Evaluating Neuronal Migration in *Celsr1* and *Wnt5a* Double Mutants

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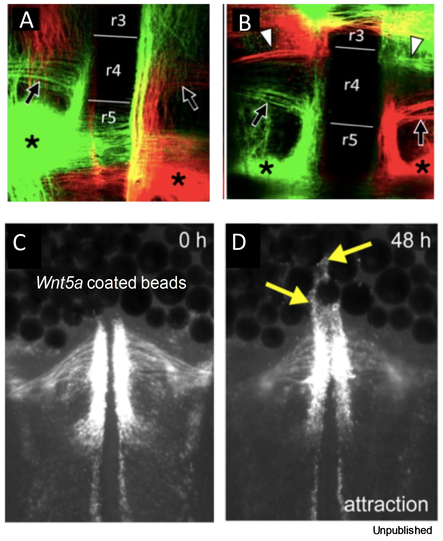
Abstract

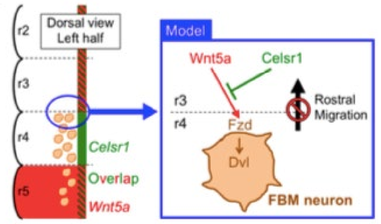
Defective neuronal migration during development can contribute to several brain disorders, including epilepsy. The goal is to understand the mechanisms of neuronal migration to help remedy these human brain disorders. Since the migration pathways of the Facial Branchiomotor (FBM) neurons are well-studied and an evolutionarily conserved process, this system will be used as the model to investigate the mechanisms involved. The current model proposes that the function of the chemoattractant Wnt5a is blocked by the membrane receptor Celsr1 to prevent inappropriate rostral migration. Previous studies with *Wnt*-soaked beads showed that excess *Wnt5a* can induce rostral migration. In addition, *Celsr1* mutants exhibited a rostral migration phenotype, suggestive of a role for Celsr1 in suppressing chemoattractant activity. To further test our model, both the *Celsr1* and *Wnt5a* genes will be knocked out and the migration phenotype will be examined. In order to generate the double knockout phenotype, the double heterozygous *Celsr1*$^{+/KO}$ *Wnt5a*$^{+/KO}$ mouse line must be generated. The lab has crossed *Celsr1*$^{+/KO}$ and *Wnt5a*$^{+/KO}$ single heterozygote mice to generate double heterozygotes. After genotyping to identify the mice, a two-factor cross will be performed to produce embryos that are homozygous mutant for both *Celsr1* and *Wnt5a*. The double mutants will be identified through genotyping performed on embryonic day 12. A corollary experiment will test whether *Celsr1* mutants exhibit enhanced rostral migration when *Wnt5a* is overexpressed.

Background



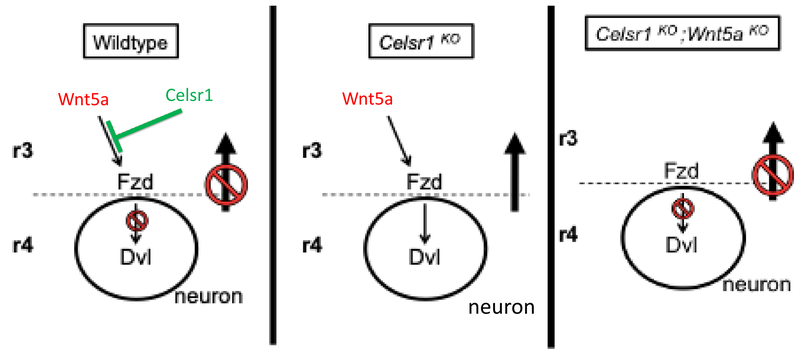
(A-B) Migratory streams were dyed to determine whether rostral migration was due to a loss in polarity or an unmasking of guidance cues
(A) WT
(B) *Celsr1*$^{KO/KO}$; the lack of axons extended through r4 is evidence that the rostral migration is due to an unmasking of local guidance cues
(C-D) Previous experiments have proven that *Wnt5a* coated beads can elicit rostral migration, supporting the hypothesis that *Wnt5a* acts as a chemoattractant

Current Model of The Roles of *Wnt5a* and *Celsr1* in Suppressing Rostral Neuronal Migration

