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Use of Non-Human Primates in Cocaine Research

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Substance abuse and addiction are serious public and personal concerns worldwide. Globally, cocaine use is estimated at 16–21 million persons (0.4%–0.5% of the overall population), with main concentrations in North America, followed by Western and Central Europe, and South America.[1] In addition to the tragic consequences at the individual level, substance abuse and addiction are a major burden for the society, with economic costs estimated to exceed $600 billion annually in the United States, including health and crime-related costs and losses in productivity.[2] In 2012, there were 1.6 million cocaine users in the United States alone, with approximately 1,800 new users per day.[3] As of 2011, cocaine continued to hold the #1 position in the United States among illicit drugs as a reason for emergency room admissions.[4]

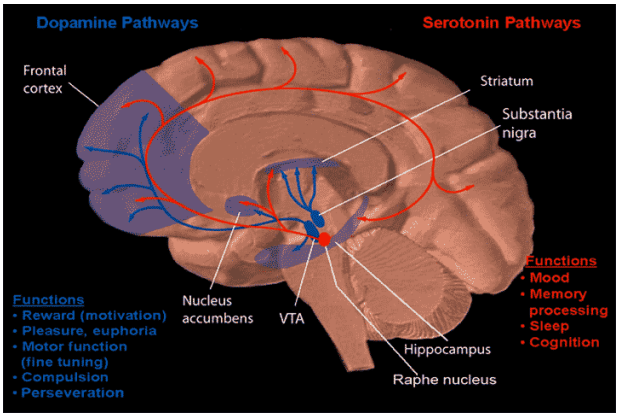
Despite extensive funding for intra- and extramural research by the National Institutes of Health over several decades, there is still no effective medical treatment for cocaine dependence and addiction. Frequently, such research projects involve animal models, including non-human primates (NHPs) which are often mentioned as "the gold standard" of animal models, due to their close relationship to humans. Between 2007 and 2013, NIH funding for projects that involved the use of NHPs in cocaine research exceeded $100 million. (NIH RePORTER search accessed 04/10/14).

As discussed below, NHPs do not appear to provide the best research model for cocaine research, nor has their use resulted in an effective human treatment. At the same time, revolutionary advances in biomedical technology have made it possible to investigate the molecular and neuronal basis of cocaine addiction and suggested possible human treatments. This paper will first provide a short overview of the biological and neuronal basis of cocaine effects. It will then consider reasons for the use of NHPs, both traditional arguments for such use, as well as countervailing considerations. Next, the paper will describe *in vitro, in silico*, and non-invasive research methods that can be used for basic research and for advanced studies on human patients. The final section will summarize the substantive points of the paper and provides recommendations for going forward.

1. Biological basis of cocaine addiction.

Cocaine addiction is now commonly accepted as a chronic, relapsing disease of the brain that is caused by the impact of the drug on the brain and modified by various environmental factors.[5] Therefore, to develop effective treatments for cocaine, we need to understand its effects on neural pathways and the molecular mechanism of its interaction with various neurotransmitters in the brain.

To date, the overall structure and pathways of the human brain have been outlined to a large extent. For example, locations for sensory and locomotor processing, emotions and memory formation, as well as judgment and self-control have been identified.[6] However, much remains yet to be clarified, particularly about the impact that activities in a given brain region, such as those induced by cocaine, have on other brain regions, and connections among the various circuits.

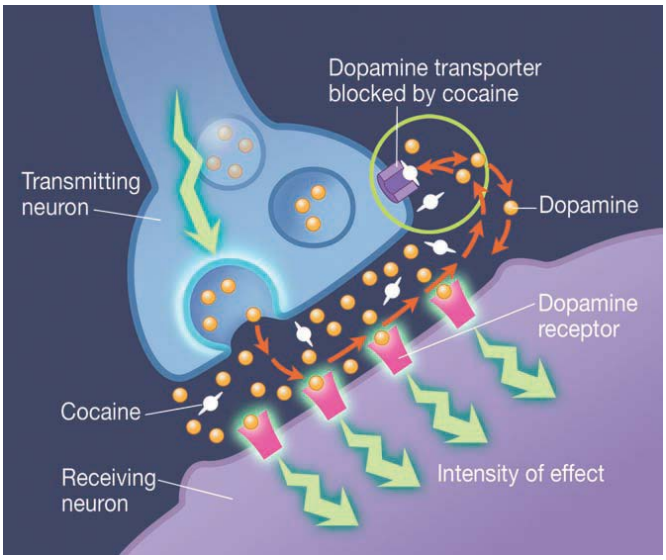


www.drugabuse.gov.

