TANGO: protein interactions traced in cells and animals

11/04/17

Technical Journal Club

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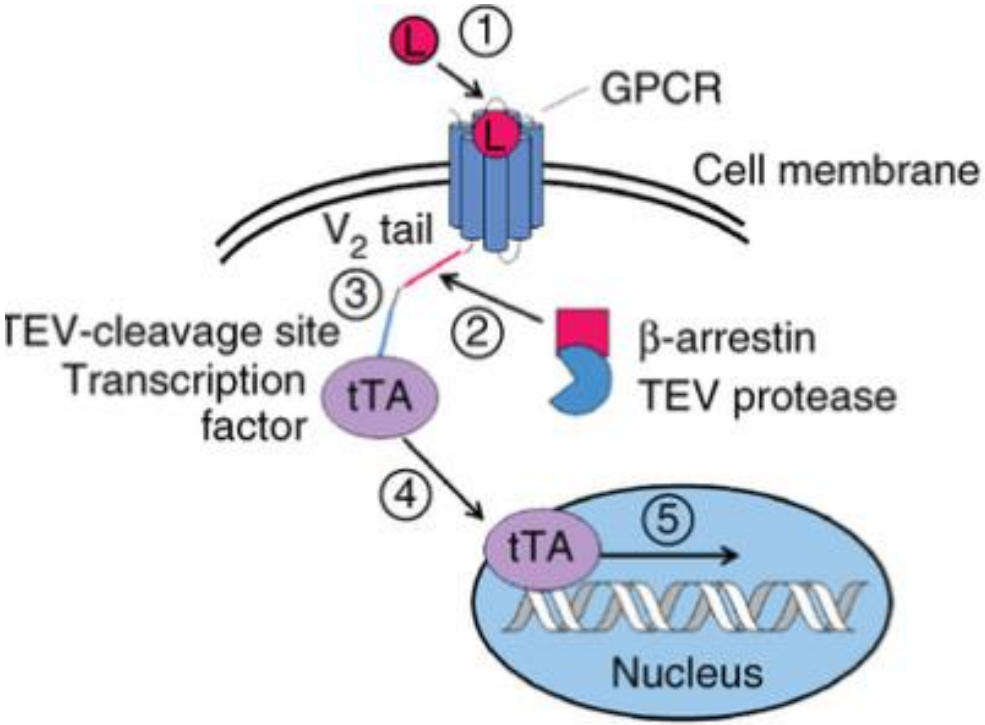
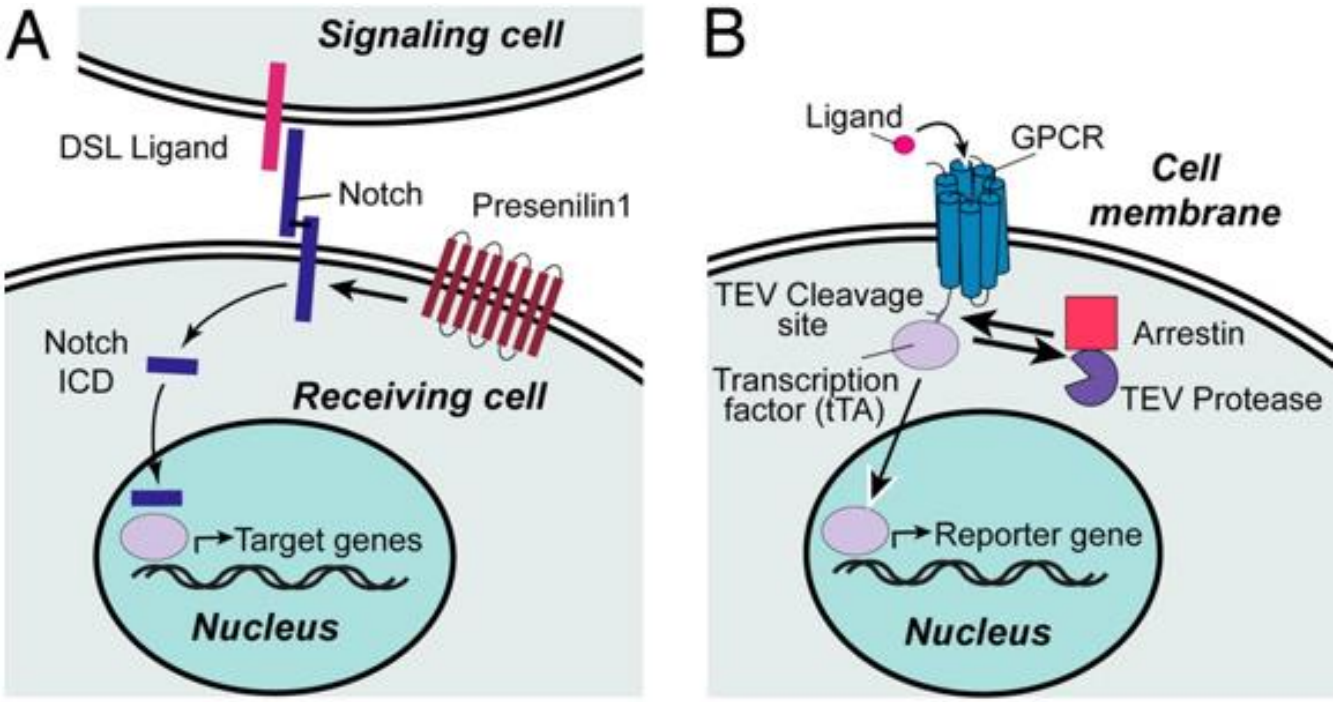
Tango

- Two proteins have to be associated to stimulate, its called TANGO
- Assay for protein-protein interaction: fusion of receptor with transcription activator
- Cleavage site (specific viral protease): Gene codes for fusion of protease with protein that activates receptor
- Adapting signaling events transformed to stable responses
- Monitor activity of different receptor classes: GPCR, Tyrosine, Steroid Hormone Receptor…



Tango: Notch receptor

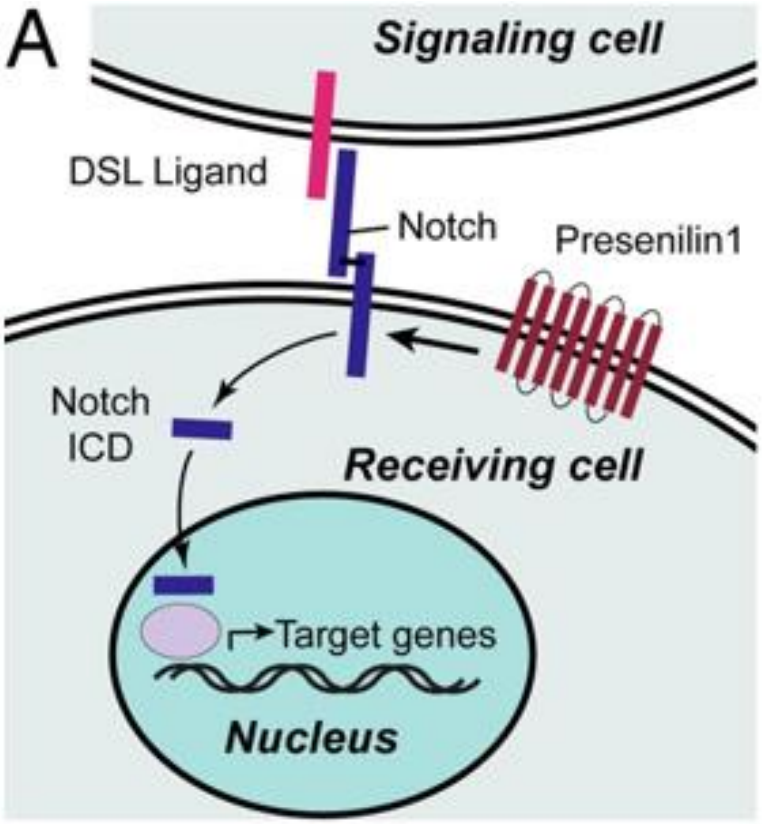
Based on Notch receptor: Ligand triggers cleavage in receptor➔ releases notch intracellular domain that translocates to nucleus➔ modulates transcription of target genes.

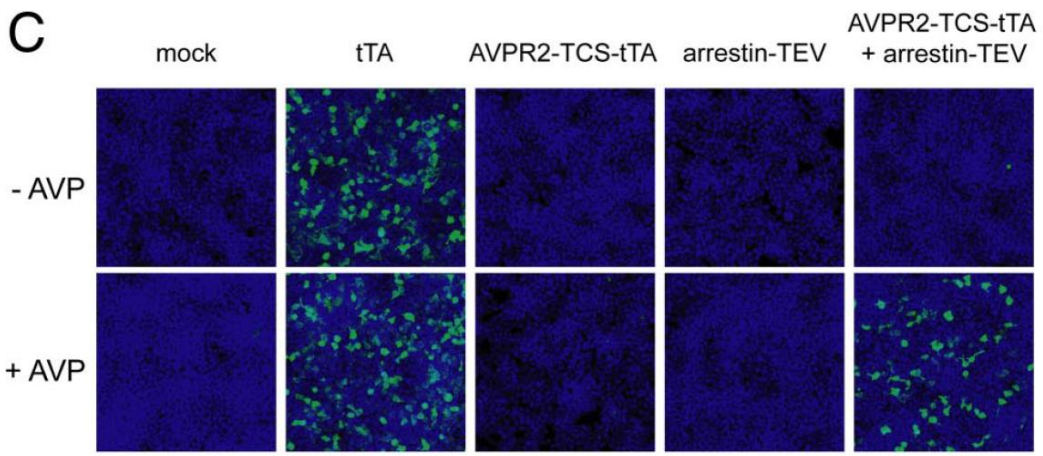


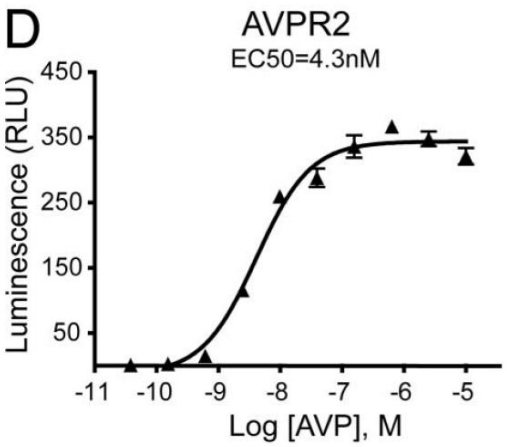
Tango: GPCR

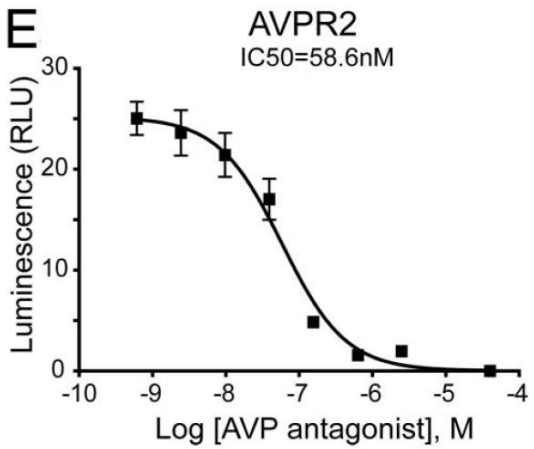
Receptor stimulation with agonist and antagonist followed cell transfection with the AVPR2 (vassopressin Rec)-TEV cleavage site (TCS)-tTA and arrestin-TEV fusions

- Ligand➔ Phosphorylation Rec➔ b-arrestin recruited-TEV





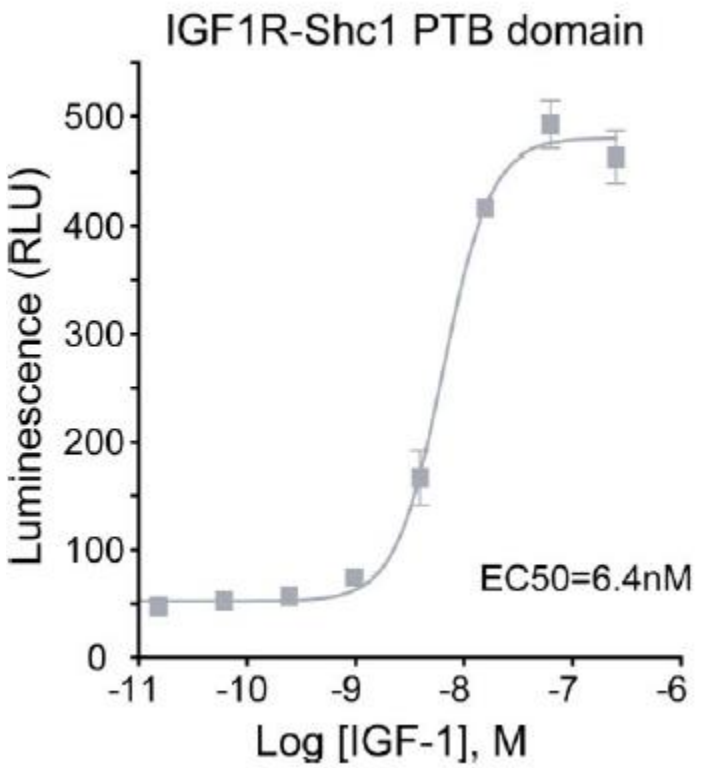


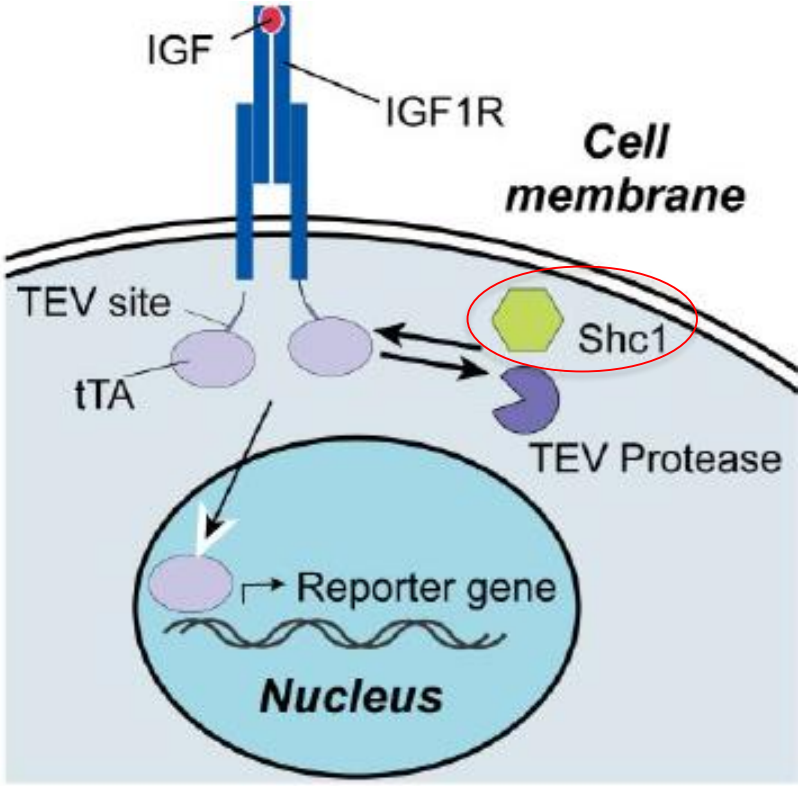


Tango: Tyrosine Kinase Rec

- Insulin-like growth factor 1 receptor interacts with Shc1 (PTB domain adaptor protein)
- Cell line containing tTA-responsive reporter gene IGF1 ligand binds

