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Drosophila strategies to study psychiatric disorders

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ABSTRACT

For decades, *Drosophila melanogaster* has been used as a model organism to study human diseases, ranging from heart disease to cancer to neurological disorders [9]. For studying neurodegenerative diseases, *Drosophila* has been instrumental in understanding disease mechanisms and pathways as well as being an efficient tool in drug discovery studies. For some better-understood disorders, such as Fragile X (a mental retardation syndrome), clinical trials are being run, based in part on translational work in flies and rodents. However, *Drosophila* is currently less used to study psychiatric disorders such as autism, schizophrenia and attention deficit and hyperactivity disorder (ADHD), despite numerous discoveries of disease susceptibility genes that could be explored by reverse genetics or miss-expression studies. This deficit might be explained by (1) a lack of reliable tests to study more complex disease (endo)phenotypes in flies, (2) difficulties in translating disease symptoms into animal models and (3) the polygenetic nature of these diseases. In this review we discuss strategies to use *D. melanogaster* to study complex psychiatric disorders such as schizophrenia, autism and ADHD. Two common features of these diseases may be defective sleep and attention mechanisms, hence calling for better methods for quantifying and screening arousal thresholds in flies.

1. Introduction

Drosophila, lovingly nicknamed 'the Golden Bug' by some *Drosophilists* [15], has been a useful model organism to study several biological processes including genetics, development, molecular and cell biology, and neuroscience. Even though the arthropod evolutionary lineage separated from the vertebrate lineage over 600 million years ago [2,131], their genetic and molecular make up is remarkably similar to vertebrates [49,141]. While flies and humans have very different body plans, they are remarkably similar on the level of biological processes. For example, the central nervous system of flies and humans are derived from a common evolutionary origin [77] and several neurobiological processes are similar in *Drosophila* and *Homo sapiens*, including membrane excitability, neuronal signalling and shared classes of neurotransmitters [118].

The *Drosophila* genome consists of approximately 16–17,000 genes [75] distributed on four pairs of chromosomes, although the 4th chromosome is so tiny it is mostly irrelevant. In comparison, the human genome has ~27,000 genes on 23 pairs of chromosomes. There is considerable overlap, as ~75% of all human disease genes have a recognizable match in the *Drosophila* genome [138]. About one third of the human disease genes, involved in a broad spectrum of diseases, have sufficiently well conserved homologues to be studied in *Drosophila* [138].

The extensive genetic toolbox of *Drosophila melanogaster* is the envy of many biologists, although psychiatrists rarely consider such tools as relevant to their field. In addition to versatile genetics allowing temporal and spatial control of any gene's expression [22], the ability to quickly generate genetic variants with altered gene expression patterns is potentially useful in studying disorders such as schizophrenia and autism, because these diseases are likely to involve complex underlying genetic effects. Lists of candidate genes involved in psychiatric disorders are growing almost weekly as more genes are discovered in genome wide association studies (GWAS) resulting from large groups of patients and matched controls. For example, an online database of schizophrenia candidate genes currently lists over 1000 genes and over 8000 polymorphisms (www.szgene.org see [3]). Screening the function of so many genes in a mammalian model can be impractical, and understanding genetic interactions among these genes is almost impossible. Yet, it is likely that heritable cognitive disorders not only result from multiple gene effects, but also multiple genetic interactions [11,35,96,135]. By making simple crosses of mutant strains and phenotyping their progeny, *Drosophila* researchers are able to unravel genetic mechanisms in ways not yet available to most vertebrate researchers, let alone psychiatrists. In addition to these small-animal benefits, *Drosophila* has a relatively simple genome with less redundancy than the human genome, so

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mutations to single genes are more likely to yield a measurable phenotype. Importantly, the fly brain has only about 100,000 neurons [148], compared to the 100 billion neurons of the human brain. The fruit fly thus seems to fit the bill on all counts for unravelling the biology of psychiatric disorders, in a simpler reductionist model.

However, there is an obvious problem with the *Drosophila* approach to psychiatry, leading to some pointed questions. To what extent can psychiatrists use fly behaviours to study what their human patients are experiencing? What exactly should we measure when screening *Drosophila* mutants for candidate genes based on human GWAS data? Perhaps because the answers to these questions are not obvious, the history of studying psychiatric disorders in *Drosophila* seems to have placed greater emphasis on genes and less on behaviour, often utilizing the same set of two or three readouts to provide the requisite behavioural histogram alongside the molecular data. We challenge this approach, and will discuss alternate behavioural strategies later in this review. First, however, we will cover the traditional approaches to studying these diseases in flies, some more successful than others.

As outlined in Fig. 1, there are three strategies to initiate a fly-based approach to studying cognitive disorders. In the first strategy (forward genetic screens), random mutations are tested for behavioural phenotypes. The second strategy (reverse genetics) uses known disorder genes, derived from patient studies, and examines their roles in an animal model. The third strategy uses animal models to test more general theories about disorders, by for example manipulating environmental variables.

In the forward genetic approach, random mutations are introduced in a population with the same genetic background, after which the resulting mutants are analysed based on their gain- or loss-of-function phenotypes. This is a powerful way to dissect molecular pathways underlying biological process. *Drosophila* genetics was pioneered a century ago by Morgan [111] and mutagenesis approaches were subsequently developed to modify the animal's DNA, by using chemicals such as ethyl methanesulfonate [50,86,87]. Thus, individual genetic lesions could be associated with phenotypes. Around the time when *Drosophila* mutagenesis was increasingly being applied to understanding physical phenotypes, Benzer and colleagues realized that this same approach could be used to link individual genes with behaviours [12] such as circadian activity rhythms [92] and olfactory learning and memory [39,47,137].

