Drosophila as a Model Organism for the Study of Neuropsychiatric Disorders

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Contents

Abstract The fruitfly *Drosophila* offers a model system in which powerful genetic tools can be applied to understanding the neurobiological bases of a range of complex behaviors. The *Drosophila* and human lineages diverged several hundred million years ago, and despite their obvious differences, flies and humans share many fundamental cellular and neurobiological processes. The similarities include fundamental mechanisms of neuronal signaling, a conserved underlying brain architecture and the main classes of neurotransmitter system. *Drosophila* also have a sophisticated behavioral repertoire that includes extensive abilities to adapt to experience and other circumstances, and is therefore susceptible to the same kinds of insults that can cause neuropsychiatric disorders in humans. Given the different physiologies, lifestyles, and cognitive abilities of flies and humans,

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many higher order behavioral features of the human disorders cannot be modeled readily in flies. However, an increasing understanding of the genetics of human neuropsychiatric disorders is suggesting parallels with underlying neurobiological mechanisms in flies, thus providing important insights into the possible mechanisms of these poorly understood disorders.

Keywords Addiction · Aggression · Autism · Courtship · Dopamine · *Drosophila* · Learning · Memory · Neuropeptides · Schizophrenia · Serotonin · Sleep

Abbreviations

ARM	Anesthesia-resistant memory
BBB	Blood–brain barrier
cAMP	Cyclic AMP
DAT	Dopamine transporter
GABA	Gamma-aminobutyric acid
LNs	Lateral neurons
NMJ	Neuromuscular junction
NPF	Neuropeptide F
NPY	Neuropeptide Y
PACAP	Pituitary adenylate cyclase-activating protein
PKA	cAMP-dependent protein kinase
SERT	Serotonin transporter
SSRI	Selective serotonin reuptake inhibitor
VMAT	Vesicular monoamine transporter

1 Introduction

Given the complexity and sophistication of behavioral phenotypes in neuropsychiatric disorders, can an apparently much simpler organism such as *Drosophila* make a useful contribution to our understanding of these disorders? At a number of levels from behavioral to molecular, the answer is certainly yes, if used appropriately. Flies and humans have found different solutions to the evolutionary struggle for survival, but their shared descent from a last common ancestor that already had a complex nervous system has left their brains and behavior with many traits in common. These shared traits include the main neurotransmitter systems, gross subdivisions of the brain along the body axis into forebrain, midbrain and hindbrain and shared behavioral traits including learning and many forms of behavioral and synaptic plasticity, circadian behavior, and some level of social behaviors. Flies offer many strengths for experimental approaches that are impossible in humans

and still difficult in other vertebrates, including their sophisticated genetics, ease of many cell biological manipulations, less gene redundancy and a very powerful toolbox of transgenic methods for neuronal circuit analysis – and for these reasons are continuing to play a pioneering role in genetic approaches to behavior. Set against this is their greater evolutionary distance from humans than vertebrate model organisms, which can manifest as lack of some key genes, lack or change of gene functions, and more divergent behavior. But as in any challenge, the key to success is to choose your weapon and exploit the best strengths of each system. For *Drosophila*, this can mean either studying a relevant defined behavior such as learning, circadian behavior, or responses to addictive substances, or increasingly, using *Drosophila* to study the cellular roles of gene products implicated by genetic mapping or genome-wide association studies in the human conditions.

2 The Architecture of the *Drosophila* Brain and Its Neurotransmitter Systems

2.1 The Fly Brain

Before looking at fly behavior and what can go wrong with it, how is the *Drosophila* brain that mediates this behavior built? The basic building blocks of the brain, neurons and glia, are found in both flies and mammals. Neurons show almost all the functional and molecular features of mammalian neurons: axons with their transport machinery, pumps, and voltage-gated channels that underlie action potential transmission, presynaptic terminals with all the machinery for synaptic vesicle release and recycling, dendrites, postsynapses with localized receptor fields and active zones. Glial cells are found intimately associated with neurons, for example often surrounding defined axon bundles and forming a blood–brain barrier (BBB) (Freeman and Doherty 2006).

With only around 10^5 neurons, the adult *Drosophila* nervous system has about one millionth as many neurons as humans have. Nevertheless, this nervous system still allows a remarkably complex behavioral repertoire, with new surprises about its capabilities continually appearing. It consists of a bilaterally symmetrical brain, which is joined to a ventral nerve cord that innervates the thorax and abdomen. Headless flies that retain only their nerve cord are capable of complex reflexive behavior, including grooming, and righting of the body if it is inverted (e.g., Ashton et al. 2001). Similarly to mammals, a segmentally repeated organization is most obvious in the nerve cord, but ontogeny and localized gene expression also show a division of the developing brain region into a protocerebrum, deutocerebrum, and tritocerebrum, which appear broadly evolutionarily homologous to the forebrain, midbrain, and hindbrain regions of vertebrates (Reichert 2005). At a more detailed level homologies between fly and mammalian brain regions are less obvious, because of the evolution of brain organizations that are adapted to their respective lifestyles.

However, molecular "evo–devo" analysis of a wider phylogenetic range of animals, including some with lifestyles less divergent from the last common bilaterian ancestor, is likely to reveal additional homologies, for example, the recent evidence for homology between the main neurosecretory regions of invertebrate brains and the hypothalamus–pituitary system of vertebrates (Tessmar-Raible 2007).

2.2 Neurotransmitter Systems

The similarities between fly and human nervous systems extend also to the main neurotransmitter systems and channels (Littleton and Ganetzky 2000), which are the targets of many pharmacological interventions relevant to neuropsychiatric conditions.

