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REVIEW

SYSTEMS NEUROSCIENCE IN *DROSOPHILA*: CONCEPTUAL AND TECHNICAL ADVANTAGES

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Abstract—The fruit fly *Drosophila melanogaster* is ideally suited for investigating the neural circuit basis of behavior. Due to the simplicity and genetic tractability of the fly brain, neurons and circuits are identifiable across animals. Additionally, a large set of transgenic lines has been developed with the aim of specifically labeling small subsets of neurons and manipulating them in sophisticated ways. Electrophysiology and imaging can be applied in behaving individuals to examine the computations performed by each neuron, and even the entire population of relevant neurons in a particular region, because of the small size of the brain. Moreover, a rich repertoire of behaviors that can be studied is expanding to include those requiring cognitive abilities. Thus, the fly brain is an attractive system in which to explore both computations and mechanisms underlying behavior at levels spanning from genes through neurons to circuits. This review summarizes the advantages *Drosophila* offers in achieving this objective. A recent neurophysiology study on olfactory behavior is also introduced to demonstrate the effectiveness of these advantages.

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Key words: *Drosophila*, systems neuroscience, genetically identified neurons, behavioral physiology, population codes, neuronal connectivity.

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INTRODUCTION

Understanding how the brain orchestrates behaviors is a major objective in systems neuroscience. This quest involves accomplishing the following tasks. First is to characterize the behavior of interest. Second is to identify the neurons and circuits responsible for the behavior. Third is to study the computations performed by these neurons. Fourth is to reveal the mechanisms underlying behavior and neural computations.

Studies using multiple organisms have uniquely contributed to advancing each of these lines of investigation. For example, primates have been invaluable in examining the neuronal computations supporting cognitive functions with physiological and psychophysical methods (Gazzaniga et al., 2013). However, it is difficult to understand the circuit mechanisms that give rise to cognition because the identity and wiring partners of recorded neurons are typically unknown. On the other hand, *Drosophila melanogaster* is suitable for understanding the mechanisms underlying behavior at the level of genes, cells, and circuits, because its neurons are identifiable and genetically tractable. In fact, *Drosophila* has been invaluable to the analysis of genetic control over behaviors (Baker et al., 2001; Vosshall, 2007) and precise neuronal wiring (Otsuna and Ito, 2006; Jefferis et al., 2007; Lin et al., 2007; Chiang et al., 2011). However, it was not a place to study neuronal computation because its central neurons had long been resistant to physiological inspection. The neurons looked too small compared to the tip of the electrode and the exoskeleton too fragile to perform recordings in individuals. Despite

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Abbreviations: ATP, adenosine triphosphate; biORN, bilaterally projecting ORN; ChR2, channelrhodopsin-2; EPSC, excitatory postsynaptic current; ORN, olfactory receptor neuron; PA-GFP, photoactivatable green fluorescent protein; PN, projection neuron; *Shi*^{ts1}, *UAS-shibire*^{ts1}; uniORN, unilaterally projecting ORN.

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these seeming hurdles, imaging techniques were successfully applied to monitor neuronal responses to sensory stimuli (Ng et al., 2002; Wang et al., 2003). Further, the application of the whole-cell patch-clamp technique to central neurons *in vivo* (Wilson et al., 2004) as well as physiological recordings in behaving adult animals (Maimon et al., 2010; Seelig et al., 2010) marked a new era of fly systems neuroscience. Therefore, the fly now provides an opportunity to combine powerful approaches in a single organism to investigate not only mechanisms but also computations supporting behavior at cellular resolution.

This review discusses the contribution the adult *Drosophila* has made to an understanding of fundamental brain functions. Recently, excellent reviews have reported the strategies (Olsen and Wilson, 2008a) and the extensive genetic toolbox (Simpson, 2009) for deciphering the fly neural circuits. Here, I will instead summarize the updated findings in the field from the viewpoint of conceptual and technical advantages unique to *Drosophila*. Each section in the main body broadly concerns individual tasks in the field introduced at the beginning. This general review is followed by the description of a work on the neural basis of odor-guided navigation to exemplify how the advantages are put to use.

FLIES DISPLAY A RICH SET OF BEHAVIORS

One may wonder whether flies exhibit sophisticated behaviors that involve complex neuronal computations in the first place. In fact, they show a variety of behaviors having resemblance to those of mammals. Flies are equipped with highly developed sensory systems. Because flies and mammals live in a similar environment, their systems are adapted to perform similar functions. For example, the olfactory circuits of flies and mammals share not only functions and computations but also a basic wiring diagram (Ache and Young, 2005; Su et al., 2009). Courtship and aggression are more intricate behaviors that involve multimodal sensory integration, memory, decision making, and exquisite motor control (reviewed in Dickson, 2008; Villella and Hall, 2008; Zwarts et al., 2012). A courting male assesses the condition of females by integrating olfactory and gustatory cues. When the male decides to court after interpreting the sensory information in the context of his past experience, he will vibrate one wing to sing a courtship song. Fighting males and females also use multiple sensory modalities to decide which action to select from a variety of agonistic behaviors. Males even establish dominance depending on their fighting records. Moreover, flies can form a long-lasting association between an odor and an electric shock or a reward, enabling the study of adaptive behaviors (reviewed in Keene and Waddell, 2007; Davis, 2011). Because automatic tracking and analysis of freely interacting flies in an arena are now routine (Branson et al., 2009; Dankert et al., 2009; Kabra et al., 2013), more intricate behaviors are expected to be discovered.

