CHAPTER TEN

Moth Sex Pheromone Olfaction

Flux and Flexibility in the Coordinated Confluences of Visual and Olfactory Pathways

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The moth doesn't really follow his nose: he follows the wind when his nose tells him to.

GONICK (1995)

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SEEING THE WIND VIA OLFACTORY AND VISUAL CONFLUENCE IN SPACE

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Protocerebral neurons connecting the mushroom body or the inferior lateral protocerebrum/LH with the lateral accessory lobe are likely to be multimodal visual-olfactory inputs

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Mushroom body-to-lateral accessory lobe protocerebral neurons

Lateral accessory lobe-to-mushroom body and other protocerebral neuropils

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CONCLUSIONS

REFERENCES CITED

Introduction

The above-mentioned quote by scientific cartoonist Larry Gonick (1995) from *Discover* magazine conveys the essence of this chapter's message. To truly appreciate how the moth sex pheromone olfactory system is constructed, we must understand that the orientational behavioral response to sex pheromone by a flying male moth is entirely a *visual* response to *wind*, i.e., its direction and speed. Olfaction does not steer the male. It merely drives a visual response to vertical, horizontal, and turn-generated rotational image-motion feedback from the edges of objects in the environment that the male uses to steer either upwind or crosswind and gauge its progress toward a pheromone-emitting female, on a sub-second basis. The olfactory and visual processing systems must be intimately associated in the moth's brain to accomplish this behavioral feat. The abstract, *odor space sensation* of "pheromone" in the brain must be placed into a real-world, four-dimensional *visual-moving-edge* context for steering while the moth is flying and suspended in a moving medium, the wind, via motion-vision.

For a detailed discussion of the maneuverings of flying male moths in response to wind-carried pheromone plumes in field and laboratory studies, see Cardé (this volume). In our chapter, we juxtapose aspects of the flying male moth's brain representation of the visual world, especially its *motion*, with the brain's reconstruction of the pheromone-odor world. We hope this confluence will put the moth sex pheromone olfactory system in context with the stereotyped flight-related behavioral responses that result in the location of a female. We discuss how the feature-extracting neuronal pathways of both the olfactory and the visual systems converge and are integrated in the higher centers of the moth brain. Apparent image-motion and molecular flux representations of pheromone odor are shown to drive sub-second, rapidly reversing turns and visually mediated "surges" to pheromone plume-strand contact. Possible neuronal networks involved in long-lasting, slower reversing turns in pockets of clean air that result in visually mediated crosswind "casting flight" are also described (see Cardé, this volume).

Seeing the Wind via Olfactory and Visual Confluence in Space

Spectral Space: Colors and Odors

The representation of the pheromone-odor world in a moth's brain involves a high-resolution integration of the pheromone's molecular component composition, its spectral sensation of "odor-blend quality," and its temporal fluctuations in intensity—*flux*—that are experienced by the pheromone-olfactory system during encounters with the variable fine-plume-strand structure of the pheromone plume itself. In the stark physical world of chemicals and electromagnetic emanations, spectral sensations of color and odor do not exist; they are only formed *within* the brain. They do not even exist in the excitations of single types of sensory neurons. They only exist in the brain in distinct and identifiable regions that we call, for the purposes of this chapter, *spectral space*.

All sensory neurons are *achromatic* and unable by themselves to discriminate stimulus quality. For example, a visual sensory neuron cannot discriminate, by itself, which wavelength of electromagnetic energy is stimulating it (e.g., discriminate color), due to a confounding of luminance intensity with spectral frequency. Because a stronger stimulus from a suboptimal part of the spectrum can excite a sensory neuron as much as a lower intensity stimulus from an optimal portion of the spectrum, action potential frequencies from an achromatic neuron do not by themselves allow spectral classification, e.g., discrimination. Similarly, each type of differentially tuned insect antennal olfactory sensory neuron (OSN) (figure 10.1A) responds in a graded manner to a panel of different odorants, and even though each OSN has an optimum at some point along that spectrum of odorants, it cannot by itself discriminate chemical quality due to a confounding of a higher concentration of a suboptimal odorant molecule with a lower concentration of an optimal molecule. Thus, insect OSNs are achromatic. The quality of an electromagnetic energy emanation or of a blend of volatile chemicals cannot be discriminated until the sensory inputs from a variety of differentially tuned achromatic sensory neurons are reported to, and cross-compared by, integrative neurons residing in higher neuronal networks. These integrative neurons can then begin to resolve the position of an odor blend, such as a sex pheromone, in spectral *odor space* (de Bruyne et al. 1999; Dobritsa et al. 2003; Hallem and Carlson 2004).

Structural Space: Flux and Flicker

Light has properties that can be sensed and used by insects to reconstruct representations of the *structural space* of the physical world. Reflected light from physical objects in the environment has different intensities of photon flux that define edge-related luminance discontinuities of objects positioned in three-dimensional (3D-structural) space. When time as a fourth dimension is added to 3D-structural space, the visual system can then detect and monitor the ON–OFF flickerings of luminance edges and their relative apparent motions as the insect moves in straight lines, turns, or is displaced by wind.

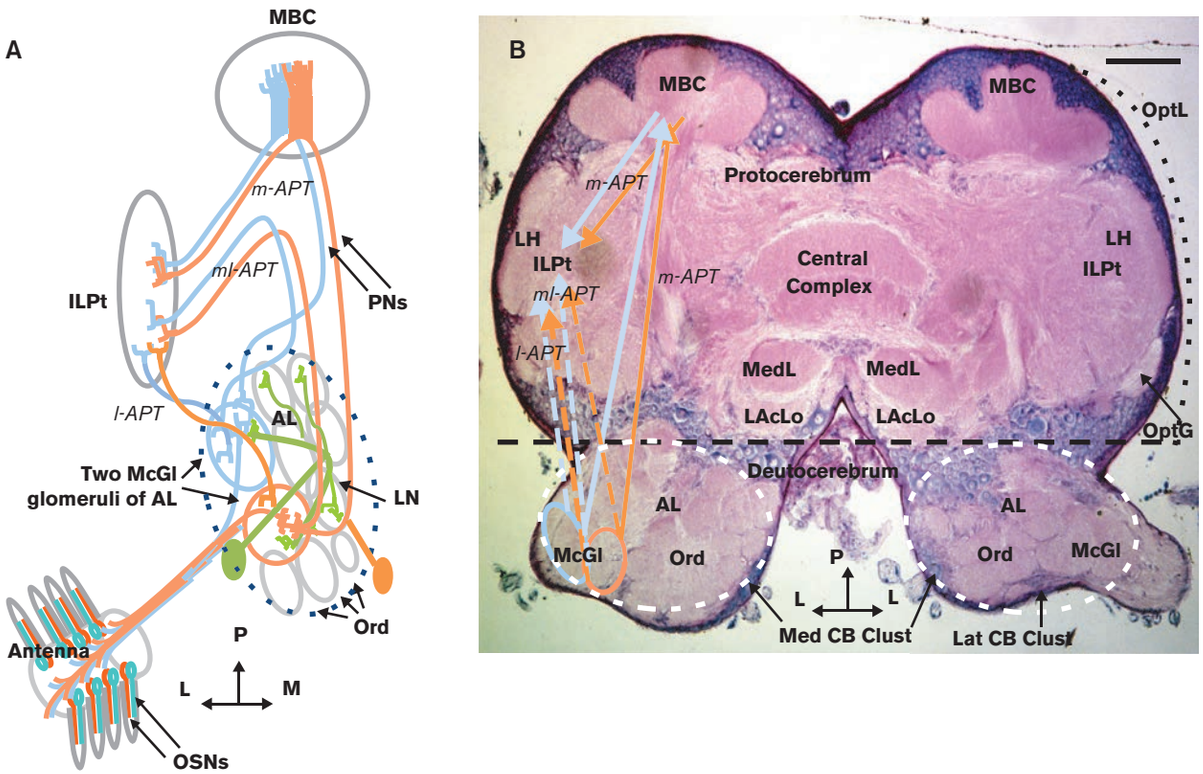

FIGURE 10.1 Neuronal architecture of lepidopteran olfactory system.

A Schematic horizontal (top-down) view of the neurons and neuropils involved in moth sex pheromone olfactory pathways. Two types of olfactory sensory neurons (OSNs, orange and blue) are depicted as being co-located in each of many antennal sensilla. In a two-component sex pheromone blend, these OSNs report the relative molecular abundances of the sex pheromone components to which they are tuned. Each type of OSN arborizes in its own pheromone-component-specific glomerulus in the macroglomerular complex (McGl) of the antennal lobe (AL). There, they each arborize with myriad local neurons (LNs, green) that shape the reports that go to protocerebral neuropils via the axons of projection neurons (PNs). Solid green oval depicts an LN's cell body residing in the lateral cell body cluster. The solid orange oval depicts a PN's cell body residing in the medial cell body cluster. Sexually isomorphic ordinary glomeruli (Ord, gray ovals) receive axons from antennal neurons tuned to general odorants (not shown). The dashed oval depicts the boundaries of the AL. PNs leaving the McGl and projecting their axons via the medial antennoprotocerebral tract (*m-APT*; arrows, blue and orange lines) send collaterals to the calyces of the mushroom body (MBCs) before continuing on to arborize in the inferior lateral protocerebrum (ILPt). PNs projecting along the mediolateral antennoprotocerebral tract (*ml-APT*) and lateral antennoprotocerebral tract (*l-APT*) send terminal arbors directly to the ILPt without visiting the MBCs. Scale bar is 100 µm.

ABBREVIATIONS: P, posterior; L, lateral; M, medial.

B Horizontal section through the brain of a male *Helicoverpa zea* showing positions of neuropils in the brain (adapted from Lee et al. 2006a). Cell bodies of neurons are stained blue; neuropils are stained pink.

ABBREVIATIONS: LAcLo, lateral accessory lobe; LH, lateral horn; MedL, medial lobe of the mushroom body; OptG, one of many optic glomeruli (others not visible) in the lateral protocerebrum receiving inputs from the optic lobe (OptL), whose approximate margin is depicted by curved dashed line. Med CB Clust, small portion of the medial cell body cluster of AL neurons; Lat CB Clust, small portion of the Lat CB Clust of AL neurons. Other abbreviations are as in (A).

NOTE: Some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

Strands of odor that have sheared off from a point-source emitter, such as a female moth's pheromone gland (see Cardé, this volume), are highly variable in their molecular concentrations and also have large pockets of relatively pheromone-free (clean) air in between the pheromone strands. Because these molecules move through the environment on the moving air masses that comprise wind, a time dimension is added that involves molecular *flux*, i.e., molecules per second that contact the insect antenna. The speed of motion of molecules on wind through the environment and by the moth's own movement through the air (its airspeed) creates further variations in molecular flux intensities contacting the antenna.

Odorant flux analysis by the insect's olfactory system involves defining the pheromone strand's temporal edges, i.e., a strand's onset upon contact with a sensillum and its offset upon departure during the moth's encounter with a clean-air pocket between strands. These odor-strand ONs and OFFs can then be integrated simultaneously with, and placed in the context of, the brain's visual representation of four-dimensional visual space-motion. In the visual system, when a change in luminance occurs sequentially across an array of photoreceptors, it provides the basis for motion detection that includes integration of direction and speed. Most of the neuronal pathways in the insect visual system are dedicated to motion detection, beginning in the lamina and then through the medulla, lobula, and lobula plate of the optic lobe (figure 10.2) to resolve the speeds and directions of image flows (Strausfeld and Campos-Ortega 1977; Strausfeld 2003, 2012). Visual motion detectors

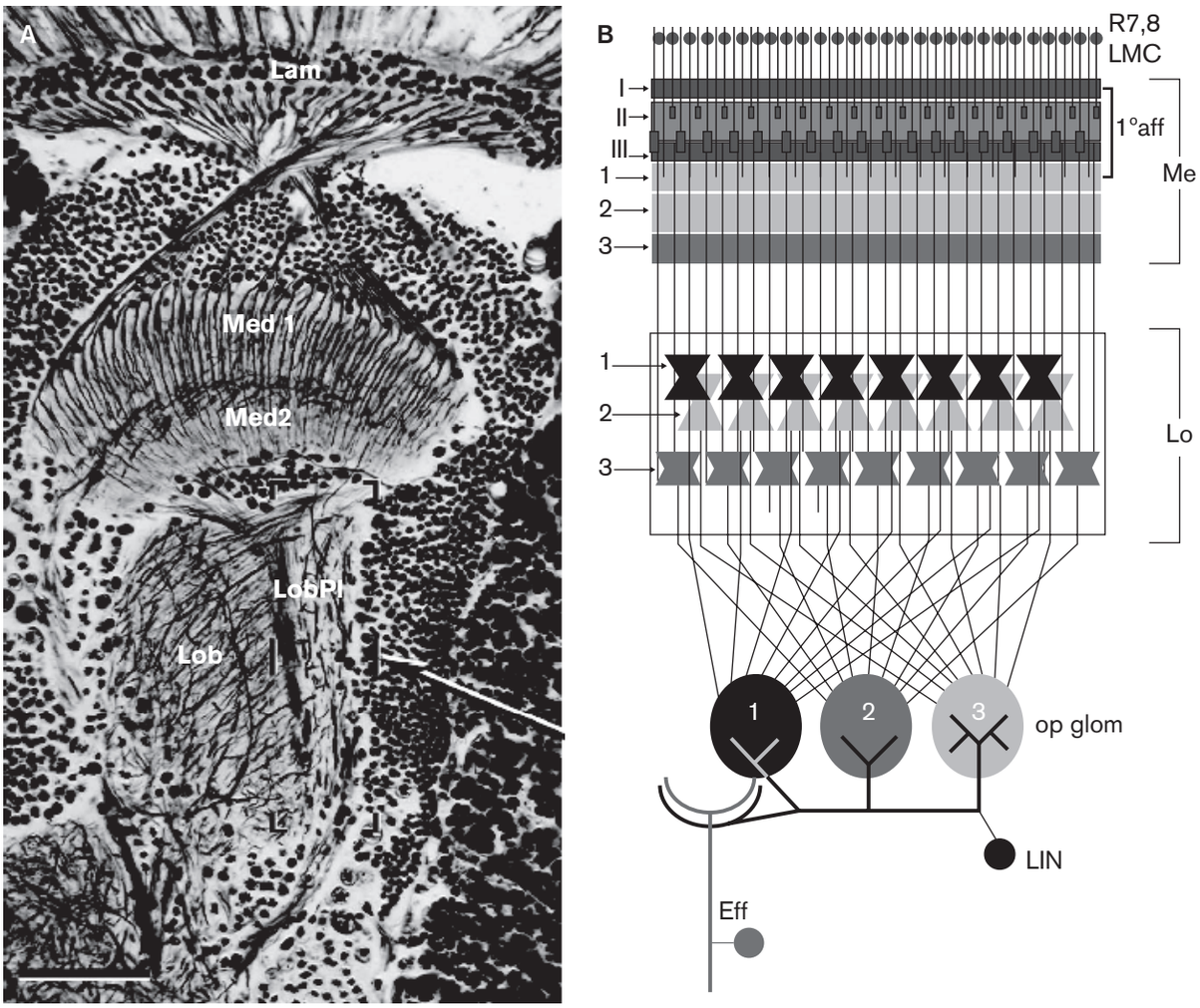

FIGURE 10.2

A Silver/cobalt preparation showing organization of the compound eye and optic lobe of a calliphorid fly (adapted from Strausfeld 2012), illustrating the retinotopic cascade of vertically ascending axons through the neuropils of the lamina (Lam), the outer and inner medulla (Med 1 and Med 2, respectively), lobula (Lob), and lobula plate (LobPl; in black brackets). Black nodules around all the neuropils are the cell bodies supplying neurites to those neuropils (black arrow from right points to cell bodies supplying the Lob Pl).

B Schematic diagram of how visual information, including directionally specific and directionally selective image-motion from different retinotopic layers via columnar connections in the optic lobe, ascends to arborize in optic glomeruli (op glom) in the lateral protocerebrum. The op glom are connected via local interneurons (LIN) that have been shown to respond to various directional image-motions (Okamura and Strausfeld 2007; Strausfeld and Okamura 2007; Strausfeld et al. 2007). They perform integrations of activities among the op glom before efferent neurons (Eff) carry this information to other protocerebral neuropils or perhaps to thoracic ganglia for motor outputs. Various horizontal, cross-integrative tangential layers in the medulla (Me) and lobula (Lo) are depicted here in various narrow- or wide-field shapes and in different shades of gray. Each optic cartridge in a retinotopic column begins at the periphery (top) with a set of primary visual afferents (1° aff) from an ommatidium, consisting of three flicker-sensitive achromatic large monopolar cells (LMC) carrying pooled synaptic inputs from receptor neurons R1–R6, plus the long visual fibers of receptor neurons 7 and 8 (R7, 8). Three op glom (1, 2, and 3) are depicted that each receive a vision-specific input from one tangential layer in the lobula. The arbors from one local interneuron (LIN) are shown that arborize with different architectures in the op glom as well as with the efferent neuron that projects its axon to other neuropils. I, II, III and 1, 2, 3 denote different narrow- and wide-field tangential neuropil layers in the lamina, medulla, and lobula that integrate at each of those layers different motion (and other) visual information across the vertical retinotopic columns transecting those layers.

SOURCE: From Strausfeld and Okamura (2007).

NOTE: Please note that some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

and odorant plume-strand flux-change detectors work together to guide the male moth in its progress through wind.

Sex Pheromone Olfaction Pathways of Moths

We define pheromone olfaction pathways as neuronal pathways that are involved with transducing, transmitting, and integrating pheromone-component stimuli to resolve the pheromone's positions in *pheromone-odor space* and *pheromone-odor time*. In the general architecture for sex pheromone olfaction (figure 10.1), pheromone-component-tuned OSNs project from the antenna to the macroglomerular complex (McGl) of the antennal lobe (AL), where they synapse with multitudes of local neurons (LNs) and projection neurons (PNs) (figure 10.1A). PN outputs from individual McGl glomeruli or combinations of glomeruli follow three different tracts to project processed pheromone-component information from the McGl to the inferior lateral protocerebrum (ILPt) of the lateral protocerebrum (LP) (figures 10.1A and 10.1B). One pathway takes a medial route through the brain and first sends collaterals to visit the mushroom body calyces (MBCs) before terminating in areas of the ILPt. The other two PN tracts are more lateral, traveling directly to the ILPt with terminal arbors there, bypassing the MBCs completely (figures 10.1A and 10.1B). OSNs responsive to general (e.g., plant-related) odorants send their axons to ordinary glomeruli in the AL, and interact synaptically with LNs there, with PNs then projecting from these ordinary glomeruli along the same three routes to the LP, except that the axons of these general odorant PNs arborize in a slightly different LP neuropil, the lateral horn (LH) (figure 10.1B).

The post-OSN, toward- or within-protocerebral pathways of sex pheromone-component-sensitive neurons thus involve three highly synaptic integrative neuropils that perform complex integration of sex pheromone-component inputs, all involving intrinsic LNs within these neuropils as well as associated efferents that project out of them. The first integrative neuropil is in the AL of the brain's deutocerebrum; it consists of networks of LNs interconnecting glomeruli within the McGl and also interconnecting McGl glomeruli with those of the entire AL, with the PNs then projecting to protocerebral neuropils. The second integrative neuropil is the mushroom body (MB) of the protocerebrum that receives inputs from the AL via PNs that arborize in the MBCs (figure 10.1B). Kenyon cells (KCs) of the MB are its intrinsic LNs and give it its shape. Their long parallel fibers extend along the length of the MB from the MBCs down through the various mushroom body lobes (MBLs). There are neurons extrinsic to the MBLs, many of which are efferent neurons that integrate information from the arrays of KCs across the MBLs and that project outgoing MB-processed information to other protocerebral neuropils. The third major pheromone-component integrative center includes the ILPt of the LP (figure 10.1B). Within the LP, any olfaction-integrating LNs of the ILPt for pheromone information or in the LH for general odorants have not yet been characterized in moths. Such LNs might be the same LNs used by insect visual systems that interconnect the tens of newly described insect "optic glomeruli" residing in the LP (Okamura and Strausfeld 2007; Strausfeld and Okamura 2007; Strausfeld et al. 2007).

