



# Mouse models of neurological disorders - a comparison of heritable and acquired traits

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## Abstract

Human neurological disorders include a wide range of illnesses which have a disproportionately high prevalence in the increasingly populous geriatric community. Any research effort directed at discovering the aetiology of neurological disease is greatly enhanced with *in vivo* models of the disease of interest. Scientific research incorporating the use of mice has advanced rapidly in the last three decades. Relatively simple to breed, maintain and train, mice have many advantages over other species for use in research. More than a century of selective breeding has provided investigators with a rich gene pool and sub-strain diversity from which to choose for their research. Thus the dramatic increase in genetic screening and gene engineering that has occurred in research in recent decades has enabled the generation of a multitude of mouse models. This review discusses the relative utility of mouse models in which a heritable or non-heritable (acquired) manipulation has been used to model a specified trait of a human neurological disorder. The techniques used in deriving useful genetic alterations or modifications and in generating acquired mouse models are outlined with examples of each provided.

## Keywords:

Mouse model, neurology, disease, inherited trait, acquired trait

1. Introduction
2. Heritable mouse models
  - a. Overexpression of a transgene
  - b. Knockout of an endogenous gene
  - c. Knockin of exogenous DNA into an endogenous gene
3. Acquired mouse models
  - a. Central injury models
  - b. Peripheral injury models
  - c. Locomotor and dyskinesia models
  - d. Models of psychoses
  - e. Gene silencing by RNA interference
4. The problem with mice
5. Summary

## 1. Introduction

Several human neurological disorders that we recognise today were initially identified and described many decades ago. For example, Alois Alzheimer first published his findings in 1906 [1], George Huntington first characterised the disease to bear his name in 1872 [2] and James Parkinson first described ‘A shaking Palsy’, which came to known as Parkinsonism in 1817 [3]. These three illnesses represent common forms of inherited neurological disease however this review will also include other neurological disorders, defined as an illness of the nervous system. Use of the term model is defined as a system whereby a therapy can be tested. The common house mouse (*Mus musculus*) has a genome with 97% homology to the human genome. It is small and easily maintained in a research services facility. It breeds well with a short gestation period and is amenable to training and testing in many *in vivo* assays. Mice have been selectively bred for features such as coat colour and for scientific research. This work was started over 100 years ago by mouse fanciers such as Abbie Lathrop and by researchers such as William Castle, a Harvard geneticist. This history of selective breeding has provided scientific investigators with a wide variety of strains to use for their research. Despite this apparent range of phenotypes, a study of 8.7 million single polynucleotide polymorphisms of inbred mouse strain revealed a surprisingly low level of genetic variability among the four main inbred mouse strains and among the original wild type mouse strains [4]. Following on from these pioneers, standardisation of the supply of mice to the research community was increased when Clarence Little opened the Jackson Laboratories in 1929 to become a world-wide resource for mouse research scientists [5]. With these resources and the complete sequencing of the mouse genome [6], the common house mouse has become the species of choice for researchers where mammalian genetic manipulation is required and, with approximately 25 million mice used in research laboratories around the world, they are the most common research mammal. This review describes how the mouse can be used to generate models of neurological disorders via genetic and non-genetic manipulations with a description of the techniques used to create selected models. The pros and cons of using the mouse as a species in which to model neurological disorders are highlighted. Not every disorder or model is covered as there are too many to include in one review, however representative examples are provided. There is an emphasis on genetically engineered models as the mouse does occupy a place of importance in this technology since few mammalian laboratory species are amenable to such genetic engineering, whereas many different laboratory species can be used to provide other, non-genetic, types of model.

Mouse models of neurological disorders can be usefully divided into whether or not the model is heritable. Human neurological disorders with a mutant gene component make ideal candidates for

modelling via gene manipulation. It follows then that human neurological disorders with an identified underlying genetic component, for example Alzheimer's disease, have been extensively modelled using genetically manipulated mouse models. Alternatively an interesting neurological phenotype may be identified as a result of a spontaneous mutation in the wild type mouse population, for example the stargazer mouse [7]. These spontaneous mutant mouse models are then bred to sustain the appropriate phenotype of interest. Clearly neurological disorders also have heritable traits that do not include mutant gene components but are well characterised risk factors for the disorder, for example the Apoe4 allele in AD. As these traits can be inherited from generation to generation they can also included as heritable trait models. Mouse models that do not carry a heritable component are focussed on replicating a phenotype characteristic of the relevant disorder. Those human disorders that do not have a defined genetic component, or in which a complex multi-gene interacting system is under investigation, are more readily modelled using non heritable mouse models that have an identified robust phenotype and are acquired by physical manipulation.

While mice have come to be used as an invaluable tool to generate models of human disorders and elegant mouse models can reproduce numerous aspects of a human disease it should be realised that, by their very nature mouse models are excellent tools to examine the function of a particular aspect of a human disorder in the context of – a mouse. As such they will never be able to reproduce the full phenotype of any human disorder and the disease relevance of the model should not be overstated [8]. However if used in appropriate context they have the power to help unravel complex biochemical and behavioural phenotypes as well as evaluate the effect of potential therapeutic interventions that are impossible to investigate otherwise. One disease area where any pre-clinical model will fail to replicate the human condition is where specifically human characteristics are involved, for example in the vocal descriptions of feeling, hallucinations and delusions manifest in mental illnesses. In such examples the disorder can be broken down into specific component phenotypes which are more amenable to modelling in mice.

Genetically manipulated mouse models model are generated by genetic engineering of the mouse genome in order to produce a desired protein expression profile. This profile may involve temporal and spatial control of the gene transcription to realise the relevant protein expression. The murine host genome is manipulated by one of two general methods which result in elevated or suppressed protein expression. Elevated protein expression can be achieved by direct micro-injection of a gene construct comprising a modified complimentary Deoxyribonucleic acid (cDNA) gene sequence (transgene) into host single cell mouse embryos. These transgenes often carry one or several mutations identified with a particular disorder and their expression under the control of a specific promoter. Protein expression can be eliminated by the 'knockout' of a gene by replacing a key sequence of the host genome with a sequence of irrelevant DNA into the loci of a targeted gene thus interfering with the gene transcription. This manipulation is carried out in genomic host DNA using

homologous recombination in embryonic stem cells and subsequent introduction into host multi-cellular blastocyst mouse embryos. These techniques are well established (see [9] for detailed methods) and have been in use for almost three decades. Initially this technology was limited to simple overexpression or knockout of a gene of interest however the technology has developed to allow temporal and spatial control of the gene manipulation with elegant tissue restricted expression of the genetic construct. In addition genetically engineered sequences of target genes of interest may be used as the irrelevant DNA in knockout mouse models, thus generating the 'knockin' mouse model. Once successfully generated and bred true, genetically manipulated mouse models may be interbred to produce all manner multiple gene mouse models.

