Advanced Cell Biology and Biotechnology

ACBB 2021/22

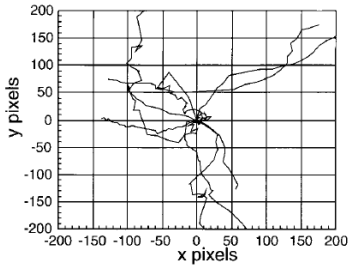
…the lecture of November 24th is about to begin…

CELL-CELL COMMUNICATION

- methods to study cell-cell communication:
  - chemotaxis & chemokinesis
  - attraction & repulsion
  - substrate preference
  - bidirectional signalling

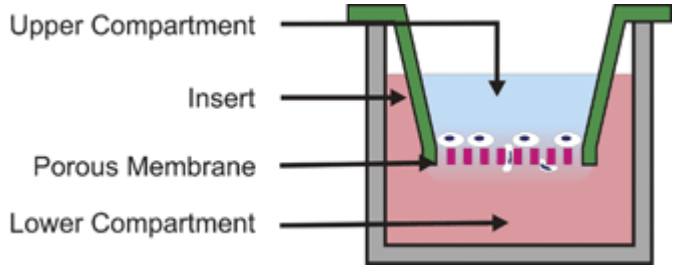
HOW TO STUDY CHEMOTAXIS AND CHEMOCHINESIS

- time lapse video microscopy

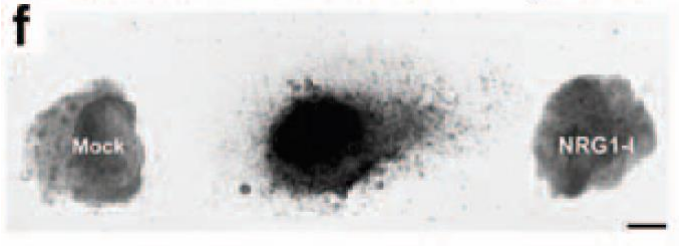


- wound healing

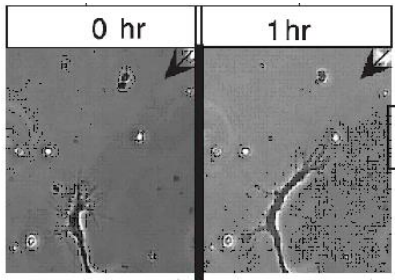
- transwell assay/Boyden's chambers



- explant migration



- turning assay



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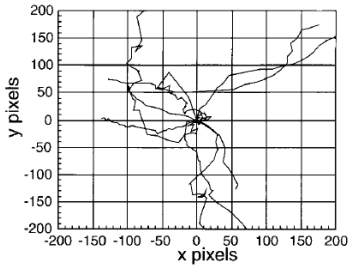

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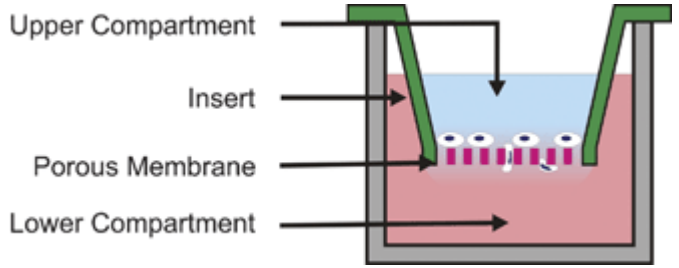

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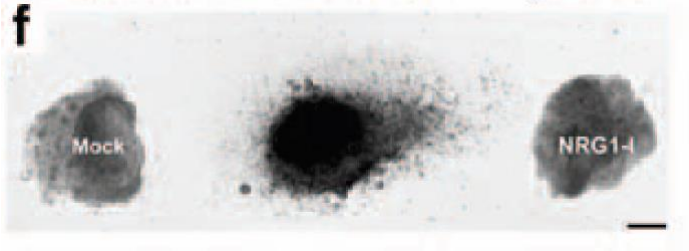

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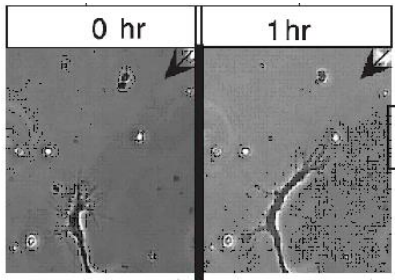
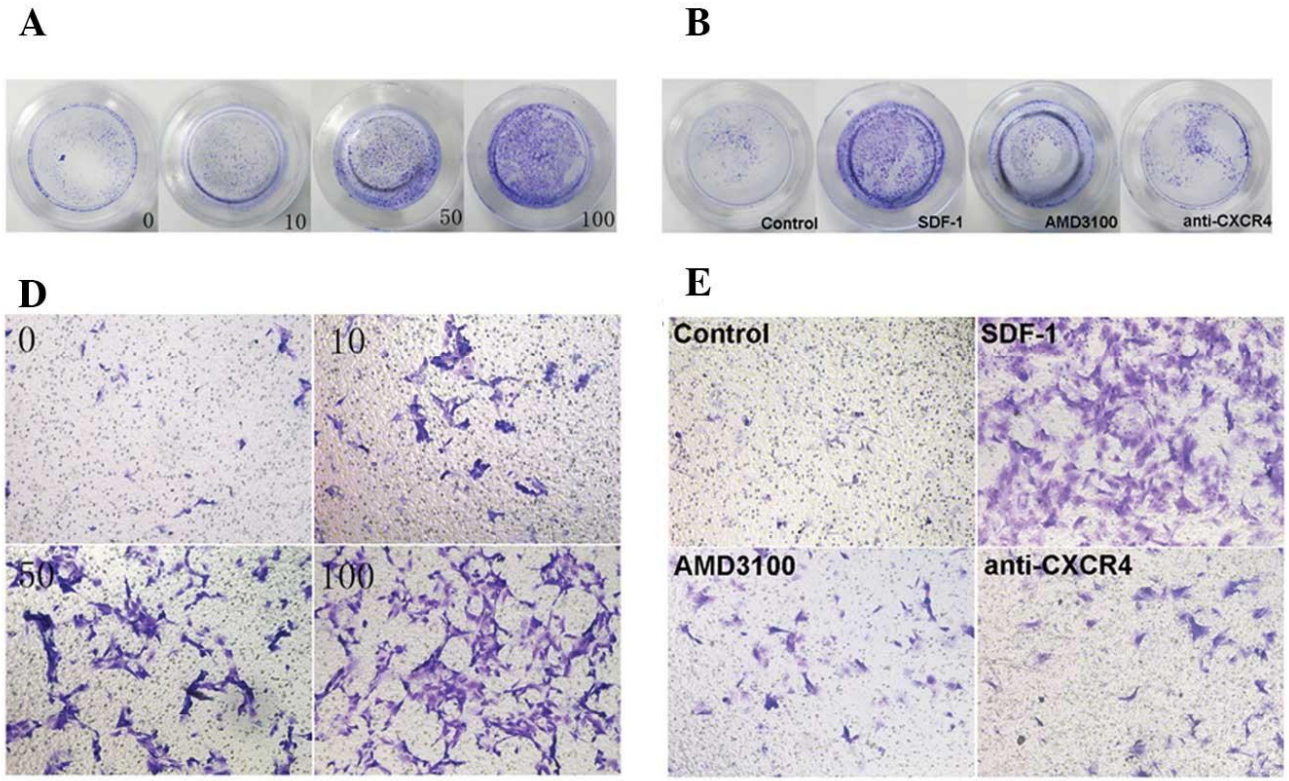
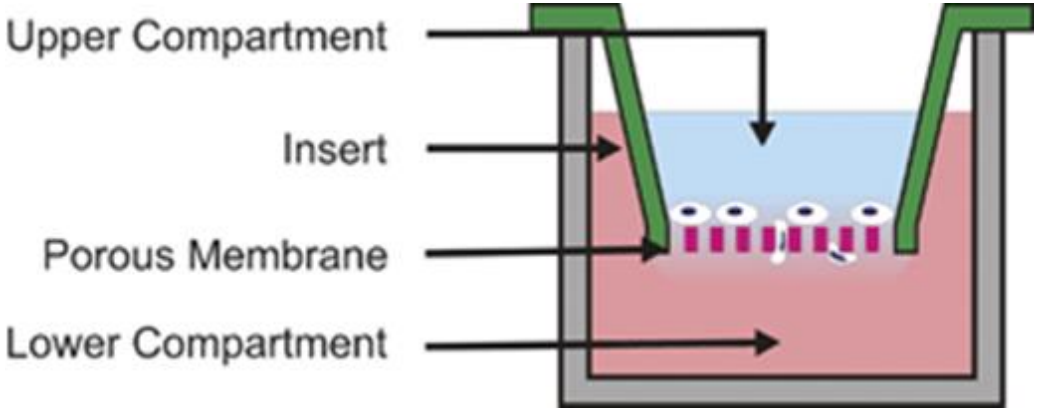


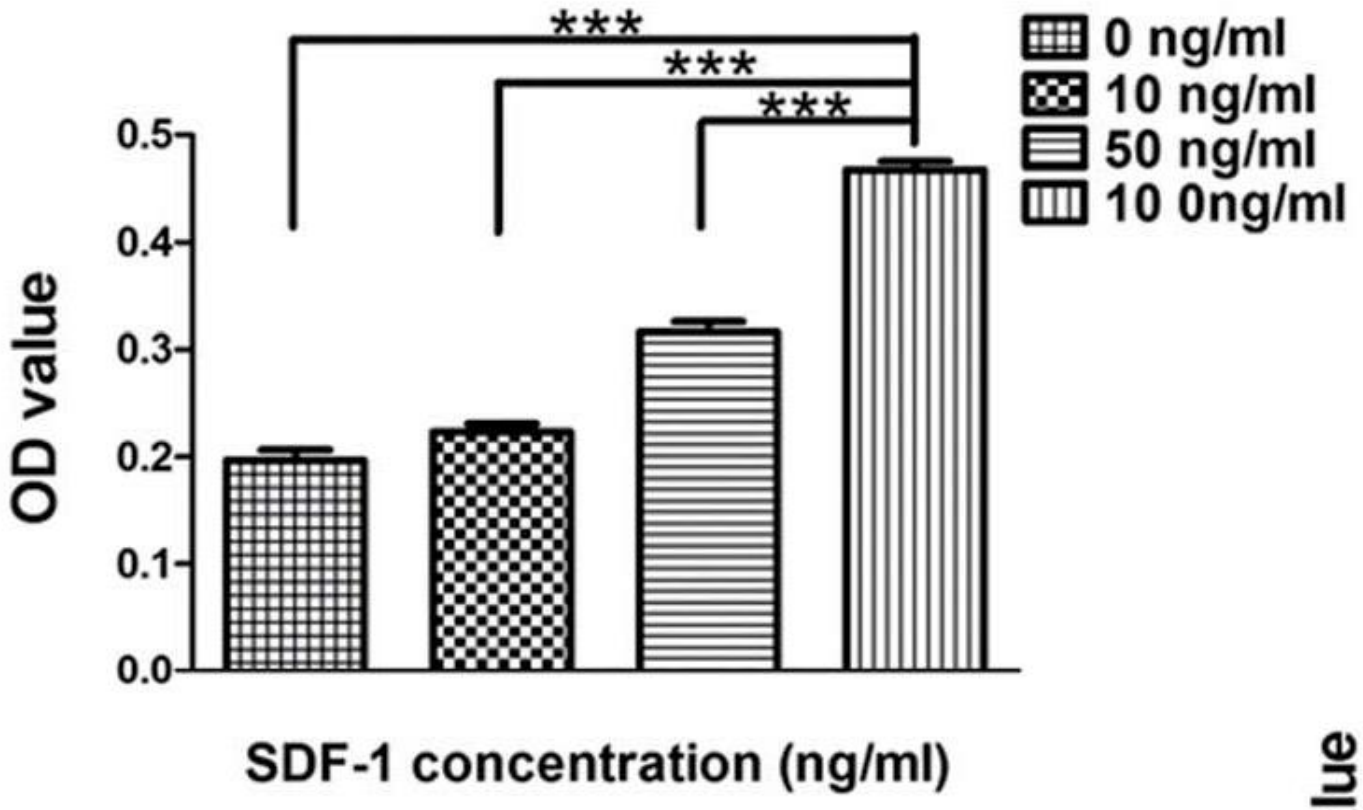
Transwell migration assay.





