# Therapeutic Cell Replacement

Steven McLoon
Department of Neuroscience
University of Minnesota

# Neuronal Death

- Neurons are lost due to four main causes:
  - Trauma
  - Toxin
  - Hypoxia (typically loss of air or blood supply)
  - Neurodegenerative disease

## Neuronal Death

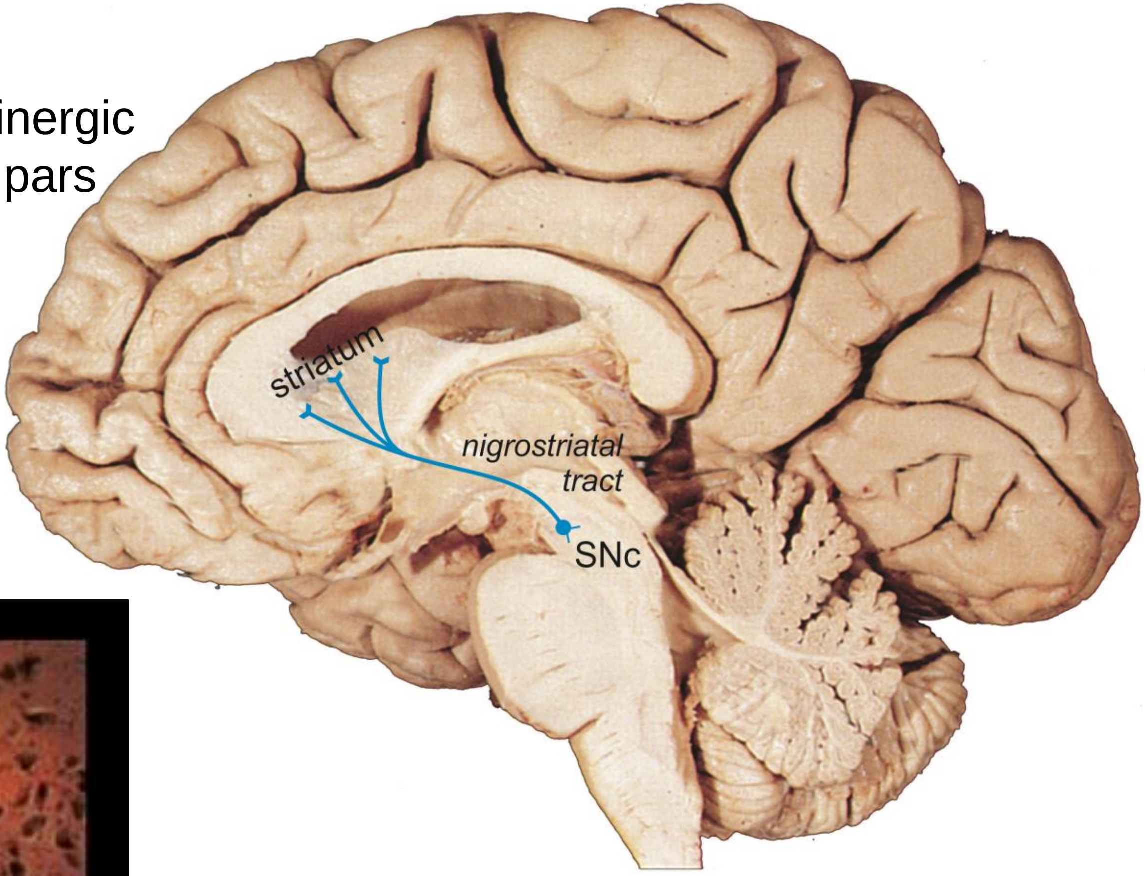
- Most common cause of trauma:
  - Auto accident!
- Common toxins:
  - Alcohol!
  - Pesticides
  - [MPTP – synthetic opiate]
- Common causes of hypoxia / loss of blood supply:
  - Heart attack
  - Local vascular obstruction (e.g. clot, arterial sclerosis)
  - Burst aneurism
  - Drowning
  - Alcohol!

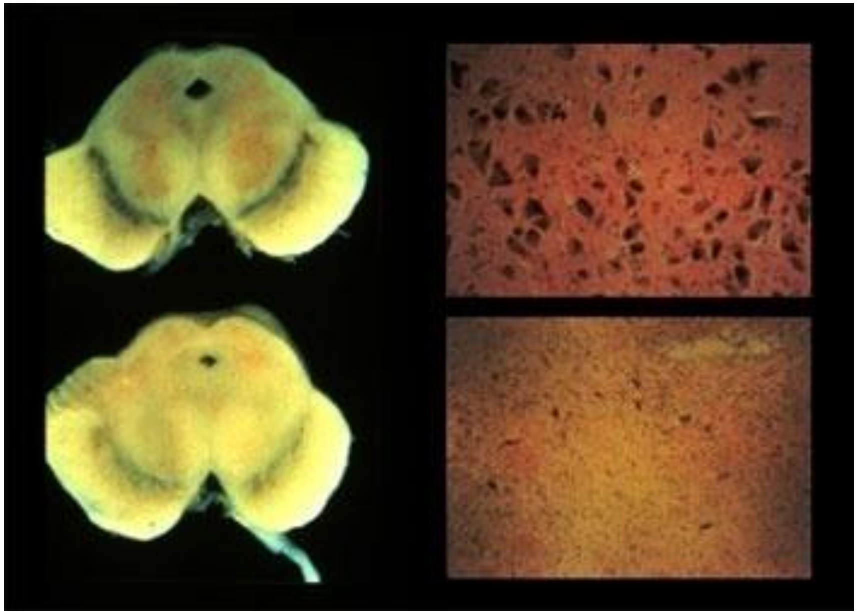
# Neuronal Death

- Common neurodegenerative diseases:
  - Parkinson's disease – dopaminergic cell loss in pars compacta of the substantia nigra
  - Amyotrophic lateral sclerosis (ALS) – motor neuron loss (upper & lower)
  - Spinocerebellar ataxia (SCA) – cerebellar neuron loss
  - Huntington's disease (chorea) – spiny neuron loss in the striatum (caudate & putamen) of the basal ganglia
  - Retinitis pigmentosa (RP) – retinal rod cell loss
  - Age-related macular degeneration (AMD) – retinal cone cell loss
  - Alzeheimer's disease – cortical neuron loss

# Neuronal Death

- Degeneration of the dopaminergic neurons in substantia nigra pars compacta (SNc) causes Parkinson's disease.