To the above-mentioned three pheromone olfaction integration centers, a fourth center might be added, the lateral accessory lobe (LAcLo), because the LAcLo has been repeatedly implicated in producing turn-reversals in flying or walking males. The paired LAcLos are located medially in the protocerebrum, ventral and slightly laterally to either side of the central complex (figure 10.1B). Inhibitory protocerebral neurons (PrtCNs) connect the two LAcLos and produce alternating "flip-flopping" of excitation related to turn-reversals during pheromone stimulation. With one exception, the LAcLo receives inputs only from purely within-PrtCNs that project there from both the ILPt and the MBLs. These PrtCNs will likely be carrying already processed pheromone-visual information to the LAcLo. However, the one exception involving the moth *Agrotis segetum* (Noctuidae) did show two pheromone-sensitive PNs projecting from the McGls *directly* to the LAcLos. These two PNs also sent collateral arbors to the ILPts on both sides of the male's protocerebrum (Wu et al. 1996). Intrinsic LNs residing within the LAcLos interconnect each LAcLo with a companion neuropil immediately ventral to it called the ventral protocerebrum (VPrtC). These LAcLo/VPrtC LNs respond to pheromone-component stimulation of the antennae by producing long-lasting excitation (LLE) in response to a single puff of pheromone. The LAcLo thus is a neuropil critical to producing premotor neuronal outputs that drive strand-initiated single turns as well as long-lasting, oscillating, clockwise–counterclockwise turn-reversals.

Visually Mediated Behavioral Responses to Pheromone

No Chemotaxis: Moths Use Two Indirect Responses to Pheromone Strands and Clean Air

For a detailed treatment of pheromone-mediated behaviors of male moths in response to pheromone plumes in the field and laboratory, see Cardé (this volume). Here, we summarize aspects of pheromone-mediated behavior for the purpose of placing these behaviors in a neuroethological context related to integrated olfactory and visual pathways.

Decades of research have resulted in the understanding that there are two major systems involved in upwind in-flight progress to a source of pheromone odor, and neither of them involves chemotaxis; i.e., there is no *direct* steering response to chemical concentration gradients. Rather, two *indirect* responses (Kennedy 1983) are now understood to be performed after encounters with the individual strands of pheromone in a plume and with the pockets of clean air between the strands: (1) pheromone-induced optomotor anemotaxis (Kennedy 1940; Kennedy and Marsh 1974), a wind-steering response to global wide-field translatory (linear) flow-field motion across or along the eyes, and (2) a pheromone-triggered, self-steered turn generator. The pheromone-triggered turns are reversed in direction (e.g., clockwise to counterclockwise) with each contact with a pheromone strand (Kanzaki et al. 1992; Mafra-Neto and Cardé 1994; Vickers and Baker 1994; Mishima and Kanzaki 1998, 1999; Iwano et al. 2010). The turn generator also drives a long-lasting program of turn-reversals in clean air that free-runs after the last contact with a pheromone strand, both with wind (Kennedy and Marsh 1974; Kanzaki et al. 1992) and without wind (Baker and Kuenen 1982). That the clockwise–counterclockwise reversals are produced by males in zero wind shows that they are "self-steered" according only to their self-generated optical image flows or to other, non-optomotor-anemotactic or nonvisual feedback (Baker and Kuenen 1982).

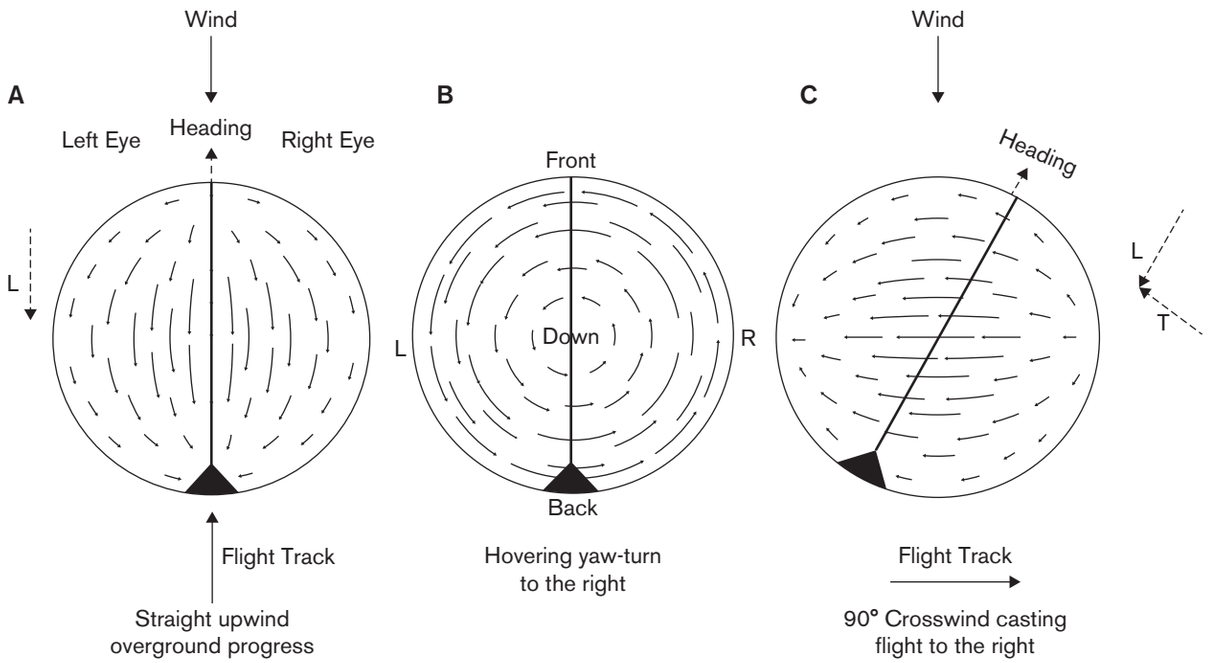

FIGURE 10.3 Visual image-flow fields experienced by the left and right eyes of a male moth flying just above a plant canopy over open ground. The lengths and directions of the vector arrows indicate the speeds and directions of the flows, with the speeds being fastest directly below the moth. Wind direction is indicated by arrows.

A Male making straight upwind progress; only longitudinal (L) front-to-back image-motion is experienced.

B Male making a clockwise turn using X-Y yaw rotation alone while keeping stationary over the ground. If the turn were to be executed by adding a clockwise roll component to the yaw-turn by reducing lift on the right side and increasing lift on the left, the flow would be more complex and involve down-to-up flow plus left-to-right flow on the right eye and up-to-down flow plus left-to-right flow on the left eye, unless the moth moved its head to minimize such flow.

C Male during a 90° crosswind casting flight-leg; the two components of image flow now include both transverse (T) cross-body and longitudinal (L) front-to-back image motion.

SOURCE: After David (1986).

Pheromone-Mediated Optomotor Anemotaxis Involves Longitudinal and Transverse Image Flow; a Turn-Reversal Involves Rotatory Image Flow

Translational image motion occurs during linear, straight-line flight tracks and is characterized by flow fields in the same direction across all the ommatidia of one eye and therefore across all the ommatidia of both eyes (figures 10.3A and 10.3C). Two types of translatory image flows are experienced by a pheromone strand-stimulated moth flying in wind. Flying straight upwind immediately after contacting a strand produces front-to-back translatory image flow along the long axis of the moth's body, called *longitudinal image flow* (figure 10.3A) (David 1986). During off-windline flight, such as during the straight-across-wind flight track legs occurring between each counterturn during clean-air casting flight, *transverse image flow* across the long axis of the moth's body also will occur (figure 10.3C) (David 1986). During these casting-flight straight legs, longitudinal flow is also present and is integrated with the transverse flow in approximately a 1:1 ratio to steer the moth's resultant flight track straight across the windline (David 1986).

The execution of a turn or loop causes *rotatory image flow* in both walking and flying moths. For example, *Bombyx mori* (Bombycidae) first zigzag and then loop for long periods after emerging into clean air after a pheromone pulse, and flying moths behave the same when they execute turns at the termini of each of their left–right straight-across-wind "casts." Turning-induced rotatory image-flow fields are characterized by opposing-direction flow fields along different portions of the eye (or, e.g., along the left vs. the right eye). In rotatory flow fields, front-to-back motion will occur along one eye, for instance, and back-to-front motion along the other eye (figure 10.3B). These opposing left-side, right-side flows across the long axis of the moth's body will be experienced by a walking moth that circles or turns from left to right or vice versa, and a flying moth that executes a turn via a yaw response (around its X-Y plane). Walking moths are not known to use any kind of image flow to steer in response to wind; they can sense wind from pressure differences across their antennae or bodies because they are fixed to the ground via tarsal contact. If a flying moth were to help execute a clockwise or counterclockwise yaw-turn by also rolling its body to the right or left, respectively (in its Y-Z plane), the flow would be more complex than illustrated in figure 10.3B, with additional rotational image flow involving up-and-down motion in that plane. If a moth were to keep its head steady while rolling or yawing its thorax and abdomen to execute a turn, rotational image flow might be minimized. Compensatory head rotation would not reduce translational flow caused by wind-induced course-track differentials, however.

Three Behavioral Responses to Pheromone Strands Coordinated by Three Protocerebral Neuropils Known to Receive Both Visual and Olfactory Inputs

Contact with a single pheromone strand involves a "pheromone ON" input during strand contact and a "pheromone OFF" input after flight into the clean air after the strand has passed. The pheromone ON stimulation produces a *resultant* flight track called an "upwind surge." The pheromone OFF (clean-air) stimulation eventually produces a resultant flight track called "casting flight" (see Cardé, this volume). However, during the transition to casting flight in long periods of clean air or during intermediate-frequency contact with plume strands in a natural point-source plume, there is an intermediate, upwind-zigzagging type of flight track. Thus, we can identify three behavioral outcomes that are mediated by the two indirect pheromone response systems due to this ON–OFF stimulation. The actual performances and integration of these two indirect response systems occur in the following temporal sequence when observed in response to a single strand of pheromone. We propose that the following protocerebral (central nervous system [CNS]) neuropils are involved in these behaviors that integrate olfaction and vision.

1. *Behavior: A turn-reversal is generated in response to the pheromone ON stimulation from the strand*. The turn-reversal is executed either clockwise to counterclockwise or vice versa in a flying or walking moth and needs no optomotor anemotactic feedback (Baker and Kuenen 1982). However, optomotor anemotaxis will polarize the turn in an upwind direction (Baker et al. 1984).
   CNS: The LAcLo/VPrtC complex generates the turn-reversal in response to a strand. A turn-reversal generator has been shown to reside in the "flip-flop" circuitry connecting the LAcLo/VPrtCs on each side of the protocerebrum of both flying (Kanzaki et al. 1991a, 1991b) and walking moths (Kanzaki and Shibuya 1986, 1992; Kanzaki et al. 1994; Kanzaki and Mishima 1996; Mishima and Kanzaki 1998, 1999; Iwano et al. 2010). The LAcLos receive inputs from the MBLs (Lei et al. 2001), and LH/ILPt (Kanzaki et al. 1991a), both of which could be sources of fast initiation of LAcLo activity after contact with a pheromone strand. In addition, a direct connection between the ALs and the LAcLos has been found in *Agrotis segetum* (Wu et al. 1996) that could also be a fast initiator of a turn-reversal. The LAcLos also receive other inputs from local interneurons that visit them as well as the optic glomeruli of the LP plus its olfactory regions (Kanzaki et al. 1991a, 1991b). A turn-reversal would generate rotatory image-motion optical feedback (figure 10.3B) from the LP to the LAcLos during the execution of a turn that reverses the direction of rotatory image flow from what had been experienced during the execution of the prior reversal.
2. *Behavior: The turn-reversal produced by contact with a strand is followed immediately by approximately direct-upwind flight*. The upwind turn-surge always involves a significant sub-second period of upwind progress (Mafra-Neto and Cardé 1994; Vickers and Baker 1994). Upwind progress (it is always an upwind-oriented turn that occurs) cannot be performed without optomotor anemotaxis that involves predominantly longitudinal, along-body-axis image flow (figure 10.3A).
   CNS: The ILPt, having LN connections with optic glomeruli in the LP, should be involved in the upwind optomotor anemotactic steering of an upwind surge after a turn-reversal. The ILPt, which receives sex pheromone-component inputs, is located in proximity to, and in potential synaptic contact with, LNs that arborize among the abundant optic glomeruli that reside in the LP (Strausfeld and Okamura 2007; Strausfeld et al. 2007). Vertical and horizontal motion detectors in the optic lobe that are known to be involved in optomotor anemotaxis feed into this system of optic glomeruli located in the LP.
3. *Behavior: Increasingly long-duration contact with clean air after a strand contact allows for the complete performance of a long-lasting motor program to play out in which spontaneous (self-steered) oscillatory turn-reversals are executed*. The reversal program involves ever-increasing periods between reversals (slower turn-reversal tempo) and increasingly greater crosswind steering angles in flying moths during casting flight. In walking moths, this relaxation of the tempo is expressed as increasingly continuous one-directional clockwise or counterclockwise looping without the faster tempo, clockwise–counterclockwise looping shifts that immediately followed contact with clean air.
   CNS: The LAcLo/VPrtC complexes on each side generate the LLE after pheromone loss that drives long-term casting flight response in flying males in clean air or long-term looping by walking males after pheromone loss. The mutually inhibitory bilateral LAcLo-to-LAcLo connections during this time drive the alternating left–right, counter-clockwise–clockwise spontaneous turn-reversals in clean air. The neurons of the LAcLo-LAcLo system exhibit an oscillatory, spontaneous flip-flopping of action potential activity manifested by increasingly longer duration flip-flopping tempos with time after loss (Kanzaki and Shibuya 1992; Kanzaki et al. 1992, 1994; Mishima and Kanzaki 1998, 1999; Wada and Kanzaki 2005; Iwano et al. 2010). Such flip-flopping will thus be the source of increasingly longer intervals between in-flight crosswind turn-reversals during casting flight by flying males. Flip-flopping tempo in clean air in walking males is manifested by the retardation of the intervals between clockwise–counterclockwise looping reversals. In flying males, the ILPt and its intimate synaptic capabilities with optic glomeruli in the LP should together be involved in a relaxation of the anemotactic vertical–horizontal image flow from mostly upwind (along the body axis) to now crosswind (blend of T and L [figure 10.3C], across-body-axis- plus along-body-axis flows).

Three Observed Behavioral Outcomes in Natural Point-Source Pheromone Plumes as a Result of Different Frequencies of Plume-Strand Contact

In a natural point-source pheromone plume, the male will receive irregular sub-second exposures to pheromone strands and clean-air pockets (Vickers et al. 2001), resulting in three characteristically observed behavioral flight-track outcomes.

1. *Straight-upwind flight: predominantly LAcLo-turn-reversal mediation*. This outcome occurs when there is such

rapid exposure to pheromone plume strands that the turn-reversals plus upwind anemotaxis triggered by pheromone strands occur so frequently that the moth can never go far left or right from the windline as it moves upwind. The rapid turn-reversals keep it aligned with the windline and flying straight up it (Mafra-Neto and Cardé 1994; Vickers and Baker 1994).

2. *Zigzagging upwind flight: mixture of LAcLo-turn-reversal mediation plus ILPt-LP-mediated resetting of the optomotor anemotactic angle to allow more transverse image motion.* This behavior will occur when the frequency of strand contact is at an intermediate level, allowing longer contacts with clean-air pockets and a transition toward casting flight during these longer clean-air pockets. This behavior is the most commonly observed resultant behavior for a male moth flying upwind in a plume and should occur because more of the turn-reversals are endogenously initiated from the flip-flop circuitry of the LAcLo and do not stem from a contact with a new pheromone strand. The increasing relaxation of the anemotactic angle to have more of a transverse component in the optomotor anemotactic circuitry of the ILPt-LP will allow the resultant flight-track angles to be angled more toward crosswind.
3. *Crosswind casting flight in long-duration clean air: a combination of the LAcLo turn-reversal generator's increasingly slower activity plus the ILPt-LP's optomotor anemotactic angle set to ca. a 1:1 ratio of transverse-to-longitudinal image flow.* With no more pheromone strands to be contacted, the turn-reversals from the endogenously operating flip-flop circuitry of the LAcLo become increasingly slower in tempo, and the anemotactic steering angle of each straight flight-track leg between reversals becomes increasingly greater (off-windline).

The Lepidopteran Visual System

Insect visual systems are constructed to render optimally a depiction of edges (luminance discontinuities) occurring in physical (structural) space and the apparent motion of these edges along, across, and around the insect's body. As such, the architecture of the insect optic lobe is organized into highly regular arrays of vertically arranged *optic cartridges* having their afferent axons ascending in parallel toward the protocerebrum from each ommatidium of the compound eye (figure 10.2) (Strausfeld 1970; Strausfeld and Blest 1970). In moths, as in flies, optic cartridges consist of columnar arrays of parallel axonal fibers synapsing multitudes of times with neurons residing in four integrative layers of visual neuropil in the optic lobe: the lamina, medulla, lobula, and lobula plate.

Research on moths' visual motion resolution within these layers (Collett and Blest 1966; Collett 1970, 1972; Strausfeld and Blest 1970; Ibbotson et al. 1991; Maddess et al. 1991; Shimohigashi and Tominaga 1991, 1999; Milde 1993; Cutler et al. 1995; Hämmerle and Kolb 1997; Wicklein and Varju 1999; Wicklein and Strausfeld 2000; Briscoe et al. 2003; Kelber et al. 2003; Stavenga and Arikawa 2006) shows that image-motion integration through these levels does not appear to differ in significant ways from that of flies, upon which most of the classic work in image-motion integrative pathways has been performed (Strausfeld and Campos-Ortega 1977; Douglass and Strausfeld 1996, 2003, 2007). As in flies, six receptor neurons (R1–R6) of the eight in each ommatidia of moths and butterflies are tuned to the same single wavelength optimum, similar to the six R1–R6 receptor neurons in fly ommatidia that synapse into several flicker-sensitive optic cartridge channels of the large monopolar cells (LMCs) in the lamina (figure 10.2B). Most of the information extracted from the environment by these achromatic neurons is devoted to delineating luminance discontinuities and their apparent motion. Thus, the lepidopteran visual system is organized similarly to the system of flies in being chiefly devoted to "achromatic" (colorless) edge detection and edge image motion (Shimohigashi and Tominaga 1991, 1999; Cutler et al. 1995; Hämmerle and Kolb 1997; Briscoe et al. 2003; Kelber et al. 2003; Stavenga and Arikawa 2006). In Lepidoptera, the other two receptor types, R7 and R8, in each ommatidium, as in flies, are tuned to two different wavelengths different also from those of R1–R6, and their inputs are integrated in the medulla and beyond with some of the R1–R6 (LMC) inputs for wavelength (color) discrimination.

Huge numbers of retinotopic reports from individual visual sensory neurons in ommatidia that view and respond to one small section of visual, structural space in the environment are integrated first in the lamina. Here, edge discontinuities in luminance and their apparent motion are resolved by elementary motion detector lamina neurons that report motion, but with no directional component.

Resolution of edges and their motions in the lamina is aided by an abundance of amacrine cells that release gamma-aminobutyric acid (GABA) and whose delays regarding changes in luminance across the retinotopic arrays of ommatidial receptor neurons provide the mechanism for elementary motion detection (Strausfeld and Campos-Ortega 1977; Strausfeld 2012). Edge-motion-detection is projected via the axons of laminar integrative neurons to the medulla.

In Lepidoptera, the medulla is where the first level of directional edge-motion information resides (Collett and Blest 1966; Collett 1970, 1972; Ibbotson et al. 1991; Maddess et al. 1991; Milde 1993). Here, the outputs of vertically arranged neurons representing small or large groups of retinotopic columns project further toward the protocerebrum, synapsing in the multitudinous horizontal layers of the next integrative regions, the lobula, the lobula plate, or both.

In the lobula, directional motion in flies is further refined to include direction-specific edge-motion as well as object shapes, sizes, and orientations (Strausfeld 2012). In moths, the lobula has similar direction-specific edge-motion abilities (Wicklein and Strausfeld 2000). These integrations are aided by inhibitory and other modulatory activities of synapses in these lobula layers. Many of these layers also include the arbors of wide-spreading centrifugal neurons that provide feedback of processed information from the protocerebrum back out to the lobula and medulla to further modulate and refine the activities along the incoming visual retinotopic pathways (Collett 1970; Milde 1993; Wicklein and Strausfeld 2000).