Behavioural phenotypes such as circadian locomotion or olfactory memory are *Drosophila*-specific, and the possible relevance of any mutants to human psychiatry can only be revealed by further studies in humans and flies. One example of this approach successfully informing psychiatric research can be found the *dunce* mutant. The *phosphodiesterase II* gene (PDE4B), which is mutant in *dunce* animals, was found by forward-genetic olfactory memory screens [47], but eventually also associated with schizophrenia [20,56,132]. Forward genetic screens have also played a crucial role in identifying genes involved in the regulation of sleep in *Drosophila* [32,33,179], and it is likely that some of these will also point to genes we should be examining in human patients, as disrupted sleep is also a recurring theme in many psychiatric disorders (see Sections 2.1–2.3 below).

Alternatively, by using reverse-genetic strategies, specific genes of interest can be targeted in two ways. When a *Drosophila* homolog of the gene of interest exists, expression levels or patterns can be altered or disrupted. On the other hand, when there is no *Drosophila* homolog, function of a human disease gene can still be studied by expressing a variant of the human gene in the fly model. This latter approach proved fruitful studying neurodegenerative disorders such as Alzheimer's disease, by expressing human tau [101] or Amyloid Beta [80] in *Drosophila* [76]. In both reverse-genetic approaches, flies are tested for neurological phenotypes correlating with the disease, such as age-associated locomotion defects or cellular degeneration visualized by imaging the fly brain [80].

In a third approach, theories of the disease etiology or disease progression can be tested, for example by exposing flies to altered environmental conditions that are proposed to play a role in the disease. The *Drosophila* Fragile X story (below) provides an excellent example, where mGLuR5 activity was linked to the disease phenotype [97], and the severity of the defect was associated with the concentration of glutamate in the diet [28]. Other potential areas of investigation could include the link between drug use and psychosis [161] or links between schizophrenia and advanced paternal age [164].

While there are theoretically three viable approaches for studying neuropsychiatric diseases in *Drosophila*, there are really two lynchpins for success. The first is the extent to which disease genes have a fly homolog. This has recently been reviewed by O'Kane [118] for schizophrenia, autism, and bipolar disorder. The second lynchpin is the ability to test a fly mutant for symptoms of the disorder. As can be seen in Fig. 1, all three strategies converge on a common experimental endpoint, where the effect of the manipulation is evaluated. Without a way to evaluate the effect of genetic manipulations, genetic malleability of a model organism, no matter how advanced, becomes irrelevant. This creates a problem for complex, heterogenic disorders, such as schizophrenia and autism that have symptoms that are hard to measure in animal models. So far, the usefulness of *Drosophila* for studying neurological and psychiatric disorders is limited by the availability of good neurophysiological or behavioural tests. This second lynchpin, the lack of relevant tests, limits the perceived usefulness of fruit flies for studying complex psychiatric disorders. To some extent, *Drosophilists* are to blame for underselling their model in this regard, by reverting too often to three trusted standards – locomotion, circadian rhythms, and olfactory learning – rather than adding new behavioural phenotypes to widen the scope of research in modelling human diseases of the brain.

In the following section, we will discuss *Drosophila* approaches to two very different categories of disease, the relatively simple case of Fragile X, contrasted to the more complex conditions such as schizophrenia, autism, or ADHD. The study of Fragile X syndrome (FXS) is a representative example that illustrates the effective use of *Drosophila* to understand a neurological disorder and develop potential interventions.

2. A *Drosophila* success story: mental retardation and Fragile X syndrome

Fragile X syndrome is the most common form of inherited mental retardation, which affects approximately 1 in 2500 human males [57] and 1 in 8000 females [128]. It is a prototypical single gene disorder that is caused by loss of function mutations in a gene called Fragile X Mental Retardation1 (fmr1), which encodes for an RNA binding protein that represses RNA translation and inhibits protein synthesis [93,120]. It is highly expressed in the human central nervous system [43].

Fragile X syndrome is characterized by mild to severe mental retardation as well as other abnormalities, including autistic behaviour, hyperactivity, attention deficit disorders, and sleep disorders. Patients also have a distinct profile of physical phenotypes, such as enlarged testes and elongated facial structures [94]. Likewise, fmr1 knockout mice show similar physical symptoms as well as a subtle defect in learning and memory [48].

Drosophila entered the stage as a model organism to study FXS when the *Drosophila* fmr1 gene (dfmr1, occasionally referred to as dfxr) was characterized [177]. dFMR1 is strongly homologous

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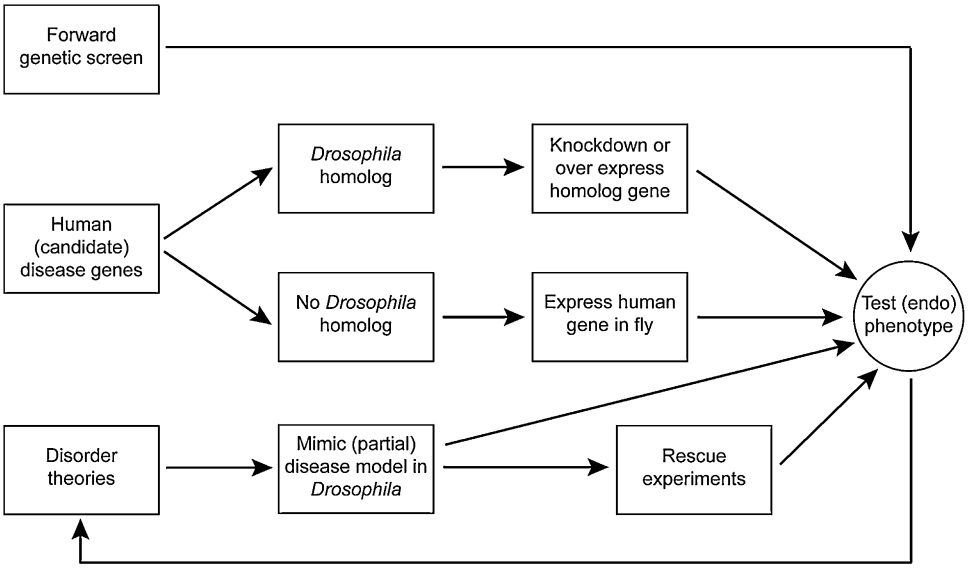