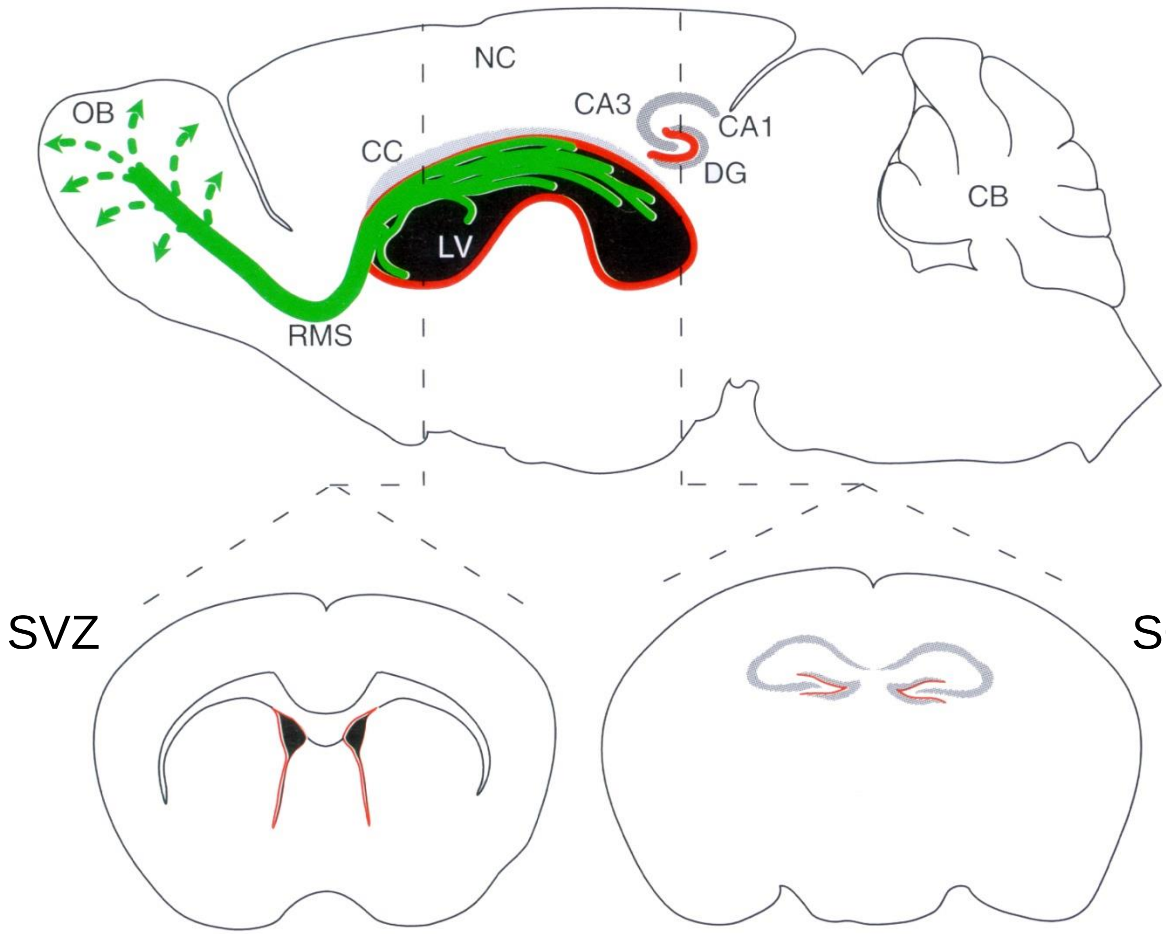



# Neuronal Death

- Death of certain neurons induces death of other neurons.

## Innate Recovery Mechanisms Following Neuron Loss

- Functional plasticity of surviving neurons.
- New neurons generated from endogenous sources.
  [minimally important if it happens at all]

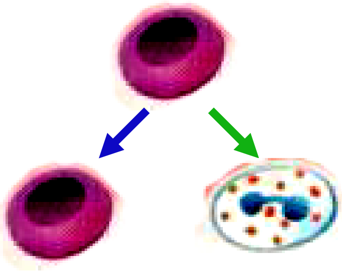


# Therapeutic Neuron Replacement

- There is tremendous need for therapeutic replacement of lost neurons.
- Transplantation offers a potential method to replace lost neurons.
- Greatest success in the laboratory and in early clinical trials has been achieved by transplanting developing neurons harvested from fetuses.
- Stem cells potentially offer an alternative source of neurons for transplantation.

# Therapeutic Neuron Replacement

- Definition of a stem cell:
  1. Capable of self renewal [indefinitely].
  2. Capable of generating multiple differentiated cell types

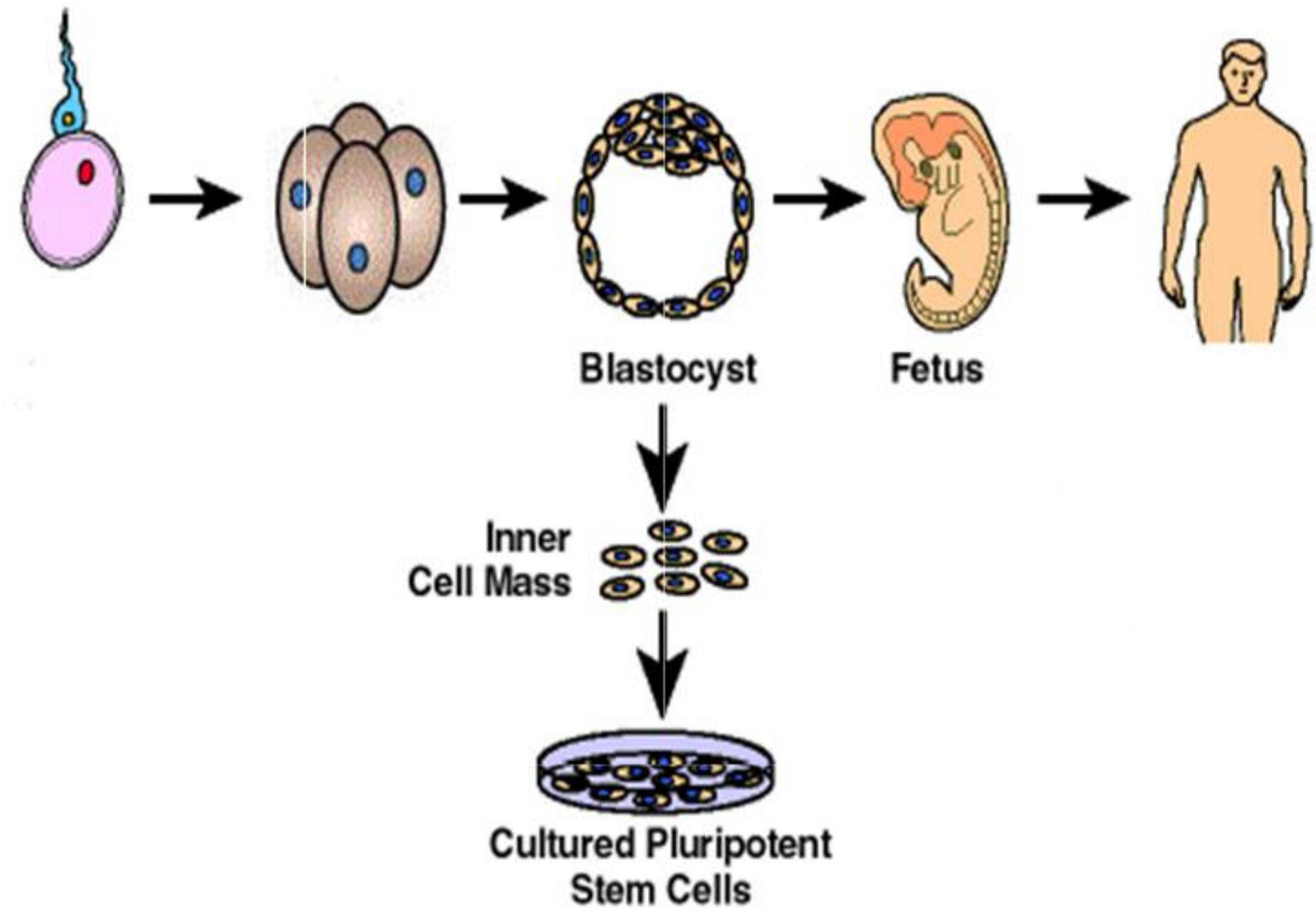


# Therapeutic Neuron Replacement

- Types of stem cells:
  - Embryonic stem cell (ESC)
  - Umbilical cord stem cell
  - Neural stem cell (NSC)
  - Other adult tissue derived stem cells
  - Induced pluripotent stem cell (iPSC)
  - Induced neurons (iN)

## Therapeutic Neuron Replacement - ESCs

- ESCs are generated from the inner cell mass of the blastula stage embryo produced by in vitro fertilization.