Upon consumption, cocaine distribution in the brain is extensive, but its principal target appears to be the dopamine-rich mesocorticolimbic pathway. This pathway, which originates from the ventral tegmental area of the mid-brain, targets a number of brain structures, including the amygdala, prefrontal cortex and nucleus accumbens, which is a central component of the brain's reward system and the focus of pleasurable experience of stimulant drugs.[7] Moreover, there is a growing consensus that repeated exposure to cocaine produces lasting neuroadaptive changes in reward and memory circuits, likely mediated by glutamate activity. The structural changes induced by drug consumption persist into abstinence. As a result, addicts have difficulty in changing their behavior, even if they understand that cocaine has a harmful effect on their health and lives.[8]

The molecular mechanism of cocaine addiction has also been increasingly clarified. For some three decades, it has been known that the main target of cocaine is the brain's dopamine (DA) system. In a healthy, non-addicted individual, neurons release dopamine into a synaptic space, so that it may activate receptors in adjacent neurons. Under normal circumstances, the extracellular DA is quickly taken up by transporters that recycle it back into the neurons. By binding to dopamine transporters, cocaine blocks the transport of dopamine back to the neurons. As a result, the levels of dopamine in the brain surge and dopamine receptors remain excessively activated. This, in turn, results in aberrant

intracellular signaling cascades, such as in the cAMP pathway, which activate gene transcription factors and result in changes in neurotransmitter availability, receptor function and regulation, electrophysiological activity, neuron morphology, and neural networks.[9,10]



http://www.drugabuse.gov/publications/research-reports/cocaine/

In less dopamine-rich brain regions, cocaine's principal targets are likely to be serotonin (SER) and norepinephrine (NE) transporters.[10] This is due to a significant overlap between the functions of monoamine transporters; for example, the NE transporter shows equal affinity for NE and DA.[11] In addition, cocaine interacts with a number of other neurotransmitters, such as glutamate, GABA, kappa-opioid, corticotrophin-releasing hormone, endocannabinoid, and endogenous opioids.[12] Cocaine also affects the brain's stress-responsive elements, as stress activates brain circuits involved in reward processing and is a significant trigger for relapse to drug-taking behaviors.[13,14]

The scientific investigation of cocaine addiction is also complex because addiction appears to have different phases, each with different neuronal location and characteristics. Thus, the initial intoxication stage, the withdrawal stage, and the preoccupation/craving stage are each thought to involve different neuronal circuits, involving reward and motivation; memory, conditioning, and habituation; executive function and inhibitory control; self-awareness; and stress reactivity.[15] Moreover, genetic, developmental, and environmental factors influence these processes and the outcome of the addiction.

2. Non-human primates in cocaine research.

NHPs have been used extensively in cocaine research at least since the 1970s. Because NHPs and other animals in laboratory settings self-administer addictive substances, it has been concluded that these animal models may be relevant for human drug abuse studies. Indeed, most cocaine NHP studies have in the recent decade been conducted using intravenous self-administration. In this model, the NHP is implanted with indwelling intravenous catheters and is trained to perform a task, such as lever pressing,

to receive an intravenous infusion of cocaine. Other self-administration studies investigate the relapse phenomenon through reinstatement studies, the environmental impact through the conditioned place preference model, or drug discrimination in order to evaluate the drug abuse liability of pharmacological substances.[16] This section explores the main issues regarding the suitability of the NHP model for human addiction of cocaine.

a. *Anatomy and physiology.*

Most animal experiments use rodents because they are comparatively inexpensive, easy to handle and allow for larger groups to study. Also, they are not covered by the Animal Welfare Act. However, some researchers argue that experiments using the NHP are warranted owing to the evolutionary closeness of NHPs to humans. There are differences between rodents and primates in the physiology of the brain's dopaminergic and noradrenergic systems, as well as the hypothalamic–pituitary–adrenal axis, which all are relevant to cocaine effects.[17] The brain of the NHP more closely approximates the human brain, particularly in areas that are central to cocaine functions, such as the ventral striatum and its connections with surrounding areas.[18] However, NHP physiology is varied, such as their size and the size of their brains.

However, although the primate brain is, in general, closer to the human than the brain of some other species, this does not mean that the NHP and human brains are necessarily comparable for the purposes of cocaine research. First, the evolutionary tracks of humans and macaquesdiverged at least 25 million years ago.[19] Since then, the human brain has undergone development in many areas that now distinguish it from the NHP brain (see discussion below on genomics).[19] Second, detailed comparisons of the brain physiology with regard to cocaine abuse is very difficult for the simple reason that both the neurological-molecular basis of addiction and the current understanding of the brain (both human and NHP) is to a large extent incomplete.[20] Third, a number of studies have noted differences in areas of the NHP and human brain that are relevant to addiction. For example, the rostral prefrontal cortex is larger and more differentiated in humans than in other primates.[21] Also, the density of dopaminergic neurons in the human substantia nigra is smaller than in the NHP, suggesting less dopaminergic regulation of the basal ganglia system compared with other species.[22] Overall, the human prefrontal cortex, which is critical to executive and social-emotional functions, differs from that of closely related primate species in terms of its organization.[23]

b. *Genetics*.

