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Perspectives on Cognitive Phenotypes and Models of Vascular Disease

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Arteriosclerosis, Thrombosis, and Vascular Biology

REVIEWS

Perspectives on Cognitive Phenotypes and Models of Vascular Disease

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ABSTRACT: Clinical investigations have established that vascular-associated medical conditions are significant risk factors for various kinds of dementia. And yet, we are unable to associate certain types of vascular deficiencies with specific cognitive impairments. The reasons for this are many, not the least of which are that most vascular disorders are multi-factorial and the development of vascular dementia in humans is often a multi-year or multi-decade progression. To better study vascular disease and its underlying causes, the National Heart, Lung, and Blood Institute of the National Institutes of Health has invested considerable resources in the development of animal models that recapitulate various aspects of human vascular disease. Many of these models, mainly in the mouse, are based on genetic mutations, frequently using single-gene mutations to examine the role of specific proteins in vascular function. These models could serve as useful tools for understanding the association of specific vascular signaling pathways with specific neurological and cognitive impairments related to dementia. To advance the state of the vascular dementia field and improve the information sharing between the vascular biology and neurobehavioral research communities, National Heart, Lung, and Blood Institute convened a workshop to bring in scientists from these knowledge domains to discuss the potential utility of establishing a comprehensive phenotypic cognitive assessment of a selected set of existing mouse models, representative of the spectrum of vascular disorders, with particular attention focused on age, sex, and rigor and reproducibility. The workshop highlighted the potential of associating well-characterized vascular disease models, with validated cognitive outcomes, that can be used to link specific vascular signaling pathways with specific cognitive and neurobehavioral deficits.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: atrophy ■ blood pressure ■ mutation ■ risk factors ■ vascular dementia

VASCULAR DEMENTIA IN HUMANS

Vascular dementia (VaD) in humans is a spectrum of cognitive disorders based on etiology, pathology, and tempo of cerebrovascular disease.[1] Challenges in defining VaD in human patients include the following: (1) VaD is not a single condition with a unifying pattern of cognitive deficits, (2) different pathologies (eg, gray matter atrophy, myelin loss) can manifest as VaD and different vascular and nonvascular etiologies can and often do co-exist, (3) cognitive deficiency syndromes can be related to a specific acute event (eg, stroke) or to repeated smaller insults (eg, small vessel disease), and (4) cognitive syndrome is, for the most part, a gradually progressive disorder that primarily affects older individuals with a spectrum of behavioral, physiological, and neurological changes that can contribute to cognitive impairment. To capture the entire spectrum of cognitive disorders and their heterogeneity, ranging from cognitive impairment to fully developed dementia, a new concept was proposed in 2011 defined as vascular cognitive impairment (VCI).[2,3] A consensus list was developed for neuropsychological evaluations of VCI which includes various cognitive assessments, imaging, and most recently other noncognitive assessments (such as alterations in gait) that have been reported to predict cognitive decline.

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Nonstandard Abbreviations and Acronyms

AD	Alzheimer disease
CADASIL	cerebral autosomal dominant arteriopathy with subcortical infarcts
cSVD	cerebral small vessel disease
NIA	National Institute of Ageing
NINDS	National Institute for Neurological Disorders and Stroke
VaD	vascular dementia
VCI	vascular cognitive impairment
VCID	vascular contributions to cognitive impairment and dementia

In a recent National Heart, Lung, and Blood Institute-funded clinical study (SPRINT [Systolic Blood Pressure Intervention Trial])[4] that assessed the potential benefits of reducing blood pressure, authors reported milder cognitive impairment in the group intensively treated to achieve a target of <130 mm Hg compared with those treated to reach <140 mm Hg systolic blood pressure. Mild cognitive impairment is indisputably a forerunner for VaD and the National Institute of Neurological Disorders and Stroke (NINDS)-National Institute of Ageing (NIA)-funded SPRINT MIND 2019 sub-study of SPRINT[5] therefore, raised the possibility that elevated blood pressure is principally responsible for many more cases of VaD than previously suspected. The American Heart Association subsequently revised the threshold for the diagnosis of hypertension from 140/90 mm Hg to 130/80 mm Hg on the basis that more effective control of blood pressure will have a significant impact in reducing the incidence of VaD. To date, however, the cellular and molecular mechanisms by which elevated blood pressure cause VaD remain poorly understood.

