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Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery

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***Abstract*——The common fruit fly, *Drosophila melanogaster*, is a well studied and highly tractable genetic model organism for understanding molecular mechanisms of human diseases. Many basic biological, physiological, and neurological properties are conserved between mammals and *D. melanogaster*, and nearly 75% of human disease-causing genes are believed to have a functional homolog in the fly. In the discovery process for therapeutics, traditional approaches employ high-throughput screening for small molecules that is based primarily on in vitro cell culture, enzymatic assays, or receptor binding assays. The majority of positive hits identified through these types of in vitro screens, unfortunately, are found to be ineffective and/or toxic in subsequent validation experiments in whole-animal models. New tools and platforms are needed in the discovery arena to overcome these limitations. The incorporation of *D. melanogaster* into the therapeutic discovery process holds tremendous promise for an enhanced rate of discovery of higher quality leads. *D. melanogaster* models of human diseases provide several unique features such as powerful genetics, highly conserved disease pathways, and very low comparative costs. The fly can effectively be used for low- to high-throughput drug screens as well as in target discovery. Here, we review the basic biology of the fly and discuss models of human diseases and opportunities for therapeutic discovery for central nervous system disorders, inflammatory disorders, cardiovascular disease, cancer, and diabetes. We also provide information and resources for those interested in pursuing fly models of human disease, as well as those interested in using *D. melanogaster* in the drug discovery process.**

I. Introduction: Drug Discovery

A. Traditional Drug Discovery

Traditionally, the drug discovery process begins by identifying a target protein that is implicated in a certain human disease and then searching for a chemical compound that can alter the function of the disease-causing protein, generally by screening a very large library of known chemical compounds, optimizing a compound by medicinal chemistry, and then testing in animal models. This brute force approach can take more than a decade and several tens of millions of dollars to identify a single promising lead compound from chemical libraries consisting of up to several million entities. Despite the investment of significant resources, the success of finding an efficacious drug to bring to market is not guaranteed, because most drug candidates eventually fail for a variety of reasons. These include unpredicted toxicity, off-target interactions leading to undesirable side effects, and therapeutic effects not translating from traditional rodent models to humans in the clinic.

Traditional high-throughput drug screening (HTS[1]) approaches are based on in vitro cell culture, biochemical assays, or receptor binding assays. To a large extent, small molecule hits identified in these assays cannot be used directly in vivo because they usually do not exhibit all the desirable characteristics for absorption, distribution, metabolism, excretion, and toxicity for the practical applications of a drug in human patients. To make these lead compounds suitable for human use, extensive medicinal chemistry optimization efforts are necessary for each lead. The failure rate of clinical products as a result of unacceptable absorption, distribution, metabolism, excretion, and toxicity characteristics is extremely high, primarily because of the poor selection of hits by test systems that have limited predictive value for clinical outcome. There are far too many examples in which HTS of several thousand to hundreds of thousands of chemicals have led to the identification of potential hits that all failed upon further testing. For example, a recent screen of 184,880 novel compounds using a "filter retardation assay" of Huntington's disease (HD) aggregates led to the identification of multiple lead compounds, including a number of benzothiazoles that inhibited polyglutamine-mediated aggregation of toxic and misfolded proteins (Heiser et al., 2002). Because riluzole, a closely related benzothiazole, had previously shown therapeutic benefit in patients with amyotrophic lateral sclerosis (Lacomblez et al., 1996), drugs from this structural class of molecules were tested for further development. In a cell culture model of aggregation, all primary hits were found to be toxic to cells, and in an animal model of HD, *none* of the compounds was of therapeutic value (Hockly et al., 2006).

It is often said that "all the low hanging fruit has been picked" when referring to the discovery of novel therapeutics. The number of new drugs coming to market year after year is substantially lower now then in years past. It is now clear that the paradigm has shifted from

[1]Abbreviations: AD, Alzheimer's disease; AED, antiepileptic drug; APP, amyloid precursor protein; APPL, APP-like; BS, bang-sensitive; CD, cardiovascular disease; CNS, central nervous system; DA, dopamine; FDA, United States Food and Drug Administration; FRET, fluorescence resonance energy transfer; FMR, fragile X mental retardation; FXS, fragile X syndrome; GFP, green fluorescent protein; HD, Huntington's disease; HTS, high-throughput drug screening; JAK, Janus kinase; PD, Parkinson's disease; Psn/PSN, presenilin; RNAi, RNA interference; SBMA, spinal and bulbar muscular atrophy; STAT, signal transducer and activator of transcription; UAS, upstream activation sequence; Y-27632, 4-[(1*R*)-1-aminoethyl]-*N*-4-pyridinyl-*trans*-cyclohexanecarboxamide.

the "one disease–one target" mentality to an understanding that nearly all diseases are multifactorial, involving many genes and proteins, each interacting with one another as well as with their environment. To take these factors into account, as well as to overcome the barrier of poor predictive value from current in vitro screening platforms, one would ideally perform primary drug screening directly in whole animals, where all relevant systems are present, not in isolation, but functioning together in an intact living organism that has high face validity with respect to human disease therapeutics. Traditional animal models, such as rodents, however, are a poor choice for a whole-animal primary screening platform. Primary screens examining the efficacy of many tens of thousands to hundreds of thousands of small molecules in rodents would be nearly impossible for many reasons, including the time necessary (especially for age-related diseases) and prohibitive costs. New directions outside the cell culture dish are needed in the drug discovery process to identify not only new therapeutics, but new targets as well.

B. New Directions

The fruit fly *Drosophila melanogaster* represents one such valid alternative in the drug discovery process. This review is an attempt to provide an overview of the advantages and uses of *D. melanogaster* in the drug discovery process. It is noteworthy that, as discussed in sections II and IV, key physiological processes are well conserved from the fly to humans. Further advantages of *D. melanogaster* include the extremely low cost of maintenance, propagation, and screening and the rapidity of studies in the fly compared with traditional mammal-based models. Not only can *D. melanogaster* be used in primary small molecule discovery validation, but they can also be an important aspect of the target discovery process by taking advantage of the sophisticated genetics available in the fly (Fig. 1). Screening for novel drugs in *D. melanogaster* enables the selection of high-quality hits that already display key features such as oral or "transdermal" availability, metabolic stability, and, most importantly, low toxicity. Such features cannot yet be adequately mimicked by cell culture or biochemical assays. Here, we briefly outline the history of and basic biology of the fly, discuss therapeutic categories of human diseases in which there are currently opportunities to use the fly, and how the fly is or can be used, and we present a list of resources and information about *D. melanogaster* for those interested in pursuing the use of the fly in their own programs.

II. *D. melanogaster* As a Model Organism

A. History

The history of the use of *D. melanogaster* in modern biological sciences is a very rich one, spanning over 100 years; therefore, it is not possible to do complete justice to it here in this forum. Nevertheless, we believe that it is important to highlight a few significant aspects of this system for perspective. For more in-depth material on this subject, we recommend the following articles and references therein: Rubin and Lewis (2000), Arias (2008), Greenspan (2008), and Bellen et al. (2010). The concept that heritable traits are carried on the chromosomes was first developed in the fly, as well as many other landmark discoveries in genetics. Indeed, the Nobel Prize for Physiology and Medicine in 1994 was awarded to Ed Lewis for his pioneering research in flies defining gene structure, as well as to Eric Weischaus and Christiane Nusslein-Volhard for their studies inves-

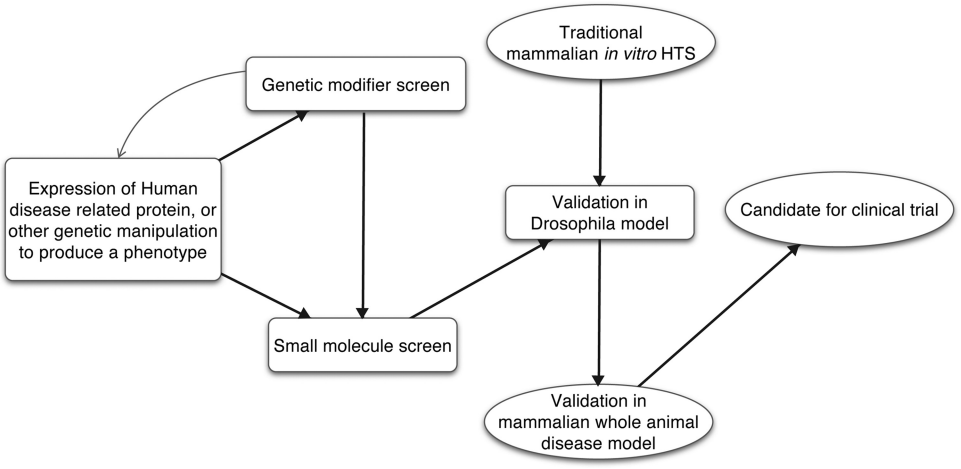

FIG. 1. *D. melanogaster* in the drug screening process. Models of human diseases are created in the fly by generation of mutants, either by mutation of the fly homolog of a human disease-related gene or by expression of the human form of the gene itself, that produce a scorable phenotype. This model can be directly screened for small molecules that rescue the phenotype or subject to genetic screens to identify modifiers of the phenotype, which represent new potential targets or models for the given disease. After initial screening, positive hits can be validated by testing in additional fly models of the disease. Significantly, these whole-animal validation studies can also be performed with the positive hits from traditional in vitro mammalian cell culture HTS to rapidly identify effective lead compounds. Drugs with efficacy in *D. melanogaster* models, however, will still need to be validated in mammalian whole-animal disease models.

tigating embryogenesis that identified a significant number of genes involved in all aspects of development. The vast majority of these genes have been subsequently found to be essential for normal mammalian development. In the modern era, *D. melanogaster* was the first major complex organism to have its genome sequenced (Adams et al., 2000). Not only did this event tremendously affect the fly world but also upon completion of the sequence of the human genome a few years later, the observed homologies between the two genomes underscored and strengthened its role as a model to understand human biology and disease processes. The fly continues to be at the forefront of biology, where genes, genetic techniques, and other discoveries are often elucidated first in the fly and then translated to mammalian systems.

B. Basic Biology

There are many salient features of the fly that make it such an attractive model to study. As mentioned previously, the fly genome has been completely sequenced and annotated, and encodes for a little more than 14,000 genes on four chromosomes, only three of which carry the bulk of the genome. It has been estimated that nearly 75% of disease-related genes in humans have functional orthologs in the fly (Reiter et al., 2001; Lloyd and Taylor, 2010). Overall identity at the nucleotide level or protein sequence between fly and mammal is usually approximately 40% between homologs; however, in conserved functional domains, it can be 80 to 90% or higher.

The fly has a very rapid life cycle. A single fertile mating pair can produce hundreds of genetically identical offspring within 10 to 12 days at 25°C. This is in contrast to traditional rodent models, in which only a handful of offspring are produced every 3 to 4 months. The fly may be considered multiple model organisms, each with its own specific advantages, defined by developmental stage: the embryo, the larva, the pupa, and the adult. The embryo is often used in fundamental developmental studies examining pattern formation, cell fate determination, organogenesis, and neuronal development and axon pathfinding. The larva, particularly the wandering third instar larva, is routinely used to study developmental and physiological processes as well as some simple behaviors such as foraging. The future adult structures of the fly are contained within the larva as imaginal discs, which are primarily composed of undifferentiated epithelium. Beginning in the late third instar larval phase and proceeding through the pupal phase, these structures undergo massive morphological changes that give rise to the final adult structures. The study of the molecular and genetic mechanisms underlying imaginal disc developmental processes in the pupa has provided significant insight not only to fly biology but also to human biology. Thus, the pupa is an appropriate model to investigate certain developmental processes.

The adult fly is a very sophisticated and complex organism not unlike higher organisms. The adult fly has structures that perform the equivalent functions of the mammalian heart, lung, kidney, gut, and reproductive tract. The brain of the adult fly is quite remarkable. More than 100,000 neurons form discreet circuits and neuropil that mediate complex behaviors, including circadian rhythms, sleep, learning and memory, courtship, feeding, aggression, grooming, and flight navigation. Significantly, the response of flies to many drugs that act within the CNS is similar to the effects observed in mammalian systems (McClung and Hirsh, 1998; Moore et al., 1998; Bainton et al., 2000; Nichols et al., 2002; Rothenfluh and Heberlein, 2002; Satta et al., 2003; Wolf and Heberlein, 2003; Andretic et al., 2008). The visual system of the adult continues to be extensively studied and has been crucial in understanding not only vision but also other key systems that include signal transduction pathways such as ras, and transient receptor potential channels, among many other processes (Ready et al., 1976; Nagaraj and Banerjee, 2004; Montell, 2005; Kumar, 2010).

Although there are many differences between flies and humans, the degree of conserved biology and physiology position *D. melanogaster* as an extremely valuable tool in the drug discovery process. We envision the role of *D. melanogaster* in target discovery, in primary high throughput screening, and in post-traditional HTS validation studies to effectively and rapidly identify small collections of higher quality hits from larger collections to then proceed with in more traditional mammalian models (Fig. 1). Significantly, the addition of the fly to the discovery process is predicted to enhance the rate of discovery at reduced costs to potentially identify new targets and therapeutics.

C. Genetics Relevant to Drug Discovery

Rather than discuss in depth the entire repertoire of genetic tools available in the fly, a brief overview of those most relevant to the drug discovery process will be presented here. First is the ease and cost effectiveness to generate a transgenic animal. Essentially, the cDNA/small interfering RNA or other genetic element desired is subcloned into a region between two inverted repeat elements (P-elements are the most common) on a DNA plasmid, and the entire construct along with a helper construct expressing a transposase is injected into the posterior germ cells of fly embryos. Of 100 embryos injected (taking only a couple of hours), 10 to 15% will result in a successful germline integration event, as visualized by a marker phenotype (typically red eyes) in the F2 generation. Once the initial transgenic DNA plasmid construct is made, the injection and selection process to obtain independent founder lines takes only approximately 6 weeks. There are several commercial sources available (see section VI) that will perform the whole procedure for less than $300 per construct, once provided with the construct.

The "workhorse" of fly transgenic models is the bipartite GAL4/UAS system, first developed by Brand and Perrimon (1993). In one parental strain, promoter regions for a particular gene drive expression of the yeast transcription factor GAL4 in defined tissues. In the other strain, GAL4 response elements (UAS) are upstream of the desired transgenic element. When the two strains are mated, the progeny express the transgene in the specific tissues defined by the GAL4 promoter element. Many modifications and enhancements of this basic system have been developed to further refine tissue specificity as well as temporal expression specificity (Roman et al., 2001; McGuire et al., 2004). A very significant resource to be used in conjunction with this technology is the Vienna Drosophila Research Center collection of UAS-RNAi responder strains. They have created a collection of RNAi knockdown strains targeting ~90% of the entire fly genome and have made it available to the research community (Dietzl et al., 2007). Together, these tools make it very easy to rapidly generate models of human disease through mutation, genetic inactivation, or misexpression of fly homologs of human disease genes or human disease genes and proteins themselves.

D. D. melanogaster *in Relation to Other Model Organisms: Zebrafish and* Caenorhabditis elegans

Two additional model organisms are useful for drug discovery, each model providing certain advantages over the other. The selection of which model organisms to use depends on the nature of disease being studied, the scientific questions being asked, and the type of small-molecule screening procedure desired. In many instances, the smaller and genetically tractable models, such as *D. melanogaster*, *C. elegans*, or *Danio rerio* (Zebrafish), can each provide critical information about genetic and cellular process underlying certain diseases in a more rapid and cost effective manner than traditional rodent-based or in vitro studies. Here, we will only briefly highlight aspects of these two additional models for comparison with the fly.

The sequencing of the *D. melanogaster*, *C. elegans,* and Zebrafish genomes have made these small animal models more applicable and useful for the study of human diseases than they were before the "genomic revolution" (Adams et al., 2000; *C. elegans* Sequencing Consortium, 1998). Significantly, approximately 75% of human disease genes have homologs in *D. melanogaster* (Lloyd and Taylor, 2010). The worm has slightly less, at approximately 65% (Sonnhammer and Durbin, 1997). Zebrafish, being a vertebrate, is predicted to have more than the fly or the worm, with most human genes having a homolog (Langheinrich U, 2003). *C. elegans* has an extremely rapid life cycle (~4 days), is prolific, and very amenable to genetic manipulation (Teschendorf and Link, 2009). Furthermore, all 302 neurons and their connections have been precisely mapped and well studied (Teschendorf and Link, 2009). The transparent nature of *C. elegans* throughout its life cycle facilitates the use of GFP fusion proteins to visualize specific cells, neurons, and synaptic connections throughout the live animal (Link et al., 2001). It is possible to directly visualize neuronal death in living worms by the morphological appearance of vacuolated neurons (Teschendorf and Link, 2009). With respect to drug discovery, *C. elegans* has been largely employed to identify moieties related to basic cellular function in screens and experiments primarily using fluorescent-based or very simple behavioral output measurements. High-throughput screens can be performed that involve worm sorters and high density plates. For example, neurodegeneration related to Parkinson's disease (PD) has been an active area of discovery. Live worms expressing GFP in dopaminergic neurons are easily scorable for severity of response to treatments including lesioning with 6-hydroxy-2-dipropylaminotetralin or overexpression of α-synuclein (Nass et al., 2002; Lakso et al., 2003). Both high-throughput genetic and low-throughput chemical screens have been employed to identify genetic modifiers and pharmacological treatments that block neurodegeneration, some of which have been validated for efficacy in mammalian systems (Nass et al., 2005; Marvanova and Nichols, 2007; Harrington et al., 2010).

There are, however, a number of limitations associated with the *C. elegans* as a model system compared with flies. *C. elegans* have fewer gene homologs in mammals, some families having no homologs at all (Rikke et al., 2000). It is noteworthy that many key organs and other physiologically relevant systems present in both the fly and human are absent from the worm. These include a sophisticated immune system and some organs, such as the heart. Another aspect of *C. elegans* that can be either advantageous or a limitation is that the worm does not have a male/female sexual system. Most worms are self-fertilizing hermaphrodites. Although the limited but well defined 302 neuron nervous system also has certain advantages, there is no centralized brain capable of mediating the repertoire of complex behaviors present in the fly that are relevant to human behaviors, precluding the use of the worm in screens involving anything but the most simple of behaviors.

Zebrafish as a vertebrate model system also provide many advantages for understanding molecular mechanisms of human disease such as neurodegeneration and cancer (Bandmann and Burton, 2010; Newman et al., 2011). Because zebrafish are vertebrates, most human genes have homologs, and the functional domains of many key proteins can be nearly 100% identical between homologs (although overall protein similarity levels are approximately 70% (Woods et al., 2000; Langheinrich, 2003). Zebrafish have been highly informative in studies investigating developmental processes because of their large transparent embryos that mature outside of the

mother. Additional advantages to the zebrafish system include rapid early embryonic development (although development time to the adult stage is comparable with that of mice), the presence of some organs truly homologous to humans (e.g., liver, kidney), a complete immune system (innate and adaptive), ease of drug administration, and a lower infrastructure cost than rodents. The small size of zebrafish allows them to be housed in a small lab space, and it is possible to get a large number of progeny (200–300 new progeny per week per pair) within a short period of time (Meeker and Trede, 2008). Unlike *C. elegans*, zebrafish display complex behaviors relevant to humans. It is noteworthy that zebrafish have been successfully used in drug discovery and chemical screening processes (Tsang, 2010). Early studies used medium-throughput approaches examining the mutagenic effects of chemicals on embryo development (Peterson et al., 2000). Examples of more recent studies include high-throughput primary screens to identify small molecules that inhibit fibroblast growth factor receptor signaling (Molina et al., 2007) and secondary validation screens of traditional HTS hits to identify modifiers of circadian activity (Hirota et al., 2010).

