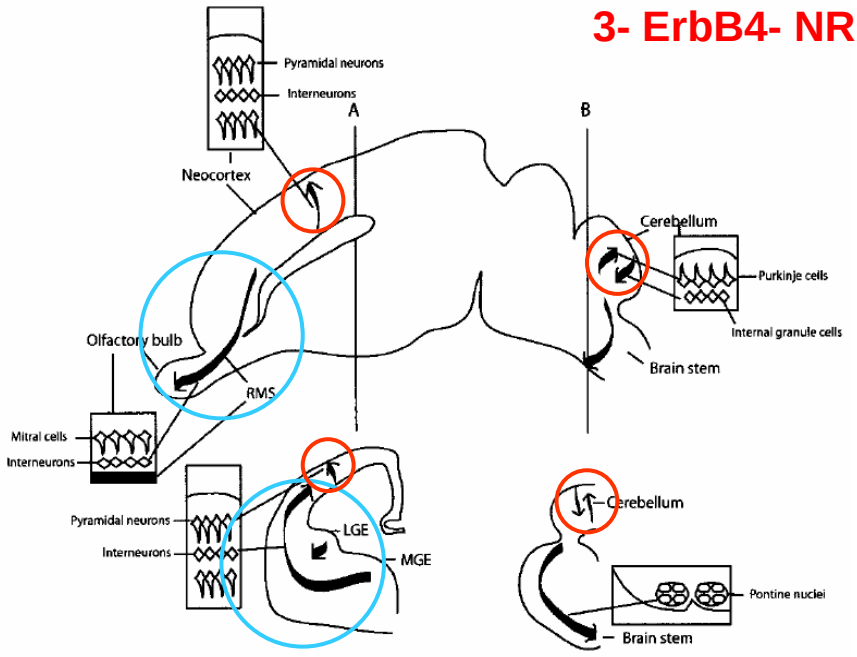
ErbB4 migrazione I parte

3- ErbB4- NRG1



NATURE NEUROSCIENCE ADVANCE ONLINE PUBLICATION

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

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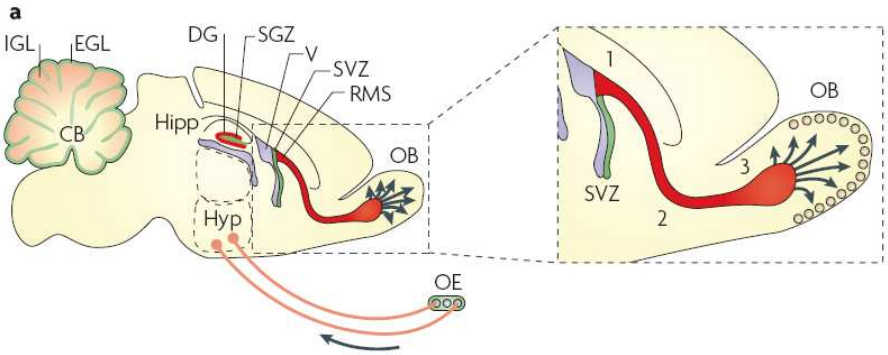
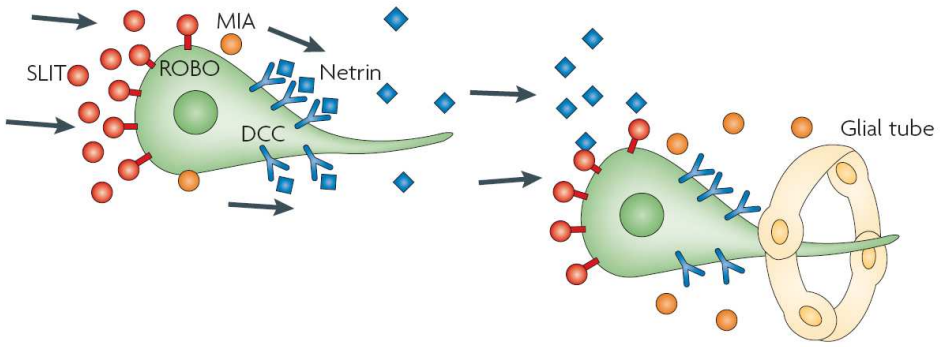


