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ORIGINAL PAPER

The diversity of lobula plate tangential cells (LPTCs) in the *Drosophila* motion vision system

Huayi Wei[1] · Ha Young Kyung[1] · Priscilla J. Kim[1] · Claude Desplan[1]

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Abstract

To navigate through the environment, animals rely on visual feedback to control their movements relative to their surroundings. In dipteran flies, visual feedback is provided by the wide-field motion-sensitive neurons in the visual system called lobula plate tangential cells (LPTCs). Understanding the role of LPTCs in fly behaviors can address many fundamental questions on how sensory circuits guide behaviors. The blowfly was estimated to have ~60 LPTCs, but only a few have been identified in *Drosophila*. We conducted a Gal4 driver screen and identified five LPTC subtypes in *Drosophila*, based on their morphological characteristics: LPTCs have large arborizations in the lobula plate and project to the central brain. We compared their morphologies to the blowfly LPTCs and named them after the most similar blowfly cells: CH, H1, H2, FD1 and FD3, and V1. We further characterized their pre- and post-synaptic organizations, as well as their neurotransmitter profiles. These anatomical features largely agree with the anatomy and function of their likely blowfly counterparts. Nevertheless, several anatomical details indicate the *Drosophila* LPTCs may have more complex functions. Our characterization of these five LPTCs in *Drosophila* will facilitate further functional studies to understand their roles in the visual circuits that instruct fly behaviors.

Keywords Motion vision · LPTC · Lobula plate · Optomotor · *Drosophila*

Abbreviations

BTF	Back to front
c.l.	Contralateral
CH	Centrifugal horizontal
ChAT	Choline acetyltransferase
dCH	Dorsal centrifugal horizontal
DMK	Denmark
dvGlut	*Drosophila* vesicular glutamate transporter
FD	Figure detection
FTB	Front to back
HS	Horizontal system
i.p.	Ipsilateral
IPS	Inferior posterior slope
LOP	Lobula plate
LPTCs	Lobula plate tangential cells
MCFO	MultiColor FlpOut
PLP	Posterior lateral protocerebrum
PS	Posterior slope
SyteGFP	Synaptotagmin eGFP
vCH	Ventral centrifugal horizontal
VS	Vertical system

✉ Claude Desplan
cd38@nyu.edu

[1] Department of Biology, New York University, New York, NY, USA

Introduction

As we navigate through a visual environment, our motion relative to our surroundings casts an optic flow pattern on our retina in the opposite direction to our self-motion. This visual feedback informs us about how we have moved. Any deviation from the set path is relayed back to our nervous system, enabling self-correction to stay on path. In dipteran flies, the visual feedback for self-motion is captured by a specific group of neurons—the lobula plate tangential cells (LPTCs) (Krapp and Hengstenberg 1996; Buschbeck and Strausfeld 1997; Borst and Haag 2002; Nordström et al. 2008).

LPTCs have elaborate arborizations in the last motion-processing neuropil of the fly optic lobe—the lobula plate. LPTCs are the major outputs from the lobula plate, projecting wide-field motion information to higher processing centers in the central brain, as well onto neck motor neurons

and descending neurons (Hausen et al. 1980; Borst and Haag 2002; Haag et al. 2007; Wertz et al. 2008, 2012; Kim et al. 2015; Suver et al. 2016). T4 and T5 neurons, which encode local motion information, are the major inputs to the lobula plate (Schnell et al. 2012; Maisak et al. 2013).

The direction sensitivity of LPTCs largely arises from integrating local motion information from presynaptic T4 and T5 neurons to derive global motion information (Schnell et al. 2012; Mauss et al. 2014; Barnhart et al. 2018). T4 neurons convey ON local motion for moving edges with luminance increase, whereas T5 neurons provide OFF local motion for luminance decrease (Maisak et al. 2013). There are four different subtypes of T4 and of T5, each encoding local motion in one cardinal direction: front to back (FTB), back to front (BTF), upward and downward (Maisak et al. 2013). The four T4 and four T5 subtypes innervate different layers of the lobula plate, which forms a four-layered structure where each layer represents motion in one direction (Maisak et al. 2013). Most LPTCs described to date innervate a specific layer of the lobula plate, and thus have an overall direction preference (Joesch et al. 2008; Schnell et al. 2010). For instance, the horizontal system (HS) cells, a set of three neurons per eye present in both *Drosophila* and the blowfly, innervate layer 1 of the lobula plate (in *Drosophila*) and prefer FTB motion (Hausen 1982a, b; Schnell et al. 2010).

In the past decades, the increasing availability of a powerful genetic toolkit in *Drosophila* has kindled a new wave of inquiries on LPTCs. Notably, studies in *Drosophila* on the role of HS in optomotor behavior and head-stabilizing behavior have demonstrated the insufficiency of silencing HS alone in abolishing horizontal tuning behaviors (Kim et al. 2017; Busch et al. 2018). These studies suggest that there are other horizontal-sensing LPTCs in *Drosophila* that contribute to these behaviors. In the blowfly, it was estimated that there are around 60 LPTCs, and they form subnetworks that drive distinctive behaviors (Hausen et al. 1980; Haag and Borst 2001, 2002; Borst and Haag 2002). In *Drosophila*, we only know about three HS neurons, VS (vertical system) neurons, a set of three neurons expressing Odd-skipped (including Hx), and a set of three neurons marked by the Foma-1 fly line (Rajashekhar and Shamprasad 2004; Joesch et al. 2008; Katsov and Clandinin 2008; Schnell et al. 2010; De Vries and Clandinin 2012; Levy and Larsen 2013; Wasserman et al. 2015). A recent EM reconstruction study added three more VS-like cells and two CH-like (centrifugal horizontal) neurons (Boergens et al. 2018). Given the similarities between the blowfly and the *Drosophila* visual systems, other LPTC subtypes are likely to exist in *Drosophila* as well. Finding these additional *Drosophila* LPTCs will enable us to answer network questions on how sensory neurons drive behavior—questions that cannot be fully addressed in the blowfly due to the lack of genetic tools.

