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Upregulation of *Arc* mRNA Expression in the Prefrontal Cortex Following Cue-Induced Reinstatement of Extinguished Cocaine-Seeking Behavior

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Abstract

Cocaine-associated cues acquire incentive motivational effects that manifest as cue-elicited craving in humans and cocaine-seeking behavior in rats. Here we examine the hypothesis that neuronal processes associated with incentive motivational effects of cocaine cues involve increased expression of the plasticity-associated gene, *Arc*. Rats trained to self-administer cocaine subsequently underwent extinction training, during which cocaine-seeking behavior (i.e., responses without cocaine reinforcement) progressively decreased. Rats were then tested for cocaine-seeking behavior either with or without response-contingent presentations of light/tone cues that had been previously paired with cocaine infusions during self-administration training. Cues elicited reinstatement of cocaine-seeking behavior and were accompanied by increased *Arc* mRNA levels in the orbitofrontal, prelimbic, and anterior cingulate cortices, suggesting *Arc* involvement in conditioned plasticity associated with incentive motivational effects of cocaine cues. Additionally, rats with a history of cocaine self-administration and extinction exhibited upregulation of *Arc* expression in several limbic and cortical regions relative to saline-yoked controls regardless of cue exposure condition, suggesting persistent neuroadaptations involving *Arc* within these regions.

Keywords

drug craving; drug conditioning; immediate early gene; incentive motivation; addiction

INTRODUCTION

In humans, relapse often involves intense craving for drug (O'Brien et al., 1993). Craving can be elicited by cues previously associated with the drug, including stimuli present during drug procurement or consumption (Childress et al., 1993; Ehrman et al., 1992; Sinha et al., 2000). In animals, exposure to cocaine-associated cues can reinstate extinguished operant responding previously reinforced with cocaine (Shaham et al., 2003; Shalev et al., 2002). Responding in the absence of cocaine reinforcement is referred to as cocaine-seeking behavior and its reinstatement by exposure to drug-associated cues is thought to reflect the incentive motivational effects of these stimuli (de Wit and Stewart, 1981).

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Several lines of evidence identify a limbic-cortical circuitry involved in cue-elicited cocaine-seeking behavior. For instance, research using expression of the immediate early gene (IEG) product Fos, as a marker of neuronal activation (Herrera and Robertson, 1996; Sharp et al., 1993), implicates a neuronal circuit comprising the nucleus accumbens (NAc), prefrontal cortex, hippocampal formation, and amygdala in the incentive motivational effects of cocaine-associated cues (Ciccocioppo et al., 2001; Crawford et al., 1995; Franklin and Druhan, 2000; Neisewander et al., 2000). Moreover, excitotoxic lesions or reversible pharmacological inactivation within these regions also supports a role of a limbic-cortical circuit in cueinduced reinstatement of cocaine-seeking behavior (Di Pietro et al., 2006; Fuchs et al., 2002, 2004a; Kantak et al., 2002; McLaughlin and See, 2003; Meil and See, 1997). A current research thrust is to identify cellular and molecular changes within these circuits, particularly as they relate to learning and memory mechanisms, given that drug addiction likely involves aberrant neuroadaptations in neuronal processing related to learning and memory (Hyman et al., 2006; Kelley, 2004; Nestler, 2002; White, 1996; Wolf et al., 2004).

Arc, also known as Arg3.1 (Link et al., 1995), is an effector IEG that is considered critical for activity-dependent plasticity and learning and memory (Guzowski, 2002; Tzingounis and Nicoll, 2006). *Arc* is rapidly and transiently transcribed by neuronal activity (Guzowski, 2002; Lanahan and Worley, 1998) and is unique among IEGs because it is primarily expressed in neurons (Rao et al., 2006; Vazdarjanova et al., 2006, but see Rodriguez et al., 2005) and is transported and localized in dendrites that receive active synaptic stimulation (Dynes and Steward, 2007; Lyford et al., 1995; Moga et al., 2004; Steward et al., 1998; Steward and Worley, 2001). Inhibiting *Arc* expression in the hippocampus attenuates long-term potentiation, a presumed correlate of learning and memory (Bliss and Collingridge, 1993; Martinez and Derrick, 1996), as well as long-term memory consolidation for spatial and inhibitory avoidance learning (Guzowski et al., 2000; McIntyre et al., 2005). Consistent with these findings, *Arc* knockout mice also exhibit long-term memory deficits for contextual and cued fear conditioning, conditioned taste aversion, and novel object recognition (Plath et al., 2006). Importantly, *Arc* induction is not thought to result from an overall response to stress, motor, or novelty processing, but instead is considered to be involved in experience-dependent learning and memory (Guzowski et al., 1999, 2001).

The potential involvement of *Arc* within circuits activated by cocaine-associated cues has not been investigated. We hypothesized *Arc* is induced in brain regions known to play a key role in the incentive motivational effects of cocaine associated cues. To examine this hypothesis, we tested rats for cue-induced reinstatement of extinguished cocaine-seeking behavior and then measured the expression of *Arc* mRNA in limbic and cortical regions.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats weighing 225–250 g at the start of the experiment were housed individually in climate-controlled colony rooms under a 12-h reverse light/dark cycle (lights off at 6:00 am). Care of the animals was in accordance with the conditions set forth in the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources Commission on Life Sciences, National Research Council, 1996).

