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Short communication

Alterations in expression and phosphorylation of GluA1 receptors following cocaine-cue extinction learning

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HIGHLIGHTS

- Cocaine-cue extinction increased and decreased, respectively, total GluA1 in vmPFC and BLA.
- Cocaine-cue extinction increased GluA1-pSer^{845} in vmPFC and NAc.
- Lever responding positively correlated with total GluA1 in NAc.
- The BLA and vmPFC appear to be loci for cocaine-cue extinction learning.
- Understanding extinction mechanisms may improve exposure therapy in cocaine addicts.

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ABSTRACT

Brain regional analyses of total GluA1 and GluA1-pSer^{845} were used to delineate plasticity of the AMPA receptor in conjunction with cocaine-cue extinction learning. Rats were trained to self-administer cocaine paired with a 2-s light cue and later underwent a single 2 h extinction session for which cocaine was withheld but response-contingent cues were presented. Control groups received yoked-saline sessions or received cocaine self-administration training without undergoing extinction training. Extinction-related increases and decreases, respectively, in total GluA1 were observed in the ventromedial prefrontal cortex (vmPFC) and basolateral amygdala (BLA). Phosphorylation of GluA1 at Ser^{845} was increased in the vmPFC and nucleus accumbens (NAc). Though total GluA1 did not change in NAc, there was a positive association between the number of responses during extinction training and the magnitude of total GluA1 in NAc. No significant changes were evident in the dorsal hippocampus. We conclude that the BLA and vmPFC, in particular, appear to be loci for the inhibition of learned behavior induced via extinction training, but each site may have different signaling functions for cocaine-cue extinction learning.

Reactivity to drug cues plays an important role in relapse to drug use. Attenuating cue reactivity may aid in maintaining abstinence and preventing a return to drug taking. Progress in understanding the brain circuits involved in extinction of drug cue reactivity has been achieved by studying cue extinction in experimental animals. Employing an animal model that explicitly extinguishes responses only in the presence of discrete drug-paired cues, our recent findings using c-Fos protein expression as a molecular correlate of neural activity in 11 brain areas suggest that sites within amygdala, prefrontal cortex, hippocampus and striatum are actively engaged during cocaine-cue extinction training [1]. We found that c-Fos protein expression was selectively increased within basolateral amygdala (BLA) and prelimbic cortex (PL) by cocaine-cue extinction learning, while the pattern of c-Fos expression within the dorsal hippocampus (DH) and infralimbic cortex (IL) implicated these sites in processing the significance of cues (whether cocaine or saline) that were present during extinction training. Within the caudate-putamen and nucleus accumbens (NAc), c-Fos expression did not differ amongst treatment groups but did positively correlate with rate of responding at the end of extinction training, suggesting that these sites may mediate motor output during extinction training. Expression of GluA2, the predominant subunit of the AMPA receptor throughout the adult brain [2], was not altered in any site examined after extinction training. In the current investigation, we focused on another key signal in synaptic plasticity underlying learning and memory, the GluA1 receptor

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Table 1
Experimental design and number of responses and infusions (mean ± SEM) during the 2 h cocaine self-administration or yoked-saline sessions at baseline.

Group	*N*	Experimental design		Baseline responses		Infusions
		Training	Test	Active	Inactive	
1	12	Cocaine	EXT	291.9 ± 31.8	25.1 ± 9.7	54.2 ± 5.8
2	6	Saline	EXT	18.5 ± 4.1	5.7 ± 1.3	N/A
3	11	Cocaine	No-EXT	273.3 ± 29.2	50.7 ± 12.1	48.6 ± 5.9
4	5	Saline	No-EXT	18.4 ± 1.7	8.2 ± 1.7	N/A

subunit [3]. Specifically, we examined the impact of cocaine-cue extinction on total GluA1 protein expression in the brain sites we previously identified as being relevant during cocaine-cue extinction training [1]. Since phosphorylation of the GluA1 subunit at serine 845 (GluA1-pSer^{845}) modulates the membrane expression of these receptors, various channel properties, and synaptic plasticity [4], we examined GluA1-pSer^{845} as well.

