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Dentate Gyrus Neurogenesis Ablation via Cranial Irradiation Enhances Morphine Self-administration and Locomotor Sensitization

Sarah E. Bulin¹, Matthew L. Mendoza¹, Devon R. Richardson¹, Kwang H. Song², Timothy D. Solberg², Sanghee Yun^{3,4}, and Amelia J. Eisch^{1,3,4,*}

¹Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas, TX 75390-9070

²Department of Radiology Oncology, University of Texas Southwestern Medical Center, Dallas, TX 75390

³Mahoney Institute of Neurosciences, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104

⁴Department of Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, Philadelphia, PA 19104

Abstract

Adult dentate gyrus (DG) neurogenesis is important for hippocampal-dependent learning and memory, but the role of new neurons in addiction-relevant learning and memory is unclear. To test the hypothesis that neurogenesis is involved in the vulnerability to morphine addiction, we ablated adult DG neurogenesis and examined morphine self-administration (MSA) and locomotor sensitization. Male Sprague-Dawley rats underwent hippocampal-focused, image-guided X-ray irradiation (IRR) to eliminate new DG neurons or sham treatment (Sham). Six weeks later, rats underwent either MSA (Sham=16, IRR=15) or locomotor sensitization (Sham=12, IRR=12). Over 21 days of MSA, IRR rats self-administered ~70% more morphine than Sham rats. After 28 days of withdrawal, IRR rats pressed the active lever 40% more than Sham during extinction. This was not a general enhancement of learning or locomotion, as IRR and Sham groups had similar operant learning and inactive lever presses. For locomotor sensitization, both IRR and Sham rats sensitized, but IRR rats sensitized faster and to a greater extent. Furthermore, dose-response revealed that IRR rats were more sensitive at a lower dose. Importantly, these increases in

* **Corresponding author:** Amelia J. Eisch, Mahoney Institute of Neurosciences and Dept Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104 USA.

Current Affiliations

SEB: Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78229-3900. TDS: Department of Radiation Oncology, University of California, San Francisco, CA. KHS: Texas Oncology PA, Fort Worth, TX.

Author Contribution

SEB and AJE conceptualized and designed the initial study. SEB, MLM, and DRR performed behavioral experiments. SEB, KHS, and TDS performed irradiation. SEB, DRR, and SY performed IHC experiments. SEB, MLM, SY, and AJE analyzed and interpreted data. SJB, SY, and AJE wrote the manuscript with input on intellectual content from MLM. All authors reviewed and approved the final version for publication.

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locomotor activity were not apparent after acute morphine administration and were not a byproduct of irradiation or post-irradiation recovery time. Therefore, these data, along with other previously published data, indicate that reduced hippocampal neurogenesis confers vulnerability for multiple classes of drugs. Thus, therapeutics to specifically increase or stabilize hippocampal neurogenesis could aid in preventing initial addiction as well as future relapse.

Keywords

Addiction; Doublecortin; Hippocampus; Neurogenesis; Opiates; X-ray

INTRODUCTION

Opiate abuse is a major problem, with over 2 million people diagnosed with prescription opioid abuse in 2013 alone (National Institute on Drug Abuse, n.d.). The study of the addicted brain has recently benefitted from a broadened focus that includes study not only of classical “reward pathway” brain regions (e.g. ventral tegmental area [VTA] to nucleus accumbens [NAc] and prefrontal cortex [PFC]) but also synaptically connected brain regions, such as the amygdala and hippocampus (Lodge and Grace, 2006; Britt et al., 2012). The hippocampus in particular is detrimentally affected by long-term drug exposure (Pu et al., 2002; Kahn et al., 2005). For example, heroin users have smaller hippocampi and reduced blood flow when compared to healthy controls (Pezawas et al., 2002). Such hippocampal drug-induced plasticity may also play a causative role, as the hippocampus of abstinent drug users is active during stimuli, like cue presentation, that can induce craving and relapse (Sell et al., 2000). As the hippocampus has a potent glutamatergic influence on NAc cell firing (Lodge and Grace, 2006; Britt et al., 2012; Bagot et al., 2015), drug- or even experience-induced hippocampal neuroplasticity may have long-term consequences for the function of the reward pathway. Indeed, of the glutamatergic NAc afferents, projections from the hippocampus uniquely synapse in the medial NAc shell where they regulate drug-induced locomotion (Britt et al., 2012). Therefore, it is important to understand hippocampal drug-induced neuroplasticity and its subsequent influence on the reward pathway.

