SUMMARY

On March 28, 2018, the National Institutes of Health (NIH) Brain Research through Advancing Innovative Neurotechnologies® (BRAIN) Initiative convened a workshop entitled *Workshop on Research with Human Neural Tissue* on the NIH campus in Bethesda, MD. The workshop brought together two dozen participants with backgrounds including neuroscience, neurology, bioethics, philosophy, and theology, along with leadership from the NIH BRAIN Initiative, to discuss the state of the science for research with *ex vivo* brain tissue and human cerebral organoids, and considerations for responsibly supporting these areas of science.

To open the workshop, Dr. Walter Koroshetz, Director of the National Institute of Neurological Disorders and Stroke (NINDS) and co-leader of the NIH BRAIN Initiative, emphasized the Initiative's goal to deliver new technologies for studying brain circuits, and the importance of proactively discussing the neuroethical implications of research. Following these opening remarks were panel presentations and discussions focused on: research with *ex vivo* brain tissue; research involving human cerebral organoids; and efforts to ensure the responsible conduct of this research with human neural tissue. To conclude the workshop, Dr. Joshua Gordon, Director of the National Institute of Mental Health (NIMH) and co-leader of the NIH BRAIN Initiative, synthesized input from participants regarding the experimental paradigms discussed during the day and the various associated neuroethical considerations.

OPENING REMARKS

Dr. Koroshetz opened the workshop by highlighting the promise and vision of the NIH BRAIN Initiative. He stated that the BRAIN Initiative is aimed at uncovering the unknowns of how the brain produces cognition, emotion, perception, and action, to provide a better understanding of the unique qualities that make us human, and to develop the knowledge and tools to enable better treatment of brain disorders. If doctors and clinicians are to treat neurological, mental, and substance use disorders more effectively, then it is vital to be able to monitor and measure the brain circuit underpinnings of these disorders. Current technology to do so is severely limited and this gap stands as a major barrier to developing therapies to normalize or compensate for brain circuit dysfunction.

To underscore the public health relevance of this mission, Dr. Koroshetz highlighted the current opioid crisis and substance use disorders as examples of circuit dysfunction. In opioid addiction the brain's reward circuits re-wire, such that the person seeks continued exposure to the drug at the expense of almost all other normally motivating factors. Dr. Koroshetz raised the following issue: how can we develop our understanding of neuroplasticity to undo the "tangles" that drive a person to repeatedly take opioids, despite numerous risks including a high risk of death? Relatedly, the lack of effective means to treat pain, especially chronic pain, led to the overprescribing of opioids and exponential rise in addiction in the U.S. Chronic pain is another classic circuit disorder for which better understanding of brain circuit function could guide much more effective treatment. Applying BRAIN Initiative neurotechnology and scientific effort to the pain and reward circuits could help alleviate these dual public health issues.

The BRAIN Initiative makes use of taxpayer dollars and works to provide a solid ethical framework for ensuring that scientific research is of the utmost value to the public it intends to serve. This understanding has been present from the start of the Initiative: the Presidential Commission for the Study of Bioethical Issues was charged with assessing neuroethical issues in light of the BRAIN Initiative in 2013. The result was *Gray Matters, volumes 1 & 2*, and a recommendation from the Commission to integrate neuroethics into the science of the BRAIN Initiative. Moreover, Dr. Koroshetz emphasized the

importance of avoiding the pull of science – and ethics – hyperbole; advancing the science is critical, and best done without unwarranted ethical or scientific hype.

Dr. Koroshetz commented that there are many examples of medicine weighing the ethical implications of applying new technologies, to eventually settle on a widely agreed upon standard of medical practice. Now, as the technologies funded by the BRAIN Initiative rapidly evolve, the accompanying guidelines for their use must similarly evolve. This workshop, focusing on a few research paradigms that make use of human neural tissue, touched on various neuroethical considerations, discussed below. It will be important to continue to revisit them and others that emerge in an interdisciplinary fashion as the science moves forward. The workshop was one step in an ongoing effort to ensure that research funded by the BRAIN Initiative is conducted according to the highest ethical standards.

In closing, Dr. Koroshetz said he felt confident that with the topics of discussion, expert consultations, and information gathered at the workshop, the NIH would be able to make proactive, well-informed decisions on ethical issues related to BRAIN Initiative-funded research going forward.

