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Steroid hormones, dietary nutrients, and temporal progression of neurogenesis

Chhavi Sood[1,2], **Susan E. Doyle**[1,2], **Sarah E. Siegrist**[1,*]

[1]Department of Biology, University of Virginia, Charlottesville, VA 22904, USA

[2]equal contribution

Abstract

Temporal patterning of neural progenitors, in which different factors are sequentially expressed, is an evolutionarily conserved strategy for generating neuronal diversity during development. In the *Drosophila* embryo, mechanisms that mediate temporal patterning of neural stem cells (neuroblasts) are largely cell-intrinsic. However, after embryogenesis, neuroblast temporal patterning relies on extrinsic cues as well, as freshly hatched larvae seek out nutrients and other key resources in varying natural environments. We recap current understanding of neuroblast-intrinsic temporal programs and discuss how neuroblast extrinsic cues integrate and coordinate with neuroblast intrinsic programs to control numbers and types of neurons produced. One key emerging extrinsic factor that impacts temporal patterning of neuroblasts and their daughters as well as termination of neurogenesis is the steroid hormone, ecdysone, a known regulator of large-scale developmental transitions in insects and arthropods. Lastly, we consider evolutionary conservation and discuss recent work on thyroid hormone signaling in early vertebrate brain development.

Keywords

steroid hormone signaling; dietary nutrients; ecdysone; PI3-kinase and growth signaling; temporal patterning; termination of neurogenesis; neural development; neural stem cells; mushroom body

Introduction

Temporal transitions are a common theme in animal development and include larval molts of many invertebrate phyla, insect and amphibian metamorphosis, and the mammalian pre-to post-natal transition and puberty. Less overt but equally important are the temporal transitions of neural stem cells and progenitors that allow for the sequential generation of different neuron types and glia over time[1–5]. Many of the mechanisms that govern

*Correspondence: ses4gr@virginia.edu.

Declaration of Interests

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metamorphic and neural stem cell transitions are shared and, in both cases, the cellular and genetic programs governing these transitions are somehow coordinated with underlying animal physiology and attuned to cues received from the outside environment.

Drosophila melanogaster has long served as a genetic model for understanding the molecular underpinnings guiding developmental transitions, including molting and metamorphosis, and it is now well appreciated that increasing systemic levels of the steroid hormone ecdysone serve to trigger these transitions [6–10]. Studies in *Drosophila* have also yielded major insights into the changes in gene expression that occur in neural stem cells over time, which allows for generation of increased neuronal diversity from a relatively small pool of stem and progenitor cells. Neuroblasts, the neural stem cells of *Drosophila*, divide asymmetrically throughout most of development to remake themselves and generate smaller daughter cells that divide once or more than once to produce neurons or neurons and glia. Neuroblasts in the central brain region are molecularly heterogeneous, however all seem to express the same or a similar set of intrinsic temporal factors in roughly the same order, yet timing of temporal transitions can vary across lineages [11–13]. Daughter cells that are produced after each division inherit the temporal program of their neuroblast mothers and pass this intrinsic program on to their neuron progeny. While many aspects of temporal patterning are shared among neuroblasts, mechanisms regulating temporal transitions during embryogenesis versus larval development appear to be quite different. Temporal patterning during embryogenesis is largely a cell-intrinsic process, while temporal patterning during larval stages, like metamorphosis, is regulated by both intrinsic and extrinsic factors.

Here, we highlight recent progress made on the role of ecdysone and dietary nutrient availability in *Drosophila* neural development, from temporal patterning to termination of neurogenesis. We discuss several mechanisms by which dietary nutrients impact ecdysone levels and signaling activity, and propose new research areas needed for better understanding. In particular, it remains unclear whether nutrient-regulated neurosecretory neurons, including the IPCs (insulin-producing cells) regulate ecdysone directly through their neurite contacts or indirectly through connections made with other neurons located in the brain, including those that make prothoracicotropic hormone (PTTH). Finally, we end by discussing evolutionary conservation, focusing on the role of thyroid hormone signaling in early vertebrate brain development.