A further approach relies on random mutagenesis rather than targeted gene manipulation. Genetically modified animals derived from these breeding programs are often discovered by phenotypic screens of thousands of mice. Spontaneous mutations have arisen in the research mouse population and these have been selectively bred to provide interesting mouse models. A good example of this is the Stargazer mouse model, used in epilepsy research, discovered at the Jackson Laboratory in 1979. Following phenotypic characterisation, where abnormal, jerky head movements were observed, the model was found to have a mutation in gamma subunit 2 of the voltage dependant calcium channel gene on chromosome 15 [7]. Useful spontaneous mutations are rare and attempts have been made to accelerate this mutagenesis with specific breeding colony experiments. One such example is the N-ethyl-N-nitrosourea (ENU) mutagenesis project [10]. This project makes use of the mutagenic properties of the ENU to introduce germ line mutations by direct intraperitoneal injection into founder mice. Once offspring derived from breeding these founder mice are screened for phenotypic abnormalities using a platform phenotype assessment and mice with interesting phenotypes are selectively bred to expand the new genetically altered line for further characterisation.

While many mouse models have a genetic component and as such have a direct link to the underlying genetic component of the disease that they are designed to represent, mouse models of neurological disorders can also be generated from pharmacological or mechanical manipulations. This approach has no heritable component and relies on an understanding of the biochemical pathways that are disturbed in the relevant disorder. A major advantage of this approach is the relative speed at which a model can be generated using wild type mice available from commercial suppliers. In addition extensive mouse breeding regimes are not required and the cost of generating genetically modified mouse models *de novo* or in-licensing them is obviated.



## 2. Heritable mouse models

A multitude of genetically altered (GA) mouse models of neurological disorders have been generated and various institutes maintain databases of the mouse model available via the World Wide Web (for examples see ([11-15])). Many articles have been published reviewing the current research efforts into a number of neurological disorders. Select reviews of models of Alzheimer disease [16] , Parkinson's disease [17] [17], Huntington's disease [18] , Tauopathies [19] Amyotrophic Lateral Sclerosis [20] , Friedreich's ataxia [21] and Duchenne Muscular Dystrophy [22] exemplify useful literature sources of information to gain an in depth understanding of current mouse models of a disorder of interest. This resource is a valuable addition to any researcher seeking to investigate the genetics of a particular disorder. It is clear however that the diverse range of phenotypes seen in mouse models developed from acquired rather than inherited characteristics means the review literature is not easily classified by disease and as such publications are limited.

### 2a. Overexpression of a transgene

A simple transgenic mouse model is generated when a transgene construct is introduced into the mouse genome and is inherited through generations of mice. The transgene is introduced into the mouse genome by micro-injection of a purified fragment of the engineered DNA into the male pronucleus of a single cell mouse embryo (figure 1a) through a very fine glass needle. Following injection the transgene is inserted into the mouse genome when the embryo undergoes mitosis. Embryos surviving this injection are transferred into the infundibulum of a pseudo-pregnant recipient dam and allowed to develop to term. Founder offspring are genotyped for incorporation of the transgene and positive transgenic mice are bred to wild type mice to further expand the colony for subsequent experimentation. The transgene comprises a complimentary DNA (cDNA) sequence encoding the gene of interest driven by a promoter directing expression to the desired tissue. In order to generate a transgenic model of a heritable disease, the cDNA should carry a mutation linked directly to the human disease. The choice of promoter is dictated by the pathological features of the disease being modelled. Many promoters are available with well characterised tissue and cell type distribution to which they drive the transgene expression [23] however many confounding factors such as integration site, copy number and mouse strain need to be considered. A comprehensive promoter selection tool would only serve to potentially mislead researchers as different promoters may produce different distribution patterns in different models. As such transgenic lines need to be investigated in isolation and characterised as such.

One disease that has been a rich source of transgenic mouse models is Alzheimer's disease (AD). While the majority of AD patients are sporadic cases and have no observed heritable genetic

component, a minority of patients do have heritable forms of the disease. These inherited forms include mutant genes as well as complex genetic traits that increase the likelihood of developing AD and both have provided researchers with invaluable tools to investigate the disease pathology. Using this genetic link many mouse models of different aspects of AD have been generated. A key pathological lesion in the brains of AD patients is the Amyloid plaque which is derived from pathological cleavage of the amyloid precursor protein (APP) by  $\beta$ - and  $\gamma$ - secretase enzymes. While a wide range of currently available AD mouse models now exists (reviewed in [16]) using a variety of genetic manipulation techniques (described below), simple transgenic mouse models of AD have proved to be useful tools to investigate disease pathology. Mouse models incorporating only APP are not models of AD, indeed no mouse model can fully replicate all facets of a human condition such as AD, though they do reproduce key features of the disease [24,25]. One early example of such a model is the PDAPP mouse in which numerous features of AD were reproduced including neuritic plaques, synaptic loss, astrogliosis and microgliosis [26]. This model has cDNA encoding the Amyloid Precursor Protein (APP) carrying the APP<sub>V717F</sub> (Indiana) mutation driven by the platelet derived growth factor (PDGF) promoter. Direct injection of this transgene into a single cell embryo led to the development of amyloid plaques and a number of phenotypes characteristic of AD including cholinergic neuropathy [27], behavioural disturbances [28] and age related learning deficits [29] that may be explained by decreased hippocampal neurogenesis [30]. While loss of synaptic and dendritic proteins was seen, no neurofibrillary tangles could be detected [31], suggesting APP alone is not sufficient to produce neurodegeneration but that amyloid pathology may occur prior to tau pathology and frank neurodegeneration as outlined in the amyloid cascade hypothesis [32]. The elevated levels of soluble A $\beta$  generated in the PDAPP mouse model not only enter the plasma but also deposit into amyloid plaques thus revealing a plaque-associated disruption of the CSF and plasma A $\beta$  dynamic equilibrium [33]. This confirmed that peripheral sequestration of the elevated levels of A $\beta$  would modulate the deposits of amyloid plaque [34]. The PDAPP mouse model replicates key pathological features seen in AD and has clear utility as a pre-clinical model of the amyloidogenic elements of the disease.

Further key features of the transgene construction may include regulatory elements that repress and/or enhance transgene expression and a reporter gene to allow spatial tracking of the transgene expression. The minimal regulatory elements required for accurate transgene expression can be confirmed *in vitro*. This is done by inserting the transgene into an appropriate neuronal cell line and screening the cell line for expressed protein of interest. In this way the transgene can be engineered to include or exclude specific gene sequences that regulate the transgene expression to give the desired expression pattern. The reporter gene can be engineered as a fusion protein with the protein of interest, allowing tracking of endogenous transcriptional activity [35] and faithfully replicating spatial expression patterns. A problem with this approach is tagging a transgene with a reporter gene may

impact on the protein function and so independent reporter expression can be achieved with an Internal Ribosomal Entry Site (IRES) element. This DNA sequence recruits the nuclear ribosomal machinery to transcribe downstream DNA but is under control of a promoter sequence upstream in the transgene construct. In this situation two gene transcripts are produced from one promoter and so a reporter protein is produced in that will not interfere with the function of the protein of interest while enabling spatial expression patterns to be followed [36].

