NICO
Neuroscience Institute Cavalieri Ottolenghi

Sara Trova
Dept. Life Sciences and Systems Biology
University of Turin

GruppoAdultNeurogenesis

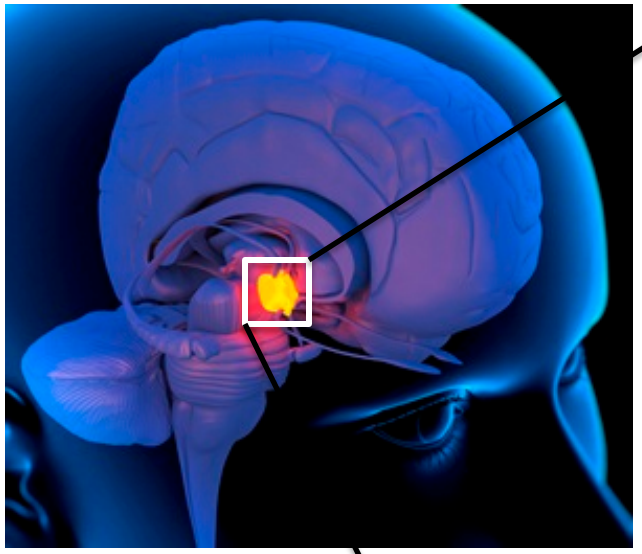
Development of neurons controlling reproduction

Master in Cellular and Molecular Biology

Developmental Neurobiology course

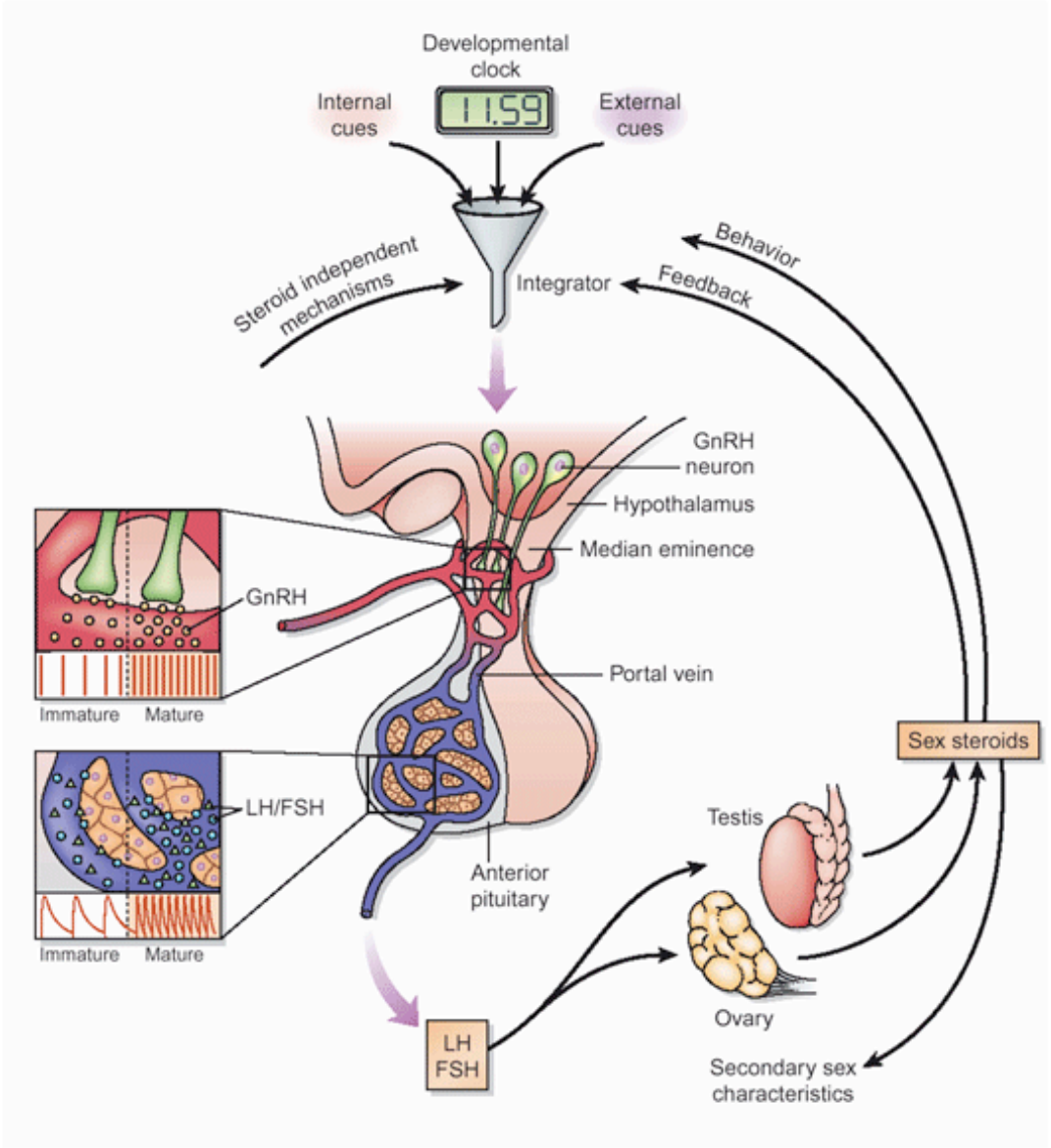
2017

Gonadotropin-releasing Hormone System
GnRH System



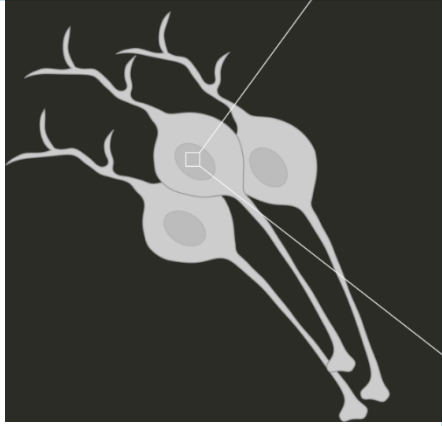
Hypothalamus
Pituitary
Gonadal axis

HPG axis



Cheryl L Sisk & Douglas L Foster, Nature Neuroscience 7, 1040 - 1047 (2004)

GnRH expressing cells



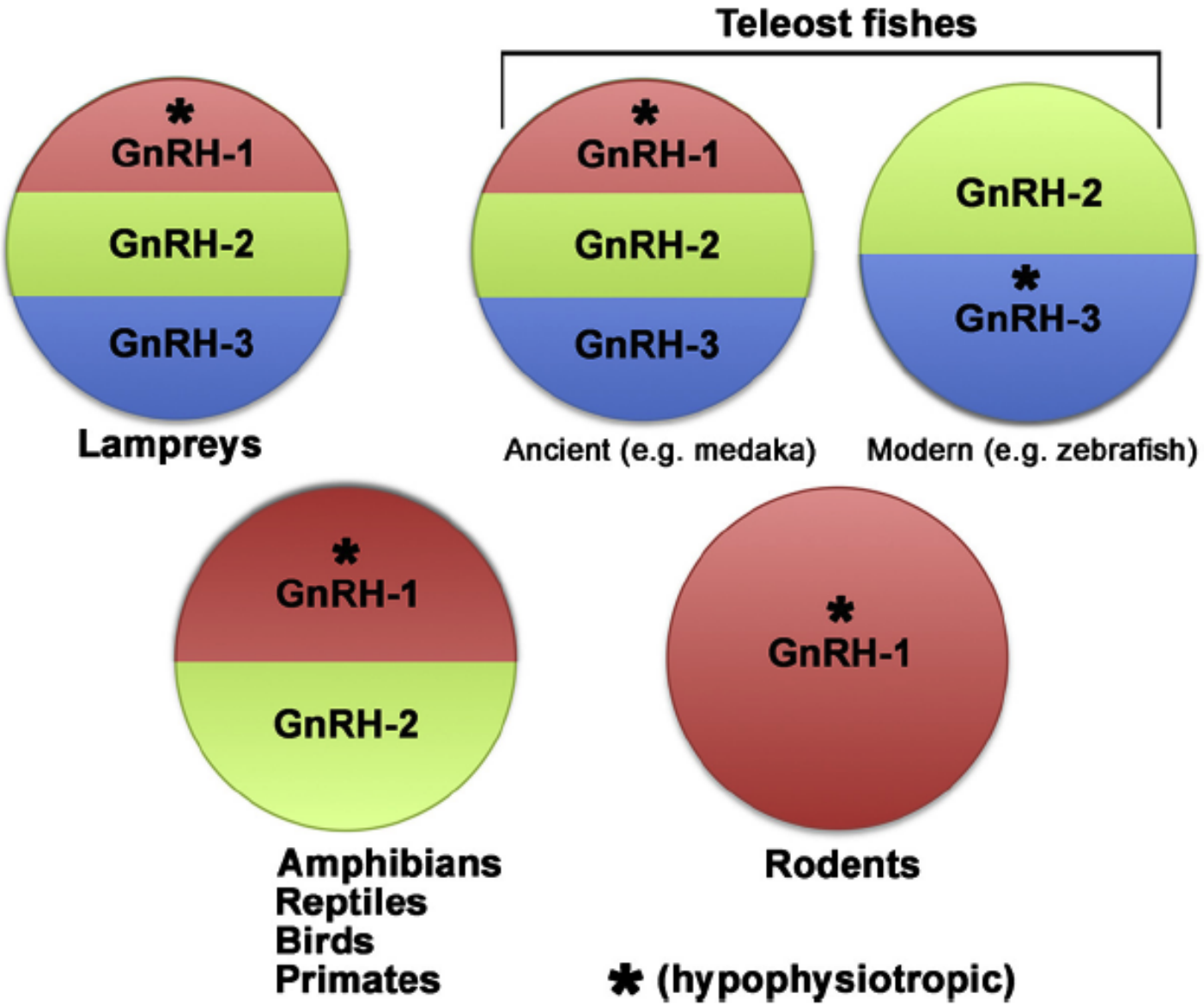
-small subset of neurons (800-2000) in mammals

-release Gonadotropin-releasing Hormone (GnRH): decapeptide

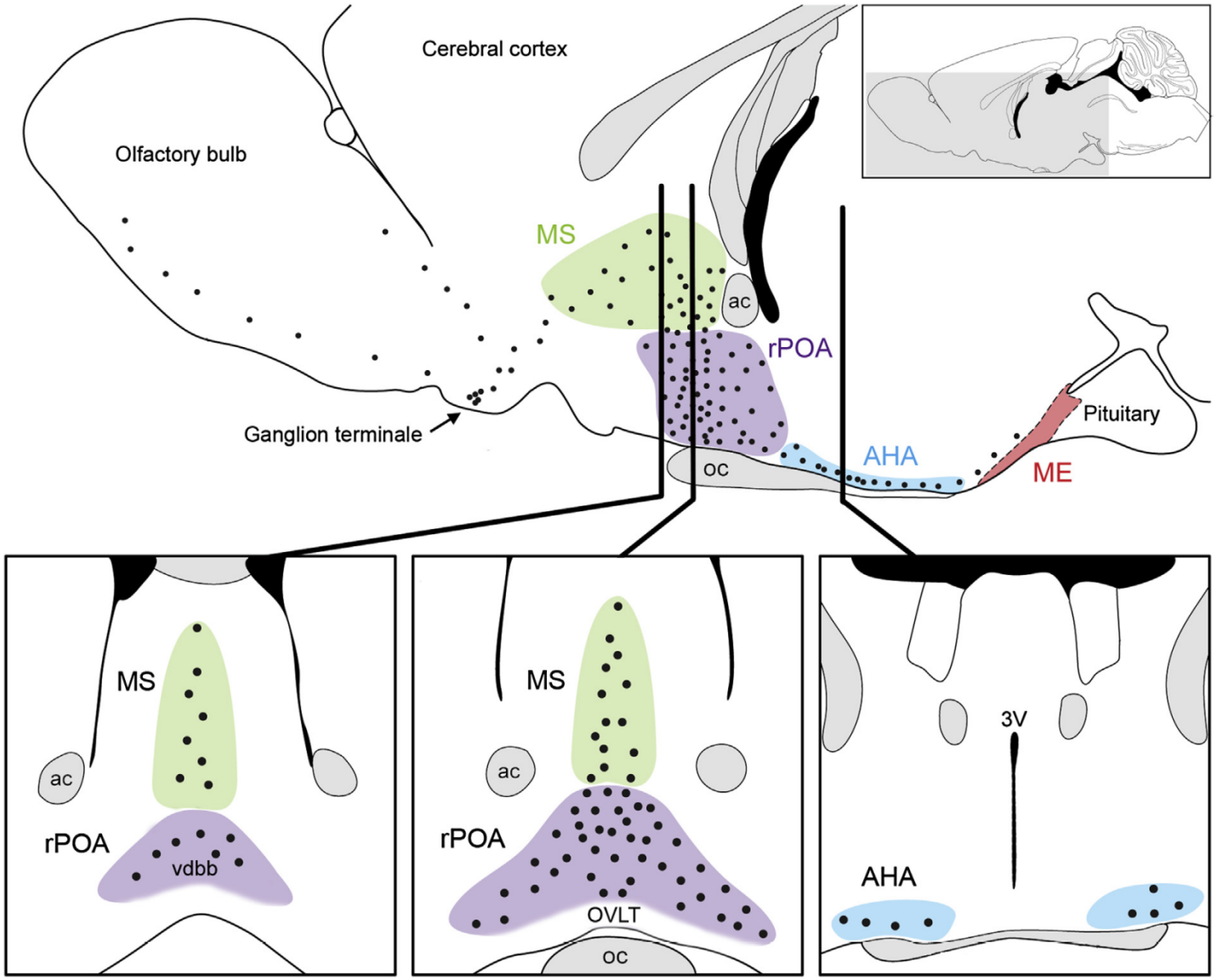
-3 isoforms encoded by 3 different genes: GnRH-1, GnRH-2, GnRH-3

-GnRH-1 (hypothalamic form) is expressed in higher vertebrates and it plays an ENDOCRINE ROLE

-GnRH-2 (mesencephalic form) and GnRH-3 (telencephalic form) mediate reproductive behaviour

