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Image-guided cranial irradiation-induced ablation of dentate gyrus neurogenesis impairs extinction of recent morphine reward memories

Phillip D. Rivera¹, Steven J. Simmons², Ryan P. Reynolds^{1,2}, Alanna L. Just¹, Shari G. Birnbaum¹, Amelia J. Eisch^{1,2,3}

¹Department of Psychiatry, UT Southwestern Medical Center, Dallas, Texas

²Department of Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

³Department of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Abstract

Dentate gyrus adult neurogenesis is implicated in the formation of hippocampal-dependent contextual associations. However, the role of adult neurogenesis during reward-based context-dependent paradigms—such as conditioned place preference (CPP)—is understudied. Therefore, we used image-guided, hippocampal-targeted X-ray irradiation (IG-IR) and morphine CPP to explore whether dentate gyrus adult neurogenesis plays a role in reward memories created in adult C57BL/6J male mice. In addition, as adult neurogenesis appears to participate to a greater extent in retrieval and extinction of recent (<48 hr posttraining) versus remote (>1 week posttraining) memories, we specifically examined the role of adult neurogenesis in reward-associated contextual memories probed at recent and remote timepoints. Six weeks post-IG-IR or Sham treatment, mice underwent morphine CPP. Using separate groups, retrieval of recent and remote reward memories was found to be similar between IG-IR and Sham treatments. Interestingly, IG-IR mice showed impaired extinction—or increased persistence—of the morphine-associated reward memory when it was probed 24-hr (recent) but not 3-weeks (remote) postconditioning relative to Sham mice. Taken together, these data show that hippocampal-directed irradiation and the associated decrease in dentate gyrus adult neurogenesis affect the persistence of recently—but not remotely—probed reward memory. These data indicate a novel role for adult neurogenesis in reward-based memories and particularly the extinction rate of these memories. Consideration of this work may lead to better understanding of extinction-based behavioral interventions for psychiatric conditions characterized by dysregulated reward processing.

*Correspondence Amelia J. Eisch, Room 402 F Abramson Pediatric Research Center, 3615 Civic Center Blvd Philadelphia PA 19104-4318. eisch@upenn.edu; eisch@email.chop.edu.

Present address: Phillip D. Rivera, Department of Biology, Hope College, Holland, MI.

CONFLICT OF INTEREST

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addiction; conditioned place preference; learning and memory; opiate; retrieval

1 | INTRODUCTION

Increasing evidence suggests that the hippocampus and its dentate gyrus subregion are important in reward-associated behavior. On a correlative level, the reward-context associations produced by morphine conditioned place preference (CPP; Bardo & Bevins, 2000; Tzschentke, 2007) modify indices of hippocampal plasticity (Zheng, Zhang, Li, Loh, & Law, 2013; Portugal et al., 2014; Rivera et al., 2015; Zhang, Xu, Zheng, Loh, & Law, 2016; Alvandi, Bourmpoula, Homberg, & Fathollahi, 2017), and after psychostimulant or opiate CPP dentate gyrus neurons are activated by re-exposure to the drug-paired context (Barr & Unterwald, 2015; Rivera et al., 2015). Notably, during CPP testing, entrance to the drug-paired context is preceded by phase-locked hippocampal theta rhythm (Takano, Tanaka, Takano, & Hironaka, 2010), suggesting the involvement of the hippocampus in reward memory retrieval of contextual cues. On a causative level, pharmacological disruptions or lesions of the hippocampus and dentate gyrus interfere with many stages of CPP, including the acquisition and retrieval of the drug/context association (Ebrahimian et al., 2016; Ferbinteanu & McDonald, 2001; Guo et al., 2016; Hernández-Rabaza et al., 2008; Hitchcock & Lattal, 2018; Katebi, Farahimanesh, Fatahi, Zarrabian, & Haghparast, 2018; Meyers, Zavala, & Neisewander, 2003; Meyers, Zavala, Speer, & Neisewander, 2006; Milekic, Brown, Castellini, & Alberini, 2006; Rezayof, Zatali, Haeri-Rohani, & Zarrindast, 2006; Sadeghi, Ezzatpanah, & Haghparast, 2016; Taubenfeld, Muravieva, Garcia-Osta, & Alberini, 2010; Zarrindast, Nouri, & Ahmadi, 2007), and interfere with other animal models of addiction-relevant behaviors (Fuchs et al., 2005; Ramirez et al., 2009; Paniccchia et al., 2018). Such studies in laboratory animals support that the hippocampus and dentate gyrus play an important role in context-dependent reward memory (Smith & Bulkin, 2014). A link between these limbic regions and reward-associated behavior is also evident in humans. For example, hippocampal function is strongly influenced by inputs from the medial temporal lobe, ventral tegmental area, and substantia nigra (Loh et al., 2016; Wittmann et al., 2005; Wolosin, Zeithamova, & Preston, 2012), and drug-associated cues that drive cravings, drug use, and relapse, also activate hippocampal circuitry (Franklin et al., 2007; Kilts et al., 2001; Koob & Volkow, 2010; McClernon et al., 2016; Schneider et al., 2001; Smolka et al., 2006). Therefore, more information on how the hippocampus and dentate gyrus influence reward-based behaviors—such as CPP—may lead to a better understanding of drug/context associations made after administration of drugs of abuse.

