Serotonin Signaling and Embryogenesis:

Physiological controls of Development

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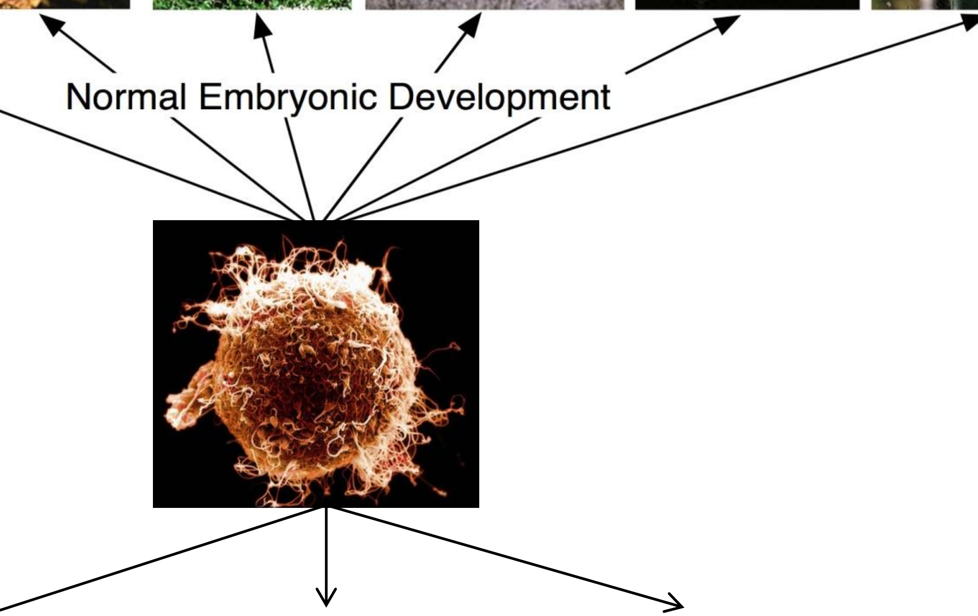
Outline

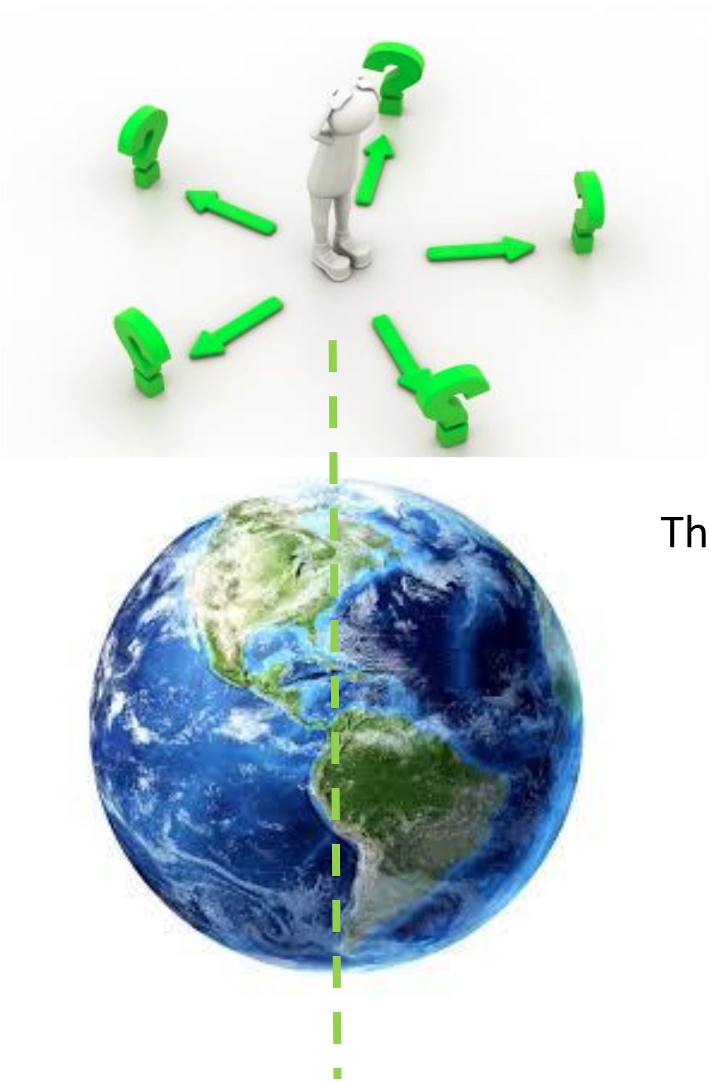
- Introduction to meaning of organ asymmetry in embryos
- Basic facts about organ asymmetry with explanation
- Clinical significance of asymmetry
- How asymmetry is set-up
  - Physiological early mechanisms
- Summary

Knowing communication and patterning during development is important



Answers critical questions of form and shape in developmental patterning of tissues and organs
- Regenerative Medicine

Determining Left-Right asymmetry is a difficult problem!



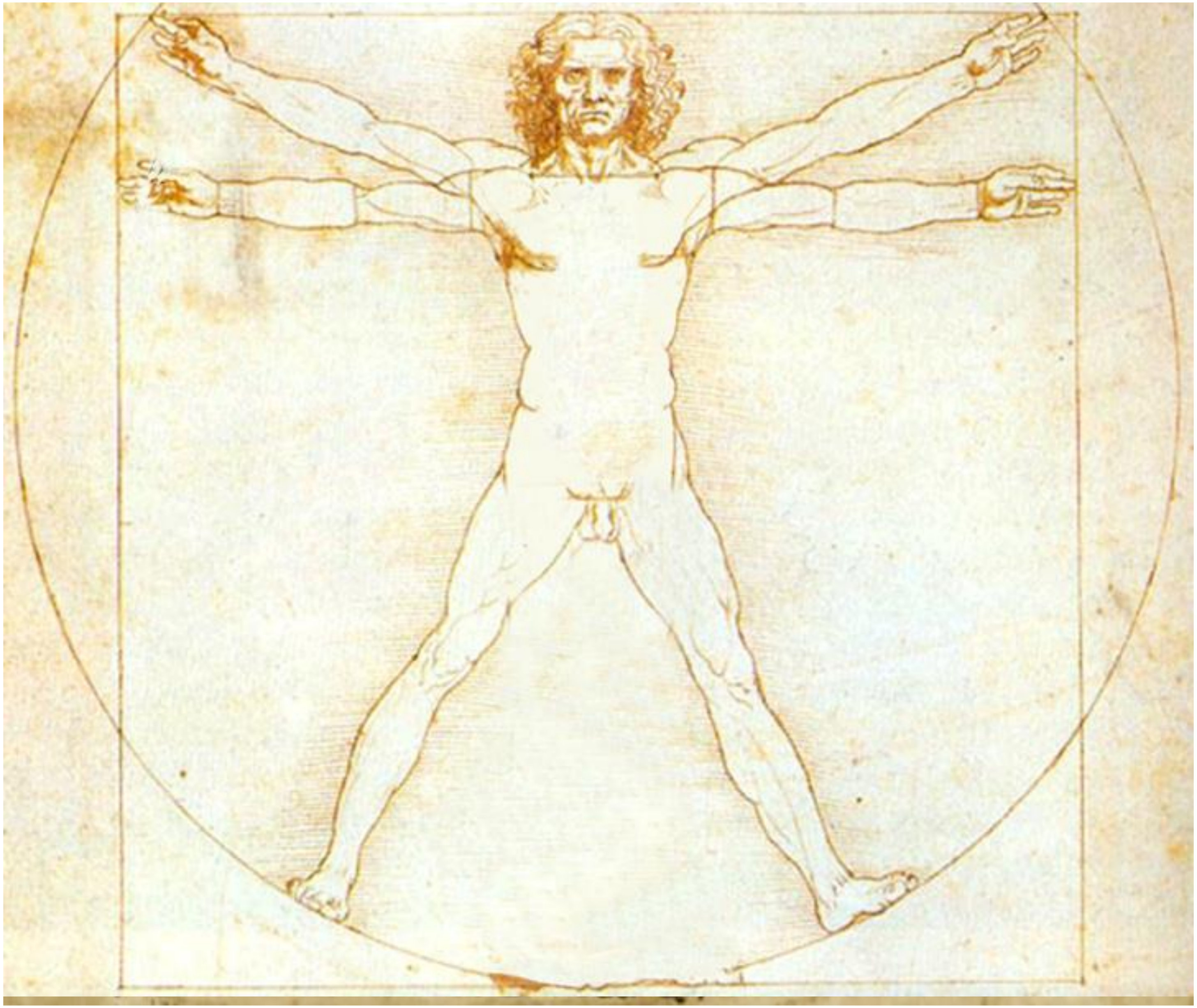
Left-Right asymmetry is defined as a consistent difference across the midline axis (not developmental noise)

There are two problems to solve.

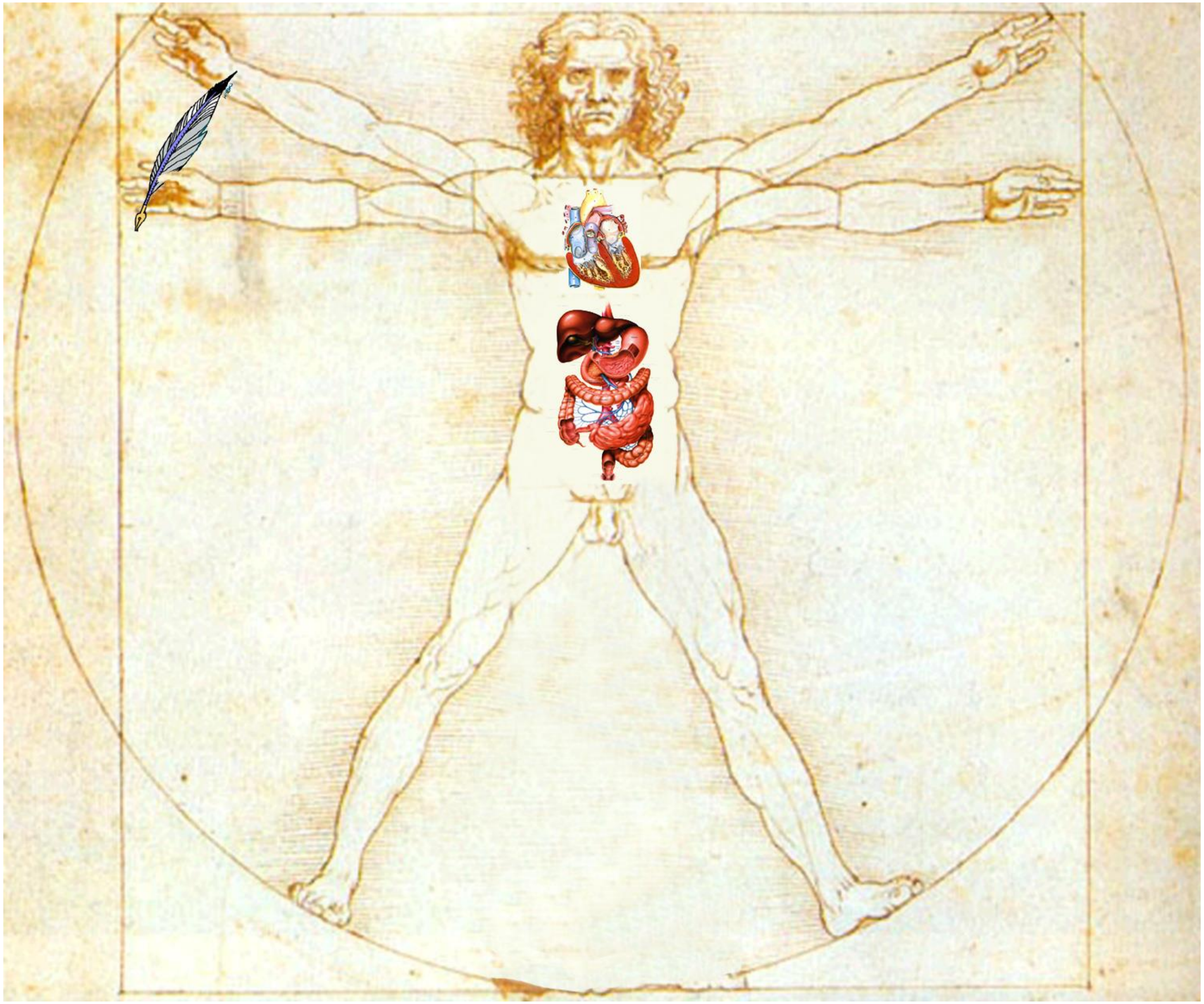
Cells need to know their left side and right side.

Cells need to know whether they are on the left side of the embryo/midline or the right side

Human Laterality



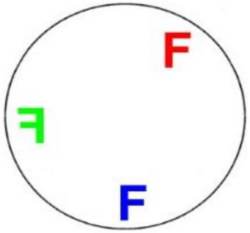
Human Laterality

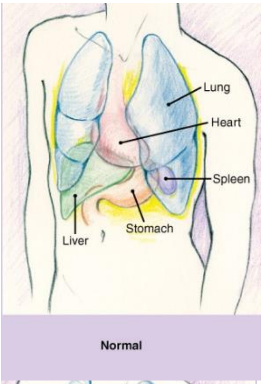


Brain, Heart and Viscera are consistently asymmetrical in all normal individual

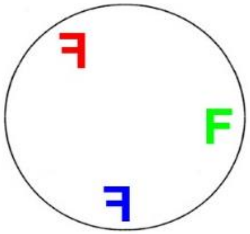
Ways that body asymmetry can go wrong:

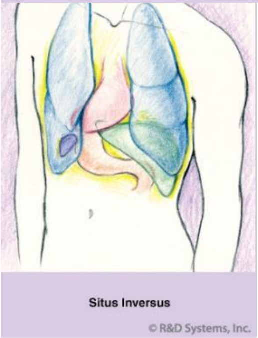
1. Situs solitus - normal situs - all organs on their correct side.





