

CHANGING DYNAMICS OF SPONTANEOUS WAVES DURING RETINAL DEVELOPMENT: A NOVEL PANRETINAL PERSPECTIVE ACHIEVED WITH THE ACTIVE PIXEL SENSOR (APS) 4,096 ELECTRODES ARRAY

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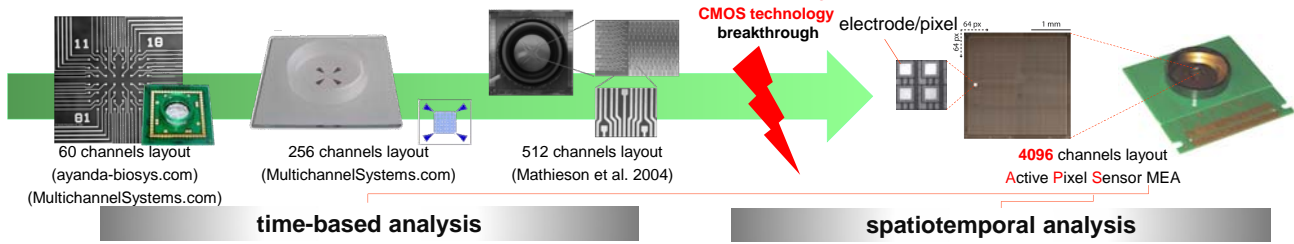
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Waves of spontaneous activity sweep across the immature retina. The spatiotemporal information encoded in these waves is believed to play a crucial role in guiding the formation of connections in the visual system. To this day, no experimental approach has provided enough accuracy to analyse wave dynamics in great detail, and mostly, to understand how the spatiotemporal features of this early retinal activity change with development. [1] Active pixel sensor array for high spatio-temporal resolution electrophysiological recordings from single cell to large scale neuronal networks. Berdondini L et al. Lab Chip 9, 2644-2651 (2009).

Using the APS MEA with 4,096 electrodes (64x64 array, 21 μm resolution) recording at

The Active Pixel Sensor MEA system

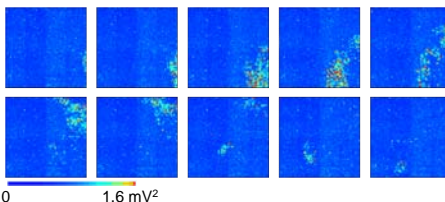


MOUSE RETINAL WAVES RECORDINGS ON THE APS MEA



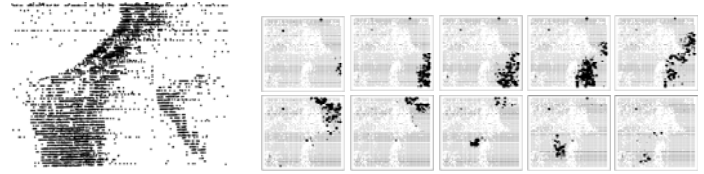
2.67 mm
The neonatal mouse retina (P10) fits almost entirely on the electrode array.

Visualization of raw data



Propagating patterns are visualized in time lapse single frames of activity raw data acquired every 0.5 s. The extracellular signals are shown in a false colour map by computing the signal variance. P10 retina.

Spike train visualization



Raster plot of detected spikes during the same activity episode. Each line represents one active channel.

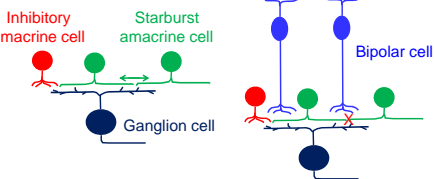
Time-lapse images of the firing rate calculated in 0.5 s bins for all channels. The size of the dots is proportional to the firing rate

DEVELOPMENTAL CHANGES IN WAVE SPATIOTEMPORAL PATTERNS

RETINAL CONNECTIVITY CHANGES DURING THE PERIOD OF RETINAL WAVES

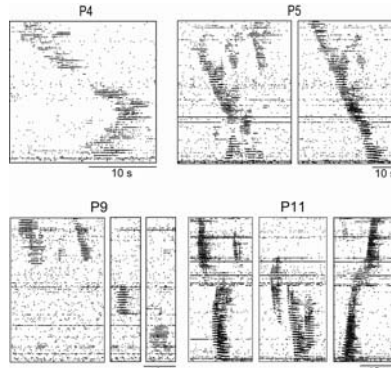
STAGE II WAVES (LATE GESTATION-P9)

STAGE III WAVES (P9-P15)



Stage II waves are generated by cholinergic starburst ACs. The activity propagates laterally between these ACs and vertically onto RGCs. Stage III starts when the vertical glutamatergic bipolar connections mature. At the same time, lateral propagation across the starburst AC network ceases.