To look for LPTC subtypes in *Drosophila*, we conducted a Gal4 driver screen based on the anatomical hallmark of LPTCs—large arborizations in the layers of the lobula plate with projections to the central brain. In this paper, we will present five different LPTC subtypes marked by fairly specific Gal4 drivers and compare them to the blowfly LPTCs. We tentatively named them after the blowfly neurons that resemble them the most in morphology: CH, H1, H2, FD1 and FD3, and V1. We also characterized their pre- and postsynaptic organizations, as well as their neurotransmitter profiles.

Methods

Fly stocks

Drosophila melanogaster was raised on standard medium at room temperature, except for crosses with the H1-Gal4 driver that were placed at 29 °C near eclosion time to boost the Gal4 expression level. Adult female progenies were dissected 2–4 days after eclosion. The Gal4 drivers used were from the Janelia Rubin collection and VDRC: R35A10-Gal4 (BSC#49897) for CH, VT045663 (VDRC#202651) for H1, R47F01-Gal4 (BSC#50318) for H2, R14C03-Gal4 (BSC#48602) for FD1 and FD3, and VT000771 (VDRC#201932) for V1. For sparse labeling, we used the flip-out tool FLEXAMP: yw, UAS-Flp; Gal80ts/Cyo; Act>y+>lexA,lexAop-myr-GFP/Tm6B (Bertet et al. 2014), and several MultiColor FlpOut (MCFO) lines: MCFO1 (BSC#64085), MCFO3 (BSC#64087), MCFO4 (BSC#64088), MCFO5 (BSC#64089) (Nern et al. 2015). To check neurotransmitter profiles, we used two transgenic lines: yw; UAS-LexADBD,LexAop-CD8GFP/cyo; GAD1^{MI09277}-p65AD/Tm6c,sb (constructed based on BSC#60322 (Diao et al. 2015; Romano et al. 2018)), and w;LexAop-nlsRFP,dvGlut^{MI04979}-QF2,QUAS-nucLacZ/CyO,DGY;ChA^{MI04508}-T2A-LexA-QFAD/TM6b, Sb, Dfd-GMR-YFP (from Matthias Landgraf) (Diao et al. 2015). Other transgenic constructs include: UAS-DenMark,UAS-Syt.eGFP; In(3L)D,mirrSaiD′D′/Tm6C,sb (BSC#33064), 10XUAS-IVS-myr::GFP (BSC#32197), 20XUAS-6XGFP (BSC#52261), and 10XUAS-IVS-mCD8::RFP (BSC#32219). BSC# = Bloomington Stock Center number. VDRC# = Vienna *Drosophila* Resource Center ID.

Staining and imaging

Dissection of adult fly brains was performed in cold PB solution. The tissues were fixed in 4% PFA for 25 min at room temperature, followed by washing with 0.1% PBT (0.1% Triton in PBS). Before primary antibody incubation, the tissues were incubated at room temperature in PBTDS (0.1% PBT

plus 5% donkey serum) for 1.5 h. For antibody stainings, all incubations were performed at 4 °C. Tissues were incubated for two nights in primary antibodies in PBTDS, washed with 0.1% PBT, then incubated in secondary antibodies in PBTDS for two nights. For MCFO staining, we performed additional tertiary antibody incubation overnight. Finally, tissues were incubated in SlowFade at 4 °C overnight, and then in fresh SlowFade for another 2 h at room temperature before mounting. Confocal microscopes (Leica SP5 and SP8) were used to image the mounted brains.

For MCFO1, the Gal4 driver lines were crossed with MCFO1 virgins. Female offspring (1 or 2 days old) were heat-shocked at 38 °C for 50 min and dissected 3 days later. For all MCFO experiments, the primary antibodies used were: mouse α-nc82 (1:25; DSHB AB_2314866), rat α-FLAG Tag (1:200; Novus AB_1625981) and rabbit α-HA Tag (1:200; CST AB_1549585). Secondary antibodies: AF405 donkey α-mouse (1:50; AB_2687445), ATTO647 donkey α-rat (1:200; ab150155), AF488 donkey α-rabbit (1:200; AB_2636877). Tertiary antibodies: DL550 mouse α-V5 (1:400; MCA1360D550GA).

For all GFP and RFP stainings, the primary antibodies used were: mouse α-nc82 (as mentioned before), chicken α-GFP (1:200; Sigma-Aldrich AB16901) or sheep α-GFP (1:200; Bio-Rad 4745-1051), and rabbit α-RFP (1:200; MBL PM005). Secondary antibodies used were: ATTO647 donkey α-mouse (as mentioned before), AF488 donkey α-chicken (as mentioned before) or α-sheep (1:200; AB_2534082), and 555 donkey α-rabbit (1:200; AB_162543).

To image the layers of lobula plate, we mounted brains on their ventral end and imaged them along the dorsal-ventral axis.

Image processing

All images were processed with the open-source software Fiji ImageJ. To enhance image clarity, we used "Subtract Background", "Despeckle", "Smooth" and "Sharpen". For all single-neuron silhouettes, we followed the neuron through an image stack, *z*-projected small portions of the stack, cleared away the irrelevant parts of the *z*-projections, and finally took the maximum of all sequentially *z*-projected images.