2. Situs inversus - complete mirror image reversal - all organs on the opposite side.





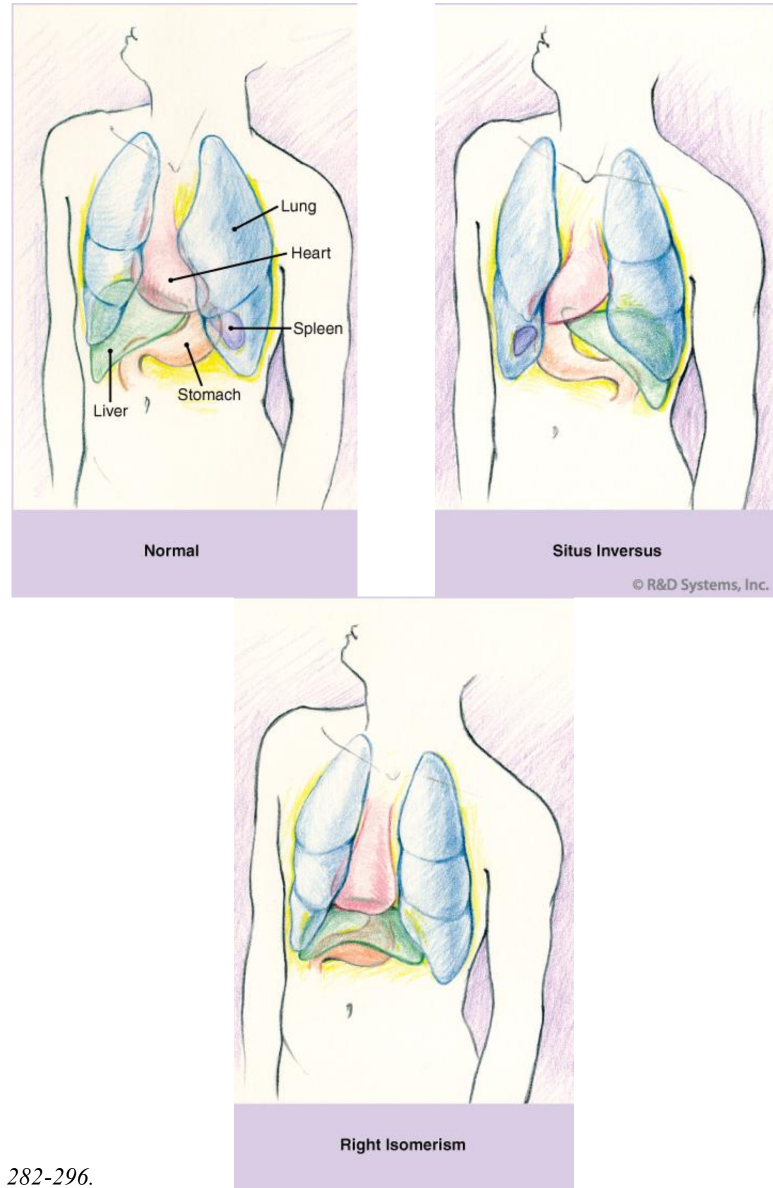
Clinical implications of LR asymmetry:

1) <u>Primary laterality syndromes</u> - *situs inversus totalis,* heterotaxia, isomerism affect more than 1 in 6,000 babies born to term. *Situs inversus totalis* involves no serious medical problems. Other laterality disturbances do.

2) Laterality defects accompany some other syndromes (holoprosencephaly, short-rib polydactily and renal-hepatic-pancreatic dysplasia syndromes).

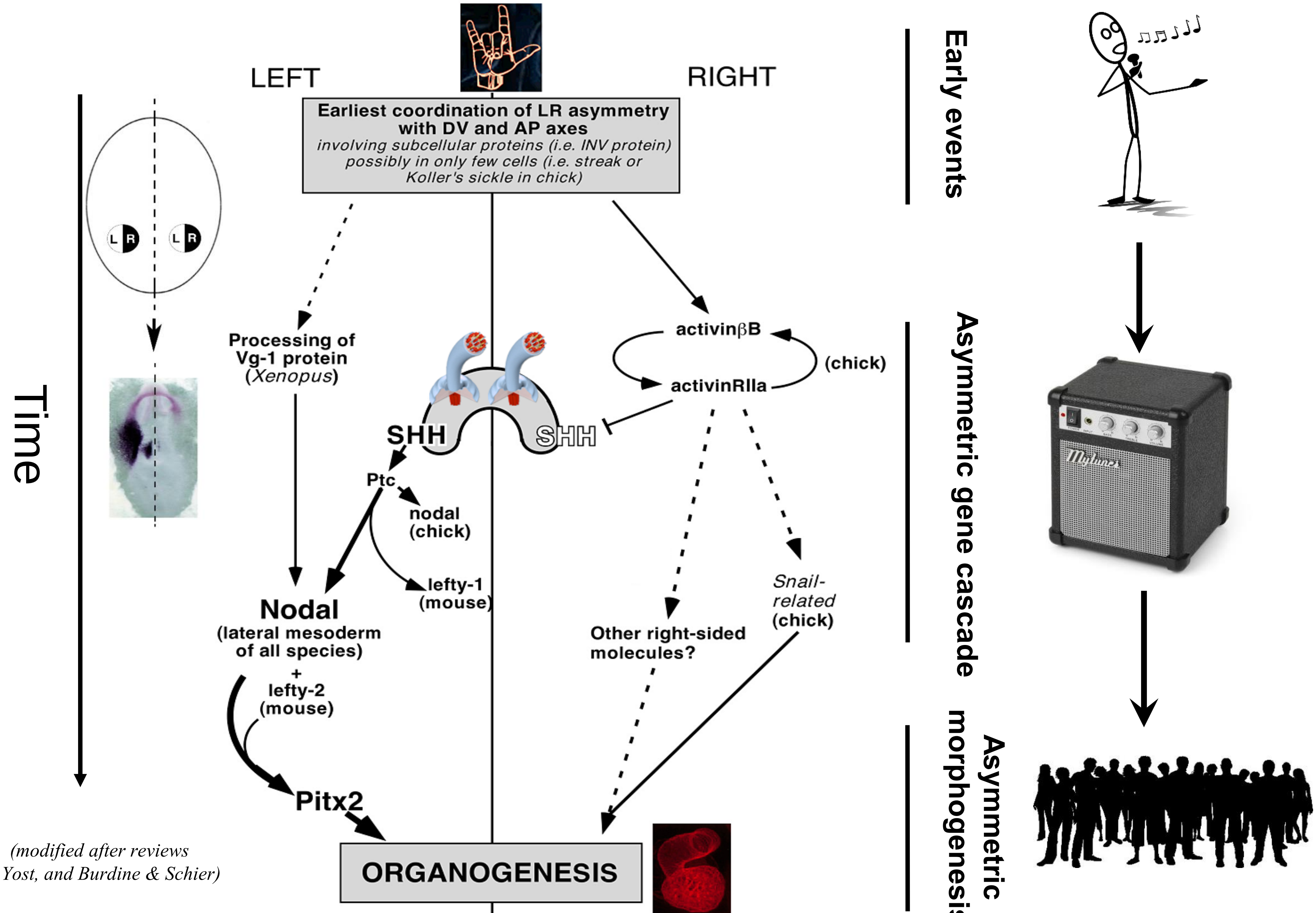
3) Some syndromes have a unilateral presentation in tissues which normally have no asymmetric characteristics (e.g., cleft lip, and Holt-Oram syndrome, which results in left-sided upper limb malformations).

4) Reversed cerebral asymmetry is associated with breast cancer.



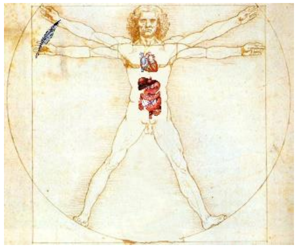
Burn, J. (1991). Disturbance of morphological laterality in humans. Ciba Foundation Symposium 162, 282-296.
Peeters, H. and Devriendt, K. (2006). Human laterality disorders. Eur J Med Genet 49, 349-62.

Asymmetry set up



(modified after reviews by Yost, and Burdine & Schier)

Understanding how Left-Right asymmetry is established



Organ Asymmetry – Only thing known until Mid-1990s

↑

Amplification steps that set up organ asymmetry

↑

Gradients of determinants (serotonin) that set up amplification steps

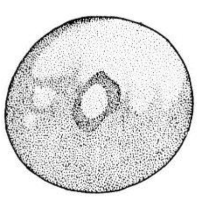
↑

Bioelectrical signals that set up the gradients of determinants

↑

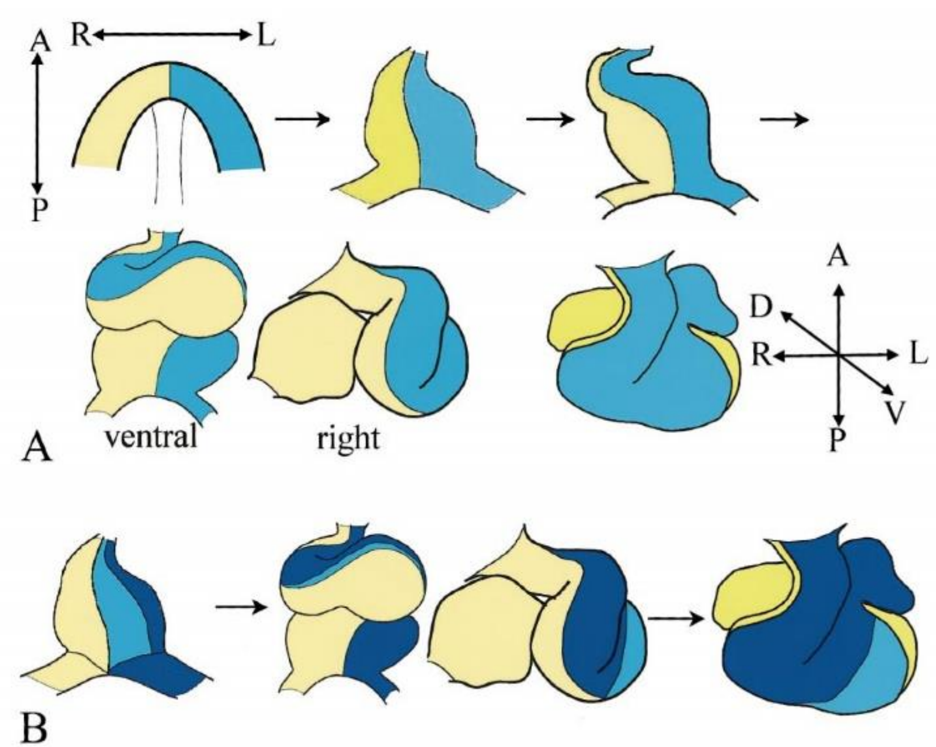
Cytoskeletal chirality that sets up bioelectrical gradient

