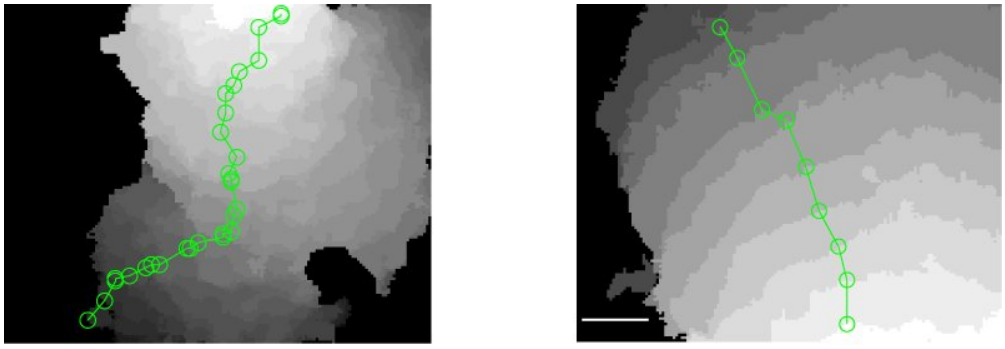
Supplemental Experimental Procedures

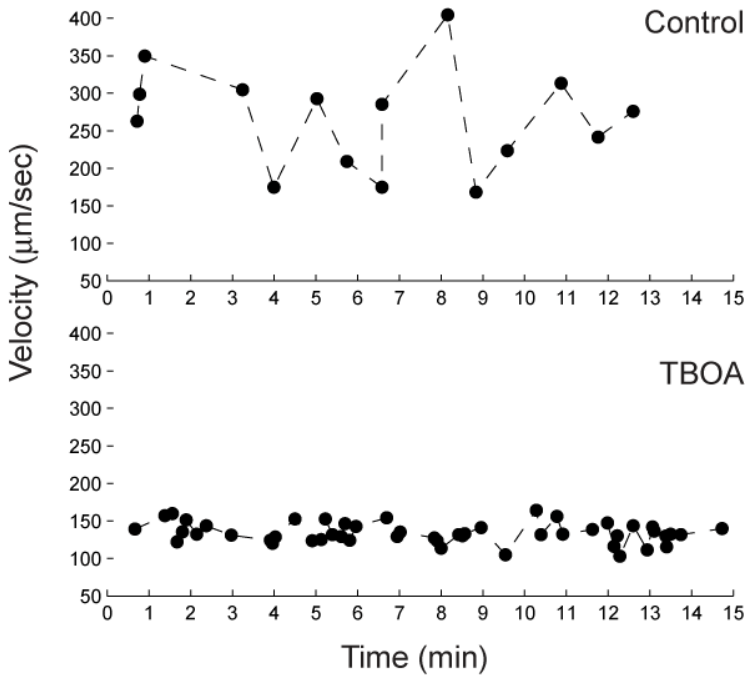
Calcium Imaging Analysis. Stage III retinal waves exhibited clusters of rapidly initiating waves, and therefore we often observed waves in regions that still had elevated calcium from a preceding wave. This property required a method of analysis that relied upon the accurate definition of wave boundaries. Therefore, fluorescence intensity image time series were processed using custom MATLAB (Mathworks) programs to define wave boundaries in the following manner. Images were spatially filtered using a two-dimensional mean filter which averaged over a 31 x 31 micron box. For each frame, the fractional change in fluorescence intensity $\Delta F/F = (F(t)-F_{avg})/F_{avg}$ was computed, where F(t) is the fluorescence intensity at time t and F_{avg} is the fluorescence intensity at each pixel averaged over the entire time series after correction for bleaching. Wave boundaries were defined as contiguous pixels that had a change in $\Delta F/F$ greater than 2% within 1 second. Contiguous regions from one frame to the next were considered to be part of one wave. In order to exclude spontaneous calcium transients in individual RGCs, we defined waves as having a minimum size of 960 μm^2. These values for the time and threshold were determined to best match the wave boundaries as determined by visual inspection. This process generated binary images of wave regions at each point in time on which subsequent analysis was compiled (Fig. 4). Inter-wave intervals were determined at 25 points on a 5X5 grid evenly spaced over the imaged region. Given our temporal filter of 1 second to reliably define waves, we could not determine inter-wave intervals of less than 2 seconds. We did not estimate the total area covered by waves because many waves initiated or ended outside of the imaged area.