One important aspect of the dentate gyrus is the process of adult neurogenesis, where adult-born granule cell neurons (ABGCs) are generated throughout life (Gonçalves, Schafer, & Gage, 2016; Kempermann, 2006; Seki, 2011). ABGCs are implicated in many aspects of hippocampal-dependent function, including stages of context-associated learning and memory, such as retrieval, forgetting, extinction, reinstatement (Akers et al., 2014; Deng, Aimone, & Gage, 2010; Kitamura & Inokuchi, 2014; Saxe et al., 2006; Suárez-Pereira, Canals, & Carrión, 2015). ABGCs have also begun to be examined for their potential role in

reward-associated learning and memory (Canales, 2007; Eisch et al., 2008; Deschaux et al., 2014; Castilla-Ortega et al., 2016; Zhang et al., 2016; Alvandi et al., 2017; Barr, Bray, & Forster, 2018). For example, ABGC deletion via image guided, hippocampal-targeted X-ray irradiation (IG-IR) directed at the hippocampus results in increased subsequent intravenous (i.v.) self-administration of cocaine and morphine in rats (Noonan, Bulin, Fuller, & Eisch, 2010; Bulin et al., 2017). Additionally, extended access to methamphetamine i.v. decreases ABGCs, and decreasing dentate gyrus neurogenesis during abstinence diminishes context-elicited reinstatement of methamphetamine-seeking (Galinato et al., 2018; Mandyam et al., 2008; Yuan, Quiocho, Kim, Wee, & Mandyam, 2011). In regards to CPP, IG-IR does not change retrieval of cocaine CPP in rat (Brown et al., 2010). In the mouse, pharmacological reduction of ABGCs prior to CPP impairs extinction of cocaine CPP, while reduction after CPP enhances retention (Castilla-Ortega et al., 2016). As distinct classes of drugs of abuse differ in their underlying longer term effects (Becker, Kieffer, & Le Merrer, 2016), it is surprising that no studies have examined whether IG-IR and subsequent loss of ABGCs in the rodent alters CPP to opiates, such as morphine.

One utility of CPP is the assessment of drug/context associations according to the memory “age,” with recent memories assessed generally <48 hr posttraining and remote memories assessed generally >1 week posttraining. In regards to context-associated memory (non-CPP experiments), there is evidence that inducible reduction of ABGCs prior to learning and memory influences both recent and remote memory (Kitamura et al., 2009; Ko et al., 2009; Saxe et al., 2006), with some studies showing that reduced ABGCs impairs the extinction of recent memory and retrieval of remote memory (Dupret et al., 2008). Few studies have examined how reduced ABGCs influence reward-associated memory. For example, reduction in ABGCs prior to cocaine CPP does not alter the retrieval of a relatively recent reward memory in rats or mice (Brown et al., 2010; Castilla-Ortega, Blanco, et al., 2016), but impairs—or prolongs—the extinction of the memory in mice (Castilla-Ortega, Blanco, et al., 2016). No studies have yet examined the role of ABGCs in extinction of recent versus remote contextual memories associated with opiate administration. Given the distinct molecular, cellular, and network underpinnings of recent versus remote memories (Bergstrom, 2016; Déry, Goldstein, & Becker, 2015; Frankland & Bontempi, 2005; Khalaf & Gräff, 2016; Kitamura et al., 2017) and the proposed role of ABGCs in memories of different “ages” (Aimone, Wiles, & Gage, 2006; Kesner et al., 2014; Kitamura & Inokuchi, 2014), CPP studies that assess the potential role of ABGCs in opiate-associated contextual memories—probed at recent and remote timepoints—are warranted.

To address the unanswered questions of whether ABGCs are involved in retrieval or extinction of recent versus remote contextual memories associated with opiate administration, we used IG-IR to ablate dentate gyrus ABGCs prior to morphine CPP. Based on work in cocaine CPP (Brown et al., 2010; Castilla-Ortega, Blanco, et al., 2016), we hypothesized that ablation of ABGCs prior to morphine CPP would not influence retrieval of a recent reward memory but would impair extinction.

