ACTUALIZACION POR TEMAS

Neurobiology of addiction: neuroanatomical, neurochemical, molecular and genetic aspects of morphine and cocaine addiction. Part III

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Summary

Drugs of abuse, including opiates and psychostimulants, induce both short and long-lasting effects on behavior and similarly exert profound changes in the neural circuits where these drugs operate producing neuronal adaptations and long term changes in synaptic plasticity. Such neuroadaptation occur as homestatic responses to excessive drug stimulation in assotiation with a specific pattern of learned behaviors induced by repeated drug stimulation. Although such neuroadaptive mechanisms may underlie several aspects of drug dependence and withdrawal, they do not account for the compulsive nature of drug seeking behavior or the tendency to relapse. Most drugs of abuse alter a set of molecular mechanisms involved in normal cellular processes that underlie the physiological aspects of learning and memory where stimulation of dopamine D1 receptors, activation of the cAMP/PKA/CREB intracellular signaling transduction pathway, alteration of gene expression, and the enhanced synaptic rearrangements currently occur in the acquisition of such behaviors. Therefore, drug addiction, as a chronic relapsing disorder, reflects novel adaptative changes occurring in specific patterns of synaptic connectivity as occurs in normal memory formation. Neural mechanisms that underlie both tolerance and sensitization might coexist and one can unmask the expression of the other after abrupt drug cessation (tolerance) or after prolonged removal of drug administration (sensitization). These effects can even persist for a long time after ending drug administration. Although many of the cellular and molecular adaptations elucidated up to date reflect those neuroadaptations that correlate with altered behavior responses during drug addiction, there are still many unsolved non response questions such as; the precise molecular events implicated in the permanente changes in neurons and neural transmission systems that are responsible for the adaptative behavioral responses during relapse, and drug seeking behavior during chronic drug addiction.

Key words: Morphine, cocaine, mesocorticolimbic system, dopamine, neuron, opioid receptors, neural transmission, addiction.

Resumen

Las drogas de abuso ilegal, tales como los alcaloides opiáceos y los psicoestimulantes, inducen cambios profundos en los circuitos neuronales donde las drogas ejercen sus efectos psicoadictivos, produciendo a corto y largo plazo importantes cambios de neuroadaptación neuronal así como cambios plásticos en la sinapsis. Estas neuroadaptaciones surgen como respuestas homestásicas que adquieren las neuronas por la continua estimulación de una droga, determinando el aprendizaje de patrones conductuales específicos. Aunque estos cambios neuroadaptativos representan cambios funcionales en el fenómeno adictivo, como es la dependencia y el síndrome de supresión, no explican los mecanismos biológicos implicados en la naturaleza compulsiva del consumo reiterado de una droga. La mayoría de las drogas de abuso alteran a largo plazo mecanismos moleculares y procesos celulares involucrados con el aprendizaje asociativo. En este contexto, la estimulación continua de los receptores dopaminérgicos

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D_1/D_3 (D_1R/D_3R), la subsecuente activación del sistema intracelular de señalamiento cAMP/PKA/CREB y la alteración progresiva de la expresión de productos genómicos, producen a largo plazo cambios morfológicos, químicos y moleculares en las neuronas dopaminérgicas, que promueven la generación de nuevos contactos sinápticos y/o remodelación de sinapsis preestablecidas.

Palabras clave: Morfina, cocaína, adicción, mesocorticolímbico, neurotransmisión, dopaminérgico, receptores opioides, neuronas.

Introduction

Chemical substances affect the brain; they influence mood, thougth and action. Many clinically used drugs, such as neuroleptics, antidepressants, lithium, levodopa, lysergic acid diethylamide (LSD) and many others, exert a profound effect on the chemistry of the brain, altering the normal and abnormal behavior of mammals, including human beings. Although many behavioral disordes in humans are related to psychiatric and movement disorders (e. g., schizophrenia, tardive diskinesia, Parkinsnson's disease), they are associated with specific disturbances in a single neurotransmitter system where a specific psychopharmacological drug impinge. Nonetheless, a myriad of neurochemical abnormalities may occur in different neurotransmission systems that directly or indirectly are involved in the pathogenesis of different behavioral abnormalities, including the abnormal behavior seen during drug addiction. Drug abuse involves a pattern of pathological use of illegal substances resulting in personal distress, drug seeking behavior, drug dependence and signs and symptoms of tolerance and withdrawal following drug cessation. Animal models of drug abuse have shown that even in the absence of physical dependence, drugs of abuse are strong reinforcers for on-going self-administration. As previously described, most drugs of abuse impinge on a specific brain circuitry circumscribed at the medial portion of the brain, namely the dopaminergic mesocorticolimbic system (Leff *et al*, 2000; Antón *et al*, 2000) where most neurochemical and molecular changes have been analyzed in acute and chronic states of addiction to opiates (e. g., morphine, heroin, codeine, methadone) and to psychostimulants (e. g., cocaine, amphetamines, and related compounds) as drug reinforces in animal models of drug self administration (Koob *et al*, 1998).

