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Gene Expression Profiling Reveals Distinct Cocaine-Responsive Genes in Human Fetal CNS Cell Types

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Abstract

Objectives—Prenatal exposure to cocaine causes cytoarchitectural alterations in the developing neocortex. Previously, we reported that cocaine inhibits neural progenitor cell proliferation through oxidative endoplasmic reticulum stress and consequent down-regulation of cyclin A, whereas cyclin A expression was increased in astrocytes. In the present study, cell type-specific responses to cocaine were further explored.

Methods—Gene expression profiles were examined in five types of cells obtained from the human fetal cerebral cortex at 20 weeks gestation. Cells were treated with 100 μM cocaine *in vitro* for 24 hr, followed by gene expression analysis using a human neural/stem cell/drug abuse-focused cDNA array, with verification by quantitative real-time RT-PCR.

Results—Cocaine influenced transcription of distinct categories of genes in a cell type-specific manner. Cocaine down-regulated cytoskeleton-related genes including *ezrin*, *γ2 actin*, *α3d tubulin* and *α8 tubulin* in neural and/or A2B5+ progenitor cells. In contrast, cocaine modulated immune and cell death-related genes in microglia and astrocytes. In microglia, cocaine up-regulated the immunoregulatory and pro-apoptotic genes *IL-1β* and *BAX*. In astrocytes, cocaine down-regulated the immune response gene *glucocorticoid receptor* and up-regulated the anti-apoptotic genes *14-3-3 ε* and *HVEM*. Therefore, cell types comprising the developing neocortex show differential responses to cocaine.

Conclusions—These data suggest that cocaine causes cytoskeletal abnormalities leading to disturbances in neural differentiation and migration in progenitor cells, while altering immune and apoptotic responses in glia. Understanding the mechanisms of cocaine's effects on human CNS cells may help in the development of therapeutic strategies to prevent or ameliorate cocaine-induced impairments in fetal brain development.

Keywords

cocaine; microarray; gene expression profiling; human fetal CNS cells; brain development

Understanding the mechanisms through which cocaine causes adverse effects on the developing brain has received considerable attention, because several hundred thousand fetuses

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per year are exposed to cocaine in the United States alone (Singer et al., 2002). Clinical studies have correlated neocortical architecture modifications and behavioral disturbances with *in utero* cocaine exposure (Chiriboga et al., 1999; Bellini et al., 2000; Singer et al., 2008). In sub-human primates, administration of cocaine at the time of neocortical neurogenesis (the second trimester) disturbs development of cerebral cortex, causing reduced neocortex volume, disturbed lamination, altered positioning of cerebral cortical neurons, and reduced density and number of cortical neurons (Lidow, 1995; Lidow and Song, 2001). Notably, these anatomical abnormalities result in neurobehavioral impairments in infant and adolescent sub-human primates similar to those seen in humans, including deficits in attention, orientation, state control, and motor maturity (He et al., 2004). In addition to the action of cocaine on neurons, interference with astroglial development in developing cerebral cortex has been reported in animal fetuses prenatally exposed to cocaine (Akbari et al., 1994; Lidow, 1995; Nassogne et al., 1998).

In vitro, cocaine has been shown to selectively induce neuronal death without affecting the survival of glial cells (Nassogne et al., 1995). We have also found that cocaine specifically inhibits proliferation of neural progenitor cells by inducing down-regulation of cyclin A2 triggered by oxidative endoplasmic reticulum stress, an effect which is not seen in neurons or microglia (Lee et al., 2008). In contrast, cocaine increases the *cyclin A2* transcript in human astrocytes (Lee et al., 2008). These data suggested that prenatal cocaine exposure may cause abnormalities in the developing cerebral cortex through multiple and divergent pathways in different types of cells.

By using a human neural/stem cell/drug abuse-focused cDNA array (hNSDA array) designed for analysis of development/maintenance of central nervous system (CNS) cell types as well as for effects of drugs of abuse, we profiled cocaine-induced gene expression changes in five types of CNS cells obtained from the developing human fetal cerebral cortex. The effects of cocaine on brain development are largely based on studies in rodent model systems, and although rodents and humans have generally similar patterns of brain development, there are some differences (Kornack and Rakic, 1998; Letinic et al., 2002). Characterization of gene expression changes induced by cocaine in primary human CNS cells *in vitro* may help to extend the understanding of cocaine's effects on brain development to the human brain. The results suggest that cocaine down-regulates cytoskeleton-related genes in progenitor cells, while modulating expression of immune and cell death-related genes in glial cells. Cocaine-induced cell-type specific alterations in gene expression may cooperate to cause overall cytoarchitectural abnormalities, by interrupting morphological development of cortical progenitor cells while altering immune and apoptotic functions of glial cells.