2 | MATERIALS AND METHODS

2.1 | Animals

Male C57BL/6J mice (6–8 weeks old, The Jackson Laboratories #000664, Bar Harbor, ME, USA) were housed at UT Southwestern Medical Center (UTSW; 4/cage, 12 hr light/dark cycle, lights on at 06:00) with ad libitum access to food and water. Mice were habituated to the vivarium for 1 week prior to CPP training. All animal procedures and husbandry were in accordance with the National Institutes of Health, Guide for the Care and Use of Laboratory Animals, and performed in IACUC-approved facilities at UTSW. Experiments were designed to minimize animal number, pain, and suffering.

2.2 | IG-IR

Small animal (IG-IR; $n = 50$) was delivered via the X-RAD 225Cx self-contained irradiation system (Figures 1 and 3; Precision X-Ray, North Branford, CT, USA) (Song et al., 2010). Briefly, the hippocampi of 7–9 weeks old mice (anesthetized with 1.5–2.5% isoflurane) received diagnostic X-rays to confirm head alignment and collimator placement (6×14 mm rectangular collimator) and were then irradiated (15 Gy; Walker et al., 2015). Sham control mice (Sham, $n = 47$) were exposed to isoflurane for a similar amount of time as IG-IR mice, but were never placed in the irradiator. Six weeks after IG-IR (a time point when ABGCs are significantly decreased; Walker et al., 2015) or Sham exposure, CPP training was performed. Mice in the dose–response study (Figure 2) never received isoflurane and were not placed inside the irradiator.

2.3 | Drug treatment

Morphine sulfate powder was provided by the National Institute on Drug Abuse (NIDA, Baltimore, MD). Morphine sulfate powder was dissolved in sterile bacteriostatic 0.9% saline (Hospira, Lake Forest, IL, USA) and administered at 15 mg/kg (subcutaneous [s.c.], Figure 1), 7 or 15 mg/kg (s.c., Figure 2), or 7 mg/kg (s.c., Figure 3) for CPP as described below. Saline controls were given sterile bacteriostatic 0.9% saline (s.c., 10 mL/kg).

2.4 | Conditioned place preference

CPP chambers were composed of three compartments: a grey compartment, middle compartment, and striped compartment, as previously described (Figure 1; Rivera et al., 2015). Grey and striped compartments were the same size with dimensions $24.5 \times 15 \times 33$ cm. The grey compartment had grey walls and large-grid wire flooring, while the striped compartment had black and white vertical striped walls with small-grid wire flooring. The middle compartment was located between the grey and striped compartments and was shorter ($12 \times 15 \times 33$ cm) with white walls and parallel bar flooring. Opaque dividers on each side of the middle compartment were lowered to either confine mice to one compartment (grey or striped) on pairing days, or lifted to allow free access to all compartments during pretest and test/extinction days. Time spent in each compartment was measured by photo beam breaks, which was collected by Med Associates, Inc. software.

During Pretest (Day 1, D1), mice were placed into the middle compartment and given free access to all three compartments for 20 min. Time spent in either grey or striped

compartments was recorded. After 20 min, mice were returned to their home cage (Rivera et al., 2015). Based on time spent in either grey or striped compartments determined from Pretest, unbiased pairing of mice to a drug context and nondrug context was performed for pairing days (D2–4; Bardo & Bevins, 2000) to bring the group sum from the calculation (nondrug context – drug context) as close to zero as possible. Any mouse with a CPP Score ± 240 s was automatically paired to receive drug in the nonpreferred context, and received pairing along with other cage mates; however, these data were not included in the analysis.

Pairing consisted of a morning saline (AM) and afternoon morphine (PM) conditioning session. During the AM session, each mouse received s.c. 0.9% saline and was immediately placed into the nondrug context for 30 min. In the PM session, each mouse received s.c. morphine and was immediately placed into the drug context for 30 min. Following the AM and PM sessions, mice were returned to their home cages (Rivera et al., 2015). Injections were only given during pairing; the retrieval and extinction sessions described below were performed with no injections (i.e., drug- and injection-free).

For retrieval of a recent reward memory, mice were placed into the middle compartment ~24 hr postpairing with free access to all three compartments for 20 min (Test, D5; Figure 1a). In a separate group of mice, retrieval of a remote reward memory was tested using the same procedure but performed instead ~3 weeks postpairing (Test, D21; Figure 1d). For extinction of recent and remote reward memories, mice were placed daily into the middle compartment and given 20 min with free access to all three compartments. Extinction testing of a recent reward memory began ~24 hr postpairing and was repeated every day for 1 week (Extinct, D5 to D11, Figure 2a) or ~3 weeks (Extinct, D5–24, Figure 3a). Extinction testing of a remote memory began 3 weeks postpairing and was repeated every day for ~3 weeks (Extinct, D25–44, Figure 3b). For retrieval and extinction sessions, time spent in each compartment was recorded.

