The neurobiology of addiction: a neuroadaptational view relevant for diagnosis

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ABSTRACT

Aims The purpose of this review is to provide a synthesis of our knowledge of the neurobiological bases of addiction relevant for the diagnosis of addiction. **Methods** A heuristic framework of neuroadaptive changes within key brain neurocircuitry responsible for different stages of the addiction cycle is outlined and linked to human studies to provide important future translational links for diagnosis. **Results** Animal studies have revealed dysregulation of specific neurochemical mechanisms (dopamine, opioid peptides) in the brain reward systems and recruitment of brain stress systems (corticotropin-releasing factor) during the development of dependence that convey vulnerability to relapse. Animal studies have implicated the prefrontal cortex and basolateral amygdala in drug- and cue-induced relapse, respectively, and the brain stress systems in stress-induced relapse. Genetic studies suggest roles for the genes encoding the neurochemical elements involved in both the brain reward and stress systems in the vulnerability to addiction, and molecular studies have identified transduction and transcription factors that may mediate dependence-induced reward dysregulation. Human imaging studies reveal similar neurocircuits involved in acute intoxication, chronic drug dependence and vulnerability to relapse. **Conclusions** Major neurobiological changes in substance abuse disorders common to human and animal studies relevant for diagnosis include a compromised reward system, overactivated brain stress systems and compromised orbitofrontal/prefrontal cortex function. No biological markers of substance abuse disorders currently exist, but there are many promising neurobiological features of substance abuse disorders that will eventually aid in the specific diagnoses of substance use, misuse and dependence.

Keywords Addiction, animal models, extended amygdala, reward, stress.

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NEUROCIRCUITRY OF DRUG REWARD, DEPENDENCE AND CRAVING

Substance dependence is a chronically relapsing disorder characterized by: (1) compulsion to seek and take the drug, (2) loss of control in limiting intake and (3) emergence of a negative emotional state (e.g. dysphoria, anxiety, irritability) when access to the drug is prevented (defined here as dependence) [1]. Addiction and substance dependence (as currently defined by the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition) will be used interchangeably throughout this text to refer to a final stage of a usage process that moves from drug use to abuse to addiction. As such, it can be defined by its diagnosis, etiology and pathophysiology as a chronic relapsing disorder. Clinically, the occasional but limited use of a drug with the potential for abuse or dependence is distinct from escalated drug use and the emergence of a chronic drug-dependent state. An important goal of current neurobiological research is to understand the neuropharmacological and neuroadaptive mechanisms within specific neurocircuits that mediate the transition from occasional, controlled drug use to the loss of behavioral control over drug-seeking and drug-taking that defines chronic addiction.

Much of the recent progress in understanding the mechanisms of addiction has derived from the study of animal models of addiction on specific drugs, such as opiates, stimulants and alcohol [2]. While no animal model of addiction fully emulates the human condition, animal models do permit investigation of specific elements of the process of drug addiction. Such elements can be defined by models of different systems, models of psychological constructs such as positive and negative reinforcement

and models of different stages of the addiction cycle. While much focus in animal studies has been on the synaptic sites and molecular mechanisms in the nervous system on which drugs with dependence potential act initially to produce their positive reinforcing effects, new animal models of components of the negative reinforcing effects of dependence have been developed and are beginning to be used to explore how the nervous system adapts to drug use. The neurobiological mechanisms of addiction that are involved in various stages of the addiction cycle have a specific focus on certain brain circuits and the neurochemical changes associated with those circuits during the transition from drug taking to drug addiction, and how those changes persist in the vulnerability to relapse [3].