MATERIALS AND METHODS

Cell Culture and Treatments

Primary human fetal CNS cells (ScienCell Research Laboratories, San Diego, CA), including neural progenitor cells, A2B5+ progenitor cells, neurons, astrocytes and microglia, were obtained from ~20-week human fetal cerebral cortexes, in accordance with principles embodied in the Declaration of Helsinki (Code of Ethics of the World Medical Association). Primary human cells were cultured as previously described (Lee et al., 2008). Human fetal CNS cultures were maintained for 8–21 days *in vitro* and treated with 100 μM cocaine hydrochloride (National Institute on Drug Abuse, Baltimore, MD) for 24 hr. The concentration of 100 μM is frequently used for *in vitro* studies of cocaine (Nassogne et al., 1995; Lee et al., 2008). Although relatively high, this concentration can be reached in the brain of chronic human cocaine users (Kalasinsky et al., 2000). Additionally, there is a dose-response relationship between the effects of cocaine and its concentration; exposure to high levels of cocaine *in utero* is correlated with the greatest impairments in human prenatal brain growth (Delaney-

Black et al., 1996; Chiriboga et al., 1999). We employed this dose to produce maximal effects, and thus to avoid finding differences between cell types based on variable sensitivity to lower doses of cocaine.

Human Neural/Stem Cell/Drug Abuse-Focused cDNA Array (hNSDA Array)

Two independent samples from individual brains were used for each group analyzed by microarray. Total RNA was extracted from human fetal CNS cultures using RNA STAT-60 (TEL-TEST, Friendswood, TX). Microarray analysis was performed using a hNSDA array containing triplicate repeats of 2400 clones, of which 56% were from the human mammalian gene collection (Strausberg et al., 1999), 33% from Research Genetics, and 11% from Invitrogen. The hNSDA array includes 2400 genes related to nervous system function, neurogenesis and neural stem cell differentiation. In addition, genes related to drug metabolism, stress signaling, cell survival and toxicity are included.

Complete materials and methods including immunocytochemistry, microarray procedure, microarray data analysis, quantitative real-time RT-PCR, and associated references are available as Supplementary methods.

RESULTS

Cell Characterization

The human neural progenitor cells used in this study were nestin-positive and A2B5-negative (> 95% of cells, data not shown), and A2B5+ progenitor cells were A2B5-positive and nestin-positive (> 90% of cells, data not shown) (Fig. 1A). A2B5+ progenitor cells can only differentiate to glial cells, oligodendrocytes and astrocytes, while neural progenitor cells can become both glial cells and neurons. The human neurons, which were MAP-2 immunopositive (Fig. 1A), were immunoreactive for glutamate (44 ± 3%), and GABA (69 ± 5%), but not for tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), or 5-hydroxytryptamine (5-HT) (data not shown). In addition, the human astrocytes and microglia were glial fibrillary acidic protein (GFAP)-positive and OX42 (CD11b/c)-positive, respectively (> 99% of cells) (Fig. 1A).

Human Neural/Stem Cell/Drug Abuse-Focused cDNA Array (hNSDA Array)

To test the ability of the hNSDA array to discriminate CNS cell types, gene expression profiles in five types of CNS cells (Fig. 1A) obtained from two independent 20-week human fetal cerebral cortexes and maintained *in vitro* were analyzed. Hierarchical clustering was employed to examine overall similarities and differences in gene expression for the five types of CNS cells. The clustering program was supplied with the averages of all individual gene z-scores, which were obtained from replicate microarray data of each cell type. A two-way clustering analysis separated the five cell types into three distinct groups (Fig. 1B). The two types of human fetal progenitor cells (neural and A2B5+ progenitors) shared a high degree of similarity in transcriptional profiles, but diverged from those of differentiated CNS neurons and astrocytes, and from hematopoetic lineage-derived microglia. The similarity between neural and A2B5+ progenitor cells, and between neurons and astrocytes was also reflected by the principal components analysis (PCA) results (Fig. 1C), in a manner similar to that seen by hierarchical clustering. The six points for each cell type represent triplicates of all 2400 genes for two independent samples. These data suggest that the hNSDA array successfully distinguished differences between CNS cell populations by gene expression profiles.

Profiling of Cocaine-Regulated Genes in Primary Human CNS Cells

The five different types of primary human CNS cells described above were treated with 100 μM cocaine for 24 hr, and RNA from two independent paired experiments was subjected to transcriptional profiling with a hNSDA array. Z test analysis identified cocaine-responsive genes that fulfilled a stringent cut-off of $p<0.05$ and $|z\ ratio| \geq 2.5$. By these criteria, numbers of genes responsive to cocaine were 15 in neural progenitor cells, 29 in A2B5+ progenitor cells, 27 in neurons, 23 in microglia, and 15 in astrocytes (Table 1).

