Optogenetics: the age of light

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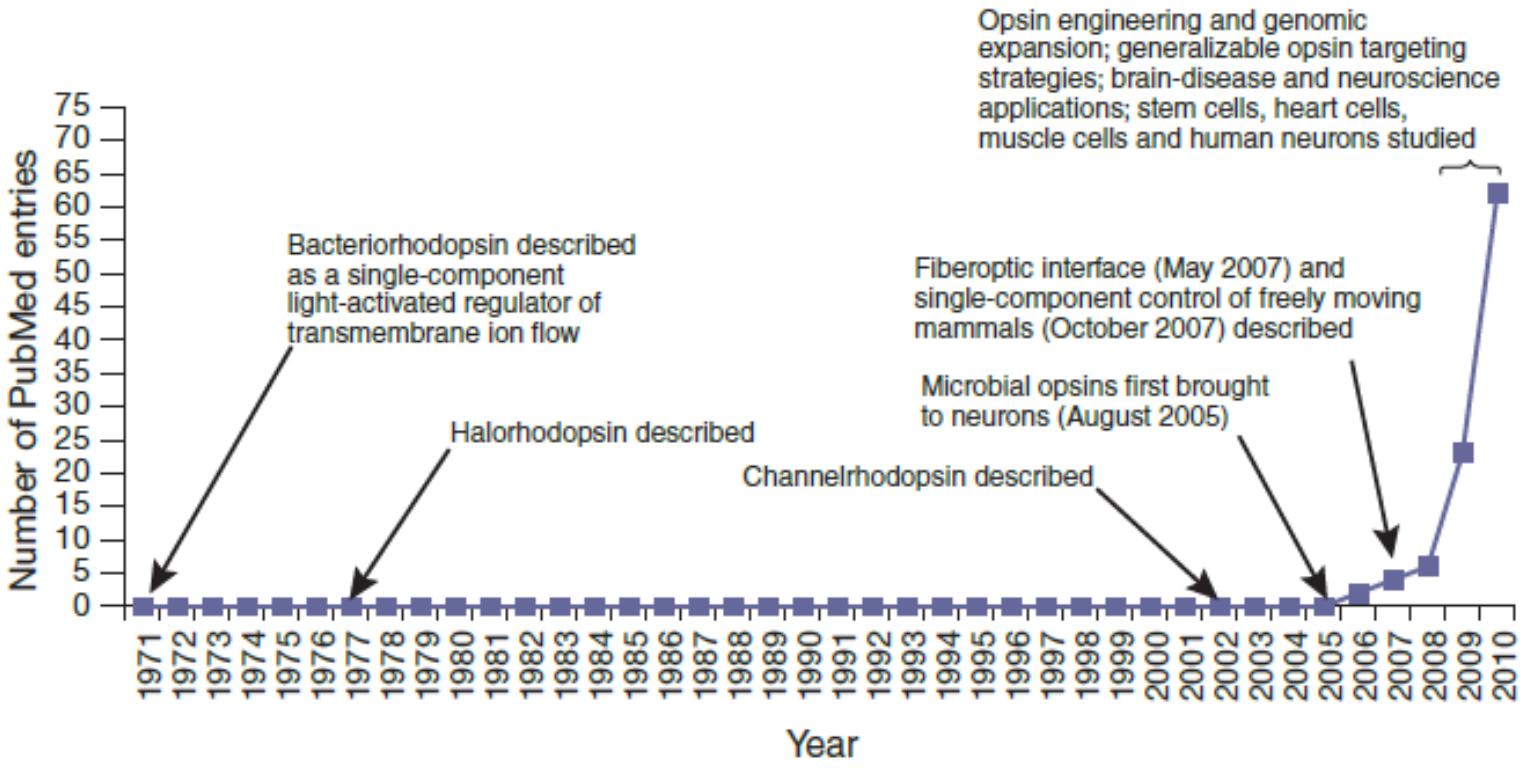
history


