Catheter Based Cell Therapy in Interventional Neuroradiology

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Ischaemic Stroke
Current Treatment Limitations

- Narrow therapeutic window (3 – 8 hs).
- Less than 5% receives tPA.
- Endovascular clot devices depends on timing.
- *After 12 hs hs there is no options.*

Outline

- Normal adult neurogenesis
- Basic concept of Stem Cells
- Injury induced neurogenesis
- Animal data
- Human studies
- Intraarterial injection of drugs and cells
- *Stroke Trial*
- *Epilepsy Trial*
- Final remarks

Outline

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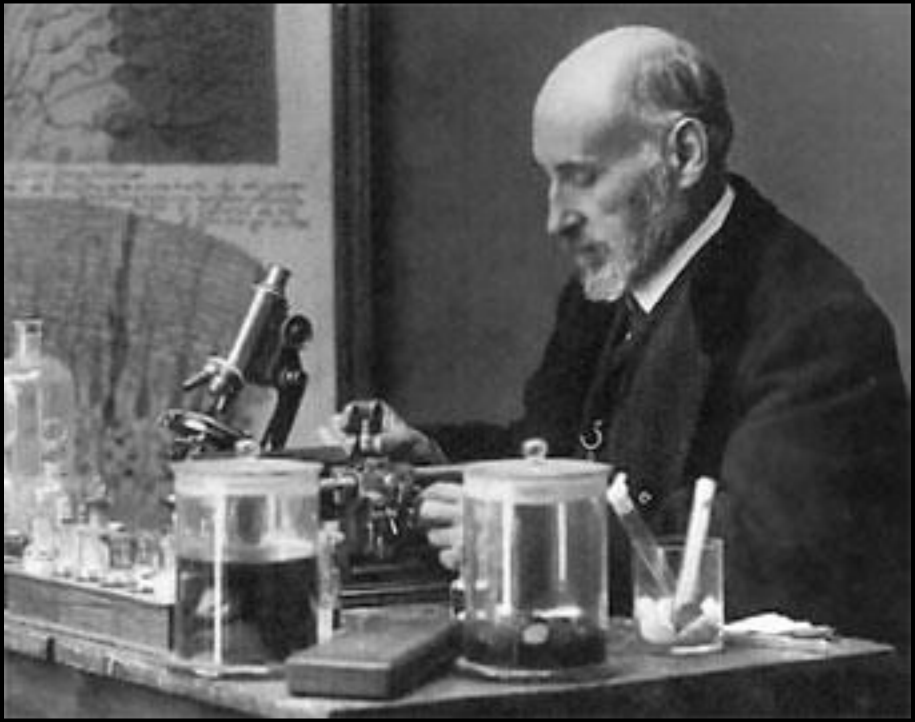
Ramón y Cajal
(1832 – 1934)



XIX - XXth Century Neurobiology Dogma

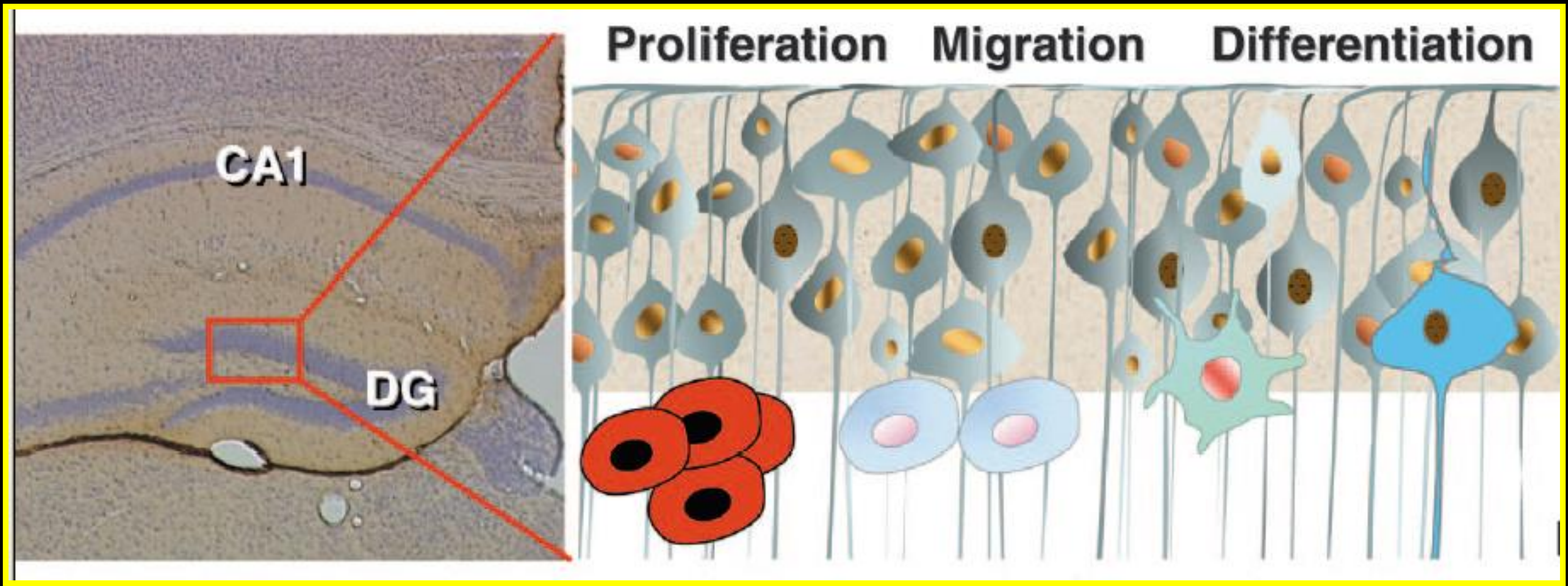
“Mature Brain cannot regenerate”

1960's
Joseph Altman's Pioneering Work

Normal Adult Neurogenesis

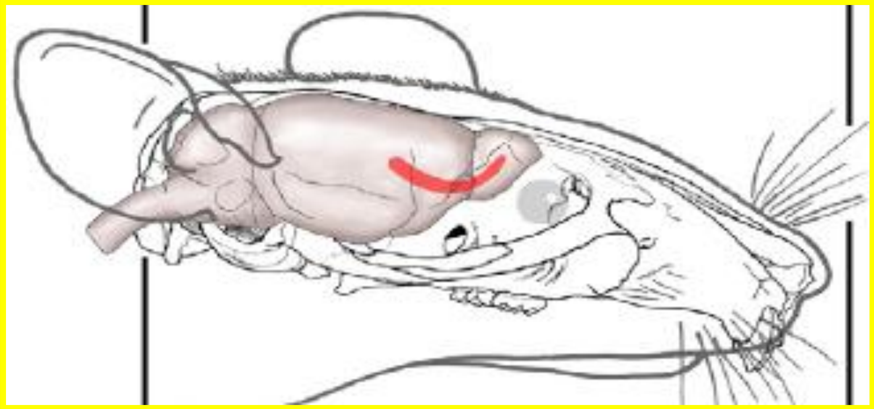
Neurogenesis in the Adult Mammalian Brain

Altman,J. & Das,G.D. Autoradiographic and histologic evidence of postnatal neurogenesis in rats.
J. Comp. Neurol. 124,319-335 (1965)



Rat Neurogenesis Areas

Dentate Gyrus **Subventricular Zone**

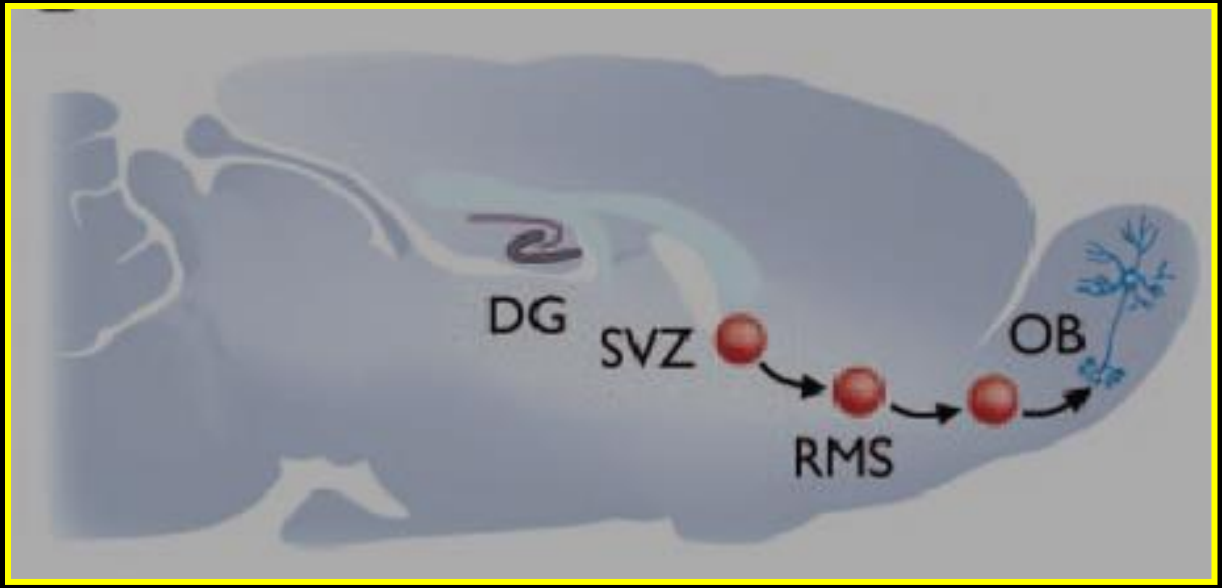


Stem Cell Migratory Stream SVZ – Olfactory Bulb

Altman J. J Comp Neurol 1969 Dec;137(4):433-57

Rat Adult Physiological Neurogenesis

- DG : up to 9000 new neurons per day
- SVZ : ≅ 30.000 new neurons per day



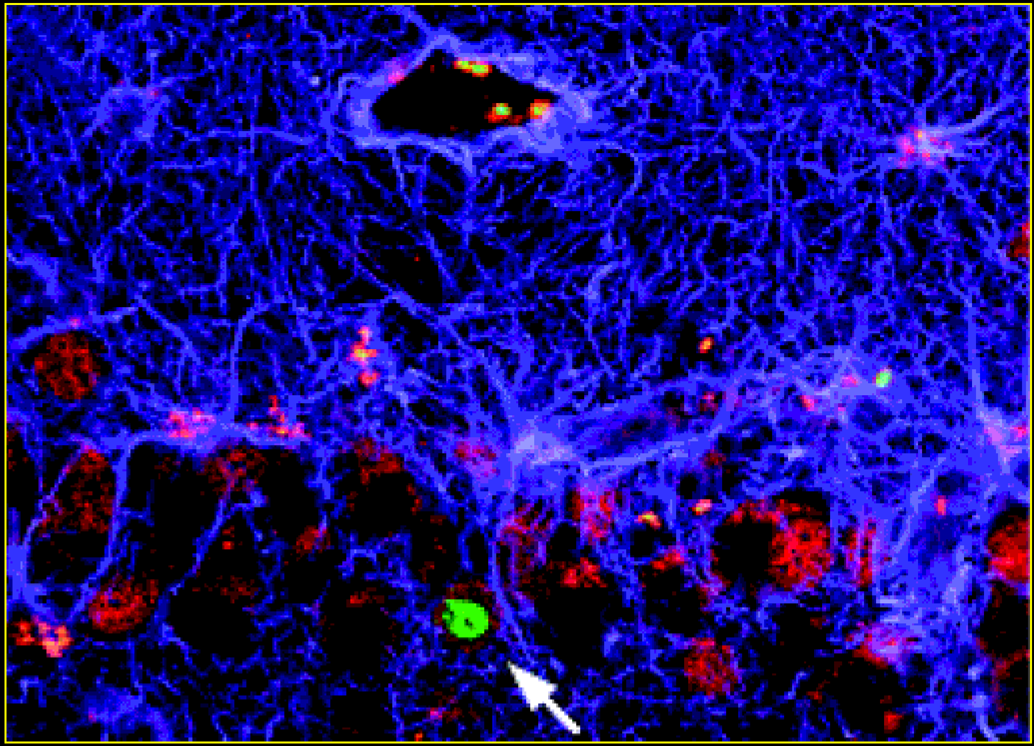
Neurogenesis in the Adult Mammalian Brain

Early Researchs and Scientific Evidences

- *1977, Kaplan et al: neurogenesis in adult rats. Confirmation by Electron Microscopy*
- *1985, Rakic: neurogenesis in primates.*
- *1992, Reynolds & Weiss: neurogenesis in mammals.*
- *1998, Gould et al: neurogenesis in adult monkeys.*

Neurogenesis in Adult Human Hippocampus

Ericksson PS, *1998 Nature Med Nov ; 4 Vol 11*



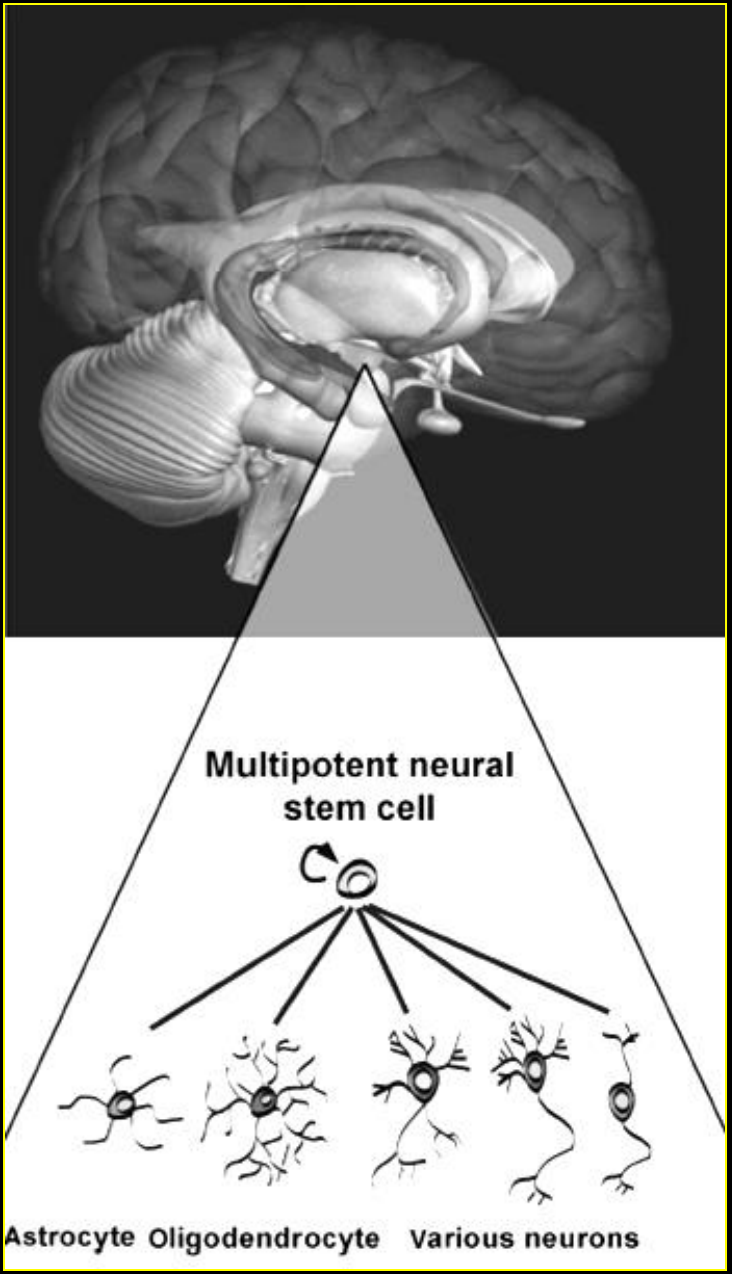
DNA BrdU labeling during Cell S phase

Neurogenesis in Humans:

Continuous physiological process.

Migration of neural precursor cells:

- Subventricular Zone
- Subgranular Zone of Dentate Gyrus
- Striatum
- Neocortex
- White matter
- *Long pathway to reach target*



Stroke in the XXIth Century
Adult Neurogenesis ?

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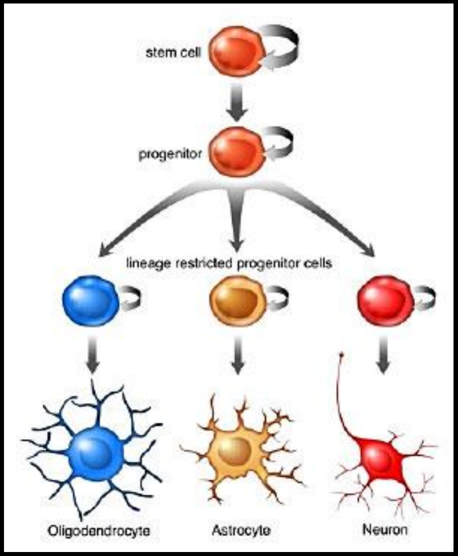
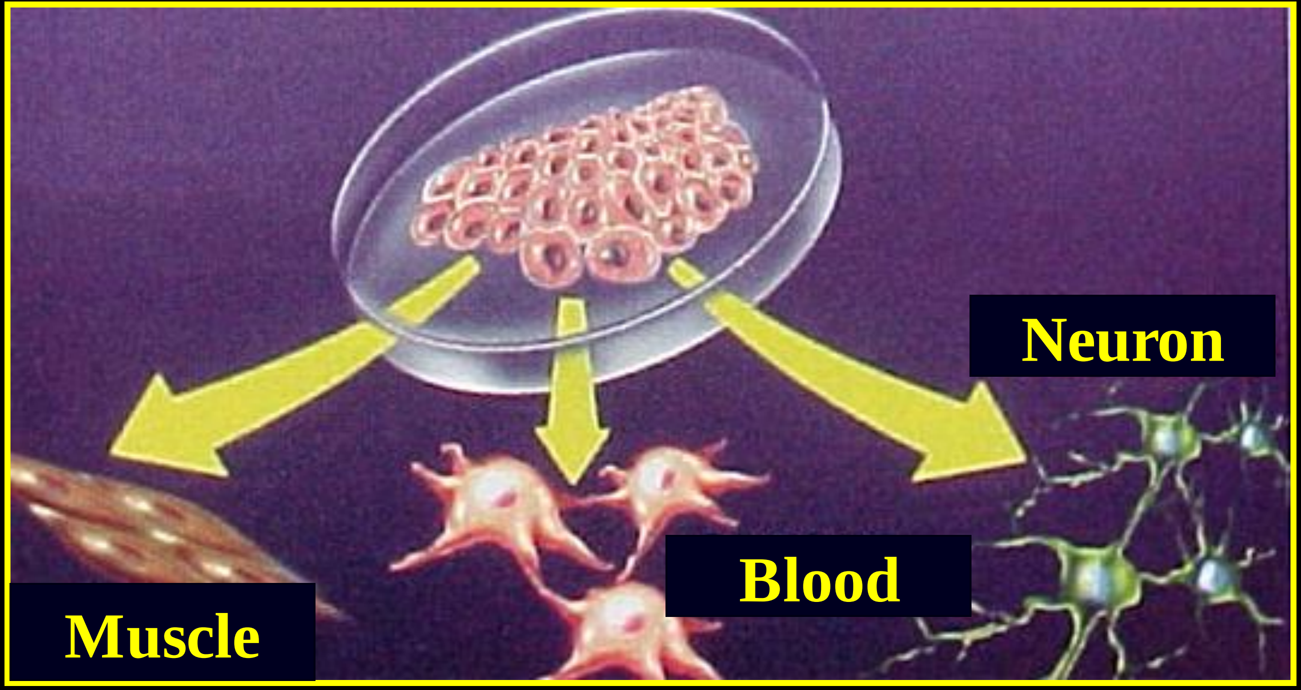
Concept of Cell Therapy

- Introducing new cells into a tissue
- Replace damage tissue.
- Specific cell or Stem Cell

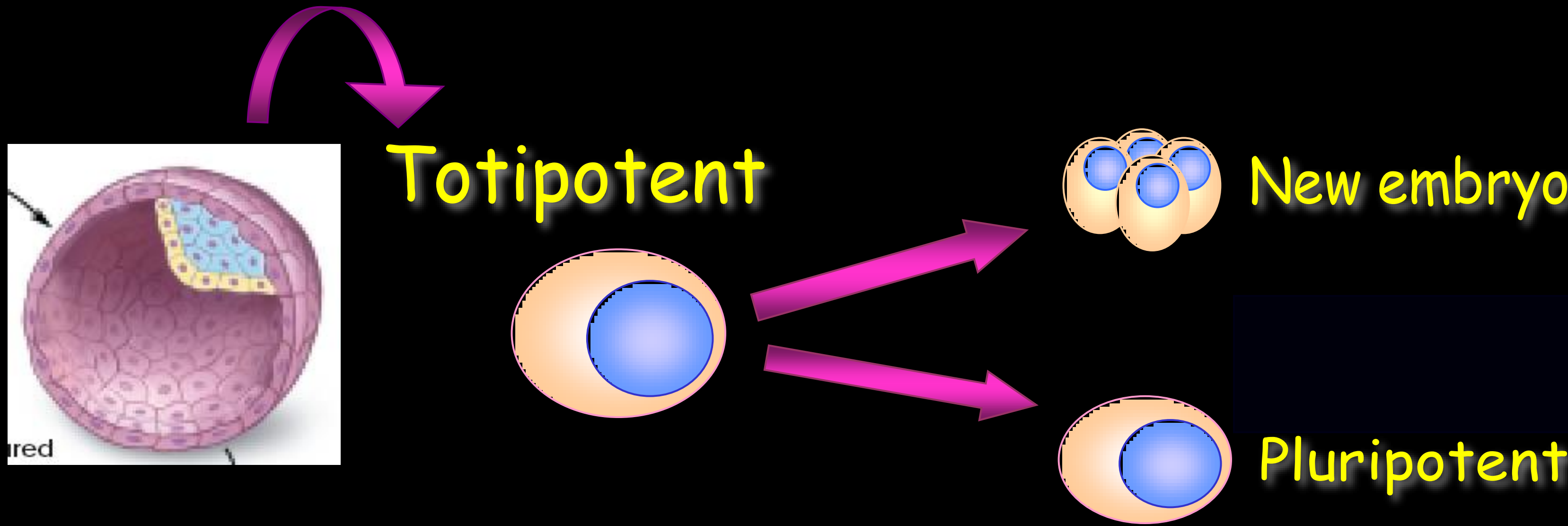


Stem Cells

- Immature pluripotential Cells.
- They can give rise to different cells and tissues.
- Proliferate from a limited source to a large number of different cells.
- Tropism for pathological areas.

