# Newest advances in neuron replacement therapy for Parkinson's Disease

## Technical Journal Club

Daniel Heinzer

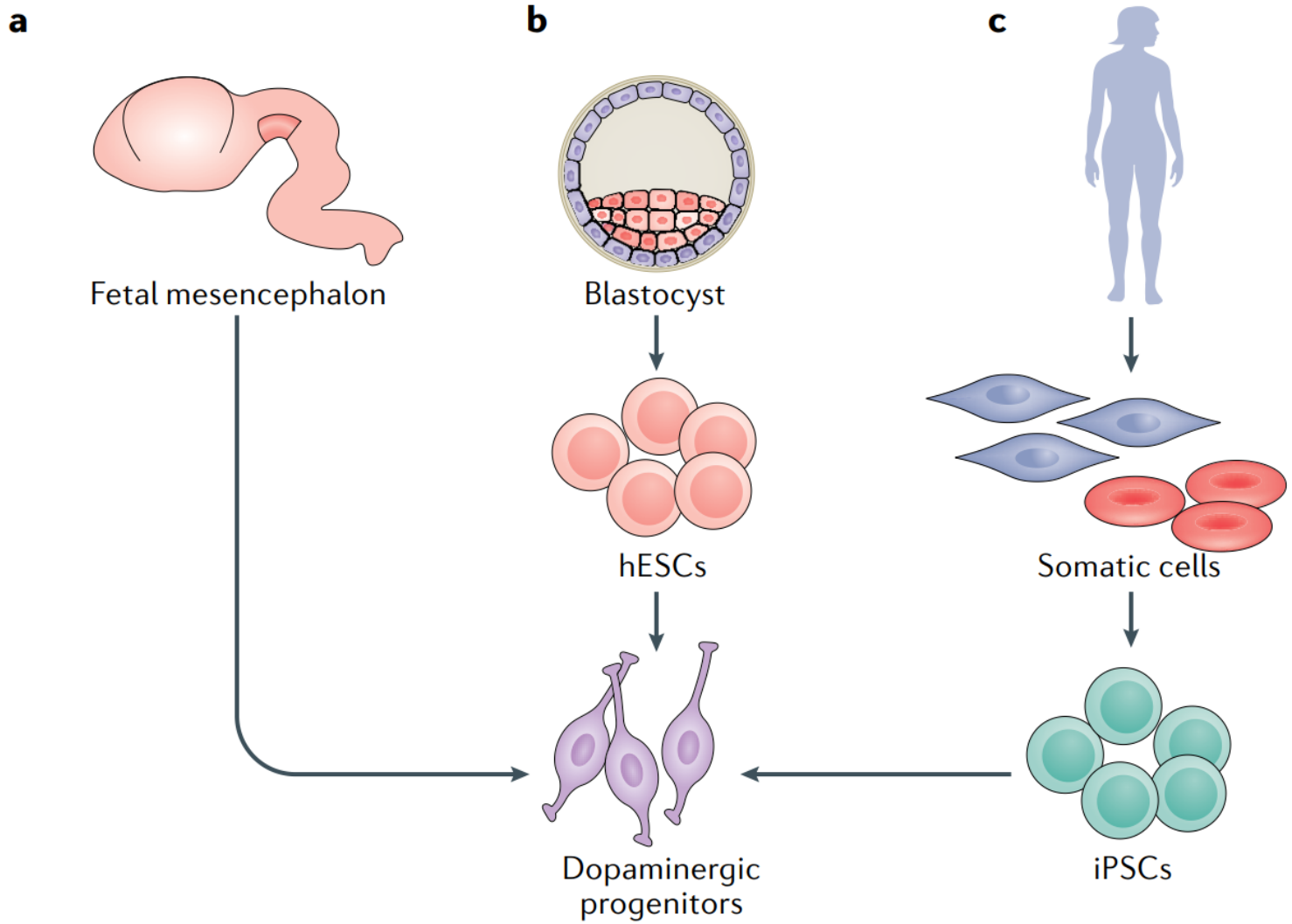
November 10th 2020

# Neuron replacement in PD – a short history

- A hallmark of Parkinson's Disease (PD) is a focal degeneration of mesencephalic dopamine (DA) neurons

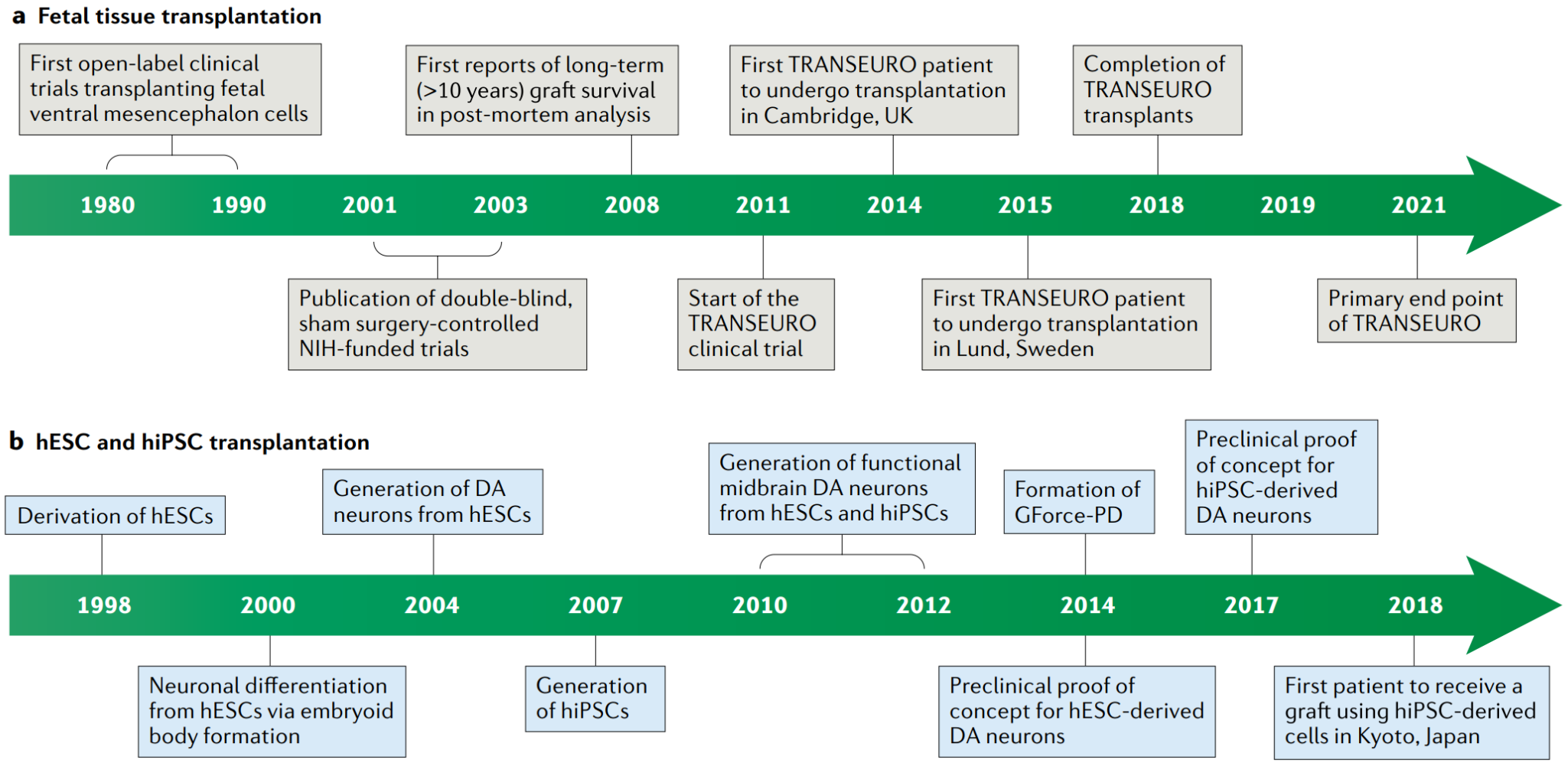
- Initial efforts of replacing degenerated neurons, alternatively to pharmacotherapy or surgical therapy (deep brain stimulation), started more than three decades ago

# Neuron replacement in PD – a short history




Parmar, M., Grealish, S. & Henchcliffe, *Nat Rev Neurosci*, 2020

# Neuron replacement in PD – a short history




Parmar, M., Grealish, S. & Henchcliffe, *Nat Rev Neurosci*, 2020

# Neuron replacement in PD – a short history

Advantage of iPSC derived cells compared to human fetal ventral Mesencephalon:

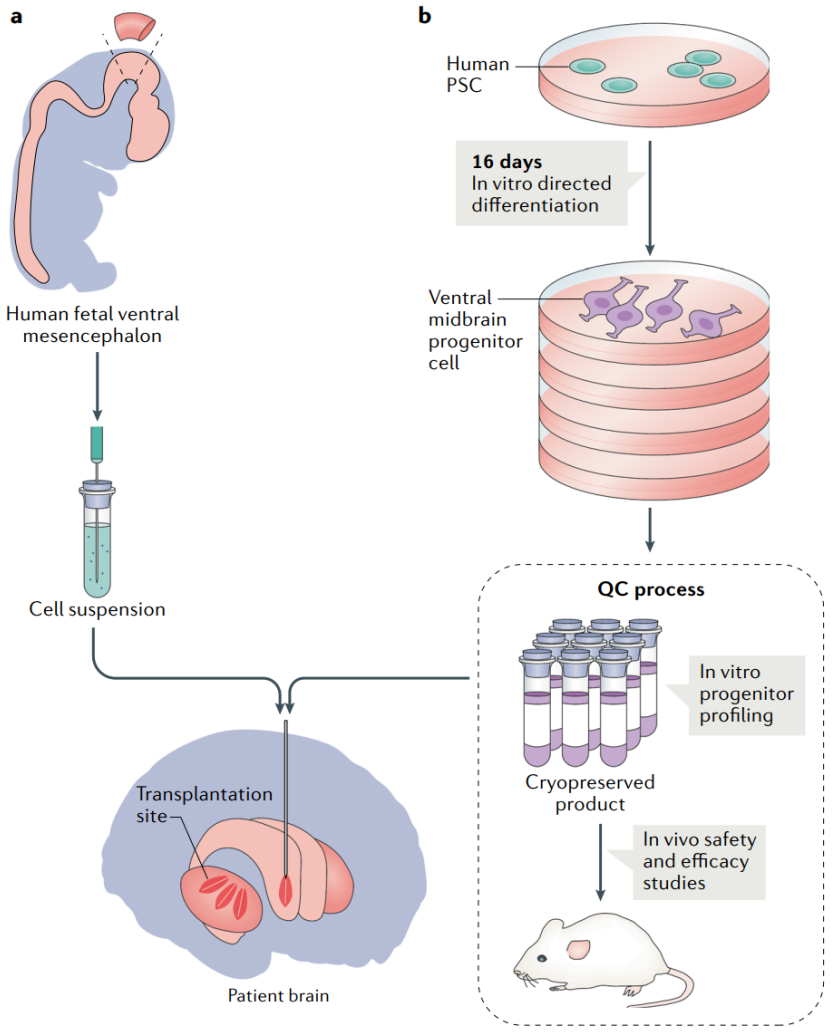
Availability

Standardized manufacture

Cryopreservation

Purity

Surgery, dosing and distribution




Parmar, M., Grealish, S. & Henchcliffe, *Nat Rev Neurosci*, 2020

# Neuron replacement in PD – a short history

Table 1 | **Academic clinical cell transplantation trials in Parkinson disease**

| Trial (NCT number) | Transplantations initiated | Donor cells (cryopreserved product) | Number of transplant recipients (age in years) | Disease duration (years) | Disease severity | Primary end point |
|---|---|---|---|---|---|---|
| TRANSEURO[a] (0189839)[66] | Completed | Human fetal VM tissue (no) | 11 (30–68) | 2–13 | Early to moderate | Efficacy |
| STEM-PD (NA)[92] | No | hESC-derived mesDA progenitors | 8 (<70) | 5–15 | Moderate | Tolerability and feasibility |
| NYSTEM-PD (NA)[78] | No | hESC-derived mesDA progenitors | 10 (45–72) | 5–15 | Severe | Safety, tolerability and feasibility |
| CiRA (NA)[93] | Yes | hiPSC-derived mesDA progenitors | 5–10 (50–69) | >5 | Severe | Safety and tolerability |
| Chinese Academy of Sciences (03119636)[161] | Yes | Stem cell-derived neural precursors | 50 (50–80) | >5 | Severe | Safety |
| Bundang CHA Hospital, Korea (01860794) | No | Human fetal VM neural precursors | 15 (18–70) | NA | Severe | Safety and tolerability |

Parmar, M., Grealish, S. & Henchcliffe, *Nat Rev Neurosci*, 2020

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

# Personalized iPSC-Derived Dopamine Progenitor Cells for Parkinson's Disease

Jeffrey S. Schweitzer, M.D., Ph.D., Bin Song, M.D., Ph.D., Todd M. Herrington, M.D., Ph.D., Tae-Yoon Park, Ph.D., Nayeon Lee, Ph.D., Sanghyeok Ko, Ph.D., Jeha Jeon, Ph.D., Young Cha, Ph.D., Kyungsang Kim, Ph.D., Quanzheng Li, Ph.D., Claire Henchcliffe, M.D., D.Phil., Michael Kaplitt, M.D., Ph.D., Carolyn Neff, M.D., Otto Rapalino, M.D., Hyemyung Seo, Ph.D., In-Hee Lee, Ph.D., Jisun Kim, Ph.D., Taewoo Kim, Ph.D., Gregory A. Petsko, D.Phil., Jerome Ritz, M.D., Bruce M. Cohen, M.D., Ph.D., Sek-Won Kong, M.D., Pierre Leblanc, Ph.D., Bob S. Carter, M.D., Ph.D., and Kwang-Soo Kim, Ph.D.

