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The Addicted Brain Craves New Neurons: Putative Role for Adult-Born Progenitors in Promoting Recovery

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Abstract

Addiction is a chronic relapsing disorder associated with compulsive drug taking and drug seeking and a loss of control in limiting intake, reflected in three stages of a recurrent cycle: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (“craving”). This review discusses the role of adult-born neural and glial progenitors in drug-seeking associated with the different stages of the addiction cycle. A review of the current literature suggests that the loss of newly born progenitors, particularly in hippocampal and cortical regions, may play a role in determining vulnerability to relapse in rodent models of drug addiction. The normalization of drug-impaired neurogenesis or gliogenesis may help reverse neuroplasticity during abstinence, and thus may help reduce the vulnerability to relapse and aid recovery.

Keywords

Prefrontal cortex; Gliogenesis; Hippocampus; neurogenesis; addiction

Introduction

Addiction to drugs of abuse has taken emotional and financial tolls on society, cutting across ages, races, ethnicities, and genders with increases in mortality, morbidity, and economic costs. Broadly defined, addiction is a chronic relapsing disorder characterized by a compulsion to seek and take drugs, a loss of control in limiting intake, and emergence of a negative emotional state during withdrawal [1]. The addiction cycle involves elements of both impulsivity and compulsivity and is composed of three stages: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (craving) (see Glossary). The study of the neurobiological bases of addiction and relapse has significantly progressed, but to date few treatments are known to reverse the drug-induced neuroplasticity changes that convey the vulnerability to relapse (see [2] for a review). Understanding the neuroplastic changes that underlie the relapse stage of addiction can help generate better treatment options for addiction.

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The ability of the brain to continuously generate new progenitors throughout adulthood may have important implications for addiction. Broadly defined, progenitors are the progeny of stem cells characterized by limited self-renewal and can survive and mature into differentiating cells, such as neurons and glia, in the brain. There are two main neurogenic areas in the adult brain: the subventricular zone (SVZ) that lines the lateral ventricles in the forebrain, which contain progenitor cells that give rise to neurons in the olfactory bulb, and the subgranular zone (SGZ) in the dentate gyrus (DG) of the hippocampus that gives rise to granule cell neurons (Figure 1). Neurogenesis has also been shown to occur in the neocortex (medial prefrontal cortex [mPFC]; Figure 2) [3–5]. Emerging evidence suggests that alterations in the rate of adult neurogenesis and gliogenesis in these brain regions, may contribute to the regulation of drug taking and drug seeking, particularly in the hippocampus and cortex. This review, therefore, focuses on altered plasticity in these regions that result from drug self-administration and considers recent findings that implicate alterations in neural and glial progenitors in the phenomenology of drug abuse.

Hippocampus and mPFC: Roles in Drug Taking and Seeking

Animal models have been developed that parallel the three stages of the addiction cycle and include various paradigms of drug self-administration for the binge/intoxication stage [6], motivational elements of withdrawal for the withdrawal/negative affect stage, and drug-, cue-, context-, and stress-induced reinstatement for the preoccupation/anticipation stage [7]. These models have been extensively used to uncover the key brain regions, brain circuitry, neurotransmitters, and neuromodulators associated with drug-taking and - seeking behavior [8, 9]. The ventral striatum (i.e., a brain region that includes the nucleus accumbens [NAc] core and shell and some nuclei of the olfactory tubercle), a terminal projection of the neural connections from the ventral tegmental area (VTA) and PFC, is considered a focal point for the reward and reinstatement associated with drug-seeking behavior [1]. The release of the neurotransmitter dopamine in these regions is considered to be significantly modulated by various drugs of abuse, particularly psychostimulants, such as cocaine, methamphetamine, and nicotine, to produce their rewarding effects. Furthermore, evidence indicates that neurotransmitters other than dopamine may also play a significant role in the rewarding effects of drugs of abuse, including opioid peptides. The NAc is also tactically situated in the brain such that it receives inputs from several other brain regions, including the mPFC, basolateral amygdala, insula, and hippocampal regions, and these inputs are hypothesized to play a key role in cue- and context-specific associations with drugs [1]. Activation of dopamine, glutamate, and corticotropin-releasing factor systems in these key brain regions are associated with drug-, cue-, and stress-induced reinstatement, respectively [10, 11].

The hippocampus and mPFC are implicated in the modulation of the reinforcing actions of drugs of abuse and play a key role in the reinstatement of drug-seeking behavior [1, 8]. Hippocampal integrity may be important for drug-context memories associated with drug reward [12] and is fundamental to the formation of context-specific memories associated with the reinstatement of drug seeking [13–16]. This hypothesis is supported by the observation that activation and inactivation of the hippocampal-subicular and hippocampal-VTA pathways, respectively, enhances and blocks drug-seeking behavior in rodents [14, 16]. Thus, it appears that the hippocampal neural plasticity that underlies learning and memory function also contributes to the modulation of reward pathways in drug addiction [17].

