Brain Research Bulletin, Vol. 53, No. 5, pp. 571–583, 2000

0361-9230/00/$–see front matter

PII S0361-9230(00)00391-9

# Behavioral transformations during metamorphosis: Remodeling of neural and motor systems

Christos Consoulas, Carsten Duch,† Ronald J. Bayline and Richard B. Levine*

*Division of Neurobiology, University of Arizona, Tucson, AZ, USA*

[Received 22 June 2000; Revised 8 August 2000; Accepted 9 August 2000]

**ABSTRACT: During insect metamorphosis, neural and motor systems are remodeled to accommodate behavioral transformations. Nerve and muscle cells that are required for larval behavior, such as crawling, feeding and ecdysis, must either be replaced or respecified to allow adult emergence, walking, flight, mating and egg-laying. This review describes the types of cellular changes that occur during metamorphosis, as well as recent attempts to understand how they are related to behavioral changes and how they are regulated. Within the periphery, many larval muscles degenerate at the onset of metamorphosis and are replaced by adult muscles, which are derived from myoblasts and, in some cases, remnants of the larval muscle fibers. The terminal processes of many larval motoneurons persist within the periphery and are essential for the formation of adult muscle fibers. Although most adult sensory neurons are born postembryonically, a subset of larval proprioceptive neurons persist to participate in adult behavior. Within the central nervous system, larval neurons that will no longer be necessary die and some adult interneurons are born postembryonically. By contrast, all of the adult motoneurons, as well as some interneurons and modulatory neurons, are persistent larval cells. In accordance with their new behavioral roles, these neurons undergo striking changes in dendritic morphology, intrinsic biophysical properties, and synaptic interactions. © 2001 Elsevier Science Inc.**

**KEY WORDS: *Drosophila*, *Manduca*, Neuromuscular, Plasticity.**

## INTRODUCTION

There are few behavioral transformations as dramatic as those observed during insect metamorphosis. Larval and adult behavior place distinct demands upon the nervous system. Most, but not all, muscles and sensory neurons are replaced by newly generated peripheral elements in the adult. In contrast, within the central nervous system (CNS), behavioral alteration is accomplished to a remarkable extent by recycling, rather than disposing of larval elements. Identified neurons undergo stereotyped and substantial changes in their intrinsic electrical properties, dendritic and axonal branching patterns, and synaptic interactions. Thus, the dramatic remodeling of the nervous system that accompanies metamorphosis creates an eminent model for more subtle forms of behavioral plasticity in other organisms [136–138,233,238–240,262].

Insects have long served as favored model systems for the analysis of neuromuscular function, neural circuits and behavior [33]. Large holometabolous insects such as the moth, *Manduca sexta*, share the advantages, such as size and accessibility to intracellular recording techniques, that are offered by locusts, cockroaches, stick insects and crickets. On the other hand, insects such as *Drosophila melanogaster* allow complementary molecular-genetic approaches to fundamental mechanisms of development that are shared among organisms. Both approaches have been exploited to study the cellular events and regulatory mechanisms of metamorphosis.

Insects that undergo complete metamorphosis (holometabolous insects) face significant behavioral and developmental challenges. Typical larval behavior includes crawling, feeding, defensive thrashing and ecdysis. By contrast, adult insects walk, fly and reproduce. Larval and adult behavior require different intrinsic properties and synaptic interactions of neurons, different contractile properties of muscles and unique transduction capabilities of sensory neurons. The circuitry and musculature underlying larval behavior must give way to that of the adult without allowing inappropriate actions to occur during the transition. One solution would be to evolve two parallel systems, which perhaps inhibit each other to allow an orderly transition. On the other extreme, recent results from the stomatogastric nervous system [78] illustrate how subtle changes in the strength of synaptic interactions or magnitude of an ionic current allow neuromodulators to alter the output of a neural network dramatically without invoking new neurons. The solution reached by holometabolous insects lies somewhere in between. Whereas cell death and neurogenesis are important components of postembryonic development within the central nervous system, many larval neurons persist to participate in significantly different behavioral patterns of the adult. These persistent neurons undergo permanent changes in structural and functional properties that go beyond those evoked by traditional modulatory influences. Understanding how specific cellular changes allow specific behavioral changes is a key goal of current research in this field, and will provide useful insight into the organizational principles of neural circuits.

The regulation of these changes also presents significant challenges. Although the most dramatic changes in cellular properties take place during the relatively quiescent pupal stage of life, these changes must not interfere with the limited behavior that is unique to this period. Moreover, larval circuits must remain functional until no

* Address for correspondence: Dr. Richard B. Levine, Division of Neurobiology, Room 611 Gould Simpson Building, University of Arizona, Tucson, AZ 85721, USA. Fax: +1-(520)-621-8282; E-mail: rbl@neurobio.arizona.edu
† Present address: Free University Berlin, Inst. Neurobiology, Königin-Luise Str. 28-30, D-14195, Berlin, Germany.

longer required and new adult behavior must be displayed at the appropriate time. Peptide hormones may activate or repress neural circuits to ensure their expression at the correct stage. Modifications in the properties of motoneurons and muscles must be coordinated. Similarly, dendritic remodeling must be coordinated with the loss of old and the formation of new synaptic inputs. Inductive interactions among cells may play an important role during metamorphosis, as they do during many phases of development in all organisms. Steroid hormones provide another regulatory mechanism which insect metamorphosis shares with the postembryonic development of other nervous systems.

This review will focus on some of the more recent advances in understanding the cellular changes and regulatory mechanisms that allow behavioral transformations during insect metamorphosis. Its goals are to identify the problems posed by the behavioral transformations of metamorphosis, describe what we currently understand about the solutions and discuss the issues that remain for the immediate future. Peripheral elements of the motor and sensory systems will be discussed first, followed by a consideration of the central nervous system. Several reviews within the past 10 years provide additional information [136–138,152,233,238–240,262]. We point, in particular, to an excellent review that provides additional information on the significant contributions that insect metamorphosis has made to our understanding of cell death as a developmental mechanism [257].

### *Remodeling of Muscles*

The most apparent behaviors of adult holometabolous insects, including flight, walking, copulation and oviposition, are accommodated by specialized appendages that develop during metamorphosis. Their function is served by striated muscles that differ in fiber number, contractile properties and innervation from their larval counterparts. The transformation is achieved by an essentially complete turnover of the larval musculature and neuromuscular junctions (Fig. 1A, see motor nerve/muscles). Some larval muscles remain physiologically active to generate the movements of the pupa, but die following adult emergence [64,237,242]. Most larval muscles, however, degenerate during the transition from the larva to pupa. Many of these muscles are associated with larval structures that degenerate, such as the prolegs, whereas others are simply not required in the adult [258]. Fiber atrophy and disorganization of contractile elements occur gradually, together with a decline in resting potential amplitude and synaptic efficacy [143, 195,196,212]. The breakdown of larval muscle fibers coincides with motor axon dedifferentiation, which is characterized by the loss of most of the larval nerve branches and motor terminals and the retraction of the primary axon into a reduced number of collateral branches that end in bulb-like terminals. During this retraction, the overall pattern of peripheral nerve branches remains relatively constant. Motoneuronal processes are associated with the epidermis, larval muscle remnants and imaginal myoblasts [36,39,47,52,60,61,177,232,246,248].

Some adult muscles differentiate using remnants of the larval fibers as a template [22,40,47,52,60,80,170,226,259]. Larval templates differentiate either directly into adult muscles (e.g., abdominal muscles in *Manduca*; [22,80]) or they are invaded by imaginal myoblasts before differentiation (e.g., one group of the flight muscles [46,52,60,128,170]. Most of the adult muscles, however, develop *de novo* from distinct populations of imaginal myoblasts that are located in imaginal discs, along nerve branches or in specific regions close to the epidermis [20,40,41,60,125,249]. During metamorphosis these cells migrate, probably using peripheral nerves and epidermal cells as guidance cues, to accumulate at the sites of adult muscle formation [39,40,47,60,61]. Myoblasts accumulate close the terminal processes of the motoneurons, where they fuse, proliferate and differentiate into striated adult muscle fibers. The retracted motor axons begin to re-expand as the myoblasts fuse and begin to differentiate. Neuromuscular transmission is functional at the earliest stages of this process [39,42]. Myotube formation and fiber striation are accompanied by the differentiation of motoneuron endings into adult presynaptic terminals [39,42,61,170].

### *Determination of Muscle Fiber Type in the Adult*

Holometabolous insects form a variety of highly specialized adult muscles that differ significantly from the rather homogeneous larval muscles. For example, larval thoracic body wall muscles that perform contractions at rates of $<0.5$ Hertz during crawling, are replaced by flight muscles that constitute 12–65% of the total mass of the animal (reviewed in [147]), and contract with frequencies up to 30 Hz. Flight muscles exhibit dramatic specializations of contractile apparatus and metabolic machinery. For instance, *Drosophila* flight muscles contain specialized forms of myosin light and heavy chains that are distinct from those of other larval and adult muscles ([23,59,202], reviewed in [162]). These specific myosin isoforms allow the two-fold increase in ATP-ase activity [222,223]. Similarly, flight muscles express a unique actin gene which is not present in other adult muscles ([66,87], reviewed in [163]). This actin isoform is required for flight muscle function in Drosophila, since its partial replacement with a larval actin isoform disrupts muscle structure and function [67]. Specializations are also extensive on the metabolic level, such that insects have been described as elite invertebrate athletes because to their flight muscle performance [265].

Larval and adult muscles, including the flight muscles of most of the species, are characterized as synchronous, due to the 1:1 relationship between neural stimuli and contraction. In contrast, dipteran flight muscles are asynchronous, showing a 1:10 ratio of neural stimuli to contractions (reviewed in [89,147,184]). This mechanism allows small flies to beat their wings with frequencies up to 1000 Hz [213]. Flight muscles have features typical of phasic arthropod muscles [8,89,250]. For example in *Manduca*, in comparison to their larval homologs, which are innervated by the same motoneurons (see below; [36,52,81]), flight muscles twitch faster, have a smaller tetanus/twitch ratio, shorter sarcomeres, regular Z-bands, smaller number of thin filaments and larger mitochondrial volume [197].

The acquisition of specialized adult muscle properties during metamorphosis may involve cellular interactions. In *Drosophila* for example, the expression of an actin isoform by a male-specific muscle is determined, not by contributing myoblasts, but by the innervating motoneuron [48,126,127]. Because the persistent larval motoneurons change their activity pattern during metamorphosis and are in contact with the muscle fibers during their development (see below), innervation and/or nerve activity may play an important role in adult fiber specification, as is true in vertebrates [72,91,203]. Similarly, in crustaceans, alteration of activity pattern by nerve stimulation can influence the physiology and morphology of the neuromuscular synapses [141]. In contrast to crustaceans, however, where tonic neuromuscular junctions differ in fine structure and function from the phasic neuromuscular junctions [9,10], larval and adult neuromuscular junctions in *Manduca* have similar ultrastructural and functional characteristics [194,197]. In contradiction to the hypothesis that the nerve can specify muscle properties, denervation early in flight muscle development did not change the ultrastructural characteristics ([171], reviewed in [170]) or actin expression [63] of the adult muscles. Thus, adult

