Neurobiology of addiction: Neuroanatomical, neurochemical, molecular and genetic aspects of morphine and cocaine addiction. Part II

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Summary

Drug addiction in humans is a chronically relapsing disorder, that impacts both society and the individual welfare. Both neurochemical and neuropharmacological studies have shown for more than a decade, that most drugs of abuse act through mechanisms that involve the mesocorticolimbic dopamine reinforcement/reward system in the CNS. These drugs, including stimulants such as cocaine and amphetamine, opiates such as morphine and heroin, and legal drugs such as alcohol and nicotine, change the neurochemical function of different neurotransmitter systems in the brain that lead to neuronal responses that converge in the increase activation of ventrotegmental dopamine neurons, and in an elevation of the extracellular dopamine concentration in the nucleus *accumbens*. Ventrotegmental dopamine neurons send axonal projections to interconnected forebrain structures that mediate many of the reinforcing, behavioral and locomotor effects of most drugs of abuse, such as the prefrontal cortex, *striatum* and nucleus *accumbens*. However, other interconnected forebrain structures, such as the amygdala, has been shown to play a crucial role in the brain reward mechanisms and motivational effects produced by these drugs. Thus, most neurochemical changes and neuroadaptations that occur during the development of drug addiction involve cellular and molecular mechanisms that affect both peptidergic and non peptidergic neurotransmission systems that impair the neurochemical function of the mesocorticolimbic neural substrate mediating the acute and chronic reinforcing actions of most drugs of abuse.

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

Resumen

La adicción a las drogas de abuso ilegal se considera hoy en día como un trastorno neuropsiquiátrico que repercute ampliamente en la salud del individuo y en el bienestar de la sociedad. Desde hace más de dos décadas, diversos estudios de investigación en el campo de la neurofarmacología y la neuroquímica han demostrado que la mayoría de las drogas de abuso actúan alterando diferentes sistemas de neurotransmisión, que modifican permanentemente las funciones químicas y moleculares de las neuronas que operan en estos sistemas. Si bien se ha demostrado en animales modelos de auto-administración de drogas psicoadictivas, que el sustrato neuroanatómico implicado directamente en el inicio, desarrollo y consolidación del fenómeno adictivo para la gran mayoría de las drogas de abuso (v.g. psicoestimulantes y alcaloides opiáceos) es el sistema dopaminérgico mesocorticolimbico; hay además otros sistemas de neurotransmisión que también parecen ser modificados funcionalmente durante el consumo crónico y reiterado de las drogas con perfil psicoadictivo (v.g., etanol, nicotina) como son, el

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sistema de transmisión GABAérgico (GABA), el sistema serotoninérgico, el sistema de transmisión opioide endogeno y otros sistemas de transmisión de naturaleza peptídica, recientemente descritos, que en conjunto modulan en forma directa o indirecta la fisiología neuronal del sistema de proyección dopaminérgico mesocorticolímbico. Por lo tanto, los cambios neuroadaptativos que ocurren a largo plazo en los diferentes sistemas de neurotransmisión que operan durante el desarrollo y el establecimiento del fenómeno adictivo a cualquier droga psicoadictiva, representa un fenómeno neurobiológico que está reflejado en su totalidad por los cambios neuroquímicos y moleculares que se establecen en los sistemas de neurotransmisión y, por ende, en las propias neuronas que operan en estos circuitos neurofuncionales.

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

Neurochemical and neuropharmacological aspects of the reinforcing actions of drugs of abuse

