Developmental Changes in the Electrophysiological Properties and Response Characteristics of *Manduca* Antennal-Lobe Neurons

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Mercer, Alison R. and John G. Hildebrand. Developmental changes in the electrophysiological properties and response characteristics of *Manduca* antennal-lobe neurons. *J Neurophysiol* 87: 2650–2663, 2002; 10.1152/jn.00452.2001. Using whole cell patch-clamp recordings, we have examined changes in the electrophysiological properties and response characteristics of antennal lobe (AL) neurons associated with the metamorphic adult development of the sphinx moth, *Manduca sexta*. Whole cell current profiles and electrical excitability were examined in dispersed AL neurons in vitro, and in medial-group AL neurons in situ in semi-intact brain preparations. Around stages 2–4 of the 18 stages of metamorphic adult development, whole cell current profiles were dominated by large outward (K<sup>+</sup>) currents. Calcium-dependent action potentials could be elicited at this stage, but only a small percentage of cells exhibited sodium spikes. From stages 3 to 10, there was a rapid increase in the proportion of AL neurons exhibiting rapidly activating, transient sodium currents, and many cells in vitro exhibited spontaneous bursts of spike activity at this time. As development progressed, action-potential waveforms became shorter in duration and larger in amplitude. Cell-type-specific differences in the prevalence of spontaneous activity, and in the electrophysiological properties and response characteristics of AL neurons, were most apparent late in metamorphosis. While a considerable amount is known about the structural development of AL neurons in *Manduca*, much less information is available about the functional development of these cells. Odor-elicited activity in antennal ORCs is detectable in electroantennogram recordings only in the last days of metamorphic adult development (Schweitzer et al. 1976), and electrical stimulation of the antennal nerve evokes little or no response in multiglomerular AL neurons prior to stage 9 (Tolbert et al. 1983). However, spontaneous activity, both in antennal nerve (sensory afferent) fibers and in AL neurons, has been reported relatively early in metamorphosis, including during critical stages of formation of glomeruli (Oland et al. 1996). Evidence suggests that early forms of electrical activity have a developmental function (see Spitzer 1991, 1994), but whether *Manduca* AL neurons exhibit characteristic forms of electrical activity that contribute to the structural and functional development of the ALs is not known. We have begun to address this question using whole cell patch-clamp recordings from AL neurons in vitro and in semi-intact brain preparations. Our results reveal dramatic changes in the electrophysiological properties and response characteristics of AL neurons associated with metamorphic adult development of the moth. We show that spontaneous activity is highly prevalent in developing AL neurons, and that developmental changes in the excitability of these cells can be influenced by the neuromodulator, serotonin (5-hydroxytryptamine, 5-HT). Characteristic forms of electrical excitability exhibited by developing AL neurons, and their modulation by 5-HT, seem likely to contribute to the functional development of the ALs of the moth.

INTRODUCTION

Olfactory receptor cells (ORCs) are born in the antennal epithelium of the sphinx moth, *Manduca sexta*, during stages 1 and 2 of the 18 stages of metamorphic adult development, and they begin almost immediately to extend axons toward the brain (Sanes and Hildebrand 1976a,b). Their arrival in the antennal lobes (ALs), late in pupal stage 3, triggers the formation of protoglomeruli (Hildebrand 1985; Tolbert et al. 1983), which develop in a lateral-to-medial wave across the AL neuropil (Malun et al. 1994). Protoglomeruli serve as a template for the development of an array of glomeruli, each of which is invaded by the dendrites of AL projection (output) neurons, the arborizations of local interneurons, and the processes of centrifugal neurons, which project to the ALs from other regions of the brain (reviewed by Boeckh and Tolbert 1993; Hildebrand et al. 1997; Oland and Tolbert 1996). During pupal stage 6, the processes of a readily identifiable serotonin-immunoreactive neuron invade the developing glomeruli (Kent et al. 1987; Oland et al. 1995), and a wave of synaptogenesis spreads across the glomerular neuropil (Oland et al. 1990). By stage 12 the structural organization of the AL appears to be complete, and the AL then bears a striking morphological resemblance to the analogous structure in the vertebrate brain, the olfactory bulb (Hildebrand and Shepherd 1997).

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METHODS

Manduca sexta (Lepidoptera: Sphingidae)

Larvae hatch from eggs and pass through five larval instars before undergoing metamorphosis from larva to pupa to adult. Metamorphic adult development can be divided into 18 stages, each of which lasts approximately 1 day and is accompanied by well-defined changes in pupal morphology (Sanes and Hildebrand 1976a,b; Tolbert et al. 1983). M. sexta were reared on an artificial diet (modified from that of Bell and Joachim 1976) and maintained at 25°C and 50–60% relative humidity under a long-day photoperiod regimen (17 h light/7 h dark).

Cell culture

AL neurons from pupae at stages 3–14 of the 18 stages of metamorphic adult development were examined in this study. Cells were either dispersed and maintained for 5–14 days in culture or examined in situ, using semi-intact brain preparations. Cells were maintained in vitro according to methods described previously by Hayashi and Hildebrand (1990). Brains removed from cold-anesthetized pupae were placed into sterile culture saline containing 149.9 mM NaCl, 3 mM KCl, 3 mM CaCl₂, 0.5 mM MgCl₂, 10 mM N-tris(hydroxymethyl)-methyl-2-aminoethane sulfonic acid (TES), 11 mM D-glucose, 6.5 g/l lactalbumin hydrolysate (GIBCO), 10% fetal bovine serum (FBS, Hyclone), 100 units/ml penicillin, and 100 μg/ml streptomycin, adjusted to pH 7 and 360 mOsm. ALs were dissected from moth brains and transferred into Hanks’ Ca²⁺- and Mg²⁺-free buffered salt solution (GIBCO) containing 0.5 mg/ml collagenase ( Worthington) and 2 mg/ml Dispase (Boehringer Mannheim) for 2 min at 37°C to dissociate the tissue, which was then dispersed by trituration with a fire-polished Pasteur pipette. Enzyme treatment was terminated by the centrifuging cells, first through 6 ml of culture-saline solution and then through the same volume of culture medium. Dissociated cells were allowed to settle and adhere to the surface of culture dishes coated with Concanavalin A (Sigma, 200 μg/ml) and laminin (Collaborative Research, 2 μg/ml). The dishes were placed in a humidified incubator at 26°C, and the cells were maintained for a maximum of 14 days in culture.

