Glossary

Non-coding RNA: Untranslated RNA species contributing to the post-transcriptional control of protein synthesis via binding and subsequent inhibition of mRNA translation

RILES or RNAi-inducible luciferase expression system: A plasmid-based system engineered to monitor the activity of endogenous RNAi machinery. The miRNA of interest suppresses the expression of a transcriptional repressor and consequently switches on the expression of a luciferase reporter gene.

degeneration. Thus, attempting to ameliorate astrocyte dysfunction in ALS by lowering the expression of miR-218 globally within the CNS would be likely to have adverse off-target effects on motor neuron health.

The pathophysiological changes contributing to neuronal injury and perturbation of glial function in ALS are complex and effective therapeutic approaches are likely to require targeting of multiple effectors simultaneously. Previous approaches, aiming to inhibit or activate a single target, have failed to deliver substantial impact on ALS disease progression in the clinic. Astrocyte involvement in ALS is characterized by a complex gain of toxic properties as well as loss of neuroprotective functions, resulting in an aberrant cellular phenotype, which is unlikely to be corrected by manipulation of a single target. In this context, engineered miRNA-like molecules with selective cellular targeting and a strong safety profile would represent useful tools in the effort to normalize multiple facets of the cellular pathophysiology underlying ALS. Hoye and colleagues have elegantly dissected through *in vitro* and *in vivo* studies one molecular mechanism contributing to the perturbed cross-talk between neurons and astrocytes in models of ALS (Fig. 1). MiR-218 has not so far emerged as a prominent candidate in studies of the expression of microRNAs in human biosamples (Waller *et al.*, 2017) and further work is required to ascertain the importance of this pathway in relation to human ALS.

Laura Ferraiuolo and Pamela J. Shaw
Sheffield Institute for Translational Neuroscience, Department of Neuroscience, University of Sheffield, 385 Glossop Road, Sheffield S10 2HQ, UK

Correspondence to: Pamela J. Shaw
E-mail: pamela.shaw@sheffield.ac.uk

doi:10.1093/brain/awy213

References

Chaudhuri AD, Dastgheyb RM, Yoo SW, Trout A, Talbot CC, Hao H, et al. TNFα and IL-1β modify the miRNA cargo of astrocyte shed extracellular vesicles to regulate neurotrophic signaling in neurons. Cell Death Dis 2018; 9: 363.

Frakes AE, Ferraiuolo L, Haidet-Phillips AM, Schmelzer L, Braun L, Miranda CJ, et al. Microglia induce motor neuron death via the classical NF-κB pathway in amyotrophic lateral sclerosis. Neuron 2014; 81: 1009–23.

Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, et al. Amyotrophic lateral sclerosis. Nat Rev Dis Prim 2017; 3: 17071.

Hautbergue GM, Castelli LM, Ferraiuolo L, Sanchez-Martinez A, Cooper-Knock J, Higginbottom A, et al. SRSF1-dependent nuclear export inhibition of C9ORF72 repeat transcripts prevents neurodegeneration and associated motor deficits. Nat Commun 2017; 8: 16063.

Hoye ML, Koval ED, Wegener AJ, Hyman TS, Yang C, O'Brien DR, et al. MicroRNA profiling reveals marker of motor neuron disease in ALS models. J Neurosci 2017; 37: 5574–86.

Hoye ML, Regan MR, Jensen LA, Lake AM, Reddy LV, Vidensky S, et al. Motor neuron-derived microRNAs cause astrocyte dysfunction in amyotrophic lateral sclerosis. Brain 2018; 141: 2561–75.

Morel L, Regan M, Higashimori H, Ng SK, Esau C, Vidensky S, et al. Neuronal exosomal miRNA-dependent translational regulation of astroglial glutamate transporter glt1. J Biol Chem 2013; 288: 7105–16.

Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol 1995; 38: 73–84.

Schratt G. MicroRNAs at the synapse. Nat Rev Neurosci 2009; 10: 842–9.

Sun S, Sun Y, Ling S-C, Ferraiuolo L, McAlonis-Downes M, Zou Y, et al. Translational profiling identifies a cascade of damage initiated in motor neurons and spreading to glia in mutant SOD1-mediated ALS. Proc Natl Acad Sci USA 2015; 112: E6993–7002.