Adult hippocampal neurogenesis is the ongoing process of adding new glutamatergic neurons to the dentate gyrus granule cell layer (DG GCL). Interestingly, many drugs of abuse, including opiates, decrease adult neurogenesis (Eisch et al., 2000; Kahn et al., 2005; Arguello et al., 2008; Fischer et al., 2008; Zhang et al., 2016). Drug-induced alterations in DG neurogenesis may contribute to addiction and relapse (Canales, 2013). Indeed, ablation of new DG neurons decreases cognitive flexibility and extinction in spatial tasks (Winocur et al., 2006; Burghardt et al., 2012). Directly relevant to a functional role for new neurons in addiction, reduction of new DG neurons increases vulnerability in a rat model of cocaine addiction (Noonan et al., 2010). However, it is unknown if this vulnerability generalizes from psychostimulants to other classes of drugs of abuse, such as opiates. In addition, it is unknown if this vulnerability occurs through altering the hippocampal influence on the reward pathway by altering drug-induced behavior or DG cellular activation.

Using image-guided cranial X-ray irradiation (IRR; Song et al., 2010), we completely ablated DG neurogenesis to test the hypothesis that decreased neurogenesis leads to greater morphine self-administration (MSA). We also hypothesized that ablation of neurogenesis would increase morphine locomotor sensitization. Locomotor sensitization reflects enhanced sensitivity within the NAc to glutamate release (Robinson and Berridge, 1993; Kalivas, 2009), and thus allows for a behavioral exploration of hippocampal-NAc circuit changes.

METHODS AND MATERIALS

Animals

Male Sprague-Dawley rats were used for all experiments (see Supplemental Information [SI]).

Drugs

Morphine sulfate was given i.v. as 0.5 mg/kg/infusion (MSA) or 0.5–10 mg/kg injection (morphine locomotor sensitization; see SI).

Cranial irradiation (IRR)

As explained in SI in detail, IRR rats were exposed to 10.63 Gy of X-ray radiation focused with a 1-cm collimeter to target the hippocampus. Sham rats were placed in the X-RAD machine, but radiation was not delivered. A subset of rats was sacrificed 5 weeks post-IRR to confirm the loss of adult-generated neural progenitors/immature neurons (via doublecortin-immunoreactive [DCX+] cell number, Fig. 1A, S1) and to qualitatively assess astrocytes and microglia (via GFAP+ and Iba1+ cells, Fig. S2). MSA rats began food training 28D post-IRR (Fig. 2A), morphine locomotor sensitization rats started locomotion testing 42D post-IRR (Fig. 3A), acute morphine locomotion rats started locomotion testing 78D post-IRR (Fig. 4A), and basal locomotion rats had two locomotor assessments 35D post-IRR and 84D post-IRR (Fig. 5A). These time points were selected to synchronize the time post-IRR when a) MSA or morphine locomotor sensitization rats began their respective experiments (42D post-IRR); and b) morphine-injected rats were sacrificed (compare Figs. 3A, 4A, and 5A).

Morphine self-administration (MSA)

A total of $n=31$ rats (Sham $n=16$, IRR $n=15$) were used for the 21D-long, 4hr/D MSA experiments (Fig. 2) as described (Noonan et al., 2010) and as detailed in SI.

Withdrawal Extinction testing (Ext)

After the completion of MSA, rats were observed twice daily for qualitative withdrawal symptoms (Cicero and Meyer, 1973). Most animals displayed diarrhea, but did not show symptoms such as wet dog shakes. After 28D of WD in the home cage, rats underwent 5D of extinction testing (Ext; Fig. 2A). Rats were placed back into their original self-administration chamber context for 4hr/D during which no drug was available. The lever presses during the 1st hour of each session is reported here as extinction (see SI).

