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Modelling Learning and Memory in *Drosophila* to Understand Intellectual Disabilities

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Abstract—Neurodevelopmental disorders (NDDs) include a large number of conditions such as Fragile X syndrome, autism spectrum disorders and Down syndrome, among others. They are characterized by limitations in adaptive and social behaviors, as well as intellectual disability (ID). Whole-exome and whole-genome sequencing studies have highlighted a large number of NDD/ID risk genes. To dissect the genetic causes and underlying biological pathways, *in vivo* experimental validation of the effects of these mutations is needed. The fruit fly, *Drosophila melanogaster,* is an ideal model to study NDDs, with highly tractable genetics, combined with simple behavioral and circuit assays, permitting rapid medium-throughput screening of NDD/ID risk genes. Here, we review studies where the use of well-established assays to study mechanisms of learning and memory in *Drosophila* has permitted insights into molecular mechanisms underlying IDs. We discuss how technologies in the fly model, combined with a high degree of molecular and physiological conservation between flies and mammals, highlight the *Drosophila* system as an ideal model to study neurodevelopmental disorders, from genetics to behavior.

Key words: intellectual disabilities, neurodevelopmental disorders, *Drosophila melanogaster*, cognition, learning and memory, genetics.

INTRODUCTION

Intellectual disabilities (IDs) are generalized neurodevelopmental disorders (NDDs) characterized by impairments in intellectual functioning, adaptive behavior and motor skills, resulting from abnormal brain development. Adaptive behavior dysfunctions affect social skills (i.e. social problem solving, social communication), conceptual skills (i.e. understand abstract ideas, solve problems, formulate processes), and practical skills (i.e. daily life, self-care) (American Psychiatric Association, 2013). The American Association of Intellectual and Developmental Disabilities (AAIDD) defines an individual with neurodevelopmental ID as presenting with an intellectual quotient (IQ) score under 70; demonstrating significant limitations in adaptive behavior; and emergence of the disability before the age of 18 (AAIDD, 2010). The Diagnostic and Statistical Manual of Mental Disorders (DSM-5, American Psychiatric Association, 2013) ranks severity degrees (i.e. mild, moderate, severe, and profound) based on similar criteria: a) impairments in intellectual functioning, b) deficits in adaptive functioning and c) the onset of these deficits during childhood. Currently, the prevalence of ID in children and adolescents is estimated to vary between 1–2% (Maulik et al., 2011; McKenzie et al., 2016).

IDs can be caused by any condition that impairs brain development before birth, during birth or in the childhood years (National Academies of Sciences and Medicine, 2015). The most common causes of IDs are chromoso-

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Abbreviations: AL, antennal lobe; ARM, anaesthesia-resistant memory; ASDs, autism spectrum disorders; CNVs, copy number variants; DANs, dopaminergic neurons; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th edition; FXS, fragile X syndrome; ID, intellectual disability; IN, interneuron; ITI, inter-trial intervals; KC, Kenyon cell; LTM, long-term memory; MB, mushroom body; MBns, mushroom body neurons; MBON, MB output neuron; MTM, middle-term memory; NDDs, Neurodevelopmental disorders; ORNs, olfactory receptor neurons; PET, positron-emission tomography; PNs, projection neurons; PSD, postsynaptic density; SCZ, schizophrenia; SNPs, single-nucleotide polymorphisms; SPECT, single-photon emission computed tomography; STM, short-term memory; TSC, tuberous sclerosis complex; WES, whole-exome sequencing; WGS, whole-genome sequencing.

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mal abnormalities such as Down syndrome (DS), monogenic causes, such as fragile X syndrome (FXS) and Rett syndrome, and complex genetic conditions such as autism spectrum disorders (ASDs), metabolic dysfunctions such as occur in hyperbilirubinemia, malnutrition, and trauma caused by complications during labor and birth. Importantly, exposure to environmental risk factors and toxins such as alcohol, mercury, cocaine, or infections during pregnancy or perinatal period, may attenuate or increase the overall risk of IDs (Grandjean et al., 1997; Morrow et al., 2006; Chokroborty-Hoque et al., 2014). ASDs seem to have important implications for IDs in a broader sense, given that between 40–61% of ASDs individuals are estimated also to have IDs (Van Naarden Braun et al., 2015).

Despite the substantial heterogeneity of clinical phenotypes and genetic contributions, there is convergence on common cellular pathways, which affect neuronal functionality, brain connectivity and, ultimately, behavior (Bagni and Zukin, 2019). IDs have a strong genetic basis, with the bulk of risk arising from *de novo* mutations and inherited genetic variation (De Rubeis et al., 2014; Iossifov et al., 2014). Unfortunately, there is a marked genetic and allelic heterogeneity (Voineagu, 2012), which makes the identification of the involved genes particularly challenging. A large number of potential ID risk genes has been identified through the analysis of copy number variants (CNVs) or single-nucleotide polymorphisms (SNPs) (Harripaul et al., 2017). Furthermore, whole-exome sequencing (WES) and whole-genome sequencing (WGS) studies have led to the identification of *de novo* and inherited variants playing an important role in the pathogenesis of IDs (Hamdan et al., 2011, 2014; de Ligt et al., 2012; Gilissen et al., 2014; Lelieveld et al., 2016; Liu et al., 2018). WES has been proven valuable by enumerating *de novo* variants in the hundreds of genes involved, for example in ASD risk and neurodevelopmental delay (De Rubeis et al., 2014; Iossifov et al., 2014; Satterstrom et al., 2020).

This wealth of information represents, however, a confounding factor since multiple potentially disease-causing variants are typically identified within a single patient and the true causative mutation has to be distinguished from many benign DNA changes. Data interpretation and validation of risk genes identified from large-scale genetics studies are also needed to distinguish between background variants and pathogenic mutations. Yet, even with the highest accuracy, a computer-based analysis can only predict the deleterious or neutral nature of human genetic variants and ultimately, an experimental validation is required. Functional tests prove the involvement of a gene in a disease, and often involve the creation of disease model systems that can greatly advance our understanding of pathogenesis. A valid disease model requires a functional nervous system and it should be amenable to both behavioral and physiological readouts that allow characterization of intellectual or social cognition. Although rodents have been largely used to address the function of specific genes in IDs, the simultaneous screening of thousands IDs genes in mice presents both economic and time constraints.

The fruit fly *Drosophila melanogaster* is an ideal animal model suited for studying ID candidate genes. Although insects and mammals have followed different evolutionary paths, they share a common bilaterian ancestor characterized by complex physiology such that *Drosophila* and mammals share many molecular, physiological and behavioral features. *Drosophila* is one of the most widely studied invertebrates and is a highly tractable genetic model organism. Cross-species studies have demonstrated that several molecular pathways regulating cellular homeostasis and functionality are remarkably conserved between fly and humans. Importantly, about 75% of the genes involved in human IDs are believed to have a functional ortholog in *Drosophila* (Reiter et al., 2001; Chien et al., 2002; Oortveld et al., 2013; Narayanan and Rothenfluh, 2016). The fly genome exhibits lower gene duplication and genetic redundancy than vertebrates. This allows the study of candidate genes without the fear of compensating effects from redundant genetic copies as occurs in mammals, though may also represent a limiting factor reflecting less complex cell-specific functional diversification of genes.

Neuronal mechanisms are conserved at the molecular level to such a point that, for example, modulators of neuronal activity such as designer receptors exclusively activated by designer drugs, DREADDs, developed in/for mammals often are also effective in flies (Becnel et al., 2013). Strong conservation is observed in the mechanisms of neuronal development and signaling, the conservation of the main classes of neurotransmitter systems, synaptic biology and physiology, as well as cellular similarities in neuronal organization in specific brain structures (Bellen et al., 2010; Ugur et al., 2016).

