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NEURONAL MIGRATION DURING DEVELOPMENT AND THE AMYLOID PRECURSOR PROTEIN

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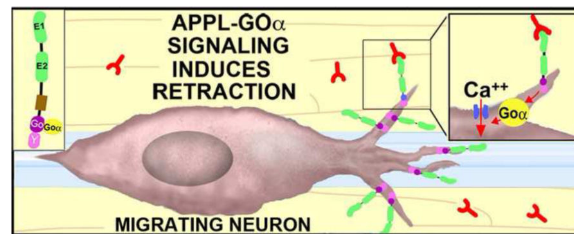
Abstract

The Amyloid Precursor Protein (APP) is the source of amyloid peptides that accumulate in Alzheimer's Disease. However, members of the APP family are strongly expressed in the developing nervous systems of invertebrates and vertebrates, where they regulate neuronal guidance, synaptic remodeling, and injury responses. In contrast to mammals, insects express only one APP ortholog (APPL), simplifying investigations into its normal functions. Recent studies have shown that APPL regulates neuronal migration in the developing insect nervous system, analogous to the roles ascribed to APP family proteins in the mammalian cortex. The comparative simplicity of insect systems offers new opportunities for deciphering the signaling mechanisms by which this enigmatic class of proteins contributes to the formation and function of the nervous system.

Graphical Abstract



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Introduction: neuronal migration and the formation of the insect nervous system

The directed migration of neurons and glia along specific pathways is a universal feature of developing nervous systems [1,2], during which cells navigate through a dynamic environment of potential guidance cues. The phenomenon of neuronal migration was first recognized in vertebrate development, where it is critical to the formation of both the central nervous system (CNS) and peripheral nervous systems (PNS) [3,4], and more modern methods have revealed extraordinary complexity in the modes of migration that give rise to the mammalian cortex [5••, 6•]. The initiation, extent, and termination of migration must be precisely regulated, and a variety of evolutionarily *conserved* guidance cues have been identified that influence particular aspects of migratory behavior [7, 8•]. The significance of the migratory process has been underscored by the numerous developmental defects and neurological diseases arising from errors in migration [9, 10••], although the precise mechanisms underlying many of these defects have proven more difficult to ascertain.

Neuronal migration also plays important roles in invertebrate nervous systems, including mollusks [11], crustaceans [12], and nematodes [13,14], where the molecular pathways regulating the migratory process can be investigated within intact organisms. Until recently, however, the contribution of migration to the formation of insect nervous systems was under-appreciated. Although the differentiation of the embryonic CNS in insects typically involves relatively small displacements of newly generated neurons from their neurogenic niches [15, 16•], more dramatic patterns of neuronal and glial migration have now been documented in both the embryonic PNS [17] and the developing adult CNS [18,19, 20••]. A particularly striking example of migration was recently identified in the developing adult visual system of Drosophila, during which streams of newborn neurons travel into the optic lobes of the brain to establish discrete layers of interneurons with position-specific characteristics [21•, 22••, 23••]. Intriguingly, this process involves Notch-dependent cell fate selection and Slit/Robo-dependent cell positioning (both of which also regulate neurogenesis in the mammalian cortex), providing an elegant illustration of how evolutionarily conserved mechanisms controlling migration play analogous roles in both insect and vertebrate nervous systems [2].

The insect Enteric Nervous System: a dramatic example of neuronal migration

The most dramatic examples of neuronal migration in insects have been described in the developing enteric nervous system (ENS). Analogous to the vertebrate ENS, the insect ENS represents a distinct division of the PNS that provides innervation to the gut and regulates digestion and metabolism [24], as well as modulating a variety of endocrine functions [25,26]. As in other organisms, the insect ENS consists of interconnected peripheral ganglia and nerve plexuses that innervate the gut musculature. In contrast to vertebrates, however, the insect ENS lies superficially on the gut, making it more amenable to direct experimental manipulations. In general, the ENS of all insect species consists of similar components: small subsets of neurons from the brain and abdominal ganglia provide some innervation to

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the anterior and posterior regions of the gut. In addition, distinct populations of enteric neurons originate from neurogenic regions in the foregut and populate enteric ganglia on the foregut (sometimes called the stomatogastric nervous system) and branching nerve plexuses with more dispersed groups of neurons on the midgut. Notably, both the foregut and midgut populations of enteric neurons achieve their mature distributions via extensive phases of migration [27], analogous to the formation of the mammalian ENS by migrating neural crest cells [28]. The following is a brief summary of the ENS in the tobacco hornworm (*Manduca sexta*) to illustrate these events (Figure 1A); however the neuroanatomy of the ENS varies dramatically in different species [29,30], reflecting the radically different digestive requirements and lifestyles needed by particular animals.

