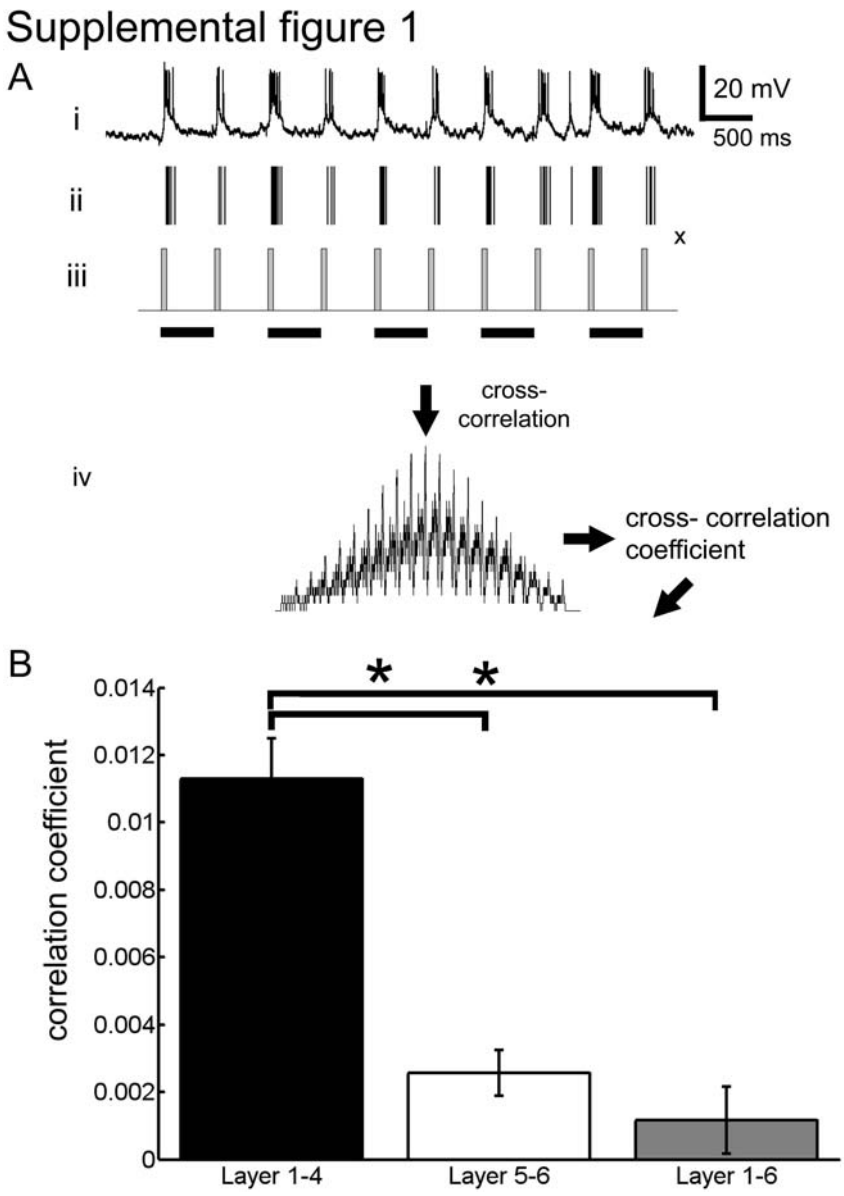
Supplemental Table 1. Classifications of the neurons based on how they respond to various stimuli.

Response type	Classification description
Significant visual response	A response two standard deviations above or below the mean background activity.
Broadly-tuned chromatic response	The same significant responses to all colors and color combinations
Narrow-tuned chromatic response	The significant responses only one or two colors
Color opponent chromatic response	Significantly different responses to the different colors and inhibition of one color response by the simultaneous presentation of another color
Non-directional motion sensitive response	Significant responses to motion regardless of direction
Directional motion sensitive response	Significant responses to a few directions of the motion stimuli over the others
Phasic-tonic response	Phasic initial responses followed by tonic activity during the light flash
Phasic	Phasic responses to the onset of the light flash
Tonic	Tonic membrane potential and spike rate increases during the light flash
On-off	Responses to the onset and offset of light flashes
Adaptation	Significant responses to the first light flash followed by reduced or no responses to the subsequent light flashes in a series of five light flashes

Supplemental Table 2. Numbers of neurons in each class displaying the listed properties.

