Impact of Neural Noise on a Sensory-Motor Pathway Signaling

Impending Collision

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Running head: Role of Noise in a Collision-Detection Circuit

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Abstract

Noise is a major concern in circuits processing electrical signals, including neural circuits. There are many factors that influence how noise propagates through neural circuits, and there are few systems in which noise levels have been studied throughout a processing pathway. We recorded intracellularly from multiple stages of a sensory-motor pathway in the locust that detects
approaching objects. We find that responses are more variable and that signal to noise ratios (SNRs) are lower further from the sensory periphery. SNRs remain low even when using stimuli for which the pathway is most selective and for which the neuron representing its final sensory level must integrate many synaptic inputs. Modeling of this neuron shows that variability in the strength of individual synaptic inputs within a large population has little effect on the variability of the spiking output. In contrast, jitter in the timing of individual inputs and spontaneous variability is important for shaping the responses to preferred stimuli. These results suggest that neural noise is inherent to the processing of visual stimuli signaling impending collision and contributes to shaping neural responses along this sensory-motor pathway.

**Keywords:** LGMD, DCMD, variability, neural noise, single neuron computation

**Introduction**

Neural pathways specialized in the detection of threats and escape must be reliable; thus it is especially important for their function to remain undisrupted by noise. Indeed, all sensory and motor systems have evolved to function with a certain amount of noise. Sensory signals are inherently noisy due to the stochastic activity of transduction channels (Baylor et al., 1980; Laughlin and Lillywhite, 1982; Rieke and Baylor, 2000) and the sensory input itself (e.g., photon noise; Fuortes and Yeandle, 1964; Scholes, 1965; Baylor et al., 1979). Because of the stochastic properties of ion channels, synaptic transmission, and other neuronal components, further noise is necessarily introduced through neural pathways (Fatt and Katz, 1952; Rosenmund et al., 1993; White et al., 2000). Yet, neurons and neural circuits carry out computations reliably, and in some
cases, optimally (Laughlin, 1981; Osborne et al., 2005; Beck et al., 2008; Sengupta et al., 2010; for related energy constraints see Niven et al., 2007).

The organization of a neural circuit can reduce noise levels in specific circumstances. If individual neurons of a population carry independent noise, then averaging will lower its level (Laughlin et al., 1987; Field and Rieke, 2002). Depending on specific circumstances, non-linear transformations such as spike thresholding and feedback loops could amplify or dampen noise (Faisal et al., 2008). Since such neural components, both noise reducing and amplifying, are widespread and interwoven in neural circuits, it is hard to predict a priori how noise levels, and conversely neural signal reliability, will change through a processing pathway.

Thus, we investigated the effects of neural noise in a sensory-motor pathway of the locust that is specialized for detecting objects approaching on a collision course and triggering escape behaviors. Earlier studies have looked at visual response variability across several stages of a neural pathway (Kara et al., 2000; Borghuis et al., 2009). However, none of them could span from the sensory periphery to neurons with direct motor system outputs, as is possible in insects.

Locusts possess a pair of identically firing visual interneurons associated with each compound eye called the Lobula Giant Movement Detector (LGMD) and Descending Contralateral Movement Detector (DCMD, Figure 1A) (O’Shea and Rowell, 1976; Rowell and O’Shea, 1976). The role of these identified neurons in detecting objects approaching on a collision course and triggering escape behaviors is well documented (Schlotterer, 1977; Rind and Simmons, 1992; Gabbiani et al., 1999; Santer et al., 2006; Fotowat et al., 2011). They respond vigorously to simulated approaching objects (looming stimuli) with a firing rate that rises smoothly, peaks, and then falls; the timing of the peak signaling when the stimulus has reached a threshold angular size on the animal’s retina.
(Gabbiani et al., 1999). The LGMD receives roughly 15,000 excitatory inputs responding to luminance changes in small areas of the visual field and inhibitory inputs whose population is thought to signal the angular size of the stimulus (Strausfeld and Nässel, 1981; Hatsopoulos et al., 1995; Gabbiani et al., 2002; 2005). The DCMD relays this information to motor neurons controlling the wings and hind legs in the mesothoracic and metathoracic (O'Shea et al., 1974; Simmons, 1980; Burrows, 1996). We also know that trial-to-trial variability in the spike trains of the DCMD can predict if and when the animal will jump (Fotowat et al., 2011). Thus, variability in the LGMD/DCMD responses is relevant to the behavioral output of this circuit.

The excitatory pathway converging onto the LGMD/DCMD has several features that could influence the amounts of variability observed at each stage and how it is propagated. There is a 6 to 1 convergence from the photoreceptors to the 2nd order visual neurons, the large monopolar cells (LMCs) of the lamina (Meinertzhagen, 1976; Strausfeld and Nässel, 1981). There is also a transition from graded-potential to spiking responses between the LMCs’ dendrites and their targets in the medulla, not unlike that between bipolar and retinal ganglion cells in the mammalian retina. Although few recordings from the medulla have been obtained (James and Osorio, 1996) and little is known about its connectivity, a divergence of approximately 1 to 5 is likely at this stage (Peron et al., 2009). Finally, there is a massive convergence of medullar inputs onto the LGMD. Thus, we wished to experimentally observe the effect of these cellular and network features on the propagation of variability and to understand how it influences the responses of the LGMD to looming stimuli. We find that the signal to noise ratio (SNR) resulting from stimulation of small parts of the visual field decreases from the periphery through the pathway, and that SNRs of responses to looming stimuli are not much higher. Modeling of the
LGMD and its inputs during looming suggests that jitter in the single facet response times is important for shaping looming responses, and that variability of the looming responses is largely independent of variability in the strengths of the summed inputs.

