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Activity-Regulated Gene Expression in Immature Neurons in the Dentate Gyrus Following Re-Exposure to a Cocaine-Paired Environment

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Abstract

Intense craving for drug and relapse are observed in addicts who are exposed to environmental stimuli associated with drug-taking behavior even after long periods of abstinence. The hippocampus is a brain region known to be involved in contextual processing, taking place predominantly in the septal hippocampus, and emotional processing, taking place predominantly in the temporal hippocampus. Conditioned place preference is an animal model of context-conditioned reward. The dentate gyrus is a hippocampal sub-region particularly important for the acquisition of cocaine-induced place preference and is a site of continuous neurogenesis, which has been implicated in the vulnerability to drug-taking behavior. Therefore, these experiments explored the role of newly generated neurons in drug reward-context association by examining the activation, as determined by expression of the immediate early gene *cfos*, of young and mature granule cells in the septal and temporal dentate gyrus of adult rats that were re-exposed to a drug-paired environment following the development of cocaine place preference. The overall level of *cfos* expression was increased in both the septal and temporal dentate gyrus of animals that developed place preference and were re-exposed to the drug paired environment compared with re-exposure to a neutral environment. Overall level of neurogenesis, as detected by the S-phase marker 5'-bromo-2'-deoxyuridine (BrdU) and the immature neuron marker double-cortin (DCX), was unaltered by cocaine conditioning. However, the number of activated new neurons (DCX + *cfos*) was greater in the temporal dentate gyrus of cocaine-conditioned rats re-exposed to the drug-paired environment as compared to those re-exposed to a neutral environment. Further understanding of the role of dentate gyrus neurogenesis on the conditioned effects of drugs of abuse may provide new insights into the role of this process in the expression of addictive behaviors.

Keywords

addiction; hippocampus; neurogenesis; reward; ventral

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INTRODUCTION

Re-exposure to environments associated with drug-taking can evoke craving capable of provoking drug-seeking behavior (Weiss, 2005; Volkow et al., 2006; Crombag et al., 2008). A model of context-conditioned reward, conditioned place preference, requires an intact hippocampus and hippocampal dentate gyrus in particular (Meyers et al., 2003, 2006; Hernández-Rabaza et al., 2008). Exposure to an environment associated with drug-taking or expression of cocaine place preference significantly increases the expression of the immediate early gene *cfos* in the rodent hippocampus and dentate gyrus (Kufahl et al., 2009; Chauvet et al., 2011). Therefore, activation of the hippocampus by a drug-associated context is linked to the expression of cocaine-induced place preference. The temporal hippocampus is critical for cue-induced and cocaine-primed reinstatement of cocaine seeking. Temporal hippocampal stimulation induces relapse to drug-seeking for cocaine (Vorel et al., 2001), while inhibition of the temporal hippocampus reduces drug-seeking behaviors (Sun and Rebec, 2003; Atkins et al., 2008). Therefore, the temporal hippocampus may be particularly important for the encoding of environmental stimuli that have strong motivational aspects, such as drug-paired stimuli. In addition to the established role of the temporal hippocampus, the septal hippocampus is also involved in drug-context association and context-induced reinstatement (Meyers et al., 2003; Fuchs et al., 2005; Meyers et al., 2006).

New neurons are continuously generated at the border between the hilus and granule cell layer (subgranular zone) of the hippocampal dentate gyrus, and many reports suggest this neurogenesis contributes to hippocampal contextual processing and memory formation depending on the maturation stage of newly generated neurons (Kee et al., 2007a; Stone et al., 2011; Gu et al., 2012; Nakashiba et al., 2012). Interestingly, impaired neurogenesis does not alter acquisition of cocaine-conditioned place preference (Brown et al., 2010). Conversely, if neurogenesis is impaired prior to cocaine self-administration training rats take more cocaine and have higher breakpoints as compared to control rats, suggesting a sensitivity to the rewarding properties of the drug (Noonan et al., 2010). There is potential for partial or complete compensation when impairment of neurogenesis occurs prior to experience (Arruda-Carvalho et al., 2011; Gu et al., 2012). Impaired neurogenesis following cocaine self-administration increases context-induced drug-seeking behavior and resistance to extinction (Noonan et al., 2010), suggesting an enhanced influence of the drug-associated environmental cues. Therefore, newly-generated neurons may be involved in drug responding and related learning since this process appears to be necessary for adapting learned responses to varying conditions (Burghardt et al., 2012; Vukovic et al., 2013).

