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REVIEW

Effects of addictive drugs on adult neural stem/progenitor cells

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Abstract Neural stem/progenitor cells (NSPCs) undergo a series of developmental processes before giving rise to newborn neurons, astrocytes and oligodendrocytes in adult neurogenesis. During the past decade, the role of NSPCs has been highlighted by studies on adult neurogenesis modulated by addictive drugs. It has been proven that these drugs regulate the proliferation, differentiation and survival of adult NSPCs in different manners, which results in the varying consequences of adult neurogenesis. The effects of addictive drugs on NSPCs are exerted via a variety of different mechanisms and pathways, which interact with one another and contribute to the complexity of NSPC regulation. Here, we review the effects of different addictive drugs on NSPCs, and the related experimental methods and paradigms. We also discuss the current understanding of major signaling molecules, especially the putative common mechanisms, underlying such effects. Finally, we review the future directions of research in this area.

Keywords Neural stem/progenitor cells · Addictive drugs · Adult neurogenesis · Proliferation · Differentiation · Survival

Abbreviations

5-HT	5-Hydroxytryptamine
AEA	Anandamide
AR	Adrenergic receptor
BDNF	Brain-derived neurotrophic factor
BLBP	Brain lipid-binding protein
bHLH	Basic helix–loop–helix
BrdU	Bromodeoxyuridine
Cdk	Cyclin-dependent kinase
CPP	Conditioned place preference
DADLE	[D-Ala^2, D-Leu^5]-Enkephalin
DCX	Doublecortin
DG	Dentate gyrus
DHT	Dihydrotestosterone
ERK	Extracellular signal-regulated kinase
GFAP	Glial fibrillary acidic protein
GPCR	G protein-coupled receptor
JNK	c-Jun N-terminal kinase
MAP	Microtubule-associated protein
MAPK	Mitogen-activated protein kinase
MDMA	3,4-Methylenedioxy-methamphetamine
METH	Methamphetamine
MPH	Methylphenidate
nAChR	Nicotinic acetylcholine receptor
NeuroD1	Neurogenic differentiation 1
Ngn2	Neurogenin 2
NSPC	Neural stem/progenitor cell
OPRD1	δ-Opioid receptor
OPRM1	μ-Opioid receptor
Pax6	Paired-box 6
PCNA	Proliferating cell nuclear antigen
PI3K	Phosphoinositide 3-kinase
pHisH3	Phosphorylated histone H3
Prox1	Prospero homeobox 1
PSA-NCAM	Polysialylated-neural cell adhesion molecule
SGZ	Subgranular zone
SVZ	Subventricular zone
Tbr	T-box brain
TRBP	TAR RNA-binding protein

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Tuj1	βIII-tubulin
VEGF	Vascular endothelial growth factor
YY1	Yin Yang 1

Introduction

The term neural stem/progenitor cells (NSPCs) is used to describe precursor cells that retain the capacity for self-renewal and give rise to neurons, astrocytes or oligodendrocytes through asymmetric cell division [1, 2]. The generation of new neurons in adult brains, or adult neurogenesis, occurs in the hippocampus and olfactory bulb of most mammalian species after fetal and early postnatal development has ceased [3]. In the process of adult neurogenesis, NSPCs give rise to mature neurons and glial cells in two major neurogenic regions [2, 4–7]. The first region is the subventricular zone (SVZ) of the lateral ventricles, where NSPCs generate new neurons that migrate to and differentiate in the ipsilateral olfactory bulb, ultimately differentiating into multiple neuronal types that participate in local circuitry [2, 8–14]. Another region is the subgranular zone (SGZ) of the dentate gyrus (DG), where robust neurogenesis continues throughout life [15, 16]. Neuroblasts and immature neurons originate in the SGZ and then migrate into the granule layer, where they integrate into the hippocampal circuitry [1, 2, 4, 5]. According to the different developmental stages of adult hippocampal neurogenesis, NSPCs can be divided into several types. Type-1 cells are radial-glia-like quiescent stem cells of the adult DG. They express glial fibrillary acidic protein (GFAP) and nestin and have several other astrocytic features [4, 17, 18]. Type-2 cells are GFAP-negative, nestin-positive and highly proliferative, with tangentially oriented short processes [4, 17]. Type-3 cells represent neuroblasts, which are doublecortin (DCX)-positive but nestin-negative cells with round nuclei [4]. Therefore, type-1 cells are defined as neural stem cells (NSCs), whereas type-2 and -3 cells are considered transiently amplifying neural progenitor cells (NPCs).

Addiction is characterized by compulsive drug taking and drug seeking behavior that takes place at the expense of other activities despite the possibility of adverse consequences [19, 20]. Among the myriad of brain functions and neural circuitry that are altered by addictive drugs, the regulation of adult hippocampal neurogenesis by addictive drugs has been revealed by both in vitro and in vivo studies [21–24]. During the past decade, it has been increasingly recognized that hippocampal function is implicated in all aspects of drug addiction such as drug memories and relapses into drug seeking behaviors. For example, dorsal hippocampus inactivation abolished the contextual reinstatement of cocaine-seeking behavior, suggesting an essential role in context-induced reinstatement of drug seeking [25]. In addition, the consolidation of morphine-induced conditioned place preference (CPP) was disrupted when protein synthesis was selectively blocked in the hippocampus [26]. When increased neurogenic differentiation 1 (NeuroD1) activity restored hippocampal neurogenesis, the extinction of morphine-induced CPP was prolonged, suggesting a positive correlation between adult neurogenesis and contextual drug memory extinction [24]. By contrast, other evidence has shown a negative correlation between drug-seeking behaviors and use and the levels of hippocampal neurogenesis. Studies on rats indicate that environmental enrichment enhanced the extinction of both amphetamine and sucrose-maintained responding [27], supporting the importance of environmental enrichment in the prevention and treatment of drug addiction [28]. Aerobic exercise, which has been proven to increase adult neurogenesis in DG [29], decreases the positive-reinforcing effects of cocaine and would possibly be an effective intervention in drug abuse prevention [30]. By contrast, factors that decrease adult neurogenesis may result in increased drug use and relapse. Stress, for example, was shown to play a complex role in triggering relapse among cocaine and heroin users [31]. It was also found in a developmental rat model that schizophrenia is associated with exaggerated cue-induced reinstatement of cocaine use [32]. The most direct evidence was shown by Noonan et al., who suppressed hippocampal neurogenesis in adult rats via cranial irradiation before drug use [33]. These results demonstrated that reduced adult hippocampal neurogenesis significantly enhanced resistance to the extinction of drug-seeking behaviors, suggesting that reduced hippocampal neurogenesis is a vulnerability factor for addiction. Although the mechanism by which adult hippocampal neurogenesis takes part in drug addiction is not yet fully elucidated, the close relationship between the two is nevertheless confirmed and well-accepted.

Because NSPCs are key players in the process of adult hippocampal neurogenesis and adult neurogenesis is regulated by a myriad of addictive drugs, it is likely that addictive drugs exert their actions on adult NSPCs, either directly or indirectly. This explains the capacity of addictive drugs such as morphine [34–36], heroin [36, 37], methamphetamine [38, 39], cocaine [40, 41], alcohol [42] and cannabinoids [43] to alter hippocampal neurogenic activity. Despite their proven effects on NSPCs, addictive drugs may target different cell types, from quiescent type-1 NSCs to type-3 neuroblasts, by modulating their proliferation, differentiation and survival. These effects in turn determine the population of the NSC pool and the neural fate specification and maturation of newborn neurons, which encompass all aspects of adult neurogenesis. More

specifically, addictive drugs can regulate NSPCs by a variety of mechanisms. For example, addictive drugs regulate adult neurogenesis by modulating cell cycling pathways, G protein-coupled receptor (GPCR) signaling cascades, mitochondrial functions, oxidative stress, or release of molecules such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) [44, 45]. Here, we review the various effects of addictive drugs on NSPCs and their underlying mechanisms, thereby elucidating the emerging roles of NSPCs in drug addiction.

Experimental methods and paradigms

Because the effects of addictive drugs on NSPCs may depend on different experimental methods and paradigms, which can lead to conflicting observations, it is necessary to elucidate the methodological issues relevant to the research on adult NSPCs. Here, we briefly describe the main methods used, including the detection of proliferative cells and differentiated cells of different lineages, along with the paradigms of in vivo experiments.

Detection of proliferative cells

Bromodeoxyuridine (BrdU), an analog of thymidine, is incorporated into the newly synthesized DNA of replicating cells during the S phase of the cell cycle and is therefore commonly used as a substitute for [^{3}H]thymidine, which has previously been used for the detection of proliferating cells in living tissues [46]. BrdU is frequently used in immunochemistry assays, both in vivo and in vitro, for its convenience in handling and compatibility with other cellular markers [5].

The protocols for BrdU labeling can vary from one another because of the doses used, the frequency of its administration, and the experimental procedures undertaken, which depend on the aims of the experiments in question. Doses may vary from 50 to 300 mg kg^{-1} day^{-1} for 3–5 consecutive days [5, 23]. The timing of the euthanization of animals is also dependent on the step of neurogenesis being investigated. For cell proliferation studies, animals are killed a short time after the last injection, whereas for differentiation and survival studies, animals are killed after a long delay (from one to several weeks), after the determination of cell lineages [5].