Development Time

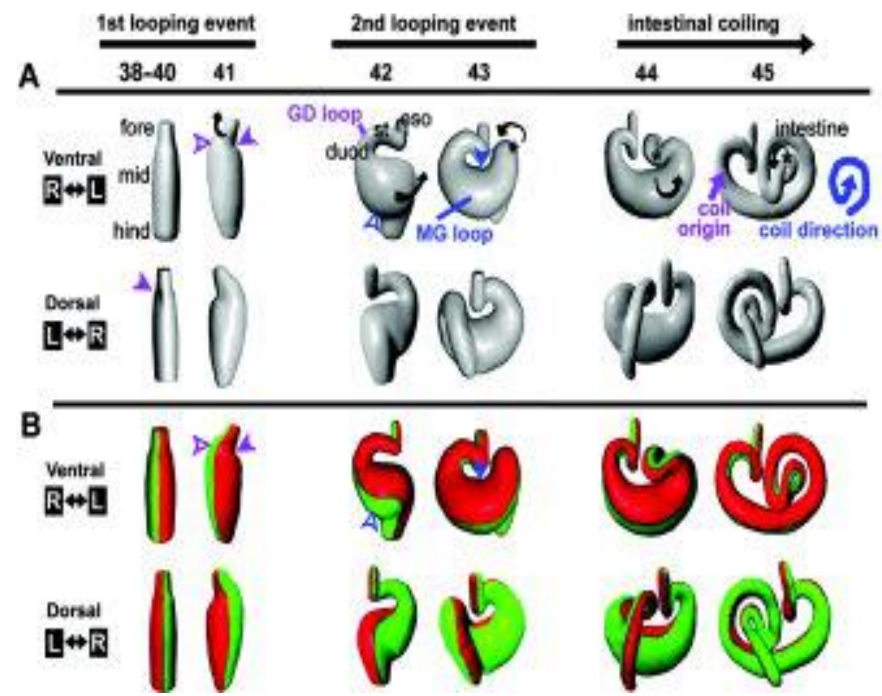


Embryo

The last phase = organogenesis



L=blue, R=yellow
Campione et al., 2001

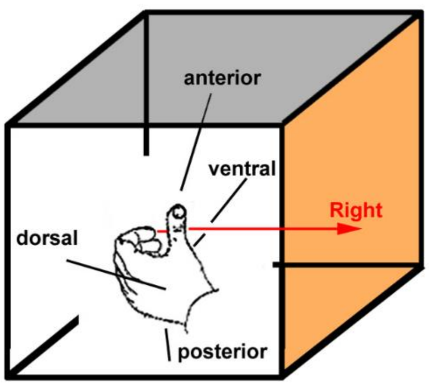


L=red, R=green
Muller et al., 2003

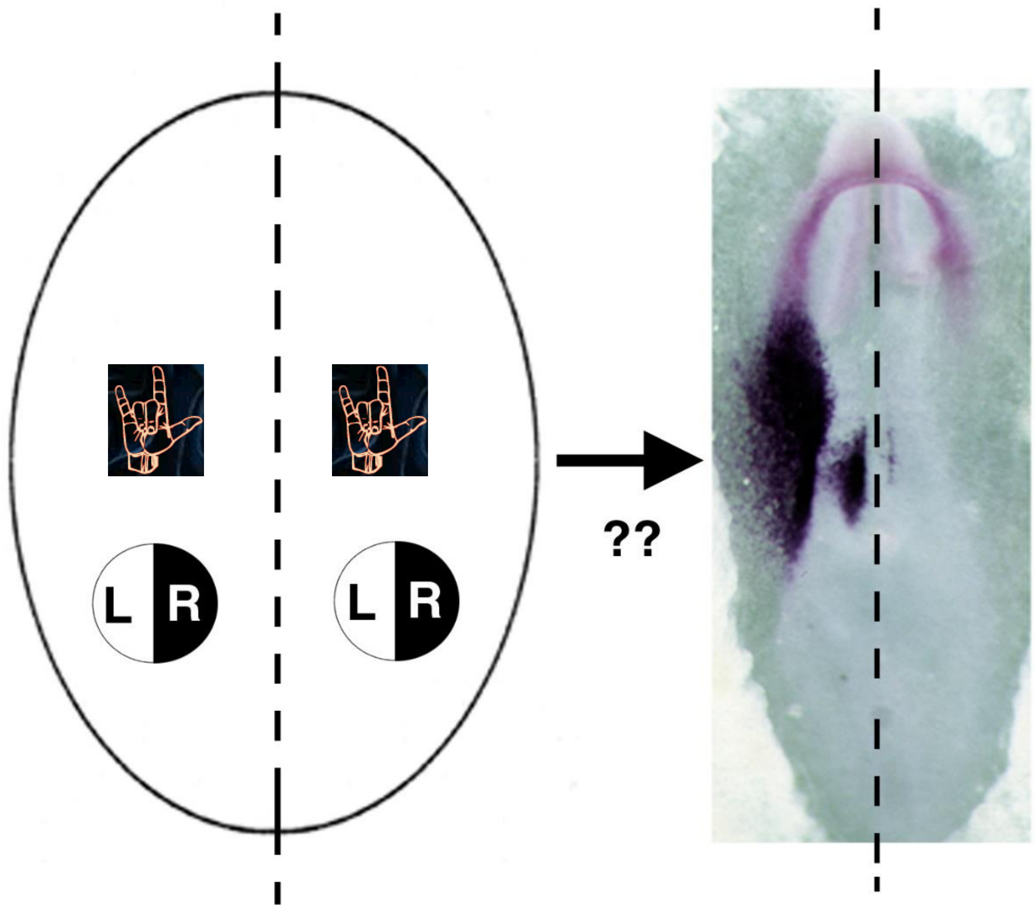
Differential cues on L vs. R sides give rise to asymmetric organs via different migration, proliferation, and tensile forces

Amplification step: Asymmetric gene expression requires knowledge of L-R position with respect to the embryo;

Going from cells' knowing which direction is L and R, to cells' knowing which side of the midline L or R they are on



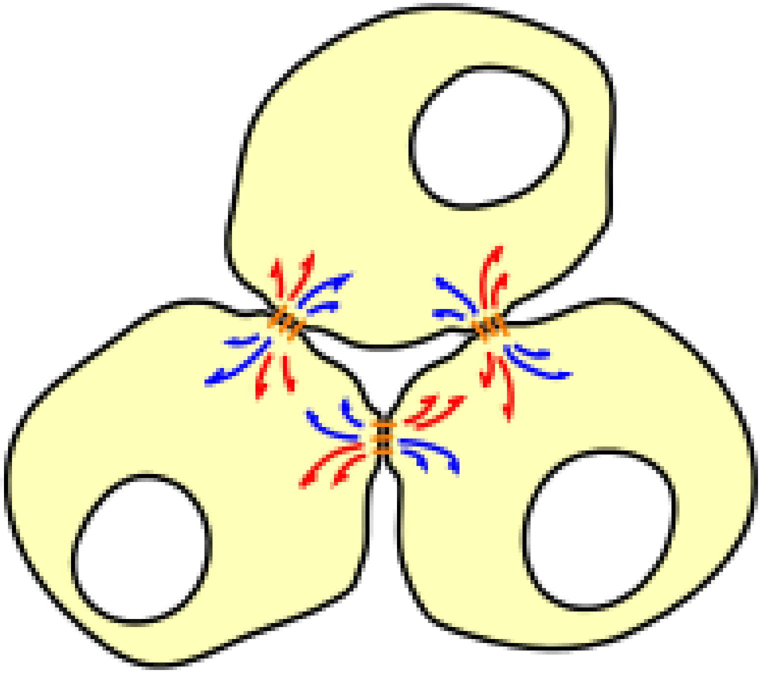
Vandenberg, L. N., Levin, M., 2009. Perspectives and open problems in the early phases of left-right patterning. Semin Cell Dev Biol. 20, 456-63

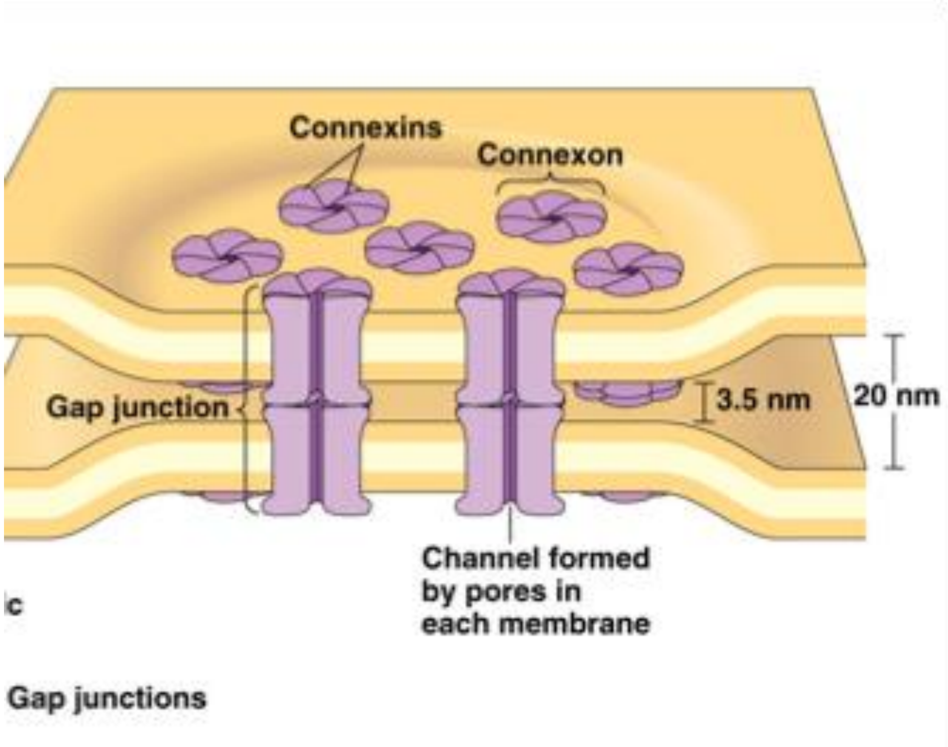


Asymmetric gene expression needs global position.

L and R side must communicate at early stages

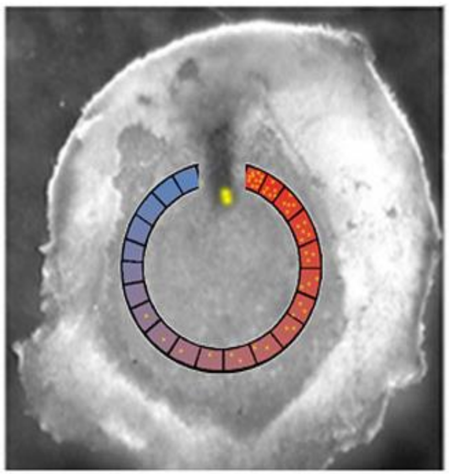
Cells communicate using Gap junctions to decide L-R identity

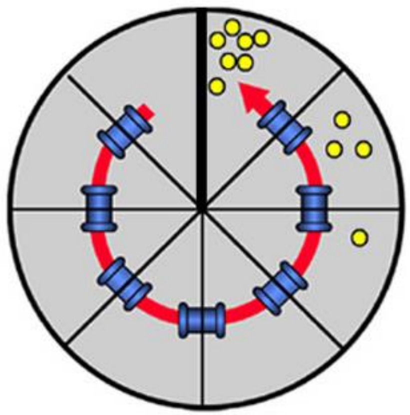




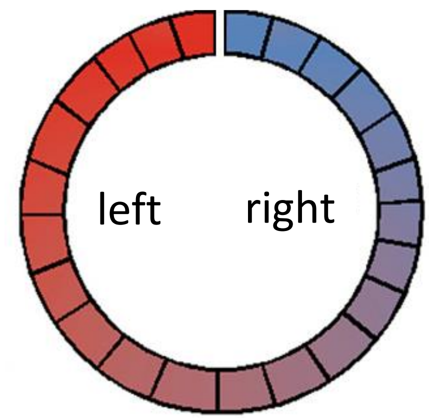
Neurons and synapses evolved by specializing these functions, first arising in somatic cells

Gap junctions mediate L-R communication





Segregation of L-R determinants through Gap junctions



A unidirectional flow of small molecules through GJs causes a net L-R asymmetric gradient across midline. This asymmetric accumulation induces asymmetric gene expression – Amplification step.

Important questions:

1] How is a unidirectional flow maintained?

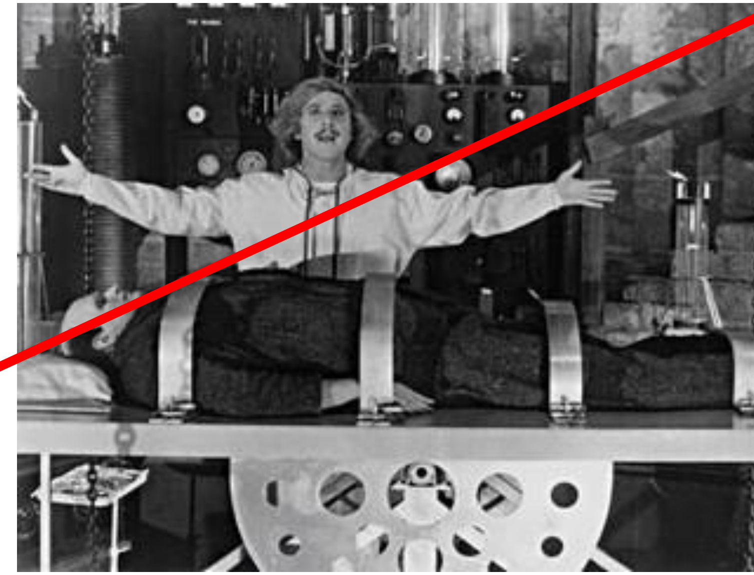
2] What is it that flows through Gap Junctions?

Levin, M., Mercola, M., 1998. Gap junctions are involved in the early generation of left-right asymmetry. Dev Biol. 203, 90-105.

Developmental Bioelectricity:

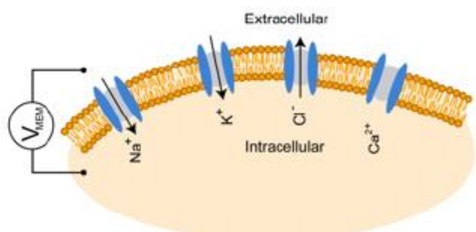
slow, steady ion fluxes, electric fields, and voltage gradients endogenously generated and sensed by all cell types

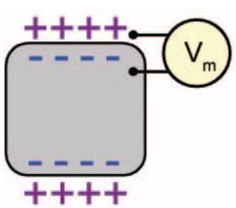
(not the rapid action potentials in classical excitable cells nor effects of environmental electromagnetic exposure)

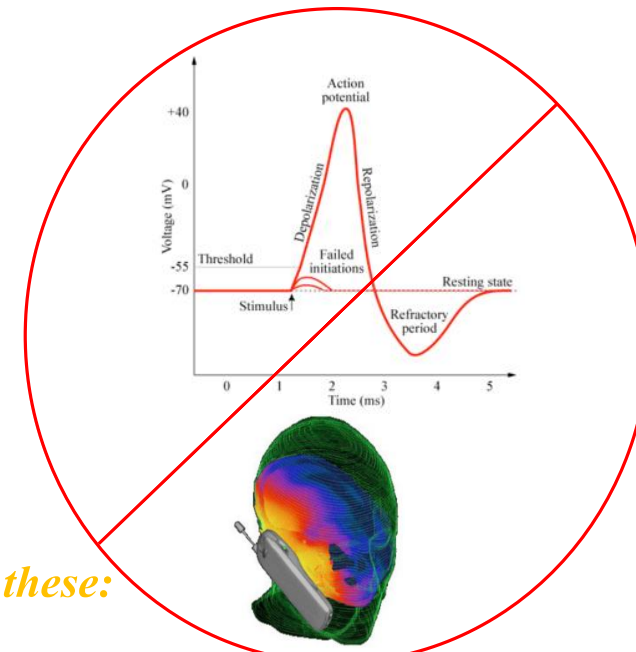


Not These…..