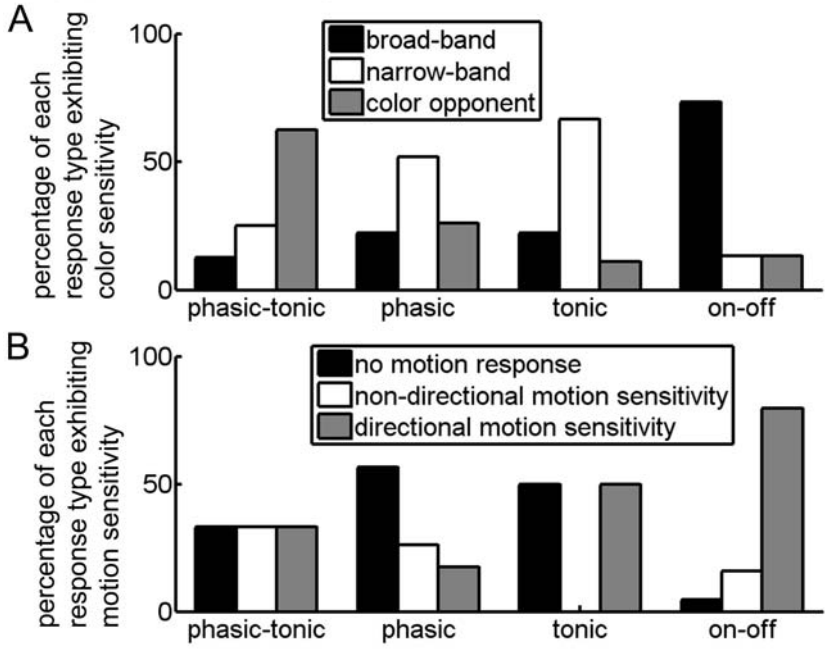
Categories	Layers 1-4 n=60	Layers 5-6 n=33	Layers 1-6 n=12
Number tested for color sensitivity	55	31	9
Broad-band sensitive	37	4	5
Narrow-band sensitive	13	12	3
Color opponent	5	15	1
Number tested for motion sensitivity	52	31	9
Non-directional motion sensitive	9	6	1
Directional motion sensitive	30	13	3
Not motion sensitive	13	12	6
Progressive motion sensitive	5	0	0
Regressive motion sensitive	7	3	0
Upward motion sensitive	9	0	0
Downward motion sensitive	9	9	1
Bidirectional progressive/regressive motion sensitive	0	0	1
Bidirectional up/down motion sensitive	0	1	1
Non-directional motion sensitive	9	6	1
Wide receptive field	14	1	5
Small receptive field	6	2	2
Spatially antagonistic receptive field	4	1	0
Receives blue, green and UV photoreceptor input	42	9	6
Receives only UV photoreceptor input	0	0	0
Receives only blue photoreceptor input	7	4	3
Receives only green photoreceptor input	0	1	0
Receives UV and blue photoreceptor input	2	10	0
Receives blue and green photoreceptor input	4	3	0
Receives green and UV photoreceptor input	1	1	0
Number tested for color and motion sensitivity	47	29	6
Both directionally motion and color sensitive	6	17	0
Number tested for adaptation and response types	58	29	11
Number of neurons exhibiting adaptation	8	20	4
Phasic-tonic response	5	3	0
Phasic	9	16	4
Tonic	2	5	4
On-off	42	5	3
Posterior superior optic tract projections	44	3	2
Posterior inferior optic tract projections	7	1	0
Anterior optic tubercle tract projections	2	8	6
Lobula optic tract projections	1	20	3
Intrinsic	4	0	0
unknown (fills out of lobula not complete)	2	1	1



