Vision restoration in photoreceptor-degenerated mice and macaque monkeys using nanowires

Jiayi Zhang (✉ jiayizhang@fudan.edu.cn)

Fudan University <https://orcid.org/0000-0002-1575-0723>

Ruyi Yang

Fudan University

Peng Zhao

Fudan University

Chenli Feng

Fudan University

Liyang Wang

Fudan University

Zhexuan Wang

Fudan University

Yingying Zhang

East China Normal University

Peng Chen

Fudan University

Mingqian Shen

Fudan University

Kaiwen Shi

Fudan University

Shijun Weng

Fudan University

Yanwen Huang

Fudan University

Chunqiong Dong

Fudan University

Fu Zeng

East China Normal University

Luoyin Fu

Fudan University

Ruoxi Ma

Fudan University

Fei Wang

Fudan University

Su Jiang

Fudan University

Wendong Xu

Fudan University

Gang Wang

Beijing Institute of Basic Medical Sciences

Gengfeng Zheng

Fudan University <https://orcid.org/0000-0002-1803-6955>

Aihua Chen

East China Normal University

Fei Yuan

Fudan University

Yuanzhi Yuan

Fudan University

Biao Yan

Fudan University <https://orcid.org/0000-0003-3252-2965>

Article

Keywords: photoreceptor degeneration, blindness, nanowire arrays, implants, nanomaterials

Posted Date: September 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-492941/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Photoreceptor degeneration diseases, such as retinitis pigmentosa and age-related macular degeneration, are major causes of blindness. Here we evaluate the performance of Au nanoparticle-coated titania (TiO₂-x) nanowire (NW) arrays as artificial photoreceptors to restore image-forming vision in blind mice and non-human primates with photoreceptor degeneration. We first demonstrated that blind mice with the subretinal implant of NW arrays were capable of detecting static, moving and flashing objects, with a visual acuity of 0.3 cpd, measured up to 9 months after the implant surgery. Using chronic in vivo calcium imaging, we continuously monitored light-induced responses of neurons in the primary visual cortex (V1) in NW-implanted blind mice for over 14 weeks. Light responses of V1 neurons first appeared at 2 days after the surgery and the number of light-responsive V1 neurons peaked 1 week after the implant surgery, and the latency of these responses decreased over time. These results collectively suggested the plasticity and improvement in the encoding of visual information in V1 after NW implant surgery. We then implanted NW arrays subretinally in macaque monkeys, showing that the NW arrays did not cause adverse responses 8 weeks post surgery. Finally, visually-guided saccades were restored in macaque monkeys with the subretinal implant of NW arrays. Our findings demonstrated the possibility to utilize nanomaterials as artificial photoreceptors to ameliorate visual deficits of patients with photoreceptor degeneration.

Introduction

Photoreceptor degeneration caused by retinitis pigmentosa (RP) or age-related macular degeneration (AMD) has long been a major cause of blindness. Despite the loss of photoreceptors in the retina, the remaining retinal cells as well as their projections to the brain are preserved from the disease. In the past decade, success in treating Leber congenital amaurosis (LCA) using AAV-based treatment has opened up opportunities in gene therapy^{1,2}. Recent studies using genetic and stem-cell based strategies have made great progress in providing proof-of-principle evidences to restore light sensitivity in photoreceptor-degenerated mice³⁻⁷. Challenges are in the lack of cell-type-specific targeting strategy in primates, biosafety issues and the requirement for alien cells to make functional connections to the remaining retinal cells⁸.

The multielectrode stimulation devices turn light signals in the external world into patterned electrical currents and stimulate the remaining retinal cells to establish visual perception in blind patients with late-stage photoreceptor-degenerating diseases⁹. Argus II, a device with 60 pixels, received market approval in both Europe and the United States. The epiretinal implant of Argus II enabled blind patients to be able to not only perceive light but also perform visual tasks¹⁰⁻¹², despite limited visual acuity and potential issues in safety profile in multicenter studies¹³. Similar issues occurred to subretinal implant Alpha AMS^{14,15}. These early efforts in retinal prosthesis were proceeded by subretinal implant IRIS II with 378-pixel electrical stimulation (each pixel is 100 μ m in diameter) in the retina¹⁶⁻¹⁸ and 1024-channel prosthesis in the V1^{19,20}, whose spatial resolution was defined by the pixel density of the device, hence limited by the semiconductor processing techniques. New materials including organic photovoltaic and injectable nanoparticles provided proof-of-principle evidence for light perception in blind mice and shed light onto the next generation retinal prosthesis with improved surgical procedure and better spatial resolution²¹⁻²⁴. However, the restoration of image-forming vision and the potential for translational application remains to be demonstrated.

We previously developed Au nanoparticle-decorated titania nanowire arrays (Au-TiO₂ NW), which converts UV, blue and green light into photocurrent to activate retinal ganglion cells²⁵. Each NW was 100 nm in diameter and 2 μm in length, with a similar physical size to photoreceptors. Light-evoked responses in the V1 and recovery of pupillary light reflex in the rod-cone range demonstrated that light perception was restored in retinal degenerated 1 knockout (*rd1*^{-/-})/cone diphtheria toxin subunit-A (*cDTA*) blind mice.

In this study, we developed Au@TiO_{2-x} NW arrays (referred to as NW array below) which exhibited an enhanced UV-visible absorbance profile compared to Au-TiO₂. We first showed that retinal ganglion cells in the NW arrays conjugated blind retina were responsive to drifting light bars as well as 2 Hz flashing light. NW-implanted blind mice had a visual acuity of 0.3 cpd. These mice also recognized static, moving and flashing objects in the two-choice box, respectively, indicating that image-forming vision was restored.

One important question rarely addressed in the retinal prosthesis is the coding of visual information as well as plasticity in the V1 after the implant surgery. We found that neurons in the V1 have an average receptive field of $402 \pm 87 \text{ deg}^2$ in the NW-implanted blind mice. Interestingly, the fraction of light-responsive V1 neurons increased by 1.44 times that in the normal mice 1 week after implant surgery, and decreased thereafter. Finally, intraocular implant surgery was conducted successfully to place NW arrays into the subretinal space of macaque monkey with photoreceptor degeneration. Visually-guided saccade was restored in the NW-implanted area.

Results

Improved photocurrent of Au@TiO_{2-x} NW arrays

The TiO_{2-x} NW arrays were prepared by hydrothermal growth and subsequent thermal annealing in H₂/Ar to create oxygen vacancies. Au nanoparticles were then in situ reduced and deposited on the NW surface to obtain Au@TiO_{2-x} NW arrays. The X-ray diffraction (XRD, Extended Data Fig.1a) pattern presented two peaks at 36.3 degrees and 63.1 degrees that matched well with rutile TiO₂ (101) and (002) planes. The NW arrays were composed of highly-dense single NW arrays with average lengths of ~ 2.5 μm and diameters of ~ 100 nm (Extended Data Fig.1b, c). High-resolution transmission electron microscopy (HRTEM) images showed the lattice spacing of the dark nanoparticles and NW arrays were 0.235 and 0.246 nm (Extended Data Fig.1c and inset), corresponding to cubic-phase Au (111) planes (JCPDS# 00-001-1172) and rutile-phase TiO₂ (101) planes (JCPDS# 01-088-1175), respectively. The spherical aberration-corrected HAADF-STEM image (Extended Data Fig.1d) showed the array of bright Ti (highlighted by blue spheres) and dark O (highlighted by red spheres) contrast on rutile TiO₂ (101) planes. The extracted intensity from the yellow dashed line of the fourth O atom greatly decreased (Extended Data Fig.1e), indicating the oxygen vacancies²⁶. The existence of oxygen vacancies was further confirmed by X-ray photoelectron spectroscopy (XPS, Extended Data Fig.1f), in which the modified Au@TiO_{2-x} presented an additional shoulder peak centered at ~ 532.5 eV²⁷.

UV-visible absorbance spectra displayed that the absorption of Au@TiO_{2-x} NW arrays ranged from 375 to 890 nm (Extended Data Fig.2a). When illuminated by UV (375/28 nm, 10 μW·mm⁻²), blue (470/20 nm, 10 μW·mm⁻²) or green (546/12 nm, 10 μW·mm⁻²) light, the average photocurrents of the newly fabricated NW arrays were

about 13000, 850 and 700 pA (Extended Data Fig.3b-d), respectively. The photocurrents increased by 5 - 7 times compared to those reported in our previous study²⁵ (Extended Data Fig.3d).

To confirm that NW arrays elicit light-evoked responses to function as artificial photoreceptors, we utilized *rd1^{-/-}/cDTA* mice (referred to as blind mice below) whose rod and cone photoreceptors were completely degenerated by P50²⁵ (Extended Data Fig.2e). We found that the retinal ganglion cells (RGCs) of blind mice retina did not respond to UV, blue or green light. When NW arrays were attached to the blind retina, the RGCs of blind mice were activated by UV (375/28 nm, 10 $\mu\text{W}\cdot\text{mm}^{-2}$), blue (470/20 nm, 10 $\mu\text{W}\cdot\text{mm}^{-2}$) or green (546/12 nm, 10 $\mu\text{W}\cdot\text{mm}^{-2}$) light, respectively (Extended Data Fig.2f).

NW-implanted blind mice recognized locations of light sources

Inspired by multiple visual tests used in human clinical trials with retinal prostheses implant^{14, 28, 29}, we designed choice-box-based mice behavioral paradigms to assess visual functions restored by NW arrays. In the choice-box, visual cues were projected onto one side of the box and the opposite side was installed with one water port (Fig.1a). The behavior experiments in mice were consisted of training and testing trials (Fig.1b). During the training trials, visual stimuli were provided after tone 1 at two nearby locations for 20 sec, and the mice were allowed to explore freely in the box. If the mice touched the target area (defined in each experiment) within 20 sec (defined as reaction time), tone 2 was provided and the mice received a water reward from the water port. A trial was defined as a “miss” trial when mice failed to touch the screen during the reaction time. When the miss rate during the training trials was less than 50% for more than 3 days, the testing trials began and the duration of the visual stimulus and reaction time was reduced to 10 sec (Fig.1b).

