Spike-frequency adaptation and intrinsic properties of an identified, looming-sensitive neuron

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Abstract

We investigated in vivo the characteristics of spike-frequency adaptation and the intrinsic membrane properties of an identified, looming-sensitive interneuron of the locust optic lobe, the lobula giant movement detector (LGMD). The LGMD had an input resistance of 4-5 MΩ, a membrane time constant of ~8 ms and exhibited inward rectification and rebound spiking following hyperpolarizing current pulses. Responses to depolarizing current pulses revealed the neuron’s intrinsic bursting properties and pronounced spike-frequency adaptation. The characteristics of adaptation, including its time course, the attenuation of the firing rate, the mutual dependence of these two variables, and their dependence on injected current, followed closely the predictions of a model first proposed to describe the adaptation of cat visual cortex pyramidal neurons in vivo. Our results thus validate the model in an entirely different context and suggest that it might be applicable to a wide variety of neurons across species. Spike-frequency adaptation is likely to play an important role in tuning the LGMD and in shaping the variability of its responses to visual looming stimuli.

Keywords: spike-frequency adaptation, LGMD, DCMD, locust, calcium, AHP.
Introduction

Spike-frequency adaptation is a widespread feature of sensory neurons. The biophysical mechanisms of spike-frequency adaptation have been extensively studied in vertebrate and invertebrate systems and often involve calcium-dependent potassium conductances (Meech, 1978; Sah, 1996). Spike-frequency adaptation has a variety of important consequences for the processing of sensory stimuli. It allows for example to emphasize changes in stimulus parameters, but can also protect neurons from firing frequency saturation and tune sensory responses to specific stimulus features, as demonstrated by several in vivo and in vitro studies (Sobel and Tank, 1994; Benda et al., 2005; Wang et al., 2003). Recently, a biophysical model of spike-frequency adaptation has been proposed (Wang, 1998; Liu and Wang, 2001) based on intracellular in vivo recordings of cat visual cortical neurons (Ahmed et al., 1998). The model made several specific predictions on how the firing frequency of adapting neurons should evolve over time and on the relation between variables characterizing spike-frequency adaptation that have not yet been tested experimentally. Although this model was based on cortical pyramidal cell data, it is likely to be widely applicable. This is due to the fact that it is based either on a simple compartmental model endowed with conductances of Hodgkin-Huxley type (Wang, 1998) that replicates the firing properties of a wide class of neurons (Pinsky and Rinzel, 1994; Mainen and Sejnowski, 1996) or on a leaky integrate-and-fire approximation (Liu and Wang, 2001; for related, phenomenological models, see Benda and Herz, 2003 and La Camera et al., 2004). We set out to experimentally test the model’s predictions on an identified interneuron in the locust visual system, the lobula giant movement detector (LGMD).

The LGMD is thought to be involved in collision avoidance and escape behavior (for review see, Gabbiani et al., 2004). It projects onto a postsynaptic target neuron called the descending contralateral movement detector (DCMD) that can easily be recorded from the animal's nerve cord (O’Shea and Williams, 1974). The connection between the LGMD and DCMD is mediated by a mixed chemical/electrical synapse (Killmann et al., 1999) and is so strong that spikes in the LGMD and DCMD are in 1:1 correspondence.
under visual stimulation (O'Shea and Williams, 1974; Rind, 1984; Gabbiani et al., 2005). The LGMD can thus be conveniently identified electrophysiologically as the unique neuron in the optic lobe whose spikes precede by ~2 ms those of the DCMD. An extensive literature based on DCMD recordings shows that both the LGMD and the DCMD are most sensitive to objects approaching on a collision course (e.g., Schlotterer, 1977; Rind and Simmons, 1992; Hatsopoulos et al., 1995). Several aspects of the computation the LGMD performs while it is tracking approaching objects have been investigated (Hatsopoulos et al., 1995; Gabbiani et al., 1999; 2001). This computation is thought to rely on a multiplicative interaction between two of the inputs impinging on the LGMD's dendritic arborizations (Gabbiani et al., 2002). Recently, the spatial receptive field structure of the LGMD has been characterized, as well as the summation properties of local excitatory inputs and the role of feed-forward inhibition on the neuron's responses to looming stimuli (Krapp and Gabbiani, 2005; Gabbiani et al., 2005). The intrinsic membrane properties of the LGMD have however not yet been studied in detail. We thus started by characterizing them prior to comparing the LGMD adaptation characteristics to those of the spike-frequency adaptation models proposed by Wang (1998) and Liu and Wang (2001). Recording from a uniquely identified neuron, such as the LGMD, as opposed to different neurons from a population reduces the number of potential noise sources. The reproducibility of our LGMD data proved advantageous to test the predictions made by these biophysical adaptation models. Furthermore, one of the model's predictions would have been very difficult to test on a heterogeneous neuronal population.
Methods

Preparation

Dissections and animal preparation were similar to those described in detail in previous publications (Gabbiani et al., 1999, 2001, 2002, 2005). Briefly, experiments were carried out on adult locusts (*Schistocerca americana*) taken from the laboratory colony 3-4 weeks after their final molt. Animals were fixed in a plastic holder and their head was bathed in locust saline. The head capsule was opened and the gut was removed. The connective contralateral to the recorded side was either cut at the level of the suboesophageal ganglion and placed in a suction electrode or recorded extracellularly with hook electrodes after cutting a window in the pronotum of the animal. The brain was exposed and desheated with fine forceps. To minimize brain movement during intracellular recordings, the mandibular muscles were sectioned and a support was placed under the brain.

Electrophysiology and data acquisition

Extracellular recordings were amplified differentially 10,000 times with standard equipment. The DCMD was easily identified as the unit with the largest extracellular action potentials. Intracellular recordings were obtained with sharp electrodes pulled with a horizontal puller (P-87, Sutter Instruments, Novato, CA) using thin-wall borosilicate glass (1.2/0.9 mm outer/inner diameter; WPI, Sarasota, FL). The pull parameters were optimized to minimize electrode resistance and thus facilitate the passage of large currents necessary for this study. The electrodes were filled with potassium acetate (2M) and their DC resistance, measured at the beginning of each experiment, ranged between 30-50 MΩ. The LGMD was identified as the unique neuron whose spikes were in one-to-one correspondence with those of the DCMD (e.g., Gabbiani et al., 2002, Fig. 4a; Gabbiani et al., 2005, Fig. 2A). As explained in the Results, we were interested in
correlating single spike shape characteristics with the properties of the current vs. spike-frequency discharge curve in individual LGMD neurons. We therefore attempted whenever possible to obtain multiple penetrations in each neuron. Following a successful penetration, the electrode was carefully retracted and subsequent attempts at impaling the LGMD were directed to dendritic locations in the optic lobe either more proximal or more distal from the spike initiation zone based on anatomical markers. Electrode withdrawal and renewed penetration did not alter the response properties of the LGMD under visual stimulation or affect its input resistance and the stability of subsequent recordings, suggesting that no measurable damage resulted from this procedure. To minimize the effects of electrode impedance, all current injection protocols were carried out using the discontinuous current clamp mode of the intracellular amplifier (Axoclamp 2B). Discontinuous current clamp sampling rates and electrode capacity compensation were adjusted on a cell-by-cell basis to allow settling of the potential to its steady-state value before sampling. Sampling rates ranged between 4 and 8 kHz and were well above the cut-off frequency set by the LGMD membrane time constant (Results). For a few recordings, a SEL10 amplifier (npi electronic, Tamm, Germany) was used at 15-20 kHz sampling rates. Current pulses were programmed using a Master 8 pulse stimulator (AMPI, Jerusalem, Israel) used to drive the external current input of the amplifier. The intra- and extracellular recordings, current traces and a voice channel were stored on digital audio tape using a professional recorder (DT800, MicroData Instrument, South Plain Field, NJ; sampling rate: 8 kHz). Subsequently, the data were transferred to a personal computer for analysis. Intracellular recordings typically lasted 40-60 mins.

