RNA interference based gene therapy for neurological disease

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Abstract
Neurodegenerative disorders represent a major class of disorders for which thus far any effective small molecule drug therapy has failed to emerge. RNA interference (RNAi), by which disease genes such as those identified for spino-cerebellar ataxia and Huntington’s disease can be specifically silenced, has great potential in becoming a successful therapeutic strategy for these diseases. RNAi has shown therapeutic value in vitro and in animal disease models and clinical trials are currently on their way. However, there are problems, such as toxicity due to non-specific silencing, generation of immune responses and over-saturation of RNAi pathway components that must be overcome in order to establish RNAi as a safe and effective therapy. Current research on the endogenous roles of RNAi, through the action of microRNAs, has offered much knowledge to optimise the exploitation of RNAi.

Keywords: RNA interference; siRNA; microRNA; gene therapy; neurodegenerative disease

INTRODUCTION
Neurodegenerative disorders present one of the major health problems faced by the ageing population of western countries especially as there is no effective therapy at present. Although it has been difficult to identify suitable drug targets for these disorders, it has been possible to identify specific genetic mutations as causative for some neurodegenerative disorders. For example, Huntington’s disease [1], fronto-temporal dementia associated with parkinsonism (FTDP) [2] and spino-cerebellar ataxia [3] are all inherited through dominant toxic or negative mutations. RNA interference (RNAi) holds much promise as a therapeutic strategy for such diseases. RNAi is a naturally occurring biological process by which a small double-stranded RNA (dsRNA) inhibits the expression of the homologous gene through a post-transcriptional mechanism [4]. RNAi, which plays a critical role in all eukaryotes, is widely used in the laboratory to evaluate gene function and has enormous potential in drug development, since it can be used to target any gene of interest. Several proof-of-concept studies have also shown that RNAi has potential in the treatment of neurological disorders. In this review, an overview of the application of RNAi to genes involved in neurological disorders is presented along with a discussion of the caveats of RNAi. At this stage, there remain significant limitations in the application of RNAi, including insufficient RNAi specificity and unpredictable off-target effects. Further, our understanding of the endogenous roles of RNAi as well as the extent of its function is as yet basic. While current studies on RNAi-based therapy for neurodegenerative disorders show exciting and encouraging results, further research is required in order to ensure that exploitation of this pathway is safe.

RNAi MECHANISM
RNAi was first described as a post-transcriptional gene regulatory mechanism in plants [5]. This finding was later extended to animals when injection of dsRNA into Caenorhabditis elegans resulted in...
specific and robust silencing of the homologous gene [6]. The RNAi pathway is conserved and functional in all eukaryotes. Since optimisation of the use of RNAi in mammalian systems [7], the RNAi pathway has been harnessed to become an enormously important research tool as well as a possible therapeutic strategy. It has been recently discovered that RNAi has several active endogenous roles. Through the action of small endogenous RNAs known as microRNAs (miRNAs), RNAi regulates the expression of key genes involved in development and differentiation as well as several other vital biological processes [8].

Figure 1: The RNAi mechanism. (A) shRNA/miRNA precursors are synthesized in the nucleus. (B) These precursors are first processed there by certain members of the RNAi pathway such as Drosha to form short hairpin structures. (C) The products are then exported into the cytoplasm by small nucleic acid exporter Exportin-5. (D) The short hairpins are then processed by Dicer in the cytoplasm to give, (E) siRNAs or miRNAs. (F) siRNAs/miRNAs are incorporated into the RNA induced silencing complex, RISC. (G) One strand of the siRNA, the guide or antisense strand, is retained in RISC and a homologous target mRNA is bound. (H) Perfectly complementary target binding leads to target degradation; whereas incomplete complementarity leads to inhibition of protein translation from the target.

The RNAi machinery is present in all eukaryotic cells (Figure 1). Double-stranded RNA precursors are first ‘diced’ by the RNase III like enzyme Dicer to form small 21–23 nucleotide double-stranded small interfering RNAs (siRNAs). These siRNAs are then incorporated into a protein complex to form the RNA induced silencing complex (RISC) [9, 10]. One of the two strands remains bound to this complex and acts as a guide strand for RISC to identify the homologous mRNA [11]. This process gives RNAi its exquisite sequence specificity [12]. Argonaute–2, one of the principal protein components of RISC then cleaves the homologous mRNA [13, 14]. A single RISC complex can catalyse the degradation of several mRNAs, making RNAi a highly efficient process.

