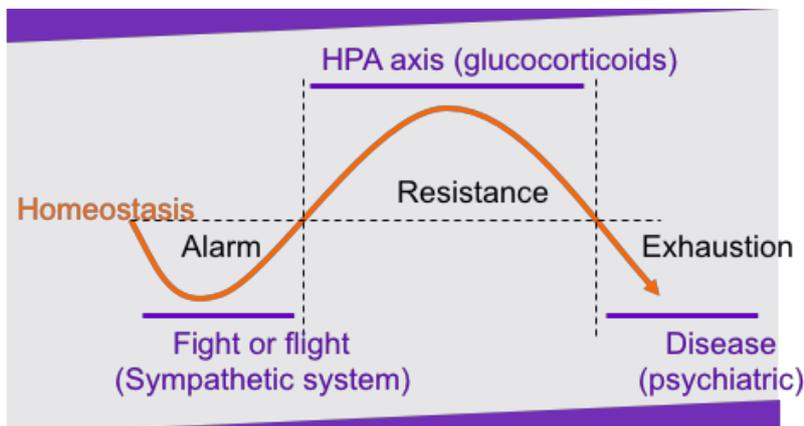


Figure 1: General Adaptation Syndrome: 3 phases: 1) Alarm: immediate reaction to a stressor triggering the fight or flight response that involves the activation of the sympathoadrenal system. 2) Resistance: If the stressor lasts, the body aims at controlling the physiological changes triggered during the alarm phase. This phase involves the HPA axis and the GCs 3) Exhaustion: if the stressor persists, homeostasis maintenance can become too high and lead to disease and death.

II. Hypothalamo–Pituitary–Adrenocortical axis and stress response

A) Description of HPA axis

The stimulation of parvocellular neurons in the paraventricular nucleus (PVN) of the hypothalamus triggers the release of two neurohormones, the Corticotropin Releasing Hormone (CRH) and the vasopressin (AVP), into the blood vessels connecting the hypothalamus and the pituitary gland (the hypophysial portal blood). Both hormones stimulate the anterior pituitary gland to produce adrenocorticotrophic hormone (ACTH), synthesized as part of a large pro-opiomelanocortin 241-amino-acid precursor. The hormonal secretion of ACTH leads to stimulation of melanocortin receptors located in the cortical part of the adrenals, which in turn synthesized GCs. ACTH, in turn, induces GCs synthesis and release from the adrenal glands, which are located at the top the kidneys. GCs regulate the function of multiple systems in living organisms e.g. neuronal activity, neurogenesis and behavioral responses, glucose metabolism in the liver, immune responses and inflammatory process, cardiovascular function, and erythropoiesis in bone marrow. Importantly, GCs can control the HPA axis activity through negative feedbacks by repressing the synthesis and the release of CRH in the hypothalamus, and of ACTH in the pituitary (Figure 2) (McEwen, 1979).

The activation of the HPA axis is under the control of different brain structures such as the prefrontal cortex (PFC), the hippocampus, and the BNST, the brainstem or the amygdala.

B) Circadian and ultradian rhythms of glucocorticoid hormone

The physiology of most organisms changes significantly between day and night. This represents a key adaptation to cope with the different environmental challenges the organism faces at different times of the day, such as changes in lighting conditions and temperature, food availability or the presence of predators. Most cells in multi-cellular organisms harbors their own cell-autonomous oscillators. The specialized suprachiasmatic nucleus in the ventral hypothalamus serves as the central pacemaker, which is known as the 'central or master clock' in mammals (Son et al., 2011) (Dickmeis, 2009), and GR signaling in peripheral cells, such as hepatocytes,

participates in the correct setting of peripheral cells with central pacemaker (Balsalobre et al., 2000).

stress response

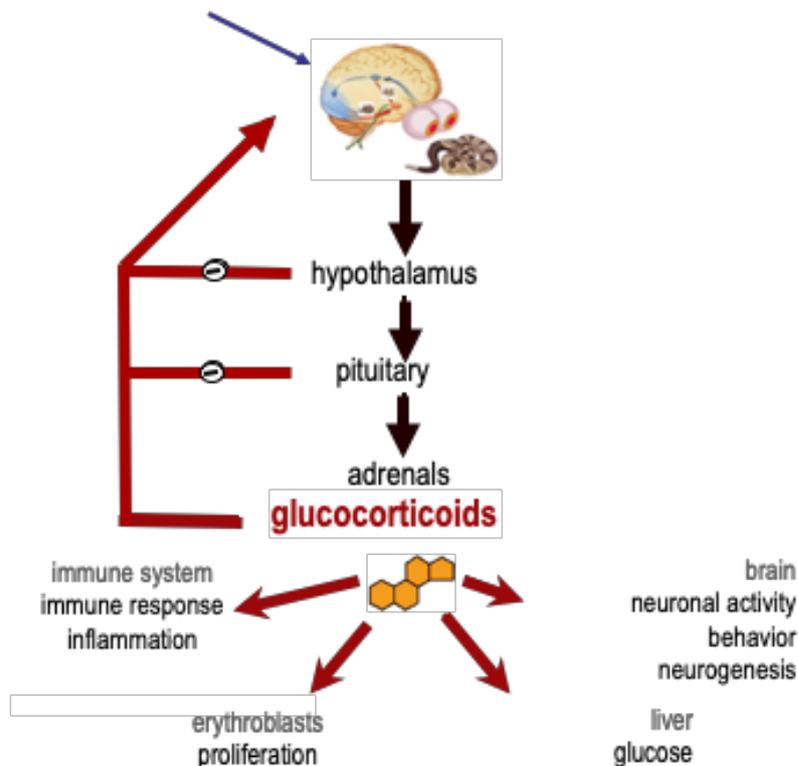


Figure 2: Description of HPA axis

HPA axis activation stimulates GC secretion from the adrenal cortex which serves both to alert the organism to environmental or physiological changes and to maintain homeostasis. GCs control their own synthesis and secretion through a set of negative feedbacks at the forebrain, hypothalamic and pituitary levels.

Under the control of the suprachiasmatic nucleus, the CRH and the AVP are secreted in the portal blood system in a circadian manner leading to a similar activity of the adrenal gland and a circadian modulation of GCs levels. This daily rhythm in adrenal activity is not yet present at birth. In rats, a significant circadian variation in plasma corticosterone concentrations is seen only from three weeks of age; in humans, the rhythm is not fully established until two or three years of age.

Adrenal GC release is pulsatile in nature, marked by an ultradian release. Pulsatility occurs under basal conditions and is maintained in the face of acute or chronic stress.

Indeed, it appears that the magnitude of a stress response can depend on where the stressor occurs with respect to an ultradian pulse: stressors imposed on the rising phase of a pulse produce greater GC responses than those occurring during a falling phase. Pulsatility is an extremely important component of HPA function, as disruption of pulses (e.g., constant GC availability) disrupts GR signaling (Herman et al., 2016).

C) In response to stress

Acute stress efficiently drives HPA stress response, and feedback mechanisms effectively terminate the response after the stressor subsides. Generally, the HPA response begins with a pulse of ACTH, beginning within minutes and lasting a relatively short period of time (30-60 min). The GC response lags in time (due to the necessity for *de novo* GC production at the adrenal) and lasts substantially longer (90-120 min). The timing of both ACTH and GCs responses are dependent on stressor modality and intensity (Herman, 2013).

As studied in rodents, mainly by ablation approaches, many brain structures that innervate directly or indirectly the PVN are involved in the control of HPA axis regulation under stress. Their roles differ depending on the nature of the stress (hemorrhage, hypoglycemia, footshock, restraint) (Herman et al., 2016). The activity of CRH-releasing neurons of the PVN is controlled by a different sets of glutamate excitatory and γ -aminobutyric acid (GABA) inhibitory inputs.

GABA exerts an inhibitory tonus on CRH-releasing neurons, primarily via GABA_A receptor binding, and this maintains low CRH neuronal activity (Radley and Sawchenko, 2011). GABAergic inputs also play a major role in limiting HPA axis responses to stress. Numerous hypothalamic nuclei send GABAergic projections to the PVN CRH neurons, including the medial preoptic nucleus, the dorsomedial hypothalamus, the BNST, and the lateral hypothalamus (Cullinan et al., 2008). Under stressful conditions, glutamatergic inputs to the PVN in areas such as the thalamus, the amygdala or the infralimbic cortex are activated, and GABAergic inhibition is suppressed, leading to HPA axis activation (Feldman and Weidenfeld, 1997). Noradrenaline contributes also to suppress GABA release, acting on α 1 adrenoceptor located at presynaptic terminals (Han et al., 2002), and potentiates glutamatergic

inputs through $\alpha 1$ adrenoceptor activation (Plotsky, 1987). In addition to glutamate, the CRH-expressing PVN neurons are also activated by brainstem catecholamine-producing pathways from the nucleus of the solitary tract, which appears to promote HPA secretory activity following physical threat such as hemorrhage, hypotension and respiratory distress, and might play a role in ACTH responses to immune challenge as well (Herman et al., 2016). Additional HPA-excitatory information come from the Raphe nuclei, which send serotonergic projections to the PVN. Most studies indicate that serotonin (5-HT) stimulates ACTH and GCs secretion through the activation of 5HT2A and perhaps 5HT1A receptors in the PVN (Herman et al., 2016).

The importance of maintaining GC secretion within tolerable limits requires efficient mechanisms for inhibiting stress-integrative PVN neurons. This process appears to be accomplished by multiple mechanism. The negative feedback exerted by GCs, which inhibits the basal expression of CRH and AVP mRNA synthesis in the PVN, and pro-opiomelanocortin gene transcription in the anterior pituitary (Castro et al., 2010; Kellendonk et al., 2002). Also, the binding of GCs to its receptor (GR) in the PVN causes rapid synthesis and release of endocannabinoids that bind CB1 receptors on presynaptic terminals, inhibiting glutamate release and thereby reducing drive onto CRH neurons (Di et al., 2003).

D) HPA axis dysregulation and depression

HPA-axis dysregulation is the scientific term for the popular syndrome known as “adrenal fatigue.” It refers to any variation of HPA axis associated with a constellation of signs and symptoms including fatigue, sleep disruption, poor exercise tolerance and recovery, low libido, brain fog, weakened immune function, and reduced stress tolerance. HPA axis dysregulation is caused by many different aspects of the modern lifestyle, including poor diet, sleep deprivation, chronic stress, lack of (or too much) exercise, and inflammation. HPA-axis activity seems to be altered in several psychiatric (such as post-traumatic stress syndrome, major depression disorders or addiction) and neurological diseases such as Alzheimer’s or, Parkinson’s diseases (Hemmerle et al., 2012), with observed elevation of GC concentration in the saliva and plasma (Du and Pang, 2015; Carroll, 1982).

Concerning depression, up to 50% of patients (80% if severely depressed) display hyperactivity of the HPA axis, increased pituitary and adrenal gland volume, and chronic high levels of GCs in saliva, cerebrospinal fluid, blood plasma and urine. In this line, the dexamethasone suppression test that allows to detect a dysfunctional HPA axis feedback, was originally applied in the late 1960s as a laboratory diagnostic tool for major depression in adults (Zunszain et al., 2011). Clinically, as observed in Cushing's syndrome, hyperactivity of the HPA-axis is highly correlated with significant increases in psychopathology, especially depression (Dorn et al., 1995). Dorn et al. found that 66% of Cushing's patients exhibited psychopathology, consisting mainly of atypical and major depression as well as anxiety disorder and suicide ideation. 3 months following treatment for hypercortisolemia, this dropped significantly to 54% and further decreased to 24% 12 months after correction (Dorn et al., 1997), suggesting a direct causative link between elevated cortisol and psychopathology.

III. The Glucocorticoid Receptor

The physiological actions of GCs are mediated by two types of receptors; the Mineralocorticoid Receptor (MR) and the GR. Both belong to the steroid receptors family (together with the progesterone, the androgen and the two estrogens receptors present in mammals), a subfamily of the nuclear receptors superfamily that includes around thirty members. GR and MR have been originally identified as transcription factors, however, it is now clear that GCs for their long-known rapid physiological effects on neuronal activity act independent from gene transcription but on the MR and GR receptors that can both interfere with intracellular signaling cascades (such as the Jun kinase and Akt ones) and be activated at the cellular membrane to modulate glutamatergic synaptic neurotransmission (de Kloet et al., 2014).

Both MR and GR can be liganded by GC and aldosterone. They however differ in their affinity for these hormones. MR display a ten-fold higher affinity for GCs than GR. Concerning their cell distribution in the organism, whereas GR is almost ubiquitously expressed, MR has a more restricted expression profile. It is found in cells engaged in salt balance of the organism. In these cells, the expression of 11 β -HSD2 rapidly oxidizes GCs into inactive metabolites making them specifically responsive to aldosterone. However, in some cell type such as pyramidal cells of the hippocampus

both receptors are present, but the degradation enzyme is not. In these cells, MR is thus a *bona fide* GC receptor (Oakley and Cidlowski, 2013; de Kloet et al., 2014).

A) Glucocorticoid Receptor structure

GR is a modular protein composed of three major domains: An N-terminal transactivation domain (NTD), a central DNA-binding domain (DBD), and a C-terminal ligand binding domain (LBD) (Kumar et al., 1999; Tronche et al., 1998) (Figure 3). In terms of both size and sequence homology the NTD represents the most variable domain among the nuclear hormone receptors. It contains a powerful transcriptional activation region; AF1/tau1/enh2 which is rich in acidic amino acid residues (Kumar et al., 2005). The NTD is highly immunogenic and contains the major known sites of phosphorylation in the GR.

The DBD, located centrally in the amino acid sequence of the GR, is the most conserved region among nuclear hormone receptors. It is composed of two highly conserved “zinc fingers”. The DBD contains amino acids that contact specific bases in GRE (Glucocorticoids Response Element) DNA sequences. The second zinc finger region stabilizes DBD:GRE interactions, and five amino acids in the second zinc finger, termed “D box”, play an important role in homodimerization at the GRE (Härd et al., 1990; Luisi et al., 1991). The DBD also interacts with certain other transcription factors, NF-AT, CREB or c-Jun for example. The C-terminal LBD is responsible for recognition and binding of steroid hormone ligands, chaperones and other proteins. In addition, the LBD contains a small but important ligand-dependent activation function (AF2) sub-domain located towards its C-terminal end which functions to complete binding surfaces for other proteins, e.g. coactivators and corepressors (Oakley and Cidlowski, 2013).

B) Activation and regulation of GR

In the cytoplasm of cells GR is present as part of a large multi-protein complex that includes chaperone proteins (hsp90, hsp70, and p23) and immunophilins of the FK506 family (FKBP51 and FKBP52; Figure 5; Grad and Picard, 2007; Pratt and Toft, 1997). These proteins maintain the receptor in a conformation that is transcriptionally inactive

but favors high affinity ligand binding. Upon binding GCs, GR undergoes a conformational change resulting in the dissociation of the associated proteins. This structural rearrangement exposes the two nuclear localization signals, and GR is rapidly translocated into the nucleus through nuclear pores.

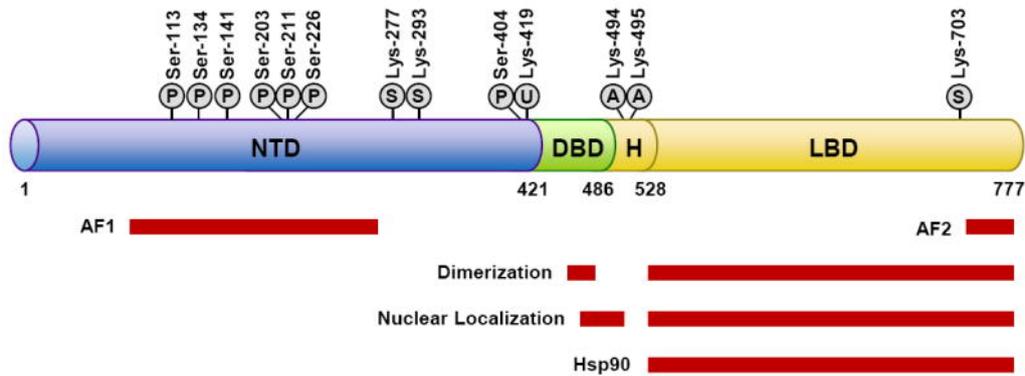


Figure 3: GR domain structure and sites of post-translational modification. The domains of GR and regions of the receptor involved in transactivation (AF1 and AF2), dimerization, nuclear localization, and hsp90 binding are pictured. Also depicted are the amino acid residues modified by phosphorylation (P), sumoylation (S), ubiquitination (U), and acetylation (A). Numbers are for human GR (Oakley and Cidlowski, 2013).

Once inside the nucleus, GR binds directly to GREs and regulates the expression of target genes (Figure 4, Freedman, 1992). Its specificity is however low and most GREs can also be bound by other steroid receptors (MR, PR and AR). The consensus GRE sequence, GGAACAnnnTGTTCT, is an imperfect palindrome that is composed of two 6-base pair half sites. GR binds this element as a homodimer or eventually heterodimers with MR, PR and AR, with each half site occupied by one receptor subunit. The GRE has been shown to mediate the GC-dependent induction of several genes and therefore is often referred to as an activating or positive GRE. However, genome-wide analyses have revealed that GR occupancy of the canonical GREs can also lead to the repression of target genes (Uhlenhaut et al., 2013). A negative GRE (nGRE) has also been recently described mediating GC-dependent repression of specific genes (Surjit et al., 2011).

Once bound to the GRE, the receptor undergoes additional conformational changes that lead to the recruitment of co-regulators and chromatin modifying enzymes that directly and indirectly modulate gene transcription rates by affecting the activity of RNA

polymerase II (Jenkins et al., 2001). Cofactors that mediate transcriptional activation include steroid receptor coactivators (SRC1-3), SWI/SNF, the histone acetyltransferases CBP/p300, and the nuclear methylase coactivator-associated arginine methyltransferase (CARM1). NCoR and SMRT are established corepressors that are recruited to GR bound to nGREs. The specific cofactors assembled and their subsequent activity are dictated by both the nature of the GC ligand and the specific GRE sequence bound by the receptor (Ronacher et al., 2009).

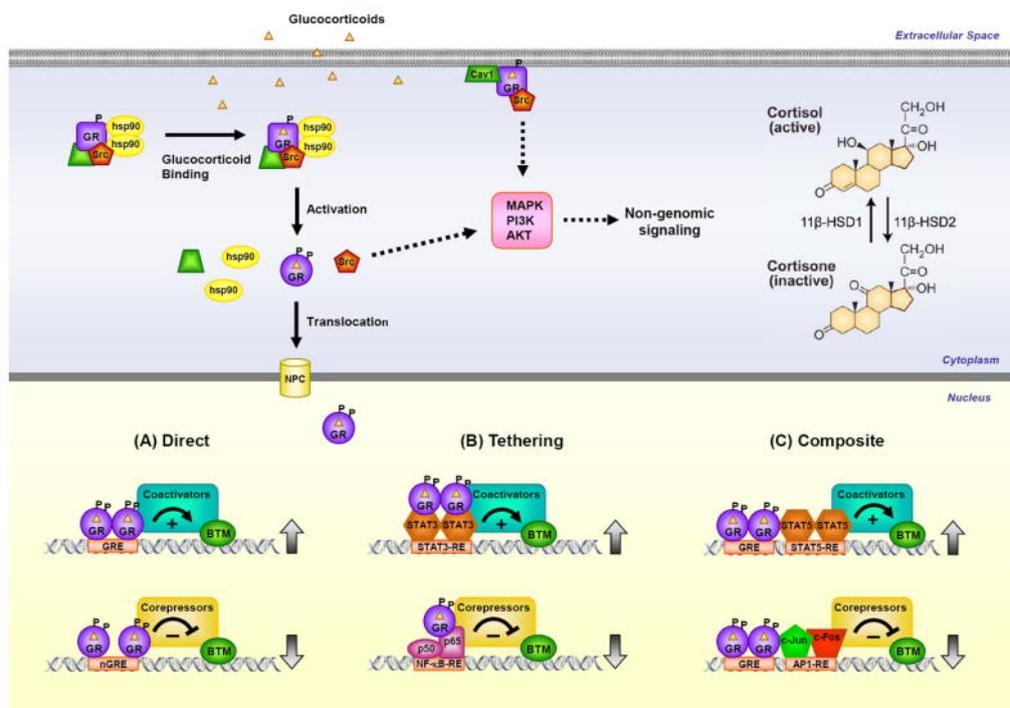


Figure 4: GR signaling pathways. GC-activated GR regulates gene expression in 3 primary ways: binding directly to DNA (A), tethering itself to other DNA-bound transcription factors (B), or binding directly to DNA and interacting with neighboring DNA-bound transcription factors (C). GR can also signal in a non-genomic manner through alterations in the activity of various kinases (Oakley and Cidlowski, 2013)

GR can also regulate the transcription of target genes by physically interacting with other transcription factors (Figure 4). The association of GR with specific members of the STAT family, either apart from or in conjunction with GRE binding, has been shown to either repress or enhance the transcription of responsive genes (Nissen and Yamamoto, 2000; Rogatsky and Ivashkiv, 2006; Tronche et al., 2004). In contrast, the interaction of GR with the pro-inflammatory transcription factors, AP1 and NF-κB, antagonizes their activity and is considered to be a primary mechanism by which GCs

suppress inflammation (Yang-Yen et al., 1990). For some genes, the repression is accomplished by GR tethering itself to these DNA-bound proteins without itself directly interacting with the DNA (Chinenov et al., 2012).

C) Role of GR in the brain

As mentioned above, the GCs regulate brain functions and behavioral responses to environmental changes. Most of what we know about the brain function of GR in the brain is coming from rodent studies. Since constitutive GR knock-out mice die soon after birth due to respiratory failure arising from impaired lung development, the study of conditional knock-out has been crucial to study the role of GR in behavior. Here, we will mainly focus on anxiety and depressive-like behaviors as well as responses to drugs of abuse which constitute the main topics of my work.

1) GR and social behavior, anxiety and depression.

GR gene inactivation in the mouse central nervous system (GR^{NesCre} mice) results in reduction of stress related behaviors such as depression-like and anxiety-like behavior (Tronche et al., 1999) and behavioral responses to cocaine (Deroche-Gamonet et al., 2003). Loss of GR function in the nervous system impairs hypothalamus-pituitary-adrenal (HPA)–axis regulation, resulting in increased GC levels that lead to symptoms reminiscent of those observed in Cushing syndrome (Tronche et al., 1999).

Therefore, more refined models have been generated and studied. GR receptor inactivation in dopaminergic D1-expressing neurons (mainly the caudate putamen, the NAc and the medial prefrontal cortex; GR^{D1Cre} mice) results in higher resistance to chronic social defeat in terms of social interaction (Barik et al., 2013). Early life stress produces a decrease in GR mRNA in the brain, with a notable reduction in the amygdala that is associated with sustained alterations in anxiety, fear and sociability-like behaviors. Lentiviral-mediated restoration of the GR mRNA deficit, specifically within the adult central nucleus of the amygdala (CeA), reverses the enduring changes in anxiety and social behavior after early life stress (Arnett et al., 2015).

2) GR and addiction

In addition to its effects on anxiety and depressive-like behaviors, the absence of GR in the central nervous system in GR^{NesCre} mice reduces the animals responses to cocaine in locomotor sensitization and self-administration paradigms (Deroche-Gamonet et al., 2003). Conversely, the overexpression of GR in the forebrain confirms leads to an increase in cocaine sensitization (Wei et al., 2004). Dopaminergic transmission within the reward system is largely implicated in the addictive properties of drugs. Targeting GR along the dopamine pathway also alter responses to psychostimulant drugs. While GR gene inactivation in dopamine neurons (GR^{DATCre} mice) does not have any effect, GR inactivation in dopaminoceptive neurons specifically reduces the self-administration, sensitization and conditioned place-preference to psychostimulants (cocaine and amphetamine) but spear the responses to opiates (Ambroggi et al., 2009; Barik et al., 2010; Parnaudeau et al., 2014).

Chapter2. Chromatin remodelers and Epigenetics

As other steroid receptors, the GR has the peculiarity to be a transcription factors that control the expression of target genes in response to a specific hormone signal. As we saw, GR can act on intracellular signaling cascades such as MAP-Kinases and Akt/PI3A pathways and, when present at the membrane, can modulate synaptic transmission, but its best-known function is its transcription factor one. It is present at the promoters or enhancers of target genes when bound to GRE DNA segments but also when interacting, via protein-protein bounds, with other transcription factors themselves bound to DNA targets like; AP1, STAT3, STAT5 (Nissen and Yamamoto, 2000; Rogatsky and Ivashkiv, 2006; Yang-Yen et al., 1990). It can intervene on the recruitment of the general transcriptional machinery, just before RNA polymerization. It can also intervene earlier, at the chromatin level, to recruit modifier enzymes that act on chromatin shaping at the protein or DNA level and modulate thus chromatin structure and chromatin accessibility of other regulatory proteins and the general transcriptional machinery (McEwen et al., 1996).

The regulation of gene expression through chromatin modification is known since the 1960's and if a vast amount of knowledge accumulated since those years demonstrated its importance for all biological functions (development, differentiation, adaptation), conceptualizing theories start to emerge. In eukaryotic cells, nuclear DNA is packaged into chromatin structures that can be modulated to generate transcriptionally active or repressed configurations in different cellular contexts and under changing environmental conditions. This remarkable plasticity is governed in part by multi-subunit protein complexes that enzymatically regulate chromosomal structure and activity. These complexes can either chemically modify the histone tails of nucleosomes, disrupt histone-DNA contacts through ATP hydrolysis or chemically modify nucleic bases in the DNA major groove.

I. Nuclear organization

In eukaryotic cells, chromatin is tightly packed in a highly organized fashion within a nucleus composed of the nucleoplasm and the nuclear envelope. There are sub-compartments in the nucleus containing factors involved in essential nuclear functions

such as DNA replication, transcription, and RNA splicing (Prasanth et al., 2010). The nuclear envelope separates nuclear functions from cytoplasmic functions and at its inner surface it provides a docking site for chromatin. The major structural elements of the nuclear envelope are the inner nuclear membrane, the outer nuclear membrane, the nuclear pore complexes, and the nuclear lamina. The lamina is comprised of a complex meshwork of proteins closely associated with the inner nuclear membrane and attached to the periphery of nuclear pore complexes and to chromatin. The main constituents of the lamina are the type V intermediate filament proteins, the nuclear lamins. Lamins are also found, in lower concentrations, distributed throughout the nucleoplasm. The organization of lamins at the nuclear periphery as well as within the nucleoplasm is influenced by numerous lamin-binding proteins (Figure 5, Dechat et al., 2008). The importance of lamins in chromatin organization is most evident in cells derived from patients suffering from various laminopathies and in the LMNA-null mouse (Table 1).

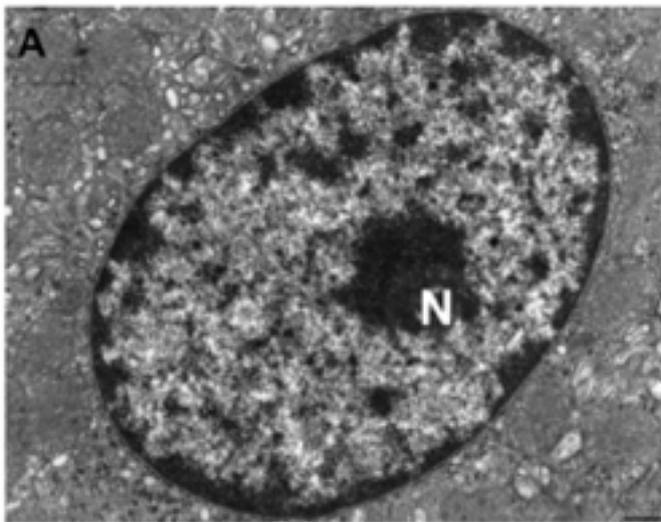


Figure 5: Electron microscopic observation of fibroblastic nucleus. Low-magnification views show peripheral heterochromatin and nucleoplasmic heterochromatic foci in the normal nucleus.

Taken together, these findings demonstrate that the mutations of lamin gene leads to global changes in the epigenetic organization of chromatin, which undoubtedly contributes to the phenotypes observed in different laminopathies, including defects in DNA repair and alterations in gene expression.

Table 1. *Changes in heterochromatin organization caused by lamin A/C mutations or deficiencies*

Mutation ^a	Species	Clinical phenotype ^b	Cell type/ tissue ^c	Chromatin phenotype ^d	Detection method ^e	Reference
Knockout	Mouse	Retarded growth rate, muscular dystrophy	MEFs and hepatocytes	Local loss of peripheral heterochromatin	Electron microscopy	Sullivan et al. 1999
			Cardiomyocytes	Fragmentation and displacement of heterochromatin	FISH with pancentromeric probe	Nikolova et al. 2004
				Peripheral and central heterochromatin clumping	Electron microscopy	
1580 G > C (R527P)	Human	AD-EDMD	Skeletal muscle	Reduced peripheral heterochromatin, heterochromatin clumps	Electron microscopy	Sabatelli et al. 2001
1444 C > T (R482W)	Human	FPLD	Dermal fibroblasts	Loss of peripheral heterochromatin	Electron microscopy	Capanni et al. 2003
1318 G > A, 1580 G > A (V440M, R527H)	Human	MAD	Dermal fibroblasts	Detachment of heterochromatin from the periphery	Electron microscopy	Lombardi et al. 2007
1580 G > A (R527H)	Human	MAD	Dermal fibroblast	Loss of peripheral heterochromatin Delocalization and solubilization of H3K9me3 and HP1 β	Electron microscopy Immunofluorescence	Filesi et al. 2005

Table 1: Changes in heterochromatin organization caused by lamin A/G mutation. ^a The DNA base pair changes and the amino acid changes (in parentheses) are shown. ^b The phenotypes are as follows: autosomal-dominant Emery-Dreifuss muscular dystrophy (AD-EDMD), familial partial lipodystrophy (FPLD), mandibuloacral dysplasia (MAD), and Hutchinson-Gilford progeria syndrome (HGPS). ^c The cell types or tissue examined are listed. ^d Alterations in chromatin organization described. (H3K9me3) Histone H3 trimethylated at Lys 9; (HP1) heterochromatin-associated protein 1; (H3K9me1) histone H3 monomethylated at Lys 9; (H3K27me3) histone H3 trimethylated at Lys 27; (H4K20me3) histone H4 trimethylated at Lys 20.

II. Structure of chromatin

Eukaryotic DNA is packaged into a complex macromolecular structure called chromatin that may facilitate compaction of the genetic material. The DNA is folded and condensed into chromatin in a dynamic manner since it still needs to be accessible to carry out key functions such as replication, transcription, and DNA repair. The first step of compaction is achieved by packaging the naked DNA into the nucleosome which consist of 174 base pairs of DNA wrapped around the histone proteins octamer containing two molecules of each core histone H2A, H2B, H3, and H4 (Luger et al., 1997). Nucleosomes are separated by approximately 50 base pairs. Histone H1 is important for higher-order chromatin formation and compaction (Robinson and Rhodes, 2006). It stabilizes the interaction of DNA with the nucleosome and prevents

ATP-dependent remodeling of the chromatin from changing the position of nucleosome (Ramachandran et al., 2003), Figure 6).

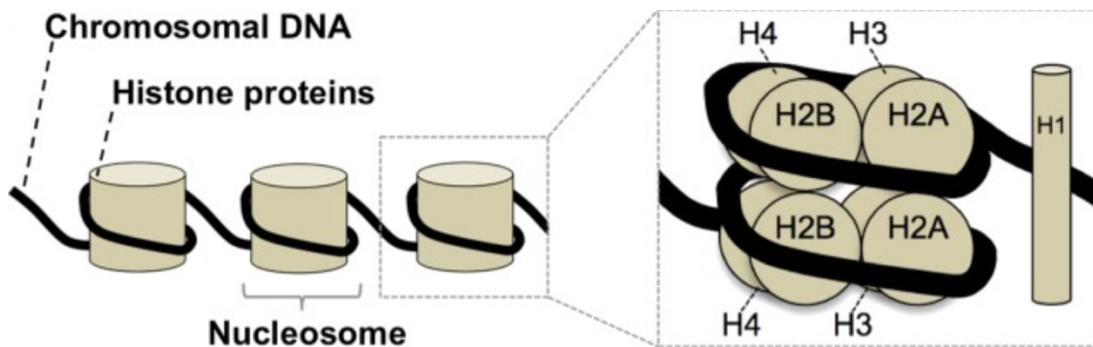


Figure 6: Structure of the chromatin (Ramachandran et al., 2003). Nucleosome consist of 174 base pairs of DNA wrapped around the histone proteins octamer containing two molecules of each core histone H2A, H2B, H3, and H4.

Histones are small basic proteins that can be covalently modified at their flexible N- or C-terminal tails (Kouzarides, 2007). Histone post-translational modifications affect their interactions with DNA, leading to alterations in chromatin structure and function (Choi and Howe, 2009; Strahl and Allis, 2000); Strahl and Allis 2000). For instance, certain histone post-translational modifications are associated with a more open and accessible chromatin conformation, while others may be found in highly compacted and transcriptionally inactive loci, hinting towards the “histone code” (Strahl and Allis, 2000). They will be described later in details.

From the functional point of view, various chromatin structures are commonly divided into euchromatin and heterochromatin. Euchromatin describes regions within the nucleus that contain actively transcribed genes. Generally, active regions contain histones that are hyperacetylated at lysine residues (Figure 7).

Constitutive heterochromatin describes the highly condensed regions of the genome that are visible as bright nuclear areas following staining with DNA dyes such as 4',6-diamidino-2-phenylindole (DAPI) and Hoechst. These regions often comprise repetitive DNA (such as satellite sequences surrounding centromeres) and are generally thought have a low density of genes (Strahl and Allis, 2000, Figure 7).

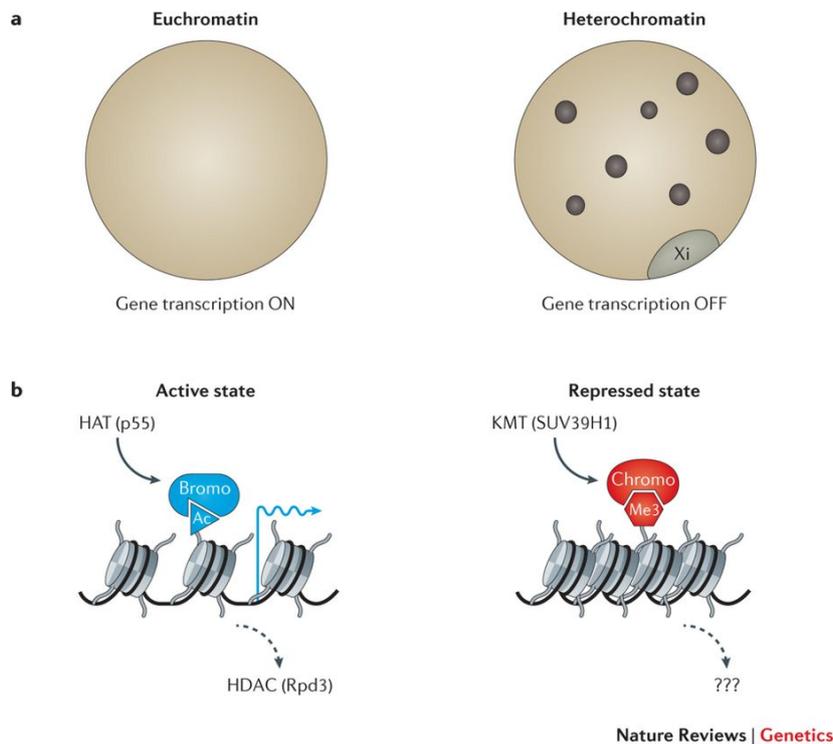


Figure 7: Euchromatin and heterochromatin (from Allis and Jenuwein, 2016). Euchromatin is a region within the nucleus that contains actively transcribed genes. Heterochromatin describes the highly condensed regions of the genome with a low density of genes.

