Tlx3 and *Tlx1* are post-mitotic selector genes determining glutamatergic over GABAergic cell fates

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by Lena Khibnik
3/8/05

Central Questions

- Molecular specification of neurotransmitter types in different neuronal populations
- Choice of excitatory vs. inhibitory fate
- Is there a “master switch” that makes a cell fate choice?

Exctation vs. Inhibition

- Glutamate is the major excitatory neurotrasmitter
- GABA is the major inhibitory neurotransmitter
- Glu and GABA rarely coexist in one neuron

Two Puzzles:

- Glu is expressed everywhere (what is Glu?)
- GABA can be synthesized from Glu in a single step (via GAD)

A bit about the system…

- Embryonic Dorsal Spinal Cord

  - neurogenesis has been extensively characterized

  - well characterized neuronal populations

- Existence of distinct excitatory and inhibitory pupulation

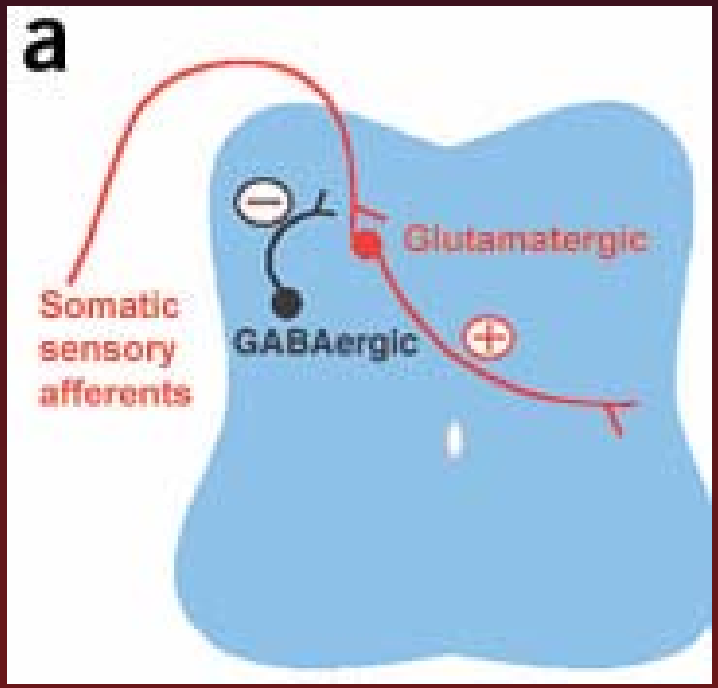
- Both types derive from a specific domain in the ventricular zone and migrate to superficial lamina in the dorsal horn

Photo removed for copyright reasons.
Spinal cord cross-section, highlighting two dorsal horn regions.

Methods

- Embryonic mouse and chick spinal cord
- Detection of gene and protein expression and co-localization via in situ hybridization and immuno staining techniques
- Genetic manipulation (knockouts; overexpression)
- Electrophysiology (whole cell patch)

How do you tell glutamatergic neuron from GABAergic?



- Glutamatergic neuron specific expression:
  - *VLUT2* = Glu transporter
- GABAergic neuron specific expression:
  - GAD = glutamic acid decarboxylase
  - *Viaat* = GABA transporter

Source: Cheng, L., et al. "Tlx3 and Tlx1 are Post - Mitotic Selector Genes Determining Glutamatergic over GABAergic Cell Fates." *Nature Neuroscience* 7 (2004): 510- 517.
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- Central question: what's the "switch" that decides cell fate for glutamatergic vs. GABAergic neurons?