2.5 | Tissue harvest, immunohistochemistry (IHC) and microscopy

Brains from mice were harvested after behavioral sessions concluded to confirm effects of IG-IR on ABGCs. Briefly, all mice received intra-cardiac perfusion with ice-cold 0.9% saline followed by 4% paraformaldehyde. Whole brains were postfixed by placement overnight in 4% paraformaldehyde at 4 °C and then cryoprotected by placement in 30% sucrose in $\times 1$ PBS, followed by placement in 30% sucrose in $\times 1$ PBS with 0.1% sodium azide until sectioning. A frozen microtome (Leica, Wetzlar, Germany) was used to collect serial sets of 30 μ m coronal sections through hippocampus-containing tissue. For IHC, tissue was washed in phosphate-buffered saline (PBS) and mounted and dried on charged glass microscope slides. Slides were submerged in hot citric acid (0.01 M; ~100 °C) for 15 min to expose antigen binding sites. After washing in PBS, a PAP pen (Millipore Sigma, St. Louis, MO) was used to create a hydrophobic barrier around tissue sections, and PBS containing 0.3% H₂O₂ was applied to tissue for 30 min at room temperature. Tissue was then washed and blocked in 3% normal donkey serum (NDS) in PBS containing 0.3% Triton X-100. Primary antibody solution (1:500 goat antiodoublecortin [DCX; Santa Cruz, Biotechnology, Dallas, TX, USA] in 3% NDS in PBS containing 0.3% Tween-20) was applied to tissue, and slides were kept in a humidified chamber overnight at room temperature. The next day,

tissue was washed with PBS, and secondary antibody solution (1:200 biontynylated donkey antgoat [Jackson Immuno Research Labs, West Grove, PA, USA] in PBS) was applied for 60 min at room temperature. After PBS rinses, an avidin-biotin solution (ABC Elite, Vector Laboratories, Burlingame, CA, USA) was applied to tissue for 60 min. Slides were then washed with PBS and exposed briefly to diaminobenzadine (Pierce DAB substrate, ThermoFisher Scientific, Waltham, MA, USA) to produce chromogenic reaction for antigen visualization. Tissue was washed in PBS, dehydrated and defatted in alcohol and Citrisolv, and was finally coverslipped using DPX. Slides were imaged using an upright brightfield microscope (Olympus BX-51 [Tokyo, Japan]) at $\times 20$ objective magnification.

2.6 | Statistical analyses

Data are reported as mean $\pm$ SEM. CPP data are either expressed as “CPP Score” ([time in morphine-paired context] – [time in saline-paired context]) or as time (s) spent in context. Extinction data are presented as single days or in averaged two-day bins when extinction length was extended. Statistical analyses were performed using Prism GraphPad software (version 7.02) and SPSS (version 24). Data were analyzed using one-, two-, or three-way analysis of variance (ANOVA) with between-subject factors of Treatment (Sham vs. IG-IR, Figures 1c,f and 3c,d), Dose (0 vs. 7 vs. 15 mg/kg, Figure 2b), Conditioned Context (nondrug context vs. drug context, Figure 3d), or Age of Memory (recent vs. remote, Figure 3c). When appropriate, the within-subjects repeated-measures (RM) ANOVA factors of Time (Pretest vs Test, Figure 1c,f), Extinction Trial (E1–20, Figure 3d) or Conditioned Context (drug context vs. nondrug context, Figure 3d) were used. To correct for violations of sphericity in the RM-ANOVAs as determined using Mauchly’s test, the Greenhouse–Geisser estimate of sphericity ($\epsilon < 0.75$) was used to correct for the degrees of freedom (Bathke, Schabenberger, Tobias, & Madden, 2009; Greenhouse & Geisser, 1959). If no violations to sphericity were found, RM-ANOVAs were further examined without correcting degrees of freedom. When significant interactions or main effects were found, significance levels for multiple post hoc comparisons were adjusted using Holm-Sidak, except for the four comparisons analyzed in Figure 3c (Recent/Sham vs. Recent/IG-IR, Remote/Sham vs. Remote/IG-IR, Recent/Sham vs. Remote/Sham, Recent/IG-IR vs. Remote/IG-IR) which were analyzed with Bonferroni in order to adjust for multiple comparisons. Statistical significance (alpha) was defined as $p < 0.05$, indicated by * in graphs with number of asterisks denoting significance level.