Waller R, Wyles M, Heath PR, Kazoka M, Wollff H, Shaw PJ, et al. Small RNA Sequencing of sporadic amyotrophic lateral sclerosis cerebrospinal fluid reveals differentially expressed miRNAs related to neural and glial activity. Front Neurosci 2017; 11: 731.

TauBI or not TauBI: what was the question?

This scientific commentary refers to 'Induction of a transmissible tau pathology by traumatic brain injury', by Zanier *et al.* (doi:10.1093/brain/awy193).

In this issue of *Brain*, Zanier *et al.* (2018) report their findings of tau pathology in a cohort of patients who survived for an average of 5 years after a severe traumatic brain injury (TBI). In parallel studies, they analyse the spreading and potential transmission of phosphorylated (P)-tau in a controlled cortical impact (CCI) model of TBI in wild-type mice (Fig. 1).

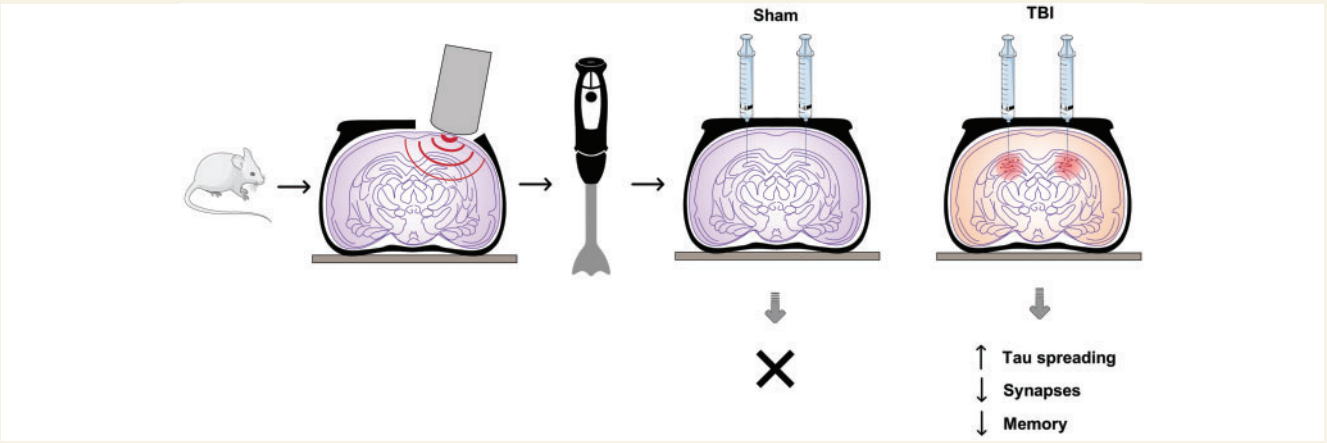

Figure 1 Schematic of the experimental design and results of Zanier *et al.* (2018). The authors describe the spreading of P-tau in human brain after TBI and in a mouse model of traumatic brain injury, the CCI model, 1 year post-injury. Following bilateral inoculation of brain homogenates from the CCI mice into wild-type mice, the authors describe the potential propagation and transmission of P-tau into neurons and glial cells of the recipient mice, leading to impaired memory and increased synaptic loss. No effect was observed in controls, whereby homogenates from sham animals were injected instead.

Over the past decade, much work has gone into determining the possible long-term consequences of multiple concussions and repetitive mild TBI, particularly in players of contact sports. This has resulted in the identification of a condition, chronic traumatic encephalopathy (CTE), which is characterized pathologically by the presence of abundant neuronal and astroglial tau in a perivascular distribution at the base of cortical sulci. The clinical phenotype associated with this pathology is mixed. However, while there seems to be a close association between repetitive mild TBI and this tau pathology, it is not clear if a single severe TBI can give rise to the same pathological changes. Here, in a cohort of 15 patients with severe TBI and 15 age-matched controls, Zanier *et al.* report the presence of tau pathology in both groups, but with the extent and distribution of the post-mortem brain pathology being greater in patients with TBI. As acknowledged by the authors, the interpretation of these findings is not straightforward, with cause and effect being difficult to establish. Age-related tau astrogliopathy (ARTAG) looks very similar to CTE in terms of tau pathology but is not associated with any clinical symptoms, so age is a significant confound. In addition, while all the TBI survivors died from non-TBI causes it is not clear whether they had any symptoms related to the original TBI. An inherent difficulty here is that head injury survivors are generally discharged into the community and lost to follow-up. Going forward, detailed tracking of these patients, with longitudinal cognitive assessments, will be required to decipher cause and effect.

