Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 75 (2003) 49–54
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

The relationship between cocaine-induced increases in NAC1 and behavioral sensitization

P.J. Wang[a], Michael Stromberg[b,c], Stephen Replenski[a], Alexander Snyder-Mackler[a], Scott A. Mackler [a,b,c,d,*]

[a]Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA
[b]Philadelphia Veterans Administration Medical Center (VAMC), Philadelphia, PA 19104, USA
[c]Department of Psychiatry, Center for Studies of Addiction, University of Pennsylvania, Philadelphia, PA 19104, USA
[d]Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Received 21 September 2002; received in revised form 22 January 2003; accepted 3 February 2003

Abstract

Repeated exposure to cocaine can cause long-term behavioral changes in mammals, including an augmented locomotor response known as behavioral sensitization. A major goal of research is the identification of molecules associated with these behaviors. NAC1, a member of the POZ/BTB transcription factor family, exhibited increased mRNA levels in the nucleus accumbens of the rat weeks after cocaine use. NAC1 exists as two isoforms, each demonstrating the ability to inhibit transcription, but to different extents. The present experiments examined the time course for both NAC1 isoforms after five consecutive days of systemic cocaine administration in male rats. Tissues were collected from several central nervous system regions and underwent Western blot analysis. There was significantly greater expression of the long isoform, lNAC1 (cocaine 1.341 ± 0.641; saline 1 ± 0.321; $P=.044$), and the short isoform, sNAC1 (cocaine 3.038 ± 2.816; saline 1 ± 0.720; $P=.001$), in the nucleus accumbens of cocaine-treated rats. The olfactory tubercle also showed a significant increase, but only in sNAC1 expression and at only one time period. No other significant differences were observed for either isoform of NAC1 in any other brain region. The expression of lNAC1 exhibited an inverse relationship with behavioral sensitization in rats 1–3 months following repeated cocaine injections predicting approximately 40% of the variance in the behavior variables ($R^2=.387$; and $P=.031$ for distance and $P=.025$ for ambulatory count). These results indicate that NAC1 expression is increased for a period of several months after chronic cocaine exposure. Furthermore, these data suggest that NAC1 may function as an endogenous inhibitor of behavioral sensitization. NAC1 represents a target for future studies examining cocaine-induced behavioral changes.

Keywords: Cocaine; NAC1; Behavioral sensitization

1. Introduction

Cocaine causes many biochemical changes and produces a wide range of behaviors, including increased locomotor activity. Neuromolecular changes in specific brain circuits appear to be responsible for the induction and expression of these behavioral changes. Most of these changes have been examined as the effect of acute drug administration, but some appear to be long-term adaptations (Koob and Bloom, 1988; Nestler, 2000, 2001).

Behavioral sensitization to cocaine refers to a behavioral model characterized by an enhanced locomotor response to a single injection of cocaine in animals with a history of prior exposure to cocaine. These behavioral changes produced by exposure to cocaine are present for many weeks following initial cocaine exposure (Kalivas et al., 1988). The cellular events producing these enduring neuroplastic and related behavioral changes have yet to be fully defined.

Previous investigations in this laboratory have begun to define the relationship between cocaine-induced behavioral sensitization and NAC1, a member of the POZ/BTB family of transcription factors. Elevated NAC1 mRNA levels have been demonstrated weeks after cocaine use in the nucleus accumbens, one brain nucleus associated with long-term changes in neuronal function resulting from cocaine admin-

* Corresponding author. Department of Pharmacology, University of Pennsylvania, 067 John Morgan Building, Philadelphia, PA 19104, USA. Tel.: +1-215-573-5724; fax: +1-215-573-2236.
E-mail address: smackler@mail.med.upenn.edu (S.A. Mackler).