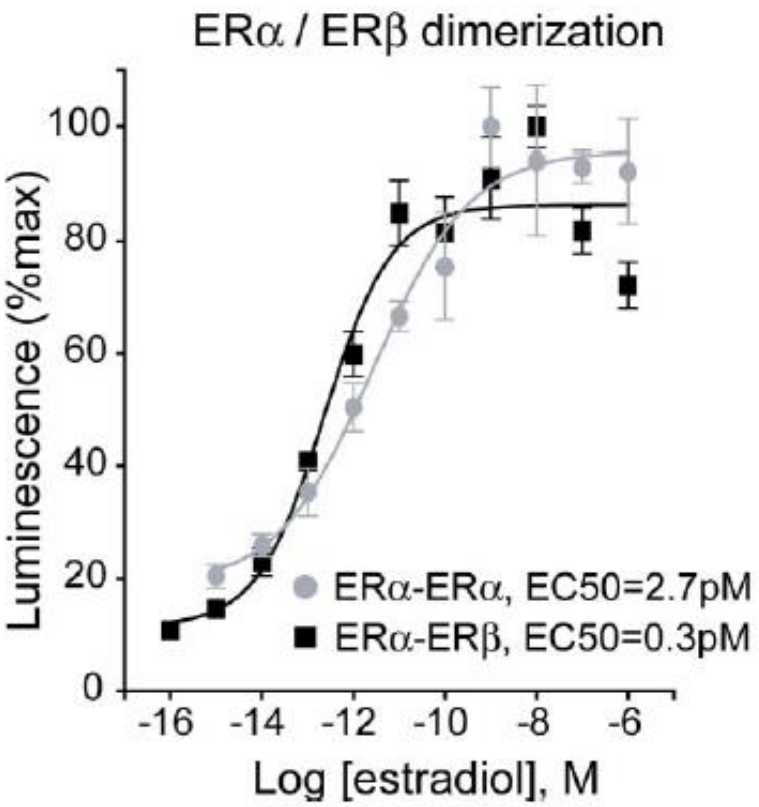
- Ligand➔ Phosphorylation Rec (PTB domain of shc1)➔Shc1 recruited-TEV

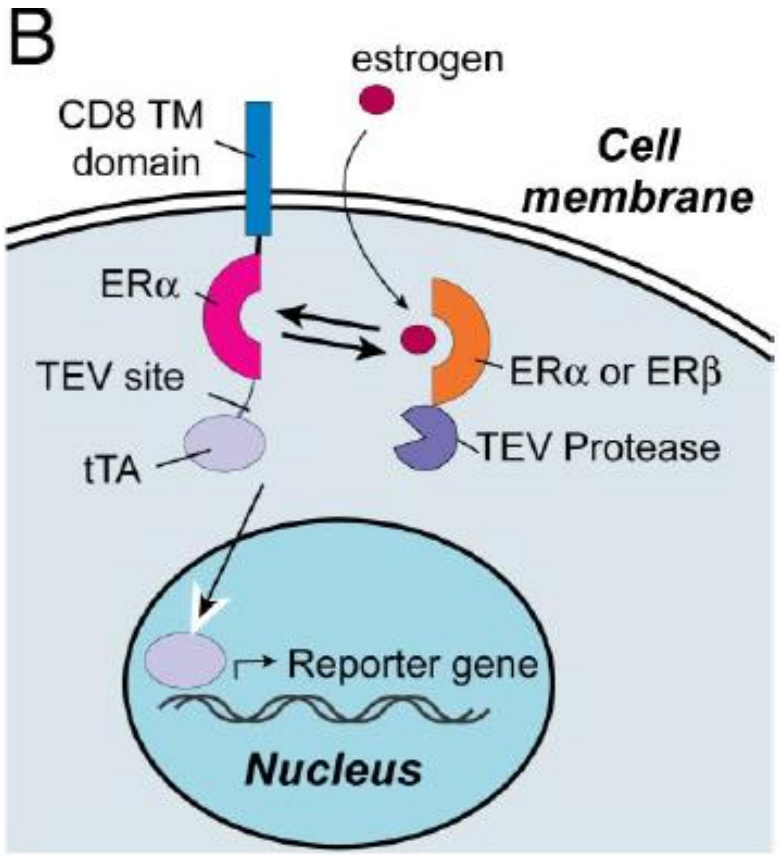




Tango: Transmembrane and intracellular receptors

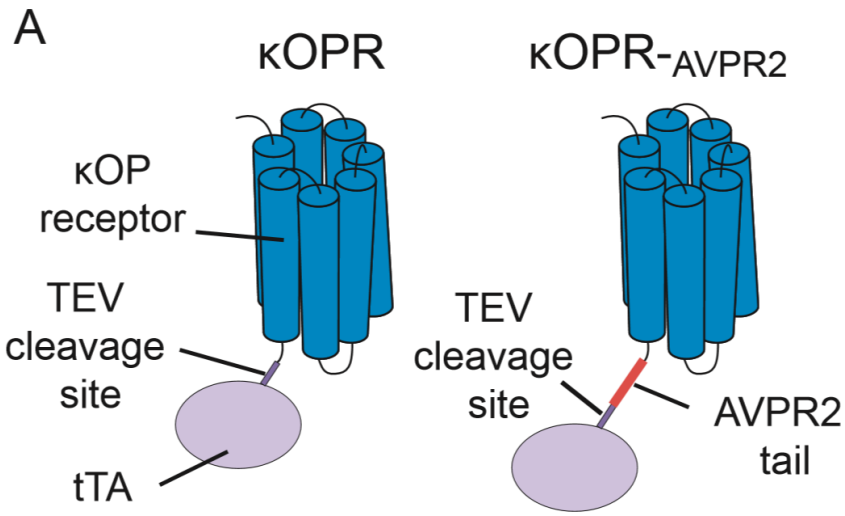
Transmembrane and intracellular receptors can be monitored (interaction of two receptor proteins)
Estrogen binds ➔ Dimers formed, enter nucleus, interact with DNA response elements and target gene transcription





Tango: optimizing sensitivity

Fusion gene (AVPR2 tail) extends the Tango assay to receptors that weakly recruit b-arrestin molecules upon activation and has allowed us to develop assays for 89 GPCR family member



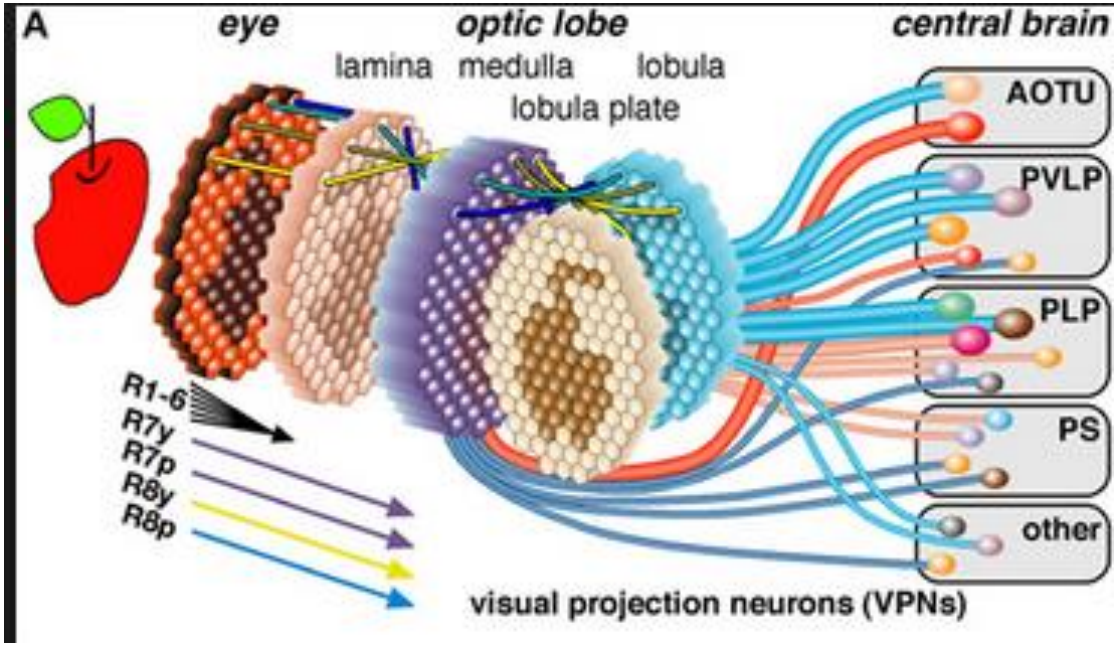
Vision in Drosophila

- Research failed to identify color opponent neurons in the optic lobe. Behavioral studies have not provided convincing evidence for color vision in the fly.
- Color vision requires the ability to distinguish light of distinct spectral composition independent of intensity
- single photoreceptor cannot distinguish different wavelengths from different intensities of light

➔ Interesting to understand how a single photoreceptor can “see” colour

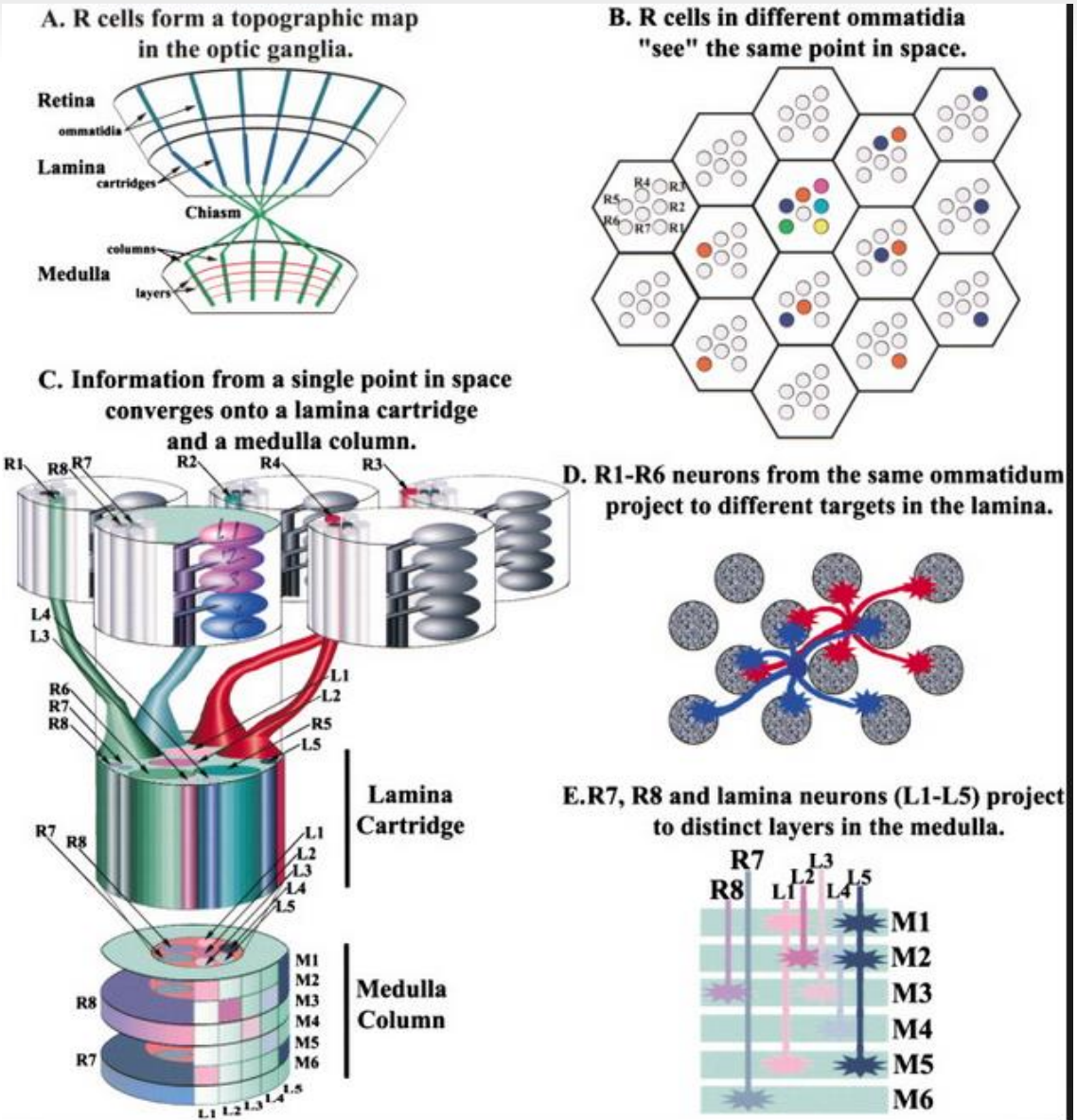
Tango-Trace: Ommatidium

- The R7 and R8 cells project axons to the same column (medulla) and the topographic organization of the columns maintains retinotopic order
- Drosophila has phototactic (movement) preference for UV light vs green light (preference gone if R7 is blocked)



(Otsuna, 2014)

Tango-Trace: core with eight photoreceptor neurons



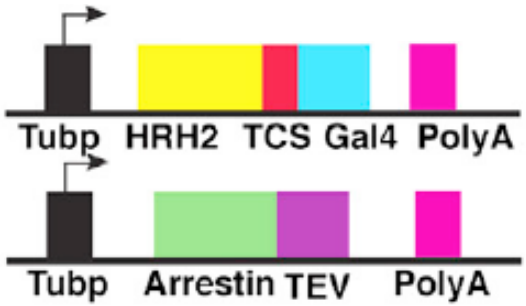
- R1-R6: achromatic neurons
- R7,8: chromatic neurons
- p/y R7,8: 4 types of inner photoreceptors: R7 (UV-sensitive rhodopsin Rh3 and Rh4:yellow) and R8 (blue-sensitive Rh5;Rh6: green)

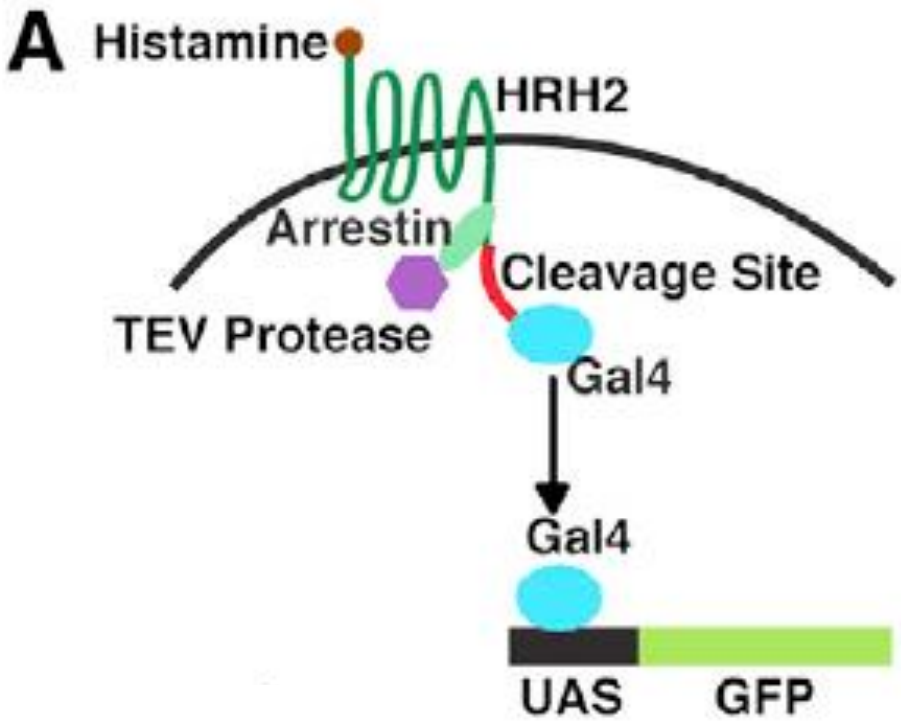
- Connections of 4 inner photoreceptors unidentified and projection of different ommatidia to what circuits?