Although BrdU staining is one of the most widely accepted methods in detecting proliferative cells, a number of disadvantages of its use have been identified. It has been reported to have a neurotoxic effect, which may alter the functioning of labeled cells. False positive and negative results are possible, especially with fewer injections [5]. Moreover, BrdU may inhibit NSPC expansion by increasing the fraction of cells in the G_0/G_1-phase of the cell cycle and may also repress neuronal and oligodendroglial differentiation and increase cell death [47]. There are several ways to overcome these problems. For example, multiple injections of a low dose may minimize false negatives and positives, as well as toxicity. The combined use of cell death markers may rule out its side effects on cell survival. More importantly, a number of endogenous markers of the cell cycle such as Ki67, PCNA, HH3 and P34cdc2 have been used in combination with BrdU. These markers help identify dividing cells by confining their expression in cells under certain periods of the cell cycle, without linking to DNA repair or to apoptotic processes [5].

Identification of cell types in adult neurogenesis

There are many cell types that are involved in adult neurogenesis, including cells under different stages of NSPC differentiation and those with different lineages after maturation. These cells can be identified according to their morphology and proliferative ability and most importantly, through the expression of markers specific to certain cell types.

Self-renewing NSPCs

The most widely used marker for self-renewing or stem-like NSPCs is nestin, which belongs to a class of filament proteins. Nestin is specifically expressed in type-1 and type-2 cells, thus distinguishing early-stage NSPCs from more differentiated cells [4, 5, 48, 49]. Sex determining region (SRY)-box 2 (SOX2) is a transcription factor essential for maintaining undifferentiated stem cells. SOX2 plays a critical role in the maintenance of embryonic and neural stem cells and is also a marker of the early developmental stages of NSPCs [48–50]. GFAP, which is a widely used astrocyte marker, is also expressed in type-1 radial-glia-like NSPs; thus, it is used to define type-1 stem cells with the coexpression of nestin [4, 5, 48, 49]. Moreover, brain lipid-binding protein (BLBP) is specifically expressed in type-1 and type-2a NSPCs [48, 49].

Immature neurons

Doublecortin (DCX) is a microtubule-associated protein expressed in type-2b, type-3 and granule cell stages. DCX is among the earliest neuronal lineage markers, the expression of which extends from the proliferation stage to the early postmitotic maturation. The polysialylated-neuronal cell adhesion molecule (PSA-NCAM), which shows an overlap with the expression of DCX, is also specific to the period from the late proliferation stage to the early

postmitotic stage [4, 5, 48, 49]. βIII-Tubulin (Tuj1) is a cytoskeletal protein expressed in all postmitotic neurons and is therefore a major marker specific for immature neurons but not other stages [5, 23, 51]. Other markers include prospero homeobox 1 (Prox1) and NeuroD, which are expressed from the type-2b stage to the late postmitotic maturation phase, when new dendrites are developed [5, 23, 48, 51, 52].

Mature cell lineages

Neuronal-specific nuclear protein (NeuN) and microtubule-associated protein (MAP2) are the two most frequently used markers for mature neurons [4, 5, 48]. These markers are both expressed throughout the postmitotic phase of newborn neurons, but their labeling is considered ambiguous as they may also react with other cell lineages [5, 53]. GFAP and calcium-binding proteins (S100β) are specific markers for mature astrocytes, but their use as such has some limitations as GFAP is also a marker of the radial-glia-like type-1 NSCs, and S100β is not expressed in all astrocytes [54]. Oligodendrocytes are often identified with O4, a marker typically expressed on the cell surface, along with myelin basic protein (MBP) and Rip, a marker for both immature and mature oligodendrocytes [55–57].

Paradigms of in vivo experiments

Drug administration paradigms

The acute drug administration paradigm means that a drug is given during one experimental session within a 24-h period, whereas the chronic paradigm means a drug is given for multiple sessions for a period of several days. In studies on adult neurogenesis, the two distinct paradigms may result in totally different observations, thus obscuring the actual effects of drugs. For example, the chronic administration of morphine and heroin decreases adult neurogenesis, but an acute injection shows no difference [36]. Similarly, repetitive administration (14 days) of cocaine decreases BrdU-positive cells, but single doses have no such effect [40, 58]. Moreover, the interval between repeated administrations is another factor that determines the overall effect of a drug. When adult rats were given intermittent (occasional) access to methamphetamine for 1 h twice a day, an initial pro-proliferative effect was produced, with no overall change in neurogenesis. However, daily (limited and extended) drug access down-regulated NSPC proliferation [21].

Therefore, it is obvious that certain drug effects may only be detectable in the chronic administration paradigm but not in the acute paradigm. Chronic drug administration, which better simulates the behavioral patterns of actual drug abusers, is thus the more convincing paradigm that should be adopted to analyze drug effects. Additionally, the interval between administrations is another noticeable factor that determines the drug effects. Because a stable drug level in the blood over a relatively long period may be advantageous for the observation of drug effects, it is deducible that the chronic daily administration paradigm is superior to the acute and intermittent paradigms in reflecting actual drug actions.

Behavioral paradigms

The rate of hippocampal neurogenesis is affected by many factors, including exercise and an enriched environment, which promote the generation and survival of newborn neurons [59, 60]. Thus, the paradigms of behavioral experiments may be crucial for controlling adult neurogenesis, thereby resulting in discrepancies among studies using differential behavioral tests. Animals that undergo certain behavioral paradigms after drug administration often show alterations in the development and survival of mature cell lineages. For example, the opioid agonist-selective regulation of adult neurogenesis was detected after the animals had undergone a training procedure for five consecutive days under the CPP paradigm [23, 24]. Similar effects were found after the animals were trained by the Morris water maze, after nicotine treatment [61]. Self-administration, a form of operant conditioning considered to be one of the most valid experimental models to investigate addiction-related behavior, may also induce detectable alterations in neurogenesis in response to various addictive drugs such as opioids [36], methamphetamine [21, 62] and nicotine [63]. Similar results were shown in a study of alcohol addiction using the voluntary drinking paradigm, which confirmed a decrease in neuronal differentiation [64]. Moreover, both environmental enrichment and voluntary physical activity induce adult hippocampal neurogenesis [65] and mediate the effect of cannabidiol by promoting the expression of the CB1 receptor [66]. By contrast, in some studies, no alterations in neurogenesis were discovered when the drugs were administered by the experimenter, without any following behavioral paradigm [67, 68]. Thus, the behavioral paradigms associated with environmental enrichment and physical activity may be crucial for detecting alterations in adult neurogenesis, especially lineage-specific differentiation and the survival of newborn neurons.

However, the evidence supporting the role of such paradigms in studies on the proliferation of undifferentiated NSPCs in the adult hippocampus is rather weak because a number of studies were able to discover the anti-proliferative effects of addictive drugs without the application of behavioral paradigms [69–71]. This may suggest

an uncertain association between behavioral factors and the self-renewal of NSPCs at an early stage. In spite of this uncertainty, it is recommended that a behavioral paradigm be carried out with or after drug addiction to elucidate the effects of addictive drugs on adult neurogenesis.

Modulation of NSPCs by addictive drugs and underlying mechanisms

Adult NSPCs are modulated by a variety of addictive drugs acting on different aspects such as proliferation, differentiation and survival. NSPCs initially undergo proliferation, during which symmetric cell divisions expand the progenitor pool, and later switch to differentiation, during which self-renewing NSPCs give rise to their offspring with different lineages and phenotypes [72]. NSPC differentiation is a crucial step in adult neurogenesis as it determines both the rate of newborn cell generation and the fate of mature cells. The initiation of differentiation and lineage determination occurs as early as type-2 cells, which feature limited self-renewal and transient amplification [4]. Cell apoptosis and survival are crucial mechanisms that modulate the number of all cell types, including all developmental stages of NSPC differentiation and neuron maturation. Mechanisms underlying cell apoptosis and survival are tightly controlled because aberrant cell death is involved in the development of a large number of pathologies [73], including drug addiction. Therefore, investigations of the proliferation, differentiation and survival of adult NSPC upon exposure to addictive drugs are important in understanding their effects on adult neurogenesis. The effects of these drugs are summarized in Table 1.

It is now clear that multiple addictive drug classes share similar roles in modulating the proliferation, differentiation and survival of adult NSPCs, but the mechanisms underlying such effects are not yet completely elucidated. In spite of the complexity implicated by the regulation of NSPCs by various drugs, the roles of some signaling molecules and pathways such as the MAPK signaling pathway, cell cycle regulatory molecules and microRNAs (miRNAs) have been identified. These signaling molecules and pathways may function independently or act in conjunction with one another to regulate NSPCs, thereby modulating adult neurogenesis. We will discuss such modulation according to different classes of drugs.