Despite certain attractive features of the zebrafish model, there are also limitations. Although the development of genetic tools available for use in the zebrafish is progressing, they are arguably not nearly as advanced as those currently available for the fly or the worm. Furthermore, although more inexpensive than rodent facilities, zebrafish do require substantially more infrastructure- and maintenance-associated costs compared with both flies and worms.

III. Considerations

A. Differences between Fly and Human

Although the bulk of this review is dedicated to describing the conserved biology between fly and human and how these similarities can be exploited in the drug discovery process, the fly is *not* a miniature person. Fundamental processes can be shared, but their implementation can be and is often very different. With regard to basic physiological and cellular processes, such as glucose utilization or receptor signaling pathways, or where the underlying cause of a human disease may be due to dysfunction of only a single gene or protein, fly models can have high degrees of conservation and face validity, facilitating primary screening and interpretation of results. For more complex processes and modeling multifactorial human diseases, the corresponding fly models usually are only able to model certain aspects of the disease, and interpretation of results are more complicated. Whereas this is especially true for models of behaviors, other contributing factors can include significant differences in physiology that produce simpler or different phenotypes in the fly. Although *D. melanogaster* models can be informative in the discovery process, having a well defined hypothesis and a thorough understanding of the limitations of the fly are absolutely critical for success.

With respect to drug discovery, a key consideration to take into account are potential differences in the pharmacokinetics and pharmacodynamics of small molecules, which may produce significant discrepancies in drug levels and tissue distribution profiles between mammal and fly. For CNS discovery, there may be blood-brain permeability differences (Stork et al., 2008; Mayer et al., 2009). Another very important issue is toxicity. Because of metabolic differences, some drugs may be toxic in flies that are not in humans and vice versa, although there seems to be a strong correlation of toxicity between the two species (Rand, 2010). Because of all these factors, and more, it is emphasized here that *D. melanogaster* can be used only as a screening platform for target discovery, primary small-molecule screening, or postscreening validation to narrow down a large pool of potential drug candidates to a much smaller pool of lead compounds that it will be absolutely necessary to validate using traditional mammalian models. Nevertheless, the incorporation of *D. melanogaster* in target discovery and HTS is predicted to enhance the rate of discovery by reducing the time necessary to identify a small collection of potentially more effective lead compounds for final validation than by traditional methods by virtue of performing the discovery phase in a whole animal following a systems-based approach.

B. Drug Delivery Issues

A natural question to ask is: “How do you give drugs to a fly?” For embryos, drugs can be administered via permeabilization (Rand et al., 2010). For larva, drugs are usually added to the solid media in which they grow for long exposures or in a dilute solution of yeast paste for shorter exposures. For adult flies, there are numerous routes of drug administration (Fig. 2). Drugs can be presented as a vapor (e.g., ethanol and cocaine) (McClung and Hirsh, 1998; Moore et al., 1998); in the food itself or from a sucrose/drug-saturated filter paper (Nichols et al., 2002); drug can be injected or dropped directly onto the exposed nerve cord of decapitated flies (Torres and Horowitz, 1998); drug can also be injected into the abdomen, where it quickly diffuses throughout the organism (Dzitoyeva et al., 2003). Potential issues determining route of administration include the taste of a drug: if a drug tastes bad, a fly is likely to not eat it. To determine whether the presence of a drug influences food intake, there are simple feeding assays that can be performed (Ja et al., 2007). If it is necessary for an unpalatable drug to be ingested, it can be included with a rewarding substrate (e.g., sucrose, banana, or yeast paste). The most high-throughput method is to dissolve drug either in normal food substrate, or agarose + sucrose (to ensure that no ingredient in the food will interfere with drug action or absorption), and aliquot into

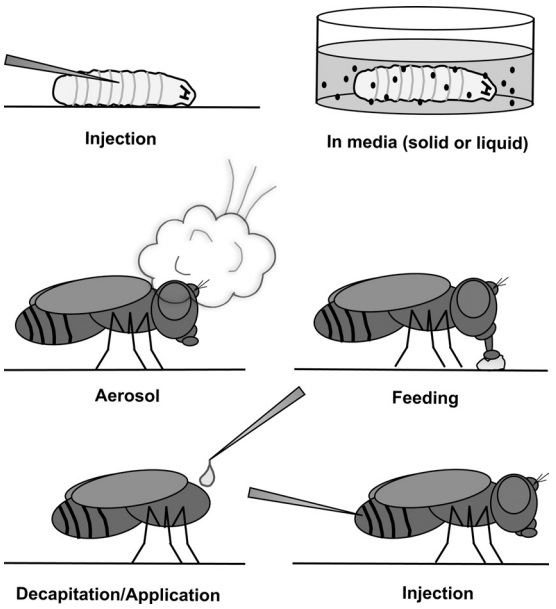

FIG. 2. Routes of drug administration. For larva (top), drug can be directly injected or drug can be mixed with media. Media can be either solid or liquid with 2% yeast paste to encourage feeding behavior. Adults can have drug delivered as an aerosol or gas, as a mixture with food substrate, as a direct application to exposed nerve cord, or as an injection. Drug administration through feeding generally has the highest throughput.

wells of a high-density plate that will contain individual animals. Physiologically effective concentrations can vary from 0.01 to 100 mM in the feeding substrate, although most studies examining the effects of drugs are in the range of ~1 to 10 mM. It must be emphasized that these are concentrations in the food; actual physiological concentrations will be much lower, and it may be necessary to examine in vivo concentrations using high-performance liquid chromatography or mass spectrometry (Kuklinski et al., 2010). It is recommended that pilot studies be performed examining three different concentrations of a known effective drug at log dilutions in the feeding substrate (0.03, 0.3, and 3.0 mM) for efficacy in a particular assay and to choose an appropriate concentration based on those results for the full screen.

There are different strategies to be considered when feeding drug to adults. One method is to starve the flies for ~16 to 18 h, and then place the flies on food substrate + drug for a few minutes. The flies will generally consume a large bolus dose of the drug. Advantages of this method include observation or measurement of the acute effects of a drug. Disadvantages include significant variability in dosage among flies as a result of various body sizes and amounts of drug ingested, as well as relatively low throughput. In our experience, drugs administered by this method begin to show behavioral effects within 10 min; maximum effects last from 15 to 60 min after feeding and full recovery occurs at 2 to 5 h. The second method is to maintain the fly on food substrate + drug for longer than 24 h, which allows for steady-state levels to be achieved before testing. This method is the highest throughput and can allow for large populations to be administered drugs. Disadvantages include possible adaptive mechanisms to prolonged exposure, such as down-regulation or desensitization of target genes or proteins. If flies are removed from the food + drug before any testing, metabolism and rate of elimination of the drug may need to be accounted for, depending on the assay, when interpreting the data.

C. Throughput

High-throughput screening by traditional methods usually involves massive parallel analysis of the effects of small molecules from a large library of 100,000 compounds or more on mammalian cells in culture in 384-well plates. An example of this is the fluorometric imaging plate reader assay, often used for the identification of molecules that alter the function of certain G-protein-coupled receptors (Sullivan et al., 1999). Throughput in *D. melanogaster* terms, for the most part, does not nearly approach that of HTS in mammalian cell culture systems. Whereas high throughput in some fly screens may approach 10,000 small molecules per month, most screens are on the order of 500 to 1000 small molecules per month. Although fly models have much lower throughput in general, one must keep in mind that, ultimately, the raw number of compounds screened from a library is not important in the discovery process; the number of quality hits resulting from the screen is the measure of success. Traditional brute-force HTS approaches can identify many "positive hits" from a large library in a short time, but the overall quality of these hits from a therapeutic standpoint is often quite poor, and significant resources must be expended to further develop the molecules before further testing in whole animals, at which point most lead candidates still will fail as a therapeutic (Gosai et al., 2010). The potential advantage of using the fly in the initial discovery process, regardless of raw throughput, is the identification of higher quality hits from fewer compounds screened. Because screening will be performed directly in the living animal, examination of the effects of the drug at the organismal level are built into the primary screen, thus significantly reducing postscreening costs to identify quality leads from the initial candidate pool. For example, drugs that seem safe in mammalian cell culture often produce unpredicted toxicity once tested in expensive rodent experiments. Many of these types of hits would not be selected for in fly screens because they would kill the fly. Regardless, if the higher throughput of traditional HTS is desired, then the fly can still perform a valuable role as a cost-effective and highly informative secondary screen on the positive hits to streamline the pool of candidates to those of higher quality before moving the entire collection to expensive rodent studies.

The actual throughput of *D. melanogaster* screens varies depending on the assay and to the degree to which it can be automated (Table 1). Although there are a number of different assays, only a few of the more prevalent ones currently used are described here to give an overall idea of what is possible. The higher throughput assays primarily depend on fully automated scoring of a visible phenotype, either live/dead, or a visible marker. Potentially one of the highest throughput quantitative strategies involves measuring the fluorescence of markers in embryos by methods similar to flow cytometry (Pulak, 2006). The same sorting technology can also be used to seed embryos into 348- or 96-well plates containing substrate and drug. Scorable HTS phenotypes suitable for automation include viability, both at the larval (do larva develop?) and pupal stages (do viable adults emerge from pupa?), as well as measurement of fluorescent markers (i.e., GFP-tagged proteins). Semiautomated and manual scoring of emerged adults are somewhat lower in throughput but are still able to screen thousands of drugs per week. These could involve, for example, examination for overt normal development or roughness of the eye. Lower throughput assays likely to be more relevant for validation of leads would include those in which a more detailed analysis of the fly is required (e.g., microscopic analysis of certain organs of the fly or biochemical analysis of the fly for levels of enzyme activity).

Behavioral assays, depending on the degree of automation, can also vary throughout from medium to low. The highest involve simple measurement of locomotor and circadian activity of individual flies in small glass capillary tubes by photo beam breaks (DAMS; Trikinetics, Waltham MA). Here, adult flies are loaded into tubes containing a food substrate and drug at one end that are placed into a 32-tube array. A single computer can measure locomotor activity in hundreds of tubes simultaneously. A small facility could screen 5000 to 10,000 small molecules in 2 to 3 months by this method. Lower throughput semiautomated methods include learning and memory assays and social interaction assays. For learning and memory, conditioned stimulus training can be automated to simultaneously train 8 to 32 small populations of flies for subsequent testing (Scott et al., 2002). A single technician and a 16- to 32-channel trainer could achieve a throughput of 25 to 50 drugs per week. Social interaction assays include aggression and courtship. Although subtle aspects of each of these interactions require a human observer, some of the more important interactions can be automated by video tracking software. For example, analysis software can simultaneously examine locomotor activity and interactions of up to 96 video channels in real time that include aspects of both aggression and courtship (Noldus, Wageningen, NL) (Branson et al., 2009; Dankert et al., 2009).

TABLE 1
Throughput in D. melangaster models

Stage	High Throughput	Medium Throughput	Low Throughput
Larvae	Lethality		Locomotor defect
	Body Size	Olfactory	Body wall contraction
	Necrotic Patches		Body wall muscle
			Response to pain
Adult	Lethality	Body weight	Life span
	Flight ability	Sleep, arousal, and rest behavior	Retinal degeneration
	Body size	Fecundity	Climbing assay
	Stress test	Aggression	Phototaxis
	Anesthesia response	Wing expansion behavior	Rotorod test
			Electrophysiology
			Prepulse inhibition
			Courtship behavior
			Feeding behavior
			Learning and memory behavior
			Response to pain
			Seizure behavior
			Visual discrimination

IV. Therapeutic Areas and Opportunities to Use *D. melanogaster* in Drug Discovery

A. Central Nervous System

1. Neurodegeneration. Neurodegenerative diseases are caused by progressive loss of specific neurons and are mostly age-related human diseases with significant pathological and clinical similarity. Persons who will develop neurodegenerative diseases are generally asymptomatic during the development of the nervous system. Many late-onset neurodegenerative diseases, including PD and HD, are associated with the formation of intracellular aggregates of toxic proteins (Taylor et al., 2002). The identification of mutations associated with familial cases of many of these neurodegenerative diseases has highlighted the significance of these pathological features and allowed investigators to develop in vitro and in vivo model systems to determine the cellular and molecular abnormalities associated with mutant gene product in many neurodegenerative diseases. These models proved to be very helpful in determining the biochemical and genetic alterations in neuronal tissues and understanding how mutant proteins cause damage to specific sets of neurons leading to distinct clinical phenotypes. A consensus has emerged regarding an underlying mechanism that contributes broadly to this class of diseases. Specifically, some proteins are more prone to misfolding into disease-causing pathological conformations that assemble into aggregates and acquire neurotoxic properties. It is believed that neurodegenerative diseases ensue when the production of neurotoxic proteins exceeds the cell's capacity for disposing of them or when neurotoxic proteins evade quality-control surveillance altogether. This concept predicts that it may be possible to develop novel approaches for treatment based on a

greater understanding of the cellular mechanism responsible for disposing of unwanted proteins.

Despite the significant contribution of human genetic studies in the identification of new genes associated with familial forms of neurodegenerative diseases, studies on human patients are of limited use for elucidating the signaling pathways and cellular processes underlying the neurodegenerative process. Often the rapid speed of the discovery of disease-causing genes is not matched by the speed of our understanding of the manner by which these mutations lead to the clinical symptoms of the disease and the mechanism of the disease progression. In addition, both ethical and technical problems pose a limit on types of genetic analysis that can be performed in human patients to determine genetic relationships among disease genes and to delineate signaling pathways. Most human neuropathological investigations use postmortem tissues, such as brain and spinal cord tissues, that almost never reflect the earliest pathologic events at the presymptomatic stage. Hence, animal models, especially *D. melanogaster*, present excellent alternatives for studying neurodegenerative disease mechanisms from early initiation events to the terminal stages.

Nevertheless, there are limitations to be aware of with fly models of neurodegeneration. *D. melanogaster* models often show striking phenotypes at early developmental stages, such as the larval, pupal, or early adult, in contrast to their human counterpart diseases that are mostly of late onset and start in the sixth or seventh decade of life (i.e., age 50–69 years). Furthermore, many of the *D. melanogaster* models rely on overexpression of the human disease-causing genes in *D. melanogaster* eyes, using eye degeneration (rough eye) as a measure of effect. Although *D. melanogaster* eyes and photoreceptor neurons have proven to be a good tool for examining the overt toxic effects of individual human disease-causing genes, eyes do not mimic the human brain with its complex circuitry and pathophysiology. *D. melanogaster* also have a much simpler immune system then mammals, limiting the study of the role of neuroinflammation in degenerative diseases. It is noteworthy that there are several significant anatomical differences between fly and human brain. For example, the fly brain has no substantia nigra, which is relevant to understanding how clinical features mediated by dopaminergic neuron loss in Parkinson's disease correlate with behavioral phenotypes. Cellular and molecular processes can also be very different in *D. melanogaster* and humans, and one or several key molecule(s) involved in mediating a disease-specific pathway could be missing in flies (e.g., α-synuclein), and there is a risk that the lessons we learn from the *D. melanogaster* model might not be biologically relevant to human disease pathways.

In the following subsections, several models of human neurodegenerative disease and their potential in the discovery arena are presented (Table 2). Each of these models shares several phenotypic commonalities in the fly, such as retinal degeneration, locomotor defect, wing phenotype, climbing defect, and reduced lifespan. Therefore, drug discovery assays aimed at identification of therapeutics for the neurodegeneration diseases discussed below can all essentially use protocols examining these common phenotypes. For example, rescue of rough eye phenotype, rescue of locomotor and climbing deficits, and restoration of normal activity. Because of their shared screening assays, our discussion of neurodegenerative models focuses on the pathophysiology of the model and not the screening process.

2. Alzheimer's Disease. Alzheimer's disease (AD) is the most common neurodegenerative disease and is characterized by progressive impairments in memory and cognitive abilities with a typical late age of onset, although the onset can be as early as fourth decade of life (i.e., age 30–39 years) in the familial forms. The disease is characterized pathologically by selective atrophy of the hippocampus and the frontal cerebral cortex. Amyloid plaques and neurofibrillary tangles are the hallmarks of AD. The main components of amyloid plaques are the Aβ-40 and Aβ-42 peptides, which are generated by proteolysis of the amyloid precursor protein (APP) via the action of β- and γ-secretase enzymes. β-Secretase activity is provided by the β-site APP-cleaving enzyme, whereas γ-secretase activity depends on a protein complex consisting of presenilin (Psn), nicastrin, aph-1, and pen-2. It is noteworthy that autosomal dominant mutations in APP, PSN-1, and PSN-2 can accelerate the age of disease onset and progression in familial AD cases. These mutations promote the generation of amyloidogenic Aβ peptides, and impairment in the trafficking of APP into protein degradation pathways may underlie the pathological accumulation of Aβ in several late-onset familial AD cases. These findings further support the amyloid hypothesis, which postulates that the accumulation of Aβ peptide is the initial event in the disease pathogenesis that may underlie synaptic failure, thereby resulting in the remarkably pure impairment of cognitive function.

