

# Extended Access to Cocaine Self-Administration Produces Long-Lasting Prefrontal Cortex-Dependent Working Memory Impairments

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Humans with drug addiction exhibit compulsive drug-seeking associated with impairment of prefrontal cortex cognitive function. Whether prefrontal cortex dysfunction is a consequence of chronic drug exposure, or mediates the transition from drug use to drug dependence, is unknown. The current study investigates whether a history of escalated vs controlled cocaine intake is associated with specific working memory impairments, and long-lasting alterations of the dorsomedial prefrontal cortex and orbitofrontal cortex in rats. Working memory was assessed in rats with a history of extended (6 h per session) or limited (1 h per session) access to cocaine (0.5 mg/kg per injection), 3–17 days after the last self-administration session, using a delayed nonmatching-to-sample task. The density of neurons, oligodendrocytes, and astrocytes was quantified in the dorsomedial prefrontal cortex and orbitofrontal prefrontal cortex 2 months after the last self-administration session. Working memory impairments were observed after a history of chronic and escalated cocaine intake, but not after repeated limited access to cocaine. Moreover, working memory impairments were correlated with a decreased density of neurons and oligodendrocytes but not astrocytes in the dorsomedial prefrontal cortex, and with a decreased density of oligodendrocytes in the orbitofrontal cortex. Considering the role of the prefrontal cortex in goal-directed behavior, the prefrontal cortex dysfunctions observed here may exacerbate the loss of control associated with increased drug use and facilitate the progression to drug addiction.

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## INTRODUCTION

A central question in drug addiction is to understand the role of the prefrontal cortex in the addiction process (Bechara, 2005; Everitt and Robbins, 2005; Kalivas and Volkow, 2005; Koob and Le Moal, 2005). Because of the difficulty in conducting prospective longitudinal studies in humans, studies with animal models with robust validity for the transition from drug use to drug dependence are critical (Ahmed and Koob, 1998; Deroche-Gamonet *et al*, 2004; Vanderschuren and Everitt, 2004). Loss of control over drug use is a central feature of addiction that has been modeled in animals. It is characterized by unsuccessful efforts to stop responding for drug despite adverse consequences (Deroche-Gamonet *et al*, 2004; Vanderschuren and Everitt, 2004) and difficulty in limiting drug intake (Ahmed and Koob, 1998; Deroche-Gamonet *et al*, 2004). Specifically,

increased drug intake is observed in rats that are given extended access to cocaine self-administration, whereas it is not observed in rats with limited access (Ahmed and Koob, 1998).

Loss of control has been attributed to a dysfunction of the prefrontal cortex, based on neuroimaging studies in humans. However, there is little evidence to date of long-lasting alterations of prefrontal cortex function in an animal model of loss of control over drug use. Despite accumulating evidence that limited drug exposure induces neuronal changes in the prefrontal cortex (Ben-Shahar *et al*, 2007; Bowers *et al*, 2004; Crespo *et al*, 2002), there is little evidence to date of long-lasting neuronal adaptations of the prefrontal cortex after escalation in cocaine intake with extended access (Ben-Shahar *et al*, 2007; Ferrario *et al*, 2005; Seiwel *et al*, 2007). Moreover, recent reports demonstrated that extended access to cocaine self-administration did not induce long-lasting impairment of prefrontal cortex cognitive function, such as response inhibition and sustained attention (Dalley *et al*, 2005, 2007).

An alternative hypothesis is that extended access to cocaine instead produces deficits in other cognitive functions relevant to decision-making and mediated by the prefrontal cortex operating under high cognitive demand

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and high-incentive conditions. A condition with high cognitive demand in this context refers to an experimental paradigm where the cognitive processes necessary to solve a task reach their capacity limits, whereas a high-incentive condition refers to an experimental paradigm that motivates a high degree of approach behavior due to the high attractiveness of the positive reinforcer. These conditions may particularly challenge the dorsomedial prefrontal cortex (dmPFC) and orbitofrontal cortex (OFC). Indeed, the dmPFC maintains stimulus representation during a delay to allow motivationally based decision-making (Narayanan and Laubach, 2006; Sakurai and Sugimoto, 1986). The OFC is critical in guiding behavior by signaling outcome expectancy when representation of the value of the expected outcome needs to be compared to an alternative response or needs to be held in memory (Schoenbaum *et al*, 2006). Moreover, working memory under a high-incentive condition has been shown to be a sensitive measure of the integrity of the prefrontal cortex (Krawczyk *et al*, 2007; Taylor *et al*, 2004).

