Striatal neuroadaptations regulated by the transcription factor Sp1 are induced by chronic cocaine exposure in rodents

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INTRODUCTION

Chronic cocaine exposure produces numerous neuroadaptations in dopamine-regulated signaling cascades that are associated with aberrant neuroplasticity and addictive-like behavior in experimental animals. To better understand the details of these drug-induced alterations, we previously used proteomics to analyze synaptoneurosomes prepared from the striatum and orbitofrontal cortex of cocaine-exposed non-human primates. A large number of regulated proteins were identified using a combination of 2-D Differential Gel Electrophoresis (DIGE) and multiplexed isobaric tagging for relative and absolute quantitation (iTRAQ). Notably, proteins that were highly enriched were found by GeneGo/MetaCore data analysis to be predominantly regulated by the ubiquitiously expressed transcription factor Sp1 (Specificity Protein 1). Cocaine-induced alterations in Sp1 activity may thus be involved in the persistent behavioral and cellular effects of prior repeated cocaine exposure. Of particular interest are published reports that Sp1 regulates several genes involved in dopamine neurotransmission (Minowa et. al., 1994; Yajima et. al., 1998; Yang et. al., 2000). However, the role of Sp1 in the neurobiological effects of chronic psychostimulant exposure is not known. Additionally, the functional consequences of post-transcriptional modifications of Sp1, whether inhibitory or excitatory, and the role that these modifications might play in Sp1's transcriptional regulation of downstream genes such as dopamine D2 receptors, has not been characterized. Here we investigated the biochemical consequences of repeated cocaine exposure on Sp1 function using several levels of analyses: 1) DNA binding activity, 2) Sp1 mRNA and protein expression, 3) levels of Sp1 cofactors/binding partners (CRSP, TFII4, TFIIF), 4) post-translational modifications, and 5) levels of Sp1-regulated target proteins. The present studies demonstrate that prior chronic cocaine exposure influences both DNA binding, Sp1 phosphorylation and the levels of its target proteins. Current experiments are examining the biochemical details of these cocaine-induced alterations in order to determine the contribution of Sp1-mediated processes to the development of aberrant cocaine-induced plasticity and behavior relevant to addiction.

METHODS

Subjects and Treatment: Male Sprague-Dawley rats received daily injections of cocaine (15 mg/kg/day i.p.) or saline for 15 days (n=10 saline, n=9 cocaine). Following 10 days of withdrawal, animals were sacrificed by rapid decapitation, and brains were fresh frozen on dry ice and stored at -80ºC unil use. Additionally, two naive, untreated adult male Sprague-Dawley rats were sacrificed as previously described.

Tissue Harvest: Frozen brains were cut into 2 mm slices using a chilled brain matrix, and bilateral punches were collected from the dorsal striatum (Bregma 1.2 mm) using a 1.5 mm punch. In the naive animals, bilateral punches were taken of the medial prefrontal cortex, orbitofrontal cortex, anterior cingulated, dorsal striatum, nucleus accumbens, hippocampus, amygdala and ventral tagmental area.

Nuclear Extraction and Sp1 Binding Assay: Nuclear and cytosolic fractions were isolated using one bilateral punch of the dorsal striatum using a Nuclear Extraction kit (Active Motif). 1.13 ug of nuclear protein per animal was used to determine Sp1 binding activity using the TransAM Sp1 transcription factor ELISA (Active Motif). Recombinant Sp1 protein (Active Motif) was used to generate a standard curve for quantification of Sp1.