Most of the behavioral aspects observed during drug addiction occur as neuroadaptive changes at the level of the individual neurons induced with prolonged exposure to the drug of abuse. Therefore, these cell adaptations in turn alter the functioning of the neural circuits implicated in drug addiction (Nestler and Aghajanian, 1997; Nestler, 1996) leading to complex behaviors that are expressed as dependence, tolerance, sensitization and craving, which characterize the addictive state (Koob and Le Moal, 1997). So, as a biological process, drug addiction implicates a time course of neuronal events that occur gradually in those neurons where drugs impinge, affecting neuronal processes, membrane receptors and a subset of intracellular protein molecules that enhance the establishment of a short and long term molecular memory that underlies the neuroadaptions occurring in drug addiction (Nestler and Aghajanian, 1997). Most of the cell and molecular adaptations induce by drugs of abuse, such as cocaine and heroin (the most prevalent illicit drugs of abuse), have been increasingly understood as short-term neuroadaptations that contribute to the transient features of an addictive state, such as somatic and motivational withdrawal symptoms, as well as changes of drug sensitivity (Nestler, 1996; Nestler and Aghajanian, 1997). Therefore, further research is needed in order to identify and characterize the long-term neuroadaptive changes in specific neural circuits and neurons during prolonged stages of drug addiction, such as craving and relapse, which persist during a long time even after drug cessation.

Short-term molecular neuroadaptations associated to drug reinforcement

In addition to the neuroadaptative changes in several neurotransmission systems that are common to chronic administration of drugs of abuse, several studies have identified the molecular changes that provide the substrate for long-term adaptation to chronic drug self-administration. Different adaptive responses at the molecular level have been observed which may account for the individual differences in susceptibility to dependence to different drugs. One of the main neuroadaptative responses that are established during chronic cocaine and amphetamine administration in *nucleus accumbens* dopamine neurons, is the activation of the D1-like receptors and the subsequent activation of intracellular signaling mechanisms that enhance an over response of neuronal activity induced by the increased synaptic dopamine concentration (Koob, *et al*, 1998; Nestler and Aghajanian, 1997). In addition, several works of research have revealed that the up-regulation of the adenosine 3', 5' monophosphate (cAMP) pathway is commonly found in several neural transmission systems during physical opiate and cocaine dependence and withdrawal. These neurochemical adaptation, as the ones occurring in the mesocorticolimbic dopamine and pontine noradrenergic neurons, have been shown to underlie most of the long term effects of chronic opiate and cocaine administration (Koob, *et al*, 1998; Nestler *et al*, 1993).

Up —regulation of cAMP was first demonstrated in neuroblastoma x glioma cells, and later in neurons in response to repeated opiate exposure (Nestler and Aghajanian, 1997). Physiologically, endogenous opiates, as well as acute opiate adminitration, inhibits cAMP pathway in neurons in different areas of the brain, whereas chronic opiate administration induces an opposite compensatory up-regulation of this intracellular regulatory pathway. This up-regulation of the cAMP pathway involves an increase in adenylyl-cyclase activity, cAMP-dependent protein kinase A (PKA) and related components of this intracellular signaling mechanism (Nestler and Aghajanian, 1997; Self *et al*, 1998). This set of neurochemical events, established during chronic opiate exposure, posits that changes in intracellular cAMP concentration represent a form of

physiological tolerance that becomes fully functional upon drug removal. Thus, these molecular changes ocurring during drug addiction contribute to features of dependence and withdrawal (Nestler and Aghajanian, 1997; Nestler, 1996). The development of dependence and tolerance to drugs of abuse is believed to result from the neuroadaptations occurring in several neurotransmission systems. Therefore, while some neural systems, such as the mesocorticolimbic dopaminergic systems, have shown to be involved in the motivational aspects of dependence (e. g., craving), other neural transmission systems seem to participate in the aversive components related to the opioid withdrawal syndrome (Christie *et al*, 1997). In support for the latter, several experimental works have demonstrated that the *locus coeruleus* (LC) play a crucial role in the expression of opioid dependence and withdrawal syndrome (Lane-Ladd *et al*., 1997; Nestler, 1996), and as such represent a first insight into what could be interpreted as a "cellular model of neuronal tolerance and dependence" (Nestler, 1996). Although the LC might be required for full or partial expression of behavioral signs of opioid withdrawal, several inconsistencies have emerged surrounding the anatomical extent to which withdrawal activation is required (Christie *et al*, 1997). Therefore, the adaptative changes might either occur in LC noradrenergic neurons or in afferent inputs that are sensitive to opioid withdrawal and which, ultimately, drives the LC neurons to produce the cellular and molecular changes previously described (Christie *et al*, 1997). Chemical lesions using selective neurotoxins (e. g., 6-hydroxydopamine or DSP4) which are capable of destroying discrete types of neurons with little effect on the surrounding neurons or fibers *in passage*, have provided evidences of the brain areas relatively involved in the expression of withdrawal behavior. Extensive lesions (>95% depletion of cortical noradrenaline) to the ascending noradrenergic fibers coming from the LC have failed to attenuate any signs of opioid withdrawal. Similarly, lesions of the LC provided evidences of the uninvolvement of this neural nucleus in opioid withdrawal. Although these pharmacological studies clearly ruled out the involvement of ascending noradrenergic projections from the LC in the withdrawal behavior, incomplete electrolytic lesions of the LC were reported to inhibit many signs of opioid withdrawal induced by intracerebroventricular (ICV) of naloxone after depleting > 60% of cortical and hippocampal noradrenergic fibers. Chronic depletion of catecholamines using other pharmacological methods also failed to inhibit naloxone-precipitated withdrawal (Christie *et al*, 1997). In general, it can be asumed that noradrenergic projections arising from the LC are not exclusively necessary for the expression of most signs of opioid withdrawal behavior, while fibers of passage, such as descending fibers arising from midbrain regions in the PAG course close to the LC region, might be relevant for the expression of withdrawal signs (Christie *et al*, 1997).