Cerebral small vessel diseases (cSVDs) are a major contributor to VCI and VaD. cSVD an umbrella term for pathologies that affect the structure or function of cerebral small vessels, are involved in one-third of ischemic strokes, the vast majority of intracerebral hemorrhages and account for about one-third of all dementia cases.[6–9] A large group of comorbidities, mainly age and hypertension but also genetic factors, are risk factors for cSVDs.[10] Among the important advances in this research field has been the identification of monogenic forms of cSVD that share many clinical, neuroimaging and pathological features with the more common multifactorial cSVDs.[11] By far, cerebral autosomal dominant arteriopathy with subcortical infarcts (CADASIL), caused by highly stereotyped dominant mutations in NOTCH3 (neurogenic locus notch homolog protein 3), a receptor predominantly expressed in mural cells of small blood vessels, is the most common monogenic cSVD and a paradigm of ischemic cSVD.[12,13]

Highlights

- Vascular cognitive impairment and dementia is a spectrum of complex set of cognitive and behavioral disorders. Etiological and pathological heterogeneity of this syndrome should be taken in consideration in animal and experimental models.
- Utilization of mice with genetically diverse backgrounds to stimulate human genetic diversity, in combination with mouse models that best mimic human disease and consideration of age-appropriate disease manifestations would be of benefit.
- Rigorous phenotypic assessments of a set of existing mouse models of vascular diseases across behavioral and cognitive assays could provide the vascular cognitive impairment and dementia field with a template or standards by which to associate certain aspects of vascular dysregulation with specific cognitive and behavioral phenotypes in mice.

There is currently no treatment available for this devastating disease. With the goal of investigating the CADASIL clinical spectrum, CADASIL-specific mouse models have been developed using transgene and knock-in technology based on specific individual human CADASIL variants.[14] However, most models only recapitulate a portion of the CADASIL-related pathology, such as altered vascular smooth muscle cell function, while strokes and white matter changes are not reproduced. There is also a lack of systematic analyses of neurobehavioral studies in mouse models to elucidate CADASIL-specific cognitive impairment. It is a major challenge to translate human cognitive impairment into a behavioral readouts that can be measured and interrogated in mouse studies. Additional approaches could potentially include more pragmatic utilization of mouse models recapitulating the subcortical VaD and frontotemporal dementia seen in CADASIL on a background of CADASIL-specific mouse models.

To best parallel the clinical syndrome of VaD in humans, animal and experimental models should be cognizant of the etiological and pathological heterogeneity of this syndrome. These models should include measures of executive function as a salient feature of the syndrome. Also, while impairments in executive function and gait anomalies may be early manifestations of VaD, deficits in attention, memory, and visuospatial domains are also core features[1,15–18] and should be measured in experimental models.

CURRENT NIA AND NINDS DEMENTIA RESEARCH STRATEGIES

To overcome historical issues of rigor and reproducibility in disease and behavioral characterization of mouse models for the study of Alzheimer disease (AD), NIA has

been supporting the Model Organism Development and Evaluation for Late Onset AD initiative, which aims to create 50 new mouse models focusing on brain pathophysiology, using CRISPR technology. Subsequently, an in-depth characterization is being conducted by dedicated centers with significant infrastructure and appropriate expertise to align mouse and human phenotypes with a focus on prioritizing the most translationally relevant phenotypes. Importantly, robust and reliable behavioral outcome measures will be evaluated in tandem with biomarkers, neuropathology, -omics, and neuroimaging.

The data from Model Organism Development and Evaluation for Late Onset AD, including mouse strains, data types, studies, and assays are available to the scientific community on the AD Knowledge Portal (https://adknowledgeportal.org).

The NINDS strategic approach is closely aligned with NIA vision and objectives. Understanding vascular contributions to cognitive impairment and dementia (VCID) is a priority identified in the National Plan to Address AD. NINDS supports mechanism-oriented VCID research such as better understanding of the neurovascular unit, the impact of cerebrovascular and cardiovascular disease insults, and the various effects of proteinopathies, metabolic disease, and immune response on cognitive function.

Together, NIA and NINDS have recognized the need for supporting multi-institutional, multidisciplinary center-based initiatives that emphasize best practices in animal model development and characterization, with milestone-driven objectives as resources for the greater research community with a focus on improving preclinical to clinical translation that emphasizes rigor, reproducibility, and Open Science principles.

EXAMPLES OF NATIONAL HEART, LUNG, AND BLOOD INSTITUTE -FUNDED VASCULAR DISEASE/DISORDER MOUSE MODELS

Although some mouse vascular disease models have been examined for cognitive behaviors, in general, it is not possible to compare results across models due to differences in methodology, genetic background, and, in some cases, poor experimental rigor and reproducibility. Measurement of cognitive phenotypes in animals involves a complex set of processes, variables, and significant resources and expertise that, while well characterized and well-understood within the neuro-cognitive scientific community, are generally not practiced effectively within the vascular biology research community.

During the workshop, various mouse models of vascular disease or disorders were presented (Table). The experimental models discussed included models of inherited small vessel disease in the brain, atherosclerosis, hypertension, cerebral cavernous malformations, and intracerebral hemorrhage. Models of inherited blood disorders, such as sickle cell anemia, and chronic lung vascular disease, such as chronic obstructive pulmonary disease were also discussed. Although one of the leading causes of VCI is stroke, discussing experimental models of hypoperfusion was not in the scope of this workshop. An increasing number of current research reports have shown that both sickle cell anemia and chronic pulmonary disease are associated with cognitive dysfunction. A recent report demonstrated cognitive deficit in a sickle cell disease mouse model.[19] Patients with chronic pulmonary disease are at higher risk of developing dementia including patients with chronic obstructive pulmonary disease.[20,21] Additionally, vascular abnormalities in this group of patients are not limited to the lung and include multi-system vascular deficiencies in other organs including brain, kidney, and heart which complicate the course and outcomes of the disease in these patients.[22] The various pros and cons with which these models accurately reflect vascular disease in humans were also discussed. In general, it was agreed that none of the models fully reflected the spectrum of vascular-induced cognitive impairment in humans, but that each of the models offered specific insights into the various basic biological processes that likely govern the development of VCID.