Morphine locomotor sensitization

A total of n=24 rats (Sham n=12, IRR n=12) were used for the morphine locomotor sensitization experiments (Fig. 3A). Locomotor activity was assessed in circular chambers, with beam break number recorded by Med Associates software, and is further detailed in SI. During the withdrawal phase of morphine locomotor sensitization, rats were observed twice daily for qualitative withdrawal symptoms (Cicero and Meyer, 1973). As with MSA rats, most morphine locomotor sensitization rat displayed diarrhea, but did not show symptoms such as wet dog shakes.

Acute morphine locomotion

A total of n=24 rats (Sham n=12, IRR n=12) were used for acute morphine locomotion experiments (Fig. 4A). Locomotor activity was assessed in circular chambers; with beam break number recorded by Med Associates software, and is further detailed in SI.

Irradiation prior to basal locomotion

A total of n=24 rats (IRR n=12, Sham n=12) were used for basal locomotor experiments (Fig. 5A). Locomotor activity was assessed in circular chambers, with beam break number recorded by Med Associates software, and is further detailed in SI.

Tissue preparation

At the end of all experiments, rats received chloral hydrate anesthesia and subsequent intracardial perfusion as previously described (Noonan et al., 2010) and as detailed in SI.

Immunohistochemistry (IHC), histology, and quantification of immunopositive cells

IHC for and stereological quantification of DCX+ was performed as described (Noonan et al., 2010) and as detailed in SI. IHC for GFAP and Iba-1 was performed as described (Moravan et al., 2011).

Statistical analysis

Data are presented as mean and standard error of the mean. Analyses were performed with GraphPad Prism (version 6.05) as detailed in SI. Grubbs outlier test was performed on all data. A complete table of statistical tests and post-hocs implemented are provided in Table S1.

RESULTS

Image-guided cranial irradiation produces a long-term ablation of adult DG neurogenesis

In order to explore the relationship between adult DG neurogenesis and morphine-related behaviors, we exposed rats to either IRR or Sham treatments six weeks prior to MSA or locomotor sensitization (Fig. 2A, 3A). Prior work indicated 2 consecutive days of 10 Gy irradiation leads to a complete ablation of neurogenesis in the dorsal hippocampus, with 50% suppression in the ventral hippocampus (Wojtowicz et al., 2008; Noonan et al., 2010). Here we used a novel diagnostic image-guided approach to accurately deliver the planned radiation dose to the hippocampus (Fig. 1A–C)(Song et al., 2010). For IRR rats, a planar X-

ray image was first taken to ensure the head was properly aligned and to target the specific Bregma (Fig. 1B). A collimator defining the radiation field was inserted, and then a second planar X-ray image was taken to confirm the target area (Fig. 1C). These diagnostic X-ray images were extremely low in dose, resulting in less than 0.03 Gy (3 cGy) in addition to their daily 10.57 Gy dose, for a total of 10.63 Gy daily. When examined 5 weeks later, IRR and Sham rats were indistinguishable based on qualitative examination of DG GCL histology, emphasizing this new X-ray irradiation technique does not lead to the gross hippocampal damage as seen in lesions studies (Hernández-Rabaza et al., 2008). While qualitatively similar based on general histology, 5 weeks post-IRR IRR rats differed from Sham rats in one notable aspect: there was a complete ablation of DCX+ immature neurons along the entire longitudinal axis of the hippocampus (Fig. 1D, Fig. S1) in IRR rats five weeks post-IRR. Thus, this new X-Ray irradiation technique produced a more thorough ablation than previously-used X-ray irradiation techniques employed in the rat. Additionally, the ablation was long-lasting, as DCX+ cells were still absent up to 4-mon post-IRR (data not shown). In addition to neurogenesis, astrocytes and microglia were also qualitatively examined. Both 5 weeks and 12 weeks post-IRR, Sham and IRR DG tissue was indistinguishable in regards to staining for GFAP+ astrocytes (Fig. S2). However, Iba-1+ myeloid cells with morphology reminiscent of reactive microglia were evident both 5 and 12 weeks post-IRR (Fig. S2), showing that - as previously published with other irradiation paradigms (Moravan et al., 2011) - there is a persistent change in the DG after this irradiation technique.