**Materials and Methods**

**Animal dissection and electrophysiology.** Locusts were mounted in a plastic holder and dissected as previously described (Peron et al., 2007). Sharp microelectrodes were used for intracellular recordings from photoreceptors, LMCs (80-240 MΩ, 2M KAc/0.5M KCl), and the LGMD (8-30 MΩ, 2M KAc/0.5M KCl or 3M KCl for voltage clamp). Intracellular signals were low-pass filtered (Vc × 10 kHz, Lc × 5 kHz) and digitized (20 kHz). Photoreceptor and LGMD recordings used borosilicate electrodes (1.2/0.8 mm and 1.2/0.5 mm outer/inner diameter, respectively; WPI Inc., Sarasota, FL) while LMC recordings used aluminosilicate (1.0 mm outer diameter; Harvard Apparatus, Holliston, MA). An Ag/AgCl wire was used as reference. Current clamp recordings were made in discontinuous current clamp (DCC; at ~25 kHz switching frequency) or bridge mode using an SEC-10LX amplifier (NPI, Tamm, Germany). Voltage-clamp recordings from the LGMD used discontinuous single-electrode voltage clamp (dSEVC; at ~25 kHz). All dSEVC electrodes had <20 MΩ resistances, and electrode resistance (bridge) or capacitance (DCC/dSEVC) were fully compensated in the bath, immediately prior to tissue penetration. The noise levels resulting from electrodes in the bath had standard deviations (SDs) of 0.15 mV for high resistance photoreceptor/LMC electrodes, 0.06 mV for LGMD DCC, and 0.18 nA for LGMD dSEVC. These values were measured before penetration of photoreceptors and LMCs and thus represent an upper bound on the electrode noise as the electrode resistance typically decreased following tissue penetration. We used these electrode noise levels to correct the corresponding spontaneous membrane potential noise measurements under the assumption that they
add independently; that is, the measured variance equals the sum of the electrode noise variance and the cell membrane potential (or current) variance. Evoked responses were not corrected for electrodenoise as it had a negligible effect on them. Intracellular recordings were obtained from the lobula and lamina through the desheathed optic lobe and from the retina through a small (~50 x 50 μm) hole just below the dorsal rim of the eye. Photoreceptor recordings were identified by their resting potential (~40 mV, depending on ambient light levels) and depolarizing responses to luminance increases. The photoreceptor recordings included in this study had characteristics of high-quality recordings: light-adapted resting potentials of 40-50 mV which dropped by an average of 17 mV when background light was removed, had strong transient responses to full contrast luminance changes (Supplementary Figure 1), and showed no appreciable change in their resting $V_m$ over ~20 minutes of recording. The extracellular potential of the lamina modulates in phase with a flashing light stimulus allowing identification of LMCs by a resting hyperpolarization and transient, anti-phase responses to light flashes (Supplementary Figure 1). In the fly, two subtypes of LMCs have been shown to generate small (<10 mV) spikes in response to light pulses when dark-adapted (Uusitalo et al., 1995). The LMC cells presented here, recorded under light-adapted recording conditions, did not exhibit such active properties. LGMD recordings were identified by the cell’s 1:1 spike correspondence with the simultaneously recorded extracellular DCMD signal (O’Shea et al., 1974). The cell was penetrated in the proximal region of the excitatory dendritic field, with spike heights varying between 20-50 mV. The LGMD is an electrotonically extended neuron receiving distributed synaptic inputs that are finely organized (Peron et al., 2007; 2009). Thus, different visual stimulation regimes will differentially affect its local membrane resistance and impact the membrane potential noise recorded by an electrode in its main excitatory dendritic branches. One of the purposes of this study was to characterize these changes and relate them to presynaptic and LGMD firing rate variability. Stable LGMD recordings could be maintained for typically >60 minutes. Extracellular signals were acquired as previously described. The procedures for intracellular LGMD recordings while presenting looming stimuli were slightly different than
above and were previously described in (Gabbiani et al., 2002).

**Visual stimulation.** Visual stimuli were generated using custom software on a PC running a real-time operating system (QNX 4; QNX Software Systems, Ottawa, Canada). Looming stimuli were presented on a CRT monitor (200 Hz, luminance range: 2 cd/m² - 90 cd/m²). The looming stimuli used were expanding dark squares on a bright background. If $\theta$ denotes the angular size of the square on the retina, the stimulus size follows $\theta(t) = 2 \cdot \tan^\dagger(l/\nu t)$, where $l$ is the half-size of the simulated object, $\nu$ is the simulated approach velocity, and $t$ is time during the approach. By convention, $\nu$ is negative for an approaching object and $t$ is 0 at the time of collision with the animal (Gabbiani et al., 1999). $\theta(t)$ is fully described by the half-size to speed ratio, $l/|\nu|$, with units of time. Assuming a constant simulated object size, the lower the $l/|\nu|$ value, the faster the object is approaching and the more suddenly it expands.

Stimulation at single ommatidium resolution was achieved by projecting an image generated using a DLP projector (LT140; NEC Corp., Tokyo, Japan) through a custom-built microscope (Jones and Gabbiani, 2010) mounted horizontally on a vibration isolated optical table (illuminance range: 4-2530 lux). Both the CRT and projector were calibrated to ensure linear, 6-bit resolution control over light levels. The ambient light level for single facet experiments was set by a ring-light mounted around the objective of the microscope. This level was constant at 490 lux, equivalent to dim daylight. Locusts stimulated using the CRT monitor were adapted to a slightly different light level, determined by the brightness of the monitor (280 lux). The mean resting $V_m$ values recorded under these two conditions were very similar, -40.4 mV and -39.1 mV for single-facet and monitor stimulated photoreceptors, respectively.

**Single facet stimuli.** Each stimulus spot was 2 x 2 pixels (5 x 5 µm) in size, positioned in the center of each ommatidium. Each stimulus was a 1,500 ms light pulse from baseline (4 lux) to a variable maximum ($\leq 2530$ lux). Since the looming stimuli that we wish to emulate are dark objects on a light background, we focus
exclusively on the responses resulting from the return of this light pulse to its dark baseline ('off' responses; Fig. 2A, B). Luminance changes had a variable duration, ranging from instantaneous (0 ms) up to 183 ms. Their time course had the profile of a cumulative Gaussian, with a standard deviation equal to ¾ of the luminance change duration. This recreates the luminance change caused by an edge moving across the approximately Gaussian spatial receptive field of photoreceptors (Wilson, 1975; Burton and Laughlin, 2003). Multiple facets or adjacent facet pairs were stimulated when recording from the LGMD (4 maximum, with at least 2 interposed facets). Each facet was stimulated less than once per minute to avoid local habituation (O’Shea and Rowell, 1976). Stimuli were presented every 5 seconds for LMC and photoreceptor experiments Trial types within all experiments were pseudo-randomly interleaved. Pseudo- looming stimuli spanned three facet rows on the eye, each 15 facets long, with each stimulus point positioned over a single facet. The 3 stimulus points in each column were stimulated simultaneously, with each column being presented a more rapid luminance change, a sequence that was designed to mimic the acceleration caused by a looming edge. See Jones and Gabbiani (2010) for more details on this stimulus and LGMD responses to it.

Data analysis and statistics. We abbreviate the standard deviation by SD and signal to noise ratio as SNR throughout. All data analysis was carried out using custom MATLAB programs (MathWorks, Natick, MA). All analyses of stimulus-evoked membrane potentials, both experimental and simulated, were performed on traces that had been median-filtered (8 ms window) to remove spikes. This window size was chosen as the shortest length able to fully exclude LGMD spikes from the traces. DCMD spikes were detected from extracellular nerve cord signals by thresholding the waveforms. Instantaneous firing rates (IFRs) were calculated by convolving individual spike trains with a Gaussian window as in (Gabbiani et al., 1999; SD = 20 ms).

We utilized nonparametric statistical tests on our experimental data (Lehmann, 1998). The
Wilcoxon rank-sum test was employed for comparisons of two independent data sets (significance level denoted by $p_w$). For comparisons of more than two conditions we used the Kruskal-Wallis test (significance level denoted by $p_{K-W}$), a nonparametric alternative to the analysis of variance (ANOVA). For the data in Figures 4 and 5, were there are multiple cell types across several conditions, we used a 2-way ANOVA to look for main effects, then verified the results with a Kruskal-Wallis test across cell types on the instantaneous (0 ms) condition and across transition durations for each cell type. Multiple comparison testing was done using Tukey's honestly significant difference (HSD) criterion to determine pair-wise differences between recording types (significance level denoted by $p_{HSD}$). Due to the low number of LMCs that we were able to stably record ($n=3$), we do not have the power to detect small differences in tests involving those recordings.

