MENTAL RETARDATION AND DEVELOPMENTAL DISABILITIES
RESEARCH REVIEWS *11: 286–294 (2005)*

Mental Retardation Genes in Drosophila: New Approaches to Understanding and Treating Developmental Brain Disorders

Linda L. Restifo*

ARL Division of Neurobiology, University of Arizona, Tucson Arizona

Department of Neurology, Arizona Health Sciences Center, Tucson Arizona

Drosophila melanogaster is emerging as a valuable genetic model system for the study of mental retardation (MR). MR genes are remarkably similar between humans and fruit flies. Cognitive behavioral assays can detect reductions in learning and memory in flies with mutations in MR genes. Neuroanatomical methods, including some at single-neuron resolution, are helping to reveal the cellular bases of faulty brain development caused by MR gene mutations. *Drosophila fragile X mental retardation 1* (*dfmr1*) is the fly counterpart of the human gene whose malfunction causes fragile X syndrome. Research on the fly gene is leading the field in molecular mechanisms of the gene product's biological function and in pharmacological rescue of brain and behavioral phenotypes. Future work holds the promise of using genetic pathway analysis and primary neuronal culture methods in *Drosophila* as tools for drug discovery for a wide range of MR and related disorders. © 2005 Wiley-Liss, Inc.
MRDD Research Reviews 2005;11:286–294.

Key Words: genetic model system; drug discovery; synaptic plasticity; mushroom body; phylogenetic conservation; mGluR; *Fmr1*; fragile X syndrome; neurogenetics

The past decade has produced a dramatic rise in publications revealing genetic and molecular mechanisms of mental retardation (MR). Nonetheless, MR remains the most common medical condition for which no specific drug treatments are available. In contrast, disorders with similar prevalence, such as heart failure and schizophrenia, stimulate vigorous drug discovery and development efforts in the academic and pharmaceutical sectors [Scatton and Sanger, 2000; Tang and Francis, 2003]. At the present time, three major factors represent obstacles to thinking of MR as treatable in the pharmacological sense. For each obstacle, the *Drosophila melanogaster* genetic model system offers valuable strategies for overcoming the challenge. First, MR disorders have diverse environmental and hereditary etiologies, diagnostic criteria vary among clinicians, and MR is often associated with other neurological manifestations [Hodapp and Dykens, 2005]. This might lead one to the pessimistic, but premature conclusion that hundreds of different drugs would be needed to treat cognitive dysfunction in the majority of MR patients. Second, in most cases, there is limited understanding of how neuronal phenotypes, if they are known at all, disrupt functional brain circuitry and thereby diminish MR patients' cognitive capacities. Third, standard bioassays, such as proliferation, apoptosis, and biochemical tests on tumor cell lines for screening potential anticancer agents, are just beginning to be developed for MR disorders [Bourtchouladze et al., 2003]. This article will summarize published studies from the *Drosophila* system relevant to the biology of MR and present arguments for its expanded use as a step toward therapeutic drug screening in mammals.

RATIONALE FOR STUDYING MR IN *DROSOPHILA*

One might wonder, "How would you recognize a mentally retarded fruit fly?" Cognitive functions can be measured in insects, and deviations from normal, whether due to hereditary or environmental factors, can be quantified. *Drosophila* manifest a wide range of experience-dependent behaviors. This behavioral plasticity has been used both to identify and to characterize genetic mutants, starting with the pioneering work of Seymour Benzer and colleagues [Dudai et al., 1976; Greenspan, 1995]. Molecular analysis of these genes has revealed that mechanisms of learning and memory are shared between vertebrates and invertebrates [Mayford and Kandel, 1999].

At the simple end of the behavioral spectrum, wild-type *Drosophila* habituate to sensory stimuli [Engel and Wu, 1996; Cho et al., 2004]. In addition, they can learn associations across sensory modalities and remember them for varying amounts of time depending on the nature and duration of the training [Connolly and Tully, 1998]. For example, flies learn to avoid an odor that has been paired with electric shock (classical olfactory conditioning) or a spatial location where they were exposed to a noxious heat stimulus (operant place learning). When the task is made more complex by the addition of irrelevant sensory stimuli, flies can still learn the relevant predictive cue [Liu et al., 1999]. In an even more complex form of behavioral plasticity, when the sexual advances of a male fruit fly are rebuffed by a

Grant Sponsor: National Institutes of Health; Grant numbers: NS28495 and HD38363.
*Correspondence to: Linda L. Restifo, MD, PhD, 1040 East 4th Street, ARL Division of Neurobiology, University of Arizona, Tucson AZ 85721-0077. E-mail: LLR@neurobio.arizona.edu
Received 31 January 2005; Accepted 31 May 2005
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/mrdd.20083

© 2005 Wiley-Liss, Inc.

Table 1. Drosophila Mental Retardation Genes[a]

Human Gene (OMIM #)	Clinical Disorder (OMIM #)	Fly Gene	Fly Phenotype Affects	References
FLNA (300017)	Periventricular heterotopia (300049)	*cheerio*	LT memory (classical associative learning)	Dubnau et al. [2003]
FMRI (309550)	Fragile X syndrome (309550)	*dfmrl*	ST memory (courtship conditioning)	McBride et al. [2005]
GNASI (139320)	Albright hereditary osteodystrophy (103580)	*G protein sα60A*	Learning (classical associative)	Connolly et al. [1996]
NFI (162200)	Neurofibromatosis 1[b] (162200)	*Neurofibromin 1*	Learning and ST memory (classical)	Guo et al. [2000]
RSK2 (300075)	Coffin-Lowry s.; MRX19 (303600)	*rsk (S6KII)*	Learning (associative: operant and classical)	Putz et al. [2004]

[a]s., syndrome; LT, long-term; ST, short-term.
[b]The cognitive deficits of *NFI* patients may result in part from interactions between *NFI* and adjacent genes [Venturin et al. 2004].

nonreceptive female, the male responds with a prolonged reduction in his courtship activity (courtship conditioning) [Tompkins et al., 1983; Mehren et al., 2004].

For each task a fly can learn, the level of initial learning and its retention during specific phases of memory, including short- and long-term, are differentially affected by single-gene mutations [Dubnau and Tully, 1998]. Moreover, different forms of learning and memory require different brain structures [Heisenberg, 2003]. Hence, while we do not know whether natural populations of fruit flies include "mentally retarded" individuals, scientists can identify flies in the laboratory that have specific, quantifiable cognitive impairments and these are likely to result from distinct biochemical and neuroanatomical mechanisms.

Despite the hundreds of millions of years of evolutionary distance that separate *Homo sapiens* and *Drosophila melanogaster*, MR-associated molecules are remarkably well conserved between the two species. In a recent comparative sequence analysis of nearly 300 molecularly identified human MR genes, we found that 87% have a highly similar relative in *Drosophila* [Inlow and Restifo, 2004]. This percentage is higher than previous reports that used the same sequence-similarity criteria to compare *Drosophila* genes and human disease genes of diverse types [Fortini et al., 2000; Reiter et al., 2001]. Furthermore, the extent and type of amino acid sequence similarity between the human MR genes and their fruit fly counterparts were sufficient in 76% to suggest that they have similar biological functions in the two species [Inlow and Restifo, 2004]. The best-characterized *Drosophila* MR gene counterpart is *Drosophila fragile X mental retardation 1* (*dfmr1*), mutations of which cause a syndrome of behavioral and cognitive deficits, neuroanatomical abnormalities, and male sterility (discussed below).