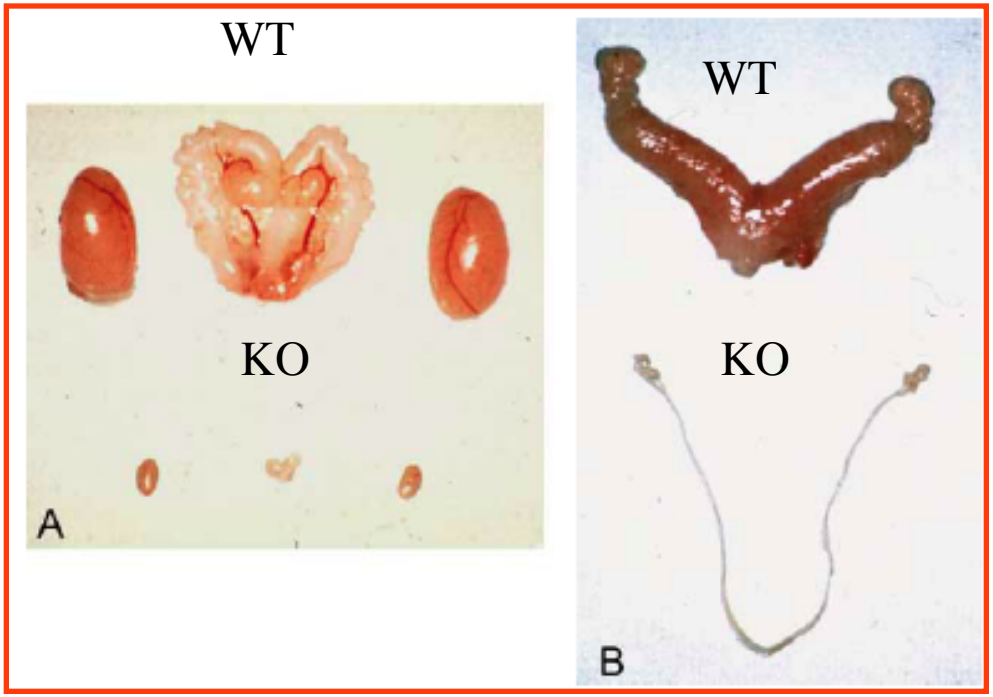

Teleost fishes
*
GnRH-1
GnRH-2
GnRH-3
Lampreys
*
GnRH-1
GnRH-2
GnRH-3
Ancient (e.g. medaka)
GnRH-2
*
GnRH-3
Modern (e.g. zebrafish)
*
GnRH-1
GnRH-2
Amphibians
Reptiles
Birds
Primates
*
GnRH-1
Rodents
* (hypophysiotropic)

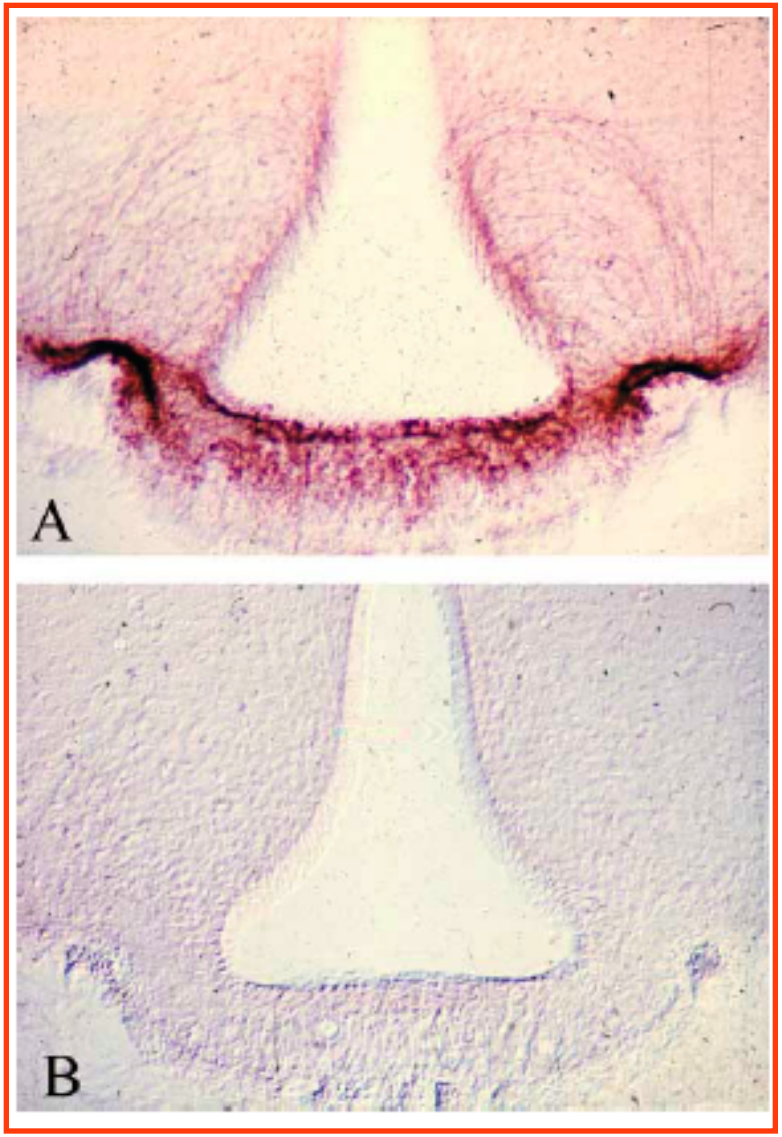
Location of GnRH-I expressing cells in an ADULT MOUSE BRAIN



GnRH-I pivotal function→ to control the HPG axis

HPG mice - Mason et al., 1986

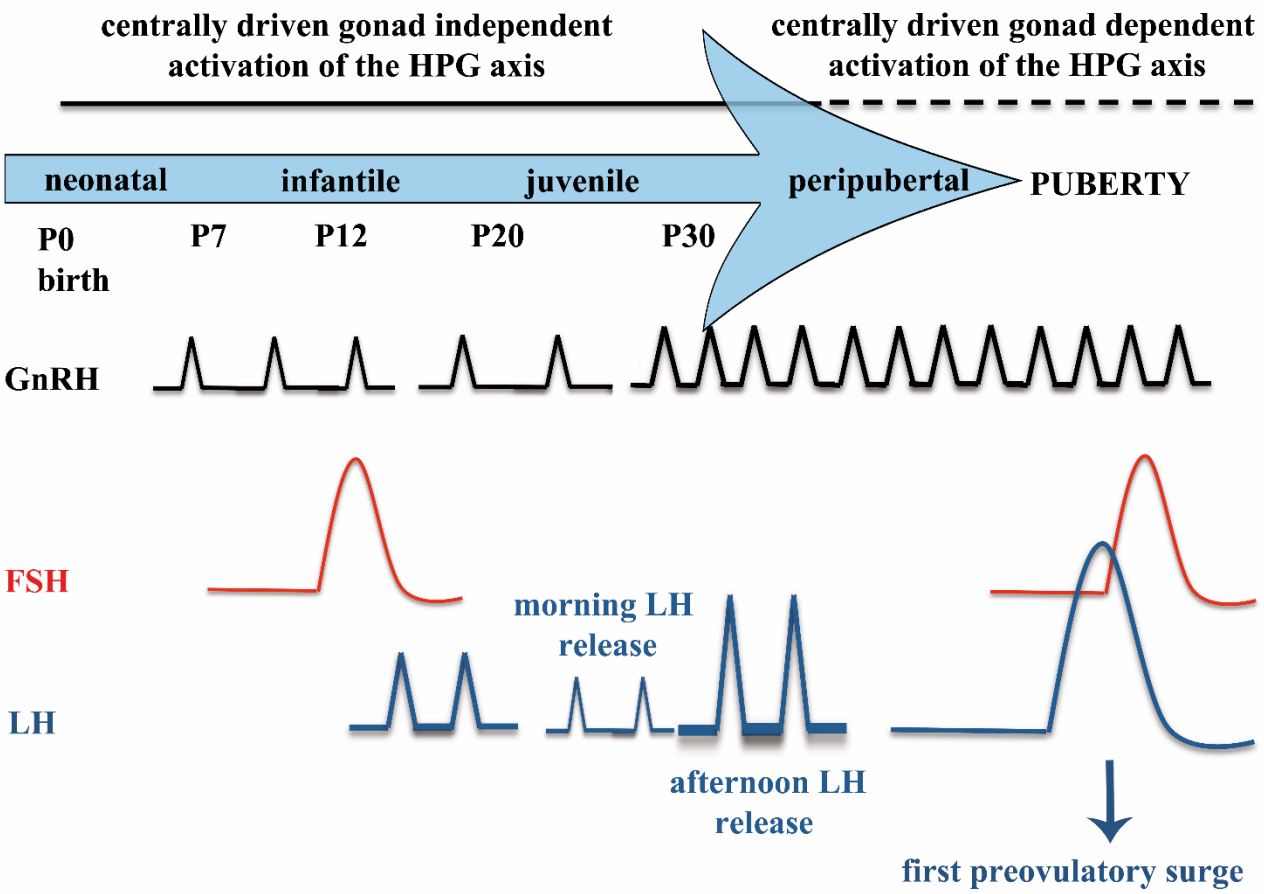




A mutation of GnRH gene (a massive 33.5 kb deletion in GAP) is responsible for the lack of GnRH secretion and subsequent infertility

No GnRH immunoreactive fibers in the median eminence of HPG mice

GnRH PULSATILE SECRETION DURING LIFEc



The phases of pubertal activation of the HPG axis in female mouse.

1) in neonatal/infantile stage GnRH and LH are low, but FSH shows a peak at P12,
2) juvenile stage LH release is higher in the afternoon compare to the morning,
3) peripubertal stage ends with the first ovulation.

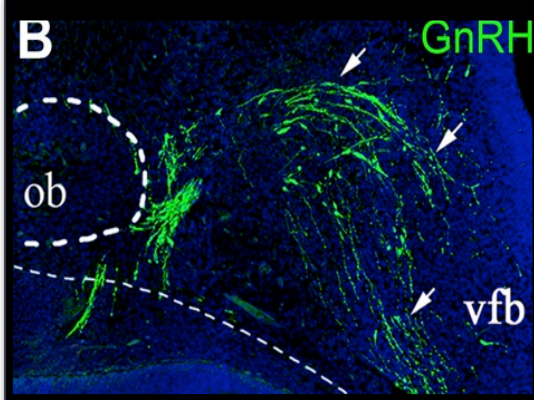
ORIGIN

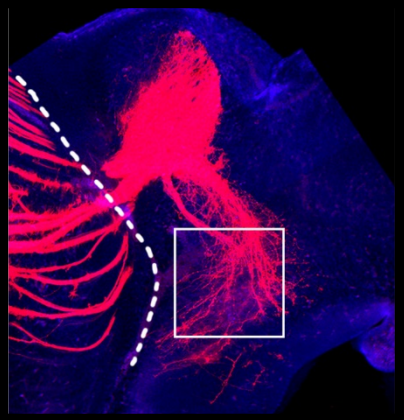
and

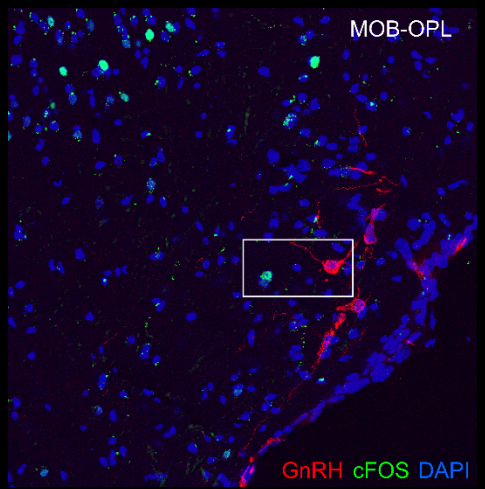
MIGRATION

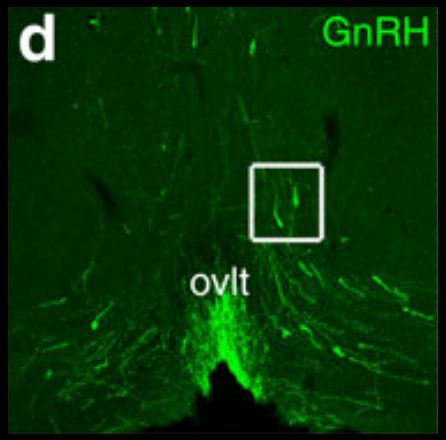
of

GnRH NEURONS









GnRH neurons originate OUTSIDE the CNS: in the OLFACTORY PLACODE

Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 8132–8136, October 1989
Neurobiology

Evidence that cells expressing luteinizing hormone-releasing hormone mRNA in the mouse are derived from progenitor cells in the olfactory placode

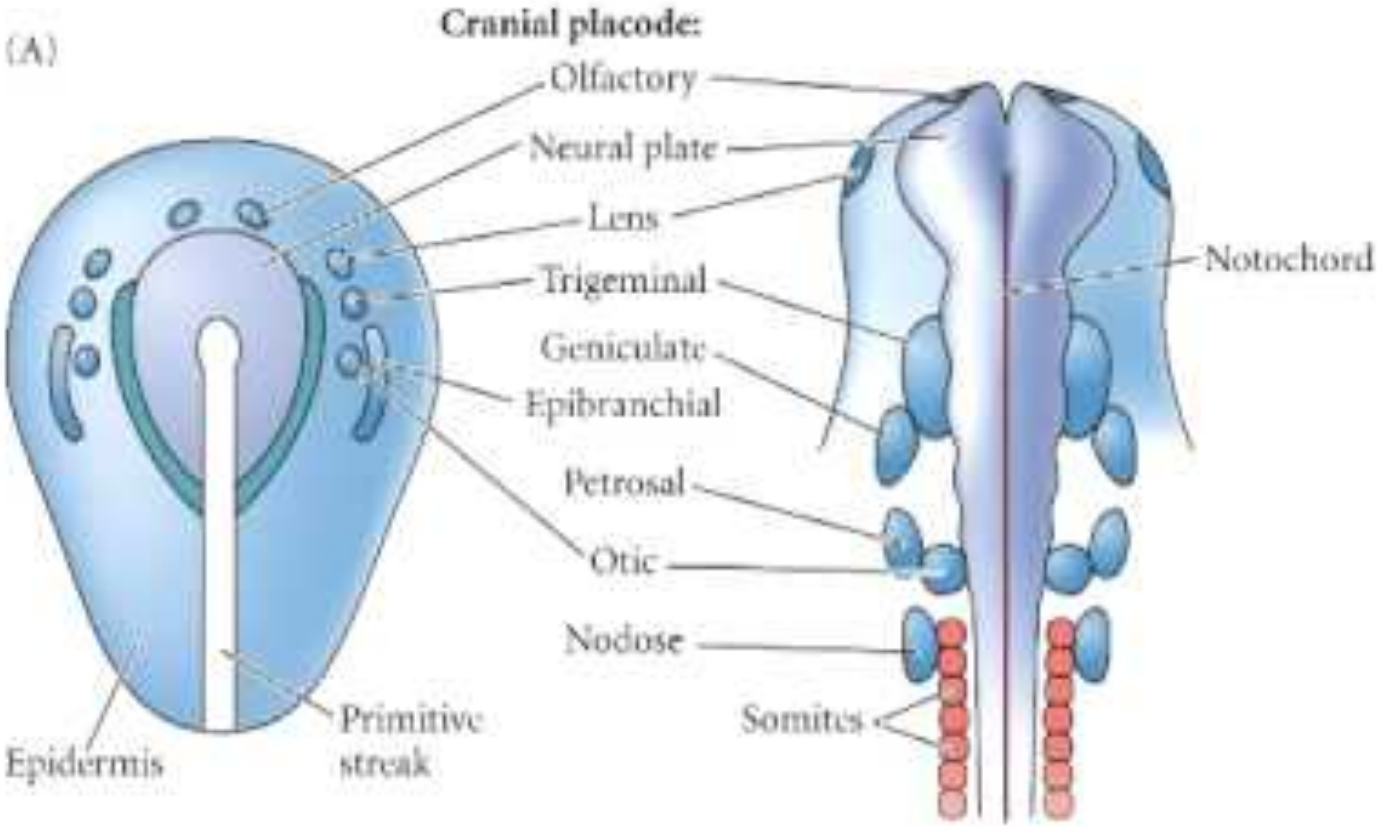
(prenatal development/*in situ* hybridization/histochemistry/immunocytochemistry/[^{3}H]thymidine autoradiography)