Intrinsic and extrinsic neuroblast temporal patterning

In embryonic neuroblasts of the *Drosophila* ventral nerve cord, the system in which temporal patterning of neuroblasts was first described, neuroblasts transition rapidly through a series of 5 transcription factors: Hunchback (Hb), Kruppel (Kr), Nubbin/Pdm2 (Pdm), Castor (Cas), and Grainy Head (Grh) [11,14–16]. This transcription factor cascade is regulated in part by feedback repression: Kr is repressed by Pdm (in some lineages), Pdm is repressed by Cas, and Cas is repressed by Grh [14,17–20]. However, other factors or mechanisms are likely required since temporal factor loss does not prevent forward progression [11,17,21–23]. Temporal patterning during embryogenesis is believed to be mostly cell-intrinsic, since neuroblasts cultured in isolation *in vitro* carry out the same temporal program as neuroblasts *in vivo* [24]. This type of cell-intrinsic regulation may be hard-wired considering the short

embryonic time period (approximately 24h), limited number of progenitor cell divisions (3–10), and constant supply of maternal nutrients. In contrast, *Drosophila* larval development takes approximately 5 days (120h), depending on environmental conditions, during which neuroblasts divide anywhere between 10 to 100 times or more depending on lineage [25,26]. Temporal patterning during larval stages may be more variable, less hard-wired, and could provide a means for *Drosophila* to better adapt to their current environment.

Larval neuroblasts in the central brain undergo a temporal transcription factor sequence that can be broadly divided into two windows of expression: (1) an early window from 0–48h after larval hatch (ALH) during which the transcription factors Castor (Cas), Seven-up (Svp), Chronologically Inappropriate Morphogenesis (Chinmo), and RNA-binding proteins Lin-28 and IGF-II mRNA-binding protein (Imp) are expressed, and (2) a later window from 60–120h ALH during which the RNA-binding protein Syncrip (Syp), and the transcription factors Broad (Br) and Ecdysone-induced protein 93F (E93) are expressed [27–31]. Recent experiments have identified ecdysone, acting through Ecdysone receptor B1 (EcRB1), as a driver of the transition from early to late temporal factor expression [30–33]. Moreover, expression of EcRB1 requires expression of the early temporal transcription factor Svp [31]. Blocking Svp prevents EcRB1 expression and results in neuroblasts that are unable to respond to ecdysone and therefore continue expressing early factors, Imp, Chinmo, and Lin-28 [31]. While it is still unknown how Svp itself is regulated, this work has provided a compelling example of how the post-embryonic neuroblast temporal cascade is regulated by an extrinsic hormonal cue.

Neuronal temporal identity and timing termination of neurogenesis

In addition to its role in larval neuroblast temporal patterning, ecdysone also regulates neuronal temporal identity and timing neurogenesis termination (Fig. 1). In the mushroom body (MB), one of the larger neuropils in the *Drosophila* brain, a high to low gradient of the transcription factor Chinmo is expressed in early- to late-born neurons, respectively, with Chinmo being required for the specification of early-born gamma and alpha’/beta’ neurons [34,35]. The Chinmo gradient in turn is generated by gradients of the neuroblast temporal factors Imp and Syp, as well as the microRNA, let-7, which is expressed in post-mitotic neurons [34]. Imp levels are highest early while Syp and let-7 are highest late and act to repress Chinmo in late-born neurons [34,36]. The transition from Imp to Syp in MB neuroblasts is regulated in part by activation of activin signaling by Myoglianin expressing glial neighbors which in turn regulates timing of Chinmo expression in mushroom body neurons [35,37,38]. Chinmo repression allows a switch in production of alpha’/beta’ neurons to alpha/beta neurons [12,36]. Let-7 is induced in post-mitotic mushroom body neurons by the large developmental ecdysone pulse that occurs during the pre-pupal to pupal phase [32]. Reducing ecdysone signaling specifically at this time eliminates let-7 expression and blocks the alpha’/beta’ to alpha/beta transition [32,35], whereas activin signaling renders the neurons competent to receive the ecdysone pulse by regulating neuronal EcRB1 expression [35,37].