Opsin engineering and genomic expansion; generalizable opsin targeting strategies; brain-disease and neuroscience applications; stem cells, heart cells, muscle cells and human neurons studied
Bacteriorhodopsin described as a single-component light-activated regulator of transmembrane ion flow
Fiberoptic interface (May 2007) and single-component control of freely moving mammals (October 2007) described
Microbial opsins first brought to neurons (August 2005)
Halorhodopsin described
Channelrhodopsin described
Number of PubMed entries
0
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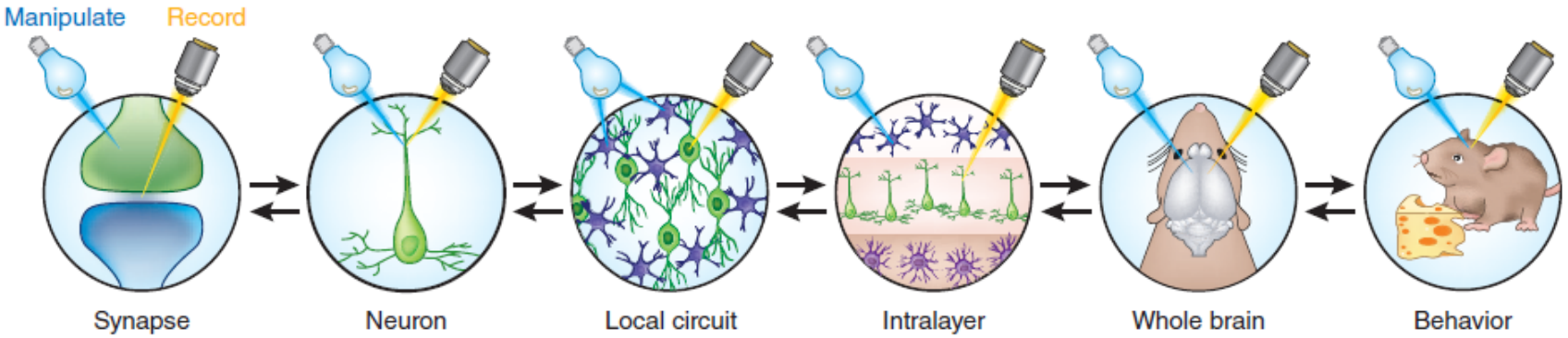
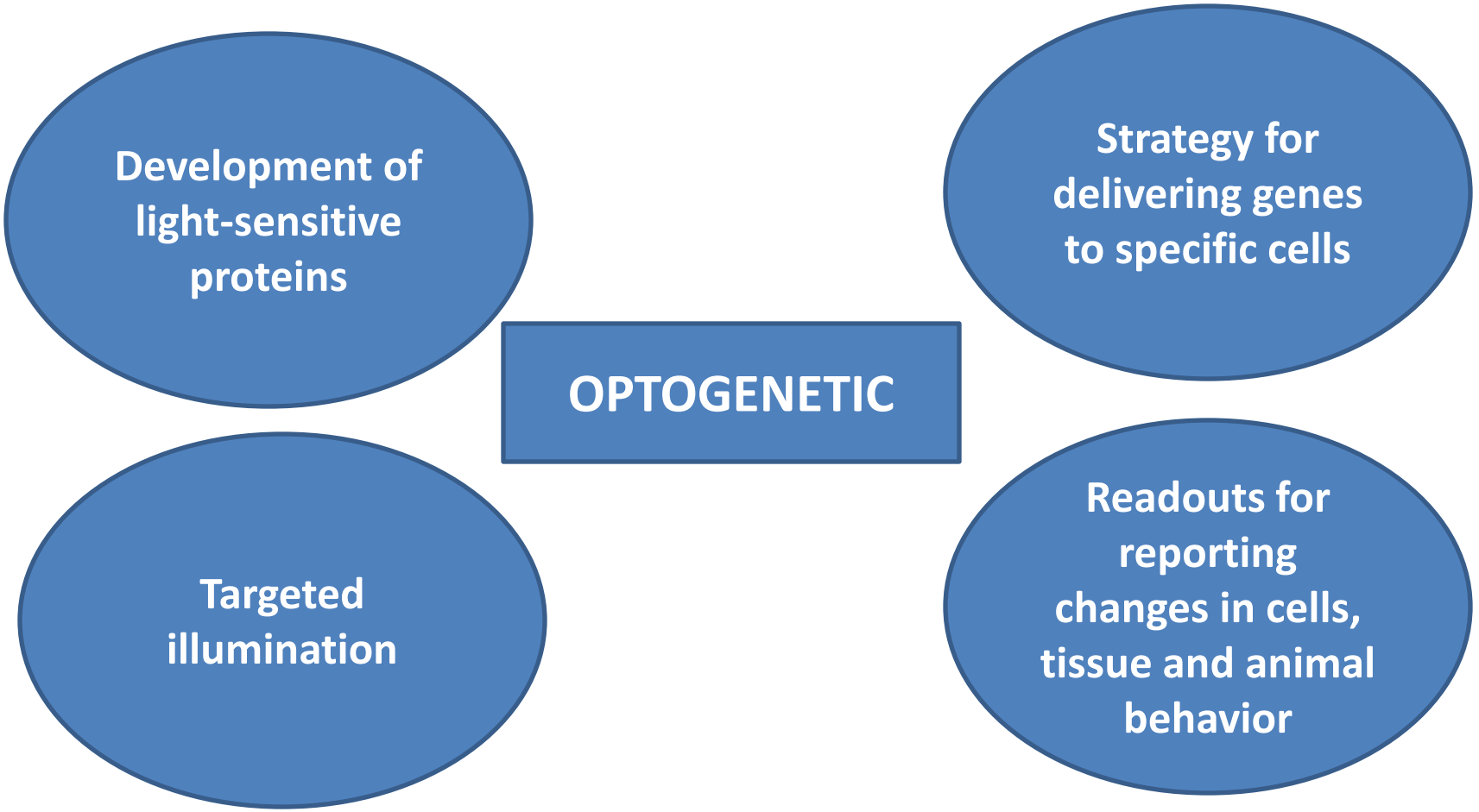
The advantages of using light

- It is noninvasive
- Can be precisely targeted
- Can be used simultaneously at multiple wavelengths and locations
- Can report the presence or activity of specific molecules



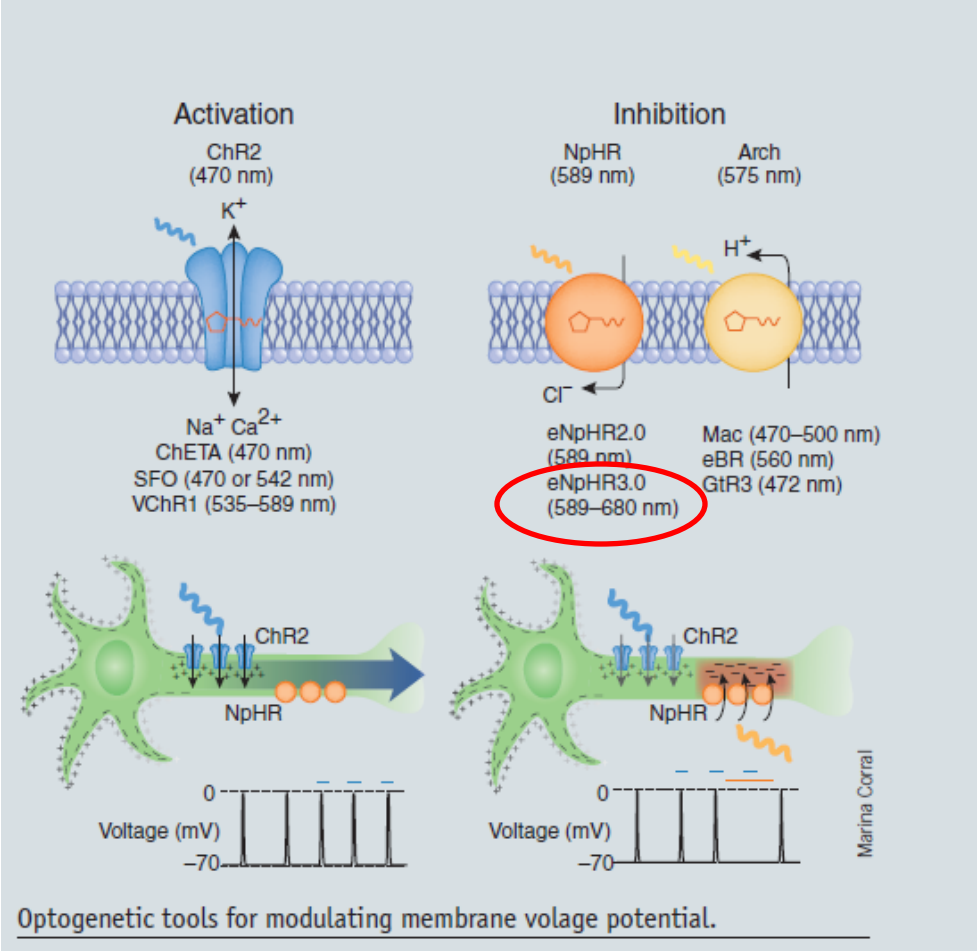


In Optogenetics, exogenus genes coding for light sensitive proteins are expressed in cells, and illuminatin is used to alter cells behavioral