Most of the genes implicated in AD pathogenesis have *D. melanogaster* homologs; e.g., the fly homolog of human APP is known as APP-like or APPL. Flies deficient for APPL demonstrate a behavioral abnormality that can be strongly suppressed by expression of a human APP transgene, indicating functional conservation between *D. melanogaster* APPL and human APP (Luo et al., 1992). As expected, however, there are some dissimilarities; *D. melanogaster* APPL lacks the amyloidogenic Aβ peptide sequence at the C terminus, and it is remains unclear whether APPL is processed in vivo like human APP. In *D. melanogaster*, the γ-secretase complex components are conserved and have been clearly implicated in the processing of Notch signaling pathways (Struhl and Greenwald, 1999; Ye et al., 1999). The γ-secretase- and presenilin-inhibiting compounds have been shown

TABLE 2
Genetic models of neurodegeneration

Diseases/Gene	Invertebrate or Animal	Phenotypes	References
Alzheimer's Disease			
β-Amyloid protein	*C. elegans*	Progressive paralysis, cytoplasmic protein accumulation, fibrillar amyloid formation	Link, 1995; Fay et al., 1998; Drake et al., 2003; Wu et al., 2006; Hornsten et al., 2007; Hassan et al., 2009
	D. melanogaster	Eye degeneration, Accumulation of amyloid plaques, reduced life span, locomotor defect, and vacuolation of the brain	Finelli et al., 2004; Crowther et al., 2005; Luheshi et al., 2007
	Zebrafish	Reduced body length, short and curly tail, defective convergent-extension movements in embryos	Joshi et al., 2009
Presenilin	*C. elegans*	Defects in neurite morphology, temperature memory, egg laying	Wittenburg et al., 2000
	D. melanogaster	Pupal lethality, dorsoscutellar bristle duplications, wing notching and wing vein defects	Seidner et al., 2006
	Zebrafish	Decreased cell proliferation and de novo neurogenesis, Irregular delineation of somites	Nornes et al., 2003; Van Tijn et al., 2009
Tau	*C. elegans*	Age-dependent progressive neurodegeneration, accumulation of insoluble tau; reduced lifespan, age-dependent progressive impairment in touch response, embryonic lethality and mechanosensory defect	Kraemer et al., 2003; Miyasaka et al., 2005; Gordon et al., 2008; Feuillette et al., 2010
	D. melanogaster	Eye degeneration, disruption of the microtubular network at presynaptic nerve terminals, axonal degeneration, neuromuscular junctions morphological defects	Williams et al., 2000; Whittman et al., 2001; Jackson et al., 2002; Mudher et al., 2004; Nishimura et al., 2004; Chee et al., 2005; Blard et al., 2007; Chen et al., 2007
	Zebrafish	Pathological hyperphosphorylation, conformational changes, and tau aggregation	Paquet et al., 2009
Parkinson's Disease			
α-Synuclein	*C. elegans*	Mitochondrial stress, dopaminergic degeneration, developmental defect, upregulation of dopamine synthesis, redistribution of dopaminergic synaptic vesicles	Lakso et al., 2003; Springer et al., 2005; Ved et al., 2005; Kuwahara et al., 2006; Karpinar et al., 2009; Hamamichi et al., 2008; Kuwahara et al., 2008; van Ham et al., 2008; Settivari et al., 2009; Cao et al., 2010
	D. melanogaster	Age+dependent loss of dopaminergic neuron and progressive climbing defect	Feany and Bender, 2000; Auluck and Bonini, 2002; Auluck et al., 2002; Coulom and Birman, 2004; Pesah et al., 2005
	Zebrafish	Zebrafish homologs of human α-synuclein are known but no animal model published yet	Sun and Gitler, 2008; Chen et al., 2009
Parkin and Pink	*C. elegans*	Hypersensitivity toward proteotoxic stress conditions, Parkin insolubility and aggregation	Springer et al., 2005
	D. melanogaster	Dopaminergic neuron loss, age-dependent motor deficits, reduced lifespan, locomotor defects, male sterility and mitochondrial pathology	Greene et al., 2003; Haywood and Staveley, 2004; Pesah et al., 2004; Cha et al., 2005; Sang et al., 2007
	Zebrafish	Dopaminergic neuron loss, reduced mitochondrial respiratory chain complex I activity, severe developmental defect	Anichtchik et al., 2008; Flinn et al., 2009; Xi et al., 2010
Triplet repeat expansion diseases			
Huntington's disease	*C. elegans*	Huntingtin-positive cytoplasmic aggregates, sensory process degeneration, axonal swelling, mechanosensory defects and perinuclear huntingtin aggregates	Faber et al., 1999; Parker et al., 2001, 2005; Brignull et al., 2006
	D. melanogaster	Axonal transport defect, lethality, neurodegeneration, behavioral and electrophysiological defects	Gunawardena et al., 2003; Romero et al., 2008
Spinal and bulbar muscular atrophy	Zebrafish	Massive neuronal apoptosis, small eyes and heads and enlargement of brain ventricle, lower jaw abnormalities; defect in iron utilization and development	Lumsden et al., 2007; Henshall et al., 2009
	C. elegans	None	Takeyama et al., 2002; Pandey et al., 2007a,b; Nedelsky et al., 2010
	D. melanogaster	Accumulation of expanded polyglutamine containing androgen receptor, protein aggregation, eye degeneration, locomotor defect	
Fragile X syndrome	Zebrafish	None	
	C. elegans	None	
	D. melanogaster	Eye degeneration, age-related cognitive impairment, abnormal circadian rhythms, courtship behavior defect, lethality, defect in synaptogenesis, spermatogenesis	Wan et al., 2000; Zhang et al., 2001; Dockendorff et al., 2002; Morales et al., 2002; Jin et al., 2007; Sekine et al., 2008; Sofola et al., 2008; Choi et al., 2010
	Zebrafish	Abnormal axonal branching, cardiomyopathy, muscular dystrophy	Tucker et al., 2006; Van't Padje et al., 2009

to induce developmental defects in *D. melanogaster* remarkably similar to those caused by genetic reduction of the notch signaling pathway (Micchelli et al., 2003). To date, there are no published studies aimed at identifying novel potential drugs for treating AD in the *D. melanogaster* model system through screening processes. Development of invertebrate models, especially *D. melanogaster* models of AD, provide excellent tools for performing drug screens to identify small molecules that can suppress the toxicity associated with Aβ accumulation and modulate the γ-secretase activity.

3. Parkinson's Disease. Parkinson's disease is the second most common age-related neurodegenerative disease and is clinically characterized by muscle rigidity, resting tremor, bradykinesia, and postural instability. PD is caused by degeneration of dopaminergic neurons in the substantia nigra region of the brain. A pathological hallmark of the disease is the formation of Lewy bodies, intracytoplasmic inclusions that are composed of α-synuclein and ubiquitin, among other proteins. Most cases of PD are sporadic with no known cause. Several familial PD cases have been identified and are caused by mutations in genes, including α-synuclein (Polymeropoulos et al., 1997), Parkin (Kitada et al., 1998), DJ-1 (Bonifati et al., 2003) PINK1, (Valente et al., 2004), LRRK2 (Paisán-Ruíz et al., 2004), and ubiquitin C-terminal hydrolase-1 (Ragland et al., 2009). Of these six PD-associated genes, α-synuclein and Parkin are the most well studied genes. Two missense mutations, A53T (Polymeropoulos et al., 1997) and A30P (Krüger et al., 1998), as well as genomic duplication and triplication of the α-synuclein gene (Singleton et al., 2003; Ibáñez et al., 2004) have been identified as causes of autosomal-dominant forms of familial PD. Parkin mutations were identified in families with autosomal recessive juvenile parkinsonism (Kitada et al., 1998), and further investigations have indicated that parkin functions as an E3 ligase (Giasson and Lee, 2003; Hattori and Mizuno, 2004; Moore et al., 2005), although additional roles in microtubule-based transport (Ren et al., 2003) and regulation of DA transporter activity have been suggested (Jiang et al., 2004). It has been hypothesized that loss of E3 ligase activity is involved in the pathogenesis of parkin-linked PD. Mutations in the LRRK2 gene have been identified as other common genetic causes of PD (Paisán-Ruíz et al., 2004; Zimprich et al., 2004). The most common mutations in LRRK2 are G2019S (Lesage et al., 2006) and G2385R (Tan and Schapira, 2008). LRRK2 is composed of multiple domains, including a GTPase domain and a kinase domain capable of exhibiting GTP-dependent phosphorylation activity (West et al., 2005). It has been shown that the disease-associated mutations of LRRK2 can increase its kinase activity and thereby its toxicity (Smith et al., 2006; West et al., 2007). DJ-1 encodes a highly conserved protein belonging to the ThiJ/PfpI superfamily. There are rare mutations reported in the DJ gene, and it has been suggested that DJ-1 is a rare cause of PD (Bonifati et al., 2003). A list of reported fly and other small animal models of PD is provided in Table 2.

4. Triplet Repeat Expansion Diseases. At present, 22 different neurological diseases are known to be caused by expansion of triplet repeats in the human genome. In 1991, two repeat expansion mutations, fragile X mental retardation syndrome (FMR1) and spinal and bulbar muscular atrophy (SBMA), were reported to produce disease phenotypes by expanded poly-amino acid tracts. The clinical phenotypes of triplet repeat expansion diseases depend on the context of the protein where the repeat expansions occur (La Spada and Taylor, 2003). Polyglutamine diseases are caused by mutations that lead to hyperexpansions of unstable CAG repeats, which are translated as glutamine in normal functioning proteins. Polyglutamine diseases are due to single-gene defects and were the first neurodegenerative models successfully created in *D. melanogaster* to use human transgenes and were generated by Nancy Bonini at the University of Pennsylvania. Polyglutamine diseases demonstrate several characteristic features in patients, such as nuclear inclusions containing the mutant protein, repeat length inversely correlated with age of onset, and age-dependent motor neuron degeneration and impairment. There are several *D. melanogaster* models of triplet repeat expansion diseases, including fragile X mental retardation with overexpression of the *FMR1* gene with various CAG repeat lengths, HD using expression of truncated wild-type and mutant forms of huntingtin/htt (Jackson et al., 2002), SCA 3 or Machado-Joseph disease expressing truncated ataxin 3 using different glutamine repeat lengths, and SBMA by expressing the human androgen receptor gene with different polyglutamine repeat lengths (Pandey et al., 2007a; Batlevi et al., 2010). All of these models demonstrated that increased poly-Gln expansion led to increased severity of degeneration, age-dependent degeneration, and repeat length-dependent protein aggregation (La Spada and Taylor, 2010). These models provided a platform to demonstrate that human disease genes can yield parallel neurodegenerative effects in *D. melanogaster*. It is noteworthy that a few studies also found that poly-Gln expression in glia can cause lethality and neurodegeneration.

5. Sleep. According to a recent Institute of Medicine report (http://www.iom.edu/sleep), at least 40 million Americans suffer from chronic, long-term sleep problems and an additional 20 million people experience occasional sleep disturbances. Sleep disorders account for an estimated $16 billion in medical costs each year, although the indirect costs due to lost productivity and other factors are largely unknown and probably much greater compared with the medical costs. Therefore, this is a very attractive area for drug discovery. *D. melanogaster* exhibits many of the behavioral characteristics of mammalian sleep, enabling the use of powerful genetic approaches to understand conserved fundamental as-

pects of sleep. As do humans, flies have a circadian activity cycle. They have a morning bout (lights on) of activity, followed by a mid-afternoon siesta period of inactivity, a late afternoon peak of activity, and relative inactivity during the night (lights off) (Cirelli, 2009). The behavior and neurological function of flies in longer bouts of inactivity more closely resemble sleep in mammals than quiet rest (Shaw et al., 2000). Significantly, wake-promoting agents such as modafinil and caffeine have similar effects in the fly, as do sleep-promoting agents such as antihistamines (Shaw et al., 2000; Hendricks et al., 2003; Andretic et al., 2008).

Over the past several years, *D. melanogaster* studies have led to the identification of novel genes (for example, *Shaker* and *sleepless*) and molecular pathways that can modulate sleep and candidate brain regions known to function in circadian regulation as well as learning and memory. *Shaker*, identified with a mutational ethylmethane sulfonate screen, encodes for the α-subunit of a tetrameric potassium channel that mediates a voltage-activated, fast-inactivating I_A current. *Shaker* loss-of-function mutant flies sleep only 2 to 4 h every day rather than 8 to 10 h (Schwarz et al., 1988). Learning and memory in these shaker mutant flies is significantly impaired, and lifespan is reduced (Schwarz et al., 1988; Cirelli et al., 2005). In contrast, *sleepless* was identified by a different approach called insertional mutagenesis (Koh et al., 2008). The *sleepless* flies, similar to the *Shaker*-null mutants, sleep only 2 h/day (significantly less than age-matched control flies), mainly because of a decrease in sleep episode duration. The *sleepless* gene encodes for a glycosylphosphatidylinositol-anchored protein with unknown function (Koh et al., 2008).

It has been demonstrated that *D. melanogaster* sleep patterns change with changes in physiology and aging. It has been shown that sleep becomes more fragmented in older flies like sleep patterns change in humans with age (Koh et al., 2006). Young flies have long, uninterrupted sleep bouts that occur mostly at night. However, sleep in older flies becomes more evenly perturbed across the 24-h day. Correlated with changes in sleep patterns during aging are changes in the strength of circadian rhythms, which suggests that the circadian clock exerts some influence over sleep consolidation. The rate of decline in the strength of circadian activity with the aging process can be altered by temperature and exposure to oxidative stress-causing agents such as paraquat (Koh et al., 2006).

Although drug discovery screens for sleep-related therapeutics have not been reported in the literature, all the tools are in place for such an initiative. Rihel et al. (2010) have successfully developed and applied a high-throughput, quantitative screen for small molecules that could alter the locomotor behavior of larval zebrafish. This is an important step toward not only identifying and characterizing psychotropic drugs involved in locomotor behavior in a whole organism but also toward dissecting the pharmacology of complex behaviors. One direction for discovery in flies could be to identify wake-promoting agents either in normal animals or in genetic models exhibiting excessive sleep. Another direction could be to identify novel sleep-promoting agents by using normal flies or genetically altered flies that have reduced sleep and assaying for increased sleep. In each strategy, HTS could be performed using arrays of photo beam-based activity arrays and software to measure circadian activity, as discussed in detail in section IV.A.5.

6. Seizure Disorders. Epilepsy was one of the first brain disorders to be described (Goldenberg, 2010), affecting more than 2 million people in the United States. Similar to all animals with complex nervous systems, including humans, electrical shock delivered to the *D. melanogaster* brain elicits seizure-like activity (Pavlidis and Tanouye, 1995; Lee and Wu, 2002). Therefore, *D. melanogaster* has been developed as a model to study seizure disorders.

There is a collection of 11 seizure-sensitive *D. melanogaster* mutants, also known as bang-sensitive (BS) paralytic mutants, that recapitulate key features of human seizures. Seizure-like behaviors prominent in these mutants becomes more obvious after a mechanical shock, such as a tap of the culture vial on the bench top (a "bang"). BS mutants display seizure-like behaviors characterized by initial seizure, temporary paralysis, and recovery seizure (Benzer, 1971; Ganetzky and Wu, 1982). The BS behavioral phenotype is fully penetrant, with electrophysiological seizure thresholds usually below 7 V (Kuebler and Tanouye, 2000). Normal flies, however, never show a BS behavioral phenotype and have electrophysiological seizure limits greater than approximately 35 V (Kuebler and Tanouye, 2000). It is noteworthy that there are several noncanonical BS mutants recently identified, including *couch potato* (*cpo*) and *kazachoc* (*kcc*). Unlike most of the original BS mutants, the BS phenotype in these noncanonical BS mutants are incompletely penetrant, and their seizure thresholds tend to be somewhat higher (11–16 V) but still significantly lower than wild-type levels (Kuebler and Tanouye, 2000).

Although the BS mutant seizure physiology resembles that observed in mammals, the BS genes do not, however, correspond to known mammalian genes involved in seizure disorders. Nevertheless, there are significant similarities between human seizures and *D. melanogaster* seizure models, providing support for the utility of the *D. melanogaster* model system for drug discovery. The importance of the *D. melanogaster* seizure model has been further strengthened by the fact that seizure-like activity spreads through the fly CNS along particular pathways that are dependent on functional synaptic connections and recent electrical activity, as do seizures in humans. Seizure-like activity in flies can also be spatially segregated into particular regions of the

CNS. It is noteworthy that *D. melanogaster* seizure phenotypes can be ameliorated by human antiepileptic drugs (AEDs) such as sodium valproate and phenytoin (which act as sodium channel blockers), gabapentin (a calcium channel blocker), and potassium bromide (a chloride channel blocker) (Kuebler and Tanouye, 2000; Tan et al., 2004; Song and Tanouye, 2006). Other therapeutics, however, including carbamazepine, ethosuximide, and vigabatrin do not have efficacy in the BS mutant strains (Reynolds et al., 2003). It is noteworthy that the anticonvulsant lamotrigine has been found to extend lifespan of *D. melanogaster* (Avanesian et al., 2010). Consistent with the mechanism of action of AEDs that primarily target sodium channels, it has been shown that mutations in sodium channels that decrease conductance can suppress seizures in flies (Reynolds et al., 2003; Tan et al., 2004).

The drug-screening strategy for seizure disorders can potentially be divided into two steps. In the first step, a library of drugs can be delivered through either the feeding or the larval bathing methods to identify effective compounds that rescue seizure-like phenotypes or paralysis. Subsequently, effective drugs can be validated more thoroughly by direct brain injection, electrophysiology, and behavioral methods. Significantly, validation of these screening approaches in a *D. melanogaster* model of seizures, where the fly contains a mutant allele of the $GABA_A$ receptor, using a panel of current AEDs has already been performed, demonstrating the potential effectiveness of this model for high-throughput AED discovery (Stilwell et al., 2006).

7. Cognitive/Psychosis/Affective Disorders. Disorders of the CNS that influence affect and cognition are complex multifactorial diseases involving genetics and environmental factors. Traditional animal models of schizophrenia and depression used in the drug discovery process are problematic because they do not model the disease state in humans, they model only certain behavioral and neurochemical aspects (Nestler and Hyman, 2010). For example, traditional models of schizophrenia employ blockade of the behavioral effects of dopaminergic agonists such as apomorphine or amphetamine, as well as short-term administration of drugs whose effects are thought to resemble psychosis, such as phencyclidine. Models of depression use forced swimming and tail suspension to identify agents able to prolong activity. Although drugs that are effective in these animal models have some efficacy in the clinic, precise therapeutic mechanisms of action remain largely unknown (i.e., atypical antipsychotics and selective serotonin-reuptake inhibitor antidepressants). There is currently a need for better animal models, as well as more effective therapeutics (Geyer, 2008; Nestler and Hyman, 2010).

Cognitive and affective disorders are generally regarded to involve disruption of key neurotransmitter systems, including dopamine, serotonin, and glutamate. Significantly, the fly CNS uses the same neurotransmitter systems to mediate many behaviors conserved with mammals, including humans. Because of this conserved neurochemistry, *D. melanogaster* can play an important role in the drug development process for CNS therapeutics. At present, the fly may be most valuable in target discovery experiments. Components of each of the neurotransmitter systems underlying particular behaviors in the fly, identified either through traditional or whole-genome analysis methods, may represent homologs of "druggable" targets in humans. One method of target discovery that holds promise is to express homologs of human genes linked to psychiatric diseases such as schizophrenia in fly brains to produce abnormal behaviors, as has been done for DISC-1, and to then perform genetic screens to identify modifiers whose human homologs may represent "druggable" targets (Sawamura et al., 2008; Furukubo-Tokunaga, 2009). In addition, the fly will be useful in post-HTS validation studies to rapidly and cost-effectively test the efficacy of compounds to block or inhibit behaviors mediated by these neurotransmitters in a whole-animal model. There are a number of behaviors and behavioral assays designed to assess function of these neurotransmitters and their receptors relevant to human neuropsychiatric disorders (Table 3).