May, 2020

# Personalized iPSC-Derived mDAPs for PD

Extensive quality check for patient derived iPSCs and iPSC derived midbrain dopaminergic progenitors (mDAPs)

**Table S4. Quality control release criteria for human iPSC working bank**

| Test | | Method | Release Criteria |
|---|---|---|---|
| DNA fingerprinting | | PCR | Same as original fibroblast |
| qRT-PCR | OCT4 | Real time PCR | Similar as hESC lines |
| | Nanog | | |
| ICC | OCT4 | Immunocytochemistry | > 90% |
| | SSEA4 | | > 90% |
| Free of pathogen | Mycoplasma test | PCR | None detected |
| | Sterility test | BacT/Alert system | No organism detected |
| Karyotype test | Chromosome analysis | Standard metaphase chromosome analysis | Intact Karyotype |

- FDA-mandated release criteria

Additionally

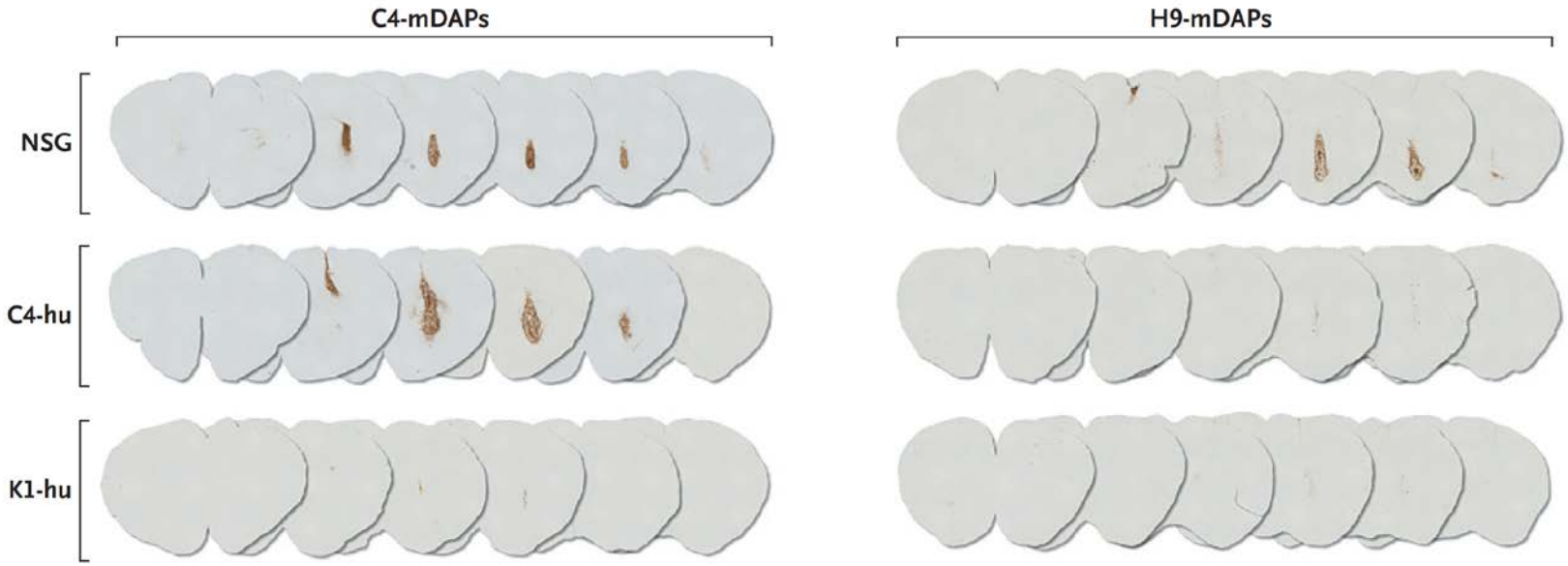
- Whole genome sequencing
- neurons derived from these progenitor cells showed dopamine secretion and electrophysiological properties in vitro characteristic of substantia nigra pars compacta dopaminergic neurons and functional efficacy in animal models similar to that of fetal midbrain-derived tissue

**Table S5. Quality control release criteria for midbrain dopaminergic progenitors**

| Target | Test | | method | Release Criteria |
|---|---|---|---|---|
| D0 | DNA fingerprinting | | PCR | Same as original fibroblast |
| | qRT-PCR | Nanog | Real time PCR | Similar as hESC lines |
| | | Oct4 | | |
| | ICC | SSEA4 | Immunocytochemistry | > 90% |
| | | OCT4 | | > 90% |
| | Free of pathogen | Mycoplasma | PCR | None detected |
| | | Sterility test | BacT/Alert | No organism detected |
| D12 | DNA fingerprinting | | PCR | Same as original fibroblast |
| | qRT-PCR | FOXA2 | Real time PCR | > 500-fold than D0 |
| | | LMX1A | | > 500-fold than D0 |
| | Free of pathogen | Sterility test | BacT/Alert system | No organism detected |
| D22 | Free of pathogen | Mycoplasma | PCR | None detected |
| D26 | DNA fingerprinting | | PCR | Same as original fibroblast |
| | qRT-PCR | FOXA2 | Real time PCR | > 500-fold than D0 |
| | | LMX1A | | > 500-fold than D0 |
| | | TH | | > 500-fold than D0 |
| | ICC | FOXA2 | Immunocytochemistry | > 55% of total cells |
| | | LMX1A | | > 55% of total cells |
| | | FOXA2/LMX1A | | > 50% of total cells |
| | | TH | | > 10% of total cells |
| | | OCT4 | | None detected |
| | | SSEA4 | | None detected |
| | | TPH2 | | < 1.0 % of total cells |
| | | 5-HT | | < 1.0 % of total cells |
| | | DBH2 | | < 1.0 % of total cells |
| | Free of pathogen | Sterility test | BacT/Alert system | No organism detected |
| D28 final release | Viability test | Viability test | Trypan Blue staining | Viability> 70% |
| | Free of pathogen | Mycoplasma | PCR | Not detected |
| | | Sterility test | BacT/Alert | No organism detected |
| | | Endotoxin | Limulus Amebocyte Lysate assay (LAL) | < 0.2EU/kg body weigh/hr |
| | | Gram Staining | Standard method | Negative |

# Personalized iPSC-Derived mDAPs for PD

Patient derived (C4) and allogeneic (H9) iPSCs were differentiated into day-28 mDAPs and grafted into the striatum of three different mice strains




hNCAM+ staining

NSG: nonobese diabetic mice with severe combined immunodeficiency and depletion of the interleukin-2 receptor γ (NOD SCID gamma mice)

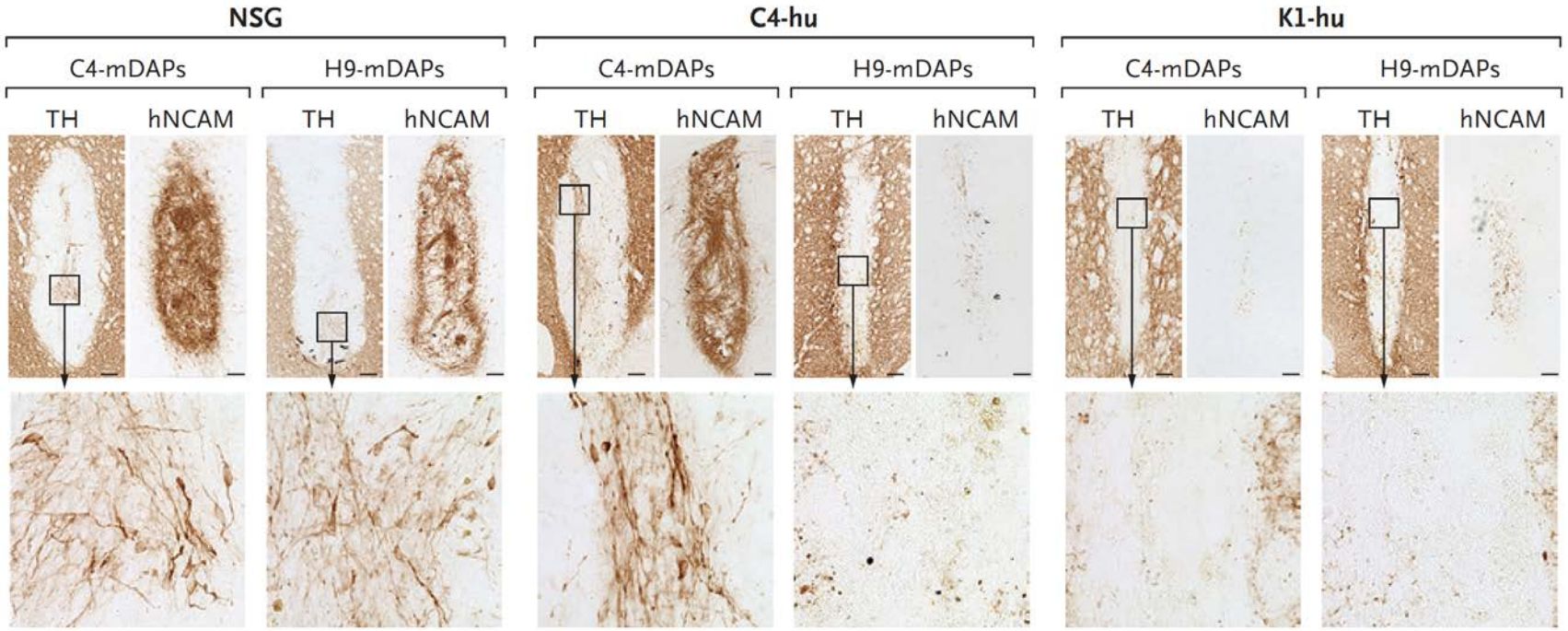
C4-hu: patient-humanized NOD SCID gamma mice

K1-hu: allogenic-humanized NOD SCID gamma mice

Two weeks after grafting, patient derived (C4) as well as allogeneic (H9) mDAPs survived in NOD SCID gamma mice.
Only patient derived (C4) mDAPs survived in patient-humanized NOD SCID gamma mice
=> Graft rejection in allogeneic humanized mice

# Personalized iPSC-Derived mDAPs for PD

Patient derived (C4) and allogeneic (H9) iPSCs were differentiated into day-28 midbrain dopaminergic progenitors and grafted into the striatum of three different mice strains




hNCAM+ staining

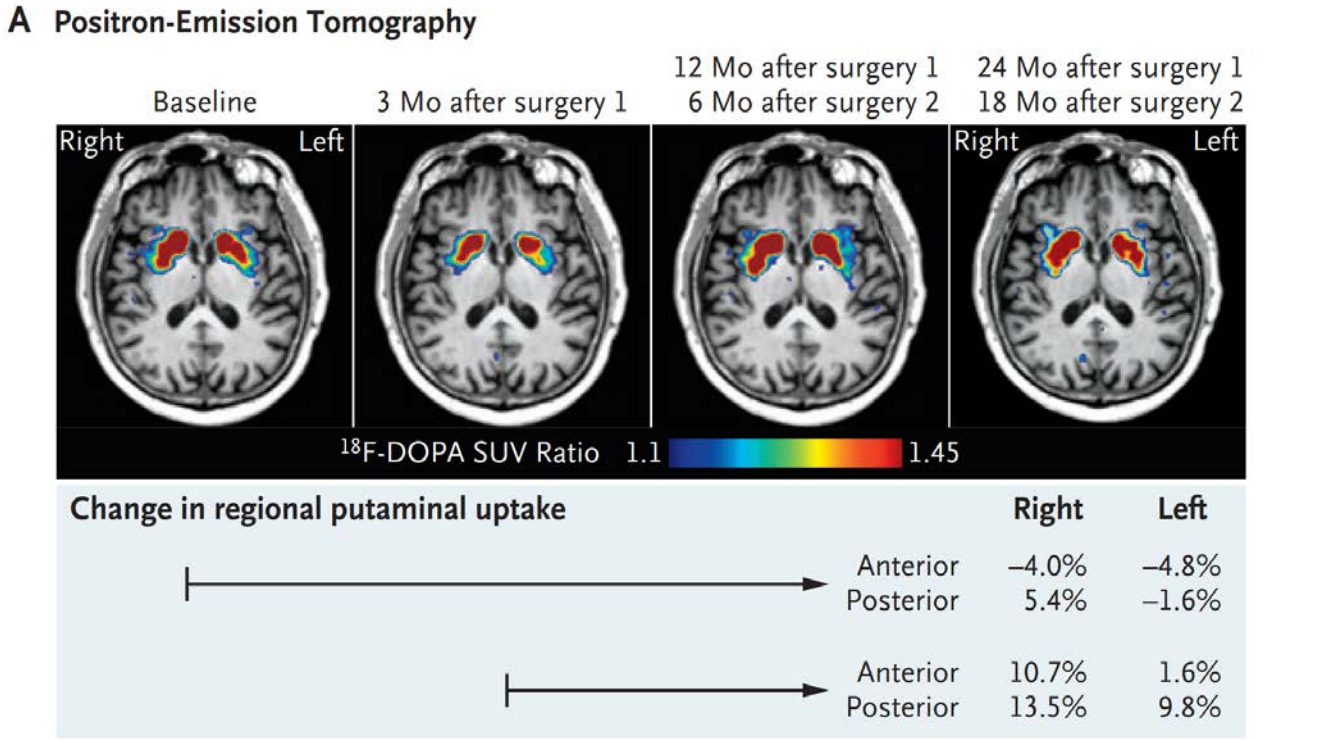
TH+ staining

Two weeks after implantation TH+ neurons could be detected

# Personalized iPSC-Derived mDAPs for PD

Computed tomographic (CT) scans were performed intraoperatively to confirm accurate placement of the cell injection in the putamen. Implantation on the left side was done 6 months prior to implantation on the right side