0091-3057/03/$ – see front matter © 2003 Published by Elsevier Science Inc.
doi:10.1016/S0091-3057(03)00040-6

istration (Cha et al., 1997). Overexpression of protein with infection using an altered virus mitigated the enhanced behavioral response of rats to repeated cocaine injections (Mackler et al., 2000). NAC1, therefore, appears to attenuate the development of this behavioral response to psychomotor stimulant drugs that have been used as analogs of specific addictive behaviors (Robinson and Berridge, 1993).

Recently, two isoforms of NAC1 were identified (Kortula et al., 2002). The isoforms, long NAC (lNAC1) and short NAC (sNAC1), differ by only 27 amino acids. Both mRNA and proteins levels showed an unequal distribution of the two forms in the adult rat brain. However, sNAC1 exhibited a significant transient increase in the nucleus accumbens 2 h following a single intraperitoneal injection of cocaine.

The effect of repeated cocaine administration on the expression of the NAC1 protein and the relationship of NAC1 expression to the augmented behavioral response over time are unknown. The objective of this study was to describe the long-term time course of NAC1 expression in response to repeated cocaine injection and its relationship to the development of neuroadaptive behaviors in the rat.

2. Materials and methods

2.1. Subjects

Forty adult male Wistar rats (275–335 g) were housed individually with food and water available ad libitum. A 12/12-h light/dark cycle was used with lights turned on at 7:00 a.m. All research works were approved by the Institutional Animal Care and Use Committee (Philadelphia VAMC) and conducted according to the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

2.2. Cocaine administration

Rats were habituated to handling for 1 h on each of 2 days before the initial injection. After habituation, rats were injected with 1.0 ml/kg saline on the first day, and daily for the next 5 days with either cocaine (15 mg/kg ip, in a volume of 1.0 ml/kg) or an equivalent volume of saline. Rats were returned to the home cage for 1 week to 3 months. At either 1 week, 2 weeks, 1 month, 2 months, or 3 months following initial treatment, rats in both cocaine ($n=4$) and saline groups ($n=4$) received a single injection of cocaine (15 mg/kg ip).

2.3. Behavioral measurements

Immediately after injections, the rats were placed in open-field activity monitors (Med Associates, Georgia, VT) for 30 min to monitor locomotor activity. These measures were calculated automatically by Med Associates software and were based on photobeam breaks. Motor activity was measured for two dimensions: distance traveled and ambulatory count.

On the first day (baseline), each rat was injected with saline and habituated to the chambers and the injection procedure. The experimental intervention began the following day and continued for 5 days. On these days, rats were injected with cocaine or saline, and placed in activity chambers for 30 min before being returned to their home cage. The final test immediately followed the final injection of cocaine for all rats (test day at each time point). The rats were sacrificed 2 days after the final cocaine injection.

2.4. Protein extraction

Rats were euthanized using CO_2 gas and the brains were immediately dissected on dry ice, removing the cerebellum, caudate–putamen, olfactory tubercle, prefrontal cortex, and nucleus accumbens. From each of these tissues, the nuclear fraction of protein was isolated as previously described (Hope et al., 1994).

2.5. Western blot analysis

Equal amounts of protein (30 μg) for each sample were separated by sodium dodecyl sulfate–7.5% polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nylon membrane. Rabbit polyclonal anti-NAC1 antibody no. 780, as previously characterized (Kortula et al., 2002), recognizes both isoforms of NAC1 and was used at a dilution of 1:400. Immunoreactive signals were detected by enzymatic chemiluminescence (ECL). Equal transfer of protein in each lane was confirmed by two methods: direct visualization with Ponceau Red, and reprobe of each membrane for the