SUSAN WRAY*, PHILIP GRANT, AND HAROLD GAINER

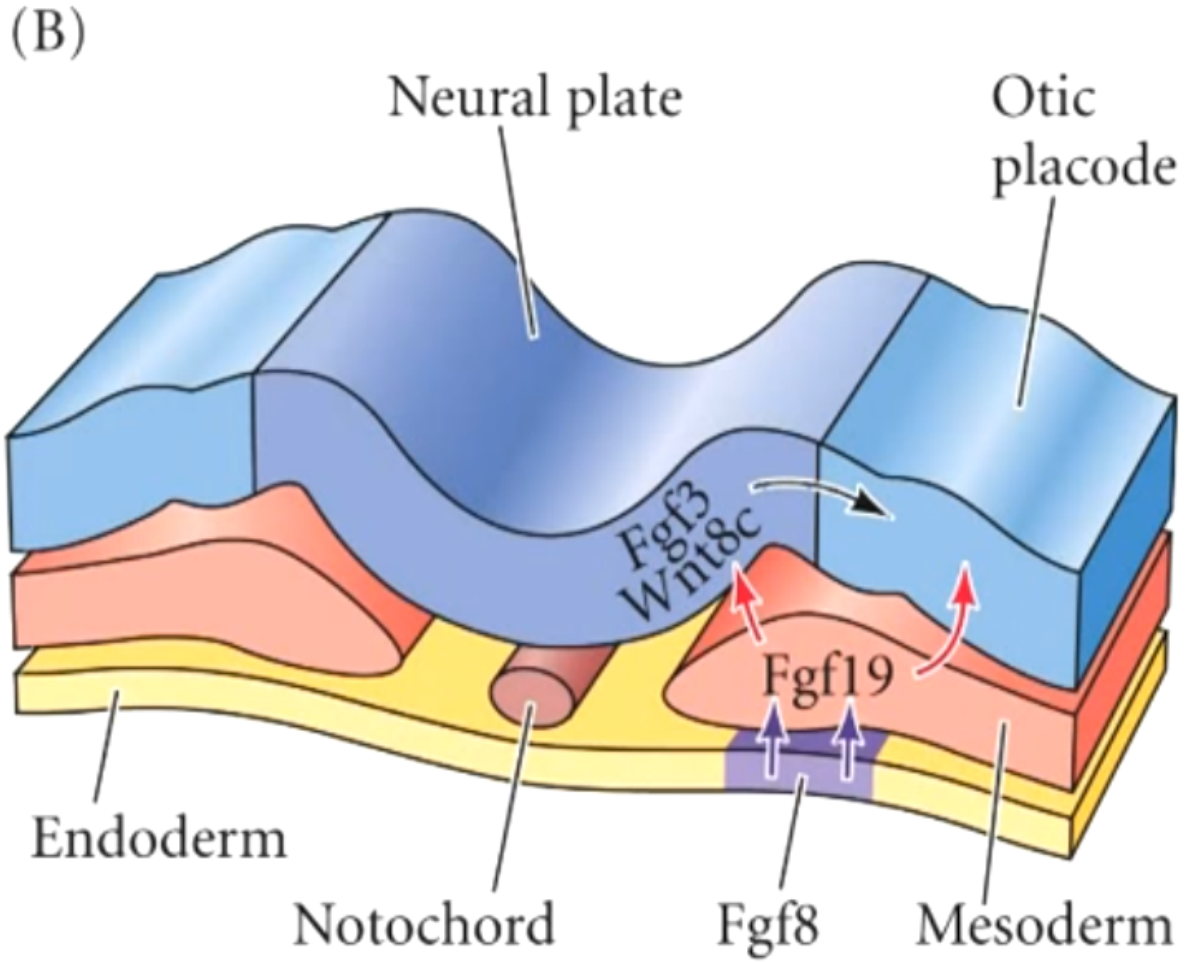
Nature. 1989 Mar 9;338(6211):161-4.

Origin of luteinizing hormone-releasing hormone neurons.Schwanzel-Fukuda M, Pfaff DW.

Cranial Placodes



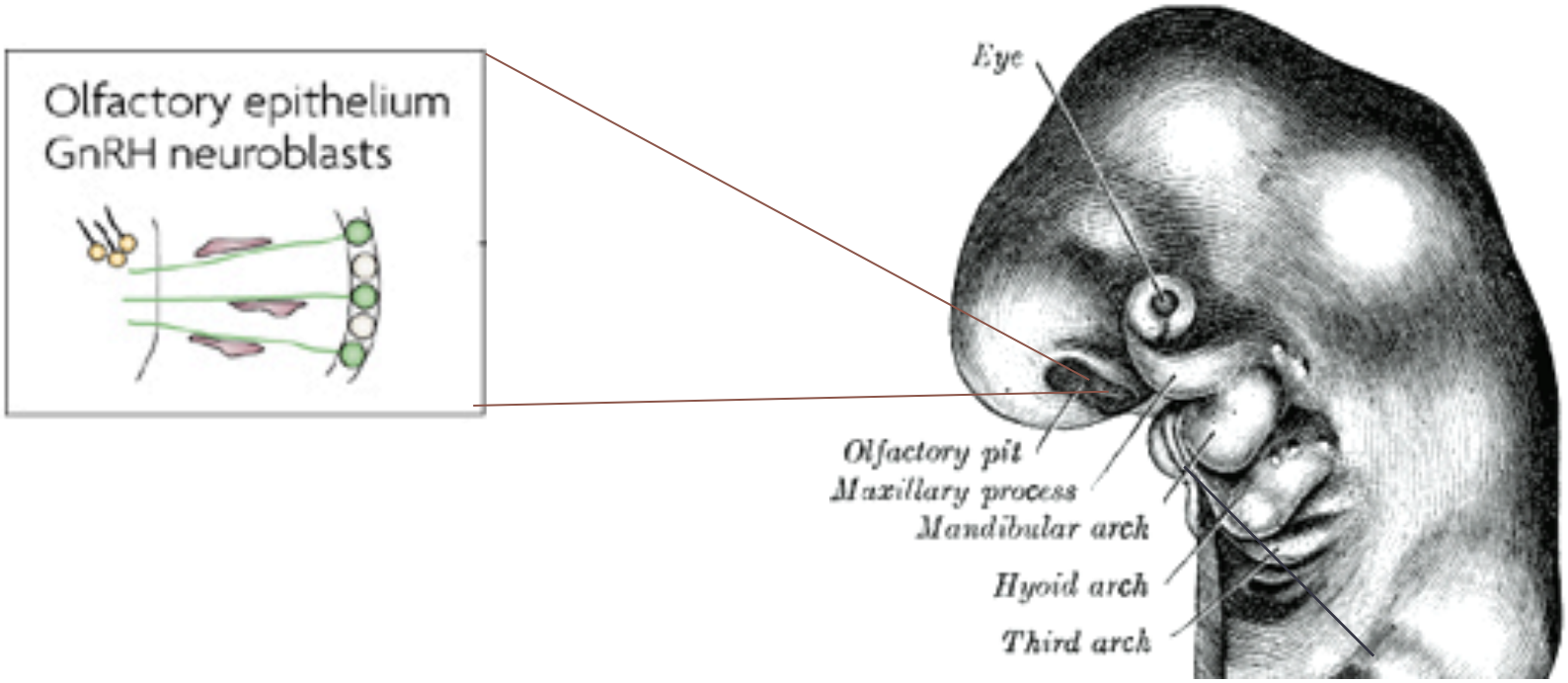
Cranial Placodes



DEVELOPMENTAL BIOLOGY, Eighth Edition, Figure 13.18 (Part 2) © 2006 Sinauer Associates, Inc.

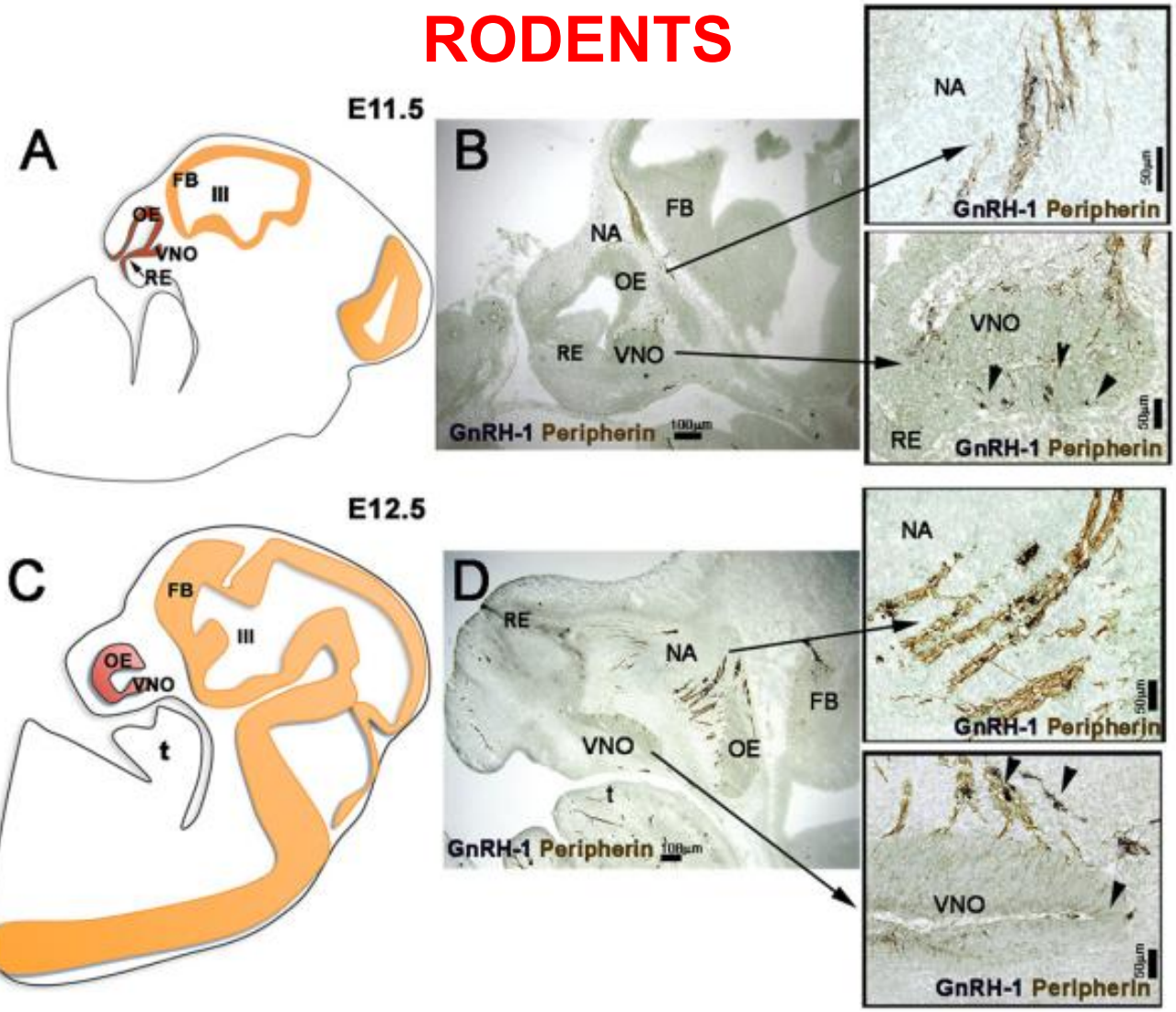
The olfactory placode

The OP are thickenings of cells lining the border of the neural plate



GnRH neurons form in a niche at the border of respiratory epithelium and vomeronasal/olfactory epithelium

RODENTS

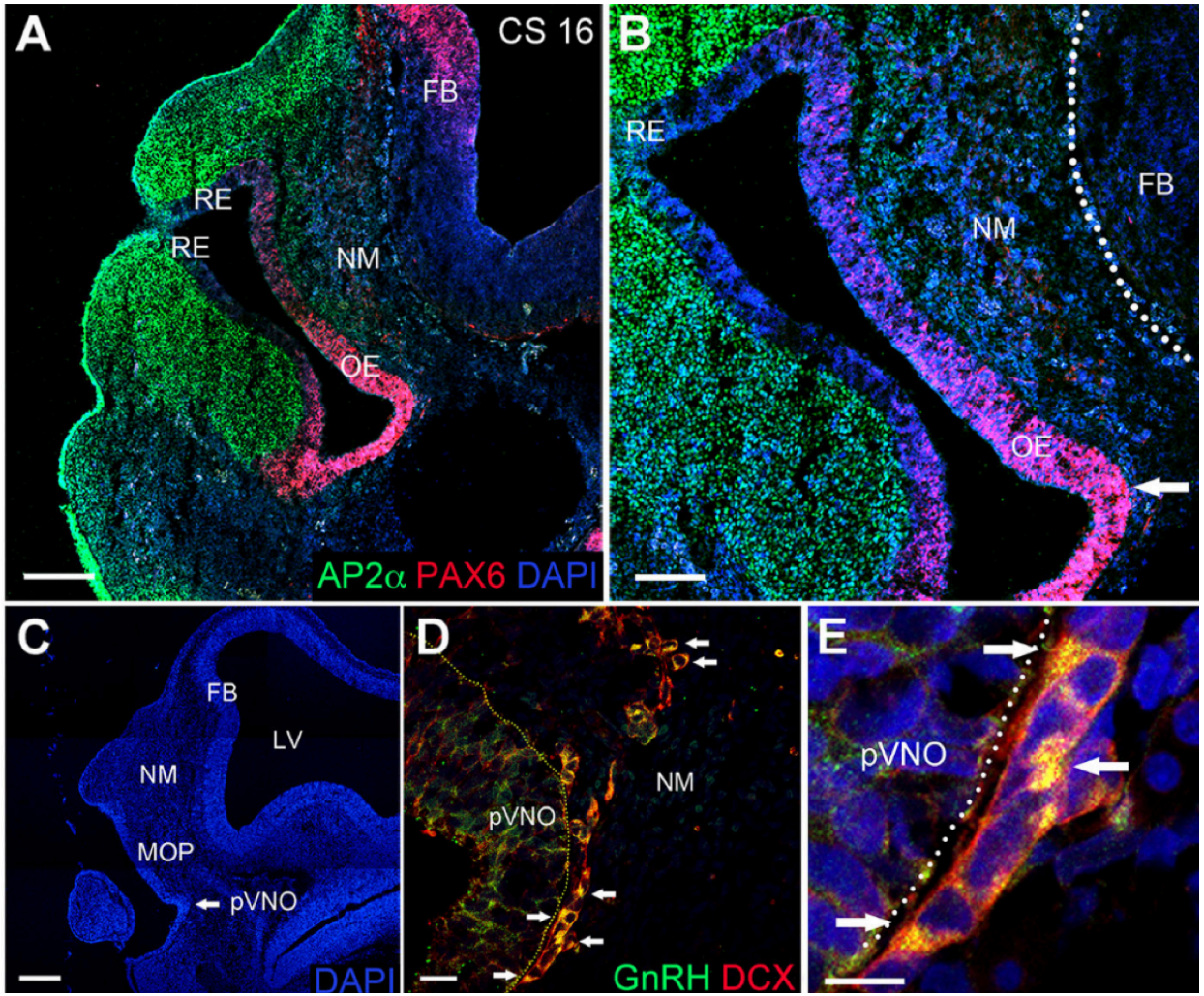


Forni and Wray., 2014

GnRH neurons form in a niche at the border of respiratory epithelium and vomeronasal/olfactory epithelium