**Current injection protocols**

Each current injection trial consisted of a square pulse lasting 500 ms. For all but one experiment, positive current pulses were preceded by a 500 ms, -2 nA negative pre-pulse to minimize possible inactivation of sodium channels. Each trial was repeated 8-10 times before selecting a new current value and two trials were separated by 15 s.
The first protocol consisted of stimulation with negative current pulses of -1, -2 and –5 nA, with 8-10 trials per value. We recorded responses to this protocol in 12 different neurons. In five experiments, we were able to obtain a second or a third penetration in the same neuron. In eight of these penetrations, the protocol could be repeated a second or third time, yielding a total of 23 measurements.

The second protocol consisted of stimulation with negative current pulses of –1 to -12 nA, -15 nA and -20 nA. We recorded responses to this protocol in 2 neurons (3 penetrations, 7 measurements).

The third protocol consisted of stimulation with positive current pulses of +1 to 10, 12, 15, 20 and 25 nA. We recorded responses to this protocol in 13 different neurons, but not all neurons could be recorded at all current values. Three neurons were recorded up to 25 nA, five up to 20 nA, one up to 15 nA, one up to 12 nA, two up to 10 nA and one up to 7 nA (Fig. 6A). We obtained multiple penetrations in three neurons for a total of 17 penetrations and 32 measurements. When the currents had been injected in increasing order during a measurement, the subsequent measurement was performed in descending order (i.e., from 25 to 1 nA) to allow detection of current injection sequence effects on the cell's response. No sequence effects (ascending vs. descending) could be observed.

Data analysis

Multiple measurements during a single penetration were used to monitor the stability and reproducibility of the recordings. Data from different penetrations were treated as independent in pooled analyses. Restricting pooled analyses to different cells did not change any of the results.

Resting membrane potential. In 11 recordings, we determined the resting membrane potential at the end of the experiment by comparing the intracellular amplifier potential reading within the neuron with that obtained immediately after retracting the electrode to the bath.
Input resistance. An estimate of input resistance was computed for current pulses of -1, -2 and -5 nA by selecting a 300 ms window for each trial, averaging the membrane potential within the window and dividing by the injected current value (Fig. 1A). Usually, the averaging window was centered at the mid-point of the pulse. In some cases, it was shifted earlier or later to avoid clearly visible excitatory postsynaptic potentials. The input resistance was then averaged across the 8-10 trials obtained for each current measurement. For depolarizing pulses of +1, 2 and 3 nA, an estimate of the input resistance was computed similarly, except that each trace was first median filtered over a 6 ms time window to suppress eventual action potentials (Fig. 1B). The averaging window typically comprised the last 300 ms of the pulse since action potentials were less likely to occur in that period (see Results). In a few trials, one or at most two action potentials were included in the averaging window, but their impact was minimal following median filtering.

Membrane and equalization time constants. The membrane and equalization time constants were computed by using the peeling method for current pulses of -1, -2 and -5 nA (Rall, 1969). The mean membrane potential averaged across 8-10 trials in response to the pulse was plotted in logarithmic coordinates after subtracting its minimum, steady-state value. The data were fitted to a straight line by least squares (Fig. 2B). The absolute value of the inverse line slope yielded the membrane time constant. The fitted line was then subtracted from the data, revealing a second, faster linear decay in logarithmic coordinates that was also fitted to a straight line to obtain the equalization time constant. We verified the accuracy of the double exponential fit by comparing it to the original mean membrane potential time course and its standard deviation obtained from repeated trials (Fig. 2A). We also verified that a direct, double exponential fit of the membrane potential time course using least squares yielded identical results (Holmes et al., 1992).

Interspike interval distributions. Interspike intervals (ISIs) were computed by subtracting successive spike occurrence times during each positive current pulse. ISIs were pooled across trials and current values. Histograms were obtained by binning the interval
distribution in 30 bins of equal size between 0 and 100 ms (Fig. 4B). The coefficient of variation (CV) of the ISI distribution was obtained by dividing the standard deviation of the ISI distribution, \( \sigma_t \), by its mean, \( t_m \) (Fig. 8A). The ISI serial correlation coefficient was computed according to the formula,

\[
\frac{1}{n-1} \sum_{i=1}^{n-1} (t_{i+1} - t_m)(t_i - t_m)
\]

and takes values between -1 and +1 (Fig. 8B). Positive values signify that ISIs longer (shorter) than the mean tend on average to be followed by similar longer (shorter) intervals. Conversely, negative values mean that longer (shorter) intervals tend to be followed by shorter (longer) intervals. A value of zero indicates uncorrelated (possibly independent) ISIs.

**Instantaneous firing frequency.** An estimate of the instantaneous firing frequency, \( f(t) \), was obtained for each trial by computing for each time, \( t \), the inverse of the ISI in which \( t \) was included. At the time of a spike, we averaged the value obtained from the preceding and following ISIs. Specifically, let \( t_1, \ldots, t_n \) be the spike occurrence times during a single trial. We set \( f(t) = 0 \) if \( t < t_1 \), \( f(t) = 0.5/(t_2-t_1) \) if \( t = t_1 \), \( f(t) = 1/(t_{i-1}-t_i) \) for \( t_{i-1} < t < t_i \), \( f(t) = 0.5/(t_{i+1}-t_i) + 0.5/(t_{i-1}-t_i) \) if \( t = t_i \), \( f(t) = 0.5/(t_{n-1}-t_{n-1}) \) if \( t = t_n \) and \( f(t) = 0 \) if \( t > t_n \). The mean instantaneous firing rate, \( f_m(t) \), and its standard deviation were obtained by averaging across trials (Fig. 5A). The instantaneous firing rate for the first ISI, \( f_{0s} \), was obtained by averaging \( 1/(t_2-t_1) \) across trials and the steady-state firing rate, \( f_{ss} \), by averaging \( f_m(t) \) over the last 100 ms of the current pulse (Fig. 5B).

**Adaptation parameters, \( \tau_{adap} \) and \( F_{adap} \).** The time constant of spike frequency adaptation, \( \tau_{adap} \), was obtained by fitting an exponentially decaying function with three free parameters (initial firing rate, \( f_{0fit} \), exponential adaptation time constant, \( \tau_{adap} \), and steady state firing rate, \( f_{ssfit} \)) to \( f_m(t) \) by least squares (Fig. 5A, D). We verified that double exponential fits did not improve significantly the fit quality, taking into account the
standard deviation of $f_m(t)$. Following Ahmed et al. (1998) and Wang (1998), the attenuation factor, $F_{adap}$, was defined as $F_{adap} = (f_0 - f_{ss}) / f_0$ (Fig. 5C).

**Mean spike height and width.** These parameters were measured in 11 neurons (14 different penetrations) using 10 isolated spikes from 10 different trials obtained in response to the lowest positive current above spiking threshold. Spike height was measured as the difference between the value of the membrane potential at the peak of the spike and the value at its inflection point during the initial depolarization leading to the spike. In practice, the inflection point was determined by finding where the first derivative of the membrane potential exceeded 5mV/ms. Spike width was then measured at half-height. The values obtained for each trial were averaged across trials to obtain the mean spike heights and widths as well as their standard deviations (Fig. 6F, Table 1).

**Afterhyperpolarization (AHP) decay time constant.** The AHP decay time constant was obtained by computing the mean membrane potential time course across trials for each current pulse leading to a peak AHP exceeding -2mV (gray traces in Fig. 7A). Currents with absolute peak AHPs < 2mV were discarded because of sensitivity to noise. Each of the mean membrane potential time courses was normalized and their mean was computed (dotted line in Fig. 7C). A single exponential was fitted to the resulting decay by least squares on the first 500 ms following a current pulse (solid line in Fig. 7C). Membrane potential values at later times were discarded as they were sensitive to noise.

