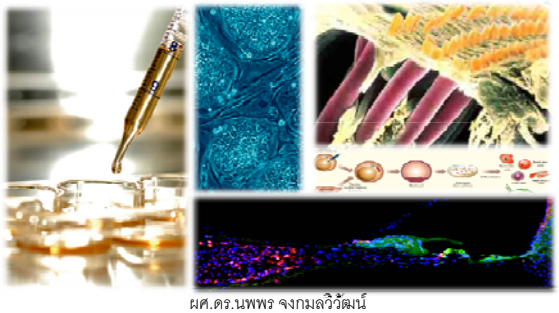
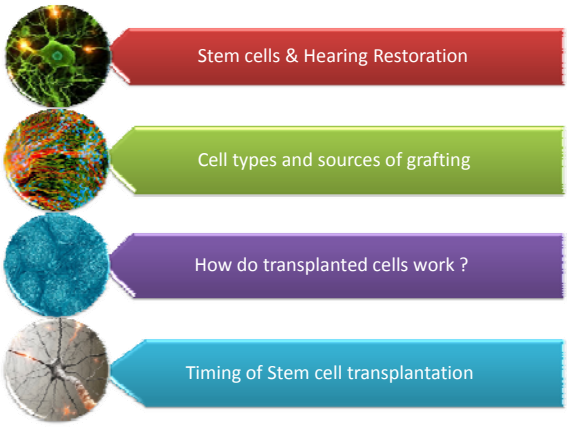
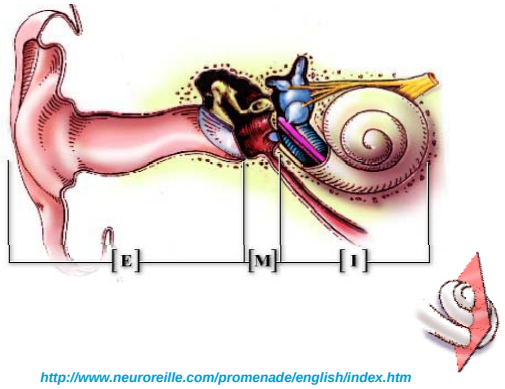
"Neural stem cells for *Hearing Impairment*."



ผศ.ดร.นพพร จงกมลวิวัฒน์
คณะสหเวชศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ
nopporn@swu.ac.th

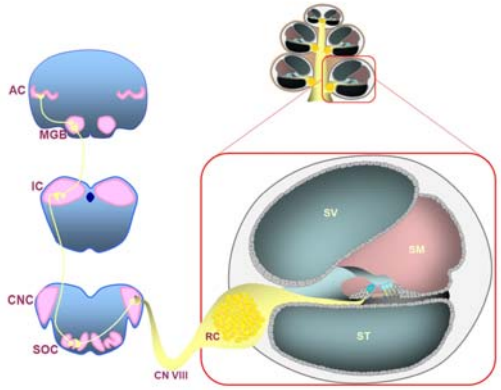


The Cochlea



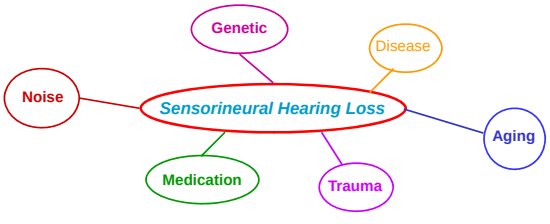
http://www.neuroreille.com/promenade/english/index.htm

