Neuroscience Research 129 (2018) 8–16

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Review article

What can tiny mushrooms in fruit flies tell us about learning and memory?

Toshihide Hige

Janelia Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, VA 20147, USA

ARTICLE INFO

Article history:
Received 15 March 2017
Received in revised form 28 April 2017
Accepted 1 May 2017
Available online 5 May 2017

Keywords:
Synaptic plasticity
Associative learning
Olfaction
Drosophila
Mushroom body

ABSTRACT

Nervous systems have evolved to translate external stimuli into appropriate behavioral responses. In an ever-changing environment, flexible adjustment of behavioral choice by experience-dependent learning is essential for the animal's survival. Associative learning is a simple form of learning that is widely observed from worms to humans. To understand the whole process of learning, we need to know how sensory information is represented and transformed in the brain, how it is changed by experience, and how the changes are reflected on motor output. To tackle these questions, studying numerically simple invertebrate nervous systems has a great advantage. In this review, I will feature the Pavlovian olfactory learning in the fruit fly, *Drosophila melanogaster*. The mushroom body is a key brain area for the olfactory learning in this organism. Recently, comprehensive anatomical information and the genetic tool sets were made available for the mushroom body circuit. This greatly accelerated the physiological understanding of the learning process. One of the key findings was dopamine-induced long-term synaptic plasticity that can alter the representations of stimulus valence. I will mostly focus on the new studies within these few years and discuss what we can possibly learn about the vertebrate systems from this model organism.

Contents

1. Introduction

A scent of fragrant flowers may give you a pleasant memory of a past spring season or may bring back a sad memory of lost love. Each of us responds to exactly the same external stimulus in a very different manner depending on our past experience. How do our nervous systems support such flexible processing of sensory information? Certain forms of experience-dependent modulation can be observed at very early stages of the sensory circuits. For example, in a mouse, repeated presentation of the same odor leads to a long-lasting shift in the odor tuning of mitral cells, which are the second-order neurons of the olfactory circuit (Kato et al., 2012). Internal states of animals can also influence the sensory coding in the early layers of a circuit. For example, in a fruit fly, starvation causes selective suppression and facilitation of signals at the level of first synaptic relay (Ko et al., 2015). However, it is generally believed that more complex forms of modulation, which involve specific association between multiple sensory stimuli, take place at the deeper, higher-order areas of the brain. One of the major challenges imposed on systems neuroscientists is to pin down the location where such flexibility is achieved and to understand how these changes are finally translated into behavior.

E-mail address: higet@janelia.hhmi.org

https://doi.org/10.1016/j.neures.2017.05.002
0168-0102/

Although studies in primate and rodent brains have provided us with useful mechanistic insights in a given brain area, the complex organization of these circuits hampers understanding of the total flow of neural transformation from sensory coding to motor output. This is where it becomes advantageous to study numerically simpler invertebrate nervous systems. In the gill-withdrawal circuit in the marine mollusk *Aplysia*, sensory neurons that are activated by skin stimulation directly synapse onto motor neurons that induce gill withdrawal. When the animal learns an association between a noxious shock on the tail and a light touch to the skin (Carew et al., 1981), it is these synapses that undergo long-term potentiation to induce a strong gill-withdrawal reflex in response to subsequent gentle tactile stimuli (Hawkins et al., 1983). Both heterosynaptic facilitation and Hebbian potentiation contribute to this form of synaptic plasticity (Hawkins and Byrne, 2015). The molecular mechanisms of the plasticity originally found in this system, including cyclic adenosine monophosphate (cAMP) pathway (Ocorr et al., 1985), were subsequently proven to be generally important in many vertebrate learning systems as well.

While the studies in *Aplysia* represent a successful example of a reductionist approach, sensory neurons in most nervous systems are connected to motor neurons through multiple intermediate circuit layers, rather than in a monosynaptic manner. Sensory information is typically represented by a population of neurons and progressively transformed over multiple circuit layers before reaching a learning center, where, in the case of associative learning, the information converges with reinforcement signals. In this more complex situation, the important questions are: What kind of sensory coding is used as input signals to the learning center? How are these signals modulated by learning? What do the output signals of the learning center represent and how are they related to behavioral changes?