**Fit of $f_0-I$ curve to that of a leaky integrate-and-fire (LIF) model.** For positive current pulses, we fitted by least squares the firing rate derived from the first ISI as a function of current, $f_0(I)$, to that of a LIF neuron with variable reset. If we denote by $v_{rest}$ the resting membrane potential (relative to an extracellular reference), $v_{th}$ the spiking threshold, $v_0$, the reset potential following a spike, $\tau$, the membrane time constant, $r_{in}$, the input resistance and $t_{ref}$ the refractory period, then the LIF firing rate as a function of injected current, $I$, is given by,
\[ f(I) = \left[ t_{\text{ref}} \log(1 - \frac{v_{\text{th}} - v_0}{r_{\text{in}} - v_0}) \right]^{-1}, \]

where \( v_{\text{th}} = v_{\text{th}} - v_{\text{rest}} \) and \( v_0 = v_0 - v_{\text{rest}} \). In those fits, both \( v_{\text{th}} \) and \( v_0 \) were allowed to vary and the other parameters were fixed. The resting membrane potential \( v_{\text{rest}} \), was set to –70 mV, \( t_{\text{ref}} \) was equal to 1.5 ms and \( \tau \) and \( r_{\text{in}} \) were determined from the responses to the hyperpolarizing current protocols.

**Leaky integrate-and-fire (LIF) model of adaptation.** We compared the adaptation time course of the LGMD firing rate to that of a LIF model similar to that studied by Liu and Wang (2001). The model includes two dynamic variables: the membrane potential, \( v(t) \), and the intracellular calcium concentration, \( x(t) \). The subthreshold dynamics of the model is described by the following two differential equations:

\[
\frac{dv}{dt} = -(v - v_{\text{rest}}) - r_{\text{in}} g_{\text{ahp}} x(v - v_K) + r_{\text{in}} I, \tag{2}
\]

\[
\frac{dx}{dt} = -\frac{x}{\tau_{\text{Ca}}}. \tag{3}
\]

The first term on the right hand side of eq. 2 represents a leak current, the second term, a potassium current whose activation depends linearly on calcium concentration and the last term, the current injected through the electrode. The second equation represents calcium extrusion with a time constant \( \tau_{\text{Ca}} \) (e.g., Traub, 1991; Helmchen et al., 1996). An action potential is generated when \( v(t) \) reaches \( v_{\text{th}} \) and the membrane potential is subsequently reset to \( v_0 \). The membrane potential stays at this value during the refractory period, \( t_{\text{ref}} \), before resuming its dynamical evolution according to eq. 2. After each action potential, the calcium concentration is incremented by \( \alpha \), i.e., \( x \rightarrow x + \alpha \) and immediately resumes its decay following eq. 3. The linear dependence of the potassium-dependent current on calcium concentration can be justified by linearizing the model of Wang (1998).
\[
g_{\text{a}h\text{p}}(x,v) = \frac{g_{\text{a}h\text{p}}}{x} - x \left( v - v_{K} \right) \approx g_{\text{a}h\text{p}} \beta (v - v_{K}),
\]

with \( k_{d} = 30 \, \mu M \) and \( \beta = 0.0267 \, \mu M^{-1} \). The approximation is very accurate for \( x < 10 \, \mu M \), as was the case in our simulations. Note that \( \tilde{g}_{\text{a}h\text{p}} = \frac{g_{\text{a}h\text{p}}}{k_{d}} \beta \) has units of conductance/concentration (in \( \mu S/\mu M \)). We set \( v_{\text{rest}} = -70 \, mV, \, v_{th} = -58 \, mV, \, v_{0} = -62 \, mV, \, \tau = 8 \, ms, \, r_{\text{in}} = 5 \, M\Omega, \, t_{\text{ref}} = 1.5 \, ms \). These parameters were determined from the analyses described above (see Results). The calcium extrusion time constant was set to \( \tau_{\text{Ca}} = 130 \, ms \), a value that will be justified in the Results. We followed Liu and Wang (2001) and set \( \alpha = 0.2 \, \mu M, \, v_{K} = -80 \, mV \). The value of \( \tilde{g}_{\text{a}h\text{p}} \) was adjusted to obtain attenuation factors similar to those observed experimentally and was set to 0.12 \( \mu S/\mu M \). Note that it is about a factor 10 higher than the value used by Liu and Wang (2001; 0.015 \( \mu S/\mu M \)). This is expected, since the input resistance of the LGMD is about a factor 10 lower than that of pyramidal neurons and the degree of adaptation in the LGMD (see Results) is similar to that of pyramidal cells (Ahmed et al., 1998). The model was simulated using a fourth order Runge-Kutta algorithm (Henrici, 1982) with a time step of 0.01 ms.

**AHP decay in the LIF adaptation model.** Following a current pulse, eqs. (2) and (3) imply that both the calcium concentration and the calcium-dependent potassium current decay exponentially towards zero with a time constant \( \tau_{\text{Ca}} \). Consequently, the membrane potential, \( v(t) \), relaxes towards its resting value, \( v_{\text{rest}} \), with a time-course that can be obtained from eq. (2) by setting \( dv/dt = 0 \) (using the approximation \( \tau << \tau_{\text{Ca}} \)). For typical average values of the peak AHP (-4 mV, see Results), the relaxation is slightly slower than an exponential decay with time constant \( \tau_{\text{Ca}} \).

Data analysis was performed with Matlab (Mathworks, Natick, MA). Data fitting was carried out using the optimization toolbox least square fitting routines. The LIF integration routine used to implement eqs. 2 and 3 was coded in C and accessed from Matlab as a Mex file to speed up simulations. In the following, standard deviations will be abbreviated by SD and correlation coefficients by \( \rho \). The Smith-Satterthwaite
procedure used in Table 1 for comparing means of distributions with unequal variances is described in Milton and Arnold (1995).
Results

We investigated the intrinsic membrane properties of the LGMD by injecting hyperpolarizing and depolarizing current pulses in 15 different neurons and recording the resulting membrane potential responses. The electrotonic properties of the LGMD have not yet been studied. We thus started by characterizing them: in vivo resting membrane potential, input resistance and membrane time constants. Next, we studied inward rectification in response to strong hyperpolarizing current pulses and classified the spiking patterns of the LGMD in response to depolarizing pulses. Finally we studied firing adaptation and its relation to the predictions made by the models of Wang (1998) and Liu and Wang (2001).

Electrotonic properties of the LGMD

The resting membrane potential of the LGMD had a typical mean value of -68 mV (SD: 6.6 mV, n=11 neurons). Figure 1A and B illustrate responses to three hyperpolarizing and depolarizing current pulses of varying amplitude, respectively. Fig. 1C illustrates the input resistance distributions derived from such experiments by averaging over 300 ms windows (Methods). Bars of different shades represent input resistance measurements for the six values of injected current. Fig. 1D gives the mean and SD of these distributions. There was a slight, but non-significant trend for the input resistance to decrease with increasing current (p>0.8, one-way ANOVA; max. difference between -5 and +3 nA: 0.8 MΩ). The overall mean input resistance pooled across all current values was 4.3 MΩ (SD: 1.6; median: 4.0).

