Human Molecular Genetics, 2012, Vol. 21, Review Issue 1 ***R72–R81***
doi:10.1093/hmg/dds318
Advance Access published on August 13, 2012

New insights into behaviour using mouse ENU mutagenesis

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Received July 21, 2012; Revised July 21, 2012; Accepted July 30, 2012

Identifying genes involved in behavioural disorders in man is a challenge as the cause is often multigenic and the phenotype is modulated by environmental cues. Mouse mutants are a valuable tool for identifying novel pathways underlying specific neurological phenotypes and exploring the influence both genetic and non-genetic factors. Many human variants causing behavioural disorders are not gene deletions but changes in levels of expression or activity of a gene product; consequently, large-scale mouse ENU mutagenesis has the advantage over the study of null mutants in that it generates a range of point mutations that frequently mirror the subtlety and heterogeneity of human genetic lesions. ENU mutants have provided novel and clinically relevant functional information on genes that influence many aspects of mammalian behaviour, from neuropsychiatric endophenotypes to circadian rhythms. This review will highlight some of the most important findings that have been made using this method in several key areas of neurological disease research.

INTRODUCTION

Neurological disease is one of the major burdens to healthcare, and in the case of neurodegenerative disorders, the incidence will undoubtedly rise with increased life expectancy. Consequently, there has been a concerted effort to understand the genetic basis of neurological disease, particularly in the post-genome era in which large population studies are highlighting new susceptibility loci for further study (1,2). The mouse has played a key role in discerning the normal function of genes in the nervous system but also the consequences of their disruption in human disease (3,4). Mice have a range of advantages as a model system in neuroscience, including their ability to perform behavioural tasks and respond to pharmacological challenges that can be extrapolated directly to human phenotypic traits (5,6).

The generation of mouse knockouts has been a commonly used approach to study gene disruption in the context of a whole organism. Recent years have seen the initiation of an internationally coordinated programme to create ES cells carrying conditional null alleles for all protein-coding genes, and this will be a valuable resource for the research community (7,8). However, in the majority of neurological disorders, a homozygous loss-of-function mutation does not occur and therefore studying a gene knockout may produce a far more severe phenotype or initiate compensatory mechanisms that are not wholly relevant to the disease in question (9). Indeed, a large proportion of human disease is caused by mutations that influence protein structure, binding affinity or function in far subtler manner than complete inactivation (10,11). In addition, it is becoming apparent that genetic variants such as single nucleotide polymorphisms (SNPs) outside of protein-coding regions are likely to play an important role in disease (1,2). Alternative mouse models are therefore vital, and random mutagenesis has become a well-established tool to elucidate both gene function and characterize novel disease pathways (12).

Several large-scale mutagenesis programs were established in the late 1990s as a systematic approach to generating new mouse models of human disease (13–15). The various strategies are reviewed extensively elsewhere (16,17), but briefly, male mice are treated with a controlled dose of *N*-ethyl-*N*-nitrosourea (ENU) that causes random point mutations to occur in their germline. Mutagenized males are then bred to wild-type females over one or two generations to generate mutant animals for analysis (16). In a phenotype-driven approach, the progeny is screened initially for abnormalities using a simple yet quantitative assessment of physiology and

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behaviour (18,19). This is followed by further analysis of specific traits or body systems of interest, as discussed in more detail below (14,15,20). Inheritance testing of the selected phenotype is then confirmed prior to positional cloning of the mutation itself. One advantage of this random mutagenesis approach is it can create a range of mutations, including hypomorphic (reduced amount of gene product), hypermorphic (increased amount of gene product) and neomorphic (altered function) alleles, in addition to those that are loss of function, such as the replacement of a coding amino acid with a premature stop codon. The mutation may also be specific for a particular isoform of the gene, which may have important functional consequences. Furthermore, the unbiased nature of the phenotyping process and the pleiotropic nature of mutations mean that novel functions can be assigned for genes that had previously only been studied using a restricted set of experiments in the knockout mouse (see below). The major bottle-neck of the phenotype-driven approach used to be the identification of the causative mutation based on genetic mapping followed by sequencing of candidate genes. With the advent of affordable next-generation sequencing technology, this notoriously laborious process can now be completed far more efficiently (21–23).