We thus investigated whether a history of escalated *vs* controlled cocaine intake is associated with specific working memory impairments and long-lasting alterations of the dmPFC and OFC in rats. To test these hypotheses, the integrity of working memory under high and low cognitive demands and high- and low-incentive conditions was assessed in rats with a history of extended or limited access to cocaine 3–17 days after the last self-administration session. Working memory under high incentive and cognitive demands was measured in food-restricted rats using a delayed nonmatching-to-sample procedure, a task sensitive to prefrontal cortical dysfunction (Divac, 1971; Brozoski *et al*, 1979; Jacobsen, 1936; Mishkin and Pribram, 1956; Simon *et al*, 1980; Aggleton *et al*, 1995; Walton *et al*, 2003). Specifically, the percentage of correct responses in this task is decreased after dmPFC lesion, particularly when a delay is used to increase the working memory load. Working memory performance under low-incentive conditions was measured in satiated rats using a novelty-induced alternation task (Gerlai, 1998, 2001; Delatour and Gisquet-Verrier, 1996; Lalonde, 2002), which is dependent on the integrity of the prefrontal cortex (Delatour and Gisquet-Verrier, 1996; Lalonde, 2002). In this task, alternation behavior is driven by a lower-incentive (novelty), instead of a highly palatable food reward. Two months after the last self-administration session, the density of the three main cellular components—neurons, oligodendrocytes, and astrocytes (Vaccarino *et al*, 2007)—was quantified in the dmPFC and OFC using immunohistochemical and stereological techniques.

## MATERIALS AND METHODS

### Animals

Thirty-one male Wistar rats (250–275 g) (Charles River, Hollister, CA) were used for all experiments. The animals were group-housed and maintained on a 12 h light/dark cycle with *ad libitum* access to food and water. All animal procedures were approved by The Scripps Research Institute Institutional Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines.

### Cocaine Self-Administration

Seventeen rats were implanted with a silastic catheter (0.3 mm inner diameter, 0.64 mm outer diameter; Dow Corning, Midland, MI) into the right external jugular vein under aseptic conditions. Methods on housing, operant boxes, and surgery have been previously described (Ahmed and Koob, 1998). After surgery and recovery, the rats were trained to self-administer 0.5 mg/kg per 100  $\mu$ l cocaine in 1 h sessions (baseline sessions) under a fixed-ratio 1 schedule for 6–20 sessions. Rats then were divided into two groups, balanced by the number of injections per session on the last baseline session. One group of rats (long-access, LgA,  $n = 7$ ) was allowed to self-administer cocaine in 6 h sessions, whereas the other group (short-access, ShA,  $n = 10$ ) was allowed to do so in 1 h sessions for a minimum of 85 sessions. Sessions were run 6–7 days per week. The LgA and ShA rats were used in a previous study examining the effect of the noradrenergic receptor ligands prazosin, UK14304, and betaxolol on the escalated rate of cocaine self-administration in rats (Wee *et al*, 2007). None of these compounds changed baseline cocaine intake in LgA and ShA rats, and rats were rebaselined for 19 days prior to beginning the present study (see Supplementary Materials and Methods for details). All rats maintained catheter patency throughout the course of the self-administration experiment; rats were not re-catheterized and were immediately excluded from the study when the patency of the catheter was not assured (6 out of 23 rats: 3 ShA and 3 LgA). An index of escalation was calculated as the average cocaine intake during the first hour of the last 30 sessions minus the cocaine intake during the first hour of the first session. Calculation of the index of escalation using different criteria (last 5, 10, or 20 sessions) led to identical results (see Supplementary Materials and Methods for more details).

### Behavioral Tasks

The delayed nonmatching-to-sample task and the novelty-induced alternation task were performed in the same apparatus. The T-maze was placed in the middle of a testing room rich in visual cues situated 50–200 cm from the maze. The walls of the T-maze were made of transparent acrylic and equipped with three removable guillotine doors. One door separated a 30 cm compartment at the beginning of the start arm. The other two doors were placed at the entrance of each goal arm and could be lowered to block entry. Dimensions of the maze were as follows: length of central stem, 60 cm; length of left/right arms, 50 cm; width, 15 cm; height, 25 cm; and length of start box, 30 cm. For a complete description of the behavioral methods, see Supplementary Information.

**Delayed nonmatching-to-sample task.** This task is sensitive to dmPFC lesion (Aggleton *et al*, 1995; Divac, 1971; Jacobsen, 1936; Mishkin and Pribram, 1956; Simon *et al*, 1980). Starting from day 4 after the last self-administration session, rats were food-deprived to 85% body weight and habituated to the T-maze until they readily ate a sucrose pellet placed at the end of each arm. After habituation, rats were trained for 10 trials/session on the delayed nonmatching-to-sample task. Each trial consists of one forced-choice

run (always rewarded and randomly chosen) and one free-choice run (rewarded only if the rat enters the opposite arm) without any delay between runs. Between runs, the maze was wiped with 30% alcohol to remove any olfactory clues. After reaching the acquisition criterion ( $>70\%$  correct responses during 2 consecutive days) a variable delay (10, 70, and 130 s) was introduced between runs, and the number of trials per session was increased to 16. Rats were tested in this protocol during 2–3 sessions corresponding to 15–17 days after the last self-administration session. The number of trials necessary to reach the acquisition criterion and the percentage of correct responses at the three different delays were calculated. Because working memory performance is affected by the delay and may mask the main effect of group (ShA, LgA vs Naive), we calculated an index that takes into account the delay-dependent decrease in working memory normally observed in naive rats. The index corresponds to the percentage change in ShA or LgA rats compared to naive rats at the 70 s delay ((ShA or LgA–Naive)/Naive  $\times$  100).