Rats irradiated prior to morphine self-administration (MSA) take more i.v. morphine and press the active lever more during extinction

To test the hypothesis that ablation of adult neurogenesis leads to a greater vulnerability to an animal model of morphine addiction, rats received either IRR or Sham prior to operant training and subsequent MSA (Fig. 2A). All IRR and Sham rats used for MSA reached food training acquisition criterion (100 pellets/D for 3 consecutive D), and 95–100% of rats reached criterion for the first time by day 3 of training (see SI). However during MSA, IRR rats pressed the active lever for morphine significantly more than Sham rats (Fig. 2B). IRR rats also took more morphine over the length of all MSA sessions, having an almost 100 mg/kg increase in total morphine intake (Fig. 2C). As hippocampal lesions may increase locomotor activity (Won et al., 2003), one explanation for greater morphine intake in IRR rats is that they may exhibit higher locomotion due to hippocampal damage. However, IRR and Sham rats had similar indices of general locomotor activity during SA sessions, such as inactive lever presses during timeout periods (Fig. 2D), suggesting again that this IRR approach does not produce gross hippocampal damage, or compulsive-like behaviors (Ripley et al., 1999).

After 28D of withdrawal, a subset of rats (Sham=8; IRR=8) was placed back into the self-administration chamber for extinction trials. IRR rats pressed more on their first day of extinction vs. Sham rats (Fig. 2E). These data suggest that IRR rats – which lack DG neurogenesis – take longer to learn that morphine is no longer available during the operant task.

Rats irradiated prior to morphine locomotor sensitization are more sensitive to the locomotor effects of repeated, daily morphine, particularly at lower doses

Locomotor sensitization is used to measure the sensitivity of the reward pathway to repeated drug exposure (Robinson and Berridge, 1993). Given that during self-administration IRR rats took more morphine and pressed the active lever more during extinction vs. Sham rats (Fig. 2), we used locomotor sensitization to measure IRR-induced behavioral activation of the reward pathway. Rats begin with baseline saline injections (Baseline Saline; Fig. 3A). Both IRR and Sham rats showed similar baseline activity, including during the first day when the locomotor context was novel (Fig. 3B). IRR and Sham rats also showed similar activity levels on their first exposure to morphine (acute exposure; Fig. 3C, D). In addition, over the 15D of morphine locomotor sensitization, IRR and Sham rats both sensitized to 5 mg/kg morphine, having the greatest number of beam breaks on D10 (Morphine locomotor sensitization; Fig. 3B, D), and having significantly higher number of beam breaks on D15 when compared to D1 (data not shown). Qualitative observational analysis during the last 5D of the sensitization phase suggested both IRR and Sham rats were sensitizing to the point of displaying stereotypical behavior. However, closer examination of the daily behavior data revealed that IRR rats sensitized significantly more than Sham rats on the day of peak morphine-induced locomotor activity (Fig. 3E), with IRR rats showing 25% more locomotion than Sham rats on D10 of exposure. Additionally, IRR rats had shorter latency to sensitization, showing significantly more morphine-induced beam breaks on D7 compared to their D1 locomotion, while Sham rats took one more day to reach significance. Overall, as shown in the highlighted session days in Fig. 3C, IRR rats have greater sensitization than Sham rats.

To assess if the differences in locomotion were the result of changes in Pavlovian conditioning rather than a locomotor response to morphine (Hinson and Poulos, 1981), rats were placed back into locomotor chambers during the withdrawal phase. Notably, the activity of IRR rats was no longer higher than Sham rats, and both IRR and Sham rats showed activity levels similar to baseline (Withdrawal Locomotion; Fig. 3F). This indicated that the increased locomotion in both IRR and Sham rats during the morphine locomotor sensitization phase relied on morphine administration, and was not contextually-conditioned locomotion. After withdrawal locomotion, a dose-response experiment was used to identify whether sensitization was enhanced in IRR rats relative to Sham rats. IRR rats showed significantly more locomotion at 1.0 mg/kg and a trend at 0.5 mg/kg vs. Sham rats (Fig. 3G). IRR and Sham rats both showed a maximum locomotor response at 3.0 mg/kg, with decreasing activity at doses higher than 3.0 mg/kg, most likely due to a shift from locomotion into stereotypic behavior. As the 1.0 mg/kg dose resulted in the largest difference in locomotion during the dose-response, we opted to this dose for a morphine challenge prior to sacrifice. However, both IRR and Sham rats showed similar locomotor response to 1.0 mg/kg on challenge day (Fig. 3H).