Fig. 2A shows the response of a single neuron within the first 60 ms of a -5 nA current pulse. Relaxation to the membrane potential steady-state value at the end of the pulse occurred in two phases. The first, rapid phase typically lasted ~1.5 ms and was followed by a slower relaxation over the next ~40 ms. Accordingly, the membrane potential time course could be fit by a double exponential (Fig. 2A, gray line) whose parameters were
determined using the exponential peeling method (Fig. 2B). The first time constant is thought to reflect rapid charge redistribution across the spatial extent of the neuron, and will be referred to as the “equalization” time constant, $\tau_e$ (Holmes et al., 1992). The second slower time constant reflects local charging of the membrane and thus corresponds to the membrane time constant, $\tau$. In the example depicted in Fig. 2A and B, $\tau = 7.8$ ms and $\tau_e = 0.3$ ms. Fig. 2C and D show histograms of the measured values of $\tau$ and $\tau_e$ averaged across 3 current values (-1, -2 and -5 nA, respectively). The means of $\tau$ and $\tau_e$ pooled across currents were 7.3 ms and 0.34 ms, respectively (SDs: 2.1 and 0.24; medians: 7.8 and 0.26). There was a trend for $\tau$ to increase with the amplitude of the pulse (one-way ANOVA, $p<0.0001$). The mean values of $\tau$ at -1, -2 and -5 nA were 6.6, 7.3 and 9.2 ms, respectively (SDs: 1.7, 1.6 and 1.9).

The LGMD is endowed with an inward rectifying current

Fig. 1A (arrowheads) suggests the presence of a small sag in the membrane potential towards the end of the -5 nA current pulse and rebound activity immediately following the pulse. The average difference in membrane potential measured over two 50 ms windows centered 100 ms and 450 ms after pulse onset was equal to 1.1 mV (SD: 1.4). This change corresponded to a significant difference in the mean membrane potentials over the two windows (t-test, $p<0.05$). When pooled across 10 neurons (15 penetrations), the average difference (1.2 mV, SD: 1.7) also corresponded to a significant difference in means (t-test, $p<0.05$). To further assess the presence of inward rectification, we injected negative currents of larger amplitude in two neurons (up to -20 nA; 3 penetrations, 7 measurements). Fig. 3A illustrates the trial-averaged and median filtered responses to such pulses for one measurement obtained in one of the two neurons. The other measurements in the same neuron (two penetrations total) and in the second neuron showed similar results. The magnitude of the sag increased monotonically with the magnitude of the pulse, which is reflected in the increased difference between the peak hyperpolarization (star in Fig. 3A, B top panel) and the hyperpolarization at the end of the pulse (gray circle in Fig. 3A, B top panel). Since the sag was seen at membrane
potentials well below the potassium reversal potential it is more likely to be associated with the opening of a mixed sodium/potassium conductance rather than with closure of a potassium conductance (Kiehn and Harris-Warrick, 1992; Halliwell and Adams, 1982). The peak rebound value is illustrated in the bottom panel of Fig. 3B (triangles; see also Fig. 3A). The abrupt increase in depolarization at -7 nA (arrow) reflects the reliable generation of a rebound spike by the LGMD at this current value. Thereafter, the number of rebound spikes increased with increasing current magnitude, reaching up to ~10 spikes at strongly hyperpolarized potentials (-20 nA, ~100 mV below rest). Similar post-pulse rebound spiking was also observed in a separate set of experiments during which five LGMD neurons were filled with Lucifer yellow by injection of large negative current pulses (Peron et al., 2003).

The LGMD is an intrinsically bursting neuron

Next, we examined the LGMD responses to depolarizing current pulses. Fig. 4A illustrates sample traces in a single neuron stimulated with pulses of 3, 4, 6 and 10 nA (from bottom to top). The current threshold eliciting spikes was typically around 3-4 nA. At threshold, the LGMD usually fired either one spike (Fig. 4A, lowest trace) or a burst of two spikes (Fig. 4A, second lowest trace). At currents above threshold, the LGMD always fired a short burst of spikes followed by isolated spikes (Fig. 4A, middle two traces). As current increased, the number of spikes in the burst typically increased and sometimes a second burst riding over a single depolarizing envelope was observed (Fig. 4A, second highest trace), followed by isolated spikes. A characteristic signature of intrinsically bursting neurons is a bimodal interspike interval (ISI) distribution, with an initial peak corresponding to intervals between spikes occurring in bursts and a second, broader peak corresponding to intervals between bursts and/or isolated spikes (see, e.g., Figs. 1 and 2 of Nowack et al., 2003). Accordingly, the interspike interval (ISI) distributions pooled across all positive current injection values were bimodal in 11 out of 13 neurons tested (17 penetrations), similar in shape to that illustrated in Fig. 4B (bottom panel). In two neurons, the ISI distributions were unimodal when pooled across all
current values. This was the case for the neuron illustrated in Fig. 4 (see panel B, top). A closer examination of the responses showed that at current values > 10 nA, the initial burst smoothly merged with subsequent isolated spikes (see Fig. 4A, top trace) thus causing the peak of the ISI distribution associated with the burst to gradually merge with the tail of the distribution. This observation was confirmed by analyzing ISI distributions for each individual current value. These ISI distributions were initially strongly bimodal and gradually became unimodal as current amplitude increased above 10 nA. Thus, when positive currents were particularly effective at driving the LGMD, the large number of action potentials obtained at high current magnitudes sometimes masked the bursting behavior seen at lower current values. This was confirmed by computing the pooled ISI distribution for current values ≤ 10 nA (corresponding to steady-state firing frequencies ≤ 50 spk/s), which were bimodal in both neurons (see Fig. 4B, bottom panel). We were also able to obtain a second penetration in one of the neurons with unimodal ISI distribution. In the second penetration, positive currents were less effective at driving the cell, presumably because the electrode was located farther away from the spike initiation zone (see next paragraph) and the ISI distribution was bimodal across all currents. Based on this experimental data, we conclude that the LGMD is an intrinsically bursting neuron, with the capacity for firing at high sustained rates.

*Adaptation of the LGMD firing frequency to sustained current pulses*

Another feature evident from Fig. 4A is that the firing rate of the LGMD adapts over the course of a current pulse. In Fig. 5A the mean instantaneous firing frequency is plotted as a function of time during current pulses of 8, 12 and 15 nA in one LGMD neuron (black lines). These curves were derived from ten current injection trials whose spike rasters are shown below the graph in Fig. 5A. The peak instantaneous firing rate, \( f_0 \), was always attained during the first interspike interval, increased with current magnitude and started to saturate for values above 10 nA (Fig. 5B). The shortest intervals reached typical values of ~1.8 ms, corresponding to peak instantaneous firing frequencies of ~550 spk/s. Thus, \( f_0 \) was a non-linear, saturating function of current and was well fit by the current - firing
frequency curve of a leaky integrate-and-fire model with refractory period and a reset potential different from rest (gray line in Fig. 5B; Methods, eq. 1). In contrast, the steady-state firing rate, $f_{ss}$, was a linear function of injected current (Fig. 5B). Thus, in the LGMD as in other neurons, adaptation results in a linear steady-state current-frequency relationship (Schwindt et al., 1997; Ermentrout, 1998; Wang, 1998). At all current values, the time course of adaptation was well fit by a single exponential function (Fig. 5A, gray lines). This yielded a time constant of adaptation $\tau_{adap}$ that increased with the magnitude of the current pulse ($\tau_{adap} = 19, 31$ and $42$ ms for pulses of $8, 12$ and $15$ nA, respectively). The attenuation of the steady-state firing rate relative to the peak firing rate was defined as $F_{adap} = (f_0 - f_{ss})/f_0$ and decreased with current pulse magnitude ($F_{adap} = 0.87, 0.78$ and $0.70$ for pulses of $8, 12$ and $15$ nA, respectively). The values of $\tau_{adap}$ and $F_{adap}$ observed in the LGMD were in the same range as those observed in pyramidal cells of cat visual cortex in vivo ($0 < \tau_{adap} < 80$ ms and $F_{adap} \geq 0.50$, Ahmed et al., 1998; see also Fig. 6 below).

