Timing the Juvenile-Adult Neurohormonal Transition: Functions and Evolution

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INTRODUCTION
Timing Metamorphosis as Compared to Puberty

Life is based on the transmission of genetic material across generations. In multicellular animals, the most frequent (and consequently the most successful) strategy is through sexual reproduction. It usually requires a juvenile-adult transition in order to reach sexual maturation. Both mammals and insects trigger this process through a complex, multi-step neurohormonal signaling that may show certain structural parallelisms (Figure 1) (1, 2). Indeed, the neuroendocrine axis is activated at the pre-pubertal and pre-metamorphic stages by the activity of a group of neurons, which transmitted the initial stimulus to the mammalian pituitary gland or the Drosophila PG (prothoracic gland). These neurons, named GnRHn (Gonadotropin Release Hormone-expressing neurons) in mammals and PTTHn (Prothoracicotropic Hormone-expressing neurons) in insects, project their axons out of the brain barrier towards the ME (median eminence) or the PG to secrete the GnRH and PTTH neuropeptides, respectively. GnRH activates its GPCR (G-protein-coupled receptor) named GnRHR in the anterior pituitary cells inducing the circulating secretion of FSH (Follicle-stimulating Hormone)
and LH (Luteinizing Hormone), which stimulates the production of steroid hormones (estrogens) in the gonads. Instead, PTTH activates its RTK (receptor tyrosine kinase) TORSO in the PG, arousing the MAPK/ERK (mitogen-activated protein kinases/extracellular signal-regulated kinases) signaling pathway, which leads to the direct stimulation of a steroid hormone (ecdysone) (3, 4). Moreover, the awakening of PTTHn and GnRHn activity at pre-metamorphic or pre-pubertal timing depends on the insect AstA/AstAR1 (AllatostatinA) and the mammalian Kiss1/Kiss1R (kisspeptin1) GPCR-dependent systems (Figure 1). The general scheme is very similar in terms of the design of the molecular players but not in the evolutive history, with the exception of AstA/kiss1 (see below). In addition to the hierarchical regulation of both axes, the different clusters of neurohormone-expressing cells also act as regulatory hubs (1, 2). For instance, GnRHn activity is controlled not only by kiss1 but also by other neuropeptides like neurokinin-B, dynorphin, NPY and the neurotransmitter GABA, among others (1).

The complexity of the mammalian neurohormonal axis (also known as hypothalamic–pituitary–gonadal -HPG- or reproductive axis) is reflected in the imbricated architecture of the cellular circuitry in contrast with the apparent simple beauty of insect one, with fewer cells and genes. Nevertheless, new players have been found recently that added complexity and solved an apparent paradox: whereas the PG down-regulation of ecdysteroidogenic enzymes arrests the developmental transition (as shown by the absence of pupariation), removing or silencing ptth or torso only creates a delay in pupariation onset. In the last decade, several studies have pointed out the PG as another node (in addition to the brain), which also integrates signals to regulate ecdysteroidogenesis. Indeed, Cruz and colleagues recently described that prothoracic EGFR (Epidermal Growth Factor Receptor) stimulated by VEIN and SPITZ ligands activate MAPK/ERK pathway in the PG, as PTTH/TORSO signaling also does (5, 6). EGF signaling might compensate for ptth loss of function (LOF). EGFR LOF causes developmental arrest, resembling the lack of ecdysone (5). The autonomous PG expression of split and vein ligand genes depends on ecdysone signaling itself, suggesting that the EGFR pathway acts as an amplifying signal of the ecdysone production in the PG, a role previously described for ecdysone/EcR signaling (7, 8). Conversely, PTTHn are controlled by developmental and environmental inputs like growing tissue damages, photoperiod, over-crowding conditions, and ecdysone itself (2, 8). PTTHn also receive inputs from other neuronal circuits like the AstAn (9) and the corazonin (crz) neurons (10). Therefore, it is suggested that PTTH might be the link between the endocrine system and the internal/external milieu and other signals like EGF and ecdysone itself are required autonomously in the PG to ensure enough ecdysone surge to provoke metamorphosis (2).