III. Epigenetics

The term, “epigenetics,” was first used to explain the complex interactions between the environment and genome that are involved in development and differentiation in higher organisms. Historically, the word “epigenetics” was used to describe events that could not be only explained by genetic principles. Conrad Waddington (1905–1975), who is given credit for coining the term, defined epigenetics as “the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being”. He forged the word from Aristoteles “Epigenesis” theory of development that was taken considering the influence of environment on embryo development and from “Genetics” since after T. Morgan it was clear that developmental genes existed and conditioned development. Epigenetics, in a broad sense, is a bridge between genotype and phenotype, a phenomenon that changes the final outcome of a locus or chromosome without changing the underlying DNA sequence. For example, even though the vast majority of cells in a multicellular organism share an identical genotype, organismal development generates a diversity of cell types with disparate, yet stable, profiles of gene expression and distinct cellular functions. Thus, cellular

differentiation may be considered an epigenetic phenomenon, largely governed by changes in what Waddington described as the “epigenetic landscape” rather than alterations in genetic inheritance (Goldberg et al., 2007; Figure 8).

Today, this term is used to refer to heritable alterations, through cell division, in higher organisms that are not due to changes in DNA sequence. By extension, it refers also to the mechanisms involved in changing gene expression, for instance long-term ones observed under environmental influences. Epigenetic modifications rather alter DNA accessibility and chromatin structure, thereby regulating patterns of gene expression. Today, a wide variety of illnesses, behaviors, and other health indicators already have some level of evidence linking them with epigenetic mechanisms, including cancers of almost all types, cognitive dysfunction, and respiratory, cardiovascular, reproductive, autoimmune, and neurobehavioral illnesses (Felsenfeld, 2014; Inbar-Feigenberg et al., 2013).

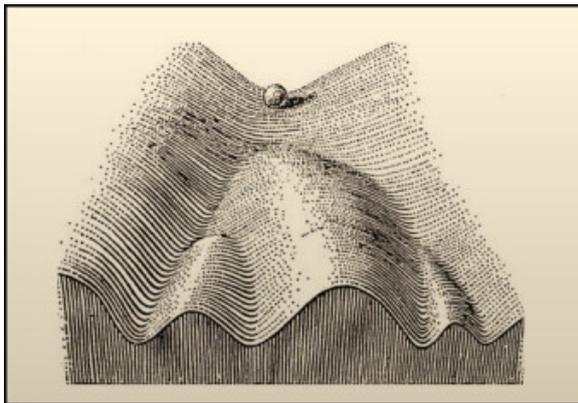


Figure 8: In 1957, Conrad Waddington proposed the concept of an epigenetic landscape to represent the process of cellular fate decision-making during development. At various points in this dynamic visual metaphor, the cell (represented by a ball) can take specific permitted trajectories, leading to different outcomes or cell fates. Figure adapted from (Goldberg et al., 2007).

Histones are basic proteins consisting of a core and an N-terminus tail composed of a loosely-structured sequence of amino acids. Posttranslational histone modifications occur primarily on the N-terminus tail, and include acetylation, methylation, phosphorylation, ubiquitination and sumoylation (Fig. 9A).

DNA methylation occurs throughout the genome but is functionally most relevant when present in sequences rich in CpG dinucleotides. Because of their chemical properties, these modifications influence the condensation of chromatin, and thereby modulate the accessibility of DNA to the transcriptional machinery (Allis and Jenuwein, 2016; Felsenfeld, 2014; Gräff and Mansuy, 2008; Weinhold, 2006).

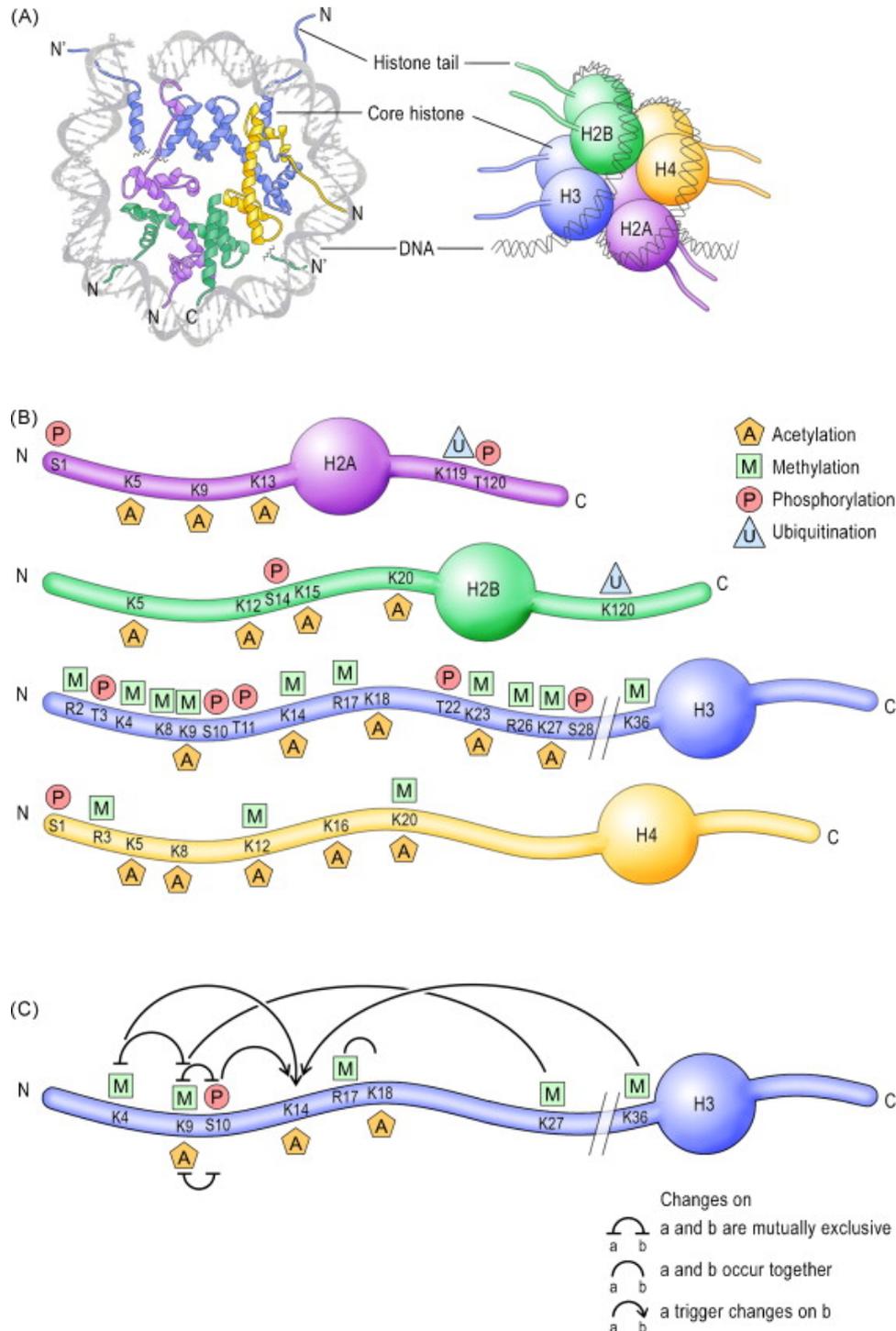


Figure 9: Epigenetic marks on histone tails and DNA. (A, left) View of the nucleosome down the DNA superhelix axis showing one half of the nucleosome structure. (Right)

Schematic representation of the four-nucleosome core histones, H2A, H2B, H3 and H4. (B) Schematic representation of the N- and C-termini of the core histones and their residue-specific epigenetic modifications. (C) Crosstalk between epigenetic modifications on the H3 N-terminus tail. Adapted from (Gräff and Mansuy, 2008)

As mentioned above, the regulation of gene expression involves chromatin modifications regulate the gene expression. For example, euchromatin in which most of active genes are present contain histones that are frequently hyperacetylated at lysine residues. As well the active genes are also enriched in methylated Lys4 and Lys79 histone H3 residues. And methylation of Arg2, Arg17 and Arg26 of H3, and Arg4 of H4, are associated with transcriptional activation (Daujat et al., 2002). Whereas, phosphorylation (notably Ser10 of H3) is associated with immediate early gene activation (Lachner et al., 2003). The hallmarks of constitutive heterochromatin include trimethylation at Lys9 of histone H3, a paucity of methylation at H3 Lys4 and trimethylation of H4 Lys20 (Kourmouli et al., 2004; Schotta et al., 2004).

An important feature of epigenetic marks that is essential for transcriptional regulation is their ability to crosstalk (Fig. 11C). Posttranslational histone modifications often act in concert, and multiple feed-forward and feed-back mechanisms involving the same nucleosome or histone, or distinct nucleosomes and histones have been identified. These cross-talks can enhance chromatin condensation when transcriptional silence is required, or chromatin opening when transcriptional activity is needed. The repertoire of DNA and histone modifications is controlled by specific enzymes that include DNA methyltransferases (DNMTs), histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone demethylases (HDMs), protein kinases (PKs), protein phosphatases (PPs), ubiquitin- and SUMO-associated enzymes (Figure 11B). These enzymes operate both independently and in synergy establishing a “histone code”, a highly dynamic and flexible chromatin marking that, in combination with chromatin-associated proteins, could stabilize the pattern of gene expression in response to given external stimuli. That way, epigenetic modifications at the level of the chromatin provide a focal point to bridge nuclear events to intracytoplasmic signaling cascades, and a potential molecular means to retain marks of prior transcriptional activity in the nuclear machinery.

Epigenetic processes are natural and essential to many organism functions, but if they occur improperly, there can be major adverse health and behavioral effects (Allis and Jenuwein, 2016; Felsenfeld, 2014; Weinhold, 2006).

A) DNA modifications (DNA methylation)

DNA methylation is defined as addition of a methyl group to cytosine within a CpG dinucleotide, thereby forming 5-methylcytosine (Bird, 1986). The term CpG refers to the base cytosine (C) linked by a phosphate bond to the base guanine (G) in the DNA nucleotide sequence. Most CpG dinucleotides in the human genome are methylated. Unmethylated CpGs are not randomly distributed, but are usually clustered together in 'CpG islands', which are in the promoter region of many genes (the region that facilitates transcription of a particular gene) (Bird, 1986; Ehrlich et al., 1982).

These modifications are achieved by two types of DNA methyltransferases. The first type, DNMT1 functions during DNA replication to copy the DNA methylation pattern from the parental DNA strand onto the newly synthesized daughter strand (Moore DL et al, 2012). DNMT3a and DNMT3b play a major role in de novo methylation, in which methylation can be performed on double-stranded DNA that is not methylated. DNA methylation is generally associated with gene silencing (Okano M et al,1999), and DNA demethylation is usually connected with gene activation (Goldberg et al., 2007).

DNA demethylation can take place as either passive or active mechanisms. Passive DNA demethylation occurs in dividing cells. Active DNA demethylation can occur in both dividing and non-dividing cells (Moore et al., 2013).

B) Histone modifications

1) Histone acetylation and deacetylation

Acetylation of histone's lysine group is highly dynamic and regulated by the opposing action of two families of enzymes, (HATs) and histone deacetylases (HDAC). Its link with gene expression is known for decades (Allfrey et al., 1964). The HATs utilize acetyl-CoA as cofactor and catalyze the transfer of an acetyl group to the ϵ -amino

group of lysine side chains. In doing so, they neutralize the lysine's positive charge and this action has the potential to weaken the interactions between histones and DNA. HDAC enzymes oppose the effects of HATs and reverse lysine acetylation, an action that restores the positive charge of the lysine. This potentially stabilizes the local chromatin architecture and is consistent with HDACs being predominantly transcriptional repressors (Bannister and Kouzarides, 2011).

2) Histone phosphorylation

Histone phosphorylation is a transfer of phosphate group from ATP to the hydroxyl group of the target amino-acid side chain. this modification adds significant negative charge to the histone that undoubtedly influences the chromatin structure. histone phosphorylation is highly dynamic process which is controlled by kinases and phosphatases that add and remove the modification, respectively (Oki et al., 2007).

3) Histone methylation

The initial publication that described histones modifications (Allfrey et al., 1964) not only observed that histones acetylation but also methylation was associated with changes in gene expression. Histone methylation mainly occurs on the side chains of lysine and arginine. Unlike acetylation and phosphorylation, however, histone methylation does not alter the charge of the histone protein. Furthermore, Methylation of lysine or arginine residues can occur in several modification states. Lysine residues can house either one (me1), two (me2) or three (me3) methyl moieties on their amine group, whereas arginine residues can carry one (me1) or two (me2) methyl groups on their guanidiny group. These defined modification states can have different and profound implications on the function of chromatin. In general, H3K4, K36, and K79 methylation are found near active transcriptional units and H3K9 and H4K20 modifications are hallmarks of silenced or heterochromatic regions. Histone arginine methylation occurs on H3R2, R8, R17 and R26 and H4R3 and has roles in defining both active and repressed chromatin states (Ng et al., 2009; Turner, 2005).

For many years, histone methylation was strangely considered a stable, static modification. Nevertheless, a number of different reactions/pathways were suggested

as potential demethylation mechanisms for both lysine and arginine (Bannister and Kouzarides, 2011).

4) Ubiquitylation and sumoylation

All of the previously described histone modifications result in relatively small molecular changes to amino-acid side chains. In contrast, ubiquitylation results in a much larger covalent modification. Ubiquitin itself is a 76-amino acid polypeptide that is attached to histone lysines via the sequential action of three enzymes, E1-activating, E2-conjugating and E3-ligating enzymes (Hershko and Ciechanover, 1998). The enzyme complexes determine both substrate specificity (i.e., which lysine is targeted) as well as the degree of ubiquitylation (i.e., either mono- or poly-ubiquitylated). For histones, mono-ubiquitylation seems most relevant although the exact modification sites remain largely elusive. However, two well-characterized sites lie within H2A and H2B. For example, H2AK119ub1 is involved in gene silencing, whereas H2BK123ub1 plays an important role in transcriptional initiation and elongation.

Even though ubiquitylation is such a large modification, it is still a highly dynamic one. The modification is removed via the action of isopeptidases called de-ubiquitin enzyme and this activity is important for both gene activity and silencing (Bannister and Kouzarides, 2011).

Sumoylation is a modification related to ubiquitylation and involves the covalent attachment of small ubiquitin-like modifier molecules to histone lysines via the action of E1, E2 and E3 enzymes. Sumoylation has been detected on all four core histones and seems to function by antagonizing acetylation and ubiquitylation that might otherwise occur on the same lysine side chain (Nathan et al., 2006).

5) Histones variants

Histone variants are deposited onto chromatin by specific histone chaperones and also interact with other chromatin modifiers. Structural differences introduced by a core histone variant typically affect interactions between histone proteins within a nucleosome, hence their stability as well as the open/compact chromatin conformation. For example, histone variants H2A.Z and H3.3 are mainly linked with an open chromatin conformation and transcriptional activity, while macroH2A deposition

stabilizes the nucleosome and is often associated with a repressive chromatin state. Consequently, replacement of canonical histones with histone variants adds another level of complexity and a distinct way of modulating chromatin function (Skene and Henikoff, 2013).

Histone variants exchange play a potential mechanism of gene regulation in response to neural activity. For example; H3.3 dramatically accumulates in neuronal and glial chromatin with age and remains highly dynamic throughout life to control cell-type specific gene expression programs and physiological plasticity. Moreover, manipulations of H3.3 in neurons, which stall its incorporation and eviction from chromatin, reveal histone turnover as a critical mediator of neuronal activity-dependent gene expression, synaptic connectivity and cognition (Maze et al., 2015).

Histone Coding

The hypothesis of a histone code suggested by Strahl and Allis in 2000, proposes that the combination of histone modifications at a certain genomic locus determines the activity state of the underlying gene (Strahl and Allis, 2000). The modifications regulate accessibility for DNA binding and regulatory proteins, such as transcription factors, either by altering the charge on the histones and thus changing histone–DNA interactions or by recruiting structural proteins. By this means, histone modifications store epigenetic information. However, the histone code is heavily debated within the epigenetic field, and it has been argued that the regulation of gene expression by various histone modifications might simply reflect a cumulative effect rather than the interpretation of a combination (Henikoff, 2005). As an extension of the initial code, theoretical ‘modification cassettes’ have been suggested to have one specific output (Fischle et al., 2003). Combinations of modifications act when they are located on the same histone tail and on different histones within a nucleosome. For example, the ubiquitylation of H2B is necessary for the methylation on lysine 79 of histone H3 (Briggs et al., 2002). The histone code was thus expanded to a ‘nucleosome code’, suggesting that the presence of all post-translational histone modifications in one nucleosome regulate the underlying DNA sequence. Particular importance is attached to so-called binary switch modules in which modifications on two neighboring residues can influence each other. An example is the methylation of lysine 9 on histone H3, which is bound by heterochromatin protein 1 (HP1). During mitosis and chromatin

condensation, serine 10 is phosphorylated, which reduces HP1 binding to H3K9me. This binding is further reduced when H3 becomes acetylated at lysine 14, which strips HP1 from the mitotic chromosomes. In addition to the inhibition of HP1 binding to the lysine 9 methylated peptide, serine 10 phosphorylation interferes with the methylation of H3K9 by SUV39H1 (Garcia et al., 2005; Rea et al., 2000).

Genome-wide mapping of individual histone modifications made it possible to detect correlations between histone modification patterns and specific states of gene activity. For example, particular modifications including H3K4me_{2,3} (histone H3 lysine4 di- and trimethylation) and H3K36me_{2,3} are frequently located in actively transcribed regions. By contrast, modifications such as H3K27me₃ and H4K20me₃ are frequently mapped to regions where transcription is repressed (Barth and Imhof, 2010) (Figure 10).

A) Nuclear topology

Eukaryotic nuclei are well-organized structures with distinct nuclear compartments. The arrangement of these compartments inside the nuclear space is termed nuclear architecture (Figure 11).

The main resident of eukaryotic nuclei is the genetic material itself, packaged in the form of chromatin. Akin to the nucleus itself, chromatin is also organized into well-defined domains called chromosomes. The chromosomes themselves occupy distinct subvolumes of the nuclear space, termed chromosome territories and the position of gene loci within each chromosome is also subject to strict regulation. (Cremer et al., 1986) In the nervous system, nuclear architecture has been shown to be distinct among different types of cells and during different stages of differentiation or development are significantly determined and regulated by chromatin (Takizawa and Meshorer, 2008).

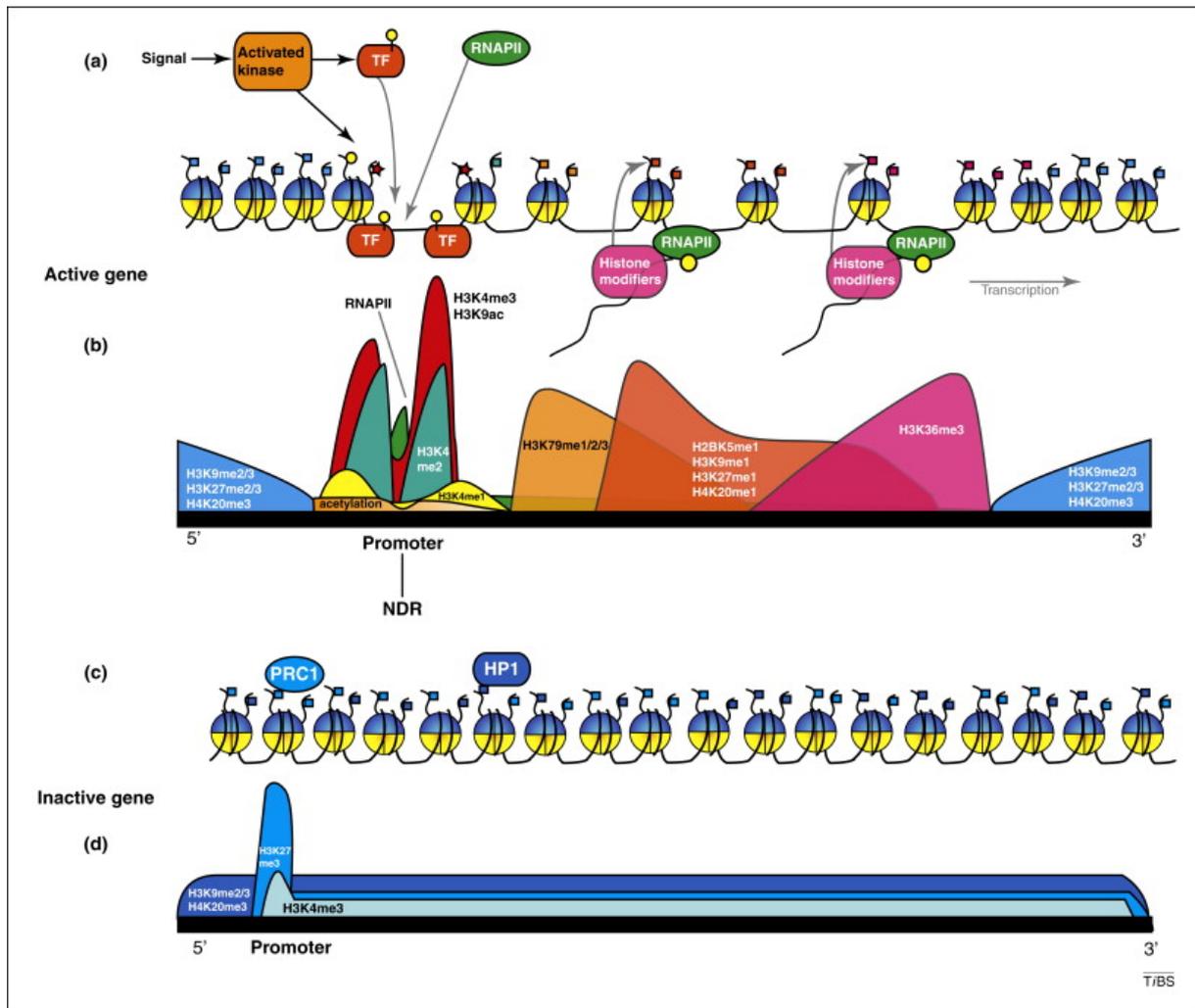


Figure 10: Distribution of histone modifications on active and silenced genes. Modification patterns differ on actively transcribed and silenced genes, which is displayed in this figure as a schematic view on nucleosomes (a and c) or modification distribution over the gene (b and d). An active gene is shown in (a) and in (b). An external signal can lead to the activation of kinases (orange) in the nucleus, which can then phosphorylate (yellow circles) histones as well as transcription factors (TF, red) to elicit an appropriate physiological response. The transcription factors will then bind DNA at the promoter and facilitate the docking of RNA polymerase II (RNAPII, green). At this point, transcription can begin. Elongating RNA polymerase II, which is highly phosphorylated on its C-terminal domain, can interact with histone modifiers (pink), for example Set2, which methylates H3K36, and thus introduce active modifications into chromatin at this locus. In particular, nucleosomes within the promoters of actively transcribed genes carry high levels of active modifications such as acetylations and methylation of H3K4. At the transcriptional start-site there is a nucleosome-depleted region (NDR) within the promoter. Active modifications such as methylation of H3K79 are present in the body of these genes. Inactive genes, as shown in (c) and (d), have a fairly even distribution of silencing modifications, such as H3K9 methylation and H4K20 methylation, whereas H3K27 methylation is enriched in the promoter. These modifications can be bound by heterochromatic proteins (blue) and, thus, this chromatin area can condense, as seen in heterochromatin. Adapted from (Barth and Imhof, 2010).

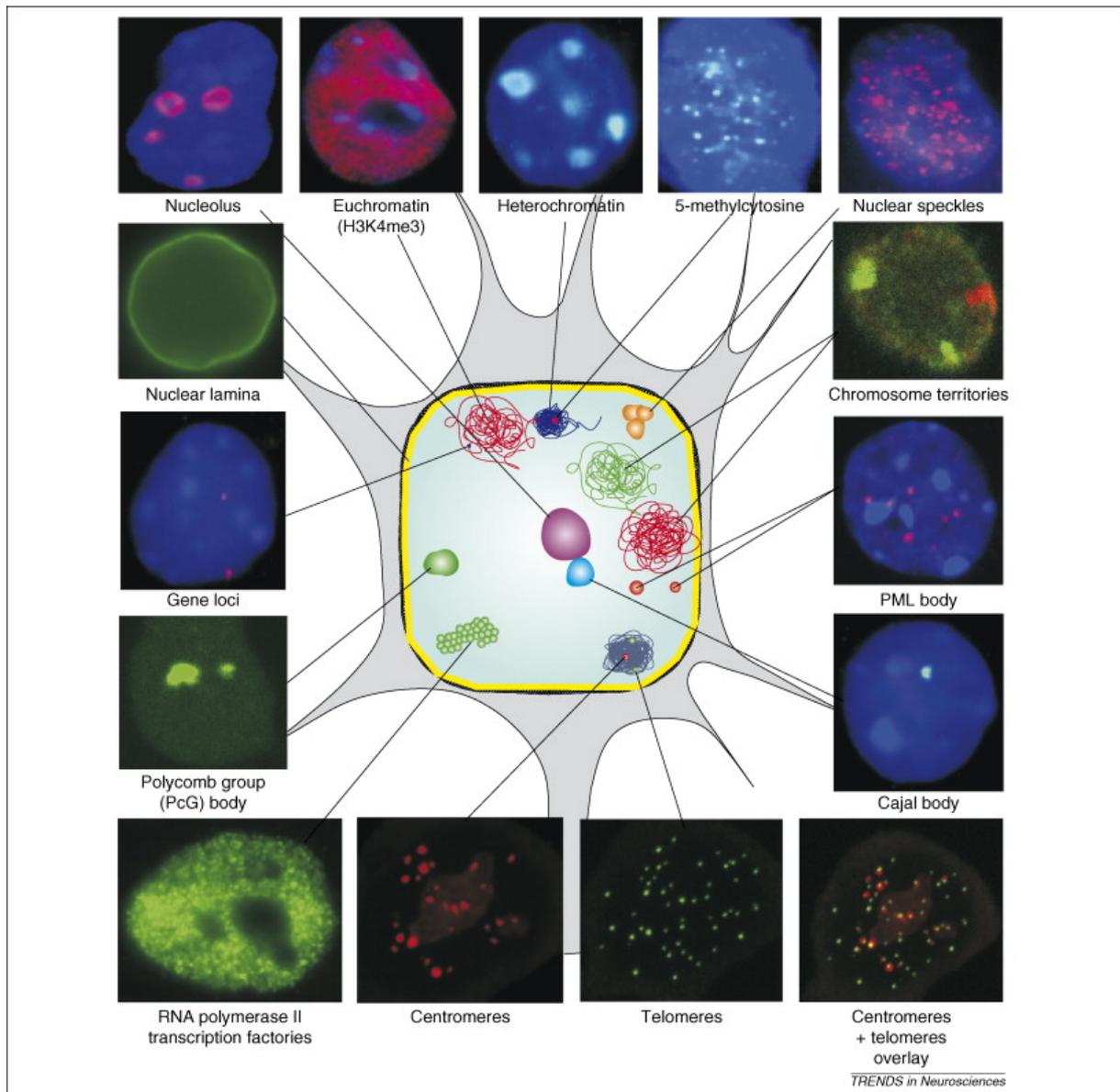


Figure 11: Nuclear architecture. Examples of common nuclear bodies are depicted. Neuronal immunofluorescence images are shown for the various nuclear domains (Takizawa and Meshorer, 2008).

Chromatin can exist in different ‘states’ as described earlier, including ‘open’ (eu-) and condensed (hetero-) chromatin. These are differentially defined by three characteristics: (1) loose or dense nucleosomal packaging euchromatin or heterochromatin, respectively, (2) specific types of post-translational histone modifications (such as acetylation), and (3) presence or absence of various chromatin regulatory proteins that either facilitate or repress transcription. For example, actively expressed genes in open chromatin show high levels of histone acetylation, with nucleosome-free intervals occupied by activator proteins (transcription factors) and the

RNA polymerase II initiation complex (Figure 12). Superimposed upon these types of nucleosomal organization is the 3-dimensional conformation of chromatin fibers and entire chromosomes, often described in terms such as ‘loopings’ or ‘globules’ and in toto referred to as the ‘3D genome’. This includes the ‘clustering’ of euchromatic and heterochromatic sequences that tend to assemble into alternating regions of approximately ~5 megabases (Mb). These ‘compartments’, positioned along the same chromosome, are able to interact with compartments from different chromosomes (Lieberman-Aiden et al., 2009). Euchromatic regions are termed ‘A compartments’ and are enriched with open/decondensed chromatin and correspond to much higher overall levels of transcription, whereas ‘B compartments’ harbor inactive and heterochromatic sequences (Rao SS et al, 2014)(Rao et al., 2014) (Figure 12). In most cell types, large clusters of heterochromatin are enriched at the nuclear periphery, in multiple pericentromeric foci in the nuclear interior and around nucleolar membranes (Padeken and Heun, 2014).

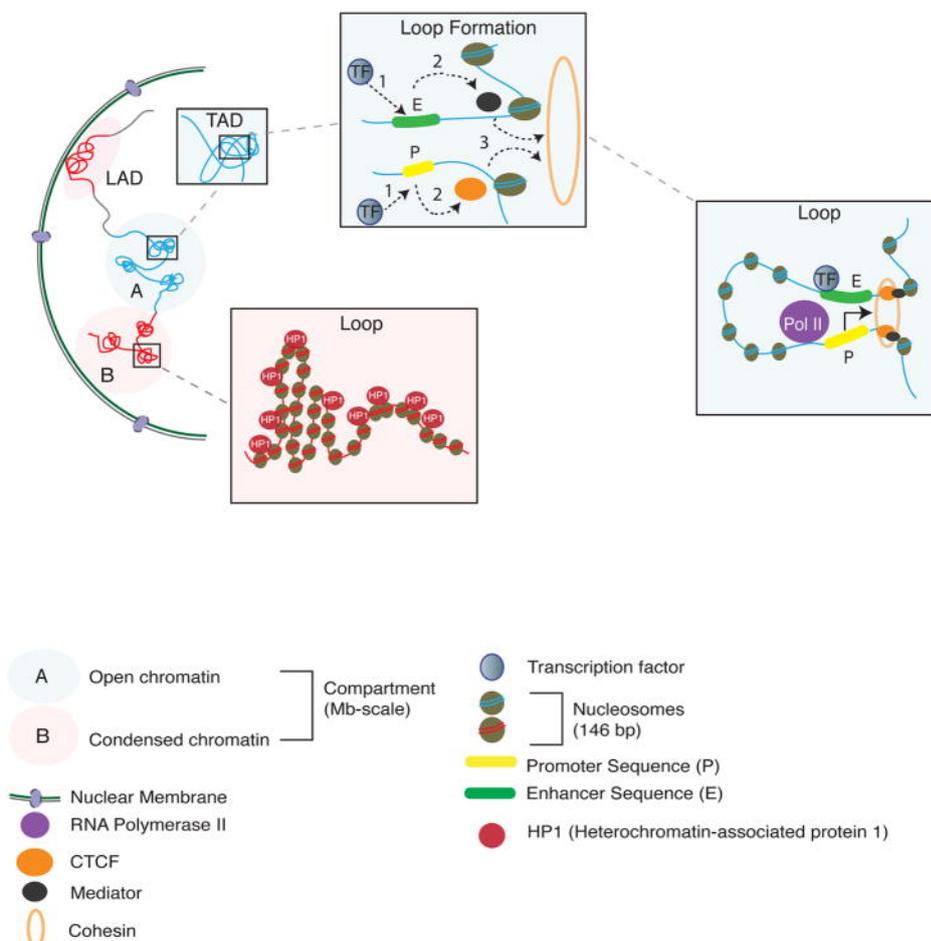


Figure 12: The 3-dimensional genome, from nucleosome to nucleus.

IV. Chromatin remodeler complex (Nucleosome remodelers)

The importance of histones and chromatin structure in the regulation of eukaryotic gene transcription has become much more widely accepted over the past few years. ATP-dependent chromatin remodeling complexes specifically recognize these histones modifications (acetylation, methylation, phosphorylation, ribosylation and ubiquitination), and through ATP hydrolysis unwrap, mobilize, exchange or eject the nucleosome, and subsequently recruiting a transcriptional apparatus to nucleosomal DNA (Cosma, 2002)

The mechanisms by which histones positioning and constitution contribute to the regulation of transcription both *in vitro* and *in vivo* have been clarify in the last decades (Vignali et al., 2000) However, chromatin itself is stable and shows limited mobility, and its dynamic properties are due to the action of chromatin-modifying and -remodeling complexes (Strahl and Allis, 2000). In this manner, chromatin structure simultaneously provides a packaging solution and a sophisticated apparatus for regulating gene expression.

A) Different ATP-dependent chromatin remodeler complexes

ATP-dependent chromatin remodeling complexes are large (>1 MDa) multi-components complexes (consisting of between 4 and 17 subunits) that are highly conserved within eukaryotes. They are characterized by the presence of an ATPase subunit belonging to the superfamily II helicase-related proteins (Singleton and Wigley, 2002). Proteins belonging to this class contain an ATPase domain that is itself comprised of two parts, the DExx and HELICc regions, which are separated by a linker. This class can be further classified into at least 4 different families (SWI/SNF, ISWI, NURD/Mi-2/CHD, and INO80) based on the additional presence of unique domains within or adjacent to the ATPase domain (Figure 13)(Tang et al., 2010).

A) SWI/SNF complex

SWI/SNF is a protein complex composed of a dozen of proteins. It was originally identified as a regulator of mating type switching (SWI) or as a requirement for growth on energy sources other than sucrose (SNF – sucrose non-fermenting) in yeast

(Neigeborn and Carlson, 1984; Peterson and Workman, 2000). The SWI/SNF complex is a large ATP-dependent chromatin remodeling complex that is highly conserved from yeast to human, which is essential for transcription regulation, genomic stability, DNA repair and many aspects of development (Kasten et al., 2011; Tang et al., 2010).

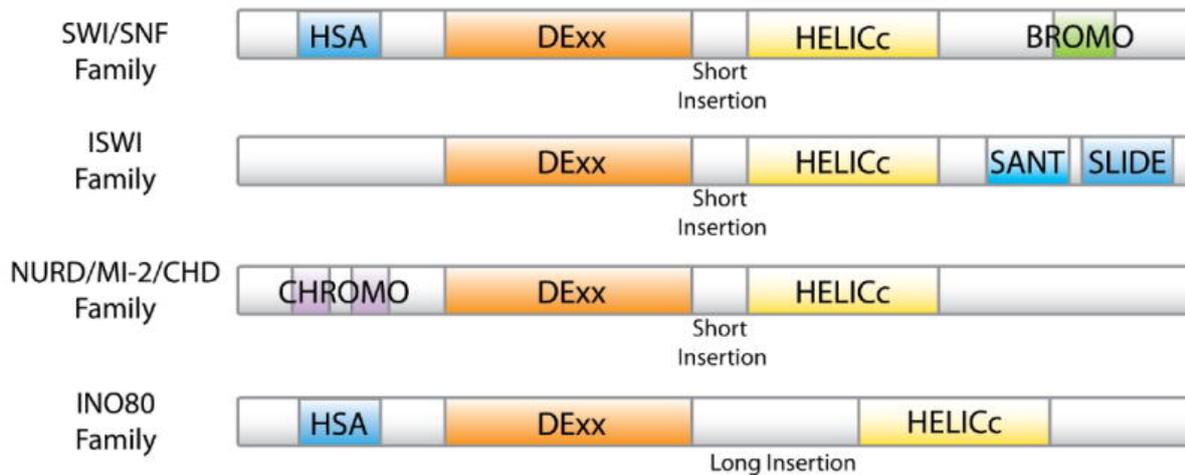


Figure 13: Classification of ATP-dependent chromatin remodeling complexes: The ATPase subunit of all the remodeling complexes belongs to the superfamily II helicase group. The ATPase always contains a DExx and a HELICc domain, spaced by a linker. The remodelers are classified into different families based on the presence of additional domains on their ATPase subunits. The SWI/SNF family contains a HSA domain, involved in actin binding, and a bromodomain important for the binding of acetylated lysines. The ISWI family contains the SANT and SLIDE domains, important for histone binding. The CHD/NURD/Mi-2 family contains a tandem Chromo domain, also used for histone binding. The INO80 family, like the SWI/SNF family, comprises a HSA domain but it is also characterized by the presence of a longer insertion between the DExx and the HELICc domains (Tang et al., 2010).