The first visual test was the light source localization test, which was conducted in human subjects implanted with Argus II or AMS alpha. 96% of patients with Argus II implant and 78% patients with AMS alpha implant performed statistically better with the device switched on^{11,29}. We implanted NW arrays subretinally into blind mice as previously shown²⁵. The LEDs with near UV, green or blue (UV LED: 375/15 nm, Green LED: 535/28 nm, Blue LED: 465/25 nm) light emissions were placed at two locations of the box (Fig.1c). The two LEDs were switched on randomly, with one LED on at one time. If the mice touched the target area (6 cm in diameter centered at the on-LED), they would receive a water reward (correct). If the mice did not touch the screen (miss) or touch areas outside the target area (wrong), they would not get a water reward (Extended Data Movie 1). The correct rate was calculated as the number of correct trials divided by the sum of correct, miss and wrong trials.

We found that the correct rate of normal mice was 94.17%, 95%, 91.11%, for UV (375/15 nm, 6.24 $\mu\text{W}\cdot\text{mm}^{-2}$), green (535/28nm, 7.83 $\mu\text{W}\cdot\text{mm}^{-2}$) and blue light (465/25 nm, 6.75 $\mu\text{W}\cdot\text{mm}^{-2}$), respectively. The correct rate of blind mice implanted with glass substrate was less than 25% for all three colors. For blind mice with NW arrays implant, the correct rate was 84.79%, 83.33%, 87.71% for UV, green and blue light, respectively (Fig.1d). There was no significant difference in the correct rate between NW-implanted mice and normal mice when light intensities were above 3.31 $\mu\text{W}\cdot\text{mm}^{-2}$ for blue light and 6.75 $\mu\text{W}\cdot\text{mm}^{-2}$ for green light (Fig.1e, f). Interestingly, we

found that 4 weeks after NW arrays implantation, the blind mice learned faster than those implanted for 1 week (Extended Data Fig.3a-d). These data suggested that NW-implanted blind mice were capable of recognizing locations of light sources.

The retinal prostheses including Argus II and AMS alpha delivered images at a frequency of 20 Hz, which leads to image fading after repetitive stimulation^{14,28,30}. In these experiments, the patients were usually head-fixed in front of display screens, hence the light source was focused on the same region of the device/retina. However, like in our choice-box experiment, patients are allowed to move around in reality, hence fading was less of an issue.

Visual motion perception in NW implanted blind mice

Detection of moving objects is an essential visual function for blind patients. Clinical studies reported that 57% of patients with Argus II implant and 56% of patients with AMS alpha implant could detect the motion direction correctly^{30,31}. We first examined whether RGCs of blind mice retina with NW arrays attached could be selectively activated by moving light bars *in vitro* (Fig.2a). We found that moving light bars (9.60 degrees in width, 6.85 degrees/sec) elicited transient spikes in the RGCs of normal mice, but not in the RGCs of blind mice (Fig.2b, c). When NW arrays were attached to the inner nuclear layer of the blind retina, a moving light bar evoked spiking activities in the RGCs of blind mice reliably (Fig.2d). Furthermore, we measured responses of the RGCs to moving light bars ranging from 0.64 to 5.76 degrees in width, and found that responses were elicited in the RGCs of normal mice and those of blind mice with NW arrays attached, but not in the RGCs of blind mice (Fig.2e, f).

To evaluate motion perception in blind mice implanted with NW arrays, we examined the performance of NW-implanted blind mice in discriminating moving light bars from static light bars in a choice-box-based behavioral test. We presented moving light bars in two designated visual stimulation areas with random temporal sequences. The mice were provided with a water reward when touching the area with a moving light bar, which was defined as a correct trial (Fig.2g, Extended Movie 2). We found that normal and NW-implanted blind mice both had a maximum correct rate of ~70% when presented with moving light bars of 5.25 degrees wide and 7.85 degrees/sec during testing trials, but the correct rate of blind mice was less than 20% (Fig.2h, Extended Data Fig.3e). For the range of moving light bars with 2.63 degrees to 7.88 degrees in width, the correct rate of NW-implanted blind mice was above 50% and significantly larger than that of blind mice as well (Fig.2h).

In Figure 2d, we noticed that RGCs of blind mice with NW arrays attached responded to moving light bars with transient spiking activities. We hence examined whether moving light bars with fast speed deteriorates the correct rate of NW-implanted blind mice. When the speed of the moving bar was between 6.54 degrees/sec and 10.47 degrees/sec and the width of the moving bar was 5.25 degrees, the blind mice with NW arrays showed similar performance with normal mice (Fig.2i). Interestingly, the correct rate of the blind mice with NW arrays was much smaller when the speed of the moving light bar decreased, but still significantly larger than that of blind mice (Fig.2i). These results suggested that NW-implanted blind mice can discriminate moving objects from static ones.

NW restored perception of flashing light in blind mice

Like other retinal prostheses, NW arrays activated the remaining cells of the inner retina by directly depolarizing their membranes, not by neurotransmitter release. The patients with PRIMA implant reported persistent perception of flickering when stimulation frequencies were from 3 to 10 Hz, and flickering disappeared when the frequency increased to 60 Hz ¹⁸.

In our previous study, we showed that RGCs in NW arrays-attached blind mice retina responded with both transient and sustained features ²⁵. Light detection experiment in Fig. 1 showed that mice were capable of detecting static light that was on for up to 10 sec, indicating that image fading was less of a concern in our mice experiments, likely due to the freely-moving feature. We first examined the temporal resolution in RGCs responses in NW-interfaced blind mice retina by stimulating the retina using whole-field near UV (375/28 nm, 10 $\mu\text{W}\cdot\text{mm}^{-2}$) flashing light with different temporal frequencies (Fig.3a). We found that the RGCs of normal mice were activated when the light was on, and remained inactivated when the light was off (Fig.3b). The RGCs of blind mice retina did not have any light-evoked responses (Fig.3c). In NW arrays-attached blind retina, the RGCs responded to each flash faithfully at 1.25 Hz stimulation (Fig.3d). We analyzed the average firing rates and the fraction of responses in the RGCs stimulated by flashlight with different temporal frequencies, and found that the firing rate and fraction of responses were significantly higher in the RGCs of normal mice than those of blind mice, but no differences were found between RGCs in normal and NW arrays-attached blind retina (Fig.3e, f).

To evaluate the temporal resolution of NW arrays restored vision in blind mice, we tested whether NW-implanted mice could distinguish flash stimulation from constant light in a choice-box-based behavioral test (Fig.3g, Extended Data Fig.3f, Extended Movie 3). We found that after training, the correct rate of blind mice with NW arrays implant was significantly higher than that of blind mice (Fig.3h). Blind mice with NW arrays implant showed similar performance as normal mice when the temporal frequency was from 2 to 3.3 Hz. When the frequency of flash stimulation was either larger than 5 Hz or smaller than 1 Hz, the correct rate of NW-implanted blind mice decreased, but still significantly higher than that of blind mice (Fig.3h).

The spatial resolution and contrast vision in NW-implanted blind mice

Visual acuity and contrast sensitivity are the gold standards for clinical assessment of visual functions. Visual acuity is associated with the ability to resolve fine details and critical to pattern recognition, while contrast sensitivity is associated with the ability to distinguish subjects from background ³². Previous clinical studies used Landolt C-rings to measure visual acuity in patients with retinal prostheses implant, and the best recorded visual acuity was 20/1260, 20/546 and 20/460 by Argus II, AMS alpha and PRIMA, respectively ^{18,30,31}. To our knowledge, contrast sensitivity was not measured in any of the retinal prosthesis in clinical trials. In recent studies, the researchers used reprogramming techniques to recover youthful epigenetic information to restore the function of RGCs in the mice with glaucoma and the visual acuity increased from 0.20 to 0.27 cpd ⁶.

To examine visual acuity and contrast sensitivity in NW arrays-implanted blind mice, we conducted an optomotor test (Fig.4a, Extended Movie 4). In normal mice, the peak of contrast sensitivity was 0.1 cpd, and the average observed contrast sensitivity was 30 (or 3.3% contrast) (Fig.4b, c). The visual acuity of normal mice was 0.424 ± 0.012 cpd. We could not observe any optomotor response in blind mice. The average contrast sensitivity of NW arrays implanted blind mice was 5 (or 20% contrast) and the contrast sensitivity peak was at 0.05 cpd. The visual acuity of NW arrays-implanted blind mice (implanted for 9 months) was 0.313 ± 0.015 cpd (about 20/1908) (Fig.4b, c), with significant improvement from blind mice. To be noted, NW arrays have been implanted in these blind mice for 9 months.

Previous studies found that sizes of receptive field are smaller where visual acuity is higher near the foveal region of the cortex in human³³. To measure the size of the receptive field in V1 neurons of NW-implanted blind mice, we conducted two-photon calcium imaging *in vivo* (Fig.4d). It was previously known that the dorsal retina (where we implanted NW arrays) retinotopically project to lateral V1^{34,35}. We first confirmed the retinotopic projection from the retina to V1 by injecting Dil in the dorsal retina and CTB 488 in lateral V1. Indeed, Dil and CTB 488 signals overlapped in the dorsal lateral geniculate nucleus (Extended Data Fig.4). GCaMP6s were expressed in V1 neurons (Fig.4e). We mapped the receptive fields of neurons in V1 using a flashing blue square on a black background, and calculated the receptive fields based on calcium signals using Gaussian fitting (Fig.4f-h). We found that the average size of V1 receptive fields in normal mice was 233 ± 20 deg², which was similar to previous studies^{36,37}. The size of V1 receptive fields was 403 ± 86 deg² in blind mice with NW arrays implant (Fig.4i-k). Together with results from the visual acuity and contrast sensitivity test, features of receptive fields in V1 neurons in NW-implanted blind mice showed that image-forming vision was largely restored in these mice.

Long-term light-evoked responses in V1 neurons of NW-implanted blind mice

Next, we aimed to investigate whether NW arrays elicited reliable light responses in V1 neurons over a long period of time. We conducted long-term two-photon recording via a glass cranial window (Extended Data Fig.5a) over V1 to continuously record activities from the same neuronal population 3, 5, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77 and 84 days after NW arrays implantation (Fig.5b, Extended Data Fig.5c, e, Extended Movie 5). The total number of recorded neurons did not show significant difference over time (Extended Data Fig.5b). Before NW arrays implantation, V1 neurons in blind mice did not respond to light (in comparison to normal mice, Fig.5c). 26.13% and 30.24% of these neurons exhibited light-evoked responses 5 days and 7 days after implant surgery (Fig.5c), and we showed the relative percentage changes in the fluorescence ($\Delta F/F_0$) trace of 28 representatively light-responsive neurons in two mice (Extended Data Fig.5d, f), confirming NW-array-mediated light responses. The fraction of light-responsive V1 neurons in NW-implanted blind mice during the 84 days of recording were significantly larger than that of before implantation (Fig.5d).