The prelimbic and infralimbic cortices of the mPFC have widespread connections to the basal forebrain, amygdala, and hypothalamus that mediate diverse functions, including attentional processing, goal-directed behavior, and working memory [18]. These functions that depend on the mPFC play a prominent role in the fundamental pathway that underlies

drug reinstatement triggered by drug priming, conditioned stimuli, and external stress [8]. Pharmacologically, excitatory inputs from the hippocampus and mPFC increase dopamine release in the NAc and VTA, which have been hypothesized to contribute to drug seeking in response to cues and context following extinction [16, 19–21]. Altogether, the hippocampus and PFC appear to play critical roles in the modulation of the reinforcing effects of drugs, and the release of neurotransmitters from key brain regions associated with cues and context are hypothesized to be essential components of the human condition of craving.

Additionally, it is well established that the neural connections from the CA1 and subiculum of the hippocampus that terminate in the prelimbic cortex of the mPFC function to facilitate the acquisition, maintenance, and independent storage and consolidation of declarative, spatial, and associative long-term memories [22]. The mPFC and hippocampus interact under a variety of functional demands, including drug taking. Such interactions may depend on both memory processes and content [1, 23] and may be critical for optimal performance [24]. Therefore, excessive drug use during abuse and addiction could compromise normal learning and memory systems and, in addition to activation of reward pathways associated with craving, may also disrupt executive control pathways, thus contributing to the impulsivity and impairment of decision making characteristic of individuals with addiction [25].

Hippocampal Neurogenesis, Drug Taking, and Drug Seeking

One possible contributor to hippocampal neural plasticity is adult neurogenesis in the SGZ of the DG. The process of neurogenesis involves stem-like precursor cells that proliferate into preneuronal progenitors, which in turn differentiate into immature neurons and eventually mature into granule cell neurons [26] (Figure 1). It is widely acknowledged that a large proportion (> 80%) of hippocampal progenitors migrate a short distance to become granule neurons in the DG [27]. Furthermore, there is sufficient evidence that supports the functional incorporation of the newly born neurons in the DG [28–30], although the hypothesis concerning the role of new DG neurons on sparse coding (facilitation of the formation of new hippocampus-dependent memories) awaits confirmation [31–33]. New DG neurons are also involved in certain aspects of addiction. Reinforcing doses of drugs self-administered by rodents decrease DG neurogenesis [34–43] (Table 1). It has been hypothesized that new DG neurons may block memories associated with the contextual reinstatement of drug seeking or enhance extinction learning [44, 45]. Thus, the reduction in spontaneous neurogenesis (i.e., a reduction in neuronal turnover) that is observed after self-administration of various drugs of abuse may result in a more robust and long-lasting memory of drug taking and seeking or decrease extinction learning. However, additional experiments are needed before the precise function of DG neurogenesis in drug taking and seeking is determined [14, 46]

The developmental stages of DG progenitors and the lineage for adult-generated DG neurons have been determined by utilizing transgenic mice that express nestin (a type VI intermediate filament protein that is highly expressed in neural progenitor cells) under the control of a green fluorescent protein (GFP) promoter (Figure 1B). Data from mouse studies indicate that proliferating cells in the postnatal DG are not homogeneous, and the process of postnatal neurogenesis is an uncoordinated cluster of developmental stages that progress in parallel, including actively dividing cells that are radial glia-like (type₁), preneuronal (type_{2a}), intermediate (type_{2b}), and early neuronal (type₃) [47] (Figure 1B). Importantly, pools of slowly dividing type₁ cells appear to be the precursors of adult-generated DG neurons. Furthermore, the distinct cell types have been individually labeled using combinations of exogenous (5-bromo-2'-deoxyuridine [BrdU]; Box 1 and Figure 2B) and endogenous (Ki-67, proliferating cell nuclear antigen, phosphorylated histone-H3, sex-

determining region Y-box 2 [Sox2]) markers of cell proliferation and cell differentiation (doublecortin [DCX], neurogenic differentiation factor 1 [NeuroD1], and polysialic acid-neural cell adhesion molecule [PSA-NCAM]) [47, 48]. By incorporating these exogenous and endogenous markers of proliferation and differentiation, critical information has been obtained in adult rat and nonhuman primate models to demonstrate a similar developmental profile of DG progenitors compared with mouse models.

