Voltage-Activated Currents From Adult Honeybee (Apis mellifera) Antennal Motor Neurons Recorded In Vitro and In Situ

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INTRODUCTION

The extraordinary behavioral ecology of the honey bee, Apis mellifera, was a focus of attention for many years (see Seeley 1985; Winston 1987), but increasing numbers of studies have now begun probing the cellular basis of honey bee behavior (for recent reviews see Erber et al. 1993b; Hammer and Menzel 1995; Mauelshagen and Greggers 1993; Menzel and Müller 1996). A significant proportion of the very extensive behavioral repertoire of the honey bee relies on sensory information that is gathered by the antennae. These multifunctional sense organs house mechanoreceptors, chemoreceptors, and receptors sensitive to temperature, humidity, and CO₂ (Esslen and Kaissling 1976), which send projections into the brain that terminate in the antennal lobes or in the antennal motor and mechanosensory centers (dorsal lobes) of the deutocerebrum (Maronde 1991; Pareto 1972; Suzuki 1975). Antennal movements in the honey bee are controlled by six muscles, four of which are located within the head and are responsible for moving the basal segment (scape) of the antenna and two of which lie within the scape and control movements of the antennal flagellum (see Snodgrass 1956). Movements of the scape are controlled by nine motor neurons, and those of the flagellum are controlled by six motor neurons (Kloppenburg 1995). The cell bodies of these neurons are located in the somata layer lateral to the dorsal lobes (DL) of the deutocerebrum and they are arranged in three clusters, two of which lie dorsal to the DL, and the third of which lies ventral to the DL. The dendritic fields of these neurons overlap extensively in the DL neuropil. Detailed neuroanatomical descriptions of the antennal muscle system, and of the motor neurons, are provided by Snodgrass (1956) and Kloppenburg (1995), respectively.

Honey bees exhibit a number of predictable antennomotor behaviors, including reflex movements of the antennae to visual stimuli (Erber and Schildberger 1980) and spatial antennal screening movements (Erber et al. 1993b). These behaviors were used to study the effects of putative neuromodulators applied to sensory and antennal motor centers of the brain (Erber et al. 1993a,b; Erber and Kloppenburg 1995; Pribbenow and Erber 1996). Injecting biogenic amines into the DL, for example, causes significant changes to normal antennal responses (Pribbenow and Erber 1996), but the cellular mechanisms that underlie these changes remain obscure, as relatively little is known about the physiological properties of antennal motor neurons and their synaptic inputs. As a first step toward addressing this issue, we have begun to explore the electrophysiological properties of antennal motor neurons in the brain of the adult worker honey bee.

In this study, whole cell patch-clamp recordings were used to examine the biophysical properties of antennal motor neurons in worker honey bees. Motor neurons were examined both in vitro as well as in intact brains, semintact brains, and in brain slices. As the biophysical properties of neurons from holometabolous insects can change dramatically during metamorphic adult development (see Mercer et al. 1996a,b, 1997), only cells from adult bees were examined in this study. Although cells in vitro are particularly amenable to analysis and can be studied under controlled conditions, extrapolating the results of in vitro studies to conditions in vivo can be problematic, as cells in vitro are removed from their native environment and are synthetically disconnected. For this reason, we have chosen to record in parallel from antennal motor neurons in vitro and in situ. Dye backfills and intracellular staining via the patch pipette enabled us to...
identify antennal motor neurons in vitro as well as in intact brains, semiintact brains, and brain slices. By using this combination of techniques we have begun to explore the basic membrane properties of honey bee antennal motor neurons. One principal objective of this study was to provide a basis for future work aimed at understanding the intrinsic properties of *Apis* antennal motor neurons and the effects of neuromodulators on neurons that drive antennal motor behaviors of the bee.

**Methods**

**Materials**

Adult worker bees were caught at the hive entrance, or at a feeding site, and immobilized by chilling for several minutes in a refrigerator (−4°C). They were then mounted in small metal or plastic tubes with their head fixed with adhesive tape and a mixture of wax–collophonium. All chemicals, unless stated otherwise, were obtained from Sigma Co (St. Louis, MO).

**Dissection and backfilling**

Details regarding the dissection and backfilling of antennal motor neurons are provided by Kloppenburg (1995). Motor neurons were backfilled by cutting the nerves close to their attachment sites in the muscles and exposing the cut ends to 2–4% Lucifer yellow (Aldrich, Molecular Probes, Sigma) for 2–5 h, or 1–2% dextran tetramethylrhodamine (lysin fixable, MG 3000; Molecular Probes) for 1 h. Both were dissolved in distilled water. After backfilling the motor neurons, the brain was removed from the head capsule and either dissociated for examination of cells in vitro or prepared for recording of motor neurons in situ.