Fig. 1. Strategies for using *Drosophila* in the study of disease. This roadmap has three entry points: genetic screens, testing disease candidate genes in *Drosophila*, and modelling theories about the etiology or ontogeny of the disorder in the fly. These three pathways all converge on a common experimental endpoint, where the effect of the manipulation is evaluated (see text for details).

to mammalian FMR1, containing identical protein domains that mediate in RNA binding [186]. Since the discovery of dfmr1, the *Drosophila* FXS model has played an important role in the elucidation of the morphological and behavioural phenotypes associated with Fragile X syndrome [14].

In the absence of dFMR1 function in *Drosophila* larvae, both neuromuscular junction neurons and dendritic arbours of larval sensory neurons exhibit an increase in branching and neuronal complexity, while over-expression of dFMR1 has the opposite effect [177]. In adult flies, dFMR1 mutants show an increase in projections and more extensive arborisation in parts of the circadian clock circuitry [45,110] as well as a midline-crossing defect in the β-lobe of the mushroom body, a large neuropil in the central brain that plays a crucial role in both short- and long-term memory [98,103,124,185]. Surprisingly for such a different animal, *Drosophila* dFMR1 null mutants also have enlarged testes, similar to human FXS patients and fmr1 knockout mice [187]. These defects suggest a similar suite of fmr1 effects in flies and humans.

Mental retardation in Fragile X patients is relative easy to diagnose. But how can a fly model of mental retardation be diagnosed? Surprisingly, dFMR1 mutants have some similar behavioural impairments as FXS humans or mice. For example, they are unable to maintain a normal circadian rhythm in constant darkness, and exhibit erratic patterns of locomotor activity [45,82,110]. Additionally, dFMR1 mutants are also impaired in higher order behaviours such as immediate recall memory, short term memory and long term memory [7,17,97] and social behaviour [45]. For the special case of Fragile X, the standard *Drosophila* behaviours were therefore quite informative. Whether social behavioural defects in a fly are comparable to social problems in human patients is debatable, although behavioural paradigms exist for studying "cultural" transmission in flies [38,102], so this interesting possibility could be further explored.

Similarities between human and fly FXS effects open the door to study potential routes for pharmacological intervention. Fly studies showed that feeding mGluR antagonists to dFMR1 mutants during development rescued defects in behaviour (e.g., courtship, learning and memory) and brain wiring (e.g., mushroom body crossover). Feeding those mutants mGluR antagonists during adulthood rescued the behavioural phenotype but not the morphology [97]. Additionally, treating FMR1 knockout mice with an mGluR5 antagonist rescued most of the behavioural phenotypes [182]. Likewise, reducing mGluR5 activity in mouse and fly model systems rescued behavioural and morphological phenotypes [14]. Currently, clinical trials are underway, investigating the possibility of using glutamate receptor antagonists, especially mGluR5 antagonists, to treat this condition in humans [13,84].

Thus, the Fragile X story demonstrates the contribution fly models can make to developing interventions, using strategies outlined in Fig. 1. First it was confirmed that knock-down of the *Drosophila* homolog of FMR1 resulted in a disease phenotype very similar to FXS patients and FMR1 knockout mice. Second, theories about mGluR5's involvement in FXS etiology were tested, confirmed and developed into a potential treatment. Furthermore, *Drosophila* research can shed more light on a mechanism beyond the initial disease-related role first assigned to it in other model systems. For example, recent research in *Drosophila* suggests a role for FMR1 in synaptic pruning during sleep [26], a crucial process required for maintaining homeostasis in brain activity. The fly model thus allows for a better exploration of the "normal" function of a molecule such as FMR1, beyond the more severe associations with the disease best studied in vertebrate models.

3. *Drosophila* as a model organism for complex psychiatric disorders

3.1. Schizophrenia

Schizophrenia is highly heritable [159], with some heritability estimates of up to ~80% [123]. However, the genetic mechanisms underlying the disorder are poorly understood. Unlike FXS, it is most likely not a single gene disorder. Instead, schizophrenia probably results from a 'common disease-rare allele' model [116,152,176,180], which proposes that a large diversity of rare genetic variants individually account for the relatively high risk of schizophrenia [117]. The most compelling evidence for this model is the many mutations (single gene mutations and copy number variations) that have been shown to co-segregate with schizophrenia and are thus possibly causal [100,145]. Over 45 distinct loci have already been implicated and it is suggested that this is merely the tip of the iceberg. Whole-genome sequencing and

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subsequent analyses with better genome coverage are likely to reveal many more cases [106,107].

Schizophrenia has a complex disease phenotype where patients display a combination of positive symptoms, such as delusions (fixed, false beliefs) and hallucinations (aberrant perceptions), negative symptoms such as reduced emotions, speech and interest and disorganized/cognitive symptoms such as disrupted syntax and behaviour [159]. Thus, one patient can be delusional, believing aliens are communicating with him through his iPod, while another patient can be almost catatonic, not eating, moving or bathing for days at a time. Both however, can be diagnosed as schizophrenic following extensive psychiatric evaluation. This heterogeneity and anthropocentrism is problematic in understanding the disorder, also because it translates poorly to animal models.