Acetylcholine is the main excitatory neurotransmitter in the *Drosophila* central nervous system (CNS), in contrast to the more limited role in the mammalian CNS. Like mammals, flies also have choline acetyltransferase, acetylcholinesterase, a vesicular acetylcholine transporter, and nicotinic (ionotropic) and muscarinic (G-protein-coupled) acetylcholine receptors that respond to nicotinic and muscarinic ligands, respectively (e.g., Reaper et al. 1998; Campusano et al. 2007). Glutamate is the excitatory neurotransmitter at the *Drosophila* neuromuscular junction (NMJ), but has a more limited role in the *Drosophila* than in the mammalian CNS. As expected, *Drosophila* have a vesicular glutamate transporter, a glutamate uptake transporter in glial cells (Rival et al. 2004), a range of ionotropic glutamate receptors (Littleton and Ganetzky 2000) that respond to some of the same ligands as mammalian receptors, including *N*-methyl D-aspartate (NMDA) receptors (Cattaert and Birman 2001). Flies also have metabotropic (G-protein-coupled) glutamate receptors that respond to mammalian receptor ligands (e.g., Zhang et al. 1999; Sinakevitch et al. 2010). As in vertebrates, GABA (gamma-aminobutyric acid) is the principal inhibitory neurotransmitter in flies, found widely throughout the brain. Flies have ionotropic GABA-A receptors, G-protein-coupled GABA-B receptors, and a vesicular GABA transporter (Enell et al. 2007). The pharmacology of these is broadly similar but not identical to that of vertebrate receptors. *Drosophila* GABA-A receptors are generally sensitive to inhibition by picrotoxin and potentiation by benzodiazepines (Buckingham et al. 2005; Wilson and Laurent 2005; Tanaka et al. 2009), but are inhibited only weakly by bicuculline (Buckingham et al. 2005). *Drosophila* GABA-B receptors are sensitive to at least one mammalian antagonist CGP54626 (Wilson and Laurent 2005).

Monoamine neurotransmitters have a variety of important roles in both *Drosophila* and vertebrates, mediated by G-protein-coupled receptors specific for each neurotransmitter. The neurotransmitter phenotype of these neurons is determined by the enzymes required to synthesize each transmitter, and by the vesicular monoamine transporter (VMAT) (Greer et al. 2005). The main types are:

Adrenergic: Octopamine and tyramine are the closest equivalents in *Drosophila* to the adrenergic neurotransmitters such as epinephrine and norepinephrine, based on

their pharmacological properties and homology between *Drosophila* octopamine receptors and vertebrate beta-adrenergic receptors (reviewed by Roeder 2005).

Dopaminergic: In humans, the dopaminergic system is the target of many addictive substances. *Drosophila* also has a dopaminergic system, comprising over a hundred neurons spread over some 15 clusters per adult brain hemisphere (Mao and Davis 2009, and references therein). The pharmacology of the *Drosophila* system is sufficiently conserved to see effects of substances including cocaine (see Sect. 3.4 below). While this makes *Drosophila* a good model for some features of addiction and dopaminergic function, the behavioral roles of dopaminergic neurons will be specific to the circuits that they belong to. For example, addictive substances often work partly by increasing the effects of dopamine in the dopaminergic mesolimbic system, which is associated with a general sensation of pleasure. On the other hand, in associative olfactory learning in flies, dopaminergic neurons that innervate the mushroom body signal an unpleasant aversive stimulus (see Sect. 3.2). Since the *Drosophila* brain has several clusters of dopaminergic neurons, it is entirely possible that some of them mediate a pleasurable stimulus, but the functions of most dopaminergic neurons in flies are not sufficiently well understood to answer this question.

Serotonergic: The human serotonergic system plays an important role in many aspects of mood regulation, affecting aggression, anxiety and depression. It is a target of a number of antidepressant therapeutics, including tricyclic antidepressants (e.g., imipramine) and selective serotonin reuptake inhibitors (SSRIs, e.g., Fluoxetine/Prozac). These potentiate the effect of serotonin by inhibiting the plasma membrane serotonin transporter (SERT), although tricyclics also target the adrenergic transporter. *Drosophila* also has a serotonergic system, with around 40 serotonergic neurons per brain hemisphere (Sitaraman et al. 2008), and a SERT that shows broadly similar but not identical pharmacological properties to human SERT (Demchyshyn et al. 1994; Petersen and DeFelice 1999). It has roles in a number of behaviors including diet (Vargas et al. 2010), sleep (Sect. 3.3) and aggression (Sect. 3.5).

Histaminergic: Histamine is the neurotransmitter used by a number of *Drosophila* neurons including photoreceptors, and is also transported by Drosophila VMAT (Romero-Calderón et al. 2006).

One important neurotransmitter system of psychiatric importance, whose effects *Drosophila* cannot model, is the cannabinoids. Initial pharmacological and sequence analysis suggested no members of the CB1 or CB2 cannabinoid receptor families in flies (e.g., McPartland et al. 2001). Although more sensitive searches subsequently identified a distantly related homolog in flies (McPartland et al. 2006), this has turned out to be an adenosine receptor (Dolezelova et al. 2007; Wu et al. 2009) and the original conclusions that flies lack cannabinoid receptors still stand. *Drosophila* has members of at least 15 classes of vertebrate neuropeptide receptors. Those of potential relevance to neuropsychiatric disorders include galanin, oxytocin/vasopressin, tachykinins, neuropeptide Y (NPY), thyrotropin-releasing hormone (TRH), bombesin/GRP, nociceptin, gastrin/cholecystokinin (Hewes and Taghert 2001; Hauser et al. 2006; Nässel and Homberg 2006). Many of these are

found in *Drosophila* interneurons and may therefore directly influence behavior. Known examples include Amnesiac, a neuropeptide similar to mammalian pituitary adenylate cyclase-activating protein (PACAP), involved in both olfactory memory and sleep (DeZazzo et al. 1999; Liu et al. 2008; Sects. 3.2 and 3.3); a pigment-dispersing factor (PDF) expressed in the lateral neurons (LNs) that regulates sleep and circadian rhythms (Sect. 3.3), and NPY, involved in behaviors including alcohol tolerance and aggression (Sects. 3.4 and 3.5).