Most drugs of abuse alter the activity of dopaminergic neurons in the mesocorticolimbic system. This system comprises a group of dopamine projecting neurons at the ventrotegmental area whose axons project to the nucleus *accumbens* and prefrontal cortex, making synaptic connections with local neurons in each brain area. Thus, addictive substances, such as morphine, heroin, cocaine, d-amphetamines, etanol, nicotine and tetrahydrocannabinols, change directly or indirectly the physiological and chemical functioning of dopamine projecting neurons by changing the chemical and molecular status of this subset of neurons (Koob et al., 1998; Stephanski et al., 1999; Shoaib et al., 1999; Watkins, et al., 1999; McBride et al., 1999; Ufer et al., 1999; De Vries et al., 1999; Alonso et al., 1999). These neurobiological changes induced by drugs of abuse produce at short and long term an over-release of dopamine from presynaptic terminals which reflects the increase of extracellular dopamine concentration at synaptic fields as have been analyzed in animal models of chronic self administration of psychostimulants and opiates exposed to *in vivo* microdialisis (Koob et al., 1998; Robbins and Everitt, 1999). Moreover, other neurotransmission systems comprising non dopaminergic neurons (e.g., GABA, glutamate, serotonin releasing neurons) in the ventral tegmental area, nucleus *accumbens*, *striatum*, hyppocampus, and amygdala (Pontieri et al., 1995; Kobb et al., 1998; Robbins and Everitt, 1999) are modified after prolonged exposure of addictive substances. The role of the serotonin transmission system in drug reinforcement has been highlighted by clinical findings (Koob et al., 1998) that have shown the close relationship existing between the dependence on drug-intake and depression (Markou, et al., 1998; Robbins and Everitt, 1999). In this context, Prozac, a drug used to treat depression, shares with cocaine similar pharmacological and molecular mechanisms of drug-cell interaction, such as its high affinity to serotonin protein transporter (Robbins and Everitt, 1999). Although neurochemical studies have demonstrated that chemical and functional interactions between serotonin and dopamine transmission systems are mutually inhibitory (Robbins and Everitt, 1999) several pharmacological and genetic studies provide evidence that such functional correlation between serotonin and dopamine transmission systems does not always seem to gel. For instance, transgenic mice lacking the 5-HT_{1B} serotonin receptor will increase self administration of cocaine when compared to non transgenic control mice expressing this serotoninergic receptor subtype under the same experimental conditions.

Similar to other drugs of abuse, sedative-hypnotics, including alcohol, produce their reinforcing actions by modifying multiple neurotransmitter systems besides the dopaminergic neural system (Engel et al., 1992), mainly, the neural site proposed to be affected by these substances and by ethanol is the GABA transmission system (Samson and Harris, 1992). These studies have been supported by pharmacological reports that demonstrate that administration of selective agonists for GABA-A and GABA-B receptors (e.g., THIP and baclofen), the development of sensitization to locomotor stimulant effects of ethanol in D8A/2J mice strain is prevented (Broadbent and Harless, 1999). Although these agonists block the acute stimulant response to ethanol in animals exposed to self-administration of ethanol (Broadbent and Harless, 1999), baclofen, but not THIP (administrated previously to ethanol) attenuates the sensitization effects of chronic ethanol consumption. These sets of results support the hypothesis that the GABA transmission system is affected by ethanol throughout the activation of both GABA-A and GABA-B receptor subtypes, emphasizing that activation of the GABA-B receptor subtype may reduce more efficiently the development of the sensitization to the locomotor stimulant effects of ethanol (Broadbent and Harless, 1999). Furthermore, neurochemical studies have shown that the GABA transmission system, under basal conditions, tonically inhibits the activity of the ventral tegmental dopamine transmission system (Koob et al., 1998). This functional interaction between the GABA and the dopamine transmission systems has been supported by pharmacological observations showing that systemic injection or acute consumption of ethanol reduces the firing rate of *pars reticulata* GABA neurons, reducing the inhibitory effect of these neurons on the ventral tegmental dopamine neurons. This drug-induced effect results in an increase of extracellular dopamine concentration in the *nucleus accumbens* (Diana et al., 1993; Merue and Guessa, 1985) as observed in rats chronically consuming low doses of ethanol (Koob et al., 1994b). Moreover, the role of the mesolimbic dopamine transmission for ethanol reinforcement has been supported by pharmacological studies that demonstrated that non dependent animals reduce lever pressing for ethanol consumption after microinjection of potent dopamine receptor antagonists into the amygdala or *nucleus accumbens* (e.g., D_1 or D_2 blocking agents) (Hyttia and Koob, 1995; Koob et al., 1994b). Although this set of observations makes quite evident that VTA-NAc dopamine transmission system make an important contribution to the reinforcing actions of ethanol, the complete destruction of this neural circuit with 6-hydroxydopamine does not alter animal response for ethanol consumption (Koob et al., 1994b). Therefore, the *nucleus accumbens*