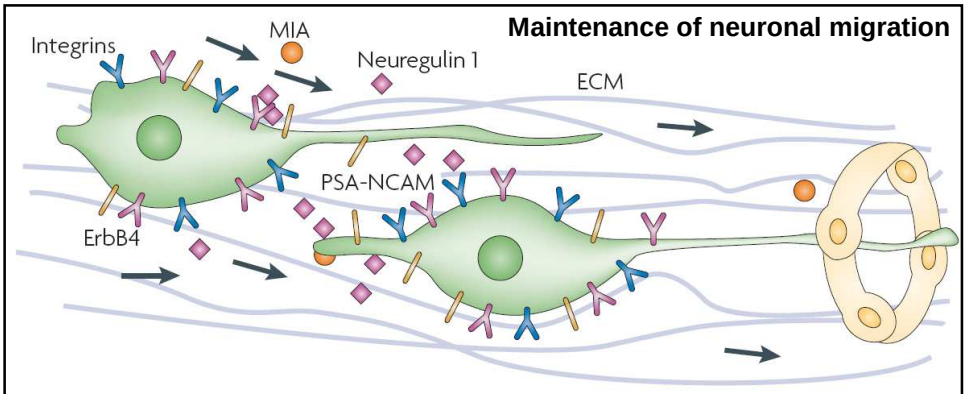
In rodent brains postnatal neuronal migration is evident in three main areas: the cerebellum (CB), the hippocampus (Hipp) and the rostral migratory stream (RMS). A small number of neurons also complete their migration into the hypothalamus (Hyp) at around the time of birth.

Initiation of neuronal migration



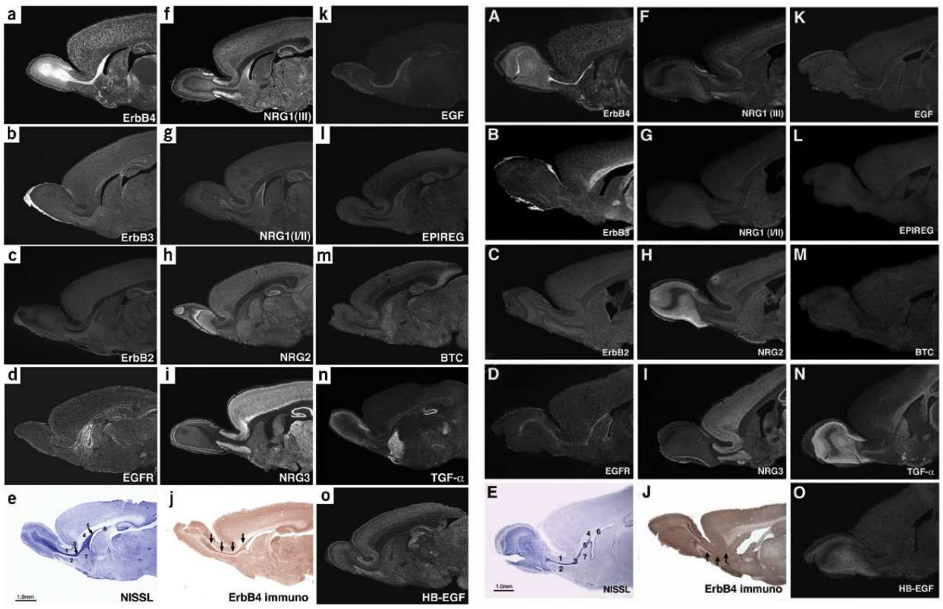
The initiation of neuronal migration is regulated by a combination of motogenic (for example, migration-inducing activity, MIA, secreted by glial tubes surrounding the neuronal chains), chemoattractive (such as netrin and deleted in colorectal carcinoma, DCC) and chemorepulsive (for example, SLIT and ROBO) signals.
Arrows indicate the direction of migration away from the subventricular zone towards the rostral migratory stream.

