New Neurons in the Dentate Gyrus Promote Reinstatement of Methamphetamine Seeking

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Chitra D Mandyam[1,2], Sucharita S Somkuwar[1], Robert J Oliver[1] and Yoshio Takashima[1,2]

[1]VA San Diego Healthcare System, San Diego, CA, USA. [2]Department of Anesthesiology, University of California, San Diego, La Jolla, CA, USA.

ABSTRACT: Addictive drugs effect the brain reward circuitry by altering functional plasticity of neurons governing the circuits. Relapse is an inherent problem in addicted subjects and is associated with neuroplasticity changes in several brain regions including the hippocampus. Recent studies have begun to determine the functional significance of adult neurogenesis in the dentate gyrus of the hippocampus, where new neurons in the granule cell layer are continuously generated to replace dying or diseased cells. One of the many negative consequences of chronic methamphetamine (METH) abuse and METH addiction in rodent and nonhuman primate models is a decrease in neural progenitor cells in the dentate gyrus and reduced neurogenesis in the granule cell layer during METH exposure. However, the number of progenitors rebound during withdrawal and abstinence from METH and the functional significance of enhanced survival of the progenitors during abstinence on the propensity for relapse was recently investigated by Galinato et al. A rat model of METH addiction in concert with a pharmacogenetic approach of ablating neural progenitor cells revealed that neurogenesis during abstinence promoted a relapse to METH-seeking behavior. Biochemical and electrophysiology studies demonstrated that an increase in neurogenesis during abstinence correlated with increases in plasticity-related proteins associated with learning and memory in the dentate gyrus and enhanced spontaneous activity and reduced neuronal excitability of granule cell neurons. Based on these findings, we discuss the putative molecular mechanisms that could drive aberrant neurogenesis during abstinence. We also indicate forebrain-dentate gyrus circuits that could assist with aberrant neurogenesis and drive a relapse into METH-seeking behavior in METH-addicted animals.

KEYWORDS: Drugs of abuse, addiction, relapse, dentate gyrus, granule cell neurons, electrophysiology

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CORRESPONDING AUTHOR: Chitra D Mandyam, 3350 La Jolla Village Dr, Building 1, Rm 6130E, San Diego, CA 92161, USA. Email: cmandyam@scripps.edu

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Introduction

Neurogenesis in the dentate gyrus (DG) is a multistep process by which stem-like precursor cells proliferate into preneuronal progenitors, differentiate into immature neurons, and subsequently mature into fully functional granule cell neurons that integrate into the hippocampal circuitry.[1] Extensive research from rodent studies suggests that the primary function of adult hippocampal neurogenesis is maintenance of neuronal turnover in the granule cell layer of the DG. In the human hippocampus, neurogenesis accounts for a 1.75% annual turnover,[2] and in the rodent brain, neurogenesis accounts for 6% of the total number of granule cell neurons.[3] Emerging mechanistic studies from rodent models indicate that adult neurogenesis is a promising bio-behavioral target in several neuropathologies as external/environmental factors concurrently modulate adult neurogenesis and functions such as cognition and memory.[1] It is well established that adult neurogenesis is modulated by inputs from almost all major neurotransmitter systems projecting from several regions of the brain, and therefore, modulators of neurotransmitter release and/or signaling affect hippocampal neurogenesis.[4] The most clearly demonstrated examples are increased neurogenesis by antidepressants and decreased neurogenesis by drugs of abuse.[5,6] However, several unanswered questions need to be addressed before a recalibration of DG function by hippocampal neurogenesis can be used as a therapeutic strategy in such neuropsychiatric conditions. For example, do granule cell neurons born during or after chronic drug experience show different electrophysiological and biochemical properties from those born in age-matched controls? If so, are these differences adaptive and help with recovery or are they maladaptive and contribute to the long-term perpetuation of deficits? Recent publication by Galinato et al[7] suggests that a subset of newly born hippocampal neurons in a model of methamphetamine (METH) addiction directly contribute to compulsive METH-seeking behavior,[7,8] thus raising new questions about the role of neurogenesis in recovery from addiction. However, technologies to address such nuanced questions are still in their nascent stages of development. For example, single-cell RNA sequencing (RNA-Seq) methods have demonstrated transcriptome-level differences between individual quiescent stem cells,[9] mature DG neurons,[10] and more importantly in stem cells responding to an ischemic challenge in the subventricular zone.[11] Furthermore, new transgenic rodent models that support conditional knockdown of adult neurogenesis will be invaluable for determining differences between new hippocampal

neurons born under physiological conditions and those following pathopharmacological challenges.[12] For example, the Nestin-TK and GFAP-TK mice that expressed thymidine kinase (TK) under a neuronal stem cell–specific promoter allowed temporal regulation of the level of neurogenesis without affecting any other neuronal or non-neuronal cell population.[13] These pharmacogenetic models helped ascertain that adult-born granule cell neurons play a role in contextual fear conditioning, spatial memory retrieval, and spatial pattern separation.[14] The recent availability of transgenic GFAP-TK rats opened up the possibility of investigating even more complex neurobehavioral questions.[15] Specifically, hippocampal neurogenesis was found to regulate sucrose preference, reward-related behavior,[15] and buffered changes in social preference during social stress, thereby contributing to reliance following a disruption in the social-dominance hierarchy.[16] These new tools can help better understand the specific role of the natural regenerative capacity of the DG and may thereby facilitate the much-needed expansion of the treatment toolkit for underserved patients with neuropsychiatric conditions.