A key element of drug addiction is how the brain reward system changes with the development of addiction, and one must understand the neurobiological bases for acute drug reward to understand how these systems change with the development of addiction [1,4]. A principle focus of research on the neurobiology of the positive reinforcing effects of drugs with dependence potential has been the origins and terminal areas of the mesocorticolimbic dopamine system, and there is compelling evidence for the importance of this system in drug reward. This specific brain circuit has been broadened to include the many neural inputs and outputs that interact with the ventral tegmental area and the basal forebrain, and as such has been termed by some as the mesolimbic reward system. More recently, specific components of the basal forebrain that have been identified with drug reward have focused on the 'extended amygdala' [3,5]. The extended amygdala is comprised of the bed nucleus of the stria terminalis (BNST), the central nucleus of the amygdala and a transition zone in the medial subregion of the nucleus accumbens (shell of the nucleus accumbens). Each of these regions has certain cytoarchitectural and circuitry similarities [6]. As the neural circuits for the reinforcing effects of drugs with dependence potential have evolved, the role of neurotransmitters/neuromodulators have also evolved, and four of those systems have been identified to have a role in the acute reinforcing effects of drugs: mesolimbic dopamine, opioid peptide, γ-aminobutyric acid (GABA), endocannabinoid.

The neural substrates and neuropharmacological mechanisms for the negative motivational effects of drug withdrawal may involve disruption of the same neural systems implicated in the positive reinforcing effects of drugs. Measures of brain reward function during acute abstinence from all major drugs with dependence potential have revealed increases in brain reward thresholds as measured by direct brain stimulation reward [7,8,9,10,11,12]. These increases in reward thresholds may reflect changes in the activity of reward neurotransmitter systems in the midbrain and forebrain implicated in the positive reinforcing effects of drugs. Examples of such changes at the neurochemical level include decreases in dopaminergic and serotonergic transmission in the nucleus accumbens during drug withdrawal as measured by *in vivo* microdialysis [13,14], increased sensitivity of opioid receptor transduction mechanisms in the nucleus accumbens during opiate withdrawal [15], decreased GABAergic and increased *N*-methyl-D-aspartate (NMDA) glutamatergic transmission during alcohol withdrawal [16–19] and differential regional changes in nicotine receptor function [20,21]. The decreases in reward neurotransmitters have been hypothesized to contribute significantly to the negative motivational state associated with acute drug abstinence and long-term biochemical changes that contribute to the clinical syndrome of protracted abstinence and vulnerability to relapse [3].

Different neurochemical systems involved in stress modulation also may be engaged within the neurocircuitry of the brain stress systems in an attempt to overcome the chronic presence of the perturbing drug and to restore normal function despite the presence of drug. Both the hypothalamic–pituitary–adrenal axis and the brain stress system mediated by corticotropin-releasing factor (CRF) are dysregulated by chronic administration of drugs with dependence potential with a common response of elevated adrenocorticotropic hormone and corticosterone and amygdala CRF during acute withdrawal from all major drugs with a potential toward abuse or dependence [22–27]. Acute withdrawal from drugs also may increase the release of norepinephrine in the BNST and decrease levels of neuropeptide Y (NPY) in the central and medial nuclei of the amygdala [28].

These results suggest, during the development of dependence, not only a change in function of neurotransmitters associated with the acute reinforcing effects of drugs (dopamine, opioid peptides, serotonin and GABA) but also recruitment of the brain stress system (CRF and norepinephrine) and dysregulation of the NPY brain antistress system [3]. Activation of the brain stress systems may contribute to the negative motivational state associated with acute abstinence [29]. Thus, reward mechanisms in dependence are compromised by disruption of neurochemical systems involved in processing natural rewards and by recruitment of antireward systems [30].

The neuroanatomical entity termed the extended amygdala [6] may thus represent a common anatomical substrate for acute drug reward and a common neuroanatomical substrate for the negative effects on reward function produced by stress that help drive compulsive drug administration. The extended amygdala receives numerous afferents from limbic structures such as the

basolateral amygdala and hippocampus, and sends efferents to the medial part of the ventral pallidum and a large projection to the lateral hypothalamus, thus further defining the specific brain areas that interface classical limbic (emotional) structures with the extrapyramidal motor system [31].