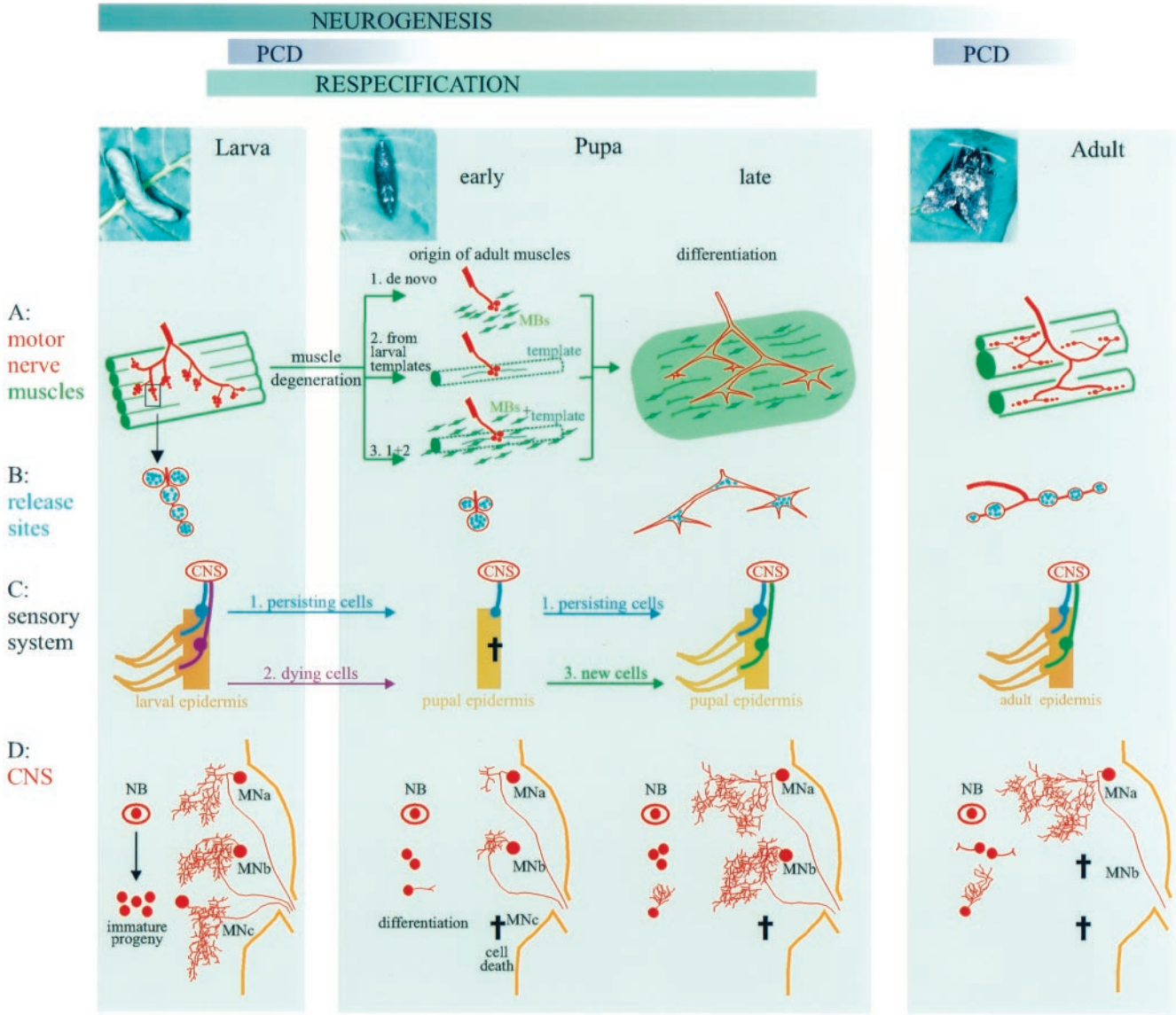


FIG. 1. Schematic summary of the metamorphic changes associated with the (A) peripheral neuromuscular system, (B) presynaptic release sites of the motor axons, (C) sensory system, and (D) central nervous system (CNS). Bars at the top indicate the time course of the three major mechanisms (neurogenesis, programmed cell death (PCD), respecification) that underlie the remodeling of the nervous and muscular systems. Photographs show the post-embryonic stages (larva, pupa, adult) of the moth *Manduca sexta*. (A) Motor nerve/muscles (motor neurons in red, muscle in green): Most of the larval muscles degenerate at the end of the last larval instar. In general three different modes of adult muscle development take place during metamorphosis. New-adult muscles develop *de novo* from imaginal myoblasts (1), or from larval muscle remnants that serve as a template (2), or from a combination of myoblasts and larval muscle remnants (3). The axons and terminals of the persistent larval motoneurons undergo severe retraction after the loss of the larval muscles but remain in the periphery to become associated with the precursors of the adult muscles. As the imaginal muscles differentiate and mature, the retracted motor axons re-expand to form new synaptic terminals. (B) Release sites: selective enlargement of motoneuron terminals (red) as indicated by the box in (A) with the sites of vesicular recycling in blue. Sites of $Ca^{2+}$-dependent synaptic vesicle release and recycling are concentrated in the intact larval and adult motor terminals and in the retracted terminals after the degeneration of the larval muscles. Sites of vesicular recycling are distributed in axonal processes of the motoneurons during the development of the adult muscles, before becoming redistributed to the new presynaptic varicosities. (C) Sensory system: Larval sensory organs (see schematic mechano-sensory hair) and most of the associated neurons degenerate during the larval-to-pupal transition (indicated by the purple sensory neuron). A few larval sensory neurons persist to the adult to innervate new sensory organs (indicated by the blue sensory neuron). The formation of new sensory organs during metamorphosis is accompanied by the birth of many new sensory neurons (indicated by the green sensory neuron) (D) CNS: Neuroblasts (NB) proliferate throughout metamorphosis to give rise to new neurons. Most of the larval motoneurons persist in the adult stage, but they undergo dendritic remodeling, as indicated by MNa. Some motoneurons survive the pupal ecdysis, but then die in the late pupal stage or after adult emergence, as indicated by MNb. A third group of motoneurons dies at the larval-to-pupal transition, as indicated by MNc.

muscles may be specified by pre-programmed precursors (muscle pioneers or founder myoblasts) [28,201,253] as are embryonic vertebrate and insect muscles ([12,13,88], reviewed in [21,72]).

*Neural Influence on Muscle Remodeling*

Despite the uncertainty of neural influences on muscle fiber type, there is strong evidence that the development of adult mus-

cles is dependent upon innervation [22,41,170]. This is in contrast to embryonic development, where the formation of the larval muscle is independent of innervation [29]. The constant close association between nerve and developing muscles (Fig. 1A) facilitates a role for the nervous system in adult myogenesis. Indeed, the possibility that orthograde interactions may influence adult muscle development was raised early in the century, when it was reported that extirpation of the CNS during metamorphosis led to the development of adults without muscles [118,170,270]. Later experiments revealed that the motoneurons play multiple roles during metamorphosis. Severing larval peripheral nerves prevents the formation of adult leg muscles in *Manduca*, and a group of indirect flight and abdominal muscles in *Drosophila* [41,48,63]. During normal development, myoblasts may migrate along peripheral nerves to reach their final destination, and may require additional signals that promote accumulation [41]. Thus motoneurons, together with epidermal cells, which direct the specification of muscle attachments [62,269], may play a role in muscle formation and patterning by providing a substrate for myoblast migration and accumulation.

An additional role of motoneurons is to control the rate of myoblast proliferation. Absence of innervation during the period when myoblasts (for muscles with *de novo* origin) or myonuclei (for muscles that derive from larval templates) normally proliferate, results in the formation of fewer and smaller muscle fibers [22,41,48,80,170]. Similarly the proliferation of myoblasts in vitro is enhanced in the presence of neurons [145]. A similar influence of the motor nerve has been suggested in birds and mammals, where primary muscle fibers differentiate in the absence of innervation, but the number of secondary myotubes is reduced severely [51,65,81,149,200]. The signals whereby motoneurons influence myoblast proliferation remain unknown. Co-culture experiments suggest that actual contact or short range nerve/myoblast interactions are required for myoblast proliferation, pointing to a cell surface and/or short-distance diffusible molecule [145]. Such a candidate is the hedgehog protein, which is released by the ingrowing retinal axons in *Drosophila* to trigger the proliferation of laminar precursor cells in the developing adult optic lobes [90]. Another possibility is that electrical activity may trigger myoblast proliferation. In aneural (peroneal muscular atrophy) and dysgenic (muscular dysgenesis) mutant mice that bear almost the proper number of primary myotubes, chronic tetrodotoxin (TTX) paralysis sharply reduces the number of secondary myotubes [6,7,77]. Muscle fiber depolarization via the nerve may be possible early during adult muscle development, because functional presynaptic vesicular release sites are present in the persistent axonal processes of motoneurons during muscle remodeling (see below; [42]). In co-cultures of *Manduca* leg myoblasts and neurons, however, TTX did not reduce myoblast proliferation [145].

### *Regulation of Motor Terminal Remodeling*

Following the degeneration of larval muscles, the axonal processes and terminal varicosities of the motoneurons retract. The retracted motor axons, however, remain in the periphery to become associated with muscle remnants, myoblasts or larval templates. Nerve retraction is followed by an initial phase of re-growth over the anlagen of the adult muscles. Nerve growth continues as motor axons branch extensively to form adult motor terminals while muscle fibers differentiate and enlarge [39,52,61,81,170,232,246]. This raises an interesting issue. What becomes of the presynaptic specializations for neurotransmitter release during the remodeling of motor terminals?

Ultrastructural studies reveal that synaptic vesicles remain in motor nerves during the degeneration and reformation of the target muscle [218]. Furthermore, synaptic vesicle proteins and sites of vesicular recycling persist in the motor terminals of retracted *Manduca* leg motoneuron axons after the loss of the larval muscles (Fig. 1B). Interestingly, release sites occur transiently in axon shafts as the re-expanded processes grow over the muscle anlagen, then finally become restricted to terminal boutons as the underlying muscle fibers mature (Fig. 1, see release sites; [42]). Similar events may accompany amphibian metamorphosis [4,82–84,214,266].

The mechanisms that regulate the dismantling and re-formation of presynaptic machinery during muscle remodeling are not well understood. Motor terminals are always in association with a target of some sort (muscles remnants, myoblasts or developing muscles), which may regulate several aspects of nerve remodeling. This hypothesis has been tested with hormonal manipulations that save the larval muscle in *Manduca*, and with target ablations in *Drosophila*. The experiments suggest that regression of axonal processes during muscle degeneration and the final phase of motor terminal growth and branching over the developing adult muscle fibers may be controlled by cues provided by the target [63,246]. In contrast, axonal regrowth over the early differentiating muscle anlagen may be target-independent [246]. Thus the survival of presynaptic specializations and function in the terminals of the retracted axons, and the progressive restriction to distal axonal processes during the formation and maturation of adult myotubes, may be attributed to target-derived cues. Similar target-dependent interactions control the survival of target-deprived terminals in vertebrates [54,55,274] and the addition of nerve branches and synaptic sites during the growth of larval muscles in *Drosophila* [32,71,112,113]. The wide distribution of synaptotagmin and sites of vesicular recycling in *Manduca* motor axons as they expand over developing muscles may be a general, target-independent, feature of developing neurons. During embryogenesis in vertebrates and *Drosophila*, presynaptic proteins are present in the growing axons of motoneurons before they reach the muscle [104,140,146,275]. Similarly, glutamate release and vesicular recycling can occur in the axons of growing hippocampal neurons in vitro before the establishment of synaptic contact [120,252] and active zones can form in the axonal processes of *Drosophila* embryonic motoneurons in the absence of muscles [186].

Is there a role for electrical activity during neuromuscular remodeling? The persistence of functional release sites as motor terminals are remodeled raises the possibility that the activity-dependent release of neurotransmitter substance or another factor from nerve terminals may be important for normal muscle development. We have already discussed the possibility that motoneurons release a factor that regulates myogenesis. At a later stage, the release of the excitatory neuromuscular transmitter (glutamate) may allow depolarization-dependent aspects of postsynaptic differentiation [30,74,113]. Nerve activity alone or in combination with target-derived cues may also play an important role in regulating several aspects of motor terminal remodeling. In *Manduca* the axonal regression and degeneration of larval neuromuscular junctions follow the withdrawal of glia from the motor terminals immediately after the cessation of activity in the neuromusclar system [196]. Flight and leg muscles in *Manduca* become spontaneously active and respond to nerve stimulation before myotube formation, as the motor axons grow over the differentiating muscle anlage [39,100,101]. The maturation of muscle responses coincides with the last phase of axonal growth, during which high-order nerve collaterals and adult neuromuscular junctions form [42]. Thus, activity may be an important factor in regulating nerve outgrowth and the formation of the neuromuscular junctions as has been shown in vertebrates [74] and in *Drosophila* during larval development [30,112,113].

*Remodeling of the Sensory System*

Hemimetabolous insects, such as locusts and cockroaches, hatch as miniature adults. They are equipped with sophisticated sensory systems that are necessary for locomotor behavior such as walking and flight [33]. Adult holometabolous insects have body forms and behaviors similar to those of hemimetabolous insects and, therefore, have similar requirements for sensory control. The larval sensory system, however, serves modalities and behaviors distinct from those in the adult. The conversion from the larval to adult sensory system during the late larval and early pupal stages involves the degeneration of all larval sensory organs and most of the associated sensory neurons, followed by the production of new adult organs and sensory neurons (Fig. 1C) [19,38,86,95,153,161, 166,225,239]. Despite this extensive turnover, some larval sensory neurons survive the pupal or even the adult molt (Fig. 1C) [38,43, 95,123,139,177,210,234,235,271]. Survival of larval sensory neurons may be important for the production of pupal and adult behavior, or for the assembly of the adult sensory system.