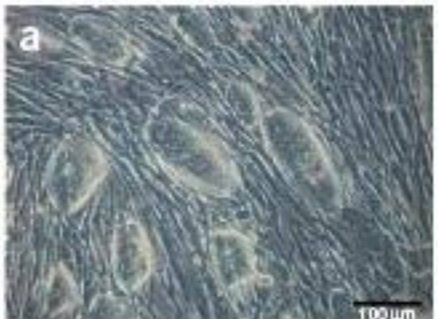


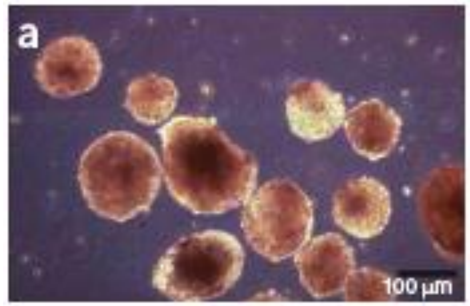
# Therapeutic Neuron Replacement - ESCs

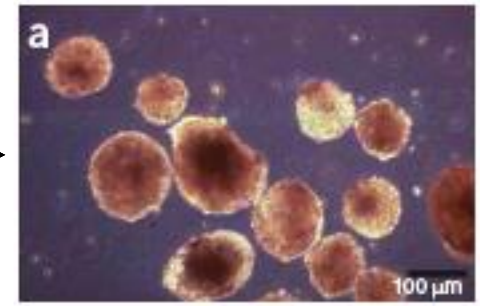
- Making neurons from ESCs:

4 days

4 days



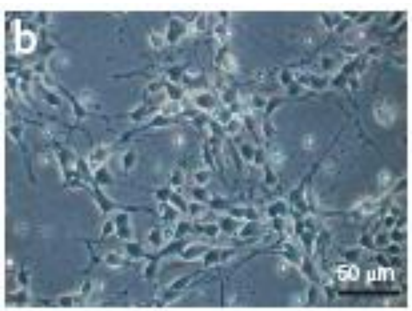



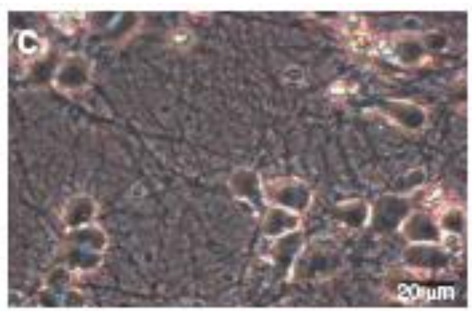

Grow ES cells on fibroblast feeders

Formation of embryoid bodies in non-adherent dishes

Add retinoic acid to enrich neural progenitors

10 days




Monolayer culture in serum-free medium to induce neuronal differentiation

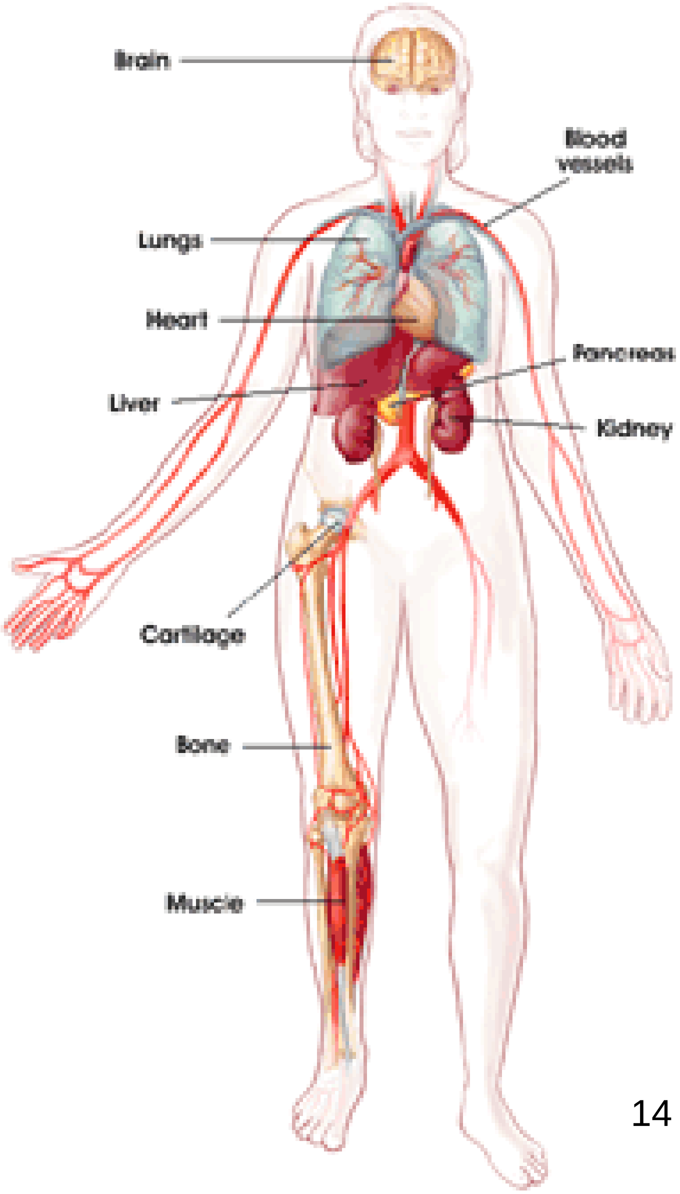
micrographs from Bibel et al. (2007)

# Therapeutic Neuron Replacement - ESCs

- Neuralized ESCs typically are heterogeneous and often form teratomas in vivo.

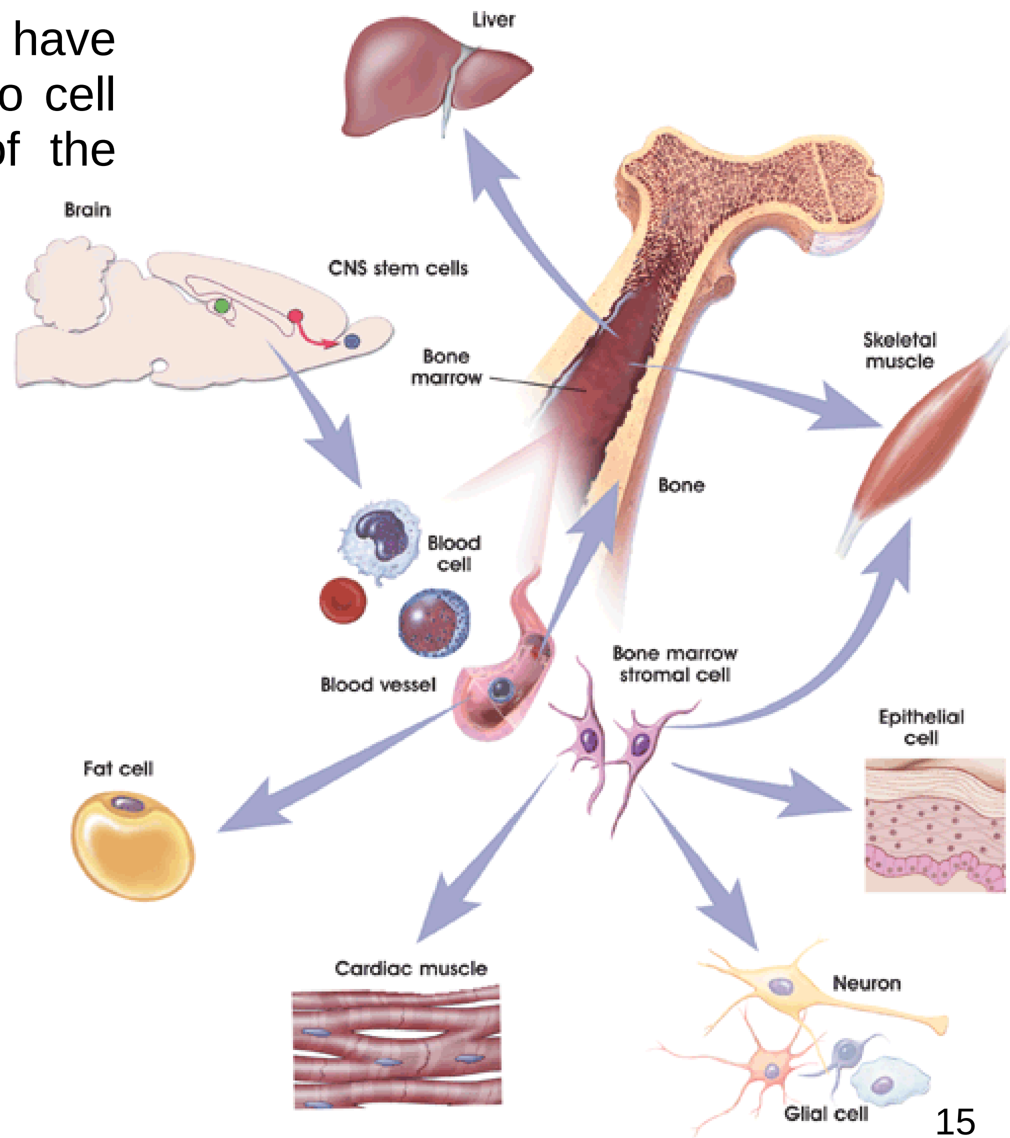
# Therapeutic Neuron Replacement – Adult Stem Cells