For cat visual cortex pyramidal neurons, a model of adaptation incorporating a variety of electrophysiological data and based on a calcium-dependent mechanism has been proposed (Wang, 1998; Liu and Wang, 2001). According to this model, calcium enters the cell through voltage-gated channels during repetitive firing and activates a calcium-dependent potassium conductance that acts as a negative feedback mechanism, decreasing the cell's firing rate. In the model, calcium is extruded from the cytoplasm with a time constant $\tau_{ca}$ (see also eqs. 2 and 3, Methods). The model explains the exponential relaxation of the firing frequency during a current pulse observed in pyramidal neurons and in the LGMD. Analysis of the model using semi-analytical and simulation techniques makes three predictions that have not yet been tested experimentally (for details see Wang, 1998; Liu and Wang, 2001). The first prediction is that the time constant of adaptation should increase linearly with injected current (top panel in Fig. 3B of Wang, 1998). The second prediction is that the attenuation factor $F_{adap}$ should decrease linearly with injected current (bottom panel in Fig. 3B of Wang, 1998). In Wang’s model, both of these predicted changes originate from a slower rate of intracellular calcium accumulation as the kinetics of the action potential becomes faster with increasing injected current amplitude. The slower rate of calcium buildup results in
turn in longer time constants of adaptation and weaker attenuations. These two predictions imply that the attenuation factor, $F_{\text{adap}}$, should depend linearly on the time constant of adaptation, $\tau_{\text{adap}}$. The third model prediction is that the inverse slope of this linear relation between $\tau_{\text{adap}}$ and $F_{\text{adap}}$ is a biophysical characteristic of the investigated neuron that matches the model’s time constant of calcium extrusion, $\tau_{\text{Ca}}$. In other words,

$$F_{\text{adap}} = 1 - \frac{\tau_{\text{adap}}}{\tau_{\text{Ca}}}.$$  \hspace{1cm} (4)

This relation can be derived analytically in the LIF model of Liu and Wang (2001, eq. 47) and holds approximately in the two-compartment model of Wang (Fig. 6D in Wang, 1998). In the latter case, eq. 4 can be explained by noting that $F_{\text{adap}}$ is nearly proportional to $\tau_{\text{adap}}$. The proportionality constant, $k$ (units: ms$^{-1}$), measures the rate of change of calcium concentration influx through voltage-gated $Ca^{2+}$ channels as a function of intracellular calcium concentration, as determined by the current injection level and instantaneous firing frequency of the cell ($k = \alpha G_{cc}$ in eq. 18 of Wang, 1998; see also his Fig. 3A, bottom panel). But, in this model, the same constant $k$ also sets the time constant of adaptation through $1/\tau_{\text{adap}} = k + 1/\tau_{\text{Ca}}$ (Wang, 1998, eq. 10). Combining these two results immediately yields eq. 4 (which corresponds to eq. 19 of Wang, 1998). Note that eq. 4 implies that $\tau_{\text{adap}}$ and $F_{\text{adap}}$ vary in antagonistic manner, i.e., as $\tau_{\text{adap}}$ increases, $F_{\text{adap}}$ decreases, as observed for the three example currents considered above. These three predictions are specific to the models considered by Wang (1998) and Liu and Wang (2001). Other biophysical models of spike frequency adaptation (see, e.g., Koch, 1999) do not necessarily fulfill them (Liu and Wang, 2001).

To test these theoretical predictions, we plotted the attenuation, $F_{\text{adap}}$, and the time constant of adaptation, $\tau_{\text{adap}}$, as a function of injected current (Fig. 5C and D). The graphs were remarkably close to linear (correlation coefficients, $\rho = 0.996$ and $-0.997$, respectively). The relation between $F_{\text{adap}}$ and $\tau_{\text{adap}}$ was also nearly linear ($\rho = -0.989$; $\tau_{\text{Ca}} = 141$ ms) in close agreement with theoretical predictions (Wang, 1998; Liu and Wang 2001). In Fig. 6A we show the peak instantaneous and steady state firing frequency
for 13 different LGMD neurons (17 penetrations). Both $f_0$ and $f_{ss}$ have similar shapes as in Fig. 5A, but there was quite a range of variability across neurons and penetrations. In particular, the slope of the steady-state firing frequency ranged from 2.5 to 13.7 spk/s/nA. Fig. 6B and C show the time constant of adaptation and the attenuation for the same recordings as a function of injected current. There was again a wide range of variability across neurons and penetrations. Except in one case (Fig. 6B arrow), the time constant of adaptation increased linearly with current magnitude and the linear relation predicted theoretically was closely followed (mean $\rho = 0.864$ across 17 penetrations). Excluding the cell arrow-marked in Fig. 6B, which had a negative correlation coefficient, yielded a mean $\rho = 0.971$. The same held true for the attenuation (mean $\rho = -0.957$ across 17 penetrations). Quite remarkably, when the attenuation was plotted as a function of the time constant of adaptation (Fig. 6D) the variability observed in B and C almost entirely disappeared and data points were tightly clustered around a single, common line. The mean $\rho$ between both variables amounted to -0.868 (17 penetrations) and was even higher (-0.957) when excluding the arrow-marked cell in Fig. 6B. In two neurons, the $F_{adap} - \tau_{adap}$ relation had a similar slope, but was slightly shifted to the right relative to the bulk of the data (arrow in Fig. 6D). A linear fit to individual $F_{adap} - \tau_{adap}$ relations yielded a mean intercept of 1.02 (SD: 0.05), very close to one, the value predicted by eq. 4, and a slope of 131 ms (SD: 23 ms). Thus, the slope of the $F_{adap} - \tau_{adap}$ relation is indeed an invariant biophysical property of the LGMD, independent of the particular animal in which the neuron was recorded and independent of the particular penetration in each LGMD neuron.

These results suggest that the variability observed in Fig. 6A-C may result from differences in the ability of current injected through the intracellular electrode to stimulate firing in the LGMD. This is to be expected if: i) the LGMD is not electrotonically compact and ii) the distance of the electrode relative to the spike initiation zone varied across recordings. The first assumption is likely true because the LGMD possesses a complex dendritic morphology (e.g., Fig. 1a of Gabbiani et al., 2002) and visual stimulation mediating inputs at different locations across the main dendrite have differential effects on the firing frequency of the LGMD (Krapp and Gabbiani, 2005). The second assumption is also likely given that in the present recordings the

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LGMD was penetrated blindly, and thus at random locations of its dendritic tree in the optic lobe. Penetrations in the dendrites at different electrotonic distances of the spike initiation zone are expected to yield spike amplitudes of varying height and width (Stuart et al., 1997). To test these assumptions directly, we took advantage of the fact that we were able to obtain in three different neurons two penetrations that led to significantly different spike heights. In all three cases the decrease in spike height was coupled with an increase in spike width, consistent with the assumption that the recording with smaller spike height was farther away from the spike initiation zone. In all three cases, the recording with the smaller spike height led to a shallower slope of the steady-state firing curve (Table 1). To further test these assumptions across our data set, we plotted in Fig. 6E the steady-state firing frequency slope as a function of spike height measured across 11 neurons (14 penetrations). We found a good correlation between spike height and steady-state firing frequency slope ($\rho = 0.81$, slope: 0.20 spk/s/nA/mV). As illustrated in Fig. 6F, the spike height was in turn negatively correlated with spike width ($\rho = -0.60$; slope: $-0.0051$ ms/mV). We conclude that differences in electrode location relative to the LGMD spike initiation zone explain to a large extent the variations observed in Fig. 6A-C across LGMD neurons and penetrations.