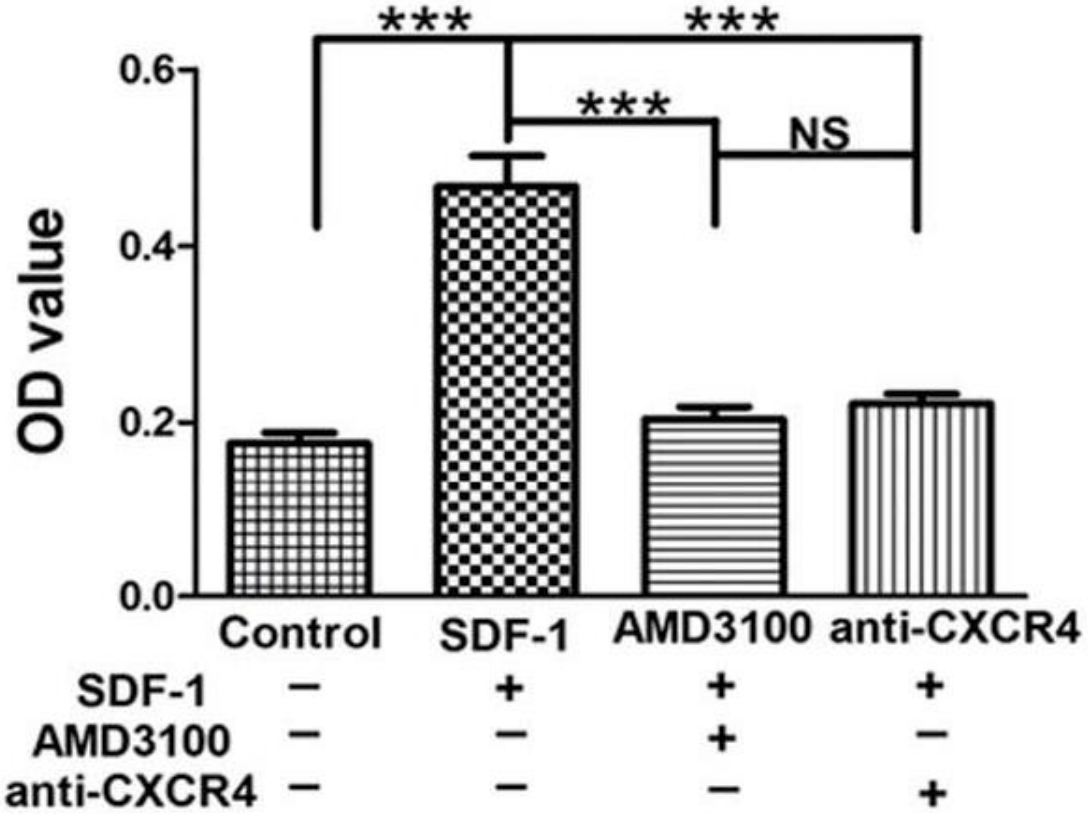
A-B - Macroscopic observation of transwell chamber (chemotaxis experiments).
D-E - Migrated cells under a light microscope.

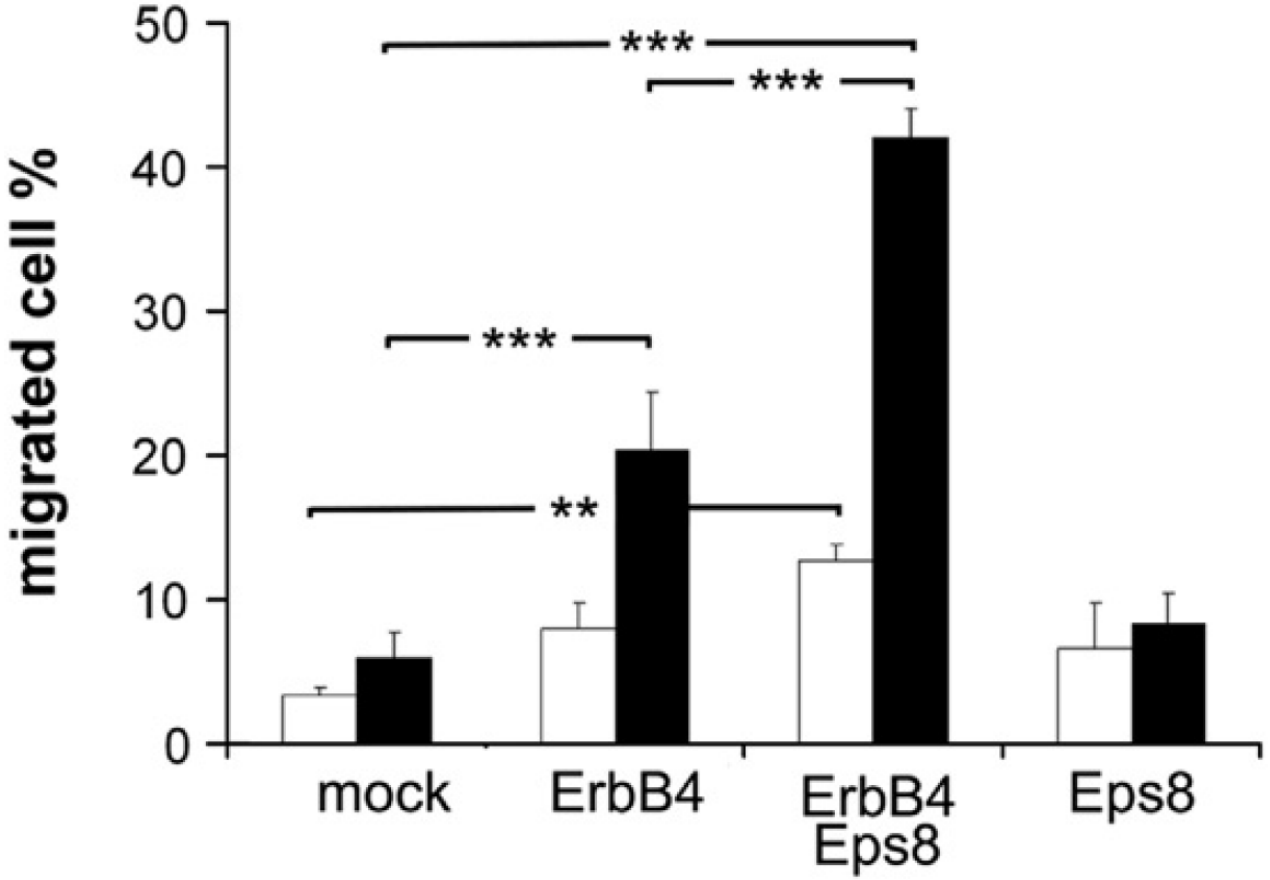
SDF-1, stromal cell-derived factor-1; BMSCs, bone marrow mesenchymal stem cells; CXCR4: SDF-1 receptor; AMD3100: CXCR4 antagonist .



*	= p < 0.05
**	= p < 0.01
***	= p < 0.001
NS	= not significant

OD = Optical density measured at 405 nm.



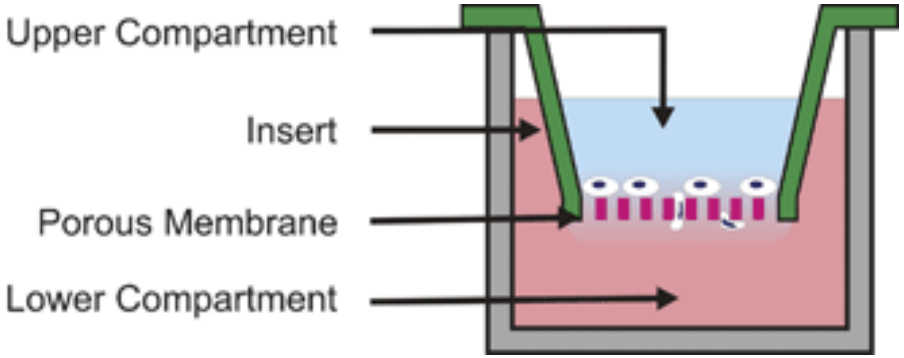


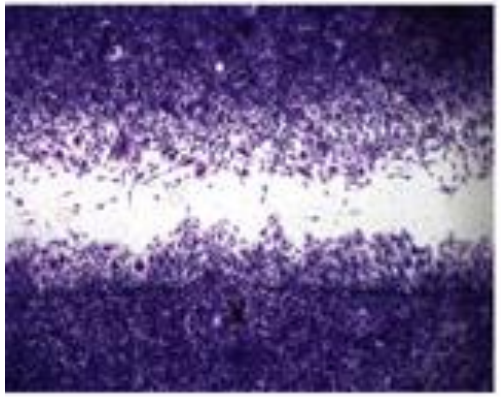
Cells were transiently co-transfected with the following combination of expression vectors: empty vectors (mock); ErbB4; ErbB4 + Eps8; Eps8.

- 48 hours after transfection, Transwell assays were performed in 2% FBS DMEM either without (white bars) or with (black bars) 5 nM NRG1.
- migration was calculated as percentage of total migrated cells.