Surgery

Catheters were implanted i.v. under sodium pentobarbital anesthesia (50 mg/kg, i.p., Abbott Laboratories, Chicago, IL) with atropine sulfate (10 mg/kg, i.p.; Sigma, St. Louis, MO) pretreatment to facilitate respiration. The catheters were inserted subcutaneously along the neck, exited through an incision across the skull, and were secured to the top of the skull using

dental acrylic and anchor screws as described by Zavala et al. (2007). Throughout the experiment, catheter patency was maintained by flushing daily with 0.1 ml bacteriostatic saline containing heparin sodium (10 U/ml; Elkins-Sinn, Cherry Hill, NJ), streptokinase (0.67 mg/ml; Astra USA, Westerborough, MA), and ticarcillin disodium (SmithKline Beecham Pharmaceuticals, Philadelphia, PA). Catheter patency was confirmed periodically by infusions of the rapid, short-acting anesthetic methohexital sodium (16.67 mg/ml, i.v.), which produces brief loss of muscle tone only when administered i.v.

Self-administration training

After 5–7 days of recovery from surgery, rats were randomly divided into groups that were either trained to press a lever reinforced by cocaine infusions (0.75 mg/kg/0.1 ml, i.v.; Cocaine group; $n = 15$) or received an equal volume of saline (Control group; $n = 12$) yoked to the schedule completions made by a rat in the Cocaine group. Training sessions occurred daily for 2 h at the same time of day across 23 consecutive days in operant conditioning chambers equipped with two levers mounted on the front wall, a cue light above one lever, a tone generator (500 Hz, 10 dB above background), and a house light mounted on the center of the back wall. The lever with the cue light was designated as the active lever and the other as the inactive lever. Each chamber was contained inside a sound attenuating chamber. Schedule completions by a cocaine rat on the active lever simultaneously activated the cue light, house light, and tone, followed 1 s later by a cocaine infusion. Control yoked rats were simultaneously presented the same stimulus complex when they received the saline infusion (0.1 ml, i.v.) contingent upon responses of their cocaine rat counterpart. Upon completion of the 6-s infusion, the cue light, tone, and infusion pump were inactivated simultaneously. The house light remained on for a 20-s timeout period during which lever presses had no scheduled consequences. Responses on the inactive lever were recorded but produced no scheduled consequences. Rats progressed from a fixed ratio (FR) 1 to a FR 11 schedule of reinforcement. A partial reinforcement schedule was chosen instead of a continuous reinforcement schedule, because it engenders more robust responding during reinstatement (Acosta et al., in press; Valles et al., 2006). Lever presses by control rats had no programmed consequences. Two days prior to self-administration training, rats were restricted to 18 g of rat chow/day to facilitate acquisition of cocaine self-administration (Carroll et al., 1981). The rats remained food-restricted until a criterion of seven cocaine infusions/h was achieved on two consecutive days, after which they were given food ad libitum for the remainder of the experiment. Control rats received the same food restriction regimen as their cocaine rat counterpart.

Extinction training

Extinction training began the day after self-administration training was completed and involved 14 daily 60-min sessions similar to self-administration sessions, except that lever presses produced no scheduled consequences. Extinction training was designed to decrease the incentive motivational and conditioned reinforcing effects of the self-administration environment in the Cocaine group. Response rate on the last extinction session was used as a baseline for assessing cue-induced reinstatement.

Cue-induced reinstatement

Prior to being tested for cue-induced reinstatement, rats were further assigned to cues or no cues conditions, counterbalanced for total number of cocaine infusions obtained during self-administration and for extinction baseline responding. Control rats were assigned to the same condition as their cocaine rat counterpart. Rats in the Cocaine-Cues group ($n = 8$) received response-contingent presentations of the stimulus complex paired previously with cocaine infusions on an FR 1 schedule during a 30-min test. This schedule was chosen because cue-induced reinstatement of extinguished responding is more robust when cues are presented on

an FR 1 schedule of reinforcement relative to a partial reinforcement schedule (Acosta et al., in press). Rats in the Saline-Cues group ($n = 6$) were simultaneously presented with the same stimulus complex contingent upon the responses of their cocaine rat counterpart. The infusion pumps were activated during the test, however, no infusions were delivered. Lever presses of rats in the Cocaine-No cues ($n = 7$) and Saline-No cues ($n = 6$) groups produced no scheduled consequences during the test session. Cocaine-seeking behavior was operationally defined as responses on the active lever in the absence of cocaine reinforcement.

Tissue preparation

Immediately after the 30-min behavioral test, rats were decapitated and their brains were removed and immediately frozen in 2-methylbutane (−30°C) and stored at −80°C. This time frame was chosen because previous results show robust *Arc* mRNA expression in rats within 30-min of being briefly exposed (5-min) to a novel environment (Guzowski et al., 1999). Sections were cut at 20 μm from different levels corresponding to +3.2, +1.6, −2.56, and −5.8 mm from bregma (Paxinos and Watson, 1998) and thaw mounted on ProbeOn Plus precleaned slides. They were placed in 4% formaldehyde for 60 min at +4°C, rinsed three times in 1× phosphate buffered saline for 5 min and dehydrated in ascending alcohols ending with 95%. Slides were air-dried completely and again stored at −80°C until processed for in situ hybridization histochemistry.