In addition to cleavage of target mRNA, RNAi can occur by two other general mechanisms. siRNAs which contain some mismatches to the target can bring about gene silencing through inhibition of translation from the homologous mRNA [15]. siRNAs can also direct transcriptional silencing by methylation of genomic DNA leading to heterochromatin formation [16]. Different Argonaute proteins might be involved in directing these different RNAi pathways; for example, Argonaute–1 directs transcriptional silencing in human cells [16]. siRNAs, as the key effector molecules, remain the common point of entry in all the RNAi pathways.
and the factors that determine which mode of silencing occurs include the degree of sequence complementarity to the target gene [17], siRNA structure and origin.

The endogenous role of RNAi is in gene regulation, directed by the cell’s own repertoire of small RNAs known as miRNAs. These are transcribed in the nucleus as single-stranded RNAs with stem-loop structures, that are then processed by the RNase III enzyme Drosha, exported from the nucleus by Exportin-5, and further processed in the cytoplasm by Dicer to form siRNA-like miRNAs [18–20]. While miRNAs can direct target mRNA cleavage if they are perfectly complementary to the target [17], typical miRNAs contain mismatches to the target sequence within the 3'UTR of the target gene, and as a result they inhibit translation from the target rather than direct its cleavage [21].

To exploit RNAi experimentally, siRNAs can be introduced into cells through a range of methods. The most basic of these is to complex the siRNAs with lipid carriers to facilitate uptake by the cell membrane. Other strategies are to encode siRNAs as stem-loop structures known as short hairpin RNAs (shRNAs), similar to miRNA genes, in a vector from which the shRNA can be expressed either transiently or stably. Both plasmid and viral vectors, such as adenoviral, lentiviral and adeno associated viral (AAV) vectors have been widely used for siRNA delivery, in vitro and in vivo.

**RNAi AND NEURODEGENERATIVE DISORDERS**

Whilst most neurodegenerative disease is sporadic in origin, in some cases specific causative mutations have now been identified. With RNAi, it is possible to envisage two therapeutic strategies. First, targeting the expression of genes implicated in disease pathology; for example, down-regulation of amyloid precursor protein (APP), or other proteins involved in the formation of plaques in Alzheimer’s disease, has been possible using RNAi. Secondly, it is possible to target a dominant disease allele, taking advantage of the sequence differences between mutant and wild-type alleles, and allowing continued expression of the wild-type allele. The latter is particularly advantageous since the maintenance of wild-type gene expression might be essential for function and survival. This RNAi approach has been investigated in a number of experimental systems in attempts to develop allele-specific gene silencing for neurodegenerative disorders.

The sS226F mutation in the muscle acetylcholine receptor subunit gene (AChR) leads to a disorder known as slow channel congenital myasthenic syndrome, therefore, knockdown of the mutant protein is postulated to be therapeutic. However, expression of the non-disease allele is essential for normal function, thus it is important to be able to selectively knockdown the mutant allele alone. Using siRNAs to target this mutation (S226F) in the disease allele, 70% knockdown of the mutant gene was achieved [22] in mammalian cell lines. This study also showed that shRNAs functioned with comparable efficiency to siRNAs, demonstrating that shRNAs had potential as a longer term therapeutic strategy. Fronto-temporal dementia with parkinsonism linked to chromosome (FTDP-17) is associated with around 25 dominant mutations in MAPT gene encoding Tau [23]. These mutations lead to the production of an abnormal form of Tau that builds up in neurofibrillary tangles and causes neurodegeneration. Miller et al. [24] designed siRNAs, and plasmid and viral expressed shRNAs that specifically targeted the V337M mutation and knocked down mutant Tau to 10% in mammalian in vitro model systems. However, this was accompanied by a 50% knockdown of wild type gene expression. Introduction of mismatches in the siRNA such that there was less affinity for the wild type allele improved the allele specificity such that the mutant was knocked down by ~80% while the wild type was knocked down by 20–30% [24, 25]. A similar strategy was used to silence the dominant mutant allele of the torsin A gene implicated in primary dystonia. By using shRNAs delivered via a feline immunodeficiency virus, mutant torsin A was knocked down by 80% with no significant effect on wild type gene expression in a neural cell model of the disease, restoring normal distribution of TorsinA [26].