Opioids

The regulation of NSPCs was most widely proven by a series of studies on addictive drugs, including opioids (Table 1). By quantifying BrdU-positive DG cells after 4 weeks of morphine administration, it was found that chronic administration of opioids such as morphine and heroin significantly diminished the cells in the granule cell layer rather than those in the hilus, indicating that opioids suppress the proliferation and survival of cells in the DG of adult rats [36]. The finding that OPRM1-knockout mice showed significantly enhanced granule survival in the hippocampus further supported the anti-survival effects of OPRM1 agonists [74]. Similar effects found with chronic morphine treatment were further confirmed in the SGZ of adult mice using BrdU along with two endogenous cell cycle markers, PCNA and phosphorylated histone H3 (pHisH3). It was found that chronic morphine treatment decreased the proliferation of adult mouse NSPCs and induced the premature mitosis of NSPCs in the SGZ by shortening the length of Gap 2 (G2)/Mitosis (M) rather than other phases of the cell cycle, suggesting an effect cell cycle modulation [69]. However, a minimal administration paradigm of buprenorphine, an opiate analgesic, was shown to reduce NSPC apoptosis in the DG of adult mice and enhance the survival of newborn cells [75]. On the other hand, repeated morphine treatment was shown to reduce cell proliferation and alter the phenotypes of DG granule cells by significantly increasing the mRNA transcription of glutamate decarboxylase-67, a GABA-synthesizing enzyme that is highly expressed in earlier rather than later stages of neuronal development. A marked rebound was observed after 1 week of withdrawal, and the return to the control level occurred after 2–4 weeks of withdrawal, suggesting that morphine inhibits NSPC proliferation and the neuronal differentiation of DG granule cells [76]. However, the regulation of SGZ proliferation by morphine largely depends on the level of morphine in the blood, which is attributed to the administration paradigm. When the pellet paradigm, which produces high and stable blood levels of morphine, was used, a significant decrease in the regulation of SGZ proliferation was detected, whereas the three injection paradigms that produced transient spikes in morphine blood levels had no significant effect [77]. By examining the key stages of NSPC maturation in the mouse SGZ using BrdU to track the newborn cells, it was found that chronic morphine administration increased the percentage of type-2b cells but decreased that of type-3 cells, indicating an in vivo role for morphine in preventing neuronal maturation and in affecting the proliferation of only type-2b and type-3 NSPCs [78] by increasing the level of VEGF but not BDNF or interleukin-1β within the neurogenic microenvironment [45]. The SGZ cells in the S-phase are most sensitive to the inhibitory effects of morphine during the initial 24 h of exposure, which may be a result of transiently increased cell death after early morphine exposure [78]. This stage-specific action is further supported by the effects of

Table 1 Effects of addictive drugs on proliferation, differentiation and survival of adult NSPCs

Drugs	Organism	Paradigm	Effects			References
			Proliferation	Neuronal differentiation	Survival	
Morphine and heroin	Rat	In vivo, acute	–			[36]
		In vivo, chronic	↓		↓	
Morphine	Mouse	In vivo, chronic	↓			[45]
Morphine	Mouse	In vivo, chronic	↓			[69]
Buprenorphine	Mouse	In vitro, chronic	↓		↑	[75]
Morphine	Rat	In vivo, chronic	↓	↓		[76]
		In vivo, withdrawal	↑			
Morphine	Mouse	In vivo, acute	–			[77]
		In vivo, chronic	↓			
Morphine	Mouse	In vitro, chronic	↓	↓		[78]
Opioid receptor antagonists	Rat	In vitro, chronic	↓	↑		[79]
Morphine	Mouse	In vitro, chronic	↑	↓	–	[23, 51]
		In vivo, chronic		↓		
Fentanyl	Mouse	In vitro, chronic	↑	–		
Opioids	Rat	In vitro, chronic	↑			[84]
Cocaine	Rat	In vivo, chronic	↓			[22]
		In vivo, withdrawal	↑	↑		
Cocaine	Rat	In vivo, acute	–			[40, 58]
		In vivo, chronic	↓		–	
Cocaine	Rat	In vivo, acute	↓			[91]
		In vivo, chronic	↓		–	
Cocaine	Human	In vivo, chronic			↓	[92]
Cocaine	Rat	In vivo, acute	–	–		[93]
		In vivo, chronic	↓	–		
		In vivo, withdrawal	↓		↓	
Cocaine	Rat	In vivo, withdrawal	↓			[94]
Cocaine	Rat	In vitro, chronic	↓			[95]
AEA	Rat	In vitro and in vivo, chronic		↓		[43]
Cannabidiol	Mouse	In vivo, chronic	↓		↑	[66]
Δ9-THC	Mouse	In vivo, chronic	–		–	[66, 67]
HU210	Rat	In vivo, chronic	–	–		[68]
CB2 agonists	Mouse	In vitro and in vivo, chronic	↑			[71]
HU210 and AEA	Rat	In vitro and in vivo, chronic	↑	↑	–	[104]
Endocannabinoids	Rat	In vitro and in vivo, chronic		↓		[106]
Cannabichromene	Mouse	In vitro, chronic		↑		[107]
WIN 55,212-2	Rat	In vivo, chronic	–			[108]
Endocannabinoids	Mouse	In vitro and in vivo, chronic	↑			[109]
Methamphetamine	Rat	In vivo, acute	↑	↑		[21]
Methamphetamine	Gerbil	In vivo, chronic	↓			[38]
Methamphetamine	Rat	In vitro, chronic	↓			[39]
		In vivo, chronic	↓	↓	↓	
Methamphetamine	Rat	In vivo, chronic	↓			[62]
		In vivo, withdrawal	↑		↑	
Amphetamine	Rat	In vivo, acute	–			[110]
Amphetamine	Rat	In vivo, chronic	–		–	[111]
		In vivo, withdrawal				

Table 1 continued

Drugs	Organism	Paradigm	Effects			References
			Proliferation	Neuronal differentiation	Survival	
Amphetamine	Mouse	In vivo, chronic	–	↑	↑	[112]
Methamphetamine	Rat	In vitro, chronic	↓	–	↓	[113]
Nicotine	Rat	In vivo, chronic	↓			[61]
Nicotine	Rat	In vivo, chronic		↑		[63]
Nicotine	Rat	In vivo, chronic	↓		↓	[114]
Nicotine	Rat	In vivo, acute	–			[115]
Nicotine	Rat	In vivo, chronic		↓	↓	[116]
Alcohol	Mouse	In vivo, chronic	↓	↓		[64]
Alcohol	Rat	In vivo, acute	↓			[70]
		In vivo, chronic	↓		↓	
Alcohol	Rat	In vivo, chronic	↓			[108]
Alcohol	Rat	In vivo, chronic			↓	[119]
Alcohol	Rat	In vivo, chronic	↓			[120]
		In vivo, withdrawal	↑			
Alcohol	Rat	In vivo, chronic	↑			[121]
Alcohol	Rat	In vivo, chronic	↓			[122]
Alcohol	Rat	In vivo, chronic	↓		↓	[123]
Alcohol	Rat	In vivo, acute	↓		↓	[124]
Alcohol	Rat	In vivo, chronic	↓			[125]
Alcohol	Mouse	In vivo, chronic	↓			[126]
Alcohol	Rat	In vivo, chronic			↓	[127]
MDMA	Rat	In vivo, chronic			↓	[127]
MDMA	Mouse	In vivo, chronic	↓			[129]
Methylphenidate	Rat	In vivo, chronic	–			[130]
		In vivo, withdrawal			↓	

–, no significant change; ↑, up-regulation; ↓, down-regulation

buprenorphine, an opiate analgesic, in decreasing the proliferation of DCX-positive neuroblasts in adult mice [75]. These results were supported by in vitro experiments as the treatment of naloxone, an antagonist of opioid receptors, was capable of inducing the neuron-preferential differentiation of rat adult NSPCs while reducing their differentiation into astrocytes and oligodendrocytes. This is indicative of the opposite effects of OPRM1 and OPRD1 agonists. The cell cycle proteins important for entering the S-phase may play a role in such effects, as indicated by their reduced levels upon treatment with opioid receptor antagonists [79]. This study exemplifies a role of MAPKs in the regulation of cell cycle proteins as the reduced levels of cell cycle proteins result from the inhibition of MAPKs induced by opioid receptor antagonists. The effects of morphine and heroin on the inhibition of neuron-preferential differentiation were also indirectly proven in SH-SY5Y cells. miR-125b, which increases the differentiation of SH-SY5Y cells with neurite outgrowth [80], is significantly down-regulated by opioids such as morphine and heroin both in vitro and in vivo and is up-regulated during neuronal differentiation [81]. The δ-opioid peptide [D-Ala^2, D-Leu^5]-Enkephalin (DADLE) shows an anti-proliferative effect, which is derived from its regulation of the cell cycle by causing an arrest of AF5 cell cycle progression at the Gap 1 (G1) checkpoint [82].