Step 1: Light –activated proteins

For modulating the membrane potential

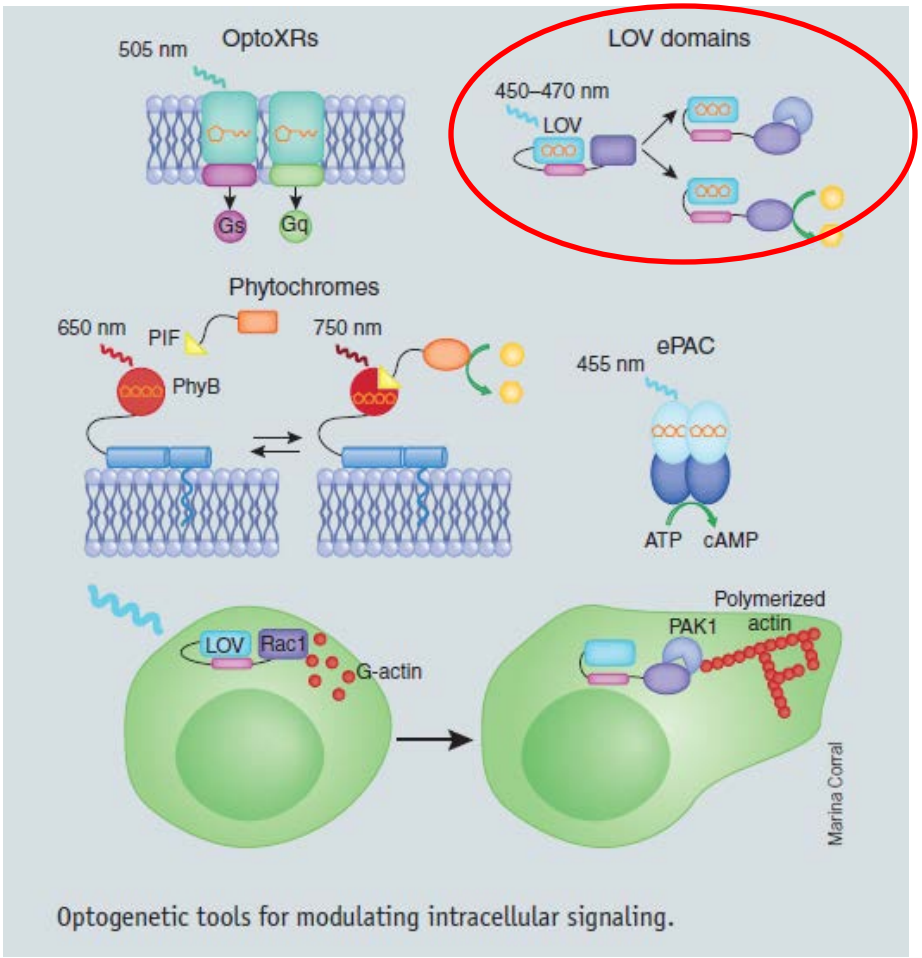
- Use chemical modified (“caged ligands”) that become active upon stimulation with light and bind exogenous receptor
- Naturally occurring genes encoding light sensitive proteins



Optogenetic tools for modulating membrane volage potential.

For mudulating cells signaling

- These tools are generally fusion of light-absorbing domains with proteins “effector” domains



Optogenetic tools for modulating intracellular signaling.

Step2: Delivering the genes

- By transfection, viral transduction or creation of transgenic animal lines.

Step3: Controlled illumination

- Integrated fiberoptic and solid-state light sources to allow specific cell types, even deep within the brain, to be controlled in freely behaving animals
- For in vivo applications, light sources coupled to optical fibers or miniaturized LEDs have been used

Step 4: Reading the outcome

- Electrodes can be used to monitor the effect of changes in membrane voltage
- Behavioral testing can be used in animals

nature biotechnology

Optogenetics enables functional analysis of human embryonic stem cell–derived grafts in a Parkinson's disease model

Julius A Steinbeck[1,2], Se Joon Choi[3], Ana Mrejeru[3], Yosif Ganat[1,2], Karl Deisseroth[4–6], David Sulzer[3,7,8], Eugene V Mosharov[3] & Lorenz Studer[1,2]

Subject

To use optogenetics to dissect the functionality of mesencephalic dopaminergic neurons derived from human embryonic stem cells transplanted in a Parkinson's disease model

Background

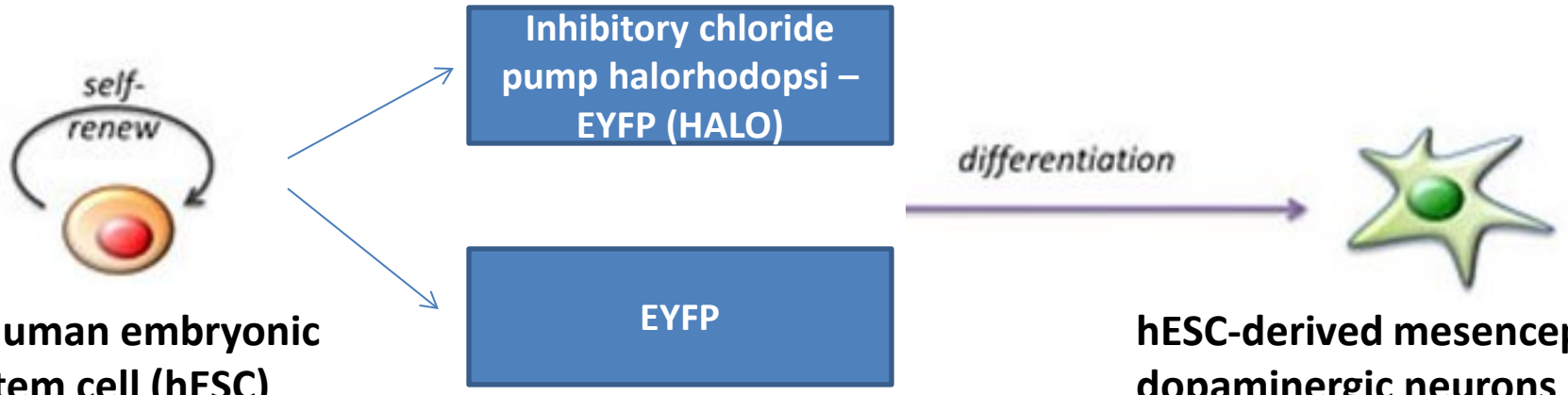
- Recent studies have shown evidence of behavioral recovery after transplantation of human pluripotent stem cell-derived neuronal cells in animal model of neurological disease

