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Subretinally injected semiconducting polymer nanoparticles rescue vision in a rat model of retinal dystrophy

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Abstract

Inherited retinal dystrophies and late-stage age-related macular degeneration, for which treatments remain limited, are among the most prevalent causes of legal blindness. Retinal prostheses have been developed to stimulate the inner retinal network; however, lack of sensitivity and resolution, and the need for wiring or external cameras, have limited their application. Here we show that conjugated polymer nanoparticles (P3HT NPs) mediate light-evoked stimulation of retinal neurons and persistently rescue visual functions when subretinally injected in a rat model of retinitis pigmentosa. P3HT NPs spread out over the entire subretinal space and promote light-dependent activation of spared inner retinal neurons, recovering subcortical, cortical and behavioural visual responses in the absence of trophic effects or retinal inflammation. By conferring sustained light sensitivity to degenerate retinas after a single injection, and with the potential for high spatial resolution, P3HT NPs provide a new avenue in retinal prosthetics with potential applications not only in retinitis pigmentosa, but also in age-related macular degeneration.

Additional access options:

Data availability

The data that support the plots within this paper together with other findings of this study are available from the corresponding author upon reasonable request.

Code availability

Custom codes and software used in this paper can be obtained from the corresponding authors on request.

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Contributions

J.F.M.-V. followed all in vivo experiments by performing electrophysiology and behavioural analyses under the supervision of F.B. and assisted in the optical coherence tomography and positron emission tomography trials; G.P., M.M. and A. Russo developed and executed the subretinal microinjection; M.M. performed optical coherence tomography analysis; G. Manfredi, J.B. and F.D.M. fabricated the NPs and characterized them under the supervision of G.L.; E.C., S.D.M., M.L.D., E.D.P., M.D. and M.B. performed the in vitro/ex vivo electrophysiological and EM experiments on HEK cells, neurons and retinal explants under the supervision of F.B.; V.C., F.T., L.E., D.S. and C.M. executed positron emission tomography experiments under the supervision of G.S.; D.S., G. Mantero and C.E. performed histological analyses under the supervision of S.D.M. and J.F.M.-V.; A. Rocchi performed the qRT–PCR experiments; J.F.M.-V., G.L. and F.B. wrote the manuscript; F.P. revised the manuscript; F.B., G.P., J.F.M.-V. and G.L. conceived, supervised and financed the project. All authors discussed the experimental results and commented on the manuscript.

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Ethics declarations

Competing interests

The P3HT NPs studied in this paper are the subject of the US patent application US 16/005248 'Eye-injectable polymeric nanoparticles and method of use therefor' by Istituto Italiano di Tecnologia and Ospedale Sacrocuore Don Calabria, with J.F.M.-V., M.M., G.P., F.B. and G.L. as inventors. The other authors declare no competing interests.

Additional information

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Extended data

Extended Data Fig. 1 P3HT nanoparticles incubated with primary neurons are not internalized.

a, Primary cortical neurons exposed to P3HT-NPs for 1 h were live stained with Cell Mask and analysed by confocal imaging. Cell Mask (cyan) labels the neuronal membrane, bisbenzimide (blue) highlights cell nuclei and the intrinsic P3HT fluorescence (red) visualizes NPs. In the middle panel, a higher magnification image of the neuron shows the co-localization of P3HT-NPs with the membrane (pink overlaid areas). The results are representative of n = 3 independent neuronal preparations. **b**, To quantify NP internalization, neurons were exposed to P3HT-NPs for either 1 h (shown) or 1 week (not shown) fixed, double immunostained for βIII-tubulin (blue) and the specific lysosomal marker LAMP1 (green) and analysed by confocal microscopy. **c**, Box plot of the extent of NPs/LAMP1 co-localization, quantified by acquiring 3D z-stack confocal images. The median P3HT NPs/LAMP1 volume ratios were 1.65% and 1.75% for 1-h and 1-week incubations, respectively. Box plots represent the median (centre line), mean (square), 25^{th}-75^{th}percentiles (box) and the limit of 3-fold the interquartile range. Sample size: n = 22 neurons from 2 independent neuronal preparations. Scale bars, 20 µm (main images); 5 µm (zoomed images).

Extended Data Fig. 2 Quantification of cell bodies in the ONL of dystrophic RCS rats at 30 and 240 DPI.

