# *Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model*

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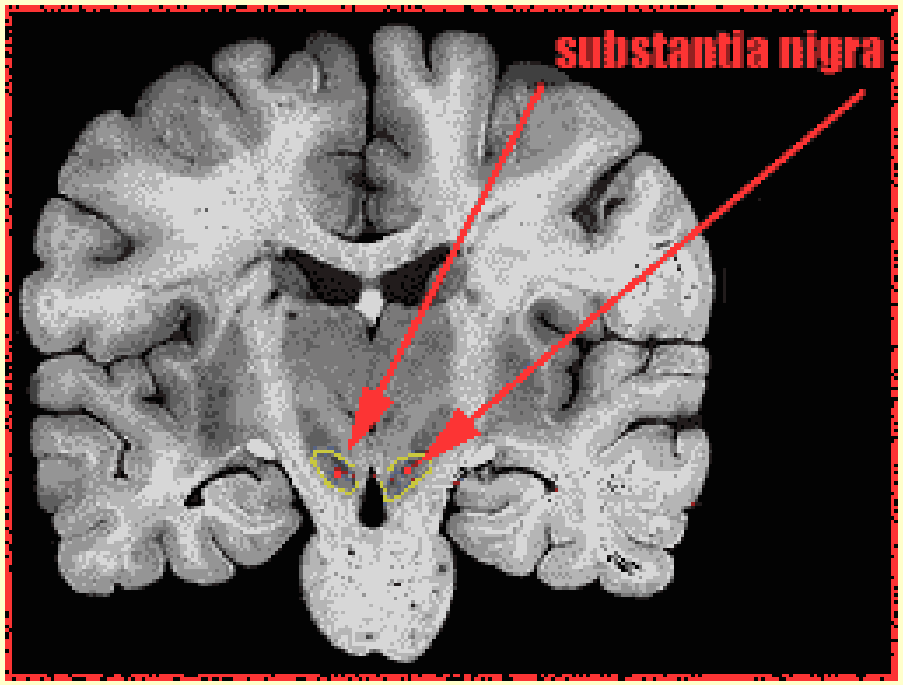
# Parkinson`s disease

- Degeneration of the Substantia nigra, loss of midbrain dopamine neurons

DA $\downarrow \rightarrow$ ACH$\uparrow \rightarrow$ 5HT $\downarrow$ NA $\downarrow$

$\Rightarrow$ Detraction of motor function as well as mental, sensory and vegetative regions



# Aim of the research

- Recent treatment with L-DOPA alleviates the symptoms and decelerates illness but the patient only gains another 10 years at most.

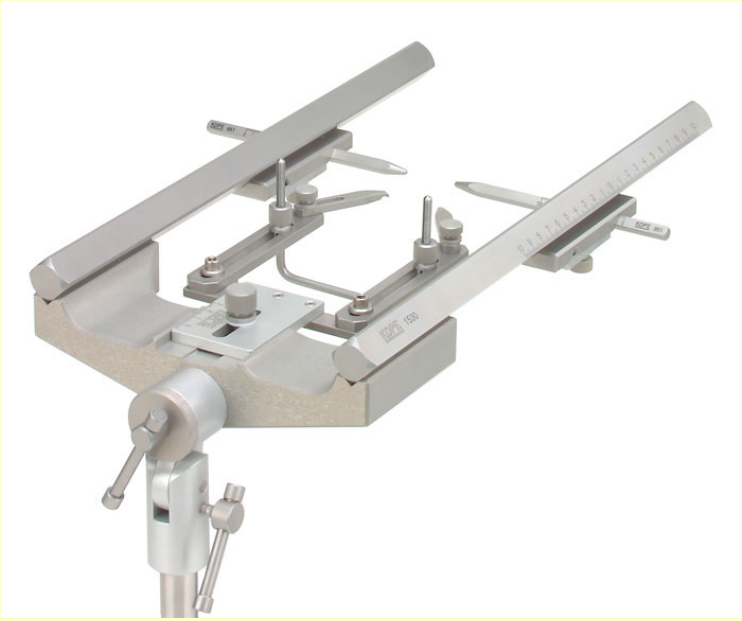
- Does development of new DA neurons via engrafting embryonic stem cells represent a sustained advancement in healing Parkinson`s disease?

