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Emergence of context-associated GluR₁ and ERK phosphorylation in the nucleus accumbens core during withdrawal from cocaine self-administration

Scott Edwards, Ryan K. Bachtell, Daniel Guzman, Kimberly N. Whisler, and David W. Self
Department of Psychiatry and Neuroscience Graduate Program, The Seay Center for Basic and Applied Research in Psychiatric Illness, UT Southwestern Medical Center, USA

Abstract

Reexposure to cocaine-associated environments promotes relapse to cocaine seeking and represents a persistent impediment to successful abstinence. Neurobiological adaptations are thought to underlie the preservation of drug-seeking behavior during protracted withdrawal periods, possibly including changes associated specifically with cocaine-paired contexts. We measured GluR₁^{S845} and ERK phosphorylation in rat striatal subregions in an animal model of cocaine relapse. Animals with cocaine self-administration experience and their yoked partners were exposed to extinction conditions for one hour in the drug-paired environmental context after one day or three weeks withdrawal to measure protein phosphorylation induced by the cocaine-paired context in the absence of cocaine reinforcement. GluR₁^{S845} (an index of PKA activity) and ERK phosphorylation increased in the nucleus accumbens core of self-administering but not yoked animals after three weeks (but not one day) withdrawal, indicating a time-dependent emergence of context-associated protein phosphorylation in this accumbens subregion. In comparison, animals trained to self-administer sucrose displayed a similar increase in ERK, but not GluR₁^{S845}, phosphorylation following reexposure to a sucrose-paired environment three weeks later, indicating that GluR₁^{S845} phosphorylation did not result solely from lever press behavior *per se*. In contrast, basal (home cage) GluR₁^{S845} phosphorylation was elevated in the nucleus accumbens shell and caudate-putamen after one day or three weeks cocaine withdrawal regardless of context exposure. These results suggest that time-dependent emergence of context-associated GluR₁^{S845} phosphorylation in the nucleus accumbens core may contribute to the persistence of cocaine-seeking behavior, whereas ERK phosphorylation may be a consequence of this behavior.

Keywords

Cyclic AMP-dependent protein kinase; ERK; rats; relapse; self-administration; striatum

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Correspondence to: David W. Self, Department of Psychiatry, UT Southwestern Medical Center, Dallas, TX 75390-9070, USA. david.self@utsouthwestern.edu.

Authors Contribution

SE conducted all behavioral and biochemical experiments with significant contributions from RKB, DG and KNW, analyzed the data and wrote the manuscript. DWS conceived and designed and cowrote the study. All authors critically reviewed content and approved the final version for publication.

INTRODUCTION

Vulnerability to cocaine relapse is a hallmark of drug addiction and can persist for several months of abstinence (O'Brien 1997). An enhancement in drug craving may underlie the potential for relapse in humans and can be elicited by exposure to cocaine-related contextual cues (Ehrman *et al.* 1992; Saladin *et al.* 2006). The ability of contextual cues to elicit relapse behavior can be modeled in rodents by reexposure to the environment where cocaine was self-administered on prior occasions (Stewart, de Wit & Eikelboom 1984). In this model, non-reinforced lever press behavior increases in a time-dependent manner from early to late withdrawal (Tran-Nguyen *et al.* 1998; Grimm *et al.* 2001), a phenomenon thought to contribute to the vulnerability to relapse. Although this model may prove difficult to translate to humans (Lu *et al.* 2004), an incubation in cue-induced cigarette craving during abstinence was recently found to exist in human smokers (Bedi *et al.* 2010).

Chronic drug use produces numerous neurobiological changes involved in regulating drug-seeking behavior, but relatively few changes have been shown to persist or arise during withdrawal concomitant with increased drug-seeking behavior. Such changes include time-dependent increases in extracellular dopamine levels, glutamate receptors and ERK activation in the amygdala (Tran-Nguyen *et al.* 1998; Lu *et al.* 2004, 2005) and increases in BDNF levels in several limbic brain regions (Grimm *et al.* 2003). In this study, we measured PKA-dependent GluR1^{S845} phosphorylation and ERK phosphorylation in neostriatal subregions following one hour reexposure to the cocaine-paired environment after early (one day) or late (three weeks) withdrawal from cocaine self-administration in rats. We compared basal and context-associated phosphorylation changes in self-administering animals with yoked animals that received an identical amount and temporal pattern of passive cocaine administration. We also measured context-associated changes in GluR1^{S845} and ERK phosphorylation in animals trained to self-administer sucrose to determine whether changes induced by the cocaine-paired context were merely the result of lever-pressing behavior.

