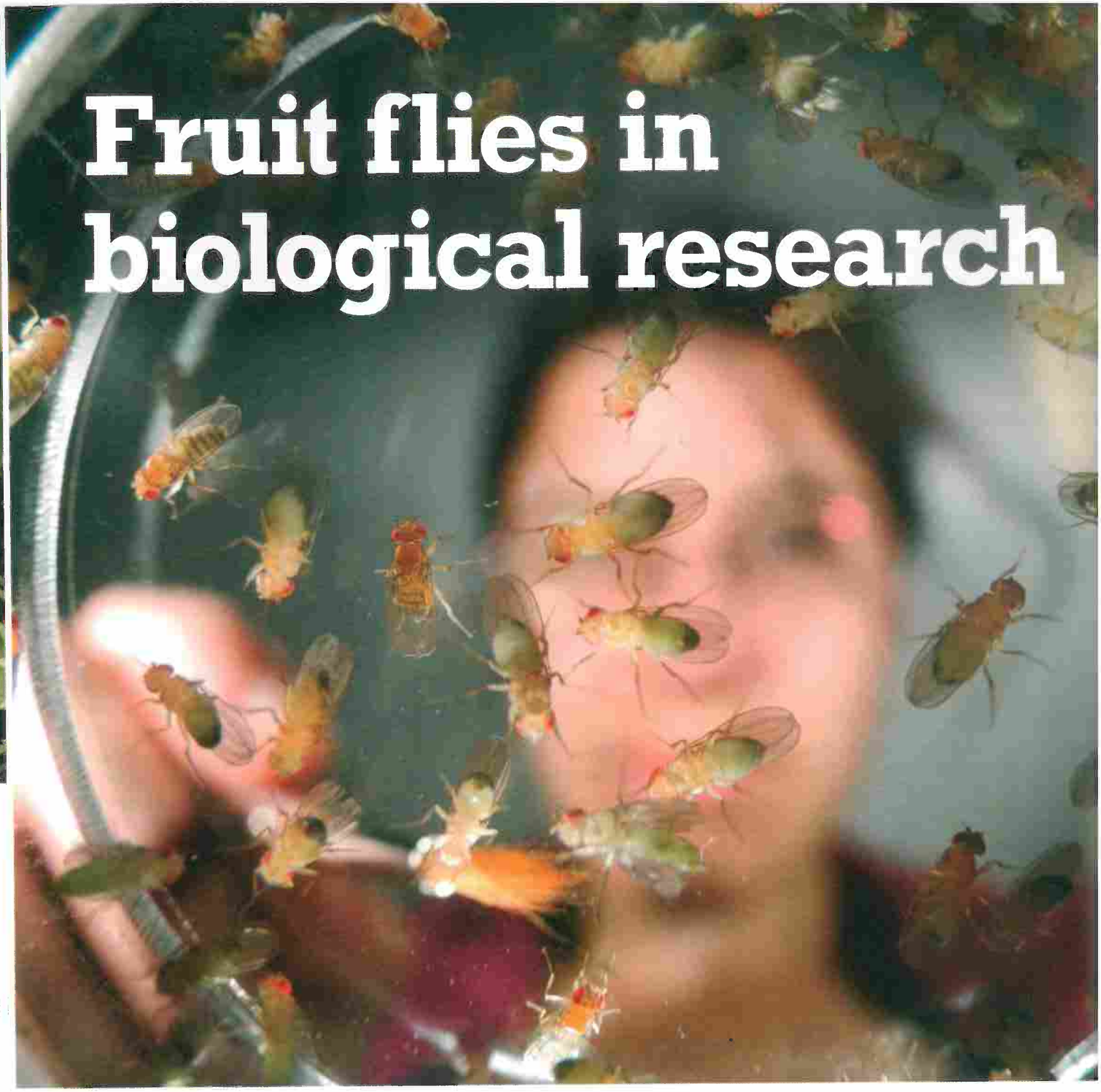
Fruit flies in biological research



Andreas Prokop

Drosophila has remained at the forefront of biological research for over a century. *Drosophila* researchers have made essential contributions to our knowledge of biology and made *Drosophila* one of the best understood organisms. Cellular and developmental neurobiologist Andreas Prokop explains why

Key words

Model organisms
Drosophila
Nervous system
Ageing
Neurodegeneration

More commonly known as the fruit fly or vinegar fly, *Drosophila melanogaster* was introduced as a genetic model organism by T. H. Morgan over 100 years ago. This fly has allowed us to understand many important biological processes and mechanisms and advanced human health research. Seven scientists have been awarded the **Nobel prize** for physiolog or medicine for their research on *Drosophila*.