SESSION I: Research with *ex vivo* brain tissue
Moderator: James Eberwine, PhD, University of Pennsylvania

A challenge to our understanding of the human brain lies in our inability to physically examine it in its entirety during a person's lifetime. This panel focused on research paradigms that make use of *ex vivo* brain tissue, or specimens extracted from animals and consenting human patients. *Ex vivo* specimens range from small cubes of tissue removed during surgical procedures, to whole animal brains.

Ex vivo tissue is particularly well-suited for studying intact brain circuits along with visualization and reconstruction of detailed cell structure and genetic code analysis. This emerging approach means that researchers can investigate genetic and electrical properties of cell types and neural circuitry at unprecedented levels of resolution. Uncovering this detailed information helps researchers to understand disorders and diseases of the nervous system, which can help inform diagnosis and treatment.

Panelists discussed some of the challenges associated with studying the connectivity and function of complex neural circuits in large mammalian brains. They covered current and emerging platforms for studying the functional properties of the human brain, including electrophysiology, morphology, transcriptomics, and detailed maps of neuronal connectivity, including at the synaptic level. Studying brain cells and their connections using these techniques allows researchers to build circuit models, which is a critical step towards the BRAIN Initiative's goal of understanding how brain circuits produce perception, cognition, emotion, and behavior. Further, applying these approaches to animal model systems enables comparative studies of neurophysiology.

Studying *ex vivo* tissue is valuable because of the extent to which this tissue maintains many aspects of the *in vivo* brain. However, it is important to note that after death, brain tissues undergo significant changes in their biophysical properties, causing the irreversible large-scale disassembling of their synaptic connections. Therefore, studying other aspects in *ex vivo* tissue, such as long-distance signaling, remains a challenge, precluding our ability to trace connections among significant numbers of neurons accurately and rendering the possibility of higher-order cognitive properties extremely remote.

SESSION II: Research with human cerebral organoids
Moderator: Arnold Kriegstein, MD, PhD, University of California, San Francisco

This panel focused on research with human brain organoids, which are 3D multicellular structures derived from induced pluripotent stem cells (iPSCs) derived from skin biopsies or blood samples. They are grown in culture systems to resemble parts of the developing human brain. These model systems represent a new avenue of research, which is still being explored and refined by scientists, but the models hold great potential for shedding light on human brain function and disease. Excitingly, these organoids hold the promise of being able to serve as better predictive models of brain disorders specific to certain genetic mutations and even individual patients, helping to translate therapeutic approaches from animal models into clinical practice. Panelists discussed approaches, for instance, to model aberrant neural migration present in some brain diseases; to grow brain organoids for extended periods of time *in vitro* to generate a fuller complement of brain cell types, including glia and blood vessels; and to compare the models with primary neural tissue in order to assess how well the models recapitulate development and function *in vivo*. The panelists also touched on limitations of these models, including the need to standardize protocols in order to decrease variability, and the fact that many brain diseases and disorders are behavioral and therefore cannot be fully modeled with brain organoids. These organoids are far from approaching the size, precise organization, and cellular complexity of an animal brain – even a simple animal like a mouse – thus, resemblance to a human brain is currently extremely limited, which is not surprising given the extraordinary complexity of the human brain. There is also an inherent limit on the extent of development possible, due to lack of blood supply. One approach to partially overcome the latter issue is to implant a human brain organoid into a host animal brain, such as a mouse brain, which may enable better modeling of human brain diseases in a physiological setting.

SESSION III: Ensuring the responsible conduct of research with human neural tissue
Moderators: Nita Farahany, JD, PhD, Duke University, and Hank Greely, JD, Stanford University
This session focused on unique neuroethical considerations associated with these areas of science, and questions that might help guide research in these areas. In summary, participants discussed three different experimental approaches: studies with brain organoids, explants of brain tissue, and whole brains studied *ex vivo*. These approaches make use of different methodologies, but prompt similar considerations which are summarized here.

Scientific promise:
Workshop participants emphasized the incredible potential of these human brain models, and discussed the ethical imperative of leveraging them to advance our understanding of human brain diseases and disorders. This urgency is also motivated by a strong premise of religion and humanism – to help those with terrible illness, and to alleviate the suffering caused by diseases and disorders of the brain. In addition, there is significant potential for public health and economic benefit from better understanding of – and better ability to treat – human brain diseases and disorders.