Results

CH-like neurons

We identified a pair of neurons that have elaborate processes in the lobula plate marked by the R35A10-Gal4 driver (Fig. 1a). Using the MultiColor FlpOut (MCFO) approach, we obtained the single-neuron morphologies of this neuronal pair (Fig. 1b). One covers the dorsal lobula plate, while the other covers the ventral portion. In contrast to their elaborate processes in the lobula plate, their limited central brain processes form a diamond shape in the inferior posterior slope (IPS). While all the processes are posterior, their cell bodies are at the anterior surface, between two antennal lobes, right across the midline (Fig. 1b). Their morphology resembles that of the blowfly centrifugal horizontal (CH) cells (Fig. 1c), which include the dorsal CH (dCH) and the ventral CH (vCH) (Eckert and Dvorak 1983). The *Drosophila* CH neurons were recently reported in an EM reconstruction study (Boergens et al. 2018). Although this EM study did not include cell body location and only traced out the major branches of the CH neurons, their partially reconstructed skeletons agree with the morphology of the CH neurons that we have identified here.

The blowfly CH cells are sensitive to FTB visual motion. This is consistent with our observation that the lobula plate arborizations of *Drosophila* CH neurons reside in layer 1 (Fig. 1d)—the layer that receives FTB local motion inputs from T4 and T5 and where HS neurons project (Maisak et al. 2013).

We examined the pre- and post-synaptic organization of CH neurons by driving DenMark—a marker for postsynaptic processes (Nicolaï et al. 2010), and synaptotagmin—a marker for presynaptic processes in these neurons (Littleton et al. 1993). While the central brain processes only express DenMark and thus appear to be purely postsynaptic, the lobula plate side expresses a mixture of both DenMark and synaptotagmin, suggesting both pre- and post-synaptic terminals. Although having mixed polarity is unusual in vertebrate neurons, it is prevalent in invertebrate neurons (White et al. 1986; Rolls 2011). In fact, an EM study reported the exact same pre- and post-synaptic organization of the blowfly vCH (Gauck et al. 1997).

CH neurons are GABAergic in the blowfly (Meyer et al. 1986; Gauck et al. 1997). To verify whether *Drosophila* CH-like cells are also GABAergic, we intersected our R35A10-Gal4 driver with a GAD1 hemi driver (see Methods for details). The intersection of these two constructs clearly labels CH cell bodies, along with other unidentified small medulla neurons (Fig. 1f).

H1-like neuron

The VT045663-Gal4 driver marks an H1-like neuron that innervates lobula plates on both sides of the brain. This driver was initially identified by Mark Frye's laboratory, which kindly shared it with us for further verification and characterization. H1-like neurons have elaborate processes that cover almost the entire lobula plate on both sides (Fig. 2a, b). The ipsilateral arborization of an H1-like

