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Levels of Neural Progenitors in the Hippocampus Predict Memory Impairment and Relapse to Drug Seeking as a Function of Excessive Methamphetamine Self-Administration

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Methamphetamine affects the hippocampus, a brain region crucial for learning and memory, as well as relapse to drug seeking. Rats self-administered methamphetamine for 1 h twice weekly (intermittent-short-I-ShA), 1 h daily (limited-short-ShA), or 6 h daily (extended-long-LgA) for 22 sessions. After 22 sessions, rats from each access group were withdrawn from self-administration and underwent spatial memory (Y-maze) and working memory (T-maze) tests followed by extinction and reinstatement to methamphetamine seeking or received one intraperitoneal injection of 5-bromo-2′-deoxyuridine (BrdU) to label progenitors in the hippocampal subgranular zone (SGZ) during the synthesis phase. Two-hour-old and 28-day-old surviving BrdU-immunoreactive cells were quantified. I-ShA rats performed better on the Y-maze and had a greater number of 2-h-old SGZ BrdU cells than nondrug controls. LgA rats, but not ShA rats, performed worse on the Y- and T-maze and had a fewer number of 2-h-old SGZ BrdU cells than nondrug and I-ShA rats, suggesting that new hippocampal progenitors, decreased by methamphetamine, were correlated with impairment in the acquisition of new spatial cues. Analyses of addiction-related behaviors after withdrawal and extinction training revealed methamphetamine-primed reinstatement of methamphetamine-seeking behavior in all three groups (I-ShA, ShA, and LgA), and this effect was enhanced in LgA rats compared with I-ShA and ShA rats. Protracted withdrawal from self-administration enhanced the survival of SGZ BrdU cells, and methamphetamine seeking during protracted withdrawal enhanced Fos expression in the dentate gyrus and medial prefrontal cortex in LgA rats to a greater extent than in ShA and I-ShA rats. These results indicate that changes in the levels of the proliferation and survival of hippocampal neural progenitors and neuronal activation of hippocampal granule cells predict the effects of methamphetamine self-administration (limited *vs* extended access) on cognitive performance and relapse to drug seeking and may contribute to the impairments that perpetuate the addiction cycle.
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INTRODUCTION

The psychostimulant drug methamphetamine is highly addictive, can be acutely lethal, and can result in long-term neuroadaptive alterations with many implications for health and well-being. Converging lines of evidence from clinical and preclinical studies have revealed that methamphetamine dependence is linked to a decreased volume of limbic-related structures (Schwartz *et al*, 2010; Simon *et al*, 2010), altered hippocampal morphology (Mandyam *et al*, 2008; Kim *et al*, 2010), and hippocampal-related deficits in learning and memory (Rogers *et al*, 1999, 2008). Given that the hippocampus is essentially involved in learning and memory processes (Black *et al*, 1977; Morris *et al*, 1982; Eichenbaum *et al*, 1992; Riedel *et al*, 1999; Scoville and Milner, 2000; Burgess *et al*, 2002) and mounting evidence that suggests a role of an important form of hippocampal plasticity, namely adult hippocampal neurogenesis in hippocampus-dependent learning and memory (van Praag *et al*, 1999; Shors *et al*, 2001; Jessberger *et al*, 2009; for reviews, see Leuner *et al*, 2006; Kim *et al*, 2011), we wished to determine whether methamphetamine-induced inhibition of hippocampal neurogenesis contributes to methamphetamine-induced impairments in hippocampus-dependent cognitive behaviors.

With respect to addiction research, clinical and preclinical studies have revealed that addictive behaviors,

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including relapse to drug taking, are regulated by mesocorticolimbic neural circuitry, which includes a projection to the hippocampal formation (Nestler, 2001; Kalivas and McFarland, 2003; Koob and Volkow, 2010). With respect to methamphetamine addiction, a few reports have suggested that the hippocampus may contribute to specific aspects of methamphetamine addiction (Ursin *et al*, 1966; Ricoy and Martinez, 2009) and relapse (Tani *et al*, 2001; Hiranita *et al*, 2006; Shen *et al*, 2006; Rogers *et al*, 2008). Thus, it appears that plasticity in the hippocampus after chronic methamphetamine use may contribute to some of the addictive behaviors associated with chronic methamphetamine use.

Recent evidence demonstrated that methamphetamine exposure alters adult hippocampal neurogenesis (Hildebrandt *et al*, 1999; Teuchert-Noodt *et al*, 2000; Mandyam *et al*, 2008). Adult hippocampal neurogenesis is a spontaneous, multi-stage process that includes the birth, survival, and integration of granule cell neurons in the subgranular zone (SGZ) of the dentate gyrus of the adult hippocampus (Altman and Das, 1965). Methamphetamine alters every stage of hippocampal neurogenesis, and these effects vary by dose, duration, pattern of drug exposure, and timing of drug exposure before labeling the neural progenitors (Hildebrandt *et al*, 1999; Teuchert-Noodt *et al*, 2000; Mandyam *et al*, 2008; Yuan *et al*, 2011). Therefore, the inhibitory effect of methamphetamine on the dynamic regenerative capacity of the adult hippocampus is now being considered a precursor of methamphetamine-induced neurodegeneration in this region (Canales, 2007, 2010).

Altogether, significant advances are being made in understanding how methamphetamine impairs adult hippocampal neurogenesis. However, it is unknown whether methamphetamine-induced inhibition of hippocampal neurogenesis correlates with methamphetamine-induced impairment of hippocampus-dependent memory. In addition, given that the hippocampus has a role in methamphetamine-seeking behavior (Hiranita *et al*, 2006), unknown is how withdrawal from methamphetamine influences the survival of hippocampal progenitors, and such studies would be important to determine whether alterations in the survival of adult-born granule neurons contributes to the reinstatement of methamphetamine-seeking behavior. Importantly, the use of various methamphetamine exposure paradigms via self-administration provides the means to pursue such correlative studies, such as whether the pattern of methamphetamine intake could be attributable to methamphetamine's differential influence on hippocampus-dependent memory, relapse behavior, and spontaneous neuroplasticity.

We thus tested the hypothesis that a history of extended access *vs* limited access methamphetamine intake is associated with specific spatial and working memory impairments dependent on the hippocampus. To test this hypothesis, the integrity of spatial memory under low-incentive conditions and working memory under high cognitive demands and incentive conditions was assessed 3–15 days after the last self-administration session in rats that self-administered methamphetamine under a fixed-ratio schedule for 1 h twice weekly (intermittent-short-access [I-ShA]), 1 h daily (short-access [ShA]), or 6 h daily (long-access [LgA]; Cryan *et al*, 2003; George *et al*, 2008). We employed a spontaneous alternation procedure that used a Y-maze (ie, a task sensitive to hippocampal dysfunction; Conrad *et al*, 1996) and a delayed nonmatching-to-sample procedure that used a T-maze (ie, a task sensitive to hippocampal and prefrontal cortical dysfunction; Gerlai, 1998, 2001; Wall and Messier, 2001; Lalonde, 2002; George *et al*, 2008; Sudai *et al*, 2011). After testing for cognitive performance, the rats underwent extinction training and drug-seeking behavior testing (ie, tasks sensitive to nucleus accumbens [NAc] core, prefrontal cortex [PFC], and hippocampal dysfunction; Hiranita *et al*, 2006; Feltenstein and See, 2008; Noonan *et al*, 2010) to test the central hypothesis that varied methamphetamine access produces differential effects on memory and relapse tasks that depend on the hippocampus and that the behavioral outcomes correlate with changes in the levels of hippocampal neural progenitors. In the context of the above studies, a different cohort of I-ShA, ShA and LgA rats was injected with 5-bromo-2′-deoxyuridine (BrdU) to label proliferating synthesis (S)-phase neural progenitors and euthanized 2 h later to measure cell proliferation and 28 d later to measure cell survival.