In sphinx moths (Sphingidae), neuroanatomical studies have shown that specific types of motion information such as "looming" and "anti-looming" (expanding or contracting image-motion parallax) are reported by neurons that arborize in the lobula, the lobula plus lobula plate, and sometimes also in the medulla (Wicklein and Strausfeld 2000). In the lobula plate of *Manduca sexta*, types of wide-spreading tangential neurons that detect and respond to vertical and horizontal image motion also have been neuroanatomically character-

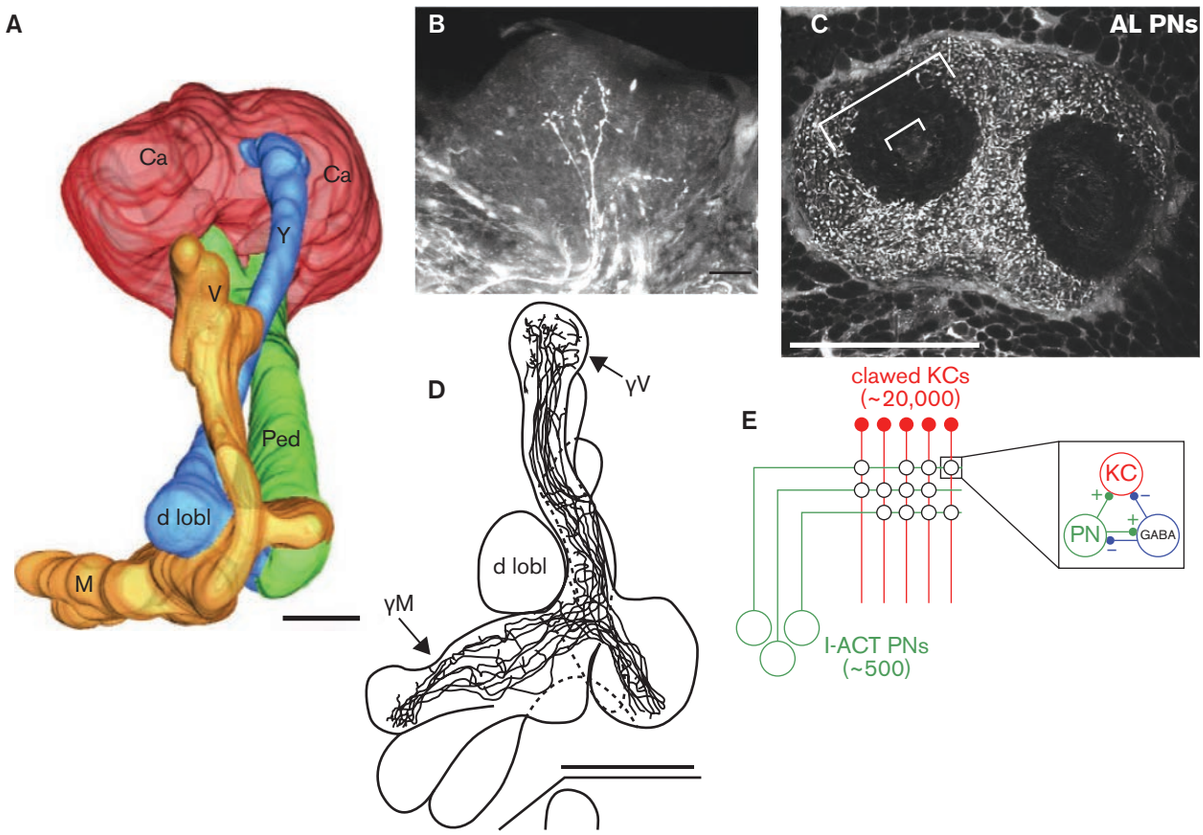

FIGURE 10.4

A Frontal view of a three-dimensional reconstruction of the mushroom body of *Spodoptera littoralis* with the calyces and lobes colored to denote the main sections. Ca, calyx; d lobl, dorsal lobulet; M, medial lobe; Ped, pedunculus; V, vertical lobe; Y, Y-lobe. Scale bar, 100 µm.

B Confocal microscope image of a dextran-stained neuron from the ipsilateral optic lobe of a *Spodoptera littoralis* that terminates with three arbors with profuse presynaptic varicosities in the extreme inner margin of the mushroom body calyx. Such neurons in *S. littoralis* and other moths may provide direct image-motion inputs to the mushroom bodies to be integrated with pheromone-strand contact excitation from antennal lobe projection neurons. Scale bar, 10 µm (from Sjöholm et al. 2005).

C Confocal microscope image of the arborizations of multitudes of antennal lobe projection neurons (AL PNs) in the outer zones of the two conjoined mushroom body calyxes of *Spodoptera littoralis*. PNs had been injected with rhodamine-dextran to aid visualization. This image highlights the lack of olfactory input to the inner zone of the mushroom body calyxes (large bracket), a zone known to receive direct input from at least some neurons from the optic lobe (see B), as well as a lack of olfactory input to the central zone (small bracket with asterisk). Scale bar, 100 µm (from Sinakevitch et al. 2008).

D Posterior view of mushroom body lobes (calyx not shown) with just a few of the multitudes of Kenyon cells that have been Golgi stained for visualization. Kenyon cells are the intrinsic (local) neurons that comprise most of the mushroom body's shape. Note the multitudes of neurites of this type of "clawed" Kenyon cell run in parallel along the lengths of the mushroom body lobes, here shown in just the vertical and medial lobes. Of many laminar sheets that comprise and run along the lengths of the mushroom body lobes, only the Kenyon cells in the "gamma" layer are depicted (γM and γV). The parallel and tightly packed arrangement of Kenyon cells, their vast numbers and arborizations with sensory projection neurons in the calyx, and their synaptic multimodal inputs and outputs with protocerebral neurons along their entire lengths in the mushroom body lobes all facilitate information processing and dispensation in the mushroom body that is essential to the life and reproduction of an insect (from Sjöholm et al. 2005).

E Depiction of how the axons of three different olfactory projection neurons (PNs) arborize in synaptic boutons with different Kenyon cells (KC) to produce odor-specific across-fiber (across-KC) patterns of activity. I-ACT PNs are the PNs of the "Inner Antenno-cerebral Tract" named by Homberg et al. (1988) that is now named "medial antennoprotocerebral tract" (*m*-APT; Galizia and Rössler 2010) (from Szyszka et al. 2005).

INSET: The details of one synaptic bouton are magnified to show how it consists of both GABA-ergic and excitatory interactions between the PN and the KCs at the synaptic junction (from Szyszka et al. 2005).

ized (Wicklein and Varju 1999). Such lobula plate neurons, called "vertically sensitive" (VS) and "horizontally sensitive" (HS), are a common feature of many dipteran and lepidopteran lobula plates. These neurons have a well-established function involving general optomotor stabilization of flight altitude and the orientation of the insect in wind with regard to the apparent horizontal motion of edges along and across the body axis.

Projections of Edge-Motion Information from the Optic Lobes to the Protocerebrum

INPUTS FROM THE OPTIC LOBES TO MUSHROOM BODY CALYXES

There is evidence in moths that direct projections from neurons in the optic lobes are sent to the MBCs (figure 10.4A) to

arborize in an "inner rim" region adjoining the arborization points of pheromone-component-sensitive PNs (figures 10.4B and 10.4C) (Sjöholm et al. 2005, 2006). Dye-filled neurons from the optic lobes were shown to terminate in this inner rim region of the MBCs in *Spodoptera littoralis* (Noctuidae) (figure 10.4B). Further work demonstrated that these inner rim areas of the MBCs in *S. littoralis* are devoid of bouton synapse inputs from olfactory PNs arriving from the AL (figure 10.4C). The inner rim was thus demonstrated to be non-olfactory, with the AL PN collaterals only arborizing in the typical lepidopteran outer rim zones of the MBCs (figure 10.4C) (Sjöholm et al. 2006; Sinakevitch et al. 2008). It was not clear from which level of the optic lobe the neurons projected (Sjöholm et al. 2005), but it likely would have been from either the medulla or the lobula. That these visual fibers arborized in the MBCs with synaptic boutons would indicate that they should be visual afferents supplying optic inputs to the MBCs rather than outgoing feedback (centrifugal) neurons modulating activity in the optic lobe. An inner rim zone of the MBCs in the cockroach *Periplaneta americana* (Blattidae) likewise receives direct inputs from the optic lobes, with an outermost layer of the calyces receiving pheromone-component-tuned PNs from the McGl and from other olfactory AL PNs (Nishino et al. 2012b).

INPUTS FROM THE LOBULA TO OPTIC GLOMERULI IN THE LATERAL PROTOCEREBRUM

Vision-related, densely grouped neuropils in the lateral and posterior LP that over the past century had been given names such as "optic foci" and "optic tubercles" have recently been recognized as comprising "optic glomeruli" (figures 10.5A–10.5C) (Okamura and Strausfeld 2007; Strausfeld and Okamura 2007; Strausfeld et al. 2007). In flies, visual information, most certainly including many types of image motion, from each of the many successive layers of the lobula (figures 10.2B and 10.6B), is now understood to be sent to individual optic glomeruli representing specific types of visual outputs from those layers (figures 10.2B and 10.6B). The collective output from within each different lobula layer is suggested to represent a pool of a certain type of visual sensation, and each glomerulus would thus represent a different pool that was gathered from a different depth of neuropil within the lobula (figure 10.6B) (Okamura and Strausfeld 2007; Strausfeld and Okamura 2007; Strausfeld et al. 2007). The 27 or more optic glomeruli known to be present in some species of flies are now recognized to populate huge volumes of each LP (figure 10.5C) (Okamura and Strausfeld 2007). It is not known whether the lepidopteran LP has the same abundance of optic glomeruli as the dipteran LP, but they indeed have been identified and diagrammed there (cf. Heinz and Reppert 2012). As in flies, the optic glomeruli of moths are in proximity to the integrative pheromone and general odor olfactory zones of the ILPt and LH (figures 10.1B and 10.5A).

KEY PLAYERS: LOCAL NEURONS INTERCONNECTING OPTIC GLOMERULI

Of further interest is that optic glomeruli have been shown to be interconnected by LNs, many of which are directionally motion selective, that are suggested to perform the same function as LNs in the AL, e.g., integrating the different activities occurring among groups of glomeruli (Strausfeld et al. 2007). The strong similarity between the architectures of AL olfactory and lateral protocerebral optic glomeruli has been recognized previously (Strausfeld et al. 2007), with the key common feature being the integration performed by LNs of the pure pools of specific olfactory (figure 10.6A) or visual information (figure 10.6B) residing in different glomeruli. Thus, an optic glomerular LN arborizing in many different glomeruli would seemingly be able to integrate the excitations occurring within those various glomerular neuropils to achieve some sort of overall, moment-to-moment visual blend sensation. For image-flow stimuli, such integrations might be an achromatic vector integration of all the different types of narrow- and wide-field image flows from the environment at any instant. These integrations might not necessarily be based upon a retinotopic representation of any specific features of the environment, but possibly upon combinations of, e.g., motion sensations related to rolling, pitching, and yawing, among other movements.

In flies, directional motion information shown to be integrated by LNs arborizing in optic glomeruli (Strausfeld et al. 2007) has been implicated as extending into other lateral protocerebral regions such as the LH, where visual information from these LNs may be further integrated with odor-related information (Strausfeld et al. 2007). Strausfeld and Okamura (2007) and Strausfeld et al. (2007) have strongly suggested that such LNs are likely to be able to integrate incoming odor-related inputs to the LH with visual information from optic glomeruli.

It is clear that the LP is emerging as an important set of neuropils in which multimodal olfactory-visual information may be occurring via the activities of LNs that arborize across optic glomeruli as well as across olfactory PN arborization locations. Thus, two of the protocerebral projection destinations of neurons carrying visual edge-motion information in moths have been identified as being the same two protocerebral regions to which sex pheromone olfactory information is processed: the MBCs and the LP.

INPUTS FROM THE LOBULA PLATE TO OPTIC GLOMERULI

The main task of the arrangement of wide-field VS and HS large tangential neurons in the lobula plate of flies and other flying insects such as moths is for general flight stabilization via optomotor feedback. It is now evident (Strausfeld et al. 2007; Strausfeld 2012) that a major portion of VS and HS lobula plate axons synapse first with LNs in the LP before these synapse with descending neurons. In *Macroglossum stellatarum* (Sphingidae), some VS and HS neurons from the lobula plate were implicated as arborizing in knots of neuropil, e.g., "optic foci," indicative of what are now known to be optic glomeruli (Wicklein and Varju 1999). These VS and HS neuronal inputs to LH neuropils were shown to be responsive to horizontal, vertical, or diagonal image motion and could be important to pheromone-mediated optomotor anemotaxis via pheromonal ILPt-LN-related integration.

Evidence for Pheromone-Stimulated Image-Flow Enhancement

There is behavioral and neuroanatomical evidence in male moths that attention to optical flow is poor unless pheromone stimulation is present. Preiss and Kramer (1983) and Preiss and Futschek (1985) demonstrated that tethered male *Lymantria dispar* (Erebidae) ignored different optical flow-

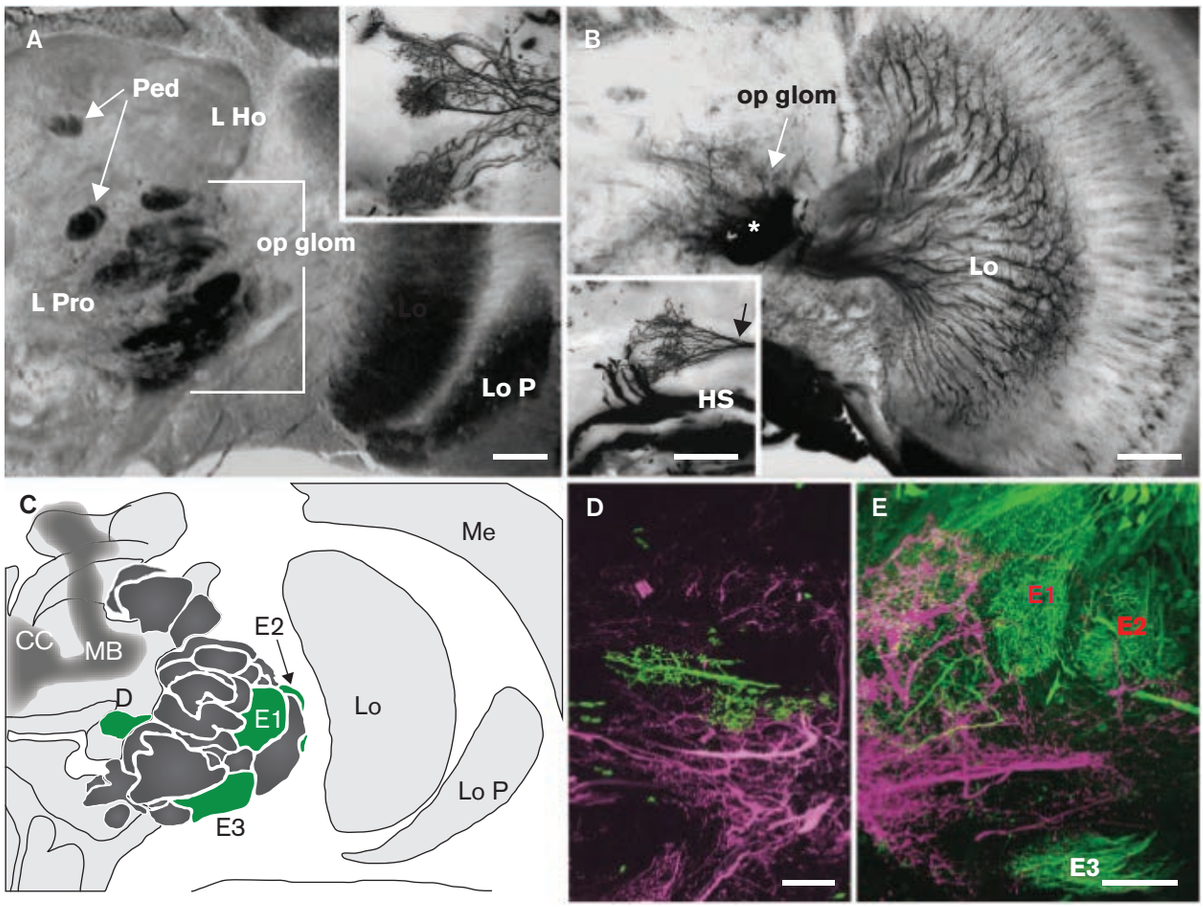

FIGURE 10.5 Optic glomeruli in the lateral protocerebrum (LPro) of the calliphorid fly *Phaenicia sericata*.

A The axons of many optic glomeruli (op glom; bracketed) from the lobula and lobula plate (Lo P) converge and terminate in knots of dense neuropils (inset) in the LPro. Op glom lie adjacent to the lateral horn (L Ho).

B When an optic glomerulus is stained with silver as is shown here, the convergence of inputs from specific retinotopic layers in the Lo is revealed. Thus, each optic glomerulus represents a specific type of visual input, perhaps image-motion specific. The inset illustrates the difference between fine retinotopic fibers ascending to a glomerulus from the Lo (arrow) versus stout fibers of the large tangential horizontally sensitive (HS) neurons from the Lo P.

C Reconstruction in top-down (horizontal) view of the entire suite of op glom that were characterized in the lateral protocerebrum of *Phaenicia sericata*, illustrating their position with regard to the central complex (CC), the ipsilateral mushroom body (MB), and Lo, Lo P, and medulla (Me).

D Double-stained confocal image of fibers in an optic glomerulus (green) amidst fibers from descending neurons (magenta) that reveal no direct connections between the two neuronal types. The optic fibers in this image are located in the green optic glomerulus labeled *D* in (C).

E This image shows as well that the fibers of descending neurons (magenta) do not synapse with the fibers (green) of three op glom labeled E1, E2, and E3, whose locations in the LP are indicated in green in (C). Thus, it appears that it may be the local neurons not visualized in these images that interconnect op glom and also provide the synapses that descending neurons articulate with.

SOURCE: From Strausfeld et al. (2007).

NOTE: Scale bars, 50 µm. Some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

related sensations indicative of the males either descending or ascending until pheromone was simultaneously presented.

But the most definitive and intriguing demonstrations of the integration of pheromone and visual flow stimuli come from studies of descending neurons in *L. dispar* by Olberg and Willis (1990) and in *Manduca sexta* by Kanzaki et al. (1991b). Descending neurons are premotor command neurons that receive inputs from the LAcLos and other protocerebral neuropils. Their axons project caudally through the subesophageal ganglion and neck toward their termination points in thoracic motor neurons in one or more of the three thoracic ganglia. The key areas in which descending neurons arborize in the protocerebrum are in the ventral medial and ventral LP (Olberg and Willis 1990; Kanzaki et al. 1991b, 1994; Wada and Kanzaki 2005; Iwano et al. 2010). These areas include both the LAcLo/VPrtC and the ILPt/LH, both of which seem to receive pheromonal, visual, and multimodal inputs.