- Transcription factors are usually involved in cell fate determination

- Find two populations of neurons in which transcription factors of interest are expressed in a mutually exclusive way

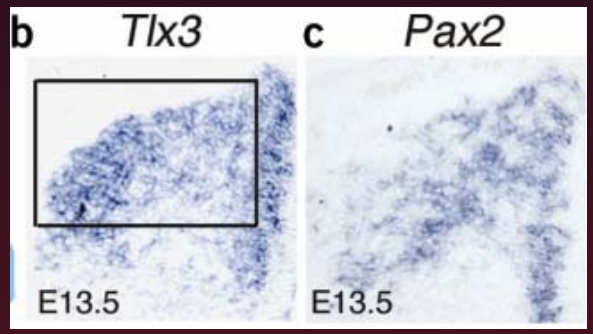
- Make sure that each transcription factor of interest is exclusively expressed in a glutamatergic or GABAergic cell population

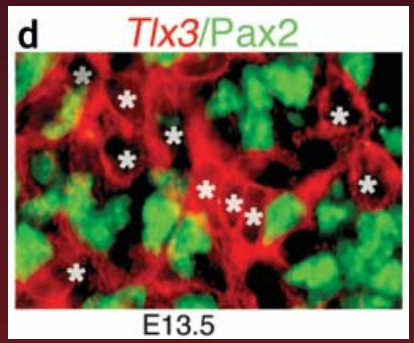
Tlx3 and *Pax2* genes fit the criteria

- *Tlx3* and *Pax2* are expressed in distinct subsets of neurons during early and late dorsal neurogenesis

- *Tlx3* and Pax2 do not coexpress

- 60% neurons in dorsal lateral spinal cord express *Tlx3* and 40% express *Pax2* (as shown by colocalization with general neuronal maker *Stmn2*)





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	Pax2⁺	VGLUT2⁺	Gad1⁺
Tlx3⁺	3.9%	96.4%	
Pax2⁺		0.6%	97.8%

Tlx3⁺	Pax2⁺	Stmn2⁺
361 ± 23	241 ± 8	606 ± 25
(59.5 ± 3.0)%	(39.9 ± 1.7)%	100%

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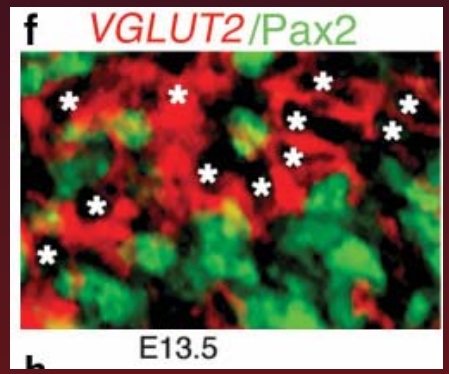
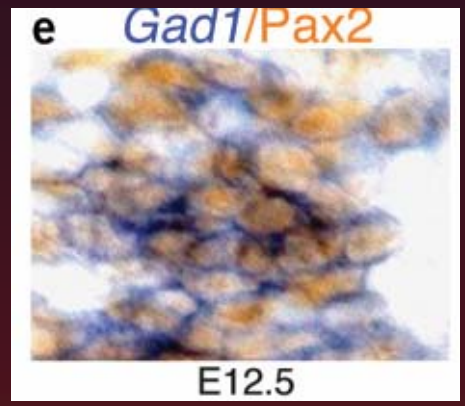
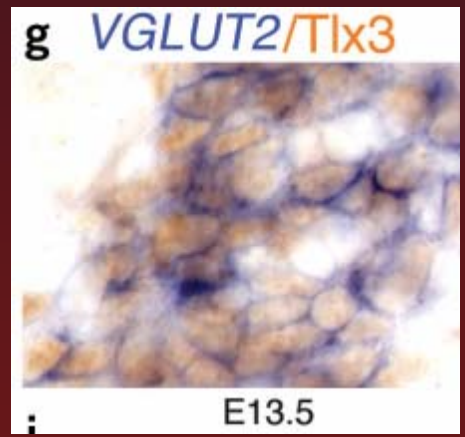
Tlx3 and *Pax2* genes fit the criteria

- 98% of *Pax2*$^{+}$ cells coexpress *Gad1*
- 99% of *Pax2*$^{+}$ cells DO NOT coexpress *VGLUT2*
- >96% of *Tlx3*$^{+}$ cells coexpress *VLUT2*