3 Neuropsychiatric Behavioral Disorders in Flies?

3.1 Fly Behavior

At a most basic level, responses to sensory stimuli include attraction or repulsion to odors, locomotor responses to movement of single objects or the entire visual field, responses to mechanical or vibrational stimuli, and responses to noxious stimuli that appear to involve a perception of pain (Vosshall 2007). These behaviors appear largely reflexive (although are usually plastic to some degree) and have proved highly amenable to the sophisticated circuit analysis that *Drosophila* now offers (Luo et al. 2008; Olsen and Wilson 2008).

However, these responsive behaviors are not the main focus of this chapter. Fly behavior cannot readily be explained as a series of responses to sensory stimuli, any more than can human behavior; and it is the higher level control, modulation and motivation of behavior that is most relevant to neuropsychiatric disorders. We are gaining an increasingly sophisticated picture of the fly's behavioral repertoire, and are thus starting to define the parameters and phenomenology of such higher order behaviors including motivation, social behavior, and some of the features of addictive behavior (Vosshall 2007). Once the phenomenology is defined, a genetic approach allows the molecular and cellular and circuit mechanisms to be defined, as well as the mechanisms of any impairment caused by genetic or pharmacological manipulations. Spatial and temporal targeting of expression enables the study of transgenes such as wild-type learning genes, proteins that can either silence or activate neuronal activity (e.g., Sweeney et al. 1995; Luo et al. 2008; Sjulson and Miesenböck 2008).

3.2 Learning and Memory

A substantial capacity for learning and memory has been demonstrated in many tests. One of the best studied examples is olfactory associative learning, in which flies can learn to associate a specific odor with either punishment or a reward. The resulting memory can be dissected on the criteria of both molecules and circuitry into a number of phases, which range from short-term memory within

seconds or minutes, to long-term memory that lasts for days (Tully et al. 1994; Isabel et al. 2004). It requires the activity of neurons in a structure called the mushroom body, which is often compared to the mammalian hippocampus, although it is not clear whether there is any evolutionary homology between the structures. While our understanding of the mushroom body circuitry is still developing, targeted genetic manipulations suggest that punishment and reward are signaled by innervation of the presynaptic compartment of mushroom body neurons by dopaminergic and octopaminergic neurons, respectively (Schwaerzel et al. 2003; Riemensperger et al. 2005; Schroll et al. 2006). Association of a conditioned olfactory stimulus with punishment or reward would then ensue in the presynaptic compartment of small subsets of mushroom body neurons that receive both the olfactory stimulus and the punishment or reward stimulus. Retrieval would ensue when the same conditioned stimulus recurs, even in the absence of the unconditioned stimulus.

Much remains to be learned about the signaling pathways that mediate the different phases of learning and retrieval, but work in flies gave some of the earliest indications that cAMP signaling plays an important role, since mutations affecting the neuropeptide PACAP, the calcium-sensitive adenyl cyclase Rutabaga, the cAMP phosphodiesterase Dunce, protein kinase A, and the cAMP-responsive transcription factor CREB, all affect learning and memory (reviewed by McGuire et al. 2005). This function of the pathway appears widely conserved phylogenetically, since cAMP signaling is central to at least some forms of learning in organisms as diverse as the mollusc *Aplysia* and mammals (Hawkins 1984; Bourtchuladze et al. 1994; Silva et al. 1998). In humans, the role of the pathway in learning or memory disorders has not been well characterized, but inhibitors of cAMP phosphodiesterase show enhancing effects in many vertebrate memory paradigms and have therefore been suggested as cognitive enhancers (Rose et al. 2005). Furthermore, a human dunce ortholog PDE4B has been implicated as a risk factor in schizophrenia-like illness (see Sect. 4.1 below; O'Tuathaigh et al. 2011; Millar et al. 2005).

3.3 Sleep and Rhythms

Sleep disorders are a feature of many degenerative and neuropsychiatric conditions, including schizophrenia, bipolar disorder, and major depression (see Krishnan and Nestler 2011). The cause-and-effect relationships between sleep disturbance, circadian rhythmicity, and other disease symptoms are complex and remain largely to be dissected. For this reason among many others, there is a compelling need to understand the nature, regulation, and functions of sleep. Since flies and humans have very different brain architectures, it came as a great surprise – but perhaps not to those aware of the sophistication of fly behavior – that flies also have a resting behavioral state that resembles sleep. The name of *Drosophila* ("dew lover") itself reflects the elevated activity of individual flies around dawn and dusk, as if sleeping at night and taking siestas during the day. This circadian inactivity is suggestive

but insufficient to meet the criteria of sleep, but it shows additional characteristics that do: flies are less responsive to sensory stimulation during inactivity; "sleep deprivation" homeostatically evokes more short resting episodes and a "sleep rebound" to make good the deprivation; some of the same molecular expression markers of sleep and wakefulness are found in flies and mammals and *Drosophila* rest and activity can be modulated by stimulants and hypnotics (Hendricks et al. 2000; Shaw et al. 2000). In other parallels to mammals, wakefulness in flies is promoted by pharmacological or genetic stimulation of dopaminergic neurotransmission, and resting by its inhibition (Andretic et al. 2005; Kume et al. 2005). Serotonergic (Yuan et al. 2006) and GABAergic (Agosto et al. 2008; Parisky et al. 2008; Chung et al. 2009) neurotransmission both promote rest.

