Automated Monitoring of *C. elegans* Movement for Neurodegeneration and Ageing: Tissue and Lifecourse Matter

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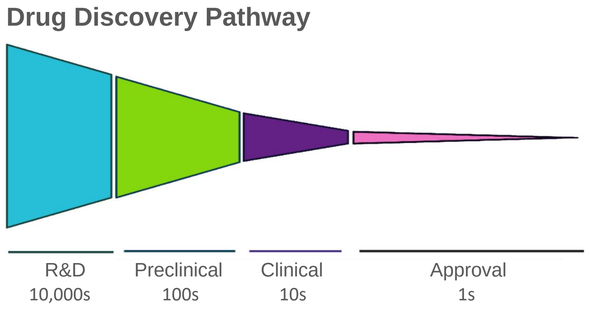
Neurodegenerative diseases: A great need and a greater challenge

Neurodegenerative disease drug development is well known for its multiple high-profile late-stage failures. While 40% of over-60s in the US show signs of preclinical Alzheimer's disease (AD, the rate of failure for disease-modifying therapies is 100% (Cummings *et al., 2019)*. Further drug development is hindered by a number of factors, including the lack of physiological and predictive animal models. Current mice models use artificially accelerated ageing, which does not reflect the natural age-related disease progression in humans, and have a large ethical footprint.

The nematode *C. elegans* has a track record of relevance to ageing pathways conserved in mice and humans (eg. IGF-1, mTOR signalling) and has been used to generate models of neurodegenerative disease through overexpression of human proteins implicated in these diseases, which leads to loss of function phenotypes that can be scored and used to test interventions.

Drug Discovery Pathway



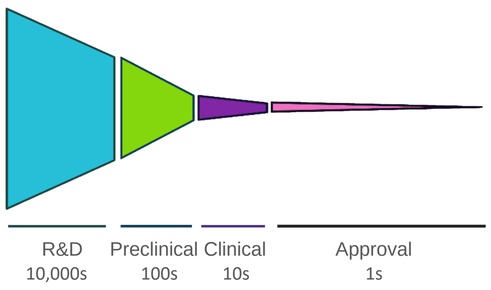
Muscle vs neurons

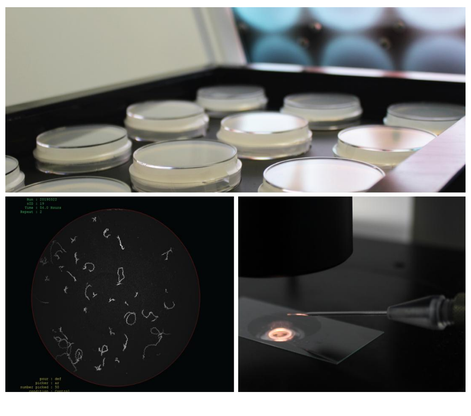
The strongest phenotypes in *C. elegans* models have arisen from overexpression in muscle cells, which gives rise to paralysis, This phenotype is relatively straightforward, albeit tedious to assess manually. Overexpression in neurons produces a range of phenotypes such changes in movement, lack of ability to move towards an attractant or explore properly. However these phenotypes are much harder to assess manually and thus the majority of studies use muscle-specific overexpression models.

Acquiring data throughout adulthood, not just old age

Age-related diseases, although most debilitating in old age, can be detected through symptoms that appear earlier in adulthood, raising the question of when to start treatment and what interventions might be effective at which stage of disease progression. Magnitude Biosciences automated imaging platform monitors worms near-continuously from early adulthood, so the precise timing of mobility decline and interventions effects can be detected.