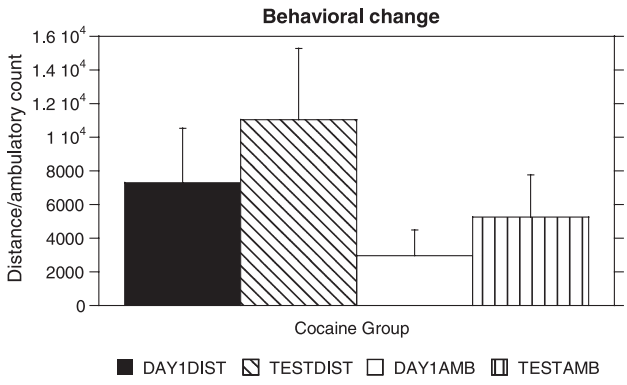

Fig. 1. Differences in behavioral response for the cocaine-treated group ($n=20$) from Day 1 to test day. Distance traveled and ambulatory counts are presented as mean ± S.D. Distance traveled was compared between Day 1 (D1) and test day (DT). Ambulatory count was compared between Day 1 (A1) and test day (AT). The data were evaluated by a paired *t* test.

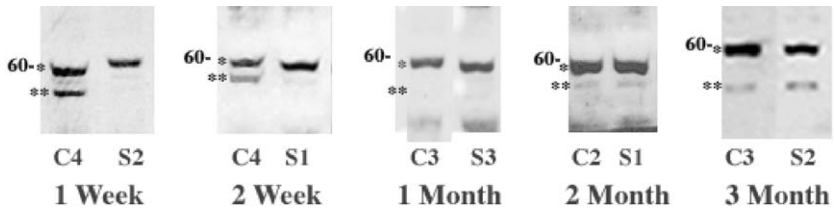

Fig. 2. Composite Western blot analysis of NAC1 antigen expression. Nuclear extracts (30 μg of protein) from the nucleus accumbens were run on 7.5% SDS-PAGE, transferred to nylon membranes, probed with NAC1 antiserum (Ab no. 780, 1:400), and visualized by ECL. All samples for each time period group were run together on the same Western gel. Representative results are shown for each time period—1 week, 2 weeks, 1 month, 2 months, and 3 months—for cocaine-treated (C) and saline-treated (S) rats, with the number of rats from each group listed beside the treatment. Both isoforms of NAC1 are observed: lNAC1 (*) and sNAC1 (**).

nuclear protein extracellular receptor kinase. Blots were first scanned into Adobe Photoshop 5.0 and protein levels were quantitated using the National Institutes of Health Image Software (v. 1.62).

2.6. Data analysis

Densities of lNAC1 and sNAC1 in the cocaine-treated group for each time period were normalized to the average respective NAC1 density for the corresponding saline-treated group. Differences in NAC1 expression by group (Cocaine, Saline) and time (1 week, 2 weeks, 1 month, 2 months, 3 months) were evaluated by two-factor analyses of variance for each brain region.

Data were analyzed for all time periods collectively and for late time periods (1–3 months). This analysis was required because of the limited number of subjects at each time point in each treatment condition. Behavioral sensitization was assessed by comparing ambulatory count and distance between Day 1 and test day for the cocaine-treated group using a paired *t* test.

Rats were operationally defined as sensitized if the measures of behavioral sensitization [distance measured on test day (DT) − distance measured on Day 1 (D1) + ambulatory count on test day (AT) − ambulatory count on Day 1 (A1)] were larger than the mean of the corresponding value for the saline-treated rats. Normalized NAC1 expression was assessed for differences by group (sensitized versus non-sensitized) using an unpaired *t* test.

Multiple linear regression was used to correlate behavior with NAC1 expression for all time periods and the late time periods.

3. Results

3.1. Behavioral sensitization

The sensitized motor response was demonstrated by both measures of locomotor activity tested, distance traveled (DT), and ambulatory count (AT) (Fig. 1). The cocaine-treated rats had significantly greater activity for DT (cocaine 11,037.366 ± 4242.19; saline 7936.412 ± 4092.535; P=.026) and AT (cocaine 5268.450 ± 2512.54; saline 3393.632 ± 2055.743; P=.015) than similarly treated, habituated, and aged rats with a saline history that were exposed to cocaine for the first time. Additionally, cocaine-treated rats had significantly greater activity on the fifth day [(DT), P=.002; (AT), P=.001] and the test day [(DT), P=.011; (AT), P=.003] than on the first day of exposure to cocaine.