MATERIALS AND METHODS

Animals

Seventy-six adult, male Wistar rats (Charles River), weighing 200–250 g at the start of the experiment, were housed two per cage in a temperature-controlled vivarium under a reverse light/dark cycle (lights off 0800–2000 hours). Food and water were available *ad libitum*. Fifty-four rats underwent surgery for catheter implantation for intravenous methamphetamine self-administration (Mandyam *et al*, 2007b). Methamphetamine hydrochloride (generously provided by the National Institute on Drug Abuse) was dissolved in physiological saline (0.9%), and methamphetamine self-administration was performed 5 days per week. For baseline training sessions, the rats were allowed to self-administer methamphetamine at a dose of 0.05 mg/kg per injection under an FR1 schedule for 8–10 sessions. After baseline training, the rats were divided into three methamphetamine access groups, balanced by the number of injections per session during the last three baseline sessions. The rats in each group were divided into Meth, Withdrawal, and Reinstatement groups balanced by their baseline performance. During the escalation period, one group of rats (LgA, $n = 18$) was allowed to self-administer 0.05 mg/kg per injection of methamphetamine for 6 h per day under an FR1 schedule, whereas the other group (ShA, $n = 18$ per group) was allowed to do so for 1 h per day under an FR1 schedule. A third group (I-ShA, $n = 18$) was exposed to methamphetamine on Mondays and Thursdays (each week). All of the rats were 19–20 weeks old when perfused (Mandyam *et al*, 2008). All procedures were performed during the dark cycle.

Sixteen to 22 h after the last methamphetamine session, some rats from all three access groups received one injection of 150 mg/kg BrdU, intraperitoneal (Boehringer Mannheim Biochemica), dissolved in 0.9% saline and 0.007N NaOH at 20 mg/ml and survived for 2 h (Meth group; Figure 1a; $n = 5$ for all methamphetamine groups; $n = 5$ for drug-naive controls) or 28 days (Withdrawal

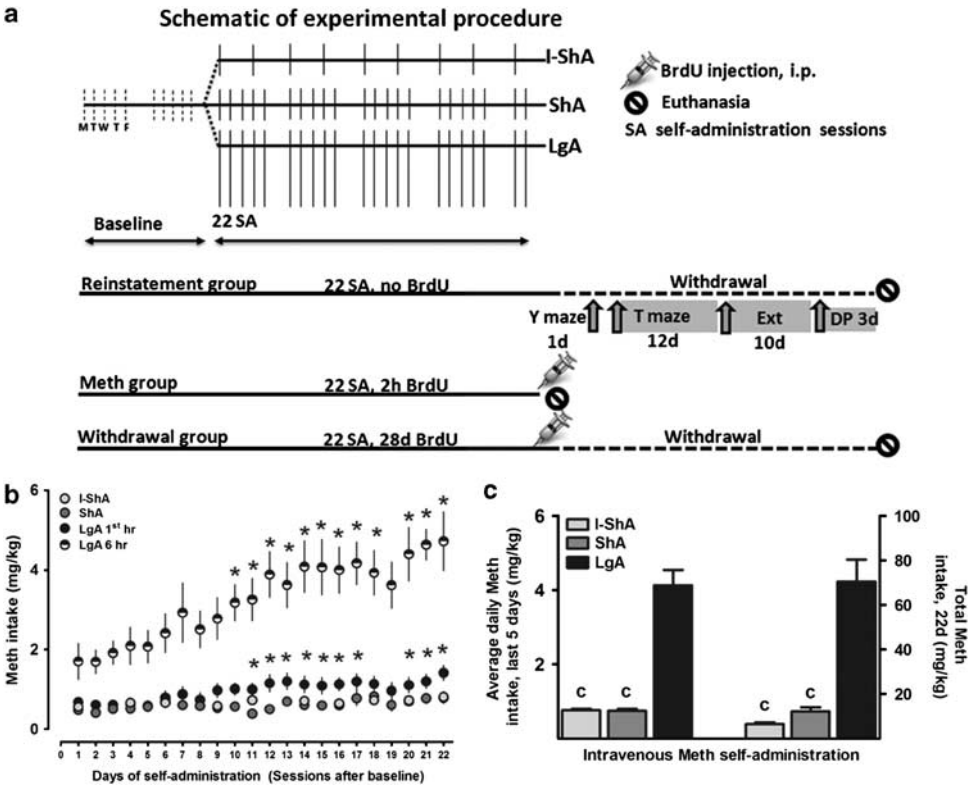

Figure 1 Time-line of methamphetamine self-administration and daily intake of methamphetamine (mg/kg). (a) Schematic representation of experimental procedure. All rats self-administered methamphetamine in 1 h baseline sessions for 10 days (indicated by dashed lines for Monday–Friday (MTWTF)). Following baseline self-administration, the rats were divided into I-ShA, ShA, and LgA groups and self-administered methamphetamine for 22 sessions. Following 22 sessions of methamphetamine, the rats were subdivided into three groups: Reinstatement (behavior group that did not receive any BrdU injections and euthanized after 29 days after the last self-administration session; I-ShA, $n=8$; ShA, $n=8$; LgA, $n=9$); Meth (methamphetamine group injected with BrdU and euthanized 2 h after injection; I-ShA, $n=5$; ShA, $n=5$; LgA, $n=5$) and Withdrawal (protracted withdrawal group injected with BrdU at the same time as the Meth group and euthanized after 28 days of withdrawal; I-ShA, $n=5$; ShA, $n=5$; LgA, $n=4$). (b) Methamphetamine intake over 22 sessions in mg/kg in I-ShA, ShA, and LgA rats after baseline sessions. (c) Average daily (left axis) and total (right axis) methamphetamine intake (mg/kg) in I-ShA, ShA, and LgA rats. $^{*}p<0.05$ in (b), compared with day 1 of escalation; $^{C}p<0.05$, compared with LgA in (c). I-ShA, $n=18$; ShA, $n=18$; LgA, $n=18$. Data are expressed as mean ± SEM.

group; Figure 1a; $n=4$–5 for all methamphetamine groups; $n=5$ for drug-naive controls), after which they were anesthetized with chloral hydrate and perfused transcardially as described previously (Mandyam *et al*, 2004). Thirty-seven rats (control, $n=12$; I-ShA, $n=8$; ShA, $n=8$; LgA, $n=9$) participated in the behavior studies. The rats were tested on the Y-maze and T-maze and for extinction behavior followed by methamphetamine-primed reinstatement (Reinstatement group; Figure 1a). The rats that underwent reinstatement were euthanized 1–2 h after the last session. Serial coronal 40 μm sections were obtained on a freezing microtome, and sections from the brain (−1.4 to −6.7 mm from bregma; Paxinos and Watson, 1997) were stored in 0.1% NaN_3 in 1 × phosphate-buffered saline at 4°C.

Spontaneous Alternation Behavior (Y-maze)

The Y-maze was performed in an automated apparatus connected to a camera and computer that monitored movement between the arms and time spent in each arm. Novelty was guided by distinct spatial cues. The experimenter was only responsible for placing the rat into the apparatus and removing the rat after the testing period. Care was taken to ensure that the rat was stress-free during testing. The Y-maze was constructed of black plastic with three arms ($61 \times 14 \times 35\,cm^3$) that extended from a central platform at a 120° angle (Figure 2a). Seventy-two hours after self-administration, each rat was placed in the middle of the maze and allowed to move freely in the three arms of the maze during a 10 min testing period. An arm entry was defined as the entry of four paws into one arm. The sequence of the arm entries was recorded using infrared beams. Alternation was defined as multiple entries into the three different arms on overlapping triplet sets. The percentage of spontaneous alternation was calculated as the ratio of the actual to possible alternations (defined as the total number of arm entries minus 2) multiplied by 100: *alternation (%) = [(number of alternation)/(total arm entries-2)]/100* (Conrad *et al*, 1996). The Y-maze task was performed by an experimenter blind to the treatment groups.

