NONLINEAR ANALYSIS OF NORMAL AND *SHAKER* K^+ CHANNEL KNOCKOUT *DROSOPHILA* PHOTORECEPTORS STIMULATED BY WHITE NOISE AND NATURAL LIGHT SIGNALS

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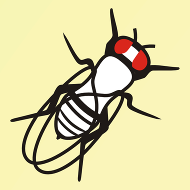
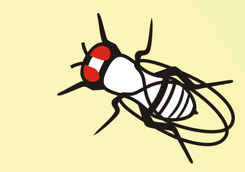
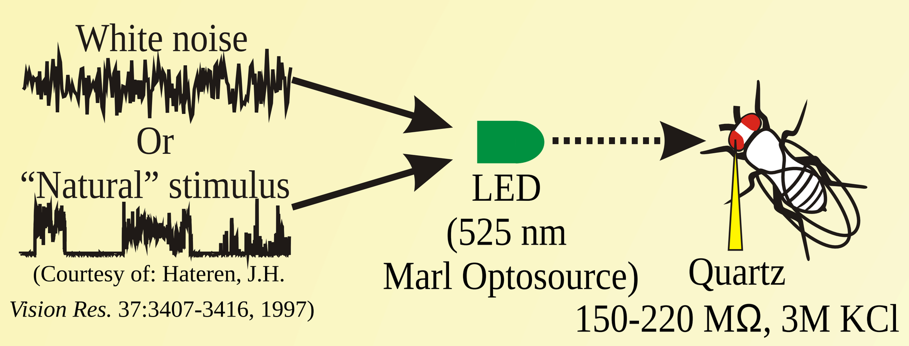




1. Methods

The wild type strain was red-eyed *Drosophila melanogaster* Oregon Red. The null mutation in the *Shaker* channel, Sh^{14}, a missense mutation in the core region resulting in non-functional *Shaker* channels was also expressed in red-eyed flies. Both strains of flies were raised at 19°C in darkness.



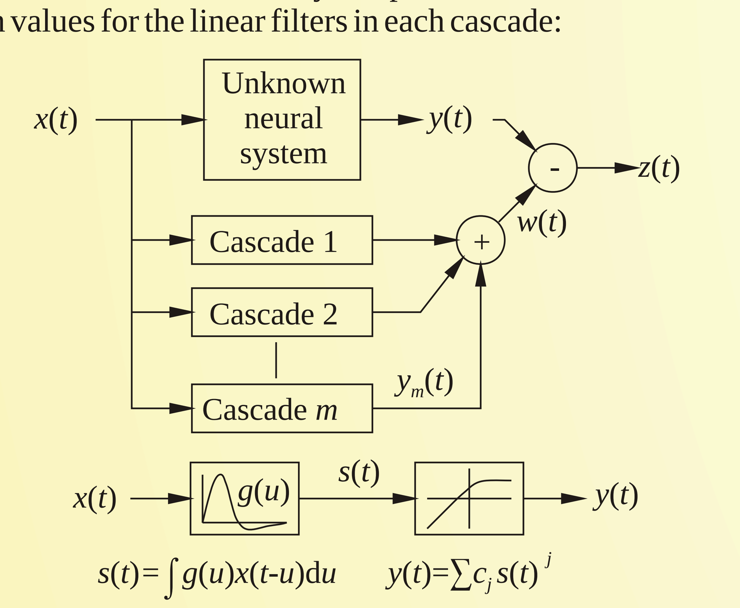
Recordings made by SEC-10L (NPI Electronic) in current-clamp mode, with switching frequencies 8-20 kHz and low-pass filtered at 500 Hz (elliptic). Temperature maintained at 25°C within 1°C accuracy using a custom-built thermocouple feedback to a Peltier device. Photoreceptors were only used if their membrane potential was below -55mV and they had at least a 45mV saturating impulse response when dark-adapted. Data acquisition, and stimulus generation by a purpose built MATLAB interface.