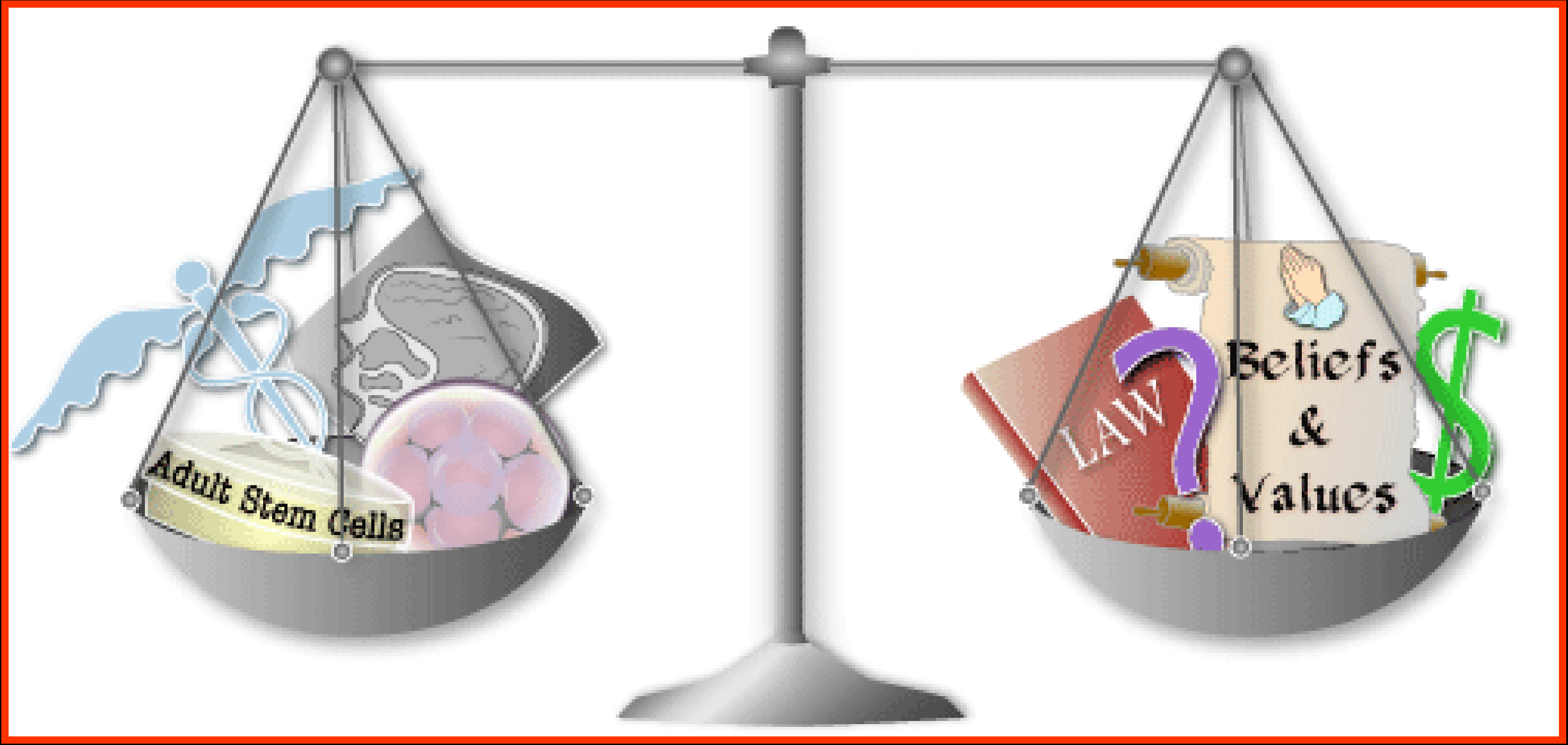


Embryonic Stem Cell
Blastocyst Inner Mass

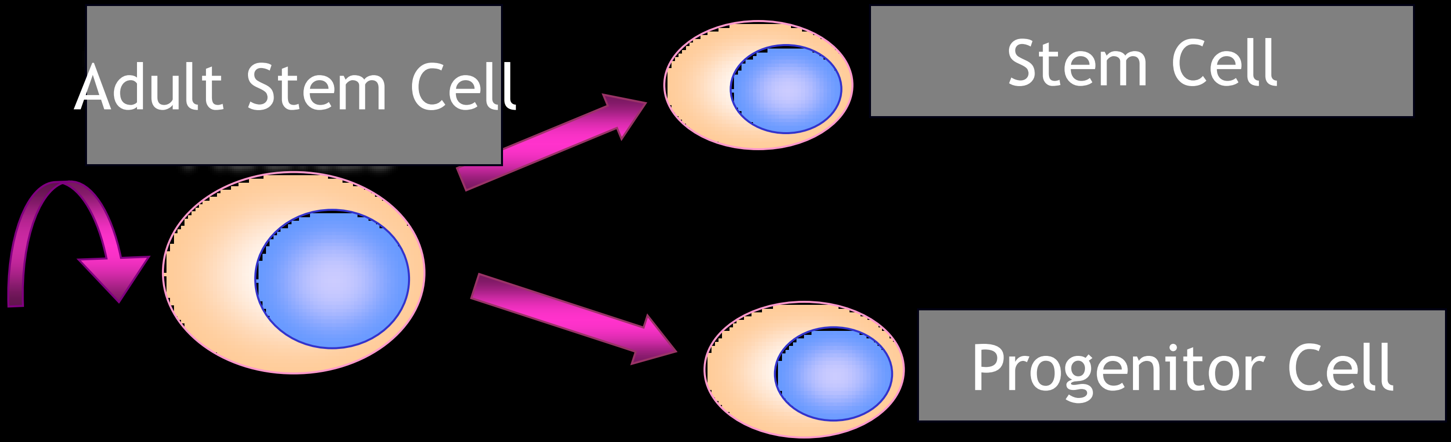


- Instability of fhenotypes
- Tumors
- Ethical problems

Alternative
Adult Stem Cells



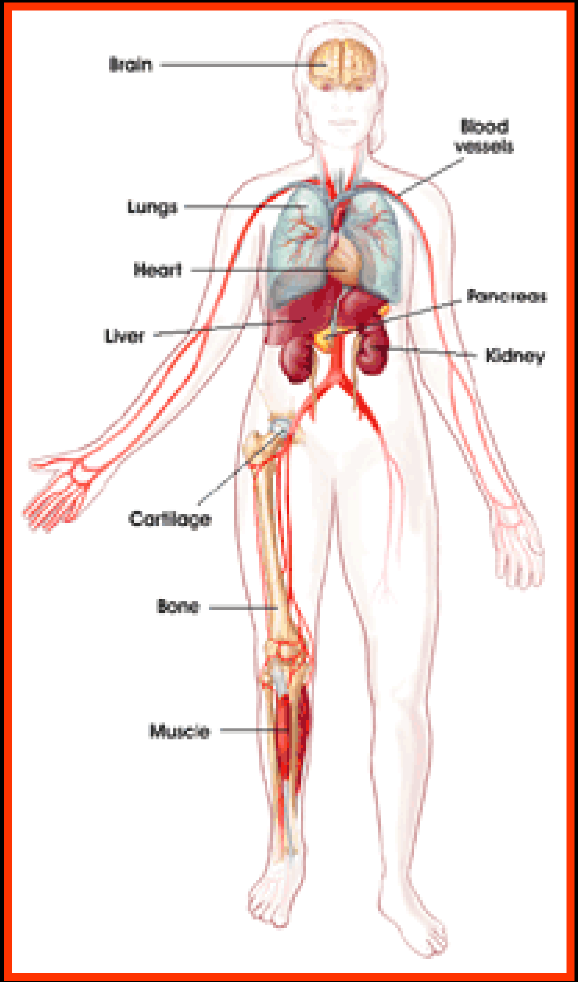
Adult Stem Cell



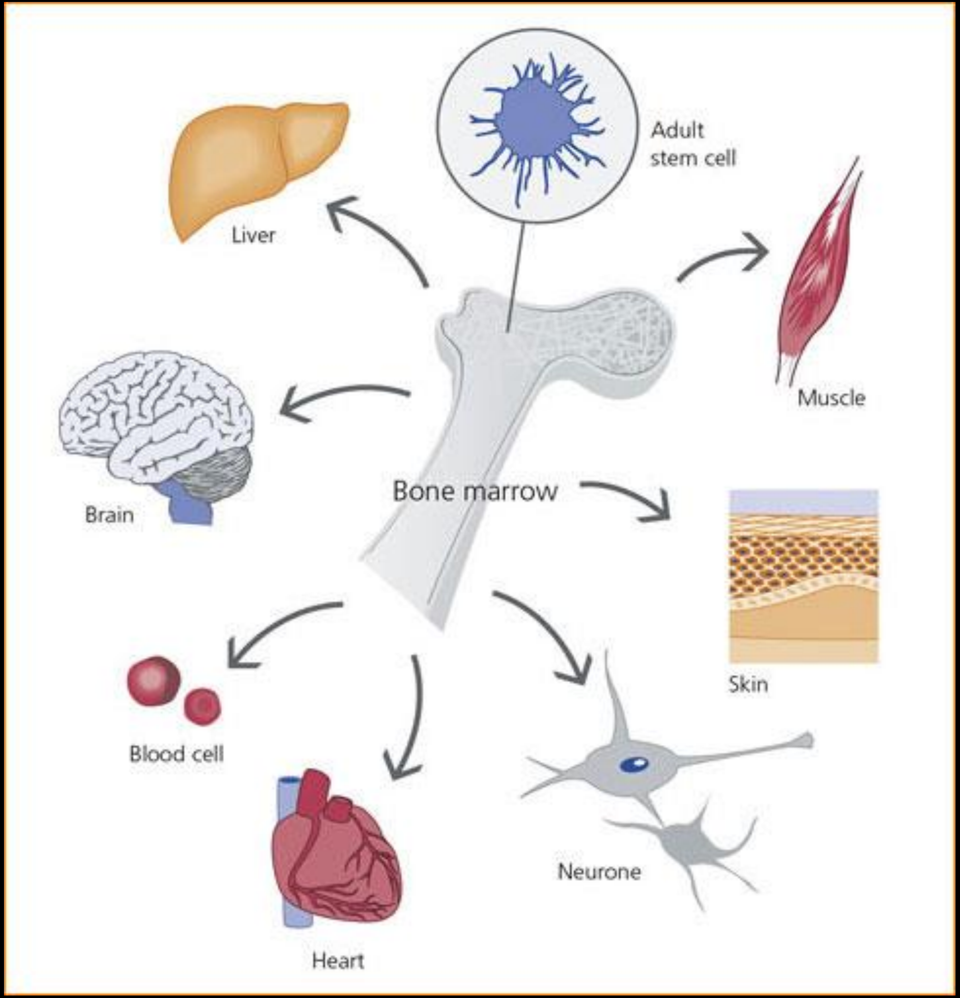
- *Undiferentiated cell*.
- Found in a *diferenciated tissue*.
- Give rise to mature cell types.

Adult Stem Cells
Physiological Sources

- *Blood*
- *Liver*
- *Skeletic muscles*
- *Pancreas*
- *Nervous system*
- *Fat*
- *Umbilical Cord*
- ***Bone Marrow***



Bone Marrow Stem Cells High Plasticity



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Injury Induced Neurogenesis

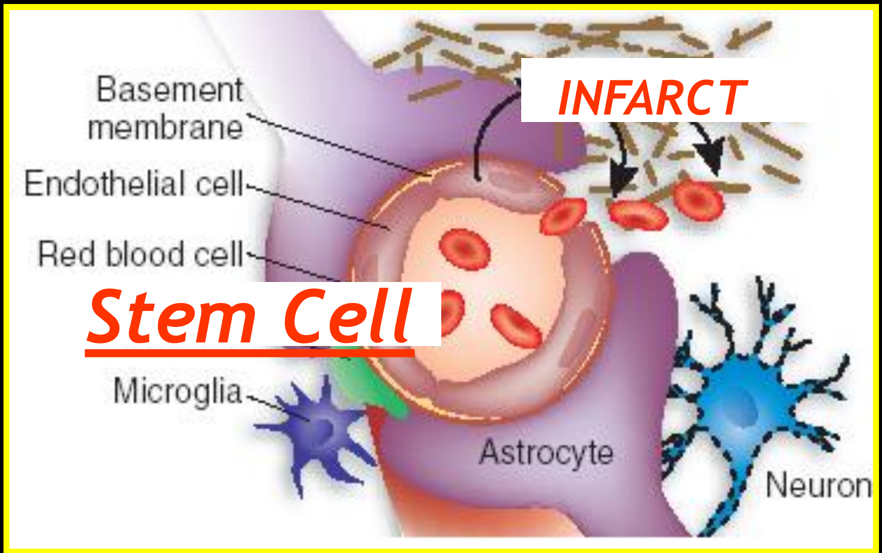
- Parent JM et al; *J Neurosci, 1997.*
- Bengzon J et al; *Proc. Natl. Acad. Sci,1997.*
- Wang Exp; *Neurol, 1998.*
- Liu J et al; *J Neuroscience, 1998.*
- Zhang RL et al; *Neuroscience, 2001.*

Cerebral Ischaemia

- Triggers a strong inflamatory response.
- Molecular Cascade.
- Expression of quimiotactic factors.
- Necrosis - apoptosis.
- Temporary Opening of the BBB.
- Recruitment of endogenous stem cells.

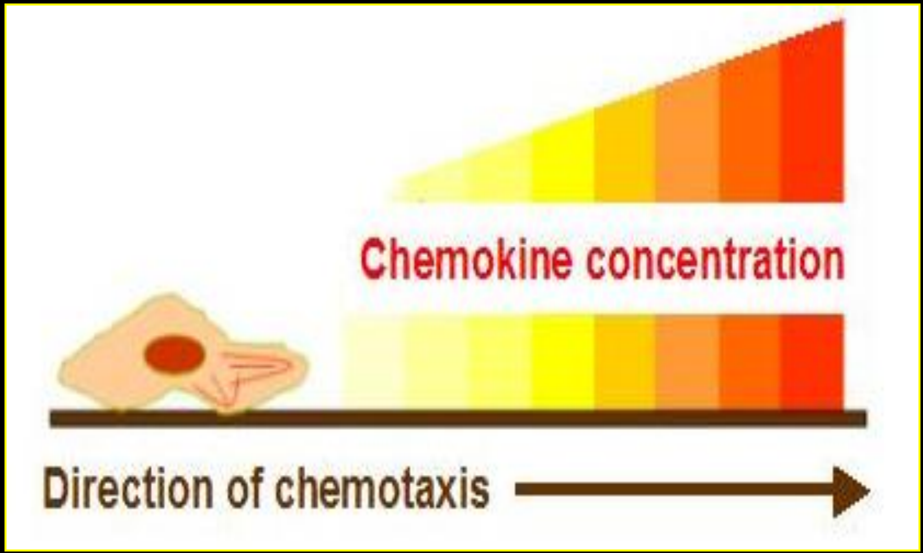
Ischaemia Recruitment of Stem Cells

Intra-vascular Route



Opening of BBB

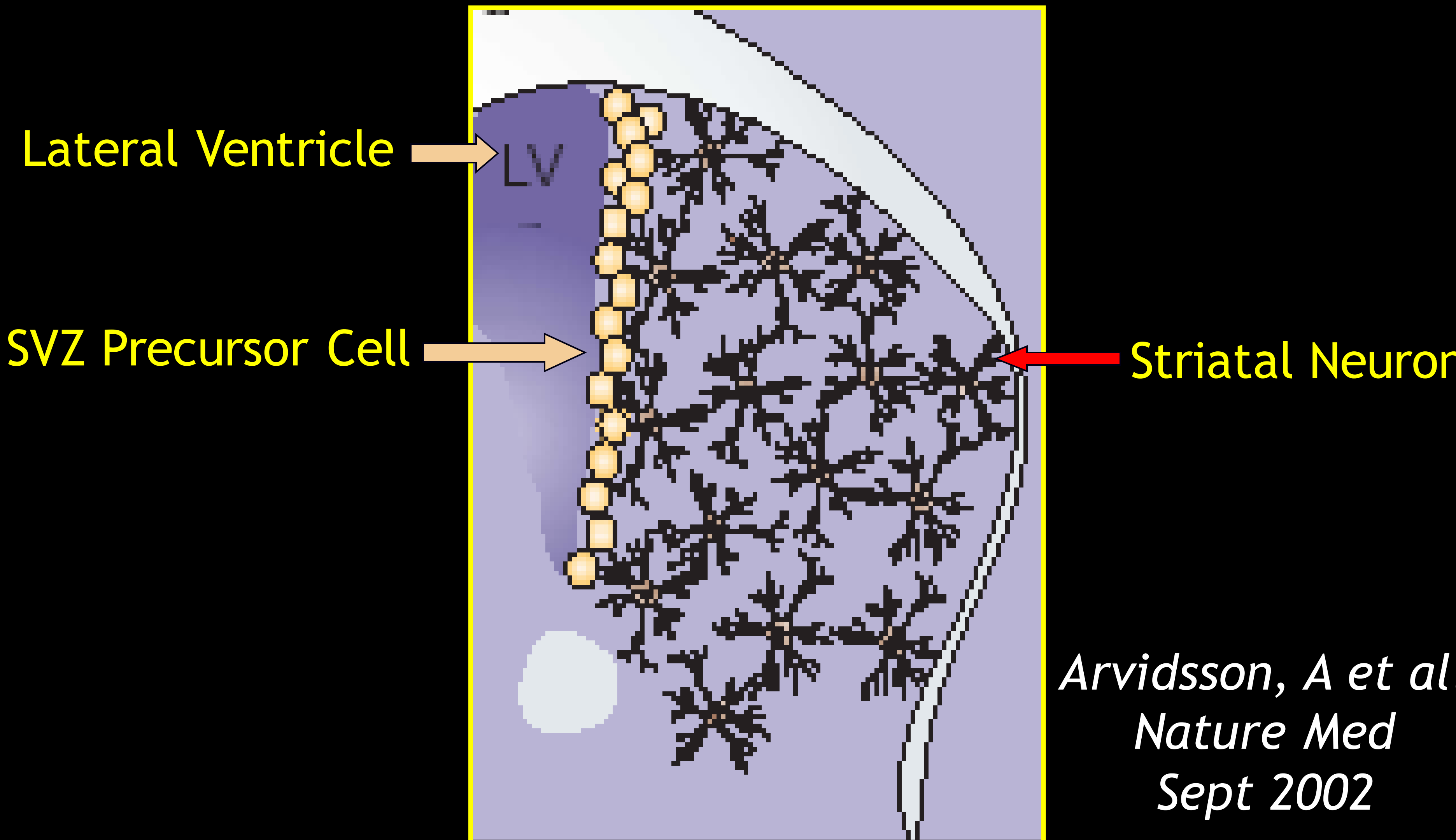
Through the Parenchyma



Injury-induced Citokynes
Metalloproteinases.
Astrocytic Tunnels.

