NEURODEGENERATIVE DISEASE

Machine learning for leg tracking

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Drosophila melanogaster is as an invaluable model to study human neurodegenerative diseases. Fruit flies can be genetically engineered to exhibit molecular features of human diseases such as Parkinson's disease (PD) and spinocerebellar ataxia type 3 (SCA3), thus increasing our understanding of the underlying mechanisms. However, to what extent these mutant flies recapitulate all clinical aspects of the disease is not clear.

"It is well known that human patients with different movement disorders exhibit stereotypic behaviours that are important for clinical diagnosis; it was not known whether fly models of different neurodegenerative diseases walked differently from one another, or whether their movements bore any similarities to the human disease," explains Sherry Shiyong Aw from the Institute of Molecular and Cell Biology in Singapore. To address these questions, Aw and her team developed a machine-learning method to track leg movements and characterize gait in fly models of PD and SCA3. Their results published in *PLoS Biology* reveal that *Drosophila* models of neurodegenerative diseases do exhibit movement signatures that recapitulate the motor phenotype seen in patients.

In a previous study, Aw and her team evaluated the climbing defects caused by mutations of a glio-protective microRNA by using the climbing assay. This test capitalizes on the natural tendency of flies to climb and has been the gold-standard to quantify the effects of genetic mutation and/or environmental condition on *Drosophila* climbing behaviour for more than two decades. Although the assay successfully identified mutants with climbing defects, it failed to quantify the subtle trembling-like behaviour characteristics exhibited by some flies. "While fly techniques for the manipulation and study of neuronal subsets were becoming increasingly sophisticated, the richness of the resultant behavioural phenotypes was not being captured at a similar detail," says Aw, adding that no method was suitable for the type of study she wanted to conduct. Therefore Aw struck up a collaboration with Li Cheng from the Bioinformatics Institute in Singapore to develop a machine-learning program to study fly movements in finer detail.

Representative FLLIT-derived walking leg traces of the respective genotypes. Reprinted with permission from Wu et al. (2019) PLOS.

Feature Learning-based LIMb segmentation and Tracking (FLLIT) was the result. FLLIT is designed to track leg positions on video recordings of freely moving flies without requiring marking the fly leg, a time-consuming process that limits experimental throughput. Researchers don't need to manually annotate images to pre-train FLLIT either—it was designed to generate its own training sets, setting this method apart from other recently developed deep-learning approaches for movement tracking.

The researchers deployed FLLIT to analyse the gait of three mutant flies: SCA3 flies expressing a mutant form of Spinocerebellar ataxia type 3 protein (SCA3 also known as Ataxin-3) with an expanded number of repeats (SCA3 fl Q84) in all neurons; PD *Drosophila* expressing human alpha-synuclein (SNCA), and homozygous *parkin* mutants, another model of PD. FLLIT showed that fly models of SCA3 and PD walked very differently; similar to what is seen in patients with SCA3, SCA3 flies exhibited repeated veering and lurching, whereas PD models showed some traits of hypokinetic movements, a feature observed in patients with PD. The two different PD models showed similarly rigid gaits even though the flies came from completely different genetic backgrounds. "This suggests that fly models of PD and SCA3 have specific, distinct and conserved gait signatures," says Aw.

SNCA overexpression in flies has been associated with dopaminergic cell degeneration. Hypothesizing that dopaminergic neuron dysfunction might be the common cause of movement disorders in both PD models, the investigators overexpressed SCA3 mutant protein only in dopaminergic neurons. The resulting gait of these mutant flies more closely

resembled that of the PD flies, suggesting that the behavioral phenotype depends on the neurons affected rather than the specific nature of the mutation.

FLLIT also helped capture previously unseen detail. Tremor—an involuntary muscle contraction that causes uncontrollable shaking—is a common feature of several neurodegenerative diseases. Leg tremor behaviour in fly degeneration models however had never been characterized before, because quantification methods were not sensitive enough to allow the measurement of these fine movements. Here, the investigators used FLLIT to study leg tremors in freely walking flies for the first time; they found that mutant flies expressing SCA3 in all neurons were the only mutant flies to exhibit tremor in this setting.

Aw explains that next, the team plans to use FLLIT to study the circuits and cellular mechanisms that underlie various distinct movement defects, including tremors, which are very prevalent in people but remain poorly understood. "With FLLIT, we can now use the fly model to study the neurogenetic mechanisms that underlie movement disorders, which are of growing importance in our aging societies," she says. "We believe that such detailed phenotyping will also be applicable for behavioural phenotype-based drug screening, for which the climbing assay may not offer enough resolution," Aw concludes, adding that she is looking for industrial collaborators to work with on such drug screening projects. The FLLIT program, readme and sample data can be found on [GitHub](#).

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