In Manduca, the first phase of ENS neurogenesis commences within three neurogenic zones within the mid-dorsal foregut epithelium (Figure 1B–D), which generates a series of neuronal and glial precursors via sequential delamination. Neurons derived from these zones then migrate anteriorly to form two foregut ganglia (frontal and hypocerebral ganglia) that are ensheathed by trailing glial populations [31]. During the second neurogenic phase, a distinct population of ~300 neurons (EP cells) invaginates from a neurogenic placode in the posterior foregut lip to form a discrete packet of post-mitotic neurons (Figure 1D–E) [32]. After spreading bilaterally around the foregut, subsets of these neurons then migrate rapidly onto the midgut via eight muscle bands to form a branching nerve plexus (the **Enteric Plexus**), (Figure 1F–G). Because of their superficial location on the gut surface, the EP cells and their processes can be readily visualized by a variety of methods throughout their differentiation (Figure 1H–J). However, unlike many neurons in the insect CNS, the migratory EP cells are not uniquely identifiable; rather, the pathways followed by individual neurons are stochastic, and only after migrating do they express particular phenotypes that are regulated in part by their final positions [33,34]. This developmental sequence of directed migration and delayed differentiation is also seen in the developing vertebrate ENS, in which enteric neurons migrate substantial distances to form the nerve plexuses of the gut while delaying their terminal differentiation until migration is largely complete [35,36].

In Manduca, the EP cells only traverse about 20% of the midgut before transitioning to axonal outgrowth and subsequent innervation of the lateral musculature, while in grasshoppers, neurons migrate along the entire length of the midgut [30]. Curiously, this aspect of ENS development has been lost in flies, whereby a substantial portion of the midgut remains uninnervated [37]. In this regard, the insect ENS offers an elegant example of how evolutionary changes in common developmental programs can mold the form and function of the nervous system, providing a rich opportunity to explore the relationship between evolution and development of the nervous system. Meticulous studies have delineated the genetic regulation of the foregut neurogenic zones in Drosophila [38•], providing new tools for investigating how gene mutations that perturb migration in the insect ENS may also contribute to congenital disorders affecting human development. In addition, a number of groups (including our laboratory) have exploited the experimental advantages of the insect ENS to define the roles of particular neuronal guidance factors and signal transduction pathways that regulate different aspects of the migratory process, including the insect ortholog of the Amyloid Precursor Protein (APPL; as summarized below).

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Amyloid Precursor Protein: a complicated protein with complex functions

The Amyloid Precursor Protein (APP) is a transmembrane glycoprotein (Figure 2A) that is strongly associated with Alzheimer's Disease (AD) but that also may serve important functions in neuronal development [39•, 40••]. Although multiple isoforms of APP are generated by alternative splicing [41], the predominant form in neurons is APP_{695}, which undergoes dynamic patterns of expression, trafficking, and cleavage by membrane-associated proteases (called secretases) [42, 43•]. In addition, APP can be processed either via the "non-amyloidogenic" (Figure 2B) or the '"amyloidogenic" pathway (Figure 2C); the latter generates β-amyloid peptide fragments ($A\beta_{40-42}$) that are thought to trigger neuronal dysfunction in AD [44, 45•]. Other APP cleavage fragments have been ascribed a bewildering array of biological activities, although their authentic functions remain controversial [41, 46•]. By comparison, growing evidence suggests that APP_{695} itself can function as a transmembrane receptor that regulates multiple aspects of neuronal motility, including migration and outgrowth, synaptogenesis, and response to injury [39•, 47••, 48•], albeit via mechanisms that are still poorly understood. Under some conditions, APP has been found to promote neuronal motility, while in other assays, APP signaling restricts growth [49, 50•]. Moreover, APP can potentially interact with dozens of binding partners and cytoplasmic proteins [51, 47••] and is subject to complex patterns of intracellular trafficking that may modulate its bioavailability [52•, 53••]. An added complication is that mammalian neurons express two closely related orthologs of APP (APLP1 and APLP2; Figure 2D) with partially overlapping biological activities [54, 55•]. Although deemed "intellectually unsatisfying" but some authors [56], these paradoxical effects are reminiscent of other guidance receptors that can both promote and restrict motile responses, depending on the developmental context [57•, 58•].

With respect to neuronal migration, compelling studies have implicated APP_{695} in regulating motile neurons within the developing mammalian cortex, during which undifferentiated neurons must travel along radial glial progenitors to reach their appropriate cortical layers [5••, 6•]. Once again, however, different experimental methods have yielded contradictory results. Genetic deletion of all three APP family proteins (APP, APLP1 and APLP2) induced a striking pattern of excessive, inappropriate neuronal migration, resulting in heterotopias near the outer layer of the cortex [59]. These results suggest that signaling by APP and its orthologs normally restricts the extent of neuronal migration. In contrast, knocking down APP expression in neuronal precursors resulted in the premature arrest of migration, suggesting that APP normally promotes migration in response to permissive cues [60]. Recent evidence demonstrating that APP family proteins also regulate the mitotic behavior of cortical progenitors may provide an explanation for these disparate results [61]. Nevertheless, deciphering how APP family proteins regulate neuronal migration within the mammalian nervous system remains an ongoing challenge.