In 2007, the DNA sequencing of the rhesus macaque genome determined that the average human-macaque DNA identity is approximately 93%.[19] NHP researchers frequently point out that the overall genetic similarity, as well as the genetic similarity of molecular targets of cocaine, such as dopamine, serotonin and norepinephrine transporters, makes the NHP a superior model in addiction research, compared to other species such as rodents.[24] It is important to note, however, that although the DNA sequencing confirms the relative phylogenetic closeness of NHPs to humans, the high percentage value only reflects the similarity in protein-coding genes. Moreover, 89% of the orthologous genes of the

human and the macaque differ at the amino acid level. In addition, many more differences exist at the level of gene expression and translation, such as insertions and deletions of individual nucleotides or DNA segments, or duplication or deletion of individual genes.[19]

The research group that sequenced the rhesus macaque DNA notes that identical DNA does not necessarily result in identical phenotypes (gene expression) in the macaque and the human, respectively. One surprising result of the analysis was the identification of several DNA mutations resulting in a disorder or disease in humans, including severe mental retardation, but not in the macaque. The scientists concluded that "it remains a possibility that the basic metabolic machinery of the macaque may exhibit functionally important differences with respect to our own." [19]

c. *Behavioral elements.*

The reasons leading to relapse in chronic human cocaine abuse are particularly important to the effort of successful treatment, and various subjective factors, such as motivation, craving, mood and drug-related dysphoria, and anhedonia, are important contributors to such relapse.[25] Therefore, a large part of the NHP use in cocaine research has involved approximations of the human behavior. For example, forced abstinence, followed by relapse and reinstatement of drug use, has been repeatedly used in many NHP studies in an effort to produce data regarding this stage of cocaine use.[26,27]

Because clinical studies have not examined effects of relapse during abstinence, NHP reinstatement experiments lack predictive validity short of such validation by a human study.[25,28] Most importantly, animal models are problematic in seeking to explain craving, which is a critical aspect of human relapse, and is therefore best addressed in human studies.[20] Other subjective characteristics are also difficult to replicate in animal studies. For example, they cannot effectively dissect the motivation to remain drug-free. [29] Also, because human studies have suggested that an impulsive personality may predispose individuals to drug use, researchers have sought to create an animal model of this characteristic.[30] However, it can only be studied in the NHP through indirect means, which may not adequately mimic the human thought and emotion.[31]

Finally, human cocaine abusers typically self-administer cocaine in a binging pattern over a period of years or decades, which is difficult to replicate in animal self-administration experiments.[32] To the extent that animal models fail to duplicate these and other aspects of human cocaine abuse, they may fall short in revealing some relevant changes in gene expression. Human study subjects, in contrast, can communicate their thoughts to the investigators, thus allowing appropriate identification and evaluation of these factors.

d. *Controlled conditions.*

Human cocaine studies usually consist of individuals who have used cocaine for some time. Many cocaine users are also current or past users of other addictive substances. Others suffer from other disorders, such as depression, ADHD, etc. and may have received medication for them. Such

comorbidities and past drug use are factors that need to be taken into account, which makes the analysis of the results more difficult. NHP investigators therefore argue that NHP studies are superior, because they are conducted without such complexities in controlled conditions, in which the drug intake and duration, diet, housing, and interaction with other primates are known.[33]

While NHP studies can systematically control study variables, such control, in itself, may produce artificial results. Controlled conditions are vastly different from the real-world conditions that influence human cocaine use.[20,34] For example, NHP research frequently employs very short drug exposures, compared to human use. Therefore, it does not fully allow the identification of neuronal adaptations that accompany long-term drug use in humans, and the intensity and duration of many adaptations may be underestimated. In addition, NHP research often uses routes of administration of cocaine that are different from the ones used by humans, although the route of administration is critical to cocaine's addictive properties.[35]

It is not uncommon to use laboratory NHPs in a number of various sequential drug experiments, possibly over many years.[36,37] Such prior drug use, even if noted in a research article, is not described in detail, and their potential effect is not discussed or identified. In addition, laboratory NHPs are routinely subjected to sedative and anesthetic drugs which may impact the same circuitry that is involved in cocaine effects.[38,39] The effect of these drugs on cocaine experiments is unknown. Thus, to a large extent, the restrictive experimental conditions for NHPs by no means guarantee that other drugs cannot influence test results.

