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Key areas where the BRAIN Initiative should begin to phase out the use of animal-based research and redirect funds towards non-animal methods.

Nerve Regeneration Research

Many neuroprotective agents have been developed that are successful in treating spinal cord injury (SCI) in animal models, but clinical trials have been disappointing. Neurologist Aysha Akhtar has described three major reasons for this failure: "differences in injury type between laboratory-induced SCI and clinical SCI, difficulties in interpreting functional outcome in animals, and inter-species and interstrain differences in pathophysiology of SCI."[1]

In their systematic review of the use of animal models to study nerve regeneration in tissue-engineered scaffolds, Angius and colleagues noted, "The large majority of biomaterials used in animal models have not progressed for approval to be tested in clinical trials in spite of the almost uniform benefit described in the experimental papers."[2] The authors lamented the low quality of described animal experiments, in that necessary detail and rationale had been omitted, making it difficult to compare data.

For example, methylprednisolone, a routinely used treatment for acute SCI, has generated inconsistent results in animal models. A systematic review examining 62 studies of the drug on a wide variety of species, from rodents to monkeys, found that 34% of the studies reported beneficial results, 58% no effect, and 8% mixed findings.[3] The results were inconsistent both among and within species, even within strains. Furthermore, the variability in results remained even when many of the study design and procedure variables were controlled. The authors pointed out numerous intrinsic differences between, and limitations of, each species/model and suggested that as a result of these immutable inter- and intra-species differences, no human-relevant animal model can be developed. They concluded that the "research emphasis should be on the development and use of validated human-based methods."

Among species, rats are particularly unsuitable for nerve repair or regeneration research. Experts have pointed out three major problems with rat models in this field:

(1) The majority of nerve regeneration data is now being generated in the rat, which is likely to skew treatment outcomes and lead to inappropriate evaluation of risks and benefits. (2) The rat is

[1] Akhtar AZ, Pippin JJ, Sandusky CB. Animal models in spinal cord injury: A review. Rev Neurosci. 2008;19(1):47-60
[2] Angius D, Wang H, Spinner RJ, Gutierrez-Cotto Y, Yaszemski MJ, Windebank AJ. A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds. *Biomaterials.* 2012;33(32):8034-8039
[3] Akhtar AZ, Pippin JJ, Sandusky CB. Animal studies in spinal cord injury: A systematic review of methylprednisolone. Altern *Lab Anim*. 2009;37(1):43-62.

a particularly poor model for the repair of human critical gap defects due to both its small size and its species-specific neurobiological regenerative profile. (3) Translation from rat to human has proven unreliable for nerve regeneration, as for many other applications.[4]

More specifically, the inconsistencies between animal models and the clinical situation include the following:

(1) healthy animals versus sick patients; (2) short versus long gap lengths (the clinical need for *large* gap repairs, while 90% of *in vivo* studies are in rats and rabbits where gap lengths are usually ≤3 cm); (3) animal models that almost always employ *mixed sensory-motor* autografts for repairing mixed defects, versus clinical repairs that almost always involve *sensory* autografts (usually sural nerve) for repairing mixed defects; (4) protected anatomical sites in animal models, versus repairs that must often cross articulating joints in humans; and (5) inbred, highly homogeneous animal strains and ages, versus diverse patient populations and ages: It is well recognized that animal models fail to mimic the human condition in terms of the *uniformity* of animal subjects used.

University of Florida biomedical engineers Mobini and colleagues add, "We are incapable of truly mimicking human neural injures in animal models because of the extensive anatomical, functional, molecular, immunological, and pathological differences between humans and frequently studied animals."[5]

Human-relevant methods such as human stem cells and clinical research can bypass these limitations and should be the focus of BRAIN Initiative funding.