Some of these vascular disease models have been evaluated for cognitive phenotypes but most have not. None have been assessed with standard protocols that enable comparisons with other vascular or AD models. Furthermore, cross-laboratory reproducibility in the same mouse models remains a gap. It was suggested that a rigorous cognitive assessment of a set of existing mouse models of vascular diseases could provide the VCID field with a template or standard by which to associate certain aspects of vascular dysregulation with specific cognitive and behavioral phenotypes in mice.

MOVING THE FIELD FORWARD

Participants identified the need for multiple animal models to advance the field because VCID is a multi-faceted disease and more frequently than not, different models highlight only a few aspects of the human disease. There was also agreement that some prioritization of models might be useful, perhaps based on the burden of disease caused by a given pathology or on other criteria.

Age is a critically important factor in the biology of VCID and its assessment. The human relevance of specific mouse models can be improved by studying VCID in aging mice. At the same time, the use of aged animals greatly increases the cost of experimentation and when coupled with the need to use both males and females can create a cost-prohibitive situation for many individual

Table. Representative Mouse Models of Vascular Disease-Related Cognitive Impairment

Disease/presenter	Disease characteristics	Model(s)	Challenges and limitations
CADASIL Manfred Boehm, MD	Rare, inherited small vessel SVD, caused by mutation in the Notch3 gene and characterized by white matter lesions, that leads to vascular dementia. Studies of human subjects identified impaired memory, decreased psychomotor skills, and loss of executive function as symptoms, suggesting it may be a front subcortical-type syndrome.	Several CADASIL/Notch3 models exist; most are transgenic	A potential approach to study subcortical vascular dementia would be to use ApoE-deficient mice subjected to bilateral cortical artery stenosis and placed on a high-fat diet, to induce hyperlipidemia.
CADASIL Anne Joutel, MD, PhD	Monogenic SVD diseases CADASIL	TgNotch3^{R169C} on a C57BL/6 background	A translationally relevant model of the most common genetic form of ischemic SVD that recapitulates the early stage of the disease and has few limitations.
ICH Anne Joutel, MD, PhD	ICH	Col4a1 mutant mice (Col4a1$^{\Delta ex41/+}$, Col4a1$^{G498V/+}$, Col4a1$^{G1064D/+}$)	Translationally relevant models of the most common genetic form of hemorrhagic SVD that faithfully recapitulate the full spectrum of the human disease; however, these models have major limitations.
CCMs Mark Kahn, MD	CCMs are thin-walled, dilated vascular malformations, caused by loss-of-function mutations in CCM genes: CM1 (*KRIT1*), CCM2, or CCM3 (*PDCD10*). CCMs can lead to neurological deficits, seizures, and hemorrhagic stroke.	Krit$^{fl/fl}$, R26-LSL, Pik3ca^{H1047R} + AAV-Cre	The first neonatal model developed was thought to be a true animal model for CCM but did not confer disease in adult animals like in humans. Addressing the deficiency of the model revealed a mechanism underlying the human disease. A new adult mouse model was developed that is now being used for preclinical testing of drugs vs CCM disease.
SCD Hyacinth Hyacinth, MD, PhD	Poor neurocognitive performance such as loss of full-scale IQ is a significant complication of SCD. Neurostructural changes, in addition to cerebral vasculopathy, are potential mechanism of cognitive and behavioral complications of SCD.	Townes sickle cell with humanized control mice	Model has been in use for over 2 decades and is well-characterized; it has documented cerebral microvascular, neuronal, and cognitive changes; the model phenotype is similar to that observed in humans and is thus highly translational; behavioral characterization has included novel object recognition and fear conditioning. However, the mice have significant motor impairments which may confound the ability to perform these tests.
Chronic lung vascular disease Kurt Stenmark, MD	COPD affects >200 million people, with cognitive impairment in up to 60 percent of certain populations. Systemic vascular inflammation is seen in the vascular COPD phenotype with a high level of XOR expression in inflammatory macrophages in the adventitial/perivascular region of the hypertensive pulmonary artery; COPD patients with pulmonary hypertension have higher levels of serum uric acid, which is associated with dementia.	Transgenic uricase KO mice	The complex metabolic environment of the human body is often not recapitulated in rodent models due to presence of an active uricase gene. Backcrossing uricase KO mice in the Denver altitude with serial tapering of an XOR inhibitor, allopurinol, led to a KO phenotype that can stay alive and breed without the necessity of allopurinol. This provided a very useful model that can mimic the deleterious effects of chronic hyperuricemia on systemic vasculature similar to humans.
Hypertension Alan Daugherty, PhD, DSc	Hypertension is the most common chronic disease in the world. The precise cause of elevated blood pressure cannot be determined in most people.	Infusion of angiotensin II in C57BL/6J, ApoE $^{-/-}$, and smooth muscle cell-specific LRP1 deletion	Role of hypertension in mouse models of cognitive disorders needs awareness of the following: Distinction of pressure per se vs multiple stimuli that can promote hypertension; regional specific effects on different vascular beds; vascular phenotypes display sex-specific effects; and background strain differences. Accurate sequential blood pressure measurements without affecting physiological condition are challenging.
Atherosclerosis Alan Daugherty, PhD, DSc	Atherosclerosis is a progressive disease with evolving pathology. Lesion formation has regional specificity; lesion composition may have regional specificity. The effects of atherosclerosis per se on cognitive impairment need to be defined.	ApoE$^{-/-}$, human apoB transgenic, and low-density lipoprotein receptor (LDLR$^{-/-}$)	ApoE$^{-/-}$ mice have cognitive impairment,[23–25] but ApoE has many effects, so the link to atherosclerosis has not been established. LDLR$^{-/-}$ mice have cognitive impairment, but the effects of hypercholesterolemia vs atherosclerosis need to be defined.