Phenotype-driven screening will not detect every potentially interesting mutant and is limited by the sensitivity of the screens themselves (24,25). Neuropathological analysis, although a key feature of the phenotyping process, may also be limited to specific tissues or histopathological methods (26). Nevertheless, cryopreservation of tissues from each mutant line allows retrospective screens to be carried out for mutations in a gene of interest; advances in the rapid detection of mutations have made it practical to use this gene-driven approach (27,28). Although there is no guarantee of any measureable phenotype in the resulting mutant, it has been calculated from pilot studies that 5000 DNA samples are sufficient to identify at least two alleles with 90% confidence (29), and ENU archives much larger than this are now available for screening (28,30). Furthermore, the fact that many thousands of mutants are generated means that multiple individual mice may contain mutations in the same gene, an allelic series (31). This facilitates detailed comparative genotype–phenotype correlations to be carried out on mice of the same genetic background which is an important consideration, particularly in behavioural neuroscience (6,32).

BEHAVIOURAL SCREENING

A number of approaches have been taken to quantify behaviour in phenotype-driven mutagenesis screens, and the major large-scale ENU programmes have focussed on a range of neurobehavioural domains for their primary screening protocols (14,15,20). Typically, high-throughput tests such as the open-field are employed as they are both rapid and electronically quantifiable. Advantageously, this test provides preliminary data on a number of parameters, including general activity in a novel environment as well as a measure of anxiety based on thigmotaxis (33). Yet even such deceptively ‘simple’ tests rely on consistency between those handling the animals and data interpretation to generate scientifically meaningful results (34,35). This is paramount when the performance of a single animal compared with a control data set may define a new mutation of importance; thus, care must be taken to define standardized protocols (32,36–38). Nonetheless, even tests that are used to model aspects of neuropsychiatric disorders can be amenable to a high-throughput screen, such as sensorimotor gating or pre-pulse inhibition (PPI). This particular paradigm quantifies the ability to respond to and filter auditory information, and is one parameter that can be analysed in mice as an endophenotype; the approximation of a similar response known to be abnormal in schizophrenia (5). Consequently, PPI and open field, along with rotarod testing for motor function and hotplate for nociception, make up the primary screen of several phenotyping pipelines (25,37,39,40).

An outlier identified from a high-throughput screen is usually taken through a more focussed and/or complementary series of secondary behavioural tests to more accurately define the phenotype of potential interest. For example, inactivity in the open field may be a consequence of anxiety or simply due to motor dysfunction, arousal or sensory defects (6). Additional physiological parameters outside of the nervous system must also be taken into account when interpreting such data; an overtly obese mouse, or one with cardiovascular defects may struggle to run on a rotarod, for instance (41,42). Another issue to consider is the statistical power of the primary screen used to isolate outliers for follow-up studies (20). Recent work based on the open-field test has demonstrated that subtler phenotypes can be reliably identified by screening an additional second generation (G2) of mutant animals. Although this entails the additional time and cost of more breeding, it was argued that the false discovery rate was dramatically reduced (43).

Despite these caveats, a considerable number of neurological ENU mutants have been successfully mapped and cloned, revealing important new insights into gene function in the nervous system (16,44). The inherent complexity of the molecular mechanisms that define the function of the brain means that many ENU mutants with abnormal neurological phenotypes are yet to be characterized. Importantly, primary screening data are usually publically available from the major centres allowing searches to be made for phenotypes of interest [see Box 1 in Gondo *et al.* (28)]. Table 1 contains a selection of the more recently identified neurological ENU mutants, and some of those that have been studied in some detail are discussed below, focussing on three key areas of neurological disease research.

MOVEMENT DISORDERS AND NEURODEGENERATION

Historically, spontaneous mouse mutants were identified during the maintenance of large breeding colonies. As a consequence, phenotypes such as ataxia represent many of the first lines to be cloned as such characteristics are particularly conspicuous (45,46). The same is true to some extent of phenotype-driven mutagenesis, where there will often be a bias towards movement defects that are both early onset and easy to recognize. For example, three independent ENU mutants in the peripheral myelin protein 22 (*Pmp22*) gene

Table 1. Examples of neurobehavioural ENU mouse mutants (2007–)