Maintenance of neuronal migration

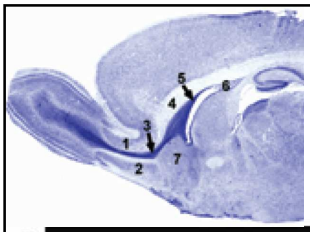


Neuroblasts migrate as chains, sliding along each other. Maintenance of chain migration is dependent on the continued motogenic activity of migration-inducing activity (MIA, orange), neuregulin 1–ErbB4 interactions (both purple), adhesion mediated by the polysialated form of neuronal cell adhesion molecule (PSA-NCAM, yellow) and extracellular matrix (ECM)–integrin signalling (blue). Arrows indicate the direction of migration towards the olfactory bulb.

The expression of the ErbB receptors in the RMS and SVZ of the forebrain of postnatal day (P) 11 and adult rats was determined using *in situ* hybridization

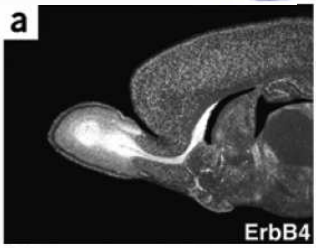


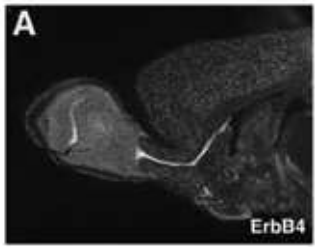
P11 **adult**



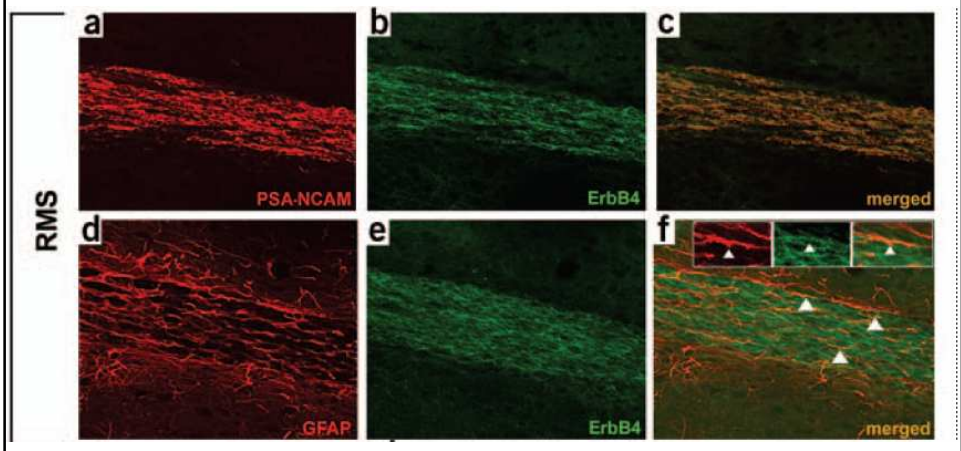
1, anterior olfactory nucleus, dorsal;
2, anterior olfactory nucleus, ventral;
3, RMS;
4, corpus callosum;
5, SVZ;
6, lateral ventricle;
7, striatum

In situ hybridization: quali informazioni ne traggo? Quali altre analisi posso effettuare?