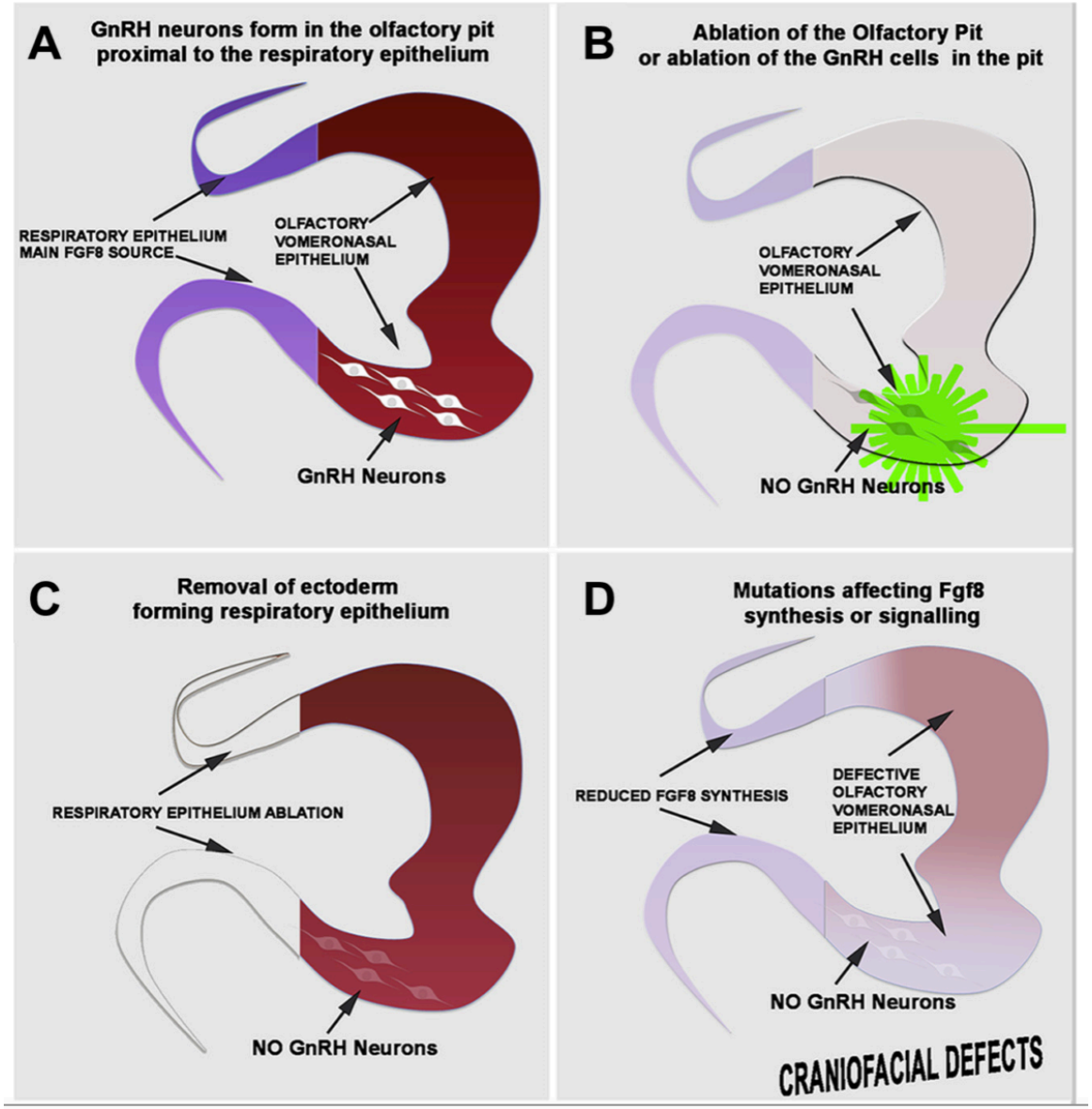
HUMANS

5th week of gestation
CS = Carnegie stage
CS 16 = 39TH day of gestation



Casoni *et al.*., 2016

Olfactory pit and putative respiratory epithelium are important for the differentiation of GnRH neurons



Ablation experiments

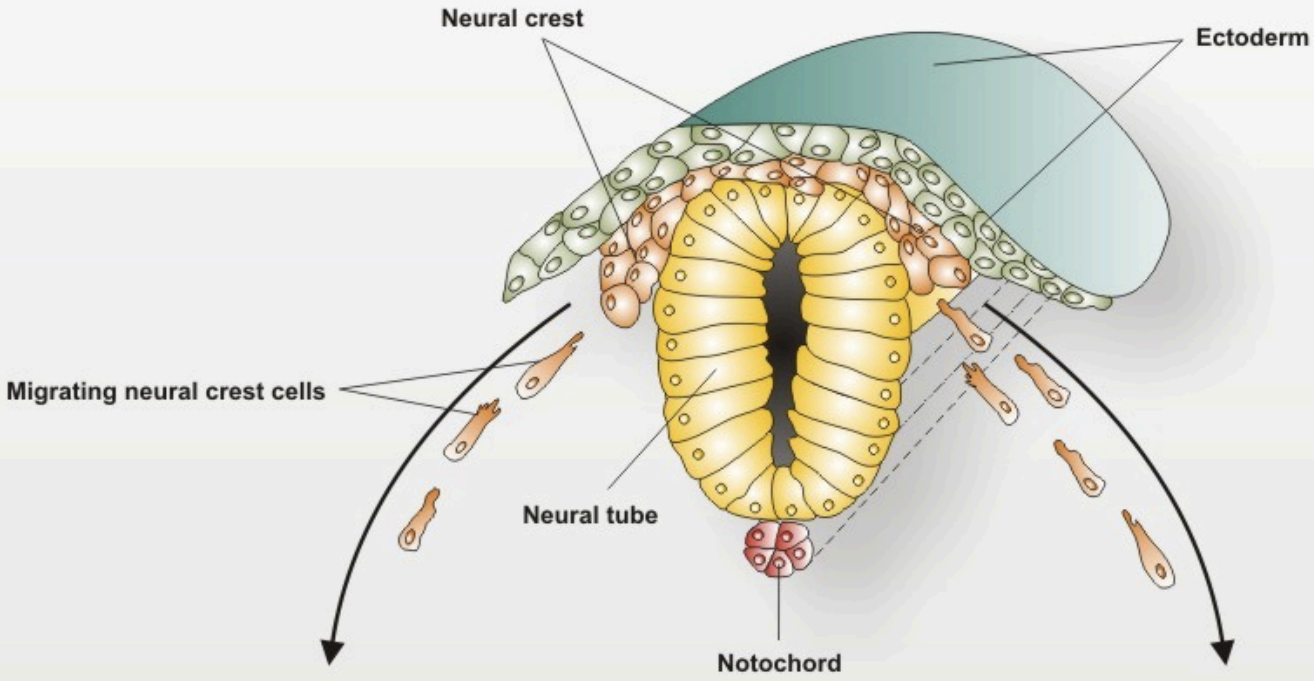
Forni and Wray., 2014

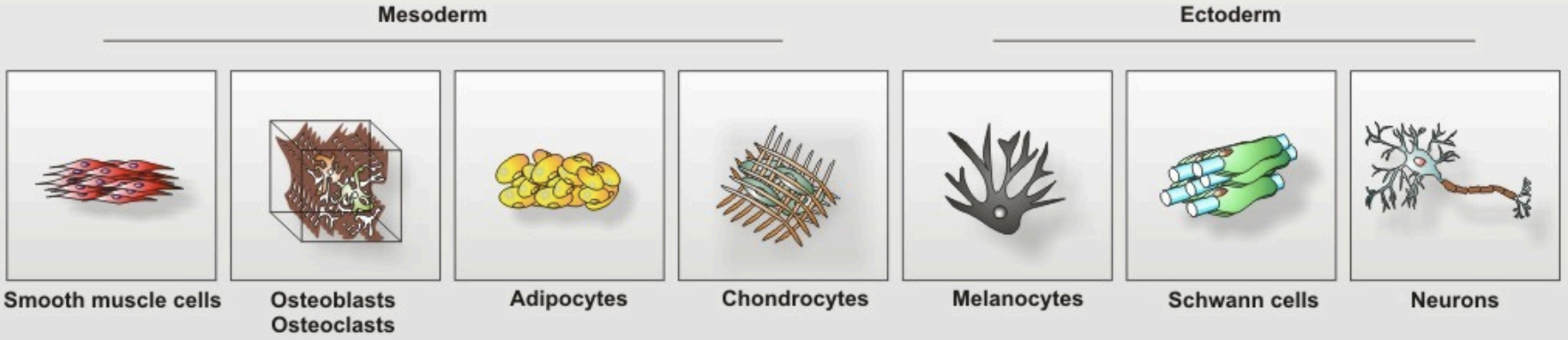
GnRH neurons origin is still debated:

- GnRH cells could originate somewhere else and then migrated and matured in the olfactory placode

- The ablated tissue (e.g. respiratory epithelium) is the source of necessary trophic factors needed for GnRH neuron differentiation or survival, rather the site of origin of these cells

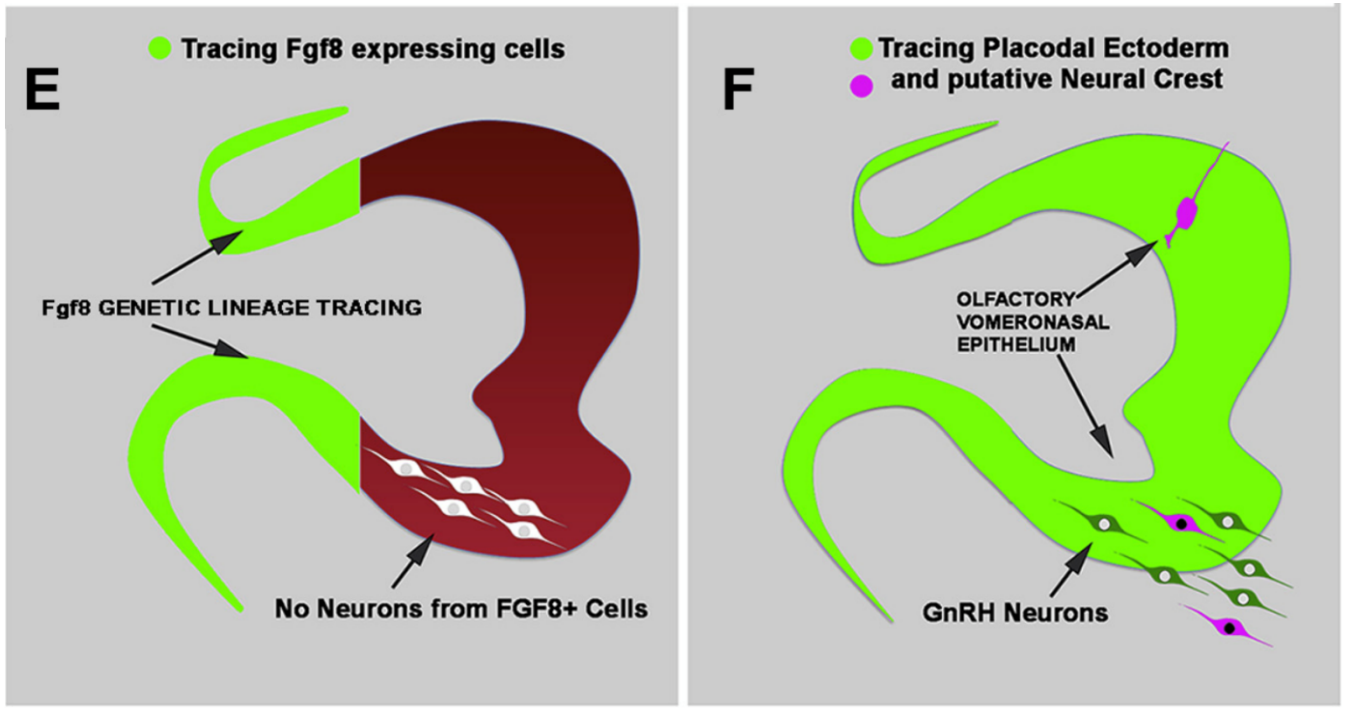
GnRH neurons could originate from NEURAL CRESTS





Cell and Lineage tracing experiments

Fgf8 producing cells of the respiratory epithelium lack neurogenic ability



Forni *et al.*, 2013

Forni *et al.*, 2011; Sabado *et al.*, 2011

Progenitor cells of putative Neural Crest origin and placodal origin contribute to GnRH cells population

Ontogenesis of GnRH neurons

GnRH-1 cells appear for the first time in the olfactory placode and they are post-mitotic. The exact origin of these cells (precursors) is still debated.