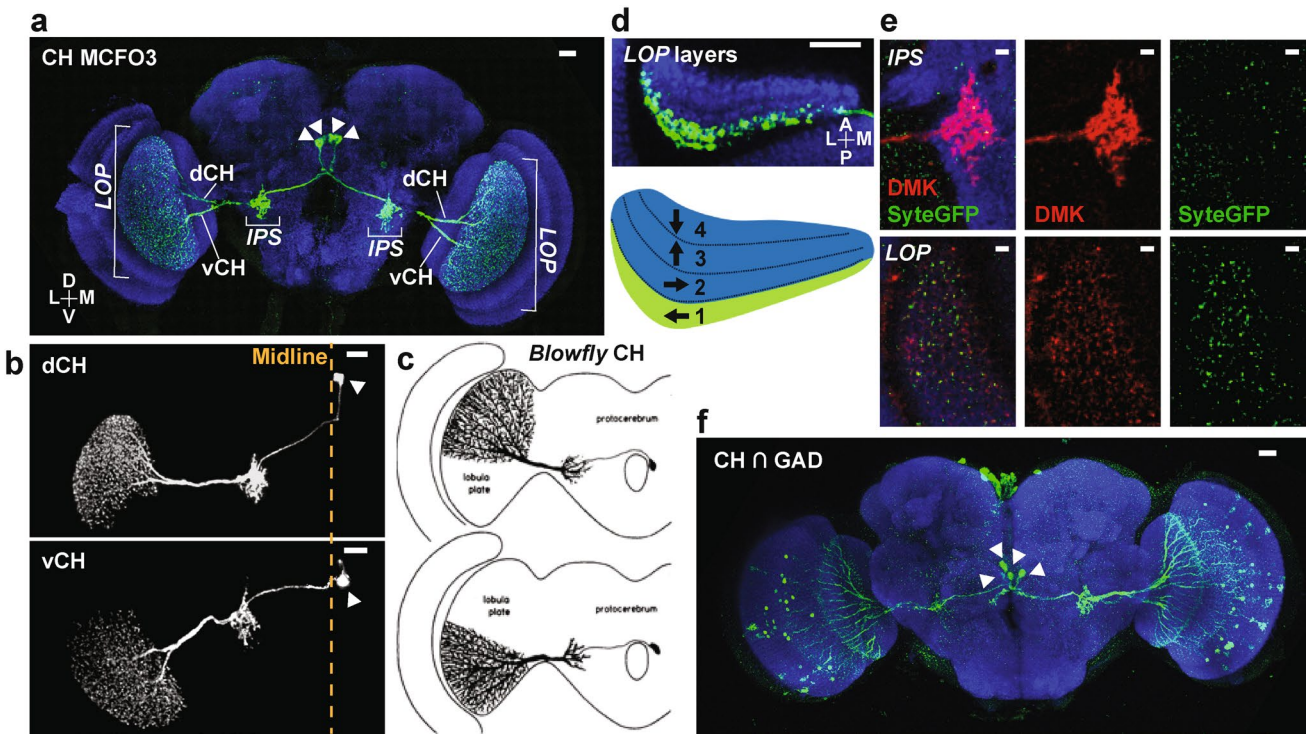

Fig. 1 Characterization of CH-like neurons. **a** A pair of CH-like neurons on each side of the brain. Arrowheads point to the cell bodies. They have processes in both the LOP and IPS. The driver R35A10-Gal4 was crossed to MCFO3 to only show the CH-like neurons (blue: anti-nc82 staining). **b** Single-cell morphology of a dCH-like neuron (up) and a vCH-like neuron (down). These silhouettes are obtained by cropping single-channel images of MCFO staining with Fiji ImageJ (up: MCFO1; down MCFO3). **c** Drawing of the blowfly CH neurons. Modified from Fig. 1 in Eckert and Dvorak (1983). **d** CH-like neurons reside in layer 1 of the LOP (upper panel: staining of LOP layers obtained by imaging the brain along the DV-axis; lower panel: schematic of LOP layers). **e** CH-like neurons have postsynaptic processes (labeled by DMK) in the IPS and a mixture of pre- and postsynaptic processes (labeled by both SyteGFP and DMK) in the LOP. **f** Intersection of R35A10-Gal4 with GAD1. Arrowheads point to the cell bodies of CH-like neurons. The intersection also captures some small medulla neurons. *MCFO* MultiColor FlpOut, *LOP* lobula plate, *IPS* inferior posterior slope, *DMK* DenMark, *SyteGFP* synaptotagmin eGFP

neuron intermingles with the contralateral projection from the H1-like neuron on the opposite side of the brain.

As this Gal4 driver is weak, it sometimes only labels the H1-like neuron on one side of the brain, which we used to clarify its single-cell morphology (Fig. 2b). Starting from one lobula plate, the main branch runs in the dorsal-anterior direction. The cell body of the H1-like neuron lies about halfway through, in the cleft between the optic lobe and the central brain, sometimes next to the lateral horn, other times next to the superior lateral protocerebrum. From there, the main branch goes further dorsal-anterior and forms an 'M' shape over the superior medial protocerebrum. It then follows an almost symmetrical path to reach the contralateral lobula plate. These morphological characteristics remarkably resemble the blowfly H1 neuron, except that the cell bodies of *Drosophila* H1-like neurons are not located in the center of the brain (Fig. 2c).

The ipsilateral lobula plate processes of the H1-like neuron express DenMark and are thus postsynaptic, whereas the contralateral lobula plate processes express synaptotagmin and are thus presynaptic (Fig. 2e). We utilized this property to examine which layer the neuron innervates on each side. The ipsilateral processes are only present in layer 2 of the lobula plate, but the contralateral processes are in both layer 1 and 2 (Fig. 2d). As layer 2 receives BTF local motion inputs (Maisak et al. 2013), these results are consistent with the blowfly H1, which prefers BTF motion (Eckert 1980).

The blowfly H1 makes excitatory connections with other LPTCs and is thus likely to be cholinergic (Haag and Borst 2001). To check the neurotransmitter expressed in *Drosophila* H1, we co-expressed the VT045663-Gal4 driver with drivers for choline acetyltransferase (ChAT) and the *Drosophila* vesicular glutamate transporter (dvGlut). ChAT is a marker for acetylcholine, the major excitatory neurotransmitter in the *Drosophila* brain. dvGlut reports the presence of glutamate, a mostly inhibitory neurotransmitter in the visual system (Mauss et al. 2015; Richter et al. 2018), although glutamate can also act as an excitatory neurotransmitter depending on which receptor is present (Li et al.