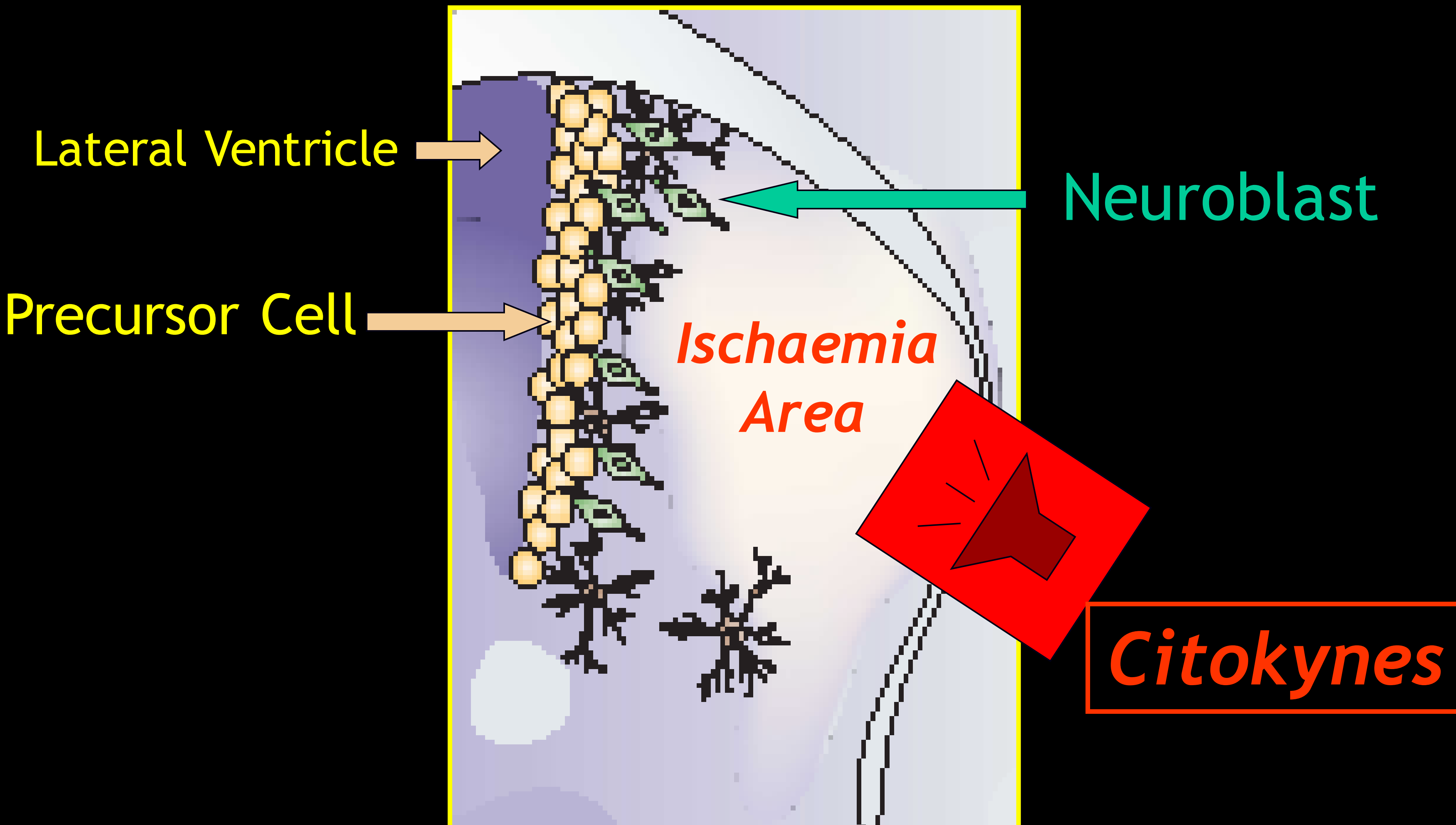
Neurogenesis in Rat Stroke Models

Neuronal Replacement from Endogenous Precursors in the Rat Adult Brain after Stroke.

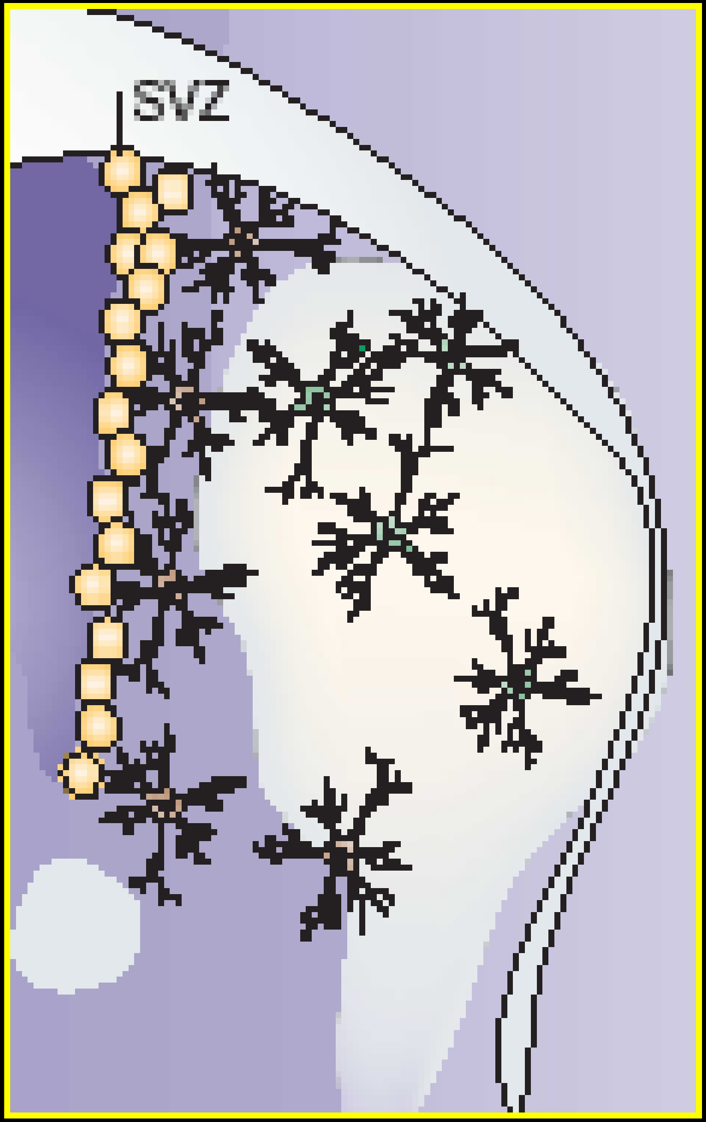


Arvidsson, A et al: Nature Med Sept 2002

Stroke
Triggers Neuroblast Migration

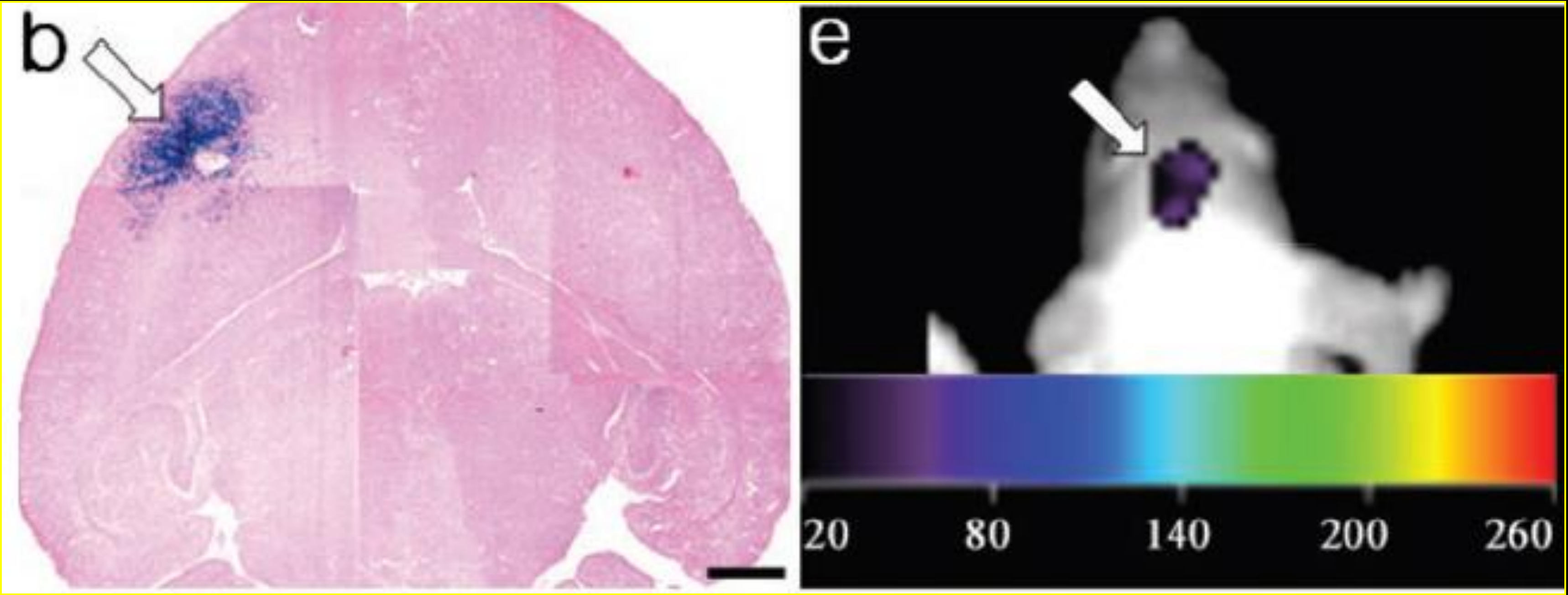


Neuroblasts → Neurons



Fraction of Dead Neurons Replacement: 0,2%

Normal Rats: Intraparenhymal Injection of Cells

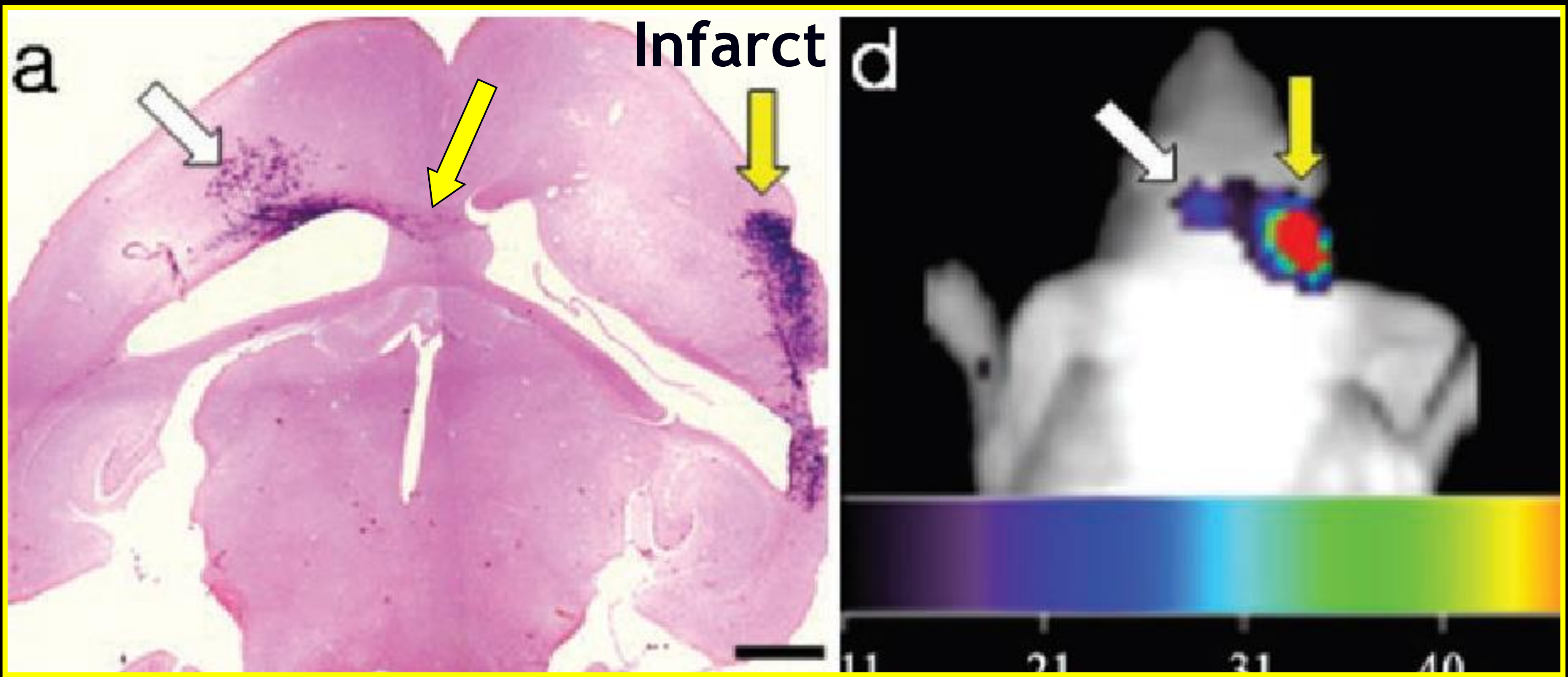


No Cell Migration

Dong-Eog King et al; Stroke 2004;35:952-957

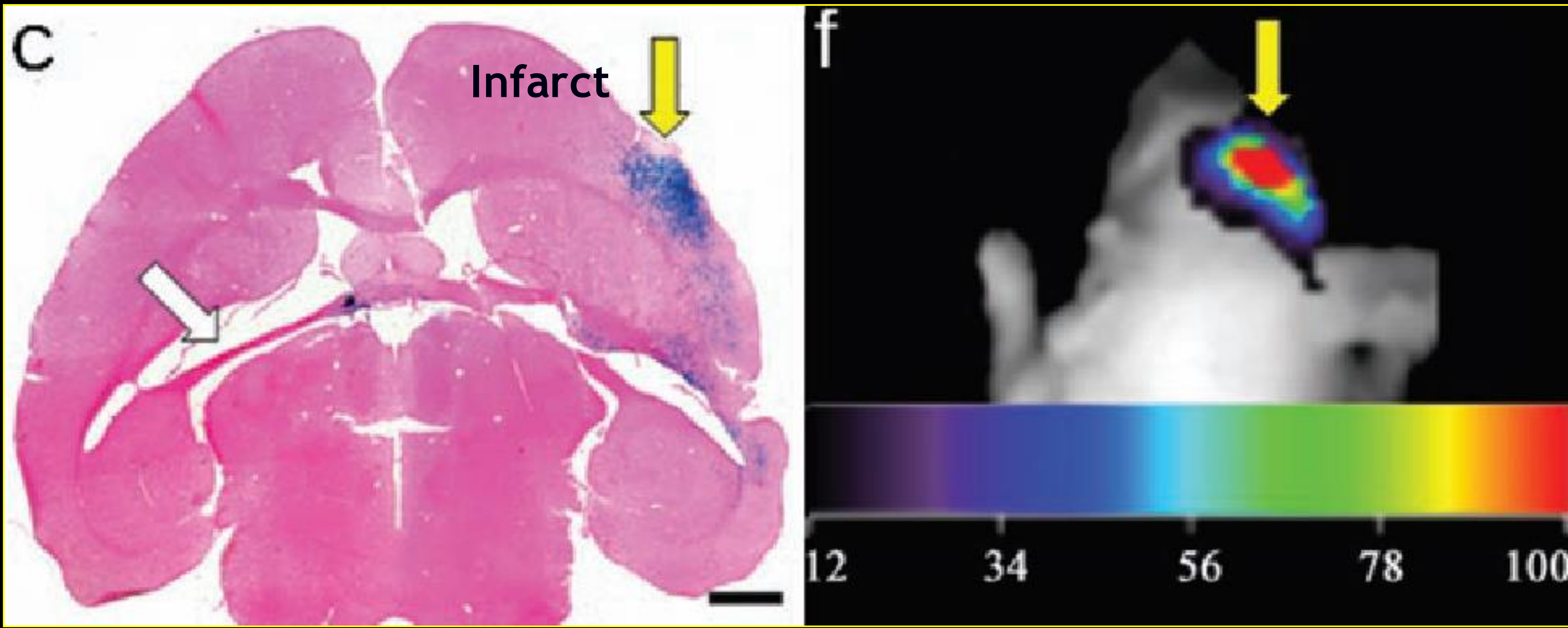
Stroke Rats: Contralateral Cell Injection of Cells

Injection Site



Cell Migration Toward Infarct

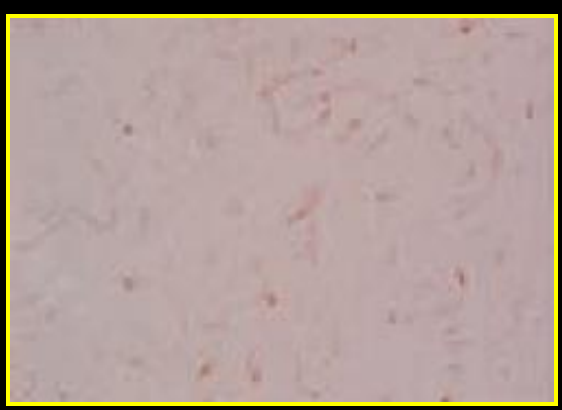
Stroke Rats: Contralateral Intraventricular Injection of Cells

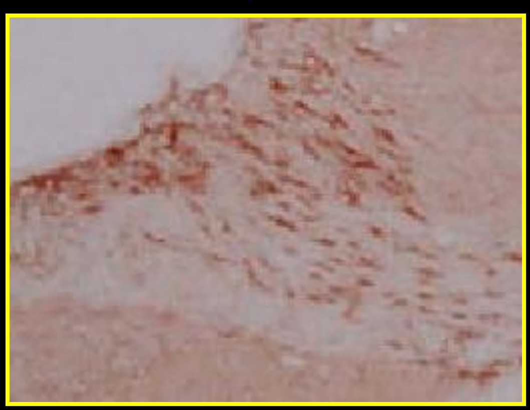


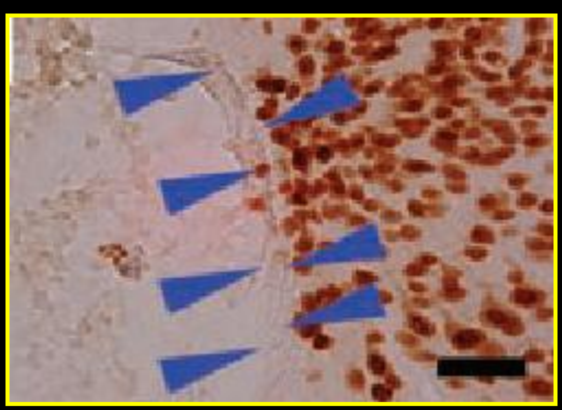
Cell Migration Through CSF Toward Infarct

Histological Analysis CD34+ Rats

Taguchi A et al 2004







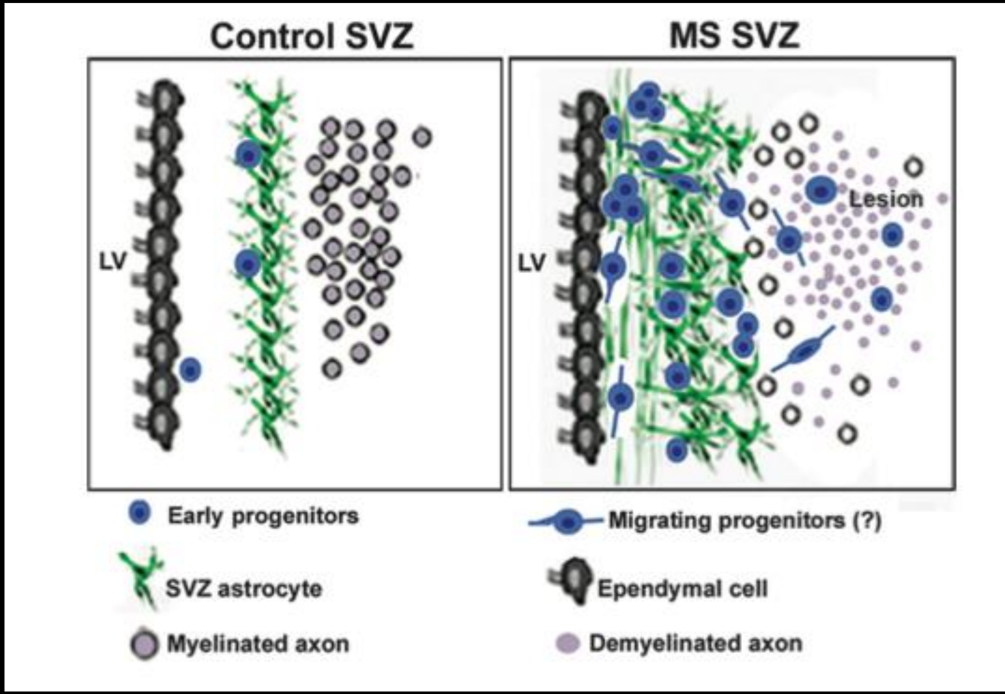
Angiogenesis   Neuroblast Migration   Differenciation

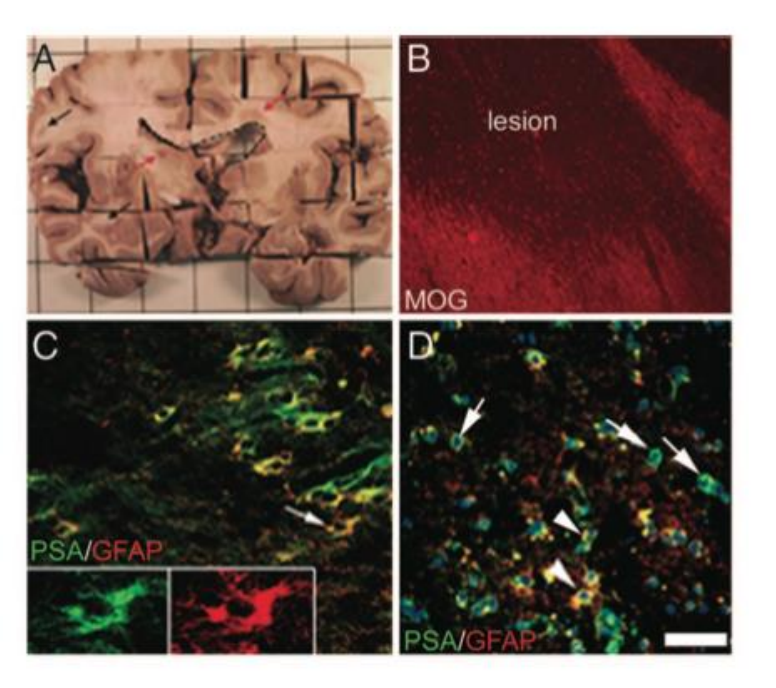
Multiple Sclerosis and Neurogenesis

Activation of the subventricular zone in multiple sclerosis: Evidence for early glial progenitors