Mutations in the Cu/Zn superoxide dismutase gene (SOD1) are linked to amyotrophic lateral sclerosis (ALS), a disorder where motor neurons in the CNS degenerate [27]. Two recent studies on RNAi-based silencing of the human SOD1 allele carrying the G93A mutation in transgenic mice reported dramatic therapeutic effects. In a transgenic mouse model of ALS that over-expressed mutant human SOD-1, injection of a lentiviral vector...
expressing shRNA against SOD-1 led to a reduction in expression of SOD-1, accompanied by improved motor neuron survival. This led to a delay in the onset of ALS symptoms by 100% and an extension of lifespan by 80% [28, 29]. This is the highest therapeutic efficacy reported so far and demonstrates the enormous therapeutic potential of RNAi. However, these results are to be interpreted with caution for two reasons. First, a number of previous therapeutic agents have shown promise in this mouse model only to disappoint in subsequent clinical trials [30]. And second, given that ~100 mutations in the SOD-1 gene have been linked to ALS, targeting a single mutation may not be the means to a widely applicable therapy. In this case, a combined RNAi/gene replacement strategy might be feasible, whereby all forms of endogenous SOD-1 are silenced by RNAi and an siRNA resistant normal version of the SOD-1 gene introduced as a replacement [31].

There are at least nine known polyglutamine disorders that are due to an expanded CAG repeat in the relevant disease gene, causing toxic aggregates of the mutant proteins, and in many cases leading to neurodegeneration. These include the huntingtin gene in Huntington’s disease and ataxin-1 in spinocerebellar ataxia type-1 (SCA1). The potential of virally expressed shRNAs to silence these genes was first demonstrated in neuronal cell models expressing a GFP-polyQ construct. siRNAs against GFP, expressed from an AAV vector successfully reduced protein aggregation in these cells. Further the ability of adenoviral expressed siRNAs to knockdown both exogenous and endogenous gene targets in vivo in mouse liver and brain was demonstrated [32]. The Davidson group subsequently went on to show knockdown of mutant ataxin-1 in heterozygous transgenic SCA1 mice using intracerebellar injections of AAV expressed shRNAs. Dramatic therapeutic effects were observed in this study; in addition to normal cellular morphology being restored and the reduction of ataxin-1 inclusion bodies in cerebellar Purkinje cells, the treated mice showed markedly improved motor coordination [33]. However, the transgenic mouse model used in this study carried the mutant human ataxin-1 transgene as well as two copies of normal mouse ataxin-1. In these mice, it was possible to specifically silence the human mutant allele. However, in a human disease situation, the shRNAs used, which targeted sequences neighbouring the CAG repeat regions would silence both the wild-type and mutant alleles i.e. they were not specific for the mutant allele. Further, targeting the CAG repeat alone is not feasible as such repeats are present in wild-type versions of several important genes, and therefore any off-target silencing of these genes could have adverse effects. One strategy to overcome this potential short-coming has been to target a single nucleotide polymorphism that segregates with the mutant allele of the polyglutamine disease gene rather than the CAG repeat mutation itself; as applied successfully for the MJD1 gene [24].

For some disorders, rather than target a causative mutation, it is advantageous to alter expression levels of genes involved in the disease pathway to restore normal function. This is illustrated in the case of development of RNAi-based therapy for Alzheimer’s disease, the most common neurodegenerative disorder. The toxic β-amyloid plaques that cause neuronal loss are formed by the increased production of β-amyloid protein from the amyloid precursor protein (APP) [34]. Therefore, knockdown of APP [25, 35] or the β-secretase enzyme (BACE1) which is involved in β-amyloid production have been explored as therapeutic targets [36, 37]. *In vivo* studies to pursue this approach have proved promising; lentiviral delivery of shRNAs targeting BACE1 reduced amyloid production, and the neurodegenerative and behavioural deficits in an APP transgenic mouse model [37].