In situ hybridization histochemistry

Slides were placed at −20°C for 30 min, dried on a warming plate and placed in proteinase K solution (100 mM Tris HCl, 50 mM ethylenediaminetetraacetic acid (EDTA)/pH to 8.0) for 10 min at 37°C. Slides were then rinsed once with diethylpyrocarbonate water and then treated with a solution of 0.1 M triethanolamine and 400:1 triethanolamine:acetic anhydride for 15 min at room temperature. Next, slides were rinsed in 2× sodium chloride/sodium citrate (SSC) at room temperature for 5 min, dehydrated in ascending alcohol, and air-dried. Sections were hybridized with a [^{33}P]UTP-labeled riboprobe 3.0 kb *Arc* (GenBank accession number U19866) kindly provided by Dr. Paul F. Worley (Johns Hopkins University, Baltimore, MD). A sense riboprobe was also generated in order to compare labeling to the antisense probe. Probes were transcribed and diluted in hybridization buffer [78.5% formamide, 52 mM Tris (pH 7.8), 3× SSC, 1× Denhardt's, 26 mM dithiothritol, 2.6 mM EDTA, 0.2 mg/ml yeast tRNA, 0.2 mg/ml salmon testes DNA, and 10% dextran sulfate] and applied to a final concentration of 3.2×10^6 cpm/slide. Following overnight hybridization at 55°C, slides were rinsed in 2× SSC for 15 min at room temperature and then treated with RNase A solution (100 mM Tris, 0.5 M NaCl, and 200 μg/ml RNase A) for 1 h at 37°C. Slides were then rinsed in 2× SSC for 10 min at room temperature, 1× SSC for 10 min at RT, 0.5× SSC for 1 h at 55°C, and ending with 0.5× SSC for 10 min at room temperature. Slides were again dehydrated in alcohol, air-dried overnight, and apposed to Kodak BioMax film for 25–30 days.

Densitometry analysis

Autoradiographs of brain sections were digitally captured using a Nikon camera (model XC-ST70). *Arc* mRNA was analyzed by examining the optical density of the films in a defined area from four sequential sections using MCID 7.0 imaging software (Interfocus Imaging, Linton, UK). Optical density values were converted to dpm/cm^2 using ^{14}C standards calibrated for ^{33}P and then converted into femtomole of probe per gram of wet weight using specific activity of the probe. Figure 1 illustrates the specific brain regions identified for densitometry analysis according to Paxinos and Watson (1998). Sections taken at +3.2 mm from bregma contained the Cg1 region of the anterior cingulate cortex (Cg1), prelimbic (PrL), infralimbic (IL), orbitofrontal (OF), and agranular insular (AgI) cortices; sections taken at +1.6 mm from bregma contained the Cg2 region of the anterior cingulate cortex (Cg2), caudate-putamen

(CPu), nucleus accumbens shell (NAcs), and nucleus accumbens core (NAcc); sections taken at −2.56 mm from bregma contained the hippocampal CA1 and CA2, central amygdala (CeA), basolateral amygdala (BlA), and lateral amygdala (LA); sections taken at −5.8 mm from bregma contained substantia nigra pars reticulata, substantia nigra pars compacta, ventral tegmental area, hippocampal CA3, dentate gyrus (DG), subiculum (Sub), and entorhinal cortex (Ent).

Statistical analysis

Data were analyzed by ANOVAs with drug history (Saline vs. Cocaine) and testing condition (No Cues vs. Cues) as between subjects variables, and day (Baseline vs. Test) and extinction days (days 1–14) as a repeated measures for behavioral measures. Sources of main effects and interactions were further analyzed using posthoc Tukey tests. Additionally, planned comparisons of *Arc* mRNA density in regions of the cortex, NAc, hippocampus, and amygdala were used to test our hypothesis that cocaine cues induce *Arc* expression based on prior research, demonstrating cue-induced increases in IEG expression in these regions (Neisewander et al., 2000; Zavala et al., 2007). Specifically, comparisons were made between the Cocaine-Cues group relative to the Cocaine-No cues and Saline-Cues groups. In addition, correlations between lever presses on the test day and the density of *Arc* mRNA labeling, as well as correlations between total cocaine (mg) intake and the density of *Arc* mRNA labeling were determined using the Pearson product-moment correlation (*r*) in brain regions where significant effects were found. All descriptive statistics are presented as mean ± SEM.

RESULTS

Self-administration

Total cocaine intake during the 23 days of self-administration training by rats in the Cocaine-No cues and Cocaine-Cues groups was 282.85 ± 33.15 and 262.21 ± 26.36 mg/kg, respectively. Cocaine intake did not vary among the two groups and was stable (variation of <12%) during the last 6 days of training with 16.67 ± 2.71 average number of daily infusions in the Cocaine-No cues and 18.35 ± 2.43 in the Cocaine-Cues groups. Number of responses/2h on the last day of self-administration training in the Cocaine-No cues and Cocaine-Cues groups, respectively, were 185.43 ± 35.56 and 187.38 ± 21.34 on the active lever and 5.57 ± 3.26 and 6.75 ± 2.69 on the inactive lever, with no statistical difference between groups in either case.

Extinction

Cocaine self-administration was followed by 14 days of extinction training to devalue the motivational significance of the self-administration environment in the cocaine rats. Responses on the active and inactive lever across extinction are presented in Figure 2. The ANOVA of active lever presses revealed a drug history by extinction day interaction ($F_{13,273} = 6.43$, $P < 0.05$). Post hoc analyses revealed that Cocaine groups exhibited higher response rates relative to the Saline groups, evident as significant differences on the first and last days of extinction (simple main effects, $P < 0.05$). Moreover, active lever presses across days of extinction for each group revealed that response rates did not differ across days of extinction in the Saline groups, but significantly declined in Cocaine groups evident as a decrease on days 7–14 relative to the first day (Tukey tests, $P < 0.05$). Variability was high for responses on the active lever in the Cocaine-Cues and Cocaine-No cues groups on the first day of extinction, and analyses revealed no significant difference between these groups ($t_{13} = 1.60$, $P = 0.13$). Responses on the inactive lever did not vary between groups.