Delayed Non-Matching-to-Sample Task (T-maze)

The T-maze procedure began 24 h after the Y-maze task and was performed as described previously (Figure 2b; George *et al*, 2008). Starting from Day 4 after the last self-administration session, the rats were food-deprived to 85% free-feeding body weight and habituated to the T-maze

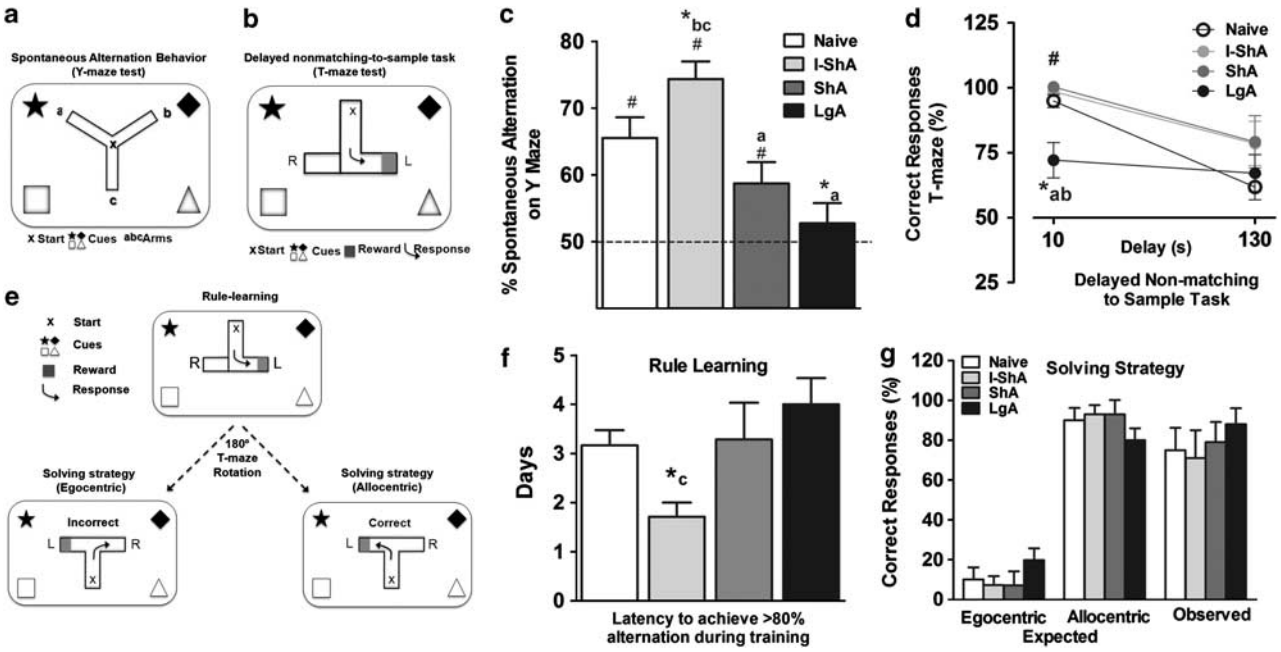

Figure 2 Effects of methamphetamine self-administration on spontaneous alternation behavior in the Y-maze and delayed non-matching-to-sample task in the T-maze. (a) Illustration of Y-maze task. (b) Illustration of T-maze task. (c) Percentage of spontaneous alternation in the Y-maze. $^{\#}p<0.05$, compared with 50% alternation (chance); $^{a}p<0.05$, compared with I-ShA; $^{*}p<0.05$, compared with naive. (d) Percentage of correct responses in the delayed non-matching-to-sample task. $^{\#}p<0.05$, compared with LgA; $^{*}p<0.05$, compared with naïve; $^{a}p<0.05$, compared with I-ShA; $^{b}p<0.05$, compared with ShA. (e–g) Rule learning and solving strategies in Reinstatement rats. (e) Illustration of rule learning and delayed non-matching-to-sample task with 180° rotation and two different possible navigational strategies—egocentric and allocentric—used to solve the delayed non-matching-to-sample task. (f) Total number of days to reach the criterion in the delayed non-matching-to-sample task (ie, latency to achieve >80% alternation during training). (g) Expected and observed results after rotation of the T-maze. The left panel represents performance expected after the rotation if the three groups had used an egocentric or allocentric strategy, based on baseline performance with the 10 s delay. The right panel represents the observed results after the rotation. Notice that all of the groups used the same allocentric strategy. I-ShA, $n=8$; ShA, $n=8$; LgA, $n=9$. Data are expressed as mean ± SEM. $^{*}p<0.05$, compared with control (panels c, d, f); $^{b}p<0.05$, compared with ShA (panels c, d); $^{c}p<0.05$, compared with LgA (panels c, f).

until they readily consumed a sucrose pellet placed at the end of each arm of the T-maze. To control for the non-specific effects of drug self-administration on behavioral performance, age-matched drug-naive (ie, without a history of self-administration) rats were tested simultaneously. I-ShA, ShA, LgA, and control rats did not differ in body weight before or during the food-deprivation period. Rats from all groups were run in parallel, in a random order, and the experimenters were blind to the group. After habituation, the rats were trained for 10 trials/session on a non-matching-to-sample task. Each trial consisted of one forced-choice run and one free-choice run (rewarded only if the rat entered the opposite arm). After reaching the acquisition criterion (>70% correct responses during 2 consecutive days), a variable delay (10 and 130 s) was introduced between the forced and free runs, and the number of trials per session was increased to 16. The first trial consisted of a 10 s delay followed by 15 trials with randomly chosen delays (10 and 130 s). The delay sequences were chosen to avoid repetition of the same delay or the same sequence and with an equal representation of each delay. The rats were tested in this protocol for 2–3 sessions that corresponded to 15–17 days after the last self-administration session. The intertrial interval (ie, the time between the second run of trial n and the first run of trial $n+1$) was fixed at 120 s to avoid proactive interference. The number of trials necessary to reach the criterion and percentage of correct responses were calculated.

Extinction Testing

After 19 days of abstinence from self-administration, the rats underwent daily 1 h extinction sessions. During the extinction sessions, the rats were not connected to the infusion apparatus. Responses on either the active or inactive lever were recorded and did not result in programmed consequences (ie, no infusions and no conditioned stimulus presentations). Extinction training consisted of a minimum of 10 sessions plus additional extinction training sessions as needed until the rats reached the extinction criterion (<20 active lever presses per session for two consecutive sessions).

Reinstatement Testing

Seventy-two hours after the final extinction session, the rats from all of the methamphetamine groups were placed into the methamphetamine-paired context (ie, the same operant box used for self-administration sessions) for 1 h, during which they were connected to the infusion apparatus to allow for a similar interaction with the spatial elements of the context as during methamphetamine self-administration training. Lever presses were used as a measure of drug seeking, and responses on either the active or inactive lever were recorded and did not result in an infusion of fluids through the catheter or other programmed consequences (ie, conditioned stimulus presentations). After the first hour

in the self-administration chamber, the rats received an intraperitoneal injection of 0, 0.5, and 1 mg/kg methamphetamine on day 1, 2, or 3 in a counterbalanced order and were put back in the same self-administration chamber after 30 min for an additional hour. Active lever responses before and after methamphetamine priming were analyzed, and methamphetamine-primed reinstatement is expressed as the total number of lever presses per animal per dose of methamphetamine after priming. All of the rats were euthanized 1–3 h post reinstatement testing. The rats that were euthanized within 90 min post testing were used for fluorescent (Fos) analysis.