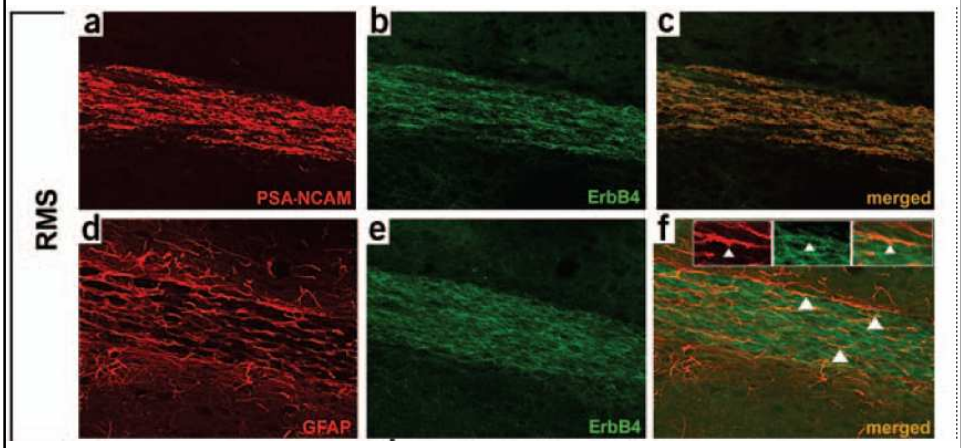


- at P11, ErbB4 is expressed at high levels in the RMS and remains detectable in these cells as they migrate into the OB
- expression persists in granule neurons in the mature OB, but at reduced levels
- ErbB4 is also expressed in scattered cells in the striatum.
- in adults, ErbB4-specific hybridization in the RMS were lower than in P11 rats

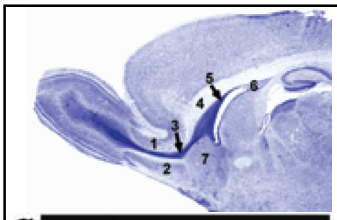


PSA-NCAM migrating neuroblast marker
GFAP^{+} glial cell marker

immunohystochemistry: quali informazioni ne traggo?



→ near-perfect colocalization of ErbB4 and PSA-NCAM in the adult RMS, indicating that this receptor was expressed by the migrating neuroblast subpopulation. ErbB4 was also detected in a small subset of GFAP$^+$ glial cells dispersed in the RMS



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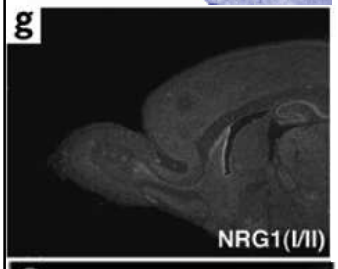
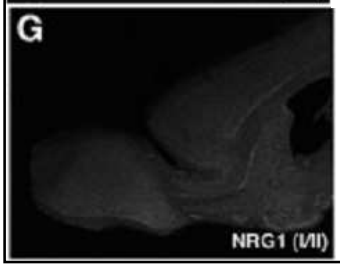
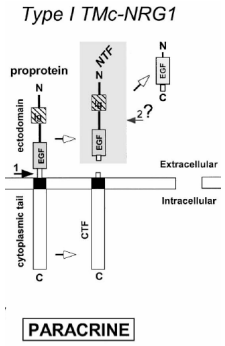
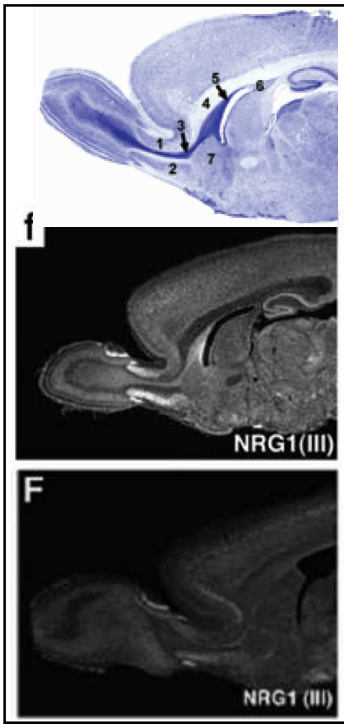




• the type I and II isoforms were detected at low levels in the stream both at P11 and in the adult and were also observed in the anterior lining of the ventricle at P11