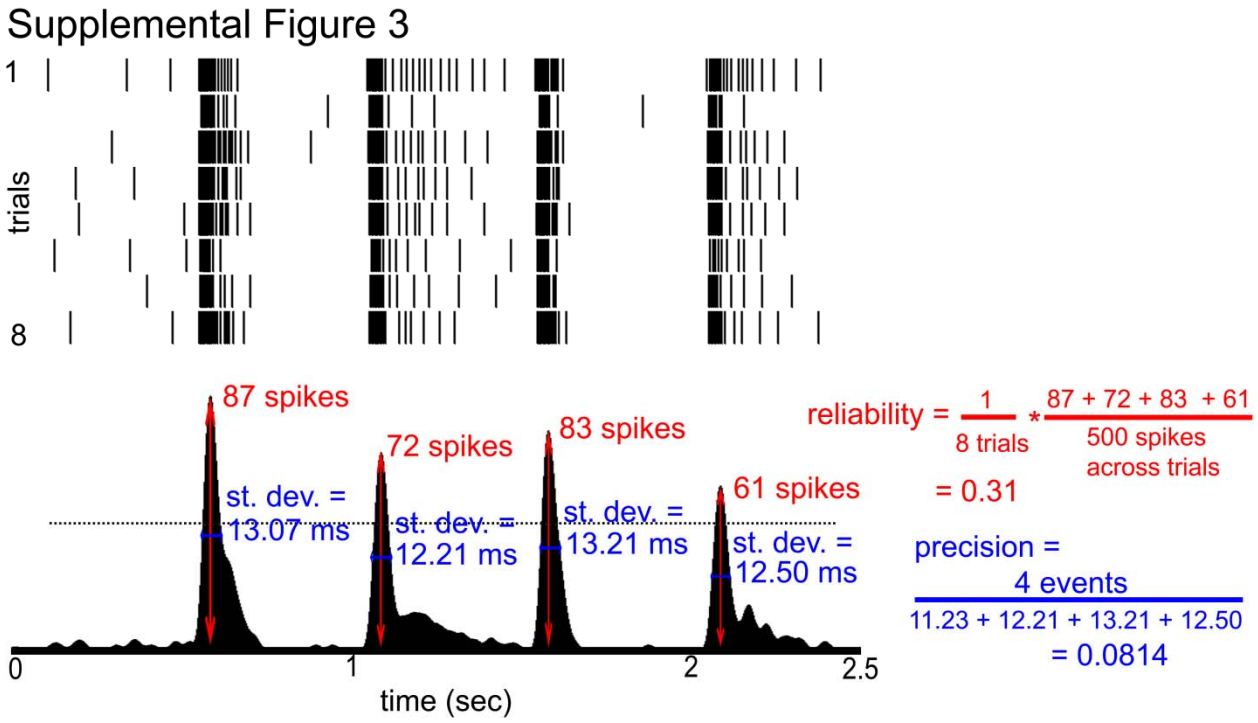
Supplemental Figure 1. Response types among the lobula layers. To confirm that there were differences in the temporal dynamics of the responses across the different layers of the lobula, we performed a cross-correlation analysis, comparing the spike times with a regular, artificial signal representing the onset and offset of the light flash stimulus (**A**). **A**. To determine how well-correlated the neural responses were to the onset and the offset of the light flashes, we performed a cross-correlation analysis of the spike times (**i** and **ii**) relative to an artificial signal

representing the onset and offset of the light flashes that included 50 ms pulses every 500 ms (**iii**). The light flashes are represented by black bars below the traces. From the intracellular recordings (**i**), we detected the spike times and represented them as rasters (**ii**). Black bars signify duration of the light flash. **B**. After performing the cross correlation (**iv**), we measured the correlation coefficient and performed statistical comparisons of the on-off correlation coefficients between responses from neurons in the different layers. We found the spike times of the Layer 1-4 neurons had significantly higher cross-correlation coefficients with the on-off signal compared to both the Layer 5-6 and the Layer 1-6 neurons (Layer 1-4 versus Layer 5-6: Mann-Whitney *U*-test statistic: 899; z-statistic: -4.3221; $p < 0.0001$; **B**). In other words, the Layer 1-4 neurons demonstrated clear on-off responses which was not as prevalent in the Layer 5-6 and Layer 1-6 neurons.