Brahim Nait-Oumesmar*†‡, Nathalie Picard-Riera*†, Christophe Kerninon*†‡, Laurence Decker*†, Danielle Seilhean*†‡, Günter U. Höglinger†§, Etienne C. Hirsch†§, Richard Reynolds¶, and Anne Baron-Van Evercooren*†‡||

4694–4699 | PNAS | March 13, 2007 | vol. 104 | no. 11



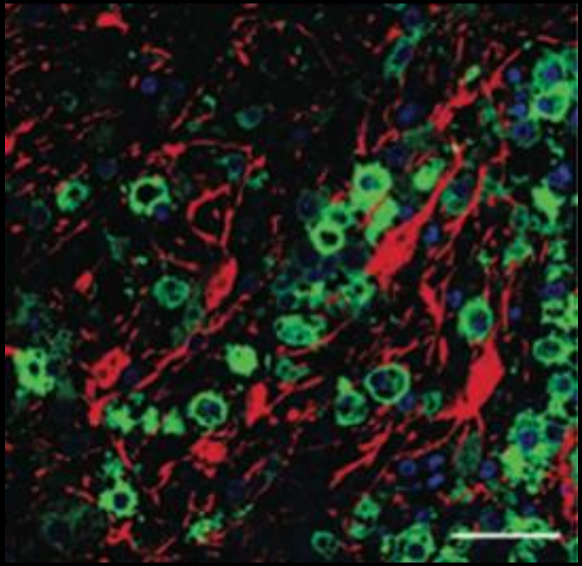


Evidence for stroke-induced neurogenesis in the human brain

Kunlin Jin*†, Xiaomei Wang†‡, Lin Xie*, Xiao Ou Mao*, Wei Zhu‡, Yin Wang§, Jianfeng Shen¶, Ying Mao‡, Surita Banwait*, and David A. Greenberg*||

13198–13202 | PNAS | August 29, 2006 | vol. 103 | no. 35

- Human stroke specimens (n=6)
- Normal brain (n=9)
- Immunohistochemistry



- *Immature Neuronal markers in the Penumbra zone and around blood vessels.*

Immigration Denied

- Non-mammalian vertebrates can regenerate large portions of brain and spinal cord
- Humans have lost this capacity during evolution
- Lost of Rostral Migratory Stream
- Resistance to accept new cells into a mature neuronal network
- Adaptation to keep neuronal population with their accumulated experience

Rakic P, Nature 2004

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Stroke Rats Models for Cell Therapy

Stem Cells and Functional Recovery

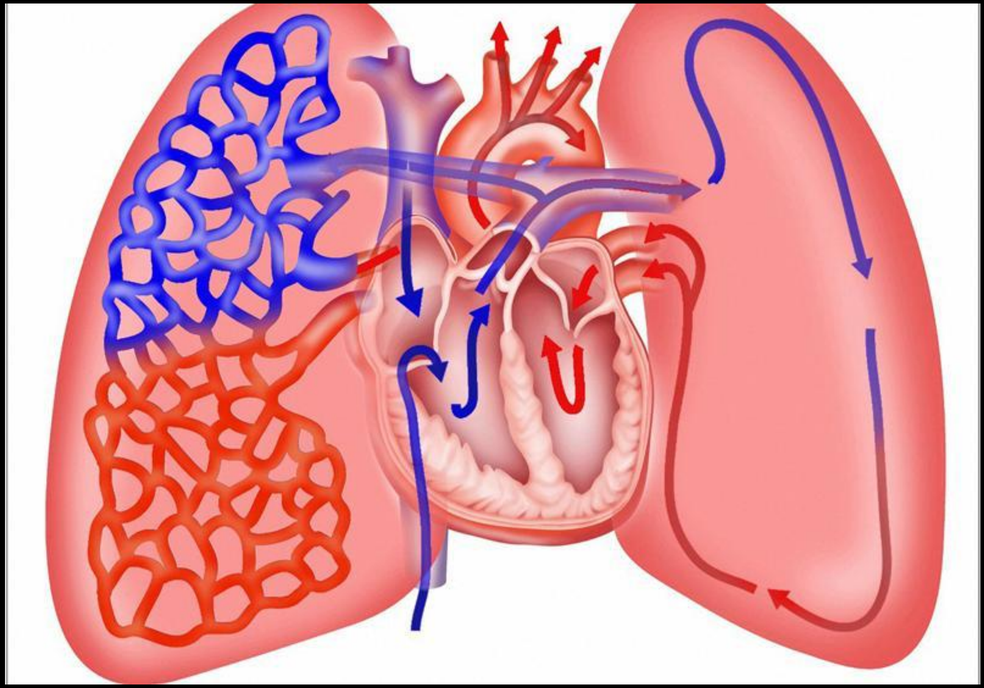
- *Neurophatology, 2003*
- *Cell Transplantation, 2001*
- *J Neurol Sci, 2002*
- *Neurology, 2002*
- *J Cereb Blood Flow Met, 2000*

Author, year	Cell source	Via
Borlongan, 1998	Human embryonal carcinoma-derived neurons (NT2N)	**Intracerebral**
Saporta, 1999	Human neuroteratocarcinoma (hNT)	**Intracerebral**
Chen, 2000	Bone marrow + BDNF	**Intracerebral**
Li, 2000	Bone marrow	**Intracerebral**
Chen, 2001	Bone marrow	**Intravenous**
Chen, 2001	Bone marrow	**Intracerebral**
Chen, 2001	Human umbilical cord	**Intravenous**
Li, 2001	Bone marrow + Z-VAD	**Intracerebral**
Li, 2001	Bone marrow	**Intracerebral**
Li, 2001	Bone marrow	**Intracarotid**
Veizovic, 2001	Immortal neuroepithelial stem cells	**Intracerebral**
Li, 2002	Human bone marrow	**Intravenous**
Modo, 2002	Transgenic murine cell (MHP36)	**Intraventricular x intraparenchymal**
Willing, 2003	Mobilized blood cells	**Intravenous**
Willing, 2003	Cord blood	**Intravenous x intracerebral**
Vendrame, 2004	Human umbilical cord	**Intravenous**

Different Responses Related to the Site of Implant

- <u>Direct puncture</u>: Invasive, poor environement, hemorrhage , epilepsy
- <u>Intravenous</u>: less invasive, , dilution and “Homing”to other tissues.
- Intraarterial

IV injection : Pulmoray Trapping
Lung cappilary network filters majority of cells



Intraarterial Injection

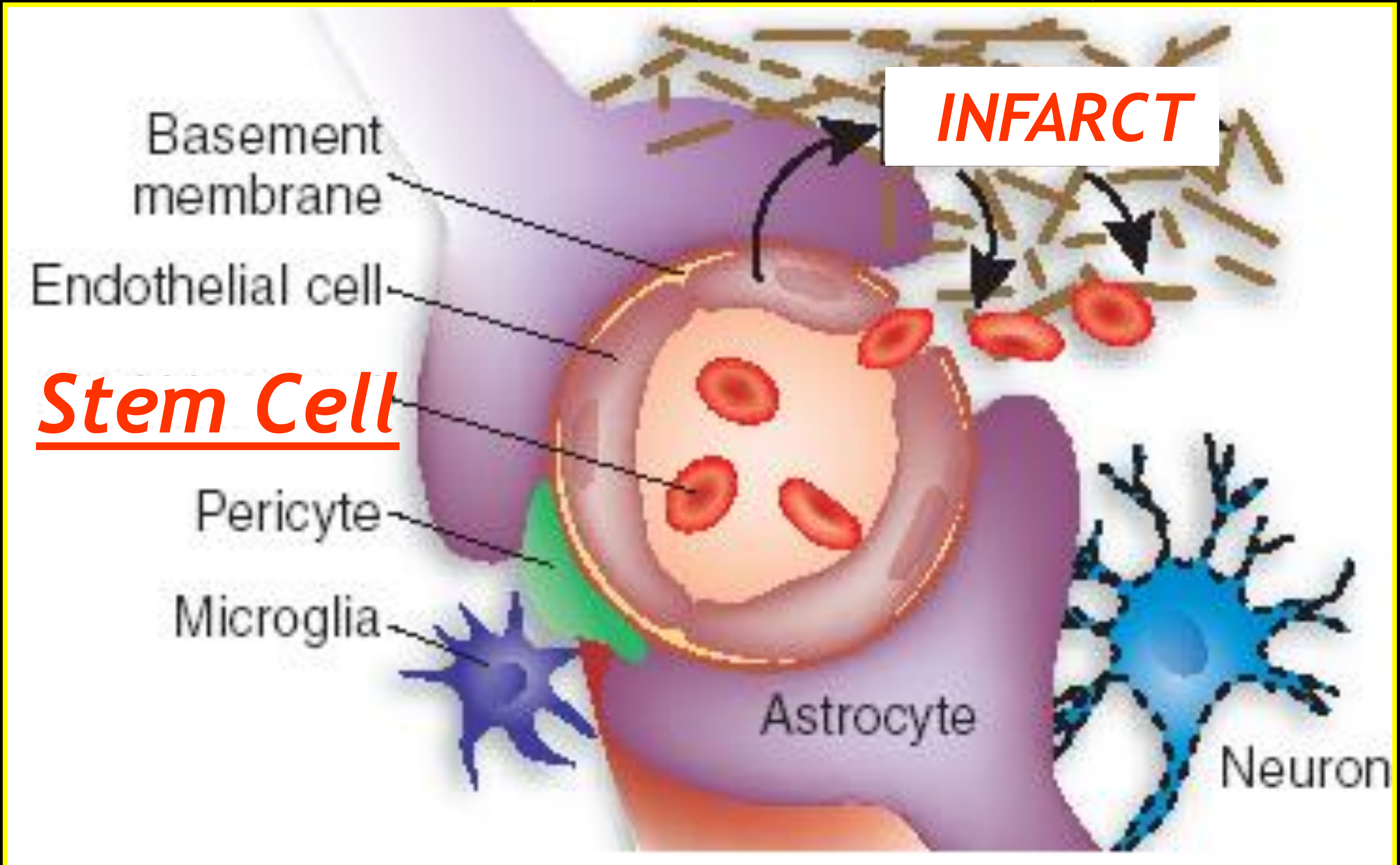
- Mimics physiological mechanisms.
- Larger volumes **(20X)** to target than any other method.
- Less “Homing” .
- Cell distribution to a wide area (Core and Penumbra).
- **Larger proportion of Cells Survival.**

21% IA X 8% IC (*Ly Y,2001*)

- Interventional procedures are safe.

Temporary Opening of BBB

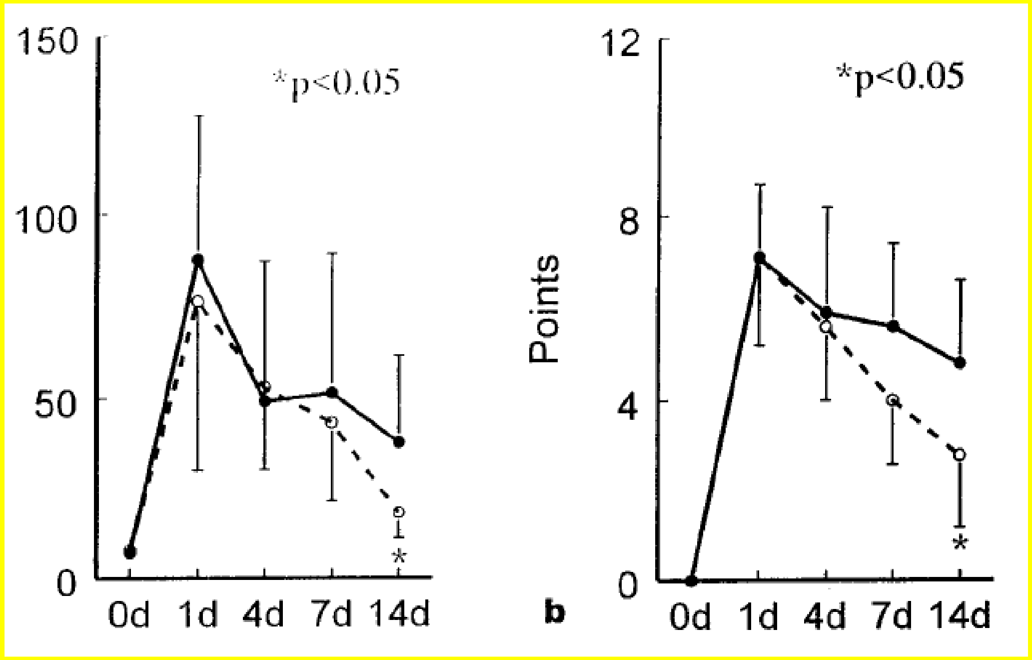
Y Li et al (2002), Shyu et al (2004)



Treatment of stroke in rat with intracarotid administration of marrow stromal cells

Y. Li, MD; J. Chen, MD; L. Wang, MD; M. Lu, PhD; and M. Chopp, PhD

- Xenotransplantation bone marrow stromal cell
- Control group.
- *Improvement in neurological tests*.



p<0,05

Neurology 2001;56:1666-1672

Dual-Modality Monitoring of Targeted Intraarterial Delivery of Mesenchymal Stem Cells After Transient Ischemia

Piotr Walczak, MD; Jian Zhang, MD; Assaf A. Gilad, PhD; Dorota A. Kedziorek, MD; Jesus Ruiz-Cabello, PhD; Randell G. Young, DVM; Mark F. Pittenger, PhD; Peter C.M. van Zijl, PhD; Judy Huang, MD; Jeff W.M. Bulte, PhD

(*Stroke*. 2008;39:1569-1574.)

- 25 stroke rats (MCA)
- Intraarterial X Intravenous injection
- Cells labeled with supermagnetic iron oxid
- *In vivo* imaging with a 4.7 T MRI
- IA injection give better cell grafts.

Shortcommings of Animal Studies

- Represent a minority of human stroke subtypes
  (1/3 of human strokes are lacunes).
- Different anatomy: < white matter
- Young healthy animals under anesthesia and with control of blood pressure, oxigen, glucose, temperature.
- Different neurological scales.

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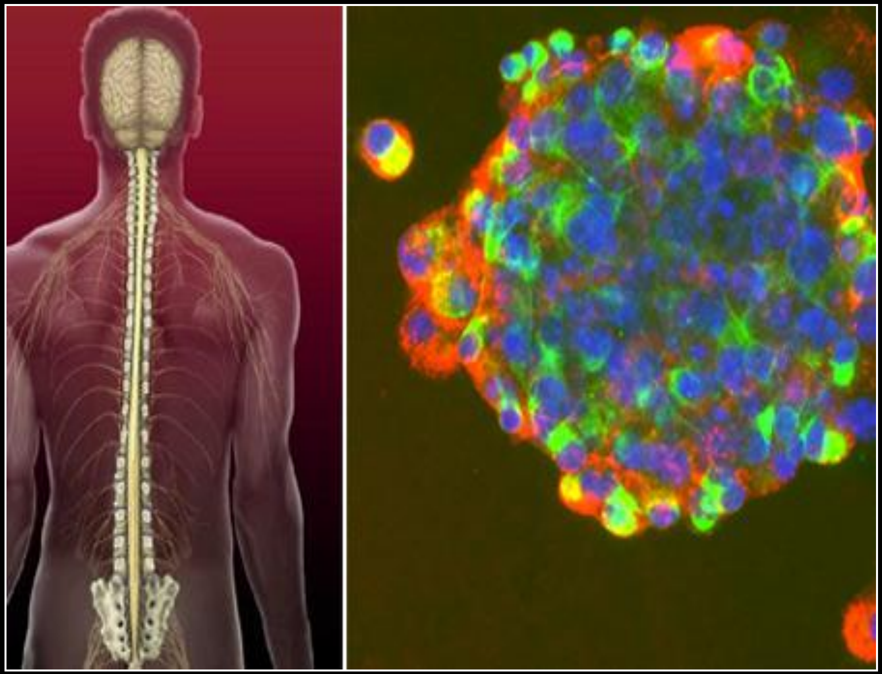
Cell Therapy for Neurological Diseases

- Parkinson's disease
- Huntington's disease
- Alzheimer
- Spinal injury
- Ischaemic stroke
- Hippocampal damage



Kordower 1995, Kondziolka 2000, Nelson 2002

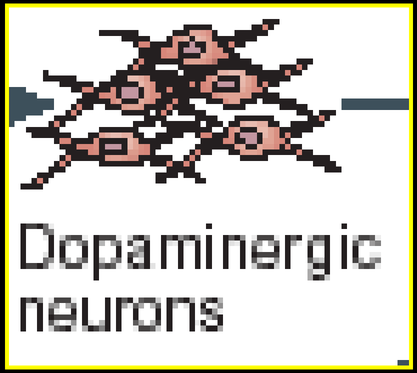
Human Studies

- Kondziolka D et al, *Neurology 2000*.
- Ickenstein GW, *AAN 2004*.
- Bang Oh, *Annals of Neurol 2005*.
- Kondziolka D et al, *J Neurosurgery 2005*.
- Shyu WC et al; *CMAJ 2006*.
- *Stems Pilot Study* (UK) Sprigg et al 2006.
- AXIS – AX 200 (G-CSF) Germany.

Neurotransplantation Differences

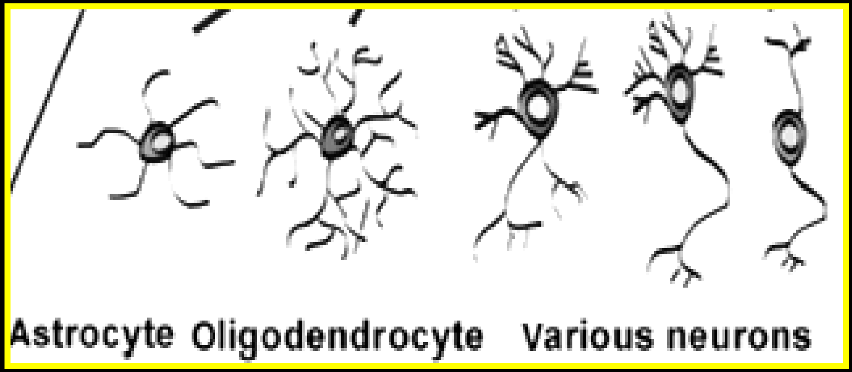
Degenerative

- Slow evolution
- Homogenous phenotype
- *Parkinson*



Ischaemia

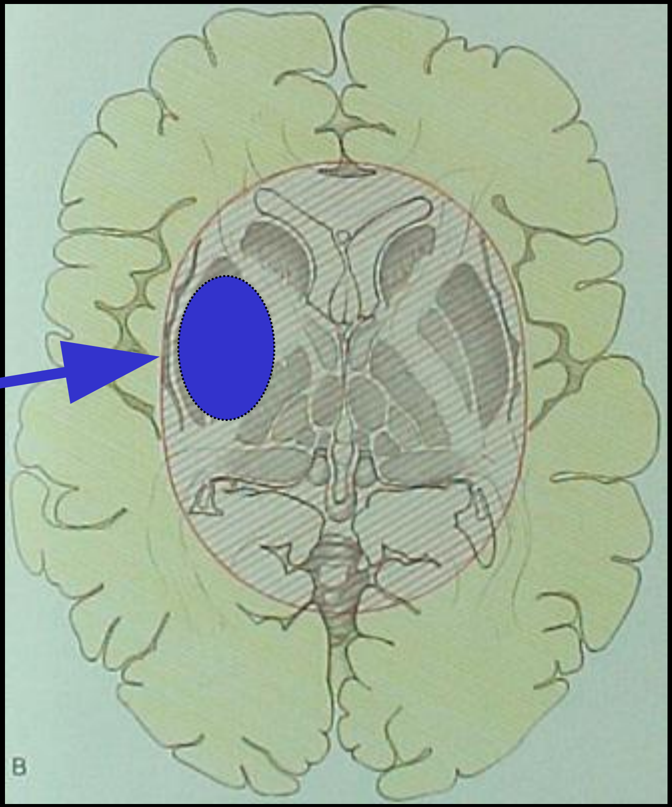
- Sudden
- Heterogenous phenotype
- Gray/White Matter
- *Stroke*



Kondziolka D et al . Transplantation of cultured human neuronal cells for patients with stroke.