Recent studies have focused on how drugs of abuse alter the process of DG neurogenesis by modifying the developmental stages of newly born adult DG progenitors and their pathway to attain a neuronal phenotype [47] (Table 1). For example, limited-access nicotine self-administration decreases the proliferation and differentiation of DG progenitors [36]. Extended-access heroin self-administration, which results in compulsive drug seeking, decreases the proliferation of DG progenitors [38]. Limited- and extended-access cocaine self-administration decreases the proliferation of DG progenitors [39, 43]. Furthermore, extended access to cocaine increases differentiation without altering the survival of progenitors [39]. Limited- and extended-access methamphetamine self-administration decreases the proliferation, differentiation, and survival of DG progenitors [35]. Intermittent-access methamphetamine self-administration, surprisingly, increases the proliferation and differentiation of DG progenitors, but such an increase in the immature neuronal population was not associated with an increase in neurogenesis [35]. Nondependent ethanol self-administration studied in both rodent and nonhuman primate models decreased the proliferation, differentiation, and survival of DG progenitors [37, 48, 49]. Excessive drinking during alcohol dependence was also found to decrease all aspects of DG neurogenesis [37, 50]. Altogether, certain neuromodulatory effects on neurogenesis appear to be particularly sensitive to the amount of daily drug intake and that a higher amount of drug intake produces more pronounced effects on DG neurogenesis.

Role of Dentate Gyrus Neurogenesis in Drug Taking and Seeking

Mechanistic approaches to address potential roles for adult-generated DG neurons in drug taking and drug seeking are currently being undertaken. For example, procedures such as low doses of irradiation have been used to ablate proliferating progenitors in the DG, and changes in the animal's environment (e.g., voluntary wheel running in the home cage) have been used to enhance the proliferation and maturation of hippocampal progenitors to provide a functional link between hippocampal neurogenesis and hippocampal-dependent memory [51]. Similarly, irradiation and wheel running have been used to examine the relationship between hippocampal neurogenesis and drug taking and seeking. Studies in rats that have used irradiation to ablate hippocampal neurogenesis before any cocaine experience have demonstrated that irradiated rats had enhanced cocaine-taking behavior, reflected by increased self-administration on a fixed-ratio schedule of reinforcement in an extended-access model compared with non-irradiated rats [46]. In other studies, voluntary wheel running before and during cocaine and methamphetamine experience reduced the maintenance of drug-reinforced behavior [52–55] and positive-reinforcing effects of cocaine compared with sedentary animals [56]. Voluntary wheel running before and during ethanol experience reduced ethanol self-administration [57] and diminished intoxicated behavioral responses to heavy binge ethanol administration compared with sedentary controls [58]. Such studies did not measure neurogenesis in the hippocampus, which would be needed to correlate DG neurogenesis with the drug-induced behavioral outcomes. However, some of the effects of wheel running on drug taking may be attributable to the neuromodulatory effects of running on DG neurogenesis [59]. Thus, increased hippocampal neurogenesis may be beneficial in diminishing the motivational impact of drugs after chronic exposure to drugs of abuse.

The effects on DG proliferation, differentiation, and neurogenesis after drug withdrawal or reinstatement to drug seeking after abstinence have been less well-studied. The studies that have addressed this issue have found that withdrawal from cocaine self-administration decreased the proliferation and enhanced the differentiation and maturation of DG progenitors compared with control animals [39, 60]. Although much more work is required to support the hypothesis of the enhanced survival of DG progenitors during withdrawal from drug exposure, one could propose that the abnormal survival of progenitors during withdrawal from the drug could be a part of the recovery process. Consistent with this hypothesis, other studies suggested that the degree of neurogenesis in the DG may affect behavioral responses after drug withdrawal. Ablation of DG progenitors by irradiation during withdrawal from cocaine self-administration delayed the extinction of cocaine-seeking behavior in rats [46]. Furthermore, voluntary wheel running before and during extinction reduced drug-primed [61, 62] and cue-induced [62, 63] cocaine-seeking behavior in rats after a period of forced abstinence. The levels of neurogenesis in the hippocampus were not measured in these studies, but, as discussed above, such findings suggest that enhanced neurogenesis after wheel running may be an underlying mechanism for such a reduction in drug-induced responses after withdrawal. Other studies have also indicated a beneficial role for hippocampal neurogenesis in reducing the vulnerability to relapse. For example, rats genetically inbred for high novelty-seeking behavior and rats exposed to early environmental stress (e.g. prenatal stress) exhibited reduced DG neurogenesis [42, 64, 65] and were prone to developing addictive behaviors [66–68]. Although more studies are needed, adult DG neurogenesis appears to be important for the maintenance of hippocampal neuroplasticity, such that reducing spontaneous DG neurogenesis during abstinence may enhance the vulnerability to relapse, and enhancing DG neurogenesis during abstinence may help reduce the vulnerability to relapse.