NEURONS AND CIRCUITS CAN BE GENETICALLY IDENTIFIED

Neurons are genetically identifiable

To understand the workings of circuits supporting these behaviors, we need to examine how individual cells within a circuit are operating. Brain circuits are harder to decipher compared to electrical circuits because no information about their elements and let alone wiring is initially available. Therefore, an effective strategy for neuroscientists is to start from identifying individual or groups of cells in order to scrutinize their functions across animals.

Cells can be identified by their location, morphology, physiology, gene expression, lineage, or any other attributes, but this procedure is especially facilitated in *Drosophila* due to two major reasons. First is numerical simplicity. There are only about 100,000 neurons in the adult *Drosophila* brain (Ito et al., 2013). The benefit of simplicity is appreciated, for example, in the olfactory circuit. Whereas there are ~2000 glomeruli in the mouse olfactory bulb, there are only ~50 glomeruli in the fly antennal lobe making them all morphologically identifiable (Couto et al., 2005; Fishilevich and Vosshall, 2005; Tanaka et al., 2012). As a consequence, the first- and the second-order olfactory neurons innervating each glomerulus also become identifiable. Second is genetic tractability. The widely used Gal4/UAS binary expression system enables expression of various genes encoded by UAS lines in cells defined by the Gal4 lines through genetic crosses (Brand and Perrimon, 1993). Over the years, a vast number of Gal4 lines labeling different subsets of neurons, collectively covering a large portion of neurons in the fly brain, have been generated (Manseau et al., 1997; Hayashi et al., 2002; Rodan et al., 2002; Pfeiffer et al., 2008, 2010; von Philipsborn et al., 2011; Jenett et al., 2012).

Most genetically tagged neurons turned out to be highly stereotypical in many respects. For example, the antennal lobe projection neurons connect with a fixed type of olfactory receptor neurons in a specific glomerulus (Jefferis et al., 2001), have stereotypical axon branching patterns (Marin et al., 2002; Wong et al., 2002), and show very similar odor tuning across animals (Wilson et al., 2004; Bhandawat et al., 2007). The few exceptions are the antennal lobe local neurons and the mushroom body Kenyon cells, which are non-stereotypical in fine-scale anatomy and physiology (Murthy et al., 2008; Chou et al., 2010; Caron et al., 2013). In any case, the ability to identify cells across animals allows us to efficiently obtain the statistics of cellular attributes. How these genetically identified neurons can be manipulated in various ways by the expression of transgenes will be thoroughly described in later sections.

A powerful extension of this approach is to tag two sets of cells in a brain separately so that, for instance, the activity of one set of cells is monitored while the property of the other is altered. This necessitates the use of another binary expression system that functions independently of the Gal4/UAS system. Such expression frameworks, namely, lexA/lexAop and Q

systems have recently been engineered (Lai and Lee, 2006; Potter et al., 2010).

The next generation transgenic lines label ever smaller subsets of neurons

A further restriction to the number of cells labeled in each transgenic line is ideal for several reasons. First, it would facilitate the characterization of wiring and function of specific cells. If fewer cells are labeled, a cell of interest can be more precisely traced in morphology and reliably targeted with electrodes. Second, it would accelerate the discovery of neurons driving the behavior. If cells of mixed types are manipulated together in a Gal4 line that elicited an interesting behavior, it will be difficult to ascribe the phenotype to particular neurons. The use of sparsely labeled lines makes the interpretation of the behavioral results straight forward, although we must remind ourselves that perturbing the activity of just a few neurons may not induce measureable phenotypes.

Two groups of laboratories have independently created the next generation transgenic lines (the GMR collection and the VT collection) in which Gal4 expression is controlled by a fragment of genomic sequences flanking neuronal genes (Pfeiffer et al., 2008, 2010; von Philipsborn et al., 2011; Jenett et al., 2012). These lines tag far fewer neurons than the previously available lines. The GMR collection contains the split-Gal4 lines that further restrict the site of gene expression (Luan et al., 2006; Pfeiffer et al., 2010). Each of these lines expresses either the DNA-binding domain or the transcription-activation domain of Gal4 in distinct spatial patterns. Because both domains must bind together to form a functional Gal4, transcription is driven only at the intersection of expression patterns of complementary split-Gal4 lines. Another very helpful feature of these Gal4 collections is the accompanying open-access database of confocal brain images showing the expression pattern of Gal4 and its broad annotation (http://flweb.janelia.org/cgi-bin/flew.cgi, http://brainbase.imp.ac.at). This provides a conceptually new, rapid, and economical way of screening for a line tagging the neurons of interest. Transgenic flies created by researchers are maintained at the stock centers around the world and shared widely in the community.

It is worth noting that mosaic methods are advantageous in terms of confining the labeling to even single neurons and covering all the neuronal lineages (Lee and Luo, 1999; Ito et al., 2013; Yu et al., 2013), but they cannot label the same cells consistently. On the other hand, specific Gal4 methods reliably label genetically defined cells in every animal.