To address the question of whether a single TBI can evoke similar tau changes in rodents, Zanier and colleagues used a CCI model. It is unlikely that a single cortical impact injury is going to cause CTE in an animal, because CTE is more typically seen with repetitive injuries or blast injuries (see review Donat *et al.*, 2017). In addition, by definition CCI is not a model of human CTE because the mouse brain is lissencephalic and has no sulci, but it may shed light on how tau pathology might arise post TBI.

Increased tau phosphorylation has been demonstrated in models of severe TBI, including CCI, fluid percussion and blast injuries. However, mild TBI triggered in closed head injury models has not been associated with increased tau phosphorylation (Mouzon *et al.*, 2014). In severe TBI it was proposed that the phosphorylated form of tau that contributes predominantly to the ensuing tau pathology and brain dysfunction (*cis* P-tau) differs from the physiological form (*trans* P-tau) owing to proline stereoisomerism (Kondo *et al.*, 2015). *Cis* P-tau was reported to spread to other brain regions, including the contralateral hemisphere, as late as 6 months after the initial injury (Kondo *et al.*, 2015). In the present study, Zanier *et al.* propose a similar propagation of tau pathology through active neuronal circuits, with the pathology remaining detectable long-term post injury in wild-type animals.

Studies of TBI performed in transgenic mice expressing human tau or in wild-type rats have demonstrated tau aggregation and/or oligomerization, contrasting with the lack of endogenous tau aggregates observed in wild-type mouse TBI (Kondo *et al.*, 2015). Therefore, the presence of endogenous mouse tau aggregates in wild-type mice observed by Zanier *et al.* is unexpected and should be analysed further. Humans and rats express all six isoforms of tau, stemming from alternative *MAPT* mRNA splicing of exons 2 and 10 to either retain or remove a specific N-terminal region, and to generate either the three repeat (3R) or four repeat (4R) microtubule binding domain. Adult mouse brain contains mainly, if not only, the 4R isoform. In addition, although human and mouse tau

proteins are quite similar, they still differ in 54 amino acids including several phosphorylation sites (Andorfer *et al.*, 2003). Therefore, the work of Zanier *et al.* and others indicates that in wild-type mice, severe TBI can lead to increased tau phosphorylation, and to the spreading of tau along neuronal circuits even long after injury, but nonetheless this process does not need to be dependent on or attributable to oligomeric forms of tau protein.

In the last few years, experimental intracerebral injection of various forms of tau protein obtained from different sources (e.g. brain tissue from patients with a tauopathy and from transgenic tau models) and of differing purity, have been shown to induce transmission and spreading of tauopathy in transgenic tau animals (see review by Goedert *et al.*, 2017). Similar results were obtained using isolated tau oligomers and fibrils as well as viral vectors that express protein tau. Most recently, a similar outcome was observed when the recipient was a wild-type mouse, but only with tau oligomers extracted from Alzheimer's disease brain (Narasimhan *et al.*, 2017) and not when wild-type mice were inoculated with recombinant tau fibrils (Sanders *et al.*, 2014). The results from Zanier and colleagues showing widespread tau pathology in wild-type mice injected with homogenates from mice that suffered TBI are therefore surprising. In fact, all studies published to date have shown that human tau is required to induce seeding of non-transgenic tau (Narasimhan *et al.*, 2017). These observations are in line with Gerson *et al.* (2016), showing limited oligomerization of protein tau in transgenic human tau mice injected with tau oligomers isolated from two rat models of TBI. Unlike in Zanier *et al.*, in all previous studies, the recipient and donor mice expressed 'exogenous' protein tau from other species, mostly together with endogenous protein tau.