**Simulations** Simulations were performed using a compartmental model of the LGMD in the NEURON simulation environment (Fig. 6A; Hines and Carnevale, 1997). The compartmental model is based on that described in (Peron et al., 2009). Briefly, it has a spike-initiation zone (SIZ) segment containing potassium and sodium conductances of Hodgkin-Huxley (HH) type, as well as a voltage-gated calcium conductance and a calcium-sensitive potassium conductance mediating spike frequency adaptation. The SIZ is connected at one end with an axon containing HH type channels, and at the other end with a passive excitatory dendritic tree (Peron and Gabbiani, 2009a). An earlier model by Wang (1998) served as the basis for the functional form and parameters of the channels, with constraints specific to the LGMD derived from our previous work (Gabbiani and Krapp, 2006; Peron et al., 2007). For further details on this modeling aspect, see Peron and Gabbiani (2009a, Supplementary Material). The rake-shaped dendritic tree, simplified from the actual anatomical shape, is where it receives retinotopically-mapped excitatory input (Krapp and Gabbiani, 2004; Peron et al., 2009). A square region of visual space from -50 to +50 deg elevation and 40-140 deg azimuth was mapped onto this dendritic tree, which has 20 straight dendritic
branches, each 20 compartments in length. Excitatory input elevation is mapped across dendrites, while azimuth is mapped along each dendrite’s length, with frontal inputs arriving more distally and posterior ones arriving proximally. Inhibitory inputs were activated in the compartments immediately proximal to the intersection of the dendritic branches but distal to the SIZ. All synaptic inputs were modeled as synaptic conductances with the time course of an alpha-function (e.g., Chap. 2 of Gabbiani and Cox, 2010).

Spontaneous synaptic activity, both inhibitory and excitatory, was generated to reproduce the level of spontaneous noise observed in LGMD current clamp recordings. The visually driven excitatory synaptic input during looming was generated using luminance changes resulting from a looming stimulus sweeping across a simulated array of facets with realistic sampling of visual space, with six synapses per facet (Krapp and Gabbiani, 2004). Each facet had a 2D Gaussian receptive field (SD = 3/4°) over which it integrated stimulus luminance. The single facet stimulation experiments were used to set the parameter values (magnitude, latency, and jitter) for individual synaptic inputs of the model. These parameters were dependent on the luminance change duration at individual facets, fitted to experimental data. In the fly, photoreceptor response dynamics have been reported to vary with location on the eye (~20%; front vs. back and side; Burton et al., 2001). Our photoreceptor recordings were carried out on the side of the eye, which also receives the bulk of inputs caused by looming stimuli approaching from the side in our simulations. Thus, slight variations in photoreceptor responses as a function of eye location are not likely to affect our modeling results. Inhibitory synaptic inputs had a time course identical to that of the area (number of facets) covered by the looming object. They were triggered with a constant delay of 70 ms after the stimulus luminance reached its midpoint at each model facet, roughly 5 ms longer than the minimal excitatory input delay. This delay is consistent with experimental inference of inhibitory timing (Gabbiani et al., 2005). Their magnitude was constant throughout the stimulus, set to produce looming responses that had similar firing rates and peak times as those in experiments. Inhibitory synaptic strength variability was set to have a SNR of 20 and jitter of 10 ms. Simulations were run 50-500 times to accurately determine the variability in
the responses. For simulations in Figure 8 without temporal jitter, a set of synaptic timings was generated and held constant for groups of 50 repetitions, for which response variability was measured. The results were then averaged for ten groups of simulations and across $I/|v|$ values. Model output was processed in the same way as experimental data. 95% confidence intervals were obtained for measurements of the model’s responses by bootstrapping (resampling 5,000 times). Code for reproducing the model and figures using model data will be deposited on ModelDB (http://senselab.med.yale.edu/modeldb).

Results

We wished to quantify the variability of neural responses throughout the excitatory visual pathway leading to the LGMD (Fig. 1A). Therefore, we recorded intracellularly from three cell types in the optic lobe of the locust: photoreceptors, large monopolar cells (LMCs) of the lamina, and the excitatory dendritic field of the LGMD itself in both current and voltage clamp configurations. This allowed us to assess neural variability in LGMD responses and – directly or indirectly – at each stage in the pathway giving excitatory input to the LGMD. The intracellular recordings were selected specifically for their stable resting membrane potentials and responses for the duration of data collection. The data come from 7 photoreceptors, 3 LMCs, 22 LGMD neurons, and 28 extracellular DCMD neurons recorded in the locust Schistocerca americana.

Different analyses of the data described in this paper have been previously published (intracellular LGMD recordings, Gabbiani et al., 2002; other recordings, Jones and Gabbiani, 2010).

Spontaneous membrane potential variability

We first established a baseline for the variability levels by quantifying the spontaneous
membrane potential noise present in the neurons. The measurements were taken from the 400 ms period immediately preceding each visual stimulus presentation (for a total of about 24 s per individual cell). We then compiled distributions of the membrane potential relative to rest for each recording (Fig. 1B-E). Mean spontaneous noise levels in LMCs were about twice that of photoreceptors (standard deviation pooled across experiments, SD = 0.43 mV and 0.24 mV, respectively; corrected for electrode noise; SD = 0.40 mV and 0.19 mV, respectively) while in the LGMD it was also about two times that of the LMCs (SD = 1.05 mV pooled across experiments; unchanged after correction for electrode noise). The spontaneous noise levels for each cell type were significantly different from each other (p_{RS} < 0.02). The membrane potential ($V_m$) distributions of photoreceptors and LMCs are quite symmetric (Fig. 1B and C), while those from the LGMD are skewed in the direction of excitatory events, which is evident in the membrane current ($I_m$) and $V_m$ traces (Fig. 1D and E). Ambient light levels during these recordings were at dim daylight levels (see Methods), so the noise levels reflect daytime conditions with an unchanging light level. Our results for photoreceptors and LMCs were generally consistent with earlier results obtained at similar light levels in photoreceptors (locust: Faivre and Juusola, 2008, Fig. 4A; fly: Burton and Laughlin, 2003) and in fly LMCs (Laughlin et al., 1987, Fig. 9). From these recordings, we find that spontaneous $V_m$ noise levels increase along the excitatory pathway leading to the LGMD.

**Membrane potential variability to single facet visual stimulation**

The unit of spatial resolution in the locust visual system is the single ommatidium (facet), with each of the eight underlying photoreceptors transducing the same light signal through a
single light collecting structure, the fused rhabdom (Shaw, 1968; Land and Nilsson, 2002).

Moving edges in the visual world cause luminance changes in each photoreceptor’s receptive field whose duration depends on the speed of motion (Jones and Gabbiani, 2010; Burton and Laughlin, 2003). To understand the variability caused by such stimuli in photoreceptors and downstream neurons, we repeatedly presented luminance decreases localized over single facets with a range of durations, as would result from dark edges moving through photoreceptor receptive fields at different speeds (top panels of Figure 2A, B). These stimuli thus mimic the range of speeds experienced by photoreceptors across the retina during a looming stimulus, since the angular velocity of its edges increases rapidly near projected collision (Gabbiani et al., 1999; Jones and Gabbiani, 2010). In LMCs, the same stimuli will stimulate the center of their receptive field, which can be expected to play a dominant role in their response to looming stimuli based on the detailed analysis of their properties available in flies (Dubs, 1982; Srinivasan et al., 1982; Shaw, 1984; Laughlin, 1994). In the LGMD, each such stimulus represents an elementary component of a looming stimulus, allowing assessment of the impact that their spatio-temporal integration has on its membrane potential and firing rate noise.