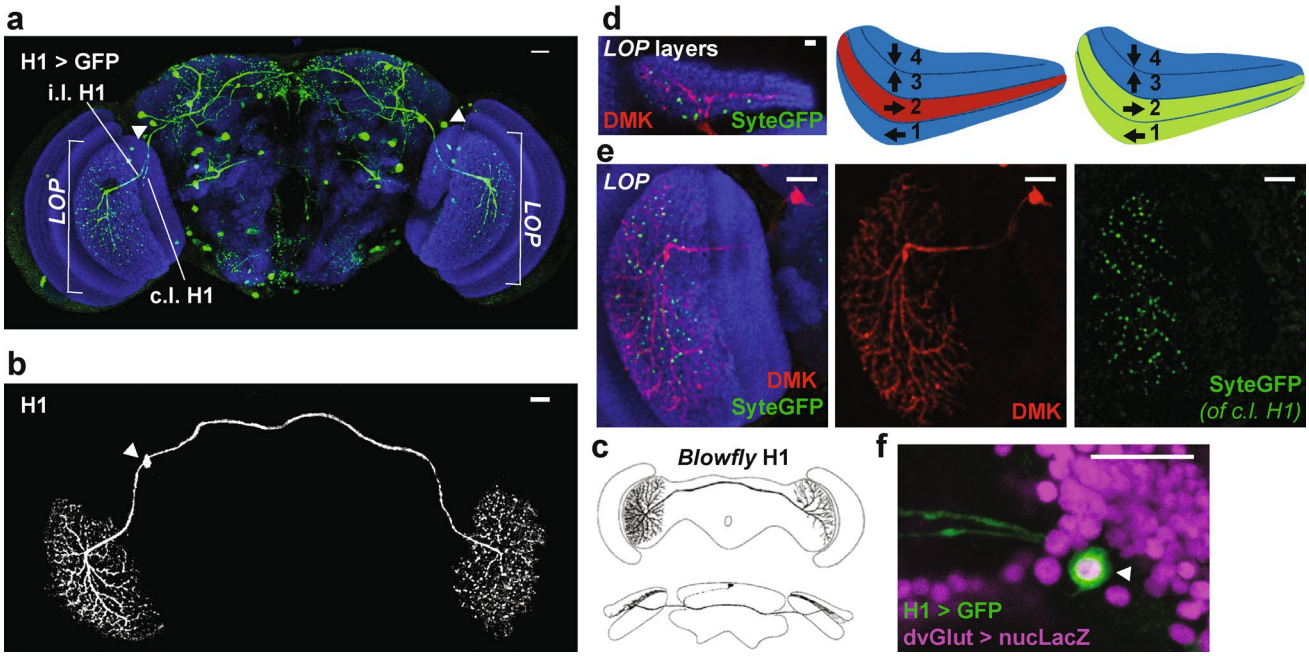

Fig. 2 Characterization of H1-like neuron. **a** Staining of VT045663-Gal4 driving 20xUAS-6XGFP. Arrowheads point to the cell bodies of H1-like neurons on each side. The ipsilateral H1-like neuron has processes that overlay the processes from the contralateral H1-like neuron. **b** Single-cell morphology of an H1-neuron. The image was obtained by cropping out the H1-like neuron from a VT045663-Gal4 > 20X UAS-6XGFP staining that happened to only label the H1-like neuron on one side of the brain. **c** Sketch of a single blowfly H1 neuron. Modified from Fig. 2 in Eckert (1980). **d** The ipsilateral processes (labeled by DMK) of H1-like neuron reside in layer 2 of the LOP. Presynaptic contralateral processes (labeled by SyteGFP) are in both layer 1 and 2 of the LOP. **e** Ipsilateral LOP processes express DMK and are postsynaptic, while contralateral processes in LOP express presynaptic SyteGFP. **f** The expression of H1-like neuronal driver colocalizes with a dvGlut driver. *i.p* ipsilateral, *c.l.* contralateral, *LOP* lobula plate, *DMK* DenMark, *SyteGFP* synaptotagmin eGFP, *dvGlut* the *Drosophila* vesicular glutamate transporter

2016). To our surprise, the *Drosophila* H1 driver colocalizes with dvGlut instead of ChAT (Fig. 2f).

H2-like neuron

We identified a Gal4 driver, R47F01 that labels a large contralateral-projecting LPTC (Fig. 3a). By cleaning up an image staining, we obtained the single-cell morphology of this neuron (Fig. 3b). Its large arborizations cover almost the entire lobula plate except the triangular corners at the dorsal and ventral tips, closely resembling the arborization pattern of the blowfly H2 neuron (Fig. 3c). The branch of the *Drosophila* H2-like neuron goes dorsal-anterior as it enters the central brain and then ventral-posterior at it reaches the contralateral IPS (Fig. 3a, b). Its cell body is located near the posterior surface, at the edge of the central brain (Fig. 3a).

The *Drosophila* H2-like neuron projects to the layer 2 of the lobula plate, which receives BTF local motion inputs (Fig. 3d). In agreement with this anatomical property, the blowfly H2 neuron responds preferentially to BTF motion (Farrow et al. 2006). The H2-like neuron expresses synaptotagmin in its contralateral IPS processes where it is thus presynaptic. Its lobula plate arborizations express a mixture of synaptotagmin and DenMark and might thus have both pre- and post-synaptic terminals. The blowfly H2 makes excitatory connections with other LPTCs (Haag and Borst 2001). In accord with this, we find that the *Drosophila* H2-like driver colocalizes with ChAT (Fig. 3f), a marker for acetylcholine—the major excitatory neurotransmitter in insect brains.

FD1-like and FD3-like neurons

We recognized two contralateral-projecting LPTCs labeled by the R14C03-Gal4 driver (Fig. 4a). We observed their single-cell morphology by crossing this driver to FLEX-AMP (a flip-out tool, see Methods for details) or MCFO5 (Fig. 4b). Both neurons have three ramifications: one in the ipsilateral lobula plate, one in the ipsilateral posterior lateral protocerebrum (PLP) and another in the contralateral IPS. Both neurons have their cell bodies at the edge of the central brain, near the posterior surface. The two neurons differ in their lobula plate arborization patterns. One neuron resembles the blowfly FD1 neuron, as it arborizes over the lateral rim of the lobula plate, but not the medial portion (Fig. 4b, c). The other neuron resembles the blowfly