Velocity of the wavefront was computed for waves covering greater than one quarter of the imaged region and lasting longer than 2 seconds. Smaller and shorter duration waves were excluded because they are difficult to distinguish from local bursts of synchronous activity. Velocity was measured by first projecting the path of the wavefront onto an isotemporal map such that the wave boundaries at each time are represented by a continuous line (Supplemental Fig. 1). The furthest distance traveled during the last frame of a wave was used as an ending point for tracing the path of the wavefront. Points along the wave path were selected by finding the shortest distance from the point at time *t* and the wavefront at time *t-1*. Wave paths were examined by eye for each wave to ensure they met the above criteria. Paths were excluded from analysis if waves propagated over regions containing patches of retina where uneven loading of calcium indicator prevented accurate measurement of the wavefront. Velocity was calculated by averaging the distance traveled between each consecutive time point then multiplying by the image acquisition rate (4Hz for all analyses of wave spatiotemporal properties). Some wavefronts propagated in a start-stop manner. For these waves pauses, where the distance between wavefronts at consecutive time points is 0, were not included in the averaging when computing velocity.

Computing velocity based on wavefronts defined by calcium imaging leads to lower values than wave velocities measured using spikes recorded on an MEA (Demas et al., 2003; Demas et al., 2006). The two methods lead to similar values for velocities for smoothly propagating waves with circular wavefronts (Supplemental Figure 2b). However, waves with more complex wavefronts, such as those that propagate in a saltatory manner (Supplemental Figure 2a) or split into two distinct wavefronts, yield different values when measured with calcium imaging and with an MEA.

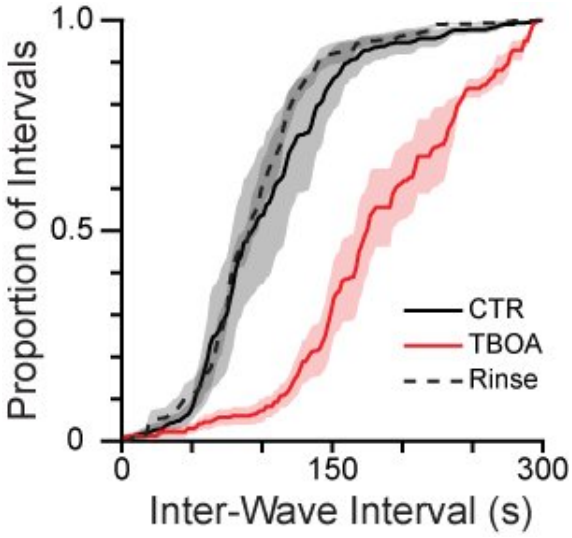


Supplemental Figure 1. Examples of images used to compute wave propagation speed Equal shades of gray represent the wave boundary at a given time, with darker shades corresponding to later time points during the wave. The width of isotemporal regions is indicative of the distance traveled during the interval between frames (250 ms). The green line represents the path of the wave and green circles represent the points along the wave boundaries where the distance measurements are taken to determine velocity (see Methods). The wave on the left has an average velocity of 116 µm/sec whereas the wave on the right has an average velocity of 313 µm/sec. Scale bar 100 µm.