As with other biological processes, one cannot assume that the mechanisms of sleep are conserved in all respects between flies and mammals. Indeed sequence homology searches (data not shown) fail to detect one important mechanism for sleep control in flies, the orexin ligand–receptor pair (reviewed by Sakurai 2007). The absence of this important neuroendocrine component from flies appears to be due to a recent evolutionary loss from the fly lineage, since orexin receptor sequences are detected in other insects (data not shown). The other parallels between fly and mammalian sleep are possibly more ancient, and strong enough for flies to a compelling model to dissect the molecular and cellular mechanisms of sleep.

Several approaches are starting to yield rapid progress. Mutagenesis screens (see Gondo et al. 2011) have recently started to yield mutants of interest. One such screen has identified mutations in the voltage-gated K^+ channel *Shaker* as causing short sleep, associated with reduced lifespan, but retaining sleep homeostasis. Another screen has identified mutations that reduce recovery sleep after sleep deprivation that affect a glycosylphosphatidylinositol-anchored protein, Sleepless/Quiver (Koh et al. 2008). Interestingly, these mutations also reduce levels of Shaker channel and currents (Koh et al. 2008). Together, the strong effects of these mutations point toward membrane excitability as a key process in sleep. As in other areas of biology, the expectation is that mutant screens will continue to generate enough biological raw material for at least a generation of researchers in the field (Harbison and Sehgal 2008 and see Gondo et al. 2011).

The powerful targeted spatial and temporal expression technologies of *Drosophila* have identified a few specific brain regions or neurons in which molecules or pathways of interest can act. First, EGFR signaling in a group of neurosecretory neurons, the pars intercerebralis, promotes sleep. Intriguingly, this structure is proposed as evolutionarily related to the hypothalamus, central to the control of sleep in mammals (Foltenyi et al. 2007). Second, different subsets of mushroom body neurons (better known for its role in learning and memory) can either promote or antagonize sleep (Joiner et al. 2006). Third, the LNs that express the neuropeptide PDF promote wakefulness and are targets for the GABA inhibition of wakefulness (Parisky et al. 2008; Sheeba et al. 2008; Chung et al. 2009). How these components interact to control all aspects of sleep and wakefulness is still unknown, but we can expect further work to "join the dots" of the circuitry in the coming years, and to develop a circuit model of how sleep can be controlled.

Finally, analysis of cellular phenotypes related to sleep in *Drosophila* has shown a striking reduction in synaptic markers in several regions of the *Drosophila* brain, which is prevented by sleep deprivation (Gilestro et al. 2009). This is consistent with sleep being a period in which unwanted synaptic connections are broken down or weakened. The molecular and genetic tools that allow this process to be monitored and manipulated promise a much better understanding of how central this process is to the nature and purpose of sleep.

In summary, *Drosophila* is emerging as a powerful model for the cellular and circuit mechanisms of sleep, which will certainly provide many testable models for the nature of human sleep, and thus provide a route to eventually unraveling the complex interrelationships between sleep disorders and a wide range of neuropsychiatric conditions.

3.4 Addiction

Addiction to psychoactive substances is a complex range of disorders that have a great human cost, and we are still far from understanding the cellular mechanisms involved. Typically, an addictive substance evokes positive reinforcement or rewarding experience, thought to be, at least in part mediated by elevating the levels or effects of extracellular dopamine in the mesolimbic dopamine system (Kauer and Malenka 2007; Heidbreder 2011 for detailed discussion). In addition, repeated exposures may result in either sensitization or tolerance, depending on the strength of exposure and intervals between exposures. Cessation of repeated exposures can impair normal physiological functions, resulting in withdrawal symptoms.

No substance has yet been demonstrated to evoke a full gamut of addiction in flies, but this could potentially improve as behavioral testing of flies becomes more sophisticated. Flies certainly show some of the elements of addictive behavior, and a number of psychoactive substances, including cocaine, nicotine and ethanol show strong behavioral effects in flies (McClung and Hirsh 1998; Bainton et al. 2000). Cocaine induces a dose-dependent range of responses, varying from increased grooming and decreased locomotion at lower concentrations, through abnormal locomotor patterns, loss of taxis behaviors, hyperkinetic activity and tremors, to total akinesia or death at higher concentrations (McClung and Hirsh 1998; Bainton et al. 2000; reviewed by Heberlein et al. 2009). Furthermore, repeated exposure to cocaine shows sensitization, with the maximum increase in response occurring when the second exposure is several hours after the first (McClung and Hirsh 1998). The behavioral response of flies to cocaine reflects the presence of its main pharmacological targets, the plasma membrane monoamine transporters including those for dopamine (DAT) and serotonin (SERT). As in mammals, dopamine plays a major role in the effects of cocaine in *Drosophila*. Pharmacological inhibition of dopamine synthesis reduces the behavioral effects of cocaine (Bainton et al. 2000) and, in an apparent contradiction, chronic blockade of both dopaminergic and serotonergic neurotransmission increases them (Li et al. 2000). The reason for the contradiction

is not clear, but may reflect the acute or chronic nature of the treatments; nevertheless, both results still implicate dopamine in the main cocaine response in flies.

The existence of such responses allows dissection of their control mechanisms in flies, for example by using powerful mutant screens, together with the powerful tools for targeted expression and circuit dissection. First, a functional circadian clock is required for cocaine sensitization in flies (Andretic et al. 1999), and this appears to reflect a target in dopaminergic neurons that innervate the PDF-expressing LNs, in which the circadian clock mechanism in turn regulates the ability of these neurons to control general locomotor activity. Ablation or impairment of LNs reduces but does not eliminate the overt behavioral effects of cocaine (Tsai et al. 2004). Remarkably, this situation is similar to that in mammals, where there is also strong interaction between cocaine and clock function (reviewed by Manev and Uz 2006); cocaine alters clock gene expression, and mutations in circadian clock gene expression significantly alter mouse responses to cocaine. Whether this similarity reflects a deep underlying conservation of the neuronal circuitry that regulates rhythmic behavior is still unclear, but it is sufficient to justify further study in flies of how circadian mechanisms contribute to the effects of cocaine and of other psychoactive drugs that target the dopaminergic system.