Potential Drug Discovery Pathway with *C. elegans*



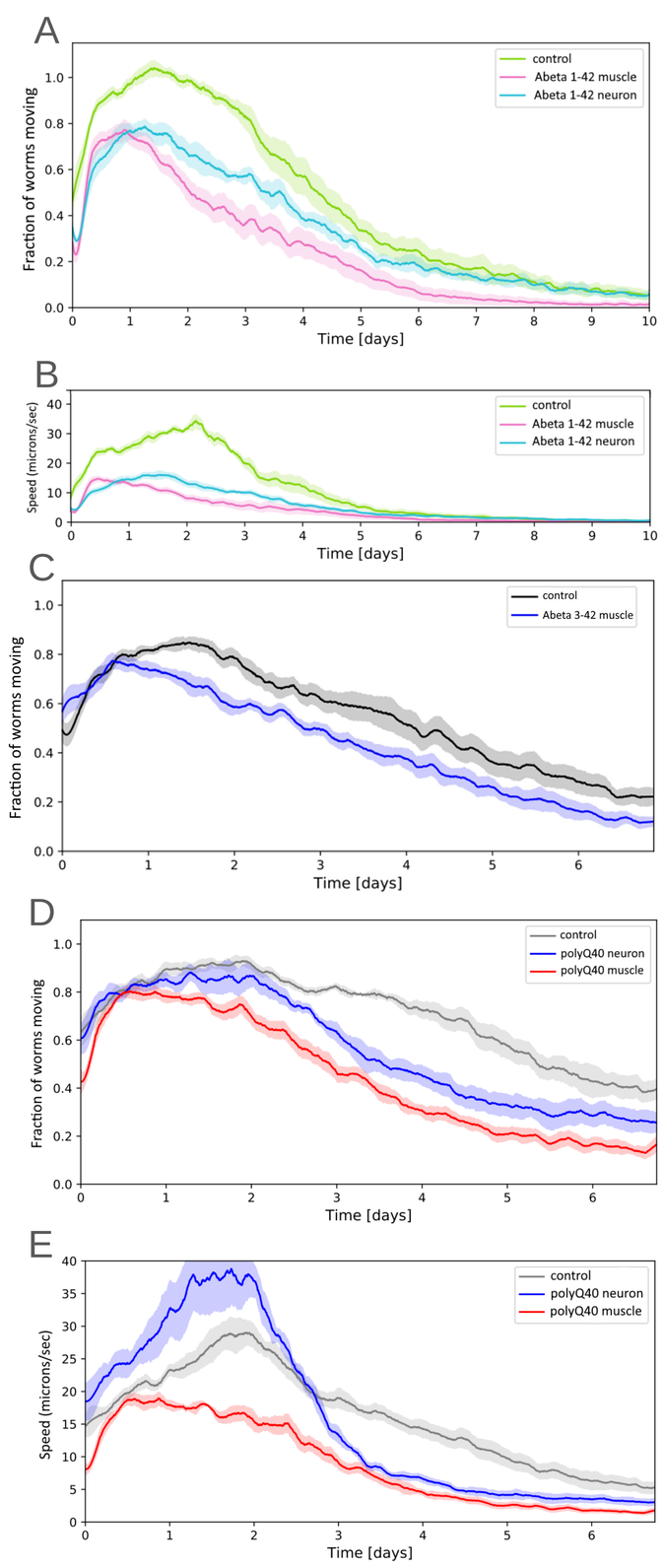


Magnitude Biosciences Automated Imaging Platform

Up to 90 Petri dishes of 30 worms each automatically tracked simultaneously by separate small cameras each controlled by a single board computer.