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Insights from an insect model: APPL and the control of neuronal migration in the ENS

APP is a member of an evolutionary ancient family of transmembrane receptors with orthologs in all higher organisms [62,63]. In contrast to mammals, insects only express one ortholog (APP-Like, or APPL); Figure 2E), which contains the same protein interaction motifs identified in APP_{695} [64–66], and that is processed by homologous classes of secretases to generate similar fragments [67,68]. Transgenic studies in Drosophila have also shown that human APP_{695} can rescue defects caused by the loss of APPL [69], while overexpression of Drosophila Aβ-like fragments induces neurodegenerative responses resembling AD [70]. However, insect APPL is only expressed by neurons [64,66], simplifying an analysis of its normal functions.

Similar to mice lacking APP, flies deleted for APPL are viable [69], but they exhibit a variety of neurodevelopmental and behavioral defects [69,71], accompanied by substantially reduced lifespans [72]. In Drosophila, APPL has been found to regulate synaptic growth at the neuromuscular junction [65], axonal targeting by developing photoreceptors, and dendritic sprouting within the metamorphosing brain [65,73,74]. Also like APP_{695}, APPL expression is substantially upregulated in response to injury [75], providing further evidence that APP family members participate in multiple aspects of neuronal motility and growth. In many instances, APPL appears to function as a transmembrane receptor, although both its cleaved ectodomain and AICD fragments have also been implicated in some of these functions [72,73,75]. In adult flies, APPL is required for associative memory [76••] and circadian clock activity [77••], supporting other evidence that perturbing the normal functions of APP may contribute significantly to the behavioral deficits that occur in AD [40••, 47••].

Does APPL play a role in regulating neuronal migration, similar to the roles ascribed to APP_{695}? To investigate this question, we adapted a well-characterized assay of neuronal migration in the developing ENS of *Manduca*, using an embryonic culture assay that permits direct manipulations and imaging of the migratory EP cells [27]. Initially, we showed that the EP cells first express APPL shortly after emerging onto the foregut, and concentrate the full-length protein in their leading processes throughout their subsequent phases of migration and outgrowth (Figure 2F–G) [66]. Based on provocative evidence that APP_{695} interacts with the heterotrimeric G protein Goα [78], we also showed that APPL co-localizes with Goα in the EP cells, and we used co-immunoprecipitation and bi-molecular fluorescence assays to demonstrate that the two proteins directly interact [66,79]. We also used an embryonic culture assay to show that inhibiting APPL expression (with antisense constructs) or Goα activity (with pharmacological reagents) induced the same distinctive pattern of ectopic migration and outgrowth onto the interband regions, compared to cultured controls (Figure 2H–I). In contrast, hyperactivating APPL-Goα signaling had the opposite effect, causing a dramatic inhibition and stalling of migration and outgrowth [79,80]. These results are analogous to the ectopic migration seen in mice lacking all three APP family proteins [59], and they substantiated our earlier studies showing that Goα signaling restricts migration via the local activation of a calcium (Ca^{2+}) current in the EP cells [81].

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Conclusions and future directions

Our results also provide new support for the model that APP family proteins function as unconventional Goα-coupled receptors that regulate neuronal migration. Within the developing insect ENS, we propose that activation of APPL signaling (by ligands associated with the interband regions) stimulates Goα-dependent Ca^{2+} influx that induces local retraction responses (Figure 3A), thereby helping to maintain the migratory neurons on their pathways. However, several outstanding issues remain to be resolved. (1). What are the ligands that regulate APPL signaling in the developing ENS? Work in mammalian systems has shown that multiple members of the Contactin family of cell adhesion receptors can directly interact with APP [82, 83•]. By comparison, insects express only a single Contactin ortholog that is expressed by glial and epithelial cells [84], and we are currently testing whether *Manduca* Contactin serves as an APPL ligand within the developing ENS. (2). Does APPL regulate neuronal migration in other regions of the nervous system? Because many examples of migration in *Drosophila* involve relatively small distances, the modulatory effects of APPL might have been previously overlooked. However, given the robust migratory patterns that were recently discovered in the developing fly visual system [21•, 22••, 23••], a renewed investigation of how APPL signaling affects optic lobe formation might provide new insight into the mechanisms controlling migration in the insect CNS. (3). How does APPL signaling promote neuronal motility in some contexts while inhibiting growth in others? Like other APP family proteins, APPL may interact with a diversity of binding partners and signaling proteins besides Contactins and Goα [71,75,85], supporting the view that APPL can be recruited into distinct signaling complexes in a context-dependent manner (Figure 3B). With the advent of improved protocols for visualizing dynamic protein interactions within neurons [86•, 87•], it may now be possible to exploit the comparative simplicity of insect models to address this challenging issue within the developing nervous system.