(Clandinin, 2002)

Tango-Trace: GFP

Photoreceptor neurons use **histamine** (HA) as NT
In Drosophila HA can be detected by using Tango-Trace: Expression of histamine receptor (human, HRH2, a GPCR)
Generate animals expressing: HRH2-TCS-Gal4 and Arr-TEV under the control of the tubulin promoter (Tubp) and reporter construct UAS mCD8-GFP
➔ visualization of their cell bodies and axonal and dendritic projections

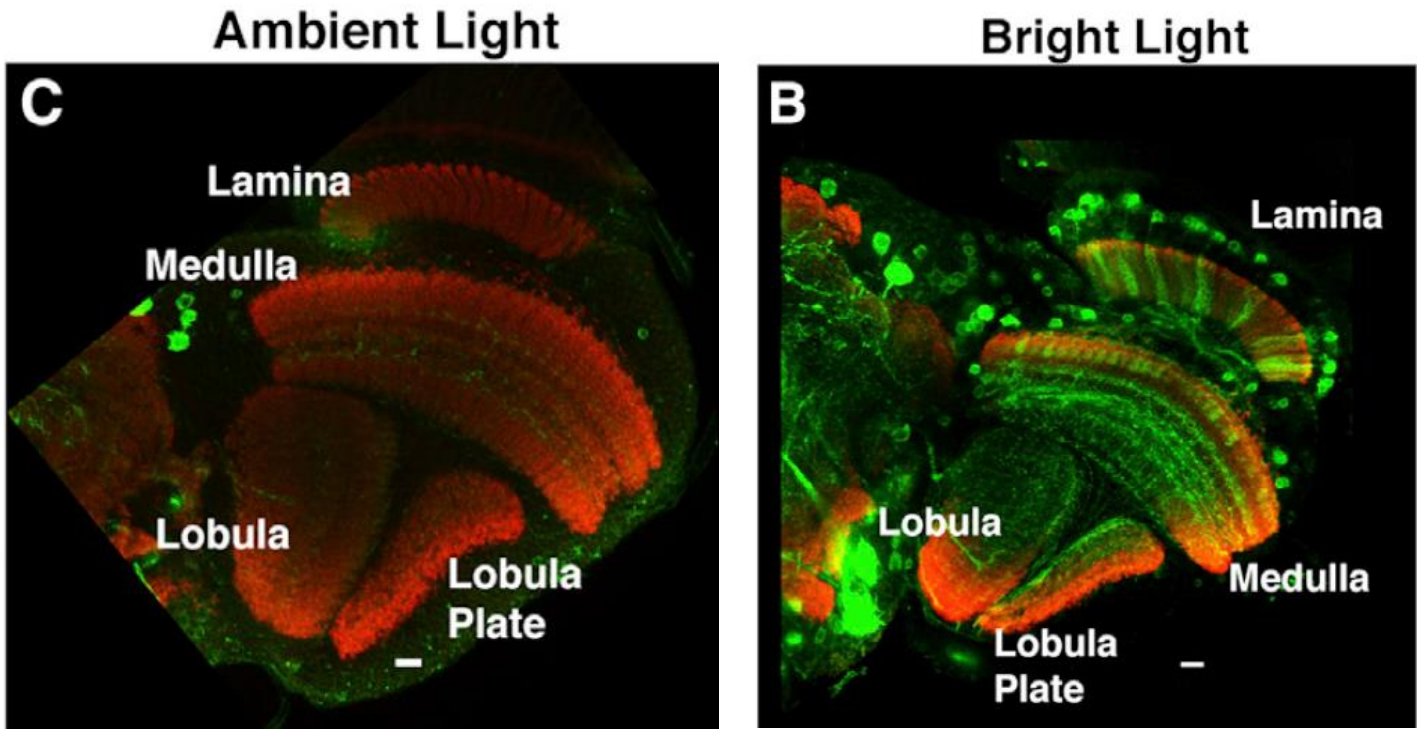




Ligand interaction results in stable transcriptional readout

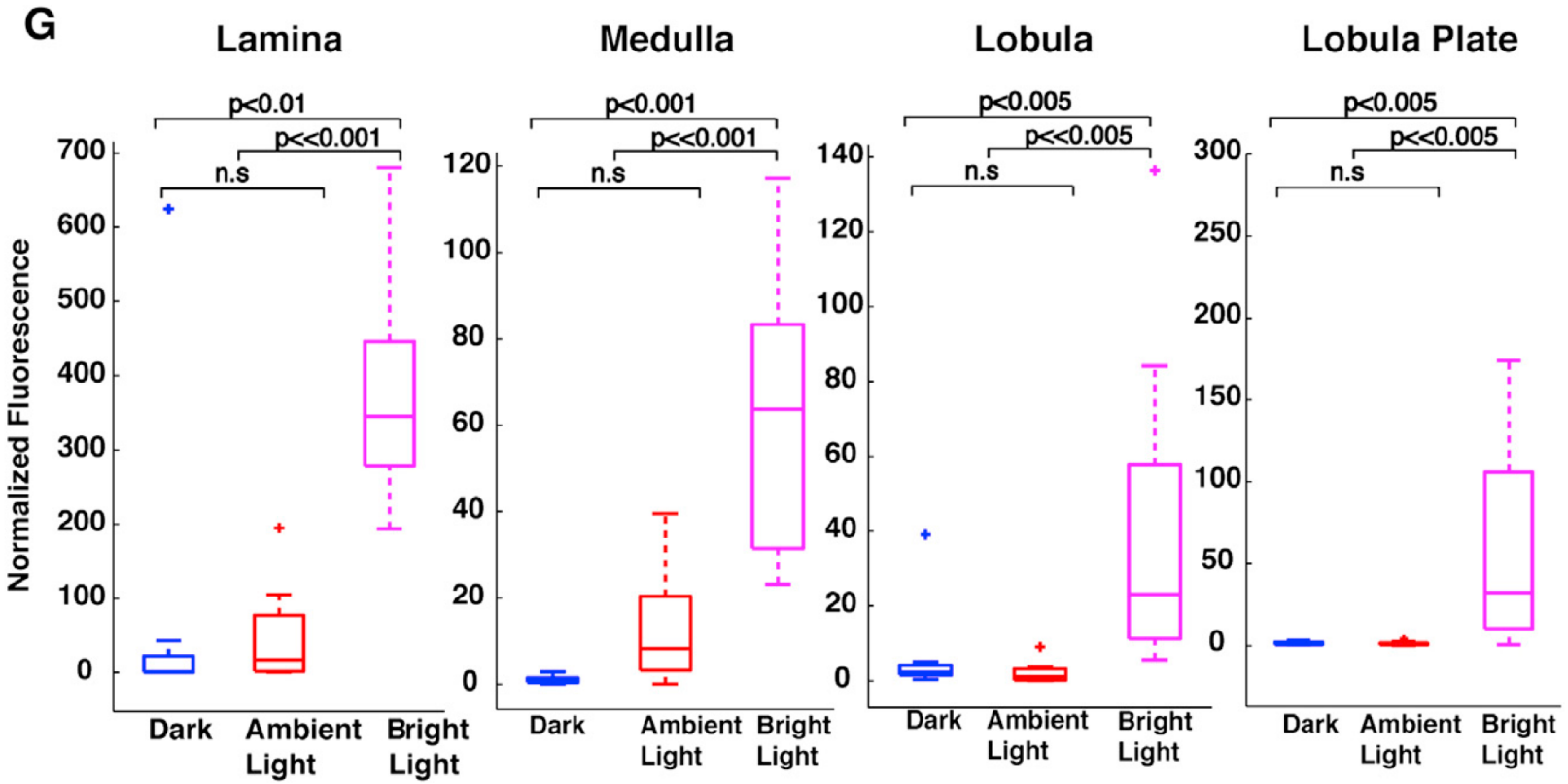
Tango-Trace: light dependent labeling

- Animals exposed to **histamine** should express CD8-GFP
- First darkness➔ Exposed 10s light flashes (3mins)
- ➔ 16hr later whole **mount Immunohistochemistry** (AB against GFP): cell bodies and axonal projections visualized in lamina and medulla
- Second fusion protein: TEV protease linked to b-arrestin
- Ligand ➔ b-arrestin recruited ➔ cleaves TCS➔ Gal4 in nucleus➔ UAS bound to GFP



Tango-Trace: Quantification of fluorescence (CD8-GFP)

- Dark- reared flies and flies reared in ambient light exhibited significantly lower levels of GFP staining
- **coordinate expression** both of HRH2-TCS-Gal4 and Arr-TEV fusion proteins

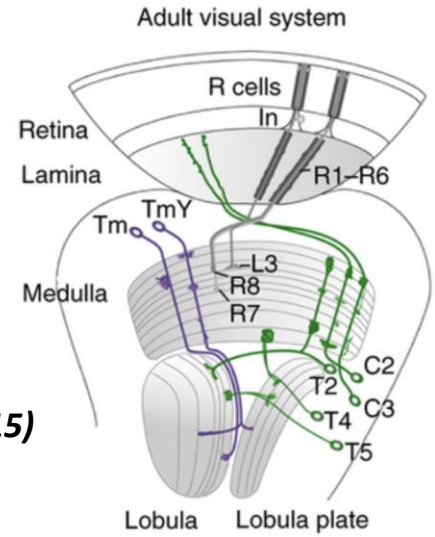


Tango-Trace: HA-Tango fly heated

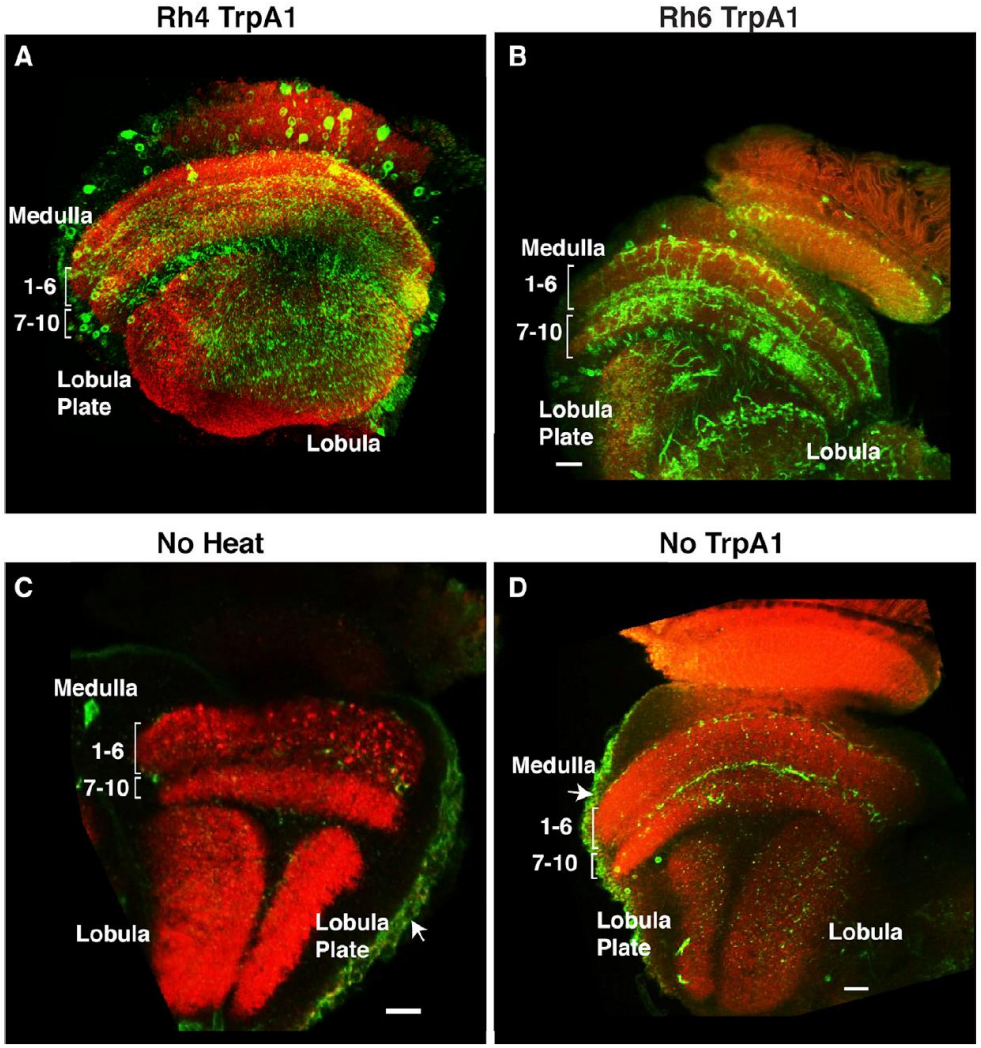
- Mosaic analysis with repressible cell marker (MARCM) clones were generated by a 30 min heat shock every day from early larval to late pupal stages during development.
- Interchromosomal recombination by heat shock during development: loss of Gal80 (transcriptional repressor) and permits the activity of gal4 driver
- Generated flies that carry the HA-Tango-Trace transgenes, along with the transgenes facilitating MARCM
- MARCM combined with Tango➔ visualize CD8-GFP expression in postsynaptic neurons (HA-Tango Flies)
- gradual warming ➔ activation of photoreceptors expressing TrpA1 (temperature sensitive channel)➔ HA release
- This strategy also labels the **pre**synaptic photoreceptors in which Rh-Gal4 drives the UASmCD8-GFP reporter and postsynaptic dendritic and axonal arbors are visualized

Tango-Trace: Projection tracing

- Promoters of four different **rhodopsins** used to drive expression of the **temperature-sensitive** cation channel, TrpA1, in p/y R7 and R8 cells
- Heat-induced HA release from yR7 cells driven by Rh4-Gal4
- Detect postsynaptic partners in the medulla and the projections of these **postsynaptic** neurons in the lobula complex