# Propagation and Preparation of ES cells

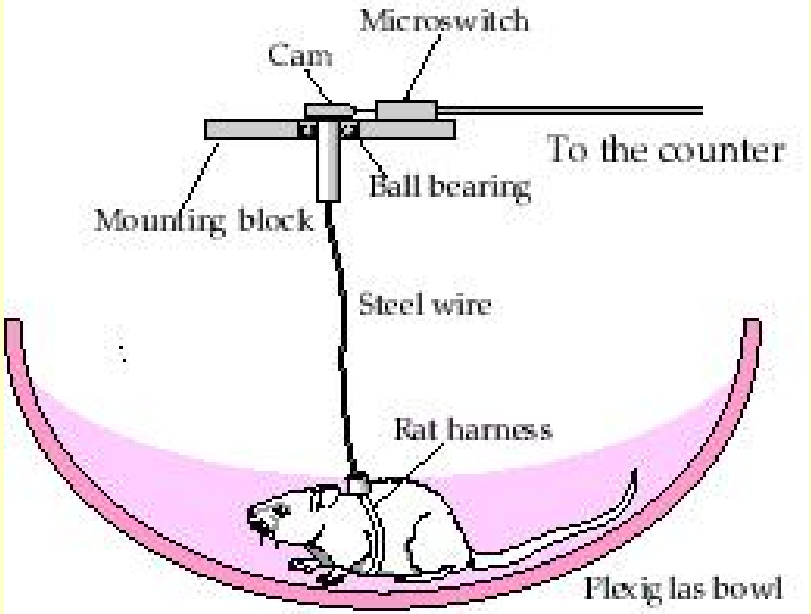
- Breeding mouse ES cell line D3 in presence of human leukemia inhibitory factor
- Trypsinization to seperate cells
- Seeding without leukemia inhibitory factor
- Again trypsinization and trituration to receive low cell densities
- Determination of viability and concentration by a homocytometer

# 6-OHDA Lesion and Amphetamine Rotations

- unilateral stereotaxic injections of the neurotoxin 6-OHDA into the median forebrain bundle, coordinates were set according to the atlas of Paxinos and Watson



- Amphetamine (4mg/kg i.p.) releases DA from the undamaged nigrostriatal nerve terminals and causes the rotation towards the lesioned side
- >500 turns ⇒ >97% DA lesion ⇒selected for grafting



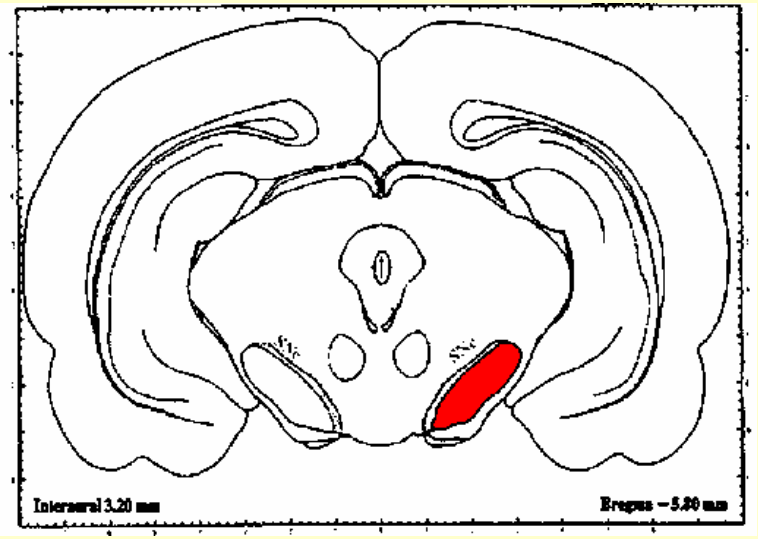
- **Transplantation procedures:**

  Stereotaxic injection of 1µl (0,25µl/min) ES cells (1000-2000) into 2 sides of the right striatum.

  25 rats received cell injection,

  13 rats received medium injection (sham surgery).