- For Parkinson's disease therapy, it has been suggested that full behavioral recovery requires functional integration of grafted dopamine neurons into disease host circuit

- In previous studies the role of grafted cells has been assessed by selective ablation of the graft

- Optogenetics allows the reversible functional manipulation of genetically and spatially defined circuits

Strategy


self-
renew
Inhibitory chloride pump halorhodopsi – EYFP (HALO)
EYFP
differentiation
Human embryonic stem cell (hESC)
hESC-derived mesencephalic dopaminergic neurons

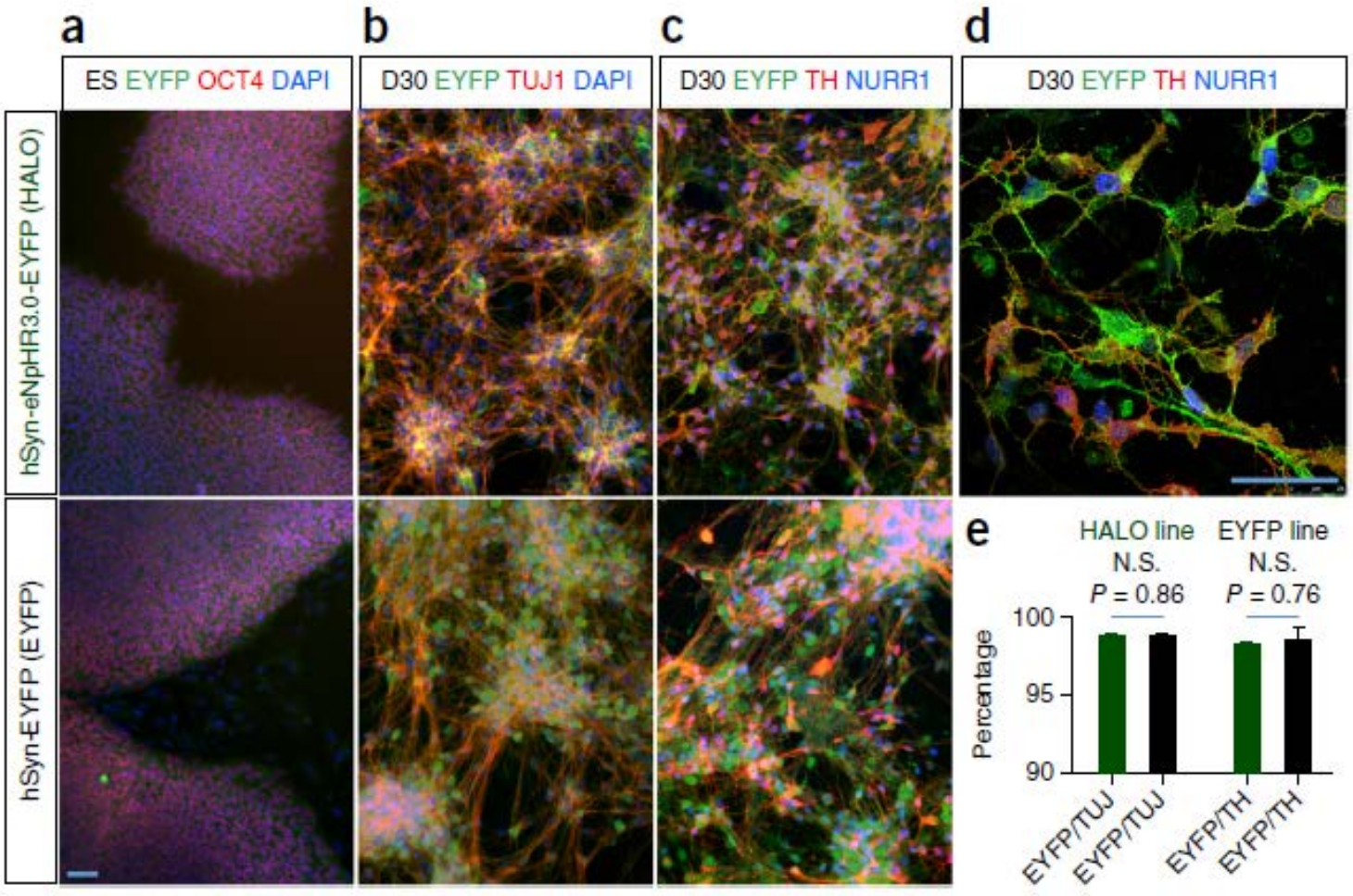


Fig 1: In vitro immunocytochemical characterizazion of opsin-expressing hESC lines and dopaminegic progeny