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HIGHLIGHTS

- Neuronal migration is essential to the formation of the insect nervous system
- The Amyloid Precursor Protein family regulates multiple types of neuronal motility
- The insect ortholog of APP (APPL) is expressed in all developing neurons
- APPL regulates neuronal migration in the insect enteric nervous system
- APP and APPL may control neuronal motility via similar molecular pathways

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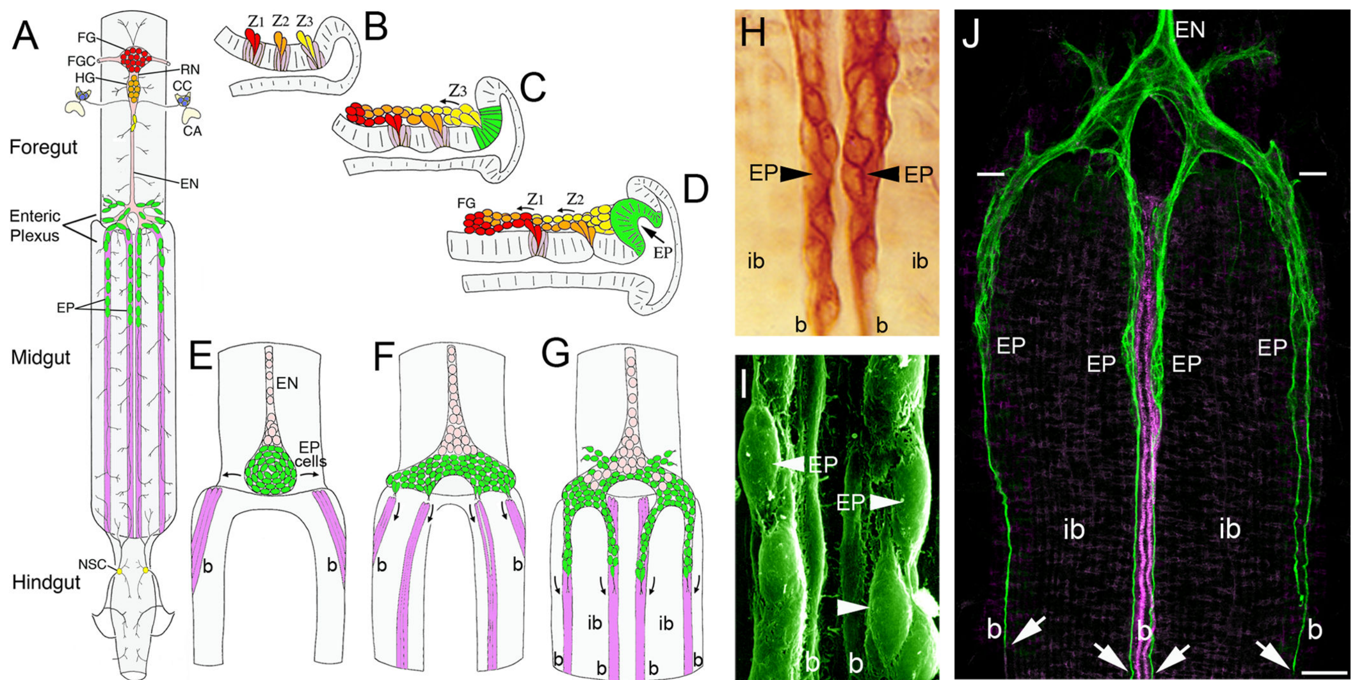

Figure 1. Embryonic development of the insect Enteric Nervous System (ENS) involves extensive patterns of neuronal and glial migration

