Sarasso *et al.*, remain central to the quantitative assessment of the degree of perilesional impairment, and, in combination with functional brain imaging and nuclear magnetic resonance tomography, could help unravel the neurochemical basis of slow waves.

In summary, Sarasso *et al.* describe a form of sleep-like activity in the area surrounding the lesioned cortex of patients with chronic focal brain injury. This finding poses a tantalizing question to clinicians and basic researchers alike about the functional significance of local sleep and its relationship to global sleep regulation. The possibility remains that the local slow waves described here represent the electrophysiological expression of a dysfunctional cortex irreversibly trapped in its default mode of activity. Alternatively, local slow waves may reflect neuroprotective or restorative processes, which contribute to recovery. Yet, an even more exciting possibility is that local sleep phenomena reflect the functional state of a brain area unable to partake in information processing, and exerting a detrimental effect on the fine-tuned global sleep-regulatory process. If this is the case, perilesional cortex is only waiting to be awoken: its reintegration into functional brain networks would then help the patient to regain lost functions. Transcranial magnetic, sensory or electrical stimulation methods could make it possible not only to probe local sleep-like mechanisms, but also to awaken the 'sleeping beauty' in the lesioned brain.

Funding

This work was supported by the Wellcome Trust PhD studentship 203971/Z/16/Z, Medical Research Council grant MR/S01134X/1 and Hertford College, University of Oxford.

Competing interests

The authors report no competing interests.

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doi:10.1093/brain/awaa396

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Increased O-GlcNAcylation prevents degeneration of dopamine neurons

This scientific commentary refers to 'O-GlcNAcylation regulates dopamine neuron function, survival and degeneration in Parkinson disease', by Lee *et al.* (doi:10.1093/brain/awaa320).

Parkinson's disease results from the progressive loss of midbrain dopamine-releasing neurons, which control voluntary movement, motivation and reward-related learning and memory. The degeneration of these specialized neurons, which is often accompanied by aggregation of α-synuclein and other proteins into Lewy bodies, leads to behavioural changes, such as shaking, stiffness, and difficulty with walking, balance and muscle coordination. But why are dopamine neurons so vulnerable to degeneration? In this issue of *Brain*, Lee and co-workers present the most comprehensive and convincing evidence to date that a post-translational