Why use fruit flies?

Drosophila has useful attributes that have fuelled it widespread use in biological research (see Figure 1 Flies are cost-effective and affordable model animal

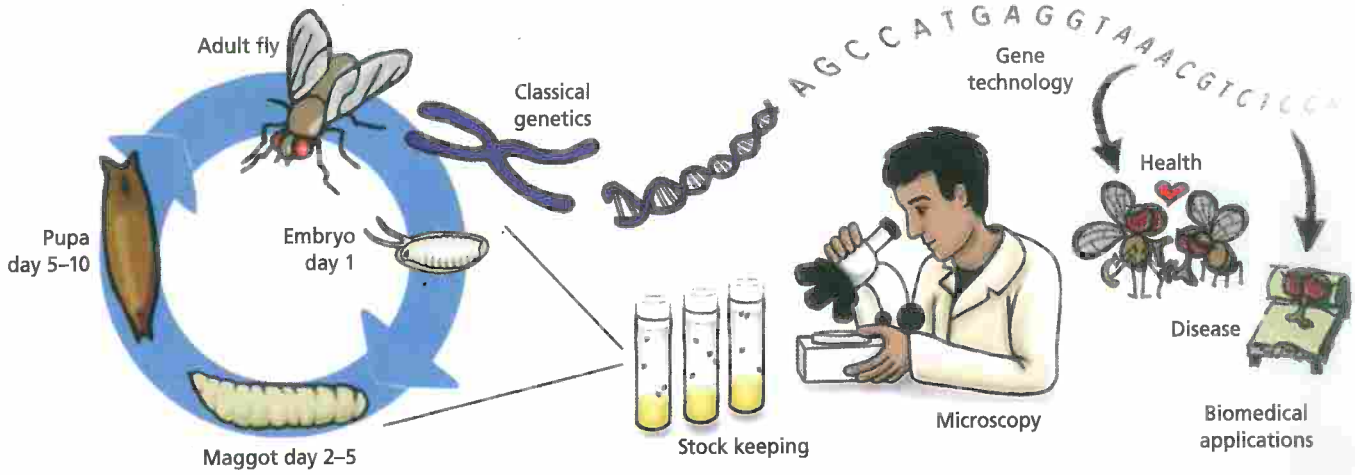


Figure 1 Research in *Drosophila* is fast and has practical advantages at many levels of the investigative process

because they are easy and cheap to breed in great numbers and they have a short generation cycle (10 days) and life span (2 months). This makes large-scale, high-throughput experiments easy. In particular, unbiased searches (called 'screens') for new genes underlying biological processes have been important drivers of modern bioscience. Highly sophisticated and efficient experimental and genetic strategies and readily available tools for virtually every fly gene (e.g. **mutations**, **transgenic flies**, **antibodies** to detect their gene products) speed up *Drosophila* research enormously. Finally, they are small, making it easy to analyse whole fly embryos or tissues in large numbers and at high resolution under the microscope.

Flies and humans have common evolutionary roots and display striking similarities at the level of their genes, cells, tissues and the biological processes underpinning their development, health and disease (see Figure 2). Of course, flies are not mini humans, but 75% of human disease genes have a match close enough to be studied in flies. This often inspires and enormously accelerates research on these genes in mice or even humans.

Using *Drosophila* to study neuronal axons

To give an example of how *Drosophila* is used in current research, let's take a look at my own research into the development and maintenance of the nervous system. This research is funded by the Biotechnology and Biological Sciences Research Council. I study nerve cells, in particular the cable-like extensions of neurones called axons that can grow up to a metre long in humans (see Figure 3). These axons transmit nerve impulses, passing them on to other neurones via synapses (see Figure 3 and Biological Sciences Review, Vol. 28, No. 2, pp. 2–5 and Vol. 28, No. 3, pp. 26–29).

