thereby generating strong expectations. There is a need to develop new therapeutic strategies for osteoporotic patients that would not only reduce bone loss, but would also allow recovery of their bone integrity while preserving their comfort and safety.

With respect to the ageing of population, osteoporosis and related fractures are a growing matter of concern for public health. Innovative approaches for the treatment of osteoporosis could therefore be of major interest not only for medical reasons, but also from a social and economical viewpoint.

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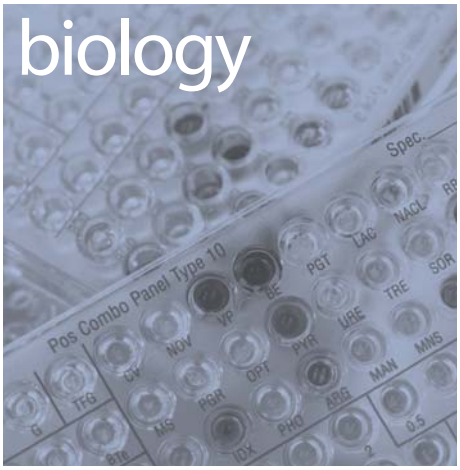


NEUROSCIENCE

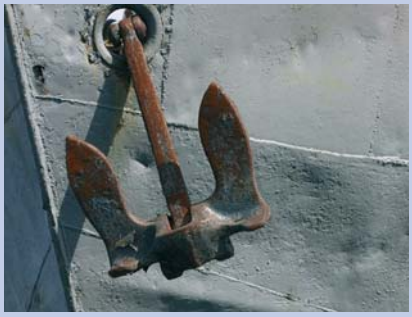
Knockdown not knockout

The discovery of candidate genes for neuropsychiatric disorders from high-throughput screens has necessitated the need for a rapid, consistent method for characterizing their function in model systems. Traditionally, this has been done using knockout or transgenic mice, but this approach is both time-consuming, technically demanding and complicated by genetic redundancy and developmental effects. The advent of RNAi technology (whereby cytoplasmic mRNAs that contain homologous sequences to a double-stranded RNA trigger are selectively destroyed) has negated many of these difficulties, but, using viral vectors, knockdown effects in the brain have, thus far, been limited to specific regions surrounding the infusion site. To ascertain the role of ubiquitously expressed genes in the brain that might affect multiple neuronal circuits, it would be desirable to have an RNAi protocol that elicited brain-wide knockdown.

Thakker and colleagues have devised and validated such a protocol in mice [1]. Briefly, through daily infusion of a double-stranded short interfering RNA (siRNA) of just 21 nucleotides into the dorsal third ventricle, the authors achieved widespread, specific knockdown of a ubiquitously expressed EGFP transgene; this knockdown effect was most pronounced adjacent to the infusion site but extended as far as the prefrontal cortex. After two weeks, knockdown was observed in the vast majority of brain regions. To confirm the efficacy of their approach the authors attempted to reduce expression of the endogenous *DAT* gene (encoding the dopamine transporter) in the ventral midbrain (i.e. far caudal to the infusion site). *DAT* mRNA levels were reduced by ~33% in this region, while protein levels were reduced by ~49%. At the functional level, this attenuation of expression gave rise to a hyperlocomotor response (quantitatively equivalent to that

CARDIOVASCULAR BIOLOGY

Anchoring proteins are multitasking



To target the action of second messenger such as cAMP, protein kinase A (PKA) and protein kinase C are specifically compartmentalized by one of the A-kinase anchoring proteins (AKAPs). This mode of regulation, used by ion channels and G-protein-coupled receptors for example, ensures that PKA is exposed to isolated cAMP gradients, which allows for efficient catalytic activation and accurate substrate selection.

Kurokawa *et al.* [2] studied the effects of Yotiao, an isoform of AKAP, on the cardiac potassium channel (IKs) and reported that Yotiao fulfills two roles in the modulation of cardiac action potential. First, in response to stimulation by the sympathetic nervous system, PKA phosphorylates KCNQ1, the α subunit of the IKs potassium channel, thereby modifying its response. Second, the authors established that Yotiao recognizes a specific motif located at the C-terminal domain of KCNQ1.

Interaction between the C-terminal domain of KCNQ1 and Yotiao modifies channel function. Moreover, this effect is only seen when the channel has been phosphorylated or if the serine involved in the phosphorylation is mutated in a negatively charged residue. This study shows that anchoring proteins such as AKAPs could have several roles. They not only recruit all the elements necessary to build specific protein complexes, they can also have a significant influence on their function.

2 Kurokawa, J. *et al.* (2004) Regulatory actions of the A-kinase anchoring protein Yotiao on a heart potassium channel downstream of PKA phosphorylation. *Proc. Natl. Acad. Sci. U. S. A.* 101, 16374–16378

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effected by a DAT inhibitor), manifest after nine days into the infusion regimen.

As the authors point out, the development of this technology, which is not hampered by the constraints associated with knockout and/or transgenic models or of virally-mediated RNAi, represents a valuable avenue towards the 'functional investigation of broadly expressed target genes implicated in neurological and psychiatric disorders'. However, caveats of the technique include the fact that the spatiotemporal dynamics of knockdown can be influenced by both proximity to the infusion site, and neuronal composition of the particular region of interest. Future work will be directed towards achieving reversible gene knockdown and coincident knockdown of multiple brain-expressed genes.

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DISEASE MECHANISMS

How West Nile Virus makes its entry into the brain

West Nile Virus (WNV) has been the cause of recent outbreaks of viral encephalitis in North America. In humans, WVN infection in elderly and immunocompromized individuals can lead to fatal encephalitis or meningitis. Both humoral and cellular immunity participate in the host defence against WVN infection. Toll-like receptors (TLRs) have an essential role in the initiation of the innate immunity. TLR3 is involved in viral recognition, but its functional role during viral infection remains unclear.

In a recent study, Wang *et al*. explore how WNV crosses the blood–brain barrier (BBB). They first show that TLR3-deficient mice have impaired production of cytokines and elevated viral loads in the blood during the first week of infection. But, surprisingly, the brains of TLR3-deficient mice show reduced levels of inflammatory cytokines, infiltrating leukocytes, infectious virus and neuropathology compared with the brains of wild-type mice. As a result, TLR3-deficient mice are more resistant to lethal WNV infection than wild-type mice.

Wang *et al*. speculate that TLR3 somehow controls viral entry into the brain. To explore this hypothesis, the authors used a dye usually excluded from the brain. They show that three days post-infection, the dye enters the brain in wild-type mice but remains excluded in TLR3-deficient mice. They further show that the use of a TLR3 ligand has the same effects on the permeability of the brain than WNV infection. Finally, TNF-α receptor-deficient mice have phenotypes very similar to the TLR3-deficient mice, suggesting that the TNF- α receptor pathway is the signaling cascade downstream of TLR3 that allows WNV to enter the brain.

These findings show the role of TLR3 as a mediator of the innate immune response that alters BBB permeability and allows free virus or virus-infected leukocytes to enter the brain. The clinical use of Tlr3 and/or TNF antagonists could be of great value for the treatment of WNV encephalitis and other viral encephalitis.

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