Neurology 2000 ; 55:565-569

- 1st clinical study in humans with Stroke.
- 12 patients (aged 44 to 75 years old)
- Old infarcts (> 6 months).
- Basal ganglia
- Stereotactic neuron implants.
- Cells from a Teratocarcinoma.
- *Functional improvement (NIH e ESC).*



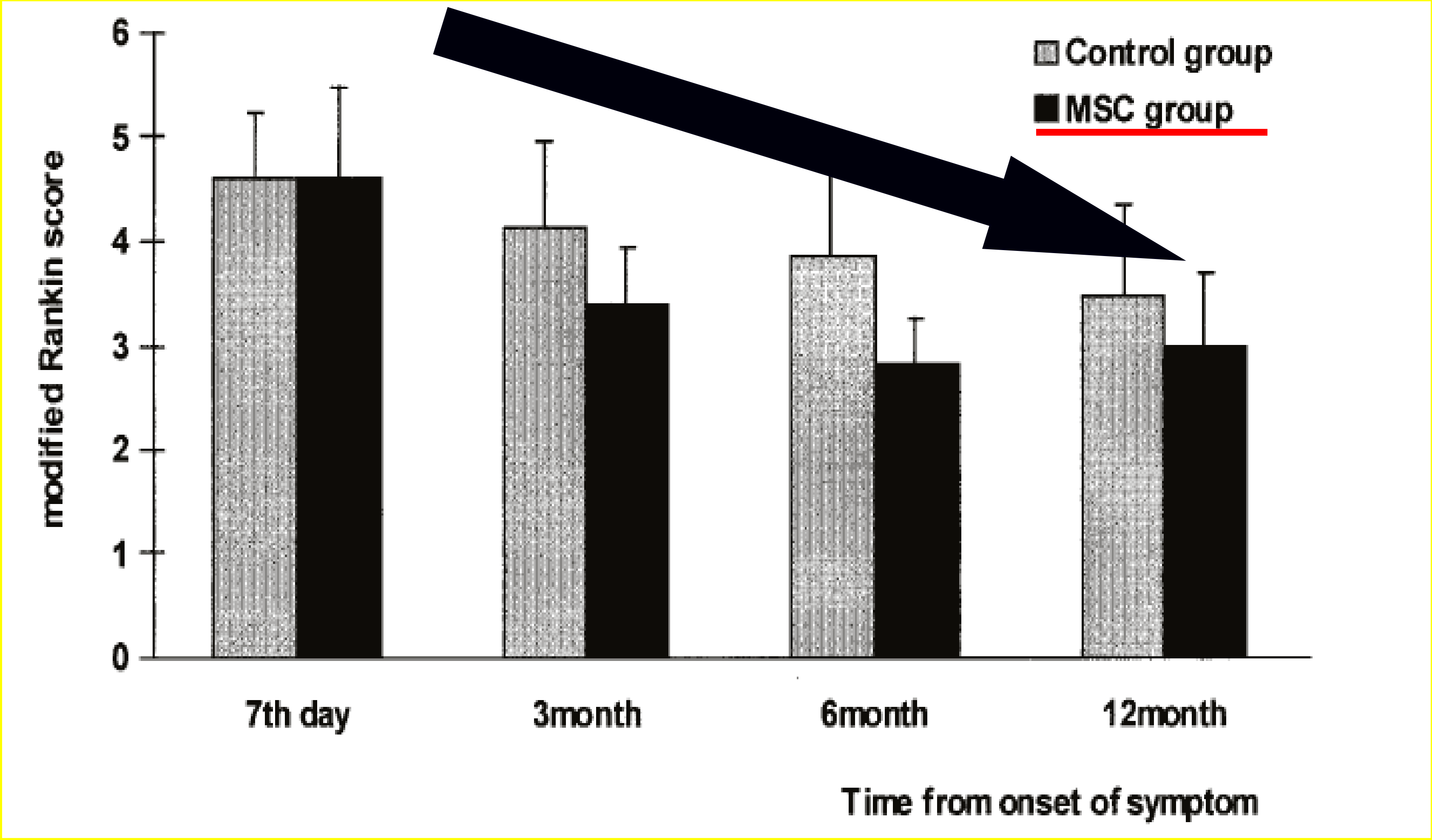
Autologous Mesenchymal Stem Cell Transplantation in Stroke Patients

Bang Oh, Lee JS, Lee PH and Lee G.

Annals of Neurol 2005;57:874

- Severe MCA infarctions, 5 treated, 25 control.
- Intravenous injection.
- Autologous MSC (1×10^8).
- Clinical and radiological follow-up of 1 year.

mRankin After Cell Therapy



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Intracarotid delivery of drugs

- Used for more than 50 years
- 1960 - Locate brain functions – WADA
- 1980 - Tumor chemotherapy
- BBB disruption
- Treatment of vasoespasm
- IA Thrombolysis - Stroke
- 2000 - Cell therapy

AJNR sept 2010

The Effect of Transcatheter Injections on Cell Viability and Cytokine Release of Mononuclear Cells

BACKGROUND AND PURPOSE: Several studies suggest that various types of cellular therapies enhance recovery after stroke in animal models. IA-based delivery of cells to the brain is under investigation for stroke, but it is unknown whether cells are injured as a result of being injected through a catheter or exposed to iodinated contrast medium or solutions containing heparin.

MATERIALS AND METHODS: We assessed the effect of catheterization with the Excelsior SL-10 catheter or exposure to heparin or iodine contrast on human bone marrow MNCs. Viability and cell injury were assessed by trypan blue exclusion, caspase-3 activity, and lipid peroxidation. Cellular function of MNCs was assessed by their production and release of VEGF, IL-10, and IGF-1.

RESULTS: Flow rates of 10 million cells from 0.5 to 2 mL/min did not alter MNC viability; however, 5 mL/min of MNCs did reduce viability by 19%. Iodine and low-dose heparin exposure did not affect cell viability; however, high-dose heparin was cytotoxic. Catheter delivery at 2 mL/min did not affect levels of VEGF, IL-10, or IGF-1.

CONCLUSIONS: MNCs do not appear to be damaged by heparin, iodine contrast, and the Excelsior SL-10 catheter at flow rates up to 2 mL/min. However, higher flow rates did reduce viability, and high-dose heparin did cause cell death.

Microcathter injection, low-dose heparin and iodine contrast do not affect cell viability up to 2 ml/min injection

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Treatment of Ischaemic Stroke by Super-selective Intra-Arterial Injection of *Stem Cells*

PHASE I Study

Raupp E, MD, PhD.

Friedrich M, MD, PhD.

Costa J, MD, PhD.

Goricochea B, MD, PhD.

Machado D, MD, PhD.

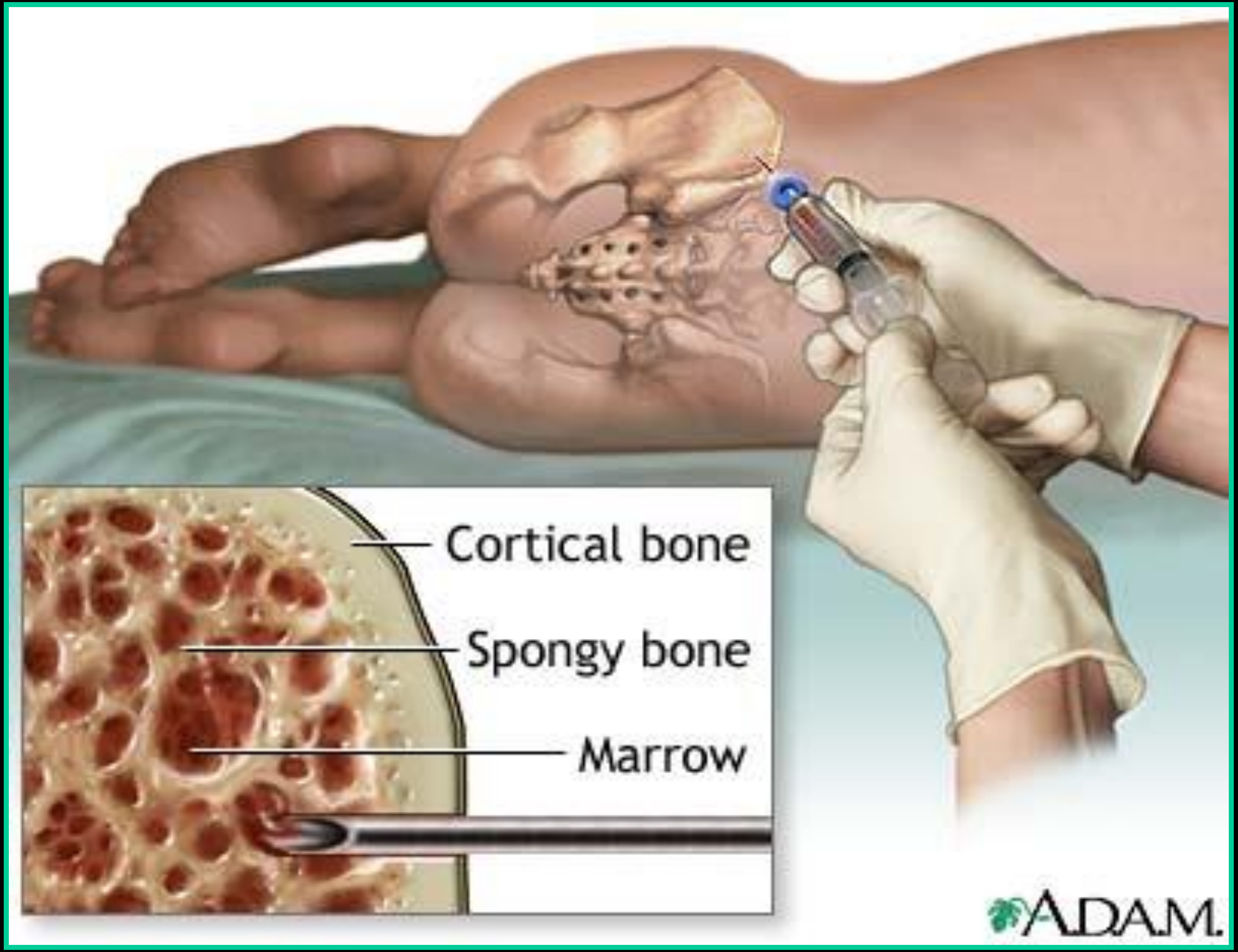
PUCRS BRASIL

Injection of Stem Cells in Large MCA Infarcts

- ✓ Phase I
- Open label
- Not randomized
- 20 patients
- No controls

- Large ischaemic stroke ≥ 1/3 MCA territory.
- Clinical and neurological stable.
- Between 3rd and 9th day from clinical onset.
- Stem Cells source: Autologous Bone Marrow.
- Middle Cerebral Artery injection.
- Follow-up: 6 months (NIH, mRankim,fMR)

PUNCTION of ILIAC BONE
≅ 50 ml



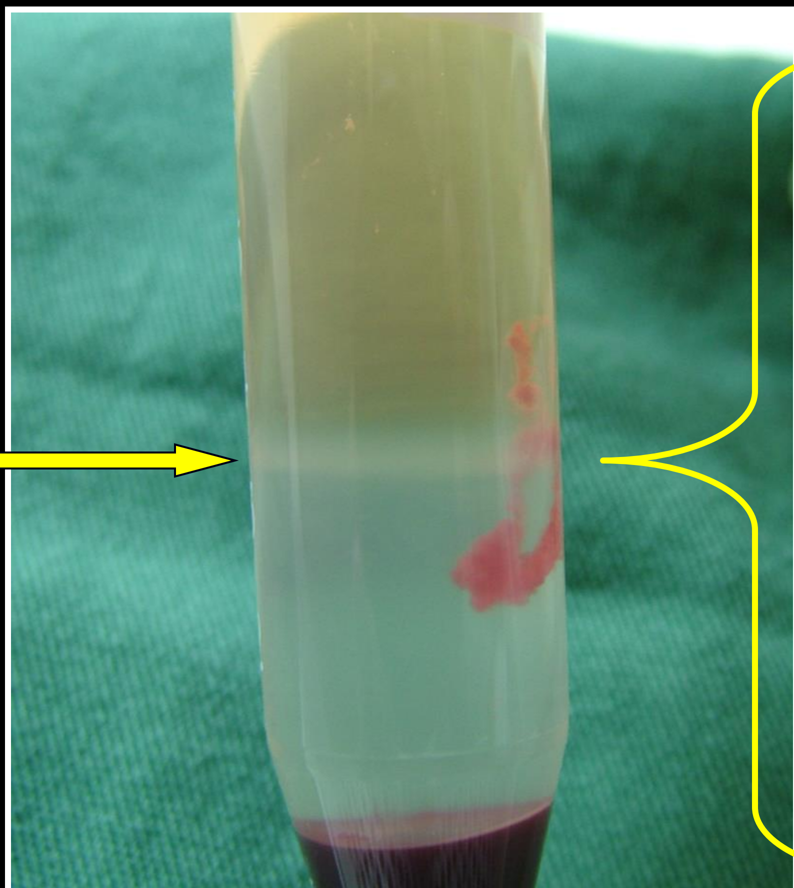

20
15
10
5

Separation of Stem Cell Fraction

- Density gradient
- Ficoll-Paque
- Minimum 4 X 10^7 MNC

Stem Cell Fraction

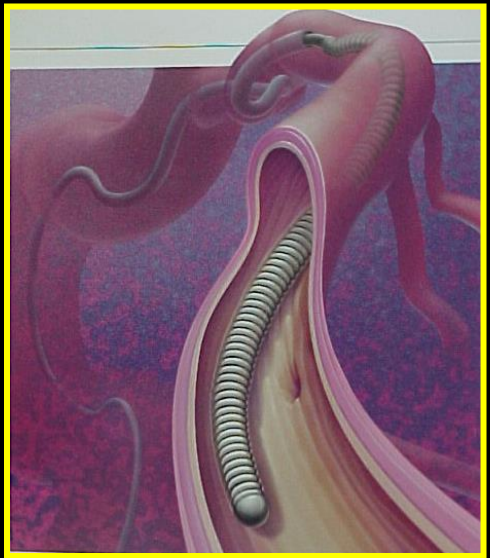
(Minimum 4X10.000.000 Mononuclear Cells)

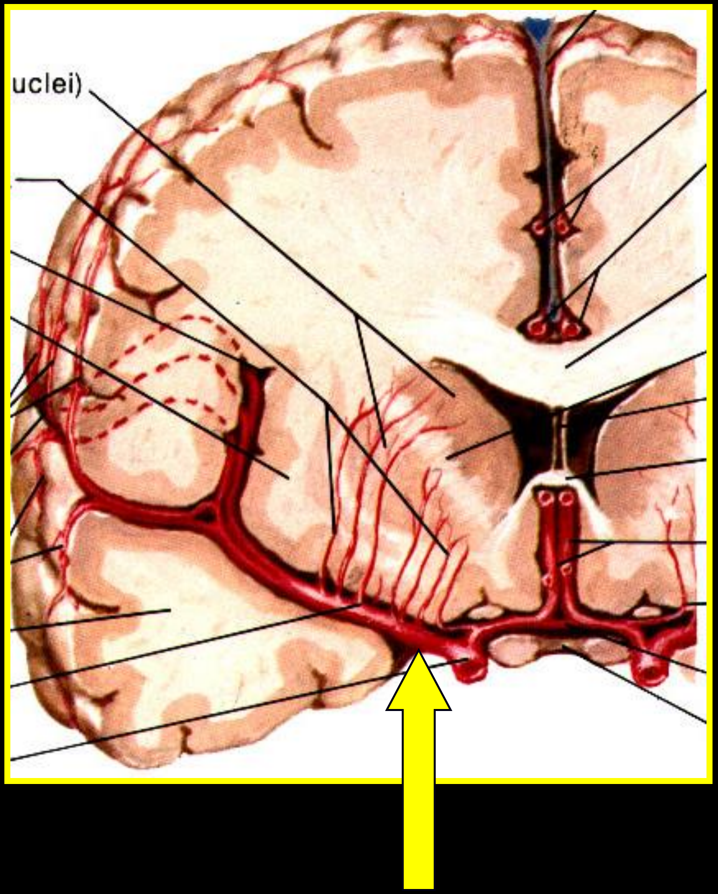


- Hematopoietic Stem cells $CD34^+$
- Mesenchymal cells $CD34^+, 117^-$
- Immature lymphocytes
- Monoblasts
- Monocytes
- *< 1% Uncommitted Stem Cells*

Intra-arterial Transplantation of Stem Cells

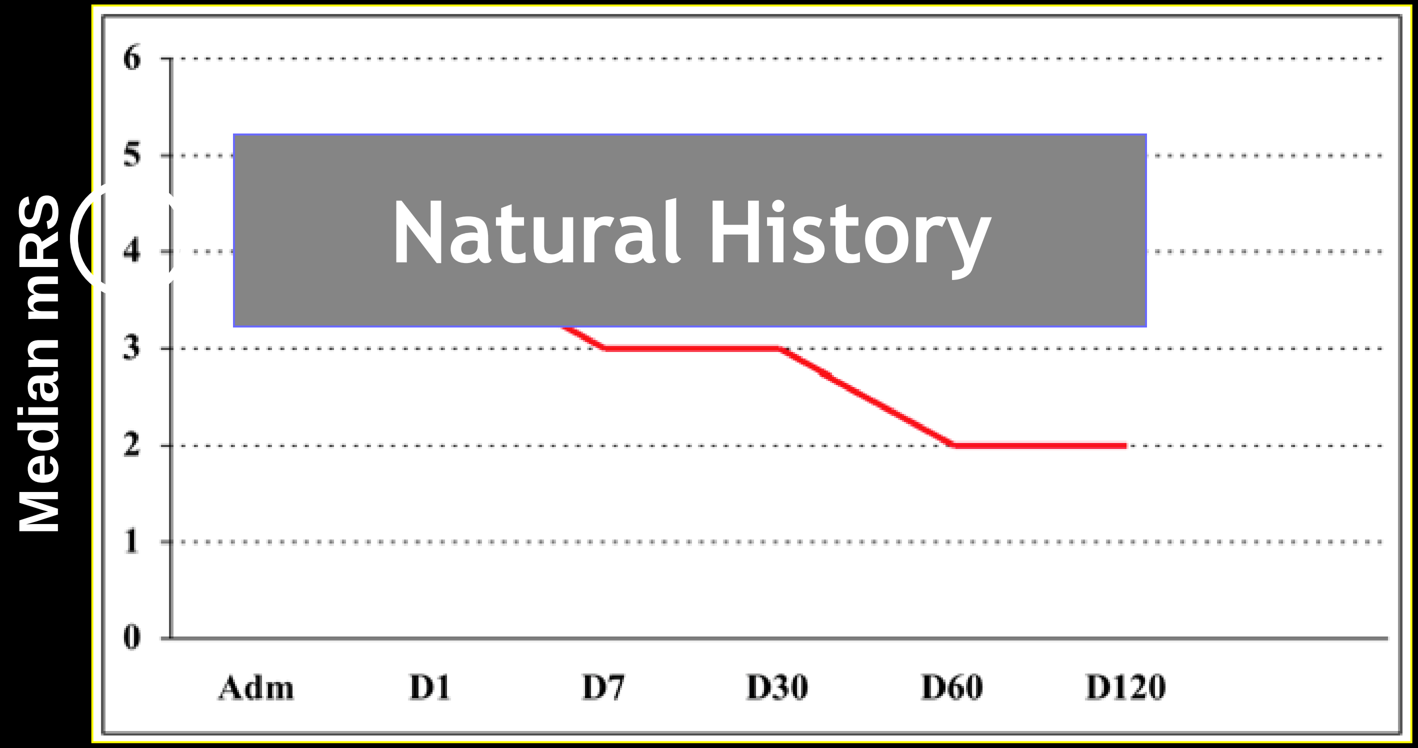
- Commom Carotid Angiography.
- Catheterization of <u>M1 segment</u>.
- Slow injection: 15ml ≅ 15 minutes.
- No post angiography.