Another enigma in the insect model comes from the difference in the observed developmental delay between the ptth null mutant (one day) and the PTTHn ablation (5 days). This suggests that PTTHn may cause electrical stimulation of the prothoracic gland to promote the release of ecdysone (11), or they may secrete another signal(s) that regulates ecdysone synthesis (12). Future studies are necessary to disclose the full PTTH neuronal function in timing the juvenile to adult transition.

**THE NEUROHORMONAL AXIS DURING JUVENILE TO ADULT TRANSITION AND FERTILITY**

Previous to adolescence and near to birth, the mammalian neurohormonal axis is active for a relatively short post-natal...
period known as mini-puberty (13, 14). This initial increase in the levels of sexual steroids is important for the correct development of gonads. Whether something similar may also happen in insects involving early pulses of ecdysone needs further investigation, although in larval stages three small ecdysone peaks of unknown function and non-related to ecdysis are described (15). After triggering puberty, the mammalian hypophysiotropic axis controls the overall pubertal process (somatic and sexual maturation) through steroid hormones. In *Drosophila*, the transition is commanded by ecdysone through the metamorphosis process. However, the role of PTTH in stimulating ecdysteroidogenesis during metamorphosis is not clear. *ptth* null mutant animals do not show any defect on the metamorphic process (12) although *ptth* transcription is highest at this stage (20 times higher than at larval or adult stages) (16). This result implies either that metamorphic PTTH does not stimulate ecdysteroidogenesis or, alternatively, that the role of PTTH in stimulating ecdysone synthesis during metamorphosis is compensated when absent (Figure 2). Moreover, *crz* or *AstA* silencing does not disturb metamorphic process itself. Therefore, whereas GnRH is essential for the pubertal process since its deficiency resulted in infertility and improper development of gonads, lack of *AstA*, *crz*, and *ptth* does not disrupt metamorphosis (like ecdysone absence does). This suggests the existence of another intrinsic/regulatory mechanism of ecdysone production in insects. Indeed, some hemimetabolous and ametabolous insects lack *ptth*, and the involvement of CRZ in their metamorphic process has not been described (17–19). Further studies should better clarify the existence of other ecdysone regulatory/intrinsic mechanism that might question the relevance of *AstA*, *crz*, and *ptth* signaling in timing Juvenile-to adult transition. Hence, the role of the PTTH-PG axis in the metamorphosis process itself remains controversial. Nevertheless, there are pieces of evidence indicating that the PTTH-PG axis is present during metamorphosis and regulates the rhythmicity of eclosion (i.e., pupal emergence) (20). PTTH acts as an intermediate player between the central clock, PDF-positive neurons, and the PG to produce ecdysone in a coordinated and timely manner to induce such rhythmic eclosion (20).

What is the role of these neurohormonal axes and their neuropeptide players in the adult stage? Are they still expressed and functional? In mammals, the pulsatile prepubertal secretion of GnRH enables the final maturation of a GnRH surge, which triggers the first ovulation (Figure 2) (21). Subsequently, the HPG axis regulates the ovulatory cycle in the female adult. In *Drosophila*, the fertility of adult females is also controlled by the ecdysone produced at the ovaries since the prothoracic gland degenerates by the end of metamorphosis (22, 23). In the adult brain, PTTH expresses in a group of neurons, but during metamorphosis, the larval brain undergoes extensive remodeling, and many new neurons are added. In this rewiring scenario, no precise analyses have been done to determine whether or not PTTH adult neurons are conserved from the juvenile larval stage. Moreover, adult PTTHn does not show

![Figure 2](image-url)
projections towards the ovaries to directly control ovary ecdysteroidogenesis and reproduction (3). These observations may imply that the adult neuroendocrine axis is not identical to the larval one. Future research is needed to determine whether PTTH controls adult ecdysteroidogenesis and reproduction as the mammalian neuroendocrine axis does.