It is worth noting that most studies leading to the conclusion that opioids, especially morphine, decreased the proliferation of NSPCs were based on in vivo experiments with chronic drug administration. Most acute in vivo drug administration and in vitro studies did not result in a decrease in proliferation, but rather, an increase in proliferation. The reason for such differences can be deduced by analyzing the studies on the mechanisms underlying the effects of morphine. First, the appropriate neurogenic microenvironment modulated by molecules such as BDNF and VEGF is more easily achieved in vivo. Second, only the chronic, not the acute administration paradigm, is

capable of producing stable blood levels of morphine, which is essential for a significant effect on NSPC proliferation. It was also hypothesized that in vivo morphine treatment may give rise to a reduction in testosterone levels, an elevation in dihydrotestosterone (DHT) levels, and an over-expression of the *p53* gene [83]. The fact that acute morphine treatment does not decrease the number of BrdU-positive cells in the SGZ of adult rats [36] can be explained by the fact that the 6-h acute paradigm does not establish stable blood levels of morphine, which is essential for a sustained alteration, such as cell proliferation [77]. As for in vitro studies, μ-opioid receptor (OPRM1) and δ-opioid receptor (OPRD1) antagonists such as naloxone, naltrindole and β-funaltrexamine were found to induce anti-proliferative effects on adult hippocampal progenitors, suggesting the in vitro proliferative actions of endogenous opioids [79]. The above finding was further demonstrated by the observation that β-endorphin and morphine increased the proliferation of NSPCs after 48 h of incubation, which was dependent on the mitogen-activated protein kinase (MAPK)-signaling pathway. This ERK signaling cascade involves the $G_{i/o}$ protein and phosphoinositide 3-kinase (PI3K) but not PKC, as indicated by the use of inhibitors [84].

The fact that GPCR induces ERK activation by two distinct and independent pathways, either the G protein- or β-arrestin-mediated pathway [85], has been widely reported during the past decade. An increasing number of studies have shown that mechanisms related to the two pathways, such as biased agonism, are extensively involved in multiple functions of GPCRs, including the opioid receptors [86, 87]. Thus, it is clear that not only ERK activation itself but also the pathways leading to ERK activation are responsible for the differential effects of addictive drugs on NSPCs.

Our recent works using hippocampal NSPCs from adult mice further elucidated the effects of opioids on NSPCs via biased agonism. Two OPRM1 agonists, morphine and fentanyl, both promote the proliferation of adult hippocampal NSPCs until the initiation of differentiation [23]. Although morphine and fentanyl are both agonists of OPRM1, only morphine was able to modulate NSPC differentiation by inducing astrocyte-preferential differentiation. This ability of morphine to control the mechanisms of cell fate determination is attributed to its regulation of the miR-181a/Prox1/Notch1 pathway, which is a result of the different mechanisms of the two agonists leading to MAPK pathway activation [23, 51]. We also evaluated the cell death effect of morphine both before and after the differentiation of mouse adult NSPCs cultured in vitro and found no significant difference between the morphine-treated group and the control group [51]. The completely different results for NSPC differentiation induced by morphine and fentanyl are due to their distinct pathways in ERK activation. Morphine activates ERKs via PKCe but not β-arrestins, and the phosphorylated ERK is distributed mainly in the cytosol. Thus, ERKs activated by morphine are capable of phosphorylating cytosolic molecules, including the HIV TAR RNA-binding protein (TRBP), which in turn stabilizes the TRBP/Dicer complex, activates the microRNA-processing machinery and facilitates the maturation of miR-181a by increasing Dicer expression. MicroRNA-181a targets the Prox1/Notch1 regulation pathway and contributes to astrocyte-preferential differentiation. On the other hand, as fentanyl activates ERKs via β-arrestins, the nucleus-translocated ERKs do not show such effects [51].

The effects of miR-190, although not yet demonstrated in NSPCs, are also worth noting because they implicate a mechanism that modulates the opioid-induced activation of NeuroD1, a crucial transcription factor of neuronal differentiation [88]. The effects of opioids on NeuroD1 activation have been thoroughly studied, although not on NSPCs, and have provided us with sufficient information on how NeuroD1 activity is modulated. Fentanyl attenuates miR-190 expression through phosphorylation of the transcription factor Yin Yang 1 (YY1), thereby facilitating NeuroD1 expression [89], which is likely to promote NSPC differentiation into immature neurons. Thus, it is likely that miR-181a and miR-190 are key mediators of two representative mechanisms that exemplify the influences of the ERK cascade on microRNA expression, at either the transcriptional or post-transcriptional levels, which in turn control opioid-induced NSPC differentiation. The regulation of miR-190 is transcriptional, as ERK inhibits the transcription of *talin2*, the host gene of miR-190, by inducing the phosphorylation of the transcription factor YY1 [89]. However, ERK does not affect miR-181a transcription as the levels of pri- and pre-miR-181a were not affected after morphine-induced ERK activation [23]. Alternatively, ERK regulates the miRNA processing machinery by TRBP, a cofactor of Dicer, and thus controls the maturation of miR-181a on a post-transcriptional level [51] and activates the miR-181a/Prox1/Notch1 pathway, which results in down-regulated neuronal differentiation. The two distinct pathways represent the main manners by which the MAPK pathway modulates miRNA expression and in turn regulates its targets.

Overall, as the complexity of the mechanisms that control NSPC proliferation, differentiation and survival is revealed, we may conclude that opioids suppress the in vivo proliferation of NSPCs with chronic drug administration but promote proliferation in in vitro assays. The effects of opioids on NSPC survival may vary among different drug types and experimental methods, and even agonists of the same opioid receptor may have differential effects that lead to distinct results.

Cocaine

Cocaine is a psychomotor stimulant shown to modulate the proliferation of NSPCs. Repetitive administration (14 days) of cocaine resulted in a significant decrease in the number of BrdU-positive granule cells in the DG of adult rats. On the other hand, the number of BrdU-positive cells showed no difference compared with the control group after 4 weeks of maturation, indicating no effect on the survival of newborn NSPCs. Single doses, however, did not have any effect [40, 58]. This was confirmed by a repetitive administration paradigm accompanied by CPP training [90] and by the observation of significantly reduced cell proliferation in the DG of adult rats in both short-term (8 days) and long-term (24 days) cocaine exposures, without any effect on the survival of newly generated cells after either paradigm of cocaine treatment [91]. However, an in vitro investigation detected significantly higher levels of lactate dehydrogenase and cytochrome *c* release in cultured human NSPCs after acute cocaine incubation (72–96 h), suggesting increased cell death and impaired survival induced by cocaine. Acute cocaine exposure was sufficient to cause a significant increase in oxidative stress in human NSPCs, which was followed by drastic apoptotic effects. This observation explains the impaired survival of NSPCs after cocaine abuse, along with compromised antioxidant capacity [92]. Further studies demonstrated that although the proliferating cells in both the SGZ and SVZ of rats decreased after 3 weeks of cocaine self-administration, the effects were reversed by 4 weeks of withdrawal. On the other hand, the number of DCX-positive immature neurons in the posterior SGZ increased after 4 weeks of either withdrawal or continued cocaine self-administration, indicating a negative effect of cocaine on neuronal differentiation [22]. These findings suggest that NSPCs in discrete stages of adult neurogenesis are regulated independently. Studies in rats with differential propensities for drug-seeking behavior further elucidated the effects of cocaine. Whereas acute cocaine treatment did not alter the cell proliferation in both groups of rats, chronic cocaine decreased the cell proliferation in rats that had low rather than high novelty-seeking behavior. However, cocaine was not capable of altering lineage-specific differentiation as the newborn neuron/glia ratio remained constant after cocaine treatment. Although cocaine withdrawal after chronic cocaine treatment did not affect the survival of immature neurons, withdrawal from cocaine treatment decreased the survival of mature neurons exclusively in rats with high novelty-seeking behavior [93]. The effects of cocaine withdrawal on NSPCs remain uncertain as Garcia-Fuster et al. found that the proliferation of rat hippocampal NSPCs decreased after 14 days of withdrawal [93, 94]. This discrepancy may be attributed to the different experimental designs and markers used. Similar results were also revealed by in vitro studies. Cocaine significantly reduced the number of AF5 cells (immortalized rat neural progenitor cells) in a dose-dependent manner by down-regulating Cyclin A, indicating a role of Cyclin A down-regulation in suppressing cell proliferation [95]. Thus, we may conclude that cocaine suppresses the proliferation of adult NSPCs and contributes to the impaired survival of NSPCs and differentiated mature neurons but may only be detectable under certain conditions. A negative effect on neuronal differentiation was also indicated by cocaine withdrawal, but more evidence is required for a conclusion.

Despite the lack of direct evidence showing the mechanisms underlying the effects of cocaine, it has been found that a series of microRNAs, which modulate NSPC proliferation and differentiation, are also regulated by cocaine (Fig. 1). For example, miR-9 inhibits NSPC proliferation and promotes neuronal differentiation by targeting the nuclear receptor TLX (homologue of the Drosophila tailless gene), which also inhibits the expression of miR-9 primary miRNA (pri-miRNA). This forms a regulatory loop that controls the balance between the proliferation and differentiation of adult NSPCs [96]. The Let-7 miRNA family, which is increasingly expressed during neural differentiation, is closely correlated to modulating the NSPC proliferation and differentiation induced by addictive drugs. Let-7d is down-regulated upon chronic cocaine exposure [97] and regulates the TLX/miRNA-9 cascade by reducing TLX expression and thus reduces neural stem cell proliferation and promotes premature neuronal differentiation [98] in parallel with cocaine's anti-proliferative effects on NSPCs. By contrast, Let-7b inhibits NSPC proliferation and promotes neuronal differentiation by regulating Cyclin D1 [99]. However, miR-124, which is also down-regulated by cocaine [97], promotes neuronal differentiation, while suppressing the proliferation of NSPCs by inhibiting the SRY-box transcription factor Sox 9 and the Notch signaling pathway [100, 101]. Therefore, it is likely that cocaine activates both Let-7d and miR-9, which target TLX and in turn control cell cycle progression. As a result, the proliferation of NSPCs is inhibited, whereas neuronal differentiation is promoted [98]. Similarly, miR-124 is also activated by cocaine and inhibits NSPC proliferation by the inhibition of Notch signaling [97, 100, 101].