Furthermore, *Drosophila* is an ideal model for understanding how the brain generates behavioral responses and dissecting the neuronal circuitries behind them: *Drosophila* behaviors are robust, shared by mammals and other vertebrates and can be easily assayed and quantified. *Drosophila* exhibits a wide range of complex and sophisticated behaviors such as circadian rhythms, sleep, learning and memory, the ability to adapt to experience and social behaviors including courtship, feeding and aggression (Konopka and Benzer, 1971; Quinn et al., 1974; Hotta and Benzer, 1976; Tully and Quinn, 1985; Hendricks et al., 2000; Shaw et al., 2000; Chen et al., 2002; Ja et al., 2007; Al-Anzi et al., 2009, 2010; Krashes et al., 2009; Lin et al., 2014b; Louis and de Polavieja, 2017).

The *Drosophila* system has been successfully utilized to dissect molecular mechanisms underlying human disorders including neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, but also neurodevelopmental disorders such ASDs and FXS (Parker et al., 2011; Tessier and Broadie, 2012; Prüssing et al., 2013; McGurk et al., 2015; Coll-Tané et al., 2019; Kanellopoulos et al., 2020).

In contrast to the other animal models, *Drosophila* mutagenesis is relatively easy and well established. The *Drosophila* research community constantly provides state-of-the-art tools to allow efficient gene and genome

engineering (Bellen et al., 2011; Venken et al., 2011b). Different strategies have been developed through the years to model human diseases using the fly model (Ugur et al., 2016). The reverse genetic approach is based on the insertion of a human mutation into the fly homolog gene. Traditionally two techniques have been used to model loss-of-function diseases, namely P-element mutagenesis and gene silencing via RNA interference combined with the binary expression systems (*i.e.* GAL4/UAS, LexA/LexAop, or QF2) (del Valle et al., 2012). These expression systems also allow the possibility of gain-of-function studies in flies since a wild type or mutant version of a human disease-causing mutation can be overexpressed (Feany and Bender, 2000). With these techniques, researchers have finely manipulated most of the genes in the *Drosophila* genome (Dietzl et al., 2007; Bellen et al., 2011; Bischof et al., 2012; Perkins et al., 2015).

The recent development of various CRISPR-based approaches for the fly (MIMIC, CRIMIC, Scarless Editing) (Venken et al., 2011a; Gratz et al., 2014, 2015a; , 2015b; Lamb et al., 2017; Li-Kroeger et al., 2018) allows researchers to model both dosage-dependent mutations (loss and gain of function) more quickly, and also provides a means to assess specific human single point mutations, mutations difficult to model with the more classical genetics approaches.

In addition to these unmatched genetic resources, researchers have put great effort into the characterization of the *Drosophila* brain transcriptome at single-cell resolution in both physiological and aging conditions (Li et al., 2017; Croset et al., 2018; Davie et al., 2018; Allen et al., 2020; Brunet Avalos et al., 2019). These atlases, together with the recently released reconstruction of the *Drosophila* "hemi-brain" connectome at synapse-level resolution (Xu et al., 2020), represent incomparable new tools to study neurobiology and to provide great insight in brain physiology. Combined, these attributes make *Drosophila* an ideal model system for screening IDs gene variants identified through large-scale genetic efforts.

Here we provide evidence that such a relatively small and simple organism as *Drosophila* can be used to identify and characterize ID-associated genes, to unravel the underlying molecular mechanisms of ID-like behaviors, and to identify druggable targets. In this review, we focused on specific behavioral assays developed to assess learning and memory abilities in flies, and discuss how the *Drosophila* system could become an optimal tool to unravel the challenges we face in understanding the underlying causes of, and treatments for, neurodevelopmental disorders.

NEUROANATOMY OF LEARNING AND MEMORY FORMATION IN FLIES

Cognitive impairment, including learning and memory deficits, is a critical hallmark of IDs (American Psychiatric Association, 2013). *Drosophila* offers a great opportunity to test complex cognitive behaviors, particularly learning and memory. Olfactory memory paradigms in *Drosophila* have been widely used for the dissection of memory formation mechanisms and the neuronal circuitries involved in such processes.

Olfactory memory formation is mediated mainly by the olfactory nervous system (Davis, 2004, 2005). Many comprehensive studies have helped to reveal the complete architecture of the olfactory circuits both in larval and adult fly stages (Strausfeld and Li, 1999; Strausfeld, 2002; Davis, 2004, 2005; Tanaka et al., 2008; Aso et al., 2014a; Eichler et al., 2017; Takemura et al., 2017; Dolan et al., 2019).

Olfactory processing starts at the antennae where the olfactory receptor neurons (ORNs) convey sensory information to the glomeruli of the antennal lobe (AL) (Fig. 1). Processed by local interneurons (INs), the odor information is transmitted to the mushroom body neurons (MBNs) by the projection neurons (PNs) (Guven-Ozkan and Davis, 2014). The mushroom body (MB) is considered the major learning and memory center able to process odor memory acquisition, consolidation and retrieval, and to translate sensory information into learned behavioral responses. For this reason, it is argued to be comparable to the mammalian hippocampus and cerebellum (Strausfeld and Hildebrand, 1999; Heisenberg, 2003; Campbell and Turner, 2010; Farris, 2011; Stevens, 2015). The MB is a complex structure composed of around 2500 neurons of three distinct types. Kenyon cells (KCs) organize their axons into three major MB compartments, the α/β, α′/β′ and γ lobes (Fig. 1) (Guven-Ozkan and Davis, 2014). MB output neurons

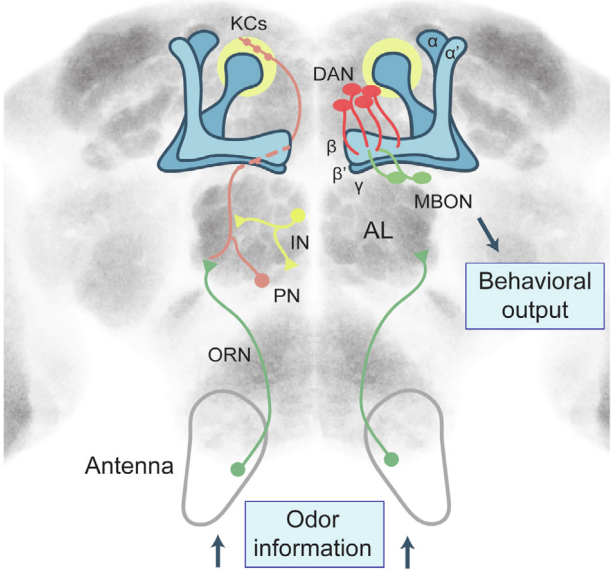

Fig. 1. Neuroanatomy of olfactory conditioning. Relevant brain areas and neuronal clusters involved in olfactory learning and memory processing. The odor information is processed from the olfactory receptor neurons (ORNs) to the antennal lobes (ALs). Local interneurons (INs) in the AL and projection neurons (PNs) convey the information to the mushroom body neurons (MBNs). PNs project their axons to innervate the Kenyon cells (KCs) in the MB. The KCs axons form parallel bundles to constitute the MB lobes, the α/β, α′/β′ and γ lobe. Abbreviations: dopaminergic neuron (DAN) and mushroom body output neuron (MBON).