Many transgenic models of Alzheimer disease have been generated incorporating APP familial mutations that replicate key clinical features of the disease pathology. A limited number of genes that carry mutations linked to AD have been discovered, one of which is the presenilin gene [37]. Presenilin is a component protein of the  $\gamma$ -secretase enzyme which, along with the  $\beta$ -secretase enzyme, cleaves the APP protein to liberate the amyloid protein fragment A $\beta$  that aggregates to form the amyloid plaque lesions found in the brains of AD patients. Transgenic mice generated incorporating this mutated gene have a selective increase in brain A $\beta$ 42 but limited further AD related pathology [38]. However when APP and PS1 lines of familial Alzheimer Disease mutant mice are interbred a more aggressive model is generated where mutant PS1 acts to accelerate the AD phenotype [39]. An even more rapid acceleration of plaque development was revealed in a quintuple gene transgenic mouse model [40] showing multiple mutations as well as multiple transgenes can model the required clinical feature. These mouse models which carry multiple transgenes and/or mutations do provide useful laboratory tools to investigate AD pathology but cannot be considered to accurately reflect AD as these multiple gene mutations have not been discovered in the human patient population.

These simple and compound transgenic lines of mice all model one neuropathological lesion found in AD, namely the amyloid plaque. While some signs of neurodegeneration have been found in these models, frank neuronal cell loss and reduced brain volume were only successfully modelled when another key pathological lesion of AD, neurofibrillary tangles (NFTs), was generated by expressing mutated microtubule associated tau (MAPT or Tau) [41]. A triple transgenic mouse model linking the mutant APP, PS1 and Tau genes has been developed and was used to clarify the relationship between A $\beta$ , synaptic dysfunction and tangles providing a useful insight into these clinical features of AD and strong support for the amyloid cascade hypothesis [42] whereby amyloid pathology precedes Tau pathology NFT and subsequent neuronal loss.

Alzheimer's disease has several mutant genes linked to the disease pathology however no single mutant mouse model of these genes is able to reproduce the full disease phenotype. In contrast insertion of an expanded repeat CAG sequence (approximately 130 repeats) in Exon of 1 the human huntingtin gene plus part of the huntingtin promoter was sufficient to produce mouse model with a progressive neurological phenotype characteristic of Huntington's disease [43]. This R6/2 transgenic

mouse model of Huntington's disease is a good example of a monogenetic mouse model that reproduces most phenotypes of a neurological disorder. The major phenotypes in this model are a constant tremor that becomes progressively worse and is exacerbated by stress during basic husbandry. The mice also exhibit severe handling-induced epileptic seizures that can last for several minutes. Through open distribution of this model many researchers have been able to explore the phenotypes displayed and found, for example, deficits in striatal based but not hippocampal based learning [44] and impaired CA3 mossy fibre LTP may contribute to impaired spatial learning deficits [45].

The introduction of modified transgenes into a mouse genome allows for a multitude of mouse models to be generated. A powerful example of this technology is the solution found when researcher wanted to overcome the problem of the effect of genetic modification during development *in utero*. In the transgenic models described above, protein expression is under control of the selected promoter. Many promoters are active during the development of the embryo *in utero* and during post partum maturation. Most neurodegenerative disorders are observed later in life and so expression of the mutant transgene during early life may have undesirable effects on development. In addition the facility to switch transgene expression on and off allows for precise temporal control of the model phenotype. This issue has been resolved elegantly using tetracycline inducible expression systems [46]. There are a number of induction systems to control mouse transgene expression [47]. The tetracycline system has advantages over other systems as it is reversible and relatively tightly controlled following the development of tetracycline controlled transcriptional silencers to eliminate transgene leakage [48]. A diverse range of transgenes and transgenic models can be derived from this technology, an elegant example of which is the Tg4510 mouse model [49]. In this model two separate lines of mice are used to provide an activator protein in a specific tissue and a responder protein driving the gene of interest. The activator in this system is tetracycline, a broad spectrum antibiotic that prevents protein synthesis by inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex, first demonstrated in mammalian cells [50]. The activator mouse line has a transcriptional activator (TA) element comprising the 'tet-Off' gene coding sequence under control of the  $\alpha$ -calcium-calmodulin-dependent kinase II (CamK2) promoter. The second mouse line has expression of a cDNA encoding a P301L Tau mutation under the control of the tetracycline operon-responsive element (TRE) (figure 2a). When mated together the two single transgene lines combine to produce bigenic offspring whereby production of the TA element is in tissue specified by the CamK2 promoter (the hippocampus and cortex [51]) which initiates transcription of the mutant tau<sub>P301L</sub> transgene. Activation of the TRE leads to the production of mutant tau<sub>P301L</sub> protein (figure 2b) and subsequent associated pathology. Further control of this gene transcription in the Tg4510 bigenic mouse line is by exogenous dietary supplement of doxycycline. Administration of this semi synthetic tetracycline antibiotic in the diet of the mice acts to effectively 'switch off' mutant tau<sub>P301L</sub> transgene expression

when the dietary doxycycline binds to the TA element thus preventing binding to the TRE and subsequent mutant tau<sub>P301L</sub> protein production. This control is reversible when doxycycline is removed from the diet the TA element is not longer bound to doxycycline and is able to bind to the TRE thus initiating tau<sub>P301L</sub> transcription (figure 2c). The Tg4510 mouse model develops progressive age-related NFTs, neuronal loss, and behavioural impairments [52]. When doxycycline is introduced into the diet the latter two phenotypes are ameliorated, however NFTs continued to accumulate. Thus in the Tg4510 model of tauopathy, neurofibrillary tangles, a key lesion observed in AD, are not sufficient to cause cognitive decline or neuronal death [49,53]. This finding could have suggested a premier role for amyloid plaques in the memory loss and neurodegeneration seen in AD, however subsequent work on Tg4510 model has linked the pre-NFT accumulation of two oligomeric aggregates with the pathogenic cascade in this mouse model [54]. This exquisite control of gene expression allows for experimental modulation of the phenotype and has led to a clearer understanding of Tau related disease pathology.