Methamphetamine Effects Neural Progenitor Cells and Neurogenesis in the DG

Newly born neurons in the granule cell layer of the DG are involved in METH addiction because reinforcing doses of drugs in vivo decrease adult neurogenesis (reviewed in Mandyam and Koob[17]). For example, experimenter-delivered and self-administered METH in rodents and nonhuman primates negatively affect proliferation, survival, and neurogenesis of neural progenitor cells (NPCs) in the DG.[18,19] Experimenter-delivered and self-administered METH produces hippocampal cell death, suggesting a potential role for cell death in an METH-induced reduction of neurogenesis. Recent studies from others and our laboratory have explored the cellular mechanisms underlying METH-induced inhibition of neurogenesis. Experimenter-delivered METH decreased the number of newly born NPCs and inhibited the proliferation and differentiation of NPCs. Cell cycle kinetic analysis demonstrates that reduced proliferation in rats that self-administered METH was attributed to reduced levels of progenitors in the synthesis (S) phase of the cell cycle without significant modifications in the length of the S phase of the cell cycle.[20] Additional findings indicate that METH self-administration in rats reduces net proliferation of progenitors and immature neurons by reducing the number of proliferating preneuronal neuroblasts and increasing the number of proliferating preneuronal progenitor cells,[20] suggesting that a decrease in the number of progenitors and immature neurons, to a large degree, is attributable to the decrease in the ability of neuroblasts to divide and produce stable progenitor cells that survive as immature neurons.[19,20] Supporting the rodent studies, new data in nonhuman primates that experienced a variety of amphetamines indicate that chronic amphetamine exposure decreases hippocampal neurogenesis by initially altering the precursor cell pool, followed by altering the transition of developmental stages of proliferating progenitors from NPCs to immature neurons and altering the neurogenic niche.[18] However, abstinence from METH in rodents increases net proliferation of progenitors and survival of newly born granule cell neurons, suggesting that cell intrinsic signals that maintain cell proliferation are differentially regulated during abstinence from METH.[21] These findings also suggest that neurogenesis in the DG could directly contribute to the propensity for relapse in METH-addicted subjects by altering the plasticity in the DG.[8]

In the context of the above hypothesis, Galinato et al[7] used a robust method to examine the direct role of adult neurogenesis during protracted abstinence from compulsive-like contextual-driven METH reinstatement in METH-addicted animals. The pharmacogenetic rat model (GFAP-TK rats[15]) was used to conditionally ablate neurogenesis in the DG.[7] Male GFAP-TK rats were trained to self-administer METH in an extended access schedule of reinforcement followed by protracted abstinence, extinction, and reinstatement. During acute withdrawal, GFAP-TK rats were administered with the antiviral drug valganciclovir (Valcyte) to induce apoptosis in actively dividing GFAP type I radial glia-like stem cells. Valcyte treatment was continued during abstinence to inhibit adult neurogenesis (indicated by significant decrease in number of cells expressing NeuroD and DCX). Valcyte treatment prevented compulsive-like contextual-driven METH seeking, and these behavioral effects correlated with reduced or abolished neurogenesis in the DG and reduced activation of plasticity-related protein calcium calmodulin kinase (CaMKII) in the DG. These findings indicate a direct role of newly born granule cell neurons in the DG in context-driven METH-seeking behavior.[7]

Context-driven METH reinstatement also alters the functional plasticity of mature granule cell neurons.[7,22] The electrophysiological findings from mature granule cell neurons indicate that these neurons have dysregulated neuronal functioning in the basal state and altered functional plasticity that correlated with context-driven METH seeking. For example, mature granule cell neurons from reinstated rats have an increased level of spontaneous glutamatergic activity as compared with controls with enhanced frequency and amplitude of spontaneous excitatory postsynaptic currents (sEPSCs). Generally, changes in the frequency of EPSCs reflect changes in the probability of glutamate release, whereas changes in EPSC amplitude and kinetics reflect change in postsynaptic glutamatergic receptor function.[23] Therefore, it is possible that context-driven METH reinstatement-induced aberrant neurogenesis could be modulating the basal synaptic properties of mature granule cell neurons.[24] Supporting this hypothesis, newly born granule cell neurons can modify excitatory synaptic transmission to mature granule cell neurons, specifically the excitatory perforant path fibers that input from the entorhinal cortex to the mature granule cell neurons. For example, an