Protein Expression of Sp1, Cofactors of Sp1, and Downstream Targets: Immunoblotting was done on nuclear and cytosolic fractions, in addition to total protein homogenates from the dorsal striatum of each subject. Standard Western blotting procedures were used. Primary antibodies used to quantify protein level included: Sp1 (Santa Cruz), Sp3 (Santa Cruz), TFIID (Santa Cruz), CRSP130 (Santa Cruz), Dopamine D1 Receptor (Abcam), Dopamine D2 Receptor (Abcam), ERK (Cell Signaling), phospho-ERK (Cell Signaling), Histone H3 (Cell Signaling), S100-beta (Abcam), and GAPDH (Advanced Immunochemical). Fluorescent secondary antibodies for rabbit and mouse primary antibody detection were used (Rockland Immunochemicals), and relative fluorescence was detected using the LiCor Odyssey imaging system (LiCor).

mRNA Expression of Sp1, Cofactors of Sp1, and Downstream Targets: Total RNA was extracted from one bilateral punch from each subject using the mirVana miRNA Isolation Kit (Applied Biosystems). Equal amounts of RNA was converted to cDNA using the SuperScript III First-Strand Synthesis System (Invitrogen). The following TaqMan (Applied Biosystems) gene expression assays were used to quantify mRNA: Sp1 (Rn00561953_m1), Dopamine Drd1a (Rn03062203_s1), Dopamine Drd2 (Rn00561126_m1), CRSP3 (Rn01537419_m1), TFIID (Rn01455646_m1), Rat actin, beta. Equal amounts of cDNA from each subject was run in triplicate for each assay according to the manufacturer's suggestions. mRNA levels were quantified using the 7500 Fast Real-Time PCR System and Sequence Detection Software (SDS). The ddCt method of analysis was used to determine relative quantification of mRNA.

Brain Region Specific Sp1 Protein Expression: As previously stated, several other brain regions were collected from two naive rats. Relative Sp1 protein expression was quantified from equal amounts of total protein in each brain region.

RESULTS

Figure 1: ***Sp1 Transcription Factor pathway interactions.***

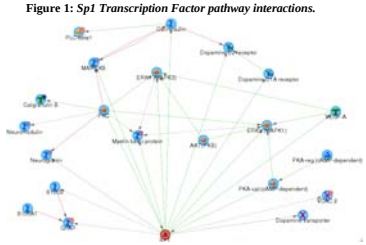


Figure 2: ***Confirmed Modification Sites on Sp1.***

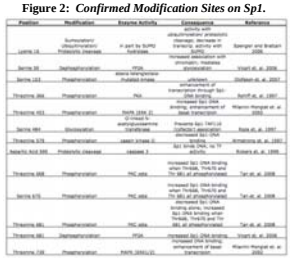


Figure32: ***Dorsal stratum tissue punch location.***

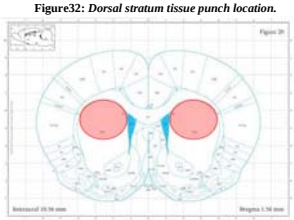


Figure43: ***Sp1 Binding Activity.***

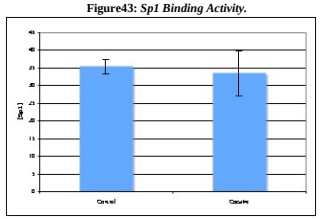


Figure 5: ***Nuclear Extract Protein Expression Quantification.*** All isoforms (non-phosphorylated, phosphorylated, modified, and attenuated) were quantified in addition to Sp3 and predicted cofactors. (#, p=0.09-0.051; *, p=0.05-0.01)

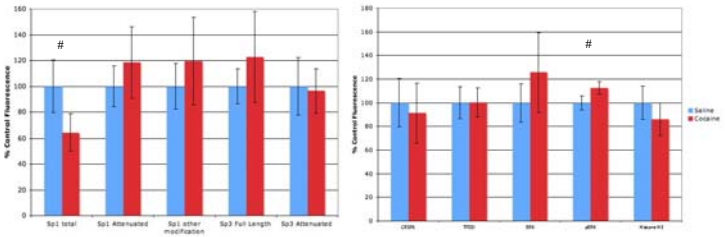


Figure 6: ***Total Cell Lysate Protein Expression Quantification.*** All isoforms of Sp1 and Sp3 were quantified. Predicted cofactors and downstream targets of Sp1 were also quantified to determine possible interactions. #, p=0.09-0.051; *, p=0.05-0.01)

