NIDA Strategic Planning –
Gene x Environment x Development Interactions (GEDI)
Co-Chairs: Naimah Weinberg and Jonathan Pollock
SPB Coordinator: Michele Rankin

Workgroup Webinar
Tuesday, May 26, 2015
3:00 p.m.

Attendees

Co-Chairs: Naimah Weinberg, Jonathan Pollock; **Extramural Workgroup Members:** Hugh Garavan, Gustavo Turecki, John Rice, Jane Costello, Bill Iacono, Eric Johnson; **NIDA Staff:** Joni Rutter, John Satterlee, Hal Gordon, Michele Rankin, Emily Einstein; **Public Participants:** Lisa Tarantino, Abraham Palmer

Welcome and Overview

Dr. Jonathan Pollock welcomed participants and provided an overview of the topics to be discussed: the scientific challenges and priorities regarding epigenetics, environment, and translational studies. Dr. Naimah Weinberg explained that the main discussion points would be captured in a draft that will be circulated to workgroup members.

Epigenetics

Dr. Gustavo Turecki shared his thoughts on the epigenetics of behavioral phenotypes.

- Epigenetics is a dynamic process that involves the understanding of how specific markers evolve over time with the acquisition of specific behaviors. He noted that it is currently impossible to conduct such studies using human brain tissue , but suggested the examination of peripheral samples over sequential periods of time might provide some insight.
- Dr. Turecki also recommended an emphasis on genome-wide epigenetic studies of addiction phenotypes that should include studies looking at methylation, hydroxymethylation, histone marks and noncoding RNA. The focus should be on brain tissue from areas already implicated in addiction – the nucleus accumbens, caudate, prefrontal cortex, and other subcortical and cortical regions.
- Research should examine the role of the different regulatory mechanisms of the genome that have already been identified, such as long noncoding RNA, in addictions. These studies should be followed by attempts to investigate to what extent the signals can be detected peripherally (from the same subjects) in order to identify potential biomarkers of addiction.
- On the topic of eQTLs, Dr. Turecki said they would be interesting to explore, particularly focusing on methylation, but he would not list that as a top priority.

Dr. Bill Iacono suggested the collection of samples from peripheral sources in longitudinal studies.

- If candidates are identified in the brain that can be successfully assayed in blood or saliva, then those samples could be used to study methylation changes with development.

- Dr. Jane Costello noted concern about the number of samples required for this approach, as well as for other workgroup suggestions for addressing GEDI research thus far, but she stated that Dr. Iacono's idea for using longitudinal population data to identify likely genes and environmental markers might be helpful in testing for environmental effects.

Dr. Hugh Garavan spoke to the potential of imaging technology.

- Dr. Garavan proposed that imaging studies might be used to try and validate some peripheral epigenetic markers in humans by assessing how they might relate to changes in brain structures and functions. He added that this process might be informed by our knowledge of brain gene expression maps. Current imaging resolution should translate to gross structural and functional changes in real time, so it should be possible to see these changes.

Dr. John Rice echoed others' suggestions and added that NIDA might draw on expertise from work being done with the human DNA methylome project.

Dr. Eric Johnson also agreed with banking longitudinal specimens.

- The challenge, he said, will be looking at differential expression and transcription by tissue type. Unfortunately, blood and brain rarely cluster together, so the focus should be on brain tissue samples and applying epigenetics to substance abuse phenotypes.
- From a developmental perspective, animal models might be particularly useful, but it is difficult to do developmental work with postmortem brains. Dr. Johnson advocated for the use of RNA-Seq, as opposed to expression arrays for the examination of multiple brain regions, due to the amount of information it produces. Integration of RNA-Seq data with genotype data will help with our interpretation, and it can improve our power to some extent by focusing on regulatory variance to detect variant phenotype associations.
- Dr. Johnson maintained that gene expression and methylation tends to yield larger effect sizes than what is typically seen for germ-line variants and association with a phenotype. He also noted that the sample size for genome-wide epigenetic studies of postmortem brain tissue between affected vs. not affected will be much smaller than typical GWAS.
- Dr. Turecki agreed, but noted the importance of *not* using the example from variation studies and (incorrectly) inferring that the same would be the case for epigenetic studies, particularly if these use different sources of information. Accordingly, one may decrease the amount of error by better focus on epigenetic variants that have a functional impact, and as such, these studies may need smaller sample sizes.