- Organs from which adult stem cells have been isolated:
  - Brain
  - Bone marrow
  - Intestine
  - Liver
  - Skin
  - Muscle
  - Testes



# Therapeutic Neuron Replacement – Adult Stem Cells

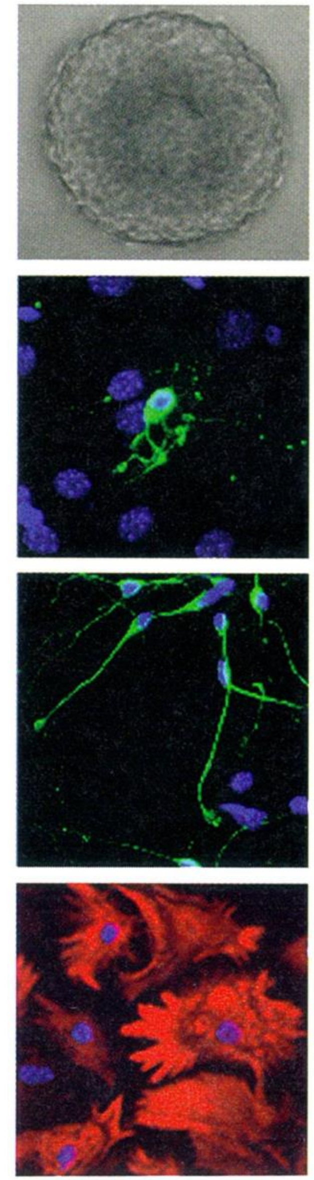
- Many types of adult stem cells have been induced to differentiate into cell types representative of each of the three primary germ layers.



## Therapeutic Neuron Replacement – Adult Stem Cells

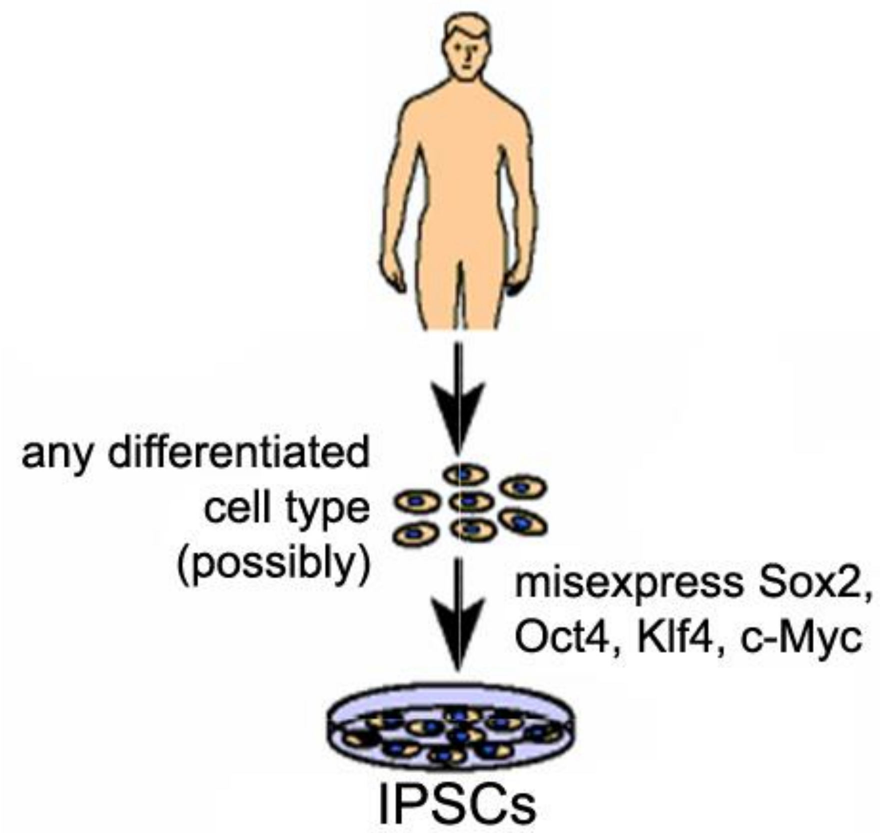
- Parts of the adult nervous system from which neural stem cells have been isolated:
  - SVZ
  - SGZ
  - Cerebellum
  - Midbrain
  - Retina
  - Spinal cord

- Neural stem cells form neurospheres in culture and give rise to neurons, astrocytes and oligodendrocytes.



# Therapeutic Neuron Replacement - iPSCs

- IPSCs can be generated (possibly) from any differentiated cell type, but usually is done with skin cells