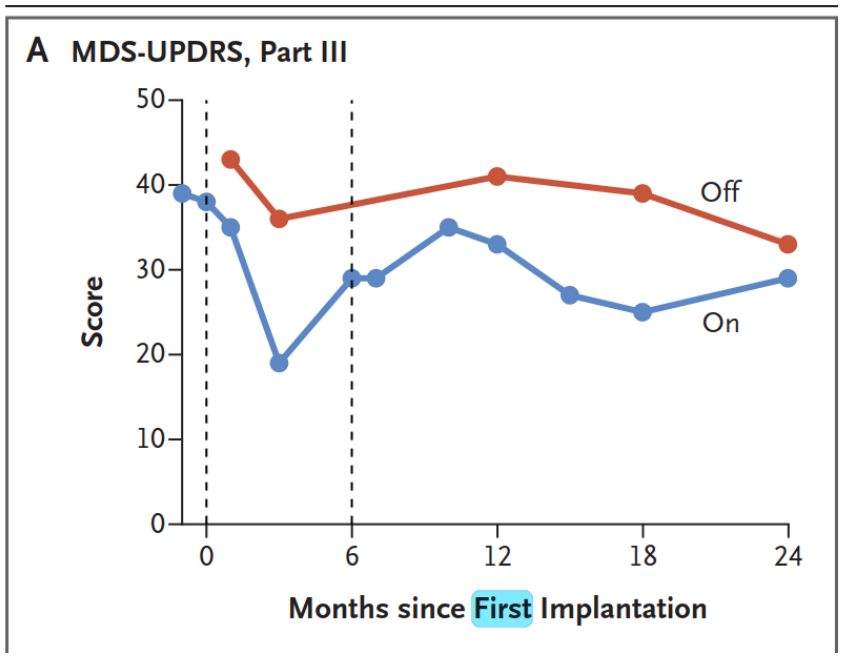
PET scan for $^{18}$F-DOPA uptake were performed at different time-points

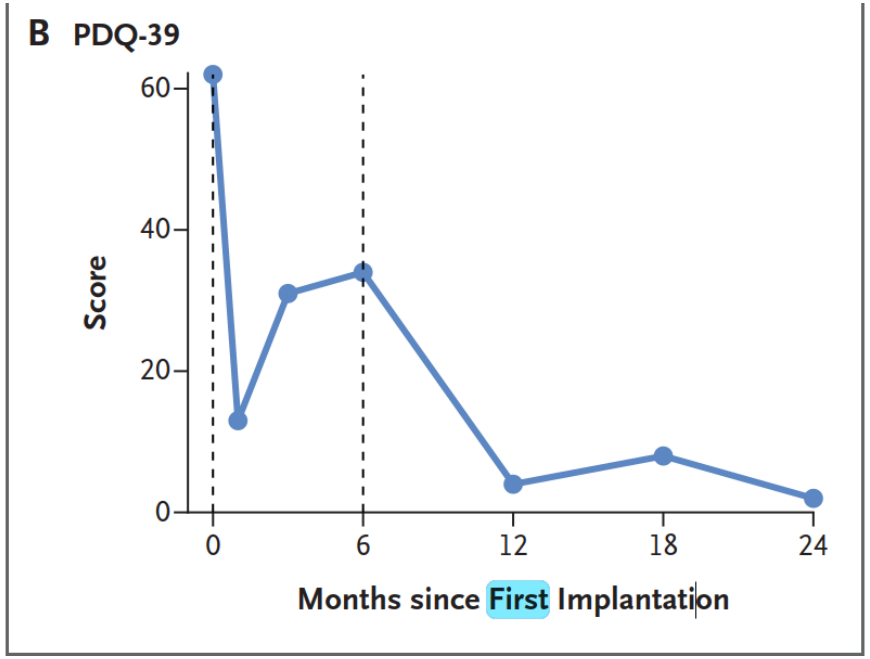



$^{18}$F-DOPA uptake increased moderately, when compared to the 3Mo after surgery 1, which was the lowest uptake measured

# Personalized iPSC-Derived mDAPs for PD

Longitudinal Clinical Assessments of Parkinson's Disease–Related Motor Function and Quality of Life




Improvements in motor assessments and patient-rated symptom

# Conclusion

- application of a personalized cell-therapy strategy using autologous, iPSC-derived dopaminergic progenitor cells in a patient with Parkinson's disease without the use of immunosuppressants

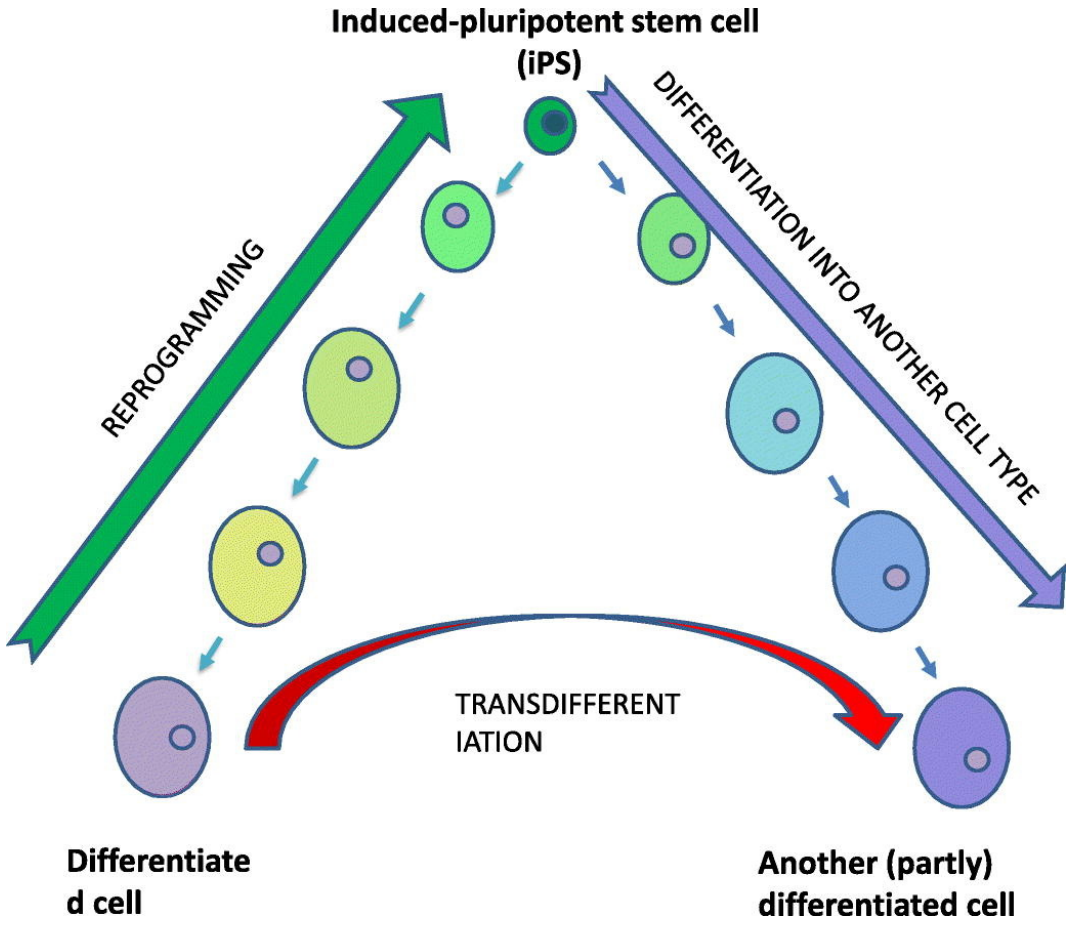
- Modest increase in $^{18}$F-DOPA uptake - PET signal

- improvements in motor assessments and patient-rated symptom scales. However, results should be interpreted with caution, because both the patient and the raters were aware of the intervention and there were no control comparisons

- after 18 to 24 months of follow-up, no observation of dyskinesias or other adverse neurologic effects

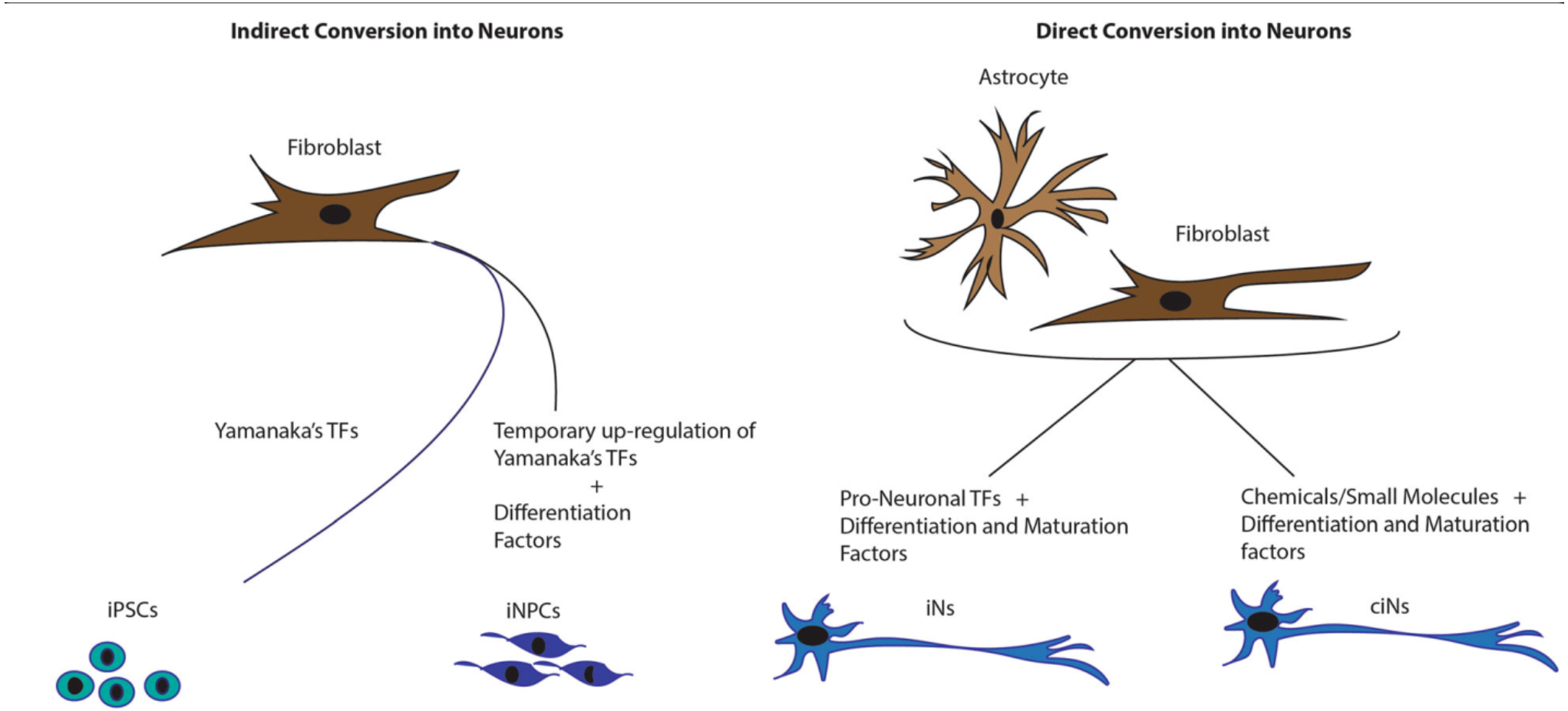
- **Proof of concept** – many more studies are necessary to evaluate every aspect of such procedures

# Alternative to cell implantation: transdifferentiation




Artur Cieślar-Pobuda *et al.,* Biochimica et Biophysica Acta, 2017

# Alternative to cell implantation: transdifferentiation




Mollinari, C., Zhao, J., Lupacchini, L. *et al., Cell Death Dis*, 2018

# Alternative to cell implantation: transdifferentiation

Pros (+) and cons (-) of transdifferentiation

Direct conversion strategy bypasses the pluripotent stem cell stages:

+ Rapid differentiation and maturation

+/- Prevents loss of specific age-related and epigenetic features (Age equivalent)

- Limited amount of cells

+ can be achieved *in vivo*

# Conceptually related papers

Cell

Volume 181, Issue 3, 30 April 2020, Pages 590-603.e16

Article

## Glia-to-Neuron Conversion by CRISPR-CasRx Alleviates Symptoms of Neurological Disease in Mice

Haibo Zhou [1, 4], Jinlin Su [1, 4], Xinde Hu [1, 2, 4], Changyang Zhou [1, 2, 4], He Li [1, 2, 4], Zhaorong Chen [1, 2, 4], Qingquan Xiao [1, 2], Bo Wang [1, 2], Wenyan Wu [1, 2], Yidi Sun [2, 3], Yingsi Zhou [1], Cheng Tang [1, 2], Fei Liu [1], Linhan Wang [1, 2], Canbin Feng [1], Mingzhe Liu [1], Sanlan Li [1], Yifeng Zhang [1] ... Hui Yang [1, 5]

