Supplementary Figure S1. Schematic illustrating photoreceptor degeneration and the concept of neurotransmitter-based stimulation. (A) The anatomy of a normal wild-type retina highlighting the organization of the primary layers including the photoreceptor outer segments (OS), outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL). (B) A schematic demonstrating the anatomical changes caused by retinal remodeling associated with photoreceptor degeneration including: 1) the loss or degeneration of the photoreceptor layer, 2) the development of an anomalous glial seal on the subretinal (top) surface, and 3) the formation of corrupt synapses. Despite these changes, the surviving bipolar and retinal ganglion cells remain intact and relatively functional. A hypothetical artificial chemical synapse chip with multiple hollow, projecting, needle-like microports is interfaced with the subretina to illustrate the concept of neurotransmitter-based stimulation, which seeks to chemically stimulate the surviving bipolar and retinal ganglion cells using native neurotransmitters such as glutamate.
Supplementary Figure S2. Photoreceptor degeneration rate in the pigmented hemizygous S334ter-3 rat. The rate of degeneration for pigmented hemizygous S334ter-3 (stars) and wild-type Long Evans rats (circles) measured by the outer nuclear layer (ONL) thickness of photoreceptors across time. As can be seen, the S334ter-3 rat displays rapid degeneration of the ONL in the first 30 postnatal days (PND) while the wild-type rat experiences a slow decline before stabilizing at a non-negligible ONL thickness. Based on the rate of degeneration exhibited by the S334ter-3 rat, we divided our experimental animals into 4 groups based on age: early stage degeneration (14-20 PND), middle stage degeneration (21-27 PND), late stage degeneration (28-35 PND), and completely blind (>50 PND). The data shown were adapted from those presented by the LaVail Laboratory.30,31
**Supplementary Figure S3. Experimental setup.** (A and B) Schematic of the experimental setup showing the arrangement of the neural recording, stimulation, and visualization components. Explanted S334ter-3 retinas were placed onto a pMEA with the RGCs in contact with the electrodes. The pMEA was inserted into a MEA amplifier situated above an inverted microscope to enable visualization of both the retina and the pMEA during experiments. The retina was perfused with oxygenated Ames medium from both the top and bottom of the pMEA, while suction was used to remove excess perfusate and ensure firm contact between the retina and the pMEA electrodes. A dedicated computer was utilized to control visual and chemical stimuli using a green LED and an 8-channel pressure injector, respectively. Glutamate injections were accomplished by fitting either a glass micropipette (A) or a multiport microfluidic device (B) into a pipette holder, which was maneuvered near the retina with a computer-controlled micromanipulator. Injections were triggered by the stimulus computer and the resulting trigger signals were acquired into another dedicated data acquisition computer along with the retinal response data recorded by the pMEA electrodes. The flow of signals and data throughout the setup is represented by the blue lines with labels above.
Supplementary Figure S4. Spatial spread of RGC responses. A comparison plot illustrating how the spatial spread of RGC responses to glutamate injections changes across different stages of degeneration. Each box-and-whisker plot displays the minimum (lower whisker), lower quartile (lower extent of black box), median (horizontal line inside box), upper quartile (upper extent of box), and maximum (upper whisker) of the distances separating glutamate-responsive RGCs from glutamate injections while the distribution of individual data points is represented as a histogram to the left of each box-and-whisker plot (bin width of 50 µm). As can be seen, the spatial spread of RGC responses were abnormally distributed because RGCs were sampled at discrete locations defined by the geometry of the MEA, which prompted the use of 2D Gaussian fitting to find the more continuous estimates of spatial spread shown in Fig. 5. However, these data show that glutamate stimulation in middle and late stage degeneration elicited significantly (p <0.05) more spatially localized responses compared with early (median of this group is the same as the lower quartile) and completely blind groups as indicated by the brackets with asterisks above the box-and-whisker plots.