Supplemental Figure 2



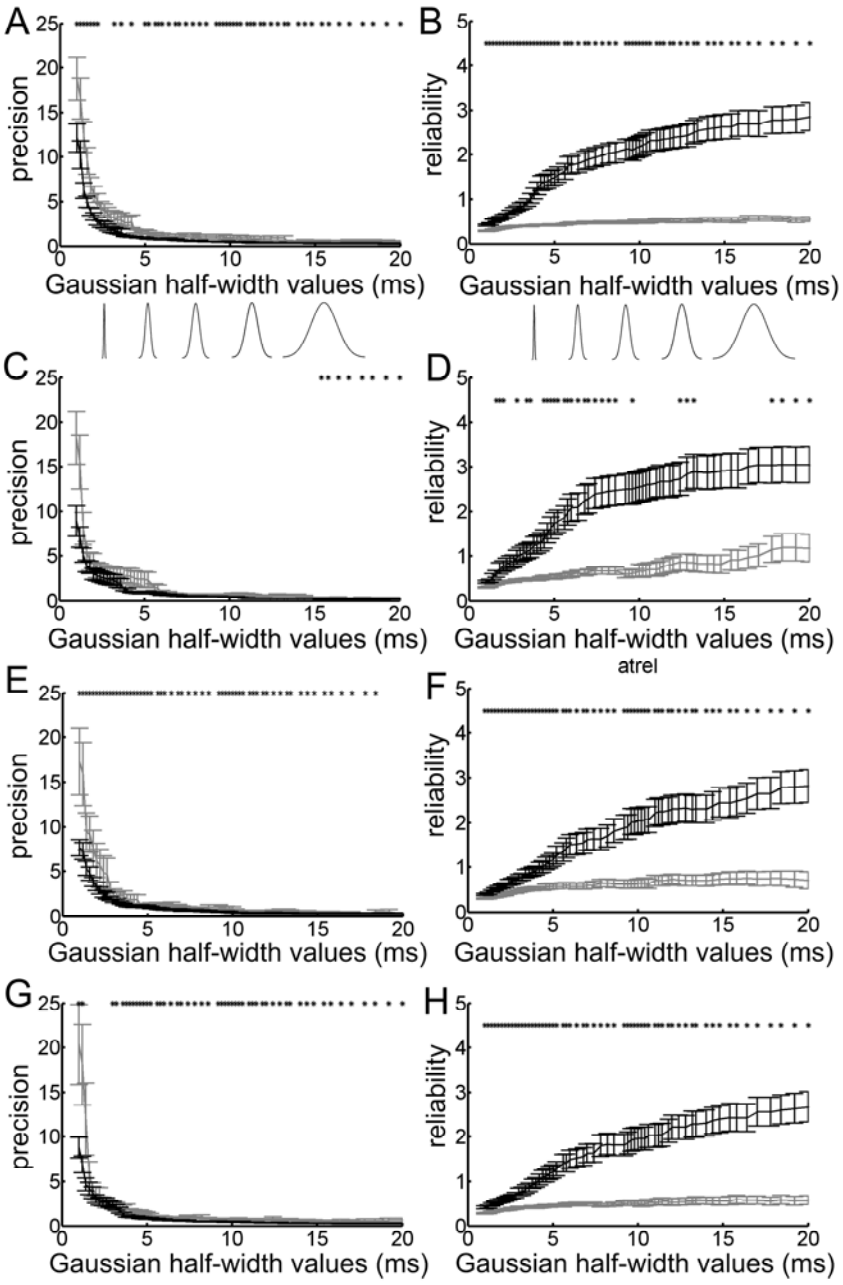
Supplemental Figure 2. The percentage of the response types that were color (**A**) or motion (**B**) sensitive. **A**. The phasic, tonic, or phasic-tonic neurons were generally color sensitive (either narrow-band or color opponent). The on-off response types were more often broad-band. **A**. The on-off responsive neurons were generally more directionally selective, while the phasic responsive neurons more often had no motion response.