Think this:



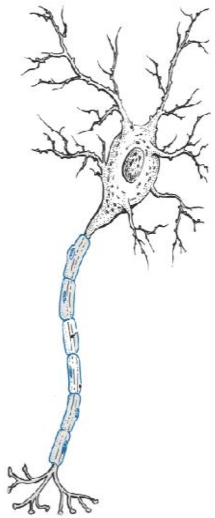


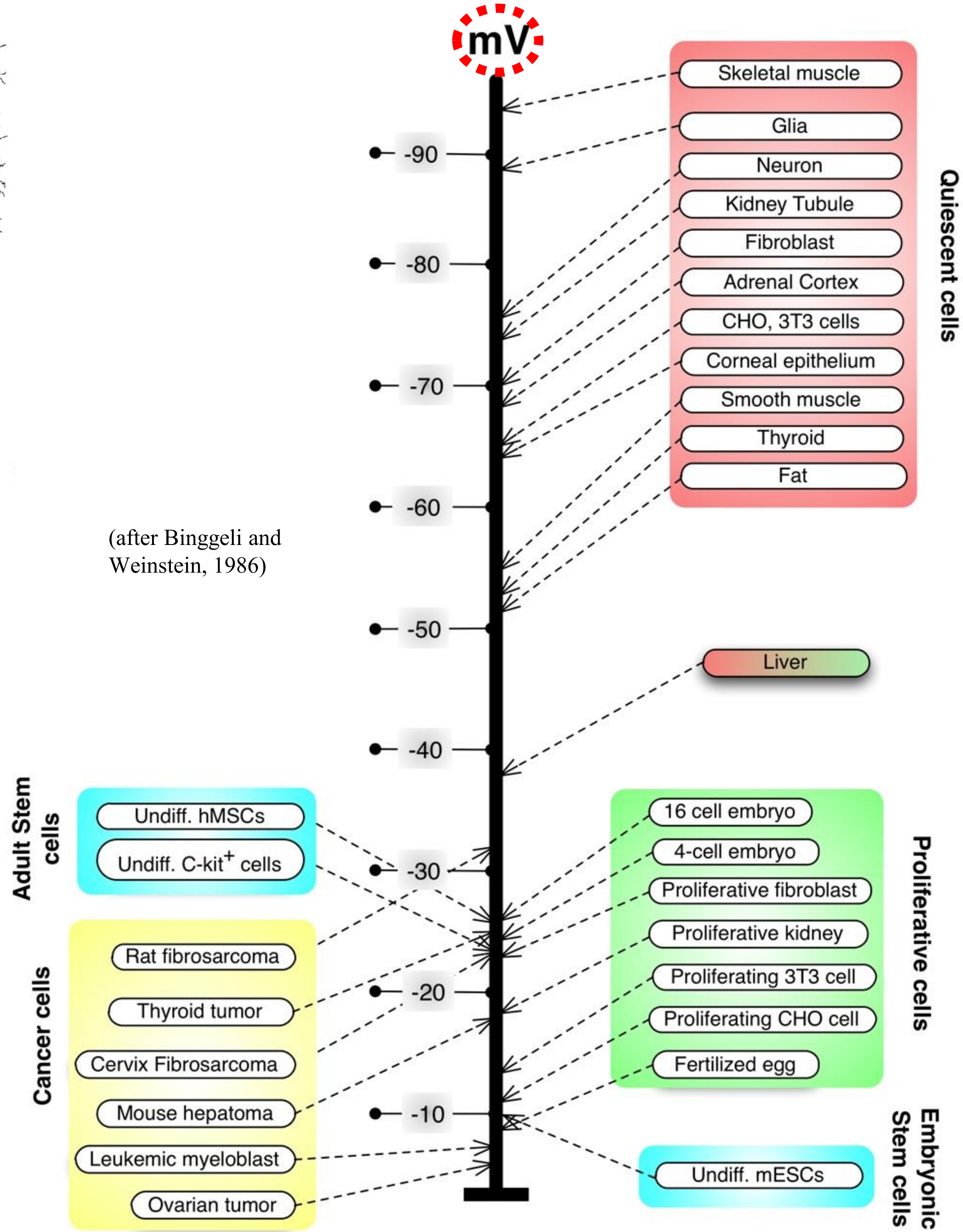


Not these:

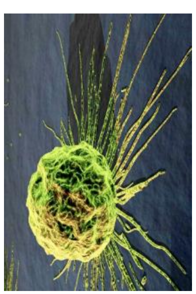
Physiol Rev. 2005 Jul;85(3):943-78

BioEssays, 2012, 34(3): 205-217





(after Binggeli and Weinstein, 1986)



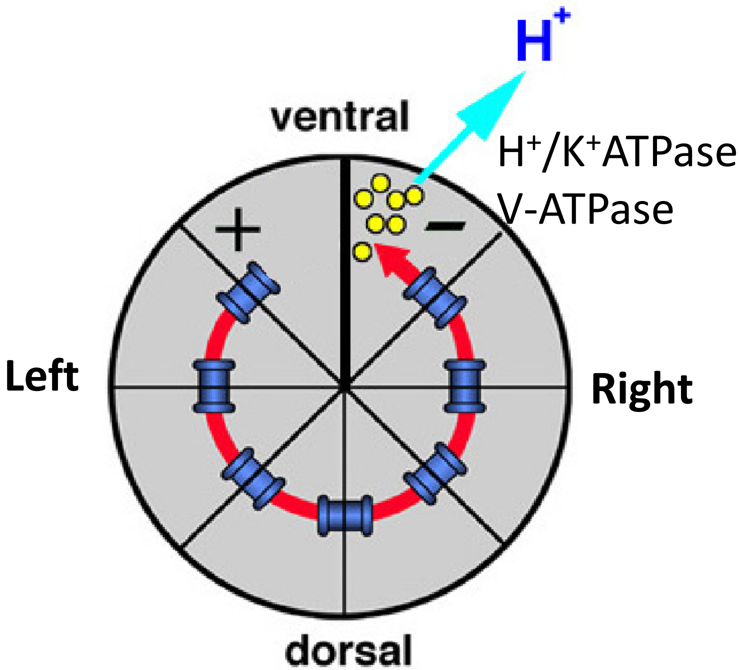
V_{mem} functionally determines cell behavior in many morphogenetic contexts.

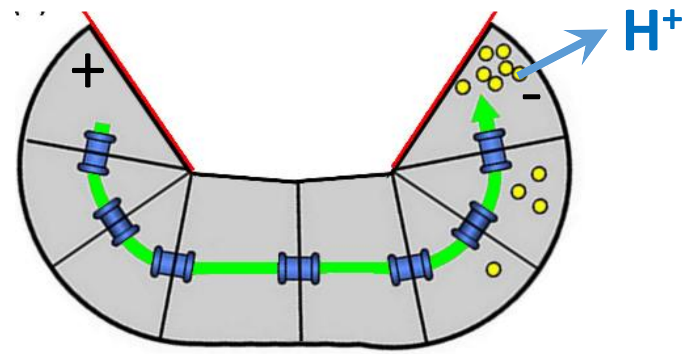
*2007, **Trends in Cell Biology**, 17(6): 262-271*

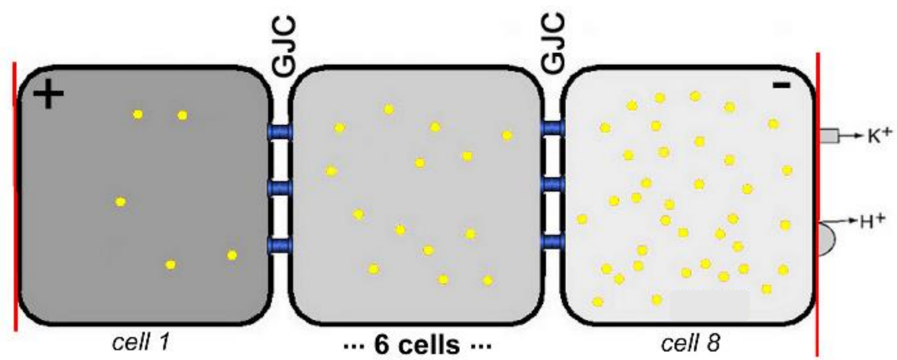
*2009, **Seminars in Cell and Developmental Biology**, 20: 543-556*

*2012, **Annual Reviews in Biomedical Engineering**, 14: 295-323*

Ion-pumps establish L-R voltage gradient across embryo







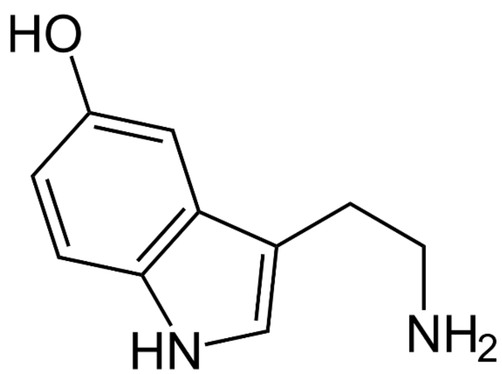
Adams, D. S., Robinson, K. R., Fukumoto, T., Yuan, S., Albertson, R. C., Yelick, P., Kuo, L., McSweeney, M., Levin, M., 2006. Early, H+-V-ATPase-dependent proton flux is necessary for consistent left-right patterning of non-mammalian vertebrates. Development. 133, 1657-1671.

Fukumoto, T., Blakely, R., Levin, M., 2005a. Serotonin transporter function is an early step in left-right patterning in chick and frog embryos. Dev Neurosci. 27, 349-63.

Fukumoto, T., Kema, I. P., Levin, M., 2005b. Serotonin signaling is a very early step in patterning of the left-right axis in chick and frog embryos. Curr Biol. 15, 794-803.

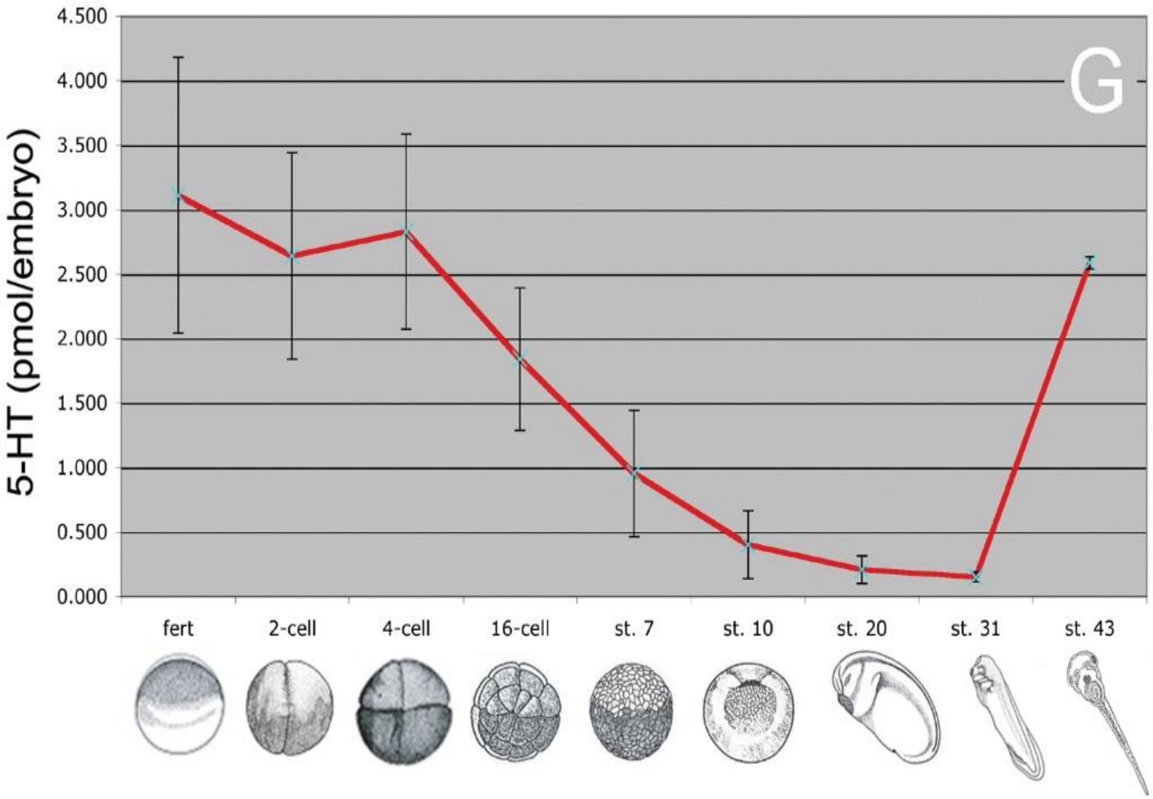
Levin, M., Buznikov, G. A., Lauder, J. M., 2006. Of minds and embryos: left-right asymmetry and the serotonergic controls of pre-neural morphogenesis. Dev Neurosci. 28, 171-85.