IRR does not increase locomotor responses to acute morphine administration

The observed behavioral plasticity seen in the locomotor sensitization and MSA experiments arose after repeated exposure to morphine. While there was no difference in locomotion after the first exposure in the morphine locomotor sensitization experiment, the time-after-

IRR and injection number may have confounded this result. Therefore, we assessed if increased locomotor activity was a by-product of irradiation and/or unique to repeated morphine exposure by examining the effects of image-guided cranial IRR on locomotor responses to acute morphine administration 12 weeks post-IRR. Rats underwent either IRR or Sham treatment, coupled with an extended recovery time, which effectively synchronized this experimental timeline to the morphine locomotor sensitization experimental timeline (Fig. 4A). First, rats underwent 5 days of baseline saline injection (Fig. 4A; i.p. 1 mg/kg). As with the morphine locomotor sensitization experiment, both Sham and IRR rats had similar baseline locomotor activity (Fig. 4B). After 5 days of baseline saline injections, all rats were subjected to a single injection of morphine (Fig. 4A; i.p. 5 mg/kg) and their subsequent locomotor activity was measured. Both Sham and IRR rats had similar locomotor responses to acute morphine administration (Fig. 4C).

IRR does not increase basal locomotor activity 5 or 12 weeks post-IRR

There is little to no evidence on the effects of post x-ray IRR recovery time length on general locomotor activity in rats (Clark et al., 2008). To ensure that our behavioral adaptations were not a byproduct of IRR or post-IRR recovery time, we examined locomotor activity in drug naive animals 5 weeks and 12 weeks post-IRR. Importantly, the 5 week time point was selected to roughly mimic the time point at which the morphine locomotor sensitization group was first exposed to the locomotor chambers and the 12 week time point was specifically selected to synchronize when all rats were sacrificed for all locomotor experiments (Fig. 5A). At both time points, Sham and IRR rats had similar levels of locomotor activity (Fig. 5B,C).

DISCUSSION

The first notable finding presented here is that image-guided, hippocampal-focused X-ray irradiation and subsequent ablation of DG neurogenesis increased vulnerability in an animal model of morphine addiction. IRR rats self-administered more morphine, and this increase was not the result of IRR-induced changes in locomotion or operant learning. Given that cranial IRR increased self-administration of both a psychostimulant (Noonan et al., 2010) and an opiate (present work) – drug classes with distinct mechanisms of action – it suggests IRR-induced ablation of DG neurogenesis may generally disrupt the hippocampal contribution to reward-based behavior. This disruption in normal behavior exhibits itself more strongly with drug-induced activation than with natural reward-induced activation as irradiated rats self-administer sucrose similarly to controls (Noonan et al., 2010), though stressed mice lacking DG neurogenesis exhibit anhedonia (Snyder et al., 2011). It is logical that DG neurogenesis would influence reward circuitry activation with all types of reward, but the disruption is more obvious when the reward pathway is activated beyond its normal scope.

Our second notable finding presented here is that IRR rats lacking DG neurogenesis sensitize more to morphine relative to Sham rats. Of particular note, IRR and Sham rats had similar locomotion at baseline and on the first day of morphine injection (acute exposure). Locomotor sensitization is a well-known behavioral paradigm that provides insight into the

long-term, drug-induced, cellular – and ultimately functional – changes in the reward pathway and related circuitry (Steketee and Kalivas, 2011). In fact, locomotor sensitization is considered to be a behavioral reflection of a hypersensitive reward pathway (Robinson and Berridge, 1993; Rademacher et al., 2007; Vanderschuren and Pierce, 2010). Additionally, the hippocampus plays a central role in locomotor sensitization. For example, sensitization will only develop in a novel context, and exposure to the sensitized context results in more c-fos expression in the hippocampus and NAc (Badiani et al., 2000; Rademacher et al., 2007). Taken together with the MSA data above, it is likely that IRR-induced ablation of DG neurogenesis results in increased sensitivity to the hedonic effects of chronic, but not acute, morphine exposure. increased hippocampal glutamate after chronic exposure to morphine, likely resulting in increased sensitivity. Interestingly, sensitization results in more morphine-induced glutamate release in the hippocampus (Farahmandfar et al., 2011), and it is reasonable to hypothesize that this is even greater in IRR rats exposed to morphine locomotor sensitization than in Sham rats exposed to the same. This increased glutamatergic signalling may ultimately result in altered glutamate transmission downstream in regions, including the NAc and PFC. Though this remains untested and is beyond the scope of this work, it would be an interesting avenue to partake in the future. Indeed, sensitization and subsequent withdrawal results in less basal glutamate in the NAc (Xi et al., 2002), setting the stage for a stronger response to drug-induced hippocampal-NAc glutamate release (Reid and Berger, 1996).

