HAMM-LIPPSTADT
UNIVERSITY OF APPLIED SCIENCES

Of mice, chicken and human induced pluripotent stem cells: studying midbrain dopaminergic neuron development and survival in the context of Parkinson's Disease

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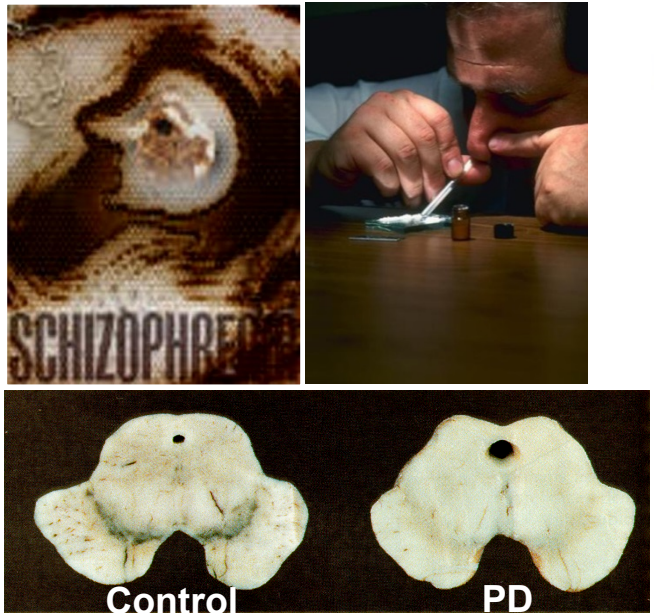
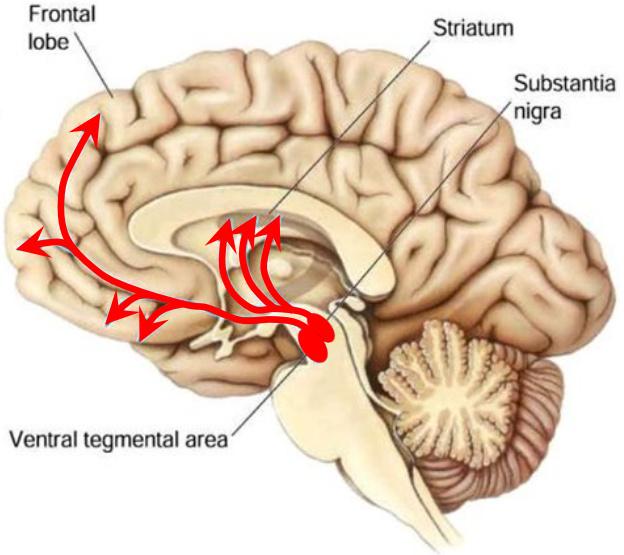
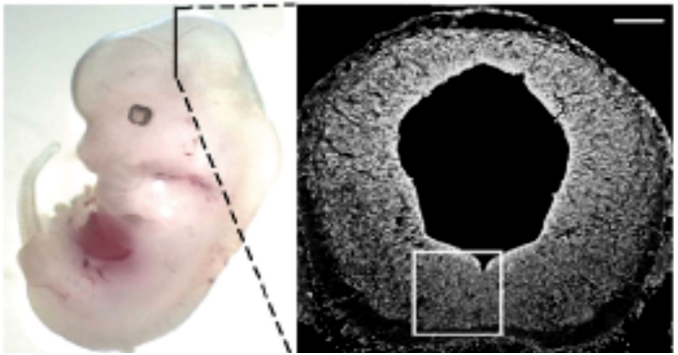
Human midbrain dopaminergic (mDA) systems



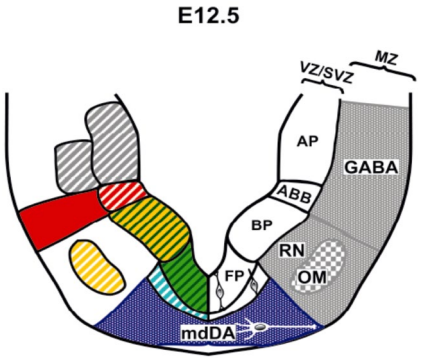


1. Mesostriatal	motor control	Parkinson‘s Disease (PD)
2. Mesocortical	cognition	Schizophrenia
3. Mesolimbic	motivation/reward	Addiction