**Article**

## Reversing a model of Parkinson's disease with in situ converted nigral neurons

https://doi.org/10.1038/s41586-020-2388-4

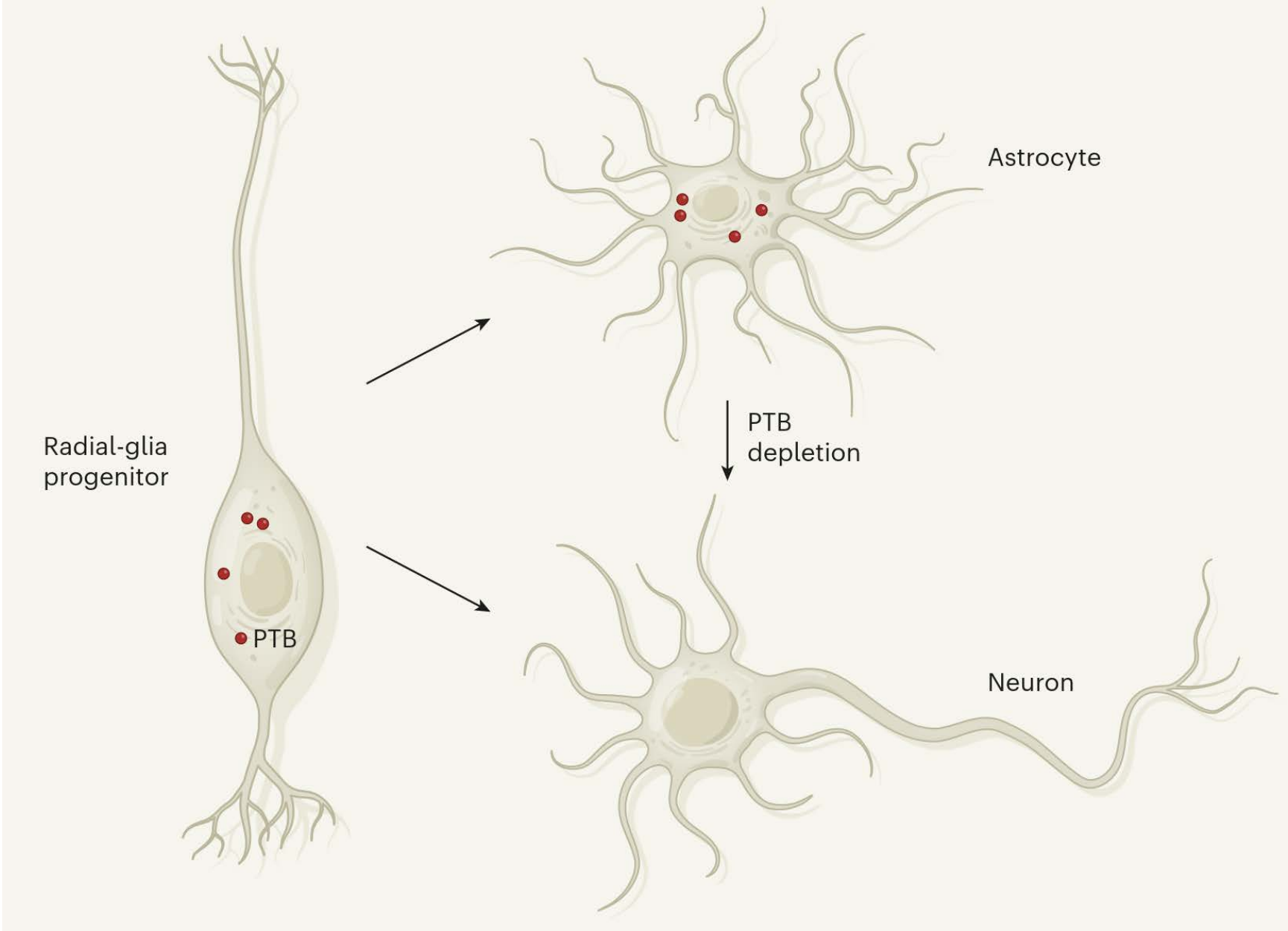
Received: 12 November 2018

Accepted: 13 May 2020

Hao Qian[1], Xinjiang Kang[2,3], Jing Hu[1,8], Dongyang Zhang[4], Zhengyu Liang[1], Fan Meng[1], Xuan Zhang[1], Yuanchao Xue[1,9], Roy Maimon[1,5], Steven F. Dowdy[1], Neal K. Devaraj[4], Zhuan Zhou[2], William C. Mobley[6], Don W. Cleveland[1,5] & Xiang-Dong Fu[1,7]

Concept:

Depletion of a single RNA binding protein, **PTB**, converts astrocytes into neurons




Ernest Arenas , Nature News and Views, 2020

Cell

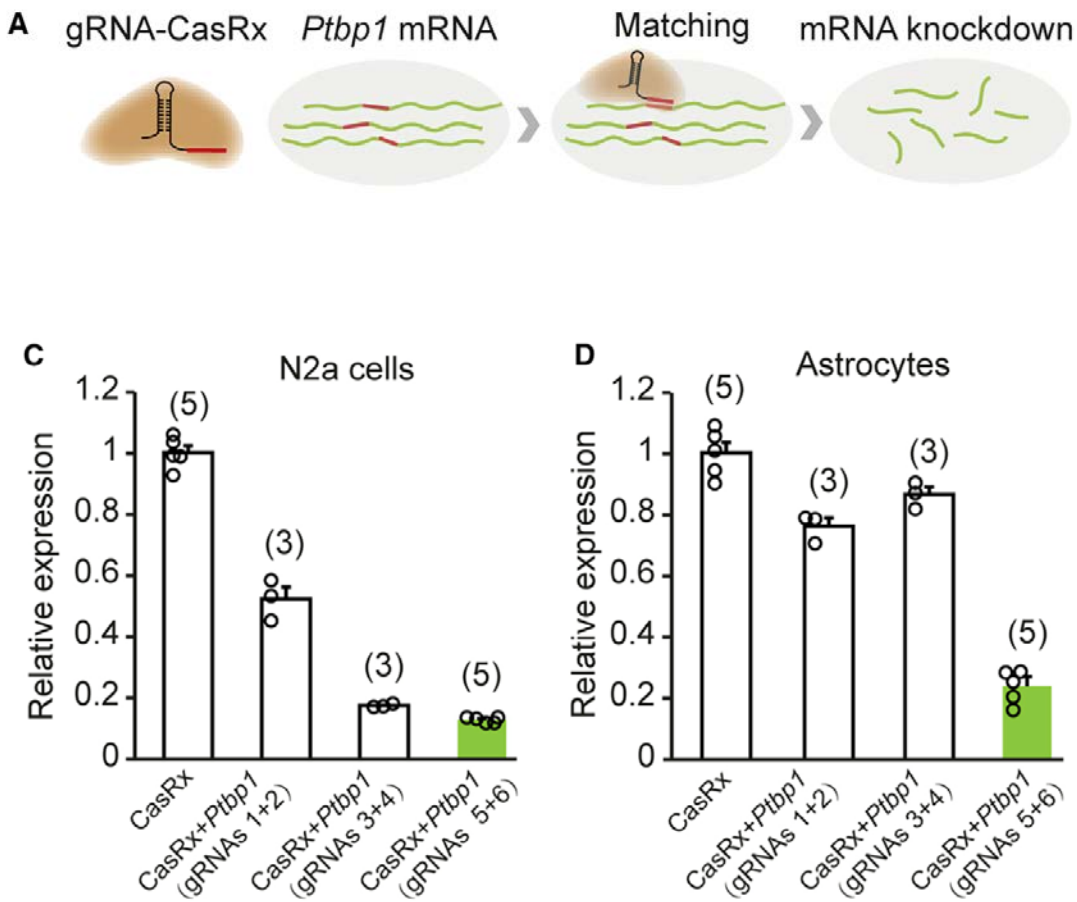
Volume 181, Issue 3, 30 April 2020, Pages 590-603.e16

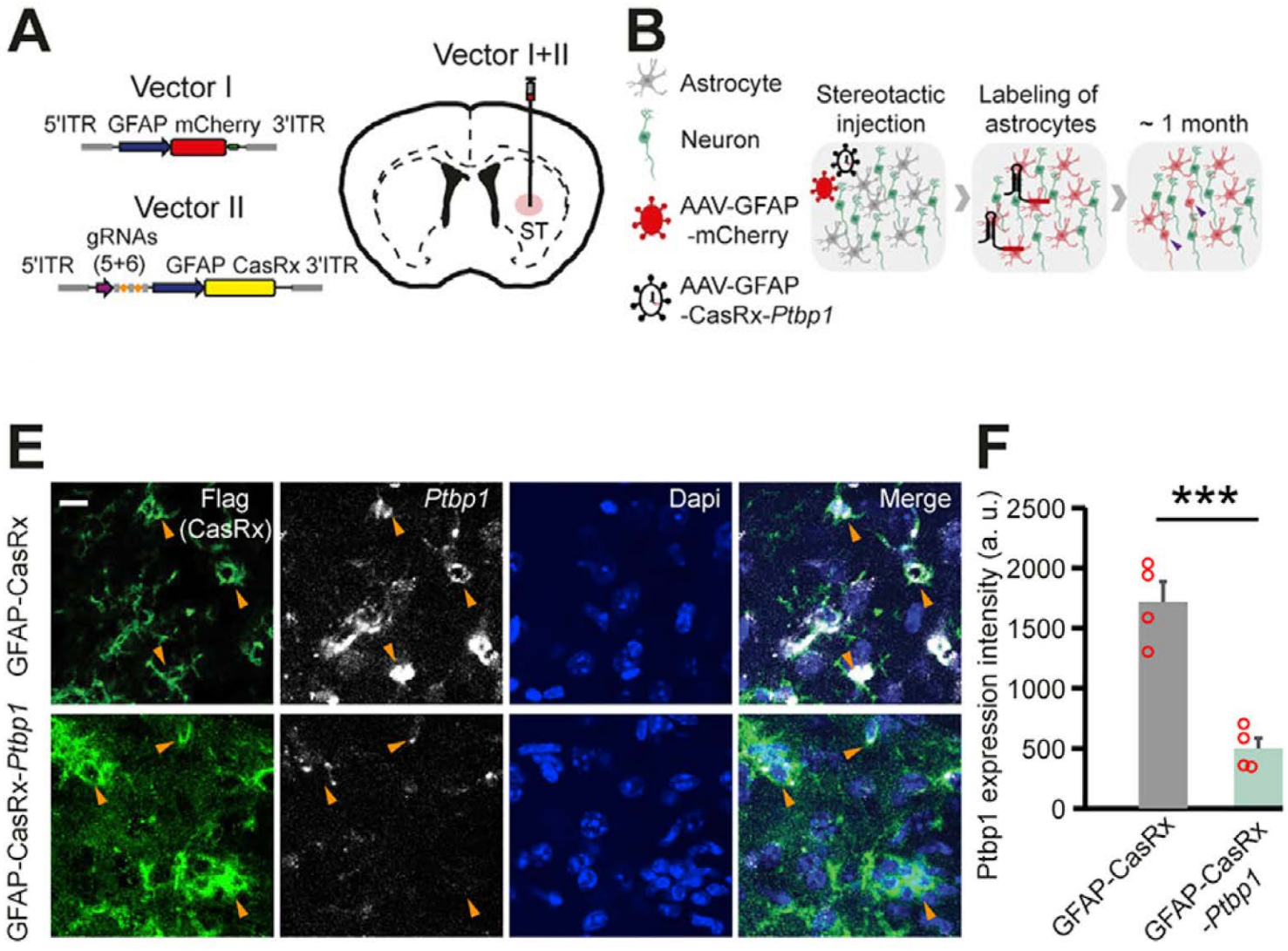
Article

# Glia-to-Neuron Conversion by CRISPR-CasRx Alleviates Symptoms of Neurological Disease in Mice

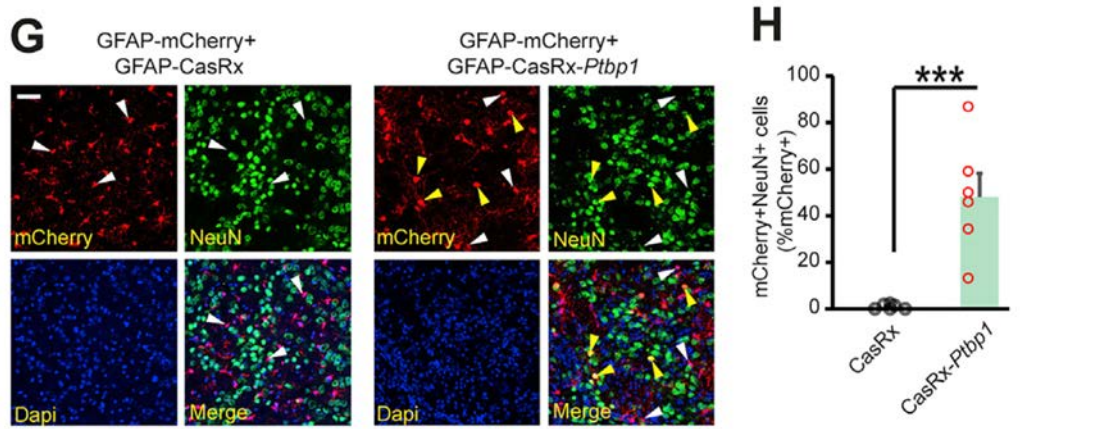
Haibo Zhou [1, 4], Jinlin Su [1, 4], Xinde Hu [1, 2, 4], Changyang Zhou [1, 2, 4], He Li [1, 2, 4], Zhaorong Chen [1, 2, 4], Qingquan Xiao [1, 2], Bo Wang [1, 2], Wenyan Wu [1, 2], Yidi Sun [2, 3], Yingsi Zhou [1], Cheng Tang [1, 2], Fei Liu [1], Linhan Wang [1, 2], Canbin Feng [1], Mingzhe Liu [1], Sanlan Li [1], Yifeng Zhang [1] ... Hui Yang [1, 5]