The boundaries of the avascular ONL were determined in transversal sections of representative healthy and dystrophic retinas dissected at 30 DPI (**a**) and 240 DPI (**c**) by histochemistry with isolectin GS-IB4 (red) staining retinal and choroidal vessels, combined

with cell nuclear staining with bisbenzimide (blue). A clear-cut thinning of the ONL associated with widening of the INL was present at both 30 DPI and 240 DPI. Quantification of the number of cell layers labelled with bisbenzimide in the ONL revealed a massive decrease of the nuclear rows of photoreceptors in all dystrophic retinas (non-injected, P3HT-NP injected or sham Glass-NP injected) already at 30 DPI (4 months-old rats; **b**), that further progressed at 240 DPI (11 months-old rats; **d**). Means ± sem are shown together with dots representing the mean value obtained for each animal from 9 samplings (3 samples/field and 3 fields/retina). *** $p < 0.001$, one-way ANOVA/Bonferroni's tests. Data are means±sem with superimposed individual experimental points. Sample size (experimental animals) @ 30 DPI: RCS-rdy, n = 13; RCS, n = 13; RCS + P3HT, n = 11; RCS + Glass, n = 14. Sample size (experimental animals) @ 240 DPI: RCS-rdy, n = 9; RCS, n = 9; RCS + P3HT, n = 8; RCS + Glass, n = 10. For exact p values, see Supplementary Table 3. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar, 100 μm.

Extended Data Fig. 3 Degeneration in dystrophic RCS retinas selectively hits recoverin-positive photoreceptors in the ONL.

a, Representative image of a retina section from n = 5 non-dystrophic RCS-rdy rats (4 months-old) stained with bisbenzimide to localize cell nuclei (red; *left panel*) and recoverin staining (white; *right panel*) to highlight both photoreceptor (pink arrows) and bipolar (green arrows) cell bodies. **b**, The frequency distribution of the cell diameter in non-dystrophic RCS-rdy retinas shows a bimodal pattern that allows distinguishing between photoreceptor (mean ± sem: 5.73 ± 0.78 μm) and bipolar (mean ± sem: 12.15 ± 0.14 μm) cell bodies. All 4 months-old dystrophic RCS groups, no matter whether treated (30 DPI) or untreated, show a single distribution of cell diameters around 12 μm, coinciding with that of bipolar cells in RCS-rdy. This shows that photoreceptors are dramatically decreased in all groups of RCS rats, while recoverin-positive bipolar cells are relatively unaffected. **c**, Pie charts representing the relative number of recoverin-positive photoreceptors and recoverin positive-bipolar cells expressed in percentage of the total cell body counts in control RCS-rdy retinas. The white areas in the RCS groups represent the percentage of photoreceptor loss. Sample size in **b,c** (experimental animals): n = 5 per experimental group (30 DPI). For each animal, images were acquired from corresponding fields in the various retinas by taking the injection site as reference point (2 slices/retina; 3 fields/slice). ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar, 50 μm.

Extended Data Fig. 4 Expression of photoreceptor-specific mRNAs in the retina of RCS rats at 30 and 240 DPI.

The mRNA levels of Rhodopsin (*Rho*; **a**), Opsin-1 short wave-sensitive (*Opn1sw*; **b**) and Opsin-1 medium wave-sensitive (*Opn1mw*; **c**) were quantified by qRT-PCR in retinal sections dissected from non-dystrophic controls (RCS-rdy) and dystrophic RCS rats that were non-injected (RCS) or injected with either P3HT-NPs or control Glass-NPs at 30 and 240 DPI. *Gapdh* and *HPRT1* were used as control housekeeping genes. Graphs show means ± sem on a semilogarithmic scale with superimposed individual points. Sample size (experimental animals): RCS-rdy (blue), n = 4; RCS

(green), n = 4; RCS + P3HT (red), n = 4; RCS + Glass (orange), n = 4 for 30 and 240 DPI groups. **p < 0.01, ***p < 0.0001, one-way ANOVA/Newman-Keuls tests *vs* the respective RCS-rdy group. For exact p values, see Supplementary Table 3.

Extended Data Fig. 5 P3HT nanoparticles do not promote proinflammatory effects in dystrophic retinas.