Five of the fruit fly counterparts of human MR genes are already known to be essential for normal learning and memory (Table 1). In two cases (*FLNA/cheerio* and *RSK2/rsk*; nomenclature is presented throughout as *human gene abbreviation/fly gene abbreviation* [see Inlow and Restifo, 2004, for additional information regarding the similarities between the human and fly genes]), the genes were identified in traditional unbiased screens for behavioral phenotypes due to loss-of-function mutations [Dubnau et al., 2003; Putz et al., 2004]. In the other cases, gain-of-function mutations (*GNAS1/Gsα60A*) or loss-of-function mutations (*NF1/Nf1* and *Fmr1/dfmr1*) were created or selected because of an interest in a specific gene and then tested for their behavioral consequences [Connolly et al., 1996; Guo et al., 2000; McBride et al., 2005]. As noted in Table 1, several different learning-and-memory paradigms were used to demonstrate deficits in the *Drosophila* mutants. In the one case for which mutants of the same gene were subjected to more than one type of test (*RSK2/rsk*), deficits were seen in both types [Putz et al., 2004]. Taken together, the data demonstrate that these *Drosophila* mutants are cognitively impaired and, even without a systematic scheme for measuring performance across the full spectrum of fruit fly abilities (i.e., a *Drosophila* "intelligence quotient test"), it is reasonable to consider the five genes involved to be "*Drosophila* MR genes." Furthermore, given the known neurological phenotypes of other MR gene counterparts in *Drosophila* [Inlow and Restifo, 2004 and references therein], it is easy to predict that many more human MR genes will eventually be shown to have conserved cognitive functions in fruit flies.

New MR genes are being published at a rate of one to three per month, but hundreds more remain to be identified [Inlow and Restifo, 2004]. Increasingly effective gene-mapping methods, as well as candidate-gene approaches, are in use to achieve these goals [Ropers and Hamel, 2005]. Morley and Montgomery [2001] proposed human counterparts of *Drosophila* learning or memory genes as candidates for genes affecting human cognition. Given the examples in Table 1, one could easily propose that all entries on the rapidly expanding list of fly learning and memory genes [Roman and Davis, 2001; Waddell and Quinn, 2001; Dubnau et al., 2003] should be considered MR gene candidates.

POWER AND POTENTIAL OF THE FLY GENETICS TOOLBOX

The experimental power and sophistication of the *Drosophila* genetic system has been detailed elsewhere [Greenspan, 1997; Sullivan et al., 2000], but several features with special relevance to developmental brain disorders warrant mention here. First, phenotypic analysis of developmental mutations is easier than in mammals. Because *Drosophila* are not viviparous, developmental phenotypes of lethal mutants are more accessible. These phenotypes provide clues to gene function that are unsuspected in hereditary disorders that cause human fetuses to die early in gestation. In addition, one can create a series of mutant alleles with a spectrum of severities to study the consequences of varying levels of residual gene function. Extremely clever methods, with origins in yeast genetics, allow the revelation of cell-autonomous neuronal phenotypes, at very high resolu-

tion, in single-cell mutant clones [Lee and Luo, 1999]. The relative simplicity of the *Drosophila* genome often permits single counterparts of a mammalian gene family to be studied without the complications of genetic redundancy that result from duplications that occurred during vertebrate evolution [Holland et al., 1994; Durand, 2003]. For example, unlike the three closely related *Fmr1* genes in mammals, only one is present in *Drosophila* [Wan et al., 2000]. On the other hand, one can not assume that the single fly gene's function represents the sum of the multiple mammalian genes' functions.

Second, the ability to regulate gene expression in time and space using transgenic *Drosophila* [McGuire et al., 2004] offers an important means of determining *when* and *where* a gene is acting to promote cognitive function—which has major implications for therapeutic intervention strategies. If transgenic rescue of a mutant fly with a wild-type allele is successful only when the transgene is active during development, then that gene is critical for functional brain asssembly but is not necessarily required in the mature animal for synaptic plasticity. Conversely, if mutant performance can be enhanced by providing the wild-type protein acutely before behavioral testing, that would indicate that the gene serves ongoing functions during adulthood. For example, the learning deficits of *Drosophila Nf1* mutants can be rescued by inducing expression of wild-type *Nf1* in the adult, suggesting that *Nf1* function during development is not essential for adult learning [Guo et al., 2000]. Similar distinctions can be made when determining the critical period for the cognition-impairing effects of dominant gain-of-function mutations. In addition, RNA interference methods can be used to induce a loss-of-function condition at specific times [e.g., Lam and Thummel, 2000] to determine the consequences of acutely eliminating a gene product in an otherwise normal animal.

Third, *Drosophila* genetics can be used to identify members of metabolic, regulatory, or signaling pathways. This results from a basic principle that mutations in genes that are members of the same biological pathway often share phenotypes. The pathway concept is important in part because, when an individual human gene has a counterpart in *Drosophila*, other members of its pathway often do as well. For instance, MR is a consequence of mutations in over a dozen genes involved in protein glycosylation, all of which have fruit fly counterparts [see Appendix of Inlow and Restifo, 2004]. Furthermore, pathways tend to intersect or converge, especially as signals are transmitted from the cell surface to regulators of enzyme activity or gene expression. For example, mutations in several highly conserved genes of the Rho-type small-G-protein signaling cascade (*OPHN1/Graf*; *GDI1/Gdi*; *ARHGEF6/rtGEF*) cause isolated MR [Ramakers, 2002]. Downstream of this pathway lies the previously mentioned MR gene *RSK2* (see Table 1) whose gene product phosphorylates the transcription factor CREB (a keystone of mammalian and invertebrate learning [Mayford and Kandel, 1999]), whose partners include CREB binding protein (CBP), product of the Rubinstein-Taybi syndrome MR gene (*CREBBP/nejire*). Genetic screens in *Drosophila* can reveal previously unknown members of a pathway and cross-pathway relationships, for instance by identifying new mutations causing synergistic enhancement or suppression of a phenotype [Bonini, 2000]. *Drosophila* CBP is an important regulator of the hedgehog pathway [Akimaru et al., 1997], whose vertebrate counterparts include several MR genes (*SHH/hedgehog*; *PTCH/patched*; *GLI3/cubitus interruptus*; *ZIC2/oddpaired*), which suggests a connection between CBP and SHH pathways in mammals that could be relevant to MR. Why do these pathway relationships matter? For the simple but powerful reason that a drug that acts on a well-placed pharmacological target could provide effective treatment for disorders resulting from mutations of many different genes throughout one complex pathway.

. . . the* Drosophila *system could also be applied to environmental causes of MR, in particular pre- or perinatal exposure to neurotoxic drugs of abuse . . .

Fourth, the *Drosophila* system could also be applied to environmental causes of MR, in particular pre- or perinatal exposure to neurotoxic drugs of abuse that alter brain development [Stanwood and Levitt, 2004]. Adult fruit flies respond to cocaine and ethanol in time- and dose-dependent ways that are reminiscent of mammalian responses, including behavioral intoxication, sensitization, and tolerance, and the genetic control of these responses is beginning to be elucidated [McClung and Hirsh, 1998; Guarnieri and Heberlein, 2003]. Extending genetic studies in *Drosophila* to the neurodevelopmental effects of ethanol and cocaine could suggest treatment strategies for acquired as well as hereditary MR. There is already one demonstration of a link, albeit a complex one, between the genetics of ethanol tolerance and the genetics of cognition in *Drosophila*. Mutations of a neuropeptide-encoding gene were independently identified in screens for memory deficits (*amnesiac* alleles) and for enhanced ethanol sensitivity (the *cheapdate* allele) [Quinn et al., 1979; Feany and Quinn, 1995; Moore et al., 1998]. The neuropeptide appears to have both developmental and adult functions because ethanol hypersensitivity could be rescued by providing the wild-type gene to adults [Moore et al., 1998], whereas rescue of the memory phenotype required wild-type gene expression during development [DeZazzo et al., 1999].