CADASIL indicates cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CCM, cerebral cavernous malformations; COPD, chronic obstructive pulmonary disease; ICH, intracerebral hemorrhage; KO, knockout; LRP1, LDL receptor related protein 1; NOTCH3, neurogenic locus notch homolog protein 3; SCD, sickle cell disease; SVD, small vessel disease; and XOR, xanthine oxidoreductase.

laboratories. Thus, center-based funded initiatives such as those being conducted by Model Organism Development and Evaluation for Late Onset AD as described above, may provide an opportunity to address these issues. Additionally, it was recognized that utilization of mice with genetically diverse backgrounds to mimic human genetic diversity will be of importance. Furthermore, genetic diversity in combination with mouse models that best mimic human disease at the genetic level (point mutations, rather than transgenics for example) taking into consideration age-appropriate disease manifestations would be of benefit.

OTHER DISCUSSION POINTS INCLUDED

- More emphasis on histological assessments and increased use of advanced functional imaging technologies, such as magnetic resonance imaging.
- Enhanced characterization of human brain vasculature from patients to better characterize pathophysiological mechanisms of disease.
- The measurement of phenotypes with no known brain pathology, such as hyperactivity and gait to set the standard for healthy to be compared with the VCID pathology.
- Increased attention to environment and animal husbandry issues as they relate to animal behavior.
- Careful analysis of confounding behaviors in mice to avoid mis-interpretation of cognitive outcomes that may be a result of other physical impairments (eg, motor function, vision).
- Focus on mouse genetic models, but with awareness that point mutations would have more distinct effects than knockout models or transgenics.

MEASURING COGNITION/COGNITIVE-LIKE PHENOTYPES IN MICE

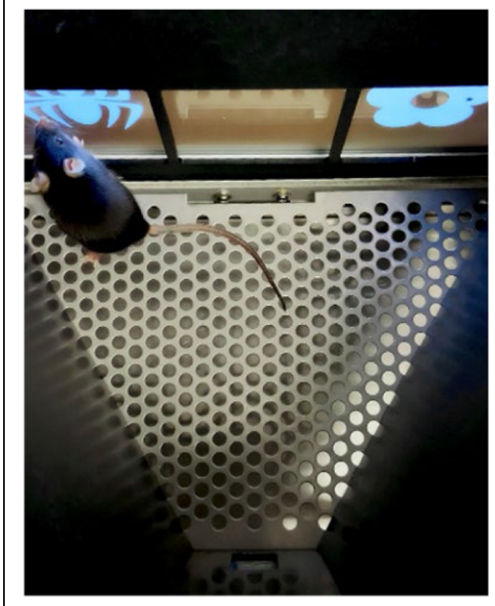
There are many challenges, limitations, and confounding factors when attempting to measure cognitive function and behavioral phenotypes in mouse models. Multiple variables can affect intra- and inter-lab reproducibility, including task type, task duration, environmental conditions, time of day, and pretest and posttest subject treatment. These many considerations need to be reported to ensure reproducibility. In addition, multiple factors can act as confounders in behavioral assays and result in misinterpretation of data, for example, aging-dependent visual impairments and hyperactivity. To ensure rigor and reproducibility, scientists should follow the Animals Research: Reporting of In Vivo Experiments guidelines[26] for experimental research, use appropriate control animals, establish a priori inclusion and exclusion criteria, predetermine sample sizes based on power analyses, randomize and counter-balance experimental conditions, and maintain data blinding until the completion of the analysis, and ensure that the personnel conducting these tests are properly trained.

