<u>**DBNBR: The Next 5 Years**</u>
1. What is the mission of your division/office?
DBNBR mission: Advancing the science of drug abuse and addiction through basic research.

2. What are your major research priorities?
DBNBR Strategic Directions (see http://www.drugabuse.gov/about-nida/organization/divisions/division-basic-neuroscience-behavioral-research-dbnbr/strategic-plan) include five main goals to promote basic research that applies state-of-the-art science and technologies to advance our understanding of the mechanisms mediating drug abuse and addiction:
- Goal 1: Validate targets and develop ligands to accelerate pharmacotherapy for drug addiction.
- Goal 2: Determine the molecular and cellular basis of vulnerability to addiction.
- Goal 3: Identify the neural circuits underlying drug addiction and their functional properties.
- Goal 4: Identify behavioral processes that underlie drug abuse and addiction.
- Goal 5: Promote cross-cutting NIDA priorities in the areas of HIV, pain, sex differences, and training.

3. What are the (3-5) most significant scientific accomplishments over the past 5 years?
I.   <u>Genes & Environment on Behavior:</u> The discovery of the association of gene variants in the *chrnb4/chrna3/ chrna5* ($\alpha 4\beta 2\alpha 5$) gene cluster on chr15q25 with nicotine dependence (Saccone et al, 2007; Amos et al. 2010, Saccone et al 2010, Thorgeirsson, 2010; TAG consortium 2010) is a major breakthrough that paved the way for functional studies of these subunit receptors. Subsequent functional analysis in animal models revealed the importance of these gene variants in these receptors (Fowler CD et al. 2011 and Flora AV et al. 2013), their specific expression in the habenulo-interpeduncular circuit (Hong LE et al. 2010), and their role in nicotine aversion and withdrawal (Fowler and Kenny, et al 2014; Antolin-Fontes et al 2014; Velasquez et al 2014). These discoveries have inspired investigators to develop new pharmacological agents for α4β2α5, such as AT-1001 (Cippitelli A et al, 2014), and positive allosteric modulators (Jin et al, 2014). Gene variants in these nicotinic receptors, as well as enzymes that metabolize nicotine are also beginning to provide clinically useful markers to guide treatment decisions (King et al 2012, Bloom et al, 2013; Bergen et al 2013; Chen et al 2014; Lerman C et al., 2015). Environmental enrichment has previously been demonstrated to reduce vulnerability to drug abuse and recent data show that access to exercise can abate the acquisition, escalation, and reinstatement of drug-seeking behavior, an effect that is more pronounced in males than in females (Smith & Pitts, 2011; Ogbonmwan et al., 2014; Zlebnik & Carroll, 2014). In addition, specific environmental factors, such as social influences, can enhance risk *or* resilience by, for example, exposing animals to a drug exposed or a drug naïve cage mate, respectively (Smith et al., 2014; Smith & Pitts, 2014). Finally, new phenotypes, such as sign-tracking vs. goal-tracking are being used to identify genetic factors and other individual differences for acquisition into the escalation phase.

II.   <u>Epigenetics and analytics of –omics data:</u> Multi-dimensional data sets provide unique insights into the molecular processes of drug abuse. Systematic integration of data sets (for example, genetic data combined with epigenetic data) to identify relevant biological factors involved in substance abuse phenotypes is increasingly important. Technologic advances have improved our ability to manipulate individual gene loci or anatomically specific brain processes showing that, for example, drug exposure influences locus-specific histone modification in particular brain regions (Heller et al. 2014). Small molecule manipulation of histone modifying enzymes and binding proteins may have promise as potential future therapeutics to treat substance abuse disorders (Maze et al. 2010; Renthal et al. 2009; Covington et al. 2011). In related work, intergenerational effects of exposure to substances of abuse by investigating multiple behavioral phenotypes, as well as genomic, epigenomic and other molecular data sets, have led to the finding that paternal cocaine exposure has protective effects on male progeny (they self-administer less cocaine) and is associated with specific epigenomic changes to the BDNF gene in the sperm of exposed males (Vassoler et al. 2013). In contrast, the offspring of adolescent males and females exposed to THC exhibit altered striatal plasticity and increased heroin seeking (Szutorisz et al. 2014). The neuroplastic changes that take place during the course of addiction may also be regulated by microRNAs. MeCP2 seems to play a key role in the dorsal striatum during escalation of drug taking by interacting with microRNA-212 to control BDNF levels (Im HI et al., 2010).