Properties of the post-pulse afterhyperpolarisation

Following a depolarizing current pulse, the LGMD membrane potential exhibited an afterhyperpolarization (AHP) before relaxing over time toward its resting value. This is illustrated in Fig. 7A where the inset shows the response to a single pulse and the following AHP time course (shaded gray area). The main panel shows the time course of the membrane potential on an expanded time scale for a range of current values (1-12nA) immediately following the termination of the pulse. The peak AHP is plotted in Fig. 7B as a function of current amplitude. The relation between these variables was close to linear ($\rho = -0.997$) in this and all other cases examined (11 neurons, 15 penetrations, 28 measurements). In the model considered in the previous paragraph, the calcium-dependent potassium current causes both spike-frequency adaptation and the post-pulse...
AHP (see also Methods, *AHP decay in the LIF adaptation model*). If this is also predominantly the case for the LGMD, one expects the potassium current to decay after a current pulse with a time constant independent of the recorded neuron and equal to $\tau_{Ca}$. Consequently, an approximately similar decay is also expected for the AHP (e.g., Schwindt et al., 1988; Sah, 1992). To test this hypothesis, we re-plotted the normalized AHP time-course in cases where the absolute peak AHP value exceeded 2mV (Fig. 7C, gray traces; obtained from those in Fig. 7A). The time-course of AHP decay was similar across current injection amplitudes and the normalized AHP averaged across current values (dotted line in Fig. 7C) could be fitted with a single exponential (Fig. 7C, black line), although these fits were typically less good than those obtained for spike-frequency adaptation. In Fig. 7D we compare the time constant of AHP decay to that obtained from the slope of the $F_{adap}$-$\tau_{adap}$ relation in 11 neurons. The mean AHP decay time constant was equal to 114 ms (SD: 37) and was slightly lower than the mean $\tau_{adap}$-$F_{adap}$ time constant (131 ms, SD: 23). There was no correlation between these two variables ($\rho = -0.03$) indicating no systematic changes across cells. These results are thus consistent with a common mechanism governing spike-frequency adaptation and AHP decay in the LGMD. Other conductances, such as inward rectification, might also contribute to a lesser extent to AHP decay, thus speeding up its dynamics (Lorenzon and Foehring, 1992).

**Interspike interval characteristics at steady-state**

As illustrated by the rasters plots shown Fig. 5A (top), the firing of the LGMD was very regular at steady-state for high firing frequencies. Correspondingly, the coefficient of variation (CV) of the ISI distribution at steady-state was between 0.1 and 0.2 for firing frequencies in excess of 50 spk/s (Fig. 8A). As the steady-state firing frequencies decreased below 50 spk/s, the variability of interspike intervals rapidly increased, with CV values between 0.2 and 0.6. When the CV is high at low firing frequencies, the theoretical models of Wang (1998) and Liu and Wang (2001) predict negative correlations between interspike intervals because randomly occurring short intervals
cause sufficient calcium entry to activate the AHP conductance, making the next ISI more likely to be longer and vice-versa (Fig. 9C of Wang, 1998; Fig. 8 of Liu and Wang, 2001). In Fig. 8B we show the ISI correlation coefficient (CC) as a function of the CV at steady state. There was a negative correlation ($\rho = -0.45$) between these two variables and the CC was indeed negative at high CVs. In contrast, when strong current pulses were dominant in driving the LGMD responses, low CVs resulted in predominantly positive CCs. This is expected for strongly periodic firing from results obtained in simplified neuronal models in the presence of weak, correlated noise (Lindner, 2004).

A leaky integrate-and-fire model fails to replicate LGMD adaptation

The linear dependence of the $\tau_{adap-I}$, $F_{adap-I}$ and $F_{adap-\tau_{adap}}$ relations observed in the LGMD were predicted in a conductance-based, two-compartment model (Wang, 1998) and in a LIF neuron (Liu and Wang, 2001) which both included a calcium-dependent adaptation current and an exponential calcium extrusion mechanism. Since an LIF neuron can be constrained from the electrophysiological data described in the previous paragraphs, we investigated whether this model is able to replicate the adaptation properties of the LGMD. Such a model cannot of course replicate more complex properties such as bursting, but has the advantage of being conceptually simple. It could thus potentially yield insight in the firing properties of the LGMD for stimuli other than current pulses. To be useful, such a model needs to work over a large fraction of the LGMD dynamic range and in particular at high firing frequencies, as observed during stimulation of the LGMD with visual looming stimuli. The inset in Fig. 9 shows a simulation for model parameters matched to the properties of the neuron depicted in Fig. 5 (current pulse amplitude: 8 nA). The firing frequency of the LIF adapts over the course of the current pulse and the model exhibits attenuation and an AHP similar to those observed in the LGMD. An important difference between the model used here and the one studied by Liu and Wang (2001) is that we had to introduce a refractory period ($\tau_{ref} = 1.5$ ms) to replicate the non-linear relation between the peak instantaneous firing rate frequency $f_0$ and current over a large fraction of the LGMD firing range (Fig. 5B, gray trace). It follows immediately from eq. (1) that the firing frequency of a LIF neuron in the
presence of a refractory period is given by \( f(I) = \left( t_{\text{ref}} + f_{\text{noref}}(I)^{-1} \right)^{-1} \), where \( f_{\text{noref}}(I) \) is the firing frequency with \( t_{\text{ref}} \) set to zero. The refractory period thus plays a negligible role for low currents \( (t_{\text{ref}} \ll f_{\text{noref}}(I)^{-1}) \), leading to an exponential decay of firing frequency over the time-course of the pulse (Fig. 9, lower black trace and gray line). For higher currents, however, the refractory period is initially predominant and sets its own time scale for firing frequency decay, thus interfering with the negative feedback mechanism mediated by the adaptation current. Accordingly, a single exponential could not fit the decay of the firing frequency observed in the model (Fig. 9, top two black traces and gray lines), in contrast to experimental findings (Fig. 5A). Thus, an LIF neuron with refractory period is unable to fit the LGMD adaptation properties over the range of firing frequencies studied experimentally. We conclude that a conductance based, compartmental model will be required for this purpose.
Discussion

This work presents the first characterization of the intrinsic membrane properties of the LGMD. It shows that the LGMD has a fast membrane time constant, a low input resistance and an extended electrotonic structure. It also reveals the presence of adaptation, of inward rectification and places the LGMD in the category of intrinsically bursting neurons (Krahe and Gabbiani, 2004). Adaptation followed remarkably well the predictions made by the two-compartment model of Wang (1998). By using a uniquely identifiable neuron, we were in particular able to verify that the relation between attenuation and the time constant of adaptation is linear and that its slope is a biophysical invariant of the LGMD. This prediction would have been very difficult to test on a heterogeneous neuronal population. Our results thus validate the model's predictions and also rule out a description of the LGMD by a simpler, leaky integrate-and-fire model.

Since we studied the electrotonic properties of the LGMD \textit{in vivo}, our measurements were presumably affected by more than pure passive membrane properties. Although we did not characterize spontaneous membrane potential fluctuations, intracellular LGMD recordings \textit{in vivo} typically exhibit a sizable number of spontaneous excitatory and inhibitory postsynaptic potentials. Thus, background activity is likely to have affected the input resistance and the membrane time constant of the cell (Bernander et al., 1991). It might also have had an impact on the resting membrane potential. Some active conductances such as the one mediating inward rectification may also be tonically active and contribute to resting membrane properties (Magee, 1998). The increase in membrane time constant with hyperpolarizing pulse amplitude would for example be consistent with rapid closing of tonically active conductances. Because the input resistance did not change significantly over longer time scales, this might have been compensated for by a slower activation of inward rectification, for instance. The estimation of electrotonic parameters may also depend on the recording technique. \textit{In vitro}, differences have been observed between sharp electrode recordings (used here) and patch-clamp recordings (Spruston and Johnston, 1992; Staley et al., 1992), but may be less conspicuous \textit{in vivo} (Borg-Graham et al., 1996). Currently, it is unknown whether such differences exist in
insect visual interneurons, because *in vivo* patch-clamp recordings have proven difficult to obtain from large wide-field cells such as the LGMD. Nonetheless, the values reported here were similar to those observed in fly tangential neurons obtained under similar circumstances (Borst and Haag, 1996). These neurons, like the LGMD, integrate on their extended dendritic arborizations a large number of local visual inputs. *In vivo* electrotonic properties are directly relevant to information processing in the context of natural visual stimuli. In particular, the low input resistance and fast membrane time constant of the LGMD allow for rapid processing of visual information. As is the case for fly tangential neurons involved in visual flight control (e.g., Egelhaaf et al., 2002; Borst and Haag, 2002), rapid visual information processing is critical to support successful collision avoidance and escape behaviors.