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Tlx3 and *Pax2* - summary

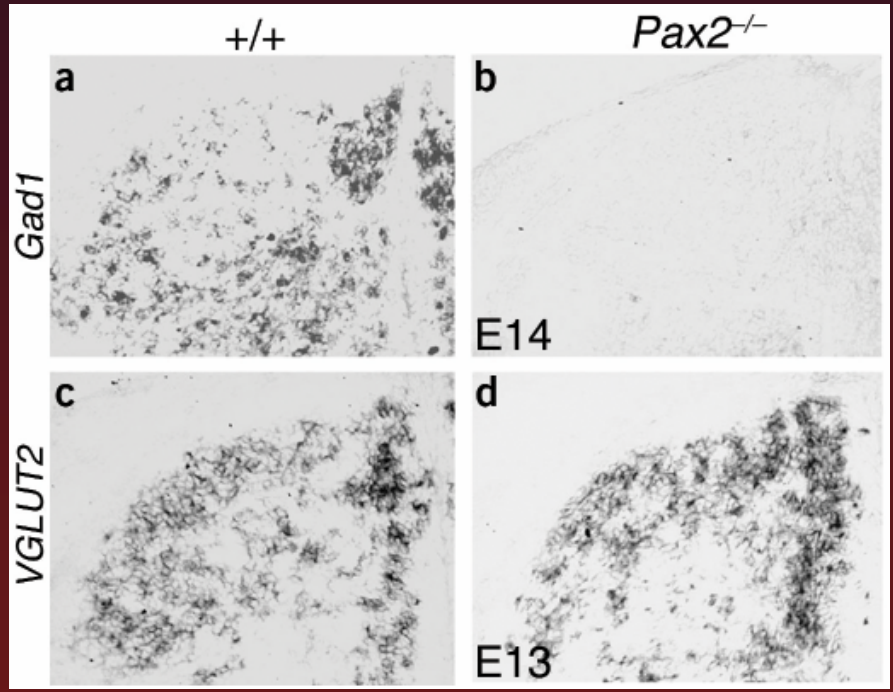
- *Tlx3* and *Pax2* are homeobox genes (transcription factors)
- Expressed in two distinct populations in a well-defined area of the dorsal horn
- Tlx3 colocalizes exclusively with glutamatergic markers, while Pax2 colocalizes exclusively with GABAergic markers

Hypothesis: *Tlx3* and *Pax2* act as selector genes to promote excitatory or inhibitory cell fate

* selector gene = genes that control fate and orchestrate cell type-specific choices for groups of cells during development

Pax2 required for GABAergic differentiation

- *Pax2*-null spinal cords lose *Gad1* expression and have reduced *Gad2/Viaat* expression
- No change in *VLUT2* expression in *Pax2* mutants
  - *Pax2* is specifically required for GABAergic differentiation



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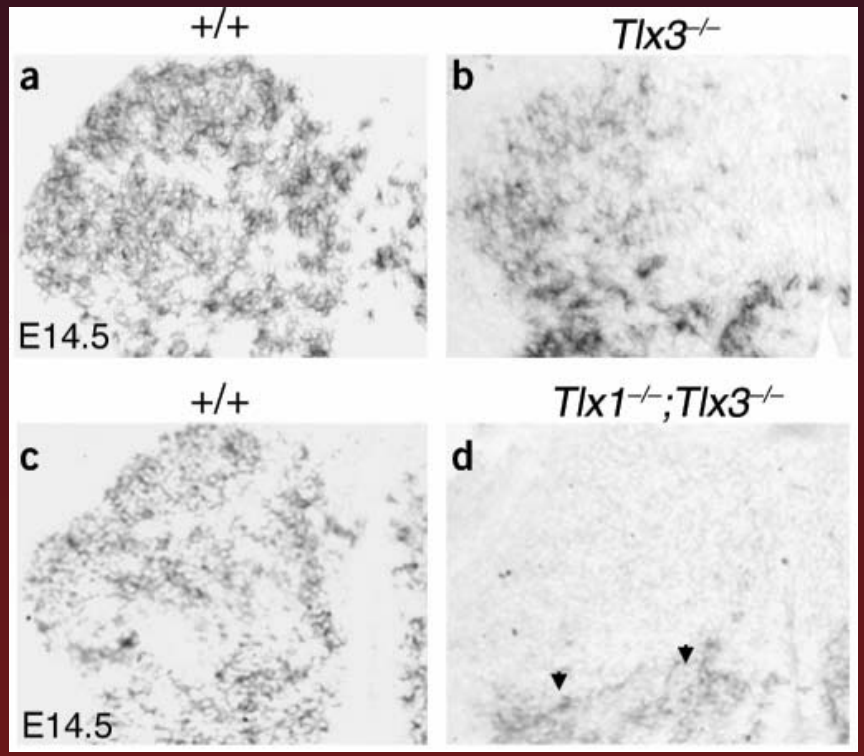
Tlx expression required for glutamatergic differentiation