Supplemental Figure 3. Computation of spike time precision and response reliability. Rastergrams are obtained from multiple trials data (here 8). The histogram across trial is computed (PSTH) and smoothed with a Gaussian curve (in this case, a Gaussian curve with a half-width peak of 40 ms). A peak detection algorithm is then used to locate peaks above 3 standard deviations of the mean level of 'noise' in the histogram (above the dotted line; pre-stimulus presentation, here between 0 and 0.4 s). These peaks are called 'events'. 'Response reliability' measures the reproducibility or consistency of the phenomenon across

trials. Response reliability is computed as the fraction of the total spikes contributing to events averaged across trials (red). 'Spike time precision' is a measure of the average temporal precision of the events. To make this measure intuitive, it is computed as the inverse of the average standard deviation at mid-height of all events (a small number means low precision, blue). A more extensive description and alternate ways of computing reliability and precision are reviewed in Tiesinga et al. (2008).

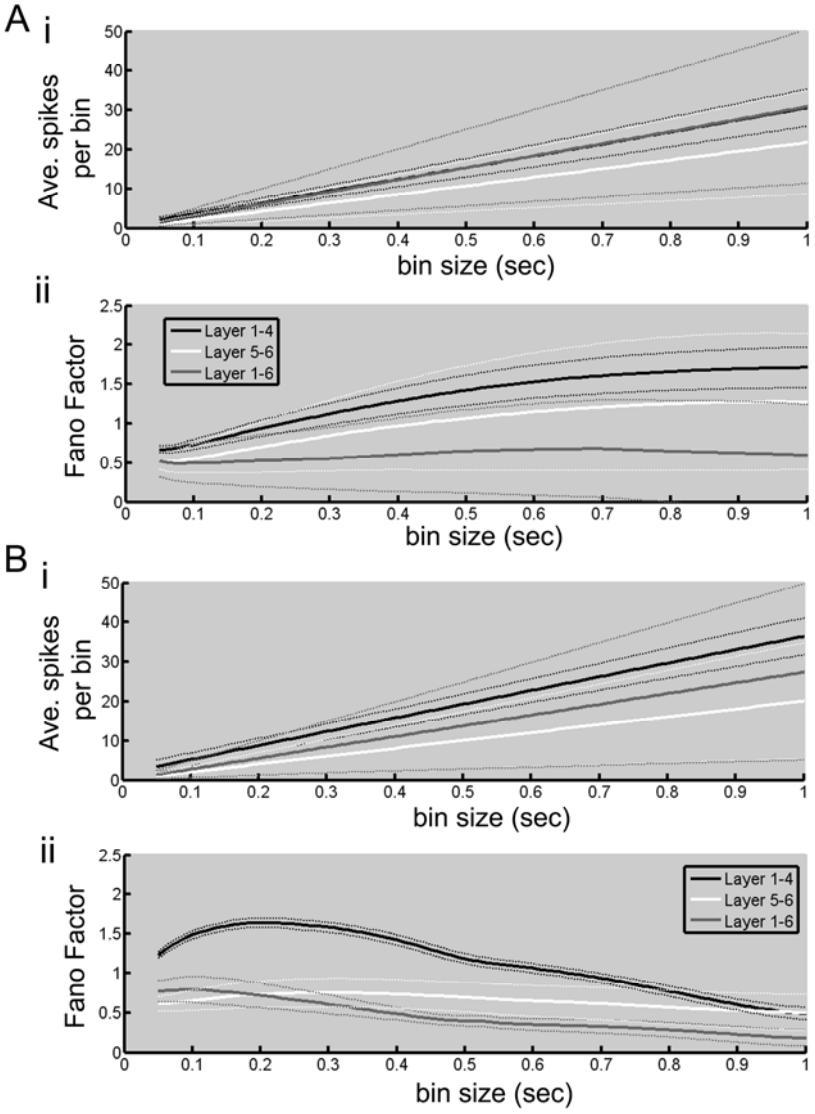
Supplemental figure 4



Supplemental Figure 4. Precision and Reliability at different time scales. While the data in Figure **7C** and **7D** were produced by convolving the PSTH of the spikes times with a Gaussian curve 8 ms wide, the next step was to examine how the convolution influenced the spike time precision and response reliability measures. **A**, **B**. The spike times during combination blue-

green-violet light flashes were convolved with several Gaussian curves of varying widths (indicated by the example curves below (**A**) and (**B**)) and the spike time precision and response reliability were measured for each convolution (**A**,**B**). **A**. Layer 5-6 neurons (black line) still had significantly higher precision values for most data points, indicated by the black asterisk above each point (where-ever there is a black asterisk: $p<0.05$). **B**. Layer 1-4 neurons had significantly higher response reliability measures across all the data points. These general trends were repeated for spike timing precision and response reliability measurements during blue (**C**, **D**), green (**E**, **F**), and violet (**G**, **H**) light flashes. However, the significantly different precision and reliability values between the Layer 1-4 and Layer 5-6 neurons depended on the color of the light flash. This variability is shown in **C**, where, for the narrow convolution curves, the precision values are not significantly different between the two layer categories. Error bars represent standard error.

Supplemental figure 5



