9. Nervous System

Jayne E. Yack & Uwe Homberg

Descriptions of the lepidopteran nervous system date back to the 17th century. Some of the earliest anatomists, equipped with crude magnifying lenses and simple dissection instruments, produced remarkably detailed and accurate renditions of the central nervous system and peripheral nerve topography. By the late 19th century, developments in histological and optical methodologies led to the first studies of the central architecture. Detailed historical reviews of the early literature have been provided by Hilton (1940), Ehnborn (1948), and Bullock & Horridge (1965).

During the past thirty years a proliferation of new methods in microscopy, intracellular staining and recording, immunohistochemistry, and molecular genetics have enabled researchers to probe further into the functional and developmental aspects of invertebrate nervous systems. The lepidopteran nervous system has become popular as a model system for studying postembryonic development (Truman 1992b; Levine 1986, 1987; Oland & Tolbert 1996), control and developmental changes of segmental reflexes (Levine & Weeks 1990; Weeks & Wood 1996; Weeks 1999), and olfactory (Hansson 1995; Hildebrand 1996) and auditory (2-11) systems.

While there are many fine works on different aspects of the nervous system in Lepidoptera, a comprehensive review of the subject has not been performed, owing, no doubt, to the magnitude of the subject. Noteworthy summaries include those of Ehnborn (1948), Bullock & Horridge (1965), and Eaton (1988). The immediate purpose of this chapter is to bring together the literature on the structure and function of the lepidopteran nervous system. The first half presents an overview of the gross anatomy of both the adult and larval nervous systems, with some consideration given to the variations between different taxonomic groups. The latter part focuses on the central nervous system, particularly the brain, with a discussion of functional systems. Other chapters in this book, namely those on Sensilla and Proprioceptors (2-10); Sound Producing and Auditory Organs (2-11); Visual Organs (2-12), and Endocrine Glands and Hormones (2-15) are directly relevant to a discussion of the nervous system.

Gross Anatomy of the Adult Nervous System

Descriptions of the anatomy of invertebrate nervous systems are frequently organized into three subdivisions, an approach which we have adopted here: (i) The Central Nervous System, comprising a dorsally located brain + suboesophageal ganglion (= cephalic nervous system) and a ventral chain of thoracic and abdominal segmental ganglia (= ventral nerve cord); (ii) The Peripheral Nervous System, consisting of mixed sensory and motor nerves and their innervation sites; (iii) The Stomodaeal Nervous System, which supplies the anterior part of the alimentary canal. The retrocerebral neuroendocrine glands, including the corpora cardiaca and corpora allata, which are closely associated with the brain, are discussed in relation to the endocrine system (see 2-15).

Lepidopteran Groundplan

The groundplan, or the plesiomorphic condition of the adult lepidopteran is typically inferred from information we have about “primitive” (non-glossatan) families, and neighbouring panorpoid orders. Comparative studies of the arthropod nervous system have shown that more primitive groups often exhibit a less concentrated ganglionic pattern (Bullock & Horridge 1965), and this is demonstrated to some degree when comparing the higher Lepidoptera with lower groups, and with the Trichoptera.

In adult Trichoptera (see Glasgow 1936; Ehnborn 1948; Korbbo 1964; Klemm 1966) the brain and suboesophageal ganglion are distinctly separated from one another, with a well developed suboesophageal ganglion lying caudal to the brain, forming part of the ventral nerve cord. The two ganglia are joined by a pair of long, well-defined circumoesophageal connectives, which form a wide orifice (= oesophageal ring) for the passage of the oesophagus, recurrent nerve, and aorta. The recurrent nerve runs below the aorta. The proto- and deutocerebrum of the brain are often discernable externally. Connectives joining the suboesophageal ganglion with the first thoracic ganglion (= cervicothoracic connectives) are also clearly separated. The ventral nerve cord typically consists of three distinct thoracic ganglia and six or seven abdominal ganglia, all joined by paired connectives (Fig. 9.1A). The degree of ganglionic fusion between the larval and imaginal forms during metamorphosis is minimal.

The most primitive Lepidoptera examined to date, namely Micropterix (Micropterigioidea) (Ehnborn 1948; Kristensen & Nielsen 1981; Kristensen 1984b), and Agathiphaga (Agathiphaga-
Fig. 9.1. A, B. Dorsal views of the adult central nervous system in (A) Trichoptera (Hydropsychidae) [After Korboot (1964)], and (B) Lepidoptera (Nymphalidae) [After Brandt (1879)]. C. A schematic overview of the adult nervous system in Lepidoptera [After Scott (1986)]. Thoracic and abdominal segments are indicated by Roman (I–III) and Arabic (1–10) numerals respectively. ANT: antenna; BR: brain; CC–CA: corpora cardiaca-corpora allata; CH: chaetosoma; FG: frontal ganglion; PB: proboscis; RN: recurrent nerve; SOEG: suboesophageal ganglion.

goidea) (Kristensen & Nielsen 1981; Kristensen 1984b; Kristensen, personal communication), differ from the Trichoptera in having a higher degree of ganglionic condensation. The circumoesophageal connectives are shortened, drawing the suboesophageal ganglion into a ventral position with respect to the brain. The oesophageal ring is still greatly enlarged compared to that of higher forms however. A free tritocerebral commissure, a primitive trait shared with the Trichoptera, is still observed (Fig. 9.3 D). The labral nerve and frontal ganglion connectives are separated at their origins on the anterior tritocerebrum. This has been considered a lepidopteran groundplan autapomorphy (1–2), but it may actually be a plesiomorphy, since a similar condition occurs in the basal trichopteran family Hydrophilidae; in contrast, other examined caddisflies have the two nerves enclosed in a common neurilemma for a long distance (Ehnbom 1948). The recurrent nerve penetrates the aorta to run inside it, until reaching the retrocerebral complex (Kristensen 1984b). The ventral nerve cord in Micropteryx and Agathiphaga resembles that of the Trichoptera in that the cervicothoracic connectives are paired, and there are three separate thoracic ganglia. There is an overall reduction in the number of abdominal ganglia, and the inter-
ganglionic connectives are fused. The five ganglionic condition of *Agathiphaga* is considered to represent the groundplan configuration for the Lepidoptera (Kristensen & Nielsen 1981; Nielsen & Kristensen 1996).

The typical ganglionic configuration for higher (Ditrysian) Lepidoptera is illustrated in Figure 9.1 B, C. The brain and suboesophageal ganglion have become closely associated, such that the circumoesophageal connectives are significantly reduced, and only discernible in histological sections. A tritocerebral commissure is no longer distinct, having become incorporated into the suboesophageal ganglion (Kristensen 1968). The oesophagus is necessarily largely constricted, to pass through the narrow oesophageal ring, but is sufficient to allow for the passage of the liquid diet of the higher Lepidoptera. Unlike for the lower Lepidoptera and Trichoptera, the cervicothoracic connectives are joined within a single mesodermal envelope, although in histological sections they are clearly discernable as two discrete bundles (Ehnbom 1948). The ventral nerve cord has become concentrated into two thoracic ganglia and four abdominal ganglia. Generally the adult central nervous system exhibits a much greater degree of ganglionic fusion than that of the larva.

**Cephalic Nervous System**

External features of the adult cephalic ganglia (brain + suboesophageal ganglion) have been described for a variety of species by Ehnbom (1948), and for *Danaus plexippus* (Ehrlich & Davidson 1961), *Heliothis zea* (Chauhan & Callahan 1967a), *Manduca sexta* (Eaton 1974, 1988), and *Papilio demoleus* (Srivasattava 1969). The general arrangement is fundamentally similar for all Lepidoptera, with the suboesophageal ganglion having moved rostrally to become fused with the brain. Some variations are observed between groups regarding the degree of fusion between the two cephalic ganglia and the relative developments and positions of their constituent lobes and nerve roots. Here we describe the major external features of the cephalic nervous system, primarily for *Manduca sexta* (Fig. 9.2), followed by an overview of the major differences observed in various taxonomic groups (Fig. 9.3).

The brain (= supraoesophageal ganglion) is the main ganglionic mass of the head, situated between the compound eyes and posterior to the large set of muscles associated with the sucking pump (Fig. 9.1 C). The three ganglionic neuro- meres of the brain, the protocerebrum, deutocerebrum, and tritocerebrum (Snodgrass 1935) have merged to a large degree. In comparison to the brain of hemimetabolous insects, as well as to that of Trichoptera, the lepidopteran brain is tilted backwards (almost 180° with respect to the neuraxis) (Hanström 1928; Schrader 1938; Pearson 1971; Homberg 1994), as is the case in the prognathous head of Coleoptera. This is already apparent in *Micropterix* (Buxton 1917), and is probably an adaptation to the dorsal position of the antennae instead of a more ventral position on the head of other insects. In *Manduca*, the protocerebrum, which functions largely to integrate information arriving from the anterior sense organs, especially the eyes, is situated in a dorsal and posterior position to the deutocere-
brum. The paired optic lobes, forming a prominent part of the protocerebrum, extend laterally, receiving information directly from the compound eyes. The principal nerves arising from the protocerebrum lead to the corpora cardiaca, mechanosensilla on the head capsule, compound eyes (the connections to the compound eyes are not called nerves, because the neuropil [lamina] sits right underneath the retina) and ocelli. The deuto- and tritocerebrum is largely represented by the two large and distinct antennal lobes that form the most anterior and dorsal aspect of the brain. It functions primarily as an association center for the antennae (Homberg et al. 1989), and gives rise to the large antennal nerves which innervate the antennal sensory organs and muscles. Species that exhibit sexual dimorphism of the antennae also exhibit dimorphism in the size of the antennal lobes (Ehnbom 1948; see discussion of antennal lobe in this chapter). In Lophocoron (Lophocoronidae), the deuto- and tritocerebral lobes are joined to one another anteriorly, forming a ‘deuto- and tritocerebral loop’ (Nielsen & Kristensen 1996). This unusual condition has subsequent been noted to be widespread among the lower Amphipod, including several Micropterygidae, and Acanthocephalidae (Kristensen, pers. comm.). The functional significance of this unusual configuration is unknown, and cannot be explained by unusually close antennal lobes (Kristensen, pers. comm.). The small tritocerebrum occupies a ventral position in the brain below the deuto- and tritocerebrum, and is difficult to detect by external inspection due to the ventral position of the suboesophageal ganglion. A pair of frontal connectives arises from the anterior edge of the ganglion, extending forward and then medially to connect with the frontal ganglion (Figs.
The labral nerve arises close to the base of the frontal connective, but unlike for the (non-hydromphid) Trichoptera, the two nerves are often independent from one another at their origin, among the numerous Lepidoptera examined by Ehnbom (1948) only the hadenine noctuid Mythimna (= Hylidulare) have them enclosed in a common neurilemma for a considerable distance. However, Ehnbom also notes that all variations ranging from complete separation to complete fusion of these 2 nerves exist in the Lepidoptera. A pair of tegumentary nerves arise from the latero-posterior edges of the tritocerebrum. In some families this nerve innervates the chaetosema (= Eltringham’s organ) (Eltringham 1925; Ehnbom 1948; Ehrlich & Davidson 1961), a structure with an unknown (but possibly mechanosensory [see 2–10]) function. Although chaetosema are not reported for Manduca (Eaton 1974), the tegumentary nerve is well developed, and innervates the vertex of the head. The circumoesophageal connectives emerge from the tritocerebral lobes and proceed posteriorly and ventrally to the suboesophageal ganglion.