Role of Olfactory Bulb Neurogenesis in Addiction

Neurogenesis in the olfactory bulb contributes to olfactory function [69], and deficits in olfactory function and sensitivity are evident in drug and alcohol dependent humans [70, 71] and these behavioral deficits may predict the propensity to relapse [72]. Recent studies have demonstrated that chronic cocaine self-administration and exposure to chronic ethanol vapors in rodents (models of drug dependence) reduce the proliferation of neural progenitors in the SVZ, a source of adult-generated olfactory neurons [39, 50]. However, protracted withdrawal from chronic drug exposure normalizes proliferation in the SVZ, albeit producing permanent changes in the SVZ neurogenic niche [39, 50]. Therefore, future studies should address whether drug-induced decreases in SVZ proliferation and consequential decreases in olfactory bulb neurogenesis produce drug-induced deficits in olfaction.

Role for Glia in Modulating Neuroplasticity Responses in the Brain

Glial fibrillary acidic protein (GFAP)- and non-GFAP-positive glia have received some attention in the past decade with respect to their possible roles in addiction. GFAP-glia in the mPFC contain cystine/glutamate antiporters [73] that maintain extracellular nonsynaptic glutamate levels [74] and provide physical support to neurons by regulating extracellular potassium and the uptake of glutamate at synapses [75]. The glutamate released by GFAP-positive glia antiporters is known to modulate neuronal metabotropic glutamate receptors and extracellular glutamate and dopamine levels in areas other than the mPFC [74]. Thus, this is one way that GFAP-positive glia can influence local synaptic activity [76]. Although neuroadaptations in mPFC GFAP-glia have not yet been clearly indicated in the reinstatement of drug seeking [74], it is a hypothesis that merits testing.

NG2 chondroitin sulphate proteoglycan (NG2)-positive glia (non-GFAP-positive glia) express AMPA receptors [77], making them antigenically distinct from GFAP-glia [75, 78]. These cells are known to play important roles in the nervous system and may in certain enriched environments support the neurogenesis of cortical progenitors [79–81]. NG2-glia are involved in the induction and expression of long-term potentiation (LTP) at neuron-glia synapses [77]. Moreover, NG2-positive glia contain voltage-gated ion channels [82] that assist with maintaining the homeostatic function of surrounding neurons in the hippocampus [77]. Other important roles of NG2-glia include draining excess ions and neurotransmitters from the extracellular space, and this activity may be important for maintaining the proliferative environment and activity of surrounding neurons [83]. Therefore, NG2-glia are likely to play a more prominent role in maintaining both synaptic neuromodulatory responses and neuroplastic responses in the adult brain after brain insults, such as addiction and relapse.

Role of Dentate Gyrus Gliogenesis in Addiction

In addition to the neuronal network that mediates most of the neuromodulatory effects associated with drug taking and drug seeking, glia-mediated nonsynaptic events, including supportive/maintenance roles, also appear to be important. In the DG, more than 10% of adult-born progenitors mature into glia, mostly into astroglia that express GFAP [84]. Notably, drugs that are reinforcing *in vivo* do not alter the number or proportion of DG progenitors that mature into GFAP-positive glia [35–39]. However, newly born DG microglia may play a role in drug withdrawal [85, 86]. Recent studies have demonstrated that withdrawal from ethanol produces immediate exaggerated microglial proliferation in the DG and several other hippocampal regions [85, 86]. These studies suggest that the hippocampus in alcohol-dependent animals is greatly susceptible to inflammation, and such pro-inflammatory events (i.e., microglial proliferation) following withdrawal may, in turn, affect DG neurogenesis and the optimal function of the hippocampus [87].

Role of mPFC Gliogenesis in Addiction

It has been demonstrated, using combinatorial labeling techniques, that a large proportion of progenitors in the adult mammalian mPFC, including the human PFC, mature into a glial phenotype (Figure 2A, B; regulated intramembrane proteolysis-positive [RIP+] oligodendrocyte, NG2+ oligodendrocyte precursor, and/or GFAP+ astrocyte), and a small proportion mature into interneurons [3–5]. The distribution of progenitors in the mPFC is suggested to be uniform, and most of the cells in the mPFC (> 50%) mature into oligodendrocyte precursors [4, 5, 88–90] (Fig. 2C). External factors have been shown to both positively (e.g., antidepressants, wheel running) and negatively (e.g., stress, drugs of abuse, ethanol) regulate cell birth and cell maturity in the mPFC [4, 37, 88, 90–94].