Mutant gene name	Mutation(s) studied	Phenotypic effects	References
Af4	V280A/+	Ataxia, cataracts, T cell development defects, adult-onset Purkinje cell death	(52,55,57)
Arcn1	I422T/I422T	Coat colour dilution, ataxia, Purkinje cell death	(128)
Atp1α3	I810N/+	Small size, seizures, elevated exploratory locomotion, sleep abnormalities	(85,88)
Cacna1a	R1255L/+	Ataxia, cerebellar atrophy	(129)
Cacna1a	R1255L/R1255L	Severe ataxia, premature death	
Disc1	L100P/+	PPI, latent inhibition defects	(71)
Disc1	L100P/L100P	PPI, working memory, latent inhibition defects, hyperactivity	
Disc1	Q31L/+	PPI defects	
Disc1	Q31L/Q31L	PPI, working memory, latent inhibition defects, depressive-like behaviour	
Disc1	Q31L/L100P	PPI defects	
Fbxl3	C358S/+	Lengthened circadian period	(102,109)
	C358/C358	Lengthened circadian period, reduced anxiety, depression-like behaviour	
Foxp2	R552H/+	Normal development, defects in motor learning	(130)
Foxp2	R552H/R552H	Severe motor impairment, developmental delay, cerebellar foliation defects, 3–4-week survival	
Foxp2	S321X/S321X	Severe motor impairment, developmental delay, cerebellar foliation defects, 3–4-week survival	
Foxp2	N549K/N549K	Late-onset cerebellar foliation defects, 3–5-month survival	
Foxc1	F107L/F107L	Cortical and skull abnormalities	(131)
Gars	C201R/+	Reduced grip strength, reduction in axonal diameter	(63)
Gars	C201R/C201R	Neurodeveopmental delay, 3-week survival	
Grin1	C844R/+	Hyperactivity, increased novelty seeking	(132)
Npc1	D1005G/+	Abnormal cholesterol metabolism, Purkinje cell loss, late-onset ataxia	(126)
ROR-alpha	C257X/C257X	Ataxia, severe cerebellar atrophy	(133)
Rtn4	R189H/R189H	Greater social preference, subtle cognitive defects	(134)
Snap-25b	I67T/+	Subtle ataxia, PPI defects, advanced circadian phase	(76,106)
Trpc3	T635A/+	Ataxia, late-onset Purkinje cell death	(59)
Jenna / Tuba1a	S140G/+	Hyperactivity, increased acoustic startle, neuronal migration defects	(135–137)
Tuba1a	D85G/+	Hyperactivity, neuronal migration defects	(138)

were some of the first to be cloned and characterized from the mutagenesis screen at MRC Harwell (14). Initially identified due to their resting tremor, these models of human peripheral neuropathy provided an excellent example of the range of phenotypic and pathological severity that can be generated by an allelic series of point mutants (47–49).

Ataxia or defects in motor co-ordination are features of many neurological disorders and are typically characterized by neurodegeneration in the cerebellum, the structure in the brain necessary for controlling motor function. Although the genetic cause for a considerable proportion of inherited ataxias is known, the pathogenic mechanisms involved are still unclear (50,51). As part of a screen to identify new models of human ataxia, the dominant ENU mutant *robotic* (*Rob*) was selected due to its jerky, ataxic gait that is apparent from as early as 3 weeks of age. Pathological analysis revealed a progressive yet highly specific loss of cerebellar Purkinje cells that begin several weeks after the onset of the ataxic gait (52). The causative mutation was subsequently identified in the gene *Af4* (ALL fused from chromosome 4, also known as *Aff1*) that at that time was known to be a putative transcriptional activator implicated in leukaemia (53). The mouse knockout of *Af4* has defects in B- and T-cell development consistent with a role in leukaemogensis, but no neurological abnormalities were reported (54); consequently, the *Rob* mouse revealed an important new function for *Af4* in the CNS that was not apparent from the null allele. Biochemical data demonstrated that the mutant protein accumulated in *Rob* Purkinje cells due to the loss of a conserved ubiqutin ligase-binding site, and that this increase in Af4 protein levels was detrimental to Purkinje cell survival (55). Understanding the proposed gain-of-function mechanism for the *Rob* mutation required detailed examination of the role of Af4 in transcriptional regulation. First, it was discovered that Af4 and related proteins are part of a large complex co-ordinating RNA polymerase II processivity and chromatin remodelling via histone methylation (56). Subsequently, transcriptional profiling of Purkinje cells from wild-type and *Rob* mice identified IGF-1 as target of the Af4-containing complex and revealed a pathogenic pathway in the mutant cerebellum (57). Significantly, dysfunction of the IGF-1 pathway is a recurrent finding in mouse ataxic mutants and human ataxia patients and thus represents a common neuropathological mechanism that may be targeted for treatment (58).