Antibodies, Immunohistochemistry, Microscopic Analysis, and Quantification

The following primary antibodies were used for immunohistochemistry (IHC): Ki-67 (1 : 1000), BrdU (1 : 400), activated caspase 3 (AC-3; 1 : 500), and c-Fos (1 : 1000). The left and right hemispheres of every twelfth (BrdU, Ki-67, AC-3) section through the rat hippocampus were slide-mounted, coded, and dried overnight before IHC. For Fos IHC, six bilateral sections that contained the dentate gyrus, two bilateral sections that contained the mPFC (including the anterior cingulate, prelimbic, and infralimbic cortices), and two bilateral sections that contains the NAc core were used for cell quantification from each rat from each group. The sections were pretreated (Mandyam *et al*, 2004), blocked, and incubated with the primary antibodies (BrdU, Ki-67, AC3, Fos) followed by biotin-tagged secondary antibodies. Immunoreactive cells in the SGZ (ie, cells that touched and were within three cell widths inside and outside the hippocampal granule cell-hilus border for 2 h BrdU, Ki-67) or granule cell layer (GCL; 28 day BrdU, AC-3, and Fos) were quantified with a Zeiss Axiophot photomicroscope ($\times 400$ magnification) using the optical fractionator method, in which sections through the dentate gyrus (-1.4 to -6.7 mm from bregma; Paxinos and Watson, 1997) were examined. Cells in the SGZ and GCL were summed and multiplied by 12 to give the total number of cells (Eisch *et al*, 2000). Fos cells in the mPFC (3.7 and 3.2 mm from bregma) and NAc core (1.2 and 1.0 mm from bregma) were examined, and cells from the left and right hemispheres that were localized in the counting frame (mPFC, 0.09 mm^2; NAc, 0.078 mm^2) were visually quantified. The total number of immunoreactive cells both in the left and right hemispheres was counted and are expressed as the total number of immunoreactive cells per mm^2 in each rat averaged across rats within a group.

Data Analysis

The methamphetamine self-administration data are expressed as the mean mg/kg per session of methamphetamine self-administration for each group of rats. The effect of the session duration on methamphetamine self-administration per session and during the first hour of a session was examined over the 22 escalation sessions using a two-way repeated-measures analysis of variance (ANOVA; session duration $\times$ daily session; SPSS software) followed by the Student–Newman–Keuls *post hoc* test. The pattern of responding for methamphetamine is expressed as the mean mg/kg per hour over 6 h sessions in LgA rats and were compared between the first and >10th escalation sessions. Differences in the rate of responding between the first and other escalation sessions were evaluated using the paired *t*-test. For the Y-maze study, the four groups (naive, I-ShA, ShA, and LgA) were used as between-subjects factors, and novelty-induced alternation in the Y-maze or self-administration sessions was used as the within-subjects factor. For the T-maze study, the four groups (naive, I-ShA, ShA, and LgA) were used as between-subjects factors, and the two delays (10 and 130 s) in the T-maze or self-administration sessions were used as the within-subjects factors. ANOVA was used to compare the Y-maze and T-maze performance with chance performance. Lever responding during extinction and during the reinstatement tests was also assessed by ANOVA. For the BrdU, Ki-67, AC-3, and Fos analyses, one- or two-way ANOVA was used when appropriate. Nonlinear regression analyses were performed using Pearson's correlation tests. The data are expressed as mean $\pm$ SEM in all graphs.

RESULTS

Escalation in Methamphetamine Intake is Evident in LgA Rats

Total and daily methamphetamine intake was significantly different between the methamphetamine groups (Figures 1b and c; average intake over 22 sessions: $F_{2,54} = 22.7$, $p < 0.0001$), and the *post hoc* analysis revealed significantly higher intake in LgA rats compared with I-ShA and ShA rats (all $p < 0.001$). Methamphetamine self-administration during the first hour was considerably higher in LgA *vs* ShA and I-ShA rats ($F_{2,54} = 7.4$, $p < 0.05$) following session 10 and was maintained until session 22 ($p < 0.05$).

Methamphetamine Self-Administration Improves Spatial Memory in I-ShA Rats and Impairs Spatial and Working Memory in LgA Rats

To test spatial memory under low-incentive conditions, rats from the control, I-ShA, ShA, and LgA rats (reinstatement group) were tested in a novelty-induced alternation task in the Y-maze (Figure 2a). Methamphetamine rats and controls exhibited a higher percentage of alternation behavior compared with chance (Figure 2c; $F_{4,45} = 13.5$, $p < 0.001$), demonstrating the incentive value of novelty. The *post hoc* analysis revealed a significantly higher response in control ($p < 0.001$), I-ShA ($p < 0.001$), and ShA ($p = 0.027$) rats compared with chance performance. LgA rats did not exhibit higher alternation compared with chance performance ($p = 0.454$). Control, ShA, and LgA rats exhibited lower alternation compared with I-ShA rats (all $p < 0.05$). LgA rats exhibited lower alternation compared with control and I-ShA rats (all $p = 0.001$), suggesting that LgA rats were impaired under low-incentive conditions. In the delayed non-matching-to-sample task (Figure 2b), the multivariate analysis revealed a Delay $\times$ Group interaction (Figure 2d; $F_{3,33} = 10.9$, $p < 0.001$). I-ShA and ShA rats exhibited normal working memory performance compared with control rats when a short delay (10 s) between the acquisition trials and test trials was used. LgA rats had significantly poorer working memory performance compared with

control, I-ShA, and ShA rats during the short delay (10 s) tests (all $p<0.001$). All of the rats performed equally well during the tests that involved a longer delay (130 s) between the acquisition trials and test trials.

Impaired Spatial and Working Memory in LgA Rats is not Attributable to Alterations in General Cognitive Ability

As I-ShA rats demonstrated an improvement in performance in the Y-maze task, we next determined whether these behavioral outcomes could be attributable to improved learning capability or improved spatial recognition in these rats (Figure 2e). I-ShA rats demonstrated a reduced latency to achieve greater than 80% alternation during the training phase of the T-maze task (Figure 2f; $F_{3,28}=3.5$, $p=0.03$) compared with control ($p=0.04$) and LgA ($p=0.004$) rats, suggesting improved learning capability. However, impaired performance on the Y-maze and T-maze in LgA rats under low-incentive and low working memory load (Figures 2c and d) was not associated with an impaired rate of learning (Figure 2f), suggesting preserved general cognitive function necessary to perform the task, such as sensation, perception, rule learning, behavioral selection, and action–outcome association. An alternative explanation for the decreased spatial and working memory performance in LgA rats is that they used a different navigational strategy to solve the Y-maze and T-maze tasks. Egocentric strategies (ie, orientation in space using proprioceptive information or intra-maze cues) and allocentric strategies (ie, orientation in space using extra-maze cues in the environment; Figure 2e; King and Corwin, 1992; Holscher and Schmidt, 1994; Nieto-Escamez *et al*, 2002) were tested. The effect of a 180° rotation of the T-maze between the acquisition and test trials was evaluated on working memory performance using a 10 s delay. The rotation shifted the position of the proximal cues in the T-maze by 180° compared with the distal cues in the room, the orientation of which were left unchanged (Figure 2e). In this case, an allocentric strategy (ie, the use of distal cues) would lead to good performance (now defined as an entrance into the same arm), whereas an egocentric strategy (ie, the use of intra-maze cues and proprioceptive information) would lead to poor performance (Figure 2e). After the rotation, all of the groups exhibited more than 90% correct responses in accordance with an allocentric strategy (Figure 2g; $F_{3,32}=10.5$, $p<0.001$), ruling out the hypothesis that a differential navigational strategy could explain the spatial and working memory impairments observed in LgA rats.