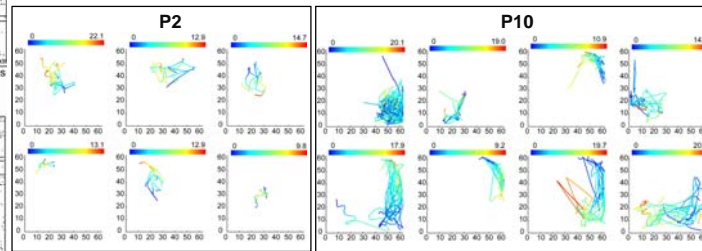
DEVELOPMENTAL CHANGES IN SPONTANEOUS ACTIVITY PATTERNS



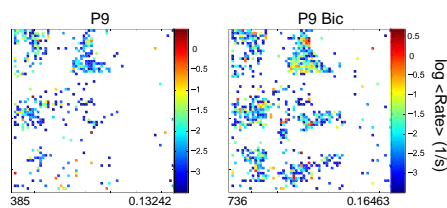
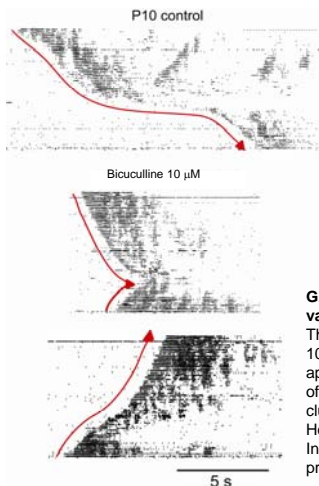
Raster plots of waves at Stage II (P4,5) and Stage III (P9,11). Stages III waves seem faster, more homogeneous and more spatially restricted.

CLUSTER ANALYSIS DETECTS REPETITIVE ACTIVITY PATTERNS

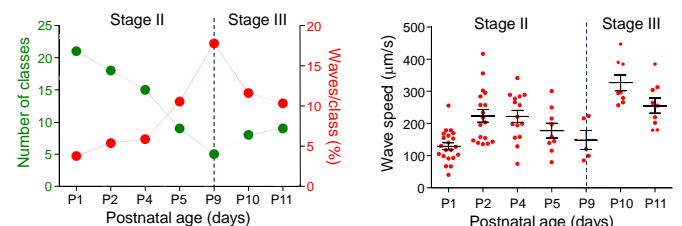
For each wave, we calculate the **Centre of Activity Trajectory (CAT)** using a temporal window of 1s and sampling every 200 ms. The CAT classification process includes an initial phase where CATs shorter than 5 CA points are discarded and the number of CAT dimensions (CA points) is equalized by means of spline interpolation. We use a k-means algorithm based on squared Euclidean distances and we remove outliers on the basis of Euclidean distance and deviation from the cluster centroid.



Clusters of CATs at P2 and P10. Clustering was performed on 30 min of spontaneous activity. For P2, only 6 out of 18 clusters in total are illustrated. For P10, all 8 detected clusters are shown. There are many different trajectories at P2, but fewer repeating ones at P10. Moreover, there is less spatial overlap between clusters at P10 than at P2: they seem to tile the retina. The temporal aspect of each CAT is represented with colour coding (blue - wave start; red - longest wave duration in the cluster). Numbers on the X and Y axes respectively represent electrode number in columns and in rows on the 64x64 array.



GABAergic signalling is not responsible for the loss in activity variability observed at Stage III. The plots above illustrate activity maps (log firing rate) obtained during 10 min of continuous recording at P9. In control conditions, the activity appears as spatially restricted clusters tiling the retina. In the presence of bicuculline, a GABA_A antagonist, the activity is stronger within the clusters, the clusters become slightly bigger and new clusters emerge. However, the overall clustering pattern remains similar. In the P10 raster plots on the left, bicuculline clearly accelerates activity propagation, but the overall propagation patterns remain unchanged.



Waves become less random with development. The number of wave classes decreases with development whilst the number of waves within each class increases. Substantial changes occur at P5, when GABA becomes involved in controlling wave propagation and beyond P9, when waves switch from cholinergic to glutamatergic control. At the same time, waves become slower at P5, when GABA becomes involved in the network, but their speed increases substantially when they become controlled by glutamate at Stage III.