Culture medium

The following were added to 500 ml of Leibovitz’s L15 medium (GIBCO): 10% FBS, 185 mg alpha-ketoglutaric acid, 200 mg fructose, 350 mg glucose, 335 mg malic acid, 30 mg succinic acid, 1.4 g TC Yeastolate, 1.4 g lactalbumin hydrolysate, 0.01 mg niacin, 30 mg imidazole, 100 μg/ml streptomycin, 100 units/ml penicillin, 1 μg/ml 20-hydroxyecdysone (Sigma), and 2.5 ml stable vitamin mix (Mains and Patterson 1973). A 5-ml stock solution of vitamin mix consists of 15 mg asparagine, 15 mg cystine, 5 mg beta-alanine, 0.02 mg biotin, 2 mg vitamin B12, 10 mg inositol, 10 mg choline chloride, 0.05 mg lipoic acid, 5 mg p-aminobenzoic acid, 25 mg fumaric acid, 0.4 mg CoA, 15 mg glutamic acid, and 0.5 mg phenol red. The medium was adjusted to pH 7 and 350 mOsm and filter-sterilized prior to use. The steroid hormone, 20-hydroxyecdysone (20-HE) has been shown to influence the growth (Oland and Hayashi 1993) and voltage-gated ionic current development (Grünewald and Levine 1998) of Manduca sexta neurons in vitro. Cell cultures with and without 20-HE were examined throughout this study, but the effects of this hormone on whole cell currents of antennal lobe neurons appear to be subtle, and further work is required to confirm their significance. As effects of removing 20-HE from the culture medium need to be examined further, this report deals only with results obtained from cells exposed to 1 μg/ml 20-HE.

Identification of cell types in vitro

Two morphologically distinct sets of AL neurons in vitro were examined in this study, Proximal Branching (PB) neurons and Rick Rack (RR) neurons (Fig. 1, A and B). These neurons have been identified elsewhere as projection (output) neurons and putative local interneurons, respectively (Hayashi and Hildebrand 1990; Oland and Hayashi 1993). AL neuron somata are located in three distinct groups lying in the lateral, medial, and anteroventral AL (Christensen and Hildebrand 1987; Homberg et al. 1988, 1989). The medial and anteroventral groups contain projection neurons, while the lateral group comprises a mixed population of local neurons and projection neurons. In this study, PB neurons were derived either from cultures produced from cells of the medial group of AL neurons alone (58%) or from cultures of whole ALs (42%). RR neurons were derived from cultures produced from cells of the lateral group alone (32%), which is enriched in local interneurons (Hayashi and Hildebrand 1990), or from cultures of whole ALs (68%). No differences between cells from cultures of whole ALs versus cells from isolated cell groups could be identified. Prior to recording, cells were maintained in culture for a minimum of 5 days, which is the time required for their distinctive morphologies in vitro to become apparent. Cells dispersed from pupae...
at a particular stage of development are referred to as being at that stage, irrespective of the number of days in vitro. To avoid developmental changes that may result from cell-to-cell contacts, only cells apparently not in contact with other neurons were selected for recording.

**AL neurons in situ**

To determine whether the electrical properties and response characteristics of AL neurons in vitro reflect those of neurons in vivo, projection (output) neurons with cell bodies located in the medial group of AL neuronal somata were examined in situ also, using semi-intact brain preparations (Fig. 1C). To aid the removal of glial cells that envelop the neuronal somata, the brain was placed for 3–5 min in enzyme (0.5 mg/ml collagenase and 2 mg/ml Dispase). The preparation was then washed thoroughly with insect saline solution (see following text) and mounted with fine pins in a dish lined with silicone elastomer (Sylgard, Dow Corning). Somata lying at the periphery of the medial cell group (MC) could be viewed with an inverted microscope and were carefully cleared of glial cells by means of a sharp glass probe mounted on a micromanipulator.

**Electrophysiological recording**

Whole cell patch-clamp recording techniques (Hamill et al. 1981) were used to examine the electrophysiological properties of neurons from pupae at stages 3–14 of the 18 stages of metamorphic adult development. Electrodyes with resistances of 1–2 MΩ were made from borosilicate glass (VWR Scientific, West Chester, CA) and filled with a solution containing (in mM) 150 K-aspartate, 5 NaCl, 2 MgCl₂, 1 CaCl₂, 11 EGTA, and 10 HEPES, and adjusted to pH 7 and 330 mOsm. Cells were viewed with an Olympus IMT-2 inverted microscope equipped with Hoffman modulation contrast optics. To facilitate the formation of high resistance (gigaohm) seals, culture medium was replaced with insect saline solution containing (in mM) 100 NaCl, 4 KCl, 6 CaCl₂, 5 d-glucose, and 10 HEPES (pH 7), adjusted to 360 mOsm with mannitol, prior to recording. Cells were continuously superfused with fresh saline solution throughout the recording period, and junction potentials were nullified prior to seal formation. To obtain whole cell recordings, light suction and brief high-voltage pulses were used to rupture the cell membrane beneath the recording electrode. The resting membrane potential was recorded immediately after rupturing the cell and monitored regularly thereafter. Recordings were made using an AxoPatch 1B amplifier (Axon Instruments, CA), and data were acquired and analyzed using pClamp 6 software (Axon Instruments, version 6.02) run on an i30486 microcomputer. Membrane currents were sampled at intervals of 100 μs and were filtered at 2 kHz with a low-pass 4-pole Bessel filter. Series-resistance (SR) compensation (60–70%) was applied, but only in cells examined in the latter part of this study (approximately 30% of the total number of cells examined). The presence or absence of SR compensation did not alter the categorization of cells according to whole cell current profile type, nor did it alter the overall response characteristics of the cells. In the absence of SR compensation, however, significant voltage errors will be present where currents measured were large. For this reason, no attempt has been made to record current-voltage (I-V) relations, times to peak current, or current densities from whole inward and outward current profiles. Linear leakage currents and capacitance artifacts were subtracted from recordings of current profiles using a P/4 protocol (see Armstrong and Bezanilla 1974) included in the data-acquisition software (Axon Instruments).

**Removing antennal afferent input to the antennal lobes**

Antennal sensory axons (approximately 300,000) provide the main input to the AL (Sanes and Hildebrand 1976a) and project exclusively to the ipsilateral lobe (Camazine and Hildebrand 1979). To examine the influence of antennal sensory input on the electrophysiological development of AL neurons, “deantennated” ALs were generated as described by Oland et al. (1990). The left antennal anlage of stage 1 pupae was removed by opening the head capsule at the point where the antenna has its origin, removing presumptive antennal tissue with fine forceps, then sealing the opening in the head with melted wax. The appearance of rapidly activating, transient sodium currents in *Manduca* AL neurons, and the shift from calcium-dependent to sodium-dependent action potentials occurs during adult metamorphosis. To determine whether this developmental shift is dependent on antennal afferent input to the ALs, the percentage of cells exhibiting sodium-dependent action potentials in AL neurons from deantennated ALs was compared with that of cells from fully afferented (control) ALs from the same animals. Action potentials were considered to be sodium dependent if they were blocked by TTX, or if they disappeared in Na⁺-free saline (Mercer et al. 1996b).