enhanced number of newly generated granule cell neurons are associated with reduced excitatory synaptic transmission to mature granule cell neurons mediated by fewer functional synapses, whereas ablation of newly born granule cell neurons is accompanied by increased excitatory synaptic transmission to mature granule cell neurons with no change in intrinsic properties and inhibition.[24] Given that newly born granule cell neurons could function to enable animals to distinguish related stimuli and events rapidly, support contextual discrimination, and participate in hippocampal memory clearance, it is tempting to speculate that endogenous alterations in the number of newly born neurons and DG excitability could contribute to memory-related disorders.[25]

Recent studies are beginning to uncover the neural circuits that regulate the function of newly born granule cell neurons. Anatomical and physiological evidence demonstrate that forebrain septal inputs directly activate the mature granule cell neurons and connect to newly born granule cell neurons, increasing the level of afferent excitatory input and thereby granule cell discharge.[26] However, the functional significance of neural circuits that regulate newborn granule cell neuron modulation of DG-dependent behaviors is unclear and is under investigation. Future studies should focus on circuitry-level analysis of newly born granule cell neurons and their role in regulating complex behavior patterns, including drug-seeking behaviors. Such studies could assist with understanding the neurobiology of addiction and open novel therapeutic strategies to treat the relapse stage of addiction.

Although regulation of newly born neurons in the DG may provide a mechanism to produce changes in addiction-associated circuitry, persistent addiction-related gene (ARG) expression may dispose these neurons to function aberrantly.[27] As such, the various levels of regulated gene expression could possibly prove to be an underlying factor as well as site for the treatment of substance use disorders. Posttranscriptional regulation is one such regulatory mechanism that has been recently implicated in addiction-related behaviors.[28,29] Posttranscriptional regulation is the regulation of newly transcribed messenger RNA (mRNA) before it is translated into a functional protein through mRNA processing, splicing, editing, and stability. The regulation of mRNA stability and translation in particular may be especially integral in neuronal function, as this regulation can be localized to specific neuronal compartments and 20% of brain-enriched genes are predicted to be targets of this mechanism.[30]

MicroRNAs (miRNAs) are one group of factors that regulate mRNA stability and translation. This process of miRNA-induced regulation occurs through complementary regions within the miRNA, termed seed regions, and its 3′ untranslated region of the targeted mRNA. Although the miRNA provides specificity in this regulatory process, it is the associated RNA-inducible silencing complex that carries out the degradation or translation repression of that mRNA.[31] Through their ability to fine tune expression patterns of hundreds to thousands of mRNAs, miRNAs can be thought of as master regulators of cellular processes. As suggested before, miRNAs may play an integral role in neuronal physiology. The expression pattern of miRNAs appears to be similar to immediate early genes, suggesting that they may play a similar role in neuronal compartment and stimulus specific responses.[32] As such, miRNAs are found to play a role in many neuronal processes such as neural development, synaptic plasticity, and behavior.

Recently, it has been found that an miRNA, miR-19, is enriched in NPCs.[33] As the NPC differentiates and migrates from the subgranular zone into the granule cell layer of the DG to become a mature granule cell neuron, miR-19 expression is diminished. This indicates that the expression pattern of miR-19 regulates the process of neurogenesis and altered patterns of expression of this miR could promote aberrant neurogenesis in the DG.[33]

Previously, we found that METH self-administration in an extended access model decreases proliferation, differentiation, and survival of NPCs in male rats.[20] The development of NPCs into immature neurons appears to be especially sensitive to escalated METH intake. Conversely, we found that protracted abstinence increased NPC development into neurons in male rats.[7,8] However, structurally these neurons appear immature, indicating aberrant neurogenesis in METH-abstinent rats.[7] This aligns with what was found by Han and colleagues where NPC miR-19 overexpression caused a stunted dendritic and generally immature neuronal phenotype. These abstinence-induced neurons appear to be necessary for context-driven METH reinstatement, as indicated by Galinato et al.[7] Therefore, it can be hypothesized that miR-19 expression may be dysregulated in NPCs in METH-addicted rats and compromised in newly born neurons following escalated patterns of METH intake. Overall, combined with the specific behavioral and neurogenic data in METH-addicted rats, investigation of NPC miR-19 and other miRs and their functional significance in increased NPC levels leading to reinstatement behavior and aberrant maturation of newly born granule cell neurons is an interesting future pursuit.

Author Contributions

CDM, SSS, RJO, and YT wrote the paper.

ORCID iD

Chitra D Mandyam https://orcid.org/0000-0002-7354-205X

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