Dr. Joni Rutter agreed that it may not be effective to study postmortem animal brains for developmental effects.

- She asked the workgroup if there was a way to look at induced pluripotent stem cells to link genetic and epigenetic information during neuron development, or if there was some other way of using those stem cells for mechanistic work, as opposed to looking at longitudinal studies only.

Dr. Turecki responded to Dr. Rutter's comments.

- He said that it is very difficult to control for so many variables and stochastic events, but using iPSCs is a viable avenue that might complement other approaches used to

investigate epigenetic effects. In addition, collecting peripheral tissue across the lifespan as a function of development of addiction phenotypes in both human and animal specimens may provide useful information.

Dr. John Satterlee pointed out that muscle tissue resembles brain more than any other tissue, including blood, but blood contains other items of interest, such as extracellular vesicles, which might be very valuable for capturing biomarkers.

- Dr. Turecki agreed with collecting more samples than less. He pointed to the difference between methylation and circulating microRNAs when analyzing blood samples, as microRNAs may be a more viable biomarkers given recent data suggesting that microRNA are excreted by cells and circulate in exosomes or in complex with proteins

Environmental Characterization

Dr. Costello explained that data harmonization involves finding a way to define an environmental factor in many different data sets. It is an extremely difficult and complex process.

- She said there are various ways to approach harmonization. One way is to conduct parallel analyses of different data sets using similar constructs, but that's only as good as the similarity of the factors being measured. All approaches will require a lot of effort and resources in order to obtain measurable amounts of power in the analyses.

Dr. Weinberg asked Dr. Costello if NIDA should follow the PhenX model for future studies.

- Dr. Costello replied that PhenX is an excellent model, as long as researchers are measuring the right thing. Environmental measures have to be evaluating the *right* aspect of the environment. She said following the PhenX model is a start, but we don't have any strong evidence that we're measuring the right things in the right ways.

Dr. Pollock asked if there was a difference between bivariate factors vs. continuous variables, and whether one or the other reduces power. Dr. Costello and Dr. Weinberg both suggested there was no way to know, but added that timing was also an issue to consider. For instance, Phoenix measures were selected to be as brief as possible.

Dr. Garavan asked if we had a common course for deconstructing the concept of "environment."

- Dr. Garavan thinks the concept needs to be deconstructed before developing adequate measures for each domain and subdomain, noting that there might be some potential for the use of new mobile technologies being developed (i.e., personalized bio/behavioral sensors).
- Environment can include stressors or opportunities, such as child abuse or peer pressure vs. such as availability of drugs, peer pressure, local culture, or parenting skills and intellectual challenges.
- Lastly, Dr. Garavan proposed studying which environmental factors contribute to resilience.

Dr. Rice and Dr. Iacono discussed a dual approach to studying genes and environment.

- Genes and environment are equally important, but the field has seen much more progress with genome measures than with environment measures. So it would be more efficient to

study genes first, and then focus on a deep characterization of the environment once the genes have been identified—and not try to analyze both at one time. Capturing large numbers of samples from a GWAS for environment (even at a superficial level) would be helpful; it could then be followed up by enriched, targeted, more deeply phenotype environmental measures on fewer subjects.

Dr. Johnson submitted that GWAS investigators have already collected a lot of information and that NIDA should examine this existing data to address the issue of which environmental variables are relevant to our question.

- Dr. Johnson proposed a broader data-sharing culture to help facilitate harmonization of data sets and help shape our future studies. While we are all required to share data through DdbGaP, what's included in that phenotype file and how many environmental measures are included tends to be quite limited.

Dr. Rutter favored incorporating animal models into some environmental characterization studies, citing recent findings on peer influence in rodent samples.