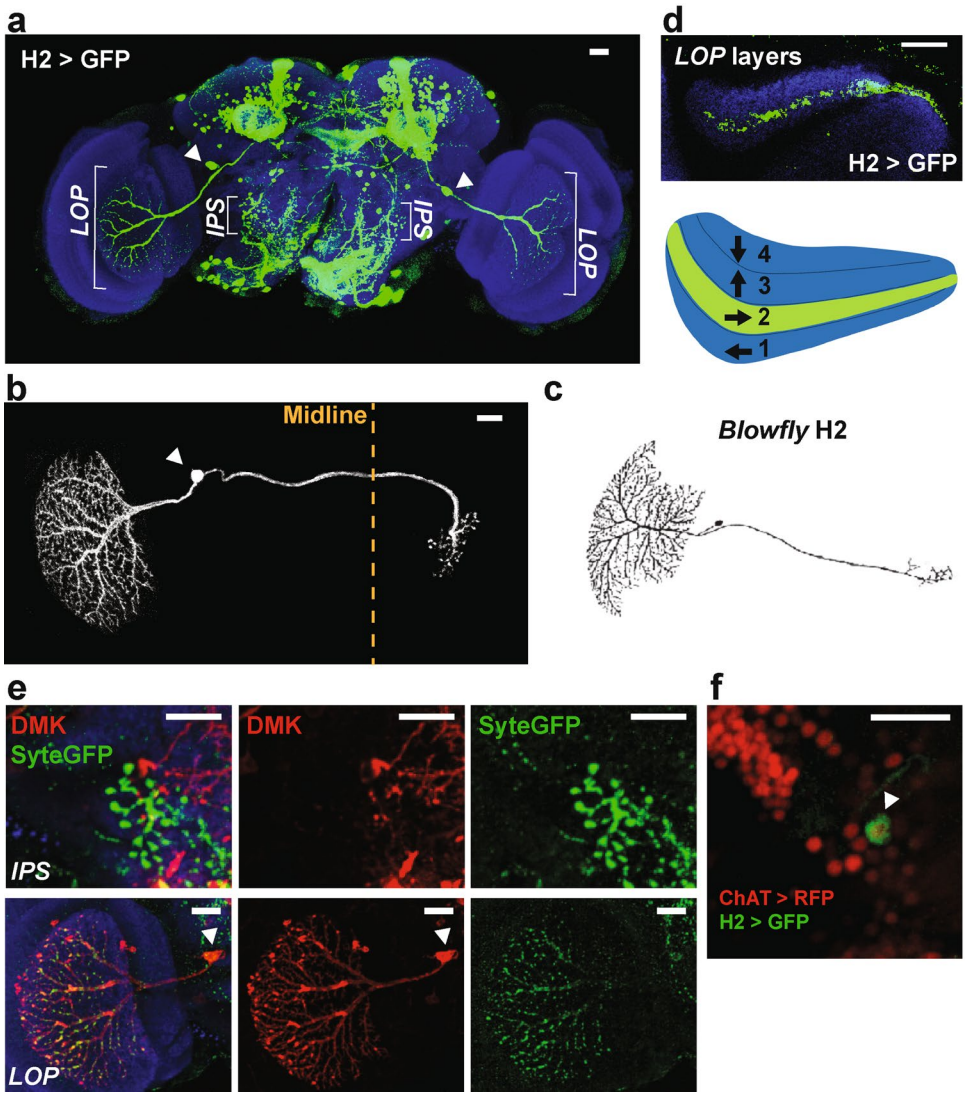

Fig. 3 Characterization of an H2-like neuron. **a** Staining of R47F01-Gal4 driving 20XUAS-6XGFP. Arrowheads point to the cell bodies of H2-like neurons on each side of the brain. Its central brain branch terminates in IPS. **b** Single-cell morphology of an H2-like neuron. The silhouette was obtained by cropping out the neuron from the staining of R47F01-Gal4 driving UAS-CD8-RFP with Fiji ImageJ. **c** Drawing of the blowfly H2 modified from Fig. 1 in Farrow et al. (2006). **d** The H2-like neuron projects to layer 2 of the LOP. **e** The LOP processes of the H2-like neuron express a mixture of postsynaptic DMK and presynaptic SyteGFP, whereas the central brain processes only express SyteGFP. **f** Expression of the H2-like neuronal driver colocalizes with the expression of ChAT. *LOP* lobula plate, *IPS* inferior posterior slope, *DMK* DenMark, *SyteGFP* synaptotagmin eGFP, *ChAT* choline acetyltransferase

FD3 neuron, as it covers the medial lobula plate but not the lateral portion (Fig. 4b, c). Interestingly, the *Drosophila* FD1-like neuron has a small branch that extends from the lobula plate to the lobula (Fig. 4b).

While the blowfly FD1 neuron prefers FTB motion (Egelhaaf 1985), the *Drosophila* FD1-like neuron innervates both layer 1 (FTB) and layer 2 (BTF) and also has faint processes in layer 3 (upwards) (Fig. 4d). The FD3-like neuron only innervates layer 2 (Fig. 4d), which is consistent with the BTF direction preference of the blowfly FD3 neuron (Egelhaaf 1985).

Both neurons express synaptotagmin, a presynaptic marker, in their contralateral IPS processes. Their lobula plate and PLP processes express DenMark, a postsynaptic marker. The driver colocalizes with ChAT in both neurons that are thus cholinergic (Fig. 4f).

A V1-like neuron

The VT000771-Gal4 driver labels a neuron that projects to the contralateral lobula plate (Fig. 5a). MCFO4 enabled us to see clearly its single-cell morphology, which resembles the blowfly V1 neuron (Fig. 5b). The *Drosophila* V1-like neuron has its cell body by the midline, at the posterior surface around the protocerebral bridge. It has dense processes in the ipsilateral posterior slope (PS). Its contralateral lobula plate projection splits up into two branches: a dorsal medial branch in layer 3 (upwards) and a ventral branch in layer 1 (FTB) (Fig. 5a).