Most of the studies showing up regulation of the cAMP pathway in the *locus coeruleus* have been shown to correlate with opiate withdrawal behaviors (Christie *et al*, 1997), and it has been shown behaviors that such event increases the intrincic firing rate of a subset of neurons in this brain stem nucleus, via the activation and permeation of ions through non selective cation channels (Kogan *et al*, 1992; Alreja and Aghajanian, 1993). The increased firing response of LC neurons during opiate withdrawal may result as a subsequent event to the increased firing of glutamaergic neurons within this brain area (Rasmussen and Aghajanian, 1989). This effect might be due to the up regulation of the cAMP pathway in primary sensory neurons which, in turn, may activate ascending excitatory inputs to the LC. Nonetheless, the degree to which LC neurons contribute to the overall expression of the opiate withdrawal syndrome is still an issue of debate; nevertheless, the neurochemical and cellular events established during opiate dependence and withdrawal whithin this brain area, have been useful as a model system to delineate the precise molecular mechanisms underlying neuronal adaptations to chronic drug exposure (Nestler and Aghajanian, 1997).

Under such context, chemical and molecular studies have provided clues about the cellular mechanisms implicated in drug addiction. Chronic opiate administration selectively up-regulates two forms of adenylyl ciclase (type I and VIII) in LC neurons. Specifically, the up-regulation of the type VIII enzyme seems to be regulated by an intracellular protein, such as the cAMP response element binding protein (CREB), one of the major cAMP regulated transcription factors in the brain (figure 1). The observation that the suppression of the nuclear expressions of this transcription factor blocks the morphine-induced increase of the adenylate cyclase type VIII enzyme is very interesting (Nestler, 1996; Nestler and Aghajanian, 1997; Reisine *et al*, 1996). In contrast, up-regulation of type I adenylyl-ciclase and of protein kinase A (PKA) is not affected by this treatment; thus it appears that up-regulation of this specific intracellular signaling pathway is under control by different cellular mechanisms (Ladd-Lane *et al*, 1997; Nestler and Aghajanian, 1997). Moreover, reduction of CREB concentration in LC neurons, after antisense oligonucleotide treatment, attenuates the activation of these cells during opiate withdrawal as well as the expression of withdrawal behaviors. Such observations have been extended in mutant mice deficient of this transcription protein factor, where selective attenuation of the expression of this protein attenuated opiate withdrawal (Maldonado *et al*, 1995).

Furthermore up regulation of cAMP pathway occurs in neurons of the *nucleus accumbens* in response to chronic administration of cocaine, opiates and alcohol (Koob *et al*, 1998) but the specific cell types within this region, which express such molecular neuroadaptations, remain unclear. The *nucleus accumbens*, as part of the mesocorticolimbic dopamine transmission system, is one of the main brain target areas of most drugs of abuse and plays a crucial role in the motivational states during the reinforcing actions of most drugs of abuse (Koob *et al*, 1998). Stimulation of D_1 dopamine receptors in *accumbens* neurons results in the activation of the stimulatory guanosine triphosphate binding protein (GS) and in the further activation of the cAMP-PKA pathway. The up-regulation of this pathway in the *nucleus accumbens* neurons results from the functional