Genetic screens in flies have also implicated the BBB in responses to cocaine. This is surprising, given the known or likely permeability of this barrier to cocaine in both mammals and flies (reviewed by Daneman and Barres 2005). Partial loss-of-function of a G-protein-coupled receptor ("Moody"), which is necessary for integrity of the BBB that is formed by junctions between glial cells, increases both cocaine and nicotine sensitivity (Bainton et al. 2000). A direct effect on drug permeability seems unlikely. The BBB appears largely intact in the mutants and the physiological basis is therefore unclear. Further investigation of this, and continuing screens for new mutants affecting fly responses to cocaine, promise further insights into the mechanisms by which it acts. In particular, this work has only scratched the surface regarding the mechanisms of cocaine sensitization, and there is no doubt room for further progress.

Probably the most widely used psychoactive substance is ethanol – but unlike cocaine, the molecular and neuronal targets for its psychoactive effects are not clear. Flies therefore offer a useful system for dissecting these effects and identifying their molecular mechanisms. Indeed, ethanol evokes a set of responses in flies that shows many similarities to its effects in humans. After initial exposure to ethanol vapor, flies first become hyperactive, but then gradually uncoordinated and sedated (Moore et al. 1998); a further effect is apparently disinhibited courtship of other males (Lee et al. 2008). On repeated exposures, these responses can show either tolerance (Scholz et al. 2000) or sensitization (Scholz et al. 2005; Lee et al. 2008), depending on the response assayed and the kinetics of exposure.

Mutant screens for flies with altered sensitivity to ethanol have revealed some of the complexity of the mechanisms, and suggest a role of a number of forms of synaptic plasticity, in defined neurons. Inhibition of cAMP (cyclic AMP)-dependent protein kinase (PKA) causes increased sensitivity to ethanol

(Moore et al. 1998). The precise cells responsible have not yet been identified, although a relatively small set of cells has been identified in which PKA inhibition by contrast decreases ethanol sensitivity (Rodan et al. 2002), indicating a variety of targets for the different components of the behavioral response to ethanol. cAMP also has an important role in mammalian responses to ethanol; for example, knockout of the AC1 calcium-sensitive adenylyl cyclase increases sensitivity of mice to ethanol-induced sedation (Maas et al. 2005), similar to the effects of loss of the equivalent enzyme in *Drosophila rutabaga* mutants (Moore et al. 1998). A further similarity to mammals is that neurons expressing or responding to the NPY homolog, NPF, also contribute to sensitivity to ethanol, without affecting responses to other sedative agents (Wen et al. 2005; Chen et al. 2008).

What of the mechanisms of tolerance, one of the processes that contributes to addiction? Genetic and pharmacological approaches have identified both a rapid tolerance induced by a single large dose of ethanol, and a longer term tolerance to prolonged exposure to a low concentration, which is dependent on protein synthesis (Scholz et al. 2000; Berger et al. 2004). Genetic screens have identified mutations that have helped to dissect the rapid tolerance phase into a component that is dependent on octopamine signaling (regarded as the fly equivalent of norepinehrine) and another component regulated by the putative transcription factor Hangover, which is also required for tolerance to other stress treatment (Scholz et al. 2005). Both of these mechanisms must regulate proteins that mediate tolerance and a good candidate for such a protein is the BK-type calcium-activated K^+ channel Slowpoke, which contributes positively to tolerance (Cowmeadow et al. 2005, 2006; Wang et al. 2009a, b). In human HEK cells BK channel properties depend on ethanol concentration (Yuan et al. 2008) and also develop tolerance to acute ethanol exposure.

In summary, analysis of *Drosophila* mutants has started both to reveal the complexity of the psychoactive effects of ethanol, and to distil some of the mechanisms out of this complexity. The recent and continuing availability of more mutants and ethanol-responsive genes (Scholz et al. 2005; Morozova et al. 2006, 2007, 2009; Bhandar et al. 2009) should help to define the cellular and circuit mechanisms further. A surprising theme emerging from some of these screens is that mutations affecting a number of cytoskeletal or cellular trafficking proteins also affect either sensitivity or tolerance to ethanol in flies, for example a RhoGAP, Homer, the endoplasmic reticulum protein Arl6IP and the cell adhesion molecule integrin (Rothenfluh et al. 2006; Urizar et al. 2007; Li et al. 2008; Bhandar et al. 2009). Since some of these are known to affect synaptic function and plasticity, this is suggestive also that structural changes in synapses may play a role in the effects of ethanol.

3.5 Social Behaviors

Many neuropsychiatric diseases have profound effects on human social behavior. Compared to humans, or even to the social insects such as hymenopterans, *Drosophila* is less obviously a social animal. Nevertheless, flies do show a variety of social

behaviors from as apparently simple ones like aggregation (Lefranc et al. 2001; Tinette et al. 2004), to complex ones like courtship and aggression. Some unexpected depths in their social behavior have emerged in recent years. The power of *Drosophila* genetics and circuit analysis tools is allowing dissection of the mechanisms of these behaviors, and while we still have much to learn, there are a few cases of striking parallels with human behavior.

