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How does a researcher choose the best rodent model for their Alzheimer's disease drug discovery study?

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Animal models aiming at studying human diseases, emerged in the 1800s and experienced a major boost during the last decades. Animal models are essential tools in biomedical research, including in dementia-related research. They aid in the development and evaluation of mechanistic hypotheses about neurological and psychiatric disorders in general and their neural substrates in particular, \textit{i.e.} the brain-behaviour relation, as well for the identification and screening of novel therapeutic approaches, most frequently drugs.

The value and applicability of any animal model is determined by different levels of validity; Aetiological validity refers to equivalent aetiologies of phenomena in the model and the human disorder, whereas face validity refers to the resemblance between the model and the situation or process being modelled. Similarity of symptoms, like age-dependent decline in various cognitive domains or the development of behavioural and psychological symptoms, is often considered in this context. Construct validity may refer to similar cellular and molecular processes in the animal model and the human patients, thereby allowing the study underlying pathophysiological mechanisms in the model. Related to preclinical research, predictive validity can be used to indicate pharmacological isomorphism — that is, can the model identify compounds with potential therapeutic effects in the
human condition [1][2]. The more levels of validity a model satisfies, the greater its value, utility and relevance to the human condition. A "perfect" model would account for aetiology, symptomatology, treatment responsiveness, and pathophysiological basis. Animal models in general do not meet all of these criteria.

Although various species across the phylogenetic tree, ranging from invertebrates like Drosophila melanogaster and Caenorhabditis elegans [3], up to non-human primates have been applied in Alzheimer’s disease (AD)-related research [4]. Invertebrate models clearly offer important experimental advantages. C. elegans nematodes are transparent, which allows the study of embryonic development and gene expression in living animals under the microscope. They also have a very short life cycle and lifespan, which allow genetic dissection of the mechanisms that affect ageing and lifespan. Similar advantages can be exploited in D. melanogaster, and moreover, fruitflies have found major application in the analysis of genetic interaction in neurological disorders, including AD, based on both classical phenotype-based genetic screens and techniques for genetic manipulation, including gene knockdown, deletion and transgenic insertions [2]. Nevertheless, rodents are unequivocally the front runners as model organisms in this field [1][2][5][6]. Rodents are easier and cheaper to house and maintain than larger mammals, and they display a fast generation time with large numbers of offspring. Moreover a highly conserved genetic homology exists between humans and rodents [7], and standardized breeding gives researchers access to various inbred strains contributing to a less variable phenotype [8]. But the most dramatic boost in rodent models of AD came with the development of embryonic stem cell technology and gene editing techniques in the mouse in the early 1980s. Gene targeting has universally revolutionized biomedical research allowing the analysis of diverse aspects of gene function in vivo and the identification of disease-causative mutations and variations. Genetically engineered mouse models have been an essential resource for modelling human disease and studying gene function since the development and further improvement of the required biological toolkit [7][9]. The development of genetically altered mice
requires a considerable amount of time and expenses, which is even further augmented by the long-term follow up of the expected progressive AD-related phenotype.

Some non-genetically altered rodent models of AD can be considered. Unlike several other mammalian species, ageing rodents do not spontaneously develop AD-like pathology, and are therefore of no use to the development of drugs targeting these neuropathological hallmarks. They do aid in uncovering the boundaries between normal and pathological ageing, and allow in-depth investigation of basic neural and neurochemical mechanisms underlying brain ageing [1][2].

Noteworthy, are the selectively bred senescence-accelerated mouse (SAM) strains, in particular the SAMP8 substrain. Latter model shows accelerated ageing, age-associated learning and memory deficits in association with amyloid-β (Aβ) deposition, as well as other pathological brain processes relevant to AD [1][2]. When a progressive phenotype is not imperative to support your research question, pharmacological, chemical and lesion-induced rodent models of AD may provide valuable options [1][2]. When focusing on the amyloid cascade theory, stating that both in familial and sporadic AD cerebral aggregation and accumulation of Aβ peptides into amyloid plaques is the main primary culprit driving AD pathogenesis, rodent models based on a single or chronic Aβ infusion are of interest. As all injection models, they largely bypass the ageing aspect of AD, but moreover, hyper-physiological Aβ concentrations are required to achieve AD-like brain changes, and thorough internal control of process-related brain changes arising from the invasive nature of the technique are pivotal [1][2].