**In vitro studies**

To examine the electrophysiological properties of isolated antennal motor neurons in vitro, brain tissue was dissected with a technique described in full by Kirchhof and Mercer (1997), based on a protocol reported by Kreissl and Bicker (1992). The deutocerebral region was dissected from the brain and dissociated by trituration. Cells were placed in modified Leibovitz L15 medium (see Kirchhof and Mercer 1997) and maintained for 1–2 days in a humidified incubator at 28°C. Antennal motor neurons backfilled with dye could be identified with an inverted microscope equipped with Hoffmann modulation contrast and epifluorescence optics (e.g., Fig. 1, B and C).

**In situ preparations**

Intact brains, semiintact brain preparations and brain slices were used for whole cell patch-clamp recordings from antennal motor neurons in situ. These preparations were similar to those used for the spiny lobster (Wachowiak and Ache 1994; Wachowiak et al. 1996) and for patch-clamp recordings in vertebrate slice preparations (Edwards et al. 1989; Sakmann et al. 1989). Such preparations offer many advantages. For example, the neurons have undergone normal development, the synaptic connectivity of the tissue remains largely intact, and it is possible to identify as well as to undertake a detailed neuroanatomical analyses of recorded neurons.

In the case of intact brain preparations, the entire brain was secured in a Sylgard-coated recording chamber (volume ~1 ml) with fine silver wire hooks, and the brain sheath above the site where the recording electrode was to be placed was removed with fine forceps. Semiintact brains, in which brain regions such as the optic lobes and/or antennal lobes was removed, were used to gain better access to and visibility of the recording site and in some cases recordings were taken also from isolated DLs. Brain slices were then mounted in small metal or plastic tubes with their head fixed with adhesive tape and a mixture of wax–collophonium. All chemicals, unless stated otherwise, were obtained from Sigma Co (St. Louis, MO).

![FIG. 1. A: antennal motor neurons, stained in vivo by backfilling with a fluorescent marker. B and C: neurons dissociated from an adult honey bee brain after 1 day in vitro. B and C show the same cells examined with Hoffmann modulation optics and epifluorescence optics, respectively. Antennal motor neurons labeled with dye could be identified with fluorescence microscopy (compare B and C). D: Bodian stained section through the dorsal lobe, the lateral soma layer and adjacent neuropils (modified from Kloppenburg 1995). E: 40-μm section of a preparation in which a single antennal motor neuron was stained via the patch pipette during a recording. The arrow is pointing to the soma. F: recording situation in a semiintact preparation of the honey bee brain. The soma of the recorded neuron is located in the lateral soma layer of the deutocerebrum. The box in D represents approximately the area shown here. Scale bars: 100 μm in A, D, and E; 50 μm in B, C, and F. Dorsal cluster of scape motor neurons (DCS), dorsal lobe (DL), lobula (Lo), soma layer (SL), ventral cluster of scape motor neurons (VCS).](image-url)
VOLTAGE-ACTIVATED CURRENTS IN ANTENNAL MOTOR NEURONS

were most often used when the motor neurons were labeled by backfilling before recording. However, slice preparations proved less useful for detailed neuroanatomical analyses and identification of recorded neurons than intact or semi-intact brain preparations.

To obtain brain slices (~150 μm) either (1) the brain was dissected out of the head capsule, embedded in a low-temperature agarose (SeaPlaque agarose, SeaPrep agarose; dissolved in saline), and then cut under cold saline with a vibratome (Technical Products) or (2) the whole head capsule was removed from the thorax, glued (acrylic glue) onto a Teflon-coated block and sectioned under cold saline with the vibratome.

Motor neurons that were backfilled with dye could be identified before recording by their fluorescence. The neurons were visualized with a fixed-stage upright microscope (Olympus, Zeiss) with long working distance objectives (×30 air or ×40 water immersion) or an inverted microscope (Olympus, Zeiss) equipped with (×40) Hoffman modulation contrast and epifluorescence optics. Neurons in unstained brain preparations, tentatively identified as motor neurons on the basis of their size and location, were stained via the recording pipette so that their identity as motor neurons could later be verified.

Whole cell recording

Whole cell recordings were performed at room temperature following the methods described by Hamill et al. (1981). Electrodes (1–4 MΩ) were fashioned from borosilicate glass (100–μl micropipettes; OD, 1.71 mm; ID, 1.32 mm; VWR Scientific) with a Flaming-Brown Puller (P-87, Sutter Instrument) and filled with a solution containing (in mM) 150 K-aspartate, 10 NaCl, 2 MgCl₂, 1 CaCl₂, 10 EGTA, 2 ATP, and 5 HEPES adjusted to pH 7 and to ~490 mosM with sucrose or mannitol.

To increase the chance of obtaining a high-quality (GΩ) seal between the recording electrode and the cell body chosen for analysis, the surface of the cell was cleaned with a small stream of saline pressure-ejected from a large diameter pipette and/or by a stream of pipette solution ejected from the recording pipette. In preparations used for in situ recordings, brief enzyme treatment (collagenase 0.5 mg/ml, dispase 2 mg/ml in saline) was also used for this purpose. During the experiments, the cells were superfused constantly with saline solution (~2 ml/min) containing (in mM) 150 NaCl, 4 KCl, 6 CaCl₂, 25 d-glucose, 10 HEPES, adjusted to pH 7 and to ~520 mosM with sucrose or mannitol.