Intravenous self-administration of methamphetamine decreased (with limited and extended access) and increased (with intermittent access) the birth and survival of mPFC progenitors [4] (Table 2), whereas intermittent, limited, and extended access to methamphetamine increased cell death in the mPFC [4]. Such findings support altered mPFC plasticity after methamphetamine exposure. Moreover, they indicate that the effects of methamphetamine on cell death (which includes both neurons and glia) are distinct compared with the changes observed with cell birth (i.e., neurogenesis is observed to a much lesser extent in the mPFC compared with gliogenesis). The progenitors reduced by limited and extended access were GFAP-positive and NG2-positive glia in the mPFC, whereas most of the progenitors induced by intermittent access were phenotyped as NG2-positive glia [4]. Although evidence suggests that increases in GFAP-positive glia occur in almost all injury types restricted to sites of neuronal loss [95], normal levels of GFAP-positive glia and enhanced

NG2-positive glial levels after intermittent access to methamphetamine could indicate otherwise. For example, an enhanced NG2-glial response could indicate a protective mechanism (i.e., characteristic central nervous system [CNS] gliosis) against methamphetamine-induced brain insult [96]. The rapid response of increased NG2-glia proliferation after intermittent methamphetamine insult could be attributable to altered glutamate release locally by glutamatergic neurons following methamphetamine exposure. This is because NG2-glial differentiation and the response to CNS insult are mediated by glutamate released by neurons and GFAP-positive glia [97]. An increase in the number of NG2-positive glia that express AMPA receptors may further produce alterations in extracellular glutamate in the mPFC, which could contribute to the neuromodulatory effects that occur via the mPFC in the other brain regions (e.g., the NAc and VTA) associated with the binge/intoxication stage of the addiction cycle. The decrease in GFAP-positive and NG2-positive glia after prolonged limited and extended access to methamphetamine could be indicative of the neurotoxic effects of methamphetamine.

Exposure to ethanol vapors after excessive drinking (i.e., operant ethanol self-administration in rodents followed by intermittent exposure to ethanol vapors over several weeks) resulted in a decrease in the birth and survival of mPFC progenitors [37]. Nondependent ethanol-drinking rats did not exhibit significant changes in cell birth and cell survival in the mPFC. Cell death was differentially regulated in both nondependent ethanol-drinking and alcohol-dependent rats, and both groups showed significantly decreased apoptosis [44], indicating a compensatory state in the mPFC in response to excessive ethanol exposure.

What can be gleaned from these two studies is that psychostimulants and ethanol alter the local homeostasis of the proliferative environment in the mPFC by decreasing the birth of newly born cells and increasing the death of existing older cells. Therefore, the involvement of the PFC in several aspects of an addiction phenotype, including an association with classical conditioning to drug exposure [98] (a phenomenon associated with relapse to drug seeking), and the response inhibition behavior maintained by the PFC may involve a key role of newly generated progenitors.

In addition to these correlative studies, a few studies have demonstrated that an altered local microenvironment in the PFC contributes to the neuroadaptations associated with the reinstatement of drug seeking. Recent studies have implicated reduced brain-derived neurotrophic factor (BDNF) levels [99] and increased phosphorylated extracellular signal-regulated kinase (pERK) [100] in the mPFC in cocaine craving and cocaine-associated memories that activate the neuronal circuitry associated with relapse. Particularly notable, wheel running during forced abstinence prevented some of the mPFC neuroadaptations, such as increases in pERK in the mPFC, and reduced cocaine craving and the reinstatement of cocaine seeking [63]. An independent study demonstrated that wheel running increased NG2- and GFAP-positive glia in the mPFC [4], although such regulation has yet to be demonstrated during forced abstinence after drug taking. Altogether, adult cortical gliogenesis appears to be altered after exposure to drugs of abuse; therefore, normalizing gliogenesis in the mPFC during abstinence may help restore some of the maladaptive neuroplastic alterations in response to drug addiction.

Therapeutic Implications: Can Enhancing Neurogenesis Promote Functional Recovery in the Addicted Brain?

Voluntary exercise appears to decrease relapse to drug seeking in some cases. The impact of voluntary exercise on abstinence from drug use has been studied in humans with regard to nicotine [101–103] and alcohol [104] addiction, with some of these studies indicating a beneficial effect. With regard to animal models of addiction, only a few studies have

assessed whether exercise after the cessation of drug use can reduce the reinstatement of drug-seeking behavior [61–63]. However, as discussed above, none of these studies addressed whether the decrease in the reinstatement of drug-seeking behavior after wheel running during forced abstinence was caused by a reversal of drug-induced neuroplastic events, such as increased neurogenesis and gliogenesis. Although one could reasonably hypothesize that this could be the mechanism, mechanistic studies that prove otherwise are currently lacking.