3.2. Difference in NAC1 levels

Expression of NAC1 in the designated brain regions for both cocaine- and saline-treated rats was determined by Western blot analysis. A composite representative blot indicating NAC1 isoforms in the nucleus accumbens is shown in Fig. 2. Normalized values for lNAC1 and sNAC1

Table 1
Normalized NAC1 means (S.D.) for each brain region (n=4 rats/group except 3 months of saline; n=3 rats/group)

Time	NAC1 isoform	Nucleus accumbens		Cerebellum		Caudate–putamen		Olfactory tubercle		Prefrontal cortex	
		Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline	Cocaine	Saline
1 week	Long	0.914 (0.188)	1 (0.315)	1.327 (0.418)	1 (0.421)	2.625 (0.401)	1 (0.834)	1.241 (0.248)	1 (0.186)	0.813 (0.182)	1 (0.221)
	Short	2.5 (2.165)	1 (0.565)	1.726 (0.790)	1 (0.477)	1.832 (0.807)	1 (0.876)	1.381 (0.418)	1 (0.397)	0.854 (0.298)	1 (0.777)
2 weeks	Long	1.362 (0.524)	1 (0.429)	0.530 (0.135)	1 (0.377)	0.538 (0.398)	1 (0.786)	0.862 (0.196)	1 (0.266)	0.958 (0.159)	1 (0.114)
	Short	0.752 (0.555)	1 (0.903)	0.606 (0.308)	1 (0.510)	0.855 (0.724)	1 (0.926)	0.829 (0.169)	1 (0.259)	1.099 (0.598)	1 (0.089)
1 month	Long	1.559 (0.984)	1 (0.138)	1.214 (0.263)	1 (0.436)	1.468 (0.104)	1 (0.053)	0.874 (0.206)	1 (0.254)	0.980 (0.365)	1 (0.499)
	Short	1.611 (2.481)	1 (0.725)	1.598 (0.975)	1 (0.800)	1.298 (0.112)	1 (0.134)	0.986 (0.066)	1 (0.124)	1.008 (0.808)	1 (0.816)
2 months	Long	1.083 (0.076)	1 (0.326)	1.060 (0.514)	1 (0.258)	1.195 (0.868)	1 (0.357)	1.059 (0.557)	1 (0.519)	1.613 (0.214)	1 (0.806)
	Short	2.997 (0.136)	1 (0.723)	1.053 (0.391)	1 (0.425)	0.915 (0.746)	1 (0.254)	1.821 (1.195)	1 (0.521)	1.104 (0.387)	1 (0.441)
3 months	Long	1.787 (0.813)	1 (0.561)	0.758 (0.255)	1 (0.362)	0.805 (0.420)	1 (0.499)	0.841 (0.399)	1 (0.260)	0.642 (0.311)	1 (0.430)
	Short	7.195 (1.271)	1 (1.180)	1.231 (0.687)	1 (0.392)	No data	No data	2.540 (0.613)	1 (0.585)	1.641 (0.142)	1 (0.585)

expression in these brain regions are presented in Table 1. There was a main effect of Group with significantly greater expression of lNAC1 (cocaine 1.341 ± 0.641; saline 1 ± 0.321; P=.044) and sNAC1 (cocaine 3.038 ± 2.816; saline 1 ± 0.720; P=.004) in the nucleus accumbens in the cocaine-treated rats (Fig. 3). The olfactory tubercle also showed a significant increase but only in sNAC1 expression in the cocaine-treated rats, and post-hoc analysis found significance at only one time period. No significant differences were observed for either isoform of NAC1 in any other brain region.

