RNAi and brain function: was McConnell on the right track?

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RNA interference (RNAi), one of the hottest topics of molecular biology research today, has unique features that are eerily reminiscent of the phenomenon of 'RNA-mediated memory transfer,' a controversial line of work that was investigated with great enthusiasm in the 1960s. If not a coincidence, then this suggests taking a new look at RNA-mediated modulation of neural function and raises the possibility that RNAi might be one of the physiologic mechanisms that regulate long-term gene expression in the brain.

In the 1960s, one of the most talked about areas of neuroscience concerned reports that chemical extracts, isolated from animals that had been subjected to classical conditioning paradigms, could enhance learning when transferred to their naïve counterparts[1]. A prominent worker in this field, James V. McConnell (1925–1990), established that planarians (flatworms) could be reliably conditioned to turn in response to light or vibration[2]. Taking advantage of the regenerative capacity of planarians, he separated the head (containing the brain) from the tail in trained animals, and reported that persistent behavioral changes were seen in animals that regenerated from either half. Furthermore, conditioning was enhanced by injecting extracts of trained planarians into naïve planarians, or (because planarians are cannibals) even just feeding them trained animals. Surprisingly, the active principle in the extract appeared to be RNA (Ref. 2).

Several groups replicated and extended these findings in planaria[1–3] and RNA-mediated transfer effects were also reported for learning in rats[3], for example in acquisition of operant responses[4] and one-way avoidance responses[5]. Although the transfer of RNA was originally envisioned as conferring specific memories on the recipient animal – an idea that is outdated according to current thinking – McConnell himself emphasized that such transfer probably perturbed molecules that are more generally involved in the build-up of learning and memory circuits (Ref. 3).

Unfortunately, not all laboratories could reproduce these effects reliably. Experimental papers on this subject tapered off by the early 1970s, with most neuroscientists having written off the work as a dead end. McConnell not only suffered a loss of scientific prestige, but ironically, as a result of his studies, he was the victim of a letter bomb sent by the Unabomber[6].

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RNA interference (RNAi)

Fast-forward to the present: the recently described phenomenon known as RNA interference, or RNAi, is being studied intensively by molecular biologists, but to our knowledge no one has pointed out that it exhibits striking parallels to McConnell's nearly-forgotten RNA-mediated memory transfer. RNAi occurs when double-stranded RNA that is expressed within, or taken up by, cells, is cleaved into smaller protein-bound fragments that hybridize to endogenous cellular sequences, resulting in selective degradation of specific endogenous mRNAs and the consequent suppression of individual gene functions[7–9] (Fig. 1). RNAi has been described for dozens of different genes in a variety of invertebrate phyla, including *C. elegans*, *Drosophila* and planarians[10–14], and in vertebrates, including zebrafish[15] and mouse[16,17].

Of the amazing features of RNAi that have attracted attention, it is relevant here to note that one can induce RNAi by simply injecting double-stranded RNA into the body cavity *in vivo*, or even by feeding animals with bacteria that express exogenous RNAs. Moreover, the inhibitory effects on gene function are not only persistent throughout the lifespan of the animal but actually become amplified over time, and after injecting RNA into a localized region of the body RNAi can spread to other regions of the body, including the germ line[11]. Only a few copies of double-stranded RNA need to be formed within a cell in order to trigger RNAi, and expression of single-stranded antisense RNAs within cells is often accompanied by the formation of sufficient quantities of double-stranded RNA to trigger RNAi.

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Thus, RNAi could potentially provide a mechanism to explain 'RNA-mediated memory transfer,' provided that the RNA preparations used in McConnell's studies were competent to trigger this phenomenon (e.g. contained double-stranded RNA or products of the RNAi process). Some of the difficulties in replicating the experiments in RNA transfer in other laboratories might reflect the peculiar requirements of RNAi, for example, double-stranded RNA has a short lifetime in cells, and its ability to elicit RNAi requires a minimum sequence length[18], therefore variations in the timing of extraction or the integrity of RNA preparations might have been crucial factors. In addition, direct injection of double-stranded RNA-containing extracts appears to elicit RNAi less reliably than expression of double-stranded RNA via transgenes[19].

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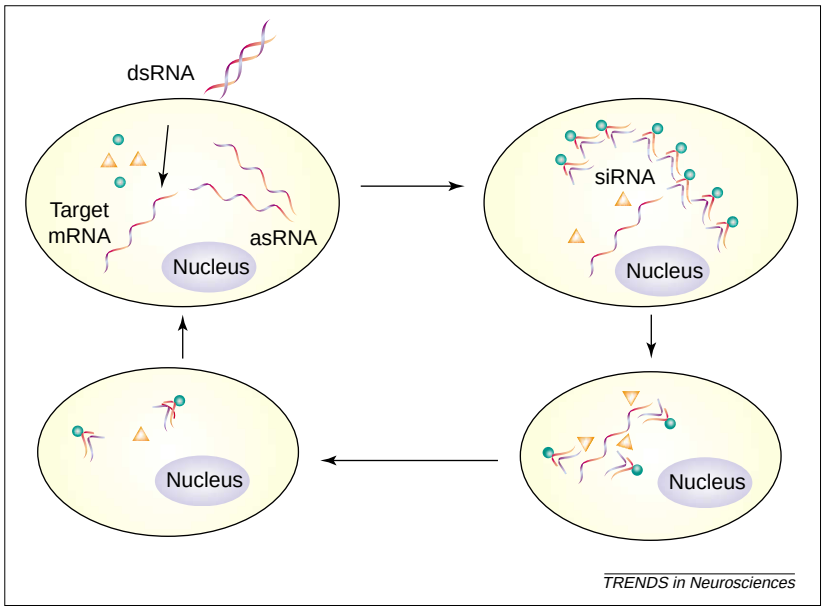