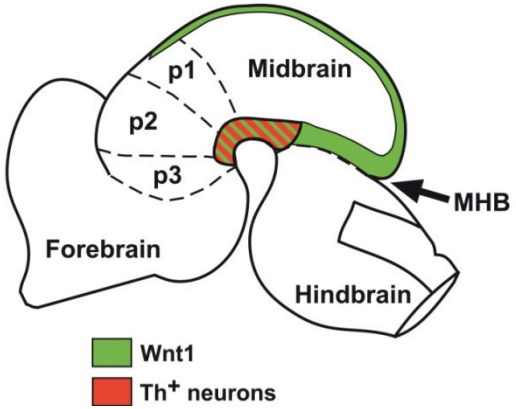
mdDA neuron development in the mouse



Ang, Development 2006



Wurst and Prakash, DA Handbook 2010



mdDA progenitor

OTX2, LMX1A/B, MSX1/2, NGN2, MASH1, EN1/2, ALDH1A1

mdDA precursor

LMX1A/B, NGN2, NURR1, EN1/2, ALDH1A1, TUBB3

mdDA neuron

LMX1A/B, NURR1, EN1/2, PITX3 TH, AADC, ALDH1A1 TUBB3

SNc DA neuron

KCND3+

VTA DA neuron

CALB1+

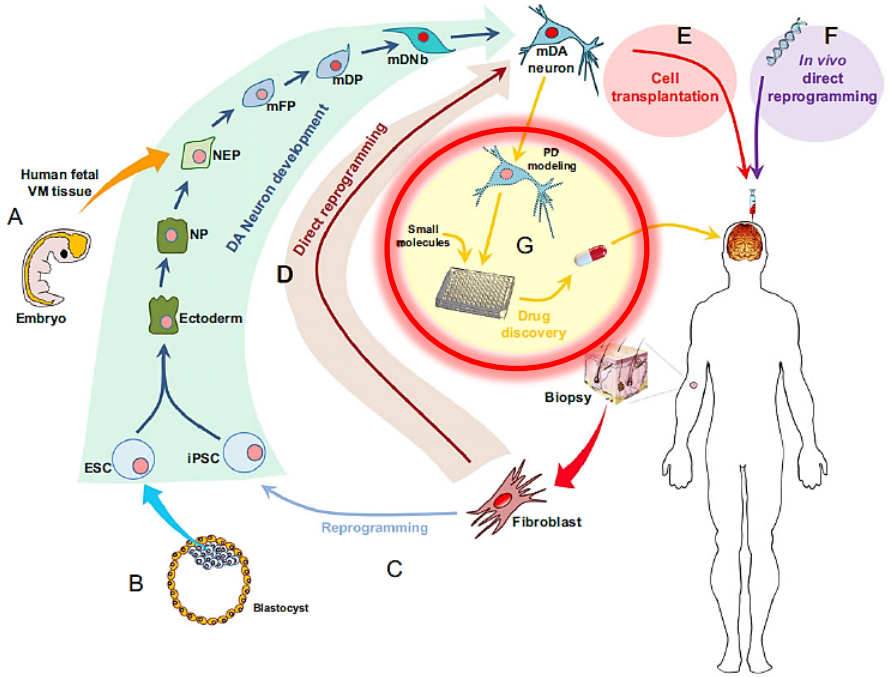
Specification

Induction

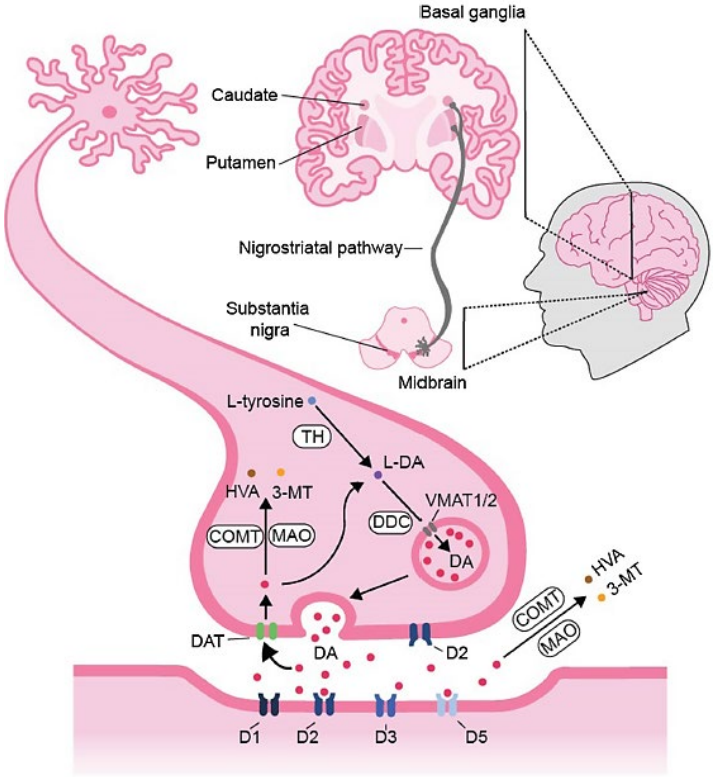
?