Standardization with well-delineated standard operating procedures is critical for high-throughput cognitive assessments and comparison across multiple models. Validated training of experimental personnel is essential as is consultation with experts in the field to ensure the most appropriate experimental design and testing battery. Commonly used traditional methods to assess cognition in mice and rats can have serious shortcomings since these approaches do not represent cognition tests in human participants. Future innovations include more video/image data, the use of artificial intelligence and neural networks for analysis, longitudinal phenotyping, and deeper, richer, more translatable data sets. Newer methodologies, including virtual technology- and machine-learning based behavioral assessments may also provide more effective alternative to traditional behavior tests.[27] One such new methodology, touchscreen cognitive testing (Figure [A]), may provide improved translation over traditional behavioral assays that can capture a spectrum of phenotypes in a standardized testing battery. Touchscreen cognitive testing is an automated, high-throughput method that enables tests of disease-relevant, high-level cognition that are identical in all important respects to those used to assess humans, such as the Cambridge Brain Sciences (cambridgebrainsciences.com) and CANTAB (https://www.cambridgecognition.com/cantab/) batteries. This method allows for flexible presentation of comprehensive tests involving visual stimuli presented at any location on a computer screen. Over 30 validated touchscreen tests (Figure [B]) are available for mice and rats to tap into disease-relevant aspects of high-level cognition including attention, memory, executive function, and motivation. These protocols are analogous to the touchscreen based CANTAB assessments presented in the clinic including delayed match and delayed nonmatch to sample, paired associates learning, pairwise discrimination and reversal learning, and 5-choice continuous performance task, among others. Protocols for an extensive battery of touchscreen tests were published in 3 invited, back-to-back papers in Nature Protocols.[28–30] Touchscreen cognitive testing enables high-throughput testing with standardization and reproducibility through computer automation, reduced human error, reduced stress on animals, and tests similar to those used with humans to increase translatability. It is also amenable to open science and data sharing. Given that no single animal model can recreate all human VCID pathologies, it is increasingly important to evaluate multiple vascular disease mouse models using a common experimental framework and to facilitate comparison of results across distinct models.

OTHER FACTORS/MEASUREMENTS THAT SHOULD BE INCLUDED

Other possible factors to consider include genetics (background, strain, and substrain,[31] rare versus common disease, transgenic versus knock-out, etc), aging, sex as a biological variable, environmental factors (feed, microbiome, lighting, background noise, time of day, handling, etc), appropriate controls (wild-type littermates), and cohorting (batching).[32] Discussants indicated all these are important factors that need to be fully reported.

The workshop participants agreed that some type of brain imaging modality should also be incorporated into any set of measurements that seeks to have a comprehensive assessment of cognition or cognitive-like phenotypes associated with vascular disease mouse models. In vivo



Rodent Touchscreen Tests

Learning and Memory:
Pairwise Visual Discrimination Learning
Object-Location Paired Associates Learning
Spatial Working Memory (the Trial-Unique Nonmatching-to-Location (TUNL) and continuous TUNL (cTUNL) task)
Location Discrimination (spatial pattern separation)
Spontaneous (Novel) Object Recognition
Habit Learning (visuomotor conditional learning)
Virtual Water Maze ('ASAT' task)
Categorization
Transitive Inference

Attention:
5-choice serial reaction time task
Continuous Performance Test (including Flanker task)
5-choice CPT
Sustained Attention Test (SAT)

Decision making:
Rodent Gambling Task
Delay Discounting
Effort-related discounting (three versions including novel Rearing-Effort Discounting task)
Meta-cognition

Cognitive Flexibility and Response Inhibition:
Reversal Learning
Set-Shifting
Extinction Learning

Emotional cognition and responding to reinforcement:
Progressive Ratio Test of motivation/apathy
Response To Negative (and Positive) Feedback (spatial and nonspatial; probabilistic and deterministic including reversal learning)
Cognitive Bias Test (optimism/pessimism)
Autoshaping (Pavlovian approach to reward; sign- and goal-tracking)

Figure. Touchscreen cognitive testing.
A, The rodent touchscreen operant chamber in use. Experimental mice respond directly to the stimuli. **B**, Rodent touch screen tests.

imaging provides significant opportunities to measure cerebrovascular functioning that can be accomplished uniquely in the mouse. Magnetic resonance imaging was the most commonly mentioned and preferred imaging modality, but again, affordable access to all laboratories can be problematic. Importantly, correlating behavioral outcomes in rodents with pathological and biochemical changes will be key for translation. It is also worth noting that not all behavioral endophenotypes in human patients have an analogous mouse behavior or reciprocally, that some mouse behaviors are not analogous to humans, for example, human bipedal gait versus mouse quadrupedal gait.[33]

HOW TO ENSURE RIGOR AND REPRODUCIBILITY IN THE OUTCOMES

Rigor and reproducibility in biomedical research are essential for discoveries to be translated into improved human health. Several recent reports, including the National Institutes of Health Advisory Committee to the Director Report on Enhancing Rigor, Transparency, and Translatability in Animal Research,[34] as well as the findings in a Nature[35] report, list factors that can improve or reduce reproducibility.