**Addition of 5-HT to the culture medium**

5-HT has a significant influence on the excitability of *Manduca* AL neurons (Kloppenburg and Hildebrand 1995; Mercer et al. 1996b). To explore the possibility that development of excitability in *Manduca* AL neurons may be influenced by this neuromodulator, RR neurons from stage 4 pupae were exposed each day to culture medium containing freshly prepared 5-HT (as the creatinine sulfate, Sigma, St. Louis, MO) at an initial concentration of 50 μM. Fresh 5-HT was applied daily because it was assumed that 5-HT would breakdown relatively quickly in culture medium. Control (untreated) cells were exposed each day to fresh culture medium without 5-HT. Data were gathered over 2 yr. To ensure that results were not biased by slight differences in media, substrate coating, or cell-dissociation procedures, each experiment included cells grown in normal culture medium (controls) as well as cells treated with 5-HT. As identical trends were apparent in the data sets from the two successive years, results were pooled for analysis.

**Statistical analysis**

χ² analysis was used to examine stage-related differences in the percentages of cells exhibiting specific types of whole cell current profile, as well as developmental changes in the percentage of neurons exhibiting sodium-dependent action potentials. Where a significant difference between stages was identified, multiple planned pair-wise χ² tests were performed to determine where the differences lay. χ² analysis was also used to examine the effects of time in culture on whole cell current distributions, and the effects of deantennation and 5-HT on the percentage of neurons exhibiting sodium-dependent action potentials.

To test the significance of differences in resting membrane potential, spike amplitude and spike threshold measurements in RR neurons and PB neurons early (stages 3–5) and late (stages 10–14) in metamorphosis, ANOVA was used. Where significant differences between groups were identified, post hoc Tukey’s tests adjusted for unequal group sizes were used to identify where the differences lay. Unless stated otherwise, a level of significance of 5% was accepted for all tests.

**RESULTS**

Electrophysiological properties and response characteristics of *Manduca* AL neurons change significantly during metamorphic adult development. Examples of responses observed in RR neurons at different stages of adult metamorphosis are shown in Fig. 2. All of the ionic currents referred to in the following descriptions of whole cell current profiles have been examined.
isolated and identified elsewhere (Kloppenburg et al. 1999; Mercer et al. 1995, 1996b), or in the accompanying paper.

**Whole cell current profile of RR neurons**

Early in development, the whole cell current profiles of RR neurons were dominated by large, slowly inactivating and/or noninactivating outward (K⁺) currents (I_{KV}) (Kloppenburg et al. 1999; Mercer et al. 1995, 1996b) that could be blocked by TEA (type 1 profiles; Fig. 2A1). The amount of inactivation apparent in the outward component of the current profile during a 100-ms voltage step was variable at this stage, but generally became less prominent in this cell type as development progressed. Around pupal stage 4, a faster-activating TEA-resistant outward component (I_{K_0}) (Kloppenburg et al. 1999; Mercer et al. 1996b) became apparent in many cells (type 2 profile; Fig. 2A2; see also accompanying paper). In addition, approximately 45% of RR neurons from pupae at stages 5 and 6 exhibited a fast-activating inward component (I_{Na}) (Mercer et al. 1996b; see also accompanying paper) in their whole cell current profile (type 3 profile; Fig. 2A3). At later stages of metamorphosis, the amplitude of this transient inward component increased significantly, and a second, rapidly activating outward component became apparent in many cells (type 4 profile; Fig. 2A4). This rapidly activating outward current differed in several respects from the transient outward current (I_{Kv}) (Kloppenburg et al. 1999; Mercer et al. 1996b) identified previously in RR neurons (see accompanying paper).

None of the current profiles described above (types 1–4; Fig. 2, A1—A4) was stage specific. However, trends in the overall occurrence of these profiles (Fig. 3) confirm that they represent a developmental sequence that progresses from profiles of type 1 to type 4. In Fig. 3, the distribution of whole cell current profiles in RR neurons maintained 5–9 days in culture (gray bars) is compared with that of cells maintained in culture for 10–14 days (white bars). χ² analysis revealed no significant differences in the current profile distributions of these two groups. For this reason, data from cells maintained 5–9 and 10–14 days in vitro were combined to compare percentages of cells exhibiting current profiles of types 1–4 at different stages of adult metamorphosis.

Around pupal stages 3 and 4 (Fig. 3A), a majority of cells exhibited type 1 current profiles. No RR neurons at these early stages of metamorphosis exhibited type 4 profiles. By pupal stages 5 and 6 (Fig. 3B), the percentage of cells exhibiting type 1 profiles had fallen, and current profiles of types 2 and 3 were more prevalent than type 1 profiles. A small percentage of cells at stages 5 and 6 exhibited type 4 current profiles. At pupal stages 8–10 (Fig. 3C), type 3 profiles predominated, and type 1 current profiles were no longer represented. The percentage of cells exhibiting type 4 profiles was significantly higher in