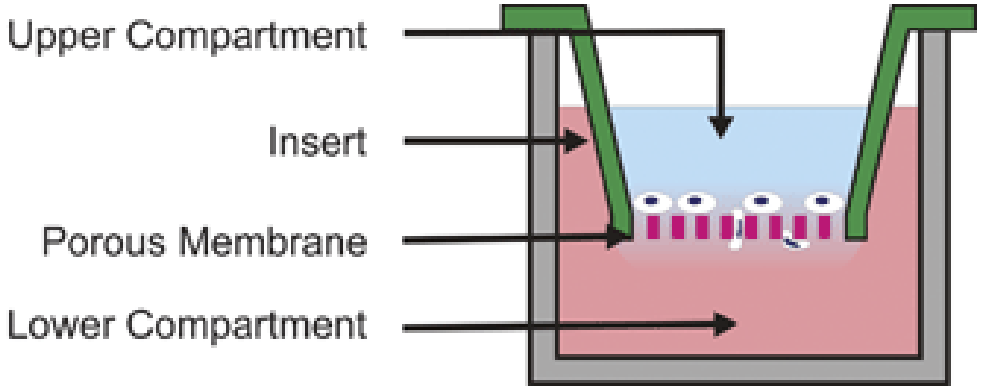
Biological triplicate experiments were carried out in a technical triplicate.

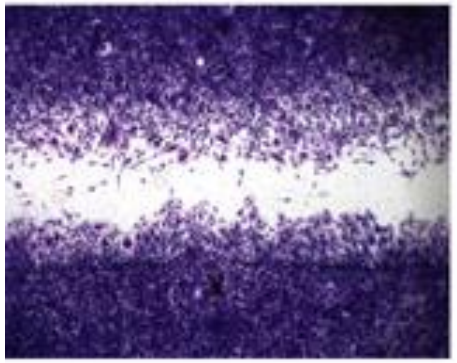
Data are represented as means + SEM **p≤0.01, ***p≤0.001.





	Boyden Chamber/Transwell Assays	2D Migration Gap Closure Assays
Analysis	Quantitative	Qualitative or Quantitative
Detection Time	Endpoint	Endpoint or Real Time
Detection Method	Plate Reader/Microscopy	Microscopy
Cell Compatibility	Choose membrane pore size to match cell type	Any
Chemoattractant Gradient	Yes	No
Sensitivity	Fair	Good
Adaptability to Automation	Poor	Good
Most Suitable Application	Measure effect of chemoattractant on migration rates	Measure differences in migration rates between treated and untreated cells





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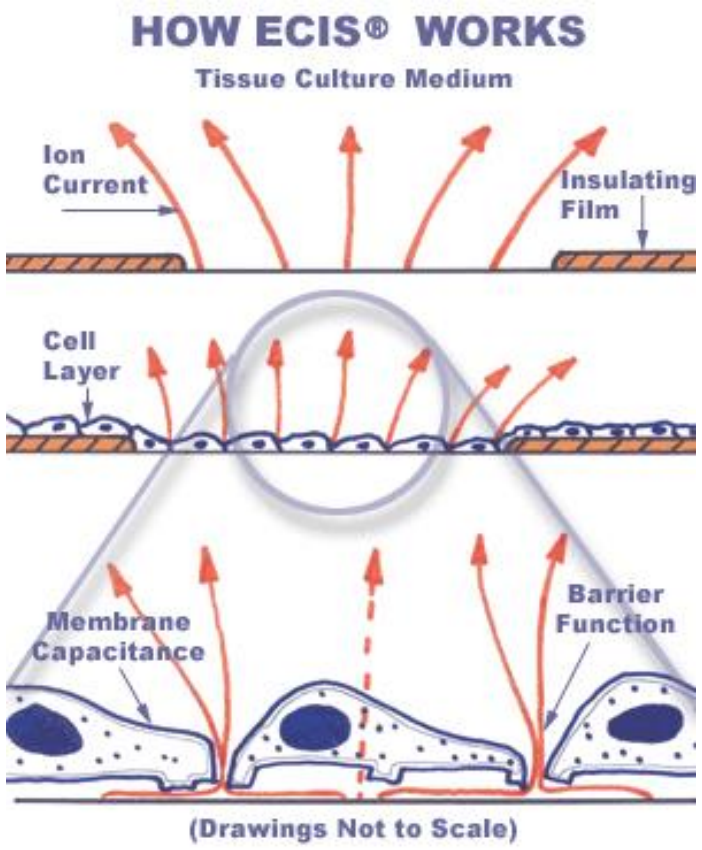
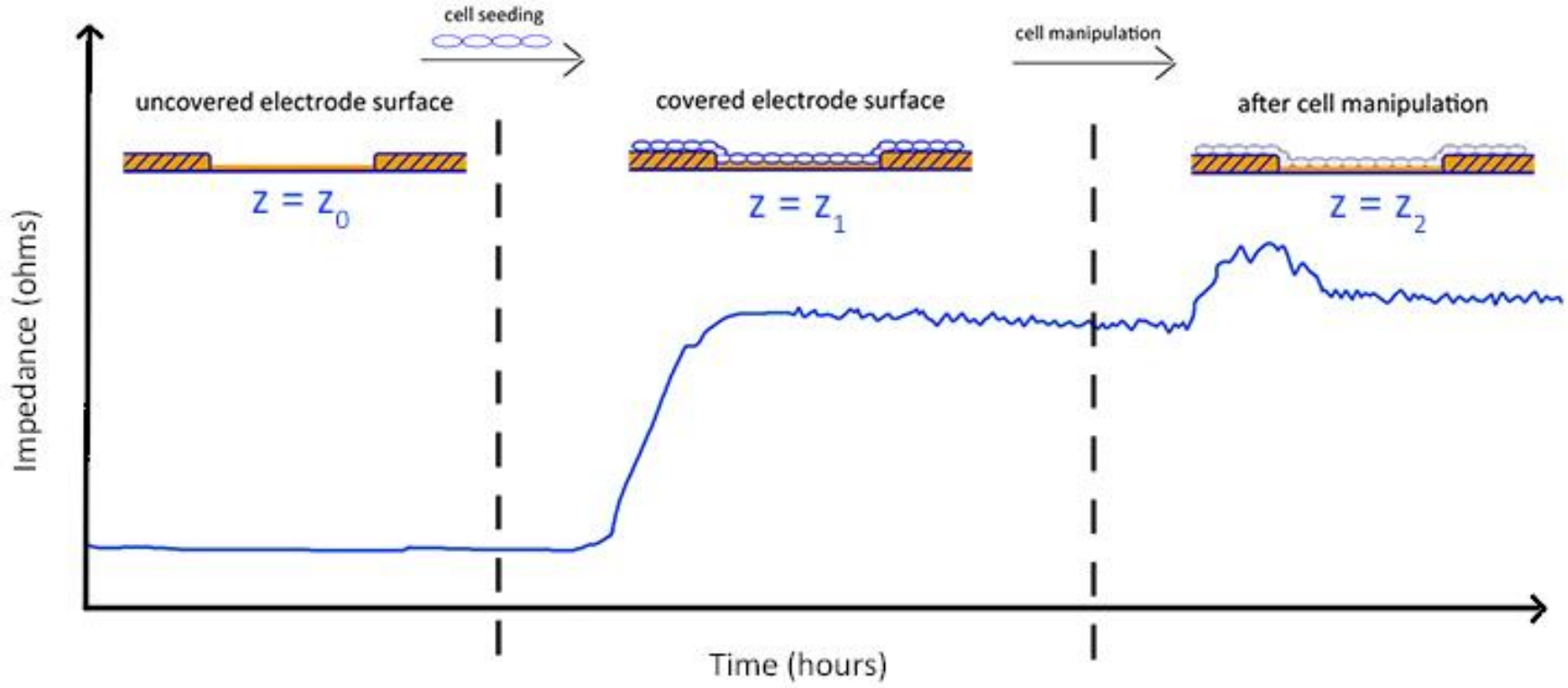
ECIS® (Electric Cell-substrate Impedance Sensing)

ECIS is a real-time, label-free, impedance-based method to study the activities of cells grown in tissue culture. These include morphological changes, cell locomotion, and other behaviors directed by the cell cytoskeleton.