Serotonin: a neurotransmitter with many roles

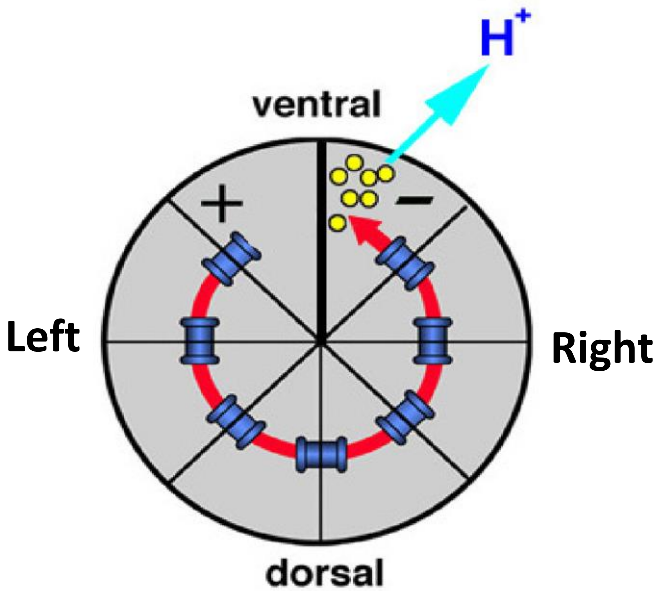


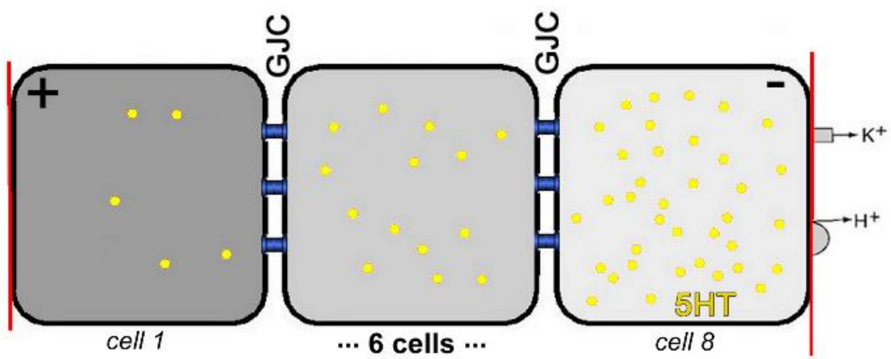
5-Hydroxy Tryptamine – 5-HT
(Serotonin)
Positively Charged

Total levels of maternal serotonin during embryogenesis

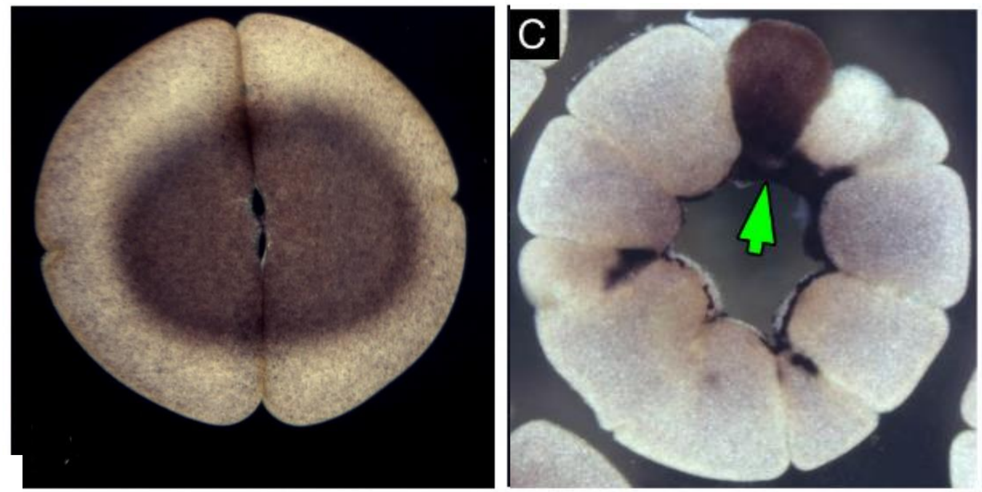


Maternal serotonin (5-HT) forms a voltage- and GJ-dependent rightward gradient in the 16-cell embryo





Serotonin in embryos



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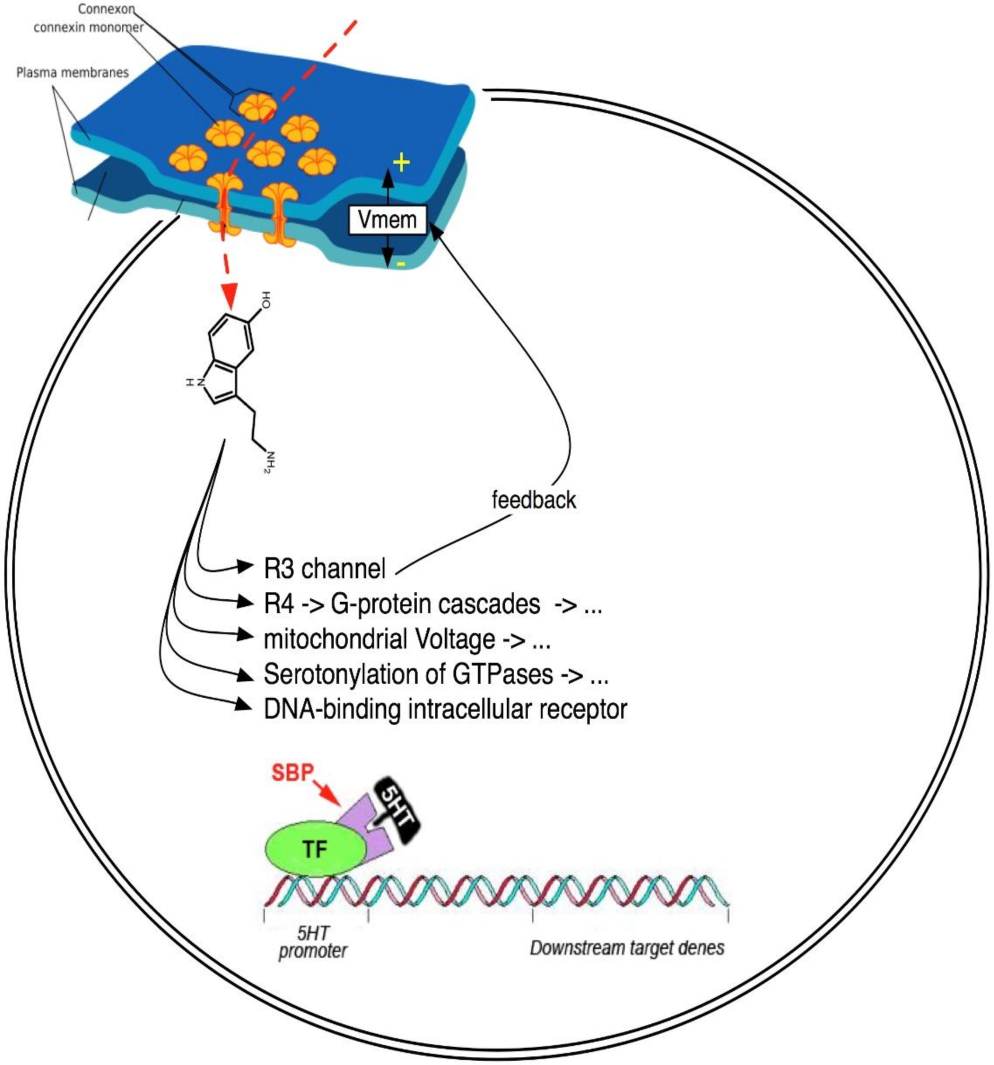
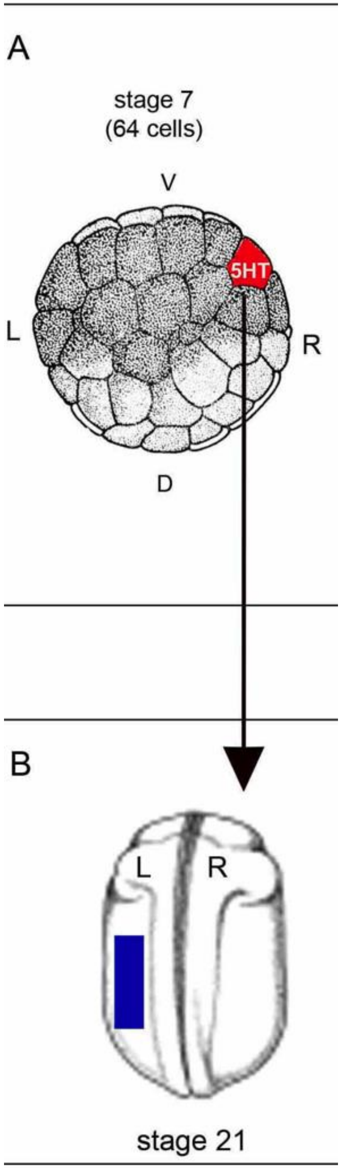
Levin, M., Buznikov, G. A., Lauder, J. M., 2006. Of minds and embryos: left-right asymmetry and the serotonergic controls of pre-neural morphogenesis. Dev Neurosci. 28, 171-85.

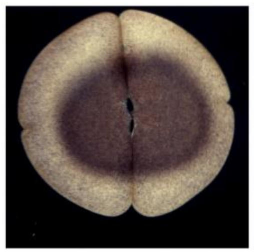
Serotonin triggers L-R asymmetric gene expression – Amplification steps

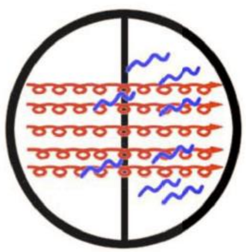




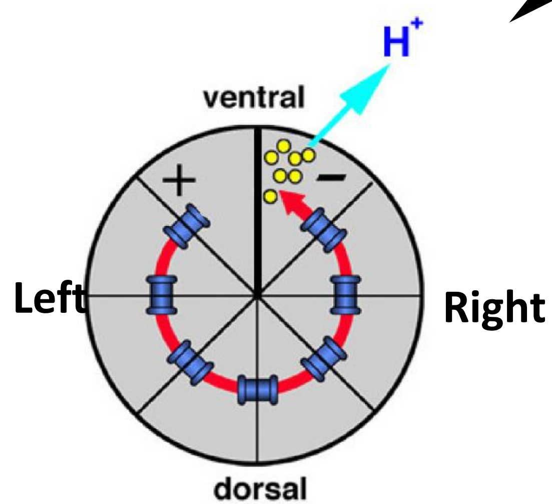
Carneiro, K., et al., and M. Levin, 2011, Histone deacetylase activity is necessary for left-right patterning during vertebrate development, BMC Developmental Biology, 11:29doi:10.1186/1471-213X-11-29

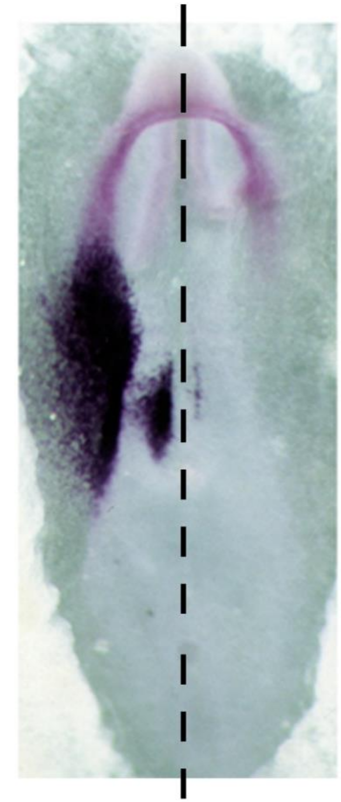
Ion pumps + GJs and unidirectional flow of 5-HT allows asymmetries generated level at single cell to be imposed on large multicellular fields in embryo





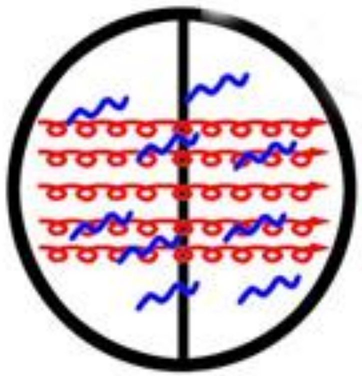
Ion pumps + GJs +5-HT



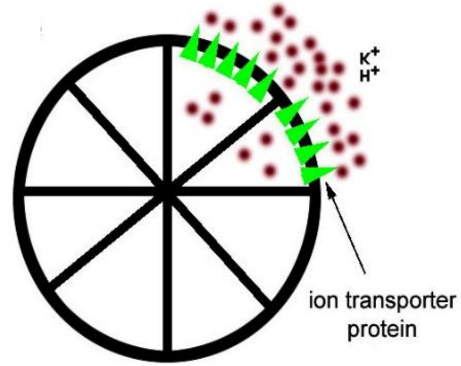


Gene expression
Amplification step

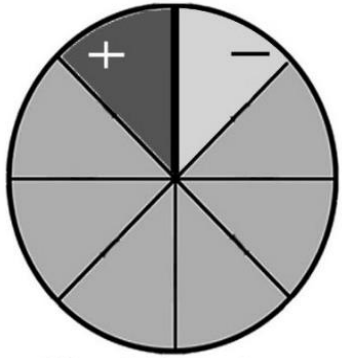
A unified model of LR pattern formation



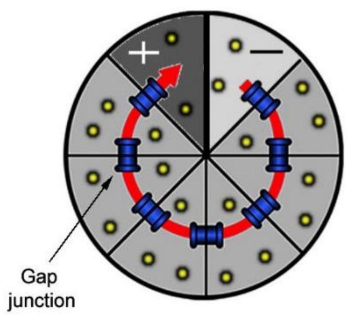
Fertilization and 1st cleavage allow LR computation to be made by a chiral component (cytoskeleton?)