**Novelty-induced alternation task.** This task is sensitive to dmPFC lesion (Delaunoy and Gisquet-Verrier, 1996; Lalonde, 2002). Three days after the last self-administration session, spontaneous alternation was assessed in the same T-maze using a method described previously (Gerlai, 1998). In this task, alternation behavior is driven by a weak reinforcer (novelty) compared to the delayed nonmatching-to-sample condition where it is driven by a strong reinforcer (sucrose under a food-deprivation state). The procedure consists of 1 forced trial followed by 14 choice trials, and the percentage of correct responses is calculated by the number of spontaneous alternations between the two goal arms during the 14 choice trials.

### Immunohistochemistry

Neurons, astrocytes, and oligodendrocytes were measured using immunohistochemistry to test the hypothesis that working memory impairments were correlated with dmPFC and OFC abnormalities. Two months after the last self-administration session, subsets of randomly selected rats (ShA,  $n = 5$ ; LgA,  $n = 5$ ; Naive,  $n = 5$ ) were anesthetized with chloral hydrate and perfused transcardially as described previously (Mandyam *et al*, 2004). Brains were sectioned coronally on a freezing microtome (American Optical Corporation, Buffalo, NY) at  $40\mu\text{m}$  through the brain and stored in  $0.1\%$   $\text{NaN}_3$  in  $1 \times$  phosphate-buffered saline (PBS) at  $4^\circ\text{C}$ . The adult rat mPFC (Figure 2a), equivalent to the human dorsolateral prefrontal cortex, spans an area of  $3\text{ mm}^3$  bilaterally along the rostral-caudal levels of the rodent brain (Gabbott *et al*, 2005). Anatomically, the mPFC is clearly distinguished from other cortical areas in bregma regions 4.20–2.70 of the adult rodent brain (Kodama *et al*, 2004). The mPFC is further divided into anterior cingulate cortex (Cg), prelimbic cortex (PrL), and infralimbic cortex (IL) subregions (Stewart and Plenz, 2006). Every ninth section through the prefrontal cortex (bregma 4.20–2.70 mm;  $360\mu\text{m}$  apart) was slide-mounted, dried overnight, and coded before immunohistochemistry, and the code was not broken until after analysis was complete. The following primary antibodies were used: neurons (mouse monoclonal

anti-NeuN, 1:50; Chemicon no. MAB377, Temecula, CA; Kempermann *et al*, 2003); astrocytes (rabbit polyclonal anti-GFAP, 1:500; Dako no. Z0334, Carpinteria, CA; Seri *et al*, 2001); and oligodendrocytes (rabbit polyclonal anti-NG2, 1:250; Chemicon no. AB5320; Dawson *et al*, 2000). Slide-mounted sections were subjected to an antigen unmasking and denaturation pretreatment step as described previously (Mandyam *et al*, 2004). Slides were incubated with  $0.3\%$   $\text{H}_2\text{O}_2$  for 30 min to remove any endogenous peroxidase activity. Nonspecific binding was blocked with  $5\%$  serum and  $0.5\%$  Triton X in  $1 \times$  PBS for 60 min and incubated with the primary antibody in  $5\%$  serum and  $0.5\%$  Tween 20 for 48 h at  $4^\circ\text{C}$ . After washing with  $1 \times$  PBS, the sections were exposed to biotinylated secondary IgG for 1 h (1:200, Vector Laboratories, Burlingame, CA). After secondary antibody incubation, slides were incubated in ABC for 1 h (catalog no. PK-6100, Vector Laboratories), and staining was visualized with DAB (catalog no. 34065, Pierce Laboratories, Rockford, IL). Sections were counterstained with Fast Red (Vector Laboratories). Omission or dilution of the primary antibody resulted in lack of specific staining, thus serving as a negative control for antibody experiments. DAB staining of the coded slides was visualized and quantified with a Zeiss Axiophot photomicroscope. Immunoreactive cells from the left and right hemispheres of bregma (4.70–4.20 for the OFC and 3.70–2.70 for Cg, PrL, and IL cortices) that were localized in the counting frame ( $0.078\text{ mm}^2$ ) were counted and represented as number of neurons per  $\text{mm}^2$ . To evaluate the accuracy of the counting technique, the density of neurons in the mPFC was counted by two blind experimenters. There was a strong correlation between the two experimenters ( $R = 0.97$ ,  $p < 0.05$ ) demonstrating the robustness of the results.