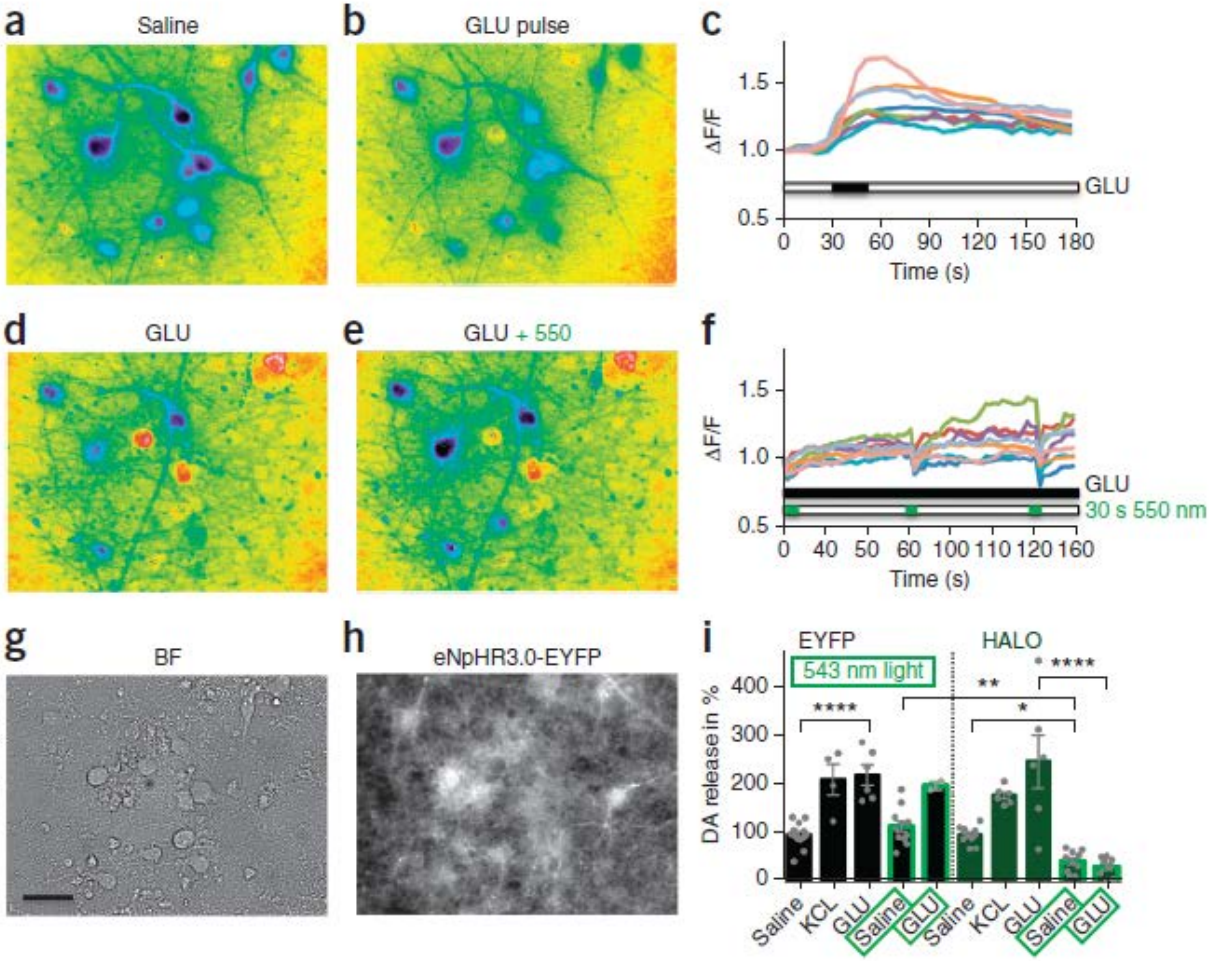
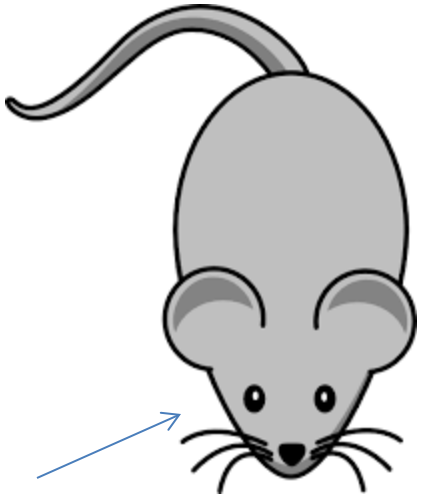