In *Manduca*, for example, specific larval sensory neurons in abdominal segments survive to the pupal stage to innervate hairs within cuticular specializations known as gin traps. The expansion of the central projections of these neurons has been correlated with the acquisition of a pupal-specific defensive reflex [15–17,132,135,254, 255]. It is not known whether the gin trap sensory neurons persist in the adult to innervate new sensory organs. Similarly, the abdominal stretch receptor organ and its associated neuron survive metamorphosis to provide information about the length or position of the abdomen to persistent motoneurons in the adult [224], and some larval femoral chordotonal organ neurons survive metamorphosis to monitor tibial movements of the adult leg [43]. Other larval sensory neurons that supply internal and external sensory organs survive metamorphosis to innervate new organs in the adult, although their function has been documented in only a few cases [38,122,123,139,210,211,231].

The persistent larval sensory neurons may help the new adult sensory neurons to navigate long distances through the complex pupal environment to reach the CNS. In invertebrate and vertebrate nervous systems, growing neural axons prefer to fasciculate with nerve pathways that had been established by earlier developing neurons, known as pioneers [18,50,57,58,111,121,144,150,178,191,206,216]. Axon guidance over long distances may be facilitated in a similar fashion during the establishment of new sensory pathways in the adults of holometabolous insects. This hypothesis is supported by the finding that the axons of specific surviving larval sensory neurons provide a scaffold for the growing adult sensory neurons in developing legs, antennae and abdomen [27,38,95,123,177,206,210,234,235]. Nevertheless, newly born sensory neurons that are located in imaginal discs and are not associated with persistent larval neurons successfully reach the CNS [235,268].

In the larvae of holometabolous insects there is a well documented relationship between the modality and peripheral position of sensory neurons and the pattern of central projections. In *Manduca* larvae, for example, the central projections of the sensory neurons innervating tactile hairs in the body wall, legs and prolegs are somatotopically organized within the ventral neuropil [106,135,179], whereas campaniform sensilla sensory neurons project to an intermediate neuropil [109]. Similarly, sensory projections are organized according to modality and cell body position in the *Drosophila* embryo [157]. In the adult forms of holometabolous and hemimetabolous insects, mechanosensory neurons project to a ventral neuropil whereas sensory neurons that are associated with proprioceptive organs terminate in an intermediate or dorsal neuropil [33,34,96,109,156,164,165,181]. Thus, for both larvae and adults, common design principles seem to exist in the organization of central projections. It has been suggested that persistent larval sensory neurons may provide cues for organizing the new sensory axon arrays in the adult CNS [27,210], although this hypothesis cannot be applied in cases where adult sensory neurons do not follow pre-existing larval pathways [176].

*Remodeling of the CNS*

The central nervous system must also be modified to accommodate the generation of stage-specific behavior. Our ability to explore the origin of adult circuitry is facilitated by the considerable information that is available about the organization of neural circuits underlying simple reflexes, walking, and flight in adult hemimetabolous insects (reviewed in [33]). Many components are required, including slow, fast and inhibitory motoneurons, different types of interneurons, modulatory neurons, and various classes of sensory organs. In hemimetabolous insects most, if not all, motor circuits are assembled during embryonic development, as strikingly demonstrated by the pharmacological induction of flight motor patterns in the flightless first instar locust [217]. Although less information about motor pattern generation is available in holometabolous insects, most of the components appear to be similar. During metamorphosis, however, the loss of the embryonically derived neuronal machinery that is used for larval behavior must go hand-in-hand with the construction of circuits for pupal and adult behavior. The conversion from the larval to the adult CNS involves two waves of degeneration of larval neurons that are not required for pupal (first wave) or for adult (second wave) behavior (Fig. 1D). Also involved are the production of new neurons from persistent neuroblasts during larval and pupal stages and the respecification of persistent larval neurons (Fig. 1D); [136,233,245,262].

Some adult neurons are born postembryonically and remain in a developmentally arrested state in the larval stages [24,238,272]. In *Manduca* and in *Drosophila* larvae, large numbers of new neurons appear in regions of the CNS that subserve adult-specific behavior. The adult second thoracic ganglion, for instance, possesses threefold more cells than it does in the larva. In contrast, cell numbers are relatively constant in the fourth abdominal ganglion [24]. Cell size measurements suggest that postembryonically generated thoracic neurons are almost exclusively interneurons [24,25]. Similarly, interneurons are produced postembryonically in higher brain centers and the thoracic neuromeres of *Drosophila* [151,154,185,209,219,239,245] and in the antennal lobe of *Manduca* [86].

The obvious hypothesis that adult-specific behavior is dependent upon these new neurons is contradicted by the behavior of *Manduca* adults in which hydroxyurea ablation of postembryonically dividing neuroblasts reduced the normal complement of thoracic neurons by more than 50%. Despite this severe deficit in new neurons, treated animals displayed walking, ecdysis and wing expansion behavior [243]. The animals could produce high frequency wing movements and fly, but most did not display sustained free flight. More detailed behavioral tests might reveal additional defects.

Most, if not all, adult motoneurons in *Manduca* are respecified larval motoneurons [26,52,107,133,134,205,229,232,241,244,247, 248,261]. The dorsolongitudinal muscle (DLM) flight motoneurons in *Drosophila* may also be respecified larval motoneurons [45,47,63,93], although direct evidence is lacking. Jaw motoneurons in amphibians provide an interesting parallel, suggesting respecification of motoneurons as a general theme among metamorphic species [3,4,14].

Many modulatory neurons also survive metamorphosis. Examples are populations of aminergic and peptidergic cells in *Manduca* [44, 49,105,182], in the blowfly [35,167,173,174], and in *Drosophila* [31,160,172,251,267]. Thus, large parts of the neuromodulatory machinery are of embryonic origin. It will be challenging to determine how these systems are respecified to act in concert with adult-specific central motor networks. During larval crawling in *Manduca*, all ef-

ferent octopaminergic ventral unpaired medial neurons are recruited by common excitatory synaptic input provided by descending pathways located in the subesophageal ganglion [99]. Their common activation may be modified during metamorphosis, however, to allow the behavior-specific differential recruitment that has been described for the homologous neurons in the adult locust (reviewed in [180]).

Due to difficulties in identification, few studies provide information on persisting interneurons [1,5,105,204,220,227,234,255]. This has become a crucial limitation to our understanding of the remodeling of behavior, because interneurons lie at the heart of neuronal networks, such as central pattern generators. In one example from *Drosophila*, however, the postembryonic differentiation of identified interneurons has been related to the acquisition of adult specific behavior. The jump-initiated escape response of adult flies requires fast electrical connections between descending giant fibers (GFs) and the tergotrochanteral motoneurons (TTMs). The GFs are born embryonically, have no larval function, show axonal growth during late larval and early pupal life, and connect to the TTMs during pupal life [1]. In addition, this system was used to identify genes involved in the formation of synaptic connections during metamorphosis [2,183,228, 236].

Too little is known about the reconfiguration of neuronal circuits during metamorphosis, although changes in proprioceptive feedback to motoneurons have been described in *Manduca*. For instance the polarity of stretch receptor input to certain abdominal motoneurons is reversed between the larval and the adult stage [133,224], perhaps correlated with the changes in abdominal flexion. Similarly, the formation of the pupal specific gin trap reflex during the larval to pupal transition coincides with changes in proprioceptive feedback to motoneurons [15–17,129,130,131,254,255]. The input of femoral chordotonal organ sensory neurons to an identified persisting leg motoneuron is modified during metamorphosis [43]. The behavioral significance of such changes, however, remains speculative.

Our understanding of central pattern generating circuits in holometabolous insects also remains sparse. Fortunately, rhythmic motor patterns such as chewing, ecdysis, flight, larval crawling and adult walking can be induced pharmacologically or with native neuropeptides in the isolated nerve cord [68,98,117,198,276]. This facilitates comparison among the motor patterns that are employed at different stages. For example, the larval crawling pattern is characterized by synchronous and rather slow activation of right and left legs within a segment [97], whereas adult walking involves an alternating gait similar to that characteristic of many insects [98]. One simple hypothesis is that new inhibitory interneurons within the thoracic ganglia [273] enforce alteration rather than synchrony of the legs. Ecdysis is a behavior that culminates the larval-larval, larval-pupal, and pupal-adult molts by allowing the old cuticle to be shed. Although ecdysis behavior is distinct at each of these stages [158,258], some elements of the underlying circuits may be shared, albeit with modifications. For example, a pair of identified ascending interneurons drive the abdominal body wall motoneurons in both larval and pupal ecdyses [168,169].

Currently, the respecification of central neurons is best understood at the level of motoneurons. Motoneurons undergo profound anatomical and physiological remodeling during metamorphosis, suggesting that their function is not simply limited to conducting spikes to the periphery. Indeed, their properties are adjusted according to the requirements of the motor network by which they are driven and to the function of their target muscle (see below). As shown mainly by work in *Manduca*, motoneurons undergo dendritic regression, which is followed by massive growth of new, adult specific dendrites during pupal development (Fig. 1D) [52,108,133,134,229,261]. Dendritic remodeling appears to be a general theme that applies to all motoneurons, including motoneurons that undergo programmed cell death [259] and efferent modulatory neurons [182]. A general assumption is that dendritic changes accommodate new synaptic input, although this has yet to be tested rigorously. In one particularly well documented example, however, the regression of proleg retractor motoneuron dendrites disconnects them from sensory input, and thereby abolishes the withdrawal reflex during late larval life [94,221]. Dendritic remodeling might also be important for modifications of the intrinsic properties of motoneurons. Structural changes influence passive electrical properties, such as time and length constants, and thus neuronal cable properties and compartmentalization [188,189,208].

The intrinsic properties of motoneurons can be modified during metamorphosis. For example, the properties of two persisting pretarsal leg motoneurons are modified in concert with functional changes of their target muscle [199]. In the larval stage the anterior pretarsal flexor motoneuron (PrtFlx-ant) innervates a slower contracting muscle bundle than the posterior pretarsal flexor motoneuron (PrtFlx-post). As is typical for slow and fast vertebrate [155] and insect motoneurons [159] the slower PrtFlx-ant motoneuron exhibits a lower firing threshold and a stronger firing response than the faster PrtFlx-post motoneuron. Interestingly, the contraction speed of the anterior as compared to the posterior muscle bundle is reversed in the adult, and concomitant with that, the PrtFlx-ant motoneuron and the PrtFlx-post motoneuron switch their relative firing responses.

A more striking example is the transformation of persistent motoneurons that innervate the adult flight muscle. The larval MN5 is a slow motoneuron which innervates a dorsal body wall muscle that participates in slow larval crawling movements. During metamorphosis it is transformed into a fast motoneuron which innervates the DLM flight muscle, providing the main force for wing downstroke during flight [36,52,197]. In accordance with its change in identity, the larval MN5 exhibits classical features of a slow insect motoneuron, whereas the adult motoneuron shows the much lower excitability of a fast motoneuron. This is due to a more hyperpolarized resting potential, more depolarized firing threshold, five-fold lower input resistance, and increased potassium conductances [53]. Such differences in excitability reflect inherent developmental changes in the membrane properties that do not simply reflect changes in dendritic membrane surface area. Potassium and calcium currents are modified in a stereotyped sequential order during pupal life, with first calcium, then potassium currents increasing significantly [53]. The behavioral function of these changes in the adult remains speculative, although calcium currents play a major role in the generation of plateau potentials [75,76,79,114,207] which, in many systems, can be induced by neuromodulators or any intervention that sufficiently reduces opposing outward currents [11,92,115,190]. Flight is strongly influenced by neuromodulators, such as octopamine, which evokes plateau potentials in locust flight interneurons [175]. Similarly, octopamine injection into the mesothoracic neuromere induces flight motor output in *Manduca* adults [37,117]. Moth flight muscles require a prolonged warm-up phase prior to flight, during which neuromodulators might prepare the CNS for flight behavior. Thus, the calcium current detected in the adult MN5 might be important in shaping its motor output, particularly if neuromodulators reduce opposing potassium currents prior to flight. A detailed characterization of the specificity of modifications of membrane currents for different types of motoneurons will be an important step towards understanding how postembryonic behavioral adjustments are accomplished.