MATERIALS AND METHODS

Subjects and surgery

Male, Sprague-Dawley rats initially weighing 275–325 g (Charles River, Kingston, RI, USA) were individually housed according to the National Institutes of Health (United States) *Guide for the Care and Use of Laboratory Animals*. All of the animals initially were maintained on a restricted diet at 85% of original body weight and trained to press a lever for sucrose pellets in operant chambers (Medical Associates, Georgia, VT, USA) on a fixed ratio 1 (FR1) reinforcement schedule. Following acquisition of lever press behavior, the animals were fed *ad libitum* prior to surgical implantation of intrajugular catheters as described previously (Edwards *et al.* 2007b).

Cocaine and sucrose self-administration procedures in rats

Self-administration and context reexposure were performed in operant test chambers as described (Edwards *et al.* 2007b). Following recovery from surgery, the animals were trained to self-administer cocaine hydrochloride (FR 1) at 500 µg/kg/50 µl infusion in daily four-hour sessions for three weeks (six days/week). During each 2.5-second infusion, a cue light above the active lever was illuminated, and the house light was extinguished, followed by an additional 12.5-second time-out (TO) period when both lights were extinguished and lever presses had no programmed consequence. The yoked cocaine animals received passive non-contingent cocaine infusions in an identical temporal pattern as their self-administering partners but without injection cues. The age- and group-matched untreated controls and the cocaine-trained animals that remained in their home cages during withdrawal were handled

daily. An additional group of animals was trained to self-administer 100 sucrose pellets/day on a similar FR1: TO 15 seconds reinforcement schedule for three weeks with identical conditioning stimuli as cocaine self-administering animals.

Measurement of protein phosphorylation in brain tissue homogenates

Either one day or three weeks after the last training session, the cocaine self-administering, yoked or sucrose self-administering animals returned to their respective operant test chambers for one hour reexposure under extinction conditions with response-contingent cues and saline injections available and were euthanized immediately afterwards along with their home-caged cohorts by microwave irradiation (5 kW, 1.5 seconds, Murimachi Kikai Co., LTD. Tokyo, Japan). The brains were rapidly dissected as previously described (Edwards *et al.* 2007a). Tissue samples were immediately homogenized by sonication in lysis buffer and stored at -80°C until determination of protein concentrations by the Lowry method. Samples of 20 μg protein were subjected to 10% SDS-polyacrylamide gel electrophoresis, followed by electrophoretic transfer to PVDF membranes (polyvinylidene; Amersham, Piscataway, NJ, USA). The membranes were blocked overnight in 5% non-fat milk at 4°C and incubated in primary antibody for pGluR $_1$ ^{S845} (1:2500; Chemicon, Temecula, CA, USA) or pERK (1:7500; Cell Signaling, Danvers, MA, USA) for 24 hours at 4°C . The membranes were washed and labeled with species-specific peroxidase-conjugated secondary (1:10 K; Bio-Rad) for one hour at 25°C . Following chemiluminescence detection (ECL plus; Amersham), blots were stripped for 20 minutes at room temperature (Restore, Thermo Scientific, Rockford, IL, USA) and reprobed for total protein levels of GluR $_1$ (1:10 K; Chemicon) or ERK (1:7500; Cell Signaling). Absence of signal after stripping was confirmed for both pGluR $_1$ ^{S845} and pERK by incubating stripped membranes with either rabbit or mouse secondary antibody alone (respectively) followed by chemiluminescence detection. Immunoreactivity was quantified by densitometry (Scion Image) under conditions linear over at least a threefold concentration range.

Data analysis

Separate gel sets composed of control and experimental animals were processed in parallel for each of four time point/environment conditions (e.g. one day WD, one day WD CTX, etc.). Densitized values were expressed as a percentage of the mean for age- and batch-matched control values (7–8/blot) to normalize data across blots. This design allows for normalization to control values across multiple blots. Phosphoprotein levels, each corrected for individual total protein levels, were compared by one-factor analysis of variance followed by protected Fishers least significant difference *post hoc* comparisons among groups or planned Student's *t*-tests comparisons with untreated controls.