Supplemental Figure 5. Spiking variability and the Fano Factor (FF). **A**. Analyses involving background activity. **A i**. The average spikes per overlapping time window were measured for the spike times during five seconds of background activity with multiple time windows of different sizes. Then, the resultant curves were averaged for each layer group and plotted against the size of the sampling time windows, with Layer 1-4 (black), Layer 5-6 (white), and Layer 1-6 (gray). The linear increase in the average number of spikes per bin indicates the variability

changed relatively independent of the mean number of spikes across the recording. **A ii**. We then performed the FF measurement on the background activity and found no significant differences in the FF curves between the layer groups. **B i**. The average number of spikes per bin was also calculated for the spiking activity during the five light flashes. **B ii**. Again there was no significant difference in the average number of spikes per bin among the layers, unlike for the light flash FF. Layer 1-4 neurons (black) had significantly higher light flash FF values for most of the time windows than Layer 5-6 (white) or Layer 1-6 (gray) neurons. (Mann-Whitney *U*-test; for comparisons within time bins between 50 and 960 ms: $p < 0.05$) The dashed lines above and below the solid lines signify standard error.

Fano Factor methods

Another temporal aspect of the neural spiking was spike time variability, which was measured using the Fano Factor (FF; Fano, 1947; Teich et al., 1996). First, we calculated the number of spikes per bin by sampling the number of spikes within a sliding, overlapping window (overlapping by 1 ms). We then calculated the FF as such:

$$FF = \sigma^2/\mu;$$

Where σ^2 is the variance of the number of spikes per bin and μ is the mean of the number of spikes per bin. Once we calculated the FF value for a window of one size (such as 50 ms), we then increased the size of the window (i.e. to 60 ms) and calculated another Fano factor value for that window size. We continued to increase the window sizes until we had FF values for a range of bin sizes. The range of time windows was from 50 ms to 1 second in 10 ms steps during LED light flash stimulation.

We performed the FF analysis on the spike times during the five seconds of activity preceding the light flash (Supplemental Fig. 3B; background FF), on the spiking times during the five consecutive light flashes at 1 Hz (Supplemental Fig. 3D; light flash FF). Repeated trains of light flashes for the same light flash color were averaged and included in the average FF curves. For statistical comparisons between groups using the FF, we performed comparisons between groups within individual sliding window sizes as well as pair-wise comparisons between the average FF curves across time windows. Statistical comparisons of measurements between cell groups or stimuli were compared using the Mann-Whitney *U*-test for all statistical comparisons.