Interestingly, the fraction of light-responsive neurons peaked around 1 week after NW arrays implantation and decreased afterwards (Fig.5d). Meanwhile, the average amplitude of calcium signals gradually increased over time, and the latency of light responses decreased over time (Fig.5e, f), indicating that neurons in V1 adapted to the stimulation by NW arrays and exhibited improvement in reliable coding of visual stimulus. 6 neurons

were tracked throughout the entire recording period, among which 3 neurons started to respond to light one month after implantation and remained responsive to light 84 days after implantation (Fig.5g), while 3 other neurons stopped to respond to light one month after implant (Fig.5h). These observations were consistent with previous studies, showing that the visual system can retain considerable plasticity beyond critical periods after blindness³⁸.

NW exhibited good biocompatibility and stability in mice and macaque monkey

To evaluate the biocompatibility of NW arrays, we examined the histology of the retina from blind mice 22 months after NW arrays implantation surgery. We examined the retinal cells at the implant site by immunostaining. We found that the retinal ganglion cells (labeled by Brn3a), bipolar cells (labeled by PKC- α), amacrine cells (labeled by HPC) did not exhibit changes 22 months after NW arrays implantation (Fig.6a). In addition, microglia cells (labeled by Iba1) were not activated. These data suggested NW arrays exhibited favorable biocompatibility in mice.

Long-term functional and behavioral experiments in NW-implanted blind mice have provided good prospects for translational study. The size of eyeballs is very different between mice and primates. Therefore, we designed NW arrays implant surgery for non-human primates (*Macaca mulatta*). We first conducted photocoagulation on the fovea and perifovea area with a 532-nm optically pumped semi-conductor laser to induce focal photoreceptors degeneration, mimicking AMD (Fig.6b). Due to the COVID-19 pandemic, we were not able to conduct surgery until 11 months after photocoagulation, by when the fixation behavior mainly mediated by macular had recovered from photocoagulation surgery (Extended Data Fig.6a), indicating that macular was no longer a good target area for NW arrays implant. A recent study indicated that subretinal implant of prosthetic device induced photoreceptor degeneration¹⁶. We identified a non-photocoagulated perifovea area for implant surgery (indicated by the white arrow in Fig.6b). To minimize the surgical trauma on the sclera and retina, we cut the NW arrays into 0.40 mm $\times$ 1.80 mm pieces, which could be delivered by 23 gauge trocars. We anesthetized the macaque monkey, performed vitrectomy, and detached the retina by subretinal injection of balanced salt solution (Lactated Ringer's solution), and made a retinotomy 3 mm in diameter. We delivered NW arrays through 23-gauge trocars using forceps and 8 pieces of NW arrays were subretinally inserted. Finally, we injected perfluoro-decalin liquid over the NW arrays to stabilize the implant and injected silicone oil into the vitreous cavity to support retinal reattachment. Photocoagulations were conducted to seal the opening in the retina from retinotomy (indicated by green arrows in Fig.6b).

We examined the NW arrays 4 days, 3, 4, 6, and 8 weeks after implant surgery by color fundus photography and found that NW arrays stayed in place (Fig.6b). We used optical coherence tomography to examine the interface between retina and NW arrays and found that the retina and NW arrays were in close contact 8 weeks after implantation (Fig.6c). There were no significant changes in RPE cells in autofluorescence images (Fig.6d). Red free fundus photography showed no vascular abnormality in the retina and surrounding tissue after NW arrays implant (Fig.6e). These data suggested that NW arrays have stable mechanical compatibility in subretinal space and good biocompatibility in the macaque monkey.

Visual stimulation elicited saccade behavior in non-human primate with NW implant

After NW arrays implantation in the right eye of non-human primate (*Macaca mulatta*), we conducted a monocular visually-guided saccade (VGS) behavioral test to examine whether NW arrays induced light-evoked responses (Fig.7a). The non-surgical eye was covered by eyeshade and the monkey was trained to focus on the central fixation point ($0.5^\circ \times 0.5^\circ$) at the beginning of the task within $7^\circ \times 7^\circ$ window of visual angle. A target point ($0.5^\circ \times 0.5^\circ$) appeared 1 sec later and the monkey was trained to shift its gaze from the central fixation point towards the target point in 1 sec. The target area was defined as the region within 15 degrees of distance from the target point and within 40 degrees of angle. The macaque monkey gets a reward for shifting its gaze into the target area, which was defined as a correct trial (Extended Data Fig.6b). To examine the normal saccade behavior, a peripheral stimulation was presented onto the normal visual field of the monkey's right eye with red, blue, or UV light (wavelength: Red: 642/18 nm, Blue: 465/25 nm, UV: 360/15 nm). Since NW arrays generate photocurrent upon UV, blue and green, but not red light, we used red LED (light intensity: $107.00 \mu\text{W}\cdot\text{mm}^{-2}$) to map out the corresponding visual field with photoreceptor degeneration caused by NW arrays implant. At the positions of polar angle of 200 to 250 degrees and eccentricity of 19 and 21 degrees where the NW arrays were implanted (Fig.7b), the saccade hit rate was 56.7% in 5 to 7 weeks after implantation and decreased to 35.7% in 10 to 12 weeks after implantation. In 14 to 15 weeks after implantation, the saccade hit rate was 12% and the monkey rarely responded to the red LED (Extended Movie 6), while the hit rate was more than 80% with stimuli in the normal region (Fig.7c, Extended Data Fig.6c-i, Extended Data Table 1). These results were consistent with previous study¹⁶ and confirmed the degeneration of photoreceptor caused by the implantation of NW arrays.

To examine the NW arrays mediated light responses, we used blue and UV LED light (light intensity: $107.00 \mu\text{W}\cdot\text{mm}^{-2}$) to stimulate the photoreceptor-degenerated area (Extended Movie 7), finding that compared to the hit rate stimulated by red light, the hit rate induced by blue or UV light was much larger than that induced by red light (Fig.7d, Extended Data Table 1). When blue or UV light was delivered, the distribution of end points was in the vicinity of the target point, but the red LED light could rarely elicit correct saccade trials (Fig.7e). When the light intensity was $107.00 \mu\text{W}\cdot\text{mm}^{-2}$, the mean hit rates elicited by blue and UV light were 72.5% and 74% respectively, while the mean saccade hit rate elicited by the red LED light was 12%. When the light intensity was $13.63 \mu\text{W}\cdot\text{mm}^{-2}$ for blue light or $7.32 \mu\text{W}\cdot\text{mm}^{-2}$ for UV light, the hit rate was below 40% (Fig.7f). These results indicated that stimulating NW arrays by blue or UV light-induced saccade behavior in a non-human primate, rescuing light responses in photoreceptor-degenerated area.

Discussion

In this study, we conducted a subretinal implant of NW arrays in photoreceptor-degenerated blind mice to restore image-forming vision. After improving the fabrication process in the NW arrays from our previous study²⁵, the light intensity threshold to elicit a perceptual response is $\sim 3 \mu\text{W}\cdot\text{mm}^{-2}$ in blue and $\sim 4 \mu\text{W}\cdot\text{mm}^{-2}$ in green, which is similar to the light intensity of normal offices and laboratories. This is similar, if not better, to the state-of-art retinal prosthetics and has shed light on the translational application of NW arrays. Nevertheless, further strategies need to be developed to enable the enhancement of visual responses in a dark environment. One possibility was the utilization of video goggles with light amplification function.

One important feature for image-forming vision is spatial resolution. In the clinical trials of subretinal implant alpha-IMS, even though a grating acuity of 3 cpd was reported in one patient, the majority of patients had low acuity and were not able to read ²⁹. In a photovoltaic Prima implant, the restored spatial resolution measured by visually evoked potentials (VEPs) in the visual cortex was 0.47 cpd in rats ¹⁷. In our study, the NW-array-implanted blind mice were capable of detecting moving light bars with a spatial resolution of 0.313 cpd. A high-density NW arrays ²¹ could potentially pave the way for restored vision with better spatial resolution.

In our previous study, we reported both ON-sustained and ON-transient responses in the RGCs recordings ²⁵, indicating that ON-sustained neurons may be responsible for static image perception while ON-transient neurons may be responsible for image changes (low frequency) and flicker fusion (20 Hz and above). In *in vitro* experiments in mice, we found that NW arrays elicited reliable responses at 3 Hz and below in ON-transient RGCs. In behavior experiments, NW-implanted blind mice also exhibited behavioral responses at 3 Hz and below. These results indicate that NW-array-mediated image-forming vision enables the perception of slow images changes at 3 Hz, with compensation in flicker fusion.

Long-term implantation of NW arrays in the subretinal space of monkeys has shed light on the translational study. Comparing to existing retinal prosthetic devices which are usually a piece of big device, NW arrays can be cut into small pieces and implanted through the small accessing port, reducing infections and post-surgery complications.

One intriguing observation from our data is the transiently enhanced responses during the first week post implant together with continuously improving response reliability in the visual cortex of blind mice. Interestingly, in the blind patients with Alpha AMS subretinal implant, the performance in grating detection 3 months after the implant was better than that 2 months after implant ¹⁵. Together, these results indicated that even in adults who are well beyond the age of the critical period, the sensory cortex retains certain levels of plasticity, which potentially leads to the functional rewiring of the visual cortex and improved visual function.

