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# Questions about the role of P3HT nanoparticles in retinal stimulation

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ARISING FROM Maya-Vetencourt et al. *Nature Nanotechnology* https://doi.org/10.1038/s41565-020-0696-3 (2020)

Maya-Vetencourt et al.[1] recently reported that poly[3-hexylthiophene] nanoparticles (P3HT NPs) injected in the subretinal space of the rat model of retinal dystrophy (the Royal College of Surgeons (RCS) model)) "mediate light-evoked stimulation of retinal neurons and persistently rescue visual function". The article also reported that the light-evoked stimulation of retinal neurons is electrical in nature and is mediated by a capacitive coupling between the NPs and the cell membrane. To support these claims, the authors performed a series of experiments that demonstrated the P3HT NPs induced a cellular response in vitro, a retinal response ex vivo and rescue the visual functions in vivo. However, a number of inconsistencies throughout the article cast doubt on these results and their interpretation, as outlined below.

## Irradiance levels and the response characteristics

**Extremely bright irradiance ex vivo and photothermal effects.** Experiments ex vivo (for example, fig. 1c–f in ref. [1]) were performed with extremely high irradiance levels (500 ms exposures at 40 mW mm$^{-2}$ with a wavelength of 540 nm). Such irradiance is nearly 250 times higher than the maximum permissible exposure (MPE) of the retina at a 540 nm wavelength[2], 0.15 mW mm$^{-2}$. An exposure of 2 J cm$^{-2}$ exceeds even the MPE for skin[3], 0.92 J cm$^{-2}$ for a pulse length of 0.5 s. It is hard to believe that such extreme exposures have no thermal effect on the cells if the NPs are efficient absorbers. Thin films of conjugated polymers have been shown to induce thermal effects on cultured neurons, brain slices and explanted retinas when exposed to comparable light levels[4]. The intracellular voltage recording in fig. 1c in ref. [1] ($i = 0$ current–clamp conditions in the retinal ganglion cells in contact with P3HT NPs irradiated at 40 mW mm$^{-2}$) demonstrates a steady rise of the cell potential by about 40 mV during the 500 ms exposure, followed by a few seconds of slow decrease. How is this related to the electrical stimulation, which, according to fig. 1h in ref. [1], had a time constant of 5 ms? The slow rise and fall of the measured potential indicate some cumulative effect with a slow dissipation, such as heating. In fact, fig. A in the Supplementary Information[1] shows a 6 °C temperature rise at an irradiance about twice higher than that used for fig. 1c. Even though the concentration of the NPs may be different in these two experiments, it is not clear how much of the heating occurred due to the NPs and how much due to just the absorption of green light in the cells or culture medium. Besides, as the details about the thermal measurement, such as the size of the pipette and its distance to the interface, as well as the laser spot size on the sample, are not specified, it is not clear how the measured temperature is related to the maximum temperature at the interface. One indication of the temperature rise could be a decrease of the action potential amplitude[5,6], which might be visible on the non-filtered spiking recordings.

**Latencies inconsistent with electrical stimulation.** Another issue concerning fig. 1c is its relation to fig. 1d, which represents a "statistical analysis of the results in Fig. 1c"[1]. If the smooth rising curve in fig. 1c in ref. [1] represents an average of multiple spiking responses during and after the 500 ms exposure, it should be similar to the peristimulus time histogram shown in fig. 1d, which exhibits a strong response at the beginning and a strong response at the end of the 500 ms exposure, with a gap in between, which is not the case in fig. 1c.

One more observation inconsistent with electrical stimulation of the retina is the latency of the retinal response shown in fig. 1f in ref. [1]. Typically, retinal and cortical responses to subretinal electrical stimulation have a lower latency than natural ones due to the lack of phototransduction[7,8]. Here, however, the median response latency exceeded 300 ms, as opposed to sub-100 ms in the natural response. The authors relate this long latency to rewiring of the RCS retinas, but this claim contradicts multiple electrode array and visually evoked potential (VEP) measurements in RCS rats[7,8].

**Contradiction between ex vivo and in vivo stimulation thresholds.** A core contradiction in Maya-Vetencourt et al.[1] is the use of many orders of magnitude higher light intensities in all the ex vivo experiments than those ultimately used in vivo. According to fig. 1e in ref. [1], the spiking activity of the retinal explants with P3HT NPs did not increase below 20 mW mm$^{-2}$, and only a very small increase in the firing rate (4.5%) occurred at the highest irradiance (40 mW mm$^{-2}$), together with a 2% increase with glass NPs. These results are in direct contradiction with the behavioural changes observed under a very dim illumination, about 5 lx, which is at least a million times dimmer than the 20 mW mm$^{-2}$ threshold measured ex vivo. (At green wavelengths, close to the peak of retinal sensitivity, 1 lx corresponds to approximately 2 mW m$^{-2}$. So, 20 mW mm$^{-2}$ = 20 kW m$^{-2}$ = $10^7$ lx.) Unlike Maya-Vetencourt et al.[1], many other publications report a similar range of subretinal stimulation thresholds ex vivo and in vivo, of approximately 1–10 μC mm$^{-2}$ for electrodes[9,10] or 0.1–1 mW mm$^{-2}$ for 10 ms pulses with various photovoltaic cells[7,11,12].

## Stimulation mechanism

**Problems with capacitive coupling at the cell–semiconductor interface.** In the circuit model presented in fig. 1g in ref. [1] and its

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description in the Supplementary Information, the P3HT particle is assumed to be connected to an electrical ground. However, in reality, the P3HT particle is electrically floating. Therefore, the circuit diagram with a floating NP and its dynamics are different from that presented in fig. 1g in ref. [1].