Transversal sections of representative retinas dissected at 30 DPI (**a**–**c**) and 240 DPI (**d**–**f**) from healthy controls (RCS-rdy) and dystrophic RCS rats that were untreated (RCS) or injected with either P3HT-NPs (RCS + P3HT) or control Glass-NPs (RCS + Glass). Sections were immunolabelled for: the astrocyte/Müller cell marker GFAP (**a,d**), the microglial marker Iba-1 (**b,e**) and the retinal trophic factor FGF2 (**c,f**). Images were acquired from corresponding fields in the various retinas by taking the injection site as reference point (2 slices/retina; 3 fields/slice). Immunostainings were merged with bisbenzimide nuclear labelling (blue). The bar plots on the right (means±sem with superimposed individual data points) report the quantitative analysis of the integrated fluorescence intensity. Dystrophic retinas display higher densities of activated astrocytes, microglial cells and FGF2-positive cells compared to RCS-rdy, as a result of the ongoing degeneration. All the RCS groups show a similar density of GFAP/Iba-1/FGF2 positive cells demonstrating that the presence of either P3HT-NPs or Glass-NPs did not promote a significant tissue inflammatory reaction. *** p < 0.001, **** p > 0.0001, *vs* RCS-rdy controls; one-way ANOVA/Dunnett's tests. Sample size (experimental animals) @ 30 DPI: GFAP 11, 10, 10, 11; Iba-1 11, 12, 12, 12; FGF2 8, 7, 7, 8; for RCSrdy, RCS, RCS + P3HT and RCS + Glass, respectively. Sample size (experimental animals) @ 240 DPI: GFAP 7, 6, 6, 7; Iba-1 8, 9, 8, 8; FGF2 4, 4, 4, 4; for RCSrdy, RCS, RCS + P3HT and RCS + Glass, respectively). For exact p values, see Supplementary Table 3. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Scale bar, 50 µm.

Extended Data Fig. 6 VEP latency in RCS rats.

a, Latency of VEPs evoked by white light flashes at 30 and 240 DPI. The electrophysiological analysis revealed that the VEP latency in RCS-rdy animals was significantly lower compared to that of RCS, RCS + P3HT-NPs or RCS + Glass-NPs at 30 DPI. The same phenomenon, albeit more pronounced, was observed at 240 DPI. The greatly increased VEP latency in aged dystrophic RCS rats is likely due to the extensive retinal rewiring that follows photoreceptor degeneration. Notably, the increased latency of dystrophic RCS rats is not rescued by P3HT-NPs at both 30 and 240 DPI. Data are means±sem. ** $p < 0.01$; *** p < 0.001 *vs* RCS-rdy controls at 30 and 240 DPI, respectively; one-way ANOVA/Tukey's tests. Sample size (experimental animals) @ 30 DPI: RCS-rdy, n = 8; RCS, n = 7; RCS + P3HT-NPs, n = 12; RCS + Glass-NPs, n = 6. Sample size (experimental animals) @ 240 DPI: n = 4 per each experimental group. **b**, Latency of VEPs evoked by white (W) or red (R) light flashes at 30 DPI from the experiment shown in Fig. 5h. No V1 response to red stimuli was detected in healthy RCS-rdy rats (indicated as infinite latency). The VEP latencies to white and red light of dystrophic RCS rats injected with P3HT-NPs were not significantly different, while they were both significantly longer that the VEP latency to white

light of healthy RCS-rdy rats. **** $p < 0.0001$; NS $p > 0.05$; one-way ANOVA/Tukey's tests. Data are means±sem with superimposed individual experimental points. Sample size (experimental animals): RCS-rdy, n = 8; RCS + P3HT-NPs, n = 7. For exact p values, see Supplementary Table 3.