Cue-induced reinstatement

Figure 3 presents the effects of response-contingent cue presentations or no cues on cocaine-seeking behavior in rats with a history of yoked saline administration or cocaine self-administration. The Cocaine-Cues group exhibited more active lever presses than all other groups (interaction, $F_{1,23} = 18.55$, $P < 0.05$ and subsequent Tukey test, $P < 0.05$). Cue-induced reinstatement was evident in the Cocaine-Cues group as a significant increase in responding on the test day relative to the last day of extinction training (Tukey Test, $P < 0.05$). Lever presses among the other groups did not differ between the extinction baseline and test day. Additionally, inactive lever presses did not differ between rats in the Cocaine-Cues and Cocaine-No cues groups, although these groups had slightly higher response rates relative to the Saline groups (main effect, $F_{1,23} = 7.02$, $P < 0.05$).

Arc *mRNA expression*—Figure 4 illustrates color-encoded images of *Arc* mRNA expression, and Figure 5 illustrates mean fmol/mg probe (±SEM) of the *Arc* signal. Regardless of cue condition, Cocaine groups had greater *Arc* mRNA levels in the Cg1, PrL, IL, OF, AgI, Cg2, Ent, BlA, Sub, CPu, and NAcc relative to saline-yoked controls ($F_{1,23} = 4.88–40.44$, $P < 0.05$). The Cocaine-Cues group exhibited increased *Arc* mRNA levels in the OF cortex relative to all other groups (interaction, $F_{1,23} = 4.56$, $P < 0.05$ and subsequent Tukey test, $P < 0.05$). There was also a difference between the Cocaine-Cues group relative to both Cocaine-No cues and Saline-Cues groups in the Cg1, OF, and PrL subregions of prefrontal cortex [planned comparisons, $t_{12–13} = 2.28–6.79$, $P < 0.05$)]. In the CeA, levels of *Arc* mRNA were lower in the Saline-No cues group relative to all other groups (interaction, $F_{1,23} = 4.59$, $P < 0.05$ and subsequent Tukey tests, $P < 0.05$).

Figure 6 portrays the scatterplot demonstrating the only significant correlation between total milligrams of cocaine taken by rats during self-administration training and the density of *Arc* mRNA, which was observed in the Cg2 subregion of the anterior cingulate cortex ($r_{13} = 0.555$, $P < 0.05$). There was no significant correlation between cocaine-seeking behavior and density of *Arc* mRNA labeling.

DISCUSSION

The findings indicate reinstatement of extinguished cocaine-seeking behavior by response-contingent cue presentations is associated with region-specific increases in mRNA of the plasticity-associated gene *Arc* in the Cg1, Prl, and OF subregions of prefrontal cortex. These increases in *Arc* mRNA were not due to sensory processing of cues because no increases in *Arc* mRNA were observed in the Saline-Cues control group that had an equal number of cue presentations during testing. Moreover, the increases in *Arc* mRNA did not result from previous exposure to cocaine self-administration or learning that took place during the extinction phase of the experiment because the effects were not observed in the Cocaine-No cues group that had a similar history of cocaine intake and extinction training in the self-administration environment. Taken together, these results suggest a cue-conditioned upregulation of *Arc* mRNA in these cortical brain regions. The present findings are in agreement with, and extend, previous work demonstrating an upregulation of *Arc* in several limbic and cortical regions, including anterior cingulate, PrL, and OF cortices following exposure to contextual cues associated with nicotine (Schiltz et al., 2005) or food (Schiltz et al., 2007) and following cue-induced reinstatement of extinguished heroin-seeking behavior (Koya et al., 2006).

Although the design of the present study does not directly address the possibility that increased motor behavior (i.e., active lever presses) in the Cocaine-Cues group may have contributed to the increases in *Arc* expression in this group, this explanation seems unlikely because functions common to subregions of the prefrontal cortex are largely related to executive processes, rather than motor behavior per se (Roberts et al., 1998; Weiss, 2005). Moreover, the lack of correlation

between *Arc* mRNA expression and cocaine-seeking behavior (i.e., active lever presses in the cocaine cues group) suggests *Arc* expression is not necessarily due to increased lever pressing. Previous research also suggests that these regions are involved in the neuronal circuitry activated by cocaine cues in humans (Childress et al., 1999; Grant et al., 1996; Wang et al., 1999) and animals (Neisewander et al., 2000; Thomas et al., 2003; Zavala et al., 2007). Moreover, pharmacological studies support a role of the medial prefrontal and OF cortices in the incentive motivational effects of cocaine-associated stimuli (Bossert et al., 2005; Kalivas and McFarland, 2003). Specifically, inactivation of the dorsal medial prefrontal cortex with tetrodotoxin or lidocaine (Di Pietro et al., 2006; McLaughlin and See, 2003) or inactivation of the OF cortex by a cocktail infusion of $GABA_A$ and $GABA_B$ agonists (Fuchs et al., 2004b) attenuates reinstatement of extinguished cocaine-seeking behavior by cocaine-associated cues. More recently, the prefrontal cortex has also been implicated in the reinstatement of cocaine-seeking behavior by contextual cues (Di Pietro et al., 2006; Fuchs et al., 2005), suggesting this region is important for both explicit and contextual conditioned stimuli in the incentive motivational effects of cocaine cues.

The cue-conditioned increases in *Arc* mRNA expression in the present study likely involve neuronal processing of motivation for drug, as well as initial extinction learning, given that the test day is the first session in which animals are under extinction conditions to the discrete cues previously paired with cocaine infusions. The latter interpretation has been suggested previously for similar increases in *Arc* observed in animals that experienced their first episode of extinction to contextual stimuli predictive of nicotine administration (Schiltz et al., 2005). This explanation is also consistent with the role of *Arc* in the initial stages of learning (Guzowski et al., 2001; Montag-Sallaz and Montag, 2003). For instance, *Arc* mRNA expression is enhanced in limbic and cortical regions of rats learning to acquire an instrumental response relative to rats that have had extensive training (Kelly and Deadwyler, 2002, 2003). The role of the prefrontal cortex, particularly the IL, in extinction learning has been well established (for recent reviews see Quirk et al., 2006; Sotres-Bayon et al., 2006), thus it was surprising that there was no change in the IL in the present study.