Cannabinoids

Cannabinoids are a class of compounds that act on cannabinoid receptors, which includes the endocannabinoids, phytocannabinoids and synthetic cannabinoids. It has been reported during the past decade that cannabinoids

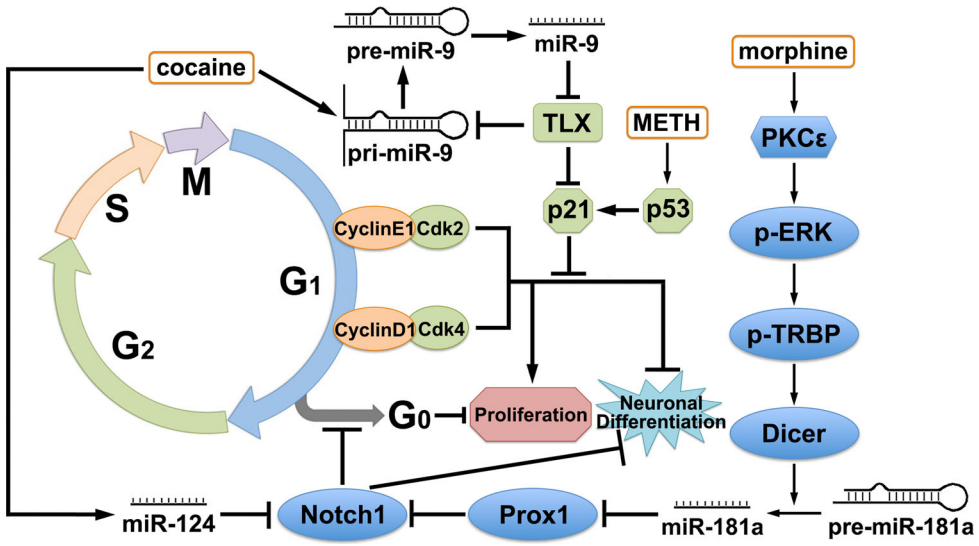

Fig. 1 Crosstalk between the different mechanisms underlying the modulation of NSPCs. The mechanisms by which morphine, cocaine and methamphetamine (METH) modulate adult NSPCs exemplify the crosstalk between the MAPK cascade, cell cycle regulation and miRNA expression induced by addictive drugs. Morphine up-regulates miR-181a by the ERK/TRBP/Dicer pathway. MiR-181a further controls Prox1/Notch1 signaling, which ultimately results in alterations of NSPC differentiation. Cocaine activates the miR-9/TLX loop, which in turn regulates p21 and further interferes with the effects of cell cycle regulatory molecules that alter proliferation and differentiation of NSPCs. On the other hand, cocaine up-regulates miR-124, which inhibits Notch1 expression, which results in cell cycle arrest. Methamphetamine activates the p53/p21 cascade, which blocks the effects of cell cycle regulatory molecules, resulting in the decreased proliferation and increased neuronal differentiation of NSPCs

modulate NSPCs with a variety of effects [102, 103]; no explicit conclusions have been reached. In vitro studies have shown that the synthetic cannabinoid HU210 and the endogenous cannabinoid anandamide (AEA) both significantly promote rat NPSC proliferation via CB1 receptors after 48 h of incubation. Moreover, in vivo experiments have confirmed that chronic, but not acute, HU210 treatment promotes NSPC proliferation in the DG of adult rats. An ERK signaling pathway that is dependent on the Gi/o protein but is independent of PI3K/Akt mediates such cannabinoid-induced proliferative effects on NSPCs. However, neither HU210 nor AEA resulted in any significant effect on neural differentiation or NSPC survival in rats, either in vitro or in vivo [104]. Another finding suggests that HU210 did not have any effect on cell proliferation or neuronal differentiation in the DG of rats [68]. However, AEA was shown to delay the appearance of the early neuronal marker Tuj1 and to decrease the expression of the mature neuronal marker NeuN after 4 days of in vitro differentiation. Similarly, in vivo assays have demonstrated that methanandamide increased the percentage of NeuN-negative cells in the DG of rats through the Rap1/B-Raf/ERK pathway, suggesting a role for the ERK signaling pathway in neuronal differentiation [43], which is likely to be dependent on G proteins and PKA [105]. The roles of cannabinoids as modulators of neural cell fate were further supported by the findings that endocannabinoids increase the number of GFAP-positive astrocytes and decrease the number of βIII-tubulin-positive neurons in rats both in vitro and in vivo [106], thereby inhibiting neuronal differentiation. It was also reported that another compound, cannabichromene, was capable of inhibiting the astrocytal differentiation of adult NSPCs in vitro [107]. Non-psychoactive CB2 cannabinoid agonists promote mouse NSPC proliferation, both in vitro and in vivo [71]. However, another active cannabinoid, Δ^9-tetrahydrocannabinol, neither affected cell proliferation in the DG of adult mice after a 3-week oral administration [67] nor induced any significant effect on NSPC survival or death in the DG of adult rats [66]. The cannabidiol did decrease cell proliferation after 6 weeks of administration of BrdU and increased neuronal cell survival after 4 weeks of BrdU injection [66]. Interestingly, a totally different result was observed in combination with alcohol consumption as the cannabinoid receptor agonist WIN 55,212-2 decreased cell proliferation in the DG [108]. In spite of these discrepancies, the pro-proliferative effect was supported by in vitro studies, as the endocannabinoids were proven to promote NSPC proliferation and neurosphere generation via the activation of the CB1 receptors [109].

Therefore, we may infer that cannabinoids play complex roles in regulating NPSC proliferation, differentiation and survival, resulting in discrepancies among different studies. These effects of cannabinoids are limited to certain types of compounds with very different mechanisms, due to the differences among the agonists of cannabinoid receptors,

the variety of markers used in their experiments, the varying paradigms of the experiments and the different periods and doses of administration.

Amphetamine and methamphetamine

Amphetamine and methamphetamine are potent CNS stimulants of the phenethylamine class and have both been proven to affect adult neurogenesis via their actions on NSPCs. Although their acute injection produced a transient and rapid decrease in the number of BrdU-positive cells in rat striatum, it had no effect on cells in the SVZ or DG [110]. Similarly, the chronic administration of D-amphetamine/amphetamine with repetitive doses did not significantly affect the proliferation or survival of NSPCs in the DG of rats [111] or mice [112], but amphetamine withdrawal reduced the survival of newly generated neurons after 4 weeks of drug treatment [111]. Moreover, amphetamine significantly increased the proportion of BrdU-positive cells that differentiated into mature neurons (indicated by the neuronal nucleus marker, NeuN), and also increased the survival of newborn neurons in a dose-dependent manner in the hippocampus of adult mice [112].

On the other hand, in spite of their chemical similarity, studies on methamphetamine showed discrepancies when compared with amphetamine. It was found that acute treatment with methamphetamine suppressed the proliferation of granule cells in the DG of adult gerbils; however, this effect was transient, as the proliferation rate was restored 36 h after the drug challenge [38]. However, later findings suggested that the intermittent (occasional access) self-administration of methamphetamine increased the number of late-phase DCX-positive cells in the SGZ of rats without exerting any significant effect on cell survival. It was also found that daily (limited and extended access) administration reduced hippocampal granule neurons and volume by decreasing the number of early-phase DCX-positive cells and significantly decreased the number of BrdU-positive cells that account for NSPC survival [21]. Thus, it is likely that acute methamphetamine treatment promotes NSPC proliferation and neuronal differentiation, whereas chronic methamphetamine exposure prevents proliferation, differentiation and survival, thereby inhibiting the maturation of newborn neurons and adult neurogenesis. By quantifying 2-h-old SGZ BrdU-positive cells, it was clear that chronic methamphetamine administration decreased the number of new hippocampal NSPCs in rats. By contrast, protracted withdrawal of methamphetamine promoted the proliferation of SGZ cells and restored the survival of impaired cells after chronic methamphetamine self-administration [62]. The in vivo findings indicating the anti-proliferation role of methamphetamine were further supported by in vitro studies using adult hippocampal progenitor cells, which found that the number of neurospheres decreased by methamphetamine treatment in a dose- and time-dependent manner [39, 113]. This anti-proliferative effect is accomplished by inhibiting cell cycle by the up-regulation of p53 and p21, which may cause G1 phase arrest and the inhibition of certain Cdk/Cyclin complexes [39]. Meanwhile, methamphetamine significantly increased NSPC death after 24–72 h of exposure in a dose- and time-dependent manner but did not impair neuronal differentiation [113].