Extending this simple idea, it follows that using this bigenic inducible mouse breeding strategy of separate activator and inducible responder mouse lines, numerous combinations are possible. When a characterised promoter providing expression in the desired tissues is used to drive expression of the TA gene and expression of a desired protein, with or without a disease linked mutation, is driven by the TRE then any protein can be expressed in any tissue for which appropriate promoters are available. An elegant combination of these technologies is demonstrated in Krt12rtTA/+/*tet*-O-LacZ mice which overexpress reporter genes in a corneal-epithelium-specific manner when induced by doxycycline [55]. Previous attempts to produce a corneal-epithelium specific mouse model were not successful so in this mouse model a gene-targeting construct containing an internal ribosomal entry site–reverse tetracycline transcription activator (IRES-rtTA) cassette was inserted into the Krt12 allele (keratin 12 gene) to produce a knock-in (see section 2c) Krt12rtTA/+ mouse line. The Krt12rtTA/+ knock-in mice were bred with *tet*-O-LacZ reporter mice to obtain Krt12rtTA/+/*tet*-O-LacZ bi-transgenic mice. The expression of the LacZ gene was induced in bi-transgenic mice by administration of doxycycline in the drinking water and chow. Validation of the desired expression pattern was demonstrated and the mouse model was employed to elucidate signalling pathways of various growth factors, cytokines and gene functions in the maintenance of homeostasis and pathogenesis in the adult mouse cornea. The model serves as an example of a genetically engineered mouse that is far removed from the wild type genome and yet is healthy and fertile and provides a valuable mouse model.

## 2b. Knockout of an endogenous gene

A gene of interest is effectively ‘knocked out’ by interfering with gene transcription leading to an inoperative protein. The formation of a completely inoperative protein is a key consideration when

designing a cloning strategy to knockout a gene of interest. To be most effective the intervening sequence (IVS) of DNA is inserted as close to the promoter as possible, preferable replacing Exon 1 of the genomic clone. In this way a truncated endogenous protein, which may have some partial function when translated, is less likely to be produced and thus lead to a partial gene knockout. Any sequence of DNA can be used to interfere with transcription of the exogenous gene locus but often a reporter gene is used as this allows the spatial distribution of the targeted gene to be followed. After the targeted gene has been appropriately engineered the entire locus is incorporated into the host genome of embryonic stem (ES) cells by homologous recombination. In order to increase the likelihood of a successful recombination approximately 2 kilobases of flanking genomic sequence is included on either side of the engineered DNA. Additional DNA sequences are used to allow positive and negative selection of ES cell clones. Examples of this may include insertion of the neomycin antibiotic resistance gene within the flanking genomic sequence as a positive selection marker and insertion of the gene encoding the Thymidine Kinase (TK) enzyme. These elements allow for positive selection of clones when the ES cells are treated with antibiotics, those that have successfully recombined to include the targeted gene will resist the antibiotic and survive due to the presence of the neomycin resistance gene but those that have not will die. Similarly those clones that have recombined at the correct place to include the targeted gene but exclude peripheral DNA sequences and the TK gene will live when treated with the antiviral pro-drug ganciclovir as it is phosphorylated by TK to produce toxic triphosphates which kill the ES cell clone.

Accurately targeted ES cell clones are cultured to a uniform colony of single cells which are collected into a hollow needle and injected into the blastocoel of a host mouse blastocyst (figure 1b) where they adhere to the inner cell mass and subsequently integrate into the body. Once recovered from the injection the blastocysts are inserted into the uterus of a recipient dam for gestation. If the coat colour of the recipient strain is different to that of the ES cell clones (for example black C57Bl/6 and agouti 129Sv) then when mature, the male offspring founder mice with approximately 50% black coat colour are mated to females to produce the first generation of mice. This percentage of coat colour minimises the contribution of the 129 background strain leading to weak, runt animals that will not breed well, while maximising the chances of selecting a male founder with germ line transmission. Tail tissue samples are collected from this first generation and analysed for the correct genotype. Mice possessing heterozygous copies of the gene are then interbred to generate homozygous mutant mice to enable full expression of the engineered gene locus.

Knockout technology is commonly used to evaluate the function of a gene of interest by removing it and testing the knockout mouse model in relevant assays. An obvious phenotype resulting from gene knockout is embryonic lethality. If the gene of interest is essential during mouse development then removing it will cause embryo fatality. This may be overcome by combining knockout technology and an inducible expression system. This was used to good effect when investigating Presenilin 1

(PS1) gene knockout mice. PS1 knockout mice die shortly after birth due to skeletal and CNS defects [56]. This prevented the study of PS1 knockout in adult mice so a conditional PS1 KO (cKO) mouse model was developed with PS1 inactivation restricted to the postnatal forebrain [57] by using Cre-Lox recombination technology. In this cKO mouse model, locus of crossover (loxP) sites were inserted either side of Exons 2 and 3 in one mouse line (fPS1) which was mated with a mouse line in which Cre (causes recombination) protein expression was driven by the CamK2 promoter. Thus *in utero* the PS1 locus was intact and the mice survived to term. Only when the CamK2 promoter became active, at approximately the third postnatal week, was the Cre protein expressed causing the loxP sites to recombine and excise Exons 2 and 3 of the PS1 locus, thus progressively removing PS1 protein expression.

## 2c. Knockin of exogenous DNA into an endogenous gene

Knockin (KI) mice are similar to knockout mice and are generated using identical technology. In knockout mouse models the intervening sequence (IVS) is irrelevant to the gene of interest however in KI mice the IVS is often relevant and is designed to be transcribed by the endogenous promoter. An example of such a model is the introduction of a mutation into the endogenous mouse genomic DNA sequence. The mutation is introduced using homologous recombination as described for knockout mice and is introduced into the host mouse genome using precisely the same techniques (figure 2b). An example of this technology is an  $APP^{NLh}/APP^{NLh}$  KI mouse model in which gene-targeting strategy was used to introduce the Swedish familial AD mutations and convert mouse A $\beta$  to the human sequence [58]. This strategy allowed the neuropathological consequences of human A $\beta$  overproduction to be evaluated longitudinally in the absence of potential mitigating effects of APP overexpression or presence of the mouse A $\beta$  peptide. A further example of this technique is a mutant leucine-rich repeat kinase 2 (LRRK2) KI mouse model. Inherited mutations in LRRK2 are a common genetic cause of Parkinson's disease and the R1441C mutation in LRRK2 has been identified in PD patients. The R1441C mutation was introduced into the murine LRRK2 locus by homologous recombination in ES cells. A positive selection element comprising the phosphoglycerine kinase promoter driving expression of the neomycin resistance gene (PGK-neo), flanked by LoxP sites (known as a floxed PGK-neo), was excised by transient transfection of Cre-expressing plasmid into the ES cells so as not to interfere with LRRK2<sup>R1441C</sup> expression. Positive ES cell clones were injected into host C57Bl/6 blastocysts and progeny tested for inclusion of the mutation. The LRRK2<sup>R1441C</sup> KI mice exhibited impaired dopaminergic neurotransmission which may represent pathogenic precursors preceding dopaminergic degeneration in PD patients [59]. Interestingly when transgenic (Tg) mice expressing LRRK2<sup>R1441G</sup> were generated using a mutated bacterial artificial chromosome (BAC) they developed a phenotype that recapitulated cardinal features of PD [60]. In contrast to the LRRK2<sup>R1441C</sup> KI mice, these mice had deficits in locomotion, dendritic degeneration of dopaminergic neurons and

tau phosphorylation. The authors attribute phenotypic differences in the two mutant R1441 LRRK2 mouse models to higher expression of mutant human LRRK2 and endogenous LRRK2 in the BAC Tg mice, background strain differences (B6/129 in KI mice and FVB in BAC Tg Mice) and the specific mutations used (R1441C and R1441G). KI mouse models often have utility in generating subtle, progressive phenotypes that may more accurately reflect the development of a chronic neurodegenerative human disorder.