Biased reporting and time pressure to publish have been cited as 2 significant causes of irreproducibility. Other factors include failure to conduct adequate statistical sample size calculations and subsequent statistical analysis, and accounting for experimental animals of both sexes. Increasingly, for experiments that seek to measure complex phenotypes and behaviors, such as cognition, it is essential to have a robust statistically justified sample size, well-defined experimental end points, appropriately chosen experimental controls instead of historical data, methods for randomization and counterbalancing, and blinded assessments.

Assay proficiency metrics and cross-lab training are important elements of rigor. There is also a need to establish reproducibly measured positive controls under the experimental conditions being used and appropriate assays for what is being measured. The use of dedicated centers to ensure standardized evaluations of complex phenotypes by fully trained personnel with well-calibrated equipment should also be considered.

FINAL THOUGHTS ON THE COMPREHENSIVE ASSESSMENT RECOMMENDATIONS

National Heart, Lung, and Blood Institute has been actively participating in and convening workshops to determine the state of science in VCID to facilitate research on key gaps and the need for new model systems to truly represent the human disease.[36,37] This workshop identified

a need for multiple vascular disease models to dissect the multifaceted aspects of VCI and dementia. In addition, the measurement of complex phenotypes, such as cognition and cognitive-like behaviors, requires careful considerations and execution by trained personnel and in consultation with experts. To achieve reliable comparisons between models, the assessments are probably most effectively done at dedicated centers that can provide the standardization and rigor needed to produce reproducible outcomes. Lessons learned from large consortiums such as the NIA-funded Model Organism Development and Evaluation for Late Onset AD centers provide an example framework for rigorous comprehensive phenotyping which can be adopted and tailored for assessment of new animal models, including vascular disease models. The phenotyping battery includes longitudinal and cross-sectional aging cohorts, up to 24 months of age, with cross-laboratory characterization including pathology, neuroimaging, biomarkers, multi-omics analyses, and behavior in well-powered cohorts of male and female genetic mouse models engineered with AD risk variants compared with their age- and sex-matched littermate controls.[38–40] Importantly, these studies are conducted un-biased with all data being reported including where no phenotype is observed.

Experimental animal models most closely aligned with known VCID risk factors should be considered high priority. Likewise, measures of VCID and executive function in humans, including processing speed, attention, and working memory, for which there are analogous mouse behavioral assays, should be prioritized. The use of newer experimental cognitive measurement technologies, such as touchscreen cognitive testing, may offer significant advantages over historical cognitive measures that have limited translational value from mouse to human.[33,41] The inclusion of imaging and histological analysis also was strongly encouraged.

The establishment of a rigorous and reproducible assessment of cognitive impairments associated with existing vascular disease models offers the prospect of being able to link specific vascular signaling pathways with specific cognitive deficits. Such a linkage may create a template or standard for a comprehensive assessment of VCID outcomes in vascular disease models, as well as an expedited conduit to the development of new therapeutic modalities.

ARTICLE INFORMATION

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REFERENCES

1. Vinciguerra L, Lanza G, Puglisi V, Fisicaro F, Pennisi M, Bella R, Cantone M. Update on the neurobiology of vascular cognitive impairment: fFrom lab to clinic. *Int J Mol Sci.* 2020;21:E2977. doi: 10.3390/ijms21082977
2. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, et al; American Heart Association Stroke Council, Council on Epidemiology and Prevention, Council on Cardiovascular Nursing, Council on Cardiovascular Radiology and Intervention, and Council on Cardiovascular Surgery and Anesthesia. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke.* 2011;42:2672–2713. doi: 10.1161/STR.0b013e3182299496
3. van der Flier WM, Skoog I, Schneider JA, Pantoni L, Mok V, Chen CLH, Scheltens P. Vascular cognitive impairment. *Nat Rev Dis Primers.* 2018;4:18003. doi: 10.1038/nrdp.2018.3
4. Wright JT Jr, Williamson JD, Whelton PK, Snyder JK, Sink KM, Rocco MV, Reboussin DM, Rahman M, Oparil S, Lewis CE, et al; SPRINT Research Group. A randomized trial of intensive versus standard blood-pressure control. *N Engl J Med.* 2015;373:2103–2116. doi: 10.1056/NEJMoa1511939
5. The SPRINT MIND Investigators for the SPRINT Research Group. Effect of intensive vs standard blood pressure control on probable dementia: a randomized clinical trial. *JAMA* 2019; 12:553–561. doi: 10.1001/jama.2018.21442
6. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol.* 2010;9:689–701. doi: 10.1016/S1474-4422(10)70104-6
7. Bosetti F, Galis ZS, Bynoe MS, Charette M, Cipolla MJ, Del Zoppo GJ, Gould D, Hatsukami TS, Jones TL, Koenig JI, et al; "Small Blood Vessels: Big Health Problems" Workshop Participants. "Small blood vessels: big health problems?": scientific recommendations of the National