Due to the condensation of the circumoesophageal connectives in the Lepidoptera, the suboesophageal ganglion merges with the brain, lying ventral to the deutocerebrum and tritocerebrum. In higher forms the exact border between the two ganglionic masses cannot be defined even in histological sections. In Manduca, several pairs of peripheral nerves arise from the suboesophageal ganglion to innervate the mouthparts (mandibular, maxillary, and labial nerves) and neck region (cervical nerves) (Eaton 1988; Davis et al. 1996; Fig. 9.2 A, B).

Ehnbom (1948) has categorized the cephalic nervous system of Lepidoptera into four main types, largely based on the form of the optic lobes: Type I, the ‘Rhopalocera type’ (Fig. 9.3 A) is characterized by greatly enlarged and rounded optic lobes that occupy the largest part of the brain volume, reflecting the importance of the visual organs in diurnal Lepidoptera. The frontal connectives are short, bringing the frontal ganglion close to the brain. Type II, the ‘Macrosyne type’ (Fig. 9.3 B) has each optic lobe divided into 2 distinct regions, and is elongated laterally. Both the proto- and deutocerebrum are well developed (the deutocerebrum especially so in the Noctuidae). The frontal ganglion is connected to the brain by very long frontal connectives. This form occurs in most Macrolepidoptera families (except for the Drepanidae and Cymatophoridae, see Type III), and some Zygaenidae. In Type III, the ‘Tineoidae type’ (Fig. 9.3 C), the whole brain appears to be compressed laterally, and the optic lobes extend dorsoventrally rather than laterally. Dorsally, the optic lobes and the large protocerebral lobes cannot be distinguished as the two have merged completely, but on the ventral surface, they can be easily distinguished by the presence of a deep groove. The frontal ganglion is situated very closely to the brain between the antennal lobes. This type occurs among the Drepanidae, and the only examined taxa of neolepidopteran micro-moths. Type IV, the ‘Micropterigidae type’ corresponds to the lepidopteran groundplan configuration discussed above and is characterized by a wide oesophageal ring, well-developed mandibular nerves (reflecting the large mandibular muscles), and a free tritocerebral commissure. This type (Fig. 9.3 D) is retained in all three non-glossatan families (1–4) now known (Kristensen & Nielsen 1983, Kristensen, pers. comm.).

Ventral Nerve Cord

The ventral ganglia of the thoracic and/or abdominal segments, with their respective nerve roots have been described for Manduca (Eaton 1974, 1988), Antheraea polyphemus (Niesch 1957), Noctua pronuba and Hepialus hamulii (Birkett-Smith 1984), and Papilio demoleus (Srivastava 1969). Comparative studies of the ventral chain include those of Brandt (1879), Cattie (1881), Petersen (1900), Peterson (1912), Nordman (1967), Srivastava & Bogawat (1969b), Mitchell & Seabrook (1973), and Kristensen & Nielsen (1981).

The meso- and metathoracic ganglia are the largest of the three original thoracic ganglia, and this may be explained by the strongly developed musculature of these segments; moreover, a fusion of the metathoracic ganglion (III) + first (I) abdominal ganglion is believed to be a prelepideopteran evolutionary event (Kristensen & Nielsen 1981). In most of the examined homoneurous Lepidoptera (Ehnbom 1948; Birkett-Smith 1984; Nielsen & Kristensen 1989, 1996) the three thoracic ganglia remain separate; extensive fusion of the mesothoracic (II), and the metathoracic (III) ganglion has so far been recorded only from the ‘primitive heptafid’ Frass. In the Heteroneura the retention of three discrete thoracic ganglia is widespread in lower-grade taxa, and has been observed even in a few representatives of some macrolepidopteran families (Notodontidae, Geometridae); however, a large pterothoracic ganglion has been formed several times independently by the partial or complete fusion of the two posterior ones. A constriction may occur in the middle of the pterothoracic ganglion, delineating an anterior (II) and posterior (III + 1 (+ 2), see below) lobe (Figs. 9.1 B, 9.4). Varying degrees of fusion may be observed even within a single family; this is the case with, e.g., the Nepticulidae, Sesiidae, Zygaenidae, Geometridae, Notodontidae and Noctuidae. In contrast, in some lineages within the higher Ditrysia, such as the butterflies and the Sphingidae, the two lobes appear consistently to have fused completely. The configuration of the intergangli-
fused, or at most (as observed in *Lophocorona*) separated by a minute space.

The abdominal ganglia lie beneath the ventral diaphragm. The primitive number of abdominal ganglia for the adult is believed to be five, as described for *Agathiphaga* (Kristensen & Nielsen 1981). Here ganglion 1 is fused with the metathoracic ganglion, the ganglia pertaining to segments 2–5 are discrete, while the fifth (terminal) ganglionic mass comprises the fused neuromeres of all following segments (6–11). The five ganglion configuration is now known also from Lophocoronidae and Mnesarchaeidae (Nielsen & Kristensen 1996), as well as from some female Psychidae and Heterogynidae. Some Hepialidae have six (Fries 1970; Nielsen & Kristensen 1989), and not five as previously reported by Brandt (1879) and Petersen (1900); here ganglion 6 remains separate from the terminal mass. Fries (1970) believed that the hepialid condition represents the plesiomorphic state, but Kristensen & Nielsen (1981) interpret this high number as an autapomorphic neotenic trait.

Most adult Lepidoptera have four abdominal ganglia. Here abdominal ganglion 2 is fused with the III+1 mass, hence the first three represent ganglia 3–5 of the larva (Fig. 9.7), and the terminal caudal ganglion, which lies in the sixth abdominal segment, is composed of the three terminal larval ganglia. Ganglionic fusion has progressed even further in a number of small-sized non-apoditransian moths: only three abdominal ganglia are present in some Micropterigidae, Eriocraniidae, Nepticulidae, Gracillariidae and Lyonetiidae, and as few as two ganglia have been reported from some Micropterigidae (Paramontria semifasciella) and Nepticuloidae (Opostega salaciella, Stigmella nylandriella). In contrast, as many as seven ganglia are present in some female Psychidae (Bourgogne 1951), certainly (like the above mentioned 5-ganglion pattern in other psychids as well as in heterogynids) reflecting an incomplete metamorphosis in these short-lived apterous insects. Sexual dimorphism in ganglion fusion is also known from some eriocraniids in which both sexes are fully winged; here females have three discrete ganglia while males have the commonplace number, four (Richards 1963; Nordman 1967; Kristensen & Nielsen 1981; Nielsen & Kristensen 1996).

The numerous obvious parallelisms in ganglion fusion evidently detract from the phylogenetic information content of this trait; individual variation has been reported even among members of the same sex of a given species (Kristensen & Nielsen 1981). Ganglion fusion may prove functionally advantageous for a variety of reasons. It has been suggested that it may result in reduced integration times between neural units (Altman & Kien 1987), and that the fusion of individual thoracic ganglia may result in increased conduction rates between units associa-
lated with flight (Amos & Mesce 1994). While this may explain the high degree of ganglionic fusion in the thorax of very strong fliers (e.g., Sphingidae) it must be noted, that it is from a tiny, weekly-flying nepticulid moth (Stigmella alyandraella) the highest ganglionic concentration (two thoracic and two abdominal masses) in adult Lepidoptera has been recorded.

Abdominal interganglionic connectives are unpaired for almost all Lepidoptera, and frequently enclosed in a common sheath. Exceptions include a neopsycheid (Kristensen & Nielsen 1981), and some Sphingidae and Saturniidae (Richards 1963).

Peripheral Nervous System

A complete description of the peripheral nervous system would include all the nerve roots and peripheral nerve branches exiting the central nervous system, including the specific locations of motor terminals and sense organs: a tremendous undertaking, indeed! It is for this reason that we see few detailed descriptions of the peripheral nervous system in Lepidoptera. Some of the more detailed studies include Niesch (1957) on the head and thorax of *Antheraea polyphemus*, Srivastava (1969) on the head, thorax and abdomen of *Papilio demoleus*, Mitchell & Seabrook (1973) on the mesothorax of *Choreutis aumejardi* (Tortricidae), Eaton (1974, 1988) on the head, thorax, and abdomen of *Manduca sexta*, and Birke-Smith (1984) on the metathorax and the first few abdominal segments of *Hepialus humuli* and *Noctua pronuba*. Beckel (1958) and Libby (1961) describe the posterior abdominal segments of *Hyalophora cecropia*, Mesne-Sabin (1975) the same of a female *Coleophora*, and Chauthani & Calahan (1967a) provide a general overview of the head, thorax and abdomen of *Heliothis zea*. Our description of the peripheral nervous system is restricted to the main nerve roots of the ventral ganglia and their general innervation fields.

Comparisons of peripheral innervation patterns between different lepidopteran taxa have revealed many similarities (cf., Eaton 1974, Birke-Smith 1984, Yack & Fullard 1990, Hasenfuss 1997, Yack et al. 1999). Variations do exist however, and without making direct comparisons, one must be cautious in establishing nerve homologies between different species. As a rule, anatomical structures of a given segment are innervated by nerves arising from the corresponding segmental ganglion. However, sometimes nerve branches override segmental boundaries, as fusions occur between nerves originating from different ganglia, or a single muscle may be innervated by nerve branches arising from different segments (e.g., Niesch 1957).

In *Antheraea* (Fig. 9.4, 9.5) and *Manduca*, the prothoracic ganglion gives rise to five pairs of peripheral nerves (IN 1–5) and one median nerve (IMN). The largest of these, IN 1 & 2, emerge from the ventral anterior edge of the ganglion, and proceed to innervate various muscles and sensory organs of the head and prothorax, including the foreleg. Three smaller branches (IN 3–5) arise from various positions along the pro-mesothoracic connective; IN 3 & 5 innervate the prothoracic ventro-longitudinal muscles, while N4 fuses with the mesothoracic nerve, INN 1. The median nerve (IMN) arises from the posterior end of the prothoracic ganglion and runs some distance along the pro-mesothoracic connectives before dividing into bilateral transverse nerves that innervate the prothoracic spiracular occlusor muscles.

Six sets of peripheral nerves and one median nerve emerge from the anterior (mesothoracic) lobe of the pterothoracic ganglion. The large anterior wing nerve (INN 1) innervates various muscles and sensory organs of the mesothorax, including the large mesothoracic dorsolongitudinal muscles, and the sensory organs at the base of the forewing. The innervation of the forewing itself has been described in some detail for a few dityrions; see Vogel (1911), Fudalewicz-Niemyzyn (1963) and references cited therein. In some butterflies, INN 1c innervates hearing organs located at the anterior edge of the forewing (Vogel 1912, Ribaric & Gogala 1996, Yack & Fullard 2000, Yack et al., 2000), and INN 1b innervates serial homologues of the noctuid auditory sensilla (Hasenfuss 1997, Yack et al. 1999). Nerves INN 2–4 arise from the ventral and lateral edges of the ganglion to innervate various pleural and dorsoventral muscles. INN 5 is the large leg nerve that arises from the ventroposterior edge of the mesothoracic ganglion, and INN 6 is a small nerve that arises from the dorsoposterior edge of the ganglion and proceeds posteriorly to innervate small pleural and leg muscles. The median (IMN) nerve arises from the junction between the two pterothoracic lobes, and the transverse nerves arising from it innervate the mesothoracic spiracles.

