Mouse models of neurodegeneration in the age of personalised medicine: know your question, know your mouse

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Neurodegenerative diseases are common, and emotional, social and financial burdens worldwide. We have unprecedented ability to create mutant mice to investigate neurodegeneration, but variability in mouse studies leads to questioning their utility. Here, we appraise mouse models for dissecting neurodegenerative mechanisms and emphasise the importance of (i) asking appropriate questions, (ii) understanding variability between and within studies. In therapeutics research, we suggest that not embracing this variability in mouse contributes to translational failure to humans. However, by addressing specific questions in bespoke mouse models and by analysing variation, we can investigate individual neurodegenerative processes, ultimately leading to precision medicine.

INTRODUCTION

Neurodegenerative diseases are common, largely untreatable and certainly incurable, and create a huge health and social load in developing and developed countries. For example, in 2015 over 46 million people had dementia (of which ~70-80% was caused by Alzheimer's disease, AD) and the overall cost came to $818 billion -- currently ~58% of those with dementia live in low or middle income countries (*1*). Of the movement disorders, for example, ~10 million people world-wide currently suffer from Parkinson's disease (PD) (*2*). These diseases are not necessarily illnesses of older age -- type 1 spinal muscular atrophy (SMA) is the biggest single genetic killer of children under 5 years of age, affecting up to 1 in 6000 new-borns, and a typical 'mid-life onset' disorder such as amyotrophic lateral sclerosis (ALS) has been described in children as young as 11 years of age (*3*). As with many other human diseases, each manifestation of neurodegeneration is likely to be personal and will probably require a tailored combination of treatments.

For decades, mice have been the mammal of choice for modelling neurodegenerative diseases because of our ability to exquisitely manipulate their DNA. However, few genetically modified mouse models fully recapitulate the human condition or have yet been instrumental in delivering treatments for neurodegeneration. This is leading some researchers to doubt the utility of mouse models in the hunt for therapies and to turn instead to alternative approaches, such as induced pluripotent stem cells (iPSCs). However, much of the uncertainty with mice stems from expecting each model to completely mimic human disease when, for example, mice live for only two years or so and exhibit differences from humans at all levels, from the genome through to neuroanatomy and behaviour. Uncertainty also arises from the apparent lack of reproducibility of results between laboratories. Indeed, confounding variability is evident not only across studies and across laboratories but also even within cohorts.

Clearly, a multiplicity of models, including genetically modified mice and 3D cellular systems, is required to understand neurodegenerative diseases. For example, human iPSCs give us cell models, and robust *in vitro* readouts that could be used for high throughput analysis such as drug screens,

which are not feasible with mice. In the future these platforms may be personalised to iPSCs from a given individual. However, mouse models remain essential because they enable us to take a holistic approach over the lifespan of an animal, giving us access to *in vivo* systemic interactions -- between cell types (for example, glia and neurons), tissues (for example, muscle and neurons), whole animal systems (for example, the immune system and the nervous system), and to developmental, metabolic and behavioural outcomes over the natural history of a disease. In particular, as many of the symptoms we are trying to treat are behavioural (for example, memory impairments, deficits in cognition), it is essential to understand how molecular, cellular and circuit changes manifest as functional changes at the level of the behaviour of the organism. Mice also allow us to model environmental effects and individual responses over a lifetime in a way that is impossible in cellular systems.

Here, (1) we argue that we have to acknowledge the differences between mice and humans and, most importantly, to tailor each mouse model to the research question being asked, in order to understand mechanism. (2) We ask why mice with the same genetic mutation have divergent phenotypes in pathological and behavioural sequelae? And can we turn this to our advantage? So that in fact, what we have hitherto seen as a problem and a lack of replicability, we now suggest is actually an important opportunity for understanding gene interactions, and gene x environment interactions, which may also underlie the variability of disease phenotypes in humans. Finally (3), we discuss briefly the failure to translate *mechanistic* findings in mice to *therapies* in humans, and suggest that this reflects the large diversity in the human clinical population that is currently not understood, nor captured in mouse studies. Embracing and understanding such variability in animal models may therefore be an essential step in the translational process. This can provide a route to a better understanding of variation in neurodegeneration in clinical populations, from which we may develop personalised therapeutics.

MICE AND HUMANS ARE DIFFERENT

Despite the remarkable similarities between us, mice are not mini-humans and humans are not large mice. There are species-specific differences in every aspect of our biology, and these can be extremely helpful for highlighting key issues in human neurodegeneration.

At the level of the genome, ~90% of mouse and human DNA falls into regions of conserved synteny, distributed between the 46 human chromosome pairs and the 40 mouse chromosome pairs. Both species have similar numbers of protein coding genes, but roughly 1% of mouse genes appear to have no human equivalent and vice versa (*4*). These genomic differences can be an issue for mouse modelling. For example, approximately 1 in 750 babies are born with trisomy of human chromosome 21 (Hsa21), resulting in Down syndrome (DS), which gives a greatly increased risk for early-onset AD (*5*). The long arm of Hsa21 has three regions of conserved synteny with mouse chromosomes 10, 16, 17 (Mmu10, 16, 17) and partial trisomies of these chromosomes have been used to model DS (*6*). However, four Hsa21 protein-coding genes appear to be absent in the mouse genome, and at least four mouse genes found in the regions of conserved synteny seem absent in humans (*7*), so mouse partial trisomies cannot fully model trisomy 21. The Tc1 mouse model, which carries a partial copy of Hsa21, was made to overcome this issue, but (at least in theory) human gene regulation and protein interaction may not be the same in a mouse cellular environment (and this chromosome is lost stochastically from mouse cells i.e. ~70% brain nuclei contain Hsa21, so modelling mosaic trisomy 21 (*8*)). So strictly speaking, no mouse can absolutely faithfully model DS. Nevertheless, analysis of phenotypes across a variety of mouse models of DS is being tremendously powerful for teasing apart the complex biology of this human chromosomal disorder, providing we take into account the characteristics of each model and ask specific questions about refined aspects of the syndrome that can be addressed in our models (*6, 9*).