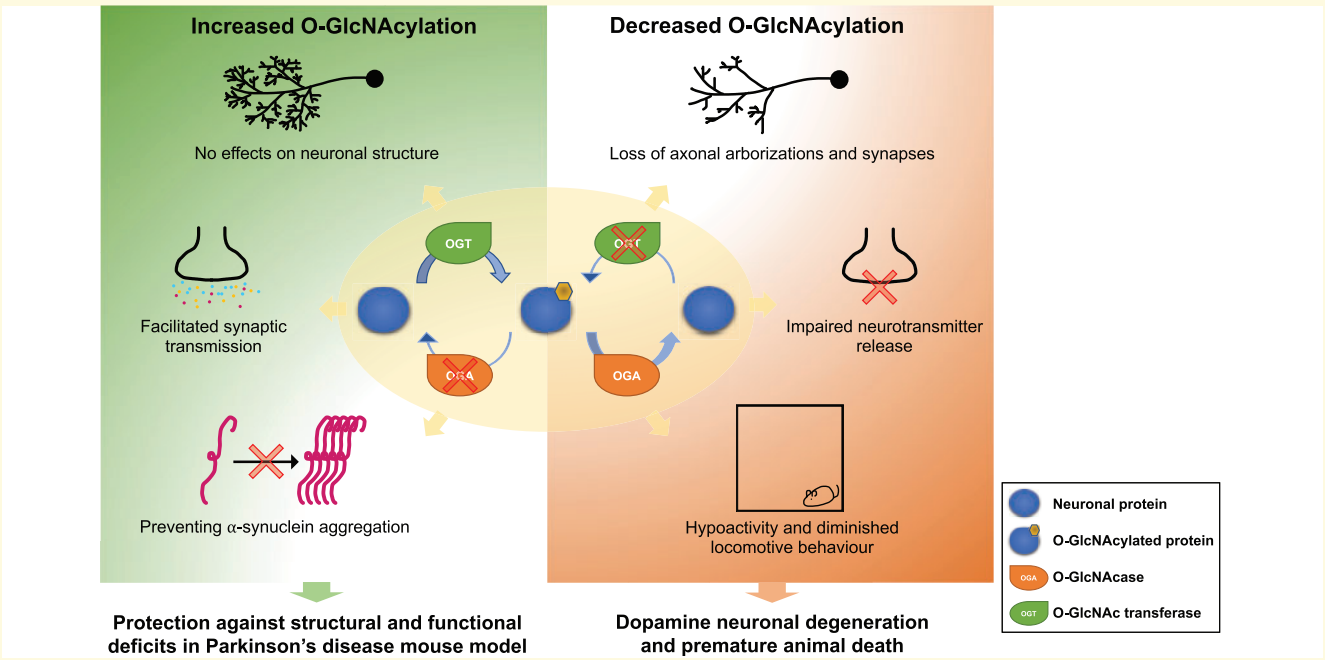

Figure 1 Increased O-GlcNAcylation (OGN) in dopamine transporter (DAT)-expressing neurons protects against Parkinson's disease pathology. *Left*: Protective effects of increased *O*-GlcNAcylation. *Right*: Pathological effects of reduced *O*-GlcNAcylation.

modification called *O*-GlcNAcylation is essential for the survival and functioning of dopamine neurons, and that a reduction in this process contributes directly to progressive Parkinson's disease (Lee *et al.*, 2020).

O-GlcNAcylation refers to the addition of a specific monosaccharide (*N*-acetylglucosamine, GlcNAc) to serine or threonine residues of nuclear and cytoplasmic proteins. The process serves as a nutrient sensor that regulates signalling, transcription, translation, and mitochondrial function in all cells (Hart, 2019). A dynamic interplay (often competitive) may be seen between *O*-GlcNAcylation and protein phosphorylation. After the pancreas, *O*-GlcNAcylation occurs most frequently in the brain, where as many as 40% of proteins are modified by this dynamic sugar, including nearly 2000 proteins in the synapse alone (Trinidad *et al.*, 2012). *O*-GlcNAcylation has been directly linked to a range of neuronal functions, including AMPA receptor trafficking, synapse formation, neuronal excitability, and long-term potentiation and memory (Lagerlof and Hart, 2014).

In recent years, more than 100 publications have implicated reduced *O*-GlcNAcylation in the brain as a causative factor in late-onset Alzheimer's disease and other tauopathies (Yuzwa and Vocadlo, 2014; Gong *et al.*, 2016; Wani *et al.*, 2017). The evidence for a direct role of *O*-GlcNAcylation in neurodegeneration associated with late-onset Alzheimer's disease, in particular, is compelling (Yuzwa and Vocadlo, 2014; Gong *et al.*, 2016; Wani *et al.*, 2017): (i) in mice, fasting decreases *O*-GlcNAcylation and concomitantly increases phosphorylation of tau at several Alzheimer's disease-associated sites; (ii) targeted brain deletion of *O*-GlcNAc transferase by Cre-Lox methods dramatically increases tau phosphorylation; (iii) paired helical filamentous tau (PHF-tau) undergoes ~20% of the *O*-GlcNAcylation of normal tau; (iv) increasing *O*-GlcNAcylation by treating rodents with a very specific *O*-GlcNAcase inhibitor, Thiamet-G, reduces tau phosphorylation and improves learning and memory; (v) the five currently mapped *O*-GlcNAcylation sites on tau are phosphorylated when tau is hyperphosphorylated; (vi) *O*-GlcNAcylation increases the non-amyloidogenic secretase processing of amyloid precursor protein (APP) via *O*-GlcNAcylation of APP and nicastrin (subunit of γ-secretase that generates amyloid-β); (vii) the *OGA* gene, which encodes *O*-GlcNAcase—an enzyme that removes *O*-GlcNAc from target proteins—is located on chromosome 10q24.1, a locus associated with late-onset Alzheimer's disease, and alternative splicing of *OGA* has been linked to Alzheimer's disease; (viii) oral administration of Thiamet-G for 36 weeks to JNPL3 tau mice, which express the most common Alzheimer's disease-causing APP mutation, markedly increased brain *O*-GlcNAcylation, and blocked cognitive decline as measured by a Morris water maze; (ix) synaptic loss is a hallmark of Alzheimer's disease and *O*-GlcNAcylation helps regulate synaptic development and receptor cycling and trafficking to the plasma membrane (Lagerlof and Hart, 2014); and (x) a recent study in mice showed that hippocampal *O*-GlcNAc transferase expression and *O*-GlcNAcylation are decreased in aged mice, and that artificially decreasing *O*-GlcNAc transferase levels in young hippocampus impairs plasticity and cognition. Strikingly, increasing *O*-GlcNAc transferase in the