Thus, despite their chemical similarities, amphetamine and methamphetamine show totally different effects on NSPC modulation. Whereas amphetamine shows no significant effect on NSPC proliferation and promotion, methamphetamine decreases both the proliferation and neuronal differentiation of adult NSPCs. As for NSPC survival, whereas conclusions can be made about the role of methamphetamine in impairing NSPC survival based on the current observations, the role of amphetamine remains elusive because of inconsistent results.

Nicotine

It has been widely accepted that nicotine, the compound responsible for tobacco addiction, has effects on the adult brain. Nicotine self-administration was shown to significantly decrease the proliferation and impair the survival of NSPCs in the DG of adult rats in a dose-dependent manner, as evidenced by a significantly increased number of pyknotic cells observed in the granule cell layer [114]. This observation was further supported by the finding that the chronic infusion of a high dose of nicotine decreased cell proliferation in the DG of adult rats, with or without water maze training [61]. On the other hand, acute nicotine treatment showed different results. Intermittent nicotine treatment had no significant effect on NSPCs in the hippocampal SGZ, although it did enhance precursor proliferation in the SVZ [115]. As for its effects on NSPC differentiation, the injection of nicotine was shown to decrease the number of PSA-NCAM- and NeuN-positive cells but had no effect on the number of GFAP-positive cells, indicating its role in neuronal inhibition but not astroglial differentiation [116]. However, it was found that extended access to nicotine self-administration and deprivation differentially regulates adult neurogenesis by increasing the number of NeuroD1-positive immature neurons in rat DG [63]. Thus, we may conclude that chronic but not acute administration of nicotine decreases adult NSPC proliferation and survival, but its effects on NSPC differentiation remain elusive.

Alcohol

Recent studies indicate that alcohol is involved in the regulation of adult neurogenesis by controlling NSPC proliferation [117, 118]. Alcohol was shown to inhibit NSPC proliferation in adult rats with the paradigms of both acute and chronic binge underlying alcoholic cognitive dysfunction. Chronic but not acute alcohol binging impaired the survival of newly divided NSPCs in the DG of adult rats 28 days after alcohol exposure [70]. The loss of DG cells was further attributed to impaired cell survival rather than proliferation as cell division was not affected 6 weeks after alcohol exposure. Therefore, alcohol may hinder the survival of newborn neurons because apoptosis was observed in the DG of alcohol-treated rats [119]. Further studies proved that the transiently decreased NSPC proliferation induced by chronic alcohol exposure was restored after weeks of abstinence [120–122]. It was also reported that after 4 weeks of chronic alcohol exposure, the proliferation of NSPCs in adult rats was reduced by approximately 50 % at various time points. This finding was accompanied by a dramatic decrease in the survival and an increase in the death of neuron-specific NSPCs, as the number of cells co-labeled with both BrdU and NeuN was reduced by over 80 % [123]. Other studies also support this anti-proliferative effect and the inhibition of neuronal differentiation in mouse DG NSPCs by alcohol exposure, as chronic binge drinking significantly decreased the number of immature DCX-positive cells by a pathway dependent on OPRM1 [64]. The negative effect of acute alcohol treatment on NSPC proliferation and survival in the DG of rats was later shown to be dose-dependent as a high dose of alcohol decreased the number of newborn neurons by approximately 50 %, a much more severe result compared with those of low and medium doses [124]. However, the observed decrease of BrdU-positive cells can be explained by an accelerated cell cycle as alcohol treatment targets and shortens the S-phase rather than other phases, which may be a cause of increased numbers of NSPCs [125]. Moreover, the effect of an alcohol binge may collaborate with cannabinoids to reach converging actions, resulting in dramatically reduced NSPC proliferation [108]. A recent study demonstrated that voluntary chronic alcohol consumption reduces BrdU retention in the SVZ but had no effect on mitotic cell types, suggesting a role of alcohol in controlling the dynamics of NSPC proliferation. These in vivo findings were supported by in vitro experiments, which found that direct exposure to high doses of alcohol significantly reduced neurosphere culture proliferation [126]. As the binge drinking of alcohol is often combined with the abuse of other addictive drugs, the combinations of alcohol with cocaine and 3,4-methylenedioxy-methamphetamine (MDMA) were also studied for their combined effects on NSPC survival. As a result, although the combination of cocaine and alcohol did not affect cell survival, the alcohol–MDMA combination significantly compromised the survival of pre-labeled hippocampal NSPCs [127]. Overall, most studies supported the idea that alcohol inhibits NSPC proliferation, neuronal differentiation and survival in the adult DG, albeit with some exceptions. The unique regulation of adult NSPCs by alcohol is shown by its cooperation with other addictive drugs such as cocaine and MDMA, corresponding to the association between binge drinking and drug abuse.

3,4-Methylenedioxy-methamphetamine (MDMA) and methylphenidate (MPH)

Apart from the major types of addictive drugs mentioned above, other drugs that have a role in modulating adult NSPCs have been studied. MDMA is a widely used drug that is a known substrate-type serotonin releaser [128]. It chronically and dose-dependently decreased the number of BrdU-positive cells in the DG of adult mice, suggesting that it decreases the NSPC proliferation rate [129]. In addition, as mentioned above, MDMA was able to impair the survival of pre-labeled hippocampal NSPCs, either independently or in combination with chronic alcohol treatment [127]. MPH, another addictive drug commonly used to treat attention-deficit/hyperactivity disorder, was also studied for its effect on NSPCs. Although there was no evidence of cell proliferation alteration after juvenile MPH exposure discovered at any time point, MPH was proven to significantly decrease the long-term survival of newborn cells in the temporal hippocampus of adult rats [130].

Summary

The proliferation, neuronal differentiation and survival of adult NSPCs in the DG are the three main processes of adult neurogenesis. Therefore, they are the three major parameters widely assessed to characterize the effects of different factors. Addictive drugs may have complicated roles in the modulation of adult NSPCs. For example, whereas most drugs decrease DG cell proliferation and survival, which agrees with the widely accepted observation that addictive drugs suppress adult neurogenesis, there are quite a few exceptions. Some drugs may have no effect on or may even promote NSPC proliferation under certain conditions. The discrepancies may be attributed to the varied mechanisms, paradigms of the experiments, methods of administration, and different doses and periods, indicating the various aspects of the effects of drugs on NSPCs and adult neurogenesis.

From the results discussed above, it can be concluded that the majority of addictive drugs negatively affect the

neuronal differentiation of adult hippocampal NSPCs. Although some exceptions were reported, these exceptions are more likely to represent aberrant changes that would contribute to addiction-related behavior and memory, rather than cause favorable effects such as promoting normal adult neurogenesis. The drugs regulate NSPC differentiation in two major ways. One way is to control the progression of developmental stages, whereas the other is to switch the lineage-specific differentiation via the mechanisms of cell fate determination. Functionally, the first mechanism modulates the balance between the NSPC pool and the mature neural cells, whereas the second regulates the proportion of newborn neurons, astrocytes and oligodendrocytes. Thus, the alternation of either mechanism induced by addictive drugs may give rise to abnormal neurogenesis that may account for addiction-related consequences.

Although each addictive drug may regulate one or several aspects of neurogenesis, the overall effect is reflected by the combination of all three processes. Thus, one may expect unparalleled effects on different processes induced by a single drug. For example, in rats with low novelty-seeking behavior, chronic cocaine treatment decreased NSPC proliferation but had no effects on survival, whereas in rats with high novelty-seeking behavior, cocaine withdrawal suppressed the survival of newborn neurons but did not affect cell proliferation [93]. It is therefore necessary to recognize the complexity underlying the actions of addictive drugs, which provide a variety of potential targets for correcting aberrant molecular and behavioral alterations induced by drug addiction.

Common mechanisms in adult NSPC regulation

Signaling molecules and cascades

According to the effects of the individual addictive drugs discussed above, it is clear that a number of signaling molecules and cascades such as MAPKs, cell cycle regulators and miRNAs play a common role in modulating NSPCs upon exposure to different drugs. Although their effects on mediating the downstream signaling of certain drugs were demonstrated separately, there is an evidence showing their involvement in the regulation of NSPCs, which, although not yet associated with drug effects, indicates similar undiscovered pathways that may connect drug effects with adult neurogenesis.

MAPKs

The MAPKs, especially the extracellular signal-regulated kinases (ERKs), are a family of widely expressed signaling molecules that regulate cell proliferation, differentiation and survival by their effects on a series of cellular functions such as mitosis, gene transfection and the activation of multiple signaling molecules. ERKs may be activated by many different extracellular stimuli, including addictive drugs; this involvement has been found in NSPC regulation induced by opioids and cannabinoids [23, 43, 51, 80, 84, 104]. There is another evidence that MAPK signaling regulates NSPCs, which has not yet been tested in the effects of addictive drugs. For example, the PI3K/Akt/CREB cascade was shown to promote proliferation and inhibit differentiation of NSPCs, which is likely to be mediated by a series of MAPKs [131]. Moreover, the involvement of ERK in adult NSPC regulation was further confirmed by the observation that both differentiation and survival were inhibited after the deletion of the ERK5 kinase [132]. There is also evidence indicating that other members of the MAPK family such as c-Jun N-terminal kinase (JNK) and p38 take part in the proliferation, differentiation and survival of adult NSPCs [133], which, although not induced by addictive drugs, nonetheless suggests the existence of similar pathways in drug addiction.