(MBONs) form synaptic connections with KCs. Dopaminergic neurons (DANs) in the MB lobes provide further regulation of KC-MBON synapses. MBONs extend their dendrites into specific regions within and beyond the MB lobes, projecting to different neuropils to support learned associative responses and to ultimately modify behavior. MBONs can be either cholinergic, glutamatergic or GABAergic. (Tanaka et al., 2008; Aso et al., 2014b; Owald and Waddell, 2015) (Fig. 1). Notably, each type of MBON is modulated by a corresponding type of DAN, thus the KC-MBON-DAN compartment represents an isolated unit for learning and memory (Takemura et al., 2017). Training pairing odors with a reward or a punishment stimulus, forms a positive- or negative-valence memory and appears to be determined by DAN responses, either potentiating or depressing KC-MBON communication. This process is believed to form the neural basis of olfactory associative learning (Heisenberg, 2003; Schwaerzel et al., 2003; Kim et al., 2007; Burke et al., 2012; Liu et al., 2012; Qin et al., 2012; Cohn et al., 2015; Hige et al., 2015; Owald and Waddell, 2015; Rohwedder et al., 2016; Kaun and Rothenfluh, 2017). Thus, dopamine mediates aversive and appetitive learning in the fly (Schwaerzel et al., 2003; Kim et al., 2007; Burke et al., 2012; Liu et al., 2012; Qin et al., 2012).

Apart from olfactory learning, the MB is also implicated in the regulation of habituation (Cho et al., 2004; Acevedo et al., 2007), olfactory discrimination (Parnas et al., 2013; Lin et al., 2014a), courtship memory (McBride et al., 1999; Montague and Baker, 2016), visual and spatial learning (Wolf et al., 1998; Ofstad et al., 2011; Vogt et al., 2014), food-seeking behavior (Tsao et al., 2018), sleep and activity cycles (Pitman et al., 2006; Guo et al., 2011; Haynes et al., 2015; Sitaraman et al., 2015) and decision making (DasGupta et al., 2014; Groschner et al., 2018). Due to its crucial role in regulating adaptive behaviors, the MB circuitry represents a suitable system to investigate ID-linked genes and their effect on neuronal circuitry and cognition.

BEHAVIORAL ASSAYS TO EVALUATE COGNITIVE DYSFUNCTIONS

Classical olfactory conditioning (Pavlovian conditioning)

The first behavioral assay to be developed in *Drosophila* was the QHB paradigm (Quinn et al., 1974), an aversive reinforced operant conditioning paradigm in which flies learn to selectively avoid one of two odors, which is previously paired by electric shock. This test allowed the identification of the first learning and memory *Drosophila* mutants in the field, *dunce*, *rutabaga*, *amnesiac,* and *turnip* (Dudai et al., 1976; Aceves-Pina and Quinn, 1979; Quinn et al., 1979; Livingstone et al., 1984). This paradigm was further elaborated into the negative associative Pavlovian conditioning assay (TQ assay), making a significant advance in the field (Tully and Quinn, 1985). In this assay, the conditioned stimulus (CS+, odor 1) occurs simultaneously with multiple electric shocks that constitute the unconditioned stimulus (US, shock). Then a second odor is presented without electric pulses (CS−, odor 2). After the training period, odor memory is tested by allowing the flies to run into either a tube containing the CS+ or CS− odor and, in each case, the number of flies are counted (Fig. 2A). This type of memory is maintained for at least 24 hours. The training cycle can be repeated with inter-trial intervals (ITI) between the training sessions (spaced training), generating an associative memory that can last 5–7 days (Tully et al., 1994). Because of its clarity and reliability, the TQ assay was widely used in many genetic studies, resulting in the dissection of numerous molecular pathways and associated circuits involved in fly learning and memory. Such studies were able to uncover and identify how olfactory memory forms into four distinct phases: short-term memory (STM), middle-term memory (MTM), anaesthesia-resistant memory (ARM), and long-term memory (LTM). For instance, *dunce* and *rutabaga* are STM genes, *amnesiac* and *DC0* are MTM genes, and *radish* is an ARM gene. LTM genes, such as *Creb2, Notch,* and *crammer* among others, require proper protein-synthesis for LTM formation (Margulies et al., 2005), a requirement also observed in mammalian systems.

Because shock is not a naturally relevant stimulus, a variant of the TQ assay has also been developed. Known as the olfactory appetitive conditioning paradigm, it uses sucrose as a reward stimulus paired with specific odors (Fig. 2A) (Tempel et al., 1983; Schwaerzel et al., 2003).

Reversal learning tasks can also be used in flies to probe cognitive flexibility capacity (Wu et al., 2012). Although flies present many types of learning such as olfactory, visual and place learning (Kahsai and Zars, 2011; Guven-Ozkan and Davis, 2014), olfactory learning has been the most widely used and proven to be the most favorable and robust.

Courtship conditioning

During courtship, male flies display a complex repertoire of behavioral modalities such as orientation, following of the female, tapping, wing extension, courtship song, licking their genitalia and copulation, to achieve reproductive success (Sokolowski, 2001; Pitman et al., 2009) (Fig. 2B). Previously mated females are for short time unreceptive to male courtship and demonstrate rejection behaviors. Following a rejection, male courtship behavior can be altered and suppressed. This behavior modification is known as *courtship conditioning* (Koemans et al., 2017). Courtship conditioning is a learning paradigm where a mated female fly teaches a male to decrease his innate courtship behavior (Siegel and Hall, 1979; Bolduc and Tully, 2009; Pitman et al., 2009). Similar to other forms of associative learning, courtship memory can last from hours to several days, depending on the training protocol (Bolduc and Tully, 2009; Krüttner et al., 2015).

Additional behavioral paradigms to study learning and memory – which are not so widely used yet are visual and motor learning in the flight simulator, olfactory conditioning of the proboscis extension reflex,