As discussed in the previous sections, despite the reduction of neurogenesis and gliogenesis in animal models of addiction that may contribute to the addiction process, the addicted brain responds to environmental factors that stimulate the neurogenic/gliogenic niche to reduce drug seeking [63, 105–108]. These studies also suggest that the hippocampus and mPFC of the addicted brain retain microenvironmental elements that revive the normal proliferation and survival of neural and glial progenitors during withdrawal. Therefore, knowledge about the cellular and molecular mechanisms that maintain the neurogenic niche *in vivo* should be beneficial for the design of new therapeutic strategies to augment endogenous neural proliferation during abstinence.

Recent work in this field has also demonstrated how the neurogenic niche can be the source of extrinsic factors that promote the maturation and survival of newly born DG progenitors (reviewed in [109]). Particularly interesting is the fact that monoamines (e.g., dopamine) play an important role in maintaining and enhancing the proliferation and maturation of hippocampal progenitors [110, 111]. This suggests that the drug-induced consequences of long-term decreases in dopamine function associated with addiction could play a significant role in drug-induced alterations in hippocampal neurogenesis. Other mechanistic studies (reviewed in [109]) have shown that certain transcription factors intrinsic to progenitors could undergo chromatin remodeling, including histone modifications. Such epigenetic modifications could control certain aspects of adult neurogenesis, such as the transition from proliferation to differentiation. Furthermore, radial glia-like stem cell-derived intrinsic factors in the neurogenic niche have been demonstrated to have nourishing and instructive effects that promote certain stages of adult neurogenesis [112–114]. Functional studies, such as the knockdown of such intrinsic factors, including wingless-type MMTV integration site family3 protein (WNT3) in the DG, have shown decreased neurogenesis and impaired hippocampal-dependent memory [115], supporting a role for the neurogenic niche in hippocampal function.

Other intrinsic factors, such as Notch [116–118], disrupted-in-schizophrenia1 (DISC1) [119, 120], and cyclin-dependent kinase (Cdk5) [121, 122], are also known to regulate the stem cell pool and affect the differentiation/maturation of hippocampal progenitors. However, it remains to be determined if the efficacy of neuronal development can be augmented by manipulating the hippocampal microenvironment, such as by enhancing the expression of the molecular targets that underlie the maintenance of the neurogenic niche in the addicted brain. Therefore, the goal of such a therapeutic approach would be to first determine whether drugs of abuse produce epigenetic changes in mature neurons or induce molecular changes in progenitor cells to inhibit neurogenesis [109]. Follow-up studies could then determine whether normalizing the neurogenic and gliogenic niche by enhancing/reducing intrinsic molecular signals during abstinence contributes to enhanced endogenous neurogenesis and reduced drug seeking. Recruitment of the endogenous progenitor cell population during the protracted abstinence/withdrawal stage may then assist in reversing the altered neuroplasticity that occurs not only in neurogenic regions, but also throughout the many other brain regions that are known to be affected after addiction to drugs of abuse.

Summary and Future Directions

The identification of adult neurogenesis in the hippocampus, cortex, and olfactory bulb, in the past two decades has shed new light on the neuroplasticity of these brain regions and, more recently, the plasticity events that are associated with drug and alcohol addiction. Future studies aimed at understanding the potential link between correlative decreases in neurogenesis and the function of these brain regions will allow us to determine whether decreases in neuro- and gliogenesis by drugs of abuse is behaviorally relevant to the process of addiction. Demonstrating correlative changes in hippocampal/prefrontal cell genesis associated with the reward and relapse phases of addiction in humans will support the hypothesis that adult neurogenesis is a vulnerability factor for addiction. This may pave the way for future therapeutic possibilities for treating drug addiction disorders that involve enhancing neural stem cell production and the functional incorporation of new neurons into affected neural circuits (Box 2).

Box 1. Detection of Adult Born Progenitors, Adult Neurogenesis, and Gliogenesis

Actively dividing progenitors in the adult mammalian brain are usually labeled with exogenously administered mitotic markers, such as [³H]thymidine, 5-bromo-2'-deoxyuridine (BrdU), 5-iodo-2'-deoxyuridine (IdU), 5-chloro-2'-deoxyuridine, and 5-ethynyl-2'-deoxyuridine (EdU) [123–126]. Exogenous mitotic markers are incorporated into DNA during the synthesis-(S)-phase of the cell cycle of actively dividing progenitors in the brain, thereby assisting with birth dating the cells. The time (hours to days to months) of euthanasia after a pulse of the mitotic marker (usually administered intraperitoneally or intravenously) determines the age of the progenitor cell when analyzed in post mortem tissue. For example, a BrdU pulse minutes to hours before euthanasia will label proliferating cells, and a BrdU pulse days to months before euthanasia will label surviving cells, thereby allowing the characterization of the kinetics, dynamics, and phenotype acquisition of newly born progenitors (Figures 1B and 2B). More recently, endogenous markers of cell proliferation and cell differentiation, such as Ki-67, proliferating cell nuclear antigen (PCNA), phosphorylated histone-H3, sex-determining region Y-box 2 (Sox2), doublecortin (DCX), neurogenic differentiation factor 1 (NeuroD1), polysialic acid-neural cell adhesion molecule (PSA-NCAM), and several others, have been used to label progenitors at distinct stages of maturation [127–129]. Combinatorial labeling with exogenous and endogenous markers has provided critical information about the morphological and functional development of progenitors in the adult brain.