However, all of these symptoms seem to result from deficits in neural processing of sensory information or stimulus salience. With this in mind, research using animal models might focus on interrogating the underlying mechanisms of salience and sensory processing, for example by investigating the role of dopamine in salience attribution, either by measuring arousal levels or by measuring sensorimotor gating processes such as prepulse inhibition [64,157]. To date, there have been no salience or sensorimotor-gating based approaches to tackling schizophrenia in *Drosophila*.

3.1.1. *Drosophila models of schizophrenia*

Currently, there are only two studies using fruit flies to investigate potential schizophrenia genes. Disrupted in Schizophrenia 1 (DISC1) is one of the most prominent schizophrenia candidate genes [105]. Unfortunately, it has no ortholog in flies. Sawamura et al. [144] expressed human DISC1 in *Drosophila* mushroom bodies, which led to increased sleep in male flies, without affecting the circadian rhythm. Although quite a meagre result, it is promising, because there is a strong connection between disrupted sleep and schizophrenia in humans. Many patients suffer from sleep onset and sleep maintenance insomnia and most studies report reduced sleep efficiency for total sleep time as well as increased sleep onset latency [23,109]. Disrupted sleep–wake cycles have also been linked to cognitive dysfunction in schizophrenia patients, where patients with a normal sleep–wake cycle performed better on frontal lobe function tasks than patients with disrupted sleep rhythms [23].

The fact that *Drosophila* DISC1 transgenics have a sleep phenotype highlights the care that must be taken in selecting the correct fly readout in such neuropsychiatric modelling (further discussed below). Sleep in *Drosophila* was only described relatively recently compared to circadian rhythms, about a decade ago [74,147]. The same devices (Trikinetics monitors, Trikinetics, MA, USA) yield activity data for both phenotypes (sleep and circadian rhythms), but only a slight tweaking of the analysis of such data (e.g., sleep quantified as cumulative 5-min bins without activity) uncovers a defect in DISC1 transgenic flies.

In another study, a forward genetic screen for mutations affecting homeostatic modulation of synaptic transmission uncovered loss of function mutations in *dysbindin*, a prominent schizophrenia candidate gene [10,90,122]. Mutants showed severe impairment of synaptic homeostatic regulation, but only a minor effect of baseline synaptic transmission in the neuromuscular junction of 3rd instar *Drosophila* larvae [44]. Despite schizophrenia not being the focus of this study, the uncovering of a schizophrenia candidate gene was most fortunate, emphasizing the usefulness of forward genetic screens to suggest mechanisms and pathways in disease, thereby complementing human association studies.

The two *Drosophila* studies mentioned above together suggest that a link might exist between synaptic homeostasis mechanisms and schizophrenia, with sleep defects being an excellent readout for failure in this regard. However, two genes do not get us very far in understanding the disease. Currently, there are over 1000 candidate genes associated with schizophrenia (www.szgene.org), calling for efficient high throughput behavioural assays to screen the fly homologues of these genes.

3.2. *Autism spectrum disorders*

Autism spectrum disorders (ASD) consist of a group of neurodevelopmental disorders that are characterized by dysfunction in reciprocal social interaction and language, as well as repetitive, stereotyped verbal and non-verbal behaviours (American Psychiatric Association, DSM IV). ASD occur four times more often in human males than in females with an overall prevalence of ~1/100 [115]. Like schizophrenia, autism spectrum disorders have a strong genetic component, but the mechanism of transmission is still largely unknown [1]. However, associations between autism and several rare monogenetic neurodevelopmental disorders (Fragile X syndrome, Angelman syndrome, tuberous sclerosis, Rett syndrome) have been described and are currently being studied in *Drosophila* as well as other animal models. These disorders have complex neurological symptoms, including cognitive disabilities and, are therefore sometimes grouped together with autism.

So far, over thirty ASD susceptibility genes have been proposed, a few of which are shared with schizophrenia [163]. ASD diagnosis leans heavily on impaired language and social interactions, symptoms that are very hard to model in animals, as for schizophrenia.

As in schizophrenia, sleep disorders are common in patients with ASD, including insomnia and lower amounts of REM sleep [139]. Sleep disorders in ASD patients are not directly linked to cognitive disorders, because patients with high functioning autism or Aspergers syndrome have a high rate of sleep disturbance as well [125]. Surprisingly, there are currently few published studies that use *Drosophila* to directly model ASD, although there is considerable speculation about connections to autism in the discussion of relevant molecules, such as synaptic adhesion molecules [8,29]. Additionally, autism spectrum disorders are not as clearly demarcated as schizophrenia, and autistic symptoms have been described in several neurodevelopmental disorders. For one, the gene underlying Fragile X, fmr1, is associated with autism and 30% of the FXS patients are diagnosed with autism [70]. Currently, several *Drosophila* groups are using the Fragile X model to study impaired social interactions [9,18,31,45], a core symptom of ASD. These social interaction models could prove fruitful for future *Drosophila* studies of other autism spectrum disorders.

3.3. *Attention deficit hyperactivity disorder*

Attention deficit hyperactivity disorder (ADHD) is a highly heritable psychiatric condition affecting ~3–5% of children worldwide [113]. Like ASD, diagnosis is based on behavioural symptoms such as impaired attention span, hyperactivity and impulsivity (American Psychiatric Association, DSM IV, 1994). Approximately 25–50% of children and adolescents with ADHD experience sleep problems [34], including insomnia, sleep onset latency and problems with sleep maintenance [150]. About 60% of children with ADHD continue to express symptoms into adulthood. Many candidate genes have been described in recent years, which have led to the discovery of disease pathways involving dopamine and serotonin [16,53,54,127].