Whole cell recordings were made with an Axopatch 1C amplifier (Axon Instruments), and cells were examined both in current-clamp and in voltage-clamp mode. Data were digitized with a TL1 or Digidata 1200 interface (both Axon instruments) and pClamp 6 software (Axon Instruments) run on a Spacecow PC with a 486 or pentium microprocessor. Stimulus protocols used in this study are described in the figure legends or in the results, where appropriate. Electrophysiological data were sampled at intervals of 100 μs and filtered at 2 kHz with a 4-pole Bessel filter. Junction potentials were nullified before seal formation. Pipette and membrane capacitances were compensated. Series resistance compensation was applied (60–80%). Additionally, in most recordings a P/4 protocol (see Armstrong and Bezanilla 1974) was used. The software programs pClamp 6, Axograph 3 (Axon Instruments), and Delta Graph 3 (Delta Point) were used for data analysis.

In brain preparations without prelabeled motor neurons, Lucifer yellow (0.1%; Aldrich, Molecular Probes, Sigma) was added to the pipette solution to stain cells examined in whole cell recording mode. It was not necessary to apply an iontophoretic current to stain the cells as sufficient dye entered the cells through the low resistance patch pipettes during the recording period. After recording, brains with Lucifer yellow stained neurons were fixed for at least 1 h in 2.5% formaldehyde in 0.1 M phosphate buffer with 3% sucrose and then dehydrated and cleared in methyl salicylate.

To confirm that the neurons stained via the recording pipette were indeed antennal motor neurons, the brains were first inspected as whole-mounts with a fluorescence microscope (Leitz). Stained neurons were then examined further in brain sections (40 μm). For sectioning, brains were embedded in Spurrs and cut with a sliding microtome (American Optical).

Isolation of currents

Currents were isolated with a combination of pharmacological blockers, appropriate voltage protocols and ion substitution. Similar protocols were used effectively for isolation of currents in Kenyon cells of the honey bee (Schafer et al. 1994). Sodium currents (I Na) were blocked by tetrodotoxin (TTX, 10⁻⁶ M) and calcium currents (I Ca) were blocked by CdCl₂ (5 × 10⁻⁵ M). To ensure that there was no residual influx of Ca²⁺ when CdCl₂ was applied, which could activate Ca²⁺-dependent channels, Ca²⁺ was substituted with Ba²⁺ in some experiments. The transient K⁺ current (I K, nomenclature adapted from Connor and Stevens 1971) was blocked with 4-aminoypyridine (4 AP, 4–5 × 10⁻⁵ M) and the sustained K⁺ current (I Ca,K) was blocked by quinidine (5 × 10⁻⁴ M). Tetraethylammonium (TEA, 2–3 × 10⁻² M) blocks I K,Na as well as calcium-activated K⁺ currents (I Ca,K). I Ba,K was also eliminated when Ca²⁺ currents were blocked by CdCl₂. In experiments in which all K⁺ currents had to be abolished, K-aspArtate was replaced by CsCl in the pipette solution. Details of recording solutions used for each set of experiments are provided in the results.

The voltage dependence of activation of I K and I Ca,K was determined by applying voltage steps from ~70 to +70 mV in 10-mV increments (holding potential ~70 mV). A 1-s prepulse to ~100 mV was applied before measuring I K to remove resting inactivation. In the case of I K and I Ca,K, peak currents were measured and then converted to peak conductances. The equilibrium potential was calculated with the Nernst equation, assuming the intracellular K⁺ concentration equals the K⁺ concentration in the pipette solution. The resulting g/V curve was fitted to a third-order (n = 3) and first-order (n = 1) Boltzmann equation of the form

\[ g/g_{\text{max}} = 1/[1 + \exp ((V - V_0)/s)]^n \]

where g max is the maximal conductance and s is a slope factor. For the third-order Boltzmann fit, V 0 is the voltage at which half-maximal activation of the individual gating steps occurs, assuming a third-order activation relation (Hodgkin and Huxley 1952). For the first-order Boltzmann fit V 0 is the voltage of half-maximal activation of the peak current. For I Ca,K and I K, the voltage dependence was analyzed with I/V plots.

Steady-state inactivation for all currents was measured from a holding potential of ~70 mV. Voltage presteps of 1-s duration were delivered with 5- or 10-mV increments starting from ~100 mV. Each prestep was followed by a test pulse from which peak currents were measured. The data, scaled as a fraction of the calculated maximal conductance (I K, I Ca,K) or maximal current (I Na), were fitted to a 1st order Boltzmann equation (Eq. 1) based on the model of (Hodgkin and Huxley 1952).

Statistical analysis

Student’s t-test were used to assess the significance of differences between mean values of parameters measured in situ and in vitro. Significance was accepted at P = 0.05.