Male Wistar rats (Crl(WI)BR; 275–300 g) were housed individually (08:00 h lights on, 20.00 h lights off) and maintained in accordance with the NIH Guide for Care and Use of Laboratory Animals and the Boston University Institutional Animal Care and Use Committee. Intravenous catheters were implanted under anesthesia as described previously [5]. Following recovery, rats were randomly assigned to either cocaine or yoked-saline groups and underwent 30 days of self-administration training. Cocaine HCl (NIDA, Bethesda, MD) was self-administered in 2 h daily sessions under a fixed ratio 5 (FR5) schedule of reinforcement, whereby each completion of 5 responses on the active lever produced an i.v. injection of 0.3 mg/kg cocaine (0.03 ml/s of a 1.6 mg/ml solution). Cocaine was infused via motor-driven syringe pumps located inside the sound-attenuating cubicle, over an approximately 2-s period and was simultaneous with a 2-s onset of the white stimulus light located over the active lever (cocaine-paired stimulus). Each rat passively receiving saline was paired to a rat self-administering cocaine. Independent of lever-press behavior, each yoked-saline rat received the same number and temporal pattern of injections and cue light presentations as the cocaine self-administering rat to which it was paired. Following self-administration training, and subsequent to a 24 h abstinence period, rats with a history of cocaine self-administration were divided into 2 groups: those receiving an extinction session (COC EXT) and those receiving an abstinence session (COC No-EXT). During the single 2 h extinction training session, lever pressing was extinguished by substituting saline for cocaine injections delivered via motor-driven syringe pumps, while maintaining response-contingent presentations of the 2-s light cue under the FR5 schedule. During the abstinence session, rats were placed in operant chambers for which levers were retracted, and without presentation of either the 2-s light cue or the delivery of cocaine. One set of yoked-saline rats received a yoked-extinction test session (SAL EXT) for which conditions used were identical to those used during training. The other set of yoked-saline rats received an abstinence session on test day (SAL No-EXT). This experimental design allowed us to determine if changes in total GluA1 and GluA1-pSer^{845} in extinguished cocaine-trained rats resulted specifically from extinction learning or from some other factor (e.g., cue exposure per se during testing or a prior history of cocaine self-administration).

Rats were sacrificed by guillotine immediately after the 2 h test session. Key brain areas were rapidly dissected using a coronal rodent brain matrix (RBM-4000C, ASI Instruments, Warren, MI) according to the method described previously [6]. The DH, NAc, BLA and ventromedial prefrontal cortex (vmPFC) were dissected immediately on ice-cooled plates from 1 mm slices, flash frozen in isopentane and stored at −80 °C. Western blot analysis was performed as previously described [7]. The assay used antibodies against GluA1ct diluted at 1:1000 (described in [7]), GluA1-pSer^{845} diluted at 1:500 (Millipore, Billerica, MA) and tubulin diluted at 1:1000 (Sigma, St. Louis, MO). Membranes were visualized using ECL (Amersham, Piscataway, NJ) and immunointensity was measured using Image J (http://rsbweb.nih.gov/ij/download.html) by a reviewer blinded to the status of the animal. To control methodologically induced variability, samples from each behavioral group were processed at the same time, such that each western batch contained tissue from each experimental group. For GluA1 or GluA1-pSer^{845} blots, a band at 110 kD was measured and quantified. All membranes were reprobed for tubulin to indicate protein loading. GluA1 values were normalized to tubulin prior to analysis. Outliers (>2 SD) were omitted from the molecular analysis. A total of four outlying values (two for GluA1 and two for GluA1-pSer^{845}) were obtained in the entire dataset.

The last five cocaine self-administration or yoked-saline sessions were used to establish baseline behavior (Table 1). Using 1-way ANOVA and post hoc Tukey analyses, significant group differences were observed for active lever responding [$F_{(3,30)} = 22.9$, $p = 0.001$]. As expected, groups receiving cocaine (Groups 1 and 3) exhibited significantly greater levels of responding ($p \leq 0.001$) than groups receiving yoked-saline (Groups 2 and 4). Infusions were not significantly different between the cocaine groups, and their inactive lever responses were ≤10% of active lever responses. Cocaine-trained rats learned to attenuate lever pressing for the motivationally salient response-contingent cues over the course of the 2 h extinction session (Fig. 1). Based on 2-way ANOVA and post hoc Tukey analyses, active lever responding was significantly altered across the four 30-min bins [$F_{(3,48)} = 4.0$, $p \leq 0.01$], an effect that was dependent on cocaine vs. saline history [$F_{(3,48)} = 4.1$, $p \leq 0.01$]. The COC EXT group exhibited significantly more active lever responding during the first 30-min bin (0–30 min) compared to the remaining bins (60–120 min; $p \leq 0.05$). The SAL EXT group had low levels of responding during the entire 2 h session.

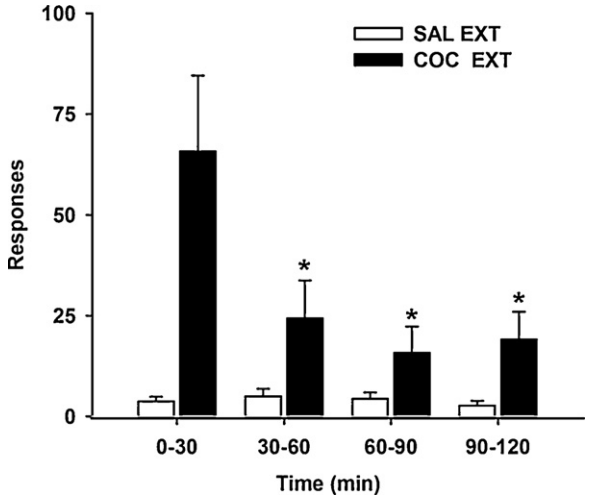

Fig. 1. Time course of cocaine-cue extinction learning in animals previously trained to self-administer cocaine ($n = 12$) or receiving yoked-saline ($n = 6$). Values are the mean ± SEM active lever responses for sequential 30 min bins over the 2 h extinction training session. * $p < 0.05$ compared to the first 30 min of extinction training.