In addition to their behavioral significance, altered membrane currents might be important signals for the structural remodeling of neurons. Embryonic and postnatal maturation of central neurons is typically accompanied by a sequential expression of different voltage-activated ionic currents leading to alterations in calcium fluxes that regulate differentiation (reviewed in [215]). In culture systems, low levels of calcium influx promote growth-cone extension and branching, but high levels of influx and prolonged internal elevation inhibit these processes (reviewed in [102,103]). During embryonic spinal

cord development, the frequency of growth-cone calcium transients is inversely proportional to the rate of axon outgrowth, with large elevations in internal $Ca^{2+}$ at pausing sites [70], causing an increase in the activity of calcineurin [124]. Similarly, calcium signals read by CAM-K II are required to limit the elaboration of neuronal arbors in the developing *Xenopus* optical tectum [277]. It is, therefore, interesting that calcium currents are downregulated in MN5 during the formation of prominent dendritic growth cones and rapid dendritic growth. The sequential modification of calcium and potassium currents allows the transient occurrence of calcium spikes, which coincide with the cessation of dendritic sprouting [53]. These data provide correlational evidence for a putative interplay between dendritic remodeling and the modification of membrane currents. Therefore, it will be important to investigate the cytosolic calcium signaling in *Manduca* motoneurons at different developmental stages, and to develop the tools to interfere with calcium signaling pathways in identified motoneurons during normal development. Moreover, the ability to correlate structural and functional events in their normal context in *Manduca* provides distinct advantages for examining the role of membrane current modifications for behavior and their possible interplay with neuronal differentiation.

### *Regulation of Motoneuron Remodeling*

Dendritic remodeling is not dependent entirely on signals from the degenerating target muscle, because surgical removal of the developing muscle or severing the motor nerve at the muscle has only subtle effects on the dendritic morphology of *Manduca* leg or flight motoneurons ([108], Duch and Bayline, unpublished observations). Laser ablations of the presumed larval target muscle of an identified flight motoneuron in *Drosophila* also reveal a subtle effect in the timing of dendritic growth [63]. Surgical removal of the imaginal leg, including the entire adult leg sensory system, also suggests that sensory inputs to motoneurons play only a subtle role in dendritic remodeling [108]. Furthermore, interganglionic interactions are not required for the dendritic modifications in *Manduca* proleg motoneurons [264]. These studies do not, however, exclude the possibility of activity dependent mechanisms, such as inputs from local interneurons, autonomous activity, and neuromodulator-induced activity.

By contrast, dendritic remodeling is affected significantly by the insect steroid hormones, the ecdysteroids [244,256,259–261,263]. Cell culture approaches suggest that ecdysteroids act directly on neurons to regulate growth and branching [138,148,187]. Similar studies using *Drosophila* mushroom body interneurons reveal a direct effect of ecdysteroids on growth [119], a finding which is particularly exciting because it opens the door for genetic approaches to investigating hormonal effects.

Rises in calcium membrane currents but not in potassium currents correspond in time with the elevation of systemic ecdysteroid titers [53]. Thus, calcium currents might be induced by increased ecdysteroid levels. This is supported by the finding that cultured leg motoneurons show an ecdysone dependent increase in calcium but not in potassium membrane currents [73]. Ecdysteroids also regulate the excitability of neurosecretory neurons in *Manduca* [85], perhaps by altering ionic currents, and steroid hormones are potent modulators of ionic currents in the vertebrate CNS [56,110,192]. Hormonal cues may act in concert with activity-dependent mechanisms [69,142]. *Manduca* motoneurons receive synaptic input and retain the ability to spike during metamorphosis. Synaptically driven action potentials propagate actively to the periphery even at stages of maximum dendritic regression [53,133,258].

## SUMMARY AND PERSPECTIVES

Changes in the periphery during metamorphosis allow the sensation of novel external stimuli and the distinct movements of the adult. The adult compound eyes and antennae provide novel sensory information that becomes necessary after metamorphosis for adult-specific behavior, such as guided flight or orientation to species-specific olfactory cues [86]. Similarly, we should anticipate that the coordinated leg and wing movements of the adult will require novel information that is provided by new and persistent mechanosensory and proprioceptive neurons. Further study of the transduction properties and synaptic connections of these sensory elements is, therefore, necessary. On the output side, rapid adult movements require coordinated changes in motor nerve and muscle. Insect metamorphosis provides a marvelous opportunity to identify mechanisms whereby motoneurons regulate myoblast proliferation and perhaps muscle fiber type and, conversely, target-derived factors that influence presynaptic terminal differentiation.

As this brief review reveals, the relative simplicity and accessibility of the insect central nervous system have enabled us to describe in detail many of the cellular changes that accompany metamorphosis. This is not a trivial accomplishment, for it is a necessary first step in our efforts to understand behavioral plasticity. Nevertheless, significant gaps persist in our ability to place these cellular changes in the context of neural circuit function. We have identified three areas in which immediate efforts will be rewarding. All require basic neurophysiological approaches, which can currently best be accomplished in *Manduca*, but will increasingly be complemented by genetic manipulations that alter the excitability, synaptic function and morphology of selected cell groups in *Drosophila*.

First, both persistent and new adult interneurons must be identified and characterized. The former provides the key to understanding whether circuits underlying behavior that is relatively similar among stages, such as ecdysis, are conserved with simple modifications or remodeled entirely during metamorphosis. The latter must be investigated to understand the emergence of unique adult behavior, such as flight.

Second, we must begin to relate changes in motoneuron and interneuron dendritic structure with the reorganization of synaptic connections. This task can begin simply enough with an investigation of monosynaptic connections between sensory neurons and motoneurons. Persistent sensory neurons may contact motoneurons on dendritic branches that are conserved through metamorphosis or may alter the location of contacts to accommodate changes in synaptic efficacy. New adult sensory neurons may contact new dendritic branches exclusively or replace lost larval inputs to conserved branches. The formation of synaptic contacts from new adult interneurons may overlap temporally with that of sensory inputs or may occur at different times relative to the formation of primary and higher-order postsynaptic dendritic branches. In any case the findings will not only help us to understand the remodeling of neural circuits, but may reveal mechanisms that regulate dendritic growth.

Third, we must continue to investigate how the intrinsic biophysical properties of identified neurons are adapted during metamorphosis to meet different behavioral demands. Recent progress in this direction with the flight motoneurons suggests that this will be a promising avenue to explore.

Insect metamorphosis has already yielded insights into how postembryonic modifications in neural circuitry are regulated. In particular, the role of steroid hormones in behavioral plasticity has been clarified significantly through the use of insect model systems. Further exploitation of primary cell culture systems will provide insights into the intracellular mechanisms through which steroid hormones induce growth, branching and new ionic currents in neurons. Moreover, *Drosophila* mutants have for several years provided

unique insights into the molecular pathways of steroid hormone action [230] and this approach will certainly transfer profitably into the nervous system [193,119], especially given recent technical advances that allow expression and manipulation of genes within small defined cell populations.

ACKNOWLEDGEMENTS

Work from the authors' laboratory was supported by the research grants from the NIH (NS28495 & NS24822) and fellowships from the Fogarty International Center (TWO4898; CC), the Deutsche Forschungsgemeinschaft (DU331/2-1; CD) and the NIH (NS07063; RJB).

## REFERENCES

1. Allen, M. J.; Drummond, J. A.; Moffat, K. G. Development of the giant fiber neuron of *Drosophila melanogaster*. J. Comp. Neurol. 397:519–531; 1998.
2. Allen, M. J.; Shan, X.; Caruccio, P.; Froggett, S. J.; Moffat, K. G.; Murphey, R. K. Targeted expression of truncated *glued* disrupts giant fiber synapse formation in *Drosophila*. J. Neurosci. 19:9374–9384; 1999.
3. Alley, K. E.; Barnes, M. D. Birth dates of trigeminal motoneurons and metamorphic reorganizaiton of the jaw myoneural systems in frogs. J. Comp. Neurol. 218:395–405; 1983.
4. Alley, K. E.; Omerza, F. F. Reutilization of trigeminal motoneurons during amphibian metamorphosis. Brain Res. 813:187–190; 1998.
5. Armstrong, J. D.; de Belle, J. S.; Wang, Z.; Kaiser, K. Metamorphosis of the mushroom bodies; large-scale rearrangements of the neural substrates for associative learning and memory in *Drosophila*. Learn. Mem. 5:102–114; 1998.
6. Ashby, P. R.; Wilson, S. J.; Harris, A. J. Formation of primary and myotubes in aneural muscles in the mouse mutant *peroneal muscular atrophy*. Dev. Biol. 156:519–528; 1993.
7. Ashby, P. R.; Pincon-Raymond, M.; Harris, A. J. Regulation of myogenesis in paralyzed muscles in the mouse mutants *peroneal muscular atrophy* and *muscular dysgenesis*. Dev. Biol. 156:529–536; 1993.
8. Atwood, H. L. Organization and synaptic physiology of crustacean neuromuscular systems. Prog. Neurobiol. 7:291–391; 1976.
9. Atwood, H. L. Synapses and neurotransmitters. In: Atwood, H. L.; Sandeman, D. C., eds. The biology of crustacea, vol. 3. New York: Academic Press; 1982:105–150.
10. Atwood, H. L.; Cooper, R. L. Functional and structural parallels in crustacean and *Drosophila* neuromuscular systems. Am. Zool. 35:556–565; 1995.
11. Bal, T.; Nagy, F.; Moulin, M. Muscarinic modulation of a pattern generating network: Control of neuronal properties. J. Neurosci. 14: 3019–3035; 1994.
12. Ball, E. E.; Goodman, C. S. Muscle development in the grasshopper embryo. I. Muscles, nerves, and apodemes in the metathoracic leg. Dev. Biol. 111:383–398; 1985.
13. Ball, E. E.; Goodman, C. S. Muscle development in the grasshopper embryo. II. Syncytial origin of the extensor tibia muscle pioneers. Dev. Biol. 111:399–416; 1985.
14. Barnes, M. D.; Alley, K. E. Maturation and recycling of trigeminal motoneurons in anuran larvae. J. Comp. Neurol. 218:406–414; 1983.
15. Bate, C. M. The mechanism of the pupal gin trap. I: Segmental gradients and the connexions of the triggering sensilla. J. Exp. Biol. 59:95–107; 1973.
16. Bate, C. M. The mechanism of the pupal gin trap. II. Closure movement. J. Exp. Biol. 59:109–119; 1973.
17. Bate, C. M. The mechanism of the pupal gin trap. III. Interneurons and the origin of the closure mechanism. J. Exp. Biol. 59:121–135; 1973.
18. Bate, C. M. Pioneer neurons in an insect embryo. Nature 260:54–56; 1976.
19. Bate, C. M. Development of sensory systems. In: Jacobsen, M., ed. Handbook of sensory physiology, vol. 9. New York: Springer-Verlag; 1978:1–53.
20. Bate, M.; Rushton, E.; Currie, D. Cells with persistent *twist* expression are the embryonic precursors of adult muscles in *Drosophila*. Development 113:79–89; 1991.
21. Bate, M.; Landgraf, M.; Bate, M. R. G. Development of larval body wall muscles. Int. Rev. Neurobiol. 43:25–44; 1999.
22. Bayline, R. J.; Khoo, A. B.; Booker, R. Innervation regulates the metamorphic fates of larval abdominal muscles in the moth, *Manduca sexta*. Dev. Genes Evol. 208:369–381; 1998.
23. Bernstein, S. I.; Hansen, C. J.; Becker, K. D.; Wassenberg, D. R. II; Roche, E. S.; Donady, J. J.; Emerson, C. P. Jr. Alternative RNA splicing generates transcripts encoding a thorax-specific isoform of *Drosophila melanogaster* myosin heavy chain. Mol. Cell. Biol. 6:2511–2519; 1986.
24. Booker, R.; Truman, J. W. Postembryonic neurogenesis in the CNS of the tobacco hornworm, *Manduca sexta*. I. Neuroblast arrays and the fate of their progeny during metamorphosis. J. Comp. Neurol. 255:548–559; 1987a.
25. Booker, R.; Truman, J. W. Postembryonic neurogenesis in the CNS of the tobacco hornworm, *Manduca sexta*. II. Hormonal control of imaginal nest cell degeneration and differentiation during metamorphosis. J. Neurosci. 7:4107–4114; 1987b.
26. Breidbach, O. The fate of persisting thoracic neurons during metamorphosis of the meal beetle *Tenebrio molitor* (Insecta: Coleoptera). Rouxs Arch. Dev. Biol. 196:93–100; 1987.
27. Breidbach, O. Metamorphic changes in the central projections of hair sensilla in *Tenebrio molitor* L. (Insecta: Coleoptera). Cell Tissue Res. 259:159–176; 1990.
28. Broadie, K. S.; Bate, M. The development of adult muscles in *Drosophila*: Ablation of identified muscle precursor cells. Development 113:103–118; 1991.
29. Broadie, K. S.; Bate, M. Muscle development is independent of innervation during *Drosophila* embryogenesis. Development 119:533–543; 1993.
30. Broadie K.; Bate, M. Activity-dependent development of the neuromuscular synapse during Drosophila embryogenesis. Neuron 11:607–619; 1993.
31. Budnik, V.; White, K. Catecholamine-containing neurons in *Drosophila melanogaster*: Distribution and development. J. Comp. Neurol. 268: 400–413; 1988.
32. Budnik, V.; Zhong, Y.; Wu, C. Morphological plasticity of motor axons in *Drosophila* mutants with altered excitability. J. Neurosci. 10:3754–3768; 1990.
33. Burrows, M. The neurobiology of an insect brain. New York: Oxford; 1996.
34. Burrows, M.; Newland, P. L. Correlation between the receptive fields of locust interneurons, their dendritic morphology, and the central projections of mechanosensory neurons. J. Comp. Neurol. 329:412–426; 1993.
35. Cantera, R.; Nässel, D. R. Postembryonic development of serotonin-immunoreactive neurons in the central nervous system of the blowfly. II. The thoracico-abdominal ganglia. Cell Tissue Res. 250:449–459; 1987.
36. Casaday, G. B.; Camhi, J. M. Metamorphosis of flight motor neurons in the moth *Manduca sexta*. J. Comp. Physiol. 112:143–158; 1976.
37. Claassen, D. E.; Kammer, A. E. Effects of octopamine, dopamine, and serotonin on production of flight motor output by thoracic ganglia of *Manduca sexta*. J. Neurobiol. 17:1–14; 1986.
38. Consoulas, C. Remodeling of the leg sensory system during metamorphosis of the hawkmoth, *Manduca sexta*. J. Comp. Neurol. 419:154–174; 2000.
39. Consoulas, C.; Kent, K. S.; Levine, R. B. Remodeling of the peripheral processes and presynaptic terminals of leg motoneurons during metamorphosis of the hawkmoth, *Manduca sexta*. J. Comp. Neurol. 372:415–434; 1996.
40. Consoulas, C.; Anezaki, M.; Levine, R. B. Development of adult thoracic leg muscles during metamorphosis of the hawkmoth *Manduca sexta*. Cell Tissue Res. 287:393–412; 1997.
41. Consoulas, C.; Levine, R. B. Accumulation and proliferation of adult leg muscle precursors in *Manduca* are dependent on innervation. J. Neurobiol. 32:531–553; 1997.
42. Consoulas, C.; Levine, R. B. Presynaptic function during muscle remodeling in insect metamorphosis. J. Neurosci. 18:5817–5831; 1998.
43. Consoulas, C.; Rose, U.; Levine, R. B. Remodeling of the femoral chordotonal organ during metamorphosis of the hawkmoth, *Manduca sexta*. J. Comp. Neurol. 426:391–405; 2000.
44. Copenhaver, P. F.; Truman, J. W. Metamorphosis of the cerebral neuroendocrine system in the moth *Manduca sexta*. J. Comp. Neurol. 249: 186–204; 1986.

