ENDOCRINE SOCIETY
Hormone Science to Health

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National Institute on Aging
Building 31, Room 5C27
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August 7, 2019

Dear Dr. Sutterer,

The Endocrine Society appreciates the opportunity to comment on the development of the NIA Longitudinal Rat Resource to study neural and behavioral trajectories of successful cognitive aging. Founded in 1916, the Endocrine Society is the world's oldest, largest, and most active organization devoted to research on hormones and the clinical practice of endocrinology. Our membership of over 18,000 includes clinical, translational, and basic scientists making discoveries to enhance our understanding of the role of hormones in healthy aging, as well as how the aging process affects endocrine systems and influences endocrine related diseases. Our members welcome the development of new resources for researchers interested in cognitive aging. In our comments, we identify some considerations relevant to behavioral assessment; measurement parameters of interest to endocrine scientists; general data management recommendations; and overarching considerations for all components of the resource.

Considerations Specific to Behavioral Assessment

One potential challenge is habituation of the animals to repeated behavioral testing in the same arena, for example, the elevated plus maze. When the interpretation of a test relies on the animal's response to a novel context, repeated tests in the same apparatus over the life of the animal might not deliver appropriate results. To address this issue, different groups of naïve animals should be run at different time points, or variations of the test designed for use at different time points, or before and after an intervention.

The document did not address whether the animals will be singly or group housed. We would avoid the use of singly housed animals for most studies due to stress on the animal. However, comparisons involving a smaller cohort of singly housed animals might be useful to study aging-related behavioral, endocrine and metabolic changes in the context of social isolation. As many researchers isolate animals for experimental concerns, the comparison of longitudinal measures in social vs singly housed animals would be very valuable to the research community.

We were encouraged by the inclusion of high-density home cage activity as part of the proposed infrastructure for the resource. We also recommend that additional social interaction testing using novel conspecifics be included in regular assessments across the lifespan of the animals. Rats are a social species and observational tests e.g., involving a new animal are straightforward, noninvasive, repeatable, and highly informative.

The longitudinal study of cognitive aging in animals is an exciting opportunity to study how early-life behaviors and functions can predict or influence later-life assessments. Behavioral assessment of juvenile and

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adolescent rats will be important in this context. Repeatable tests could be conducted e.g. every 4 months, to track changes in performance.

Measurement Parameters of Interest to Endocrine Research

The implementation of this new resource is an opportunity for investigators to explore how nutrition, metabolism, and endocrine signaling influence cognitive aging over the course of the lifespan. In this context, we recommend that the consistency or variation of nutrition over the lifespan be explicitly addressed in the design of the resource as this will have an impact on other measurements. Metabolic parameters, such as animal body weight and composition, can be easily assessed noninvasively and should be measured repeatedly as animals age, taking care to consider the estrous cycle in females. Additionally, oral glucose tolerance tests given at regular intervals (e.g., every 3 to 6 months) would provide valuable data on aging and insulin resistance.

Noninvasive biological sampling should include hair, urine, and feces at least every 4-6 months and more often pre- and peri-pubertally. These samples can allow for assessment of relevant hormonal markers for e.g., stress and metabolic activity. For stress assessment, the time of day should be standardized for sample collection. Useful information about the relationship between the HPA-axis and healthy aging could be gathered periodically by measuring corticosterone and its metabolites from feces following a behavioral challenge expected to activate the stress response.

To prepare for postmortem sampling and tissue preparation, terminal tissue and blood measurements should be conducted at the same time of day. We recommend that consideration be given to removing food from the cages for a minimum of two hours pre-sacrifice, although optimally for six hours to avoid variability that could arise from meal-based effects that would influence many endocrine parameters. The estrous cycle stage for females should at a minimum be recorded, with the aim of sacrificing at a consistent stage when possible. Post-mortem brain collection and preparation should be approached thoughtfully and with consideration for new and emerging technologies that could enable advanced imaging studies. Traditional approaches such as flash-freezing and/or perfusion of whole brains should be considered, as well as dissection/preparation of specific regions of interest immediately after sacrifice for high-throughput sequencing, epigenetics, metabolomics and other studies. Of the newer histology and imaging techniques (CLARITY, iDISCO, MERFISH, Slide-Seq, etc.), some can be used on routinely collected fresh frozen specimens, while others require more specialized handling of the specimens following collection. Therefore, we recommend engaging with labs using these techniques ahead of time to ensure a diversity of specimen collection that will allow researchers to use the tissue for most modern research applications.

General Data Management Recommendations

As NIA develops the database that will house the information gathered over the course of the study, we encourage prioritizing searchability and development of built in data visualization tools to help researchers explore the available data. Data should be made available at the level of the individual animal whenever possible to enable the most detailed analysis. It will also be helpful to identify similarities between studies based on common data elements proposed by other working groups, such as the Traumatic Brain Injury Preclinical Working Group, supported by the NIH (NINDS) and DOD common data elements group.

As a final point, while we appreciate that the focus of the resource is on noninvasive approaches, there are valuable data that could be gathered using invasive approaches on a subset of animals. To that end, we encourage considering the use of implantable technologies (in vivo electrophysiology and EEG recordings, calcium imaging, microdialysis, venous catheters for repeated blood sampling, etc.), even on a small subset of animals at selected timepoints. We would also be encouraged by opportunities to examine various parameters following the loss of gonadal hormones +/- replacement.

Overarching Considerations for Studies

We strongly support the consideration of sex as a biological variable, the investigation of sex-specific effects, and careful consideration of the hormonal status of the animal at the time of assessment. All measurements need to be standardized to the time of day e.g., to account for diurnal adrenal and androgen cycles, in addition to circadian rhythms inherent in all cells and organs. For females, and for studies involving both sexes, timing of assessments with respect to the estrous cycle should be considered. Circadian exposures in general should be monitored, and the temperature and humidity of the facility be recorded continuously. We are particularly excited about the opportunity to assess the hypothalamus and pituitary in relation to the natural history of the animal, in addition to the hypothalamic-pituitary-adrenal-axis response to stress.

We note that Long-Evans rats will be exclusively used in this resource, and while there may be features of this strain that are well-suited to these studies, we note the potential loss of variability and generalizability that might arise due to strain-specific effects. The different available rat strains vary widely in their innate behavioral characteristics and also some endocrine parameters, such as the hypothalamic-pituitary-adrenal axis response to stress. We encourage NIA to explain their rationale for the use of this specific strain and to consider incorporating other rat strains into this initiative.

In conclusion, we hope that our comments are helpful in the design, development, and implementation of the NIA Longitudinal Rat Resource and we thank you for the opportunity to share our perspective. We look forward to additional details about the resource and opportunities for our member scientists and colleagues to partner with NIA on impactful new research emerging from this initiative. If we can be of any further assistance, please contact Joseph Laakso, PhD, Director of Science Policy at jlaakso@endocrine.org.

Sincerely,

E. Dale Abel MD PhD

President, Endocrine Society