### Statistical Analysis

Results were analyzed with SPSS software using an analysis of variance (ANOVA) or Student's *t*-test. Normality, equal variance, and sphericity were tested before ANOVA to ensure validity. For the cocaine intake data, a multivariate analysis was used instead of an ANOVA because the assumption of sphericity was violated, and the within-subjects factor contained more than two levels (Cole and Grizzle, 1966; Maxwell and Delaney, 1990). The three groups (Naive, ShA, and LgA) were used as between-subjects factors and the three delays (10, 70, and 130 s) or self-administration sessions as within-subjects factors. *Post hoc* Newman–Keuls and Pearson's correlation tests were used when necessary. Student's *t*-tests were used to compare the behavioral performance to chance. The level of significance was set at  $p < 0.05$ . Data are shown as mean  $\pm$  SEM.

## RESULTS

### Extended, but not Limited Access to Cocaine Induces Working Memory Impairments Under High-Incentive Conditions

Extended access to cocaine self-administration produced a progressive escalation of drug intake that was not observed with limited access ( $F_{4,60} = 4.2$ ,  $p < 0.01$ ; Figure 1a). LgA rats exhibited increased cocaine intake during the first hour of

each session, starting from the second week of self-administration that remained stable until the end of the experiment. The index of escalation (difference in cocaine intake between the last 30 sessions and the first session) also was higher in LgA than in ShA rats ( $t_{15} = -2.15$ ,  $p < 0.05$ ; Figure 1b). Unequal blocks of data were used in Figure 1 to represent the most important phases of the self-administration study (baseline, acquisition of escalation, stabilization of escalation). Calculation of the index of escalation using different criteria (last 5, 10, or 20 sessions) led to identical results (see Supplementary Materials and Methods for details).

In the delayed nonmatching-to-sample task, a multivariate analysis revealed a Delay  $\times$  Group interaction ( $F_{2,28} = 4.6$ ,  $p < 0.05$ ; Figure 2a). LgA and ShA rats exhibited normal working memory performance compared to naive rats when a short delay (10 s) between acquisition trials and test trials was used (all  $p$ 's  $< 0.05$  vs chance; Figure 2a). However, increasing the delay from 10 to 70 and 130 s

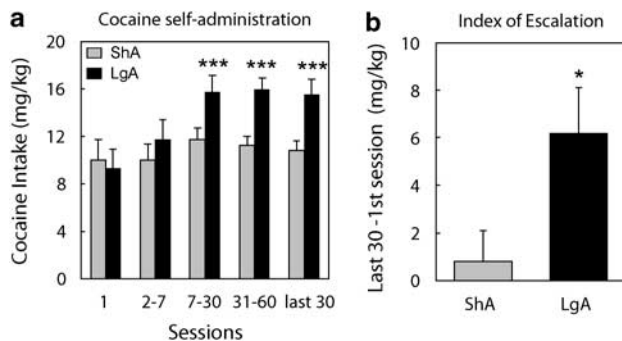
revealed a robust deficit in LgA rats (Group effect at 70 and 130 s delay:  $F_{2,28} = 4.0$ ,  $p < 0.05$ ). The working memory impairments observed at the 70 s delay were still significant after controlling for the effect of delay in naive rats (Group effect:  $F_{2,28} = 5.9$ ,  $p < 0.01$ ; Figure 2b). Furthermore, working memory performance in ShA and LgA rats with the 70 and 130 s delay was negatively correlated with the index of escalation of cocaine intake ( $r = -0.74$ ,  $p < 0.001$  and  $r = -0.64$ ,  $p < 0.01$ , respectively; Figure 2c). In contrast, the index of escalation was not correlated with working memory performance with the 10 s delay ( $r = 0.01$ , NS (not significant); Figure 2c) or with baseline performance during training ( $r = -0.12$ , NS, data not shown).

To test working memory under low-incentive conditions, the three groups were tested in a novelty-induced alternation task. The three groups exhibited a lower percentage of alternation behavior compared to the previous paradigm ( $67 \pm 3$  vs  $90 \pm 2\%$ ,  $t_{27} = 6.7$ ,  $p < 0.001$ ), confirming the lower incentive value of novelty. LgA rats exhibited similar performance compared to ShA and naive rats ( $F_{2,28} = 0.2$  NS; Figure 2d), suggesting that LgA rats were not impaired under low-incentive conditions. Moreover, the percentage of correct responses in this task was not correlated with the index of escalation ( $r = 0.09$ , NS, data not shown).