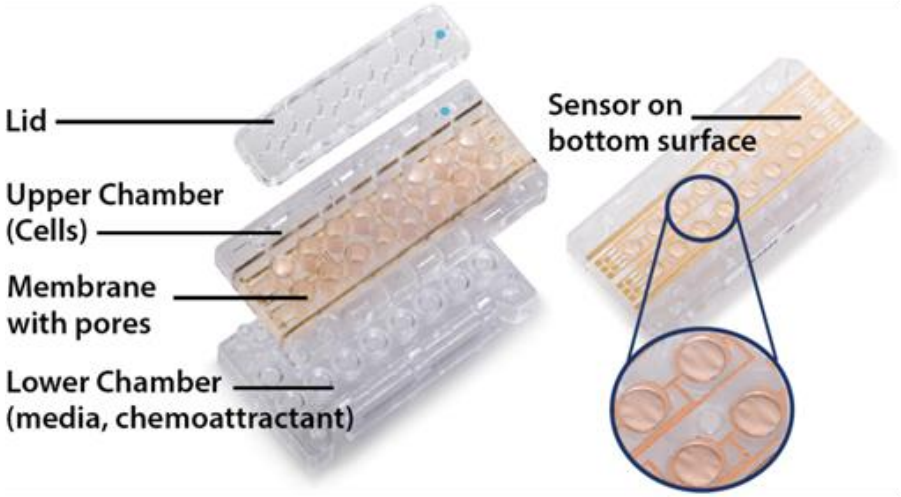




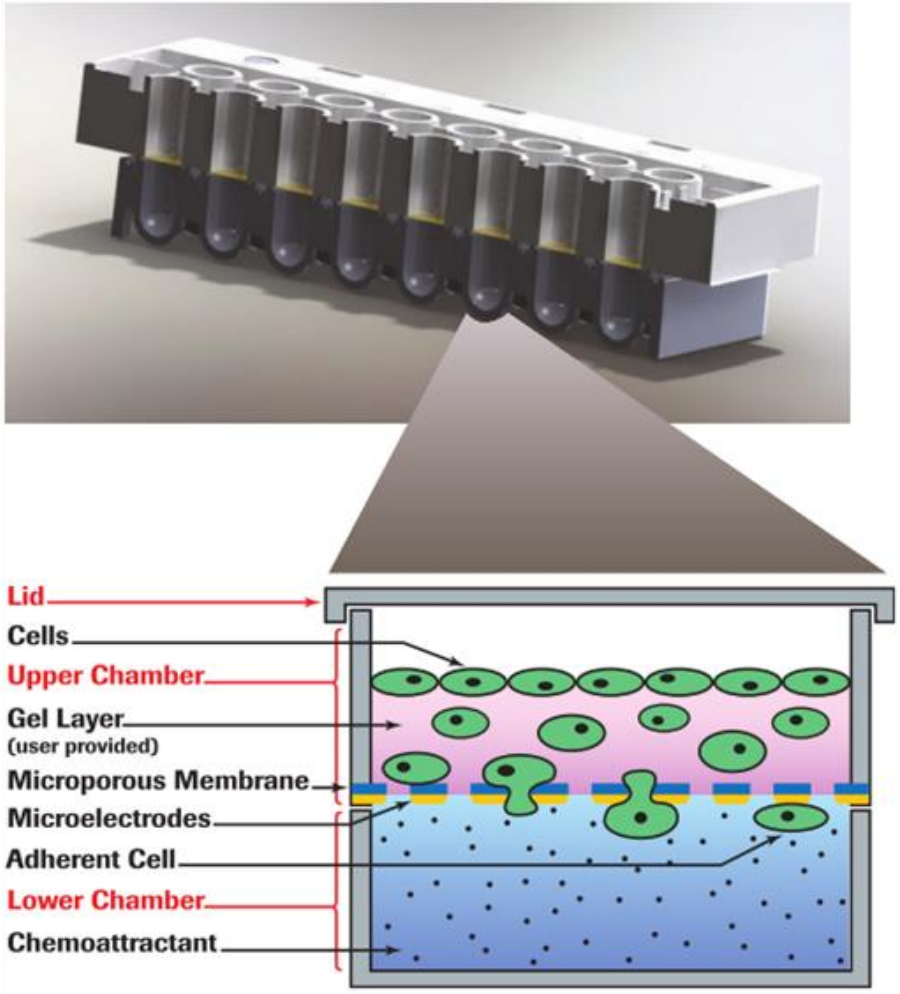
When cells attach to the electrodes, they act as insulators increasing the impedance. As cells grow and cover the electrodes, the current is impeded in a manner related to the number of cells covering the electrode, the morphology of the cells and the nature of the cell attachment.

When cells are stimulated to change their function, the accompanying changes in cell morphology alter the impedance. The data generated is impedance versus time.

Quantitative real-time analysis of cell invasion/migration.



B

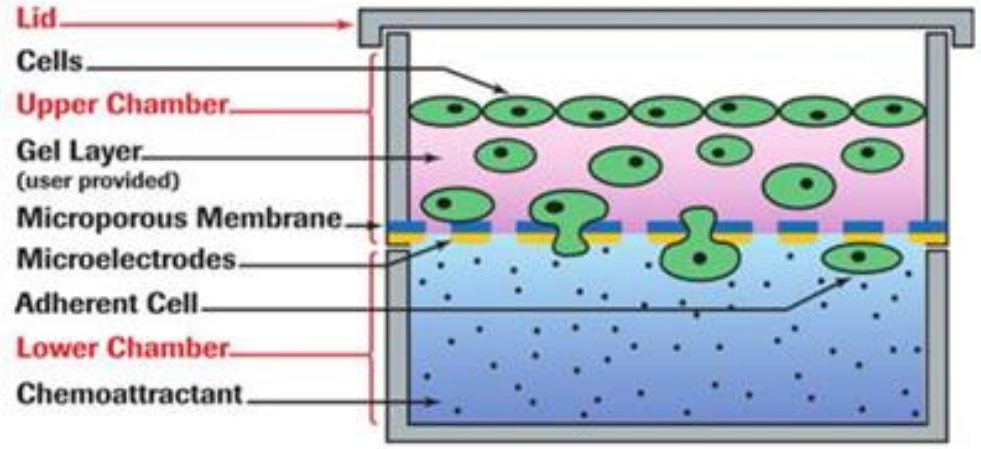


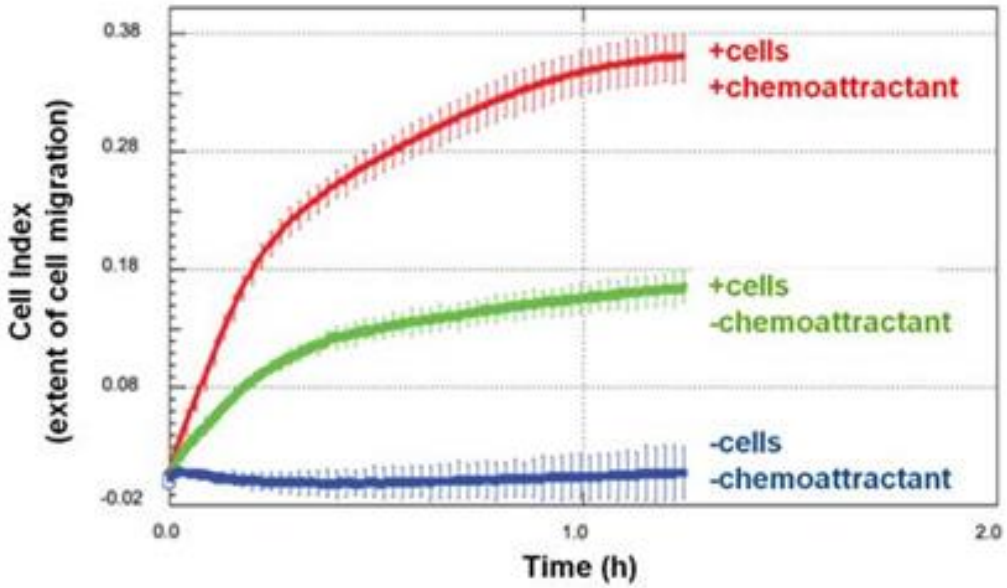
xCELLigence® RTCA DP system

The figure illustrates the upper and lower chambers *for a single well*. The bottom surface of the upper chamber is composed of a microporous membrane that cells can migrate through. Gold electrodes on the underside of this membrane detect the presence of adherent cells.

For a simple migration assay (not illustrated here) the cells being monitored would be plated directly onto the membrane. For an invasion assay (shown here), cells are plated on top of a basement membrane matrix, a cellular monolayer, or some combination thereof.

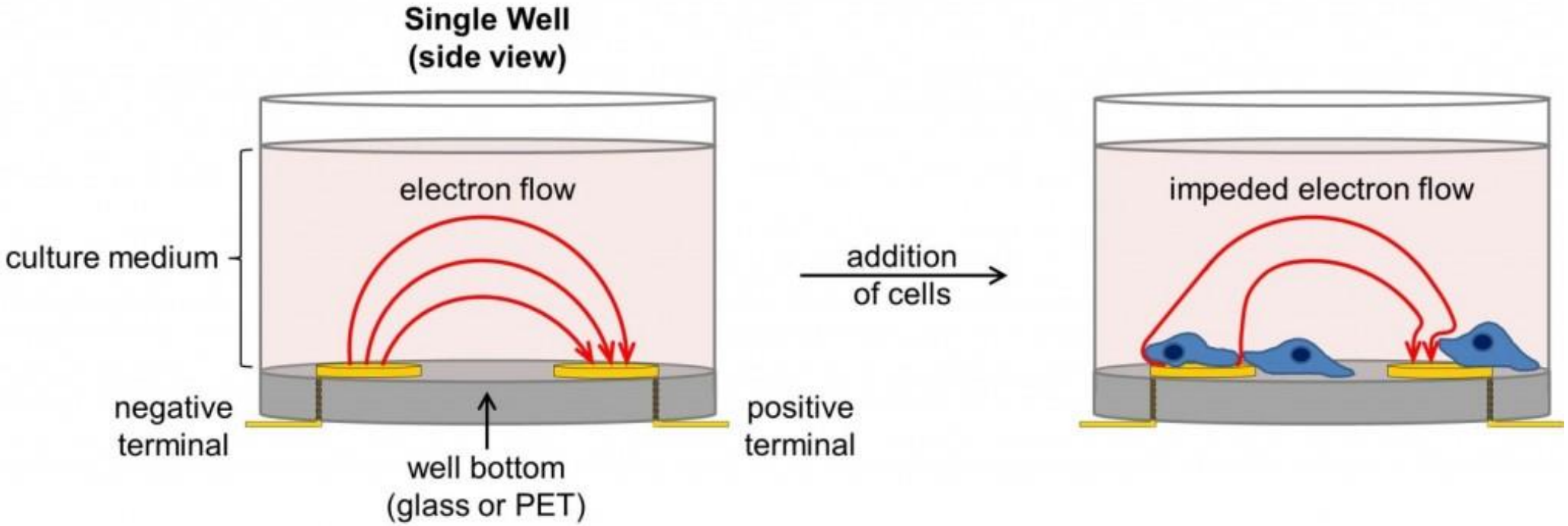
Quantitative real-time analysis of cell invasion/migration.





As cells move from the upper chamber towards chemoattractant in the lower chamber, they pass through a membrane containing 8 µm pores and then adhere to gold impedance microelectrodes**.** The resultant **change in impedance signal perfectly correlates with the number of cells** attached to these electrodes, enabling collection of highly reproducible data over time ranges spanning from minutes to days.

- **negative control; blue line**: in the absence of cells the impedance signal is unchanged over the 75 minutes of the assay;
- **green line**: in the absence of chemoattractant some cells migrate through the porous membrane;
- **red line**: in the presence of chemoattractant in the lower chamber, migration is significantly stimulated.



Set of gold microelectrodes fused to the bottom surface of a microtiter plate well.
When submersed in an electrically conductive solution (such as buffer or standard tissue culture medium), the application of an electric potential across these electrodes causes electrons to exit the negative terminal, pass through bulk solution, and then deposit onto the positive terminal to complete the circuit. Because this phenomenon is dependent upon the electrodes interacting with bulk solution, the presence of adherent cells at the electrode-solution interface impedes electron flow.
The magnitude of this impedance is dependent on the number of cells, the size and shape of the cells, and the cell-substrate attachment quality.
Importantly, neither the gold microelectrode surfaces nor the applied electric potential (22 mV) have an effect on cell health or behavior.