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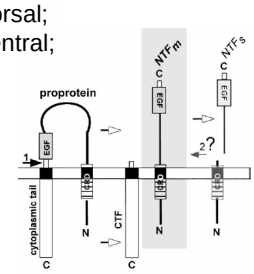
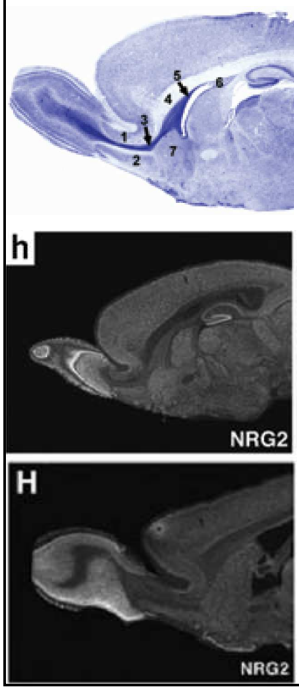


- the type III form was expressed at high levels in the RMS at P11 and was particularly noticeable in the regions surrounding the RMS, including in the anterior olfactory nucleus, in the internal granule cell layer as well as in the mitral cell and glomerular layers in the OB

- in the adult, the type III isoform was detected in the same regions, but at reduced levels



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- NRG2 was present at very low levels in the stream and at high levels in the internal granule cell, external plexiform and glomerular layers of the OB at P11 and in the adult.

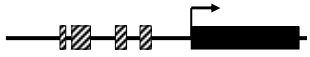
These studies showed that:

- ErbB4 is expressed by the migrating neuroblasts and at least some of the proliferating precursors of the SVZ

- multiple potential ligands are located in the forebrain region, where they may influence neuroblast proliferation, migration and differentiation through ErbB4

Conditional knock-outs

inactivate a gene only in specific tissues and at certain times during development and life.

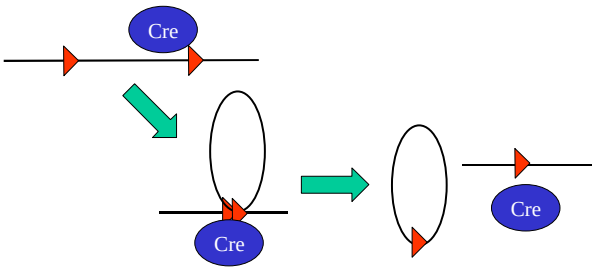


Promoter element **Cre**

Cre-lox technology

Cre – a site-specific recombinase enzyme from the P1 phage.

Recognises a 34bp DNA sequence *loxP* =



Your gene of interest is **flanked by 34 bp *loxP* sites (floxed).**

Where CRE recombinase is expressed at least once

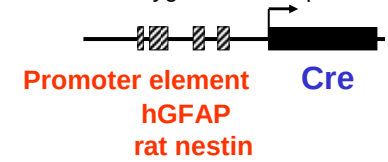
Gene between loxP sites is removed

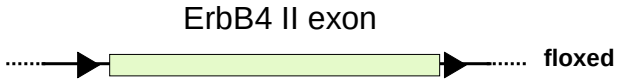
CONDITIONAL DELETION OF ErbB4

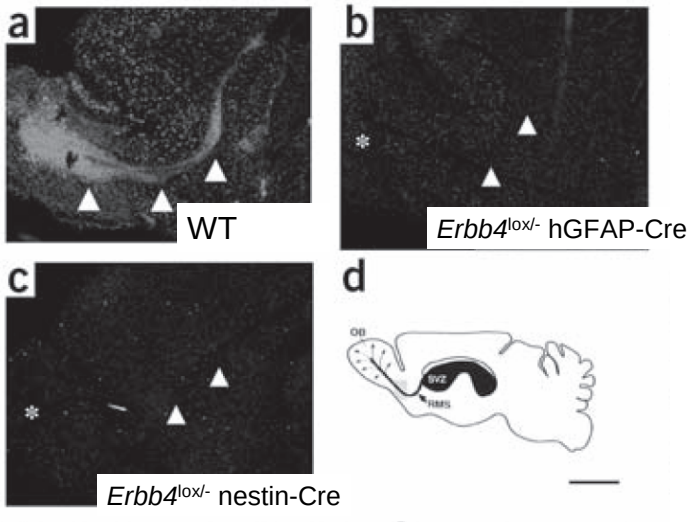
- lines of conditional null mice were generated that lacked ErbB4 expression in the CNS to evaluate the role of this receptor in the SVZ and RMS