Olberg and Willis (1990) performed recordings on descending neurons from the severed cervical (neck) connectives of male *L. dispar* ventral nerve chords (figure 10.7A). Seventeen

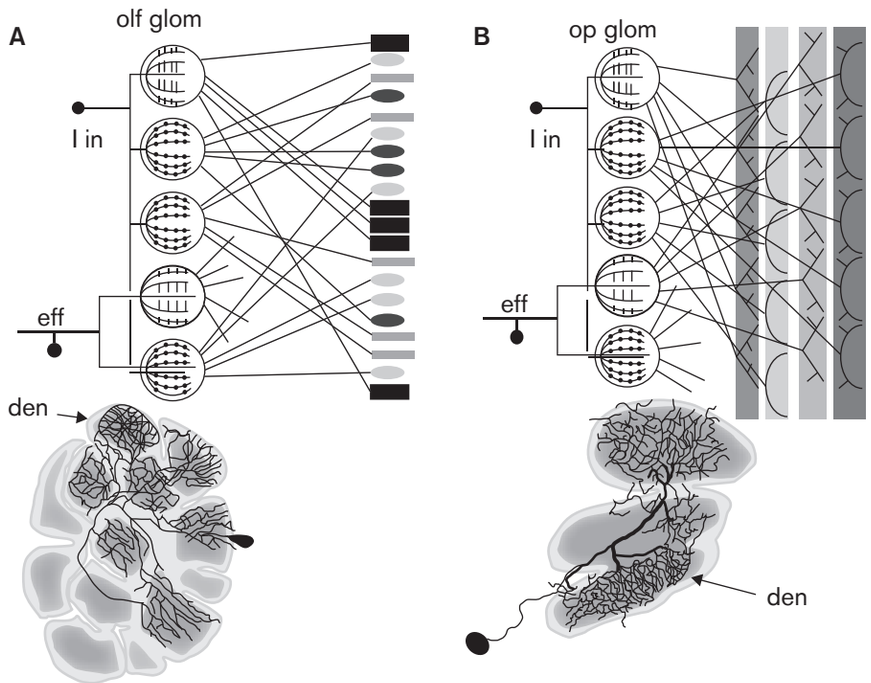

FIGURE 10.6 Schematic diagrams illustrating the similarities between olfactory and optic glomeruli that were recognized by Strausfeld et al. (2007) and the importance of local neurons in integrating the activities occurring among glomeruli in both olfactory and visual systems.

A Each olfactory glomerulus (olf glom) receives inputs from a specific type of identically odorant-tuned olfactory sensory neuron (differently shaped and shaded geometric figures at right). A local interneuron (l in) is illustrated as arborizing in different ways in different glomeruli to modulate and integrate the activities among the glomeruli. Olfactory system efferent neurons (eff) as depicted here are the projection neurons that arborize in different olfactory glomeruli and convey their integrated excitations to the mushroom body and inferior lateral protocerbrum/lateral horn in the protocerebrum. The arborization architecture of an olfactory local neuron in the glomeruli of an antennal lobe is illustrated in the lower left (den, dendrites of the local interneuron).

B Each optic glomerulus (op glom) is illustrated here as receiving vision-specific inputs, including image-motion-specific inputs, from different layers of the lobula, illustrated in different shades of gray on the right. A local interneuron (l in) is shown arborizing in the optic glomeruli, with an efferent neuron (eff) conveying the integrated outputs to other protocerebral neuropils or to thoracic ganglia. Arborization architecture of a visual local interneuron distributing its dendrites (den) among three optic glomeruli is illustrated in the lower right.

SOURCE: From Strausfeld et al. (2007).

NOTE: Please note that some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

of 70 pheromone-responding descending neurons responded to visual left–right motion in front of the male, and nine of these neurons were also directionally motion sensitive (responding only to either leftward or rightward movement of a visual grid pattern). Five of these neurons exhibited significantly heightened response to the visual grid motion in their preferred direction when 100 ng of *L. dispar* pheromone had been simultaneously presented to the male (figure 10.7B). The initial arborization locations of the pheromone-enhanced directional motion-sensitive neurons were in the posterior LP before they projected their axons caudally to the pterothoracic ganglia (figure 10.7A).

Kanzaki et al. (1991b) found descending neurons in *M. sexta* that responded with LLE of the greatest intensity in response to the optimal two-component pheromone-blend ratio compared to a single component. Several of the neurons were multimodal (visual-olfactory), being not only responsive to the pheromone blend but also to lights-on and lights-off, as well as to hand movement from the researchers. A few of these multimodal neurons changed firing status *only* when pheromone and visual stimuli were presented closely together in time. Thus, many of the descending neurons were visual-olfactory multimodal neurons, exactly the type of neuron that should be important to pheromone source location by a flying male moth. Kanzaki et al. (1991b), and others (Li and Strausfeld 1999; Strausfeld and Li 1999; Strausfeld 2003), have pointed out that this multimodal integration will most likely have already occurred in protocerebral neuropils before being synaptically transmitted to descending neurons and then to pterothoracic ganglia motor neurons.

Response to Pheromone Strands Requires High-Speed Temporal Integration of Pheromone Spectral Odor Space with Visual Inputs

High-temporal-resolution pathways that represent changes in molecular flux at the OSN level up through higher centers in

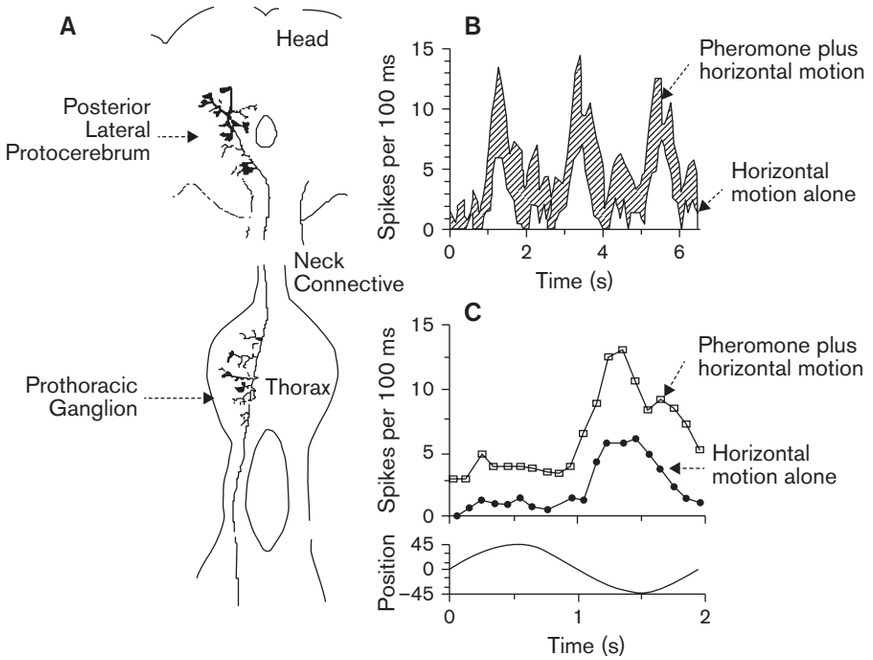

FIGURE 10.7 Descending neuron in *Lymantria dispar* that exhibited heightened responsiveness to the horizontal motion of a bar pattern across its eyes when sex pheromone was presented simultaneously with the motion.

A Top view of the stained descending neuron whose postsynaptic arbors in the posterior lateral protocerebrum projected and arborized with presynaptic arbors in the thoracic ganglia on the ipsilateral side.

B Response of this neuron to 1/sec rightward bar motion alternating with 1/sec leftward motion. Lower (white) curve is the action potential frequency of the neuron in clean air with peaks every 2 sec during rightward bar motion. Upper (darkened) curve is the response of the neuron to the same bar motion in the presence of pheromone.

C A single cycle of bar motion (lower curve) with bar position through time indicated at 45° to the left or right in front of the moth. Middle curve shows action potential frequency in response to the bar in clean air and upper curve is the response of the same neuron to the same bar motion in the presence of pheromone.

SOURCE: Adapted from Olberg and Willis (1990).

the brain should be a crucial part of successful, sustained upwind flight to the source. Upwind flight behavior in response to a species' pheromone blend is known to be elicited on a strand-by-strand, clean-air-pocket-by-clean-air-pocket basis (Mafra-Neto and Cardé 1994; Vickers and Baker 1994). Optimal upwind progress toward a pheromone source also includes a high-temporal-resolution assessment of the positions of pheromone strands in spectral odor space. Contact by a flying male with a single conspecific pheromone strand tainted with a heterospecific behavioral antagonist results in a truncated upwind turn-reversal compared to a longer duration reversal after contact with a strand of the pure pheromone alone (Vickers and Baker 1997; Quero et al. 2001). Similarly, upwind flight is compromised even if the components of the correct blend are emitted separately in a staggered manner such that they arrive in separate, interleaved strands but at what should be a behaviorally optimal sub-second frequency (Vickers and Baker 1992). Thus, temporal resolution of strand ONs and OFFs with apparent image motion is not the only aspect involved in response to pheromone. The visual reaction to wind, the latency to a turn-reversal, or both depend also on the position of the single pheromone strand in pheromone-odor space.

Response Properties of Neurons and Neuropils along Moth Sex Pheromone Olfaction Pathways

The sex pheromone blends of moths are usually comprised of two, sometimes three (or more), chemical components that evoke upwind flight and source location when they are emitted at precise ratios (see Allison and Cardé, this volume). Each of the pheromone OSNs that is tuned to respond optimally to each component is an achromatic neuron, and nearly all of the neurons that conduct pheromone-component information further up into higher levels of the brain are also achromatic (i.e., they have not integrated pheromone-component blend-ratio information even at these deeper projection zones of the protocerebrum). When the inputs of two or three types of pheromone-component-tuned achromatic neurons are integrated eventually by higher order neurons, these neurons are producing *chromatic* pheromone-odor sensations that can be pinpointed by the moth at some location in spectral odor space. It has become increasingly clear, however, that very few chromatic pheromone-blend-sensitive neurons have been shown to exist in the male moth brain. The majority of the

neurons involved in the sex pheromone olfaction pathways of moths have been found to remain achromatic, even deep into the protocerebrum.

Trichoid Sensilla

The functional unit on the antennae of male moths for acquiring, detecting, and processing molecular flux is the *sensillum trichodeum*. The neuroanatomies and biochemical interactions occurring in trichoid sensilla have been discussed in expert manner in Leal (this volume). We add to that overview by providing perspective regarding the various important roles these sensilla play in reporting the ONs and OFFs from pheromone strands in plumes that need to be integrated with vision for optimal performance of pheromone-mediated flight.

Trichoid sensilla are organules (Lawrence 1966) that have been shaped by evolution as flux detectors and not concentration measurers (Kaissling 1998), very likely due to the need of the male moth to behaviorally respond as rapidly as possible to individual pheromone strands and the pockets of clean air between strands that comprise the fine-grained structure of an airborne pheromone plume (Baker and Haynes 1987; Baker 1990; Kaissling 1990; Mafra-Neto and Cardé 1994; Vickers and Baker 1994). For reporting changes in flux, a flux detector does not allow the system to come into equilibrium, but rather concentrates the molecules at onset and rarifies them at offset (Kaissling 1998). Flux detectors thus accentuate the onset and offset of the signal. OSNs are the reporters of highly processed pheromone-component flux information and operate as the final step in a complex chain of biochemical events within the sensillum (see Leal, this volume) for transducing chemical stimuli into electrical impulses toward the brain.

Pheromone-Component-Responsive Olfactory Sensory Neurons

Pheromone-component-tuned OSNs of male moths reside in the tens of thousands of trichoid sensilla on male moth antennae and by far dominate the antenna in their abundance. Each type of OSN that is tuned specifically to one of the pheromone components of that species' blend projects with 100% fidelity to a dedicated, component-specific glomerulus within the male-specific McGl of the AL (figure 10.1A, AL) (Hansson et al. 1991, 1995; Ochieng' et al. 1995; Todd et al. 1995; Berg et al. 1998, 2005; Lee et al. 2006a, 2006b). Trichoid sensilla with their OSNs must adsorb, transport, and transduce to action potentials the vanishingly small numbers of molecules. Through their spike frequencies, on a subsecond pheromone-plume-strand basis, the OSNs differentially tuned to each component report the relative molecular abundances of their particular components in each strand to their target glomeruli in the McGl.

One stereotypical feature of OSNs is that their dendrite diameter (thickness) is positively correlated with the relative abundance of the component to which they are tuned. In sensilla housing co-compartmentalized OSNs (for which dendrite diameters can be directly compared), the major component-tuned OSNs have a larger action potential amplitude and their dendrites are wider in diameter (Hallberg et al. 1994); thus, they also have a greater dendritic surface area than the dendrites of minor component-tuned OSNs. Baker et al. (2012) suggested that this phenomenon is due to the OSNs tuned to a major component needing to have a higher working range of flux-handling ability to accommodate the higher ranges of ups and downs in molecular flux than do the OSNs tuned to a minor component. The ratios of some two-component pheromone blends are as severe as 100:1 major component to minor component; thus, OSNs tuned to the major component must handle strand-to-clean-air flux ranges (100 vs. 0) of the major pheromone component in each strand that are 100 times what needs to be handled (1 vs. 0) by the minor pheromone-component-tuned OSNs in these same strands. The larger diameter would not necessarily make the major component-tuned OSNs more sensitive to that component, but rather would allow these OSNs to accurately report both the major component's extremely high peak fluxes in strong strands and its very low or zero fluxes in weak strands or clean-air pockets in the plume (Baker et al. 2012).

OPTIMAL RESOLUTION OF PHEROMONE IN BOTH ODOR SPACE AND ODOR TIME BY CO-LOCALIZATION OF OLFACTORY SENSORY NEURONS WITHIN SINGLE TRICHOID SENSILLA

Across all moth species, certain pairs of differentially tuned pheromone-component-sensitive OSNs are co-compartmentalized within the same trichoid sensillum (figure 10.1A, OSNs). This co-compartmentalization is found whether the OSNs are involved in detecting and reporting conspecific pheromone components whose ratios are crucial for optimal attraction, or they are involved in detecting and reporting heterospecific pheromone components that behaviorally antagonize attraction. The reasons for such close and stereotypical pairings have been proposed to be due to the need for optimizing the reporting of blend-ratio quality (odor space resolution) as well as the optimal reporting of synchronous arrival of components (odor time resolution) (Todd and Baker 1999; de Bruyne and Baker 2008). The best way to optimize both odor space and odor time reporting of strands is to place the two differentially tuned sensors as close together as possible so they can report molecular flux changes on a subsecond basis from the same spot at the same time (Baker et al. 1998; Todd and Baker 1999; de Bruyne and Baker 2008).

Synchronous arrival of pheromone components to an OSN in a sensillum would indicate that they are in the same strand and thus will have originated from a conspecific female's pheromone gland. Conversely, an asynchronous arrival of pheromone-related compounds will indicate that the compounds are in separate, interleaved strands that thus must have originated from two separate females, one of which may be a heterospecific female (Todd and Baker 1999; de Bruyne and Baker 2008).

The sex pheromones of hundreds of species of moths, especially in the Tortricidae, Crambidae, and Yponomeutidae, rely on extremely narrow differences in pheromone-component ratios of the same components common to many species in these families (see Allison and Cardé, this volume; Löfstedt et al., this volume). These definitive narrow-ratio ranges achieve a unique communication channel for each species. In these particular families, OSNs that are tuned to the two most vital pheromone components in a blend, and whose narrow range of ratios are critical to male attraction, are usually

paired within the same sensillum. Such pairings will allow the two-component ratios from strands having both strong or weak flux to be equally well reported. These pairings also can allow high-fidelity reporting from OSN pairs in sensilla on one end of the antenna receiving a strong portion of a strand as well as pairs on a different portion of the antenna receiving a weaker flux from the same strand (Todd and Baker 1999).

For species in other families, such as the Noctuidae, blend ratios do not seem to be as critical for optimal conspecific attraction, and they can vary across quite a large range before attraction becomes reduced at the extremes. In noctuids, co-localization of major and minor sex pheromone-component-tuned OSNs in the same sensillum has not yet been demonstrated (De Bruyne and Baker 2008). It remains to be seen whether this has constrained the evolution of finer blend-ratio tuning or rather is the result of the lack of a need to have such fine-grained odor space–odor time resolution.

However, even in the Noctuidae, OSNs that respond to heterospecific pheromone components antagonistic to upwind flight behavior are co-localized within the same sensillum along with at least one type of OSN tuned to one of the conspecific pheromone components. Such OSNs are often broadly tuned to more than one heterospecific ligand and serve as a sort of catchall for heterospecific behavioral antagonists (cf. Grant et al. 1988; Hansson et al. 1990, 1994; Almaas and Mustaparta 1991; Almaas et al. 1991; Todd et al. 1992; Ljungberg et al. 1993; Berg et al. 1998, 2005; Cossé et al. 1998; Baker et al. 2004; Linn et al. 2007; Koutroumpa et al. 2014).

The temporal resolution of odor is exquisitely fine-grained even when OSNs do not co-reside in the same sensillum. The asynchronous arrival of two conspecific sex pheromone components has been shown to reduce the success of upwind male flight compared to when the two components arrive synchronously in each strand (Vickers and Baker 1992). In *Heliothis virescens* (Noctuidae), when the minor sex pheromone component, Z9-14Ald, was alternated in puffs at 5/sec with the major component, Z11-16Ald, also puffed at 5/sec, for a total puff-frequency of 10/sec (staggered), source contact by males was significantly lower than the complete blend co-emitted synchronously from the same cartridge at 5/sec. This meant that for this species, separate pheromone components arriving asynchronously and separated by only 0.1 sec did not produce the same quality odor space sensation as they did when they arrived simultaneously on the antenna. In *H. virescens*, the major- and minor-component-tuned OSNs, although not co-localized in the same sensilla (see Hillier and Baker, this volume), are housed in neighboring sensilla in the same row and are thus nearest sensillar neighbors, as close together as possible on an intersensillar basis (Lee and Baker 2008). The distance between the two types of differentially tuned OSNs in different sensilla was found to be only 7.4 µm, whereas the nearest sensillum in the next row was 26.9 µm, nearly four times as far (Lee and Baker 2008).

The odor space–odor time resolution provided by OSNs in response to heterospecific pheromone-component antagonists also has been shown to be extremely high. The heterospecific antagonist to *Helicoverpa zea* (Noctuidae) male attraction, Z11-16Ac, is not as antagonistic to *H. zea* upwind flight if it arrives in strands separated by even just 1 ms (or 1 mm) from strands of the two-component pheromone, Z11-16Ald plus Z9-16Ald (Baker et al. 1998; Fadamiro et al. 1999). The OSN tuned to Z11-16Ac is a small-spiking OSN that is housed in the same sensillum as the larger-spiking OSN tuned to the Z9-16Ald pheromone component (Cossé et al. 1998).

Antennal Lobe Local Neurons

The AL of the male moth is its primary olfactory center. After information leaves the AL to synapse in protocerebral neuropils, it will most certainly be integrated with additional stimuli from other modalities. The AL is an exceedingly complex structure, both morphologically and physiologically. It may rival portions of the insect optic lobe with regard to the complexity of layers upon layers of cross-linked LN–LN neuronal processing circuits (Strausfeld 2012). LNs can temporally shape the outputs of achromatic PNs as well as change the ranges of their molecular receptive fields. Changes in the strengths or polarities (inhibition vs. excitation) of LN synaptic connections can take activities of the antennal reports of OSNs and diminish or broaden the outgoing "views" of odor space that are transmitted to the MBCs by achromatic PNs (Martin et al. 2011).

The incoming signals of each of the differentially tuned OSNs to their respective target glomerulus in the McGl are integrated via synaptic interactions of "intrinsic" neurons of the AL, the LNs, that have no axons and so do not project outputs exiting the AL. Multiple layers of LNs in the McGl and the rest of the AL process many features of incoming information about odorant flux and the relative abundances of odorant molecules in as yet incompletely understood ways. The neuronal activity outcomes of these myriad LN interconnectivities can only be assessed by painstaking and precise physiological studies of the output activities of PNs in the context of their AL glomerular arborization locations. Their projection destinations in the MBCs and ILPt also need to be identified to characterize the type of pheromonal information transfer. The LNs, which function as laterally spreading amacrine cells, can impose lateral inhibition on OSN axons, on other LNs, and on PNs in neighboring glomeruli as well as within their own glomerulus (Anton and Homberg 1999).

In addition to their varied morphologies and arborization patterns within and between glomeruli in the AL, LNs have more recently been found to be quite dynamic in their physiologies. A new generation of studies has shown, in several different moth families, that the processing of pheromone in the McGl and plant odorants in the ordinary glomeruli of the AL is not as segregated as was previously thought: tortricids *Cydia pomonella* (Yang et al. 2004; Trona et al. 2010, 2013) and *Grapholita molesta* (Varela et al. 2011); bombycid *Bombyx mori* (Namiki et al. 2008); and sphingid *Manduca sexta* (Reisenman et al. 2008; Heinbockel et al. 2013). Outputs of McGl PNs can be altered by mixtures of pheromone-plus-plant odorants due to LN interactions that reshape the PN outputs, sometimes in an asymmetric manner favoring increased pheromone-PN output but not plant volatile-PN output. Sometimes, a pheromone-plus-plant volatile blend has been found to suppress the output of the pheromone-tuned McGl PNs, as in male *Agrotis ipsilon* (Noctuidae) (Chaffiol et al. 2012; Deisig et al. 2012), but to increase its temporal acuity to respond to sub-second blend-odor pulses compared to pheromone alone (Chaffiol et al. 2012). Dekker and Barrozo (this volume) provides a complete treatment of this subject.