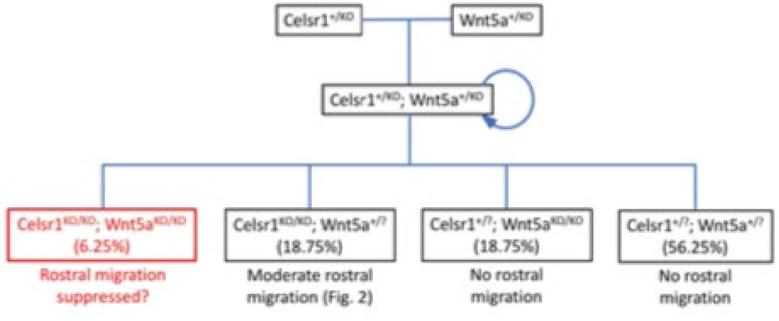


Celsr1 regulates caudal migration into r5 and r6. As shown in the right, it is expressed in the floorplate rather than within neurons.
The current model proposes that *Celsr1* acts to block *Wnt5a* from inciting rostral migration. Previous experiments have demonstrated that *Celsr1*^{KO} mice exhibit rostral migration, specifically at the r3/r4 boundary

One Prediction of Current Model- Rescue of Migration Defect in Double Mutants

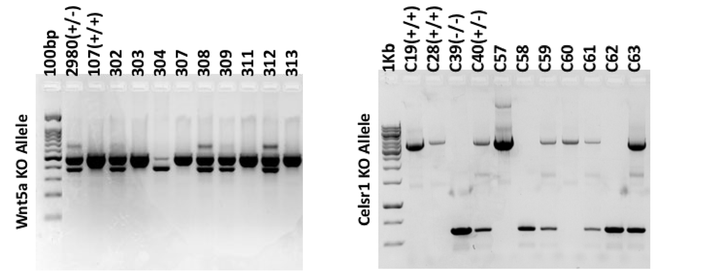


Experimental Crosses to Generate Double Mutant Embryos



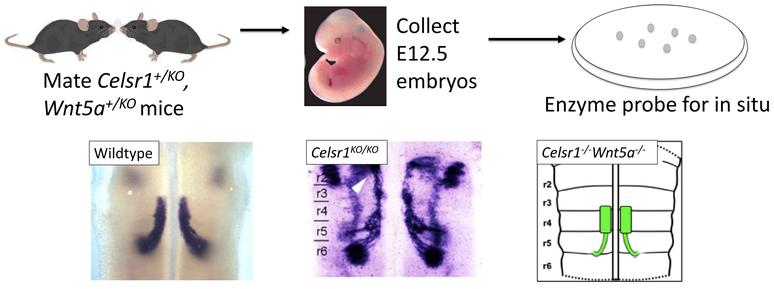
Celsr1 and *Wnt5a* single heterozygous lines will be crossed to generate double heterozygotes *Celsr1*$^{+/KO}$ *Wnt5a*$^{+/KO}$ mice. Mice will be mated to each other in a two-factor cross to produce double mutant embryos.

Genotyping Protocol to Identify *Celsr1* and *Wnt5a* Mutants



- In the *Wnt5a* PCR, wildtype alleles will generate an 800-900bp product and mutant alleles will generate a 700bp product. 302, 304, 308, 309, and 312 are samples of mice with mutant alleles.
- In Celsr1 PCR, wildtype alleles will generate a 3,000bp product and mutant alleles will generate a 400bp product. C58 and C62 mice are homozygous mutant, C59, C61, and C63 are heterozygous, and C57 and C60 are homozygous wildtype.

Examining Rostral Phenotypes



Embryos are collected at embryonic day 12 and genotyped to identify double mutants. Double mutants and control embryos will be processed for in situ hybridization with Tbx20 probe to examine rostral migration phenotypes

Future Experiments

Testing whether ectopic expression of *Wnt5a* in *Celsr1* mutants leads to rostral migration would help solidify the role of *Wnt5a* as a chemoattractant. Previous experiments have shows that *Wnt5a* soaked beads can overcome inhibitory factors and induce rostral migration

Acknowledgements

Supported by NIH grant R21 NS108367 and MARC IMSD Fellows program's National Institute of General Medical Science (NIGMS) a component of the National Institute of Health (NIH) grant R25GM056901