This KI technology has also enabled the development of a new generation of mouse models in which a specified endogenous locus is targeted to allow expression of the gene of interest. This method allows for stable, single copy gene integration at a known locus. A bigenic mouse model of AD incorporating CAMK2 promoted APP<sub>swe/lon</sub> and Tau<sub>301L/406W</sub> mutant transgenes was generated by knocking into the HPRT locus. This model was crossed with a human PS1 transgenic mouse line to generate a trigenic mouse model named PLB1 [61]. The proposed advantage of such a model is that it provides a more subtle phenotype when compared to existing models generated by pronuclear injections or cross-breeding and the model is not hampered by transgenic procedure-related and disease-unrelated alterations.



### 3. Acquired mouse models

Mouse models expressing a phenotype that is acquired from direct manipulation and do not transmit the phenotype from generation to generation can be classified not by the heritable genotype they carry but by the phenotype they produce. The major advantage of these models is the broad nature of the phenotypes produced that are simply not possible to model using gene engineering. Progressive neurological disorders often have an impact on specific neuronal substrates at different stages of the disorder. Thus a clear understanding of the neuropathology and molecular mechanism underlying the disease to be modelled are essential when generating acquired mouse models of neurological disorders. This targeting primarily takes the form of direct interference with a specific CNS structure via a selective physical lesion or global interference with the CNS via systemic pharmacological administration.

#### 3a. Central injury models

The mouse brain is small when compared to other pre-clinical species however skilled neurosurgeons can target neuronal substrates in the mouse brain with a high level of accuracy and deliver models that reproduce particular aspects of a disease. Resources such as the Atlas of the Mouse Brain [62] or the Allen Mouse Brain Atlas [63] provide valuable neuro-anatomical maps making such precise lesions possible. It is important to note that, while different laboratories publish results using a particular lesion model, the exact method of inducing the model including the materials and substances used, the concentration, formulation, duration and route of administration of the toxin and the mouse strain used all have a bearing on the reproducibility of the model.

Excitotoxic lesions are derived from neuronal exposure to high concentrations of glutamate (or glutamate analogues such as NMDA, ibotenic acid) interacting with its selective membrane receptors. Glutamate receptors can be divided into either G protein coupled (metabotropic receptors) or cation channel coupled (ionotropic receptors) receptors. Ionotropic glutamate receptors can be further divided into three major types named after their selective agonists: N-methyl-D-aspartate (NMDA),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptors. Once activated, these receptors produce neurotoxicity by inducing a massive intracellular influx of  $\text{Ca}^{2+}$  which can activate a number of biochemical pathways leading to irreversible alterations and ultimately cell death [64]. Glutamate receptor ion channels are expressed throughout the mouse CNS as in all mammals [65]. While gene engineering has been used to manipulate the levels of glutamate receptors in vivo [66], the application of exogenous non-competitive antagonists of glutamate NMDA receptors, for example phencyclidine (PCP), ketamine and dizocilpine, has been used to change both human and animal behaviour and induce schizophrenia-like phenotypes [67]. Interestingly antagonism of NMDA

receptors (NMDAR) in a chronic, low dose PCP treatment regime in C57Bl/6 mice led to a consistent impairment in spatial learning and working memory without any apparent sensorimotor impairments [68]. This has been proposed as a mouse model of the cognitive deficits seen in schizophrenia patients, however as more is understood about the function of NMDAR in disturbed cognitive function then it may prove useful in modelling the expression of cognitive decline in other disorders.

Neurodegeneration is a key diagnostic feature of AD and while several neural networks contribute to the AD phenotype [69], the hippocampus and para-hippocampal formations are specifically implicated in short term memory both in AD and in normal aging [70]. As in transgenic mouse models that target overexpression of the A $\beta$  peptide to the hippocampus, a physical lesion of the structure would also be expected to lead to impaired memory function. This was found to be the case when selective memory deficits were found in different strains of mice with hippocampal lesions [71]. Such lesions are produced with stereotaxic surgery, whereby predefined coordinates from the Lambda and Bregma cranial suture intersections, and are used to orientate the mouse brain with respect to these visible skull landmarks. This allows very precise delivery of cytotoxic molecules to the specified brain region (figure 3a). Using this technology NMDA was delivered to the hippocampus of 12 week old mice (figure 3b) to generate a hippocampal lesion mouse model in which all of the hippocampus and dentate gyrus except the most ventral portions were removed. These mice had impaired spatial cognition but were spared on non-spatial tasks [72].

Intact NMDAR function is essential for spatial learning and memory and for hippocampal synaptic plasticity [73]. In addition altered NMDAR function has been linked to the pathways that underlie the A $\beta$  modulated disruption of long term potentiation in the hippocampus [74]. In aging, it has been suggested that it is NMDAR function, receptor subunit composition, and/or the environment in which the receptor interacts in the hippocampus which contribute to memory decline [75]. Based on the importance of the NMDAR in hippocampal function, mouse models in which NMDAR function is manipulated would be potential useful models of impaired memory. A comprehensive comparison of the behavioural effects of eight NMDAR antagonists in the rat found that the NMDA antagonists tested produced very diverse effects on the expression of instrumental action, from a basic ability to respond on one lever for reinforcement to the ability to respond conditionally on response alternatives associated with different reward probabilities. Other aspects of responding were left intact but based on these findings the authors suggest caution when using NMDARs to model psychoses such as those seen in schizophrenia [76].

### 3b. Peripheral injury models

Spatial learning is impaired following controlled cortical impact in the mouse as a model of traumatic brain injury [77]. The neurobiology underlying this model has a significant inflammatory component.

This brain inflammatory response has provided a mouse model of neuroinflammation useful for investigating the consequences of head trauma and stroke in humans [78]. Increased cytokine expression can be achieved in transgenic mouse models [79]. However the known limitations of developmental expression of such transgenes and the multitude of coincident CNS responses during ischemic brain degeneration [80] or cerebral trauma mean they are more comprehensively modelled in an acquired mouse model. Interestingly recent studies have indicated that the mediators of neuroinflammation following ischemic brain injury stimulate amyloid precursor protein metabolism by upregulation of  $\beta$ -secretase and therefore are able to establish a vicious cycle linking key components of AD with brain ischemia and neuroinflammation [81].