3 | RESULTS

3.1 | IG-IR does not affect retrieval of recent and remote morphine-context reward memories

Six weeks post-IG-IR or Sham treatment, mice were trained on morphine CPP (15 mg/kg, s.c.) and recent reward memory was examined ~24 hr postpairing (Figure 1a). To examine the influence of hippocampal IG-IR on retrieval of recent and remote reward memories, we first validated that ABGCs (DCX⁺ cells) were evident in the dentate gyrus of Sham mice and absent throughout the dentate gyrus of IG-IR mice (Figure 1b). When the CPP Scores for recently-probed reward memory were examined by two-way ANOVA between Sham and IG-IR groups, there was a main effect of Time ($F_{1,25} = 72.93$, $p < 0.001$, Figure 1c) with no

main effect of Treatment ($F_{1,25} = 0.7663$, $p > 0.05$) or interaction ($F_{1,25} = 0.5999$, $p > 0.05$). These data suggest that the acquisition and retrieval of a recent morphine-context reward memory does not require ABGCs.

In a separate cohort of mice, retrieval of a remote reward memory was then examined 3 weeks postpairing in Sham and IG-IR mice (Figure 1d,e). There was a main effect of Time ($F_{1,19} = 23.97$, $p < 0.05$), but no main effect of Treatment ($F_{1,19} = 3.803$, $p > 0.05$) and no significant interaction ($F_{1,19} = 0.679$, $p > 0.05$) (Figure 1f). These data show that, similar to a recent reward memory (Figure 1a–c), retrieval of a remote morphine-context reward memory was intact 6 weeks post-IGIR (Figure 1d–f).

3.2 | Extinction of a recent reward memory after CPP training with 7 or 15 mg/kg

Prior to performing extinction testing with IG-IR and Sham mice, we assessed the dose–response of initial extinction learning in naïve (i.e., did not receive Sham or IG-IR treatment) mice. Naïve mice were trained in CPP using 0, 7, and 15 mg/kg of morphine and the extinction of recent reward memory was examined (Figure 2). The CPP Score within each dose was examined across ~1 week of extinction (D5–D11; Figure 2a). When examining Averaged Extinction CPP Scores, one-way ANOVA revealed a significant main effect of Dose ($F_{2,18} = 23.92$, $p < 0.05$), and post hoc analysis showed a significant difference in the 7 and 15 mg/kg groups compared to 0 mg/kg control (all P s < 0.05 , Figure 2b). These data show that morphine led to the acquisition of reward memory regardless of dose used during conditioning (7 and 15 mg/kg). Neither group showed preference on the last extinction trial (data not shown) suggesting extinction of a reward memory occurs after ~7 extinction trials (Rutten, van der Kam, De Vry, & Tzschentke, 2011). Due to a potential ceiling effect from using 15 mg/kg morphine, a 7 mg/kg morphine dose was used for the remainder of the experiments.

3.3 | IG-IR impairs extinction of a recent morphine-context reward memories

Previous research has shown that a reduction in the number of dentate gyrus ABGCs prior to cocaine CPP impairs extinction this recent reward memory (Castilla-Ortega, Blanco, et al., 2016). Therefore, we examined if IG-IR similarly impaired extinction of recent and remote reward memories after morphine CPP. Mice received IG-IR or Sham treatment 6 weeks prior to morphine CPP and underwent extinction of the recent or remote reward memory (Figure 3a,b, respectively). A RM three-way ANOVA revealed no significant interactions for Time $\times$ Treatment ($F_{9,36} = 0.521$, $p > 0.05$), Time $\times$ Age of Memory ($F_{9,36} = 0.524$, $p > 0.05$), or Time $\times$ Treatment $\times$ Age of Memory ($F_{9,36} = 0.923$, $p > 0.05$; data not shown). As there was no difference in the within- or between-subjects RM three-way ANOVA, the data were collapsed and averaged over extinction trials 1–20 to compare overall group differences (Figure 3c) (Bardo, Miller, & Neisewander, 1984; Sotres-Bayon, Bush, & LeDoux, 2007) as was conducted in the prior experiment (Figure 2b). Two-way ANOVA revealed no main effect of Treatment ($F_{1,36} = 1.228$, $p > 0.05$) or Age of Memory ($F_{1,36} = 2.835$, $p > 0.05$), but an interaction of Treatment $\times$ Age of Memory ($F_{1,36} = 5.303$, $p < 0.05$) (Figure 3c). Post hoc analyses revealed that Sham mice in the remote memory group had higher Averaged Extinction CPP Scores versus Sham mice in the recent memory group ($p < 0.05$, Figure 3c).