Inward rectification has been reported in many vertebrate and invertebrate neurons (e.g., Golowasch and Marder, 1992; Kiehn and Harris-Warrick, 1992; Lüthi and McCormick, 1998). Originally, inward rectifying conductances have been linked to bursting (Jahnsen and Llinas, 1984). More recently, inward rectification has been shown to shorten the window of synaptic integration (Magee, 1998; Poolos et al., 2002; Migliore et al., 2004). The role played by inward rectification in the LGMD during synaptic integration of visual inputs is under study. Inward rectification may also play a role in shaping the bursting properties of the LGMD. In our experiments, the hyperpolarizing pre-pulses preceding depolarizing current pulses should have activated little additional inward rectification (Fig. 3). In one experiment, we studied the responses to depolarizing current pulses without applying hyperpolarizing pre-pulses and observed no differences in the bursting characteristics of the LGMD. The role of inward rectification in synaptic integration will also depend on the parameters characterizing the underlying conductance (see Wang, 1994, Liu et al., 1998, Hill et al., 2001 and Poolos et al., 2002 for very different models), and on its distribution within LGMD's dendritic tree (Migliore and Sheperd, 2002).

An alternative mechanism that could explain bursting in the LGMD is the electrical load imposed by its extended dendritic compartments on the spike initiation zone. High
dendritic loads are likely to contribute to bursting in cortical neurons (Mainen and Sejnowski, 1996) and induce bursting in Wang’s model (1998, Fig. 5C). Notably, this mechanism should not interfere with the model’s predictions. A detailed reconstruction of LGMD’s dendritic tree (Peron et al., 2003) and a compartmental model derived from it will be required to test this hypothesis. Active conductances, like a persistent sodium current may also contribute to bursting in the LGMD (Gabbiani et al., 2002), as in other neurons (e.g., Wu et al., 2005).

The LGMD exhibits pronounced adaptation in response to depolarizing current pulses. The degree of adaptation is comparable to that observed in cortical pyramidal neurons in vivo (Ahmed et al., 1998). The firing characteristics of cortical neurons have been investigated particularly thoroughly and categorized according to several distinct classes (McCormick et al. 1985; Schwindt et al., 1997; Nowak et al., 2003). Typically, many cortical neurons that exhibit spike-frequency adaptation are regular spiking, although adaptation is also observed in intrinsically bursting neurons. The LGMD is both intrinsically bursting and strongly adapting. Thus, its firing characteristics may lie at an intermediate level between these two categories. Bursting and adaptation are likely to explain several of the properties of LGMD spike trains observed in response to looming stimuli. When an object approaches the animal on a collision course, the spike patterns of the LGMD typically consist initially of short bursts of spikes followed by more prolonged periods of silence (Fig. 1 of Gabbiani et al., 1999 and of Gabbiani et al. 2005). This pattern of firing could readily be explained by the interplay between intrinsic bursting and adaptation mechanisms activated by visually driven synaptic inputs. Spike-frequency adaptation is also likely to contribute to the cell's preferential tuning to looming objects. This can be illustrated by considering the properties of looming objects as opposed to translating ones. The edge of a looming object is continuously expanding, and thus activates an increasing number of excitatory synaptic inputs. This should effectively counteract the onset of spike-frequency adaptation in the LGMD. In contrast, the edges of a translating object activate a constant number of synaptic inputs, and are thus expected to be subject to adaptation over relatively short time scales, similar to current pulses of constant amplitude. These ideas can be tested experimentally by
manipulating the intracellular calcium concentration of the LGMD \textit{in vivo} during visual stimulation.

The predictions made by the model of Wang (1998) were remarkably well reproduced in the LGMD. In addition, the variability in the slopes of the $\tau_{\text{adap}}$ and $F_{\text{adap}}$ vs. $I$ curves could to a large extent be explained by variability in the location of the electrode relative to the spike initiation zone. The residual variability in the slope of the $F_{\text{adap}}$ vs. $\tau_{\text{adap}}$ curve around its mean value ($\sim 130$ ms) may result from genuine variations in calcium dynamics from cell to cell. In this case, one would expect a correlation between the time constant derived from the slope of the $F_{\text{adap}}$ vs. $\tau_{\text{adap}}$ curve and the rate of decay of the AHP following a current pulse. No such correlation was detected (Fig. 7D), suggesting that systematic cell-to-cell variations, if present, lie within experimental noise. Because spike-frequency adaptation over the time scales reported here is mediated in a vast majority of neurons by calcium-dependent potassium currents (Sah, 1996), it is likely that the same mechanism is at work in the LGMD. Specifically, we predict calcium transients to decay with a time constant of $\sim 130$ ms within LGMD's dendritic tree. We base this prediction on the fact that the slope of the $F_{\text{adap}}$-$\tau_{\text{adap}}$ relation is a biophysical invariant, corresponding to the effective time constant of calcium extrusion in Wang's model. In the model, spatial variations in local calcium dynamics can be detected by the presence of multiple adaptation time constants. The mono-exponential decay reported here thus suggests fairly homogeneous calcium entry/extrusion and calcium-dependent potassium mechanisms across LGMD’s main dendrites, as recently reported in hippocampal neurons (Ngo-Anh et al, 2005).

The LIF model of Liu and Wang (2001) failed to reproduce the dynamics of adaptation over the full firing range of the cell, because of the addition of an absolute refractory period. This feature was necessary to replicate the saturation of the first ISI vs. current curves observed experimentally (Fig. 6A), but significantly affected the dynamics of adaptation at high firing frequencies. Clearly, a simple threshold and an absolute refractory period represent only a crude approximation to more realistic compartmental models (Ermentrout, 1998; Liu and Wang, 2001). Nonetheless, the LIF model
successfully approximates the firing characteristics of cortical pyramidal neurons (Rauch et al., 2003). This may be in part due to the fact that pyramidal neurons often fire at lower frequencies (Simons and Carvell, 1989). In any case, Wang’s model appears sufficiently general to capture the properties of spike-frequency adaptation in the LGMD, a neuron quite different from those originally used to constrain it. We suggest that it might therefore encapsulate fundamental properties of spike-frequency adaptation over time scales of a few hundreds of milliseconds.
Acknowledgments

Thanks to J. Niven and S. Huston for comments.

Grants

Supported by a DFG travel grant (H.G.K.), an Alfred P. Sloan Fellowship (F.G.) and an NIMH R01 grant (F.G.).
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### Table 1.