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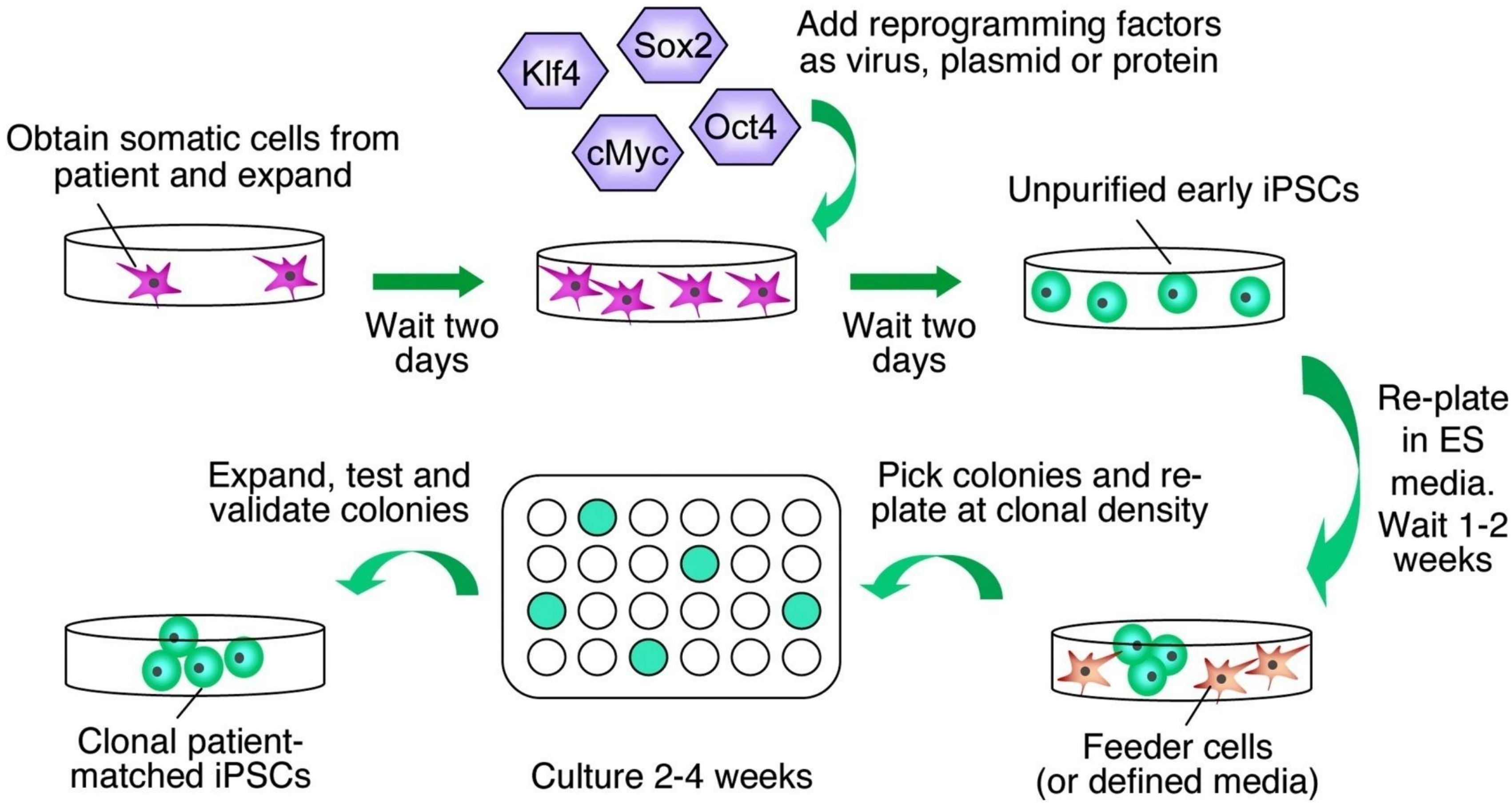
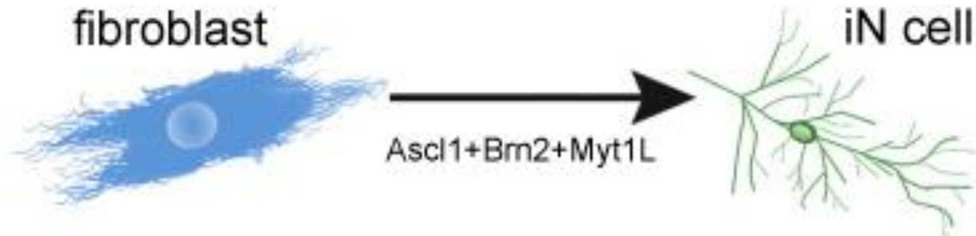
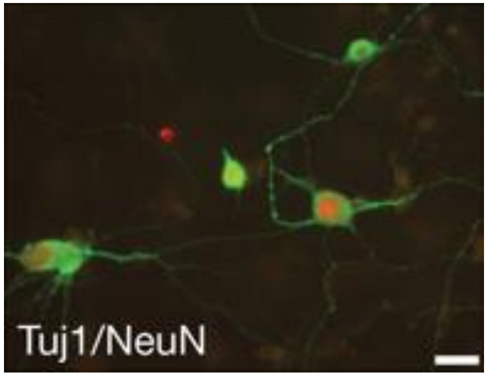


## Therapeutic Neuron Replacement – iN Cells

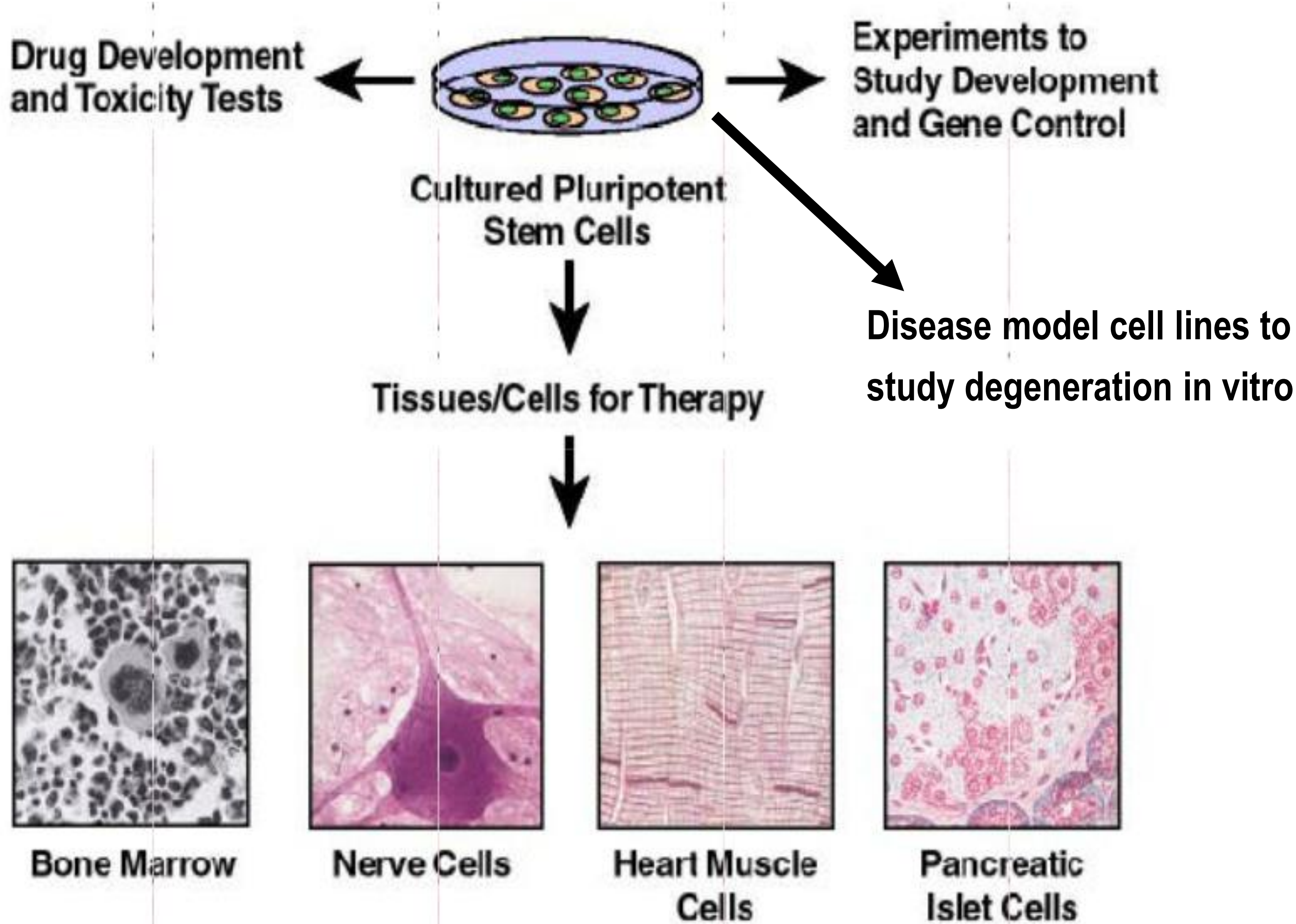



- Differentiated fibroblasts have been directly converted to neurons without going through a pluripotent step by misexpression of the transcription factors Brn2, Ascl1 and Myt1L (BAM factors).