In *S. Cerevisiae*, as in drosophila and Humans, there are two versions of the chromatin complex called SWI/SNF and RSC (for Remodeling the Structure of Chromatin). RSC is founded more in the cell comparing to SWI/SNF. RSC is essential for cell growth while SWI/SNF is not. The catalytic subunit of yeast SWI/SNF is the Swi2 or Snf2 protein and its paralog in RSC is the Sth1 subunit (Du et al., 1998).

In drosophila, the two forms of chromatin remodeler complexes are called BAP-complex (Brahma Associated Protein) and PBAP-complex (Polybromo-associated BAP) which both comprise the same ATPase subunit, named Brahma. In Human, similarly, these two complexes are called BAF complex (Brg1 Associated Factors) and PBAF complex (Polybromo-associated BAF) and can contain one of the two distinct

ATPase subunits hBrm (human Brahma) or Brg1 (Brahma-related Gene 1) (Tang et al., 2010).

Brg1 and Brm activities are partially the same but they have different expression profiles; Brg1 protein levels are relatively constant in all cells whereas Brm protein concentrations increase during cellular differentiation (Kadam and Emerson, 2003). *In vivo* studies suggest that these proteins exhibit distinct non-redundant biological functions. Interestingly, genetic inactivation of Brg1 or Brm in mice resulted, depending on the tissues in moderate to severe deregulation of cellular processes such as proliferation. Brg1-deficient mice die at the pre-implantation stage, suggesting that Brg1 is essential for early development. Moreover, Brg1 heterozygotes were found to be predisposed to tumor development. Conversely, Brm null mice were found to be viable, fertile, although these mice showed increased expression of Brg1 in certain (brain, liver, spleen, kidney, thymus, lung and hearts) tissues and displayed aberrant cell cycle regulation (Reyes et al., 1998).

B) SWI/SNF dependent nucleosome remodeling mechanism

The SWI/SNF complex binds to the nucleosome at two separate locations: an internal location ~2 turns from the nucleosomal dyad (the center of the nucleosome around which there is an overall pseudo two-fold symmetry), and an external site that involves a DNA linker that is near the nucleosome-entry site (Figure 14). The SWI/SNF remodeling complexes break down the link between histones and DNA by using the energy of ATP hydrolysis to slide the DNA around the nucleosome. By this the histone-DNA contacts broken thereby creating a transient DNA loop that propagates around the nucleosome and resolves when it, thereby increasing the accessibility of DNA-binding factors to their targets. (Tang et al., 2010).

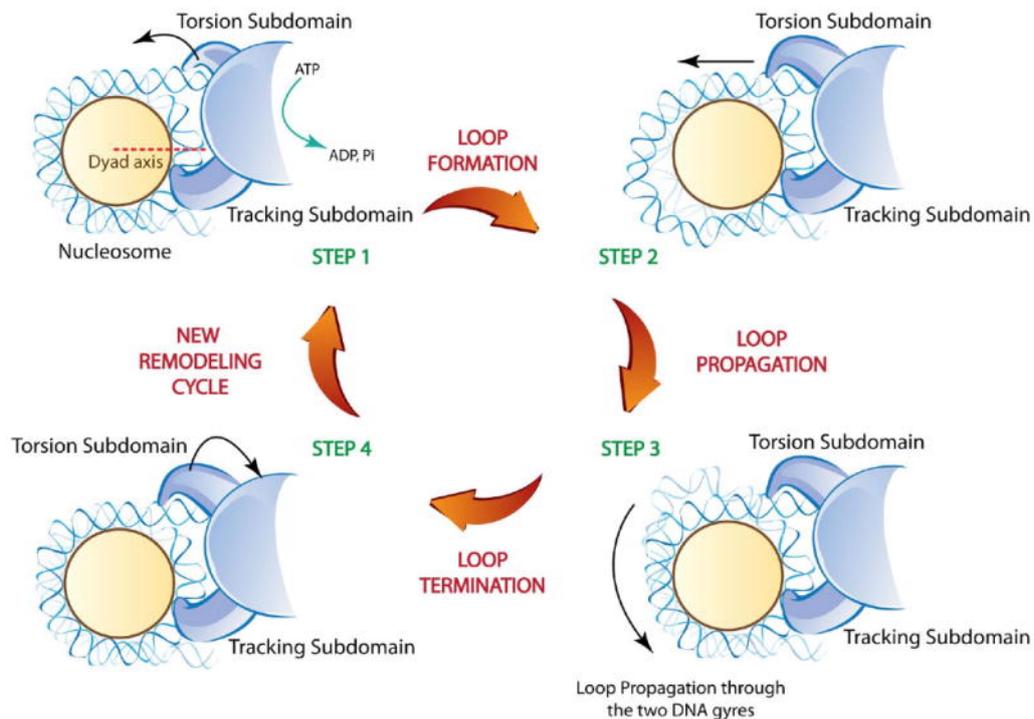


Figure 14: Schematic representation of the SWI/SNF dependent nucleosome remodeling process: Four steps are proposed. First, the remodeler binds the nucleosome core with its ATPase domain engaging the DNA ~2 turns from the dyad (step 1). Upon ATP-dependent hydrolysis, the torsion sub-domain generates a DNA loop that translocates through the tracking subdomain and the dyad (Step 2-3). The loop resolves when it reaches the exit site on the other side of the nucleosome (Step 4). The combination of these steps results in nucleosome repositioning. The complex is then ready for a new remodeling cycle (Step1) (Tang et al., 2010).

c) Biological role of SWI/SNF complex (BAF) in mammal's neurons

SWI/SNF complex has been reported to play essential epigenetic roles in many biological processes. Since the consequences of some of the mutations of the SWI/SNF (*snf2/snf3/snf5*) encoding genes are suppressed by deletions of genes encoding for histone 2A and 2B, suggesting that SWI/SNF complexes were possibly involved in histone binding and chromatin organization (Hirschhorn et al., 1992). Also, it has been shown that SWI/SNF is implicated in regulating critical cellular processes by interacting with a wide variety of proteins such as differentiation and cell cycle arrest (Klochender-Yeivin et al., 2002) (Figure 15).

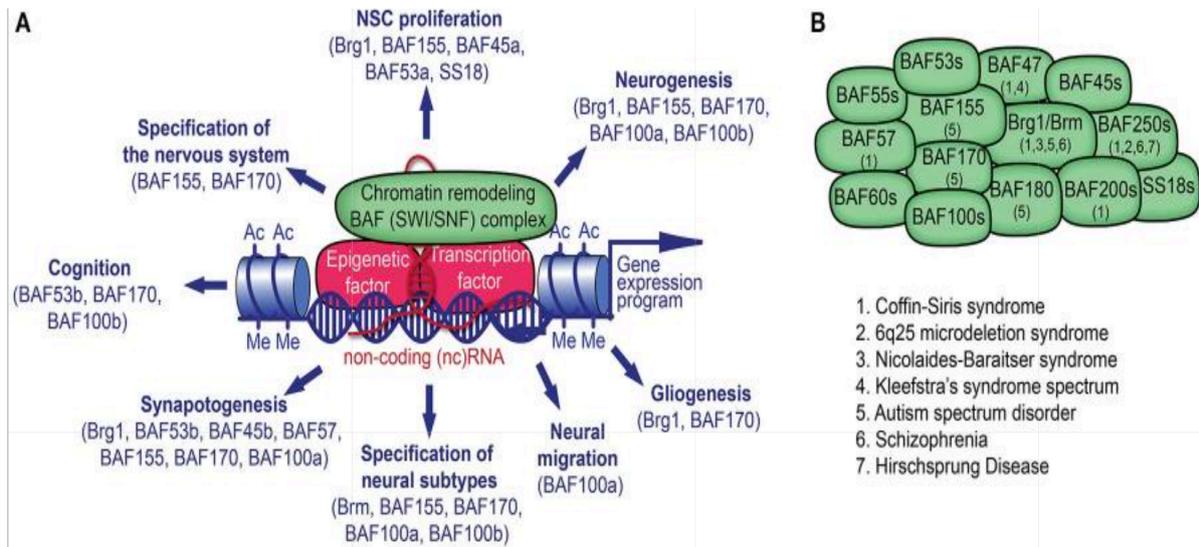


Figure 15: Chromatin remodeling BAF (mSWI/SNF) complex in neural development and disorders (Sokpor et al., 2017).

Many studies show that specific BAF complexes can instruct specific cell fates *in vivo*. For instance, the epigenetic landscape in embryonic stem (ES) cells include specific ES cell BAF (esBAF) complex needed for maintenance of their proliferative (capacity and pluripotency). esBAF complex is composed of Brg1, BAF60a/b, BAF155, and BAF250a (Kaeser et al., 2008). After receiving the appropriate stimuli, ES cells differentiate into neural stem cells (NSCs). Accordingly, reconstitution of the esBAF produces neuronal progenitor BAF (npBAF) complex. npBAF complex play a critical role in the self-renewal of neural progenitors, and is composed of either a Brg1 or Brm core ATPase, a BAF155:BAF155 homodimer or BAF155:BAF170 heterodimer, and BAF250 paralogs (BAF250A or BAF250B) (Lessard et al., 2007). As neural development advances, also neural progenitors differentiating into neurons BAFs (nBAF) is involved in remodeling the post-mitotic neuronal chromatin and play an essential role in dendritic morphogenesis (Son and Crabtree, 2014). (Figure 16)

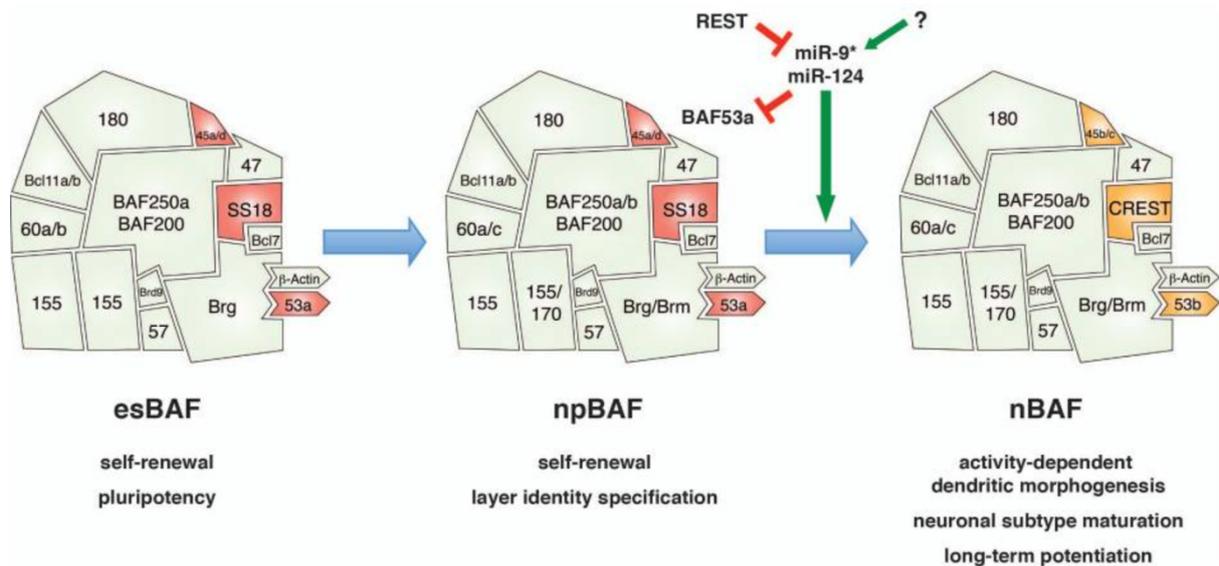


Figure 16: BAF complex assemblies in neural development. From Son and Crabtree, 2014.

SWI/SNF complex is important during neural development in mammals, including the establishment and maintenance of neural fates and functionality. In primary cell culture, lack Brg1 in neuronal stem cell (NSC) results in impaired proliferative and their self-renewal capacity. , conditional deletion of Brg1 under Nestin promoter in mouse NSCs at E10.5 led to reduced brain size, consequent to diminished proliferation and depletion of neural precursor population (Lessard et al., 2007). Also, it has been shown that double conditional knock-out of BAF155 and BAF170 subunits in mice result in an increase in the repressive epigenetic marks (H3K27me2/3), an overall reduction in active chromatin marks (H3K9Ac), and to a severely perturbed growth of cortical structures (Narayanan et al., 2015).

In primary culture, it has been shown that the mice lacking Baf53b (Baf53b^{-/-} neurons) have a severe reduction in activity-dependent dendritic outgrowth in hippocampal neurons (Wu et al., 2007). Recent works on BAF53b dominant-negative mutant (BAF53b Δ HD) and BAF53b heterozygous null (Baf53b^{+/-}) mice suggest that BAF53b is important for memory consolidation and synaptic plasticity in the hippocampus and NAc (Vogel-Ciernia et al., 2013; White et al., 2016). Specific knockdown of BAF53b in lateral amygdala impairs long-term memory formation of auditory fear conditioning with normal basal synaptic transmission and spine structures (Yoo et al., 2017).

D) Brg1 and Brm; central catalytic ATPase subunits

The Brg1 molecule is over 1600 residues long and contains several conserved motifs and domains defined through sequence homology (Figure 17).

The HSA domain that lies between the QLQ and ATPase. Adjacent to the HSA domain, the BRK domain is unique to multicellular organisms. BRK domains are also found in a subset of the chromodomain helicase remodeling proteins from the Snf2 family and often in pairs. The ATPase homology that follows them occupies approximately one third of the Brg1 sequence and contains two separate Rec-A like domains (DEXHc and HelicC) that are found paired in all Snf2 family members joined by linkers of varying length. Following the ATPase region, a small AT hook motif is thought to act as a DNA binding motif. Such motifs are found in many DNA binding and chromatin interacting proteins and often act in concert with additional DNA interacting domains. Finally, the BROMO domain of Brg1 can interact with acetylated histones, though its affinity is weak when compared to BROMO domains from other proteins suggesting the Brg1 BROMO domain interacts with histones in the context of additional interactions (King et al., 2012).

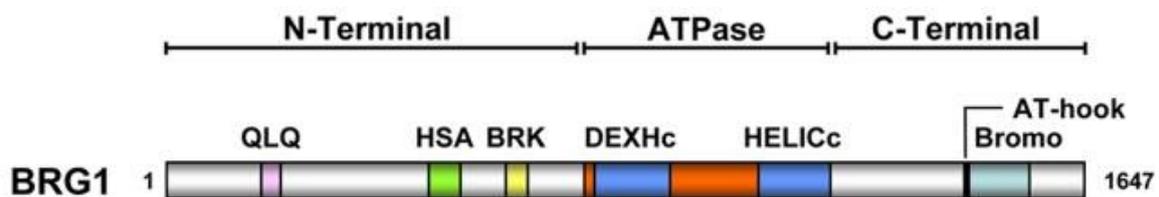


Figure 17: Domain architecture of Brg1. QLQ domain conserved motif is postulated to be mediating in protein interactions. HSA domain predicted to bind with DNA and is often found associated with helicase. BRK domain's function is unknown. DEXHc domain associated with DEXH box helicase, involved in DNA and RNA unwinding. HELICc domain found in a wide variety of helicase and helicase related proteins, sharing the ability to unwrap the DNA using the energy of ATP hydrolysis. AT-Hook is a small DNA-binding motif cooperative with their DNA binding activities and facilitating changes in the structure of the DNA. BROMO domain can interact with acetylated histone. (modified from (Trotter and Archer, 2008).

1) Brg1 and Brm complexes and interacting proteins

Mammalian SWI/SNF complexes contains either BRM or BRG1 which are usually associated with approximately 10-12 of BRG1 associated factors (BAF) subunits or other proteins involved in regulation of gene expression (Wang et al., 1996). Furtherly, mammalian SWI/SNF complexes can be subdivided into the BAF and PBAF complexes which can be distinguished by presence of specific subunits, BAF250a/b or BAF180, found in BAF or PBAF, respectively (Lemon et al., 2001; Nie et al., 2000; Yan et al., 2005)(Figure 18).

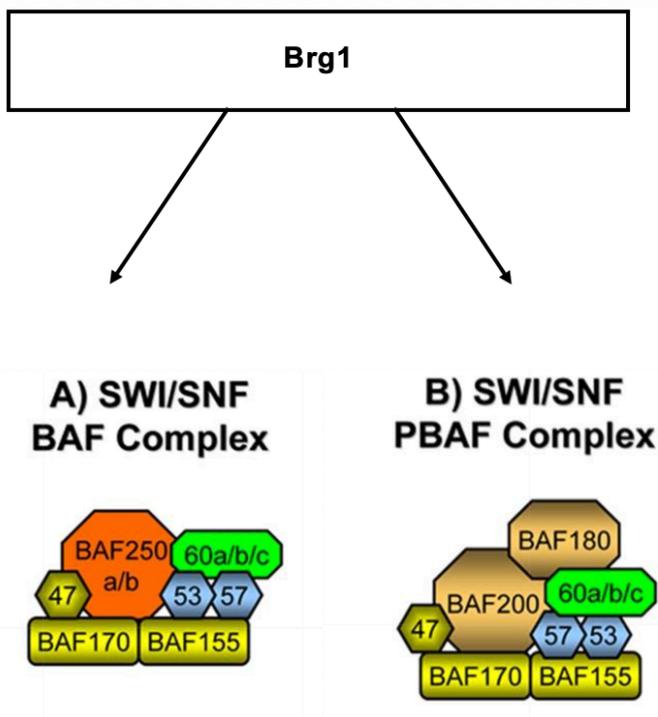


Figure 18: The chromatin remodeling complexes containing BRG1. BRG1 is the catalytic central ATPase of SWI / SNF remodeling complexes BAF and PBAF. The BAF complex is characterized by the presence of BAF250, whereas the PBAF complex contains BAF180 and BAF200.

The role of the BAF proteins within the remodeling complex may be to target or stabilize nucleosomes in a particular conformation that facilitates Brg1 remodeling activity. It has been shown *in vitro* that BRG1 alone is able to induce chromatin remodeling, and by addition of core SWI/SNF complex subunits, BAF170, BAF155 and BAF47 allows for optimal chromatin remodeling by increasing BRG1 remodeling activities (Phelan et al., 1999).

2) Role of Brg1 and Brm in transcription control of nuclear receptors

Brg1 and Brm can be targeted to many different DNA-binding motifs within gene-specific promoters and enhancers. It has been demonstrated that nuclear receptors-mediated activation of a chromatin template requires SWI/SNF remodeling activity

through the recruitment of its ATPases (Brm or Brg1), which are highly homologous and mutually exclusive subunits (Fryer and Archer, 1998; Trotter and Archer, 2007). The Brm SWI/SNF ATPase is an important coregulator of GR-mediated repression. In contrast, the Brg1 ATPase is thought to only play a minor role in the repression of GR target genes. Brm knockdown decreases GR occupancy at GR-activated Brm-dependent genes and has disparate effects on GR occupancy at GR-repressed Brm-dependent genes (Engel and Yamamoto, 2011; John et al., 2008). (GR), progesterone receptor (PR), estrogen receptor (ER), and androgen receptor (AR) are all type 1 nuclear receptors known to associate with the Brg1 complex and to require its chromatin remodeling activity for proper function (Fryer and Archer, 1998; Ichinose et al., 1997; Li et al., 2006).

a) Transcriptional activation

Steroid hormones act through a group of high affinity receptors that regulate transcription by binding to hormone response elements (HREs) located within the promoters of target genes, which themselves are organized with nuclear proteins to form chromatin. The steroid inducible mouse mammary tumor virus (MMTV) promoter is a model system classically used to identify the mechanism of action of steroid hormones. The MMTV promoter is assembled into a phased array of 6 nucleosomes that are specifically positioned in rodent cells. Induction of transcription by GCs is accompanied by the appearance of a hypersensitive region in the proximal promoter which allow the GR to interact with chromatin remodeler complex such as Brg1 to modify the chromatin conformation and recruit other transcription factors including nuclear factor 1 (NF1) and the octamer transcription factor (OTF) in hormone dependent manner (Archer et al., 1995; Richard-Foy and Hager, 1987).

The SW-13 cell line which is derived from a small-cell carcinoma of the adrenal cortex, was used to study GR-mediated chromatin remodeling of the MMTV promoter by Brg1. These cells lack a functional SWI/SNF remodeling complex due to the absence of Brg1 and hBrm proteins while they express the BAF proteins required for complex formation. In the absence of Brg1, the GR is unable to activate transcription from the MMTV promoter despite the presence of other ATPases such as ISWI. It has also been proven that the Brg1 complex is required for GR-mediated chromatin remodeling and the

transcriptional activation of a stably maintained MMTV promoter (Trotter and Archer, 2004).

Other techniques such as ultrafast UV-laser crosslinking have provided insight on the progression and the possible mechanism of GR-mediated chromatin remodeling by SWI/SNF at the MMTV promoter. Using this assay, the highly dynamic interactions between GR and MMTV are dependent on GR-directed hSWI/SNF remodeling of the template and require the presence of ATP. (Nagaich et al., 2004).

The observation that in T47D/A1-2 breast cancer cells expressing both GR and PR, GR activation leads to transcriptional upregulation from both stable MMTV promoter and transiently transfected MMTV chloroamphenicol acetyl-transferase reporter while PR activation leads only to increased transcription from transiently transfected templates reveals a major difference between GR and PR activity (Archer et al., 1994; Lee and Archer, 1994). The PR antagonist ORG31710, like progestin, triggers PR binding to HREs as well as Brg1. This sequesters the Brg1 complex and strongly diminishes the GR-Brg1 interaction which is leading to inhibit GR-mediated transcriptional activation (Fryer and Archer, 1998).

b) Connecting BAFs bridge GR and BRG1

With the MMTV promoter used as a model, it has been reported that GR-mediated transactivation of MMTV is a bimodal, two-step process. First, the interaction of GR with the Brg1 complex is required to allow chromatin remodeling, which is an obligatory step prior to activation of MMTV transcription from a chromatin template. But the interaction between GR and BRG1 is not direct and relies on connecting BAF proteins to bridge Brg1 and the hormone receptor. BAF proteins that participate in receptor bridging interactions include BAF250, BAF60a, BAF57, and BAF53a (Hsiao et al., 2003; Nie et al., 2000; Trotter and Archer, 2008). BAF250 is able to bind GR through its C-terminal domain (Nie et al., 2000; Trotter and Archer, 2008). This binding event is evident from both *in vivo* immunoprecipitation and *in vitro* pull-down assays between GR and the BAF250 C-terminus (Nie et al., 2000). GR-induced transcriptional activation is diminished in cells expressing the BAF250 C-terminal truncation mutant. A specific role for BAF250a during GR signaling is consistent with observed increases in GR-responsive transcription with BAF250a overexpression (Inoue et al., 2002).

While, the BAF60a GR-binding region has been localized to its N-terminus (residues 4–128 of 515), which also binds the PR, ER (Belandia et al., 2002). The N-terminus of BAF60a is not only responsible for Brg1 and GR interaction, but also allow it to bridge Brg1 and other core BAF subunits like BAF170 and BAF155 (Hsiao et al., 2003).

c) Transcriptional repression

The role of Brg1 has been widely associated with transcriptional activation; however, studies indicate the remodeling protein can play critical roles in gene silencing through interactions with a variety of transcriptional corepressors (Gaston and Jayaraman, 2003; Underhill et al., 2000).

Complexes purified from mammalian extracts demonstrate Brg1 associates with the mSin3A/HDAC complex that is implicated in transcriptional repression of a variety of genes (Sif et al., 2001). The Brg1 complex associates with the *c-fos* gene promoter via its interaction with specific protein 1(Sp1). In unstimulated neurons, Brg1 recruits Rb, which in turn recruits HDAC1 and mSin3A to the promoter to suppress *c-fos* gene expression (Qiu and Ghosh, 2008).

Negative feedback regulation of the HPA axis by the GR in the pro-opiomelanocortin gene is exerted by trans-repression between GR and the orphan nuclear receptors related to NGFI-B. Requirement for the SWI/SNF chromatin remodeling protein BRG1 and its ATPase activity in this mechanism is critical for formation of stable complexes between GR and NGFI-B, and between GR and Histone deacetylase 2 (HDAC2). Promoter recruitment of both GR and HDAC2 are GC dependent and are associated with decreased acetylated histone H4 at the promoter and throughout the gene. It also shown that 50% of pituitary adenomas from Cushing disease patients or dogs have misexpression of either Brg1 or HDAC2 in tumor tissue but not in adjacent normal pituitary tissue (Bilodeau et al., 2006).

BRG1 has been shown to interact with retinoblastoma tumor suppressor to form a repressor complex which inhibits cell cycle proteins such as cyclin A, D1 and E (Giacinti and Giordano, 2006).

V. Implication of Chromatin or nuclear protein complex in neurodevelopmental disorders and psychiatric diseases

A) Implication of SWI/SNF complex in neurodevelopmental disorders and psychiatric diseases

The ATP-dependent BRG1/BRM associated factor (BAF) chromatin remodeling complexes are crucial in regulating gene expression by controlling chromatin dynamics. Proper functioning of the BAF complexes plays critical roles in neural development, including the establishment and maintenance of neural fates and functionality. Indeed, recent human exome sequencing and genome-wide association studies have revealed that mutations in BAF complex subunits are linked to neurodevelopmental disorders such as Coffin-Siris syndrome, Nicolaides-Baraitser syndrome, Kleefstra's syndrome spectrum, Hirschsprung's disease, autism spectrum disorder, and schizophrenia.

Coffin-siris syndrome: is a rare congenital abnormality with clinical characteristics such as intellectual disability, progressive coarsening of the face, hypertrichosis, frequent infections, feeding difficulties, and hypoplasia of the 5th distal phalanges and fingernails (Santen et al., 2012). Recently, comprehensive human exome sequencing and genome-wide association studies have showed that about 110 of 188 cases of Coffin-Siris syndrome reported carry a mutation in genes encoding BAF complex subunits. These genes include BAF250A, BAF250B, Brm, Brg1, BAF47, BAF5, and BAF200 (Bramswig et al., 2015; Santen et al., 2012; Tsurusaki et al., 2012).

Nicolaides-baraitser syndrome: is characterized by intellectual disability with impaired speech, sparse scalp hair, prominence of the interphalangeal joints and distal phalanges due to decreased subcutaneous fat, characteristic coarse facial features, microcephaly, and seizures. Exome sequencing has been shown that Nicolaides-baraitser syndrome is associated with Brm gene (also called SMARCA2) missense mutation (Van Houdt et al., 2012).

6q25 microdeletion syndrome: is an uncommon congenital and genetic disease characterized by intellectual disability with developmental delay, microcephaly, hearing impairment, distinct dysmorphic features, visual impairment, and corpus callosum

agenesis. Mutations of BAF250b gene were reported to be linked with this syndrome (Ronzoni et al., 2016).

Kleefstra's syndrome spectrum: phenotypic features for this syndrome spectrum include intellectual disability, childhood hypotonia, behavioral anomalies, synophrys, and midface hypoplasia. Mutations in BAF47, and euchromatin histone methyl transferase 1 (EHMT1) genes could lead to KSS (Kleefstra et al., 2012).

Autism spectrum disorder: Autism is a neurodevelopmental disorder characterized by deficits in social skills, speech problems, and restricted and repetitive behaviors. It is well established that a combination of genetic and environmental factors cause autism. Recent exome sequencing analyses of autism patients have revealed mutations in genes coding for BAF complex components such as BAF155, BAF170, BAF180, BAF250b, and BAF100a subunits (Basak et al., 2015; Neale et al., 2012).

Schizophrenia: is a chronic, severe, and disabling brain disorder that affects ~0.3–0.7% of the world's population. It is characterized by delusion, thought disorder, auditory hallucination, reduced emotional expression and social engagement, disorganized speech, and lacking motivation. The causes of schizophrenia include genetic and/or environmental factors (Zai et al., 2017). In addition to genetic mutations, epigenetic alteration has also been shown to be involved in schizophrenia (Tsankova et al., 2007). Single nucleotide polymorphism within Brm gene associated with schizophrenia in a screening study of Japanese population (Koga et al., 2009).

B) Implication of other nuclear proteins in neurodevelopmental disorders and psychiatric diseases

The Fragile X Syndrome: is a genetic disease inherited through the X chromosome, which was described for the first time in 1943 by Martin and Bell. It is actually considered the most common inherited cause of intellectual disability and the second most prevalent cause after Down syndrome. Affected men have a classic phenotype characterized by long face, large and protruding ears and macro-orchidism. The Fragile X Syndrome is caused by an abnormal expansion in the number of the trinucleotide CGG repeats located in the 5' UTR in the fragile X mental retardation 1 gene (FMR1) at Xq27.3. It is a dynamic mutation with expansion of the CGG repeat in each generation moving from the premutation range of 55 to 200 repeats and

expanding to a full mutation when pass on by a women to her children (Saldarriaga et al., 2014).

Rubinstein-Taybi syndrome: is an extremely rare disease and was first described in 1963. RSTS is primarily characterized by poor postnatal height-weight growth, intellectual disability, microcephaly, dysmorphic facial features, broad thumbs, and big first toes. Approximately half of patients mutations in the gene encoding cyclic-AMP-regulated enhancer binding protein (CBP) in 16p13.3 (Milani et al., 2015).

Rett syndrome: is a severe neurodevelopmental disorder that contributes significantly to severe intellectual disability in females worldwide. It is caused by mutations in MECP2 in the majority of cases, but a proportion of atypical cases may result from mutations in CDKL5. Mutations in MECP2 appear to give a growth disadvantage to both neuronal and lymphoblast cells, often resulting in skewing of X inactivation that may contribute to the large degree of phenotypic variation. MeCP2 was originally thought to be a global transcriptional repressor, but recent evidence suggests that it may have a role in regulating neuronal activity dependent expression of specific genes such as Hairy2a in *Xenopus* and Bdnf in mouse and rat (Weaving et al., 2005).

Other mutations in genes encoding nuclear proteins causing different neurodevelopmental diseases are listed in table 2.

Disease	Chromatin defect	Clinical features
Rubinstein–Taybi syndrome ^{62,70,109}	Heterozygous mutations in <i>CBP</i>	Autosomal dominant inheritance; mental retardation, abnormal facial features, blunted growth
Fragile X syndrome ^{62,105}	Hypermethylation of DNA at the <i>FMR1</i> and <i>FMR2</i> promoters, caused by trinucleotide repeat expansion	X-linked inheritance; most common inherited form of mental retardation, signs of autistic behaviour, macrocephaly, long and narrow face with large ears, macroorchidism, hypotonia
Coffin–Lowry syndrome ^{70,106,107}	Mutation in <i>RSK2</i> , which can interact with CREB and CBP and can phosphorylate H3 <i>in vitro</i> ¹⁰⁶	X-linked inheritance; psychomotor retardation, craniofacial and skeletal abnormalities
Rett syndrome ^{61,70–72}	Mutations in <i>MeCP2</i>	X-linked, predominantly affecting girls; pervasive developmental disorder associated with arrested brain development, cognitive decline and autistic-like behaviour
Alpha-thalassemia/mental retardation syndrome, X-linked (<i>ATR-X</i>) ⁷⁰	Mutations in the <i>ATRX</i> gene encoding XH2 — a member of SWI/SNF family of proteins; defective chromatin remodelling thought to downregulate the α -globin locus	X-linked inheritance; mental retardation, haemolytic anaemia, splenomegaly, facial, skeletal and genital anomalies
Immunodeficiency–centromeric instability–facial anomalies syndrome (ICF) ⁷⁰	Mutations in <i>Dnmt3B</i> ; hypomethylation at centromeric regions of chromosomes 1, 9 and 16	Autosomal recessive; mild mental retardation, marked immunodeficiency, facial anomalies
Myotonic dystrophy ¹⁰⁷	Abnormal CTG repeat expansion at the 3' UTR of the <i>DMPK</i> gene favours chromatin condensation, affecting the expression of many neighbouring genes	Autosomal dominant; mild mental retardation, myotonia, abnormal cardiac conduction, insulin-dependent diabetes, testicular atrophy, premature balding
Prader–Willi syndrome ¹⁰⁸	Rare forms caused by abnormal imprinting (DNA methylation) of paternal chromosomal region 15q11–13	Mild mental retardation, endocrine abnormalities
Angelman syndrome ¹⁰⁸	Rare forms caused by abnormal imprinting (DNA methylation) of maternal chromosomal region 15q11–13	Cortical atrophy, cerebellar dysmyelination, cognitive abnormalities

CBP, CREB binding protein; CREB, cyclic AMP-response element binding protein; DMPK, DM1 protein kinase; *Dnmt3B*, DNA methyltransferase 3B; *FMR1*, fragile X mental retardation protein 1; *MeCP2*, methyl-CpG-binding protein 2; *RSK2*, ribosomal S6 kinase 2; SWI/SNF, mating switching and sucrose non-fermenting complex; UTR, untranslated region; XH2, X-linked helicase 2.

Table 2: Mutation within genes encoding nuclear proteins in neurodevelopmental disorders and psychiatric diseases

Chapter 3: Reward System, addiction, and depression

Midbrain dopaminergic neurons in the substantia nigra pars compacta and the VTA regulate extrapyramidal movement, motivation, reward associations, and habit learning. Dysfunctions in dopaminergic circuitry have been implicated in several neuropsychiatric disorders, including addiction, depression and schizophrenia, whereas selective degeneration of dopamine neurons in substantia nigra pars compacta is a key neuropathological feature in Parkinson disease. In the following sections, I will focus on the so-called brain reward system which includes the VTA dopamine neurons projecting to distinct brain regions including the NAc core and shell, the medial part of the striatum and the prefrontal cortex (PFC). I will then describe the cellular and molecular changes occurring within these brain circuits participating to the responses to stress exposure in a model of depression (the repeated social defeat stress) or to cocaine.

I. The reward system

The reward system was first evoked in 1954 by Olds and Milner. They noted that in rats, electrical stimulation of certain areas of the brain had a positive reinforcing effect (Olds and Milner, 1954). The reward system includes the mesolimbic and mesocortical pathway (Phillips AG et al, 2008). The mesolimbic dopaminergic system comprises the VTA dopamine neurons and their projection areas that include the NAc, the olfactory tubercle, the amygdala and the hippocampus. The mesocortical dopaminergic system includes the VTA dopamine neurons projecting to prelimbic, cingulate and perirhinal cortices (Figure19). Because of the overlap between these two systems they are often collectively referred to as the mesocorticolimbic system (Wise, 2004, 2005).

The mesocorticolimbic pathway and a specific set of the pathway's output neurons (i.e., D1-D2 type medium spiny neurons (MSNs) within the NAc play a central role in the neurobiology of depression and addiction (Koob and Simon, 2009; Nestler et al., 2002). Hence, we will focus on the structural and functional changes within these circuits in the context of preclinical models of depression and addiction (Van den Heuvel and Pasterkamp, 2008).