RESULTS

All recordings presented in this paper were from cells identified as antennal motor neurons by morphological characteristics (Kloppenburg 1995). Before recording, neurons...
that were stained by backfilling were identified in situ (e.g., Fig. 1A) or in vitro (e.g., Fig. 1, B and C) with fluorescence microscopy. Cells examined in situ, selected initially on the basis of their size and the location of their cell bodies (see Figs. 1, D and F), were confirmed to be motor neurons by analyzing the morphology of the cells after they were stained via the recording pipette (Fig. 1E). Whole cell patch-clamp recordings in current- and in voltage-clamp mode were used to analyze, in parallel, the electrophysiological properties of antennal motor neurons in vitro and in situ.

Resting membrane potentials of antennal motor neurons, measured immediately after breaking into the cells, were found to be similar in vitro and in situ, and ranged between −40 and −60 mV. These values may differ slightly from the true resting potential because pipette solution diffuses into the cell as soon as the cell membrane is ruptured (Pusch and Neher 1988). Action potentials could be elicited from antennal motor neurons in vitro with depolarizing current pulses (Fig. 2A), but spontaneous activity was not observed in these cells. In contrast, antennal motor neurons in situ showed bursts of activity, as well as strong synaptic input (Fig. 2B). Action potentials recorded in vitro and in vivo were TTX (10⁻⁷ M) sensitive. In cells with resting potentials around −40 mV, depolarizing pulses often failed to elicit action potentials. However, spikes could usually be generated in these cells if depolarization was preceded by a hyperpolarizing pulse (≥100 ms; ≤−70 mV), suggesting voltage inactivation of Na⁺ channels in cells with low resting potentials (see Fig. 6E). Voltage-clamp recordings were used to examine voltage-gated currents that are likely to contribute to the action potentials observed in these cells.

Depolarizing voltage steps from a holding potential of −70 mV elicited a fast transient inward current followed by a transient and a sustained outward current (Fig. 3A). Not only the outward currents (Fig. 3B) but also the inward currents (Fig. 3C) represented several ionic currents in combination (Fig. 3, A–C; see arrows). By using ion substitution, standard pharmacological agents, and appropriate voltage protocols several components of the inward and outward currents were isolated.

**Outward currents**

The transient inward current (Iₙa) could be blocked with the sodium channel blocker TTX (10⁻⁷ M) and Ca²⁺ currents were blocked by CdCl₂ (5 × 10⁻⁵ M). Experiments with varying intra- and extracellular potassium concentrations (data not shown) suggested that K⁺ was the major

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**FIG. 2.** A and B: current-clamp recordings from identified antennal motor neurons in vitro (A) and in an intact brain preparation (B). Motor neurons in vitro showed no spontaneous activity, but generated over- and undershooting action potentials during depolarizing current injection. Neurons from intact brain preparations showed spontaneous bursts. The action potentials shown in B are recorded during a burst. Spike waveform was difficult to evaluate because of strong synaptic input to the cells in situ.

**FIG. 3.** Whole cell recordings (in vitro) of voltage-activated currents from 3 neurons under different pharmacological conditions. Holding potential was −70 mV. A: inward and outward currents recorded in CdCl₂ (5 × 10⁻⁵ M). Potential was stepped from −70 to +70 mV in 10-mV increments. B: outward currents recorded in the presence of TTX (10⁻⁷ M) and CdCl₂ (5 × 10⁻⁵ M). Potential was stepped from −70 to +70 mV in 10 mV increments. C: Inward currents recorded in the presence of TEA (3 × 10⁻² M) and 4AP (4 × 10⁻³ M). In addition, K⁺ in the pipette solution was replaced by Cs⁺. Potential was stepped from −70 to −10 mV in 5-mV increments.

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charge carrier of the outward current that remained. Blockade of the Ca\(^{2+}\) or Na\(^{+}\) currents decreased the magnitude and altered the form of the \(K^+\) current profiles, indicating that Ca\(^{2+}\)- and Na\(^{+}\)-sensitive \(K^+\) currents were present in these cells. However, in this study we focused on two voltage-activated \(K^+\) currents, \(I\) a transient current (\(I_A\), Fig. 4) and 2) a sustained current (\(I_{K(V)}\), Fig. 5). Both currents were apparent in all of the investigated neurons. However, a comparison of the \(K^+\) current profiles revealed that the ratio between \(I_A\) and \(I_{K(V)}\) varied.