- *VLUT2* expression is reduced in *Tlx3* mutants
- *VLUT2* expression is ABSENT in *Tlx1/3* compound mutants
  - *Tlx3* and *Tlx1* are required for glutamatergic differentiation



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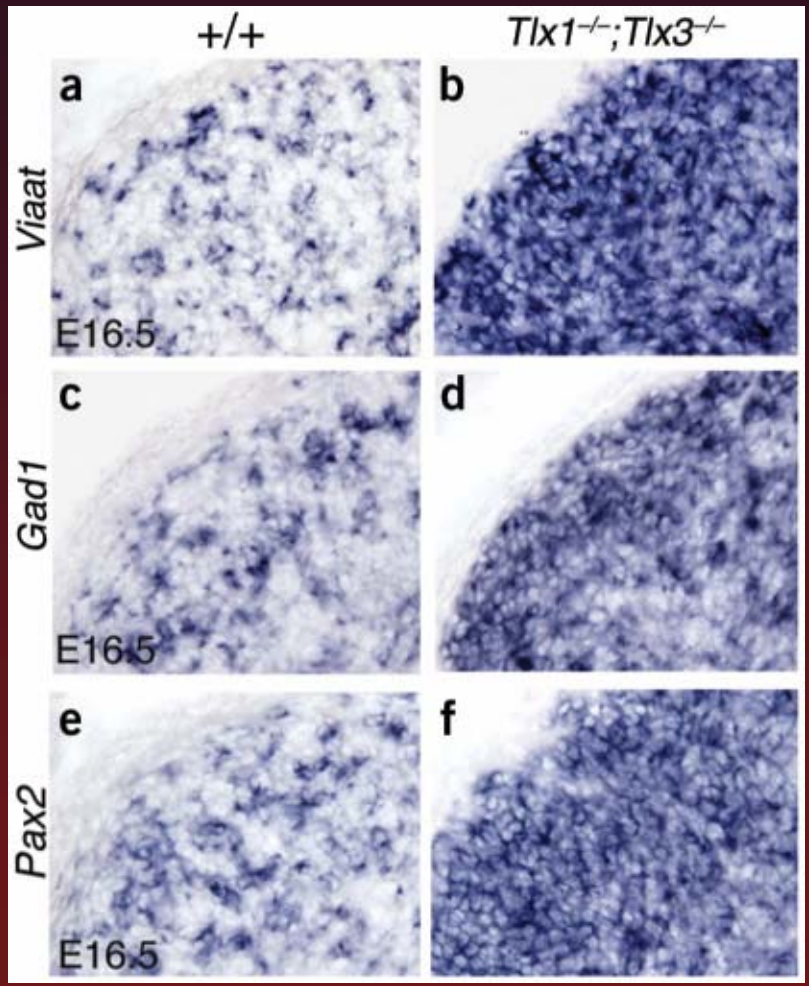
Are Tlx ‘selector’ genes?