CNS inflammation and demyelination are seen in the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS). This model requires peripheral administration of myelin antigens emulsified in adjuvant to elicit a T-cell mediated immune response. It is characterised by CNS inflammation targeting the spinal cord leading paralysis, initially in the hind limbs and progressing to the forelimbs [82]. Different mouse strains and different antigen combinations can be used to elicit different levels of inflammation and demyelination. Systematic immuno- and histopathological comparisons of popular mouse EAE models on the C57Bl/6 background have been used to advance understanding of the complexity of MS. Specifically antigens comprising myelin basic protein-proteolipid protein (MBP-PLP) fusion protein (MP4)-, myelin oligodendrocyte glycoprotein (MOG) peptide 35-55- and PLP peptide 178-191 – induced onset of EAE with 6, 12 and 15 days respectively. The reason for these differences is unclear, though CD8+ T-cell could play an important role [83]. In addition to the immunological differences the behaviour of EAE mice has been studied to further understand the behavioural phenotypes seen in MS patients. When EAE was induced in SJL mice sickness behaviour symptoms including anorexia, loss of body weight, reduced social exploration, and decreased preference for sucrose solution were measured. These symptoms were most prevalent during the acute phase of the disease but abated in later phases and recovery of these symptoms preceded recovery from other neurological symptoms. It was suggested that these behavioural readouts could be used as a model of the symptoms of depression seen in MS patients [84].

### 3c. Locomotor and dyskinesia models

Parkinsonism can be induced in rodents by direct intracerebroventricular infusion of the dopaminergic/noradrenergic selective neurotoxin 6-hydroxydopamine (6-OHDA) into the substantia nigra [85]. While this model has been developed in mice [86], it has more commonly been used in rats to good effect when studying the locomotor disturbances seen in PD patients [87]. Interestingly infusion of 6-OHDA in neonatal rat pups generates a model of Lesch–Nyhan syndrome, an inherited

disorder caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase. The neurological symptoms are similar to those seen in Huntington's disease and include facial grimacing, involuntary writhing, and repetitive movements of the arms and legs. Another method of modelling the dopaminergic depletion seen in PD is to give a subcutaneous dose of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is a lipophilic molecule that crosses the blood brain barrier where it is metabolised to generate the neurotoxin 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>). MPP<sup>+</sup> acts by interfering with oxidative phosphorylation in mitochondria and selectively kills dopaminergic cells of the substantia nigra [88]. This preclinical model is also useful to assay potential treatments for PD.

Psychotic patients receiving long term neuroleptic treatment go on to develop Tardive Dyskinesia (TD) in 15-20% of cases. TD is characterised by repetitive involuntary facial movements that can persist after treatment has ceased. This oral dyskinesia can be modelled in the rat by subcutaneous treatment with the catecholamine depleting agent, reserpine [89]. Increased chewing, twitching and tongue protrusion are quantifiable features of the robust phenotype in this model and it is used to investigate the effects of potential therapies for Parkinson's disease as dopamine antagonists alleviate the TD seen in patients on chronic neuroleptic treatment and TD is indistinguishable for the L-Dopa induced dyskinesia seen in Parkinson's disease patients. Administration of the D2 antagonist spiroperidol dose dependently blocks the dyskinesia [89], mimicking the effect of L-dopa in PD patients. This model is used in mice to show that the atypical neuroleptic resperidone, successfully reversed reserpine or haloperidol induced oral-facial movements [90] in line with clinical studies in schizophrenic patients. Interestingly the  $\gamma$ -aminobutyric acid (GABA) B agonist Baclofen also abolished reserpine induced oral facial movements reinforcing the GABAergic involvement in hypofunction of this aspect of TD [91].

Further evidence of GABAergic involvement in oral-facial dyskinesias has been observed in the Huntington disease research [92]. Oral-facial dyskinesias were observed when rats were treated with the mitochondrial toxin 3-nitropropionic acid (3-NPA) which also produced a preferential degeneration of medium-sized spiny GABAergic neurons but spared interneurons and afferents, as observed in HD striatum. Huntington's disease (HD) is a good example of a disease which has been modelled in both an acquired mouse model and in a heritable mouse model. The R6/2 mouse model as described in section 3a has been generated to reproduce the pathological features of HD. 3-NPA is an irreversible suicide inhibitor of the mitochondrial enzyme succinate dehydrogenase (SDH). This enzyme forms part of the mitochondrial electron transport chain and 3-NPA acts to cause acute metabolic stress and subsequent cell death. Systemic administration of 3-NPA leads to localised striatal neurodegeneration with a preferential loss of (GABA)ergic spiny projection neurons mimicking features of HD [93]. Acevedo-Torres *et al* used Quantitative Polymerase Chain Reaction to compare mitochondrial damage in the striatum of 3-NPA treated animals with that of the R6/2 transgenic mouse line and found increased damage compared to nuclear DNA in both models [94].

The mechanisms underlying striatal vulnerability in HD are unknown [95], but these models serve as important examples of how heritable and non heritable approaches can synergise to help to understand a disease pathology.

### 3d. Models of Psychoses

In rodents attenuation of the startle response by Pre-Pulse Inhibition (PPI) is used as a model of sensory motor gating mechanisms in the brain. Human schizophrenic patients have an impaired PPI response and thus the rodent PPI model is often used to investigate the effects of potential novel therapeutics, often in combination with glutamatergic receptor blockade [96,97]. It has been shown that rat pups removed from their mother for 1 day at post natal day (PND) 6 or 9 had a reduced PPI response associated with hyperactivity of the dopaminergic system involving the hypothalamic-pituitary-adrenal (HPA) axis [98]. The disturbances in the HPA system, the dopamine system, hippocampus and long-term behavioural effects modelling deficits seen in mental patients led the authors to propose 24 hr maternal deprivation model to be a 'schizophrenia-like' neuro-developmental animal model [99]. This maternal separation model has been extended to mice where decreased anxiety, learning and memory dysfunction, deficits in behavioural flexibility as well as a 20% loss of neurons in the dentate gyrus were seen in PND 9 pups subjected to a single 24 hour maternal separation [100].

### 3e. Gene silencing by RNA interference

Rather than engineering an endogenous gene locus to lower or remove the expression of a particular gene of interest, protein expression from an intact gene can be prevented by interfering with the RNA generated from the transcribed DNA. During protein production, typically DNA is transcribed into a specific type of RNA that codes for the gene called messenger RNA (mRNA). This mRNA is then translated into a primary protein within ribosomes in the cell. This primary protein is subjected to a series of post-translational modifications to become a functional protein. RNA interference (RNAi) is a useful technique to provide a model in which the mRNA transcribed from the gene of interest is targeted with exogenous RNA administration. First demonstrated in *Caenorhabditis elegans* [101], RNAi works when exogenous double stranded RNA (dsRNA) is introduced into the host cells where it acts to destabilise or degrade targeted complementary mRNA, thereby effectively silencing targeted gene expression. One promising use for this technology is systematic large scale RNAi silencing of the genes in a molecular pathway of interest and examining the effect on downstream gene expression. Further work has identified micro RNAs (miRNAs) which regulate endogenous gene expression and in vertebrates are thought to comprise 1% of animal genes [102]. These miRNAs can regulate the expression of hundreds of RNAs and several have been shown to be associated with

different neurological disorders and even subtle changes in neuronal organisation can impact on cognitive and psychological functions [103]. As a method of manipulating gene expression this technology represents an advantage over engineering the endogenous genome as much of the work is completed *in vitro* and delivered to the adult mouse to generate the model. Extensive breeding and genotyping regimes are not required, though the manipulation is not carried through the germ line.