2. Parallel casade analysis

40,000 data pairs were analyzed as the input and output of an unknown nonlinear dynamic system with light intensity as the input, $x(t)$, as a function of time, t, and the receptor potential as output, $y(t)$, represented by the first three terms of a Volterra functional series:

$$y(t) = K_0 + \int_{-\infty}^{\infty} K_1(u)x(t-u)\,du + \int_{-\infty}^{\infty}\int_{-\infty}^{\infty} K_2(u,v)x(t-u)x(t-v)\,du\,dv$$

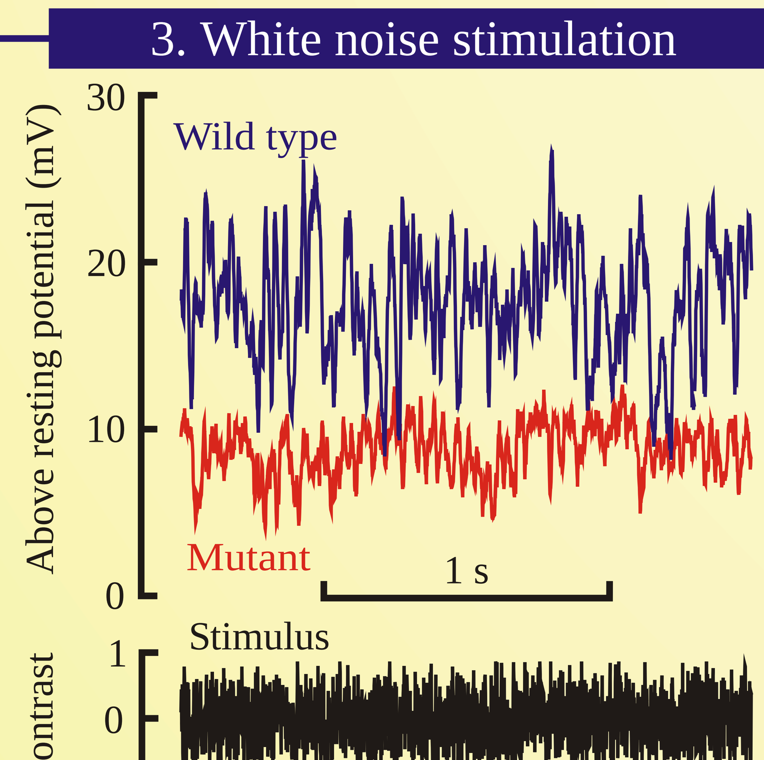
Kernel values were obtained by the parallel cascade method, using random values for the linear filters in each cascade:



Mean square error (*MSE*) between Volterra series and experimental data was obtained from:

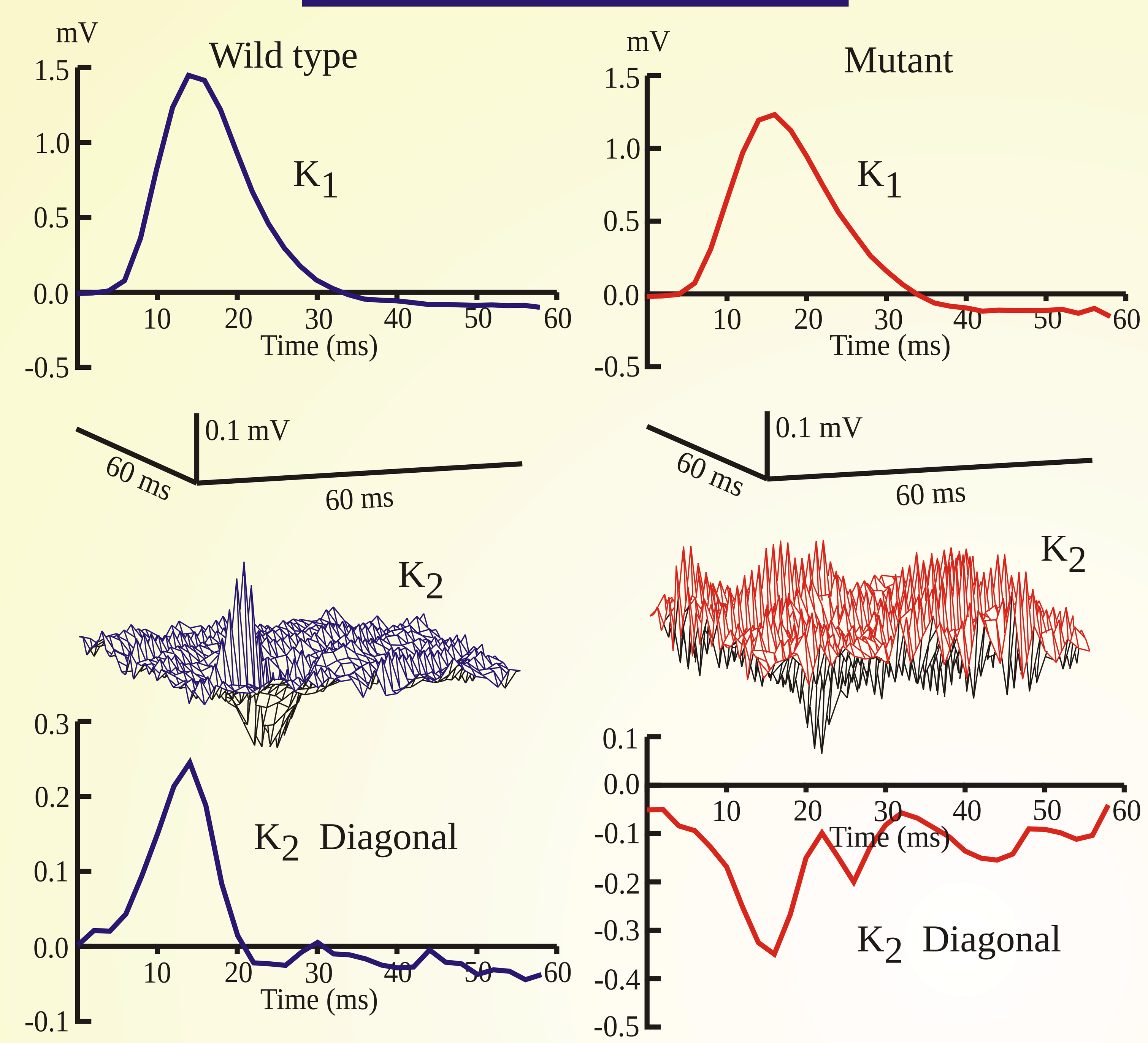
$$MSE = 100\,\frac{\overline{(y(t)-y_s(t))^2}}{\overline{y^2(t)} - (\overline{y(t)})^2}$$

3. White noise stimulation



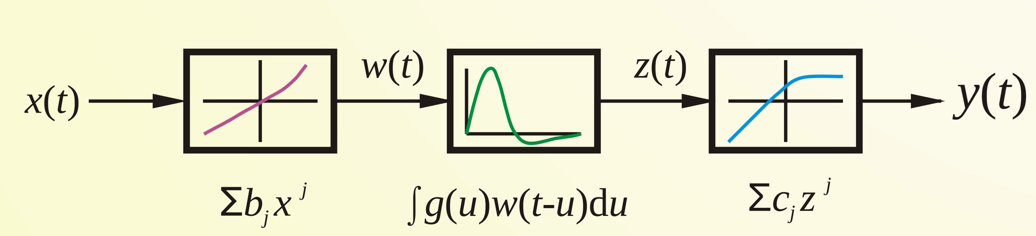
Intracellular membrane potential responses (depolarization above resting potential) of wild type and mutant photoreceptors to identical white noise modulated light stimulus. Note the larger light-induced mean depolarization and larger fluctuating response of the wild type (blue) photoreceptor compared to the Sh^{KS133} mutant (red)