RESULTS

Figure 1a illustrates the cocaine treatment regimens for experimental groups used in the analysis of protein phosphorylation levels. The self-administering animals developed stable cocaine intake by the third week of training with mean daily cocaine intake (days 13–18) averaging 46.0 mg/kg and 49.7 mg/kg for the one-day and three-week groups, respectively. Cocaine-seeking behavior (drug-paired minus inactive lever responses) during context reexposure trended higher with withdrawal time, from an average of 56.1 non-reinforced lever presses after one day withdrawal to 78.5 lever presses after three weeks withdrawal (Fig. 1a). While not significantly different, this elevation is comparable with other studies using similar levels of cocaine training and withdrawal times (for review, see Lu *et al.* 2004). Lower levels of right and left lever press responses were found in yoked animals when reexposed to the test chambers after one day (left, 9.6 ± 4.1 ; right, 18.2 ± 7.3) and three weeks withdrawal (left, 25.9 ± 7.1 ; right, 15.7 ± 4.2).

Tissue punches of nucleus accumbens (NAc) core, shell and caudate-putamen (CPu) were collected from coronal brain slices as depicted in Fig. 1b. All observed changes involved regulation of protein phosphorylation status since there were no significant alterations in the total amount of GluR₁ or ERK protein in any brain region studied. In addition, we previously reported that neither GluR₁^{S845} nor ERK phosphorylation was altered in these striatal subregions in the saline self-administering animals upon immediate removal from the test chambers when compared with untreated controls (Edwards *et al.* 2007a), indicating that changes reported here were not caused by handling, surgical or other testing procedures.

BASAL AND CONTEXT-ASSOCIATED GLUR₁^{S845} PHOSPHORYLATION AFTER EARLY AND LATE COCAINE WITHDRAWAL

There were no changes in basal (home cage) GluR₁^{S845} phosphorylation in the NAc core after one day or three weeks withdrawal when compared with the untreated controls (Fig. 2a). Similarly, one hour reexposure to the cocaine-paired context had no effect on GluR₁^{S845} phosphorylation in the NAc core after one day withdrawal despite the fact that the animals engaged in substantial lever press behavior. In contrast, after three weeks withdrawal, reexposure to the cocaine-paired context increased GluR₁^{S845} phosphorylation by 31% in the animals trained to self-administer cocaine ($F_{2,37} = 3.317$, $P = 0.047$) but not in their yoked partners, indicating a reinforcement-related emergence of context-associated GluR₁^{S845} phosphorylation between early and late cocaine withdrawal.

Conversely, Fig. 2b shows that basal GluR₁^{S845} phosphorylation significantly increased by 19–20% in the NAc shell after one day withdrawal in both the self-administering and the yoked animals ($F_{2,17} = 3.479$, $P = 0.05$), and this increase persisted after three weeks withdrawal in self-administering animals ($T_{22} = 2.526$, $P = 0.019$). A similar and persistent 22–24% increase in basal GluR₁^{S845} phosphorylation was found in the CPu after one day ($F_{2,33} = 3.79$, $P = 0.03$) and three weeks ($F_{1,17} = 5.676$, $P = 0.029$) cocaine withdrawal (Fig. 2c). No further increases in GluR₁^{S845} phosphorylation were detected in the NAc shell and CPu when the animals were reexposed to the cocaine paired context; in fact, reexposure to the context abolished basal increases in GluR₁^{S845} phosphorylation in the NAc shell after one day withdrawal. Together, these results indicate that context-associated increases in GluR₁^{S845} phosphorylation are found specifically in the NAc core subregion after an extended withdrawal period, and only in the animals self-administering cocaine.

CONTEXT-ASSOCIATED ERK PHOSPHORYLATION AFTER LATE BUT NOT EARLY COCAINE WITHDRAWAL

Similar to GluR₁^{S845} phosphorylation, reexposure to the cocaine-paired context increased ERK phosphorylation by 68% in the NAc core after three weeks but not one day withdrawal (Fig. 3a) and only in the self-administering but not the yoked animals ($F_{2,37} = 5.729$, $P = 0.007$). However, in contrast to GluR₁^{S845} phosphorylation, a similar time-dependent increase in ERK phosphorylation was found in the NAc shell after three weeks withdrawal in both self-administering (73%) and yoked (50%) animals after one hour reexposure to the cocaine-paired context (Fig. 3b; $F_{2,36} = 7.89$, $P = 0.001$). There were no changes in basal ERK phosphorylation in the NAc in cocaine withdrawal, while a moderate 36% increase in basal ERK phosphorylation was found in the CPu after one day withdrawal exclusively in the self-administering animals ($T_{22} = 2.347$, $P = 0.028$). This increase in basal ERK phosphorylation was not further augmented by context reexposure (Fig. 3c). However, prominent and context-specific increases in ERK phosphorylation in the CPu were found after three weeks withdrawal in both the self-administering (97%) and the yoked (62%) animals ($F_{2,36} = 8.284$, $P = 0.001$) when basal levels of ERK phosphorylation had returned to normal.