Fig. 1. RNAi occurs when double stranded RNA (dsRNA) is either taken up by cells or is formed by endogenous antisense RNA (asRNA) hybridizing to a target messenger RNA (mRNA). The dsRNA is cleaved to small interfering RNAs (siRNA) that associate with RNA-specific proteins (circles) and bind to the target mRNA, and is consequently cleaved and degraded by RNAi-specific nucleases (triangles).

Does RNAi participate in regulating changes in gene expression in brain?

Why does it matter whether McConnell's findings were caused by RNAi? The key observation is that many genes have been shown to express endogenous antisense RNA transcripts within cells in a wide range of invertebrate and mammalian species[20], including genes that are expressed in neural tissue, such as basic fibroblast growth factor. Expression of these antisense RNA transcripts appears to be involved in regulating the abundance of the corresponding sense transcripts[20]. At least one example, the SCA8 transcript, has been described which is primarily expressed in the brain and is an endogenous antisense transcript that interacts with mRNA encoding an actin-organizing protein, KLHL1,

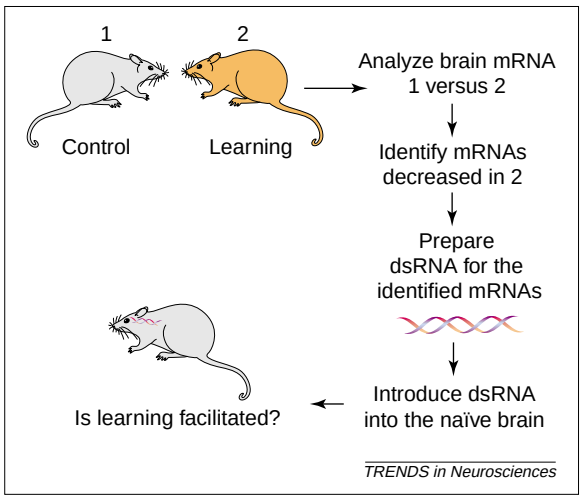

Fig. 2. A test for whether RNAi can modulate brain function is shown here for rodents. Alternatively, such experiments can be pursued in *Drosophila*, a species in which both RNAi (Refs 12,13) and learning and memory[28] can readily be studied.

on the opposite strand[21]. Thus, if double-stranded RNAs were formed within neural cells, this would provide a potential substrate for triggering RNAi under physiologic conditions resulting in silencing the expression of specific genes in the brain. Such silencing would be expected to be long-lasting, perhaps even self-amplifying, compatible with a role in learning and memory. Indeed, genes that regulate long-term changes in synaptic plasticity are now thought to underlie not only simple forms of learning, such as classical and operant conditioning, but also the types of conscious memory that most people refer to as 'memory' in everyday language[22].

A model of how RNAi could operate in the adult mammalian brain (Fig. 2) can be tested as follows.

(1) Can a set of genes be identified that are silenced in a long-lasting manner within the brain following learning paradigms? Several neural genes including those encoding cFOS, cAMP-dependent protein kinase regulatory subunit and neurogranin have, indeed, been shown to be strongly downregulated in cerebral cortex following prolonged enrichment training in mice[23], although the full spatial and temporal extent of this downregulation have, as yet, not been examined.

(2) Can it be shown that one or more of these genes actively modulate learning and/or memory? This can be carried out by suppressing the expression of these genes (one at a time) in naïve animals, and testing whether learning or recall are subsequently facilitated. At least three gene knockout strains of mice have been reported [deficient in ryanodine receptor type 3 (Ref. 24), cannabinoid CB1 receptors[25] and nociceptin receptors[26]] in which spatial learning and/or LTP are facilitated, although to our knowledge no one has examined whether the endogenous transcripts are downregulated during learning in wild-type mice.

(3) Can it be shown that RNAi is an effective means of silencing expression of these genes in neurons, either by injecting double-stranded RNAs directly into the brain or by expressing double-stranded transgenes? It is probable that the relevant enzymatic machinery to support RNAi is present in brain, because RNAi has been produced in embryonic neurons in *Drosophila*[27] and mature neurons in *C. elegans*[19].

(4) Can it be verified that RNAi is the specific mechanism by which these genes are silenced physiologically? The answer to this question requires evidence that corresponding endogenous antisense RNA transcripts are dynamically induced within the CNS when animals are subjected to learning paradigms or other challenges, that they hybridize to their sense counterparts, and that RNAi degradation products are formed.

As molecular biologists learn more about the mechanisms of RNAi in simpler systems, it will become easier to assess the likelihood that RNAi occurs physiologically within the brain. However, we believe that the history of science is itself a rich source of ideas that that can suggest new experimental questions for today's researchers.

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