Transcription factors involved in the olfactory placode induction:

1) OTX-1 e 2 (orthodentical homeobox 1-2)
2) Pax-6: Defective development of the olfactory structures, lack of GnRH-1 cells (Dellovade et al., 1998; Skynner et al., 1999)
3) Mash-1, Math4A, NeuroD (precocious olfactory markers)
4) Olfs e GATA-4 (late olfactory markers)

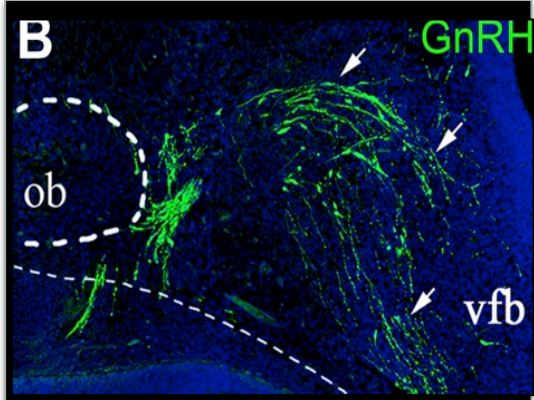
ORIGIN

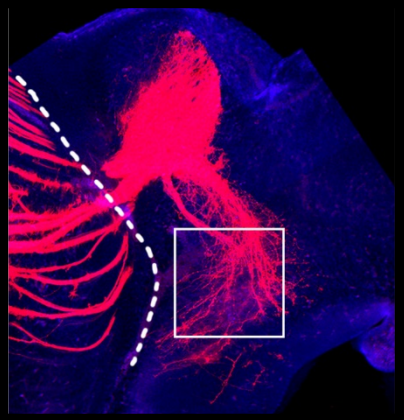
and

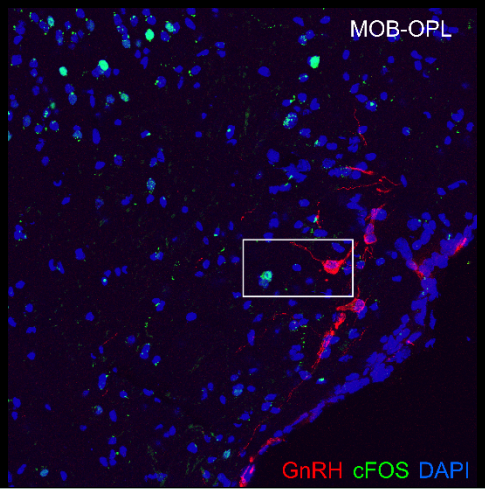
MIGRATION

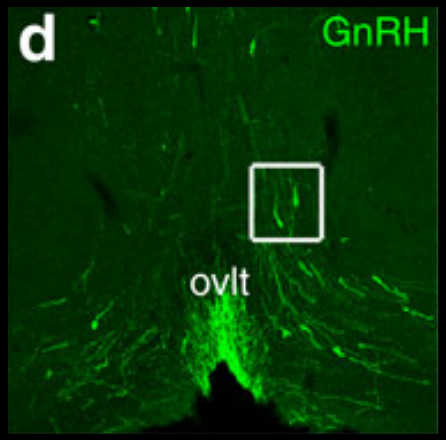
of

GnRH NEURONS

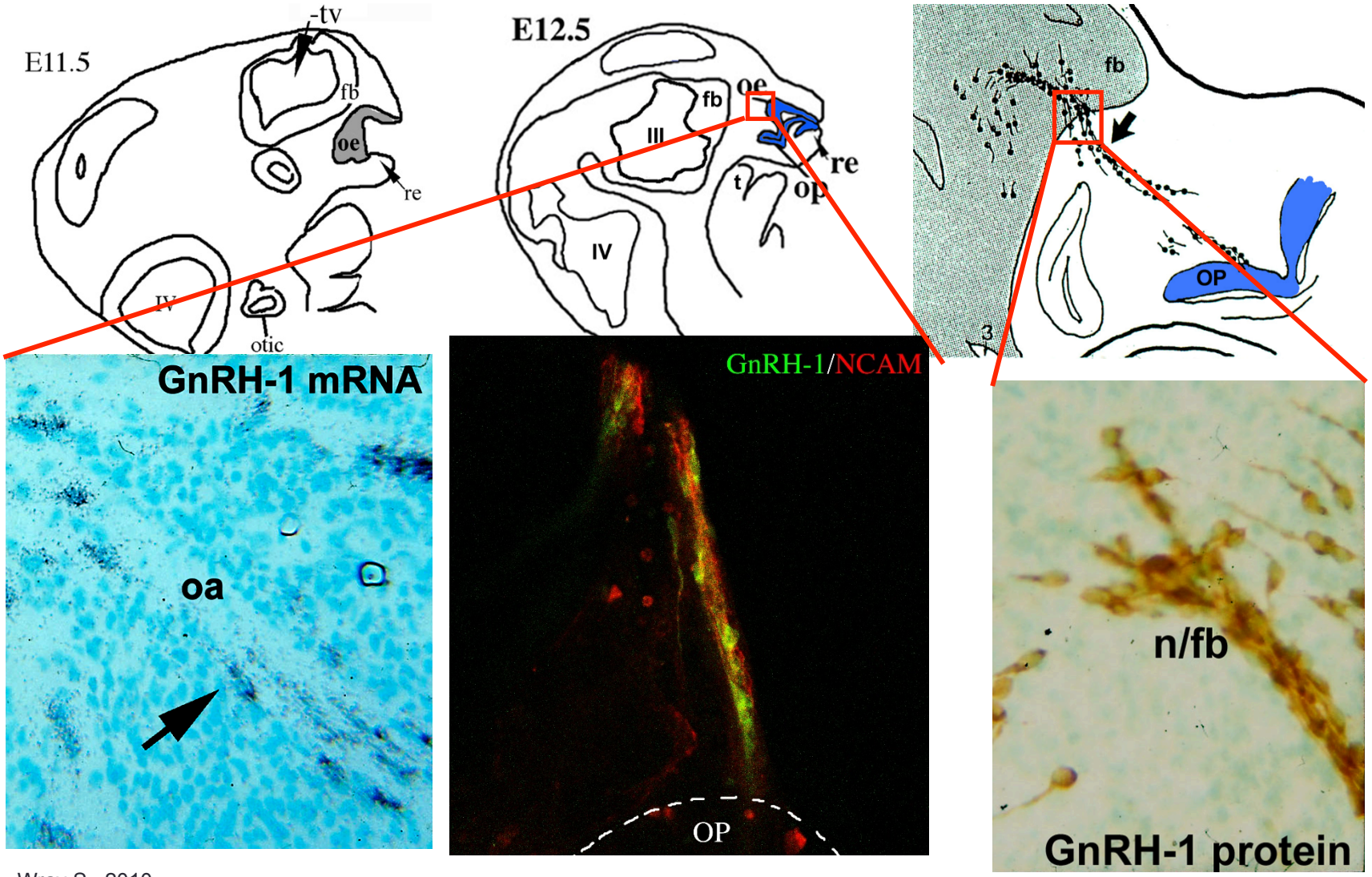








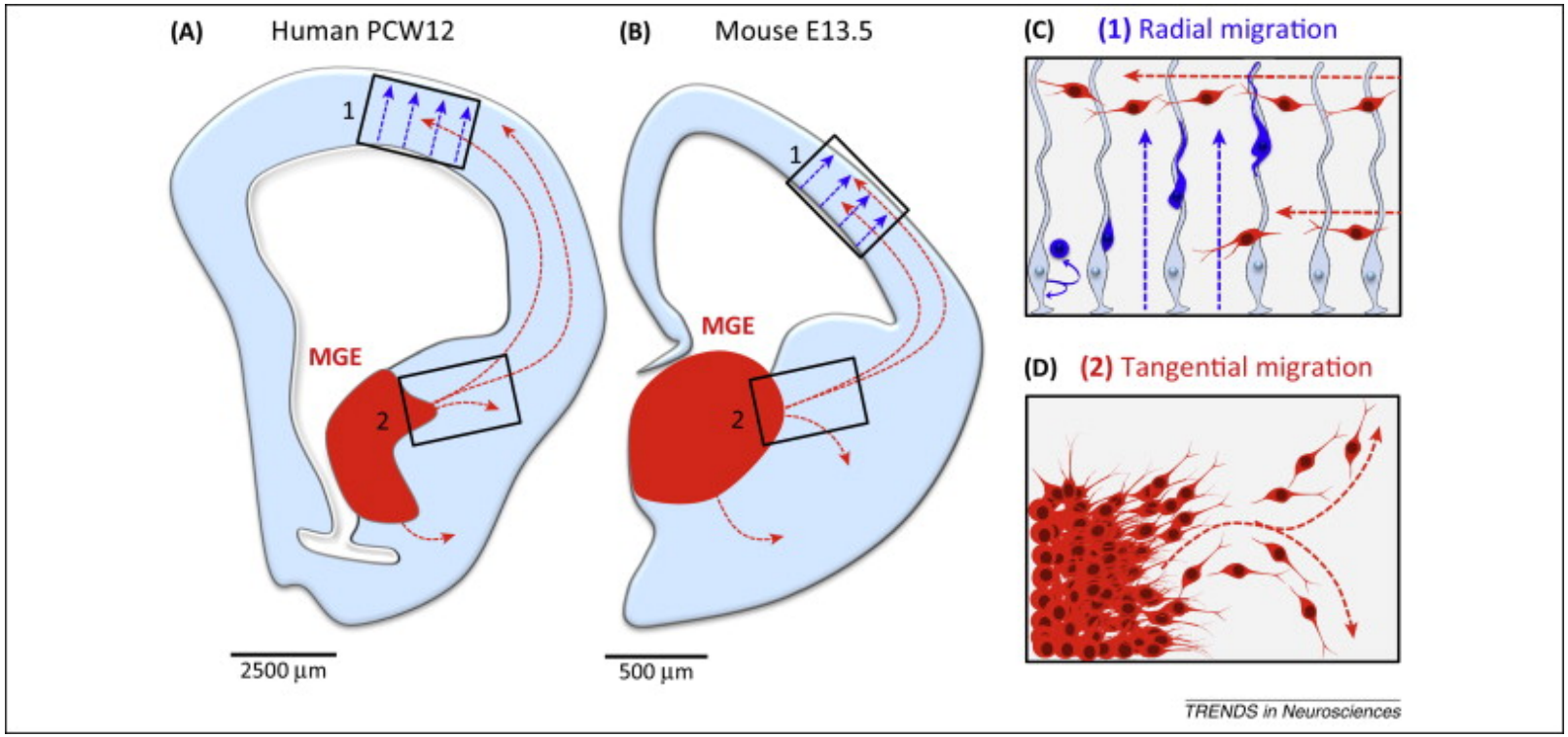
GnRH neurons migrate from nose to brain through an axophylic migration



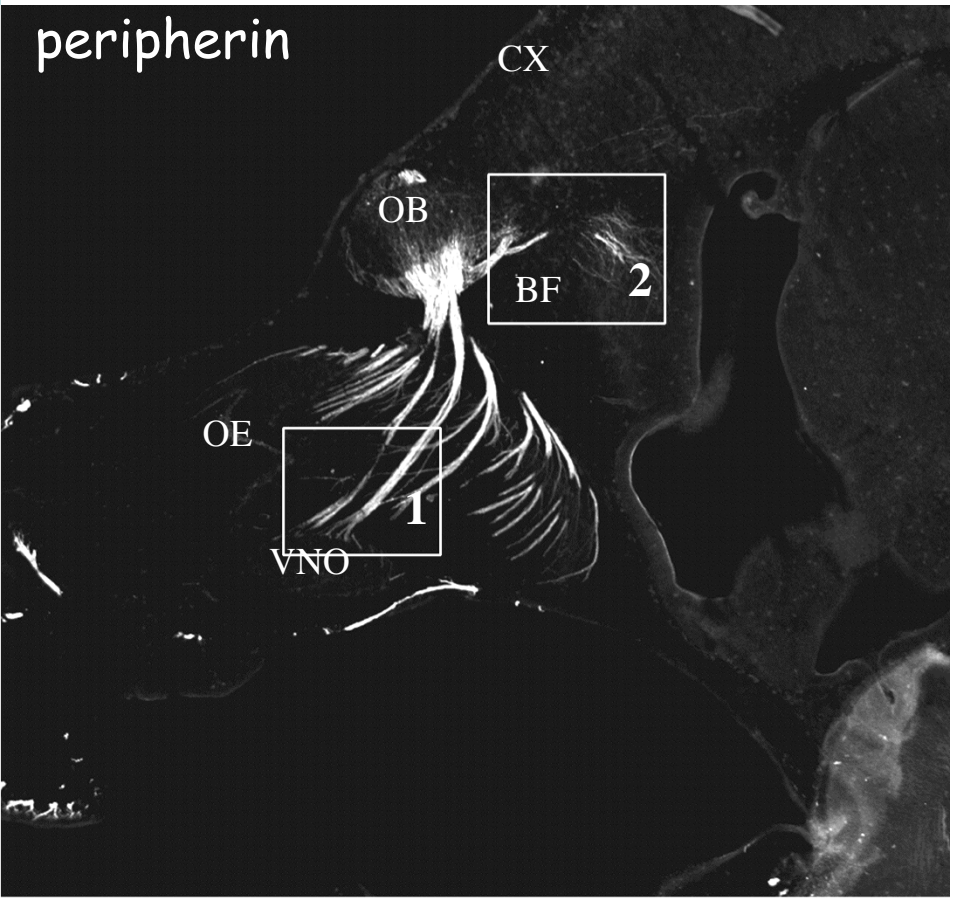
Wray S., 2010

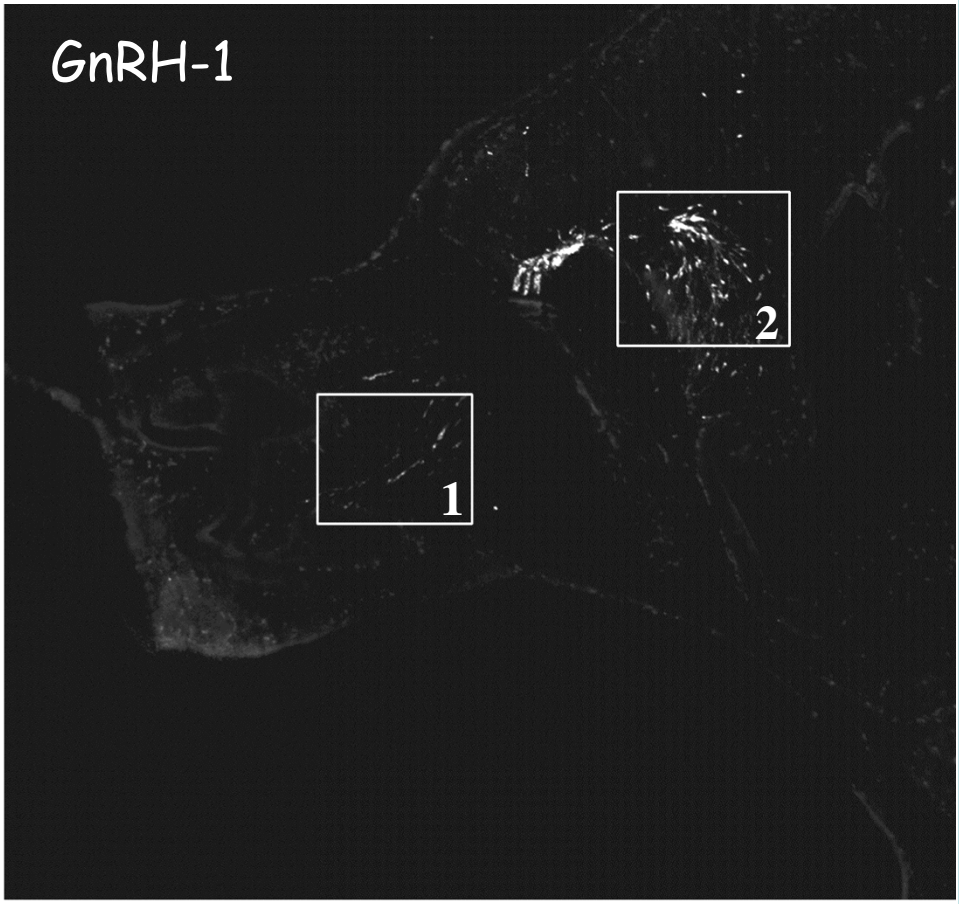
GnRH neurons migration:

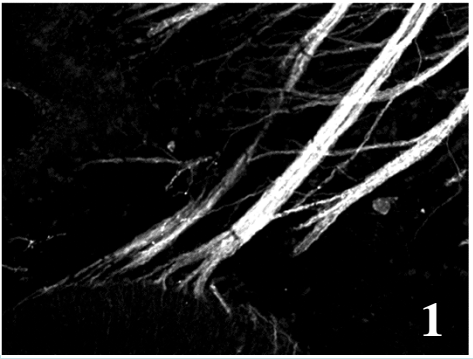
tangential migration
that involves a pathway support

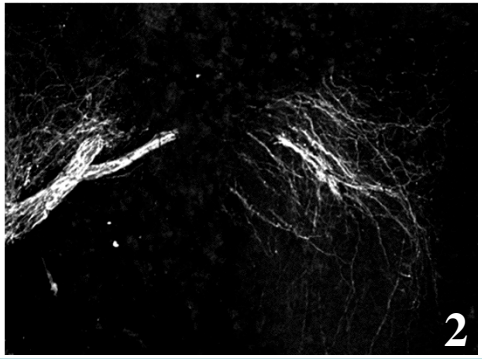


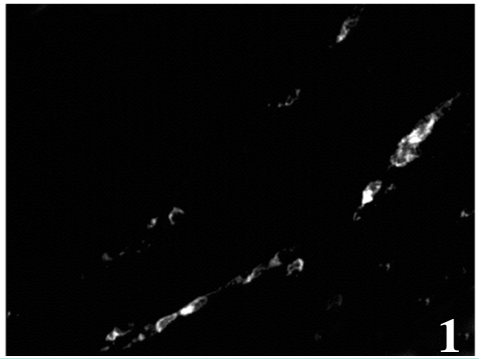
GnRH migratory pathway
rodents

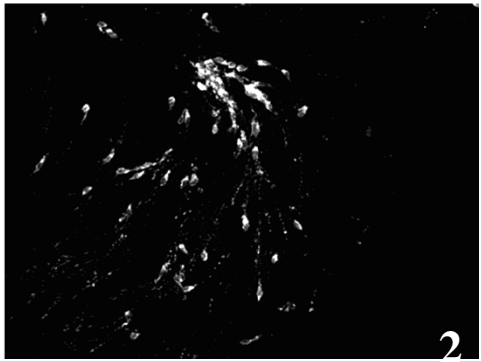




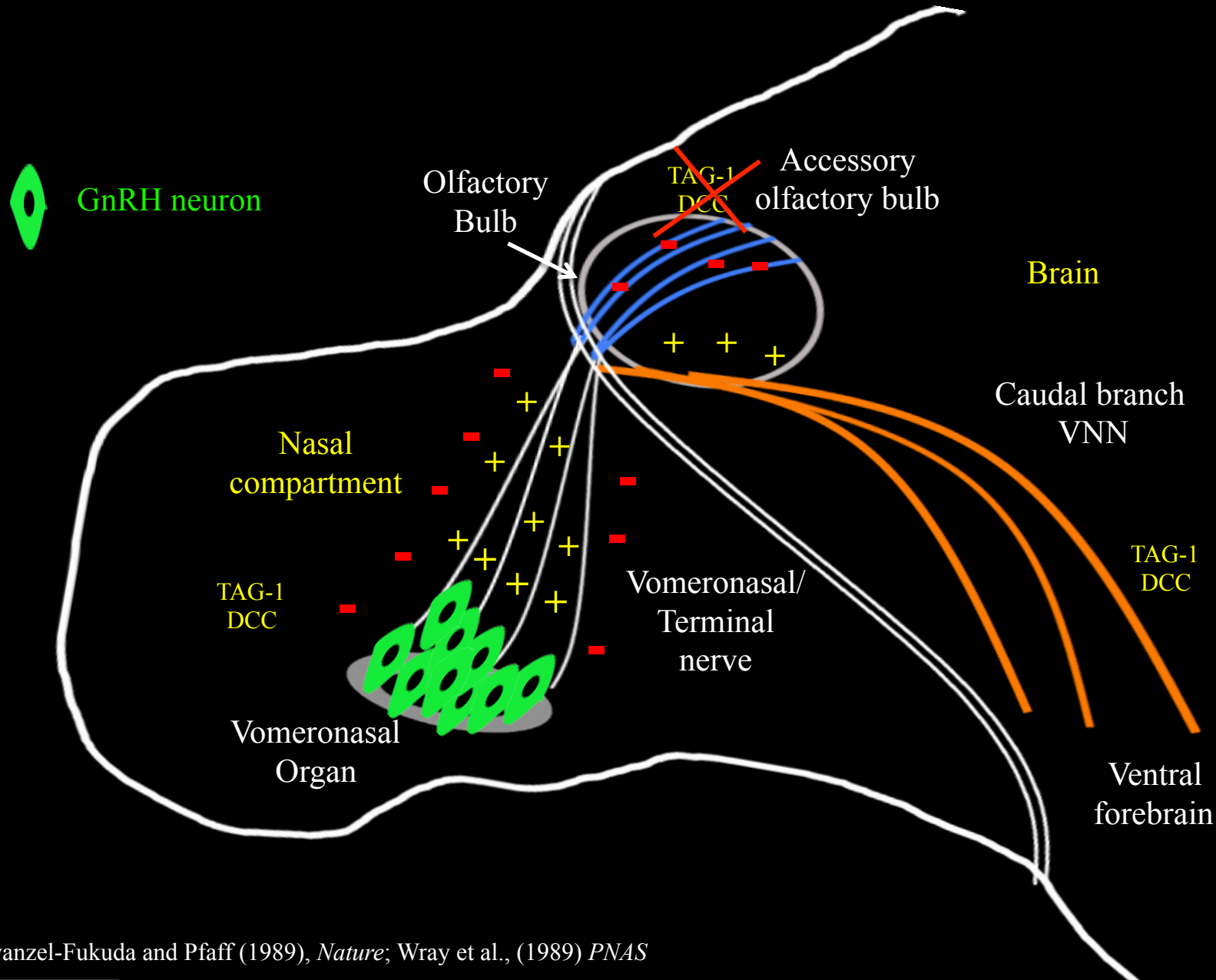






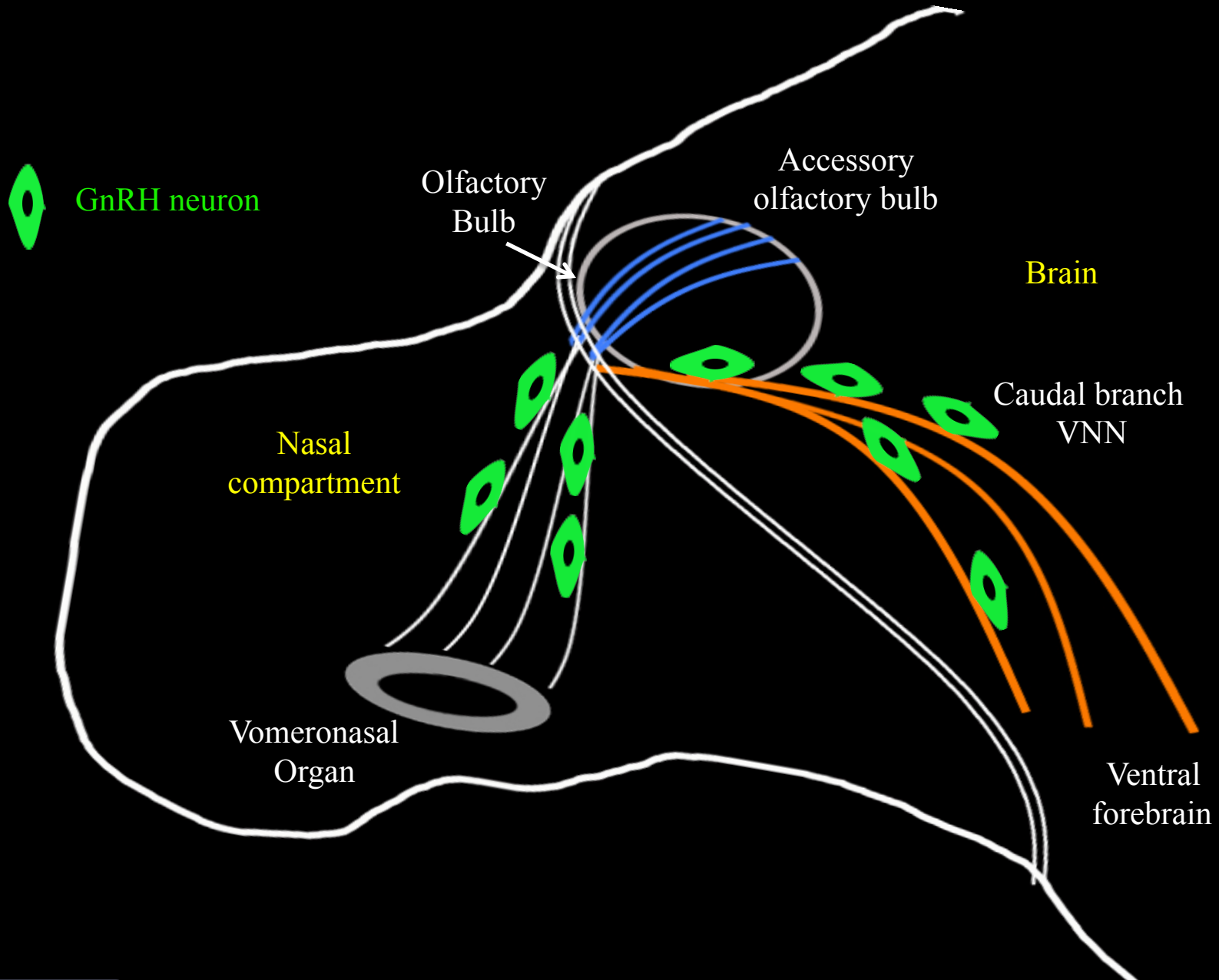


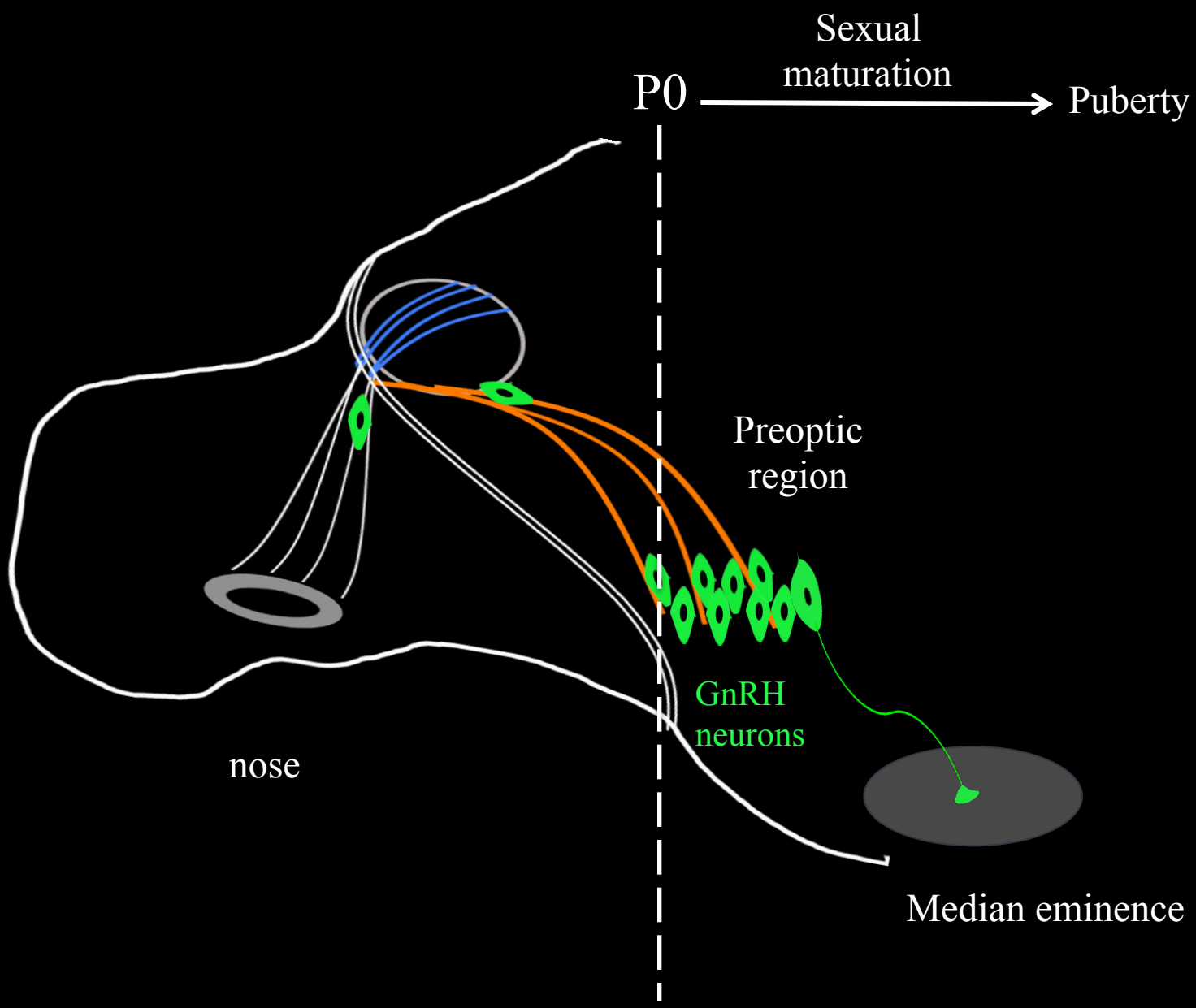
GnRH neurons migrate from nose to brain through an axophylic migration