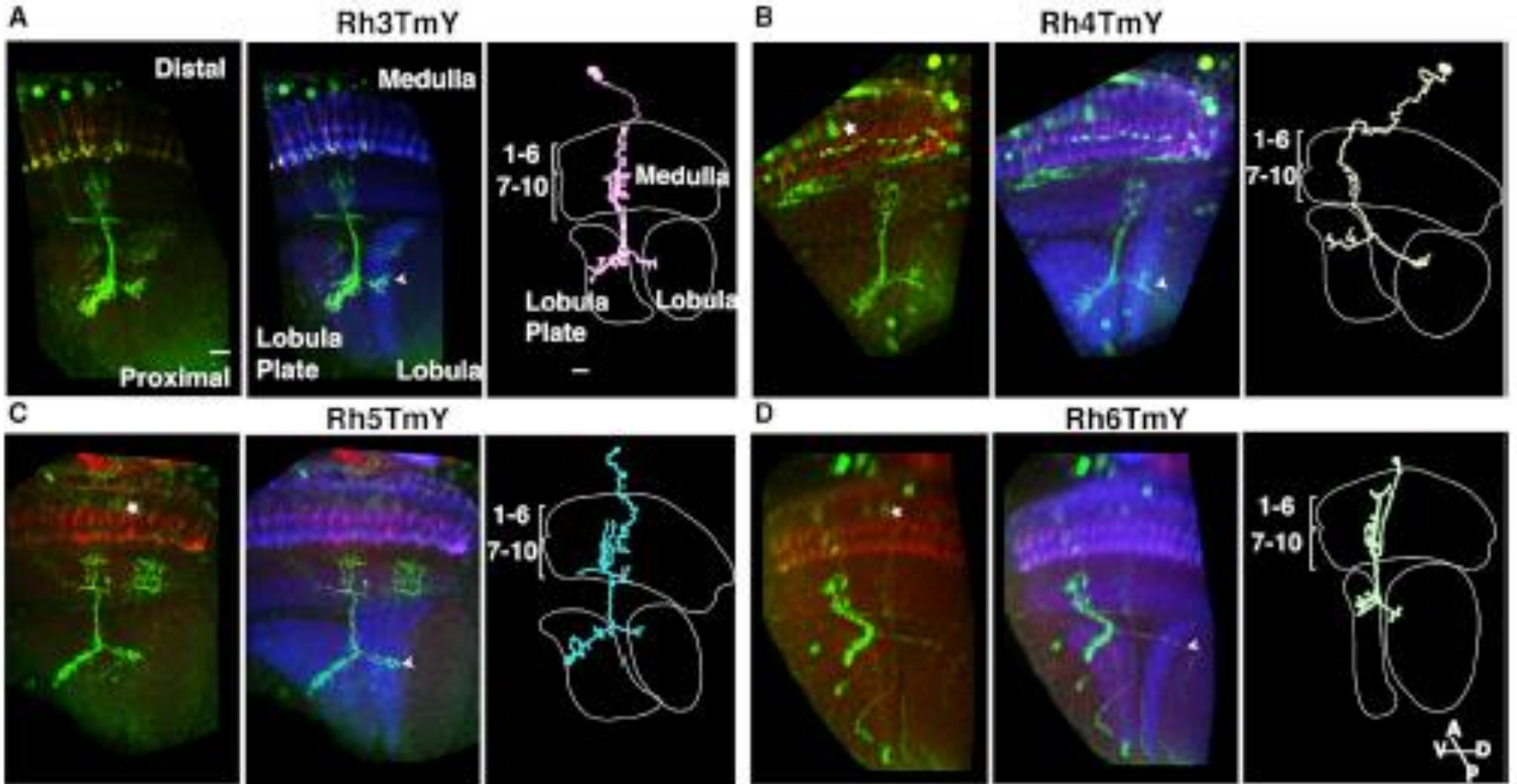


(Apitz, 2015)



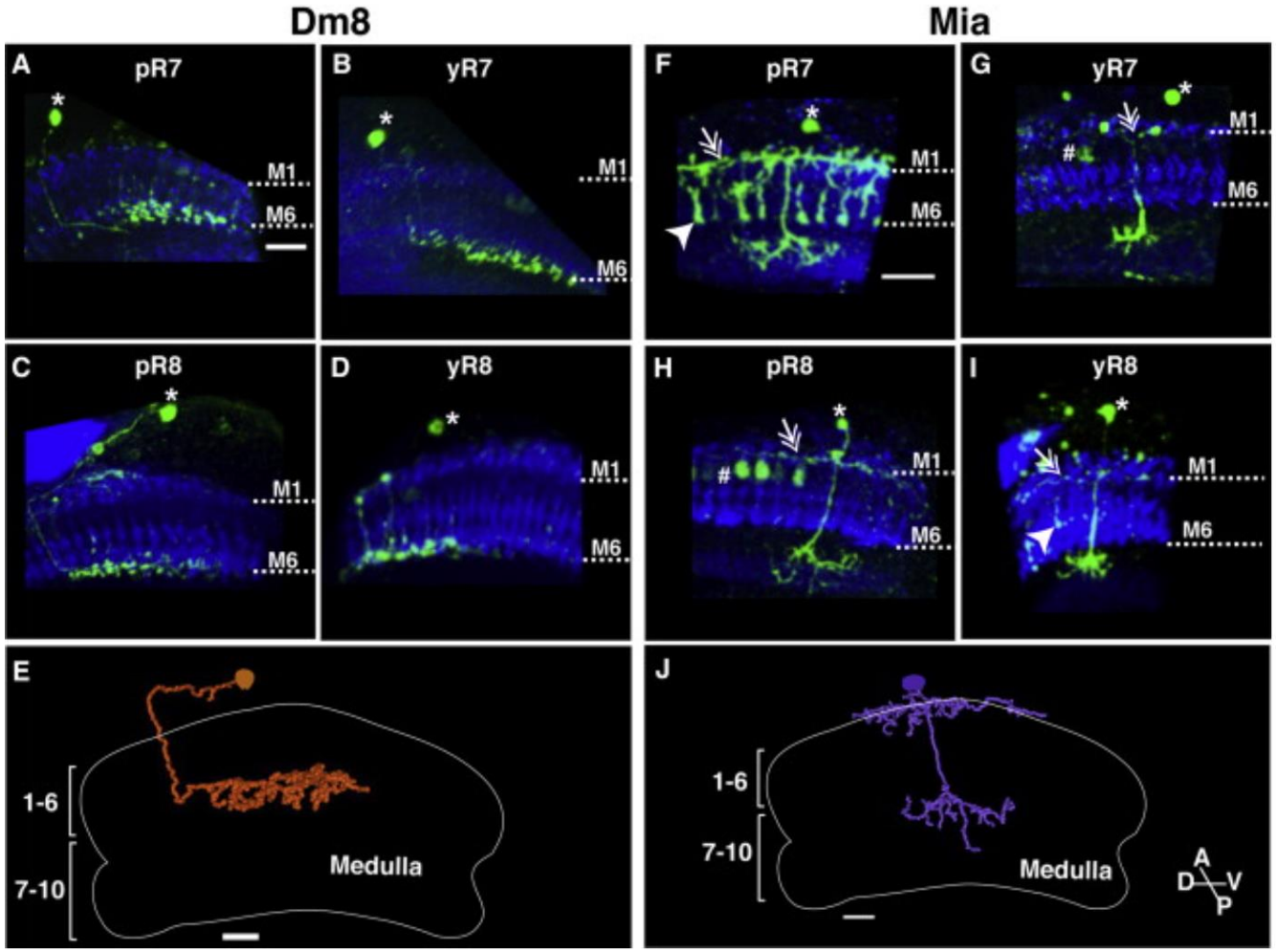
Tango-Trace: distinct branching

- **photoreceptors**, p/y R7 and R8 each synapse on one of four **distinct TmY cells**. distinct branching that characterize the four TmY cell types
- Tracking strategy labels postsynaptic partners of R7 and 8 photoreceptors and R7, 8 cells themselves= examine projections from TmY into labeled column



Tango-Trace: Neurite tracing of rare cells

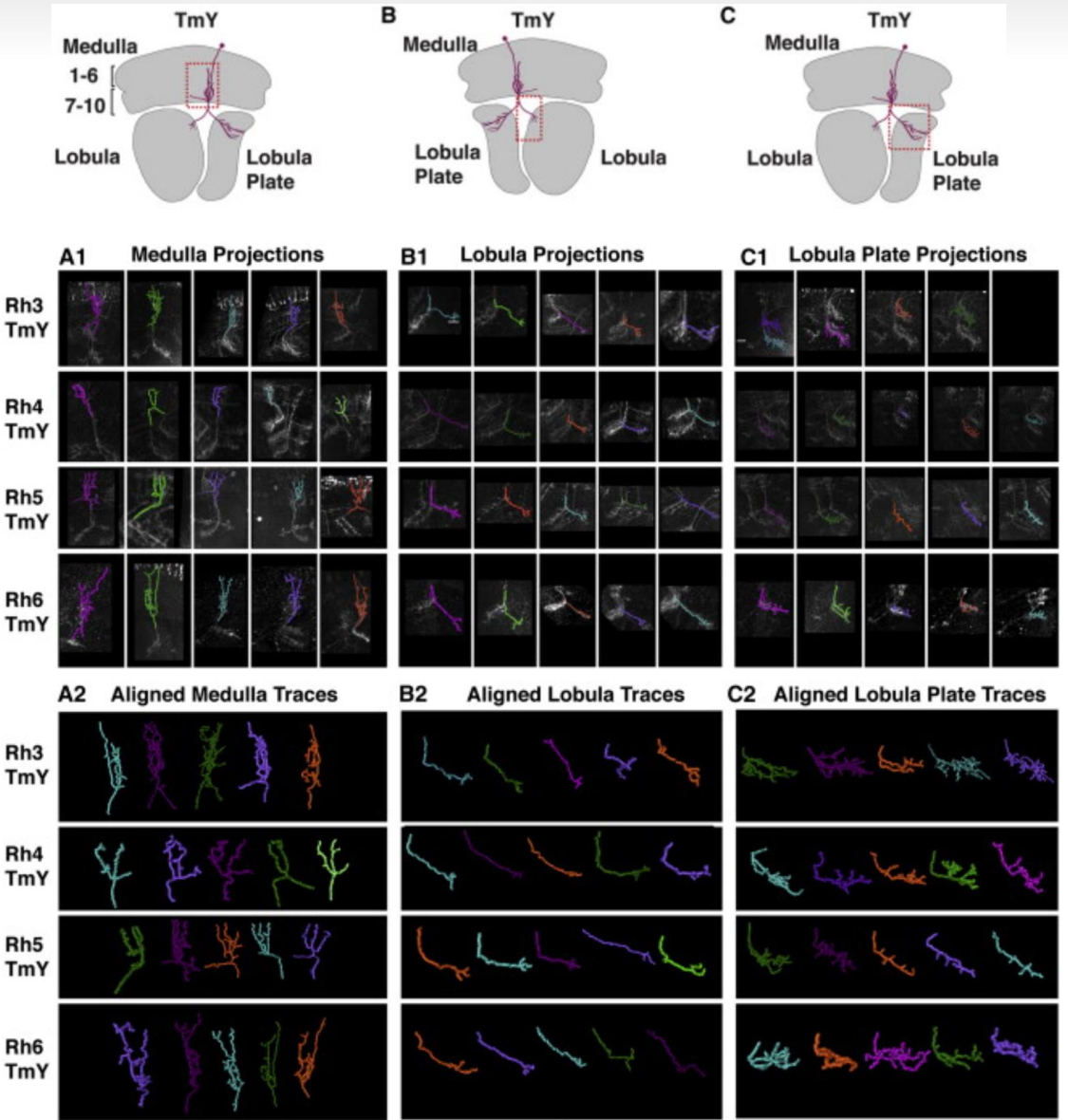
Dm8 is a wide-field amacrine cell contacting 13–15 columns in layer 6 of the medulla and was identified as a postsynaptic target of p/y R7 and R8
contact Mia, a narrow-field amacrine cell contacting 4–5 columns in layer 8 of proximal medulla



Tango-Trace: projection pattern

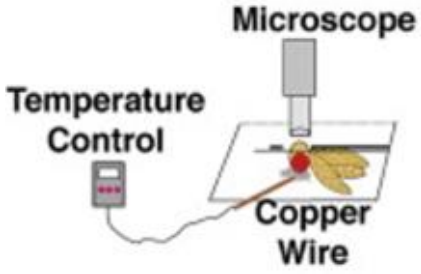
similarity in projection pattern is observed among the four different TmYs medullary projections of the TmY cells cluster into four groups that correspond to their four presynaptic partners

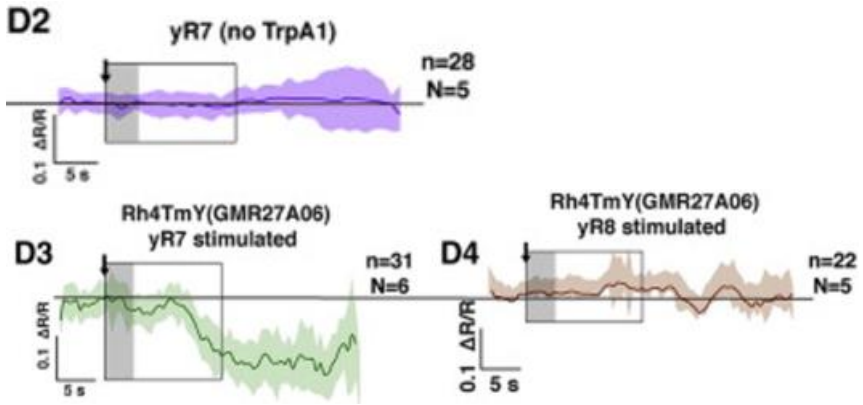
- A TmY cell, postsynaptic to a given photoreceptor type also innervates columns that receive input from different photoreceptors

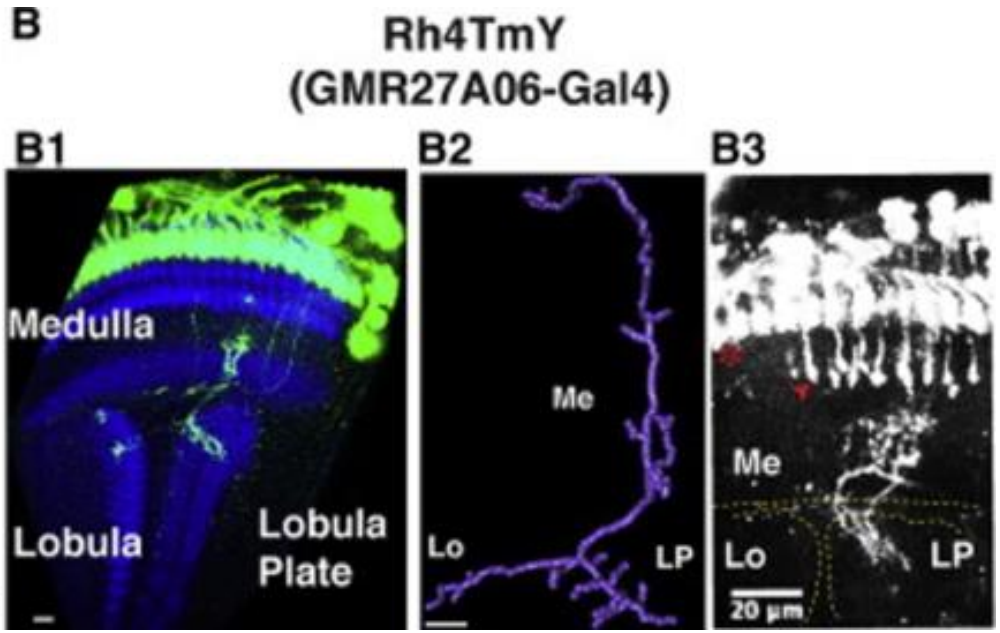


Tango-Trace: Imaging responses of TmY cells

- Enc**oded calcium indicator** expressed cells postsynaptic to yR7 and in yR7 photoreceptors cells using HA-Tango-Trace
- Expose eye to local heat with a temperature controlled copper wire
- The heat-sensitive channel, dTrpA1, is expressed in the individual photoreceptors and calcium responses were recorded (2Photon) in the postsynaptic partners labeled by Tango-Trace in response to heat activation (stimulating yR7 and R8 cells)
- Immuno-stained to depict CD8GFP labeling with anti-GFP (green), neurophil (blue), photoreceptor (red)