Extended Data Fig. 7 Light-evoked metabolic activation of V1 is rescued in dystrophic RCS rats injected with P3HT nanoparticles.

a, Representative brain images of basal metabolic activity acquired in the four experimental groups at 240 DPI. A map of the rat brain showing the location of V1 (blue areas) and a pseudo-colour scale corresponding to the average SUV of ^{18}F-FDG uptake over the scanned areas are shown on the left. **b**, Quantitative analysis of the average SUV in the V1 volumes of interest (~1 mm^3) demonstrates a significant increase in light evoked V1 metabolic activity in RCS rats injected with P3HT-NPs at 240 DPI. Box plots in **b** represent the median (centre line), mean (square), 25^{th}-75^{th} percentiles (box) and the limit of 3-fold the interquartile range. * $p < 0.05$; ** $p < 0.01$; one-way ANOVA/Tukey's tests. Sample size (experimental animals): RCS-rdy, n = 10; RCS, n = 12; RCS + P3HT-NPs, n = 14; RCS + glass-NPs, n = 11. For exact p values, see Supplementary Table 3.

Extended Data Fig. 8 Correlation map of all the variables studied in the four experimental groups of RCS rats.

Each column and each row describe a different variable, with squares on the map representing the correlation between row and column variables ("photoreceptor", "inflammation" and "visual function" variables). Analysis by the Pearson's correlation coefficient revealed the existence of a strong correlation among all the variables, suggesting the use of a Principal Component Analysis decomposition. Sample size (experimental animals) @ 30 DPI: RCS-rdy, n = 5; RCS, n = 5; RCS + P3HT-NPs, n = 5; RCS + Glass-NPs, n = 6. Sample size (experimental animals) @ 240 DPI: RCS-rdy, n = 4; RCS, n = 4; RCS + P3HT-NPs, n = 4; RCS + Glass-NPs, n = 4. PLR, pupillary constriction; VEPs, VEP amplitude; GFAP, astrocyte immunoreactivity; IBA-1, microglia immunoreactivity; FGF2, fibroblast growth factor immunoreactivity; PhRecCounts, recoverin-positive photoreceptor cell bodies; ConesCounts, cone arrestin-positive cone cell bodies; ONL cell rows, bisbenzimide-stained nuclear rows in the ONL; Acuity, visual acuity; Dark persistence, percentage time in the dark in the light–dark box behavioural test.

Extended Data Fig. 9 Component analysis for morphological and functional parameters in individual animals at 30 and 240 DPI.

3D plot of RCS morphological and functional parameters as a function of 3 reduced variables at 30 and 240 DPI. The x, y, z variables are indicators of visual performances (PLR, VEPs, Acuity, Dark persistence), photoreceptor cell counts (rod, cone and cell rows in the ONL, as reported in Supplementary Fig. 14) and the inflammatory state of the retina (GFAP, Iba-1 and FGF2 immunoreactivities, as reported in Supplementary Fig. 15), respectively. The scatter plots report the individual responses at 30 (**a**) and 240 (**b**) DPI within the four experimental groups. The shaded areas represent the highest values (up to 0.001) of the probability density function of the calculated clusters. The dashed lines and their terminal points represent the projections

of each cluster centroid onto the origin planes. Sample size (experimental animals) @ 30 DPI: RCS-rdy (blue; n = 5), RCS (green; n = 5), RCS + P3HT-NPs (red; n = 5) and RCS + Glass-NPs (orange; n = 6). Sample size (experimental animals) @ 240 DPI: RCS-rdy (blue; n = 4), RCS (green; n = 4), RCS + P3HT-NPs (red; n = 4) and RCS + Glass-NPs (orange; n = 4).

Supplementary information

Supplementary Information

Supplementary text, Tables 1–3, Figs. 1–16 and refs. 1–17.

Reporting Summary

Supplementary Video 1

Pupillary reflexes in bilaterally injected and dark-adapted animals at 30 DPI evoked by a light stimulus of 5 lux. Videos are image sequences obtained with a Hamamatsu camera at 5 Hz frame rate. Representative examples of the four experimental groups (RCS-rdy; RCS; RCS + P3HT NPs; RCS + glass NPs) are shown.

Supplementary Video 2

Pupillary reflexes in bilaterally injected and dark-adapted animals at 240 DPI evoked by a light stimulus of 5 lux. For further details, see caption to Supplementary Video 1.

Supplementary Video 3

Escape latency in the light–dark box test at 30 DPI. A representative example of the light (5 lux)-evoked escape behaviour is shown for each experimental group (RCS-rdy; RCS; RCS + P3HT NPs; RCS + glass NPs).

Supplementary Video 4

Escape latency in the light–dark box test at 240 DPI. For further details, see caption to Supplementary Video 3.

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