Methods

Synthesis of TiO_{2-x} NW and Au@TiO_{2-x} NW arrays

In a typical synthesis, 0.5 mL tetrabutyl titanate (TBOT) was added into the mixture solution of 15 mL deionized water and 13 mL hydrochloric acid (HCl), and stirred to form a transparent solution. Then, the solution and fluorine-doped tin oxide (FTO) coated glass substrate with hydrophilic surface were transferred into a 50-mL Teflon-lined stainless-steel autoclave, and heated to 150°C for 12 h. Then it was annealed at 550°C for 3 h with a heating rate of 5°C·min⁻¹ in air. To introduce oxygen vacancies into the TiO_2 lattice, the as-synthesized TiO_2 was further annealed at 350°C in 5% H_2/Ar atmosphere for 8 h, with a flowing rate of 150 ~ 200 standard cubic centimeters per minute (sccm) to obtain the TiO_{2-x} NW arrays with oxygen vacancies. Afterwards, Au nanoparticles were coated on the TiO_2 NW arrays. In brief, the FTO substrate with TiO_{2-x} NWs was immersed into the 0.01 M HAuCl_4 aqueous solution, and the pH was tuned to ~ 4.5 by adding 0.2 M NaOH aqueous solution. After incubating for 2 h, the FTO substrate was taken out, dried and annealed at 300°C under Ar atmosphere for 2 h with a heating rate of 5 °C·min⁻¹.

For *in vivo* test, the FTO substrate was etched using a mixed solution of H₂SO₄/HF (40 wt%) with the volume ratio of 13:2 at 85°C until the total thickness of the substrate was less than 100 µm.

Animals and genotyping

Mice were reared with 23 ± 2°C room temperature, 60 ~ 65% relative humidity, and a 12 h light/12 h dark cycle. The wild-type (C57BL/6J) mice were purchased from the Slac Laboratory Animal Co. (Shanghai, China). *rd1*^{-/-}/*cDTA* lines were maintained via crossbreeding *rd1* knockout mice and *cDTA* transgenic mice. Cone diphtheria toxin subunit-A (cone-*DTA*) positive and homozygous *rd1*^{-/-} mice were sorted by genotyping methods described in our previous work ²⁵. All the mice used in this study were older than 8 weeks. All animal treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the animal facility at Fudan University.

One male rhesus monkey (*Macaca mulatta*) aging 12 years old and weighing 8 kg (in East China Normal University, Shanghai) was used in this study. This study was carried out under the recommendations of the Institutional Animal Care and Use Committee at East China Normal University. The protocol was approved by the Institutional Animal Care and Use Committee at East China Normal University.

Patch-clamp recording of retinal ganglion cells

Mice were anesthetized with isoflurane (1 ~ 2% at 0.5 ~ 1.0 L/min) and then treated with enucleation of the eye. The eye was placed in oxygenated Ranger's solution (in mM, NaCl 124, KCl 2.5, CaCl₂ 2, MgCl₂ 2, NaH₂PO₄ 1.25, NaHCO₃ 26, and glucose 22, pH 7.35; oxygenated with 95% O₂ and 5% CO₂) quickly and retina was dissected and placed in on a filter paper (Merck Millipore, Burlington, USA) in the recording chamber. Subsequently, NW arrays were attached to the inner nuclear layer of the blind retina. After a glass pipette (5 ~ 10 MΩ) was pulled by P-97 micropipette puller (Sutter Instruments, Novato, USA) filled with internal solution (in mM, potassium gluconate 105, KCl 5, CaCl₂ 0.5, MgCl₂ 2, EGTA 5, HEPES 10, Mg-ATP 4, GTP-Na 0.5, and sodium phosphocreatine 7, PH 7.4), *in vitro* patch clamp system was used to record action potentials of RGCs via MultiClamp 700B patch-clamp amplifier (Molecular Devices, San Jose, USA) and digitizer Digidata 1440 (Molecular Devices, San Jose, USA) under DIC microscope (Zeiss, Oberkochen, Germany) ³⁹.

Visual stimuli for patch clamp recording of RGCs were provided by mercury lamp (X-Cite 120PC Q, LUMEN DYNAMICS, Ontario, Canada) and Digital Micromirror Device (DMD) (wavelength: 400 nm). To investigate the response of RGCs to light with different wavelengths, mercury lamp generates UV, blue and green light, and provides the photo-stimulation via a × 40 water-immersive objective. DMD was used to present moving, static and flash visual stimuli. The moving light bar appears 10 times in 30 sec, while static light bar remains bright for 30 sec. The velocity of the moving light bar used is 6.85 degrees per sec. Different widths of the moving light bar (0.64, 1.28, 1.92, 2.56, 3.20 and 3.84 degrees) were used to study the spatial resolution capacity of

mice's RGCs. Different temporal frequencies (5.00, 2.5, 1.57, 1.25, 1.00, 0.5 Hz) of a flash stimulus ($180 \times 180 \mu\text{m}^2$) were used to study the temporal resolution capacity of mice's RGCs.

Clampfit (Axon, Scottsdale, USA) was used to analyze the action potentials of RGCs and Prism 6 (Graph pad, San Diego, USA) was used to analyze firing rates and the fraction of responses. Firing rate is the number of action potentials, which occurred during visual stimulation, over the stimulation time (1.4 sec). Fraction of responses is calculated as the rate of the action potentials in ten times of visual stimulation.

Implant surgery in mice

The *rd1*^{-/-}/*cDTA* mice (blind mice) aged 8 to 10 weeks were anesthetized with isoflurane (1 ~ 2% at 0.5 ~ 1.0 L/min). The eye-ball was fixed in a suitable position for implantation by suturing the upper and lower eyelids. A small incision was made on the dorsal sclera, and 1 ~ 2 μL of 0.9% sodium chloride solution was injected rapidly and carefully with nano project to create the space for NW arrays implantation. This step was prone to bleeding and required prompt compressions and other effective methods for hemostasis. Expanding the incision at an angle of 45 ~ 60 degrees to the nasal-temporal axis to leave enough space for NW arrays implant, which was depended on the size of the substrate size of NW arrays. The typical size of implant NW arrays was 0.5 ~ 1 mm^2 , which were sterilized by steam under high pressure. NW arrays were clamped by tweezers and inserted carefully into the cut along the direction of the incision under the microscope. After the implant, washed the eye-ball with 0.9% sodium chloride solution and removed the suture, applied erythromycin eye ointment finally.

Choice-box-based Behavioral Test

The choice-box (240 mm $\times$ 240 mm $\times$ 180 mm) is composed of acrylic sheets, a water reward system (Kamoer, Shanghai, China), which is composed of water pipe, water port, and water pump, and an audio signal system, including a buzzer. Visual stimuli appear at two positions in the visual stimulation panel (one side of the box opposite to the water port). LED visual stimulation, water reward system and the audio signal system are all controlled by an Arduino board.

Choice-box-based behavior test is divided into adaptation phase, training phase and testing phase⁴⁰. During the adaption phase, mice undergo water restriction 3 days before the training phase. During the training phase, mice can first freely explore and acclimatize themselves to the environment of the choice box for 5 ~ 10 min before the test starts. Subsequently, an audio signal is sent to indicate the mice of the upcoming visual stimulus, which then appear at two random positions in the visual stimulation panel. The stimulation duration and reaction time are both 20 sec. Within the stimulation duration, the mice need to touch the corresponding position of the target visual stimulus either by their paws or noses. Afterward, another audio signal will be given and the mice can obtain a water reward at the water port. If the mice touch the visual stimulation panel before the stimulation appears, the next trial will pause until the mice leave the panel. If the mice do not touch the visual stimulation panel within the stimulation duration, another visual stimulation trial will proceed. The

interval of each trial is at least 10 sec, and 40 trials are carried out daily. The testing phase is similar to the training phase, except that the stimulation duration and the reaction time are reduced to 10 sec, and the interval between each trial is at least 5 sec.

Different visual stimulations were used in our study in different experiments. LED light with different wavelengths and light intensities was used. LED visual stimuli appear at one of two positions randomly in the visual stimulation panel. Mice need to touch within 6 cm in diameter centered at the on-LED in the visual stimulation panel as the center to get water reward. In the testing phase, UV LED light (375/15 nm, 6.24 $\mu\text{W}\cdot\text{mm}^{-2}$), blue LED light (465/25 nm, light intensity: 6.75, 3.31, 2.93, 1.53 and 0.64 $\mu\text{W}\cdot\text{mm}^{-2}$), and green LED light (535/28 nm, light intensities: 7.83, 4.20, 2.04, and 1.15 $\mu\text{W}\cdot\text{mm}^{-2}$) were used.

Moreover, both moving and static light bar were used, presented by a projector (CB-S41, EPSON, Suwa Japan). The moving light bar stimulus appears at one of the two positions randomly in the visual stimulation panel, and a static light bar stimulus appears at the other position simultaneously. Mice can get a water reward if they touch within the moving range of the moving light bar. In the testing phase, static or moving light bar stimulus with different widths and velocities (width of light bar: 2.63, 5.25, 7.57, 7.88, and 3.94 degrees; velocity of moving light bar: 5.23, 6.54, 7.85, 9.18, and 10.47 degrees/sec) were used.

Finally, a flash stimulus and constantly bright stimulus were used, presented by a projector (CB-S41, EPSON, Suwa, Japan). A flash stimulus (6 cm $\times$ 6 cm square) appears at one of the two positions randomly in the visual stimulation panel, and a constantly bright stimulus appears at the other position simultaneously. Mice need to touch the position of flash stimulus to get a water reward. In the testing phase, temporal frequencies of 1, 1.25, 2, 3.33 and 5 Hz were used for the flash stimulus.

If mice touch the corresponding area of the visual stimulation panel within the required time, it is a correct trial. If mice do not touch the visual stimulation panel, it is considered as a miss trial. The wrong trial refers to the case when mice touch the non-corresponding position of the visual stimulation panel. When the correct rate of *rd1^{-/-}/cDTA* mice, which have been implanted with Au@TiO_{2-x} NW arrays, reaches 70% and maintains stability for 3 days, the testing phase starts.

Visual acuity and contrast sensitivity measurement

To measure the visual acuity and contrast sensitivity, we developed a system with 4 LCD screens arranged in a quadrangle, a cylindrical mice platform (height: 12 cm, radius: 4 cm) and a camera on the top of the system to record the experiment. Visual stimulus was generated by Python, using the Psychopy toolbox ⁴¹. There are two alternating moving directions (0 degrees, 180 degrees) of the stimulus, lasting 10 sec for 3 times.

Mice were placed one at a time on the platform and the animals were allowed to move freely. When a grating perceptible to the mouse was showed on the screen, the mouse normally stopped moving its body and would begin to track the grating with reflexive head movements accompanying the rotation. Before the test started, all animals were habituated by handling and placing them on the platform for several minutes at a time. If the

animal slipped or jumped off the platform during the course of testing, it was simply returned to the platform and testing was resumed.

