



# Metamorphosis of the Central and Peripheral Nervous System (CNS and PNS) in Insects

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## Abstract

Formation of the nervous system in adult insects requires two separate but consecutive developmental events; embryogenesis and metamorphosis. During embryonic development, neural stem cells are formed and these cells give rise to a large number of neurons that constitute functional juvenile nervous system. This system develops continuously during post-embryonic development and then major restructuring of the nervous system takes place during metamorphosis. We have been particularly interested in the mechanisms underlying metamorphosis-associated remodeling of the nervous system. Here we will review past and recent discoveries associated with the nervous system development in the fruit fly, *Drosophila melanogaster*.

## Formation of the larval CNS

Upon fertilization, the egg cell undergoes rapid nuclear and cellular division. Shortly after the initial completion of cell divisions, cells in the ventral neuroectoderm are genetically programmed to take two different fates; epithelial vs. neurons. Through Notch-Delta mediated lateral inhibition, specific groups of neuroectodermal cells are delaminated and become Neuroblasts (NB). As a result, there are 30 distinct NBs formed in each hemi-segment in *Drosophila* Ventral Nerve Cord (VNC) at stereotypic positions and their uniqueness is determined by spatio-temporal cues [1]. Every NB divides asymmetrically, giving rise to specific numbers of neurons, together of which forms a larval CNS [2,3]. Interestingly, the numbers of division cycle of each NB are genetically predetermined, resulting in heterogenous numbers of neurons born from each NB. For instance, NB7-3 divides only three times and gives rise to 6 neuronal precursor cells, two of which are eliminated *via* Notch-induced apoptosis. By comparison, NB7-1 divides many more times and produces about 40 neurons [4-7]. One of the NB7-3 progeny is vCrz neurons and NB3-5 lineage was reported to give birth to CCAP neurons these two groups of peptidergic neurons have been excellent model systems for understanding the apoptotic cell death mechanisms during metamorphosis, as described later (Figure 1) [8,9].

Terminal differentiation or programmed death of NBs following their final cell division are the major factors determining the ultimate number of neurons derived from each NB [10-12]. In addition, survival of these neurons depends on the target-driven neurotropic stimulus, as neurons that failed to receive the neurotropic signals are programmed to be eliminated [13,14]. Such stochastic death of excessive embryonic cells has been well documented in the vertebrate CNS as well in which nearly 50% of embryonic neurons do not get enough trophic signals and thus are programmed to die [15-17].

## CNS Metamorphosis

At the end of larval growth, extensive genetic reprogramming orchestrated by a steroid hormone, ecdysone, modifies the body patterns including the CNS in order to accommodate adult life styles. In holometabolous insects such as the fruit flies that undergo complete metamorphosis (i.e., egg-larva-pupa-adult); larval organs are degenerated in conjunction with de novo formation of adult ones in pupa. We refer to the metamorphosis-associated cell death as 'metamorphoptosis' [17].

In contrast to complete degeneration of larval organs during metamorphosis, the larval CNS is sculptured extensively to form adult CNS. Two major cellular events occurring in the metamorphosing CNS are: (1) remodeling of persisting neurons. This event involves pruning of neurites followed by extension of new axons and dendrites to establish adult-specific neuronal architecture. (2) Programmed apoptotic death of obsolete larval neurons. These neurons are likely to perform functions specific to larvae or pupae, and then are eliminated as their roles are not required

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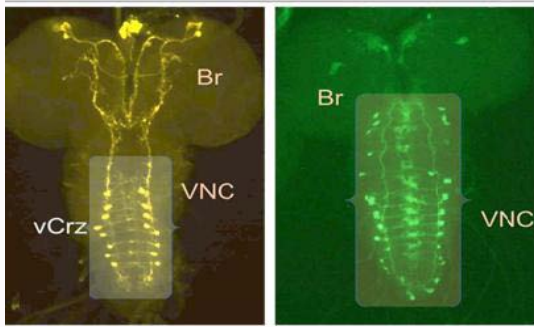
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**Figure 1:** Two different peptidergic doomed neurons in the larval CNS. (Left) Corazonin immunoreactive neurons. vCrz neurons that are programmed to die shortly after the onset of metamorphosis are highlighted (Death Class-I). (Right) GFP reported CCAP neurons. The neurons inside highlighted are undergo apoptotic death after adult eclosion (Death Class-III).

in ensuing life stages. In both remodeling and apoptosis, ecdysone plays a key role.

The best-known case of remodeling is the Mushroom Body Gamma (MB) neurons. In the larval CNS, these neurons send bifurcating axons in two directions, dorsal and medial. Around 4 hours to 6 hours After Prepupal Formation (APF), axons start show the first histological sign of axon remodeling, and then all medial and dorsal axons are fragmented and completely removed by 18 hours APF [18,19]. The pruning of MB neurons seems to be initiated *via* activation of TGF-beta type-I receptor Baboon and type-II receptors, Punt and Wit [20]. These receptors are activated by gli-derived ligand Myoglianin, and then the activated receptors induce expression of ecdysone receptor B1 (EcR-B1) isoform [21]. A recent study further showed that an immunoglobulin superfamily protein, Plum, facilitates the Myoglianin signaling to up-regulate EcR-B1 expression [22]. The pruning event also requires Ultraspiracle (Usp), suggesting that EcR-B1: Usp heterodimer, a canonical receptor for ecdysone, is a key regulator for inducing pruning-associated cytological events such as the disassembly of the microtubules [19]. Despite these studies, it is unclear what factors act downstream of the EcR-B1: Usp. It was initially thought that axonal degeneration mimics apoptosis cytologically; however, MB axonal fragmentation does not seem to involve apoptotic machinery, as no evidence found for the role of caspases, the key apoptosis executioners [19]. This study, however, showed that Ubiquitin Proteasome (UPS)-mediated protein degradation is essential for the axonal pruning. More studies are needed to clarify the downstream pathway of EcR-B1: Usp.