The FatiGO web tool, which extracts gene ontology terms that are significantly represented by groups of genes (Al-Shahrour et al., 2004), revealed that cocaine influenced distinct categories of genes in a cell-type specific manner (Supplementary Fig. 1). Supplementary figure 1 represents the ten most highly-represented categories influenced by cocaine for each type of cell. Although there were some categories that were altered across all or most of the five cell types (e.g., transcription, cellular protein metabolism, and phosphate metabolism), some ontology terms appeared to unique to certain cell types. Cocaine tended to down-regulate cytoskeleton-related genes including *ezrin* (*villin 2*), γ*2 actin* (*actin, gamma 2, smooth muscle, enteric*), α*3d tubulin* (*tubulin, alpha 3d*), and α*8 tubulin* (*tubulin, alpha 8*) in neural and/or A2B5+ progenitor cells (Tables 1A and 1B). Among these, ezrin was strongly down-regulated by cocaine in both neural (z ratio = − 3.1) and A2B5+ progenitor cells (z ratio = −4.7). In addition, cocaine down-regulated several transcription factors related to nervous system development such as *DLX6* (*distal-less homeobox 6*), *BRN-3A* (*POU domain, class 4, transcription factor 1*), and *PAX5* (*paired box gene 5*) in neurons (Table 1C). On the other hand, cocaine modified immunomodulatory and cell survival-related genes in microglia and astrocytes (Supplementary Fig. 1). Cocaine up-regulated the immunocytokine and pro-apoptotic genes *IL-1*β (*interleukin 1, beta*) and *BAX* (*BCL2-associated X protein*) in microglia (Table 1D), but down-regulated the immune response gene *glucocorticoid receptor* (*nuclear receptor subfamily 3, group C, member 1*), and up-regulated the anti-apoptotic genes *14-3-3* ε (*tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide*) and *HVEM* (*tumor necrosis factor receptor superfamily, member 14*) in astrocytes (Table 1E). Thus, the effect of cocaine varies for different types of CNS cells that are involved in cerebral cortex development.

Chromatin remodeling has been suggested to be involved in cocaine-induced gene expression changes in neurons (Kumar, 2005). Exploration of cocaine-regulated genes using WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt/), which aligns groups of genes along chromosomes, did not show a significant clustering of genes up-or down-regulated by cocaine.

The oPOSSUM web tool (http://burgundy.cmmt.ubc.ca/oPOSSUM/) can be used to identify transcription factor binding sites that are over-represented in the promoters of sets of genes. oPOSSUM was used to search for transcription factor binding sites common to multiple genes up-or down-regulated by cocaine. Six transcription factors, MZF1_5–13, SRF, Arnt, SP1, USF1, and Mycn, were identified as cocaine-induced transcriptional changes in the various cell types (Supplementary Table 2). Further analysis would be required to determine if these transcription factors are involved in the modulation of expression of these genes by cocaine.

Quantitative Real-Time RT-PCR

Quantitative real-time RT-PCR analyses of the *ezrin*, γ*2 actin*, α*8 tubulin*, *DLX6*, *IL-1*β , *BAX*, *glucocorticoid receptor*, *and 14-3-3* ε gene products were performed for all five cell types. The quantitative real-time RT-PCR results were very similar to the results of the microarray (Table 2). Cocaine decreased expression of *ezrin*, γ*2 actin* and α*8 tubulin*, in neural progenitor cells (0.16 ± 0.05, 0.27 ± 0.02, and 0.32 ± 0.12, respectively) and A2B5+ progenitor cells (0.38 ± 0.07, 0.52 ± 0.05, and 0.23 ± 0.05, respectively), decreased expression of *DLX6* (0.23 ± 0.11)

in neurons, and increased expression of *IL-1*β (3.70 ± 0.19) and *BAX* (8.97 ± 2.63) in microglia. In astrocytes, decreased expression of *glucocorticoid receptor* (0.39 ± 0.03) and increased expression of *14-3-3* ε (2.10 ± 0.33) were confirmed.

Although cocaine-induced down-regulation of α*8 tubulin* and γ*2 actin* (in neural and A2B5+ progenitor cells, respectively) did not fulfill our threshold criteria for positive transcripts from the microarray data (both genes $p<0.001$ but $|z\ ratio|<2.5$), quantitative real-time RT-PCR confirmed that both were significantly down-regulated by cocaine. Similarly, we found that cocaine significantly decreased *cyclin A2* in neural and A2B5+ progenitor cells, and increased *cyclin A2* in astrocytes (z ratio: neural, −1.0; A2B5, −1.1; astrocytes, 2.0). Although these changes did not meet the 2.5 z ratio criterion used in the present study, they were nonetheless statistically significant and consistent with similar changes found previously by quantitative RT-PCR (Lee et al., 2008). While it was necessary to employ the very restrictive 2.5 z ratio criterion due to the small sample size, this also would have minimized false-positives. However, only the transcripts most markedly affected by cocaine would have been detected in the present study.

