The effect of lithium on Frontotemporal Dementia

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Introduction:

Frontotemporal Dementia (FTD) is a neurodegenerative disease highly prevalent in those under 65. The most common genetic cause of FTD is a hexanucleotide repeat expansion in the 72nd ORF region on chromosome 9, known as C9orf72. The resultant toxicity is due to the production of dipeptide repeat proteins, which are synthesised by the non-canonical non-ATG initiated translation. The non-ATG initiation site is introduced within the hexanucleotide expansion in C9orf72. A model with 36 hexanucleotide repeats was developed in *Drosophila melanogaster*, which could be inducibly expressed using the Gal4-UAS system. Previous research has shown that lithium, which can ameliorate Aβ toxicity in Alzheimer's, may also mitigate toxicity observed in C9-expressing flies. Two key genes, Cdk5 and Sgg, are vital in normal neuronal function, cell signalling, and gene expression. The downregulation of these contribute to the pathophysiology of FTD, which affects the lifespan of flies expressing downregulated Cdk5 and Sgg. Their protein products, kinase enzymes, are also known targets for lithium, making them ideal for this study.

Aims of the project

The overall aim of this project is to investigate the effect of lithium on the lifespan of C9orf72-expressing *Drosophila melanogaster.* Specifically,

- To set up stocks of three genotypes of flies: downregulated Cdk5, downregulated Sgg and "wildtype", and to induce C9-linked FTD.
- To set up, run and analyse lifespan assays of these flies, both with and without lithium

Methods used

To achieve these aims, 100 female virgins from each relevant stock were collected. These were crossed with C9-expressing males in a 4:1 ratio; 25 males were placed into each mating cage. To practise the techniques, in my first week I collected virgins and set up a mating cage from the v-w+ stock only. In the second week, I collected and set up cages for all three stocks (to generate comparable results). The cages were tipped on the next day, and I collected eggs on the 2 subsequent days (day 2 and 3). I collected the eggs by adding phosphate-buffered saline and drawing up the suspension using a cut-tip pipette. I deposited these eggs into bottles containing food and incubated at 25℃ for approximately 10 days, until the flies had hatched and mated. At this point, I collected females from the bottles, taking care to select against flies expressing TM6b, a balancer chromosome. If selected, these would be unaffected by the C9 gene, and thus impact results. From each genotype, I wanted to split flies into 10 vials of 15 flies (150 in total) for each of four conditions, either exposed to RU-486 (which induced the C9 gene), exposed to Lithium, both, or neither. In other words, I required 600 flies from each of v-w+, cdk5 downregulated and Sgg downregulated. These vials were then placed into Drosoflipper frames, to allow for scoring and tipping of lifespans. I scored the number of dead flies and tipped the lifespan assays for each condition every two days, putting my results into a pre-formatted Excel sheet.

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Results and outcomes

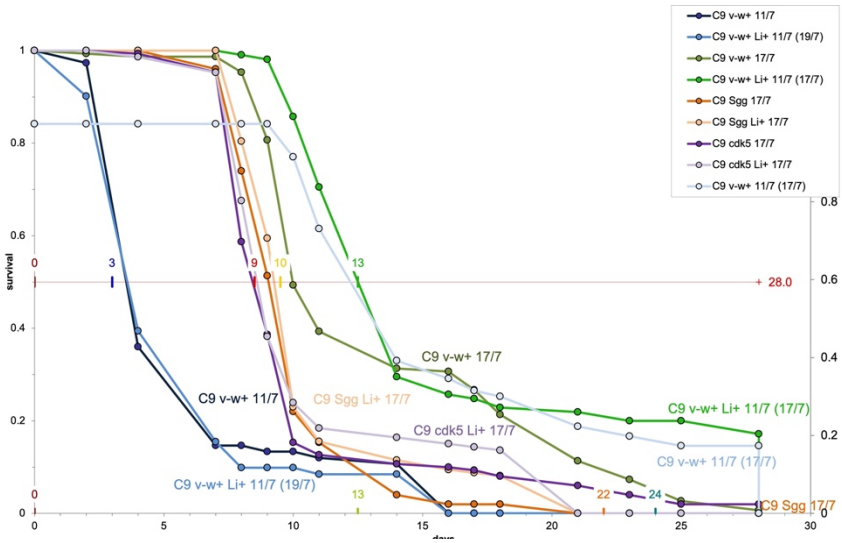

Figure 1 Survival plot of flies on RU+

In my hands, I found that lithium made a statistically significant improvement to the lifespan of C9 cdk5 flies (P=0.012). However, the other conditions did not experience a significant difference under lithium. This could be due to a number of reasons, such as experimental design and the inherent variability expected when using living organisms as model systems. There could also have been flaws in the execution; for example, we had issues with collecting an adequate number of flies for certain conditions (see below).

Future directions

In the future it may be possible to further studies to determine why lithium did not have the intended effect, as previous studies have shown its efficacy in similar scenarios.

Departures from original plan

Despite collecting seemingly enough eggs, I was unable to fulfil the required 600 flies from each genotype (see breakdown above) when collecting from the cages I set up in my second week. I was able to get 10 vials of 15 flies for each of my RU- conditions (C9 cdk5 Li-, C9 cdk5 Li+, C9 Sgg Li-, C9 Sgg Li+) except for C9 v-w+. I also got 10 x 15 flies for the following conditions on RU+: C9 cdk5 Li-, C9 cdk5 Li+, C9 Sgg Li-, C9 Sgg Li+ and C9 v-w+. To supplement the data I would obtain for C9 v-w+ flies on RU+, I used flies I had collected from my first cage, which I had split evenly into an RU+Li- and an RU-Li- condition. The ones I had previously on RU+Li-, I split between RU+Li-, and RU+Li+, giving me 5x15 flies for each, and reserved ~100 for a western blot. The ones I had previously on RU-Li-, I split evenly for RU+Li- and RU+Li+. This gave me flies that had been on RU+ since splitting (and some put on lithium late) and flies that were put on RU+ late (with and without Lithium).

Value of studentship

This experience was incredibly valuable to me. Not only did I learn advanced laboratory techniques such as western blotting and qPCR, but I developed a new-found appreciation for the lab environment, further confirming my future goals. I have gained important experience in *Drosophila* genetics and husbandry procedures and put my theoretical knowledge to practical use. I felt honoured to have contributed to research in this field, despite an inconclusive outcome.

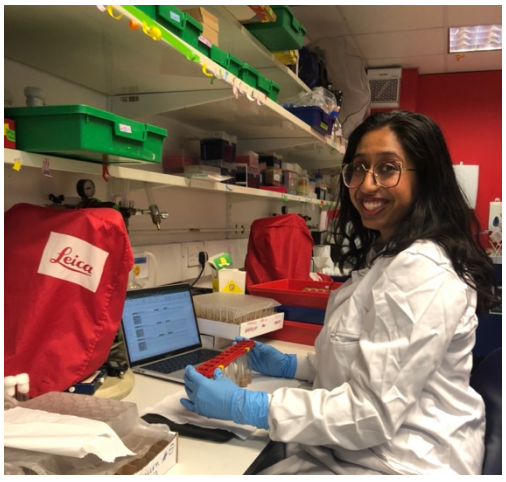

Figure 2 Tanisha tipping lifespans.