BOX 1 | COMPARISON OF DIFFERENT *IN VITRO* MIGRATION ASSAYS

	In vitro scratch assay	ECIS	Boyden chamber	Microfluidics-based system
Cell type suitable for analysis	Adherent cells, monolayer of cells are required	Adherent cells, monolayer of cells are required	Adherent cells	Adherent cells
Sample pool for analysis	Population or individual cells	Population or individual cells	Population of cells	Primarily for individual cells
Ability to track individual cells	Yes	Yes	No	Yes
Gradients	No	Possible for ECM	Yes for both ECM and soluble factors	Yes for both ECM and soluble factors
Sample size in one assay	One condition per tissue culture plate	Up to 96 different conditions for one experiment	Up to 96 different conditions for one experiment	One condition per assay chamber
Complexity of equipment set up	Extremely easy	Commercial plates are available, easy to set up	Commercial chambers are available, easy to set up	Nanofabrication facilities required, difficult to set up
Incubation time before the assay	1–2 days	1–2 days	None	None
Time of the assay	Cell types-dependent (average 14 h)	Cell types-dependent (average 10 h)	Cell types-dependent (average 6 h)	Cell types-dependent (average 4 h)
Data collection and analysis	Cell counting with microscope, and image processing hardware/software	Special-designed devices and software for detection	Cell counting with microscope, and image processing hardware/software	Cell tracking with microscope, and image processing hardware/software
Cost of equipment	No extra cost than routine tissue culture, inexpensive	Commercial instruments and plates, expensive	Boyden chambers and filter membranes, inexpensive	Nanofabrication facilities are required, very expensive

ECIS (Electric Cell-substrate Impedance Sensing)

HOW TO STUDY CHEMOTAXIS AND CHEMOCHINESIS

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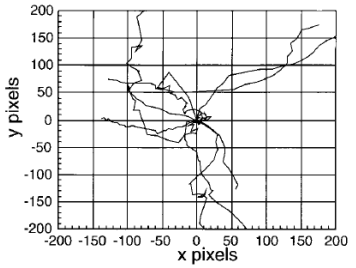

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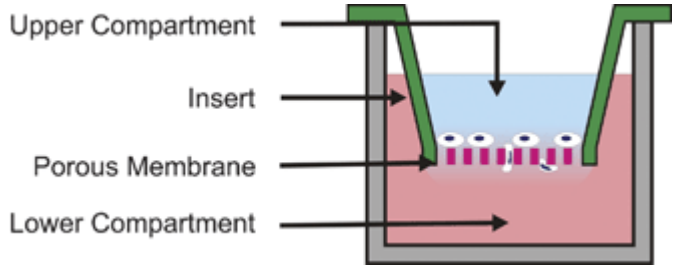
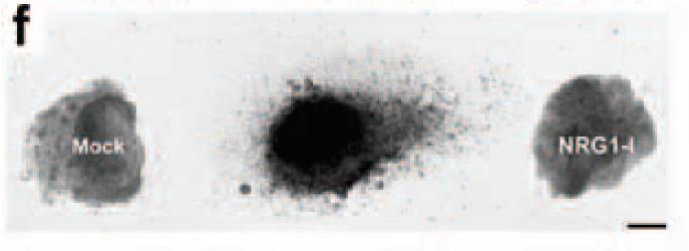


- explant migration



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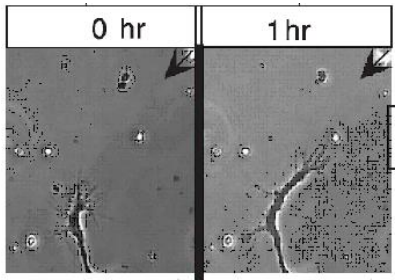
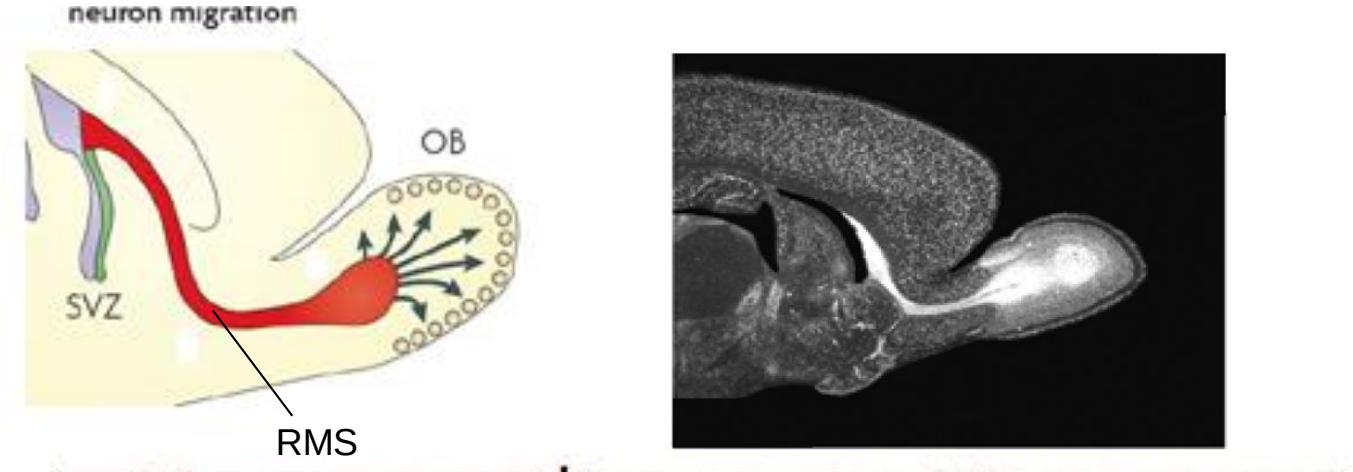
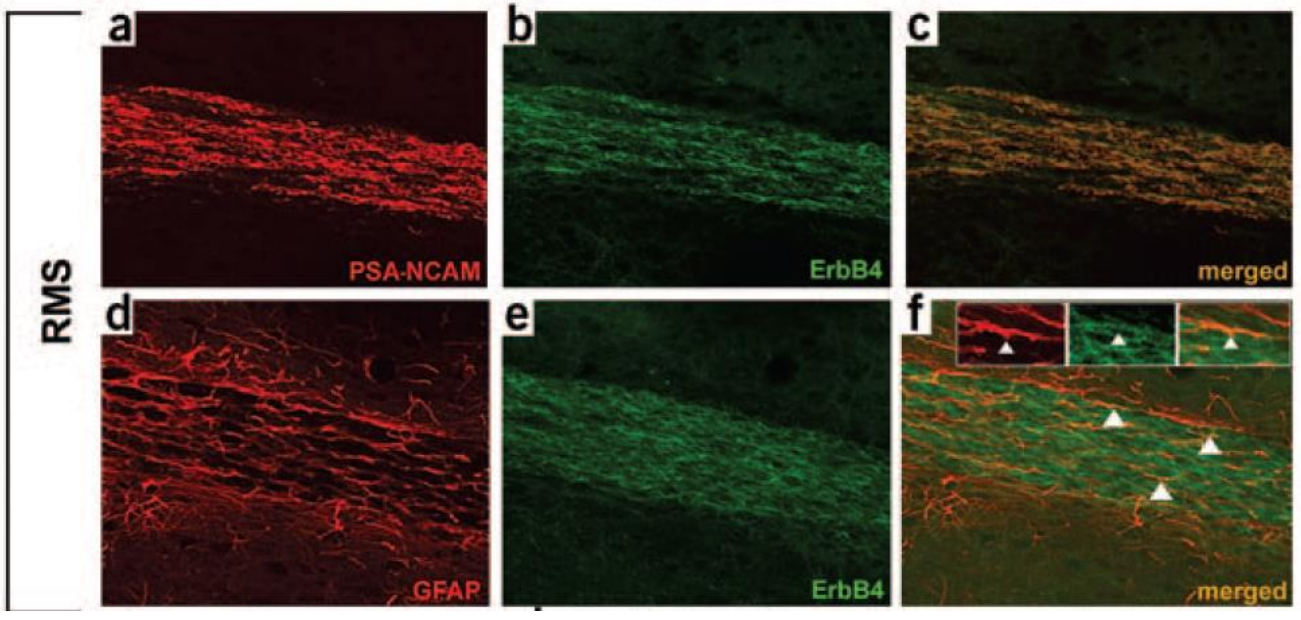


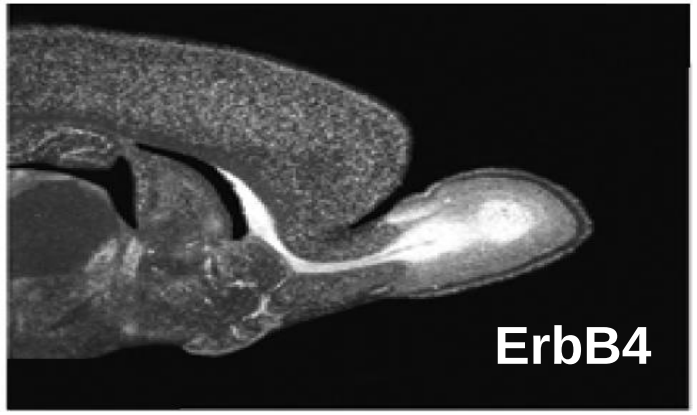
Receptor tyrosine kinase ErbB4 modulates neuroblast migration and placement in the adult forebrain

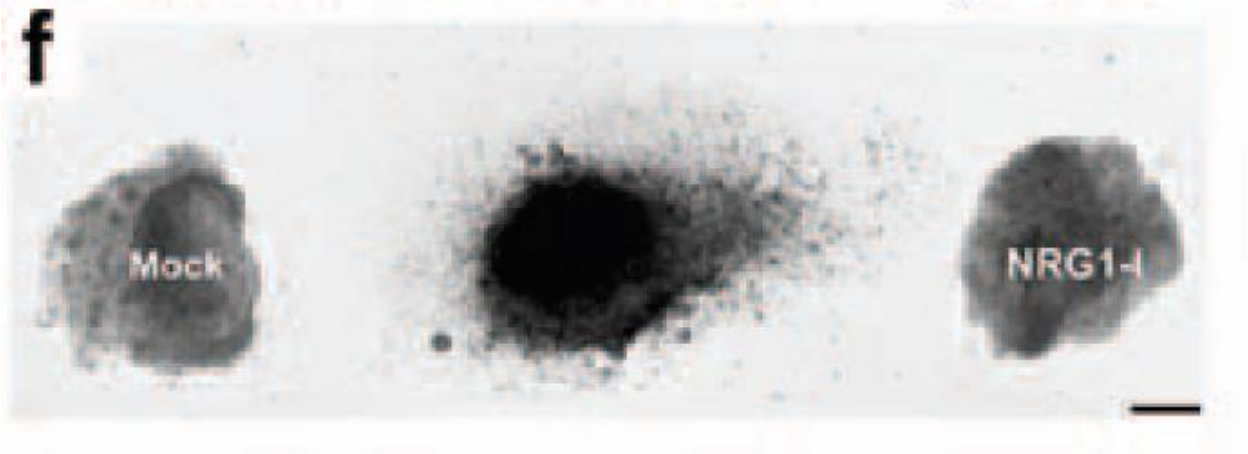
E S Anton[1,8], H T Ghashghaei[1,8], Janet L Weber[2], Corey McCann[1], Tobias M Fischer[2], Isla D Cheung[2], Martin Gassmann[3], Albee Messing[4], Rudiger Klein[5], Markus H Schwab[2,6], K C Kent Lloyd[7] & Cary Lai[2]