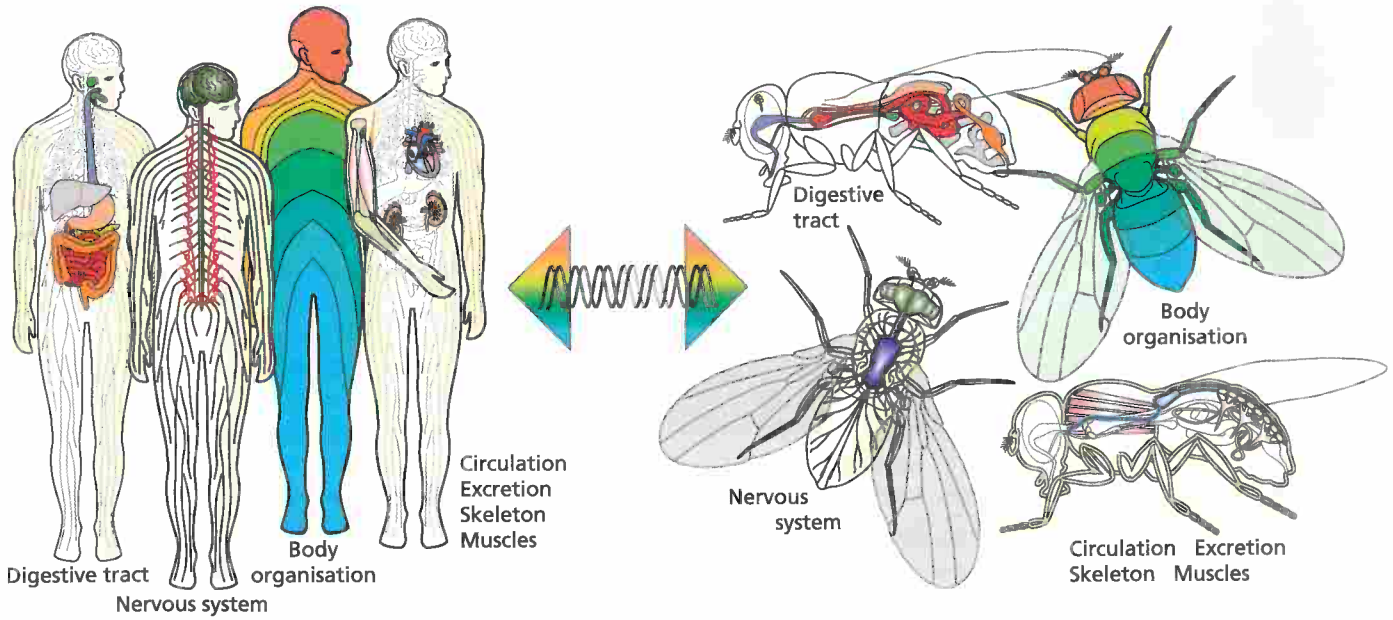


Figure 2 For most human organs there is a match in flies, and common genes tend to regulate their development, organisation and function