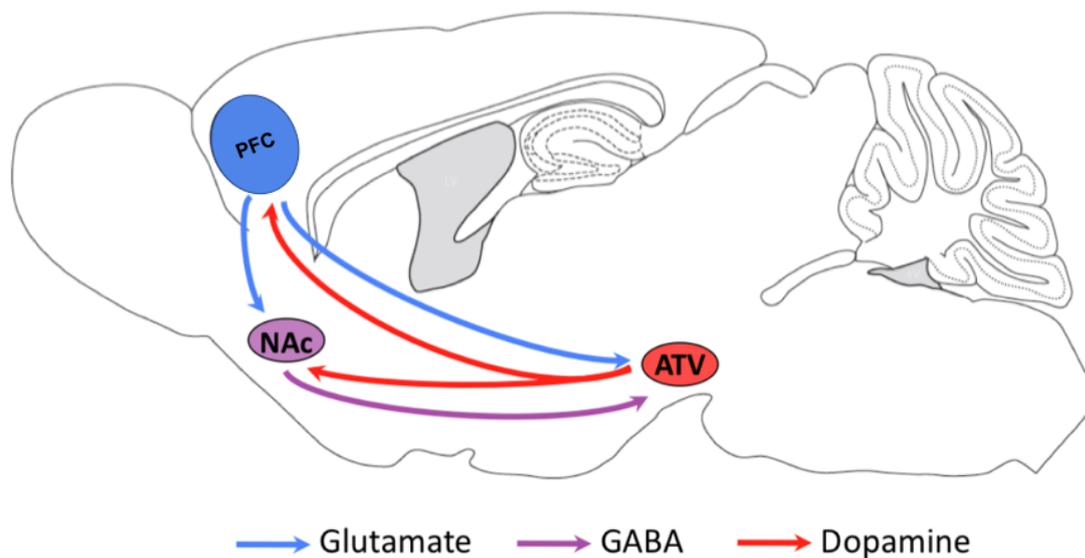


Figure 19: Location of the main connections of the mesocorticolimbic system in mice. The dopamine system is characterized by interconnected neural networks comprising various brain regions. The VTA, the NAc and the prefrontal cortex (PFC) are at the base of this system.

A) The Brain structures of the reward system

1) Nucleus Accumbens

The striatum is involved in the attribution of a motivational value of stimuli and in the motor response of the organism. This region is considered to be key for the integration of sensorial information and in the coordination of behavioral responses to the environmental stimuli. The striatum can be divided according to a ventro-dorsal gradient with the ventral part called NAc being involved in rewarding and emotional processes (Cardinal et al., 2002). The main neuronal type of the NAc are GABAergic MSNs, which in rodents constitute 90–95% of all striatal neurons. In addition to MSNs, the NAc contains three groups of interneurons: the cholinergic interneurons, the GABAergic interneurons and the somatostatin/nitric oxide–expressing interneurons. Each group constitutes 1–3% of all striatal neurons (Nicola et al., 2000) (figure 20). The NAc can be divided in two sub-regions, the core and the shell, differing in their morphological and neurochemical properties as well as their connections and functions (Heimer et al., 1991).

The NAc core

The NAc core receives dopaminergic afferences mainly from the lateral part of the VTA. It receives glutamatergic inputs from the PFC (mainly the prelimbic, cingulate and agranular insular cortices) and the central thalamic nuclei as well as GABA inputs from lateral-ventral pallidum (Berendse and Groenewegen, 1990; Berendse et al., 1992a; Groenewegen et al., 1993) . In terms of output, the core sends GABA projections to the lateral VTA but also to the dorso-lateral part of the ventral pallidum and to the substantia nigra pars reticulata (Berendse et al., 1992a; Heimer et al., 1991). From these sets of projections, the NAc core can be functionally associated with the dorsal striatum and is rather involved in the motor phase of behaviors.

The NAc shell

The NAc shell can be subdivided into two parts, the medial and the lateral. The lateral shell can be associated with the NAc core based on its sets of inputs and outputs (Ikemoto, 2007; Voorn et al., 2004). The medial part of the NAc shell receives dopamine inputs from the medial part of the VTA and glutamatergic inputs from the infralimbic and medial orbitofrontal cortices, the basolateral amygdala, the subiculum and the paraventricular thalamic nucleus (Berendse and Groenewegen, 1990; Berendse et al., 1992a; Groenewegen et al., 1987; Krettek and Price, 1978; Moga et al., 1995; Russchen and Price, 1984). GABA innervation of the medial shell also arises from the medial part of the ventral pallidum (Groenewegen et al., 1993). The MSNs from the shell projects to the postero-medial and lateral parts of the VTA where they provide a feedback onto dopaminergic neurons as well as to the medial part of the ventral pallidum (Heimer et al., 1991; Zahm and Heimer, 1990). Finally the NAc shell also sends projections to the lateral hypothalamus and the periaqueductal gray matter which are involved in emotional and rewarding processing of new stimuli (Berendse et al., 1992b; Heimer et al., 1991).

On the contrary to the NAc core, the medial part of the shell is closely associated with the limbic and hypothalamic structures attributing the emotional value of the stimuli and allowing the central nervous system to be awake. It is also involved in the motivational effects of drugs as well as in the formation of associations between

reinforcing stimuli and the environment (Bassareo et al., 2003; Ito et al., 2004; Sellings and Clarke, 2003).

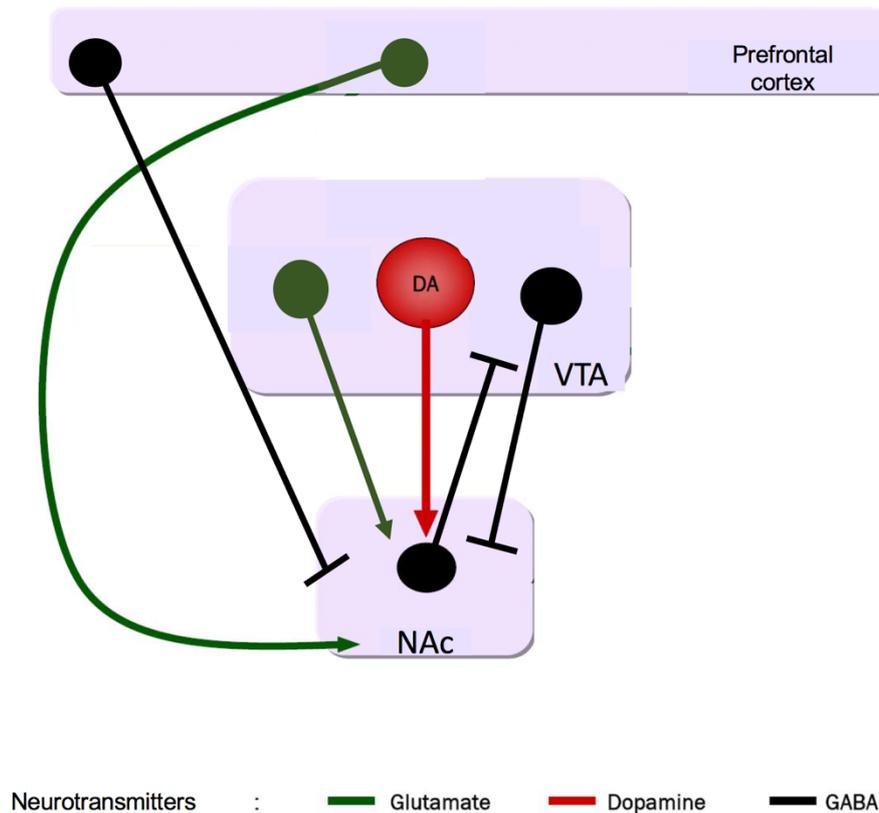


Figure 20: Main nucleus accumbens mesocorticolimbic connections in the reward system. NAc sends GABAergic projections to VTA, and receives glutamatergic, dopaminergic and GABAergic innervations from the VTA and glutamatergic and GABAergic innervations from the PFC (NAc: Nucleus Accumbens, VTA: Ventral Tegmental Area).

2) Prefrontal cortex

The PFC is a cortical area located in the anterior frontal lobe, including several subdivisions such as the prelimbic, the infralimbic, the cingulate, the orbitofrontal and the agranular insular cortices. Each of these areas is interconnected with different sets of subcortical structures and are therefore involved in distinct functions. The PFC is composed 10-15% of GABA interneurons and 80-85% of pyramidal projecting neurons that are glutamatergic (Barbas, 2015). It is reciprocally interconnected with the basolateral amygdala, the mediodorsal thalamus and with the VTA. In addition, it receives inputs from the hippocampus and sends projections to the NAc and the dorsal

striatum (Cardinal et al., 2002; Schoenbaum et al., 2006). From its sets of connections, the PFC is considered as a key structure for cognitive control (e.g. planning, attention, problem-solving, error-monitoring, decision-making, social cognition, and working memory) (Bechara, 2004; Cardinal et al., 2002; Schoenbaum et al., 2006)(Figure 21).

Research implicates the ventromedial and dorsolateral PFC as key neural substrates underlying depression. Functional imaging studies associate depression with opposite patterns of activity in these areas: hypoactivity in dorsolateral but hyperactivity in ventromedial PFC. Similarly, lesion studies in humans demonstrate opposite effects of damage to these areas on depression with dorsolateral PFC lesions exacerbating depressive symptoms whereas ventromedial PFC lesions diminish depressive symptoms (Koenigs and Grafman, 2009). Finally, imaging studies in addictive behaviors have identified a key involvement of the PFC both through its regulation of limbic reward regions and its involvement in higher-order executive function (for example, self-control, salience attribution and awareness) (George and Koob, 2010; Goldstein and Volkow, 2011).

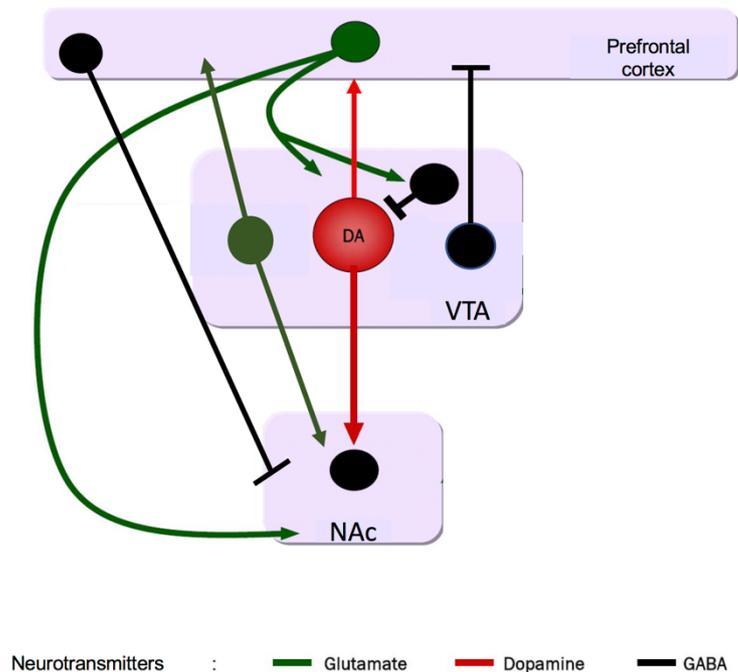


Figure 21: Main connections of the prefrontal cortex in the mesocorticolimbic system. PFC sends glutamatergic projections to the NAc and VTA, receives dopaminergic afferents from VTA.

3) The amygdala

The amygdala comprises several interacting sub-regions. The central (CeA) and basolateral amygdala (BLA) are involved in emotional processes and are particularly sensitive to stress-exposure. The Lateral portion of the amygdala is the main input area to the amygdaloid complex. It receives sensory inputs from via the cortex or the thalamus. The CeA is the output region and innervate the brainstem, the hypothalamus, the VTA and the SNc. It plays therefore an important role in the expression of innate and acquired emotional responses. The BLA projects to the PFC and the NAc playing a key role in the reward system. Of note these two nuclei receive inputs from the PFC and the VTA (Cardinal et al., 2002; LeDoux, 2007; See et al., 2003).

The BLA plays a key role in fear conditioning and it is assumed that this structure receives sensory information to which it would give an emotional value that would be then transmitted to the CeA. The CeA would then allow the freezing reaction by acting on the hypothalamic and brainstem nuclei (LeDoux, 2000). The amygdala is not only involved in fear responses but may also be involved in “positive” stimuli. For example, in humans, it has been shown to be involved in the development of emotional reaction to conditioned stimuli such as money (Bechara et al., 1999). It is noteworthy that in rodents, an reinforcement of the BLA-NAc and CeA-VTA pathways are observed during an approach behavior to stimuli associated with drugs (Ciano and Everitt, 2004; Everitt et al., 1999). Finally the amygdala is also critical for self-administration relapse after re-exposure to drugs-associated stimuli (See et al., 2003).

4) The VTA Dopamine neurons

Dopaminergic neurons are distributed in the central nervous system in nine major nuclei distributed in the midbrain (A8, A9 or substantia nigra, A10 or VTA, thalamus (A11), hypothalamus (A12 to A15) and olfactory bulbs(A16) (Björklund and Dunnett, 2007). These dopaminergic nuclei ensure diverse function including perception, the regulation of some pituitary hormones, thermoregulation, memory, motor function and motivation. The midbrain nuclei ensure these last three functions (Berridge, 2007; Cenci, 2007; Schultz, 2007). Here we will mainly focus on the dopaminergic neurons

from the VTA which have been extensively studied in the fields of depression and drug addiction.

B) Behavioral functions of VTA dopamine neurons

The VTA is a heterogeneous midbrain structure that plays a major role in regulating different adaptive brain functions related to reward and motivation processing. It is predominantly composed of dopaminergic neurons (55%–65%), while the rest are mainly GABAergic 30%, and a small portion of glutamatergic cells (5%) (Hnasko et al., 2012; Steffensen et al., 1998).

Dopamine neurons in the VTA can exhibit three different states. The hyperpolarized inactive state is due to the high activity of GABAergic neurons projections from ventral pallidum to the VTA that inhibit the release of dopamine. The release of that inhibitory control of the ventral pallidum by the activation of the GABA inputs from the NAc to this structure leads to a tonic activity state during which the dopamine neurons show a slow and regular firing rate. Finally, the phasic activity state is crucially dependent on glutamatergic afferents input to the VTA and is characterized by bursts of action potentials (Grace et al., 2007). Dopamine neurons with a high firing frequency selectively projects to the PFC, the NAc core and the medial shell as well as to the BLA. In contrast, slow-firing dopamine midbrain neurons only project to the lateral shell of the NAc and the dorsolateral striatum (Lammel et al., 2008).

Dopamine neurons in the VTA have long been linked with the processing of reward. Most dopamine neurons show short phasic activation after primary various appetitive stimuli or rewards and this activation varies according to the value and delay of the reward (Roesch et al., 2007). It is noteworthy also that some dopamine neurons (about 14%) show a phasic activation when primary aversive stimuli are presented. Interestingly, after repeated pairings of visual and auditory cues followed by reward, dopamine neurons change the time of their phasic activation from just after the time of reward delivery to the time of cue onset. But if the reward is not delivered at the appropriate time after the onset of the cue, dopamine neurons are depressed markedly below their basal firing rate exactly at the time that the reward should have been delivered (Schultz, 1998; Schultz et al., 1997). These results suggest that dopamine plays a role in reward processing and learning and code for reward prediction error. Of

note, social interaction and related behaviors also activate the dopamine system suggesting that they are not different from other types of rewards such as food (Gunaydin et al., 2016).

1) Dopamine receptors

Dopamine interacts with membrane receptors belonging to the family of seven transmembrane domain G-protein coupled receptors. Their activation leads to the formation of second messengers, and the activation or repression of specific signaling pathways. Five different subtypes of dopamine receptors have been cloned from different species. Based on their structural and pharmacological properties, a general subdivision into two groups has been made: the D1-like receptors, which stimulate intracellular cAMP levels, comprising D1 and D5, and the D2-like receptors, which inhibit intracellular cAMP levels, comprising D2, D3, and D4 receptors (Missale et al., 1998). cAMP production activates PKA and thus the activation of the D1 and D2 receptors can modulate neuronal excitability by altering the properties of a number of different voltage-dependent ion channels (Nicola et al., 2000).

D1 receptors are expressed at a high level of density in the nigrostriatal, mesolimbic, and mesocortical areas, such as the caudate-putamen, NAc, substantia nigra, olfactory bulb, amygdala, and frontal cortex, as well as at lower levels in the hippocampus, cerebellum, thalamic areas, and hypothalamic areas. The highest levels of D2 dopamine receptors are found in the striatum, the NAc, and the olfactory tubercle. D2 receptors are also expressed at significant levels in the substantia nigra, VTA, hypothalamus, cortical areas, septum, amygdala, and hippocampus (Beaulieu and Gainetdinov, 2011). Dopamine D1 receptors are highly concentrated in dendritic spines including spine heads and the postsynaptic density of neurons where they can interact with other receptors and influence signaling mechanisms involved in the function of spines. D2 receptors are involved in both pre- and postsynaptic inhibition. These auto-receptors are located on the soma and dendrites of midbrain dopamine neurons as well as on their axon terminals in projection areas. The activation of these receptors decreases both the excitability of dopamine neurons and the release of dopamine (Ford, 2014; Tepper et al., 1997). The D1 and D2 receptors play major role in addiction and depression (McCreary and Marsden, 1993; Varela et al., 2011).

2) Expression of D1 and D2 receptors in the stratum

The principal population of neurons in both the striatum and the NAc are projection GABAergic MSNs. Broadly speaking, the population of MSNs in the striatum can be divided into two types: those expressing D1 receptors and those expressing D2 receptors. Each of the two MSNs cell types represents approximately 50% of MSNs population and both D1 and D2-expressing MSNs are 'randomly' distributed throughout the striatal volume. Furthermore, these two cell types are part of two different neural pathways.

In the dorsal striatum, the D1-expressing MSNs predominantly send inhibitory projections to the globus pallidus internal and the substantia nigra pars reticulata. This is referred to as the 'direct pathway' or 'D1 pathway'. The D2-expressing MSNs predominantly send inhibitory projections to the globus pallidus external which sends inhibitory projections to the substantia nigra pars reticulata and the sub-thalamic nucleus. The subthalamic nucleus sends excitatory projections back to all structures in the basal ganglia, including the globus pallidus interna/substantia nigra pars reticulata. This pathway is referred to as the 'indirect pathway' or 'D2 pathway' (Nicola et al., 2000; Keeler et al., 2014; Soares-Cunha et al., 2016). Of note, these pathways are not entirely segregated. First, about 20 to 25% of MSNs within dorsal striatum expresses both D1 and D2 (Nicola et al., 2000). Also, the direct pathway has been shown to send collaterals to the globus pallidus external (Cazorla et al. 2014). In the rat, 37% projected exclusively to the external segment of the globus pallidus ("pure" indirect pathway), whereas only 3% projected only to the entopeduncular nucleus and substantia nigra pars reticulata ("pure" direct pathway). The same pathways also exist in the NAc. The D1-expressing MSNs predominantly project to the VTA while the D2-expressing MSNs project mainly to the ventral pallidum that sends projections to the dorsal thalamus and to the VTA (Bock et al., 2013; Yawata et al., 2012). In this region, only around 10% of MSNs co-express D1 and D2. Also, recent work has shown that about 50% of neurons in the the dorsal ventral pallidum receives inputs from both D1 and D2 MSNs thus revealing a lack of complete segregation between the D1 and D2 pathways (Kupchik et al., 2015) (Figure 22).

Although they are not perfectly segregated, the direct and indirect pathways have been shown to have opposite functions and are often referred as the go and no-go pathways

respectively. Indeed, the chemo-genetic ablation of D2-expressing MSNs in the whole striatum or restricted to the dorsomedial striatum results in a hyper-locomotor phenotype (Durieux et al. 2009, Durieux 2012). Conversely, the optogenetic stimulation of this pathway induces freezing and decreases locomotor initiations (Kravitz et al. 2010; Cazorla et al. 2014). On the other side, the ablation of D1 MSNs induces a hypo-locomotion even when restricted to the dorsomedial striatum (Durieux et al. 2012) while optogenetic stimulation of this pathway reduces freezing and increases locomotor activity (Kravitz et al. 2010; Cazorla et al. 2014). Of note, calcium-imaging studies have challenged this idea of go/no-go pathway by showing that action initiation is preceded by a concurrent activation of both D1 and D2 MSNs (Cui et al. 2013). Finally, the direct and indirect pathways also have opposite effects on responses to drugs of abuse with the D1 activation enhancing behavioral responses to cocaine and D2 activation decreasing it (Ferguson et al. 2011, Kravitz et al. 2012).

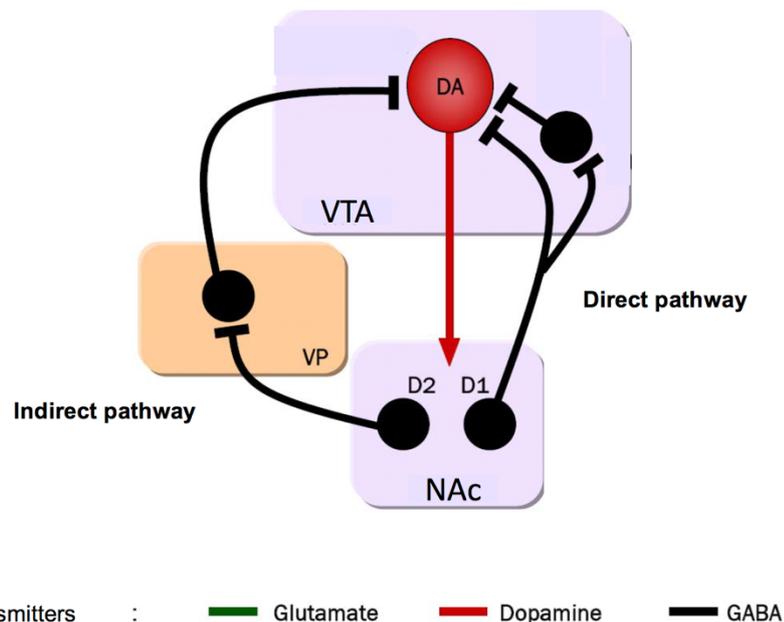


Figure 22: Direct and indirect dopamine pathway: Direct pathway project to the substantia nigra (SN), and the internal segment of the globus pallidus express high levels of D1 receptor, whereas the indirect pathway projects to the external segment of the globus pallidus express high levels of D2 receptor.

II. Depression, addiction and the meso-cortico-limbic system

It has become more and more evident that the dopamine system plays a key role in the response to stress exposure. Indeed, numerous studies have reported that dopamine release is increased in response to aversive stimuli in mice, which suggests that dopamine is likely to be involved in motivation and attention processes underlying the behavioral response to relevant stimuli, whether aversive or rewarding. Disruption in the dopamine system has been implicated in numerous psychiatric and neurological disorders, including major depressive disorders, addiction, schizophrenia and Parkinson's disease. In this part, we will focus on depression and addiction and on some pre-clinical models that I used during my thesis work. These models include the repeated social defeat for depression, and behavioral responses to cocaine including locomotor sensitization and conditioned placed preference (CPP).

A) Depression and addiction

1) Depression

Depression, known also as major depressive disorder, is a common and serious mood disorder. Those who suffer from depression experience persistent feelings of sadness and hopelessness and lose interest in activities they once enjoyed. Aside from the emotional problems caused by depression, individuals can also present physical symptoms such as chronic pain or digestive issues (Krishnan and Nestler, 2008; Palazidou, 2012; Willner et al., 2013, Figure 23).

About one in six individuals in the United States will have major depression during their lifetime. Epidemiologic studies show that roughly 40%–50% of the risk for depression is genetic (Fava and Kendler, 2000). Vulnerability to depression is not only due to genetic factors, but also due to non-genetic factors as diverse as stress and emotional trauma, viral infections (e.g., Borna virus), and even stochastic processes during brain development have been implicated in the etiology of depression (Fava and Kendler, 2000).

A) Five or more out of nine symptoms (including at least one of depressed mood and loss of interest or pleasure) in the same 2-week period. Each of these symptoms represents a change from previous functioning state.

1. Depressed mood (subjective or observed); can be irritable mood in children and adolescents.
2. Loss of interest or pleasure.
3. Change in weight or appetite.
4. Insomnia or hypersomnia.
5. Psychomotor retardation or agitation (observed).
6. Loss of energy or fatigue.
7. Worthlessness or guilt.
8. Impaired concentration or indecisiveness.
9. Thoughts of death or suicidal ideation or attempt.

B) Symptoms cause significant distress or impairment.

C) Episode not attributable to a substance or medical condition.

Note 1: Criteria A–C represent a major depressive episode (MDE).

Note 2: Clinical judgement is inevitably required to distinguish if MDE is present in addition to a normal response to a significant loss.

D) Episode not better explained by a psychotic disorder.

E) There has never been a manic or hypomanic episode.

Note 3: Exclusion E does not apply if (hypo)manic episode was substance induced or attributable to medical condition.

Figure 23: DSM-5 criteria for major depressive disorder

2) Addiction

Addiction is now generally recognized as a chronic disease of the brain that involves progressive development, relapse, and the potential for fatality if not treated. It is

difficult to provide an exact definition of addiction. The latest edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V, ASAM, (FAACT, 2012) lists eleven criteria that characterize an addict and proposes different levels of intensity of dependence according to the number of criteria presented (Figure 24).

People of any age, sex or economic status can become addicted to a drug. Certain factors can affect the likelihood and speed of developing an addiction including genetic predisposition, mental health disorder such as depression, attention-deficit/hyperactivity disorder (ADHD) or post-traumatic stress disorder. Peer pressure is also a strong factor in starting to use and misuse drugs, particularly for young people. As mentioned earlier, stress is also an important risk factor (Ducci and Goldman, 2012; Sinha, 2011). It should be noted that recently, the American Society of Addiction Medicine (ASAM) has broadened the concept of addiction and included other type of addictions such as gambling, sex, shopping, or food addiction (FAACT, 2012).

Substance use disorders span a wide variety of problems arising from substance use, and cover 11 different criteria:

1. Taking the substance in larger amounts or for longer than you're meant to.
2. Wanting to cut down or stop using the substance but not managing to.
3. Spending a lot of time getting, using, or recovering from use of the substance.
4. Cravings and urges to use the substance.
5. Not managing to do what you should at work, home, or school because of substance use.
6. Not managing to do what you should at work, home, or school because of substance use.
7. Not managing to do what you should at work, home, or school because of substance use.
8. Not managing to do what you should at work, home, or school because of substance use.
9. Not managing to do what you should at work, home, or school because of substance use.
10. Not managing to do what you should at work, home, or school because of substance use.
11. Not managing to do what you should at work, home, or school because of substance use.

Two or three symptoms indicate a mild substance use disorder; four or five symptoms indicate a moderate substance use disorder, and six or more symptoms indicate a severe substance use disorder.

Figure 24: DSM V Criteria for Substance Use Disorders

B) Animal models

1) Depression model: the repeated social defeat stress

Researchers have used various forms of chronic stress to induce behavioral adaptations relevant to depression. These include chronic unpredictable stress, early life stress such as maternal separation, restraint stress, or repeated social defeat. During my PhD, I focused on the repeated social defeat stress model that is known to induce enduring behavioral and molecular changes. In that model, an intruder mouse (the experimental mouse) is placed directly within a resident CD1 mouse home cage. After 5 min of social defeat, the intruder is maintained in the cage separated from the aggressor by a perforated divider for 24 h. For each subsequent daily defeat, the experimental mouse is exposed to a novel aggressor's home cage. This treatment leads, in two thirds of the animals (termed 'susceptible'), to the development of marked social avoidance. Consistently, social defeat produces a subgroup of animals (termed 'resilient') corresponding to approximately one third of the entire population that does not develop social avoidance. In addition to social avoidance, repeated social defeat is known to trigger an increase of despair behavior in the forced swim test and a decrease of sucrose preference. Importantly, these depressive-like behaviors have been shown to be sensitive to antidepressant drugs. Finally, repeated social defeat also durably increases anxiety levels in both susceptible and resilient mice which can be explained by increased levels of GCs as suggest the increase of adrenal gland weight in this model. (Berton et al., 2006; Golden et al., 2011).

2) Behaviors related to addiction

Several behaviors can be assessed in order to test the sensitivity of mice to drugs of abuse. Of note, none of these behaviors can be considered as a model of addiction per-se but they can give important insights on some elements of the disease.

Locomotor Sensitization

The word sensitization refers to a progressive and persistent increase in a drug effect produced by repeated drug administration. In terms of drugs of abuse, cocaine, amphetamines, and morphine, all cause a progressive increase in the locomotor

activity in animal models (Paulson and Robinson, 1995; Vezina and Stewart, 1989). This phenomenon can be maintained for a long time, since the maximum activity reached can be found during a re-injection of the drug after a long weaning. For example, amphetamine sensitization persists for at least 1 year after the withdrawal in rats (Paulson et al., 1991). Interestingly, the sensitization may be influenced by many factors such as environment, context, age, sex, or genetic background (Anagnostaras and Robinson, 1996, 1996; Belluzzi et al., 2004; Valjent et al., 2006a).

Conditioned Place Preference (CPP)

CPP is a simple form of classic conditioning or Pavlovian conditioning, a learning process that involves either positive or negative associations between two stimuli (Tzschentke, 1998). The CPP procedure generally consists of three phases: pre-conditioning, conditioning, and testing. During the pre-conditioning, the animal is allowed to move freely throughout a test apparatus with two chambers with distinct visual patterns and textures. At this time, animal's initial preference is measured, and the researcher may assign treatment pairings in a biased or unbiased design. In an unbiased design, subjects are randomly assigned regardless of their initial preferences. In a biased design, the initially non-preferred side is paired with the test drug. During conditioning, one chamber of the apparatus is paired with the drug, whereas the other side is paired with vehicle injection. This training involves multiple pairings of each contextually distinct compartment with the drug or vehicle over a period of several days. After the conditioning, preference is tested in a drug-free state by measuring the amount of time spent in each chamber. The choice of one context over the other is said to impart information regarding the drug-induced motivational state. If the drug is "rewarding," the subject is expected to spend more time in the drug-paired environment, thus producing CPP (Bardo and Bevins, 2000). Conversely, if the drug induces a negative state, the subject will avoid the paired context, producing a place aversion (Mucha et al., 1982).

Self-administration

To measure the behavior of self-administration the animals are placed in a cage equipped with two holes or two levers: the first, active, in which they need to make a response (either a lever press or a nose poke) to obtain the administration of a dose

of drug; the second, called inactive, is linked to an injection of saline solution. It is an operant conditioning that uses the motivational properties of the drug as a positive reinforcement (Goeders et al., 1986).

C) Neuronal plasticity induced by social defeat and cocaine

Dopaminergic neurons in the reward circuit play a crucial role in mediating stress and drugs responses. Drugs and stress do not only activate this brain system, they also induce long-term changes in the neuronal connections of these circuits. These changes are coined by the term of neuronal plasticity. An increase in synaptic efficiency is called Long Term Potentiation (LTP), while a decrease is termed Long Term Depression (LTD). Synaptic plasticity is essential for development of learning but aberrant plasticity can also be triggered by stress exposure and drugs of abuse. Here I will mainly focus on plasticity processes induced by repeated social defeat and cocaine within the VTA and the NAc. This does not rule out the fact that plasticity processes also occur in other brain regions of the mesocorticolimbic circuits.

1) Plasticity in the VTA

Repeated social defeat triggers an enduring increase in phasic activity of VTA dopaminergic neurons in susceptible mice (Berton et al., 2006). Optogenetic-mediated phasic stimulation of VTA-to-NAc dopamine pathway has been shown to enhance mice susceptibility to a sub-threshold social defeat that normally does not induce social avoidance (Chaudhury et al., 2013). Conversely, quinpirole mediated decrease of dopamine neurons firing make mice resilient to social defeat (Barik et al. 2013). The increase of dopamine neurons firing following repeated social defeat has been linked to an increased release of brain-derived neurotrophic factor (BDNF) to projection regions, including the NAc (Berton et al. 2006; Krishnan et al., 2007). BDNF potentiates dopamine release in the NAc through activation of TrkB receptors on dopaminergic nerve terminals. Local deletion of the BDNF gene in the VTA exert an antidepressant-

like effect by opposing the development of social avoidance behavior in defeated mice (Berton et al., 2006).

Different types of drugs of abuse cause an increase in dopamine levels in the brain regions including the NAc indicating that the mesolimbic dopaminergic system plays a role in drug addiction. The changes in excitatory synapses can be monitored by measuring the ratio of AMPAR-mediated excitatory postsynaptic currents (EPSCs) to NMDAR-mediated EPSCs (the AMPAR/NMDAR ratio). It has been found that a single exposure to cocaine caused a large increase in this ratio in the VTA dopamine cells when measured 24 hours later in brain slices due to a calcium-dependent upregulation of GluR1 AMPARs (Ungless et al., 2001). Insertion of GluR2-lacking AMPARs making them permeable to calcium has been shown to be key for the increase of AMPA/NMDA ratio and depotentiation along with removal of GluR2-lacking synaptic AMPARs could be achieved through the activation of metabotropic receptors mGluR1 (Bellone and Lüscher, 2005, 2006). Also, recent evidence showed that repeated cocaine could potentiate the NAc D1 expressing MSN-to-VTA GABA interneuron synapse ultimately leading to a disinhibition of dopamine neurons (Bocklisch et al., 2013).

2) In the NAc

The NAc is a key mesolimbic interface, receiving input from the hippocampus, PFC, AMG, and VTA (O'Donnell et al., 1999). The stress-associated changes in limbic plasticity, VTA dopamine release, and reward-related behaviors point to the NAc as a potentially important locus of stress-induced plasticity. Studies showed that stress exposure enhances synaptic strength in the NAc shell. Indeed, two days of cold-water stress in mice triggers an increase AMPAR/NMDAR ratio in the NAc shell, but not in the core MSNs. The change in AMPAR/NMDAR ratio was accompanied by increased AMPAR mEPSC (Excitatory post synaptic current) amplitude without a corresponding change in mEPSC frequency. These increases were blocked by intraperitoneal pre-administration of GR antagonist RU486 (Campioni et al., 2009). Considering social defeat, recent studies showed that D1-MSNs display reduced mEPSC frequency along

with an increased intrinsic excitability which is dependent on *Egr3* while D2-MSNs exhibit an increase of mEPSCs frequency in susceptible mice. Repeated optogenetic activation of D1-MSNs promoted resilience to Chronic social defeat (CSDS) while the stimulation of D2 neurons had the opposite effect (i.e. increased sensitivity to a subthreshold stress (Francis et al., 2015; Francis et al., 2017). In addition, CSDS has been shown to increase AMPA/NMDA ratio in the intralaminar thalamus-to-NAc pathway and the inhibition of these circuits decreases NAc stubby spine density and promote behavioral resilience (Christoffel et al., 2015). In comparison, increasing the activity of mPFC-NAc inputs via optogenetic stimulation promotes resilience to CSDS (Christoffel et al., 2015; Bagot et al., 2015).

NAc and its associated circuitry has also a key role in many forms of reward-dependent learning and drugs of abuse are thought to exert their reinforcing effect partly through the hijack of these circuits (Nestler, 2001). NAc as mentioned earlier receives glutamatergic input from hippocampus, basolateral amygdala, as well from the PFC. Unlike the VTA, a single *in vivo* dose of cocaine has been reported to cause no change of plasticity in the NAc. However chronic cocaine treatment has been shown to induce a decrease of AMPA-mediated current in the NAc shell MSNs at the synapse from PFC afferent during withdrawal (Thomas et al., 2001). Of note, plasticity induced by cocaine in the NAc can be be-directional depending on the synapse studied and on the protocol of cocaine administration (Kourrich et al., 2007; Pascoli et al., 2011, 2014). In the past decade, the contributions of NAc D1-MSN and D2-MSN cell populations to cocaine-associated behaviors have been explored. Withdrawal from cocaine self-administration has been shown to increase AMPA/NMDA ratio in the synapses from the ventral hippocampus to NAc D1 MSNs while the opposite was observed in synapses from PFC to NAc D1 MSNs. Linking these forms of plasticity and cocaine self-administration, optogenetic reversal of these phenomenon abolished drug seeking (Pascoli et al., 2014).