STRIATAL ERK BUT NOT $\text{GluR}_1^{\text{S845}}$ PHOSPHORYLATION WITH REEXPOSURE TO A SUCROSE-PAIRED CONTEXT

To determine if context-associated changes in protein phosphorylation were simply related to increased lever press behavior, we measured GluR_1 and ERK phosphorylation induced by one hour reexposure to the test chambers in the animals trained to self-administer sucrose pellets for three weeks on an identical reinforcement schedule. Three weeks after the last sucrose self-administration session, reexposure to the sucrose-paired context elicited 119.4 ± 23.8 lever press responses compared with 78.5 ± 23.9 responses in the cocaine-trained animals after three weeks withdrawal. Reexposure to the sucrose-paired context induced substantial (60–72%) increases in ERK phosphorylation in the NAc core ($T_{14} = 2.200$, $P = 0.046$), shell ($T_{14} = 2.769$, $P = 0.016$) and CPu ($T_{14} = 2.388$, $P = 0.032$), but there were no significant changes in $\text{GluR}_1^{\text{S845}}$ phosphorylation (Table 1). These data indicate that time-dependent increases in $\text{GluR}_1^{\text{S845}}$ phosphorylation elicited by the cocaine-paired context can be dissociated from lever press behavior, whereas increases in ERK phosphorylation may be consequential to this behavior.

DISCUSSION

Previous studies found that cocaine-seeking behavior elicited by reexposure to the cocaine-paired environment increases in a time-dependent manner during withdrawal from cocaine self-administration (Tran-Nguyen *et al.* 1998; Neisewander *et al.* 2000; Semenova & Markou 2003). Consistent with these studies, we found a 40% increase in mean cocaine-seeking behavior from early (one day) to late (three weeks) withdrawal during initial reexposure to the self-administration test chambers in animals self-administering cocaine in daily four-hour sessions for three weeks. Other studies, however, found no change (Marinelli *et al.* 2003) or even decreases in cocaine seeking over time with reexposure to cocaine-conditioned contextual cues after a single self-administration session (Ciccocioppo, Martin-Fardon & Weiss 2004). Lu and colleagues (Lu *et al.* 2004) have postulated that this time-dependent increase in drug seeking (termed ‘incubation’) may, in part, depend on the magnitude of prior drug exposure and intensifies with even longer withdrawal periods.

We measured changes in basal and context-associated protein phosphorylation in striatal subregions in withdrawal from cocaine self-administration and compared these changes with yoked animals that received identical cocaine exposure during training but did not engage in cocaine-seeking behavior. However, yoked animals do form Pavlovian contextual associations with passive cocaine exposure that could elicit conditioned responses that alter striatal protein phosphorylation. Moreover, changes in protein phosphorylation that are unique to self-administering animals could reflect neural events that elicit cocaine-seeking behavior or that result from the act of lever press behavior itself. Therefore, we used an environment paired with prior sucrose self-administration to determine whether protein phosphorylation also would increase concomitant with non-reinforced lever press behavior in the absence of cocaine-associated contextual stimuli.

Of the three striatal subregions and two protein substrates examined, only PKA-dependent phosphorylation of $\text{GluR}_1^{\text{S845}}$ in the NAc core subregion was related specifically to cocaine-seeking behavior but independent of the act of lever pressing. Thus, $\text{GluR}_1^{\text{S845}}$ phosphorylation failed to increase in yoked animals that received cocaine in a non-response-contingent manner or in sucrose-trained animals that engaged in even greater lever press behavior. Moreover, context-associated increases in $\text{GluR}_1^{\text{S845}}$ phosphorylation were evident only after late but not early withdrawal, coinciding with time-dependent increases in cocaine-seeking behavior, and there were no changes in basal $\text{GluR}_1^{\text{S845}}$ phosphorylation in the NAc core in cocaine withdrawal. We previously found that cocaine-induced $\text{GluR}_1^{\text{S845}}$ phosphorylation in the NAc core is reduced by ~50% immediately following chronic

cocaine self-administration (Edwards *et al.* 2007a), possibly explaining the lack of context-associated GluR₁^{S845} phosphorylation after only one day withdrawal.