**Transient \(K^+\) current (\(I_A\))**

To measure \(I_A\) (Fig. 4) the cells were bathed in saline containing 10\(^{-7}\) M TTX, 5 \(\times\) 10\(^{-5}\) M quinidine, and 5 \(\times\) 10\(^{-5}\) M CdCl\(_2\). \(I_A\) activated at voltages above -40 to -30 mV. This current was transient and decayed due to inactivation during a maintained depolarizing voltage step (Fig. 4, A and B). Inactivation of \(I_A\) could be removed by hyperpolarization. The conductance/voltage relationship for steady-state activation (Fig. 4D) was determined from the peak currents evoked by each voltage step. These showed typical voltage dependence for activation for \(I_A\) and were fitted to a third- and first-order Boltzmann equation (Eq. 1). Fit parameters from 13 neurons collectively showed a voltage for half-maximal activation for each of the individual gating steps of -25.5 \(\pm\) 3.0 mV (\(s = -26.9 \pm 4.3\) mV). The parameters measured in vitro (\(V_A = -26.1 \pm 3.3\) mV; \(s = -28.0 \pm 4.6\); \(n = 7\)) and in situ (\(V_A = -24.8 \pm 2.8\) mV; \(s = -25.5 \pm 3.9\); \(n = 6\)) were not significantly different (\(P > 0.05\)). These values corresponded to a half-maximal activation of the peak current at +8.8 \(\pm\) 5.1 mV (\(s = -18.8 \pm 2.3\); \(n = 13\)). The voltage dependences of steady-state inactivation (Fig. 4, C and E) were well fitted by a 1st order Boltzmann equation (Eq. 1). The mean voltage for half-maximal inactivation measured from 13 neurons was -43.2 \(\pm\) 3.0 mV (\(s = +7.2 \pm 1.6\)). The parameters measured in vitro (\(V_A = -42.4 \pm 3.7\) mV; \(s = 7.5 \pm 1.4\); \(n = 7\)) and in situ (\(V_A = -44.1 \pm 2.4\) mV; \(s = 6.9 \pm 1.9\); \(n = 6\)) were not significantly different (\(P > 0.05\)).

**Sustained \(K^+\) current (\(I_{K(V)}\))**

To measure \(I_{K(V)}\) (Fig. 5) the cells were bathed in saline containing 10\(^{-7}\) M TTX, 4 \(\times\) 10\(^{-3}\) M 4AP and 5 \(\times\) 10\(^{-5}\) M CdCl\(_2\). \(I_{K(V)}\) activated with voltage steps above -50 to -40 mV. The current was sustained and did not decay during a maintained depolarizing voltage step (Fig. 5, A and B). The conductance/voltage relations for voltage activation (Fig. 5C), determined from the maximal currents evoked by each voltage step, showed a typical voltage dependence for activation of \(I_{K(V)}\) and were fitted to a third- and to a first-order Boltzmann equation (Eq. 1). Fitted curves from 11 neurons showed a mean voltage for half-maximal activation for each of the individual gating steps of -16.7 \(\pm\) 3.0 mV (\(s = -21.0 \pm 2.3\) mV). The parameters measured in vitro (\(V_A = -17.1 \pm 4.1\) mV; \(s = -20.6 \pm 2.7\); \(n = 6\)) and in situ (\(V_A = -15.9 \pm 1.0\) mV; \(s = -21.4 \pm 1.9\); \(n = 5\)) were not significantly different (\(P > 0.05\)). These values corresponded to a half-maximal activation of the peak current at +11.2 \(\pm\) 4.6 mV (\(s = -15.0 \pm 1.4\); \(n = 11\)). \(I_{K(V)}\) showed little or no inactivation even with depolarization steps lasting 1 s or longer, and there was no detectable voltage dependence of steady-state inactivation (Fig. 5D).

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**Fig. 4.** Characterization of the transient potassium current (\(I_A\)). A and B: current traces for steady-state activation of \(I_A\) from motor neurons in vitro (A) and in situ (B). The holding potential was -70 mV. After prepulses to -100 mV (1 s), the voltage was stepped from -50 mV to +70 mV in 15-mV increments. C: current traces for steady state inactivation of \(I_A\) (in situ). The holding potential was -70 mV. Test pulses (+20 mV) were preceded by 1 s pulses between -90 mV and -30 mV in 10 mV increments. D and E: conductance/voltage curves for steady state activation (D) and inactivation (E) of \(I_A\) measured from neurons in vitro (filled symbols) and in situ (open symbols). Conductances were calculated assuming \(E_K = -91.6\) mV (see METHODS). Values are expressed as a fraction of the calculated maximal conductance. D: curves from 13 neurons are fits to a 3rd-order Boltzmann equation with the mean parameters: \(V_A = -25.5 \pm 3.0\) mV; \(s = -26.9 \pm 4.3\). The parameters measured in vitro (\(V_A = -26.1 \pm 3.3\) mV; \(s = -28.0 \pm 4.6\); \(n = 7\)) and in situ (\(V_A = -24.8 \pm 2.8\) mV; \(s = -25.5 \pm 3.9\); \(n = 6\)) were not significantly different (\(P > 0.05\)). E: data for inactivation from 13 neurons were individually fitted to a 1st-order Boltzmann equation. The mean parameters were \(V_A = -43.2 \pm 3.0\) mV; \(s = +7.2 \pm 1.6\). Parameters measured in vitro (\(V_A = -42.4 \pm 3.7\) mV; \(s = -7.5 \pm 1.4\); \(n = 7\)) and in situ (\(V_A = -44.1 \pm 2.4\) mV; \(s = -6.9 \pm 1.9\); \(n = 6\)) were not significantly different (\(P > 0.05\)).
**Inward currents**

To analyze the inward currents, outward currents were blocked by substituting K\(^{+}\) in the pipette solution with Cs\(^{+}\) and adding 3 \(\times\) 10\(^{-7}\) M TEA and 4 \(\times\) 10\(^{-7}\) M 4AP to the extracellular solution. The remaining inward current consisted of a transient component and a more slowly activating/ inactivating component (Fig. 3C). By using pharmacological blockers and ion substitution, both components could be separated and identified. The transient component was a sodium current (Fig. 6) and most of the sustained component was a calcium current (Fig. 7).