LOCAL NEURON MORPHOLOGY

LNs have diverse morphologies, and as pointed out by Martin et al. (2011), they transmit pheromone-component flux information through inhomogeneous interglomerular

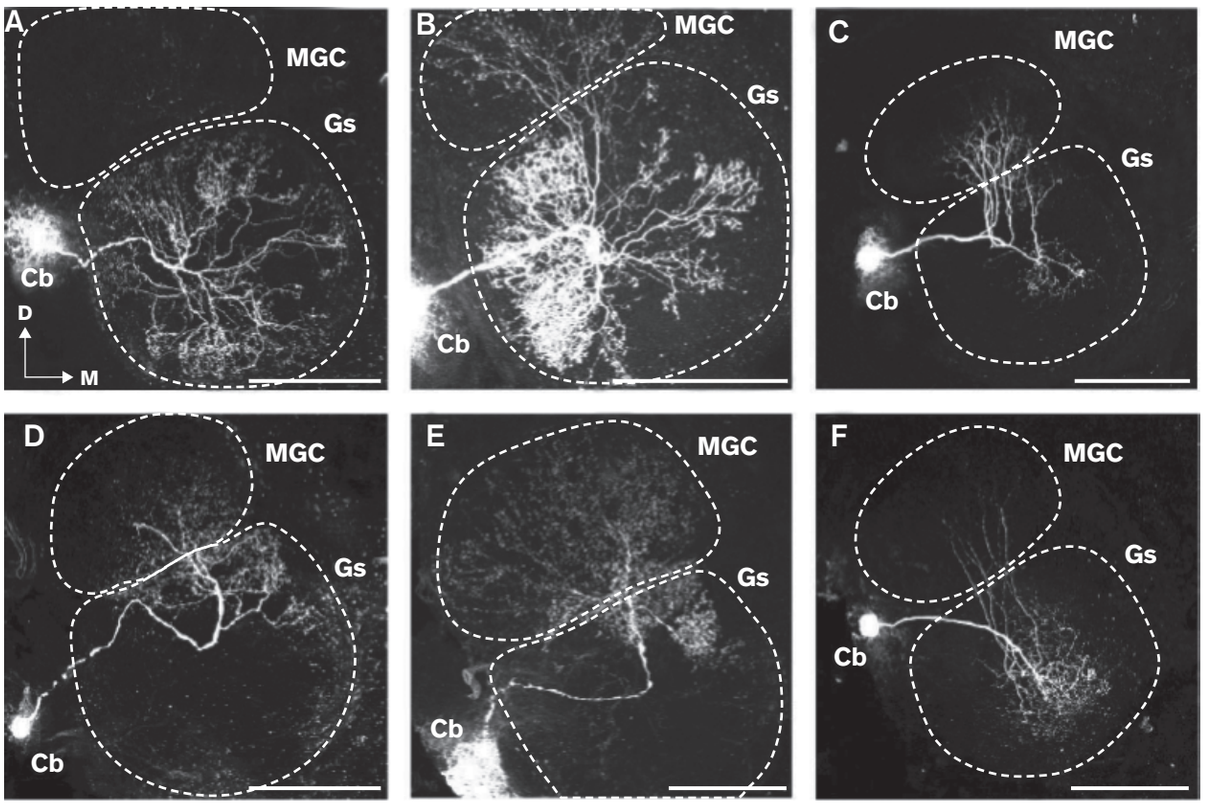

FIGURE 10.8 Examples of the wide variety of morphologies exhibited by the antennal lobe local neurons of *Bombyx mori*, with wide-field global as well as asymmetric and sparse local neuron arborizations occurring among ordinary (Gs) and macroglomerular complex (MGC) glomeruli. (A) Some local neurons arborize only among ordinary glomeruli and do not visit the MGC. (B–F) Various combinations of wide-field versus narrow-field, symmetric versus asymmetric local neuron arborizations. Cb, cell body. Arborizations may be sparse or profuse (C and D vs. A and B). Local neurons may exhibit extreme differences in the intensities of the varicosities of their arbors both from one antennal lobe zone to another, e.g., ordinary glomerulus zone to MGC as in (B) and (E) and within only one zone (A). Hundreds of local neurons are estimated to be in the antennal lobes of *Manduca sexta* and *B. mori*, so it is noteworthy that so much variation in local neuron morphology can be exhibited by just this handful of stained local neurons.

SOURCE: From Seki and Kanzaki (2008).

NOTE: All scale bars, 100 µm. Please note that some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

connections and either excitatory or inhibitory synapses across varied ensembles of glomeruli. Some wide-field LNs interconnect McGl glomeruli with ordinary glomeruli across the entire AL (figures 10.8A and 10.8B) and may be involved in the upregulation or downregulation of overall "gain" (global sensitivity-activity levels) of the entire ensemble of glomeruli (Martin et al. 2011). Other LNs, denoted as "relay LNs" (Strausfeld 2012), have more localized interconnections with particular glomerular ensembles (figures 10.8C–10.8F) (Matsumoto and Hildebrand 1981; Christensen et al. 1993; Anton and Hansson 1995; Seki and Kanzaki 2008; Reisenman et al. 2011). These more restricted interconnections may serve to sharpen interglomerular ratios of activity via inhibitory GABA transmission or to heighten activity in one direction, e.g., from plant odor-plus-pheromone blends to heighten McGl glomerular excitation while either suppressing or not affecting ordinary glomerular excitation (Namiki et al. 2008; Trona et al. 2010; Chaffiol et al. 2012). More global LN networks in the AL may serve to broaden or narrow the ranges of PN output activities exiting ensembles of AL glomeruli (Martin et al. 2011) via their up- or down-modulation by biogenic amines or neuropeptides from centrifugal neurons (Anton and Homberg 1999) that visit the AL from areas deeper within the protocerebrum (Homberg et al. 1988). The arborization patterns of LNs *within* the volume of a glomerulus and the varied types of fine vesiculations of their dendrites are also highly variable. Some LNs arborize only at the perimeter of a glomerulus, some only in the core, and some in only one hemisphere of the glomerulus or the other, whereas other LNs invest the entire glomerular volume (Seki and Kanzaki 2008; Reisenman et al. 2011).

LOCAL NEURON PHYSIOLOGY: GABA-ERGIC LATERAL INHIBITION

The majority of AL LNs in moths are inhibitory GABA-ergic (GABA-releasing) neurons and impose lateral inhibition on neighboring glomeruli and on the neurites within individual glomeruli (Hoskins et al. 1986; Waldrop et al. 1987; Homberg et al. 1988, 1990; Seki and Kanzaki 2008; Reisenman et al. 2011; Heinbockel et al. 2013). Combinations of cross-glomerular LN synaptic inhibition via their impositions on various combinations of other McGl AL neurons are able to produce PN outputs to protocerebral centers that convey a more precise temporal representation of pheromone odor-

strand flux (Waldrop et al. 1987; Christensen and Hildebrand 1988, 1997; Christensen et al. 1993, 1998, 2000, 2003; Heinbockel et al. 1999, 2004, 2013). However, precise "tracking" of major pheromone-component strands (pulses) has been demonstrated to occur in PNs of other species without any extra temporal sharpening occurring from the addition of secondary pheromone components via LN-related lateral inhibition (Vickers et al. 1998; Lei and Hansson 1999; Vickers et al. 2001, 2005; Vickers 2006).

Lateral inhibition by LNs that improve the ability of AL PNs to follow pulsatile stimulation with pheromone components has been shown to occur in LNs arborizing only in ordinary glomeruli, with no arborizations in the McGl (Heinbockel et al. 2013). These ordinary glomeruli-spanning LNs must thus be being inhibited by other LNs that arborize in the McGl and synapse with them in some way (Heinbockel et al. 2013). Using multielectrode recording techniques, Christensen et al. (1993) showed that LNs have the ability to excite otherwise silent PNs in specified glomeruli via GABA-ergic LN–LN synaptic disinhibition. Lateral inhibition imposed on another inhibitory LN can thus disinhibit an output PN and create a different output pattern using the same hardwiring but with different GABA-related outcomes according to which odorant blend is presented to the antenna.

Although GABA-ergic inhibitory LNs have been most commonly described thus far, there are indications that there are other types of LNs that use other neurotransmitters that may not be inhibitory, even releasing different types of neurotransmitters and neuropeptides that can shift subsequent PN activities within the AL in different directions (Homberg et al. 1990; Berg et al. 2007; Utz et al. 2008). Berg et al. (2007) found several neuropeptides present in the AL LNs of *Heliothis virescens*, including A-type allatostatins, *Manduca sexta* allatotropin, FMRFamide-related peptides, and tachykinin-related peptides.

LOCAL NEURON PHYSIOLOGY IS MALLEABLE AND INFLUENCED BY AGE, MATING STATUS

The PN outputs of male *Agrotis ipsilon* (Noctuidae) in response to pheromone or plant odorants were shown to change significantly depending on the physiological state (e.g., mated vs. unmated) or age (young vs. old) of the moth. These differences in AL LN modulations of PN outputs were proven to be due at least in part to alterations in juvenile hormone (JH) levels (Anton and Gadenne 1999; Anton et al. 2007).

In further studies with *A. ipsilon*, during a refractory period after copulation, males were shown to lose interest in mating (Barozzo et al. 2011). During the 24 h after mating, the pheromone sensitivity of AL PNs decreased, but OSNs responsive to pheromone components remained at the same level of sensitivity, as did AL neurons processing plant-related odors (Barrozo et al. 2010, 2011). The mating-induced modulation of sensitivity thus was occurring at the AL level and was restricted to PN outputs that resulted from AL LN–PN processing. After mating, JH concentration drops to a level comparable to that of immature males, so here again JH seems to be playing an important role.

McGl PN sensitivity to pheromone can also increase within minutes due to preexposure to pheromone, which again seems to orchestrate LN modulation of AL activity along with sensitization of OSNs due to preexposure (Anderson et al. 2003; Guerrieri et al. 2012). In experiments involving male *Spodoptera littoralis* preexposed to pheromone, Guerrieri et al. (2012) found that the major pheromone-component-specific McGl glomerulus became significantly enlarged after behaviorally relevant preexposures; extra synaptic interactions there could explain an observed increased behavioral response by preexposed males. Together, these findings indicated that modulation of AL LNs can increase the overall gain of PNs and help the male become more sensitive to calling females in a matter of minutes after first receiving a brief pheromone exposure.

A final consideration regarding the output malleabilities of the LN networks in the AL is the knowledge that centrifugal neurons (Homberg et al. 1988) originating in regions of the protocerebrum, including the pars intercerebralis and subesophageal ganglia, project back out to the AL. These neurons are known to release various neuromodulators, such as histamine, dopamine, serotonin, and neuropeptides, that can change the input–output gain of AL LNs, or shift the balance of neurotransmission activity between and among asymmetric LNs of the AL (Homberg et al. 1990; Homberg and Hildebrand 1991). Thus, the AL is not just a relay station for more refined blend-ratio reporting and precise temporal renderings of pheromone-strand onsets and offsets. Rather, the interactions among LNs can be adjusted and affect the outputs of PNs that conduct action potentials to higher protocerebral centers, significantly altering the olfactory information that is transmitted to these integrative deep-brain locations.

Antennal Lobe Projection Neurons

After all the highly complex processing that goes on in the AL from various interactions between OSN inputs, LN–LN interactions, and LN–PN interactions, the outputs from the McGl glomeruli are carried by PNs from the deutocerebral AL level of the brain to two higher olfactory centers in the protocerebrum: the MB, arborizing in its calyces, and the ILPt.

RECONCILING RESULTS OF VARIOUS STUDIES ON MOTH PROJECTION NEURON MORPHOLOGIES AND PHYSIOLOGIES

The study of moth protocerebral neuroanatomy is an extremely difficult and slow-going endeavor. It is rare that a researcher can impale a PN and record from it for a long enough period of time to run through a full palette of stimuli, and then inject a visible dye with sufficient success to trace its anatomy from the McGl to either the MBC or the ILPt. In addition, only a few research groups, with a few moth species, have incorporated rapidly pulsed pheromone-component stimulation into their multi-pheromone-component-and-blends stimulus regimes, with the majority using just a single stimulus pulse. Thus, it is difficult to compare complete neuroanatomical results across species because attention to temporal aspects of the stimulus has not routinely been addressed by all research groups. It is common for staining of neurons to seem to have been accomplished only to find later that it failed or was incomplete.

Thus, it is not surprising that tens of thousands of PNs of various species have been impaled over the decades, but very few have had their physiological response profiles to pheromone components and pulsatile stimulations fully characterized and in addition had their full anatomies revealed

through complete staining to protocerebral destinations along their entire lengths. Inability to accurately visualize the structural details of the neuropil destinations targeted even by completely stained PNs is another factor that has limited more precise descriptions of the targeted neuropils and perhaps the functions of many of these PNs' transmissions.

Most of the earlier studies, such as on *Manduca sexta* (Christensen and Hildebrand 1988; Kanzaki et al. 1989), traditionally impaled pheromone-sensitive PNs from the medial cell cluster of the AL, an area that has been found throughout the decades to be highly populated with the cell bodies of PNs that use the medial antennoprotocerebral tract (*m*-APT) route to the MBCs and then to the ILPt. However, the departure routes of a few of these medial cell cluster PNs in which complete staining was accomplished has shown that a small percentage of these PNs bypass the MBCs and use the mediolateral antennoprotocerebral tract (*ml*-APT) route (cf. Seki et al. 2005; Namiki et al. 2013). Thus, assessing a stained neuron as having a cell body in the medial cell cluster and exiting on what appears to be the *m*-APT (to first visit the MBCs) might not always be correct.

Results from many moth species have shown that when recording from PNs whose cell bodies reside in the lateral cell cluster of the AL, pheromone-sensitive PNs nearly always will project via the *ml*-APT or lateral antennoprotocerebral tract (*l*-APT) directly to the ILPt and terminate there (Vickers et al. 1998; Vickers and Christensen 2003; Vickers 2006; Kárpáti et al. 2008, 2010). However, only more recently has more attention been paid to recording from lateral cell cluster PNs, and researchers are thus starting to gather more information about the physiologies of these *l*-APT PNs, which seem to have a greater tendency to be blend synergist (chromatic) PNs.

PROJECTION NEURON MORPHOLOGIES: THREE AXONAL TRACTS TO PROTOCEREBRAL NEUROPILS

The major PN tract leading to the protocerebrum is the *m*-APT (Galizia and Rössler 2010) (figure 10.1), formerly known as the inner antenno-cerebral tract (Homberg et al. 1988). This is also the route on which most of the neurophysiological recordings and stainings of pheromone PNs have been performed. These PNs, whose cell bodies characteristically reside in the medial cell cluster of the AL (toward the midline of the brain), send axons toward the MB, with collaterals visiting and arborizing in the MBCs before their main axons continue on to terminate in the ILPt.

The MB is the dominant, most distinctive neuropil in the protocerebrum of insects (Strausfeld 2003, 2012; Farris 2005; Strausfeld et al. 2009). In the Lepidoptera, the MB on either side of the protocerebrum has a complex structure with prominent, paired, conjoined calyces occurring dorsally on the pedunculus (stalk) (figures 10.4A and 10.4C) (Pearson 1971; Sjöholm et al. 2005, 2006; Rø et al. 2007; Sinakevitch et al. 2008; Fukushima and Kanzaki 2009). The pedunculus furcates vertically and medially into vertical (alpha), medial (beta), and gamma lobes of the MBLs. In the Lepidoptera, there is another distinct MBL, the Y-lobe (figure 10.4A) (Pearson 1971; Sjöholm et al. 2005, 2006; Rø et al. 2007; Sinakevitch et al. 2008; Fukushima and Kanzaki 2009).

Tens of thousands of intrinsic KCs run in parallel along the lengths of the MBs, from the calyces down through the ends of the lobes (figure 10.4D). The MB is somewhat similar to the AL in that it consists primarily of intrinsic LNs, but in the MB these intrinsic cells appear to us as highly organized arrays of KCs that, because of their number and density, give the MB its characteristically stalked, multilobed shape (figure 10.4A). The swollen region of KCs comprising the MB calyces has repeatedly been shown to be the major MB reception area for sensory afferents in insects (Strausfeld 2003, 2012; Farris 2005; Strausfeld et al. 2009). However, the MBLs have more recently been identified as also being significantly involved in receiving many types of sensory and multimodal inputs (Strausfeld 2003, 2012; Farris 2005; Strausfeld et al. 2009).

Pheromone-sensitive PNs that arborize in the outer zone of the MBCs do so using tiny synaptic connections that are considered to comprise "microglomeruli" and are called boutons (figures 10.4C and 10.4E), each with excitatory–inhibitory PN-GABA-ergic integrative capabilities (Szyszka et al. 2005). The synaptic pattern of PN/KC boutons on the array of KC parallel fibers (figures 10.4D and 10.4E) has the potential to produce great numbers of different cross-fiber patterns on KCs from the inputs of many PNs, each with their own bouton distributions and different physiological response profiles. This vast array of achromatic temporally precise pheromone-component PN input patterns can be integrated across KCs to resolve pheromone-odor time and odor space as well as be integrated with any visual image-motion inputs that might arrive at the MB directly from the optic lobe (figures 10.4B and 10.4C).

A large proportion of the KCs in moth MBs are GABA-ergic along their lengths of the MBLs, as well as in the MBCs, and should impose sharpening and "sparsening" of the integrated olfactory pattern in the MBCs to be transmitted through the lobes (Szyszka et al. 2005). There are many other neuromodulatory and neurotransmitter substances present in KCs; so, highly complex integration of odor, visual, and other modality inputs should be expected throughout the MB. Recurrent neurons also are known to provide feedback between the lobes and calyces and can thus modulate the activities of the KCs throughout the MBCs (Sjöholm et al. 2005; Rø et al. 2007; Sinakevitch et al. 2008; Fukushima and Kanzaki 2009). All of these activities add to the ability of the MB to be a highly refined integrator of temporally precise achromatic pheromone-component information into many types of outputs, possibly even strand-specific chromatic odor space resolution that can be transmitted to other protocerebral neuropils.

A second McGl PN route is out from the McGl glomeruli through the *ml*-APT (Galizia and Rössler 2010) (figure 10.1), formerly known as the middle antenno-cerebral tract (Homberg et al. 1988). These PN axons directly visit the ILPt and terminate with arborizations there, bypassing the MBs. They seem to have their cell bodies in either the lateral or the medial cell clusters (Vickers et al. 1998; Vickers and Christensen 2003; Vickers 2006; Kárpáti et al. 2008, 2010; Namiki et al. 2013).

The third route taken by PN axons that leave McGl glomeruli is called the *l*-APT (Galizia and Rössler 2010; figure 10.1), formerly called the outer antenno-cerebral tract (Homberg et al. 1988). The cell bodies of these PNs characteristically lie in the lateral cell cluster of the AL and their axons project directly to the ILPt, terminating with arborizations there and again bypassing the MBs (Vickers et al. 1998; Vickers and Christensen 2003; Vickers 2006; Kárpáti et al. 2008, 2010; Namiki et al. 2013).

Because all pheromone-component-specific information arrives at the ILPt from the three main APT pathways and the PNs of only one pathway visit the MBCs, it is becoming increas-

ingly clear that the ILPt of the protocerebrum is important for pheromone olfaction (Anton et al. 1997; Kanzaki et al. 2003; Kárpáti et al. 2008, 2010) and deserves an increasingly intense research effort to unravel more knowledge about male moth pheromone olfaction and behavior. In *Ostrinia nubilalis* (Crambidae), Anton et al. (1997) found that the arborizations of PNs in the ILPt were more extensive and profusely arborizing than in other moth species, and they remarked that this could mean that *O. nubilalis* PNs terminating in the LP might have a greater opportunity to be involved in "multimodal" integration there. Strausfeld (cf. 2003, 2012; Strausfeld et al. 2007) has emphasized that the LP, including the LH and ILPt, should be a place where olfactory and visual image-motion information is likely to be integrated because of the multitudes of optic glomeruli connected by networks of LNs residing there. Because the ILPt and LH, unlike the MB and its calyces, are comprised of ill-defined neuropil features, it has been difficult to delineate different subzones within them and thus to decipher anatomically how pheromone and general odors might be integrated there.

