

Drug Target and Drug Discovery

Aktogen has developed a fruit fly based automated behaviour screening technology that aims to accelerate the discovery of novel drugs for Central Nervous System (CNS) disorders. Aktogen was founded in October 2003 and has been funded to date by private investment and a DTI SMART Award. The company is currently conducting its first service contract work for the University of Cambridge, and is in discussion with large and medium-sized pharmaceutical companies to initiate further collaborative projects. The first applications of our technology is in neurodegenerative and neuropsychiatric disorders (NPDs), including schizophrenia, which affect more than 1 in 100 people all over the world. Existing drugs in this market have limited effectiveness and unwanted side effects. Aktogen's technology allows initial fast screening of potential drug candidates in vivo and furthermore enables it to directly link genetic information to these conditions. Aktogen uses the fruit fly to accelerate the discovery of drug targets and potential drugs. Our particular edge is the development of automated systems for fast screening of complex behaviour phenotypes that have relevance for human CNS disorders.

Although, our first application is in neuropsychiatric and neurodegenerative disorders, the technology will be readily adaptable to other CNS conditions such as Parkinsonism, drug abuse, sleep disorders, chronic pain, safety pharmacology, etc. *Drosophila* show a wide repertoire of behaviour, for example several forms of learning, including habituation. Habituation is a non-associative learning mechanism whereby an initial response wanes with repeated stimulation.

Habituation and pre-pulse inhibition (PPI) are measures of sensorimotor gating, a process by which irrelevant information is filtered from the environment and brain processes. Habituation and PPI deficits of startle have been identified as features of the NPDs. In spite of decades of efforts, the genetic and biological basis of NPDs is still not understood. A genome-wide habituation or PPI mutant screen in *Drosophila* would greatly facilitate the discovery of underlying genes, and the products of those could serve as targets for drug development.

While developing its platform, Aktogen is offering platform services and initiating collaborations with pharmaceutical companies. The offered services include

- A. behaviour mutant (genetic) screens related to human CNS diseases (drug target screens)
- B. behaviour testing of *Drosophila* strains mutated in potential human CNS disease genes
- C. drug feeding tests in the behaviour assay (ideally in "humanised" flies)

The main driver of Aktogen is the inventor of the company's core technology, Dr. Zoltan Asztalos, founder and experienced behaviour geneticist. Aktogen is supported by an internationally recognised Scientific Advisory Board, including Professors Michael Ashburner, Mark Geyer (Univ. California, CA, USA), Jay Hirsh (Univ. Virginia, VA, USA), Michael Owen (Univ. Cardiff, UK), Trevor Robbins (Univ. Cambridge, UK), and Tim Tully (Helicon Therapeutics, NY, USA).

The fruit fly as human CNS disorder model

Summary

- Existing CNS disorder models are known to be poorly predictive
- The available mammalian models are expensive and low-throughput
- Additional biological information could facilitate the discovery of novel drugs for the treatment of CNS disorders
- The fruit fly, *Drosophila m.* offers remarkable biological similarity to humans, sophisticated genetic tools, economy and large scale in drug target and drug screens
- Several *Drosophila* CNS disorder models, including neurodegenerative and neuropsychiatric disorders (NPDs) are already partially validated
- Aktogen is building an automated fruit fly behaviour platform for screening drug targets and drugs relevant for CNS disorders
- As the first application, *Drosophila* neuropsychiatric disorder (NPD) model(s) will be developed, validated and used for drug discovery in the behaviour system
- Aktogen's proposed technology is 100 times faster and more cost effective than the mammalian "schizophrenia models"

Searching for novel drugs to treat CNS disorders, including schizophrenia

The enormous complexity of the human brain does not allow us to clearly understand the cause of CNS disorders, including neurodegenerative disorders, Parkinson's disease, drug addiction, neuropsychiatric disorders (NPDs), etc. Several different approaches have been developed to discover drugs for the treatment of these conditions. The traditional hit and miss approach has been replaced with more directed ones. For these, one either needs to identify potential drug target(s) and/or test(s) for the condition of interest. Naturally, most experiments cannot be conducted in humans, and so model systems have to be established. The test systems always have to include intact animals as there

is no reliable biochemical or cellular model for these complex disorders.