Finally, and perhaps most important, enthusiasm for using *Drosophila* in the study of MR is bolstered by the recent development of fly models of neurodegenerative disorders and successful drug treatment to normalize behavioral and anatomical phenotypes [Bonini and Fortini, 2003]. *Drosophila* models of Parkinson's disease include both transgenic flies expressing *α-synuclein* with a human disease-associated dominant mutation [Feany and Bender, 2000], as well as wild-type flies exposed to the pesticide rotenone [Coulom and Birman, 2004]. In the former case, as in humans with familial Parkinson's disease, mutant α-Synuclein protein aggregates within dopaminergic brain neurons, causing adult-onset degeneration and locomotor abnormalities [Feany and Bender, 2000]. Overexpression of Parkin protein suppresses α-Synuclein–induced neurodegeneration in both cultured mammalian neurons and mutant flies [Petrucelli et al., 2002; Haywood and Staveley, 2004]. In addition, geldanamycin, a modulator of stress-response pathways, prevents age-dependent dopaminergic cell loss in these *α-synuclein* transgenic flies [Auluck and Bonini, 2002].

Equally compelling data come from the *Drosophila* model of Hunting-

ton's disease (HD), created by expression of human mutant Huntingtin protein with a polyglutamine-tract expansion, which causes repeat-length–dependent neuronal degeneration [Jackson et al., 1998]. Three biochemical approaches to modulating HD pathology have similar beneficial effects in rodent and *Drosophila* models [Steffan et al., 2001; Hockly et al., 2003; Ravikumar et al., 2004; Steffan et al., 2004]. In addition, the fly system has begun to suggest new pharmacological strategies and specific chemical compounds that could ultimately yield effective treatments for neurodegenerative diseases in humans [Auluck and Bonini, 2002; Auluck et al., 2002; Kazantsev et al., 2002].

CASE IN POINT: *DROSOPHILA FRAGILE X MENTAL RETARDATION 1 (dfmr1)*

Fragile X syndrome (FXS), a very common MR disorder with cytogenetic and neurodevelopmental manifestations, was covered in detail in a recent dedicated issue of this journal edited by Crnic and Hagerman [see MRDDRR volume 10, issue 1, 2004]. FXS patients have loss-of-function mutations of *Fmr1*, which causes a distinctive profile of cognitive deficits of varying severity, along with enlarged testes, characteristic facies, and, in some patients, other neurological features such as autism, epilepsy, and disordered sleep [Hagerman, 2002; Cornish et al., 2004]. Evidence from both mammalian and fly systems strongly supports a working model in which FMRP's primary neuronal function is selective RNA binding and transport to dendrites, for the purpose of translational control—mainly repression—of specific proteins in a synaptic activity–dependent manner [Darnell et al., 2004; Willemsen et al., 2004]. This would put FMRP, and localized translation, at the center of synaptic plasticity mechanisms in both developing and mature animals.

Activation of metabotropic glutamate receptors (mGluR), specifically the Gp1 class, regulates FMRP localization and perisynaptic translation [Weiler et al., 1997, 2004; Todd et al., 2003; Antar et al., 2004]. In addition, mGluR-mediated long-term depression at hippocampal synapses is *increased* in *Fmr1* mutant mice [Huber et al., 2002]. These observations have led to the proposal that FXS neurobehavioral manifestations result from excessive mGluR signaling [Bear et al., 2004], which suggests that Gp1 mGluR antagonists could provide effective treatment for the disorder.

The *Drosophila* gene product, dFMRP, is very similar in sequence and domain organization to its mammalian counterparts, with ≥70% amino acid identity in the RNA-binding KH domains and ribosome-interaction domain [Wan et al., 2000]. It is found throughout the central nervous system (CNS) and peripheral nervous system (PNS) of developing and mature animals, primarily in the cytoplasm of (probably all) neuronal somata [Zhang et al., 2001; Morales et al., 2002; Lee et al., 2003; Pan et al., 2004], but not in glia. Immunoelectron microscopy localization methods would be required to rule out small amounts of dFMRP in neuronal nuclei or near postsynaptic sites, as was found in mammals [Feng et al., 1997].

The neurological **phenotypes of *dfmr1* mutants include behavioral and cognitive deficits**. The most compelling of these, in terms of comparison to human FXS, is very rapid memory loss in the courtship conditioning assay, recently reported by McBride and Jongens and their colleagues. After rejection by an unreceptive female during a 1-hour training period, mutant males reduce their courtship activity to the same degree as do wild-type males, demostrating intact learning [McBride et al., 2005]. However, within minutes thereafter, the mutants resume courtship at their prior baseline level, revealing a profound short-term memory deficit. This inability to suppress a behavior might also represent a form of perseveration. Short-term memory for complex tasks is one of the specific weaknesses of the human FXS cognitive profile [Cornish et al., 2004]. In addition, lack of behavioral inhibition, which often results in perseveration, has been proposed as a fundamental underlying deficit in FXS [Cornish et al., 2004]. Because *dfmr1* mutant performance on other tasks has not yet been reported, it is too early to conclude anything about the scope and specificity of the cognitive deficit. Nonetheless, the nature of the memory deficit in the fly mutants suggests a qualitative overlap with FXS patients' cognitive weaknesses. Another major recent advance has been the pharmacological rescue of the *dfmr1* memory deficit by treatment with mGluR antagonists [McBride et al., 2005], discussed below.

Drosophila dfmr1 mutants also have circadian rhythm defects, notably poor rhythmicity of locomotor behavior. Because the eclosion of the locomotor rhythm is intact, (the emergence of the adult fly from its protective cocoon-like structure at the end of metamorphosis) the locomotor rhythm defect reflects abnormal output of the circadian clock rather than a broken central oscillator [Dockendorff et al., 2002; Inoue et al., 2002; Morales et al., 2002]. This phenotype is reminiscent of the aberrant sleep patterns and elevated melatonin levels found in FXS males [Gould et al., 2000]. Finally, "courtship interest" is reduced in *dfmr1* mutant males [Dockendorff et al., 2002], but the residual level can still be reduced further by courtship conditioning [McBride et al., 2005]. As with the memory defect, courtship interest was restored by mGluR blockade during development or adulthood, whereas the activity rhythm phenotype was not [McBride et al., 2005].

In humans, *Fmr1* mutations do not cause major disruptions in brain structure, although modest quantitative changes have been noted by magnetic resonance imaging (MRI) in some regions and these changes correlate with cognitive deficits [Hessl et al., 2004]. However, at the single-neuron level (using the Golgi impregnation technique on postmortem brain tissue), cortical neurons in several regions have abnormal dendrites, with spines showing excessive numbers and length [Rudelli et al., 1985; Hinton et al., 1991; Wisniewski et al., 1991; Irwin et al., 2001]. Similar studies in the *Fmr1* mutant mouse confirmed these findings, as well as excessive dendritic branching [Comery et al., 1997; Irwin et al., 2002; Galvez et al., 2003]. Given that spines are postsynaptic sites and that dendrites undergo complex alterations during development [Cline, 2001], it is not surprising that a "subtle" morphological change would cause serious functional consequences. The abnormal spine appearance has been interpreted as immaturity or stalled development, possibly due to insufficient pruning [reviewed in Beckel-Mitchener and Greenough, 2004], but it may reflect synaptic loss [Bear et al., 2004]. Moreover, real-time analyses of GFP-labeled cortical neurons of *Fmr1* mutant mice are more consistent with abnormal timing and speed of dendritic development [Nimchinksy et al., 2001], suggesting that this issue needs further investigation. Note that neither axonal projection patterns nor axon terminal-arbor morphology has been examined in *Fmr1* mutant mammals.