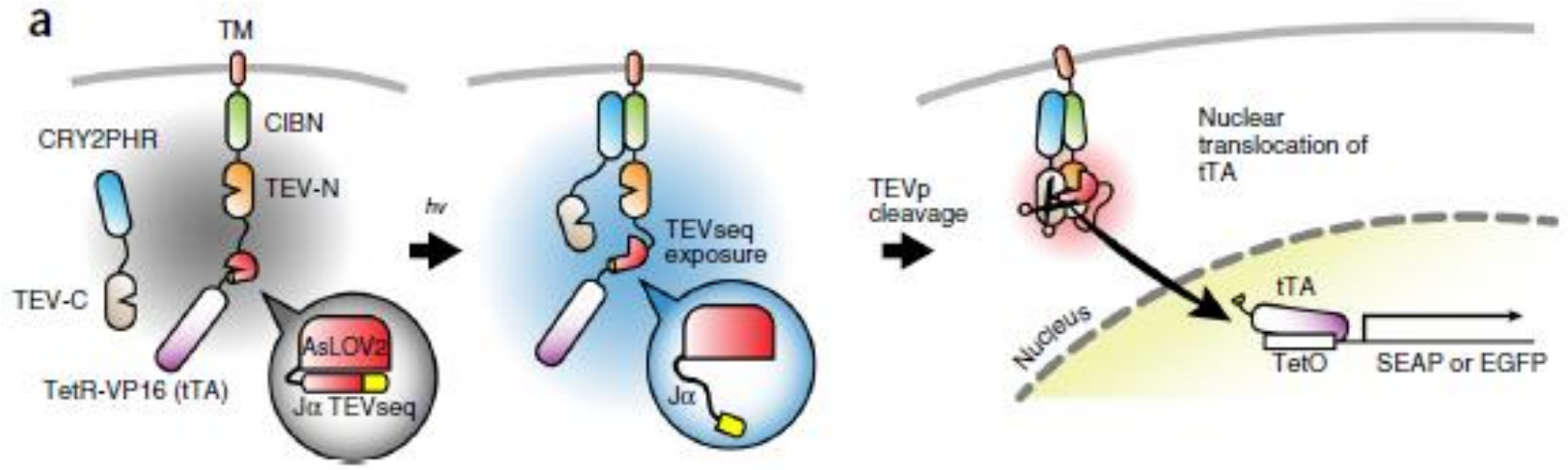


Summary

- HA-Tango-Trace labels specific functional connections in the visual system
- LIMIT: Although each TmY innervates two to three distinct columns, the photoreceptor in only one of these columns was labeled by the Tango-Trace strategy

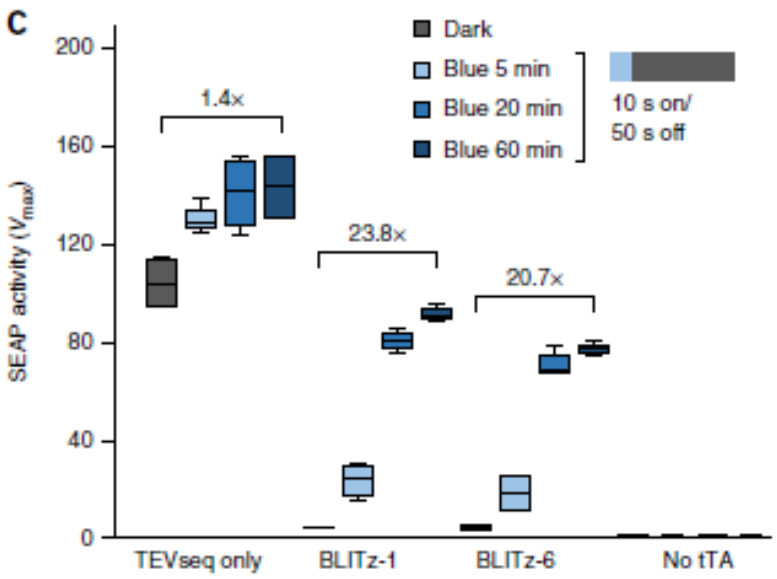
iTango: light-switch control system

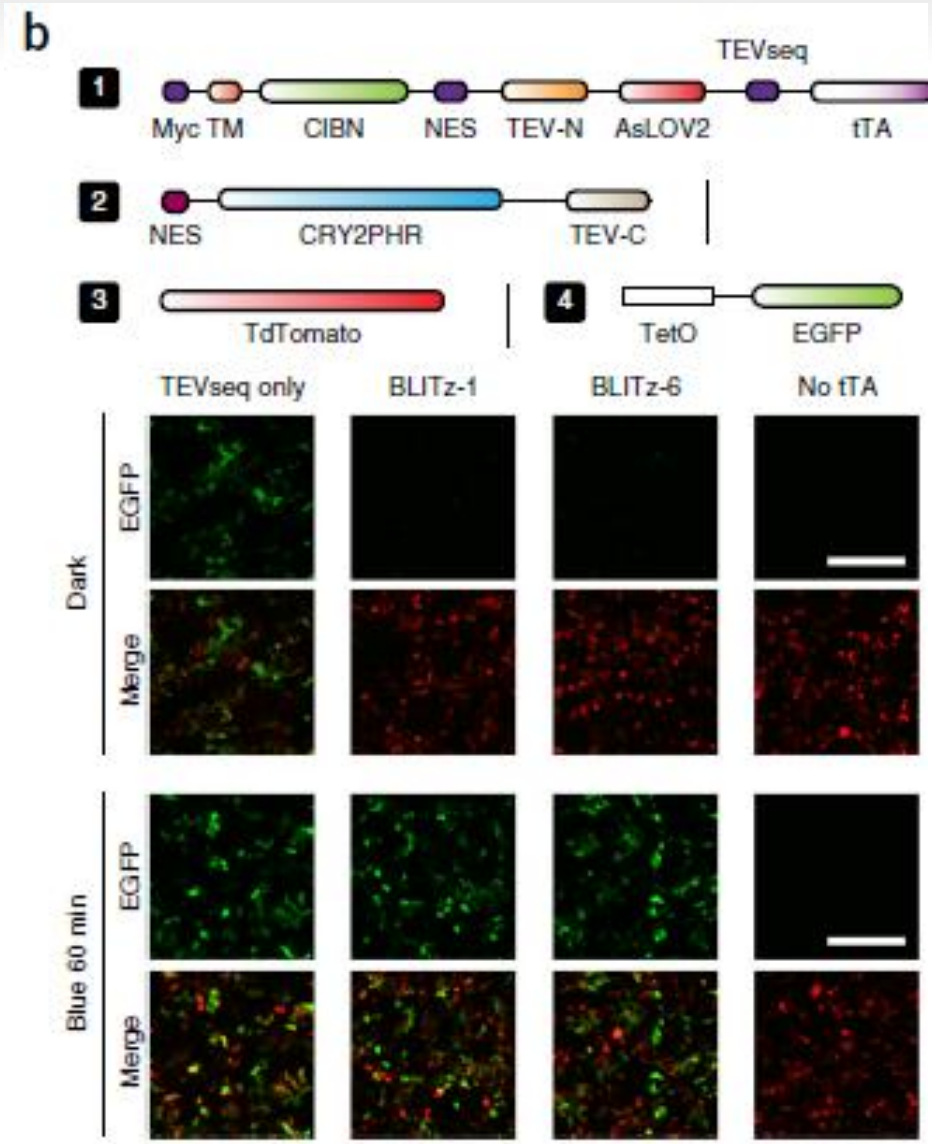
- **two-component** light-switch control system, named **B**lue-**L**ight-**I**nducible **T**EV protease (BLITz)
- TEV-C and TEV-N cant bind without light
- Replaced TEV with **TEV-C** to reduce leakiness originated form intrinsic affinity of C and N terminal fragments
- Light: CIBN and CRY2PhR interact ➔ TEV-C and TEV-N interact and cleave TEV seq
- AsLOV2= new light inducible dimer
- BLITz relies on protein-protein interaction



iTango and BLITZ

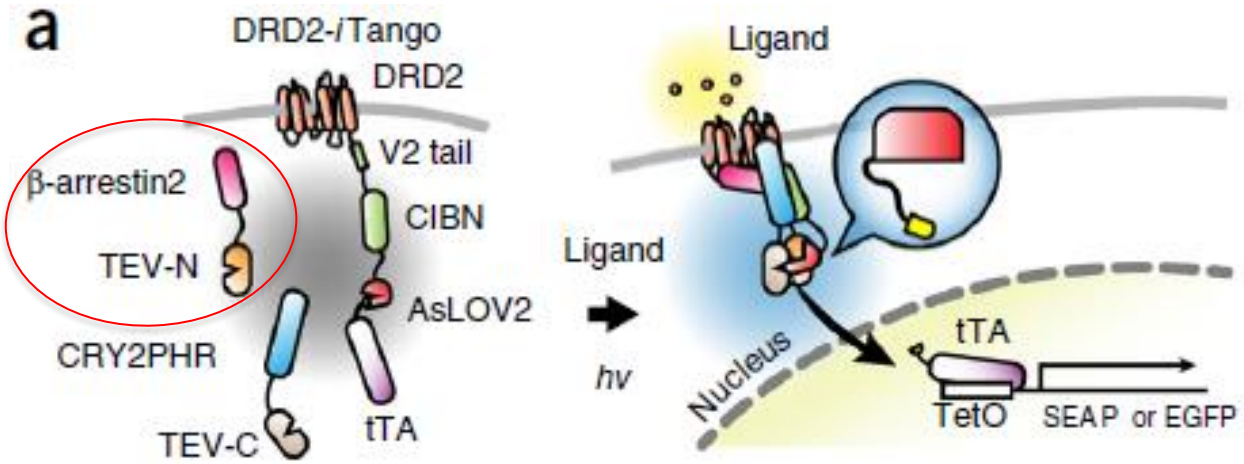
- BLITZ1 as light-gating module
- Gene expression increased with longer exposure time





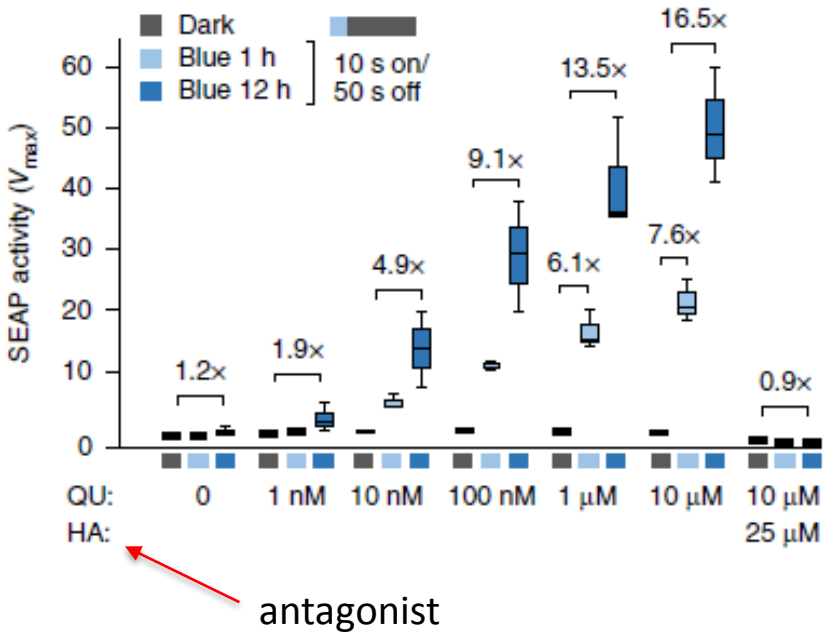
iTango: two-step activation design

Both ligand and light should be present
Ligand binding➔ b-arrestin TEV-N recruited, not cleaved
Blue light➔ TEV-C forms protease and cleaves TEV Seq ➔ Transcription activator, reporter gene expression

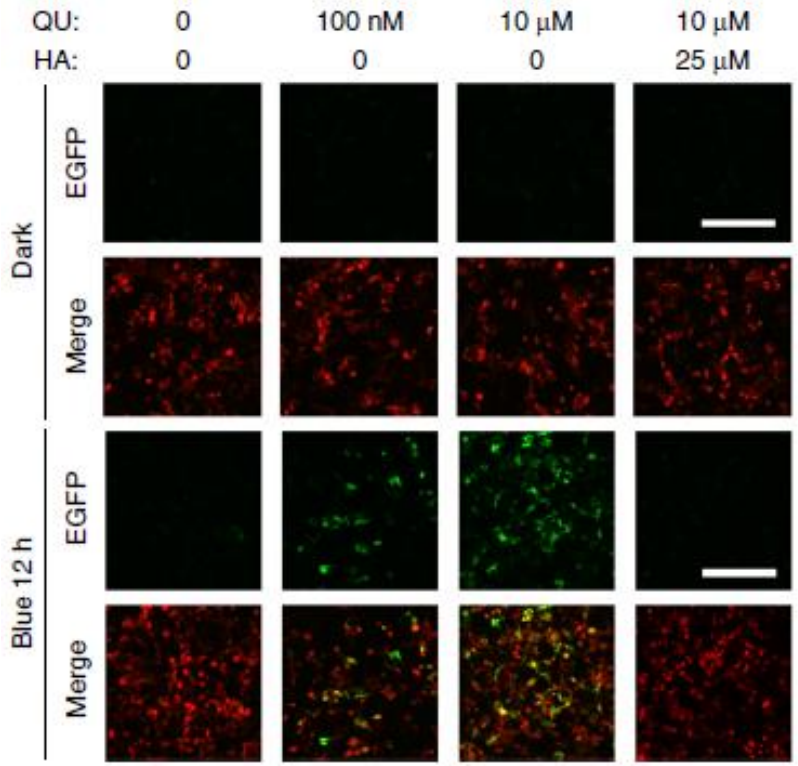


iTango: two-step activation design

iTango (with D2R domain) construct expressed in HEK cells and treated with D2R agonist ➔ SEAP reporter (dark: no gene expression; blue light; gene expression)
Secreted embryonic alkaline phosphatase (SEAP) = study promoter activity or gene expression