Immunohistochemistry

Mice were deeply anesthetized using overdose isoflurane for euthanasia and perfused transcardially with physiological saline, followed by 4% paraformaldehyde (PFA) using a perfusion pump. Brains were kept in 4% PFA for fixation at 4°C overnight and then dehydrated in 30% sucrose until the sample sunk to the bottom. After embedded and frozen, brains' tissue was sectioned into 30-μm coronal slices in a cryostat (Leica CM 1950, Leica, Wetzlar, Germany). The slices were washed with Tris-buffered saline (TBS) 5 times (5 min each), covered with coverslips and mounted to photograph by a fluorescence microscope (A1R, Nikon, Tokyo, Japan).

With regard to immunohistochemistry of mice's retina, the retina was fixated in 4% PFA for 4 ~ 7 h at 4°C, after enucleation of eye and dissection of the retina. After fixation, the retina was dehydrated using 10%, 20% and 30% sucrose solution respectively. After dehydration, the retina was embedded in OCT compound at -80°C for more than 2 h, and subsequently sectioned into 14 μm slices in a cryostat (Leica CM 1950, Leica, Wetzlar, Germany). After rinsed by TBS and immersed in 0.5% Triton-X-100 for 20 min, slices were incubated in a blocking solution consisting of DST for 20 h. Afterward, slices were incubated by primary antibody (anti-Choline Acetyltransferase and anti-Brn3a, Millipore, Massachusetts, USA, 1:200) diluted in blocking solution overnight. The next day, slices were washed 3 times (5 min each), and secondary antibody (Donkey anti-Goat conjugated to Alexa Fluor 594, Jackson ImmunoResearch, West Grove, USA, 1:300) diluted in blocking solution was used to cover the slices for 2 h in darkness. Slices were then stained with 1:3000 DAPI after rinsing, and finally rinsed, air-dried, and coverslipped. A fluorescence imaging microscope (A1R, Nikon, Tokyo, Japan) was used to obtain fluorescence images of the slices. The fluorescence image was then processed using Adobe Photoshop CC 2018.

Intravitreal injection

The mice were anesthetized with isoflurane (1 ~ 2% at 0.5 ~ 1.0 L/min). Subsequently, 69 nL Dil Stain Perchlorate (1.5mg/mL, D282, Invitrogen, USA) was injected into the nasal dorsal retina of *rd1^{-/-}/cDTA* mice, in which the NW arrays were typically implanted, using a Nanoject II (Drummond scientific company, Broomall, USA). Following surgery, mice were placed on a heating pad to recover and monitored for postoperative health.

Surgery for CTB-488 and GCaMP6s virus injections in primary visual cortex

CTB-488 (2 mg/μL, C22841, Life Technology, USA) was injected into the medial V1 in *rd1^{-/-}/cDTA* mice which had been treated with intravitreal injection. Four coordinates used for targeting V1 were as follows: 3.2 mm posterior and 2.0 mm lateral, 3.6 mm posterior and 2.0 mm lateral, 3.2 mm posterior and 2.5 mm lateral, 3.6 mm posterior and 2.5 mm lateral of bregma, at depths of 0.45, 0.33 and 0.20 mm depth (60 nL in each deep)

using a Nanoject II (Drummond scientific company, Broomall, USA). Following surgery, mice were placed on a heating pad to recover and monitored for postoperative health.

To prepare animals for recording, we injected an adeno associated virus (AAV) that expresses GCaMP into the visual cortex^{42,43} in *rd1^{-/-}/cDTA* mice. At the time of surgery, all mice were 2 months old and anesthetized with isoflurane (1 ~ 2% at 0.5 ~ 1.0 L/min). We removed the skin above the surface of the brain and dried the skull using air. To express GCaMP specifically in visual cortex neurons, we unilaterally injected AAV2/8-hSyn-GCaMP6s (Taitool Bioscience Co., LTD, Shanghai, China) into the right V1. To target the V1 layer 2/3, we made a small craniotomy and injected virus in three sites (2.1 mm lateral and 3.2 mm posterior, 2.5 mm lateral and 3.2 mm posterior, 2.3 mm lateral and 3.6 mm posterior of bregma) at depths of 0.40 and 0.25 mm using a Nanoject II (Drummond scientific company, Broomall, USA). At each depth, 100 nL was delivered manually in 25 × 4 nL steps, with approximately 20 sec pauses between steps. Virus injection lasted several minutes, and then the injection pipette was slowly removed over the course of several minutes.

Cranial window implantation

Surgery for cranial window implantation was performed 6 ~ 8 weeks after virus injections when most neurons exhibited cytosolic-only GCaMP6 expression^{44,45}. Mice were anesthetized with isoflurane (1 ~ 1.5% at 1 ~ 1.5 L/min). The depth of anesthesia was monitored by the pinch withdrawal reflex throughout the surgery. Core body temperature maintained at 37 ~ 38°C using a heating pad. Eyes were protected from dehydration during the surgery with eye ointment. The scalp overlaying the visual cortex was removed, the center of the glass coverslip was positioned above right V1, 2.3 mm lateral and 1.3 mm anterior of lambda. To gain optical access to the cortex, a 2.5 mm diameter craniotomy was performed. After removing the skull flap, the cortical surface was kept moist with physiological saline (0.9% NaCl). The dura was left intact and any occasional bleedings were immediately stopped with Gelfoam (Fu Kang Sen Medical Equipment Co., LTD, Guilin, China). A 3 mm diameter glass coverslip sterilized in ethanol (0.1 mm thickness) was fixed on the brain using cyanoacrylate-based glue (3M Animal Care Products, St. Paul, USA) to gently compress the underlying cortex. After waiting for fifteen minutes for this glue to dry, we applied dental cement (Super Bond C&B, Japan) to attach a fixed bar allowing for subsequent head fixation during subsequent imaging, and glass coverslip to the cranium. After waiting for twenty minutes for the dental cement to cure, the surgery was completed. Mice were recovering on a heating pad for one hour and returned to their home cage after being injected with ceftiofur sodium by intraperitoneal (5 mg/kg body weight, Quan Yu Biotechnology Animal Pharmaceutical Co., LTD, Shanghai, China) and dexamethasone sodium phosphate by intramuscular (0.1 mg/kg body weight, Quanyu Biotechnology Animal Pharmaceutical Co., LTD, Shanghai, China) mixture dissolved in saline.

Two-photon microscope setup and neuronal imaging

Recordings of neuronal activity were performed with Olympus FluoView FVMPE-RS upright two-photon laser-scanning system with an Olympus XL Plan N25 × /1.05 WMP ∞/0.23/FN/18 dipping objective (Olympus, Tokyo, Japan). Two-photon excitation was performed using 920 nm MAITAI eHPDS-OL laser (Mai Tai, Spectra-

Physics, Santa Clara, USA), and emitted fluorescence was detected through a 495 ~ 540 nm bandpass filter. For imaging of GCaMP6s expression, fields of view were imaged with a resolution of 512 × 512 pixels at 30 Hz. Imaging sessions lasted 2 ~ 3 h and included 1 ~ 2 h of effective imaging time.

On the day of blue LED stimulus recording mice were placed in a stereotaxic apparatus, in which the animal's head was rigidly held in a specially designed holder in the awake state, during the imaging sessions. Anesthesia was maintained throughout receptive field stimuli recording with isoflurane (0.5 ~ 1.0% at 1 ~ 1.5 L/min), and body temperature was monitored continuously to judge the animal's health and maintain proper anesthesia. We administered topical 3% sodium hyaluronate eye drops to prevent drying eyes during the recording.

Visual stimulus for two-photon Calcium imaging.

Blue LED was used as light sources for the light-responsive experiment. LED was located 6 cm away from the mouse eye. Each experiment trial started with a blank period for 20 ~ 25 sec randomly, and a LED stimulation was subsequently turned on for 1 sec, repeated 6 times.

Stimuli used in receptive field mapping were generated by Python, using the Psychopy toolbox ⁴¹. The screen was shown adjusted to be 45 degrees from the anteroposterior axis, 12 cm from the mouse's eye, subtending 96 and 80 degrees of the horizontal and vertical visual field, respectively. We used a sparse noise stimulus, consisting of blue squares presented on a black background along a grid of 6×5 squares to map RFs, each square is ~ 16×16 degrees. The squares were presented for either 1 sec with a 10-sec interval for the anesthetized mice with 8 repetitions.

Analysis of *in vivo* calcium imaging data

In vivo calcium movies were pre-processed in python using a custom-built pipeline based on CalmAn package ⁴⁶ for large-scale calcium imaging data analysis. Movies were motion-corrected using a rigid registration method to remove motion artifacts ⁴⁷. Calcium activity traces of single neuron were extracted from the registered movie using a constrained non-negative matrix factorization (CNMF) framework ⁴⁸. Spatial correlation thresholds for ROI detection were set to 0.85, and the signal to noise ratio for accepting a component was set to 2.50. The automatic detection was manually screened to ensure correct segmentation of somatic calcium activity. This pipeline generated a set of spatial footprints and temporal traces for each animal on each day of recording. The relative percentage changes in fluorescence ($\Delta F/F$) trace were used in further analyses.

Light responsive neurons identification

To identify light-responsive neurons, we defined a pre-stimulus period as the 2 sec window (60 frames) preceding the stimulus onset, and the baseline of the trial is the mean $\Delta F/F$ during the pre-stimulus period. We also defined a post-stimulus period as the 5 sec window (150 frames) following the stimulus onset. A neuron was considered responsive to the stimulus if the maximum $\Delta F/F$ during the post-stimulus period was more than five times SD above the baseline and the time to decay half-peak must over 10 frames in more than 50% of the trials. **Mean $\Delta F/F$ amplitude, latency.** To estimate an ROI's $\Delta F/F$ mean amplitude during its activation, we isolated the $\Delta F/F$ traces during each light-evoked spiking segment and used the maximum $\Delta F/F$ value at each segment as a measurement of the $\Delta F/F$ amplitude during that segment. The time point after the stimulus and firstly exceeded the threshold was used as the latency. The average of these maximal values and time points for light-responsive neurons were computed as the mean $\Delta F/F$ and latency. To compare the changes of latency and mean $\Delta F/F$ amplitude, we took the data recorded before implantation and the 3rd day after implantation as the standard of latency and mean $\Delta F/F$ amplitude, respectively. **Fitting spatial RFs.** The retinotopic organization of single neuron was assessed by measuring the average $\Delta F/F$ response to each of the 30 stimulus positions (6×5 grid) with 8 repetitions. First, these data were interpolated by a 2D bilinear interpolation. Afterward, and fit by least-squares regression with a two-dimensional Gaussian Model. Neurons whose receptive field center within 5 degrees from the edge of the screen were selected for further analysis.

