Gene expression evidence for remodeling of lateral hypothalamic circuitry in cocaine addiction


*Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA 92130; 1Laboratoire de Neuropsychobiologie des Desadaptations, Université Victor Segalen Bordeaux 2, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5541, 33076 Bordeaux, France; 2Dipartimento di Scienze della Salute, Instituto Universitario di Scienze Motorie, Piazza Lauro De Bosis 15, 00194 Rome, Italy; and 3Behavioral Neuroscience Branch, Intramural Research Program, National Institute on Drug Abuse, National Institutes of Health, 5500 Nathan Shock Drive, Baltimore, MD 21224

Communicated by Floyd E. Bloom, The Scripps Research Institute, La Jolla, CA, May 27, 2005 (received for review December 28, 2004)

By using high-density oligonucleotide arrays, we profiled gene expression in reward-related brain regions of rats that developed escalated cocaine intake after extended access to cocaine (6 h per day). Rats allowed restricted daily access to cocaine (only 1 h) that displayed a stable level of cocaine intake and cocaine naive rats were used for controls. Four analysis methods were compared: Affymetrix MRIOARRAY SUITE 4 and MIROARRAY SUITE 5, which use perfect-match-minus-mismatch models, and DCHIP and RMA, which use perfect-match-only models to generate expression values. Results were validated by RT-PCR in individual animals from an independent replication of the experiment. A small number of genes was associated with escalated cocaine intake (ESC genes). Unexpectedly, of the brain regions examined [prefrontal cortex, nucleus accumbens, septum, lateral hypothalampus (LH), amygdala, and ventral tegmental area], the LH was the most transcriptionally responsive in escalation of cocaine intake. Most of the ESC genes identified are also expressed during synaptogenesis and synaptic plasticity and include genes that code for several presynaptic and postsynaptic proteins involved in neurotransmission. These results suggest that LH intrinsic circuitry underlies a structural reorganization during escalation of cocaine use. This remodeling of LH circuitry could contribute to the chronic deficit in reward function that has been hypothesized to drive the transition to drug addiction. Results also support the value of using multiple analysis strategies to identify the most robust changes in gene expression and to compensate for the biases that affect each strategy.

A current challenge for the neuroscience of drug addiction is to understand the molecular mechanisms responsible for the development of compulsive drug use (1). Such a transition is generally associated with a pattern of escalating drug use whereby consumption increases over time and becomes increasingly difficult to control. In the present study, we investigated gene expression changes associated with drug addiction by using an animal model that demonstrates escalation of cocaine intake. In this model, drug intake gradually escalates when daily access to the drug is increased to >6 h [long access (LgA)] (2, 3), whereas with only 1 h of access per day [short access (ShA)], drug intake remains low and stable over time. The difference in cocaine consumption between ShA and LgA animals has been hypothesized to model the difference drawn by clinicians between controlled and compulsive drug use (2, 3). This view is supported by recent results showing that escalation in cocaine intake in LgA animals is associated with an increased motivation to seek and to take cocaine and with a persistent downregulation of brain reward function when compared with ShA animals (3–5). Thus, escalation of cocaine intake in LgA rats appears to replicate the behavioral and neuroadaptative changes associated with the development of addiction (1, 6).

The elucidation of gene expression changes in the present model may provide unique clues to the molecular mechanisms behind the reward dysfunction that drives the transition to compulsive cocaine use. By using high-density oligonucleotide arrays, we profiled gene expression changes in several reward-related brain regions in LgA rats in comparison with ShA rats and cocaine naive controls. Results were extensively validated by RT-PCR in individual animals from an independent replication of the experiment. The results suggest that the lateral hypothalamus (LH) in particular undergoes a structural reorganization during escalation of cocaine use. This remodeling of LH circuitry could contribute to the chronic deficit in reward function that has been hypothesized to drive the transition to drug addiction (3, 7).