dopamine transmission system does not seem to participate as an essential neuroanatomical substrate as it does for other drugs of abuse (e.g., cocaine, morphine and heroin) in ethanol reinforcement (Koob et al., 1994b; Hyttia and Koob, 1995). In addition, supporting the GABA transmission system on the reinforcing actions of ethanol, neurochemical reports have provided evidences that microinjection of benzodiazepines into the brain (e.g., RO-15-4513, inverse agonist) reverses some of the behavioral effects of ethanol, reducing oral ethanol self-administration in rats in a dose-dependent manner (Samson and Harris, 1992). In addition, pharmacological manipulations that change the brain serotonin synaptic availability in subcortical areas of the mammal's brain (e.g., rat) reduce ethanol consumption (Sellers et al., 1992) since serotonin reuptake blockers as well as selective antagonists of the serotonin-3 and serotonin-2C receptors induce a decrement in ethanol consumption in animals exposed to drug self administration paradigms. This set of results shows that the complexity of neurochemical interactions of several neurotransmission systems does take place in the reinforcing actions of ethanol as demonstrated in serotonin, GABA and dopamine neurotransmission systems (LeMarquand, et al., 1994).

So far, it seems clear from pharmacological and neurochemical reports that the mesocorticolimbic dopamine transmission system does not exclusively contributes to the reinforcing actions of ethanol as well as of other neurotransmission systems that might be directly or indirectly altered by drugs of abuse (Koob et al., 1994, 1998). For instance, two well documented neurotransmission systems that indirectly participate in the reinforcing actions of cocaine and opiates, are the endogenous dynorphin and enkephalin opioid systems (Koob et al., 1998; Cappendijk et al., 1999; Comings et al., 1999). Neurochemical studies have shown that neurons that synthesize and release dynorphyn A, dynorphin B, Met-enkephalin and Leu-enkephalin and substance P peptides are activated in the caudal *striatum* and septal areas of the rat brain during chronic administration of cocaine (Hyman et al., 1996) or in heroin treated animals (Cappendijk et al., 1999). Likewise, neurons that synthesize and express the antiopioid peptide, NPFF, are similarly activated after chronic administration of morphine and heroin (Malin et al., 1990; Lake et al., 1992). Moreover, neurochemical studies have reported that neuropeptides that modulate the nociceptive transmission in the CNS of mammals, regulate the mesocorticolimbic dopamine transmission in the *nucleus accumbens* and decrease the activity of ventral-tegmental dopamine neurons in animals challenged to cocaine administration, who subsequently develop hyperlocomotion effects induced by the reinforcing actions of cocaine (Murphy and Maidment, 1999a; Murphy et al., 1996; Narayanan and Maidment, 1999c). Based on dual-probe microdialysis experimental designs on anesthetized rats, it has been shown that the endogenous ligand for the ORL-1 (opioid receptor-like) receptor, the heptadecapeptide *Orphanin FQ/nociceptin* (OFQ/NOC) reduces *nucleus accumbens* dialysate dopamine levels when injected into the ventrotegmental area (VTA). Simultaneous measurements of dialysate amino acid content showed that this neuropeptide induces a significant increase of GABA and glutamate in the *nucleus accumbens* during infusion of the neuropeptide into the VTA (Murphy and Maidment, 1999a). On this basis, infusion of GABA-A receptor antagonists, such as bicuculline, transiently blocked the suppressive action of Orphanin FQ on the *accumbens* dopamine levels. This set of results provides new insights that nociceptin inhibits the dopamine neuronal activity in the ventral tegmental area, reducing the dopamine transmission system in the *nucleus accumbens* through a mechanisms that may involve an induced overflow of GABA (Murphy and Maidment, 1999a). Furthermore, when OFQ/NOC is bilateral administered into the VTA prior to the challenge of repeated cocaine self-administration in male rats (5-10 min), a transient decrease of the hyperlocomotor response to cocaine (15-30 min) is observed on the first trial of cocaine administration. However, repeated intra-VTA administration of the OFQ/NOC neuropeptide, 3 days before animals are challenged to cocaine administration trials, a sensitized response is obtained to single doses of cocaine given subsequently for 5-7 days later. These studies provide evidence that Orphanin FQ produce a short-term decrease in the activity of ventral tegmental dopamine neurons, inducing a rapid tolerance and inadequate mechanisms that prevent the development of cocaine sensilization (Narayanan and Maidment, 1999). Similar results have been obtained after intracerebroventricular (ICV) administration of this neuropeptide in rodents during conditioned place aversion or preference paradigms induced by subcutaneous administration of morphine. These studies provide information that Orphanin FQ significantly reduces the development of place preference to morphine in animals exposed to chronic administration of morphine (Narayanan and Maidment, 1999).