The **cellular neural phenotypes of *dfmr1* mutant flies** are qualitatively similar to the mammalian ones, with a common—but not universal—theme of dendritic and axonal overgrowth in CNS and PNS, as well as pathfinding errors

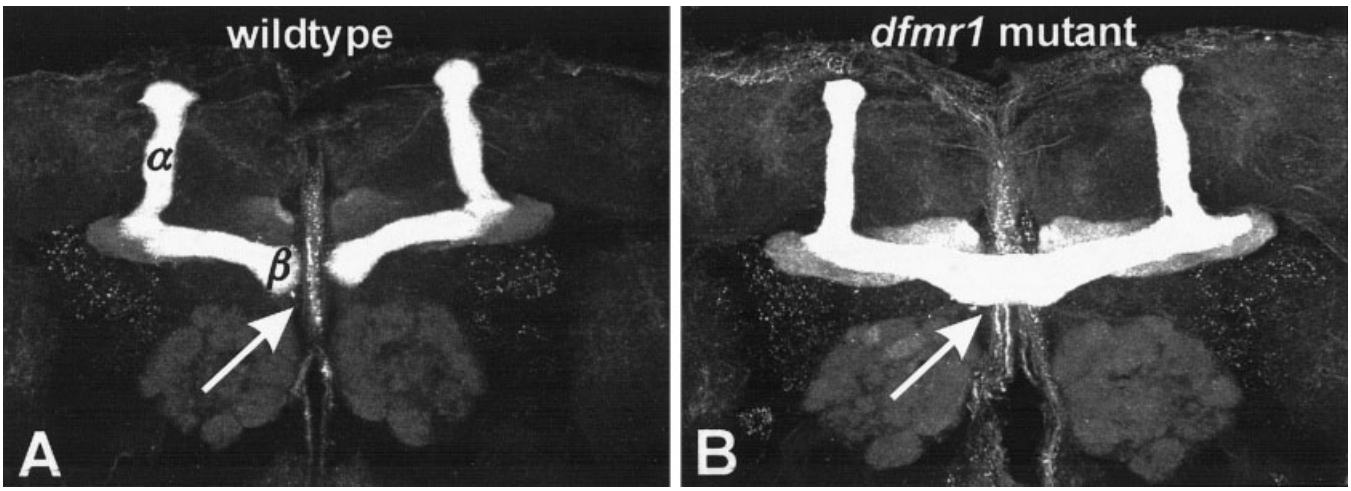

Fig. 1. Abnormal mushroom body structure in *dfmr1* mutants. Confocal micoroscopy analysis using anti-Fasciclin II to visualize the mushroom body α and β lobes. In the wildtype (A), the β lobes approach, but stop before, the midline (arrow). In the *dfmr1* mutant (B), the β-lobe axons have grown across the midline and fused with the contralateral fibers (arrow). [adapted from Michel et al., 2004].

[Zhang et al., 2001; Dockendorff et al., 2002; Morales et al., 2002; Lee et al., 2003; Michel et al., 2004; Pan et al., 2004]. The only neurons for which both axonal and dendritic morphologies have been examined in *dfmr1* mutants are those of the mushroom bodies, a brain region well known for its role in many forms of associative learning and memory [Zars, 2000; Roman and Davis, 2001; Heisenberg, 2003]. Carlos Michel and Robert Kraft in my lab found a specific developmental defect during the metamorphic transition [Michel et al., 2004], when hundreds of mushroom body α/β neurons are born and differentiate [Lee et al., 1999]. Wild-type α/β axons bifurcate at right angles and project into vertical (α) and medial (β) lobes. In *dfmr1* mutants, axons project into the lobes at the appropriate developmental stage but, as they approach the midline, they fail to detect or to obey a "stop " signal [Michel et al., 2004]. Rather, they continue growing into the contralateral hemisphere, resulting in the appearance of β-lobe midline fusion, observable by confocal microscopy with appropriate fluorescent markers (Fig. 1). Three lines of evidence from other labs suggest that this mushroom body phenotype is the cause, at least in part, of the above-mentioned memory deficit. First, prevention of mushroom body development results in poor courtship conditioning memory [McBride et al., 1999]. Second, the *Drosophila* memory mutant *linotte* has the same mushroom body phenotype [Moreau-Fauvarque et al., 1998]. Third, the α/β lobes are required for memory retrieval [McGuire et al., 2001].

How severe is β-lobe fusion in a fly relative to mammalian brain defects? Given the differences in absolute size and structural organization between mammalian and insect brains, the comparison is difficult. Nonetheless, roughly speaking, the β lobes are comparable to a large bilaterally symmetric pair of axon tracts that can be visualized by MRI or histological staining, e.g., the internal capsule. In other words, if FXS brains had a defect comparable to β-lobe fusion, it would be relatively easy to detect without morphometric analysis. This, along with the other neuronal phenotypes (see below), suggests that *dfmr1* mutants are neurologically more severely affected than are human or mouse *Fmr1* mutants.

Pan and colleagues [2004] used genetic techniques to create mosaic *Drosophila* in which single mushroom body neurons were *dfmr1* mutant and surrounded by wild-type brain tissue. The mutant neurons had excessive and disordered branching of axons and dendrites. Conversely, overexpression of wild-type *dfmr1* in mushroom body neurons reduced dendritic and axonal complexity to less than those of wildtype [Pan et al., 2004]. These data provide strong evidence that *dfmr1* normally functions to limit the exuberance of axonal and dendritic branching, consistent with earlier observations in the PNS (see below). However, there is an important caveat when interpreting phenotypes in such mosaic animals, exemplified by the report that single-neuron *dfmr1* mutant clones in the β lobes do not show midline crossing [Pan et al., 2004]. This discrepancy provides an important clue, suggesting that the whole-brain mutant phenotype [Michel et al., 2004] is not-cell-autonomous, but rather results from cell–cell interaction. The cell-autonomy issue has broader implications, for instance, for understanding the phenotypic variation of many X-linked MR disorders in heterozygous human females, whose brains are mosaic due to X-chromosome inactivation [e.g., Braunschweig et al., 2004]. Furthermore, while it is tempting to assume that mushroom body neuron dendrites and axons have excessive branching in whole-animal *dfmr1* mutants, it would be prudent to determine their structure directly by using Golgi or another high-resolution method.

Elsewhere in the brains of *dfmr1* mutants, neurite extension defects were seen in DC neurons, which are important for emergence of the adult after metamorphosis, and in the circadian-pacemaker LNv neurons [Morales et al., 2002]. Both types of neurons also showed pathfinding or projection errors, but their phenotypes differed in that DC neurons frequently showed reduced axon extension and ectopic branching, while the LNv neurons showed axon overextension but only rarely [Morales et al., 2002]. Given the complexity of CNS anatomy, the presence of phenotypes affecting many neuron types and the apparently nonautonomous behavior of α/β axons at the midline raise the nontrivial question of which phenotypes are primary and which are secondary to other defects.

In the PNS of *dfmr1* mutant larvae, axon terminals at the neuromuscular junction are larger than normal, with similar increases in branch and synaptic bouton numbers [Zhang et al., 2001]. In other words, the junction is larger, but proportionately so. Neurotransmission from the larger junctions is also increased, indicating a roughly proportionate in-

crease in functional synapses [Zhang et al., 2001]. On the sensory side, the phenotype is slightly different. Dendritic arbors of mutant larval body wall sensory neurons show increased higher-order branching, but remain restricted within their usual segment boundaries. Hence, the overall footprint of the arbor is normal, but within that region there are more terminal branches [Lee et al., 2003]. For both the motor neuron axon terminals and the sensory neuron dendrites, overexpression of wild-type dFMRP induces the corresponding opposite phenotypes, consistent with the conclusion that *dfmr1* wild-type function is to limit the extent and complexity of a neuron's terminal arbors, whether axonal or dendritic.

Given these neuronal defects, it is not surprising that movement is adversely affected by *dfmr1* mutations. Specifically, larval crawling is abnormally circuitous (although normal in speed), another indication of a more prominent neurological phenotype than in mammals. One might guess that this results from a combination of motor and sensory defects, but it can be rescued by selective expression of wild-type *dfmr1* in the sensory neurons, suggesting that the altered locomotion is due primarily to aberrant sensory input to the motor system [Xu et al., 2004]. Adult flight performance is reduced in *dfmr1* mutants [Zhang et al., 2001], but the underlying cause is unknown.