(**A**), Schematic drawing of the ENS and associated neurosecretory organs in the larval stage of the tobacco hornworm *Manduca sexta* (modified from [27]). The primary ganglion on the foregut is the frontal ganglion (FG; red), connected to the overlying brain lobes by paired frontal ganglion connectives (FGC). Several nerve branches extend anteriorly onto the pharynx, while the recurrent nerve (RN) extends posteriorly to the hypocerebral ganglion (HG; orange), situated below the brain. In Manduca, the hypocerebral ganglion initially forms during embryogenesis but then becomes closely opposed to the frontal ganglion and is no longer readily distinguished in later stages. The HG is also connected to the paired *corpora cardiaca* (CC; blue), the primary neurosecretory organs of the brain, which are adjacent to the *corpora allata* (CA; the source of Juvenile Hormone). From the HG, the esophageal nerve (EN) extends posteriorly along the length of the foregut, giving rise to short nerve branches that innervate the foregut musculature. Near the foregut-midgut boundary, the esophageal nerve connects with the enteric plexus that spans the foregut-midgut boundary, which includes nerve branches extending along radial muscles on the foregut and major nerves that extend along eight well-defined muscle bands that lie superficially on the midgut (purple). The enteric plexus contains a population of ~300 distributed neurons (EP cells; green), which includes intermingled groups of neurons expressing a variety of morphological and transmitter phenotypes. The EP cells occupy positions along the anterior 20% of the midgut, and extend long axons posteriorly along the muscle bands with sparse lateral branches that provide a diffuse innervation of the interband midgut musculature. The hindgut is innervated by branches of the proctodeal and rectal nerves that originate in the terminal abdominal ganglion of the ventral nerve cord. Branches of the proctodeal nerve also extend onto the posterior midgut and contain several peripheral neurosecretory cells (yellow). (**B–C**), Neurogenesis of the developing ENS in Manduca

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(after [31]). Panels show lateral views of the foregut midline; anterior is to the left, dorsal is to the top. When raised at 25°C, Manduca embryogenesis is complete in 100 hr (1 hr = 1 hour post-fertilization, or hpf). **(B),** By ~24 hpf, three neurogenic zones (Z1, Z2, & Z3) have formed in the dorsal foregut epithelium, which give rise to a series of mitotically active precursor cells via sequential delamination. Precursors giving rise to neurons typically divide only once (or occasionally twice) after delaminating, similar to midline precursors in the embryonic CNS. (**C**), By 28 hpf, streams of zone-derived cells have begun to migrate anteriorly along the foregut, while the remaining zone 3 cells delaminate as a group. The epithelium surrounding the original position of zone 3 subsequently differentiates into a distinct placode that will form the EP cells (green). (**D**), By 33 hpf, migrating zone cells have begun to form the frontal ganglion (FG), while the remaining zone 2 cells delaminate as a group. The EP cell placode has also begun to invaginate from the EP cell packet (described below). Zone 1 continues to generate cells until almost 40 hpf (not shown); late-emerging zone cells derived from all three zones tend to become glial precursors that remain mitotically active throughout much of embryogenesis and establish the glial sheath surrounding the foregut nerves and ganglia. (**E–F**), Formation of the midgut enteric plexus; panels show dorsal views of the developing ENS at the foregut-midgut boundary (after [88]). **(E)**, By 40 hpf, the EP cells (green) have invaginated *en mass* from their neurogenic placode located within the posterior dorsal lip of the foregut (**D**, green). The neurons then commence a bilateral spreading phase of migration (arrows) that almost completely encircles the foregut, adjacent to the foregut-midgut boundary. Concurrently, subsets of longitudinal muscles on the midgut (magenta) begin to coalesce into eight well-defined bands as dorsal closure of the midgut proceeds. Anteriorly, the EP cell packet is in continuity with the developing esophageal nerve (EN), which contains populations of proliferating glial precursors (pink; derived from zone 3) that will subsequently ensheath the enteric plexus. (**F**), By 55 hpf, the EP cells have almost completely surrounded the foregut, and subsets of the neurons have aligned with each of the midgut muscle bands (only the dorsal four are shown). (**G**), By 58 hpf, subsets of EP cells have begun to migrate in a chain-like manner along the midgut muscle bands; smaller subsets also migrate onto radial muscles of the foregut (muscles not shown). Proliferating glial cells (pink) subsequently migrate along the pathways established by the neurons, thereby ensheathing the branches of the enteric plexus. (**H**), Magnified view of EP cell groups migrating on the mid-dorsal band pathways (at 58 hpf) of an embryo immunostained with an antibody recognizing all isoforms of the cell adhesion receptor Fasciclin II (Fas II). The migratory neurons and underlying muscle bands (b) express transmembrane Fas II (TM-Fas II), while the trailing glial cells express GPI-linked Fas II. The migratory EP cells and their processes remain primarily confined to their band pathways while avoiding the adjacent interband musculature (ib). (I), Scanning electron micrograph showing the migratory EP cells on the mid-dorsal band pathways (b) of an embryo at 65 hpf. (**J**), Lower magnified view of the developing ENS at 62 hpf, in an embryo that was immunostained with anti-TM-Fas II (green). TM-Fas II immunoreactivity in the mid-dorsal muscle bands is shown in magenta to better distinguish the EP cell processes (after [79]). At this stage, the EP cells have migrated ~200 μm and have begun to extend fasciculated axons (arrows) more posteriorly along the muscle bands (b). Throughout this developmental period, the EP cells avoid the adjacent interband regions (ib), extending

terminal branches onto the lateral musculature only after migration and axogenesis is complete (~80 hpf). Scale = 20 μm in (**H**); 5 μm in (**I**); 60 μm in (**J**).