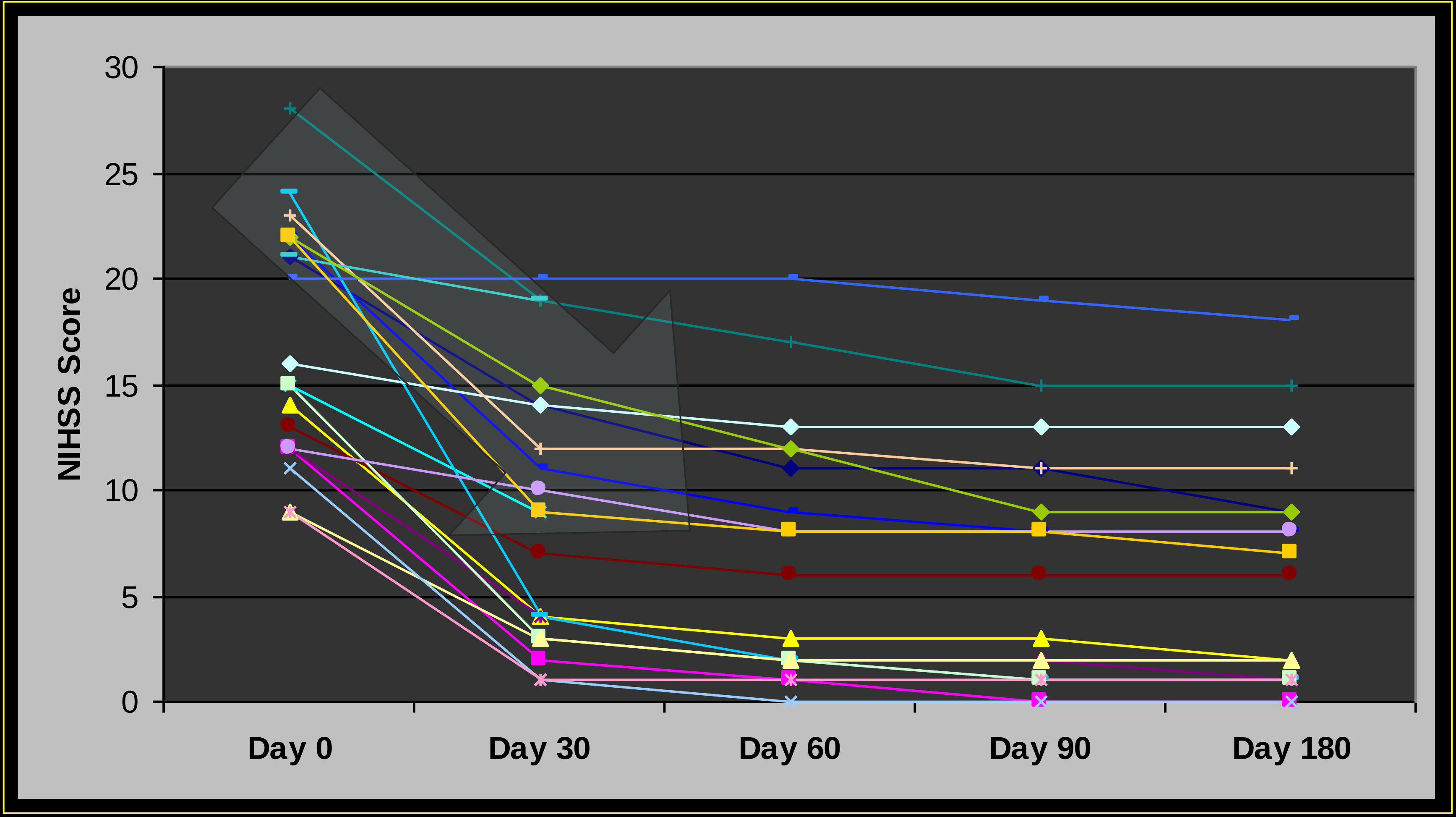


MCA Injection of 60.000.000 Mononuclear Cells

Modified Ramkin Scale
20 patients



NIHSS 6 Months



Cell Transplantation, Vol. 21, Supplement 1, pp. S13–S21, 2012

0963-6897/12 $90.00 + .00
DOI: http://dx.doi.org/10.3727/096368912X612512
E-ISSN 1555-3892
www.cognizantcommunication.com

Intra-Arterial Infusion of Autologous Bone Marrow Mononuclear Cells in Patients With Moderate to Severe Middle Cerebral Artery Acute Ischemic Stroke

Maurício A. G. Friedrich,*† Maurer P. Martins,*† Mariana D. Araújo,* Charles Klamt,*† Leonardo Vedolin,* Bernardo Garicochea,* Eduardo F. Raupp,* Jeber Sartori El Ammar,* Denise Cantarelli Machado,* Jaderson C. da Costa,* Raul G. Nogueira,‡ Paulo Henrique Rosado-de-Castro,§ Rosalia Mendez-Otero,¶ and Gabriel R. de Freitas¶

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¶Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Intra-Arterial Bone Marrow Mononuclear Cells in Ischemic Stroke

A Pilot Clinical Trial

Francisco Moniche, MD; Alejandro Gonzalez, PhD; Jose-Ramon Gonzalez-Marcos, PhD; Magdalena Carmona, PhD; Pilar Piñero, PhD; Ildefonso Espigado, MD; David Garcia-Solis, PhD; Aurelio Cayuela, PhD; Joan Montaner, PhD; Cristina Boada, PhD; Anna Rosell, PhD; Maria-Dolores Jimenez, PhD; Antonio Mayol, MD; Alberto Gil-Peralta, PhD

Cell Transplantation, Vol. 23, Supplement 1, pp. S57–S64, 2014
Printed in the USA.

0963-6897/14 $90.00 + .00
DOI: http://dx.doi.org/10.3727/096368914X684934
E-ISSN 1555-3892
www.cognizantcommunication.com

Intra-Arterial Bone Marrow Mononuclear Cell Transplantation Correlates With GM-CSF, PDGF-BB, and MMP-2 Serum Levels in Stroke Patients: Results From a Clinical Trial

Francisco Moniche,* Joan Montaner,†‡ Jose-Ramon Gonzalez-Marcos,* Magdalena Carmona,§ Pilar Piñero,¶ Ildefonso Espigado,§ Aurelio Cayuela,# Irene Escudero,* Francisco-Javier de la Torre-Laviana,* Cristina Boada,‡ Anna Rosell,‡ Antonio Mayol,¶ Maria-Dolores Jimenez,* Alberto Gil-Peralta,* and Alejandro Gonzalez¶

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#Clinical Research Services, Hospital Universitario Virgen del Rocío, Seville, Spain

Neurological Improvement Mechanisms

- Proven but poorly understood.
- Interruption of apoptotic sequences.
- Protection to Penunbra.
- Immunomodulation.
- Release of trophic factors – Angiogenesis.
- Increase of sinaptic activity.
- Support of axonal regeneration.
- Increases cerebral plasticity.
- *Celular replacement ??(Unlikely in Acute Phase).*

Angiographic Demonstration of Neoangiogenesis After Intra-arterial Infusion of Autologous Bone Marrow Mononuclear Cells in Diabetic Patients With Critical Limb Ischemia

Rafael Ruiz-Salmeron,* Antonio de la Cuesta-Diaz,* Manuel Constantino-Bermejo,* Immaculada Pérez-Camacho,* Francisco Marcos-Sánchez,* Abdelkrim Hmadcha,†‡ and Bernat Soria†‡

*Hospitales Universitarios San Lázaro and Virgen Macarena, Sevilla, Spain
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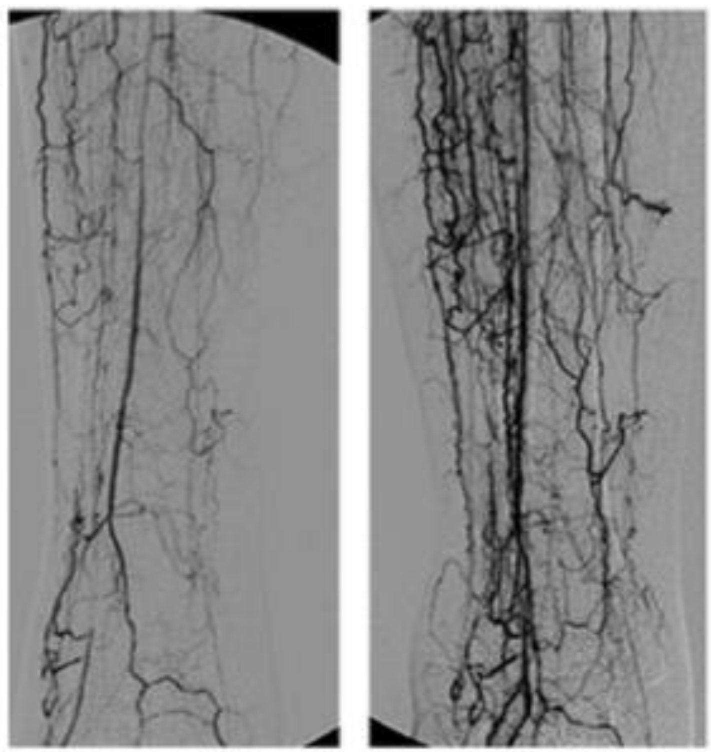
‡CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain



Baseline

3 months follow-up (early frames)

On Going Trials of Autologous Stem Cell Therapy for Stroke

Over 40 Trials currently

Spain - HU Asturias, MCA stroke, intraarterial , $CD34^+$ cells

France - UH Grenoble, IV injection, carotid territ. stroke

UK - Imperial College London, intraarterial $CD34^+$ cells, MCA stroke

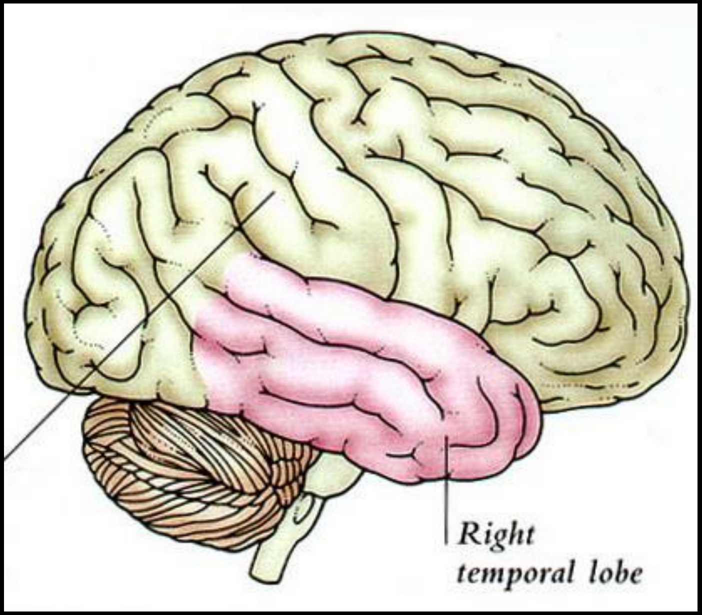
China - UH China, MCA stroke, direct puncture, $CD34^+$ cells

US - UTx (Houston), MCA stroke, IV injection

Outline

- Basic concept of Stem Cells
- Normal adult neurogenesis
- Injury induced neurogenesis
- Cell therapy for neurological diseases
- Intraarterial injection of drugs and cells
- Animal data
- Human studies
- *Stroke Trial*
- ***Epilepsy Trial***
- Final remarks

Temporal Lobe Epilepsy (TLE)

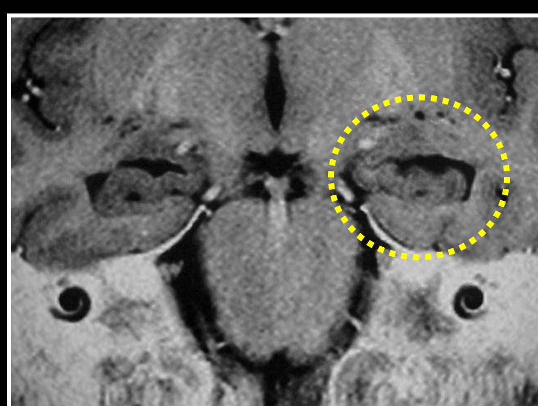


Temporal Lobe Epilepsy (TLE)

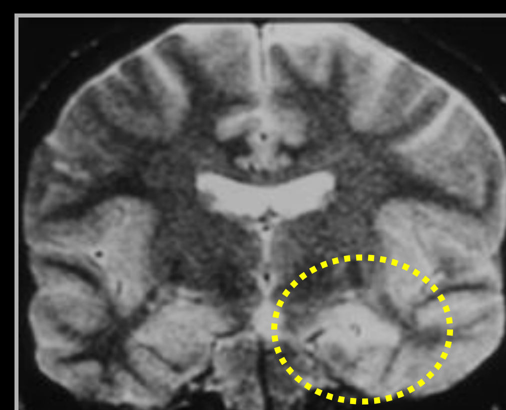
- 40% of patients with epilepsy.
- Most commom and intractable form of epilepsy.
- Learning and memory impairments.
- 30% have chronic seizures (Partial seizures).
- 40% are resistant to medication.

TLE - Diagnosis

- History of recurrent temporo-limbic seizures.
- Problems of verbal or non-verbal memory.
- Interictal EEG with 70% or more *discharges in one temporal lobe and/or slow temporal acticity*
- <u>Neuroimaging typical findings</u>:



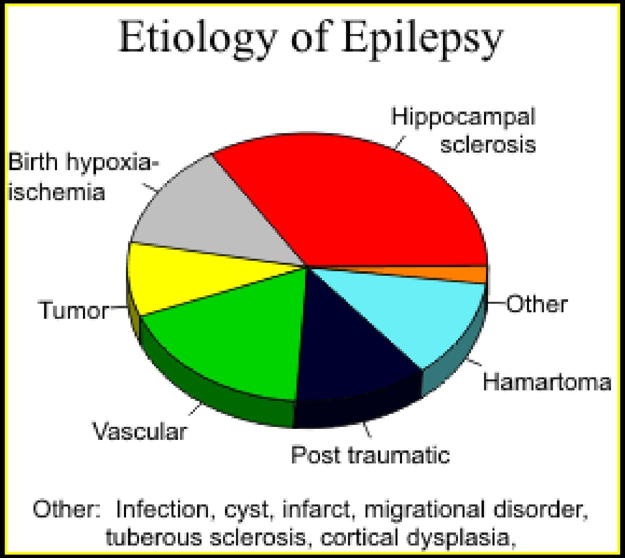
Atrophy

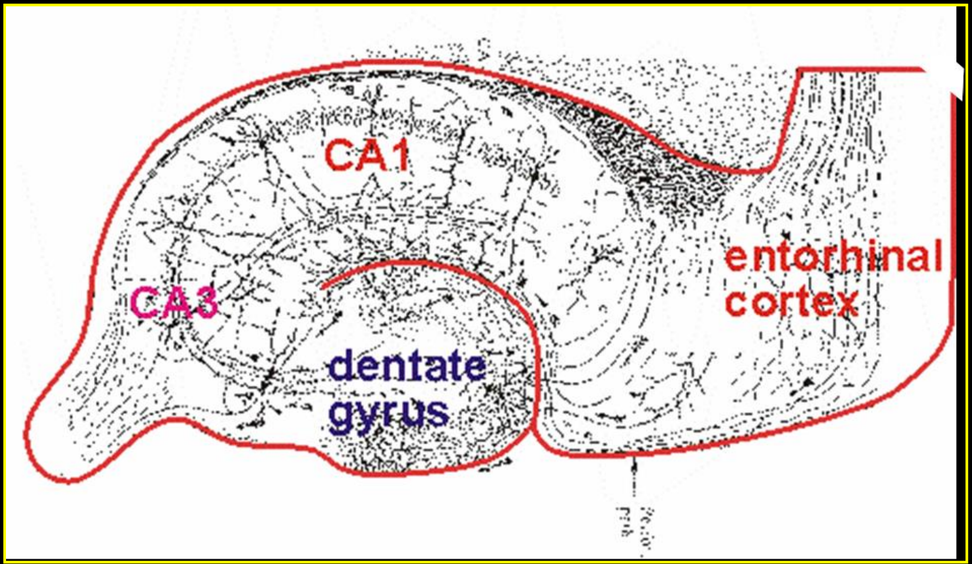


–T2 signal

TLE - Hippocampal Sclerosis Pathology

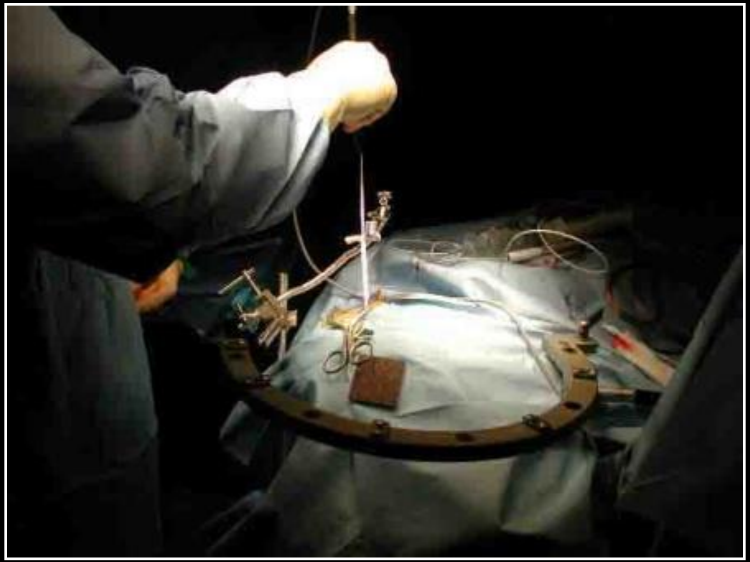
- Hippocampal neuronal loss and gliosis(CA1).
- *Decline dentate neurogenesis.*





TLE - Types of Treatment

Politeraphy



Surgery

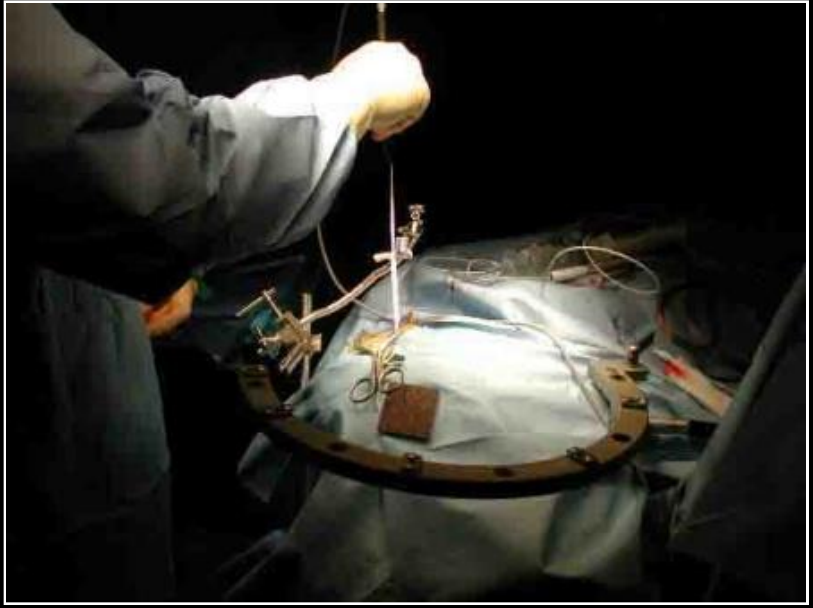
Politherapy

- 40% do not respond.
- Does not change the course of disease.
- ↓ Memory/cognition.
- Low quality of life.
- Risk of sudden death.

  (3X normal pop)

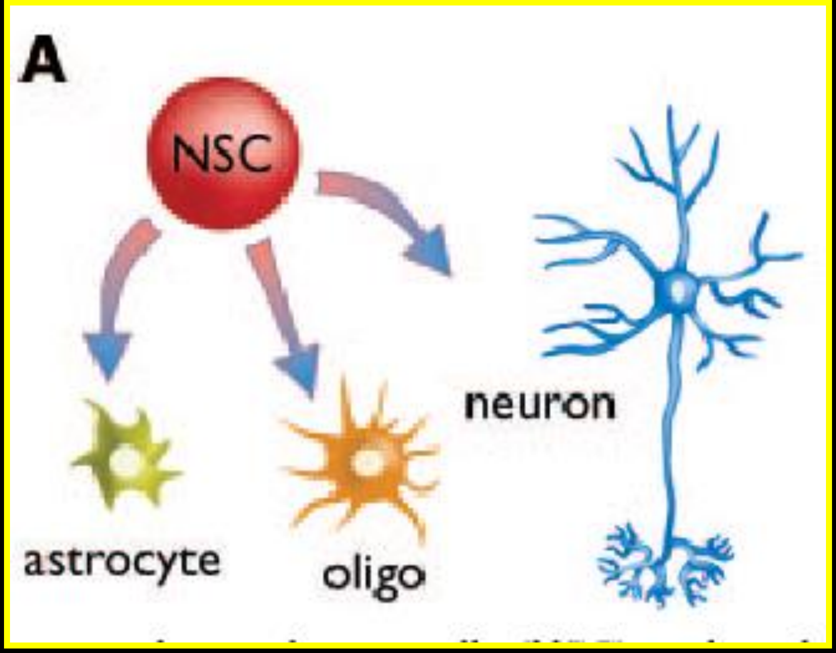
Amigdalo-hippocampectomy

- Pat. with no response to medication.
- 0-3% mortality , 0 – 25% morbidity.
- 20% – 30% keep medication after surgery.
- Does not improve memory and cognition.



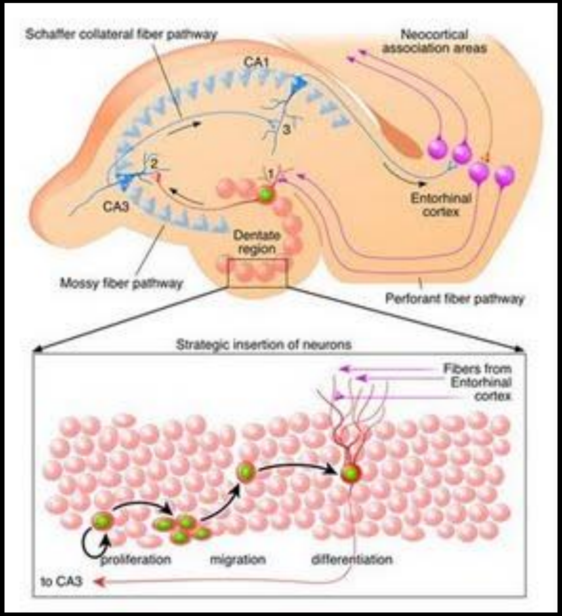
Why Search New Tx in TLE?