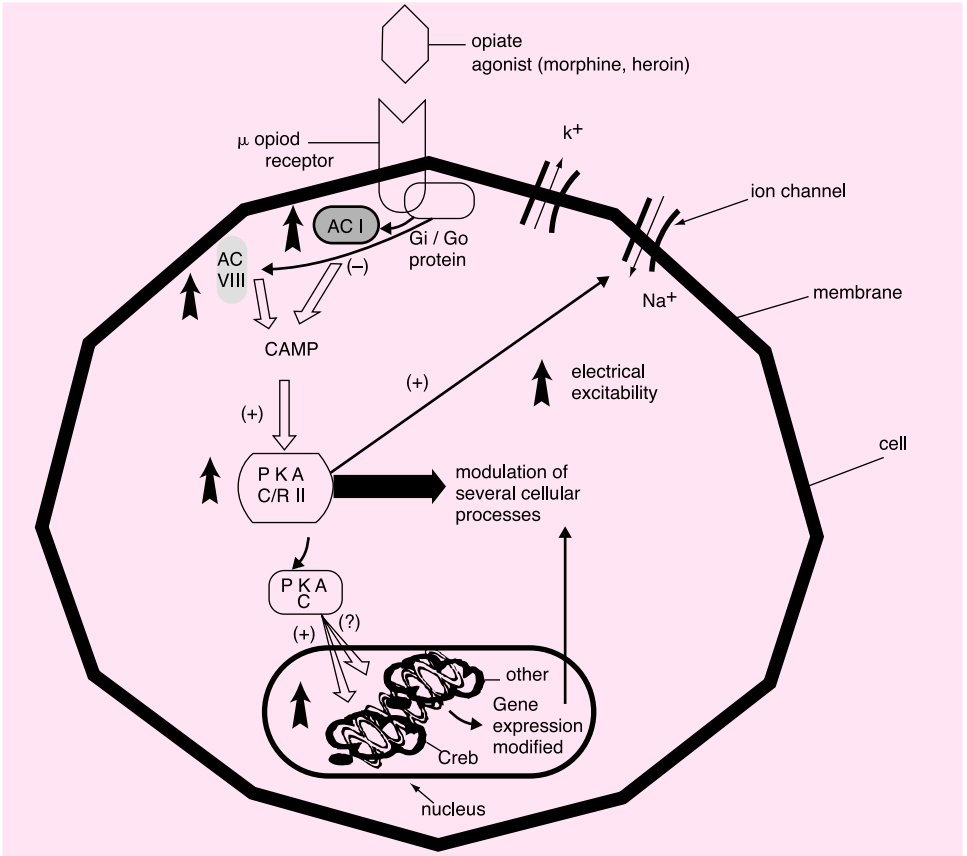

Figure 1. *Actions of drugs of abuse affecting the intracellular signaling pathway in mesocorticolimbic dopaminergic neurons.* Most drugs of abuse affect several molecular systems within the cell, thus affecting the neural transmission system where peptide and non peptide transmitters operate. The illustration shows how opiates acutely inhibit dopamine neurons by increasing the inwardly rectifying K^+ channel current, and decreasing the Na^+ dependent inward current after activation of the μ opioid receptor subtype coupled to Gi/Go proteins subtypes. Activation of Gi/Go proteins produce subsequent inhibition of Adenylyl cyclase. Enzyme inhibition decreases cAMP concentration and reduces PKA activity in the phosphorilation state of other subset of proteins (ion channels and pumps) as well as in additional proteins involved in cell processes responsible for the neuronal excitability. As a result of the drug activation of the μ opioid receptor and the cAMP/PKA cascade inhibition, the CREB phosphorilation state (nuclear transcription factor) is also reduced. Chronic administration of morphine and heroin induces an increased concentration of type I and VIII adenylyl cyclase (AC I/VIII), PKA catalytic (C) and regulatory (RII) subunits, as well as phosphorilated proteins such as nuclear transcription factors like CREB. This set of molecular changes is partially implicated in the altered phenotype of the drug addicted state. Up regulation of AC-VIII/PKA signaling pathway is mediated by CREB, whereas up regulation of AC-I and PKA subunits are mediated by a CREB-independent mechanism (?). Up regulation of this signaling system, as well as of other phosphoproteins, contributes to the altered neuronal excitability and to the cellular expresion of mechanisms such as tolerance, dependence and withdrawal (Adapted from Nestler and Aghajanian, 1997, modified by the first author of this review).

supersensitivity of D_1 dopamine receptors expressed in this area, following chronic administration of cocaine or other kind of psychostimulants. Thus, stimulation of the cAMP pathway results in the activation of protein kinase (PKA) which, in turn, phosphorilates voltage gated Na^+ channels, and in the increased response of the neuronal activity (White *et al*, 1998). Several works have updated that the up-regulation of cAMP pathway in the *nucleus accumbens* opposes drug reinforcement mechanisms that contribute to the aversive state during withdrawal (Self and Nestler, 1995). Thus, stimulation of PKA counteracts the rewarding effects of cocaine. This suggests the existence of a neuronal mechanism of tolerance (Self *et al*, 1998). Conversely, several evidences have suggested the hypothesis that up-regulation of cAMP could simultaneously enhance a sensitization response to the locomotor-activating effects of stimulants (Cunningham *et al*, 1993).

Different experimental works have demonstrated that the increased PKA activity leads to the increased phosphorylation of CREB, which in turn mediates many of the effects of cAMP and PKA on the gene expression (Gonzales *et al*, 1998; Cole *et al*, 1995). For instance, chronic administration of opiates or psychostimulants have been shown to alter CREB expression or its phosphorylation in *nucleus accumbens* and in other related striatal regions, affecting the expression of genes coding for several opioid peptides (Nestler and Aghajanian, 1997). Although these sites represent potential targets for CREB in these neural regions during drug

addiction, gene regulation by CREB would be important for establishing a long-term memory and neural plasticity as has been recently hypothesized by several authors (Martin and Kandel, 1996; Kandel, 1997).