3.3. Correlation of NAC1 expression and behavior

In order to determine the relationship between NAC1 isoform expression and sensitization, the levels of both lNAC1 and sNAC1 were compared to behavioral sensitization. Rats were categorized as "sensitized" or "not sensitized" in the whole group including all time periods as well as in the late group (1, 2, or 3 months). In the whole group with 20 cocaine-treated rats, 11 were classified as not sensitized and 9 were classified as sensitized. When considering the late group with 12 cocaine-treated rats, seven were classified as not sensitized and five were classified as sensitized. Rats characterized as "not sensitized" had significantly higher lNAC1 expression in the nucleus accumbens than rats characterized as "sensitized" for the late time periods (not sensitized 1.872 ± 0.732; sensitized 0.922 ± 0.146; P=.013; Fig. 4). Additionally, rats that were high expressers of lNAC1 (>2) demonstrated no behavioral sensitization. A similar relationship was not observed with sNAC1 expression or for any other brain region.

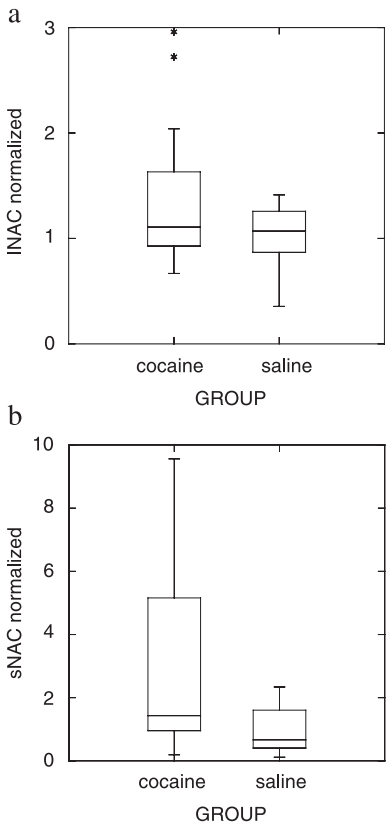

Fig. 3. Box plot of NAC1 isoform expression in the nucleus accumbens of cocaine-treated (n=20) and saline-treated rats (n=19). Levels of NAC1 were measured by Western blot analysis and evaluated by unpaired t test. (a) lNAC1 (P=.044). (b) sNAC1 (P=.004).

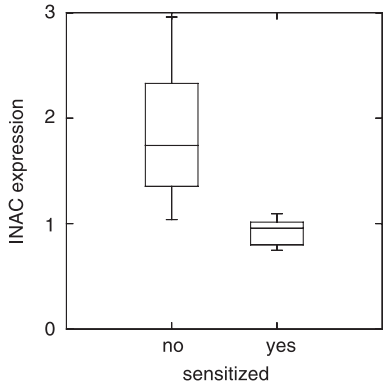

Fig. 4. Box plot of lNAC1 expression in the late group (1–3 months) of cocaine-rechallenged rats categorized by behavioral sensitization (n=12). Rats were classified as behaviorally sensitized (yes or no), as previously defined. Normalized lNAC1 expression in the nucleus accumbens was determined by Western blot analysis and evaluated by t test (P=.013).

Regression analysis was used to model the behavior variables of sensitization as a function of lNAC1 expression. This analysis revealed some trends in the whole group, but no significant differences. However, in the late group, lNAC1 expression predicted approximately 40% of the variance in the behavior variables. As shown in Fig. 5, R^2=.387 and P=.031 for distance. A significant difference was also determined for ambulatory count (R^2=.410 and P=.025). In addition, both correlations were negative, demonstrating an inverse relationship between NAC1 expression and behavioral sensitization, as hypothesized.