Moonwaker (*Mwk*), a second dominant ataxic mutant characterized from the same ENU screen, also displays gait defects around weaning age, although Purkinje cell loss in this case is later in onset from around 6 months of age (59). The causative mutation was identified in the *Trpc3* gene, encoding the transient receptor potential cation channel C3 (TRPC3). The point mutation occurs in a highly conserved cytoplasmic linker region of TRPC3, resulting in altered gating and increased activity of the channel as a gain-of-function mechanism (59). Significantly, Purkinje cell morphology is impaired in the *Mwk* mouse, which is consistent with the highly specific expression of *Trpc3* in Purkinje cells during their dendritic development. Genetic ablation of this channel in the mouse also causes gait defects (60); however, the *Mwk* ENU mutant defines a novel role for TRPC3 in both the development and survival of Purkinje cells. Attempts have since been made to identify human *TRPC3* mutations in ataxia. Although no proven causative mutations have been described to

date (61), a polymorphism influencing the methylation of *TRPC3* was reported in idiopathic ataxia, although its frequency did not reach the significance in the population analysed (62).

Phenotypic ENU screens are also able to identify more subtle motor abnormalities. For example, a mouse originally identified due to its reduced grip strength contains a mutation in glycyl t-RNA synthetase or GARS (63). *Gars*$^{C201R/+}$ heterozygous mutants show a reduction in the axon diameter of peripheral nerves and some muscle denervation, but no axonal degeneration. *Gars*$^{201R/201R}$ homozygous mutants, however, only survive for a few weeks with major defects in the CNS observed. Importantly, this gene is mutated in Charcot-Marie-Tooth type 2D (CMT2D) disease, a form of dominantly inherited motor neuropathy. The relatively mild phenotype in *Gars*$^{201R/+}$ mice differs from the more severe *Gars*^{Nmf249} dominant mutant that also contains a point mutation (P279K) in the same gene (64). These mice display an early-onset loss of peripheral nerve axons and abnormal morphology of the neuromuscular junction. Interestingly, a gene-trap insertional mutant—that is essentially a complete gene knockout—shows no dominant phenotype, suggesting that haploinsufficiency is not the cause of neuropathy in the two-point mutants (64). To investigate this further, both point mutants were crossed with transgenic lines over-expressing either wild-type or mutant forms of *Gars.* These studies demonstrated that the dominant neuropathy phenotype observed in *Gars*$^{201R/+}$ and *Gars*$^{Nmf249/+}$ mice is caused predominantly by a dose-dependent gain of function that is not alleviated by over-expression of the wild-type protein (65). Considering that dominant mutations in three other tRNA synthetase genes cause neuropathies similar in pathology to CMT2D, these mouse lines will continue to be valuable models of the heterogenous nature of these disorders.

NEUROPSYCHIATRIC DISEASE

The significant genetic component of psychiatric disorders is well-documented and many susceptibility genes are conserved in rodents, although it is accepted that examining specific symptoms related to emotions or psychotic behaviour cannot be realistically 'modelled' in the mouse (66,67). Therefore, research has relied on the aforementioned endophenotypes to analyse specific pathophysiological features of psychiatric disease by studying mutations in genes directly associated with a particular disorder, relevant neurobiological processes such as neurotransmission, or behavioural responses to pharmacological agents known to modulate particular symptoms (6,67–69). Perhaps a reflection of the complex aetiology of psychiatric disease and interpretation of behavioural assays in mice, relatively few ENU mutants have been characterized in this area; however, some important examples have been identified.