Materials and Methods

Behavioral Procedures. Male Wistar rats (280–340 g) were prepared with a chronic i.v. catheter and were food-restricted 5 days later and trained for 7 days to press a lever to obtain food pellets. Two days after food training, 20 rats were tested for cocaine self-administration during two consecutive phases: a screening phase (1 day) and an escalation phase (18 days). Eight more rats were exposed to the same experimental manipulations as the other rats, except that they were not exposed to cocaine. During the screening phase, the 20 rats tested for self-administration were allowed to self-administer cocaine during only 1 h on a fixed-ratio schedule where one lever press results in one cocaine infusion (250 μg per injection in a volume of 0.1 ml delivered in 4 sec), after which two balanced groups with the same mean weight and mean cocaine intake were formed. During the escalation phase, one group had access to cocaine self-administration for only 1 h per day (ShA rats) and the other group for 6 h per day (LgA rats). Four of the 20 rats allowed to self-administer cocaine were discarded from the study either because of a failure to reach the criterion for acquisition of cocaine self-administration (n = 3) (i.e., at least eight injections per h) or because of inconsistent within-session intake for several days (n = 1), leaving eight rats per group.

Brain Dissection. Forty-eight hours after the last self-administration session, drug-naive, ShA, and LgA rats (n = 8 per group) were

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GluR, glutamate receptor; GNB1, G protein β-subunit 1; GRIP, GluR-interacting protein; ICSS, intracranial self-stimulation; LgA, long access; LH, lateral hypothalampus; MAP, microtubule-associated protein; MAP, microtubule-associated protein; MAL, microarray suite; MM, mismatch; NAS, nucleus accumbens; PM, perfect match; SEP, septum; SH, short access; VTA, ventral tegmental area.

Data deposition: The microarray data reported in this paper have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus database (NCBI GEO accession no. GSE 3016).

**To whom correspondence should be addressed at: Department of Neuropharmacology, The Scripps Research Institute, 10550 North Torrey Pines Road, CVN-12, La Jolla, CA 92037. E-mail: psanna@scripps.edu.

© 2005 by The National Academy of Sciences of the USA
killed in random order to obtain tissue samples from six reward-related brain regions. Animals were perfused with 10% RNAlater (Ambion, Austin, TX) in 0.1 M PBS under anesthesia by CO2 narcosis. Brains were sliced with a wire brain slicer (Research Instruments and Manufacturing, Corvallis OR) and dissected with the assistance of a rat brain atlas (8). A 14-gauge needle constructed from a spinal tap needle and equipped with a plunger to facilitate the transfer of the dissected tissue was used to collect the nucleus accumbens (NAC), the LH, the septum (SEP), and the ventral tegmental area (VTA). The medial prefrontal cortex and the amygdaloid complex were dissected free-handedly with established anatomical landmarks (8).

**Microarray Procedures.** Total RNAs of regions of interest were prepared with an RNeasy miniprep kit according to manufacturer’s (Qiagen, Valencia, CA) protocol. RNA was quantified by using the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), and quality was assessed with a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and with agarose gel electrophoresis. Depending on the brain region, between 1 and 5 μg of total RNA was used to prepare double-stranded cDNA with a cDNA synthesis kit (GIBCO/BRL) for first and second strand. Biotinylated cRNA was generated from these cDNAs with a BioArray high-yield RNA transcript labeling kit (Enzo Life Sciences). It was then purified with RNeasy spin columns (Qiagen) and fragmented before hybridization (9). Hybridization mixtures were boiled at 99°C, loaded on Affymetrix Neurobiology RNU34 chips and hybridized at 45°C for 16 h. Washes were performed on a Fluidics Station with the manufacturer’s (Affymetrix) recommended wash solutions, and the chips were stained with a streptavidin–phycoerythrin conjugate for fluorescence detection. After staining, chips were scanned with an Affymetrix GeneArray Scanner 2500. For the amygdaloid complex and medial prefrontal cortex, hybridizations were run in quadruplicate (four independent pools of two animals each were hybridized on four RNU34 chips); for smaller regions, such as NAc and LH, four RNU34 chips were hybridized with duplicate hybridizations of two independent pools of four animals each; for VTA and SEP, we ran three replicate hybridizations of single pools each of eight animals. Results were extensively validated by RT-PCR in brain regions of individual animals, and cDNAs were obtained as described above. Sixty-five chips in the experiment (10). With RMA, we subtracted the background from PM values, performed quantile normalization, and generated log2-transformed expression values. Negative expression levels occurring in MAS4 (12, 14) were converted to 0.01 rather than 0 to avoid calculation artifacts, such as fold change ratios equal to infinity (15). The following three empirically defined additional filtering criteria were also applied: a minimum fold change of 1.4 for MAS4 and MAS5 data and 1.2 for DCHIP and RMA, consistent with the criteria of other studies (16–19); genes labeled absent in all chips by the MAS “detection call” algorithm were filtered out in all four analysis strategies; and genes that did not display an average expression level of at least 100 in at least one condition (after scaling to a target intensity of 250) were also excluded in MAS4 and MAS5. This minimum expression level filter was not applied to DCHIP or RMA because of their lower levels of noise. These empirical filters were validated by initial RT-PCR results. Gene lists were annotated by use of National Center for Biotechnology Information ENTREZ Nucleotide and Unigene databases and National Institutes of Health DAVID (20). Biological themes were explored with National Institutes of Health EASE (21) and the Kyoto Encyclopedia of Genes and Genomes (22).