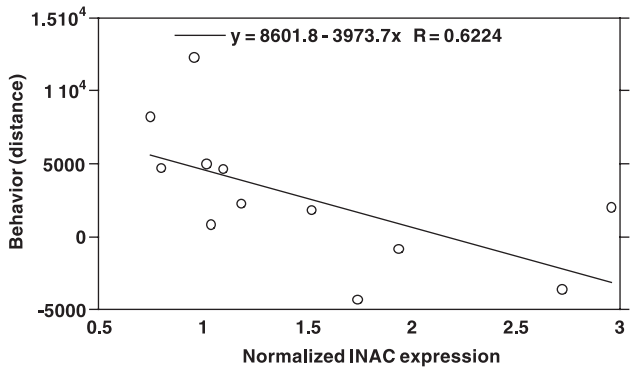

Fig. 5. Correlation of lNAC1 expression and behavior sensitization. The behavioral measurement for the distance traveled between first exposure to cocaine and test day 1–3 months after last exposure and normalized lNAC1 expression were analyzed by linear regression. (R = – 0.629, P=.028). High expressers (normalized lNAC1 values of >1.75) demonstrate no behavioral sensitization.

There were no significant relationships between either isoform of NAC1 and behavior for any other brain region.

4. Discussion

4.1. Behavioral sensitization

Behavioral sensitization is the process by which repeated administration of a psychomotor stimulant drug such as cocaine produces a progressively greater and enduring behavioral response characterized by enhanced motor activity. Predictably, in this study, the rats considered as a group developed behavioral sensitization to cocaine administration. While there was variability in the present sensitization data, this is consistent with previous studies, and is not entirely due to strain-, hormonal-, or context-specific factors (Hooks et al., 1991; Cailhol and Mormede, 1999). This variability seen at the behavioral level reflects an underlying neuroplasticity, which may be modulated, in part, by differences in expression of NAC1 produced by interaction with drug.

4.2. Differences in NAC1 levels

The hypothesis that the NAC1 levels will correlate inversely with the amount of activity was supported by the data in the present experiments. This relationship appeared to be limited to the long isomer, lNAC1. The lNAC1 levels were high when activity was low and were low when activity was high. The average normalized lNAC1 level was the same in both the sensitized and saline rats, whereas the average lNAC1 level was almost twofold higher in those rats showing no evidence of sensitization following exposure to cocaine. This suggests that expression of lNAC1 functions to prevent those neuroadaptations resulting from repeated cocaine injections. These findings confirm previous studies demonstrating that reduction of NAC1 expression in the nucleus accumbens by microinjection of NAC1 antisense oglionucleotides potentiated the expression of behavioral sensitization (Kalivas et al., 1999), while virally mediated overexpression of NAC1 in the nucleus accumbens attenuated the development of behavioral sensitization (Mackler et al., 2000). The final cocaine injection would not explain the increased NAC1 levels for two reasons. First, both NAC1 mRNA (Cha et al., 1997) and protein (Kortula et al., 2002) levels returned to baseline within a day following a single cocaine injection. Second, and more importantly, each group that had received saline injections for five consecutive days received cocaine on the last day of open-field monitoring.

There appears to be a differential expression of the two NAC1 isoforms in response to drug exposure. Previous work has demonstrated that only sNAC1 expression increased rapidly and transiently in the nucleus accumbens 2 h following a single acute intraperitoneal cocaine injection (Kortula et al., 2002). Whereas in the present study, only lNAC1 expression was increased with repeated administration of cocaine and was associated with a lack of behavioral sensitization.

A possible source for the differential expression of NAC1 isoforms could be differential regulation by the transcription factor complex, activator protein (AP-1). NAC1 gene expression is controlled by an AP-1 site (Mackler et al., 2002). Furthermore, there is considerable work identifying the transcription factors induced following cocaine administration (reviewed in Hope, 1998; Torres and Horowitz, 1999). These transcription factors, Fos, Jun, and other leucine zipper proteins, can dimerize to form AP-1 complexes, which bind in the major groove of DNA at AP-1 sites. Acute administration of cocaine caused the rapid and transient (1–4 h) induction of several Fos family members (c-Fos, FosB, Fra-1, and Fra-2) in the nucleus accumbens (Graybiel et al., 1990; Hope et al., 1994; Moratalla et al., 1996). AP-1 binding was increased in the nucleus accumbens for both acute and chronic cocaine administration (Hope et al., 1992), and has also been observed in human brain endothelial cells in vitro (Hope et al., 1994; Lee et al., 2001). Chronic cocaine administration also induces Fos isoforms in the nucleus accumbens, including a stable Fra, delta FosB (Hope, 1998), and produces an accumulation of the chronic AP-1 complex (Hope, 1996).