Surgical procedures for NW arrays implantation in monkey

Monkey (*Macaca mulatta*) was anesthetized with intramuscular tiletamine hydrochloride and zolazepam hydrochloride (Zoletil 50, 0.1 mL/kg body weight, Virbac S.A., France) after intramuscular atropine (0.5 mg/mL, 0.1 mL/kg body weight, Heulton Animal Pharmaceutical Co., LTD, Sichuan, China) 20 min later and isoflurane inhalation maintained general anesthesia during retinal implant surgery. Mydriasis in the right eye was induced by tropicamide (5 mg/mL, [Santen Pharmaceutical Co., LTD](#), Osaka, Japan). Lateral canthotomy was performed to allow 23-gauge trocars (Carl Zeiss Meditec AG, Jena, Germany) for vitrectomy and a retinal bleb was created by subretinal injection of balanced salt solution¹⁶. NW implants (5 pieces of implantation in all, 0.4 mm width and 1.8 mm length for each piece) were inserted subretinally through 3 mm-wide sclerotomies (Constellation vision system, Fort Worth, USA). The detached retina was reattached with injection perfluorocarbon liquid (Bausch & Lomb, Rochester, USA) and silicone oil (Arciolane3000, Arcadophta SARL, France). Retinal laser photocoagulation was operated at the border of the retinotomy^{49,50}.

Eyes Examinations for Monkey

One to two drops of Compound Tropicamide Eye Drops (Santen Pharmaceutical Co., LTD, Shiga Plant, Japan) were instilled into monkeys' eyes to dilute its pupil 3 times and 1 h before the examination began. Monkey was injected with atropine (0.5 mg/mL, 0.1 mL/kg body weight, Heulton Animal Pharmaceutical Co., LTD, Sichuan, China) and then anesthetized with intramuscular injection of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil 50, 0.1 mL/kg body weight, Virbac S.A., France). After anesthetization, Monkey was then placed prone in a supporting apparatus to receive eye examinations.

Color, Red-free, and autofluorescence fundus photography (TRC-50DX, TOPCON, Tokyo, Japan) of monkey's right eye were taken before photocoagulation, 11 months after photocoagulation, 4 days, 3, 4, 6 and 8 weeks after implantation with Au@TiO_{2-x} NW arrays. Optic Coherence Tomography (OCT) (Cirrus HD-OCT 4000, Zeiss, Oberkochen Germany) was also used to scan the retinal structure and the corresponding places of Au@TiO_{2-x} NW arrays in the retina of the right eye of the monkey. OCT images were taken 4 days, 3, 4, 6 and 8 weeks after implantation with Au@TiO_{2-x} NW arrays. Silt lamp (Oculus Optikgerate GmbH, Wetzlar, Germany) was used to examine the monkey's lenses, cornea and anterior chamber by providing direct visualization.

Recording of eye position and visually-guided saccade task

The monkey was seated in a chair which placed on a platform in front of the screen (the distance between the monkey's eyes and the screen is 30 cm) and looked flat at the central fixation point. A lightweight acrylic cap was implanted for head stabilization chronically. Monkey's head was fixed by a custom-made head holder to keep the head stable during experiment. Eye position was monitored and digitalized by an infrared eye system, including an eye-tracking camera (above the center of the screen), infrared illuminator and Wise Center software for real-time recording of traces of pupil's position. Visual stimulations were presented by red 642/18 nm), blue (465/25 nm) and UV (360/15 nm), the stimuli in this task were generated by TEMPO experiment control system (Reflective computing, Olympia, WA, USA), Neuron task software and Arduino. A variable DC power supply was used to control the light intensity of the LED light.

During visual-guided saccade task trials, the monkey maintained fixation for 1000 msec after fixation onset. After the fixation point (0.5° × 0.5°) disappeared, a red, blue, or UV target point (0.5 degrees), with a centrifugal rate from the fixation point of 17 to 21 degrees (interval 2 deg) and a pole angle of 0 to 350 degrees (interval 10 degrees) appeared. The animal needed to make a saccade to the target point within 1 sec of the onset of the target point for a fluid reward.

To calculate the saccadic endpoint, we calculated the velocity changes of eye movement trajectories using self-written python code and determined the mean eye position in a time window when the eye position was stationary (50 ~ 100 msec after the peak velocity) as the previous study²⁰. The experiment animal was considered to have completed one correct saccade if the angle between the endpoint of the saccade and the target point was less than 40 degrees and the pitch was within 15 degrees. The animal was rewarded immediately when it made a correct saccadic eye movement. With regards to normalized saccadic endpoints location indicated by θ and ρ in the polar coordinate, θ is the angular difference between the saccadic endpoints and target points, and ρ is the normalized distance between the saccadic endpoints and target points

$$= \frac{\sqrt{x_{\text{saccadic endpoint}}^2 + y_{\text{saccadic endpoint}}^2}}{\text{Eccentricity of target point}}).$$

Statistical analysis

The differences were tested by Prism (Graphpad, San Diego, CA). Comparisons between two groups were made by unpaired and paired two-tailed student's t-test, and comparisons between three or more groups were made by one-way ANOVA. Results of the choice-box-based behavioral test were analyzed by two-way repeated-measures (RM) ANOVA followed by the Tukey post-hoc test. Results of patch-clamp recording and visually-guided saccade test were analyzed by one-way repeated-measures ANOVA followed by the Dunnett post-hoc test. With regards to the fitting model, we used log-linear regression to fit the data. $P < 0.05$ was considered statistically significant. Data are presented as mean $\pm$ SEM.

Data availability

Data used from this study are available from the corresponding author upon request.

Code availability

Custom-written codes used to analyze data from this study are available from the corresponding author upon request.

Declarations

Acknowledgments The authors thank the following funding agencies for supporting this work: the NSF of China (31771195, 81790640, 82021002, 82071200, 22025502), a Shanghai Municipal Science and Technology Major Project (2018SHZDZX01) and ZJLab, Key Scientific Technological Innovation Research Project of the Ministry of Education, Sanming Project of Medicine in Shenzhen (SZSM202011015), Shanghai Hospital Development Center (SHDC2020CR4007), Shanghai Health and Family Planning Commission (20164Y0096, 20184Y0184).

Author contributions J.Z., B.Y. and Y. Y. conceived the study. J.Z. and B.Y. wrote the paper. R.Y. performed the behavioral experiments of mice and monkeys, refined the techniques, and analyzed the data, with help from P.Z., K.S., L.F., R.M., C.P. and G.Z. synthesized the Au@TiO_{2-x} NW arrays. P. Z and R.Y. developed two-photon microscope setups and chronic calcium imaging. Z.W., S.W. and G.W. performed *in vitro* patch clamp experiments. L.W., M.S., C.D., F.Y. and Y.Y. conducted the surgical procedures for NW arrays implantation in monkey. C.F., Y.Z., A.C., F.Z. and Y.H. worked on monkeys' eyes examinations. S.J., W.X. and F.W. worked on choice-box-based behavioral device assembly. All authors discussed the results and commented on the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to J.Z., B.Y., or Y.Y.

References

- 1 Maguire, A. M. *et al.* Safety and efficacy of gene transfer for Leber's congenital amaurosis. *The New England journal of medicine* **358**, 2240-2248, doi:10.1056/NEJMoa0802315 (2008).
- 2 Cascella, R. *et al.* Towards the application of precision medicine in Age-Related Macular Degeneration. *Prog Retin Eye Res* **63**, 132-146, doi:10.1016/j.preteyeres.2017.11.004 (2018).
- 3 Zhou, H. *et al.* Glia-to-Neuron Conversion by CRISPR-CasRx Alleviates Symptoms of Neurological Disease in Mice. *Cell* **181**, 590-603.e516, doi:10.1016/j.cell.2020.03.024 (2020).
- 4 Mahato, B. *et al.* Pharmacologic fibroblast reprogramming into photoreceptors restores vision. *Nature* **581**, 83-88, doi:10.1038/s41586-020-2201-4 (2020).
- 5 Hoang, T. *et al.* Gene regulatory networks controlling vertebrate retinal regeneration. *Science (New York, N.Y.)* **370**, doi:10.1126/science.abb8598 (2020).
- 6 Lu, Y. *et al.* Reprogramming to recover youthful epigenetic information and restore vision. *Nature* **588**, 124-129, doi:10.1038/s41586-020-2975-4 (2020).
- 7 Maeder, M. L. *et al.* Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. *Nature medicine* **25**, 229-233, doi:10.1038/s41591-018-0327-9 (2019).
- 8 Roska, B. & Sahel, J. A. Restoring vision. *Nature* **557**, 359-367, doi:10.1038/s41586-018-0076-4 (2018).
- 9 Sahel, J. A., Bennett, J. & Roska, B. Depicting brighter possibilities for treating blindness. *Science translational medicine* **11**, doi:10.1126/scitranslmed.aax2324 (2019).
- 10 Humayun, M. S. *et al.* Visual perception elicited by electrical stimulation of retina in blind humans. *Archives of ophthalmology (Chicago, Ill. : 1960)* **114**, 40-46, doi:10.1001/archopht.1996.01100130038006 (1996).
- 11 da Cruz, L. *et al.* Five-Year Safety and Performance Results from the Argus II Retinal Prosthesis System Clinical Trial. *Ophthalmology* **123**, 2248-2254, doi:10.1016/j.ophtha.2016.06.049 (2016).
- 12 Wilke, R. *et al.* Spatial resolution and perception of patterns mediated by a subretinal 16-electrode array in patients blinded by hereditary retinal dystrophies. *Investigative ophthalmology & visual science* **52**, 5995-6003, doi:10.1167/iovs.10-6946 (2011).
- 13 Mills, J. O., Jalil, A. & Stanga, P. E. Electronic retinal implants and artificial vision: journey and present. *Eye (London, England)* **31**, 1383-1398, doi:10.1038/eye.2017.65 (2017).
- 14 Stingl, K. *et al.* Interim Results of a Multicenter Trial with the New Electronic Subretinal Implant Alpha AMS in 15 Patients Blind from Inherited Retinal Degenerations. *Front Neurosci* **11**, 445,

doi:10.3389/fnins.2017.00445 (2017).