Terminal differentiation / survival

PSC-based approaches to DA-associated neuropsychiatric diseases

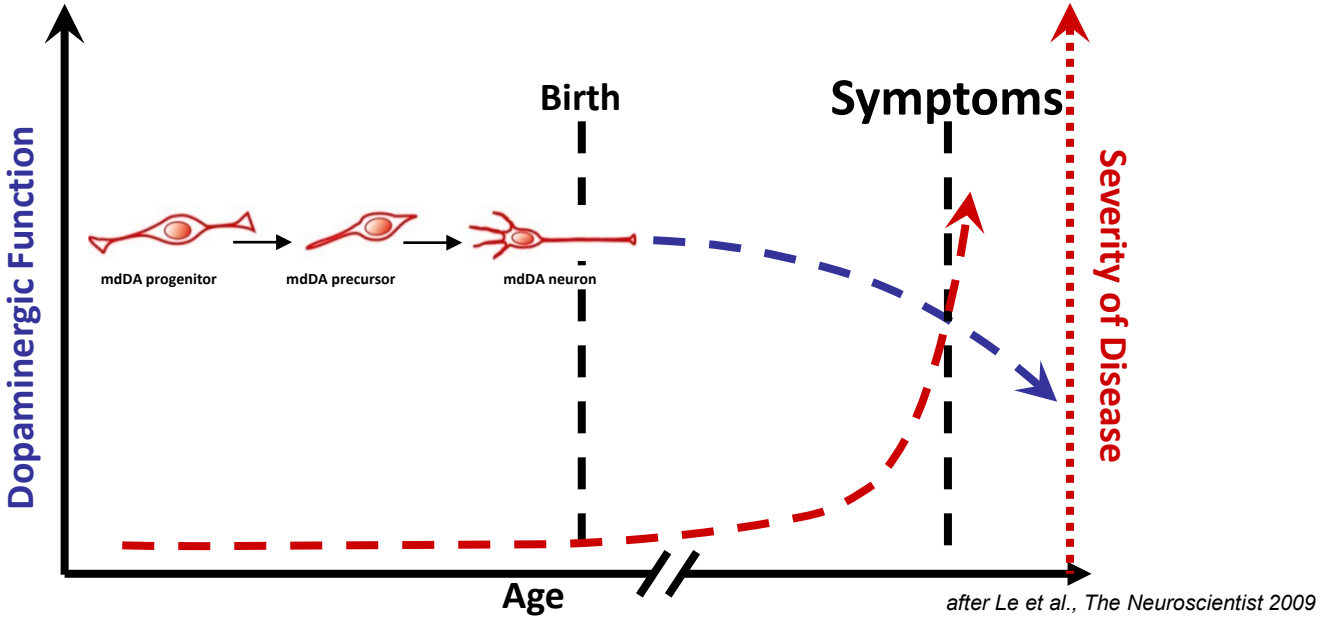


Arenas et al., Development 2015



Lopes et al., Neuromol Med 2017

PD is an age-related progressive neurodegenerative disease



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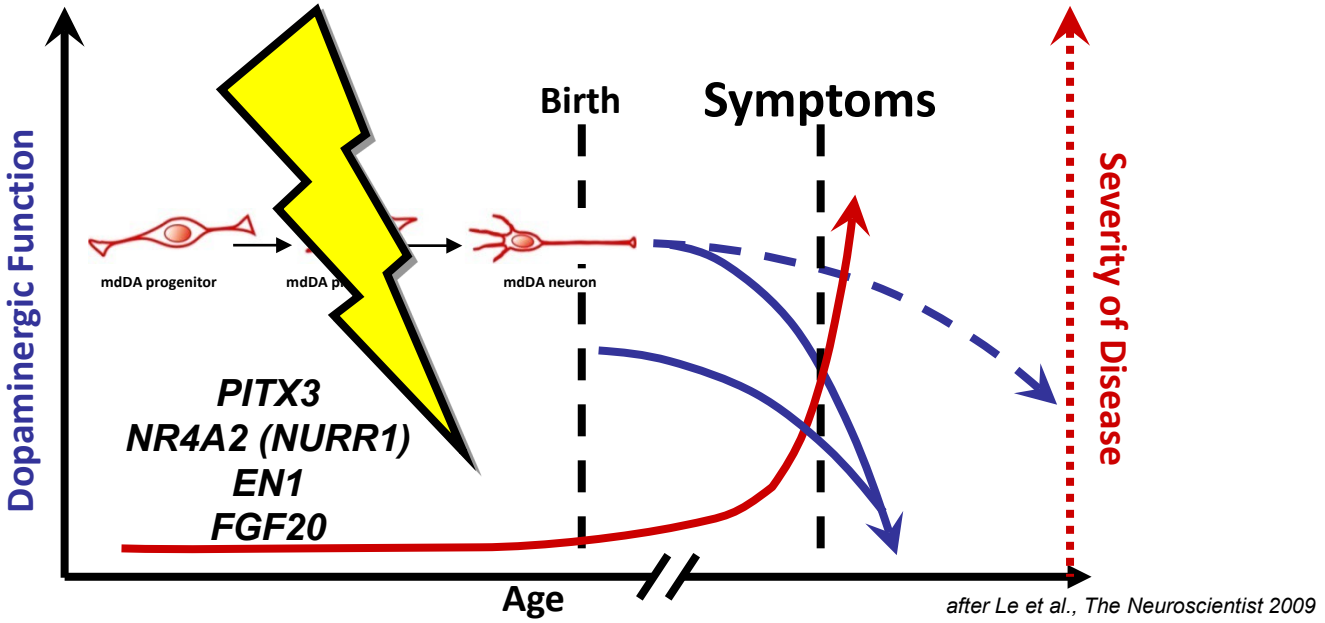
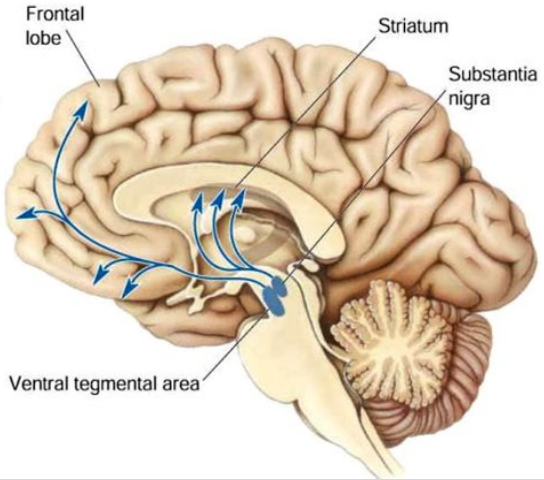
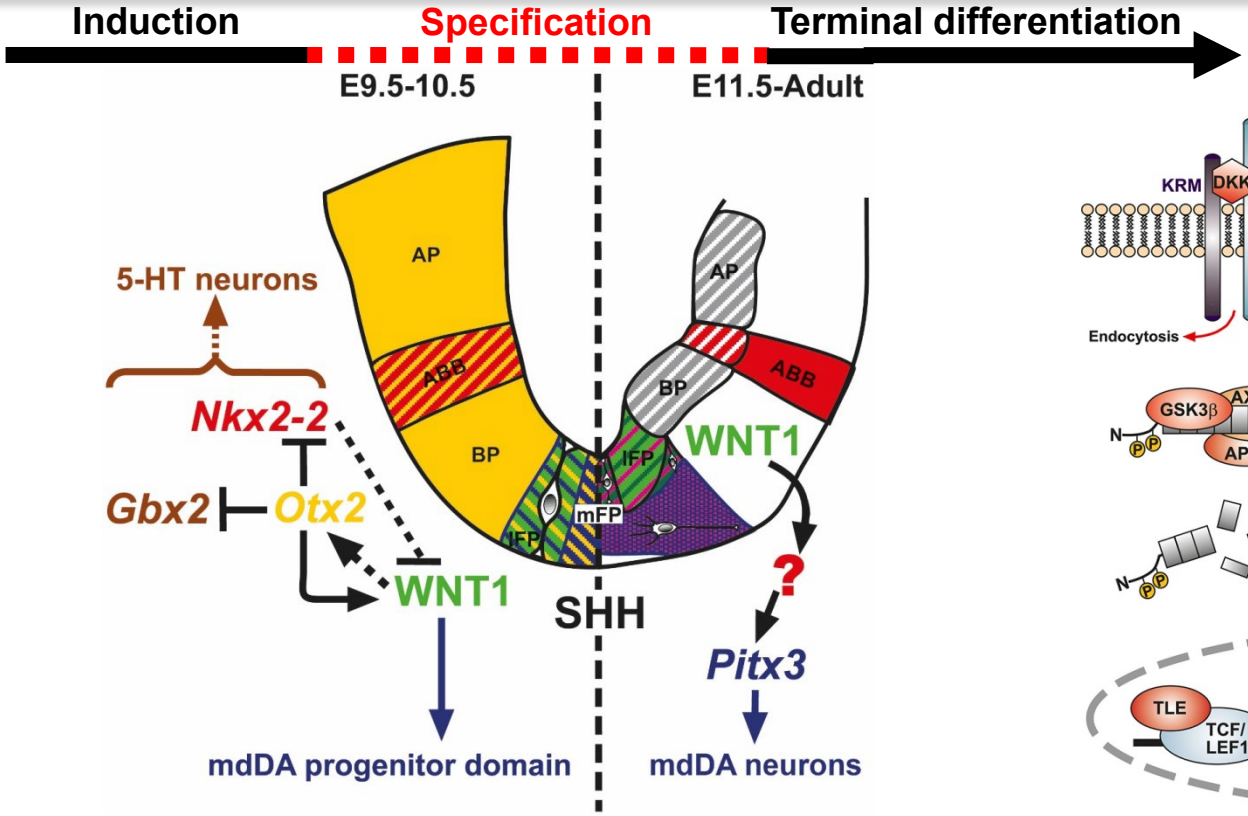