Animal models of 'craving' involve the use of drug-primed reinstatement, cue-induced reinstatement or stress-induced reinstatement in animals that have acquired drug self-administration and have then been subjected to extinction from responding for the drug [2]. Most evidence from animal studies suggests that drug-induced reinstatement is localized to the medial prefrontal cortex/nucleus accumbens/ventral pallidum circuit mediated by the neurotransmitter glutamate [32]. In contrast, neuropharmacological and neurobiological studies using animal models for cue-induced reinstatement involve the basolateral amygdala as a critical substrate with a possible feed-forward mechanism through the prefrontal cortex system involved in drug-induced reinstatement [33,34]. Stress-induced reinstatement of drug-related responding in animal models appears to depend on the activation of both CRF and norepinephrine in elements of the extended amygdala (central nucleus of the amygdala and BNST) [35,36].

In summary, three neurobiological circuits have been identified that have heuristic value for the study of the neurobiological changes associated with the development and persistence of drug dependence. The acute reinforcing effects of drugs of abuse that comprise the binge/intoxication stage of the addiction cycle most probably involve actions with an emphasis on the extended amygdala reward system and inputs from the ventral tegmental area and arcuate nucleus of the hypothalamus. In contrast, the symptoms of acute withdrawal important for addiction, such as negative affect and increased anxiety associated with the withdrawal/negative affect stage, most probably involve decreases in function of the extended amygdala reward system but also a recruitment of brain stress neurocircuitry. The craving stage, or preoccupation/anticipation stage, involves key afferent projections to the extended amygdala and nucleus accumbens, specifically the prefrontal cortex (for drug-induced reinstatement) and the basolateral amygdala (for cue-induced reinstatement). Compulsive drug-seeking behavior is hypothesized to be driven by ventral striatal–ventral pallidal–thalamic–cortical loops (Fig. 1) [37].

MOLECULAR AND CELLULAR TARGETS WITHIN THE BRAIN CIRCUITS ASSOCIATED WITH ADDICTION

Acknowledging that all drugs of abuse share some common neurocircuitry actions, namely inhibition of medium spiny neurons in the nucleus accumbens either through dopamine or other G_i-coupled receptors, the search at the molecular level has led to examining how repeated perturbation of intracellular signal transduction pathways leads to changes in nuclear function and altered rates of transcription of particular target genes. Altered expression of such genes would lead to altered activity of the neurons where such changes occur, and ultimately to changes in neural circuits in which those neurons operate.

Two transcription factors in particular have been implicated in the plasticity associated with addiction: cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) and ΔFosB. CREB regulates the transcription of genes that contain a CRE site (cAMP response element) within the regulatory regions and can be found ubiquitously in genes expressed in the central nervous system such as those encoding neuropeptides, synthetic enzymes for neurotransmitters, signaling proteins and other transcription factors. CREB can be phosphorylated by protein kinase A and by protein kinases regulated by growth factors, putting it at a point of convergence for several intracellular messenger pathways that can regulate the expression of genes.

Much work in the addiction field has shown that activation of CREB in the nucleus accumbens, one part of the brain reward circuit, is a consequence of chronic exposure to opiates, cocaine and alcohol, and deactivation in the central nucleus of the amygdala, another part of the reward circuit. The activation of CREB is linked to the activation of the 'dysphoria'-inducing κ opioid receptor binding the opioid peptide dynorphin and has led one researcher, Eric Nestler, to argue:

> There is now compelling evidence that up-regulation of the cAMP pathway and CREB in this brain region (nucleus accumbens) represents a mechanism of 'motivational tolerance and dependence': these molecular adaptations decrease an individual's sensitivity to the rewarding effects of subsequent drug exposures (tolerance) and impair the reward pathway (dependence) so that after removal of the drug the individual is left in an amotivational, dysphoric, or depressed-like state [38].

In contrast, decreased CREB phosphorylation has been observed in the central nucleus of the amygdala during alcohol withdrawal and has been linked to decreased NPY function and consequently the increased anxiety-like responses associated with acute alcohol withdrawal [39]. These changes are not necessarily mutually exclusive and point to transduction mechanisms that could produce neurochemical changes in the neurocircuits outlined above as important for breaks with reward homeostasis in addiction.