Genes for opioid peptides contain CRE sites (specific sequences of DNA on which CREB acts) which are known to be affected by drugs of abuse via CREB phosphorylation. For instance, gene coding for the preprodynorphin protein is known to be regulated by CREB *in vitro* (Cole *et al*, 1995), and repeated cocaine administration increases its expression in the *nucleus accumbens* and in the dorsal *striatum* (Hurd *et al*, 1993). Several experimental works have established that CREB regulates dynorphin expression in the rat *nucleus accumbens in vivo* as has been demonstrated in rats overexpressing CREB, which in turn have an important increase in dynorphin mRNA, and conversely, rats overexpressing mutant form of CREB transcription factor (mCREB) show a decrease in dynorphin mRNA (Carlezon *et al*, 1998). In support for such studies, pharmacological studies have shown that animals exposed to *in vivo* injection of kappa opioid receptor agonists (kappa opioid receptor subtype is known to bind with high affinity and selectivity dynorphins peptides) into the shell of the *nucleus accumbens* induce an aversion behavior to repeated self administration of cocaine (Bals-Kubik *et al*, 1993). Conversely, by blocking brain kappa opioid receptors with irreversible specific antagonists, such as norBNI, after ICV injection in rats who overexpress HSV-CREB, the aversive effects associated with self administration of cocaine were completely blocked, which suggest that the increase of neuronal expression of dynorphin induced by CREB plays a crucial role in the aversion effects to cocaine and other drugs of abuses as well (Carlezon *et al*, 1998). These results support other experimental evidences that show that over expression of CREB in *nucleus accumbens* decreases the rewarding effects of cocaine, and low doses of the drug result to be aversive to animals undergoing drug self administration. Conversely, overexpression of the mutant form of this transcription factor (mCREB) in the rat *nucleus accumbens*, increases the rewarding effects of cocaine, and similar results were observed after ICV injection of the kappa opioid receptor antagonist, norBNI, in rats over expressing this mutant form of the CREB protein (Carlezon *et al*, 1998). These set of results indicates that k opioid receptors are involved in determining reward or aversion mechanisms to cocaine, and suggests that the regulatory transcriptional effect mediated by CREB in *nucleus accumbens* neurons could play a significant role as a "drug reward rheostat" by regulating the gene expression of proteins and peptides such as dynorphins (Carlezon *et al*, 1998).

These data propose that a sequence of intracellular events brought out by an initial drug administration, culminates in an altered gene transcription and gene expression, to which subsequent drug exposure determines the subjective effect of the drug; either to act as a positive reinforcer having a reward effect, or as a negative reinforcer resulting in an aversive effect (Carlezon *et al*, 1998). In such context, repeated exposure to cocaine causes an up-regulation of dynorphin expression throughout the stimulation of the cAMP-PKA pathway, which results in the increased functional activity of CREB and in the alteration of the gene expression. Such events result from the initial and continuous stimulation of D_1 dopamine receptors in neurons of the *nucleus accumbens* (Carlezon *et al*, 1998; Cole *et al*, 1995). In addition, repeated exposure of cocaine would enhance an increased release of dynorphin, that in turn would inhibit local dopamine release by acting through k opioid receptors located at presynaptic terminals on mesocorticolimbic dopaminergic neurons that innervate the *nucleus accumbens* (Shippenberg and Rea, 1997; Spanagel *et al*, 1990). Therefore, decreased release of dopamine would make the drug aversive or would tend to unmask other neuronal responses induced by the drug that opposes the rewarding effect of the drug itself (Ritz *et al*, 1987; Carlezon *et al*, 1998).

Several studies have shown that the *striatum* expresses a high density of D_1 and D_2 dopamine receptors, whereas high concentration of D3 dopamine receptors are localized preferentially in regions of the ventral *striatum* (Bordet *et al*, 1997; Mansour *et al*, 1995). These dopamine receptors subtypes are expressed mostly in striatal spiny neurons (e. g., D_1 cells and D_2 cells) that project to the *globus pallidus* and *substantia nigra* as well (Berke and Hyman, 2000). Neurons expressing D_1 dopamine receptors are coupled to Gs/Go protein, which in turn stimulates adenylate cyclase giving up an intracellular increase in cAMP, and the resultant activation of PKA, which phosphorilates a number of various substrates, including L-type calcium channel, sodium and potassium channels, NMDA glutamaergic receptors subtypes, and several transcriptions factors (e. g., CREB, Fos and JUN) as well as many different intracellular signaling components (Berke and Hyman, 2000; Cepeda *et al*, 1998; Cantrell *et al*, 1999; Hernández-López *et al*, 1997). Neurons expressing D_2 dopamine receptors produce opposite effects to the activation of D_1 dopamine receptors, as they seem to be coupled to Gi/Go protein and, therefore, inhibit adenylate cyclase, enhancing the activation of the inwardly rectifying potassium channel. Striatal D_2 dopamine receptors are tonically stimulated by basal levels of dopamine, and this neurotransmission system is relevant for motor control behavior. The observation that enhanced dopamine release can be observed in dissociated striatal slices, altering the signal transduction pathway in dopamine terminals is interesting (Kantor *et al*, 1999). Moreover, ICV administration in mice of either D2 antagonist or of drugs that produce vesicle dopamine depletion (e. g., reserpine), causes the disinhibition of the cAMP-PKA-CREB pathway and the induction of immediate early genes (IEGs) in D_2 striatal cells (Adams *et al*, 1997; Konradi and Heckers, 1995; Konradi *et al*, 1994); this effect is blocked by the coadministration of D_2 agonist (Dragunow *et al*, 1990; Cole and Di Fligia, 1994). Mice lacking D_1 dopamine receptors do not show any gross motor abnormalities (Drago *et al*, 1994; Xu *et al*, 1994) but may have an important role in the dopamine dependent learning processes; D_2 receptor activity might be crucial for the expression of dopamine dependent behavioral patterns (Berke and Hyman, 2000). However, activation of both