<u>Low quality of life</u>



Neurogenesis after Seizure

- Induced seizures in rats
- Hippocampal neurogenesis
- Repair mechanism



Hellsten, et al 2000

Madsen, et al 2000

Neurogenesis after Seizures

Status Epilepticus

↓

Increase Neurogenesis

↓

Repair Mechanism

Chronic Epilepsy

↓

Hippocampal Damage

↓

Decreases Neurogenesis

Parent et al, J Neurosci 1997

Control of Crisis with Cell Therapy
Pre Clinical Studies

- Kokaia et al, 1994 *Exp Neurol*
- Loscher, et al 1998 *Trends in Neuroscience*
- Eglitis, et al 1999 *Neuroreport*
- Madsen, et al 2000 *Biol Psychiatry*
- Chu,et al 2004 *Brain Res*
- Costa, et al 2006 *ISSR Annual Meeting*
- Veturini, et al 2008 *UFRGS Postgrad Neurosci*

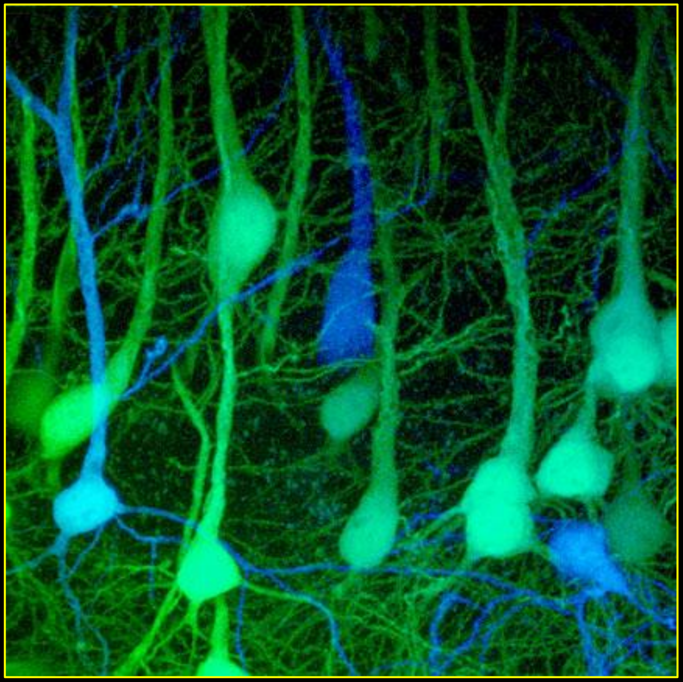
Preclinical Studies

Rat Pilocarpine Model of TLE

Transplantation of transgenic mice *stem cells*

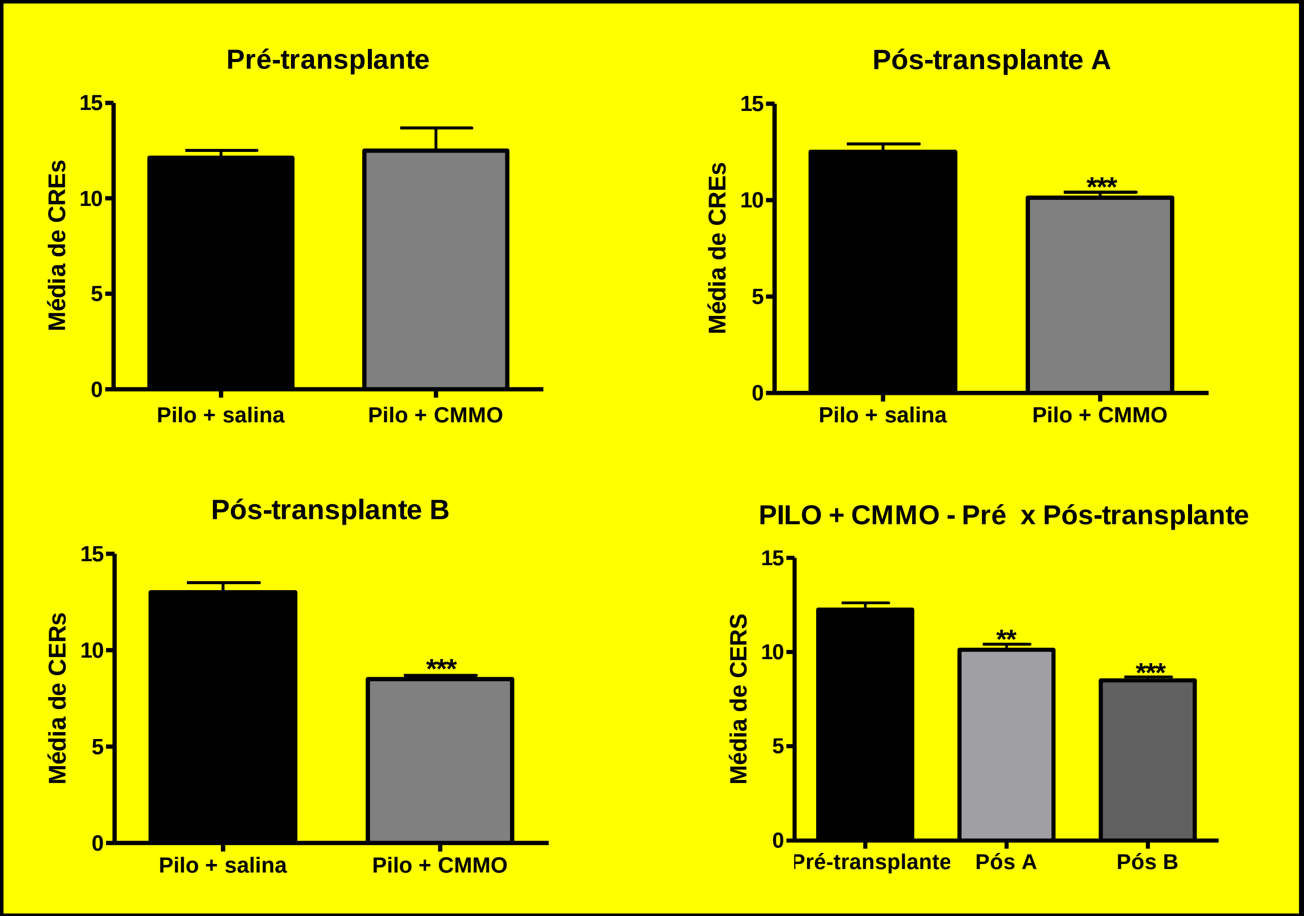
Clogh 1996, Eglitis 1999, Chu 2004, Costa 2006

Histology: Migration of stem sells to the damaged hippocampus



Costa J, 2006

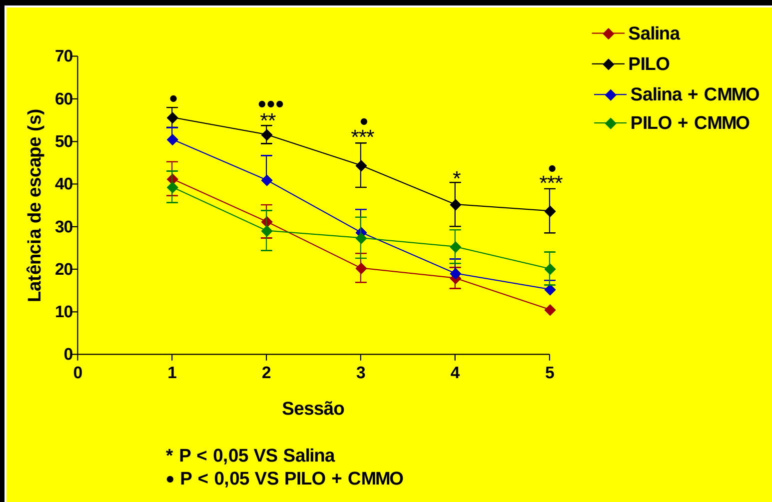
Frequency of Crisis

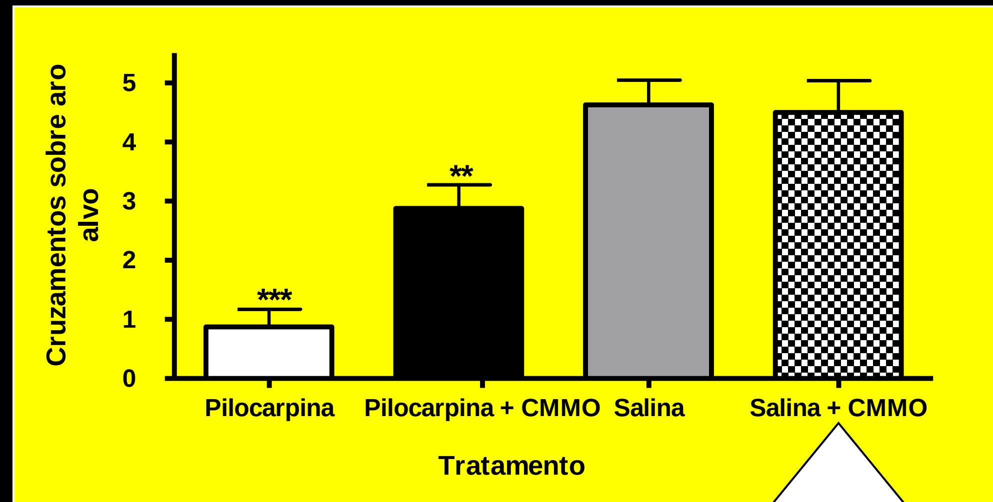


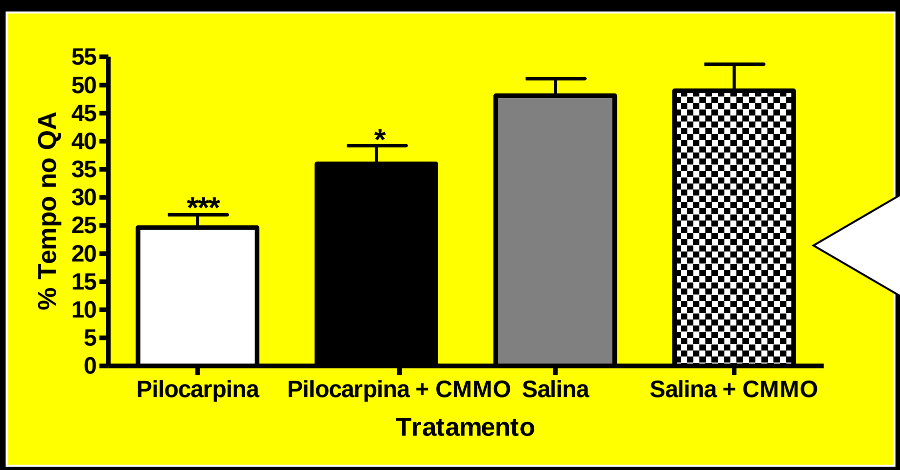
Control of Crisis

Costa J, 2006

Cognition







Costa J, 2006

Rationale of Stem Cell Therapy in TLE

- Imbalance between excitatory / inhibitory neurotransmiter system.
- Resulting in decreased inhibition (GABA).
- Replace dead neurons (GABA-ergic neurons).
- Control of crisis.
- Improvement of cognition.

West and Arvidsson 2000
Madsen, et al. 2000

Treatment of Temporal Lobe Epilepsy by Intra-Arterial Injection of Stem Cells

PHASE I Study

Raupp E, MD, PhD.

Costa J, MD, PhD.

Goricochea B, MD, PhD.

Machado D, MD, PhD.

Portuguez M W,

Vedolin L, MD

PUCRS Porto Alegre BRASIL

Stem Cell in TLE
Objectives

- 20 patients;
- Temporal Lobe Epilepsy with Hippocampal Sclerosis.
- Evaluate safety of Intraarterial injection of Autologous Adult Bone Marrow Mononuclear Stem Cells.
- Intraarterial injection in Posterior cerebral artery.

Patient Selection

- Engel's scale of crisis frequency.
- ECG and blood tests.
- Neurological examination.
- NeuroPsycological examination.
- Neuroimaging studies (MR).
- Video-EEG.

Safety Criteria

- Clinical/Neurological complications during endovascular procedure and follow-up.
- Modification of crisis frequency according to Engel's Scale.
- EEG alterations during endovascular procedure.
- Modifications in MR imaging (Volumetry).
- Compare previous clinical status after 3 and 6 months

Inclusion Criteria

1. Age > 18 years old.
2. Mesial Temporal Lobe Epilepsy refractory to clinical treatment.
3. Neuroimaging compatible with Hippocampal Sclerosis.
4. Informed consent term.

Exclusion Criteria

- Neoplasms.
- Autoimmune diseases.
- Neurovegetatives Diseases.
- Acute Cardiac Insufficiency.
- Primary Hematological diseases.
- Osteopathy that increases the risk of bone punction.
- Coagulopathy.
- Hepatic Insufficiency.
- Moderate Renal Insufficiency (creatinine > 2 mg/dl)
- Dependence on organic support (circulatory or pulmonary).
- Pregnancy.
- Participation in another clinical study.
- Impossibility of vascular access.

Patients Profile n=20

	Sexo	1ª CC não febril (anos)	CC febril na infância	Duração de crise (anos)	História Familiar
1	Feminino	35	Sim	5	Não
2	Feminino	32	Não	26	Sim
3	Masculino	8	Não	29	Não
4	Masculino	2	Não	40	Não
5	Masculino	10	Não	30	Sim
6	Feminino	18	Sim	30	Sim
7	Feminino	20	Não	30	Não
8	Masculino	1	Não	26	Sim
9	Feminino	2	Não	39	Sim
10	Masculino	16	Sim	8	Sim
11	Masculino	13	Não	16	Não
12	Masculino	7 meses	Não	27	Não
13	Masculino	12	Sim	30	Não
14	Masculino	15	Sim	30	Não
15	Masculino	42	Não	20	Não
16	Feminino	11	Sim	19	Sim
17	Masculino	43	Não	9	Sim
18	Feminino	4	Não	47	Não
19	Feminino	3	Sim	29	Não
20	Masculino	9	Sim	18	Não

12 males
8 Familial history
8 had febrile seizure infancy

Ethics

- Research Ethics Committee of our Institution (# 0931/07)
- National Committee of Ethics and Scientific Research (# 947/2007, CAAE 0194.0.002.000-07).

ClinicalTrials.gov
Protocol Registration System

Protocol Registration Receipt
06/08/2009

Autologous Bone Marrow Stem Cells Transplantation in Patients With Temporal Lobe Epilepsy

This study is currently recruiting participants.
Verified by Instituto do Cerebro de Brasilia, June 2009

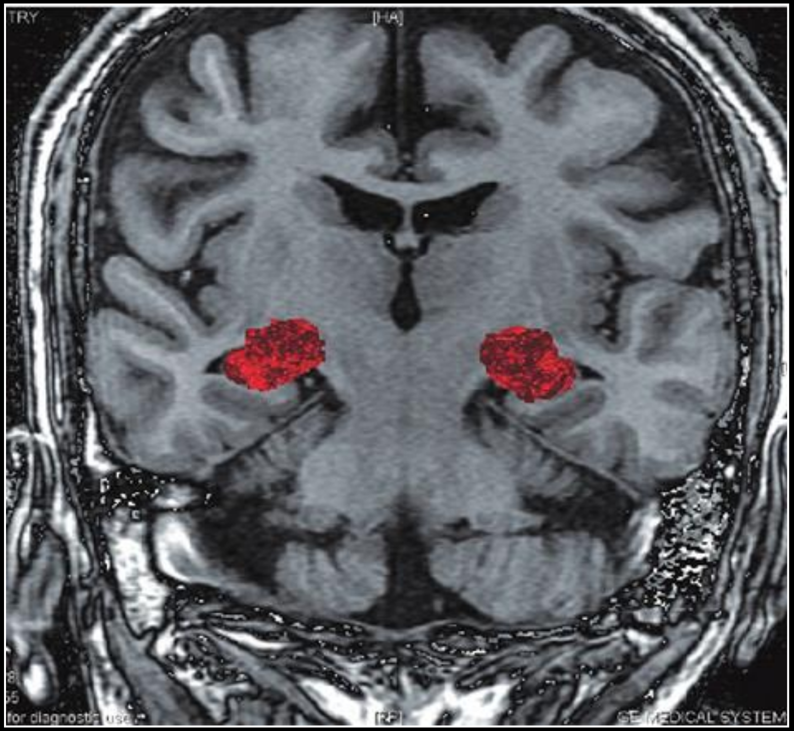
Sponsored by:	Instituto do Cerebro de Brasilia
Information provided by:	Instituto do Cerebro de Brasilia
ClinicalTrials.gov Identifier:	NCT00916266

Neuropsycology Evaluation

- Verbal memory
- Visual memory
- Language

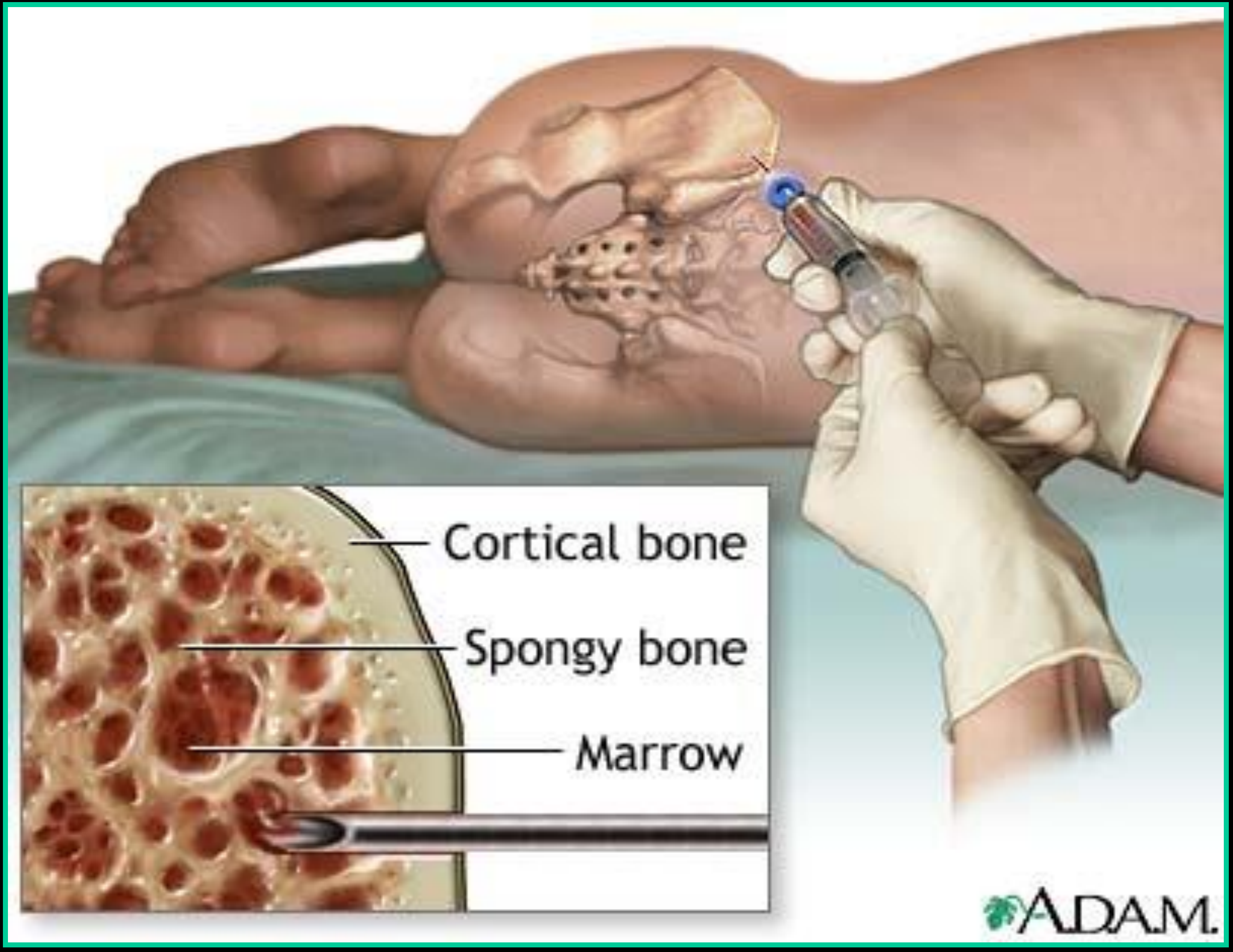
MR Hippocampal Volumetry

1.5 T Image Pro Plus V 4.5.1



Video EEG

PUNCTION of ILIAC BONE
≅ 50 ml

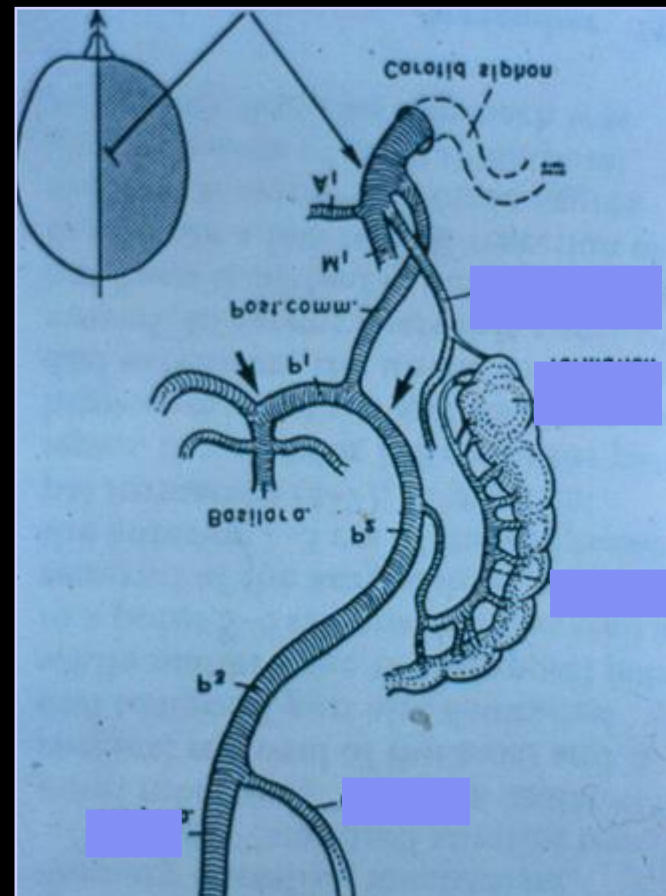



20 ml
15
10
5

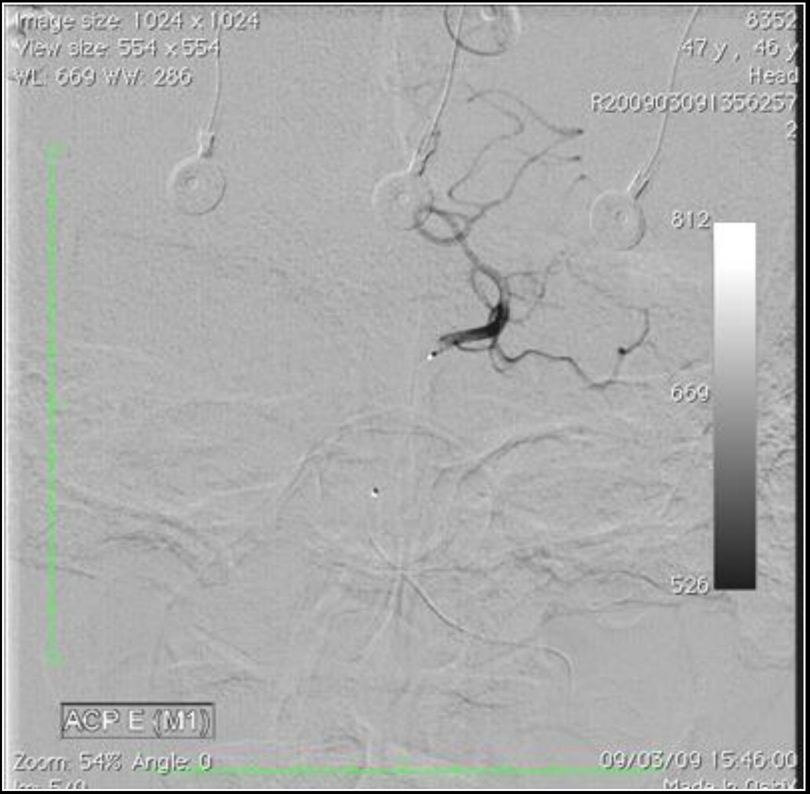
Separation of Stem Cell Fraction

- Density gradient
- Ficoll-Paque
- Minimum 4 X 10^7 MNC

Injection of Cells in the Posterior Cerebral Artery (P1-P2)