3.3.1. *Drosophila models of ADHD*

Although the stage seems set to use *Drosophila* to further investigate this disorder, hardly any work has been published so far, possibly due to a lack of adequate behavioural tests. One way of studying this disease in flies was recently suggested by van Swinderen and Brembs [172] who described ADHD-like behaviour

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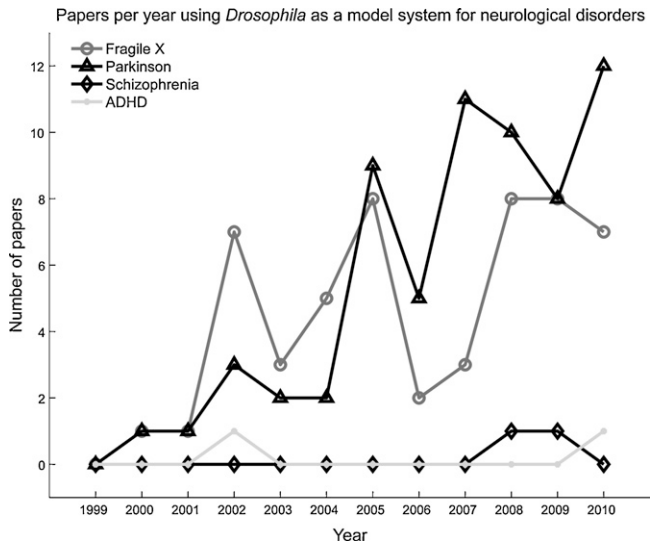

Fig. 2. Papers per year using *Drosophila* as a model organism to study Fragile X syndrome, Parkinson's disease or schizophrenia. Whereas FXS and PD studies show a steady increase in using *Drosophila* as a model organism, the fruit fly is hardly used at all to study more complex psychiatric disorders. There are currently three times as many reviews philosophizing about the use of *Drosophila* as a model organism to study schizophrenia or autism than actual research papers. ADHD papers show a trend similar to schizophrenia.

in *radish*[1], a *Drosophila* memory consolidation mutant that displayed hyperactivity and increased distractibility in a number of behavioural paradigms, compared to wild type flies. Some of these behaviours could be returned to wild-type levels by administering methylphenidate (Ritalin), a drug used to treat ADHD in patients.

An earlier study explored the connection between another classic *Drosophila* mutant and ADHD. Based on the idea that small differences in protein kinase G (PKG) activity leads to either active (*rover*) or more passive (*sitter*) foraging behaviour in *Drosophila* [121], de Luca et al. used this finding to investigate an assumed involvement of human homologue PKG in adult ADHD, because both fly PKG mutants and ADHD patients show increased locomotor activity. Yet, no direct involvement was found [41]. A common behavioural phenotype in the flies and humans (e.g., hyperactivity) does not necessarily suggest a common genetic cause, and any such assumption illustrates a potential pitfall in the use of *Drosophila* to understand neurological disorders. Similarly, because a drug used to treat a disease called ADHD in humans also corrects a phenotype in flies (as in the former study) does not mean that mutant flies are necessarily suffering from attention deficits. Such reasoning is of course flawed, and more work is instead required to prove a connection between fly models and that disease in humans.

4. Problems with studying psychiatric diseases in *Drosophila*

How successful has *Drosophila* research been in tackling psychiatric diseases? A review of the research output on fly models of different cognitive disorders is quite informative towards illustrating successes or failures in using *Drosophila* to study neuropsychiatric diseases (Fig. 2). When looking at the body of literature on *Drosophila* models of neurodegeneration, established models such as Fragile X or Parkinson's disease (PD) show similarly productive publication patterns, although for PD there are currently no clinical trials based on *Drosophila* data. The first papers were published around the year 2000, followed by a steady increase in publications that used genetic mutants of *Drosophila* to model the disease symptoms (and to identify the molecular and physiological basis of the disease). The focus then gradually shifted from investigating disease etiologies and describing neurophysiological and behavioural phenotypes in the fly model to using validated *Drosophila* models to test and evaluate treatment options.

The literature pattern for *Drosophila* models of mental disorders such as autism and schizophrenia looks very different than for PD or FXS. As discussed above, there are currently only two studies linking candidate disease genes with phenotypes in flies. However, the number of review/opinion papers discussing the possibility of using *Drosophila* to study these disorders [25,61,71,81] currently outnumbers the actual research papers [44,144]. Likewise, a recent review about animal models of schizophrenia only discusses rodent models [183]. This state of affairs raises the question: why isn't more progress being made in providing a *Drosophila* solution to studying diseases such as ADHD, autism, and schizophrenia? Candidate genes have been identified for each of those three disorders, which should provide inroads to the development of fly models. We propose that a limiting factor might be the availability of appropriate behavioural assays, especially assays relating to attention-like behaviour. Theories about human disorders can be used to predict altered behaviour in fruit fly mutants mimicking that disorder to a certain degree (see the dFMR1 story above). However, without a solid theory explaining altered behaviour in both fly mutants and human patients, behavioural similarities between the species are not sufficient to assume that a similar mechanism underlies this behaviour. What solid theory might we propose as a lynchpin for future *Drosophila* investigations on these complex disorders?