Genetic methods illuminate the neuronal wiring diagram

One of the next steps after identifying and characterizing individual neurons is to delineate the connectivity among these neurons in order to understand the mechanisms that confer specific functions to a circuit. The most precise and exhaustive method to obtain the neuronal wiring diagram is connectomics using serial electron microscopy. The connectome has been reconstructed in the *Drosophila* medulla (Takemura et al., 2013). However, this method requires massive resources, is not practical to perform on multiple brains, and does not reveal the functional properties of each synaptic connection. Another way to create connectivity maps is to superimpose the confocal microscopy images of single neurons labeled in different brains on a common frame of reference (Jefferis et al., 2007; Lin et al., 2007). Thousands of neuronal clones were generated and their morphology was registered to the standard brain to obtain a whole-brain wiring diagram (Cachero et al., 2010; Yu et al., 2010; Chiang et al., 2011). This gave insight into the interaction among brain regions, but because of the variability inherent in brains and registration processes, it did not provide information about the precise connectivity between individual neurons.

An alternative approach with a potential to find functional cellular connections is to illuminate the structure of physically overlapping neurons using photoactivatable green fluorescent proteins (PA-GFP, Datta et al., 2008). The fluorescence of PA-GFP increases dramatically after photoconversion with a pulsed laser. The beauty of PA-GFP is threefold. First, because it is activated by two-photon excitation, a pan-neuronal Gal4 line can be used to express it ubiquitously and illuminate any neurons in a brain with cellular resolution. Second, the entirety of the neuronal structure can be labeled even with partial activation. PA-GFP activated just in dendrites, soma, or axons diffuses to the tip of every neurite (Datta et al., 2008). Therefore, for instance, the knowledge of a dendritic region alone is sufficient to discover the target region of a neuron. It is even possible to find the soma of downstream neurons by photoactivating their dendrites close to the pre-labeled axonal endings of input neurons. Third, because PA-GFP undergoes photoconvertion *in vivo* and diffuses rapidly, the illuminated neurons can be targeted with electrodes to test the functional connectivity among them.

One study applied this strategy to complete the circuit from pheromone-sensing olfactory receptor neurons (ORNs) to the putative fourth-order neurons descending to the ventral nerve cord, which control body movement (Ruta et al., 2010). Electrophysiological recordings showed that these physically proximal neurons labeled by PA-GFP do communicate, although the presence of another neuron in between could not be ruled out. PA-GFP was also used to find a large portion of third-order olfactory neurons in the lateral horn and to eventually characterize the synaptic transmission between a pair of identified neurons (Fisek and Wilson, 2014). The connectivity between the antennal lobe neurons and the lateral horn neurons was found to be stereotypical. These experiments demonstrated the potential of this method to reveal any functional connection in the brain.

VARIOUS TRANSGENES HELP TO DISSECT THE FUNCTION OF CIRCUITS

Inhibiting neuronal activity

A fundamental way of characterizing the functional role of neurons and circuits is to examine the consequences

following their manipulation. Accordingly, various transgenes have been developed to manipulate the individual circuit elements. In general, necessity of the targeted neurons for a behavior is shown by the positive effect of neuronal silencers, while sufficiency is shown by the positive effect of neuronal activators.

Killing the cells by expressing a variety of transgenes is a straightforward method to eliminate neuronal activity. Toxins including diphtheria toxin A from bacteria (Lin et al., 1995) and ricin A from castor plant seeds (Hidalgo and Brand, 1997) induce cell death by inhibiting protein synthesis. Proapoptotic genes such as *reaper*, *grim*, and *head involution defective* induce programed cell death by activating caspases (Zhou et al., 1997; Wing et al., 1998). To ensure the normal development of the animal, their expression can be initially suppressed by a temperature-sensitive Gal80, which represses Gal4-dependent transcription at a permissive temperature (McGuire et al., 2003). However, despite the effectiveness in earlier stages, these genes switched on from the adult stage act slowly or even fail to cause behavioral phenotypes in some cells (Thum et al., 2006).

Neural function can be more specifically inhibited by blocking synaptic transmission. Tetanus toxin light chain abolishes action potential-evoked neurotransmitter releases by cleaving neuronal synaptobrevin, a component of vesicular release machinery. While the effect of tetanus toxin is irreversible, a thermo-sensitive transgene *UAS-shibire^{ts1}* (*Shi^{ts1}*) blocks synaptic transmission reversibly by disabling vesicular endocytosis only at a restrictive temperature (Kitamoto, 2001). Therefore, by controlling the temperature, synaptic communication can be terminated and restored at a certain time in adulthood. Because *Shi^{ts1}* is remotely activated by heat, it is easily applicable to freely behaving flies. *Shi^{ts1}* was, for instance, used to better understand the circuit for olfactory memory. The mushroom body was recognized to be necessary for memory (de Belle and Heisenberg, 1994; Connolly et al., 1996), but it was not known which subset of Kenyon cells in this brain region was required at each phase of memory processing. By expressing *Shi^{ts1}* in subsets of Kenyon cells and shifting the temperature to a restrictive range during acquisition, consolidation, or retrieval of memory, it was demonstrated that each set of cells was necessary at distinct phases of memory formation (Dubnau et al., 2001; McGuire et al., 2001; Krashes et al., 2007). These experiments suggested that specific parts of the circuit are dynamically recruited in turn to support the brain function. It was further shown using a similar approach that distinct subsets of dopaminergic neurons are conveying different information about punishment and reward to the mushroom body during conditioning (Burke et al., 2012; Liu et al., 2012).