Courtship. Flies have sophisticated courtship behaviors based on visual, auditory and pheromonal cues, all involving high level neuronal control (recently reviewed by Dickson 2008). These behaviors ensure that males will court only females of the right species, that only unmated females are receptive to males and that males learn to avoid repeating unproductive courtship encounters, e.g., with mated females. In addition to these obvious behaviors, more subtle social behaviors related to courtship are being uncovered. For example production of courtship pheromones and the behaviors that they control are sensitive to social context (Kent et al. 2008; Krupp et al. 2008); and it has recently been discovered that *Drosophila* females can copy the mate choice preferences of other females (Mery et al. 2009), an ability that would evoke amazement even when found in vertebrate species.

The highly species-specific nature of courtship obscures any mechanistic similarities between fly and human sexual behavior. However, genetic manipulations of the sexual identity of neurons in flies can have profound effects on sexual behavior (e.g., Clyne and Miesenböck 2008; Rideout et al. 2010), illustrating the extent to which such behaviors are under genetic control – notwithstanding the complexity of nature versus nurture arguments. The need for sexually dimorphic behavior must be as evolutionary ancient as sexually dimorphic multicellular animals and the genetic and circuit analysis tools of *Drosophila* are arguably turning it into the animal in which we can best understand the neural control of sexual behavior. Flies therefore offer at least a perspective that will be informative for neuronal control of human sexual behavior, even if direct mechanistic similarities are as yet not obvious.

Learning. While initial analysis of associative learning failed to find any group influences on individual learning, more sophisticated analyses have found a strong influence of social context on some forms of learning and memory in *Drosophila* (Chabaud et al. 2009). A form of long lasting memory, anesthesia-resistant memory (ARM), can be induced by massed training in an aversive learning olfactory choice paradigm. Remarkably, retrieval performance of individuals in this paradigm is enhanced by testing in the presence of other trained individuals; whether the basis for this is purely behavioral or is pheromonal is unknown.

Aggression. *Drosophila* is not normally thought of as an aggressive animal, but the potential of arthropods to show aggressive behavior is obvious in insects such as ants or wasps and in crustacea such as crabs and lobsters. To apply the genetic and circuit tools of *Drosophila* to the study of aggression, attempts have been made to design scenarios in which aggression can reproducibly be generated in *Drosophila*. For example, *Drosophila* males will fight each other in the presence of an immobilized (headless but living) female (Chen et al. 2002), and females will fight in the presence of a limited food source (Nilsen et al. 2004). Aggression can be modified

by experience and hierarchical relationships among flies develop on repeated encounters (Yurkovic et al. 2006).

Targeted expression, circuit analysis, mutant and pharmacological approaches have identified some surprising similarities between the neurobiology of fly and human aggression. Male aggressive behavior is almost completely abolished in the absence of the neurotransmitter octopamine, considered to be the invertebrate equivalent of norepinephrine (Hoyer et al. 2008) and a small set of octopaminergic neurons has been identified that mediates aggression (Zhou et al. 2008). Moreover, serotonin promotes aggression in both flies and mammals, and NPF (or NPY in mammals) inhibits aggression. All these similarities suggest that aggressive behavior is an evolutionarily conserved behavioral repertoire with a conserved neural basis. It still remains to be seen whether the major regulatory neurons are also evolutionarily conserved and homologous in both mammals and flies. However, the similarities that are already established provide a good basis for further work on flies, that can be used to generate models of the molecular, cellular and circuit mechanisms that control aggression in mammals including humans.

4 Human Neuropsychiatric Disorders in *Drosophila*?

It is clear that *Drosophila* has a wide and sophisticated behavioral repertoire that has enough in common with other animals including humans, to be a powerful model system for the molecular and circuit mechanisms of these behaviors, and for impairments in them. However, the most common human neuropsychiatric disorders have very complex effects on a range of higher human behaviors. Can these be modeled in flies?

4.1 Schizophrenia

Schizophrenia is a complex and heterogeneous disorder, the best known symptoms of which include hallucinations and delusions, and which may overlap with other disorders including bipolar disorder. Since it is currently impossible to conceive how to identify these behavioral phenomena in flies, modeling these aspects of the disease in flies is not feasible. However, these higher level behavioral phenomena must to some extent reflect underlying neurobiological processes. Consistent with this, schizophrenia has a strong genetic component and some convincing susceptibility loci have started to emerge, thus providing an opportunity to use flies to understand the functions of the affected genes (see also Gondo et al. 2011; O'Tuathaigh et al. 2011). Causative neurobiological processes are still only vaguely defined, although emerging themes include impairment of connectivity between the prefrontal cortex and temporal lobe, and subtle dysfunction of glutamatergic and dopaminergic synapses (see also O'Tuathaigh et al. 2011). The complexity of the

disease mechanisms is reflected in the length of time taken to map even a few loci that are associated with the condition (Stefansson et al. 2008; The International Schizophrenia Consortium 2008; Walsh et al. 2008), and in the recent identification of large number of loci that make small contributions to disease susceptibility (Stefansson et al. 2009; The International Schizophrenia Consortium 2009). However, now that risk genes are being identified, *Drosophila* has considerable potential to illuminate how these genes might underlie the cellular and neurobiological basis of the condition.

DISC1 and PDE4B. Disappointingly, one of the strongest schizophrenia candidate genes, *DISC1* (Chubb et al. 2008), has no obvious ortholog in flies. DISC1 is a large predicted coiled-coil protein that diverges relatively rapidly in evolution, with likely orthologs in lower chordates showing only barely detectable homology to human DISC1 (data not shown). Therefore, either DISC1 is specific to the deuterostome lineage of animals (including humans), or protostomes including *Drosophila* might have a DISC1 ortholog, but one that is too divergent to be identified by sequence homology searches. However, flies do have well studied homologs of some known DISC1 binding partners, including the cAMP phosphodiesterase PDE4B, itself also implicated in psychiatric illness (Millar et al. 2005). The *Drosophila PDE4B* ortholog, *dunce*, is a learning and memory mutant (reviewed by McGuire et al. 2005), and the molecular and circuit mechanisms of how *dunce* and related genes contribute to neuronal and behavioral plasticity are the subject of intense study (see Sect. 3 above on learning and memory). If a *Drosophila* DISC1 ortholog emerges in future, then flies will offer a wealth of neurobiological context in which to study its function.