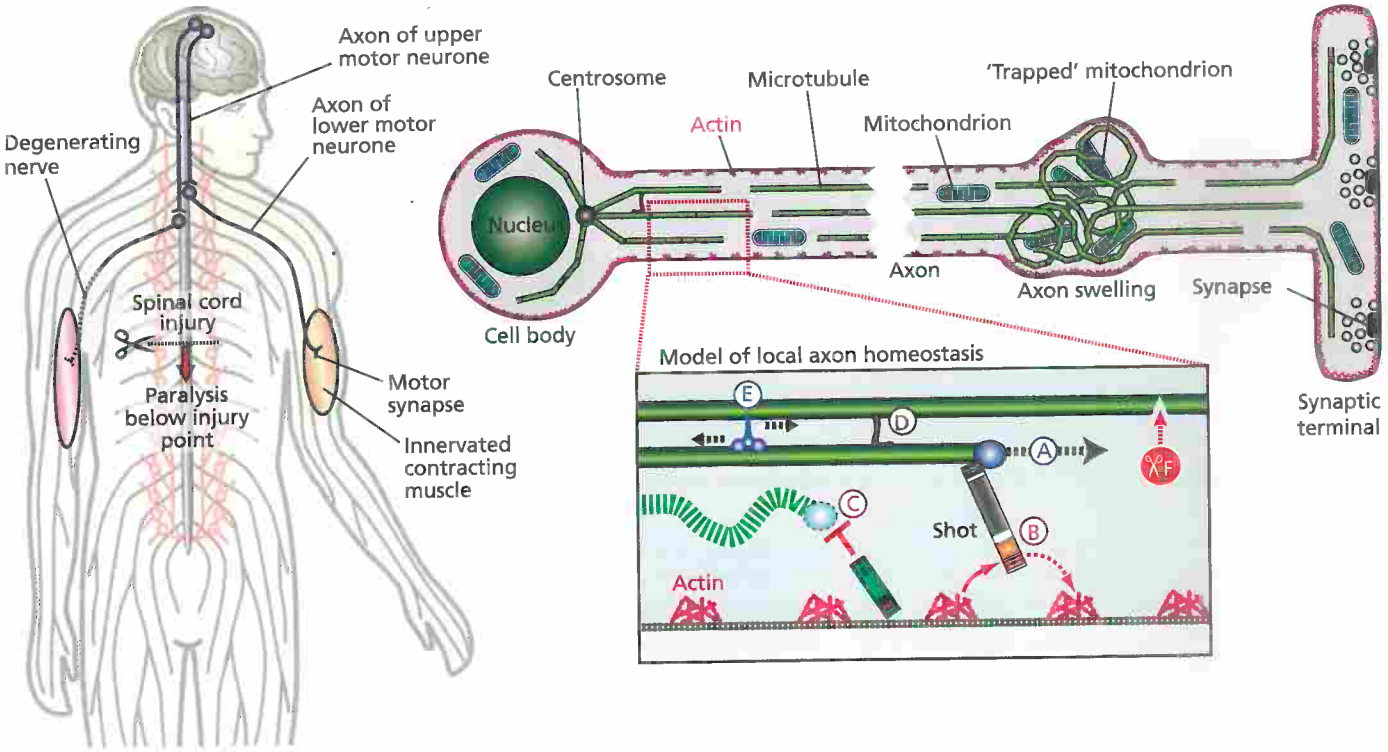


Figure 3 Axons are the cable-like extensions of nerve cells that wire the brain. If axons are damaged by injury, or degenerate, the muscles innervated by them become paralysed. Bundles of microtubules (green bars) form the axon structural backbones, which are maintained by different sets of proteins (A–F, see main text for details) to prevent the formation of harmful axon swellings. The green stippled line represents an 'off track' microtubule leaving the straight bundle

Terms explained

Antibodies The mammalian immune system uses antibodies to detect and eliminate harmful intruders. When mammals (e.g. rabbits) are injected with proteins from flies (or other species), they produce antibodies against these proteins. These antibodies can be isolated from the rabbit blood and used to detect the localisation of these proteins in flies (as shown in Figure 4).

Coma State of unconsciousness that can be caused by axonal damage in the brain stem through injury.

Mutation A defect in the DNA sequence of a gene.

Neurodegenerative disease A disease that accelerates the loss of neural elements beyond a body's capacity to compensate; symptoms of these diseases are more likely to manifest in old age (see also **http://tinyurl.com/pqmytn7**). Examples of neurodegenerative diseases mentioned in this article are:

- **Alzheimer's disease**: decay of the brain, leading to dementia
- **Charcot-Marie-Tooth disease**: affecting primarily sensory nerves and leading to loss of touch sensation and body control
- **Hereditary sensory and autonomic neuropathy**: loss of the neurones of the sensory and non-voluntary nervous system
- **Hereditary spastic paraplegias**: progressive stiffness, primarily in the lower limbs, as a result of motor neurone decay
- **Motor neurone disease**: loss of upper and/or lower motor neurones, eventually leading to paralysis
- **Parkinson's disease**: primarily loss of specific nerve cells in the midbrain region, leading to loss of body coordination

Nobel prize The highest award given to researchers.

Spinal cord injury Damage to the spinal cord that tends to cause irreversible paralysis below the point of injury.

Transgenic flies Flies that carry an artificially introduced DNA fragment in their genome. They are usually generated to study gene functions.

During development, these axons grow alon precise pathways, thus forming the circuits tha control our bodies and coordinate our behaviours The importance of these delicate axons i exemplified by the paralysis that results fron **spinal cord injury** (see Figure 3) or **coma** after hea injury. During normal ageing we lose about 50% of our axons, a rate that is greatly accelerated i **neurodegenerative diseases** such as **Alzheimer** and **Parkinson's** disease.