One of the most challenging conditions is the group of neuropsychiatric disorders, including schizophrenia, obsessive-compulsive disorder, etc. The most widely used animal NPD models are established in rodents. Although it is not believed that these animals can be, for example, schizophrenic, but they can carry similar biological defects. There are behavioural markers, which are shared between patients suffering in the disorders and the animal models. Unfortunately, the slow, labour intensive and expensive behavioural testing in mammals, poses one of the bottlenecks in the drug discovery process.

In order to reduce time, expense and ethical concerns, Aktogen proposes to use the fruit fly, *Drosophila melanogaster*, to complement the rodent models in drug discovery for the treatment of neuropsychiatric and other CNS related disorders.

Fruit flies and humans?

Humans share a large degree of biological similarity with simpler organisms. Detailed sequence analysis indicates that 75% of the human disease genes in the "Online Mendelian Inheritance in Man" database have strong corresponding protein sequence matches to *Drosophila* genes.

There are convincing examples of how *Drosophila* can be useful as a living test tube to study human gene function. By expressing the human α -synuclein protein in fruit flies it is possible to evoke the symptoms of Parkinson's disease, even though there is no homologue of this gene in *Drosophila*. Administration of five different prototype drugs used against Parkinson's disease in humans resulted in significant improvement of these symptoms in the fly.

In other experiments Helicon Therapeutics Inc. (NY, USA), in collaboration with the Cold Spring Harbor Laboratory (NY, USA) found at least one drug, currently entering Phase 1 clinical trials, that specifically enhanced long-term memory in fruit flies and in mice. This example clearly shows the utility of the fruit fly in the drug discovery process, even for higher brain functions such as long-term memory formation.

On the basis of such experiments, the fruit fly is already becoming a successful model system for human Parkinson's disease, neurodegenerative diseases (e.g. Alzheimer's disease), drug abuse (cocaine, ethanol), cancer, etc.

Flies are practical

The fruit fly, *Drosophila melanogaster* has a short generation time of ten days, is easy to maintain and large numbers can be handled in parallel. The complete *Drosophila* genome sequence is in the public domain. Many of the predicted 13,500 genes have been mutated, and the mutant lines are maintained in international public stock centres (see Matthews et al., 2005)

A plethora of advanced genetic tools are available for the manipulation of the *Drosophila* genome. One of the best used one in target validation is the transgenic RNAi technology. These tools make fast gene identification, targeted knock-out or knock-in experimental techniques are well established.

Flies have specific advantages

- Being simple can be an advantage. In about 60% of mouse gene knock-out strains, the expected disease phenotype is not observed because there are several other similar genes that maintain quasi-normal function. In the fruit fly most genes have only a single copy, and so gene knock-outs more frequently show a phenotype.
- Multiple gene lesions can be simultaneously introduced into fly strains with relative ease. This can be particularly important in NPDs, for which combination of different gene alleles are believed to be responsible.
- Introduction of human gene(s) into the fly can generate "humanized flies" that carry the exact drug target required in drug development.
- Not least, conducting experiments in insects saves more valuable animal life and attracts less ethical concerns. Importantly, based on its complex central nervous system consisting of about 100,000 neurons, *Drosophila* show a wide repertoire of behaviour, e.g. several forms of learning, including habituation. Habituation is a non-associative learning mechanism whereby an initial response wanes with repeated stimulation.