Two "pheromone-component-specific" achromatic PNs were identified in *Agrotis segetum* and found to be bilateral in their projections to both the contralateral and the ipsilateral LAcLos, also arborizing in corresponding ILPts on both sides (Wu et al. 1996). Thus far, these PNs are the only PNs in any moth species that have been found to directly connect the AL with the LAcLo and provide a potentially "reflexive" pathway (Wu et al. 1996) to behavioral response.

PROJECTION NEURON MORPHOLOGIES: ODORANT-SPECIFIC SYNAPTIC REGIONS IN THE MBCs

In moths, achromatic pheromone-component-sensitive PNs leaving the McGl along the *m*-APT route send collaterals to first arborize in the MBCs before continuing on to terminate with arborizations in the ILPt. In the MBCs, the PN collaterals synapse with multitudes of microglomeruli (Szyszka et al. 2005) on the intrinsic neurons of the MB, the KCs at characteristic locations around the outer rim zone of the MBCs (figures 10.4C and 10.9A–10.9C).

In *Bombyx mori*, these PN arborizations can be profuse (figure 10.9A) or sparse (figure 10.9B), depending on whether a bombykol pheromone component or bombykal behavioral-antagonist McGl glomerulus was the arborization origin of that PN (figure 10.9) (Seki et al. 2005; Namiki et al. 2013). Bombykol is the only definitive pheromone component known for *B. mori* (Butenandt et al. 1959). Bombykal was later identified from female *B. mori* glands, but curiously, it was shown to be behaviorally antagonistic when added to bombykol (Kaissling et al. 1978). Bombykal has distinct OSN pathways to the "cumulus" of the McGl that differ from the bombykol-tuned OSNs that arborize in the "toroid" of the McGl. The McGl glomerular integration of bombykal inputs with those of bombykol may have more to do with bombykal being a heterospecific antagonist (Daimon et al. 2012) than a *B. mori* pheromone component. Regardless, it is now also known that PNs of *B. mori* traveling along the *m*-APT from either the bombykal-sensitive cumulus or the bombykal–bombykol-sensitive "horseshoe" glomeruli of the McGl have synaptic boutons on the KCs of the MBC that exhibit broad overlap with boutons of PNs carrying general odorant information from AL ordinary glomeruli (figures 10.9B, 10.9C, and 10.10). In contrast, bombykol (pheromone)-responding PNs projecting to the

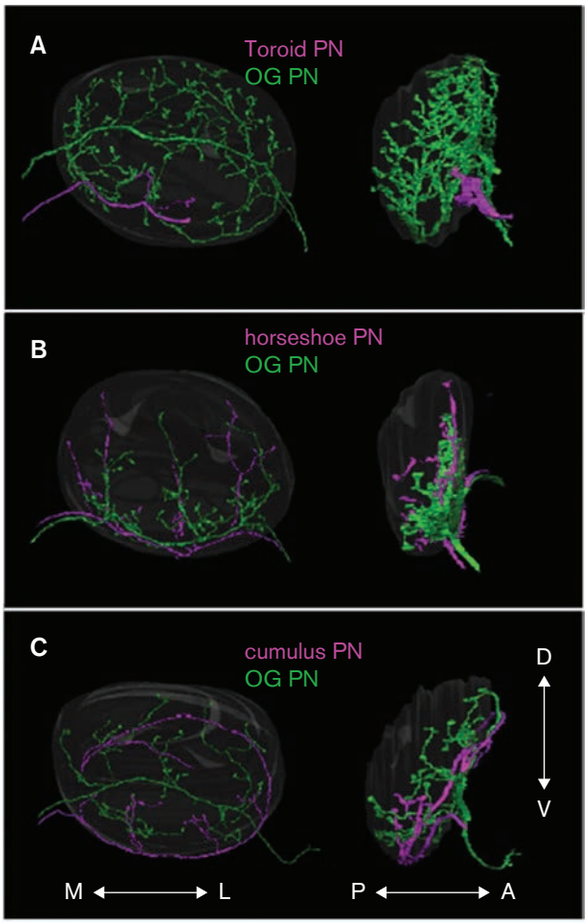

FIGURE 10.9 Confocal microscope images (A–C) of *Bombyx mori* projection neuron (PN) arborization locations in the mushroom body calyxes showing different arborization destinations of PNs carrying different pheromone-component-related information. Pheromone-component-related PNs projecting from glomeruli of the macroglomerular complex are labeled in green to compare with the arborization locations of plant-volatile-sensitive PNs (OG PN) projecting from ordinary glomeruli in the antennal lobe that are labeled in magenta. Top-down (horizontal) views of the mushroom body calyxes are on the left and side views (vertical) of the same preparations are on the right. Note the highly restricted varicose arbors of the PN from the toroid glomerulus of the macroglomerular complex (Toroid PN, green neurites in A) that reports bombykol-related activity, which do not overlap to any large degree with the arbors of neurons projecting from ordinary glomeruli in the antennal lobe (magenta neurites). Compare this arborization pattern to that of the PN from the cumulus of the macroglomerular complex (Cumulus PN, green arbors in B) that reports bombykal activity. The PN from the cumulus has more profuse varicose MBC arbors than the bombykol-reporting PN from the toroid glomerulus and these overlap with the arbors from an ordinary glomerulus PN (magenta in B). The mushroom body calyx arbors of the PNs from the horseshoe glomerulus of the macroglomerular complex (green neurites in C) also overlap to a much greater degree with the mushroom body calyx arbors of PNs projecting from ordinary glomeruli than do the mushroom body calyx arbors of the toroid PNs (A).

ABBREVIATIONS: M, medial; L, lateral; P, posterior; A, anterior; D, dorsal; V, ventral.

SOURCE: From Namiki et al. (2013).

NOTE: Some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

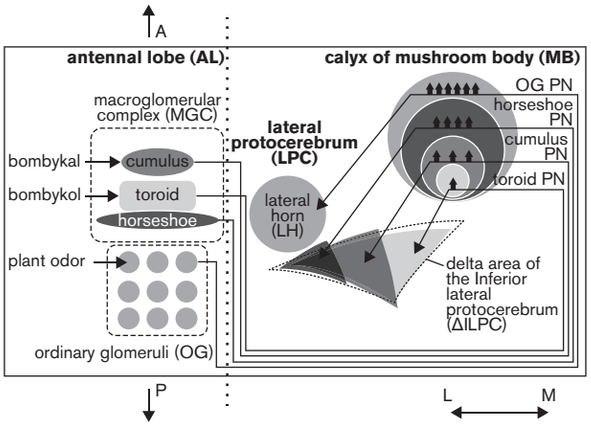

FIGURE 10.10 Illustration of the different semiochemical-related projection destinations that are now known for the medial antennoprotocerebral tract projection neurons (PNs) of *Bombyx mori* that are responsive to bombykol pheromone (toroid PN); bombykal (cumulus PN); and to either bombykol or bombykal or both (horseshoe PN) as well as to plant odorants (OG PN). PNs that arborize in the toroid glomerulus of the macroglomerular complex (MGC) and are responsive to bombykol project collaterals to a restricted area of the mushroom body calyx that arborize there before the main axons terminate with arbors in a more medial area of a pheromone-component-specific region ("delta area") of the inferior lateral protocerebrum, here called the ΔILPC. PNs arborizing in the cumulus of the MGC that are responsive to bombykal, a pheromone-related behavioral antagonist, send axon collaterals that arborize in a broader area of the mushroom body calyces before projecting their main axons to terminate with arbors in a more lateral region of the ΔILPC. A third type of PN that arborizes in the MGC does so in the horseshoe glomerulus, but usually it also has other arbors in the cumulus. This third type is responsive to combinations of bombykol and/or bombykal and sends collaterals to arborize in a still broader region of the mushroom body calyces before terminating with arbors in a lateral region of the ΔILPC. PNs responsive to plant-related volatiles that arborize in one, or in large combinations of various OG project collaterals to an even broader area of the mushroom body calyxes. These plant-volatile-sensitive PNs do not send terminal arbors to the ILPC, but rather terminate in another distinct lateral protocerebral neuropil called the lateral horn (LH).

ABBREVIATIONS: L, lateral; M, medial; A, anterior; P, posterior.

SOURCE: From Namiki et al. (2013).

NOTE: Some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

MBCs from the toroid glomerulus of the McGl have boutons arborizing within a narrow zone of the MBCs and have little overlap with MBC boutons from cumulus, horseshoe, or ordinary glomerulus PNs (figures 10.9A and 10.10). Similarly, in *Periplaneta americana*, two distinctive McGl PN arborization destinations in two subregions of the MBCs were observed, with one of the PN types responding to the major and the other responding to the minor pheromone component of this species (Nishino et al. 2012a).

PROJECTION NEURON MORPHOLOGIES: ODORANT-SPECIFIC SYNAPTIC REGIONS IN THE ILPt

In *Bombyx mori*, the same *m*-APT PNs carrying achromatic information about different pheromone components to distinctly different zones of the MBCs continue on to terminate with arborizations in different and slightly distinctive subregions of the ILPt (figure 10.10) (Seki et al. 2005; Namiki et al. 2013). This discovery informs us that in moth pheromone-olfactory systems, there must be further integration needed at the ILPt protocerebral level for the separate incoming pheromone-component-specific inputs. Local integrative neurons (LNs) may process this information to produce higher precision information within the ILPt about the chromatic quality (blend ratios) of the pheromone odor, the temporal aspects of its intermittency, or both. The ratios of excitation coming from the achromatic PNs differentially tuned to pheromone components reaching the ILPt might be integrated to produce chromatic pheromone-blend outputs via the activities of ILPt/LH LNs, or perhaps via optic-olfactory multimodal LNs arborizing in the ILPt/LH and optic glomeruli of the LP (Okamura and Strausfeld 2007; Strausfeld et al. 2007; see "The Lepidopteran Visual System" above). Chromatic pheromone-blend information would then be sent from the ILPt out to other protocerebral areas, e.g., the MBLs or the LAcLo via trans-protocerebral PNs, or to thoracic ganglia for motor output via descending neurons synapsing in with ILPt/LH LNs in the posterior LP.

GENERAL ODORANT-TUNED PROJECTION NEURON MORPHOLOGIES

General odorant-tuned PNs of all moth species send their axons out of the AL from ordinary glomeruli to the protocerebrum via the *m*-APT, *ml*-APT, and the *l*-APT. Unlike pheromone-sensitive PNs, however, the arborization destination of general odorant-tuned PNs in the LP is the LH, an area of neuropil adjacent and dorsal to the ILPt (cf. Anton and Hansson 1994; Seki et al. 2005; Namiki et al. 2013). In all moth species, PNs exiting ordinary glomeruli via the *m*-APT send collaterals that first arborize in the MBCs and then continue on to terminate in the LH (Namiki and Kanzaki 2011; Namiki et al. 2013) (figure 10.10).

PROJECTION NEURON PHYSIOLOGIES: ANTENNOPROTOCEREBRAL TRACTS ARE RELATED TO CONVEYANCE OF ODOR TIME vs. ODOR SPACE INFORMATION

Here, we explain the use of different PN tracts exiting the McGl of male moths based on their functional physiologies, regardless of the numbers of AL glomeruli in which they arborize. Our approach contrasts with overviews that have attempted to link PN glomerular arborization anatomies in the AL to being "uniglomerular" or "multiglomerular." Such overviews have characterized the axons of uniglomerular PNs arborizing in a single AL glomerulus as using predominantly *m*-APT, whereas multiglomerular PNs arborizing in two or more glomeruli have been characterized as using *l*- or *ml*-APT (Galizia and Rössler 2010; Martin et al. 2011).

One reason for functionally characterizing a PN before linking it to one of the APT routes that it takes is that the molecular range of responsiveness of even uniglomerular achromatic PNs can be increased or decreased by LN activities in the AL to include greater collective inputs from differentially odorant-tuned, interconnected glomeruli (see figure 3 of Martin et al. 2011). Even though these PNs may be uniglomerular and seemingly should respond to just one odorant, they now respond to many more odorants due to LNs increasing the gathering of OSN activities from within greater numbers of

glomeruli (see figure 3 of Martin et al. 2011). Also, despite their increased molecular range of responsiveness, such PNs remain achromatic, although reporting now across a broader molecular range along a graded, two-dimensional odorant spectrum. Such adjustments in the molecular receptive range of a PN will not change its MBC arborization location in the protocerebrum; they will only change how narrow or broad its view of pheromone-odor space is in making its report to those same locations.

It is becoming increasingly clear that there is usually little correspondence between the number of McGl glomeruli in which a PN arborizes, the APT route on which it travels or its responsiveness to one versus more than one pheromone component. For example, in heliothine moth species, PNs have been found that exit the McGl along the *m*-APT, respond only to the major pheromone component, and yet have multiglomerular McGl arborizations (Christensen et al. 1991; Vickers et al. 1998; Vickers 2006). Hansson et al. (1994), working with *Agrotis segetum*, likewise found multiglomerular McGl PNs using the *m*-APT, yet these PNs responded only to single components. They also found multiple-component-responding *m*-APT-projecting PNs that had only a uniglomerular arborization in the McGl. The lack of correspondence between multiglomerular or uniglomerular McGl arborizations and subsequent PN response profiles was further confirmed in this species by Wu et al. (1996).

In *Trichoplusia ni* (Noctuidae) (Anton and Hansson 1999), *Spodoptera littoralis* (Anton and Hansson 1995), *Ostrinia nubilalis* (Anton et al. 1997), *Bombyx mori* (Kanzaki et al. 2003), *Cydia pomonella* (Trona et al. 2010), and *Grapholita molesta* (Varela et al. 2011), a proliferation of non-correspondences between the number of McGl glomerular arborization locations and PN response characteristics has been similarly noted. In most of these studies, responses to pheromone components were even observed coming from PNs that arborized in ordinary glomeruli, with many instances of PN responses to plant volatiles that arborized in McGl glomeruli.

Thus, we think that it may be more instructive to examine how well PNs respond temporally to rapid pheromone pulses of single pheromone components versus blends, or how much of a dynamic range they have in response to various pheromone components and plant volatiles, rather than whether they are uniglomerular or multiglomerular in AL glomeruli, as outlined via two points below regarding odor time and odor space.

1. Odor time: the m-APT carries achromatic, high-fidelity plume-strand- and clean-air-pocket temporal information to the MBC, then to the ILPt

The *m*-APT, the most commonly traveled of the three APT routes and which first includes a visit to the MBCs, should be involved in the generation of turn-reversal upwind surges in response to plume-strand contact. The PNs that project their axons along the *m*-APT have been the ones most commonly shown to be able to "follow" rapid sub-second pulses of pheromone components or blends of components. Depending on the species, they may originate in only one, or in several of the many McGl glomeruli, or may even have additional arborizations in ordinary glomeruli as described above. Like the columnar visual neurons in the medulla and lobula of the optic lobe, most of these PNs carry high-resolution achromatic olfactory edge-motion (temporal strand-edge) information to higher brain centers.

In the *m*-APT PNs of *Manduca sexta*, *Heliothis virescens*, *H. subflexa* (Noctuidae), *Helicoverpa zea*, *A. segetum*, and *A. ipsilon*, pulsed pheromone-component stimulation was used to examine the temporal acuity of pheromone-sensitive PNs leaving McGl glomeruli. All but two of the fully anatomically characterized *m*-APT PNs that were examined were achromatic, responding to only one pheromone component or several, but with no greater-than-additive action potential frequencies exhibited when two or more pheromone components were presented. In all of these studies, the PNs could "follow" and lock-on to at least 5-Hz short pulses (*H. virescens*: Vickers et al. 1998, 2001; Vickers and Christensen 2003; Vickers 2006; *H. zea*: Vickers et al. 1998; *H. subflexa*: Vickers and Christensen 2003; Vickers 2006; *M. sexta*: Christensen and Hildebrand 1988, 1997; Heinbockel et al. 1999, 2004; *A. ipsilon*: Chaffiol et al. 2012; and *A. segetum*: Lei and Hansson 1999). Several studies showed that the phase-locking to the pulses was sharpened, i.e., exhibited greater temporal acuity, when a blend of two or more components was puffed (Christensen and Hildebrand 1997; Vickers et al. 1998). Enhanced temporal resolution to 5-Hz pulses was found in *A. ipsilon m*-APT PNs when a blend of pheromone plus a plant odorant, heptanal, was puffed compared to the pheromone alone (Chaffiol et al. 2012).

Double impalements of *m*-APT PNs of *M. sexta* innervating the different glomeruli of the McGl showed that two PNs branching in the same McGl glomerulus display a more synchronized firing pattern than those branching in different glomeruli (Christensen and Hildebrand 1988, 1997; Christensen et al. 1998; Heinbockel et al. 1999, 2004, 2013; Martin et al. 2013). PNs in different glomeruli also tend to inhibit each other's activities. Such LN-related lateral inhibition was shown to produce an increased temporally precise tuning of responses to pheromone pulses. The temporal patterns observed are formed in the AL, and they seem to depend on interactions between *m*-APT PNs and LNs. Experiments suppressing the action of inhibitory LNs showed that pulse-following acuity was abolished in *m*-APT PNs (Christensen and Hildebrand 1988, 1996; Christensen et al. 1998; Lei et al. 2009) and that upwind flight in a pheromone plume to its source was hampered (Lei et al. 2009).

The crisp, phasic-bursting response pattern observed in *m*-APT PNs when all pheromone components are present thus seems to be a prerequisite for optimal orientation in a pheromone plume. In further experiments with *M. sexta*, when the natural pheromone blend was mimicked, using a 1:1 ratio of bombykal (*E,Z*-10,12-hexadecadienal) to a behaviorally effective synthetic hydrocarbon analog of the natural aldehyde second component (*E,E,Z*-10,12,14-hexadecatrienal), the pulse-tracking bursts of action potentials from the PNs were sharper and more accurate in response to the sub-second odor pulses than when either component was pulsed alone (Heinbockel et al. 2004). Further studies showed that a 2:1 blend of the two actual pheromone components (using the triene aldehyde this time and not the hydrocarbon analog, again mimicking the natural blend) created a synchronous firing of the *m*-APT PNs arborizing in the same McGl glomerulus compared to when single components or off-blends were used (Martin et al. 2013). A blend-enhanced synchronous firing of PNs exiting the same glomerulus, rather than an elevation of action potential frequency by such PNs was suggested to be a mechanism for pheromone-blend-quality encoding by such neurons (Martin et al. 2013).

The relationship between some observed highly regular oscillatory patterns called local field potentials (LFPs) resonating between the protocerebrum and the AL due to their

circuitries, and the possible synchronization of PN responses to plume strands, was studied previously (Heinbockel et al. 1999; Christensen et al. 2000, 2003; Vickers et al. 2001). Vickers et al. (2001) found that the male moth pheromone-processing system relies not on LFP oscillations, but rather on non-LFP-related excitations of PNs that are directly caused by the arrival of each temporally irregular sub-second plume strand. The precise reactions to plume strands were behaviorally assessed and temporally monitored via electroantennograms, and the *m*-APT PN responses were recorded, all within natural point-source plumes (Vickers et al. 2001).

The temporal sharpening that occurs in the AL in response to blends and its output in the McGl-PNs of the *m*-APT is similar to improved edge-resolution of the visual system due to lateral inhibition (Strausfeld and Campos-Ortega 1977; Strausfeld 2003). Multiple layers of AL LNs shorten the temporal differences between the rise and fall times of the firing of PNs to each pheromone puff and thereby sculpt the temporal edges of the firings. Thus, the predominant type of information that travels along the *m*-APT PNs up to the MBCs involves numerous achromatic reports from differentially tuned PNs related to variations in plume-strand flux.

We should remember that these temporally sharp reports by *m*-APT PNs to the MBCs go on to terminate in the ILPt. In the MBs, integration into chromatic blend outputs on either a strand-by-strand or a time-averaged basis may be occurring. However, these temporally acute *m*-APT reports also may be processed in the ILPt for integrating plume-strand encounters with perhaps any achromatic or chromatic blend-quality reports that are sent to the ILPt from the other two APT pathways.