Of all the genes implicated in schizophrenia, Disrupted-in-Schizophrenia 1 (*DISC1*) is one of the most prominent, with both the *DISC1* locus and polymorphic variants in the *DISC1* gene associated with the disorder (70). This gene was therefore selected for gene-driven screening in a mouse ENU mutant archive, in which two missense mutations were identified (71). Interestingly, both lines showed a range of behavioural deficits although few were shared; the Disc1-Q31L homozygous mutant displayed depressive-like behaviour, whereas the Disc1-L100P homozygous line showed abnormalities related to schizophrenia, such as PPI and cognitive defects. These schizophrenic endophenotypes were reversible with antipsychotic treatment (71) as well as an inhibitor of GSK3, a known signalling molecule that interacts with Disc1 during neurodevelopment (72). Detailed pathological studies have since identified morphological defects in the dendrites of L100P cortical neurons (73). These two mutants show how point mutations in a single gene can cause a heterogeneous behavioural profile. As a caveat to this work, an independent group has been unable to reproduce either the depressive or sensorimotor gating abnormalities in these mutants (74); this could be explained by the additional generations of backcrossing to the wild-type (C57BL/6) strain carried out prior to testing or differences in the behavioural protocols, for example (75).

The dominant *blind-drunk* (*Bdr*) mutant was originally identified due to its subtle gait disturbance and positional cloning isolated a missense mutation in the neuronal isoform of SNAP-25, part of the core SNARE complex essential for exocytosis (76). The mutation causes increased binding affinities within the SNARE complex and disrupted exocytotic vesicle recycling *in vivo*, limiting neurotransmission under continued stimulation (76). Behavioural testing of these mice showed no overt cognitive deficits, but an impaired PPI response. Importantly, the study of SNARE proteins has been limited by the neonatal lethality of knockout lines (77,78); *Bdr* therefore provides valuable new insights into the role of the SNARE complex and aberrant neurotransmission relevant to psychiatric disease due to the relatively subtle nature of the mutation. Furthermore, SNAP-25 itself has been implicated in schizophrenia from a range of genetic (79), pathological (80,81) and functional (82) studies of the synapse.

Bipolar disorder shares some overlapping symptoms with schizophrenia as part of a spectrum of psychotic disorders, although it is associated more readily with both manic and depressive episodes. Many genes have been implicated in bipolar disorder, although similar to schizophrenia, it is unlikely that a single genetic factor will be causative (83,84). In a phenotype-driven screen, the *Myshkin* (*Myk*) dominant mutant was selected due to its small size in addition to the presence of spontaneous, recurrent convulsive seizures and was originally presented as a model of epilepsy (85). A missense mutation in the Na^+,K^+-ATPase (NKA) α3 isoform (*Atp1a3*) caused an approximate 40% reduction of the enzyme activity in the *Myk* brain; this gene has been implicated both in bipolar disorder and rapid-onset dystonia parkinsonism (86,87). When examined in more detail, the behavioural phenotype in *Myk* mice also included highly elevated exploratory locomotion and sleep abnormalities in addition to enhanced sensitivity to D-amphetamine; significantly, these mania-related behaviours could be attenuated with mood stabilizers such as lithium (88). Studies of primary neurons suggested that enhanced calcium-dependent signal-transduction pathways downstream of NKA may underlie the behavioural phenotypes and are

potential therapeutic targets (88). *Myk* mice therefore provide important insights into neuronal signalling in mania, particularly as epilepsy and bipolar disorder can be co-morbid in humans (89).

Non-genetic influences are undoubtedly a major risk factor in psychiatric disease, ranging from prenatal stress to adverse life events during adolescence (90,91). In recent years, increasing attention has been paid to how best to incorporate such elements into behavioural experiments in mice to understand the synergy between genes and the environment while potentially improving the construct validity of such models (92–94). Building on work in wild-type rodents, there is now a range of gene × environment paradigms being applied to mutant mice, and ENU lines have provided useful examples of these methods.