Focus on axon structure

To understand axons in health and disease, I stud their structure and how they are formed fron parallel bundles of microtubules (see Figure and Biological Sciences Review, Vol. 28, No. pp. 15–19). Microtubules are structural protei polymers arranged into hollow filaments ~25 n in diameter. They are constantly elongatin and shrinking in a process that is regulated b microtubule-binding proteins (see Figure 3 Studying these proteins is important becaus mutations in genes encoding them often cau neurodegenerative diseases in humans. Laborato experiments can reveal how these protei influence microtubule dynamics in principle. B it is difficult to understand how they function i health and disease, because they cooperate with complex regulatory processes that are difficult take apart and study.

This can be more easily achieved in neurones of the fruit fly. We can put neurones from *Drosophila* embryos into culture and grow them for 6 hours to study axon development, or grow them for up to 30 days to study axon maintenance. Using microscopy, we can visualise these axons in great detail (see Figure 4). To study the role of specific proteins in axon organisation and maintenance, we culture neurones from *Drosophila* embryos carrying mutations in the genes encoding specific proteins. One key advantage of using *Drosophila* for this work is the speed and ease with which we can look at the effects of many different mutant genes, alone or in combination, to investigate their functions and cooperation during axon development.

Using this strategy we have developed a new concept of axon biology that we call the 'model of local axon homeostasis' (see Figure 3; homeostasis is Greek for 'staying in balance'). Microtubules are constantly renewed through steady-state turnover involving their removal and re-polymerisation (A in Figure 3). By default, these newly forming microtubules would arrange into chaotic criss-crossed networks (axon swelling in Figure 3), if there were no microtubule-regulating proteins

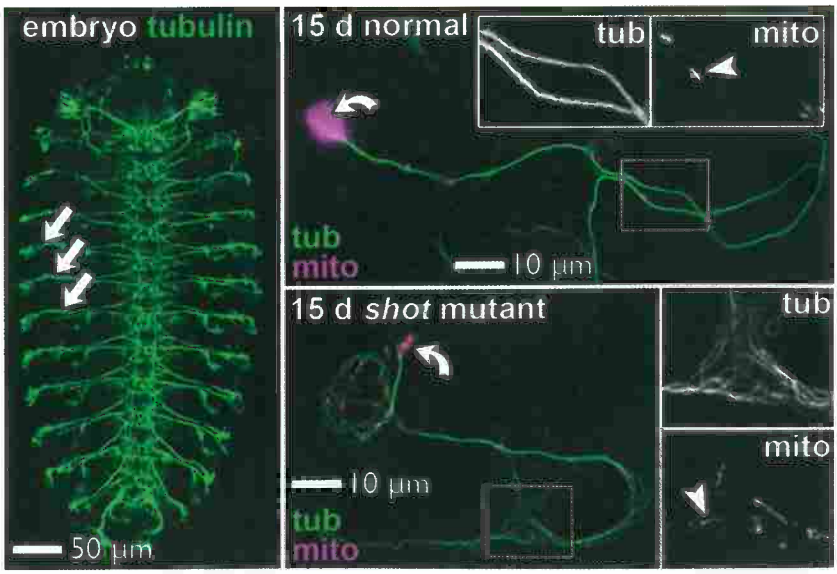


Figure 4 Left: Visualising axonal microtubules (tubulin, green) in a fly embryo shows the regular pattern of motor nerves (arrows). **Right:** A normal fly neurone at 15 days in culture (curved arrow points at cell body) shows bundled microtubules and slightly elongated mitochondria (mito, arrowhead). In neurones lacking Shot (lower panel), microtubules are disorganised and mitochondria abnormally shaped and distributed. Boxed areas represent two-fold magnified close-ups showing one stain at a time (images were generated using antibodies linked to fluorescent stains)

to ensure ordered bundle organisation through a number of mechanisms. For example, we showed that the Short stop (abbreviated 'Shot') protein can guide the extension of newly forming microtubules along actin at the axon surface (B in Figure 3), thus laying them out into regular bundles. Another protein sits at the axon surface and, if microtubules leave their bundle and go off-track, it can capture and eliminate them (C in Figure 3), thus correcting any errors that occur during Shot-mediated guidance. Another set of proteins cross-links microtubules with one another and stabilises them, thus maintaining bundles once they are formed (D in Figure 3).