The pre-pupal to pupal ecdysone pulse also triggers the majority of central brain neuroblasts to shift their metabolic program from glycolysis to oxidative phosphorylation (Fig. 1B) [39].

Ecdysone, in conjunction with Mediator, a multi-subunit transcriptional co-activator, induces transcription of a number of key metabolic enzymes that cause the switch, leading to reductions in neuroblast growth rates and termination of neurogenesis [39]. The temporal factors, Imp and Syp, both regulate Mediator and restrict its activity to late staged neuroblasts [13]. Syp also promotes accumulation of the differentiation factor Prospero and growth-inhibited neuroblasts with increasing Prospero are reported to terminally differentiate, however some likely undergo apoptosis as well [13,40,41]. While the majority of neuroblasts cease divisions during early pupal stages, a small subset, the mushroom body (MB) neuroblasts, which generate the mushroom body neurons, divide several days longer and undergo an autophagic/apoptotic cell death just before animals emerge from their pupal cases as young adults (Fig. 1) [41,42]. Ecdysone signaling and neuroblast temporal factors also regulate timing of MB neurogenesis termination [42], however it is unclear which of the ecdysone pulses triggers termination and whether ecdysone triggers early to late temporal factor expression, as is the case for non-MB NBs [30,31]. Nevertheless, Imp inhibits premature expression of the late factors, Syp and E93 in MB neuroblasts, while Syp inhibits Imp and promotes E93, and E93 downregulates PI3-kinase growth signaling in MB neuroblasts to induce autophagy [42]. When E93, a pipsqueak transcription factor family member and ecdysone responsive gene, is knocked down, autophagy fails and MB neurogenesis continues transiently into adulthood [40]. While all neuroblasts express E93 at the time of their disappearance, only the MB neuroblasts require E93 to terminate neurogenesis. The reason for this difference is not clear and could be due to differences in mechanisms used for termination, autophagy/apoptosis versus differentiation, or to differences in systemic nutrient levels at the time of lineage termination.

Dietary nutrient availability, neurogenesis timing, and ecdysone production

While dietary nutrients are required for animal growth and timing developmental transitions, how dietary nutrients impact neural development and neurogenesis is less understood. A number of evolutionarily conserved growth signaling pathways become active when dietary nutrients are available, including insulin/PI3-kinase and TOR-kinase [43–47]. In neuroblasts, this leads to their reactivation from developmental quiescence during early larval stages, which occurs in a coordinated manner, dependent on growth of cortex glial with tracheal neighbors [48–53]. During early larval stages, dietary nutrients also regulate neuroblast proliferation rates, however, once animals pass ‘critical weight’, a developmental checkpoint that senses nutrient levels, neuroblast proliferation can continue independent of dietary nutrients [51,52,54,55]. Nutrient-independent neuroblast proliferation during late larval stages requires Anaplastic lymphoma kinase (Alk), but whether ecdysone is also required is not yet known. PI3-kinase levels decline in neuroblasts prior to their elimination during pupal stages and in MB neuroblasts declining PI3-kinase leads to increased nuclear Foxo which triggers termination of MB neurogenesis via autophagy [41,42].

Beyond fueling neuroblast cell divisions, dietary nutrients also impact ecdysone production and signaling activity. Ecdysone is synthesized from cholesterol by steroidogenic enzymes in the prothoracic gland (PG), part of the larger ring gland, located adjacent and anterior to the two brain hemispheres (Fig. 2A). Cells of the PG take up cholesterol and store it for later use in lipid droplets (Fig. 2C) [56–58]. When dietary nutrients are limited or autophagy

inhibited, cholesterol-rich droplets accumulate in the PG leading to reductions in ecdysone synthesis [56–58]. Transcription of steroidogenic enzymes, including *phantom* (*phm*) and *disembodied* (*dib*), is also nutrient-regulated and increasing insulin signaling activity in the IPCs or the PG itself leads to increased *phm* and *dib* levels and increased ecdysone synthesis [59–62]. Conversely, when dietary nutrients are limited, Foxo, a transcription factor and PI3-kinase responsive gene relocates to the nucleus to inhibit *phm* and *dib* transcription resulting in reduced ecdysone synthesis [63]. Ecdysone, once produced, is released systemically into the circulating blood-like hemolymph, where it is converted by peripheral tissues into active 20-hydroxyecdysone (20E) (Fig. 2A) [64]. Conversion of ecdysone to 20E requires the enzyme, Shade, whose expression depends on dietary amino acids [65,66].