DISCUSSION

Neonatal exposure to cocaine causes both morphological and neurobehavioral alterations in the developing human brain (Chiriboga et al., 1999; Bellini et al., 2000; Singer et al., 2008). The mechanisms involved in the adverse developmental effects of cocaine are, however, not fully known. Novikova and coworkers identified Wnt/cadherin and multiple apoptosis-signaling pathways as being involved in cocaine's actions on brain development from gene expression profiling of the cortex of cocaine-treated mouse fetuses (Novikova et al., 2005a, 2005b). Nonetheless, the cell type or types and cell-type specific gene alterations related to developmental effects of cocaine are unknown.

Administration of cocaine during the second trimester only, the period when neocortical neurogenesis is most active, is capable of disturbing neocortical cytoarchitecture in non-human primates (Lidow et al., 2001). Cocaine has previously been shown to induce distinct effects on different types of CNS cells (Nassogne et al., 1995; Lee et al., 2008). We identified several cell type-specific effects of cocaine, including modulation of cytoskeleton-related transcripts in progenitor cells; CNS development-related transcription factors in neurons; and immune and cell survival-related transcripts in glial cells.

Cytoskeleton Organization and Biogenesis

Transcripts involved in cytoskeletal organization and biogenesis were down-regulated by cocaine in both neural and A2B5+ progenitor cells. One of these cytoskeleton-related transcripts is *ezrin*, a member of the ERM (ezrin-radixin-moesin) family of membrane-cytoskeleton linker proteins regionally expressed in the developing neuroepithelium (Gimeno et al., 2004). In cultured neurospheres, ezrin was specifically localized in filopodia of adherent neuronal progenitor cells (Gronholm et al., 2005). Ezrin, in addition to its role in the regulation of cell shape, adhesion, and migration, is also involved in cellular signaling pathways, like Rho and PI3-kinase (Louvet-Vallee, 2000). Ezrin may also be involved in early embryonic development (Dard et al., 2001).

A number of transcripts encoding subunits of actin and tubulin filaments, such as γ*2 actin*, α*3d tubulin*, and α*8 tubulin*, were down-regulated by cocaine in neural and/or A2B5+ progenitors. In eukaryotic cells, microtubule and actin filaments interact to form the cytoskeletal network involved in determination of cell architecture, mitosis, motility, and differentiation. Increased rates of cytoskeletal protein synthesis occur during chick brain development (Bryan et al., 1981). In contrast, cytoskeletal components are reduced in the

frontal cortex of patients with neurodegenerative diseases, including Alzheimer's disease, Down's syndrome, and Pick's disease (Pollack et al., 2003). Down-regulation of cytoskeletal genes in progenitor cells by cocaine might lead to disturbances in neural differentiation and migration.

Transcription Factors Related to CNS Development

Genes encoding transcription factors related to nervous system development, such as *DLX6*, *BRN-3A*, and *PAX5*, were down-regulated in neurons by cocaine. Among these, *DLX6*, a member of a homeobox transcription factor gene family homologous to the distal-less (*Dll*) gene of Drosophila, is primarily expressed in subpopulations of neurons in the developing forebrain with roles in forebrain development (Merlo et al., 2000; Panganiban and Rubenstein, 2002). Down-regulation of these transcription factors in neurons by cocaine might be a consequence of cocaine-induced cytoskeleton derangement in neural progenitors, which may impact ensuing cortical neurogenesis.

Immune Response and Apoptosis

Genes involved in immune response and apoptosis were specifically changed by cocaine in glial cells. Cocaine up-regulated the proinflammatory cytokine *IL-1*β and the pro-apoptotic regulator *BAX* in microglia. IL-1β, produced by activated microglia, exerts a number of effects on cell proliferation, differentiation, and apoptosis in the brain (Gibson et al., 2004). BAX, a member of BCL2 family, functions as an apoptotic activator by permeabilizing the outer mitochondrial membrane, leading to the release of apoptogenic proteins such as cytochrome c (Er et al., 2006). In fact, cocaine-induced up-regulation of Bcl2 family genes such as Bcl-x (L) in the brains of cocaine self-administering rats has been reported (Ahmed et al., 2005), and could reflect apoptotic pressure in the brain. It is also interesting to note that increased expression of Bcl-x(L) has been shown to stimulate synaptic transmission (Jonas et al., 2003).

Microglia are detected in the developing CNS during the period of neurogenesis (Ashwell, 1991). One role of microglia in the developing CNS is thought to be to eliminate dead cells and debris by phagocytosis. A developmental role of microglia, in directing migration and differentiation of embryonic neural progenitor cells, has also been suggested (Aarum et al., 2003). Therefore, abnormal activation and/or apoptosis of microglia by cocaine might interrupt microglial orchestration of neurogenesis during development.