Methamphetamine Self-Administration Increases S-phase Cells in I-ShA Rats and Decreases S-phase Cells in LgA Rats in the Hippocampal Dentate Gyrus

Cell proliferation experiments determined whether enhanced/impaired performance on the Y-maze correlated with increases or decreases in the number of neural progenitors in the SGZ of the dentate gyrus. After 16-22 h the methamphetamine session I-ShA, ShA, LgA, and drug-naive control rats from meth group were injected with BrdU and euthanized 2 h later to label S-phase cells (Figure 1a). BrdU IHC revealed a normal pattern of S-phase cells in the dentate gyrus, with BrdU cells in the SGZ as reported previously (Mandyam *et al*, 2007a; Noonan *et al*, 2008). BrdU cells at this proliferation time point appeared small, irregular, and in clusters in the control and all methamphetamine groups (Figure 3a). Cell quantification of S-phase BrdU-positive cells demonstrated that methamphetamine significantly altered the number of BrdU cells (Figure 3b; $F_{3,19}=11.5$, $p<0.001$). Specifically, I-ShA rats exhibited an increased number of BrdU cells compared with controls and the other methamphetamine groups (all $p<0.05$), and LgA rats had reduced BrdU cells compared with controls and I-ShA rats (all $p<0.05$).

Total Amount of Methamphetamine Self-Administered Negatively Correlates with Cell Proliferation in the Dentate Gyrus and Spontaneous Alternation on the Y-maze

As the extent of alternation on the Y-maze and cellular proliferation in the dentate gyrus varied across rats in the methamphetamine groups, the percentage of alternation on the Y-maze and 2 h old BrdU cells were compared with the total amount of methamphetamine intake over 22 sessions in the methamphetamine rats using linear regression analysis. There was a significant correlation between performance on the Y-maze task and amount of methamphetamine consumed ($p<0.001$, $r=0.43$) and cell proliferation and the amount of methamphetamine consumed

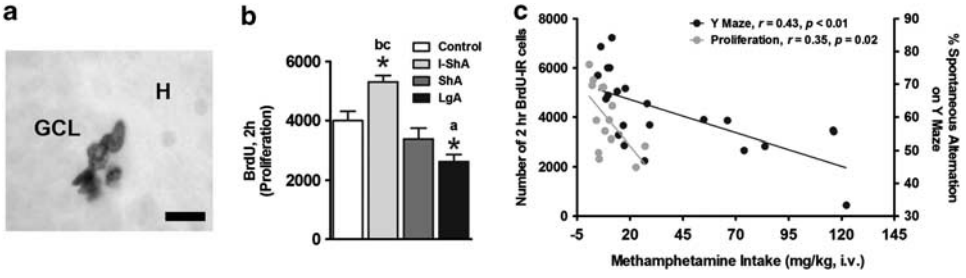

Figure 3 Effects of methamphetamine self-administration on cell proliferation. (a) Photomicrograph of proliferating cells in the SGZ of the hippocampal dentate gyrus labeled with a BrdU antibody. BrdU-labeled 2-h-old proliferating cells are visible as irregularly shaped cells in a cluster. GCL, granule cell layer; H, hilus. Scale bar in (a) = 20 μm. (b) Quantitative analysis of BrdU cell counts from Meth rats from serial coronal sections. Total number of BrdU cells per hippocampus from each access group. Control, $n=5$; I-ShA, $n=5$; ShA, $n=5$; LgA, $n=5$. Data are expressed as mean ± SEM; $^{*}p<0.05$, compared with control; $^{a}p<0.05$, compared with I-ShA; $^{b}p<0.05$, compared with ShA; $^{c}p<0.05$, compared with LgA. (c) Linear regression analysis of methamphetamine self-administration-induced changes in proliferation and cognitive behavior. Total number of 2 h BrdU-labeled cells per hippocampus per animal (gray circles, left axis) and percent spontaneous alternation per animal (black circles, right axis) plotted against total methamphetamine consumed during 22 self-administration sessions. I-ShA, $n=5$–8; ShA, $n=5$–8; LgA, $n=5$–9.

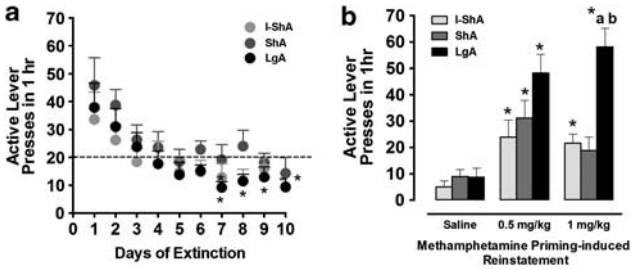

Figure 4 Extinction and reinstatement of methamphetamine seeking. (a) Decreased lever responding on the previously methamphetamine-paired lever in daily 1 h extinction trials. No significant differences were seen across access conditions. The extinction criterion was reached after day 6 in all groups (*$p<0.05$, compared with day 1 of extinction responding). (b) Methamphetamine-primed reinstatement expressed as active lever presses after methamphetamine priming. *$p<0.05$, compared with saline responding; [a]$p<0.05$, compared with I-ShA rats; [b]$p<0.05$ compared with ShA rats). I-ShA, $n=8$; ShA, $n=8$; LgA, $n=9$. Data are expressed as mean ± SEM.

($p=0.02$, $r=0.35$) when all of the methamphetamine rats were combined (Figure 3c).

Protracted Withdrawal from Methamphetamine Self-Administration Produces Methamphetamine-Primed Reinstatement of Methamphetamine-Seeking Behavior in I-ShA, ShA, and LgA Rats, and this Behavior is Enhanced in LgA Rats

Following the cognitive tests, all of the methamphetamine rats in the reinstatement group underwent several extinction sessions in their operant chambers. During the extinction sessions, methamphetamine was no longer available, and drug-paired lever responding readily declined across daily extinction sessions (Figure 4a). The extinction session duration was maintained at 1 h for all of the groups, and responses during the sessions gradually declined to the criterion of $\leqslant 20$ lever presses (significant main effect of Day, $F_{18,172}=7.7$, $p<0.001$), which was reached with 6–10 days of training (Figure 4a; $p<0.05$ *vs* day 1). All rats extinguished before reinstatement testing.

All methamphetamine rats showed significant methamphetamine-primed reinstatement (Figure 4b; $F_{4,45}=14.4$, $p<0.001$). A significant difference between methamphetamine groups was seen during methamphetamine-primed reinstatement (Figure 4b; $F_{4,45}=4.8$, $p=0.01$). I-ShA, ShA, and LgA rats showed significant methamphetamine-primed reinstatement after 0.5 mg/kg methamphetamine priming (all $p<0.05$). After the 1.0 mg/kg dose of methamphetamine, LgA rats demonstrated greater reinstatement than I-ShA and ShA rats (all $p<0.05$).