Another criticism of NHP cocaine research and drug self-administration research in general is that the NHPs might self-administer drugs because they are raised in an artificial and impoverished environment. Such study conditions hardly model the environment that gives rise to human cocaine abuse and maintains such abuse, and may well distort the resulting study results.[40] For example, NHPs are usually housed individually in small steel cages that do not provide room for any species-typical exercise and without ethologically appropriate contact with their own species. Environmental enrichment is either lacking or inadequate, and NHPs are without control of their circumstances, particularly regarding repeated medical procedures that are not without physical and mental pain and distress. There is no guarantee that such circumstances would not produce effects that are equally, if not more, confounding than polydrug use found in a human study group.

Difficulties cited with human cocaine studies can be overcome with proper study planning. For example, it is sometimes said that it is difficult to find out the health status of persons at a time that predates their drug use, or to measure changes in the early phases of the use.[29] Investigators cannot overcome this problem by recruiting individuals who are not past drug users, while there is no such obstacle in NHP studies.[17] However, this impediment to human studies is not insurmountable, as experience in other disease investigations demonstrates (HIV-AIDS, hepatitis B and C, etc.). These investigations recruit disease-naïve study subjects in high-risk populations, where a relatively large number of individuals will eventually present with the disease or disorder that is being investigated.[41,42] Although large clinical trials are more expensive than experiments using a few captive animals, more limited

human laboratory studies are already used effectively, avoiding the problem of translation of animal studies.

e. Small study numbers and statistical validity.

Statistical analysis is a critical part of interpreting results of biomedical animal experiments, but it requires sufficient numbers of study animals. Valid statistical analysis evens out random effects and detects patterns that individual or small-group experiments do not disclose. Indeed, it is now recognized that small studies simply are not likely to produce true and reliable results.[43] However, NHP addiction studies typically involve only a handful of individuals per group [44] (n = 3-4) [45] (n = 4), and therefore cannot produce results that have statistical validity. Nevertheless, it is not unusual that a commentary on a small study present its results in a manner that suggests general applicability.[24] Human clinical trials, on the other hand, involve thousands of participants that offer statistical confidence in the observed results. Even human laboratory studies typically consist of more participants than NHP studies.

An example from the schizophrenia research, which has no effective treatment, illustrates the benefit of studies involving large numbers of participants. Over the years, various genetic studies had identified some 30 DNA locations that appeared to be predictive of the disease. However, a recent systematic genome-wide association study (GWAS) of nearly 35,000 patients disclosed 108 locations where certain genetic types were more frequent than in healthy controls. Significantly, a large majority of these loci are new, that is, not previously associated with schizophrenia. Conversely, some loci that previously appeared predictive of the disease, could not be confirmed in this larger study.[46] Similarly, a GWAS of human cocaine abusers could have potential to identify underlying genetic factors and suggest directions for developing treatments in a more rational manner than testing of individual hypotheses – no matter how well-informed – in NHPs.

f. Duplicative projects.

Even a cursory review of past NHP research published in the peer-reviewed scientific literature indicates that frequently, such cocaine experiments give the appearance of duplication. For example, NHP research is often undertaken with respect to treatments for which human laboratory studies or clinical trials have already taken place and provided answers to the questions that are posed in an NHP experiment.

Using the NIH Clinical Trials database (http://www.clinicaltrials.gov/), we reviewed all clinical trials that had tested drugs for cocaine treatment. The purpose was to determine to what extent, according to the publicly available data, the preclinical research had used NHPs to test these drugs. We identified 277 clinical trials, but, with many trials using the same compound, in the end 101 separate treatments for cocaine abuse had undergone clinical trials. The large majority, 76 treatments or about 75%, involved drugs that had already been approved by the U.S. Food and Drug Administration (FDA) for the treatment of another neurological or psychiatric disorder. A search of peer-reviewed literature then showed that NHP experiments are reported for 40 of these 76 drugs. However, 55% of the NHP-tested cocaine treatments (22 out of 40 drugs) obtained results that were either in part or whole different from results

in human laboratory or clinical studies. In addition, it is noteworthy that for about 85% of NHP-tested drugs, such testing continued after results from relevant human clinical or laboratory studies had already been reported in scientific literature.

3. Other methods.

In just three decades, advances in biotechnology have revolutionized research in every area of life sciences, including neurosciences, in a way that makes *in vitro* and *in silico* research increasingly sophisticated and effective. These advances benefit basic science that elucidates the properties and mechanisms of the underlying pathophysiology, such as describing functions of transporters and receptors of biogenic amines in cocaine addiction.[47] On the other hand, genetic studies and non-invasive imaging strategies have made it possible to study addiction directly in human patients. Innovative combinations of various *in vitro* and *in silico* studies may, in effect, not only replace *in vivo* experiments, but produce better results. Moreover, even more effective methods are being developed and are expected to be available in the near future.