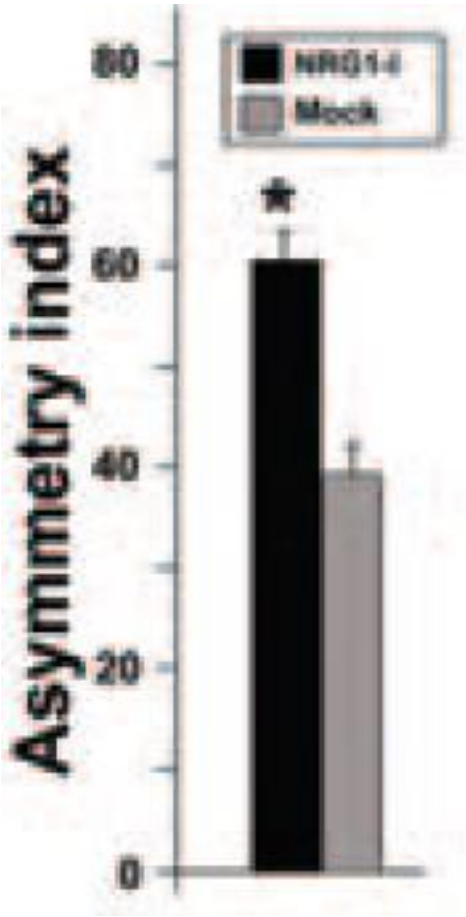




in vivo ErbB4 expressing neurons are attracted by soluble NRG1



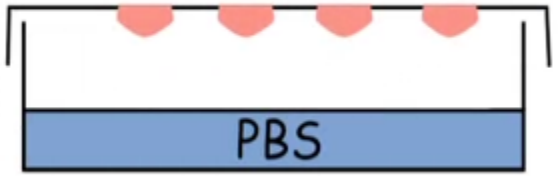


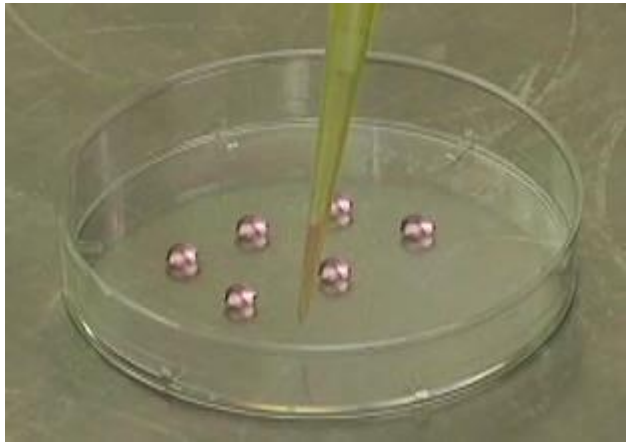


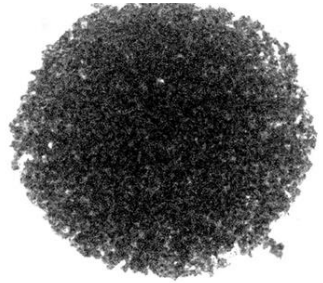
(Anton *et al*, 2004)

How can you obtain cell aggregates expressing a ligand?

HANGING DROP TECHNIQUE

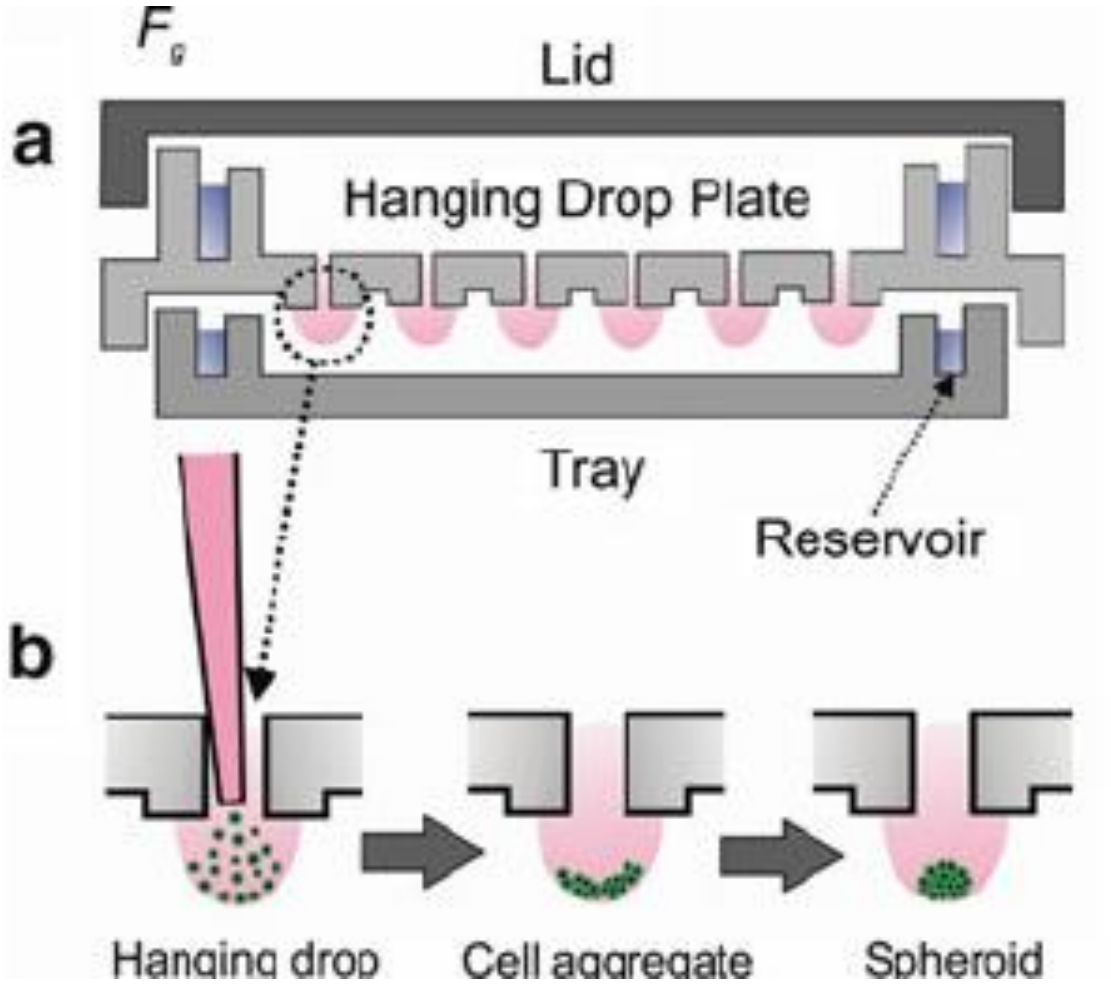


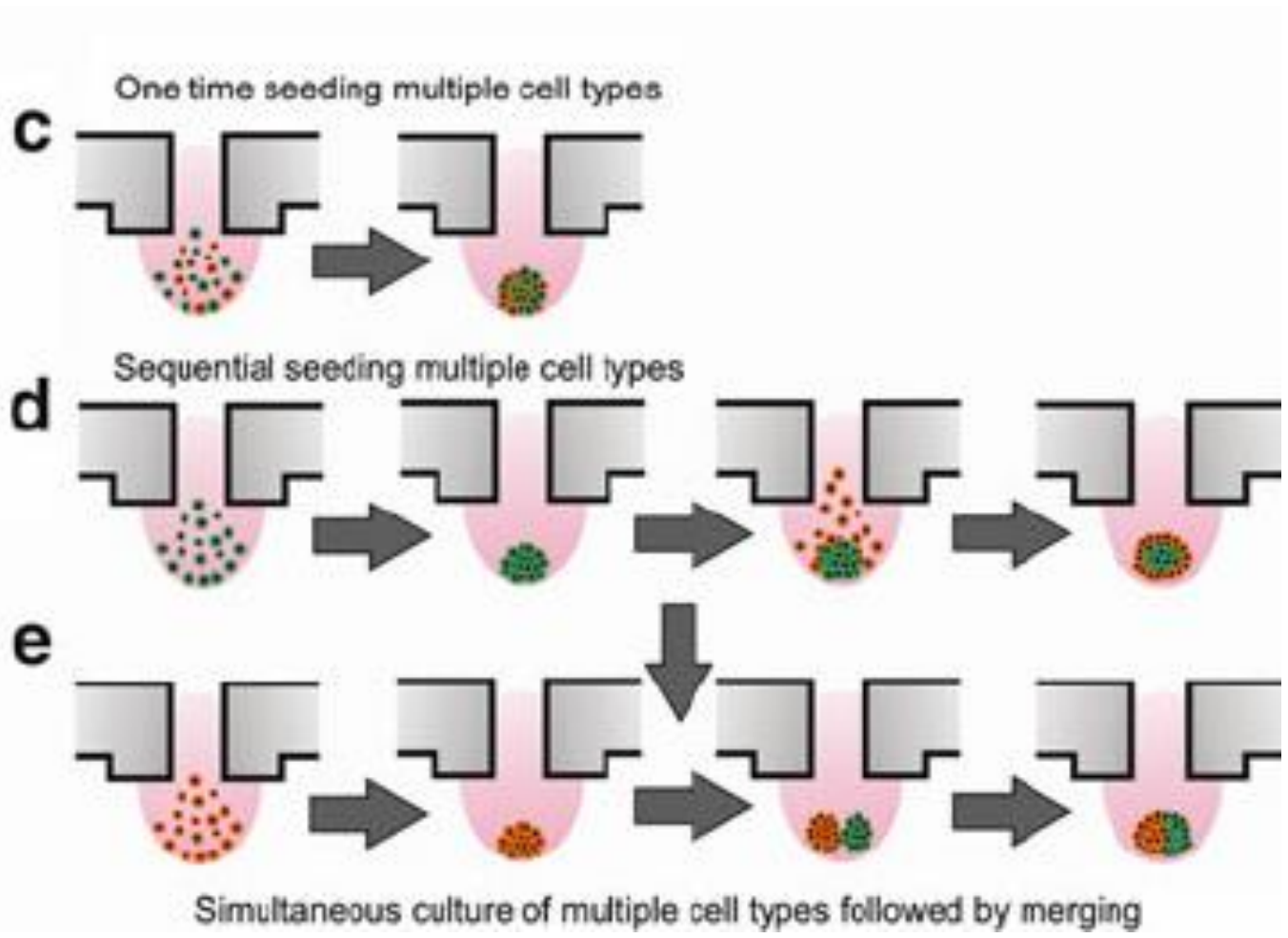




Cells are previously transiently transfected to obtain expression and release of the soluble factor that must be tested.