#### 4. The problem with mice

When describing mouse models the impact of the background strain on which the relevant model is generated is a key consideration. For acquired mouse models the choice of mouse strain is important though previous characterisation of different mouse strains can provide a guide for subsequent experiments. As an example four different mouse strains responded with very different behavioural responses and showed variable responses to CNS injury when Kainic Acid was infused into the CNS [104]. Interestingly, of those tested, only the FVB/N mouse strain showed apparent spatial learning and memory deficits following CNS lesion and yet, as the authors note, the FVB/N mouse strain is almost blind [105] making data generated from most visual based behavioural tasks un-interpretable for this strain. Even within a single mouse background, strain phenotypic differences can depend on the precise nature of the model. In evaluating 4 mouse models of Diabetic Neuropathy (DN), all on a C57Bl/6 background strain, different responses were recorded when diabetes was induced. Diabetes was confirmed in mouse models of streptozotocin (STZ)-induced diabetes, spontaneous type 1 diabetes [B6Ins2] and 2 models of spontaneous type 2 diabetes [B6-db/db, BKS-db/db] diabetes. Despite persistent hyperglycemia, the STZ-treated and B6Ins2 mice were resistant to the development of DN. In contrast DN developed in both type 2 diabetes models though the B6-db/db mice required an increased fat diet while the BKS-db/db mice developed severe DN and remained hyperglycemic on standard mouse chow [106]. In addition when different mouse strains were exposed to a middle cerebral artery occlusion, differences in susceptibility to cerebral ischemia were found to be related to different cerebral vascular anatomy in the wild type mouse lines tested [107]. Thus when a novel mouse strain is used to generate an acquired mouse model which may be subjected to behavioural characterisation, it is prudent to evaluate the physical attributes of the model using a platform phenotype assessment such as SHIRPA [108] which has shown clear strain differences in all of the levels of platform phenotyping [109].

Further considerations to be aware when embarking on a mouse model research effort include the health status of the mouse colony, husbandry practices, the environment in which the mice are maintained and the breeding history of the colony. Most animals suffer from infections at some time in their life however pathogenic infections can have a significant impact on mouse models, particularly chronic neurodegenerative models or those with a depressed immune response. While

biological research facilities aim to keep animals in top health conditions, outbreaks of infection do occur and the decision as to whether or not to cull existing mouse models is a difficult one, particularly heritable models where rederivation into a clean area of the facility can be very costly and have a major impact on project timelines. Quantifying the benefits of maintaining a clean colony is difficult however the Mary Lyon Centre (MLC) of the Medical Research Council (MRC) Harwell reported on the outcome of a 30-month programme to rederive 310 specific pathogen-free mouse strains. The MLC used embryo rederivation and hysterectomy performed in isolators to facilitate the containment and decontamination of two mouse hepatitis virus (MHV) infection outbreaks. Rederivation of the colony eliminated a number of viral, worm, protozoan and mite infection. The improved microbiological status yielded notable benefits for mouse health and welfare and the science at MRC Harwell. Among several improvements early weight loss associated with inflammatory bowel disease in a mouse model of Huntington's disease and early weight loss in male mice mutagenised with ENU were markedly reduced or eliminated [110]. Animal husbandry practices can combine with background strain to impact on such conditions as environment enrichment [111], noise levels [112] and while decreased stocking density may have a negative impact [113], increased stocking density may not be an issue [114]. Maintenance of colony records is standard husbandry practice in large mouse breeding establishments but often smaller breeding efforts do not keep adequate records. This is crucial in avoiding problems with maintaining the colony of a heritable mouse model as it is all too easy for genetic drift or inbreeding depression to influence to breeding performance of the colony [115]. The key is to be aware that the influence of these factors on the phenotype of mouse model under consideration and control for them appropriately.

## 5. Summary

Making mouse models of human neurological disorders incurs a significant investment of resources for any research organisation. The return on this investment is a deeper understanding of the disorder aetiology and/or a preclinical model that is effective in predicting a clinical outcome for a given therapeutic agent. Pre-clinical models of disease need to be readily available, robust and develop the desired phenotype relatively rapidly. Chronic mouse models, particularly of the cognitive deficits observed in neurodegenerative disorders, may take several months or years even, to display a robust phenotype. As such, when considered in a pharmaceutical industry setting, these chronic models are simply impractical for use in screening the efficacy of potential therapeutics. Thus mouse models with a rapidly expressed phenotype are more suitable as pre-clinical models and popular models are those with non-behavioural readouts in genetically altered mouse models or acute responses in acquired mouse models.

The extensive library of mutant mouse models will continue to expand as new molecules linked to specific disease are discovered and ever more elegant methods of controlling elevated or depleted gene expression are developed. As with any good research the key requirement of adequate experimental controls will become more relevant as more laboratories access this powerful technology to generate their own models and the potential for false positive results is increased. Conversely as multiple research centres conduct experiments on existing mouse models and provide further validation with positive data derived from different research institutes, then the model in question gains in trust among investigators as a compelling model of the relevant disease phenotype. Similarly as the basic neurobiology of disparate neurological disorders is revealed common molecular pathways are likely to become clearer. Once initiated by a diagnostic lesion, many neurological disorders have failures in common transmitter neuronal systems that are amenable to testing in acquired mouse models.

Mouse models of neurological disorders are produced because they are amenable to treatments with potential therapeutic agents. As heritable mouse models more accurately reproduce the specific lesions seen in a wide variety of diseases, they are more relevant in a research effort employing a disease modification strategy. Acquired mouse models are often not designed to reproduce neurological lesions but model failures in the underlying neurotransmitter systems concurrent in a range of disease. As such they are more applicable for use in research efforts targeting symptomatic therapies. The challenge to mouse modellers is to specify appropriate background strains and controlled environments in which to test their favourite model and to publish both the negative and positive results of such specifications.