The current study also allows critical discussion of some methodological issues. These include immunohistochemical staining to detect tau phosphorylation and aggregation with the AT8 antibody that is known to react with phosphorylated human tau in pathological brain (Petry *et al.*, 2014). However, AT8 also reacts in physiological and reversible conditions, like hypothermia, and is extremely prominent in hibernation. Furthermore, special care has to be taken with AT8 on mouse brain sections, where it reacts with great non-specificity compared to human brain sections (Petry *et al.*, 2014). Therefore, the staining of P-tau with AT8 in wild-type mice should be further analysed by other methods, including immunohistochemistry for established pathological epitopes, e.g. PHF1 or MC1, and additionally by histological staining, e.g. Gallyas silver, Thiazine red, or similar.

One more factor that is likely to be extremely important in the regional spreading of CNS pathology in general, and in TBI in particular, is inflammation. Microglial activation is evident soon after TBI and can persist for years (Donat *et al.*, 2017). Of note, sites of microglial activation often coincide with neuronal degeneration and axonal abnormalities. Imaging studies have documented the spreading of inflammation into brain regions distant from the injury site, including the thalamus (Donat *et al.*, 2017). In addition, a number of reports support the notion that increased neuroinflammation exacerbates tau phosphorylation. Microglia have also been implicated in the propagation of tauopathy by mechanisms involving or mediated by exosomes (Asai *et al.*, 2015). Therefore, to rule in or out the role of glial cells in the regional spreading of tau pathology and synaptic loss, protein tau should be immunodepleted from the homogenates before injection. One final issue to consider is the crude homogenates (10% in phosphate-buffered saline) that were isolated and injected into the brains of wild-type mice. These homogenates were not further purified or analysed, other than being selected for '... the highest amounts of P-tau by western blot ...'. Most likely, these brains also contained the highest levels of other, as yet undefined mouse brain constituents that might play a secondary or even primary role in the observed induction of P-tau neuropathology, defined by AT8 immunostaining. Further studies will be needed to answer these and other open questions in this important area of neurodegenerative disease.

Magdalena Sastre,[1] Fred Van Leuven[2] and Steve M. Gentleman[1]

1 *Division of Brain Sciences, Imperial College London, London W12 0NN, UK*
2 *Experimental Genetics Group-LEGTEGG, Department of Human Genetics, KU Leuven, B-3000, Leuven, Belgium*

Correspondence to: Magdalena Sastre
E-mail: m.sastre@imperial.ac.uk

doi:10.1093/brain/awy225

Acknowledgements

We thank Dr Cornelius K. Donat and Miss Damaris Ribeiro-Rodrigues for their assistance with the figure.

Funding

ISSF Wellcome Trust (reference 105603/Z/14/Z) and the Alzheimer's Research UK Imperial College London Network Centre Grant (ARUK-NC2017-IMP).

Competing interests

The authors report no competing interests.

References

Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker KL, Barde YA, et al. Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. J Neurochem 2003; 86: 582–90.

Asai H, Ikezu S, Tsunoda S, Medalla M, Luebke J, Haydar T, et al. Depletion of microglia and inhibition of exosome synthesis halt tau propagation. Nat Neurosci 2015; 18: 1584–93.

Donat CK, Scott G, Gentleman SM, Sastre M. Microglial activation in traumatic brain injury. Front Aging Neurosci 2017; 9: 208.

Gerson J, Castillo-Carranza DL, Sengupta U, Bodani R, Prough DS, DeWitt DS, et al.

Tau oligomers derived from traumatic brain injury cause cognitive impairment and accelerate onset of pathology in htau mice. J Neurotrauma 2016; 33: 2034–43.
Goedert M, Eisenberg DS, Crowther RA. Propagation of tau aggregates and neurodegeneration. Annu Rev Neurosci 2017; 40: 189–210.
Kondo A, Shahpasand K, Mannix R, Qiu J, Moncaster J, Chen CH, et al. Antibody against early driver of neurodegeneration cis P-tau blocks brain injury and tauopathy. Nature 2015; 523: 431–6.
Mouzon BC, Bachmeier C, Ferro A, Ojo JO, Crynen G, Acker CM, et al. Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model. Ann Neurol 2014; 75: 241–54.
Narasimhan S, Guo JL, Changolkar L, Stieber A, McBride JD, Silva LV, et al. Pathological tau strains from human brains recapitulate the diversity of tauopathies in nontransgenic mouse brain. J Neurosci 2017; 37: 11406–23.
Petry FR, Pelletier J, Bretteville A, Morin F, Calon F, Hébert SS, et al. Specificity of anti-tau antibodies when analyzing mice models of Alzheimer's disease: problems and solutions. PLoS One 2014; 9: e94251.
Sanders DW, Kaufman SK, DeVos SL, Sharma AM, Mirbaha H, Li A, et al. Distinct tau prion strains propagate in cells and mice and define different tauopathies. Neuron 2014; 82: 1271–88.
Zanier ER, Bertani I, Sammali E, Pischiutta F, Chiaravalloti MA, Vegliante G, et al. Induction of a transmissible tau pathology by traumatic brain injury. Brain 2018; 141: doi: 10.1093/brain/awy193.