To examine the significance of early life events combined with a synaptic mutation, a variable prenatal stress protocol was applied to the *Bdr Snap-25* mutant and the offspring were assessed for their behaviour as adults (95). Significantly, the sensorimotor gating (PPI) deficits observed in non-stressed mutants were markedly enhanced by stress *in utero* and social interaction abnormalities were observed only in *Bdr* animals from stressed dams (95). These results showed for the first time that combining a synaptic mouse point mutant with a prenatal stressor paradigm produces both modified and previously unseen phenotypes relevant to the study of psychiatric disorders. Disc1-L100P and -Q31L mutants have also been further studied in the context of a postnatal stress paradigm. Chronic social defeat (CSD), a correlate of psychosocial stress in humans, was applied to both heterozygous mutant lines and, interestingly, each responded differently to the CSD stressor (96). Measures of anxiety were influenced by CSD in the Q31L line, whereas social interaction was affected in the L100P mutant (96). The exact pathophysiological mechanisms that underlie such gene × environment interactions are still unclear, but these studies serve to illustrate that ENU mutants with subtle behavioural defects are highly suited for assessing this phenomenon.

CIRCADIAN BEHAVIOUR

Circadian rhythms are of vital importance to mammalian physiology, and as a consequence, disruption of these daily cycles can have profound effects on human health. Indeed, studies are beginning to define a direct association between disrupted rest/activity patterns and an increasing number of neurological disorders, including psychiatric and neurodegenerative disease (97–99). In the mouse, there are well-established methods for analysing aspects of circadian behaviour, predominantly based around quantifying wheel-running activity under differential lighting conditions (100). Screening ENU mutants using these methods, although not as high-throughput as other behavioural screens, has been particularly successful; a considerable number of lines displaying abnormally long or short active periods or aberrant responses to shifts in the light/dark cycle have been identified (101). Of those that have been characterized at a mechanistic level, the *Afterhours* (*Afh*) mutant displayed considerably lengthened activity profiles under constant lighting conditions, with a free-running period of approximately 27 h (102,103). *Afh* contains a loss-of-function mutation in the E3 ubiquitin ligase FBXL3, causing a reduction in the normal degradation of cryptochrome (Cry) proteins. A post-translational feedback loop containing Cry and period (Per) is essential for normal pacemaking in the suprachiasmatic nucleus (SCN), and it was shown that the imbalance of normal Cry levels resulted in an extended period of the core clock in these mutants (102,103). By crossing the *Afh* line to a casein kinase (*Csnk1e*) mutant that causes destabilization of Per, it was recently shown that the circadian period is finely controlled by both Cry and Per proteins independently as opposed to the mutual stabilization model predicted by *in vitro* experiments (104).

Although the mechanisms that link disturbed sleep/wake profiles and psychiatric disease are unclear, it is intuitive that the multiple brain regions disrupted in conditions such as schizophrenia are likely to overlap with those involved in the complex processes of sleep and the maintenance of circadian rhythms (105). In view of the schizophrenic endophenotypes in the *Bdr* mouse (see above), this mutant line was also screened for circadian activity (106). The behavioural data showed an advanced phase of activity under light/dark cycles, but also fragmentation of the profiles, somewhat reminiscent of the disturbed sleep/wake patterns observed in schizophrenia (107). Interestingly, the timing of activity was normal under constant light or dark, suggesting that the core clock in *Bdr* was functioning correctly. This was confirmed by molecular analysis of the SCN, although expression of the signalling neuropeptide arginine vasopressin (Avp) was phase advanced in line with the activity data. These results suggest that disrupted neurotransmission in the SCN due to the *Bdr* Snap-25 mutation influences important hypothalamic outputs, potentially causing desynchrony between the brain and the periphery (106). Due to the undoubted anatomical overlap between complex behavioural domains, it is also becoming clear that mouse mutants originally identified from circadian screens are likely to show additional behavioural abnormalities (108); indeed, homozygous *Afh* mutants also show hyperactivity, reduced anxiety and depression-like behaviour, suggesting this mutant may also model some aspects of mania related to disrupted circadian rhythms (109).

FUTURE PROSPECTS

The examples above illustrate the complex and varied behavioural phenotypes that can be generated by either phenotype- or genotype-driven ENU mutagenesis. Another practical application of ENU mutants is for identifying new loci that either suppress or enhance a disease phenotype of interest, also known as a modifier screen. Successfully utilized in lower organisms (110), this method is beginning to be applied in the mouse (111). This strategy may be particularly suited to neurodegenerative disease models, where performance in a simple motor-coordination task would provide the baseline data and used to identify outliers in a genetic cross with a population of random mutants (16). In particular, the identification of a suppressor mutation may help identify new therapeutic targets or disease mechanisms. A prime example of this phenomenon using a single ENU mutant line is the