In *Antheraea*, three pairs of nerve roots (INN 1,2,3) and one median nerve emerge from the metathoracic ganglion. INN 1 arises dorsally and midway along the pterothoracic ganglion, and proceeds caudally to innervate the large metathoracic dorso-longitudinal muscles and the wing sensory organs. In the Noctuoidea, INN 1b innervates the metathoracic tympanal organ (Treat 1959), while in several atympanate groups, this branch innervates homologous sensory structures believed to represent evolutionary precursors to the noctuid auditory sensilla (Hasenfuss 1997, Yack et al. 1999). INN 2 arises ventrally from the ganglion and innervates the muscles and sensory organs of the metathoracic leg. INN 3 [= IN 1 of Eaton (1974, 1988)] combines with the anterior nerve of the first abdomi-
Fig. 9.5. Schematic drawings of the thoracic nervous system in *Athetaeccerus (= TELEA) polyphemus* [From Nüesch (1957)].

A. Lateral view of the right half of the head and thorax showing the major nerves, muscles and aorta. B. Major nerves of the head and thorax. Nerves emanating from various ganglia are delineated as such: Short dashes, suboesophageal ganglion; dots, prothoracic ganglion; solid lines, mesothorax; long dashes, metathorax. Ao: aorta; ant: antennal muscles; An: anal vein of wing; Cea: corpus cardiacum-allatulum; cd: cervicodorsal muscles; cc: cervicoventral muscle; C-Sc: costal-subcostal wing vein; ccx: coxal muscle; d: dorsal neck muscles; dl: dorsolongitudinal muscle; dv: dorsoventral muscle; Fg: frontal ganglion; F: furca; G: brain; Gs: intersegmental muscle; Mx: maxillary muscle; Md: midgut; Mn: median nerve; Mo: mouth opening; Nr: recurrent nerve; Oes: oesophagus; P: pleural muscle; Pd: pleurodorsal muscle; Ph: pharynx muscle; Pr: pleuroventral muscle; R M-Cu: radial-medial-cubital wing veins; S: suboesophageal ganglion; Sp: spiracular opener muscle; Sc: spiracular closer muscle; Sp: spina; St: sternopleural muscle; T: attached to the tergum; Teg: tegulum; tr: transversal (Diaphragm muscle); v: ventrally attached to the head; vc: ventrolongitudinal muscle.

...nal segment and extends caudally to innervate the metathoracic dorsoventral muscles and abdominal dorsolongitudinal muscles. The median nerve of the metathorax arises from the interganglionic connective and extends posteriorly to innervate the first abdominal spiracle. The original
ganglionic swellings of the first two larval abdominal segments have become fused with the pterothorax in the adult (Fig. 9.7), and their respective nerve branches may arise from the posterior end of the pterothoracic ganglion, or along the interganglionic connective, depending on the species. In the Geometroidae, the abdominal tympanic organ is innervated by 1N1, which arises from the posterior edge of the pterothoracic ganglion (Hasenfuss 1997).

Each of the independent abdominal ganglia (A 3.4.5) send out two pairs of lateral nerves (a dorsal and ventral nerve), and a caudal median nerve. The large dorsal nerves, which correspond to the wing nerves of the pterothorax, exit the ganglion anterolaterally and innervate the pleural and tergal region of its corresponding segment. The dorsal nerve also receives side branches from the transverse nerve of the preceding segment. The ventral nerves leave the ganglion lateroanteriorly to innervate the ventral longitudinal musculature. The ventral nerve also connects with the transverse nerve of the following segment. The median nerve travels posteriorly along the interganglionic connective to the anterior edge of the next posterior segment, where it splits into two transverse nerves that innervate the spiracular muscles of that segment.

Several pairs of nerves emerge from the terminal ganglion (= fused segmental neuromeres 6–11) to innervate various muscles and sensory organs associated with reproductive, posterior gastro-intestinal, and genital structures; in the very unusual case of Opostega (Nepticuloiden-Opostegidae) all terminal nerve branches have a long common stem (Kristensen & Nielsen 1981). Differences in the number, size, and innervation patterns of peripheral branches have been observed, depending on the species and/or sex (Ruckes 1919; Libby 1961; Chauhan & Callahan 1967a; Srivastava & Bogawat, 1969a; Mesnier-Sabin 1973; Eaton 1988).

The Stomatogastric Nervous System

The stomatogastric nervous system (also termed enteric, stomadeal, or visceral nervous system) innervates the musculature and related structures associated with the alimentary tract. It develops from neurogenic zones within the foregut epithelium (Kobayashi & Ando 1983; Copenhaver & Taghert 1989, 1991), and is thus distinct from, although closely associated with the central nervous system and retrocerebral organs. Gross anatomical descriptions of the adult stomatogastric nervous system are provided by Bickley (1942), Ehnborn (1948), Eastham & Eassa (1955), Niesch (1957), Srivastava (1969), Eaton (1988), and Miles & Booker (1998). The larval stomatogastric nervous system is described later in this chapter.

In adult Lepidoptera, as for most other insects, the principal components of the stomatogastric nervous system are the frontal ganglion, the frontal (= procurent) nerve, the frontal ganglion connectives, and the recurrent nerve (Figs. 9.1 C, 9.2 B, C, 9.3). The frontal ganglion, as its name implies, lies anterior to the brain and may sit in a dorsal or ventral position, depending on the species (Ehnborn 1948). It connects to the anterior tritocerebrum by a pair of frontal connectives which may be very short, so that the frontal ganglion is adjacent to the brain (e.g. Phymatopus Fig. 9.3 C), or elongate (e.g. Hadena, Fig. 9.3 B). As previously mentioned, in some Macrolepidoptera the frontal connective fuses with the ipsilateral labral nerve to form a common labro-frontal root. A single frontal nerve arises from the anterior edge of the frontal ganglion and divides into anteriorly and posteriorly directed branches which innervate the compressors and dilators of the cibarium, and the suboesophageal ganglion, respectively. The frontal ganglion in the adult is necessary for the activity of the cibarial pump during feeding, the swallowing of air and molting fluid during eclosion (Miles & Booker 1998), and post-feeding weight loss (Buschman & Nelson 1990). A single median recurrent nerve arises posteriorly from the caudal edge of the frontal ganglion, sending branches to the cibarial pump and oesophageal constrictor muscles. It passes through the oesophageal ring of the brain and through the aorta (Kristensen 1984b) until it reaches the level of the corpora cardiaca, to which it extends a pair of lateral nerve branches. The recurrent nerve may then send ramifications to, or innervate a hypocerebral ganglion, which generally consists of a small number of cells (Ehnborn 1948; Copenhaver, pers. comm.). In Manduca, some of the hypocerebral ganglion neurons project locally onto the foregut musculature, while others project anteriorly into the frontal ganglion and out the frontal ganglion connectives toward the brain (Copenhaver & Taghert 1991). The recurrent nerve (sometimes referred to as the oesophageal nerve after leaving the hypocerebral ganglion) continues posteriorly to innervate the foregut muscles and salivary glands, and then continues through the anterior section of the midgut, where it forms a large plexus over the alimentary canal. In some species, these cells may be organized into one or more small ganglia.

Gross Anatomy of the Immature Nervous System

Larval Nervous System

The larval and adult stages of Lepidoptera exhibit remarkable differences in their morphology
and behaviour. The larva is mainly designed for crawling and feeding, while the adult is designed for mating and reproduction, with particularly well-developed visual and olfactory senses, and flight mechanisms. These differences are to some extent reflected in the overall markedly different external anatomy of their respective nervous systems.

Many studies have been published on the gross anatomy and peripheral nerve topography of the larval nervous system. Detailed works include Lyonet (1762) for *Cossus*, Cattie (1881) for * Sphinx* and * Acherontia*, Peterson (1912) and Eaton (1988) for * Manduca sexta (= Protoparce carolina auct.*), DuPorte (1915) for * Spilota obliqua*, Swaine (1920) for *Sthenopis thule*, Kuwana (1932) for *Bombus*, Hilleman (1933) for *Papilio polyxenes*, Libby (1959) for *Hyalophora cecropia*, Dzierzynski (1957) for several lower heteroneuran families, Srivastava (1959) for *Leucinodes orbonalis*, Berestynska-Wilczek (1966) for *Antispila stachymantha*, Singh (1973, 1974) for * Sonta (= Philosamia auct.*) ricini, and Birket-Smith (1984) for *Hepialus humuli* and *Noctua pronuba*. The sensory trunk innervation of *Galleria mellonella* is compared with that of a basal trichopteran larva in a major study by Hasenfuss (1973, 2–5).

The most generalized condition of the central nervous system encountered in larval Lepidoptera is as unsegmented as in any insect, consisting of a brain, a separate subesophageal ganglion, three thoracic ganglia and eight abdominal ganglia (Fig. 9.6 A). Ganglionic fusion may happen not only at metamorphosis, but during larval development as well (see below).

The brain is situated along the dorsal wall of the pharynx. It is usually located in the anteroventral portion of the head, but in prononymously prognathous caterpillars it is, together with the subesophageal ganglion, partly or entirely displaced into the thorax, and the pharyngeal connectives therefore form marked swirls around the tentorial bridge. This configuration occurs in the Micropterigidae and is also characteristic of many leafmining micro-moth caterpillars (Dziurzynski 1957, Berestynska-Wilczek 1966, Kristensen & Nielsen 1983, Kristensen 1984a). The protocerebral lobes are prominent, but the deutocerebrum and tritocerebrum are not visible externally, giving the brain a simple bilobed appearance (Fig. 9.6 A,B). Already in the Micropterigidae and Agathiphaga the brain lobes are elongated anteriorly; this is thought to represent the larval groundplan condition (Kristensen 1984a and pers. comm.), and the configuration is retained throughout the order. The visual system is simple, and the optic lobes poorly developed compared with those of the adult. As for the Trichoptera and the lower Lepidoptera, a free tritocerebral commissure is typically evident in the larval brain (Denis & Bitsch 1973; Kristensen 1984a; Eaton 1988).

Main nerve branches of the brain include the lateral and frontal nerves, which are united for a considerable distance in *Agathiphaga* (Kristensen 1984a) and * Sthenopis thule* (Swaine 1920), but separate from their roots in *Manduca* (Eaton 1988), the optic nerves, which innervate the stemmata, and the antennal nerves, which are significantly smaller than for the adult (Nordlander & Edwards 1968). The circumoesophageal connectives are long and well developed to pass around the large larval pharynx.