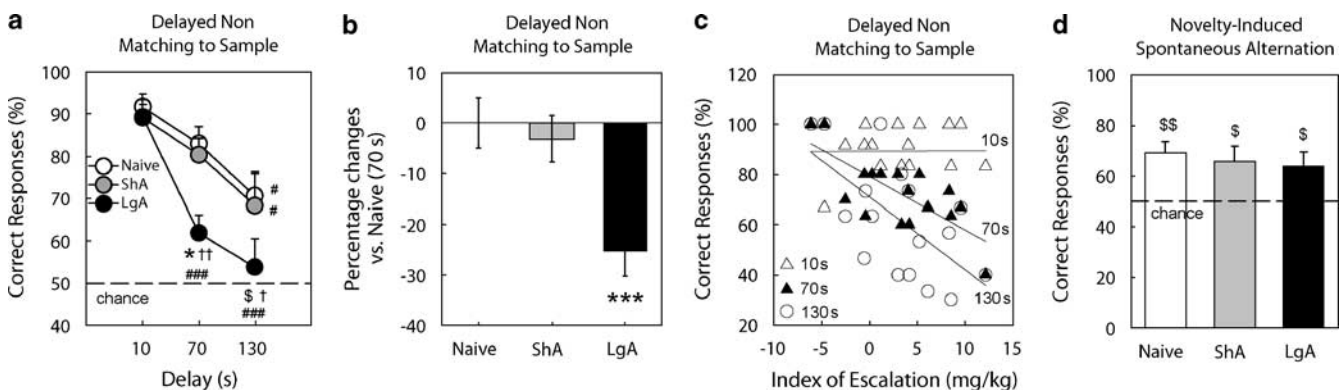
### Extended Access to Cocaine does not Impair General Cognitive Abilities

Working memory impairments observed in LgA rats under high working memory load (Figure 2b) were not due to general cognitive impairments or impairments of other cognitive functions necessary to perform the task, such as sensation, perception, rule learning, behavioral selection, and action–outcome association. Indeed, the three groups learned the delayed nonmatching-to-sample task at a similar rate ( $F_{2,28} = 0.08$ , NS; Figure 3a) and exhibited similar performance with the 10 s delay (Figure 2a).

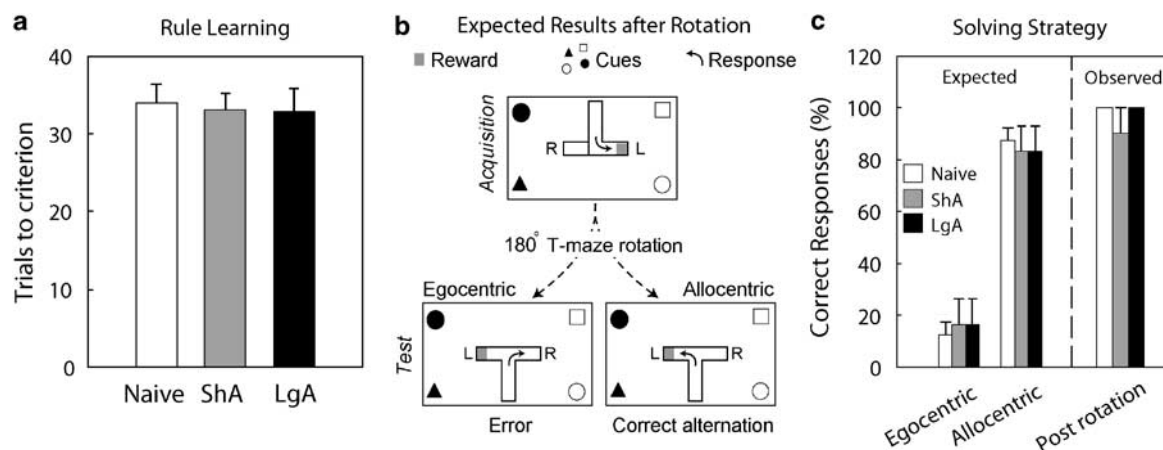
An alternative explanation for the decreased working memory performance in LgA rats is that they used a different navigational strategy to solve the task. Egocentric strategies (ie orientation in space using proprioceptive



**Figure 1** (a) Effect of long-access (LgA) vs short-access (ShA) to cocaine on cocaine intake during the first hour of self-administration. \*\*\* $p < 0.001$  vs first session. Sessions 1–14 are also represented in this figure of Wee et al (2007). (b) Index of escalation of cocaine intake in ShA and LgA rats, calculated by subtracting the cocaine intake during the first session from the average cocaine intake during the last 30 sessions of self-administration (only the intake during the first hour of each session was used in the calculation). \* $p < 0.05$  vs ShA.