As illustrated in the middle panel of Fig. 2A, photoreceptor membrane potential changes tracked changes in light levels and hyperpolarized to luminance decreases. The slopes of their membrane potential changes depended on the duration of the luminance change (Supplementary Figure 1A-C; Jones and Gabbiani, 2010). Under our recording conditions, LMCs are also graded potential neurons, with largely transient responses that are of the opposite polarity to those of photoreceptors (Laughlin et al., 1987; Juusola et al., 1995). They thus depolarized in response to the same luminance decreases and the size of this transient response also varied with the duration
of the luminance change (Supplementary Figure 1D-F; Jones and Gabbiani, 2010). The LGMD responded with transient depolarization and sometimes spiking to both single facet luminance increases and decreases (Fig. 2B, middle panel; Supplementary Figure 1G-I). The synaptic currents recorded in voltage clamp to the same stimuli were also transient and excitatory (Fig. 2F of Jones and Gabbiani, 2010).

In order to examine the temporal profile of the variability in each cell’s responses, we computed the inter-trial standard deviation of the response for each point in time. We then averaged these traces across all recordings of each type. The bottom panels of Figure 2A, B shows the resulting response SD in photoreceptor and LGMD recordings. The inter-trial SD starts at levels slightly greater than those measured for the spontaneous period. In both cell types, variability increases with the strength of the response. To quantify the relationship between membrane potential and variability, we further plotted these two measures against each other for each point in time during the stimulus presentation. Figure 2C shows this analysis for LGMD recordings. The black cloud shows each time point, with the blue line showing the mean variability as a function of membrane potential in 1.25 mV bins. Figure 2D shows the mean variability for our photoreceptor, LMC, and LGMD recordings. This analysis reveals that the LGMD variability increases as a function of response magnitude more quickly than in photoreceptors or LMCs. The differences in baseline variability are also evident in the vertical positions of these relationships. We thus conclude that membrane potential noise in response to single facet visual stimulation also increases along the excitatory pathway leading to the LGMD. Signal to noise ratios (SNRs) of specific response features along the excitatory pathway
While membrane potential change and variability are correlated, we cannot tell from the previous analysis what aspects of the responses are variable. In addition, our goal is to focus on those aspects that are important for the encoding of looming stimuli under the bright light, high contrast conditions characterizing our looming stimuli. Based on these premises, we measured several features of single trial responses selected for their relevance to the encoding of looming stimuli along the pathway. In photoreceptors we measured the slope of the response, since it carries information on edge speed that is extracted in the peak LMC responses (Juusola et al., 1995; Jones and Gabbiani, 2010). Accordingly, we also measured $V_r$ peaks of LMCs, as well as $V_m$ and $I$ peaks of the LGMD. The timing of single facet responses synchronizes inputs impinging onto the LGMD and plays a role in its tuning to looming stimuli (Jones and Gabbiani, 2010). We thus measured the response onset in photoreceptors, and the timing of peak responses in LMCs and the LGMD. Finally, the width of the single facet response is one of the factors that determine their summation within the LGMD. We therefore measured the full width at half height (FWHH) for the LMC and LGMD responses, as well as the response duration for photoreceptors. Examples of single trial traces and these measurements are given in Figure 3A. For each stimulus condition we then computed a mean, standard deviation, and signal to noise ratio, $SNR = mean/SD$, for each of these measures. We then compared these quantities across the different stages of the LGMD visual pathway to track changes in the encoding of their associated features. The signal to noise ratios we define also characterize performance in related signal detection tasks (e.g., Chaps. 24 and 25 of Gabbiani and Cox, 2010; or Chap 4 of Wickens, 2002), although this is not central to the subsequent analysis.

The normalized response strengths (slope or peak) for each condition are shown in Figure
3B and the corresponding SNRs in Fig. 3C. Response strength falls off as the single facet luminance changes become slower, with photoreceptor slopes dropping off more sharply than responses in downstream cells. The dependence of response strength on luminance change duration for voltage clamp and current clamp measures in the LGMD are quite similar. The SNRs of response strengths to single facet stimuli are smaller for recordings further along the visual processing pathway (2-way ANOVA, p = 0), and individual comparisons yield significant differences between photoreceptors and the LGMD I_m and V_m (p_{FSD} < 0.05). Photoreceptor slope SNRs were typically in the range of 10-25 (median = 22.9, across all conditions), whereas the SNR of LGMD response peaks were on average ~5 (median = 4.0). The peak SNR, however, was not statistically dependent on the speed of the luminance change for any recording type (p_{cov} > 0.36).

To consider the possibility that this observed SNR difference might merely be due to the response feature measured (slope versus peak) in different recording types, we calculated the slope SNRs for our LMC and LGMD V_m responses. We did not find higher SNRs (median = 3.1 and 1.2 for LMC and LGMD, respectively). Similarly, calculating the SNR based on response peaks for photoreceptors yields very similar values as for the slope (median peak SNR = 22.6).

The magnitudes, variability, and SNRs of the other response features, timing and width, are shown in Figure 4. These SNRs, like those calculated from response magnitudes, provide a useful metric of how reliable their associated response features are. The two basic questions that we wished this data to answer were: 1) Does the SNR/variability of these features change through the visual pathway? If so, between which cell types? And, 2) Does the SNR/variability change with the luminance change duration? We find that the SDs of response timing and width (Fig 4B, E) increase through the visual pathway, and that the SNRs of response timing and widths (Fig 4C, F)
change as well (across cell types: $p_{KW}=1.9\times10^{-3}$ for the SD of response timing at instantaneous luminance changes; response width SD: $p_{KW}=3.3\times10^{-4}$; timing SNR: $p_{KW}=6.6\times10^{-10}$; response width SNR: $p_{KW}=1.9\times10^{-3}$). For both features, photoreceptors had lower levels of variability and higher SNRs than both LGMD $I_m$ and $V_m$ ($p_{HSD} < 0.05$). The LGMD $I_m$ and $V_m$ did not exhibit any significant differences with each other, and individual tests did not find differences with LMCs except for the LGMD $I_m$ in the case of response timing SD and SNR ($p_{HSD} < 0.05$).

Response timings and widths increase with luminance change duration for photoreceptors and the LMCs (Fig. 4A and D). LGMD response timings increase with luminance change duration, but response widths are constant. We observe a large dependence on both cell type and luminance change duration for the variability in response timing (Fig 4B). Response timing variability is dependent on luminance change duration for all recording types (LMC: $p_{KW}=0.01$, others: $p_{KW} < 4.71.7\times10^{-6}$). The SNRs of both measures (Fig. 4C and F) were in most cases unchanged across the range of luminance change durations, the only exception being the SNR of the LGMD $I_m$ response width ($p_{KW} = 1.7\times10^{-4}$).