The *subesophageal ganglion* is a well-developed separate ganglionic mass that lies in the anteroventral portion of the head or in the prothorax, thus forming part of the ventral chain (Fig. 9.6 C). The main subesophageal nerve roots are the large mandibular, maxillary, and labial nerves, which arise from the anterior or antero-lateral portion of the ganglion. The subesophageal ganglion connects via paired cervicothoracic connectives to the prothoracic ganglion. A fusion of the subesophageal and first thoracic ganglia is recorded to take place during larval life in *Cossus* (Petersen 1900), but usually the larval thoracic ganglia are discrete.

The ganglia of the ventral nerve cord typically have two pairs of lateral nerve roots (except for the eighth abdominal ganglion) and one unpaired median nerve root. In the prothoracic ganglion, an antero-lateral nerve and a postero-lateral nerve innervate the muscles and sensory organs of the tergal/pleural and sternal regions of the body respectively. A median nerve preceding the prothoracic ganglion is not usually present, but has been demonstrated in some specimens of *Sthenopis thule* and *S. argenteomaculatus* (Swaine 1920). The median nerve root arises from the postero-dorsal edge of the prothoracic ganglion, and extends along the interganglionic connective to the mesothoracic ganglion, where it bifurcates into paired transverse nerves that innervate the prothoracic spiracles. In the meso- and metathoracic ganglia, the antero-lateral nerve sends a short branch that fuses with the transverse nerve of the preceding segment. The postero-lateral nerve root innervates the sternal regions, and also sends a small branch that connects with the transverse nerve of that segment. The transverse nerves of the mesothoracic ganglion travel to the neighbourhood of the rudimentary metathoracic spiracles, while those of the metathoracic ganglion travel caudemally to innervate the spiracles of the first abdominal segment. Connectives between the thoracic ganglia are typically paired, but varying degrees of fusion have been observed (Swaine 1920, 1921).

A distinct ganglionic mass is associated with each of the first six abdominal segments, and the nerve root patterns correspond to those described for the thoracic ganglia, with two pairs of lateral trunks, and a single median nerve that arises from the dorsal and posterior region of the
ganglion. In some cases, the median nerve of the abdominal segment continues right to the following ganglion, and the transverse nerves arise directly from the anterior edge of the ganglion (Snodgrass 1935). Connectives between the abdominal ganglia are typically unpaired, but
have been noted to be separated to varying degrees in some species (Swaine 1921).

The terminal ganglionic masses, located in the seventh abdominal segment, comprise the seventh and eighth abdominal ganglia. Nerve roots arising from the seventh ganglion are arranged similarly to those of the first six segments. The eighth ganglion, representing the fusion of probably four primitive neuromeres (8–11), has three pairs of lateral nerves that emerge from the caudal region of the ganglion, and one median nerve. The nerve branches are elongated, and take a postero-folateral course to innervate the lateral segments of the caterpillar and the posterior end of the alimentary canal (Fig. 9.6A, C). The degree of fusion between the last two abdominal ganglia ranges from complete separation which is prevalent in (at least the early instars of) the examined microlepidoptera-grade taxa (Swaine 1920, 1921; Nordman 1967; Friese 1970), to complete fusion as in Papilio (Hilleman 1933; Swaine 1921), while in other forms (e.g. Manduca), the two are separated by an obvious constriction (Eaton 1988). In several cases the ganglia are discrete in early instars but fuse later in larval life. Closely related taxa may differ in this respect; this is, for instance, the case with congeneric species of the lyconid genus Leucoptera (Nordman 1967) as well as with some neighbouring genera of nymphaline butterflies (Newport 1832, 1834; Ingles 1928).

Descriptions of the larval stomatogastric system are provided by Peterson (1912), Swaine (1920), Hilleman (1933), Kuwanan (1935), Snodgrass (1935), Bickley (1942), Drektrah et al. (1966), Edwards (1968), Eaton (1988), Coppenhaver & Taghert (1989), Žitnah et al. (1989), and Miles & Booker (1994). As for the adult, the frontal ganglion lies anterior to the brain, connecting to the latter by paired frontal connectives. The larval frontal ganglion is necessary for peristaltic movements of the gut. In fifth instar Manduca larvae (Coppenhaver & Taghert 1991) and Galleria mellonella (Žitnah et al. 1989), and late embryonic Neomicropteryx nippokensis (Kobayashi & Ando 1983), a distinct hypocerebral ganglion lies just caudal to the frontal ganglion (Fig. 9.6C, D). The recurrent nerve continues posteriorly along the dorsal surface of the foregut, giving off several fine branches to the pharynx and oesophagus. It then terminates in a fine network of nerves, named the enteric plexus in Manduca (Coppenhaver & Taghert 1989), which innervates the musculature of the midgut (Fig. 9.6C, D). As observed in the adult condition, a considerable amount of variability appears to exist in the number, size, and position of the small ganglia associated with the stomatogastric system in different species.

Metamorphosis

As for other holometabolous insects, the nervous system of Lepidoptera undergoes a number of notable changes during metamorphosis (Levine 1986). Gross anatomical changes, whereby the central ganglia change their sizes and positions, and may consolidate to form the adult nerve cord, have been described by Newport (1832, 1834) for Sphinx ligustri, Brandt (1879) for Pieris and Aglais; Ingles (1928) for Aglais antiopa, Ashhurst & Richards (1964) for Galleria mellonella, Chathani & Callahan (1967b) for Heliothis zeas, Singh & Srivastava (1973) for Philosamia ricini. Ali (1973) and Tsujimura (1983) for Pieris sp., and Amos & Mesce (1994) for Manduca sexta. Following is a brief overview of the gross anatomical transformations of the brain and ventral nerve cord. The metamorphic changes associated with the central architecture is discussed later in this chapter.

Overall, the gross anatomical changes to the lepidopteran CNS in 'typical' higher Lepidoptera include a marked expansion of the brain, posterior tilt as the deutocerebrum grows and moves upward, fusion of the suboesophageal ganglion with the tritocerebrum, and the consolidation of the thoracic ganglia (from 3 to 2), and abdominal ganglia (from 7 to 4). Reorganization of the central nervous system generally begins within hours prior to or following pupation, and proceeds rapidly thereafter. In Manduca the pattern of ganglionic fusion and the sequence of events generally occurs in a stereotyped pattern (Fig. 9.7, Amos & Mesce 1994). An overall shortening of the nerve cord is already evident several hours prior to pupal ecdysis. By four hours following ecdisis, the suboesophageal ganglion appears twice its original size, and the paired cervicothoracic connectives are drawn closer together. These events are associated with the elongation and enlargement of the surrounding ganglionic sheath, which presumably facilitates the fusion between neural elements. At 24 hours further shortening of the suboesophageal connectives occurs, and the 3 ganglia (A 1, 2 and T 3) that eventually form the posterior lobe of the pterothoracic ganglion begin to merge. The neuronal cell bodies and neuropil of A 1 and A 2 migrate anteriorly to join T 3, leaving their original ganglionic sheaths vacant. In the adult condition, the original nerve roots from A 1 & 2 remain in their original position, now leaving the thoracic abdominal connective. By the end of 3 days following pupal ecdisis these ganglia have become 95% fused. By five days T 2 begins to migrate toward T 3, and the last 2 abdominal ganglia (A 6 and A 7 + 8) have merged by 84%. By 6 days the ganglia are more or less recognizable in their adult form, although further minor changes occur between this time and eclosion. The final size of the pterothoracic ganglion is larger than all other ganglia, but smaller than the 4 ganglia that form it put together. The sequence of events and/or pattern of ganglionic translocation may differ between taxonomic groups (cf. Ali 1973; Singh & Srivastava 1973).
Functional Organization of the Brain

Overviews of the internal organization of the lepidopteran brain exist for a variety of species, but a cellular analysis of particular brain areas has been performed only on selected species, most notably Manduca sexta, Antheraea polyphemus, Bombyx mori, Papilio spec., and Sphinx ligustri. In the brain as well as the ventral nerve cord, neuronal cell bodies form a peripheral cortex around central neuropil, which consists of synaptic areas and fiber tracts and lacks neuronal cell bodies (Fig. 9.8). The three divisions of the brain, the proto-, deuto- and tritocerebrum are largely fused and, particularly the extension of the small tritocerebrum can be estimated only by the central branching areas of the tritocerebral nerves. The protocerebrum, by far the largest division of the brain, is highly compartmentalized (Fig. 9.8). The optic lobe consists of four neuropils, the lamina, medulla, lobula, and lobula plate. In the central part of protocerebrum, the mushroom bodies and the central complex are the two most conspicuous neuropil areas (Fig. 9.8). The paired mushroom bodies are composed of calyces, pedunculus, and several lobes. The central complex is a group of neuropils in the center of the brain encompassing the posterior protocerebral bridge and the central body (Fig. 9.8). The mushroom bodies and the central complex are embedded in surrounding protocerebral neuropils, which provide connections with sensory areas (optic and antennal lobe), efferent neurons (neurosecretory cells, antennal motor neurons), de- and ascending pathways. The deutocerebrum consists of the antennal lobe, the primary brain center for processing olfactory information, and the ventrally adjacent antennal mechanosensory and motor center involved in the control of antennal movements (Fig. 9.10). The nearly 180° dorso-posterior tilt of the neuraxis of the brain is readily reflected internally. Thus, the calyces of the mushroom bodies point backwards, the pedunculi run nearly horizontally, and the antennal lobes occupy a fronto-dorsal instead of a fronto-ventral position in the brain (Fig. 9.8). This brain tilt does not extend to the optic lobes, which maintain their usual orientation with the two optic chiasms restricted to the horizontal plane (Fig. 9.8). The following review of the central nervous system is organized primarily with respect to functional aspects rather than adhering to the structural organization of the brain and ventral nerve cord.
Visual System

Four distinct visual systems have been described in Lepidoptera, each originating from different anlagen and serving different and distinct roles: (i) the compound eyes-optic lobes essential for visual image formation and processing, (ii) a pair of dorsal ocelli, (iii) abdominal photoreceptor organs involved in copulation of certain species, and (iv) larval stemmatal photoreceptor organs.

Optic Lobes. Corresponding to the highly developed compound eye, the optic lobes of Lepidoptera are conspicuous and particularly large in diurnal species with apposition eyes (Rhopalocera-type brain) (Bretscheider 1924 b; Ehnbom 1948). The optic lobes are composed of the lamina, the medulla/accessory medulla, and the lobular complex (from distal to proximal, Fig. 9.8). The three neuropils are connected via columnar neurons forming chiasmatib fiber crossings in the horizontal plane (Fig. 9.9). As in Diptera, Coloeoptera and Trichoptera, the lobular complex consists of two distinct neuropil masses, the lobula and the lobula plate (Bretscheider 1924 b; Strausfeld & Blest 1970; Homberg et al. 1987; Homberg & Hildebrand 1989 b; Ichikawa 1994 a, b). Of the nine photoreceptor cells comprising each ommatidium, six terminate in the lamina and three in the medulla (Nowikoff 1931; Strausfeld & Blest 1970; Gordon 1985; Ribi 1987; Shimongiashi & Tominaga 1991, 1999). The organization of the optic lobe beyond the photoreceptor level, studied in Manduca, Sphinx, Papilio, Parnara, and Pieris reveals a variety of neuronal cell types including up to five types of lamina monopolar cells (Gordon 1985; Ribi 1987; Shimongiashi & Tominaga 1999), a large number of other columnar neurons (Fig. 9.9; Strausfeld & Blest 1970), tangential neurons with projections into the median protocerebrum, bilaterally projecting neurons connecting the medullae and/or lobulae, and centrifugal elements (Strausfeld & Blest 1970; Homberg et al. 1987, 1990; Homberg & Hildebrand 1989; Ibottson et al. 1991; Milde 1993; Ichikawa 1994 a, c; Wickel & Strausfeld 2000). Immunocytochemical studies in Manduca suggest that γ-aminobutyric acid (GABA) is a prominent neurotransmitter in the visual system (e.g. in the homologs of the dipteran C2 transmedullary neurons and in CH centrifugal neurons of the lobula plate (Homberg et al. 1987)), while serotonin, histamine, and the
neuropeptide FMRFamide may be additional neuromediators of centrifugal neurons (Homburg & Hildebrand 1989a, b, 1991; Homburg et al. 1990). Histamine, as in all other arthropods, appears to be the neurotransmitter of photoreceptor neurons (Homburg 1994).