In addition to the remodeling process in some neurons, other larval neurons are programmed to die during metamorphosis. Interestingly, these doomed neurons do not disappear at the same metamorphic stage. Instead, they show highly diverse timing of death during metamorphosis, indicating heterogeneity of molecular cell death mechanisms.

We propose three classes of doomed neurons based on their death timing. Death Class (DC)-I includes the neurons that die shortly after the onset of metamorphosis. The best-known model system in this class is the eight pairs of corazonin-producing neurons (vCrz) in the VNC (Figure 1). Using an *in vivo* Caspase Sensor (Casor), these neurons are shown to initiate apoptotic program as soon as larvae turn into white prepupae, and then they are completely degenerated within 6 hours to 7 hours APF [23-26]. Genetic and transgenic

studies have shown that both EcR-B1 and B2 isoforms are required to activate caspase dependent apoptotic events. Further analyses revealed essential role for the Usp and the grim cell death gene [6]. Together, it is likely that an ecdysone pulse at the end of larval growth activates EcR-B: Usp nuclear receptors, which induce expression of grim. Grim proteins antagonize caspase inhibitor DIAP1, thereby unleashing destructive power of the caspases. Despite these studies, it is yet to be understood the regulatory mechanisms underlying grim expression.

Other identified neurons in the Death Class-I are eleven pairs of CEv motoneurons and two pairs of CED neurons in the ventral segments [26]. Quite interestingly, although the death timing of these neurons is similar to that of vCrz neurons, our data did not support the roles played by EcR (unpublished data). These results suggest that the apoptotic mechanisms of the CED/v neurons are quite different from those of vCrz neurons.

Death Class-II is the larval neurons in which their apoptosis is triggered by a small ecdysone pulse at 12 hours APF, which marks prepupa-to-pupa transition. Only one group, RP2 motoneurons in the VNC, is well characterized for this class. The RP2 neurons are eliminated at 15 hours to 20 hours APF, and as observed for vCrz neuron death, RP2 apoptosis is triggered by ecdysone signaling through EcR-B isoforms [27].

Death Class-III includes the neurons that survive during metamorphosis but undergo apoptosis following adult emergence (a.k.a. eclosion). An example is a heterogenous group of ~300 neurons (type-II) that are characterized by a high level of EcR-A expression, otherwise their neurochemical identities are unknown. Most type-II neurons undergo apoptotic death within 24 hours after eclosion [28,29]. Another group in this class is the neurons producing CCAP neuropeptide in the VNC (Figure 1). Most of CCAP neurons also die of apoptosis along with the type-II neurons [17]. Quite interestingly, in stark contrast to the case shown for DC-I and II, DC-III neurons are protected by ecdysone signaling during larval and pupal development. Because of high level EcR-A expression in these neurons, EcR-A is suggested to play a role for the provision of protection from premature apoptotic death. It is likely that ligand-bound EcR functions as a repressor of the cell death gene grim during the metamorphosis (unpublished results). At eclosion, very low ecdysone titers initiate apoptotic program, most likely due to de-repression of grim by unoccupied EcR [30]. These results highlight opposite roles of EcR for orchestrating two different fates of larval neurons during metamorphosis; pro-apoptosis (DC-I and II) vs. anti-apoptosis (DC-III).

## Metamorphosis of the Peripheral Nervous System (PNS)

A majority of larval sensory neurons are degenerated during metamorphosis and replaced with adult-specific neurons [31,32]. However, some groups of Dendritic Arborization (da) neurons survive into adulthood [22]. These da neurons undergo extensive dendritic remodeling processes involving first removal of dendritic arbors *via* severing, followed by fragmentation and clearance during 5 hours to 16 hours APF [33]. As is the case for axonal degeneration in the MB neurons, dendrite breakage of the da neurons is mediated by EcR-B1: Usp complex and UPS [34]. Moreover, UPS degrades DIAP1, leading to the local activation of a caspase Dronc [35,36]. In conjunction with this, it is notable that production of a DIAP1

antagonist, Hid, is elevated in the *da* neurons *via* down-regulation of translational repressors Nanos and Pumilio [37]. Additional genetic studies identified Sox14 (HMG transcription factor) as a downstream target of EcR: Usp and Mycal as a downstream of Sox14 [33]. Mycal possibly alters cytoskeletal arrangement or dynamics, which can be a prerequisite to dendritic pruning.

In summary, reconstruction process of the nervous system during metamorphosis is constituted by the remodeling of persisting neurons, apoptosis of obsolete neurons, and neurogenesis of adult specific neurons (not discussed here). Although the former two cellular events are regulated commonly by ecdysone signaling, responses of neurons are quite diverse depending on the neuronal identities; partial removal of neurites (axonal degeneration in MB neurons and dendritic pruning in *da* sensory neurons) and total destruction of neurons at different metamorphic phases (DC-I, II, III). It is still not understood how the same hormonal signal during metamorphosis triggers such diverse cell-type specific responses and how modes of EcR signaling are differentially executed. Molecular identification and functional characterizations of the EcR downstream factors will shed more light on the diverse mechanisms ongoing in the metamorphosing nervous system [38,39].

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