Gene copy number may differ between humans and mice. For example, humans have *SMN1* and up to 4 copies of *SMN2* (survival motor neuron 1 and 2) genes that are paralogs, lying within a duplicated region on chromosome 5. *SMN2* has a single base pair difference from *SMN1* that results in alternative splicing such that only up to 20% of *SMN2* transcripts encode functional protein. Mutations in SMN1 result in Spinal Muscular Atrophy in which the lower motor neurons gradually die, and the rate of death is broadly inversely related to the amount of functional SMN2; in the most severe form children die before the age of four. Mice have been essential for studying SMA. However, the mouse has only one *Smn* gene and heterozygous *Smn* knockouts are fully viable whereas homozygous null mutants die early in embryogenesis (*10*). Importantly, SMA has been modelled successfully by placing human *SMN2* into *Smn* null mice ((*10*) and papers therein). Now a range of genetically different SMA mouse models exists, each with different patient mutations, or with a mouse *Smn* gene mutated to resemble human *SMN2*. Each model has different advantages and disadvantages, such as reduced severity but a longer time in which to study disease processes (*10*). These models, including conditional mutants, have greatly helped to dissect the timing of neuronal loss and to identify which neuronal populations are at risk. Furthermore, these models have been critical for therapy development, including conventional and genetic therapies (*10, 11*).

At the level of gene expression, the ENCODE project, which examined regulatory regions of mouse and human genomes in multiple tissues/cell types, demonstrated transcript profiles of a large number of human and mouse genes, and much of the *cis*-regulatory landscape, including DNAse1 hypersensitive sites, are different between the two species (*12-15*). Mouse and human also have different splice variants, and humans produce a greater number of splice isoforms (average of 3.4 isoforms per gene) compared with mice (2.4 isoforms per gene) (*16*). Splice isoform differences are particularly important in diseases such as the most common genetic form of frontotemporal dementia (FTD) (*17*), which is caused by mutations in the *MAPT* (microtubule-associated protein tau) gene that encodes the protein TAU. Transgenic mice have been made using human *MAPT* cDNAs and these have been helpful for pinning down mechanism but mostly cannot address the issues of the FTD that is caused by abnormal TAU splice isoform ratios. However, now mice with the complete human *MAPT genomic* region exist and these have the various *human* TAU splice variants. Importantly for developing therapies, such mice respond to antisense oligonucleotide (ASO) treatment by switching isoforms, so giving insight into tauopathies and new models for developing therapies for FTD (*18*).

Non-protein coding DNA may, or may not, be well-conserved between human and mouse, and yet mutations in such regions can be causative for neurodegeneration, for example, as in the intronic mutation in the *TAF1* gene that results in X-linked Parkinsonism Dystonia (*19*) or the tRNA changes that lead to neuronal death (*20*). Non-coding changes also *modulate* disease outcomes -- by 2017 over 3,000 different genome-wide association studies (GWASs) had reported >30,000 SNP-disease associations, the vast majority of which lie in non-coding regions (*21*), indicating the importance of gene regulation in disease, including neurodegeneration (*20, 22*).

Surprisingly small differences between human and mouse amino acid sequences can have large effects on phenotype. Humans with three copies of the wildtype *APP* (amyloid precursor protein) gene succumb to early onset AD, whereas three copies of wildtype mouse *App* does not lead to similar amyloid deposition (*5*). Human and mouse APP are highly homologous but the 17 amino acid differences between them include three key residues that affect how APP protein forms amyloid deposits and this may in part explain why human and mouse respond differently to three 'doses' of wildtype APP (*23*). Studying these differences may help shed light on how primary sequence affects amyloid deposition. Similarly, human and mouse superoxide dismutase 1 (SOD1) proteins have a few key amino acid differences, likely making the human protein more prone to aggregation than the mouse equivalent (*24*). The $SOD1^{D83G}$ mutation causes autosomal dominant ALS in humans but in mice

the picture is very different. A mouse with the identical nucleotide and amino acid mutation (*Sod1*^{D83G}) only had progressive motor neuron loss when homozygous, and then with only mild upper motor neuron loss (~20% by 29 weeks of age) and a similar loss (23%) in lower motor neurons by 15 weeks of age. Remarkably, after these timepoints motor neuron loss stops and motor neuron numbers remain stable for at least a year, although accompanied by a severe peripheral neuropathy (*25*). Dissecting the phenotype of the *SOD1*^{D83G} mouse showed motor neuron axonal degeneration and cell body degeneration were separate phenomena (at least initially), potentially giving an insight into the human disorder SOD1-ALS and separate loss-of-function and gain-of-function effects arising from a single mutation, even though the mouse does not fully model human ALS (*25*).

Cellular and/or metabolic pathways may be common to both species but may have a different importance in each. The *Hexa* (hexosaminidase subunit alpha) null mouse was made to model the lysosomal storage disorder Tay-Sachs disease, which in humans is caused by loss of HEXA function. In finding out why the null mouse does *not* overtly model this deadly childhood disorder, researchers teased out an alternative metabolic pathway that is present in mice but of less importance in humans, although it could nevertheless still be used as a possible therapeutic route for this disease (*26*). This highlights an important point that humans and mice have evolved to fill different niches and so major biochemical/metabolic/physiological pathways may be different between us. For example, compared to humans, mice have a higher proportion of metabolically active tissues such as liver, and larger deposits of brown fat, which are essential for thermoregulation (*27*). Other differences include: cell membranes that have more polyunsaturated fatty acid in mouse than in humans (*28*); our different dietary requirements -- mice can synthesise vitamin C, whereas humans cannot (*27*); fundamental differences within the mouse and human immune systems (*27*) which will be essential to take into account as we learn more of the connections between immunology and neurodegeneration.

Genes themselves may be more important to one species than the other: while we mostly share essential genes that are lethal if deleted, *SOD1* is essential in humans but not in mice (*29*), and null mice survive whereas no null humans have been reported. This is important when modelling SOD1-ALS, as the majority of mutations result in some loss of superoxide dismutase 1 activity, and possibly the outcome in human motor neurons is more severe than in mouse motor neurons (*30*).

Differences in gross morphology are also important for our modelling of disease, for example, size is closely correlated with metabolic rate in mammals (*27*). With respect to the gut and the microbiome the relative length of the small intestine to the colon is larger in mice than in humans, probably because the cecum is important for microbial fermentation of foods (*27, 31*). Mice and humans have clear neuroanatomical differences in brain and spinal cord, less so in the peripheral nervous system. In the brain, correspondence between human and mouse regions is largely characterised by histological staining patterns and the connections between regions, but there are many key differences. For example, the human brain has substantially more white matter than that of the mouse – the amount of white matter increases as a cubic function of the size of an animal because more axons are needed to innervate the body (*32*). Furthermore, we are largely visual animals, whereas mice rely more heavily on other sensory inputs, such as olfaction.