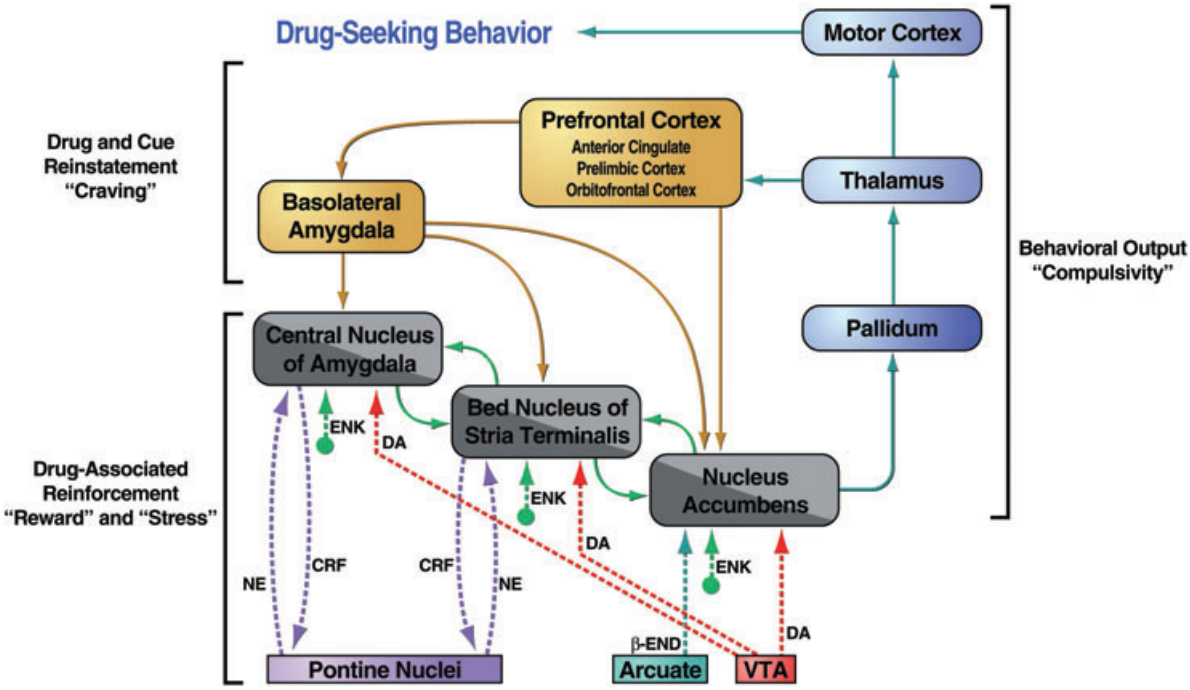

Figure 1 Key common neurocircuitry elements in drug-seeking behavior of addiction. Three major circuits that underlie addiction can be distilled from the literature. A drug-reinforcement circuit ('reward' and 'stress') is comprised of the extended amygdala, including the central nucleus of the amygdala, the bed nucleus of the stria terminalis and the transition zone in the shell of the nucleus accumbens. Multiple modulator neurotransmitters are hypothesized, including dopamine and opioid peptides for reward and corticotropin-releasing factor and norepinephrine for stress. The extended amygdala is hypothesized to mediate integration of rewarding stimuli or stimuli with positive incentive salience and aversive stimuli or stimuli with negative aversive salience. During acute intoxication, valence is weighted on processing rewarding stimuli, and during the development of dependence aversive stimuli come to dominate function. A drug- and cue-induced reinstatement ('craving') neurocircuit is comprised of the prefrontal (anterior cingulate, prelimbic, orbitofrontal) cortex and basolateral amygdala with a primary role hypothesized for the basolateral amygdala in cue-induced craving and a primary role for the medial prefrontal cortex in drug-induced craving, based on animal studies. Human imaging studies have shown an important role for the orbitofrontal cortex in craving. A drug-seeking ('compulsive') circuit is comprised of the nucleus accumbens, ventral pallidum, thalamus and orbitofrontal cortex. The nucleus accumbens has long been hypothesized to have a role in translating motivation to action and forms an interface between the reward functions of the extended amygdala and the motor functions of the ventral striatal–ventral pallidal–thalamic–cortical loops. The striatal–pallidal–thalamic loops reciprocally move from prefrontal cortex to orbitofrontal cortex to motor cortex, leading ultimately to drug-seeking behavior. Note that for the sake of simplicity, other structures are not included, such as the hippocampus (which presumably mediates context-specific learning, including that associated with drug actions). Also note that dopamine and norepinephrine both have widespread innervation of cortical regions and may modulate function relevant to drug addiction in those structures. DA, dopamine; ENK, enkephalin; CRF, corticotropin-releasing factor; NE, norepinephrine; β-END, β-endorphin (reproduced with permission from Koob & Le Moal [37])