Shrirao and colleagues at Rutgers University recommend microfluidic devices, which are "adaptable for modeling a wide range of injuries" and provide advantages over traditional *in vivo* and *in vitro* experiments by "allowing researchers to (1) examine the effect of injury on specific neural components, (2) fluidically isolate neuronal regions to examine specific effects on subcellular components, and (3) reproducibly create a variety of injuries to model TBI and SCI."[6] For example, MIMETAS scientists collaborating with scientists from Leiden University and Utrecht University developed a three-dimensional motor neuron model using iPSC-derived motor neurons that allows for directed neurite growth and separation of axons from soma and dendrites to advance the study of motor neuron disease and nerve regeneration mechanisms.[7] Researchers at the University of Texas Health Science have developed cerebral organoids that can be used to study human-specific pathological changes induced by TBI. Their model is being used to simulate the controlled cortical impact procedures commonly used to create traumatic brain injuries in rodents and other animals.[8]

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[4] Kaplan HM, Mishra P, Kohn J. The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. *J Mater Sci Mater Med*. 2015;26(8):226.
[5] Mobini S, Song YH, McCrary MW, Schmidt CE. Advances in ex vivo models and lab-on-a-chip devices for neural tissue engineering. *Biomaterials.* 2019;198:146-166.
[6] Shrirao AB, Kung FH, Omelchenko A, et al. Microfluidic platforms for the study of neuronal injury in vitro. *Biotechnol Bioeng.* 2018;115(4):815-830.
[7] Spijkers XM, Pasteuning-Vuhman S, Dorleijn JC, Vulto P, Wevers NR, Pasterkamp RJ. A directional 3D neurite outgrowth model for studying motor axon biology and disease. *Sci Rep*. 2021;11(1):2080.
[8] Ramirez S, Mukherjee A, Sepulveda S, et al. Modeling Traumatic Brain Injury in Human Cerebral Organoids. *Cells*. 2021;10(10):2683.

Neurodegenerative Disease Research

There is ample literature documenting the limitations of animal models of neurodegenerative diseases, including Alzheimer's (AD), Parkinson's (PD), Huntington's (HD), and amyotrophic lateral sclerosis (ALS). To date, no animal model has been able to recapitulate all aspects of these complex, human-specific diseases. In a bioinformatic analysis comparing transcriptional signatures of human AD, PD, HD, and ALS with mouse models of these diseases, Stanford scientists made the following findings:

> [M]ost available mouse models of neurodegenerative disease fail to recapitulate the salient transcriptional alterations of human neurodegeneration and … even the best available models show significant and reproducible differences compared to human neurodegeneration. Although the reasons for the poor transcriptional performance of mouse models varied, the unifying theme was the failure of mouse models to exhibit the variety and severity of diverse defects observed in human neurodegeneration.[9]

Scientist and policymakers are realizing that neurodegenerative research strategies should be more human-relevant, and that funding should be allocated away from animal studies and toward more promising techniques involving patient-derived induced pluripotent stem cell models, "omic" technology (genomics, proteomics, etc.), *in silico* models, neuroimaging, and epidemiological studies.[10]

These tools are already being used to advance our understanding of neurodegenerative disease. For example, proteomic analysis of post-mortem brain tissue from patients with AD, PD, and varying forms of dementia have allowed researchers to identify a molecular fingerprint for dementia[11] as well as to study the role of myelin- and oligodendrocyte-related protein expression changes in different hippocampal subfields in myelin loss and subsequent cognitive decline in AD.[12] Researchers at USC, UCLA, and UCI recently used 2-[18F]fluoro-3(2(S) azetidinylmethoxy) pyridine (2FA) PET imaging to compare nicotinic cholinergic receptor binding in brains regions of patients with AD, individuals with mild cognitive impairment, and healthy age-matched controls and how binding differences related to cognitive abilities in these groups.[13]

Human-based, *in vitro* tools are also significantly advancing understanding of neurodegenerative diseases. For example, researchers at Dongguk University and the University of Pennsylvania have created 3-dimensional midbrain organoids of LRRK2-associated PD that exhibit increased

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[9] Burns TC, Li MD Mehta S, Awad AJ, Morgan AA. Mouse models rarely mimic the transcriptome of human neurodegenerative diseases: A systematic bioinformatics-based critique of preclinical models. *Eur J Pharmacol*. 2015;759:101-117

[10] Pistollato F, Ohayon EL, Lam A, et al. Alzheimer disease research in the 21st century: Past and current failures, new perspectives and funding opportunities. *Oncotarget.* 2016;7(26):38999-39016.

[11] Bereczki E, Branca RM, Francis PT, et al. Synaptic markers of cognitive decline in neurodegenerative diseases: a proteomic approach [published correction appears in Brain. 2019 Jun 1;142(6):e24]. *Brain*. 2018;141(2):582-595.