Synthesis of ecdysone is also regulated by a number of neuropeptides produced in neurosecretory neurons located in the brain (Fig. 2B,C). Many of these neurosecretory neurons innervate the PG directly as well as make contact with each other, and other neurons in the brain, suggesting that a neural circuit may function to control ecdysone levels and coordinate ecdysone production with environmental cues, including nutrient availability. Some of these neurosecretory neurons include those that make prothoracicotropic hormone (PTTH), the IPCs that synthesize and secrete *Drosophila* insulin-like peptides, serotonin producing neurons (SE0PG), and neurons that make Allatostatin-A (AstA) (Fig. 2B). PTTH, once released, binds to and activates the tyrosine kinase receptor, Torso in PG cells, leading to Ras/Raf/Erk MAP kinase activation, inhibition of *Drosophila* Hormone Receptor 4, and increased synthesis of ecdysone [67–70]. IPCs synthesize and secrete four of the eight *Drosophila* insulin-like peptides that bind to and activate PI3-kinase signaling in response to dietary nutrient availability [71–74]. SE0PG neurons innervate the PG directly and when nutrients are limited, SE0PG neurite arborization is reduced as well as is the levels of ecdysone [75]. The neurons that make AstA connect IPCs with the PTTH neurons. IPCs and PTTH neurons both express the Allatostatin A receptor (AstAR1) and when AstA binds AstAR1, secretion of Dilp-2 (Drosophila insulin-like peptide 2) and PTTH is increased respectively [76]. Whether any of the neurosecretory neurons communicate directly with each other to regulate ecdysone production remains an open question.

Evolutionary conservation between ecdysone and thyroid hormone in regulation of neurogenesis

Just as ecdysone triggers metamorphic changes and affects brain development in insects, thyroid hormones in vertebrates do the same [77–79]. Tissue levels of both hormones are tightly controlled through the action of solute carrier family transporters, and both activating and deactivating enzymes [80,81]. Ecdysone and thyroid hormone both signal through nuclear hormone receptors that heterodimerize and interact with cofactor complexes to regulate gene expression. EcR and thyroid hormone receptor (TR) cluster in the same subfamily of nuclear hormone receptors [82] and both heterodimerize with the same partner, Ultraspiracle (Usp) in *Drosophila* and Retinoid X receptor (RXR) in vertebrates. Usp and RXR are homologues and in vertebrates Usp stimulates TR to bind DNA [83,84]. Interestingly, Mediator was first purified from HeLa cells bound to TR-α/RXR [85]. Whether TR-α/RXR/Mediator, like EcR/USP/Mediator in *Drosophila*, induces changes in

metabolism that trigger progenitor exit from cell cycle remains an open question. In tadpoles, thyroid hormone increases neural progenitor proliferation and promotes differentiation of newborn neurons, while in humans, TR-α inhibits premature differentiation of cortical progenitors [86,87]. A recent study using stem cell derived human retinal organoids revealed a novel mechanism by which tissue levels of TH are modulated by temporal expression of TH transporter and deiodinase enzymes, resulting in a developmental switch from S cone to L/M cone photoreceptor fate [88]. While these studies clearly indicate that thyroid hormone signaling is important for neural development, progenitor proliferation, and cell fate determination, the transcriptional targets of TR in neural progenitors remains largely unknown. Interestingly, TH and let-7 levels are both elevated in zebrafish embryos at 2–3 days post-fertilization, raising the possibility that TH induces let-7 transcription as is the case for EcR in *Drosophila* [89].