**RT-PCR Validation of Microarray Results.** For RT-PCR validation, an independent experiment was conducted (n = 5 in all groups) in the same manner described above (‘Behavioral Procedures’). RNA was extracted from the aforementioned brain regions of individual animals, and cDNAs were obtained as described above. Sixty-five

![Fig. 1. Effects of access time to cocaine self-administration on drug intake and brain gene expression levels. (A) Escalation of i.v. cocaine consumption in rats. Rats had access to cocaine for either 1 h (ShA rats, n = 8) or 6 h per day (LgA rats, n = 8). Data represent the mean (±SEM) number of cocaine injections obtained during the first hour of each daily self-administration session. * Different from ShA rats; P < 0.05, tests of simple main effects after appropriate two-way analyses of variance. (B) Total number of probe sets per brain region that significantly changed in LgA and ShA rats compared with drug-naive control rats (CSA genes, white bars) and probe sets that significantly changed in LgA rats from ShA and drug-naive rats (ESC genes, black bars). AMY, amygdaloid complex; MPF, medial prefrontal cortex.](https://www.pnas.org/cgi/doi/10.1073/pnas.0504438102)
genes (represented by 78 probe sets) were tested by RT-PCR in cDNA samples from individual animals. Primers were designed with BEACON DESIGNER software (PREMIER Biosoft International, Palo Alto, CA). iQ SYBR Green Supermix (Bio-Rad) was used in a 25-μl reaction volume with a MyiQ real-time PCR detection system (Bio-Rad) using 0.2-ml, 96-well, thin-wall PCR plates from Bio-Rad. The relative amounts of mRNA were normalized to β-actin with the exception of the VTA for which Na+/K+-ATPase α1-subunit gene (Atp1a1) was used because its expression proved more invariant than β-actin in this region.

Results

The experimental design for this study comprised three groups of rats (n = 8) with different daily access to a continuous schedule of i.v. cocaine self-administration (0.25 mg per injection). 0 h access per day (drug-naive control rats), 1 h access per day (ShA rats), or 6 h access per day (LgA rats) (see Materials and Methods). As expected, in rats allowed to self-administer cocaine, the duration of access dramatically influenced cocaine intake. Within 18 days, cocaine intake in the first hour in LgA rats rose to a level almost 2-fold greater than that observed in ShA rats, which remained stable over time (Fig. 1A). Total cocaine intake in LgA rats also increased over the same period from an initial average of 48 to an average of 126 cocaine injections over the 6-h period. Gene expression profiling was then performed for each dissected brain region by using Affymetrix rat neurobiology arrays. This array is stable over time (Fig. 1A). The diagonal lines in each graph represent the 1.4-fold cutoffs used in the analysis of MAS4 and MAS5 data (see Materials and Methods for details). In this plot, deviations from the center within the area defined by the diagonal lines indicate changes in ShA and LgA rats from controls (CSA genes). Points outside the area defined by the diagonal lines indicate genes that differed in LgA from ShA and control rats (ESC genes).