The studies presented here aimed to determine whether there are regional differences in activation of mature or newly generated neurons of the septal versus temporal dentate gyrus in response to a drug-associated environment. Therefore, *cfos* expression in the granule cell layer and subgranular zone of the septal and temporal dentate gyrus was measured in rats that acquired cocaine-induced place preference and were re-exposed to the drug-paired environment or a neutral environment.

MATERIAL AND METHODS

Animals and Treatments

Twenty-four adult male Sprague-Dawley rats (Charles River, 225–250 g at the start of the experiment) were used in the following experiments. Rats were housed 2 per cage, maintained on a 12 h light/dark cycle, and provided food and water ad libitum. Animal use procedures were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals (8th edition, 2011), and approved by the Institutional Animal Care and Use Committee of Temple University. To label adult-born granule cells, all rats were given 2 injections of the S-phase marker 5-bromo-2'-deoxyuridine (BrdU; Sigma, 200 mg/kg/injection ip, dissolved at 20 mg/ml in saline, 0.007N NaOH) spaced 6 h apart (Barr et al., 2010). Beginning two weeks after BrdU injections, rats were handled daily for 5 days. Three weeks following BrdU injections, rats underwent cocaine conditioned place preference (Fig. 1A). This delay was to ensure all BrdU-labeled cells were the same age as one another (~4 weeks) at the time of testing (Kee et al., 2007a; Snyder et al., 2009a).

Conditioned Place Preference

A biased conditioned place preference procedure was used with a two compartment place preference apparatus (San Diego Instruments, San Diego, CA). All animals underwent a 30 min pretest at 12 pm prior to conditioning, in which rats had free access to both compartments of the apparatus and time spent in each compartment was recorded. The compartment that the rat spent the lesser amount of time in during the pretest was designated as the cocaine-paired compartment for the conditioning sessions. Rats were randomly assigned to one of three groups ($n=8$ /group), saline-S (administered saline in both compartments+re-exposed to a saline-paired compartment), cocaine-S (cocaine-conditioned +re-exposed to the saline-paired compartment), and cocaine-C (cocaine-conditioned+re-exposed to the cocaine-paired compartment). The rats were conditioned twice daily for 7 days. Each rat received saline (1 ml/kg, ip) injection in the morning at 9 am and was confined to the preferred compartment for 30 min. In the afternoon, rats were injected with cocaine (20 mg/kg, ip) or saline (1 ml/kg, ip) at 2 pm and confined to the nonpreferred compartment for 30 min. On day 8, rats had free access to both compartments in a drug-free state at 12 pm for 30 min and time in each compartment was recorded. The difference in seconds between time spent in the cocaine-paired chamber on test day and time spent in the same, originally non-preferred compartment during the pretest was used to measure place conditioning. Therefore, a positive number is indicative of a conditioned place preference. The day after testing, rats were confined to the nonpreferred side (saline-S group), saline-paired side (cocaine-S), or cocaine-paired side (cocaine-C) at 12 pm for 30 min and after 90 min transcardially perfused (2 h after being placed in chamber; Soderman and Unterwald, 2008).

Immunofluorescence and Quantification

All animals were perfused with phosphate-buffered saline followed by 4% paraformaldehyde. The entire bilateral hippocampus was extracted and postfixed in paraformaldehyde for an additional 24 h. A randomly selected hemisphere of hippocampus was divided into thirds (Fig. 1B) and sections of the septal one-third and temporal one-third

hippocampus were cut at 40 μ m using a vibratome. About 80 sections were obtained per region and quantification was performed on every 10th section for a total of eight sections per region per animal. Sequential fluorescent labeling was performed for cfos, followed by BrdU or doublecortin (DCX), on free floating sections. DCX is marker of immature neurons (<4 weeks; Rao and Shetty, 2004), and therefore identifies a population of newly generated neurons within an overlapping age range whereas BrdU is incorporated into a discrete population of cells labeled the day of BrdU injections (~4-weeks-old at time of perfusion).