Concluding remarks

The control of temporal patterning during *Drosophila* post-embryonic development involves the sensing of environmental cues, such as nutrient availability by sensory cells and tissues and the translation of these cues into signals that regulate the production of the steroid hormone ecdysone by the prothoracic gland. Ecdysone impacts temporal transitions through effects on RNA-binding proteins (Imp and Syncrip), micro-RNAs such as let-7, and temporal transcription factors such as E93. In this review, we have highlighted recent studies demonstrating specific effects of ecdysone on regulation of the larval neuroblast temporal transcription factor cascade, neuronal cell fate, and termination of neurogenesis during pupal stages. Vertebrate thyroid hormones share many molecular and functional similarities with insect ecdysone. However, an important unanswered question is whether thyroid hormones also regulate temporal patterning of neural progenitors. Finally, extrinsic nutrient cues are known to regulate production of both ecdysone and thyroid hormones, but how these cues are sensed and integrated by different cells and tissues in order to coordinate progenitor proliferation and tissue growth remains unclear.

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HIGHLIGHTS

1. Steroid hormone signaling regulates developmental transitions and neural stem cell temporal patterning and neurogenesis timing in *Drosophila*.
2. Neural stem cells respond to the steroid hormone, ecdysone, in a lineage-specific manner.
3. Dietary nutrients regulate ecdysone production and developmental timing, yet it remains unclear whether dietary nutrients affect neural stem cell temporal patterning.
4. Thyroid hormones in vertebrates play similar roles to ecdysone in insects, yet whether thyroid hormones regulate temporal patterning of neural progenitors remains unknown.