In contrast to microglia, cocaine down-regulated the immunoreactive gene *glucocorticoid receptor*, while up-regulating the anti-apoptotic genes *14-3-3* ε and *HVEM* in astrocytes. The glucocorticoid receptor, a ligand-activated transcription factor, has previously been reported to be down-regulated by cocaine in human astrocytoma cells (Malaplate-Armand et al., 2005). Activation of glucocorticoid receptors causes anti-inflammatory and immunosuppressive actions through inhibition of the pro-inflammatory transcription factor NF-kappa B activity, by induction of I kappa B alpha (Auphan et al., 1995). *14-3-3* ε mediates an anti-apoptotic signal through interaction with Bad, resulting in retention of Bad in the cytoplasm, preventing subsequent cytotoxic interaction with Bcl-x(L) in mitochondria (Won et al., 2003). HVEM, a member of the TNF-receptor superfamily, protects cells against TNF-α-mediated apoptosis by inhibiting caspase-3 and caspase-8 activation (Matsui et al., 2002). Astrocytes, the guiding structures for migrating neurons during brain development, provide maintenance, support, and protection to neurons (Kirchhoff et al., 2001). Changes of immunological and cell survival signaling of astrocytes by cocaine might affect their structural and trophic functions.

Monoaminergic System Transcripts

Cocaine induces its primary effects in the brain through monoaminergic systems. Nevertheless, the expression of *dopamine receptors (D1-5), adrenergic receptors (α-1A, 1B, 1D, 2A, 2B, β-1, 3), serotonin receptors (1E, 1F, 2A-C, 3A, 3B, 4, 5A, 6,7),* and all three types of monoamine transporters including *dopamine, norepinephrine, and serotonin transporters* were not changed by cocaine in our CNS cell cultures, at least at the transcriptional level (Supplementary Table 3). The only neurotransmitter-related transcript which was changed by cocaine was *dopamine beta-hydroxylase (DBH),* which catalyzes the synthesis of norepinephrine from dopamine in noradrenergic neurons. *DBH* was decreased (z ratios = −3.0 and −3.2) in both neural and A2B5+ progenitor cells (Tables 1A, 1B and Supplementary Table 3). Cocaine also tended to increase *DBH* transcript expression in microglia (z ratio = 3.4), although this difference was not significant by our criteria. The functional significance of DBH in neural and A2B5+ progenitor cells is, however, unknown.

Clinical Relevance

Cocaine exposure in utero is associated with multiple and prolonged behavioral and neurological changes. In addition to slower intrauterine growth, these babies are born with a smaller head circumference and neurological abnormalities such as hypertonia and tremor (Chiriboga et al., 1999). Persistent developmental effects of fetal cocaine exposure have also been found (Singer et al., 2008). Despite neurological indications, relatively little is known about the direct effect of cocaine in the developing brain, and the mechanisms by which prenatal cocaine exposure causes CNS injury are not clear. We have studied cocaine-induced changes in gene expression patterns in order to obtain a better understanding of the mechanisms involved in the adverse effects of in utero cocaine exposure.

CONCLUSION

The present study indicates that cocaine can directly alter gene expression profiles in a cell-type specific manner, even in non-dopaminergic, non-serotonergic CNS cells. We found that cocaine down-regulates cytoskeleton-related genes in neural and A2B5+ progenitor cells, but modulates immune response and cell death-related genes in glial cells (Fig. 2). These data suggest that exposure of infants to cocaine could have adverse effects on the development of both neural progenitor cells and glia.

We previously found that cocaine inhibits neural progenitor cell proliferation, and that this effect was due to oxidative endoplasmic reticulum stress consequent to P450 metabolism of cocaine, leading to phosphorylation of EIF2α, up-regulation of ATF4, and down-regulation of cylin A (Lee et al., 2008). Although this effect of cocaine is likely to contribute to developmental brain injury, it does not necessarily comprise the entire reason for cocaine's adverse developmental effects. It is necessary to understand the effects of cocaine on brain cell types other than neural progenitors, as well as obtaining a more comprehensive survey of the molecular effects of cocaine.

This study provides a general survey of the molecular effects of cocaine on the major cell types which are present in the developing brain, using primary human cells *in vitro*. At the present time, which of these molecular changes contribute to the adverse effects of cocaine on brain development is unknown. Future studies will focus on determining which if any of these effects are due to P450 metabolism of cocaine and consequent oxidative stress, how these various molecular events are interrelated, and which of these effects contribute to the adverse effects of cocaine on brain development. If the molecular events which lead to cocaine-induced developmental brain injury can be determined, it may be possible to develop drugs which can be used to prevent this form of developmental damage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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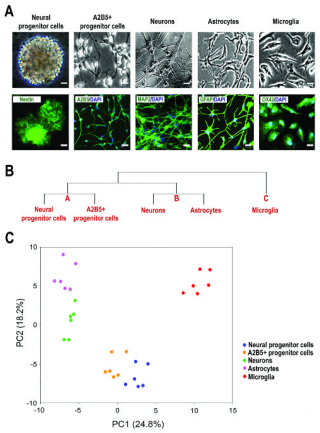

FIGURE 1.