Most LPTCs have postsynaptic terminals in the lobula plate, and presynaptic terminals in the central brain, presumably receiving local motion information from the lobula plate and projecting wide-field motion information to higher

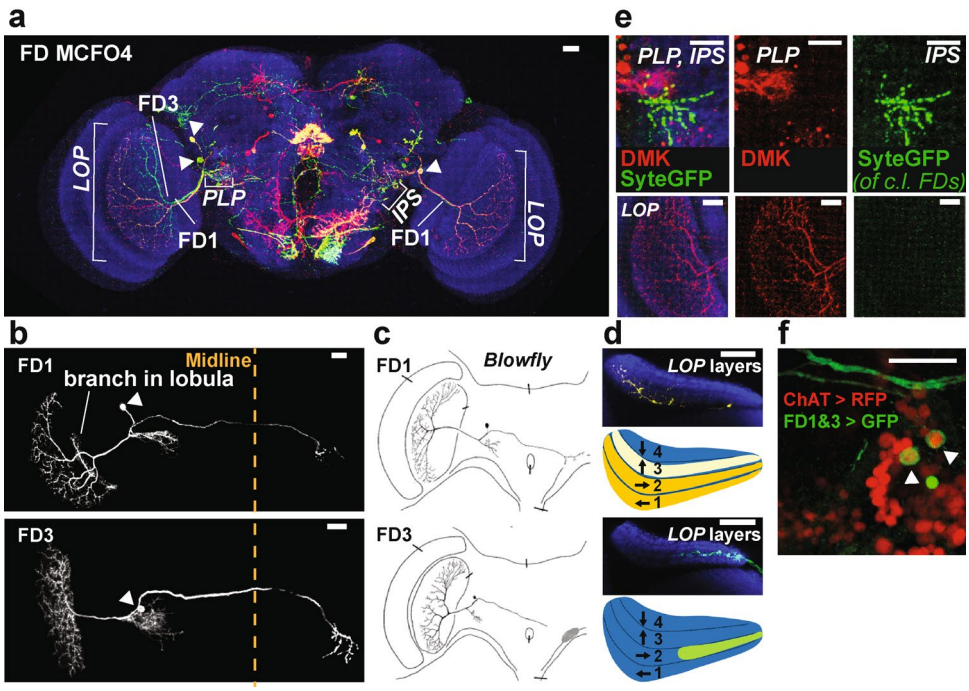

Fig. 4 Characterization of FD1-like and FD3-like neurons. **a** Staining of R14C03-Gal4 crossed to MCFO4. Two FD1-like neurons are labeled on both sides of the brain. An FD3-like neuron is labeled on the left side. In the central brain, both neurons have ipsilateral branches in the PLP and contralateral projections to the IPS. **b** Single-cell morphology of an FD1-like neuron (upper panel) and an FD3-like neuron (lower panel). These images are generated by cropping the neurons from sparse labeling staining obtained through crossing VT045663-Gal4 with FLEXAMP (a flip-out tool, see "Methods"; upper panel) or MCFO5 (lower panel). **c** Drawing of the blowfly FD1 and FD3 modified from Figs. 6a and 13, respectively, in Egelhaaf (1985). **d** FD1-like neuron occupies mainly layer 1 and 2 of the LOP, and only sparsely innervates layer 3. FD3-like neuron occupies layer 2 only. **e** Both FD1-like and FD3-like cells express postsynaptic DMK in the LOP and in the ipsilateral IPS. Their projections to the contralateral central brain express presynaptic SyteGFP. **f** Expression of FD1-like and FD3-like neurons colocalizes with the expression of ChAT. *MCFO* MultiColor FlpOut, *LOP* lobula plate, *IPS* inferior posterior slope, *PLP* posterior lateral protocerebrum, *DMK* DenMark, *SyteGFP* synaptotagmin eGFP, *ChAT* choline acetyltransferase

processing centers in the central brain. But this V1-like neuron has the opposite organization: it only expresses synaptotagmin—a presynaptic marker—in the lobula plate, and only DenMark—a postsynaptic marker—in the PS (Fig. 5d). This is consistent with the blowfly V1, which receives information from the axons of ipsilateral VS neurons in the central brain and relays that information to the contralateral lobula plate (Kurtz et al. 2001). The *Drosophila* V1-like neuron is cholinergic as its driver colocalizes with ChAT (Fig. 5f).

Discussion

We characterized five LPTC subtypes in *Drosophila* and named them based on their morphological similarities to the blowfly LPTCs. They are CH, H1, H2, FD1 and FD3, and V1. Despite the gross morphological resemblances, they all have small differences from their likely blowfly counterparts. CH, H1 and V1 have close resemblances except for the locations of their cell bodies. The *Drosophila* FD1 has lobula plate processes that cover more ventral regions than the blowfly FD1, and it also has a branch in the lobula, which is atypical for LPTCs (Fig. 4b, upper panel). However, the dissimilarities could arise from the ambiguity in the description of the blowfly LPTC morphologies, which were mostly based on sketches of dye-filled neurons.

The morphologies and anatomical locations of the five *Drosophila* LPTCs described here largely align well with our knowledge on the direction preference of these neurons in the blowfly. Among the five neurons, the CH neurons closely match their likely blowfly counterparts: they are both likely to prefer FTB motion, are GABAergic, and have mixed pre- and post-synaptic terminals in layer 1 of the lobula plate (Fig. 1). As we have limited profiles on the other four subtypes in the blowfly, further functional studies in *Drosophila* will be required to confirm their functional resemblance.