Box 2. Outstanding questions

- Do epigenetics play a role in mediating alterations in hippocampal neurogenesis induced by drugs of abuse?
- Do drugs of abuse distinctly alter intrinsic signals that maintain neurogenesis vs. gliogenesis?
- Can imaging human neurogenesis become feasible with improved technology?
- Does reduced hippocampal neurogenesis by drugs of abuse lead to cognitive impairments in human addicts?
- Do current therapeutic strategies in humans that incorporate physical activity during abstinence alter neurogenesis and gliogenesis to reduce craving?

- Do current or novel therapeutic strategies in humans that alter neurogenesis and gliogenesis reduce craving?

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Glossary

Addiction	Chronic relapsing disorder characterized by a compulsion to seek and take drugs, loss of control in limiting intake, and emergence of a negative emotional state during withdrawal.
Compulsivity	Behaviorally defined as the persistent reinitiation of habitual acts in responding in the face of incorrect responses in choice situations or preservation of responding in the face of adverse consequences.
Drug Craving	Memory of the pleasant aspects of a drug superimposed on a negative emotional state.
Drug Dependence	In animal models, manifestation of a withdrawal syndrome.
Drug Relapse	Reinstatement of drug seeking in previously detoxified individuals. This can occur in individuals after detoxification and long periods of abstinence, despite sincere efforts to refrain; behavior can be provoked by stress, cues, or contexts previously associated with drug use.
Impulsivity	Behaviorally defined as a predisposition toward rapid, unplanned reactions to internal and external stimuli without regard for negative consequences.
Negative Reinforcement	A process by which removal of an aversive stimulus (e.g., negative emotional state of drug withdrawal or a footshock) increases the probability of a response.
Place Conditioning	A procedure whereby the effects of drugs are paired with distinct environments, and the animal changes its subsequent preference for that distinct environment.
Positive Reinforcement	A process by which presentation of a stimulus (usually pleasant; e.g., pleasurable effects of a drug) increases the probability of a response.
Reinstatement of Drug Seeking	Animal model that investigates relapse to drug seeking. Learned self-administration behavior is extinguished by explicit nonreward, and subjects are later tested for their ability to reinstate drug-seeking behavior (e.g., lever pressing in the operant chamber) in response to a priming stimulus (i.e., drug, cue, context, or stressor).
Self-Administration	Arbitrary instrumental action, such as lever pressing, to gain access to positive reinforcers, such as food or drugs of abuse.

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Figure 1.

(A) Coronal section through the adult rat brain at bregma -3.6 highlighting the hippocampal dentate gyrus (yellow with black and gray shaded regions). The hippocampal trisynaptic pathway is indicated: perforant path (PP) connections in violet, mossy fiber (mf) connections in pink, and Schaffer collaterals (Sch) connections in green. The dentate gyrus is subdivided into the molecular layer (Mol), granule cell layer (GCL, gray), and hilus (H, yellow). The subgranular zone (SGZ) is indicated as a black hatched area between the granule cell layer and the hilus of the dentate gyrus. A granule cell neuron is indicated in the GCL, which is magnified in (B) to depict the various developmental milestones of hippocampal neural stem cells. (B) Schematic of the hippocampal GCL demonstrating the

sequence of preneuronal, early neuronal, and postmitotic cell types during postnatal neurogenesis. Cells are born as type-1 radial glia-like stem cells and slowly divide to produce type-2 cells. Rapidly dividing type-2 cells differentiate into immature neuron type-3 cells and finally into a mature granule cell neuron. Various endogenous markers of proliferation (Sox2, Ki-67) and differentiation (DCX, PSA-NCAM, NeuroD1) can be used in combination with the astrocytic marker GFAP to determine the cell type of proliferating cells.

Figure 2.