Dysbindin (*DTNBP1*). *Drosophila* has been highly informative on functions of dysbindin that may be relevant to a causative role in schizophrenia. Dysbindin, encoded by the *DTNBP1* locus, is a binding partner of dystrobrevin and a component of the BLOC-1 complex that has an emerging role in trafficking in the endosomal–lysosomal pathway (reviewed by Ryder and Faundez 2009). It has been implicated in schizophrenia in a number of linkage and association studies and the condition is associated with lowered levels of dysbindin expression (Straub et al. 2002; Ross et al. 2006). Strikingly, loss-of-function mutations in *Drosophila* dysbindin were recovered in a screen for mutations affecting presynaptic homeostatic upregulation of glutamatergic release at the NMJ, in response to acute blockade of postsynaptic receptors (Dickman and Davis 2009). *dysbindin* mutations showed only a modest effect on baseline neurotransmission, but severe impairment of this homeostatic regulation, which is likely to be a key process in regulating the strength of synaptic transmission to an appropriate level. This finding provides a plausible model of how dysbindin mutations might cause neurobiological defects that can lead to the behavioral symptoms of schizophrenia and suggests that defects in homeostatic regulation of glutamatergic transmission rather than in basal transmission mechanisms are a causative factor (among others) in the condition. The fly NMJ is a highly amenable system to study synaptic regulation, including processes known to involve the endosomal–lysosomal system (e.g., Sweeney and Davis 2002; Wucherpfennig et al. 2003; Wang et al. 2007) and offers many possibilities to test

whether other schizophrenia susceptibility genes affect synaptic homeostasis, or other aspects of synaptic regulation.

22q11 deletion. Deletions in the 22q11 region are associated with increased susceptibility to schizophrenia (Karayiorgou et al. 1995). The relative contributions of genes in this region remain to be determined, but strong candidates include *COMT1*, which encodes the dopamine-metabolizing enzyme catechol-*O*-methyltransferase (Gothelf et al. 2005) and proline dehydrogenase, which encodes a mitochondrial enzyme that mediates one potential pathway for glutamate biosynthesis (Gogos et al. 1999). Despite having a functional dopaminergic system, *Drosophila* has no ortholog of COMT1, since sequence homology searches using human COMT1 detect more similar homologs in organisms as distant as bacteria and fungi than in flies (data not shown). The *Drosophila* homolog of proline dehydrogenase is *sluggish-A*; mutations in this gene do not affect basal glutamatergic synaptic transmission, although they cause adult locomotor defects and mild neurodegeneration (Shayan et al. 2000; Fergestad et al. 2008), which are suggestive of some subtle neuronal dysfunction. *Sluggish-A* mutants may therefore deserve testing for synaptic phenotypes similar to those of *dysbindin* mutants.

Neuregulin (*NRG1*). Among the other major susceptibility loci (Ross et al. 2006), neuregulin (encoded by *NRG1*) also has a *Drosophila* homolog, the EGF-like protein known as Vein. This has been implicated in multiple developmental processes (www.flybase.org) but so far has not been linked to synaptic phenotypes, although this deserves to be investigated further in light of the above findings on *Drosophila* dysbindin function.

DAOA encodes a short 153 amino-acid residue protein that is evolving rapidly, with no orthologs yet detected outside primates (Chumakov et al. 2002).

Other regions are associated recurrently as copy number variants with schizophrenia (Stefansson et al. 2008; The International Schizophrenia Consortium 2008; Walsh et al. 2008). One of the smallest of these, 15q11.2, contains only four candidate genes, three of which have *Drosophila* orthologs that are required for normal synaptic development and function. First, mutations in the *Drosophila* ortholog of *CYFIP1* result in aberrant axon pathfinding and synaptogenesis, apparently by affecting its ability to antagonize the action of actin cytoskeletal regulator Rac1 and the Fragile X protein ortholog FMR1 (Schenk et al. 2003). Second, the single *Drosophila* ortholog of *NIPA1* (causative for a dominant form of hereditary spastic paraplegia) and *NIPA2* regulates synaptic organization by inhibiting BMP receptor endocytic trafficking and signaling (Wang et al. 2007); interestingly, schizophrenic symptoms are observed in some subgroups of hereditary spastic paraplegia patients, but this has not yet been noted for patients carrying *NIPA1* mutations (McMonagle et al. 2006).

In conclusion, the usefulness of *Drosophila* as a model to gain mechanistic insights into schizophrenia is likely to grow. Expecting flies to be a simple model for schizophrenia is too naïve. Nevertheless, as more susceptibility genes are identified there is a growing need to understand their neurobiological role and *Drosophila* offers a wealth of possibilities for this. As the neurobiological basis underlying schizophrenia and related conditions emerges, *Drosophila* offers the

opportunity to study the relevant neurobiological phenomena in a model with powerful genetic and cell biological tools.

4.2 Other Developmental Disorders: Bipolar Disorder, Autism

As in the case of schizophrenia, to expect mutant or transgenic flies that can be considered as simple models of these genetically and phenotypically complex conditions is overly naïve. Nevertheless, there is sometimes a wealth of understanding from flies on the neurobiological roles of the emerging collection of risk alleles.