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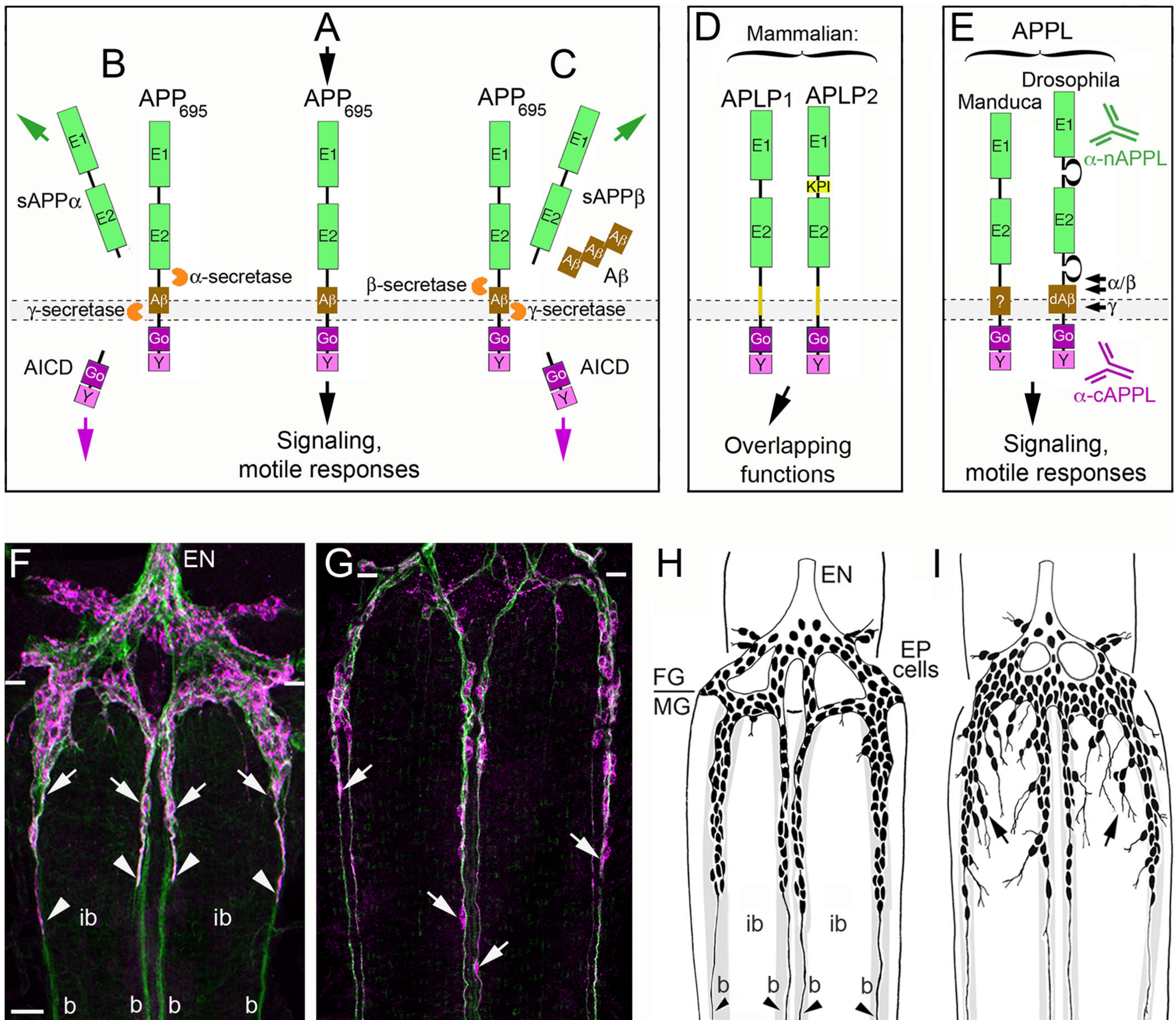

Figure 2. The insect ortholog of Amyloid Precursor Protein regulates neuronal migration in the developing ENS

(**A–E**), the structure and processing of APP family proteins is similar in insects and mammals. (**A**), human APP_{695} (containing 695 amino acids) has the topology of a type-1 transmembrane glycoprotein, consisting of two extracellular protein interaction domains (E1 and E2); a transmembrane domain that contains the Aβ cleavage fragment; and a short cytoplasmic tail that contains highly conserved binding domains for the heterotrimeric G protein Goα (Go) and a tyrosine-based sorting motif (Y). A wide variety of potential binding proteins and ligands have been identified that can interact with the E1–E2 extracellular domains, while numerous intracellular adapter and signaling proteins besides Goα (are capable of interacting with the cytoplasmic domains. Studies in a variety of systems have shown that APP_{695} is capable of functioning as a transmembrane receptor, whereby activation with candidate ligands can induce signaling responses that modulate