Tetanus toxin light chain and *Shi^{ts1}* affect chemical synapses but spare electrical synapses. To alter the communication through electrical synapses or excitability of the neuronal membrane, several types of potassium channels have been overexpressed in the cell. An increase in the potassium conductance hyperpolarizes the resting membrane potential toward the reversal potential of potassium ions and shunts synaptic currents both of which make cells more difficult to fire. Successfully applied channels include a human inwardly rectifying potassium channel Kir2.1 (Baines et al., 2001; Paradis et al., 2001), a *Drosophila* truncated open-rectifier potassium channel dORK-ΔC (Nitabach et al., 2002), and a modified *Drosophila* Shaker potassium channel EKO (White et al., 2001).

Enhancing neuronal activity

To excite the cells, sodium channels can be expressed instead of potassium channels. NaChBac is a voltage-dependent bacterial sodium channel whose measured property suggested its contribution to increase the excitability of the cell (Nitabach et al., 2006). A note of caution is that the actual effect of its chronic overexpression on membrane potential dynamics can be complex in some cells (Sheeba et al., 2008). A ligand-gated channel would allow phasic cellular activation. $P2X_2$ is an ionotropic purinoceptor gated by adenosine triphosphate (ATP). ATP released from caged-ATP by ultraviolet light activated $P2X_2$ expressed in the giant fiber system and evoked typical escape behaviors (Lima and Miesenbock, 2005).

More recently, a blue-light-sensitive cation channel Channelrhodopsin-2 is used to excite neurons with higher temporal resolution (ChR2; Nagel et al., 2003; Fenno et al., 2011; Packer et al., 2013). It opens immediately after the application of light and closes upon termination of light. Activation of ChR2 was able to generate spike trains in the ORNs, which in turn elicited behavior resembling that evoked by an odor (Suh et al., 2007). Although ChR2 was generally ineffective in exciting central neurons in the brain, the red-light-sensitive channelrhodopsin ReaChR (Lin et al., 2013; Inagaki et al., 2014) and Chrimson (Klapoetke et al., 2014) overcame this problem likely because the fly cuticle is more transparent to red than blue light (Inagaki et al., 2014).

Another potent activator of neurons is the *Drosophila* transient receptor potential channel dTrpA1, a thermosensitive cation channel, which opens above certain temperature (Hamada et al., 2008). Although precise temporal control of temperature is difficult, dTrpA1 has been so far more practical than ChR2 because heat can readily penetrate the cuticle and, unlike blue light, does not interfere with vision. Artificially activating neurons is also effective in searching for a neuron whose activation triggers a sequence of behaviors. By expressing dTrpA1 in various groups of cells using a large set of Gal4 lines, one study successfully uncovered the Fdg neuron that drives a particular movement of the proboscis and the pharyngeal pump mirroring feeding behavior (Flood et al., 2013a,b). It was further shown that the dTrpA1-expressing Fdg neuron can be driven at cellular resolution with heat provided by a pulsed laser.

INVESTIGATING THE COMPUTATIONS AND MECHANISMS

Physiological methods can be applied with ease

In order to understand the computations performed by circuits, it is essential to record neural activity.

Electrophysiology and imaging complement each other in measuring neural activity. These techniques became applicable to intact *Drosophila* about a decade ago and continue to be refined. Extracellular recordings with sharp electrodes or cell-attached glass electrodes measure the spikes without altering the resting membrane potential of a cell or the composition of the cytosol. Alternatively, whole-cell patch-clamp recording ruptures part of the membrane, but has additional merits in that it is more sensitive, can control the membrane potential, and reveal how synaptic inputs and intrinsic properties interact to produce spike outputs. In other words, it is possible to study the mechanisms of signal transformation in a neuron. The list of electrophysiologically-analyzed central neurons has expanded to include the antennal lobe projection neurons (Wilson et al., 2004) and local neurons (Wilson and Laurent, 2005), the mushroom body Kenyon cells (Turner et al., 2008), the lateral horn neurons (Ruta et al., 2010; Kohl et al., 2013; Fisek and Wilson, 2014), the lobula plate neurons (Joesch et al., 2008), auditory neurons (Tootoonian et al., 2012; Lehnert et al., 2013), and the central complex neurons (Weir et al., 2014). These identified cells can be unambiguously targeted by genetically marking them with fluorescent proteins. Because cell bodies lie at the surface of the brain surrounding the neuropil, it is relatively easy to make whole-cell recordings under direct visual guidance.

Given that intracellular recordings are typically made from a soma that is somewhat electrotonically distant from the dendrite, these methods cannot assess the true magnitude and spatial impact of synaptic inputs. Imaging techniques are useful in this respect due to their ability to access the activity of local neuronal structures. They are also suitable for simultaneously recording from many neurons (see next section). Because genetically-encoded calcium indicators work well in flies, calcium imaging has become routine to record both dendritic and axonal activity in various neurons (Wang et al., 2003; Marella et al., 2006; Kamikouchi et al., 2009; Yorozu et al., 2009; Seelig et al., 2010; Gruntman and Turner, 2013; Li et al., 2013; Maisak et al., 2013; Seelig and Jayaraman, 2013; Strother et al., 2014). The genetically-encoded voltage indicator ArcLight has reported subthreshold events and action potentials in neurons in an intact fly (Cao et al., 2013). However, data must be interpreted with caution because optical methods are less sensitive than electrophysiological methods and reflect only one aspect of neuronal excitation.