All antibodies were diluted in phosphate-buffered saline containing 0.03% Triton X-100 and 2% normal donkey serum. For cfos/BrdU double-immunolabeling, sections were first incubated with rabbit anti-cfos antibody (1:10,000; Chemicon; at 4°C) followed by Alexa568 donkey antirabbit secondary antibody (1:200; Molecular Probes; 2 h at room temperature). Sections were treated with 1N HCl at 60°C for 60 min to denature DNA and expose BrdU (Barr et al., 2010). Sections were then incubated with rat anti-BrdU antibody (1:100; Abcam; overnight at 4°C) followed by Alexa488 donkey antirat secondary antibody (1:200; Molecular Probes; 2 h at room temperature). Immunolabeling for cfos/DCX was done similarly, using rabbit anti-cfos (1:10,000; Chemicon; at 4°C) and mouse anti-DCX (1:200; Santa Cruz; at 4°C) followed by Alexa568 donkey antirabbit and Alexa488 donkey antimouse.

The total numbers of cfos-, BrdU-, or cfos+DCX-labeled cells were counted within the granule cell layer and subgranular zone (defined as a two-cell-body-wide zone on the border of the granule cell layer and hilus) of the same sections blind to treatment using the fractionator method within a bioquantification software system (Bioquant TCW 98, Nashville, TN; Encinas and Enikolopov, 2008; Coggeshall and Lekan, 1996). Nuclei were counted on 10 $\times$ 2D images and verified as cfos⁺ if the signal filled the nucleus and stood out clearly compared to surrounding tissue (Kee et al., 2007b). Every BrdU⁺ and DCX⁺ cell was examined for cfos expression with a Nikon epi-fluorescent microscope with a 60 $\times$ oil immersion lens moving in and out of focus throughout the granule cell layer. Potential double-labeled cells were then further examined through the depth of the section using a Leica TCS SP5 confocal microscope to confirm colocalization. To quantify DCX⁺ cells, which have many overlapping projections, the irregular region of interest tool and videocount area array option of the Bioquant software was utilized. Threshold values were selected based on mean levels of background immunostaining. The threshold values were stored in the computer program for consistent auto-measurement of the immunostained slides. Percent area of immunoreaction product was calculated by dividing the videocount area containing pixels at or above the defined background threshold by the videocount area of the total number of pixels in the chosen field (granule cell layer and subgranular zone), and multiplying by 100 (Ehlers et al., 2013; Briones and Woods, 2013; Miller et al., 2014).

Statistical Analysis

Statistical analyses were performed using Sigmaplot 12.5 software, with significance set at $P < 0.05$. Conditioned place preference and numbers of cfos, BrdU, DCX, and double-labeled cells within the septal or temporal dentate gyrus were analyzed using one-way analysis of

variance (ANOVA). Significant main effects were followed by Student-Newman-Keuls (SNK) *post hoc* test for multiple comparisons.

RESULTS

Development of Cocaine-Induced Conditioned Place Preference

Rats underwent conditioning for seven days with 20 mg/kg, ip cocaine. The data in Figure 2 represent mean preference scores for rats in each experimental condition. One-way ANOVA revealed a significant difference between groups ($F_{2,24}=7.920$; $P=0.003$). *Post hoc* analysis showed that conditioned place preference was established in the two groups of cocaine-conditioned animals versus saline controls (saline-S vs. cocaine-S, $P=0.006$; saline-S vs. cocaine-C, $P=0.002$, Fig. 2), and there was no difference between the two cocaine-conditioned groups (cocaine-S vs. cocaine-C, $P > 0.05$).

Septal and Temporal Dentate Gyrus Neurogenesis

The level of BrdU labeling within the dentate gyrus was sparse, with greatest number of labeled cells just within the inner granule cell layer. The number of BrdU-positive cells (Fig. 3A) was compared within the septal or temporal granule cell layer of cocaine and saline conditioned rats. There were no differences within each region, septal (Fig. 3B, $F_{2,21}=0.0864$, $P=0.918$) or temporal (Fig. 3C, $F_{2,21}=3.197$, $P=0.064$) granule cell layer, between experimental groups. The level of DCX-labeling (Figs. 4A,C) was also compared within the septal (Fig. 4B) or temporal (Fig. 4D) dentate gyrus of cocaine and saline conditioned rats. Level of DCX labeling was comparable in the septal ($F_{2,19}=1.687$, $P=0.215$) or temporal ($F_{2,18}=2.598$, $P=0.105$) granule cell layer of all experimental groups. Therefore, levels of neurogenesis were comparable across experimental groups for both four-week-old BrdU⁺ and immature (<4 week, DCX⁺) neurons.