D) Molecular changes

The extracellular signal-regulated kinases (ERK) cascade

The MAPK/ERK pathway is a signaling cascade involving series of kinases (ras, raf, MEK) leading to the activation by phosphorylation of ERK. The activated form of ERK translocate into nucleus and leads to the activation of transcription factors CREB (cyclic AMP response element protein) via MSK1 or ribosomal protein S6 kinase (RSK) and Elk1. This signaling pathway allow the expression of immediate early genes such as c-Fos, FosB, Arc and Egr1 which allow a subsequent transcriptional cascade (Figure 25).

Both stress exposure and cocaine injection can induce the activation of the ERK signaling pathway in specific brain regions of the mesocorticolimbic circuits. This pathway underlies neuronal plasticity and behavioral changes induced by stress and drug exposure (Girault et al., 2007; Iñiguez et al., 2010; Lu et al., 2006). The activation of the MAPK/ERK pathway and its behavioral effects have been thoroughly studied in response to drugs. ERK1/2 phosphorylation is induced by a single injection of cocaine, but also other types of drugs of abuse, in the PFC, striatum and the NAc (Valjent et al., 2005). The activation of ERK by cocaine in the striatum and NAc is restricted mainly to D1-expressing MSNs and relies on the concomitant activation of D1 and NMDA receptors and on the potentiation of NR2B-containing NMDAR by D1 receptor (Pascoli et al., 2011; Valjent et al., 2000). This potentiation of NMDAR leads to an increase of intracellular calcium which activate the Ca²⁺/Calmoduline pathway triggering the activation of the MAPK/ERK pathway (Kim et al., 1998; Vanhoutte et al., 1999).

At the behavioral level, the inhibition of ERK by the systemic injection of a MEK inhibitor SL327 decreases cocaine behavioral sensitization and this effect may be due to an inhibition of this pathway within the VTA (Pierce et al., 1999; Valjent et al., 2006b). The MAPK/ERK is also key for cocaine CPP. The injection of SL327 before conditioning blocks cocaine CPP (Valjent et al., 2000). Local injection of another MEK inhibitor (U0126) in the NAc core just before the test, also blocks the CPP (Miller and Marshall, 2005). ERK in brain regions such as the hippocampus and PFC have been shown to modulate depressive like behaviors in a variety of pre-clinical models (Mao and Wang,

2019). Less is known about the direct role of ERK in response to repeated social defeats although the functions of its downstream targets such as CREB and Δ FosB have been extensively studied. However one study indicates that the overexpression of ERK2 in the VTA increases sensitivity to CSDS while blocking its activity has the opposite effect (Iñiguez et al., 2010).

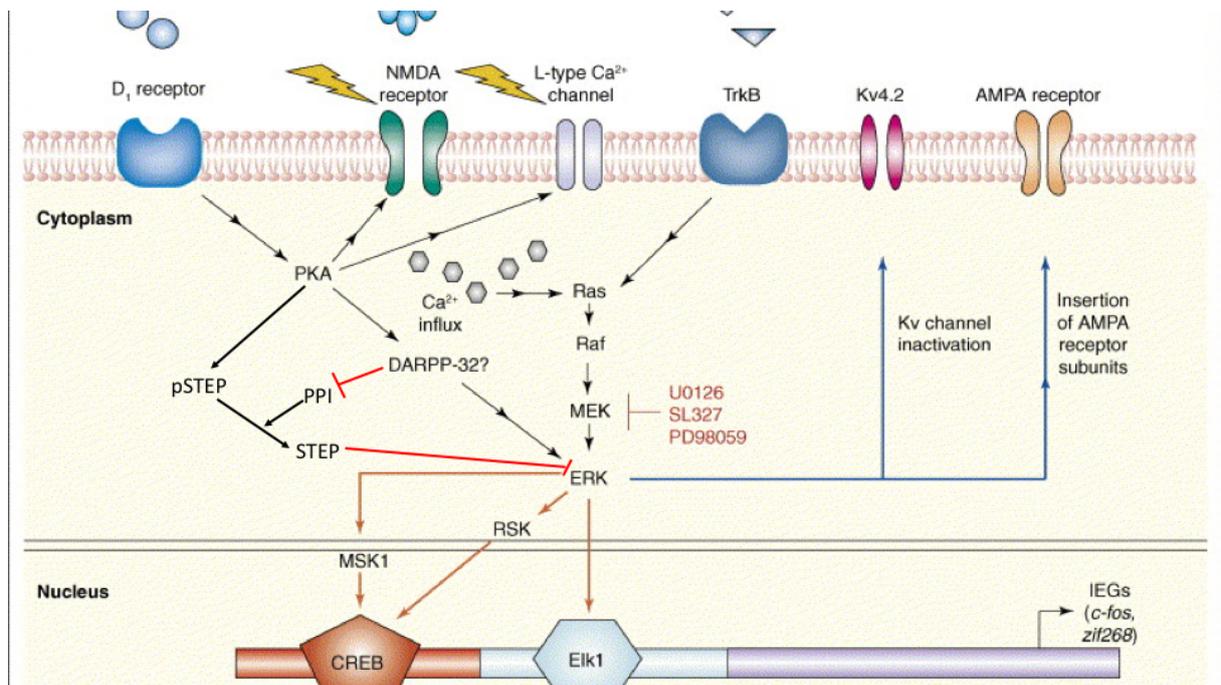


Figure 25: ERK signaling cascade. Stress and Cocaine-induced activation of D1 receptors, leading to activation of L-type Ca^{2+} channels and NMDA receptors via PKA (Protein kinase A), NMDA receptors are also activated by glutamate. Activation of NMDA receptors and L-type Ca^{2+} channels result in enhanced Ca^{2+} influx, which is crucial for activation of Ras. Ras–Raf–MEK signaling results in ERK phosphorylation. Also, D1 receptors phosphorylate DARPP-32 via PKA (Valjent et al., 2005). Phosphorylated DARPP-32 can act as potent inhibitor of the protein phosphatase PP-1, which dephosphorylates another phosphatase, the striatal-enriched tyrosine phosphatase (STEP). Dephosphorylation of STEP activates its phosphatase activity, thus allowing STEP to dephosphorylate ERK. pERK translocate into nucleus and leads to activation of the transcription factors CREB via MSK1 or ribosomal protein S6 kinase (RSK) and Elk1. Activation of these transcription factors leads to the induction of the transcription factor intermediate-early genes *c-fos* and *zif268*. (modified from Lu et al., 2006)

CREB and upregulation of the cAMP pathway

CREB and related proteins were described originally as transcription factors that mediate effects of the cAMP second messenger pathway on gene expression. CREB

binds to CRE sites as a dimer and activates transcription only when both subunits are phosphorylated on their Ser 133 residue. CREB can be phosphorylated on Ser 133 by protein kinase A (PKA), Ca²⁺/calmodulin-dependent protein kinase IV, or protein kinases regulated by growth factor–ras pathways (Mayr and Montminy, 2001; Shaywitz and Greenberg, 1999).

CREB has been shown to modulate reward and addictive behaviors. Increased CREB function in the NAc decreases an animal's sensitivity to the rewarding effects of morphine and cocaine, whereas decreased CREB function has the opposite effect (Carlezon et al., 1998, Barrot et al., 2002). CREB produces its behavioral effects via the regulation of other genes (Mayr and Montminy, 2001). Chronic administration of cocaine or other stimulants induces dynorphin expression in the NAc, and this induction is dependent on CREB (Cole et al., 1995, Carlezon et al., 1998). Dynorphin is known to dampen reward mechanisms in the NAc via the activation of κ opioid receptors in dopamine neurons (Shippenberg and Rea, 1997; Figure 26). The inactivation of κ opioid receptors by using an antagonist blocks the ability of CREB activity to dampen the cocaine reward (Carlezon et al., 1998, Pliakas et al., 2001). Upon phosphorylation, CREB also induces the expression of immediate early genes such as FosB and its truncated form Δ FosB which will be discussed in the following part and c-Fos. The inactivation of this latter gene in D1-expressing MSNs has been shown to reduce the behavioral responses to cocaine (Zhang et al., 2006). CREB is also induced in response to stress exposure and the inactivation of CREB within the NAc has been shown to reduce social avoidance after social defeat but also to block the enhancement of stress-sensitivity by cocaine exposure (Covington et al., 2011a).

Δ FosB

Δ FosB is a member of the Fos family of transcription factors. These proteins dimerize with a Jun family member to form the activator protein-1 (AP-1) transcription factor complexes, which bind to AP-1 sites present within the regulatory regions of many genes (Morgan and Curran, 1995). Acute stress and acute administration of several types of drugs of abuse causes the rapid (1–4 hour) induction of several Fos family members (c-Fos, FosB, Fra-1, Fra-2) in the NAc and in dorsal striatum. All of these

Fos family proteins, however, are highly unstable and return to basal levels within hours (8-12 hours) of drug administration (Berton et al., 2006; Hope et al., 1992; Young et al., 1991). Truncated form of FosB called Δ FosB is induced only slightly by acute stress or drug exposure. However, Δ FosB begin to accumulate with repeated stress and drug administration in many regions of the reward system owing to their high stability and eventually become the predominant Fos-like protein (Hope et al., 1994; Lobo et al., 2013; Vialou et al., 2010). In the striatum and the NAc, this induction seems to be specific to the D1-expressing MSNs and this cellular pattern of induction is specific for addictive drugs (Kelz and Nestler, 2000; Moratalla et al., 1996). The mice that overexpress Δ FosB in the hippocampus show greater sensitivity to the behavioral effects of cocaine in locomotor activity, conditioned place preference, and self-administration assays (Chen et al., 2000).

Δ FosB expression has been shown to be significantly reduced in the NAc of human depressed patients. In preclinical models, NAc Δ FosB expression is significantly enhanced in mice that are resilient, but not susceptible to CSDS. The virally-mediated overexpression of Δ FosB in the NAc promoted resilience to CSDS (Vialou et al., 2010). Comparatively, Δ FosB expression is enhanced in D1-MSNs of resilient mice and enhanced in D2-MSNs of susceptible mice in both NAc core and shell regions (Lobo et al., 2013). The overexpression of Δ FosB decreased excitatory synaptic strength and increased silent synapses on D1 MSNs in these regions. In contrast, the overexpression of Δ FosB decreased silent synapses onto D2 MSNs in the NAc shell, but not in the core. Furthermore, Δ FosB increased the density of immature spines in D1 MSNs but not D2 MSNs (Grueter et al., 2013). Rac1 expression, which is also significantly decreased in human depressed patients, has been shown to mediate changes in stubby spine formation in mice via an epigenetic mechanism (Golden et al., 2013).

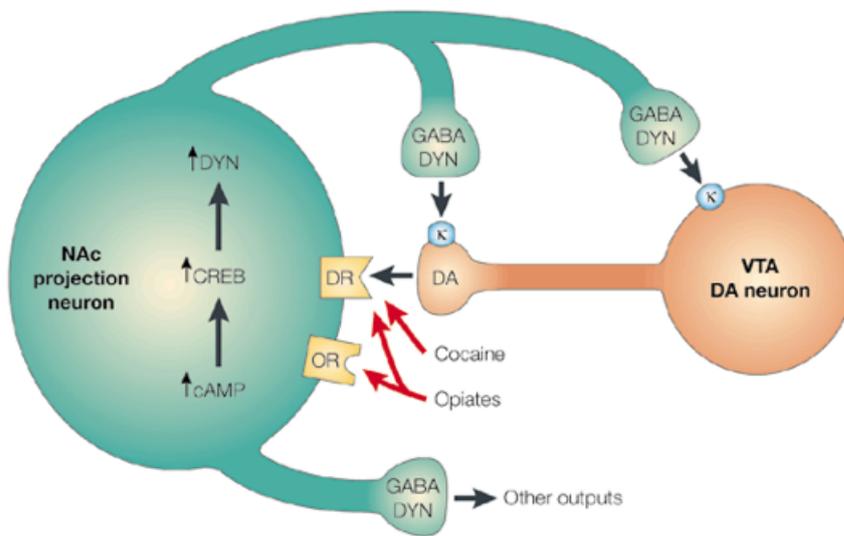


Figure 26: The figure shows a dopamine (DA) neurons of the VTA innervating a class of GABA (γ -aminobutyric acid) projection neuron from the NAc that expresses dynorphin. Dynorphin constitutes a negative feedback mechanism in this circuit: dynorphin, released from terminals of the NAc neurons, acts on κ -opioid receptors (κ) located on nerve terminals and cell bodies of the dopamine neurons to inhibit their functioning (Nestler, 2004)

Calcium/calmodulin-dependent protein kinase II (CaMKII) is necessary for LTP induction (Lisman et al., 2002). It has been shown that Δ FosB binds the CaMKII α gene promoter in the NAc and that this binding increases after mice are exposed to CSDS. Paradoxically, chronic exposure to the antidepressant fluoxetine reduces binding of Δ FosB to the CaMKII α promoter and reduces CaMKII expression in NAc. These effects are through an epigenetic mechanism of reducing acetylation and increasing H3meK9 methylation (Robison et al., 2014).

E) Epigenetics changes

Environmental events and behavioral experience interact with an individual's genetic constitution to induce epigenetic changes at particular gene loci that shape neuronal plasticity and behavior. Some of these changes can be very stable and even persist for a lifetime. An increasing number of studies have been interested in the role of epigenetic changes in drug and stress-induced behavioral impairment. So far, the net effect of these modifications is hard to picture and different results and interpretations

stem from the different brain regions studied but also from the scale of the studies going from one particular gene promoter to whole genome modifications.

1) DNA methylation

Chronic social defeat increases the expression of the DNA methyltransferase 3a in the NAc and its overexpression enhances depression-like behavior while local inhibition with an inhibitor has the opposite effect (LaPlant et al., 2010). Chronic social defeat stress has been reported to cause a decrease in DNA methylation at the *Crh* gene promoter and a sustained upregulation of *crh* gene expression in the PVN of susceptible mice. Specific knockdown of CRH from the PVN attenuated stress-induced social avoidance after CSDS (Elliott et al., 2010). Furthermore, Conditional knockout of MeCP2 (methyl CpG binding protein-2) in the PVN, which reduces the repression of certain methylated genes, induces an abnormal physiological stress response (Fyffe et al., 2008). Interestingly, female rats exposed to chronic mild stress show increased DNA methylation at the *crh* gene promoter in the PVN, suggesting sex-specific alterations in HPA axis activity (Sterrenburg et al., 2011).

Genetic background influences the outcome of stress exposure in animals and humans, and this could be mediated in part by DNA sequence-directed differences in epigenetic modifications in response to stress. Chronic mild stress increases anxiety- and depression-like behaviors in BALB/C mice, but no effect in C57BL/6 mice. Both mouse strains show an increase in DNA methylation and MeCP2 binding at the *Gdnf* promoter in the NAc after chronic stress however, BALB/C mice display decreased GDNF expression in the NAc while C57BL/6 mice show an increased expression. These differences are due to the fact that in BALB/C mice, MeCP2 interacts with HDAC2 to decrease H3 acetylation and concomitantly represses *Gdnf* transcription, whereas the association of MeCP2 with CREB in C57BL/6 mice enhance *gdnf* expression in the NAc of C57BL/6 mice (Uchida et al., 2011).

Unfortunately, few studies show how DNA methylations modulate drug addiction. Acute cocaine treatment causes an upregulation of DNMT3A and DNMT3B gene expression in the NAc. Acute and repeated cocaine administration induces hypomethylation at the *FosB* gene promoter, a change that has been associated with

transcriptional upregulation of *fosB* in the NAc (Anier et al., 2010). Local knockout of DNMT3a within the NAc, or local infusion of the DNMT inhibitors increases behavioral responses to cocaine, whereas DNMT3a overexpression in NAc has the opposite effect, reminding the results obtained with CSDS (LaPlant et al., 2010). In addition, NAc knockout of MeCP2 (methyl CpG binding protein-2) enhances amphetamine reward (Deng et al., 2010). In contrast, chronic cocaine increases MeCP2 expression in dorsal striatum, where local knockdown of the protein attenuates cocaine self-administration (Im et al., 2010).

2) Histone Acetylation

Histone acetylation is dynamically regulated after CSDS. Indeed, repeated social defeats causes a transient decrease, followed by a persistent increase, in levels of acetylated H3 in the NAc. This persistent increase in H3 acetylation is associated with decreased levels of HDAC2. Showing the impact of histone acetylation on responses to stress exposure, infusion of HDAC inhibitors into the NAc reverses the stress-induced social avoidance in defeated mice (Covington et al., 2009). Other brain regions show different modulations of histone acetylation. As such, CSDS causes a transient increase in the levels of acetylation of H3 in the hippocampus and the amygdala, followed by a persistent decrease. Infusion of HDAC inhibitor into the hippocampus does not restore social interaction behavior after CSDS. Interestingly, social avoidance is reversed when HDAC inhibitor is infused directly into amygdala (Covington et al., 2011b).

Histone acetylation is the most studied chromatin modification in models of drug abuse. Acute exposure to cocaine increases global levels of acetylated histone H4 at the *c-Fos* promoter in the NAc whereas repeated cocaine injection increases levels of H3 acetylation at BDNF and *Cdk5* promoters (Kumar et al., 2005). Little is known about the intracellular signaling pathways through which stimulants leads to such global changes in histone acetylation, including which of numerous subtypes of HATs and HDACs are responsible for modifying the various Lysine residues that display regulation. The HAT, CBP, and Class II HDAC, HDAC5, have been implicated (Levine et al., 2005; Renthal et al., 2007). Studies investigating the effects of pan-HDAC inhibition on psychostimulant-induced behavioral plasticity have yielded conflicting

results, with some studies reporting that systemic or intra-NAc HDAC inhibition enhances the behavioral effects of cocaine or amphetamine, and other studies reporting changes in the opposite direction (Kalda et al., 2007; Kumar et al., 2005; Levine et al., 2005; Renthall et al., 2007; Schroeder et al., 2008). One study showed that local knockdown of HDAC1 in the NAc significantly reduces cocaine locomotor sensitization without any effect on the response to the first cocaine injection. In contrast, local knockdown of HDAC2 or HDAC3 has no effect on cocaine-induced behaviors (Kennedy et al., 2013). However, another study reported a facilitation of extinction of cocaine CPP in mice with a NAc inactivation of HDAC3 (Malvaez et al., 2013). Finally, homozygous HDAC5 knockout mice show a hypersensitivity to cocaine (Renthall et al., 2007).

3) Histone methylation

Both repeated social defeat and cocaine have been shown to downregulate the expression of G9a methyltransferases in the NAc which catalyzes H3K9me2, a major transcription repression mark. G9a overexpression within the NAc not only made mice resilient to repeated social defeat but also counteracted the additive effect of cocaine exposure potentially through an inhibition of the ERK signaling pathway and of CREB activation (Covington et al., 2011b; Maze et al., 2010). Also, blocking the activity of G9a in the NAc potentiates behavioral responses to cocaine, whereas increasing G9a function exerts the opposite effect (Maze et al., 2010; Sun et al., 2012). G9a appears to be a critical control point for epigenetic regulation in the NAc, and generally opposes the induction of Δ FosB (Robison and Nestler, 2011), while Δ FosB in turn suppresses G9a expression (Maze et al., 2010; Sun et al., 2012).

In addition, CSDS also increases H3K27me3 levels and DNA methylation at the *Bdnf* gene promoter in hippocampus of susceptible mice (Roth et al., 2011; Tsankova et al., 2007).

4) Other histone modifications

Only few studies examined the role of chromatin remodelers in the responses to stress exposure or drugs of abuse. Recently CSDS has been shown to increase ACF

expression (ATP-utilizing chromatin assembly and remodeling factor) in the NAc in susceptible mice. Furthermore, suppression of ACF in NAc in susceptible mice exerts antidepressant-like actions (Sun et al., 2015). Concerning SWI/SNF complexes, the pharmacological inhibition of Brg1 in the NAc suppresses cue-induced reinstatement of cocaine seeking, while its overexpression exacerbates cocaine-seeking behavior (Wang et al., 2016).

Rational of my thesis

The aim of my thesis was to study the role of BRG1 and BRM containing SWI/SNF complex on stress- and drug-induced maladaptive behavior. This work stems from two different lines of research, one involving recent studies about chromatin remodelers and their potential role in psychiatric disease pathophysiology and one involving findings on GR studies carried out by the lab in the past 10 years.

Epigenetic mechanisms underlying stress-mediated vulnerability to psychiatric disorders?

Stress is a well-known risk factor for most psychiatric diseases including depression and addiction. These diseases are characterized by life-long behavioral impairment that are thought to be underlined by long-lasting neuroadaptations in several brain regions among which the meso-cortico-limbic circuit. In the past decade, many studies have pointed toward epigenetic modifications and resulting maladaptive genes expression in these circuits as molecular underpinnings (Nestler et al., 2016). These epigenetic modifications include both histone and DNA modifications such as acetylation or methylation which modify the level of compaction of chromatin and modulate the accessibility of genes promoters by the transcriptional machinery (Bannister and Kouzarides, 2011). However, chromatin by itself has limited mobility and requires ATP-dependent remodeling to effectively move. Chromatin remodeling complexes ensure that function and while their role in cell division and differentiation has been well-characterized, their influence on brain function is still understudied. It is of key importance since as mentioned, genome-wide association studies have linked mutations in genes encoding for subunits of chromatin remodeler complexes with brain neurodevelopmental disorders (Maze and Nestler, 2011; Sokpor et al., 2017). In preclinical models, a recent study has shown that mice susceptible to repeated social defeat but also patients with depression have an upregulation of ACF, a subunit of the ISWI chromatin remodeler complex, within the NAc. Also, the overexpression of ACF (BAZ1A plus SMARCA5) within the NAc made mice more sensitive to social defeat while knocking it down made them more resilient. Of note, increased ACF in mice was associated with altered nucleosome positioning and a repressed transcription of key genes involved in the sensitivity to social defeat (Sun et al., 2015). Considering the

SWI/SNF complex, one study has shown that intra-NAc inhibition of BRG1 decreases cocaine self-administration relapse while NAc Brg1 overexpression has the opposite effect (Wang et al., 2016). These data strongly suggest an involvement of Brg1 containing chromatin remodeler complexes in drug and stress-related maladaptive behavioral responses and my thesis project directly follow-up on this hypothesis.

What are the molecular mechanisms involved in the action of the glucocorticoid receptor on behavioral responses to drugs and stress exposure?

Our team has highlighted the role of GR in drug and chronic social defeat behavioral responses. More specifically, mice deprived of GR within the dopaminergic neurons (GR^{D1Cre} mice) have reduced behavioral responses to cocaine including self-administration (Ambroggi et al., 2009), conditioned place preference and locomotor sensitization (Barik et al., 2010). Along with these behavioral differences, GR^{D1Cre} mice exhibit a reduced spontaneous firing of dopamine neurons within the VTA potentially due to an altered feed-back from the NAc MSNs (Ambroggi et al., 2009; Parnaudeau et al., 2014). These data brought some insight on the mechanisms underlying stress-mediated increased vulnerability to drugs of abuse. Of note, all these results were observed under basal conditions, however the inactivation of GR in dopaminergic neurons also reduces social aversion induced by repeated social defeat and blocks the long-term enhancement of dopamine neurons activity normally occurring after such stress-exposure (Barik et al., 2013).

GR is a nuclear receptor that can function as a transcription factor and thereby regulate genes expression. As mentioned, in order to regulate transcription, GR can interact among others with chromatin remodelers and more particularly with the SWI/SNF complex which is composed of one ATPase motor subunit being either Brm or Brg1 and associated factors called BAFs. In fact, both Brm and Brg1 have been shown to potentiate GR transcriptional activity in cell culture (Engel and Yamamoto, 2011; Fryer and Archer, 1998; Johnson et al., 2008; Muchardt and Yaniv, 2001) and whole-genome scale studies have shown that in the 3T3 fibroblastic cell line, around 40% of GR-stimulated genes and 11% of GR-repressed genes require the function of Brg1-containing SWI/SNF complexes (John et al., 2008). Thus, the main hypothesis of my thesis work was that GR could regulate behavioral responses to either drugs or stress-

exposure through this interaction with SWI/SNF complexes. If so, the inactivation of Brg1 or Brm from dopaminergic neurons should decrease behavioral sensitization and conditioned place preference to cocaine and prevent mice to develop social aversion after repeated social defeats.

Part II: Results

BRG1 containing SWI/SNF chromatin remodeler complex in dopaminoceptive neurons is required behavioral responses to stress.

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Abstract

Stress exposure is a risk factor for most psychiatric diseases and clinical studies along with studies using animal models pointed at changes in gene expression involving epigenetic modifications within mesocorticolimbic brain circuits as potential underlying mechanisms. Besides chemical chromatin modifications, chromatin reorganization is essential to efficiently modulate genes expression. So far, the role of chromatin remodeling in stress-induced long-term behavioral impairment has been understudied. Brahma (BRM) and Brahma-Related Gene 1 (BRG1) are ATPase subunits of the SWI/SNF chromatin remodeling complex which is known to potentiate nuclear receptors transcriptional activity. Here we show that the inactivation of Brg1 gene within dopaminoceptive neurons, and the constitutive inactivation of *Brm* gene lead to an almost complete resilience to chronic social defeat stress and strongly decreases the behavioral responses to cocaine. These mutations do not affect dopamine neurons activity, but deeply reduce the induction of immediate early genes by either cocaine treatment or social defeat, and the phosphorylation of histone H3S10 in striatum and nucleus accumbens medium spiny neurons. These results posit the SWI/SNF complex within the mesocorticolimbic system as an important factor in the vulnerability to both stress and drugs of abuse and in stress-mediated maladaptive genes-expression.

Introduction

Chronic stress exposure is a well-known risk factor for most psychiatric diseases including depression and addiction. In the past decade, intensive effort has been made to understand the mechanisms through which stress exposure can lead to life-long behavioral impairment. These persistent behavioral changes are underlined by long-lasting neuroadaptations which are thought to arise from maladaptive genes expression. In this context, the focus has been naturally brought onto epigenetic mechanisms and compelling evidence show that transcription factors¹ but also histone modifications²⁻⁴ and DNA methylations⁵ within the meso-cortico-limbic system regulate the vulnerability to chronic stress exposure and the development of depression-like behaviors in mice.

Stress exposure induces a rapid release of glucocorticoids hormones from the adrenal glands. Through their binding to the glucocorticoid receptor (GR), a ubiquitously expressed transcription factor, they play a key role in the transcriptional response to stress exposure thereby facilitating the organism's ability to cope with environmental changes. However, abnormal glucocorticoids levels are frequently associated with psychiatric diseases such as depression or addiction⁶. Our previous work strongly suggests that this pathological stress-response involves the GR within the dopamine pathway. More specifically, we showed that selective GR gene inactivation in dopaminergic neurons (including the striatum, the nucleus accumbens (NAc), and the prefrontal cortex (PFC)) in mice blocks the development of social aversion induced by repeated social defeat and the associated activation of dopamine neurons in the ventral tegmental area⁷. We also showed that the same manipulation decreased the behavioral responses to cocaine and amphetamine⁸⁻¹⁰.

GR modulates genes expression through a diversity of strategies. It can interact with components of the basal transcriptional machinery but also recruit histone modifiers or chromatin remodeler complexes such as the mating-type switching/sucrose non-fermenting (SWI/SNF) complex¹¹⁻¹². These chromatin remodeling complexes use the energy of ATP to disrupt nucleosome DNA contacts, making possible the move of nucleosomes along DNA and the removal or the exchange of nucleosomes. By this way, they facilitate the accessibility of DNA or chromatin to proteins that can modulate transcription¹³. Mammalian SWI/SNF are multi-subunit complexes that contain either

Brahma (BRM) or Brahma-related gene 1 (BRG1) as the central ATPase subunit, associated with 10-12 BRM/BRG1 associated factors¹⁴.

While there is compelling evidence for the involvement of DNA and histone modifying enzymes in behavioral impairment induced by stress and drug exposure, very little is known concerning the potential role of chromatin remodeling complexes. A recent study has shown that the upregulation of ACF chromatin remodeling complex within the NAc is key for the development of depressive-like behavior in mice subjected to chronic stress exposure and for reinforcement to cocaine^{15, 16}. In addition, BRG1 in the same brain region has been recently involved in cocaine relapse¹⁷. Here, we show that BRG1 in dopaminergic neurons and BRM are essential for the development of social avoidance and anxiety after repeated social defeat in mice, a preclinical model of depression. Mice lacking either BRG1 or BRM also exhibit decreased responses to cocaine in locomotor sensitization and conditioned place preference paradigms. These phenotypes appear to be independent from any changes of dopamine neurons activity or cell signaling transduction at the post-synaptic level within the striatum or the NAc. However, we found that the absence of BRG1 leads to a decrease of stress- and drug-induced immediate early genes within these structures. At a larger scale, the absence of BRG1 also decreases the Serine 10 phosphorylation of the histone 3 and increases heterochromatin surface within the NAc indicating a reduction of transcriptional activity. Altogether, these results posit the SWI/SNF complex within the mesocorticolimbic system as an important factor in the vulnerability to both stress and drugs of abuse. They further suggest that these chromatin remodelers play a key role in the maladaptive transcriptional response to stress and drug exposure and the ensuing pathological behaviors.

Material and Methods

Animal Breeding

Animals were bred and raised under standard animal housing conditions, at 22°C, 55% to 65% humidity, with a 12-h light/dark cycle (7 AM/7 PM) and had free access to water and a rodent diet. All experiments were performed in accordance with French (Ministère de l'Agriculture et de la Forêt, 87-848) and the European Directive 2010/63/UE and the recommendation 2007/526/EC for care of laboratory animals. Mice were 2 to 4-month-old males. Experiments were carried out during the light phase.

Mice carrying an inactivated BRM gene, generated by homologous recombination, have been previously described¹⁸. Briefly, an internal exon of *Brm* gene (also denominated *Smarca2*), corresponding to amino acids 120-276, was disrupted by the insertion of a neomycin cassette. This led to the absence, in BRM^{-/-} mice, of any functional BRM protein able to assemble in SWI/SNF complexes. To inactivate *Brg1* gene in dopaminoceptive neurons, we crossed mice carrying a conditional allele of *Brg1* gene, sensitive to the Cre recombinase (*Brg1*^{L2})¹⁹, with TgYAC-D1aCre mouse line^{20, 21}. The *Brg1*^{L2} allele contains *LoxP* sites flanking two internal exons. Their excision results in the deletion of the sequence encoding amino acid residues 814 (threonine) to 872 (lysine) and the creation of a frameshift in exon 4 with a stop codon at amino acid position 819. The remaining protein produced from the disrupted gene will encode a C-terminally truncated protein lacking the ATP hydrolysis site.

DNA from mice tails was extracted and analyzed by PCR. For *Brm*⁺ and *Brm*⁻ alleles the primers were CCTGAGTCATTTGCTATAGCCTGTG (sense strand), CTGGACTGCCAGCTGCAGAG (reverse strand) and CATCGCCTTCTATCGCCTTC (reverse strand in the neomycin cassette). The amplified bands corresponding to the wild-type and the mutated allele are 310 and 700 bp in length, respectively). *Brg1*⁺ and *Brg1*^{L2} alleles were detected using GATCAGCTCATGCCCTAAGG (sense strand) and GCCTTGTCTCAAAGTATAAG (reverse strand), primers giving rise to 241bp and 313bp DNA segments, respectively. To detect the D1Cre transgene, primers were GCCTGCATTACCGGTCGATGCAACGA (sense strand) and GTGGCAGATGGCGCGGCAACACCATT (reverse strand). The amplified band was 700 bp long.

Animals were bred on a mix genetic background derived of 129SvEv and C57BL/6 mouse strains. For experiments in which BRM, BRG1 and compound mutants were compared (essentially cocaine sensitization), animals were bred following a strict breeding scheme. $Brm^{+/-}Brg1^{+/L2}TgYACD1aCre$ males and females, issued from a mating between $Brm^{-/-}Brg1^{L2/L2}TgYACD1aCre$ males and C57BL/6 females, were mated to obtain $Brm^{+/+}Brg1^{L2/L2}$ females and $Brm^{+/+}Brg1^{L2/L2} TgYACD1aCre$ males, as well as $Brm^{-/-}Brg1^{L2/L2}$ females and $Brm^{-/-}Brg1^{L2/L2} TgYACD1aCre$ males. These individuals were then mated to generate experimental males that were first cousins: control mice ($Brm^{+/+}Brg1^{L2/L2}$, denominated $Brg1^{LoxP}$ thereafter), single mutants ($Brm^{-/-}Brg1^{L2/L2}$ and $Brm^{+/+} Brg1^{L2/L2} TgYACD1aCre$, denominated $BRM^{-/-}$ and $BRG1^{D1Cre}$ respectively thereafter) and compound mutants (denominated $BRM^{-/-}BRG1^{D1Cre}$ thereafter). For the other experiments, each model was compared to its own control littermates.

Co-immunoprecipitation assays and western blots

Whole striata were dissected and homogenized in 200 μ l de radio-immunoprecipitation assay (RIPA) buffer (50 mM Tris- HCl, pH 7.6, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, 0.5% sodium deoxycholate, 2 mM EDTA, 0.2 mM sodium orthovanadate and 1X protease inhibitor complete cocktail (EDTA-Free, Roche, 11873580001)). Samples were centrifuged for 30 min at 13,000 rpm and cell lysates were subjected to a second centrifugation for 20 min. 150 μ l of lysate from $GR^{loxP/loxP}$ male striata were precleared with 20 μ l of protein-A sepharose beads (Sigma, 82506) by 4 h incubation at 4°C. Immunoprecipitation was performed by incubating overnight 50 μ l of the supernatants each containing the following antibodies a) GR (a rabbit polyclonal M20 antibody raised against the N-terminal region, 1:1000 dilution, Santa Cruz Biotechnology,) b) BRG1 (mouse monoclonal, 1:1000, Santa Cruz, sc-17796) and c) BRM (rabbit polyclonal 1:500, abcam, ab15597). The following day, the supernatants were washed three times with 1 ml PBS/Tween, each precipitate was resuspended in 60 μ l of 2X Laemmli Buffer. 20 μ l of the immunoprecipitated fractions and 20 μ g of striata lysates from GR^{D1Cre} and $GR^{loxP/loxP}$ mice were denatured at 95°C for 5min. The immunoprecipitated samples were centrifuge briefly to separate the beads. All the samples were then separated on a 10% SDS-PAGE gel and transferred to a nitrocellulose membrane (Bio-Rad, 162-0112). The membranes were incubated in a blocking solution (5% milk in TBS-T: 20mM Tris-HCl, pH 7.6, 137 mM NaCl, and 0.1% Tween 20) for 1h at room

temperature and then incubated with anti-GR antibody in a 5% BSA in TBS-T overnight at 4°C. Membranes were washed 3 times with TBS-T, then incubated with peroxidase-conjugated donkey anti-rabbit secondary antibody (1:1000, Jackson Laboratories, 711-035-152) diluted in a milk solution 5% BSA in TBS-T for 1 h at room temperature and rinsed 3 times in TBS-T. The proteins were detected using enhanced chemiluminescence (ECL) detection kit (GE HealthCare, RPN2235).