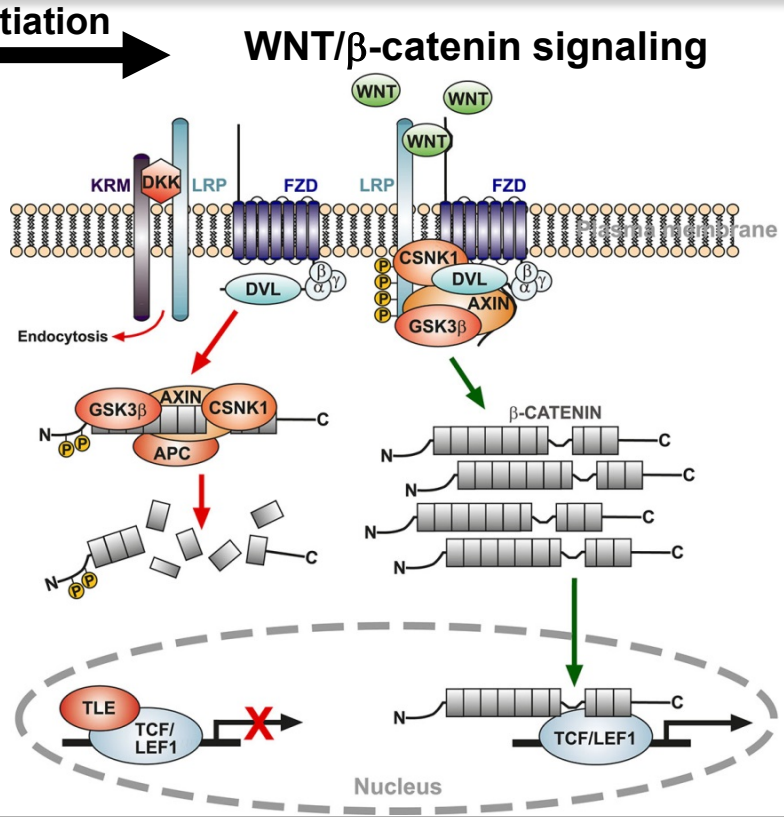
- ***PITX3* polymorphisms: sporadic and early-onset PD** (Fuchs et al., 2009; Bergman et al., 2010; Le et al., 2011; Haubenberger et al., 2011; Guo et al., 2011)
- ***NR4A2* polymorphisms/mutations: sporadic and familial PD** (Le et al., 2003; Zheng et al., 2003; Xu et al., 2002; Grimes et al., 2006)
- ***EN1* polymorphisms: sporadic PD** (Fuchs et al., 2009; Haubenberger et al., 2011)
- ***FGF20* polymorphisms: familial and sporadic PD** (van der Walt et al., 2004; Mizuta et al., 2008; Wang et al., 2008; IPDGC & WTCCC2, 2011; Pan et al., 2012)



Genetic mouse models

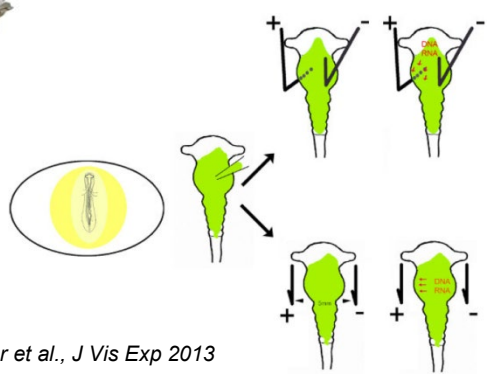
WNT1 controls two different steps in the generation of mdDA neurons *in vivo*



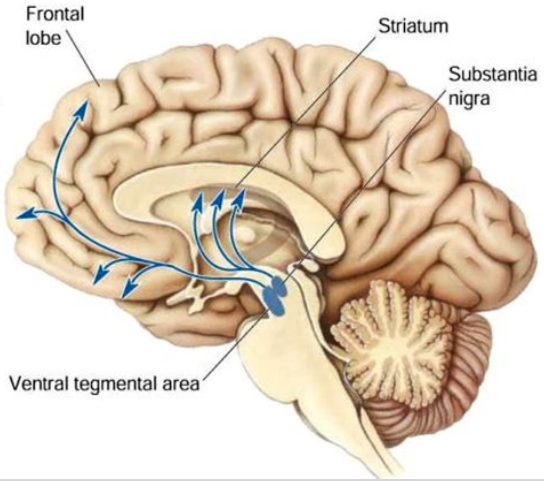


Prakash et al., Development 2006
Omodei, et al., Development 2008
Di Giovannantonio, et al., Dev Biol 2013

Joksimovic et al., Nat Neuroscience 2009
Tang et al., Development 2009
Tang et al., J Neurosci 2010