The Cochlea

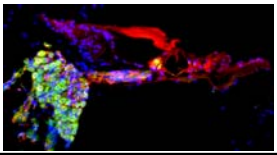


Jongkamonwiwat et al., CDT 2010

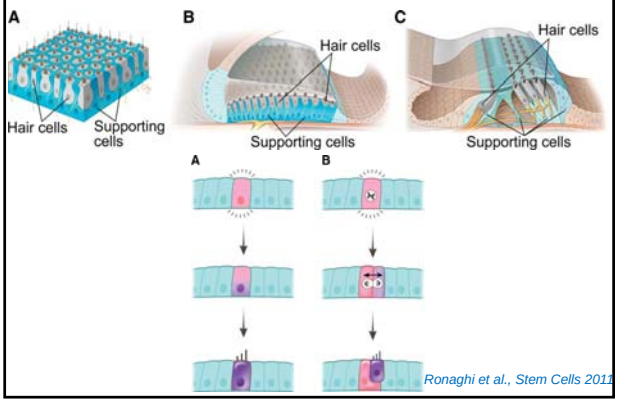
Hearing Impairment



Abnormalities in the hair cells of the organ of Corti in the cochlea

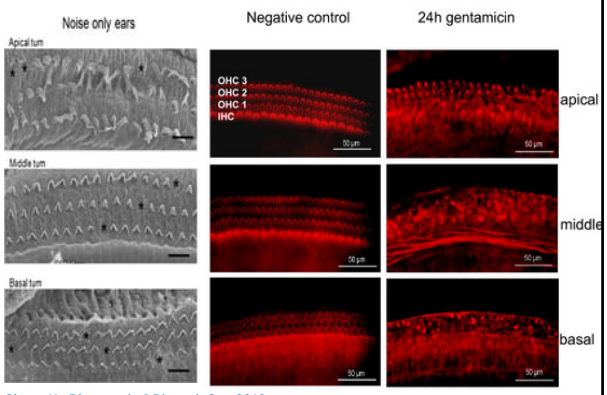


The Cochlea: Avian & Mammalian



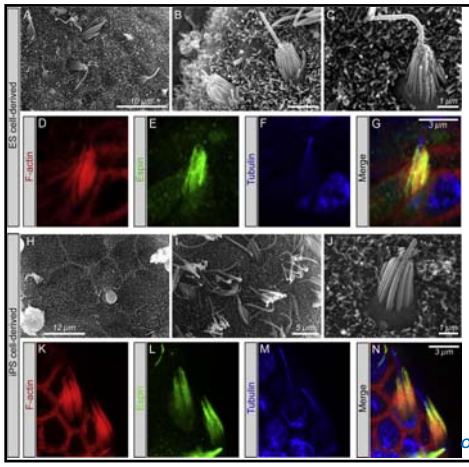
Ronaghi et al., Stem Cells 2011

The Cochlea: Avian & Mammalian



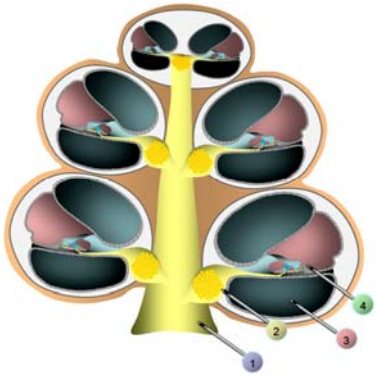
Chung-Ku Rhee et al., J Biomed. Opt. 2012 Yang et al., JBS 2009

Hair cell from ESCs & iPSCs



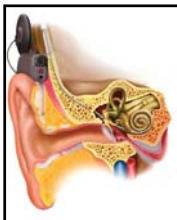
Oshima et al., Cell 2010

The routes of hair cell transplantation

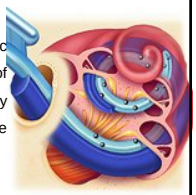


Jongkamonwiwat & Rivolta (2011) Regenerative Medicine: From protocol to patients, Springer

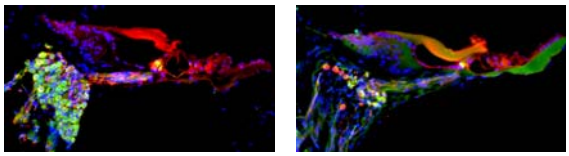
Cochlear Implant



Surgically implanted electronic device that provides a sense of sound. Directly stimulating any functioning auditory nerves inside the cochlea with an electric field.



People with mild or moderate sensorineural hearing loss are generally not candidates for cochlear implantation.

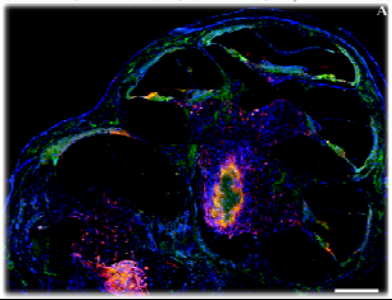


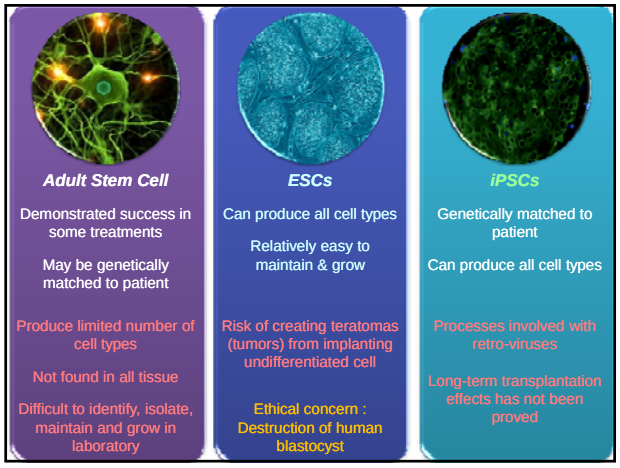
LETTER

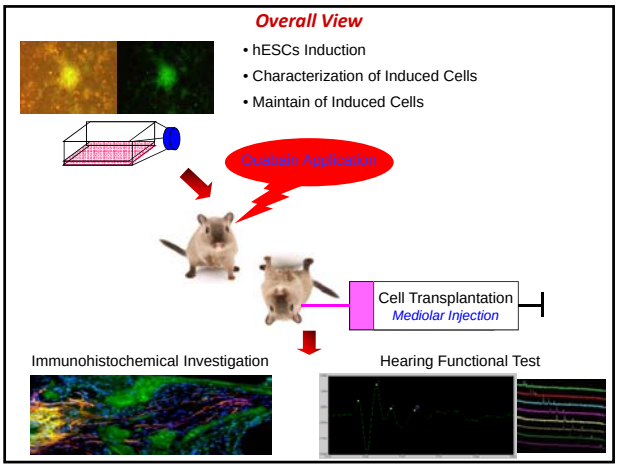
doi:10.1038/nature11415

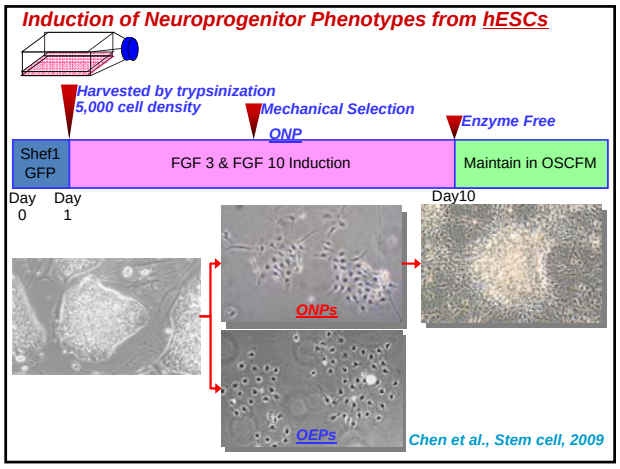
Restoration of auditory evoked responses by human ES-cell-derived otic progenitors

