IJIREEICE

ISSN (Online) 2321 – 2004
ISSN (Print) 2321 – 5526

INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH IN ELECTRICAL, ELECTRONICS, INSTRUMENTATION AND CONTROL ENGINEERING
Vol. 4, Issue 4, April 2016

Identification and Analysis of Neural Activity and the Visual System in Drosophila

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Abstract: The task of the visual system is to translate light into neuronal encoded information. This translation of photons into neuronal signals is achieved by photoreceptor neurons (PRs), specialized sensory neurons, located in the eye. Upon perception of light the PRs will send a signal to target neurons, which represent a first station of visual processing. Increasing complexity of visual processing stems from the number of distinct PR subtypes and their various types of target neurons that are contacted. The visual system of the fruit fly larva represents a simple visual system (larval optic neuropil, LON) that consists of 12 PRs falling into two classes: blue-sensitive PRs expressing Rhodopsin 5 (Rh5) and green-sensitive PRs expressing Rhodopsin 6 (Rh6). These afferents contact a small number of target neurons, including optic lobe pioneers (OLPs) and lateral clock neurons (LNs). We combine the use of genetic markers to label both PR subtypes and the distinct, identifiable sets of target neurons with a serial EM reconstruction to generate a high-resolution map of the larval optic neuropil.

Keywords: Drosophila, Lateral Clock Neurons (LNs), Neural Activity, Photoreceptor Neurons (PRs), Fruit Fly Larva.

INTRODUCTION

The photoreceptor neurons (PRs) of the eye transform lightinduced signals into neuronal information, which is transmitted to the higher order neurons in visual information processing. Depending on which sensory.receptor gene is expressed in a PR, the neuron will become sensitive to a certain spectrum of light. In the case of PRs sensory receptor genes are rhodopsin. In the mammalian visual.

System integration of visual sensory information already occurs in the retina, while in insects PRs connect to target neurons in the optic Ganglia in the brain. In the fruit fly Drosophila melanogaster the adult compound eye is composed of about 800 unit-eyes, called ommatidia. Each ommatidium comprises eight PRs, which are subdivided into six outer PRs (R1–R6) and two inner PRs (R7 and R8). Outer PRs are required for motion detection and dim-light vision and express the broad-wavelength sensitive photoreceptor protein Rhodopsin1.

The two inner PRs are required for colour discrimination and colour vision and express an array of four different rhodopsin genes (rh3, rh4, rh5 or rh6). Axonal projections of the PRs terminate in the optic ganglia of the adult brain (Borst, 2009; Sanes and Zipursky, 2010). The adult insect brain is traditionally subdivided into the central brain and the optic lobe. The optic lobe consists of four neuropil compartments; the outer two, lamina and medulla, receive input from retinal photoreceptors; the inner two, lobula and lobula plate, connect the medulla to the visual centres ("optic foci") of the central brain. In line with this functional diversification outer and inner PRs connect to target neurons in distinct optic neuropils the lamina (outer PRs) and medulla (inner PRs), respectively (Morante and Desplan, 2004).

Compared to the rather complex organization of the adult visualsystem the larval visual system of is comparably simple. The larval eye (Bolwig's organ; BO) is only composed of 12 PRs and can be further divided into two PR subtypes (Daniel et al., 1999; Green et al., 1993).Eight PRs express the green-sensitive Rh6, while the remaining fourPRs express the blue-sensitive Rh5 (Sprecher et al., 2007; Sprecher and Desplan, 2008). PRs of the larval eye extend their axons into thelarval optic neuropil (LON), a small neuropil compartment and firstcenter for visual information processing in the larval brain. Larval PRs provide two essential functions for animal behaviour: first in rapid light avoidance and second for circadian rhythm control (Mazzoni et al.2005). In order to gain a more detailed and complete view on thedevelopment and anatomy of LON-innervating neurons we have initiated a project where we combine electron microscopy with confocal microscopy. We integrate anatomical findings of a 3D reconstructed serial TEM (transmission electron microscopy) stack with molecular and genetic markers data gathered from confocal microscopic stacks. This paper presents the first in a series analysing neuronal ultrastructure and connectivity of the LON. We provide here an overview of the developmental changes of the LON from first instar larva to the adult fly. We introduce different elements of the LON and their ultrastructure and connectivity within the LON, focusing in particular on the terminal arborisation of the photoreceptor neurons providing sensory input to the LON.