**Figure 2** (a) Percentage of correct responses in the delayed nonmatching-to-sample task. # $p < 0.05$  and ### $p < 0.001$  vs 10 s; \* $p < 0.05$  vs short-access (ShA); † $p < 0.05$  and †† $p < 0.01$  vs naive; ‡NS vs chance. (b) Working memory impairments estimated after controlling for the effect of delay in naive rats. Percentage changes vs naive rats at the 70 s delay. \*\*\* $p < 0.001$  vs ShA and naive. (c) Correlation between the index of escalation (Figure 1b) and the percentage of correct response at the 10 s delay ( $r = 0.01$ , NS), 70 s delay ( $r = -0.74$ ,  $p < 0.001$ ), and 130 s delay ( $r = -0.64$ ,  $p < 0.01$ ). Lines represent linear regression at 10, 70, and 130 s. (d) Percentage of correct responses in the novelty-induced spontaneous alternation task (§ $p < 0.05$  and §§ $p < 0.01$  vs chance). Data represent mean  $\pm$  SEM.



**Figure 3** (a) Total number of trials to reach the criterion in the delayed nonmatching-to-sample task. (b) Illustration of the two possible navigational strategies used to solve the delayed nonmatching-to-sample task. (c) 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. Note that the three groups used the same allocentric strategy. Data represents mean  $\pm$  SEM.

information or intra-maze cues; Figure 3b) are more sensitive to prefrontal cortex lesion than allocentric strategies (ie orientation in space using extra-maze cues in the environment; Figure 3b; King and Corwin, 1992; Nieto-Escamez *et al*, 2002). To test this hypothesis, we evaluated the effect of a 180° rotation of the T-maze between acquisition and test trials 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 to the distal cues in the room that were left unchanged (Figure 3b). In this case, an allocentric strategy (use of distal cues) would lead to good performance (now defined as an entrance into the same arm), whereas an egocentric strategy (ie use of intra-maze cues and proprioceptive information) would lead to poor performance (Figure 3c, left panel). After the rotation, all groups exhibited more than 90% correct responses in accordance with an allocentric strategy ( $F_{2,28} = 1.0$ , NS) (Figure 3c, right panel), ruling out the hypothesis that a differential navigational strategy could explain the working memory impairments observed in LgA rats.

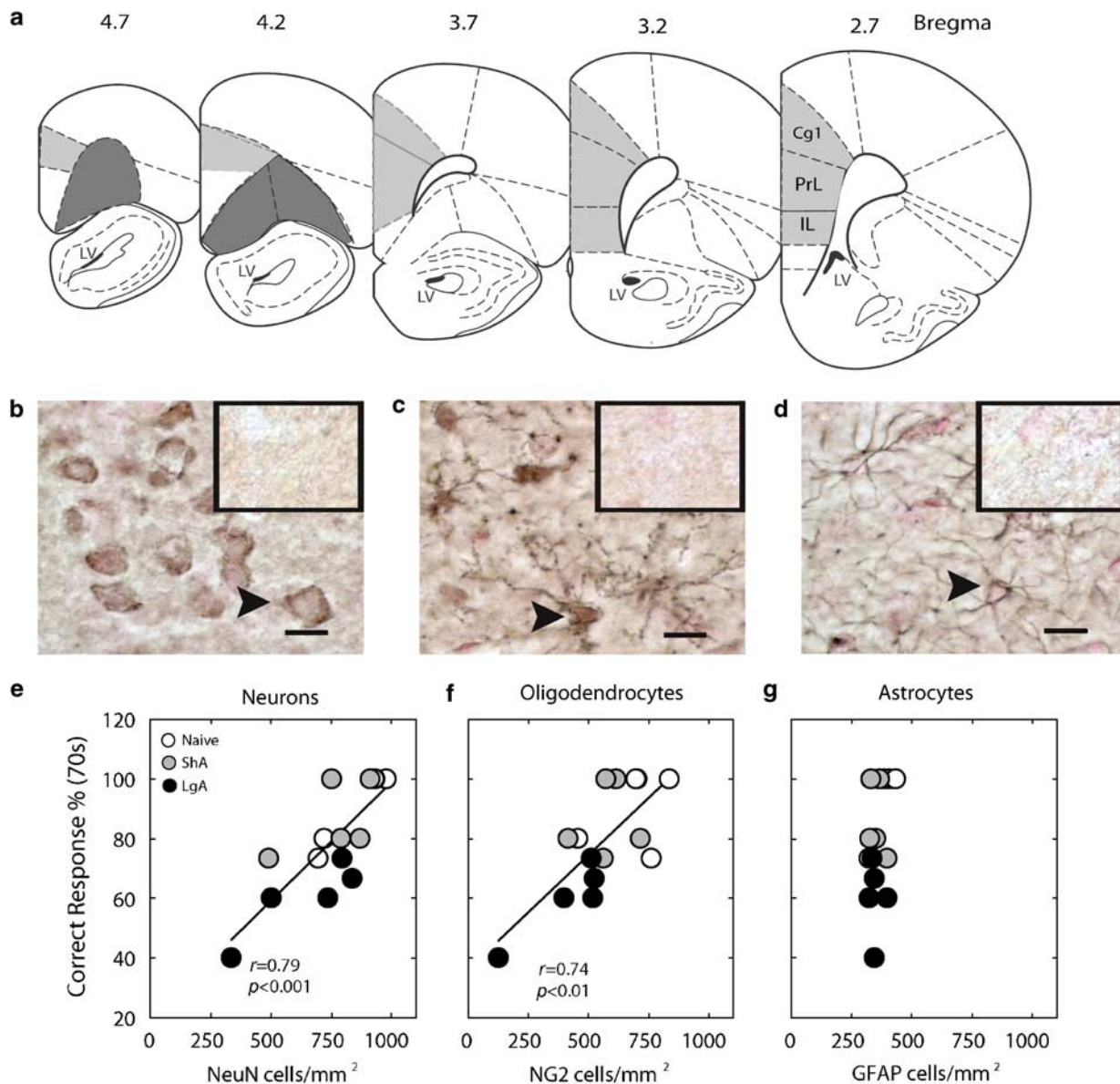
### Prefrontal Cortex Alterations Predict Working Memory Impairments