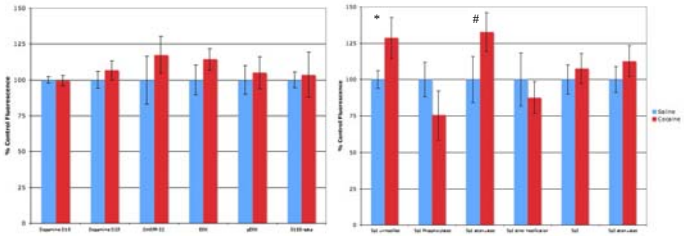


Figure 7: ***Quantitative Real-Time PCR.*** qPCR was done on Sp1, confirmed cofactors of Sp1, and genes we predicted to be under the transcriptional control of Sp1. No changes in mRNA were found between cocaine and saline treated groups using the ddCt method and beta-actin as the endogenous control.

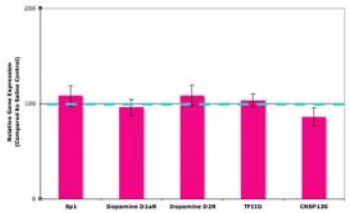
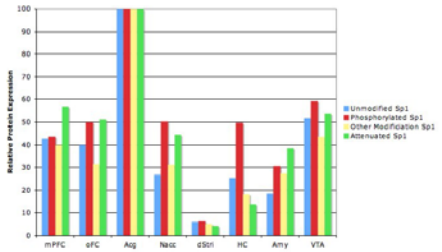


Figure 8: ***Brain Region Specific Relative Sp1 Protein Expression.***



CONCLUSIONS

Based on established literature and our unpublished dats, we expected to observe dysregulation of dopamine receptors in the dorsal striatum, however no changes were found in D1 or D2 receptors on the protein or mRNA level in this brain region.

There was no change observed in Sp1 binding activity after cocaine treatment, which was not the expected outcome, since we had previously observed an increase in Sp1 binding activity in non-human primates. This may be explained by a species difference, or more likely, a difference in Sp1 localization. There was a trend for a decrease in Sp1 in the nuclear fraction that did not correlate to total Sp1 protein or mRNA levels after cocaine treatment. So perhaps, there is enhanced Sp1 activity in rats after cocaine treatment, but there is less available Sp1 in the nucleus to affect transcription.

Further findings also suggest that there may be a shift in localization of Sp1. In the nuclear fraction, we observed a trend for a decrease in the unmodified species of Sp1, while in the whole cell lysate, there was a significant increase in the same species of Sp1. A trend for increase was also found in the attenuated form of Sp1 (product of proteolysis). If Sp1 is indeed being transported from the nucleus, this could explain the decrease in Sp1-DNA binding, and Sp1's ability to exert transcriptional control. Previous literature has shown that there is an increase in Sp1 in the cytosol following inhibition of acetylglucosaminyltransferase, which glycosylates Sp1 (Dauphinee et. al., 2005).

It is known that glycosylation is regulated through glutamine, and cocaine is potentially affecting glutamine metabolism (Baker et. al., 2003). One possible mechanism for Sp1 transport is that cocaine treatment and withdrawal results in reduced extracellular glutamate, leading to a decrease in acetylglucosaminyltransferase, which in turn would mean less glycosylated Sp1. Unglycosylated Sp1 would then be transported to the cytosol, rendering it inactive.

Ongoing studies seek to further elucidate post-translational modifications on Sp1 as a consequence of cocaine exposure, and what role they play in Sp1 cellular localization, transcriptional activity and control over downstream genes. We also want to examine in depth, the importance of Sp1 in psychostimulant drug abuse through knockdown and over-expression of Sp1 *in vivo*.

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SUPPORT

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