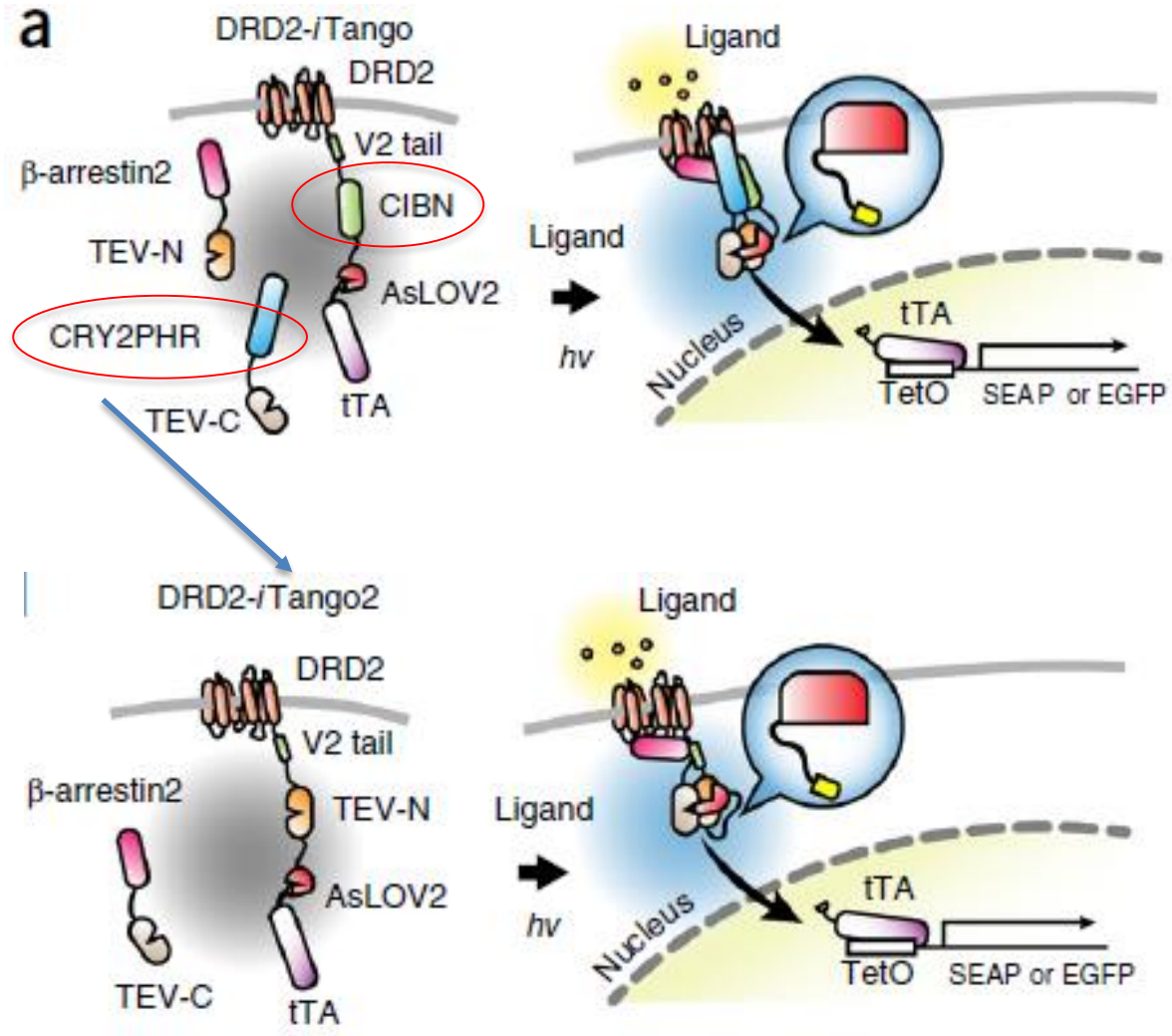
EGFP-reporter expression assay



B-arrestin2 protein in cytosol is critical for good SNR

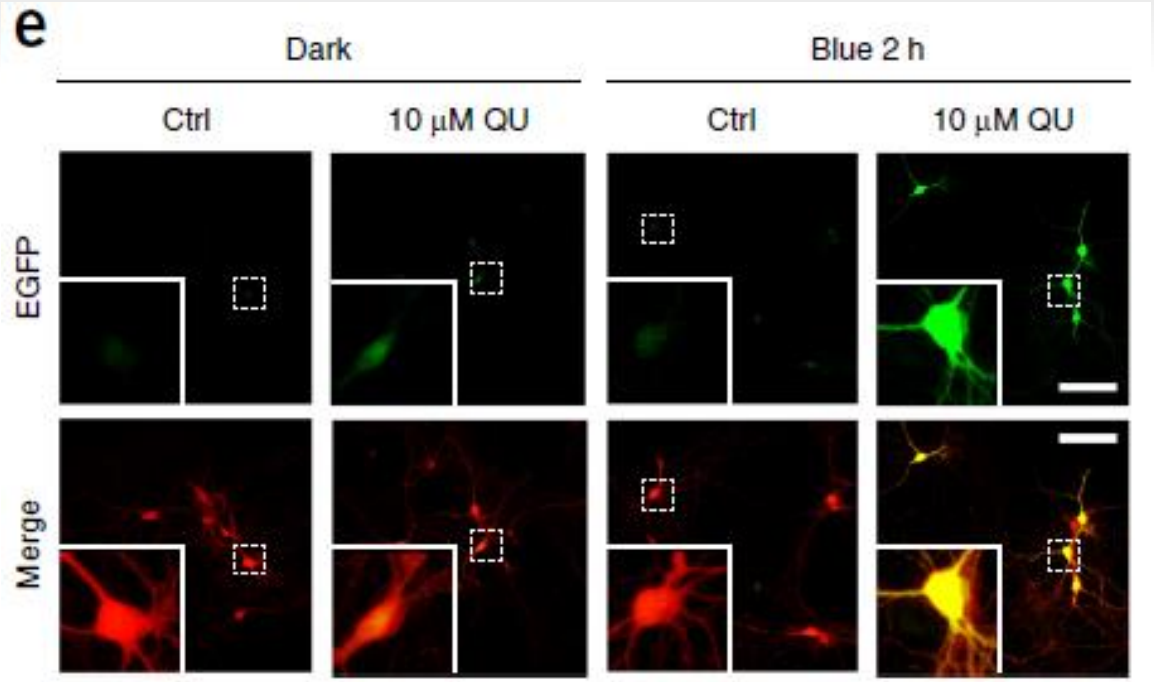
iTango2: in neurons

Lack of CRY2PHR–CIBN light switch: efficient formation of protein complex

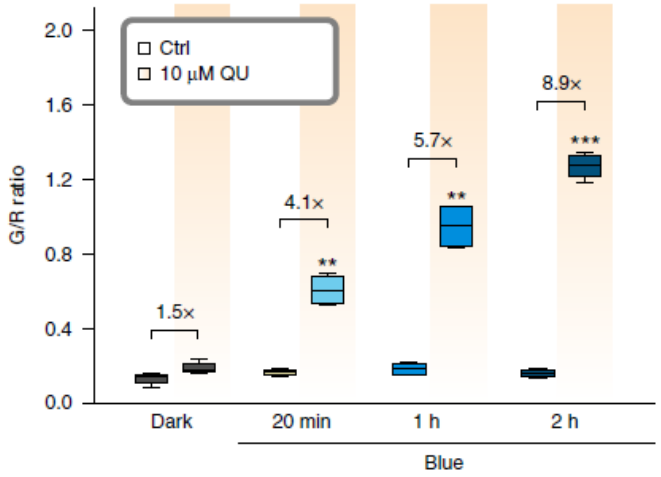


iTango2: in neurons

Rat HC culture neurons transfected with iTango2



SNR corresponding to an ~900% increase. conventional Tango system, the same experiment yielded only a 50% increase in gene expression.



iTango2: in neurons

DrD2-iTango2 construct (barrestin TEVC Tomato, TRE EGFP reporter) In mouse cortical pyramidal neurons (slice cultures).

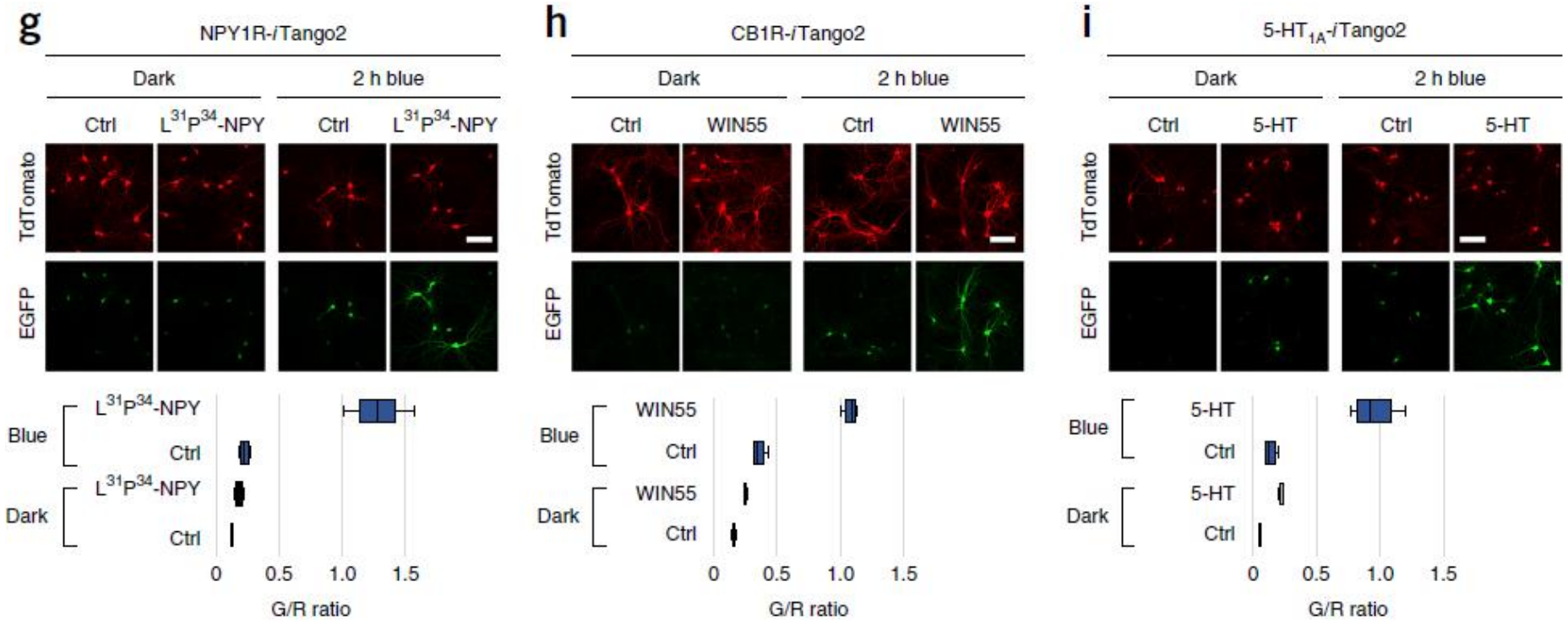
Check if iTango2 interacts with endogenous pathways:

➔ Dendirtic spine number unachanged (vs nontransfected neurons)

➔ Electrophysiology unchanged (AMPAR EPSCs normal)

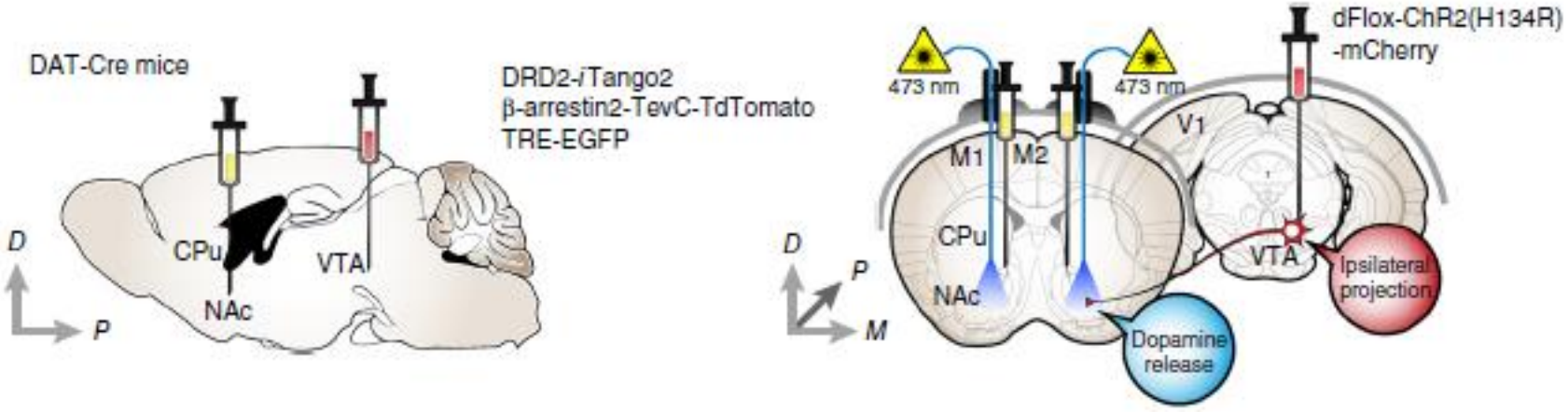
iTango2: different neurmodulators

Cannabinoid receptor, serotonin receptor…



iTango2: in vivo labeling

Injecting DRD2-iTango2 viuses into nucleus accumbens and AAV-dFlox mCherry into VTA
DA neuron projections are unilateral: Expecting left hemisphere “light only”, right hemisphere “Dopamine release”