- Analysis of GABAergic markers in Tlx mutants revealed:
  - Expanded expression of Viaat, Gad1 and Gad2



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Mechanisms of GABAergic expansion

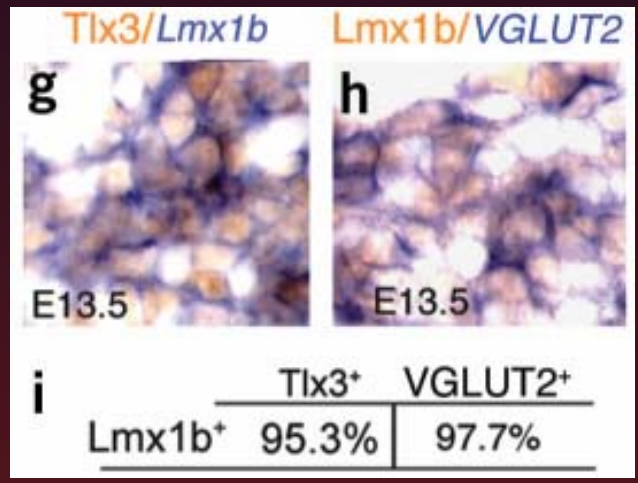
- Expansion of GABAergic population (neurogenesis)
  - Unlikely: dealing with postmitotic neurons and no increased cell death

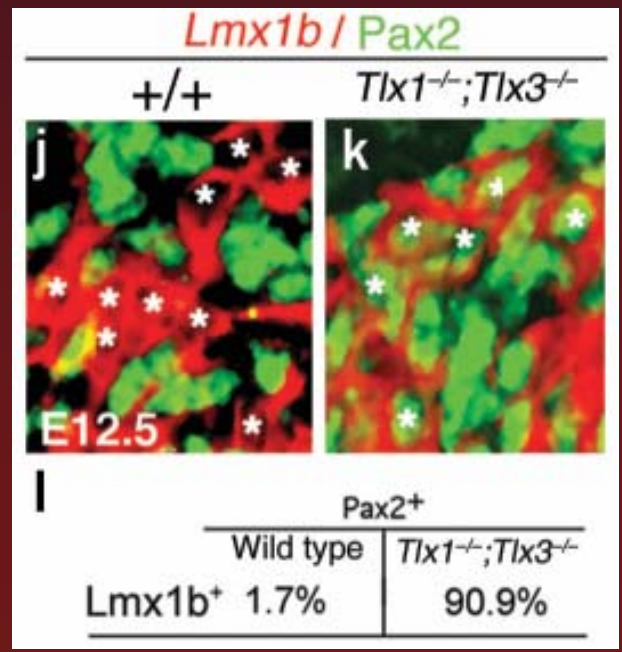
- Transformation of glutamatergic into GABAergic cells – Tlx and Pax genes are responsible for cell fate determination

Glutamatergic cells revert to GABAergic cell fate

- LIM-class *Hox* gene *Lmx1b* normally coexpresses with *Tlx3* and *VGLUT2*, but not with *Pax2* (<2%)
- *Lmx1b* is still present in *Tlx* mutants (marker for Glu cells)

- In *Tlx* mutants *Pax2* and *Lmx1b*$^{+}$ coexpressed in >90% of cells





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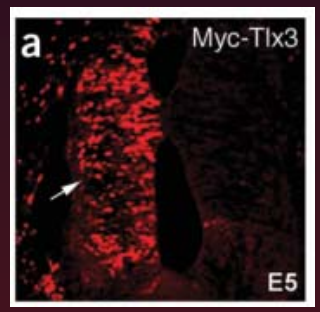
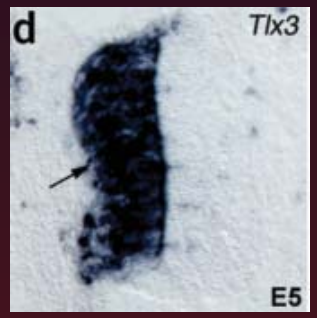
Does *Tlx3* suppress GABAergic fate?