  6 rats showed no graft survival, 5 rats died because of teratoma-like tumors.



- **Immunosuppression:**

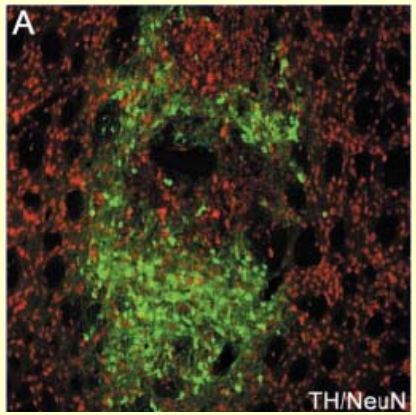
  by injections of cyclosporine A.

- **Cell Counting:**

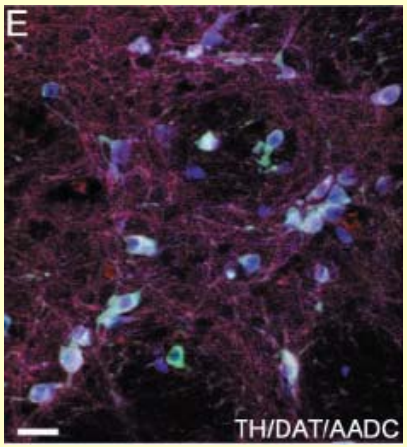
  3- dimensional Abercrombie method with a fluorescence detecting confocal laser microscope.

# Detection of DA neurons

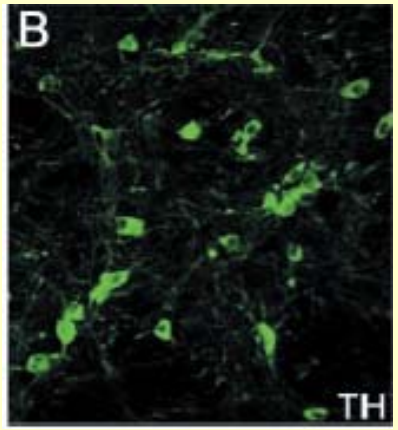
- TH-positive cells (*green*) coexpressed the neuronal marker NeuN (*red*):



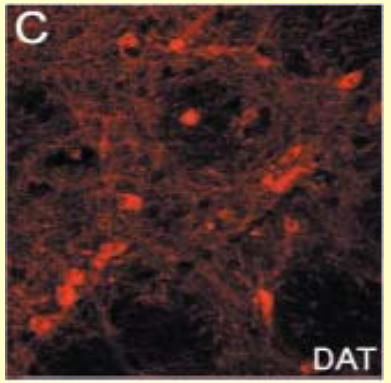

- Triple labeling (*white*) TH/DAT/AADC:



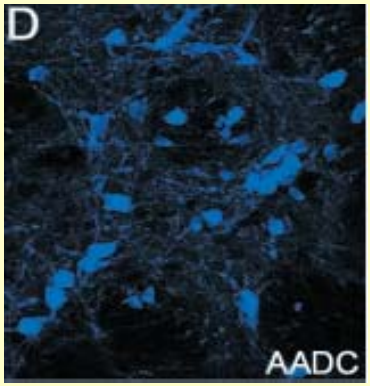

- TyrosineHydroxylase -positive cells:



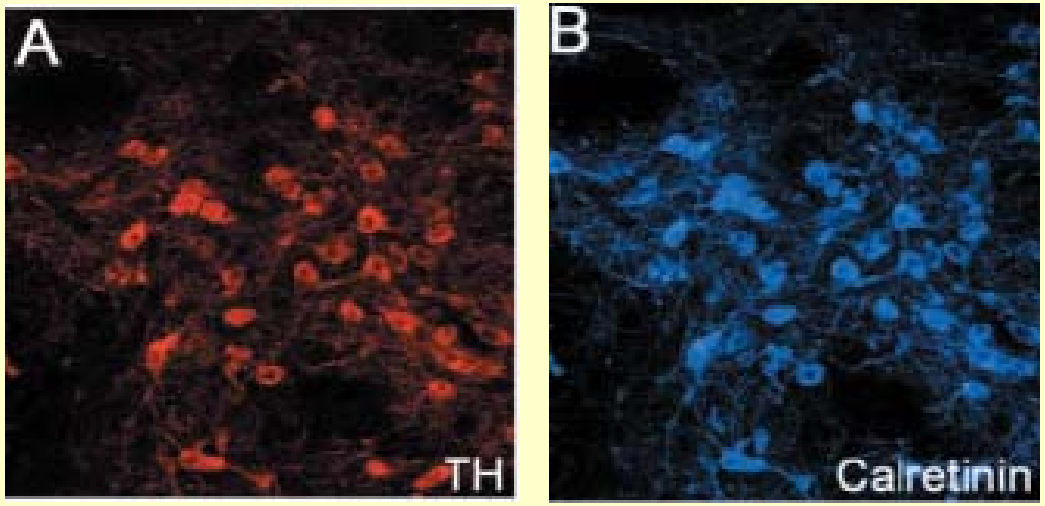

- TH cells coexpressing DATransporter:




- TH cells coexpressing AromaticAcidDecarb oxylase:



# Detection of DA neurons in the ventral striatal region (A10)

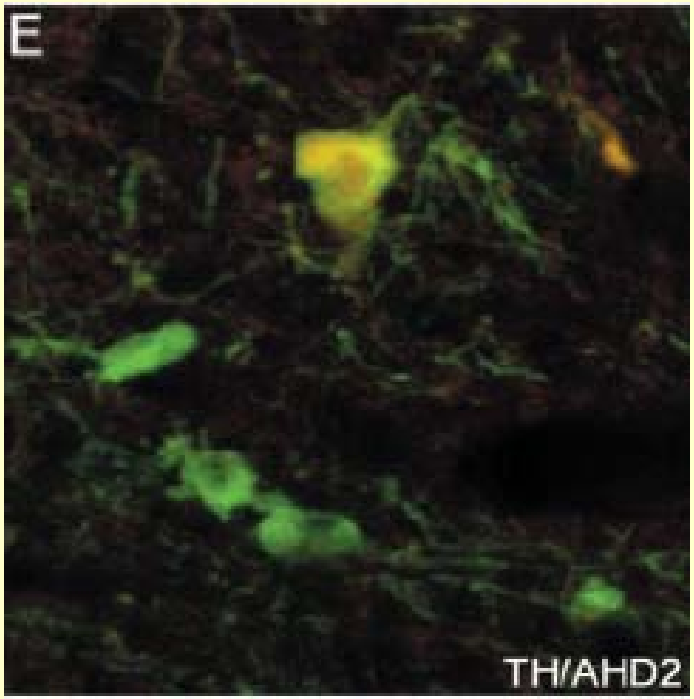



- calretinin and calbindin are typical DA neuron markers

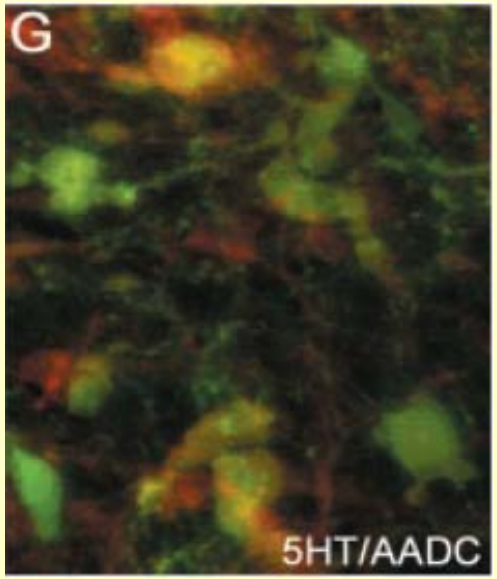
- *purple* cells show coexpression of all 3 DA neuron specific markers