**CAVEATS OF RNAi-BASED THERAPY**

**Delivery of siRNA**

Effective delivery of nucleic acid-based therapeutic molecules to the CNS remains a limiting issue for RNAi. The CNS is a difficult organ to target because of its delicate nature, highly complex architecture, post-mitotic neurons and the presence in almost all regions of a blood brain barrier (BBB). Systemic delivery of unmodified siRNAs to the CNS is currently precluded, since siRNAs do not readily cross the BBB, are rapidly degraded *in vivo* by endonucleases and filtered through the renal system. Thus, naked siRNAs have been delivered by direct injection into brain but mixed results have been observed. Intrastriatal infusions at nanomolar levels of naked siRNAs against dopamine D1 receptors in the rat failed to result in significant knockdown, in contrast to 76% knockdown achieved *in vitro* [38].
In another study, infusion of high doses of siRNA against the dopamine transporter gene into the ventricular CSF resulted in significant knockdown in adjacent regions and dorsolaterally distant regions [39]. siRNAs have also been delivered to the brain by electroporation into localized regions such as the visual cortex or hippocampus, resulting in significant gene knockdown [40]. In cellular studies, siRNAs are routinely delivered to cells complexed with cationic lipids, in the form of liposomes. This procedure has shown some success in vivo in mouse brain, where picomolar quantities of siRNA were sufficient to show knockdown of the transiently expressed reporter gene target [41]. However, the disadvantages are that the lipid reagents used typically show dose-dependent toxicity and delivery is inefficient and not targeted. However, liposomes can be modified with ligands in order to achieve targeted delivery. For tissue specific delivery in vivo, Partridge et al. [42] constructed receptor-specific pegylated liposomes carrying ligands to recognize specific receptors on intracranial human brain tumours in mouse and rat models. In these, were encapsulated plasmids expressing shRNAs against the EGF receptor that achieved 90% knockdown in vivo and a life span extension of nearly 90% in the rodent models [42, 43]. Zimmerman et al. [44] recently delivered siRNA duplexes against apoB complexed with lipid as stable nucleic acid lipid particles (SNALPs) to the liver of primate models. A single dose of this siRNA resulted in silencing of apoB for over 11 days in vivo and caused a substantial reduction in the levels of serum cholesterol and low-density lipoproteins, resulting in clinically relevant knockdown in a non-human primate. Such a delivery approach has yet to be evaluated in the CNS, but this study and those above serve to demonstrate the therapeutic potential of RNAi and suggest that siRNAs could be the next major class of therapeutic drugs.

In order to achieve long-term, stable gene silencing, naked siRNAs are unsuitable as they typically produce only transient effects in vivo, and are rapidly degraded. For long-term stability, chemical modification of the siRNA to protect it from degradation appears to be a successful strategy. Based on methods that have been developed for antisense RNA and ribozymes [45], siRNAs have been modified on the RNA backbone, at the phosphorothioate linkages or the 2′OH of the sugar in order to increase stability without compromising activity. Czauderna et al. used a 2′O-Methyl modification on a few internally placed bases in the siRNA and reported significantly increased stability without any loss of RNAi function [46]. Similarly, 2′O-allylated siRNA and phosphorothioated siRNAs show increased stability in vivo [47]. The efficacy of chemically stabilized siRNA has been proven both in cultured mammalian cells [48] and in vivo complexed with lipid delivery agents [49-51]. Chemically modified siRNAs have been shown to be successful in preclinical studies [49] and are currently being used in clinical trials for treatment of macular degeneration [52]. An important advantage of this approach is that certain chemical modifications have been shown to reduce off-target effects, a significant limitation in the application of RNAi. siRNAs can have off-target effects on targets with seed matches, i.e. with nucleotide matches at positions 2–7 of the antisense siRNA strand. 2′-O-methyl ribosyl substitution at position 2 of the antisense strand has been shown to significantly reduce such off-target effects [53] by preventing silencing of incompletely matched targets with seed region complementarity. Thus, solving the problem of targeted siRNA delivery will require further studies in chemical modification and siRNA pharmacokinetics, treating these molecules much like conventional pharmacological reagents. An advantage of this approach with respect to expressed shRNAs is that the latter have a different set of delivery limitations, related more to those of traditional gene therapy reagents.

In order to achieve long-term gene silencing, an alternative approach is for siRNAs to be expressed transiently or stably within the cell. To achieve this, siRNAs can be transcribed from plasmid vectors in the form of short hairpin RNAs (shRNAs), similar to miRNA precursors, under the control of specific promoters, such as the RNA Pol III promoters U6 or H1 [54-58]. The shRNAs consist of the sense strand of the siRNA followed by a short 6–8 nucleotide loop sequence which is followed by the antisense strand of the siRNA. These shRNAs are then processed by Dicer to yield siRNA effector molecules. The advantage of using plasmid vectors is that shRNA expression can potentially be regulated with the use of tissue-specific or inducible promoters, such as a Tet-responsive promoter [57]. This allows knockdown to be controlled in a spatio-temporal manner. A new generation of highly potent shRNAs have been designed on miRNA precursors,
such that siRNA production mimics the natural miRNA production process [59].