An ideal place to decipher the population code

Information is thought to be encoded in the activity of an ensemble of neurons. Due to its small size, the *Drosophila* brain presents an outstanding opportunity to access the activity of all the neurons engaged in a particular task. One approach to achieve this aim is to use a set of sparsely labeling transgenic lines that collectively label the cells in a specific brain region. Because *Drosophila* neurons are identifiable, complementary findings from different animals can be assembled to obtain the full picture. To give one successful example, the work of several labs has provided a nearly complete representation of odors in the first layer of the fly olfactory circuit (de Bruyne et al., 1999, 2001; Couto et al., 2005; Fishilevich and Vosshall, 2005; Yao et al., 2005; Hallem and Carlson, 2006; van der Goes van Naters and Carlson, 2007; Benton et al., 2009; Silbering et al., 2011). Each ORN type was mapped to a specific glomerulus using Gal4 lines, each of which mimics the expression pattern of a particular olfactory receptor. Electrophysiological recordings have characterized the odor tuning of most of the ORNs. These fly lines and data sets have greatly assisted both experimentalists and theorists to further our knowledge of olfactory processing in the antenna and in the antennal lobe (Wilson, 2013). One such case is the study on neuronal gain control in the antennal lobe. It has been known that neurons normalize their responses depending on the activity of other neurons (Carandini and Heeger, 2012), indicating the importance of considering the activity of all the relevant neurons. By utilizing the knowledge of ORN tuning, Olsen et al. (2010) differentially activated feedforward and lateral input to the recorded second-order projection neuron (PN). Through this approach, they created the model of normalization that predicts the response of PNs. They and others have further revealed that lateral presynaptic inhibition is the mechanism of this normalization (Root et al., 2008; Olsen and Wilson, 2008b).

Another way to examine the activity of a group of neurons is to use imaging techniques that can scan through multiple neurons in the same brain. Notably, this method better captures how neurons co-vary in activity over time. This is important because some information is hypothesized to be encoded in the coordinated activity of neurons such as oscillations and sequential excitation. For functional imaging, the numerical simplicity and physical compactness of the fly brain is an advantage. The fly brain occupies only $600 \times 350 \times 300$ microns. Therefore, any brain region is within the reach of two-photon microscopy and it can be scanned at a high frame rate. The latest versions of genetically-encoded calcium indicators are sensitive enough to detect individual spikes in some cells types (Akerboom et al., 2012; Chen et al., 2013). Thus, the time seems ripe for deciphering the population codes in the fly brain.

Using a pan-neuronal Gal4 driver, a recent study imaged the dendritic responses of all the central complex ring neurons to visual stimuli (Seelig and Jayaraman, 2013). They found that the dendrites of these neurons are arranged retinotopically and exhibit orientation tuning. The same Gal4 line was used to examine the visual responses from all the 10 layers in the medulla of the optic lobe (Strother et al., 2014). This method revealed layer-specific responses that match the anatomically known parallel pathways for processing light on and off stimuli. Another study imaged the tuning of elementary motion detectors with a lobula plate driver (Maisak et al., 2013). The detectors were classified into ON cells and OFF cells, and each cell type was further subdivided into neurons tuned to one of the four cardinal directions

suggesting that this experiment has revealed all the detector types. Just as in zebrafish larva (Ahrens et al., 2012, 2013), a comprehensive imaging approach will likely to be increasingly informative in *Drosophila.*

Neuronal activity can be measured in behaving animals

The role of neurons can be inferred from the relationship between neural activity recorded in restrained animals and behavior displayed by freely moving animals in the same environmental condition. However, this correlational approach is not applicable to the study of neurons having motor or cognitive functions. Moreover, neural activity is modulated by behavioral or attentional states of the animal. This state-dependent modulation can be found even in relatively early sensory processing (Maimon, 2011). Therefore, to understand how neural circuits function in different contexts, it is ultimately necessary to record neural activity in behaving animals.

Several groups have recently shown that simultaneous monitoring of physiology and behavior is feasible in flies. Individual flies were tethered to a stage that maintained the exposed brain under saline while keeping the body dry and unrestrained to allow navigation in a visual arena (Chiappe et al., 2010; Maimon et al., 2010; Seelig et al., 2010; Suver et al., 2012; Weir et al., 2014). Whole-cell patch-clamp recording showed that the resting membrane potential and visual responses of motion-processing interneurons in the lobula plate increased during flight (Maimon et al., 2010; Suver et al., 2012). Mechanistically, this was suggested to be due to a stronger synaptic input to the neurons. It was further shown that neurons expressing octopamine, an insect equivalent of norepinephrine, are involved in this state-dependent modulation because pharmacological application of octopamine in quiescent flies mimicked the physiological change observed during flight and octopamine neurons projecting to the lobula plate increased their activity upon initiation of flight (Suver et al., 2012). Motion-processing interneurons displayed similar properties in flies walking on a ball under a two-photon microscope. Their dendritic calcium response to visual motion was strengthened and their tuning to temporal frequency was shifted to a higher rate when the flies were walking compared to resting (Chiappe et al., 2010). The ability to analyze the co-variability between neural activity and behavior is invaluable to reveal the contribution of neurons to any brain functions besides sensorimotor integration.