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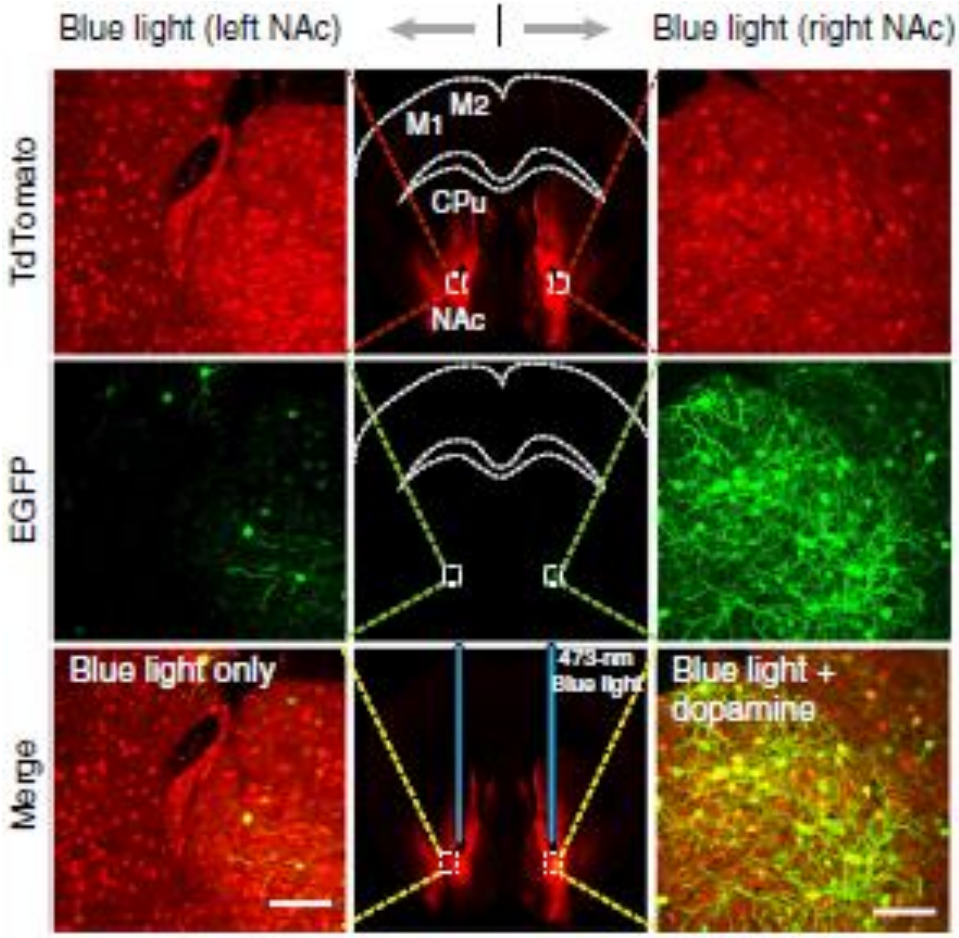
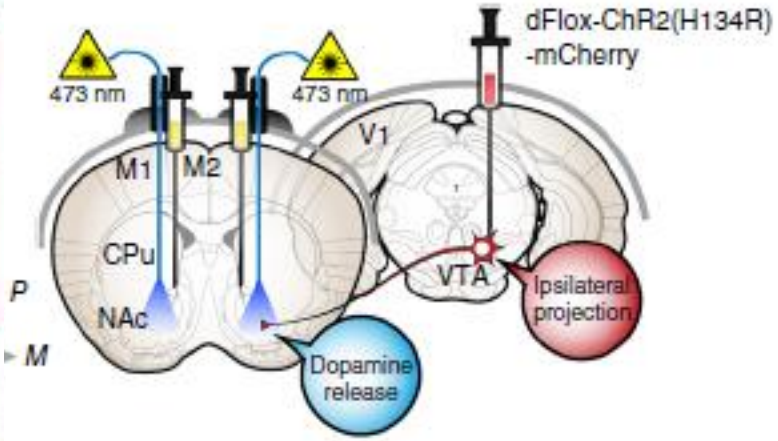
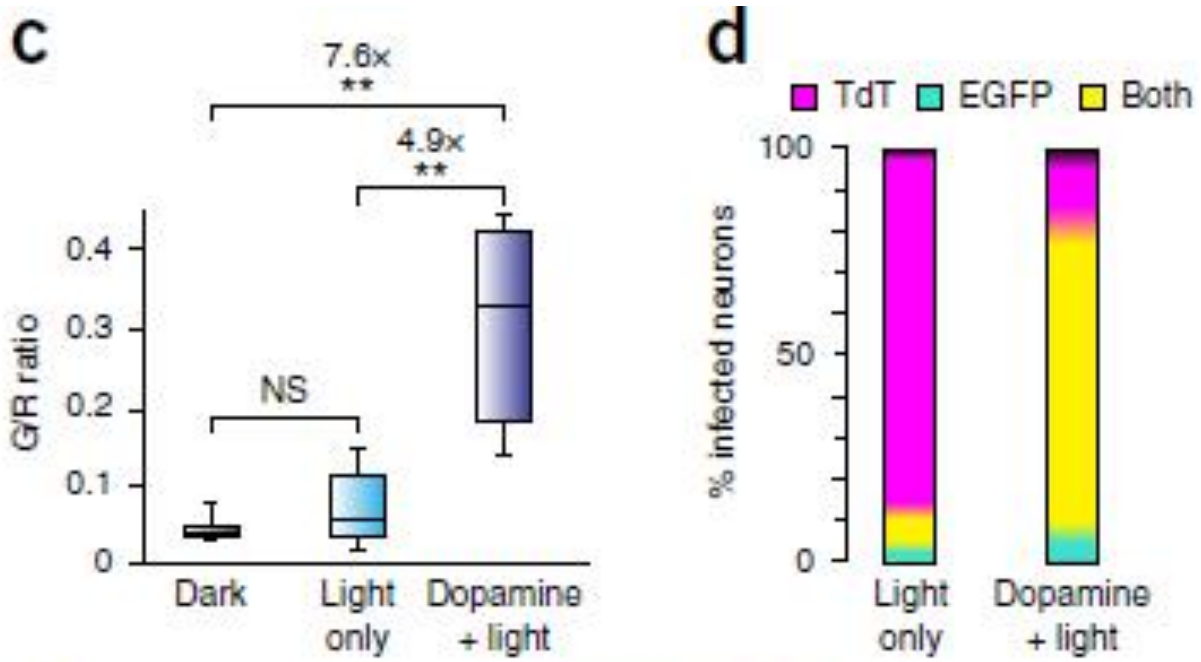
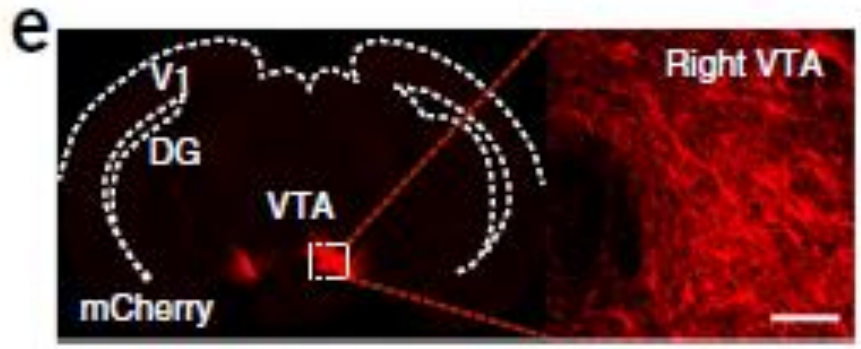




iTango2: detection of endogenous DA release

DRD2-*i*Tango2 is sufficiently sensitive to detect endogenous phasic DA release *in vivo*

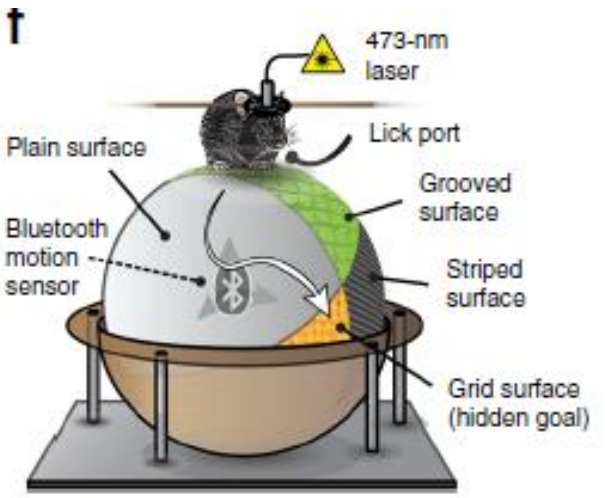


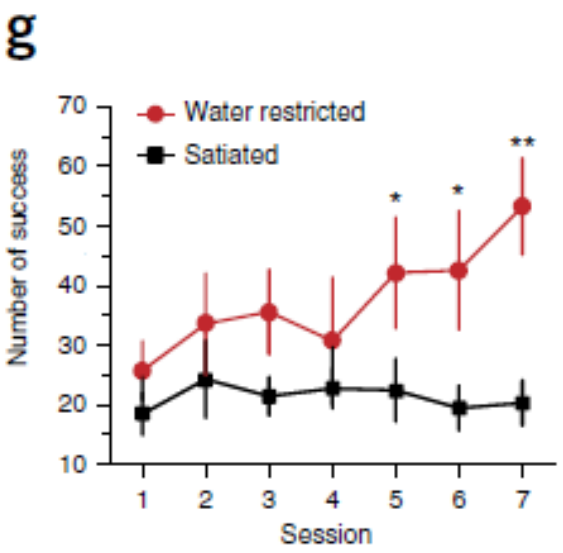


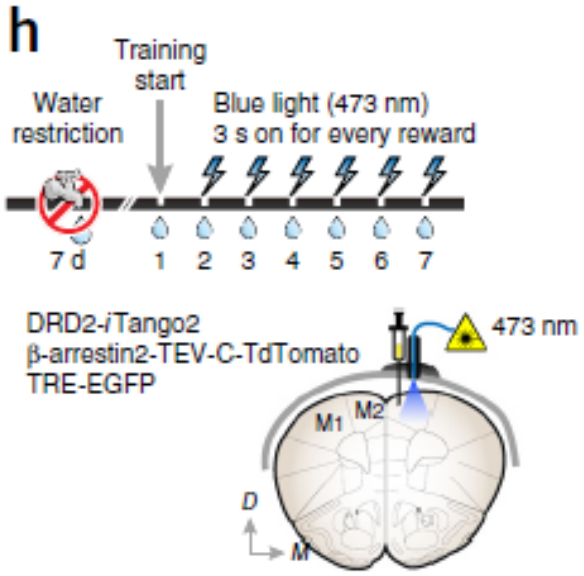
ChR2 expression in DA neurons confirmed in a posterior coronal section from the same mouse

iTango2: behavioral studies

4 sections on the surface of ball maze: plain, grooved, gridded, striped (sensory cues)
Sensory cues associated with reward (grid surface= water reward)



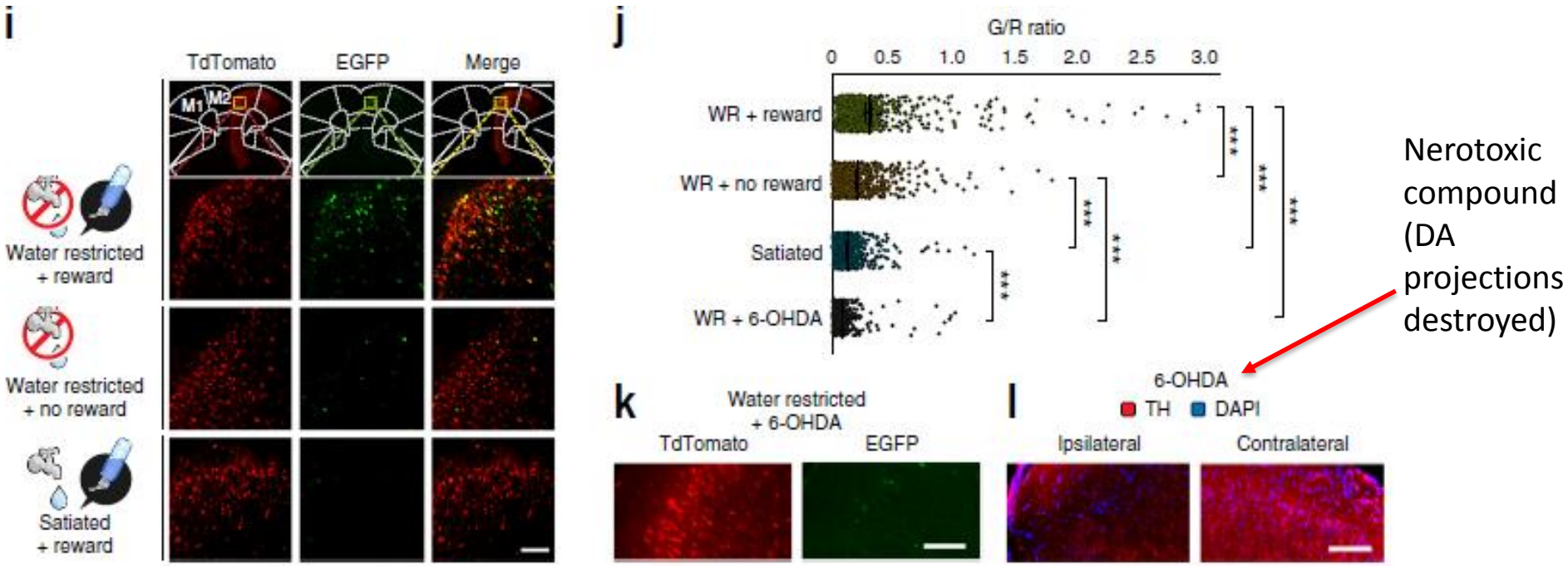




injected DRD2-iTango2 into the premotor cortex (M2) + blue light for 3 s whenever rewards were delivered

iTango: behavioral studies

EGFP expression with DA release and blue light

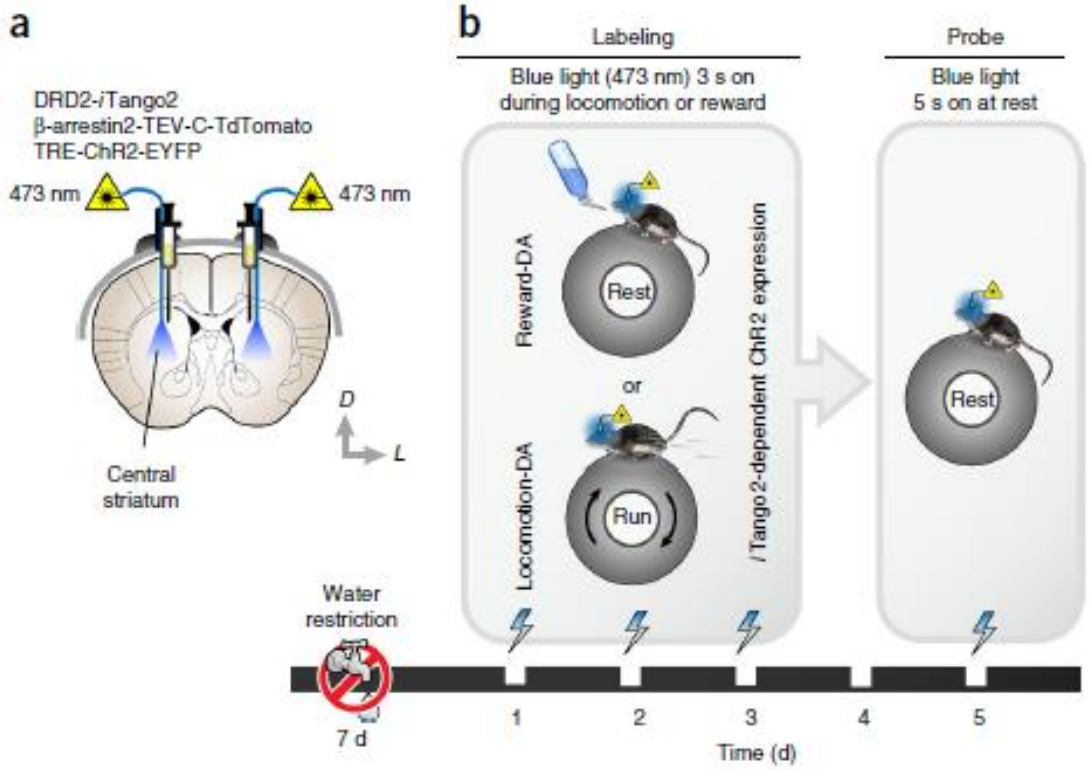


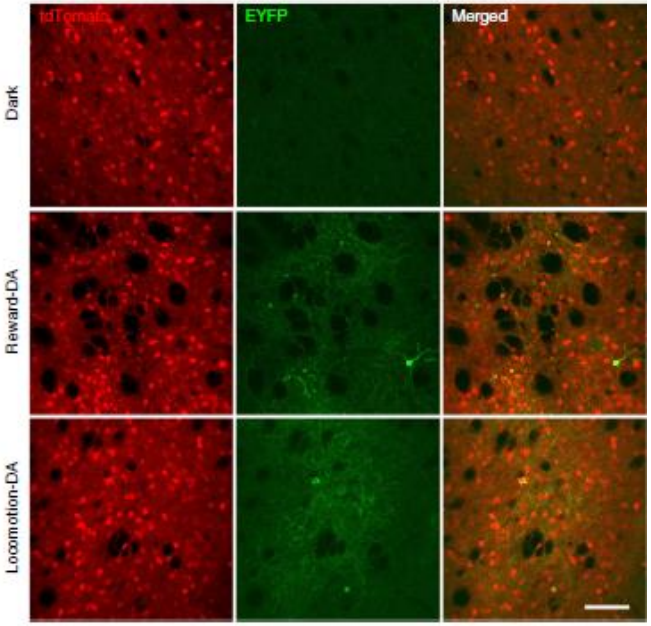
Nerotoxic compound (DA projections destroyed)

iTango system enables the visualization of neuromodulation action with high spatiotemporal precision in awake behaving animals