45. Costello, W. J.; Wyman, R. J. Development of an indirect flight muscle in a muscle-specific mutant of *Drosophila melanogaster*. Dev. Biol. 118:247–258; 1986.
46. Crossley, A. C. The morphology and development of the Drosophila muscular system. In: Ashburner, M.; Wright, T. R. F., eds. The genetics and biology of Drosophila, vol. 2b. London/New York/San Francisco: Academic Press; 1978:499–560.
47. Currie, D. A.; Bate, M. The development of adult abdominal muscles in *Drosophila*: Myoblasts express *twist* and are associated with nerves. Development 113:91–102; 1991.
48. Currie, D. A.; Bate, M. Innervation is essential for the development and differentiation of a sex-specific muscle in *Drosophila melanogaster*. Development 121:2549–2557; 1995.
49. Davis, N. T.; Homberg, U.; Dircksen, H.; Levine, R. B.; Hildebrand, J. G. Crustacean cardioactive peptide-immunoreactive neurons in the hawkmoth *Manduca sexta* and changes in their immunoreactivity during postembryonic development. J. Comp. Neurol. 338:612–627; 1993.
50. Dodd, J.; Morton, S. B.; Karagogeous, D.; Yamamoto, M.; Jessell, T. M. Spatial regulation of axonal glycoprotein expression on subsets of embryonic spinal neurons. Neuron 1:105–116; 1988.
51. Donoghue, M. J.; Sanes, J. R. All muscles are not created equal. Trends Genet. 10:396–401; 1994.
52. Duch, C.; Bayline, R. J.; Levine, R. B. Postembryonic development of the dorsal longitudinal flight muscle and its innervation in *Manduca sexta*. J. Comp. Neurol. 422:1–17; 2000.
53. Duch, C.; Levine, R. B. Remodeling of membrane properties and dendritic architecture accompany the postembryonic conversion of a 'slow' into a 'fast' motoneuron. J. Neurosci. 20:6950–6961; 2000.
54. Dunaevsky, A.; Connor, E. A. Long-term maintenance of presynaptic function in the absence of target muscle fibers. J. Neurosci. 15:6137–6144; 1995.
55. Dunaevsky, A.; Connor, E. A. Stability of frog motor nerve terminals in the absence of target muscle fibers. Dev. Biol. 194:61–71; 1998.
56. Dunlap, K. D.; McAnelly, M. L.; Zakon, H. H. Estrogen modifies an electrocommunication signal by altering the electrocyte sodium current in an electric fish, *Sternopygus*. J. Neurosci. 17:2869–2875; 1997.
57. Edwards, J. S.; Chen, S.-W. Embryonic development of an insect sensory system, the abdominal cerci of *Acheta domesticus*. Rouxs Arch. Dev. Biol. 186:151–178; 1979.
58. Eisen, J. S.; Myers, P. Z.; Westerfield, M. Pathway selection by growth cones of identified motoneurones in live zebrafish embryos. Nature 320:269–271; 1986.
59. Falkenthal, S. V.; Graham, M.; Wilkinson, J. The indirect flight muscle of *Drosophila* accumulates a unique myosin alkali light chain isoform. Dev. Biol. 121:262–272; 1987.
60. Fernandes, J.; Bate, M.; VijayRaghavan, K. The development of the indirect flight muscles of *Drosophila*. Development 113:67–77; 1991.
61. Fernandes, J.; VijayRaghavan, K. The development of indirect flight muscle innervation in *Drosophila melanogaster*. Development 118:215–227; 1993.
62. Fernandes, J.; Celniker, S. E.; VijayRaghavan, K. Development of the indirect flight muscle attachment sites in *Drosophila*: Role of the PS integrins and the *stripe* gene. Dev. Biol. 176:166–184; 1996.
63. Fernandes, J. J.; Keshishian, H. Nerve-muscle interactions during flight muscle development in *Drosophila*. Development 125:1769–1779; 1998.
64. Finlayson, L. H. Normal and induced degeneration of abdominal muscles during metamorphosis in the *lepidoptera*. Q. J. Microsc. Sci. 97:215–234; 1956.
65. Fredette, B. J.; Landmesser, L. T. A reevaluation of the role of innervation in primary and secondary myogenesis in developing chick muscle. Dev. Biol. 143:19–35; 1991.
66. Fyrberg, E. A.; Mahaffey, J. W.; Bond, B. J.; Davidson, N. Transcripts of the six *Drosophila* actin genes accumulate in a stage- and tissue-specific manner. Cell 33:115–123; 1983.
67. Fyrberg, E. A.; Fyrberg, C. C.; Biggs, J. R.; Saville, D.; Beall, C. J.; Ketchum, A. Functional nonequivalence of *Drosophila* actin isoforms. Biochem. Genet. 36:271–287; 1998.
68. Gammie, S. C.; Truman, J. W. Neuropeptide hierachies and the activation of sequential motor behaviors in the hawkmoth, *Manduca sexta*. J. Neurosci. 17:4389–4397; 1997.
69. Garcia, D. E.; Cavalie, A.; Lux, H. D. Enhancement of voltage-gated calcium currents induced by daily stimulation of hippocampal neurons with glutamate. J. Neurosci. 14:545–553; 1994.
70. Gomez, M. G.; Spitzer, N. C. In vivo regulation of axon extension and pathfinding by growth-cone calcium transients. Nature 397:350–355; 1999.
71. Gorczyca, M.; Augart, C.; Budnik, V. Insulin-like receptor and insulin-like peptide are localized at neuromuscular junctions in *Drosophila*. J. Neurosci. 13:3692–3704; 1993.
72. Grinnel, A. D. Dynamics of nerve-muscle interaction in developing and mature neuromuscular junctions. Physiol. Rev. 75:789–834; 1995.
73. Grünewald, B.; Levine, R. B. Ecdysteroid control of ionic current development in *Manduca sexta* motoneurons. J. Neurobiol. 37:211–223; 1998.
74. Hall, Z. W.; Sanes, J. R. Synaptic structure and development: The neuromuscular junction. Neuron 10:99–122; 1993.
75. Hancox, J. C.; Pitman, R. C. Plateau potentials drive axonal impulse bursts in insect motoneurons. P. Roy. Soc. Lond. B 244:33–38; 1991.
76. Hancox, J. C.; Pitman, R. C. Plateau potentials in insect motoneurons can be driven by synaptic input. J. Exp. Biol. 171:15–42; 1993.
77. Harris, A. J. Embryonic growth and innervation of rat skeletal muscles. I. Neural regulation of fiber numbers. Philos. Trans. R. Soc. Lond. B Biol. Sci. 293:257–297; 1981.
78. Harris-Warrick, R. M.; Marder, E. Modulation of neural networks for behavior. Annu. Rev. Neurosci. 14:39–57; 1991.
79. Hartline, D. K.; Russell, D. F. Endogenous burst capability in a neuron of the gastric mill pattern generator of the spiny lobster *Panulirus interruptus*. J. Neurobiol. 15:345–364; 1984.
80. Hegstrom, C. D.; Truman, J. W. Steroid control of muscle remodeling during metamorphosis in *Manduca sexta*. J. Neurobiol. 29:535–550; 1996.
81. Heinertz, R. Untersuchungen am thorakalen Nervensystem von *Antheraea polyphemus Cr.* (*Lipidoptera*) unter Berücksichtigung der Metamorphose. Rev. Suisse Zool. 83:215–242; 1976.
82. Herrera, A. A.; Banner, L. R.; Nagaya, N. Repeated, *in vivo* observation of frog neuromuscular junctions: Remodeling involves concurrent growth and retraction. J. Neurocytol. 19:85–99; 1990.
83. Herrera, A. A.; Werle, M. J. Mechanisms of elimination, remodeling, and competition at frog neuromuscular junctions. J. Neurobiol. 21:71–98; 1990.
84. Herrera, A. A.; Banner, L. R.; Werle, M. J.; Regnier, M.; Nagaya-Stevens, N. Post-metamorphic development of neuromuscular junctions and muscle fibers in the frog *Cutaneous pectoris*. J. Neurobiol. 22:15–28; 1991.
85. Hewes, R. S.; Truman, J. W. Steroid regulation of excitability in identified insect neurosecretory cells. J. Neurosci. 14:1812–1819; 1994.
86. Hildebrand, J. G.; Rössler, W.; Tolbert, L. P. Postembryonic development of the olfactory system in the moth *Manduca sexta*: Primary afferent control of glomerular development. Semin. Cell Dev. Biol. 8:163–170; 1997.
87. Hiromi, Y.; Hotta, Y. Actin gene mutations in *Drosophila*; heat shock activation in the indirect flight muscles. EMBO J. 4:1681–1687; 1985.
88. Ho, R. K.; Ball, E. E.; Goodman, C. S. Muscle pioneers: Large mesodermal cells that erect a scaffold for developing muscles and motoneurones in grasshopper embryos. Nature 301:66–69; 1983.
89. Hoyle, G. Muscles and their neural control. New York: Wiley; 1983.
90. Huang, Z.; Kunes, S. Hedgehog, transmitted along retinal axons, triggers neurogenesis in the developing visual centers of the *Drosophila* brain. Cell 86:411–422; 1996.
91. Hughes, S. M.; Salinas, P. C. Control of muscle fiber and motoneurons diversification. Curr. Opin. Neurobiol. 9:54–64; 1999.
92. Hultborn, H.; Kiehn, O. Neuromodulation of vertebrate motoneuron membrane properties. Curr. Opin. Neurobiol. 2:770–775; 1992.
93. Hummon, M. R.; Costello, W. J. Induced disruption in the connectivity of an identified neuron in the *Drosophila* ts mutant *shibire*. J. Neurosci. 7:3633–3638; 1987.
94. Jacobs, G. A.; Weeks, J. C. Postsynaptic changes at a sensory-to-motoneuron synapse contribute to the developmental loss of a reflex behavior during insect metamorphosis. J. Neurosci. 10:1341–1356; 1990.
95. Jan, Y. N.; Ghysen, A.; Christoph, I.; Barbel, S.; Jan, L. Y. Formation of neuronal pathways in the imaginal discs of *Drosophila melanogaster*. J. Neurosci. 5:2453–2464; 1985.
96. Johnson, S. E.; Murphey, R. K. The afferent projection of mesothoracic