III.   Neuroplasticity of Withdrawal, Incubation, and Relapse: Neuroplasticity is defined as the ability of the nervous system to respond to intrinsic or extrinsic stimuli by reorganizing its structure, function and connections (Cramer et al., 2011). *Withdrawal/Incubation:* Recent studies of neuroplasticity have benefited from the use of animal models that correlate neuronal alterations with specific behavioral stages of addiction, such as the heightened risk of cue-induced cocaine seeking ("incubation of craving") that occurs during prolonged withdrawal. The incubation phenomenon is relevant to human users who maintain abstinence but exhibit increased vulnerability to cue-induced relapse. In the nucleus accumbens (NAc) cocaine withdrawal generates "silent," immature synapses primarily NMDA-type glutamate receptors. Silent synapses mature in a circuit-dependent manner during incubation by recruiting AMPA-type glutamate receptors. During incubation, the infralimbic-mPFC to NAc shell circuit recruits calcium permeable-AMPA receptors (CP-AMPARs), whereas the prelimbic mPFC to NAc core circuit (pmPFC-core) recruits non-CP-AMPA receptors. These functional adatpations have different consequences on the activity between these two circuits. More importantly, blocking synapse maturation within the former pathway potentiates incubation whereas blocking maturation within the latter pathway inhibits incubation (Ma et al., 2014). The timing of synapse maturation seems to be a critical aspect and one that could be exploited as a target to prevent incubation—and thereby, a mechanism to prevent relapse (Heller et al. 2014, Loweth JA et al, 2014). *Relapse:* Drug-induced relapse is mediated by activity within the pmPFC or the ventral tegmental area (VTA) to the NAc core circuits. Extinction/withdrawal from cocaine induces an increase in neuronal excitability and an increase in dendritic spine morphology (spine head diameter) within the NAc core that are further augmented by subsequent cocaine exposure and activation of the mPFC circuit, yet are attenuated by drug-induced activation of the VTA circuit (Shen et. al. 2014). Coupled with recent studies demonstrating that exposure to environmental enrichment (EE) during withdrawal induces neuroplasticity associated with reduced risk of relapse to drug-seeking behavior (Chauvet C, et al. 2012), these findings suggest that the transition period from withdrawal-to-incubation-to-relapse is critical and may provide an optimal period for intervention.

IV.   Role of Glia and the Importance of Dendritic Spines: The role of glia and microglia was thought to play a secondary role in the function of the nervous system; however a convergence of data point to a more direct role. Recent work highlights the remarkable role that microglia and astrocytes play in sculpting neural circuitry during development by secreting factors involved in forming and eliminating synapses (Clarke and Barres, 2013; Schafer et al, 2012). Other work suggests that memories associated with drug use are supported by structural and functional plasticity driven by F-actin polymerization in post-synaptic dendritic spines at excitatory synapses, providing novel therapeutic targets for stimulants (Young EJ et al. 2014).

V.   Technologies for enhancing drug development: Accurate knowledge of molecular structures is a prerequisite for rational drug design and structure-based functional studies. In 2012, the crystal structures of the opioid system receptors were solved (Manglik et al., 2012; Granier S et al., 2012; Wu et al., 2012; Thompson AA et al., 2012). Researchers have since been able to explore the way new ligands might interact within the binding pockets of these receptors (Filizola M and Devi LA, 2013). Other technologic advances include optogenetics (Tye and Diesseroth, 2012) and Designer Receptors Exclusively Activated by Designer Drugs (DREADDs; Zhu H and Roth BL. 2014) to control neural activity and behavior, as well as cell-based neurotransmitter fluorescent engineered reporters, called CNiFERs, to monitor native transmitter release in freely-moving animals (Muller A et al., 2014).

4. What (3-5) scientific questions d   you want to answer, including 1-2 "bold/big" ones?

Building on the accomplishments and advances of the last five years, the DBNBR envisions using *integrative approaches* to better understand the *neuroplasticity of addiction* and to identify *novel intervention strategies.*

Question 1: Integrative Approaches—Towards an "Addictome"
Integrating data from genomic, epigenomic, behavior, neurobiological, environmental, and other areas to produce the phenotypes associated with the stages of drug abuse and addiction is challenging. What is the best way to

generate or assimilate a diverse, interoperable collection of multi-scale data sets that can be mined by the scientific community and visualized in a user-friendly, 4D-framework to discover novel relationships and scientific knowledge for the stages of addiction? The Addictome is a collection of all data types representing the internal and external influences that contribute to an individual's propensity for drug abuse, organized by key transitional stages within the trajectory of addiction. It enables investigation into how the Addictome varies across individuals and establishes a platform and a knowledgebase that helps identify and characterize those important differences.