The olfactory circuit in the fruit fly, *Drosophila melanogaster*, offers one of the ideal models to address these questions. First, the *Drosophila* nervous system consists of only $\sim 10^5$ neurons, much less than vertebrate systems (e.g. $\sim 10^8$ in a mouse, $\sim 10^{11}$ in a human). Yet, it clearly uses a population of neurons to encode sensory stimuli in a given circuit layer, which makes the system more comparable to vertebrate ones. Importantly, such population coding is commonly involved in complex behaviors like sensory discrimination and learning. Second, there are numerous genetic tools available for manipulating and labeling the circuit. These tools enable physiological recordings from identified neurons, both with electrophysiology and with functional imaging, as well as behavioral tests for the functions of specific neurons. Third, anatomical information of the circuit is rapidly accumulating not only at the light microscopic level but also at the ultra-structural level. In this review, I will summarize recent findings on the mechanisms of Pavlovian olfactory learning in *Drosophila*. The brain area in focus is the mushroom body (MB). The MB is regarded as a central brain structure for associative olfactory learning, and therefore there has been a long history of research in multiple fields, including behavioral genetics, neuroanatomy and physiology. I will skip most of these historical aspects because they have been summarized elsewhere multiple times (Heisenberg, 2003; Davis, 2005; McGuire et al., 2005; Keene and Waddell, 2007; Waddell, 2013). Because of the limited space, I will also leave unmentioned many of the excellent studies in other insects that made great contributions for understanding the MB functions.

2. From sensory input to sparse coding

The organization of the early layers of the *Drosophila* olfactory circuit shows a striking similarity to that in vertebrates (Hildebrand and Shepherd, 1997; Fig. 1). Olfactory receptor neurons (ORNs) expressing the same receptor converge onto the same glomerulus (Vosshall et al., 2000), which is the input neuropil structure of the antennal lobe, the first brain area of the olfactory circuit. There are about 50 glomeruli in each antennal lobe (Laissue et al., 1999). Each glomerulus contains dendrites of several projection neurons (PNs), which are the only neurons that send axons to higher-order brain areas. The dendrites of the most PNs are confined to a single glomerulus (Stocker et al., 1990), and therefore PNs largely inherit their odor-tuning patterns from the ligand specificity of the olfactory receptors expressed in their presynaptic ORNs. PNs are actually more broadly tuned to odors in comparison to ORNs (Wilson et al., 2004; Bhandawat et al., 2007) due to the properties of ORN-PN synapses and lateral interactions between glomeruli (Wilson, 2013). Nonetheless, tuning patterns of the same class of PNs are as highly stereotyped across different individual flies as ORNs (Wilson et al., 2004; Murthy et al., 2008). Excellent reviews on the detailed olfactory processing in these early layers of the circuit are available elsewhere (Wilson and Mainen, 2006; Masse et al., 2009; Wilson, 2013; Kazama, 2015).

Information from different olfactory channels starts to converge at the next stage of the circuit (Fig. 1). Most of the excitatory PNs project to both MB and lateral horn (LH) (Tanaka et al., 2004; Jefferis et al., 2007; Lin et al., 2007). In both areas, PN activity is read out as a combinatorial code. Converging connectivity patterns from PNs to a given type of LH neurons are stereotyped across different animals (Fişek and Wilson, 2014). As a result, tuning patterns of the same type of LH neurons are also highly stereotyped, although the breadth of tuning varies widely across different types of neurons. However, the situation is very different in the MB. The principal neurons of the MB are called Kenyon cells (KCs). The MB in each hemisphere contains about 2000 KCs (Aso et al., 2009; 2014a), each of which receives input from, on average, 7 PNs (Turner et al., 2008; Caron et al., 2013). The connectivity between PNs and KCs is probabilistic, rather than deterministic (Caron et al., 2013; Gruntman and Turner, 2013). This makes it unpredictable which KC responds to which odors (Murthy et al., 2008). In general, KCs are narrowly tuned to odors, and only about 6% of total KCs reliably respond to a given odor (Turner et al., 2008; Honegger et al., 2011). This sparse format of sensory representations is a widely observed feature in the MBs in other insects (Perez-Orive et al., 2002; Szyszka et al., 2005; Ito et al., 2008; Demmer and Kloppenburg, 2009) as well as in vertebrate cortical areas (Hromádka et al., 2008; Jadhav et al., 2009; Poo and Isaacson, 2009; Stettler and Axel, 2009) and is advantageous for accurate memory formation (Olshausen and Field, 2004). It maximizes memory capacity by minimizing the overlap between representation patterns of different stimuli. Indeed, the sparse coding in the MB is demonstrated to be important for associative olfactory learning (Campbell et al., 2013; Lin et al., 2014a). Notably, in vivo calcium imaging of odor responses across the KC population almost perfectly predicted odor identity (Campbell et al., 2013; Hige et al., 2015b). Thus, through the first three layers of the circuit, olfactory representations are transformed from a dense to a sparse format that is now suitable for memory-related functions. So then, how is the sensory information further transformed in the next layer? How does learning induce changes in sensory signals? To address these questions, let's look at the circuit organization of the output side of the MB.

3. Circuit organization of the mushroom body

Pioneering studies on neuroanatomy of the MB proposed a highly modular structure of the MB circuit (Ito et al., 1998; Li and Strausfeld, 1999; Strausfeld, 2002; Frambach and Schürmann, 2004; Sjöholm et al., 2006; Tanaka et al., 2008; Mao and Davis, 2009). Recent comprehensive work in *Drosophila* confirmed and