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neuronal motility. (**B**), In the non-amyloidogenic pathway of APP processing, APP is first cleaved by α-secretases at a juxtamembrane site within the Aβ domain, which releases a soluble/secreted ectodomain fragment (sAPPα) and a short transmembrane C-terminal fragment (CTF; not shown). CTF fragments are then rapidly cleaved by the γ-secretase complex (containing presenilins) to produce a cytoplasmic APP intracellular domain (AICD) and a small "p3" peptide of no apparent significance (not shown). (**C**), In the amyloidogenic pathway, APP is first cleaved by β-secretase (BACE) to generate a slightly shorter sAPPβ ectodomain fragment and a slightly longer CTF fragment containing the Aβ peptide (not shown). This intermediate fragment is then rapidly cleaved by the γ-secretase complex to generate an identical AICD fragment and β-amyloid peptide fragments ($A\beta_{40-42}$) of varying lengths that accumulate in the brain with aging. Secreted sAPP ectodomain fragments have been ascribed a variety of functions (both beneficial and harmful to neurons), including activation of APP signaling (via interactions with the transmembrane holoprotein); AICD fragments have been shown to induce changes in gene transcription (analogous to the Notch intracellular domain; NICD), although the biological significance of these activities remains under debate. (**D**), In addition to APP, vertebrates also express to closely related orthologs: APP-Like Protein 1 & 2 (APLP1 and APLP2). Both family members contain extracellular and intracellular protein interaction domains that are closely similar to these domains in APP and have been shown to have partially overlapping functions within the nervous system. (**E**), Insects only express a single APP family protein, APPL (APP-Like). They also contain similar extracellular and intracellular domains that share considerable sequence conservation with human APP_{695}, including 100% conservation within the Go domain (required for direct interactions with Goα; [79]). Drosophila APPL has also been shown to contain an Aβ-like fragment (dAβ) that is generated by sequential cleavage of APPL by endogenous β- and γ-secretases [70]). Antibodies specific for the n-terminal (α-nAPPL) and c-terminal (α-cAPPL) regions of APPL have been generated that can distinguish the distribution of the holoprotein from its cleavage fragments. (**F–G**), The embryonic ENS of Manduca at different developmental stages, labeled with anti-TM-Fas II (green) and anti-cAPPL (magenta). (**F**), At 58 hpf, TM-Fas II is expressed by both the EP cells and their muscle band pathways on the midgut (b). The migratory EP cells also strongly express APPL (arrows) as they travel onto the bands while largely avoiding the adjacent interband regions (ib). Previous studies have shown that transmembrane APPL traffics into their leading processes (arrowheads), where it interacts with Goα [79]). (**G**), By 65 hpf, the EP cells have transitioned from migration to axon outgrowth, but they continue to robustly express APPL in their cell bodies (arrows) and advancing growth cones (out of the field of view). Paired white hatchmarks indicate the foregut-midgut boundary; scale bar = 30 μm. (**H–I**), examples of the ENS in embryos that were opened to expose the developing ENS prior to the onset of EP cell migration (~50 hpf) and allowed to develop for an additional 18 hr (through the periods of migration and outgrowth). At the completion of the culture period, embryos were fixed and immunostained with anti-Fas II to reveal the extent of migration and outgrowth, and analyzed by *camera lucida* methods. (**H**), Embryo that was treated with control antisense morpholino constructs with no known gene targets in insects; EP cell migration and axon outgrowth (arrowheads) was largely confined to the normal band pathways. (**I**), Embryo that was treated with antisense morpholino constructs specific for *Manduca* APPL mRNA; although EP cells that maintained strong contact with their bands migrated and extended

axons normally along these pathways, a substantial number of neurons migrated and extended processes inappropriately onto the interband regions (black arrows). A similar pattern of ectopic migration was caused by inhibiting the heterotrimeric G protein Goα or by blocking Goα-dependent Ca^{2+} influx [79,81].