[1212] Gao, Y., Liu, J., Wang, J., Liu, Y., Zeng, L. H., Ge, W., & Ma, C. (2022). Proteomic analysis of human hippocampal subfields provides new insights into the pathogenesis of Alzheimer's disease and the role of glial cells. *Brain pathology (Zurich, Switzerland)*, e13047. Advance online publication.

[13] Sultzer DL, Lim AC, Gordon HL, Yarns BC, Melrose RJ. Cholinergic receptor binding in unimpaired older adults, mild cognitive impairment, and Alzheimer's disease dementia. *Alzheimers Res Ther*. 2022;14(1):25.

α-synuclein, a pathological signature of LRRK2 patients absent in animal models.[14] Researchers at the University of Sheffield and the University of Luxembourg are using a humanoid organoid model of PD to study the effect of PINK1 deficiency, a genetic condition associated with early onset PD, on dopaminergic differentiation in the midbrain.[15] A team of researchers at the University of Central Florida have developed a human neuromuscular junction-on-a-chip, the first of its kind, which can be used for toxicity testing of drugs designed to treat neuromuscular diseases, such as ALS and spinal muscular atrophy.[16]

Modern, human-relevant research tools are also being used conjunctively to study the interacting roles of RNA-binding protein TDP-43 and single nucleotide polymorphisms in the *UNC13A* gene in neurodegenerative disease. Recently, researchers at the National Institute of Neurological Disease and Stroke (NINDS) and the University College London Queen Square Motor Neuron Disease Centre studied cells derived from patients with ALS and frontotemporal dementia (FTD) and identified a cryptic exon in the risk gene *UNC13A* following TDP-43 binding protein depletion.[17] Similar findings were reported by researchers at Stanford University, who found that TDP-43 represses cryptic exon splicing in *UNC13A* but TDP-43 reduction led to the inclusion of a cryptic exon in *UNC13A.*[18] As these authors point out, "This cryptic exon inclusion event—similar to that of STMN2—is not conserved in mouse, so will require studies in human neuron models to test whether blocking *UNC13A* cryptic splicing is sufficient to rescue phenotypes associated with loss of TDP-43 function." These critical findings combined the use of genome wide association studies (GWAS), RNA sequencing, CRISPR inhibition, post-mortem tissue analysis, and iPSC technology to reveal a crucial molecular pathway and potential treatment target associated with both ALS and FTD.

Neuropsychiatric and Neurodivergent Research

Animal models of neuropsychiatric disorders and neurodivergence lack critical aspects of model validity, including the following: (1) construct validity, meaning that the mechanistic underpinnings creating the observed symptoms in animals are different from those that lead to the disorder in humans; (2) face validity, meaning that animals lack the ability to "recapitulate important anatomical, biochemical, neuropathological, or behavioral features of a human disease";[19] and (3) predictive validity, meaning that results from experiments on animals don't reliably translate into similar results in humans. No single animal model is able to replicate all aspects of a particular condition, and features of human behavior representing hallmarks of these disorders cannot be produced or properly assessed in animals.

[14] Kim H, Park HJ, Choi H, et al. Modeling G2019S-LRRK2 Sporadic Parkinson's Disease in 3D Midbrain Organoids. *Stem Cell Reports*. 2019;12(3):518-531.
[15] Brown SJ, Boussaad I, Jarazo J, et al. PINK1 deficiency impairs adult neurogenesis of dopaminergic neurons. *Sci Rep*. 2021;11(1):6617.
[16] Santhanam N, Kumanchik L, Guo X, et al. Stem cell derived phenotypic human neuromuscular junction model for dose response evaluation of therapeutics. *Biomaterials.* 2018;166:64-78
[17] Brown AL, Wilkins OG, Keuss MJ, et al. TDP-43 loss and ALS-risk SNPs drive mis-splicing and depletion of UNC13A [published online ahead of print, 2022 Feb 23]. *Nature*. 2022;10.1038.
[18] Ma XR, Prudencio M, Koike Y, et al. TDP-43 represses cryptic exon inclusion in the FTD-ALS gene UNC13A [published online ahead of print, 2022 Feb 23]. *Nature*. 2022;10.1038
[19] Nestler EJ, Hyman SE. Animal models of neuropsychiatric disease. *Nat Neurosci*. 2010;13(10):1161-1169.