Legs-at-odd-angles (*Loa*) mouse that contains a mutation in the cytoplasmic dyenin heavy chain (*Dync1h1*) (112). While crossing *Loa* mice to model of Huntington's disease enhances the pathological phenotype by slowing clearance of Huntingtin protein (113), the neurodegenerative phenotype of the SODG93A model of amyotrophic lateral sclerosis (ALS) is significantly suppressed (114). Preliminary data from an ENU screen for modifiers of K3 mice, a model of Alzheimer's disease (AD), have recently been described (115). This model expresses mutant tau protein and has been well studied for pathological and behavioural abnormalities (116); consequently, a simple battery of motor tests could be used to define the K3 phenotype. A number of mutant pedigrees, including one that partially rescued the tremor observed in K3 mice, were described, although the identification of this potential suppressor mutation or the analysis of additional cognitive phenotypes was not reported (115). Such studies therefore rely on a robust and quantifiable phenotypic assay for the disease model in the first instance; however, this is a potentially very valuable and unbiased approach to understanding how in the interaction between genes can influence pathogenic pathways.

While detailed optimization and standardization of ENU mutant phenotyping is undoubtedly important, due to obvious practical and financial constraints, primary screening is typically carried out on adult mice between 8 and 18 weeks of age (25,37). As a consequence, with particular reference to age-related disorders such as neurodegeneration, potentially valuable mouse models that display late-onset phenotypes or pathology would not be detected. To address this issue, a small number of ageing ENU screens have been established, with the aim of testing cohorts of mutants at several time points up to 18 months of age or beyond [for examples, see http://www.har.mrc.ac.uk/research/functional-genomics-and-disease-mechanisms-section/disease-model-discovery-and-translatio-2 and Johnson (117)]. It is well-established that the behavioural response in ageing mice can be quite different from younger animals (118), and issues of general health and weight must also be taken into account (119). However, these challenging and time-consuming studies are a very important way of identify genes and pathways involved in age-related disease.

Finally, looking further ahead, advances in sequencing technology and data analysis will have an important role to play in the exploitation of ENU mutants, not just at the positional cloning stage. Gene-driven screens are currently limited to screening small numbers of loci for an allelic series of mutations; however, it will become financially feasible in the coming years to sequence the entire genome of all archived mutants. Another approach would be to focus on the mouse exome or even the transcriptome from archived tissue (120). Although this would dramatically reduce the amount of data to generate and analyse, with the realization that non-protein-coding sequences also play such a key role in the function of the CNS (121,122), a genome-wide database of mutants would maximize the potential of the ENU mutagenesis resource that numbers over 50 000 individually archived lines. Indeed, ENU mutations in regulatory non-coding regions causing distinct phenotypes have already been described (123,124).

CONCLUSION

As more is revealed regarding the complexity and multi-factorial nature of human neurological disease, access to models that recapitulate aspects of the behavioural and/or pathological abnormalities associated with these disorders is essential. With the ability to carry out longitudinal studies to identify early biomarkers and by focussing on specific endo-phenotypes, the mouse will continue to be a vital tool to identify disease pathways in neurodegenerative and psychiatric disease research. ENU mutagenesis has already generated a significant number of new clinically relevant mouse models, and technological improvements in phenotyping and sequencing strategies will only help improve the efficiency of their identification. Access to point mutants will also complement gene knockout studies to model the heterogeneous range of human disease phenotypes. For example, the well-studied mouse model of the lysosomal storage disorder Niemann-Pick type C (NPC) does not express any NPC protein and serves as a model for the severe infantile forms of this disease ($Npc1^{nih}$) (125); however, a recently described ENU mutant ($Npc1^{nmf164}$) provides a more practical model of the more common yet later-onset NPC pathology that is caused by missense mutations (126). Furthermore, the number of potential genetic variants that lead to disease susceptibility is ever-expanding, including novel SNPs or *de novo* copy number variants that will drive future mouse modelling experiments (127) as well as epigenetic and environmental factors. Therefore, in combination with transgenics and conditional knockouts, ENU mutagenesis will continue to play an important role in the identification and characterization of mutations that underlie human neurological disease.

Conflict of Interest statement. None declared.

FUNDING

The authors wish to thank the financial support of the UK Medical Research Council.

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