It is worth considering how similar fly and mammalian phenotypes need to be in order to validate the fly system—although it will be some time before a clear answer emerges. Take, for example, the fragile X testis phenotype. Both mammalian and fly mutants have testicular enlargement. In the former case, it occurs after puberty and is due to Sertoli cell hyperplasia [Slegtenhorst-Eegdeman et al., 1998], whereas in the latter it is seen transiently in young adults and caused by disorganized bundles of spermatids [Zhang et al., 2004]. Behavioral phenotypes are more difficult to compare. Is the reduced courtship interest of *dfmr1* mutant males [Dockendorff et al., 2002] a reflection of reduced libido or is it a counterpart of social anxiety and avoidance seen in FXS males [Cornish et al., 2004]? For now, I would argue that the phenotypic details may matter less than the molecular pathways underlying them, for, if mechanisms are similar, then drug-based treatments should be similar as well.

The recent successful **pharmacological rescue of *dfmr1* phenotypes** takes the fly model of FXS one giant step forward. When *dfmr1* mutant larvae are fed a diet supplemented with one of several mGluR antagonists (including both competitive and noncompetitive agents), the resulting adult flies show normal memory in the courtship conditioning assay and normal mushroom body morphology [McBride et al., 2005]. The further result of that study, showing that memory can be rescued by treating flies only as adults, is striking for two reasons. First, the developmental mushroom body defect is not reversed by the adult-treatment protocol, which is hardly surprising given the bulk of misdirected axonal projections that would have to be retracted. Second, and most important, the ability to achieve behavioral rescue by treating "postnatally"—without needing to "fix" a major morphological defect and without needing to replace the defective gene—gives great hope for effective postnatal therapies for developmental brain disorders. One is reminded of the extraordinary plasticity of young human brains that enables their functional reorganization after major trauma such as hemispherectomy [Vargha-Khadem et al., 1997].

. . . the ability to achieve behavioral rescue by treating "postnatally"—without needing to "fix" a major morphological defect and without needing to replace the defective gene—gives great hope for effective postnatal therapies for developmental brain disorders.

Biochemical studies revealed that **dFMRP exerts translational control through RNA interference mechanisms** [reviewed in Siomi et al., 2004], and this led to similar findings for the mammalian protein. *Drosophila* FMRP associates with an RNA-induced silencing complex (RISC), through which cells destroy specific transcripts, and is also involved in microRNA pathways of translational repression [Caudy et al., 2002; Ishizuka et al., 2002; Schenck et al., 2003; Jin et al., 2004]. These studies led to the finding that mammalian FMRP associates with the microRNA pathway [Jin et al., 2004]. This is a clear case of the fly system leading, rather than merely following, the field.

Functionally relevant protein partners and target transcripts of dFMRP have also been identified using a combination of genetic and biochemical approaches. The first of these was *futsch*, which encodes the microtubule-associated protein MAP1B and has an NMJ mutant phenotype that is opposite of the *dfmr1* phenotype; in double mutants, the two mutations suppress each others' effects and the resulting NMJ is close to normal [Zhang et al., 2001]. This led to the demonstration that dFMRP associates with *futsch* RNA and negatively regulates its translation [Zhang et al., 2001]. Two independent biochemical screens identified mammalian MAP1B transcripts as FMRP-associated [Kaytor and Orr, 2001]. Similarly, dFMRP associates with the fly counterpart of human CYFIPs (*cy*toplasmic *F*MRP-*i*nteracting *p*roteins), which has mutant phenotypes that are generally opposite those of *dfmr1* [Schenck et al., 2003]. In the sensory neurons whose dendritic arbors are controlled by dFMRP, the protein binds and negatively regulates transcripts encoding the GTPase Rac1 [Lee et al., 2003] and a degenerin/epithelial sodium channel family member [Xu et al., 2004]. Recently, a genetic screen for mutations that interact with *dfmr1* revealed Lgl, encoded by *lethal(2) giant larvae*, as a close associate of FMRP in both flies and mice [Zarnescu et al., 2005]. Together, the available data point to considerable mechanistic similarities between fly and mammalian FMRP function.

In summary, neuroanatomical, behavioral, biochemical, and pharmacological data provide compelling support for the use of *Drosophila* as a genetic model system for fragile X syndrome and, indirectly, for other hereditary MR disorders as well.

PROSPECTS FOR DRUG DISCOVERY AND DEVELOPMENT

In light of the structural and functional similarities between human MR genes and their fruit fly counterparts, and the recent report of successful treatment of *dfmr1* mutants, there is an extraordinary opportunity to use the *Drosophila* system as a drug-screening tool for MR. There are two general strategies for mon-

itoring effects of an MR-relevant drug: rescue of a mutant phenotype (whether at the biochemical, neuroanatomical or behavioral level) or modulation of the function of an identified drug target. In the first case, one need not know specific drug targets in advance and the mechanism of action is a secondary matter. In the second strategy, one needs an assay for the activity of the known target.

In *Drosophila*, bioassays based on whole animals or on cultured cells each have particular advantages. For whole animals, there are multiple options for routes of drug administration, including feeding, topical application, injection, and aerosol exposure [e.g., Hirsh, 2000]. One can then assess the resulting behavior and brain structure as well as more general outcomes, such as viability, longevity, and fecundity. On the other hand, *in vitro* cell-based assays have the advantage of bypassing the anatomical barriers around the CNS, from integument and gastrointestinal tract to circulatory system and blood–brain barrier, structures that may differ considerably between insects and mammals. Furthermore, compared with whole-animal assays, cell-based assays are more likely to be amenable to automation and, hence, to higher-throughput screening methods.

Cellular assays can be divided into those using established cell lines and those using primary cells or tissues freshly explanted from wild-type or mutant animals. The Schneider-2 (S2) cell line, which can express transfected genes, has revealed molecular mechanisms of cell adhesion [Hortsch and Bieber, 1991], much as *Xenopus* oocytes have done for ion channel function. *Drosophila* Neuroglian, whose human counterpart, L1 cell adhesion molecule (*L1CAM/Nrg*), is defective in a complex set of MR disorders [Weller and Gartner, 2001], controls axon pathfinding by regulating adhesive interactions between neurons [Hall and Bieber, 1997; Hortsch et al., 1998]. S2 cells expressing mutant forms of Neuroglian could be used to screen for drugs that restore cell adhesion. When S2 cells grow on a flat substrate they can be induced to send out long, thin processes, and this property has recently been exploited to demonstrate that dFMRP-containing ribonucleoprotein granules are transported along microtubules in the neurite-like processes [Ling et al., 2004]. For now, this has been important for understanding dFMRP transport mechanisms, but it suggests that S2 cells with processes may provide a drug-screening tool for MR-related functions.

Primary culture methods have the obvious advantage of allowing the size, shape, and growth dynamics of actual neurons from intact animals to be assayed. We developed culture methods for genetically marked *Drosophila* mushroom body neurons harvested at specific developmental times. *In vitro*, these developmentally plastic neurons grow a neurite arbor with distinctive morphometric features and respond with greater outgrowth to physiological levels of an endogenous steroid hormone [Kraft et al., 1998]. Moreover, mutant neuronal phenotypes, including stunted growth, excessive growth, and altered branching structure, can be detected [Kraft R, Escobar M, Kurtis J, Restifo L, manuscript in preparation]. If mutations in *Drosophila* MR genes disrupt neuronal morphology *in vitro*, then cell culture could be used as a screening assay for drugs that normalize them.

CLOSING REMARKS: WHAT IS NEEDED TO OPTIMIZE THE VALUE OF THE FLY SYSTEM?