2. Odor space: the very small amount of chromatic pheromone-blend information projecting out of the AL is carried more by ml- and l-APT PNs than by m-APT PNs

The type of PNs described by Vickers et al. (1998) as "blend synergist" PNs is the type we are calling chromatic PNs. Chromatic, pheromone-blend synergist PNs exhibit a level of action potential frequency in response to a blend of pheromone components that is significantly greater than the sum of the excitations elicited by the same components presented individually. These PNs constitute a tiny proportion of the thousands of PNs that have been recorded from thus far. The few pheromone-blend chromatic PNs that have been described are thus capable of sending to protocerebral neuropils at least a primitive rendering of the blend's position in odor space as a result of only AL-level integration. Chromatic blend synergist PNs seem to be absent in some species. Out of hundreds of recordings and complete stainings by the Kanzaki and Hildebrand groups, no chromatic pheromone-blend PNs have been found in either *M. sexta* or *B. mori*, respectively. However, because *B. mori* seems to use bombykol as a one-component pheromone communication system, with bombykal only serving as a heterospecific behavioral antagonist, it is understandable why efforts to understand the effects of bombykol plus bombykal blends have been unfruitful.

PROJECTION NEURON PHYSIOLOGIES: POOR TEMPORAL PLUME-STRAND RESOLUTION BY CHROMATIC PROJECTION NEURONS?

When chromatic pheromone PNs have been fully neuroanatomically characterized, a slight majority (three of five) have been shown to use the *ml*- or *l*-APT and project to arborize in the ILPt. These PNs were found in *Heliothis virescens* (Vickers et al. 1998). When challenged with 5-Hz pulsed blend stimulation, these chromatic PNs were very poor at following pheromone-blend pulses, whereas nearly all the achromatic PNs following the *m*-APT in this same species did so extremely well (Vickers et al. 1998). Interestingly, there was also a lack of ability to resolve 5-Hz pulses by two chromatic blend synergist PNs of *H. subflexa* and *H. virescens* hybrids (these PNs have not been anatomically fully characterized; Vickers and Christensen 2003).

In both *H. virescens* (Vickers et al. 1998) and *Agrotis segetum* (Hansson et al. 1994), one completely stained and physiologically characterized chromatic pheromone-blend PN was found that used the *m*-APT. The chromatic PN in *H. virescens* following the *m*-APT was also unable to resolve 5-Hz pheromone pulses. The PN in *A. segetum* was not challenged with pulsed stimulation (Hansson et al. 1994). Both of these PNs were shown to send collaterals that arborized in the MBCs and axons that continued on to terminate with their arbors in the ILPt (Hansson et al. 1994; Vickers et al 1998).

Although the projection routes have not been characterized for some PNs, it is possible to hypothesize the projection routes on the basis of whether a PN's cell body is located in the medial or the lateral cell cluster of the AL. Lateral cell cluster PNs have been found to nearly always use either the *ml*-APT or *l*-APT routes (Anton et al. 1997; Kárpáti et al. 2008, 2010) and thus only arborize in the ILPt, not the MBCs. Thus far, no PN has been found having a lateral cell body cluster that projects along the *m*-APT. For PNs shown to have their cell bodies in the medial cluster, there is a bit more uncertainty. In some cases (e.g., *Bombyx mori*), a small percentage of medial cluster cell body PNs leaving via what appears to be the *m*-APT divert their paths and instead use the *ml*-APT, bypassing the MBCs with terminal arbors directly in the ILPt (Kanzaki et al. 2003; Namiki et al. 2013).

Out of many hundreds of recordings from moth PNs with cell bodies in the medial cell cluster, two chromatic pheromone PNs were characterized in *Helicoverpa zea* (Christensen et al. 1991), two in *H. subflexa* (Vickers and Christensen 2003), one in *H. virescens* (Christensen et al. 1995), one in *Spodoptera littoralis* (Anton and Hansson 1995), and seven in *A. segetum* (Hansson et al. 1994; Wu et al. 1996; Hartlieb et al. 1997; Lei and Hansson 1999). It is unknown how many of these chromatic PNs actually followed the *m*-APT.

Most of the PNs from other species having cell bodies in the lateral cluster and whose axons can be inferred to use the *l*- or *ml*-APT have been characterized as chromatic blend synergist PNs. Much of this latter data comes from studies on the PNs of *Ostrinia nubilalis* (Anton et al. 1997; Kárpáti et al. 2008, 2010). These PNs were not challenged with pulsed pheromone stimuli, so their temporal acuity in response to blends is unknown.

A few unstained or incompletely stained chromatic pheromone PNs have been shown to be able to discriminate blends differing from each other only by 5–10% in composition ratios of their pheromone components (Wu et al. 1996; Anton et al. 1997). This precision of blend-ratio discrimination was exhibited by chromatic PNs in both *A. segetum* (Wu et al. 1996) and *O. nubilalis* (Anton et al. 1997). These results suggest that there has been some selection pressure for odor space discrimination to take place at the AL level before the information is transmitted to the protocerebrum. It also highlights once again the subtleties of the complex integrative networks of LNs, OSNs, and PNs at this level.

PROJECTION NEURON PHYSIOLOGIES: GABA-ERGIC PROJECTION NEURONS USING THE ml-APT

The neurotransmitter used by most PNs is understood to be acetylcholine (Hoskins et al. 1986; Homberg et al. 1988, 1990; Seki and Kanzaki 2008; Seki et al. 2005; Reisenman et al. 2011); acetylcholine will transmit excitatory inputs to the MBCs as well as to the ILPt. Two immunocytochemical studies from *Manduca sexta* and *Heliothis virescens*, however, have indicated that many, and possibly most, of the PNs traveling over the *ml*-APT to the ILPt exhibit GABA-like immunoreactivity (GLIR) (Hoskins et al. 1986; Berg et al. 2009). These PNs terminate in the ILPt without continuing on to the MBCs. However, Hoskins et al. (1986) note that this is a tract that also showed the majority of PNs being cholinergic, and they suggested that it is possible that the *ml*-APT PNs might be both cholinergic and GABA-ergic. If *ml*-APT PNs are able to be GABA-ergic, then enhanced discrimination of pheromone-blend ratios and a sharpening of temporal synchrony in the ILPt may be aided by the integration of these inhibitory PN inputs arriving at the ILPt and excitatory inputs arriving there via the *ml*- and *l*-APTs.

The Lateral Accessory Lobe: Site for Counterturn Generation, Convergence of Multimodal Olfactory-Visual Inputs, and Descending Premotor Neurons

The LAcLos are paired neuropils in the midline of the protocerebrum slightly anteroventral to the CC (figures 10.1, 10.11A, and 10.11B). The LAcLos have a large degree of neuronal connectivity with the CC (Strausfeld and Hirth 2013), and they also receive inputs from multimodal neurons projecting to them from other protocerebral neuropils. The LAcLos are key premotor centers in the insect brain that promote the execution of many behaviors, including pheromone-mediated counterturning (Iwano et al. 2010). Pheromone-sensitive neurons of the LAcLo of several moth species arborize with premotor descending neurons in the ventrolateral and inferior lateral protocerebral neuropils (Olberg 1983; Olberg and Willis 1990; Kanzaki et al. 1991b, 1994; Kanzaki and Shibuya 1992; Wada and Kanzaki 2005; Iwano et al. 2010). These neuropils are also key sites where pheromone-olfactory and visual image-motion information is likely to become integrated, either from direct optic inputs to the LAcLo or via multimodal PrtCNs (Strausfeld et al. 2007).

The LAcLo on each side of the protocerebrum is connected via intrinsic LNs to a corresponding VPrtC and forms a LAcLo/VPrtC complex (figure 10.11C) (Iwano et al. 2010). The LAcLo/VPrtC LN network on either side of the protocerebrum is indicated as being the source of the neuronal circuit's LLE. This LLE produces prolonged behavioral excitation by setting up fast-recurrent oscillation between the LAcLo and the VPrtC to drive a continuous LLE output in efferent bilateral LAcLo-to-LAcLo neurons in response to a brief pheromone pulse (Iwano et al. 2010). For both *Manduca sexta* and *Bombyx mori*, the LLE is optimally evoked when the species' pheromone is presented, compared to an off-ratio of pheromone components or of antagonists and plant odor (Kanzaki et al., 1991b, 1994; Kanzaki and Shibuya 1992; Wada and Kanzaki 2005; Iwano et al. 2010).

The bilateral LAcLo/VPrtC neurons connecting each LAcLo/VPrtC unit on either side of the protocerebrum are GABA-ergic and inhibitory (Iwano et al. 2010); they have been shown to impose an inhibitory LLE to suppress the LLE of the corresponding LAcLo/VPrtC unit on the other side. Thus, a currently dominating LLE produced by one side becomes overtaken and "flipped" to the off-position, by the new dominance of the other side's LLE from its LAcLo/VPrtC unit. The flip-flop occurs either spontaneously after a long time of firing, such as during a sustained period of post-pheromone clean air, or else by the arrival of a new pheromone strand (Iwano et al. 2010).

Thus, the brief excitation in one LAcLo/VPrtC from a pheromone strand contacting either or both of the antennae appears to be able to trigger an immediate turn-reversal-related flip-flop of firing activity in the pair of bilateral LAcLo-to-LAcLo neurons. The pheromone strand will also simultaneously initiate the LLE that will drive a many-seconds-long oscillating series of flip-flops during exposure to clean air after the strand. In *B. mori*, an intermediate time frame, high-frequency, endogenous flip-flopping is also expressed in the activities of bilateral LAcLo neurons (Iwano et al. 2010) that is correlated with short-term zigzagging upwind walking in the 200–300 ms between straight-upwind walking and long-term looping in clean air. In flying moths, such a several-hundred-millisecond period would be related to the period of quick zigzag turn-reversals in clean air during the transition to long-term casting flight after a single upwind turn-surge in response to a single strand (Mafra-Neto and Cardé 1994; Vickers and Baker 1994). In most moth species, the counterturning during casting flight occurs with a more or less regular, oscillatory tempo, but in a few species, such as *M. sexta* (Willis and Arbas 1991) and *Lymantria dispar* (Kuenen and Cardé 1994), the counterturn tempos during clean-air casting are much less regular.

The sub-second reaction of a counterturn in response to a pheromone plume strand would seem to imply a very fast connection between the contact of peripheral OSNs with a strand and the LAcLo. There is one direct pheromone-olfactory input that has been found in *Agrotis segetum* by Wu et al. (1996). They reported two "pheromone-component-specific" PNs from the McGl that projected to the contralateral as well as the ipsilateral LAcLos and ILPts on both sides of the protocerebrum. We do not know whether such AL PNs exist in other moth species, but their presence in *A. segetum* is informative in perhaps showing in at least one species the presence of a direct communication channel with the LAcLo-turn generator in response to contact with individual plume strands. Such a direct connection between the AL and the LAcLo has never been found in *M. sexta* or *B. mori*, despite thousands of AL PNs having been recorded from and stained.

Protocerebral Neurons Connecting the Mushroom Body, Inferior Lateral Protocerebrum, and Lateral Accessory Lobe

Inferior Lateral Protocerebrum-to-Mushroom Body and Lateral Horn-to-Mushroom Body Protocerebral Neurons

Some pheromone-olfactory PrtCNs connecting the ILPt with the MBC have been found in *Bombyx mori* (Namiki et al. 2013). One type of neuron, called "ΔILPC" neurons (figures 10.12A and 10.12B), has smooth postsynaptic connections within the ILPt and blebby presynaptic outputs to the same outer collar region of the MBC that corresponds with the

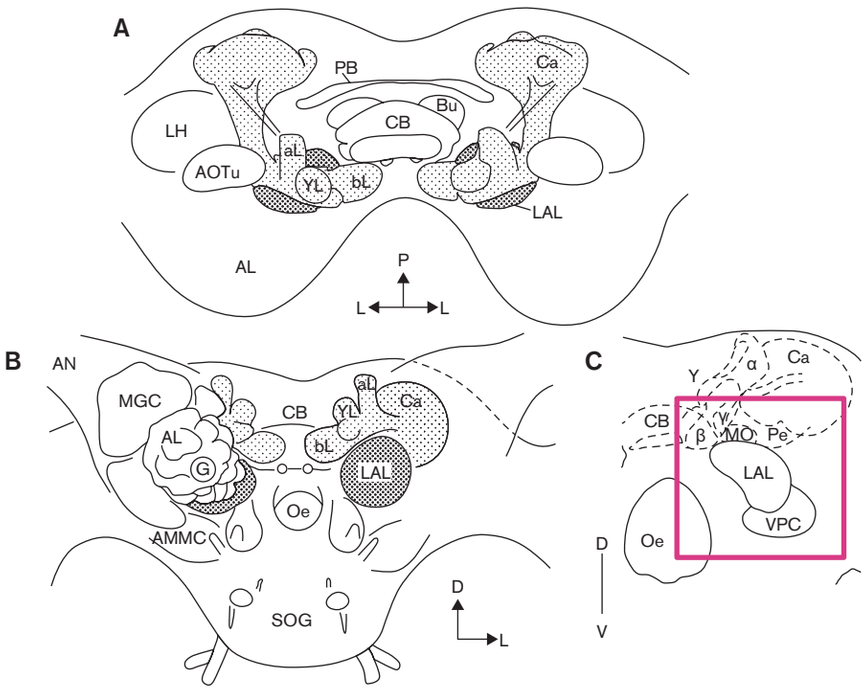

FIGURE 10.11 Position of the lateral accessory lobes of *Bombyx mori* with respect to other protocerebral neuropils, including the ventral protocerebral neurons that synapse with the lateral accessory lobes and promote long-lasting excitation that drives long-lasting, clean-air looping behavior in walking *B. mori* males.

A Vertical (top-down) view of the protocerebrum, which is the same perspective as in figure 10.1B. The positions of the lateral accessory lobes (LAL) on each side of the central body (CB) and posterior to the medial (bL), vertical (aL), and Y-lobes (YL) of the mushroom body can be clearly seen. The anterior positioning of the antennal lobe (AL), the dorsal-anterior positioning of one prominent optic glomerulus (the anterior optic tubercle [AOTu]), and the ventro-posterior positioning of the lateral horn (LH) are depicted as well.

ABBREVIATIONS: Ca, mushroom body calyx; PB, protocerebral bridge; Bu, buttress; L, lateral; P, posterior.

B Frontal (anterior) view of the *Bombyx mori* brain showing one of the anteriorly situated antennal lobes (ALs) with one of its glomeruli noted (G) and its magroclomerular complex (MGC), and the positions of the ALs and MGC with respect to the more posterior LAL, which is not labeled but is the dark gray neuropil oval on that side. The more ventral position of the LAL with respect to the medial (bL), vertical (aL), and Y-lobes (YL) of the mushroom body and also its calyces (Ca) also can be seen.

ABBREVIATIONS: CB, central body; Oe, esophagous; SOG, subesophageal ganglion; AMMC, antennal mechanosensory and motor center. D, dorsal; L, lateral.

C Frontal view (same as in B) of the *Bombyx mori* protocerebrum showing the position of the neuropil of the ventral protocerebrum (VPC) whose neurons synapse with the LAL to produce LLE on that side.

ABBREVIATIONS: α, vertical lobe; β, medial lobe; γ, gamma lobe; Y, Y-lobe; Pe, pedunculus; Ca, calyx of the mushroom body; MO, median olive.

SOURCE: (A) and (B) are from Kanzaki and Shibuya (1992); (C) is from Iwano et al. (2010).

NOTE: Some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

MBC arborization locations of *m*-APT PNs that have projected there from the McGl. Thus, the ΔILPC neurons appear to be pheromone-related feedback or feed-forward neurons from the ILPt to the MBC.

Another type of PrtCN, named LH neurons (figures 10.12C and 10.12D), connects the plant volatile-related LH region of the LP with the MBCs (Namiki et al. 2013). These LH-MBC neurons have smooth postsynaptic connections in the LH and arborize with presynaptic blebs in the same regions of the MBCs that receive inputs from plant volatile-related PNs leaving ordinary glomeruli from the AL (figures 10.12C and 10.12D) (Namiki et al. 2013). GABA immunostaining of many more of the LH neurons showed that the neurons connecting the LH with the MBCs did not exhibit GLIR and would thus not be inhibitory. They could possibly be feedback or feed-forward neurons communicating LH-integrated plant volatile-related information to the MBCs' plant volatile reception regions. Although these ΔILPC and LH PrtCNs have been shown thus far to be only olfactory, they have not yet been challenged with visual or mechanosensory stimuli; so, it is possible that these PrtCNs may be capable of sending multimodal visual-olfactory information to the MBCs.

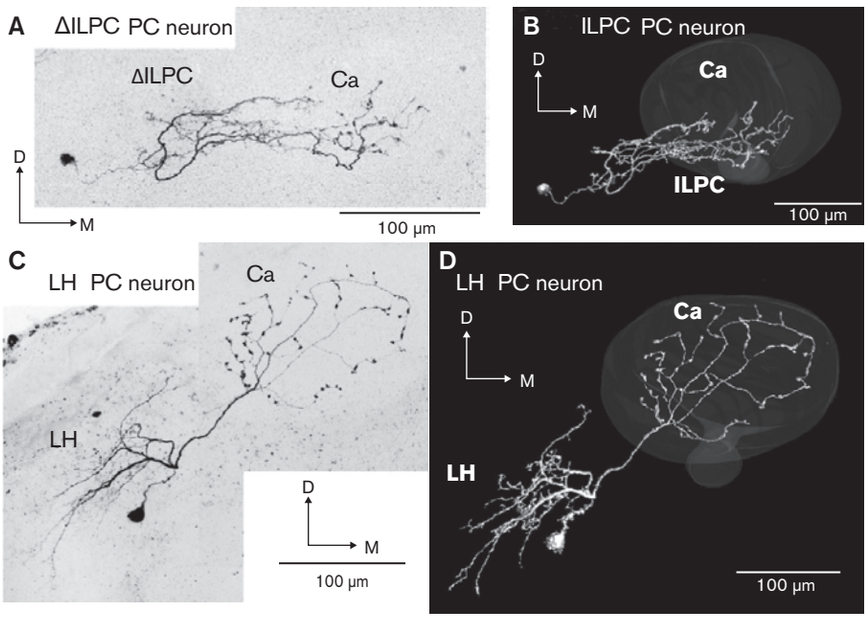

FIGURE 10.12 Examples of protocerebral neurons (PC neurons) that connect either the inferior lateral protocerebrum (ILPC) or the lateral horn (LH) with mushroom body calyxes.

A Confocal image of a "ΔILPC" PC neuron that has smooth arbors in the pheromone-component-targeted region (delta region) of the inferior lateral protocerebrum (ΔILPC) and then has varicose arbors in the mushroom body calyxes. Smooth arbors are considered postsynaptic, and varicose arbors are considered as being presynaptic. Therefore, this type of PC neuron should be transmitting pheromone-related or even multimodal pheromone-plus-visual information from the ΔILPC to the mushroom body calyxes.

B Three-dimensional reconstruction of this neuron with its targeted mushroom body calyx visualized for better perspective of its arbors in the mushroom body calyxes.

C Confocal image of a "LH" PC neuron. It has smooth arbors in the LH of the lateral protocerebrum and varicose arbors in the mushroom body calyxes. Because the LH is the target of projection neurons from ordinary antennal lobe glomeruli that transmit plant-volatile-related information, this "LH" PC neuron is likely to be transmitting plant odor information, or even plant odor-plus-visual information to the mushroom body calyxes from the LH.

D Three-dimensional reconstruction of this neuron with its targeted mushroom body calyx visualized for better perspective of its arbors.

ABBREVIATIONS: Ca, calyx of the mushroom body; D, dorsal; M, medial.

SOURCE: From Namiki et al. (2013).