**LGMD SNRs vary little with stimulus type**

The LGMD has a much larger receptive field than cells earlier in the visual pathway and responds much more robustly to its preferred looming stimuli than to single facet stimuli. Thus, there is the possibility that it encodes its preferred stimuli more reliably, resulting in looming responses having higher SNR values than those to single facet stimuli. To examine this possibility, we compared the SNRs of LGMD responses to single-facet stimuli (0 ms condition) with those evoked by two types of stimulated approach, looming and pseudo-looming stimuli (see Methods).
Both of these stimuli activate much larger portions of the retina and contained accelerating
motion, for which the LGMD is selective (Simmons and Rind, 1992; Peron and Gabbiani, 2009b;
Jones and Gabbiani, 2010). This comparison is shown in Figure 5. The looming and pseudo-
looming stimuli had slightly different \( l/|v| \) values, so we grouped the responses into two speed
categories, very rapid approaches and slower ones (\( l/|v| \approx 10 \) and 40 ms, respectively). Since these
stimuli produced significant LGMD spiking, we were able to compute the peak SNR for both the
instantaneous firing rates and the underlying \( V_m \), allowing us to contrast them.

The peak SNR values for firing rates were similar across stimulus types both for fast and
slow approaches and were similar to those of the single-facet \( V_m \). The SNRs for looming \( V_m 
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responses were higher than for single facet responses (slow: \( p_{RS} = 0.017 \), fast: \( p_{RS} = 0.008 \)), but also
higher than those for looming firing rates (slow: \( p_{RS} = 0.04 \), fast: \( p_{RS} = 0.002 \)). Consequently, the
peak SNR of the LGMD spiking output was no different than the peak SNR of strong single facet
responses (slow: \( p_{RS} = 0.48 \), fast: \( p_{RS} = 0.27 \)). There was no significant elevation in the peak SNR
values of pseudo-loom responses (\( V_m \) or IFR) relative to single-facet responses. Thus, the LGMD's
preferred stimuli, which activate many single facets, do not evoke more reliable spiking responses
than the membrane potential deflections resulting from single facet inputs.

Modeling LGMD looming responses

We built a model of the LGMD visual pathway to address the following question: given the
variability observed in individual neurons to the single facet signals, how reliable should the
LGMD responses to looming stimuli be? The model consisted of a realistic sampling of visual
space, which was used to trigger a set of appropriately timed and weighted synaptic inputs, based
on the luminance time course at each model facet, to a compartmental model of the LGMD. This model had an elaborated, though simplified, dendritic structure (Fig. 6A; Peron et al., 2009).

Spontaneous and visually evoked inputs were modeled independently. Spontaneous activity was randomly generated at a constant rate, excitation and inhibition balanced, to produce the level of spontaneous membrane variability found in vivo (Fig. 6B). We determined the appropriate level of excitatory drive by simulating single facet visual stimuli and adjusting the synaptic strengths to produce responses evoking one or two action potentials, as in many of our recordings (Fig. 6B).

Variability of the synaptic input strength and timing for each facet was also chosen to closely match the single facet responses observed in vivo.

We next constructed a set of simulated looming evoked responses (Fig 6C). The pattern of synaptic input to looming stimuli was determined using the properties of single facet responses recorded in the LGMD under voltage clamp (Figs. 3 and 4). Specifically, excitatory inputs were triggered by luminance changes beginning at each individual facet, with magnitudes and latencies that were variable throughout the loom and based on the speed of luminance changes occurring at each individual facet in the model. Inhibitory input latencies and magnitudes were constant throughout the stimulus, triggered with a constant delay after the stimulus luminance reached its midpoint. The variability in the excitatory synaptic parameters was set to match that observed during single facet stimulation (Fig. 6B), and the inhibitory variability was set to similar levels.

Further details of the model are given in Methods. This pattern of synaptic stimulation preserved stimulus induced correlations between different facet inputs to the LGMD, which have been shown to play a role in its tuning to looming stimuli (Jones and Gabbiani, 2010). It does not take into account possible interactions between adjacent facet inputs, but such interactions have not
been found to impact LGMD responses (Jones and Gabbiani, 2010). Another dynamic lateral
inhibitory interaction (O’Shea and Rowell, 1975) is also likely to play a minor role in the
responses of the LGMD to looming stimuli (Gabbiani et al., 2002).

We found that this simple input structure produced responses that well matched those of
the LGMD. The response time course follows the same rise and fall in firing rate, with the peak
time linearly related to the size to speed ratio \( \beta / |v| \) of the stimulus (Fig. 6D). Also, the variability
in the timing of the peak firing rate increases with \( \beta / |v| \), consistent with DCMD recordings (Fig.
6F; Gabbiani et al., 1999). The range of \( \text{SD}_{\text{peak time}} \) values observed in model responses was also
similar to those observed in vivo. The \( \text{SNR}_{\text{peak}} \) of the response \( V_m \) and IFR depart slightly from the
data: they are about twice as high as those observed in vivo. The relationship between the two is
however the same, with the \( \text{SNR}_{\text{peak}} \) of the \( V_m \) being higher than the \( \text{SNR}_{\text{peak}} \) of the IFR (Fig 6E).

We thus conclude that the model adequately reproduces the LGMD responses to looming stimuli.

**Impact of input variability on model output**

The model includes temporal jitter in the synaptic inputs to the LGMD, as observed in the
single facet response data (Fig. 5A). For instantaneous luminance changes, the variability of the
LGMD \( V_m \) peak time was 3.9 ms (median), therefore we set the minimum jitter of the synaptic
inputs to produce a similar jitter in single facet simulations (\( \text{SD}_{\text{jitter}} = 6 \) ms). This peak time
variability increased when the luminance changes were slower (Figure 4B), and we incorporated
this into our model using the fitted slope of the linear relationship between the \( \text{SD}_{\text{peak time}} \) and the
luminance change duration, \( (0.19; \text{ dimensionless since both variables have units of time}) \). If we
ignore the experimental relationship, instead setting the synaptic jitter to be constant and low
throughout the looming stimulus, then the model fires bursts of spikes early in the stimulus (Fig. 7A). This contrasts with the gradual buildup seen in vivo. Such bursting behavior is consistent with LGMD responses to a modified looming stimulus in which luminance changes early in the stimulus occur more quickly than in a normal looming stimulus (‘constant-rate looming’ in Fig. 4 of Jones and Gabbiani, 2010).

We also included variability in synaptic input strengths, as might arise from variability in quantal number, quantal size, stochastic receptor activation, or other synaptic transmission parameters. We initially set the variability of the excitatory synaptic strength (SD_{syn} = mean_{syn} /SNR_{syn}) to be roughly equal to that of the LGMD single facet responses, SNR_{syn}=5. However, the model’s responses are quite insensitive to variability in the underlying synaptic input strengths, as shown in Figure 7B and C. Varying the parameter SNR_{syn} caused a significant change in the reliability of single facet responses (data not shown), but did not cause a significant change in any of the aspects of the model’s response to looming that we examined. This insensitivity can be partially explained by the number of synaptic inputs the model receives. The model LGMD, at the time of its firing rate peak, receives ~40 independently variable synaptic inputs, resulting in the trial mean input being ~6.3 (√40) times less variable than the individual inputs.