Fig 2: mature mesDA-rich cultures are incubated with the calcium dye Fura-2

Transplant functionality in vivo



Unilateral 6-hydroxydopamine lesions

>6 ipsilateral rotation/min in response to amphetamine

3 weeks

2x 105 hESCs-derived mesDA-rich cells

16 weeks

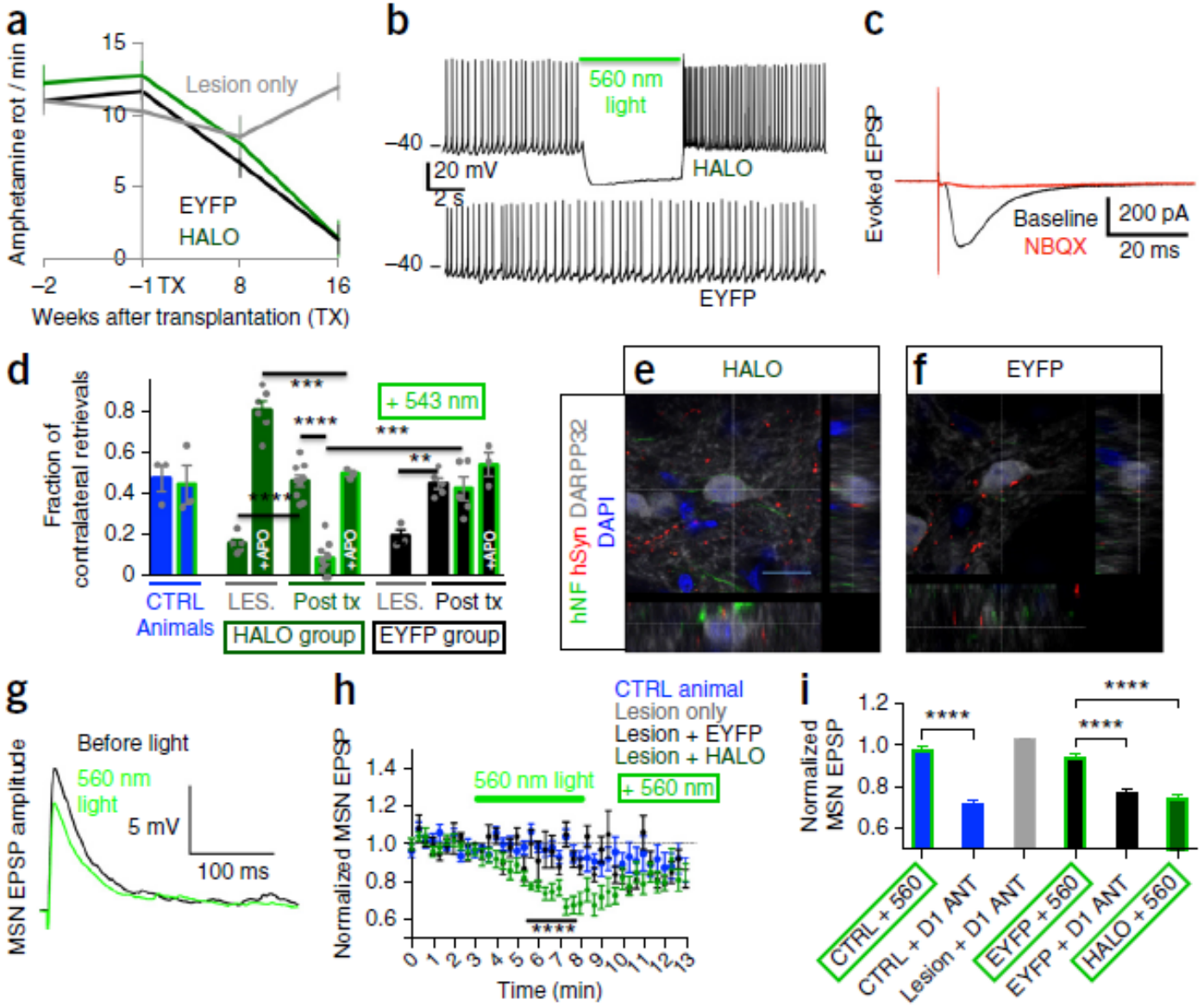
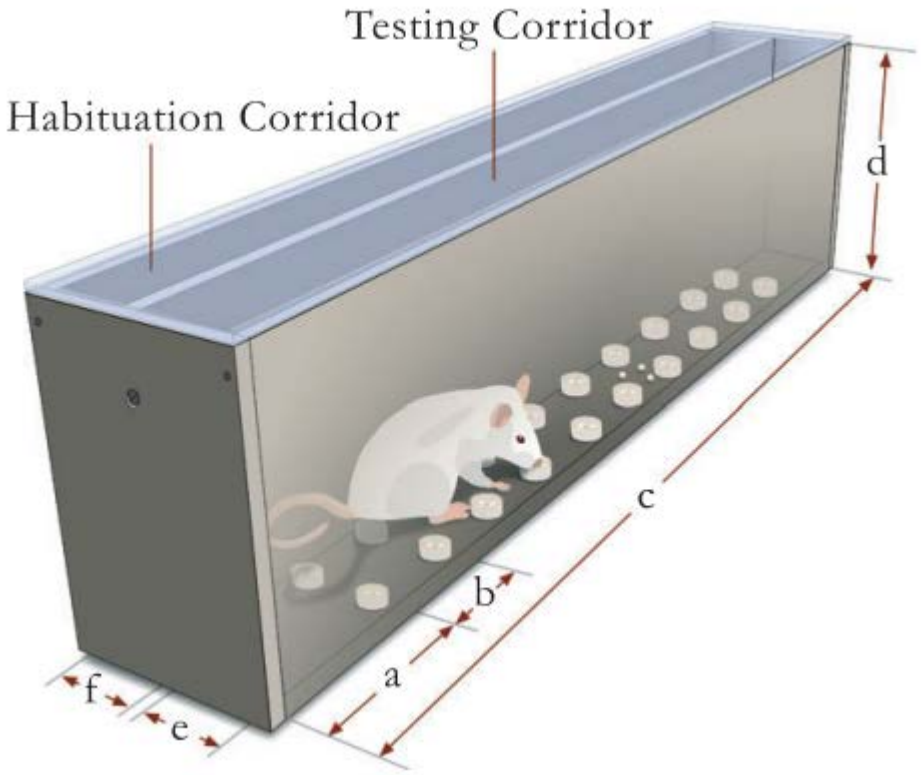


Fig 3: Behavioral, physiological and morphological assessment of graft functional connectivity

The corridor test



Recovered animals were implanted with a fiber optic cannula for light delivery

Animals were habituated to the corridor for three consecutive days

Outcomes

- The results demostrate that mesDA-rich graft are capable of modulating glutamatergic synaptic transmission onto striatal MSNs

- This study demostrates the utility of optogenetics to dissect the mechanisms underlying hESC graft function in preclinical model of Parkinson's disesase

LETTER

doi:10.1038/nature14128

Optogenetic control of organelle transport and positioning

Petra van Bergeijk[1]*, Max Adrian[1]*, Casper C. Hoogenraad[1] & Lukas C. Kapitein[1]

Subject

Create a tool to modulate locally the distribution of specific organelles with spatiotemporal accuracy, by using light-sensitive heterodimerization to recruit specific cytoskeletal motor proteins