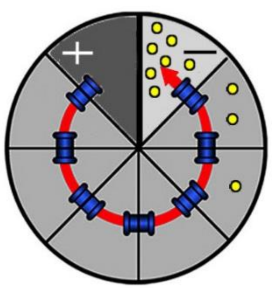
This leads to asymmetric localization of ion channels and pumps



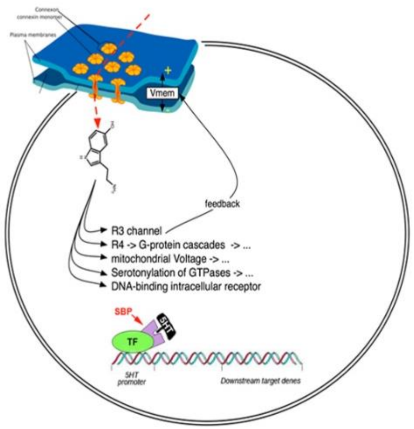
Differential ion exchange with the outside world leads to a LR voltage gradient



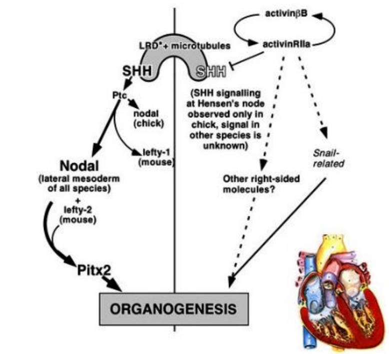
DV-patterning pathways (Wnts, etc.) set up a large-scale GJC region surrounding a zone of isolation. Small molecule determinants are randomly distributed.



The existing voltage difference electrophoreses charged small molecule determinants in a preferred direction, subject to gap junctional selectivity.



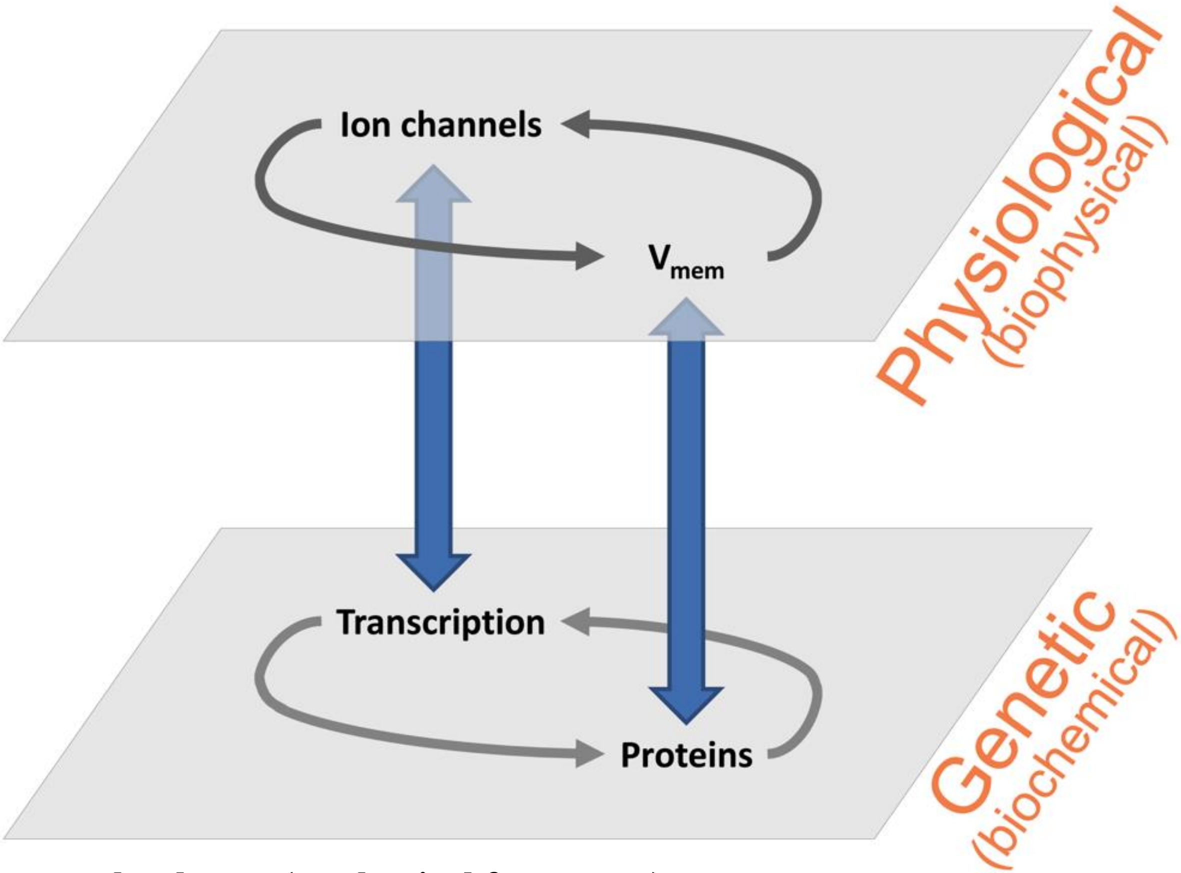
Asymmetric gene expression driven by serotonin signaling



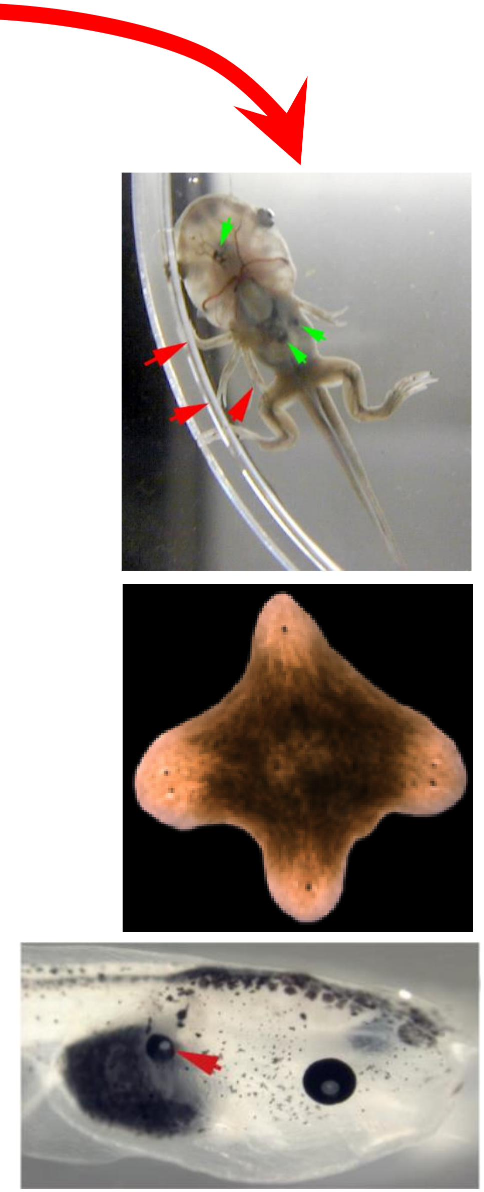
Early asymmetric markers initiate cascade of asymmetric signaling molecules which in turn guide the chiral morphogenesis of the viscera

Levin, M., Palmer, A. R., 2007. Left-right patterning from the inside out: widespread evidence for intracellular control. Bioessays. 29, 271-87.
Levin, M., 2006. Is the early left-right axis like a plant, a kidney, or a neuron? The integration of physiological signals in embryonic asymmetry. Birth Defects Res C Embryo Today. 78, 191-223.

Instructive Shape Information Resides in Bioelectrical Networks among all Cell Types

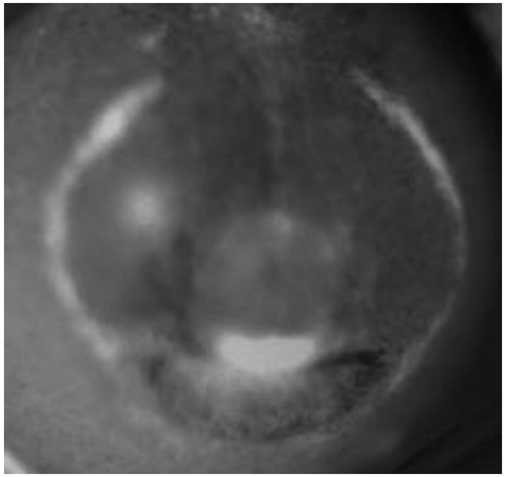


+ other layers (mechanical forces, etc.)



Bioelectric Prepattern Determines Transcriptional Domains during craniofacial morphogenesis

Dany Adams

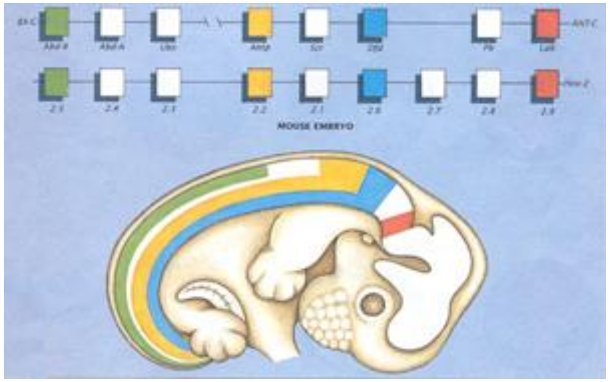


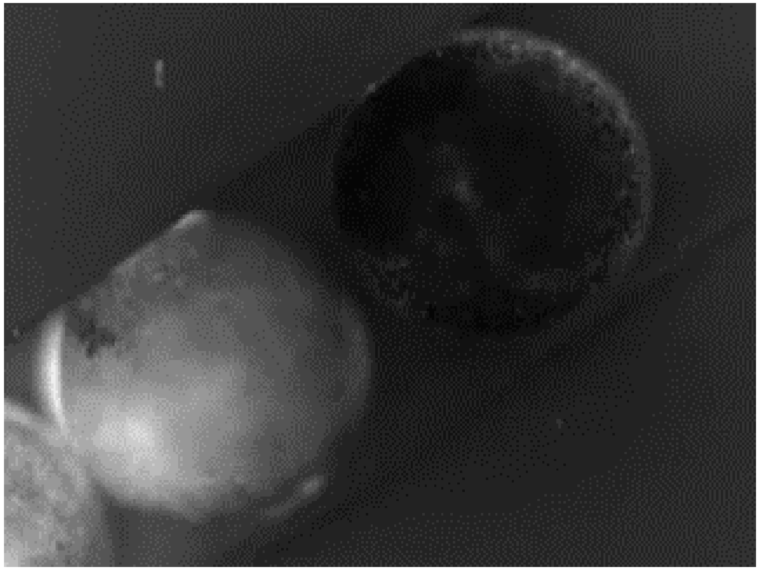
Physiological Prepattern underlying (controlling) gene expression:

O'Donnell et al., 2006

Pax6, Frizzled, Wnts, BMPs

Genetic Prepattern underlying (controlling) subsequent morphology



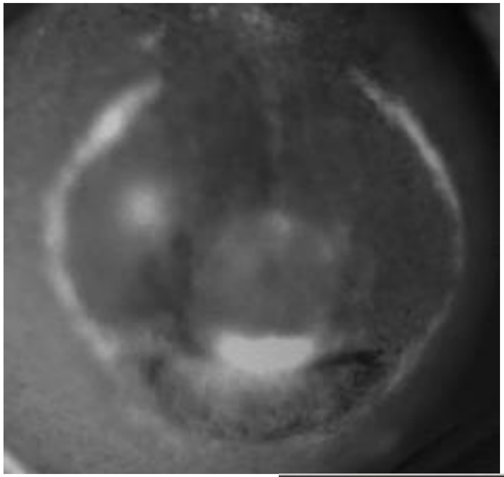


Transmembrane voltage reporter dye during craniofacial patterning:

Developmental Dynamics (2011), 240:1889–1904

Frog embryo's "Electric Face" as prepattern

Endogenous V_{mem} distribution



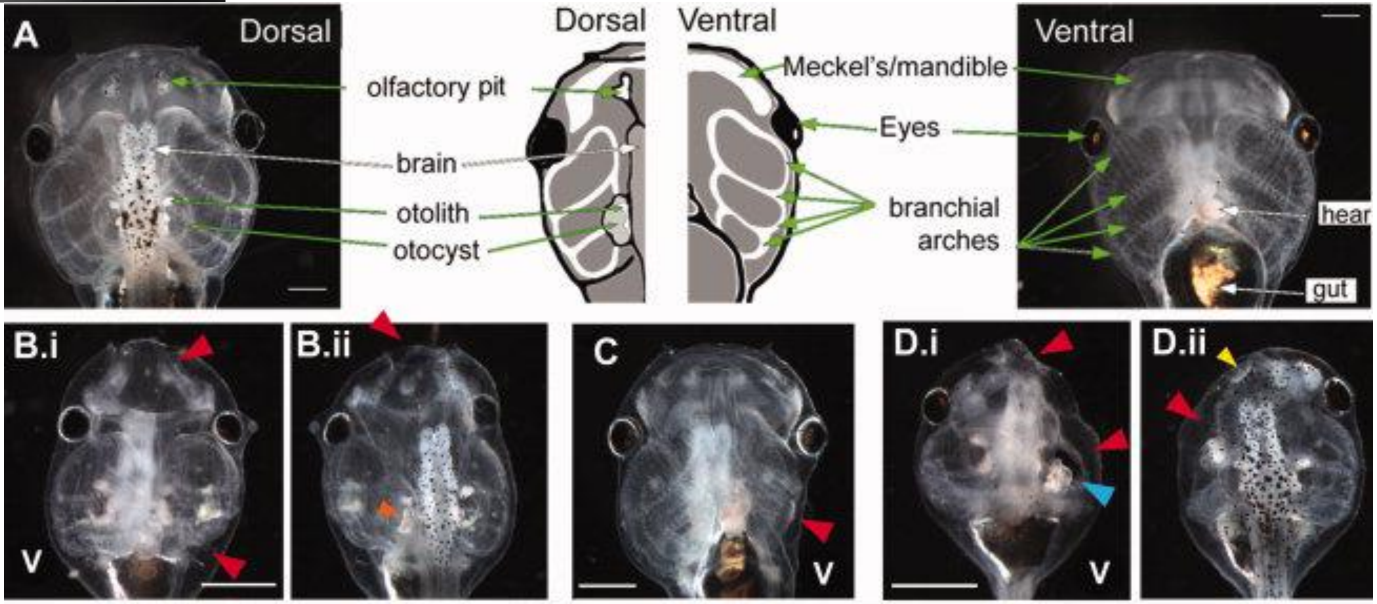
Change V_{mem} pattern using ANY channel

↓

Altered gene expression

↓

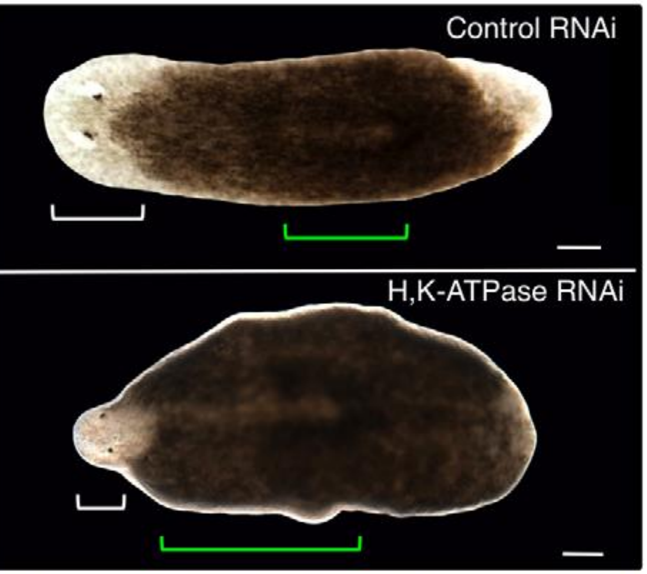
Altered craniofacial anatomy



2011, Dev Dyn, 240:1889-1904

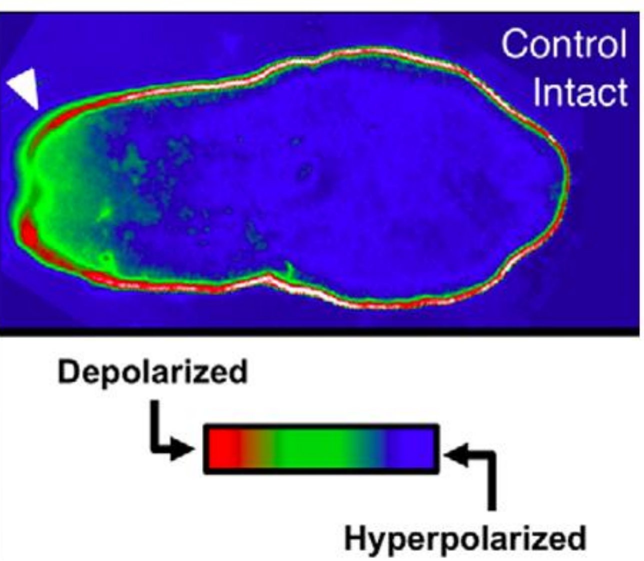
Bioelectric signals also sculpt planarian and human faces

Development, (2013), 140: 313-322



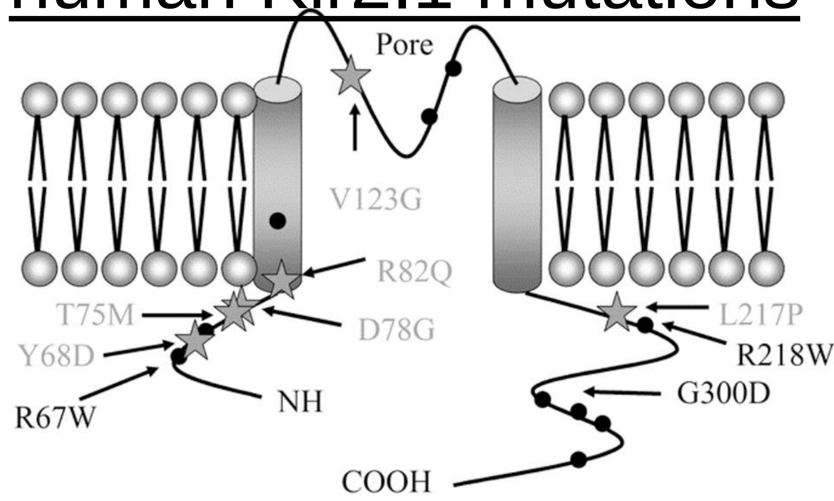
normal head

head with wrong proportions



normal head bioelectric pattern

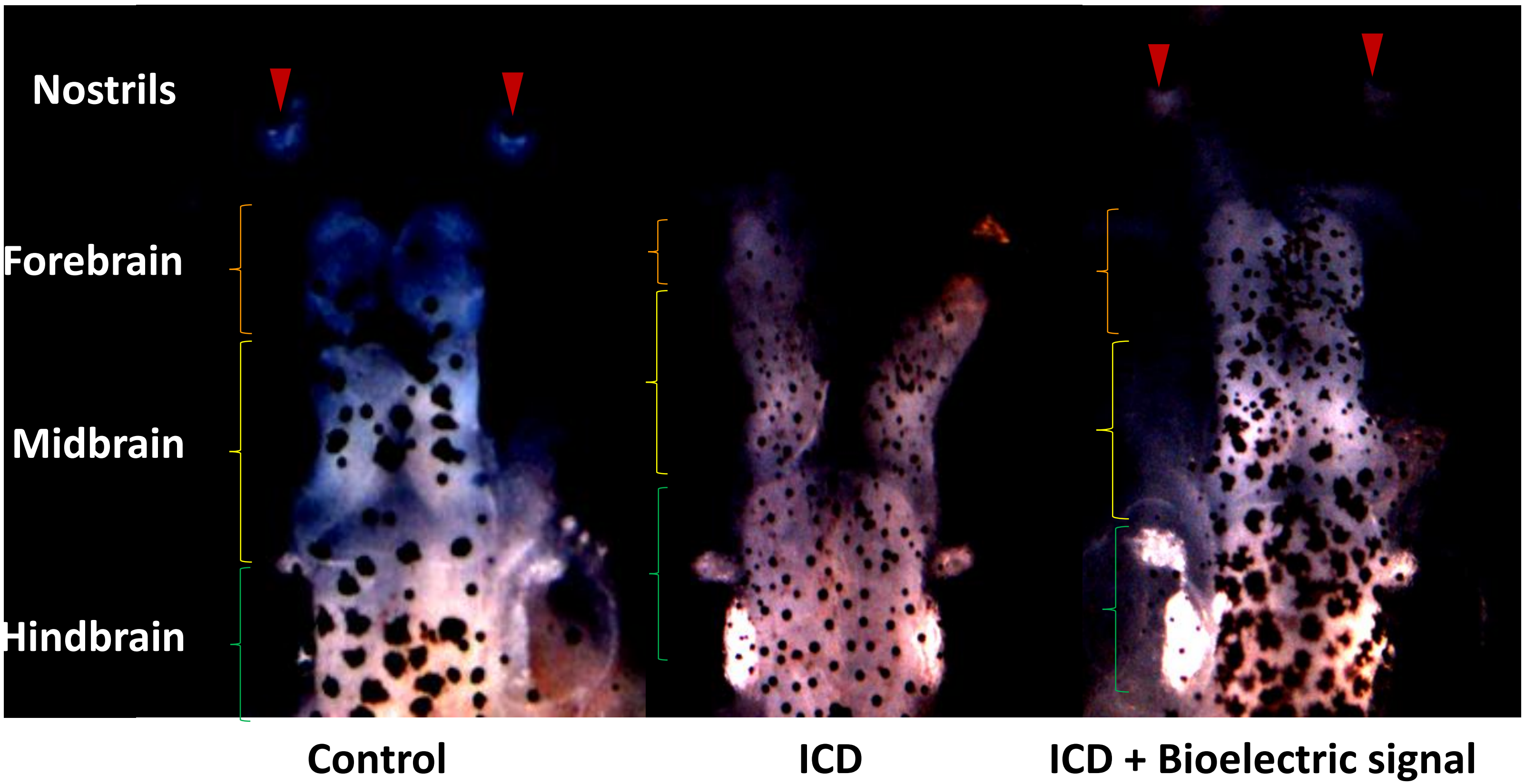
human Kir2.1 mutations



Mutated Kir2.1 channel causes Andersen-Tawil Syndrome in human babies

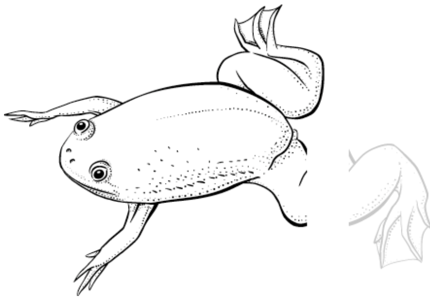
Showing low set ears, micrognathia and retrognathia

Reinforcing bioelectrical signals rescues active notch-induced brain defects

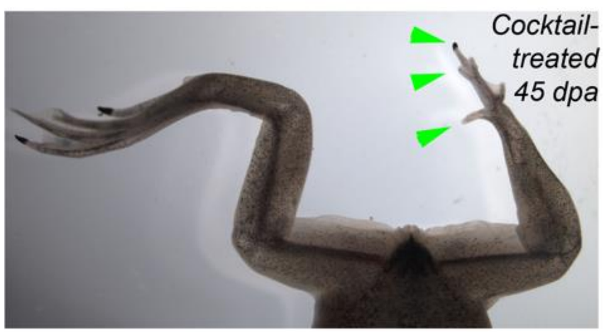


Pai et al 2015 J Neurosci. 35(10):4366-85

Bioelectric signals can triggering regeneration of complex structures

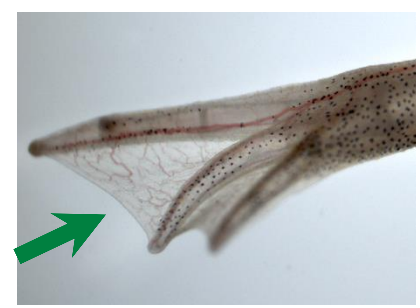


Hind-leg amputation



Outgrowth with distal patterning induced (and still growing)

The regenerated leg has both sensation and mobility:



Implications

Serotonin and ion channels have important functions in development

1) Careful of drugs - SSRIs, anti-epileptics, anti-arrythmics during pregnancy

2) Opportunity for electroceuticals --- To fix defects

Brief Report

Placental Passage of Antidepressant Medications

Victoria Hendrick, M.D.
Zachary N. Stowe, M.D.

Results: Antidepressant and metabolite concentrations were detectable in 86.8% of umbilical cord samples. The mean ratios of umbilical cord to maternal serum concentrations ranged

Published in final edited form as:
Clin Pharmacol Ther. 2009 December ; 86(6): 672–677. doi:10.1038/clpt.2009.201.