**FIG. 2.** Electrophysiological properties of RR neurons at different stages of adult metamorphosis. Ionic currents contributing to the whole cell current profiles are described in the accompanying paper. A: recordings under voltage clamp. Voltage was stepped from −80 to +50 mV in 10-mV increments from a holding potential of −70 mV. Onset and offset of voltage steps in A1−A4 is indicated by the black bar beneath the traces shown in A: A1: recording from a stage 3 RR neuron. Large, slowly inactivating and/or noninactivating potassium currents dominate the whole cell current profile of RR neurons early (stages 2 and 3) in development (type 1 current profile). A2: recording from a stage 4 RR neuron. A rapidly activating potassium current (I_{K_0}) (Kloppenburg et al. 1999; Mercer et al. 1996b) became apparent in the whole cell current profiles of many cells (arrow) around pupal stage 4 (type 2 current profile). A3: recording from a stage 6 RR neuron. Small-amplitude, fast-activating sodium currents (I_{Na}) (Mercer et al. 1996b) were apparent (arrow) in the whole cell current profiles of approximately 45% of RR neurons from pupae at stages 5 and 6 (type 3 current profile). I_{Na} became more prevalent and more prominent as development progressed. A4: recording from a stage 14 RR neuron. As I_{Na} amplitude increased, a prominent, fast-activating outward current (arrow), which differed pharmacologically from that seen at earlier stages of development (see accompanying paper), became apparent also in the whole cell current profile of RR neurons (type 4 current profile). Large amplitude currents present in cells at late stages of metamorphosis made it difficult to obtain adequate clamping of cells, as evidenced here in the small oscillations in the outward current profile. This problem was solved, at least in part, by examining in isolation, ionic currents that contribute to the whole cell current profiles (see accompanying paper). B1−B4: current-clamp recordings from the same cells as in A1−A4, respectively. Responses to a 600-μs depolarizing current pulse (0.5−1 nA) reveal changes in spike waveform associated with developmental changes in the whole cell current profile. Onset and offset of current pulses in B1−B4 is indicated by the black bar below the traces shown in B. B1: Around stages 2−3 of metamorphosis, RR neurons showed no spike activity in response to injections of depolarizing current. The sag in the response (arrow) to depolarization could be blocked by TEA (see Fig. 4A). B2: around pupal stage 4, prior to the appearance of a fast inward component to the whole cell current profile (see, A3), depolarizing current pulses induced small-amplitude, Ca²⁺-dependent action potentials in the cells (see also Fig. 4B). B3: the appearance of a fast inward component to the whole cell current profile (A3) coincided with the appearance of Na⁺-dependent action potentials in the cells (see also Fig. 4C). B4: action-potential waveform late (stage 14) in development. Action potentials became larger in amplitude and shorter in duration as development progressed.
cells from pupal stages 12–14 (Fig. 3D) than at all earlier stages of development and, as in cells from pupae at stages 8–10, no type 1 current profiles were observed.

Development of action-potential waveform

Recordings from RR neurons examined under current clamp revealed changes in spike waveform associated with the development of the whole cell current profile (Fig. 2B). Immature neurons exhibiting type 1 current profiles (Fig. 2A1) responded to injection of positive current with a depolarizing shift in membrane potential that sagged to more polarized potentials during the current pulse (Figs. 2B1 and 4A1). The sag was abolished by the K⁺-channel blocker TEA (Fig. 4A2), indicating that outward movement of K⁺ ions accounted for this response. Neurons with type 2 whole cell current profiles exhibited small amplitude spikes in response to depolarizing current pulses (Figs. 2B2 and 4B1). These spikes remained intact in Na⁺-free medium (Fig. 4B2) but were blocked by the addition of Cd²⁺ to the perfusate (see Fig. 4B3). The appearance of small, rapidly activating inward currents in the whole cell current profile of RR neurons (Fig. 2A3), coincided with the appearance of Na⁺-dependent action potentials in the cells (Figs. 2B3 and 4C1). In the presence of Cd²⁺, Na⁺-dependent action potentials remained intact, but their amplitude was generally reduced and spike-duration increased (Fig. 4C2). Na⁺ spikes could be blocked by TTX (Fig. 4C3), or by placing the cells in Na⁺-free saline (Mercer et al. 1996b; see also accompanying paper). Sodium spikes became larger in amplitude and shorter in duration as development progressed (Fig. 2, B3 and B4; see also Fig. 8C).

Development of repetitive firing behavior

A characteristic feature of the Ca²⁺-dependent action potentials observed in RR neurons early in metamorphosis was the progressive reduction in amplitude of these spikes during prolonged depolarizing current pulses (Figs. 5A). In contrast, the action potentials observed in immature neurons at stages 5 and 6 were significantly higher than the percentage of cells exhibiting type 1 or type 2 current profiles at stages 8 and 10 (χ²₁ = 17.48; P < 0.0006). By stage 8, type 1 profiles were no longer observed. A majority of cells exhibited type 1 or type 2 profiles, and significantly more cells displayed type 3 profiles than profiles of type 4. Distribution of current profiles in RR neurons at stages 12–14 (χ²₁ = 33.679; P < 0.0001). A significant difference in the percentage of cells exhibiting type 1 profiles was significantly higher than the percentage of cells displaying type 2 profiles was, in turn, significantly higher than the percentage exhibiting types 3 or 4. The percentage of cells exhibiting type 2 current profiles in cells maintained 5–9 days in culture (gray bars) was presented separately from those of cells maintained 10–14 days in vitro (white bars). χ² analysis revealed no significant differences relating to time in culture. To compare percentages of cells exhibiting current profiles types 1–4 at different stages of metamorphosis, data from cells maintained 5–9 and 10–14 days in vitro were combined. For each graph, the significance of differences between groups is represented by letters that appear above the bars. Groups that do not differ significantly share at least 1 letter; groups that differ significantly do not share a letter. A Distribution of current profile types in RR neurons from pupal stages 3 and 4. A significant difference in the percentage of RR neurons exhibiting current profiles types 1–4 was identified at this stage (χ²₁ = 53.21; P < 0.0001). Pair-wise comparisons revealed that the percentage of RR neurons exhibiting type 1 profiles was significantly higher than the percentage of cells displaying type 2 current profiles. The percentage of cells displaying type 2 profiles was, in turn, significantly higher than the percentage exhibiting profiles of types 3. No type 4 profiles were observed at the onset of metamorphic adult development. B: distribution of current profiles in stage 5 and 6 RR neurons. A significant difference in the percentage of RR neurons exhibiting current profiles of types 1–4 was identified also in cells from pupae at stages 5 and 6 (χ²₁ = 54.91; P < 0.0001). At this time, the percentage of cells with type 1 profiles was significantly lower than the percentage of cells displaying either type 2 or type 3 current profiles. Type 4 profiles were observed at this stage, but only in a small proportion of cells. C: distribution of current profiles in RR neurons at stages 8–10 (χ²₁ = 17.48; P < 0.0006). By stage 8, type 1 profiles were no longer observed. A majority of cells exhibited type 1 or type 2 profiles, and significantly more cells displayed type 3 profiles than profiles of type 4. D: distribution of current profiles in RR neurons at stages 12–14 (χ²₁ = 33.679; P < 0.0001). Late in metamorphosis, the percentage of cells exhibiting type 4 current profiles increased significantly compared with earlier stages of development. There were no type 1 profiles observed, and significantly more cells exhibited type 3 profiles than profiles of type 2. Taken together these results indicate that whole cell current profiles in these cells appear in a developmental sequence that progresses from type 1 to type 4. Numbers of cells examined are shown in E.
pulses elicited Ca$^{2+}$ (see Fig. 2B3). Blocked by Cd$^{2+}$ into cells to retain the resting membrane potential at the level recorded prior to addition of blocker. Prior to the appearance of fast, transient Na$^{+}$ currents, depolarizing current pulses elicited Ca$^{2+}$-dependent action potentials in many cells. B2: perfusion with Na$^{+}$-free saline did not block these Ca$^{2+}$ spikes. B3: calcium spikes were blocked by Cd$^{2+}$. C1: recording from a stage 8 RR neuron. C2: the appearance of small, rapidly activating inward currents (see Fig. 2A3) coincided with the appearance of small amplitude Na$^{+}$-dependent action potentials in the cells (see Fig. 2B1). C2: Na$^{+}$ spikes could still be elicited in the presence of the Ca$^{2+}$-channel blocking agent, CdCl$_2$. In CdCl$_2$, the latency of spike onset was generally reduced, and spike duration was increased. As depolarizing effects of Ca$^{2+}$ sometimes masked small amplitude Na$^{+}$ spikes, current was injected into cells to retain the resting membrane potential at the level recorded prior to the addition of CdCl$_2$. C3: Na$^{+}$ spikes were blocked by TTX. * Membrane potential maintained at the level recorded prior to adding blocker.