Intraarterial Superselective Cell Transplantation



P1 – P2

Clinical Cases

Case 1

- 43 y, F
- ***4 - 12*** partial complex crisis/month
- Politherapy
- MR : left hippocampal atrophy
- *10 months follow-up*

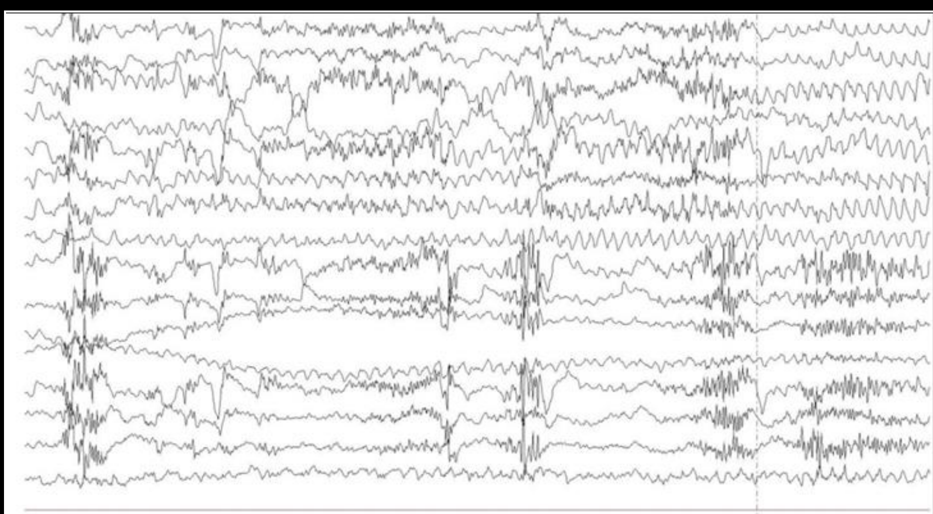
Case 2

- 53 y, F
- ***4 - 6*** partial complex crisis/month
- Politeraphy
- MR : left hippocampal atrophy
- *6 months follow-up*

Case 1

Frequency of Crisis

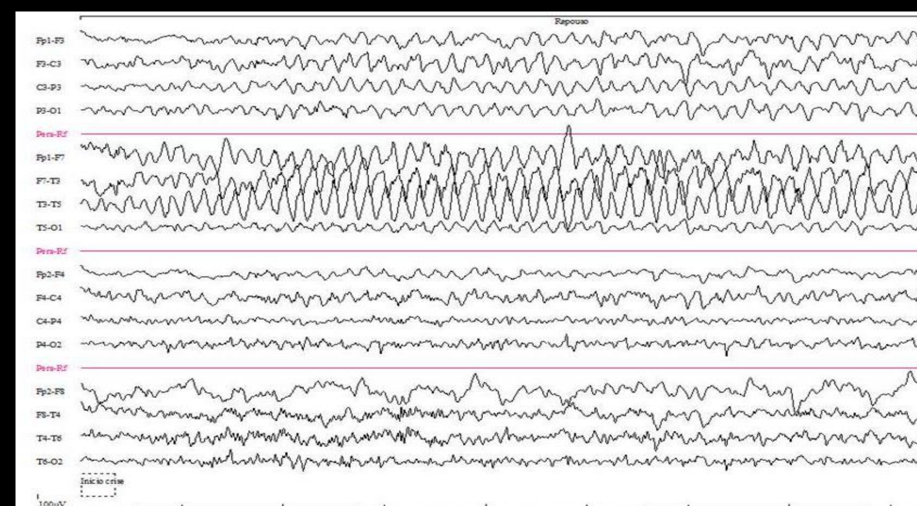
Pre -Transpl: **4 - 12/month**
Post 10 months : **01**



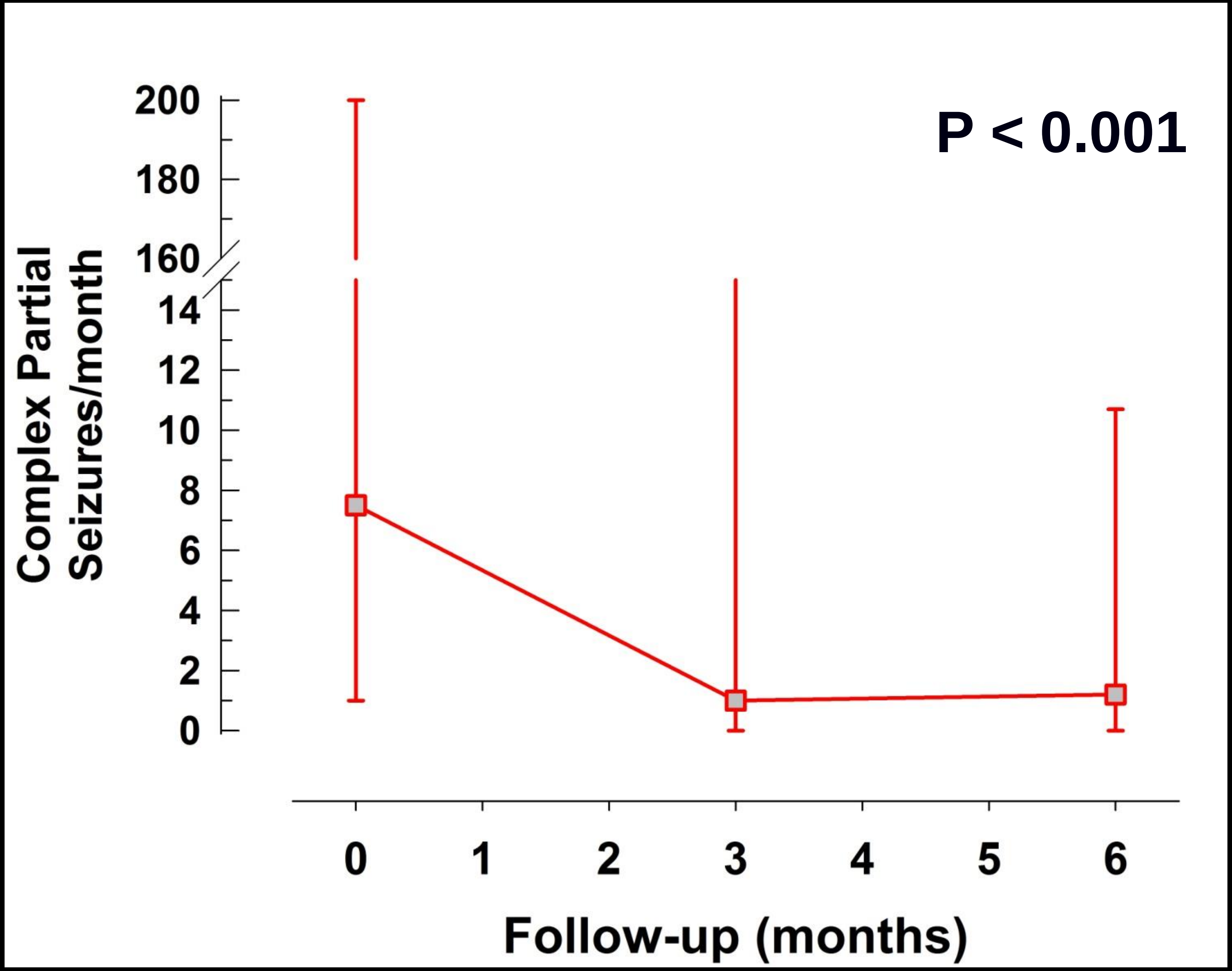
Case 2

Frequency of Crisis

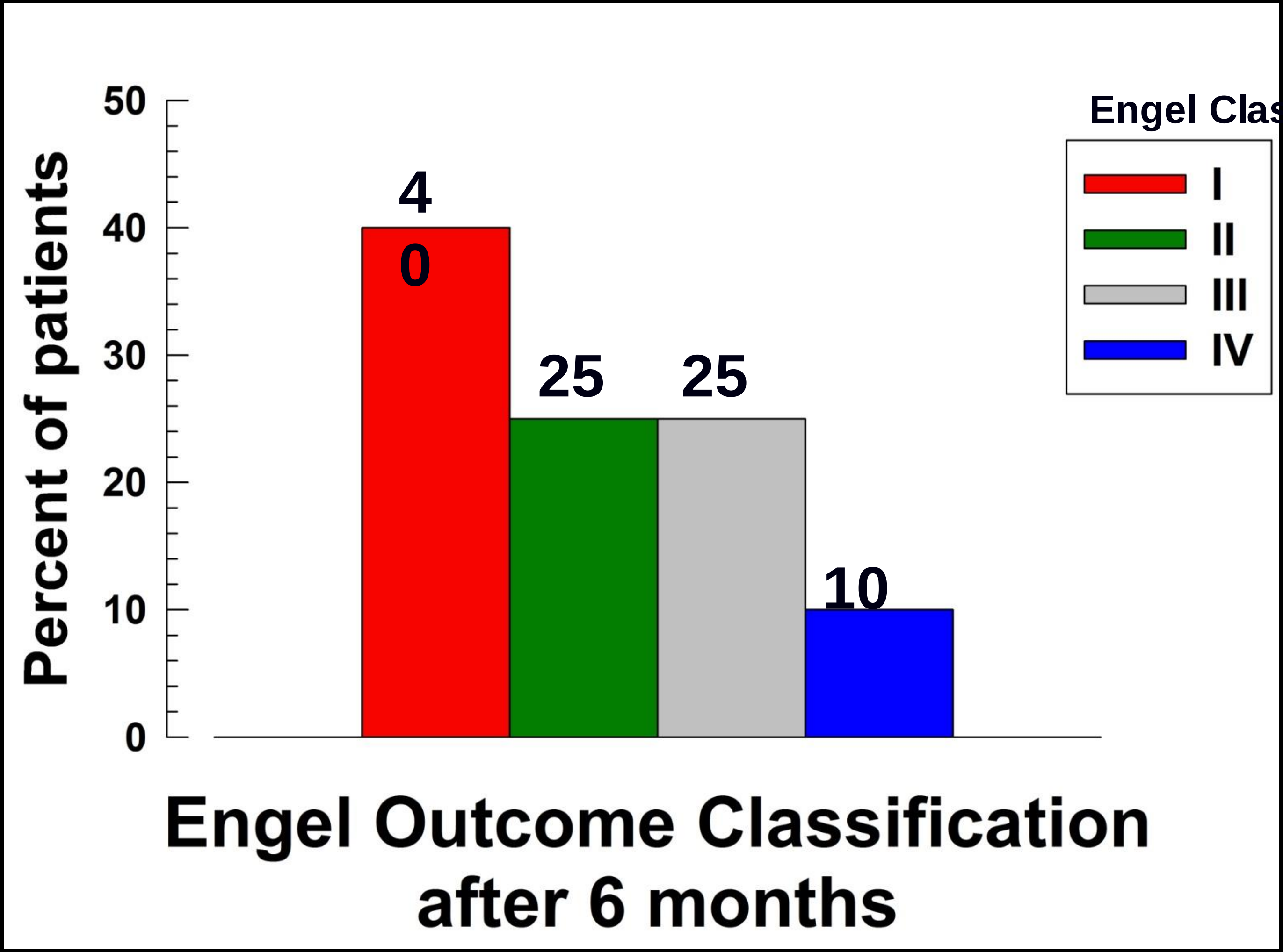
Pre - Transpl: **4 - 6/month**
Post 06 months: **none**



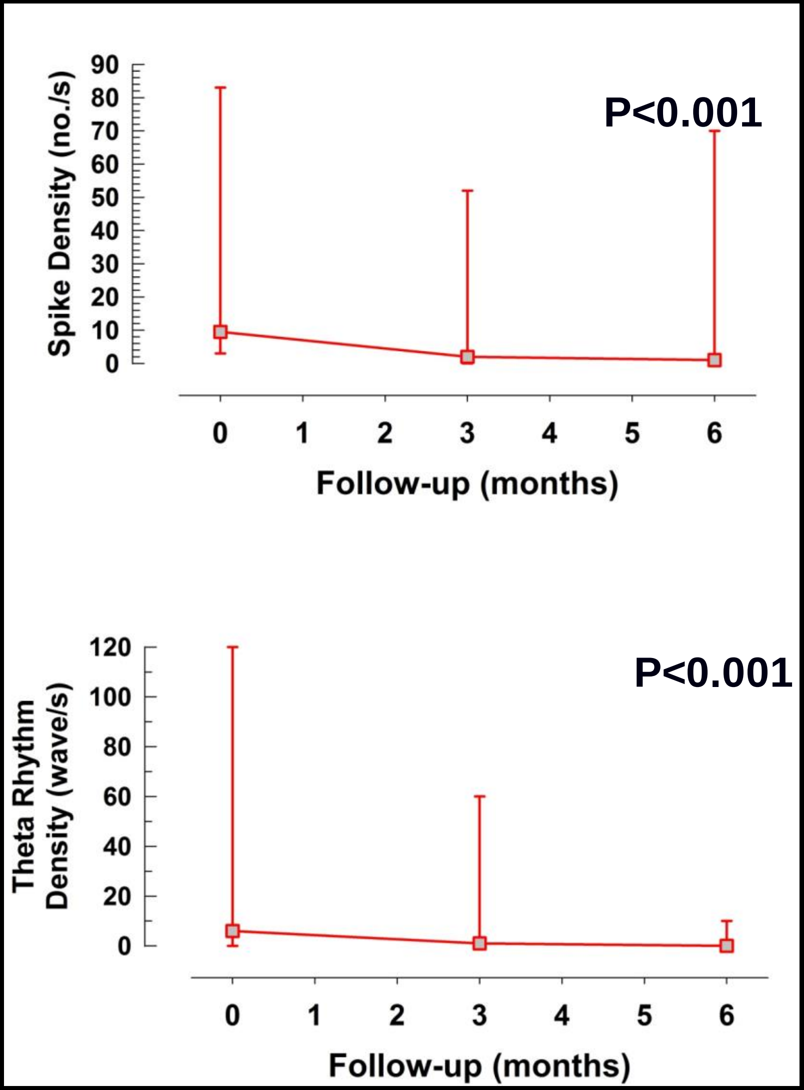
Number of Crisis / Month



Reduction of Number of Crisis
Engel's Scale

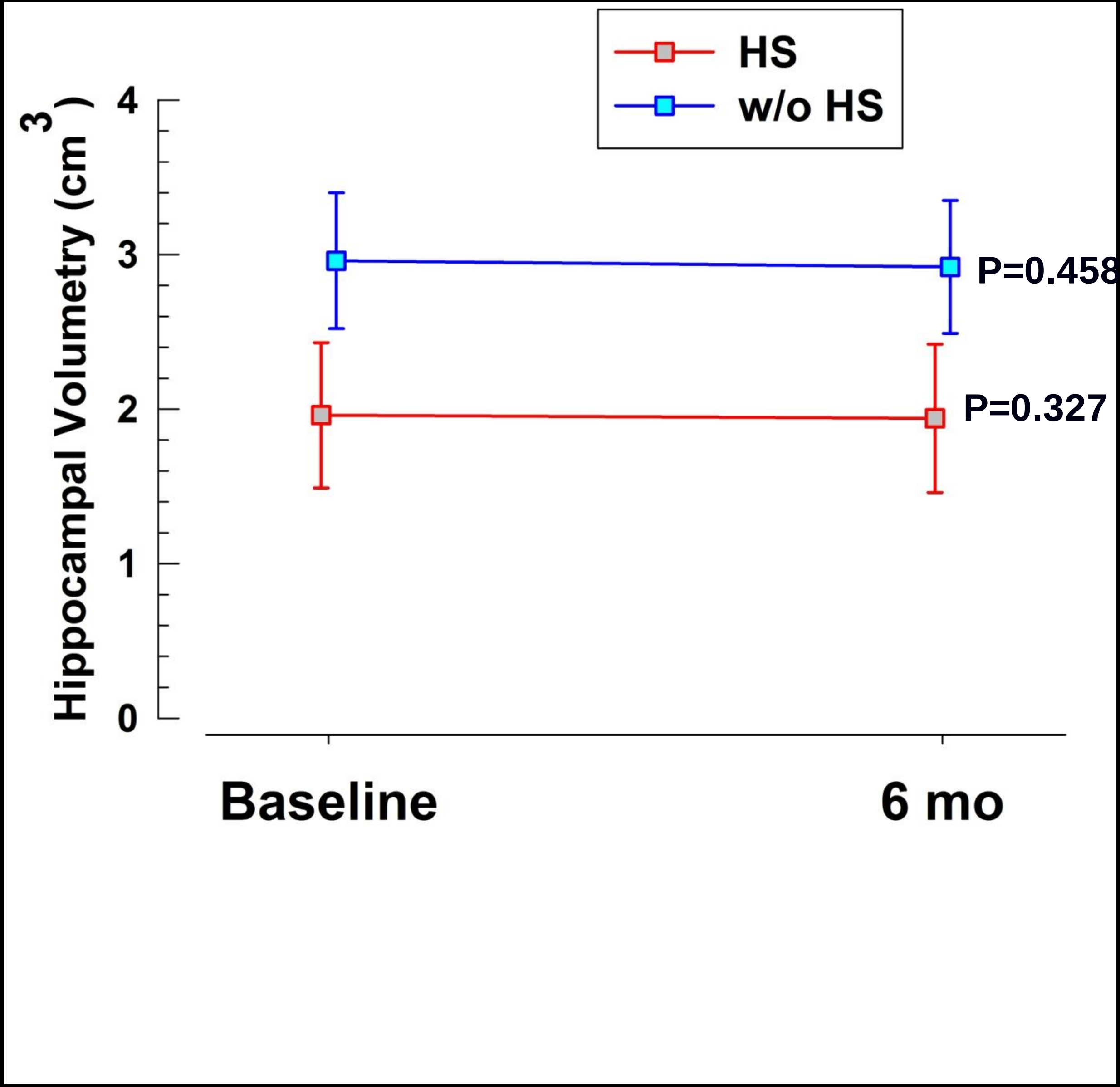


EEG



Spike Density (no./s)
90
80
70
60
50
40
30
20
10
0
P<0.001
0
1
2
3
4
5
6
Follow-up (months)
Theta Rhythm Density (wave/s)
120
100
80
60
40
20
0
P<0.001
0
1
2
3
4
5
6
Follow-up (months)

Hippocampal Volumetry



Conclusions

- Feasible and safe in 20 patients
- No clinicals complications
- No deaths
- According to animal epilepsy models stem cell transplantation seems to control seizures activity in humans with TLE.
- No seizure worsening
- Decreased seizure frequency: 67% Engel I
- No hippocampal changing
- No new EEG abnormalities
- Decreased theta and spike density

Final Remarks

- Stem cell therapy in humans is reproducing animal results.
- Stem cells can be easily isolated from various sources of autologous tissues.
- Stem cells protect acutely injured tissues and regulate and/or restore loss cells.
- Intraarterial transplantation has many advantages over IV and direct puncture.

MUCHAS GRACIAS