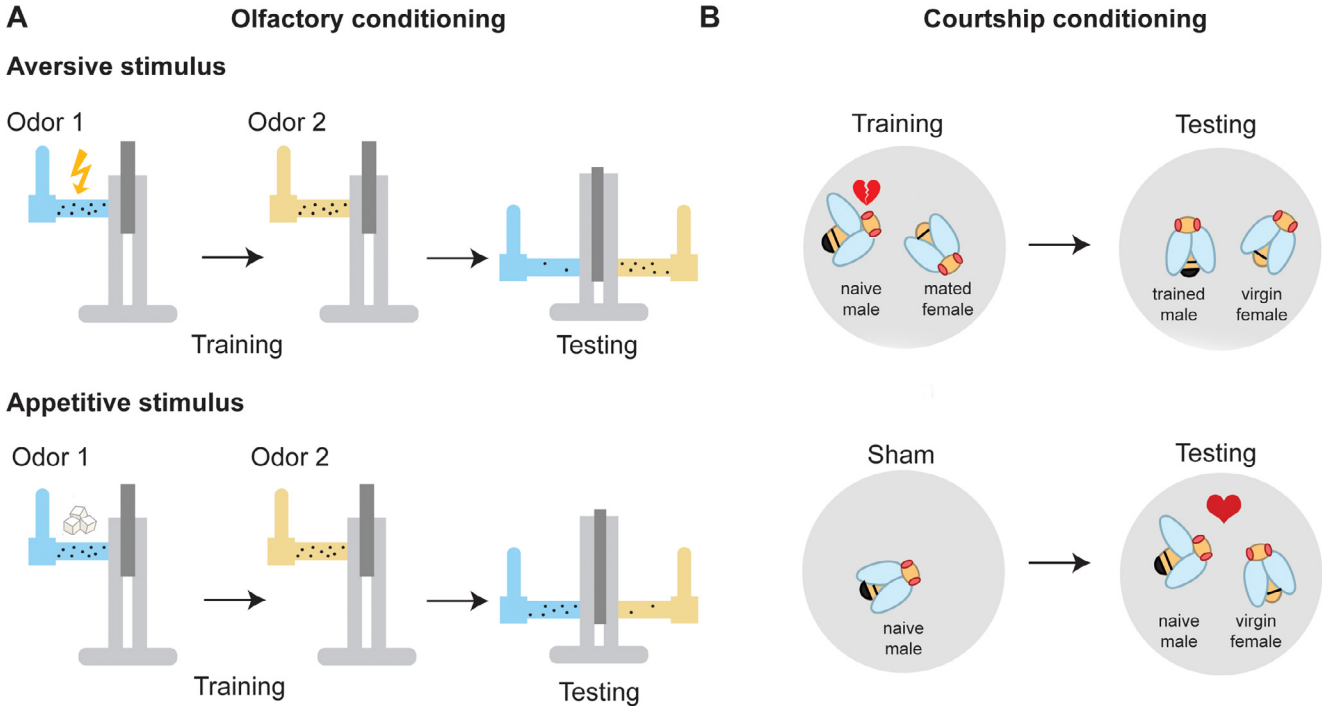

Fig. 2. Classical olfactory and courtship conditioning assays in *Drosophila melanogaster*. **(A)** In olfactory conditioning, a group of flies is trained on the upper part of a T-maze for (1) aversive conditioning using electric shock or (2) appetitive conditioning using sucrose. After training, flies are transferred to lower part of the T-maze for testing. Black spots represent individual flies. **(B)** In courtship conditioning, a naïve male fly is placed with a mated female or alone (sham) during the training period. Trained and sham males are transferred to another chamber with a virgin female and the courtship index is measured between trained and sham flies.

spatial orientation learning, aversive phototactic suppression, and heat box among others (Pitman et al., 2009).

MUTATIONS IN THE FLY ORTHOLOGS OF HUMAN ID GENES EXHIBIT LEARNING OR MEMORY DEFICITS

Next-generation sequencing has led to enormous progress in identifying genetic variants associated with IDs. Many of these genes and their pathways have already been studied using the *Drosophila* model (Table 1). Here, we review work on selected genes and pathways, in which *Drosophila* has helped in the understanding of the molecular mechanisms underlying cognitive dysfunctions in IDs, highlighting deficits in neurotransmitter systems, the MAPK signaling pathway, anchoring and scaffolding proteins and protein homeostasis.

Neurotransmission

The dopaminergic system. The dopaminergic system appears to be affected in ASDs (Paval, 2017) and schizophrenia (SCZ) (Brisch et al., 2014), among other neurodevelopmental disorders. Importantly, CNVs and SNPs in genes encoding various proteins such as dopamine receptors, the dopamine transporter DAT and enzymes involved in dopamine synthesis and catabolism have been found in patients with ASD, SCZ and associated comorbidities (Nguyen et al., 2014). In ASDs, reduced release of DA in the prefrontal cortex and reduced activation of DA neurons in the mesocorticolimbic and nigrostriatal circuits have been reported. These affected brain circuitries are involved in high-order brain function, from reward and motivation-related behaviors to goal-directed behavior and reinforcement during learning and memory (Wise, 2004; Paval, 2017). SCZ, in contrast, is associated with increased presynaptic dopamine release due to increased dopamine synthesis (Howes et al., 2015).

In *Drosophila*, the dopaminergic system is implicated in a wide range of behaviors, including learning and memory (Kim et al., 2007; Berry et al., 2012; Aso and Rubin, 2016). Notably, genes involved in DA homeostasis from synthesis to secretion and catabolism are conserved between flies and humans, allowing the use of the fly model to investigate the effects of DA signaling dysregulation (Yamamoto and Seto, 2014). Great efforts have been put into the morphological and functional characterization of the *Drosophila* DA circuitries of the MBs (described above) involved in both learning and forgetting (Berry et al., 2012, 2018; Aso and Rubin, 2016). Studies in *Drosophila* have shown that memory retention is affected upon abrogation of dopamine transporter DAT (Zhang et al., 2008), aversive olfactory behavior is altered upon abrogation of the tyrosine hydroxylase ortholog *Ple* (Riemensperger et al., 2011), and loss of function of the

Table 1. Human ID-associated genes studied in *Drosophila*

Human gene	Associated disease	Function	Fly gene	Fly model(s)	References
DISC1	Schizophrenia	Scaffold protein	//	Overexpression of the human *DISC1*: *UAS-hDisc1*	(Furukubo-Tokunaga et al., 2016)
DSCR1	Down syndrome	Serine/ threonine phosphatase	*Sarah/nebula* (CG6072)	Hypomorph mutants: Sra^{1}, Sra^{2}; knocking-down of *Drosophila nebula*: UAS-$Sra^{JF02557}$.	(Chang et al., 2003; Shaw et al., 2015)
DTNBP1	Schizophrenia	Synaptic transmission	*Dysbindin* (CG6859)	Hypomorph mutant: $Dysb^{e01028}$; overexpression of *Drosophila* and human *dysbindin*: *UAS-Ddysb, UAS-Hdysb;* knocking-down of endogenous *dysbindin*: UAS-$Dysb^{NIG.6856R}$.	(Shao et al., 2011; Mullin et al., 2015)
DYRK1A	Down syndrome	Serine/ threonine protein kinase	*Minibrain* (CG42273)	Hypomorph mutants: mnb^{1}, mnb^{2}, mnb^{3}, mnb^{4}, knocking-down of the endogenous *mnb: UAS-*mnb^{RNAi}.	(Heisenberg et al., 1985; Kacsoh et al., 2019)
CDH7	Charge syndrome	Chromodomain Helicase DNA Binding protein	*Kismet (CG3696)*	Knocking-down of endogenous *kis: UAS-*kis^{GD4510}*; UAS-*kis^{GD1633}.	(Melicharek et al., 2010)
CYFIP1	Autism Spectrum Disorders / Schizophrenia	Cytoplasmic FMRP interacting protein 1	*Sra-1/Cyfip (CG4931)*	Null allele: *Sra-*$1^{85.1}$.	(Woo et al., 2019)
FLNA	Periventricular Nodular Heterotopia	Cytoskeleton	*Cheerio* (CG3937)	Gain of function: $cher^{joy}$; hypomorphyc allele: $cher^{EPS\Delta 15}$	(Bolduc et al., 2010)
FMR1	Fragile X syndrome	RNA binding protein	*Fmr1* (CG6203)	Null mutant: $Fmr1^{3}$, $Fmr1^{B55}$, amorphic allele: $Fmr1^{\Delta 50M}$, $Fmr1^{5\text{-}HA\text{-}1014}$, $Fmr1^{CB\text{-}0950\text{-}3}$ knocking-down of endogenous *Fmr1*: *UAS-*$Fmr^{HMS00248}$, *UAS-*$Fmr1^{GD1288}$, *UAS-* $Fmr1^{JF02634}$; overexpression of *Drosophila Fmr1*: *UAS-Fmr1*.	(McBride et al., 2005; Bolduc et al., 2008; Kanellopoulos et al., 2012; Doll and Broadie, 2015, 2016; Gross et al., 2015; Dong et al., 2016; Bienkowski et al., 2017)
GABARB3, *GABABR5*	Autism Spectrum Disorders	$GABA_A$ receptor	*Rdl* (CG10537)	Knocking down of the endogenous *Rdl*: UAS-$Rdl^{dsRNA.2\text{-}7.UAS}$; *UAS-*$Rdl^{dsRNA.8\text{-}10.UAS}$	(Liu et al., 2009)
HOMER1, *HOMER2*, *HOMER3*	Autism Spectrum Disorders	Scaffold protein	*Homer* (CG11324)	Amorphic allele: $homer^{R102}$.	(Diagana et al., 2002)
NLGN1, NLGN2, NLGN3, NLGL4, NLGN4Y	Autism Spectrum Disorders	Adhesion protein	*Nlg1* (CG31146), *Nlg2* (CG13772), *Nlg3* (CG34127), *Nlg4* (CG34139)	Null allele: $Nlg4^{MB03367}$.	(Dong et al., 2016)
NRXN1, *NRXN2*, *NRXN3*	Pitt-Hopkins-like syndrome 2, Tourette syndrome	Adhesion protein	*Nrx-1* (CG7050)	Null allele: *Nrx-*$1^{\Delta 83}$*; Nrx-*$1^{HP35068}$; knocking down of the endogenous *Nrx-1: Nrx-*$1^{JF02652}$.	(Zeng et al., 2007; Dong et al., 2016)
NF1	Neurofibromatosis Type 1	GTPase-activating protein	*Nf1* (CG8318)	Null mutants: $NF1^{p1}$, $NF1^{p2}$, $Nf1^{E1}/Nf1^{E2}$; hypomorph mutant $NF1^{c00617}$; heat-shock inducible transgenic fly *hsNF1*; overexpression of *Drosophila* wild type *Nf1*: *UAS-dNF1*; overexpression of human wild type and mutant of NF1: *UAS-hNF1, UAS-*$hNF1^{L847P}$, *UAS-*$hNF1^{R1276P}$, *UAS-*$hNF1^{K1423E}$, *UAS-*$hNF1^{GRD1}$, *UAS-*$hNF1^{GRD2}$, *UAS-*$hNF1^{Cterm}$, *UAS-*	(The et al., 1997; Guo et al., 2000; Tong et al., 2002; Hannan et al., 2006; Ho et al., 2007; Buchanan and Davis, 2010; Gouzi et al., 2011)