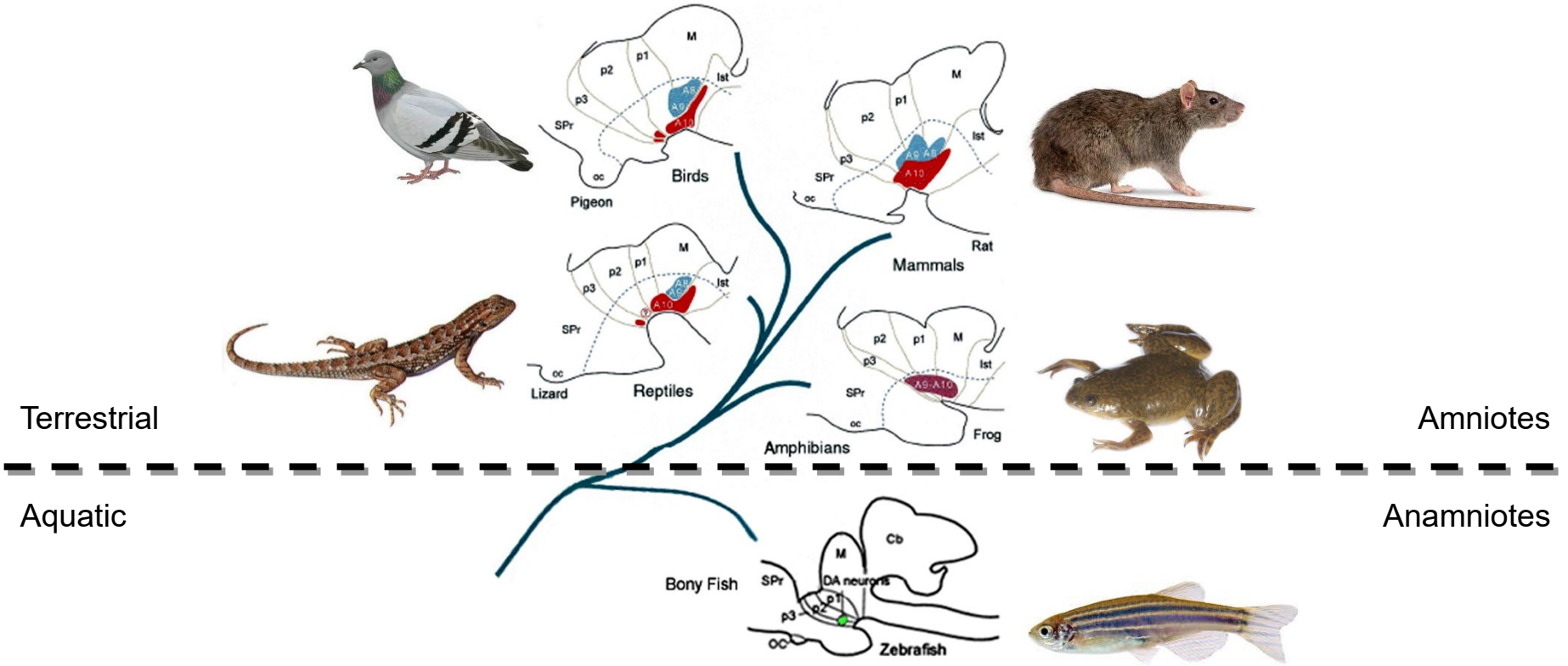


Huber et al., J Vis Exp 2013



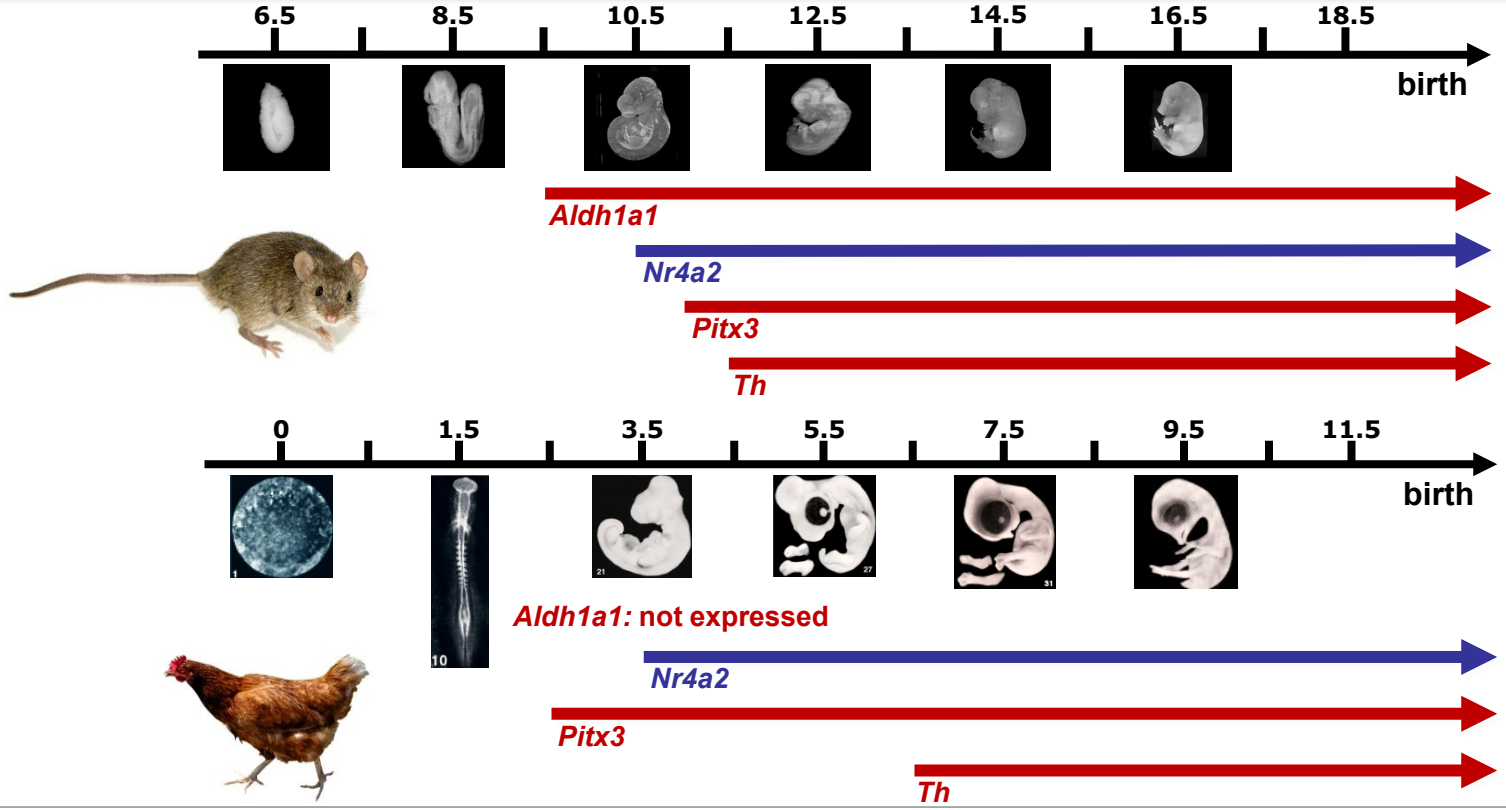
Chicken *in ovo* electroporation

mdDA neurons appeared at the tetrapod transition during evolution



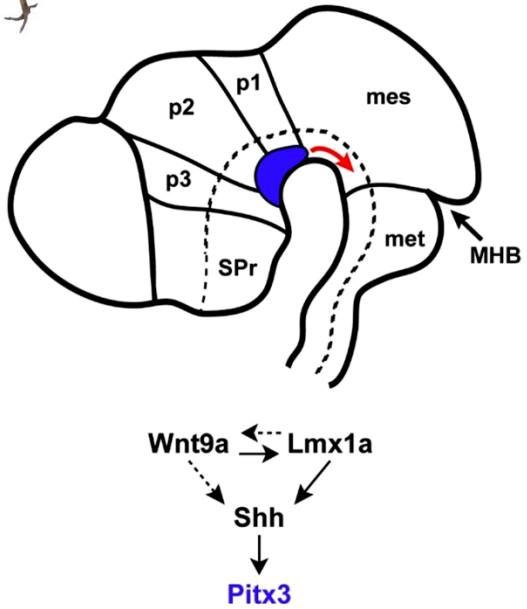
modif. Marín et al., TINS (1998)