(A) The adult rat medial prefrontal cortex (mPFC), which is equivalent to the human dorsolateral PFC, spans a 3 mm³ area bilaterally along the rostral-caudal levels of the rodent brain [18]. Anatomically, the mPFC is clearly distinguishable from other cortical areas in bregma regions 3.7-2.2 of the adult rat brain [92]. A coronal section through the adult rat brain at bregma 2.7 highlights the mPFC in tan color. The mPFC is further divided into the anterior cingulate cortex (Cg1), prelimbic cortex (PrL), and infralimbic cortex (IL) subregions [130]. LV, lateral ventricle. (B) The mPFC contains proliferating cells (black filled circles; one cell in a rectangle box is enlarged and is a cycling cell in yellow) that mostly mature into glia (oligodendrocytes in blue and astrocytes in green) and endothelial

cells (blue cells in blood vessels) or neurons (red) to a lesser extent. The percentage of mature phenotypes reflect data pooled from various reports (see below) of 20-day or older BrdU-IR cells that were co-labeled with various markers for immature (NG2; Rip) or mature (PLP, proteolipid protein; O4, oligodendrocyte marker clone O4) oligodendrocytes, mature astrocytes (GFAP; S100 β , S100 calcium binding protein B), endothelial cells (RECA, pan-endothelium marker), or mature neurons [neuronal nuclease (NeuN)]. (C) Twenty-day-old or older BrdU-immunoreactive cells were colabeled with various markers for immature (NG2, Rip) or mature (PLP, O4) oligodendrocytes, mature astrocytes (GFAP, S100 β), endothelial cells (RECA), immature neurons (DCX), or mature neurons (NeuN). The ratio of labeled phenotypes (y-axis) from various reports [4, 5, 88–92, 94] using adult rat brain tissue (x-axis) is indicated. Such findings indicate that there is a large variability between studies, although most studies found that NG2+ cells constituted the majority of BrdU labeled cells, whereas NeuN+ cells were relatively rare.

Hippocampal Neurogenesis is altered after self-administration of various drugs of abuse and after withdrawal/relapse in animal models of drug addiction.

Table 1

Stage	Source of reinforcement	Animal models	Hippocampal Neurogenesis ¹			Refs
			Proliferation	Immature neurons	Mature neurons	
Binge/Intoxication	Positive reinforcement	Conditioned place preference				
		MDMA	--	ND	--	40
	Drug self-administration	Cocaine	--	ND	--	41
		Methamphetamine				
		Intermittent access (1h/d, 2d/w)	↑	↑	--	35
		Limited access (1h/d, 5d/w)	↑	↑	↑	35
		Cocaine				
		Limited access (1h/d, 7d/w)	↓	ND	↓	43
		Extended access (>4h/d, 5d/w)	↓	↑	--	39
		Nicotine				
		Limited access (1h/d, 5–7d/w)	↓	↓	ND	36
Withdrawal/Negative affect	Negative reinforcement	Heroin				
		Extended access (6h/d, 7d/w)	↓	ND	ND	36
		Alcohol				
		Nondependent drinking (30m/d, 5d/w)	↓	↓	↓	37, 48
		Conditioned place aversion		no data		
		Increased self-administration during dependence				
		Methamphetamine				
		Extended access (6h/d, 5d/w)	↓	↓	↑	35
		Cocaine				
		Extended access (>4h/d, 5d/w)	--	--	ND	60
Relapse	Conditioned positive and negative reinforcement	Alcohol				
		Excessive drinking during dependence	↓	↓	↓	37, 50
		Cue-induced reinstatement		no data		
		Stress-induced reinstatement		no data		
		Protracted abstinence/withdrawal				

Stage	Source of reinforcement	Animal models	Hippocampal Neurogenesis ¹			Refs
			Proliferation	Immature neurons	Mature neurons	
		Extended access cocaine (14d)	↓	↓	ND	60
		Extended access cocaine (28d)	--	↑, --	--, ND	39, 60

¹ --, no change;
↑, increase;
↓, decrease;
ND, not determined.

Table 2

Gliogenesis in the mPFC is altered after self-administration of various drugs of abuse and after withdrawal in animal models of drug addiction¹

Stage	Source of reinforcement	Animal models	mPFC Gliogenesis ²	Survival	GFAP-glia	NG2-glia	Refs
Binge/Intoxication	Positive reinforcement	Conditioned place preference	no data				
		Drug self-administration					
		Methamphetamine					
		Intermittent access (1h/d, 2d/w)					
		Limited access (1h/d, 5d/w)					
Withdrawal/Negative affect	Negative reinforcement	Alcohol	no data				
		Nondependent drinking (30m/d, 5d/w)					
		Conditioned place aversion					
		Increased self-administration during dependence					
		Methamphetamine					
		Extended access (6h/d, 5d/w)					
		Alcohol					
		Excessive drinking during dependence					

¹The effects of relapse on gliogenesis in the mPFC have not yet been studied.

²--, no change;

↑ increase;

↓ decrease;

↓ND, not determined.