We further examined Time in Context (instead of difference scores as was analyzed in Figure 3c) using a mixed model three-way ANOVA with independent variables of Conditioned Context, Time (Extinction Trial), and Treatment (Zhang, Ma, & Yu, 2012). Mauchly's test indicated that the assumption of sphericity had been violated ($\chi^2[54] = 197.9, p < 0.05$), and therefore degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity ($\epsilon = 0.526$). The three-way RM ANOVA of recent memory revealed a main effect of Conditioned Context ($F_{1,36} = 27.0, p < 0.05$), no interaction of Treatment $\times$ Time ($F_{5,26,189.5} = 0.053, p > 0.05$), but a two-way interaction of Conditioned Context $\times$ Time ($F_{5,26,189.5} = 35.89, p < 0.05$) and a three-way interaction of Treatment $\times$ Conditioned Context $\times$ Time ($F_{5,26,189.5} = 3.46, p < 0.05$) (Figure 3d). Post hoc analysis revealed Sham mice spent more time in drug versus nondrug conditioned context from Extinction Trial 1–2 through 7–8, while IG-IR mice spent more time in drug versus nondrug conditioned context from Extinction Trial 1–2 through 15–16 (all P s < 0.05 , Figure 3d). Taken together, these data suggest that ABGCs play a role in the extinction of a recent reward memory.

4 | DISCUSSION

Prior to the present work, it was unknown if dentate gyrus ABGCs were involved in the retrieval or extinction of recent versus remote reward-associated memories in the context of opiate use. Of particular relevance to the current work, ABGCs appear to play roles in the temporal separation of events (Aimone et al., 2006; Rangel et al., 2014), discrimination of similar contexts (Guo et al., 2011; Rangel et al., 2014; Tronel et al., 2012), memory consolidation (Kitamura & Inokuchi, 2014), and in the extinction of reward memories (Deschaux et al., 2014; Castilla-Ortega, Blanco, et al., 2016; Galinato et al., 2018). These roles fit with the known role of the hippocampus and brain regions upstream of the hippocampus, dentate gyrus, and ABGCs in the processing of recent and remote memory (Hales et al., 2018). The current study was designed in part to examine if ABGCs play a role in extinction process of morphine-context reward memories when probed at recent and remote timepoints. Using IG-IR mice trained using morphine CPP (15 mg/kg), we demonstrate that acquisition and retrieval of recent and remote reward memories were preserved in both Sham and IG-IR groups (Figure 1). In addition, mice that received either dose (7 or 15 mg/kg) of morphine during CPP pairing were able to both express CPP (Figure 2) and extinguish CPP learning (data not shown). However, relative to Sham mice, IG-IR mice were impaired (as determined by two-way but not three-way ANOVA) in the extinction of recent—but not remote—reward-associated memories (Figure 3). As discussed below, our data with morphine CPP are generally consistent with work on other drugs of abuse showing that ablation of ABGCs does not influence retrieval of recent or remote reward memory. However, the ability of IG-IR to interfere with the extinction of recent morphine reward memories is novel, and suggests that ABGCs have a functional role in the maintenance (persistence) of a recently-formed morphine-associated reward memory.

4.1 | Retrieval of recent and remote drug-associated contexts are unchanged after IG-IR

In the present study, the retrieval of recent and remote morphine-context reward memories is unchanged after ablation of ABGCs using IG-IR. This is in agreement with the few studies

that have examined ABGC function in relation to reward. For example, ABGC ablation prior to CPP does not interfere with retrieval of a recent cocaine-associated context memory (CPP; Brown et al., 2010; Castilla-Ortega, Blanco, et al., 2016). In operant tasks, hippocampal IG-IR enhances the reinforcing efficacy of morphine and cocaine when self-administered by rats (Bulin et al., 2017). However, there is less agreement among the larger number of studies that have examined ABGC function in relation to fear- and contextual-associated memories. Some studies show ABGCs contribute to the retrieval of recent or remote fear- and contextual-memories (Drew, Denny, & Hen, 2010; Dupret et al., 2008; Imayoshi et al., 2008; Saxe et al., 2006; Snyder, Radik, Wojtowicz, & Cameron, 2009; Warner-Schmidt, Madsen, & Duman, 2008; Winocur, Wojtowicz, Sekeres, Snyder, & Wang, 2006), while other studies do not (Akers et al., 2014; Clark et al., 2008; Drew et al., 2010; Dupret et al., 2008; Groves et al., 2013; Kitamura et al., 2009). Among the negative findings, work suggests that retrieval of a remote fear-associated memory is mediated by distinct brain regions in Sham versus irradiated mice (neocortex vs. hippocampus) (Kitamura et al., 2009). This is one of many studies that challenges the classical consolidation model of memory where the hippocampus is a time-limited structure (Moscovitch et al., 2005), but one of the few that specifically highlights a role for ABGCs in the multiple trace theory of memory. Our present work does not test which neural substrates drive the recent versus remote memories of a morphine-associated context in Sham versus IG-IR mice. However, through the lens of the multiple trace theory of memory, the retrieval of recent and remote morphine-associated contextual memories in our IG-IR mice should not be interpreted to mean that the dentate gyrus does not play a role in these memory processes. Rather, further studies are warranted to explore whether—as has been shown with fear memory (Kitamura et al., 2009)—IG-IR-induced loss of ABGCs shifts the neural substrates involved in drug-associated memory retrieval, and to clarify the mechanisms causing such a potential shift.