Lifespan is another important difference when considering mouse models of mid-life/old-age neurodegeneration. For example, the widely-used mouse strain C57BL/6J typically lives for just over two years, compared to, say, people in Japan who have an average (male and female combined) life expectancy of greater than 83 years. A mouse succumbing to disease at one year of age may be in late middle-age (depending on the strain) whereas at three-months -- which is often when we study mice -- that animal may be still a young adult. Moreover, ageing in mice does not necessarily reflect the same processes as in humans (*33*).

For both humans and mice we need to carefully tease apart effects of normal ageing from effects of neurodegeneration. For example, this is an issue when investigating the AD that arises in DS, because premature ageing is also part of the clinical picture of DS (*34*). Moreover, a related point for mouse models is that individual inbred mouse lines may have alleles that predispose them to progressive phenotypes as the mice age, such as retinal degeneration, hearing loss, and other defects, even before any mutation is placed onto these strains (*35*). There are many apocryphal stories of researchers testing mice with visual cues, unaware that both littermate controls and mutants have sight loss. Therefore, we need to know about the normal ageing characteristics of our mice, as well as ourselves, while investigating neurodegenerative phenotypes.

Undoubtedly one of the most difficult areas to tackle for neurodegeneration studies is that of behaviour. Nevertheless, while behavioural changes are highly variable between people affected by neurodegenerative diseases, there are common patterns such as memory loss or disinhibition, depending on the disorder. This is such a difficult area to appraise and yet so important; it will be discussed in depth later in this review.

WHICH MUTATION? WHICH MOUSE MODEL?

While the majority of human neurodegenerative diseases are sporadic, meaning that we are all at risk (usually that risk increases with age), there are often rarer, familial, monogenic forms of disease that enable us to create animal models to study underlying mechanisms. For example, up to 40% of FTD and 10% of ALS are due to mutations in single causative genes. Up to 5% of AD is inherited in an autosomal dominant manner from mutations in one of three genes (*APP*, presenilin 1 *PS1*, presenilin 2 *PS2*); approximately 15% of PD occurs in people with a family history of the disease and up to 2% of all PD is due to causative (usually dominant) mutations in single genes. Causative alleles can vary greatly in different populations: the G2019S mutation in Leucine-rich repeat kinase 2 (*LLRK2*) accounts for ~37% of familial PD in some Arab groups (*36*) but is rare in Taiwan (*37*). At the other end of the spectrum, nearly 100% of Huntington disease (HD) arises from triplet repeat expansion in the Huntington gene, *HTT*.

Deciding which mutation to put into a mouse to model a specific neurodegenerative disease has often been a pragmatic choice of what is the fastest mutation to make, and what is likely to result in the most aggressive change found in humans, so that we may have the best chance of producing a quantifiable phenotype in mouse. However, given the range of tools now available for genome engineering, we can take a more considered approach to creating mouse models to answer specific questions about mechanism, without expecting a perfect mimic of human disease. Here we discuss the range of *genetic* models available. Mouse models created using recombinant adeno-associated viral (AAV) or lentiviral vectors, for example, are also giving new insight into disease mechanisms; this is a rapidly developing field that we do not discuss further here, and which has implications for studying mechanism and for downstream gene therapy approaches. Another area that will be extremely important for dissecting neurodegenerative mechanisms is the increasing use of human-mouse chimeric animals, in which specific cell types are human and can be studied *in vivo* in the mouse (for example, (*38*)). This research platform brings its own challenges, including novel ethical issues, and is a very rapidly expanding area that we touch on only briefly below.

Mouse modelling – what is the question?

There are a few key questions that reoccur with all neurodegenerative diseases. For example, specific proteins are usually deposited – but is protein deposition a disease causing, or a disease response mechanism, or both? Why do neurons die? Causative genes are usually ubiquitously expressed, so why do only particular neurons and/or synapses die, usually in a well-described pattern? Does disease arise from loss- or gain-of-function, or both? What determines timing of symptom onset? Is a

neurodegenerative disease cell autonomous? How does disease spread? What determines time to death? Why do these disorders progress from pre-symptomatic to symptomatic to end-stage? Why does incidence tend to increase with ageing? What pathways produce variation in severity (and penetrance of genetic disease) and can these give us routes to therapeutic modulation? What is the connection between histopathological changes and altered behaviour? What is the therapeutic window? Is neurodegenerative disease reversible?

To help answer these questions we can now create mouse models to order, with exquisite accuracy. These include knockins, genomically humanised models, transgenics, chemical mutants, conditional mutants, inducible mutants, chromosome engineered mutants, transchromosomic mutants, each giving different types of information about neurodegenerative processes (**Table 1: which disorder, which question, which mouse model?**). In creating a mouse model of neurodegeneration we therefore first need to consider 'Which question are we trying to answer?' and then 'Which mouse model would best provide that answer?'

For research into early neurodegenerative processes we can work with mice expressing disease genes at physiological levels, which may give slowly progressing, prodromal, phenotypes that do not reach end-stage within the lifespan of a mouse. However, this may not be helpful for trying to test therapeutics targeted to later stages of disease, which is often when patients come into clinic for the first time. Developing biomarkers or predictors of disease (for example, blood biochemicals, neuroimaging modalities, behavioural changes) may require access across disease stages, using different models to address diagnostic issues and to monitor therapeutic outcomes. Endophenotypes (characteristics associated with a disorder but not necessarily a direct outcome of that disorder) may help with such issues but need careful validation. Mouse models can also indicate whether disease is reversible even at late stage (*39*); or whether even treating non-neuronal tissue is helpful -- for example, we think of SMA as a neurological deficit but therapies derived from studying the whole mouse, now target the liver because pre-clinical studies of liver pathology show efficacy in a mouse model of severe SMA (*40*).

The most widely used models of dominant neurodegenerative disease are still transgenic mice, created by injecting DNA into the pronucleus of a fertilized mouse egg. The DNA inserts randomly often creating additional, unintended mutations -- so multiple founder lines are required to exclude phenotypes arising from random insertion -- and usually concatemerises so the animal has multiple copies of the transgene and thus overexpresses the protein of interest. A study of 40 well-cited transgenic strains, many of which are used in neurodegeneration research, showed about half had mutations at the insertion site, although little phenotypic outcome appears associated with these insertional mutation events (*41*). Injected constructs are often cDNAs under non-endogenous promotors that do not express with exactly the same pattern as the endogenous gene, and only express the splice isoform encoded by the cDNA. Alternatively, Bacterial Artificial Chromosomes (BACs) may be injected to create transgenic mice, and have the advantages that (1) they have large insert sizes (up to ~200kb) of genomic DNA that may include the entire promotor/exon/intron architecture of a single gene (or genes), (2) therefore they may express the complete set of human splice isoforms, and (3) they tend to integrate in fewer than three copies, so ameliorating overexpression effects.