appearance of Na$^{+}$ spikes in the cells coincided with the development of repetitive firing behavior in response to intracellular injections of depolarizing current (Fig. 5B). Interestingly, many cells in vitro exhibited spontaneous activity at this stage (Fig. 12). In response to depolarizing current pulses, RR neurons with type 3 current profiles exhibited trains of action potentials that showed little or no spike frequency adaptation: that is the frequency of spikes remained relatively unaltered during the entire spike train (Fig. 5C). However, adaptation of spike activity became increasingly apparent in RR neurons as adult metamorphosis progressed (Fig. 5D), and occurred rapidly in cells exhibiting type 4 whole cell current profiles (Fig. 5E). Cells with type 4 whole cell current profiles did not exhibit spontaneous activity.

Development of rebound excitation

In contrast to spike-frequency adaptation, which was observed only in relatively mature RR neurons, responses of RR neurons to hyperpolarizing current pulses changed early in development (Fig. 6). RR neurons from stage 3 pupae, the majority of which displayed type 1 whole cell current profiles, showed little or no response to hyperpolarizing current pulses (Fig. 6A4), whereas in cells exhibiting type 2 current profiles, the membrane potential sagged toward more depolarized potentials during the hyperpolarizing current pulse (Fig. 6B). Hyperpolarizing pulses were followed in these cells by prolonged afterdepolarization of the membrane that was sometimes accompanied by small-amplitude spikes similar to those described earlier (see Figs. 2B2 and 4B1). In RR neurons exhibiting type 3 current profiles, hyperpolarizing current pulses induced rebound excitation and spike activity (Fig. 6C). Rebound excitation was apparent also in RR neurons with type 4 current profiles, but the magnitude of the sag was variable, and rebound spike activity was much briefer in these cells than in cells exhibiting type 3 current profiles (Fig. 6D).

PB neurons versus RR neurons

To determine whether developmental changes similar to those observed in RR neurons (putative local interneurons) occur in other cell types, whole cell current profiles and cell excitability were examined also in PB neurons, which belong to the second major category of AL neurons, known as projection neurons. PB neurons were maintained in vitro for similar periods of time as RR neurons. As in RR neurons, no significant differences were identified between the whole cell...
current pro-
files distributions of cells maintained for 5–9 days versus 10–14 days in culture (data not shown).

PB neurons exhibited three main types of whole cell current profile. Early in development (stages 3–4), the whole cell current profiles of PB neurons, like RR neurons, were dominated by large outward K\(^+\) currents (PB type 1 current profile), which usually (but not always) showed pronounced inactivation during a 100-ms depolarizing voltage step (Fig. 7A1). While inactivation during depolarizing voltage steps was less prominent in RR neurons early in development (Fig. 2A1) than in PB neurons (Fig. 7A1), the appearance of the current profile early in metamorphic adult development could not be used as a reliable predictor of cell type. However, differences in the electrophysiological properties and response characteristics of RR neurons and PB neurons became more obvious as development progressed. PB type 2 profiles were characterized by the presence of a small-amplitude, rapidly activating inward component of the whole cell current profile (Fig. 7A2), whereas large-amplitude inward currents, together with large-amplitude outward currents that showed pronounced inactivation during the voltage step, were typical of PB type 3 current profiles (Fig. 7A3). Changes in spike waveform were less dramatic in PB neurons than in RR neurons, but as in RR neurons, metamorphosis was accompanied by an increase in spike amplitude and a fall in spike duration in PB neurons (Fig. 7, B1 and B2). In contrast to RR neurons, PB neurons from pupae late (stage 14) in metamorphosis showed little or no spike frequency adapta-
tion (accommodation) during prolonged injections of depolarizing current. Moreover, spike activity often continued after the cessation of the depolarizing current pulse in these cells (Fig. 7C).

Results suggested that there may be significant differences between PB neurons and RR neurons in resting membrane potential (RMP), spike amplitude, and spike threshold, particularly late in metamorphosis. However, subtle changes in the culture medium during the course of the study, and factors such as the time when measurements were taken after rupturing the cell membrane, could have had an impact on the measurement of such properties. For this reason, a subset of neurons maintained and examined under identical conditions was used to examine major shifts in RMP, spike threshold, and spike am-

![Image](http://jn.physiology.org/Downloadedfrom)
plitude. PB and RR neurons early in development (stages 3–5) were compared with cells examined late (stages 10–14) in metamorphosis (Fig. 8). As each group contained cells exhibiting a range of whole cell current profiles, trends revealed using this approach are likely to be a conservative indication of changes occurring at the single-cell level.

Significant differences in RMP between cell groups were identified. While there was no significant difference early in development between the mean RMP in PB and RR neurons (Fig. 8A1), as development progressed the membrane potential in RR neurons became significantly more polarized. A similar trend in PB neurons was not statistically significant. For comparison, the RMP was examined also in RR neurons grouped according to their whole cell current profiles. The mean RMP recorded for RR neurons exhibiting RR type 1 current profiles was significantly smaller than in cells displaying RR type 3 or RR type 4 current profiles (Fig. 8A2). Differences in spike threshold and spike amplitude were also identified between RR neurons and PB neurons. As the RMP increased in RR neurons, the threshold for spike initiation fell to a more hyperpolarized level, and there was a significant increase in spike amplitude in these neurons (Fig. 8, B and C). A similar shift in spike amplitude was apparent in PB neurons but was not statistically significant.