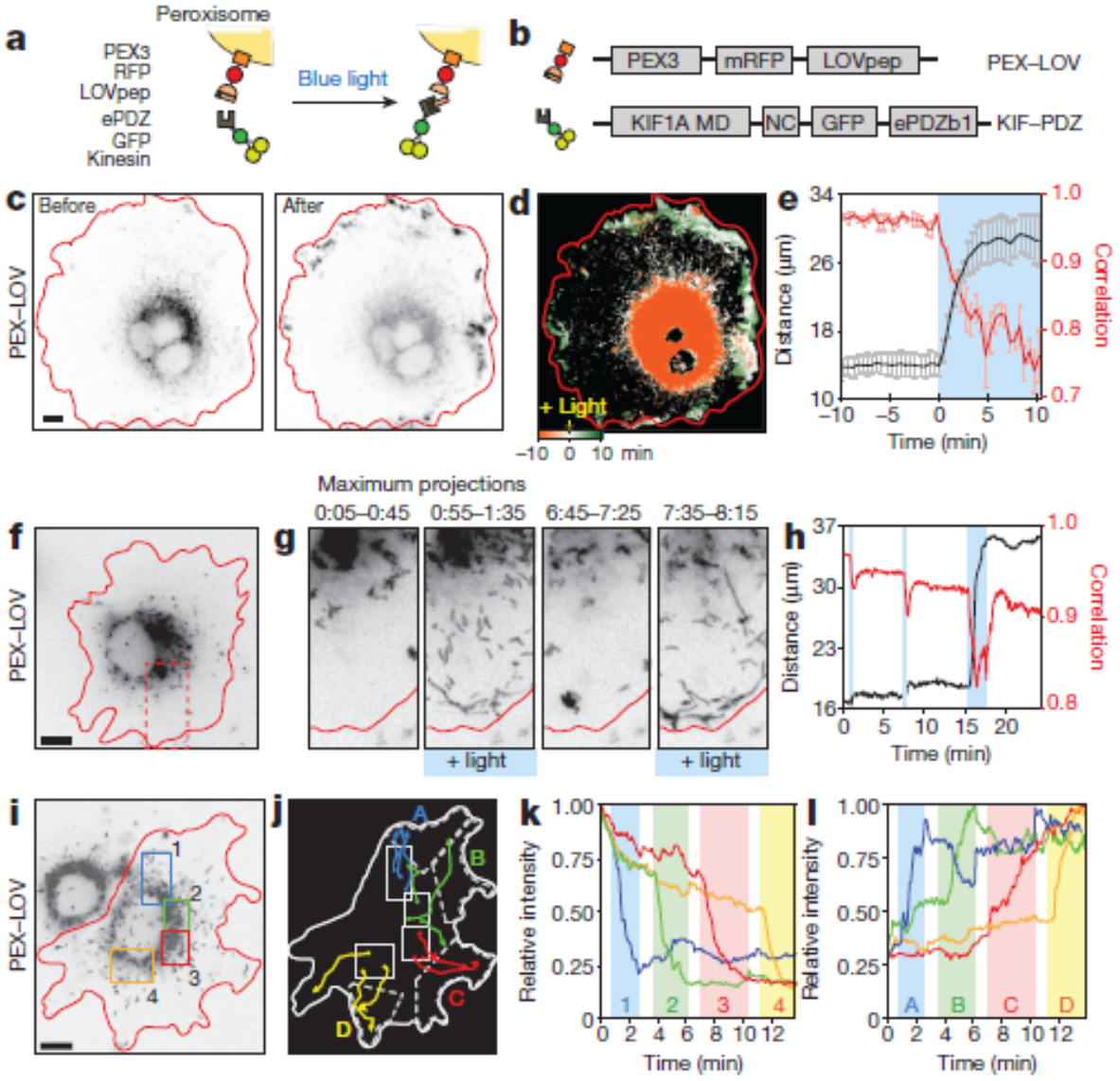


Fig 1: Local and reversible activation of microtubule-based transport with light

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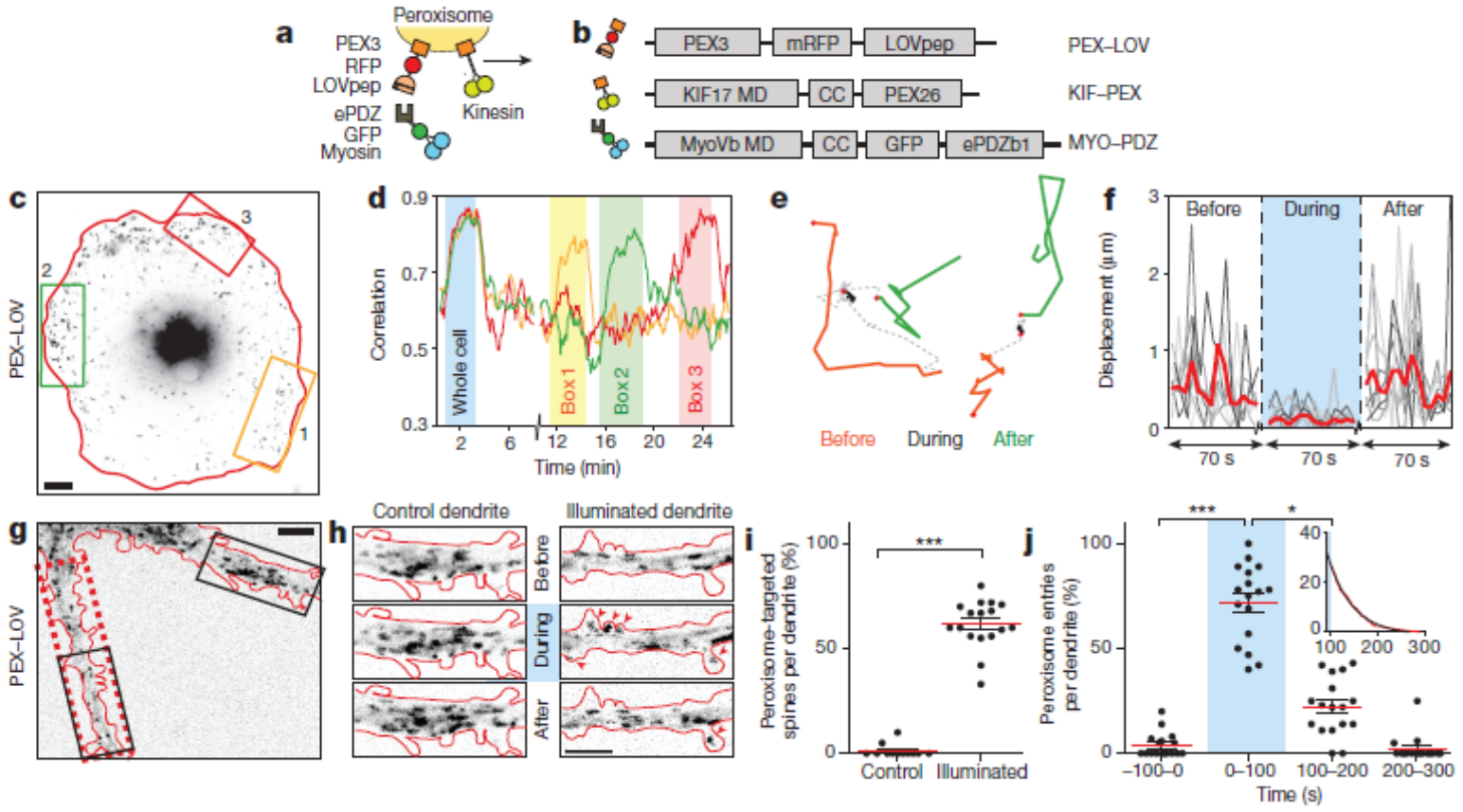

Fig 2: Light- induced myosin-Vb recruiment anchors organelles or targets them into dendritic spines