Protracted Withdrawal from Methamphetamine Self-Administration Increases the Proliferation and Survival of Progenitors in LgA Rats in the Dentate Gyrus of the Hippocampus

As methamphetamine increased S-phase BrdU cells in I-ShA rats and decreased S-phase BrdU cells in LgA rats (Figure 3b), we then examined whether protracted withdrawal from methamphetamine self-administration for 4 weeks would normalize SGZ proliferation and cell survival in I-ShA, ShA, and LgA rats. BrdU was given to the withdrawal group at the same time as the meth group, whereas meth group rats were killed 2 h later, withdrawal group rats were killed 4 weeks later, corresponding to the same time frame when reinstatement group rats were euthanized (Figure 1a). Thus, BrdU cells in the withdrawal group represented surviving, not proliferating, S-phase cells. Mature BrdU cells in withdrawal group rats were round and less clustered and presented more punctate BrdU staining (Figure 5a), typical of SGZ cells 4 weeks after BrdU injection (Cameron and McKay, 2001; Dayer *et al*, 2003). As BrdU proliferating cells increased in I-ShA rats and decreased in LgA rats compared with controls (Figure 3b), a higher number of mature BrdU cells in I-ShA rats and a lower number of mature BrdU cells in LgA rats were expected after withdrawal. Protracted withdrawal increased the number of mature BrdU cells (Figure 5c; $F_{3,18}=3.4$, $p=0.05$), with LgA rats having more BrdU cells compared with I-ShA rats ($p=0.03$). An explanation for the surprisingly normal level of mature BrdU cells in I-ShA and LgA rats (Figure 5c) after protracted withdrawal could be the altered rate of survival of BrdU S-phase cells. To test this possibility, and because BrdU was injected on the same day in rats in the meth and withdrawal groups (Figure 1a), the ratio of S-phase BrdU cells to mature BrdU cells was calculated in all rats. Methamphetamine self-administration had a significant effect on the rate of survival of BrdU progenitors (Figure 5d; $F_{3,18}=9.8$, $p=0.001$), with LgA rats having more cells that survived compared with control ($p=0.001$), I-ShA ($p<0.001$), and ShA ($p=0.005$) rats. ShA rats also exhibited enhanced cell survival compared with I-ShA rats ($p=0.046$).

To examine whether protracted withdrawal altered the proliferation of SGZ cells, actively dividing SGZ cells in the withdrawal group were detected with an antibody against Ki-67, an endogenous marker of proliferation that is used interchangeably with short timeframe BrdU labeling to detect proliferating cells (Kee *et al*, 2002). Ki-67 cells were irregularly shaped and showed immature morphology that was consistent with proliferating cells and were often clustered (Figure 5b; Mandyam *et al*, 2008). Protracted withdrawal increased the number of Ki-67 cells in all of the methamphetamine groups, with a significant effect of drug on cell numbers (Figure 5e; $F_{3,18}=3.7$, $p=0.05$). Control rats had fewer Ki-67 cells compared with I-ShA ($p=0.01$), ShA ($p=0.02$), and LgA ($p=0.05$) rats. Cell death increased after withdrawal, reflected by cell counts for AC-3 cells ($F_{3,21}=4.3$, $p=0.02$), with LgA and I-ShA rats having more AC-3 cells compared with controls (all $p<0.05$; controls, 72 ± 19; I-ShA, 265 ± 124; ShA, 90 ± 20; LgA, 319 ± 143).

Methamphetamine Priming-Induced Reinstatement of Methamphetamine Seeking Enhances Neuronal Activation of Granule Cell Neurons in LgA Rats without Effecting Proliferation of SGZ Progenitors

After demonstrating the distinct effects of methamphetamine self-administration *vs* protracted withdrawal on the number of proliferating cells and adult-generated mature BrdU cells, we then explored whether reinstatement of methamphetamine seeking during protracted withdrawal

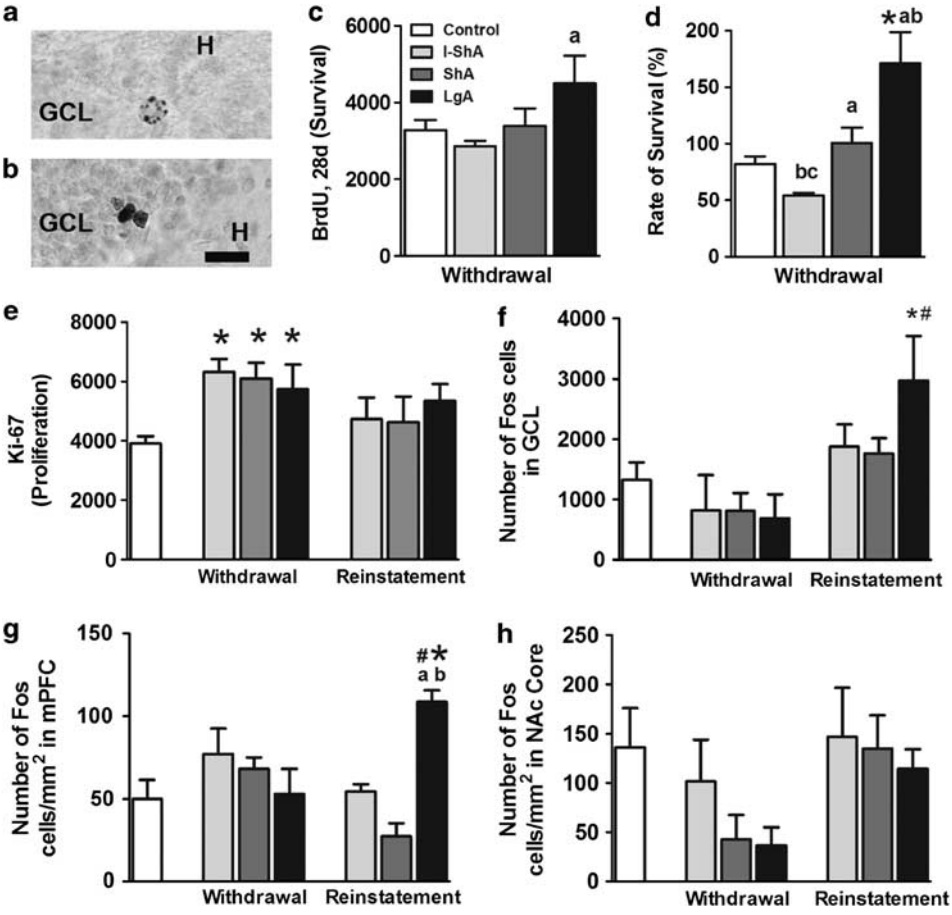

Figure 5 Effects of protracted withdrawal from methamphetamine self-administration and methamphetamine-primed reinstatement on proliferation, survival, and neuronal activation in the dentate gyrus, mPFC, and NAc core. (a) Photomicrograph of mature 28-day-old BrdU cells in the GCL of the hippocampal dentate gyrus. Mature BrdU cells are round to oval in shape, resembling surrounding granule cell neurons. (b) Photomicrograph of proliferating Ki-67 cells in the SGZ of the hippocampal dentate gyrus. Ki-67 cells are irregular and often found in clusters of cells that resemble the immature morphology of proliferating progenitors. GCL, granule cell layer; H, hilus. Scale bar = 15 μm in (a) and 30 μm in (b). (c) Quantitative analysis of total number of 28-day-old BrdU cells in the dentate gyrus; $^{a}p<0.05$, compared with I-ShA. (d) Proportion of 2 h BrdU cells (meth group) that survived to become BrdU neurons (withdrawal group) in all access groups. The rate of survival was calculated as the ratio of withdrawal group BrdU cell counts to meth group BrdU cell counts. $^{*}p<0.05$, compared with control; $^{a}p<0.05$, compared with I-ShA; $^{b}p<0.05$, compared with ShA; $^{c}p<0.05$, compared with LgA. (e) Quantitative analysis of total number of Ki-67 cells in the dentate gyrus from withdrawal group rats and reinstatement group rats. $^{*}p<0.05$, compared with controls. (f–h) Quantitative analysis of total number of Fos-labeled cells in the GCL of the dentate gyrus (f), mPFC (g), and NAc core (h). $^{*}p<0.05$, compared with controls; $^{a}p<0.05$, compared with I-ShA; $^{b}p<0.05$, compared with ShA; $^{\#}p<0.05$, compared with withdrawal rats from the same access group. Control, $n=5$–13; I-ShA, $n=5$–8; ShA, $n=5$–8; LgA, $n=4$–9. Data are expressed as mean ± SEM.

differentially affects proliferation. Reinstatement group rats from all of the methamphetamine groups learned to extinguish operant responses and were subsequently tested for methamphetamine-primed reinstatement. Because these rats were not injected with BrdU, proliferating cells were quantified by Ki-67 labeling (Figure 5b). Reinstatement of methamphetamine seeking normalized the number of Ki-67 cells that were increased during protracted withdrawal (Figure 5e; all $p>0.05$).