Other neurobiological aspects of drug addiction include the neuroendocrine studies that have been undertaken to investigate the hormonal and behavioral responses to physical and chemical stressors that occur during drug dependence and withdrawal. The activation of the hypothalamus-pituitary-adrenal axis has been recognized for long as the main neurosecretory system modulating several aspects of drug dependence and withdrawal in humans (Kreek, 1987). This neuroendocrine system is characterized by its ability to secrete peptide hormones in response to physiologic and chemical stressors modulating many of the behavioral and physiological responses related to stress-inducing factors, anxiety and pain in mammals. Animal models exposed to cocaine, nicotine and ethanol self-administration paradigms show important anxiogenic-like responses after cessation of chronic administration of drugs, that are reverted following intra-cerebroventricular of CRF (corticotrophin-releasing-factor) antagonist (Koob et al., 1998). Thus, anxiogenic-like effects induced by ethanol withdrawal would enhance the physiological release of CRF-ACTH/β-endorphin and stress related substances from adrenal gland that are reversed by administration of low doses of CRF

antagonist into the central nucleus of the amygdala (Koob, 1996). Similar results have been obtained by microinjecting similar doses of the same antagonist that reverses the aversive effects of morphine withdrawal in animal models of opiate self-administration (Koob, 1996). In addition, neurochemical observations obtained by *in vivo* microdialysis studies have shown that ethanol, cocaine and THC (tetrahydrocannabinol) administration induces an increase in the extracellular CRF during drug withdrawal (Merlo-Pich et al., 1995; Rodriguez de Fonseca et al., 1997). Therefore, CRF activation may represent one of the many common neurophysiological mechanisms that might contribute to the motivational effects and subjective symptoms (e.g., increased stress and negative affect) that characterizes the development and establishment of drug dependence (Koob, 1996).

Besides the chemical changes that occur in the opioid and non opioid peptide transmission systems during drug addiction in the CNS of mammals and humans, a new putative peptide transmission system has been recently discovered that encompasses functional roles in sensory processing, brain development, stress, drug reinforcement and reward. This peptide transmission system, referred to as CART transmission system (cocaine and amphetamine regulated transcripts) (Kuhar and Dall Vechia, 1999) has been defined on the neurochemical basis that the mRNA transcript products generate new neuronal and extra neuronal peptides that modulate physiological processes, such as feeding behavior, stress and development of drug addiction. These peptides are highly abundant in specific cell groups in subcortical areas of the mammal's brain, including the human, as *in situ* hybridization studies have revealed for the CART mRNA sublocalization in discrete areas of the rodent and human brain (Gautvik et al., 1996). For example, CART mRNA localization occurs in some ganglion cells in the retina, mitral cells in the olfactory bulb, which suggest that protein products of CART mRNA may be involved in the regulation of sensory processing (Kuhar and Dall Vechia, 1999). More interesting is the fact that CART mRNA are highly abundant in specific nuclei of the rat hypothalamus (Douglass et al., 1995), supporting the initial biochemical studies regarding the identification and isolation of CART peptide fragments (e.g., a short fragment consisting of 116 amino acids and a long fragment consisting of 129 amino acids) from sheep hypothalamus (Spiess et al., 1981). Moreover, immunohistochemical studies revealed that CART peptides are highly expressed in the paraventricular, arcuate nuclei and median eminence of the hypothalamus, as well as in the posterior and anterior lobe of the pituitary, including adrenal medulla: structures known to contain the CART mRNA (Douglass et al., 1995; Couceyro et al., 1997). The demonstration that these peptides occur in the hypothalamic-pituitary-adrenal axis supports their physiological role as neurohormones controlling several parameters of stress induced by emotional and physiological factors, including the anxiogenic-states induced by acute suppression of drug intake (Kuhar and Dall Vechia, 1999). Besides the modulatory actions of these peptides in controlling feeding behavior, and their putative role as neurotrophic factors, these peptides have been shown to increase the expression of nuclei transcript factors (e.g., FOS protein) in neurons segregated to specific hypothalamic nuclei (Vrang et al., 1999) and to exert amphetamine and cocaine like effects in the rat *nucleus accumbens* (Grace et al., 1998).