**Sodium currents (\(I_{Na}\))**

To measure \(I_{Na}\) (Fig. 6) the cells were bathed in saline containing 4 \(\times\) 10\(^{-7}\) M 4AP, 3 \(\times\) 10\(^{-2}\) M TEA, and 5 \(\times\) 10\(^{-5}\) M CdCl\(_2\). In the pipette solution K\(^{+}\) was replaced with Cs\(^{+}\). \(I_{Na}\) could be blocked by TTX (10\(^{-7}\) M) and was eliminated when NaCl in the extracellular solution was substituted by choline chloride. \(I_{Na}\) activated very rapidly and decayed rapidly due to inactivation during a maintained depolarizing voltage step (Fig. 6, A and B). Once inactivated, Na\(^{+}\) channel inactivation had to be removed by hyperpolarization. In many recordings, both in vitro and in situ, \(I_{Na}\) appeared abruptly as the amplitude of the depolarizing voltage steps was increased, indicating imperfect voltage control (see DISCUSSION). The current/voltage relation for steady-state activation (Fig. 6D) was determined from the peak currents evoked by each voltage step. This curve showed a typical voltage dependence for activation of \(I_{Na}\). The current was activated at command potentials more positive than −35 mV with a maximum around −5 mV. The current decreased during more positive test pulses as they approached the sodium equilibrium potential (+68 mV, calculated with the Nernst equation, assuming the intracellular Na\(^{+}\) concentration equals that of the pipette solution). The voltage dependence of steady-state inactivation (Fig. 6, C and D) was well fitted with a first-order Boltzmann equation (Eq. 1). Fit parameters from nine neurons were \(V_{A} = −72.6 \pm 3.7\) mV; \(s = 6.7 \pm 1.9\) and in situ (Fig. 6, A and B). Like the transient component, the sustained component could be eliminated by TTX (10\(^{-7}\) M).

**Calcium current (\(I_{Ca}\))**

To measure \(I_{Ca}\) (Fig. 7) the cells were bathed in saline containing 10\(^{-7}\) TTX, 4 \(\times\) 10\(^{-3}\) M 4AP, and 3 \(\times\) 10\(^{-2}\) M TEA. In the pipette solution K\(^{+}\) was replaced with Cs\(^{+}\). \(I_{Ca}\) could be blocked by CdCl\(_2\) (5 \(\times\) 10\(^{-5}\) M) and was eliminated when CaCl\(_2\) in the extracellular solution was substituted by MgCl\(_2\). \(I_{Ca}\) activated relatively quickly and decayed during a maintained depolarizing voltage step (Fig. 7, A, B, and F). \(I_{Ca}\) consisted of a fast and a slow (or non) inactivating component (Fig. 7F). The inactivation kinetics varied between cells. The current/voltage relationship (Fig. 7A) was determined by plotting the peak currents evoked by each voltage step. This curve showed a typical voltage dependence for activation of \(I_{Ca}\). The current was activated at command potentials more positive than −45 to −40 mV, with a maximum around −15 mV. The current decreased during more positive test pulses as they approached the calcium equilibrium potential (≫ +45 mV, estimated with the
FIG. 6. Characterization of the transient sodium current ($I_{Na}$). A and B: current traces for steady-state activation of $I_{Na}$ from motor neurons in vitro (A) and in situ (B). In both cases, the holding potential was $-70 \text{ mV}$ and the voltage was stepped from $-65 \text{ mV}$ to $-5 \text{ mV}$ in 5-mV increments. C: current traces for steady-state inactivation of $I_{Na}$ (in vitro). The holding potential was $-70 \text{ mV}$. Test pulses to $-10 \text{ mV}$ were preceded by 1-s pulses ranging from $-95 \text{ mV}$ to $-45 \text{ mV}$ in 5-mV increments. D: current/voltage curves for steady state activation of $I_{Na}$ measured from 9 neurons, 5 cells in vitro (filled symbols) and 4 cells in situ (open symbols). The current is activated at command potentials more positive than $-35 \text{ mV}$ with a maximum around $-5 \text{ mV}$. E: current/voltage curves for steady-state inactivation of $I_{Na}$ measured from 8 neurons. The data were fitted to a 1st-order Boltzmann equation with the following mean parameters: $V_A = -72.6 \pm 3.7 \text{ mV}$, $s = 6.7 \pm 1.9$. The parameters measured in vitro (solid symbols; $V_A = -73.4 \pm 3.6 \text{ mV}$; $s = 7.3 \pm 2.1$; $n = 4$) and in situ (filled symbols; $V_A = -71.7 \pm 3.0 \text{ mV}$; $s = 6.1 \pm 1.9$; $n = 4$) were not significantly different ($P > 0.05$).