- 15 Edwards, T. L. *et al.* Assessment of the Electronic Retinal Implant Alpha AMS in Restoring Vision to Blind Patients with End-Stage Retinitis Pigmentosa. *Ophthalmology* **125**, 432-443, doi:10.1016/j.ophtha.2017.09.019 (2018).
- 16 Prevot, P. H. *et al.* Behavioural responses to a photovoltaic subretinal prosthesis implanted in non-human primates. *Nat Biomed Eng* **4**, 172-180, doi:10.1038/s41551-019-0484-2 (2020).
- 17 Lorach, H. *et al.* Photovoltaic restoration of sight with high visual acuity. *Nat Med* **21**, 476-482, doi:10.1038/nm.3851 (2015).
- 18 Palanker, D., Le Mer, Y., Mohand-Said, S., Muqit, M. & Sahel, J. A. Photovoltaic Restoration of Central Vision in Atrophic Age-Related Macular Degeneration. *Ophthalmology* **127**, 1097-1104, doi:10.1016/j.ophtha.2020.02.024 (2020).
- 19 Beauchamp, M. S. *et al.* Dynamic Stimulation of Visual Cortex Produces Form Vision in Sighted and Blind Humans. *Cell* **181**, 774-783 e775, doi:10.1016/j.cell.2020.04.033 (2020).
- 20 Chen, X., Wang, F., Fernandez, E. & Roelfsema, P. R. Shape perception via a high-channel-count neuroprosthesis in monkey visual cortex. *Science* **370**, 1191-1196, doi:10.1126/science.abd7435 (2020).
- 21 Gu, L. *et al.* A biomimetic eye with a hemispherical perovskite nanowire array retina. *Nature* **581**, 278-282, doi:10.1038/s41586-020-2285-x (2020).
- 22 Nelidova, D. *et al.* Restoring light sensitivity using tunable near-infrared sensors. *Science* **368**, 1108-1113, doi:10.1126/science.aaz5887 (2020).
- 23 Maya-Vetencourt, J. F. *et al.* Subretinally injected semiconducting polymer nanoparticles rescue vision in a rat model of retinal dystrophy. *Nat Nanotechnol* **15**, 698-708, doi:10.1038/s41565-020-0696-3 (2020).
- 24 Acaron Ledesma, H. *et al.* An atlas of nano-enabled neural interfaces. *Nat Nanotechnol* **14**, 645-657, doi:10.1038/s41565-019-0487-x (2019).
- 25 Tang, J. *et al.* Nanowire arrays restore vision in blind mice. *Nature communications* **9**, 786, doi:10.1038/s41467-018-03212-0 (2018).
- 26 Li, J. *et al.* Nanoscale stacking fault-assisted room temperature plasticity in flash-sintered TiO(2). *Science advances* **5**, eaaw5519, doi:10.1126/sciadv.aaw5519 (2019).
- 27 Wang, G., Wang, H., Ling, Y., Tang, Y. & Li, Y. Hydrogen-Treated TiO₂ Nanowire Arrays for Photoelectrochemical Water Splitting. *Nano letters* **11**, 3026-3033 (2011).
- 28 Geruschat, D. R. *et al.* An analysis of observer-rated functional vision in patients implanted with the Argus II Retinal Prosthesis System at three years. *Clinical & experimental optometry* **99**, 227-232, doi:10.1111/cxo.12359 (2016).

- 29 Stingl, K. *et al.* Artificial vision with wirelessly powered subretinal electronic implant alpha-IMS. *Proceedings. Biological sciences* **280**, 20130077, doi:10.1098/rspb.2013.0077 (2013).
- 30 Stingl, K. *et al.* Subretinal Visual Implant Alpha IMS–Clinical trial interim report. *Vision Res* **111**, 149-160, doi:10.1016/j.visres.2015.03.001 (2015).
- 31 Humayun, M. S. *et al.* Interim results from the international trial of Second Sight's visual prosthesis. *Ophthalmology* **119**, 779-788, doi:10.1016/j.ophtha.2011.09.028 (2012).
- 32 Xiong, Y.-Z. *et al.* Relationship Between Acuity and Contrast Sensitivity: Differences Due to Eye Disease. *Investigative ophthalmology & visual science* **61**, 40-40, doi:10.1167/iovs.61.6.40 (2020).
- 33 Harvey, B. M. & Dumoulin, S. O. The relationship between cortical magnification factor and population receptive field size in human visual cortex: constancies in cortical architecture. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **31**, 13604-13612, doi:10.1523/JNEUROSCI.2572-11.2011 (2011).
- 34 Wang, Q. & Burkhalter, A. Area map of mouse visual cortex. *The Journal of comparative neurology* **502**, 339-357, doi:10.1002/cne.21286 (2007).
- 35 Sterratt, D. C., Lyngholm, D., Willshaw, D. J. & Thompson, I. D. Standard anatomical and visual space for the mouse retina: computational reconstruction and transformation of flattened retinæ with the Retistruct package. *PLoS computational biology* **9**, e1002921, doi:10.1371/journal.pcbi.1002921 (2013).
- 36 Roth, M. M. *et al.* Thalamic nuclei convey diverse contextual information to layer 1 of visual cortex. *Nature neuroscience* **19**, 299-307, doi:10.1038/nn.4197 (2016).
- 37 Samonds, J. M., Feese, B. D., Lee, T. S. & Kuhlman, S. J. Nonuniform surround suppression of visual responses in mouse V1. *Journal of neurophysiology* **118**, 3282-3292, doi:10.1152/jn.00172.2017 (2017).
- 38 Kalia, A. *et al.* Development of pattern vision following early and extended blindness. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 2035-2039, doi:10.1073/pnas.1311041111 (2014).
- 39 Boinagrov, D. *et al.* Upper threshold of extracellular neural stimulation. *Journal of neurophysiology* **108**, 3233-3238, doi:10.1152/jn.01058.2011 (2012).
- 40 Li, X. *et al.* Serotonin receptor 2c-expressing cells in the ventral CA1 control attention via innervation of the Edinger-Westphal nucleus. *Nature neuroscience* **21**, 1239-1250, doi:10.1038/s41593-018-0207-0 (2018).
- 41 Peirce, J. W. PsychoPy–Psychophysics software in Python. *Journal of neuroscience methods* **162**, 8-13, doi:10.1016/j.jneumeth.2006.11.017 (2007).
- 42 Fang, Q. *et al.* A Differential Circuit via Retino-Colliculo-Pulvinar Pathway Enhances Feature Selectivity in Visual Cortex through Surround Suppression. *Neuron* **105**, 355-369.e356, doi:10.1016/j.neuron.2019.10.027 (2020).

- 43 Carrillo-Reid, L., Han, S., Yang, W., Akrouh, A. & Yuste, R. Controlling Visually Guided Behavior by Holographic Recalling of Cortical Ensembles. *Cell* **178**, 447-457.e445, doi:10.1016/j.cell.2019.05.045 (2019).
- 44 Rossi, L. F., Harris, K. D. & Carandini, M. Spatial connectivity matches direction selectivity in visual cortex. *Nature* **588**, 648-652, doi:10.1038/s41586-020-2894-4 (2020).
- 45 Rasmussen, R., Matsumoto, A., Dahlstrup Sietam, M. & Yonehara, K. A segregated cortical stream for retinal direction selectivity. *Nature communications* **11**, 831, doi:10.1038/s41467-020-14643-z (2020).
- 46 Giovannucci, A. *et al.* CalmAn an open source tool for scalable calcium imaging data analysis. *eLife* **8**, doi:10.7554/eLife.38173 (2019).
- 47 Pnevmatikakis, E. A. & Giovannucci, A. NoRMCorre: An online algorithm for piecewise rigid motion correction of calcium imaging data. *Journal of neuroscience methods* **291**, 83-94, doi:10.1016/j.jneumeth.2017.07.031 (2017).
- 48 Pnevmatikakis, E. A. *et al.* Simultaneous Denoising, Deconvolution, and Demixing of Calcium Imaging Data. *Neuron* **89**, 285-299, doi:10.1016/j.neuron.2015.11.037 (2016).
- 49 Matsuo, T. *et al.* Visual Evoked Potential Recovery by Subretinal Implantation of Photoelectric Dye-Coupled Thin Film Retinal Prosthesis in Monkey Eyes With Macular Degeneration. *Artificial organs* **42**, E186-e203, doi:10.1111/aor.13120 (2018).
- 50 Tu, H. Y. *et al.* Medium- to long-term survival and functional examination of human iPSC-derived retinas in rat and primate models of retinal degeneration. *EBioMedicine* **39**, 562-574, doi:10.1016/j.ebiom.2018.11.028 (2019).

Figures

Figure 1

Choice-box-based behavioral test for the localization of light sources. a, Schematics of Au@TiO_{2-x} NW arrays implanted blind mice in a choice-box-based behavioral system. b, Training protocol for the choice-box-based behavioral test. The stimulation duration and reaction time were both 20 sec in the training phase. Miss rate was the number of miss trials divided by the number of all trials. The testing phase started when the miss rate was below 50% for 3 days, in which the stimulation duration and reaction time was reduced to 10 sec. Tone 1 and 2 were provided during a trial start and water reward, respectively. c, Schematics of choice-box-based behavioral test with LED light stimulus. The correct rate was calculated as the number of correct trials divided by the sum of all trials. d, Correct rate of normal mice (n = 3), blind mice with glass implant (n = 3) and blind mice with NW arrays implant (n = 4) in choice-box-based behavioral test with UV (375/15 nm, 6.24 μW·mm⁻²), green (535/28 nm, 7.83 μW·mm⁻²) and blue LEDs (465/25 nm, 6.75 μW·mm⁻²). e-f, Correct rate of normal mice (n = 3), blind mice with glass implant (n = 3) and blind mice with NW arrays implant (n = 4), in choice-box-based behavioral test using blue and green LED stimulus with different light intensities (blue LED intensities: 0.64 ~ 6.75 μW·mm⁻², green LED intensities: 1.15 ~ 7.83 μW·mm⁻²). * indicates a significant difference between blind mice with NW arrays and blind mice with glass implant, # indicates a significant difference between blind mice with NW arrays and normal mice (* and #, P < 0.05, *** and ###, P < 0.001, one-way ANOVA for d, two-way RM ANOVA for e and f). Data are shown as mean ± SEM.