4.2 | Diminished extinction of recent, but not remote, reward memories after IG-IR

Our most interesting finding is that IG-IR mice have impaired extinction (enhanced persistence) of a recent reward memory, as is evident when comparing time spent in the morphine-conditioned context between Sham and IG-IR groups (Figure 3d). While several studies suggest that morphine itself enhances extinction of spatial memory by way of affecting ABGCs (Fan et al., 2018; Zheng et al., 2013), a causal relationship between ABGCs and the persistence of a morphine-related memory had not been tested prior to our work presented here. Our results with IG-IR and morphine CPP add to prior work showing that reduced ABGCs disrupt recent extinction of (a) cocaine CPP (Castilla-Ortega, Blanco, et al., 2016; Deschaux et al., 2014), (b) cocaine i.v. self-administration (Noonan et al., 2010), (c) methamphetamine i.v. self-administration (Galinato et al., 2018), and (d) morphine self-administration (Bulin et al., 2017). Interestingly, pharmacological enhancement of adult hippocampal neurogenesis also enhances extinction of a cocaine place memory in mice (Ladrón de Guevara-Miranda et al., 2018). Together, these data support that diminished ABGCs interfere with the neural mechanisms necessary for extinction of a recent—but not remote—reward-associated memory.

Our present work did not assess the mechanism as to why remote memory extinction is invulnerable to ABGC ablation. However, recent and remote memories are qualitatively

distinct; recent memories contain more specific contextual information, while remote memories tend to generalize more contextual information over time (Sekeres, Moscovitch, & Winocur, 2017; Wiltgen & Silva, 2007). Taken together with prior publications on ABGCs and pattern separation (i.e., the ability to separate similar temporal and contextual information), it is possible that decreased adult neurogenesis decreases pattern separation or supports generalization of the contextual cues of the recent reward-associated memory thus decreasing extinction (Aimone et al., 2006; Nakashiba et al., 2012; Suárez-Pereira et al., 2015). An alternative or perhaps complementary hypothesis is based on memory clearance (Akers et al., 2014; Frankland, Köhler, & Josselyn, 2013), where decreased adult neurogenesis increases memory persistence thus decreasing extinction. In support of this, enhancing adult hippocampal neurogenesis by voluntary exercise diminishes retention (persistence) of a contextual fear memory relative to sedentary control mice (Gao et al., 2018). Regardless of the mechanism, our data raise the possibility that driving the production of ABGCs soon after the formation of a reward-associated contextual memory may speed extinction.

Our results also build on other learning and memory work which show that adult-generated neurons do not have a role in extinction of remote memory (Deng, Mayford, & Gage, 2013; Ko et al., 2009). The lack of involvement of new neurons in remote memory extinction may be due to the gradual decay of hippocampal memory dependence over time (Kitamura et al., 2009). However, some memories always remain hippocampal-dependent, even at remote timepoints (Martin, de Hoz, & Morris, 2005). Therefore, future studies could explore if ablation of ABGCs alters hippocampal-dependency of remote memories. If remote memories are not hippocampal-dependent, other brain regions might contribute to extinction of a remote reward-associated memory, such as the neocortex or specifically the prefrontal cortex (PFC) (Kitamura et al., 2009; Peters, Kalivas, & Quirk, 2009; Sotres-Bayon, Sierra-Mercado, Pardilla-Delgado, & Quirk, 2012; Suzuki et al., 2004). Indeed, silencing of ventromedial PFC pyramidal neurons prevents remote extinction (~3 weeks posttraining) of a CPP-induced cocaine memory in mice (Van den Oever et al., 2013). Taken together, if a reward memory becomes hippocampal-independent, targeting other brain regions—such as the PFC, anterior cingulate cortex, or potentially the amygdala (Xue et al., 2014)—may help advance extinction-based treatments for remote reward-associated memories.