4. White noise - kernels



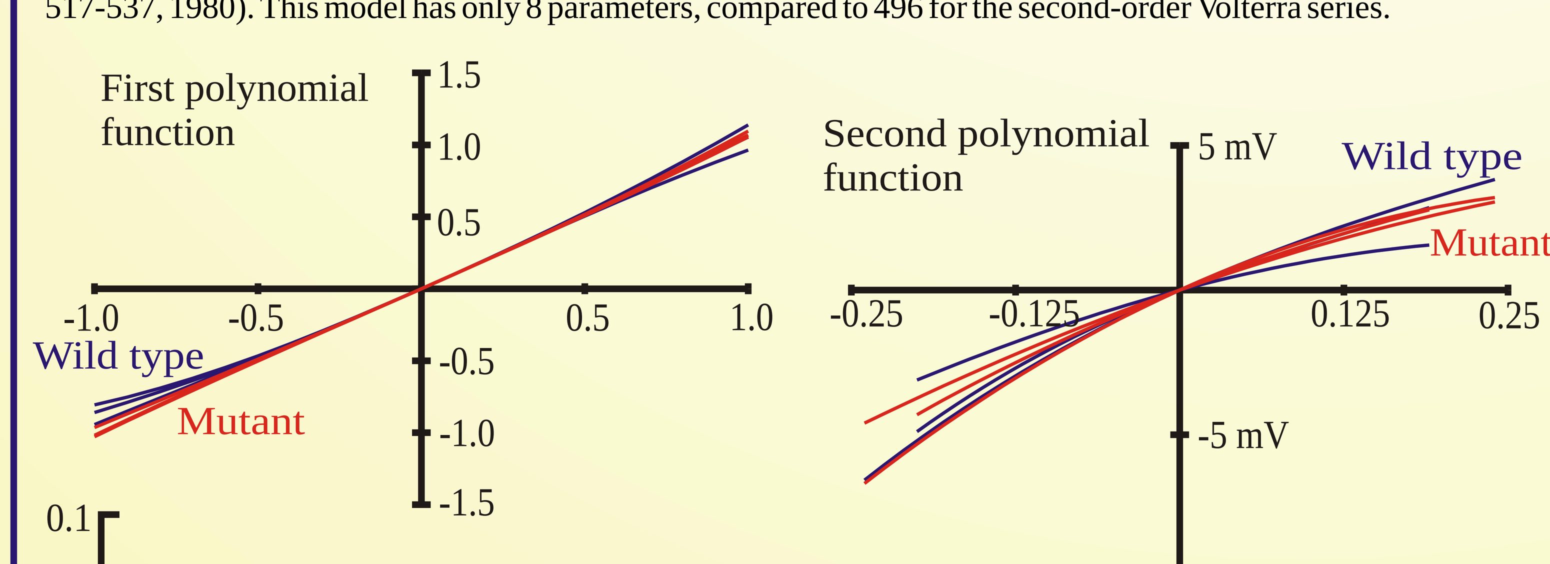
First- and second-order kernels for phototransduction in wild type and mutant *Drosophila* photoreceptors.
Upper row: first-order kernels, $K_1(u)$. Middle row: perspective plots of second-order kernels, $K_2(u,v)$.
Bottom row: diagonals of second-order kernels, $K_2(u,u)$.
Wild type diagonals were dominated by a positive peak, representing nonlinear amplification of the signal during the early phase of the response. However, the second-order kernel also showed significant, later off-diagonal negative values.
Mutant diagonals did not have the positive peak. Instead, there was a negative peak, continuous with slower and broader, off-axis negative values, having a similar distribution to those seen in wild type receptors.