Cell cycle regulators

The cell cycle represents a series of event leading to the division and duplication of cells and is divided into several phases, namely Gap 0 (G0), Gap 1 (G1), Synthesis (S), Gap 2 (G2) and Mitosis (M). Two key classes of regulatory molecules, Cyclins and Cyclin-dependent kinases, are responsible for the regulation of the cell cycle [134]. Growing evidence indicates there is an association between cell cycle lengths and the modulation of NSPCs [72], agreeing with the findings found with addictive drugs, including opioids, cocaine, methamphetamine and alcohol [39, 69, 79, 82, 95, 98, 125]. Because mitosis is a crucial step of cell proliferation, it is obvious that factors that controlling the cell cycle may also control NSPC proliferation. The correlation between adult NSPC differentiation and the cell cycle was also shown by the observation that the S-phase and the total cell cycle length were shortened [135]. Moreover, there is evidence that the duration of the G1 phase is a key factor that regulates cell fate [136, 137]. Thus, the targeting of certain cell cycle phases is a common strategy used extensively by a number of addictive drugs in regulating NSPC proliferation.

After the regulative effects of addictive drugs on the cell cycle phases were revealed, further research aimed to determine their regulatory molecules, such as the Cyclins and Cdks, and the pathways leading to such results. For example, it was observed that the lengthening of the G1 phase by the inhibition of the Cdk2/Cyclin E1 complex is sufficient to switch the fate of NSPCs from proliferation to

neuronal differentiation [136, 137]. Similarly, the overexpression of the Cdk4/Cyclin D1 complex increased NSPC proliferation, whereas the inhibition of the complex resulted in G1 phase lengthening and enhanced neuronal differentiation [138]. Another regulatory molecule, Cyclin D2, is also a positive regulator of G1 progression and thus controls cell fate by functioning as a switch between NSPC proliferation and neuronal differentiation [139]. However, another analysis suggested that the G1 phase was not the only phase correlated with NSPC differentiation because it was also shown that the S-phase was involved [140]. Interestingly, it was reported that instead of regulating the cell cycle, the Cdks were capable of directly phosphorylating the proneural transcription factor Neurogenin 2 (Ngn2), thereby promoting neuronal differentiation [141]. These reports suggest that in addition to their roles in the regulation of cell proliferation, the cell cycle regulatory molecules contribute to modulating NSPC differentiation via multiple mechanisms. Although some results were obtained from cells other than adult NSPCs, they are nonetheless informative, and it is likely that these mechanisms are universal.

MicroRNAs

MicroRNAs are a family of non-coding small RNAs that post-transcriptionally control gene expression by leading to the transcriptional inhibition or degradation of their target mRNAs. They are shown to take part in multiple biological processes, including proliferation and differentiation of adult NSPCs [142, 143]. So far, a number of members of the miRNA family such as miR-9, miR-124, miR-125b, miR-181a and Let-7d have been shown to take part in the modulation of adult NSPCs induced by addictive drugs, especially opioids and cocaine [23, 51, 80, 96–99]. These studies suggest the roles of a series of miRNAs in NSPC regulation mediated by addictive drugs, and because of the relative abundance of the total pool of miRNAs and their targets, we are likely to discover a vast field of miRNA-mediated NSPC modulation beyond our current knowledge through further investigation.

Prox1/Notch1

On the account of the complexity of the mechanisms that control adult neurogenesis in response to addictive drug exposure, we expect to see many more mechanisms and pathways take part in this process, far beyond the scope of the major mechanisms discussed above. The Prox1/Notch1 cascade, for example, is a key player in the fate determination of NSPCs and adult neurogenesis [144]. It has been widely accepted that Notch1 regulates adult NSPCs by maintaining type-1 cells in the SGZ [145] and by promoting astroglial but not neuronal differentiation [146, 147]. These effects of Notch1 may be attributed to its ability to enable the prolonged exposure of Sonic hedgehog by regulating the subcellular locations of the receptor, Patched1 [148]. Prox1 is exclusively expressed in DG cells and promotes the differentiation and survival of NSPCs in the adult hippocampus by inhibiting *Notch1* gene expression [149, 150]. As we have recently reported, the Prox1/Notch1 cascade is required for morphine-induced alteration of NSPC differentiation [23].

Pax6/Ngn2/Tbr2/NeuroD1/Tbr1

The transcription factor paired-box 6 (Pax6) and the basic helix–loop–helix (bHLH) transcription factors Ngn2 and NeuroD1 are important for proliferation and differentiation of NSPCs [151, 152]. Pax6 is a multifunctional regulator of NSPCs as it may promote either NSPC proliferation or neuronal differentiation in a context-dependent manner [153]. Ngn2 is expressed in neuronal progenitor cells and is required for DG cell proliferation and development [154] as it directs granule neuroblast production and amplification [155]. NeuroD1, on the other hand, is essential for the differentiation and survival of granule cells [152]. Although these three transcriptional factors may regulate NSPCs independently, they are able to define a cascade with two other factors, T-box brain gene 1 and 2 (Tbr1 and Tbr2), in the sequence of Pax6-Ngn2-Tbr2-NeuroD1-Tbr1 [153], which plays a crucial role in regulating adult SGZ NSPCs [156].

Crosstalk between different modulators

The finely tuned regulation of NSPCs in vivo or in vitro suggests a comprehensive system composed by a series of mechanisms that interact with one another rather than functioning independently. Therefore, the crosstalk between different signaling molecules and pathways should be intensively studied for in-depth analyses. As shown in Fig. 1, major addictive drugs modulate the balance between the proliferation and differentiation of adult NSPCs by the crosstalk of different mechanisms. The MAPK pathway is a key player as it is a combination of the receiver of multiple extracellular stimuli and the regulator of other mechanisms. This effect was also confirmed on embryonic NSPCs as the activation of ERK and JNK2 increased Cyclin D1 expression [157]; however, this has not yet been shown in adult NSPCs after addictive drug exposure.

Apart from the MAPK cascade, Notch is also a regulator of the cell cycle. Notch signaling is capable of mediating the self-renewal of adult NSPCs via the expression of the *presenilin1* gene, which controls the cell cycle length

[158]. Thus, the cell cycle and Notch signaling may collaborate to serve as a switch that regulates the balance between the proliferation and differentiation of adult NSPCs. Decreased Notch signaling increases cell cycle exit and thus inhibits cell proliferation, whereas increased Notch signaling decreases cell cycle exit and promotes cell proliferation. Additionally, studies using embryonic NSPCs have revealed the crosstalk between Notch and growth factors that modulate NSPC proliferation and differentiation [159], which may infer the involvement of such mechanisms in adult NSPCs. On the other hand, the regulatory molecules of the cell cycle also interact with other pathways. It was found that Cdks promote neuronal differentiation by directly phosphorylating and activating Ngn2, thereby revealing the correlation between cell cycle lengthening and neuronal differentiation [141].

On account of the large and growing variety of miRNAs reported during the past decade, it is likely that miRNAs may take part in almost all effects induced by addictive drugs through their interactions with other signals. At least 35 up-regulated miRNAs have been found to take part in alcohol-induced effects by regulating mechanisms such as the cell cycle and apoptosis [160], and 11 miRNAs were reported to play regulatory roles in opioid pharmacology [161]. However, only a few miRNAs have been shown to participate in the effects of addictive drugs on adult NSPCs by interacting with other mechanisms. Studies on other cells may also provide other information about the correlation between miRNAs and other mechanisms. Thus, the crosstalk between different mechanisms serves as a mediator that transfers the complex stimuli of different addictive drugs to the final outcome reflected by alterations of NSPCs and adult neurogenesis.

Putative common pathways and mechanisms of NSPC regulation by addictive drugs

The GPCR/miRNA modulating pathway

Despite the various mechanisms that control adult NSPCs, we can nevertheless identify crucial factors that are commonly involved in modulation of NSPCs by addictive drugs. Because of the large variety of GPCRs expressed on NSPCs, including those regulated by addictive drugs, either directly or indirectly, it is likely that the signaling of GPCRs is essential in the regulation of adult neurogenesis [162]. These GPCRs consist of receptors that bind to specific addictive drugs such as opioid receptors and cannabinoid receptors, along with those that bind to neurotransmitters and neuromodulators such as dopamine, serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine. On the other hand, miRNAs are widely involved in all aspects of adult neurogenesis regulated by different addictive drugs, as discussed above. Therefore, we would like to suggest that receptors (mainly GPCRs) and miRNAs represent the key players in the common pathway activated by addictive drugs that modulate adult NSPCs. Their interaction is likely to summarize the underlying mechanism.