NOTE: Some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

Protocerebral Neurons Connecting the Mushroom Body or the Inferior Lateral Protocerebrum/LH with the Lateral Accessory Lobe Are Likely to Be Multimodal Visual-Olfactory Inputs

Only a single pheromone-olfactory-only type of direct connection between McGl AL PNs and the LAcLo has been found thus far, suggesting that the majority of inputs to the LAcLo in moths are likely to come from olfactory-visual multimodal PrtCNs from within the protocerebrum itself. Comprehensive neuroanatomical studies of PrtCNs of *Periplaneta americana* have elucidated how multimodal moth PrtCNs might integrate visual and pheromone-olfactory inputs (Li and Strausfeld 1997, 1999; Strausfeld and Li 1999). These studies demonstrated that there are many multimodal neurons extrinsic to the MB (e.g., non-KC protocerebral interneurons synapsing with the MB and carrying information directly to or away from the MB) that are responsive to the *P. americana* pheromone and to visual stimuli. These PrtCNs were shown to arborize in discrete tufts in the vertical and medial MB lobes as well as in other neuropils, such as the LH/ILPt neuropils (Li and Strausfeld 1997).

Many of these visual-, olfactory-, and sound-responsive extrinsic MB neurons were efferents that projected from the MB and terminated in the LP (Li and Strausfeld 1999). Li and Strausfeld (1999) strongly suggested that such neurons relay information from the MB to the LP about the "sensory context" of olfactory, visual, and other stimuli where the LP can process and integrate it with, for instance, olfaction-only inputs from AL olfactory PNs (Li and Strausfeld 1999). Several different response types of these PrtCNs were found to have stereotypical arborization regions on the MB lobes. For instance, visual-olfactory multimodal MB efferents from the alpha lobes (= vertical lobes) of different individuals were found to send axons to medial protocerebral neuropil (Li and Strausfeld 1997). Conversely, multimodal MB efferents from the beta lobes (= medial lobes) sent axons to the ILPt and superior LP (Li and Strausfeld 1999). Multimodal efferent neurons

recurrently connecting the vertical with the medial lobes were found that projected out to the ILPt (Li and Strausfeld 1999).

Instead of the MBCs just receiving unimodal olfactory or visual sensory pathway inputs, it is now clear that they can receive multimodal inputs from PrtCNs as well. In *P. americana*, many multimodal visual-olfactory afferent PrtCNs project from visual-olfactory neuropils to arborize with inputs to the MB pedunculus and calyces (Strausfeld and Li 1999). Nishino et al. (2012a, 2012b) recorded from and stained a PrtCN of *P. americana* that responded to both odor and visual stimuli whose neurites originated in both the medulla of the optic lobe and the LH and then projected to arborize in both the inner- and outer-rim zones of the MBCs.

Inferior Lateral Protocerebrum/Lateral Horn-to-Lateral Accessory Lobe Protocerebral Neurons

In moths, there are several examples of PrtCN synaptic connections between the pheromone olfaction-related ILPt/LH and the LAcLo, and also between the MB and the LAcLo. Kanzaki et al. (1991a) found a pheromone-responsive PrtCN in *Manduca sexta* that arborized with smooth arbors in the ILPt/LH of the ipsilateral side of the brain from where antennal pheromone stimulation came, and projected with varicose arbors to the LAcLo/VPrtC neuropils on both the ipsilateral and contralateral sides (figure 10.13A). This PrtCN responded with brief excitation to the pheromone, and considering the analysis and model of Iwano et al. (2010) for *Bombyx mori*, this type of ILPt-LAcLo PrtCN could be implicated as providing pheromone-olfactory input from the ILPt to trigger LLE in the LAcLo/VPrtC neurons on one side as well as a turn-reversal-related flip-flop by bilateral LAcLo neurons upon contact with a pheromone strand.

Local Pheromone-Sensitive Protocerebral Neurons Connecting the Lateral Protocerebrum, Lateral Accessory Lobe, and Optic Glomeruli

Another LAcLo-visiting PrtCN in *Manduca sexta* that was excited by pheromone was determined to be an "LN" (figure 10.13B) that invested smooth arbors in the LP on one side only (Kanzaki et al. 1991a). This local PrtCN that produced a brief burst of action potentials in response to pheromone projected with varicose arbors to the LAcLo, the ventral LP, optic glomeruli (which had not yet been recognized as such) called "posterior optic foci," and the ventral medial protocerebrum. A few other LN PrtCNs also arborized in the LAcLo of one side and visited other optic glomeruli, intermingling either along the "anterior optic tubercle" or with "posterior optic foci" on that side (figure 10.13C) (Kanzaki et al. 1991a).

These types of ProC LNs may be involved with integrating pheromone-olfactory inputs to the ILPt and optical image flow from optic glomeruli, to trigger LLE/flip-flop-related, turn-reversals. Because no optical stimuli were used during this study (Kanzaki et al. 1991a), it cannot be determined for certain whether these PrtCNs would be multimodal pheromone-visual neurons (Kanzaki et al. 1991a). However, because numerous descending neurons in a companion study of *M. sexta* (Kanzaki et al. 1991b) were found to be multimodal, responding to hand movement, lights-on and lights-off, and pheromone stimulation, it may be inferred that the source of this multimodality was in PrtCNs, such as those that were found by Kanzaki et al. (1991a).

In *M. sexta* PrtCNs, Lei et al. (2013) found a highly pheromone-responsive PrtCN that arborized with smooth processes in the VPrtC and sent a neurite to the lobula of the contralateral optic lobe. They suggested that this PrtCN might be involved in multimodal integration of visual and pheromonal stimuli, although visual stimuli were not tested.

Mushroom Body-to-Lateral Accessory Lobe Protocerebral Neurons

In *Agrotis segetum*, amidst the large number of pheromone-responsive PrtCNs that were recorded from and stained, one PrtCN was found that visited both the LAcLo and the MBLs (figure 10.13D) (Lei et al. 2001). This neuron responded to all pheromone components, could follow 3-Hz pulses of pheromone stimulation, but displayed LLE in response to plant odors or the pheromone behavioral antagonist. It exhibited dense, smooth (postsynaptic) arbors in discrete bulbous areas in two portions of the MB lobes, bifurcated also with arbors in the MB medial lobes, and projected to the LAcLo where it exhibited varicose (presynaptic) blebby synapses (figure 10.13D) (Lei et al. 2001). Such a neuron could be transmitting plume-strand-related pheromone flux information integrated with optic flow-field information to descending neurons found by Lei et al (2001) in their *A. segetum* preparations that exhibited smooth, spiny postsynaptic arbors in the LAcLo.

Similarly, in *Bombyx mori*, quite a few of the LAcLo/VPrtC bilateral neurons found to exhibit only brief excitation or inhibition were shown to receive inputs from extrinsic, efferent MB lobe neurons (Iwano et al. 2010). These MB inputs to the LAcLo/VPrtC bilateral neurons came from either the alpha or beta lobes of the MB (Iwano et al. 2010).

In *Manduca sexta*, Kanzaki et al. (1991a) found some PrtCNs out of the many that were recorded from that had arbors in the MB lobes, but none of these neurons visited the LAcLo. One such PrtCN exhibited LLE and invaded not only two distinctly different zones at the junction of all the MB lobes and the pedunculus but also arborized in the LP, which would include olfaction-vision-related areas of the LH and ILPt. The other few PrtCNs arborizing in the MB lobes all exhibited brief excitation to pheromone and appeared to be wide-ranging protocerebral LNs. Some of these exhibited characteristic tuft-like arbors, called "glomeruli" by Kanzaki et al. (1991a), in a particular MB zone of the lobes. Some tufts such as these have been found in extrinsic MB neurons of *Periplaneta americana* and *Apis mellifera* (Apidae) and can be either outgoing efferent PrtCNs or incoming afferents depending upon the types of spiny or varicose synaptic connections these extrinsic neurons have with the KCs of the MBLs (Farris 2005). Regardless, for *M. sexta* there thus far appears to be a greater direct set of connections with the ILPt and LAcLos than between the MB and LAcLos.

Thus, there is some evidence for *B. mori* and *A. segetum* that temporally sharpened and perhaps quickly integrated visual-olfactory outputs from the MBLs might be received directly by the LAcLo/VPrtC to help trigger counterturns and LLE to visually stabilize crosswind casting behavior. These connections also could explain the attainment of an upwind flight orientation due to optomotor longitudinal image motion during a single surge. In heliothine moths, a single upwind surge is known to be directed quickly upwind and to persist for longer durations when the correct pheromone blend is used rather than a blend of pheromone tainted with a trace amount

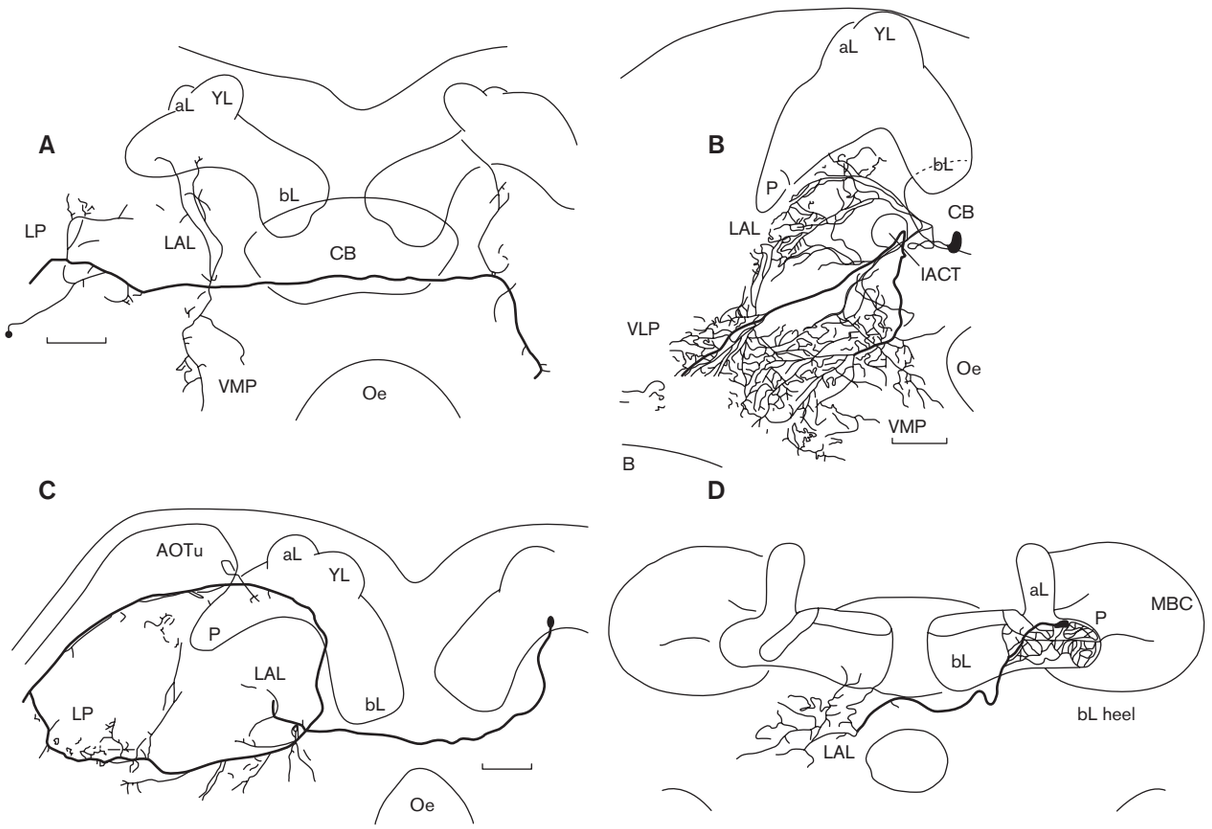

FIGURE 10.13 Reconstructions of stained protocerebral neurons of *Manduca sexta* (A–C) (Kanzaki et al. 1991b) and *Agrotis segetum* (D) (Lei et al. 2001) that respond to their conspecific sex pheromones and innervate the lateral accessory lobe/ventral protocerebrum with varicose arbors. These neurons receive inputs with their smooth arbors that originate either in the lateral protocerebrum (A–C) or next to the mushroom body lobes (D). Reconstructions are all illustrated in frontal view.

A This protocerebral neuron has smooth arbors in the lateral protocerebram (LP) ipsilateral to the antenna receiving sex pheromone stimulation and projects with varicose arbors to the ipsilateral and contralateral lateral accessory lobe (LAL)/ventral protocerebrum.

ABBREVIATIONS: aL, mushroom body vertical lobe; bL, mushroom body medial lobe; YL, mushroom body Y-lobe; CB, central complex; Oe, esophagus; VMP, ventromedial protocerebrum.

B A protocerebral neuron considered by Kanzaki et al. (1991a) to be a "local neuron" (not bilateral) with smooth arbors in the ipsilateral LP and projecting to the ipsilateral LAL, ventro-lateral protocerebrum (VLP), and optic glomeruli (the "posterior optic foci").

ABBREVIATIONS: P, mushroom body pedunculus; IACT, inner antennocerebral tract (nomenclature of Homberg et al. 1988, for the *m*-APT).

C This protocerebral neuron arborized in the LP and responded to pheromone stimulation on the antenna ipsilateral to this LP arborization but contralateral to its cell body (black oval at right). It arborized also in the LAL and visited the length of the optic glomerulus known as the anterior optic tubercle (AOTu).

D This pheromone-responding protocerebral neuron in *Agrotis segetum* arborized with varicose arbors in the contralateral LAL, having come from the mushroom body, where it had smooth arbors in two dense tufts in the "heel" near the base of the medial lobe and pedunculus (bL heel). It also bifurcated to visit the medial lobe in two places before projecting to arborize in the contralateral LAL (Lei et al. 2001).

NOTE: Scale bars (B, C), 100 µm. Some abbreviations (labels) are different from the abbreviations in our text for the same structures; we have retained the original abbreviations from the cited work.

of behavioral antagonist (Vickers and Baker 1997; Quero et al. 2001); so, it is possible that chromatic blend information is integrated by the MBs before at least a small proportion of such MB-PrtCN efferents project to the LAcLo.

Lateral Accessory Lobe-to-Mushroom Body and Other Protocerebral Neuropils

Iwano et al. (2010) found that LLE, pheromone-responding bilateral LAcLo neurons seemed to be involved in sending outputs to other protocerebral regions, including one bilateral LAcLo neuron that sent its output to the beta lobes of the MB. Other such LLE-exhibiting LAcLo bilateral neurons sent arbors to dorsal protocerebral areas, including one such neuron that also sent arbors to the "posterior slope," an area in which optic glomeruli LNs can intermingle with the axons of lobula–lobula plate visual neurons involved with optomotor regulation of flight and other visual image–motion inputs (Wicklein and Strausfeld 2000).

Protocerebral Chromatic Pheromone-Blend Neurons

In *Manduca sexta* and *Agrotis segetum*, a surprisingly small proportion of PrtCNs have been found that respond preferentially to their pheromone blends, considering that these are

two species for which blends are significantly better at eliciting upwind flight of males than individual components (Kanzaki et al. 1991a; Lei et al. 2001, 2013). Thus, at this higher integrative level of the brain, the majority of pheromone information being transmitted involves individual pheromone components and not the blend itself; only a small percentage of PrtCNs convey chromatic information about pheromone-blend quality. With regard to temporal plume-strand information, one study (Lei et al. 2001) on *A. segetum* PrtCNs showed that only a few of these neurons could respond to pulsed pheromone-component stimulation up to 3 Hz; the majority were only able to follow pulses at 1 Hz.

Conclusions

The sex pheromone olfactory system of flying male moths is designed for sub-second pheromone-plume-strand and clean-air processing so that quick steering reactions to the wind can be made via visual image-flow inputs from the optic lobes. The sex pheromone olfactory pathways of male moths converge on many of the same protocerebral neuropils as edge-motion-sensitive optic pathways, such that the two non-chemotactic, indirect behavioral responses to pheromone strands and clean air can be precisely executed: (1) a turn-reversal plus optomotor anemotaxis in response to an individual pheromone strand and (2) an endogenous program of repetitive turn-reversals plus optomotor anemotaxis that plays out in pockets of clean air between strands.

The neuropils receiving pheromone-olfactory-only and edge-motion-vision-only information from primary sensory neuropils are olfactory glomeruli in the AL and optic glomeruli in the LP, respectively. Although these two neuropils appear to be highly separated from each other, residing in two different brain regions, the AL's first-order-processed pheromone information in fact projects to the LP along three different PN axonal routes, so is not separated even early on from optic flow-field information. Pheromone information thus should be able to be integrated with visual edge-motion information via LNs that populate the LP and synapse among optic glomeruli as well as with pheromone-sensitive AL PNs terminating in the ILPt.

The MBCs are also a site of convergence of visual and pheromone-component olfactory information, and may play a role in mediating in-flight behavioral responses to pheromone plume strands and clean air. The MBCs appear to be the preferred neuropil for receiving highly phasic, strand-related fast-tempo inputs from AL PNs projecting along the medial-antennoprotocerebral tract. As such, the MB may be able to provide outputs to other protocerebral neuropils, such as the LAcLos, that can drive strand-triggered turn-reversals related to the upwind surges made by males when contacting an individual pheromone strand.

The ILPt receives the same highly phasic strand-related pheromone-component PN inputs as the MBCs, but it also preferentially receives inputs from two other PN pathways (the mediolateral- and lateral-antennoprotocerebral tracts) that seem to be more involved with carrying integrated, less temporally precise pheromone odor-blend information with less emphasis on phasic, flux-related pheromone-component information. As such, the ILPt and its potential optic glomeruli LN interconnections make it appear to be a site more strongly associated with long-lasting, clean-air casting flight behavior driven by the correct pheromone blend. Casting flight is a highly optomotor anemotactically guided behavior that depends upon a precise maintenance of a balanced ratio of transverse and longitudinal image flows that reverses in polarity between each endogenously generated turn-reversal.

Thus, at present, we would propose that the protocerebral neuropils that are more medially located, e.g., the mushroom bodies and the LAcLos, are more likely to be involved with promoting straight-upwind flight via the rapid phasic generation of turn-reversals and optomotor anemotactic longitudinal flows upon plume-strand contacts. The more laterally located neuropils of the LP connected with the LAcLo's self-generated flip-flop oscillator appear more likely to be involved with long-term, crosswind casting flight in clean air. Prolonged casting flight requires an LLE-related after effect of the correct pheromone blend to optimally drive the LAcLo flip-flop circuit's endogenous turn-reversal-related program. It also requires a precise integration of longitudinal plus transverse image flows, and such flows are likely to be integrated by optic glomerular LNs that also can synapse with the pheromone-blend-sensitive areas in the ILPt.

Protocerebral neuropils are interconnected by neurons that are most likely all multimodal-responding neurons (Strausfeld 2003, 2012). Thus, the LAcLos' turn-generating outputs may be orchestrated in concert with integrated pheromone-plus-visual-edge-motion inputs from both the mushroom bodies and the LP. Some pheromone-sensitive PrtCNs have been described in moths that have their synaptic origins along the MBLs and terminal arbors in the LAcLos. Other PrtCNs have been described that originate in the LP and have their terminal arbors in the LAcLos. It remains to be seen what such neurons would do if they were to also be challenged with visual motion stimuli.

It is not surprising that pheromone-sensitive PrtCNs such as these were not also presented with visual stimuli because researchers in insect olfaction in the past have not been aware that the response to odor by a flying insect is nearly entirely a *visual* response. Strausfeld et al. (2007) emphasized that an organized regime of multimodal stimuli should be used on the LNs connecting optic glomeruli in the LH and LP to wholly understand their full complements of stimulus-related behavior. They also predicted that these lateral protocerebral LNs known to invade optic glomeruli should prove to be odor- as well as visual-responsive.

We hope that an awareness of this type of multimodal stimulus regime can be applied more often in future neuroethological work, such as when undertaking difficult and time-limited recordings from AL PNs, or even OSNs during antennal single-cell recordings, to at least rule out definitively any types of centrifugal neuron motion-vision or olfactory feedback influencing these more peripheral neurons. Surprises may be in store that will inform us more completely about the degree to which motion-vision and pheromone olfaction may be integrated to modulate all levels of the male moth sex pheromone olfactory system. Attention also should be paid to what we now know to be the significant amount of malleability of integrative neuropils, such as those comprising the AL. Neuronal responses to pheromone components from these neuropils can be influenced by the male's age, mating status, or recent preexposure to stimuli, in addition to the admixing of host plant odorants with pheromone components. These factors all have the potential to change these neurons' reports that characterize their current views of odor time and odor space.

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