Humanness:
The brain confers on us certain abilities that make us human, while simultaneously making each of us unique. In light of the cutting-edge neurotechnologies presented at the workshop, and their capacity to begin unlocking the mysteries of human brain circuit function, there was extensive discussion of what, specifically, makes us human. For instance, is it sentience, i.e. the capacity to have subjective experiences, or something more, such as self-awareness? Relatedly, there was emphasis on the embodiment of human brains as critical to human identity. Fundamentally, the brain computes, as do computers, but it is the embodiment of a human brain within a human being, who, further, exists within

a social context, that is critical; this allows a human brain to be shaped by experience and to generate a human identity. The group discussed memory, and the importance of memory in generating personal identity in association with an individual narrative history. Brain organoids derived from human iPSCs have no apparent mechanism to retain memories from the fibroblast donor. With *ex vivo* human brain tissue, perhaps one day technology could become sophisticated enough that donor memories could be found in such tissue – but this capability does not exist at present nor appear likely in the near future. From a religious perspective, participants emphasized the importance of humility in the face of unknowns, the fact that we do not fully understand consciousness, and that questions remain about whether consciousness is purely reducible to physical processes happening within the brain. Finally, there was discussion of experimental approaches leveraging integration of human cells into brains of non-human mammals; for instance, studies in which a human brain organoid is implanted into the brain of a non-human mammal, to enable the growth of more sophisticated brain organoid models that can provide new insights into human brain disorders. The group discussed various reasons why such approaches are unlikely to make the animal more human-like and emphasized that such research should be informed by careful consideration of societal benefit and animal welfare. Institutional Animal Care and Use Committees (IACUCs), which would be required to approve any such experimental protocol, indeed exist to assess animal welfare in scientific research. The group agreed that such human/animal chimera experiments have important scientific potential, should be attended to regarding ethical implications as the science advances, and suggested a central question to consider is: What kind of functional modification of the animal brain would matter morally and why?

Moral considerability vs. moral status:
As discussed at the workshop, human brain organoids are already human in a biological sense. What functions or capacities would need to be present in a brain organoid to lend moral significance? Workshop participants discussed a framework that explores a distinction between moral status and moral considerability. An entity, for instance, a human being, has moral status if the entity has interests that matter morally to some degree for the entity's own sake, and this also suggests the entity can be wronged. A related but distinct concept is moral considerability, which prompts care and consideration for an entity, but not in the interests of the entity itself. For example, human tissue discarded as medical waste, such as a diseased gallbladder, has moral considerability and must be disposed of appropriately, but is not capable of being wronged and does not have moral status.

How then might models of the human brain fit in this framework? The group discussed whether moral considerability or moral status might be more appropriate for human brain models, and that even moral considerability may be sufficient to prompt consideration of boundaries for research (see *informed consent*, *stewardship*, and *oversight* below). There was also discussion of why a human brain organoid might have moral consideration, and whether other types of human organoids, such as kidney or liver organoids, do or do not. Participants noted that sentience, or perhaps even the capacity for sentience, is often considered a critical moral marker.

Metrics for assessing sentience or consciousness (or the capacity for either):
The workshop participants discussed different types of awareness, including sentience, or the capacity to have pleasant or unpleasant experiences, which is widely agreed to be characteristic of all vertebrate animals. This is reflected in U.S. Public Health Service policy regarding the welfare of vertebrate animals used in research, while regulations in the European Union include cephalopods as well. The group also discussed whether it might matter morally if an entity has the capacity for sentience, even if it lacks sentience. A key question discussed was: how would a researcher know if a human brain organoid has developed sentience, especially in an *in vitro* setup where the neural tissue is not connected to typical

sensory input and motor output, nor able to communicate? A suggestion was offered that this might be an area for potential research, to inform revisiting these questions in the future. The group indicated that given the current state of the art in human brain organoid research, the relative simplicity of the organoids – in terms of number of neurons, and connectivity – makes it extremely unlikely that any sort of higher order process, such as sentience, is present.