Transgenic models expressing multiple copies of a mutant gene may be helpful for accelerating mid-life/late onset disease (if mutant protein levels and disease onset positively correlate), which is necessary for studying later stages of neurodegeneration. However, phenotypes may arise as a result of overexpression per se, rather than the effects of mutation within the transgenic DNA, as has been reported in the *SOD1*^{G93A} transgenic model of ALS, for example, (*42*). Nevertheless, this strain is used to model SOD1-ALS, allowing scientists access to rapidly progressing and later neurodegenerative

processes, but it is particularly important that strains like this are compared to a mouse control overexpressing similar levels of the human wildtype protein – in this case, the equivalent wildtype human SOD1 overexpressing transgenic (*43*).

The utility of transgenics is exemplified in the prion disease field. Transgenic mice overexpressing a human prion gene were instrumental in showing that bovine spongiform encephalitis (BSE) could be transmitted between species (i.e. potentially to humans). Notably, had transgenic overexpressors *not* been used, this result would have been missed because the time course of disease would have been longer than the lifespan of the mouse (*44*).

In terms of gene targeting, *knockout* mice have DNA sequences removed such that the targeted gene no longer functions. Such mice may be conditional or inducible such that the gene loses function at genetic triggers, or can be induced under experimental conditions because of hormone or drug administration, either by injection or in the drinking water. In addition to understanding gene function these animals can be extremely helpful for determining if late stage neurodegeneration is reversible, by switching off the relevant gene mutation at specific timepoints (*45*).

Knockin mice have a sequence such as a mutation or mutant gene precisely targeted into their genome at a specific locus. Sequences may have exogenous promotors and can be placed into 'parking spots' such as the *Rosa26* locus, which are safe places to insert foreign DNA without disrupting other genes. Sequences may also be targeted by homologous recombination or by CRISPR/Cas9 and other technologies, into their orthologous mouse locus, such that they are expressed at physiological levels by the mouse gene promotor. Because gene expression is therefore lower than in transgenic models, knockin mice tend to have longer times to disease onset and a slower, less aggressive course of disease, which may give us access to early disease stages. For example, a knock-in model of dominant ALS expresses the mutant protein VAPB (vesicle-associated membrane protein B/C) at physiological levels, and with a much slower disease course than transgenic overexpressing animals (*46*); these mice have dysfunctional motor neurons but no signs of motor neuron loss. Despite the lack of motor neuron death in this model, it gives us access to early disease processes and potentially to biomarkers -- which are badly needed for many human neurodegenerative diseases. Equally, mouse models of PD may not give the full human clinical picture, but can give access to the prodromal syndrome including behavioural abnormalities (*47*), although it can be difficult to tease out repair and/or compensation responses from true disease processes as found in humans.

Knockin models are particularly important if the gene of interest is dosage-sensitive, i.e. more than the normal two copies gives rise to a phenotype that is not necessarily related to the disease under study. For example, point mutations in the *FUS* gene (fused in sarcoma) can cause ALS. As *FUS* is a dosage-sensitive gene, transgenic *FUS* mice do not model FUS-ALS well, whereas knockin models expressing pathogenic *FUS* mutations at physiological levels are excellent models of ALS, giving access to early stage disease processes; other ALS genes are also exquisitely dosage sensitive (*45, 48, 49*).

Another class of knockin mice are the genomically humanised models, in which either whole genes in mouse are replaced with human genes (*50, 51*), or key residues are changed into human amino acids from the mouse sequence in the hope of producing more accurate phenotypes for human disease. These include the now widely used APP^{NL-F} and APP^{NL-FG} models of AD amyloid deposition (*52*) in which critical amino acids have been changed from mouse to human residues, so making the amyloid beta (Aβ) fragment more 'aggregatable'.

Mutant mouse models can be generated with random mutations in the genome, such as those arising from chemical mutagenesis (*53*), which may give unexpected insights into gene dysfunction at physiological levels. For example, a chemically induced point mutation which turned out to be within

the microRNA, miR-96, was shown to give rise to progressive hearing loss in mice (*54*) and this finding was then replicated in humans (*55*).

Other types of mouse model include those created by chromosome engineering, which enables us to move massive blocks of DNA around the genome and to delete and duplicate huge regions. This allows us to model, for example, chromosomal full or partial aneuploidy (*6, 56*), or large-scale copy number variation (*57*).

A different type of model is provided by chimeric mice, which are made from two different cell lines and can be extremely helpful in understanding cell autonomous processes. For example, human neurons have been transplanted into both a mouse model of AD and wildtype animals. Notably, only the human neurons in the AD mice developed amyloid plaques indicating that cell death is dissociable from tau tangle formation (*38*). In another example, chimeric mice comprising both wildtype and mutant SOD1 (causative for ALS) expressing mouse neurons, gave the first strong evidence that ALS is a non-cell autonomous disorder (*58*). Somatic mutation, which may also cause neurodegenerative disease, can be modelled by yet other types of mice with mosaic expression of key genes (*59*). It is highly likely that the use of chimeric mice to model neurodegeneration, for example, with human immune systems, or specific human neurons, will increase greatly in the future.

Another advantage of working with mouse models to understand neurodegeneration, is our ability to cross independent models and study the double mutant progeny to tease out disease networks, as in a recent study of demyelinating and axonal neuropathy, and specific genes that modify these phenotypes in mouse models of Charcot-Marie-Tooth diseases (*60*), and a study of the effects of ApoE4 on tau-mediated neurodegeneration (*61*). Similarly, such classical genetic crosses allow us to uncover novel genetic interactions, such as between mutant SOD1 in ALS models and the cytoplasmic dynein heavy chain (*62-64*).

Clearly, one single mouse model for any given disease is unlikely to provide all the outcomes needed to understand both the underlying early- and late-stage molecular mechanisms and to develop therapies and biomarkers. We need a tailored range of different animal models to address different questions about pathogenesis and treatment, and fortunately we are now in a position to produce the bespoke mice we need.

THE IMPORTANCE OF VARIABILITY:
LEARNING FROM DIFFERENT PHENOTYPES THAT ARISE FROM THE SAME MUTATION

Genetic models enable us to study phenotype in a highly controlled system under highly controlled conditions, and can help tease out how individual variation arises and how it impacts on disease – but only provided rigorous statistical and unbiased, blinded approaches to analysis are taken (*65, 66*). This variation may highlight key cellular pathways, as exemplified in human GWASs, and it provides a potential basis for dissecting individual responses to disease and therapeutics. However, as with humans, we still understand comparatively little of what causes variation in individual mice.