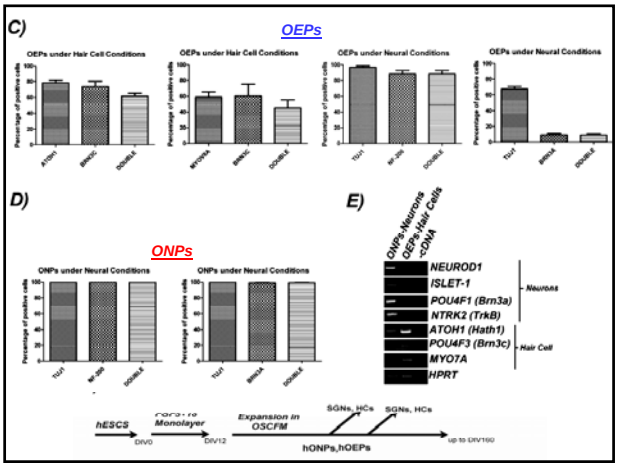
Wei Chen[1,2]*, Nopporn Jongkamonwiwat[1,2,3]*, Leila Abbas[1,2], Sarah Jacob Eshtan[1,2], Stuart L. Johnson[2], Stephanie Kuhn[2], Marta Milo[2], Johanna K. Thurlow[1,2], Peter W. Andrews[1,2], Walter Marcotti[2], Harry D. Moore[1,2] & Marcelo N. Rivolta[1,2]

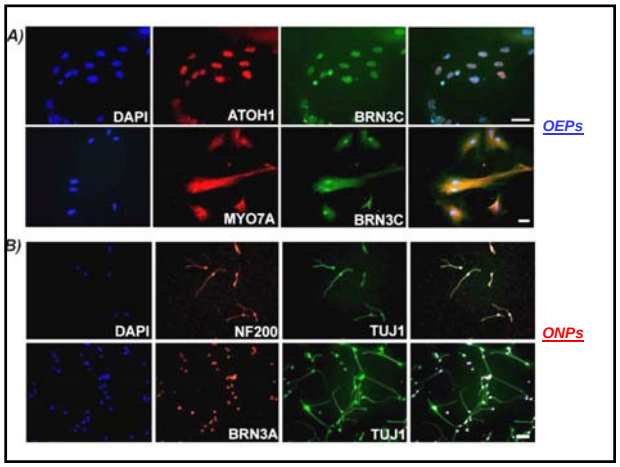


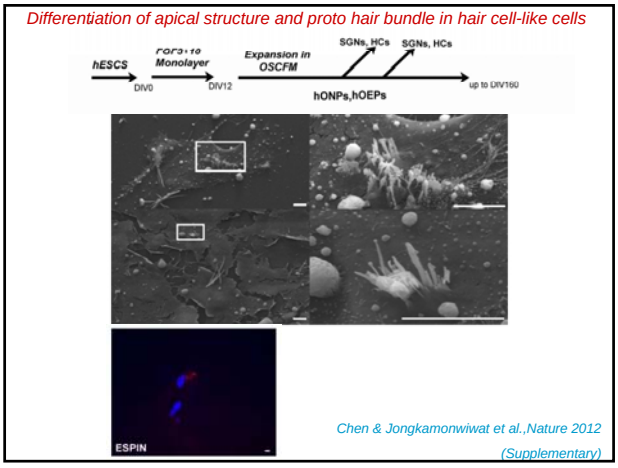


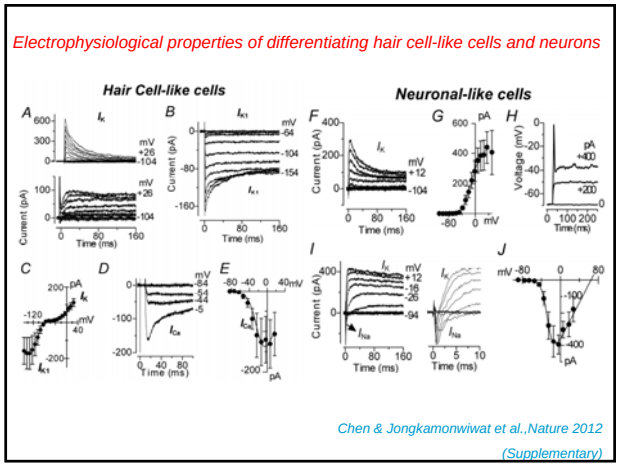

Overall View
• hESCs Induction
• Characterization of Induced Cells
• Maintain of Induced Cells
Ouabain Application
Cell Transplantation
Mediolar Injection
Immunohistochemical Investigation
Hearing Functional Test