Moreover, for electrical coupling to an adjacent cell, a NP must have a positive and a negative pole that face the cell and the medium, respectively. It is not clear how the charges will separate and how will they do it asymmetrically—positive towards the cell and negative towards the medium or vice versa—if there are neither doping areas nor electrodes on the NP surface. Different light intensities on two sides of a semiconductor layer do not create two poles either. Also, if the charges separate radially inside the particle (as mentioned in the Supplementary Information[1]) such that its surface acquires a negative charge during illumination as its centre becomes positive, an electric field will exist only within the particle itself, between the negative surface and a positive centre. Even if the particle acquires a non-compensated charge during the illumination (a monopole), it will be surrounded by the counter ions in the electrolyte, which neutralizes it within a Debye layer, which is about 1 nm thick in saline—much thinner than the cleft.

According to fig. 1h in ref. 1 [1], the charging time was about 5 ms and the electric current stopped flowing in the medium much after that. Therefore, it is not clear how cells were stimulated during the continuous illumination of 500 ms in fig. 1 in ref. [1], and even for longer exposures during the behavioural experiments.

Another problem is that photovoltage such a particle can produce is much lower than the values modelled in fig. 1h in ref. [1]: pure P3HT in solid-state diodes under simulated solar irradiation has been shown to generate photovoltage of around 20 mV (ref. [13]). Similarly, on pure P3HT electrochemical interfaces irradiated with green light, photovoltage also does not exceed 20 mV (refs. [14,15]), and the extracellular voltage step will be even lower if the coupling to the medium is capacitive. A doping area or an interface with other active materials, deemed crucial for an efficient neurostimulation with P3HT-based photovoltaic prostheses[16], is missing in these NPs.

Even if the NP is efficiently capacitively coupled to a cell, its effect on the cell potential will be minute due to the very small area of the interface. For example, capacitance of the cell membrane in front of the 200-nm-radius NP is about 1.2 fF. Even assuming no conductive losses due to a high seal resistance, with a 30 mV voltage step across such a capacitor, it will attract $3.5 \times 10^{-17}$ C of charge. Distributed over the rest of the 10-µm-wide cell membrane, such a charge will generate a voltage change of about 12 µV—a thousand times lower than the threshold of the opening of any voltage-sensitive ion channels, which is about 10–15 mV (ref. [17]). Moreover, with a seal resistance of 8 MOhm (according to Supplementary Table 1[1]), the resistor–capacitor discharge time of such a capacitor is about 10 ns, a million times shorter than the 10 ms time constant shown in fig. 1g in ref. [1].

**Location of the NPs in vitro and in vivo.** According to ref. [1], one of the key features for the cellular stimulation by NPs is a very tight (<20 nm) contact with the cell membrane (fig. 1b). However, fig. 2 in ref. [1] shows that, in vivo, the P3HT NPs are located in the outer plexiform layer, several micrometres away from the target cells in the inner nuclear layer.

## Residual natural vision

Figure 5c,e in ref. 1 [1] shows that the VEP amplitude decreased with age in both the control (RCS and RCS + glass) and the treated (RCS + P3HT) groups, from about 80–240 µV at 30 days post injection (DPI) to about 5–18 µV at 240 days. However, according to fig. 2 in ref. [1], the number of cells in the inner nuclear layer did not decrease much between the day 30 and day 240. This indicates that VEP was unlikely to be induced by the NP stimulation of the bipolar cells. Rather, the authors perceived the gradually declining natural photoreceptor-mediated vision in the degenerating retina. In fact, fig. 2c in ref. [1] demonstrates the preservation of many photoreceptor nuclei in the outer nuclear layer at day 240, which supports the fact that RCS retina still exhibit very robust visual responses. Similarly, the strata of cells labelled INL in Extended Data fig. 5d–f in ref. [1] is much thicker than that of the healthy inner nuclear layer in the left column (RCS-rdy), and hence might include the remaining photoreceptors.

Surprisingly, according to fig. 5 in ref. [1], VEP in healthy rats (RCS-rdy) also decreased with age by an order of magnitude—from about 200 µV at day 30 to 20 µV at day 240. In contrast, we observed pretty stable VEPs over the lifetime of the wild-type rats[18,19].

Most importantly, in all the in vivo experiments in the article[1], as well as in the previous study from the same group[20], the control group exhibited substantial VEP and very robust behavioural responses. Anatomical evidence of outer nuclear layer thinning is not enough to claim the absence of natural vision. For example, human retinitis pigmentosa patients with a barely discernible outer nuclear layer in optical coherence tomography and no detectable electroretinogram still exhibit some vision, so the role of a few remaining photoreceptors in the preservation of sight should not be underestimated.

The absorption spectrum of P3HT largely overlaps with that of the retinal photoreceptors. For an unambiguous demonstration of prosthetic vision, animals with no remaining visual responses should be used. Other researchers on P3HT-based photovoltaic retinal prostheses have carefully addressed this point by using animal models insensitive to light, as demonstrated in control experiments, or by using pharmacological assays[16,21,22].

## Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41565-021-01044-6.

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## Author contributions

All the authors co-wrote the paper.

## Competing interests

D.P. is a consultant to Pixium Vision, a company that develops a photovoltaic retinal prosthesis. His patents about this technology are licensed to Pixium Vision by Stanford University. E.D.G. and D.G. declare no competing interests.

## Additional information

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