The density of neurons (NeuN immunoreactive cells; Figure 4b) and oligodendrocytes (NG2 immunoreactive cells; Figure 4c) in the dmPFC was positively correlated with working memory performance (NeuN:  $r = 0.79$ ,  $p < 0.001$ , Figure 4d; NG2:  $r = 0.74$ ,  $p < 0.001$ , Figure 4e). Working memory performance also was correlated with oligodendrocyte density in the OFC ( $r = 0.54$ ,  $p < 0.05$ , data not shown). No correlation was found between working memory performance and neuronal density in the OFC ( $r = 0.00$ , NS). Finally, the density of astrocytes (glial fibrillary acidic protein-positive cells; Figure 4d) in the dmPFC (Figure 4f), and the OFC (data not shown) was similar in the three groups and was not correlated with working memory performance (dmPFC:  $r = 0.39$ , NS; OFC:  $r = -0.42$ , NS).

### DISCUSSION

The present study demonstrates that extended access to cocaine self-administration induces an escalated pattern of cocaine intake associated with an impairment of working memory and a decrease in the density of dmPFC neurons that lasts for months after cocaine cessation. LgA and ShA rats exhibited a high percentage of correct responses in the delayed nonmatching-to-sample task under low cognitive demand (delay  $< 10$  s). LgA and ShA rats refrained from visiting the previously reinforced arm and chose the opposite arm to obtain a reward, demonstrating apparently intact executive control function. However, increasing the working memory load (ie close to the capacity limit of working memory), by increasing the delay from 10 to 70 and 130 s, revealed a robust working memory deficit in LgA rats. Furthermore, the magnitude of escalation of cocaine intake was negatively correlated with working memory performance in ShA and LgA rats with the 70 and 130 s delays, but not with the 10 s delay or with baseline performance during training, demonstrating that the relationship between escalation of cocaine intake and behavioral performance in this task was restricted to working memory performance under high cognitive demand.

Moreover, when tested under a low-incentive condition (ie using novelty instead of highly palatable food as a reinforcer), no difference was observed between LgA, ShA, and naive rats, suggesting that high incentive and high cognitive demand are required to reveal working memory impairments in LgA rats. Also, the working memory impairments observed in LgA rats cannot be explained by use of a different navigational strategy used to solve the task (egocentric vs allocentric). Behavioral performance after rotation of the T-maze demonstrated that all three groups used an allocentric strategy to solve the task. Thus, extended access to cocaine self-administration induced long-lasting impairments of working memory under high cognitive demand and high-incentive conditions that can be predicted by the increase in cocaine intake.



**Figure 4** (a) Schematic of rat brain coronal sections (bregma 4.7–2.7) showing areas of the dmPFC (shaded in light gray; Cg1, anterior cingulate; PrL, prelimbic; IL, infralimbic) and OFC (shaded in dark gray), lateral ventricle (LV) (schematic adopted from Paxinos and Watson, 1997). (b–d main panel) Qualitative representative images of (b) NeuN-positive neurons, (c) NG2-positive oligodendrocytes, and (d) glial fibrillary acidic protein-positive astrocytes from one control drug-naïve rat. The arrowhead in (b)–(d) (main panel) points to an immunoreactive cell. (b–d, inset) Negative control images obtained after omitting the primary antibody. Scale bar = 10 μm. (e–g) Correlation between the number of (e) neurons, (f) oligodendrocytes, and (g) astrocytes in the mPFC and working memory performance with the 70 s delay ( $n = 15$ , Pearson's correlation).