In all of the brain regions studied, the majority of genes whose expression levels were affected by a history of cocaine self-administration were not differentially affected by the pattern of cocaine intake (stable/moderate in ShA rats vs. escalating/excessive in LgA rats). The diagonal lines in each graph represent the 1.4-fold cutoffs used in the analysis of MAS4 and MAS5 data (see Materials and Methods for details). These data support the value of using multiple analysis strategies both to identify the most robust changes in gene expression, and to compensate for the biases that affect each strategy, as noted by others (23, 24).

Examples of genes involved in structural and functional plasticity that were induced in the LH of LgA rats with escalated drug intake include δ-catenin, microtubule-associated protein (MAP)-1a and the fibroblast growth factor receptor (Table 1). δ-Catenin is a postsynaptic density neuron-specific catenin expressed at high level in the cortex, basal ganglia, and midbrain catecholaminergic nuclei (see the SYMATLAS database of the Genomics Institute of the Novartis Research Foundation at http://symatlas.gnf.org/SymAtlas), and it is involved in dendritic branching (25, 26). The absence of δ-catenin leads to mental retardation in humans and mutant mice (25, 26). It is also involved in the organization of postsynaptic density where it interacts with scaffolding proteins involved in the targeting and trafficking of ionotropic NMDA and metabotropic glutamate receptors (Fig. 3 and Table 1) (27, 28). Additionally, the α1, α3, and β2 isoforms of Na+/K+-ATPase,
which are induced during peripheral nerve regeneration (29), were also induced in the LH. The α3 isofrom of Na+/K+-ATPase has been linked to bipolar disorder (30). Decreases in MAP-2 and neurofilament light chain in the NAc (Table 1) could be the result of trophic changes in these regions, as suggested by Nestler and colleagues (31). Increased expression of apoptotic and antiapoptotic gene products, such as Bel-2 and related genes, was observed in various regions (Table 1).

Among the genes in the neurotransmitter receptors, enzymes, and signal transduction group, the NMDA receptor subunit 2D (NR2D) was increased in rats with escalated levels of cocaine intake. This subunit is predominantly expressed during development and confers slow channel kinetics to the NMDA receptors (32–34). The slow deactivation of the embryonic subunits is believed to lower the temporal threshold for coincidence detection favoring synaptic strengthening during development (32–34). Extrasynthetically located NR2D receptors have been demonstrated (35, 36). It has been proposed that such extrasynaptic NR2D receptors mediate glutamate trophic actions rather than contribute to neural transmission (34). Thus, the increased expression of the embryonic NR2D subunit in the LH of cocaine-escalating rats could also be a hallmark of plastic structural rearrangements.

The PDZ (postsynaptic density-95/Drosophila disk large-tumor suppressor zona occludens 1) domain-containing glutamate receptor (GluR)-interacting protein (GRIP)2 was also increased in the LH (Table 1). GRIP2 binds to the C terminus of the GluR2 and GluR3 subunits of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and is enriched in synaptic plasma membrane and postsynaptic density fractions, and its expression relatively late in development parallels the expression of AMPA receptors (37, 38). The complex formed by GluR2 and GRIP1 (a protein highly homologous to GRIP2 expressed at earlier developmental stages) has been shown to be capable of directly interacting with kinesin heavy chains in dendrites, acting as motors for AMPA receptors (39). MAP-1a was also induced in the LH of LgA rats (Table 1). This interaction has been proposed to contribute to AMPA receptors targeting to synapses (40). Interestingly, GRIP1 has also been shown to bind to MAP-1a and MAP-1b in the yeast two-hybrid assay, an interaction that could play a role in AMPA receptors’ dynamics (40). Other proteins involved in trafficking of glutamatergic receptors and synaptic plasticity, such as Homer (41) and postsynaptic density-95 (42–44), have been shown to be involved in cocaine’s actions in other brain regions and models of cocaine exposure, suggesting that cocaine-induced adaptations affect multiple genes involved in glutamatergic receptor trafficking.