D_1 and D_2 receptors by increased dopamine concentration or selective dopamine agonnists may have synergistic effects on neuronal activity, gene expression and behavior (Hu and White, 1997), that would result in the activating basal ganglia pathway from the coordinated actions of D_1 and D_2 receptor stimulation (Wise *et al*, 1996). Activation of intracellular signaling pathway produced by D_1 receptor stimulation causes a variety of cellular responses varying in time. Some of this neuronal responses may enhance a sensitivity to neurotransmitters as occurs in altered behavioral responses to drugs. For example, acute exposure to amphetamines induce over minutes a rapid phosphorylation and internalization of striatal D_1 receptors associated with a decreased cAMP response to subsequent D_1 stimulation (Dumartin *et al*, 1998). This type of rapid neuroadaptation may be responsible for the first cocaine administration producing an initial acute tolerance to the drug. Prolonged activation of D_1 receptors leads to an altered gene expression, as occurs with the increased neural expression of dynorphin in striatal D_1 cells (Gerfen *et al*, 1990; Cole *et al*, 1995). As previously decribed, dynorphin acting on kappa opioid receptors on dopamine nerve terminals, cause a decreased release of dopamine (Spanagel *et al*, 1992). Thus, many of the effects of the psychostimulants may converge in altering gene expression, which reflects that compensatory neuronal adaptations occur in order to equilibrate the over-stimulation of neurotransmitter receptors. Over expression of dynorphin in striatal cells (e. g., *nucleus accumbens* neurons) results during prolonged cocaine exposure, and such event may reflect the neuroadaptive mechanism used by neurons in order to blunt normal dopamine transmission (Steiner and Gerfen, 1996). These neuroadaptive mechanisms operating during drug addiction could contribute to the negative emotional state (v. g., dysphoria, anxiety, irritability) displayed during drug withdrawal, due to dynorphin acting on kappa receptors, in the same way as kappa agonist produces an aversive response behavior in both rats and humans during the administration of psychostimulants (Koob *et al*, 1998; Shippenberg *et al*, 1993; Shippenberg and Rea, 1997). Prolonged stimulation of D_1 receptors induces an increase in both dynorphin expression and over-expression of CREB (in a phosphorylated state) in the ventral *striatum*. However, dozens of different mRNA increase, besides dynorphin mRNA, in ventral *striatum* after prolonged stimulation of D_1 dopamine receptor by dopamine agonist or cocaine (Spangler *et al*, 1996) but they fade away in a couple of days after drug cessation. Most neuroadaptative changes produced by addictive drugs occur as reversible homeostatic mecanisms occurring in different areas of the brain, as exemplified by the increase of dynorphin expression in the *nucleus accumbens* during withdrawal. Therefore, most neuroadaptative changes induced by a drug of abuse result form hemostatic responses (Koob *et al*, 1998; Hyman, 1996; Rasmussen *et al*, 1990). So, when drug administration is withdrawn, neural systems gradually return to their basal level of normal responses, which can take either from minutes to weeks, depending on the homeostatic response involved. However, none of these mechanisms seem to lapse for long-periods before addicted subjets come to a relapse (Berke and Hyman, 2000).

Other neuroadaptative changes occurring during psychostimulant administration, refers to those alterations in postsynaptic responsiveness to dopamine, involving some forms of drug sensitization (Nestler, 1996). Although sensitization to psychostimulants does not readily correlate with long-lasting changes in dopamine receptor mRNA or increased protein levels, psychostimulants cause detectable changes in the intracellular signaling pathway, altering G protein levels or other components of the same signaling pathway (Striplin and Kallivas, 1993), as has been shown to up regulate the cAMP pathway in dopaminergic neurons (Terwilliger *et al*, 1991; Nestler, 1996). Such results have been corroborated with some results demonstrating long lasting changes (even one month after the last cocaine administration) in the increased coupling of D_1 receptors to adenylate cyclase (Sala *et al*, 1995) and the inhibition of the evoked firing glutamaergic striatal neurons in anaesthetized rats after priming animals with cocaine (Henry and White, 1995).