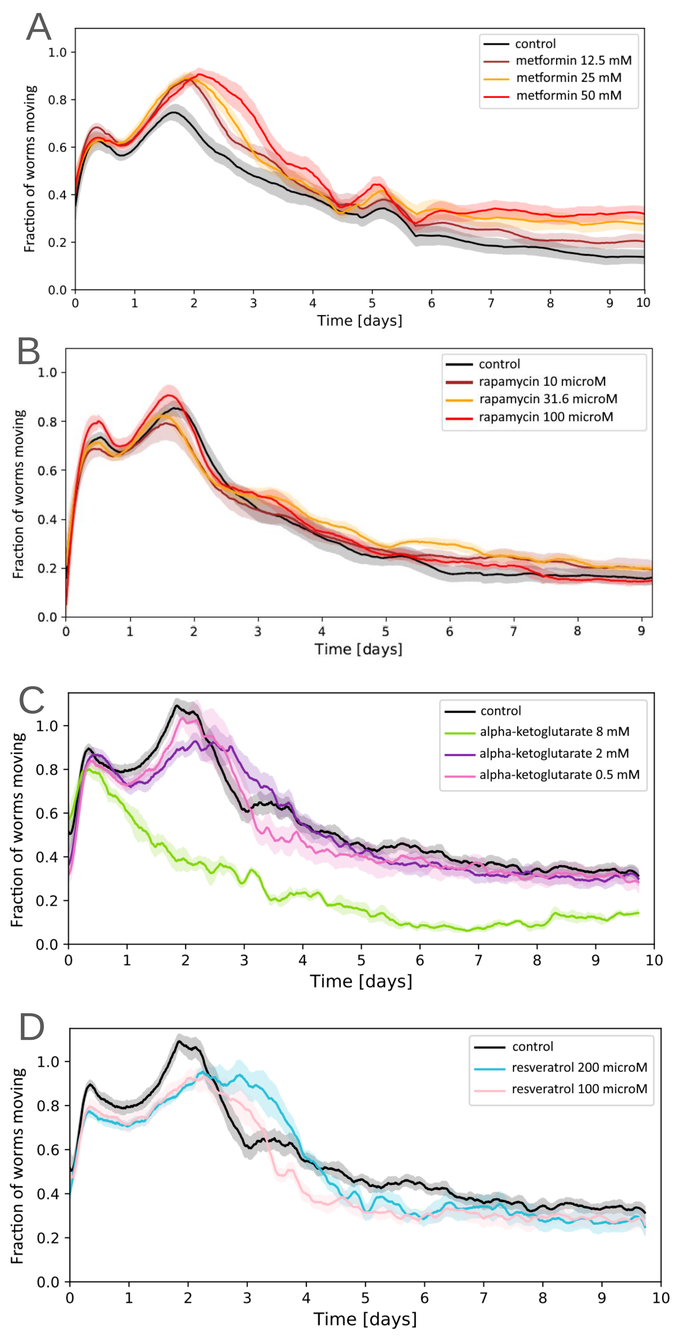
- Near-continuously movement tracking: images taken every 0.8 seconds for 160 seconds, repeated every 5 minutes, for up to 10 days of worm adulthood.
- Non-invasive: no mechanical disruption, no abrupt changes in lighting or temperature.
- Multiple mobility parameters: worm speed, position, percentage moving, population fragmentation by speed, speed decline over time, chemotaxis, exploration, paralysis, increases in population size in fertile worms.
- Standardised reagents, protocols and schedules for manual worm maintenance prior to automated assays
- Assays monitored remotely

Top: Petri dish array set for illumination and image acquisition. Bottom Left: Representation worm tracks recording. Bottom Right: Micro-injection needle for transgenic strain generation.



LEFT- Figure 1: Mobility differences with tissue expression in muscle vs neuron-expressing neurodegenerative disease models of *C. elegans*.

C. elegans strains expressing amyloid beta 1-42 in either their muscle or neurons (Alzheimer 's disease model) both show marked decreases in mobility (A) and speed (B) compared to control,. Although this decline is greater is the muscle-expressing strain, the neuron-expressing strain is the more physiological model. By contrast a muscel-expressing strain of amyloid-beta 3-42 shows a smaller decline in mobility(C) , as expected from the known absence of its pathological involvement in humans. *C. elegans* strains expressing poly-glutamine repeats in either their muscle or neurons (Huntington's disease model) also both show decreases in mobility, with again the muscle-expressing strain showing the strongest effect. (D). The effect of tissue expression on speed is profound, with i.e. increased speed in early adulthood in muscle-expressing worms while speed is decreased throughout in neuron-expressing worms (E).



RIGHT - Figure 2: Time-dependent effect of ageing interventions across the adulthood of *C. elegans*.

Exposing a wild-type *C. elegans* strain to a range of ageing interventions already exemplified in other models (mice and/or humans) reveals age-specific effect s on mobility. The diabetes drug metformini ncreases mobility in a dose-dependent manner throughout adulthood (A), while rapamycin (inhibitor of the mTOR pathway) show s beneficial effects only in old age and at a narrow concentration window (B). Alpha ketoglutarate, which show reduced activity in Alzheimer's, has a marginal beneficial effect at a narrow time window in middle-age but a marked toxicity at higher doses (C). Likewise, resveratrol, a plant phenol linkied with sirtuins and which has been shown by some to slow down progression of mild to moderate Alzheimer's (Turner *et al.*, 2015), has a beneficial effetc in middle0age, followed by an earlier decline in mobility (D).

References:
Cummings et al., 2019. *Alzheimer's Research and Therapy*. The ''rights'' of precision drug development for Alzheimer's disease.
Turner et al., 2015. *Neurology*. A randomized, double-blind, placebo-controlled trial of resveratrol for Alzheimer disease.