Several mechanisms can explain the observed differences between lNAC1 and sNAC1 levels. These include differential gene expression, mRNA stability, and protein synthesis and/or degradation. These mechanisms are not mutually exclusive. Earlier work indicates that differences in mRNA levels may account for unequal lNAC1 and sNAC1 protein amounts (Kortula et al., 2002). Thus, it is interesting that these cocaine-induced changes in components of the AP-1 complex could result in the differential expression of lNAC1. There may also be interactions with other *cis*-acting sequences in the promoters and with other transcription factors. This complicated interaction with the AP-1 site has been observed with matrix metalloproteinase gene expression (Benbow and Brinckerhoff, 1997). Therefore, additional alterations in gene expression that are induced by each acute administration during repeated cocaine exposure could lead to the accumulation of lNAC1 expression.

4.3. Temporal relationship of increased NAC1 expression and protection against sensitization

The present study also demonstrates a time course of NAC1 expression that is consistent with the long-term changes that are seen during the development of sensitization. For all of the statistical tests, the groups of rats were separated into "all times" and "late groups" (only 1, 2, and 3 months). Only the "late groups" had a significant amount of the variability in behavior explained by lNAC1 expression. In the late groups, the relationship between NAC1 expression and behavioral changes was more robust and statistically significant. This established a temporal relation-

ship between increased NAC1 expression and protection against sensitization.

The expression of behavioral sensitization (Kalivas et al., 1988) and attempts to reinstate cocaine self-administration (Grimm et al., 2001) can increase with time, even months after the initial exposure. This time course is similar to the increased NAC1 levels in the present study. The inverse relationship between lNAC1 and behavioral sensitization suggests that lNAC1 limits the extent of the augmented locomotor responses. This has been supported by viral overexpression work (Mackler et al., 2000). The protective role of NAC1 could be useful in ameliorating the long-term effects of drug use, especially after drug administration has discontinued. As NAC1 is also expressed in the human genome, the possible use in human intervention is intriguing. Currently, pharmacotherapy for cocaine dependence is problematic as there are many aspects of addiction that must be considered. Blocking the reward aspect of addiction does not address the craving and low hedonic function apparent in periods of abstinence. The lack of biological markers for reversal of the neuroadaptations associated with cocaine addiction in animal models is also problematic (reviewed in Dackis and O'Brien, 2002). NAC1 may present a potential pharmacological agent or a useful biomarker for reversal of the neuroadaptations associated with cocaine addiction.

5. Summary

Rats chronically injected with cocaine were more active than similarly treated control rats that were injected with cocaine acutely on the test day. Both isoforms of NAC1 were expressed more strongly in those rats exposed to cocaine than in rats exposed to saline in the nucleus accumbens but not in other regions of the brain. However, the effect on behavioral changes appeared to be confined to the long isoform of NAC1. Behavioral responses to repeated injections of cocaine were inversely related to the amount of lNAC1 in the nucleus accumbens. Nonsensitized rats had significantly higher lNAC1 counts than the sensitized rats. A stronger relationship between lNAC1 and behavioral sensitization was exhibited in the 1-, 2-, and 3-month groups. The expression of lNAC1 after chronic exposure to cocaine appears to protect rats from developing behavioral sensitization as a result of repeated cocaine injection. Therefore, NAC1 may limit long-term neuroplastic adaptations to cocaine use.