Long term molecular and cellular neuroadaptations in drug reinforcement

Most neuroadaptations occurring at the molecular and cellular level after repeated drug administration are shorlived adaptations that end relatively soon after drug cessation, in contrast to the long-lasting adaptations observed in animals and humans that endure from weeks to months after drug discontinuation (Nestler and Aghajanian, 1997). Similar to the biological models of long-term memory, long-lived adaptations induced by prolonged drug exposure involve changes in gene expression that ultimately lead to morphological and chemical changes in neurons and in the neural transmission systems implicated in drug action and reinforcement. These changes include permanent alterations in the neuron structure and in the number of synaptic connections formed by individual neurons. Though psychostimulants induce short-term changes in the gene expression of several molecules in striatal D_1 cells (Cole *et al*, 1992; Douglass *et al*, 1995; Berke *et al*, 1998), most mRNAs expressions return to baseline levels withing a few hours or days after drug exposure (Berke *et al*, 1998; Wang *et al*, 1995). In such context, acute exposure to psychostimulants, such as cocaine and amphetamines; to opiates, such as morphine and heroin, or to nicotine, have been shown to induce transiently the expression of certain family of transcription factors (e. g., Fos and Jun) in the *nucleus accumbens* and in the related striatal regions (Nestler and Aghajanian, 1997). Conversely, chronic drug exposure desensitizes the ability of these proteins to be induced and “brings up” a gradual accumulation of different Fos related proteins (Nestler, 1996). Long term changes in gene expression as occurs in several brain nuclei, including the ventral *striatum* (Cha *et al*, 1997), have been shown to result from post-translational modified protein products of the Fos gene, referred as “chronic Fos related antigens” (Chronic FRAs) (Hope *et al*, 1994). Chronic FRAs have

been identified as several isoforms of the *Δ-FosB* transcription protein, where one of these isoforms represents a truncated spliced form of the *fosB* gene (Chen *et al*, 1997; Hope *et al*, 1994). Chronic FRAs are relatively stable proteins that are accumulated in the brain during repeated drug exposure, as they seem to increase for up to 4 weeks during drug treatment. Moreover, these gene protein products seem to alter the ability to induce the expression of other genes that are regulated by other kinds of transcription factors, such as the AP-1 protein complex, after continuous drug stimulation (Hope *et al*, 1994). Thus, these molecules seem to alter subsequent patterns of psychostimulant induced gene expression (Berke and Hyman, 2000). Although the specific target genes for *Δ-FosB* isoforms still remains to be elucidated, several evidences point out the importance of these isoforms in the behavioral plasticity in response to drugs of abuse, as has been reported recently (Nestler and Aghajanian, 1997). For instance, mice lacking the *fosB* gene show enhanced locomotor activity and reinforcing responses to cocaine (Hiroi *et al*, 1997), and conversely, transgenic mice overexpressing *Δ-FosB* gene products in the *striatum* show an altered behavioral sensitivity to cocaine (Kelz *et al*, 1999). Long term changes in gene expression seem to be crucial for the persistence of some forms of sensitization to drugs of abuse in addictive humans, however, no experimental evidences have been shown for the up or down regulation of mRNAs of protein products in the brain, that lasts long enough to account for such changes in drug sensitization (Berke and Hyman, 2000).

In general, transcription factors represent the most plausible neuroadaptative mechanisms that underlie drug induced neural-plasticity in the mammal's brain, including the human (Nestler and Aghajanian, 1997).

Synaptic plasticity in drug reinforcement

Drug addiction and drug seeking behaviors rely on the association of specifics cues which implies that alternative associative learning processes might be involved. As an associative learning process, drug addiction and addictive drugs, such as cocaine and opiates, are coupled to an increase of dopamine in the *nucleus accumbens* and to the activation of dopamine receptors subtypes D_1 and D_3 (Berke and Hyman, 2000; Le Foll *et al*, 2000), as they are coupled to the cAMP/PKA/CREB intracellular signaling cascade pathway. More striking is the observation that this signaling pathway is particularly implicated in physiological processes implicated in memory formation and neuronal plasticity in diverse species (e. g., fruit flies, mollusks and mice) (Berke and Hyman, 2000; Silva *et al*, 1998). D_1 receptors have an important role in hippocampal long term potentiation (LTP), the most striking physiological model of synaptic plasticity. LTP is formed when simultaneous depolarization of pre and postsynaptic neurons induces the activation and opening of NMDA receptors, followed by calcium entry into the cell and enhancement of the strength of synaptic connectivity (Malenka and Nicoll, 1993). In such context, several electrophysiological experiments have shown that in discrete regions of the hippocampus, CA_1 pyramidal cells can spread LTP to neighboring cells limited to an area within 150 mm aside from the potentiated cell (Bonhoeffer *et al*, 1989; Schuman *et al*, 1994; Harris *et al*, 1992). Moreover, no monosynaptic connections or gap junctions were shown to occur between neighboring cells during the spreading of LTP, which allowed to conclude that the potentiation must spread via axonal input from one cell forming synapses on dendrites of the adjacent cells (Bliss *et al*, 1993; Malinow *et al*, 1994). Moreover, if LTP spreads out to neighboring cells via the input of axons, there must be retrograde messengers released from the potentiated neighboring cell to signal presynaptic axons when LTP has occurred (Harris and Kater, 1994). Different candidates for retrograde signals do exist ranging from proteins that span the synaptic cleft (v. g., integrin type molecules) to small molecular weight diffusing molecules (e. g., nitric oxide, arachidonic acid, platelet activating factor) (Harris, 1995). LTP in hippocampal neurons can persist for up to 2-3 hrs (late phase of LTP), and requires increased stimulation of postsynaptic cAMP, phosphorilation of CREB; gene transcription and new protein synthesis (Berke and Hyman, 2000). The observation that D_1 agonist and the activators of a cAMP cascade can induce the late phase of LTP (L-LTP) (Frey *et al*, 1993; Huang and Kandel, 1995), is an interesting fact. Furthermore, the D_1 agonist can prevent depotentiation of potentiated synapses (Otmakhova and Lisman, 1998) and, conversely, the blockade of D_1 receptors prevents hippocampal L-LTP maintenance (Huang and Kandel, 1995) as it was recently shown that D_1 knockout mice do not show L-LTP (Matthies *et al*, 1997). Therefore, besides the fact that D_1 receptors are implicated in the psychostimulant reinforcement during drug addiction, D_1 receptor in the hippocampus may act as a gateway in synaptic plasticity formation, enhancing the changes involved in long lasting synaptic strength. In addition, dopamine receptors may be involved in the modification of synaptic strength as a consequence of the psychostimulant induced-increase of dopamine in the *nucleus accumbens*. Thus, the increase level of dopamine may act as a reinforcement learning signal in the *striatum* (Wickens and Kotter, 1996; Beker and Hyman, 2000).