Our recent works clearly exemplify the existence of the receptor/miRNA module in the morphine-activated signaling pathway that regulates NSPC differentiation [23, 51]. Morphine binds to and activates OPRM1, a member of the GPCR family, which in turn promotes the maturation of miR-181a via the ERK/TRBP/Dicer pathway [51]. MicroRNA-181a further controls Prox1/Notch1 signaling, which ultimately results in alterations of NSPC differentiation [23]. In addition, morphine inhibits the activity of CaMKIIα and the subsequent NeuroD1 phosphorylation by activating OPRM1, thus decreasing NeuroD1 activity and the subsequent expression of NeuroD1 targets, such as Doublecortin, that are known to regulate the differentiation and maturation of new born neurons [52]. Thus, morphine can regulate adult neurogenesis via the activation of OPRM1, with or without changes in miRNA levels. Although GPCR activation and miRNA regulation are not yet fully elucidated for other addictive drugs, their effects are implied in the common involvement of GPCRs and miRNAs in the regulation of NSPCs. Cocaine, for example, binds differentially to the dopamine, 5-HT and norepinephrine transport proteins and prevents their re-uptake, thus increasing the concentration of the three neurotransmitters [163]. Therefore, cocaine may indirectly induce alterations of signaling pathways via the receptors of dopamine [164, 165] and 5-HT [166], which are both GPCRs. It is possible that these GPCRs mediate the cocaine-induced up-regulation of pri-miR-9 and miR-124, thereby regulating NSPC proliferation and differentiation. Similarly, other addictive drugs such as amphetamine, methamphetamine, cannabinoids and alcohol are capable of directly or indirectly activating GPCRs [167–169] and altering the expression of miRNAs [160, 170–173], though their roles in regulating adult NSPCs have not been fully elucidated. Nicotine is an exception because the nicotinic acetylcholine receptor (nAChR) is an ion channel-linked receptor but is not a member of the GPCR family [174]. However, it also alters the expression of a series of miRNAs [175]; therefore, the receptor/miRNA module may nonetheless mediate the effects of nicotine on NSPCs. These findings suggest a role for the receptor/miRNA module in the regulation of adult NSPCs by addictive drugs, which remains to be further elucidated by future investigations.

Biased agonism

Biased agonism, or the functional selectivity of a receptor, is a ligand-dependent selectivity for certain signal transduction pathways using the same receptor, especially a member of the GPCR family. It is a common phenomenon when a receptor has several possible alternative pathways and ligands [176], which occurs with a series of GPCRs, including receptors for opioids, dopamine and 5-HT. [177, 178]. As for the signaling of addictive drugs, OPRM1 is a well-studied GPCR with biased agonism. The different pathways of ERK phosphorylation selected by morphine and fentanyl, two OPRM1 agonists, result in distinct effects in the miR-181a/Prox1/Notch1 pathway, and consequently, the differentiation of NSPCs [23, 51]. Although the involvement of biased agonism is not yet confirmed in the modulation of NSPCs by other addictive drugs, there is evidence that it may be a general mechanism that controls adult neurogenesis in response to various drugs. Cannabinoids, for example, are a group of selective ligands of the cannabinoid receptors, CB1 and CB2, both of which are GPCRs [179]. The biased agonism effects of CB1 ligands were demonstrated earlier on the activation of G_i isoforms as WIN activates all of the G_i subtypes, whereas other ligands activate only some G_i isoforms. Moreover, different CB1 agonists, including WIN, HU210, CP55940 and AEA, showed different efficacies for the $G_{i/o}$ and G_s signaling pathways [180]. It was later found that chronic CP55940 treatment up-regulated the β-arrestin2-ERK interaction and β-arrestin2 expression via CB2 in the prefrontal cortex of rats, which further contributed to the up-regulation of 5-HT_{2A} receptors. Other selective CB2 ligands showed similar effects, which were inhibited by CB2 antagonists, indicating the functional selectivity of β-arrestin2-mediated pathways [181]. Furthermore, it was recently revealed that CB1 receptors are subject to ligand-biased signaling and allosterism, as shown by the distinct biased signaling profiles of different cannabinoid agonists. For instance, whereas WIN 55,212-2 showed little preference for the inhibition of cAMP and the phosphorylation of ERK1/2, anandamide, methanandamide, CP55940 and HU210 were biased toward cAMP inhibition. Thus, the biased agonism of cannabinoid receptors may contribute to distinct downstream effects of different cannabinoid agonists, including their effects on proliferation, differentiation and survival of adult NSPCs.

Although other addictive drugs do not directly bind to GPCRs, it is confirmed that their pharmacological effects are accomplished via certain GPCRs [64, 164–169]. Therefore, the biased agonism of GPCRs may regulate their effects on NSPCs. For example, amphetamine-induced locomotor activity is inhibited in β-arrestin2 knockout mice, indicating an essential role for β-arrestin2 for the signaling mechanisms of dopamine [182]. However, although the β-arrestin2 knockout does not affect the acquisition of CPP in response to cocaine, the reconsolidation of CPP is impaired. Moreover, propranolol, a nonselective blocker of the β-adrenergic receptor (β-AR), inhibits the reconsolidation of CPP and conditioned fear memory, suggesting that the β-AR/β-arrestin/ERK pathway, rather than the G_s protein/PKA pathway, regulates the memory reconsolidation of addictive drugs [183]. Thus, we may infer that biased agonism is a universal mechanism that regulates various pharmacological effects in response to distinct addictive drugs, including the modulation of adult NSPCs.

Conclusions and prospects

So far, the available evidence outlines the effects of major addictive drugs on the proliferation, differentiation and survival of adult NSPCs, along with the underlying mechanisms that interact with one another. The interaction among these mechanisms is reflected in the alterations of NSPCs, which contribute to the effects of addictive drugs on adult neurogenesis. Although the adverse effects of addictive drugs are now widely accepted and the suppression of adult hippocampal neurogenesis seems to be a universal property of all drugs, we did discover positive drug effects that promote the proliferation, differentiation and survival of NSPCs under certain conditions, which appear to be unparalleled with their anti-neurogenic consequences. However, although NSPCs are the structural basis of adult neurogenesis, we should fully recognize the complexity of the mechanisms that correlate with the modulation of both NSPCs and neurogenesis before simply reaching a conclusion on the effects of these drugs. Due to the complexity of drug addiction, it is likely that adult neurogenesis is indispensable for the establishment of addiction-related memory and behavior, which rely on the aberrant pro-proliferative or neuronal differentiation effects of addictive drugs.

In spite of the abundant studies on the mechanisms that are involved in the modulation of NSPCs, only a few of these mechanisms have been confirmed to take part in the regulation of adult NSPCs induced by addiction. However, it is not surprising that mechanisms revealed using embryonic NSPCs or mature neural cells, or induced by other non-addictive stimulants, may also be applicable to the actions of addictive drugs on adult NSPCs. Thus, these confirmed mechanisms should be examined in future research on the molecular and cellular mechanisms controlling adult neurogenesis. By extensively elucidating these underlying mechanisms, we will be capable of finding new targets essential for studying addiction vulnerability on the cellular level.

As a complex recurrent process, drug addiction is often evaluated through its behavioral consequences such as drug seeking and taking, drug memory, abstinence and the relapse to drug seeking [184]. On the other hand, molecular and cellular mechanisms that control NSPCs and adult neurogenesis in response to drug stimuli have been comprehensively studied, as discussed earlier. However, how these alterations of NSPCs on the molecular and cellular levels would contribute to behavioral consequences still remains elusive. Therefore, we should focus on the correlation between the cellular effects of addictive drugs such as the alterations of the proliferation, differentiation and apoptosis of adult NSPCs, and the behavioral phenomena. The integration of pharmacological approaches and behavioral models is crucial for the further understanding of this area of research. This future direction of investigation should be able to provide us with sufficient information on how NSPCs play an essential role in mediating drug-induced behavioral regulation, and this could subsequently lead to new approaches in the treatments for addiction-related disorders.

So far, studies in the field of adult neurogenesis are based on rodent models or primary cultured cells, but the practical application of this knowledge calls for the extension of these studies into humans. Although the traditional labeling of proliferative cells is difficult to apply in humans because of the toxicity of this labeling, a new strategy to retrospectively determine the age of cells has been developed [185]. Moreover, developmental stages and marker profiles in adult neurogenesis, which were originally studied in rodents, have been extended to human hippocampal tissue by investigating the pattern of DCX-positive neuroblasts across the lifespan from 0 to 100 years [186]. The similar neural exchange rates of adult mice and humans suggest their extents of neurogenesis are comparable [185]. As for the effects of addictive drugs, it has been reported that cocaine increases cell death in adult human NPCs by inducing oxidative stress [92], agreeing with the results observed in rodent studies. However, studies on other drugs, such as antidepressants, showed differential effects on the proliferation of DG cells [187], implying different cellular mechanisms between rodents and humans, in spite of their many similarities [188]. Thus, although rodent studies on addictive drugs and adult neurogenesis have valuable implications for human brain functions after drug abuse, new strategies should be developed to access the mechanisms in humans in parallel with the currently used animal models. These intriguing findings will undoubtedly contribute to our knowledge of adult neurogenesis in response to drug abuse and other human diseases.

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