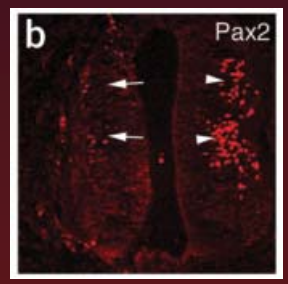
- Misexpressed mouse *Tlx3* in a chick spinal cord via electroporation with a Myc-tagged mouse *Tlx* expression construct

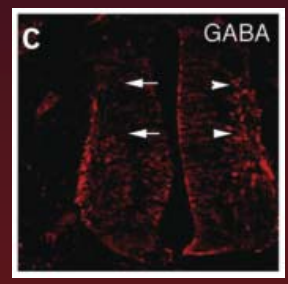
- Later analysis revealed repressed Pax2 protein expression on the electroporated side of the neural tube (along with reduced GABA and *Gad1* immunostaining)

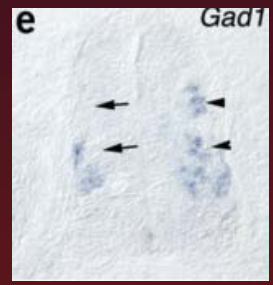
- Expansion of *VLUT2* expression





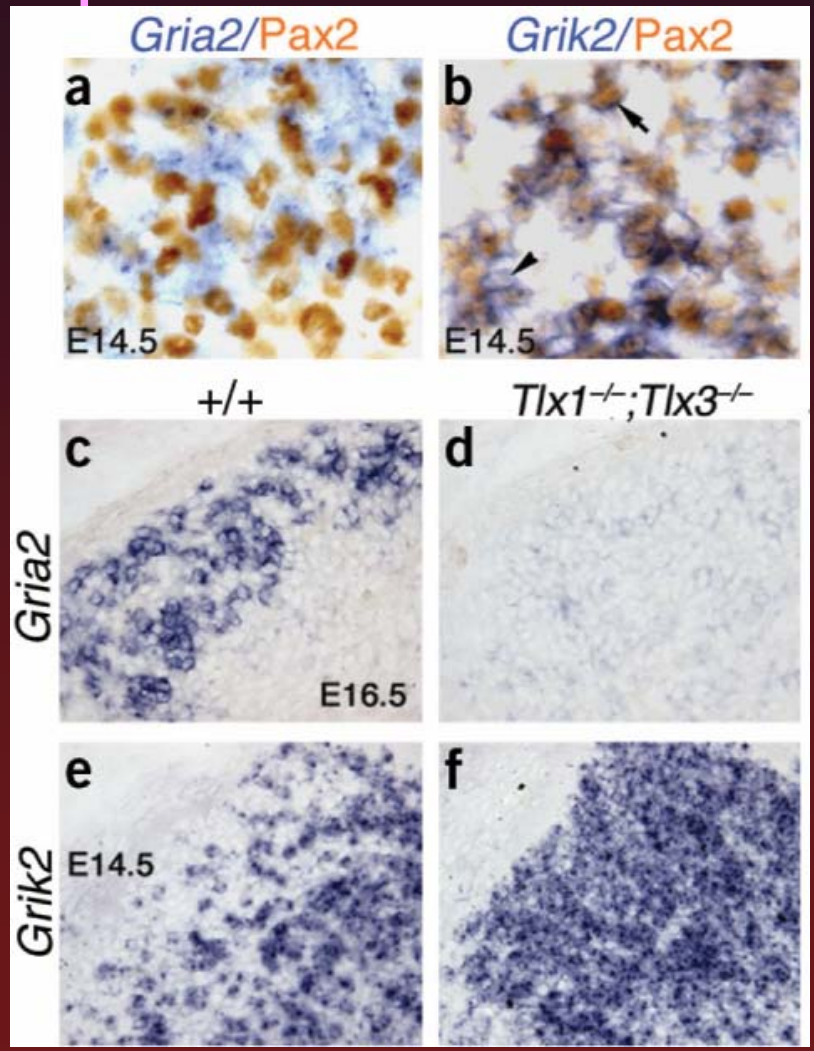






Tlx acts as a switch to activate the entire profile of cell-type specific markers