Recordings from cells from the medial group of AL neurons in situ

To determine whether cells in vitro reflect developmental changes occurring in vivo, recordings were made in situ from medial-group cells (MC; Fig. 1C). Cells in this group contain only projection (output) neurons (Homberg et al. 1989). Over 50% of the PB neurons examined in this study were derived from cultures of cells prepared from this group (see METHODS). Typically, the amplitude of currents recorded from MC neurons in situ was smaller than in either of the two cell types examined in vitro. However, the response characteristics and whole cell current profiles of MC neurons in situ (Fig. 9) strongly resembled those of PB neurons in vitro (Fig. 7). As for PB neurons, the whole cell current profiles of MC neurons in situ fell into three major groups: MC type 1 profiles were dominated by outward currents that sometimes, but not always (75%), exhibited a rapidly activating transient component in addition to a slowly inactivating and/or noninactivating component (Fig. 9A1). MC type 2 profiles had, in addition, a small-amplitude rapidly activating transient inward component (Fig. 9A2), and MC type 3 profiles were characterized by large-amplitude currents that showed pronounced inactivation of the outward component during the depolarizing voltage step (Fig. 9A3). As development progressed, action-potential waveform increased in amplitude and became shorter in duration (Fig. 9B). In addition, MC neurons in situ, like PB neurons in vitro, responded to prolonged injections of depolarizing current with trains of action potentials that showed little or no spike frequency adaptation, and spike activity often outlasted the duration of the current pulse (Figs. 9C).

Stage-related changes in whole cell current profile distributions of PB and MC neurons

PB neurons from pupae at stages 3 and 4 exhibited current profiles of types 1 and 2 only (Fig. 10A1); no type 3 profiles were observed this early in metamorphosis. The same was true of MC neurons examined in situ (Fig. 10B1). By stages 5 and 6, a small percentage (<10%) of PB neurons exhibited type 3 current profiles (Fig. 10A2), but current profiles of types 1 and 2 remained equally prevalent in these cells. No type 3 profiles were observed in MC neurons at pupal stages 5 and 6 (Fig. 10B2), but as in PB neurons, a similar percentage of cells at this stage exhibited type 1 and type 2 profiles. By stage 8 (Fig. 10A3), type 1 current profiles were less prevalent in PB neurons than at earlier stages of metamorphosis and type 2 profiles predominated at this stage. In pupae at stages 8–10, the per-
percentage of MC neurons exhibiting type 1, type 2, and type 3 current profiles was similar (Fig. 10B3). At the latest stages of metamorphosis examined in this study (pupal stages 12–14), type 1 current profiles were absent in PB neurons (Fig. 10A). While this was not the case in MC neurons, type 2 and type 3 profiles were observed more frequently in MC neurons at stages 12 and 14 than type 1 profiles (Fig. 10B4). Taken together, these results indicate that the whole cell current profiles observed in PB neurons in vitro, and in MC neurons in situ, represent a developmental sequence that progresses from current profiles of types 1–3. There were no significant differences between the overall proportions of PB neurons and MC neurons exhibiting type 1 profiles (38% in situ, n = 32; 34% in vitro, n = 117), type 2 profiles (55% in vitro, n = 117; 47% in situ, n = 32), or type 3 profiles (11% in vitro, n = 117; 15% in situ, n = 32).

**Factors affecting the electrophysiological development of AL neurons**

In all three cell types examined in this study, the percentage of cells exhibiting Na⁺-dependent action potentials increased as development progressed (Fig. 11A). Interestingly, Na⁺ spikes were observed earlier in metamorphosis in PB neurons (stage 3) than in RR neurons (stage 4). Consistent with this observation, rapidly activating, transient Na⁺ currents were already apparent in over 50% of PB neurons from pupae at stages 3 and 4, whereas none of the RR neurons examined from stage 3 pupae and only about 15% of stage 3 and stage 4 RR neurons, collectively, exhibited currents of this type. In RR neurons, a significant increase in the percentage of RR neurons exhibiting Na⁺-dependent action potentials occurred between stages 3–4 and stages 5–6 of development. This was not the case in PB neurons or in MC neurons. The possibility that direct or indirect contact with primary-afferent sensory cells might trigger the development of Na⁺ currents in these neurons was examined using animals in which antennal sensory input to one AL had been removed early (stage 2) in metamorphic adult development. However, no significant difference was observed between percentages of cells exhibiting Na⁺-dependent action potentials in deantennated ALs and in fully afferented (control) ALs (Fig. 11B). In contrast, the timing of the appearance of Na⁺ currents in these cells did appear to be affected by the neuromodulator, 5-HT. A significantly higher percentage of stage 4 RR neurons exhibited Na⁺-dependent action potentials following daily exposure to 5-HT than did controls grown in normal culture medium without exposure to 5-HT (Fig. 11C).

**Spontaneous activity in developing AL neurons**

Spontaneous activity was recorded in all three cell groups examined in this study (Fig. 12) and appeared to be most prevalent in cells midway through metamorphosis. Of 12 RR neurons examined for spontaneous activity between pupal stages 4 and 10, seven exhibited spontaneous spikes (Fig. 12A). However, spontaneous activity was detected less frequently in more mature RR neurons. In RR neurons exhibiting type 4 current profiles (n = 6), for example, no spontaneous activity was observed. In PB neurons (n = 8), spontaneous activity was apparent early (Fig. 12B) and late (Fig. 12C) in metamorphosis, even in cells displaying PB type 3 current profiles. Properties of cells that contribute to this activity are being examined currently (Mercer et al. 2000) and will be considered in detail elsewhere. In MC neurons, bursts of “spontaneous” activity were first observed around pupal stage 6. However, spike amplitudes overall were much smaller in MC neurons in situ than in cells in vitro (compare Fig. 12, C and D). If generated some distance from the recording electrode, small-amplitude bursts of spontaneous activity may have escaped detection because of signal attenuation.
**Discussion**

Our results reveal for the first time that the biophysical properties and excitability of *Manduca* AL neurons change dramatically during metamorphic adult development, and moreover, that AL neurons exhibit characteristic forms of electrical excitability that could contribute significantly to the structural and functional development of the antennal lobes. Developmental events, as well as their time course, appear to be cell-type specific. However, all three cell groups examined in this study (PB neurons, RR neurons, and MC projection neurons in situ) exhibited similar developmental trends, which can be summarized as follows. 1) Early in metamorphosis, low-threshold Ca<sup>2+</sup> spikes could be elicited from the cells, but only a small percentage of cells exhibited Na<sup>+</sup>-dependent action potentials. 2) Around the time when subunits of synaptic neuropil (glomeruli) form in the ALs, there was a rapid increase in the percentage of AL neurons exhibiting Na<sup>+</sup>-dependent action potentials, and many cells were spontaneously active at this time. 3) As development progressed, action-potential waveforms became larger in amplitude and shorter in duration. 4) While injections of hyperpolarizing current produced little or no response at the onset of metamorphosis (pupal stages 2 and 3), relatively early in development (around pupal stages 4 and 5) most cells began responding to hyperpolarizing current pulses with rebound depolarization. Later in development, rebound depolarization was generally accompanied by spike activity. The magnitude and prevalence of these responses became less predictable late in metamorphosis (stages 12–14), particularly in RR neurons.