Glossary

O-GlcNAc transferase (OGT): Highly conserved enzyme that covalently attaches *O*-GlcNAc to thousands of proteins in all cells. OGT is highly abundant in neurons.

O-GlcNAcase (OGA): Highly conserved enzyme that selectively removes *O*-GlcNAc from serine and threonine residues on proteins. The combined action of OGT and OGA cause the cycling of *O*-GlcNAc, analogous to the cycling of phosphate residues on proteins.

O-GlcNAcylation (OGN): The cycling of *N*-acetylglucosamine on serine and threonine residues of proteins, which serves as a major nutrient sensor in all cells. The rate of cycling on and off proteins is site-dependent.

Paired helical filamentous tau (PHF-tau): Tau, which is an important microtubule bundling protein, forms insoluble fibres (PHF-tau) when it is hyperphosphorylated in Alzheimer's disease and other tauopathies. In normal brain, tau is modified by *O*-GlcNAc, which helps prevent its phosphorylation and aggregation.

Thiamet-G (TMG): A highly specific inhibitor of OGA, the enzyme that removes *O*-GlcNAc. TMG analogues readily cross the blood–brain barrier and show promise for the treatment of neurodegenerative disease.

hippocampus rescues these cognitive impairments, even in elderly mice (Wheatley *et al.*, 2019).

However, with the exception of *in vitro* studies showing that *O*-GlcNAcylation of α-synuclein prevents its aggregation (Zhang *et al.*, 2017; Levine *et al.*, 2019), few studies have examined whether changes to OGN could also contribute to the degeneration of dopamine neurons in Parkinson's disease. In their *tour de force* paper, Lee *et al.* therefore used targeted genetic manipulations of *O*-GlcNAcylation in murine dopamine neurons, along with pharmacological manipulations in mice, to increase or decrease *O*-GlcNAcylation. They combined these manipulations with biochemical and proteomic analyses of *O*-GlcNAcylation proteins in the midbrain dopamine area and striatum, and with analyses of dopamine neuronal function (Lee *et al.*, 2020). The results revealed that *O*-GlcNAcylation is essential for the survival and maintenance of the midbrain dopamine system. Downregulation of *O*-GlcNAcylation in dopamine neurons caused severe motor defects, whereas upregulation of *O*-GlcNAcylation produced no adverse effects, but improved function at dopaminergic synapses.

Lee *et al.* (2020) explored the roles of *O*-GlcNAcylation in dopamine neurons and in the aetiology of Parkinson's disease from many different angles. They performed targeted deletion of OGT in dopamine transporter (DAT)-expressing neurons and found that reduction of *O*-GlcNAcylation led to massive loss of axons, loss of axonal arborizations and loss of synapses in the dopamine neurons targeted, which was followed by degeneration and premature death. When *O*-GlcNAcylation was reduced in DAT neurons, the release of several neurotransmitters at synapses was also severely reduced. In contrast, targeted knockdown of OGA in DAT neurons increased *O*-GlcNAcylation and had no deleterious effects on neuronal structure or function; in fact it increased synaptic transmission. In other animal studies, knockout of OGT in DAT neurons led to reduced activity and reduced locomotor behaviour. To better understand the molecules involved in these effects, Lee *et al.* performed proteomic/glycomic analyses of the midbrain dopamine area and the striatum. They identified >900 *O*-GlcNAcylation proteins, including neurotransmitter transporters, synaptic adhesion molecules, postsynaptic density (PSD) proteins, GABA receptors and glutamate receptors. The authors then asked if increased *O*-GlcNAcylation could protect mice from Parkinson's disease pathology induced by injection of an adeno-associated virus (AAV) bearing a mutant form of α-synuclein (A53T). They found that targeted knockdown of OGA not only increased *O*-GlcNAcylation, but also protected the mice from Parkinson's disease pathology. They posited that upregulation of *O*-GlcNAcylation *in vivo* may make dopamine neurons less susceptible to pathological insults in Parkinson's disease by blocking the aggregation of α-synuclein; a possibility supported by earlier *in vitro* studies (Levine *et al.*, 2019). Behavioural analyses of the mice showed that increased *O*-GlcNAcylation in dopamine neurons normalized the behavioural abnormalities in this Parkinson's disease model. Finally, using this same murine model, the authors showed that a specific *O*-GlcNAcase inhibitor, Thiamet-G, increased *O*-GlcNAcylation and effectively prevented and ameliorated Parkinson's disease pathology.