Physiological studies have investigated central mechanisms of color vision, visual field properties, binocular interaction, movement detection, and distance perception. Evidence for color vision both in moths and in butterflies is supported by the demonstration of three to five different spectral classes of photoreceptor cells (see 2–12), and by recent behavioral learning experiments (Kelber & Pfaff 1999; Kelber & Hénique 1999; Kinoshita et al. 1999). Color-specific responses in unidentified interneurons, including color-opponent neurons, narrow and broad band chromatic responses as well as more complex chromatic properties were demonstrated in a variety of butterflies by Swihart (1969, 1970, 1972a,b) and Schürmperli (1975). Single cell recordings in moths (Manduca, Sphinx, Noctua) and butterflies (Papilio) revealed that neurons tuned to self motion (directionally selective responses to large field motion) are already present at the level of the distal medulla (Collett 1970, 1971b; Ibbottson et al. 1991; Maddess et al. 1991; Milde 1993; Ichikawa 1994a). Recordings in the hawkmoth Macroglossum stellatarum (Wicklein & Varju 1999) demonstrated large-field directionally selective motion-sensitive neurons in the lobula plate and showed that the lobula plate may be organized into directionally specific layers similar to the organization in Diptera (reviewed by Hausen 1993). Many of the recorded neurons were centrifugal elements with cell bodies in the midbrain and axonal projections to the medulla. Recordings from a variety of butterflies suggest that, as in flies, motion-vision is colour-insensitive and is driven mainly by green or blue photoreceptors (Horridge et al. 1984; Singarajah 1988). Tuning to object motion (directionally-selective responses to small-field stimuli) were characterized by Collett (1971a, 1972) and found in recordings from the anterior optic tract of Sphinx. Recordings in hawk moths (Manduca, Sphinx) revealed looming-sensitive interneurons with selective responses to approaching or receding stimuli (Collett 1972; Wicklein & Strausfeld 2000). These units are thought to be involved in distance perception during hovering flights while feeding on flowers.

Ocellar system. Lepidoptera possess a pair of dorsal ocelli (see 2–12), which in some families, e.g. sphingids, are reduced and in part internalized (Eaton 1971; Dickens & Eaton 1973, 1974). Unlike in most other insects (reviewed by Goodman 1981; Mizunami 1994, 1999), ocellar receptor fibers studied in Trichophasia and Manduca do not make synapses with second order neurons in a peripheral ocellar plexus, but send axonal fibers into the dorsal protocerebrum (Eaton &
Pappas 1977; Pappas & Eaton 1977; Homberg & Hildebrand 1991). In *Trichophasia*, as in many other insects, large ocellar interneurons project to the posterior proctocerebrum (Pappas & Eaton 1977), but in *Manduca*, they are completely missing (Homberg & Hildebrand 1991). Evidence from *Manduca* suggests that ocellar photoreceptors as in other insects are histaminergic (Homberg & Hildebrand 1991). Ablation of ocelli in *Trichophasia* led to delayed flight initiation at dusk, suggesting that the ocelli are important for detecting changes in illumination for the diurnal control of motor activities (Sprint & Eaton 1987).

**Larval Visual System.** Lepidoptera larvae have a pair of simple larval eye clusters, usually comprising six ommatidial stemmata (reviewed by Toh & Tateda 1991; Gilbert 1994; Ichikawa 1999; see 2–12). In *Papilio*, each stemma consists of seven retinula cells, comprising green, blue and UV-sensitive cells; three to four of these send axonal processes to the larval lamina and the remainder, to the larval medulla (Ichikawa & Tateda 1984). Electrophysiological recordings from visual interneurons in the larval medulla of *Papilio* revealed an antagonistic center-surround organization of visual fields (Ichikawa 1986, 1992), and a variety of chromatic responses including color opponency (Ichikawa 1986, 1990, 1991a; reviewed by Gilbert 1994; Ichikawa 1999).

**Metamorphosis of the Visual System.** In Lepidoptera as in other holometabolous insects, the adult compound eye develops from an epidermal eye imaginal disc during metamorphosis, while the adult optic lobe originates from two aggregates of neuroblasts, the optic lobe anlagen (Schrader 1938; Panov 1960; Nordlander & Edwards 1969b; Meinertz-Hagen 1973). The outer optic lobe anlage gives rise to neurons of the lamina and medulla and the inner optic anlage, to neurons of the lobula (Nordlander & Edwards 1969b; Monsma & Booker 1996a). Recent work in *Manduca* showed that proliferation of neuronal precursors in the optic lobe is closely controlled by juvenile hormone, ecdysteroids, and by local production of nitric oxide (Monsma & Booker 1996b; Champlin & Truman 2000). While the stemmata and larval visual system in the trichopteran caddisfly are retained into adulthood (Hagberg 1986) considerable degeneration takes place in Lepidoptera. The external structure of the larval eyes of moths and butterflies disappears at pupation but photoreceptors remain as pigmented remnants at the posterior edge of the optic lobe (Sánchez & Sánchez 1926; Nordlander & Edwards 1968, 1969b; Homberg & Hildebrand 1989a; Ichikawa 1991b, 1994b). Work in *Papilio* showed that these photoreceptors remain photosensitive in the adult (Ichikawa 1991b). The larval medulla is transformed into the accessory medulla of the adult optic lobe (Homberg & Hildebrand 1994; Ichikawa 1994b), but some of the larval visual interneurons, immunostained with an antiserum against γ-aminobutyric acid, grow new processes and become tangential neurons of the medulla (Homberg & Hildebrand 1994; Ichikawa 1994b).

**Chemo- and Mechanosensory Systems**

**Antennal Lobe.** The *olfactory system* has been particularly well studied in a number of Bombycoidea (*Antheraea, Bombyx, Lymantria, Manduca* and Noctuoidea *Agris, Heliothis, Helicoverpa, Spodoptera, Trichophasia*), with very limited comparative data from other families (Han- son 1995, 1999). The primary olfactory brain area, the antennal lobe, receives direct input from olfactory receptor neurons of the antenna as well as from CO$_2$ receptors on the labial palps (see 2–10). Olfactory receptor neurons make contacts with second-order neurons in spheroidal neuropill structures, termed *antennal-lobe glomeruli* (Homberg et al. 1989; Anton & Homberg 1999). In all Lepidoptera studied, the glomeruli form a peripheral layer around a central coarse neuropil of the antennal lobe. The number, size, and arrangement of glomeruli is species-specific: 66 glomeruli have been mapped in male *Manduca* (Rospars & Hildebrand 1992); 67 in *Manduca brassicae*, 62 in *Pieris brassicae* (Rospars 1983), and 55–60 in *Bombyx mori* (Koontz & Schneider 1987). In addition to olfactory receptor neurons, three types of interneurons arborize in the antennal lobe (Fig. 9.10): (i) local interneurons interconnect many glomeruli, but have no axonal process; (ii) different types of projection neurons arborize in one glomerulus, a few glomeruli, or all glomeruli and send axonal projections to various brain areas, most prominently the mushroom body, the inferior protocerebrum and the lateral horn of the brain; and (iii) a small number of centrifugal neurons have dendritic processes outside the antennal lobe and send axonal projections into one or both antennal lobes (Homberg et al. 1989; Anton & Homberg 1999). While local and projection neurons have cell bodies in well-defined cell clusters of the antennal lobe, the cell bodies of most centrifugal neurons lie outside the antennal lobe (Fig. 9.10; Homberg et al. 1989; Anton & Homberg 1999).

Owing to the importance of pheromone perception for mate finding, the olfactory system shows a prominent sexual dimorphism in moths (Anton & Homberg 1999) but apparently not in the butterfly *Pieris* (Rospars 1983). Sex pheromones, mixtures of aliphatic alcohols, aldehydes, or acetates, have been identified in more than 400 species of Lepidoptera (Arn et al. 1992, 1999). Sex pheromones as long-distance attract-
Fig. 9.10. Neuronal cell types in the antennal lobe of Manduca sexta. A. Frontal and B. horizontal diagram of the brain. AC: anterior cluster of antennal-lobe neurons; aL: a-lobe of the mushroom body; AL: antennal lobe; AMMC: antennal mechanosensory and motor center; AN: antennal nerve; bL: b-lobe of the mushroom body; Ca: calyces of the mushroom body; CF: centrifugal neuron; ILP: IMP: inferior lateral, inferior median protocerebrum; LCI, LII: lateral cluster I and II of antennal-lobe neurons; LR: lateral horn of the protocerebrum; L/MGC: local neuron innervating the macrogglomerular complex; LPO: afferents from the labial palp organs; LPOG: glomeruli innervated by afferents from the labial palp organs; MC: medial cluster of antennal-lobe neurons; MGC: macrogglomerular complex; MR: mechanosensory receptor fiber; Oe: oesophageal foramen; OR: olfactory receptor fiber; P: pedunculus of the mushroom body; Pla(G), Pla(MGC): type a projection neuron of the inner antennio-cerebral tract innervating an ordinary glomerulus; the macrogglomerular complex (MGC). PM: projection neuron of the inner antennio-cerebral tract; PO a.c: types a and c projection neurons of the outer antennio-cerebral tract. Scales = 100 µm. After Homberg et al. (1989).

In this context, antennal receptors are usually emitted by females. They are detected by males through a large number of pheromone-specific olfactory receptor neurons (Hansson 1995; Anton & Homberg 1999). Pheromone receptor fibers project into a specific set of male antennal-lobe glomeruli, termed the macrogglomerular complex. In bombycoids, the macrogglomerular complex is a tri- to tetrapartite structure consisting of a “cumulus,” and two to three “toroids” (Bretschneider 1924 a, b; Koontz & Schneider 1987; Homberg et al. 1995; Heimbuck & Hildebrand 1998). In noctuids and pyralids, in contrast, the macrogglomerular complex is composed of a cumulus plus two to six spheroidal satellite glomeruli (reviewed by Anton & Homberg 1999). Female-specific glomeruli have been found in Manduca, Bombyx, and Mamestra (Rospars 1983; Koontz & Schneider 1987; Rößler et al. 1998; Rospars & Hildebrand 2000), and recent work in Manduca showed that neurons in one of these glomeruli respond particularly well to the common plant odor linalool which is also a component of a male pheromone in another moth, Trichoplusia ni (King et al. 2000).