Figure 2

In vitro patch-clamp recording of RGCs of NW arrays-attached blind retina and choice-box-based behavioral test with moving light bars. a, Schematics of in vitro patch-clamp recording of the RGCs in NW arrays-attached blind mice retina. b-d, RGC responded to moving light bars stimulus (width: 9.6 degrees, speed: 6.85 degrees/sec) in normal, blind and NW arrays-attached blind retinas. e-f, Average firing rates and fraction of responses of RGCs in normal, glass-attached and NW arrays-attached retinas using moving light bars with width ranging from 0.64 to 5.76 degrees. Normal mice: 9 RGCs from 6 retinas. Blind mice: 10 RGCs from 8 retinas. Blind mice with NW arrays attached: 16 RGCs from 10 retinas. g, Schematics of the choice-box-based behavioral test using moving light bar and static light bar stimulus. h-i, The correct of normal mice (n = 3), blind mice with glass implant (n = 3) and blind mice with NW arrays implant (n = 4) in choice-box-based behavior test with moving light bars of different widths and speeds (width: 2.63 ~ 7.88 degrees, speed: 5.23 ~ 10.47 degrees/sec). * indicates a significant difference between blind mice with NW arrays and blind mice with glass implant, # indicates a significant difference between blind mice with NW arrays and normal mice (* and #, $P <$

0.05, ** and ##, $P < 0.01$, *** and ###, $P < 0.001$, one-way ANOVA for e and f, two-way RM ANOVA for h and i). Data are shown as mean $\pm$ SEM.

Figure 3

In vitro patch clamp recording of RGCs of NW arrays-attached blind retina and choice-box-based behavioral test with flashing light. a, Schematics of in vitro patch clamp recording of the RGCs in NW arrays-attached blind mice retina. t indicates the interval of stimuli, which was equal to the duration of stimuli. b-d, Flashing light (duration: 0.40 sec, 1.25 Hz, 18.09 $\mu\text{W}\cdot\text{mm}^{-2}$) responses in normal, blind and NW arrays-attached blind retinas. e-f, Average firing rate and fraction of response of normal, glass-attached and NW arrays-attached retinas using flash visual stimulus with temporal frequency ranging from 0.50 to 5.00 Hz. Temporal frequency was calculated as 1 divided by t . Normal mice: 7 RGCs from 6 retinas. Blind mice: 6 RGCs from 4 retinas. Blind mice with NW arrays attached: 15 RGCs from 8 retinas. g, Schematics of choice-box-based behavioral test with

flashing light. t indicates the duration of stimuli, which was equal to the interval of stimuli. h , Correct rate of normal mice ($n = 3$), blind mice with glass implant ($n = 3$) and blind mice with NW arrays implant ($n = 4$) in choice-box-based behavior test with different temporal frequency flashing light stimulus (temporal frequency: 1.00 ~ 5.00 Hz). * indicates a significant difference between blind mice with NW arrays and blind mice with glass implant, # indicates a significant difference between blind mice with NW arrays and normal mice (** and ##, $P < 0.01$, ###, $P < 0.001$, one-way ANOVA for e and f, two-way RM ANOVA for h). Data are shown as mean $\pm$ SEM.

Figure 4

Optomotor test and receptive fields in V1 neurons of blind mice with NW arrays implant. a, Schematics of the optomotor test. The mice were placed on a platform at the center of four screens displaying grating stimuli. A video camera is used to monitor the animal's behavior from above. The grating was turned clockwise or counterclockwise. When the grating was rotated, the mouse tracked the drifting grating with head and neck movements. b, Contrast sensitivity of normal mice ($n = 5$), blind mice with glass implant ($n = 3$), and mice with NW arrays implant ($n = 4$) in optomotor test with different spatial frequencies of grating stimulus. c, The spatial frequency of normal mice ($n = 5$), blind glass-implant mice ($n = 3$), and NW-implanted mice ($n = 4$) in optomotor test. d-e, Schematics of receptive field mapping using two-photon calcium imaging recording of V1 neurons through the cranial window. The position of the cranial window corresponds to the NW implant in the retina (nasal-dorsal) retinotopically. f, Example fluorescence traces of neurons in normal mice (left) and blind mice with NW arrays implant (right) of a single button (individual trace in gray, average in black) ordered according to stimulus position. g, Responses of example neurons in Fig.4f to blue squares. The red line indicates the outline of the receptive field. h, Receptive fields in Fig.4g fitted with bilinear interpolation and least-squares regression with a two-dimensional Gaussian model. i-j, Distribution of receptive fields of normal mice and blind mice with NW arrays implant. Each ellipse curve represents the outline of the receptive field of one V1 neuron. k, Areas of the receptive field of V1 neurons in normal mice (red, 233.19 deg², 111 cells from 4 mouse) and blind mice with NW arrays implant (purple, 402.74 deg², 13 cells from 3 mouse). (***, $P < 0.001$, one-way ANOVA for c, and student's t-test for k). Data are shown as mean $\pm$ SEM.

Figure 5

Long-term in vivo two-photon calcium imaging in V1 neurons of blind mice with NW arrays implant. a, Schematics of two-photon calcium imaging in V1 neurons. b, 4 example neurons (indicated with green circles) 2 days before implant surgery and 3, 5, 7, 28, 56, 84 days after NW arrays implant surgery. c, Mean $\Delta F/F$ trace of 6 trials with blue LED stimulus (465/25, 6.75 $\mu W \cdot mm^{-2}$) in normal mice, blind mice, blind mice with NW arrays implant at 5 and 7 days after implant surgery. The data in the same row of the three heatmaps were from the same neuron. The red dotted line indicates the onset of the light stimulus. d-f, Fraction of light-responsive neurons, average event amplitude and latency of normal mice (3 imaging sites from 3 mice), blind mice before and after NW arrays implant (4 imaging sites from 3 mice) (*, $P < 0.05$, paired t-test for d). g-h, The fluorescence trace of 4 example neurons (Cell 1-4 in Fig.5b) Data are shown as mean $\pm$ SEM.

Figure 6

Immunofluorescence of NW-implanted blind mice retina and ophthalmological characterization of non-human primate with NW arrays implant. a, Representative immunofluorescence staining of blind mice retina with NW arrays implant for 22 months. Brn3a, RGCs. PKC-a, bipolar cells. HPC, amacrine cells. iba-1, microglia. PNA, cone cells, and cone domains of the interphotoreceptor matrix. b, Color fundus photograph of the right eye of the monkey before photocoagulation, 10 days and 11 months after photocoagulation, 4 days, 3, 4, 6, and 8 weeks after implantation. Eight pieces of NW arrays were subretinally implanted in the monkey's right eye. The turbid places in the fundus photograph after implantation were mainly due to the use of silicon oil in the implantation surgery. The white arrows indicate the perifovea area for implant surgery, and the green arrows indicate the region for photocoagulation to seal the opening in the retina. c, The retinal structure and the interface between the retina and NW arrays in OCT images of monkey's right eye 3, 4, 6, and 8 weeks after implantation. d, Fundus autofluorescence imaging of monkey's right eye 3, 4, 6, and 8 weeks after implantation. e, Red-free mode fundus photography of monkey's right eye 3, 4, 6, and 8 weeks after implantation.

Figure 7

Visually-guided saccade task for a non-human primate with NW arrays implant. a, Schematics of visually-guided saccade (VGS) behavioral task for macaque monkey with NW arrays implant. The monkey needs to gaze at the fixation point centered in the screen for 1 sec, and saccade to the target point within 1 sec. b, NW arrays in fundus photograph and corresponding regions in the visual field (polar angle of 200 to 250 degrees and eccentricity of 19 and 21 degrees). c, Illustration of mean hit rate in VGS task with red LED at the target point before implantation (left) and 14 ~ 15 weeks after implantation (right). d, Illustration of mean hit rate in VGS task with blue LED (left) and UV LED at the target point (right) 14 ~ 15 weeks after implantation. e, Distribution of normalized saccadic endpoints location using red (left), blue and UV (right) LED stimulus 14 ~ 15 weeks after implantation. The location of endpoints was normalized using the distance and included angle from the fixation point to target point as the standard in different trials. LED stimuli were presented at red grids regions in Fig.7b. f, Left: Hit rate of VGS task with red, blue and UV LED stimulus in NW arrays-implanted regions (Eccentricities: 19 degrees, polar angles: 240 and 250 degrees; Eccentricities: 21 degrees, polar angles: 220 ~ 240 degrees) for different light intensities (7.32 ~ 107.00 $\mu\text{W}\cdot\text{mm}^{-2}$). Right: Mean hit rate of VGS task using red, blue and UV LED stimulus (107.00 $\mu\text{W}\cdot\text{mm}^{-2}$) in NW arrays-implanted regions before implantation (99.11 $\mu\text{W}\cdot\text{mm}^{-2}$) and 14 ~ 15 weeks after implantation (107.00 $\mu\text{W}\cdot\text{mm}^{-2}$). (***, $P < 0.001$, one-way RM ANOVA for f). Red LED, 642/18 nm. Blue LED, 465/25 nm. UV LED, 360/15 nm. Data are expressed as mean $\pm$ SEM.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ExtendedDataMovie7.Visualguided saccade task with Blue LED stimulus.mp4](#)
- [ExtendedDataMovie1.Choice box based behavioral test with green LED light stimulus.mp4](#)
- [ExtendedDataMovie3.Choice box based behavioral test with flash visual stimulus.mp4](#)
- [ExtendedDataMovie6.Visualguided saccade task with Red LED stimulus.mp4](#)
- [ExtendedDataMovie4.Visual acuity and contrast sensitivity test.mp4](#)
- [ExtendedDataMovie5.Light evoked responses in V1 neurons through two-photon calcium imaging recording.mp4](#)
- [ExtendedDataMovie2.Choice box based behavioral test with moving visual stimulus.mp4](#)
- [ExtendedDataFigures.docx](#)