Effects of Drug-Associated Environment on cfos Expression Within the Septal and Temporal Dentate Gyrus Granule Cell Layers

The immediate-early gene *cfos* was used to measure neuronal activation in the septal (Fig. 5A) and temporal (Fig. 5B) dentate gyrus following exposure to cocaine- or saline-paired environments. A one-way ANOVA found significant main effect of treatment on *cfos* labeled cells in both the septal dentate gyrus (Fig. 5C, $F_{2,18}=5.006$, $P=0.022$) and temporal dentate gyrus (Fig. 5D, $F_{2,23}=8.869$, $P=0.004$). *Post hoc* analysis showed that cocaine conditioned rats that were re-exposed to the cocaine-paired environment (cocaine-C) had significantly more *cfos*⁺ cells as compared to saline-conditioned rats and cocaine-conditioned rats re-exposed to the saline-paired environment (cocaine-S) in both septal (cocaine-C vs. saline-S, $P=0.031$; cocaine-C vs. cocaine-S, $P=0.027$) and temporal (cocaine-C vs. saline-S, $P=0.031$; cocaine-C vs. cocaine-S, $P=0.003$) dentate gyrus. These results indicate that exposure to the environment associated with cocaine administration activated granule cell neurons in the septal and temporal dentate gyrus.

Effects of Drug-Associated Environment on cfos Expression in Immature Neurons in the Septal and Temporal Subgranular Zone

To determine the activation of newly generated neurons by exposure to an environment associated cocaine exposure, neurons positive for both DCX⁺cfos⁺ in the septal or temporal subgranular zone were quantified. Figure 6C shows representative labeling of an immature neuron with both cfos (Fig. 6A) and DCX (Fig. 6B). The number of DCX⁺cfos⁺ double-labeled neurons was not different between groups in septal subgranular zone (Fig. 5D, $F_{2,17}=2.5$, $P=0.116$). There was a significant main effect of treatment on DCX⁺cfos⁺ double-labeled neurons in temporal subgranular zone (Fig. 6E, $F_{2,17}=4.778$, $P=0.025$). *Post hoc* analysis showed no difference between cocaine-S and saline-S controls (*post hoc* $P=0.689$). However, cocaine-C rats had more DCX⁺cfos⁺ double-labeled neurons in temporal subgranular zone than both saline-S (*post hoc* $P=0.030$) and cocaine-S (*post hoc* $P=0.027$) controls. These results suggest that immature neurons are activated by exposure to cocaine environmental cues. Very low numbers of confirmed BrdU⁺cfos⁺ cells were identified (data not shown). This prevented reliable comparisons between treatment groups using the BrdU marker.

DISCUSSION

The present study demonstrates that rats that develop cocaine-induced conditioned place preference show activation of the septal and temporal regions of the dentate gyrus when re-exposed to the cocaine-paired environment. In addition, activated immature neurons in the temporal subgranular zone of the dentate gyrus of cocaine-conditioned rats re-exposed to the drug-paired environment were more numerous as compared to cocaine-conditioned rats re-exposed to a neutral environment.

The present findings add to the increasing number of reports that have identified the hippocampus and dentate gyrus as a critical region involved in drug-associated responses and drug-seeking behaviors. Exposure to conditioned cues and aversive stimuli are powerful triggers of drug cravings and relapse, and are associated with activation of limbic brain regions, including the hippocampus, in both humans and rodent models (Neisewander et al., 2000; Kilts et al., 2001). Although output projections and particular functions vary along the septo-temporal axis (Moser and Moser, 1998; Kjelstrup et al., 2002), the entire hippocampus appears to be involved in the processing of contextual information (Rudy and Matus-Amat, 2005; McDonald et al., 2006; Kjelstrup et al., 2008; Komorowski et al., 2013). The increased cfos expression in the septal and temporal dentate gyrus in response to re-exposure to the environment associated with cocaine in the present study is in accordance with previous studies showing increased septal dentate gyrus cfos expression associated with expression of cocaine place preference (Chauvet et al., 2011) and increased cfos expression in temporal hippocampus positively correlated with cue-induced cocaine seeking (Zhou et al., 2013). Results from the present study demonstrate that cfos activity is specific to the environment previously paired with cocaine exposure.