Histology

Mice were deeply anaesthetized with pentobarbital (Centravet, France) and transcardially perfused with cold phosphate buffer (PB: 0.1 M Na₂HPO₄/NaH₂PO₄, pH 7.4), followed by 4% PFA. Brains were post-fixed overnight in 4% PFA-PBS. Free-floating vibratome sections (30 µm) were rinsed three times with PBS (10 min) and incubated (120 min) in PBS-BT (PBS-Triton 0,1%, 0.5% BSA,) with 10% normal goat serum (NGS). Sections were incubated overnight (4°C) in PBS-BT, 1% NGS, with primary antibody. Sections were then rinsed in PBS and incubated (2 h) with secondary antibodies anti-rabbit or anti-mouse Alexa488 (1:1000, Invitrogen), and/or anti-rabbit or anti-mouse CY3 (1:1000, Invitrogen). Sections were then rinsed with PBS (3x10 min) and mounted using Vectashield Mounting Medium with DAPI (Vector Laboratories). Images were acquired using a fluorescence microscope equipped with an Apotome module (Zeiss, Axiovert 200M) and confocal microscope (Leica TCS SP5 AOBS). The primaries antibodies we used include a rabbit polyclonal Cre antibody (dilution 1:3000)²², a rabbit polyclonal NeuN (1:200, Millipore, MABN140), a mouse monoclonal BRG1 (1:1000, Santa Cruz, sc-17796), a rabbit polyclonal BRM (1:500, Abcam, ab15597), a mouse monoclonal c-Fos antibody (1:1000, Santa Cruz, sc-166940), a rabbit polyclonal Egr1 (1:500, Abcam, ab133695), a rabbit polyclonal phospho-ERK (1/400, Cell Signaling Technology, 4370), a mouse monoclonal H3S10P (1:1000, Millipore, 05-598), and a mouse monoclonal Lamin B antibodies (1/1000, Abcam, ab16048).

Social defeat and ensuing sociability, anxiety and depressive like behaviors

For all tests, animals were daily transferred in a dedicated room one to two hours before the tests for habituation. For each test, chambers of the apparatus were cleaned with alcohol 10% and dried with paper towels between each trial.

Repeated social defeat and social interaction

Social defeat was performed as previously described⁷. Six-month-old CD1 breeder male mice were screened for their aggressiveness. BRM, BRG1 mutants, and their respective control littermates were subjected to 10 consecutive days of social defeat. Each defeat consisted of 5 min of physical interactions between the resident mouse (CD1) and the intruder (experimental mouse). The rotation schedule was set to exclude a repeated defeat by an already encountered resident. Following this, the intruder remained for another 24 h in the resident's home cage that was partitioned in half by a perforated transparent polycarbonate, which allowed visual, auditory, and olfactory communication whilst preventing direct contact. Undefeated mice were handled and rotated daily and housed 2 per cage with the polycarbonate partition separating the cage in 2 halves.

Social interaction was studied 24 h after the last defeat (day 11) in a low luminosity environment (30 lux). Undefeated and defeated mice were introduced for 150 s in a corner of an open-field (40cmx40cmx25cm) containing in one side an empty perforated polycarbonate box ("no target" condition). Immediately after this, mice were rapidly removed and an unfamiliar CD1 mouse was placed in the box ("target" condition) and mice were re-exposed to the open-field for another 150 s. The time spent in the interaction zone surrounding the polycarbonate box was recorded and used as an index of social interaction.

Three chambers test

Sociability was also measured on day 12-14 in a three-chambered box (300 x 150 x 150 mm per compartment) with opening (50 x 50 mm) in each delimiting wall. The test is divided in 2 phases, each lasting 5 min. During the habituation phase, the challenged mouse was restrained to the central chamber, with the two doorways closed. Doors were then opened and the challenged individual could either visit the compartment with an unfamiliar adult male mouse (C57BL/6J) placed in a perforated transparent plastic box or the other compartment containing a similar empty box. The amount of time mice spent in direct interaction with each box was scored.

Elevated zero-maze

Elevated zero-maze was performed on day 15-18 after last defeat. The maze is a circular track (width 7 cm, diameter outer 47 cm) elevated 80 cm from the floor and made of grey polyvinyl chloride (PVC). It was divided in four sections of equal lengths: two opposite open portions and two closed portions with PVC walls, 15 cm in height. At the start, mice were placed in front of a closed portion. Their locomotion was recorded for 10 min. The latency to enter into the open portion, the number of entries and the time spent in the open parts were quantified. A mouse was considered in the open portion when the 4 paws were introduced. The intensity of the light was set to 30 lux in the open parts.

Dark-light test

The dark-light test was performed on day 18 after last defeat. The dark-light box was 45x20x25 cm, separated into two compartments connected by a central aperture (5x5 cm). The dark compartment (black PVC, 15 cm) was covered. The lit compartment (white PVC, 30 cm) was illuminated at an intensity of 30 lux. At the start, individuals were placed in the dark compartment and the latency to enter and time spent in the lit compartment were recorded for 10 min. A mouse was considered to be in the lit area when its 4 paws were inside.

Sucrose preference test

SPT was performed on Day 19-24 after last defeat. Prior to beginning testing, mice were habituated to the presence of two drinking bottles for at least 3 days in their home cage. In order to detect possible side preference in the drinking behavior, the water intake was measured daily in both bottles. After the acclimatization, the mice were separated (one mouse per cage) and presented to two drinking bottles: one containing 1% sucrose and the other water for 3 days in their home cage. Water and sucrose solution intake were measured daily by weighing the bottles. The position of two bottles was switched daily to reduce any confound produced by a side bias. Sucrose preference was calculated as a percentage of the volume of sucrose intake over the total volume of fluid intake and averaged over the 3 days of testing.

Forced-swim test

FST was performed on day 25. Mice were placed into a glass cylinder (40 cm tall, 12 cm diameter,) filled to a depth of 10 cm with water (23 °C). Test session lasted 6 min and mice were reassessed 24 h later. Sessions were videotaped and immobility, movements and escape manually scored. The total length of immobility was measured for 6 min, during three 2 min-intervals. Mice were considered immobile when floating passively, doing only slight movements of limbs and/or tail, without major head movements.

Contextual Fear Conditioning

For context conditioning, animals were placed into a conditioning chamber and allowed to habituate for 3 min, followed by three consecutive tone and foot shocks (85 dB 30 s, 0.4 mA shock at the last 2 s) separated by 30 s. Animals remained in the chamber for 1 min after the delivery of the last foot shock. Contextual fear conditioning was measured 24 h after in the same chamber, and freezing was scored for a total of 6 min. Sessions were recorded and freezing was automatically scored. Freezing was defined as complete absence of movement, except for respiration. Cued fear conditioning was measured 24 h after contextual fear conditioning. After changing the context of the chamber (ground, walls, shape, odor and light color) animals were placed into a new chamber and allowed to habituate for 3 min, followed by tone for 3 min, and freezing was scored for the first 3 min and compared with the last 3 min.

Behavioral responses to cocaine

Spontaneous locomotor activity and drug sensitization

Locomotor activity was assessed in circular chamber (4,5 cm width, 17 cm external diameter) crossed by four infrared captors (1,5 cm above the base) placed at every 90° (Imetronic, Pessac, France). Locomotor activity was scored automatically when animals interrupted two successive beams, having traveled a quarter of the circular corridor. The spontaneous locomotor activity was measured during 180 minutes following a first introduction into the locomotor apparatus. For locomotor sensitization to cocaine (Sigma-Aldrich, Saint-Quentin Fallavier, France), mice were habituated to the apparatus for 3 hours for 3 consecutive days and received a saline injection on Days 2 and 3 after 90 minutes of recording. On days 4 to 7 animals were daily injected

with either cocaine (10 mg/kg, *i.p.*) or saline. Following 6 days of withdrawal, all mice received an acute challenge injection of cocaine (10 mg/kg, *i.p.*).

Conditioned Place Preference

The conditioned place preference apparatus consisted of two chambers (10 x 25 x 20 cm) with distinct visual and tactile cues connected by a neutral area. On Day 1 (preconditioning), mice were placed in the neutral area allowed to explore the apparatus freely for 18 min. The time spent in each chamber was measured. On Days 2, 4, and 6, cocaine-paired mice received a cocaine injection (10 mg/kg, *i.p.*) and were confined to one chamber for 20 minutes. On days 3, 5, and 7, cocaine-paired individuals received saline in the opposite chamber and were also confined for 20 minutes. Saline-paired animals received saline in both chambers. During the post-conditioning (Day 8), mice (in a drug free state) were allowed to explore both chambers freely for 18 minutes. The CPP scores were expressed as the increase of time spent in the paired chamber between the post- and the preconditioning sessions.

In vivo electrophysiological recordings

Adult male mice (6-12 weeks) were anesthetized with chloral hydrate (8%), 400 mg/kg *i.p.* supplemented as required to maintain optimal anesthesia throughout the experiment, and positioned in a stereotaxic frame. A hole was drilled in the skull above midbrain dopaminergic nuclei (coordinates: 3 ± 1.5 mm posterior to bregma, 1 ± 1 mm [VTA] lateral to the midline)²³. Recording electrodes were pulled from borosilicate glass capillaries (with outer and inner diameters of 1.50 and 1.17 mm, respectively) with a Narishige electrode puller. The tips were broken under microscope control and filled with 0.5% sodium acetate. Electrodes had tip diameters of 1-2 μ m and impedances of 20–50 M Ω . A reference electrode was placed in the subcutaneous tissue. The recording electrodes were lowered vertically through the hole with a micro drive. Electrical signals were amplified by a high-impedance amplifier and monitored with an oscilloscope and an audio monitor. The unit activity was digitized at 25 kHz and stored in Spike2 program. The electrophysiological characteristics of dopamine neurons were analyzed in the active cells encountered when systematically passing the microelectrode in a stereotaxically defined block of brain tissue including the VTA (1). Its margins ranged from -2.9 to -3.5 mm posterior to bregma (AP), 0.3 to 0.6 mm

mediolateral (ML) and 3.9 to 5 mm ventral (DV). Sampling was initiated on the right side and then on the left side. Extracellular identification of dopamine neurons was based on their location as well as on the set of unique electrophysiological properties that distinguish dopamine from non-dopamine neurons in vivo (2): (i) a typical triphasic action potential with a marked negative deflection; (ii) a long duration (>2.0 ms); (iii) an action potential width from start to negative trough > 1.1 ms; (iv) a slow firing rate (<10 Hz and >1 Hz).

Electrophysiological recordings were analyzed using the R software (<https://www.r-project.org>). Dopamine cell firing was analyzed with respect to the average firing rate and the percentage of spikes within bursts (%SWB, number of spikes within burst divided by total number of spikes). Bursts were identified as discrete events consisting of a sequence of spikes such that: their onset is defined by two consecutive spikes within an interval <80 ms whenever and they terminate with an inter-spike interval >160 ms. Firing rate and %SWB were measured on successive windows of 60 s, with a 45 s overlapping period on a total period of 300 s.

Quantification of IEGs and pERK in response to cocaine and acute social defeat.

Immediate early genes expression of *c-Fos* and *Egr1* were quantified as previously described⁹. Mice either received an acute injection of 10 mg/kg cocaine or were exposed for 5 minutes to social defeat. The respective controls received either a saline injection or were similarly handled without aggression. The mice were deeply anesthetized with pentobarbital 1 hour after and went through transcardiac perfusion. The immunostainings were performed on 4 free-floating sections equally spaced along the rostro-caudal axis and encompassing the striatum and the NAc. The images were acquired using a fluorescence microscope equipped with an Apotome module (Zeiss, Axiovert 200M) and the number of c-Fos and Egr1 positive nuclei were quantified using Image J. Phospho-Erk was quantified the same way except that the mice were anesthetized and their brain sampled 10 minutes after the acute social defeat.

Quantification of nuclear H3S10P, GR and BRG1 distribution in response to acute social defeat.

Nuclear levels and distribution of H3S10P, GR and BRG1 were quantified 1 hour after a 5 minutes social defeat (acute condition) or the day after 10 repeated social defeats (chronic condition). Control mice were handled in parallel, without aggression. The

images were acquired using a confocal microscope. Quantification has been performed automatically on 3D stacks using Image J.

Primary striatal cultures

Striata were dissected out from E14 (BRG1^{D1cre} or control littermates), mouse embryos and striatal cultures were prepared as previously described²⁴, separately for each individual. Briefly, after trypsinisation of the tissue in trypsin (Gibco), EDTA solution for 15 min at room temperature (RT), cells were incubated for 5 min at RT in fetal calf serum complemented with and DNase I (Worthington), followed by mechanical dissociation using a P1000 Gilson pipette. Cells were then suspended in neurobasal medium supplemented with B27 (Invitrogen), 500 nM L-glutamine, 60 µg/ml penicillin G and 25 µM β-mercaptoethanol (Sigma Aldrich) and plated at a density of 1,000 cells per mm² into 8-µwell plates (Biovalley) coated with 50 µg/ml poly-D-lysine (Sigma Aldrich). Cultures were kept in at 37°C in a humidified atmosphere with 5% CO₂. Culture medium was changed at DIV6 and live calcium imaging performed the day after.

Calcium imaging

At DIV7, cultured neurons were loaded for 35 min at RT with a calcium probe Fluo-4 and pluronic F 127 (v/v; 50%; Invitrogen) diluted to 1/500 in a glutamate-free recording medium (129 mM NaCl, 4 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM Glucose, 10 mM HEPES). Neurons were washed three times in this recording medium before imaging. Acquisitions were performed at 36.2°C every second, prior to and during treatments with the indicated doses of (R)-(+)-SKF38393 (Sigma Aldrich) and/or L-glutamic acid (Calbiochem), with a spinning disk confocal microscope (Leica) using a 40X oil immersion objective. Fluorescence intensity was analyzed in the soma of all neurons present in a given field by using the ImageJ software. Viability of the neurons was tested after stimulation with a high dose of glutamate (10 µM), which yields high and sustained calcium responses (not shown), at the end of image acquisition. Data are presented as means ± s.e.m. of $\Delta F/F$, $([F_t - F_0])/F_0$ where F_t and F_0 are the fluorescence intensities after and before stimulation. Representative pictures of the $\Delta F/F$ signal at the pic of the responses were prepared with Matlab. Calcium imaging experiments

were independently performed on 5 littermates and 5 to 23 μ -wells were analyzed for each pharmacological treatment.

Statistical analyzes

Data are presented as means \pm s.e.m. Statistical analysis was carried out using either one-way or two-way ANOVA followed by Bonferroni's multiple comparisons. Unpaired Student's t-test were used for the nuclear shape quantification, fear-conditioning data, for the frequency of dopamine firing rates and for the calcium imaging analysis. Wilcoxon test was used for the spike in burst analysis.

Results

GR and BRG1 interact in NAc MSNs

BRG1/BRM containing SWI/SNF complexes interact with GR in various tissues and cell lines²⁵⁻²⁷. The three proteins are widely expressed, and we observed by co-immunostainings that BRG1 is co-expressed with GR in cells nuclei within most brain regions including within the striatum and NAc medium spiny neurons (MSNs; Figure 1a) and that BRM and BRG1 are also co-expressed. Co-immunoprecipitation from whole striata lysates further confirmed that in brain cells too GR physically interacts with BRG1/BRM containing complexes (Figure 1b). Indeed, GR is present within the immunoprecipitated proteins interacting with BRG1 (lane 3) or BRM (lane 4), using respective antibodies. This result shows that GR and BRG1 containing complex can interact in striatal extract, however it does not indicate the degree of interaction within striatal neurons. To further evaluate the proportion of GR possibly interacting with BRG1 containing complexes, we analyzed GR-BRG1 co-localization within NAc MSNs nuclei using confocal imaging. To assess whether co-localization was altered in response to stress exposure, we performed this analysis in control mice, and in challenged animals one hour after an acute social defeat or one day after social defeats repeated daily for 10 days. BRG1 and GR proteins had a puncta distribution within the nuclei, with foci containing BRG1 (green), GR (red), or both proteins (yellow; Figure 1c). GR foci numbers significantly increased 1 hour after a single defeat (from 247 ± 24 to 416 ± 42 foci per nucleus) and tended to remain high one day after chronic social defeat. On the other hand, BRG1 levels transiently increased after an acute defeat (from 80 ± 9 to 175 ± 27 BRG1 foci) and came back to unstressed levels after repeated defeats (85 ± 5). Only a small quantity of foci contained both GR and BRG1 (2.6 ± 0.5 foci per nucleus, in control animals, see arrow heads). However, the colocalization significantly increased 1 hour after an acute defeat (9.2 ± 0.7 foci per nucleus) and remained high in mice that went through repeated social defeats (7.9 ± 0.6 foci per nucleus) suggesting a potential involvement of GR/BRG1 interaction in responses to stress exposure (Figure 1d).

Characterization of mice lacking BRG1 in dopaminoceptive neurons or BRM

We previously showed that GR gene inactivation in dopaminoceptive neurons (GR^{D1Cre} mice) prevents the appearance of social aversion after chronic social defeats⁷. To assess the function of BRG1 and BRM in the behavioral adaptations to repeated social

defeat, we generated mice deprived of BRG1 in the same cell populations (BRG1^{D1Cre} mice). To address the role of BRM, we used constitutive BRM mutant mice (BRM^{-/-}). We assessed *in situ* the efficiency of genes inactivation in the striatum and the NAc of both models (Figure 2a). In control mice both BRG1 and BRM proteins are present within MSNs nuclei (Figure 2a, upper row). In BRG1^{D1Cre} mice, BRG1 is efficiently recombined from most MSNs (Fig 2a, middle row). Overall these mice show a similar profile of gene recombination to that of GR^{D1Cre} mice. In other words, *Brg1* gene is inactivated in most MSNs of the dorsal striatum and the NAc, as well as in pyramidal neurons of deep cortical layers (data not shown), a profile coherent with that expected when using the YAC-D1aCre transgenic line²¹. In BRM^{-/-} mice, the staining for BRM was totally absent from all cells (Figure 2a, lower row). We also generated compound mice, harboring the two mutations. As expected, most MSNs are deprived of both BRG1 and BRM (Figure S2a). SWI/SNF complexes control the proliferation rate and the differentiation process of different cell types¹⁴. We thus studied whether the absence of BRG1 from dopaminoceptive neurons or of BRM would have affected gross anatomy of the striata and cortice and observed no cytoarchitectural alteration on Nissl staining from BRG1^{D1Cre} (Figure 2b, middle row) or BRM^{-/-} mice (Figure 2b, lower row). We quantified the number of neurons within the dorsomedial striatum and the NAc using NeuN staining and found no difference between controls and BRG1^{D1Cre} or BRM^{-/-} mice (Figure 2c). The number of neurons was also quantified in older animals (24 months old) and here again, we did not observe any difference between control and BRG1^{D1Cre} mice (Figure S1b). Similarly, we did not observe any change in neurons number within the PFC (data not shown). This absence of effects might be due to the fact that in BRG1^{D1Cre} animals recombination occurs late in post-mitotic cells. Recent report has shown that BRG1 may control nuclear shape in non-tumorigenic epithelia cell line due to its impact on chromatin dynamics²⁸. Using nuclear envelope Lamin B staining and confocal imaging, we found that BRG1^{D1Cre} mice exhibited a significant increased percentage of abnormally shaped nuclei with an increased number of grooved (about 6% in controls and 11% BRG1^{D1Cre} mice) and multi-lobed nuclei (about 2% in controls and 10% in BRG1^{D1Cre} mice) compared to control mice (Figure 2d).

BRG1^{D1Cre} mice blocks behavioral impairment induced by repeated social defeat

To assess the behavioral response to stress-exposure in our models, we used the repeated social defeat paradigm, a widely used pre-clinical model of depression²⁹

(Figure 3a). Social defeat repeated for 10 days during encounters with stronger CD1 males induces long-lasting social aversion that can be measured in an open field in which a CD1 mouse never encountered before is placed within a perforated transparent box. In this test, control undefeated mice typically spend more time in the vicinity or interacting when a CD1 mouse is within the box than when the box is empty (Figure 3b, upper row). After social defeats, a subset of the defeated mice classically exhibits a significant social aversion which manifests by an increased time spent in the corners of the open field when a CD1 is within the box (Figure 3b, middle row). In absence of any mouse in the perforated box, both controls and BRG1^{D1Cre} mice, defeated and not, spent similar time investigating the empty box (Figure S3a). Control mice that underwent social defeats showed a significant decrease in social interaction compared to non-defeated animals (Figure S2a) which was obvious while looking at the social avoidance z-score that accounts for the time of interaction and the time spent in the corners of the open field (z-score -1.19 ± 0.16 in undefeated control mice; 0.11 ± 0.23 in defeated control mice; Figure 3c). Strikingly, mice deprived of BRG1 in dopaminergic cells kept interacting normally with the CD1 mouse despite the social defeats (Figure 3b, lower row, S2a). Indeed, BRG1^{D1Cre} mice did not exhibit any social avoidance after repeated defeats as shown by the avoidance z-score (-1.29 ± 0.13 in undefeated BRG1^{D1Cre} mice vs -1.07 ± 0.21 in defeated BRG1^{D1Cre} mice, Figure 3c). The same phenotype was observed in BRM^{-/-} mice, meaning that these mice also exhibited a normal social interaction despite the repeated social defeats (Figure S2b). Overall, while only 54% of control mice could be considered as resilient to social defeat (e.g. spending more time interacting with the other mouse than with the empty box), 96 % and 89 % of BRG1^{D1Cre} and BRM^{-/-} respectively were found to be resilient (Figure S2c). This result was confirmed in another social interaction task (the 3 chambers test) in which the experimental mice are put in presence of an unknown mouse from a different strain than the aggressors (C57BL/6 mice; Figure 3d). Undefeated mice (either controls or BRG1^{D1Cre}) showed a preference for interacting with a congener rather than an empty perforated box (50.1 ± 3.6 s with the object vs 69.4 ± 3.8 s with the mouse for controls, 52.0 ± 3.8 vs 71.0 ± 5.1 for the BRG1^{D1Cre} mice). Following repeated social defeat, control mice failed to show such preference (51.9 ± 6.3 s with the object vs 47.3 ± 6.8 s with the mouse) whereas BRG1^{D1Cre} mice still interacted with the unknown mouse (43.3 ± 5.5 s vs 77.0 ± 8.6 s; Figure 3d). Repeated social defeat classically induces an increase in anxiety-like behavior as observed in control mice in

both elevated O-maze and dark light box tests (Figure 3f left and right panel, respectively). Although they show a trend toward more anxiety under unstressed conditions, BRG1^{D1Cre} mice did not exhibit that increase after repeated social defeat (Figure 3f). BRM^{-/-} displayed a different phenotype compared to BRG1^{D1Cre} mice and had an increase of anxiety-like behaviors comparable to that of their control littermates after repeated social defeat in an elevated O-maze (Figure S2d). Concerning depression-like behaviors other than social aversion social defeat did not produce any change in sucrose preference and that parameter was identical in control and BRG1^{D1Cre} mice (Figure S2e). Forced-swim test did not reveal any change in despair-like behavior in controls and BRG1^{D1Cre} mice whether they were defeated or not (Figure 3e). We did not observe any difference for locomotor activity in any group of mice (Figure S2f). Finally, since acute and repeated social defeat is known to have an impact on the HPA axis activity, we measured the weight of adrenal glands and thymus as a proxy for average glucocorticoid production. As previously observed^{7, 29}, repeated social defeat induces a significant increase of adrenal gland weight and that increase was similar in both control and BRG1^{D1Cre} mice (2.97 ± 0.16 mg to 4.07 ± 0.06 mg in control, and 2.78 ± 0.26 mg to 4.07 ± 0.24 mg in BRG1^{D1Cre} mice; Figure S3a). No changes of thymus weight (Figure S3b) or body weight (not shown) were observed after repeated social defeat, and no differences were found between BRG1^{D1Cre} mice and their control littermates. Altogether, these data demonstrate that BRG1 in dopaminoceptive neurons is key for the long-term behavioral impairment induced by chronic stress-exposure. Of note, these results were not due to general impairment in fear learning. Indeed, in a fear conditioning paradigm (Figure S4a), controls and BRG1^{D1Cre} mice showed similar freezing percentage during the conditioning phase (Figure S4b) or in response to either a context (Figure S4c) or a cue previously associated with foot shocks (Figure S4d).

BRG1 in dopaminoceptive neurons modulates responses to cocaine

In humans, stress-exposure is a well-known risk factor for drug addiction and many studies in animal models have shown that behavioral responses to drugs are sensitive to stress exposure and glucocorticoids^{30, 31}. Since SWI/SNF complexes seem key for behavioral adaptation to social defeat, we wanted to assess whether it could possibly also modulate other stress-related behaviors, such as responses to drugs of abuse. We first examined the locomotor sensitization to repeated injections of cocaine. We

showed that the locomotor response to an acute injection of 10 mg/kg cocaine in saline pre-treated animals was identical between controls and BRG1^{D1Cre} mice (Figure 4a and 4c). Daily injection of 10mg/kg cocaine induced a significant locomotor sensitization in control mice since they showed an enhanced activity after a challenge injection of cocaine compared to saline pre-treated animals. However, the same treatment failed to induce any locomotor sensitization in BRG1^{D1Cre} mice which displayed the same response whether they received repeated treatment with cocaine or saline (Figure 4b and 4c). Although less dramatic, a decreased sensitization was also found in BRM^{-/-} mutant mice compared to their control littermates while mice with the two mutations showed an absence of locomotor response to either acute or chronic cocaine treatment (Figure S5a-c).

To confirm the participation of SWI/SNF complexes into behavioral responses to cocaine, we assessed cocaine rewarding effect in a conditioned-place preference paradigm. During pre-conditioning, mice showed no preference for any of the compartment of the place-preference box (Figure 4d). After pairing one compartment to cocaine and the other to saline, control mice developed a classical place-preference for the compartment associated with cocaine compared to the one associated with saline (score of -4 ± 41.4 s for saline-paired compartment and 355 ± 13.2 s for cocaine-paired compartment, Figure 4e). Although BRG1^{D1Cre} mice also showed a place preference for the compartment associated with cocaine injections, it was markedly lower than the one of control mice (score of 67.6 ± 33.2 s for saline-paired compartment vs 229.2 ± 18.7 s vs for cocaine-paired compartment, Figure 4e). Overall, these results show that SWI/SNF complexes in the dopamine reward pathway are required in the behavioral changes induced by drugs of abuse.

Dopaminergic firing in response to chronic social defeat

Repeated social defeat induces a long-lasting increase of dopamine neurons firing activity in the ventral tegmental area²⁹. We previously showed that this increase is likely to be causally linked with the appearance of social aversion and requires GR in dopaminergic neurons⁷. We thus examined whether the inactivation of BRG1 within dopaminergic neurons could also alter the change of dopamine neurons activity observed after social defeat. We thus measured dopamine neurons firing within the ventral tegmental area of control and BRG1^{D1Cre} mice in basal conditions or after chronic social defeat. In control mice, social defeat induced a significant increase of

dopamine neurons firing rate (from 3.5 ± 0.21 Hz in unstressed animals to 4.3 ± 0.28 Hz after social defeat) and a significant increase of burst activity ($13.8 \pm 2.8\%$ of spike in burst in unstressed vs $27.0 \pm 4.2\%$ in stressed animals; Figure 5a). Surprisingly, a similar basal activity and the same increase of dopamine neurons firing was observed in BRG1^{D1Cre} mice with a firing rate going from 3.2 ± 0.18 Hz to 4.8 ± 0.31 Hz after and a percentage of spike in burst going from 12 % to 28 % after social defeat (Figure 5b). Basal firing of dopamine neurons was unchanged in both BRG1^{D1Cre} and BRM^{-/-} mice compared to respective control animals (Figure S6a and b). However, compound mutant mice deprived of both BRG1 and BRM in dopaminergic neurons exhibited a marked reduction of dopamine neurons firing rate and burst activity compared to controls similar to that observed in GR mutant animals (Figure S6c)⁸. These results suggest that the behavioral phenotypes observed in absence of BRG1 within dopaminergic neurons are unlikely due to changes of dopamine neurons activity. It is noteworthy however that compensatory between BRM and BRG1 may be in play for the regulation of dopamine cells firing.

BRG1 in dopaminergic neurons is essential for transcriptional responses to repeated social defeat and cocaine treatment

Since dopamine neurons activity remained unaltered in BRG1^{D1Cre} mice, and could not therefore account for behavioral changes, we investigated for differences at the post-synaptic level, within the MSNs. Stress exposure, as cocaine injections, activate the Extracellular Signal-Regulated Kinase (ERK) pathway, leading to the induction of immediate early genes expression, such as *c-Fos* and *Egr1*. We tested whether immediate early genes induction by social defeat could be altered in absence of BRG1. In control mice, an acute social defeat strongly enhanced the expression of both *c-Fos* and *Egr1* within the dorsomedial striatum, the NAc core and the NAc shell. The induction of *c-Fos* gene expression, was significantly reduced within the dorsomedial striatum and the NAc shell in BRG1^{D1Cre} mice (Figure 6a), and no significant induction of *Egr1* gene expression was observed in BRG1^{D1Cre} mice for any structure tested (striatum, NAc core and shell, Figure 6b). The expression of *c-Fos* gene was also induced by an acute injection of cocaine in control mice whereas in BRG1^{D1Cre} mice, this induction was largely abolished although an increased *c-Fos* gene expression was overall observed in response to a saline injection (Figure S7). These decreases of immediate early genes expression are unlikely due to an ineffective ERK pathway

activation. Indeed, an acute social defeat was able to induce similar levels of ERK1/2 phosphorylation in the NAc of both controls and BRG1^{D1Cre} mice (Figure 6c). Calcium responses play a central role in MSNs neurons response to glutamate and dopamine and in the MAP kinase pathway induction. We assessed in primary striatal cultures, calcium responses to glutamate stimulations and to glutamate and dopamine D1 receptors agonist co-stimulations as an *in vitro* model of MSNs activation by cocaine²³. Calcium signal were increased with increasing doses of glutamate application in a similar way in both controls and BRG1^{D1Cre} mice (Figure S8a-e). In addition, the integration of dopamine D1 receptors and glutamate signals were also identical in controls and in BRG1^{D1Cre} as shown by calcium imaging after glutamate and SKF co-stimulations (Figure S8f-g).

These results suggest that the absence of BRG1 dampen the induction of immediate early genes by either social defeat or cocaine administration while signal integration in MSNs is unimpaired. To have a more global view of transcriptional defects in BRG1^{D1Cre} mice, we assessed for the induction of H3 serine 10 phosphorylation levels after an acute social defeat. In control mice, acute social defeat triggered a significant increase of H3S10P positive foci within NAc MSNs nuclei revealing a global activation of transcription by stress-exposure. Strikingly, this increase was abolished in absence of BRG1, suggesting a general lack of transcriptional response (Figure 7a). Using DAPI staining, we also assessed the level of heterochromatin within controls and BRG1^{D1Cre} mice MSNs nuclei which reflects the amount of transcriptionally silent regions. While social defeat did not have any impact per se on heterochromatin levels, we observed a general increase of heterochromatin surface in BRG1^{D1Cre} mice (Figure 7b). Altogether, these data indicate that BRG1 is important to unlock transcription and enable the cells to respond transcriptionally to environmental challenges.

Discussion

In this article, we investigated the role of SWI/SNF chromatin remodelers in the behavioral and transcriptional responses to stress exposure using mouse models with a genetic inactivation of both of the central ATPase subunits of these complexes, BRG1 and BRM. We showed that the selective ablation of *Brg1* gene within dopaminoceptive neurons and the constitutive deletion of *Brm* gene blocked the social aversion induced by repeated social defeat. SWI/SNF complexes are essential for behavioral responses to cocaine, as we showed in both locomotor sensitization and conditioned place preference. Importantly, these behavioral changes were not due to general impaired memory. BRG1^{D1Cre} mice showed indeed normal contextual and cued fear memory. They were not involving changes of dopamine neurons activity within the ventral tegmental area but might likely be due to impaired transcriptional responses at the post-synaptic level. In absence of BRG1 from dopaminoceptive neurons, animals display decreased stress-induced expression of immediate early genes *c-Fos* and *Egr1* within the striatum and the NAc, despite a normal activation of MSNs ERK signaling pathway and a preserved glutamate-mediated calcium responses and integration of D1 receptor-glutamate signals. At a global level, in absence of BRG1, we observed a blunted induction of Serine 10 phosphorylation of H3 histones after social defeat and a global increase of heterochromatin surface within NAc MSNs reflecting, most probably, a general lack of transcriptional activation. These data highlight the importance of SWI/SNF chromatin remodeler complexes in the transcriptional and behavioral plasticities in response to environmental challenges.

Repeated social defeat is a widely used preclinical model for depression. It has been repeatedly shown to induce anxiety, social avoidance and anhedonia and antidepressant treatment has been shown to normalize some of these behavioral impairments²⁹. Given the long-term nature of the behavioral changes triggered by stress exposure, many studies focused on long-term changes in gene expression mechanisms, such as epigenetic ones as potential molecular underpinnings for stress-induced maladaptive behaviors and depression³². Chronic social defeat increases the expression of the DNA methyltransferase 3a in the NAc and its overexpression enhances depression-like behavior while local inhibition with an inhibitor has the opposite effect⁵. Early stress in both rat and mice have also been shown to alter DNA methylation in specific genes promoters such as the one coding for the arginine

vasopressine in the paraventricular nucleus, the GR and the BDNF in the hippocampus and PFC^{33, 34}. Of note changes of methylation have been reported in post-mortem brain samples from suicide completers including subjects with severe child abuse^{35, 36}. Repeated social defeat also leads to changes of histone acetylation levels in several brain regions including the NAc and local injections of histone deacetylase (HDAC) inhibitors within the NAc or the PFC, ultimately leading to increased acetylation, reverse the social avoidance triggered by repeated defeats^{4, 37}. In general, systemic inhibition of histone deacetylation produces antidepressive-like effects comparable to that of regular antidepressant such as fluoxetine³⁸. On the opposite, pharmacological activation of HDACs enhances the anxiety and depressive-like effects of early life stress³⁹. Histone methylation has also been shown to be involved in long-term behavioral changes induced by stress and drug-exposure. Both repeated social defeat and cocaine have been shown to downregulate the expression of G9a methyltransferases in the NAc which catalyzes Lysine 9 histone3 dimethylation (H3K9me2), a major transcription repression mark. G9a overexpression within the NAc not only made mice resilient to repeated social defeat but also counteracted the additive effect of cocaine exposure potentially through an inhibition of the ERK signaling pathway and of CREB activation⁴.

Epigenetic modifications participate to the elaboration of a transcriptional response to environmental challenges and stress exposure may under certain circumstances push that response toward a pathological path. However, chromatin by itself shows limited mobility and needs the action of chromatin remodeling complexes which can specifically recognize histone modifications and through ATP hydrolysis unwrap, mobilize, exchange or eject the nucleosome, to subsequently recruit the transcriptional apparatus to nucleosomal DNA⁴⁰. So far, only few studies investigated the role of chromatin remodeling complexes in the pathophysiology of psychiatric disorders. In that regard, it is of note that recent human exome sequencing and genome-wide association studies have linked mutations in subunits of the SWI/SNF complex including BRM and BRG1 to neurodevelopmental disorders such as Coffin-Siris syndrome⁴¹⁻⁴³, Nicolaides-Baraitser syndrome⁴⁴, autism spectrum disorder⁴⁵, and schizophrenia⁴⁶. In addition, meta-analyses of GWAS studies pointed at BRG1 as one of the central player. At the preclinical level, the expression of BRG1 and complex formation with SMAD3 within the NAc has been shown to increase in rats following

withdrawal from cocaine self-administration. Intra-accumbal pharmacological inhibition of BRG1 attenuated cocaine-relapse while virally-mediated NAc overexpression enhanced this behavior¹⁷. In addition, the NAc inactivation of BAZ1B, a subunit of ACF complexes also decreases cocaine self-administration¹⁶. Our results are complementary since the inactivation of BRG1 in dopaminergic neurons including the NAc decreases the locomotor sensitization and the conditioned place preference to cocaine. Also, *Brm* gene inactivation in mice have been previously reported to have decreased pre-pulse inhibition and social interaction⁴⁶. This last finding is not consistent with what we found since BRM mutant mice showed a similar social behavior than their control littermates in an open field and further showed enhanced social interaction after social defeat compared to defeated controls.