Induction of Neuroprogenitor Phenotypes from hESCs
Harvested by trypsinization
5,000 cell density
Mechanical Selection
ONP
Enzyme Free
Shef1 GFP
FGF 3 & FGF 10 Induction
Maintain in OSCFM
Day 0
Day 1
Day10
ONPs
OEPs
Chen et al., Stem cell, 2009

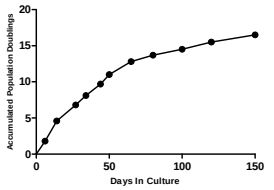

C)
OEPs
D)
ONPs
E)
NEUROD1
ISLET-1
POU4F1 (Brn3a)
NTRK2 (TrkB)
ATOH1 (Hath1)
POU4F3 (Brn3c)
MYO7A
HPRT
Neurons
Hair Cell
hESCS
Monolayer
Expansion in OSCFM
hONPs,hOEPs

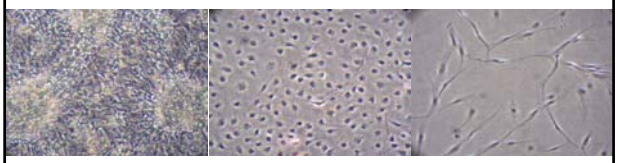

A)
DAPI
ATOH1
BRN3C
MYO7A
OEPs
B)
NF200
TUJ1
BRN3A
ONPs


Differentiation of apical structure and proto hair bundle in hair cell-like cells
hESCS
Monolayer
Expansion in OSCFM
hONPs,hOEPs
ESPIN
Chen & Jongkamonwiwat et al.,Nature 2012
(Supplementary)


Electrophysiological properties of differentiating hair cell-like cells and neurons
Hair Cell-like cells
Neuronal-like cells
Chen & Jongkamonwiwat et al.,Nature 2012
(Supplementary)

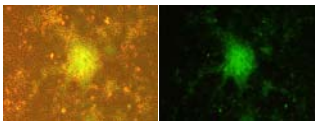
Population Doubling of Induced SHEF1-GFP

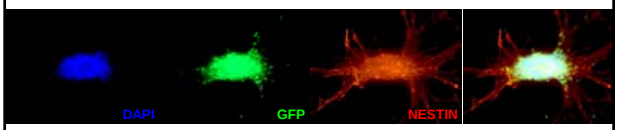


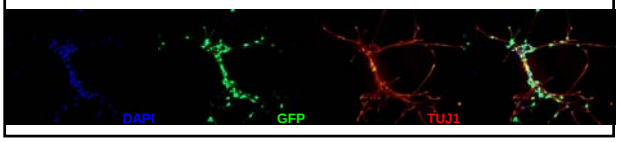


Chen & Jongkamonwiwat et al.,Nature 2012

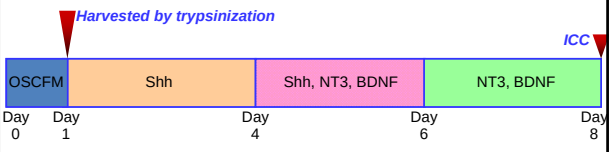
Induced SHEF1-GFP: Early Stage

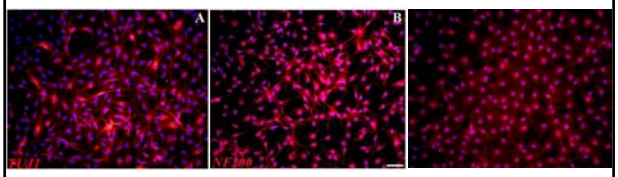




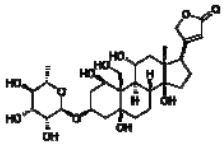


Induced SHEF1-GFP: Neuralizing Media





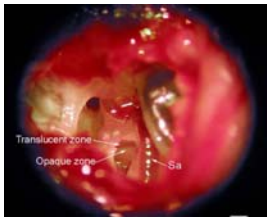
Deafness induction and Cell transplantation



Ouabain induces apoptotic cell death in type I spiral ganglion neurons.

Lang H. et al., 2005

Ouabain: Na^+/K^+ ATPase Inhibitor



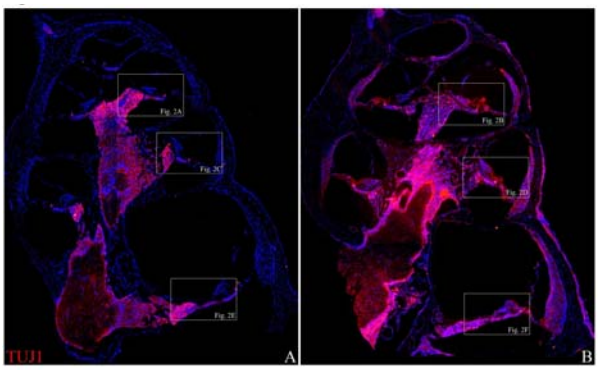
Application Dose: 20µl of 1mM Ouabain (Sigma,O-3125) in NSS