Habituation and PPI deficits are indicators of neuropsychiatric disorders Habituation and pre-pulse inhibition (PPI) are measures of sensorimotor gating, a process by which irrelevant information is filtered from the environment and brain processes (Braff & Geyer, 1990). Habituation and PPI deficits of startle have been identified as features of the neuropsychiatric disorders. As a model of gating PPI is proposed to have face, construct and predictive validity.

Rodent pharmacological "schizophrenia models" employ three classes of drugs to disrupt PPI. The target receptors of these drugs, as well as the genes knocked out in mouse genetic "schizophrenia models" are nearly all present in the fruit fly, together with many other molecular steps in the neural information processing pathways (neurotransmitter receptors, ion channels, transporters, cell adhesion molecules, kinases, transcription factors, etc.).

In spite of decades of efforts, the genetic and biological basis of neuropsychiatric disorders is still not understood. A

genome-wide habituation or PPI mutant screen in *Drosophila* would greatly facilitate the discovery of underlying genes, and the products of those could serve as targets for drug development.

Further validation of fruit fly neuropsychiatric disorder models

Schizophrenic fruit fly?

Naturally, it is not possible to mimic NPDs in their completeness in an animal model, including the fruit fly. The same biological problem manifests differently in various organisms depending on their level of complexity. What a *Drosophila* model can best offer is the reproduction of the basic functional deficit(s) that cause the conditions in humans, and assays that reflect human behaviour more closely than cultured cells ever can, but allow a much higher throughput than is possible in higher (and more expensive) model organisms. If one can validate a behavioural marker in the model system that reflects those molecular/cellular deficits, the marker then can be used to discover further, novel molecular pathways and their affecting drugs. Because of its link to NPDs, we propose habituation and PPI performance as a measure of NPD related defects in the fruit fly.

Partially validated

Potential *Drosophila* NPD models are already partially validated. Two of the many habituation studies clearly indicate that dopaminergic systems play an important role in habituation in the fruit fly, similar to mammals and humans. Wittekind and Spatz (1988) reported that injection of dopamine into the head capsule of wild-type flies decreases landing response habituation. Neckameyer (1998) found that depletion of dopamine levels in adult flies diminishes habituation of a male to immature male courtship conditioning paradigm. This effect was rescued by additional L-Dopa administration.

The other piece of validation comes from genetic mutant analysis. Synapsins are synaptic vesicle proteins and have been identified as susceptibility genes in schizophrenia (synapsin 2 and 3, Vawter et al., 2002) and epilepsy (synapsin 1, Garcia et al., 2004). *Drosophila* has only one highly complex synapsin gene and the effects of mutation were recently studied. Flies lacking the synapsin gene are apparently healthy and do not show obvious anatomical defects. Nevertheless, tests in the mutant revealed impaired performance in various kinds of learning assays including olfactory jump reflex habituation (Godenschwege, 2004).

Validation by drug feeding

For the validation of fruit fly neuropsychiatric disorder models the same psychotic drug classes will be applied on the flies as in the rodent "schizophrenia models" (dopamine agonists, serotonin agonists and NMDA/glutamate antagonists) to test for any disruption of habituation/PPI. If this is successful, we will apply well-known typical (e.g. haloperidol, chlorpromazine) and atypical (e.g. clozapine, risperidone) anti-psychotic drugs along with the psychotic ones to test for any "curing" effect of the latter.

Validation by genetic models

The possible molecular defects in humans leading to NPDs are only emerging through genetic marker association studies (disorder susceptibility gene mutations or changes in their expression). We will test the effects of schizophrenia (e.g. neuregulin, dysbindin, synapsin, metabotropic glutamate receptor; for review see Harrison and Weinberger, 2005), and bipolar disorder susceptibility gene orthologues (DARPP-32, tachykinin 1; Ogden et al., 2004) on habituation/PPI in the fruit fly. We will also combine genetic manipulation and drug feeding validation studies.

The results of these genetic lesion and drug application studies will indicate the extent of utility of *Drosophila* NPD models

Available fruit fly jump habituation systems

For the completion of the large number of validation experiments the availability of an automated behaviour platform is obligatory.