Another recent study has shown that mice susceptible to repeated defeat but also patients with depression display, within the NAc, an upregulation of ACF, a subunit of ISWI, another chromatin remodeler complex. Furthermore, the overexpression of ACF (BAZ1A plus SMARCA5) within the NAc made mice more sensitive to social defeat whereas knocking it down made them more resilient. Increased ACF was associated with altered nucleosome positioning and a repressed transcription of key genes involved in the sensitivity to social defeat¹⁵. Our findings are similar in that the inactivation of BRG1 within dopaminergic neurons or of BRM, the other ATPase subunit of the SWI/SNF complex, made mice resilient to repeated social defeat. However, while ACF seems to have a repressive impact on gene transcription, BRG1 has been generally associated with transcriptional activation among others through its interaction with nuclear receptors^{27, 47}. Here we show that BRG1 gene inactivation prevents immediate early genes induction while leaving the MAPK signal transduction intact. In other words, SWI/SNF complex is needed to induce immediate early genes *c-Fos* and *Egr1*.

In primary cortical cell culture, BRG1 through its interaction with CREST (Calcium Responsive Transactivator), a neuron specific member of SWI/SNF complexes has been shown to be key for CBP (CREB binding protein) recruitment and ensuing *c-Fos* gene transcription upon calcium influx⁴⁸. This same mechanism could be at play within the striatum and the NAc explaining the decreased *c-Fos* gene induction in response to social defeat or cocaine in absence of BRG1. At rest, BRG1-CREST complex rather

inhibits *c-Fos* transcription by the recruitment of a phospho-Retinoblastoma-HDAC repressor complex⁴⁸. This could explain the trend toward higher levels of *c-Fos* in saline injected BRG1^{D1Cre} mice compared to controls. Of note, BRG1 and CREST also binds to the *Arc* and *Egr1* promoters which could also explain the lack of *Egr1* induction in our study. The lack of *c-Fos* and *Egr1* gene induction could partly explain the behavioral phenotype of BRG1^{D1Cre} mice. Indeed, the induction of both factors have been shown to be crucial for behavioral responses to cocaine⁴⁹⁻⁵⁰, although *Arc* has been recently shown to have the opposite effect⁵¹. Also, *c-Fos* gene expression is under the control of CREB whose inactivation in the NAc has been shown to reduce social avoidance after social defeat⁴. On the other side, *Egr1* is under the control of Elk1 activation and the systemic injection of a blood-brain-barrier permeable peptide that inhibits ERK-mediated activation of Elk1 has been recently shown to induce resilience to social defeat in mice⁵². Finally, *Egr3* has been shown to be induced in mice susceptible to repeated defeat and reduced *Egr3* mRNAs levels in NAc D1-expressing MSNs prevented the appearance of depressive-like behaviors along with the stress-induced dendritic atrophy and the altered activity of this neuronal population⁵³. *Egr3* mRNAs reduction also lowered cocaine sensitization and conditioned place preference similar to what we observed here in BRG1^{D1Cre} mice⁵⁴. Interestingly, these effects of *Egr3* knock-down are selective for the D1-expressing MSNs since D2-expressive MSN knockdown of *Egr3* have an opposite effect⁵⁴. In our mice, the Cre expression is larger than the canonical D1-expressing MSN population⁷. Thus, inactivation of BRG1 restricted to D1- and D2-expressing MSNs population may also yield opposite effects.

Also beyond the scope of the present study, it would be of interest to assess the impact of BRG1 inactivation on neuronal morphology and activity in different brain areas and cell types including the D1- and D2-expressing MSNs. Indeed, BRG1-associated factor BAF53b has been shown to be key for dendritic growth in culture of hippocampal neurons⁵⁵. In addition, the same factor has been shown to be key for hippocampal-dependent memory and synaptic plasticity^{56, 57} and knock down of that subunit in the lateral amygdala has been shown to impair fear memory⁵⁸.

SWI/SNF complex containing either BRM or BRG1 are essential interaction partners of nuclear receptors, among which the GR. The interaction between GR and BRM or BRG1 is not direct but relies on connecting BAF proteins including BAF250, BAF60a,

BAF57, and BAF53a²⁵⁻²⁷. In cell culture, both BRM and BRG1 potentiate GR transcriptional activity^{13, 59-61} and studies at a genome scale have shown that in the fibroblastic 3T3 cell line, around 40% of GR-stimulated genes and 11% of GR-repressed genes require the function of BRG1-containing SWI/SNF complexes⁴⁷. In the present study we observed only limited nuclear co-localization between BRG1 and GR foci in the nuclei of striatal and NAc MSNs. However, the degree of co-localization significantly increased after a single defeat and remained enhanced one day after repeated defeats. The interaction between GR and BRM could not be examined due to the lack of adequate antibodies. However, a role for the interaction between GR and BRG1/BRM in the modulation of stress-related behaviors is suggested by the very similar behavioral phenotypes we reported here and the ones previously observed in GR^{D1Cre} mice^{7, 9}. These results suggest that the development of depressive-like behaviors after social defeat and the sensitivity to drugs of abuse may involve an interaction of GR with BRG1 containing SWI/SNF complexes. However, some data in the present study do not match what was previously shown in GR^{D1Cre} mice. Indeed, the inactivation of GR gene in dopaminergic neurons leads to a decrease of dopamine neurons spontaneous firing under basal conditions, as well as after repeated social defeats. In contrast, the inactivation of *Brg1* and *Brm* genes in the same cell population does not. Several possibilities could explain this difference. GR and BRG1 have functions that are independent from each other, and the control of presynaptic dopamine could require a GR-dependent mechanism that do not involve BRG1 or BRM. This could be at the level of gene expression control but could also involve non transcriptional functions of GR such as interferences with signaling cascades or with synaptic activity via plasma membrane-bound GR^{62, 63}. An alternate possibility is that BRM and BRG1 may compensate for each other gene inactivation. This would be in line with our observation that double gene inactivation leads to a decrease of dopamine firing rate and number of spikes in bursts in BRG1^{D1Cre}BRM^{-/-} mice. More work will be needed in order to clearly know which genes and which functions are under the control of GR-SWI/SNF complexes. The present results along with previous work on GR mutants bring one more piece to the understanding of the molecular mechanisms underlying stress-induced maladaptive behavior and could be relevant for the pathophysiology of depression and drug addiction.

Conflict of interest

The authors declare no conflict of interest.

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Contributions

AZ and SP performed experiments and the analysis. CB performed the cocaine sensitization and condition place preference under the supervision of JB. RDdC and FM performed and analyzed the dopamine neurons recordings under the supervision of PF. ESJ performed and analyzed the calcium imaging in primary neuronal culture under the supervision of PV. SK performed the co-immunoprecipitations. SP and FT conceived the experiments and wrote the manuscript.

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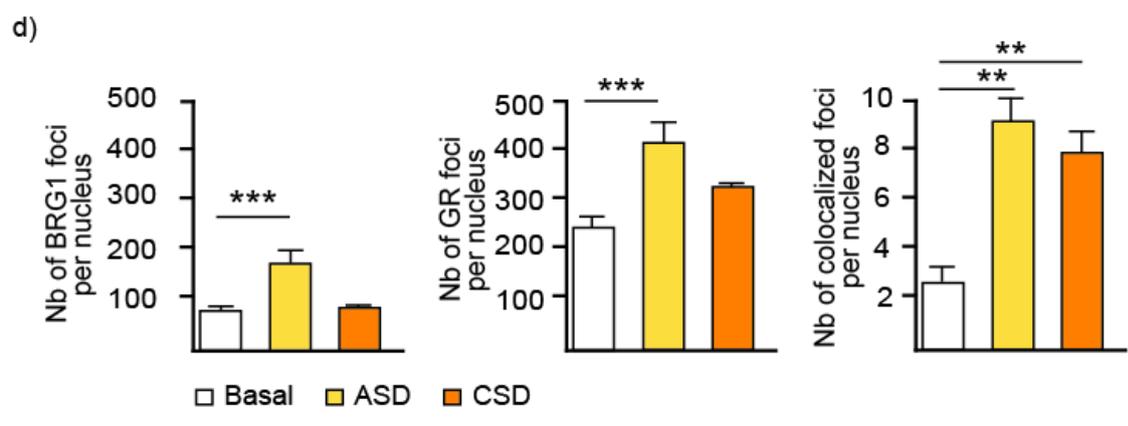
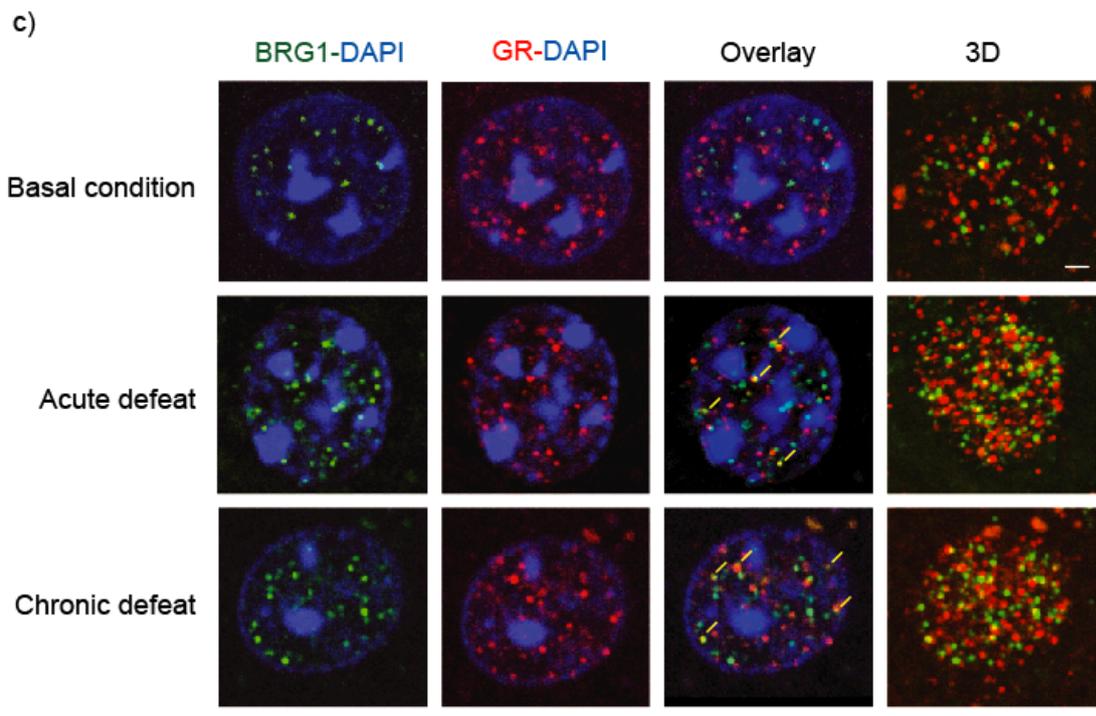
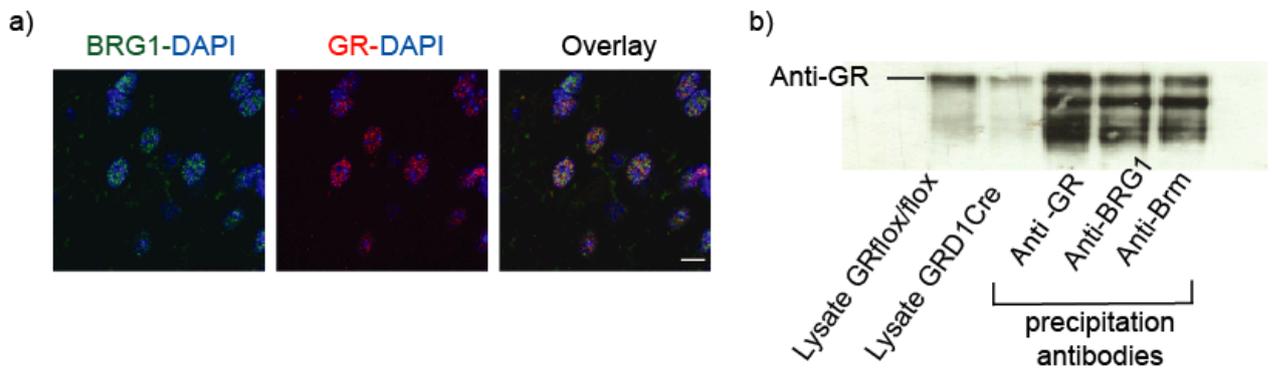


Figure 1: Colocalization of BRG1 and GR within the nucleus accumbens under basal conditions or after stress exposure. (a) Colocalization of BRG1 (in green) and GR (in red) in medium spiny neurons nuclei within the nucleus accumbens (NAc). Scale bar = 10 μ m **(b)** Co-immunoprecipitation of GR, BRG1 and BRM in whole striatum extracts. Lane 1 and 2 correspond to GR detection in whole striatum lysates from GR^{loxP/loxP} control mice and GR^{D1Cre} mice respectively. Lane 3, 4 and 5 correspond to GR detection after immunoprecipitation with GR, BRG1 and BRM antibodies, respectively. **(c)** High magnification of BRG1 (green) and GR (red) colocalization in nucleus accumbens medium spiny neurons in mice undefeated, 1 hour after social defeat or 24 hours after repeated (10 days) social defeat. Each picture presents one nucleus. The three first columns correspond to one stack for BRG1-DAPI, GR-DAPI and overlay stainings with the yellow bars pointing at foci in which BRG1 and GR colocalize. The last column corresponds to the nuclei 3D images. Scale bar = 2 μ m **(d)** Quantification of number of BRG1 (left chart), of GR and of BRG1 and GR colocalization foci in nucleus accumbens medium spiny neurons nuclei in mice undefeated, 1 hour after social defeat or 24 hours after repeated (10 days) social defeat. *p<0.05, **p<0.01, ***p<0.001. n=10 nuclei in 4 mice per condition. Data are represented as mean \pm s.e.m.

Figure 2

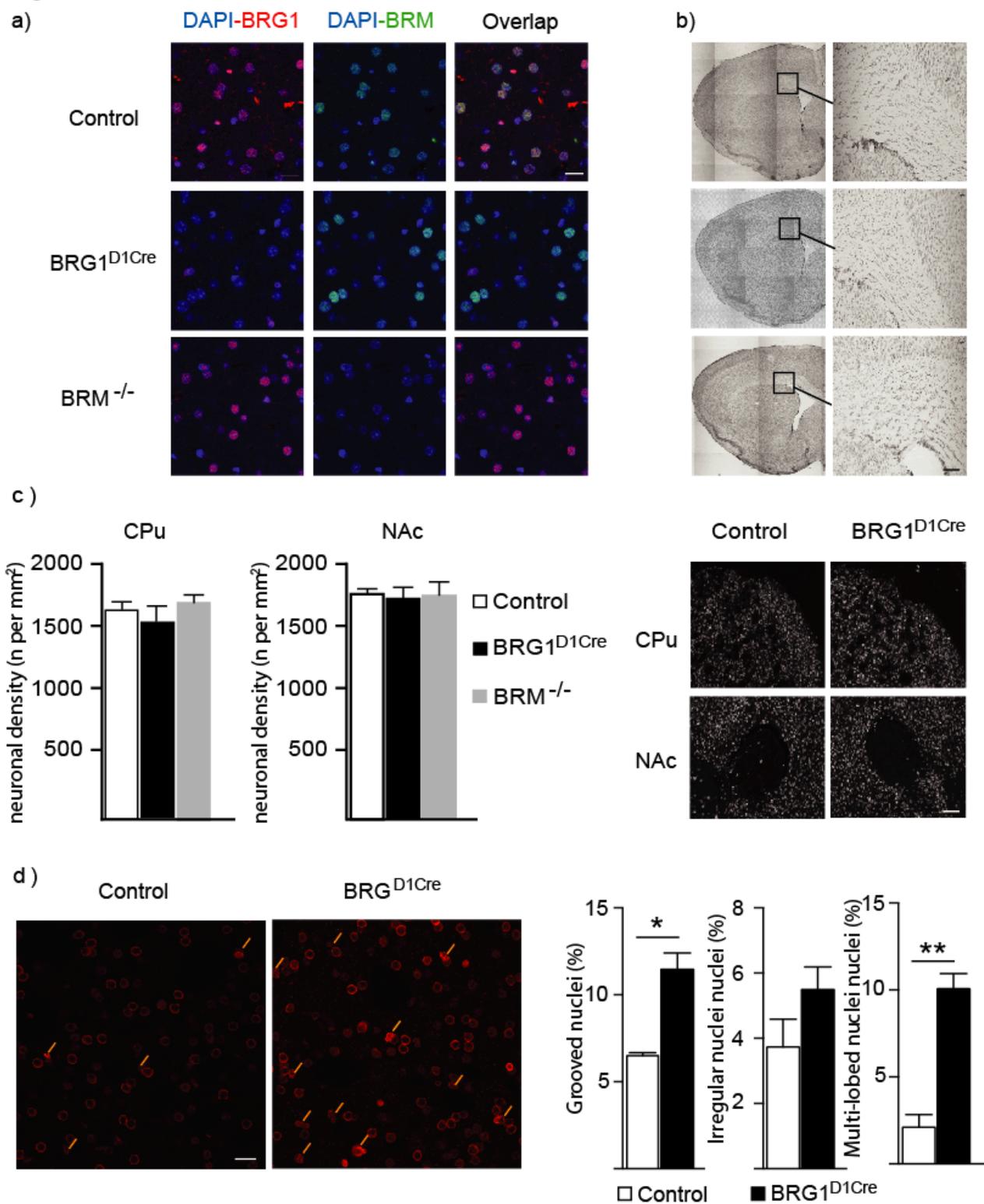
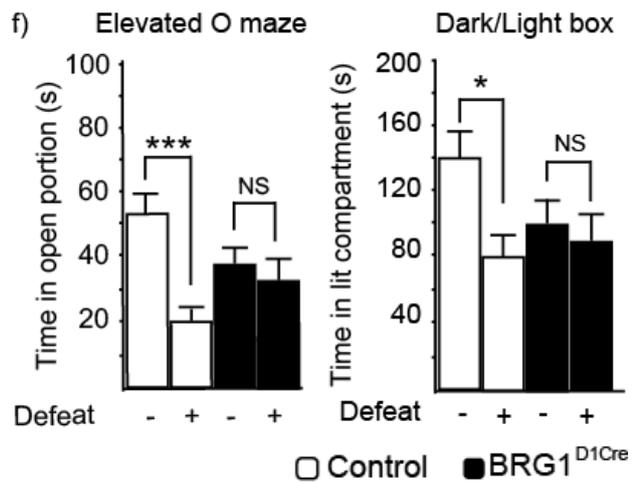
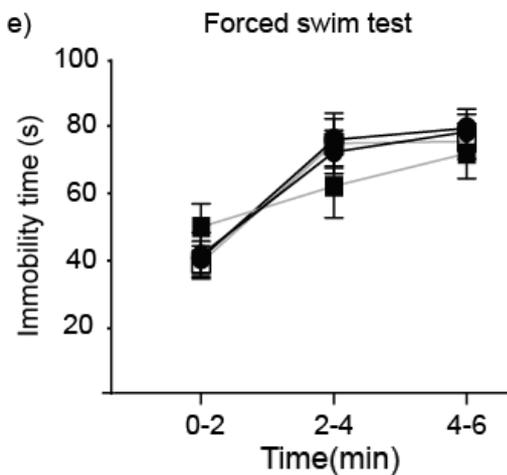
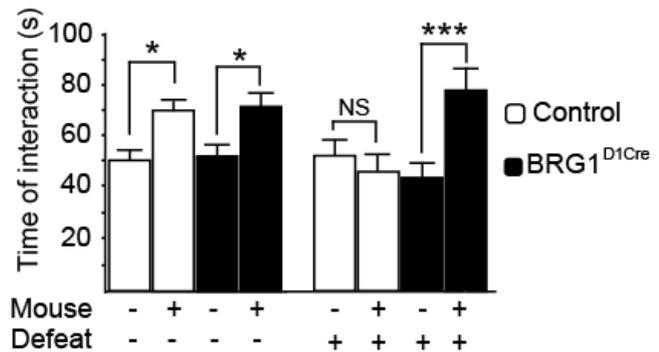
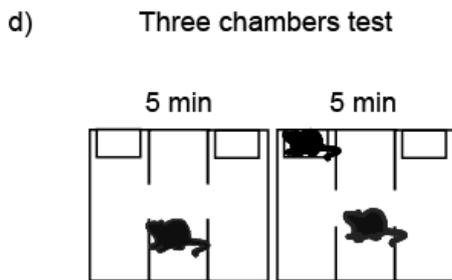
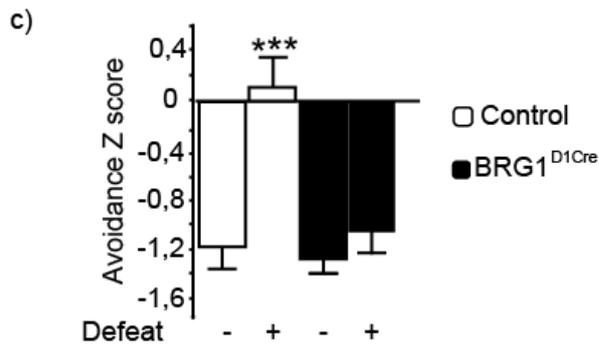
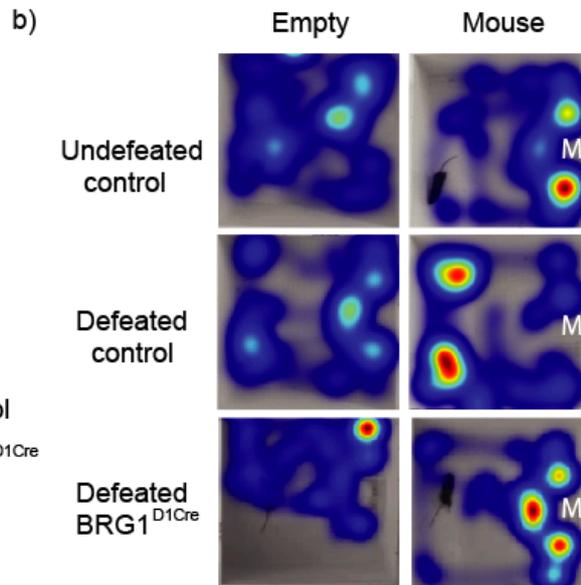
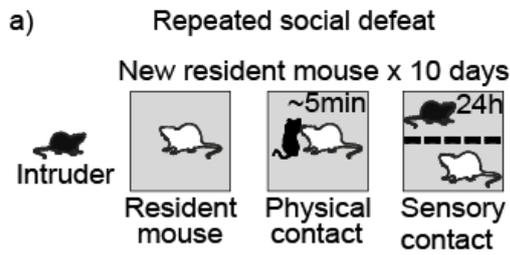


Figure 2: Genetic inactivation of BRG1 in dopaminoceptive neurons in BRG1^{D1Cre} mice and of BRM in the whole organism in BRM^{-/-} mice. (a) BRG1 (red) and BRM (green) co-staining within the nucleus accumbens of control (upper row), BRG1^{D1Cre} (middle row), and BRM^{-/-} (lower row) mice. Scale bar = 25 μ m **(b)** Nissl staining at 4X and 10X in control (upper row), BRG1^{D1Cre} (middle row), and BRM^{-/-} (lower row) mice. Scale bar = 70 μ m **(c)** Neuronal density using NeuN staining in the dorsomedial striatum and nucleus accumbens of control (n=3), BRG1^{D1Cre} (n=3) and BRM^{-/-} mice (n=3). Scale bar = 70 μ m **(d)** Nuclear abnormalities (pointed by yellow bars) in the nucleus accumbens of control and BRG1^{D1Cre} mice using Lamin B staining with percentage of grooved, irregular and multilobed nuclei (n=3/3 mice). Scale bar = 25 μ m. Unpaired t-test *p<0.05, **p<0.01. Data are represented as mean \pm s.e.m.



□ Control undefeated ○ BRG1^{D1Cre} undefeated
 ■ Control defeated ● BRG1^{D1Cre} defeated

Figure 3: Behavioral effects of repeated social defeat in control and BRG1^{D1Cre} mice. (a) Schematic view of the repeated social defeat paradigm. (b) Representative hitmaps (red color means increased spent time) of social interaction in undefeated controls (upper row), defeated controls (middle row) and defeated BRG1^{D1Cre} (lower row). White M: mouse (c) Avoidance Z score in undefeated and defeated controls and BRG1^{D1Cre} mice. Undefeated control n=25, defeated control n=24, undefeated BRG1^{D1Cre} n=25, defeated BRG1^{D1Cre} n=22. (d) Left panel: schematic view of the three chambers interaction test. Right panel: social interaction in the three chambers test. (e) Immobility time in a forced swim test in control undefeated (n=12), defeated control (n=12), undefeated BRG1^{D1Cre} (n=13) and defeated BRG1^{D1Cre} (n=13). (f) Anxiety levels in the elevated O maze (left chart) and in the dark-light box test (right chart). Undefeated control n=25, defeated control n=24, undefeated BRG1^{D1Cre} n=25, defeated BRG1^{D1Cre} n=22. NS: not significant, *p<0.05, ***p<0.001. Data are represented as mean ± s.e.m.

Figure 4

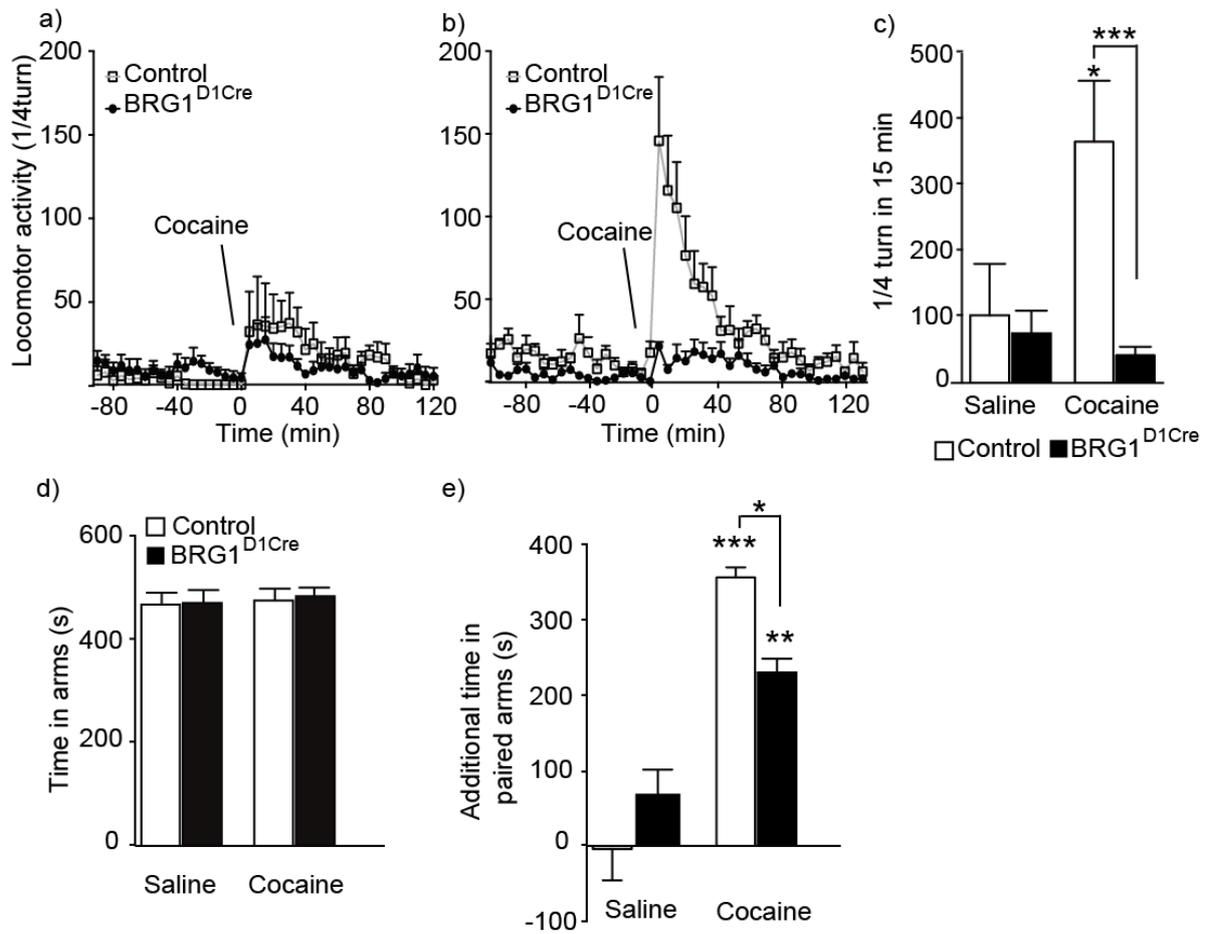


Figure 4: Behavioral responses to cocaine in control and BRG1^{D1Cre} mice. (a) Locomotor response (by 5 min blocks) to a cocaine injection (10mg/kg) in saline pre-treated controls (n=4) and BRG1^{D1Cre} mice (n=7). **(b)** Locomotor response (by 5 min blocks) to a cocaine injection (10 mg/kg) in cocaine pre-treated control (n=6) and BRG1^{D1Cre} mice (n=8). **(c)** Locomotor sensitization (cumulated activity during 15 min after injection) to cocaine in controls and BRG1^{D1Cre} mice. Saline pre-treated controls n=4, saline pre-treated BRG1^{D1Cre} n=7, cocaine pre-treated controls n=6, cocaine pre-treated BRG1^{D1Cre} mice n=8. **(d)** Time spent in each compartment of the conditioned place preference setup (future saline and cocaine-paired compartments) during the preconditioning phase. Control n=18, BRG1^{D1Cre} n=17 **(e)** Additional time spent in the saline- and cocaine-paired compartment after conditioning in controls and BRG1^{D1Cre} mice. Saline- and cocaine-conditioned controls n=9 for each, saline- and cocaine-conditioned BRG1^{D1Cre} mice n=8 and n=9 respectively. *p<0.05, **p<0.01, ***p<0.001. Data are represented as mean ± s.e.m.

Figure 5

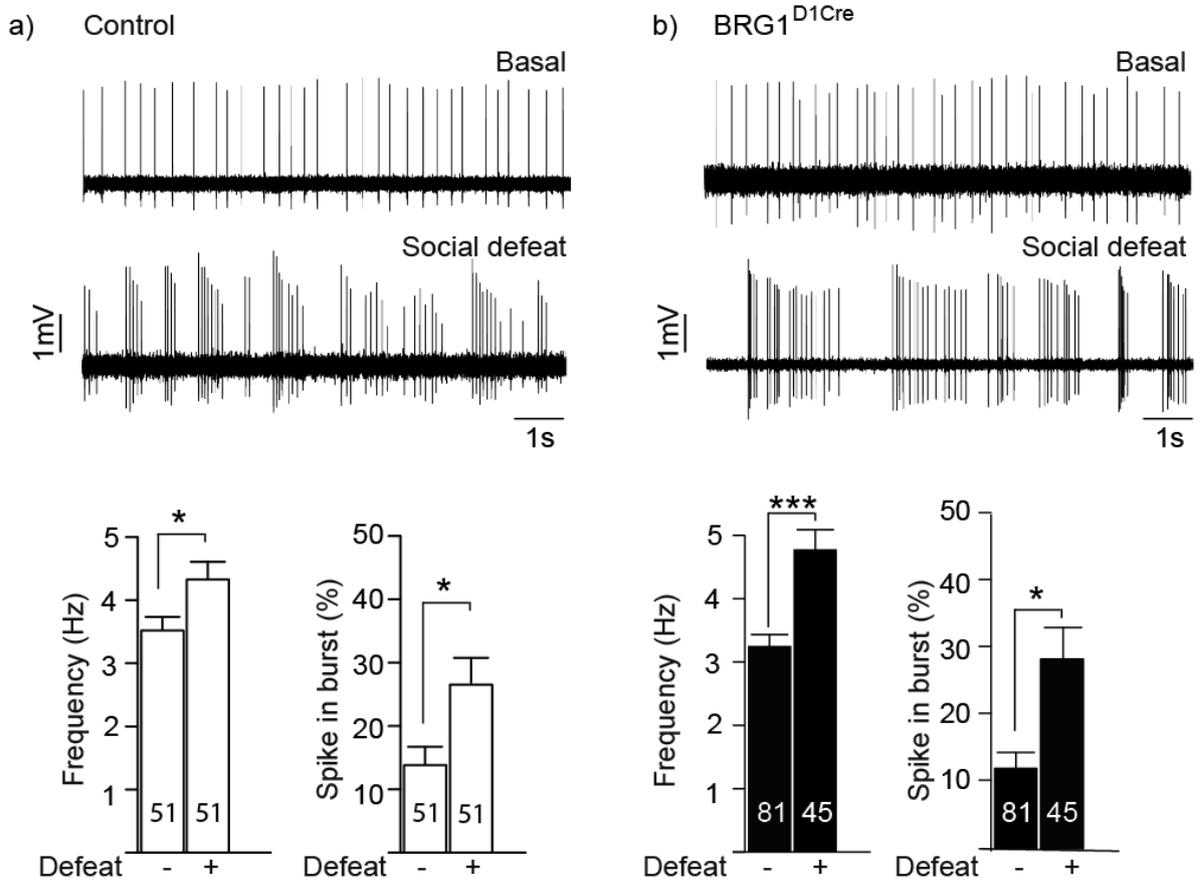


Figure 5: Consequences of repeated social defeat on ventral tegmental area dopamine neurons spontaneous activity in control and BRG1^{D1Cre} mice. (a) Representative traces of dopamine neurons activity in undefeated (upper trace) and defeated (lower trace) control mice. Firing frequency (left chart) and percentage of spike in burst (right chart) in undefeated (n=51 neurons) and defeated (and n=51 neurons) control mice. T-test (for frequency) and Wilcoxon test (spike in burst) *p<0.05. **(b)** Representative traces of dopamine neurons activity in undefeated (upper trace) and defeated (lower trace) BRG1^{D1Cre} mice. Firing frequency (left chart) and percentage of spike in burst (right chart) in undefeated (n=81 neurons) and defeated (n=45 neurons) BRG1^{D1Cre} mice. T-test (for frequency) and Wilcoxon test (spike in burst) *p<0.05, ***p<0.001. Data are represented as mean ± s.e.m.