Research in rodents provides some ideas in this regard. For decades, rodents have been the model system of choice to study brain disorders, and many tests have been developed to study the behavioural consequences of genetic manipulations in mice. If it is already difficult to diagnose a psychiatric patient, how does one recognise a psychiatric animal model? Psychiatric disorders are perhaps too complex to model in their entirety in animals, and positive symptoms such as hallucinations and delusions are simply not measurable in non-humans. Therefore, research into susceptibility genes using rodent models embraced the endophenotype strategy [89,165]. Endophenotypes are specific restricted features of the disease that reflect the action of genes underlying a disorder, even in the absence of diagnosable pathology [21,69]. Also, because the genotype responsible for the endophenotype is presumably less complex than the genotype underlying the whole disease, studying endophenotypes is a viable reductionist approach to unravel complex disease pathways.

The latest behavioural endophenotyping assays for rodent models of different psychiatric disorders have been discussed extensively elsewhere [52,89,149,183]. These behavioural assays share several common themes. Many are tests of higher cognitive processes such learning and memory, decision making and social interactions as well as several low level processes such as locomotor activity, sensorimotor gating and sleep. Even though there is no established set of behavioural assays to study aspects of psychiatric disorders in *Drosophila*, there are many fly paradigms that provide insight into those higher and lower level processes whose defects are associated with mental disorders. These must be chosen carefully (as in the sleep/circadian dichotomy discussed above) to best reflect what we expect is being compromised in a mutant. Any behaviour will not do – it has to be tied to a theory of the disease.

5. Solving the problem with appropriate behavioural assays

A closer examination of behavioural symptoms for psychiatric disorders suggests that several seem to be a consequence of impaired sensory filtering mechanisms. The most obvious examples are delusions and hallucinations, where a patient tries to make

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sense of aberrant salience attributed to random events in the environment [88,166].

A well known phenomenon of sensory filtering that is present in humans and other animals is prepulse inhibition (PPI). PPI is a sensory motor gating mechanism where a relatively weak stimulus, the prepulse, suppresses the motor response to a strong, startle-eliciting stimulus when the prepulse precedes the startle stimulus by a brief duration. It occurs throughout the animal kingdom and has, besides rodents [24,65,156] and humans [157] been described in molluscs [60,108], crickets [55] and non-human primates [85]. PPI is impaired in patients with schizophrenia [21,64,157] and is a widely used endophenotype in rodent models of schizophrenia.

In humans, PPI is thought to be governed by central inhibitory mechanisms that strongly influence the structure and cohesiveness of thought [130,155,158]. So, PPI illustrates how a low level filtering problem, measurable by simple startle phenotypes, could underlie the infinitely more complex and subjective phenotype of incoherent thoughts, a phenotype that is impossible to translate to an animal model. PPI has been found to exist in many animals – from molluscs to humans – therefore quite likely to be present in *Drosophila*, but remaining to be to be demonstrated to date.

PPI is perhaps only one aspect of the broader issue of arousal thresholds. One common theme underlying several cognitive disorders in humans is that they affect arousal levels, or responsiveness to stimuli. As we have seen earlier, measuring sleep seems to be a promising new measure to uncover effects in candidate gene approaches. We suggest that this is because the classic sleep metrics [147], or sleep rebound, or increased arousal thresholds [79,147] tap into a fundamental mechanism related to how an animal responds to (or suppresses responses to) stimuli in the environment.

The simplest approach to measure arousal thresholds may be to quantify responsiveness of flies to increasing levels of a physical stimulus (mechanical, light, or odour), and thereby quantify an arousal threshold in a strain. Startle induced-locomotion, an increase in locomotor activity after a startling stimulus (such as a puff of air), is a promising way to measure arousal thresholds, as an increase in stimulus intensity results in a proportional increase in locomotor activity [95].

We have noted above a recurrent connection between neuropsychiatric diseases and sleep phenotypes. The criterion that best characterizes sleep in animal models is increased arousal thresholds. When an animal is asleep, a stronger stimulus is required to evoke a behavioural response. Yet, when an animal is awake, arousal thresholds are also dynamically regulated to direct an animal's responses to appropriate stimuli, while suppressing responses to others.

Sleep and locomotor activity are behaviours that are very well suited for high throughput screening [74,79,147]. Fortunately, both sleep and locomotion can be quantified in similar ways, either by counting beam crossings in an infrared device or by using video based activity monitoring [66,188]. Any sleep study in *Drosophila* should ideally be accompanied by some arousal threshold testing, and it is likely that these combined metrics (sleep metrics and arousal thresholds) might provide the best and most efficient description of cognitive defects in fly models of human diseases of the brain.

How could a simple readout such as fly sleep tap into the more complex cognitive processes that appear to fail in human neuropsychiatric diseases? Sleep and attention may for example be mechanistically related through suppression mechanisms in the fly brain, hence the apparent success of sleep in uncovering schizophrenia and FXS gene effect in flies. At the heart of this sleep measure, however, are altered arousal thresholds. Other assays might also address this fundamental aspect of fly behaviour (i.e., how responsive is the animal to stimuli), and perhaps bring the investigator closer to aspects of attention-like behaviour relevant to cognitive dysfunction.

Selective attention refers to the ability to focus on one stimulus while suppressing other competing stimuli [134]. This capacity allows for effective learning, and consequently appropriate action selection. Clearly, any disturbance of attention-like processes is likely to produce maladaptive behaviour, such as is seen in most psychiatric patients. Thus, a variety of conditions, from schizophrenia to autism, can all be characterized to some extent as failures of attention processes, or failures on how a patient allocates perceptual resources to their world – external or internal. A behavioural handle on attention in *Drosophila* would go a long way to providing an ideal endophenotype for modelling human psychiatric diseases.