Sustained Neurobehavioral Effects of Exposure to SSRI Antidepressants During Development: Molecular to Clinical Evidence

TF Oberlander[1,2], JA Gingrich[3], and MS Ansorge[3]

PNAS

Rare copy number variations in congenital heart disease patients identify unique genes in left-right patterning

Khalid A. Fakhro[a,b], Murim Choi[a,b], Stephanie M. Ware[c], John W. Belmont[d], Jeffrey A. Towbin[c], Richard P. Lifton[a,b,1], Mustafa K. Khokha[a,e,1], and Martina Brueckner[a,e,1]

[a]Department of Genetics, [b]The Howard Hughes Medical Institute, and [e]Department of Pediatrics, Yale University School of Medicine, New Haven, CT 06520; [c]Department of Pediatrics, Cincinnati Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229; and [d]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030

Contributed by Richard P. Lifton, January 3, 2011 (sent for review September 21, 2010)

Selective Serotonin Reuptake Inhibitor Exposure Alters Osteoblast Gene Expression and Craniofacial Development in Mice

James J. Cray Jr*[1], Seth M. Weinberg[2], Trish E. Parsons[2], R. Nicole Howie[3], Mohammed Elsalanty[3,4,5], and Jack C. Yu[4,6]

Thank you to:

Principal Investigator:

Michael Levin

Post-docs:

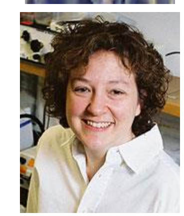
Gary McDowell – Establishment of left-right asymmetry
Celia H-R, and Justin Guay - apoptosis, sodium flux, tail and appendage regeneration
Junji Morokuma - gap junctions and planarian stem cells
Douglas Blackiston - melanocytes and K^+ channels
Vaibhav Pai - ion flux and eye/brain induction, dynamics of bioelectrical networks
Daniel Lobo - symbolic modeling of regeneration
Juanita Mathews -

Students:

Fallon Durant – V_{mem} and planaria regeneration
Brook Chernet – V_{mem} and oncogene-mediated tumor formation
Maria Lobikin - V_{mem} as a regulator of metastasis

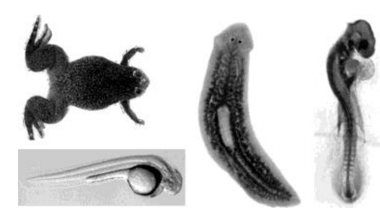
Technical assistance:

Rakela Lubonja - lab manager
Erin Switzer - animal husbandry
Joan Lemire, Jean-Francois Pare - molecular biology



Collaborators:

Dany Adams - V-ATPase in asymmetry, regeneration, craniofacial patterning
Christopher Martyniuk – microarray and gene analysis
David Kaplan - human MSC differentiation and biodome sleeves
Douglas Brash - cancer
Paul C. W. Davies - top-down causation models
John Y. Lin - optogenetics control of V_{mem}



Model systems: tadpoles, worms, zebrafish, and chick embryos

Reagents:

Erik Jorgensen, Trevor Smart, Laurent Bernheim, Henry Lester, Ed Boyden,
Thomas Knopfel, M. Montero-Lomeli, M. Lu - ion transporter constructs

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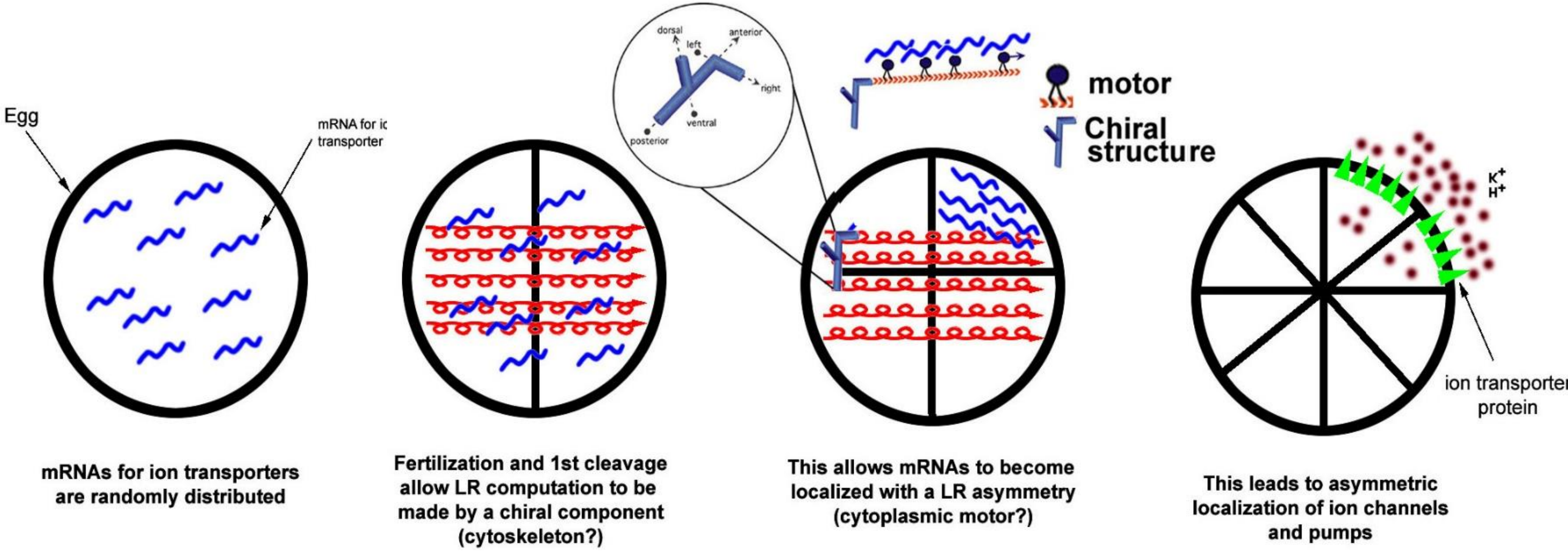
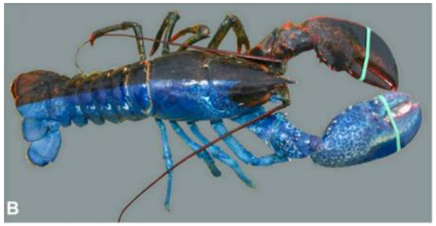
Inspiration: Lionel Jaffe, a pioneer of bioelectricity


time for questions

Cytoskeletal direction orients asymmetric placement of ion pumps.



Bilateral gynandromorphs suggest midline is linked to first cleavage



Levin, M., Palmer, A. R., 2007. Left-right patterning from the inside out: widespread evidence for intracellular control. Bioessays. 29, 271-87.

Levin, M., 2006. Is the early left-right axis like a plant, a kidney, or a neuron? The integration of physiological signals in embryonic asymmetry. Birth Defects Res C Embryo Today. 78, 191-223.

Ion channels are implicated by genetic screens

Protein	Morphogenetic role or LOF phenotype	Species	Reference
TMEM16A chloride channel	Tracheal morphogenesis	Mouse	(Rock et al., 2008)
Kir7.1 potassium channel	Melanosomes development	Zebrafish	(Iwashita et al., 2006)
Cx41.8 gap junction	Pigmentation pattern	Zebrafish	(Watanabe et al., 2006)
Cx45 gap junction	Cardiac defects (cushion patterning)	Mouse	(Kumai et al., 2000; Nishii et al., 2001)
Cx43 gap junction	Fin regeneration	Zebrafish	(Hoptak-Solga et al., 2008)
Cx43 gap junction	Oculodentodigital dysplasia (ODDD), heart defects (outflow tract and conotruncal), left-right asymmetry	Human, mouse	(Britz-Cunningham et al., 1995; Debeer et al., 2005; Ewart et al., 1997; Pizzuti et al., 2004; Reaume et al., 1995)
Kir2.1 potassium channel	Wing patterning	Drosophila	(Dahal et al., 2012)
Cx43 gap junction	Fin size and pattern regulation Craniofrontonasal syndrome	Zebrafish Mouse	(Davy et al., 2006; Iovine et al., 2005; Sims et al., 2009)
Cx43 gap junction	Osteoblast differentiation in bone patterning	Mouse	(Civitelli, 2008)
Kir2.1 potassium channel	Craniofacial morphogenesis (Andersen-Tawil syndrome) and limb patterning	Mouse	(Bendahhou et al., 2003; Dahal et al., 2012)
CFTR chloride channel	Bilateral absence of vas deferens	Human	(Uzun et al., 2005; Wilschanski et al., 2006)
Girk2 potassium channel	Cerebellar development	Mouse	(Hatten et al., 1986; Patil et al., 1995; Rakic and Sidman, 1973a, b)
GABA-A receptor (chloride channel)	Craniofacial patterning (Angelman Syndrome, cleft palate) and limb defects	Mouse	(Culiat et al., 1995; Homanics et al., 1997; Miller and Becker, 1975; Wee and Zimmerman, 1985)
KCNH2 K^+ channel	Cardiac patterning	Mouse	(Teng et al., 2008)
NHE2 Na^+/H^+ exchanger	Epithelial patterning	Drosophila	(Simons et al., 2009)
V-ATPase proton pump	Wing hair patterning	Drosophila	(Hermle et al., 2010)
KCNQ1 potassium channel	Abnormalities of rectum, pancreas, and stomach	Mouse	(Than et al., 2013)
KCNQ1 potassium channel	Inner ear defects - Jervell and Lange-Nielsen syndrome	Human, mouse	(Casimiro et al., 2004; Chouabe et al., 1997; Rivas and Francis, 2005)
Kir6.2 potassium channel	Craniofacial defects	Human	(Gloyn et al., 2004)
NaV channels	Spina bifida, heart, CNS, and neck defects	Human	(Fonager et al., 2000)
NaV 1.5, Na^+/K^+-ATPase	Cardiac morphogenesis	Zebrafish	(Chopra et al., 2010; Shu et al., 2003)
Innexin gap junctions	Foregut development, cuticle (epithelial) patterning	Drosophila	(Bauer et al., 2002; Bauer et al., 2004)

channelopathies

(huge under-estimate due to compensation/redundancy)

Ion Translocator Protein	Species	Reference
NaV1.5 sodium channel	Human	(House et al., 2010b; Onkal and Djamgoz, 2009)
EAG-1 potassium channel	Human	(Pardo et al., 1999)
KCNK9 potassium channel	Mouse	(Pei et al., 2003)
Ductin (proton V-ATPase component)	Mouse	(Saito et al., 1998)
SLC5A8 sodium/butyrate transporter	Human	(Gupta et al., 2006)
KCNE2 potassium channel	Mouse	(Roepke et al., 2010)
KCNQ1 potassium channel	Human, mouse	(Lee et al., 1997; Than et al., 2013; Weksberg et al., 2001)
SCN5A voltage-gated sodium channel	Human	(House et al., 2010a)
Metabotropic glutamate receptor	Mouse, Human	(Martino et al., 2012; Song et al., 2012; Speyer et al., 2012)

Ion channel/pump oncogenes

If cilia initiate asymmetry de novo, and are a widely-relevant, global LR symmetry compass, how good is the fit to actual data?

Experimental question:	**cilia model predicts:**	**cytoplasmic model predicts:**	**chromatid segregation model predicts:**	**experimental result:**
Should mutation of kinesin, dynein, MTOC, and PCP proteins randomize LR?	YES	YES	YES	YES
Should viscosity changes at the node randomize LR?	YES	no, unless cilia amplify	no, unless cilia amplify	YES
Should chick embryos have asymmetric gene expression before node forms?	NO	YES	YES	YES
Should frog embryos establish asymmetric gradients long before cilia form?	NO	YES	YES	YES
Should disruption of cytoskeleton or of physiological asymmetries only during the first couple of cleavages randomize LR?	NO	YES	yes	YES
Should chick node receive LR information from lateral tissue?	NO	YES	NO	YES
Should embryos (human & newt) randomize if split at the 2 cell stage?	NO	YES	yes	YES[1]
Should the brains of primary ciliary dyskinesia patients have normal laterality?	NO	YES	YES	YES
Should any mutants exist with abnormal ciliary flow but normal LR asymmetry?	NO	YES	YES	YES
Should animals and plants with no cilia and no node be able to establish asymmetry using some of the same molecules as ciliated vertebrates?	NO	YES	YES	YES
Should organizers induced past the first few cleavages be randomized?	NO	YES	YES	YES

Origin of asymmetry:	Centriole + PCP	Centriole + PCP	Mitotic apparatus + PCP
Amplification by:	Cilia-driven fluid flow in node during gastrulation	Intracellular localization of bioelectric machinery at cleavage stages	PCP-aligned differential chromatid segregation

So,

Many of the mouse genetic studies do not distinguish among the two models

Cilia definitely appear to do something, perhaps a parallel, later pathway?

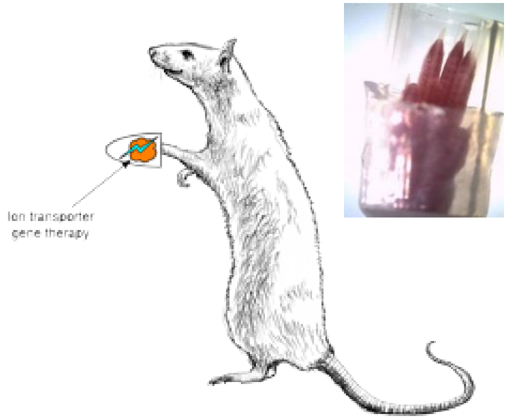
New Concepts, Tools
(next steps for us)

- Target morphology encoding in bioelectrical networks as a true memory (incorporating ideas from cognitive science)

- Spontaneous symmetry-breaking in bioelectrical networks (modeling of self-organizing voltage patterns in tissues for guided self-assembly in synthetic biology)

- Optogenetics for non-excitable cells - cracking the bioelectric code by reading/writing electrical patterns in vivo

- Biomedical applications
  - cancer diagnosis, suppression
  - wearable bioreactors for rat limb regeneration



- AI tools for a new Bioinformatics of Shape