AN EXAMPLE STUDY: NEURAL MECHANISMS OF ODOR LOCALIZATION

Taking into consideration the strengths of *Drosophila* as a model organism, it is well suited for addressing biological questions involving olfaction. Flies show robust responses to odors and ORNs, PNs, and the synapses between them are genetically identifiable as well as physiologically accessible. Below, I describe how a recent study revealed the neural basis of odor localization utilizing many of the said advantages.

Animals compare bilateral inputs to localize odors

Localizing sensory cues is critical for survival because mere detection is insufficient for deciding the direction one should proceed or escape. It is easier to appreciate the importance of bilateral input in visual depth perception (Cumming and DeAngelis, 2001) and sound source localization (Grothe et al., 2010) because two eyes and ears are physically set apart and we are fully aware of these abilities. What about the nose that sits in the middle of the face? There are two nostrils and despite their close alignment, humans (von Bekesy, 1964; Porter et al., 2005, 2007) and rats (Rajan et al., 2006) can use inter-nasal cues to localize odor sources. Flies are not an exception. Their two odor-sensing antennae are just several hundred microns apart, but walking and flying *Drosophila* are able to turn toward the antenna that is more strongly stimulated (Borst and Heisenberg, 1982; Duistermars et al., 2009). The mechanisms underlying this ability had long remained unknown until a study shed some light on them (Gaudry et al., 2013).

Testing the circuit mechanism

In order to precisely control the spatiotemporal structure of odor stimulation, Gaudry et al. (2013) made individual tethered flies walk on a spherical treadmill and observed their responses to odorized air delivered to one or both antennae (Fig. 1A_1). Flies biased their turns when one antenna was preferentially stimulated, whereas they showed no bias upon bilateral antennal stimulation (Fig. 1A_2). What form of circuitry transmits the differential input to the brain?

The olfactory circuit of a fly is very similar to that of vertebrates (reviewed in Ache and Young, 2005; Su et al., 2009). ORNs expressing the same odorant receptor converge to a neuropil structure called a glomerulus in the antennal lobe, a brain region analogous to the vertebrate olfactory bulb. There they connect to the second-order PNs, which send the processed signals to the deeper brain regions. One characteristic of the fly ORNs is that, unlike those in mammals or other insects, most of them project axons to both hemispheres in the brain. The simplest mechanism underlying odor localization is that the unilaterally projecting ORNs (uniORNs) send asymmetric information (Fig. 2A); however, they are small in number and their combined receptive field would likely permit only a limited number of odors to be localized. Therefore, the first question was whether the bilaterally projecting ORNs (biORNs) are sufficient to support odor lateralization.

To address this question, one biORN type innervating a particular glomerulus was preferentially activated in two ways. The first was to use an odor that binds with high affinity to only one ORN type among all the characterized ORNs. This odor induced biased turning, which was lost in a mutant where biORNs were silenced, indicating that uniORNs alone are not sufficient to localize the odor (Fig. 1B_1). The second was an optogenetic method to more strictly confine the activation to one ORN type. ChR2 was expressed in a single ORN type, and light was applied to one of the antennae. A brief flash of light was sufficient to bias the