Human depressive disorders, for example, are characterized, in part, by a generalized feeling of sadness, hopelessness, and despair. In an effort to measure "despair" in rodents, the most commonly used behavioral test is the forced swim test, in which a rat or mouse is placed in a container of water with no way to escape and no place to rest out of the water. Naturally, the animal will spend some time swimming and trying to find a way out of the stressful situation but will eventually become immobile and float. The time spent swimming may be extended by giving the animal some forms of human antidepressant drugs, a finding that led some scientists to assert that less time spent immobile was a sign that animals were less "depressed" and that more time spent immobile meant they were more "depressed," as if they had "given up" and were in despair.

However, as has now been widely discussed in the scientific literature, immobility in the forced swim test may simply be an animal's adaptation to their situation and should not be used to determine their mood.[20] Individual animals who are quicker to float save their energy and are less likely to sink, meaning that those who pick up on this sooner and spend less time struggling may simply be learning this adaptive behavior more readily. Time spent swimming versus floating is also influenced by an animal's strain as well as experimental variances, such as water depth and temperature.[21,22,23]

In August 2021, a PETA neuroscientist and her psychologist collaborator published a paper that discredited the use of the forced swim test as a screen for antidepressant drugs. In the study, they examined the use of this test by the world's top 15 pharmaceutical companies and found that for 109 compounds used in forced swim test experiments, most of which purportedly showed "antidepressant-like effects" in the test, none are currently approved for market.[24]

In a series of citation analyses, researchers have demonstrated that human medical papers in the field of major depressive disorder rarely cite results from experiments on rats or monkeys, two of the most common species used in this field, and more frequently relied on the results of research using human cells and human biological data.[25,26,27] A similar failure of animal studies to contribute to clinical knowledge has been noted with bipolar depression research,[28] and animal studies have been cited as the primary source of attrition (failure of drugs) in neurobehavioral

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[20] Molendijk ML, de Kloet ER. Immobility in the forced swim test is adaptive and does not reflect depression. *Psychoneuroendocrinology*. 2015;62:389-391.
[21] De Pablo JM, Parra A, Segovia S, Guillamón A. Learned immobility explains the behavior of rats in the forced swimming test. *Physiol Behav*. 1989;46(2):229-237.
[22] Jefferys D, Funder J. The effect of water temperature on immobility in the forced swimming test in rats. *Eur J Pharmacol*. 1994;253(1-2):91-94.
[23] Lucki I, Dalvi A, Mayorga AJ. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology (Berl)*. 2001;155(3):315-322.
[2424] Trunnell ER, Carvalho C. The forced swim test has poor accuracy for identifying novel antidepressants. *Drug Discov Today*. 2021;26(12):2898-2904.
[25] Carvalho C, Varela SAM, Marques TA, Knight A, Vicente L. Are *in vitro* and *in silico* approaches used appropriately for animal-based major depressive disorder research? *PLoS One*. 2020;15(6):e0233954.
[26] Carvalho C, Peste F, Marques TA, Knight A, Vicente LM. The contribution of rat studies to current knowledge of major depressive disorder: Results from citation analysis. *Front Psychol*. 2020;11:1486.
[27] Carvalho C, Herrmann K, Marques TA, Knight A. Time to abolish the forced swim test in rats for depression research? *J Appl Anim Ethics Res*. 2021;1-9.
[28] Kato T, Kasahara T, Kubota-Sakashita M, Kato TM, Nakajima K. Animal models of recurrent or bipolar depression. *Neuroscience*. 2016;321:189-196.

clinical trials.[29] Nevertheless, thousands of published papers ignore these warnings and use the forced swim test to draw erroneous conclusions about an animal's mood[30] or the potential effects of compounds on human depressive disorders.

In addition to having poor validity, experiments on animals for neuropsychiatric conditions are of poor quality. In a survey of 121 animal studies claiming to investigate attention deficit hyperactivity disorder (ADHD), only five were found to be in any way relevant to the hypotheses of the human medical papers in which they were cited. The authors of the survey concluded that "animal research has contributed very little to contemporary understanding of ADHD."[31]

Significant differences in physiology between humans and other animals likely account for a large percentage of failed translation. For example, the gene encoding tyrosine hydroxylase, the enzyme involved in the formation of dopamine, was found to be regulated in an entirely different manner in humans than it is in mice.[32] Misregulation of tyrosine hydroxylase has been implicated in several psychiatric illnesses, such as bipolar disorder and schizophrenia. In a 2019 study published in *Nature*, 64 researchers analyzed the brains of mice and humans and found substantial species differences in types of brain cells and the ways they produce proteins critical to neuropsychiatric function. The authors noted numerous "failures in the use of [the] mouse for preclinical studies" because of "so many [species] differences in the cellular patterning of genes."[33]