In the past years, we have established an olfactory jump reflex habituation paradigm in fruit fly in which we were able to identify learning mutant phenotypes. To make this largely manual system more effective in mutant screens, a semi-automated version of the paradigm was developed. This system uses the EthoVision image-processing software to follow 16 flies simultaneously. 500 mutant lines have already been tested in this paradigm and at least one novel habituation mutant has been isolated (Sharma et al., 2007).

Development of a novel integrated habituation/PPI system

To reduce cost and complexity, a novel idea has been introduced. A "light-off" jump response habituation phenomenon has been previously observed both at behavioural and electrophysiological level in *Drosophila*. The fruit fly jump response can be detected in a novel way, which allows high efficiency and automation. In this system, response habituation can be measured, and we also have established a PPI paradigm. Aktogen has already developed a prototype of this system and submitted a patent application for it.

In experiments employing hundreds of automated habituation assays, fly breeding, collection and drug application become bottlenecks. We are currently experimenting with simple fly sorting devices and plan to develop automated fly

collection assemblies. With the help of Prof. Jay Hirsh (Univ. Virginia, VA, USA), we will build a high-throughput drug application system based on the design recently published in his laboratory (Lease & Hirsh, 2005).

With such an integrated behaviour system we ultimately aim to achieve a throughput of several 1,000 behaviour tests a day (equivalent of several drug dose-response curves) compared to 10 to 12 rodent behaviour tests a day in a typical laboratory.

Potential applications of validated behaviour platform

The proposed integrated behaviour system will be used for primary testing of mutant and drug treated fly stocks. The pre-selected strains can then be analysed further, in an image detection based behaviour system, for more detailed description of behaviour. Some disease conditions or drug effects will not allow the flies to jump, and so visual detection of fly behaviour will be essential in those cases. With a set of five automated systems, plus a visual detection based system, up to 10,000 habituation or 50,000 PPI assays could be performed in one year, which is a hundred times faster than similar rodent behaviour tests.

Drug screening

Aktogen will be able to add value to both the drug discovery and drug development processes. Using our automated behaviour system and appropriately chosen genetic disease models, we can test the effects of very high numbers of drugs on higher cognitive functions.

Drug target screens

Aktogen can also unlock the potentials of un-examined drug target collections of pharmaceutical companies. With the available genetic technology we will be able create and test hundreds of genetic disease models of choice.

Safety pharmacology

The relative complexity of the fruit fly brain and the high-throughput of our behaviour screens will allow Aktogen to take a share in the toxicological assessment of drug libraries, in line with the increasing significance of predictive toxicology in drug development.

Genetic mutant screens

To find the elusive genes behind NPDs, one could mutate each gene in a given genome and identify in a test system whether the disrupted genes are implicated in neuropsychiatric disorders. Knocking out all genes in mammals and testing the mutants' complex behavioural phenotypes would incur a huge amount of effort, cost and time. Aktogen is aiming to do this in the much more amenable fruit fly. Aktogen will attempt testing all the available, appropriate mutant stocks from stock centres and other sources, and may generate its own mutant collection. Unlike mammalian genomes, the fruit fly genome rarely has multiple genes for similar functions, making a single-gene mutation screen far more attractive as a method for implicating specific loci in neuropsychiatric phenotypes. As a complementary and more directed approach, we plan to use RNA interference (RNAi) studies on selected genes, especially on those that are already considered as candidate genes in NPDs. As the neuropsychiatric disorders are almost certainly mediated by polygenic variation, RNAi technology is particularly suitable for impairing multiple gene products, in parallel.

Further conditions of interest

Although habituation is one of the simplest types of learning process, through its measurement one can gain information about many different brain processes. These include general neural activity, excitability, motivation, information processing (gating), learning, memory and their potential role in other cognitive processes.