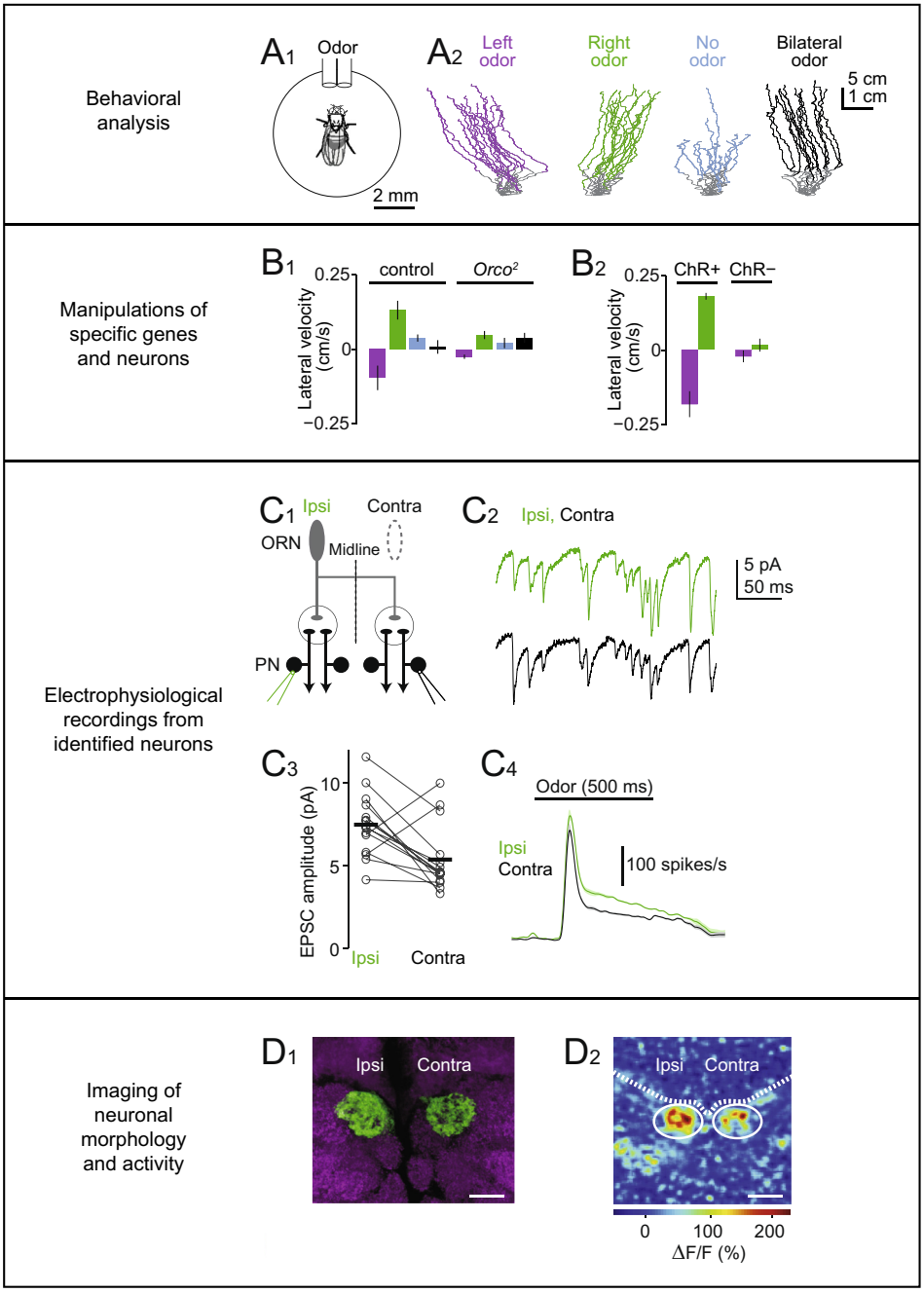

Fig. 1. A series of methods applicable to flies. (A) Behavioral analysis. (A_1) Schematic of a fly walking on a spherical treadmill. Odors can be applied to individual or both of the antennae. (A_2) Trajectories of a representative fly in response to unilateral, bilateral, or no odor application. Flies bias their turns toward the side of the stimulated antenna. Gray lines indicate navigation during the pre-odor period. (B) Manipulations of specific genes and neurons. (B_1) An odor that preferentially activates biORNs in glomerulus DM6 evokes biased turns in control flies. This biased navigation is lost in the *Orco*^{2} mutant (Larsson et al., 2004) where biORNs are silenced. Colors represent conditions as in (A_2). (B_2) Activation of ChR2-expressing ORNs in just one glomerulus in one antenna is sufficient to bias the turns. This behavior was not observed in flies without the expression of ChR2. Magenta bar corresponds to left antennal activation and green corresponds to right antennal activation. (C) Electrophysiological recordings from identified neurons. (C_1) Schematic of the fly olfactory circuit and the recording configuration. Most ORNs innervate a pair of glomeruli bilaterally, one in each hemisphere where they synapse onto PNs. In these experiments, one antenna was removed to lateralize the ORN input. (C_2) A representative dual recording from an ipsilateral and a contralateral PN in glomerulus DM6. All the spontaneous EPSCs in the two cells are synchronized. (C_3) The average spontaneous EPSC amplitude is larger in ipsilateral PNs (glomerulus DM6). (C_4) Ipsilateral PNs fire at a higher rate in response to an odor (glomerulus DM6). (D) Imaging of neuronal morphology and activity. (D_1) Neuronal synaptobrevin-GFP was expressed in DM6 ORNs. One antenna was removed 3 days prior to the experiment to let it degenerate. Therefore, the fluorescence originates only from ORNs in the remaining antenna. Fluorescent level is higher in the ipsilateral glomerulus. Care must be taken to interpret this result because the antennal lobe circuit undergoes plastic changes days after antennal removal (Berdnik et al., 2006; Kazama et al., 2011). Magenta shows the neuropil structures. Scale bar = 10 μm. (D_2) A representative odor-evoked response of a calcium indicator GCaMP3 (Tian et al., 2009) expressed in DM6 ORNs. The change in fluorescence was higher in the ipsilateral glomerulus. Scale bar = 10 μm. Figure panels were modified with permission from Gaudry et al. (2013).

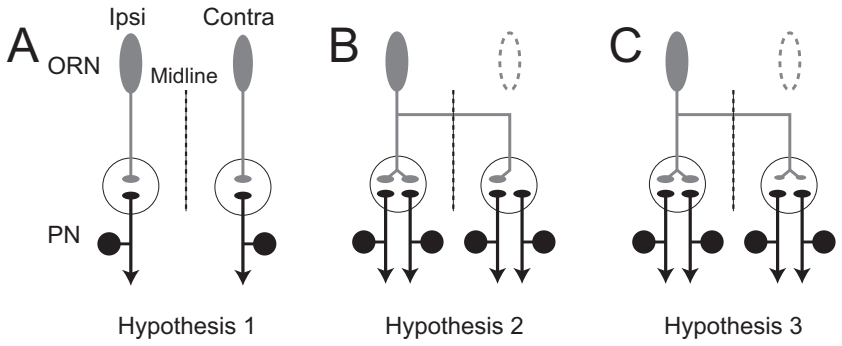

Fig. 2. Possible mechanisms underlying odor lateralization. (A) Hypothesis 1. ORNs exclusively innervating the ipsilateral glomeruli send asymmetric signals. (B) Hypothesis 2. Each biORN contacts a larger number of PNs in the ipsilateral side compared to the contralateral side. (C) Hypothesis 3. Each biORN contacts all the PNs within the glomerulus, but with stronger synaptic strength in the ipsilateral side.

running direction (Fig. 1B_2). These results suggest that odor localization is mediated by biORNs but not uniORNs.

