



Neurodegeneration Discovery Solutions

Brainwave-Discovery collaborative research

Brainwave-Discovery Ltd. is a new company based in Edinburgh, Scotland, that is dedicated to providing commercially focussed high-level research services to customers seeking to develop drugs for CNS disease treatment. We combine the unique analytical power of the *Drosophila* genetic system with proprietary automated *in vivo* neurophysiology and behaviour assessment, and strong synthetic biology and bioinformatics skills.

The combination of our skills, and the proven suitability of the *Drosophila* system for analysing molecular mechanisms in neurodegeneration, defines a unique opportunity for customers to make novel and rapid advances in drug development for these major diseases.

This document provides a brief overview of how Brainwave-Discovery Ltd. can help customers accelerate their neurodegeneration drug development.

Drosophila is a proven model system for human neurodegenerative diseases

A large body of evidence has shown that the neuronal cell death processes known to underlie many human neurodegenerative diseases can be faithfully modelled in the *Drosophila* brain.

Examples:

- Alzheimer's: Abeta toxicity¹⁻⁴ and tau toxicity^{5,6}
- Parkinson's: α -synuclein toxicity^{7,9}, and neurodegeneration due to loss of the PARK gene functions parkin¹⁰⁻¹² and PINK1^{13,14} as in man. The first demonstration that parkin and PINK1 are in the same pathway was in flies¹⁰
- Huntington's and other polyQ repeat protein toxicity¹³⁻¹⁸
- ALS/Lou Gehrig's disease: human mutant SOD1 toxicity¹⁹, VAPB-related dysfunction / neurodegeneration^{20,21}

The high level of development of genetic and molecular tools and short life cycle in *Drosophila* accelerates R&D. This is already leading to much more rapid understanding of molecular mechanisms of known human neurodegeneration pathways than has proved possible using mammalian cell systems or transgenic rodent systems.

Neurodegenerative cell death mechanisms are conserved between *Drosophila* and man.

The remarkable preservation between insects and man of cell death mechanisms initiated by human disease proteins is illustrated by the following striking examples:

- Relative protein toxicity for different neuronal types can be preserved. For example, in both *Drosophila* and man, dopaminergic neurons are preferentially affected by α -synuclein overexpression, even when a general neuronal promoter is used ⁷. This indicates that common molecular components are involved in the cell death process in both species.
- In some cases insect neuron phenotypes under neurodegenerative protein challenge are much more like the human than are rodent neuron phenotypes. The most striking examples are those of familial Parkinson's disease: loss of function mutations in genes encoding parkin and PINK1, give rise to clear neurodegeneration with mitochondrial involvement in the fly ¹⁰⁻¹² and man, but primarily affect dopamine release with minor mitochondrial effects in the mouse ²²⁻²⁴.
- Recent evidence (2009) indicates that the generation of an Alzheimer's-related toxic peptide by specific protease action is conserved between *Drosophila* and man ²⁵.

An example: Alzheimer's disease in the fly

Since Alzheimer's disease represents the greatest unmet, and growing, therapeutic need, we will look at this in some more detail in a *Drosophila* context.

Crucially, the entire molecular machinery that is known to be central to the toxicity mechanisms of Alzheimer's disease (AD) is present in fly neurons. *Drosophila* contains homologues of APP and all four components of the γ -secretase complex ²⁵⁻²⁹, and expression of human APP in *Drosophila* results in cleavage by endogenous γ -secretase activity. It also has an orthologue of the presenilin-interacting protein ubiquilin ³⁰, the gene for which is also implicated in genetic causation of late onset Alzheimer's. *Drosophila* has been used successfully for studying presenilin functions and the roles of presenilins and ubiquilin in neurodegeneration. Early work implied that there was no equivalent of BACE in flies, but recent data contradicts this. In fact, *Drosophila* has been shown to produce an equivalent peptide to Abeta 42 ²⁵: we note that Abeta 42 has normal functions when expressed at physiological levels.

The importance of these facts is that the proteins implicated in human Alzheimer's disease are part of a strongly conserved system. The same cell biological processes are going on in the fly and human neurons. For example, the expression of a human AD associated variant of ubiquilin in *Drosophila* causes an earlier onset, and more severe adult-onset eye degeneration when compared with wild-type human ubiquilin ³¹, indicating that the fly neuron reacts in the same way as the human neuron. Thus, flies engineered by Brainwave-Discovery to express human components of the Alzheimer's system would have human drug-relevant targets embedded within a functionally homologous cell biological response system.

Drosophila neurons are sensitive to Abeta toxicity, and to tau-mediated toxicity. Targeted expression of Abeta 42 caused neurodegenerative phenotypes, amyloid deposits, and learning deficits, whereas Abeta 40 expression only caused learning deficits ^{1,3}, confirming a requirement for the Abeta 42 peptide in pathology as in human neurons. There is a well-established *Drosophila* tau toxicity model ⁵, which has perhaps most strikingly been used to validate - in flies - the relevance to tau toxicity of genes identified in a mouse expression screen for genes that have altered expression when mouse neurons are challenged by a human toxic tau mutant protein ³². It was shown that a protein upregulated in the mouse was able to degrade human tau and to reduce tau toxicity in the fly tauopathy model ³². Recently tau has been re-integrated into the paradigm of neuronal death in Alzheimer's disease.

General molecular cell biology of neurons is strongly conserved between *Drosophila* and man.

Drosophila neurons use the same basic molecular systems as human neurons. Evolutionary conservation of molecular systems has been shown both by bioinformatic analysis and in some instances by functional replacement of fly proteins with human proteins. A recent example relevant to neurodegenerative disease is

the successful complete replacement of the fly protein DVAP-33A with its human homologue VAPB, with maintenance of function at the neuromuscular junction³³ : VAPB variants are implicated in motor neurone disease causation. Moreover, at least 75% of known human disease genes have orthologues in *Drosophila*³⁴.