- mice in which the second exon of the gene ErbB4 was flanked by *lox*P sites were mated with transgenic mice that expressed Cre recombinase under the control of either the human GFAP (hGFAP) promoter or the rat nestin promoter and enhancer

- to maximize the number of cells with a complete loss of ErbB4 function, they mated the mice carrying *lox*P sites (*ErbB4*^{lox}) with the mice carrying Cre recombinase in a background that is heterozygous with respect to the null allele of ErbB4 (Gassmann).



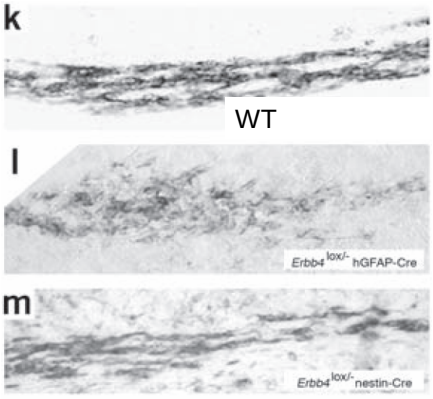




In situ hybridization using a probe specific to *Erbb4* exon 2 did not detect any cells in either the RMS or the OB of P12 *Erbb4*$^{lox/-}$ nestin-Cre or *Erbb4*$^{lox/-}$ hGFAP-Cre mice

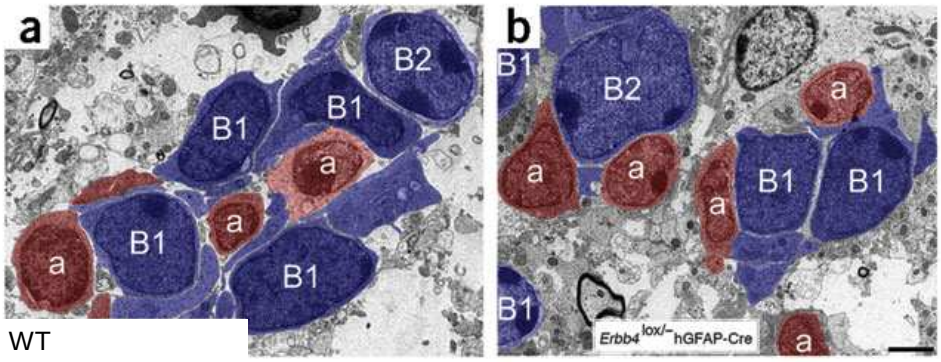
Altered RMS in conditional mutants of ErbB4

The mid-RMS was labeled with antibodies to PSA-NCAM, which mark neuroblasts that are typically organized as clusters of chains