**TRANSITIONAL NEUROHORMONES IN THE ADULT**

In contrast with the reduced number of larval AstA-expressing neurons, adult AstA expresses in several neurons and endocrine cells (23). The existence of several AstA-producing cell populations suggests distinct AstA roles in adult physiology, including behavior. The most conserved and studied function of AstA/AstAR signaling during adulthood refers to feeding and hunger regulation (23, 24). The activity of AstA-expressing neurons provides a satiety signal and regulates systemic insulin signaling. Lowering AstA levels in enteroendocrine secretory cells (EESs) from the gut induces early midgut senescence and signaling. Lowering AstA levels in enteroendocrine secretory neurons provides a satiety signal and regulates systemic Insulin signaling also has an impact on anxiety-like behaviors, which may be GnrH/estrogen-mediated or exclusively Kiss1R-mediated in the hippocampus (39). Kiss1 regulates reproduction-related behaviors such as male sexual preference mediated by GnrH neurons, although a copulatory behavior like lordosis (the arching of female back in response to male copulation) is controlled by Kiss1 independently of Kiss1 (40). A possible relation of AstA with anxiety-like or addiction behaviors remains to be studied in Drosophila models of such behaviors. The only suggestive data is that high levels of AstA induce aggressiveness in Africanized honeybees (41).

In the adult brain, ecdysone regulates circadian rhythmicity through Let7 (although it is not clear where ecdysone is produced) (42). pth mRNA is also detected in adult heads, with levels that change following a 24-h cycle (42). It has been proposed that PTTH controls adult ecdysone production participating in a regulatory feedback loop of the circadian clock although down-regulating pth receptor torso does not produce any rhythmic alterations in the locomotion activity (20, 42), a classical readout of circadian rhythm in some insect and mammalian models. According to a pth reporter that reflects faithfully larval and pupal expression, pth expression in the adult is restricted to the Ellipsoid Body, a part of the Central Complex associated with sleep regulation (3, 27). This expression pattern is compatible with a role for pth in sleep, although this possibility remains unexplored until now. In adult stages, PTTH behavioral function requires further studies, as well as its role in biological rhythms and steroid production.

Conversely, the adult role of CRZ has been studied in much more detail in regards to stress signaling metabolism and reproduction-related behaviors (43). crz is expressed in the somata of neurosecretory cells, among others. Down-regulation of crz in these cells or crzR in the IPCs (Insulin Producing Cells)
prolongs survival in flies exposed to starving conditions, and also affect lipid and glucose metabolism (44). The knockdown of crzR in salivary glands and fat body (functionally equivalent to mammalian fat cells and liver) causes similar metabolic phenotypes, including increased triacylglyceride levels and feeding, after exposure to different stress conditions (45). Furthermore, in the marine bristle worm Platynereis dumerilii CRZ signaling coordinates metabolic state with the lunar phase in order to achieve the final size and induce sexual maturation (46). Intriguingly, crz- and crzR- expressing neurons in the abdominal ganglia regulate sperm transfer and copulation duration in males (47). The activation of CRZ signaling is sufficient to mimic ejaculation-caused reward, driving appetitive memories and reducing ethanol consumption (48). It was previously shown that CRZ regulates ethanol sensitivity and detoxification (43).

The presence of many GnRH neurons outside the hypothalamus in transparentized human fetuses, and the expression of GnRHR in several adult brain structures (which include the cortex, spinal cord, cerebellum, and hippocampus) suggest that GnRH signaling plays additional roles even after adolescence (49, 50). Most of them depend on its estrogenic activity, like fertility. Low GnRH levels and high GnIH (Gonadotropin Inhibitory Hormone, the main GnRH antagonist), correlate with the severity of human insomnia, probably through circadian estrogen regulation (51, 52). The role of estrogens in sleep and metabolism has been extensively studied and is described elsewhere (53). In addition, given the increasing evidence that GnRH signaling has neurotrophic, neuroprotective, and regenerative functions, it has been proposed that combined therapy of GnRH with Growth Hormone may be beneficial following neural damage (50). For instance, GnRH signaling in the female hippocampus regulates the synaptic plasticity through an estrogen-mediated mechanism (54).