NEURAL ACTIVITY

Neuroscientists have made impressive advances in understanding the micro scale function of singleneurons and the macro scale activity of the human brain. One can probe molecular and biophysical aspects of individual neurons and also view the human brain in actionwith magnetic resonance imaging (MRI) or magneto encephalography (MEG). However, the mechanisms of

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perception, cognition, and action remain mysterious because they emerge from the real-time interactions of large sets of neurons in densely interconnected, widespread neural circuits.

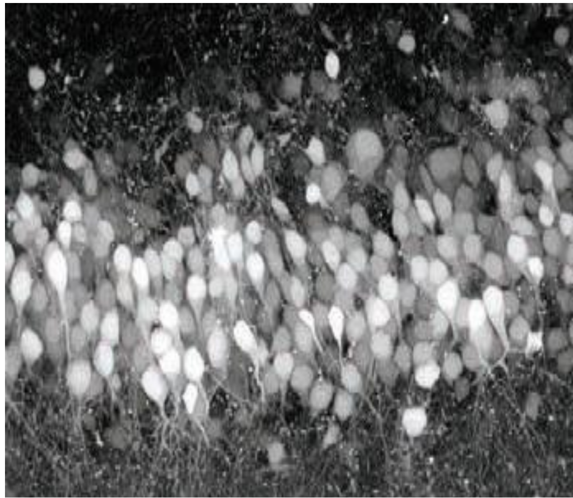

Fig 1 Neural activity

Neuronal activity in the hippo campus. Activity isshown by two-photon calcium imaging.It is time for a large-scale effort in neuroscienceto create and apply a new generation of tools to enable the functional mapping and control of neural activity in brains with cellular and millisecond resolution. This initiative, the Brain Activity Map (BAM), could put neuroscientists in a position to understand how the brain produces perception, action, memories, thoughts, and consciousness and be a major step toward a complete understanding of brain function and dysfunction. The function of neural circuits is an emergent property that arises from the coordinated activity of largenumbers of neurons. To capture this, we propose launching a large-scale, international public effort, the Brain Activity Map Project, aimed at reconstructing the full record of neural activity across complete neural circuits. This technological challenge could prove to be an invaluable step toward understanding fundamental and pathological brain processes.

MATERIALS AND METHODS

Drosophila strains and genetics:

Dissection and analysis of the brain were done as previouslydescribed (Sprecher et al., 2007). Secondary antibodiesused for confocal microscopy were Alexa-488, Alexa-555, and Alexa- 647 antibodies generated in goat (Molecular probes), all at 1:300 – 1:500 dilution.

Serial TEM acquisition and processing:

First instar larval brains were dissected and then fixed and embedded in epon resin as described in Johnson et al. (2009). A block containing a single brain was trimmed with a glass knife, and60-nm serial sections were cut on a Leica UC8 ultra tome. Ribbons ofsections were collected onto single-slot copper grids with pioloformmembranes. Sections were contrasted with 8% uranyl acetate and inReynold's lead citrate (Johnson et al., 2009). Serial sections wereimaged automatically with the software Leginon (Suloway et al., 2005) driving an FEI 12 electron microscope. A collection of partially overlapping images was acquired for each serial section.Images were automatically contrast-corrected and automatically montage within and across sections using the open source softwareTrakEM2 (Cardona et al., 2010). Each neuron and glial cell wasmanually reconstructed in 3D with computer-assisted segmentationtools provided by TrakEM2. Reconstructed neurons were rendered in3D with the software ImageJ 3D Viewer (Schmidt et al., 2010).

Anatomical methods:

In lucky cases, it can be possible to identify candidate cellular components of a circuit simply by visually screening Gal4 lines. The Gal4/UAS technique provides a way to drive ectopic gene expression in a genetically defined subpopulation of cells (see Refs [20] for reviews). When crossed to a green fluorescent protein (GFP) reporter line, a Gal4 line will sometimes label only a small number of neurons. Gal4 labeling can also be further restricted according to a neuron's lineage [19]. If the GFP-labelled neurons reside in a highly ordered brain region, their participation in a circuit can sometimes be obvious.