In addition to the lack of applicability of animal neuropsychiatric models to the human condition, animals used in these experiments suffer immensely. To induce "depression," experimenters subject them to uncontrollable pain through electric shocks or chronic stressors such as restraining them for extended periods of time, starving them or denying them water, tilting their cages, forcing them to live in wet bedding, shaking them, or disrupting their circadian rhythms. Animals are often made to live in complete isolation from other members of their species, bullied and physically assaulted by other animals, deprived of parental care, and subjected to genetic or surgical manipulations in an effort to induce a depressed or altered mental state. To quote Dutch animal behaviorists van der Staay, Arndt, and Nordquist, "If evidence accumulates that the intended goal/purpose cannot be reached, then one should consider abandoning further development of the model."[34] This group also points out that in all cases, "benefits must outweigh the ethical costs of the animals. These costs include pain and suffering, distress and death."[35]

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[29] Garner JP. The significance of meaning: Why do over 90% of behavioral neuroscience results fail to translate to humans, and what can we do to fix it? *ILAR J*. 2014;55(3):438-456.
[30] Molendijk, de Kloet
[31] Carvalho C, Vieira Crespo M, Ferreira Bastos L, Knight A, Vicente L. Contribution of animal models to contemporary understanding of attention deficit hyperactivity disorder. *ALTEX*. 2016;33(3):243-249.
[32] Jin H, Romano G, Marshall C, Donaldson AE, Suon S, Iacovitti L. Tyrosine hydroxylase gene regulation in human neuronal progenitor cells does not depend on Nurr1 as in the murine and rat systems. *J Cell Physiol*. 2006;207(1):49- 57.
[33] Hodge RD, Bakken TE, Miller JA, *et al*. Conserved cell types with divergent features in human versus mouse cortex. *Nature*. 2019;573(7772):61-68.

[34] van der Staay FJ, Arndt SS, Nordquist RE. Evaluation of animal models of neurobehavioral disorders. *Behav Brain Funct*. 2009;5:11.
[35] *Ibid*.

Funds should be allocated to more relevant, human-based experimental models, such as computational modeling using already well-defined biomarkers[36] and the use of patient-specific stem cells for personalized medicine, which "affords the ability to generate neuronal cell-based models that recapitulate key aspects of human disease"[37] and can be used in drug discovery. Complex diseases like schizophrenia are ideal disorders "to model through stem cell approaches due to … heterogeneous, complex genetics that are hard to recapitulate in animal models."[38]

Recent developments in the field of human neuropsychiatric research include the following:

- A research group at Johns Hopkins Bloomberg School of Medicine used stem cell–derived "mini-brains" to study the effects of an antidepressant drug on neurons in the developing human brain.[39]
- University of California–San Diego scientists created organoids using reprogrammed cells from patients with a specific genetic mutation strongly linked to autism to study early brain development.[40] The authors noted that mouse models of this genetic mutation have phenotypes that are the opposite of what is observed in humans[41] and that a "patient-derived model will be more ideal and more beneficial than looking at the mouse."[42]
- At Brown University, neuroscientists and engineers conducted the first-ever study of electrical activity in the brains of people with obsessive-compulsive disorder over an extended period of time while the participants were in their homes, going about daily living.[43] Along with behavioral biomarkers, the team used machine learning to examine correlations between real-life behavioral measures and brain signals. This research can be used to help guide adaptive deep brain stimulation treatments for this population.
- Scientists in Tokyo used a combination of brain imaging and machine learning to create a diagnostic algorithm for autism, schizophrenia, and psychosis based on brain scans.[44]
- A team of Indian and Canadian researchers used artificial intelligence and functional magnetic resonance imaging data to develop a diagnostic tool that can predict schizotypy in first-degree relatives of patients with schizophrenia with 87% accuracy.[45]