Neurodegenerative diseases

Our fly-derived model predicts that different mechanisms work together towards one common goal: the maintenance of microtubule bundles. Since nothing biological is perfect, there is a possibility that this machinery will fail and cause harmful axon swellings with disorganised, curled microtubule arrangements where mitochondria get trapped, leading eventually to axon degeneration (see Figure 3). Axon swellings are frequently found in the ageing brain. This offers one explanation as to why we lose 50% of our axons as we age. We predict that such swellings become more frequent if the microtubule machinery is weakened by mutations in microtubule regulators, potentially explaining why several of these mutations cause paralysis through nerve degeneration (see Figure 3). For example, mutations in the microtubule regulators spastin (which cleaves microtubules — F in Figure 3), kinesin or dynein (two microtubule-associated motor proteins driving transport and generating forces — E in Figure 3) cause **hereditary spastic paraplegias** or **Charcot-Marie-Tooth disease**. Mutations in dynactin (which partners with dynein) cause **motor neurone disease**, and mutations in dystonin cause **hereditary sensory and autonomic neuropathy** (dystonin is the human protein that corresponds to *Drosophilia* Shot — B in Figure 3) and, as in *Drosophila*, dystonin-deficient mice show microtubule disorganisation. A mutation of human dystonin affecting its interaction with microtubules was recently shown to cause the same type of neurodegeneration as shown in mice, and our model offers a plausible explanation.

In conclusion, our model derived from work in flies predicts that microtubule disorganisation may be a potential common disease mechanism fo mutations in microtubule-regulating genes. Usin flies, we test, challenge and refine this model, an then perform focused experiments in mice to tes whether our ideas hold true in higher animals Even if future work proves aspects of our mode wrong, work in the fly will have provided innovativ ideas to influence and advance concepts of nervou system ageing and degeneration.

This is just one example of how *Drosophil* remains an important pillar in the process c scientific discovery and continues to spearhead ne research trends as a constant generator of ideas an conceptual understanding.

Further reading

http://tinyurl.com/jbs52y3 Two 'Small fly, big impact' YouTube movies describing the origins and importance of fly research (part 1 — 'Why the fly?') and how research in flies can help us to understand disease and find potential treatments (part 2 — 'Making research fly').

https://droso4schools.wordpress.com The 'Why fly?' page explains the advantages of *Drosophila* in research. The 'Organs' page compares tissues and organs of flies and humans with helpful overview images; the L1 tab explains the use of flies for neurodegeneration research; the L3 tab explains the working of nerve cell networks. Other tabs on this site provide curriculum-relevant biology sample lessons, as well as information on the biology of alcohol and statistics.

www.flyfacility.ls.manchester.ac.uk/forthepublic The Manchester Fly Facility has put together additional information for the public and school teachers: the 'Why the fly?' tab complements the information on droso4schools, providing simple facts, non-specialist books and over 80 lay articles about fly research; the 'Outreach Resources' page provides many other exciting links to *Drosophila*-specific information and resources.

www.prokop.co.uk/Research/LAYMAN/1-brain-intro.html An eight-page layman's guide to principles of neuronal circuits and synapses, also explaining how flies are used to study them.

www.youtube.com/watch?v=E_r-mfMc610 A short film explaining the axon model of local homeostasis.

http://tinyurl.com/z5bu8hc This blog explains why *Drosophila* is not only great for research but also a powerful teaching tool for biology lessons.

Brookes, M. (2001) *Fly: the Unsung Hero of Twentieth-Century Science*, Ecco; explains the history and importance of *Drosophila*.

Andreas Prokop is professor of cellular and developmental neurobiology at the Faculty of Life Sciences at The University of Manchester. As well as his work described here, he is engaged in schoolwork using *Drosophila* as a modern, curriculum-relevant teaching tool, which makes biology lessons lively and memorable.

Key points

- *Drosophila* has been used as a model organism in biomedical research for over 100 years, leading to seven Nobel prizes in physiology or medicine.
- *Drosophila* has advanced our understanding of fundamental biology in many areas, most of them highly relevant for understanding important diseases
- Axons are the cables that wire the brain. They are actively maintained to prevent nerve degeneration.
- The mechanisms maintaining axons can be studied in flies. These studies have led to a novel concept of local axon homeostasis.