# Expression of  neuronal markers in the substatia nigra

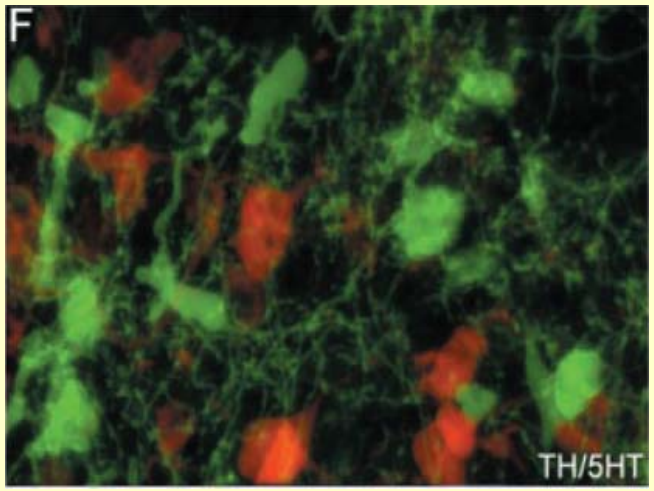
- TH positive neurons (*green*) coexpressing the A9 (substantia nigra) marker aldehyde dehydrogenase 2 (AHD 2, *yellow* coexpression)




- 5-HT neurons (*green*) also coexpressed aromatic acid decarboxylase (*yellow*)

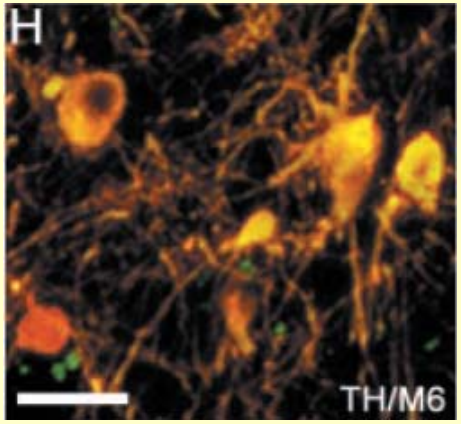



- 5-HT neurons (*green*) and TH neurons (*red*)

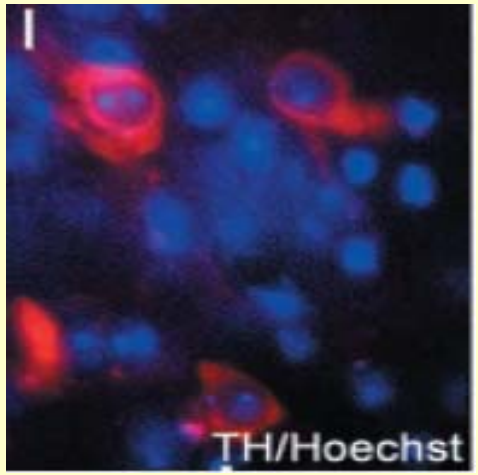


# Identification of mouse ES cell-derived DA neurons

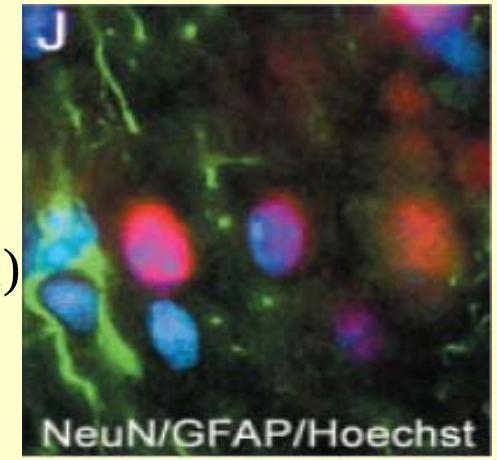
- Mouse specific M6 antibodies (*yellow* coexpression with TH)