Viral delivery of expressed shRNAs in vivo holds much promise as a therapeutic strategy given the high efficiency of viral gene delivery and the ability of many viral systems to transduce non-dividing cells, such as differentiated neurons of the CNS. Lentiviruses and AAVs with a tropism towards brain have been used in several studies, including those discussed in the previous section [33]. These vectors are currently the major RNAi expression vectors being used in preclinical studies of neurodegenerative disease for several reasons, including; their safety features, relatively low immunogenicity and stability of target gene silencing. However, viral siRNA delivery is associated with similar challenges to those faced by traditional gene therapy methods. These include the issues of titre-dependent toxicity, immune responses against vector component epitopes and non-specific genome integration, all of which require further advances in vectorology, rather than siRNA technology.

**Specificity**

Long dsRNAs cause widespread off-target silencing in mammalian cells through the interferon pathway, whereas dsRNAs shorter than 30 nucleotides were originally thought to confer greater specificity [60]. Recent reports, however, show otherwise. Two separate studies looking at the effects of siRNA-based gene silencing analysed global mRNA expression profiles of cultured human cells transfected with siRNAs and reported widespread silencing of off-target genes [61, 62]. High concentrations of the siRNA were especially associated with widespread off-target effects that could be observed at the mRNA level. A recent concern is that siRNAs could exert effects on off-target genes through the miRNA pathway (summarized in Figure 2). Such effects would typically be undetectable at the mRNA level, as miRNAs cause inhibition of translation from their target mRNAs as opposed to degradation, therefore, an siRNA with three to four mismatches causes suppression of its target gene mainly at the protein level [15]. As few as six or seven complementary bases in the seed region (positions 2–8) are sufficient for miRNAs to exert their effects [63, 64]. It is quite possible that either the sense or antisense strand of the siRNA could have miRNA like effects on off-target genes to which they are complementary by a sufficient number of bases. One study investigated this and identified widespread and divergent changes

![Figure 2: Sources of off-target effects. Sources of off-target effects include: (A) Over-saturation of RNAi pathway components, such as Exportin-5. (B) Induction of interferon response from siRNA/shRNA, or toxicity from siRNA/shRNA delivery agents. (C) Reduced production of miRNAs due to competition with shRNA production. (D) Competition between siRNAs/miRNAs for RISC components and RISC loading. (E) Loading of RISC with the anti-guide strand, leading to recruitment of non-target mRNAs. (F) Non-specific silencing of non-target mRNAs through recognition with anti-guide strand or due to incomplete matches with guide strand. (G) Incomplete function of miRNAs due to competition for RNAi resources, leading to global alterations in gene expression patterns.](https://academic.oup.com/bfg/article-abstract/6/1/40/272507)
in the protein levels of untargeted genes as a result of siRNA silencing [65], and further found that this was not adequately represented at the mRNA level. A computational study revealed hundreds of potential target sequences on which these siRNAs could have had miRNA-like effects. These off-target effects could cascade downstream, causing divergent changes in the levels of unrelated proteins. Off-target effects can be minimized by selecting fewer and more potent siRNAs with minimal matches to off-target genes. Furthermore, chemical modification of the base at position 2 in the guide strand of the siRNA has been shown to greatly reduce off-target effects [53]. Several software packages have been designed to analyse siRNAs for off-target effects and can be used for more effective siRNA design [66].