Methamphetamine-primed reinstatement produced a significantly greater number of Fos-positive cells in the GCL of the dentate gyrus compared with protracted withdrawal (Figure 5f; $F_{6,37}=2.9$, $p=0.02$). Specifically, reinstatement group LgA rats had a greater number of Fos-positive cells compared with controls ($p=0.007$) and withdrawal group LgA rats ($p=0.001$). Reinstatement group I-ShA and ShA rats did not exhibit significant changes in Fos cells compared with controls and withdrawal group I-ShA and ShA rats.

Methamphetamine Priming-Induced Reinstatement of Methamphetamine Seeking Enhances Neuronal Activation in the mPFC in LgA Rats

Methamphetamine-primed reinstatement produced a significantly greater number of Fos-positive cells in the mPFC compared with protracted withdrawal (Figure 5g; $F_{6,39}=5.5$, $p<0.05$). Specifically, reinstatement group LgA rats had a greater number of Fos-positive cells compared with control ($p<0.005$), I-ShA ($p=0.001$), ShA ($p<0.001$), and withdrawal group LgA rats ($p=0.002$). Reinstatement group I-ShA and ShA rats did not exhibit significant changes in Fos cells compared with controls and the withdrawal group I-ShA and ShA rats. No significant

differences in Fos cell counts were seen in the NAc core region (Figure 5h).

Protracted Withdrawal-Induced Enhancement of Mature BrdU Cells in the Dentate Gyrus and Active Lever Responses during Reinstatement of Methamphetamine Seeking Positively Correlate with Total Amount of Methamphetamine Consumed

As the extent of methamphetamine-primed reinstatement and mature BrdU cells in the dentate gyrus varied across methamphetamine rats in the reinstatement and withdrawal groups, active lever presses during reinstatement testing and 28-day-old BrdU cells were compared with the total amount of methamphetamine intake over 22 sessions using linear regression analysis. There was a significant correlation between active lever responses during methamphetamine-primed reinstatement and the amount of methamphetamine consumed ($p<0.001$, $r=0.54$) and between mature BrdU cells and the amount of methamphetamine consumed ($p<0.001$, $r=0.64$) when all of the methamphetamine rats were combined (Figure 6a).

Fos Expression in the Dentate Gyrus and mPFC Positively Correlates with Active Lever Responses during Reinstatement to Methamphetamine Seeking

As the extent of methamphetamine-primed reinstatement varied across methamphetamine rats in the reinstatement group, active lever presses during reinstatement testing were compared with the number of Fos-labeled neurons in the GCL, mPFC, and NAc core in the methamphetamine rats using linear regression analysis. There was a significant correlation between active lever presses and the number of Fos-labeled granule neurons ($p=0.005$, $r=0.65$) and mPFC neurons ($p=0.008$, $r=0.62$) when all of the methamphetamine rats were combined (Figure 6b).

DISCUSSION

The present results highlight how varied access to methamphetamine self-administration alters memory and cognitive performance dependent on the hippocampus and cellular proliferation in the dentate gyrus of the hippocampus. Regression analysis of methamphetamine intake and behavioral performance and cellular proliferation demonstrated that alterations in spontaneous plasticity maintained by adult neural stem cells can predict cognitive performance dependent on the hippocampus as a function of total methamphetamine consumed. Withdrawal from methamphetamine self-administration enhanced methamphetamine seeking following priming in LgA rats compared with ShA and I-ShA rats, suggesting that the magnitude of methamphetamine seeking following priming is directly related to the amount of intake during prolonged methamphetamine self-administration (Yan *et al*, 2007; Rogers *et al*, 2008). Enhanced methamphetamine seeking following withdrawal correlated with increases in the survival of hippocampal neural progenitors and the neuronal activation of hippocampal granule cell neurons, suggesting that adult hippocampal plasticity may impact hippocampal function in general and relapse in particular (Noonan *et al*, 2008; Garcia-Fuster *et al*, 2011).

Intermittent and daily access to methamphetamine self-administration was performed to mimic the intermittently low and continued high levels of methamphetamine during recreational use in humans that may distinctly affect memory and cognitive function. With regard to psychomotor/cognitive performance in humans, there is general consensus about enhanced cognitive performance with acute (recreational) methamphetamines in methamphetamine-exposed individuals (Johnson *et al*, 2000, 2005; Hart *et al*, 2003; Silber *et al*, 2006) and deficits in cognitive performance in methamphetamine addicts (Scott *et al*, 2007; Parrott *et al*, 2011). The current preclinical findings of enhanced spatial memory in the Y-maze and learning in the T-maze by I-ShA rats and impaired performance in LgA rats, therefore, generally agree with the clinical results (Rogers *et al*, 2008; Reichel *et al*, 2011). Although merely speculative, our finding of cognitive performance in I-ShA, ShA, and LgA rats supports the hypothesis that the reinforcing effects of stimulants are increased when performance is perceived to be improved following drug administration (Silverman *et al*, 1994; Comer *et al*, 1997; Jones *et al*, 2001; Stoops *et al*, 2005), enhancing drug intake with increased access to the drug and mimicking the 'loss of

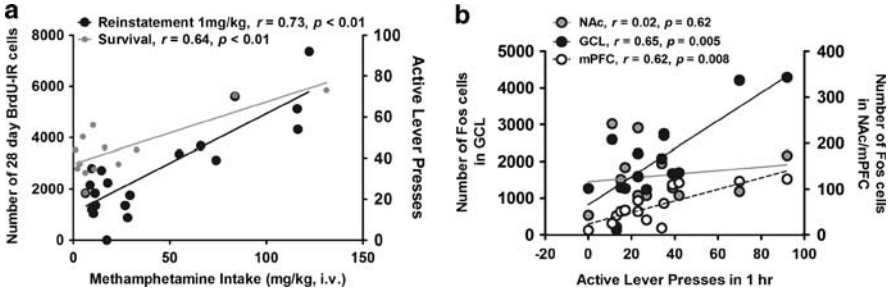

Figure 6 Linear regression analysis. (a) Protracted withdrawal from methamphetamine self-administration-induced changes in cell survival and methamphetamine-primed reinstatement. Total number of 28-day-old BrdU-labeled cells per dentate gyrus per rat (gray filled circles, left axis) and difference in active lever presses (saline *vs* 0.1 mg/kg methamphetamine priming) during reinstatement testing (black filled circles, right axis) plotted against total methamphetamine consumed during 22 self-administration sessions. I-ShA, $n=5$–8; ShA, $n=5$–8; LgA, $n=5$–9. (b) Total number of Fos-labeled cells per dentate gyrus (black filled circles, left axis), mPFC (white filled circles, right axis), and NAc core (gray filled circles, right axis) per rat plotted against difference in active lever presses (saline *vs* 0.1 mg/kg methamphetamine priming) during reinstatement testing 60–90 min before euthanasia. I-ShA, $n=5$; ShA, $n=5$; LgA, $n=4$.

control' over drug use observed in human addicts (Koob *et al*, 2004; Morgan and Roberts, 2004).