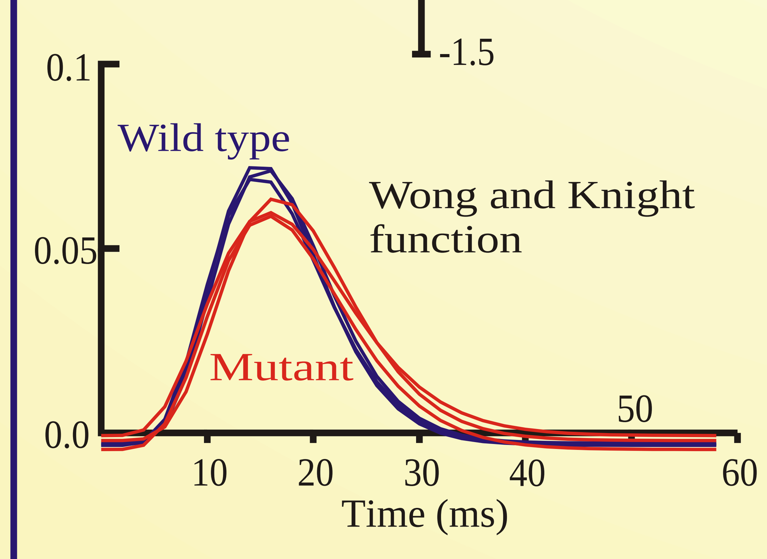
5. White noise - NLN Model



$$g(t) = \frac{1}{n!\tau}\left(\frac{t}{\tau}\right)^n e^{-t/\tau}$$

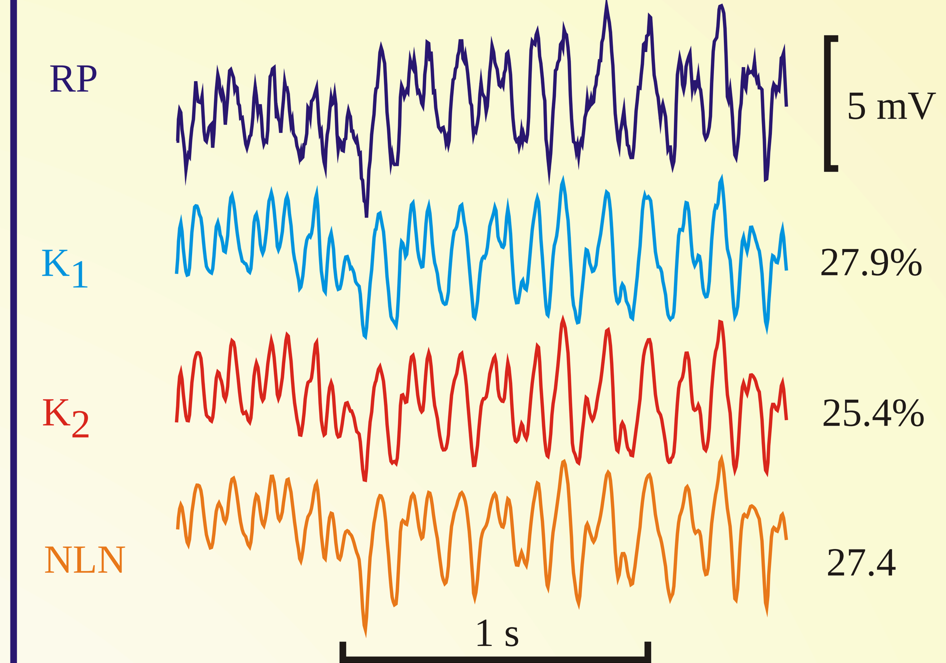
A simple nonlinear parametric model of phototransduction, consisting of two static third-order polynomial functions cascaded with a linear filter based on the Wong and Knight model of phototransduction (*J Gen Physiol* 76: 517-537, 1980). This model has only 8 parameters, compared to 496 for the second-order Volterra series.