Differences in the spatiotemporal expression profile of mdDA neuron markers between chicken and mice

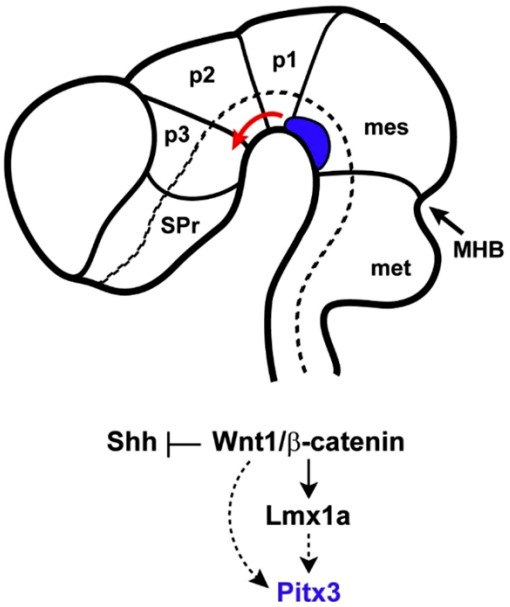


Klafke et al., Development 2016

Differences in the genetic regulation of mdDA neuron markers between chicken and mice



B



Klafke et al., Development 2016

Species-specific (evolutionary) similarities and differences in mdDA neuron development

1. Similarities:

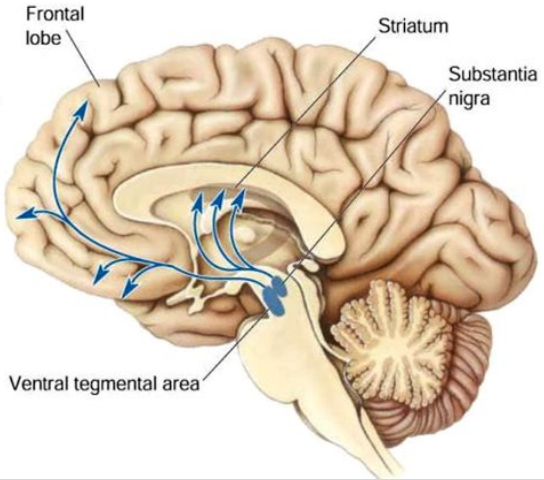
- Chicken and mice transcription factor (*Nr4a2*, *Pitx3*) and enzyme (*Aldh1a1*, *Th*) gene sequences are very conserved and the corresponding proteins most likely have the same biochemical functions.

2. Differences:

- Transcriptional (and posttranscriptional/posttranslational?) regulation and epistatic relationships of these genes/signaling pathways have diverged between chicken and mice.

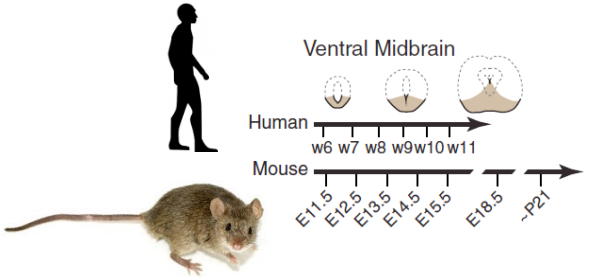
- Consequently, the spatiotemporal expression patterns of these genes in the brain differ considerably between chicken and mice.

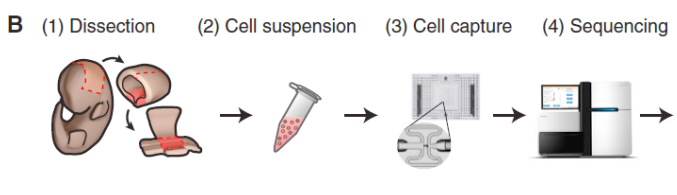
☞ **Developmental (preclinical) studies in animal models may have only very limited translational value for the human situation.**

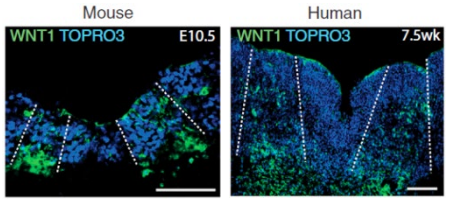


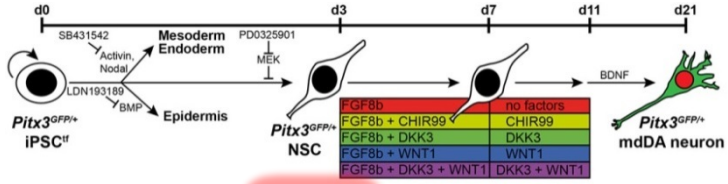
Genetic mouse models

Differences in gene regulatory signaling levels for mdDA neuron generation between mice and humans?

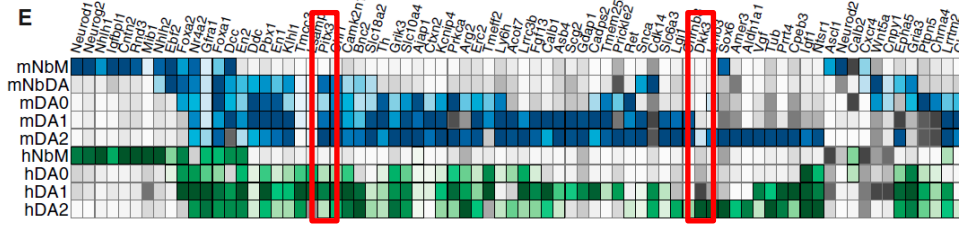


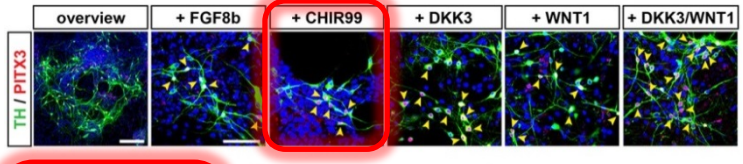


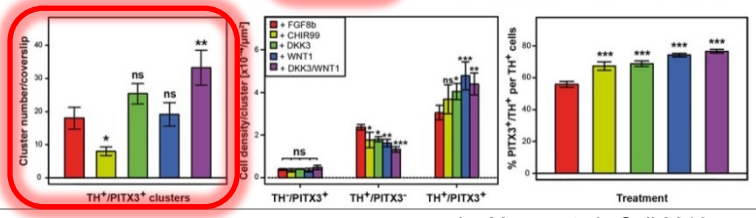




Similar gene expression:

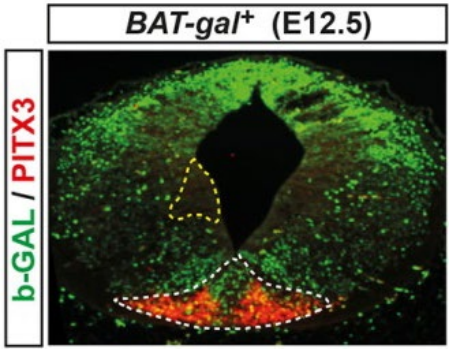






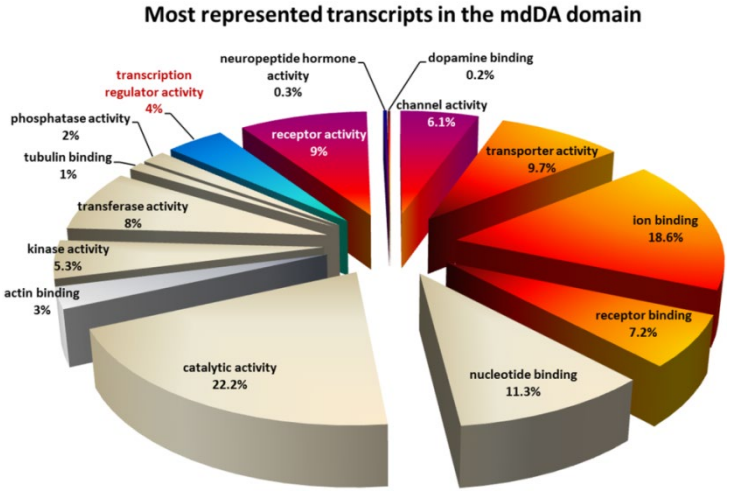
La Manno et al., Cell 2016
Pertek et al., Mol Biotechnol 2014
Fukusumi et al., J Neurosci 2015

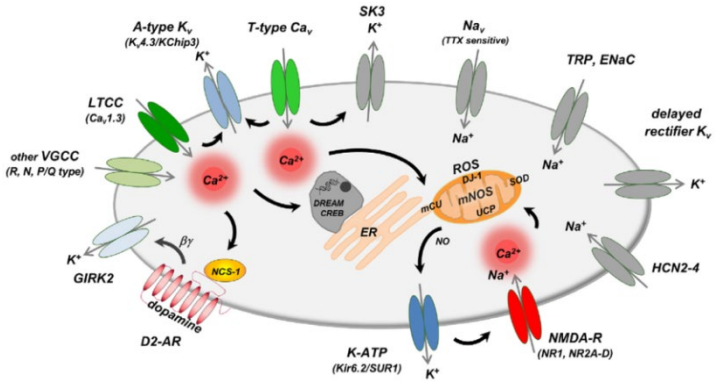
Transcriptome profiling of developing WNT-responsive mdDA neurons



Laser microdissection

Affymetrix Mouse Gene 1.0 ST arrays



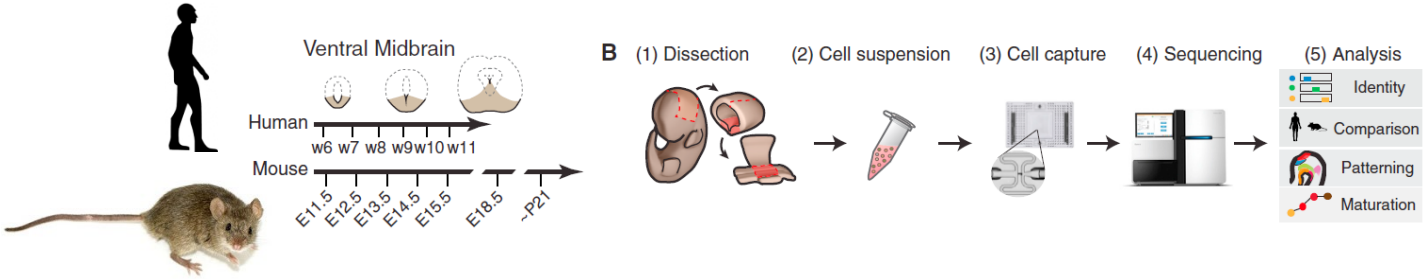


Dragicevic et al., Neuroscience 2015

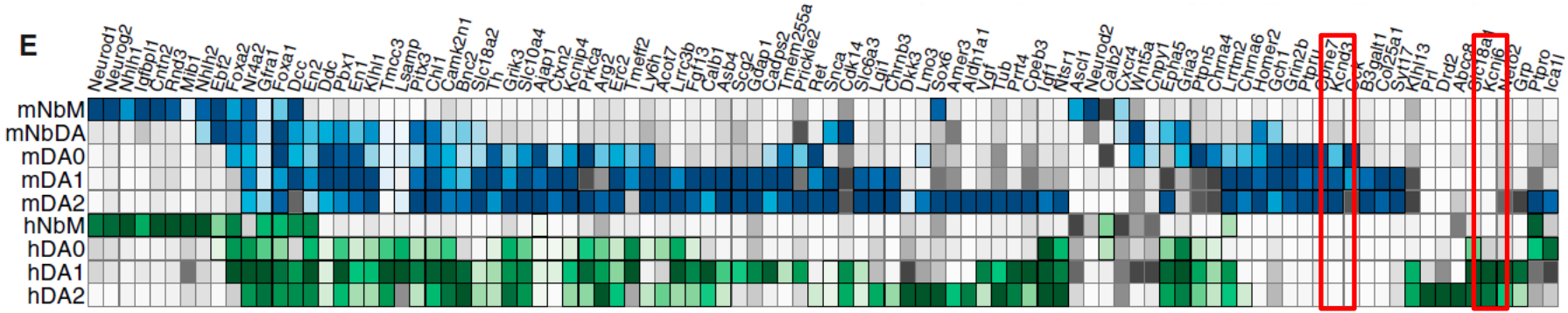
☞ ~51% of the transcripts enriched in the WNT-responsive mdDA domain at E12.5 encode ion channel, receptor and transporter proteins.

Götz et al., Acta Physiol 2016

Differences in the spatiotemporal expression profile of activity-related mdDA neuron genes between mice and humans?



Distinct gene expression:



La Manno et al., Cell 2016

Species-specific (evolutionary) similarities and differences in mdDA neuron development

1. Similarities:

- WNT1/b-catenin signaling appears to be crucial for mdDA neuron generation in mice and humans.