bristle hairs in the cricket *Acheta domesticus*. J. Comp. Physiol. A 156:369–379; 1985.
97. Johnston, R. M.; Levine, R. B. Locomotor behavior in the hawkmoth *Manduca sexta*: Kinematic and electomyographic analyses of the thoracic legs in larvae and adults. J. Exp. Biol. 199:759–774; 1996.
98. Johnston, R. M.; Levine, R. B. Crawling motor patterns in isolated larval nerve cords of *Manduca sexta*. J. Neurophysiol. 76:3178–3195; 1996.
99. Johnston, R. M.; Consoulas, C.; Pfluger, H. J.; Levine, R. B. Patterned activation of unpaired median neurons during fictive crawling in *Manduca sexta* larvae. J. Exp. Biol. 202:103–113; 1999.
100. Kammer, A. E.; Rheuben, M. B. Adult motor patterns produced by the moth pupae during development. J. Exp. Biol. 65:65–84; 1976.
101. Kammer, A. E.; Kinnamon, S. C. Maturation of the flight motor pattern without movement in *Manduca sexta*. J. Comp. Physiol. 130:29–37; 1979.
102. Kater, S. B.; Mattson, M. P.; Cohan, C.; Connor, J. Calcium regulation of the neuronal growth cone. Trends Neurosci. 11:315–321; 1988.
103. Kater, S. B.; Mills, L. R. Regulation of growth cone behavior by calcium. J. Neurosci. 11:891–899; 1991.
104. Kelly, A. M.; Zacks, S. I. The fine structure of motor endplate morphogenesis. J. Cell Biol. 42:154–169; 1969.
105. Kent, K. S.; Hoskins, S. G.; Hildebrand, J. G. A novel serotonin-immunoreactive neuron in the antennal lobe of the sphinx moth *Manduca sexta* persists throughout postembryonic life. J. Neurobiol. 18:451–465; 1987.
106. Kent, K. S.; Levine, R. B. Neural control of leg movements in a metamorphic insect: Sensory and motor elements of the larval thoracic legs in *Manduca sexta*. J. Comp. Neurol. 271:559–576; 1988.
107. Kent, K. S.; Levine, R. B. Neural control of leg movements in a metamorphic insect: Persistence of larval leg motor neurons to innervate the adult legs of *Manduca sexta*. J. Comp. Neurol. 276:30–43; 1988.
108. Kent, K. S.; Levine, R. B. Dendritic reorganization of an identified neuron during metamorphosis of the moth *Manduca sexta*: The influence of interactions with the periphery. J. Neurobiol. 24:1–22; 1993.
109. Kent, K. S.; Fjeld, C. C.; Anderson, R. Leg proprioceptors of the tobacco hornworm, *Manduca sexta*: Organization of central projections at larval and adult stages. Microsc. Res. Tech. 35:265–284; 1996.
110. Kerr, D. S.; Campbell, L. W.; Thibault, O.; Landfield, P. W. Hippocampal glucocorticoid receptor activation enhances voltage-dependent calcium conductances: Relevance for brain aging. Proc. Natl. Acad. Sci. USA 89:8527–8531; 1992.
111. Keshishian, H.; Bentley, D. Embryogenesis of peripheral nerve pathways in grasshopper legs. I. The initial nerve pathway to the CNS. Dev. Biol. 96:89–102; 1983.
112. Keshishian, H.; Chiba, A.; Chang, T.; Halfton, M.; Harkins, E.; Jarecki, J.; Wang, L.; Anderson, M.; Cash, S.; Halpern, M.; Johansen, J. Cellular mechanisms governing synaptic development in *Drosophila melanogaster*. J. Neurobiol. 24:757–787; 1993.
113. Keshishian, H.; Broadie, K.; Chiba, A.; Bate, M. The *Drosophila* neuromuscular junction: A model for studying synaptic development and function. Annu. Rev. Neurosci. 19:545–575; 1996.
114. Kiehn, O. Plateau potentials and active integration in the 'final common pathway' for motor behavior. Trends Neurosci. 14:68–73; 1991.
115. Kiehn, O.; Harris-Warrick, R. M. Serotonergic stretch receptors induce plateau properties in a crustacean motoneuron by a dual-conductance mechanism. J. Neurophysiol. 68:485–495; 1992.
116. Kimura, K. I.; Truman, J. W. Postmetamorphic cell death in the nervous and muscular systems of *Drosophila melanogaster*. J. Neurosci. 12:403–411; 1990.
117. Kinnamon, S. C.; Klaassen, L. W.; Kammer, A. E.; Claassen, D. Octopamine and chlordimeform enhance sensory responsiveness and production of the flight motor pattern in developing and adult moths. J. Neurobiol. 15:283–293; 1984.
118. Kôpec, S. The influence of the nervous system on the development and regeneration of muscles and integument in insects. J. Exp. Zool. 3:15–25; 1923.
119. Kraft, R.; Levine, R. B.; Restifo, L. L. The steroid hormone 20-hydroxyecdysone enhances neurite growth of *Drosophila* mushroom body neurons isolated during metamorphosis. J. Neurosci. 18:8886–8899; 1998.
120. Kraszewski, K.; Mundigl, O.; Daniel, L.; Verderio, C.; Matteoli, M.; De Camilli, P. Synaptic vesicle dynamics in living cultured hippocampal neurons visualized with CY3-conjugated antibodies directed against the lumenal domain of synaptotagmin. J. Neurosci. 15:4328–4342; 1995.
121. Kuwada, J. Y. Cell recognition by neuronal growth cones in a simple vertebrate embryo. Science 233:740–746; 1986.
122. Lakes-Harlan, R.; Pollack, G. S.; Merritt, D. J. From embryo to adult: Anatomy and development of a leg sensory organ in *Phormia regina*, Meigen (Insecta: Diptera). I. Anatomy and physiology of a larval "leg" sensory organ. J. Comp. Neurol. 308:188–199; 1991.
123. Lakes-Harlan, R.; Pollack, G. S.; Merritt, D. J. From embryo to adult: Anatomy and development of a leg sensory organ in *Phormia regina*, Meigen (Insecta: Diptera). II. Development and persistence of sensory neurons. J. Comp. Neurol. 308:200–208; 1991.
124. Lautermilch, N. J.; Spitzer, N. C. Regulation of calcineurin by growth cone calcium waves controls neurite extension. J. Neurosci. 20:315–325; 2000.
125. Lawrence, P. A. Cell lineage of the thoracic muscles of *Drosophila*. Cell 29:493–503; 1982.
126. Lawrence, P. A.; Johnston, P. The genetic specification of pattern in a *Drosophila* muscle. Cell 29:493–503; 1984.
127. Lawrence, P. A.; Johnston, P. The muscle pattern of a segment of *Drosophila* may be determined by neurons and not by contributing myoblasts. Cell 45:505–513; 1986.
128. Lebart-Pedebas, M. C. Origin and development of the dorso-ventral flight muscles in *Chironomus* (Diptera: Nematocera). Tissue Cell 22: 199–211; 1990.
129. Lemon, W. C.; Levine, R. B. Role of proprioceptive input during abdominal bending reflex motor activity in the tobacco hawkmoth *Manduca sexta*. Biol. Bull. 185:314–316; 1993.
130. Lemon, W. C.; Levine, R. B. Multisegmental motor activity in the segmentally restricted gin trap behavior in *Manduca sexta* pupae. J. Comp. Physiol. A 180:611–619; 1997.
131. Lemon, W. C.; Levine, R. B. Segmentally distributed metamorphic changes in neuronal circuits controlling abdominal bending in the hawk moth *Manduca* sexta. J. Comp. Physiol. A 180:597–610; 1997.
132. Levine, R. B. Expansion of the central arborizations of persistent sensory neurons during insect metamorphosis: The role of the steroid hormone, 20-hydroxyecdysone. J. Neurosci. 9:1045–1054; 1989.
133. Levine, R. B.; Truman, J. W. Metamorphosis of the insect nervous system: Changes in morphology and synaptic interactions of identified neurons. Nature 299:250–252; 1982.
134. Levine, R. B.; Truman, J. W. Dendritic reconstruction of abdominal motoneurons during metamorphosis of the moth, *Manduca sexta*. J. Neurosci. 5:2424–2431; 1985.
135. Levine, R. B.; Pak, C.; Linn, D. The structure, function, and metamorphic reorganization of somatotopically projecting sensory neurons in *Manduca sexta* larvae. J. Comp. Physiol. A 157:1–13; 1985.
136. Levine, R. B.; Weeks, J. C. Hormonally mediated changes in simple reflex circuits dring metamorphosis in the moth, *Manduca sexta*. J. Neurobiol. 21:1022–1036; 1990.
137. Levine, R. B.; Morton, D. B.; Restifo, L. L. Remodeling of the insect nervous system. Curr. Opin. Neurobiol. 5:28–35; 1995.
138. Levine, R. B.; Weeks, J. C. Cell culture approaches to understanding the actions of steroid hormones on the insect nervous system. Dev. Neurosci. 18:73–86; 1996.
139. Lewis, F. P.; Fullard, J. H. Neurometamorphosis of the ear in the gypsy moth, *Lymantria dispar*, and its homologue in the earless forest tent caterpillar moth, *Malacosoma disstria*. J. Neurobiol. 31:245–262; 1996.
140. Littleton, J. T.; Bellen, H. J.; Perin, M. S. Expression of synaptotagmin in *Drosophila* reveals transport and localization of synaptic vesicles to the synapse. Development 118:1077–1088; 1993.
141. Lnenicka, G. A.; Atwood, H. L.; Marin, L. Morphological transformation of synaptic terminals of a phasic motoneurons by long-term tonic stimulation. J. Neurosci. 6:2252–2258; 1986.
142. Lnenicka, G. A.; Arcaro, K. F.; Calabro, J. M. Activity-dependent development of calcium regulation in growing motor axons. J. Neurosci. 18:4966–4972; 1998.
143. Lockshin, R. A. Degeneration of insect intersegmental muscles—Electrophysiological studies of populations of fibers. J. Insect Phys. 19:2359–2372; 1973.
144. LoPresti, V.; Macagno, E. R.; Levinthal, C. Structure and development of neuronal connections in isogenic organisms: Cellular interactions in