- Use of CRISPR-CasRx, to directly degrade the mRNA of Ptbp1

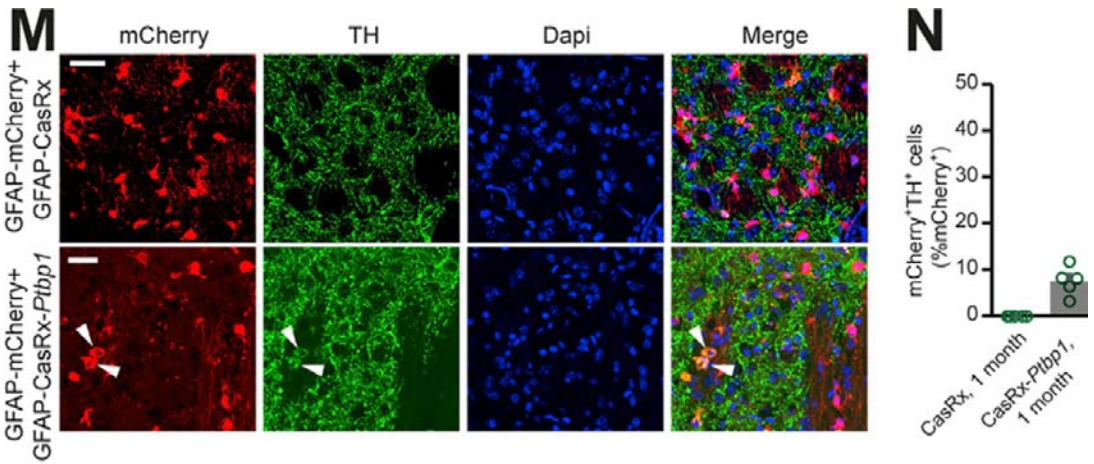

A
gRNA-CasRx
Ptbp1 mRNA
Matching
mRNA knockdown
C
N2a cells
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
(5)
(3)
(3)
(5)
CasRx
CasRx+Ptbp1 (gRNAs 1+2)
CasRx+Ptbp1 (gRNAs 3+4)
CasRx+Ptbp1 (gRNAs 5+6)
D
Astrocytes
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
(5)
(3)
(3)
(5)
CasRx
CasRx+Ptbp1 (gRNAs 1+2)
CasRx+Ptbp1 (gRNAs 3+4)
CasRx+Ptbp1 (gRNAs 5+6)



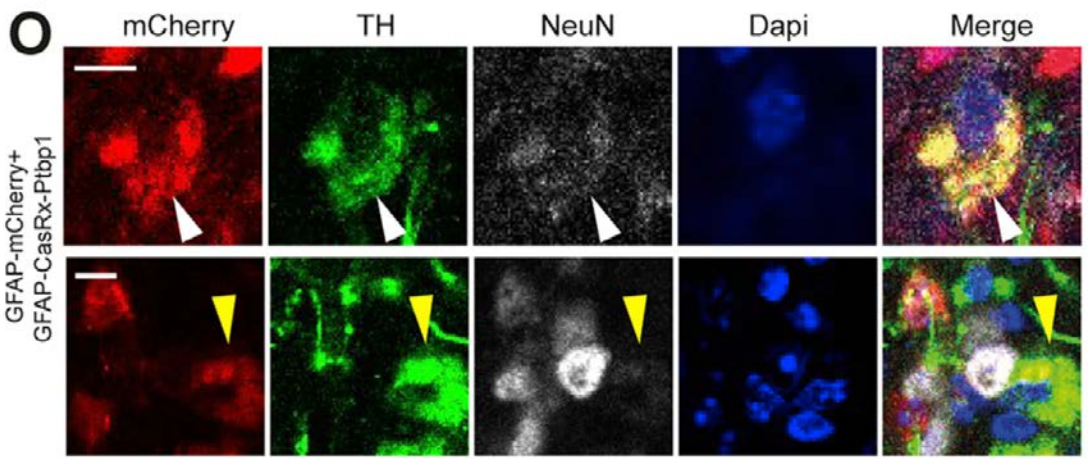

1 week after injection




1 month after injection

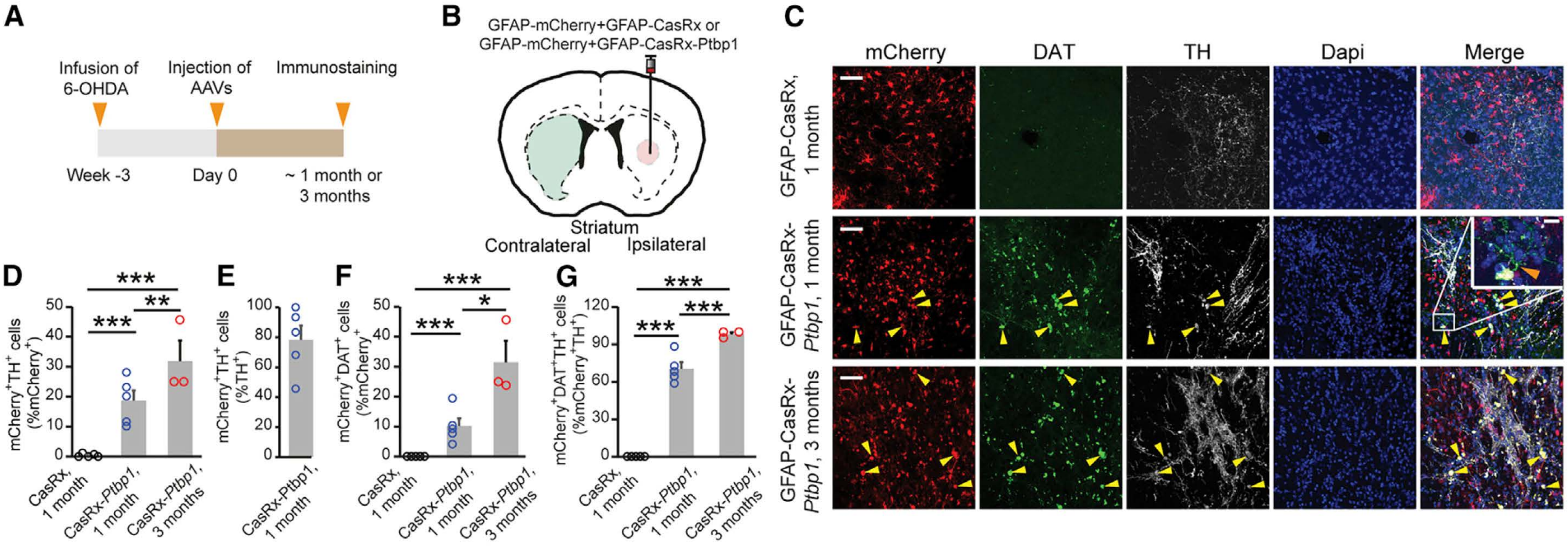



1 month after injection




Striatum

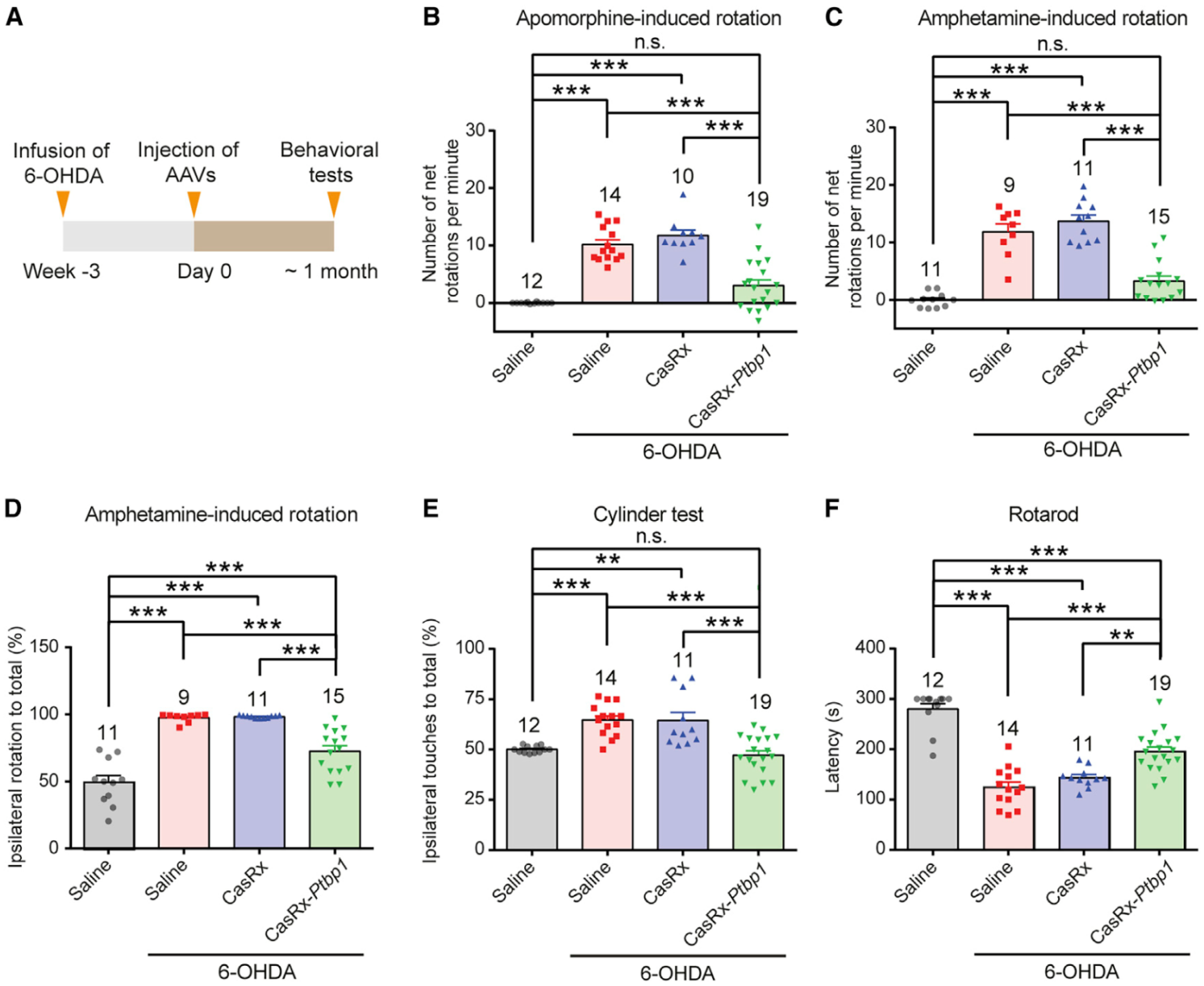
7.5% of transduced cells show a positive stainig for mCherry+TH+




Mouse model of PD generated by unilateral infusion of 6-OHDA

Downregulation of Ptbp1 in striatal astrocytes converted them into TH+ neurons.
Empty vector shows no efficient replacement of 6-OHDA abrogated TH+ cells in the striatum

# Induced neurons in the striatum can alleviate the symptoms in the 6-OHDA-induced PD mouse model



# Short synopsis

- Ptbp1 downregulation suffices to convert astrocytes into neurons, also *in vivo*

- Depending on the glia cell type, environmental cues or both, the conversion leads to different neuronal types:
  - As presented here, Ptbp1 downregulation in the striatum can give rise to TH+ cells
  - Knockdown of Ptbp1 converts Müller glia into retinal ganglion cells in the mature retina

- Conversion of striatal Astrocytes into neurons can alleviate motor symptoms in a chemically induced mouse model of PD

Article

# Reversing a model of Parkinson's disease with in situ converted nigral neurons

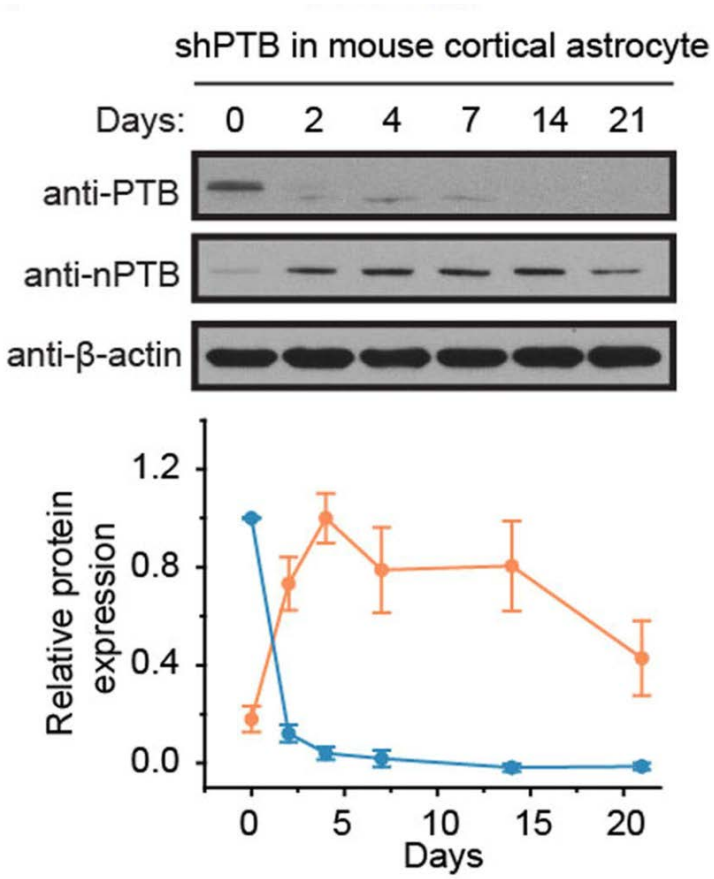
https://doi.org/10.1038/s41586-020-2388-4