Physiological studies, largely on bombycoid, noctuid and pyralid species have focused on the processing of sex pheromones in the antennal lobe (reviewed by Hildebrand 1996; Mustaparta 1996; Hansson & Christensen 1999). Work in Manduca, Trichoplusia, Spodoptera, Heliothis, and Agrotis revealed an odotopic organization of the macrogglomerular complex, i.e. each subunit of the macrogglomerular complex receives input from receptors tuned to a specific component of the pheromone blend (Hansson et al. 1991; Mustaparta 1996; Vickers et al. 1998; Hansson & Christensen 1999; Galizia et al. 2000). Projection neurons with ramifications in single macrogglomerular-complex glomeruli, therefore, respond to single pheromone components, while neurons with ramifications in several macrogglomerular-complex glomeruli respond to a specific blend of pheromone (Hansson et al. 1991; Mustaparta
Antennal Mechanosensory and Motor System. Mechanoreceptor fibers from the flagellum and from Johnston’s organ and Böhm bristles (see 2–10) on the basal segments of the antenna bypass the antennal lobe and enter the second sub-division of the deutocerebrum, the antennal mechanosensory and motor center (AMMC), also termed “dorsal lobe” or “posterior antennal center” (Fig. 9.10; Koontz & Schneider 1987; reviewed by Homberg et al. 1989). Flagellar mechanoreceptors in *Bombus*, *Lymnantria* and *Antheraea* terminate in the AMMC (Koontz & Schneider 1987), similar to most antennal mechanoreceptors in *Manduca*, but certain fibers in *Manduca* continue to the suboesophageal ganglion or to the ventral nerve cord (Hildebrand et al. 1980). Mechanoreceptors in *Manduca* stain heavily for acetylcholinesterase, and thus, may be cholinergic (Stengel et al. 1990; Homberg et al. 1995). The intrinsic and extrinsic muscles of the antenna of *Manduca* are innervated by at least 12 motoneurons (Kloppenburg et al. 1997). Their dendritic fields are confined to the AMMC with extensive overlap with the projections of antennal mechanoreceptors, suggesting that local circuits are involved in the control of antennal movements.

Larval Antennal Center. Larval antennae are minute and bear no resemblance to their counterparts in the adult (reviewed by Zacharuck & Shields 1991; Keil 1999). The physiology and ultrastructure of larval antennal sensilla have been studied in a few species (e.g. *Bombus*: Morita & Yamashita 1961; Ishikawa et al. 1969). In *Manduca*, receptor fibers enter a small larval antennal center which is substructured into noduli. Small numbers of projection neurons, local neurons, and a single serotonin-immunostained neuron, which persists into adulthood, have been found (Homberg & Hildebrand 1994; Kent & Hildebrand 1987; Kent et al. 1987; Itagaki & Hildebrand 1990). Physiological recordings in *Manduca* revealed broad tuning of olfactory interneurons and integration of olfactory and mechanosensory stimuli at early stages of central processing (Itagaki & Hildebrand 1990).

Development of the Antennal Lobe. The development of the adult antennal lobe has been studied in *Ephesia* (Schrader 1938), *Danaus* (Nordlander & Edwards 1970), and in a series of more recent studies on *Manduca* by Tolbert, Hildebrand and coworkers (reviewed by Oland & Tolbert 1996; Hildebrand et al. 1997; Salecker & Malun 1999). During larval stages, a few neuroblasts (5 in *Manduca*) give rise to adult-specific neurons which grow processes into the larval antennal center (Nordlander & Edwards 1970; Sorensen 1993). Mitotic activity ceases soon after pupation. The antennal neuropil loses its nodular appearance around pupation (Schrader 1938;
Nordlander & Edwards 1970) and, in *Manduca*, GABA immunostaining disappears from the neuropil (Homberg & Hildebrand 1994). Formation of glomeruli in *Manduca*, which starts at about 30% of metamorphic development, depends critically on ingrowing receptor fibers from the antenna (Schneiderman et al. 1982; Tolbert & Siriani 1990; Kössler et al. 1999). A subsequent envelope of the developing protoglomeruli by glial cells, which proliferate in response to the developmental hormone 20-hydroxyecdysone, is another essential factor for glomeruli formation and stabilization (Oland et al. 1988, 1998; Tolbert & Oland 1990; Oland & Tolbert 1996; Hildebrand et al. 1997; Salecker & Malun 1999). Synapse formation and fine tuning largely occurs during the second half of metamorphosis (Oland & Tolbert 1996).

Functional Organization of the Protocerebrum

The protocerebrum can be regarded as the principal association center of the nervous system integrating sensory information, particularly from the antennae and compound eyes, coordinating motor output through descending neurons, and controlling the endocrine and stomatogastric systems. While most brain areas have not been studied in any detail, some attention has been given to the arrangement, branching patterns and functional roles of neurosecretory cells in the superior protocerebrum (see 2–15), central pathways and mechanisms of olfactory and visual integration, organization of the mushroom body and central complex, the localization of the internal circadian clock, and the structure and physiology of descending pathways connecting the brain to the ventral nervous system. The distribution of neurotransmitter candidates, including GABA, serotonin, histamine, and peptides related to FMRFamide has been mapped in *Manduca sexta* (Homberg et al. 1987, 1990; Homberg & Hildebrand 1989a, 1991). Prominent visual projections from the optic lobes and ocelli extend to the posterior brain, overlapping with ramifications of descending neurons, and to the anterior optic tubercle (Pappas & Eaton...
1977; Homberg et al. 1987; Ibbotson et al. 1991; Ichikawa 1994a, c; Milde 1993; Wicklein & Varju 1999). Jawaowski (1963) noted that the anterior optic tubercle of Lepidoptera, particularly in nocturnal species, is large. As in Trichoptera (Ehnborn 1948), it is divided into two main parts, one of which has a striking glomerular appearance (Jawowski 1963). Olfactory projections from the antennal lobe innervate the mushroom bodies (see below), and then terminate in the lateral horn of the protocerebrum (Kanzaki & Shibuya 1986a; Homberg et al. 1988; Christensen et al. 1991; Anton & Hansson 1994; Hansson et al. 1994; Anton et al. 1997), and the inferior median protocerebrum (Homberg et al. 1988; Wu et al. 1996). In Manduca, projection neurons from ordinary glomeruli and from the macroglomerular complex have topographically distinct projections in the lateral brain (Homberg et al. 1988, 1989; Kanzaki et al. 1989). Another brain area involved in olfactory processing is the lateral accessory lobe. In Bombyx and Manduca bilateral neurons connecting both lateral accessory lobes show excitatory responses to pheromone that outlast the stimulus by up to several seconds (Kanzaki et al. 1991a; Kanzaki & Shibuya 1992; reviewed by de Belle & Kanzaki 1999). The lateral accessory lobes are, furthermore, involved in the production of characteristic flip-flop activity observed in certain descending neurons (reviewed by Kanzaki 1996).

Mushroom Bodies. In comparison to Trichoptera (Ehnborn 1948), the mushroom bodies of Lepidoptera appear more elaborate (Buxton 1917; Bretschneider 1924a, b; Pearson 1971). Pearson (1971) provided the most detailed study on Sphinx in comparison with several other species. In Micropterix calthella, the calyces have been described as a single globular mass (Buxton 1917) similar to their appearance in Trichoptera (Ehnborn 1948). In higher bombycoids, noctuids, and butterflies, the calyces consist of a double cup-shaped neuropil with the walls of the two adjacent cups fused (Figs. 9.8, 9.11; Bretschneider 1924a, b; Jawowski 1963; Pearson 1971; Homberg et al. 1988). The anteriorly running stalk (pedunculus) divides into three lobes, which have been termed by Pearson (1971) as α-, β-, and γ-lobe. A second lobe system, apparently peculiar to Lepidoptera has been noted by Bretschneider (1924b) and described in detail by Pearson (1971). A second small stalk, termed Y-tract (or “secondary pedunculus”), originates from the calyces and forms a Y-shaped lobe (Y-lobe) in the notch between α- and β-γ-lobe (Fig. 9.11). If Buxton's (1917) “ascending branch/trunk” of the mushroom body of Micropterix refers to the α-lobe, and the “inner root” to the parallel β-γ-lobes, then the “posterior branch” might be the Y-lobe/tract, which contacts the α-lobe slightly more dorsally than in the more advanced species. In the butterfly Pieris, Pearson (1971) did not find a Y-lobe/tract, suggesting that they have fused with the pedunculus and lobes of the mushroom body (Pearson 1971). Golgi preparations in Sphinx, Pieris and other species revealed a variety of extrinsic and intrinsic neuronal cell types (Pearson 1971: Figs. 9.11 C, D). The intrinsic Kenyon-cells have extensive dendritic processes, often covering the whole calyx area, and axonal fibers with small side branches in the lobes (Pearson 1971: Fig. 9.11 C). Extrinsic input fibers enter the calyces via several tracts from the antennal lobe and from other brain areas (Pearson 1971; Homberg et al. 1988: Figs. 9.10 B, 9.11 D). In Manduca, a group of fibers in the protocerebro-calyceal tract connects the α-, β-, and γ-lobes back to the calyx area; the neurons are GABA-immunoreactive and, thus, might provide a negative feedback loop within the mushroom bodies (Homberg et al. 1987). Similar connections have also been described in the honeybee (Bicker et al. 1985). The prominent connections of the mushroom bodies with the antennal lobe argue, as in other insects, for a key role in olfactory processing. Intracellular recordings from a few mushroom body output neurons in Manduca, correspondingly, showed excitatory responses to pheromone (Kanzaki et al. 1991a).

Central Complex. The central complex consists of the central body and the protocerebral bridge (Fig. 9.8). Both structures are well developed in Lepidoptera (Buxton 1917; Homberg et al. 1987; Homberg & Hildebrand 1991), but no specific features have been noted. The central body is divided into two major subunits commonly called upper and lower division of the central body (Fig. 9.8 C). The former has been further subdivided in Manduca into fan-shaped body and superior arch (Homberg et al. 1987, 1990; Homberg & Hildebrand 1989 a). Prominent connections to the lateral accessory lobes and, via the anterior bundles, to the superior protocerebrum are already evident in Micropterix (tracts m and mm in Buxton 1917). Prominent GABA-immunostained tangential fibers connect the upper and lower division of the central body of Manduca to the lateral accessory lobes (Homberg et al. 1987); similar fibers have been found in all insects studied so far (e.g. Homberg et al. 1999). Additional transmitter candidates mapped immunocytochemically in Manduca include serotonin and histamine in tangential neurons and FMRFamide-related peptides in columnar neurons of the central complex (Homberg & Hildebrand 1989 a, 1991; Homberg et al. 1990).