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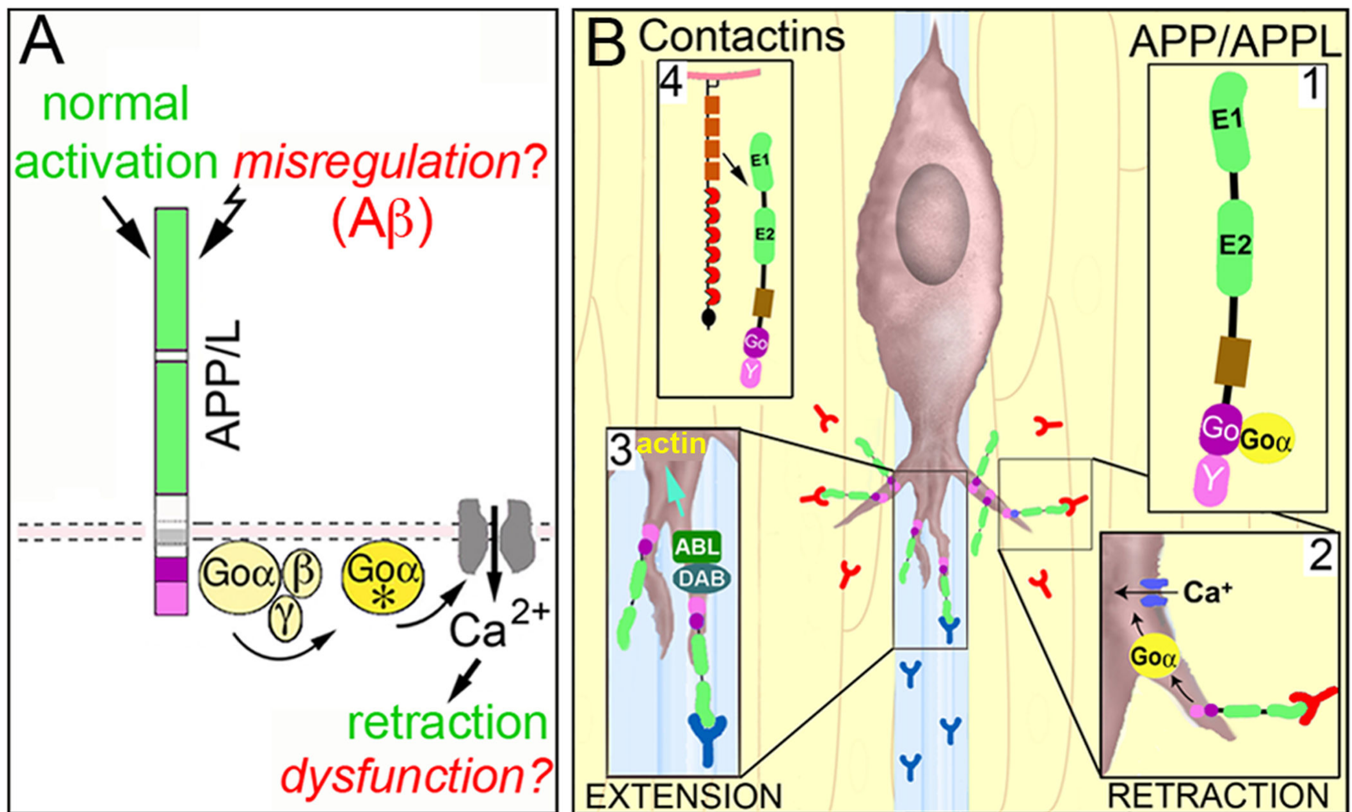

Figure 3.

Proposed model for how APP family proteins regulate the motile behavior of developing neurons in response to context-dependent guidance cues. (**A**), Stimulation of human APP (or insect APPL) by endogenous ligands activates the heterotrimeric G protein Goα (Goα*), which in turn induces Goα-dependent effectors (including Ca^{2+} influx) that alter cytoskeletal dynamics required for filopodial retraction. During normal development, this signaling pathway helps restrict inappropriate neuronal migration and outgrowth, and might also regulate synaptic pruning. In neurodegenerative conditions like Alzheimer's Disease, multiple factors (including Aβ) might induce the misregulation of APP signaling, provoking Goα hyperactivation and Ca^{2+} overload that results in neuronal dysfunction and death. (**B**), Within the developing ENS of *Manduca*, APPL acts as a Goα-coupled receptor (**B_1**) for ligands encountered by the migratory EP cells when they extend filopodia off their normal band pathways. Stimulation of APPL induces the local activation of Goα within filopodia (**B_2**), resulting in Goα-dependent Ca^{2+} influx (via a voltage-independent Ca^{2+} current). In turn, Ca^{2}-dependent modulation of the actin cytoskeleton results in filopodial retraction, helping to keep the neurons on their correct band pathways. A variety of potential ligands associated with the ensheathing glial cells and interband musculature might trigger APPL-Goα signaling, including insect Contactin (**B_4**). However, in other neurons (and in other regions), ligands associated with permissive regions might activate different APP/L-linked signaling pathways that promote growth. For example APP interactions with the adapter protein Disabled (DAB) can induce the activation of Abl kinase [71], which might enhance actin remodeling to promote outgrowth (**B_3**). In this manner, APP family proteins can

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function as "molecular hubs", capable of regulating different types of motile responses in a context-dependent manner.