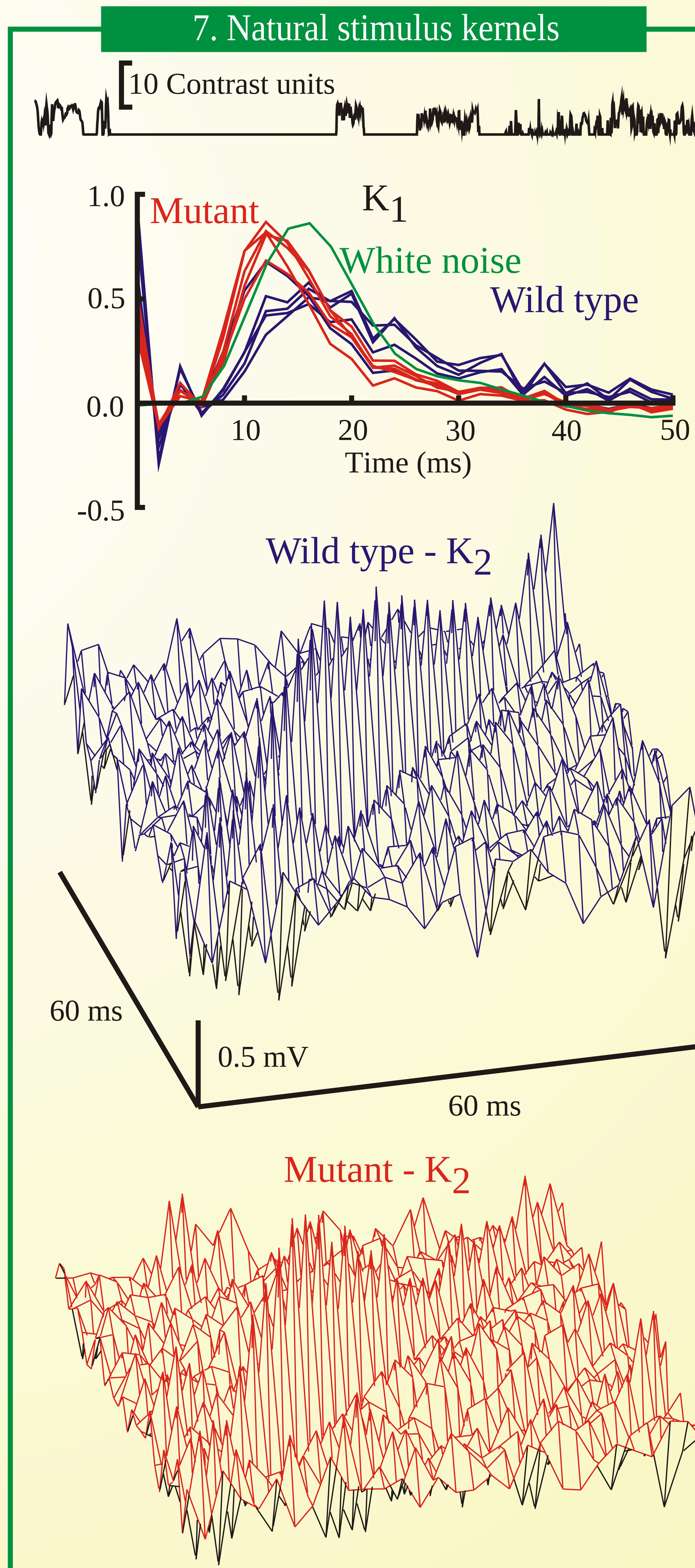
NLN model for three wild type (WT) and three Sh^{14} mutant *Drosophila* photoreceptors. The first static nonlinearity gave mild nonlinear compression of the signal at negative contrast values. The compression was always greater for WT than mutant receptors. The linear filter for WT receptors was always faster and larger than for mutant. The second static nonlinearity compressed increasing response at all output values, but there were no detectable differences between WT and mutant receptors. The only parameters with absolute significance are the dimensionless contrast input and membrane potential.