**When are AL neurons fully mature?**

Developmental shifts in the biophysical properties of AL neurons were not confined to specific time points but generally spanned several stages of metamorphic adult development. This is consistent with the finding that AL glomeruli do not develop simultaneously, but rather, in a lateral-to-medial wave across the AL neuropil (Malun et al. 1994). At what stage the electrophysiological properties and response characteristics of *Manduca* AL neurons can be considered fully mature is not yet entirely clear. While whole cell current profiles of MC neurons in situ appear to be similar at stage 14 to those observed in the

**FIG. 10. Developmental changes in the occurrence of whole cell current profiles types 1–3 observed in PB neurons (gray bars, A1–A4) and in MC neurons (white bars, B1–B4).** For each graph, the significance of differences between groups is represented by letters that appear above the bars. Groups that do not differ significantly share at least 1 letter; groups that differ significantly do not share a letter. Trends apparent in the whole cell current profile distributions of PB neurons and MC neurons at different stages of adult metamorphosis indicate that whole cell current profiles appear in a developmental sequence that progresses from type 1 to type 3. A1—A4: significant differences in the percentage of PB neurons exhibiting current profiles of types 1–3 were identified at stages 3 and 4 (χ<sup>2</sup> = 55.30; P < 0.0001), stages 5 and 6 (χ<sup>2</sup> = 26.12; P < 0.0001), and at stages 12 to 14 (χ<sup>2</sup> = 10.09; P < 0.0006). No significant difference was identified between percentages of cells displaying different current profiles around pupal stages 8–10 (χ<sup>2</sup> = 4.88; P = 0.09), a probable reflection of low n values examined at these stages. Early in adult metamorphosis, type 1 and type 2 profiles were predominant and no (A1), or very few (A2) type 3 profiles were encountered. As development progressed there was a marked shift to a predominance of type 2 and type 3 profiles (A3 and A4) with type 1 profiles absent at late stages of metamorphosis (A4). The same trends are apparent in MC neurons (see B1–B4). B1–B4: significant differences in the percentage of MC neurons exhibiting current profiles of types 1–3 were identified at stages 3 and 4 (χ<sup>2</sup> = 4.2; P = 0.04) and at stages 5 and 6 (χ<sup>2</sup> = 7.0; P = 0.008). No significant differences between groups of MC neurons at stages 8–10 (χ<sup>2</sup> = 2.1; P = 0.15) or at stages 8–10 (χ<sup>2</sup> = 2.63; P = 0.11) were identified, a probable reflection also of low n values at these stages. Numbers of cells examined in this component of the study are shown in C.
Whole cell current profiles of MC neurons and PB neurons were strikingly similar, supporting the view that both cell types belong to the same major category of AL neuron, namely projection (output) neurons. RR neurons, on the other hand, are thought to be local AL interneurons (Hayashi and Hildebrand 1990; Oland and Hayashi 1993), the arbors of which are restricted to the AL neuropil. Presumably, differences in the electrophysiological properties and response characteristics of PB and RR neurons reflect the different roles played by these neurons in the AL. That not all putative local interneurons have current profiles identical to those exhibited by RR neurons (Hayashi and Hildebrand 1990) probably reflects the diversity of local interneurons in the insect AL (e.g., Christensen et al. 1993; Flanagan and Mercer 1989).

What triggers developmental changes in electrical excitability?

Ultrastructural and electrophysiological studies of the synaptic circuitry of the ALs suggest that there is little direct interaction between sensory axons and uniglomerular projection neurons in adult insects (Boeckh and Tolbert 1993; Christensen et al. 1993). Nonetheless, AL neurons exposed in vivo to sensory axons prior to dissociation develop significantly more branching in vitro than do neurons taken from deantennated ALs (Oland and Hayashi 1993). This and other studies (reviewed by Oland and Tolbert 1996) suggest that interactions between AL neurons and ORC axons are developmentally important. As Na⁺-dependent action potentials were detected in PB neurons at an earlier stage of metamorphosis than in RR adult moth (Kloppenburg et al. 1999), detailed comparisons with adult neurons have yet to be undertaken.

In vivo, each stage of metamorphic adult development lasts approximately 1 day, but clearly this is not the case for AL neurons in vitro. Whole cell currents of cells from pupae at early stages of metamorphosis remained immature, even after 14 days in vitro. Moreover, times in culture >5 days had little, if any, impact on the current-profile distributions of cells. The whole cell current profile distributions of PB neurons and same-stage MC neurons also suggest that if AL neurons continue to undergo development in vitro, they do so at a significantly slower rate than in vivo.

![Image](http://jn.physiology.org/)

**FIG. 11.** Percentages of cells exhibiting Na⁺-dependent action potentials. A: developmental changes in the percentages of RR neurons, PB neurons and MC neurons displaying Na⁺ spikes. In all 3 cell types, the percentage of cells exhibiting Na⁺-dependent action potentials increased as development progressed (RR neurons, \( \chi^2 = 46.53; P < 0.0001 \); PB neurons, \( \chi^2 = 11.42; P = 0.01 \); MC neurons \( \chi^2 = 4.09; P = 0.043 \)). Sodium spikes were apparent at an earlier stage of development (stage 3) in PB neurons than in RR neurons (stage 4). B: the percentage of cells exhibiting Na⁺ spikes was not affected by removing antennal sensory input to the AL ("deantennation") early in metamorphosis. A comparison of cells from deantennated ALs with those from fully afferented (control) ALs revealed no significant differences in the prevalence of Na⁺ spikes, either in RR neurons \( (\chi^2 = 0.01; P = 0.924) \) or in PB neurons \( (\chi^2 = 0.02; P = 0.879) \). C: the percentage of stage 4 RR neurons exhibiting Na⁺-dependent spikes was significantly higher in cells exposed daily to 50 \( \mu \)M 5-HT than in cells grown in 5-HT-free culture medium \( (\chi^2 = 6.64; P = 0.01) \).