Circadian Clock. Circadian rhythms have been studied in a variety of Lepidopteran species (reviewed by Page 1985), but their neural bases have only been investigated in two saturniids. Transplantation experiments showed that the
circadian pacemaker controlling rhythmic flight activity and the gating of adult eclosion in Antherea pernyi and Hyalophora cecropia resides in the central brain (Truman 1972, 1974; Truman & Riddiford 1970, Fig. 9.12). Synchronization of animals with transplanted midbrains to light-dark rhythms suggests that entrainment of the circadian system occurs through extraretinal photoreceptors in the central brain (Truman & Riddiford 1970). A similar location of the circadian clock has been suggested for some Diptera, but work in Drosophila and most other insect groups provided evidence for neurons associated with the accessory medulla in the optic lobe as pacemaker neurons (reviewed by Helfrich-Förster et al. 1998). A homologue of the Drosophila period gene, an essential molecular element of the circadian clock, was recently cloned from A. pernyi heads (Reppert et al. 1994). Both the PER-protein and another molecular clock marker, TIM, showed a daily rhythm in abundance in four pairs of lateral neurosecretory cells, suggesting that these neurons are circadian clock neurons (Sauman & Reppert 1996).

**Descending Neurons.** Intersegmental interneurons which connect the brain to the ventral nerve cord have been studied in relation to optomotor responses and pheromone-guided flight behavior. In the butterfly Heteronympha, recordings from the cervical connective revealed neurons with directional responses to small moving objects (Sun 1993), but not to background motion. In contrast, neurons affected by wide-field image motion were characterized in the cervical connective of Manduca (Rind 1983b) and Macroglossum (Kern 1998). In Manduca an identified movement detector neuron descending from the brain to the thoracic ganglia was sensitive to yawing movements (Rind 1983b). The neuron provided subthreshold input to ipsilateral flight motoneurons involved in yaw optomotor responses (Rind 1983c).

Descending neurons responding to pheromone have been studied in Manduca and Bombyx (Olberg 1983; Kanzaki et al. 1991b, 1994; Kanzaki & Shibuya 1986b; Mishima & Kanzaki 1999). Neurons with ramifications in the lateral accessory lobes show a peculiar response pattern termed “flip-flopping” (reviewed by Kanzaki 1996). The neurons have a high and a low frequency firing state and, in response to sequential pheromonal stimulation, switch back and forth between these two states. The flip-flop activity is correlated with and may underlie the self-generated upwind zigzagging approach of male moths while tracking plumes of female sex pheromone (Olberg 1983; Kanzaki & Mishima 1996; Mishima & Kanzaki 1998, 1999). Some descending neurons in Manduca, Bombyx and Lymantria integrate several sensory modalities including pheromone, optomotor and mechanosensory information (Olberg 1983; Olberg & Willis 1990; Kanzaki et al. 1991b).

**Larval and Pupal Brain.** Brain development has been studied in butterflies (Hanström 1925; Nordlander & Edwards 1968, 1970), bombycoids...
(Panov 1959; Granger et al. 1989; Homberg & Hildebrand 1994), and the pyraloid *Ephesia* (Schraer 1938). The mushroom bodies are readily discernable in newly hatched larvae, but the Y-lobes and Y-tract have not been described. In *Manduca*, GABA-immunoreactive neurons of the protocerebro-calycetal tract have not been seen in the larva (Homberg & Hildebrand 1994). The protocerebral bridge is present from the first larval instar, but the central body is greatly reduced. It has been reported absent in caterpillars of *Pieris* and in the first two larval instars of *Danaus* (Hansström 1925; Nordlander & Edwards 1970). In *Antheraea* and *Manduca*, and apparently *Ephesia*, however, a small upper division of the central body is present (Schraer 1938; Panov 1959; Homberg & Hildebrand 1994). During larval life, the brain grows in size owing to constant division of neuroblasts and in-growth of fibers from newly born neurons into the neuropil (Schraer 1938; Nordlander & Edwards 1969a, 1970). These postembryonic neurons, however, do not become functionally mature in the larva and accordingly, the distribution and number of neurons immunostained for the neurotransmitters GABA and serotonin remain constant during larval development (Granger et al. 1989; Homberg & Hildebrand 1994). Major changes in brain organization occur during metamorphosis, including in-growth of fibers into the midbrain from the optic and antennal lobes, as well as transient and permanent changes in transmitter phenotype. In *Manduca*, neurons of the brain transiently express nitric-oxide dependent cyclic guanosine monophosphate (Schachtner et al. 1998). Major groups of GABA-immunostained neurons are first visible midway through metamorphosis including tangential neurons of the lower division of the central body (Homberg & Hildebrand 1994).

Internal Organization and Metamorphic Changes

Segment specific differences in the organization of the ventral nervous system are already present in the larva but become more pronounced during metamorphosis. Studies by Panov (1963) on *Antheraea* and Heywood (1965) on *Pieris* have produced some evidence for postembryonic neurogenesis in the ventral nervous system but a more detailed study exists only for *Manduca*. From the larva to the adult, the number of neurons in the first thoracic ganglion increases about 3-fold, but in the unfused abdominal ganglia, neuronal cell numbers decrease by more than 50% (Booker & Truman 1987; Booker et al. 1996; Truman 1996). In addition to differentiated neurons, the larval ganglia contain a segment-specific number of neuroblasts, which produce nests of imaginal ganglion cells during larval life. At the onset of metamorphosis, certain larval neurons and some imaginal nest cells die and a second wave of neuronal cell death, particularly in segmental abdominal ganglia, occurs after adult eclosion (Truman 1983; Truman & Schwartz 1984). Surviving imaginal nest cells differentiate and are incorporated into the adult ventral nervous system. Expression of transmitter phenotype (GABA, peptides related to small cardioactive peptide) in postembryonic neurons is lineage dependent and segment-specific (Witten & Truman 1991a, b, 1998). In contrast to interneurons, apparently all motoneurons are born embryonically (reviewed by Levine 1986, 1987). These include pairs of peripherally projecting ventral median neurons in the abdominal ganglia which have also been described in *Antheraea* (Brookes & Weevens 1988; Pflüger et al. 1993). Certain motoneurons degenerate at the start of metamorphosis or after adult ecdysis, but others are modified for the innervation of adult musculature (Fig. 9.13; reviewed by Levine 1987; Kent & Levine 1988 b). During metamorphosis, sex-specific differences develop in the terminal abdominal ganglia due to sexual differences in neuroblast number and progeny (Booker & Truman 1987; Thorn & Truman 1994a), sex-specific cell death (Giebultowicz & Truman 1984; Thorn & Truman 1994 b) and sexual dimorphic remodelling of larval motoneurons (Thorn & Truman 1989). The control of developmental events in the ventral nervous system through the hormone 20-hydroxyecdysone has been studied in detail (reviewed by Weeks & Truman 1986; Levine et al. 1995; Truman 1996; see 2–15). A neuroanatomical atlas of the adult thoracic ganglia of *Manduca* (Suder et al. 1992; Suder 1994) revealed many similarities to the organization of thoracic ganglia in the locust (Tyrer & Gregory 1982).

Ventral Nervous System

Neural circuits for segmental reflexes and patterned motor activity such as walking, flight, feeding, breathing, calling, and mating reside primarily in the ventral nerve cord. *Manduca sexta* has become one of the most prominent model systems for studies on the neural mechanisms governing these behaviors and on the changes in neuronal circuits that underlie the reorganization of behavior during metamorphosis (reviewed by Truman & Schwartz 1982; Levine 1986; Levine & Weeks 1990; Weeks & Levine 1992; Tullitz et al. 1991: Weeks & Wood 1996; Truman 1992 b, 1996; Weeks 1999). Since comparative information from other species is severely limited, this section will draw mostly from data on *Manduca* concerning the organization and metamorphic changes of neural circuits in the ventral nervous system involved in a variety of sensory-motor programs.

Feeding

Insect mouthparts are controlled by the subesophageal ganglion (reviewed by Blaney & Sim-
monds 1987). Accordingly, larval taste, olfactory, and mechanosensory receptors on the maxillae and labial-hypopharyngeal complex of Manduca extend axonal projections primarily into the suboesophageal ganglion, but some fibers project to the brain or prothoracic ganglion (Kent & Hildebrand 1987; Griss 1990). The response profiles of taste receptors and their involvement in larval feeding behavior has been studied in Pieris, Mamestra, Manduca, Spodoptera, and a few other species (reviewed by Blaney & Simmonds 1987; Kent & Hildebrand 1987), but central mechanisms of foodplant recognition and discrimination are unexplored.

The neural basis of larval feeding behavior with particular emphasis on chewing and swallowing has only been studied in Manduca (Griss 1990; Griss et al. 1991; Rohrbacher 1994a, b; Miles & Booker 1994; Bowd & Wyse 2000). Mandibular muscles involved in chewing are innervated by 20 motoneurons, 12 motoneurons to the mandibular opener and 8 to the mandibular closer muscle (Griss 1990). Rhythmic chewing behavior is generated in the suboesophageal ganglion (Griss et al. 1991) and parts of the neural network associated with the central pattern generator circuit for chewing have been elucidated (Rohrbacher 1994a, b). Swallowing movements of the foregut are largely controlled by the frontal ganglion (Bell 1986; Miles & Booker 1994). It generates rhythmic patterns of squeeze movements and peristalsis of the foregut and contains all foregut muscle motoneurons (Miles & Booker 1994).

During metamorphosis, the feeding apparatus in Lepidoptera-Glossata is restructured from chewing mandibles and a simple foregut to a proboscis with a cibarial pump allowing nectar-feeding (Miles & Booker 1998). In a variety of moth and butterfly species, stimulation of the antennae, proboscis, or tarsi with sugar water elicits uncoiling of the proboscis and feeding (Friggs & Friggs 1956; Hartlieb 1996; Fan et al. 1997; Miles & Booker 1998). As in the honeybee, this proboscis extension reflex can be conditioned to odors (Hartlieb 1996; Fan et al. 1997), but the neural basis of this reflex and odor conditioning have not been studied. Proboscs movements are likely controlled by neurons of the suboesophageal ganglion (Pieris: Eastham & Eassa 1955), and work in Manduca showed that the frontal ganglion, as in the larva, contains the foregut motoneurons which drive the activity of the cibarial pump (Miles & Booker 1998).

Crawling – Walking

Larval locomotion (crawling) consists of waves of motor activity in body wall muscles in combination with sequential flexion and extension movements of the thoracic legs and abdominal prolegs, when present. Adults, in contrast, move their legs in a typical tripod gait for walking. Little attention has been given to the intersemmal coordination of locomotor behavior, but recent work in Manduca showed that application of the muscarinic agonist pilocarpine to isolated larval nerve cords induces motorneuron activity strikingly similar in timing and patterning to crawling motor activity in intact animals (Johnston & Levine 1996; Johnston et al. 1999). These experiments suggest that crawling is produced by a central pattern generating network in the nervous system.

Control of Thoracic Legs. Larvae of most Lepidoptera possess, on each of the three thoracic segments, a pair of segmented legs which are used for grasping movements and walking. In larvae of Manduca, leg motoneurons arborize in lateral leg neuropil and in a dorsomedial region of the corresponding thoracic ganglion (Kent & Levine 1988a). Mechanosensory hairs on the thoracic body and leg surface have somatotopic projections in the ganglion and, when stimulated, elicit reflexive leg movements which are apparently involved in grasping movements during crawling (Kent & Levine 1988a). While most leg sensory organs degenerate (Consoulas 2000), larval motoneurons persist during metamorphosis, but undergo considerable remodelling of their dendritic arborizations (Kent & Levine 1988b, 1993), peripheral processes (Consoulas et al. 1996; Consoulas & Levine 1998), as well as changes in membrane properties (Rose & Levine 2000) as they innervate new adult leg muscles.