Given that context-associated GluR₁^{S845} phosphorylation in the NAc core can be dissociated from lever press responding, these changes may reflect neural events that trigger drug-seeking behavior elicited by context reexposure. One potential mechanism could involve context-associated dopamine transients (Stuber, Wightman & Carelli 2005) and activation of D1 receptors that increase PKA-mediated GluR₁^{S845} phosphorylation in NAc neurons (Mangiavacchi & Wolf 2004). In D1 dopamine receptor-containing NAc neurons, this effect would potentially mediate an enhancement of cocaine-seeking behavior (Anderson *et al.* 2008) while increasing GluR₁ function in D2 receptor-containing neurons inhibits cocaine seeking (Bachtell *et al.* 2008). PKA-mediated phosphorylation of GluR₁ both potentiates AMPA receptor currents (Roche *et al.* 1996) and facilitates membrane insertion of new AMPA receptors (Mangiavacchi & Wolf 2004), phenomena that, in fact, occur in the accumbens after late but not early withdrawal from cocaine self-administration (Conrad *et al.* 2008). Context-associated increases in GluR₁^{S845} phosphorylation selectively in the NAc core is consistent with a role for AMPA receptors in the NAc core, and not in the shell, in eliciting cocaine-seeking behavior (Park *et al.* 2002; McFarland, Lapish & Kalivas 2003). Along these lines, Martin *et al.* (2006) found an impairment in long-term depression in the NAc core, but not in the shell, after three weeks withdrawal and only in self-administering and not yoked animals.

In contrast to context-associated changes, basal PKA-mediated GluR₁^{S845} phosphorylation increased in the NAc shell and CPu after one day withdrawal in self-administering animals and remained elevated for at least three weeks. This increase also occurred to a lesser extent in yoked animals, indicating a generalized response to chronic cocaine exposure. These data suggest that persistent increases in the amount of PKA produced by chronic cocaine administration (Terwilliger *et al.* 1991; Lu *et al.* 2003) translate into functional increases in PKA-mediated phosphorylation *in vivo*. Interestingly, elevated basal GluR₁^{S845} phosphorylation in the NAc shell was abolished by reexposure to the cocaine-paired context after one day withdrawal, potentially relating to reductions in NAc shell dopamine levels when cocaine self-administering animals are initially exposed to extinction conditions (Neisewander *et al.* 1996).

Striatal ERK phosphorylation is regulated by convergent dopamine and glutamate signaling (Valjent *et al.* 2005) and is hypothesized to play a central role in the persistence of cocaine craving in withdrawal (Lu *et al.* 2006). In the present study, ERK phosphorylation increased in all striatal subregions following reexposure to the cocaine-paired context after three weeks withdrawal. In the NAc shell and CPu, this effect was not specifically related to cocaine-seeking behavior since it also occurred in yoked cocaine animals, and ERK phosphorylation was strongly induced in all three subregions during reexposure to a sucrose-paired environment. While these results suggest that ERK phosphorylation may be due to generalized behavioral activation in the cocaine- and sucrose-paired environments, the fact that ERK phosphorylation in the NAc was induced at late rather than early withdrawal is remarkably similar to incubation of context-associated ERK phosphorylation in the amygdala (Lu *et al.* 2005). Furthermore, Schumann & Yaka (2009) found time-dependent increases in accumbens pERK after withdrawal from non-contingent cocaine only in animals that exhibited behavioral sensitization. Previous studies found that ERK signaling in the NAc is necessary for expression of a cocaine-conditioned place preference (Miller & Marshall 2005), consistent with context-associated ERK activation in both the yoked and the self-administering animals in our study. The NAc core conveys Pavlovian influences that augment instrumental behavior (Hall *et al.* 2001) and is critical for the expression of a conditioned approach responses (Cardinal *et al.* 2002). Moreover, transient ERK activation

in the NAc is involved in reconsolidation mechanisms that maintain the conditioned rewarding effects of cocaine-paired environments (Valjent *et al.* 2000, 2006; Miller & Marshall 2005). In comparison, ERK signaling within the dorsal striatum has been shown to mediate the performance of specific goal-directed instrumental actions (Shiflett, Brown & Balleine 2010). Thus, context-associated striatal ERK phosphorylation after late withdrawal could facilitate the impact of Pavlovian (contextual) influences on cocaine-seeking behavior and contribute to the maintenance of these associations over prolonged periods of cocaine abstinence.