The GTPase-activating protein neurofibromin was induced in the LH of LgA rats. Neurofibromin is involved in the regulation of the activity of the low-molecular-weight G protein Ras (45, 46) and cAMP levels (46). Neurofibromin deficiency is associated with impaired synaptic plasticity and cognitive impairments (46, 47). Increased neurofibromin is expected to result in reduced ERK activation (47) and higher cAMP levels (48). The G protein β-subunit 1 (GNB1; also known as rGβ1), was found to be down-regulated in the LH of escalating rats. GNB1 is enriched in the brain, is up-regulated by cocaine and amphetamine in the shell region of the NAc, and it is required for behavioral sensitization induced by repeated administration of psychostimulants (49). GNB1 mediates the fast voltage-dependent inhibition of N-type Ca2+ channels produced by many G protein-coupled receptors (50). Therefore, decreased GNB1 could result in greater neurotransmitter release. GNB1 also inhibits adenylyl cyclase and reduces cAMP levels (51). Therefore, increased neurofibromin and decreased GNB1 could synergize in increasing accumulation of cAMP. Interestingly, GNB1 was increased in the present study in the SEP of LgA rats (Table 1) and in the VTA of SHA and LgA rats (data not shown).

The expression of various presynaptic proteins found at excitatory synapses was affected (generally increased) in the LH of LgA rats (Fig. 3 and Table 1), including proteins involved in vesicle docking and neurotransmitter release, such as Rab-3A, synaptotagmin-3 and -11, syntaxin-1, and some potential regulator of their function, PKCγ (Fig. 3 and Table 1). The presynaptic protein synaptophysin, which is increased by plasticity of excitatory synapses (52), was also induced in the LH (Fig. 3 and...
Fractalkine also reduces glutamate neurotransmission, and its expression is a chemokine predominantly expressed in the brain and is believed to be induced as an adaptive mechanism at times of excessive neuronal stimulation (54). PNAS vol. 102 no. 32 11537

Ahmed et al.

Discussion

The goal of the present study was to identify genes specifically associated with escalated and compulsive cocaine intake (ESC genes) by using a microarray-based transcriptional approach. Four different strategies were used to analyze the gene expression data obtained. RT-PCR of individual animal samples from an independent replication of the experiment was used to validate the genes identified. The main finding of the present study is that the expression levels of only a small fraction of genes changed significantly in association with drug intake escalation (ESC genes). ESC genes varied across different brain regions, suggesting that different components of the reward circuitry undergo specific adaptations during escalation of cocaine self-administration. The most dramatic changes were observed in the LH. This observation points to a previously underappreciated importance of this brain region in the development of drug addiction.

Data Analysis Considerations. Multiple strategies for the analysis of microarray data have been devised. However, optimal protocols for gene expression analysis in specific experimental settings remain to be defined. In the present study, we have analyzed expression levels obtained with two versions of Affymetrix analysis software and with SAM analysis of DCHIP- and RMA-generated PM-only expression values. The MAS4 and RMA software use PM-minus-MM model to subtruct nonspecific binding from expression values, subtract background, and normalize the data by globally scaling to an arbitrary target intensity; these programs also have additive signal algorithms (12, 14). The MAS4 output incorporates an anti-log of robust average to transform negative expression levels, which occur with and without a limitation of MAS4 (12, 14). Conversely, DCHIP normalizes individual probes across arrays to a computed “invariant set” of genes, determined by ranking and iteratively comparing the PM probes across all of the arrays in the experiment or across experiments (10, 59). The arrays in the experiment are then scaled to the median array in the experiment. Signal values are generated with a multiplicative algorithm called Model-Based Expression Indices (10, 59). RMA uses a robust average of log2-transformed background-corrected PM intensities combined with a quantile normalization method (11, 12). The use of PM-only expression values in the latter two methods as opposed to the PM-minus-MM model used in the MAS algorithms is motivated by the observation that some MM probes can be unreliable indicators of nonspecific binding (10). The comparison of four microarray analysis strategies performed here suggests that all of the analysis strategies used have validity and supports the usefulness of using multiple analysis strategies to identify the most robust changes in gene expression, as noted by others (23, 24), and to compensate for the biases that affect each strategy (24).