Figure 6

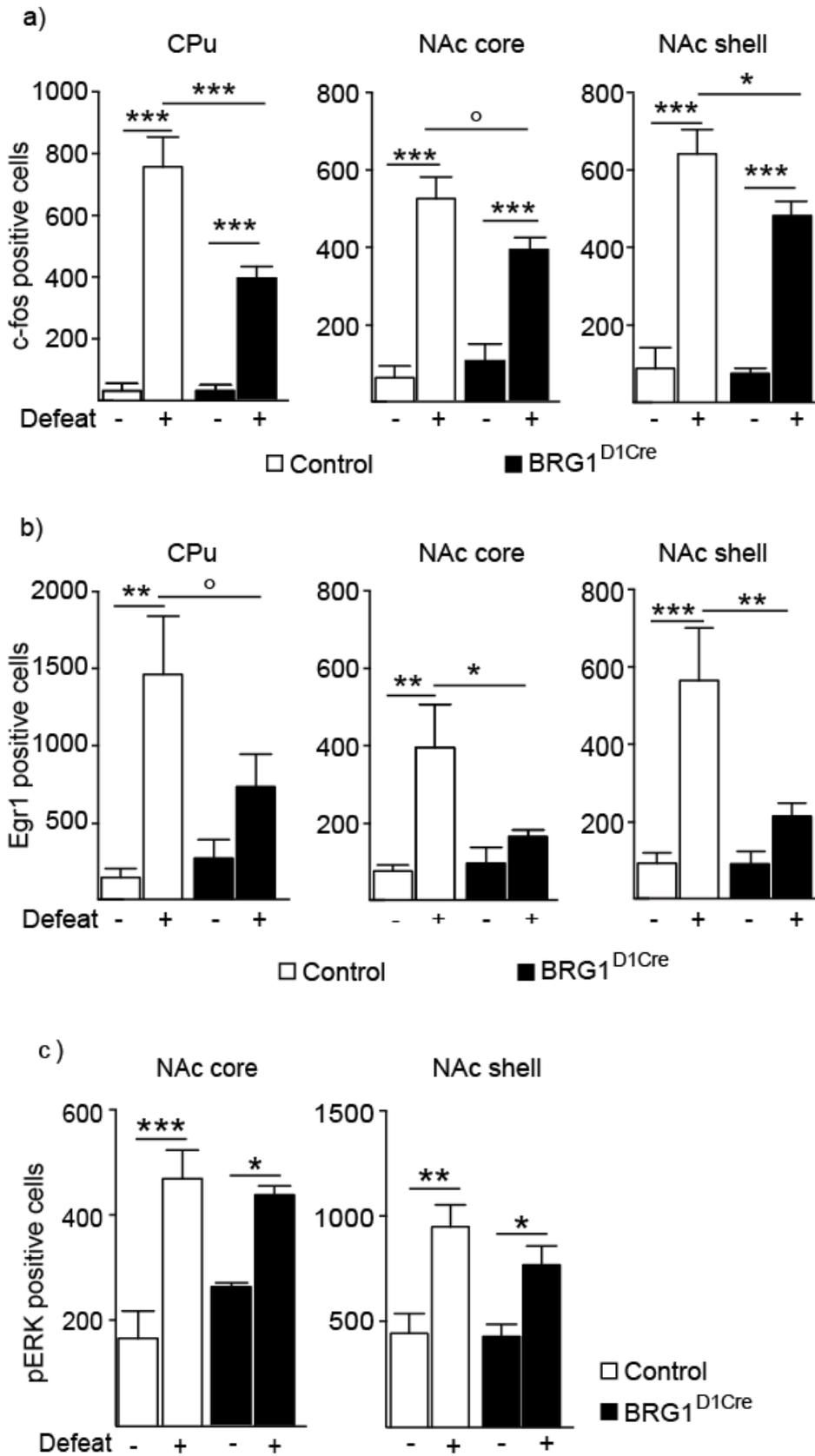
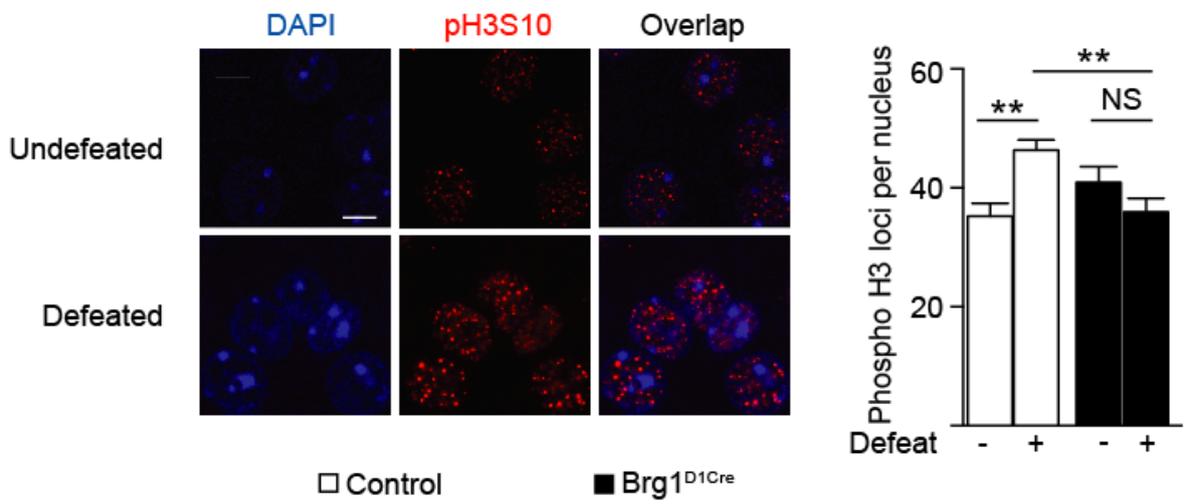


Figure 6: Immediate early genes induction and ERK pathway activation in response to a single social defeat in control and BRG1^{D1Cre} mice. (a) c-Fos gene induction by social defeat in control and BRG1^{D1Cre} mice in the dorsomedial striatum (CPu, left chart), nucleus accumbens core (middle chart) and shell (right chart). Undefeated control n=6, defeated control n=6, undefeated BRG1^{D1Cre} n=6, defeated BRG1^{D1Cre} n=6. **(b)** *Egr1* gene induction by social defeat in controls and BRG1^{D1Cre} mice in the dorsomedial striatum (CPu, left chart), nucleus accumbens core (middle chart) and shell (right chart). Undefeated control n=6, defeated control n=6, undefeated BRG1^{D1Cre} n=6, defeated BRG1^{D1Cre} n=6. **(c)** ERK phosphorylation after social defeat in controls and BRG1^{D1Cre} mice in the dorsomedial striatum (CPu, left chart), nucleus accumbens core (middle chart) and shell (right chart). Undefeated control n=5, defeated control n=5, undefeated BRG1^{D1Cre} n=4, defeated BRG1^{D1Cre} n=5. °p<0.1, *p<0.05, **p<0.01, ***p<0.001. Data are represented as mean ± s.e.m.

Figure 7

a)



b)

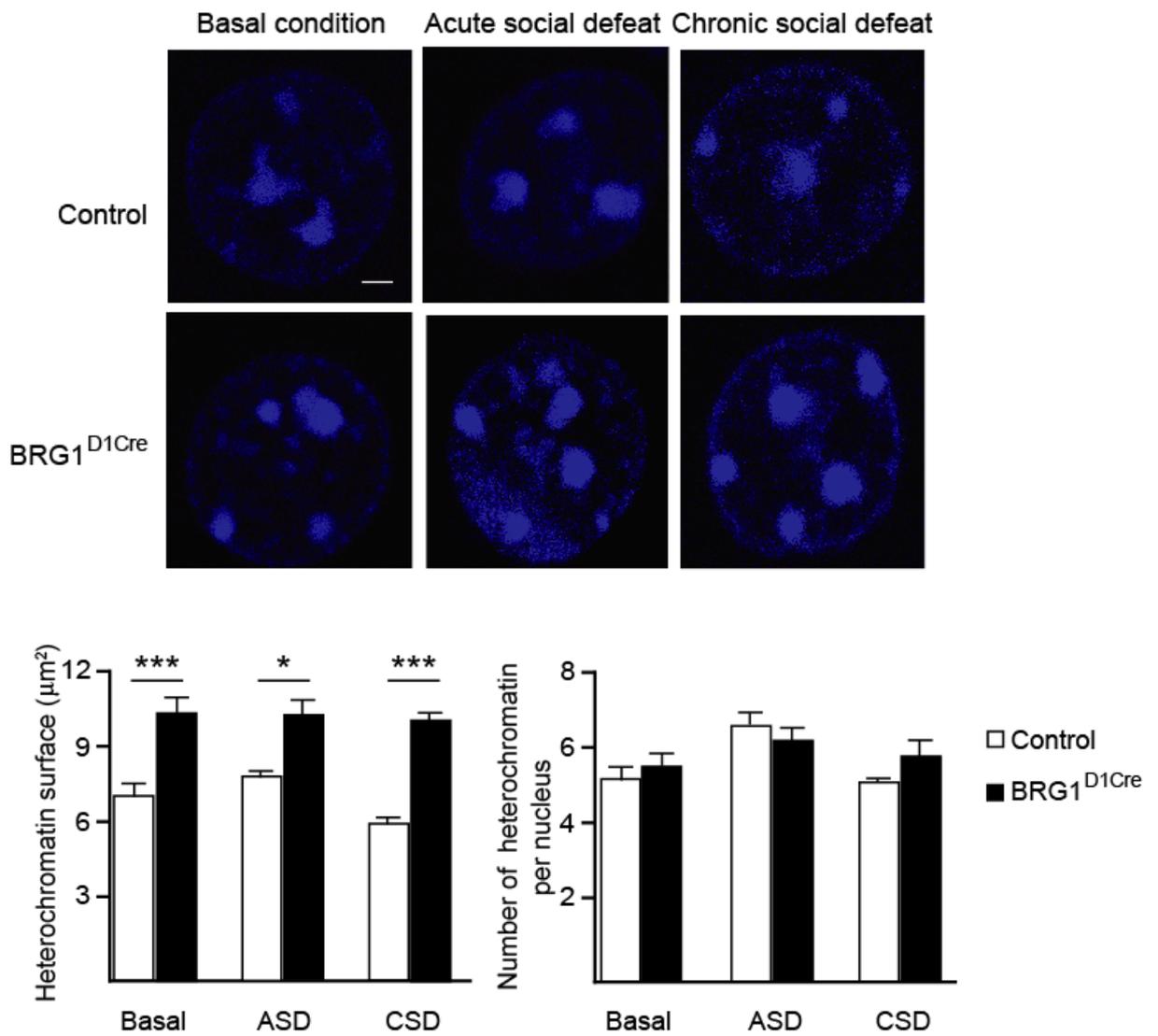
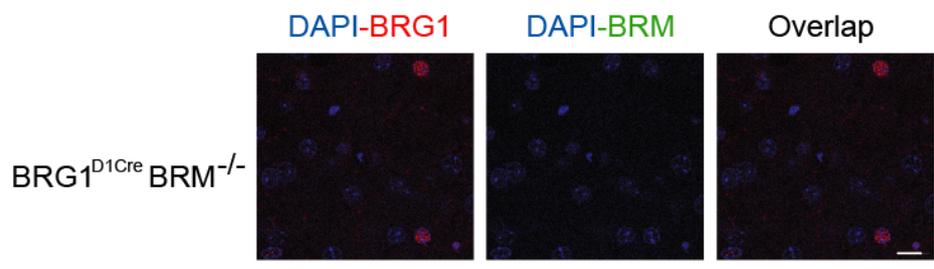


Figure 7: Induction of serine10 phosphorylated form of histone H3 by acute social defeat and heterochromatin size in control and BRG1^{D1Cre} mice. (a) Representative H3S10P stainings (red) in the nucleus accumbens of control mice undefeated and defeated (left panel). Induction of S10-PhosphoH3 by social defeat in control and BRG1^{D1Cre} mice (right panel). Undefeated control nuclei n=26 (5 mice), undefeated Brg1^{D1Cre} nuclei = 26 (5 mice), defeated control nuclei = 26 (5 mice), defeated Brg1^{D1Cre} nuclei = 26 (5 mice). Scale bar = 25 μ m. **(b)** Representative DAPI staining in nuclei from control and BRG1^{D1Cre} mice undefeated, after a single defeat or after repeated social defeat (upper panel). Total surface of heterochromatin and number of heterochromatin areas in control and BRG1^{D1Cre} mice undefeated, after a single defeat or after repeated social defeat. Scale bar = 2 μ m. *p<0.05, **p<0.01, ***p<0.001. Data are represented as mean \pm s.e.m.

Figure S1

a)



b)

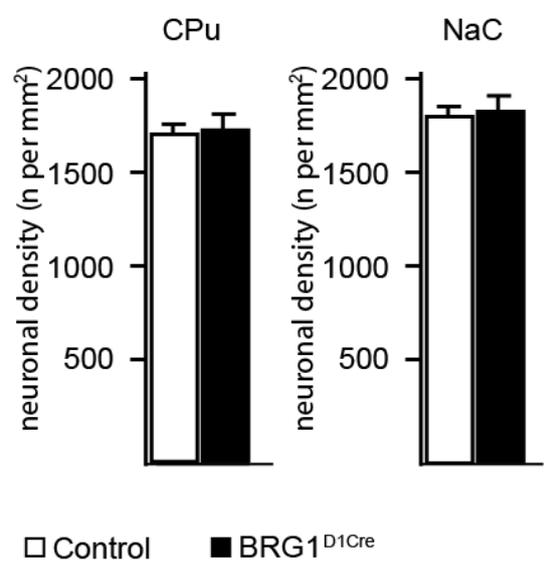


Figure S1: Genetic inactivation of BRG1 and BRM in compound mutants and neuronal density in 24 months old mice. (a) Genetic inactivation of BRG1 in dopaminoceptive and of BRM in the whole organism in BRG1^{D1Cre} BRM^{-/-} double mutant mice. Scale bar = 25 μ m. **(b)** Neuronal density using NeuN staining in the dorsomedial striatum (CPu on the left) and nucleus accumbens (NAc on the right) of 24 months old control (n=3) and BRG1^{D1Cre} (n=3) mice. Data are represented as mean \pm s.e.m.

Figure S2

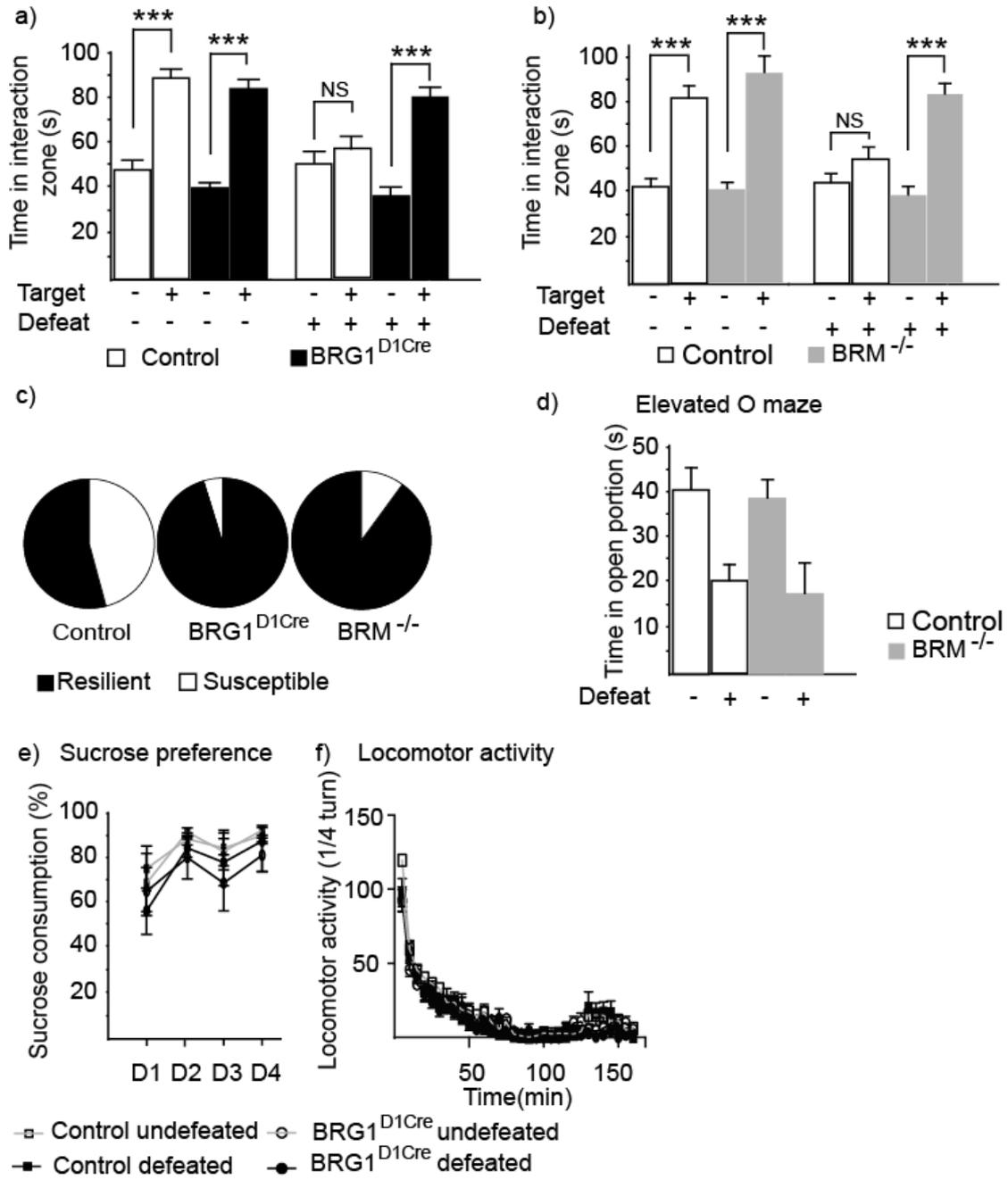


Figure S2: Behavioral consequences of repeated social defeat in controls and BRG1^{D1Cre} and BRM^{-/-} mice. (a) Effect of repeated social defeat on social interaction time in control and BRG1^{D1Cre} mice. Undefeated control n=25, defeated control n=24, undefeated BRG1^{D1Cre} n=25, defeated BRG1^{D1Cre} n=22. **(b)** Effect of repeated social defeat on social interaction time in control and BRM^{-/-} mice. Undefeated control n=17, defeated control n=19, undefeated BRM^{-/-} n=9, defeated BRM^{-/-} n=16. **(c)** Percentage of resilient (in black) and susceptible mice (in white) in defeated controls (n=41), defeated BRG1^{D1Cre} (n=22) and defeated BRM^{-/-} mice (n=16). **(d)** Effect of repeated social defeat on sucrose preference in control and BRG1^{D1Cre} mice. Undefeated control n=7, defeated control n=8, undefeated BRG1^{D1Cre} n=7, defeated BRG1^{D1Cre} n=8. **(e)** Effect of repeated social defeat on locomotor activity in control and BRG1^{D1Cre} mice. Undefeated control n=9, defeated control n=6, undefeated BRG1^{D1Cre} n=9, defeated BRG1^{D1Cre} n=9. **(f)** Anxiety levels in the elevated O maze in controls and BRM^{-/-} mice. NS: not significant, ***p<0.001. Data are represented as mean±s.e.m.

Figure S3

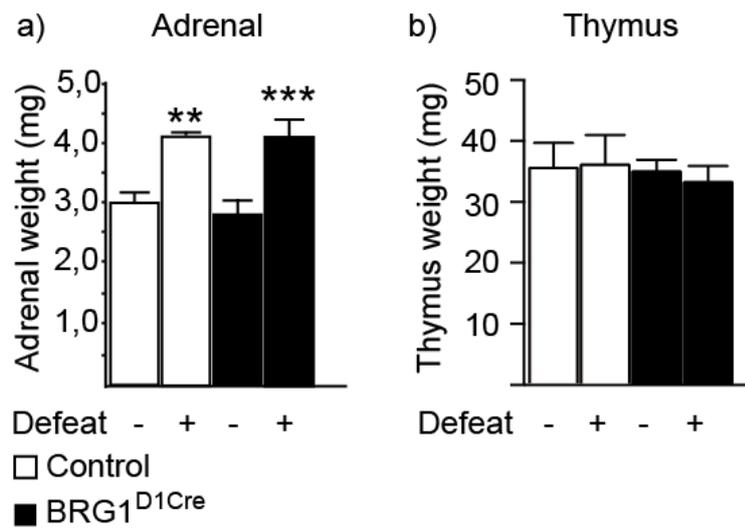


Figure S3: Effect of repeated social defeat on thymus and adrenal weight in controls and BRG1^{D1Cre} mice. **(a)** Adrenal glands weight in undefeated control n=11, defeated control n=7, undefeated BRG1^{D1Cre} n=9, defeated BRG1^{D1Cre} n=9. **(b)** Thymus weight in undefeated control n=10, defeated control n=5, undefeated BRG1^{D1Cre} n=9, defeated BRG1^{D1Cre} n=9. **p<0.01, ***p<0.001. Data are represented as mean ± s.e.m.

Figure S4

a)

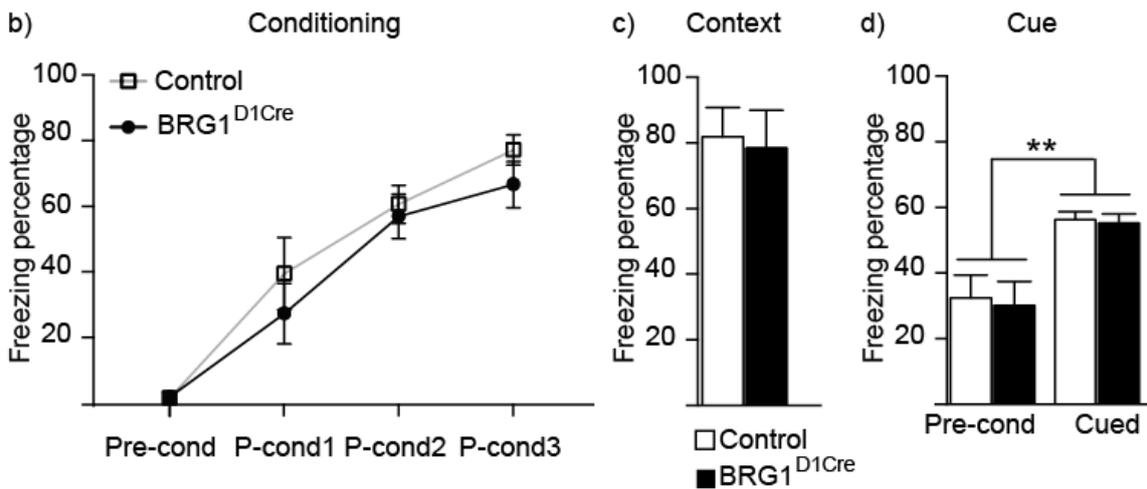
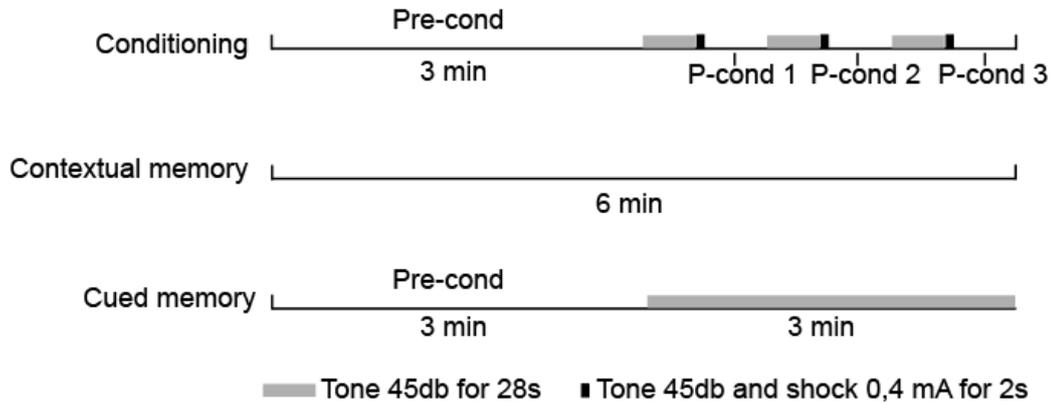


Figure S4: Fear memory in controls (n=8) and BRG1^{D1Cre} mice (n=8). (a) Schematic of the fear conditioning protocol with the conditioning (up), the contextual memory (middle) and the cued memory (bottom). (b) Freezing percentage during the conditioning in response to the three foot-shocks. (c) Freezing percentage during the contextual fear memory test. Unpaired t-test $p > 0.05$. (d) Freezing percentage during the cued fear memory test. $**p < 0.001$. Data are represented as mean \pm s.e.m.

Figure S5

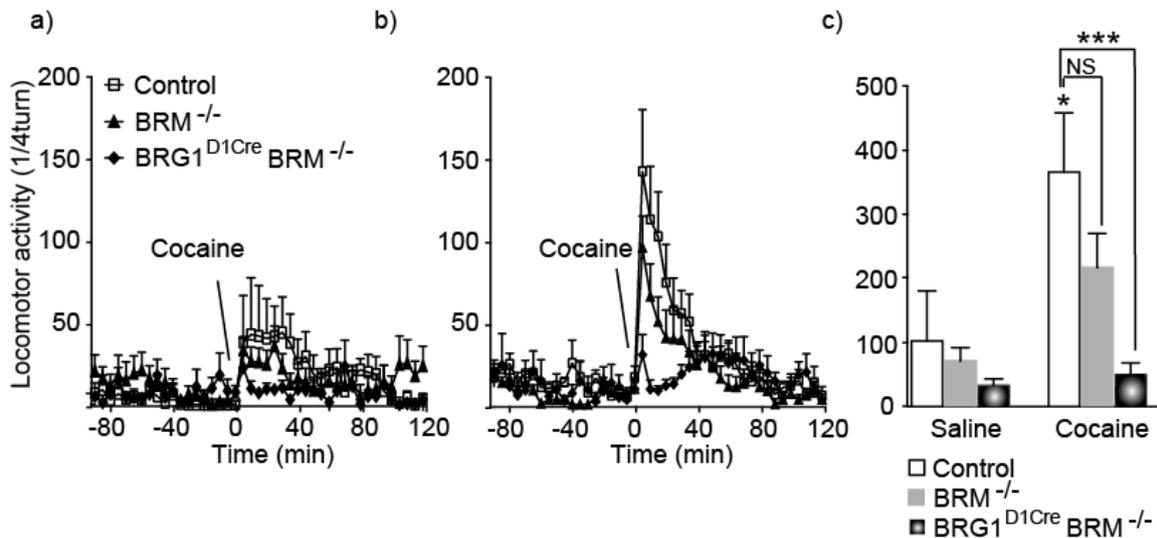


Figure S5: Behavioral responses to cocaine in BRM^{-/-} and BRG1^{D1Cre}BRM^{-/-} mice. (a) Locomotor response (by 5 min blocks) to a cocaine injection (10mg/kg) in saline pre-treated controls (n=4, same as Figure 4), BRM^{-/-} (n=8) and BRG1^{D1Cre}BRM^{-/-} mice (n=6) mice (n=7). (b) Locomotor response (by 5 min blocks) to a cocaine injection (10mg/kg) in cocaine pre-treated control (n=6, same as Figure 4), BRM^{-/-} (n=7) and BRG1^{D1Cre}BRM^{-/-} (n=8) mice. (c) Locomotor sensitization (cumulated activity during 15 min after injection) to cocaine in control, BRM^{-/-}, and BRG1^{D1Cre}BRM^{-/-} mice. Saline pre-treated controls n=4, saline pre-treated BRM^{-/-} n=8 and BRG1^{D1Cre}BRM^{-/-} mice (n=6), cocaine pre-treated controls n=6, cocaine pre-treated BRM^{-/-} n=7 and BRG1^{D1Cre}BRM^{-/-} mice (n=8). NS: not significant, *p<0.05, ***p<0.001. Data are represented as mean ± s.e.m.

Figure S6

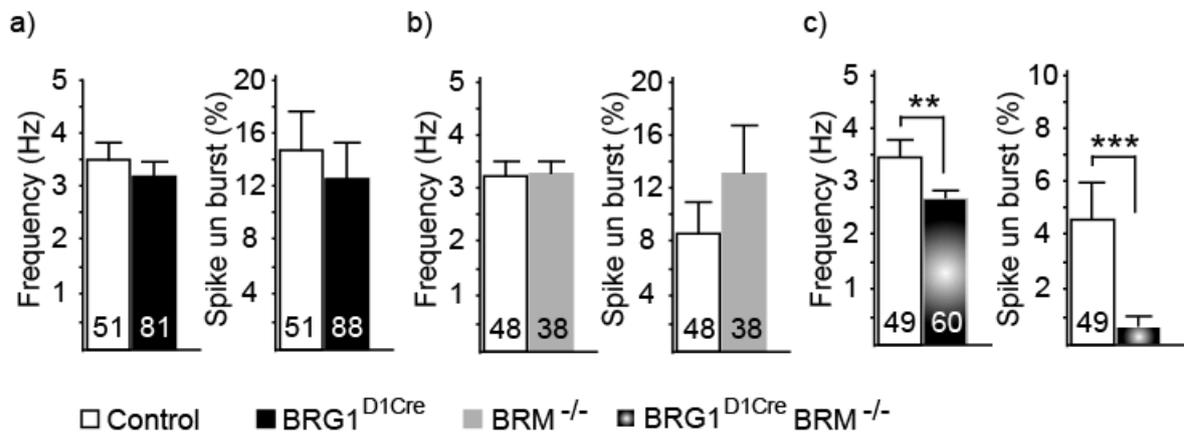


Figure S6: Basal spontaneous activity of ventral tegmental area dopamine neurons in control, BRG1^{D1Cre}, BRM^{-/-} and BRG1^{D1Cre}BRM^{-/-} mice. (a) Firing frequency (left chart) and percentage of spike in burst (right chart) in control (n=51) and BRG1^{D1Cre} mice (n=81; data from Figure 5a and b). **(b)** Firing frequency (left chart) and percentage of spike in burst (right chart) in control (n=48) and BRM^{-/-} mice (n=38). **(c)** Firing frequency (left chart) and percentage of spike in burst (right chart) in control (n=49) and BRG1^{D1Cre}BRM^{-/-} mice (n=60). T-test (for frequency) and Wilcoxon test (spike in burst) **p<0.01, ***p<0.001. Data are represented as mean ± s.e.m.

Figure S7

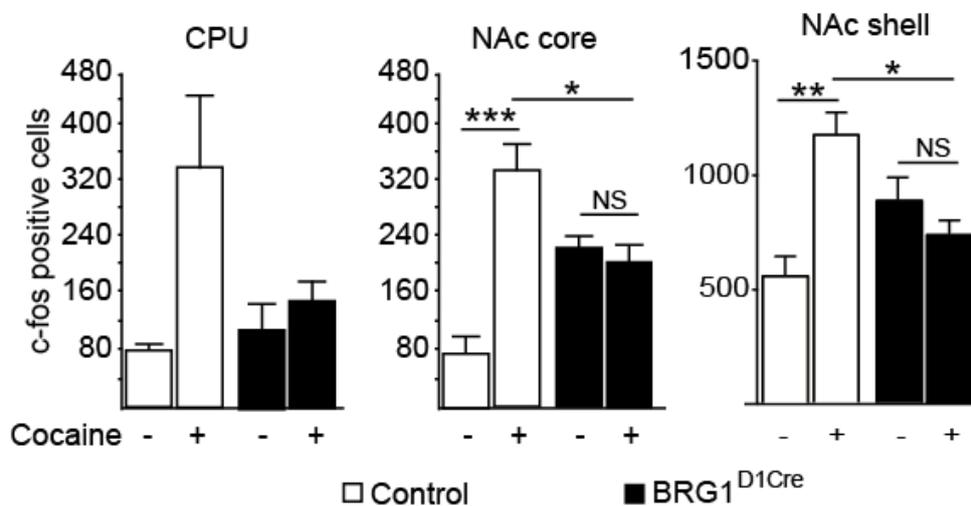


Figure S7: c-Fos gene induction by social defeat in controls and BRG1^{D1Cre} mice in the dorsomedial striatum (CPu, left chart), nucleus accumbens core (middle chart) and shell (right chart). Saline-treated control n=3, cocaine-treated control n=6, saline-treated BRG1^{D1Cre} n=3, cocaine-treated BRG1^{D1Cre} n=6. *p<0.05, **p<0.01. Data are represented as mean \pm s.e.m.

Figure S8

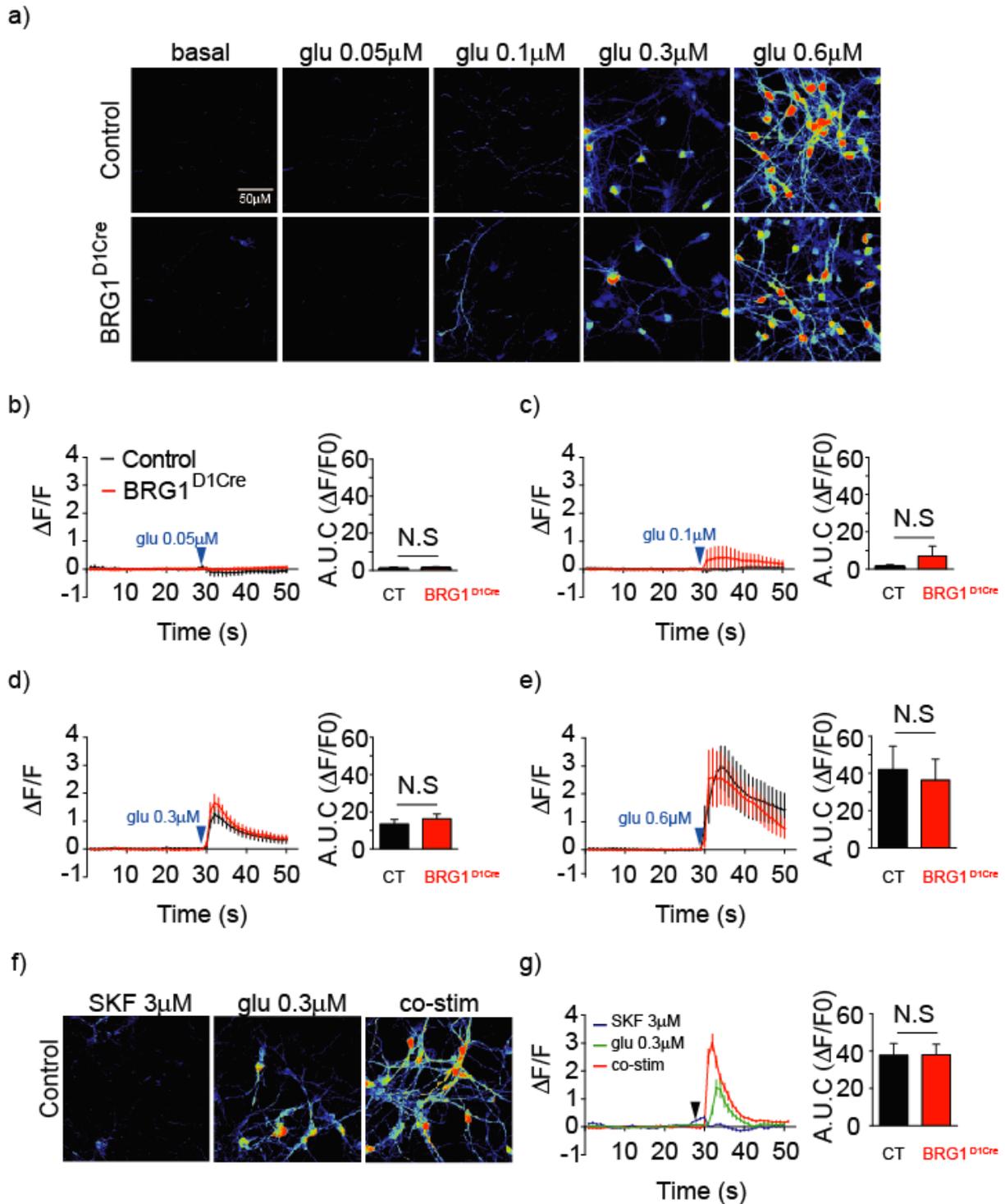


Figure S8: Figure legend: Deletion of Brg1 in D1R-MSN preserves glutamate-mediated calcium responses as well as the integration of D1R and glutamate signals. (a) Representative confocal images of $\Delta F/F$ calcium signals obtained at the peak of the responses during glutamate applications at the indicated doses in WT (top panels) or Brg1^{D1Cre} (bottom panels) cultured striatal neurons. **(b-e)** Left panels: dynamics of calcium signals before (0-30 sec) and during (30 to 50 sec) glutamate

applications at the indicated concentrations measured from WT (black) or Brg1^{D1cre} (red) neurons. Right panel: quantifications of the corresponding area under the curves (AUC) for WT (black bars) and Brg1^{D1cre} (red bars) neurons. **(f)** Representative pictures of the calcium responses elicited by a D1R agonist SKF38393 (3 μ M) and glutamate (0.3 μ M), applied separately, or together (co-stim) in WT neurons. **(g)** Right panel: corresponding calcium profiles illustrating the D1R-mediated facilitation of glutamate-mediated calcium transient in WT neurons. Left panel: Quantifications of the AUC of calcium transients triggered by the co-stimulation paradigm in WT and Brg1^{D1cre}. Note that the amplitude of the responses elicited by the co-stimulation is similar for both genotypes. n.s: not significant.

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