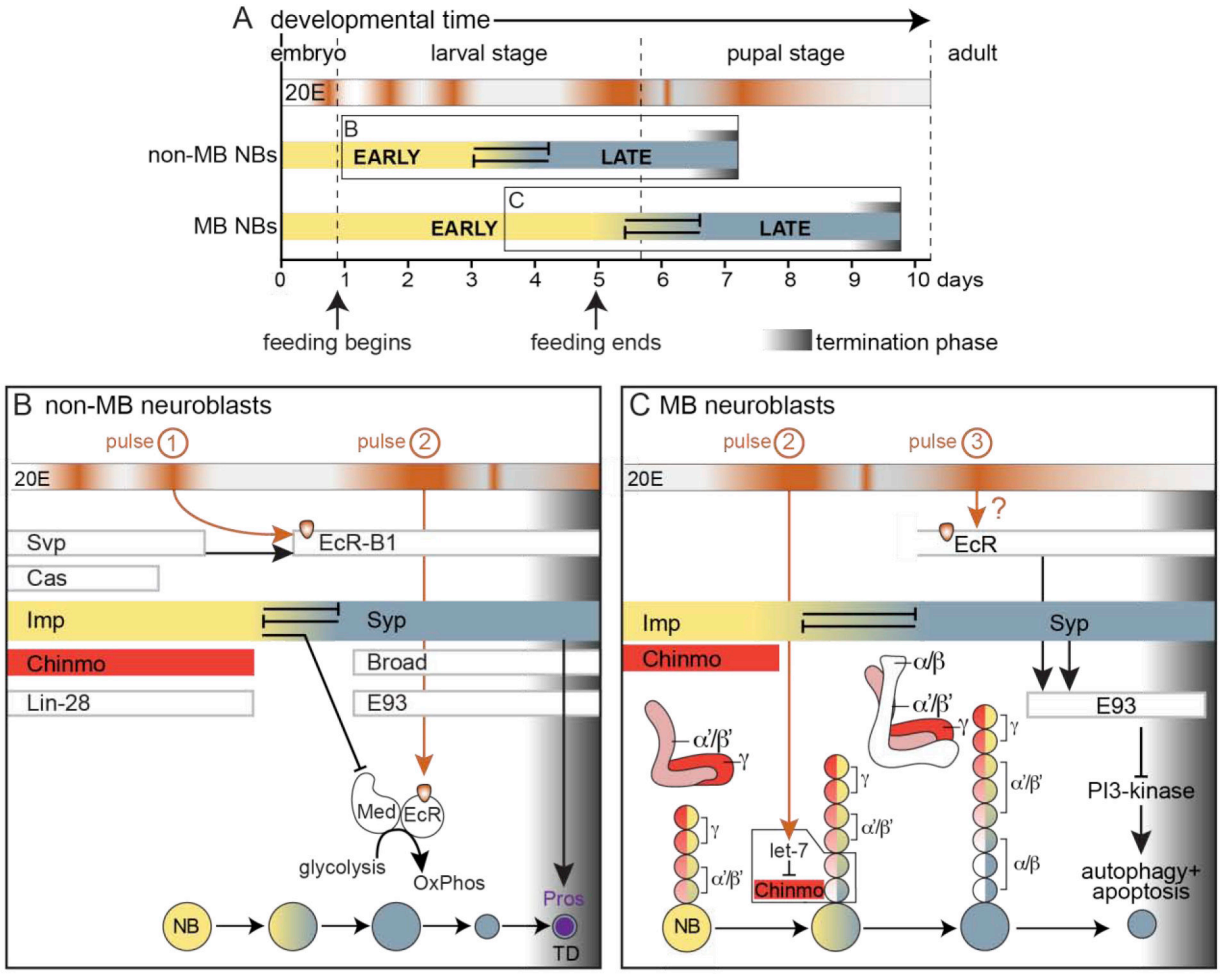

Figure 1: Schematic of *Drosophila* temporal patterning and neurogenesis timing during development and points of ecdysone control.

(A) Overview of developmental timing, pulsed systemic levels of 20-hydroxyecdysone (20E), and timeframe of Mushroom body and non-Mushroom body neurogenesis with changes in early to late temporal factor expression within each of the lineages. **(B)** Temporal transcription factors expressed in non-MB neuroblasts. Neuroblasts respond to ecdysone (pulse 1) after Svp induces expression of EcR-B1, resulting in a switch from expression of Imp, Chinmo, and Lin-28 to Syp, Broad, and E93. Imp/Syp restrict Mediator complex activity to late larval stages, and in response to another ecdysone pulse (pulse 2), EcR/Med triggers neuroblasts to switch from glycolysis to oxidative phosphorylation. Syp also promotes Prospero accumulation, leading to terminal differentiation of growth-inhibited non-MB neuroblasts. **(C)** In early-born neurons of the mushroom body, Imp promotes expression of Chinmo, which is required for specification of gamma and alpha'/beta' neurons. Later, Syp and ecdysone-regulated Let-7 repress Chinmo in late-born neurons, leading to specification of the alpha/beta neurons in response to ecdysone (pulse 2). Termination of neurogenesis involves the coordinate actions of the intrinsic temporal factors Imp, Syp, and E93 (ecdysone-induced protein 93F) in response to EcR signaling. Whether

MB neuroblasts respond to a late ecdysone pulse (pulse 3) to terminate neurogenesis is not known.