Despite the powerful genetic technology afforded by the *Drosophila* system, a number of additional or modified tools would greatly boost its value as a model for MR and other neurobehavioral disorders. In particular, a "standardized neurological exam" for evaluation of mutants would be a major advance. One can imagine two versions, starting with a relatively simple exam that could be performed in any *Drosophila* laboratory. This would be analogous to a screening exam performed by a nonspecialist physician, covering all the major neurological functions. The importance of multisystem assays is exemplified by the study of associative learning, which requires an assessment of sensory acuity and reactivity to the punishing stimulus in order to interpret behavioral deficits [Connolly and Tully, 1998]. While it would be desirable to devise a test not requiring sophisticated equipment, one can envision a maze-like gadget within which environmental stimuli could be controlled and fly behavior observed. Ideally, the basic test would include a behavioral overview, reminiscent of the Mini-Mental State Exam [Bassuk and Murphy, 2003], an imperfect but rapid screening test of cognitive function that can also be used to follow individuals over time. If abnormalities were detected in the mutants on the basic test or if subtle defects were suspected based on gene expression patterns or other information, then the mutant strain could be referred to a specialty lab for further analyses. Many of the behavioral tests currently in use for *Drosophila* measure population responses (although courtship conditioning is assayed on individual males). Emphasis on additional single-fly tests, especially those that could be repeated on the same individual, e.g., to monitor effects of aging, would be a particularly good investment.

Another area that is poised to make rapid and very important advances is functional neuroanatomy. Until very recently, the understanding of localization of function in the *Drosophila* brain has been comparable to that in the mammalian brain in the mid-20th century. The correlations made between brain morphological phenotypes and behavioral deficits in mutants were analogous to structure/function studies based on postmortem studies of stroke and tumor patients many decades ago—an essential first step, but allowing only a crude, low-resolution map of functional zones with lots of question marks between them. In part because of its small size, *D. melanogaster* has not undergone extensive neural circuit analysis, at the level of either anatomical or functional connectivity. There has been a great temptation to extrapolate from studies in larger insects, but insects comprise extraordinarily diverse taxa and many of the cross-species assumptions have never been tested. Thus, it is very exciting that optical imaging and *Drosophila* genetic technology have recently converged to allow direct monitoring of neuronal activity in living preparations exposed to various stimuli [Fiala et al., 2002; Ng et al., 2002]. During the classical olfactory conditioning paradigm, one can observe rapid, short-lived spread of synaptic activity into new regions within the antennal lobes [Yu et al., 2004]. At the moment, these innovative methods have been used only for superficial regions of the brain, but that is likely to change with the incorporation of higher-sensitivity and higher-resolution microscopy.

In conclusion, the obstacles to MR therapeutics are formidable but not insurmountable. The perspective of phylogenetic conservation provides much cause for optimism that novel therapeutic strategies will emerge from a synergistic synthesis of advances in human and *Drosophila* neurogenetics. ■

ACKNOWLEDGMENTS

Research in the author's lab is funded by NIH NS28495 and HD38363. The author also thanks the John Merck Fund for its previous scholarship support through its Program in Developmental Disabilities in Children. J. Inlow and R.

Kraft provided helpful comments on the manuscript. Discussions with T. Jongens have also been much appreciated.

REFERENCES

Akimaru H, Chen Y, Dai P, et al. 1997. *Drosophila* CBP is a co-activator of cubitus interruptus in hedgehog signalling. Nature 386:735–738.

Antar LN, Afroz R, Dictenberg JB, et al. 2004. Metabotropic glutamate receptor activation regulates fragile X mental retardation protein and *FMR1* mRNA localization differentially in dendrites and at synapses. J Neurosci 24: 2648–2655.

Auluck PK, Bonini NM. 2002. Pharmacological prevention of Parkinson disease in *Drosophila*. Nat Med 8:1185–1186.

Auluck PK, Chan HY, Trojanowski JQ, et al. 2002. Chaperone suppression of alpha-synuclein toxicity in a *Drosophila* model for Parkinson's disease. Science 295:865–868.

Bassuk SS, Murphy JM. 2003. Characteristics of the Modified Mini-Mental State Exam among elderly persons. J Clin Epidemiol 56:622–628.

Bear MF, Huber KM, Warren ST. 2004. The mGluR theory of fragile X mental retardation. Trends Neurosci 27:370–377.

Beckel-Mitchener A, Greenough WT. 2004. Correlates across the structural, functional, and molecular phenotypes of fragile X syndrome. Ment Retard Dev Disabil Res Rev 10:53–59.

Bonini NM. 2000. *Drosophila* as a genetic tool to define vertebrate pathway players. Methods Mol Biol 136:7–14.

Bonini NM, Fortini ME. 2003. Human neurodegenerative disease modeling using *Drosophila*. Annu Rev Neurosci 26:627–656.

Bourtchouladze R, Lidge R, Catapano R, et al. 2003. A mouse model of Rubinstein–Taybi syndrome: defective long-term memory is ameliorated by inhibitors of phosphodiesterase 4. Proc Natl Acad Sci USA 100:10518–10522.

Braunschweig D, Simcox T, Samaco RC, et al. 2004. X-Chromosome inactivation ratios affect wild-type MeCP2 expression within mosaic Rett syndrome and *Mecp2*$^{-/+}$ mouse brain. Hum Mol Genet 13:1275–1286.

Caudy AA, Myers M, Hannon GJ, et al. 2002. Fragile X-related protein and VIG associate with the RNA interference machinery. Genes Dev 16:2491–2496.

Cho W, Heberlein U, Wolf FW. 2004. Habituation of an odorant-induced startle response in *Drosophila*. Genes Brain Behav 3:127–137.

Cline HT. 2001. Dendritic arbor development and synaptogenesis. Curr Opin Neurobiol 11:118–126.

Comery TA, Harris JB, Willems PJ, et al. 1997. Abnormal dendritic spines in fragile X knockout mice: maturation and pruning deficits. Proc Natl Acad Sci USA 94:5401–5404.

Connolly JB, Roberts IJH, Armstrong JD, et al. 1996. Associative learning disrupted by impaired G_s signaling in *Drosophila* mushroom bodies. Science 274:2104–2107.

Connolly JB, Tully T. 1998. Behaviour, learning and memory. In: *Drosophila*: a practical approach, Second edition. Roberts DB, editor. Oxford: Oxford University Press. p 265–317.

Cornish K, Sudhalter V, Turk J. 2004. Attention and language in fragile X. Ment Retard Dev Disabil Res Rev 10:11–16.

Coulom H, Birman S. 2004. Chronic exposure to rotenone models sporadic Parkinson's disease in *Drosophila melanogaster*. J Neurosci 24:10993–10998.

Crnic LS, Hagerman R. 2004. Preface: fragile X syndrome—frontiers of understanding gene–brain–behavior relationships. Ment Retard Dev Disabil Res Rev 10:1–2.

Darnell JC, Warren ST, Darnell RB. 2004. The fragile X mental retardation protein, FMRP, recognizes G-quartets. Ment Retard Dev Disabil Res Rev 10:49–52.

DeZazzo J, Xia S, Christensen J, et al. 1999. Developmental expression of an *amn*(+) transgene rescues the mutant memory defect of *amnesiac* adults. J Neurosci 19:8740–8746.

Dockendorff TC, Su HS, McBride SM, et al. 2002. *Drosophila* lacking *dfmr1* activity show defects in circadian output and fail to maintain courtship interest. Neuron 34:973–984.

Dubnau J, Chiang AS, Grady L, et al. 2003. The staufen/pumilio pathway is involved in *Drosophila* long-term memory. Curr Biol 13:286–296.

Dubnau J, Tully T. 1998. Gene discovery in *Drosophila*: new insights for learning and memory. Annu Rev Neurosci 21:407–444.

Dudai Y, Jan YN, Byers D, et al. 1976. *dunce*, a mutant of *Drosophila* deficient in learning. Proc Natl Acad Sci USA 73:1684–1688.