a. *Advances in cell culture.*

Cell culture-based research provides an increasingly effective means for formulating strategies for treatments for disorders, such as substance abuse and addiction. Critical discoveries about receptors, transporters and signal transduction in neural cells and cocaine addiction have been accomplished by *in vitro* techniques. Both immortalized cell cultures and primary cell and tissue cultures have their advantages, such as having a dynamic structure, allowing for electrophysiological measurements and pharmacological manipulation.[48,49] Cultures composed of different types of neurons are starting to elucidate properties of circuits.[20] Heterologous expression systems take up foreign DNA and express it readily, allowing, for example, genetic re-engineering studies on dopamine and other monoamine transporter systems.[50-52]

In the past decade, stem cell technology has developed at a rapid pace in various areas of research, including neuroscience.[53] A particularly promising technique is induced pluripotent stem cells (iPSC), which are reprogrammed adult cells.[54] This technology may be particularly useful for human disease modeling, as the disease characteristics can be then studied in neural cells derived from patients with the particular disease. As most neuropsychiatric disorders have a strong genetic component, human iPSCs thus provide a clear advantage over animal models for studying the effect of human genetic background on the disease.[55] Importantly, they may offer a superior method for determining which drugs are effective in humans or in specific patients.[56,57] While stem cell technology in addictive disease research is not yet as far along as it is, for example, in Parkinson's disease and in other neurodegenerative diseases, it could be a critical tool for designing new therapeutic strategies for cocaine or other drug addiction. For example, neurogenesis appears to be negatively impacted by cocaine abuse, and therefore, stem cell technology might be used to augment neurogenesis particularly during the critical abstinence period.[58]

In addition to traditional two-dimensional cell cultures, *in vitro* methods are able to provide increasingly realistic models through 3-D cell aggregations.[54] Recently, scientists announced a new 3-D artificial brain tissue that exhibits electrical activity and responsiveness resembling signals seen in the intact brain. Among other things, this tissue mimics the layers of the human brain cortex with different types of neurons, and expresses genes involved in neuron growth and function. It is expected that this construct will be used in investigating various brain injuries and disorders.[59]

In vitro electrophysiological methods allow detailed analysis of subcellular macromolecules of neurons. For example, voltage and calcium fluctuations that inform on neural activity can be tracked by dyes and by genetic methods. Imaging (such as light, fluorescence and electron microscopy) makes it possible to visualize subcellular structures on a nanometer scale, and genetic fluorescence tagging further clarifies the movement and functions of these structures.[60] One of the latest additions to the research arsenal is the use of cryoelectron microscopy to image ligand-gated channels of ionotropic glutamate receptors.[61] Such receptors are thought to be key to the development and maintenance of addiction.[8]

Microfluidics is another revolutionary technology that can be applied to neuroscience. It permits the control of fluidic micro-environments surrounding an individual neuron and investigation of cell-cell interaction.[62] While retaining many of the advantages of cell cultures, it also allows manipulation beyond traditional cultures. For example, it can be used to mimic aspects of brain microenvironments, such as the development of cortico-striatal synaptic connections, basic 3-D environments, and co-cultures.[63,64] Currently, published literature does not yet show the use of this technology in addiction research, but the area is being developed by increasingly more investigators.[65]

b. *Human tissues and imaging.*

The study of postmortem human brains is the oldest method to study brain structure, and rapidly improving technology has provided powerful means of studying the electrophysical and chemical properties of human neural cells and tissue.[63] Autopsied brains can be used for extensive physiological, pathological and chemical analyses and are used to reconstruct detailed 3D models. A number of brain banks currently operate in the U.S., so that post-mortem human brains are reasonably well available. Studies involving neural tissue eventually need to include human brain as a central component, because the critical molecular events may not occur in other tissues or species, and *in-vitro* or *in-silico* analysis alone is insufficient.[66]

Research on the effects of cocaine on the human brain has abundantly availed itself of post-mortem brain tissue. For example, it has been used in analyzing the effect of dopamine transmission in various regions of the brain.[67,68] Alterations in energy metabolism, mitochondria and oligodendrocyte function, cytoskeleton and related signaling, and neuronal plasticity have been identified in an addicted human brain, as well as differences in transcriptional regulation.[69] Post-mortem analysis may also suggest new physiological causes of addiction, such as induction of innate immunity, and thereby point to new treatments.[70]

With respect to the use of living human brain in cocaine abuse research, various imaging methods are essential to addiction research. In the past few decades, live imaging has provided invaluable data about brain anatomy and tissue composition; biochemical, physiological and functional brain processes; neurotransmitters; energy use and blood flow; and drug distribution and kinetics in the living brain.[71,72] Various imaging modalities provide different types of information and may often be combined to yield structurally and functionally complete data.[73] Importantly, imaging studies can be performed on human beings safely and more conveniently than on nonhuman animals.