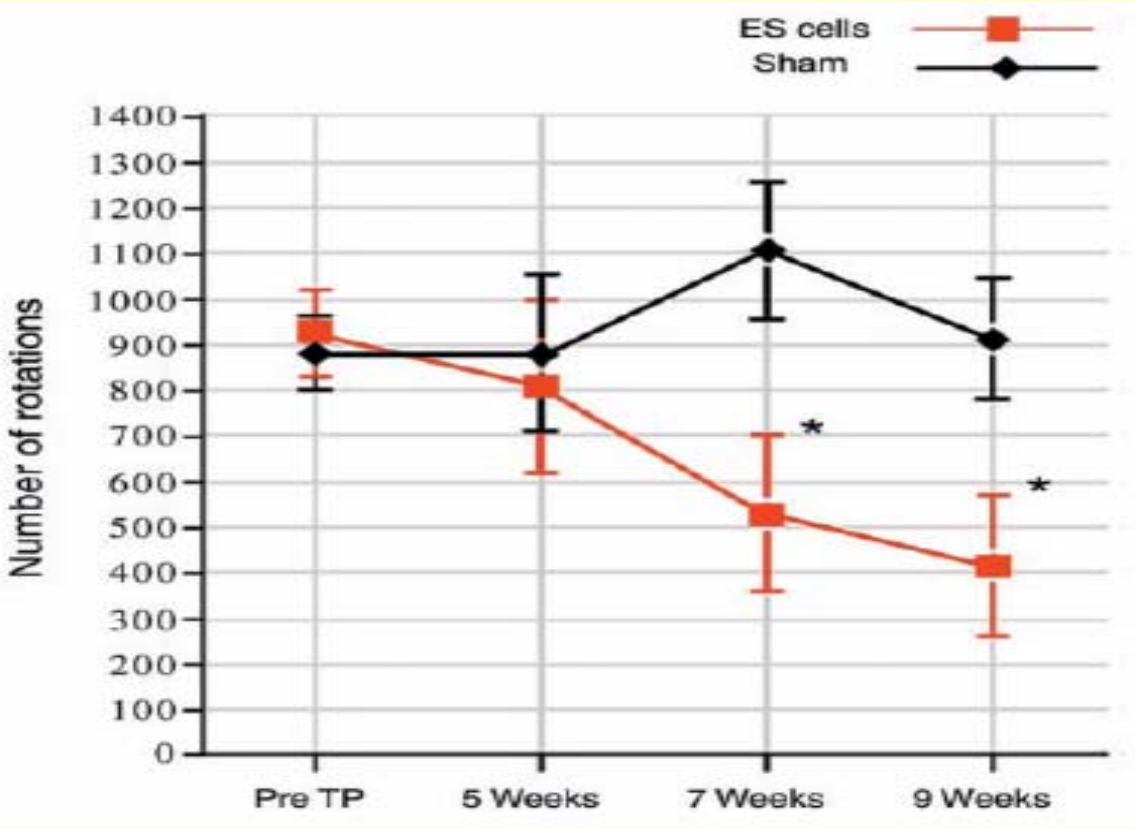

- Mouse specific inclusions after Hoechststaining (*blue*), coexpression with TH (*red*)



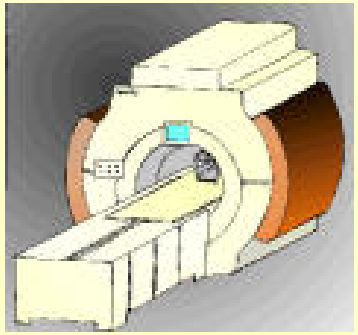

- Astrocytes (glial fibrillary acidic protein-positive, *green*) and neurons (NeuN, *red*), that show mouse specific intranuclear fluorescent inclusions (*blue*)



# Statistical Analysis of the rotational behavior




- Significant decrease in amphetamine-induced turns of animals with ES cell neuronal grafts
- posttransplantational number of rotations decreases in comparison to pretransplantation of ES cell engrafted animals