HANGING DROP TECHNIQUE





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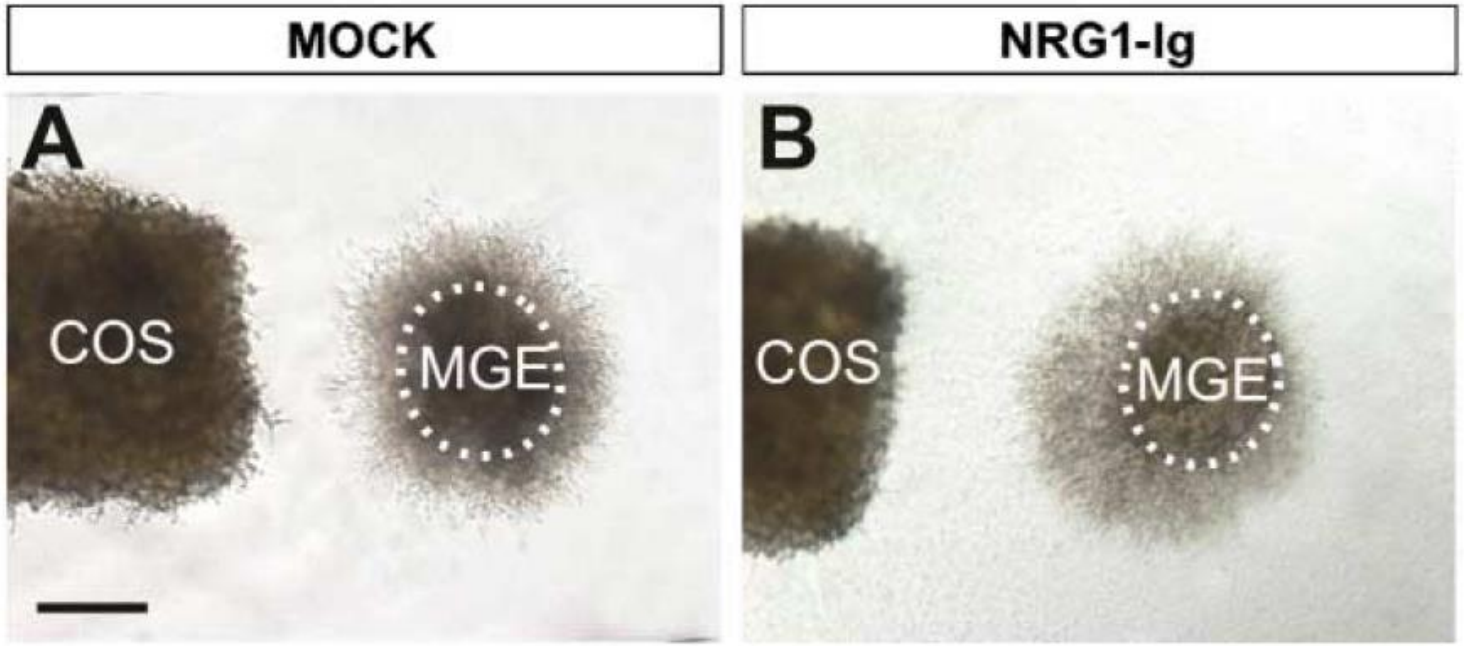
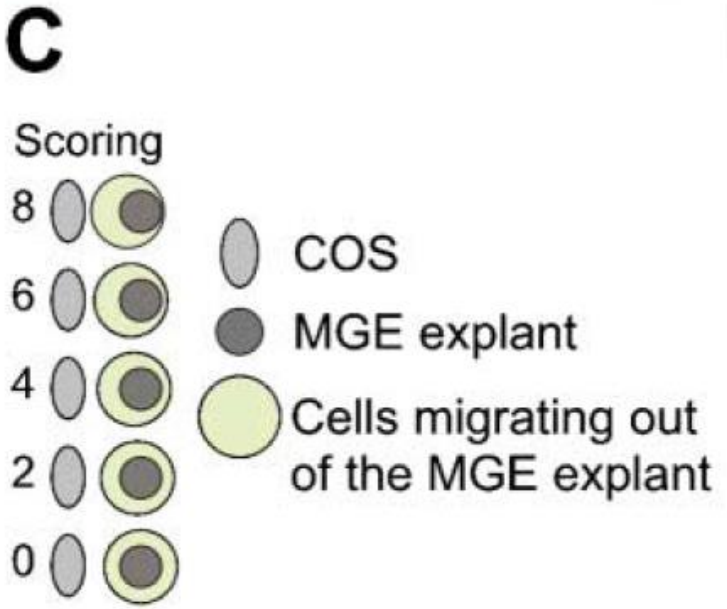
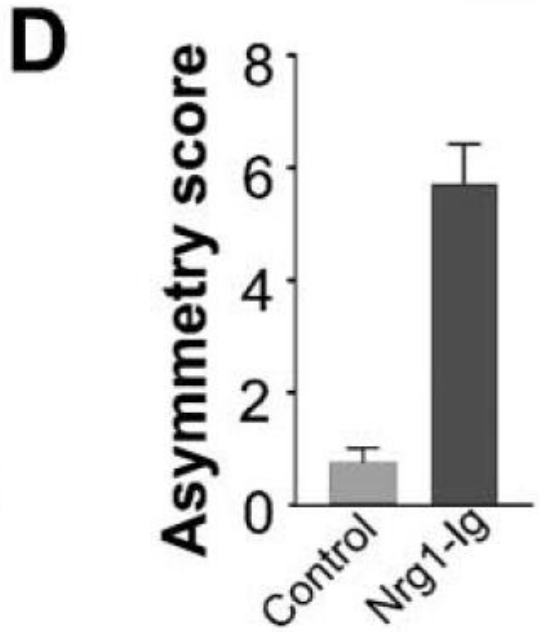
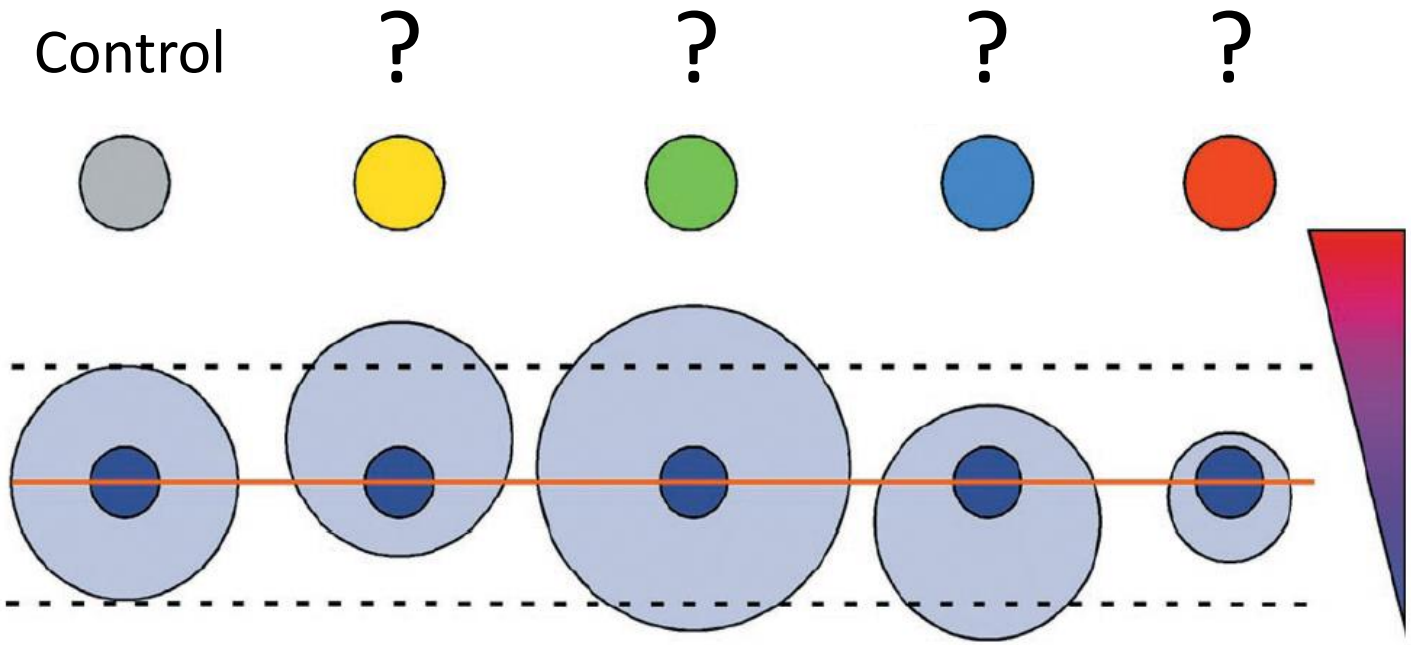




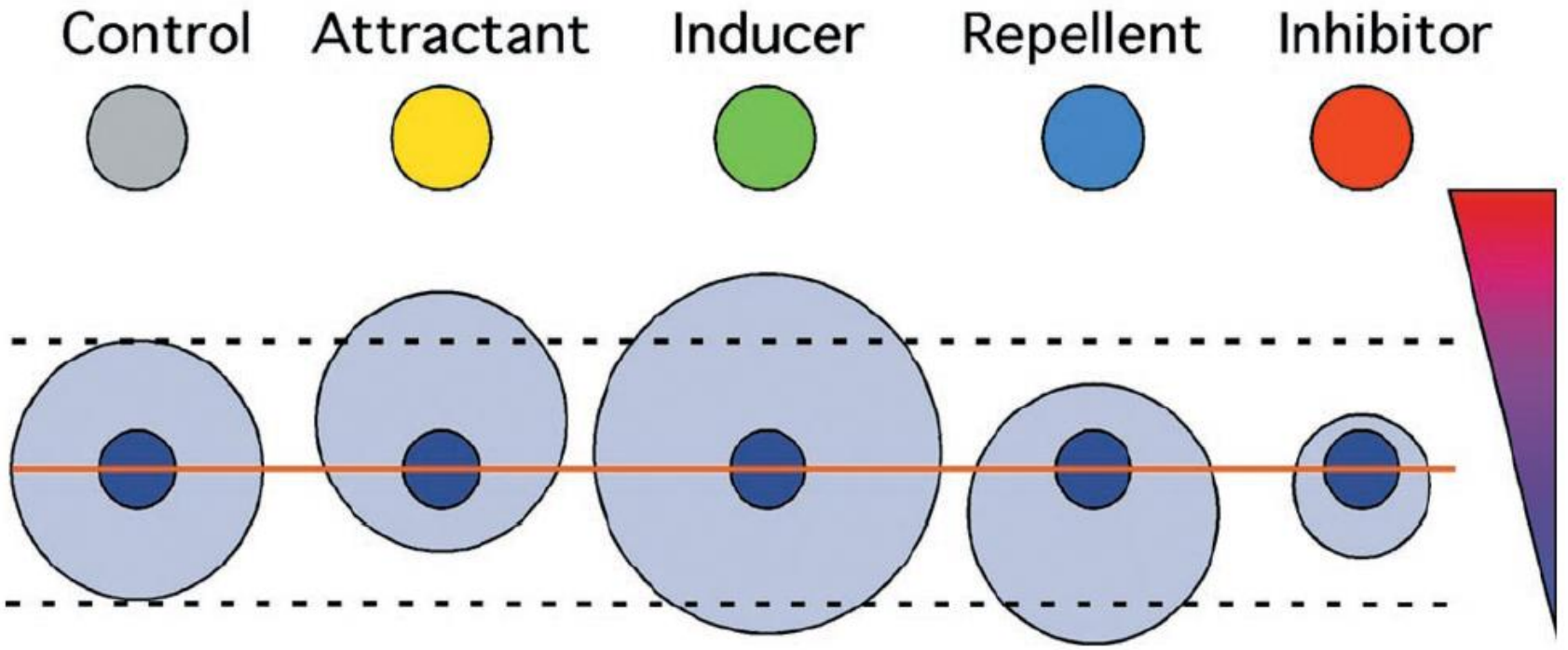


(Flames *et al,* 2004)

What is “blind analysis”?