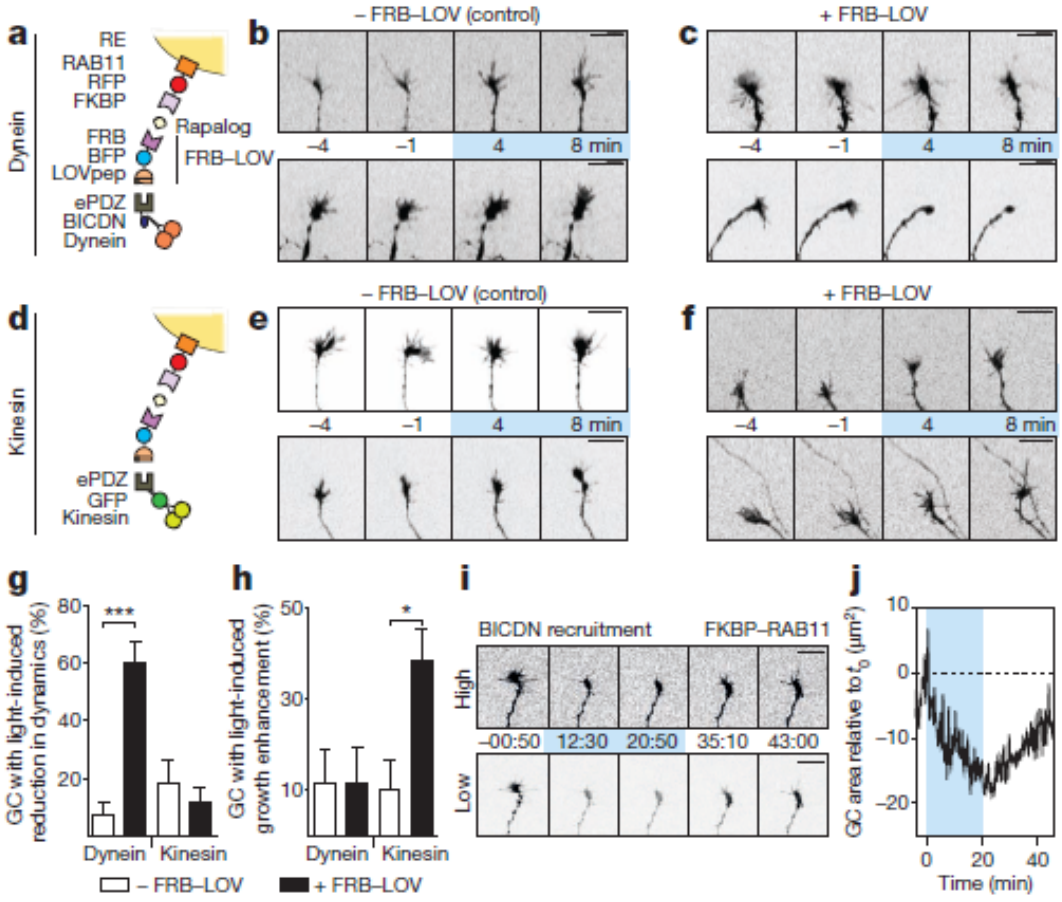

Fig 3: Motor-based redistribution of recycling endosomes modulates axon outgrowth

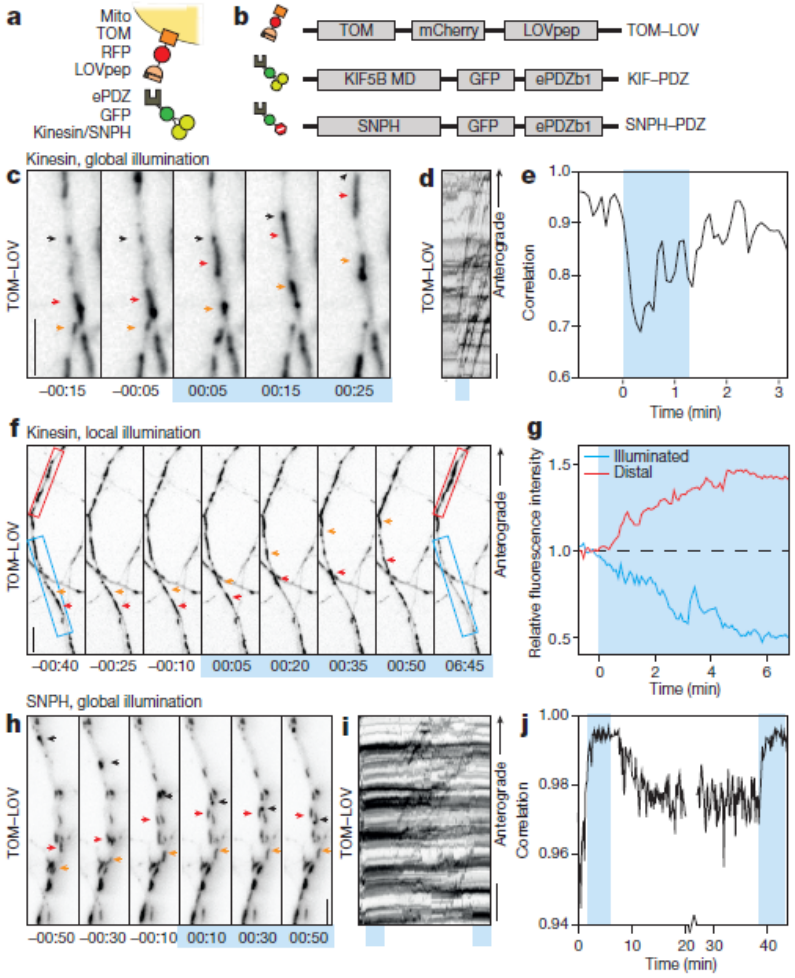

Fig 4: Altering mitochondrial dynamics through recruitments of motors and anchors

Outcome

- They established optically-controlled intracellular transport by using light=sensitive heterodimerization to recruit specific cytoskeletal motor proteins to selected cargos

The good

- The ability to target probes to genetically defined cell types and subcellular compartments
- The fact that optogenetic activators and inhibitors can be expressed in the same cells is crucial for testing both necessity and sufficiency

The bad

- The level of stimulation risks driving neuronal responses outside the physiological range
- Light stimulation and optogen expression are not uniform across the target neurons population

The ugly

- The implementation of optogenetic probes can itself perturb the system being investigated

Thank you……