CHAPTER 9

Clonal Unit Architecture of the Adult Fly Brain

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Abstract

During larval neurogenesis, neuroblasts repeat asymmetric cell divisions to generate clonally related progeny. When the progeny of a single neuroblast is visualized in the larval brain, their cell bodies form a cluster and their neurites form a tight bundle. This structure persists in the adult brain. Neurites deriving from the cells in this cluster form bundles to innervate distinct areas of the brain. Such clonal unit structure was first identified in the mushroom body, which is formed by four nearly identical clonal units each of which consists of diverse types of neurons. Organised structures in other areas of the brain, such as the central complex and the antennal lobe projection neurons, also consist of distinct clonal units. Many clonally related neural circuits are observed also in the rest of the brain, which is often called diffused neuropiles because of the apparent lack of clearly demarcated structures. Thus, it is likely that the clonal units are the building blocks of a significant portion of the adult brain circuits. Arborisations of the clonal units are not mutually exclusive, however. Rather, several clonal units contribute together to form distinct neural circuit units, to which other clones contribute relatively marginally. Construction of the brain by combining such groups of clonally related units would have been a simple and efficient strategy for building the complicated neural circuits during development as well as during evolution.

Introduction

The fly brain consists of a complicated meshwork of neural circuits.[1,2] Each neuron projects to and arborises in its distinct subareas. Visualisation of specific subtypes of neurons, either by antibody staining or by expression of reporter genes, suggests that, although certain variability is observed in the number of the labelled cells, the projection patterns of the labelled neurons are rather stereotyped in the adult brain.[3-5] Molecular mechanisms underlying the formation of such complicated but stereotyped neural architecture have been studied extensively during the past few decades. Neurons are generated by asymmetric division of the stem cells called neuroblasts.[6,7] Each neuroblast gives birth to a series of clonal progeny during neurogenesis. The brain is therefore composed of "families" of clonally related cells. In this chapter, we examine how such lineage-dependent groups of neurons contribute to the formation of the elaborated neural circuits of the adult fly brain.

Structure of the Adult Brain

Before discussing the relationship between clones and neural network, we will briefly overview the general structure of the adult fly brain (for structure and development of the larval brain, see chapter by V Hartenstein et al). The adult brain is a mass of neurons that is about 500 μm wide, 200 μm thick and 250 μm tall. It consists of three parts, the central brain and an optic lobe on either side. The latter is the lower-order sensory centre specialised for visual information processing,[8,9] whereas the former contains lower-order centres of other sensory modalities (olfactory,

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etc.) as well as integrative and associative centres and higher-order motor control centres. Figures 1A,B show sections of a silver-stained adult fly brain. The area near the brain surface is occupied by the rind, or cortex, where cell bodies of all the neurons are confined (yellow areas). Unlike vertebrates, insect neurons have no synapses around their cell bodies. Thus, there are no synapses in the rind. All the brain neurons are monopolar, sending single neurites (cell body fibres) deeper into the brain and form synaptic connections[2] (Fig. 1C). The area occupied by these fibres and synapses is called the neuropile.

The thickness of the rind is different depending on the area of the brain. It is thickest in the area called the lateral cell body region (LCBR), which is between the central brain and the optic lobe (Fig. 1A,B). The rind is thin in the areas where the underlying neuropiles are protruded. Especially, there are essentially no cell bodies in the anteriormost surface area of the suboesophageal ganglion (SOG), antennal lobe (AL), ventrolateral protocerebrum (vlpr) and the anterior inferiorlateral protocerebrum (aimpr) (Fig. 1D). The ventral area of the posterior brain has no cell bodies, either, because this area is occupied by the cervical connective that houses the descending and ascending neural fibres to and from the thoracic ganglion (Fig. 1E). The diameter of the neural cell bodies tend to be smaller in the optic lobe and in the area above the calyx (ca) of the mushroom body (MB) than in other areas of the central brain (Fig. 1E).

Neurites generally form arborisations in several areas along their trajectories (Fig. 1C). The arborisations that are closest from the cell bodies are called the primary arborisations and those that are farthest are the terminal arborisations. In a simplistic view, the primary arborisation is often regarded as "postsynaptic dendrites" or "input areas," whereas the terminal arborisation is often called "presynaptic axon terminals" or "output areas." Though this is true in some cases, the situation is often more complicated. For example, many projection neurons that convey olfactory information from the AL to the second-order olfactory centres (the MB and the lateral horn, LH) have presynaptic sites not only in their terminals in the MB and LH but also in their dendrites in the AL (R Okada and KI, unpublished observation). Kenyon cells of the MB have postsynaptic sites not only in the calyx, which is supposed to be the input area of the MB, but also in the lobes, which is regarded as its output area.[10] Thus, pre and postsynaptic sites may in various cases co-exist in the same branches of neurites. Presynaptic sites in the primary arborisations may function for emitting local feedback signals and postsynaptic sites in the terminal arborisations might receive local modification signals for their output. On the other hand, there are indeed some neurons in which pre and postsynaptic sites are preferentially distributed in the proximal and distal areas of the neurites, respectively.[8] The direction of information therefore is not self evident from the projection pattern alone. Because the term "dendrite" often infers its role as input sites, care should be taken when using this word for referring to certain primary arborisations.

The brain consists of neurons and glial cells. Figure 1F,G show cross sections of the brain labelled for synaptic areas (with monoclonal antibody nc82[11]) and glial processes (with GFP driven by the glial specific repo-GAL4 driver.) The rind is contributed extensively by the processes of cell body glia (or cortex glia),[12] which ensheath each neural cell body. As explained before, synapses exist only in the neuropile. By comparing Figure 1A and 1G, which show the sections of the same level of the brain, it is clear that the neuropile areas that are occupied by large tracts of neural fibres (bundles of thick lines in Fig. 1A) are devoid of synapses (black areas in Fig. 1G). These tracts are covered by the processes of the neuropile glial cells.

The neuropile glia also separate the borders between major brain areas. For example, the borders around the AL, MB and the central complex, as well as the border between the suboesophageal ganglion (SOG) and the supraoesophageal ganglion, are covered by the glial sheath. Glial processes, however, do not always demarcate borders between functional areas of the neuropile. For example, although the MB is covered extensively by glial processes, there is no glial sheath structure between the LH—the other second-order olfactory centre—and the surrounding neuropiles. Similarly, although the anterior half of the ventrolateral protocerebrum (vlpr) is clearly demarcated by glial processes, the border between its posterior half and neighbouring neuropiles is more ambiguous.