Implications of the Remodeling of LH Circuitry in Cocaine Addiction.

The LH is a heterogeneous hypothalamic region whose function and neuronal organization are still poorly understood (60). LH comprises intrinsic neuronal populations and circuitry that are bidirectionally connected by the medial forebrain bundle to key reward-related forebrain regions, such as the NAc. The LH is a brain region that is highly rewarding when electrically stimulated (61, 62). Intracranial self-stimulation (ICSS) in the LH is potentiated by drugs of abuse, including cocaine, amphetamine, and mediated excitatory neurotransmission (55, 56). Also worth noticing is that fractalkine has been found to be up-regulated in the brain tissue from patients with HIV-1 encephalitis (57) and to potentially induce the migration of monocytes across endothelial cells (58). Although fractalkine is neuroprotective to cultured neurons exposed to HIV, it has also been proposed to contribute to HIV invasion of the CNS (58). Therefore, increased fractalkine induction by cocaine intake could contribute to the pathogenesis of HIV invasion of the CNS in human cocaine addicts.
fensine, morphine, heroin, and nicotine (63–69). Conversely, decreased ICSS reward in the LH is seen in drug-dependent animals when cocaine has been eliminated from the body and transient gene expression changes are likely to have subsided. It is widely believed that protracted gene expression changes induced by chronic drug self-administration are key in craving and dependence (1). Changes in brain gene expression at this withdrawal time point are likely to reflect longer-lasting adaptations that may underlie the maintenance of escalated cocaine use. Future studies will be required to extend these findings to longer withdrawal periods to assess the dynamics and persistence of these neuroadaptations.

In conclusion, the present multianalyses study reports a list of strong candidate genes specifically associated with the development of compulsive cocaine intake. The differential regulation of several genes related to structural plasticity in the LH of animals with escalated cocaine intake but not in rats with stable cocaine intake suggests that a remodeling of LH circuitry involving synaptogenesis and neurogenesis contributes to the transition to cocaine addiction. Future studies need to focus more on this understudied brain region by specifically targeting the genes identified in this study.

This work was supported by National Institutes of Health Grants DA13821, DA017208, and DA044398. D.L. and V.R.-C. were partially supported by Training Grant AA007454 (Neuropsychopharmacology–Multidisciplinary training). S.H.A. was supported by Centre National de la Recherche Scientifique, and V.R.-S. was supported by Italian Ministry of Health Commission for the Monitoring and Control of Doping Grant N 2002-18.

Experimental Design Considerations. Several methodological issues should be considered in interpreting the present data. Increased access to cocaine self-administration results in increasing the levels of operant experience and of drug exposure, both of which could affect the behavioral and molecular changes observed here. Although plausible, a role of operant experience in cocaine intake escalation is unlikely. In fact, extended exposure to cocaine without changes in operant experience is sufficient to produce escalated levels of cocaine self-administration (78). Additionally, gene expression changes were assessed after a period of cocaine withdrawal (48 h) when cocaine has been eliminated from the body and transient gene expression changes are likely to have subsided. It is widely believed that protracted gene expression changes induced by chronic drug self-administration are key in craving and dependence (1). Therefore, changes in brain gene expression at this withdrawal time point are likely to reflect longer-lasting adaptations that may underlie the maintenance of escalated cocaine use. Future studies will be required to extend these findings to longer withdrawal periods to assess the dynamics and persistence of these neuroadaptations.

In conclusion, the present multianalyses study reports a list of strong candidate genes specifically associated with the development of compulsive cocaine intake. The differential regulation of several genes related to structural plasticity in the LH of animals with escalated cocaine intake but not in rats with stable cocaine intake suggests that a remodeling of LH circuitry involving synaptogenesis and neurogenesis contributes to the transition to cocaine addiction. Future studies need to focus more on this understudied brain region by specifically targeting the genes identified in this study.