The molecular changes associated with long-term changes in brain function as a result of chronic exposure to drugs of abuse have been linked to changes in transcription factors, factors that can change gene expression and produce long-term changes in protein expression and, as a result, neuronal function. While acute administration of drugs of abuse can cause a rapid (within hours) activation of members of the Fos family, such as c-*fos*, FosB, Fra-1 and Fra-2 in the nucleus accumbens, other transcription factors (isoforms of ΔFosB) accumulate over longer periods of time (days) with repeated drug administration. Animals with activated ΔFosB have exaggerated sensitivity to the rewarding effects of drugs of abuse. Nestler has argued that ΔFosB may be a sustained molecular 'switch' that helps to initiate and maintain a state of addiction. How changes in ΔFosB that can last for days can translate into vulnerability to relapse remains a challenge for future work [38].

Genetic and molecular genetic animal models have provided a convergence of data to support the neuropharmacological substrates identified in neurocircuitry studies. High-alcohol-preferring rats have been bred that show high voluntary consumption of alcohol, increased anxiety-like responses and numerous neuropharmacological phenotypes, such as decreased dopaminergic activity and decreased NPY activity [40,41]. In an alcohol-preferring and -non-preferring cross, a quantitative trait locus was identified on chromosome 4, a region

to which the gene for NPY has been mapped. In the inbred preferring and non-preferring quantitative trait loci analyses, loci on chromosomes 3, 4 and 8 have been identified which correspond to loci near the genes for the dopamine D_2 and serotonin $5HT_{1B}$ receptors [42].

Advances in molecular biology have led to the ability to inactivate systematically the genes that control the expression of proteins that make up receptors or neurotransmitter/neuromodulators in the central nervous system using the gene knock-out approach. Knock-out mice have a gene inactivated by homologous recombination. A knock-out mouse deficient in both alleles of a gene is homozygous for the deletion and is termed a null mutation (–/–). A mouse which is deficient in only one of the two alleles for the gene is termed a heterozygote (+/–). Transgenic knock-in mice have an extra gene introduced into their germ line. An additional copy of a normal gene is inserted into the genome of the mouse to examine the effects of overexpression of the product of that gene. Alternatively, a new gene, not normally found in the mouse, can be added, such as a gene associated with specific pathology in humans. Wild-type controls are animals bred through the same breeding strategies involving mice that received the transgene injected into the fertilized egg (transgenics) or a targeted gene construct injected into the genome via embryonic stem cells (knock-out) but lacking the mutation on either allele of the gene in question. While such an approach does not guarantee that these genes are the ones that convey vulnerability in the human population, they provide viable candidates for exploring the genetic basis of endophenotypes associated with addiction [43].

Notable positive results with gene knock-out studies in mice have focused on knock-out of the μ opioid receptor, which eliminates opioid, nicotine and cannabinoid reward and alcohol drinking in mice [44]. Opiate (morphine) reinforcement as measured by conditioned place preference or self-administration is absent in μ knock-out mice, and there is no development of somatic signs of dependence to morphine in these mice. Indeed, to date all morphine effects tested, including analgesia, hyperlocomotion, respiratory depression and inhibition of gastrointestinal transit are abolished in μ knock-out mice [45].

