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Reducing hippocampal cell proliferation in the adult rat does not prevent the acquisition of cocaine-induced conditioned place preference

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ABSTRACT

Neurogenesis is important for developing certain forms of memory. Recently, hippocampal cell proliferation has been implicated in the development of drug addiction, an extreme form of emotional/motivational pathological memory. Aiming to explore the role of hippocampal neural cell proliferation in cocaine-induced conditioned place preference (CPP), we treated rats with whole brain X-irradiation, which substantially decreases the number of progenitor cells in the subventricular zone of the lateral ventricles and subgranular zone of the dentate gyrus. Surprisingly, there was no difference in the expression of cocaine-induced CPP. These results suggest that the existing neural network, rather than potential new neural circuits mediated by adult neurogenesis, is sufficient for the acquisition of cocaine-induced CPP.

Cocaine addiction is characterized by compulsive drug use despite adverse consequences with a high prevalence of relapse following withdrawal [1]. Persistent drug taking is thought to usurp reward-related learning and memory processes resulting in neuroadaptations that ultimately may lead to a hypersensitivity to cocaine-related cues [40]. Studies examining cocaine administration have primarily focused on signaling cascades [17,25], growth factors [3,34], spine morphology [31,35] and physiological processes associated with changes in synaptic plasticity [15,19], thus influencing learning and memory processes. Within the last decade experiments have now shown that drugs of abuse (ethanol [7], nicotine [36], methamphetamine [39] and cocaine [9,27,43]) impact neural cell proliferation, which, ultimately may impact synaptic strength [6]. Recently it has been documented that cranial irradiation prior to cocaine self-administration significantly increases cocaine self-administration but has no impact on sucrose self-administration [26]. The goal of this study was to determine whether a decrease in cell proliferation in neurogenic niche prior to cocaine-induced conditioned place preference (CPP) training would impact the spatially associated cocaine task similarly to its operant counterpart.

Radiation-based disruption of cell proliferation has been established in adult rats to examine the behavioral role of adult neurogenesis [13,42]. The acute responses of neurogenic cells in the subgranular zone (SGZ) of the hippocampal dentate gyrus as well as in the subventricular zone (SVZ) of the lateral ventricles after exposure to radiation showed that proliferating precursor cells and immature neurons undergo apoptosis shortly after irradiation [23]. Long-term effects after exposure to radiation revealed reduction in generation of new neurons [13,21,42]. However, reports dealing with recovery of proliferative potential following brain X-irradiation are inconsistent [13,32]. Some results show that after irradiation proliferating neural precursor cells and immature neurons decrease in numbers [32,42] and that such changes are persistent [32], whereas other results show a full or partial recovery of neural stem cells after X-irradiation [13].

Nonetheless, our results show that X-irradiation significantly decreased the number of progenitor cells in the SGZ, SVZ and olfactory bulb (OB). However, rats with such a disruption of neural cell proliferation exhibited normal cocaine-induced CPP. Thus, our results suggest that acquisition of cocaine-induced CPP is independent of the potentially new neuron-mediated circuitry reorganization.

Male Sprague–Dawley rats weighing 280–300 g were used for all experiments. Experiments were conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and experimental protocols were approved by the University Animal Care and Use Committee. Animals were housed in groups of two per cage with free access to food and water in a temperature- and humidity-controlled room with a 12 h light cycle. After irradiation, animals were singly housed.

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Cocaine hydrochloride was obtained from the Drug Supply Program of National Institute on Drug Abuse and dissolved as the weight of the salt to a final concentration of 12 mg/mL.

All CPP studies were conducted during the same time of day (11:00 am–1:00 pm). The proposed studies employed a three-compartment CPP apparatus (Med Associates, Inc., St. Albans, VT) as previously described [4]. Briefly, rats were subdivided into four groups, each of the four groups was then subdivided again into groups that had either 10 days or 60 days of recovery from X-irradiation before CPP commenced: rats with cocaine administration (sham cocaine, $n=8$ for 10-day group; $n=7$ for 60-day group), with irradiation and saline administration (irradiated saline, $n=7$ for 10-day group; $n=5$ for 60-day group), with irradiation and cocaine administration (irradiated cocaine, $n=8$ for 10-day group; $n=7$ for 60-day group), and with saline administration (sham saline, $n=8$ for 10-day group; $n=8$ for 60-day group). The X-irradiated groups were anesthetized i.p. with zyket (ketamine 87 mg/kg + xylazine 13 mg/kg) and subsequently subjected to whole brain X-irradiation (2.00 cm × 0.75 cm as outlined in Fig. 1B) ≈5 gy of X-irradiation per exposure under the guidance of the radiologists at the Washington State University clinic. Exposures were conducted 2–3 days apart for a total of ≈15 gy. A subset of sham animals ($n=4$) also received i.p. zyket to ensure there was no non-specific effect of the anesthesia on cell proliferation. The preconditioning phase began either 10 days or 60 days after X-irradiation. Rats were preconditioned for the cocaine place preference procedure as follows. Animals were placed into the central compartment of one of eight boxes. The animals were free to explore all three-compartments for a 15-min period. The time spent in each compartment was analyzed by automated software. Two preconditioning days were conducted, and the second day was used for determining preference (initial preference day).