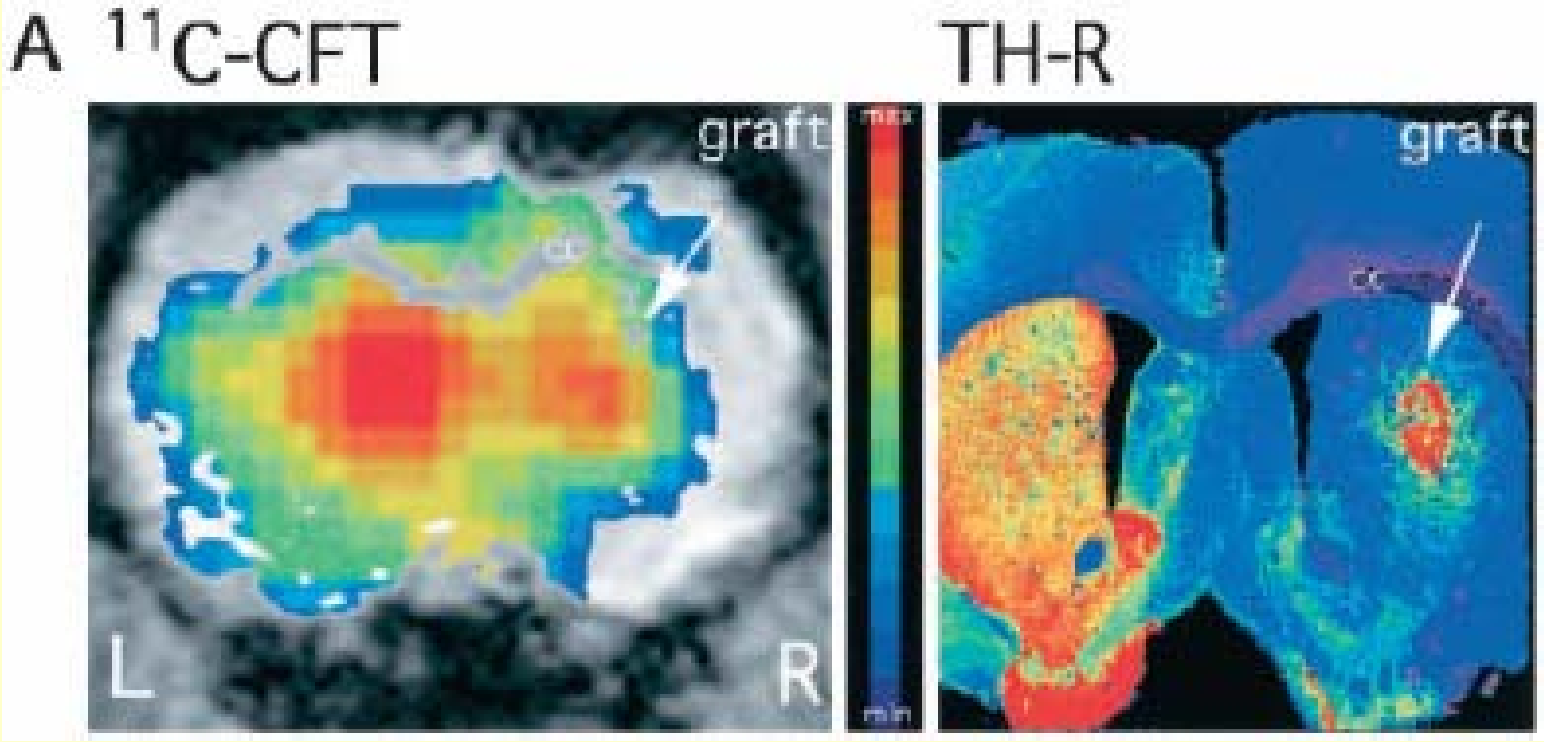


# Functional assay

How can you measure metabolic changes in the brain caused by DA release in response to amphetamine?