Bipolar disorder shows an overlapping spectrum of phenotypes with schizophrenia, and although it has a strong genetic component, the individual loci are as yet even less well defined than for schizophrenia. However, the two conditions share some susceptibility genes (The International Schizophrenia Consortium 2009; Craddock and Sklar 2009), meaning that flies should yield comparable insights into the mechanisms of bipolar disorder as for schizophrenia.

Autism and autism spectrum disorders also present similar difficulties in diagnosis and mechanistic study to schizophrenia and bipolar disorder, presenting with a range of features including impaired social awareness and communication. Again there is a strong genetic component, but no compelling single mechanism (Abrahams and Geschwind 2008). A small proportion of autism cases can be ascribed to a heterogeneous collection of specific loci, and genome-wide screens are now identifying additional susceptibility regions as single-nucleotide polymorphisms and copy-number variants (Glessner et al. 2009; Wang et al. 2009a; Weiss et al. 2009).

Mechanisms for the human disorders are still elusive, but since many of the susceptibility loci encode synaptic proteins, this points at as yet undefined defects in synaptogenesis or synaptic organization (Abrahams and Geschwind 2008; Glessner et al. 2009; Wang et al. 2009a; Weiss et al. 2009). Some loci encode synaptic cell surface molecules such as cadherins, neurexins and neuroligins, the L-type calcium channel CACNA1C, the neurexin-like protein Caspr2 (encoded by *CNTNAP2*) known for its role in organizing protein localization at nodes of Ranvier, and semaphorin 5A (*SEMA5A*), best known for its role in axonal guidance, the postsynaptic density protein Shank3, and the Fragile X protein FMR1. Ube3A protein was recently shown to regulate postsynaptic receptor trafficking (Greer et al. 2010), and Tsc1 and Tsc2 regulate dendritic spine size and synapse function (Tavaziole et al. 2005). *MECP2*, responsible for a syndromic form of autism, Rett Syndrome, encodes a methylcytosine-binding protein with a predicted role in regulation of chromatin conformation and gene expression. Interestingly, *MECP2* expression in glia influences the morphology and function of adjacent neuronal dendrites (Ballas et al. 2009).

Despite the growing characterization of *Drosophila* social behavior it would be highly speculative to model the behavioral aspects of autism spectrum disorders in flies. However, the main value of flies lies in their usefulness as a tool to understand

the functions of susceptibility genes and studies with fly mutants point to functions for many of these genes in synapse development and differentiation:

- Neurexin-1 is required to establish normal synaptic architecture, including apposition of the presynaptic active zone with the postsynaptic receptor field; loss-of-function mutants show defects in this architecture, and moderate reductions in synaptic strength (Li et al. 2008).
- Neurexin-IV (Caspr2) has a role in neuron–glia interactions, e.g., in glial migration, ensheathment, and subdivision of groups of commisural axons (Stork et al. 2009; Wheeler et al. 2009).
- Much of what we know about the role of FMR1 in regulating the synaptic cytoskeleton and in translational control comes from flies (Zarnescu et al. 2005). Work in *Drosophila* continues to reveal new roles of FMR1, e.g., in regulation of sleep and rhythms (Bushey et al. 2009; Gatto and Broadie 2009). The availability of whole-organism phenotypes for *FMR1* mutants have also recently allowed a screen for small molecules that rescue *FMR1* loss-of-function phenotypes (Chang et al. 2008) – an approach with future potential for other genes implicated in neuropsychiatric disorders.
- Levels of semaphorins specify positional targeting of axons during development (e.g., Zlatic et al. 2009).

4.3 Mood, Stress, and Attention

Mood disorders such as depression and anxiety also have complex etiologies, with both environmental causes such as stress, and genetic contributions (see Krishnan and Nestler 2011). They involve systems including the serotonergic system and a number of neuropeptides (reviewed by Leonardo and Hen 2006). Comparable behavioral conditions are hard to identify in *Drosophila*, although there may be some parallels. For example, NPY helps to counteract the behavioral responses of stress in humans (Eaton et al. 2007); recently it has been shown that the homologous NPF system in *Drosophila* inhibits avoidance responses to external noxious stimuli, at least in part by negatively regulating neuronal excitation that results from activation of TRP channels by such stimuli (Xu et al. 2010). As discussed above (Sect. 2.2) *Drosophila* also has a serotonergic system, although the functions of most of its serotonergic neurons are unknown, and at this point in time it can only be speculated on whether they mediate any higher level mood states. Given the emerging realization of the sophistication of higher order fly behaviors, it would not be surprising if such states existed, although detecting them will require equally sophisticated behavioral experimental approaches. Regardless of progress on this front, association studies will doubtless identify novel genetic influences on human mood disorders (e.g., McMahon et al. 2010) and flies will be invaluable in analyzing their underlying neurobiological function, as they have for other disorders.

5 Conclusions

In spite of their small size and apparent simplicity, *Drosophila* show a range of sophisticated behaviors that have much in common with what are regarded as "simple" human behaviors that can be impaired in neuropsychiatric disorders, including behaviors as fundamental as Pavlovian learning and sleep. Here, the powerful genetic and circuit analysis tools of *Drosophila* allow investigation of the mechanisms of these behaviors and of how they can be impaired. Higher order human behaviors, that are impaired in some of the most devastating neuropsychiatric disorders, cannot be modeled so simplistically in flies, but even here there is potentially much to learn from them. Flies may display some of the underlying simpler component behaviors (such as ethanol tolerance), or may provide mutant phenotypes in homologs of susceptibility genes (such as dysbindin) that can provide important clues on underlying neurobiological mechanisms of the human condition. Future use of *Drosophila* in the field is likely to increase, first from increasing understanding of higher order fly behaviors, and secondly from the use of flies to understand the functions of the growing number of susceptibility genes emerging from genome-wide association studies.

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