Nevertheless, the anatomical details of these five LPTCs support the observation in the blowfly that LPTCs are not purely output neurons passing wide-field motion information to the central brain. V1 is likely to serve as a feedback neuron to the visual system, as it gets information from its

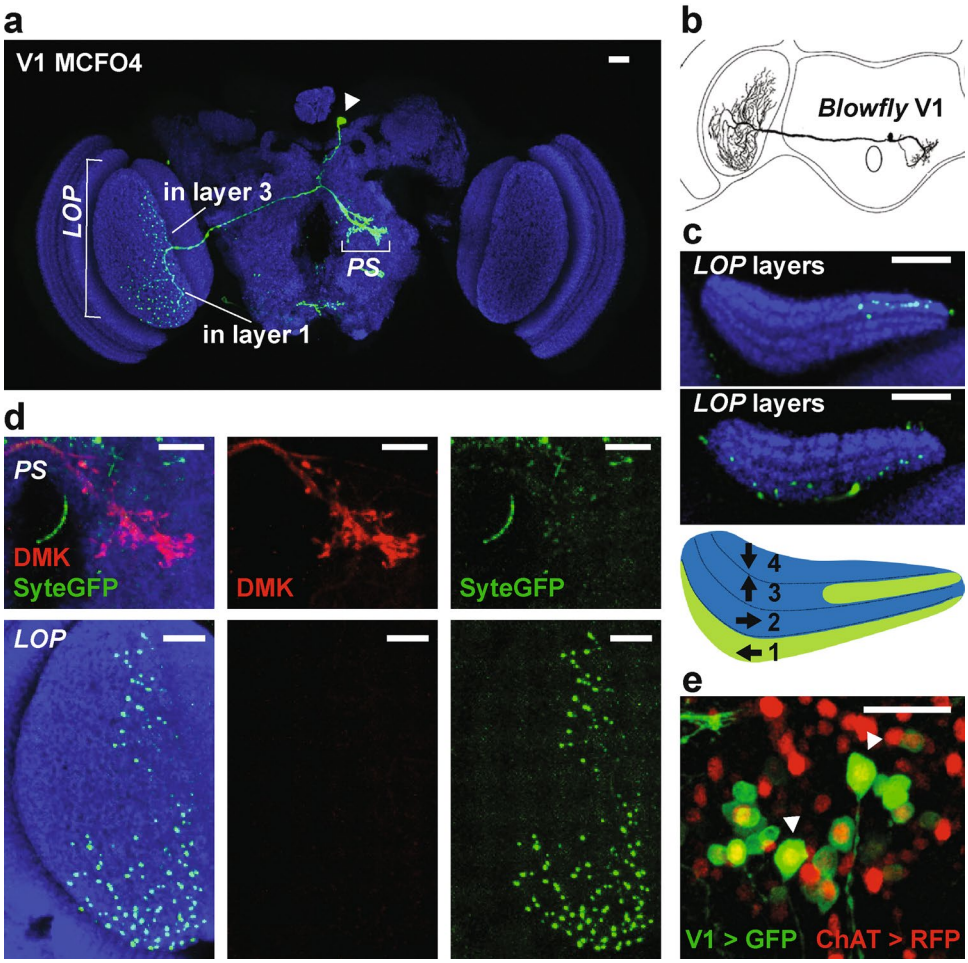

Fig. 5 Characterization of a V1-like neuron. **a** Single-cell morphology of a V1-like neuron. The image was obtained by crossing VT000771-Gal4 with MCFO4. The neuron has two branches in different parts of LOP, and its central brain processes are in PS. **b** Sketch of the blowfly V1 modified from Fig. 1a in Haag and Borst (2008). Copyright 2008 Society for Neuroscience. **c** The dorsal LOP branch of the V1-like neuron is in layer 3 of the LOP, whereas the ventral LOP branch is in layer 1. **d** The ipsilateral processes in the central brain express postsynaptic DMK, while the projection to the contralateral LOP expresses presynaptic SyteGFP. **e** Expression of V1-like neuronal driver colocalizes with the expression of ChAT. *MCFO* MultiColor FlpOut, *LOP* lobula plate, *PS* posterior slope, *DMK* DenMark, *SyteGFP* synaptotagmin eGFP, *ChAT* choline acetyltransferase

dendrites in the central brain and projects to the lobula plate, potentially communicating with other LPTCs there. H1, with dendritic processes in one lobula plate and axonal processes in the other, could coordinate between the two optic lobes to enable neuronal communications across the entire visual space. CH and H2 are more complex with mixed dendritic and axonal terminals in the lobula plate. Lastly, both FD1 and FD3 have dendritic terminals in the PLP in additional to their dendrites in the lobula plate.

LPTCs are thought to be highly adaptive to the behavior profiles of different fly species (Buschbeck and Strausfeld 1997). As the blowfly is much larger and faster than *Drosophila* and they have very different food sources and habitats, the two fly species may differ in their LPTCs. Without a deeper understanding of the differences in their naturalistic behaviors, we can only speculate about the functions of these five *Drosophila* LPTCs based on their likely blowfly counterparts.

Yet, with the information presented in this paper alone, we can suggest that the functions of LPTCs are perhaps more complex than previously imagined. H2 has mixed dendritic and axonal terminals in the lobula plate. This was never reported nor speculated in the blowfly literature. This could imply either interspecies differences or undiscovered postsynaptic partners of H2 in the lobula plate. Furthermore, FD1 has an additional branch in the lobula, which was not described in the blowfly literature. It is unclear what kind of input it receives from the lobula and how that adds to its overall ability to differentiate between figure and ground.

Our characterization adds a detailed description of five LPTC subtypes to our existing knowledge of the *Drosophila* LPTCs. We also present a list of LPTC-Gal4 drivers that are relatively clean in the optic lobes, which could be used for patch-clamp recordings, calcium imaging and some limited behavior studies. Recent studies on LPTCs have pointed to the need to understand their network effect in mediating behaviors, especially the horizontal network that controls horizontal tuning behavior in optomotor paradigms and head motion during walking and flying (Fujiwara et al. 2017; Kim et al. 2017; Busch et al. 2018). In this paper, we provide drivers and morphological knowledge to at least three more LPTC subtypes (CH, H1, H2) in the horizontal network, facilitating future functional inquiries on how visual feedback networks mediate fly behaviors.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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