Rationale/Significance: There is a critical need to integrate data from diverse sources to maximize knowledge generation and reduce redundancy. There is also a strong desire to ensure that critical scientific discoveries are replicable and validated to ensure that we have a strong foundation on which to build. Finally, data sets generated for specific questions could be repurposed and used to answer additional questions through integrated secondary analysis by additional investigators. Given tight fiscal times it is critical that we maximally extend the data generated to prioritize future research endeavors.

BIG & BOLD: The Addictome. As a first step in this direction, it is strategically prudent to embark on a pilot Addictome Portal. The Addictome Portal will provide data coordination, visualization, and analysis tools for a collection of scientifically compelling Addictome data generated by NIDA researchers and addiction relevant information generated beyond NIDA. This portal moves NIDA towards managing all data generated through investigator-initiated studies as a whole to enable data mining and identify emergent opportunities across seemingly disparate data sets. This concept both anticipates and positions data generated from NIDA supported science to be aligned with NIH-level and BD2K data repositories.

Question 2: Neuroplasticity of Addiction

Which neuro- glial- adaptations occur with abstinence from chronic drug use that heighten risk of relapse, which impart resilience to relapse, how do interventions for treating drug abuse disorders reverse or compensate for these adaptations to maintain abstinence, and can the brain fully recover normal function after chronic drug use?

Rationale/Significance: Drug addiction is a chronic, relapsing disorder that is characterized by the transition from voluntary, casual use to compulsive, uncontrollable use. This behavior results from distinct molecular, functional and structural adaptations which alter neuronal function as a person progresses along the addiction trajectory. However, whether an individual transitions through the stages of addiction depends upon the consequences and functional interactions of neuro-glial adaptations that are influenced by, for example, the type of drug used, the age of drug use, the sex of the individual, an individual's genetic and environmental influences, their peers, enriched or impoverished environment, and social status/dominance that, in sum, may confer increased risk of, or resilience to, addiction.

Question 3: Novel Intervention Strategies

What are the key transition points during the progression of addiction that can be characterized to identify malleable processes for targeted interventions?

Rationale/Significance: The general trajectory of addiction proceeds from casual use, to escalated use and a diagnosis of addiction, with subsequent cycles of withdrawal, abstinence, and relapse to drug use. In thinking about interventions to break the cycle, there are at least three important considerations: 1) effective interventions and treatments are likely to differ at different stages of this trajectory; 2) individual differences, including early life experience, sex, genetics, temperament, age, and co-morbid conditions are likely to differentially affect the biological factors underlying transitions along this trajectory; and 3) there are likely to be specific points along this trajectory at which the brain is particularly malleable, and where the right type of intervention might prevent continuation of drug taking and transition to an addicted state or "incubation of craving" and relapse.

BIG & BOLD (Note that this is based on Questions 2 & 3): Preventing relapse is a key objective in treating drug addiction. One goal is to understand the myriad of neuro-glial adaptations that occur throughout withdrawal and abstinence, and determine the relative contributions of these in enhancing drug craving and seeking that increase relapse vulnerability. Can these neuro-glial adaptations induced by interventions that prolong abstinence be exploited as a means for inducing full recovery of brain function after chronic drug use? Ultimately, this research should inform novel molecular targets and behavioral approaches towards regulating

neuroplasticity to prevent relapse—towards the development of "synaptoceuticals," which would target and modulate the structure of synapses to improve their function.

**See Also the Genetics Workgroup Strategic Plan submitted separately.*

5. How will you g   about answering these scientific questions and what will you nee   to d   so? (i.e., actions to be taken, resources needed, collaboration with other divisions/ICs, etc.)

Question 1: Integrative Approaches—Towards an "Addictome"

The Addictome can be conceived of as the collective data from the community of addiction researchers as a whole. The Addictome Portal could include features to facilitate better interactions among addiction researchers to tackle particular interdisciplinary areas of investigation. The Addictome would enhance NIDA activities in three major areas: a) Scientific management, b) Hypothesis generation, c) Advancing discoveries toward improvements in diagnosis and treatment of substance use disorders (SUDs) and associated co-occurring diseases, and d) Identifying scientific gaps and opportunities, areas of agreement and congruence (which will help to improve the overall reproducibility of the NIDA research enterprise) and unprofitable scientific areas by making negative results more transparent.