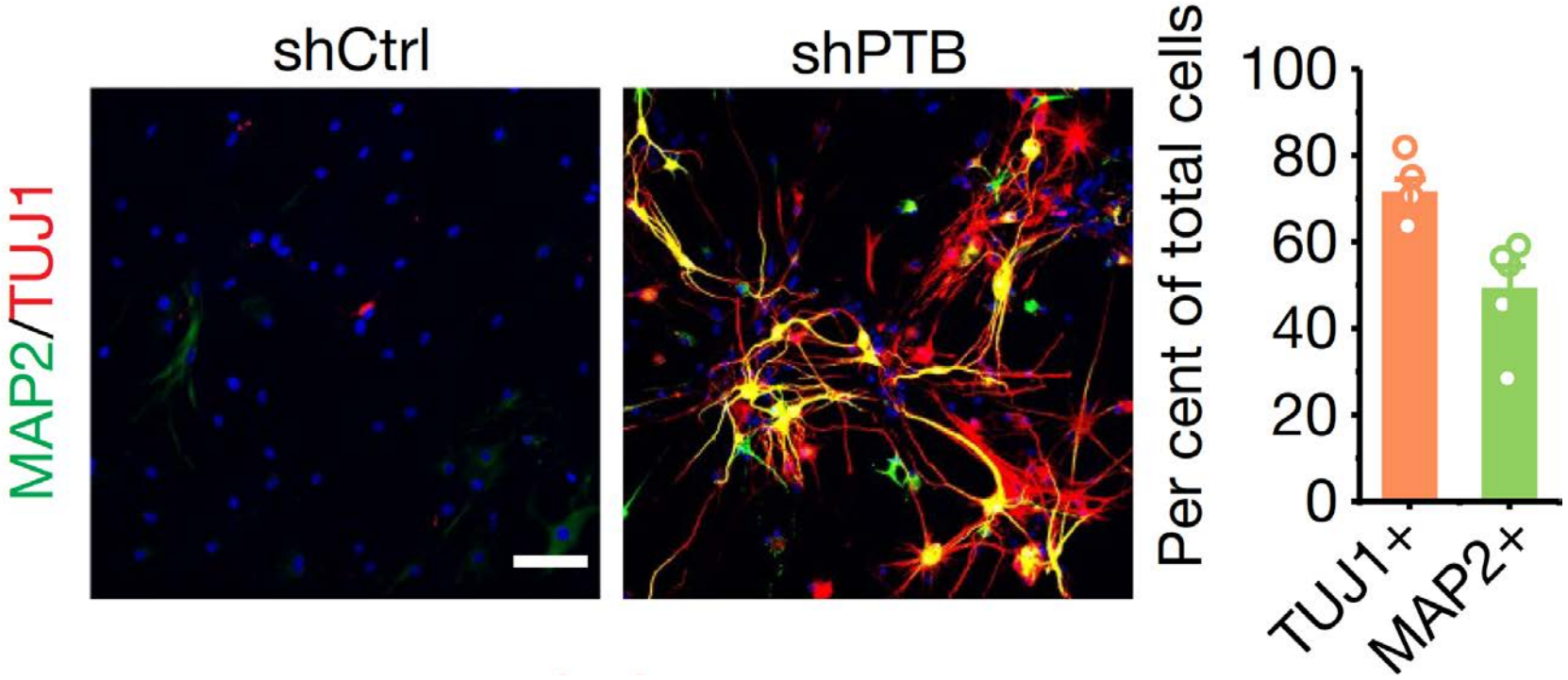
Received: 12 November 2018

Accepted: 13 May 2020

Hao Qian[1], Xinjiang Kang[2,3], Jing Hu[1,8], Dongyang Zhang[4], Zhengyu Liang[1], Fan Meng[1], Xuan Zhang[1], Yuanchao Xue[1,9], Roy Maimon[1,5], Steven F. Dowdy[1], Neal K. Devaraj[4], Zhuan Zhou[2], William C. Mobley[6], Don W. Cleveland[1,5] & Xiang-Dong Fu[1,7] ✉

- Use of shRNAs, to directly degrade the mRNA of Ptbp1

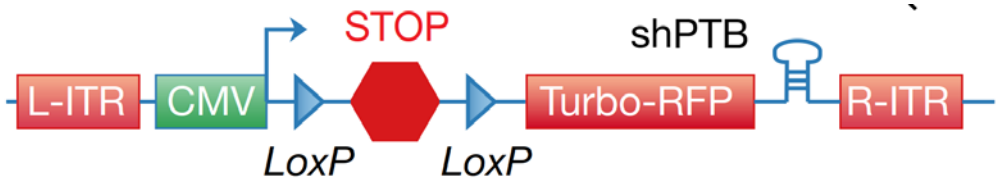



Isolated mouse cortical astrocytes were transduced with lentivirus harboring a shRNA against Ptbp1

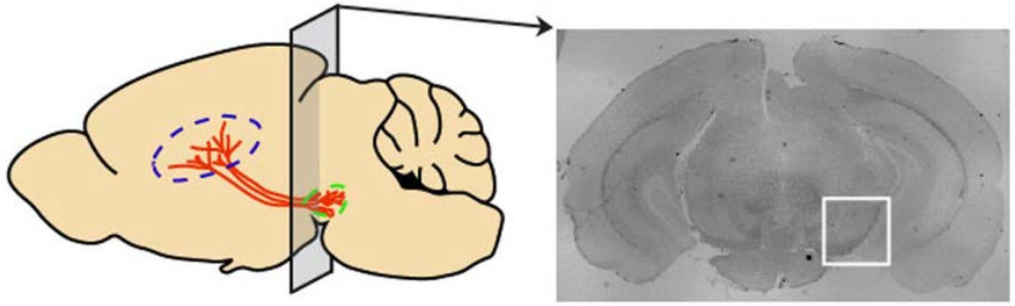
Downregulation of PTB as well as expression of pan-neuronal markers four weeks after transduction

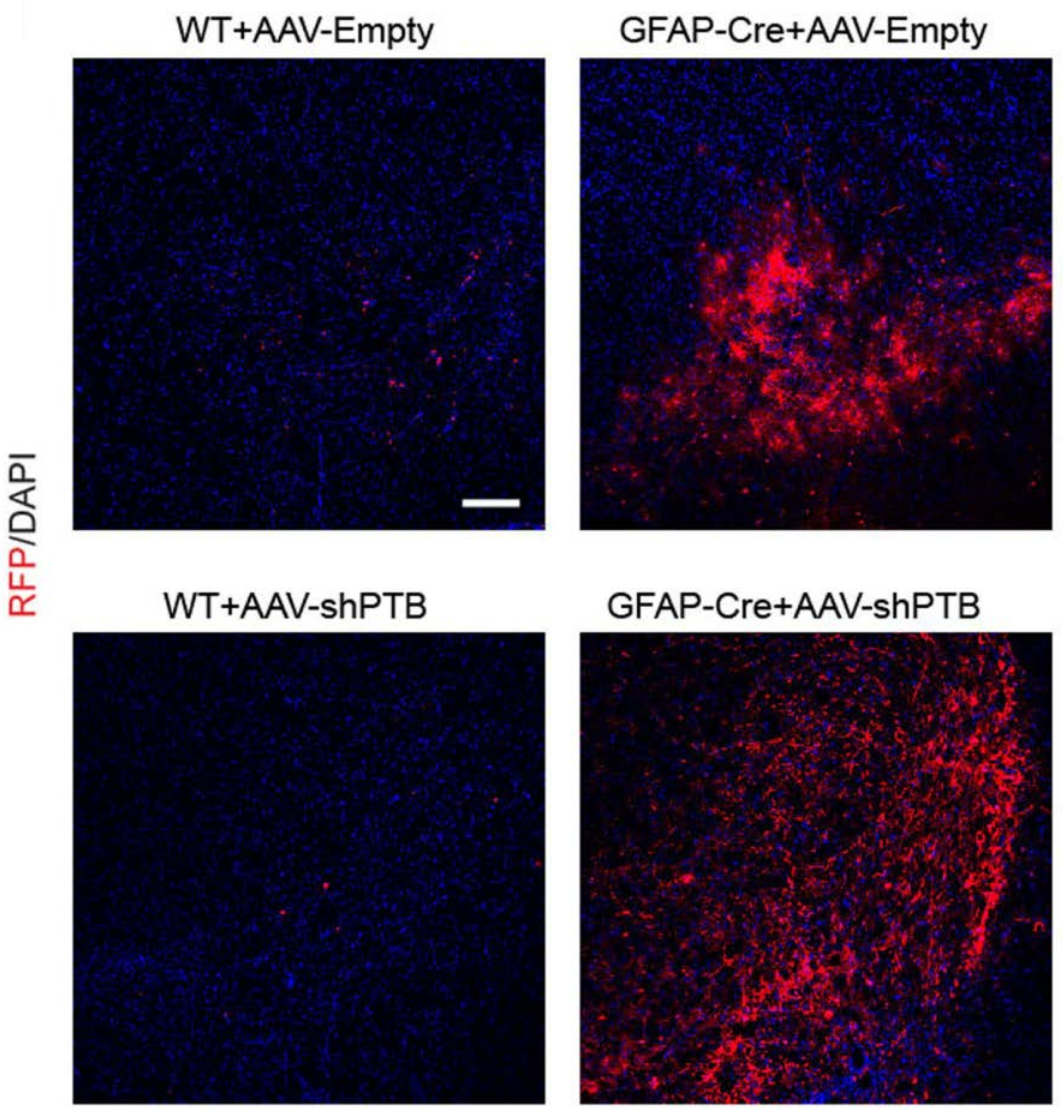
Direct conversion of astrocytes in a mouse brain using an AAV-construct