COMPARISON OF NEUROHORMONAL AXES AND THEIR EVOLUTIONARY ORIGIN

One controversial issue is how similar the overall design of GnRHn and PTTHn neuroendocrine hubs is since both trigger very similar processes, puberty and metamorphosis, respectively. In humans, GnRHN unmyelinated projections have unipolar or bipolar morphology that functions as a dendrite and axon simultaneously, a structure named dendron (55). These projections travel to the ME of the pituitary gland covering distances over 1–3 mm (56). Once in the ME, GnRHN projections branch into multiple short axons with “specialized” neuroterminals boutons that target the blood vessels from the closed portal vasculature. There, GnRH is released to act on the anterior pituitary gland (57). In Drosophila, PTTHn projections also have a very simple unipolar morphology that functions simultaneously as a dendrite and axon and travels a long distance of up to 500 µm to reach the PG (3, 58). PTTHn projections branch into multiple short axons with “specialized” terminal boutons that target individual PG cells (12). GnRHN exhibit pulse and surge modes of activity to control fertility. Whereas the LH surge requires functional soma-proximal dendrites and distal dendron, the distal dendron integrates synaptic information to drive pulsatile GnRH secretion (59). Recent studies indicate the presence of regulatory mechanisms controlling GnRHN and PTTHn projections into the ME/PG, respectively (9, 60, 61). Actually, various genes involved in the Semaphorin signaling, like semaphorin-3A (Sema3A), neuropilin (Nrp1 or Nrp2) coreceptors, and its receptor plexin-A1 (PlexA1), have been implicated in the guidance of GnRH neuronal migration, its survival and adult GnRHN plasticity affecting fertility (60, 62–66). Whereas PTTH neuronal development and migration towards the PG needs to be elucidated, a recent study suggests that PTTH neurons are also suffering from axon remodeling during the juvenile period (9). Drosophila Semaphorin signaling is well conserved, and it has also been implicated in axon guidance and synaptic plasticity (67). Interestingly, silencing the homologous of PlexA1 (pLexB receptor) in the PG caused a delayed onset of metamorphosis, suggesting that a similar mechanism regulates PTTH neuronal growth (68). Further studies should investigate the involvement of Drosophila Semaphorin signaling pathway in PTTH neuroterminal remodeling, migration towards the PG or survival, expanding or not the similarities between PTTH and GnRH neuronal development and physiology.

Unlike Drosophila PTTHn, which directly innervate the prothoracic gland itself, Lepidoptera PTTHn release PTTH into the hemolymph from their specialized nerve endings in the corpus allatum (CA) (69). The CA is the endocrine organ that produces juvenile hormone (JH), the primary negative regulator of PTTH in Lepidoptera Manduca sexta. JH drops upon the organism attainment of a critical weight allowing PTTH titters to rise and induce metamorphosis (70). In Drosophila, the function of JH on ecdysone synthesis has been the center of controversies. Indeed, removal of total JH by CA ablation or the use of different null mutants does not advance metamorphosis as expected by releasing the potential JH inhibitory effect on ecdysteroidogenesis (71). However, the silencing of the JH receptor exclusively in the PG induces ecdysone biosynthesis and triggers the precocious initiation of pupariation (72, 73). It has been proposed that this difference reflects a secondary effect of JH in the Insulin-mediated growth pathway, which antagonizes the direct timing effect on the PG. Further studies are needed to better clarify this point. Differences in the neuroendocrine connectivity between Drosophila and Manduca might reflect species-specific variances in ecdysone regulation. Indeed, in other insects like Bombyx mori, PTTHn also innervates the CA, raising the possibility that PTTHn projections towards the CA is a general trait in insects (74). Furthermore, Drosophila PTTH functions as a neuropeptide on the PG, whereas Lepidoptera PTTH acts as a circulating hormone (3, 12, 69). Indeed, PTTH in Manduca sexta and Bombyx mori shows more similarities with the mammalian GnRH, which does not directly reach the hypophysis and acts as a secreted hormone into the circulating portal vessels.