Related to cognition and cognitive disorders is the process of learning and memory. The study of learning and memory in the fly has a long and rich history (Quinn et al., 1974; Tully and Quinn, 1985). Indeed, many of the molecular mechanisms underlying learning and mem-

TABLE 3
Neurotransmitter-related behaviors

NT/Receptor	CNS-Related Behavior	Reference
Serotonin	Feeding, aggression, courtship, sleep, learning and memory	Dierick and Greenspan, 2007; Sitaraman et al., 2008; Alekseyenko et al., 2010; Neckameyer, 2010
$5\text{-HT}_{1A\text{-like}}$	Aggression, sleep, learning and memory	Yuan et al., 2005; Johnson et al., 2008, 2009
5-HT_2	Circadian, aggression, visual processing	Nichols and Sanders-Bush, 2002; Nichols, 2007; Johnson et al., 2008
5-HT_7	Learning and memory, courtship and mating	Johnson et al. 2010; C. D. Nichols, unpublished data
Dopamine	Locomotor activity, arousal, circadian	Foltenyi et al., 2007; Hirsh et al., 2010
D1	Learning and memory, prepulse inhibition	Lebestky et al., 2009; Waddell, 2010
D2	Locomotor activity, arousal	Draper et al., 2007
Glutamate	Social interaction, learning and memory	Grosjean et al., 2008
GABA	Sleep, circadian, learning and memory	Chung et al., 2009; Hamasaka et al., 2005; Davis, 2005
Acetylcholine	Learning and memory, circadian	Gu and O'Dowd, 2006; Hamasaka et al., 2007

ory in mammalian systems were first elucidated in the fly. The fly has short- and long-term memory involving acquisition, consolidation, and recall (Margulies et al., 2005). Sophisticated learning and memory assays have been developed to examine olfactory, appetitive, and place conditioning using both aversive and rewarding conditioned stimulus protocols (Davis, 2005; Sitaraman et al., 2008; Krashes et al., 2009). Neurotransmitters critical for proper learning and memory include dopamine, acetylcholine, GABA, serotonin, and glutamate (Gu and O'Dowd, 2006; Liu et al., 2007; Wu et al., 2007; Sitaraman et al., 2008; Waddell, 2010). Aside from the use of *D. melanogaster* for target discovery in basic science studies, flies can be potentially used in primary or validation studies for the identification of "cognitive enhancers" to be used as therapeutics for diseases that impair learning and memory or even age-related decline in learning and memory (Scott et al., 2002).

Although the fly brain is complex, composed of distinct neuropil with functional specialization and many conserved neurotransmitters and advanced behaviors as described above, it is not a human brain, and there are obviously significant differences. Therefore, certain features of the system need to be considered when designing screens and interpreting data. First and foremost, the fly brain is not capable of producing higher order cognitive behaviors that are associated with thought, affect, and other features that give can rise to uniquely human neuropsychiatric disorders. The fly may be most informative in elucidating molecular and genetic mechanisms and in small-molecule discovery for therapeutics relevant to specific behaviors that are associated with neuropsychiatric disorders (e.g., aberrant aggression, sleep, memory) rather than serve as a holistic model for disorders. Whereas the fly has most of the neurotransmitters found in mammals, there are key differences. For example, the fly does not have an adrenergic system and contains neither epinephrine nor norepinephrine nor the α and β adrenergic receptors. Instead, the fly uses octopamine, a trace amine in humans, as a major neurotransmitter that roughly performs similar physiological functions as the mammalian adrenergic neurotransmitter system (Evans and Maqueira, 2005). Furthermore, the fly does not have the full complement of receptors that mammals have for each conserved neurotransmitter. For example, there are six families of GPCR serotonin receptors in mammals and only three in flies, and five dopamine receptor families in mammals but only two in flies (for review, see Nichols, 2006). Moreover, the neurotransmitters themselves can be used differently than in mammals to regulate behaviors. In mammals, the primary excitatory neurotransmitter in the brain is glutamate, and at the neuromuscular junction, it is acetylcholine, but in the fly, the role of these two neurotransmitters are reversed (for review, see Nichols, 2006).

B. Cancer

In the past, cancer research has been conducted almost exclusively in mammalian-based systems ranging from tissue culture to whole-animal studies. Recently, however, the fly has been increasingly used as a model system. Perhaps one of the greatest contributions of the fly to the study of cancer biology was the elucidation of the Ras signal transduction cascade more than 20 years ago in the fly visual system (Simon et al., 1991; Olivier et al., 1993; Nagaraj and Banerjee, 2004). Each of the major components of this pathway have been found to be conserved in mammalian cells. At a fundamental level, cancer can be thought of as a misregulation of signaling events within a cell that leads to abnormal growth and proliferation. Depending on the type and nature of the cancer, the underlying mechanisms of the abnormal proliferation are varied and often remain elusive. Therefore, potential therapeutics are likely to be dependent on a detailed understanding of individual types of cancers. A so-called "magic bullet" effective against all or many types of cancer may still be possible, however, with further research into the nature of abnormal cell proliferation.

The majority of cancers in humans are derived from epithelial cells (Christofori and Semb, 1999), making these types of tumors significant targets for therapeutics. Accordingly, there are a number of fly models being developed to study epithelial cell-derived cancers that could and are being translated to a discovery platform. These models include not only proliferative phenotypes but metastatic and invasive ones as well. The challenge here is to develop effective screening paradigms that are able to identify agents able to prevent or inhibit proliferation and metastasis. One effective strategy has been to misexpress either the *D. melanogaster* version of a human signaling molecule linked to tumors, or the human protein itself, in the eye of the fly. The repeating "crystalline" nature of the eye make it highly susceptible to even slight perturbations in development, which usually manifest as a rough or disorganized phenotype easily scored by simple observation (Cagan and Ready, 1989). For example, Cagan and colleagues (Vidal and Cagan, 2006) misexpressed the fly homolog for the Ret receptor tyrosine kinase (implicated in human multiple endocrine neoplasia type 2), dRet, in a constitutively active form and produced a rough eye phenotype. They used this fly to both perform modifier screens to identify interacting factors as well as to validate efficacy of a small molecule inhibitor of Ret in vivo (Vidal and Cagan, 2006). Additional epithelial models using morphological changes in adult structural phenotypes have also been developed for discovery of molecules targeting the EGF receptor/ras pathway (Aritakula and Ramasamy, 2008), and E-cadherin (Pereira et al., 2006).

Alternative approaches have involved higher throughput strategies using larva and pupae. For example, one exciting model is a high-throughput platform examining

pupal viability as a measure of tumor suppression. In this model, invasive tumors and ultimately cell death at the pupal phase are produced from the expression of both a constitutively active form of Ras and a mutant of the tumor suppressor scribble together in imaginal discs (Pagliarini and Xu, 2003; Humbert et al., 2008; Wu et al., 2010). It is noteworthy that these flies were also engineered so that the tumors express the marker protein GFP, allowing for visual quantification of tumor size and metastasis. Assays are conducted in 96-well plates with a small number of larva seeded per well, with drug present in the media. After 5 days, a sucrose solution is added to the wells, and the dead larvae float to the top, where the GFP intensity, as a measure of tumor growth, can be measured by microscopy.

Another high throughput screening system relies upon flies with gain of function raf or a dominant negative allele of Notch, that each exhibit abnormal cell growth of midgut epithelium as a model for asymmetric stem cell division related cancers (Micchelli and Perrimon, 2006; Januschke and Gonzalez, 2008). For this assay, raf or Notch mutant flies that express luciferase in gut epithelial cells are maintained on 96-well plates on media containing test drug, and then homogenized and assayed for luciferase activity as a measure of abnormal proliferation.

Additional opportunities for discovery lie in other types of cancers, including those derived from blood cells. Much work has been performed demonstrating conservation in blood cell development between flies and humans, including the study of lozenge/Runt-related transcription factors (Braun and Woollard, 2009) and JAK/STAT signaling (Bina et al., 2010) in hematopoietic cells; however, high-throughput assays for therapeutic discovery relevant to blood cancers such as leukemia remain to be developed. Nevertheless, there are limitations of the fly in cancer research. Whereas fundamental molecular mechanisms underlying tumorigenesis and metastasis can probably be efficiently probed in *D. melanogaster*, the fly is not able to model many types of tumors that are common in humans, such as those related to specific tissues (e.g., prostate, ovarian, or breast cancer).

C. Cardiovascular

Cardiovascular disease (CD) and related illnesses are the leading cause of death in the United States, and therefore a highly desirable area for development of new and more effective therapeutics. Recent work has indicated that the fly can be used successfully in the discovery process for CD. A key consideration to keep in mind is that cardiovascular diseases are for the most part complex multifactorial disorders that involve heredity as well as environmental factors, and that whereas certain aspects of CD can be modeled in the fly to yield informative results, the inherently complex nature of the cardiovascular system in humans presents certain limitations in the fly for accurate modeling. For example, the fly heart has only one cardiac chamber and has no coronary arteries.

Fly heart development depends on a set of genes conserved up through mammals (Bryantsev and Cripps, 2009; Reim and Frasch, 2010), and sophisticated tools have been developed, including tomography, to allow its function to be probed in detail (Choma et al., 2006; Null et al., 2008; Bradu et al., 2009). Various forms of dysfunction that include structural defects, arrhythmias, and cardiomyopathies are known to occur in natural populations of flies (Ocorr et al., 2007c). Many of these effects can be age-related, and even result in cardiac failure in the fly (Ocorr et al., 2007a,b). Together, these aspects of the fly heart and its function indicate that the fly can be a valid model for the study of aspects of mammalian CD and an important tool in the process to discover new therapeutics (Wolf and Rockman, 2008; Wu and Sato, 2008; Akasaka and Ocorr, 2009). Significantly, the beating fly heart can be observed through a traditional dissection microscope for analysis. An excellent resource for protocols on visualization, dissection, and electrophysiological recording from larva heart is a publication from Robin Cooper and the accompanying video tutorial (Cooper et al., 2009). Using these methods, it is possible to easily examine the effects of pharmacological agents on heart function (Dasari and Cooper, 2006; Dasari et al., 2007; Neckameyer et al., 2007). Additional tools to facilitate examination of the heart include GAL4 drivers that can be used to express GFP in the heart, allowing for real-time observation of function with conventional epifluorescence or confocal microscopy (Wu and Sato, 2008; Alayari et al., 2009; Vogler and Ocorr, 2009).

So where does the fly fit in the overall scheme of the discovery process for CD? One important role is in the discovery of new targets through genetic methods to identify components crucial for heart function (Kim et al., 2010; Neely et al., 2010) for which subsequent traditional small-molecule discovery can then be performed against. There is also a role in the validation process of positive hits from more traditional screens to assess the actions of particular drugs on cardiac function using low-throughput methods (Akasaka and Ocorr, 2009). Given the recent advances in genetic and imaging tools available to examine fly heart function, it is hoped that higher throughput methods will be soon developed enabling this powerful model to be used for small molecule discovery.

D. Inflammation/Infectious Disease

D. melanogaster have a very sophisticated immune response that current research demonstrates is highly relevant to the understanding of human inflammatory conditions. Flies are constantly exposed to pathogens within their environment, largely in the form of bacteria, both as larvae and as adults. In response to patho-

gen challenge, antimicrobial peptides are released through two primary pathways that involve evolutionarily conserved components, including Toll and Toll-like receptors, as well as nuclear factor-κB, tumor necrosis factor-α, and JAK/STAT signaling. Whereas *D. melanogaster* has a sophisticated innate immune system, largely evolved to combat bacterial and fungal pathogens, the fly does not have an adaptive immune system. Therefore, a potentially significant limitation is that the fly is not an appropriate model for the study of antibody- and lymphocyte-dependent adaptive immune defenses. The following articles are recommended for more comprehensive reviews of the basic physiology of the inflammatory response and immune signaling in the fly: Kauppila et al. (2003), Ferrandon et al. (2007), Wu and Silverman (2007), and Hetru and Hoffmann (2009).

Although it is conceivable that multiple human inflammatory conditions can be modeled and used in the discovery process, the *D. melanogaster* model for asthma, which is the most common chronic inflammatory disease of the lung, is arguably the most advanced. The *D. melanogaster* respiratory system is the trachea, which consists of approximately 10,000 interconnected and branching tubules. Significantly, there are many conserved genes and regulatory components between trachea development in the fly and lung development in mammals (Liu et al., 2003; Horowitz and Simons, 2008). Airway epithelial cells form the trachea, and they are the first line of defense against airborne pathogens. Unlike mammalian airways, the *D. melanogaster* trachea is much simpler and consists of only one type of epithelial cell (Whitten, 1957; Horowitz and Simons, 2008). Because there is only one type of epithelial cell, it is essentially a cell culture model within an intact organism, and an immune response initiated from any one part of the tracheal system is identical to that initiated from another. Inflammatory responses in the trachea to pathogens include Toll, tumor necrosis factor-α, c-Jun NH_2-terminal kinase, and JAK/STAT signaling activity (Wagner et al., 2008).

Opportunities therefore exist to further develop and use *D. melanogaster* in the asthma therapeutic discovery process (Roeder et al., 2009). One area where the fly shows particular promise is in target discovery. A number of genetic tools are available, including GAL4 drivers that can drive transgene expression specifically in the trachea (Shiga and Tanaka-Matakatsu, 1996; Liu et al., 2003). One method for exploration is to use these strains to drive small interfering RNA elements in the trachea to selectively knockdown expression of genes whose human homologs are important for airway physiology and the development of asthma to produce an abnormal physiological phenotype (Roeder et al., 2009). Genes and proteins identified by forward genetics and modifier screens that rescue these mutant phenotypes represent starting points for traditional high-throughput small-molecule discovery for drugs that would beneficially influence function of not only the fly protein but also the human protein. Another role in target discovery, although more indirect, would be to use the fly as a platform to validate novel genes and proteins identified from human whole-genome association and next-generation sequencing studies (Moffatt et al., 2007) for function in airway epithelial cells and the trachea. Both of these approaches to target discovery have the potential to identify and validate key components of airway function that represent "druggable" targets for asthma therapeutics.

E. Metabolic Disorders and Diabetes

Obesity and related disorders such as diabetes are a significant health problem in the United States. Two thirds of the adult population is overweight, and nearly 4% of the population has diabetes. Accordingly, this is an attractive area for drug discovery. Although the fly has not yet been used in the drug discovery process for this area, recent advances in the understanding of metabolic processes, glucose homeostasis, and endocrinology in the fly have poised *D. melanogaster* as a valid model relevant to human metabolic disorders and diabetes that can be used in the therapeutic discovery arena. Although the molecular mechanisms and signaling underlying metabolic processes are conserved to a degree, a potential limitation of the fly here is that the structures mediating these processes are quite different. For example, the fly does not a have a pancreas that secretes insulin or a liver. Furthermore, unlike for mammals, it is not possible to feed flies a "Western diet" and have them become obese and develop metabolic syndrome.

In the fly, there are neurosecretory cells (Nässel and Winther, 2010) in the brain that secrete insulin, as well as additional secretory cells that secrete a glucagon analog that together exhibit physiological and genetic parallels to the vertebrate endocrine axis (Wang et al., 2007; Haselton and Fridell, 2010). Ablation of the adult insulin-secreting cells can lead to increased glucose levels in the hemolymph (the "blood" of the fly), increased circulating lipids, and resistance to starvation, among other phenotypes (Baker and Thummel, 2007; Haselton and Fridell, 2010). Fat cells and the fat body in *D. melanogaster* perform functions similar to those of the mammalian liver and are regulated by insulin through mechanisms conserved in mammalian systems in terms of metabolism and triglyceride and glycogen storage (DiAngelo and Birnbaum, 2009).

With respect to the use of flies as a model to study diabetes, flies express a homolog of the human sulfonylurea receptor that, along with the Ir potassium channel, forms an ATP-sensitive potassium channel to regulate release of certain fly hormones, including a fly hormone with glucagon-like function (adipokinetic hormone) (Kim and Rulifson, 2004). It is noteworthy that antidiabetic sulfonylurea drugs, including glyburide and tolbutamide, affect glucose homeostasis in the fly through interactions with the ATP-sensitive potassium channel

on neurosecretory cells (Nasonkin et al., 1999; Kim and Rulifson, 2004). It is also noteworthy that flies deficient in insulin production demonstrate a delay in development as well as small body size, both as larvae and as adults (Rulifson et al., 2002; Kaplan et al., 2008; Ruaud and Thummel, 2008). Body size is an easily scorable phenotyp and may be useful in the development of high thr-oughput screens for identification of small molecules able to rescue insulin secretion-deficient mutants. Therefore, flies may potentially be useful in the discovery, screening, and validation phases for diabetes/metabolic disorder therapeutics. Therapeutic classes amenable for discovery may be limited, however. For example, sulfonylurea drugs have efficacy in insulin-deficient flies, but other classes of therapeutics, such as metformin have not been demonstrated to be effective.

V. Successful Examples of *D. melanogaster* in the Drug Discovery Process

As briefly addressed above, there have been several published reports in which the fly has been used for both primary screens and secondary validation of biologically active compounds for therapeutic discovery for a wide range of human diseases, ranging from neurodegeneration to cancer. In this section, we will go into more detail with a few specific examples of successful screens and how the fly was employed, with a focus on discovery in the neurodegeneration arena.

Fragile X syndrome (FXS) is a neurodegenerative disease that has been successfully modeled in flies (Jin et al., 2007; Sofola et al., 2007). Deletion of the human fragile X mental retardation gene (*FMR1*) ortholog in *D. melanogaster* (*Fmr1*) recapitulates several phenotypes associated with fragile X syndrome in humans, including defects in synaptogenesis, courtship behavior, and spermatogenesis (Wan et al., 2000; Zhang et al., 2001; Dockendorff et al., 2002; Morales et al., 2002). In an attempt to identify potential therapeutics, this *D. melanogaster* model was used as a primary screening platform to probe a drug library of 2000 FDA approved compounds (Spectrum Collection compound library; MicroSource Discovery Systems, Gaylordsville, CT) (Chang et al., 2008). For this screen, Chang et al. (2008) followed a high-throughput approach. *Fmr1*(−/−) mutant embryos were sorted using flow cytometry methods, with 12 embryos automatically dispensed into individual wells of 96-well plates, where each well contained a 40 mM concentration of an individual drug from the Spectrum library dissolved in the food substrate. To score rescue of the rate of lethality that occurs before puparium formation that is associated with deletion of the *Fmr1* gene, the percentage of live pupae and adults in each well was counted manually. Of the 2000 compounds tested in this assay, 61 were found to rescue at least some of the lethality associated with *Fmr1*(−/−) flies, and the top 25% of these hits (15 of 61) were selected for further validation based on their more robust effects. Follow-up validation studies included dose response experiments using the same assay, where 9 of these 15 compounds showed a dose-dependent effect for rescuing the lethality associated with the *Fmr1*(−/−) flies. It is noteworthy that three of these were related to the GABA signaling pathway, which is a major pathway underlying many of the symptoms of FXS (Krogsgaard-Larsen et al., 2000; Reith et al., 2006; Chang et al., 2008). These hits were GABA itself, nipecotic acid (a GABA reuptake inhibitor), and creatinine (a potential activator of the $GABA_A$ receptor). It is noteworthy that treatment with bicuculline (a $GABA_A$ receptor antagonist) rescues the lower dendritic spine density associated with knockout of the *Fmr1* gene in the mouse model of FXS (Selby et al., 2007). It remains to be seen whether nipecotic acid or creatinine, or any of the other drugs identified from this fly screen, are effective in the mouse model. Nevertheless, this screen clearly demonstrates that *D. melanogaster* models of human diseases and assay systems are well suited for the drug discovery process. Significantly, multiple high-quality hits were identified from screening only 2000 small molecules, and these compounds can serve as potential candidate compounds (alone or in combination) for developing therapeutic interventions of fragile X syndrome, although they will need to be further validated in a mammalian model system before moving to human clinical trials.