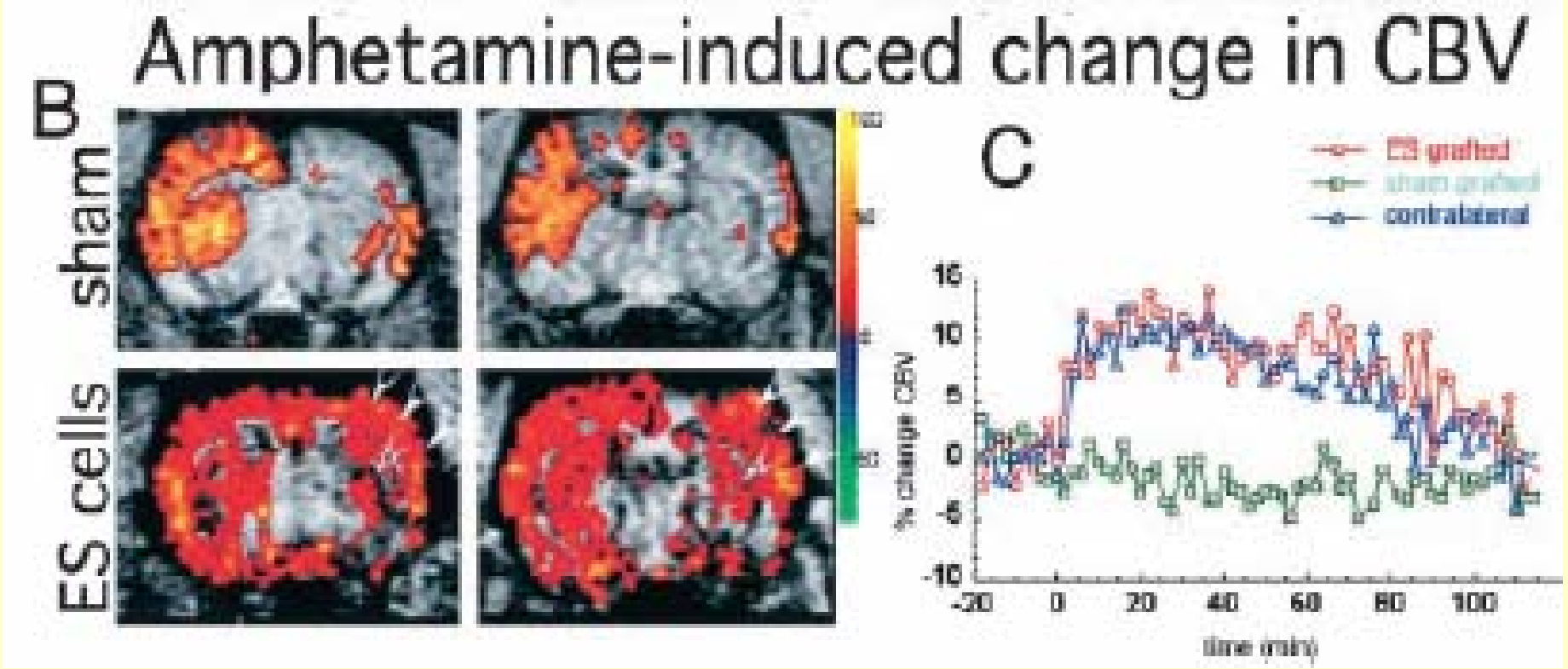
→ PET (positron emission tomographie); produces slices of living organisms and shows biochemical and physiological actions via detecting activated substances

→ MRI (magnetic resonance imaging); shows metabolic activities  of cerebral cross sections by detecting changes of a magnetic field




- [$^{11}$C]CFT, a specific DAT ligand showed specific binding in the right stiatum (*left*).

- The increase of [$^{11}$C]CFT binding was correlated with the presence of postmortem TH-immunoreactive neurons in the graft (*right*).




- Percentage of change in rCBV in response to amphetamine for control (*upper*) and an ES cell-derived graft (*lower*).

- The response on the grafted (*red line*) and the normal (contralateral) (*blue line*) striata was similar, no changes in sham grafted animals (*green line*).

# Main results/Summary

- ES cells differentiate into mesencephalic dopaminergic-like phenotypes after transplantation to the adult 6-OHDA-lesioned brain and become integrated into the host circuitry.

- 6-OHDA-lesion results in a complete absence of CBV regulation and induces motor asymmetry of the animals in response to amphetamine.

⇒ Parkinson rats show behavioral recovery, reduced asymmetry and improved physiological & functional response to DA-releasing agents after ES cell grafts

# Discussion and Perspectives

- There are a lot of factors that could be responsible for the neuralizing effect such as *noggin*, *follistatin*, *cerberus* and *chordin*, but the reasons for grafted ES cells specifically becoming DA neurons in vivo are not known. Low cell density benefits the development into neurons.

- This form of Parkinson`s disease in rat is man-made. The reasons why dopaminergic neurons degenerate at the „real“ form of PD are not known. Won`t these destroying factors kill the new developed DA cells as well?