As a further example, an extensive comparison of post-synaptic density complexes in the mouse and *Drosophila* has been carried out in work involving Brainwave-Discovery Ltd. staff. Using both bioinformatics and proteomics methods, Emes et al (2008)³⁵ showed that the complement of proteins at the synapse are remarkably similar in profile between mouse and fly. Much of the evolutionary change observed relates to ancestral gene duplication with fewer in the fly. The remaining differences are largely accounted for by protein components of myelin, which is not found in insects, and to genetic drift of cell adhesion molecules which makes them unrecognisable by current methods. Thus the apparent 50% fit is in fact likely to be more in the region of 90%. Thus 90% of the molecules in this key complex central to learning, memory, neuronal survival and death, are recognisably conserved from flies to mammals.

In summary, flies lack some of the complexity of protein variants of the mammalian brain, which is an advantage for analytical work aimed at specifying drug targets, but share the same underlying molecular cell machinery.

Neurodegeneration is best studied in a living brain, not in cells in vitro

In many cases, the response of the intact organism is not fully recapitulated in cell line models. Obvious problems are that interacting physiological pathways and responses (e.g., neurotransmitter circuitry and interactions with support cells) are not present, non-autonomous cellular influences are removed, and the molecular biology of the cells is often altered by modifications such as those used to immortalise cells. Thus cells in culture may not reflect in vivo pathology correctly, conclusions based on their use must be checked carefully, and drugs developed with cell systems may not act as expected in vivo. Meaningful assessment of the impact of pathogenic proteins is difficult in in vitro systems. It is also very likely that cells in culture represent a subset of cells that do not reflect the diversity of neurons of the adult brain.

For example, the study of the relative pathogenicity of a set of familial presenilin Alzheimer's mutations gave human-correlated data in *Drosophila* having failed to do so in cell lines³⁶.

Drosophila offers a fast way of assessing anti-neurodegenerative drug effects in a living brain with a proven track record of human-equivalent sensitivity to human toxic proteins.

Brainwave-Discovery Ltd.: linking neurodegeneration models to automated neurophysiology and behaviour assessment

Brainwave-Discovery specialises in humanising *Drosophila* neurons. We can make a fly strain expressing any human neurodegeneration toxin construct that customers require. We can extend current models, both by technical improvement and by engineering in multiple human toxic pathway components. Importantly, Brainwave-Discovery can provide an additional advantage to customers, by using our proprietary rapid Brainwave screen as an early-warning system for neurodegeneration, allowing anti-neurodegenerative effects of drugs to be assessed even more rapidly.

Brainwave-Discovery has a unique way of measuring neuronal activity – our proprietary system senses light output from cells of the *Drosophila* brain that have been engineered to emit light in proportion to calcium levels. We do not just measure calcium levels, but analyse very large regular calcium fluctuations that occur spontaneously in Kenyon cells of the mushroom bodies³⁷ – we have shown that this system is an order of magnitude more sensitive than simple calcium level sensing in detecting responses to CNS active drugs. The mushroom bodies are also the main site for associative learning and memory in flies³⁸, which is a component of our favoured automated behaviour assay, courtship conditioning.

So how can we link these technologies to neurodegeneration?

In *Drosophila* we can target the expression of multiple gene complexes to particular neurons very easily. In this way we can both deliver a human neurodegeneration toxic protein to a neuron of our choice, leaving the rest of the brain intact, and in parallel modify the expression of any protein(s) whose role in neurodegeneration we wish to investigate. Intrinsic *Drosophila* proteins can be removed from the system, if necessary, by RNAi knock-down, or more permanently by gene replacement. A wide range of technologies are available to modify and study the fly nervous system^{39,40}.

Therefore, we can deliver human neurodegeneration toxin constructs to essentially any cell type in the fly brain (including dopaminergic neurons). Other proteins can be added to or taken out of the system at will. This connects our rapid Brainwave physiological and behavioural pharmacology assays to neurodegeneration.

The early stages of neurodegenerative processes typically affect synaptic terminals, leading to transmission deficiencies and circuit disruption. Therefore we predict that we will get modified Brainwave outputs, and changed learning behaviour, even in early very stages of neuron dysfunction, well before cell death occurs, and these can be monitored automatically. This will allow very rapid assays of drug effects on neurodegeneration to be developed, and will be an ideal system in which to test and develop drugs that interfere with the neurodegeneration process.

Brainwave for neurodegeneration offers:

- Flexibility – We can rapidly design and deploy new single and multi-gene models, often unhindered by IP restrictions placed on mammalian models, and we can design and generate pathways for you.
- Speed – answers in days and weeks rather than months and years
- Throughput – much higher than any other in vivo neurodegeneration model
- Lower cost – with no need for expensive, highly regulated mammal models, shorter life cycle and lower amounts of lead compound required, we can significantly reduce the cost of neurodegeneration R&D.
- Immense further R&D capacity – a laboratory in a fly, not just an assay

How would a collaborative research contract be developed?

- The customer defines the goals
- Our experienced scientists meet or video-conference with yours to define the best way to design the constructs and the experimental analysis
- On the basis of these discussions, Brainwave-Discovery Ltd. makes a contract proposal, which is then negotiated and completed.
- Brainwave-Discovery takes the contract from design to data output.
- Downstream compound screens can be in-licensed to you or contracted out to Brainwave-Discovery under strict confidentiality.

Contact: info@brainwave-discovery.co.uk

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