6. White noise predictions and MSEs



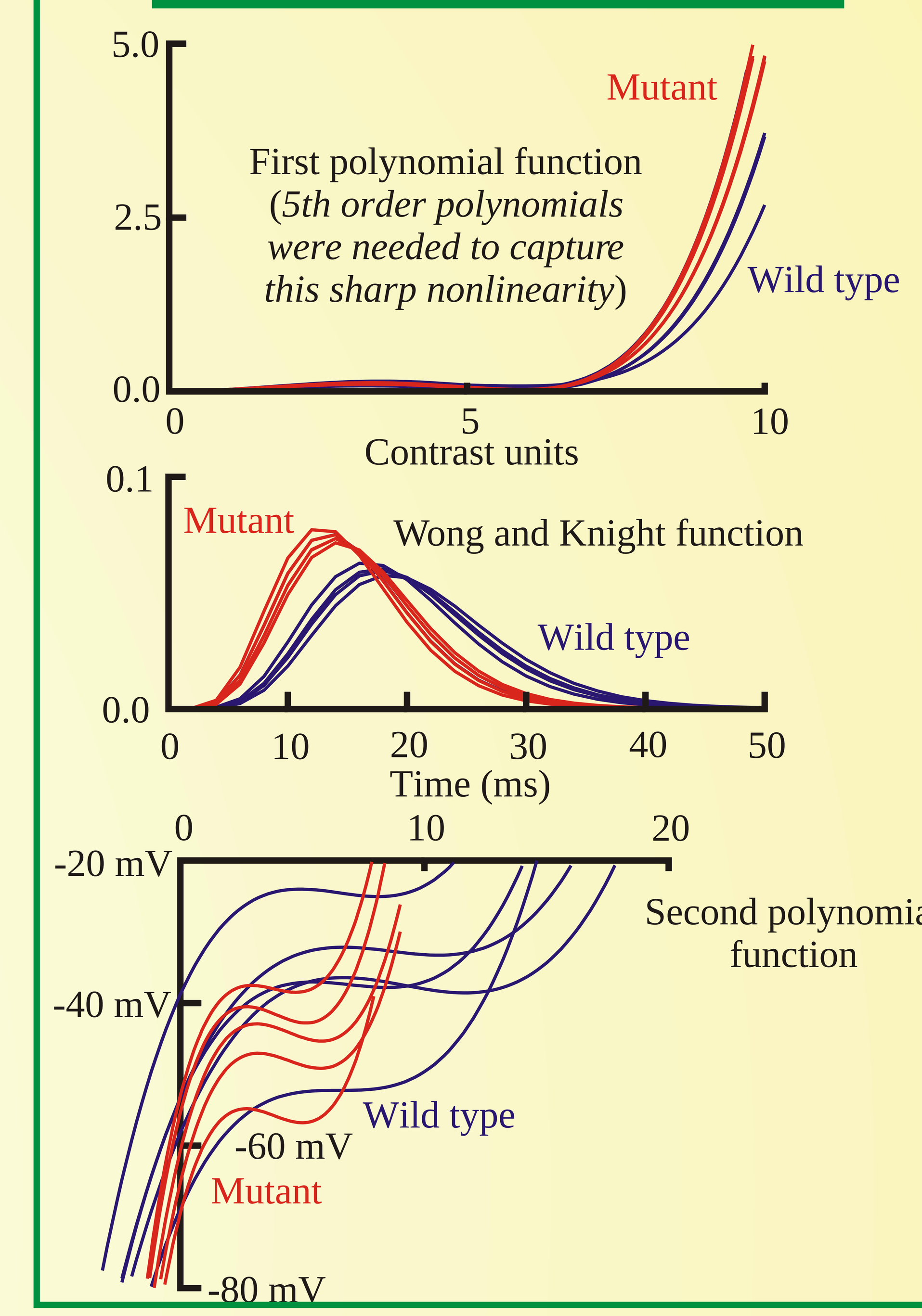
Experimental and predicted responses in wild type photoreceptors. MSE values for Mutant flies were: 47.1%, 41.2% and 42.5%.