Applied to RW membrane for 30 minutes

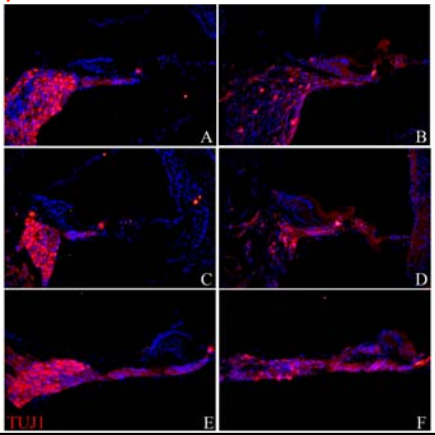
Removed by small piece of cotton wick

Lang H. et al., 2008

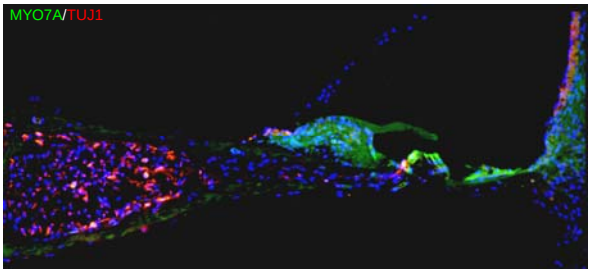
Comparison between Ouabain Treated and Untreated Cochlea



Comparison between Ouabain Treated and Untreated Cochlea

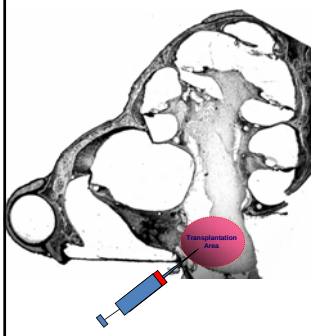


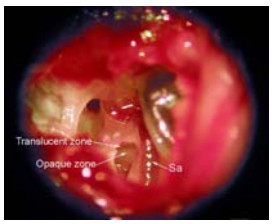
Persistence of Hair cells in Ouabain treated cochlea



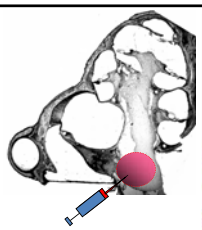
Ouabain induces apoptotic cell death in type I spiral ganglion neurons.

Deafness induction and Cell transplantation



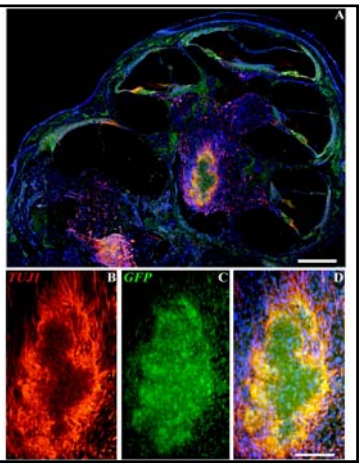


- A Nanoliter Microinjection System (WPI) with 33gauge needle
- 3 µl (~5x10⁴) of cell suspension in DMEM store at 4°C
- Daily injection of cyclosporine (15 mg/kg s.c.) starting 1 day before surgery and terminating the day before sacrifice.

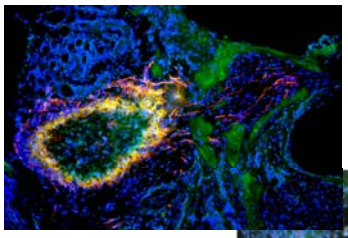


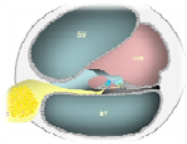
Short Term Transplantation:
Forming of Ectopic ganglion
in the cochlear modiolus

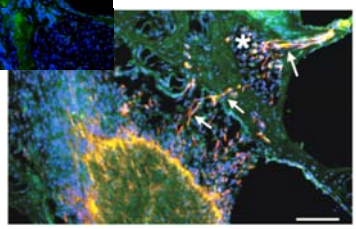
Neuronal Differentiation of Transplanted cells



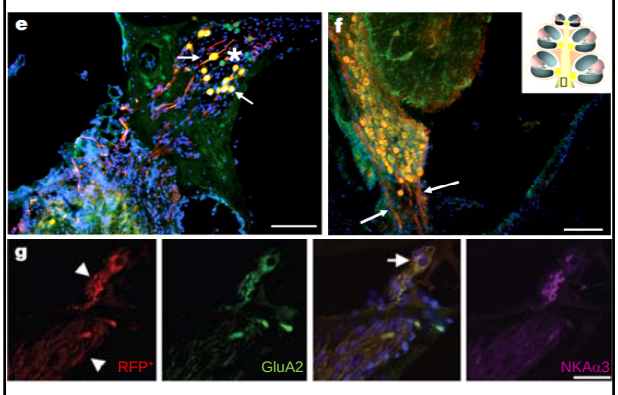
Immunohistochemistry Staining after Transplantation