Yet, how to study attention-like processes in flies is not obvious [167]. To date, there have been two convincing approaches to address attention in *Drosophila* [169]. The first approach is rather archaic, but still the best: the *Drosophila* flight arena [73]. In this paradigm, tethered flies "report" their visual choices by modulating their flight dynamics, and clever presentation of competing stimuli can reveal much about attention-like states in fruit flies [142]. In a different approach, recordings taken from the fly brain as it is exposed to competing visual stimuli also reveals evidence for attention-like states in *Drosophila* [172–174]. In this brain recording paradigm, it was shown that flies do not even have to perform (e.g., by flight behaviour) to display selection and suppression of visual stimuli, as well as associated attention dynamics [172]. A combination of both paradigms, tethered flight choices and brain recordings, provides the best evidence to date for visual selective attention in *Drosophila* [160]. These attention phenotypes would be obvious measures to consider in any characterization of candidate genes relevant to human cognitive disorders.

One problem with the above-mentioned measures of selective attention in *Drosophila* is that they are not amenable to the large-scale genetic screening that seems to be called for by the hundreds of candidate genes proposed by human GWAS datasets. Each experiment is on a single fly, which requires considerable preparation time. Worse, most fly mutants do not fly reliably, so it would not always be possible to measure an attention-like behaviour in a particular strain. A solution to this problem would be to screen for some aspect of fly attention using another, more high-throughput behavioural assay, and then confirm attention-like phenotypes in the single animal assays. Fortunately, a number of such paradigms now exist, each measuring responsiveness to stimuli in some way. One assay to quickly screen for defects in visual attention is the optomotor maze paradigm, which measures responsiveness to moving visual stimuli [168]. In this assay, groups of flies walk through a series of choice points while being exposed to visual stimuli. Increased responsiveness in this assay has been associated with *failure* of attention-like processes [171,173]. An automated version of this paradigm makes it especially appropriate to high-volume genetic screens [51].

Other *Drosophila* behavioural assays also measure various features of arousal, and this includes a variety of learning and memory paradigms. There are several assays to study different types of learning, such as olfactory learning [40,133], place learning [58,119]. Likewise, a battery of tests exists to measure social behaviours such as aggression [6,30], social interactions [18], mate choice copying [102] or social learning [143,184].

5.1. Modelling positive, negative and cognitive symptoms of schizophrenia in animals

Consensus in the current literature states that it is impossible to measure positive symptoms such as hallucinations and delusions in animals. The same goes for negative symptoms associated with schizophrenia, such as reduced emotions and interests.

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However, the terminology used to describe schizophrenic symptoms is anthropocentric (psychosis = abnormal condition of the mind, schizophrenia = split mind). Likewise, as outlined by Kapur [88], when a psychotic patient seeks help, patient–doctor interactions mainly take place on a mind or behavioural level of description, where the patient describes symptoms and the clinician tries to diagnose using DSM-IV criteria. However, theories about schizophrenia mainly operate on a neurobiological level, while interventions are pharmacological in nature.

This problem, where schizophrenia is treated as a human specific disease because it operates on the mind/behavioural level, adds unnecessary complications to modelling disorder in animals. One way to circumvent this anthropocentric view is to approach schizophrenia as a salience dysregulation syndrome, hence our proposed solution to focus on arousal thresholds in flies. Many positive psychotic experiences constitute a fundamental alteration in salience attribution. Delusions are a cognitive effort to make sense of these experiences, while hallucinations occur when aberrant salience is attributed to internal representations. In this view, dopamine is proposed to play a crucial role in modulating salience [88,166]. In *Drosophila*, dopamine appears to also play a prominent role in setting arousal thresholds across different behavioural states [5,95,170]. Treating psychosis as a salience dysregulation syndrome allows it to be translated to experimental conditions that can be measured in animal models.

Drosophila is an excellent model organism to study the potential role of dopamine in salience attribution. A good starting strategy would be to manipulate dopamine levels, either in the adult fly or during development, and test flies for altered attentional states, using either a behavioural [142] or an electrophysiological approach [167,168,174], ideally followed by pharmacological interventions that rescue the behavioural phenotype. Thus, manipulating signalling pathways provides an inroad to better understand the extent to which dysregulation of these pathways contribute to a disease phenotype.

6. Discussion

We propose that quantifying low level sensory filtering impairments is the best starting point for modelling complex psychiatric disorders in *Drosophila*. Instead of trying to replicate complex behavioural phenotypes such as impaired cognitive abilities or dysfunctional social interactions, it might be more fruitful to study their underlying processes, such as impaired attention-like processes and altered arousal thresholds. This approach would allow *Drosophila* researchers to circumvent the difficulties of trying to mimic highly anthropocentric symptoms in an animal model, while utilizing the many genetic and neurobiological similarities between man and fly.

Drosophila models have been instrumental towards better understanding the biology of several neurological and neurodegenerative disorders such as Fragile X mental retardation, Alzheimer's disease [36] and Parkinson's disease [178]. However, fruit flies are still debutantes when it comes to studying psychiatric disorders such as schizophrenia, ADHD and autism spectrum disorders. These disorders are more complex, with a complicated underlying genetic architecture and heterogenous symptoms that can be difficult to model in other species. There are lists of candidate genes available for all three disorders, and given that ~75% of all human disease genes have a fly homolog, there should be many susceptibility genes that can be tested in flies. However, the success of testing prospective fly mutants depends crucially on which behavioural paradigms are used.