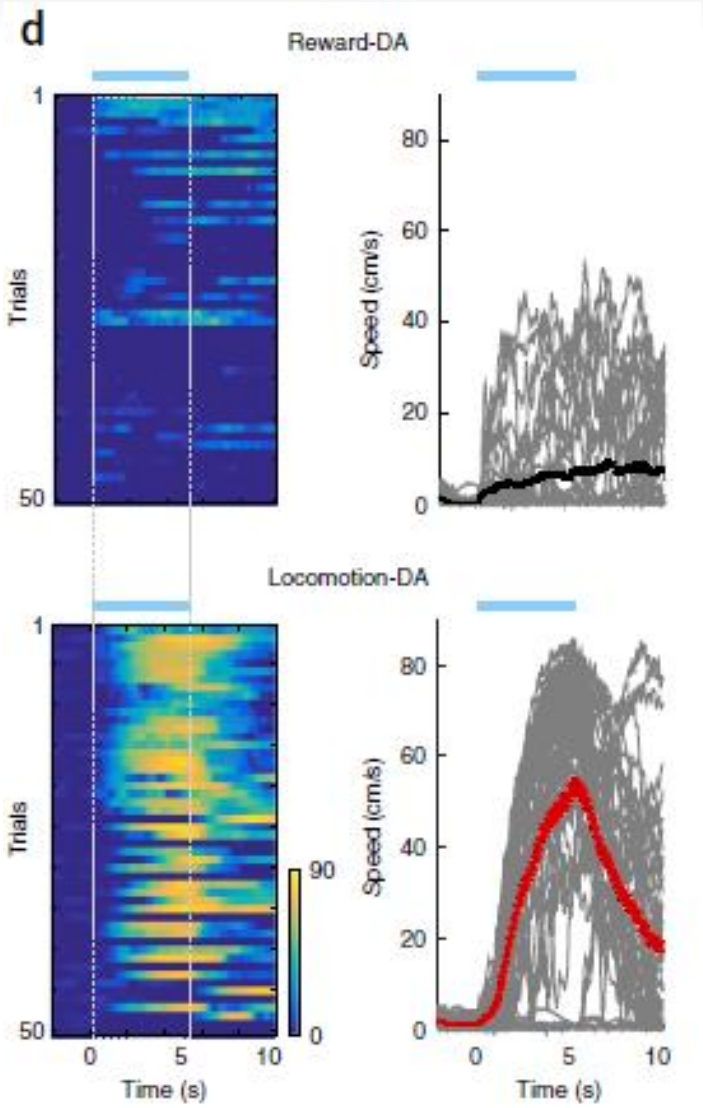
iTango: manipulation of neuronal activity

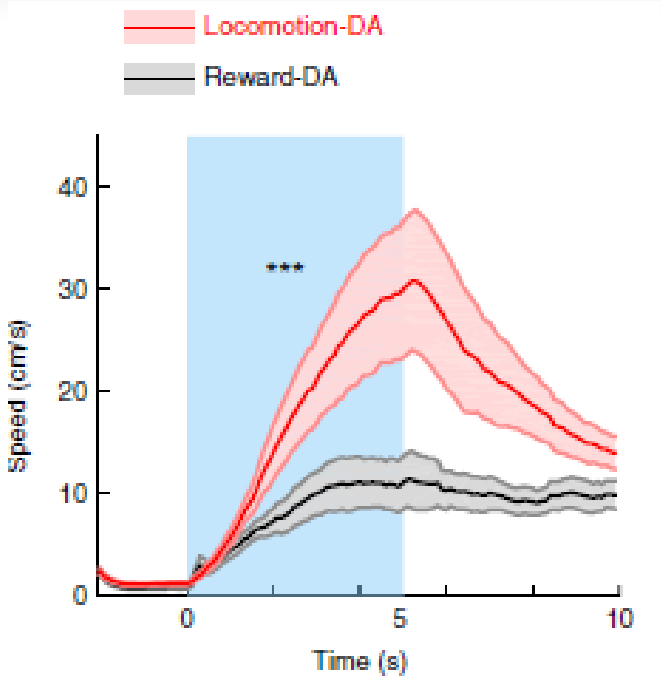
manipulation of neuronal activity by optogenetic effectors
Viruses were injected in central striatum and induced ChR2 expression with light during locomotion or reward-related (reported to be activated by DA)





iTango: channelrhodopsin triggered locomotion



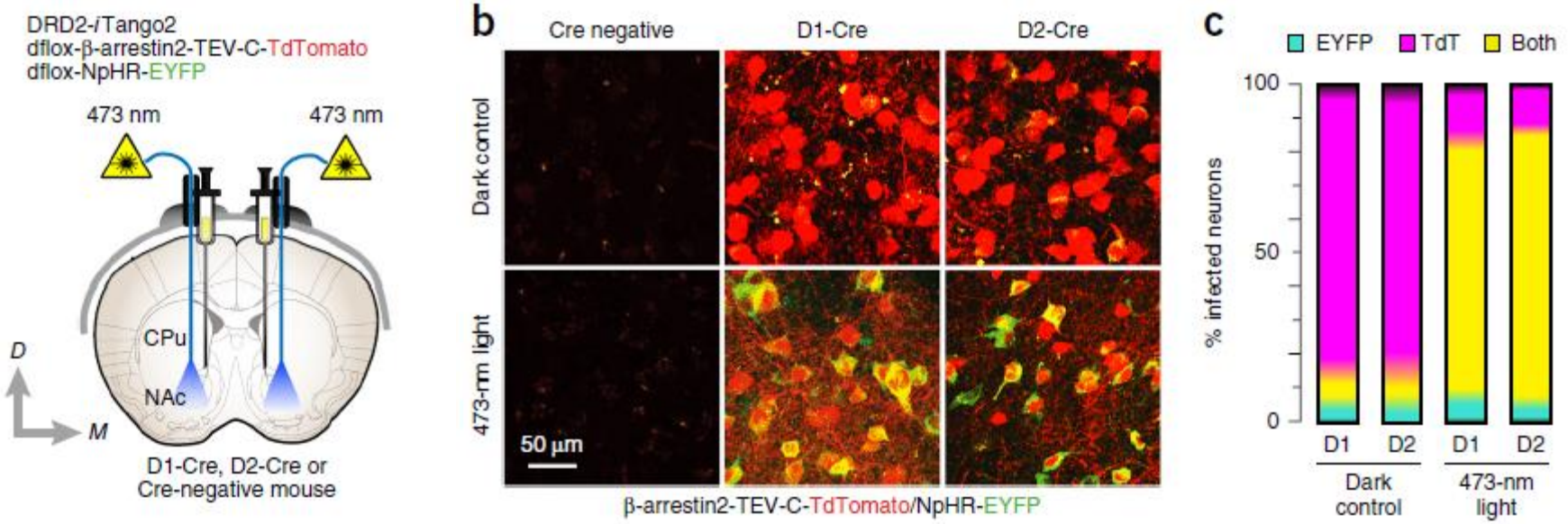


Channelrhodopsin activated with blue light
➔locomotion and no locomotion triggered dependent on neuronal population

distinguish behaviorally relevant subpopulations of neurons in behaving animals and to test the sufficiency of eliciting behaviors.

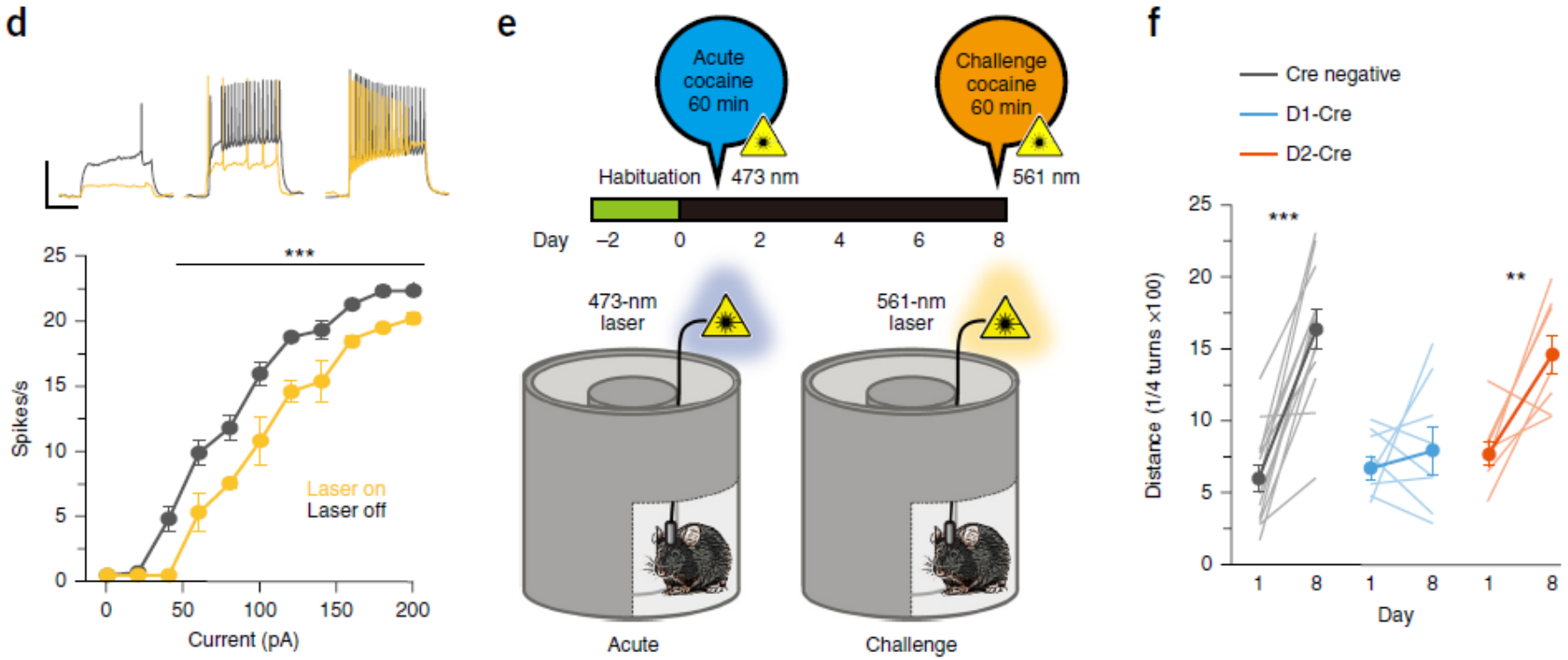
iTango: cocaine induced locomotion

Flox and Cre: controls, blue light necessary for expression
D2-coupled DA-receptor sensing system to induce the expression of halorhodopsin (eNpHR), an light-activated proton pump that inhibits cell firing



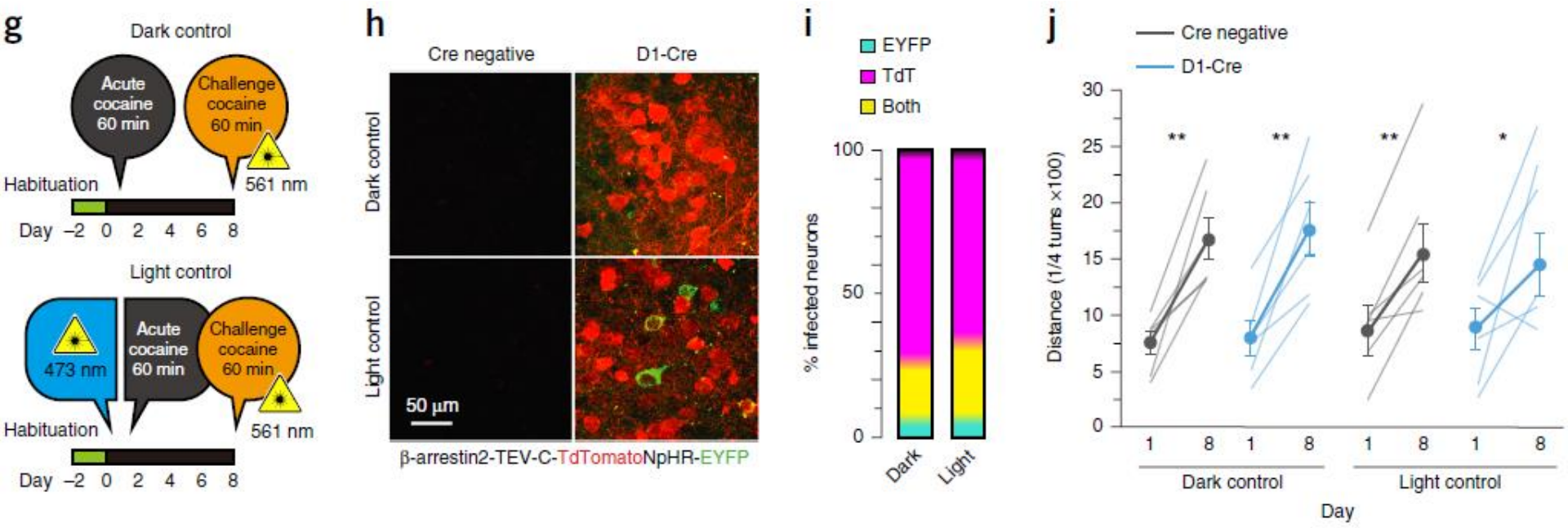
iTango: locomotor sensation assay

Slices from NAc of Cre-positive mice exposed to blue light and cocaine
Ex vivo slices: electrophysiology ➔ light inhibited firing of EYFP-positive cells
Injection of cocaine with blue-light➔ locomotor response
After 7 days of withdrawal: challenge dose of cocaine➔ locomotion suppression in D1-Cre



iTango 2017

Dark control or asynchronized light and cocaine



manipulate and thus assess the behavioral relevance of a temporally and genetically identified population of neurons.

high SNR and precise spatiotemporal

Summary

Tango

- converts a transient interaction into a stable and amplifiable reporter gene signal to record the activation of a receptor **without interference from endogenous** signaling pathways

Tango-Trace

- fast and reliable light-inducible technique, with a high SNR, that can be used to monitor and manipulate neuromodulatory signaling events

iTango

- iTango system enables the visualization of neuromodulation action with high spatiotemporal precision in awake behaving animals
- high SNR and precise spatiotemporal resolution
- monitor protein interactions in a cell with a high degree of selectivity and sensitivity

Thank You