Schwanzel-Fukuda and Pfaff (1989), *Nature*; Wray et al., (1989) *PNAS*

Migration of GnRH neurons @ E14.5-E16.5



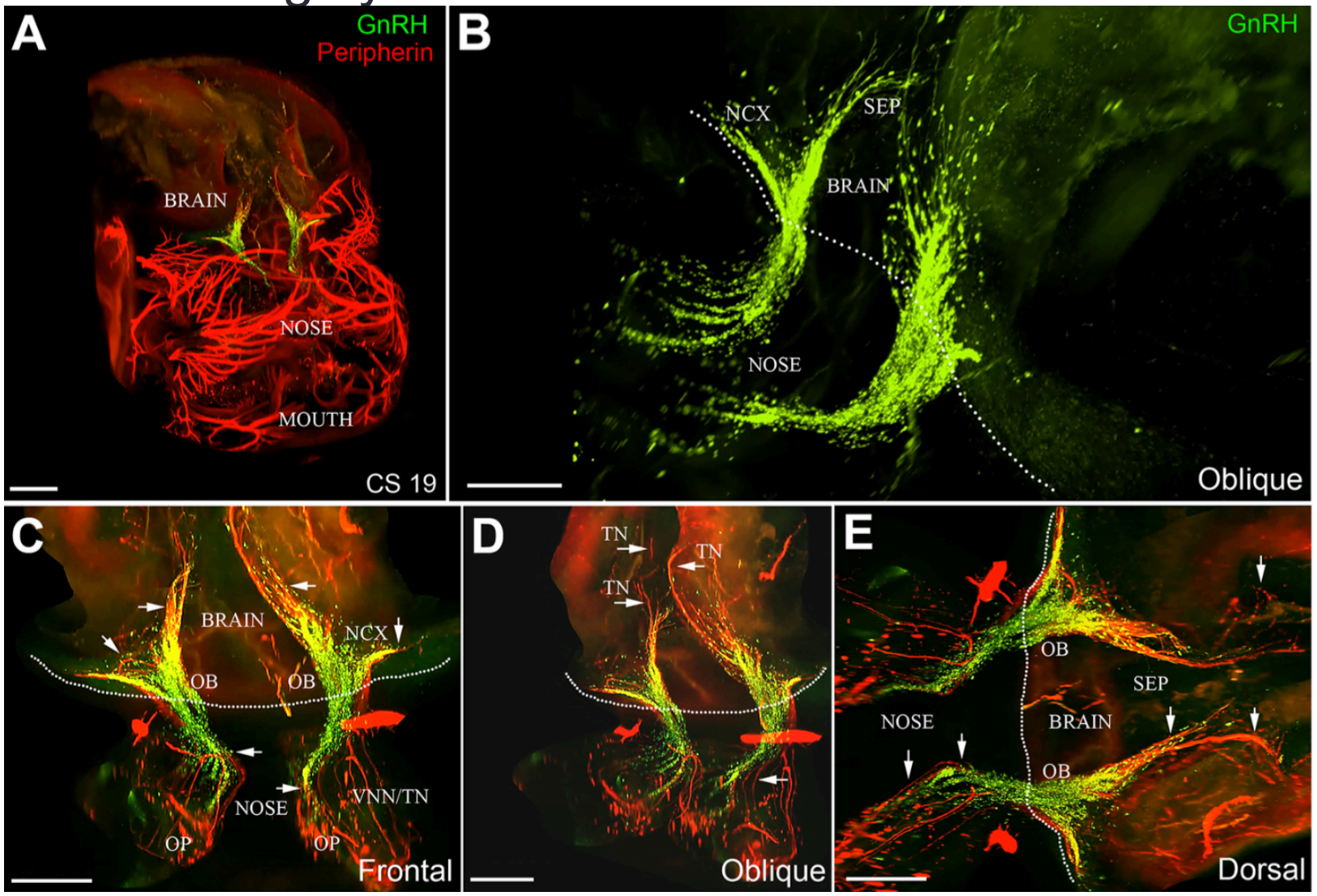


Alterations in the development of this system or in the secretion of GnRH are associated with the reduction or failure of sexual competence

GnRH migratory pathway

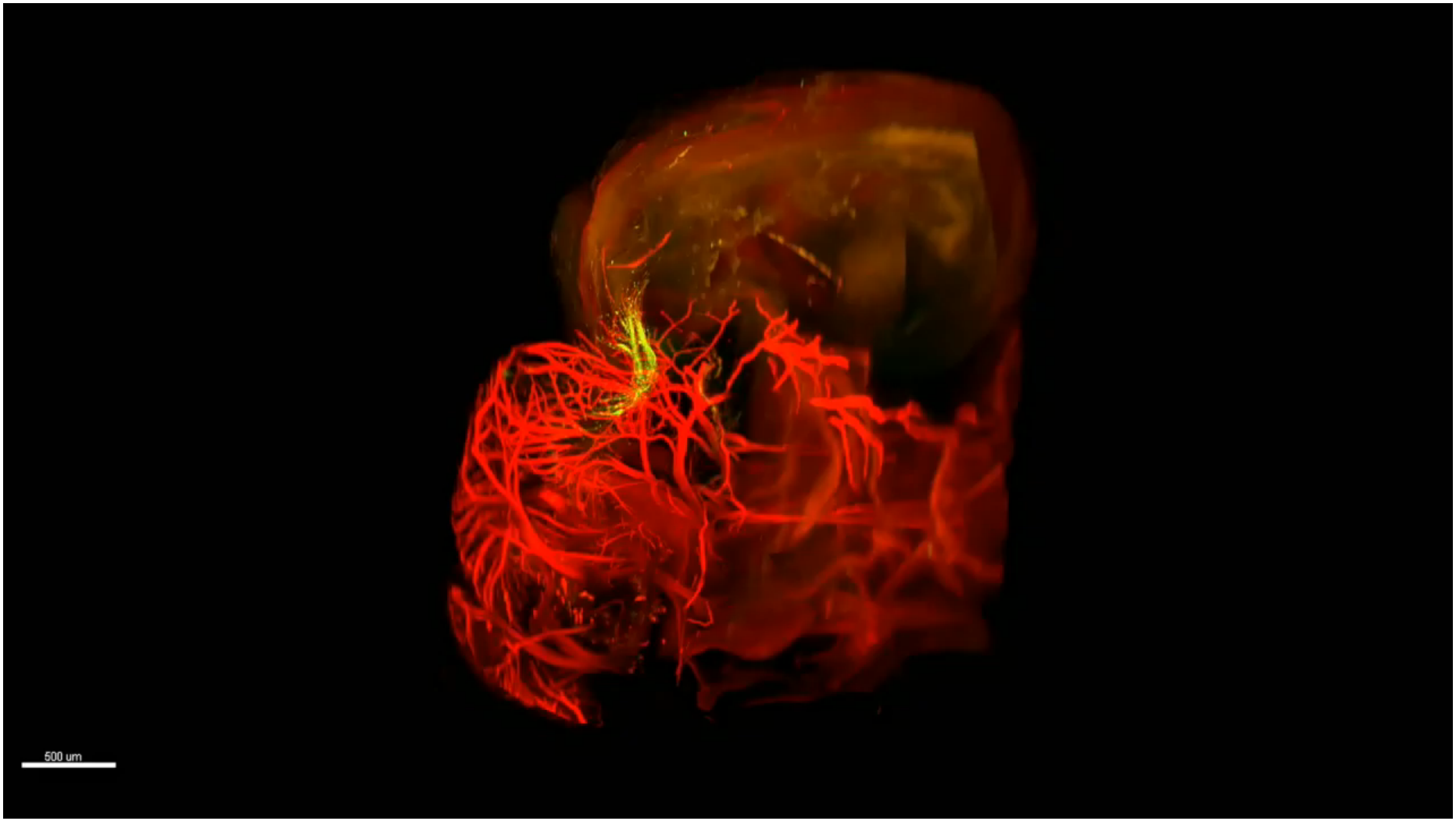
Humans

Highly conserved across evolution



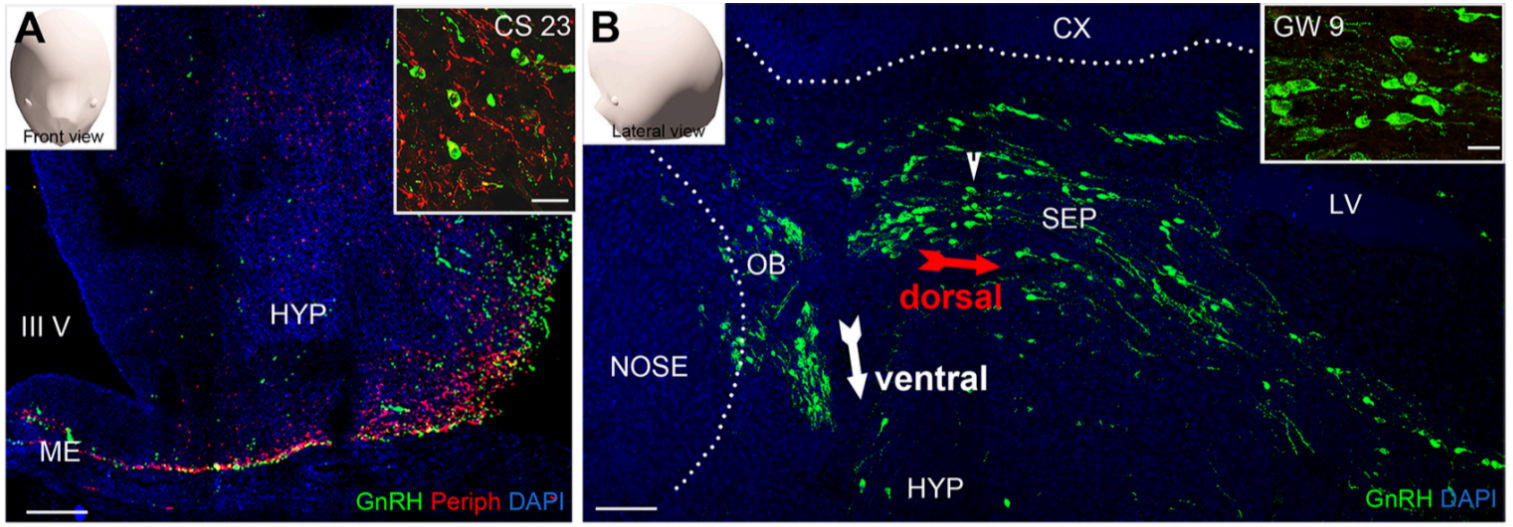
Casoni *et al.*, 2016

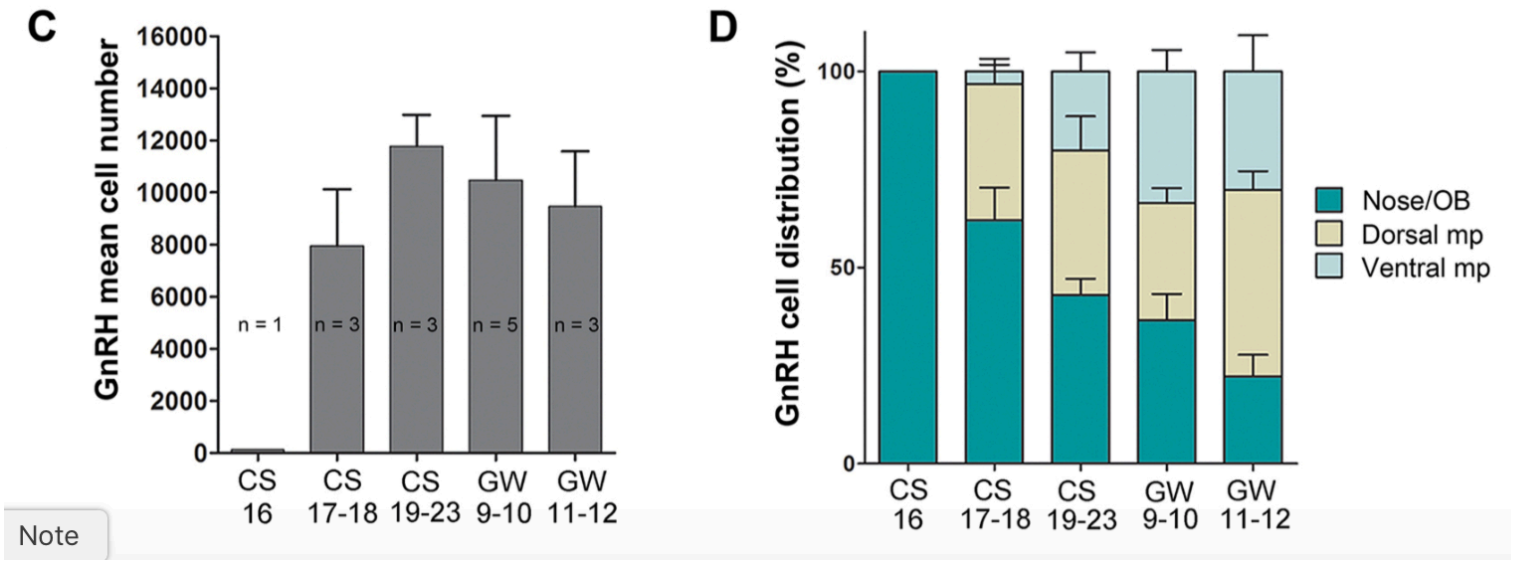
Light-sheet laser scanning microscopy; 3DISCO (optical clearing technique) humans