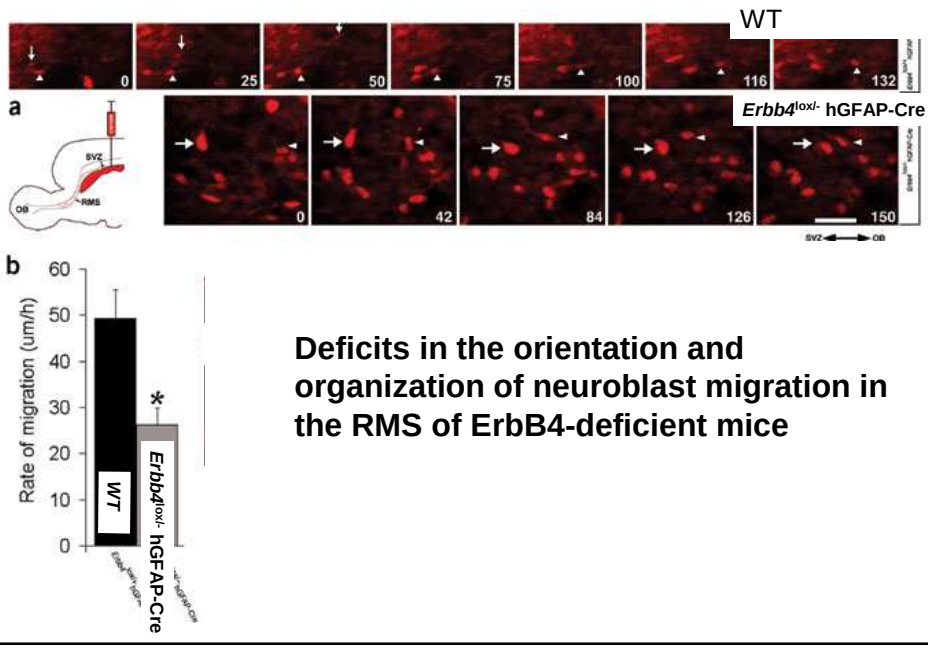
• the PSA-NCAM^{+} cells in the mutant mice formed fragmented chains that did not have the normal interdigitated and contiguous appearance

Integrity of glial tubes is disrupted in the RMS after ErbB4 deletion



• to determine how these gross morphological differences were reflected at the cellular level, electron microscopic ultrastructural analyses was carried out using 1.0-µm-thick sections of the RMS. The close apposition of migrating cells (A cells) and the glial-like cells (B1, B2) that normally ensheath them was greatly reduced in the *Erbb4*$^{lox/-}$ hGFAP-Cre mice

• the loss of ErbB4 seems to disrupt the characteristic 'glial tube' organization found in the normal adult RMS



Deficits in the orientation and organization of neuroblast migration in the RMS of ErbB4-deficient mice

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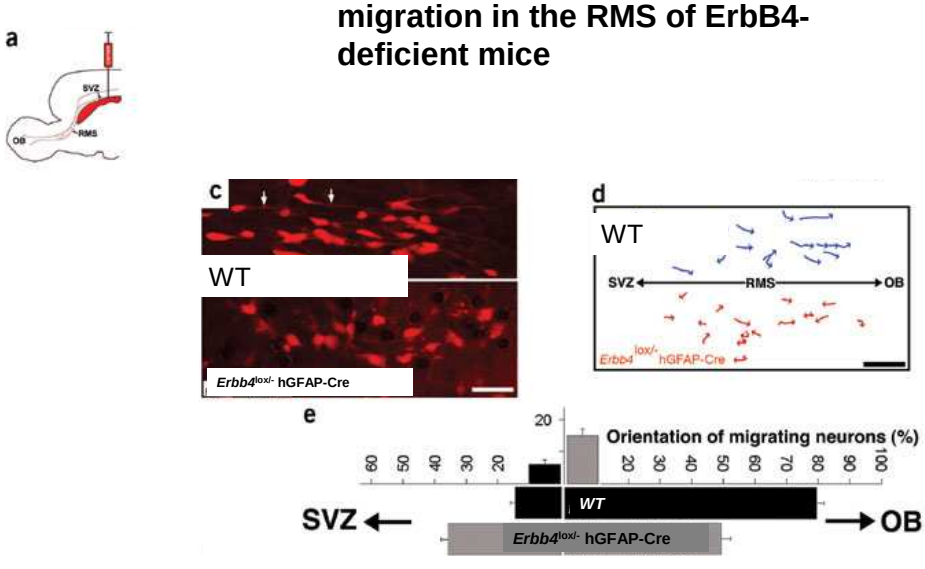
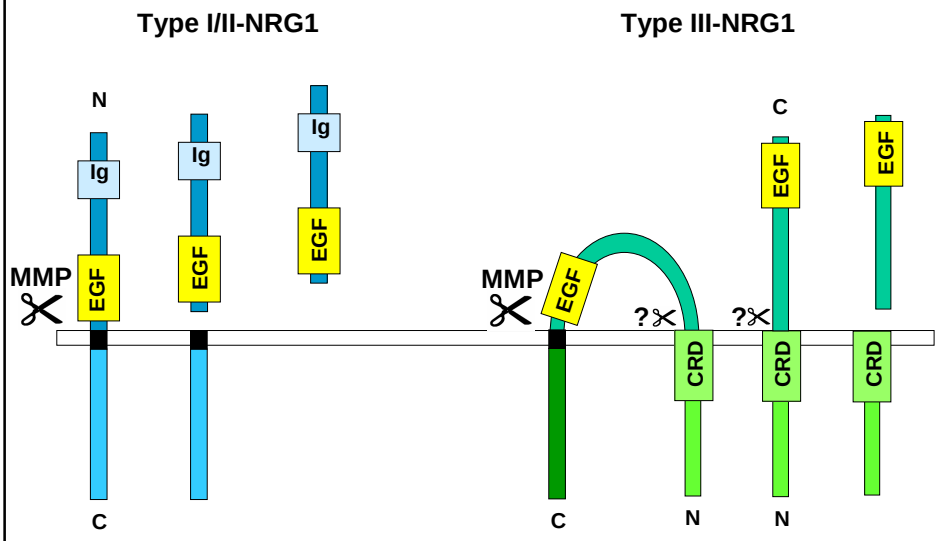