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[36] Siekmeier PJ. Computational modeling of psychiatric illnesses via well-defined neurophysiological and neurocognitive biomarkers. *Neurosci Biobehav Rev*. 2015;57:365-380.
[37] Haggarty SJ, Silva MC, Cross A, Brandon NJ, Perlis RH. Advancing drug discovery for neuropsychiatric disorders using patient-specific stem cell models. *Mol Cell Neurosci*. 2016;73:104-115.
[38] Adegbola A, Bury LA, Fu C, Zhang M, Wynshaw-Boris A. Concise review: Induced pluripotent stem cell models for neuropsychiatric diseases. *Stem Cells Transl Med*. 2017;6(12):2062-2070.
[39] Zhong X, Harris G, Smirnova L, *et al*. Antidepressant paroxetine exerts developmental neurotoxicity in an iPSC-derived 3D human brain model. *Front Cell Neurosci*. 2020;14:25.
[40] Urresti J, Zhang P, Moran-Losada P, *et al*. Correction: Cortical organoids model early brain development disrupted by 16p11.2 copy number variants in autism. *Mol Psychiatry*. 2021;26(12)7581.
[41] *Ibid*.
[42] Dattaro L. Protein inhibitor normalizes neuronal migration in organoid model of autism. SpectrumNews.org. Published September 1, 2021. Accessed February 8, 2022. https://www.spectrumnews.org/news/protein-inhibitor-normalizes-neuronal-migration-in-organoid-model-of-autism.
[43] Provenza NR, Sheth SA, Dastin-van Rijn EM, *et al*. Long-term ecological assessment of intracranial electrophysiology synchronized to behavioral markers in obsessive-compulsive disorder. *Nat Med*. 2021;27(12):2154-2164.
[44] Yassin W, Nakatani H, Zhu Y, *et al*. Machine-learning classification using neuroimaging data in schizophrenia, autism, ultra-high risk and first-episode psychosis. *Transl Psychiatry*. 2020;10(1):278.
[45] Kalmady SV, Paul AK, Greiner R, *et al*. Extending schizophrenia diagnostic model to predict schizotypy in first-degree relatives. *NPJ Schizophr*. 2020;6(1):30.

Owing to the psychological distress inherent in animals provoked to display neuropsychiatric disease tendencies and the inapplicability of the results to humans, we recommend that the use of animals in such studies be ended.

Stroke Research

According to researchers at the Institute for Stroke and Dementia Research in Munich, "More than 1000 neuroprotective compounds have been tested in rodent models with the aim to improve stroke outcome. … Indeed, many agents reduced brain damage (in most cases measured as decreased infarct volume) in rodent models of experimental stroke. Out of these candidates approximately 50 neuroprotective agents were tested in more than 100 clinical stroke trials, but none has improved outcome in clinical stroke patients."[46] Experts in the field admit, "animal models of stroke mimic at best less than 25 percent of all strokes."[47]

In a 2017 review,[48] Clemens Sommer, M.D., of the University Medical Center at Johannes Gutenberg University Mainz, details the following aspects of animal experimentation that limit the translatability of animal-based stroke research to the clinical setting:

- Most animals studied in stroke research have lissencephalic, or smooth, brains, unlike the gyrencephalic brains of humans.
- The expression of certain signaling molecules differs between rodents and humans in three types of brain cells—neurons, astrocytes, and microglia—both at baseline and in response to oxygen deprivation.
- In humans, ischemic damage to the white matter of the brain is important in the prognosis of stroke, but white matter content in humans is much higher than in other animals, meaning that a major factor in stroke outcomes for humans cannot be accurately compared in animal models.
- Blood vessels in the brain have a different anatomy in humans compared to other animals; even strains of rodents differ in their vascular framework potentially impacting the pathophysiology of the ischemic cascade.
- Ischemic stroke typically occurs in heterogeneous elderly patients with comorbid conditions, whereas animal stroke experiments are predominantly carried out in young, healthy, male, inbred animals.
- Immune system differences between humans and other species are drastic. Sommer describes this as follows:

> [T]he percentage of neutrophils in mice and rats is about 10–20% compared to 50–70% in humans, while the opposite situation is seen for lymphocytes, which comprise about 50–100% in rodents compared to 20–40% in humans, respectively. Moreover, there is only a minimal intersection of whole-genome mRNA and microRNA expression in leukocytes from rodents versus humans at both baseline and after stroke, raising the question whether rodents are acceptable models at all for the human immune system after stroke.