7. Natural stimulus kernels

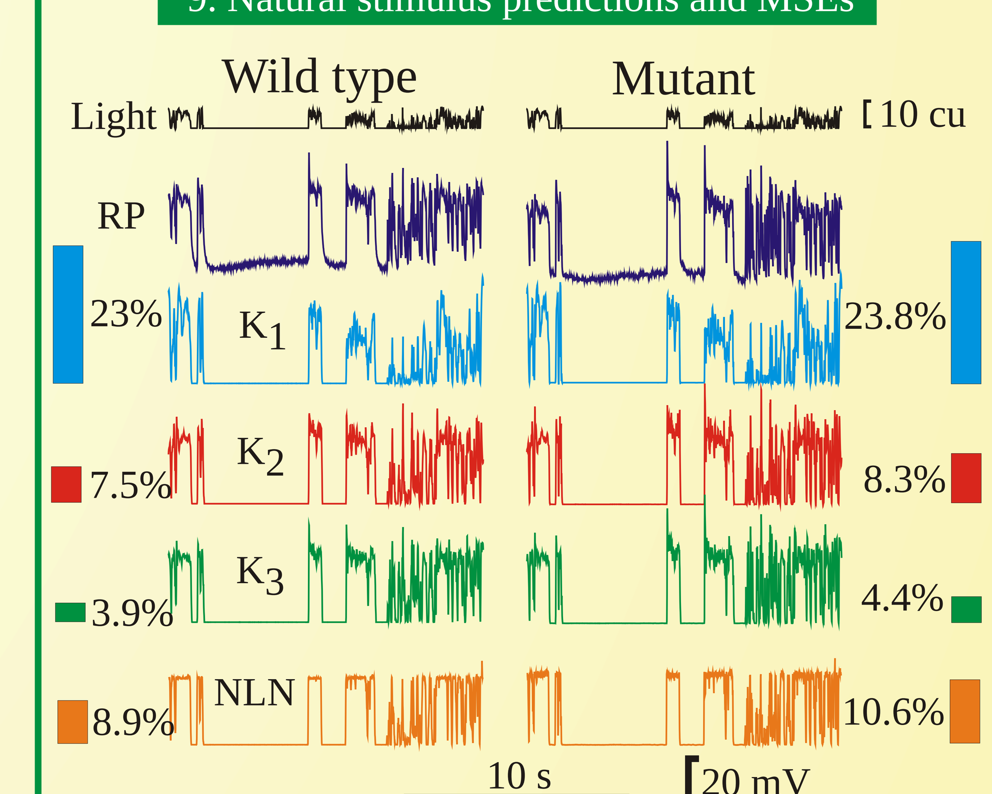


First- and second-order kernels from natural stimulation (top trace). Mutant flies showed larger and faster first-order responses under these conditions (a wild type first-order kernel with white noise stimulation is shown in green).
The prominent diagonal ridge in both second-order kernels is characteristic of a linear filter following a static nonlinearity.

8. Natural stimulus LNL models



9. Natural stimulus predictions and MSEs



10. Conclusions

- Volterra series models of *Drosphila* photoreceptor responses gave large errors with white noise stimulation.
- Addition of second-order kernels only reduced the error by ~2%.
- Mutant flies had larger errors, presumably due to the lower signal-to-noise ratio of *Shaker* mutants.
- NLN models with only 8 parameters were equally useful.
- Volterra series models were better with natural stimulation but third-order kernels were needed to give errors <5%.
- NLN models with only 10 parameters gave similar errors to second-order Volterra models.
- Wild type and Shaker mutant flies gave similar errors under these conditions, suggesting that *Shaker* does not improve signal-to-noise during natural stimulation.
- NLN models for natural stimulation contained strong nonlinearities, including rectification.
- One nonlinearity featured a negative slope region, suggesting an active conductance. This region shifted its operating range in *Shaker* mutants.

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