This work was supported by National Institutes of Health Grants DA13821, DA017208, and DA044398. D.L. and V.R.-C. were partially supported by Training Grant AA007454 (Neuropsychopharmacology–Multidisciplinary training). S.H.A. was supported by Centre National de la Recherche Scientifique, and V.R.-S. was supported by Italian Ministry of Health Commission for the Monitoring and Control of Doping Grant N 2002-18.

Experimental Design Considerations. Several methodological issues should be considered in interpreting the present data. Increased access to cocaine self-administration results in increasing the levels of operant experience and of drug exposure, both of which could affect the behavioral and molecular changes observed here. Although plausible, a role of operant experience in cocaine intake escalation is unlikely. In fact, extended exposure to cocaine without changes in operant experience is sufficient to produce escalated levels of cocaine self-administration (78). Additionally, gene expression changes were assessed after a period of cocaine withdrawal (48 h) when cocaine has been eliminated from the body and transient gene expression changes are likely to have subsided. It is widely believed that protracted gene expression changes induced by chronic drug self-administration are key in craving and dependence (1). Therefore, changes in brain gene expression at this withdrawal time point are likely to reflect longer-lasting adaptations that may underlie the maintenance of escalated cocaine use. Future studies will be required to extend these findings to longer withdrawal periods to assess the dynamics and persistence of these neuroadaptations.

In conclusion, the present multianalyses study reports a list of strong candidate genes specifically associated with the development of compulsive cocaine intake. The differential regulation of several genes related to structural plasticity in the LH of animals with escalated cocaine intake but not in rats with stable cocaine intake suggests that a remodeling of LH circuitry involving synaptogenesis and neurogenesis contributes to the transition to cocaine addiction. Future studies need to focus more on this understudied brain region by specifically targeting the genes identified in this study.

This work was supported by National Institutes of Health Grants DA13821, DA017208, and DA044398. D.L. and V.R.-C. were partially supported by Training Grant AA007454 (Neuropsychopharmacology–Multidisciplinary training). S.H.A. was supported by Centre National de la Recherche Scientifique, and V.R.-S. was supported by Italian Ministry of Health Commission for the Monitoring and Control of Doping Grant N 2002-18.

Experimental Design Considerations. Several methodological issues should be considered in interpreting the present data. Increased access to cocaine self-administration results in increasing the levels of operant experience and of drug exposure, both of which could affect the behavioral and molecular changes observed here. Although plausible, a role of operant experience in cocaine intake escalation is unlikely. In fact, extended exposure to cocaine without changes in operant experience is sufficient to produce escalated levels of cocaine self-administration (78). Additionally, gene expression changes were assessed after a period of cocaine withdrawal (48 h) when cocaine has been eliminated from the body and transient gene expression changes are likely to have subsided. It is widely believed that protracted gene expression changes induced by chronic drug self-administration are key in craving and dependence (1). Therefore, changes in brain gene expression at this withdrawal time point are likely to reflect longer-lasting adaptations that may underlie the maintenance of escalated cocaine use. Future studies will be required to extend these findings to longer withdrawal periods to assess the dynamics and persistence of these neuroadaptations.

In conclusion, the present multianalyses study reports a list of strong candidate genes specifically associated with the development of compulsive cocaine intake. The differential regulation of several genes related to structural plasticity in the LH of animals with escalated cocaine intake but not in rats with stable cocaine intake suggests that a remodeling of LH circuitry involving synaptogenesis and neurogenesis contributes to the transition to cocaine addiction. Future studies need to focus more on this understudied brain region by specifically targeting the genes identified in this study.

This work was supported by National Institutes of Health Grants DA13821, DA017208, and DA044398. D.L. and V.R.-C. were partially supported by Training Grant AA007454 (Neuropsychopharmacology–Multidisciplinary training). S.H.A. was supported by Centre National de la Recherche Scientifique, and V.R.-S. was supported by Italian Ministry of Health Commission for the Monitoring and Control of Doping Grant N 2002-18.