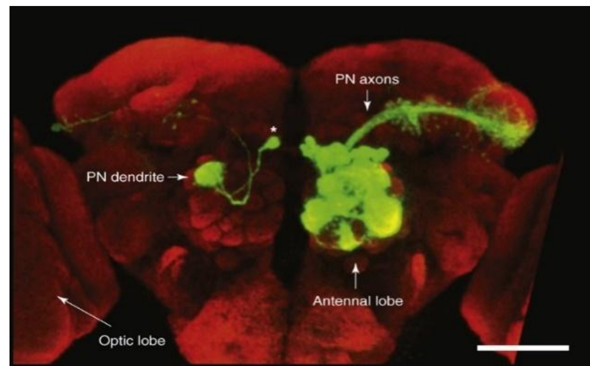

Fig 2 anatomical methods

This approach has been used to describe cellular components of three orderly circuits – the antennal lobe [12], mushroom body [13] and optic lobe [14].

Behavioural methods:

Historically, it has been more common to search for the genes rather than the neurons involved in a behaviour. However, gene identification via mutant screens can alsoprovide a handle for identifying neurons if the population of neurons expressing the gene is not too large or diverse. Cell-specific rescue experiments can be used to identify the specific set of neurons in which the functional gene is required for normal behaviour. This method has been used to identify neurons involved in circadian rhythms [15], courtship , temperature regulation [16] and learning and memory . A second of type of behavioural screen directly probes neurons rather than genes. In this screen, many candidate Gal4 lines are crossed with a UAS-transgene that silencesneurons (either by blocking neurotransmitter release, inhibiting spiking or killing the cells), and progeny are screened for defective behaviours. The goal is to identify neurons that are required for the behaviour (Figure 2b). A silencing screen should be particularly effective at identifying bottlenecks in a neural

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circuit (e.g. motorneurons). But if neural codes are distributed across large ensembles of central neurons, none of which are strictly required for behavioural performance, this approach might not yield a comprehensive set of important circuit elements. It will be interesting to see what kinds of neurons turn up in these screens. Onlya few large-scale screens have been reported so far but already some novel and intriguing circuit elements have emerged from these efforts.
One difficulty with this approach is the fact that many Gal4 lines label large and diverse sets of neurons. In this case, the number of neurons expressing the silencing UAS transgene can be narrowed down using combinatorial methods for transgene expression. Additionally, a project is under way to produce an enhancer library of Gal4 lines that label relatively small subsets of neurons.

Physiological methods:
A third approach is to use functional imaging. The idea is to express a fluorescent activity reporter (like G-CaMP) under the control of a pan-neuronal Gal4 line, or else a more restrictive Gal4 line arising from a screen. Imagingstimulus-evoked fluorescence in these flies should revealall the (labelled) neurons that are activated by a particularstimulus. However, the available genetically encoded activity sensors still lack sensitivity meaning that this method will reveal only neurons with high firing rates. This method could also be useful in identifying neurons that are downstream from a genetically identified set of neurons. For this type of experiment, the sensory stimulus could be bypassed with a genetically strategies for identifying neurons in sensory and behavioural circuits. (a) A broadly expressed promoter is used to express PA-GFP in a large population ofcandidate neurons Photo stimulation of the neuropil region containing the axons of known presynaptic neurons (box) increases PA-GFP fluorescence in thedendrites of postsynaptic neurons.

Activated GFP molecules diffuse out of the dendrite and into the cell body, revealing the location of the stomata of postsynaptic neurons Recordings can be made from these neurons by using the GFP signal to target a patch electrode. (b) In this approach, many different Gal4 lines are used to express a silencing agent such as tetanus toxin. If a behavioural defect is observed, this indicates that the cells labelled by the Gal4 line were somehow involved in the behaviour.

Comparison:
The optic neuropil of the early larva (LON) is formed by the sensory terminals of the larval eye (Bolwig's organ), aswell as endings of several small populations of primary neurons that transmit visual input to the central brain. In this early larval stage the LON is enclosed by the epithelial optic anlage that is situated at the ventrolateral surface of the brain (Figs. 3A, B). The optic anlage comprises an outer part (OOA), a curved hemi cylindrical structure, and an inner part (IOA), located medially adjacent to the outer optic anlage (Figs. 3A, B, D).