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Table 1 (*continued*)

Human gene	Associated disease	Function	Fly gene	Fly model(s)	References
SHANK1, *SHANK2*, *SHANK3*	Autism Spectrum Disorders	Scaffold protein	*Prosap* (CG30483)	*hNF1*^{Nterm}. Amoprhic allele: *Prosap*^{8k}; *Prosap*^{749}.	(Wu et al., 2017)
SLC6A3	Autism Spectrum Disorders	Dopamine transporter	*DAT* (CG8380)	Null allele: *DAT*^{fmn}; overexpression of *Drosophila DAT*: *UAS-dDAT*.	(Zhang et al., 2008)
RHEB	Tuberous Sclerosis	GTPase-activating protein	*Rheb* (CG1081)	Overexpression of *Drosophila* Rheb: *UAS-Rheb*.	(Brown et al., 2012)
RPS6KA3	Autism Spectrum Disorders	Ribosomal protein S6 kinase II	*RSK* (S6KII) (CG17596)	Null allele: *S6kII*$^{gn-P1}$.	(Putz et al., 2004; Neuser et al., 2008)
TSC1	Tuberous Sclerosis	Chaperone/ ATPase inhibitor activity	*Tsc1* (CG6147)	Point mutation: *Tsc1*^{R460X}; knocking down of the endogenous *Tsc1*: *Tsc1*^{JF01262}.	(Dong et al., 2016)
UBE3a	Angelman syndrome	Protein ubiquitination	*Ube3a* (CG6190)	Null allele: *Ube3a*^{EP3214}; point mutation: *Ube3a*$^{C941A.UAS}$; amorphic allele: *Ube3a*^{15B}; overexpression of *Drosophila Ube3a*: *Ube3a*$^{UAS.cWa}$; knocking down of endogenous *Ube3a*: *Ube3a*^{GD450}; *Ube3a*^{JF03406}.	(Chakraborty et al., 2015; Dong et al., 2016)

two dopamine receptors Dop1R1 and Dop2R impairs both learning and memory consolidation (Kim et al., 2007; Qin et al., 2012; Scholz-Kornehl and Schwärzel, 2016).

The serotonergic system. Alterations in the serotonergic system appear to play a significant role in the pathogenesis of ASDs (Kinast et al., 2013; Muller et al., 2016). Serotonin (5-HT) has been widely associated with circuitries regulating anxiety, aggression, mood regulation, impulsivity, sleep, stress and learning, all behaviors affected in IDs (Sodhi and Sanders-Bush, 2004; Lesch and Waider, 2012; Olvera-Cortes et al., 2013). Aberrant serotonin levels in blood and dysfunction of serotonergic circuitries have been reported in ASD (Schain and Freedman, 1961). Functional brain imaging techniques, such as positron-emission tomography (PET) and single-photon emission computed tomography (SPECT) have provided insights into serotonin levels in IDs. Altered receptor and/or serotonin transporter levels were reported throughout the brain in adults with ASDs and the medial frontal cortex of children with ASDs (Makkonen et al., 2008; Nakamura et al., 2010). Further, rare amino acidic variants of the serotonin transporter (*SERT*) have been hypothesized to increase susceptibility to ASDs (Sutcliffe et al., 2005). Interestingly, pharmacological studies in humans revealed that lower serotonin function impaired long term memory and cognitive flexibility, suggesting that the lower serotonin signaling observed in IDs could underlie the cognitive disturbances in such individuals (Schmitt et al., 2006).

The majority of the components of the serotonergic system are conserved in flies, modulating numerous behaviors including sleep, courtship, associative learning and place memory (Yuan et al., 2006; Sitaraman et al., 2008; Becnel et al., 2011; Pooryasin and Fiala, 2015; Liu et al., 2019). The serotonin receptors present in *Drosophila*, such as 5-HT1$_A$ and 5-HT1$_B$, are expressed in the MBs and implicated in learning and memory. Both pharmacological and genetic manipulations of serotonergic receptors were shown to impair olfactory learning and memory processing (Johnson et al., 2011).

The GABAergic system. Neuronal excitatory and inhibitory balance is essential for proper brain function, and alterations in inhibitory signaling have been associated with several neuropsychiatric disorders including FXS, Rett syndrome, ASDs, epilepsy, and SCZ (D'Hulst and Kooy, 2007; Selby et al., 2007; Sgadò et al., 2013; Braat and Kooy, 2015; Gao and Penzes, 2015; Nelson and Valakh, 2015; Chiapponi et al., 2016; de Jonge et al., 2017; Davenport et al., 2019). GABAergic signaling is pivotal in the regulation of synaptic plasticity which in turn is the leading mechanistic model of learning and memory (Heaney and Kinney, 2016).

In *Drosophila*, the GABAergic circuitry is crucial in the learning and memory process and in memory formation. GABAergic neuronal clusters in the AL glomeruli orchestrate the inputs to MBs that regulate learning. Furthermore, GABAergic inputs to the MB, such as the anterior paired lateral neurons and the dorsal paired medial neurons, are implicated in behavioral flexibility, learning and memory consolidation (Lin et al., 2014a;

Liu and Davis, 2009; Pitman et al., 2011; Ren et al., 2012; Wu et al., 2012). In the MB lobes, GABAergic output neurons are coupled to DANs to create a dual feedback circuitry that shapes dopaminergic signaling to promote appetitive long-term memory consolidation. Notably, this mechanism shares a great deal of similarity with the mammalian mesolimbic rewarding system in which inhibitory neuron feedback prevents the overactivity of dopaminergic neurons (Pavlowsky et al., 2018). In addition to GABAergic circuits, GABAergic receptors have also been implicated in learning and memory. The *Drosophila* $GABA_A$ receptor, *Resistance to dieldrin* (*Rdl*), highly expressed in the MB, has been shown to negatively modulate olfactory associative learning and memory acquisition in both overexpression and knockdown studies (Liu et al., 2009).