the development of the optic lamina of *Daphnia*. Proc. Natl. Acad. Sci. USA 70:433–437; 1973.
145. Luedeman, R.; Levine, R. B. Neurons and ecdysteroids promote the proliferation of myogenic cells cultured from the developing adult legs of *Manduca sexta*. Dev. Biol. 173:51–68; 1996.
146. Lupa, M. T.; Hall, Z. W. Progressive restriction of synaptic vesicle protein to the nerve terminal during development of the neuromuscular junction. J. Neurosci. 9:3937–3945; 1989.
147. Marden, J. H. Variability in the size, composition, and function of insect flight muscles. Annu. Rev. Physiol. 62:157–178; 2000.
148. Matheson, S. F.; Levine, R. B. Steroid hormone enhancement of neurite outgrowth in identified insect motor neurons involves specific effects on growth cone form and function. J. Neurobiol. 38:27–45; 1999.
149. McClennan, I. S. Neural dependence and independence of myotube production in chicken hindlimb muscles. Dev. Biol. 98:287–294; 1983.
150. McConnell, S. K.; Ghosh, A.; Shatz, C. J. Subplate pioneers and the formation of descending connections from cerebral cortex. J. Neurosci. 4:1892–1897; 1994.
151. Meinertzhagen, I. A. Sleeping neuroblasts. Curr. Biol. 3:904–906; 1993.
152. Kent, K. S.; Consoulas, C.; Duncan, C.; Johnston, R. M.; Luedeman, R.; Levine, R. B. Remodelling of neuromuscular system during insect metamorphosis. Amer. Zool. 35:578–584; 1995.
153. Meinertzhagen, I. A. Fly photoreceptor synapses: Their development, evolution, and plasticity. J. Neurobiol. 20:276–294; 1989.
154. Meinertzhagen, I. A.; Piper, S. T.; Sun, X. J.; Frohlich, A. Neurite morphogenesis of identified visual interneurons and its relationship to photoreceptor synaptogenesis in the flies, *Musca domestica* and *Drosophila melanogaster*. Eur. J. Neurosci. 12:1342–1356; 2000.
155. Mendell, L. M.; Collins, W. F.; Munson, J. B. Retrograde determination of motoneuron properties and their synaptic input. J. Neurobiol. 25:707–721; 1994.
156. Merritt, D. J.; Murphey, R. K. Projections of leg proprioceptors within the CNS of the fly *Phormia* in relation to the generalized insect ganglion. J. Comp. Neurol. 322:16–34; 1992.
157. Merritt, D. J.; Whitington, P. M. Central projections of sensory neurons in the *Drosophila* embryo correlate with sensory modality, soma position, and proneural gene function. J. Neurosci. 15:1755–1767; 1995.
158. Mesce, K. A.; Truman, J. W. Metamorphosis of the ecdysis motor pattern in the hawkmoth, *Manduca sexta*. J. Comp. Physiol. A 163:287–299; 1988.
159. Meyer, D.; Walcott, B. Differences in the responsiveness of identified motoneurons in the cockroach: Role for the motor program for stepping. Brain Res. 178:600–605; 1979.
160. Monastirioti, M.; Gorczyca, M.; Rapus, J.; Eckert, M.; White, K.; Budnik, V. Octopamine immunoreactivity in the fruit fly *Drosophila melanogaster*. J. Comp. Neurol. 356:275–287; 1995.
161. Monsma, S. A.; Booker, R. Genesis of the adult retina and outer optic lobes of the moth, *Manduca sexta*. I. Patterns of proliferation and cell death. J. Comp. Neurol. 367:10–20; 1996.
162. Morgan, N. S. The myosin superfamily in *Drosophila melanogaster*. J. Exp. Zool. 273:104–117; 1995.
163. Mounier, N.; Sparrow, J. C. Muscle actin genes in insects. Comp. Biochem. Physiol. 105B:231–238; 1993.
164. Murphey, R. K.; Bacon, J. P.; Johnson, S. E. Ectopic neurons and the organization of insect sensory systems. J. Comp. Physiol. A 156:381–389; 1985.
165. Murphey, R. K.; Possidente, D. R.; Vandervorst, P.; Ghysen, A. Compartments and topography of leg afferent projections in *Drosophila*. J. Neurosci. 9:3209–3217; 1989.
166. Murray, M. A.; Schubiger, M.; Palka, J. Neuron differentiation and axon growth in the developing wing of *Drosophila melanogaster*. Dev. Biol. 104:259–273; 1984.
167. Nässel, D. R.; Ohlsson, L. G.; Cantera, R. Metamorphosis of identified neurons innervating thoracic neurohemal organs in the blowfly: Transformation of cholecystokinin-like immunoreactive neurons. J. Comp. Neurol. 267:343–356; 1988.
168. Novicki, A.; Weeks, J. C. A single pair of interneurons controls motoneuron activity during pre-ecdysis compression behavior in larval *Manduca sexta*. J. Comp. Physiol. A 176:45–54; 1995.
169. Novicki, A.; Weeks, J. C. Developmental attenuation of *Manduca* pre-ecdysis behavior involves neural changes upstream of motoneurons and relay interneurons. J. Comp. Physiol. A 186:69–79; 2000.
170. Nüesch, H. Control of muscle development. In: Kerkut, G. A.; Gilbert, L. I., eds. Complete insect physiology, biochemistry and pharmacology. Oxford: Pergamon Press; 1985:425–452.
171. Nüesch, H.; Bienz-Isler, G. Die Entwicklung denervierter Imaginalmuskein bei *Antheraea polyphemus* (Lep.) und die Frage der entwicklungsfordernden Nervenwirkung. Zool. Jb. Anat. 89:333–350; 1972.
172. O'Brien, M. A.; Schneider, L. E.; Taghert, P. H. In situ hybridization analysis of the *FMRFamide* neuropeptide gene in *Drosophila*. II. Constancy in the cellular pattern of expression during metamorphosis. J. Comp. Neurol. 304:623–638; 1991.
173. Ohlsson, L. G.; Nässel, D. R. Postembryonic development of serotonin-immunoreactive neurons in the central nervous system of the blowfly. I. The optic lobes. Cell Tissue. Res. 249:669–679; 1987.
174. Ohlsson, L. G.; Johansson, K. U. I.; Nässel, D. R. Postembryonic development of Arg-Phe-amide-like and cholecystokinin-like immunoreactive neurons in the blowfly optic lobe. Cell Tissue Res. 256:199–211; 1989.
175. Orchard, I.; Ramirez, J. M.; Lange, A. B. A multifunctional role for octopamine in locust flight. Annu. Rev. Entomol. 38:227–249; 1993.
176. Palka, J. Neuronal specificity and its development in the *Drosophila* wing disc and its derivatives. J. Neurobiol. 24:788–802; 1993.
177. Paspalas, C.; Consoulas, C.; Theophilidis, G. The fate of specific motoneurons and sensory neurons of the pregenital abdominal segments in *Tenebrio molitor* (Insecta: Coleoptera) during metamorphosis. Rouxs Arch. Dev. Biol. 202:204–213; 1993.
178. Peinado, A.; Zipser, B.; Macagno, E. R. Segregation of afferent projections in the central nervous system of the leech *Hirudo medicinalis*. J. Comp. Neurol. 301:232–242; 1990.
179. Peterson, B. A.; Weeks, J. C. Somatotopic mapping of sensory neurons innervating mechanosensory hairs on the larval prolegs of *Manduca sexta*. J. Comp. Neurol. 275:128–144; 1988.
180. Pflüger, H. J. Neuromodulation during motor development and behavior. Curr. Opin. Neurobiol. 9:683–689; 1999.
181. Pflüger, H. J.; Bräunig, P.; Hustert, R. The organization of mechanosensory neuropiles in locust thoracic ganglia. Philos. Trans. R. Soc. B Biol. Sci. 321:1–26; 1988.
182. Pflüger, H. J.; Witten, J. L.; Levine, R. B. Fate of abdominal ventral unpaired median cells during metamorphosis of the hawkmoth, *Manduca sexta*. J. Comp. Neurol. 335:508–522; 1993.
183. Phelan, P.; Nakagawa, M.; Wilkin, M. B.; Moffat, K. G.; O'Kane, C. J.; Davies, J. A.; Bacon, J. P. Mutations in *shaking-B* prevent electrical synapse formation in the *Drosophila* giant fiber system. J. Neurosci. 16:1101–1113; 1996.
184. Pringle, J. W. S. Insect flight. Cambridge: Cambridge University Press; 1957.
185. Prokop, A.; Technau, G. M. Normal function of the *mushroom body defect* gene of *Drosophila* is required for the regulation of the number and prolifeation of neuroblasts. Dev. Biol. 161:321–337; 1994.
186. Prokop, A.; Landgraf, M.; Rushton, E.; Broadie, K.; Bate, M. Presynaptic development at the Drosophila neuromuscular junction: Assembly and localization of presynaptic active zones. Neuron 17:617–626; 1996.
187. Prugh, J. K.; Della Croce, K.; Levine, R. B. Effects of the steroid hormone, 20-hydroxyecdysone, on the growth of neurites by identified insect motoneurons *in vitro*. Dev. Biol. 154:331–347; 1992.
188. Rall, W. Theoretical significance of dendritic trees for neuronal input-output relations. In: Reiss, R. F., ed. Neural theory and modeling. Stanford, CA: Stanford University Press; 1964:73–94.
189. Rall, W. Time constants and electrotonic length of membrane cylinders and neurons. Biophys. J. 9:1483–1508; 1969.
190. Ramirez, J. M.; Pearson, K. G. Octopamine induces bursting and plateau potentials in insect neurons. Brain Res. 549:332–337; 1991.
191. Raper, J. A.; Bastiani, M. J.; Goodman, C. S. Pathfinding by neuronal growth cones in grasshopper embryos. II. Selective fasciculation onto specific axonal pathways. J. Neurosci. 3:31–41; 1983.
192. Rendt, J. M.; Toro, L.; Stefani, E.; Eruklar, S. D. Progesterone increases calcium currents in myometrial cells from immature and nonpregnant adult rats. Am. J. Physiol. 262:C293–C301; 1992.
193. Restifo, L. L.; White, K. Mutations in a steroid hormone-regulated gene disrupt the metamorphosis of the central nervous system in Drosophila. Dev. Biol. 148:174–194; 1991.
194. Rheuben, M. B. Quantitative comparison of the structural features of