The parallels between the findings of Lee *et al.* on the role of *O*-GlcNAcylation in Parkinson's disease and the many similar studies on the role of *O*-GlcNAcylation in Alzheimer's disease are striking. They suggest that reduced glucose utilization or uptake in parts of the ageing brain, leading to reduced *O*-GlcNAcylation of many brain proteins, contribute to fundamental mechanisms underlying neurodegeneration. This raises the exciting possibility that drugs that increase *O*-GlcNAcylation in the brain could be effective in preventing neurodegeneration and memory loss caused by both Alzheimer's disease and Parkinson's disease.

Funding

G.W.H. and C.-W.H. are supported by NIH R01GM116891 and by the Georgia Research Alliance.

Competing interests

The authors report no competing interests.

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doi:10.1093/brain/awaa398

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Neglected cytotoxic T cell invasion of the brain: how specific for Parkinson's disease?

This scientific commentary refers to 'CD8 T cell nigral infiltration precedes synucleinopathy in early stages of Parkinson's disease', by Galiano-Landeira *et al.* (doi:10.1093/brain/awaa269).

The holy grail for understanding the pathogenesis of Parkinson's disease is to identify the earliest brain changes occurring before loss of the dopamine neurons and abnormal aggregation of the synaptic protein α-synuclein that defines the disease. This was the aim of Galiano-Landeira and co-workers in this issue of *Brain* (Galiano-Landeira *et al.*, 2020). By studying the brains of people with α-synuclein pathology who did not have clinical Parkinson's disease and analysing T cell infiltration into the dopamine cell region, they found that those with incidental Lewy body disease had significant cytotoxic T cells in the absence of dopamine cell loss. Only a single previous report in 2009 had assessed cytotoxic T cells in Parkinson's disease and found they were plentiful in the dopamine cell region (Brochard *et al.*, 2009), a finding that had not been followed-up or replicated until now. In addition to carefully quantifying the amount of dopamine neuron loss and α-synuclein pathology in association with T cell infiltration, Galiano-Landeira and colleagues phenotyped the T cells to provide more evidence for any functional consequences. This is an important and timely study that can inform on how peripheral immune cells impact on neurodegeneration in patients with Parkinson's disease.

This new study confirmed the earlier work of Brochard *et al.* (2009) in patients with Parkinson's disease that first showed T cell infiltration into the dopamine cell region of the midbrain. The numbers of cytotoxic CD8 T cells are substantially greater than the numbers of helper CD4 T cells, as also previously demonstrated (Brochard *et al.*, 2009). A major advance with this new study is the analysis of a greater variety of cases with pathology, including cases with pathology but no clinical disease. There was a robust relationship between the increased numbers of cytotoxic T cells and the loss of dopamine neurons, supporting the idea that the cytotoxic T cells are directly relevant to the loss of these neurons (Galiano-Landeira *et al.*, 2020). However, this study does not attain the holy grail of determining whether cytotoxic T cells initiate the killing of dopamine cells before α-synuclein deposition for the reasons below.

The main reason is the type (and number, $n = 9$) of incidental Lewy body disease cases studied. Three types of cases were assessed. Stage I cases had α-synuclein in the olfactory bulb but not the midbrain dopamine region ($n = 4$), stage II also had α-synuclein Lewy body pathologies in the midbrain dopamine region ($n = 3$), and stage III also had α-synuclein Lewy body pathologies in limbic brain