Control of Larval Prolegs. Abdominal prolegs (2–5) in lepidopteran larvae are used for grasping and crawling. The neural circuitry for the control of proleg movements and its metamorphic fate have been studied for the prolegs on abdominal segments three through six of Manduca (reviewed by Weeks & Wood 1996). Stimulation of the planta hairs on the tip of each proleg evokes a proleg withdrawal reflex through segmentally organized mono- and polysynaptic connections with identified motoneurons to the two planta retractor muscles (Weeks & Jacobs 1987; Streichert & Weeks 1995; Sandstrom & Weeks 1991, 1996). Synaptic transmission from sensory to motor neurons occurs through nicotinic and muscarinic acetylcholine receptors (Trimmer & Weeks 1989, 1993; Trimmer 1994), and neural mechanisms underlying habituation and dishabitation of the reflex have been studied (Weeks & Wood 1996; Wiel & Weeks 1996; Wood et al. 1997). Corresponding to proleg degeneration late in larval life, the sensory neurons and motoneurons degenerate shortly after pupation (Weeks & Ernst-Uttschneider 1989). The accessory planta retractor motoneurons in segment A 3 and A 4, and their homologues in A 2, however, are respecified for pupal
specific functions, including control of hemolymph circulation in the developing wing, and die after adult emergence (Weeks & Erns-

Control of Abdominal Movements

Neuronal circuits underlying abdominal postural movements have been studied in Manduca sexta (reviewed by Levine 1987; Levine & Weeks 1990). Metamorphic changes in a stretch reflex have received particular attention (Fig. 9.13). In the larva, the reflex counteracts lateral flexions of the abdomen. The motor neuron MN-1 to the dorsal external oblique muscle (DEO 2) is excited by an abdominal stretch receptor ipsilateral to its target muscle and is inhibited by the contralateral receptor (Fig. 9.13; Levine & Truman 1982, 1985). After degeneration of DEO 2 during the pupal stage, MN-1 innervates the new dorsal external muscle (DE 4) of the adult. MN-1 acquires new dendritic arborizations in the ganglion and receives novel excitatory input from the contralateral stretch receptor (Fig. 9.13). These changes correspond to a shift in the function of MN-1 from an involvement in right/left movements in the larva to a role in dorsal/ventral movements in the adult (Levine 1987). Specific

metamorphic changes in arborizations and muscle innervations have also been studied for abdominal motoneurons to other muscles of the body wall (Levine & Truman 1985; Truman & Reiss 1988).

Slow directionally-specific abdominal flexion reactions in Manduca larvae are elicited by stimulation of mechanoreceptive hairs covering the abdominal surface (Levine et al. 1985; Waldrop & Levine 1989). The sensoryafferent projections (Levine et al. 1985) as well as the dendritic arborizations of the involved intersegmental muscle motoneurons in the ganglion are somatotopically organized (Fig. 9.14), which probably underlies the directionality of the response (Levine 1987). After pupation, this response is modified segment-specifically. In pupal segments A 5–7 it is modified to the pupal-gin trap reflex, a rapid bending of the abdomen toward the side of stimulation and closing of gin traps (Bate 1973a–c). Changes in the neuronal circuit underlying this modification as well as the multisegmental coordination of the motor response have been studied by Waldrop & Levine (1989, 1992) and Lemon & Levine (1997a, b).

Flight

Flight motor activity in Manduca is controlled by a central pattern generator network. In a

![Fig. 9.13. Metamorphic changes in dendritic arborizations and neuronal circuitry. A. During metamorphosis, the abdominal motoneuron MN-1 of Manduca sexta grows a new dendritic tree contralateral to its axon. B. In the larva, MN-1 is inhibited indirectly by a stretch receptor contralateral to its target. Top: single stimuli to the stretch receptor cause an inhibitory postsynaptic potential, while a burst of stimuli causes prolonged inhibition (bottom). In the adult, however, the same stretch receptor excites the motor neuron through monosynaptic connections that form upon the newly acquired dendrites. In the late pupal stage, single stretch receptor action potentials cause a biphasic response in MN-1, with a new excitatory postsynaptic potential preceding the inhibitory response. However, a burst of stimuli still inhibits the motoneuron. SR: stretch receptor. Scales A = 100 μm; B (top) = 5 mV/100 msec; B (bottom) = 10 mV/500 msec. Reprinted from Levine & Truman (1982) by permission from Nature (Nature 299: 250–252) copyright © 1982 Macmillan Magazines Ltd.
Control of Ecdysis Behavior

Cuticle shedding at ecdysis is a behavior which is largely orchestrated by a neuromodulatory cascade involving pre-ecdysis triggering hormone, ecdysis triggering hormone and eclosion hormone (Truman 1992a; Zitnian et al. 1999; Zitnian & Adams 2000; see 2-15). In Manduca larvae and pupae, two sequences of pre-ecdysis behavior (pre-ecdysis I and II, which result in cuticle loosening) have been distinguished from subsequent ecdysis behavior (cuticle shedding) (Miles & Weeks 1991; Novicki & Weeks 1995, 1996; Zitnian & Adams 2000). The behavioral sequence of adult ecdysis studied in Manduca and Hyalophora shows certain modifications from the larval pattern (reviewed by Truman 1992a). Larval pre-ecdysis behavior is characterized by rhythmic dorso-ventral compressions of the abdomen. The behavior is controlled by abdominal motoneurons MN-2 and MN-3 which innervate the tergo-pleural and anterior lateral external muscles (Miles & Weeks 1991). A neural network generating the rhythm is located in the terminal abdominal ganglion (Novicki & Weeks 1993) and conveys rhythmic input to all abdominal motoneurons MN-2 and 3 via a pair of ascending interneurons (Novicki & Weeks 1995).

Following pre-ecdysis behavior, larval and pupal ecdysis involves anteriorly running peristaltic contractions of the abdomen caused by rhythmic activity of intersegmental and tergopleural muscles and their motoneurons (Weeks & Truman 1984). The motor program is activated through release of the neuromodulator “crustacean cardioactive peptide” from sets of segmentally arranged interneurons (group 27/704; Gammie & Truman 1997a, b). These neurons are activated by the action of ecdysis triggering hormone on the cephalic ganglia (Zitnian et al. 1999; Zitnian & Adams 2000), and, probably in addition, by the release of eclosion hormone from descending brain neurons (VM neurons) into the ventral ganglia (Gammie & Truman 1999). The neuronal machinery underlying ecdysis behavior is largely preserved through metamorphosis (Mesce & Truman 1988), but after adult ecdysis, the intersegmental muscles, their motoneurons and the group 27/704 neurons die within 36 hours after adult eclosion (Ewer et al. 1998), while the VM neurons, containing eclosion hormone, persist during adult life (Riddiford et al. 1994).

Reproductive Behavior: Calling – Mating – Oviposition Behavior

Sexual attraction in moths involves acoustic (Conner 1999) and chemical communication. Calling through pheromones in male and female Lepidoptera consists of extrusions of the last abdominal segments for exposure of sex pheromone glands (see 2-13). In most species, pheromone biosynthesis appears to be controlled hor-
monally, but evidence in *Heliothis, Helicoverpa, Manduca*, and *Spodoptera* argues for a neural or mixed control of the gland (reviewed by Christensen & Hildebrand 1995; Marco et al. 1996; Teal et al. 1999). Pheromone gland extrusion and pheromone release in *Manduca* is controlled by neural outputs from the brain-subsophageal ganglion (Itagaki & Conner 1986; Christensen et al. 1994), and suppression of pheromone production after mating in several species is induced by neural signals ascending the ventral nerve cord, which inhibit the release of pheromonomorphic neuropeptides from the subsophageal ganglion (e.g., Foster 1993; Ichikawa et al. 1996).

The neural control of mating has only been studied with respect to the peculiar presence of genital photoreceptors not found in any other group of insects so far, and their involvement in copulation. A genital light response has been demonstrated in a variety of butterfly families but not in Sphingidae, Bombycidae and Amauridae (Arikawa et al. 1980; Arikawa & Aoki 1982). Detailed investigations in *Papilio xuthus* showed that two pairs of UV/violet-sensitive photoreceptors occur on the genitalia of adult males and females but not in larvae (Arikawa & Aoki 1982; Miyako et al. 1993). One of the photoreceptors of males directly inhibits genital motoneurons and appears to be involved in controlling the correct position of the female’s vagina for penis insertion (Arikawa & Aoki 1984; Arikawa 1993; Arikawa et al. 1997; see 2-12).

The behavioral sequences of oviposition and sensory cues involved in host plant finding have been studied in several species (2-18) reviewed by Ramasswamy (1988), but the neural control of the behavior is unexplored.

**Concluding Remarks**

While comparative data on the gross morphological layout of the nervous system allow us to draw some conclusions about evolutionary trends within the Lepidoptera, this is generally not possible with regard to the internal organization of the brain and neuronal circuits and physiological mechanisms underlying sensory processing, control of behavior, and neural development. All of these aspects have been studied so far only in a few, often closely related species, most prominently in the sphinx moth *Manduca sexta*. *Manduca* has, in fact, become one of the most prominent insect model systems for the analysis of postembryonic development and olfactory integration. There exists already a vast literature on the nervous system of this species, so that often only reviews could be cited, and pioneering progress is continuing at a fast pace. This wealth of information on the nervous system of a single lepidopteran species has already begun to stimulate comparative research on other species, particularly on the olfactory system and, hopefully will attract researchers to start comparative research also on other functional aspects of the nervous system.

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Addendum

Important references published after completion of the manuscript include an analysis of the neural control of heart reversal in the hawkmoth *Manduca sexta* (Dulcis et al. 2001). Work on the role of genital photoreceptors in *Papilio xuthus* demonstrates that in females these photoreceptors have a crucial role in oviposition but not during mating (Arikawa & Takagi 2001). Studies on the control of ecdysis in *M. sexta* are complemented by Fuse & Truman (2002), who further characterize the role of the suboesophageal and thoracic ganglia in modulation of ecdysis.

Several accounts advanced understanding of lepidopteran olfactory mechanisms. A three-dimensional atlas of olfactory glomeruli was provided for two species of tobacco budworm moths, *Helicoverpa assulata* and *Heliothis virescens* (Berg et al. 2002). Sadek et al. (2002) show that single plant volatile compounds are not represented within a single glomerulus in the antennal lobe of the noctuid *Spodoptera littoralis*. Research in *Manduca sexta* shows that discharge rates of antennal lobe neurons closely match intermittent odor stimulation (Vickers et al. 2001) and suggest that the temporal tuning of output from each glomerulus is enhanced by reciprocal interglomerular inhibition (Lei et al. 2002). Anton & Hansson (2001) provided further evidence for an involvement of the lateral accessory lobes in moth olfaction.