Evidence suggests dopamine (DA) D1 receptor stimulation is necessary for the expression of *Arc* (Fosnaugh et al., 1995; Fumagalli et al., 2006; Granado et al., 2007; Wirtshafter, 2007; Yamagata et al., 2000) and DA D1 receptors play a critical role in cocaine-seeking behavior elicited by cocaine cues (Alleweireldt et al., 2002, 2003; Khroyan et al., 2000). Accordingly, DA D1 receptors within the prefrontal cortex may have contributed to the conditioned increases in *Arc* mRNA levels in animals engaged in cue-induced reinstatement of extinguished cocaine-seeking behavior in the present study. Although the role of D1 receptors in the prefrontal cortex in cue-induced reinstatement have not been directly examined, evidence suggests this region may represent a final common pathway for cue, cocaine, and stress reinstatement (Capriles et al., 2003; Neisewander et al., 2000; Rebec and Sun, 2005). Thus, given that DA D1 receptors within the prefrontal cortex are important in the reinstatement of cocaine-seeking behavior by cocaine priming injections (Park et al., 2002; Sun and Rebec, 2005), foot-shock stress (Capriles et al., 2003), and reinstatement of cocaine-conditioned place preference (Sanchez et al., 2003), it is likely that DA D1 receptors play a similar role in cue-induced reinstatement of operant cocaine-seeking behavior and contributed to the increased expression of *Arc* mRNA in the present study.

The lack of increase in *Arc* mRNA labeling in the hippocampus and amygdala in the Cocaine-Cues group is surprising because previous research has identified these regions as part of the neuronal circuitry activated by cocaine-associated stimuli (Ciccocioppo et al., 2001; Crawford et al., 1995; Franklin and Druhan, 2000; Neisewander et al., 2000; Zavala et al., 2007) and manipulations of these regions confirm their role in cue-elicited cocaine-seeking behavior (Fuchs and See, 2002; Kantak et al., 2002; McLaughlin and See, 2003; Meil and See, 1997;

Rogers and See, 2007; Sun and Rebec, 2003). It is important to note that the lack of evidence for changes in *Arc* expression in the hippocampus and amygdala does not preclude a role of *Arc* in these regions in cue-induced reinstatement. For instance, it is possible that the lack of increase in *Arc* expression in these regions in the present study may be due to the use of a discrete stimulus rather than contextual stimuli during reinstatement testing. Indeed, *Arc* expression is increased in the BlA and hippocampus after exposure to contextual cues associated with food or nicotine (Schiltz et al., 2005, 2007). Moreover, the changes observed in the latter studies were evident after a 45-min testing session, whereas the present study examined changes in *Arc* mRNA expression after a 30-min testing session. Thus another possible explanation is that changes in *Arc* expression in the amygdala and hippocampus may require extended exposure to the testing environment, compared with that of the prefrontal cortex, and was therefore not observed in the present study. Alternatively, cue-elicited cocaine-seeking behavior may involve molecular signaling pathways that induce other IEGs such as Fos or *Zif268* (Lee et al., 2004, 2005; Neisewander et al., 2000; Thomas et al., 2003; Zavala et al., 2007).

The present findings demonstrate an increase in *Arc* mRNA levels in the Cg1, PrL, IL, OF, AgI, Cg2, Ent, BlA, Sub, CPu, and NAcc in rats with a history of cocaine self-administration, regardless of whether they were exposed to cues on the test day. These findings may represent morphological changes associated with repeated exposure to cocaine, considering *Arc* is thought to be involved in cytoskeletal rearrangements (Fujimoto et al., 2004; Lyford et al., 1995). This notion is consistent with morphological and functional long-term changes observed in relevant brain circuits after administration of drugs of abuse (Robinson et al., 2001; Robinson and Kolb, 1997, 2004). Indeed, increases in *Arc* are evident after systemic injections of a variety of psychostimulants, including cocaine (Fosnaugh et al., 1995), methamphetamine (Kodama et al., 1998; Yamagata et al., 2000), methylphenidate (Chase et al., 2007), and amphetamine (Gonzalez-Nicolini and McGinty, 2002; Klebaur et al., 2002). More importantly, chronic injections of cocaine upregulate expression of *Arc* in the striatum, an effect that lasts up to 14 days after the last day of cocaine administration (Fumagalli et al., 2006).

Alternatively, the upregulation of *Arc* mRNA in rats with a history of cocaine self-administration may have resulted from cocaine withdrawal or extinction training. In the present study, rats underwent 14 days of abstinence following cocaine self-administration, thus the upregulation of *Arc* mRNA may have resulted from cocaine withdrawal, or resulted from extinction training that occurred during the abstinence period. Consistent with the first possibility, prolonged periods of abstinence from cocaine are associated with increases in motivation for cocaine (Grimm et al., 2001; Neisewander et al., 2000; Tran-Nguyen et al., 1998) and are accompanied by several neuroadaptations including alterations in DA D3 receptors (Neisewander et al., 2004), glutamate receptor subunits (Lu et al., 2003, 2005a; Tang et al., 2004), brain-derived neutrophic factor (Grimm et al., 2003), and extracellular signal-regulated kinase (Lu et al., 2005b). Consistent with the second possibility, extinction training is also associated with unique neuroadaptive changes (Self and Choi, 2004), such as upregulation of AMPA glutamate receptor subunits (Sutton et al., 2003).