Acknowledgements

This work was supported by the Veterans Administration (DVA-MR-393) and the NIH (DA 11809-03). The authors appreciate the editorial efforts of Jennifer Phillips, PhD, and Lynn Snyder-Mackler, PT, ScD.

References

Benbow U, Brinckerhoff CE. The AP-1 site and MMP gene regulation: what is all the fuss about? Matrix Biol 1997;15:519–26.

Cailhol S, Mormede P. Strain and sex differences in the locomotor response and behavioral sensitization to cocaine in hyperactive rats. Brain Res 1999;842:200–5.

Cha X-Y, Pierce RC, Kalivas PW, Mackler SA. NAC1, a rat brain mRNA, is increased in the nucleus accumbens three weeks after chronic cocaine self-administration. J Neurosci 1997;17:6864–71.

Dackis CA, O'Brien CP. Cocaine dependence: the challenge for pharmacotherapy. Curr Opin Psychiatry 2002;15:261–7.

Graybiel AM, Moratallam R, Robertson HA. Amphetamine and cocaine induce drug-specific activation of the c-*fos* gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci USA 1990;88:1291–5.

Grimm JW, Hope BT, Wise RA, Shaham Y. Neuroadaptation. Incubation of cocaine craving after withdrawal. Nature 2001;412:141–2.

Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB. Individual differences in amphetamine sensitization. Pharmacol Biochem Behav 1991; 38:467–70.

Hope BT. Novel transcription factors are induced by chronic cocaine treatment. Ann NY Acad Sci 1996;801:1–12.

Hope BT. Cocaine and the AP-1 transcription factor complex. Ann NY Acad Sci 1998;844:1–6.

Hope B, Kosofsky B, Hyman SE, Nestler EJ. Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. Proc Natl Acad Sci USA 1992;89:5764–8.

Hope BT, Nye HE, Kelz MB, Self DW, Iadarola MJ, Nakabeppu Y, et al. Induction of long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. Neuron 1994;13:1235–44.

Kalivas PW, Duffy P, DuMars LA, Skinner C. Neurochemical and behavioral effects of acute and daily cocaine. J Pharmacol Exp Ther 1988; 245:485–92.

Kalivas PW, Duffy P, Mackler SA. Interrupted expression of NAC1 augments the behavioral responses to cocaine. Synapse 1999;33:153–9.

Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. Science 1988;242:715–23.

Kortula L, Wang PJ, Lewis DM, Neustadter JH, Stromberg MF, Mackler SA. Differences in expression, actions and cocaine regulation of two isoforms for the brain transcriptional regulator NAC1. Neuroscience 2002;110:421–9.

Lee YW, Hennig B, Fiala M, Kim KS, Toborek M. Cocaine activates redox-regulated transcription factors and induces TNF-alpha expression in human brain endothelial cells. Brain Res 2001;920:125–33.

Mackler SA, Kortula L, Cha X-Y, Koebbe MJ, Fournier KM, Bowers MS, et al. NAC1 is a brain POZ/BTB protein that can prevent cocaine-induced sensitization in the rat. J Neurosci 2000;20:6210–7.

Mackler SA, Homan YX, Conti AC, Korutla L, Blendy JA. Cloning and promotor analysis of mouse Nac1 gene program no. 500.15. 2002 Abstract viewer/itinerary planner. Washington (DC): Society for Neuroscience; 2002. Online.

Moratalla R, Vallejo M, Elibol B, Graybiel AM. D1-class dopamine receptors influence cocaine-induced persistent expression of Fos-related proteins in striatum. Neuroreport 1996;8:1–5.

Nestler EJ. Genes and addiction. Nat Gen 2000;26:277–81.

Nestler EJ. Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2001;2:119–28.

Robinson TE, Berridge KC. The neural basis of drug craving: an incentive–sensitization theory of addiction. Brain Res Brain Res Rev 1993; 18:247–91.

Torres G, Horowitz JM. Drugs of abuse and brain gene expression. Psychol Med 1999;61:630–50.