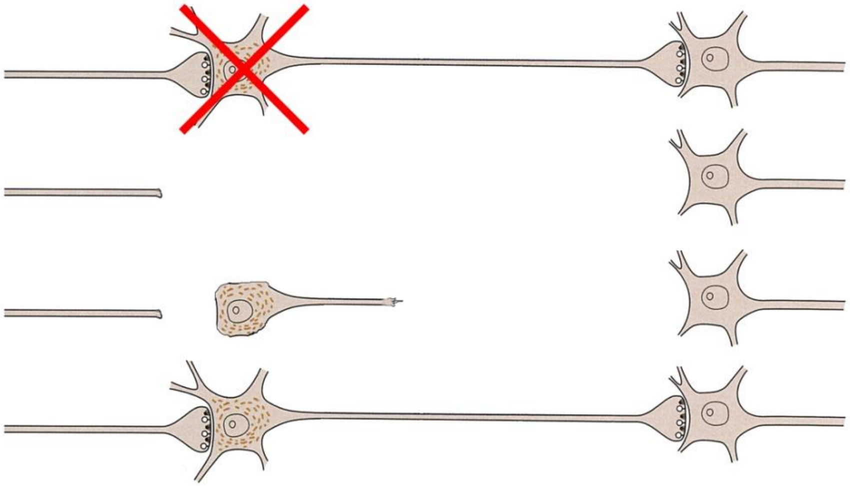
e.g. Zhang Y et al. (2013) Neuron 78:785



# Multiple Uses for Stem Cells



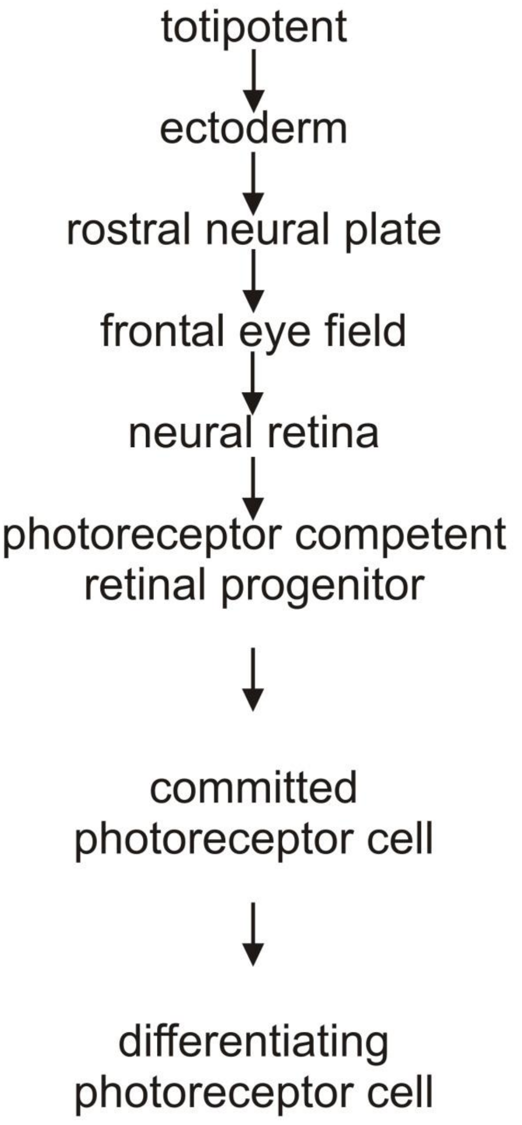
# Therapeutic Neuron Replacement



- Successful neuron replacement will require:
  - appropriate donor cell type
  - purified donor cell population at the proper stage of development
  - delivery of new cells to the proper location
  - survival of afferent & target cell populations
  - growth of axons from new cells to appropriate targets
  - formation of new synapses between new axons & target cells
  - connection of original afferents to new cells
  - myelination of the new axons

## Therapeutic Neuron Replacement

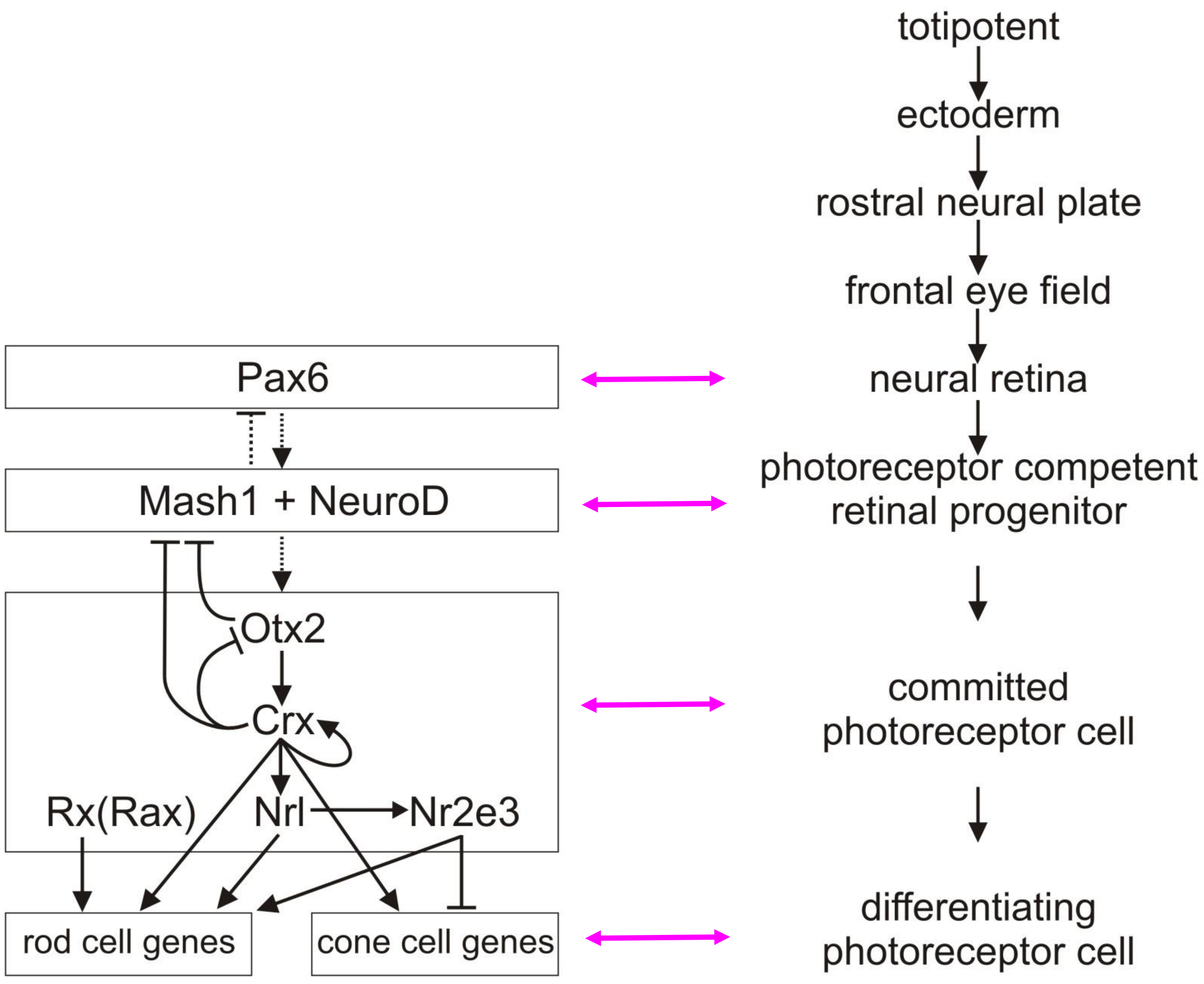
- Methods of inducing desired phenotype:
  - Stepwise recapitulation of development by culturing stem cells in different cytokines.
  - Transfect cells with the fate determining transcription factors.