Age, gender and genetic background are well-known and significant sources of variation in mouse studies. For example, a single well-defined mutation can result in markedly different phenotypes when bred onto different inbred lines, which have homogeneous (within the inbred line) but different (between inbred lines) genetic backgrounds (*67*). Not surprisingly, therefore, breeders go to great lengths to try to maintain a standard background and avoid genetic drift over time (*68*). In working with different sub-strains of individual inbred lines, we may have expectations that sub-strains used in different laboratories will be similar to each other, whereas in fact they can be markedly different;

for example, the widely used C57BL/6J and C57BL/6N sub-strains, have some notable phenotypic differences such as reduced motor performance in the /6N compared to the /6J sub-strain (*69*).

Genetic background effects can in fact give great biological insight; this common source of variation can be used to help identify modifier loci (*70*). For example, a modifier locus for human Dravet syndrome was identified using the strain dependent epilepsy phenotype of a mouse *Scn1a* (sodium voltage-gated channel alpha subunit 1) mutation (*71*). Another example comes from studies of the *Cacna1c* (calcium voltage-gated channel subunit alpha 1 C) and of *Tcf7l2* (transcription factor 7 like 2) genes, which have been implicated in human studies of psychiatric disorders and in type 2 diabetes respectively. Crosses of null mutations in these genes onto 30 different inbred lines showed that opposite effects from the same allele can occur, depending on the genetic background, and that sex could also have a marked effect on phenotype (*72*). Thus, if we confine ourselves to studying one inbred line, or one sex, we may miss the panoply of variation arising from a single mutation. Rather surprisingly the genetic background in a mouse cross can also include 'inert' control elements such as the tetracycline transactivator which in theory only exert an effect when activated, but in practice may independently affect phenotype (*73*).

Other sources of variation such as *parent of origin* effects (*74*) (which may warrant phenotyping wildtype offspring of mutant mice) and contribution from transgenerational inheritance (*75, 76*) may be important sources of variation for some phenotypes. Similarly, even when closely defined hybrid lines are used in highly controlled environments, *litter of origin* effects (i.e. phenotype depends on which litter a mouse comes from) may arise and so mice from the same litters should be distributed across experimental cages), as shown in a carefully controlled study for therapeutic trials in a mouse modelling aspects of HD (*77*).

It is important to identify and understand the many other sources of phenotypic variation so that we can fully characterise our animals, and thus better understand human biology and pathology. This may be crucial for understanding gene interactions, and also for gene x environment interactions, both of which can exert huge influences on disease aetiology and progression. Here we briefly discuss some of the conditions leading to phenotypic variability between studies.

Life history, well-being and stress

Life history is an important determinant of disease, for a mouse and for a human. For example, the level of adversity in the developmental environment biases protective stress or anxiety responses in adulthood, to better adapt an organism to the environment into which it is born (resilience) (*78-80*). Notably, this may not always be helpful later in life if there is a mismatch between the developmental and adult environments. Mice, like humans, are affected by early life trauma, which can alter brain structure, produce epigenetic and genomic effects and lead to neuropsychiatric phenotypes later in life (*81-86*). Stress can also influence the *in utero* environment such as when effects of maternal inflammatory responses lead to brain and behaviour abnormalities in offspring (*87*).

Indeed, stress is a hugely important determinant for pathology and behavioural sequelae in neurodegenerative disorders. Stress and the hypothalamic-pituitary-adrenal axis (HPA-axis) are key determinants of levels of circulating corticosteroid hormones, which can have powerful effects on neurodegenerative phenotypes. For example, stress, either from chronic isolation or as the result of an acute challenge, can lead to increased levels of Aβ peptide in brain interstitial fluid in a transgenic mouse model of Aβ deposition used to study AD (*83*).

Social interactions play a role in mouse and human well-being, and can markedly affect phenotypes. Single housing of social animals such as mice causes stress and aberrant behaviours (*88*). Furthermore,

social hierarchies and dominant/submissive relationships may have a profound impact on mouse well-being and so, for example, the population density of a cage may affect phenotype (*89*). Likewise, human relationships are important for outcomes in neurodegeneration. For example, in an ALS patient cohort, being married was associated with an 8-month median longer survival time than in single individuals (*90*). Interestingly, it now appears that the genetic status of those around an individual may also be important for its well-being. Notably, in mice several traits from wound healing to anxiety are significantly affected by the genotype of cagemates (*91*).

Ambient temperature is a source of variability we rarely consider because mice are generally housed at temperatures of between 20-26 ^{o}C. However, this may be too cold, as mice have activated thermogenesis at these temperatures to maintain normal body temperature. This mild chronic cold stress affects tumour formation and metastasis, potentially confounding our understanding of these processes – which may also be true for neurodegenerative mechanisms (*92*) and for metabolic pathways (*93*). It is worth noting that some researchers, including those engaged in therapeutics studies, are moving to keep their mice, inbred strains or outbreds, in environments that as closely as possible mimic those of wild mice, and this can have large effects on many aspects of phenotype (*94*).

Circadian rhythms and sleep

Circadian rhythms can affect experimental and pathological outcomes in neurodegeneration research (*95*). In fact, we collect tissue samples only at specific times of day to avoid variations in gene expression arising from circadian rhythms -- for example, normal daily cycles in body temperature affect alternative splicing of transcripts (*96*). Similarly, behavioural testing is usually carried out at the same time of day within an experiment in order to minimize variability. However, as a consequence, we may miss phenotypic variation across the 24-hour cycle. This may be particularly important as disruption of normal circadian rhythms can significantly impact biochemical pathways underlying neuropathology and the expression of their behavioural sequelae (*97*).

Related to circadian effects, is the importance of sleep and sleep disturbance in the development of neurodegenerative phenotypes. For example, sleep plays a critical role in the clearance of Aβ from the brain (*98*). Thus, sleep deprivation and/or sleep disruption may impact on Aβ pathways and exacerbate disease phenotypes and progression by affecting Aβ removal from the brain (*99*). Moreover, sleep disruption per se may also directly exacerbate cognitive impairments arising from neuropathological changes, similar to its putative effects in neuropsychiatric conditions (*100*).