In general, activation of the cAMP signaling pathway and increase of cAMP and CREB phosphorilation in D_1 striatal cells induce the expression of genes which in turn, modify the activity of the whole neuron, similarly to what occurs during the hippocampal LTP that involves the subsequent modification of specific synapses.

In extension, just as both dopamine and glutamate receptors are involved in synaptic plasticity in the hippocampus, both receptors and neuronal inputs to the *striatum* cooperate in the induction of gene expression and behavioral changes. Selective stimulation of D_1 receptors in the *striatum* causes a slight induction of IEG expression (Robertson *et al*, 1992). But cortical stimulation (v. g., auditory stimuli or stimulation of auditory cortex) may increase the striatal IEG expression after D_1 receptor activation by systemic injection of dopaminergic agonists (Arnauld *et al*, 1996). Similarly, amphetamine injection in an animal exposed to a novel

environmental cue increases the activity of cortical areas and enhances an increased induction of IEG as well as behavioral sensitization to the drug, without affecting the level of dopamine release from striatal cells (Badiani *et al*, 1998). Interestingly, drugs of abuse, such as cocaine, amphetamine, nicotine and morphine, induce IEG expression in ventral *striatum*. This event is blocked by either D_1 receptor or NMDA receptor antagonists (Kiba and Jayaraman, 1994; Liu *et al*, 1994). Mutant mice lacking D_1 receptors do not show increase of IEG expression, and locomotor sensitization to psychostimulants is diminished and does not change in a dose dependent manner (Moratalla *et al*, 1996; Crawford *et al*, 1997). Similar results have been observed after systemic injection of NMDA receptor antagonist, where development of conditioned-locomotor response to a psycho stimulant associated enviroment is prevented (Wolf and Khansa, 1991). Moreover, D1 receptor agonists induce a significant increase of cAMP and of the phosphorilation of CREB in cultured striatal cells, which result to be blocked by the NMDA receptor antagonist and calcium removal (Kondari *et al*, 1996; Das *et al*, 1997). Intracellular calcium affects CREB phosphorilation via the mitogen activated protein kinase pathway (MAPK) (Xing *et al*, 1996). This molecular mechanism is important in the synaptic plasticity and for enhancing certain forms of learning, as it has recently been shown that *in vivo* stimulation of cortical areas causes and increases the activation of MAPKs in the striatal cells (Impey *et al*, 1998; Sgambato *et al*, 1998). This phenomenon is dependent of the NMDA receptor activation and of the calcium entry into the strial cells (Vincent *et al*, 1998). Although psychostimulants cause a rapid and transient induction of different genes in D_1 striatal cells, many of them show an overlapping distribution with genes that are expressed in LTP (*homer-1a, narp*, and *arc*) and are involved in the regulation of the synaptic function (Cole *et al*, 1989; Brakeman *et al*, 1997; O'Brien *et al*, 1999). Chronic administration of psychostimulants causes increased dendritic spine density and branched spines in both the *nucleus accumbens* and the prefrontal cortex, similar to the morphological changes that occur in the late phases of hippocampal LTP, where formation of new synaptic connections are known to occur (Engert and Bonhoeffer, 1999; Robinson and Kolb, 1997). Opposite to this, morphological changes have been shown after dopamine denervation, where decrease in striatal dendritic spine density and asymmetric synapses are observed (Ingham *et al*, 1989, 1993, 1998; Meredith *et al*, 1995).

Therefore, prolonged psychostimulations of D_1 receptors in *nucleus accumbens* dopamine neurons may underline a mechanism of synaptic plasticity as a form of learning process. One theory proposes that the proper activation of a specific synapses may signal and "tag" that particular synaptic connection to further respond for a long-lasting change brought up by molecular signals coming from the nucleus (Frey and Morris, 1997; Martin *et al*, 1997b). In addition, at certain stages during drug addiction, several mechanisms may be involved in the synaptic "tagging" of those dopaminergic neurons. Dopamine may be one of the crucial mechanisms, acting as a reinforcement signal, inducing long-lasting changes on gene expression (Berke and Hyman, 2000) as well as enhancing long-term associated neuroadaptative changes in the neural circuits implicated in drug addiction.

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