The DBNBR proposes a four step pilot phase using DBNBR data, with addition of trans-NIDA relevant data types at a later stage. The first critical step is to support workshops to identify metadata elements and to establish the infrastructure to evolve ontologies that ensure Addictome data discoverability. To best utilize resources in the pilot phase, data efforts could be constrained to collecting data specific to a well-defined critical time period (e.g. withdrawal-to-incubation-to-relapse). A second step will be to implement a multi-pronged strategy to require and incentivize researchers to share their data. Step three will be to create an Addictome Coordinating Center to: a) develop a portal for available data sets, b) establish workflows and automation to wrangle and curate data into user-friendly, discoverable formats, c) provide open source analytical tools and software, and d) enable user-friendly, cloud-based data visualization interfaces. Step four will be to encourage the scientific community to perform secondary data analysis to make novel scientific discoveries on datasets too large to move to personal computing devices. Effective data sharing will provide information about currently unreported negative results, increasing transparency and efficiency.

Question 2: Neuroplasticity of Addiction

Substance use and abuse induces structural and functional adaptations within the brain that occur at each stage of the addictive process. Studying critical drug-induced neuro-glial adaptations that contribute to phenotypes such as heightened risk of drug-craving and relapse after abstinence and withdrawal, adopts a translational approach that necessarily combines human research with validated animal behavioral models. Data should be obtained in real-time, across multiple dimensions of analyses and address compelling questions, such as:

a)   The functional consequences of neuro-glial interactions during drug abstinence and relapse.
b)   Exploit the differences between high and low risk phenotypes to elucidate underlying neuroplastic mechanisms and determine neuro-glial adaptations subserving behavioral interventions to guide pharmacotherapy development or approaches.
c)   Identify the functional contributions of emotional, cognitive, motivational, learning, decision-making and impulsivity/disinhibition processes during withdrawal and relapse.
d)   Define structural and functional changes in synaptic function, including GABAergic and cholinergic neurotransmission, neuropeptide signaling, and other under-studied neurotransmitter systems.
e)   Explore neuroplastic substrates induced to maintain homeostatic function (e.g. energetics and glymphatics) from neuroplastic substrates that contributes to behavior change (e.g. craving, relapse).

Question 3: Novel Intervention Strategies

A tractable approach to addressing the question above is to focus efforts on two time periods where there is the most evidence that interventions could alter the course of the addiction trajectory, and also where drug users are most likely to seek or be referred to treatment:

A. **Transitioning from casual to escalated, compulsive use.** This is a period during which younger users are likely to be (or should be) treated without necessarily having an addiction diagnosis, and where interventions could not only reduce drug use, but possibly offer protection against return to drug use. Are there protective factors known from studies of vulnerability that could be bolstered to confer resilience in otherwise vulnerable individuals?

    a. Exploit known phenotypes that escalate more readily compared to those that don't (e.g. sign-tracking/goal-tracking); test behavioral and environmental manipulations in an attempt to alter vulnerability (including in combination with pharmacotherapies).

    b. Continue to characterize intrinsic or innate vulnerability factors (e.g. neurobiological, genetic, psychological, microbiomes, etc.) and environmental influences (e.g. enrichment, nurturing, exercise, social, etc.) that can be manipulated to alter behavioral trajectories, with a focus on the nexus of environmental interventions and individual differences or behavioral phenotypes.

    c. Identify neurobiological mechanisms that confer protection/resilience and which might suggest targets to be manipulated by pharmacology, or serve as mechanistic biomarkers to predict behaviors.

    d. Develop computational models of behavioral and neurobiological processes that predict varying addiction trajectories that can be used to test hypotheses about intervention strategies.

B. **Abstinence and incubation.** In a variety of rat studies, there appears to be a time period (2-4 weeks) after last drug exposure when considerable neurobiological changes occur, some of which have been shown to underlie the "incubation" phenomenon. If these neurobiological processes could be prevented or reversed by targeted interventions, it might be possible to prevent the "incubation of craving" believed to underlie relapse. The ideal intervention would be a short-term pharmacological, behavioral, or other treatment (e.g. transcranial magnetic stimulation, or other brain stimulation) that would "re-set" causative molecular or cellular processes and prevent the transition to a chronic, relapsing condition. Here, development of new behavioral assessments of incubation of craving – or behavioral indicators of other potential mechanisms driving continued drug seeking and taking are needed to complement studies using responding during extinction conditions. Additionally, understanding subsequent cycles of withdrawal, abstinence and relapse is needed to better explore the questions such as: does incubation re-set with successive cycles, or does it continue to increase in intensity?

6. What are the greatest challenges in attaining your goals and how d   you intend to address them?

<u>Challenge #1 Analytics and Promoting a Culture of Data Sharing.</u> Two major challenges are: 1) The analysis and efficient utilization of "big data" and 2) working with investigators and other stakeholders to promote a strong culture of data sharing. Implicit in this conceptual framework is the need to work closely with Office and Division Directors across NIDA to identify areas of common need and to enable research development where the findings can accelerate beyond DBNBR.