Durand D. 2003. Vertebrate evolution: doubling and shuffling with a full deck. Trends Genet 19:2–5.

Engel JE, Wu C-F. 1996. Altered habituation of an identified escape circuit in *Drosophila* memory mutants. J Neurosci 16:3486–3499.

Feany MB, Bender WW. 2000. A *Drosophila* model of Parkinson's disease. Nature 404:394–398.

Feany MB, Quinn WG. 1995. A neuropeptide gene defined by the *Drosophila* memory mutant *amnesiac*. Science 268:869–873.

Feng Y, Gutekunst CA, Eberhart DE, et al. 1997. Fragile X mental retardation protein: nucleocytoplasmic shuttling and association with somatodendritic ribosomes. J Neurosci 17: 1539–1547.

Fiala A, Spall T, Diegelmann S, et al. 2002. Genetically expressed cameleon in *Drosophila melanogaster* is used to visualize olfactory information in projection neurons. Curr Biol 12: 1877–1884.

Fortini ME, Skupski MP, Boguski MS, et al. 2000. A survey of human disease gene counterparts in the *Drosophila* genome. J Cell Biol 150: F23–F30.

Galvez R, Gopal AR, Greenough WT. 2003. Somatosensory cortical barrel dendritic abnormalities in a mouse model of the fragile X mental retardation syndrome. Brain Res 971: 83–89.

Gould EL, Loesch DZ, Martin MJ, et al. 2000. Melatonin profiles and sleep characteristics in boys with fragile X syndrome: a preliminary study. Am J Med Genet 95:307–315.

Greenspan RJ. 1995. Flies, genes, learning, and memory. Neuron 15:747–750.

Greenspan RJ. 1997. Fly pushing: the theory and practice of *Drosophila* genetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Guarnieri DJ, Heberlein U. 2003. *Drosophila melanogaster*, a genetic model system for alcohol research. Int Rev Neurobiol 54:199–228.

Guo HF, Tong J, Hannan F, et al. 2000. A neurofibromatosis-1-regulated pathway is required for learning in *Drosophila*. Nature 403:895–898.

Hagerman RJ. 2002. The physical and behavioral phenotype. In: Fragile X syndrome: diagnosis, treatment, and research, 3rd Edition. Hagermann RJ, Hagermann PJ, editors. Baltimore: John Hopkins University Press, p 3–109.

Hall SG, Bieber AJ. 1997. Mutations in the *Drosophila* neuroglian cell adhesion molecule affect motor neuron pathfinding and peripheral nervous system patterning. J Neurobiol 32: 325–340.

Haywood AF, Staveley BE. 2004. Parkin counteracts symptoms in a *Drosophila* model of Parkinson's disease. BMC Neurosci 5:14.

Heisenberg M. 2003. Mushroom body memoir: from maps to models. Nat Rev Neurosci 4:266–275.

Hessl D, Rivera SM, Reiss AL. 2004. The neuroanatomy and neuroendocrinology of fragile X syndrome. Ment Retard Dev Disabil Res Rev 10:17–24.

Hinton VJ, Brown WT, Wisniewski K, et al. 1991. Analysis of neocortex in three males with the fragile X syndrome. Am J Med Genet 41: 289–294.

Hirsh J. 2000. Exposing *Drosophila* to neuroactive drugs. In: *Drosophila* protocols. Sullivan W, Ashburner M, Hawley SR, editors. New York: Cold Spring Harbor Laboratory Press, p 617–623.

Hockly E, Richon VM, Woodman B, et al. 2003. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. Proc Natl Acad Sci USA 100:2041–2046.

Hodapp RM, Dykens EM. 2005. Measuring behavior in genetic disorders of mental retardation. Ment Retard Dev Disabil Res Rev 11: 340–346.

Holland PW, Garcia-Fernandez J, Williams NA, et al. 1994. Gene duplications and the origins of vertebrate development. Dev Suppl:125–133.

Hortsch M, Bieber AJ. 1991. Sticky molecules in not-so-sticky cells. Trends Biochem Sci 16: 283–287.

Hortsch M, Homer D, Malhotra JD, et al. 1998. Structural requirements for outside-in and inside-out signaling by *Drosophila* neuroglian, a member of the L1 family of cell adhesion molecules. J Cell Biol 142:251–261.

Huber KM, Gallagher SM, Warren ST, et al. 2002. Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci USA 99:7746–7750.

Inlow JK, Restifo LL. 2004. Molecular and comparative genetics of mental retardation. Genetics 166:835–881.

Inoue S, Shimoda M, Nishinokubi I, et al. 2002. A role for the *Drosophila* fragile X-related gene in circadian output. Curr Biol 12:1331–1335.

Irwin SA, Idupulapati M, Gilbert ME, et al. 2002. Dendritic spine and dendritic field characteristics of layer V pyramidal neurons in the visual cortex of fragile-X knockout mice. Am J Med Genet 111:140–146.

Irwin SA, Patel B, Idupulapati M, et al. 2001. Abnormal dendritic spine characteristics in the temporal and visual cortices of patients with fragile-X syndrome: a quantitative examination. Am J Med Genet 98:161–167.

Ishizuka A, Siomi MC, Siomi H. 2002. A *Drosophila* fragile X protein interacts with components of RNAi and ribosomal proteins. Genes Dev 16:2497–2508.

Jackson GR, Salecker I, Dong X, et al. 1998. Polyglutamine-expanded human *huntingtin* transgenes induce degeneration of *Drosophila* photoreceptor neurons. Neuron 21:633–642.

Jin P, Zarnescu DC, Ceman S, et al. 2004. Biochemical and genetic interaction between the fragile X mental retardation protein and the microRNA pathway. Nat Neurosci 7:113–117.