[46] Roth S, Liesz A. Stroke research at the crossroads—where are we heading? *Swiss Med Wkly.* 2016;146:w14329
[47] Sutherland BA, Minnerup J, Balami JS, Arba F, Buchan AM, Kleinschnitz C. Neuroprotection for ischemic stroke: Translation from the bench to the bedside. *Int J Stroke.* 2012;7(5):407-418.
[48] Sommer CJ. Ischemic stroke: Experimental models and reality. *Acta Neuropathol*. 2017;133(2):245-261.

Human-based models of stroke do not suffer from these species-inherent deficiencies. Scientists at Louisiana State University have written that a "key benefit of *in vitro* systems is the opportunity to work with human cells, as such Werth *et al*., utilized the brain slice method in human cortical slices to provide the first direct evidence of glutamate receptor involvement in ischemic injury in the human brain."[49] Physicians and chemists at the University of Duisburg–Essen, in Germany, are cultivating six different human cell types to create mini-brains for use in stroke research and drug discovery.[50] At the Wake Forest Institute for Regenerative Medicine, a brain organoid of this type has already been created and was validated in stroke experiments after the model showed clinically accurate responses to known drugs.[51]

Neurosurgeons and biomedical engineers at Stanford University and Johns Hopkins University teamed up to create a neurovascular unit on a microfluidic chip that they are using to assess the restorative potential of stem cell therapies for use in ischemic stroke recovery.[52] In the Netherlands, the company MIMETAS has also created a neurovascular unit–on-a-chip that can be used for basic stroke research and drug discovery[53] and computational scientists at the University of Amsterdam have developed an *in silico* trial platform that can be used to assess treatment of acute ischemic stroke using clinical parameters of virtual patients.[54] Clinical researchers are now utilizing artificial intelligence to improve stroke prevention, detection, and care.[55,56]

Substance Abuse Research

Fundamental aspects of nonhuman animals make them inappropriate for the study of human addiction. First, the use of and addiction to drugs of abuse in humans is a vastly complex experience, one that has been impossible to mimic using animals in a laboratory setting.[57] It has been argued that attempts to model human disorders such as addiction in nonhuman animals, especially rodents, are "overambitious" and that the "'validity' of such models is often limited to superficial similarities, referred to as 'face validity' that reflect quite different underlying phenomena and biological processes from the clinical situation."[58]

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[49] Holloway PM, Gavins FN. Modeling ischemic stroke in vitro: The status quo and future perspectives. *Stroke*. 2016;47(2):561-569.

[50] Wiesmayer P. "Mini-brains" to replace mouse model in stroke research. InnovationOrigins.com. https://innovationorigins.com/en/mini-brains-to-replace-mouse-model-in-stroke-research. Published July 21, 2021. Accessed February 9, 2022.

[51] Nzou G, Wicks RT, VanOstrand NR, *et al*. Author Correction: Multicellular 3D neurovascular unit model for assessing hypoxia and neuroinflammation induced blood-brain barrier dysfunction. *Sci Rep*. 2020;10(1):20384

[52] Wevers NR, Nair AL, Fowke TM, *et al*. Modeling ischemic stroke in a triculture neurovascular unit on-a-chip. *Fluids Barriers CNS*. 2021;18(1):59.

[53] Miller C, Padmos RM, van der Kolk M, *et al*. In silico trials for treatment of acute ischemic stroke: Design and implementation. *Comput Biol Med*. 2021;137:104802.

[54] Miller C, Padmos RM, van der Kolk M, *et al*. In silico trials for treatment of acute ischemic stroke: Design and implementation. *Comput Biol Med*. 2021;137:104802.

[55] Gunda B, Neuhaus A, Sipos I, *et al*. Improved stroke care in a primary stroke centre using AI-decision support [published online ahead of print, February 8, 2022]. *Cerebrovasc Dis Extra*. 2022;10.1159/000522423.

[56] Guo Y. A New Paradigm of "Real-Time" Stroke Risk Prediction and Integrated Care Management in the Digital Health Era: Innovations Using Machine Learning and Artificial Intelligence Approaches. *Thromb Haemost*. 2022;122(1):5-7.

[57] Tzschentke TM. Where do we stand in the field of anti-abuse drug discovery? *Expert Opin Drug Dis*. 2014;9(11):1255-1258.

[58] Stephens DN, Crombag HS, Duka T. The challenge of studying parallel behaviors in humans and animal models. *Curr Top Behav Neurosci*. 2013;13:611-45.