Another pending question in the field is whether or not genes involved in the mammalian and insect neuroendocrine
circuitry are evolutionary related, given the evolutionary distance (Figure 3A). Several phylogeny studies propose that the insect AstA system is evolutionary related to the mammalian GAL and KISS1, the mammalian gatekeeper of puberty (75–79). Indeed, gal and kiss emerged from the same ancestral gene precursor, and most likely this divergence occurs before protostome and deuterostome separation since kisSR sequences have been found in protostomes such as annelids and mollusks (Figure 3A) (78–80). Based on neuropeptide receptor analysis, the AstA system was initially evolutionarily associated with GAL (Figure 3B) (75, 76). Functional studies in the Drosophila AstA system support this evolutionary relation since AstA participates in similar biological processes as GAL (sleep, food intake, metabolism, gut physiology, and others; see above). Nevertheless, more recent phylogeny and gene synteny studies on the receptors and mature peptides might indicate that AstAR and KISS1R emerged after GALR gene divergence from the common ancestral gene (Figure 3C) (77, 78). This model is also reinforced by our finding that AstA regulates the juvenile-to-adult transition, as Kiss1 does in mammals (9). Moreover, KISS signaling in the non-chordate deuterostome sea cucumber Apostichopus japonicus regulates both reproductive and non-reproductive (glucose metabolism) functions as the AstA system does in Drosophila, hinting to a possible dual ancestral role for KISS/AstA signaling (81). These data favor the hypothesis that AstA may be the true kiss1 orthologue while gal is a kiss1 paralog gene. Further studies are required to determine the precise mammalian AstA orthologous and paralogous conclusively.

Whereas AstA and Kiss1 are evolutionarily related, GnRH and ptth are non-homologous genes. PTTH, a cysteine knot protein, was the first hormone/neuropeptide identified in insects (82). It is part of the noggin/noggin-like family, an extracellular
regulator of BMP (Bone Morphogenetic Protein) signaling first characterized in vertebrates (2). However, pth has evolved from a noggin-like gene exclusively in insects being a common component of holometabolous, with no conservation in other insect species or in vertebrates (19). On the other hand, the PTTH receptor Torso (4) is an RTK, similar to the large numbers of RTKs present in animal genomes, making difficult its precise identification for evolutionary studies. However, some phylogenetic relationship analyses determine that torso appeared before the divergence of lophotrochozoans and ecdysozoans but not after the divergence of deuterostomes and protostomes (Figure 3A) (19).

GnRH neuropeptide and its receptor GnRHR do exist in Arthropoda, which include insects. Evolutionary analyses suggest that a common ancestral gene in the Bilateria family gave rise to GnRHR and crzR (corazonin receptor) through gene duplication; after that crzR was lost in the vertebrates (Figure 3D) (83, 84). The second gene duplication in Arthropoda from GnRHR gave rise to Adipokinetic Hormone (AKHR) and AKH/CRZ-related peptide Receptors (ACPR), the latter being lost in Drosophila (not shown). Functional experiments uncovered an essential role of AKH in energy/metabolism homeostasis, acting as the equivalent of the mammalian glucagon, and providing evidence that AKH does not function as the ortholog of GnRH (85). Accordingly, circuity analyses in Drosophila demonstrated that AKH positive neurons do not show a direct innervation to the prothoracic gland as PTTH neurons do or as GnRH neurons connect with the pituitary gland (86). Interestingly, crz is expressed in a group of neurons (CRZn) that project towards both the PTTHn and PG. Early in development crzR is expressed and functional in PTTHn and not in the PG, thus regulating systemic growth PTTHn-mediated but not the metamorphosis timing (10). These observations may indicate that the CRZ-PTTH circuit is more likely a recent innovation in insects. Alternatively, a cross-talk between CRZ neurons and AstAn may exist, which would suggest a more persuasive homology between the mammalian and insect neuroendocrine systems than previously thought (10).