Selective deletion of the genes for expression of different dopamine receptor subtypes and the dopamine transporter has revealed significant effects to challenges with psychomotor stimulants [46,47]. Dopamine D_1 receptor knock-out mice show no response to D_1 agonists or antagonists and show a blunted response to the locomotor-activating effects of cocaine and amphetamine. D_1 knock-out mice also are impaired in their acquisition of intravenous cocaine self-administration compared with wild-type mice. D_2 knock-out mice have severe motor deficits and blunted responses to psychostimulants and opiates, but the effects on psychostimulant reward are less consistent. Dopamine transporter knock-out mice are dramatically hyperactive but also show a blunted response to psychostimulants. Although developmental factors must be taken into account for the compensatory effect of deleting any one or a combination of genes, it is clear that D_1 and D_2 receptors and the dopamine transporter play important roles in the actions of psychomotor stimulants [48].

BRAIN IMAGING CIRCUITS INVOLVED IN HUMAN ADDICTION

Brain imaging studies using positron emission tomography with ligands for measuring oxygen utilization or glucose metabolism or using magnetic resonance imaging techniques are providing dramatic insights into the neurocircuitry changes in the human brain associated with the development and maintenance and even vulnerability to addiction. These imaging results bear a striking resemblance to the neurocircuitry identified by human studies. During acute intoxication with alcohol, nicotine and cocaine there is an activation of the orbitofrontal cortex, prefrontal cortex, anterior cingulate, extended amygdala and ventral striatum. This activation is often accompanied by an increase in availability of the neurotransmitter dopamine. During acute and chronic withdrawal there is a reversal of these changes with decreases in metabolic activity, particularly in the orbitofrontal cortex, prefrontal cortex and anterior cingulate, and decreases in basal dopamine activity as measured by decreased D_2 receptors in the ventral striatum and prefrontal cortex. With limited studies, cue-induced reinstatement appears to involve a reactivation of these circuits resembling acute intoxication [49–51]. Two strongly represented markers for active substance dependence in humans across drugs of different neuropharmacological actions are decreases in prefrontal cortex metabolic activity and decreases in brain dopamine D_2 receptors that are hypothesized to reflect decreases in brain dopamine function.

CONCLUSIONS

Much progress in neurobiology has provided a heuristic neurocircuitry framework with which to identify the neurobiological and neuroadaptive mechanisms involved in the development of drug addiction. The brain reward system implicated in the development of addiction is comprised of key elements of a basal forebrain macrostructure termed the extended amygdala and its connections. Neuropharmacological studies in animal models of addiction have provided evidence for the dysregulation of

specific neurochemical mechanisms in specific brain reward neurochemical systems in the extended amygdala (dopamine, opioid peptides, GABA and endocannabinoids). There also is recruitment of brain stress systems (CRF and norepinephrine) and dysregulation of brain antistress systems (NPY) that provide the negative motivational state associated with drug abstinence. The changes in reward and stress systems are hypothesized to remain outside a homeostatic state, and as such convey the vulnerability for development of dependence and relapse in addiction. Additional neurobiological and neurochemical systems have been implicated in animal models of relapse with the prefrontal cortex and basolateral amygdala (and glutamate systems therein) being implicated in drug- and cue-induced relapse, respectively. The brain stress systems in the extended amygdala are directly implicated in stress-induced relapse. Genetic studies to date in animals suggest roles for the genes encoding the neurochemical elements involved in the brain reward (dopamine, opioid peptide) and stress (NPY) systems in the vulnerability to addiction, and molecular studies have identified transduction and transcription factors that may mediate the dependence-induced reward dysregulation (CREB) and chronic-vulnerability changes (ΔFosB) in neurocircuitry associated with the development and maintenance of addiction. Human imaging studies reveal similar neurocircuits involved in acute intoxication, chronic drug dependence and vulnerability to relapse.

While no exact imaging results necessarily predict addiction, two salient changes in established and unrecovered substance-dependent individuals that cut across different drugs are decreases in orbitofrontal/prefrontal cortex function, decreases in brain dopamine D_2 receptors and overactive brain stress systems. No biochemical markers are sufficiently specific to predict a given stage of the addiction cycle, but changes in certain intermediate early genes with chronic drug exposure in animal models show promise of long-term changes in specific brain regions that may be common to all drugs of abuse. Although there are no biological markers of substance abuse disorders on the immediate horizon, there are many promising and continually evolving biological and neurobiological features of substance use disorders that eventually will aid in the specific diagnoses of substance use, misuse and dependence.

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