For example, magnetic resonance imaging (MRI) and functional MRI (fMRI) are entirely non-invasive methods and respectively produce detailed structural images and depict brain activity.[74] Functional MRI can correlate neural activity to mental operations, such as cognition, learning, and memory and allows correlating "subjective" experiences, such as drug "high," craving, to brain areas that are involved in such processes.[75-77]

Positron emission tomography (PET) enables study of compounds in the brain that are of physiological and pharmacological significance, even in the nano- to picomolar range. Despite its use of radio-isotopes, this technology is relatively benign owing to the isotopes' short half-lives. Since the 1990s, human PET imaging has significantly contributed to our understanding of the neurochemical basis of cocaine addiction with respect to other neurotransmitters and metabolism.[78] For example, among many PET studies conducted on cocaine-dependent persons, dopamine concentrations in a synapse and its associated behavioral effects have been studied in humans.[79] Single-proton (photon) emission computed tomography (SPECT) is similar to PET technology in its main outlines and is increasingly used to investigate neurotoxicity of medications, including cocaine treatments,[80] because of its lower cost compared to PET.

In addition to the above imaging modalities, other techniques can provide more information about cocaine's effects in human subjects. Magnetic resonance spectroscopy (MRS) measures chemical composition of the brain, as well as the concentration and kinetics of certain compounds (such as drugs), or abundant neurotransmitters, such as gamma-aminobutyric acid (GABA) that are implicated in cocaine dependence.[81] Diffusion MRI, or diffusion tensor imaging (DTI), in turn, can trace the structure of neural networks in the brain.[82] Transcranial magnetic stimulation (TMS) allows experiments in humans through the use of a magnetic coil. In cocaine research, a number of clinical trials and laboratory studies have attempted to use this technique as a treatment modality.[83] Electroencephalograhy (EEG) and magnetoencephalography (MEG) provide a picture of the brain's electric and surface magnetic activity, respectively, and are noninvasive. In cocaine research, EEG measurements in humans have been correlated, for example, to the length of treatment and reactivity to drug cues.[84,85]

Different imaging techniques can be combined to produce an aggregate, highly detailed picture of the structure and activity of a human brain. For example, if a PET image of the activity of dopamine receptors is combined with an MRI that provides detailed information about the brain structure, the resulting activity image can provide information about the anatomical location of that activity. Similarly,

PEG and MEG measurements can be combined with other imaging techniques, such as MRI and fMRI, to provide information about the brain's states and correlating such information with other measurements. Moreover, recent major research initiatives, such as the U.S. Brain Initiative (http://www.braininitiative.nih.gov) and the E.U. Human Brain Project (http://www.humanbrainproject.eu), are embarking on a comprehensive discovery of the human neural morphology and connections and on developing new technology, such as non-invasive tools to study the human brain.

c. *Genetic and proteomic studies.*

In the past decades, familial gene studies already suggested that substance abuse has a strong genetic basis, particularly regarding vulnerability to drug abuse and dependence on a drug. We now know that close to half of the risk for becoming addicted can be traced to our genes.[86] Rapidly advancing knowledge and technology of molecular genetics and proteomics promise to contribute increasingly to our understanding of human susceptibility to, and molecular mechanisms of, cocaine addiction, and might lead to effective treatment and prevention candidates.

In addition to individual candidate gene studies, microarray technology allows investigators to study the expression of tens of thousands of genes and likely pathways associated with cocaine addiction.[87] Such expression analysis makes it possible to identify, on a genome-wide basis and without preconceived theories, likely relevant genes and pathways associated with cocaine addiction. Alternatively, microarray expression studies may focus on a subset of genes, such as transcription factors, or microRNAs, that are likely to have a broad impact on the regulation of genes.[88,89]

Studies on cocaine-influenced gene expression have been largely conducted in rodents, but some information has been gathered from human genomic studies using post-mortem brain tissue from individuals who have been using cocaine. [90] Limited studies have also been conducted on NHP brain tissue, after the NHPs had been killed for the purpose of the study.[91] The first NHP microarray study on cocaine-related genes was conducted on cynomolgus monkeys, more than a dozen years ago.[92] However, given the state of technology at the time, the microarray was not large and was using human-based primers; moreover, the description of the results was also truncated, and consequently, the study has been of limited scientific use. In contrast, several microarray studies on human brain tissue have subsequently been conducted. For example, in 2003-2004, three publications described cocaine-related gene expression in chronic and overdose users.[69,93,94] Several other cocaine use-related genomic studies of the human brain have appeared subsequently,[68,95-99] as well as a number of studies dealing with specific genes affecting brain metabolism.[100,101]