To further dissect which sources of variability contributed to the output variability of our model, we ran the simulations while eliminating specific sources of variability (Fig. 8). Eliminating variability in the excitatory synaptic strength (SD_{syn} = 0, ’F-Exc Gsyn’) changed the SNR_{peak} and SD_{peak time} measures very little, consistent with responses being insensitive to the value of SNR_{syn}. Keeping the synaptic timings constant (’F-Exc Jitter’) made a much larger difference which contributed most of the decrease in variability observed when both the synaptic strength
and timing variabilities were eliminated ('F-Exc Var'). Taking away inhibitory input variability
('F-Inh Var'), of both synaptic timing and strength, also had a relatively small effect on the
model's output variability. The strongest source of variability came from the spontaneous activity
('Spontaneous Only'), accounting for about 35% of the $SD_{\text{peak time}}$ and capping the $\text{SNR}_{\text{peak}}$ at about
150% of its value in the full variability model. Thus, synaptic strength variability levels within the
range of those observed \textit{in vivo} are not important for determining looming response SNR in the
model. Instead, the firing rate SNR and variability in the timing of the peak is determined in a
large part by the ongoing spontaneous activity and jitter in the timing of stimulus driven inputs.

Discussion

We have shown that both spontaneous and response variability increase along the visual
processing pathway that includes the LGMD and DCMD. The reliability of several aspects of
responses drops, including strength, width, and timing. The variability of response timing also
depends on luminance change duration, a single facet proxy of edge speed. Increased luminance
change duration, as would result from a slower moving stimulus, evokes responses with greater
temporal jitter. At the level of the LGMD, summation of many inputs does boost response
reliability, but spiking output from the neuron is no more reliable than the underlying $V$, from
single facet inputs. Modeling the LGMD excitatory input structure as simply reflecting the
properties of single facet luminance changes, with inhibition corresponding to stimulus size,
recreates many of the LGMD’s looming response properties. Manipulating single sources of
variability in the model identify jitter in LGMD excitatory inputs and spontaneous activity as the
main drivers of LGMD’s spike rate variability.
Increase in spontaneous noise through the visual pathway

We observed that the spontaneous membrane potential gradually became more variable as we recorded farther along the visual pathway. The membrane noise increased from the photoreceptors to the LMCs. Each LMC receives input from 6 photoreceptors, which might cancel out a portion of photoreceptor noise, assuming their independence and adequate averaging at the level of LMCs. Although locust photoreceptors are electrically coupled within a single ommatidium, the 'bumps' resulting from individually resolved photon absorption events are uncorrelated in dark-adapted conditions, suggesting functional independence (Lillywhite, 1977; 1978). Additionally, in flies electrical coupling between photoreceptor terminals within a lamina cartridge has been suggested to reduce coupling due to extracellular potential changes (Weckström and Laughlin, 2010) by allowing changes in intracellular potential to match those in the extracellular space (van Hateren, 1986). However, detailed studies of synaptic transmission at the photoreceptor-LMC synapse in the fly have shown it to have a high gain (Laughlin et al., 1987; Juusola et al., 1995). Further, LMC cutoff frequencies are higher than those produced by photoreceptor transduction noise, meaning that both the signal and noise are subject to amplification (Laughlin et al., 1987). These factors may contribute to the noise increase observed in our experiments. We also found spontaneous noise to be larger in the LGMD than in LMCs. This most likely results from the relatively high median size of single spontaneous excitatory postsynaptic membrane potentials deflections (~0.75 mV; see Fig. S5 in Peron et al., 2009).

Decrease in single facet SNR through the visual pathway
We observed that the SNRs of single facet visual responses decreased markedly as we recorded further from the visual periphery. This was true when measuring response peaks, timings, and widths (Figs. 3 and 4). These SNR decreases were mostly evident in comparisons between LGMD recordings and those from photoreceptors. This does not however preclude a modest change in SNR between photoreceptors and LMCs, since we were able to obtain only a small number of stable LMC recordings despite much effort (3 cells from 60 animals). The reason for their extreme difficulty is unclear; to our knowledge, their are only two previous reports of locust LMC recordings prior to the ones carried out here (Shaw, 1968; James and Osorio, 1996). Although the paucity of data from LMCs warrants caution in interpreting the results, their overall trend well fits the pattern seen along the pathway from photoreceptors to LGMD current clamp data.

Studies in the fly visual system concluded that the SNR of their LMCs is higher than those of single photoreceptors due to an averaging of photoreceptor variability (~160% in light-adapted conditions; Fig. 10 of Laughlin et al., 1987; Fig. 3 of de Ruyter van Steveninck and Laughlin, 1996). In these studies, the SNR was either defined from sensitivity measurements for contrast steps eliciting responses close to membrane potential noise threshold (Laughlin et al., 1987), or through time-averaged wide-band random stimuli optimized to maximize information rates (de Ruyter van Steveninck and Laughlin, 1996). Whereas these studies characterized contrast coding (in two very different regimes), the present study used light steps at a fixed maximal contrast with time-varying transition speeds. Thus, our SNRs describe how reliably the luminance transition speed or duration is conveyed by various features of the neural responses at successive stages of the visual system, and cannot be directly compared with the SNRs of these studies. Furthermore,
the SNR improvement observed in fly LMCs may originate from independent photoreceptor
sampling related to the neural superposition structure of the eye (Braitenberg, 1967; Kirschfeld,
1967). The impact of the apposition structure of the locust eye and of intra-facet photoreceptor
coupling (Shaw, 1967; 1969; Lillywhite, 1978) on signal improvement at the level of the LMCs
remains to be determined.

We did not see any change in SNR between $I_m$ and $V_m$ within the LGMD. We know that
there are active conductances in the LGMD dendrites open around the resting potential, such as
hyperpolarization activated cation channels (Dewell and Gabbanini, unpublished observations).
This suggests that these conductances do not introduce significant amounts of noise.

One potential source of the decrease in SNR observed throughout the pathway is the
transition from graded potential to spiking neural responses at the level of the medulla. Such a
decrease would be consistent with the fact that information rates measured in fly photoreceptors
and LMCs are much higher than those found in spiking neurons (de Ruyter van Steveninck et al.,
1997; see also DiCaprio et al., 2007 for a graded potential/spiking neuron comparison in another
system). Confirmation of an eventual SNR decrease at that level will have to await
electrophysiological recordings from medullary neurons synaptically connected with the LGMD.
Such recordings have not proven practical up to this point, but would clearly provide critical
information on the functioning of the excitatory pathway leading to the LGMD.

Analogous decreases in SNR, or increases in variability, through multiple stages of a visual
pathway have been observed in other systems. Kara et al. (2000) observed that the Fano Factor
(FF), i.e., the ratio of across-trial spike count variance to spike count mean, increases from the
retina to the lateral geniculate nucleus to primary visual cortex. That study, while reporting
cortical FFs that were lower than many others in the literature, measured equivalent SNR values lower than what we observe in the LGMD single facet responses (2-3.75 at ~40 Hz firing rate). It has also been found that along the pathway from cones to bipolar cells to retinal ganglion cells (RGCs), sensitivity to small luminance changes declines through the pathway, approximately 4-fold at each stage (Borghuis et al., 2009). These declines were mainly attributed to fluctuations in synaptic vesicle release at both synapses and spike generation within the RGCs. Decreases in SNR through a processing pathways are not inevitable, however, with various studies describing behavior that seems to be limited by noise present in the sensory system, far from motor outputs (Osborne et al., 2005; Churchland et al., 2006).