Sleep represents a promising screening phenotype that, if properly probed, should address changes in arousal threshold during day and night. Disrupted sleep seems to be a recurring theme in many psychiatric disorders, including schizophrenia, ASD and ADHD. Likewise, in bipolar disorder both sleep disruption and circadian rhythm disturbances are very common, both during mania and depression [72].

Sleep is thought to play an important role in memory consolidation [126,153] and several studies have demonstrated links between sleep and neuronal plasticity [19,59,62,104,140]. Sleep is proposed to play a role in synaptic homeostasis, where synaptic connections formed during the day are downscaled during sleep [162]. The speculative relationship between sleep and synaptic plasticity is now evident in the *Drosophila* model [67,146]. In fruit flies, synaptic size or number increased while being awake and decreases only once flies are allowed to sleep. Likewise, a richer wake experience resulted in a greater sleep need [62] as well as an increase in dendritic spines that are subsequently eliminated during sleep – a process that is under control of the gene dFMR1 [26].

Psychiatric disorders such as schizophrenia, autism spectrum disorders, and Alzheimer's disease have been linked to deficiencies in dendritic spine numbers [129]. In young ASD patients, the number of spines increases more than in healthy children, and stays elevated throughout adult life. Additionally, brains of ASD patients are characterized by increased connectivity in local circuits and decreased connectivity between brain regions [112]. In schizophrenia patients, dendritic spine numbers decrease during adolescence as synapses are eliminated. Spine loss and volume decrease has been reported for the dorsolateral prefontral cortex, [68], the auditory cortex [154], and the hippocampus [91,151]. Likewise, in Alzheimer's disease there is a dramatic decrease in spines in the hippocampus and throughout the cortex during late adulthood, suggesting that this reduction may underlie the accompanying cognitive decline [42]. There thus seems to be an interesting correlation between disrupted sleep in psychiatric disorders and abnormal numbers of dendritic spines. However, it remains to be seen whether sleep disorders are caused by altered brain connectivity or whether altered brain connectivity is a result of disrupted sleep patterns. A recent study showing that it is now possible to induce sleep on demand in *Drosophila* [46] suggests that the fly model might shed some light on cause and effect between sleep disorders and cognitive disorders.

6.1. Future directions

Having tentatively settled on what phenotypes are most appropriate for studying neuropsychiatric diseases in flies, let us now return to the underlying complexity of the genetics and how fly-based approaches might prove ideal for resolving genetic contributions to the diseases. Most psychiatric disorders are caused by multitudes of susceptibility genes, and variations in gene expression are as likely to be involved as actual mutations in the protein sequence of genes [27,83,99,136]. Mapping these gene networks is a vast undertaking, requiring the pooled resources of many labs working together. Genome-wide association studies have been instrumental in discovering candidate genes for schizophrenia and autism. Technological advances in GWAS, and decreased sequencing costs combined with screening populations of tens of thousands of patients, are expected to uncover additional associations [63]. However, the GWAS approach has statistical limits for rare variants [4], as well as for uncovering statistical interactions among genes.

Armed with an efficient and relevant behaviour quantifying arousal thresholds in *Drosophila*, how might one proceed to make sense of the multitude of GWAS candidates from the human literature? Two strategies present themselves, brute force or an approach grounded on some theory. A brute force approach would involve working through a list of candidate genes by, for example, knocking

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down their expression one-by-one in an RNAi-type screen [114]. Of course, knocking down one candidate at a time will not always result in a detectable phenotype. However, when a significant phenotype does occur, the knocked down gene is likely to play an important role in the biology of the disorder, and it could function as a node in a network of susceptibility genes, thereby providing an inroad to the underlying disease mechanism. An alternative, perhaps more focused, approach would organize the lists of susceptibility genes into networks, based on data or theories on how these genes might interact, or how they may be organized into different functional groups. Such networks would, by their organization, suggest the most relevant nodes to start mutagenizing in flies, namely the most central ones. There are several possibilities here, each with their own value: each disease might reveal overlapping gene sets, and these might suggest the most promising starting points for fly research [37]. Alternatively, protein–protein interaction networks might be organized based on reported physical interactions among these candidates and information gleaned from available bioinformatics resources [78]. Such a protein–protein interaction network of known disorder candidate genes and their interacting partners could also reveal which genes are more promiscuous, and thus which are more likely nodes of failure in psychiatric disease, to be then functionally tested using *Drosophila* endophenotypes (Charles Claudianos, personal communication).

But then what? Surely, we would not want to have progressed from a long list in humans to a shorter list in flies, without explaining anything. Again, two different strategies would present themselves at this point. The first, most obvious to *Drosophilists*, would be to proceed to understand the biology of a well-chosen gene and its effects on the brain. This is best exemplified by FMR1, the Fragile-X gene, which, alongside causing mental retardation, is now being placed into the broader context of synaptic homeostasis [26]. The alternative strategy is to exploit *Drosophila* genetics to understand how these genes might control an endophenotype in concert, through interactive effects. Such an approach would postpone understanding the biology of any gene until we understand the genetic architecture of the endophenotype involved. For complex neuropsychiatric diseases such as schizophrenia, this additional step may be invaluable. Evolution of most diseases, just like the evolution of any phenotype, is likely to have resulted in large part from altered genetic interactions producing altered gene expression patterns, rather than from single mutations with a direct phenotypic consequence [181]. Classic epistasis experiments in *Drosophila*, involving simple crosses between candidate genes to elucidate pathways, or quantitative genetic designs such as the diallel cross to highlight non-additive interactions [175], would highlight functional gene networks controlling arousal thresholds in flies. These select networks could then be specifically probed in human disease datasets, thereby closing the loop from human to fly and back to human again.

Conflict of interest

The authors declare that there are no conflicts of interest.

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