Proteomics allows us to identify proteins – products of gene expression – that are present in a brain region that is involved in addiction. Their presence or absence in a given region helps to identify and examine pathways that are critical to cocaine addiction. There are now methods to purify and analyze large numbers of proteins at a time, the principal ones being the two-dimensional differential in-gel electrophoresis (2D-DIGE), and isotopic labeling reagents, together with mass spectrometry. The latter

uses ionization to enable very accurate measurement of the mass of peptides or proteins.[60] In 2005, cocaine-induced changes in the expression of a glutamate receptor were compared in the NHP and human brain.[102] A 2D-DIGE proteomic analyses on cocaine-associated changes in the human,[103] and rhesus monkey brain then followed.[104]

Overall, more than 100 genes have been shown to be altered by cocaine abuse in humans. These include genes that have potentially wide impact, such as extracellular matrix proteins; receptors; ion channels; and transporters (primarily the dopamine transporter, a primary target of cocaine in the brain); signal transduction; mitochondrial function and transcription factors.[105] Specifically, changes in genes for the intracellular cascade mediator cAMP,[103] deacetylases that may affect epigenetic changes,[98] and GABAergic genes have been identified in cocaine addicts.[99]

In the rhesus monkey, cocaine use has appeared to increase the cAMP-dependent protein kinase A (PKA) and other potential transcriptional regulators, as well as glutamate receptors and phosphorylation of NMDA-related structures.[92] In a direct comparison of cocaine-induced changes in ionotropic glutamate receptor subunits in the human and rhesus, some changes were noted in the nucleus accumbens, but not in the putamen, in both species. However, increases in various types of glutamate receptors were not identical among the two species. Some ambiguity results from the fact that there is little information on the relative abundances of ionotropic glutamate receptor subunits in the NHP brain.[102]

These genomic and proteomic studies represent only initial results of the use of these technologies in cocaine studies. Direct comparison of the results between different species, and even among different human studies, has been complicated by differences in their scope, cohorts, and protocols. Further human studies with sufficient sample numbers can be expected to produce additional and more specific results, which can be used in developing treatment strategies.

4. Conclusions.

Cocaine abuse is a serious global health problem and significant resources have been devoted to discovering the mechanism of addiction and designing treatments for human patients. However, no effective treatment exists today for cocaine abuse, nor is the physiological and neural basis for it in humans fully understood. Nor does it appear that the same studies conducted on humans and other species produce comparable results. This suggests that the use of current animal models, including the NHP, is not an effective research strategy for cocaine addiction. This paper provides an overview of relevant considerations for such reappraisal.

The primary reasons for using NHPs are their biological proximity to humans, and the fact that they will self-administer cocaine in laboratory settings. However, as discussed above, even NHPs do not accurately mimic human cocaine addiction, which is a complex physiological and neuropsychological condition. As a matter of fact, the differences between NHPs and humans are significant. First, the evolution of each species has resulted in many biological differences, including the structure of prefrontal cortex and the density of dopaminergic neurons in the human brain. Apart from physiology,

complex behavioral factors, such as motivation for use and persistence to remain drug-free, are critical to the success of human treatment, but can be modelled in NHPs only with difficulty.

Second, NHP studies are commonly justified by the ability of researchers to tightly control the conditions during the experiment, while human studies typically involve comorbidities, and past drug use may be difficult to verify. Nonetheless, NHP experiments also introduce conditions whose influence on cocaine pathophysiology is not well understood. NHPs are typically used in many different experiments, sometimes over a number of years, involving cocaine and other psychoactive drugs. The potential effect of such repeated prior use might be equally confounding to the experimental results as polydrug use by humans. Moreover, NHPs are repeatedly subjected to sedation and anesthesia, potentially affecting the same neurotransmitters and brain circuits as cocaine. The environmental impoverishment, lack of exercise, and stressful laboratory conditions may also affect relevant neurophysiology of NHP experiments.

Does the continued generous funding of NHP experiments promote human health in the most effective manner? In the past seven years, the NIH has provided grants in excess of $100 million for experiments that use NHPs in cocaine research. In addition to the issues discussed above, this line of research presents a number of issues, including: (1) NHP experiments are conducted in small test groups of animals, which does not provide statistical validity and the results of which cannot readily be used by other researchers; (2) Compared to other research modalities, NHP experiments are relatively expensive; (3) For the past several decades, NHP experiments frequently appeared duplicative of prior studies, but yet with minimal variation, which does not allow confirmation or reproduction of past experiments. In sum, the past results of funding NHP experiments in this area do not suggest either a scientifically or economically efficient research paradigm. Going forward, the cost-benefit ratio of continued funding of NHP research of cocaine research by taxpayer funds warrants a more stringent review.