# Induction of Retinal Cell Phenotypes by Cytokines

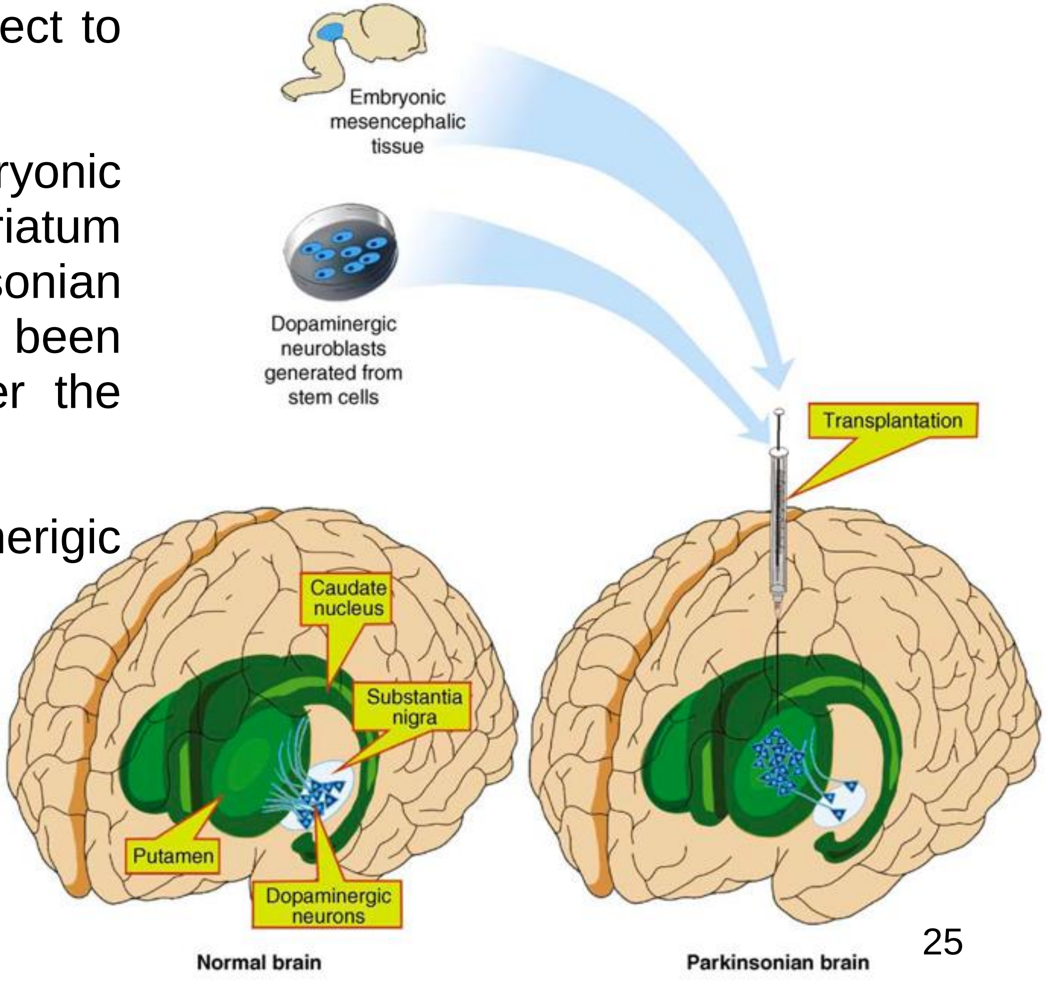
- Cytokine treatments reported to induce retinal cell fates:
  - Taurine
  - Retinoic acid
  - Noggin + Dickkopf + IGF1 (3-7day), add bFGF (3wk)

# Transcription Factors that Specify Photoreceptors

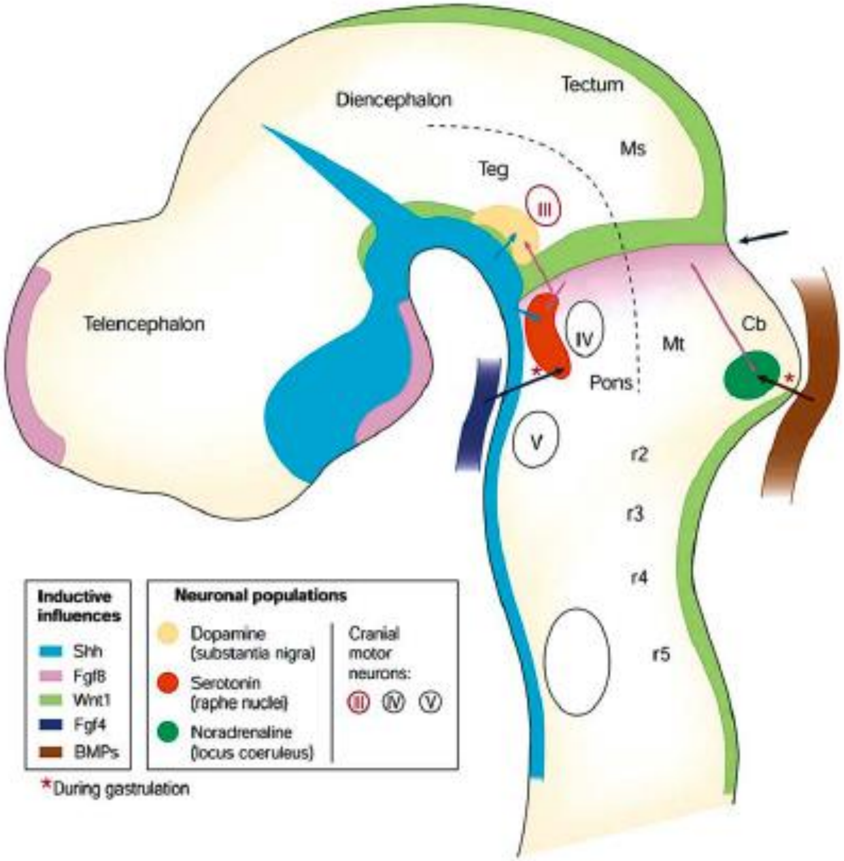


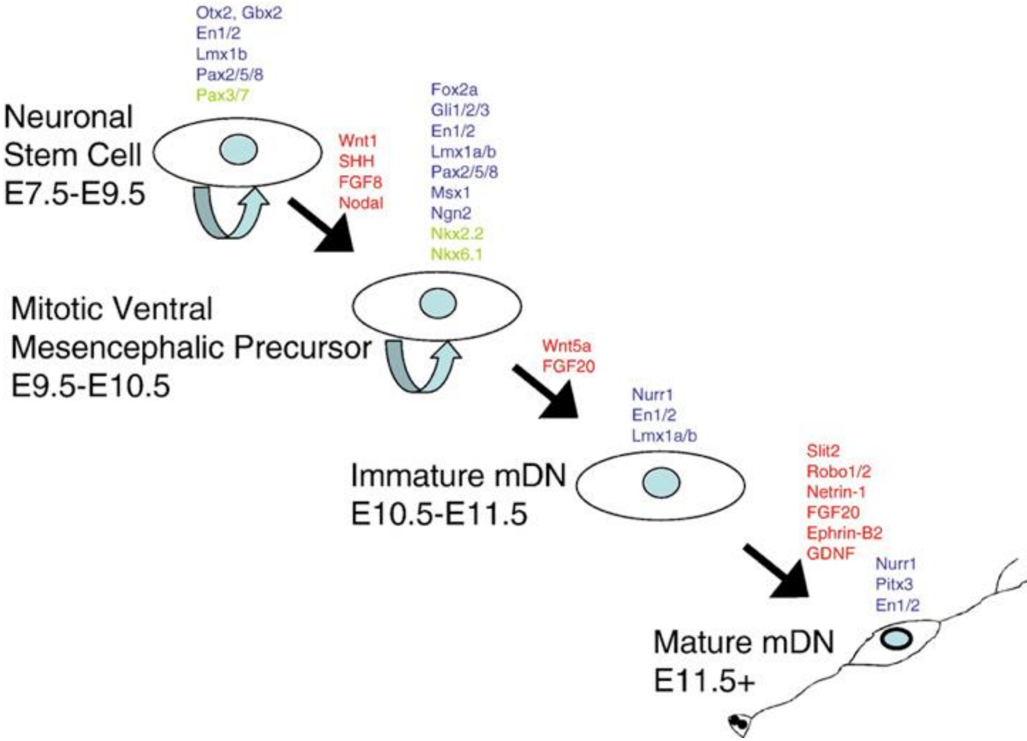
# Generation of Dopaminergic Neurons from Stem Cells

- Aim: Transplant dopaminergic neurons into the striatum, to replace the input from the degenerated midbrain cells that normally project to the striatum.
- Transplantation of embryonic mesencephalic tissue to the striatum is effective in eliminating Parkinsonian symptoms in animals, and has been used in over 400 patients over the past 20 years.
- A stem cell source of dopaminerigic neurons is needed.