In preparations labelled with a synaptic marker, the LON appears as a synapse-rich, narrow process extending from the ventrolateral surface of the central brain (Fig. 3C).

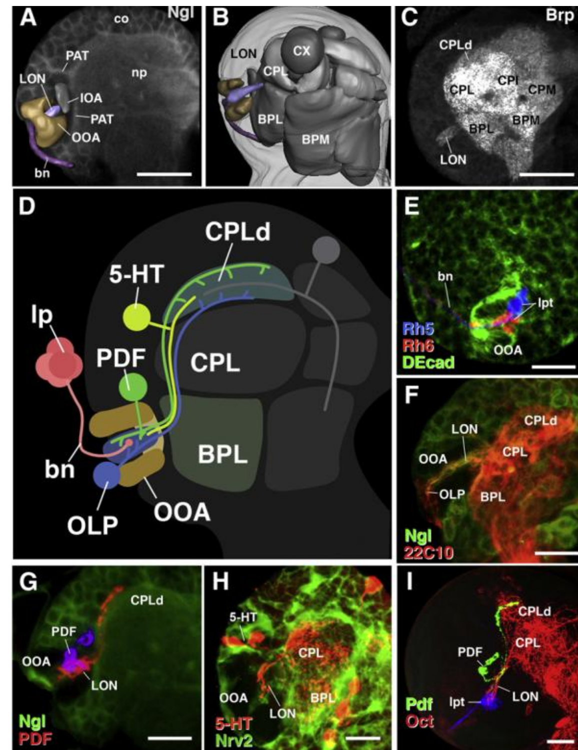

Fig 3 surface of the central brain

The twelve sensory PR axons of the larval eye enter the outer optic anlage hemi cylinder laterally and terminate in the LON (Figs. 3D, E). Larval PRs and their axons form two classes: oneclass of four cells expresses rh5; the other class of eight cells expressesrh6. As shown in Figs. 1E and described in more detail below, axonterminals of these two classes occupy largely non-overlapping territories in the LON. We found that at least two populations of neurons are postsynaptic to larval PRs (see below). One population is comprised of three optic lobe pioneer neurons (OLP) per hemisphere, the other the four neurons expressing the neuropeptide pigment dispersing factor (PDF)per hemisphere. These four neurons belong to the circadian clockcircuit and are termed lateral neurons (LNs). Cell bodies of the OLPs,which flank the optic anlage laterally are derived from thesame embryonic placode (called optic lobe placode (Green et al., 1993)) that also gives rise to optic anlage itself (Chang et al., 2003). OLP axons fasciculate with PR axons as they enter the optic neuropil from laterally (Campos et al., 1995; Nassif et al., 1998; Tix et al., 1989). OLP axons exit the LON medially and enter the central brain neuropil. The LNs form part of a central brain lineage located in the dorsal brain cortex (Fig. 1G). LNs cell body fibres project ventrally through an opening in the outer optic anlage (Figs. 1D, G); after entering the optic neuropil.

CONCLUSION

The function of neural circuits is an emergent property that arises from the coordinated activity of largenumbers of neurons. To capture this, we propose launching a large-scale, international public effort, the Brain Activity Map Project, aimed at reconstructing the full record of neural activity across complete neural circuits. This technological

challenge could prove to be an invaluable step toward understanding fundamental and pathological brain processes.

The virtues of Drosophila as a model for systems neuroscienceare easy to grasp. However, the limitations of this model organism receive less public attention. Some of these limitations might be swept aside by future breakthroughs, but others might be intrinsic to the fly. One problem is that the small size of the Drosophila brain will make it extremely challenging to perform electro physiological measurements in the behaving fly. Functional imaging might offer a solution to this problem, but imaging is even more sensitive than electro physiology to movements of the preparation. Because neural circuitscan be highly stereotyped in an enormous amount simply by correlating neural activity in one individual fly with the behaviour of a different individual. However, without simultaneous measurements, it will not be possible to correlate trial-to-trial fluctuations in neural activity with variations in behavioural performance.

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