For lineage tracing

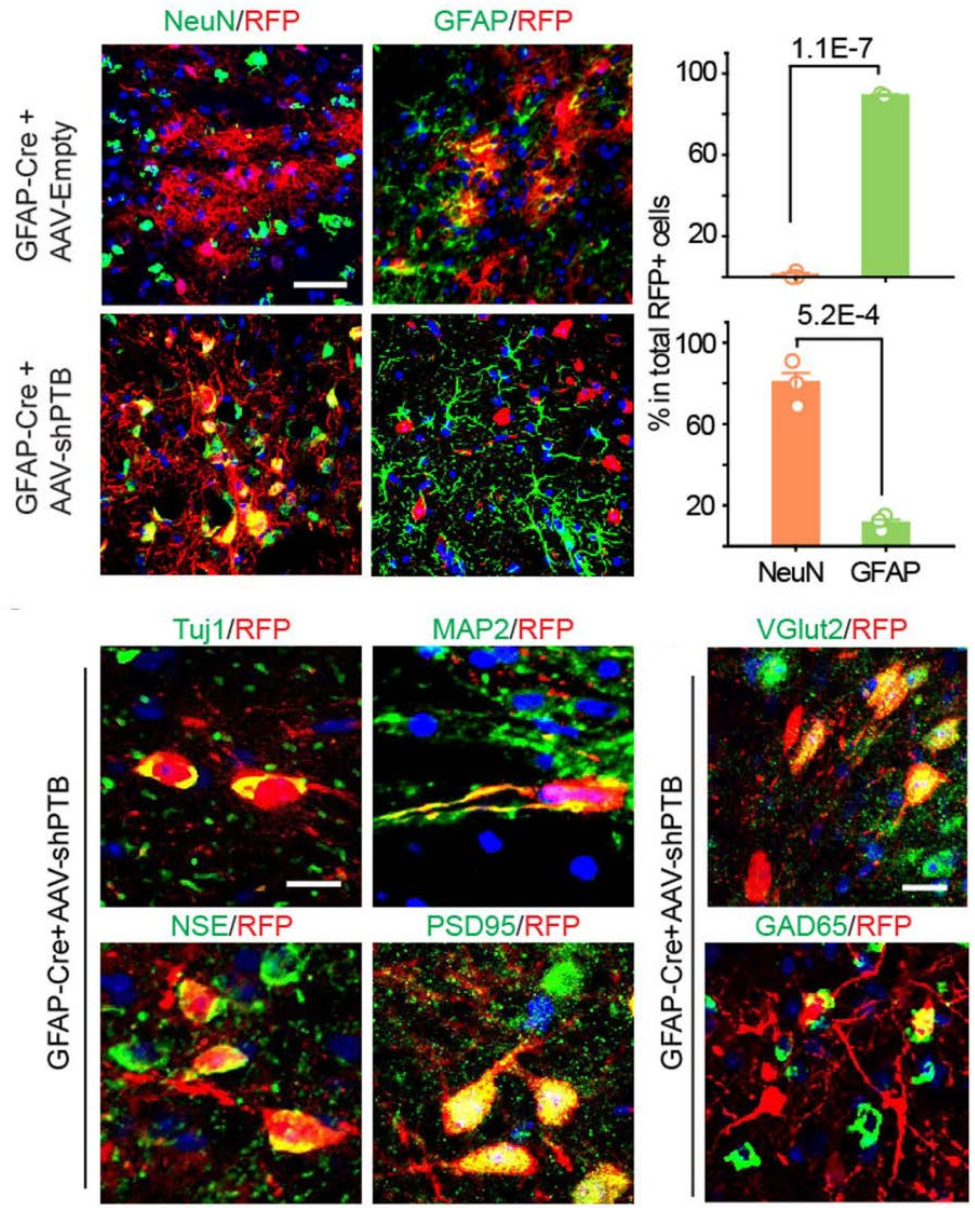
AAV injection into the substantia nigra

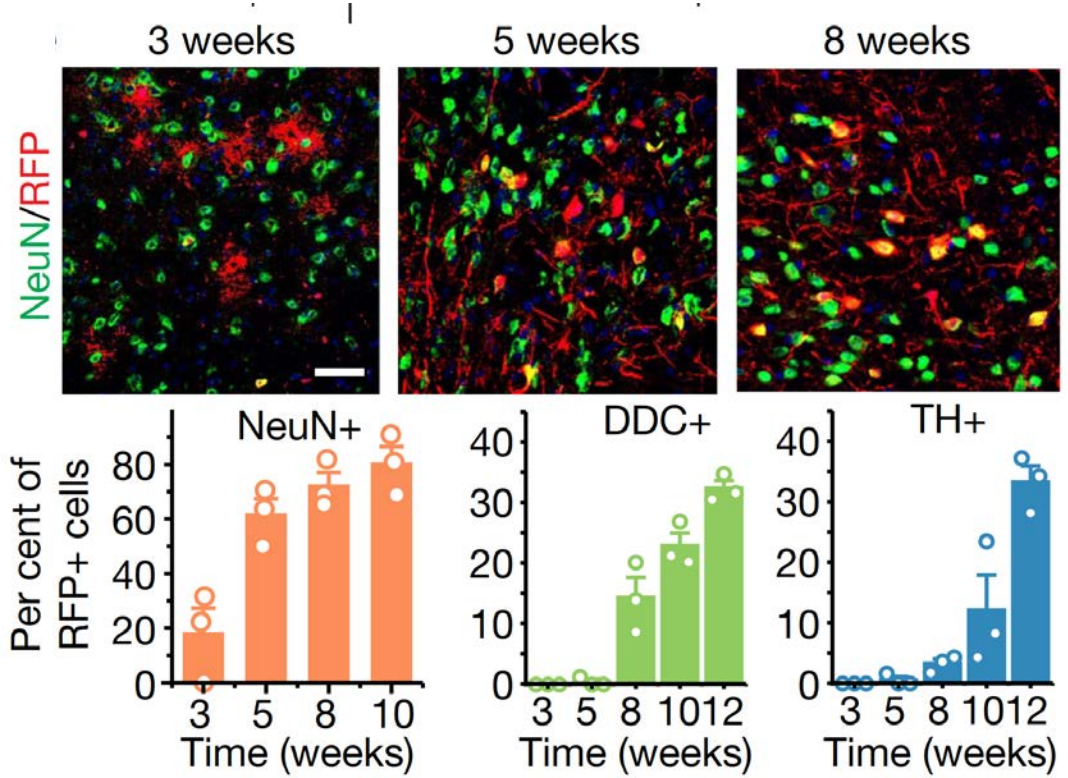





RFP expression in cells of mice expressing Cre under the GFAP promoter – further investigation of cell characteristics

Direct conversion of astrocytes in a mouse brain using an AAV-construct

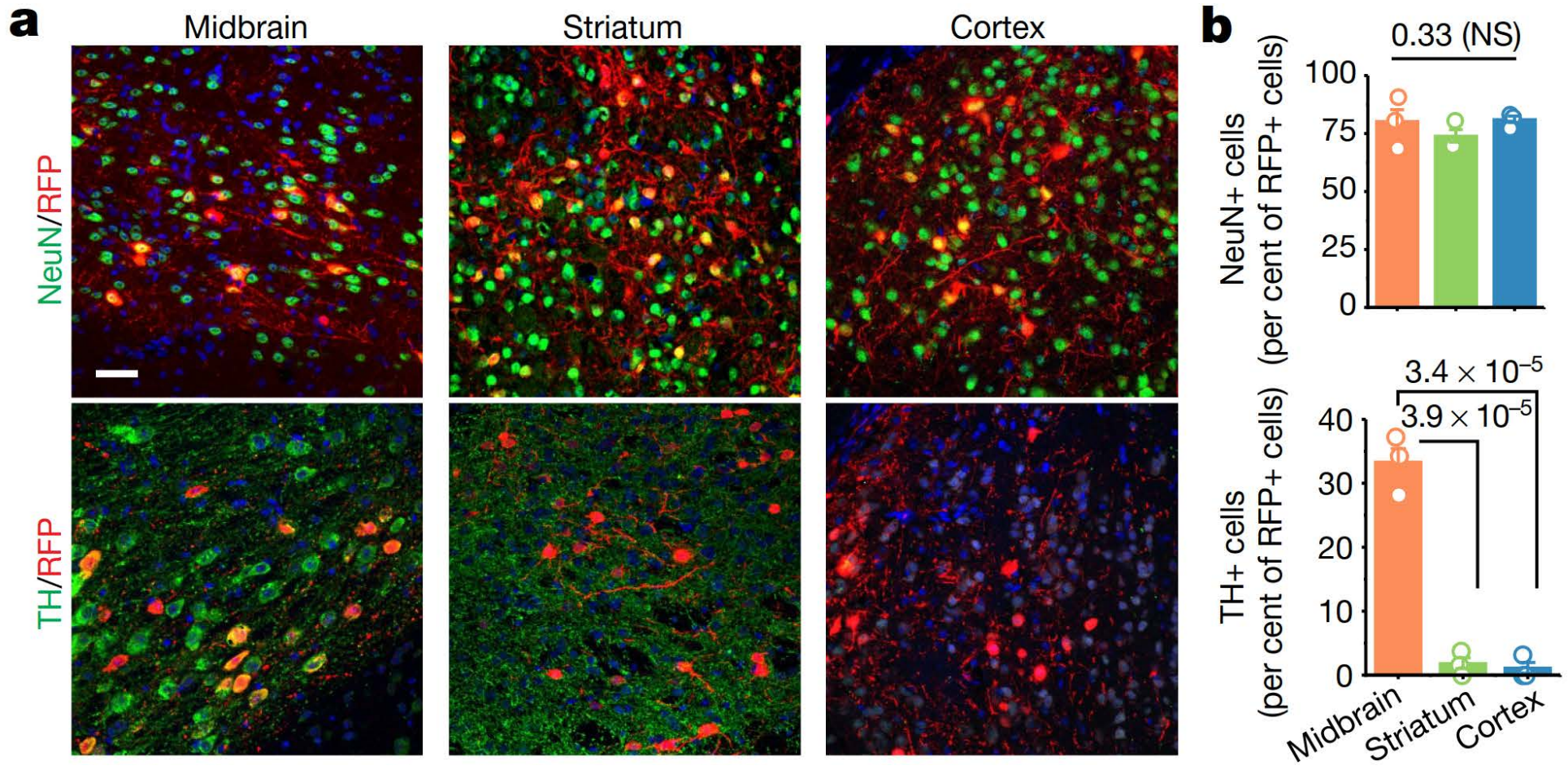



Ten weeks after injection, most cells transduced with shPTB lost their GFAP expression but expressed NeuN and additional pan-neuronal markers as well as subtype specific markers

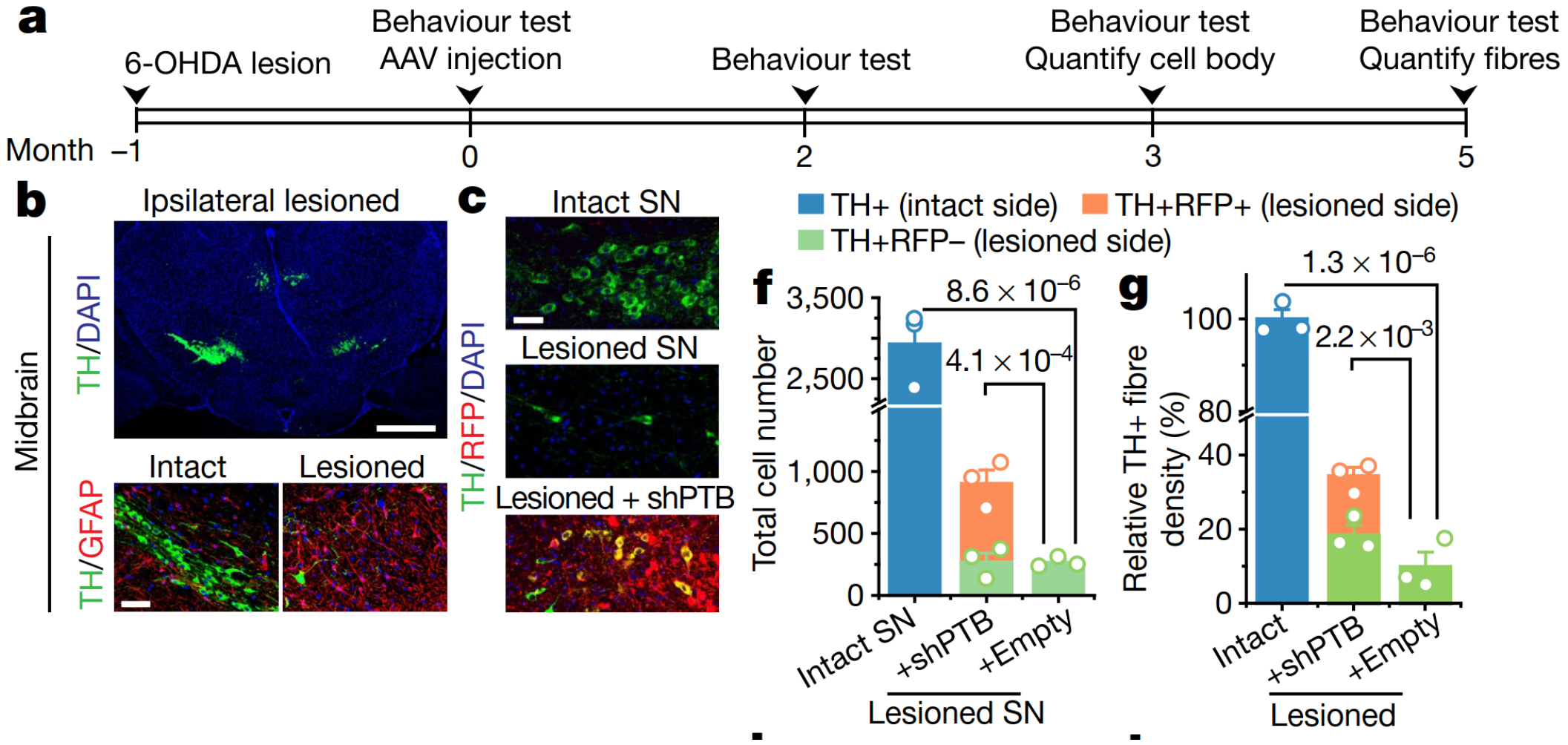
They further investigated the amount of cells that started to express TH, as well as DDC

Region specificity of neuronal conversion



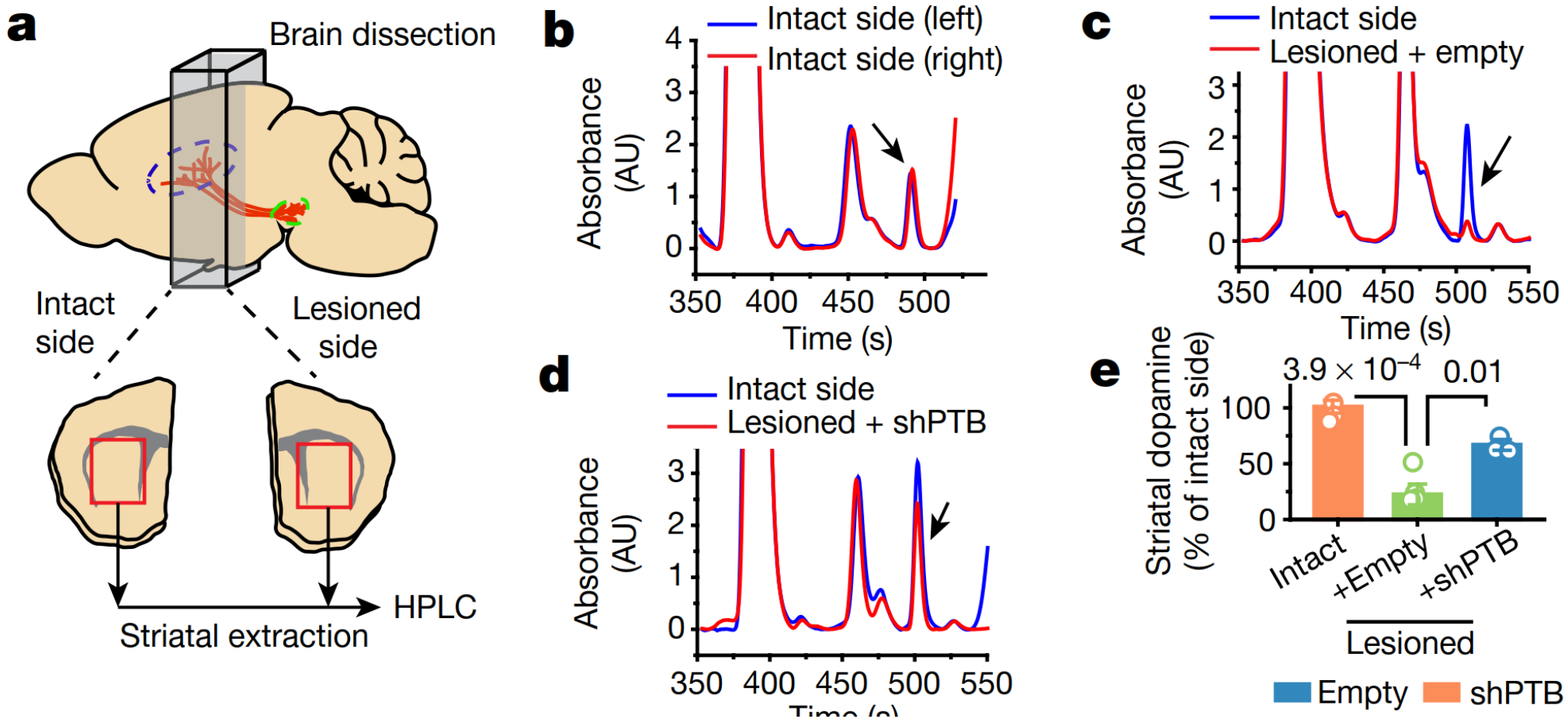

Injection of AAVs into three different brain regions
Based on the NeuN staining, conversion efficiency in the three region was similar ~75%
Only the injection in the midbrain produced TH+ cells