Clustering of transcriptional profiles for five distinct types of human fetal CNS cells. (A) Phase contrast and immunostaining images of human fetal CNS cells. Five different types of human primary cells from cerebral cortex (8–21 days *in vitro*) were characterized by the CNS cell type-specific markers including nestin (green) for neural progenitor cells, A2B5 (green) for A2B5+ progenitor cells, MAP2 (green) for neurons, GFAP (green) for astrocytes and OX42 (green) for microglia. DAPI nuclear staining is shown in blue. All scale bars are 25 μm, except marker expression of neural progenitor cells and morphology of A2B5+ progenitor cells are 15 μm, and marker expression of A2B5+ progenitor cells, morphology and marker expression of astrocytes are 35 μm. (B) Hierarchical clustering result, showing that the two progenitor cell types, neural progenitor cells and A2B5+ progenitor cells, shared a high degree of similarity in transcriptional expression patterns (sublevel A). Two differentiated CNS cell types, neurons and astrocytes, also shared a high degree of similarity (sublevel B). Microglia showed a pattern of transcription different from the other four types of CNS cells. (C) Principal components analysis differentiating the five cell types.

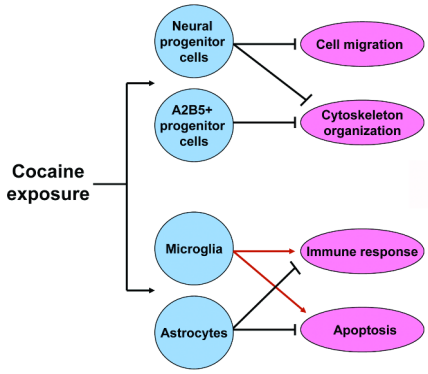

FIGURE 2.

Schematic diagram illustrating the cell type-specific action of cocaine on gene transcription. Alterations in cytoskeleton-related gene expression in multipotent cortical progenitor cells by cocaine may interrupt morphological development and migration of progenitors. Microglia provide neuronal immunomodulatory functions and play a developmental role during neurogenesis, and astrocytes provide trophic, metabolic and structural support for neurons. Cocaine may derange immunoregulatory functions and survival of glial cells. Activation of each specific response is denoted by →, inhibition of each specific response is denoted by ⊣.

Table 1

Cocaine-induced gene expression in human CNS cells.

A. Neural progenitor cells

Gene Name	*Gene Symbol*	*GenBank*	*Z ratio*
amyloid beta (A4) precursor-like protein 2	APLP2	BC000373	12.9
chloride channel Ka	CLCNKA	BC035373	8.7
unc-51-like kinase 1	ULK1	NM003565	7.9
neurogranin (protein kinase C substrate, RC3)	NRGN	BC002835	6.1
insulin-like growth factor 1 receptor	IGF-1R	NM000875	2.9
syndecan binding protein (syntenin)	SDCBP	BC013254	2.8
Kruppel-like factor 8	KLF8	BC031355	2.6
phospholipase C, beta 2	PLCB2	BC009009	−2.6
actin, gamma 2, smooth muscle, enteric (γ2 actin)	ACTG2	BC012617	−2.7
NADH dehydrogenase (ubiquinone) flavoprotein 1, 51kDa	NDUFV1	BC008146	−2.7
dopamine beta-hydroxylase (dopamine beta-monooxygenase)	DBH	BC017174	−3.0
villin 2 (ezrin)	VIL2	BC013903	−3.1
protein kinase, Y-linked	PRKY	BC074851	−6.5
general transcription factor IIIC, polypeptide 3, 102kDa	GTF3C3	BC015995	−10.1
nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	NFATC1	BC033848	−11.4

B. A2B5+ progenitor cells

Gene Name	*Gene Symbol*	*Genbank*	*Z ratio*
fibroblast growth factor 13	FGF13	BC034340	8.1
nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	NR3C1	BC015610	3.6
homer homolog 3	HOMER3	BC012113	3.5
destrin (actin depolymerizing factor)	DSTN	BC009477	3.2
filamin A, alpha (actin binding protein 280)	FLNA	BC014654	2.8
insulin-like growth factor binding protein 4	IGFBP4	BC016041	2.7
opioid growth factor receptor	OGFR	BC014137	2.5
growth arrest and DNA-damage-inducible, alpha	GADD45A	BC011757	2.5
galactosidase, alpha	GLA	BC002689	−2.5
DR1-associated protein 1 (negative cofactor 2 alpha)	DRAP1	BC010025	−2.6
bone morphogenetic protein 8b (osteogenic protein 2)	BMP8B	BC023526	−2.6
glutamate receptor, ionotropic, N-methyl D-asparate-associated protein 1 (glutamate binding)	GRINA	BC041788	−2.6
sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3G	SEMA3G	BC098104	−2.6
protein phosphatase 1, catalytic subunit, beta isoform	PPP1CB	BC002697	−2.6
enolase 2 (gamma, neuronal)	ENO2	BC002745	−2.8
GNAS complex locus	GNAS	BC002722	−2.9
homeobox B5	HOXB5	BC008940	−2.9
v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	ERBB3	BC002706	−2.9
SRY (sex determining region Y)-box 10	SOX10	BC007595	−3.0
chloride channel 6	CLCN6	BC050457	−3.1
dopamine beta-hydroxylase (dopamine beta-monooxygenase)	DBH	BC017174	−3.2