How can you define the behaviour of these explants?



The *top colored circles* represent aggregates of cells secreting putative regulators of migration. The *small blue circles* represent explants, and the *larger light-blue circles* represent the migrating cells. The size of the circle signifies the number of cells; their location relative to the *inner circle* shows the preferred direction of migration. The *orange line* shows the separation between the distal and proximal hemispheres; the *dashed lines* facilitate the comparison between control and experimental points. The *triangle* on the *right* depicts an expected concentration gradient of the molecules.

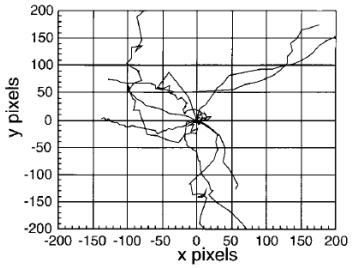
COS Cell aggregates

COS7 cell aggregates expressing *Gfp*, *Nrg1-Ig*, or both were prepared by diluting transfected cells with Matrigel in a 1:1 proportion.
After jellification, COS cell aggregates were cut with a scalpel in small cubes with dimensions of approximately 400 µm. In control experiments, a control vector was transfected together with *Gfp* into COS7 cells. The sequences of the cDNAs used for expression of type I NRG1 (Nrg1-Ig) and type III NRG1 (NRG1-CRD) correspond to Nrg1-typeI1a (accession number AY648976) and Nrg1-typeIII1a (accession number AY648975), respectively.

(Flames *et al,* 2004)

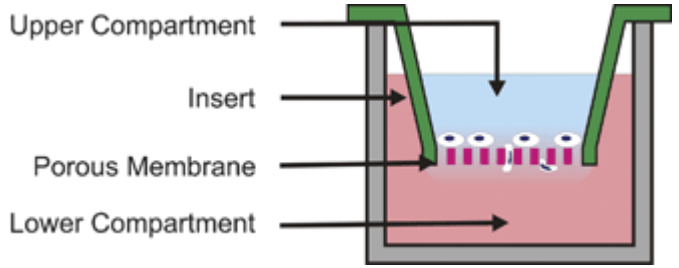
HOW TO STUDY CHEMOTAXIS AND CHEMOCHINESIS

- time lapse video microscopy

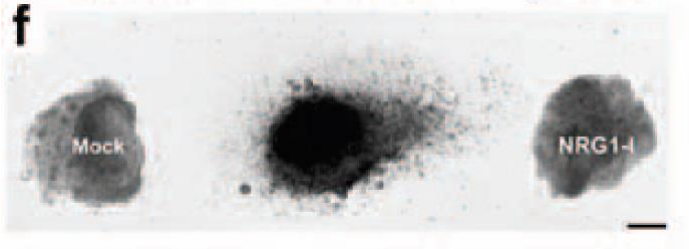


- wound healing

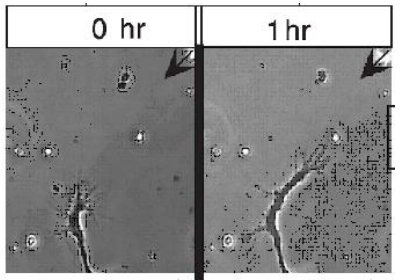
- transwell assay/Boyden's chambers

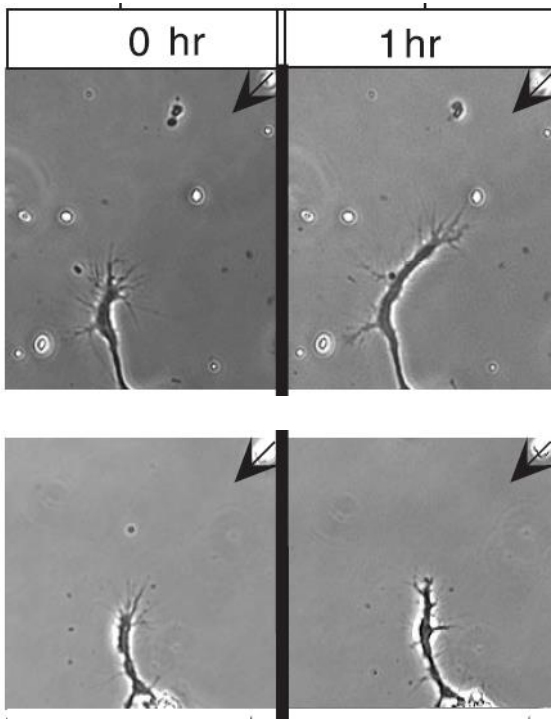


- explant migration

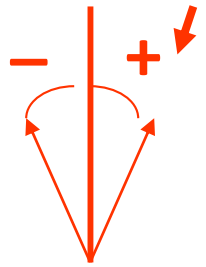


- turning assay



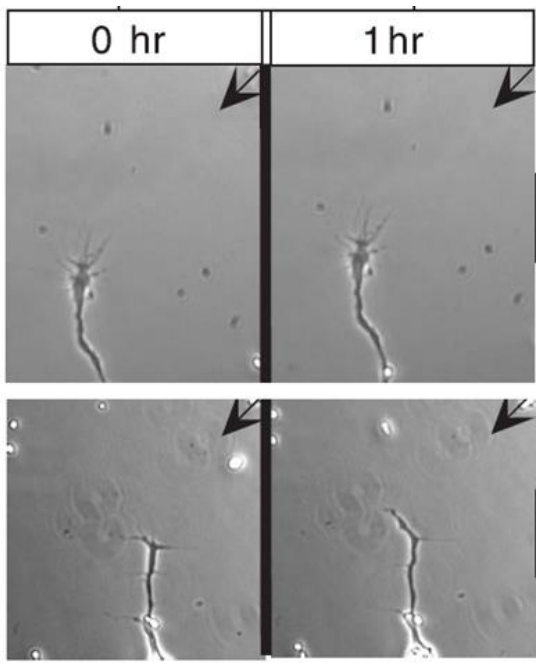


turning angle

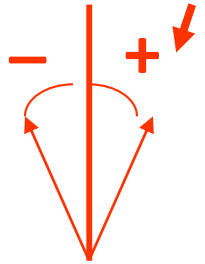


TURNING ASSAY

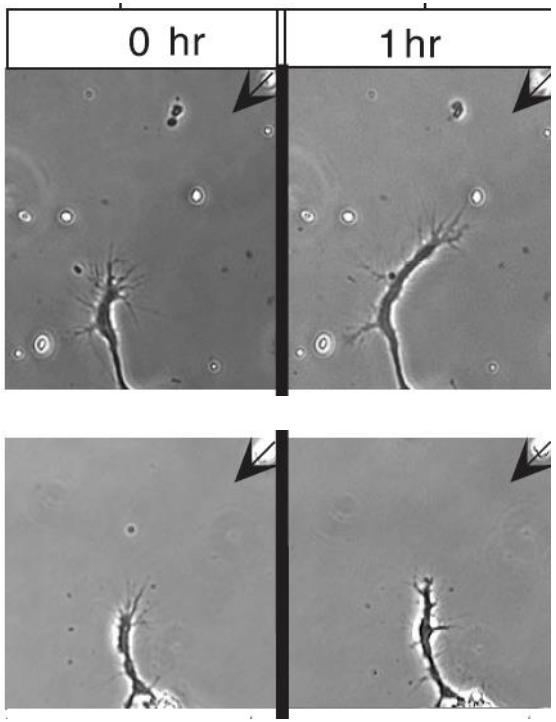
?



turning angle

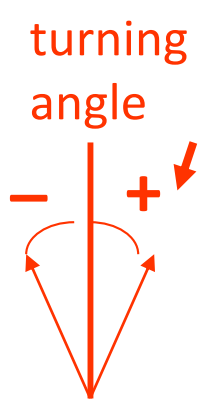


TURNING ASSAY

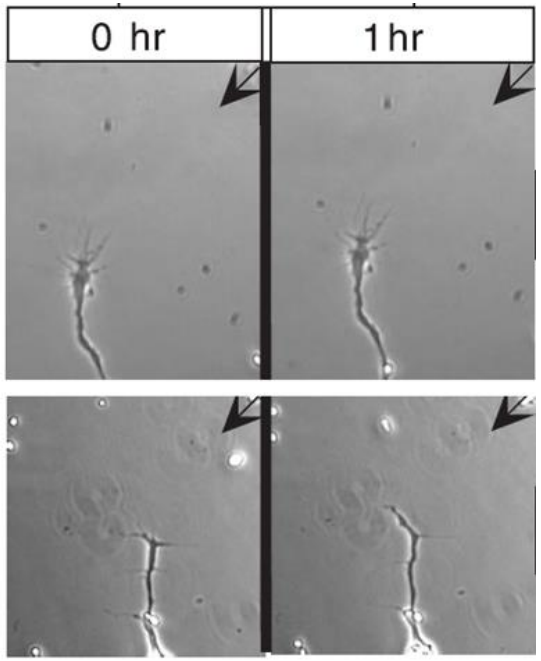


When growth cones of neurons from stage 22 Xenopus embryos are exposed to a gradient of netrin1 for 1 hour, they turn toward the source.

The same axons exposed to a gradient of Slit2 protein did not show a directional response.

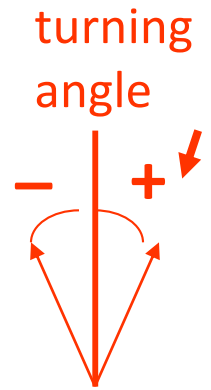


TURNING ASSAY



The axons of spinal neurons obtained from stage 28 Xenopus embryos did not show any response to netrin1.

The same axon were repelled by Slit2.



TURNING ASSAY