Gene-targeting techniques have given rise to an elaborate armamentarium of AD-relevant rodent models; A regularly updated and extensive overview of genetically modified models can be consulted at the Alzforum website [10]. These models can be largely divided into three clusters: a) Models with altered amyloid-β (Aβ) production based on amyloid precursor protein (APP) mutations, like e.g. the PDAPP, Tg2576 and APP23 model; b) models based on the modulation of secretases, i.e. presenilin (PSEN1 and PSEN2) mutation models, like e.g. PS1(M146L) and PS1(M146V) mice; and c) tau or microtubule-associated protein tau (MAPT)-based models, like e.g. JNPL3(P301L) and hTau.P301S.
Also models combining several of these genetic modifications are of interest, like e.g. the APPPS1, 3xTg, and 5xFAD model [10]. Also models based on late-onset AD genetic risk factors, such as apolipoprotein E or triggering receptor expressed on myeloid cells 2 (TREM2), as well as transgenic lines based on other aetiological hypotheses, e.g. mutated human a-synuclein models, human cyclooxygenase-2 overexpression models, anti-nerve growth factor mice can be considered [10][11]. Few genetically altered rat models of AD have been developed as well [10]. Although novel molecular tools, like the bacterial CRISPS/Cas9 system (referring to clustered regularly interspaced short palindromic repeats and CRISPR-associated genes), promote in vitro genome editing, the ability of CRISPR/Cas9 to directly target any gene of interest in the embryonic genome holds great promise to faster, less expensively and more reliably generate in vivo models of neurodegenerative diseases like AD [10][12].

Importantly, in preclinical drug screening one should always consider interspecies differences in neurochemistry that may hinder the success rate of the CNS drug discovery pipeline, including rodent versus human brain differences (i) in specific neurotransmitter circuit wiring; (ii) in the pharmacology of a particular compound for target subtypes; and (iii) in drug metabolism [13].

**Expert opinion**

It is obvious that a researcher has a myriad of AD-relevant rodent models at his or her fingertips. One of the most important, and at the same time, unquestionably the most challenging task, is choosing the best AD model, or more correctly, the most appropriate model for a particular research question. As we all realize, AD models are partial representations of a complex human brain disease, and most probably will never fully recapitulate the entire human clinical and pathological picture. Quite often researchers are confronted with the choice between models that reproduce cardinal pathological features of the disorders caused by mechanisms that may not necessarily occur in the patients versus models that are based on known aetiological mechanisms that may not reproduce all clinical features. In case of preclinical drug screening, we are convinced that the primary model of choice should depend on the mode of action and the molecular target of the compound under investigation;
for example, not every model will display neuronal loss and the same degree of neurodegeneration as in human AD. Also the required treatment window will determine whether one opts for a model with fast or more slowly developing brain pathology and/or symptomatology. Practical considerations should also take into account the read-out methodology and outcome parameters. The larger rat brain may for example be the better choice for in vivo small animal imaging. Concerning genetically modified models, a researcher should make an informed choice about the pros and cons of various strategies for the development of transgenic animals and other gene-targeting techniques in order to minimize unwanted variation, and to maximize fidelity to the target pathology and disease. Knock-in models have fully preserved native expression patterns and splicing at physiological levels, whereas the spatial and temporal expression of a particular splice variant in a transgenic model may deliver the desired robust pathology level. The reliability of preclinical drug trials does not merely depend on the quality of the model itself or on the pharmacokinetics and pharmacodynamics of the administered compound. A significant part depends on methodological elements of the study design, including animal-related aspects, such as background strain, age and gender, housing and husbandry conditions. But also experimental aspects, such as the inclusion of proper control groups, the choice of appropriate behavioural paradigms and the testing environment, as well as the experience and skills of animal experimenters, should be considered. An interesting case study to define best practices for the selection and validation of cognitive and functional endpoints to improve study design in preclinical AD drug discovery has been presented in an AD-relevant mouse model [14].

Even after taking all these aspects into consideration, uncritical and premature extrapolation of animal model findings to the human condition may remain unreliable and dangerous. Only a multi-tier approach, from in silico over in vitro to in vivo, as well as patient- or human material-based research, delivers complementary insights that, when leveraged, reliably expand our knowledge and understanding of AD pathophysiology and treatment options. All animal models may be valid when used appropriately and in a complementary manner. As such, reliable drug discovery for AD, and by
extension for any disease of interest, should leverage the knowledge gained via multi-tiered research and apply a multi-species and multi-model approach in preclinical drug screening.

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References


   This review paper summarizes and critically evaluates currently available animal models, and discusses their value to the Alzheimer drug discovery pipeline. Models dealt with include spontaneous models in various species, including senescence-accelerated mice, chemical and lesion-induced rodent models, and genetically modified models developed in Drosophila melanogaster, Caenorhabditis elegans, Danio rerio and rodents.


   This review paper critically evaluates the genetic and non-genetic strategies used in Alzheimer's disease modelling, discussing their strengths and limitations, as well as new opportunities for the development of better models for the disease.


**Database providing information about selected rodent models of neurodegenerative disease, including Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis.**

Available at: [https://www.alzforum.org/research-models/alzheimers-disease](https://www.alzforum.org/research-models/alzheimers-disease) [Last accessed 12 July 2019].

*The Alzforum website hosts a regularly updated and extensive database of genetically modified models of Alzheimer’s disease and related disorders with an excellent search function, description of the models and related references, as well as information on where to obtain a particular model.*