In addition to cocaine, we found that NAc ERK phosphorylation was induced by reexposure to a sucrose-paired environment, consistent with a recent report where NAc ERK phosphorylation was induced by a sucrose-paired auditory cue (Shiflett *et al.* 2008). In contrast, the sucrose-paired context (and sucrose seeking) failed to increase GluR₁^{S845} phosphorylation in the NAc. While many neural substrates recruited by natural and drug reward-related cues are shared, cocaine and natural rewards activate distinct neuronal ensembles within the NAc (Carelli, Ijames & Crumling 2000). In addition, a recent study found c-Fos activation in the NAc core (but not shell) during expression of a cocaine- and not food-conditioned place preference (Zombeck *et al.* 2008). Thus, cocaine-paired environments may elicit greater glutamatergic responses in the NAc core via PKA-dependent GluR₁ trafficking, ultimately leading to enhanced neuronal ERK activation in prolonged cocaine withdrawal.

These distinct region- and substrate-specific changes suggest that both GluR₁^{S845} and ERK phosphorylation could contribute to cocaine-seeking behavior in withdrawal. PKA-dependent GluR₁^{S845} phosphorylation in the NAc core is most closely associated with prior cocaine self-administration experience and the elicitation of cocaine seeking independent of instrumental consequences. Future work is needed to determine whether the context-associated GluR₁ phosphorylation persists or intensifies with even longer withdrawal periods. Taken together, these findings underscore the notion that withdrawal from cocaine self-administration is a dynamic state characterized by the emergence of late-forming changes in brain function that could exacerbate the propensity for cocaine relapse in prolonged abstinence.

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Figure 1.

Timeline depicting cocaine administration regimens and withdrawal times in the yoked (Cocaine YK) and the self-administering (Cocaine SA) animals. Animals were euthanized by microwave fixation after one day and three weeks cocaine withdrawal (WD), with and without one-hour reexposure to the cocaine-paired context (CTX) where time-dependent increases in cocaine seeking was observed (right) (a). Schematic representation of regional brain dissections collected from 1.5-mm thick coronal slices. NAc core (NAC), shell (NAS) and CPu (CP) in anterior–posterior coordinates depicting the posterior side of brain slices (Paxinos & Watson 1998)(b)

Figure 2.

Basal and context-associated striatal GluR₁^{S845} phosphorylation following withdrawal from active versus passive cocaine administration. Context-associated GluR₁^{S845} phosphorylation is found exclusively in the NAc core of cocaine SA but not YK animals after three weeks withdrawal (WD) (a). In contrast, basal (home cage) GluR₁^{S845} phosphorylation increases following one day or three weeks WD in the NAc shell and CPu in cocaine SA animals and in the cocaine YK animals at one day (shell) or three weeks (CPu) (b, c). Data reflect the mean ± SEM of phosphorylated/total protein ratios expressed as a percentage of untreated controls ($n = 7-24/\text{group}$). Asterisks indicate values differ from controls by Fisher's LSD tests ($*P < 0.05$, $**P < 0.01$) or by planned Student's t -tests ($^+P < 0.05$). Bars depict pooled comparisons. Basal GluR₁^{S845} phosphorylation at one-day WD was reported previously (Edwards *et al.* 2007a)

Figure 3.

Context-associated striatal ERK phosphorylation following withdrawal from active versus passive cocaine administration. Context-associated ERK phosphorylation is found in the NAc core of cocaine SA animals and in the shell of both the cocaine SA and YK animals after three weeks WD (a, b). Context-associated ERK phosphorylation is found in the CPu in both cocaine SA and YK animals after three weeks WD animals, while basal ERK phosphorylation increases after one day in the cocaine SA animals (c). Data reflect the mean $\pm$ SEM of phosphorylated/total protein ratios expressed as a percentage of untreated controls ($n = 7-24$ /group). Asterisks indicate values differ from controls by Fisher's least significant difference tests (* $P < 0.05$, ** $P < 0.01$) or by planned Student's t -tests ($^+P < 0.05$)

Table 1

Regulation of protein phosphorylation by sucrose seeking.

Phosphoprotein	NAC	NAS	CPU
pGluR _I ^{S845}	5 ± 5	-8 ± 6	15 ± 16
pERK	72 ± 30 [*]	63 ± 16 [*]	60 ± 25 [*]

^{*} $P < 0.05$.

Values indicate % Δ from control.