As varied access to methamphetamine distinctly altered hippocampus-dependent memory and because hippocampal neurogenesis within the hippocampal formation was proposed to contribute to the acquisition and retention of certain hippocampus-dependent memories (Leuner *et al*, 2006), a different cohort of methamphetamine rats was injected with BrdU to determine whether hippocampus-dependent behavioral outcomes correlated with changes in the levels of hippocampal neural progenitors. We previously found increased neural progenitors and immature neurons in the dentate gyrus in I-ShA rats, even after 42 sessions of self-administration (Mandyam *et al*, 2008). We hypothesized that the increases in the number of neural progenitors would be evident at an earlier time point that coincided with the time point that analyzed behavioral performance. Our results demonstrate that I-ShA rats have an increased number and LgA rats have a decreased number of BrdU progenitors in the dentate gyrus, and methamphetamine-induced alterations in BrdU progenitors correlate with methamphetamine-induced alterations in hippocampus-dependent memory (Figure 3c). These results, therefore, highlight the novel role of drug-induced adult neuroplasticity in cognitive function. Although care must be taken in comparing correlations of dentate gyrus progenitors and cognitive performance from drug self-administration *vs* non-drug studies (van Praag *et al*, 1999; Santarelli *et al*, 2003), our findings may provide insights into some important functions of spontaneous hippocampal plasticity that were previously underappreciated. Nevertheless, I-ShA methamphetamine-induced new neurons may transiently participate in memory storage and eventually outlive their usefulness for cognitive performance, perhaps becoming important for some other functions, including methamphetamine-seeking behavior.

The general pattern of extinction responding after withdrawal from methamphetamine self-administration was similar in all methamphetamine groups (Figure 4a). However, methamphetamine priming increased methamphetamine seeking, the magnitude of which was proportional to the amount of methamphetamine intake (Yan *et al*, 2007; Rogers *et al*, 2008). The analysis of mature BrdU cells and proliferating Ki-67 cells in the dentate gyrus in rats in the withdrawal groups addressed the possibility that the increased methamphetamine-primed reinstatement in LgA rats was likely related to differences in persistent neuroplastic effects produced during protracted withdrawal after LgA methamphetamine (Noonan *et al*, 2008; Garcia-Fuster *et al*, 2011). As I-ShA methamphetamine increased and LgA methamphetamine decreased BrdU progenitors, we hypothesized that protracted withdrawal would normalize the effects on proliferation and would not affect the survival of progenitors in all groups. Notably, protracted withdrawal maintained the enhanced proliferation in I-ShA rats, reflected by increased Ki-67 cell numbers. Enhanced proliferation during self-administration and withdrawal in I-ShA rats did not produce an increase in mature BrdU cells. Importantly, the lower number of mature BrdU cells was attributable to a reduced rate of BrdU survival in I-ShA rats, suggesting withdrawal-specific effects on later stages of neuronal development, including the maturation and differentiation of granule neurons (Noonan *et al*, 2008; Garcia-Fuster *et al*, 2011).

In LgA rats, protracted withdrawal increased proliferation, reflected by increased Ki-67 cells. The enhanced proliferation could be attributable to a compensatory effect (ie, decreased cell death) that might have occurred for the decreased population of progenitors (2 h BrdU cells) during self-administration. The analysis of AC-3 cells rules out this possibility because protracted withdrawal increased apoptosis in LgA rats. Alternatively, the modulation of the cell division of progenitors, such as the increased rate of symmetric division, could be responsible for the enhanced number of progenitors during protracted withdrawal (Noonan *et al*, 2008) and therefore the normal number of mature BrdU cells in LgA withdrawal group rats. As proliferation was not measured in LgA rats during early withdrawal and mature BrdU cells mostly reflect the survival of progenitors born during BrdU injection, an alternative explanation for the normal number of mature BrdU cells in LgA rats could be compensatory changes that occur in the survival rate of progenitors born during BrdU injection. The ratio of BrdU progenitors to mature BrdU cells demonstrates that protracted withdrawal after LgA methamphetamine increased the rate of survival of BrdU progenitors (Figure 5c and d). This withdrawal-specific effect in LgA rats is worth noting because this maladaptive change could function to return the homeostasis of granule cell neuron turnover, or incubate drug, and drug-context associations relating to drug seeking (Vorel *et al*, 2001; Hiranita *et al*, 2006; Rademacher *et al*, 2006; Shen *et al*, 2006; Zhou and Zhu, 2006; Lasseter *et al*, 2010; Noonan *et al*, 2010; Luo *et al*, 2011). However, further functional exploration of this putative enhanced survival is warranted, as well as identification of other neuroadaptations during protracted withdrawal that may regulate the hippocampus and hippocampus-dependent relapse behavior (Tani *et al*, 2001; Hiranita *et al*, 2006; Zhou and Zhu, 2006; Lasseter *et al*, 2010; Noonan *et al*, 2010; Garcia-Fuster *et al*, 2011).

We next determined whether relapse behaviors following noncontingent exposure to methamphetamine produces changes in dentate gyrus neural progenitors and whether these changes are altered compared with protracted withdrawal-induced changes. Methamphetamine seeking normalized Ki-67-proliferating progenitors in all methamphetamine groups (Figure 5e). The normalization of the protracted withdrawal-induced enhancement of Ki-67 can be hypothesized to be an immediate effect of methamphetamine priming because the regulation of Ki-67 is immediate following any exposure to a novel stimuli (Kee *et al*, 2002; Brown *et al*, 2003). It is also possible that increases in proliferation and survival in LgA rats were withdrawal-specific, and these changes might be altered in reinstated rats that experience learning and methamphetamine-seeking behavior during protracted withdrawal (Gould *et al*, 1999; Neisewander *et al*, 2000). Therefore, one minor limitation of the present results is the multiple test design. Inevitably, the fact that cellular analysis was conducted in the reinstatement group of rats that performed multiple behavioral tests (ie, cognitive followed by relapse) suggests that some caution is warranted.

Lastly, we examined whether methamphetamine-primed reinstatement of methamphetamine seeking differentially regulates neuronal activation in methamphetamine groups

with varied access in key brain regions that encompass the mesocorticolimbic dopamine system and compared the changes with protracted withdrawal. The mPFC, NAc core, and hippocampal regions were analyzed based on research from methamphetamine-seeking studies that demonstrated mechanistic and correlative evidence of the involvement of these brain regions in methamphetamine relapse (Hiranita *et al*, 2006, 2008). Specifically, the hippocampal dentate gyrus was also examined (Noonan *et al*, 2008, 2010; Garcia-Fuster *et al*, 2011). We determined whether Fos expression was altered because Fos, a product of the immediate early gene c-*fos*, is transiently induced by cocaine and amphetamines (Ruskin and Marshall, 1994; Neisewander *et al*, 2000; Ciccocioppo *et al*, 2001; Gross and Marshall, 2009). The present study demonstrated increases in Fos protein expression by methamphetamine-primed seeking in the mPFC and dentate gyrus, and these increases were related to methamphetamine-seeking behavior (Figure 6b). However, the present study failed to observe changes in Fos protein expression in the NAc core, supporting some studies that also did not observe changes in NAc Fos levels after exposure to cocaine-paired stimuli (Brown *et al*, 1992; Crawford *et al*, 1995). For example, previous studies reported that increases in Fos protein expression in the NAc after acute drug administration exhibit tolerance after repeated administration (Graybiel *et al*, 1990; Brown *et al*, 1992; Hope *et al*, 1992; Moratalla *et al*, 1996; Neisewander *et al*, 2000), suggesting that discrepancies in Fos expression may be attributable to differences in the amount or schedule of drug experience, drug dose, length of withdrawal period with or without drug-seeking behavior before testing, and anatomical subregion analyzed.

Altogether, the present study defined the novel and distinct impact of varied methamphetamine self-administration access and subsequent withdrawal and methamphetamine-seeking behavior during withdrawal on neural proliferation, survival, and neuronal activation of granule cell neurons. Future studies should seek to clearly understand intermittent *vs* extended access methamphetamine-induced alterations in the hippocampal neurogenic microenvironment. These studies may point to a critical and mechanistic role of the dentate gyrus neurogenesis in drug-induced memory impairment, drug craving, and ultimately relapse, perhaps via selective facilitation of recall of drug-context associations that stimulate relapse behaviors at the expense of recall of non drug memories.

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DISCLOSURE

The authors declare that, except for the income received from the primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service, and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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