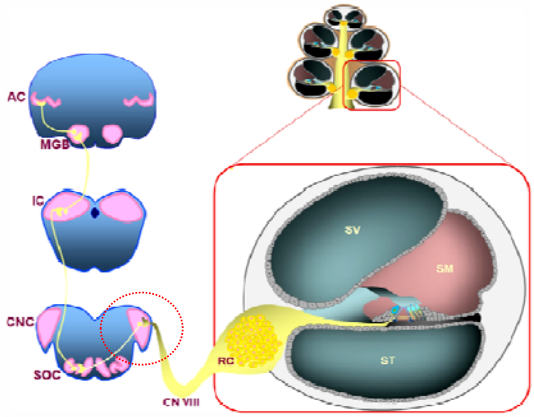




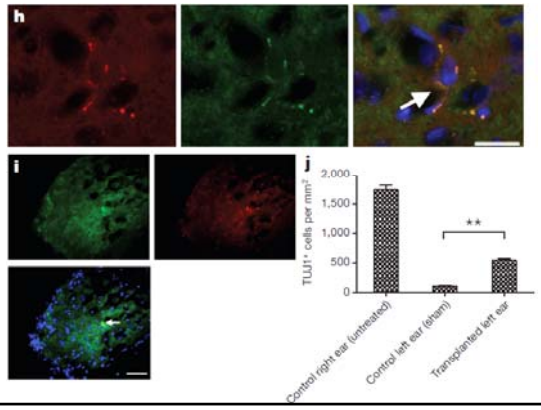
Immunohistochemistry Staining after Transplantation



Reorganization at the level of brainstem auditory nucleus

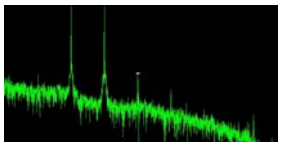


Immunohistochemistry Staining after Transplantation

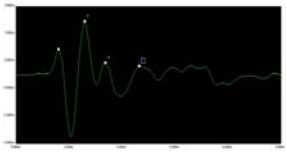


Hearing Measurement

Auditory brainstem responses (ABR) and Distortion product otoacoustic emissions (DPOAE) have been applied to the evaluation of peripheral auditory function.



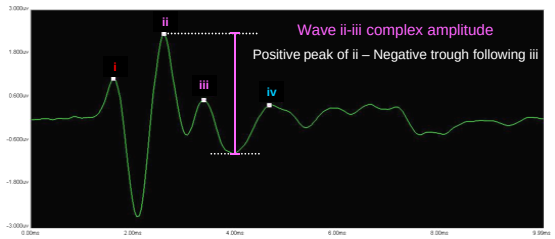
2f1-f2 DPOAE



Click evoked ABR testing

The purpose of this study was to measure click evoked ABR testing and 2f1-f2 DPOAE as detectors of sensorineural damage induced by ouabain treatment and also trace the recovery after transplantation.

Waveform nomenclature

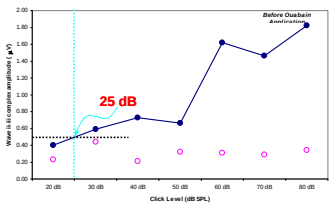


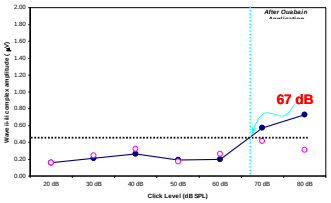
Wave i: The wave occurring at I-2 ms

Waves ii and iii: The complex double-waves at 2-3.5 ms

Wave iv: The large wave at 4-5 ms

Burkard et al.(1993)



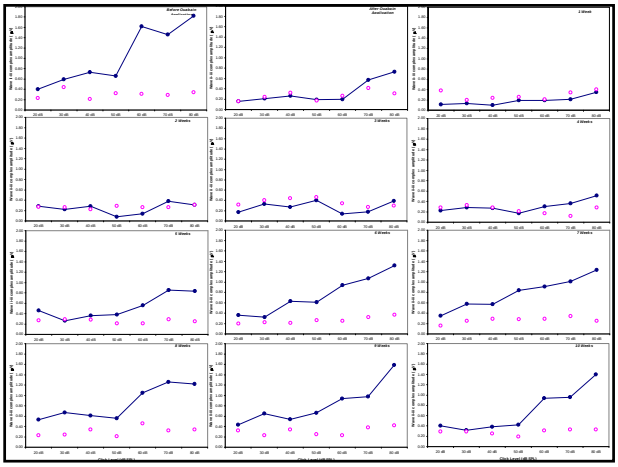


ABR Threshold:

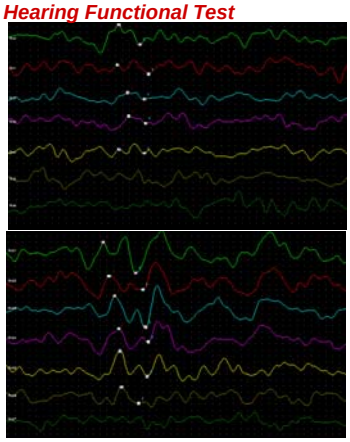
Stimulus level that evoked a peak-to-peak voltage 2 S.D. above the mean background activity.