Rats that underwent cocaine conditioning did not show differences from saline controls in levels of BrdU or DCX. Repeated cocaine exposure can reduce new cell proliferation in the dentate gyrus but does not alter the long-term survival of newborn cells (Yamaguchi et al.,

2004; Domínguez-Escribà et al., 2006; Noonan et al., 2008). The dose of cocaine used in this study (20 mg/kg, ip for 7 days) did not significantly alter numbers of newly generated neurons (BrdU⁺) that were 3-weeks-old at the time of first cocaine exposure and may have been already integrated into the dentate circuitry, or immature (DCX⁺) neurons (<4-week-old) measured two days following cocaine exposure. Studies investigating dendritic morphology have shown no differences in numbers of dendrites or dendritic arbor length of DCX labeled cells in rat dentate gyrus following 8 or 24 days of 20 mg/kg ip cocaine (Domínguez-Escribà et al., 2006) nor in cell number and dendritic morphology following three weeks of cocaine self-administration (Noonan et al., 2008). In the present study, more DCX⁺ neurons in the temporal dentate subgranular zone were activated in cocaine-conditioned rats re-exposed to the drug paired environment. The specific function of these activated new neurons is presently unknown. A central question of adult neurogenesis is how the integration of a small number of new neurons can affect the hippocampal network and influence function and behavior. It has been suggested that unique properties of new neurons such as enhanced excitability allows these neurons to function differently from the existing neural network (Ge et al., 2007; Marín-Burgin and Schinder, 2012). These properties are understood to be present in cells between 4 and 6 weeks of age, promoting their integration (Gu et al., 2012). In the current study, we found lower levels of activated 4-week-old neurons (BrdU⁺ in the current study) than activated immature DCX⁺ neurons. The activated DCX-expressing neurons observed are likely 2- to 3-weeks-old as this is when newly-generated neurons receive functional input from existing circuits and begin to express immediate early genes (Jessberger and Kempermann, 2003; Schmidt-Hieber et al., 2004; Snyder et al., 2009b, 2012). Depletion of DCX⁺ cells does not affect recall of an established spatial task but impairs reversal learning (Vukovic et al., 2013). Therefore, it is possible that the activated DCX⁺ neurons in the present study are involved in new learning, perhaps due to absence of the drug during the reexposure session, rather than recall of the prior drug-associated experience. Future, more extensive studies, using multiple cell cycle markers or multiple thymidine analog markers, aimed at the specific contribution of newborn neurons at different ages may more clearly assess the role of the neurogenic process in drug-context memory formation.

These new neurons are likely involved in processing that occurs within strong emotive contexts such as a drug-paired environment since the temporal hippocampus has a role in affect and motivation (Sahay and Hen, 2007; Fanselow and Dong, 2010; Gruber and McDonald, 2012). Therefore, an alternative explanation may be related to the anxiogenic effects of cocaine withdrawal since re-exposure occurred two days following the last injection of cocaine, a time when cocaine withdrawal-induced anxiety is high (Rudoy and Van Bockstaele, 2007). The formation of drug-context association is important for subsequent withdrawal responses linked to the same context, and disruption of hippocampal activity inhibits this effect (Taubenfeld et al., 2010). Rats conditioned with cocaine and re-exposed to cocaine-associated cues display greater anxiety-like behavior immediately after re-exposure to the context or after re-exposure following 10 days of withdrawal (DeVries and Pert, 1998; Erb et al., 2006). Glutamatergic activation of the temporal hippocampus is important for the expression of anxiety-like behaviors (Barkus et al., 2010; Nascimento Hackl and Carobrez, 2007). Several studies have correlated reduced neurogenesis to

increased anxiety-like behaviors (Earnheart et al., 2007; Bergami et al., 2008; Revest et al., 2009) and antidepressants that reduce anxiety also stimulate dentate gyrus neurogenesis (Malberg et al., 2000; Encinas et al., 2006; Boldrini et al., 2009). Impaired neurogenesis specifically in the temporal dentate gyrus eliminates reductions in latency to feed following stress in the novelty suppressed feeding paradigm by the antidepressant fluoxetine (Wu and Hen, 2014). Therefore, neurogenesis within the temporal hippocampus may be necessary to express proper levels of anxiety within an appropriate context.

Additionally, adult hippocampal neurogenesis has been linked to regulation of the hypothalamo-pituitary-adrenal (HPA) axis (Schloesser et al., 2009; Surget et al., 2011; Snyder et al., 2011). Since, the temporal hippocampus modulates neuroendocrine and behavioral responses to stimuli (Herman et al., 1998; Mueller et al., 2004), and exposure to cocaine-associated contextual cues produces a conditioned increase in blood corticosterone concentrations (DeVries et al., 1998), these neurons may be involved in the regulation of the HPA axis due to increases in corticosterone release evoked by the cocaine-associated environment.