Informed consent:
The group discussed informed consent as it pertains to the models discussed at the workshop – for instance, donating skin cells that are used to generate iPSCs, which might in turn be developed into a brain organoid; or donating for research explants of brain tissue removed during surgery, for detailed study. One participant suggested an empirical study on whether donors have concerns about their iPSCs being used to grow brain organoids. If so, it may be worth considering incorporating additional information and options in the informed consent process for skin biopsy donation for iPSCs.

Stewardship:
Discussion revolved around several aspects of stewardship of human brain models (whether human brain organoids or *ex vivo* human brain tissue), such as unique considerations with respect to duration and conclusion of studies. Another issue discussed was disposal of tissue, and best practices may vary with the different models. There are existing U.S. federal and state laws to govern disposal of samples as medical waste, but there may someday in the distant future be unique considerations for these models since they involve human neural tissue.

Brain death:
Related to research with *ex vivo* human brain tissue, there was discussion of potential implications for our current understanding of brain death. Dr. Bernat explained that the clinical definition of brain death assumes irreversible cessation of the neuronal networks responsible for brain function. Importantly, this does not suggest the absence of all cellular activity of the brain, but rather, an absence of clinical functions of the brain. If, in the distant future, technology is developed that can preserve some degree of functionality in *ex vivo* human brain tissue, this may be viewed as reversing, to some limited extent, what was previously considered irreversible neuronal damage, and might inform our understanding of brain death.

Oversight:
The workshop participants discussed whether existing oversight infrastructure can address these various ethical considerations. For instance, per U.S. federal regulations, Institutional Review Boards (IRBs) review informed consent forms as part of their work of reviewing human subjects research, and IACUCs provide oversight regarding animal welfare in scientific research. Nevertheless, the group suggested the models discussed at the workshop may raise new, unique issues for which IRBs and IACUCs may need additional guidance. Research with human brain organoids or *ex vivo* human brain tissue currently falls outside the scope of human subjects research, and various participants suggested it may benefit from institutional oversight of donor informed consent, duration of studies, conclusion of studies, and disposal of tissue. Dr. Greely noted that California is unique among states in that its stem cell research oversight bodies review research involving all types of stem cells, not just embryonic stem cells, so this might be an approach to providing oversight for research with human brain organoids.

Avoiding hype:
Many people have unique concerns and sensitivities about their brains, as compared with other organs of the body. The models discussed at the workshop raise complex neuroethical questions and it is in the

best interests of all stakeholders to discuss both the science and the ethics as clearly and accurately as possible - and to avoid sensationalizing either. The group noted that concerns may stem from the science itself, or from the perception of the science, and both need consideration and discussion.

Collaborative ethics as a model:
Dr. Lunshof presented collaborative ethics as a model to consider, based on her experience as an ethicist working in a laboratory doing cutting edge research with iPSCs. Embedding an ethicist in a research lab enables ongoing dialogue between scientists and ethicists in research groups. Further, this promotes ethics that is responsive to the dynamics of research, and can include both philosophical and practical ethics, with the emphasis shifting dynamically in response to the science. The group discussed this as a possible model for continuing efforts to integrate neuroethics into neuroscience research.

CLOSING REMARKS
Dr. Gordon closed the meeting by re-emphasizing that the human brain models discussed at the workshop warrant continued forward progress, with thoughtful consideration of the various neuroethical implications discussed throughout the day. He summarized that the questions are not necessarily limited to the particular experimental methods used, but rather, lie in the potential for model systems to develop or exhibit higher cognitive functions such as sentience, which would raise the issue of moral considerability or moral status as discussed above. There is a clear consensus that the current model systems available for *ex vivo* brain research are quite rudimentary, and far from raising any of these concerns. He also noted the importance of continuing to consider metrics that might delineate the acceptable boundaries on these experimental approaches, to ensure that the models are complex enough to be scientifically useful, but not so complex that they raise major ethical questions.

Regarding oversight, he summarized that based on the current state of the science, it might be best to consider offering guidance to IACUCs and IRBs, for instance regarding informed consent for studies collecting skin biopsies for generation of iPSCs. Finally, Dr. Gordon thanked all presenters and attendees for the thoughtful discussion, noting that he and Dr. Koroshetz would leverage the workshop's discussion to inform responsibly supporting these areas of science.