- GluR2 (encoded by *Gria2*) normally expressed in glutamatergic cells and do not colocalize with *Pax2*
- GluR6/7 (encoded by Grik2/3) are expressed in GABAergic cells and colocalize with *Pax2*
- In *Tlx* mutants, *Gria2* expression is lost, while Grik2/3 expression becomes uniform



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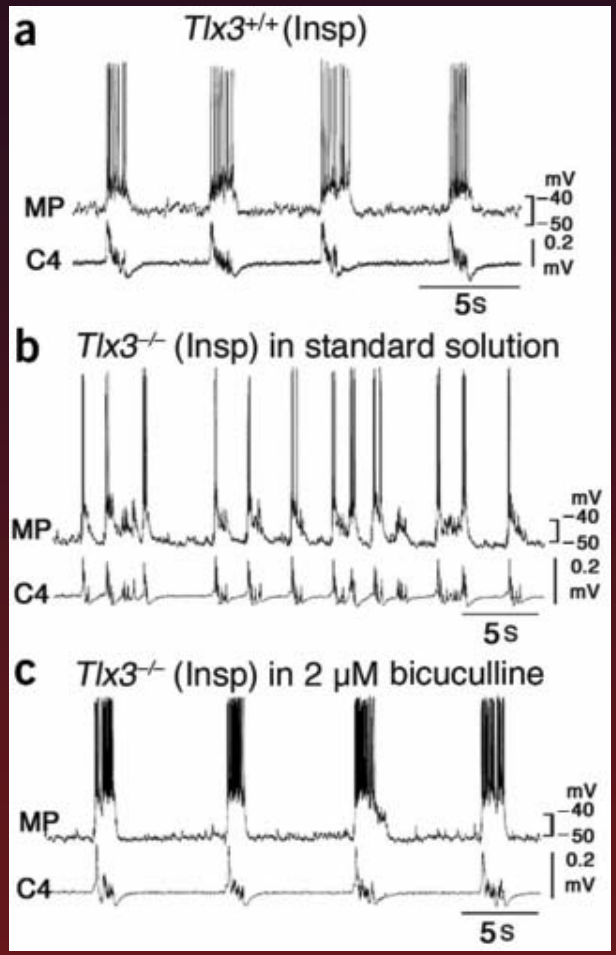
A Functional Connection

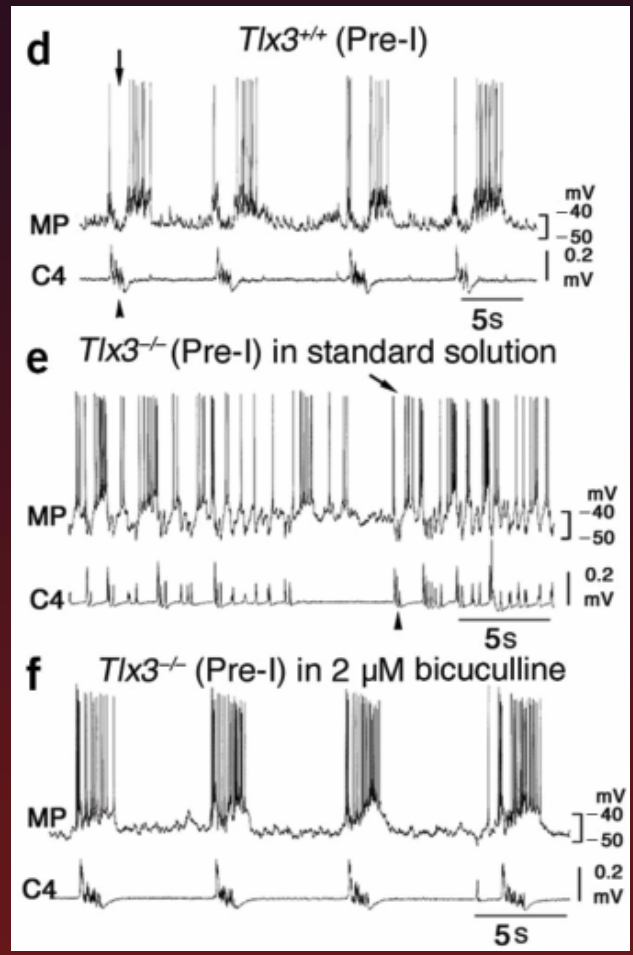
- Do neurons transformed from glutamatergic to GABAergic exhibit true inhibitory behavior?
- Used a simple circuit (brainstem-to-spinal cord prep) to study firing properties of two interconnected oscillators involved in generation of respiratory rhythms (in a Tlx mutant has a clearly defined respiratory failure phenotype)
- Exhibit normal connectivity as indicated by normal resting membrane potential and input resistances and presumably have expansion of GABAergic cell population similar to the dorsal horn