The cognitive dysfunctions observed in LgA rats under a high, but not low, incentive condition, and in humans with cocaine addiction (Hester and Garavan, 2004), may result from an imbalance between a hypoactive cognitive system that controls decision-making under a high cognitive demand situation and an overactive incentive salience system (Bechara, 2005). The working memory impairment in this task also may be explained by an increase of perseveration or compulsion, a prominent feature of prefrontal cortex lesions (Brush *et al*, 1961; Mishkin, 1964; Pribram, 1961).

The density of neurons and oligodendrocytes in the dmPFC was positively correlated with working memory

performance. The lower the density of neurons or oligodendrocytes in the dmPFC, the more severe the working memory impairment. Working memory also was correlated with the density of oligodendrocytes in the OFC, suggesting that OFC alteration after escalated drug intake may play a role in working memory deficits. However, no correlation was found between working memory performance and neuronal density in the OFC, suggesting that OFC neurons may be less vulnerable to the deleterious effects of chronic cocaine exposure than dmPFC neurons. Finally, it is unlikely that the decrease of neurons and oligodendrocytes in the prefrontal cortex resulted from a global decrease of prefrontal cortex volume. The density of

astrocytes in the dmPFC and OFC was similar in the three groups and was not correlated with working memory performance.

The correlations between working memory and density of neurons and oligodendrocytes in the dmPFC confirm reports showing that lesions of the dmPFC affect performance in the delayed nonmatching-to-sample task, particularly when longer delays are used to increase working memory load (Aggleton *et al*, 1995; Walton *et al*, 2003). The role of the OFC in the delayed nonmatching-to-sample task has been less studied and definitive conclusions cannot be drawn, but our results show that the density of oligodendrocytes in the OFC was correlated with working memory performance, suggesting that the OFC also may be involved in this task.

Considering that cocaine withdrawal is associated with low dopaminergic tone in the dmPFC (Sorg *et al*, 1997; Williams and Steketee, 2005), and that low dopaminergic tone in the dmPFC induces working memory impairments in this task (Mizoguchi *et al*, 2000; Simon *et al*, 1980), it is likely that the working memory impairments observed in LgA rats resulted from both decreased dopaminergic tone and a decreased number of neurons/oligodendrocytes in the dmPFC. Similar neural changes may be responsible for the cognitive impairments observed in humans with drug addiction (Goldstein and Volkow, 2002; Pfefferbaum *et al*, 1998; Bechara, 2005; Franklin *et al*, 2002; Jentsch and Taylor, 1999; Rogers and Robbins, 2001).

There is accumulating evidence that repeated passive exposure to psychostimulants leads to deficits in tasks that depend on intact prefrontal cortex function (Fletcher *et al*, 2005, 2007; Roesch *et al*, 2007; Schoenbaum *et al*, 2004; Schoenbaum and Setlow, 2005). Moreover, withdrawal from repeated passive cocaine or amphetamine treatments also decreases firing rate and bursting activity of dmPFC neurons (Nogueira *et al*, 2006; Homayoun and Moghaddam, 2007) and abolishes dopamine D<sub>2</sub> receptor-mediated regulation of dmPFC excitability (Nogueira *et al*, 2006) and membrane bistability of dmPFC neurons (Trantham *et al*, 2002). These reports suggest that chronic cocaine self-administration, even with limited access, may impair behavioral performance in prefrontal cortex-dependent tasks. Surprisingly, we did not find any working memory impairments in ShA rats, suggesting that the prefrontal cortex dysfunctions observed in previous studies after passive administration of cocaine may not adequately model the neuroadaptations occurring during chronic self-administration, and that alterations of prefrontal cortex function after cocaine use are not inevitable but may be observed only when subjects exhibit the increased drug intake associated with extended access.

Cognitive dysfunctions observed in humans with drug addiction may also result from preexisting abnormalities of the prefrontal cortex. Indeed, extended access to cocaine self-administration in rats does not induce long-lasting impairments of cognitive function known to depend on the prefrontal cortex, such as response inhibition and sustained attention (Dalley *et al*, 2005, 2007), whereas trait impulsivity precedes the onset of drug use and facilitates the progression to drug addiction (Dalley *et al*, 2007). However, our results demonstrate that independent of any premorbid condition (because rats were randomly assigned to the three

groups), extended access to cocaine self-administration by itself may cause severe working memory impairments associated with prefrontal cortex damage, suggesting that a significant contribution to prefrontal cortex dysfunction also may be a consequence of chronic drug use.

In summary, this study demonstrates that extended access to cocaine self-administration induced an escalated pattern of cocaine intake associated with long-lasting damage to the prefrontal cortex. Considering the role of the prefrontal cortex in goal-directed behavior, particularly with regard to mediating delays in reinforcement, prefrontal cortex dysfunction may decrease the ability to self-regulate (reflecting a loss of control), thus contributing to the compulsive nature of the addiction process, and reflect—and possibly be responsible for—the transition from drug use to drug dependence.

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## DISCLOSURE/CONFLICT OF INTEREST

The authors declare that, except for 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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