The only brain region for which there was a correlation between *Arc* mRNA and the total cocaine intake during self-administration training was the Cg2 subregion of anterior cingulate cortex (see Fig. 6). The lack of correlational changes in other regions exhibiting *Arc* upregulation may have resulted from a lack of power, given the low number of animals that were examined. The relationship between *Arc* and cocaine intake in the Cg2 suggests that cocaine self-administration may underlie the neuroplasticity observed in this region. Furthermore, the results suggest heterogeneity of function of *Arc* expression among subregions of the prefrontal cortex, with *Arc* changes in the PrL, OF, and Cg1 involved in neuroplasticity

associated with cue-induced reinstatement and changes in Cg2 associated with cocaine self-administration.

In conclusion, the present study found support for our hypothesis that cue-elicited cocaine-seeking behavior is associated with potential sites of Arc-mediated neuroplasticity. Contrary to our predictions, however, these effects were found only in the Cg1, PrL, and OF cortex and not in the amygdala or hippocampus. Although not the focus of the present study, the design and results also revealed potential sites of Arc-mediated neuroplasticity in Cocaine groups, regardless of cue exposure, in the Cg1, PrL, IL, OF, AgI, Cg2, Ent, BlA, Sub, CPu, and NAcc. These effects may have resulted from chronic cocaine exposure, withdrawal from cocaine, or extinction training. Further research elucidating the role of *Arc* in these processes will be important for understanding cocaine addiction and relapse.

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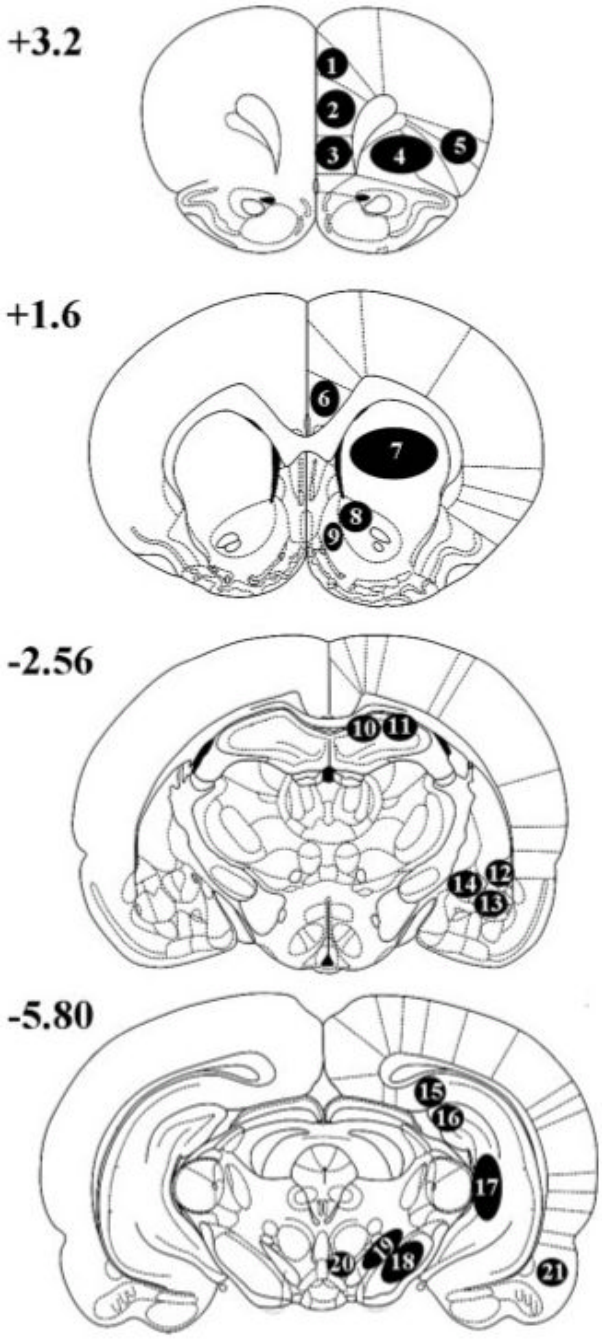

Fig. 1.

Schematic of coronal sections of the rat brain (+3.2, +1.6, −2.56, and 5.88 mm from Bregma) adapted from Paxinos and Watson (1998) representing regions analyzed for *Arc* mRNA labeling. Numbers in the sections represent the regions analyzed as follows: (1) Cg1 region of the anterior cingulate cortex (Cg1); (2) prelimbic cortex (PrL); (3) infralimbic cortex (IL); (4) orbitofrontal cortex (OF); (5) agranular insular cortex (AgI); (6) Cg2 region of the anterior cingulate cortex (Cg2); (7) caudate-putamen (CPu); (8) nucleus accumbens core (NAcc); (9) nucleus accumbens shell (NAcs); (10) hippocampal CA1 region (CA1); (11) hippocampal CA2 region (CA2); (12) lateral amygdala (LA); (13) basolateral amygdala (BlA); (14) central amygdala (CeA); (15) subiculum (Sub); (16) dentate gyrus (DG); (17) hippocampal CA3

region (CA3); (18) substantia nigra pars reticulata (SNR); (19) substantia nigra pars compacta (SNC); (20) ventral tegmental area (VTA); and (21) entorhinalcortex (Ent).