NRG1 isoforms



(modified from Falls, 2003)

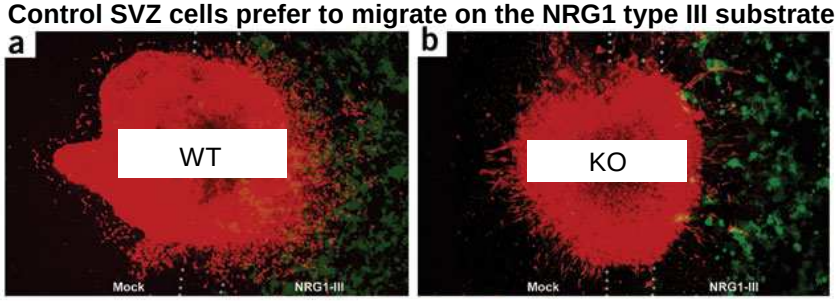
• the expression of types I and type III NRG1 isoforms in the stream suggested that they could modulate neuroblast migration through ErbB4 by potentially serving as permissive guidance cues, chemotropic agents or motogens

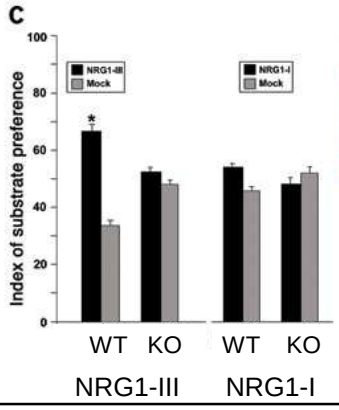
Quale saggio si può fare per valutare l'affinità nei confronti di un certo substrato?

• the expression of types I and type III NRG1 isoforms in the stream suggested that they could modulate neuroblast migration through ErbB4 by potentially serving as permissive guidance cues, chemotropic agents or motogens

• to determine if these isoforms provided a permissive substratum for migration, CMTMR-labeled SVZ explants were placed in between adjacent strips of COS cells expressing NRG1 type I and control (mock-transfected) cells or adjacent strips of COS cells expressing NRG1 type III and control cells

Control SVZ cells prefer to migrate on the NRG1 type III substrate





• the substrate preference of the neuroblasts migrating out of the SVZ explant was evaluated

• SVZ cells had a strong preference for COS cells expressing NRG1 type III, but not for mock-transfected cells or COS cells expressing NRG1 type I

• NRG1 type III at the cell surface may provide a permissive, guidance substratum for neuroblast migration towards the OB