In conclusion, re-exposure to an environment recently associated with cocaine increases the number of cfos positive cells in both the septal and temporal dentate gyrus to a greater degree than exposure to a neutral environment, while the number of activated immature neurons is significantly increased only in the temporal dentate gyrus of these animals. Expression of immediate early genes by newly generated neurons has been associated with experience-dependent integration of new neurons into hippocampal networks related to memory (Trouche et al., 2009; Veyrac et al., 2013). Since disruption of neurogenesis following cocaine experience conserves drug seeking (Noonan et al., 2010; Deschaux et al., 2012), and enhanced neurogenesis following cocaine experience is associated with enhanced extinction (Mustroph et al., 2011), new dentate gyrus neurons may facilitate hippocampal networking that mediates learning during cocaine conditioning, withdrawal, and extinction. It is unknown whether the activated immature neurons in the present study play a specific role in drug-context association or represent new learning in the absence of the psychoactive effects of the drug. Future studies should investigate the role of new neurons during each of these phases.

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FIGURE 1. Experimental design (A) Experimental timeline. All rats received two injections of BrdU; three weeks later rats underwent conditioning sessions followed by a preference test on Day 29. One day later, rats were confined to the cocaine- or saline-paired side then perfused for immunofluorescence labeling 90 min later. (B) The hippocampus was divided into thirds and the septal one-third (S) and temporal one-third (T) were sectioned. Example sections were labeled with a mature neuronal marker (NeuN). White lines outline granule cell layer and yellow lines represent the subgranular zone of the dentate gyrus. Scale bar=200 μ m. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 2.

Cocaine administration produced a significant place preference. Two groups of rats conditioned with cocaine (20 mg/kg ip) for seven days showed a significant place preference toward their cocaine-paired side as compared to saline controls that received saline in both compartments. Data represented as means $\pm$ SEM of seconds of post-test minus pretest scores ($N=8$ /group). *Significantly different as compared to rats administered saline on both sides ($P<0.05$).

FIGURE 3.

Numbers of 4-week-old (BrdU+) cells in septal and temporal granule cell layer are unaffected by cocaine administration. (A) Representative photomicrograph of a cell labeled by 5'-bromo-2'-deoxyuridine (BrdU). The numbers of BrdU labeled cells were similar between the experimental groups in the septal (B) and temporal (C) dentate gyrus. Mean $\pm$ SEM; $N=8$ rats/group. Scale bar=50 μ m. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 4.

Levels of immature (DCX+) neurons in septal and temporal subgranular zone/granule cell layer are unaffected by cocaine administration. Representative photomicrograph of doublecortin (DCX) labeled neurons in the septal (A) and temporal (C) subgranular zone/granule cell layer. The amount of DCX labeling was similar in all treatment groups in both septal (B) and temporal (D) subgranular zone/granule cell layer. Scale bar=200 μ m. Mean $\pm$ SEM; $N=8$ rats/group. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 5.

Re-exposure to the drug reward-associated environment increased cfos expression within the septal and temporal granule cell layers. Representative photomicrographs of cfos labeled nuclei in the (A) septal and (B) temporal dentate gyrus (cfos-labeled nuclei indicated by white arrowheads in the granule cell layer). Scale bars=200 μ m, inset=25 μ m. The number of cfos-positive nuclei was greater in cocaine-conditioned rats re-exposed to the cocaine-paired environment compared to cocaine-conditioned rats re-exposed to saline-paired environment or saline-conditioned rats, in both septal (C) and temporal (D) granule cell layer (* P <0.05). Mean $\pm$ SEM, (N =8 rats/group). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIGURE 6.

Re-exposure to cocaine-associated environment activates immature neurons in the temporal subgranular zone. Representative photomicrographs of the same section through the granule cell layer showing (A) a cfos positive nucleus, (B) DCX labeled neurons, and (C) both antigens in the merged image. Cell exhibiting both DCX and cfos can be seen at the white arrowhead. GCL=granule cell layer Scale bar=25 μ m. Cocaine-C rats had a similar number DCX⁺cfos⁺ neurons as both saline-S and cocaine-S controls in the septal region (D), but a larger number in the temporal (E) granule cell layer. *Significant from saline and cocaine-S groups ($P<0.05$). Means+SEM are shown for all groups. ($N=8$ /group). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]