MAPK/ERK pathway

Neurofibromatosis type 1 (NF1). Neurofibromatosis (OMIM #162200) is a genetic disorder characterized by multiple tumors, café-au-lait spots, macrocephaly and bone dysplasia (Brems et al., 2009). Additionally, most individuals with NF1 exhibit cognitive and behavioral difficulties: learning disabilities occur in up to 40% of the cases (Torres Nupan et al., 2017) and the prevalence of ADHD and ASD is increased in NF1 individuals (Hyman et al., 2005). NF1 encodes for neurofibromin, a GTPase-activating protein that negatively regulates RAS/MAPK pathway activity by accelerating the hydrolysis of Ras-bound GTP (Fig. 3). Loss or reduction of neurofibromin leads to Ras over-activation, which in turn regulates signaling pathways including MAPK, PI3K, PKB and mTOR. Ras over-activation has been suggested as an explanation for multiple tumors observed in NF1 patients (Brems et al., 2009; Banerjee et al., 2011). The *Drosophila* NF1 protein is highly conserved, with a 60% sequence identity at protein level (The et al., 1997) and Table 1. Interestingly *NF1* mutant flies show deficiencies in short and long term memory (Guo et al., 2000; Ho et al., 2007). Multiple studies have indicated that NF1-dependent learning in *Drosophila* involves the cyclic adenosine monophosphate (cAMP) pathway, and genetic interaction between NF1 and the rutabaga-encoded adenylyl cyclase (Rut-AC) has been demonstrated (Guo et al., 2000). The work of Buchanan and Davis, 2010 further characterized the role of NF1/Rut-AC in olfactory memory, and demonstrated that NF1 in α/β MB neurons are required for memory acquisition and Rut-AC is also required for memory stability. In addition, similar to the mammalian system, NF1 in flies modulates RAS activity downstream of the *Alk* Receptor Tyrosine Kinase (RTK). Alk regulates NF1-controlled neuronal Ras/ERK pathways, and downregulation of Alk enhances learning, long term memory and rescues the olfactory learning defects of *NF1* mutants (Gouzi et al., 2011, 2018; Walker and Bernards, 2014).

The MAPK-activated ribosomal S6 kinase (RPS6KA3/RSK2). In humans, loss-of-function mutations in *RPS6KA3/RSK2* are known to cause Coffin-Lowry syndrome (OMIM #303600). Coffin–Lowry syndrome is a syndromic form of X-linked mental disorder, characterized by cognitive impairment, reduction of physical movements and skeletal anomalies in males. Female carriers are more often mildly affected. Cognitive deficits have been observed in both males and female individuals with variable severity (Marques Pereira et al., 2010). RSK2 mutations cause premature translation termination resulting in complete loss of function of the mutant allele or in the modification of its activity by altering, for example, its phosphorylation sites, ATP binding sites, or ERK docking site (Marques Pereira et al., 2010).

RSK2 is a serine-threonine protein kinase that acts at the end of the MAPK-ERK signaling pathway cascade activated by G protein-coupled receptors (GPCRs) (Fig. 3) and is implicated in the activation of substrates involved in cell-cycle, cellular differentiation and homeostasis (Hauge and Frodin, 2006). For example, RSK2 regulates phosphorylation of the cAMP-response element binding protein (CREB), a key nuclear mediator of long-term memory formation (Silva et al., 1998). Gene transcription controlled by CREB represents an important molecular switch, controlling the transition from short-term, protein synthesis-independent memory formation to long-term, protein synthesis-dependent memory storage (DeZazzo and Tully, 1995; Martin et al., 1997; Alberini, 2009).

In *Drosophila*, there is only one *RSK* gene, demonstrating the greatest homology with the human *RSK2* gene (60–63% sequence identity at protein level) (Wassarman et al., 1994 and Table 1.). Interestingly, in *Drosophila*, *RSK* negatively regulates spine morphology in synaptic boutons by inhibition of ERK signaling (Fischer et al., 2009) and its full deletion leads to defects in learning (Putz et al., 2004; Neuser et al., 2008), indicating that its function in the brain is conserved across species.

Scaffold and cell adhesion proteins

Scaffold proteins are modular proteins that assemble multimolecular signaling complexes, playing a major role in the organization of the postsynaptic signal machinery. They regulate receptor positioning and trafficking, axonal pathfinding and neuronal communication (de Bartolomeis and Fiore, 2004). Scaffold proteins in excitatory neurons are localized at the postsynaptic density (PSD), a specialized matrix of the postsynaptic terminals, where scaffold proteins play a critical role in glutamatergic neurotransmission. The role of PSD scaffolds proteins in the CNS have been deeply reviewed (see, for example, (Emes and Grant, 2012; Verpelli et al., 2012; Gao et al., 2013). Not surprisingly mutations in those proteins have often been found in patients with IDs.

SHANK/ProSAP family proteins. SHANK/ProSAP family proteins (SHANK1, SHANK2, SHANK3) have emerged as risk factors for ASDs (Pinto et al., 2010; Sato et al., 2012; Boccuto et al., 2013 and Table 1.). The human SHANK3 maps to the critical region of

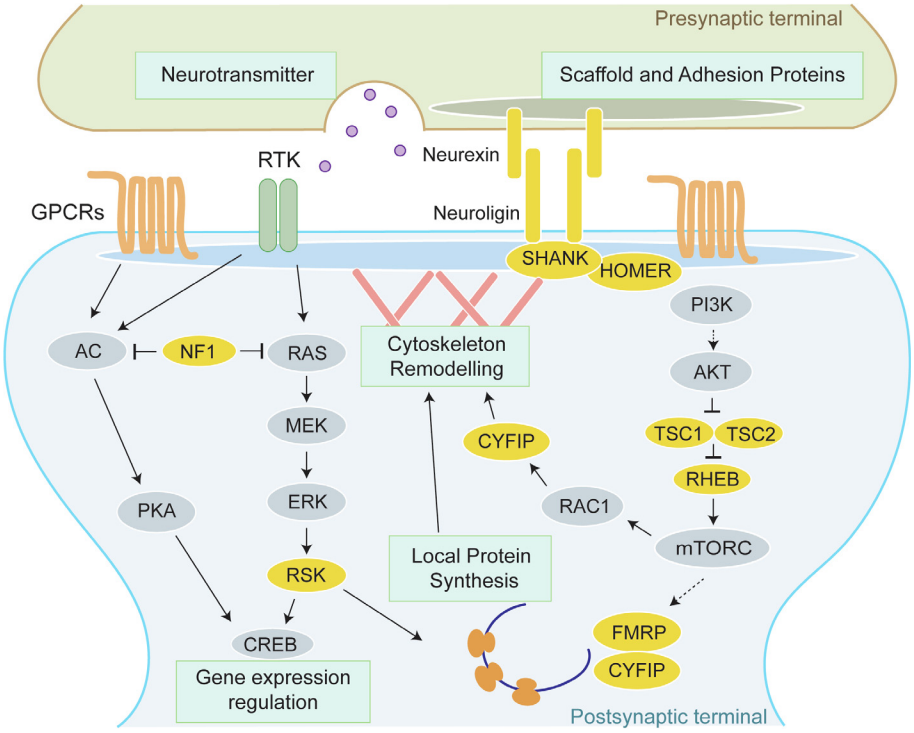

Fig. 3. Schematic overview of selected genes and pathways implicated in learning and memory processing in *Drosophila* melanogaster. Mutations impairing neurotransmission, receptor signaling cascade, protein synthesis and gene expression have been commonly found in human cognitive deficits. *Drosophila* mutants for ID-linked genes exhibit learning and memory impairments, implicating those pathways in learning and memory. The proteins that are discussed in more detail in this review and have been studied in *Drosophila* in the contest of learning and memory, are highlighted in yellow.