In addition to serving as a primary screening platform, the fly can also be extremely valuable as a secondary validation screen subsequent to traditional in vitro HTS. Using the fly in this manner to rapidly narrow down larger collections of hits to smaller and higher quality collections of leads for subsequent medicinal chemistry optimization and testing in rodent models will likely save considerable resources compared with proceeding with all primary hits in expensive optimization and rodent experiments and will ultimately facilitate the overall discovery process.

For example, in an attempt to identify new therapeutics for SBMA, an in vitro fluorescence resonance energy transfer-based cellular aggregation assay was performed against a library of 2800 biologically active compounds (Annotated Compound Library, Brent R. Stockwell, Columbia University, New York, NY) that assessed the ability of a drug to inhibit polyglutamine protein aggregation in mammalian cells. In this screen, there were 739 positive hits that reduced protein aggregation by more than 30% (Pollitt et al., 2003). It is noteworthy that treatment with one of the strongest hits, 4-[(1*R*)-1-aminoethyl]-*N*-4-pyridinyl-*trans*-cyclohexanecarboxamide (Y-27632), could robustly suppress neurodegeneration in a *D. melanogaster* model of polyglutamine disease (Pollitt et al., 2003). None of the compounds identified, however, had yet been approved for clinical use by FDA (Pollitt et al., 2003). Encouraged by the results from this first screening effort, where some of the more effective drugs

had efficacy in fly models of polyglutamate disease, the same group subsequently undertook another screen and tested 4000 biologically active compounds that contained a large number of FDA-approved drugs in the same fluorescence resonance energy transfer-based assay (Desai et al., 2006). These compounds were obtained from three different sources that included the Annotated Compound Library (ACL) of biologically active small molecules of diverse structure (2800 compounds), a kinase inhibitor collection (300 compounds provided by Dr. Kevan Shokat, University of California at San Francisco, San Francisco, CA), and the National Institute of Neurological Disorders and Stroke Custom Collection of FDA-approved drugs and natural products (1040 compounds).

The ten positive hits from this screen were subsequently tested in a *D. melanogaster* model of Huntington's disease, a poly-Gln degenerative disease similar to SBMA. This involved scoring a progressive photoreceptor neurodegeneration phenotype caused by expression of expanded Huntingtin protein (htt Gln93) in *D. melanogaster* photoreceptor cells as the measure, where degeneration can be quantified by the pseudopupil technique (Franceschini and Kirschfeld, 1971; Steffan et al., 2001). The pseudopupil technique is a simple assay that involves decapitating a fly, using a drop of clear nail polish to mount the head on a glass slide, and then shining a light through the back of the head to observe the general organization of seven visible rhabdomeres (the membranous structures of the photoreceptor neurons that contain the opsins) in the retina across several ommatidium (the individual "unit" that insect eyes are composed of). Degeneration in fly eye models of neurodegeneration perturbs the overall structure of the retina, leading to a progressive loss of pseudopupil organization that correlates with the degree of degeneration. At least 200 pseudopupils were scored from treated and untreated groups and photographed with a dissecting microscope (Steffan et al., 2001). It must be emphasized that treating, preparing, and scoring 200 flies and their retinas requires far less effort and resources than treating, preparing, and scoring even only a fraction of this number of rodent retinas or brains. Significantly, three out of the five drugs found to be effective in alleviating neurodegeneration in the fly were already approved by the FDA (nadolol, fosfanol, and levonordefrin) (Desai et al., 2006). As such, these are high-quality hits, requiring far less resources to bring to market as repurposed therapeutics for neurodegeneration than completely novel drugs needing to go through extensive toxicology testing and early-phase clinical trials demonstrating safety. These three FDA-approved drugs are, unfortunately, associated with some toxicity and are not approved for long-term therapy that would be necessary for patients with HD. The results of these screens, however, can guide further drug development and medicinal chemistry efforts to identify or generate less toxic analogs that may be useful for Huntington's disease therapeutics (Zhang et al., 2005; Desai et al., 2006). Although the five hits from this screen need to be further validated in rodent models of HD, these studies emphasize that secondary screening in flies is a practical method to follow to narrow larger collections of hits to smaller but higher quality collections to proceed with for further development.

In another successful example of *D. melanogaster* in the drug discovery process, the fly was used as an initial whole-animal validation platform of a lead compound immediately after traditional in vitro HTS. This strategy can serve as an informative, economical, and rapid final "flight check" of lead candidates before medicinal chemistry optimization and/or whole-animal rodent studies to identify potential red flags or issues that might preclude further costly steps in its development. For this, Apostol et al. (2003) initiated a large drug screen using a library of 16,000 compounds (Chembridge, San Diego, CA) to identity potential new drugs that can inhibit polyglutamine protein-mediated aggregation using yeast expressing htt-103Q-EGFP as a primary platform. Htt-103Q-EGFP is cytotoxic in yeast and cells expressing mutant huntingtin protein grow poorly (Meriin et al., 2002). The primary screen identified nine compounds that significantly increased the mutant yeast's growth. These chemicals were then tested in in vitro mammalian cell culture models, where four were found to reduce polyglutamine-mediated aggregation in PC12 cells as well as COS cells (Apostol et al., 2003; Zhang et al., 2005). These four compounds, as well as three structural analogs, were next tested for their ability to inhibit aggregation in mouse hippocampal slice culture. The slices were from the brains of R6/2 transgenic animals that ubiquitously express human htt exon 1 with >150 polyglutamine repeats (HD 150Q), and have several similar neurological and biochemical features conserved with HD (Mangiarini et al., 1996). One compound, C2-8, structurally analogous to one of the original four hits, was found to be very effective at reducing aggregation in the mutant hippocampal slices (Zhang et al., 2005). The next step in the development process was to test the efficacy of this drug in a whole-animal model. A fly model of HD was chosen for initial tests, where C2-8 was found to be very effective in suppressing neurodegeneration (Zhang et al., 2005). Based upon these results, such effective compounds can be moved forward for testing in rodent whole-animal models with added confidence for success. In this strategy, if lead candidates are not found to be effective or prove toxic in an unexpected manner, then they may have similar outcomes in rodent whole-animal models, and their advancement through the pipeline could be paused for further investigation before investing significant resources to move the drug to rodent model testing.

VI. Resources

There are several online resources available for Drosophila geneticists (fly pushers) that provide crucial information about available mutant alleles, RNAi knockdown lines, human disease homologs in *D. melanogaster*, and whole-genome sequences. The most useful and comprehensive internet-based resource for the fly community, and those considering using flies, is Flybase (http://flybase.org/). Flybase also provides links to other stock centers and to virtually every relevant Drosophila information web site available. Following the link to Flybase below are additional internet resources arranged in alphabetical order.

A. Flybase and Other Internet Resources

The primary source of genetic and genomic information on *D. melanogaster* can be obtained from Flybase, a comprehensive "one-stop shop" for information and data on *D. melanogaster*, and new *D. melanogaster* researchers are encouraged to become familiar with this web site. Flybase provides a comprehensive and integrated view of data obtained from the published scientific literature, sequence databanks, and large-scale data providers of *D. melanogaster* material resources (such as mutant stocks or cell lines). Also included at Flybase are information on genes, annotation, gene sequences, transgene constructs, and their insertions in experimental genomes, and references to the literature. Most of the other important *D. melanogaster*-related web sites are linked at Flybase, making it easier for fly researchers to easily obtain or reach much of the available information from one site.

- Flybase: http://flybase.org/.
- Berkeley Drosophila Genome Project (home of the fly genome project): http://www.fruitfly.org.
- Drosophila Interaction Database (CuraGen) (assembled gene and protein interaction data from a variety of sources): http://www.droidb.org/DBdescription.jsp.
- *Drosophila melanogaster* Exon Database (a database that contains information on *D. melanogaster* exons presented in a splicing graph form): http://proline.bic.nus.edu.sg/dedb.
- Drosophila Polymorphism Database (a collection of all the existing polymorphic sequences in the *Drosophila* genus): http://dpdb.uab.es/DPDB/dpdb.asp.
- Drosophila Population Genome Project (accurate and complete description of genomes of collections of individuals from populations of *D. melanogaster* worldwide): http://www.dpgp.org.
- Drosophila Species Genomes (a comparative database of *Drosophila* species): http://insects.eugenes.org/DroSpeGe/.
- DrosDel Drosophila Isogenic Deficiency Kit (a collection of isogenic *Drosophila* strains containing deletions covering most of the genome): http://www.drosdel.org.uk.
- Drosophila Genetic Resource Center (Kyoto) (stock center and information database based in Japan): http://www.dgrc.kit.ac.jp/en/index.html.
- Drosophila Genomics Resource Center (resource center that provides cDNA clones, vectors, and cell lines): http://dgrc.cgb.indiana.edu.
- East Asian Distribution Center (provides antisera to segmentation gene proteins): http://www.nig.ac.jp/labs/DevGen/segmentation.
- Flybrain (an online atlas and database of the Drosophila nervous system): http://flybrain.neurobio.arizona.edu.
- FlyChip (provides *D. melanogaster* microarrays and screening services): http://www.flychip.org.uk.
- FlyEx (a database of segmentation gene expression): http://urchin.spbcas.ru/flyex.
- FlyMine (an integrated database for *D. melanogaster* and *Anopheles* spp. genomics): http://www.flymine.org.
- FlyMove (images and movies of *D. melanogaster* development): http://flymove.uni-muenster.de.
- FlyPNS (a database specializing in the embryonic and larval peripheral nervous system): http://www.normalesup.org/~vorgogoz/FlyPNS/page1.html.
- FlySNP (high density genome-wide map/database of single nucleotide polymorphisms): http://flysnp.imp.univie.ac.at.
- FlyTrap (stock collection of random enhancer trap strains with associated expression patterns in the UK): http://www.fly-trap.org.
- FlyTrap (stock collection of gene trap GFP strains at Yale): http://flytrap.med.yale.edu.
- Gene Disruption Project P-Screen Database (searchable database of gene disruption strains): http://flypush.imgen.bcm.tmc.edu/pscreen.
- Homophila (human disease to *D. melanogaster* gene database): http://superfly.ucsd.edu/homophila.
- Interactive Fly (a large database of information regarding all aspects of fly development with links to other important resources): http://www.sdbonline.org/fly/aimain/1aahome.htm.
- WWW Virtual Library–Drosophila (a list of links to various *D. melanogaster* online resources): http://www.ceolas.org/fly.

B. Stocks and Reagents/Services

The *D. melanogaster* public stock centers are valuable resource for obtaining a variety of tools (mutant strains, RNAi strains, balancers, and deficiency kits) for research. Among these stock centers, the Bloomington Drosophila Stock Center at Indiana University is the largest commonly used stock center by fly geneticists.

- The Bloomington Drosophila Stock Center at Indiana University: http://flystocks.bio.indiana.edu/.

- Drosophila RNAi Screening Center: http://flyrnai.org/RNAi_index.html.
- Drosophila TILLING Project: http://tilling.fhcrc.org/fly/.
- Drosophila Affymetrix GeneChip Arrays: http://www.affymetrix.com/.
- Duke University Model System Genomics: http://www.biology.duke.edu/model-system/FlyShop/index.html.
- Exelixis Drosophila Stock Collection at Harvard Medical School: http://drosophila.med.harvard.edu.
- Fly stocks of National Institute of Genetics (NIG-FLY): http://www.shigen.nig.ac.jp/fly/nigfly/index.jsp.
- Gene Disruption Project Database: http://flypush.imgen.bcm.tmc.edu/pscreen/.
- Kyoto Institute of Technology, Japan: http://kyotofly.kit.jp/cgi-bin/stocks/index.cgi.
- Vienna Drosophila Resource Center (RNAi strain collection): http://www.vdrc.org.

1. Injection/Transgenic Production. Besides the public stock centers, there are several private companies that provide routine services for microinjection of DNA to generate transgenics, screening for florescent phenotypes, and balancing of transgenic flies (The BestGene Inc., Genetic Services Inc., and Rainbow Transgenic Flies Inc).

- Genetic Services:
- http://www.geneticservices.com.
- The BestGene: http://www.thebestgene.com.
- Rainbow Transgenic Flies: http://www.rainbowgene.com/services.html.

2. Companies Performing Preclinical Screening in Flies. There are several companies that have been using *D. melanogaster* as a primary platform for screening for therapeutics for human diseases and behaviors such as learning, cognition, neurodegenerative diseases, diabetes, and cancer.

- Aktogen (15 years of experience in performing *D. melanogaster* behavioral test for learning and memory, also actively involved in drug discovery of human CNS-related disorders): http://www.aktogen.co.uk.
- Brain-wave Discovery (provides contract-based services for drug discovery projects related to learning/cognition, neurodegeneration, and novel target analysis): http://www.brainwave-discovery.com/index.htm.
- En Vivo Pharmaceuticals (focused on discovering and developing drugs for CNS-related disorders, particularly Alzheimer's disease and schizophrenia): http://www.envivopharma.com.
- Genescient Corp (drug discovery for aging related disorders using *D. melanogaster* as a model system): http://www.genescient.com/.
- Medros Pharmaceuticals (drug screening for cancer metastasis and diabetes-related disorders using *D. melanogaster*): http://www.medrospharma.com.
- Molecular Libraries Program: Pathways to Discover (this is an National Institutes of Health-sponsored program for discovering drugs through HTS that can modulate a given biological pathway or disease state): http://mli.nih.gov/mli/mlpcn/.

C. Conferences and Courses

1. Conferences. There are many scientific platforms where the fly researchers can present their data and discuss new ideas. The Annual Drosophila Research Conference is considered the largest international meeting for the Drosophila community. In general, most of the other genetic meetings organize separate sessions on animal models that allow fly researchers to present their work.

- American Society of Human Genetics: http://www.ashg.org/2010meeting/.
- Annual *Drosophila* Research Conference: http://www.drosophila-conf.org/2011/.
- European *Drosophila* Neurobiology Conference: http://www.meeting.co.uk/confercare/neurofly2010/.
- Cold Spring Harbor Laboratory Neurobiology of Drosophila: http://meetings.cshl.edu/meetings/dros09.shtml.
- EMBO Conference Series: the molecular and developmental biology of *Drosophila*: http://cwp.embo.org/cfs3–10-03/.
- EMBO symposia: Functional Neurobiology in Minibrains: from Flies to Robots and Back Again: http://www.esf.org/activities/esf-conferences/details/2010/confdetail324.html.
- London Fly Meeting (biennial): http://www.lfm2010.net/.
- Model organism to human biology: http://www.mohb.org/2010/.
- *Swiss Drosophila* meeting: http://www.unifr.ch/zoology/assets/files/rev_Program-2010.pdf.

2. Courses. There are few courses on basics of *D. melanogaster* genetics regularly offered by the Cold Spring Harbor Laboratory and the Wellcome foundation. These courses are offered to a limited number of selected candidates and often offer a scholarship to defer the cost of the course.

- Drosophila Genetics and Genomics: http://www.wellcome.ac.uk/Education-resources/Courses-and-conferences/Advanced-Courses/Courses/WTX027650.htm.
- Neurobiology of *Drosophila* – Cold Spring Harbor Laboratory, NY: http://meetings.cshl.edu/courses/c-dros10.shtml.

D. Useful Books for Drosophila Research

There are several books on Drosophila that can also be used for teaching basic fly genetics to new researchers.

Among these books, *Fly Pushing: The Theory and Practice of Drosophila Genetics* by R. J. Greenspan, is the most popular.

- Ashburner MA, Golic KG, and Hawley RS (2004) *Drosophila: A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Ashburner M and Wright TR (1979) The Genetics and Biology of Drosophila. Academic Press, San Diego, CA.
- Bate M and Martinez AA (1993) *The Development of Drosophila melanogaster*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Demerec M (1994) *Biology of Drosophila.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Echalier G (1997) *Drosophila Cells in Culture.* Academic Press, San Diego, CA.
- Greenspan RJ (1997) *Fly Pushing: the Theory and Practice of Drosophila Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Hartenstein V (1995) *Atlas of Drosophila Development.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Roberts DB (1998) *Drosophila: A Practical Approach.* Oxford University Press, New York, NY.
- Zhang B, Freeman MR, and Wadde S (2010) *Drosophila Neurobiology: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

E. Small-Molecule Libraries

Below are listed sources of small molecule libraries potentially suitable for *D. melanogaster* screens. These are provided by small- to medium-size pharmaceutical companies involved in drug development process for a variety of human diseases. These companies produce or provide diverse classes of chemicals and sell them individually or in collections to the researchers for further testing. Among these, MicroSource, Inc provides several useful drug libraries such as "The Spectrum Collection" (1280 FDA-approved drugs collection) that cover a broad range of biologically active and chemically diverse compounds. It is noteworthy that each of the drugs in the Spectrum collection are already FDA-approved, potentially reducing preclinical testing necessary for new therapeutic uses while repurposing the drug.

- MicroSource Inc. (A leading provider of synthetic compounds as well as FDA approved drugs and natural products in 96-well plate format): http://www.msdiscovery.com/home.html.
- ChemBridge Corporation (offers drug libraries with over 700,000 diverse compounds and target based compounds): http://www.chembridge.com/products.html.
- Prestwick Chemical Library (Offers contractual drug discovery services and provides several small molecular libraries with diverse classes of compounds): http://www.prestwickchemical.fr/index.php?pa=26.
- ActiMol (Provides predesigned chemical libraries of 100,000 compounds in micro plate and vial format): http://www.actimol.com/.
- Comgenex (in addition to providing a diverse classes of chemical libraries of 200,000 compounds, this company also provides technical expertise to bridge the gap from genomic/proteomics to novel drug targets): http://www.rdchemicals.com/targeted-compound-libraries/comgenex.html.
- Analyticon Discovery (provides purified compounds and semisynthetic compounds from natural products and the drug libraries, has over 25,000 compounds): http://www.ac-discovery.com/index.php.
- Enamine (one of the largest providers of screening compounds for HTS, building blocks, custom synthesis and molecular modeling): http://www.enamine.net/.
- Life Chemicals (this company has a collection of 736,000 drug-like compounds and can provide several small molecule libraries with diverse compounds for drug discovery): http://www.enamine.net/.
- TimTech (leading provider of synthetic organic and natural compounds, targeted libraries, building blocks. and custom synthesized compounds for drug screening): http://www.timtec.net/.
- Vitas-M Laboratory Ltd. (provider of large organic compounds for drug discovery): http://www.vitasmlab.com/.
- Zelinsky Institute Inc. (source of organic compound libraries for biological screening, provider of building blocks and custom synthesis): http://www.zelinsky.com/.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Pandey and Nichols.