Rational therapy design and development is best grounded on systematic genetic, molecular, and cellular strategies. A better understanding of cocaine's biological action is necessary for developing effective treatments, and molecular and cellular research strategies are an indispensable part of intelligent drug design.[106-108]

Innovative methodologies, such as the Adverse Outcome Pathway which is revolutionizing the toxicology field , promise new and productive ways of examining existing knowledge base of various substances and their effects on metabolism. The Organization for Economic Cooperation and Development (OECD), the U.S. Environmental Protection Agency (EPA), and the European Union Joint Research Centre are developing the necessary infrastructure to host a unified 'knowledge base' of adverse outcome pathways (AOPs) that covers the broad spectrum of biological pathways that are likely to be involved in human health and ecological risk assessment: the Adverse Outcome Pathway Knowledge Base (AOP KB). [109-112] This approach could be applied to disease models, including cocaine addiction, as scientists and clinicians have already gathered data about cocaine addiction pathways, some of which are integrated in a KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway map.

Further work needs to be done to integrate this information in a common and accessible database where sequences of molecular changes within the cells leading to the development of the clinical conditions could be listed. This approach would allow for new information to be added as it is discovered to get a broader understanding of the disease, or of specific populations, and to identify new therapeutic targets. Importantly, this would avoid duplicative research projects in the future.[113]

Biotechnology is advancing exponentially, such that many current methods would have been unimaginable even two decades ago. On a molecular level, genetic studies now provide leads for therapy development, and genetic engineering allows formulation of new types of treatment. On a cellular level, a number of promising new research methods are being developed, which also promise new therapies: Induced pluripotent stem cells can be cloned both from embryonic stem cells and neural cells of human patients; 3-D tissue cultures and neural microfluidics provide enhanced tools for studying basic properties of interacting neural cells and circuits. Extensive new initiatives are set to explore human neurology in detail and to develop new technology to study the human brain.

New research strategies can already be complemented effectively by non-invasive laboratory and clinical studies in human cocaine patients, who undoubtedly provide the best models for the complex mechanisms of addiction. The many forms of imaging technology provide increasingly accurate views of the pathophysiology of the human brain, such as it can be seen at various stages of cocaine addiction. On the other hand, post-mortem tissue from cocaine overdose victims can be studied through elaborate molecular, genetic and proteomic tools, such as those used in gene expression and epigenetic studies. While humans and NHPs have common neural characteristics, even limited gene and protein expression analyses have demonstrated substantive differences, such as regarding glutamate metabolism that is increasingly regarded as a critical part of addictive behavior. To the extent that undertaking human studies may sometimes require more effort than NHP experiments, there is no reason to assume that our scientists would not rise to the challenge if given the opportunity and resources.

Finally, in considering the need to use NHPs in cocaine research, scientific arguments should not be alone on the table.[114] Ethical concerns are an inextricable part of the issue in a developed and civilized nation such as the United States. Although the U.S. Government has not rigorously implemented the so-called 3R principles, it is usually concluded that the existing set of laws and regulations regarding the care and use of laboratory animals embraces these principles.[115] These principles consist of --

- *Replacement.* Use of non-animal systems or less-sentient animal species to partially or fully replace animals;
- *Reduction.* Reduction in the number of animals utilized to the minimum required to obtain scientifically valid data; and
- *Refinement.* Use of a method that lessens or eliminates pain and/or distress and therefore enhances animal well-being.[116]

The minimally incremental nature of NHP research in the past suggests that the same or better results could well be obtained by replacing NHPs with other methods. The duplication that is apparent in U.S.-funded NHP projects suggests that the current procedures may not effectively observe the principle of

reduction. Nor do the somewhat opaque publication methods ensure that these U.S.-funded research projects would readily allow their results to be used by other researchers, and thereby reduce the need of repetitive experiments. With respect to refinement, the physiological and neural closeness of NHPs to humans argues that NHPs deserve to have their physical and psychological needs taken into account better than in the current practice.

The time is ripe for a re-evaluation of the use of NHPs in substance addiction research. A useful antecedent for such a process can be seen in the Committee set up by the Institute of Medicine of the U.S. National Academies and the National Research Council ("the IOM Committee").The Committee examined the necessity of continued use of the chimpanzee in biomedical research and established criteria according to which one should evaluate any proposed future use of the chimpanzee in biomedical research. [117] Subsequently, it has been proposed that a group of diverse stakeholders be set up to critically analyze current uses of other NHPs, the viability of alternative models, and the economic issues involved. [118]

In sum, the necessity and effectiveness of NHP use for cocaine addiction warrants a comprehensive reappraisal. Past results in this respect do not appear to promise significant future gains. On the other hand, new research strategies have come along that offer sophisticated basic research possibilities in vitro and in silico. Non-invasive human studies elucidate human biology and behavior in cocaine abuse more faithfully than NHP studies do. Last but not least, ethical considerations call for a better implementation of the 3R principles.

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