FIG. 7. Characterization of the calcium current ($I_{Ca}$). A and B: current traces for steady-state activation of $I_{Ca}$ from motor neurons in vitro (A) and in situ (B). The holding potential was $-70 \text{ mV}$ and voltage was stepped from $-70 \text{ mV}$ to $+20 \text{ mV}$ in 5-mV increments. C: current traces for steady-state inactivation of $I_{Ca}$ (in vitro). The holding potential was $-70 \text{ mV}$. Test pulses to $-10 \text{ mV}$ were preceded by 1-s pulses ranging from $-95 \text{ mV}$ to $-10 \text{ mV}$ in 5-mV increments. D: current/voltage curves for steady-state activation of $I_{Ca}$ measured from 8 neurons, 4 cells in vitro (filled symbols) and 4 cells in situ (open symbols). The current is activated at command potentials more positive than $-45$ to $-40 \text{ mV}$ with a maximum around $-15 \text{ mV}$. E: current/voltage curves for steady-state inactivation of $I_{Ca}$ measured from 8 neurons, 4 cells in vitro (filled symbols) and 4 cells in situ (open symbols). $F$: inactivation of $I_{Ca}$ (in vitro) during a 1,185 ms long voltage pulse to $-20 \text{ mV}$ from a holding potential of $-70 \text{ mV}$. 
Nernst equation and assuming the intracellular Ca\(^{2+}\) concentration \(\approx 1\) mM). Generally \(I_{Ca}\) decreased by \(>50\%\) within several minutes. This rundown increased with the number, duration and especially the amplitude of the depolarizing voltages. Substituting calcium with barium increased the maximum amplitude of the current, indicating that the channels were more permeable to Ba\(^{2+}\) than to Ca\(^{2+}\). This increase in conductance often led to loss of voltage control, therefore, Ba\(^{2+}\) currents were not examined in detail.

Steady-state inactivation of \(I_{Ca}\) was measured from a holding potential of \(-70\) mV. Voltage steps (1 s) from \(-100\) mV to \(-10\) mV in 5-mV increments were followed by test pulses to \(-10\) mV. Steady-state inactivation increased with the size of the depolarizing prepulse.

**Discussion**

By examining adult antennal motor neurons in vitro, as well as in isolated brain preparations, we believe this study provides valuable information about the electrophysiological properties of antennal motor neurons in the bee. Performing biophysical experiments on cells in vitro, as well as in situ, enabled us to take advantage of the easy access and controlled conditions provided by in vitro studies, and to demonstrate the physiological relevance of these results by examining cells in their native environment, with their synaptic connections largely intact. We expect this parallel approach to be useful also for studying neuromodulatory and developmental mechanisms in the insect brain. However, it is important to keep in mind that the present study provides only a small window on the biophysical properties of antennal motor neurons.

The voltage-activated currents isolated from *Apis* antennal motor neurons were similar to equivalent currents found in other insect preparations. For example, the voltage operating range of the transient sodium current observed in antennal motor neurons is similar to transient Na\(^+\) currents observed in *Drosophila* neurons (Byerly and Leung 1988; O'Dowd and Aldrich 1988; Saito and Wu 1991), in honey bee Kenyon cells (Schäfer et al. 1994), and in leg motor neurons of the sphinx moth, *Manduca sexta* (Hayashi and Levine 1992). In this study, \(I_{Na}\) often appeared abruptly during gradual increases in the amplitude of depolarizing voltage steps, indicating imperfect space clamp and suggesting that at least some of the Na\(^+\) channels are some distance from the soma membrane. Although freshly dissociated cells do not have large processes, Na\(^+\) channels may be expressed on newly formed processes that extend from the cell soma within hours of plating (see Tribut et al. 1991).

In antennal motor neurons, \(I_{Na}\) activates and inactivates rapidly and is blocked by relatively low concentrations of 4AP. \(I_{Na}\) is half-maximally activated at around +9 mV \((s = -19)\). This is 30–70 mV more positive than described for \(I_{Na}\) in blowfly monopolar cells (Hardie and Weckström 1990), *Drosophila* photoreceptors (Hardie 1991; Hevers and Hardie 1995), and cockroach DUM neurons (Grolleau and Lapied 1995), and is slightly more negative to values described in honey bee Kenyon cells (Schäfer et al. 1994). The value for half-maximal inactivation of \(I_{Na}\) in antennal motor neurons \((-43\) mV; \(s = 7\)) is close to that described in Kenyon cells of the honey bee (Schäfer et al. 1994), and is 10–60 mV more positive than found for \(I_{Na}\) in blowfly monopolar cells (Hardie and Weckström 1990), *Drosophila* photoreceptors (Hardie 1991; Hevers and Hardie 1995), cockroach DUM neurons (Grolleau and Lapied 1995) and in many cultured *Drosophila* CNS neurons (Saito and Wu 1991; Solé and Aldrich 1988).