References

Adams MD, Celniker SE, Holt RA, Evans CA, Gocayne JD, Amanatides PG, Scherer SE, Li PW, Hoskins RA, Galle RF, et al. (2000) The genome sequence of *Drosophila melanogaster*. *Science* **287:**2185–2195.

Akasaka T and Ocorr K (2009) Drug discovery through functional screening in the Drosophila heart. *Methods Mol Biol* **577:**235–249.

Alayari NN, Vogler G, Taghli-Lamallem O, Ocorr K, Bodmer R and Cammarato A (2009) Fluorescent labeling of Drosophila heart structures. *J Vis Exp* doi:10.3791/1423.

Alekseyenko OV, Lee C, and Kravitz EA (2010) Targeted manipulation of serotonergic neurotransmission affects the escalation of aggression in adult male *Drosophila melanogaster*. *PLoS ONE* **5:**e10806.

Andretic R, Kim YC, Jones FS, Han KA, and Greenspan RJ (2008) Drosophila D1 dopamine receptor mediates caffeine-induced arousal. *Proc Natl Acad Sci USA* **105:**20392–20397.

Anichtchik O, Diekmann H, Fleming A, Roach A, Goldsmith P, and Rubinsztein DC (2008) Loss of PINK1 function affects development and results in neurodegeneration in zebrafish. *J Neurosci* **28:**8199–8207.

Apostol BL, Kazantsev A, Raffioni S, Illes K, Pallos J, Bodai L, Slepko N, Bear JE, Gertler FB, Hersch S, et al. (2003) A cell-based assay for aggregation inhibitors as

therapeutics of polyglutamine-repeat disease and validation in Drosophila. *Proc Natl Acad Sci USA* **100:**5950–5955.
Arias AM (2008) *Drosophila melanogaster* and the development of biology in the 20th century. *Methods Mol Biol* **420:**1–25.
Aritakula A and Ramasamy A (2008) Drosophila-based in vivo assay for the validation of inhibitors of the epidermal growth factor receptor/Ras pathway. *J Biosci* **33:**731–742.
Auluck PK and Bonini NM (2002) Pharmacological prevention of Parkinson disease in Drosophila. *Nat Med* **8:**1185–1186.
Auluck PK, Chan HY, Trojanowski JQ, Lee VM, and Bonini NM (2002) Chaperone suppression of alpha-synuclein toxicity in a Drosophila model for Parkinson's disease. *Science* **295:**865–868.
Avanesian A, Khodayari B, Felgner JS, and Jafari M (2010) Lamotrigine extends lifespan but compromises health span in *Drosophila melanogaster*. *Biogerontology* **11:**45–52.
Bainton RJ, Tsai LT, Singh CM, Moore MS, Neckameyer WS, and Heberlein U (2000) Dopamine modulates acute responses to cocaine, nicotine and ethanol in Drosophila. *Curr Biol* **10:**187–194.
Baker KD and Thummel CS (2007) Diabetic larvae and obese flies-emerging studies of metabolism in Drosophila. *Cell Metab* **6:**257–266.
Bandmann O and Burton EA (2010) Genetic zebrafish models of neurodegenerative diseases. *Neurobiol Dis* **40:**58–65.
Batlevi Y, Martin DN, Pandey UB, Simon CR, Powers CM, Taylor JP, and Baehrecke EH (2010) Dynein light chain 1 is required for autophagy, protein clearance, and cell death in Drosophila. *Proc Natl Acad Sci USA* **107:**742–747.
Bellen HJ, Tong C, and Tsuda H (2010) 100 years of *Drosophila* research and its impact on vertebrate neuroscience: a history lesson for the future. *Nat Rev Neurosci* **11:**514–522.
Benzer S (1971) From the gene to behavior. *JAMA* **218:**1015–1022.
Bina S, Wright VM, Fisher KH, Milo M, and Zeidler MP (2010) Transcriptional targets of Drosophila JAK/STAT pathway signalling as effectors of haematopoietic tumour formation. *EMBO Rep* **11:**201–207.
Blard O, Feuillette S, Bou J, Chaumette B, Frébourg T, Campion D, and Lecourtois M (2007) Cytoskeleton proteins are modulators of mutant tau-induced neurodegeneration in Drosophila. *Hum Mol Genet* **16:**555–566.
Bonifati V, Rizzu P, Squitieri F, Krieger E, Vanacore N, van Swieten JC, Brice A, van Duijn CM, Oostra B, Meco G, et al. (2003) DJ-1(PARK7), a novel gene for autosomal recessive, early onset parkinsonism. *Neurol Sci* **24:**159–160.
Bradu A, Ma L, Bloor JW, and Podoleanu A (2009) Dual optical coherence tomography/fluorescence microscopy for monitoring of *Drosophila melanogaster* larval heart. *J Biophotonics* **2:**380–388.
Brand AH and Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118:**401–415.
Branson K, Robie AA, Bender J, Perona P, and Dickinson MH (2009) High-throughput ethomics in large groups of Drosophila. *Nat Methods* **6:**451–457.
Braun T and Woollard A (2009) RUNX factors in development: lessons from invertebrate model systems. *Blood Cells Mol Dis* **43:**43–48.
Brignull HR, Moore FE, Tang SJ, and Morimoto RI (2006) Polyglutamine proteins at the pathogenic threshold display neuron-specific aggregation in a pan-neuronal *Caenorhabditis elegans* model. *J Neurosci* **26:**7597–7606.
Bryantsev AL and Cripps RM (2009) Cardiac gene regulatory networks in Drosophila. *Biochim Biophys Acta* **1789:**343–353.
Cagan RL and Ready DF (1989) The emergence of order in the Drosophila pupal retina. *Dev Biol* **136:**346–362.
Cao P, Yuan Y, Pehek EA, Moise AR, Huang Y, Palczewski K, and Feng Z (2010) Alpha-synuclein disrupted dopamine homeostasis leads to dopaminergic neuron degeneration in *Caenorhabditis elegans*. *PLoS One* **5:**e9312.
C. elegans Sequencing Consortium (1998) Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282:**2012–2018.
Cha GH, Kim S, Park J, Lee E, Kim M, Lee SB, Kim JM, Chung J, and Cho KS (2005) Parkin negatively regulates JNK pathway in the dopaminergic neurons of Drosophila. *Proc Natl Acad Sci USA* **102:**10345–10350.
Chang S, Bray SM, Li Z, Zarnescu DC, He C, Jin P, and Warren ST (2008) Identification of small molecules rescuing fragile X syndrome phenotypes in Drosophila. *Nat Chem Biol* **4:**256–263.
Chee FC, Mudher A, Cuttle MF, Newman TA, MacKay D, Lovestone S, and Shepherd D (2005) Over-expression of tau results in defective synaptic transmission in Drosophila neuromuscular junctions. *Neurobiol Dis* **20:**918–928.
Chen X, Li Y, Huang J, Cao D, Yang G, Liu W, Lu H, and Guo A (2007) Study of tauopathies by comparing Drosophila and human tau in Drosophila. *Cell Tissue Res* **329:**169–178.
Chen YC, Cheng CH, Chen GD, Hung CC, Yang CH, Hwang SP, Kawakami K, Wu BK, and Huang CJ (2009) Recapitulation of zebrafish sncga expression pattern and labeling the habenular complex in transgenic zebrafish using green fluorescent protein reporter gene. *Dev Dyn* **238:**746–754.
Choma MA, Izatt SD, Wessells RJ, Bodmer R, and Izatt JA (2006) Images in cardiovascular medicine: in vivo imaging of the adult *Drosophila melanogaster* heart with real-time optical coherence tomography. *Circulation* **114:**e35–e36.
Choi CH, McBride SM, Schoenfeld BP, Liebelt DA, Ferreiro D, Ferrick NJ, Hinchey P, Kollaros M, Rudominer RL, Terlizzi AM, et al. (2010) Age-dependent cognitive impairment in a Drosophila fragile X model and its pharmacological rescue. *Biogerontology* **11:**347–362.
Christofori G and Semb H (1999) The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. *Trends Biochem Sci* **24:**73–76.
Chung BY, Kilman VL, Keath JR, Pitman JL, and Allada R (2009) The GABA(A) receptor RDL acts in peptidergic PDF neurons to promote sleep in Drosophila. *Curr Biol* **19:**386–390.
Cirelli C (2009) The genetic and molecular regulation of sleep: from fruit flies to humans. *Nat Rev Neurosci* **10:**549–560.
Cirelli C, Bushey D, Hill S, Huber R, Kreber R, Ganetzky B, and Tononi G (2005) Reduced sleep in *Drosophila Shaker* mutants. *Nature* **434:**1087–1092.
Cooper AS, Rymond KE, Ward MA, Bocook EL, and Cooper RL (2009) Monitoring heart function in larval *Drosophila melanogaster* for physiological studies. *J Vis Exp* doi: 10.3791/1596.
Coulom H and Birman S (2004) Chronic exposure to rotenone models sporadic Parkinson's disease in *Drosophila melanogaster*. *J Neurosci* **24:**10993–10998.
Crowther DC, Kinghorn KJ, Miranda E, Page R, Curry JA, Duthie FA, Gubb DC, and Lomas DA (2005) Intraneuronal Abeta, non-amyloid aggregates and neurodegeneration in a Drosophila model of Alzheimer's disease. *Neuroscience* **132:**123–135.
Dankert H, Wang L, Hoopfer ED, Anderson DJ, and Perona P (2009) Automated monitoring and analysis of social behavior in Drosophila. *Nat Methods* **6:**297–303.
Dasari S and Cooper RL (2006) Direct influence of serotonin on the larval heart of *Drosophila melanogaster*. *J Comp Physiol B* **176:**349–357.
Dasari S, Viele K, Turner AC, and Cooper RL (2007) Influence of PCPA and MDMA (ecstasy) on physiology, development and behavior in *Drosophila melanogaster*. *Eur J Neurosci* **26:**424–438.
Davis RL (2005) Olfactory memory formation in Drosophila: from molecular to systems neuroscience. *Annu Rev Neurosci* **28:**275–302.
Desai UA, Pallos J, Ma AA, Stockwell BR, Thompson LM, Marsh JL, and Diamond MI (2006) Biologically active molecules that reduce polyglutamine aggregation and toxicity. *Hum Mol Genet* **15:**2114–2124.
DiAngelo JR and Birnbaum MJ (2009) Regulation of fat cell mass by insulin in *Drosophila melanogaster*. *Mol Cell Biol* **29:**6341–6352.
Dierick HA and Greenspan RJ (2007) Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. *Nat Genet* **39:**678–682.
Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblauer S, et al. (2007) A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. *Nature* **448:**151–156.
Dockendorff TC, Su HS, McBride SM, Yang Z, Choi CH, Siwicki KK, Sehgal A, and Jongens TA (2002) Drosophila lacking dfmr1 activity show defects in circadian output and fail to maintain courtship interest. *Neuron* **34:**973–984.
Drake J, Link CD, and Butterfield DA (2003) Oxidative stress precedes fibrillar deposition of Alzheimer's disease amyloid beta-peptide (1–42) in a transgenic *Caenorhabditis elegans* model. *Neurobiol Aging* **24:**415–520.
Draper I, Kurshan PT, McBride E, Jackson FR, and Kopin AS (2007) Locomotor activity is regulated by D2-like receptors in Drosophila: an anatomic and functional analysis. *Dev Neurobiol* **67:**378–393.
Dzitoyeva S, Dimitrijevic N, and Manev H (2003) Gamma-aminobutyric acid B receptor 1 mediates behavior-impairing actions of alcohol in Drosophila: adult RNA interference and pharmacological evidence. *Proc Natl Acad Sci USA* **100:**5485–5490.
Evans PD and Maqueira B (2005) Insect octopamine receptors: a new classification scheme based on studies of cloned Drosophila G-protein coupled receptors. *Invert Neurosci* **5:**111–118.
Faber PW, Alter JR, MacDonald ME, and Hart AC (1999) Polyglutamine-mediated dysfunction and apoptotic death of a *Caenorhabditis elegans* sensory neuron. *Proc Natl Acad Sci USA* **96:**179–184.
Fay DS, Fluet A, Johnson CJ, and Link CD (1998) In vivo aggregation of beta-amyloid peptide variants. *J Neurochem* **71:**1616–1625.
Feany MB and Bender WW (2000) A Drosophila model of Parkinson's disease. *Nature* **404:**394–398.
Ferrandon D, Imler JL, Hetru C, and Hoffmann JA (2007) The Drosophila systemic immune response: sensing and signalling during bacterial and fungal infections. *Nat Rev Immunol* **7:**862–874.
Feuillette S, Miguel L, Frébourg T, Campion D, and Lecourtois M (2010) Drosophila models of human tauopathies indicate that Tau protein toxicity in vivo is mediated by soluble cytosolic phosphorylated forms of the protein. *J Neurochem* **113:**895–903.
Finelli A, Kelkar A, Song HJ, Yang H, and Konsolaki M (2004) A model for studying Alzheimer's Abeta42-induced toxicity in *Drosophila melanogaster*. *Mol Cell Neurosci* **26:**365–375.
Flinn L, Mortiboys H, Volkmann K, Köster RW, Ingham PW, and Bandmann O (2009) Complex I deficiency and dopaminergic neuronal cell loss in parkin-deficient zebrafish (*Danio rerio*). *Brain* **132:**1613–1623.
Foltenyi K, Andretic R, Newport JW, and Greenspan RJ (2007) Neurohormonal and neuromodulatory control of sleep in Drosophila. *Cold Spring Harb Symp Quant Biol* **72:**565–571.
Franceschini N and Kirschfeld K (1971) [Pseudopupil phenomena in the compound eye of drosophila]. *Kybernetik* **9:**159–182.
Furukubo-Tokunaga K (2009) Modeling schizophrenia in flies. *Prog Brain Res* **179:**107–115.
Ganetzky B and Wu CF (1982) Indirect suppression involving behavioral mutants with altered nerve excitability in *Drosophila melanogaster*. *Genetics* **100:**597–614.
Geyer MA (2008) Developing translational animal models for symptoms of schizophrenia or bipolar mania. *Neurotox Res* **14:**71–78.
Giasson BI and Lee VM (2003) Are ubiquitination pathways central to Parkinson's disease? *Cell* **114:**1–8.
Goldenberg MM (2010) Overview of drugs used for epilepsy and seizures: etiology, diagnosis, and treatment. *P T* **35:**392–415.
Gordon P, Hingula L, Krasny ML, Swienckowski JL, Pokrywka NJ, and Raley-Susman KM (2008) The invertebrate microtubule-associated protein PTL-1 functions in mechanosensation and development in *Caenorhabditis elegans*. *Dev Genes Evol* **218:**541–551.
Gosai SJ, Kwak JH, Luke CJ, Long OS, King DE, Kovatch KJ, Johnston PA, Shun TY, Lazo JS, Perlmutter DH, et al. (2010) Automated high-content live animal drug screening using *C. elegans* expressing the aggregation prone serpin α1-antitrypsin Z. *PLoS One* **5:**e15460.
Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, and Pallanck LJ (2003) Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proc Natl Acad Sci USA* **100:**4078–4083.
Greenspan RJ (2008) The origins of behavioral genetics. *Curr Biol* **18:**R192–R198.
Grosjean Y, Grillet M, Augustin H, Ferveur JF, and Featherstone DE (2008) A glial