Institutes of Health Workshop. *J Am Heart Assoc*. 2016;5:e004389. doi: 10.1161/JAHA.116.004389
8. Iadecola C, Duering M, Hachinski V, Joutel A, Pendlebury ST, Schneider JA, Dichgans M. Vascular cognitive impairment and dementia: JACC Scientific Expert Panel. *J Am Coll Cardiol*. 2019;73:3326–3344. doi: 10.1016/j.jacc.2019.04.034
9. Ferrante EA, Cudrici CD, Boehm M. CADASIL: new advances in basic science and clinical perspectives. *Curr Opin Hematol*. 2019;26:193–198. doi: 10.1097/MOH.0000000000000497
10. Joutel A. Prospects for diminishing the impact of nonamyloid small-vessel diseases of the brain. *Annu Rev Pharmacol Toxicol*. 2020;60:437–456. doi: 10.1146/annurev-pharmtox-010818-021712
11. Haffner C, Malik R, Dichgans M. Genetic factors in cerebral small vessel disease and their impact on stroke and dementia. *J Cereb Blood Flow Metab*. 2016;36:158–171. doi: 10.1038/jcbfm.2015.71
12. Chabriat H, Joutel A, Dichgans M, Tournier-Lasserve E, Bousser MG. Cadasil. *Lancet Neurol*. 2016;7:643–653. doi: 10.1016/S1474-4422(09)70127-9
13. Chabriat H, Joutel A, Tournier-Lasserve E, Bousser MG. CADASIL: yesterday, today, tomorrow. *Eur J Neurol*. 2020;27:1588–1595. doi: 10.1111/ene.14293
14. Manini A, Pantoni L. CADASIL from bench to bedside: disease models and novel therapeutic approaches. *Mol Neurobiol*. 2021;58:2558–2573. doi: 10.1007/s12035-021-02282-4
15. Bir SC, Khan MW, Javalkar V, Toledo EG, Kelley RE. Emerging concepts in vascular dementia: a review. *J Stroke Cerebrovasc Dis*. 2021;30:105864. doi: 10.1016/j.jstrokecerebrovasdis.2021.105864
16. Gorelick PB, Furie KL, Iadecola C, Smith EE, Waddy SP, Lloyd-Jones DM, Bae HJ, Bauman MA, Dichgans M, Duncan PW, et al; American Heart Association/American Stroke Association. Defining optimal brain health in adults: a presidential advisory from the American Heart Association/American Stroke Association. *Stroke*. 2017;48:e284–e303. doi: 10.1161/STR.0000000000000148
17. Verghese J, Lipton RB, Hall CB, Kuslansky G, Katz MJ, Buschke H. Abnormality of gait as a predictor of non-Alzheimer's dementia. *N Engl J Med*. 2002;347:1761–1768. doi: 10.1056/NEJMoa020441
18. Mielke MM, Roberts RO, Savica R, Cha R, Drubach DI, Christianson T, Pankratz VS, Geda YE, Machulda MM, Ivnik RJ, et al. Assessing the temporal relationship between cognition and gait: slow gait predicts cognitive decline in the Mayo Clinic Study of Aging. *J Gerontol A Biol Sci Med Sci*. 2013;68:929–937. doi: 10.1093/gerona/gls256
19. Hardy RA, Rached NA, Jones JA, Archer DR, Hyacinth HI. Role of age and neuroinflammation in the mechanism of cognitive deficits in sickle cell disease. *Exp Biol Med (Maywood)*. 2021;246:106–120. doi: 10.1177/1535370220958011
20. Dodd JW. Lung disease as a determinant of cognitive decline and dementia. *Alzheimers Res Ther*. 2015;7:32. doi: 10.1186/s13195-015-0116-3
21. Liao KM, Ho CH, Ko SC, Li CY. Increased risk of dementia in patients with chronic obstructive pulmonary disease. *Medicine (Baltimore)*. 2015;94:e930. doi: 10.1097/MD.0000000000000930
22. Polverino F, Celli BR, Owen CA. COPD as an endothelial disorder: endothelial injury linking lesions in the lungs and other organs? (2017 Grover Conference Series). *Pulm Circ*. 2018;8:2045894018758528. doi: 10.1177/2045894018758528
23. Masliah E, Mallory M, Ge N, Alford M, Veinbergs I, Roses AD. Neurodegeneration in the central nervous system of apoE-deficient mice. *Exp Neurol*. 1995;136:107–122. doi: 10.1006/exnr.1995.1088
24. Gordon I, Grauer E, Genis I, Sehayek E, Michaelson DM. Memory deficits and cholinergic impairments in apolipoprotein E.-deficient mice. *Neurosci Lett*. 1995;199:1–4. doi: 10.1016/0304-3940(95)12006-p
25. Oitzl MS, Mulder M, Lucassen PJ, Havekes LM, Grootendorst J, de Kloet ER. Severe learning deficits in apolipoprotein E-knockout mice in a water maze task. *Brain Res*. 1997;752:189–196. doi: 10.1016/s0006-8993(96)01448-5
26. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *Exp Physiol*. 2020;105:1459–1466. doi: 10.1113/EP088870