Exercise and environmental enrichment

Levels of physical exercise play an important role in modulating brain function. Exercise results in the release of trophic factors including BDNF (*101*), and promotes neurogenesis in the dentate gyrus of the hippocampus (*102-104*), a brain area strongly implicated in neurodegeneration and its behavioural sequelae. Exercise, such as voluntary wheel running in mice, enhances glymphatic influx which may be pro-cognitive (*105*). Environmental enrichment can also have an enormous impact on the development of behavioural and histopathologial trajectories in mouse models of neurodegenerative disease. For example, the onset of cerebral volume reduction and motor disorders are significantly delayed in an HD mouse model if the animals are exposed to a stimulating environment from four weeks of age (*106, 107*). This may parallel the concept of 'cognitive reserve,' such that people who frequently perform cognitively demanding tasks may be less likely to suffer from deterioration of brain function (*108*).

Diet, the microbiome and inflammation

An advantage of working with mice is to be able to study the effects of diet on any given phenotype (*109*). However, often scientists in labs may not even be aware of any variation in mouse diet. Many mouse chows contain soy and so may result in a relatively large intake of phytoestrogens, potentially influencing disease outcomes such as anxiety or pain. Exposure to phytoestrogens *in utero* also has effects on disease outcome. However, the amount of soy and therefore phytoestrogens can vary between batches of chow, thus potentially adding to variability in phenotypes (*110*). This may be relevant to modelling disorders such as ALS in which oestrogen may have a protective effect.

Alterations in the gut microbiome, whether derived from diet or otherwise, can also have a great influence on biochemical, neural and behavioural phenotypes (*111, 112*), and lab mice have a gut microbiome that is notably different from their wild relatives (*113*). Strikingly, treatment with gut bacteria from PD patients exacerbated motor deficits, microglia activation and pathology in an alpha-synuclein overexpressing transgenic mouse, an effect which, in turn, could be ameliorated by antibiotic treatment (*114*). Efforts are now being made to define the effects of wild mouse gut microbiota in promoting host fitness (*113*).

Similarly, the overall health status of the mouse colony can greatly affect the cellular components of the innate and adaptive immune systems, which may again affect phenotypes in mouse models (*115*). CNS inflammation has long been established as important in the progression of neurodegenerative disorders (*116*). Recently, it has been found that systemic inflammation also plays a key role (*117*). The adaptive pathways by which signals of systemic inflammation are communicated to the brain have now been well described, and it is becoming increasingly clear that excessive or prolonged activation of these pathways is detrimental to the brain (*87*), and can accelerate and/or exacerbate neurodegenerative conditions. Thus, health status may be an important determinant of the rate of progression of pathology in a given mouse model of neurodegenerative conditions. Indeed, this may relate to a much bigger story about how the health status of human subjects impacts on disease aetiology and progression. In particular, infection and inflammation may be key drivers in disease processes, including neurodegenerative disease (*59, 118*).

Making sense of behaviour

A further major source of variation across laboratories and across studies comes from the behavioural phenotyping of mice. Often the use of different behavioural tasks, and different testing paradigms and protocols in different laboratories, means that researchers are not actually assessing the same psychological processes in their mouse studies, and hence different results can be obtained. Even small differences in experimental protocol (such as amount and/or nature of any pretraining, duration and/or nature of stimuli, inter-trial interval, motivational state) can have a major influence on the way rodents might solve a particular task and hence the sensitivity to a given experimental manipulation. This reflects the multiple memory mechanisms and cognitive processes underlying complex behaviours in both rodents and humans. Again, understanding the sources of this variability across studies, in terms of behavioural outcomes, may shed light on disease processes.

A key related question when using genetically modified mouse models of neurodegenerative disorders is whether the behaviour we are studying in our rodents is accurately modelling the appropriate behaviour in humans? Therefore it is essential to identify the psychological process that is disrupted in a given mouse model, to determine whether this is the same psychological process that underlies the impairment in human patients. A clear understanding of these psychological processes will also greatly aid in identifying the underlying neural circuits and mechanisms that are affected in the model. Importantly, this requires the characterisation of the mouse across several behavioural tests, which allows the precise nature of the impairment to be inferred by comparing what the mouse can and cannot do. It is not possible to determine the key psychological process that is disrupted in a disease model from studying mice in just a single task.

In a number of situations, classical rodent assays of cognitive behaviour may not always model the human cognitive process as intended (see Figure 1). For example, do deficits in Morris watermaze performance in mouse models of neurodegeneration always indicate memory impairment? Is working memory as studied in rodents on win-shift maze tasks (for example, the radial maze or T-maze) really the equivalent of working memory in humans as studied with paradigms like the N-back task or the digit span task (*119*)? Is contextual fear conditioning in mice measuring episodic memory or conditioned anxiety? Examples from basic science experiments have highlighted these issues but the questions raised from these studies also likely apply to tests with neurodegenerative mouse models, demonstrating the importance of extending the behavioural test batteries to provide a more comprehensive and precise description of any cognitive and psychological deficits.

For example, for watermaze acquisition it is imperative to include the appropriate control tasks that are well matched in terms of sensorimotor and motivational task demands (visual acuity, emotionality, stress, swim time, difficulty, etc.), and then also to include a battery of spatial memory tests that allow the generality of any findings from the watermaze to be ascertained in different behavioural paradigms that are unaffected by performance factors like thigmotaxis (continually swimming close to the side wall of the pool) and floating behaviour. Both of these are often clear and present confounds in watermaze studies with mouse models of neurodegeneration. If a mouse watermaze deficit is due to thigmotaxis (possibly as the result of altered anxiety), any drug treatment developed from such an assay may be unlikely to remedy cognitive symptoms in patients. Conversely, however, increased thigmotaxis and/or enhanced floating behaviour likely reflect important behavioural responses to a given aversive situation. Understanding these behaviours, and the contribution that a particular genetic change and/or its neuropathological sequelae make to these phenotypes, may provide important information about emotionality phenotypes (such as depression, anxiety) that can occur in neurodegenerative conditions in human patients.

Phenotyping is a group activity

Importantly, we emphasise that the best way of working with mice, which also likely involves using the fewest animals, is to work collaboratively with scientists with expertise in different areas, from physiology to genetics to endocrinology, transcriptomics to behaviour to developmental biology, immunology to neuroimaging to protein chemistry, in concert with clinical experts from each neurodegenerative disease. Multidisciplinary teams enable us to maximise what we learn from each model, including pleiotropic effects (separating these from co-morbidities, for example), and not just to have a narrow focus on a specific cell type of interest. Each mouse mutant should undergo broad phenotyping of the sort provided by the International Mouse Phenotyping Consortium pipeline (*66*), as well as the in-depth drilling down into mechanism from the disease specialists.

Metabolomic studies provide a good example of why studying neurodegeneration should not only be the work of neuroscientists. One of the most distressing aspects of ALS and HD is weight loss and this may correlate with poor clinical outcome. Furthermore, low body mass index is thought to be a risk factor for ALS (*120*). In ALS we know there are lipid disturbances in both humans and mouse models but why these arise remains unknown. This could turn out to be an important avenue of research for disease modifying treatments.