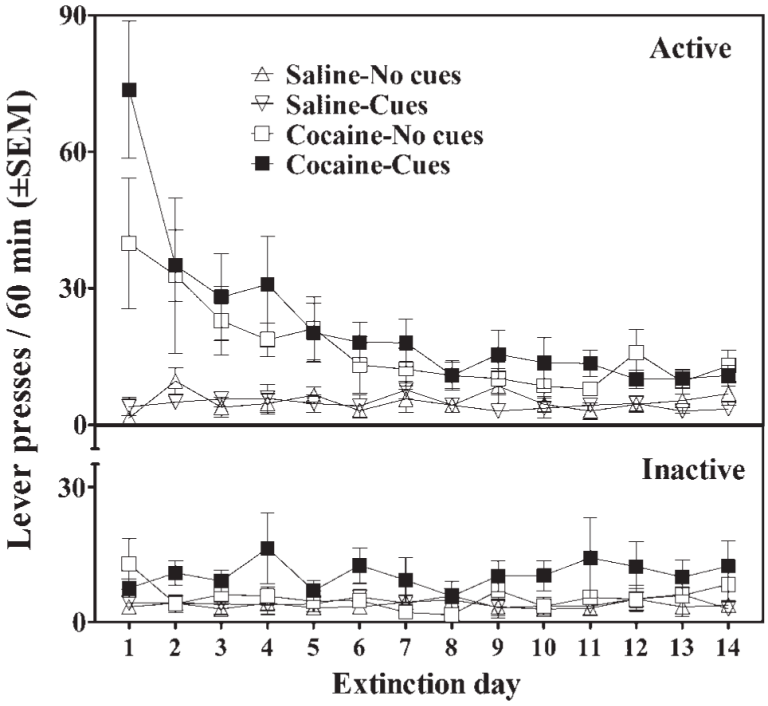

Fig. 2.

Lever presses on the active (top) and inactive (bottom) levers across the 14 days of extinction training in rats with a history of yoked saline administration (Saline) or cocaine self-administration (Cocaine). Animals were later assigned to groups that received cues contingent upon responses of a Cocaine rat (Cues) or received no cues (No Cues) on the test day for cue-induced reinstatement. As expected, the Cocaine groups exhibited a decline in active lever presses across extinction days and they exhibited more active lever presses across extinction days compared with Saline groups. Importantly, there were no significant differences between the Cocaine-Cues and Cocaine-No cues groups in their responding on the active or inactive levers. There were no group differences in inactive lever responses.

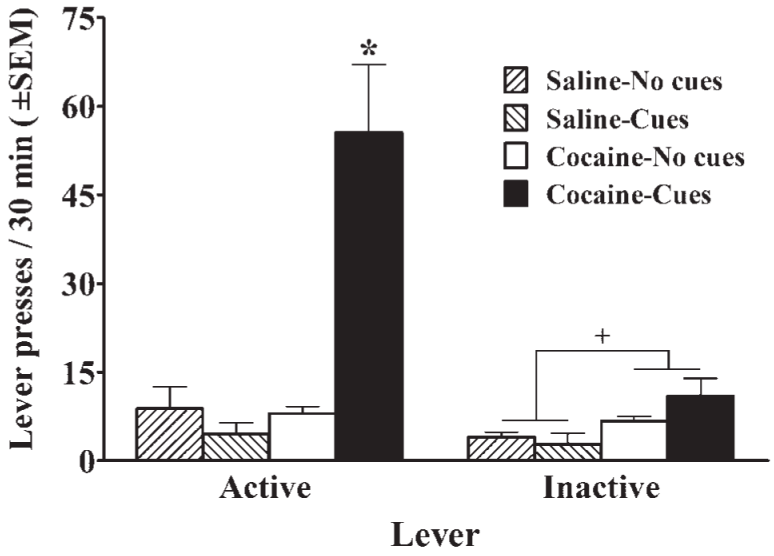

Fig. 3.

Lever presses on the active and inactive levers on the test day for reinstatement of extinguished cocaine-seeking behavior. Rats with a history of yoked saline administration (Saline) or cocaine self-administration (Cocaine) and 14 days of extinction training underwent testing for cocaine-seeking behavior during which cues were presented contingent upon responses of a Cocaine rat (Cues) or were not available (No Cues). * represents a difference from all other groups (Tukey test, $P < 0.05$). + represents a main effect of drug history ($P < 0.05$).