- MP = membrane potential
- C4 = neuronal activity

- Short burst duration
- Arrhythmic firing patterns in both populations

- Normal firing is restored by application of GABA antagonist, bicuculline (also by picrotoxin and low Cl-)



- Respiratory failure results from excess GABA inhibition

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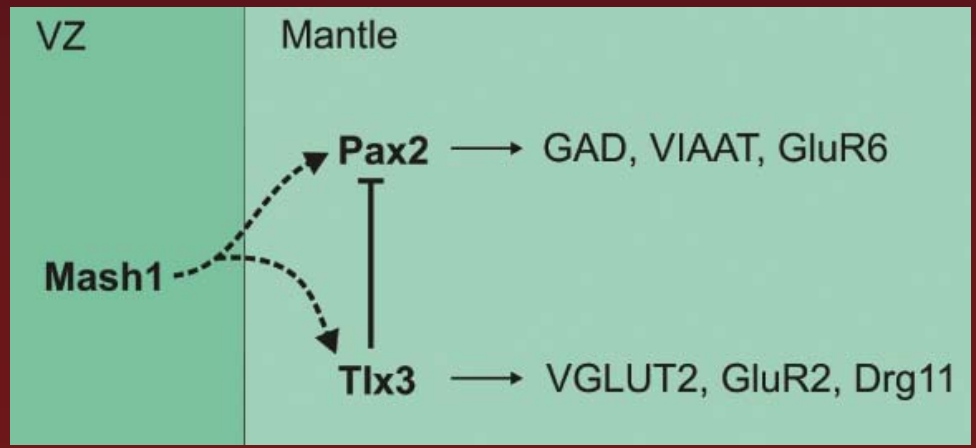
To summarize…

- *Tlx3* and *Tlx1* act as selector genes, promoting glutamatergic over GABAergic differentiation in the dorsal embryonic spinal cord:
  - *Tlx3* is expressed in Glu neurons (with loss of specific Glu markers in *Tlx* mutants)
  - *Tlx* genes are able to repress GABAergic cell fate, with reversible expansion of GABAergic cells in *Tlx* mutants
  - Ectopic *Tlx3* expression is sufficient to repress endogenous GABAergic differentiation and induce glutamatergic cell development

- *Pax2* is involved in acquisition of GABAergic cell phenotype in dorsal embryonic spinal cord
  - But….loss of *Pax2* is not accompanied by expansion in glutamatergic cells
  - *Pax2* probably controls the latest stages of GABAergic differentiation program



Weaknesses and Further Directions

- Evidence is largely correlational, direct molecular interactions are still unknown; is this regulation direct or indirect? What is the pathway of glutamatergic cell fate onset and how is the switch from glutamatergic to GABAergic cell fate accomplished?
- What induces expression of *Tlx3* and *Pax2*?

- How is glutamatergic/GABAergic cell differentiation regulated in other parts of the nervous system? Why is it advantageous to have different mechanisms in different parts of the system?