- She suggested this might help advance our understanding of the more mechanistic aspects of GxExD. Others workgroup members agreed with this strategy.

Dr. Hal Gordon said that it was crucial to study the interplay of timing and environment and wondered if capturing broader measurements in the beginning could provide a strong platform to build upon.

- One option might focus on characterizing adverse environments at particular points in development, compared to other times of development. Another approach might compare positive environment vs. negative environment. He said trying to study too many environmental factors at once would be too complex.
- Dr. Rutter pointed out that this could be done with animal models.

<u>Animal to Human Integration</u>

Dr. Garavan spoke to the challenge of funding for translational research.

- He said that researchers need to demonstrate excellence in both areas for grant applications but that often is not the case. Additionally, trying to integrate both domains requires compromise, which might spark friction between the two camps and can also result in superficial findings. Dr. Costello and another workgroup member agreed with this observation.
- Dr. Weinberg suggested it might require a special review or announcement.

Dr. Turecki offered that there are different ways to think about animal/human study designs: combined studies and translational.

- Combined studies look at both animal and human in parallel. Translational studies work off results from animal research (in which there is a wealth of existing data) and focus on human design. Dr. Turecki suggested a great need for cross-talk between the animal and human camps so that more integrated investigations can be conducted.

- Dr. Turecki said that NIDA should consider promoting networks of researchers; develop grants that will allow the two camps to work together on common projects; and promote conferences to foster dialogue.

Dr. Rice stated that there are many uses for animal models, but pointed to results from past mouse QTL studies on alcohol consumption as an example of an *un*successful integration model.

Dr. Iacono suggested that researchers run into problems when they try to study both animal and human models concurrently in the context of discovery. However, if you end up with a good gene candidate that's confirmed, and you have a good homolog model in a different species, you could then examine mechanisms involved with environment and development, and look for parallels across species.

Dr. Johnson agreed with Dr. Iacono's description of best-case scenario, citing the Cogent project as an example of a successful animal model that can be used to satisfy reviewers, build up R01-level projects for both animals and humans, get researchers to interact and share findings for translation, and lead to a more mechanistic understanding of the initial GWAS results.

Public Comments

- Dr. Lisa Tarantino suggested that many grants speak to a translational component, but it is often included only as a token line item to perhaps satisfy a review requirement, and the design is not really fully developed or followed up on. She also pointed out that both animal and human studies are in the very early stages of *identifying* genes that are relevant to addiction, so we don't really have enough information from either to look at common circuits, and that's what we want, not specific genes. Once we get to know more about the underling pathways in human candidate genes, we can actually start to integrate with animal models better than we have before.
- Dr. Tarantino remarked that new approaches, new populations, and new genomics tools are allowing us to identify genes more quickly in mouse QTLs than in the past. They are doing discovery work, and she believes that is where the animal models will be the most useful. So instead of thinking about it as a GWAS for epigenetics and needing high numbers of samples to get to significant loci, we have examples from animal models. She pointed out that it is easier to do environmental manipulations with animals and much easier to get brain tissue. Researchers can start to look at specific genes that are identified in the animal models, and then the human sample sizes won't need to be so large.

Next Steps

NIDA program staff will draft sections for priority recommendations. All workgroup members are welcome to review the draft and provide comment, but the following extramural members volunteered to complete the first-round edits:

- Dr. Gustavo Turecki—epigenetic approaches
- Dr. Bill Iacono—improved phenotyping
- Dr. John Rice—gene identification
- Dr. Jane Costello—environment characterization.

Dr. Pollock will draft the human-animal section for full workgroup review.

- Dr. Weinberg asked workgroup members to keep in mind the cross-cutting themes when reviewing the draft recommendations document.
- Dr. Pollock asked workgroup members to send comments about the resources needed to accomplish the goals for all the sections covered so far. He also requested feedback on the GxE interplay.

Next Meeting

The next WebEx Event is scheduled for Tuesday, June 9, at 3 p.m.