The conditioning stage began 1 day after the last preconditioning test. A cocaine or saline injection was given each day. Animals received four saline (1 mL/kg, i.p.) and four cocaine (12 mg/kg, i.p.) pairings in an alternating fashion and were confined to the assigned compartment for a 25-min period. The saline sham group was given only saline prior to confinement in alternating compartments for 8 days.

The test day for cocaine-induced CPP was done 1 day after the last training day. Each animal was placed into the central compartment and had free access to all compartments over a 15-min test period [4]. A preference score was calculated as time spent in cocaine-paired chamber minus initial preference for that chamber.

To determine the effects of radiation and cocaine treatment on the cell proliferation in the SGZ, SVZ and OB, rats received four i.p. injections (50 mg/kg) 5-bromo-2′-deoxyuridine (BrdU) every other day starting at 35 (10-day group) or 85 day (60-day group) of the experiment (Fig. 1C). Next, rats were anesthetized (at day 45 or 95, Fig. 1C) and perfused transcardially with saline followed by 4% paraformaldehyde in buffered saline (pH 7.4). The brain was removed, processed, and sectioned using a Leica cryostat. Thirty-micrometer-thick sections were stored at 4 °C in cryoprotectant solution until needed. Steamer/citrate antigen retrieval method was used for BrdU staining [44]. Free-floating sections were immunostained using the following primary antibodies and working concentrations: mouse anti-BrdU (1:100; Sigma, B8434). Subsequently, the sections were incubated 2 h at room

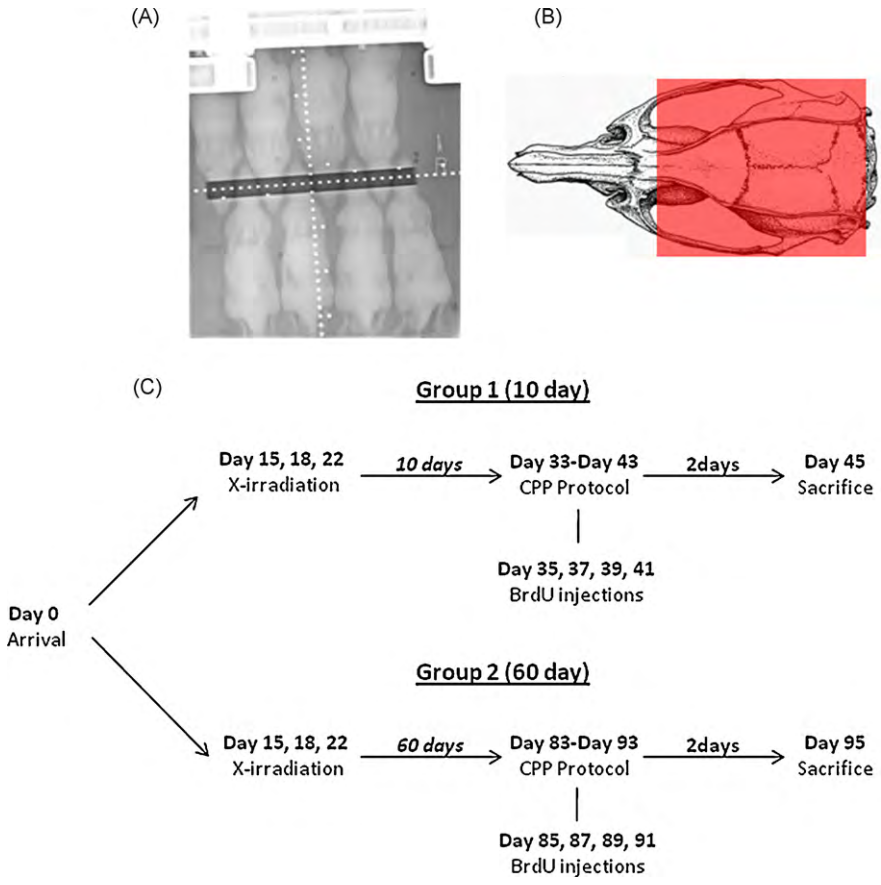

Fig. 1. Photograph illustrating the extent of X-irradiation and a schematic depicting the experimental design. (A) Representative picture of how the rats were aligned during the X-irradiation procedure. The dark quadrant indicates the region of the rats' head that were exposed to X-irradiation. (B) Schematic adapted from Paxinos and Watson [30] indicating the region of the skull that was irradiated (red box). (C) Timeline of the experimental procedure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)