An alternative mechanism behind increased MSA and morphine locomotor sensitization after IRR would be decreases in hippocampal inhibitory tone, leading to increased excitability that may influence downstream regions involved with reward circuitry. For example, recent work shows decreasing hippocampal interneuron number can strongly increase spontaneous dopaminergic firing (Perez and Lodge, 2013). Also, as interneurons are increasingly appreciated as potent regulators DG neurogenesis (Song et al., 2013), more work is needed to understand the relationship between hippocampal interneurons and neurogenesis as a potential mechanism behind ablation-induced alteration of hippocampal output. In sum, while it is unclear what drives the increased and faster onset of hypersensitivity to morphine after X-ray-induced ablation of DG neurogenesis, we hypothesize it may be due to less inhibitory tone in the DG, which results in greater subsequent activation of the reward pathway. This hypothesis is ripe for testing, particularly with the development of approaches to inducibly delete or silence adult DG neurons (Dhaliwal and Lagace, 2011) and inducibly activate select cell populations the hippocampal and reward circuitry through the use of optogenetics or DREADDs (Deisseroth, 2012).

Our third notable finding is that IRR rats took longer than Sham rats to extinguish both MSA and sucrose training. There were two examples of this presented here, both in the MSA experiment. First, in the first 4D of MSA, IRR rats pressed the morphine-paired lever 25% more than Sham rats, presumably taking longer to learn that the previously sucrose-paired lever was now paired with morphine. Second, during the first day of extinction, IRR rats pressed the formerly morphine-paired lever 40% more than Sham rats, again presumably taking longer to learn that this lever no longer delivered morphine. One explanation for the increased lever pressing is that IRR rats have deficits in extinction learning and/or in cognitive flexibility, both hallmarks of addiction (Baldacchino et al., 2012). It is important to

note that the higher lever pressing during the first day of extinction could instead be attributed to the higher morphine-taking behavior seen during the last of the regular MSA sessions. However, this would only explain the deficit on the first day of extinction, but not the elevation of intake seen during the transfer from sucrose to MSA, as all animals received equal amounts of sucrose training (Lattal and Lattal, 2012).

In support of IRR-induced extinction deficits, fear- and contextual-learning studies suggest DG neurogenesis is important in extinction (Ko et al., 2009), where old knowledge must be suppressed in order to learn something new, and also in cognitive flexibility, which is the ability to ignore previous information when circumstances change, and learn new information (Burghardt et al., 2012). In this regard, our study is the first to illustrate IRR-induced cognitive flexibility deficits in an animal model of addiction. It has been argued that ablation of adult neurogenesis leads to an overall deficit in learning rather than in extinction, as some studies have seen deficits in spatial and contextual learning (Shors et al., 2002). However, we found no deficits in the initial learning of the operant task, but only deficits in extinction/cognitive flexibility. If, as our study suggests, IRR rats indeed have deficits in extinction/cognitive flexibility, this would suggest that strategies to bolster neurogenesis might accelerate extinction learning or prevent relapse. In support of this, DG neurogenesis protects against relapse in an animal model of cocaine addiction (Deschaux et al., 2014). While cognitive flexibility has been indirectly linked to hippocampal neurogenesis (Burghardt et al., 2012), the vast majority of work indicates a PFC mechanism for cognitive flexibility. This suggests that the hippocampal-PFC pathway may also be detrimentally affected after irradiation. Thus altered DG neurogenesis may robustly influence PFC structure and function, and future studies are warranted to explore this as-yet unknown link.