<table>
<thead>
<tr>
<th>Penetration 1</th>
<th>Penetration 2</th>
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<tr>
<td><strong>Height</strong></td>
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<td><strong>Height</strong></td>
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<tr>
<td><strong>Slope</strong></td>
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<tr>
<td><strong>Height</strong></td>
<td>30.2 mV, SD: 1.3</td>
</tr>
<tr>
<td><strong>Width</strong></td>
<td>1.05 ms, SD: 0.09</td>
</tr>
<tr>
<td><strong>Slope</strong></td>
<td>6.6 spk/s/nA</td>
</tr>
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</table>

Mean spike width, height and SDs (n=10) in three neurons recorded in two different locations and corresponding slopes of the steady-state firing frequency curves as a function of injected current. Heights and widths in penetrations 1 and 2 were always significantly different (t-test, Smith-Satterthwaite procedure, p<0.01).
Figure Legends

Figure 1. Input resistance of the LGMD. A: Membrane potential hyperpolarization (top) evoked in response to square current pulses (bottom) of -1, -2 and -5 nA, respectively. Each membrane potential trace is an average over 10 stimulus presentations in a single neuron. The two arrowheads indicate the slight sag and rebound activity for the –5 nA current injection, respectively. B: Membrane potential depolarization in a single neuron evoked in response to square current pulses of 1, 2 and 3 nA, respectively. In addition to trial averaging as in A, each membrane potential trace was first median-filtered to suppress action potentials. C: Histogram of input resistance values derived from A and B for negative and positive current pulses (top and bottom panels, respectively). Negative current pulse data were obtained in 12 neurons (16 different penetrations) and positive current pulse data in 13 neurons (18 different penetrations). D: Average input resistance (mean, SD) as a function of injected current derived from the histograms illustrated in C.

Figure 2. Membrane time constant of the LGMD. A: Membrane potential hyperpolarization (top) to a -5 nA current pulse (bottom). The mean and SD across 10 stimulus repetitions are illustrated by the solid and dotted black lines, respectively. The gray line is a double exponential fit with two time constants, \( \tau = 7.8 \text{ ms} \) and \( \tau_e = 0.3 \text{ ms} \) obtained using the peeling method illustrated in B. B: Time constant estimation through exponential peeling. The membrane time constant (\( \tau \), bottom and left axes) was obtained by fitting the logarithm of the membrane potential minus its minimum steady state value (black dots) to a straight line (black). \( \tau \) is the absolute value of the inverse fit line slope. The equalization time constant (\( \tau_e \), top and right axes, gray data points and fit line) was obtained in the same manner, after subtraction of the fitted black straight line (\( \log(v_{peel}) \)) from the experimental data. C: Histogram of \( \tau \) values averaged across 3 currents (-1, -2 and -5 nA) obtained in 11 neurons (17 penetrations). D: Histogram of values obtained for \( \tau_e \) (same data sample as in C). In C and D downward pointing arrows indicate mean \( \tau \) and \( \tau_e \) values, respectively.
Figure 3. Inward rectification and post-stimulus rebound activity in the LGMD. A: Membrane potential deflection in response to negative square current pulses of increasing magnitude (-1 to -12, -15, and -20 nA, respectively). Each trace is averaged across 10 stimulus presentations and was median filtered to suppress rebound spikes. The star, grey circle and black triangle indicate the peak and end-pulse hyperpolarization as well as the maximum rebound activity to the -20 nA square pulse, respectively. B: Top panel shows peak and end-pulse hyperpolarization (star and grey circles, respectively, derived from A) as a function of injected current. The bottom panel shows the peak membrane potential post-stimulus rebound (black triangle in A) as a function of injected current. Arrow indicates sudden jump in rebound activity, caused by a spike.

Figure 4. LGMD bursting in response to depolarizing current pulses. A: The top four traces illustrate the intracellular membrane potential recorded in a single neuron in response to +3, 4, 6 and 10 nA current pulses (bottom), respectively. Action potentials are truncated and only the first 280 ms of the pulse are shown. At threshold (lower membrane potential trace) the LGMD often fires an isolated action potential. For higher currents (middle two traces) one or two short bursts of spikes are followed by isolated action potentials. At very high current intensities (top trace), the leading burst merges with the subsequent isolated action potentials and cannot be unambiguously separated from them. Note the increasing temporal separation of action potentials over the course of the pulse (adaptation). B: Top panel is a histogram of interspike interval duration (≤100 ms) over the entire range of currents tested (1-10, 12, 15 and 20 nA) in the neuron illustrated in A. The bottom panel is a histogram of interspike interval duration (≤100 ms) for current pulses leading to ≤50 spk/s mean spike frequency at the end of the pulse (3-10 nA).

Figure 5. Adaptation of the LGMD spike frequency during depolarizing current pulses. A: LGMD spiking response to depolarizing current injections of 8, 12 and 15 nA. The top panel depicts the instantaneous firing frequency of the LGMD ($f_m(t)$, black lines) as a function of time (in decreasing order of magnitude). Time zero indicates the positive current pulse onset. The three gray lines are exponential fits to the instantaneous spike frequency. Bottom rasters illustrate spike occurrence times (ticks) for each of the three
current pulses. Each line corresponds to a single current pulse (10 pulses per current value). B: Mean instantaneous spike frequency for the first interspike interval (triangles) and at steady-state (circles) as a function of current magnitude. The gray line is a fit to the spike frequency curve of a LIF neuron with reset different from rest (see eq. 1, Methods; \( r_{in} = 5 \, \text{M}\Omega, \, \tau = 8 \, \text{ms}, \, v_0 = -62 \, \text{mV}, \, v_{th} = -58 \, \text{mV} \)). C: Attenuation factor, \( F_{adap} = (f_0 - f_{ss})/f_0 \), as a function of current magnitude. D: Time constant of adaptation, \( \tau_{adap} \), derived from exponential fits (A) as a function of current magnitude. E: Attenuation factor, \( F_{adap} \), as function of the adaptation time constant, \( \tau_{adap} \).

**Figure 6.** Adaptation characteristics measured in 13 LGMD neurons. A: Mean spike frequency derived from the first interspike interval and steady-state spike frequency as a function of current amplitude. Data presented as gray lines in A-D and marked by black arrows in B and D were obtained from the same three neurons. Data from 17 penetrations are illustrated in A-D. B: Time constant of adaptation as a function of current magnitude. Arrow indicates the single neuron for which the time constant of adaptation decreased with injected current. C: Attenuation factor, \( F_{adap} \), as a function of current magnitude. D: Attenuation factor, \( F_{adap} \), as a function of adaptation time constant, \( \tau_{adap} \). Arrow indicates the two neurons whose curves are slightly offset from the rest of the data. E: Steady-state spike frequency slope (derived from A) as a function of spike height. 9 neurons (14 penetrations) are illustrated in E and F. F: Spike width as a function of spike height. Vertical and horizontal error bars denote SDs and are sometimes too small to see.

**Figure 7.** Characteristics of the AHP in the LGMD. A: Plot of the AHP time course following current pulse offset (illustrated by gray area in inset) for depolarizing currents of 1-12 nA. Time zero denotes the time of pulse offset. Each trace is an average across 10 stimulus presentations. The asterisk denotes the peak AHP amplitude for a current pulse of 12 nA. Data plotted in gray were used to prepare panel C. B: Peak AHP (derived from A, star) as a function of current magnitude. C: Normalized AHP time course (peak AHP set to one) for currents of 3-12 nA. The dotted black line is the mean across all traces. The black solid line is a single exponential fit to the dotted black line. D: Time constant
derived from $\tau_{adap} - F_{adap}$ plots (see Fig. 6D) as a function of the time constant of AHP decay (derived from C) in a sample of 11 neurons (15 penetrations, 28 measurements).

Figure 8. Interspike interval variability and correlation at steady-state. A: Coefficient of variation of the interspike interval distribution at steady-state as a function of the steady-state spike frequency. B: Interspike interval correlation coefficient as a function of interspike interval coefficient of variation. Data from 12 different neurons (15 penetrations).

Figure 9. Modeling of LGMD adaptation using a leaky integrate-and-fire (LIF) neuron. Plot of instantaneous spike frequency as a function of time for three current pulses (8, 12 and 15 nA, as in Fig. 5A). Time zero denotes positive current pulse onset. Gray lines are exponential fits to the simulated firing rate. Inset illustrates the response of the model for an 8 nA current injection.
Gabbiani et al., Figure 6
Gabbiani et al., Figure 7
Gabbiani et al., Figure 9