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**Figure 1.** Methods of generating genetically modified mouse models. **a)** Schematic representation of three types of transgene construct used to generate transgenic mouse models by microinjection into the male pronucleus of a host single cell embryo. The dotted line represents expansion of the pronucleus on injection of the construct. i) GOI + reporter fusion protein construct, ii) simple GOI construct, iii) GOI and reporter generated independently using an IRES element. **b)** Schematic representation of the steps used to generate knockout or knockin mouse models. The endogenous GOI is disrupted with an exogenous IVS, incorporated into the genome of an embryonic stem cell by HR and appropriately targeted clones are injected into the blastocoel of a host blastocyst where they adhere to the inner cell mass and are incorporated into the soma.

Abbreviations: GOI: Gene Of Interest; IRES: Internal Ribosomal Entry Site; I-P: Isolate and Purify; FS: Flanking Sequence; HR: Homologous Recombination; Sel<sup>n</sup>: Selection with positive and negative markers; IVS: Intervening Sequence; ES Cells: Embryonic Stem Cells

**Figure 2.** Schematic representation of steps required to generate a bigenic mouse model using the tetracycline ‘off’ controlled expression system. **a)** Separate mouse lines carrying activator and responder elements. **b)** GOI switched on. Bigenic mouse model in which protein generated by expression of the TA binds to the TRE leading to transcription of the GOI. **c)** GOI switched off. Exogenous doxycycline binds to the TA stopping transcription of the GOI.

Abbreviations: TA: Transcriptional Activator; GOI: Gene Of Interest; TRE: Tetracycline Responsive Element; Dox: Doxycycline;

**Figure 3.** Representation of key structures used to generate acquired mouse models. **a)** Schematic diagram of a mouse skull showing a plan view of the external orientating features of Lambda and Bregma in context of the eye sockets and sagittal suture. **b)** Schematic diagram of the mouse brain showing a transverse section through the brain to illustrate key brain areas and the needle positioning required to deliver a toxic insult to the hippocampus. Dotted line indicates the approximate location of the transverse section of the brain represented in 3b. Figures reproduced with permission from The Mouse Brain in Stereotaxic Coordinates, Franklin & Paxinos, 3<sup>rd</sup> Edition, page IX and plate 48. (C) Elsevier 2008.

Figure 1a

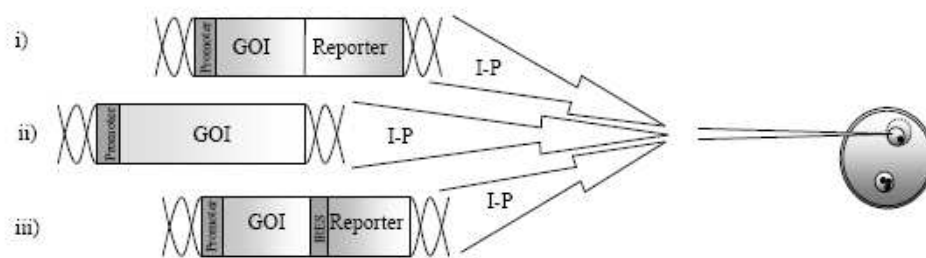


Figure 1b

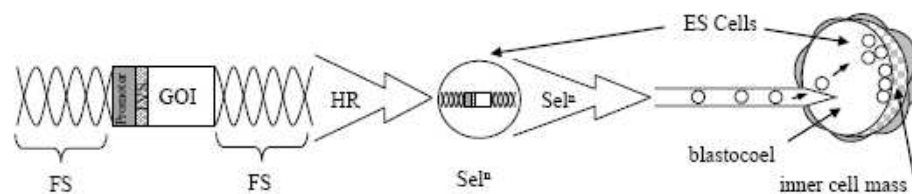


Figure 2a

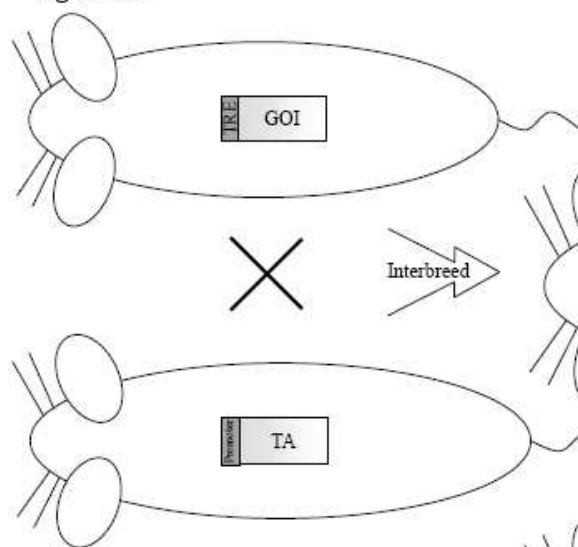


Figure 2b

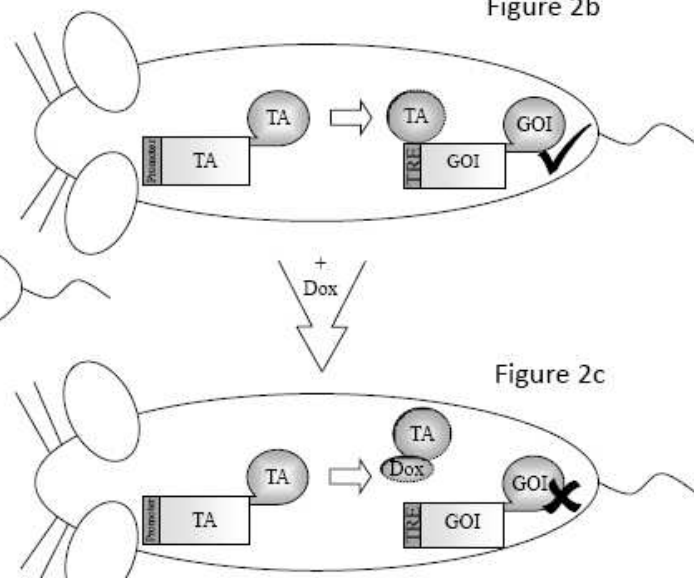


Figure 2c

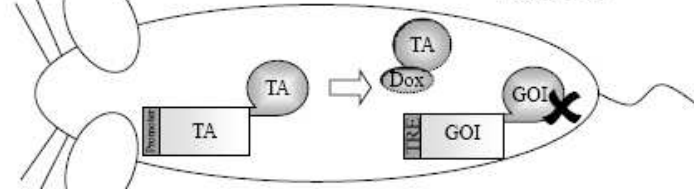


Figure 3a

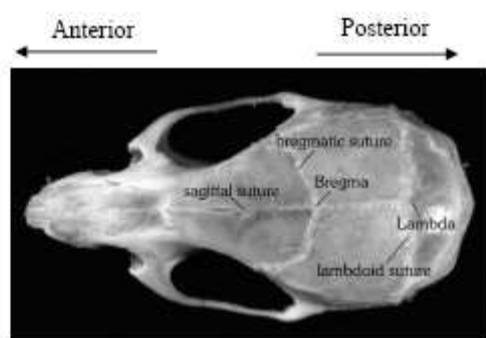


Figure 3b