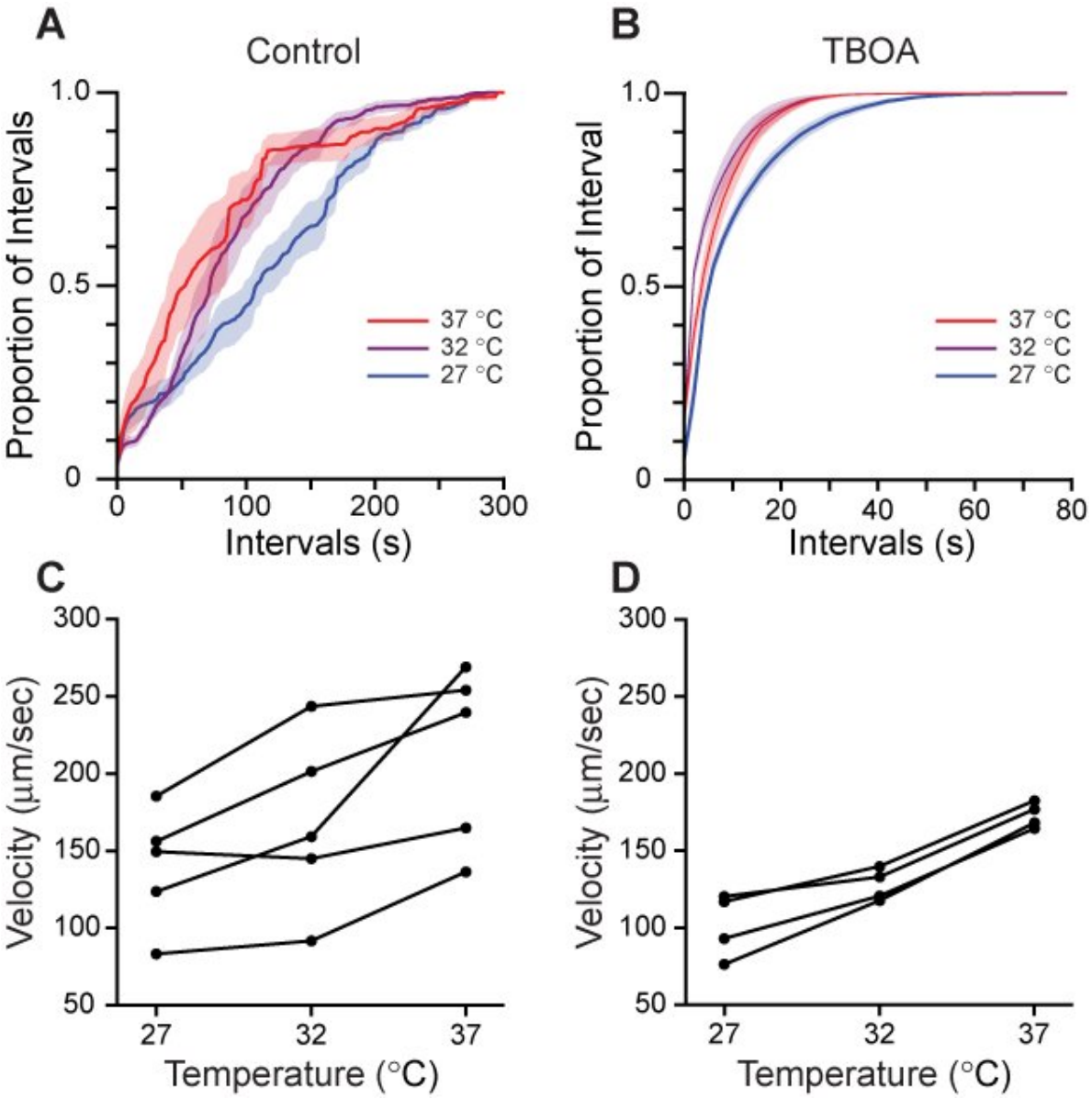


Supplemental Figure 2. Blocking glutamate transporters decreases the variability of wave propagation speed

Plots of propagation speed vs. time in a single retina. Each dot represents one wave. Top, control; bottom, 25 µM TBOA.



Supplemental Figure 3. TBOA reduces wave frequency during Stage II waves
Cumulative histogram summarizing effect of 25 μM TBOA on inter-wave intervals during Stage II waves. n=5 retinas, aged P5-P6. Black is control, red is 25 μM TBOA, dotted line is rinse.



Supplemental Figure 4. Temperature effects on spatiotemporal properties of Stage III waves

(**A**) Cumulative histogram summarizing effects of different bath temperatures on inter-wave intervals. Shaded areas represent standard error of the mean.

(**B**) Cumulative histogram summarizing effects of different bath temperatures on inter-wave intervals in the presence of 25 µM TBOA.

(**C**) Effects of temperature on propagation speed. Individual dots represent single retinas; lines connect a retina's speed at three different temperatures.

(**D**) Effects of temperature on propagation speed in the presence of 25 µM TBOA. Note there is a reduction in the across-retina variability in the presence of TBOA as described in Figure 5. Since the effects of changing temperature on the spatiotemporal properties of waves follows the same trends in the presence of TBOA, we cannot differentiate effects of temperature on glutamate transporters from effects on other physiological processes.

Supplemental Movie Legends

Supplemental Movie 1

Pseudo-colored fluorescence image movie of Oregon Green Bapta1-AM loaded retina in control ACSF. The time series corresponds to the wave images and ΔF/F trace in Figure 4 (b and c). Intensity in the green channel corresponds to fluorescence intensity of the calcium indicator. The red channel indicates the binarized wave front (see Methods). Movie is 5x real-time.

Supplemental Movie 2

Pseudo-colored fluorescence image movie of Oregon Green Bapta1-AM loaded retina during bath application of 25 μM TBOA. Retina is the same as in Supplemental Movie 1. Coloring and framerate are as in Supplemental Movie 1.