Besides the neurochemical findings that several neuropeptides regulate different states of drug addiction including the POMC (Proopiomelanocortin protein precursor) derived peptide hormones (e.g., *β*-endorphin and ACTH) that regulate anxiogenic-like responses and stressful conditions (Elias y col., 1998), other neuronal release substances, localized in several areas of the mammal's brain, have been implicated in drug reinforcement and reward and have strengthened the hypothesis that neurotransmission systems besides the mesocorticolimbic dopamine transmission system are modified by chronic administration of drugs of abuse. These neurotransmission systems as describe above, include the gabaergic transmission system in the *nucleus accumbens*, whose neurons receive important dopaminergic inputs from the ventral-tegmental area, and the *locus coeruleus*, the major noradrenergic transmission system in the brain, located on the floor of the fourth ventricle in the anterior pons. This small group of neurons provided a widespread noradrenergic innervation to virtually all areas of the brain and spinal cord implicated in major functions such as the animal's state of arousal (e.g., attention, vigilance and autonomic tone) as well as regulation of different stress conditioning situations (Nestler et al., 1996; Koylu y col., 1999). This area of the brain has been implicated in somatic opiate withdrawal behaviors as confirm by several pharmacological and electrophysiological evidences showing that this group of cells increase their intrinsic firing during opiate withdrawal (Nestler, 1992; Nestler et al., 1993; Nestler, 1996, 1997; Koob et al., 1992, 1998).

Nevertheless, most neurochemical changes that occur in several neurotransmission systems during drug addiction have been shown to structure a common neural circuitry termed as the *extended* amygdala (Alheid and Heimer, 1988; Heimer and Alheid, 1991; Koob, 1999a). This neuroanatomical substrate contains specific component where neurochemical events and neuropharmacological actions mediate the acute reinforcing actions of most drugs of abuse as well as the negative reinforcement of compulsive drug administration associated with drug dependence (Koob et al., 1998; Koob, 1999a; Koob, 1999b). This neural substrate is composed by separate entities of basal forebrain structure, such as the bed nucleus of the stria terminal's, the central medial amygdala, the posterior medial part of the *nucleus accumbens* (e.g., posterior shell) and the sublenticular *substantia innominata*. Although these neural areas share common cytoarchitectural features, histochemical and morphological similarities (Heimer and Alheid, 1991; Koob et al., 1998; Koob, 1999b) they receive afferent connection from limbic structures such as the basolateral amygdala, hyppocampus and limbic cortices, midbrain and lateral hypothalamus. The efferent projections coming out from this neural complex include the ventral *pallidum*, medial ventral tegmental area, brain stem and lateral

hypothalamus (Koob, 1999 b). Moreover, it has been documented that the lateral and medial divisions of the septal area are implicated in spatial learning processes during self administration of morphine in mice (Cazala et al., 1998). Therefore, the extended amygdala may be considered as a macrostructure that functionally relies on specific neural circuits that interconnect specific brain areas interfacing classical limbic structures involved in the emotional and motivational aspects of drug reinforcement and reward with the extrapyramidal motor system that regulates most of the behavioral and locomotor effects of acute and chronic drug addiction (Koob et al., 1998, Koob, 1999 a).