22q13.3 deletion syndrome and is the major contributor to the pathology Phelan-McDermid syndrome (OMIM #606232). The key clinical features associated with this disease are developmental delay, hypotonia, severe cognition deficits and ASD (Zwanenburg et al., 2016). Interestingly, a meta-analysis of SHANK mutations in ASDs has revealed a gradient of severity in cognitive impairments, in which SHANK3 mutations were mainly found in individuals with ASDs combined with moderate to severe ID (Leblond et al., 2014). The only fly member of the SHANK family, Shank/Prosap, regulates the formation, organization, and plasticity of excitatory synapses. As in mammals, Shank is enriched at glutamatergic synapses of fly neuromuscular junctions (Harris et al., 2016). Studies using the *Drosophila* system have underlined Shank roles in regulating the morphology of the synaptic boutons in the MB and mediating olfactory stimuli response (Wu et al., 2017).

Homer family proteins. The Homer family of adaptor proteins consists, of three members in humans, HOMER1, HOMER2 and HOMER3, each with several splice variants. Homer1b/c and Homer2, in rodents, are predominantly associated in the PSD to type I mGluRs, IP3Rs and Ryanodine receptors as well as various kinases, including ERK and PI3K (Gao et al., 2013). *HOMER1* rare variants have been identified in cases of non-syndromic ASDs (Kelleher et al., 2012). In *Drosophila*, a single *Homer* homolog has been identified, which is predominantly upregulated during long-term memory after spaced training (Miyashita et al., 2012). Furthermore, mutations affecting Homer functionality lead to severe courtship conditioning defects (Diagana et al., 2002).

DISC1. Another scaffolding protein that interacts with proteins involved in the dopamine signaling cascade is encoded by the *Disrupted-in-Schizophrenia 1 (DISC1)* gene, a risk factor for SCZ (Porteous et al., 2014 and Table 1.). *DISC1* contains two SNPs associated with SCZ that have been linked to gray matter abnormalities in different brain areas and altered hippocampal function during cognitive tasks (Johnstone et al., 2011). Although the *DISC1* gene is not conserved in the fly genome, *Drosophila* has homologs for 93% of the DISC interactome (e.g. *nudE* – the fly homolog of *NDEL1*, and *dysbindin* – the fly homolog of *DTNBP*), allowing *Drosophila* to serve as a tool to study genetic interactions within this complex (Furukubo-Tokunaga et al., 2016). Overexpression of *DISC1* in larval MB neurons significantly impairs associative olfactory memory due to axonal and dendritic branching defects. Importantly, *Drosophila* studies allowed the identification of the amino-terminal domain (amino acid 46–290) of DISC1 as being relevant for memory suppression (Furukubo-Tokunaga et al., 2016 and Table 1.).

Neurexins (NRXNs) and neuroligins (NLGNs) family proteins. Neurexin and their binding partners, the neuroligins, are central organizing molecules at synapses that work as adhesion proteins and regulate pre- and post- synapse maturation and activity (Scheiffele et al., 2000; Graf et al., 2004; Varoqueaux et al., 2006; Südhof, 2017). Three neurexin genes (*NRXN1*, *NRXN2* and *NRXN3*), and five neuroligin genes (*NLGN1*, *NLGN2*, *NLGN3*, *NLGN4* and *NLGN4Y*) are present in the human genome. Recurrent mutations in neurexin genes have been identified in individuals with ASDs and neuropsychiatric disorders (Südhof, 2008; Kasem et al., 2018; Feng et al., 2006; Wang et al., 2018) and Table 1. NRXN1 mutations have also been implicated in Pitt-Hopkins-like syndrome 2 (OMIM #614325), an autosomal recessive intellectual disability syndrome characterized by severe cognitive deficits (Zweier et al., 2009). Furthermore, CNVs for several neuroligin genes have been reported in ASDs and SCZ cohorts (Laumonnier et al., 2004; Blasi et al., 2006; Glessner et al., 2009; Sun et al., 2011a; Parente et al., 2017). *Drosophila* has only a single *α-neurexin* homolog (*Nrx-1*) and four neuroligin genes: *Nlg1, Nlg2, Nlg3* and *Nlg4*. The interaction between *Nrx-1* with *Nlg1*

and *Nlg2* has been shown to regulate the development of fly glutamatergic synapses (Sun et al., 2009, 2011b; Banovic et al., 2010; Chen et al., 2012). Interestingly knock-down studies for *Nrx-1* and *Nlg4* in flies have revealed severe cognition defects in the reversal-learning, i.e. cognitive flexibility (Dong et al., 2016).

Protein homeostasis

Fmr1 encodes the fly ortholog of the human FMRP protein, an RNA-binding protein whose loss causes the FXS. The majority of the patients with FXS (OMIM #300624) show a CGG-triplet expansion in the 5′ UTR of the *FMR1* gene, which leads to hypermethylation and transcriptional silencing of the gene (Verkerk et al., 1991; Bagni et al., 2012) and Table 1. FXS represents the most common monogenic cause of intellectual disability and the prevalence due to the full mutation is approximately 1 in 4000 males and 1 in 8000 in females (Bagni and Zukin, 2019). Individuals with FXS exhibit phenotypes that include mild to severe ID, autistic-like behaviors and neurodevelopmental deficits. FXS individuals might also present cognitive executive dysfunctions in working memory, cognitive flexibility, attentional set-shifting and planning (Hooper et al., 2008). FMRP regulates mRNA metabolism at multiple levels, namely transport, translation and stability with a key role at synapses (Fig. 3) (Bagni and Zukin, 2019). Because the *Fmr1* deficiency in flies can recapitulate core features of FXS, this model has provided major insights into the pathophysiological mechanisms of the disease. Similar to what has been reported in individuals with FXS, *Fmr1* mutant flies show neurodevelopmental defects such as axonal and synaptic dysmorphogenesis. Several studies have reported developmental defects in the MBs of *Fmr1* mutants. Specifically, the β-lobe axons of the MB do not terminate in the midline, leading to the formation of fused lobes (Pan et al., 2004; McBride et al., 2005; Bolduc et al., 2008; Coffee et al., 2010, 2012; Gatto and Broadie, 2011). In parallel with the MB structural defects, *Fmr1* mutants exhibit cognitive behavioral dysfunctions such as short and long term memory impairments (McBride et al., 2005; Bolduc et al., 2008; Kanellopoulos et al., 2012; Gatto et al., 2014; Dong et al., 2016). Learning and memory deficits in the *Fmr1* mutant fly models have been extensively investigated, allowing the identification of key molecular mechanisms involved in the behavioral phenotype. Using genetic and pharmacological tools, it was found that the effects of increased mGluR levels upon FMRP reduction are linked to reduced cAMP, leading to associative learning and memory deficits (Kanellopoulos et al., 2012). In agreement, courtship conditioning deficits in *Fmr1* mutants could be rescued pharmacologically using the metabotropic glutamate receptor 5 (mGluR5)-specific antagonist 2-methyl-6-phenylethynyl-pyridine (MPEP) (McBride et al., 2005). Additionally, in aversive conditioning and the associated reversal-learning task, *Fmr1* mutant flies showed strong reversal-learning defects due to the inability to activate Rac1-dependent forgetting (Dong et al., 2016).