TRANSLATION: THE FINAL FRONTIER

Despite the hundreds of mouse models of human neurodegenerative disease, we still have no cure for any major form of neurodegeneration, and extremely limited treatments for just a handful of these diseases. This is not a review about Translation; many insightful articles have been written on moving

forward from mouse studies of disease *mechanism*, and the difficulties of translating *therapies* that modulate disease in mouse to successful human clinical trials (for example, (*27, 121-124*)). Here, we suggest that variability – mouse and human - may be an important factor in current translation failures -- but also a source of potential insight.

What is translation?

The translation phrase 'from Bench to Bedside' encompasses two separate processes: (1) to discover pathomechanism, and (2) to develop and trial new therapies. In our view, mouse models have great utility for dissecting biological processes but so far have not proven particularly useful or practicable for producing treatments that work in humans (with notable exceptions, including the success of ASO therapies (for example see (*125*)). At present, therefore, the major and proven utility of mouse models lies in identifying mechanism; their potential utility (or lack of) as drug screening tools may only become apparent once we have a better understanding of both (i) pathomechanisms, and (ii) why current attempts at translation have so often failed. We argue that the answer to the latter question may partly lie within our appreciation of the variability in our mouse studies and also the great variability within the human clinical population.

From mouse and mechanism to medicine: confounding variability beleaguers translation

In biomedical studies we generally only look at a snapshot of disease; for example, for technical or financial reasons we study genetic mutations on one or two inbred mouse lines only, or worse, on ill-defined outbred lines that are usually a lot more inbred than expected (*126*) (although outbreds certainly have their uses (*127*), and abuses (*128*)). We tend to study single gene mutations, even though most human disease is a mix of genes plus environment, and even the rarer monogenic disease forms may show synergistic effects between unrelated loci (*129*).

As scientists, our cultural viewpoint is to minimise variation within our studies in order to maximise our chances of identifying significant differences between experimental and control conditions. To this end, we generate genetically modified mice on homogenous genetic backgrounds, and maintain these animals in constant environmental conditions. It follows necessarily that any important discoveries arising from these animal studies, particularly with respect to therapeutics that modulate biochemical pathways and networks, may only be relevant to a limited subgroup of the clinical population, especially in diseases that are mostly sporadic.

It is not surprising, therefore, that when attempts are made to translate from findings in animal models to the much more heterogenous human clinical population (which of course will vary tremendously in terms of age, gender, genetic background, environment, life history), these attempts have failed consistently. Put another way, the homogeneity of approach which benefits reproducibility and increases statistical power in preclinical studies (and yet which is still remarkably difficult to carry out in mice) may come at the cost of reducing generalizabilty when it comes to translation of therapeutics from mouse to human (*122*).

This is an issue which is not exclusive to animal models of neurodegeneration, but instead reflects a general problem in making the crucial step between pre-clinical studies in animals and clinical trials for many human disorders, as has been comprehensively highlighted in the stroke field (*122, 130*) as well as in neurodegeneration studies (*131*). Researchers have made suggestions for improving this process, such as running mouse studies across several independent laboratories, in order to mimic multi-centre clinical trials in humans. Nevertheless, at the heart of this issue remain both the inherent variability of pre-clinical mouse studies from the many sources we mention earlier in this article, and, possibly even more importantly, the pronounced variability as we move into the human clinical patient population. Crucially, therefore, embracing rather than rejecting variability in our mouse studies, and then understanding both its sources and its underlying mechanisms, could be of great benefit for

successful translation to clinical sub-groups. Moreover, it seems likely to us that questions regarding the utility (or otherwise) of mouse models for (i) determining pathomechanisms relevant for human neurodegenerative conditions, and (ii) ultimately as drug screening tools, can only be properly answered once we have addressed this central issue of variability.

THE FUTURE: UNDERSTANDING HUMAN NEURODEGENERATION THROUGH A HOLISTIC APPROACH TO MOUSE MODELS

While currently we have few treatments for human neurodegeneration, and a relatively limited understanding of the mechanisms underlying neuronal death, we are also racing into a new era of personalised medicine. By working with the appropriate mouse models to address specific questions, and by examining causes of variation in these models, we can help identify diverse mechanisms for neuronal dysfunction and death. Variation may be particularly important for neurodegenerative disorders, given the heterogeneous nature of many of these conditions, and the current drive to identify biomarkers that allow stratification of patient subgroups and markers for early diagnosis and intervention.

It is impossible to standardise all experimental conditions across laboratories, but instead we need to recognise variation and use it as a source of insight. Variation has hitherto been seen as a problem and something that should be diminished and reduced at all costs. Here we suggest that variation could be an opportunity that may allow us to understand and identify disease mechanisms and risk factors, and at the same time elucidate treatment strategies, on an individual by individual basis. Indeed, embracing and understanding variation may be of great benefit for translation.

Of course identifying the source(s) of variation will not be straightforward but nevertheless, we suggest an agenda for the future of working with mouse models of neurodegeneration:

(i) models should be studied in response to a specific need – animals with slow disease progression and relatively mild phenotypes that will be of use for understanding early disease pathogenesis may not be useful for trials of therapeutics developed for late stage human disease;

(ii) funding agencies and scientists should take a more sophisticated approach to each model and not expect complete recapitulation of the human disease – mouse models may often be better suited as tools for understanding the role of a specific gene or protein, or a specific aspect of the disease process, rather than as primary drug-screening tools;

(iii) to capture how variation arises, we need considerably more detail than is often provided in current manuscripts. In an attempt to ensure mouse phenotypes are fully defined in the literature, the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) introduced a checklist of information required for any paper describing animal research; adherence to the resulting 'ARRIVE guidelines' (Animal Research: Reporting of In Vivo Experiments) is required by many journals. Working to the ARRIVE guidelines should mean some sources of variability are clearly reported (*123*) and so we can begin to understand their effects on phenotype. Furthermore, we need the refereeing process to be rigorous in ensuring accurate reporting of such experimental detail. However, more than anything, as a scientific community we need to change our attitude to the variability in our data.

(iv) we need to create long-term metadata repositories in which data are deposited and made available to the wider community for critical analysis and further phenotypic screening.

New approaches to working with mice are on their way. For example, Home Cage analysis enables us to capture aspects of behaviour not seen when humans are around (*132*), and new informatics

approaches including machine learning could help us find new phenotypes from the immense amount of data generated by Home Cage analysis. Developing ontologies for human-mouse phenotypes, in combination with new imaging and computational methods to help cross-reference between mouse and human will greatly enrich our understanding of human – and mouse – biology, and neurodegenerative processes, but only if we also take variation into account.