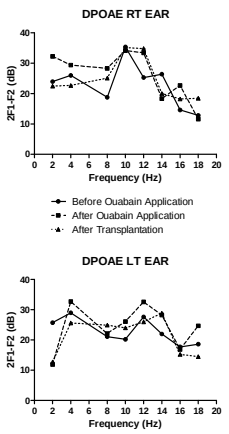
Mean Background = 0.31 μV
S.D.= 0.08
2S.D.=0.16
2 S.D.+ Mean Background= 0.47

Mean Background = 0.27 μV
S.D.= 0.09
2S.D.=0.18
2 S.D.+ Mean Background= 0.45

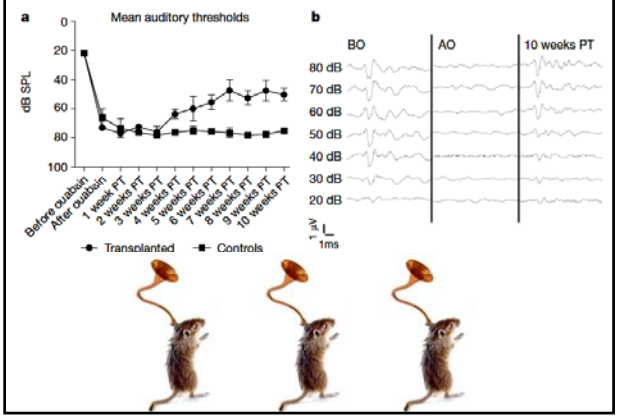


Hearing Functional Test





Hearing Functional Test



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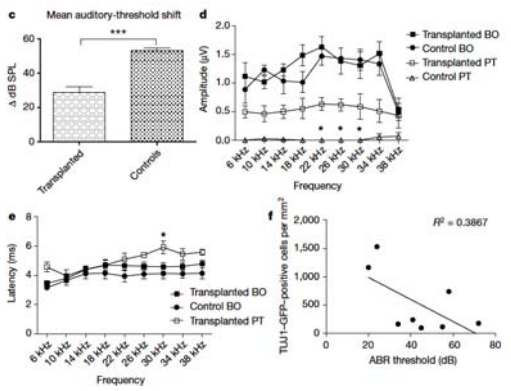

Transplanted cells Quantification

	Untreated ear (right)		Ouabain-treated ear (left)			Surviving neurons (%)
	TUJ1+ mm-2	Periph. + mm-2	TUJ1+ mm-2	Periph.+ mm-2	% Periph. +	% total (Type I)
Apical Turn	1848.73 ± 187.5	105.2 ± 5.9	88.7 ± 30.5	85.7 ± 13.6	96.6%	4.8 % (0.16%)
Mid Turn	1773.1 ± 93.9	169.6 ± 19	127.2 ± 11.7	101.9 ± 31.5	80.1%	7.1 % (1.41%)
Basal Turn	1607.77 ± 205.4	134.1 ± 28.9	121.5 ± 33.5	101.2 ± 3.6	83.3%	7.5% (1.25%)
Mean Total	1743 ± 71.5	136.3 ± 18.6	112.5 ± 11.9	96.2 ± 5.2	86.7%	6.4% (0.94%)

	Control animal (untreated, right ear)	Control animal (ouabain, left ear)	Transplanted animals (left ear)	
	TUJ1+ mm-2	TUJ1+ mm-2	TUJ1+ mm-2	% TUJ1+/GFP+
Apical Turn	1848.73 ± 187.5	88.7 ± 30.5	498.17 ± 166.8	95.36 ± 1.9
Mid Turn	1773.17 ± 93.9	127.2 ± 11.7	603.35 ± 226.8	95.09 ± 1.3
Basal Turn	1607.77 ± 205.4	121.5 ± 33.5	537.56 ± 302.3	94.25 ± 2.3
Mean Total	1743 ± 71.5	112.5 ± 11.9	546.4 ± 30.68	94.9 ± 0.3

From 5x10⁴ transplanted cells

The University Of Sheffield.

Centre for Stem Cell Biology

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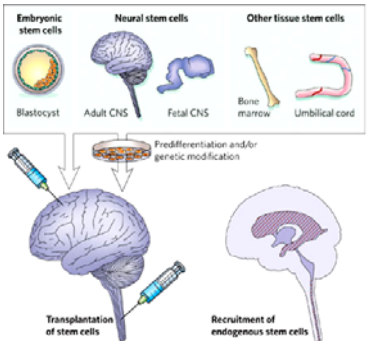
Factors influence transplantation

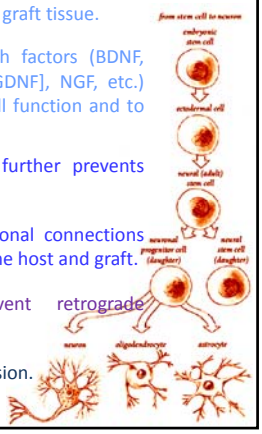
The goal of optimizing these variables is to maximize the number of surviving functional cells present at the appropriate site of injury.

- *Timing*
- *Niche*
- *Administration route*
- *Immunosuppression*
- *Predifferentiation*
- *Pretreatment*
- *Scaffolds*
- *Controls*



How do transplanted cells work?

1. Neurotransmitters released from the graft tissue.
2. Release of the neurotrophic/growth factors (BDNF, glial derived neurotrophic factor [GDNF], NGF, etc.) acting as local pumps to support cell function and to prevent cascade of apoptosis.
3. Regenerating neuronal population further prevents subsequent cell death.
4. Re-establishment of local interneuronal connections and synaptic connectivity between the host and graft.
5. Limit glial reaction and prevent retrograde degeneration.
6. Improvement of regional oxygen tension.
7. Cell differentiation and integration.



Stroke
Journal of the American Heart Association
American Stroke Association
A Division of American Heart Association

Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS) : Bridging Basic and Clinical Science for Cellular and Neurogenic Factor Therapy in Treating Stroke

The STEPS Participants

The *Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS)* *meeting was organized* to bring together clinical and basic researchers with industry and regulatory representatives to assess the critical issues in the field and to create a framework to guide future investigations.