B. A2B5+ progenitor cells

Gene Name	*Gene Symbol*	*Genbank*	*Z ratio*
guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type	GNAL	BC050021	−3.3
natriuretic peptide precursor A	NPPA	BC005893	−3.9
tubulin, alpha 8 (α8 tubulin)	TUBA8	BC074827	−4.1
villin 2 (ezrin)	VIL2	BC013903	−4.7
Fas apoptotic inhibitory molecule 3	FAIM3	BC006401	−5.5
tubulin, alpha 3d (α3d tubulin)	TUBA3D	BC057810	−5.6
E2F transcription factor 4, p 107/p130-binding	E2F4	BC033180	−6.0
S100 calcium binding protein G	S100G	BC112174	−6.8

C. Neurons

Gene Name	*Gene Symbol*	*Genbank*	*Z ratio*
cadherin 3, type 1, P-cadherin (placental)	CDH3	BC014462	2.7
signal sequence receptor, alpha (translocon-associated protein alpha)	SSR1	BC007710	2.6
sema domain, immunoglobulin domain (Ig), and GPI membrane anchor, (semaphorin) 7A	SEMA7A	BC101643	2.6
choline kinase beta	CHKB	BC024286	−2.5
solute carrier family 9 (sodium/hydrogen exchanger), member 1 (antiporter, Na+/H+, amiloride sensitive)	SLC9A1	BC012121	−2.5
paired box gene 5 (B-cell lineage specific activator)	PAX5	NM016734	−2.5
G protein-coupled receptor 132	GPR132	BC004555	−2.6
activin A receptor, type IIB	ACVR2B	BC096243	−2.7
protein tyrosine phosphatase, receptor type, B	PTPRB	BC051329	−2.7
latent transforming growth factor beta binding protein 2	LTBP2	BC032434	−2.8
G protein-coupled receptor kinase 5	GRK5	BC018116	−2.8
fibroblast growth factor 5	FGF5	BC071227	−2.8
v-raf murine sarcoma viral oncogene homolog B1	BRAF	BC038966	−2.9
SMAD specific E3 ubiquitin protein ligase 1	SMURF1	BC008574	−2.9
POU domain, class 4, transcription factor 1 (BRN-3A)	POU4F1	BC041859	−2.9
CD34 antigen	CD34	BC03146	−2.9
COP9 constitutive photomorphogenic homolog subunit 4 (Arabidopsis)	COPS4	BC004302	−3.0
netrin G1	NTNG1	BC030220	−3.0
activator of basal transcription 1	ABTI	BC048812	−3.1
distal-less homeobox 6	DLX6	BC069363	−3.1
EPH receptor B4	EPHB4	BC004264	−3.1
Kruppel-like factor 5 (intestinal)	KLF5	BC042131	−3.1
gap junction protein, beta 5 (connexin 31.1)	GJB5	BC004379	−3.2
ataxin 1	ATXN1	BC011026	−3.2
ubinuclein 1	UBN1	NM016936	−3.2
ring finger protein 150	RNF150	BC101992	−3.6
calcium-sensing receptor (hypocalciuric hypercalcemia 1, severe neonatal hyperparathyroidism)	CASR	BC104999	−3.8

D. Microglia

Gene Name	*Gene Symbol*	*Genbank*	*Z ratio*
BCL2-associated X protein	BAX	BC014175	7.8