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Table 1: Which disorder, which question, which mouse model?

Figure 1: Behaviour; the final frontier

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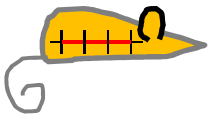
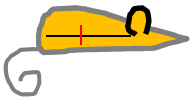
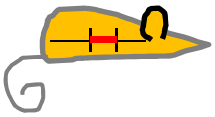
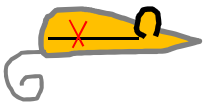
Table 1. Mouse models of neurodegenerative disease and the questions they may address

Mouse model	Possible phenotypic characteristics	Some questions and uses
Transgenic Carrying multiple copies of a transgene or BAC or other DNA construct. 	Expressivity of the phenotype likely depends on the number of transgene copies. If high copy, likely to be an early-onset aggressive phenotype. Possible artefacts from over expression.	What are disease mechanisms? can late stage disease be treated? Ideally use a transgenic overexpressing the wildtype protein of interest at the same level as the mutant protein as a control for overexpression artefacts. Can we develop biomarkers?
Knockin model Mutation in the mouse genome that replaces mouse sequence with another sequence (mouse or human) and is expressed at physiological levels. 	Appearance of the phenotype can be in the middle to late stage of mouse life, and might be relatively mild, depending on the mutation.	What are early disease mechanisms? when does disease start including protein deposition? Can we develop therapies for pre-symptomatic disease? Can we develop biomarkers?
Genomically humanised model A type of knock in mouse in which a genomic region – an exon, whole gene, chromosome region, has been replaced by the equivalent human genomic region. 	Appearance of the phenotype can be in the middle to late stage of mouse life, as the mouse is expressing the gene as physiological levels, but this is unpredictable as replacing mouse regulatory elements with human elements may alter expression.	What are early disease mechanisms? when does disease start? Do we see human protein deposition? Can we develop therapies for pre-symptomatic disease? Can we develop therapies for splicing defects? Can we develop biomarkers?
Chromosome engineered model A chromosomal region is duplicated or deleted, usually many Mb. 	Expressing two mouse endogenous genes plus one human gene from the chromosome regionof interest. Likely relatively mild phenotypes although unpredictable.	Useful for mapping genes/loci involved in aneuploidy phenotypes. What is disease mechanism?
Transchromosomic model A human chromosome is added to the mouse genome 	Expressing two mouse endogenous genes plus one human gene from the chromosome of interest. Likely relatively mild phenotypes although unpredictable.	What are early disease mechanisms? when does disease start? Can we develop therapies for pre-symptomatic disease? Can we develop therapies for reducing expression of entire

		chromosomes? Can we develop biomarkers?
Chimeras, mouse:mouse, human:mouse Animals created from two different cell lines, possibly mouse:mouse or mouse:human 	Potentially slow developing phenotypes as genes are expressing at endogenous levels.	How does disease spread? is it cell autonomous? Are multiple cell types involved, and if so, which one is key for treatment?
Inducible, conditional models Expresses (inducible) or deletes (conditional) a sequences at a specific time or in a specific tissue according to genetic or chemical signals 	Not necessarily a disease model, more for investigating disease mechanisms.	Which cell types are key for disease? Is disease reversible? What happens when particular cell types/tissues are affected? What is the timing? Can we treat disease by focussing on one cell type?
Knock out model Mutation in the mouse genome that destroys the function of a gene 	May model loss of function disease.	What are loss of function effects? How early are these seen? Can they be reversed by genetic or other therapies? Can we develop biomarkers?

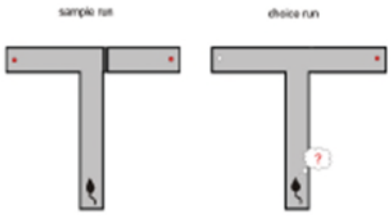
Caveat 1: mouse models are not predictable and we need to make the mouse to learn about the outcome.

Caveat 2: phenotypes depend on genetic background and environment.

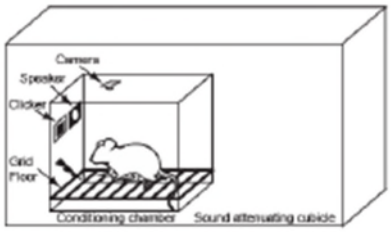
Figure 1.

The Morris watermaze as a test of long-term memory in mouse models of neurodegeneration

Testing working memory in rodents



What is contextual fear conditioning measuring?



The Morris watermaze: Do impairments in watermaze performance in some mouse models of neurodegenerative disorders really reflect deficits in learning and memory? There are difficulties associated with testing mice in the open-field watermaze task such as thigmotaxis, floating behaviour and fatigue following prolonged swim times, all of which are often more pronounced in genetically modified animals. For example, do thigmotaxic mice that swim around the perimeter of the watermaze repeatedly trial after trial really have a spatial learning and memory impairment? Are they even trying to find the platform? Would the mice be impaired on an alternative test of long-term spatial memory which is not potentially confounded by thigmotaxis? The distance that the platform is located from the side wall of the pool may even be an important experimental variable in such studies. For example, D-amino acid oxidase(DAO) knockout mice which have increased synaptic plasticity exhibit better watermaze performance than controls when the platform is in the periphery of the watermaze, but perform worse than controls when the platform is centrally located (*101*). **Testing working memory in rodents**: Working memory in rodents on win-shift maze tasks (for example, spatial working memory on radial maze and T-maze) may not equate to working memory in humans (as in N-

back or digit span tasks). Instead, rodents may often solve these tasks using familiarity judgements based on short-term habituation processes. Studies in genetically modified mice that lack the GluA1 subunit of the AMPA receptor, and as a result exhibit deficits in synaptic plasticity, illustrate how impairments in short-term habituation can look like impairments in working memory (*102, 107*). **What is contextual fear conditioning measuring?** Contextual fear conditioning is often taken as a mdoel of hippocampus-dependent, rapid, one trial, episodic-like memory encoding. However, there are a number of potential problems with this, including generic issues with interpreting freezing data in animals that exhibit marked locomotor hyperactivity, including many mouse models of neurodegenerative disease. Furthermore, contextual fear conditioning deficits may in some instances reflect changes in conditioned anxiety rather than impairments in spatial/episodic memory encoding. Evidence from genetically modified mouse models of deficient adult neurogenesis support this possibility (*133*).