slow and fast neuromuscular junctions in *Manduca*. J. Neurosci. 5:1704–1716; 1985.
195. Rheuben, M. B. Degenerative changes in the muscle fibers of *Manduca sexta* during metamorphosis. J. Exp. Biol. 167:91–117; 1992.
196. Rheuben, M. B. Degenerative changes in the structure of neuromuscular junctions of *Manduca sexta* during metamorphosis. J. Exp. Biol. 167: 119–154; 1992.
197. Rheuben, M. B.; Kammer, A. E. Comparison of slow larval and fast adult muscle innervated by the same motoneuron. J. Exp. Biol. 84:103–112; 1980.
198. Rohrbacher, J. Fictive chewing activity in motoneurons and interneurons of the suboesophageal ganglion of *Manduca sexta* larvae. J. Comp. Physiol. A 175:629–637; 1994.
199. Rose, U.; Levine, R. B. Comparison of identified leg motoneuron structure and function in larval and adult *Manduca sexta*. J. Comp. Physiol. 186:327–336; 2000.
200. Ross, J. J.; Duxon, M. J.; Harris, A. J. Neural determination of muscle fiber numbers in embryonic rat lumbrical muscles. Development 100: 395–409; 1987.
201. Roy, S.; VijayRaghavan, K. Homeotic genes and the regulation of myoblast migration, fusion, and fibre-specific gene expression during adult myogenesis in *Drosophila*. Development 124:3333–3341; 1997.
202. Rozek, C. E.; Davidson, N. *Drosophila* has one myosin heavy-chain gene with three developmentally regulated transcripts. Cell 32:23–34; 1983.
203. Salmons, S.; Sreter, F. A. Significance of impulse activity in the transformation of skeletal muscle type. Nature 263:30–34; 1976.
204. Sandstrom, D. J.; Weeks, J. C. Reidentification of larval interneurons in the pupal stage of the tobacco hornworm *Manduca sexta*. J. Comp. Neurol. 308:311–327; 1991.
205. Sandstrom, D. J.; Weeks, J. C. Segment-specific retention of a larval neuromuscular system and its role in a new, rhythmic, pupal motor pattern in *Manduca sexta*. J. Comp. Physiol. A 183:283–302; 1998.
206. Sanes, J. R.; Hildebrand, J. G. Origin and morphogenesis of sensory neurons in an insect antenna. Dev. Biol. 51:300–319; 1976.
207. Schwindt, P. C.; Crill, W. E. Membrane properties of cat spinal motoneurons. In: Davidoff, R. A., ed. Handbook of the spinal cord. New York: Decker; 1984:199–242.
208. Segev, I. Single neurone model: Oversimple, complex and reduced. Trends Neurosci. 15:414–421; 1992.
209. Selleck, S. B.; Steller, H. The influence of retinal innervation on neurogenesis in the first optic ganglion of *Drosophila*. Neuron 6:83–99; 1991.
210. Shepherd, D.; Smith, S. A. Central projections of persistent larval sensory neurons prefigure adult sensory pathways in the CNS of *Drosophila*. Development 122:2375–2384; 1996.
211. Smith, S. A.; Shepherd, D. Central afferent projections of proprioceptive sensory neurons in *Drosophila* revealed with the enhancer-trap technique. J. Comp. Neurol. 364:311–323; 1996.
212. Sonea, I. M.; Rheuben, M. B. Degenerative changes in the function of neuromuscular junctions of *Manduca sexta* during metamorphosis. J. Exp. Biol. 167:61–89; 1992.
213. Sotavalta, O. Recording of high wing-stroke and thoracic vibration frequency in some midges. Biol. Bull. 104:439–444; 1953.
214. Sperry, D. G.; Grobstein, P. Postmetamorphic changes in the lumbar lateral motor column in relation to muscle growth in the toad, *Bufo americanus*. J. Comp. Neurol. 216:104–114; 1983.
215. Spitzer, N. C. A developmental handshake: Neuronal control of ionic currents and their control of neuronal differentiation. J. Neurobiol. 22: 659–673; 1991.
216. Stanier, D. Y. R.; Gilbert, W. Pioneer neurons in the mouse trigeminal system. Proc. Natl. Acad. Sci. USA 87:923–927; 1990.
217. Stevenson, P. A.; Kutsch, W. Demonstration of functional connectivity of the flight motor system in all stages of the locust. J. Comp. Physiol. A 162:247–259; 1988.
218. Stocker, R. F.; Nüesch, H. Ultrastructural studies on neuromuscular contacts and the formation of junctions in the flight muscles of *Antheraea polyphemus* (Lep.). I. Normal adult development. Cell Tissue Res. 159: 245–266; 1975.
219. Stocker, R. F.; Tissot, M.; Gendre, N. Morphogenesis and cellular proliferation pattern in the developing antennal lobe of *Drosophila melanogaster*. Rouxs Arch. Dev. Biol. 205:62–75; 1995.
220. Stocker, R. F.; Himbeck, G.; Gendre, N.; de Belle, J. S. Neuroblast ablation in *Drosophila* P[GAL4] lines reveals origins of olfactory interneurons. J. Neurobiol. 32:443–456; 1997.
221. Streichert, L. C.; Weeks, J. C. Decreased monosynaptic sensory input to an identified motoneuron is associated with steroid-mediated dendritic regression during metamorphosis in *Manduca sexta*. J. Neurosci. 15: 1484–1495; 1995.
222. Takahashi, S.; Takano-Ohmuro, H.; Maruyama, K. Regulation of *Drosophila* myosin ATPase activity by phosphorylation of myosin light chains. I. Wild-type fly. Comp. Biochem. Physiol. 95B:179–181; 1990.
223. Takahashi, S.; Takano-Ohmuro, H.; Maruyama, K.; Hotta, Y. Regulation of *Drosophila* myosin ATPase activity by phosphorylation of myosin light chains. II. Flightless *mfd*- fly. Comp. Biochem. Physiol. 95B:183–185; 1990.
224. Tamarkin, D. A.; Levine, R. B. Synaptic interactions between a muscle-associated proprioceptor and body wall muscle motor neurons in larval and adult *Manduca sexta*. J. Neurophysiol. 76:1597–1610; 1996.
225. Taylor, B. J. Sexually dimorphic neurons in the terminalia of *Drosophila melanogaster*: I. Development of sensory neurons in the genital disc during metamorphosis. J. Neurogenet. 5:173–192; 1989.
226. Taylor, H. M.; Truman, J. W. Metamorphosis of the abdominal ganglia of the tobacco hornworm, *Manduca sexta*. Changes in populations of identified motor neurons. J. Comp. Physiol. 90:367–388; 1974.
227. Technau, G.; Heisenberg, M. Neural reorganization during metamorphosis of the corpora pedinculata in *Drosophila melanogaster*. Nature 295: 405–407; 1982.
228. Thomas, J. B.; Wyman, R. J. Mutations altering synaptic connectivity between identified neurons in *Drosophila*. J. Neurosci. 4:530–538; 1984.
229. Thorn, R. S.; Truman, J. W. Sex-specific neuronal respecification of the genital segments of the tobacco hornworm moth *Manduca sexta*. J. Comp. Neurol. 284:489–503; 1989.
230. Thummel, C. S. Flies on steroids—Drosophila metamorphosis and the mechanisms of steroid hormone action. Trends Genet. 12:306–310; 1996.
231. Tissot, M.; Gendre, N.; Hawken, A.; Störtkuhl, K. F.; Stocker, R. F. Larval chemosensory projections and invasion of adult afferents in the antennal lobe of *Drosophila*. J. Neurobiol. 32:281–297; 1997.
232. Tissot, M.; Gendre, N.; Stocker, R. F. *Drosophila* P[GAL4] lines reveal that motor neurons involved in feeding persist through metamorphosis. J. Neurobiol. 37:237–250; 1998.
233. Tissot, M.; Stocker, R. F. Metamorphosis in *Drosophila* and other insects: The fate of neurons throughout the stages. Prog. Neurobiol. 62:89–111; 2000.
234. Tix, S.; Minten, J. S.; Technau, G. M. Pre-existing neuronal pathways in the developing optic lobes of *Drosophila*. Development 105:739–746; 1989.
235. Tix, S.; Bate, M.; Technau, G. M. Pre-existing neuronal pathways in the leg imaginal discs of *Drosophila*. Development 107:855–862; 1989.
236. Trimarchi, J. R.; Murphey, R. K. The shaking-$B^2$ mutation disrupts electrical synapses in a flight circuit in adult *Drosophila*. J. Neurosci. 17:4700–4710; 1997.
237. Truman, J. W. Programmed cell death in the nervous system of an adult insect. J. Comp. Neurol. 216:445–452; 1983.
238. Truman, J. W. Metamorphosis of the CNS of *Drosophila*. J. Neurobiol. 21:1072–1084; 1990.
239. Truman, J. W. Metamorphosis of the insect nervous system. In: Gilbert, L. I.; Tata, J. R.; Atkinson, B. G., eds. Metamorphosis: Postembryonic reprogramming of gene expression in amphibian and insect cells. San Diego: Academic Press; 1996:283–320.
240. Truman, J. W. Steroid receptors and nervous system metamorphosis in insects. Dev. Neurosci. 18:87–101; 1996.
241. Truman, J. W.; Reiss, S. E. Dendritic reorganization of an identified motoneuron during metamorphosis of the tobacc hornworm, *Manduca sexta*. Science 192:477–479; 1976.
242. Truman, J. W.; Schwartz, L. M. Steroid regulation of neuronal death in the moth nervous system. J. Neurosci. 4:274–280; 1984.
243. Truman, J. W.; Booker, R. Adult-specific neurons in the nervous system of the moth, *Manduca sexta*: Selective chemical ablation using hydroxyurea. J. Neurobiol. 17:613–625; 1986.
244. Truman, J. W.; Reiss, S. E. Hormonal regulation of the shape of identified motoneurons in the moth, *Manduca sexta*. J. Neurosci. 8:765–775; 1988.
245. Truman, J. W.; Taylor, B. J.; Awad, T. A. Formation of the adult nervous

system. In: Bate, M.; Martinez Arias, A., eds. The development of *Drosophila melanogaster*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1993:1245–1275.
246. Truman, J. W.; Reiss, S. E. Neuromuscular metamorphosis in the moth *Manduca sexta*: Hormonal regulation of synapse loss and remodeling. J. Neurosci. 15:4815–4826; 1995.
247. Tsujimura, H. Metamorphosis of wing motor system in the silk moth, *Bombyx mori*: L. (Lepidoptera: Bombycidae): Anatomy of the sensory and motor neurons that innervate larval mesothoracic dorsal musculature, stretch receptors, and epidermis. Int. J. Insect Morphol. Embryol. 17: 367–380; 1988.
248. Tsujimura, H. Metamorphosis of wing motor system in the silk moth, *Bombyx mori*: Origin of wing motor neurons. Dev. Growth Differ. 31:331–339; 1989.
249. Ursprung, M.; Conscience-Egli, D.; Wallimann, T. Origin of leg muscle during *Drosophila* metamorphosis. Proc. Natl. Acad. Sci. USA 69:2812–2813; 1972.
250. Usherwood, P. N. R. The nature of 'slow' and 'fast' contractions in the coxal muslces of the cockroach. J. Insect Physiol. 8:31–52; 1962.
251. Valles, A. M.; White, K. Serotonin-containing neurons in *Drosophila melanogaster*: Development and distribution. J. Comp. Neurol. 268: 414–428; 1988.
252. Verderio, C.; Coco, S.; Fumagali, G.; Matteoli, M. Calcium-dependent glutamate release during neuronal development and synaptogenesis: Different involvement of ω-AgaIV A and ω-CTx-GVIA sensitive channels. Proc. Natl. Acad. Sci. USA 92:6449–6453; 1995.
253. VijayRaghavan, K.; Gendre, N.; Stocker, R. F. Transplanted wing and leg imaginal discs in *Drosophila melanogaster* demonstrate interactions between epidermis and myoblasts in muscle formation. Dev. Genes Evol. 206:46–53; 1996.
254. Waldrop, B.; Levine, R. B. Development of the gin trap reflex in *Manduca sexta*: A comparison of larval and pupal motor responses. J. Comp. Physiol. A 165:743–753; 1989.
255. Waldrop, B.; Levine, R. B. Intersegmental interneurons serving larval and pupal mechanosensory reflexes in the moth *Manduca sexta*. J. Comp. Physiol. A 171:195–205; 1992.
256. Weeks, J. C. Time course of hormonal independance for developmental events in neurons and other cell types during insect metamorphosis. Dev. Biol. 124:163–176; 1987.
257. Weeks, J. C. Steroid hormones, dendritic remodeling and neuronal death: Insights from insect metamorphosis. Brain Behav. Evol. 54:51–60; 1999.
258. Weeks, J. C.; Truman, J. W. Neural organization of peptide-activated ecdysis behaviors during the metamorphosis of *Manduca sexta*. II. Retention of the proleg motor pattern despite loss of the prolegs at pupariation. J. Comp. Physiol. A 155:423–433; 1984.
259. Weeks, J. C.; Truman, J. W. Independent steroid control of the fates of motoneurons and their muscles during insect metamorphosis. J. Neurosci. 5:2290–2300; 1985.
260. Weeks, J. C.; Truman, J. W. Steroid control of neurons and muscle development during the metamorphosis of an insect. J. Neurobiol. 17: 249–267; 1986.
261. Weeks, J. C.; Ernst-Utzschneider, K. Respecification of larval proleg motoneurons during metamorphosis of the tobacco hornworm, *Manduca sexta*: Segmental dependence and hormonal regulation. J. Neurobiol. 20:569–592; 1989.
262. Weeks, J. C.; Levine, R. B. Postembryonic neuronal plasticity and its hormonal control during insect metamorphosis. Annu. Rev. Neurosci. 13:183–194; 1990.
263. Weeks, J. C.; Davidson, S. K.; Debu, H. G. Effects of a protein synthesis inhibitor on the hormonally mediated regression and death of motoneurons in the tobacco hornworm, *Manduca sexta*. J. Neurobiol. 24:125–140; 1992.
264. Weeks, J. C.; Davidson, S. K. Influence of interganglionic interactions on steroid-mediated dendritic reorganization and death of proleg motor neurons during metamorphosis in *Manduca sexta*. J. Neurobiol. 25:535–554; 1994.
265. Wegener, G. Flying insects: Model systems in exercise physiology. Experientia 52:404–412; 1996.
266. Wernig, A.; Herrera, A. A. Sprouting and remodelling at the nerve-muscle junction. Prog. Neurobiol. 27:251–291; 1986.
267. White, K.; Hurteau, T.; Punsal, P. Neuropeptide-FMRFamide-like immunoreactivity in *Drosophila*: Development and distribution. J. Comp. Neurol. 247:430–438; 1986.
268. Whitlock, K. E.; Palka, J. Development of wing sensory axons in the central nervous system of *Drosophila* during metamorphosis. J. Neurobiol. 26:189–204; 1995.
269. Williams, C. J. A.; Caveney, S. A gradient of morphogenetic information involved in muscle patterning. J. Embryol. Exp. Morph. 58:35–61; 1980.
270. Williams, C. M.; Schneiderman, H. A. The necessity of motor innervation for the development of insect muscles. Anat. Rec. 113:560; 1952.
271. Williams, D. W.; Shepherd, D. Persistent larval sensory neurons in adult *Drosophila melanogaster*. J. Neurobiol. 39:275–286; 1999.
272. Witten, J. L.; Truman, J. W. The regulation of transmitter expression in postembryonic lineages in the moth *Manduca sexta*. I. Transmitter identification and developmental acquisition of expression. J. Neurosci. 11: 1980–1989; 1991.
273. Witten, J. L.; Truman, J. W. Distribution of GABA-like immunoreactive neurons in insects suggests lineage homology. J. Comp. Neurol. 398: 515–528; 1998.
274. Yao, Y. M. Maintenance of axon terminals at synaptic sites in the absence of muscle fibers. In: Reiet, P. J.; Bunge, R. P.; Seil, F. J., eds. Current issues in neural regeneration research. New York: Alan R. Liss, Inc.; 1988:167–178.
275. Yoshihara, M.; Rheuben, M. B.; Kidokoro, Y. Transition from growth cone to functional motor nerve terminal in *Drosophila* embryos. J. Neurosci. 17:8408–8426; 1997.
276. Zitnan, D.; Kingan, T. G.; Hermesmann, J. L.; Adams, M. E. Identification of ecdysis-triggering hormone from an epitracheal endocrine system. Science 271:88–91; 1996.
277. Zou, D. J.; Cline, H. T. Postsynaptic calcium/calmodulin-dependent kinase II is required to limit elaboration of presynaptic and postsynaptic neuronal arbors. J. Neurosci. 19:8909–8918; 1999.