27. Sato M, Kawano M, Mizuta K, Islam T, Lee MG, Hayashi Y. Hippocampus-dependent goal localization by head-fixed mice in virtual reality. *eNeuro*. 2017;4:ENEURO.0369–ENEU16.2017. doi: 10.1523/ENEURO.0369-16.2017
28. Oomen CA, Hvoslef-Eide M, Heath CJ, Mar AC, Horner AE, Bussey TJ, Saksida LM. The touchscreen operant platform for testing working memory and pattern separation in rats and mice. *Nat Protoc*. 2013;8:2006–2021. doi: 10.1038/nprot.2013.124
29. Horner AE, Heath CJ, Hvoslef-Eide M, Kent BA, Kim CH, Nilsson SR, Alsiö J, Oomen CA, Holmes A, Saksida LM, et al. The touchscreen operant platform for testing learning and memory in rats and mice. *Nat Protoc*. 2013;8:1961–1984. doi: 10.1038/nprot.2013.122
30. Mar AC, Horner AE, Nilsson SR, Alsiö J, Kent BA, Kim CH, Holmes A, Saksida LM, Bussey TJ. The touchscreen operant platform for assessing executive function in rats and mice. *Nat Protoc*. 2013;8:1985–2005. doi: 10.1038/nprot.2013.123
31. Sukoff Rizzo SJ, McTighe S, McKinzie DL. Genetic background and sex: impact on generalizability of research findings in pharmacology studies. *Handb Exp Pharmacol*. 2020;257:147–162. doi: 10.1007/164_2019_282
32. Sukoff Rizzo SJ, Silverman JL. Methodological considerations for optimizing and validating behavioral assays. *Curr Protoc Mouse Biol*. 2016;6:364–379. doi: 10.1002/cpmo.17
33. Silverman JL, Nithianantharajah J, Der-Avakian A, Young JW, Sukoff Rizzo SJ. Lost in translation: at the crossroads of face validity and translational utility of behavioral assays in animal models for the development of therapeutics. *Neurosci Biobehav Rev*. 2020;116:452–453. doi: 10.1016/j.neubiorev.2020.07.008
34. ACD Working Group on Enhancing Rigor, Transparency, and Translatability in Animal Research. June 11, 2021. https://acd.od.nih.gov/documents/presentations/06112021_RR-AR%20Report.pdf. [Online]
35. Six factors affecting reproducibility in life science research and how to handle them. 2017. https://www.nature.com/articles/d42473-019-00004-y. [Online]
36. Snyder HM, Corriveau RA, Craft S, Faber JE, Greenberg SM, Knopman D, Lamb BT, Montine TJ, Nedergaard M, Schaffer CB, et al. Vascular contributions to cognitive impairment and dementia including Alzheimer's disease. *Alzheimers Dement*. 2015;11:710–717. doi: 10.1016/j.jalz.2014.10.008
37. Zlokovic BV, Gottesman RF, Bernstein KE, Seshadri S, McKee A, Snyder H, Greenberg SM, Yaffe K, Schaffer CB, Yuan C, et al. Vascular contributions to cognitive impairment and dementia (VCID): A report from the 2018 National Heart, Lung, and Blood Institute and National Institute of Neurological Disorders and Stroke Workshop. *Alzheimers Dement*. 2020;16:1714–1733. doi: 10.1002/alz.12157
38. Kotredes KP, Oblak A, Pandey RS, Lin PB, Garceau D, Williams H, Uyar A, O'Rourke R, O'Rourke S, Ingraham C, et al. Uncovering disease mechanisms in a novel mouse model expressing humanized APOEε4 and Trem2*R47H. *Front Aging Neurosci*. 2021;13:735524. doi: 10.3389/fnagi.2021.735524
39. Oblak AL, Lin PB, Kotredes KP, Pandey RS, Garceau D, Williams HM, Uyar A, O'Rourke R, O'Rourke S, Ingraham C, et al. Comprehensive evaluation of the 5XFAD mouse model for preclinical testing applications: a MODEL-AD study. *Front Aging Neurosci*. 2021;13:713726. doi: 10.3389/fnagi.2021.713726
40. Oblak AL, Forner S, Territo PR, Sasner M, Carter GW, Howell GR, Sukoff-Rizzo SJ, Logsdon BA, Mangravite LM, Mortazavi A, et al; The MODEL-AD; Consortium. Model organism development and evaluation for late-onset Alzheimer's disease: MODEL-AD. *Alzheimers Dement (N Y)*. 2020;6:e12110. doi: 10.1002/trc2.12110
41. Nithianantharajah J, McKechanie AG, Stewart TJ, Johnstone M, Blackwood DH, St Clair D, Grant SG, Bussey TJ, Saksida LM. Bridging the translational divide: identical cognitive touchscreen testing in mice and humans carrying mutations in a disease-relevant homologous gene. *Sci Rep*. 2015;5:14613. doi: 10.1038/srep14613