**Generation of immune response**

While dsRNA longer than 30 nucleotides provokes a strong interferon response through the dsRNA dependant protein kinase response (PKR), shorter dsRNAs have been shown to be non-immunogenic [67]. However, at least five studies to date report an siRNA-induced interferon response [61, 68–71]. This response appears to vary between species and cell type; in one study, an siRNA generated no interferon response in mice, whereas the same siRNA caused a potent response in human monocytes [72]. In certain cases, the immune response has been proved not to be due to the siRNA itself, but to the method of siRNA generation or delivery. For example, in the studies by Kariko et al. [70] and Sledz et al. [71] that reported an immune response, the siRNAs were generated by *in vitro* transcription using T7 RNA polymerase. This polymerase adds a 5’ triphosphate to its transcripts that has been shown to generate a strong immune response [73]. However, interferon responses are generated directly against double-stranded siRNAs. Two separate studies originally reported that siRNAs can activate dendritic cells through Toll-like receptors (TLRs), a subset of which (TLR3, TLR7, TLR8 and TLR9) recognizes foreign double-stranded nucleic acids [68, 74]. Hornung et al. [68] recently identified a nine nucleotide sequence in an siRNA that activated the TLR7, and thereby caused an immune response. Judge et al. [69] have also found such immunostimulatory motifs in siRNAs. Such knowledge now allows siRNAs to be designed with reduced likelihood of immunostimulatory effects [75]. Potentially, additional such immunostimulatory motifs could exist, and therefore further work is necessary to continue to improve siRNA design and safety.

**Interference with endogenous silencing mechanisms**

The RNAi pathway has myriad endogenous functions through the action of miRNAs that are just being uncovered. About a third of the genome is currently thought to be regulated by miRNAs [76]. miRNAs control several important biological functions ranging from tissue differentiation, development of the CNS [77] to regulation of insulin release [78]. The adult mouse CNS shows specific patterns of miRNA expression [79] suggesting they have an important role in maintaining CNS function. The specific functions of miRNAs are currently being identified, for example miR-134 has been identified to control dendritic spine development in rat hippocampal neurons by inhibiting the expression of the protein LimK1 [80]. With continued improvements in siRNA design, enhancing preferential incorporation into RISC, it is possible that the innate small miRNAs may be prevented from carrying out their normal functions as required. This has been shown to be the case with exogenous siRNAs i.e. siRNAs can compete with one another for the RISC machinery, limiting the ability of lower potency siRNAs to carry out silencing [81]. It is thus conceivable that exogenous siRNAs could saturate the RNAi system. A recent study reported fatality in mice due to over-saturation of the RNAi system. In these experiments shRNAs against six separate gene targets were expressed in the liver at high levels from AAV vectors. Mortality was found in a high proportion of cases, and morbidity was associated with the down-regulation of liver-specific miRNAs. This suggested competition between expressed shRNAs and endogenous miRNAs, one explanation for which was found to be saturation of nuclear RNA exporter, exportin-5, by shRNAs, leading to down-regulation of miRNA expression [82].

More fundamentally, a functional RNAi pathway appears to be essential in mammalian cells; Dicer knockout in mice is embryonically lethal [83]. Further, mutations in fragile X mental retardation protein (FMRP) are linked to Fragile X syndrome, a common neurodevelopmental disorder [84]. That FMRP is one of the players in the RNAi pathway...
and that miRNAs are involved in CNS development leads to the hypothesis that a faulty RNAi pathway may underlie the pathogenesis of this disease [84], and potentially several others. Thus, in addition to concerns of major side-effects due to over-saturating the RNAi pathway, it is possible that subtle alterations could arise from interfering with critical miRNA-based functions, which could have adverse effects and generate subtle phenotypes, particularly in the CNS. Therefore, a more detailed understanding of the RNAi pathway, its endogenous functions, its regulation and the processing of exogenous therapeutic siRNAs in the CNS will be important before RNAi therapies can proceed safely.

### CONCLUSION AND EXPERT OPINION

RNAi has been used with great success to knock down the expression of selected target genes, with therapeutic value both in vitro and in animal models of neurodegenerative disease. This has led to speculation about siRNAs and RNAi-based therapy being a major class of ‘drugs’ in the near future. While RNAi shows great promise and these speculations could well be realized it is necessary to keep in mind that RNAi-based therapy exploits an important natural pathway. Current research shows that small RNA-based gene regulation is important in maintaining the integrity of biological systems, especially in the mammalian CNS, and extensive research is now being conducted in this field. On gaining a better understanding of the RNAi pathway and its function, it will be possible to optimise the exploitation of this pathway in a way that is safe and effective. As more is learnt about the RNAi pathway in the CNS, in particular about miRNAs, their structure and the processing of miRNAs by the RNAi machinery in neurons, it will become increasingly possible to design therapeutic siRNAs that mimic many of the natural attributes of miRNAs and yet avoid the potential adverse effects that might arise from targeting this critical cellular pathway.

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### References