**FIG. 12.** Spontaneous activity recorded in *Manduca* AL neurons. Recordings were obtained in 2.5 s "episodes" using pClamp6 acquisition software. Spontaneous activity appears at random points during an episode and sometimes outlasted the duration of the episode. All membrane depolarizations shown here are spontaneous events. A: spontaneous activity was prevalent in RR neurons around pupal stages 5–10. However, no spontaneous activity was detected in RR neurons exhibiting RR type 4 current profiles. B and C: spontaneous activity recorded in PB neurons at relatively early (B) and late (C) stages of metamorphosis. The stage 10 PB neuron exhibited a PB-type 3 current profile. D: "Spontaneous" activity in a stage 10 MC neuron in situ. Whether electrical activity in MC neurons in situ is autogenic, or the result of synaptic input has yet to be clarified.
neurons, and previous studies had shown that dendritic processes of projection (output) neurons invade the developing glomeruli one stage earlier in metamorphosis (pupal stage 5) than the processes of multiglomerular local AL interneurons (Malun et al. 1994; Oland et al. 1990), we examined the possibility that direct or indirect contacts with antennal sensory axons might be responsible for triggering developmental changes in the electrical excitability of AL neurons. Our analysis of RR neurons suggests that removal of sensory input from the antenna early in metamorphosis has little, if any, impact on the development of Na⁺-dependent action potentials in these cells. However, deantennated lobes are not entirely devoid of input from primary sensory neurons. A small number of sensory axons from sensilla in the labial pit organ enter the deantennated lobe and terminate, as they do in fully afferented lobes, in a single glomerulus located in the ventromedial region of the lobe (Kent et al. 1986, 1999; Malun et al. 1994). It seems unlikely that this small input alone triggers normal developmental changes in the biophysical properties of AL neurons. However, despite the profound structural abnormalities that occur in deantennated ALs (Oland et al. 1990), whole cell current profiles and response characteristics of RR neurons from deantennated and fully afferented ALs were remarkably similar.

**Do changes in electrical excitability play a role in development?**

Spontaneous activity in developing *Manduca* AL neurons coincides with critical periods of glomerular development and synaptogenesis (Oland et al. 1996; present investigation), but whether such activity contributes to the development of the ALs has yet to be established. Oland et al. (1996) report that injection of TTX into the hemolymph of *Manduca* immediately prior to the arrival of ORC axons at the AL has no obvious affect on the dendritic branching patterns of AL neurons or the overall structure of the AL (Oland et al. 1996). While TTX was used in these experiments to prevent spontaneous activity through blockade of Na⁺ currents in the cells, results of the present investigation reveal that characteristic forms of electrical activity exhibited by AL neurons at the time of glomerular development and synaptogenesis not only involve long-duration Na⁺-dependent action potentials, but also Ca²⁺ spikes. Calcium signals provide a trigger for a variety of developmental events (e.g., Desarmenien and Spitzer 1991; Gruol et al. 1992; Mattson et al. 1988; Spitzer et al. 1995; see also recent reviews by Spitzer 1991, 1994; Spitzer and Ribera 1998). It is probable that spontaneous activity involving long-duration Na⁺-dependent action potentials and Ca²⁺ spikes alters intracellular Ca²⁺ levels in immature *Manduca* AL neurons. Such signals could have a significant impact on the growth and elaboration of dendritic arbors, the formation of synapses within developing glomeruli, and the maturation of synaptic connectivity in the ALs. Preliminary studies reveal that developmental changes in cell excitability show a strong temporal correlation with the morphological development of *Manduca* AL neurons and their dendritic arbors (Mercer et al. 1997).

**5-HT modulation of developing AL neurons**

One of the principal differences between cells that undergo developmental changes in cell excitability and those that do not resides in the time course of maturation of K⁺ currents (see Spitzer 1994; Spitzer and Ribera 1998). We have shown previously that 5-HT modulates at least two distinct K⁺ currents in *Manduca* AL neurons (Kloppenburg et al. 1999; Mercer et al. 1995, 1996b) and also affects the growth of these cells in vitro (Mercer et al. 1996a). In other neuronal systems, intracellular Ca²⁺ levels (Goldberg et al. 1992) and developmental events such as neurite outgrowth (Budnik et al. 1989; Goldberg and Kater 1989; Goldberg et al. 1990; Haydon et al. 1984, 1987; Whitaker-Azimta 1991) and synaptogenesis (Chabakov et al. 1986; Goldberg and Kater 1989) have been shown also to be influenced by this modulatory amine. In *Manduca*, a readily identifiable 5-HT-immunoreactive neuron invades the developing AL glomeruli at pupal stage 6 (Kent et al. 1987; Oland et al. 1995), and, as in many regions of the vertebrate nervous system (Lauder 1990, 1993), the processes of the 5-HT-containing cell are well positioned to influence developmental events in the AL (Kent et al. 1987; Oland et al. 1995). As a result of adding 5-HT to the culture medium, the percentage of RR neurons exhibiting Na⁺-dependent action potentials early in metamorphosis increased significantly. This suggests that in addition to its effects on neurite outgrowth (Mercer et al. 1996a) and cell excitability (Kloppenburg and Heinboekel 2000; Kloppenburg and Hildebrand 1995; Mercer et al. 1995, 1996b), 5-HT can exert influence on the electrophysiological development of *Manduca* AL neurons. Whether 5-HT affects ion channel expression directly, or indirectly via 5-HT modulation of currents that contribute to the normal electrophysiological development of *Manduca* AL neurons, has yet to be determined. Either way, if changes in electrical excitability have a developmental function, modulatory actions of 5-HT on immature AL neurons will have a significant impact also on the development of the ALs. In adult moths, synaptic contacts involving the 5-HT-immunoreactive neuron in each AL are predominantly, but not solely, output synapses from the 5-HT-containing cell (Sun et al. 1993). If as suggested elsewhere, synapses in the developing nervous system release fewer quanta of neurotransmitter per action potential than in the adult (e.g., Campbell and Shatz 1992), relatively high cell input resistances and lower current levels needed to reach firing threshold in immature AL neurons, both of which are promoted by 5-HT, might serve also to enhance synaptic transmission in the developing ALs.

While not all neurons undergo developmental changes in electrical excitability, many do. For example, in the rat neocortex (McCormick and Prince 1987), hippocampus (Schwartz-kroin and Kundel 1982), cerebellum (Gardette et al. 1985), and spinal cord (Baccaglini 1978; Fulton 1987; Spitzer 1991; Walton and Fulton 1986; Westbrook and Brenneman 1984), action potentials become briefer in duration and larger in amplitude during postnatal development, and in many cases, as in *Manduca* AL neurons, these changes are accompanied by a shift in resting membrane potential and a fall in cell input resistance. There is intense current interest in the developmental regulation of cell excitability and its role in the development of the CNS. *Manduca sexta* provides an excellent model system for such studies. Voltage-gated ionic currents and their...
contribution to developmental changes in action potential waveform in *Manduca* AL neurons are described in the accompanying paper.

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