In support of the role of the brain areas that integrate the extended amygdala, pharmacological evidences have shown that acute administration of major drugs of abuse, including cocaine/heroin combinations (Speedball) in rats, induces an important increase or synergistic elevations of extracellular dopamine concentrations in the shell of the *nucleus accumbens* (Hemby et al., 1999; Pontieri et al., 1995). Moreover, the shell of the *nucleus accumbens* has been shown to be particularly sensitive to the cocaine antagonist acting on the D1 dopamine receptor subtype (Caine et al., 1995), and the ventromedial shell of this brain area has been shown to express high level of the dopamine D3 receptor subtype mRNA (Diaz et al., 1995), which has been targeted in new pharmacological treatments to ameliorate the reinforcing properties of cocaine (e.g., cocaine craving) (Caine et al., 1997). In such context, the development of new selective drugs acting as a partial dopamine agonists (e.g., BP-897) at the D3 receptor subtype, has demonstrated to be useful in reducing self administration of cocaine and drug reinforcement (Pilla et al., 1999, Aston-Jones and Druhan, 1999). While most dopamine agonists activate several dopamine receptor subtypes in a non selective manner, this drug acts preferentially in D3 receptors showing a high affinity but low intrinsic activity on this receptor subtype (Pilla et al., 1999). So, at low dopamine activity, as seen during cocaine withdrawal, this drug stimulates D3 receptor, and conversely, at high dopamine activity, antagonizes the enhanced dopamine responses that occur during chronic cocaine addiction (Aston-Jones and Druhan, 1999). Parallel to these pharmacological observations, similar experiments have demonstrated that the central nucleus of the amygdala has a role in ethanol reinforcement. Administration of GABA antagonists or opioid peptide antagonists into the central nucleus of the amygdala can attenuate self-administration of oral ethanol (Hyttia and Koob, 1995). In addition, similar experimental observations have shown that microinjection of GABA agonists into the central nucleus of the amygdala decreased self-administration of ethanol in animals dependent of the same substance (Roberts et al., 1996). This data presumptuously proposes that the GABAergic system may be functionally altered to respond more efficiently to agonists during development of drug dependence (Koob et al., 1998). Therefore, the extended amygdala may be an important substrate responsable for the neurochemical changes that occur in the brain reward system in association with drug dependence (Koob et al., 1998). However, it may be speculated that besides the experimental observations that have demonstrated the neurochemical changes in specific neural circuits during drug addiction, other neurochemical systems also may be engaged within the neurocircuitry of the extended amygdala in order to overcome the chronic presence of perturbing drugs in the extracellular space, and to restore the normal function of the implicated neural circuitry affected by the presence of psychoactive drugs (Koob and Bloom, 1988). This hypothesis has been supported by pharmacological observations showing that neurochemical changes in the CRF peptidergic transmission pathway occur in drug-dependent animals during acute

TABLE 1
Neuroanatomical and neurochemical substrates por the reinforcing actions of drugs of abuse

Drugs of abuse	*Neurotransmission systems*	*Brain areas*	*Receptors*
Cocaine and amphetamines	Dopamine Serotonin OFQ/NOC ?	Nucleus accumbens Amygdala Nucleus accumbens	D1, D2, D3 5HT-1B ORL-1 VTA
Opiates Morphine and heroin	Dopamine GABA Opioid peptides OFQ/NOC ?	VTA Nucleus accumbens Nucleus accumbens Nucleus accumbens VTA	D1, D2 GABA-A, GABA-B MU opiod receptor ORL-1
Nicotine	Dopamine Opioid peptides?	VTA Nucleus accumbens	D1, D2 MU Opioid receptor
Etanol	Dopamine Opioid peptides Serotonin GABA Glutamate	VTA Nucleus accumbens Amygdala Amygdala, pars reticulata	D1, D2 MU opioid receptor 5HT-3, 5HT-2c GABA-A, GABA-B NMDA

VTA: VENTRAL TEGMENTALAREA. ORL-1: Opioid receptor like-1. D1, D2: Dopamine receptors subtypes 1 and 2. 5HT-3: 5HT-2c: Serotonin receptors subtypes. (Adapted from Koob et al., 1998 and modified by authors (B. Arias and P. Leff) for this publication)

drug withdrawal (Koob et al., 1994a, 1994b). Thus, neuronal secretion of CRF in the hypothalamus and in the extrahypothalamic areas of the brain (e.g., central nucleus of the amygdala, parabrachial area, bed nucleus of the stria terminalis, *locus coeruleus*, olfactory bulb) may mediate many of the neurochemical and behavioral aspects of stress associated with drug abstinence (Koob, 1999b).

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