\(I_{K(A)}\) in antennal motor neurons also resembles delayed-rectifier type K\(^+\) currents described elsewhere. It is TEA sensitive, activates more slowly than \(I_{Na}\) and shows little or no voltage inactivation. However, the half-maximal voltage for activation of \(I_{K(A)}\) \((+11\) mV; \(s = -15\)) observed in this study is 10–70 mV more positive than values reported for the delayed K\(^+\) currents described in blowfly monopolar cells (Hardie and Weckström 1990), *Drosophila* photoreceptors (Hardie 1991; Hevers and Hardie 1995) and in cockroach mechanoreceptors (Torkkeli and French 1995).

\(I_{Ca}\) in antennal motor neurons consists of a fast and a slow inactivating component. It activates at voltages above \(-45\) to \(-40\) mV and has its maximum at \(-15\) mV. This is similar to Ca\(^{2+}\) currents described in *Drosophila* neurons (Byerly and Leung 1988; Saito and Wu 1991), in honey bee Kenyon cells (Schäfer et al. 1994), and in *Manduca* motor neurons (Hayashi and Levine 1992). In this study, the inactivation kinetics of \(I_{Ca}\) were very variable between recordings. The reason for this variability is unclear, but could be due to differential expression of different Ca\(^{2+}\) channel types, or the presence of residual currents carried by ions other than calcium. Substituting calcium with barium increased the maximum amplitude of the \(I_{Ca}\), indicating that the investigated channels are more permeable to barium than to Ca\(^{2+}\), as described for Ca\(^{2+}\) channels in other cell types (see Hille 1992). In most of the experiments, \(I_{Ca}\) showed considerable rundown, which in other cells is attributed to calcium-dependent inactivation or regulation of \(I_{Ca}\) (e.g., Schäfer et al. 1994). In our experiments the rundown was accelerated by increasing the number, duration and especially, the amplitude of the depolarizing voltage pulses. This suggests that at least some of the rundown is voltage dependent.

During this study we gathered preliminary evidence for the presence of several other currents that were not described in detail. While recording the transient sodium current, we usually observed a persistent TTX-sensitive component. Persistent sodium currents were described in *Drosophila* neurons (Saito and Wu 1991) and also in the honey bee (Schäfer et al. 1994). However, to confirm that the persistent current observed in *Apis* motor neurons is carried by sodium ions, further experiments are necessary. During experiments in which Ca\(^{2+}\) or Na\(^+\) currents were blocked, we observed a reduction in amplitude and change in the form of the K\(^+\) currents, indicating the presence of Ca\(^{2+}\) and Na\(^+\) sensitive potassium currents in the cells. Ca\(^{2+}\)-dependent K\(^+\) currents (Schäfer et al. 1994 [honey bee]; Thomas 1984, Torkkeli and French 1995 [cockroach]; Wegener et al. 1992 [locust]; Zufall et al. 1991 [sphinx moth]) and Na\(^+\) dependent K\(^+\) currents (Dale 1993 [*Xenopus*]; Grolleau and Lapied 1994 [cockroach]) were found in many preparations.

The four voltage-activated currents analyzed in this paper were present in all of the cells examined, and no obvious differences in the parameters of the single currents were identified from cell to cell. However, the ratio of the currents, especially of the two K\(^+\) currents, can vary between motor
neurons. Although not addressed in detail in this study, this finding suggests that there might be subtypes of motor neurons with different intrinsic properties. Extracellular recordings of fast and slow muscle potentials (Pribbenow 1994) suggest the presence of different classes of antennal motor neurons in the honey bee. From other insect antennal motor systems (Bauer and Gewecke 1991; Honegger et al. 1990; Pribbenow 1994) and from insect motor systems in general (see Hoy 1974), we know that within any one system there are usually different classes of motor neurons, which differ in their physiological properties. In insect antennal motor systems, the number of motor neurons generally exceeds the number of muscles (Bauer and Gewecke 1991; Honegger et al. 1990; Kloppenburg 1995). If insect skeletal muscles are innervated by three or more motor neurons, there is often a slow fiber, a fast fiber and an inhibitory unit represented (see Hoy 1974). Neuronal properties are largely determined by the types of ion-channels expressed and by the rate of channel expression for different channel types. It will be interesting in the future to determine with in situ recordings from identified motor neurons if different ratios of currents can be attributed to different types of antennal motor neurons, possibly reflecting their different physiological properties and function.

This study is an important first step toward understanding the intrinsic properties of antennal motor neurons and is the first to use in vitro studies in combination with recordings from identified antennal motor neurons in situ. By using this approach, it is hoped that future studies will not only provide a more complete description of the biophysical properties of these cells, but also reveal cellular differences between populations of motor neurons that may relate to their performance of different tasks.

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