Replenishing lost DA neurons in a disease model



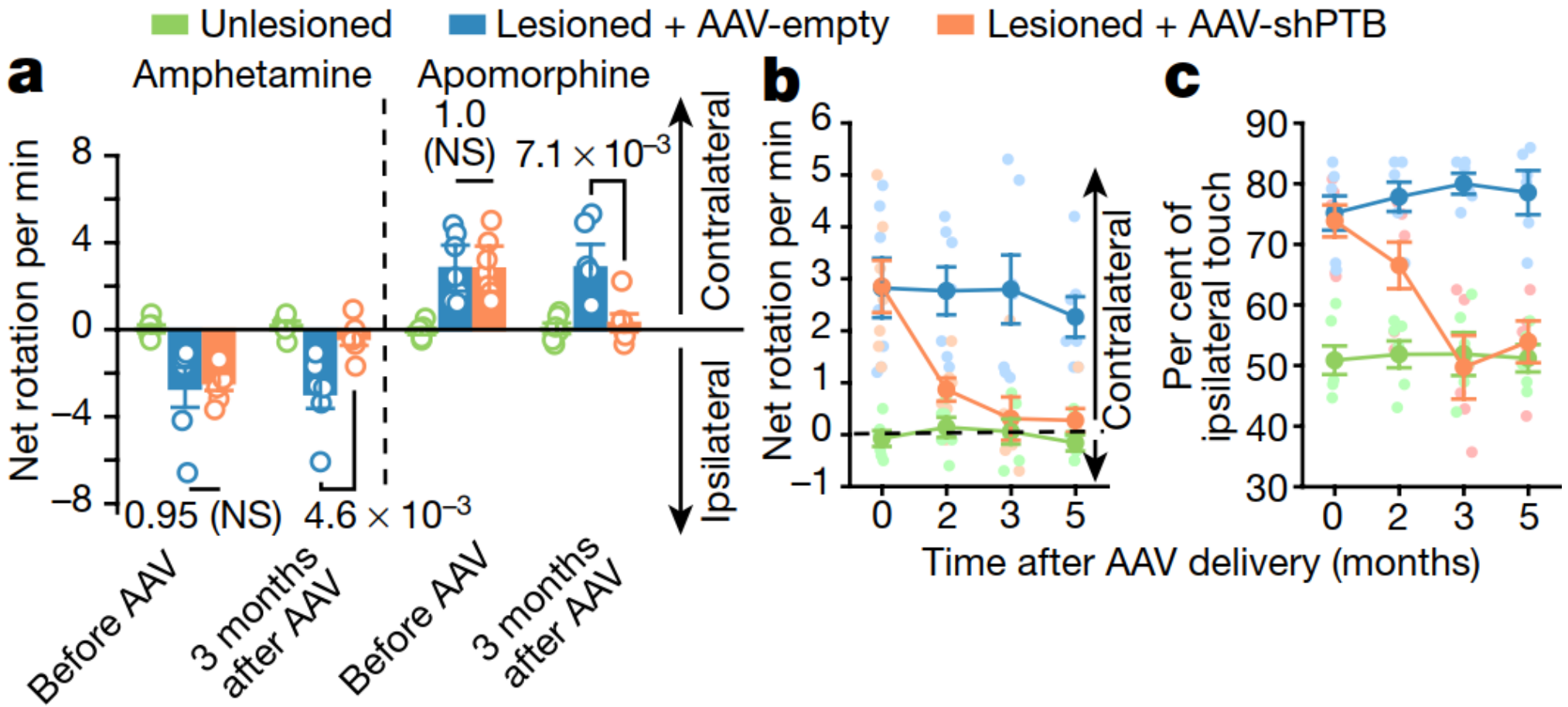

AAV-shPTB injection could replenish TH+ neuron and fibers in the subtantia nigra

Restoration of striatal dopamine



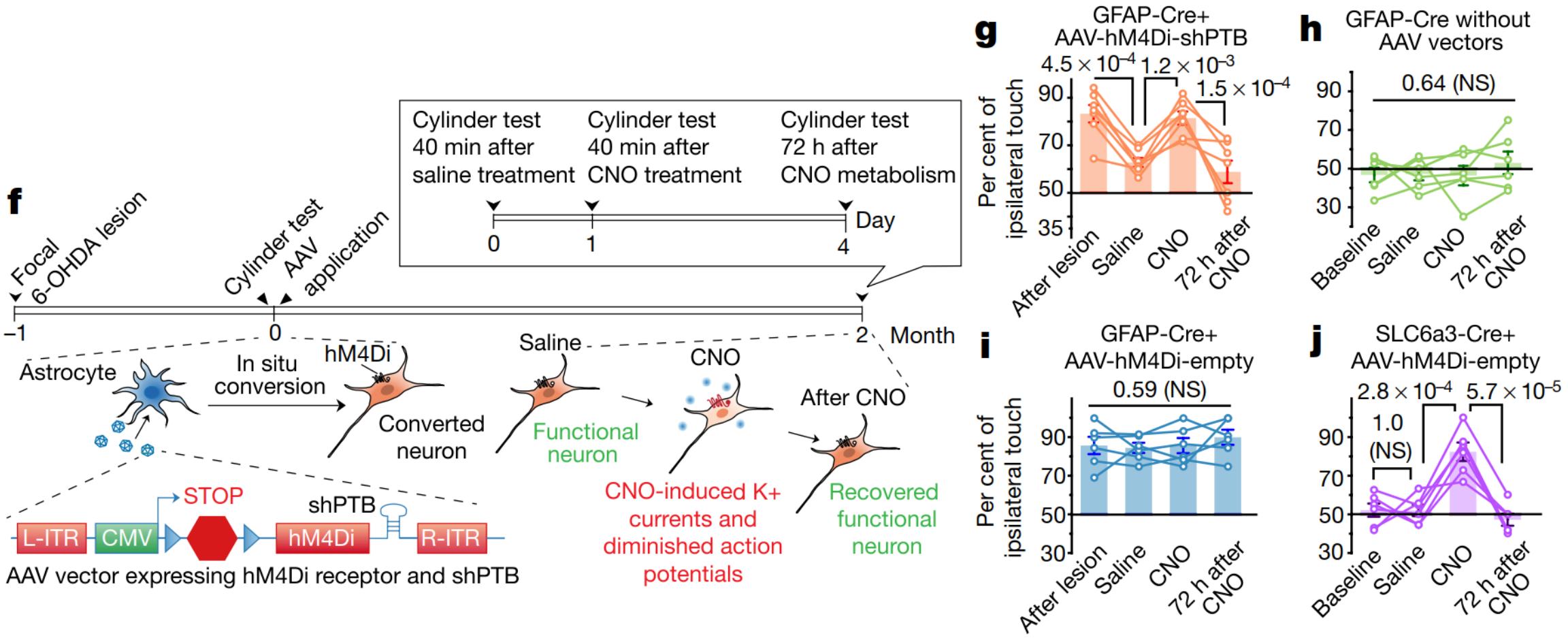

AAV-shPTB injection could restore parts of the dopamine released into the striatum

Reversing disease-relevant motor phenotypes




AAV-shPTB injection could restore the 6-OHDA induced motor functions

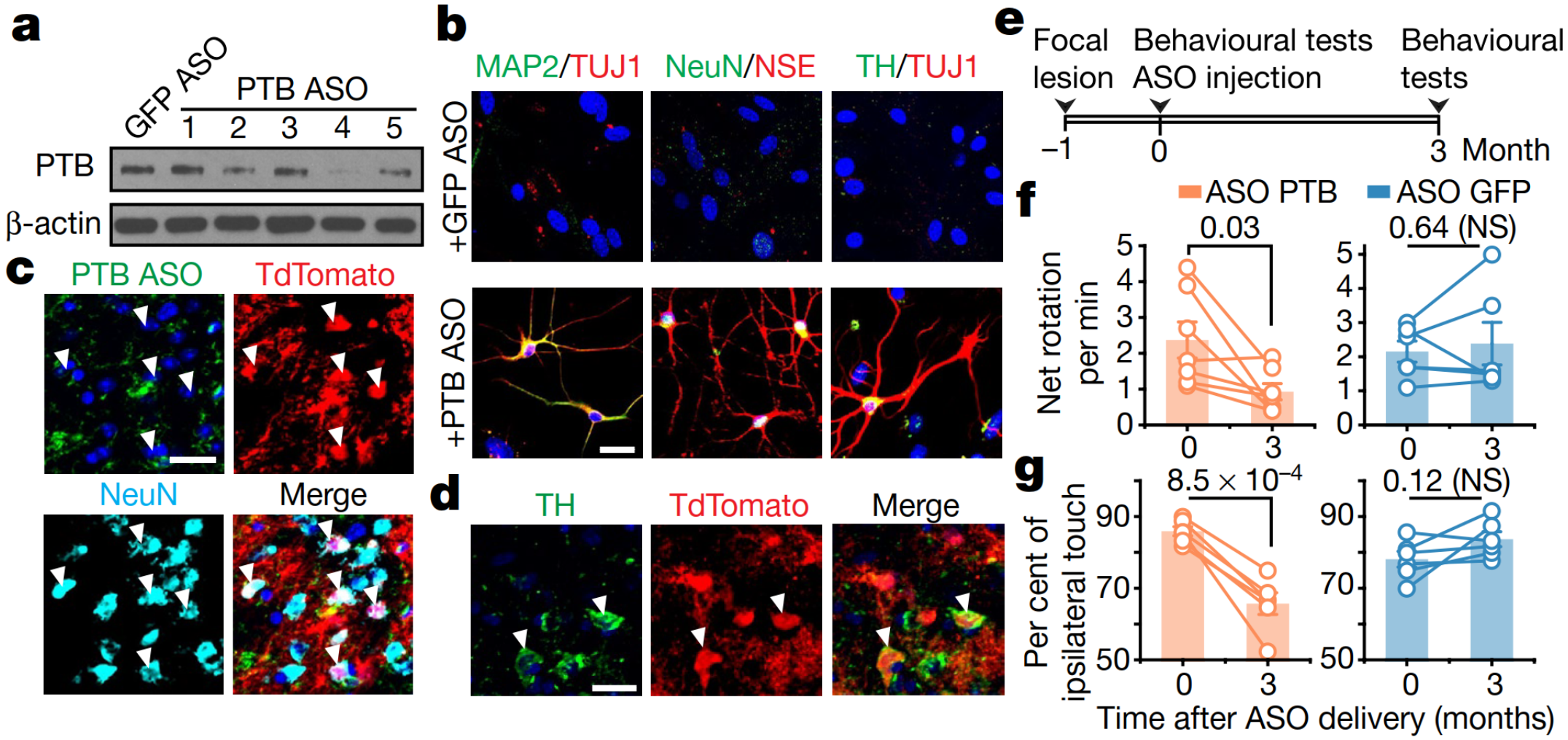
Chemogenetic analysis of new DA neurons




To ensure that the recovery of the motor functions comes from the new DA neurons, they created a new vector containing hM4Di, which is an engineered inhibitory muscarinic receptor variant responding to clozapine-N-oxide (CNO)

CNO leads to the supression of electrical activity in the expressing cells

ASO-based neuronal conversion and rescue




ASO treatement showed the same effect in the disease model

# Conclusions:

- Cell replacement therapy for Parkinson's Disease is still advancing, first trials with iPSC derived neurons are under its way
  - Big challenges, especially the use of autologous derived stem cells would be a big hurdle (QC etc.)
  - If allogeneic cells could be applied, therapy would become more feasible

- Newer approach of transdifferentiation might be more widely applicable
  - Although already successfully applied *in vivo*, many questions remain:

    - age-related limits of reprogramming
    - understanding potential adverse effects caused by local astrocyte depletion
    - specifically targeting regions that harbour vulnerable neurons
    - detecting potential side effects due to mistargeted neurons

Also, how would the replaced neurons overcome the actual pathological mechanisms going on in a patient

Thank you for your attention!