# Generation of Dopaminergic Neurons from Stem Cells

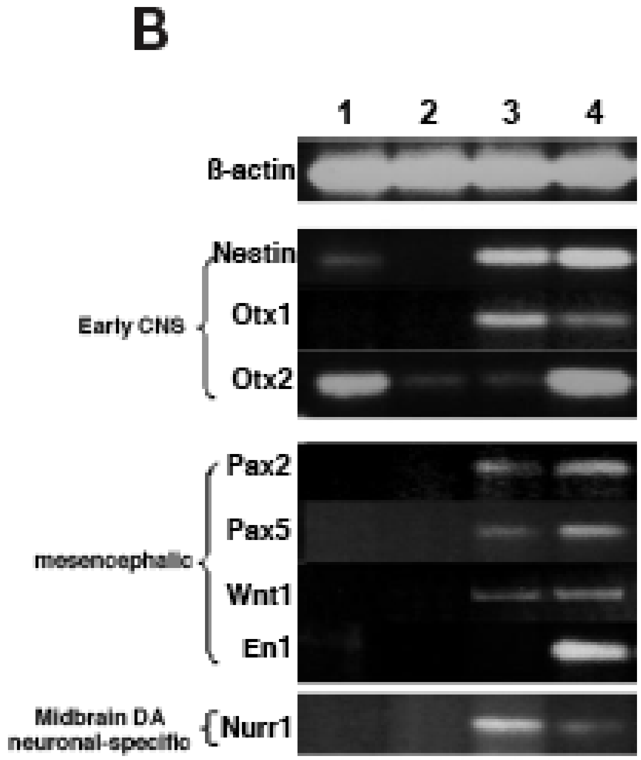


## Generation of Dopaminergic Neurons from Stem Cells

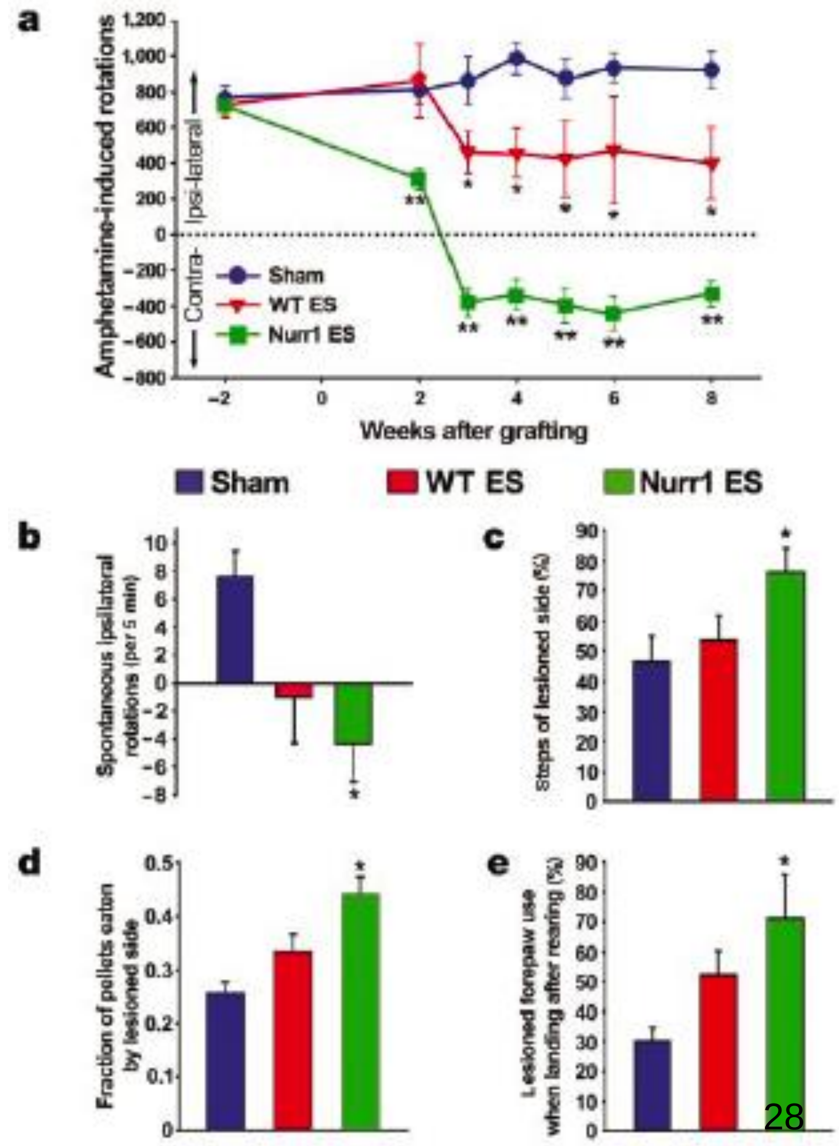
- Steps in generating dopaminergic neurons:

1. Expand undifferentiated ESCs.
2. Generate EBs in suspension culture for 4 days.
3. Plate EBs in serum-free medium for 8 days to select neural progenitor cells.
4. Grow cells in FGF2 + Shh + FGF8 on laminin for 6 days.
5. Withdraw FGF2 to induce differentiation.



## Generation of Dopaminergic Neurons from Stem Cells

- Grafted Nurr1+ cells derived from ESCs resulted in behavioral improvement in a rat model of Parkinson's disease.
- Similar results were obtained in MPTP treated monkey.
- Similar results were obtained using dopaminergic neurons produced from iPS cells.



# Generation of Dopaminergic Neurons from Stem Cells

Dopaminergic neurons produced from iPS cells were implanted into a person with Parkinson’s disease. At Kyoto University, 2.4 million cells were injected into 12 sites in the striatum.

Press conference (as reported by Japan Times)
9 Nov 2018

## Therapeutic Glial Cell Replacement

- Glial cell failure/death is at the root of several diseases:
  - e.g. Multiple sclerosis – lose of CNS myelin
- Inducing stem cells to acquire a glial cell fate for therapeutic use is an active area of research.

# Therapeutic Glial Cell Replacement

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## Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis

**Stefano Pluchino*, Angelo Quattrini†‡, Elena Brambilla*, Angela Gritti§, Giuliana Salani*, Giorgia Dina†, Rossella Galli§, Ubaldo Del Carro‡, Stefano Amadio‡, Alessandra Bergami*, Roberto Furlan*‡, Giancarlo Comi‡, Angelo L. Vescovi§ & Gianvito Martino*‡**

** Neuroimmunology Unit—DIBIT, † Neuropathology Unit, § Stem Cell Research Institute, and ‡ Department of Neurology and Neurophysiology, San Raffaele Hospital, via Olgettina 58, 20132 Milano, Italy*

**Widespread demyelination and axonal loss are the pathological hallmarks of multiple sclerosis. The multifocal nature of this chronic inflammatory disease of the central nervous system complicates cellular therapy and puts emphasis on both the donor cell origin and the route of cell transplantation. We established syngenic adult neural stem cell cultures and injected them into an animal model of multiple sclerosis—experimental autoimmune encephalomyelitis (EAE) in the mouse—either intravenously or intracerebroventricularly. In both cases, significant numbers of donor cells entered into demyelinating areas of the central nervous system and differentiated into mature brain cells. Within these areas, oligodendrocyte progenitors markedly increased, with many of them being of donor origin and actively remyelinating axons. Furthermore, a significant reduction of astrogliosis and a marked decrease in the extent of demyelination and axonal loss were observed in transplanted animals. The functional impairment caused by EAE was almost abolished in transplanted mice, both clinically and neurophysiologically. Thus, adult neural precursor cells promote multifocal remyelination and functional recovery after intravenous or intrathecal injection in a chronic model of multiple sclerosis.**

## Therapeutic Glial Cell Replacement

- A clinical trial, which is on-going, is to inject ESC derived oligodendrocytes into patients.

## Trophic Effect of Stem Cells

- Transplantation of stem cells slows the death of the remaining host neurons in several disease models.