Variability of looming responses

The amount of variability observed in neuronal responses can depend on the parameters of the input it receives (Faivre and Juusola, 2008). These factors include how strongly the stimulus drives the neuron (Tolhurst et al., 1983; Faivre and Juusola, 2008) and stimulus dynamics (Warzecha et al., 2000; but see Schaette et al., 2005). In the LGMD/DCMD, variability has earlier been analyzed mainly in the dark-adapted state, to very different stimuli that those used here (Barker, 1993). We found that the peak SNR of the membrane potential in response to looming stimuli is larger than to single facet stimuli. Since looming stimuli activate the LGMD much more robustly than single facet stimuli, it is not entirely surprising that the SNRs of looming responses are larger than those of single facet responses. However, the peak SNR for looming stimuli was unchanged when considering firing rates instead of $V_m$. This suggests that the spike generation process within the LGMD introduces additional noise, at least during looming stimuli. Variability
in spike threshold is a possible source of this additional noise (Azouz and Gray, 1999; Chacron et
al., 2007). Consistent with this idea, both the LGMD and our model exhibit spike frequency
adaptation (SFA), which dynamically shifts the cell’s f-I curve based on past spiking. Indeed our
model LGMD responses reproduce this drop in SNR from $V_m$ to firing rate and removing SFA
from the model, by removing the $K_{Ca}$ current, yields SNR values for the IFR that are higher than
those for the $V_m$ (data not shown).

Looming responses in the LGMD result from the summation of thousands of inputs over
the course of the stimulus. The number of inputs activated over time, both excitatory and
inhibitory, grows due to both the expanding area of the stimulus and increasing angular speed.
Using a compartmental model of the LGMD allowed us to study how variability in the individual
inputs shapes the variability of the LGMD response. These simulations show that, surprisingly,
the variability in the strength of individual facet inputs do not play a strong role in determining
the SNR of the LGMD peak firing rate or peak membrane potential. Instead, the looming
variability is largely determined by the variability from spontaneous input arriving onto the
LGMD and temporal jitter of the excitatory inputs.

The model allowed us to examine the importance of LGMD input timing variability in
shaping the looming responses by running the simulations with and without a luminance change
duration dependent jitter. We observe in our single facet responses that the variability in response
latency from trial to trial increased with luminance change duration (Fig 4). Thus, early on during
an object’s approach (or a simulated approach) the angular velocity of its edges is slow, and the
object takes a long time before it’s edge reaches a new photoreceptor’s receptive field (RF), since
locust photoreceptor RFs are $\sim 3^\circ$ wide with centers separated on average by $1-2^\circ$ (mean $= \sim 1.5^\circ$;
Krapp and Gabbiani, 2004). The high amount of jitter in the synaptic inputs to the LGMD originating from different facets allows the LGMD’s firing rate to climb smoothly, rather than evoke a series of bursts triggered by synchronous inputs when the stimulus reaches new visual fields. Supporting this notion, subtle bursting to stimulus changes can be observed in LGMD responses to “constant-rate” looming stimuli, a variant of a looming stimulus that stimulates each facet of the eye with a roughly equal duration luminance change that should evoke LGMD excitation with less temporal jitter than normal looming. If such bursting were to occur in vivo to looming stimuli, escape behaviors would likely be grossly mistimed (see below).

**Relationship with behavior**

It was recently shown that trial-to-trial variability in the LGMD/DCMD spike trains affect jump escape responses evoked by looming stimuli (Fotowat et al., 2011). Specifically, the timing of the locust’s jump correlates strongly with the time of DCMD peak firing rate such that jumps occur approximately 70 ms after the DCMD reaches its peak. Additionally, after the onset of the energy storage phase preceding the jump, called co-contraction (Burrows, 1996; Santer et al., 2005), both the DCMD spike count and the regularity of DCMD spiking are predictive of the animal jumping. Thus, the stimulus speed dependence of temporal jitter contributes to the production of DCMD firing patterns capable of driving downstream motor neurons important for jumping. Also, the timing of the co-contraction is partially dependent on the DCMD reaching a firing rate threshold. Very bursty LGMD spike trains, like those of the model with constant jitter, are likely to cross that threshold at very different times than in the normal case, possibly evoking mistimed jumping if such spike trains were produced in intact locusts. Behavioral
experiments with stimuli designed to induce such bursting DCMD firing could be carried out to
further test this idea. Although there is strong evidence that the DCMD and its ipsilateral
homolog play an important role in the generation of jump collision avoidance behavior to
looming stimuli measured in the laboratory (Fotowat et al., 2011), other pathways will also likely
contribute to escape behaviors under more natural conditions, such as those involving giant wind
sensitive neurons (e.g., Chap 10 of Burrows, 1996).

In conclusion, we have shown that neuronal response variability through a visual pathway
in the locust increases, and that the SNR along the pathway for single facet signals decreases.
Summation of many of these inputs within the LGMD during looming boosts the SNR slightly,
but spike generation lowers it again. Modeling suggests that variability in the magnitudes of
individual synaptic inputs contributes little to trial-to-trial response variability. Instead, both
ongoing spontaneous activity and variability in the latencies of synaptic input shape LGMD
responses. Spontaneous activity influences the SNR of the LGMD output around its peak time,
while timing variability both enables a smoothly rising firing rate during looming and helps
determine the variability of the response peak, both attributes of the response important for
behavioral output. Taken together, these results suggest that noise, especially temporal response
variability, contributes to shaping the neural responses elicited by looming stimuli along this
sensory-motor pathway.

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Figure Legends
Figure 1. Spontaneous variability observed throughout an excitatory visual pathway involved in collision detection. A Schematic illustration of the pathway with its main anatomical and signaling properties. The excitatory input is successively relayed from photoreceptors to LMCs, which both respond in a graded manner under our stimulus conditions, and medullary T-cells before impinging on the large dendritic fan of the LGMD (green). The two smaller dendritic fields receive inhibitory inputs (red). B Top: two 400 ms long traces illustrating the spontaneous membrane potential variability of a typical photoreceptor. Bottom: histogram showing the distribution of membrane potentials for the same cell relative to the resting potential. C Spontaneous membrane potential traces and histogram for a recording from a large monopolar cell (LMC). D, E Similar traces and histograms of spontaneous membrane currents (voltage clamp) and membrane potentials (current clamp) in the LGMD. SD: standard deviation for the illustrated experiments.

Figure 2. Time course and dependence of variability on membrane potential during single facet stimulation. A, B Top traces show the stimulus luminance presented to a single facet over time. The luminance is initially bright and returns to its background (backgnd) value, causing the ‘off’ response along the visual pathway. Darker colors denote slower luminance changes; brighter are faster. The middle traces depict correspondingly colored mean photoreceptor (A) and LGMD (B) responses, averaged over all cells. The mean membrane potentials are given relative to rest. Photoreceptors are initially depolarized since the initial light level is above its background value. The bottom traces show the intertrial standard deviation of the responses, averaged across
recordings. C Response means and standard deviations for the LGMD membrane potential (from B) have been plotted against each other, with each black point showing a time sample (selected at 2.5 ms intervals). The blue line shows the average resulting standard deviation within 1.25 mV mean response bins, for bins that contain > 0.05% of the data. The dashed red line indicates the spontaneous noise level (Fig. 1E). D Same analysis as in C for photoreceptors (green), LMCs (red), and the LGMD V. (blue; replotted from C).