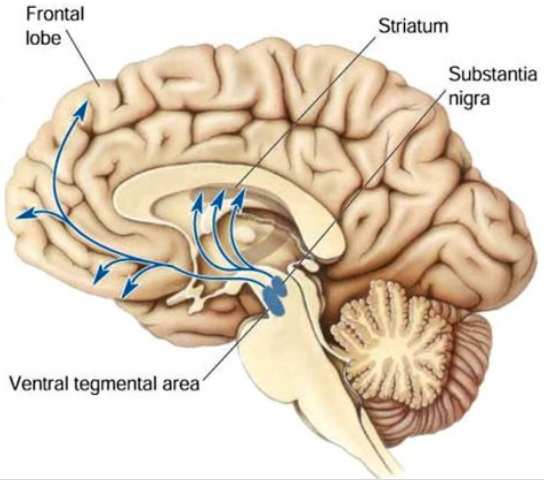
- Spatiotemporal expression patterns of critical components and target genes of this signaling pathway are at least similar if not identical in mice and humans.

2. Differences:

- Human mdDA neuron development appears to require a much higher WNT/b-catenin signaling dosage compared to mouse.

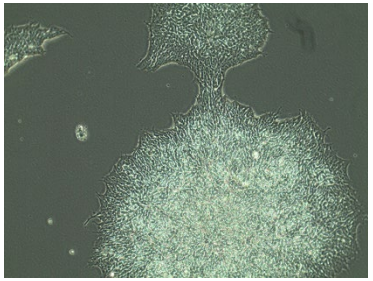
- Spatiotemporal expression patterns of electrophysiological activity-related and potential WNT/b-catenin target genes appear to differ between mice and humans (?).

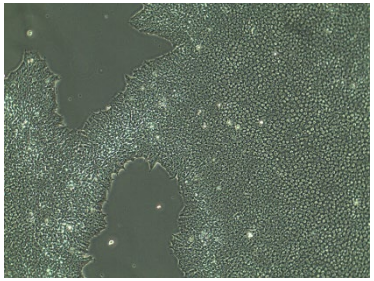
☞ **Assessment of gene expression patterns, regulatory (signaling) pathways and physiological aspects in the human condition (stem cells and tissues) appears mandatory for any translational approach in human mdDA neuron development.**

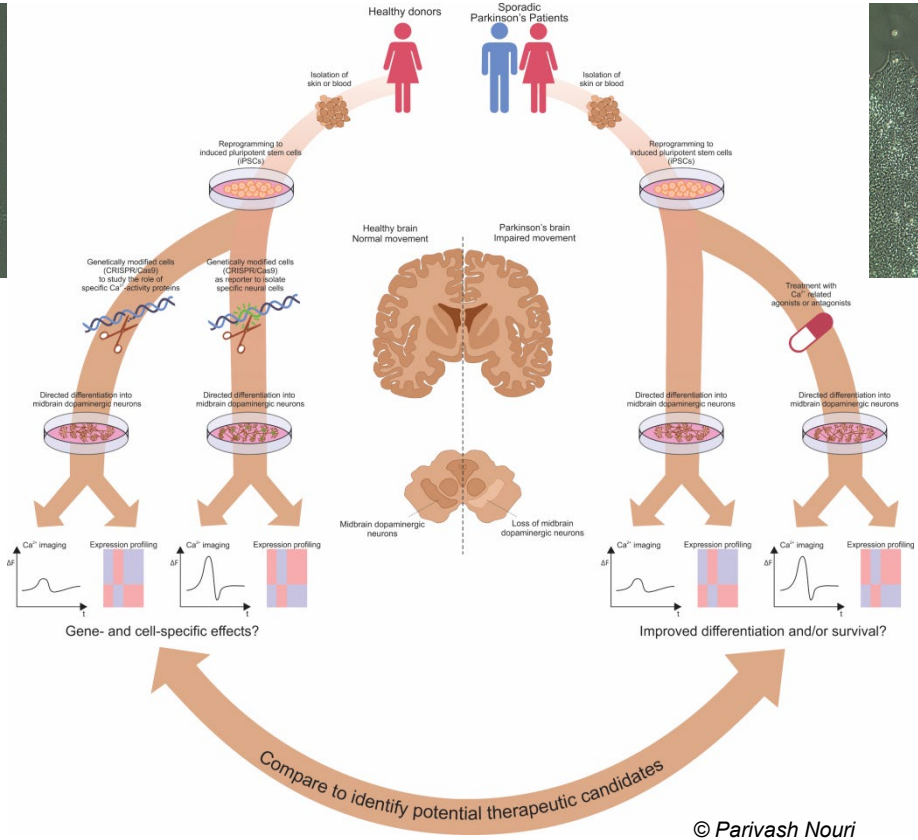

Frontal lobe
Striatum
Substantia nigra
Ventral tegmental area

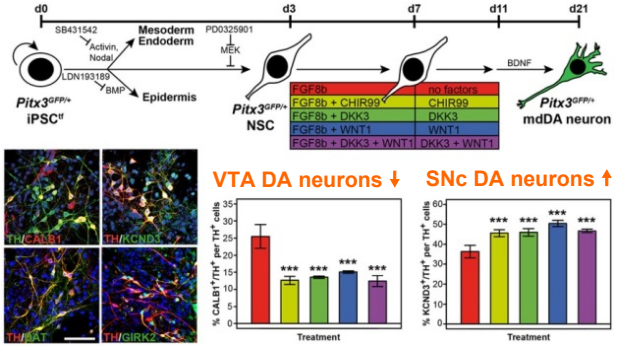
Human iPSCs

Calcium activities in mdDA neuron development: DACalON









Fukusumi et al., J Neurosci 2015

Lessons from mice, chicken and human iPSCs:

1. **Despite (very) similar sequences and structures as well as biochemical functions of many mdDA neuron-associated genes and/or proteins among more or less closely related animal species, crucial (post-?) transcriptional and/or (post-?) translational regulatory and/or physiological processes are/may be different!**
   - Wrong conclusions about genetic, epistatic and physiological impacts on phenotypic outcome.

2. **Analyses of human organs, tissues and/or cells thus appear mandatory to draw the right conclusions required for any therapeutic approach to human disease.**
   - Organ/tissue/cell availability?
   - Ethical and legal issues?

3. **Can organs, tissues and/or cells replace an entire organism on the systemic level?**
   - Emergent properties of a whole organism/body compared to single organs, tissues, cells.
   - Exhaustive efficacy and safety (preclinical) testing of potential therapeutic agents: possible in just cells, tissues, organs?

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