<u>Potential resolutions:</u> In addition to requiring data registration with the Addictome, data sharing must be incentivized by developing ways for researchers to receive tangible career or scientific discovery benefits from depositing and sharing their data. Incentives could include small supplements, citation of deposited datasets for use or quality, authorship credit, and credit towards tenure or promotion decisions at universities. We propose that investigators include any extra costs for data sharing in their grant applications. Benefits of this investment will be in making data widely available to researchers, reducing costs and increasing the efficiency of publicly funded research.

<u>Challenge #2 Increased Coordination of NIDA Research Across Divisions.</u> In addition to transitioning to a culture of generating, cataloging, and sharing big data, current needs include creating and applying new animal models of chronic drug administration, and understanding their clinical validity to enhance the translational potential of the science we support. These challenges are addressed by fostering collaborations between behavior experts with the

needed paradigms with molecular, genetic, physiology, and clinical efforts. As the ABCD study launches, this coordination and integration is critical for reproducing, validating, and maximizing the effort.

Potential resolutions: Create "think tank" like spaces for cross-divisional efforts for each of the research priorities. These groups would discuss milestones, develop focused initiatives, encourage transdisciplinary research, and conduct portfolio analyses to ensure comprehensive and well-integrated approaches. Another suggestion is to earmark 20% of divisional funding allocations to investigator-initiated applications that fall under the NIDA strategic plan priorities, but may not make the increasingly tighter priority score cut-offs. Lastly, a more specific example could be to support a PAR (NIDA review) for translational collaborations between DBNBR and other divisions to fund R21/R33 mechanisms where the R21 starts in DBNBR and the R33 transitions across divisions for research to develop and test novel clinical, prevention or early stage drug abuse intervention questions for humans that are inspired by findings in the animal research literature—or vice versa (could also consider the UH2/UH3 and K18 mechanisms).

Challenge #3 Portfolio Analyses and Evaluation—Establishing Metrics of Success for the Strategic Plan. A big challenge will be to identify the right metrics to measure the success of key elements of the strategic plan to show clear progress as we move forward.

Potential Resolutions: Obtain concurrence and engagement of key thought leaders in addiction, and other areas. Enhance administrative data systems to facilitate portfolio analysis. Some examples: Question 1: success might be the development of "Addictome.org" that hosts and serves addiction-related datasets from DBNBR and NIDA-supported research. Question 2: New science in the area of plasticity will provide insights that result in new interventions, treatments, and therapeutics, such as thinking of therapies that target discreet structure and function of synapses, i.e. synaptoceuticals. Question 3: If successful, there will be at least two new opportunities at one or more stages of addiction for intervention, either behavioral or pharmacological.

7. How will your plans take into account current NIH priorities? (e.g., health disparities, sex/gender differences, BRAIN, reproducibility, big data/data sharing)

Across NIH, questions proposed here are relevant for datasets from other ICs and trans-NIH projects including Common Fund, CRAN (ABCD Study), BRAIN, BD2K, Precision Medicine Initiatives, and from other national and international projects. The Addictome could be designed and used for systems biology studies that might reveal interactions between SUDs and co-occurring conditions such as HIV, psychiatric disease, pain, sex/gender, disparities, etc. Data from each of the strategic questions could be significantly informed by, and feed into, the NIH BD2K project, such that BD2K resources can be quickly adopted and leveraged by NIDA researchers. In addition, the same programs addressing NIH priorities are generating exciting new technologies that can be adopted, or even developed, in addiction research to advance NIDA's mission. Some examples:
- Single neuron and glia analyses show heterogeneity within addiction pathways, requiring sub-population and sub-circuits level analysis to understand age-gender-ethnicity-specific and substance specific addiction.
- The development of emerging transformative research platforms will dramatically advance patient specific cellular and molecular studies of addiction:
  o Employ iPSCs derived from addicted individuals for 3-D neuronal cultures to recapitulate brain and psychiatric disorders (Choi et al., 2014; Chen et al., 2014; Doers et al., 2014; Williams et al., 2014), as well as cerebral organoid cultures for brain development disorders (Lancaster et al., 2013; Lancaster and Knoblich, 2014), and uses for gene editing, drug screening, neural circuit formation, and plasticity.
  o Exploitation of genome editing technologies (e.g. CRISPR-Cas9/TALEN) to generate genetic models to rigorously validate genetic, epigenetic, and other addiction-relevant molecular discoveries.
  o Exploitation of new technologies (e.g. light sheet microscopy, CLARITY, and brain expansion technologies) to improve visualization of brain cell types and their connections.