STEP I

Table 1. Considerations for Animal and Human Testing of Cellular Therapy

Mechanism of action
Trophic factors
Cell replacement
Local environment support
Other
Pathological substrate
Ischemic versus hemorrhagic
Peri-infarct versus intra-infarct
Location of pathology
Cortical versus subcortical
Brainstem
Timing after stroke
Acute
Subacute Chronic

Table 2. Guidance on Cell Delivery Approaches

1. Establish compatibility of cells with delivery device and determine optimal cell density and delivery volume necessary for efficacy
2. Intracerebroventricular: requires further safety and feasibility study
3. Direct intracranial injection: may be most suitable for neural stem cells
4. Intra-arterial: requires demonstration that cells do not lead to microembolism and brain infarcts
5. IV: cells may need homing signal to brain; demonstration that cells do not cause organ toxicity or interfere with organ physiology

Table 3. Guidance on Devices to Assist in Cell Delivery

1. Establish cell compatibility with device and establish maximal cell density and delivery volume
2. Establish animal and human compatibility
3. Biodegradability for implanted devices with long-term safety evaluation

Table 4. Guidance on Dosing

1. Determine MTD from the literature
2. Determine dose–response curve
3. Initial clinical trials should be based on animal studies of the optimal dose
4. Dose ranges will likely be negotiated with FDA and historical MTD

MTD indicates maximum tolerated dose.

STEP II

Stem Cell Therapy as an Emerging Paradigm for Stroke (STEPS) II

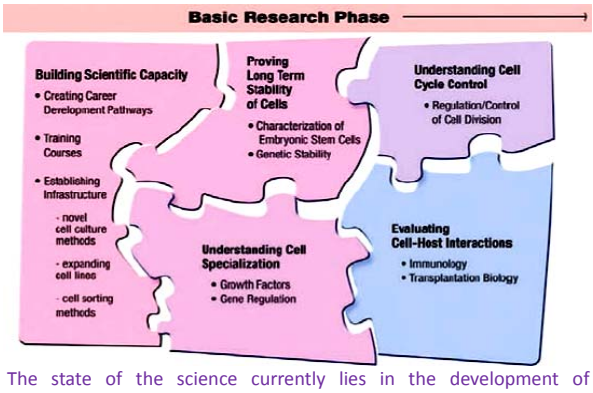
Sean I. Savitz, Michael Chopp, Robert Deans, S. T. Carmichael, Donald Phinney and Larry Wechsler

Drafted new recommendations to create a framework to guide future investigations in cell-based therapies for stroke

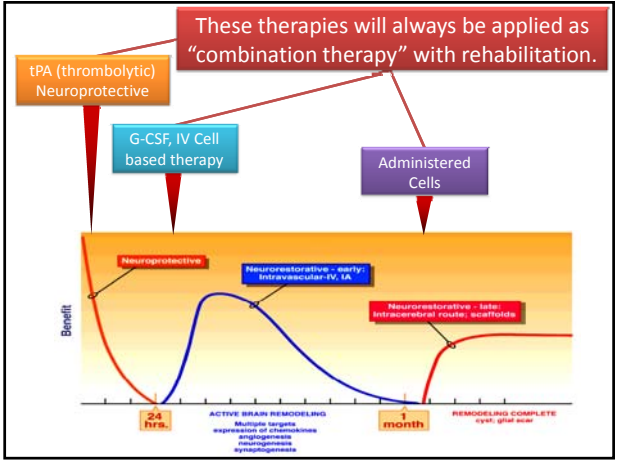
Table 1. Guidance on Cell Delivery Approaches

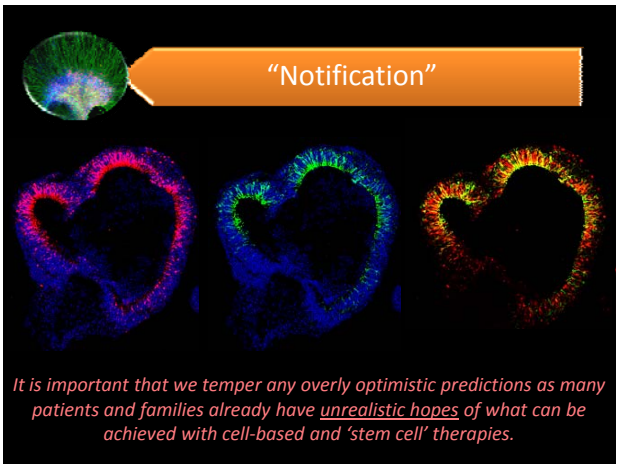
1. Establish compatibility of cells with delivery device and determine optimal cell density and delivery volume necessary for efficacy
2. Intracerebroventricular: requires further safety and feasibility study
3. Direct intracranial injection: may be most suitable for neural stem cells
4. Intra-arterial: requires demonstration that cells do not lead to microembolism and brain infarcts
5. Intravenous: cells may need homing signal to brain; demonstration that cells do not cause organ toxicity or interfere with organ physiology

The scientific challenges of human stem cells



The state of the science currently lies in the development of fundamental knowledge of the properties of human pluripotent cells.





It is important that we temper any overly optimistic predictions as many patients and families already have unrealistic hopes of what can be achieved with cell-based and 'stem cell' therapies.