Second, the pharmacokinetic actions of drugs are different among species. For example, "the rate of metabolism of MDMA [street name: Ecstasy, E, or Molly] and its major metabolites is slower in humans than rats or monkeys, potentially allowing endogenous neuroprotective mechanisms to function in a species specific manner."[59] Pharmacokinetic differences between humans and "model" animals likely explain why the neurotoxicity seen in rodents after MDMA administration has not been observed in the clinical setting.[60] Since MDMA is being explored not only because of its illegal use as a recreational drug but also for its potential use as a therapeutic, accurate knowledge regarding its safety in humans is paramount.

Third, serious flaws in experimental design of addiction experiments greatly skew interpretation of their results. In the human experience with drugs, the user chooses to consume the addictive substance. They choose it over other substances or activities that they may find rewarding. Animals in laboratories are typically not given this option. When they are, the vast majority of them will choose an alternative reward, such as sugar, over the drug of abuse.[61] This holds true for primates as well as mice and rats.[62] Even in animals with very heavy previous drug use, only about 10% would continue to give themselves a drug when they had the option to make another rewarding choice.[63] In a review on the "validation crisis" in animal models of drug addiction, French neuroscientist and addiction researcher Serge Ahmed asserts that the lack of choice offered to animals in these experiments elicits "serious doubt" about "the interpretation of drug use in experimental animals."[64]

The nonhuman animal has been called a "most reluctant collaborator" in studying alcohol addiction and has been noted to have a "determined sobriety" that the experimenter must fight against in order to overcome "their consistent failure to replicate the volitional consumption of ethanol to the point of physical dependency."[65] National Institute of Mental Health researchers reason that "it is difficult to argue that [drug self-administration by rodents] truly models compulsion, when the alternative to self-administration is solitude in a shoebox cage."[66]

Despite the prevalence of addiction research conducted on animals, "drugs that effectively curb opioid or psychostimulant addiction by promoting abstinence and preventing relapse have yet to be developed" and "very little clinical development is currently ongoing."[67] The data from animal studies were promising in certain drug classes, but these have either failed to be effective in human trials or not been tolerated well by humans, a negative outcome that was not predicted by animal trials.[68]

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[61] Ahmed SH. Validation crisis in animal models of drug addiction: Beyond non-disordered drug use toward drug addiction. *Neurosci Biobehav Rev*. 2010;35(2):172-184.
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[63] *Ibid*.
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[65] Ramsden E. Making animals alcoholic: Shifting laboratory models of addiction. *J Hist Behav Sci*. 2015;51(2):164- 194.
[66] Hyman SE, Malenka RC. Addiction and the brain: The neurobiology of compulsion and its persistence. *Nat Rev Neurosci*. 2001;2(10):695-703.
[67] Tzschentke.
[68] *Ibid*.

Non-invasive human research methods can provide us with answers to the questions that nonhuman animals, in their distaste for drugs of abuse, are fundamentally unable to answer. Rutgers University Robert Wood Johnson Medical School researchers recently authored a review article describing how the use of human induced pluripotent stem cells (iPSC) can provide a "unique opportunity to model neuropsychiatric disorders like [alcohol use disorders] in a manner that … maintains fidelity with complex human genetic contexts. Patient-specific neuronal cells derived from [induced pluripotent stem] cells can then be used for drug discovery and precision medicine."[69]

Human-relevant, non-animal research on alcohol use disorder is being carried out by scientists at the University of Connecticut, who recently used stem cells donated by alcoholic and non-alcoholic subjects to study the effects of alcohol on a specific receptor in the brain that is targeted by alcohol. Their results were at odds with some of the findings from animal experiments.[70] At Rutgers, scientists used patient-derived cells to generate neural cell types specific to individuals in which they could study alcohol's effects on various aspects of cell physiology. Their results demonstrated a role for neuronal inflammation in the pathophysiology of alcohol use disorder.[71] Researchers at the National Institute on Drug Abuse are using three-dimensional neocortical organoids to study the effects of prenatal cocaine exposure on the developing human brain.[72] Scientists at the Medical College of Wisconsin are using human iPSC-derived organoids to study the mechanisms of ethanol-induced gene dysregulation on the development of fetal alcohol spectrum disorders.[73] Other investigators are using human iPSCs to study the effects of alcohol on the human liver.[74]

In addition, the funds used to support ineffective and wasteful substance abuse studies in animals could instead be used to aid effective and directly human-relevant drug prevention, rehabilitation, and mental health programs.

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