Your brain scan may be a reflection of your genes

This scientific commentary refers to 'Molecular properties underlying regional vulnerability to Alzheimer's disease pathology', by Grothe *et al.* (doi:10.1093/brain/awy189).

Over the past few decades, MRI and FDG-PET have given us a window into the neurodegenerative sequelae of Alzheimer's disease. More recently, the development of other imaging methods has made the pathological hallmarks of Alzheimer's disease visible within the living brain. PET imaging of the amyloid burden in the brain started with the discovery of Pittsburgh compound B (PiB) about 15 years ago (Klunk *et al.*, 2003; Mathis *et al.*, 2003), and imaging of tau has been possible in the last 5 years thanks to the development of AV-1451 (Xia *et al.*, 2013; Chien *et al.*, 2014). From the very first amyloid image, it became apparent that amyloid accumulation in the brain is diffuse, often present in cognitively unimpaired elderly people, and is also seen in cognitively normal younger people. These observations were further supported by autopsy data, at least in the elderly. This was surprising because the longstanding neurodegenerative biomarker findings in MRI and FDG had demonstrated regional patterns of abnormality that were very distinct signatures of different disease phenotypes in dementia syndromes (Grimmer *et al.*, 2013; Whitwell *et al.*, 2013). In this issue of *Brain*, Grothe and co-workers have attempted to further investigate the disagreement between amyloid deposition patterns and regions of neurodegeneration within the brain by testing for gene expression associations in the differing imaging patterns (Grothe *et al.*, 2018).

In their study, Grothe *et al.* investigated PET and MRI data in 76 patients with Alzheimer's disease dementia and 126 healthy controls enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI). They excluded cognitively normal individuals with biomarker evidence of brain amyloidosis. To test gene expression associations with a hypothesis-driven experiment, they correlated the gene expression profiles for *APP* and *MAPT* genes found in a cortical transcriptome map generated from the Allen Human Brain Atlas with their findings of amyloid distribution using PET and neurodegeneration imaging with MRI. They then derived regional gene expression profiles from brain-wide microarray measurements provided by the Human Brain Atlas of the adult human brain transcriptome that includes data from 3700 brain regions of six normal adults aged 24–57 years. To explore the genetic properties underlying regionally selective vulnerability to amyloid deposition and neurodegeneration, Grothe and co-authors used gene set enrichment analysis (GSEA) of the data. They explored 497 functional pathways from the human reactome database and 1036 gene sets from gene ontology using the GSEA method to determine if predefined gene sets have an association with imaging biomarker phenotypes.

In agreement with previous reports, the current study showed a lack of correlation between the regional distributions of brain amyloid and neurodegeneration. Further, the authors showed that the expression profiles for *APP* and *MAPT* genes on the cortical transcriptome map coincided with the respective findings of amyloid imaging with PET and neurodegeneration imaging tracked by MRI and FDG-PET. On GSEA, in the regions of amyloid deposition, gene sets that regulate various aspects of protein synthesis, response to viral infection and immunity were negatively enriched. Other gene sets that were negatively enriched represent pathways of mitochondrial respiration, including the citric acid cycle and oxidative phosphorylation. In the brain regions vulnerable to neurodegeneration, GSEA revealed 11 positively enriched gene sets encoding: 'cellular differentiation and neurite formation', 'extracellular signal-regulated kinase (MAPK/ERK) pathways', and 'proteoglycan metabolism'. Further neurodegeneration-specific genes coding for diverse classes of molecules jointly involved in developmental processes such as neurite outgrowth,