It is tempting to speculate that an ancestral common neurohormonal axis regulating metamorphosis existed in Urbilateria (the putative common ancestor of protostomes and deuterostomes), which would probably have an initial ciliated free-swimming (pelagic) larval phase (Figure 3E) (87). This axis would diverge to mammalian KISS1/GnRH and insect AstA/CZR. Not surprisingly, non-chordate deuterostomes like sea cucumber and starfish have functional KISS and GnRH signaling, respectively (81, 88), and the metamorphosis of an ancient chordate like the Ascidian Ciona is triggered by GABA-induced GnRH release (89). In this scenario, PTTH/TORSO signaling might have been co-opted later on during insect evolution and somehow substituted the transitional developmental function of CRZ (Figure 3E). In contrast, CRZ kept its role in systemic growth and food intake during starvation-induced stress response (67). Supporting this hypothesis, in the diptera-like Oriental fruit fly (Batrocera dorsalis) CRZ signaling regulates juvenile-adult transition, although pth/torso genes are also present in the genome with no discernable role on pupariation (90, 91). Also, the initiation of Manduca ecdysis depends on CRZ activity, and the role of pth on Manduca pupariation is firmly established (82, 92). In summary, we propose that a functional neuroendocrine axis comprising ancestral KISS/GnRH-like signaling, which regulated juvenile-adult transition and probably also stress-related metabolism, existed in Urbilateria (Figure 3E). Despite posterior modifications in other functions and the incorporation of new genes, this surprising conservation among phyla would mean that separating immature juveniles and sexually mature adults might be a successful and most likely unique evolutionary event. It will be of interest to explore this hypothesis further.

**CONCLUSIONS AND FUTURE PERSPECTIVES**

The genetic network involved in the developmental switch display several functions in adulthood. While puberty and metamorphosis present common features regarding the central role of the brain controlling steroid production to initiate the developmental transition, the neuroendocrine axis looked simpler in insects than in mammals. The central neurons controlling gland hormone production involves GnRH and PTTH signaling pathways, with no sequence homology between GnRH/GnRHR and pth/torso genes. These observations would suggest that both neurohormonal axes are just functionally analogous. However, we recently found that Ast is the Drosophila homolog for kisspeptin1, thus challenging this simple view. Moreover, a recent report on Drosophila crz together with novel evolutionary aspects of kiss-GnRH systems indicated that puberty and metamorphosis might be homologous processes. These data open the question of whether the neuroendocrine axis represents an evolutionary convergence or an evolutionary conserved mechanism timing Juvenile-to-adult transition between mammals and insects. Even if there are clear similarities between pubertal and metamorphic mechanism, several differences are also present.

In mammals, the adult HPG axis control steroid production to regulate fertility. In Drosophila, whereas it is not clear whether or not PTTH or CRZ regulate steroid production, steroid (ecdysone) does control adult fertility. The neuroendocrine gene network has also been found to regulate adult physiology and behavior both in mammals and Drosophila. GAL/KISS and AstA are involved in feeding/metabolism and sleep regulation, although with differential relevance. This may indicate not only the maintenance of putative ancestral metabolic roles but also the acquisition of novel functions. So the question is if we can model any dysregulations of the neuroendocrine axis or associated complex human pathologies (such as anxiety and addiction) in a simpler model system like Drosophila.

Future research might lead to a better understanding of the endocrine network evolution and confirm (or refute) the common origin of the neuroendocrine axis that controls the developmental transition to adulthood. Distinguishing similarities and differences in the juvenile to adult transition between mammals
and invertebrates is an essential step in the process of using model organism to allows the identification of target elements to assay potential remedies to multifactorial ailments.

AUTHOR CONTRIBUTIONS

CB: writing – original draft. BG-M: writing – original draft. DD: writing – original draft. NR: conceptualization, funding acquisition, project administration, writing – original draft. FM: conceptualization, funding acquisition, project administration, writing – original draft. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: DD was employed by company Sartorius Netherlands BV. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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