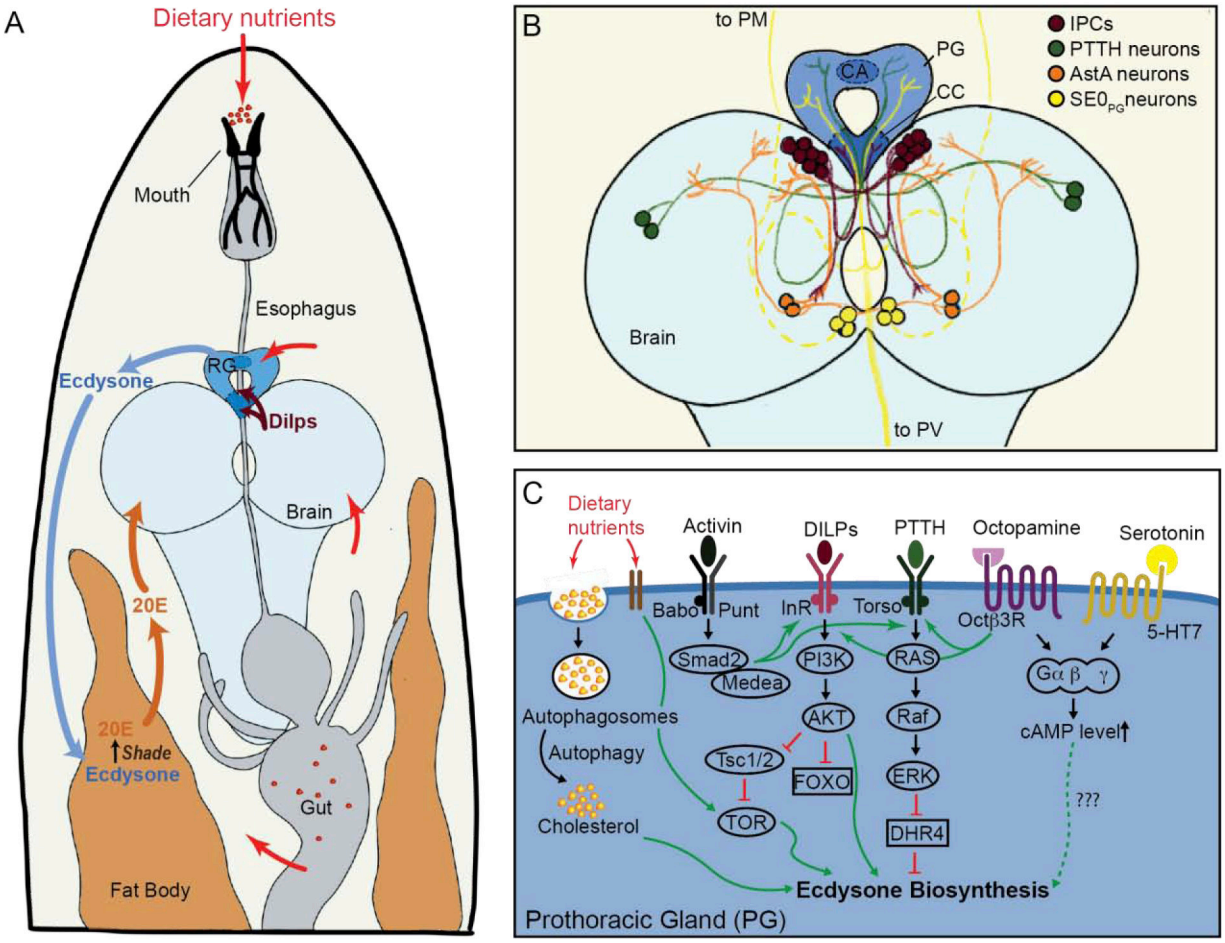

Figure 2: Schematic of ecdysone production and systemic release in response to dietary nutrient availability.

(A) Uptake of dietary nutrients (red) in response to animal feeding, results in release of *Drosophila* insulin like peptides (Dilps) from the insulin producing cells (IPCs). Dilps trigger ecdysone biosynthesis in the prothoracic gland (PG) which is part of the larger ring gland (RG). Uptake of dietary nutrients also trigger production of enzyme *shade* in the fat body and other peripheral tissues. Ecdysone released from the PG is converted to 20E by the enzyme *shade*. 20E binds to EcR/Usp heterodimer to regulate ecdysone responsive genes in tissues. **(B)** Representation of different neurons involved in regulating ecdysone biosynthesis. The PTTH neurons and the serotonergic (SE0PG) neurons innervate the prothoracic gland and the IPCs innervate the corpora cadiaca (CC). The IPCs and the PTTH neurons express Allatostatin A receptor, and the Allatostatin A producing neurons innervate the IPCs and PTTH to regulate release of Dilps and PTTH respectively. CA, corpora allata; PM, pharyngeal muscles; PV, proventriculus. **(C)** A schematic of different signaling pathways in the PG that regulate ecdysone biosynthesis. Dietary cholesterol stored in the form of lipid droplets in autophagosomes provide the building blocks for ecdysone production. Dilps released from the IPCs activate PI3-kinase and dietary amino acids activate TOR pathway to promote ecdysone synthesis. PTTH released by the PTTH neurons activates the downstream Ras/Raf pathway to promote ecdysone synthesis. The bioamines,

octopamine (released by cells of the PG) and serotonin (released by the SE0PG neurons) activate Octb3R and 5-HT7 to increase cAMP levels and increase ecdysone. Activin as well as Octopamine/Octb3R impinge on insulin signaling and PTTH driven Ras/Raf signaling to regulate ecdysone production [90,91].