Kaytor MD, Orr HT. 2001. RNA targets of the fragile X protein. Cell 107:555–557.
Kazantsev A, Walker HA, Slepko N, et al. 2002. A bivalent Huntingtin binding peptide suppresses polyglutamine aggregation and pathogenesis in *Drosophila*. Nat Genet 30:367–376.
Kraft R, Levine RB, Restifo LL. 1998. The steroid hormone 20-hydroxyecdysone enhances neurite growth of *Drosophila* mushroom body neurons isolated during metamorphosis. J Neurosci 18:8886–8899.
Lam G, Thummel CS. 2000. Inducible expression of double-stranded RNA directs specific genetic interference in *Drosophila*. Curr Biol 10: 957–963.
Lee A, Li W, Xu K, et al. 2003. Control of dendritic development by the *Drosophila fragile X-related* gene involves the small GTPase Rac1. Development 130:5543–5552.
Lee T, Lee A, Luo L. 1999. Development of the *Drosophila* mushroom bodies: sequential generation of three distinct types of neurons from a neuroblast. Development 126:4065–4076.
Lee T, Luo L. 1999. Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22:451–461.
Ling SC, Fahrner PS, Greenough WT, et al. 2004. Transport of *Drosophila* fragile X mental retardation protein-containing ribonucleoprotein granules by kinesin-1 and cytoplasmic dynein. Proc Natl Acad Sci USA 101:17428–17433.
Liu L, Wolf R, Ernst R, et al. 1999. Context generalization in *Drosophila* visual learning requires the mushroom bodies. Nature 400: 753–756.
Mayford M, Kandel ER. 1999. Genetic approaches to memory storage. Trends Genet 15:463–470.
McBride SM, Guilianai G, Choi C, et al. 1999. Mushroom body ablation impairs short-term memory and long-term memory of courtship conditioning in *Drosophila melanogaster*. Neuron 24:967–977.
McBride SMJ, Choi CH, Wang Y, et al. 2005. Pharmacological rescue of synaptic plasticity, courtship behavior and mushroom body defects in a *Drosophila* model of Fragile X syndrome. Neuron 45:753–764.
McClung C, 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, Le PT, Davis RL. 2001. The role of *Drosophila* mushroom body signaling in olfactory memory. Science 293:1330–1333.
McGuire SE, Roman G, Davis RL. 2004. Gene expression systems in *Drosophila*: a synthesis of time and space. Trends Genet 20:384–391.
Mehren JE, Ejima A, Griffith LC. 2004. Unconventional sex: fresh approaches to courtship learning. Curr Opin Neurobiol 14:745–750.
Michel CI, Kraft R, Restifo LL. 2004. Defective neuronal development in the mushroom bodies of *Drosophila fragile X mental retardation* 1 mutants. J Neurosci 24:5798–5809.
Moore MS, DeZazzo J, Luk AY, et al. 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, et al. 2002. *Drosophila* fragile X protein, DFXR, regulates neuronal morphology and function in the brain. Neuron 34:961–972.
Moreau-Fauvarque C, Taillebourg E, Boissoneau E, et al. 1998. The receptor tyrosine kinase gene *linotte* is required for neuronal pathway selection in the *Drosophila* mushroom bodies. Mech Dev 78:47–61.
Morley KI, Montgomery GW. 2001. The genetics of cognitive processes: candidate genes in humans and animals. Behav Genet 31:511–531.
Ng M, Roorda RD, Lima SQ, et al. 2002. Transmission of olfactory information between three populations of neurons in the antennal lobe of the fly. Neuron 36:463–474.
Nimchinsky EA, Oberlander AM, Svoboda K. 2001. Abnormal development of dendritic spines in *FMR1* knock-out mice. J Neurosci 21:5139–5146.
Pan L, Zhang YQ, Woodruff E, et al. 2004. The *Drosophila* fragile X gene negatively regulates neuronal elaboration and synaptic differentiation. Curr Biol 14:1863–1870.
Petrucelli L, O'Farrell C, Lockhart PJ, et al. 2002. Parkin protects against the toxicity associated with mutant alpha-synuclein: proteasome dysfunction selectively affects catecholaminergic neurons. Neuron 36:1007–1019.
Putz G, Bertolucci F, Raabe T, et al. 2004. The *S6KII* (rsk) gene of *Drosophila melanogaster* differentially affects an operant and a classical learning task. J Neurosci 24:9745–9751.
Quinn WG, Sziber PP, Booker R. 1979. The *Drosophila* memory mutant *amnesiac*. Nature 277:212–214.
Ramakers GJ. 2002. Rho proteins, mental retardation and the cellular basis of cognition. Trends Neurosci 25:191–199.
Ravikumar B, Vacher C, Berger Z, et al. 2004. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet 36:585–595.
Reiter LT, Potocki L, Chien S, et al. 2001. A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. Genome Res 11:1114–1125.
Roman G, Davis RL. 2001. Molecular biology and anatomy of *Drosophila* olfactory associative learning. Bioessays 23:571–581.
Ropers HH, Hamel BCJ. 2005. X-linked mental retardation. Nat Rev Genet 6:46–57.
Rudelli RD, Brown WT, Wisniewski K, et al. 1985. Adult fragile X syndrome. Clinico-neuropathologic findings. Acta Neuropathol (Berl) 67:289–295.
Scatton B, Sanger DJ. 2000. Pharmacological and molecular targets in the search for novel antipsychotics. Behav Pharmacol 11:243–256.
Schenck A, Bardoni B, Langmann C, et al. 2003. CYFIP/Sra-1 controls neuronal connectivity in *Drosophila* and links the Rac1 GTPase pathway to the fragile X protein. Neuron 38:887–898.
Siomi H, Ishizuka A, Siomi MC. 2004. RNA interference: a new mechanism by which FMRP acts in the normal brain?. What can *Drosophila* teach us? Ment Retard Dev Disabil Res Rev 10:68–74.
Slegtenhorst-Eegdeman KE, de Rooij DG, Verhoef-Post M, et al. 1998. Macroorchidism in *FMR1* knockout mice is caused by increased Sertoli cell proliferation during testicular development. Endocrinology 139:156–162.
Stanwood GD, Levitt P. 2004. Drug exposure early in life: functional repercussions of changing neuropharmacology during sensitive periods of brain development. Curr Opin Pharmacol 4:65–71.
Steffan JS, Agrawal N, Pallos J, et al. 2004. SUMO modification of Huntingtin and Huntington's disease pathology. Science 304:100–104.
Steffan JS, Bodai L, Pallos J, et al. 2001. Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in *Drosophila*. Nature 413:739–743.
Sullivan W, Ashburner M, Hawley SR, editors. 2000. *Drosophila* protocols. New York: Cold Spring Harbor Laboratory Press.
Tang WH, Francis GS. 2003. Novel pharmacological treatments for heart failure. Expert Opin Investig Drugs 12:1791–1801.
Todd PK, Mack KJ, Malter JS. 2003. The fragile X mental retardation protein is required for type-I metabotropic glutamate receptor-dependent translation of PSD-95. Proc Natl Acad Sci USA 100:14374–14378.
Tompkins L, Siegel RW, Gailey DA, et al. 1983. Conditioned courtship in *Drosophila* and its mediation by association of chemical cues. Behav Genet 13:565–578.
Vargha-Khadem F, Carr LJ, Isaacs E, et al. 1997. Onset of speech after left hemispherectomy in a nine-year-old boy. Brain 120:159–182.
Venturin M, Guarnieri P, Natacci F, et al. 2004. Mental retardation and cardiovascular malformations in *NF1* microdeleted patients point to candidate genes in 17q11.2. J Med Genet 41:35–41.
Waddell S, Quinn WG. 2001. What can we teach *Drosophila*?. What can they teach us? Trends Genet 17:719–726.
Wan L, Dockendorff TC, Jongens TA, et al. 2000. Characterization of dFMR1, a *Drosophila melanogaster* homolog of the fragile X mental retardation protein. Mol Cell Biol 20:8536–8547.
Weiler IJ, Irwin SA, Klintsova AY, et al. 1997. Fragile X mental retardation protein is translated near synapses in response to neurotransmitter activation. Proc Natl Acad Sci USA 94:5395–5400.
Weiler IJ, Spangler CC, Klintsova AY, et al. 2004. Fragile X mental retardation protein is necessary for neurotransmitter-activated protein translation at synapses. Proc Natl Acad Sci USA 101:17504–17509.
Willemsen R, Oostra BA, Bassell GJ, et al. 2004. The fragile X syndrome: from molecular genetics to neurobiology. Ment Retard Dev Disabil Res Rev 10:60–67.
Weller S, Gartner J. 2001. Genetic and clinical aspects of X-linked hydrocephalus (L1 disease): Mutations in the *L1CAM* gene. Hum Mutat 18:1–12.
Wisniewski KE, Segan SM, Miezejeski CM, et al. 1991. The Fra(X) syndrome: neurological, electrophysiological, and neuropathological abnormalities. Am J Med Genet 38:476–480.
Xu K, Bogert BA, Li W, et al. 2004. The *fragile X-related* gene affects the crawling behavior of *Drosophila* larvae by regulating the mRNA level of the DEG/ENaC protein pickpocket1. Curr Biol 14:1025–1034.
Yu D, Ponomarev A, Davis RL. 2004. Altered representation of the spatial code for odors after olfactory classical conditioning: memory trace formation by synaptic recruitment. Neuron 42:437–449.
Zarnescu DC, Jin P, Betschinger J, et al. 2005. Fragile X protein functions with Lgl and the PAR complex in flies and mice. Dev Cell 8:43–52.
Zars T. 2000. Behavioral functions of the insect mushroom bodies. Curr Opin Neurobiol 10: 790–795.
Zhang YQ, Bailey AM, Matthies HJ, et al. 2001. *Drosophila* fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. Cell 107:591–603.
Zhang YQ, Matthies HJ, Mancuso J, et al. 2004. The *Drosophila* fragile X-related gene regulates axoneme differentiation during spermatogenesis. Dev Biol 270:290–307.