How, then, can biORNs transfer asymmetric input to their cognate PNs? One possibility was unequal innervation of PNs by each ORN. If each ORN contacted a larger number of PNs in the ipsilateral side compared to the contralateral side, the signal would be preferentially transmitted to the ipsilateral antennal lobe (Fig. 2B). To reveal the pattern of connectivity between ORNs and PNs within a glomerulus, we had previously performed dual recordings from pairs of PNs in different hemispheres but innervated by the same ORN types (Fig. 1C_1; Kazama and Wilson, 2009). Because a spike in an ORN will always evoke a fast excitatory postsynaptic current (EPSC) in a PN (Kazama and Wilson, 2008), a bias in the number of ORNs connected to each PN can be assessed by counting the number of fast EPSCs in a pair of PNs (Figs. 1C_1, 2B). Surprisingly, recordings showed that virtually all the EPSCs occurred synchronously in two PNs (Fig. 1C_2). These results demonstrated that the connectivity between ORNs and PNs is all-to-all: each ORN diverges onto every PN within a glomerulus. Therefore, the second hypothesis was also rejected (Fig. 2B).

Testing the synaptic mechanism

Now the potential mechanism was narrowed down to asymmetric synaptic interactions in the antennal lobe (Fig. 2C). Upon close inspection of EPSCs in sister PNs, Gaudry et al. (2013) have found that the average amplitude was larger in ipsilateral PNs compared to that in contralateral PNs (Fig. 1C_3). Thus, a spike originating in one ORN had a stronger impact on the ipsilateral PNs. Accordingly, the odor-evoked spikes were generated earlier and at a higher rate in the ipsilateral PNs (Fig. 1C_4).

This difference in synaptic strengths could stem from pre- and/or postsynaptic factors. Two lines of evidence supported a presynaptic origin. First, a synaptic vesicular protein tagged with a fluorescent protein was more abundant in ipsilateral side when this construct was expressed in ORNs (Fig. 1D_1). Second, odor-evoked calcium responses in the ORN axon terminals were again larger in ipsilateral side (Fig. 1D_2). The magnitude of calcium response was not altered by pharmacological blockade of neurotransmitter receptors making the postsynaptic contribution unlikely. In conclusion, odor localization is mediated by an asymmetric neurotransmitter release from each ORN onto PNs in the opposite hemispheres (Fig. 2C).

The ability to identify and functionally probe neurons was fully capitalized on to reach this conclusion. All the glomeruli as well as ORNs and PNs innervating them are identifiable. A set of transgenic lines exists to label neurons in a particular glomerulus. Recording from two PNs belonging to the same glomerulus would be a formidable task without these genetic lines. The knowledge about odor tuning for many ORN types has accumulated due to the fact that the same neurons can be studied repeatedly. It is because of these unique features that the understanding of sensory processing in flies is especially advanced in the olfactory circuit (Wilson, 2013).

CONCLUSIONS AND PERSPECTIVES

In summary, many neurons and circuits are identifiable in the fly brain. Neurons can be genetically labeled and controlled to examine their roles in generating specific behaviors. Electrophysiology and imaging techniques are applicable to study the computations performed by individual neurons, and even the entire ensemble of relevant neurons in a particular region, due to the numerical simplicity and physical compactness of the brain. A large set of transgenic lines has been developed with the aim of manipulating ever smaller number of neurons in various ways. The combination of these attributes and tools provides us with a unique opportunity to better understand the neuronal and circuit basis of behavior.

Flies live in the same environment as us, assessing sensory cues and executing actions. Their brain uses similar neurotransmitters, channels, and wiring modules as those in mammalian brains. Therefore, it is perhaps not surprising that our brains employ similar computations and mechanisms in some cases. The fly will continue to be useful for understanding the principles and especially the mechanisms of basic brain functions. It will also be an interesting challenge to

inquire into the fly's hidden repertoire of higher order capabilities. It is tempting to speculate that the nearly unexplored protocerebra occupying the large portion of the fly central brain are responsible for these higher order functions. Comparative studies across animals should indicate the qualitative difference in cognitive functions that are executable by brains with different complexity. Some evidence already suggests that flies are able to form a kind of working memory (Neuser et al., 2008) and make simple decisions (Pick and Strauss, 2005; Maimon et al., 2008). *Drosophila* may thus help reveal the fundamental computations that support at least the primitive forms of cognitive abilities. Even if the algorithms behind these computations turn out to be specific to flies, it will be fascinating to discover an efficient algorithm that can be implemented with a small number of computational units.

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