D. Microglia

Gene Name	*Gene Symbol*	*Genbank*	*Z ratio*
prominin 1	PROM1	BC012089	4.7
integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	ITGA3	BC015344	4.5
GATA binding protein 1 (globin transcription factor 1)	GATA1	BC009797	4.1
Rho-associated, coiled-coil containing protein kinase 1	ROCK1	BC041849	3.4
histone deacetylase 1	HDAC1	BC000301	3.3
POU domain, class 4, transcription factor 2	POU4F2	NM004575	3.1
ELK1, member of ETS oncogene family	ELK1	BC048296	3.1
interleukin 1, beta (IL-1β)	IL1B	BC008678	3.1
synaptotagmin XI	SYT11	BC004291	3.1
RNA terminal phosphate cyclase-like 1	RCL1	BC001025	3.0
guanylate binding protein 1, interferon-inducible, 67kDa	GBP1	BC002666	2.9
phosphoilpase D1, phophatidylcholine-specific	PLD1	BC068976	2.8
dual specificity phosphatase 13	DUSP13	BC009778	2.8
mitogen-activated protein kinase 12	MAPK12	BC015741	2.7
heparan sulfate proteoglycan 2 (perlecan)	HSPG2	BC033152	2.5
pyruvate dehydrogenase kinase, isozyme 3	PDK3	BC015948	2.5
mitogen-activated protein kinase kinase kinase kinase 4	MAP4K4	BC010909	−2.8
transient receptor potential cation channel, subfamily A, member 1	TRPA1	NM007332	−3.0
gremlin 1, cysteine knot superfamily, homolog (Xenopus laevis)	GREM1	BC069525	−3.2
collapsin response mediator protein 1	CRMP1	BC007898	−3.2
FK506 binding protein 12-rapamycin associated protein 1	FRAP1	NM004958	−3.7
S100 calcium binding protein G	S100G	BC112174	−11.6

E. Astrocytes

Gene Name	*Gene Symbol*	*Genbank*	*Z ratio*
chromobox homolog 3 (HP1 gamma homolog)	CBX3	BC000954	2.9
activin A receptor, type IB	ACVR1B	BC000254	2.8
solute carrier family 1 (neutral amino acid transporter), member 5	SLC1A5	BC000062	2.8
gamma-aminobutyric acid (GABA) A receptor, gamma 2	GABRG2	BC036030	2.7
cytochrome P450, family 2, subfamily B, polypeptide 6	CYP2B6	BC067430	2.6
tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide (14-3-3 ε)	YWHAE	BC001440	2.6
phosphoilpase C-like 1	PLCL1	BC101531	2.6
catenin (cadherin-associated protein), alpha 2	CTNNA2	BC040458	2.5
RAP1B, member of RAS oncogene family	RAP1B	BC000176	2.5
tumor necrosis factor receptor superfamily, member 14 (herpesvirus entry mediator, HVEM)	TNFRSF14	BC029848	2.5
collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)	COL7A1	NM000094	−2.8
anti-Mullerian hormone receptor, type II	AMHR2	NM020547	−3.1
glutamate receptor, ionotropic, kainate 5	GRIKS	BC037358	−4.2
nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	NR3C1	BC015610	−5.7
Fas apoptotic inhibitory molecule 3	FAIM3	BC006401	−7.5

Table 2

Comparison between quantified values from the Micorarray and quantitative real-time RT-PCR

Gene Name	Methods	Neural progenitor cells	A2B5+ progenitor cells	Neurons	Microglia	Astrocytes
Ezrin	*Z ratio* by Microarray	−3.09	−4.75	0.14	0.36	−0.79
	Relative expression by RT-PCR	0.16±0.05***	0.38±0.07***	1.09±0.12	1.19±0.26	1.00±0.16
γ2 *actin*	*Z ratio* by Microarray	−2.68	−1.99	−0.98	1.01	−1.29
	Relative expression by RT-PCR	0.27±0.02**	0.52±0.05**	1.10±0.26	1.06±0.34	1.15±0.26
α8 *tubulin*	*Z ratio* by Microarray	−2.40	−4.12	0.37	−0.40	−0.36
	Relative expression by RT-PCR	0.32±0.12**	0.23±0.05***	1.29±0.20	1.33±0.18	1.19±0.34
DLX6	*Z ratio* by Microarray	−0.07	−0.96	−3.09	0.30	−0.18
	Relative expression by RT-PCR	0.80±0.12	0.89±0.08	0.23±0.11*	0.74±0.15	0.95±0.18
*IL-1*β	*Z ratio* by Microarray	−0.05	0.77	1.95	3.08	1.12
	Relative expression by RT-PCR	1.09±0.22	0.87±0.08	1.28±0.06	3.70±0.19**	1.01±0.12
BAX	*Z ratio* by Microarray	−0.36	−0.32	−0.11	7.80	1.71
	Relative expression by RT-PCR	1.09±0.30	0.94±0.09	1.02±0.11	8.97±2.63*	0.87±0.19
Glucocorticoid receptor	*Z ratio* by Microarray	0.92	3.62	3.31	0.75	−5.72
	Relative expression by RT-PCR	0.88±0.08	2.34±0.45*	1.28±0.48	1.14±0.20	0.39±0.03***
14-3-3 ε	*Z ratio* by Microarray	0.75	−0.78	1.29	−0.03	2.57
	Relative expression by RT-PCR	1.01±0.10	0.94±0.30	1.11±0.22	0.95±0.16	2.10±0.33*

Data in red indicate significant differential expression compared to each respective control.

See “Methods” for detailed calculations of “*Z ratio* by Microarray”, “Relative expression by RT-PCR” represents ratios of cocaine-treated samples to respective controls.

* $p<0.05$

** $p<0.01$

*** $p<0.001$ compared to each control.