amino-acid transporter controls synapse strength and courtship in Drosophila. *Nat Neurosci* **11:**54–61.
Gu H and O'Dowd DK (2006) Cholinergic synaptic transmission in adult Drosophila Kenyon cells in situ. *J Neurosci* **26:**265–272.
Gunawardena S, Her LS, Brusch RG, Laymon RA, Niesman IR, Gordesky-Gold B, Sintasath L, Bonini NM, and Goldstein LS (2003) Disruption of axonal transport by loss of huntingtin or expression of pathogenic polyQ proteins in Drosophila. *Neuron* **40:**25–40.
Hamamichi S, Rivas RN, Knight AL, Cao S, Caldwell KA, and Caldwell GA (2008) Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model. *Proc Natl Acad Sci USA* **105:**728–733.
Hamasaka Y, Rieger D, Parmentier ML, Grau Y, Helfrich-Förster C, and Nässel DR (2007) Glutamate and its metabotropic receptor in Drosophila clock neuron circuits. *J Comp Neurol* **505:**32–45.
Hamasaka Y, Wegener C, and Nässel DR (2005) GABA modulates Drosophila circadian clock neurons via GABAB receptors and decreases in calcium. *J Neurobiol* **65:**225–240.
Harrington AJ, Hamamichi S, Caldwell GA, and Caldwell KA (2010) *C. elegans* as a model organism to investigate molecular pathways involved with Parkinson's disease. *Dev Dyn* **239:**1282–1295.
Haselton AT and Fridell YW (2010) Adult *Drosophila melanogaster* as a model for the study of glucose homeostasis. *Aging* **2:**523–526.
Hassan WM, Merin DA, Fonte V, and Link CD (2009) AIP-1 ameliorates beta-amyloid peptide toxicity in a *Caenorhabditis elegans* Alzheimer's disease model. *Hum Mol Genet* **18:**2739–2747.
Hattori N and Mizuno Y (2004) Pathogenetic mechanisms of parkin in Parkinson's disease. *Lancet* **364:**722–724.
Haywood AF and Staveley BE (2004) Parkin counteracts symptoms in a Drosophila model of Parkinson's disease. *BMC Neurosci* **5:**14.
Heiser V, Engemann S, Bröcker W, Dunkel I, Boeddrich A, Waelter S, Nordhoff E, Lurz R, Schugardt N, Rautenberg S, et al. (2002) Identification of benzothiazoles as potential polyglutamine aggregation inhibitors of Huntington's disease by using an automated filter retardation assay. *Proc Natl Acad Sci USA* **99:**16400–16406.
Hendricks JC, Kirk D, Panckeri K, Miller MS, and Pack AI (2003) Modafinil maintains waking in the fruit fly drosophila melanogaster. *Sleep* **26:**139–146.
Henshall TL, Tucker B, Lumsden AL, Nornes S, Lardelli MT, and Richards RI (2009) Selective neuronal requirement for huntingtin in the developing zebrafish. *Hum Mol Genet* **18:**4830–4842.
Hetru C and Hoffmann JA (2009) NF-kappaB in the immune response of Drosophila. *Cold Spring Harb Perspect Biol* **1:**a000232.
Hirsh J, Riemensperger T, Coulom H, Iché M, Coupar J, and Birman S (2010) Roles of dopamine in circadian rhythmicity and extreme light sensitivity of circadian entrainment. *Curr Biol* **20:**209–214.
Hirota T, Lee JW, Lewis WG, Zhang EE, Breton G, Liu X, Garcia M, Peters EC, Etchegaray JP, Traver D, et al. (2010) High-throughput chemical screen identifies a novel potent modulator of cellular circadian rhythms and reveals CKIα as a clock regulatory kinase. *PLoS Biol* **8:**e1000559.
Hockly E, Tse J, Barker AL, Moolman DL, Beunard JL, Revington AP, Holt K, Sunshine S, Moffitt H, Sathasivam K, et al. (2006) Evaluation of the benzothiazole aggregation inhibitors riluzole and PGL-135 as therapeutics for Huntington's disease. *Neurobiol Dis* **21:**228–236.
Hornsten A, Lieberthal J, Fadia S, Malins R, Ha L, Xu X, Daigle I, Markowitz M, O'Connor G, Plasterk R, et al. (2007) APL-1, a *Caenorhabditis elegans* protein related to the human beta-amyloid precursor protein, is essential for viability. *Proc Natl Acad Sci USA* **104:**1971–1976.
Horowitz A and Simons M (2008) Branching morphogenesis. *Circ Res* **103:**784–795.
Humbert PO, Grzeschik NA, Brumby AM, Galea R, Elsum I, and Richardson HE (2008) Control of tumourigenesis by the Scribble/Dlg/Lgl polarity module. *Oncogene* **27:**6888–6907.
Ibáñez P, Bonnet AM, Débarges B, Lohmann E, Tison F, Pollak P, Agid Y, Dürr A, and Brice A (2004) Causal relation between alpha-synuclein gene duplication and familial Parkinson's disease. *Lancet* **364:**1169–1171.
Ja WW, Carvalho GB, Mak EM, de la Rosa NN, Fang AY, Liong JC, Brummel T, and Benzer S (2007) Prandiology of Drosophila and the CAFE assay. *Proc Natl Acad Sci USA* **104:**8253–8256.
Jackson GR, Wiedau-Pazos M, Sang TK, Wagle N, Brown CA, Massachi S, and Geschwind DH (2002) Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila. *Neuron* **34:**509–519.
Januschke J and Gonzalez C (2008) Drosophila asymmetric division, polarity and cancer. *Oncogene* **27:**6994–7002.
Jiang H, Jiang Q, and Feng J (2004) Parkin increases dopamine uptake by enhancing the cell surface expression of dopamine transporter. *J Biol Chem* **279:**54380–54386.
Jin P, Duan R, Qurashi A, Qin Y, Tian D, Rosser TC, Liu H, Feng Y, and Warren ST (2007) Pur alpha binds to rCGG repeats and modulates repeat-mediated neurodegeneration in a Drosophila model of fragile X tremor/ataxia syndrome. *Neuron* **55:**556–564.
Johnson O, Becnel J, and Nichols CD (2008) Serotonin 5-HT(2) and 5-HT(1A)-like receptors differentially modulate aggressive behaviors in *Drosophila melanogaster*. *Neuroscience* **158:**1292–1300.
Johnson O, Becnel J, and Nichols CD (2009) Serotonin 5-HT1A-like, 5-HT2, and 5-HT7 receptors modulate learning and memory in drosophila. 50th Annual Drosophila Research Conference; 4–8 Mar 2009; Chicago IL. Abstract 599B. The Genetics Society of America, Bethesda, MD.
Joshi P, Liang JO, DiMonte K, Sullivan J, and Pimplikar SW (2009) Amyloid precursor protein is required for convergent-extension movements during Zebrafish development. *Dev Biol* **335:**1–11.
Kaplan DD, Zimmermann G, Suyama K, Meyer T, and Scott MP (2008) A nucleostemin family GTPase, NS3, acts in serotonergic neurons to regulate insulin signaling and control body size. *Genes Dev* **22:**1877–1893.
Karpinar DP, Balija MB, Kügler S, Opazo F, Rezaei-Ghaleh N, Wender N, Kim HY, Taschenberger G, Falkenburger BH, Heise H, et al. (2009) Pre-fibrillar alpha-synuclein variants with impaired beta-structure increase neurotoxicity in Parkinson's disease models. *EMBO J* **28:**3256–3268.
Kauppila S, Maaty WS, Chen P, Tomar RS, Eby MT, Chapo J, Chew S, Rathore N, Zachariah S, Sinha SK, et al. (2003) Eiger and its receptor, Wengen, comprise a TNF-like system in Drosophila. *Oncogene* **22:**4860–4867.
Kim IM, Wolf MJ, and Rockman HA (2010) Gene deletion screen for cardiomyopathy in adult Drosophila identifies a new notch ligand. *Circ Res* **106:**1233–1243.
Kim SK and Rulifson EJ (2004) Conserved mechanisms of glucose sensing and regulation by Drosophila corpora cardiaca cells. *Nature* **431:**316–320.
Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, and Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature* **392:**605–608.
Koh K, Evans JM, Hendricks JC, and Sehgal A (2006) A Drosophila model for age-associated changes in sleep:wake cycles. *Proc Natl Acad Sci USA* **103:**13843–13847.
Koh K, Joiner WJ, Wu MN, Yue Z, Smith CJ, and Sehgal A (2008) Identification of SLEEPLESS, a sleep promoting factor. *Science* **321:**372–376.
Kraemer BC, Zhang B, Leverenz JB, Thomas JH, Trojanowski JQ, and Schellenberg GD (2003) Neurodegeneration and defective neurotransmission in a *Caenorhabditis elegans* model of tauopathy. *Proc Natl Acad Sci USA* **100:**9980–9985.
Krashes MJ, DasGupta S, Vreede A, White B, Armstrong JD, and Waddell S (2009) A neural circuit mechanism integrating motivational state with memory expression in Drosophila. *Cell* **139:**416–427.
Krogsgaard-Larsen P, Frølund B, and Frydenvang K (2000) GABA uptake inhibitors. Design, molecular pharmacology and therapeutic aspects. *Curr Pharm Des* **6:**1193–1209.
Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M, Kösel S, Przuntek H, Epplen JT, Schöls L, and Riess O (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet* **18:**106–108.
Kuebler D and Tanouye MA (2000) Modifications of seizure susceptibility in Drosophila. *J Neurophysiol* **83:**998–1009.
Kuklinski NJ, Berglund EC, and Ewing AG (2010) Micellar capillary electrophoresis—electrochemical detection of neurochemicals from Drosophila. *J Sep Sci* **33:**388–393.
Kumar JP (2010) Retinal determination the beginning of eye development. *Curr Top Dev Biol* **93:**1–28.
Kuwahara T, Koyama A, Gengyo-Ando K, Masuda M, Kowa H, Tsunoda M, Mitani S, and Iwatsubo T (2006) Familial Parkinson mutant alpha-synuclein causes dopamine neuron dysfunction in transgenic *Caenorhabditis elegans*. *J Biol Chem* **281:**334–340.
Kuwahara T, Koyama A, Koyama S, Yoshina S, Ren CH, Kato T, Mitani S, and Iwatsubo T (2008) A systematic RNAi screen reveals involvement of endocytic pathway in neuronal dysfunction in alpha-synuclein transgenic *C. elegans*. *Hum Mol Genet* **17:**2997–3009.
La Spada AR and Taylor JP (2010) Repeat expansion disease: progress and puzzles in disease pathogenesis. *Nat Rev Genet* **11:**247–258.
La Spada AR and Taylor JP (2003) Polyglutamines placed into context. *Neuron* **38:**681–684.
Lacomblez L, Bensimon G, Leigh PN, Guillet P, and Meininger V (1996) Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet* **347:**1425–1431.
Lakso M, Vartiainen S, Moilanen AM, Sirviö J, Thomas JH, Nass R, Blakely RD, and Wong G (2003) Dopaminergic neuronal loss and motor deficits in *Caenorhabditis elegans* overexpressing human alpha-synuclein. *J Neurochem* **86:**165–172.
Langheinrich U (2003) Zebrafish: a new model on the pharmaceutical catwalk. *Bioessays* **25:**904–912.
Lebestky T, Chang JS, Dankert H, Zelnik L, Kim YC, Han KA, Wolf FW, Perona P, and Anderson DJ (2009) Two different forms of arousal in Drosophila are oppositely regulated by the dopamine D1 receptor ortholog DopR via distinct neural circuits. *Neuron* **64:**522–536.
Lee J and Wu CF (2002) Electroconvulsive seizure behavior in Drosophila: analysis of the physiological repertoire underlying a stereotyped action pattern in bang-sensitive mutants. *J Neurosci* **22:**11065–11079.
Lesage S, Dürr A, Tazir M, Lohmann E, Leutenegger AL, Janin S, Pollak P, Brice A, and French Parkinson's Disease Genetics Study Group (2006) LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. *N Engl J Med* **354:**422–423.
Link CD (1995) Expression of human beta-amyloid peptide in transgenic *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **92:**9368–9372.
Link CD, Johnson CJ, Fonte V, Paupard M, Hall DH, Styren S, Mathis CA, and Klunk WE (2001) Visualization of fibrillar amyloid deposits in living, transgenic *Caenorhabditis elegans* animals using the sensitive amyloid dye, X-34. *Neurobiol Aging* **22:**217–226.
Liu L, Johnson WA, and Welsh MJ (2003) Drosophila DEG/ENaC pickpocket genes are expressed in the tracheal system, where they may be involved in liquid clearance. *Proc Natl Acad Sci USA* **100:**2128–2133.
Liu X, Krause WC, and Davis RL (2007) GABAA receptor RDL inhibits Drosophila olfactory associative learning. *Neuron* **56:**1090–1102.
Lloyd TE and Taylor JP (2010) Flightless flies: Drosophila models of neuromuscular disease. *Ann NY Acad Sci* **1184:**e1–e20.
Luheshi LM, Tartaglia GG, Brorsson AC, Pawar AP, Watson IE, Chiti F, Vendruscolo M, Lomas DA, Dobson CM, and Crowther DC (2007) Systematic in vivo analysis of the intrinsic determinants of amyloid beta pathogenicity. *PLoS Biol* **5:**e290.
Lumsden AL, Henshall TL, Dayan S, Lardelli MT, and Richards RI (2007) Huntingtin-deficient zebrafish exhibit defects in iron utilization and development. *Hum Mol Genet* **16:**1905–1920.
Luo L, Tully T, and White K (1992) Human amyloid precursor protein ameliorates behavioral deficit of flies deleted for Appl gene. *Neuron* **9:**595–605.
Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton

M, Trottier Y, Lehrach H, Davies SW, et al. (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* **87:**493–506.
Margulies C, Tully T, and Dubnau J (2005) Deconstructing memory in Drosophila. *Curr Biol* **15:**R700–R713.
Marvanova M and Nichols CD (2007) Identification of neuroprotective compounds of caenorhabditis elegans dopaminergic neurons against 6-OHDA. *J Mol Neurosci* **31:**127–137.
Mayer F, Mayer N, Chinn L, Pinsonneault RL, Kroetz D, and Bainton RJ (2009) Evolutionary conservation of vertebrate blood-brain barrier chemoprotective mechanisms in Drosophila. *J Neurosci* **29:**3538–3550.
McClung C and Hirsh J (1998) Stereotypic behavioral responses to free-base cocaine and the development of behavioral sensitization in Drosophila. *Curr Biol* **8:**109–112.
McGuire SE, Roman G, and Davis RL (2004) Gene expression systems in Drosophila: a synthesis of time and space. *Trends Genet* **20:**384–391.
Meeker ND and Trede NS (2008) Immunology and zebrafish: spawning new models of human disease. *Dev Comp Immunol* **32:**745–757.
Meriin AB, Zhang X, He X, Newnam GP, Chernoff YO, and Sherman MY (2002) Huntington toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1. *J Cell Biol* **157:**997–1004.
Micchelli CA and Perrimon N (2006) Evidence that stem cells reside in the adult Drosophila midgut epithelium. *Nature* **439:**475–479.
Micchelli CA, Esler WP, Kimberly WT, Jack C, Berezovska O, Kornilova A, Hyman BT, Perrimon N, and Wolfe MS (2003) Gamma-secretase/presenilin inhibitors for Alzheimer's disease phenocopy Notch mutations in Drosophila. *FASEB J* **17:**79–81.
Miyasaka T, Ding Z, Gengyo-Ando K, Oue M, Yamaguchi H, Mitani S, and Ihara Y (2005) Progressive neurodegeneration in *C. elegans* model of tauopathy. *Neurobiol Dis* **20:**372–383.
Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufe A, Rietschel E, et al. (2007) Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* **448:**470–473.
Molina GA, Watkins SC, and Tsang M (2007) Generation of FGF reporter transgenic zebrafish and their utility in chemical screens. *BMC Dev Biol* **7:**62.
Montell C (2005) The TRP superfamily of cation channels. *Sci STKE* **(272):**re3.
Moore DJ, Zhang L, Troncoso J, Lee MK, Hattori N, Mizuno Y, Dawson TM, and Dawson VL (2005) Association of DJ-1 and parkin mediated by pathogenic DJ-1 mutations and oxidative stress. *Hum Mol Genet* **14:**71–84.
Moore MS, DeZazzo J, Luk AY, Tully T, Singh CM, and Heberlein U (1998) Ethanol intoxication in Drosophila: genetic and pharmacological evidence for regulation by the cAMP signaling pathway. *Cell* **93:**997–1007.
Morales J, Hiesinger PR, Schroeder AJ, Kume K, Verstreken P, Jackson FR, Nelson DL, and Hassan BA (2002) Drosophila fragile X protein, DFXR, regulates neuronal morphology and function in the brain. *Neuron* **34:**961–972.
Mudher A, Shepherd D, Newman TA, Mildren P, Jukes JP, Squire A, Mears A, Drummond JA, Berg S, MacKay D, et al. (2004) GSK-3beta inhibition reverses axonal transport defects and behavioural phenotypes in Drosophila. *Mol Psychiatry* **9:**522–530.
Nagaraj R and Banerjee U (2004) The little R cell that could. *Int J Dev Biol* **48:**755–760.
Nasonkin I, Alikasifoglu A, Ambrose C, Cahill P, Cheng M, Sarniak A, Egan M, and Thomas PM (1999) A novel sulfonylurea receptor family member expressed in the embryonic Drosophila dorsal vessel and tracheal system. *J Biol Chem* **274:**29420–29425.
Nass R, Hahn MK, Jessen T, McDonald PW, Carvelli L, and Blakely RD (2005) A genetic screen in *Caenorhabditis elegans* for dopamine neuron insensitivity to 6-hydroxydopamine identifies dopamine transporter mutants impacting transporter biosynthesis and trafficking. *J Neurochem* **94:**774–785.
Nass R, Hall DH, Miller DM 3rd, and Blakely RD (2002) Neurotoxin-induced degeneration of dopamine neurons in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **99:**3264–3269.
Nässel DR and Winther AM (2010) Drosophila neuropeptides in regulation of physiology and behavior. *Prog Neurobiol* **92:**42–104.
Neckameyer WS, Coleman CM, Eadie S, and Goodwin SF (2007) Compartmentalization of neuronal and peripheral serotonin synthesis in *Drosophila melanogaster*. *Genes Brain Behav* **6:**756–769.
Neckameyer WS (2010) A trophic role for serotonin in the development of a simple feeding circuit. *Dev Neurosci* **32:**217–237.
Nedelsky NB, Pennuto M, Smith RB, Palazzolo I, Moore J, Nie Z, Neale G, and Taylor JP (2010) Native functions of the androgen receptor are essential to pathogenesis in a Drosophila model of spinobulbar muscular atrophy. *Neuron* **67:**936–952.
Neely GG, Kuba K, Cammarato A, Isobe K, Amann S, Zhang L, Murata M, Elmén L, Gupta V, Arora S, et al. (2010) A global in vivo Drosophila RNAi screen identifies NOT3 as a conserved regulator of heart function. *Cell* **141:**142–153.
Nestler EJ and Hyman SE (2010) Animal models of neuropsychiatric disorders. *Nat Neurosci* **13:**1161–1169.
Newman M, Verdile G, Martins RN, and Lardelli M (2011) Zebrafish as a tool in Alzheimer's disease research. *Biochim Biophys Acta* **1812:**346–352.
Nichols CD (2006) *Drosophila melanogaster* neurobiology, neuropharmacology, and how the fly can inform central nervous system drug discovery. *Pharmacol Ther* **112:**677–700.
Nichols CD (2007) 5-HT2 receptors in Drosophila are expressed in the brain and modulate aspects of circadian behaviors. *Dev Neurobiol* **67:**752–763.
Nichols CD and Sanders-Bush E (2002) A single dose of lysergic acid diethylamide influences gene expression patterns within the mammalian brain. *Neuropsychopharmacology* **26:**634–642.
Nichols CD, Ronesi J, Pratt W, and Sanders-Bush E (2002) Hallucinogens and Drosophila: linking serotonin receptor activation to behavior. *Neuroscience* **115:**979–984.
Nishimura I, Yang Y, and Lu B (2004) PAR-1 kinase plays an initiator role in a temporally ordered phosphorylation process that confers tau toxicity in Drosophila. *Cell* **116:**671–682.
Nornes S, Groth C, Camp E, Ey P, and Lardelli M (2003) Developmental control of Presenilin1 expression, endoproteolysis, and interaction in zebrafish embryos. *Exp Cell Res* **289:**124–132.
Null B, Liu CW, Hedehus M, Conolly S, and Davis RW (2008) High-resolution, in vivo magnetic resonance imaging of Drosophila at 18.8 Tesla. *PLoS ONE* **3:**e2817.
Ocorr K, Akasaka T, and Bodmer R (2007a) Age-related cardiac disease model of Drosophila. *Mech Ageing Dev* **128:**112–116.
Ocorr K, Perrin L, Lim HY, Qian L, Wu X, and Bodmer R (2007b) Genetic control of heart function and aging in Drosophila. *Trends Cardiovasc Med* **17:**177–182.
Ocorr KA, Crawley T, Gibson G, and Bodmer R (2007c) Genetic variation for cardiac dysfunction in Drosophila. *PLoS ONE* **2:**e601.
Olivier JP, Raabe T, Henkemeyer M, Dickson B, Mbamalu G, Margolis B, Schlessinger J, Hafen E, and Pawson T (1993) A Drosophila SH2-SH3 adaptor protein implicated in coupling the sevenless tyrosine kinase to an activator of Ras guanine nucleotide exchange, Sos. *Cell* **73:**179–191.
Pagliarini RA and Xu T (2003) A genetic screen in Drosophila for metastatic behavior. *Science* **302:**1227–1231.
Paisán-Ruíz C, Jain S, Evans EW, Gilks WP, Simón J, van der Brug M, López de Munain A, Aparicio S, Gil AM, Khan N, et al. (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron* **44:**595–600.
Pandey UB, Batlevi Y, Baehrecke EH, and Taylor JP (2007a) HDAC6 at the intersection of autophagy, the ubiquitin-proteasome system and neurodegeneration. *Autophagy* **3:**643–645.
Pandey UB, Nie Z, Batlevi Y, McCray BA, Ritson GP, Nedelsky NB, Schwartz SL, DiProspero NA, Knight MA, Schuldiner O, et al. (2007b) HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. *Nature* **447:**859–863.
Paquet D, Bhat R, Sydow A, Mandelkow EM, Berg S, Hellberg S, Fälting J, Distel M, Köster RW, Schmid B, et al. (2009) A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. *J Clin Invest* **119:**1382–1395.
Parker JA, Arango M, Abderrahmane S, Lambert E, Tourette C, Catoire H, and Néri C (2005) Resveratrol rescues mutant polyglutamine cytotoxicity in nematode and mammalian neurons. *Nat Genet* **37:**349–350.
Parker JA, Connolly JB, Wellington C, Hayden M, Dausset J, and Neri C (2001) Expanded polyglutamines in *Caenorhabditis elegans* cause axonal abnormalities and severe dysfunction of PLM mechanosensory neurons without cell death. *Proc Natl Acad Sci USA* **98:**13318–13323.
Pavlidis P and Tanouye MA (1995) Seizures and failures in the giant fiber pathway of Drosophila bang-sensitive paralytic mutants. *J Neurosci* **15:**5810–5819.
Pereira PS, Teixeira A, Pinho S, Ferreira P, Fernandes J, Oliveira C, Seruca R, Suriano G, and Casares F (2006) E-cadherin missense mutations, associated with hereditary diffuse gastric cancer (HDGC) syndrome, display distinct invasive behaviors and genetic interactions with the Wnt and Notch pathways in Drosophila epithelia. *Hum Mol Genet* **15:**1704–1712.
Pesah Y, Burgess H, Middlebrooks B, Ronningen K, Prosser J, Tirunagaru V, Zysk J, and Mardon G (2005) Whole-mount analysis reveals normal numbers of dopaminergic neurons following misexpression of alpha-Synuclein in Drosophila. *Genesis* **41:**154–159.
Pesah Y, Pham T, Burgess H, Middlebrooks B, Verstreken P, Zhou Y, Harding M, Bellen H, and Mardon G (2004) Drosophila parkin mutants have decreased mass and cell size and increased sensitivity to oxygen radical stress. *Development* **131:**2183–2194.
Peterson RT, Link BA, Dowling JE, and Schreiber SL (2000) Small molecule developmental screens reveal the logic and timing of vertebrate development. *Proc Natl Acad Sci USA* **97:**12965–12969.
Pollitt SK, Pallos J, Shao J, Desai UA, Ma AA, Thompson LM, Marsh JL, and Diamond MI (2003) A rapid cellular FRET assay of polyglutamine aggregation identifies a novel inhibitor. *Neuron* **40:**685–694.
Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, et al. (1997) Mutation in the α-synuclein gene identified in families with Parkinson's disease. *Science* **276:**2045–2047.
Pulak R (2006) Techniques for analysis, sorting, and dispensing of *C. elegans* on the COPAS flow-sorting system. *Methods Mol Biol* **351:**275–286.
Quinn WG, Harris WA, and Benzer S (1974) Conditioned behavior in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* **71:**708–712.
Ragland M, Hutter C, Zabetian C, and Edwards K (2009) Association between the ubiquitin carboxyl-terminal esterase L1 gene (UCHL1) S18Y variant and Parkinson's Disease: a HuGE review and meta-analysis. *Am J Epidemiol* **170:**1344–1357.
Rand MD (2010) Drosophotoxicology: the growing potential for Drosophila in neurotoxicology. *Neurotoxicol Teratol* **32:**74–83.
Rand MD, Kearney AL, Dao J, and Clason T (2010) Permeabilization of Drosophila embryos for introduction of small molecules. *Insect Biochem Mol Biol* **40:**792–804.
Ready DF, Hanson TE, and Benzer S (1976) Development of the Drosophila retina, a neurocrystalline lattice. *Dev Biol* **53:**217–240.
Reim I and Frasch M (2010) Genetic and genomic dissection of cardiogenesis in the Drosophila model. *Pediatr Cardiol* **31:**325–334.
Reiter LT, Potocki L, Chien S, Gribskov M, and Bier E (2001) E A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. *Genome Res* **11:**1114–1125.
Reith ME, Zhen J, and Chen N (2006) The importance of company: Na^+ and Cl^- influence substrate interaction with SLC6 transporters and other proteins. *Handb Exp Pharmacol* **175:**75–93.
Ren Y, Zhao J, and Feng J (2003) J Parkin binds to alpha/beta tubulin and increases their ubiquitination and degradation. *J Neurosci* **23:**3316–3324.
Reynolds ER, Stauffer EA, Feeney L, Rojahn E, Jacobs B, and McKeever C (2003) Treatment with the antiepileptic drugs phenytoin and gabapentin ameliorates seizure and paralysis of Drosophila bang-sensitive mutants. *J Neurobiol* **58:**503–513.
Rihel J, Prober DA, Arvanites A, Lam K, Zimmerman S, Jang S, Haggarty SJ, Kokel