Figure 3. Variability and SNR of response features to single facet stimuli. A Representative traces for each cell type showing the trial-by-trial variability of responses to an instantaneous luminance decrease at a single facet (0 ms duration). The stimulus occurs at the time the traces begin. Trial averaged responses are shown in black and 3 individual trials in grey. Measured features are marked in red for each trial. For photoreceptor traces they are the response slopes, from 25%-75% of the response height. For LMC and LGMD traces they are the peaks (+) and response widths (full width at half-height, FWHH). The signal to noise ratio (SNR) is noted for the peak and slope features for the experiments shown. It is defined as the mean divided by the standard deviation of each measured feature. B Relative response strengths (photoreceptor slopes; peak heights for other recording types) to single facet luminance changes of varying duration. For each box plot, the central horizontal line denotes the median and the box extent depicts the 25th (p25) to 75th (p75) percentile data range. Whiskers show the range of data not considered outliers, and plus signs represent outliers, i.e., points more than 1.5(p75-p25) times away from lower (p25) or upper (p75) data quartile. Responses are normalized to the mean of the population distribution (at 0 ms duration) for each recording type (photoreceptors: 5.5 mV/10 ms; LMCs: 7.2 mV; LGMD L:
2.0 nA; LGMD V. : 9.3 mV). CSNRs as a function of luminance change duration for the recording types. Box plots as in B. The dashed line shows the mean SNR of the LGMD V. peak for pseudo- looming stimuli, a stimulus type that targets 45 facets in a pattern that mimics some aspects of a looming stimulus. There is a significant effect of recording type at 0 ms luminance change duration (asterisk and square brackets; \( p_{\text{corr}} = 0.0023 \), corrected for multiple comparisons using HSD criterion).

**Figure 4. Variability of response widths and timing.** A, D Distributions of latencies (photoreceptor response onset, response peak for other recordings) and response widths (FWHH) for different recording types. B, E Distributions of the standard deviation in each experiment for these measures. C, F Corresponding SNRs for the same measures. Box plot conventions as in Figure 4B.

**Figure 5. Comparison of LGMD variability for several stimulus types.** Distributions of SNR_{\text{peak}} values are shown for single facet, pseudo- looming, and looming stimuli. A Shows the values for looming stimuli with an \( |l|/|v| \) of 10 ms and pseudo- looming stimuli with an equivalent \( |l|/|v| \) value of 15 ms. The single facet SNR_{\text{peak}} distributions for 0 ms luminance changes is shown plotted for comparison. B Shows the values in response to slower approaching stimuli. Looming stimuli had an \( |l|/|v| \) of 40 ms for the IFR measurements and 30 ms for V. measurements. Pseudo- looming had an equivalent \( |l|/|v| \) of 50 ms. Pseudo- looming stimuli in A and B are the “fast” and “medium” speed stimuli described in Jones and Gabbiani (2010). Asterisks denote significance \( p_{\text{corr}} < 0.05 \). Box plot conventions as in Figure 4B.
Figure 6. Modeling LGMD responses. A Schematic of the active compartmental model of the LGMD used. Excitatory input impinges retinotopically onto the rake-shaped dendritic tree and inhibitory synapses are made onto the dendritic segment immediately proximal to the rake. The model is modified from the one used in Peron et al. (2009). Details can be found in Methods. B Single facet responses from the model. Short trains of synaptic conductances (3 events at 200 Hz) from 6 inputs are triggered at the marked onset time, in grey, with 4 ms temporal jitter. Individual synaptic conductances are drawn from a normal distribution with an SNR of 10 and a mean adjusted to obtain responses of a strength similar to those observed in vivo. Rasters are shown from 20 repetitions (top), the model Vm for 5 repetitions in grey with the mean model Vm in black (middle), and the SD of the membrane potential (bottom). Synaptic parameter values were chosen to closely match the strength and variability of responses in vivo. C Looming responses in the model. Top traces show the angular size of the square looming stimulus for three parameter values (θ/|v| = 10, 40, and 80 ms in green, red, and blue respectively). Middle shows spike trains from the stimulated LGMD for 100 simulations from each stimulus. Bottom shows the Gaussian-convolved instantaneous firing rates with envelopes showing the SD. D Relationship of the looming stimulus parameter and timing of the peak rate. Model peak time means and SDs (error bars) are shown (black) with the best-fit linear relationship (black dashed line; fit slope: 4.5, intercept -31.0 ms, angular threshold: 25.2°). The experimentally observed linear relationship in Jones and Gabbiani (2010) is shown in grey. E The SNR of the firing rate and Vm peaks as a function of θ/|v|. F The SD of the peak times for IFR and Vm. Error bars in E, F show bootstrapped
95% confidence intervals for plotted values.

**Figure 7. How input variability shapes LGMD model responses.** A Shows the spiking and instantaneous firing rates of the LGMD model if single-facet luminance change dependent jitter is removed. Instead, the synaptic times are jittered in the model by amount drawn from a normal distribution with an SD set at a constant value of 4 ms. B, C The variability of LGMD model responses is insensitive to changes in the variability of the underlying synaptic input strength. The SNR of the peak firing rate (B) and SD of the time of the peak firing rate (C) are shown for simulations where the synaptic input strength has different levels of variability, as parameterized by the SNR of the excitatory synaptic input strength (SNR\text{exc}). Darker lines indicate higher SNR values. Measurements using both the $V_a$ (orange-yellow) and IFR (grays) are shown. Values are compiled using 100 simulations per condition. Error bars show bootstrapped 95% confidence intervals on the values.

**Figure 8. Sources of variability in the LGMD model.** The model was run with specific sources of variability eliminated. 'Full' (F) refers to the model with all sources of variability. Other conditions eliminate variability in excitatory synaptic $g_{\text{exc}}$ (F-Exc Gmax), excitatory synaptic timing (F-Exc Jitter), both excitatory timing and $g_{\text{exc}}$ variability (F-Exc Var), the inhibitory variability (F-Inh Var), and all visually driven variability (Spontaneous Only). Variability was estimated for groups of 50 simulated approaches, and averaged over 10 groups. A The relative SNR\text{exc} resulting from elimination of sources of variability. The height of each bar shows the difference from the 'Full' condition, normalized by the difference between 'Full' and 'Spontaneous
Only. The SNR<sub>out</sub> (averaged over \( l/|v| \)) values of the 'Full' condition are 14.0 and 9.9 for V<sub>r</sub> and IFR, respectively, while the SNR<sub>out</sub> values for the 'Spontaneous Only' condition are 19.0 and 14.0.

The proportion that the SD<sub>out</sub> value is decreased from that of the 'Full' condition for each condition shown. Bar height indicates the difference from full model SD<sub>out</sub> for each condition, divided by the full model SD<sub>out</sub> value (SD<sub>out</sub> - SD<sub>out</sub>\textsuperscript{real}/SD<sub>out</sub>). SD<sub>out</sub> values for the full model were 38.8 and 45.5 ms for V<sub>r</sub> and IFR respectively (averaged over all 3 \( l/|v| \) values).
Jones and Gabbiani, Figure 1
Jones and Gabbiani, Figure 2
Jones and Gabbiani, Figure 3
Jones and Gabbiani, Figure 4
Jones and Gabbiani, Figure 5
Jones and Gabbiani, Figure 6
Figure 7

A. Plot showing firing rate over time relative to collision.

B. Graph depicting SNR peak against I/I/{v} with IFR and V_m.

C. Graph illustrating SNR_syn against I/I/{v} with peak timing SD.
Jones and Gabbiani, Figure 8