A potential caveat with any work that utilizes X-ray radiation—even when image-guided, as used here—is that irradiation induces an inflammatory response. It is unclear how the IG-IR used here influences inflammation, and to what extent IR-induced inflammation may drive our behavioral results. We selected a dose previously used for cranial ^{137}Cs IR which ablates DCX^+ cells, increases a microglial marker 3 months post-IR, but does not elevate other key indices of inflammation (Jenrow et al., 2010; Moravan, Olschowka, Williams, & O'Banion, 2011). It is likely that our 15 Gy dose of IG-IR also drives microglia, since 10 Gy of cranial X-ray IR induces microglial activity 1 month post-IR (Kitamura et al., 2009). Given the multifaceted role that microglia play in shaping neuronal structure and function (Lacagnina, Rivera, & Bilbo, 2017; Salter & Stevens, 2017), it will be important to delineate if and how microglia interact with ABGCs in regard to proper recent extinction of reward-context associated memory.

4.3 | Future directions and conclusion

Our work suggests that hippocampal dentate gyrus adult neurogenesis is important for extinction of a recent reward memory associated with morphine administration. Given that adult neurogenesis is believed to be involved in the temporal separation of events (Aimone et al., 2006; Deng, Saxe, Gallina, & Gage, 2009; Rangel et al., 2014) and extinction learning is temporally distinct from other forms of learning and memory (Suzuki et al., 2004), future studies should examine if other brain regions and their interactions with cortical engrams (Cowansage et al., 2014; Kitamura et al., 2017; Suzuki et al., 2004) impact extinction learning. Clarifying the mechanisms through which ABGCs influence the persistence of reward-associated contextual memories may improve the development of extinction-based therapies for disorders characterized by maladaptive associative learning, including substance use disorders.

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FIGURE 1.

Image guided, hippocampal-targeted X-ray irradiation (IG-IR) does not affect retrieval of recent or remote reward memories. (a and d) Timeline of morphine conditioned place preference (CPP) experiment and schematic of treatment groups. Six weeks after IG-IR or Sham treatment, mice were trained on morphine CPP (15 mg/kg, s. c.) for 4 days (D1–4) and tested (a) 24 hr or (d) 3 weeks later to examine recent and remote reward memories, respectively. (b and e) The dentate gyrus of Sham and IG-IR mice was examined for doublecortin (DCX), a marker of immature neurons, immediately after (b) recent and (e) remote memory retrieval. Scale bars: 100 μ m. (c and f) CPP Scores were examined during retrieval of (c) recent ($n = 11$ –16/group) and (f) remote ($n = 8$ –13/group) reward memory in Sham and IG-IR mice, where a main effect of Time was found (see Section 3 for details).

Data are presented as mean $\pm$ *SEM*. * $p < 0.05$ main effect of Time. DC = drug context; NDC = nondrug context

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FIGURE 2.

Morphine (7 and 15 mg/kg) produces conditioned place preference (CPP) that is susceptible to extinction in C57BL/6J mice. (a) Timeline of morphine CPP extinction experiment and schematic of treatment groups (0, 7, or 15 mg/kg morphine). Mice were given a Pretest (D1) followed by morphine CPP (D2–4) and began testing 24 hr later for recent reward memory extinction. (b) The Averaged Extinction CPP Scores for each dose. * $p < 0.05$ Holm-Sidak post hoc based on significant main effect of Dose ($F_{2,18} = 23.92$, $p < 0.05$) compared to 0 mg/kg group. $N = 8$ –10/group. DC = drug context; NDC = nondrug context

FIGURE 3.

Image guided, hippocampal-targeted X-ray irradiation (IG-IR) diminishes extinction learning of recent—but not remote—morphine-context reward memory. (a and b) Extinction timelines of (a) recent and (b) remote morphine-context reward memory in Sham and IG-IR treatment groups. Six weeks post-IG-IR or Sham treatment, mice were trained on morphine conditioned place preference (CPP; D2–4, 7 mg/kg). (a) 24 hr or (b) 3 weeks later mice underwent extinction testing for ~3 weeks to assess extinction of a (a) recent reward memory or (b) remote reward memory. (c) The Averaged Extinction CPP Scores for the experiments assessing recent ($n = 9–11$ /group) or remote ($n = 14–15$ /group) reward memory. $*p < 0.05$ Bonferroni post hoc comparing Sham-Recent and Sham-Remote. (d) The total time spent in the DC or NDC ~3 weeks extinction of recent reward memory in Sham and IG-IR treatment groups. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ Holm-Sidak post hoc comparing Sham-DC and IG-IR-DC to respective Pretest score. Data are presented as mean $\pm$ SEM. DC = drug context; NDC = nondrug context