Upregulated protein synthesis has been observed in human tissues isolated from FXS individuals (Hoeffer et al., 2012; Jacquemont et al., 2018) and correct translation is pivotal for memory acquisition and stabilization (Hernandez and Abel, 2008). Similar to humans, protein synthesis is dysregulated in *Fmr1* mutant flies and, most interestingly, low concentrations of the protein synthesis inhibitor cycloheximide ameliorate the long-term memory defects of *Fmr1* mutant flies (Bolduc et al., 2008).

FMRP has been shown to interact with several proteins at synapses including the Cytoplasmic FMRP Interacting proteins 1 and 2 (CYFIP1/SRA-1 and CYFIP2) in mammals and in flies (Kobayashi et al., 1998; Schenck et al., 2001, 2003; Napoli et al., 2008) and Table 1 and Fig. 3. CYFIP1 has emerged as a significant risk factor for SCZ and ASDs (Tam et al., 2010; Leblond et al., 2012; Zhao et al., 2013; Purcell et al., 2014; Stefansson et al., 2014; Waltes et al., 2014; Vanlerberghe et al., 2015; Wang et al., 2015; Butler, 2017; Williams et al., 2020). Copy number variations of the 15q11.2 BP1-BP2 region encompassing the *CYFIP1* gene have been associated with increased risk for ID and psychiatric problems, including SCZ, ASDs, attention deficit hyperactivity disorder, Alzheimer's disease and obsessive–compulsive disorder (Stefansson et al., 2008; van der Zwaag et al., 2010; Cooper et al., 2011; Ghani et al., 2012; Cafferkey et al., 2014). Specifically, cognitive assessment in a cohort of adult BP1-BP2 deletion carriers and their non-carrier family members revealed deficits in cognition and specifically in grammatical reasoning, arithmetic reasoning, and working memory in BP1-BP2 deletion carriers (Woo et al., 2019). In *Drosophila,* a single *CYFIP/Sra-1* gene has been identified (Schenck et al., 2001, 2003). *CYFIP* haploinsufficiency in flies causes impaired associative learning in a negative reinforcement paradigm, mimicking the cognitive deficits observed in the human deletion carriers (Woo et al., 2019). Recently it was shown that *Drosophila Cyfip* mutants exhibit an excessive synthesis of mitochondrial proteins that ultimately affects mitochondrial function and altered group behavior (Kanellopoulos et al., 2020).

Mammalian target of rapamycin (mTOR) is a key regulator of cellular processes, including cell growth, protein synthesis and synaptic functions (Sato, 2016). Abnormalities in the TSC-Rheb-mTOR pathway (Fig. 3) are highly prevalent in ASDs (Borrie et al., 2017; Rosina et al., 2019). Tuberous sclerosis complex (TSC1) (OMIM #191100) is a dominantly inherited multisystem syndrome resulting in benign hamartomatous tumors and neurological disorders, including epilepsy, grey matter abnormality, cognitive disorders and ASDs (Curatolo et al., 2015 and Table 1). ID is significantly associated to TSC with a prevalence of up to 44% (Joinson et al., 2003). Verbal and visual recall memory and spatial working memory are all significantly impaired in TSC individuals (Ridler et al., 2007).

The TSC1–TSC2 complex regulates mTOR activity through modulation of Rheb, a small GTPase (Manning and Cantley, 2003). The Tsc-Rheb-mTOR pathway is highly conserved between flies and mammals, and *Drosophila* studies have given insights into the dysregulated

molecular mechanisms in TSC. Similarly to the mammalian system, in flies, *Tsc1* and *Tsc2 (gig)* regulate cell growth, proliferation and organ size (Potter et al., 2001). *Tsc1* mutations strongly affect memory formation. Indeed flies with *Tsc1* downregulation display behavioral inflexibility indicated by impaired reversal-learning, showing an inability to forget the first aversive odor association after training with a second aversive odor (Dong et al., 2016). Rheb was originally discovered in *Drosophila* genetic screens for novel regulators of cell growth (Saucedo et al., 2003). Overexpression of Rheb in the MB causes abnormal morphology of the axonal lobes and defective memory due to mTOR over-activation (Brown et al., 2012).

Another gene with a role in protein homeostasis that is implicated in ID is *UBE3A*. Mutations in *UBE3A* cause many of the characteristic features of Angelman syndrome (AS) (OMIM #105830) such as severe mental disability, fits of laughter, epilepsy, ataxia and specific dysmorphic features (Williams et al., 2006) and Table 1. *UBE3A* encodes an E3 ubiquitin-protein ligase E6-associated protein that has functions in learning and memory, neuronal and synapse development, and synaptic plasticity (Wu et al., 2008; Greer et al., 2010). In neuronal cells, *UBE3A* is imprinted and only the maternal copy is expressed (Chamberlain and Lalande, 2010). *Drosophila Ube3a* shares high homology with the human ortholog, allowing the use of the fly system to investigate the molecular mechanisms behind the neurologic defects (Lu et al., 2009). AS patients suffer from severe cognitive impairment that is recapitulated by the fly system as well, and olfactory conditioning assays have revealed strong memory deficits after spaced training due to the failure to form long term memory (Wu et al., 2008). *Ube3a* mutant flies also display strong impairments in reversal-learning and the inability to activate Rac1-dependent forgetting (Dong et al., 2016).

FUTURE PERSPECTIVES

The need to understand the underlying causes of intellectual disabilities is urgent. Currently, 1–2% of the population is estimated to be affected by IDs, and the incidence is increasing (AAIDD, 2010). While recent advances in human genetics have greatly increased our capacity to detect genetic variants, it remains difficult to discern causal events among the multitude of benign variations that are also identified.

To study ID pathogenesis, animal models are needed that can be manipulated at will. Mammalian models such as rodents are widely used, but experiments in mice – not to speak of higher mammals – are very time demanding and cost intensive. However, *in-vivo* genetic screens in *Drosophila* are very feasible and show great promise in identifying causal ID gene variants.

We have reviewed how research using the *Drosophila* model has offered key insights in the field of IDs, contributing to the understanding of basic mechanisms affected in several IDs, and we have provided demonstrations that highlight the utility of *Drosophila* as a competent model for translational research. The genetic basis of intellectual and social cognition is reasonably conserved between fly and human, and the power of fly genetics is unique. Both the generation time of the animal and the cost of animal breeding and maintenance are a fraction of other animal models (e.g. rodents). Given the genetic tractability of flies, and the wealth of tools available for their genetic manipulation, numerous studies can now be performed in flies to more rapidly discover the molecular mechanisms by which human mutations cause disease phenotypes.

We envision that the understanding of ID pathogenesis will be based not on a single gene/protein function but multiple genes and different (synaptic) pathways. Since the simultaneous study of many mutant lines is possible in *Drosophila*, this will allow clustering of individual mutations into common pathways, ultimately helping to identify key druggable targets.

In addition, drug targets can be screened and validated with ease in *Drosophila*. For certain fly models of human ID such as FXS among others, it has been successfully shown that the cognitive deficits can be ameliorated with genetic or pharmacological interventions (McBride et al., 2005; Bolduc et al., 2008; Kanellopoulos et al., 2012). Thus, flies can serve as a platform for pharmacological screening tests using multiple mutant lines, including with genes that have not yet been extensively studied, to advance the field towards the design of successful treatment strategies.

Functional investigation of human mutated genes and variants using the fly model will allow a better understanding of the molecular and behavioral pathophysiology of neurodevelopmental disorders. The use of *Drosophila*, together with other ‘‘simple” animal models such as *C. elegans* and zebrafish, will also help to push translational research forward (van der Voet et al., 2014; Coll-Tané et al., 2019).

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