D, Rubin LL, Peterson RT, et al. (2010) Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science* **327:**348–351.
Rikke BA, Murakami S, and Johnson TE (2000) Paralogy and orthology of tyrosine kinases that can extend the life span of *Caenorhabditis elegans*. *Mol Biol Evol* **17:**671–683.
Roeder T, Isermann K, and Kabesch M (2009) Drosophila in asthma research. *Am J Respir Crit Care Med* **179:**979–983.
Roman G, Endo K, Zong L, and Davis RL (2001) P[Switch], a system for spatial and temporal control of gene expression in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* **98:**12602–12607.
Romero E, Cha GH, Verstreken P, Ly CV, Hughes RE, Bellen HJ, and Botas J (2008) Suppression of neurodegeneration and increased neurotransmission caused by expanded full-length huntingtin accumulating in the cytoplasm. *Neuron* **57:**27–40.
Rothenfluh A and Heberlein U (2002) Drugs, flies, and videotape: the effects of ethanol and cocaine on Drosophila locomotion. *Curr Opin Neurobiol* **12:**639–645.
Ruaud AF and Thummel CS (2008) Serotonin and insulin signaling team up to control growth in Drosophila. *Genes Dev* **22:**1851–1855.
Rubin GM and Lewis EB (2000) A brief history of Drosophila's contributions to genome research. *Science* **287:**2216–2218.
Rulifson EJ, Kim SK, and Nusse R (2002) Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* **296:**1118–1120.
Sang TK, Chang HY, Lawless GM, Ratnaparkhi A, Mee L, Ackerson LC, Maidment NT, Krantz DE, and Jackson GR (2007) A Drosophila model of mutant human parkin-induced toxicity demonstrates selective loss of dopaminergic neurons and dependence on cellular dopamine. *J Neurosci* **27:**981–992.
Satta R, Dimitrijevic N, and Manev H (2003) Drosophila metabolize 1,4-butanediol into gamma-hydroxybutyric acid in vivo. *Eur J Pharmacol* **473:**149–152.
Sawamura N, Ando T, Maruyama Y, Fujimuro M, Mochizuki H, Honjo K, Shimoda M, Toda H, Sawamura-Yamamoto T, Makuch LA, et al. (2008) Nuclear DISC1 regulates CRE-mediated gene transcription and sleep homeostasis in the fruit fly. *Mol Psychiatry* **13:**1138-1148.
Schwarz TL, Tempel BL, Papazian DM, Jan YN, and Jan LY (1988) Multiple potassium-channel components are produced by alternative splicing at the *Shaker* locus in *Drosophila*. *Nature* **331:**137–142.
Scott R, Bourtchuladze R, Gossweiler S, Dubnau J, and Tully T (2002) CREB and the discovery of cognitive enhancers. *J Mol Neurosci* **19:**171–177.
Seidner GA, Ye Y, Faraday MM, Alvord WG, and Fortini ME (2006) Modeling clinically heterogeneous presenilin mutations with transgenic Drosophila. *Curr Biol* **16:**1026–1033.
Selby L, Zhang C, and Sun QQ. (2007) Major defects in neocortical GABAergic inhibitory circuits in mice lacking the fragile X mental retardation protein. *Neurosci Lett* **412:**227–232.
Settivari R, Levora J, and Nass R (2009) The divalent metal transporter homologues SMF-1/2 mediate dopamine neuron sensitivity in *Caenorhabditis elegans* models of manganism and parkinson disease. *J Biol Chem* **284:**35758–35768.
Shaw PJ, Cirelli C, Greenspan RJ, and Tononi G (2000) Correlates of sleep and waking in *Drosophila melanogaster*. *Science* **287:**1834–1837.
Shiga Y and Tanaka-Matakatsu M (1996) A nuclear GFP/β-galactosidase fusion protein as a marker for morphogenesis in living Drosophila. *Dev Growth Differ* **38:**99–106.
Simon MA, Bowtell DD, Dodson GS, Laverty TR, and Rubin GM (1991) Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. *Cell* **67:**701–716.
Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, et al. (2003) α-Synuclein locus triplication causes Parkinson's disease. *Science* **302:**841.
Sitaraman D, Zars M, Laferriere H, Chen YC, Sable-Smith A, Kitamoto T, Rottinghaus GE, and Zars T (2008) Serotonin is necessary for place memory in Drosophila. *Proc Natl Acad Sci USA* **105:**5579–5584.
Sekine T, Yamaguchi T, Hamano K, Siomi H, Saez L, Ishida N, and Shimoda M (2008) Circadian phenotypes of Drosophila fragile x mutants in alternative genetic backgrounds. *Zoolog Sci* **25:**561–571.
Smith WW, Pei Z, Jiang H, Dawson VL, Dawson TM, and Ross CA (2006) Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat Neurosci* **9:**1231–1233.
Sofola OA, Jin P, Qin Y, Duan R, Liu H, de Haro M, Nelson DL, and Botas J (2007) RNA-binding proteins hnRNP A2/B1 and CUGBP1 suppress fragile X CGG premutation repeat-induced neurodegeneration in a Drosophila model of FXTAS. *Neuron* **55:**565–571.
Sofola O, Sundram V, Ng F, Kleyner Y, Morales J, Botas J, Jackson FR, and Nelson DL (2008) The Drosophila FMRP and LARK RNA-binding proteins function together to regulate eye development and circadian behavior. *J Neurosci* **28:**10200–10205.
Song J and Tanouye MA (2006) Seizure suppression by shakB2, a gap junction mutation in Drosophila. *J Neurophysiol* **95:**627–635.
Sonnhammer EL and Durbin R (1997) Analysis of protein domain families in *Caenorhabditis elegans*. *Genomics* **46:**200–216.
Springer W, Hoppe T, Schmidt E, and Baumeister R (2005) A *Caenorhabditis elegans* Parkin mutant with altered solubility couples alpha-synuclein aggregation to proteotoxic stress. *Hum Mol Genet* **14:**3407–3423.
Steffan JS, Bodai L, Pallos J, Poelman M, McCampbell A, Apostol BL, Kazantsev A, Schmidt E, Zhu YZ, Greenwald M, et al. (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. *Nature* **413:**739–743.
Stilwell GE, Saraswati S, Littleton JT, and Chouinard SW (2006) Development of a Drosophila seizure model for in vivo high-throughput drug screening. *Eur J Neurosci* **24:**2211–2222.
Stork T, Engelen D, Krudewig A, Silies M, Bainton RJ, and Klämbt C (2008) Organization and function of the blood-brain barrier in Drosophila. *J Neurosci* **28:**587–597.
Struhl G and Greenwald I (1999) Presenilin is required for activity and nuclear access of Notch in *Drosophila*. *Nature* **398:**522–525.
Sullivan E, Tucker EM, and Dale IL (1999) Measurement of $[Ca^{2+}]$ using the Fluorometric Imaging Plate Reader (FLIPR). *Methods Mol Biol* **114:**125–133.
Sun Z and Gitler AD (2008) Discovery and characterization of three novel synuclein genes in zebrafish. *Dev Dyn* **237:**2490–2495.
Takeyama K, Ito S, Yamamoto A, Tanimoto H, Furutani T, Kanuka H, Miura M, Tabata T, and Kato S (2002) Androgen-dependent neurodegeneration by polyglutamine-expanded human androgen receptor in Drosophila. *Neuron* **35:**855–864.
Tan JS, Lin F, and Tanouye MA (2004) Potassium bromide, an anticonvulsant, is effective at alleviating seizures in the Drosophila bang-sensitive mutant bang senseless. *Brain Res* **1020:**45–52.
Tan EK and Schapira AH (2008) Uniting Chinese across Asia: the LRRK2 Gly2385Arg risk variant. *Eur J Neurol* **15:**203–204.
Taylor JP, Hardy J, and Fischbeck KH (2002) Toxic proteins in neurodegenerative disease. *Science* **296:**1991–1995.
Teschendorf D and Link CD (2009) What have worm models told us about the mechanisms of neuronal dysfunction in human neurodegenerative diseases? *Mol Neurodegener* **4:**38.
Torres G and Horowitz JM (1998) Activating properties of cocaine and cocaethylene in a behavioral preparation of *Drosophila melanogaster*. *Synapse* **29:**148–161.
Tsang M (2010) Zebrafish: A tool for chemical screens. *Birth Defects Res C Embryo Today* **90:**185–192.
Tucker B, Richards RI, and Lardelli M (2006) Contribution of mGluR and Fmr1 functional pathways to neurite morphogenesis, craniofacial development and fragile X syndrome. *Hum Mol Genet* **15:**3446–3458.
Tully T and Quinn WG (1985) Classical conditioning and retention in normal and mutant *Drosophila melanogaster*. *J Comp Physiol A* **157:**263–277.
Valente EM, Abou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, et al. (2004) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. *Science* **304:**1158–1160.
van Ham TJ, Thijssen KL, Breitling R, Hofstra RM, Plasterk RH, and Nollen EA (2008) *C. elegans* model identifies genetic modifiers of alpha-synuclein inclusion formation during aging. *PLoS Genet* **(3):**e1000027.
Van Tijn P, Paridaen JT, Van Rooijen C, and Zivkovic D (2009) Zebrafish models for familial Alzheimer's disease. *6th European Zebrafish Genetics and Development Meeting*; 15–19 July 2009; Rome Italy. Abstract 293. Adria Congrex srl and Meetitaly, Rimini, Italy.
Van't Padje S, Chaudhry B, Severijnen LA, van der Linde HC, Mientjes EJ, Oostra BA, and Willemsen R (2009) Reduction in fragile X related 1 protein causes cardiomyopathy and muscular dystrophy in zebrafish. *J Exp Biol* **212:**2564–2570.
Ved R, Saha S, Westlund B, Perier C, Burnam L, Sluder A, Hoener M, Rodrigues CM, Alfonso A, Steer C, et al. (2005) Similar patterns of mitochondrial vulnerability and rescue induced by genetic modification of alpha-synuclein, parkin, and DJ-1 in *Caenorhabditis elegans*. *J Biol Chem* **280:**42655–42668.
Vidal M and Cagan RL (2006) Drosophila models for cancer research. *Curr Opin Genet Dev* **16:**10–16.
Vogler G and Ocorr K (2009) Visualizing the beating heart in Drosophila. *J Vis Exp* doi: 10.3791/1425.
Waddell S (2010) Dopamine reveals neural circuit mechanisms of fly memory. *Trends in neurosciences* **33:**457–464.
Wagner C, Isermann K, Fehrenbach H, and Roeder T (2008) Molecular architecture of the fruit fly's airway epithelial immune system. *BMC Genomics* **9:**446.
Wan L, Dockendorff TC, Jongens TA, and Dreyfuss G (2000) Characterization of dFMR1, a *Drosophila melanogaster* homolog of the fragile X mental retardation protein. *Mol Cell Biol* **20:**8536–8547.
Wang S, Tulina N, Carlin DL, and Rulifson EJ (2007) The origin of islet-like cells in Drosophila identifies parallels to the vertebrate endocrine axis. *Proc Natl Acad Sci USA* **104:**19873–19878.
West AB, Moore DJ, Biskup S, Bugayenko A, Smith WW, Ross CA, Dawson VL, and Dawson TM (2005) Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci USA* **102:**16842–16847.
West AB, Moore DJ, Choi C, Andrabi SA, Li X, Dikeman D, Biskup S, Zhang Z, Lim KL, Dawson VL, et al. (2007) Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. *Hum. Mol. Genet* **16:**223–232.
Whitten J (1957) The post-embryonic development of the tracheal system in *Drosophila melanogaster*. *Q J Microsc Sci* **98:**123–150.
Williams DW, Tyrer M, and Shepherd D (2000) Tau and tau reporters disrupt central projections of sensory neurons in Drosophila. *J Comp Neurol* **428:**630–640.
Wittenburg N, Eimer S, Lakowski B, Röhrig S, Rudolph C, and Baumeister R (2000) Presenilin is required for proper morphology and function of neurons in *C. elegans*. *Nature* **406:**306–309.
Woods IG, Kelly PD, Chu F, Ngo-Hazelett P, Yan YL, Huang H, Postlethwait JH, and Talbot WS (2000) A comparative map of the zebrafish genome. Genome Res **10:**1903–1914.
Wolf FW and Heberlein U (2003) Invertebrate models of drug abuse. *J Neurobiol* **54:**161–178.
Wolf MJ and Rockman HA (2008) *Drosophila melanogaster* as a model system for genetics of postnatal cardiac function. *Drug discovery today Disease models* **5:**117–123.
Wu CL, Xia S, Fu TF, Wang H, Chen YH, Leong D, Chiang AS, and Tully T (2007) Specific requirement of NMDA receptors for long-term memory consolidation in Drosophila ellipsoid body. *Nat Neurosci* **10:**1578–1586.
Wu L and Silverman N (2007) Fighting infection fly-style. *Fly* (*Austin*) **1:**106–109.
Wu M and Sato TN (2008) On the mechanics of cardiac function of Drosophila embryo. *PLoS ONE* **3:**e4045.
Wu M, Pastor-Pareja JC, and Xu T (2010) Interaction between Ras(V12) and scribbled clones induces tumour growth and invasion. *Nature* **463:**545–548.
Wu Y, Wu Z, Butko P, Christen Y, Lambert MP, Klein WL, Link CD, and Luo Y (2006) Amyloid-beta-induced pathological behaviors are suppressed by Ginkgo biloba extract EGb 761 and ginkgolides in transgenic *Caenorhabditis elegans*. *J Neurosci* **26:**13102–13113.

Xi Y, Ryan J, Noble S, Yu M, Yilbas AE, and Ekker M (2010) Impaired dopaminergic neuron development and locomotor function in zebrafish with loss of pink1 function. *Eur J Neurosci* **31:**623–633.
Ye Y, Lukinova N, and Fortini ME (1999) Neurogenic phenotypes and altered Notch processing in *Drosophila* Presenilin mutants. *Nature* **398:**525–529.
Yuan Q, Lin F, Zheng X, and Sehgal A (2005) Serotonin modulates circadian entrainment in Drosophila. *Neuron* **47:**115–127.
Zhang X, Smith DL, Meriin AB, Engemann S, Russel DE, Roark M, Washington SL, Maxwell MM, Marsh JL, Thompson LM, et al. (2005) A potent small molecule inhibits polyglutamine aggregation in Huntington's disease neurons and suppresses neurodegeneration in vivo. *Proc Natl Acad Sci USA* **102:**892–897.
Zhang YQ, Bailey AM, Matthies HJ, Renden RB, Smith MA, Speese SD, Rubin GM, and Broadie K (2001) Drosophila fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. *Cell* **107:**591–603.
Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, Kachergus J, Hulihan M, Uitti RJ, Calne DB, et al. (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron* **44:**601–607.