A reasonable methodological concern of this study – and of all studies that utilize irradiation – is the nonspecific nature of cranial irradiation. X-ray radiation kills dividing cells and suppresses DG neurogenesis, but it also induces inflammation and can compromise neuronal synapses and dendrites, vasculature integrity, and the neurogenic niche (Wojtowicz, 2006; Raber, 2010). Given that our qualitative analysis of Iba-1 staining suggests a persistent activation of microglia post-IRR (Fig. S2), additional studies are clearly needed to test our hypothesis of the influence of DG neurogenesis on the reward pathway. For example, this hypothesis merits testing in future studies via methods of temporary ablation (optogenetics) or silencing (DREADDS) of newborn neurons at key points during the addiction process. However, there are three reasons why we still favor the hypothesis that the enhanced morphine reward shown here is at least in part due to suppressed DG neurogenesis. First, these experiments were performed at a time post-IRR when astrocyte number and morphology were similar between Sham and IRR rats (Fig. S2 and Noonan et al., 2010). Second, the dose of irradiation used here had no obvious influence on operant learning, locomotion, or other behaviors that would be sensitive to damage of hippocampal neurons or gross interference with the neurogenic niche (present data and (Wojtowicz et al., 2008)). Third, there is the concern is that IRR has disrupted the blood-brain-barrier, allowing for higher levels of morphine in the brains of IRR rats. However, the increase in MSA parallels previous work where cocaine self-administration was increased post-IRR with brain levels of cocaine unaltered by the irradiation, suggesting this is not the case. Future work specifically assessing integrity of the blood-brain-barrier after this new image-guided

irradiation approach would be needed to definitively address this possibility. Finally, the IRR-induced enhanced behaviors shown here were achieved with complete ablation of DG via image-guided irradiation, yet our prior work with IRR-induced enhanced cocaine SA was achieved with complete ablation in the dorsal but only 50% ablation in the ventral DG (Noonan et al., 2010). The similar findings with different drugs and different levels of neurogenesis minimally do not contradict the idea that neurogenesis suppression is a common denominator of these studies. Given the more selective targeting available with the image-guided cranial irradiation used here, it would be valuable to specifically assess hippocampal physiology, microanatomy, and a broader spectrum of behaviors after such directed ablation.

While limited comparison can be made between our work and those performed with other drugs of abuse and approaches to ablate neurogenesis, it remains notable that both 50% reduction (Noonan et al., 2010) and 100% ablation of ventral DG neurogenesis (present work) enhance drug reward. As the ventral hippocampus both sends afferents to the NAc and contributes to functions closely linked to addiction – like anxiety and response to stress – (Siegel and Tassoni, 1971; O’Leary and Cryan, 2014), one might expect a more robust effect in morphine-related behaviors when more of the ventral DG neurogenesis is ablated. It is possible that there is an under-appreciated role of the dorsal DG in regulation of the reward pathway, particularly through indirect connections (e.g. via the stress axis or PFC). Indeed, recent work supports a role for dorsal hippocampal neurogenesis in some behavioral responses to antidepressants (Wu and Hen, 2014). While we hypothesize that it is the IRR-induced ablation of new neurons in the ventral DG that primarily contributes to the enhanced morphine reward shown here, this warrants testing with discrete ablation of dorsal vs. ventral DG neurogenesis (Wu and Hen, 2014).

In sum, we report that image-guided cranial X-ray IRR and subsequent ablation of DG neurogenesis increases drug intake and results in extinction deficits in an animal model of morphine addiction (MSA), and increases locomotor sensitivity to repeated morphine. These data underscore a role for neurogenesis in the regulation of the reward pathway and behaviors linked to addiction. As these data show reduced hippocampal neurogenesis confers vulnerability for multiple classes of drugs, therapeutics to specifically increase or stabilize hippocampal neurogenesis (Petrik et al., 2012) could aid in preventing initial addiction as well as future relapse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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A Cranial Image-Guided Irradiation Ablates Adult Hippocampal Neurogenesis

Fig. 1.

Fig. 2.

Fig. 3.

A Cranial Image-Guided Irradiation Before Acute Morphine Locomotion

B

C

Fig. 4.

Fig. 5.