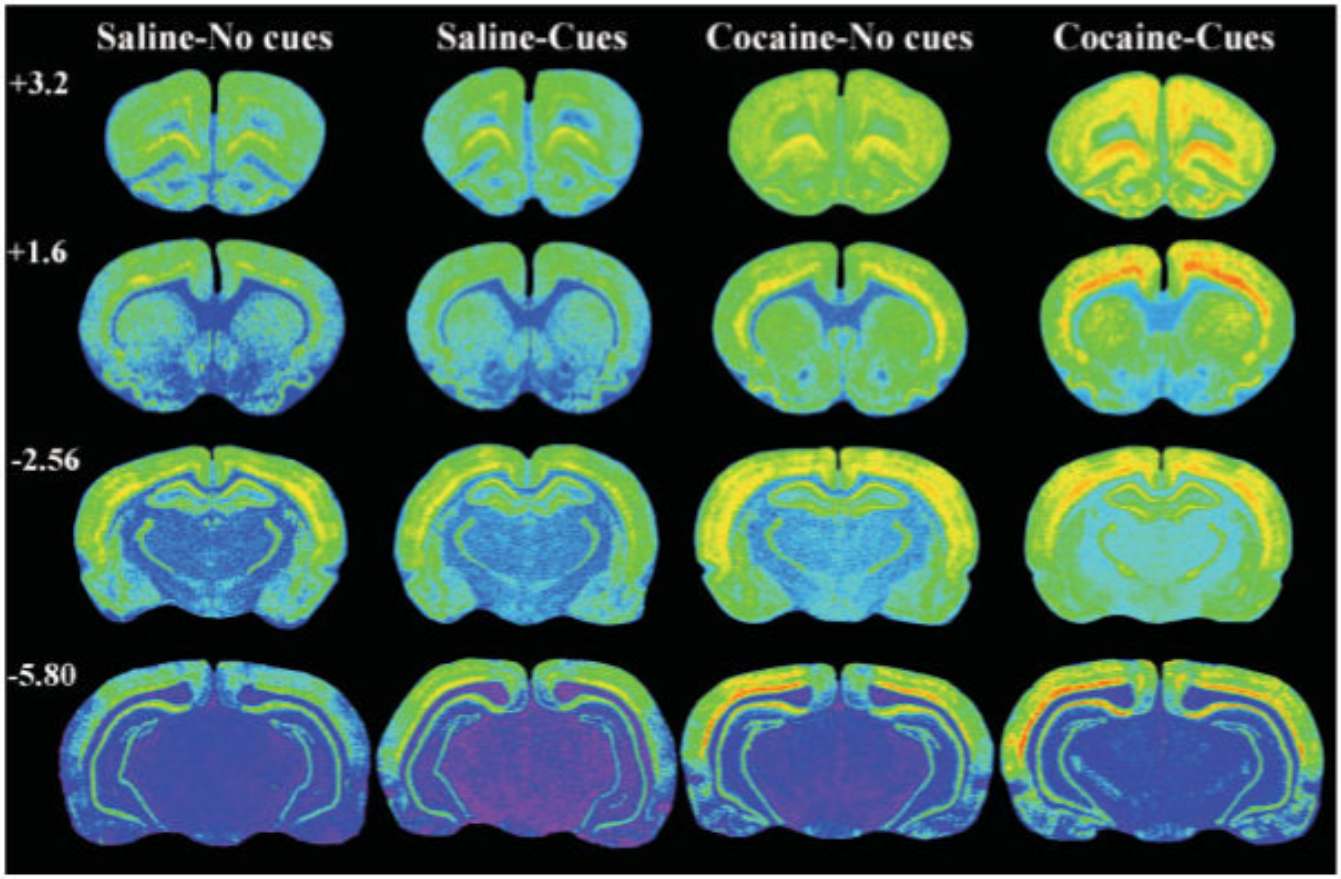

Fig. 4.

Color-encoded images of representative coronal sections illustrating *Arc* mRNA levels in the brain, designated by distance from bregma (mm) on the left of the images (see Fig. caption 3 for explanation of group labels). The expression of *Arc* mRNA hybridization from lowest to highest is represented by purple, blue, green, yellow, orange, and red, respectively.

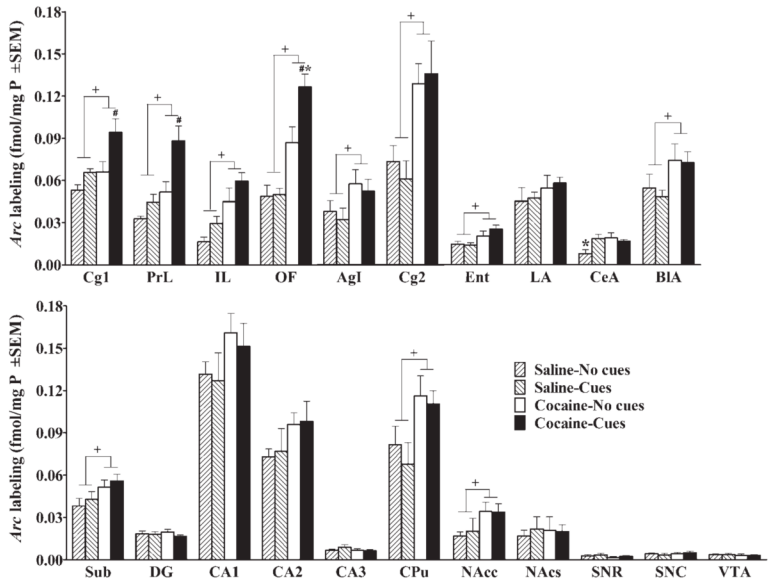

Fig. 5.

Arc mRNA hybridization across the 21 regions examined (see Fig. 1 caption for definition of brain region abbreviations and Fig. 3 caption for explanation of group labels). * represents a difference from all other groups (Tukey test, $P < 0.05$); + represents a main effect of drug history ($P < 0.05$); # represents a difference from both Cocaine-No cues group and Saline-Cues group (planned comparison, $P < 0.05$).

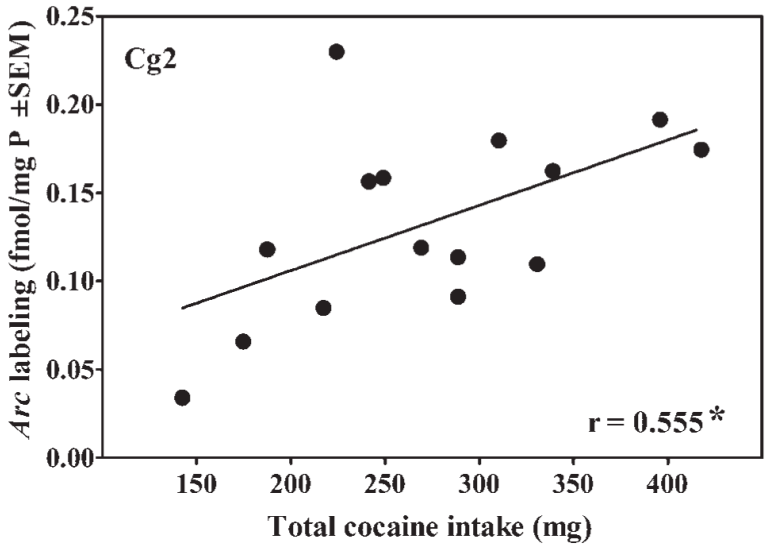

Fig. 6.

Scatter plot of the relationship between total cocaine intake (mg) during the 23 days of self-administration and the density of *Arc* mRNA labeling in the Cg2 subregion of the anterior cingulate cortex. *Significant correlation ($P < 0.05$).