Casoni *et al*., 2016

A dorsal and a ventral migratory stream humans



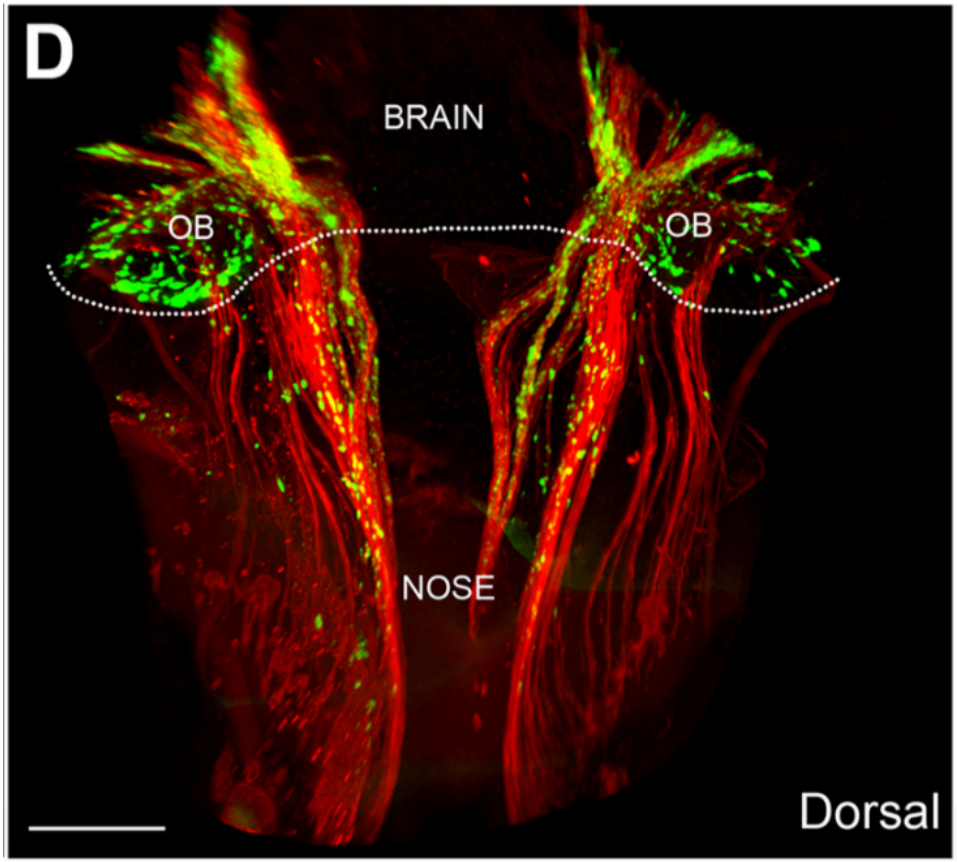


Note

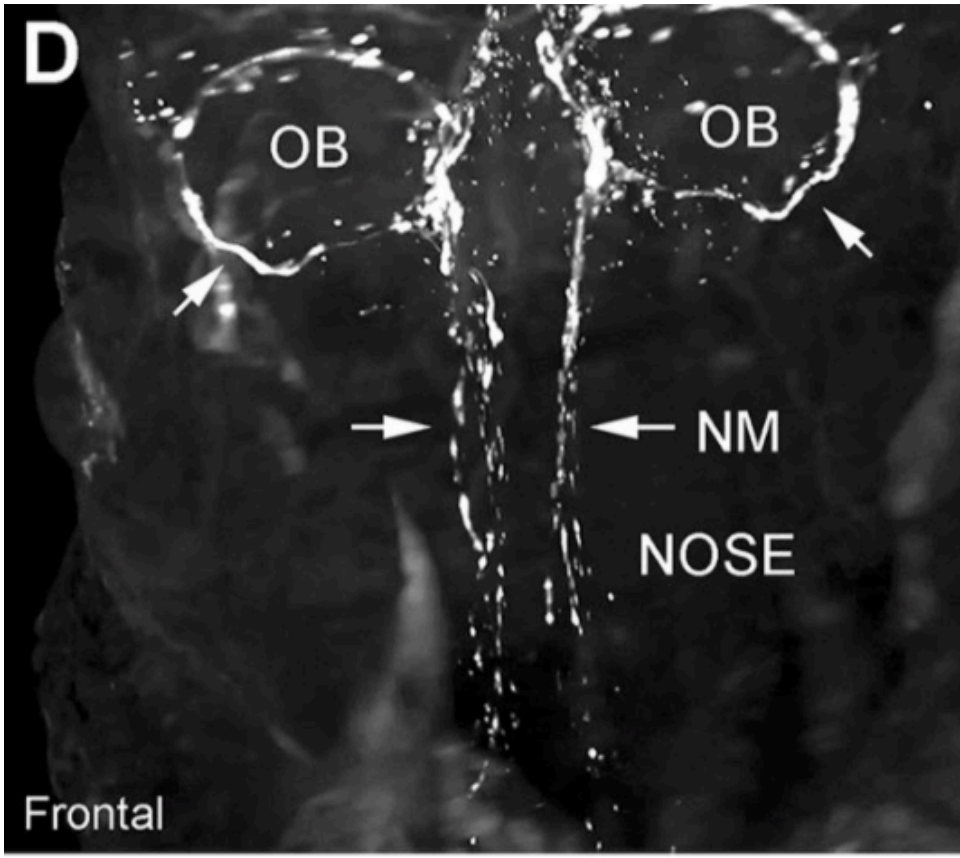
Casoni *et al.*, 2016

A ring-like distribution around the olfactory bulb

Human GW 9



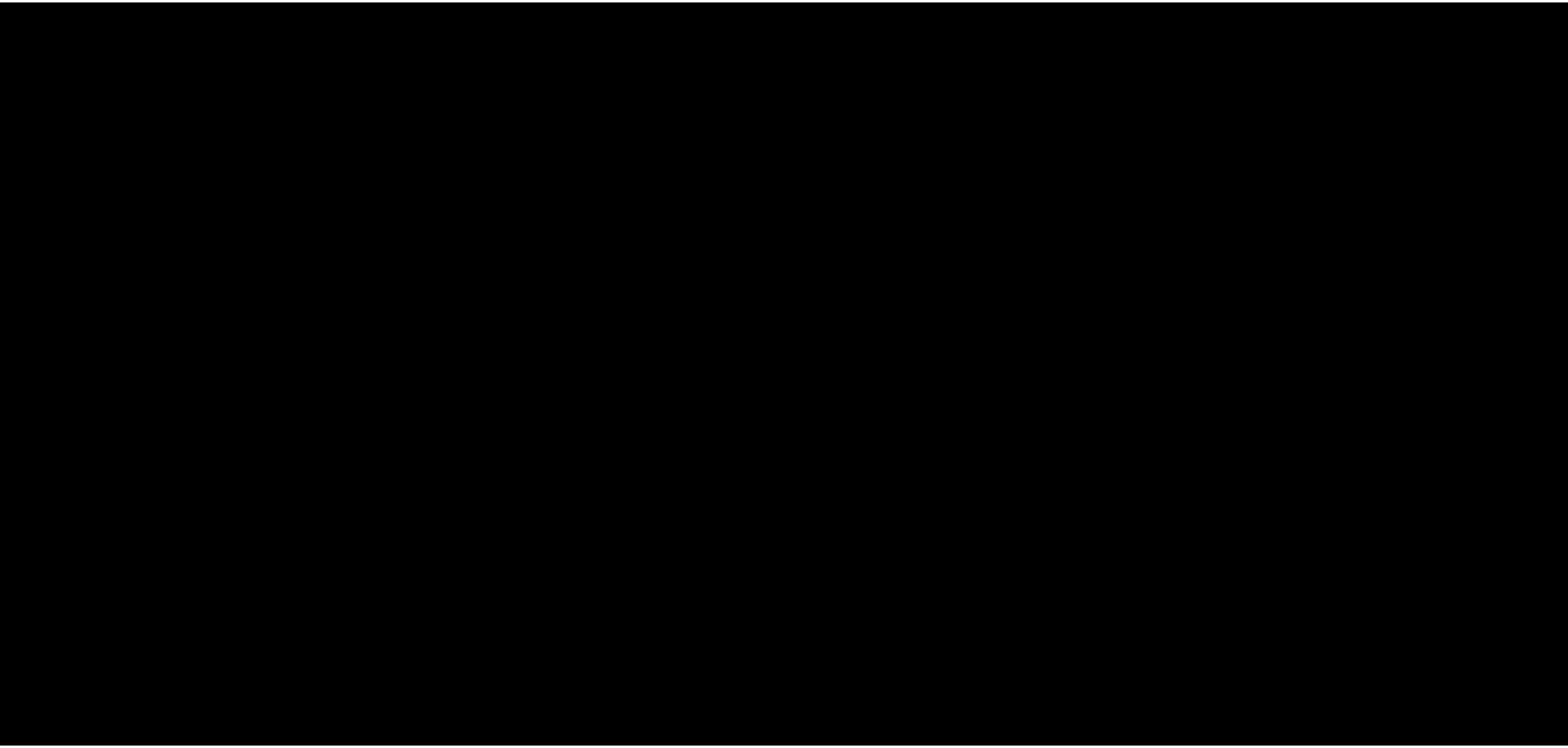
Mouse E16



Casoni *et al*., 2016

GnRH neurons distribution in adulthood

P90 male mouse brain



Casoni *et al*., 2016

Molecules involved in the migration of GnRH-1 cells

1. DIFFUSIBLE MOLECULES:

Semaphorins ←

NETRIN

SDF-1

NEUROPILINS

HGF/SF

2. ADHESION MOLECULES:

N-CAM (Neural Cell Adhesion Molecule)

PSA (Polysialic Acid)

NELF (Nasal Embryonic LHRH Factor)

axonal surface glycoprotein TAG-1

3. NEUROTRANSMITTERS/ PEPTIDES:

GABA

Glutamate

CCK (Cholecystochin)

1. Diffusible molecules:

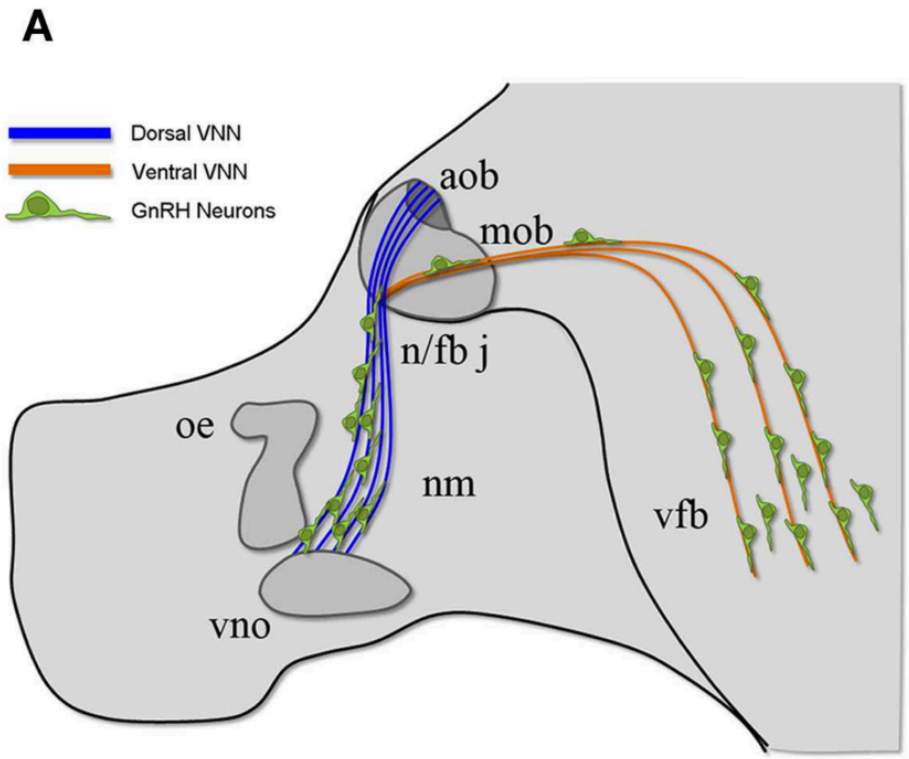
Semaphorins

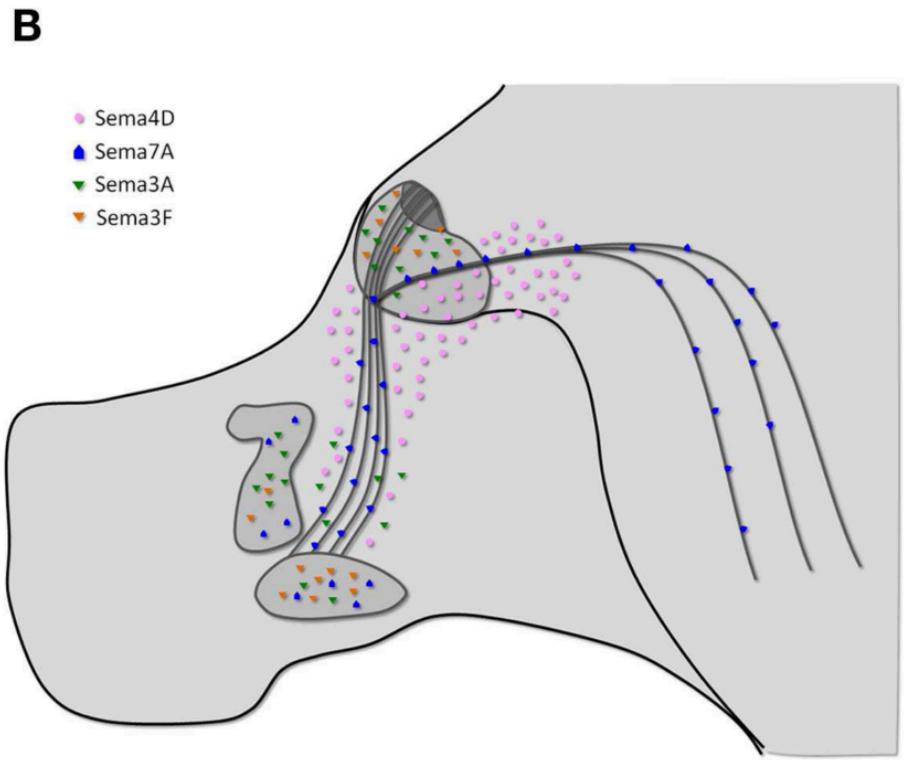
FIGURE 1 | Schematic representation of the protein structure of semaphorins and their receptors. Semaphorins are represented in their classification into eighth classes. Class 1 and 2 semaphorins are found in invertebrates. Class 3–7 semaphorins are found in vertebrates. Both semaphorins and plexins are characterized by Sema domains. Additional domains present in semaphorins and plexins include PSI domains (plexin, semaphorin, and integrin) and immunoglobulin (Ig)-like domains. The structural conserved domains are drawn in different shapes and colors as indicated in the figure. Domains abbreviations: PSI, plexin semaphorin integrin; IPT, Ig-like Plexin Transcription factors; Ig-like, immunoglobulin like; CUB, complement C1r/C1s, Uegf, Bmp1; FV/VIII, coagulation factor V/VIII homology like; MAM, meprin like; GPI, glycosylphosphatidylinositol.

Messina and Giacobini, Neuroendocrinology 2013

1. Diffusible molecules:

Semaphorins in guidance of GnRH neuronal migration



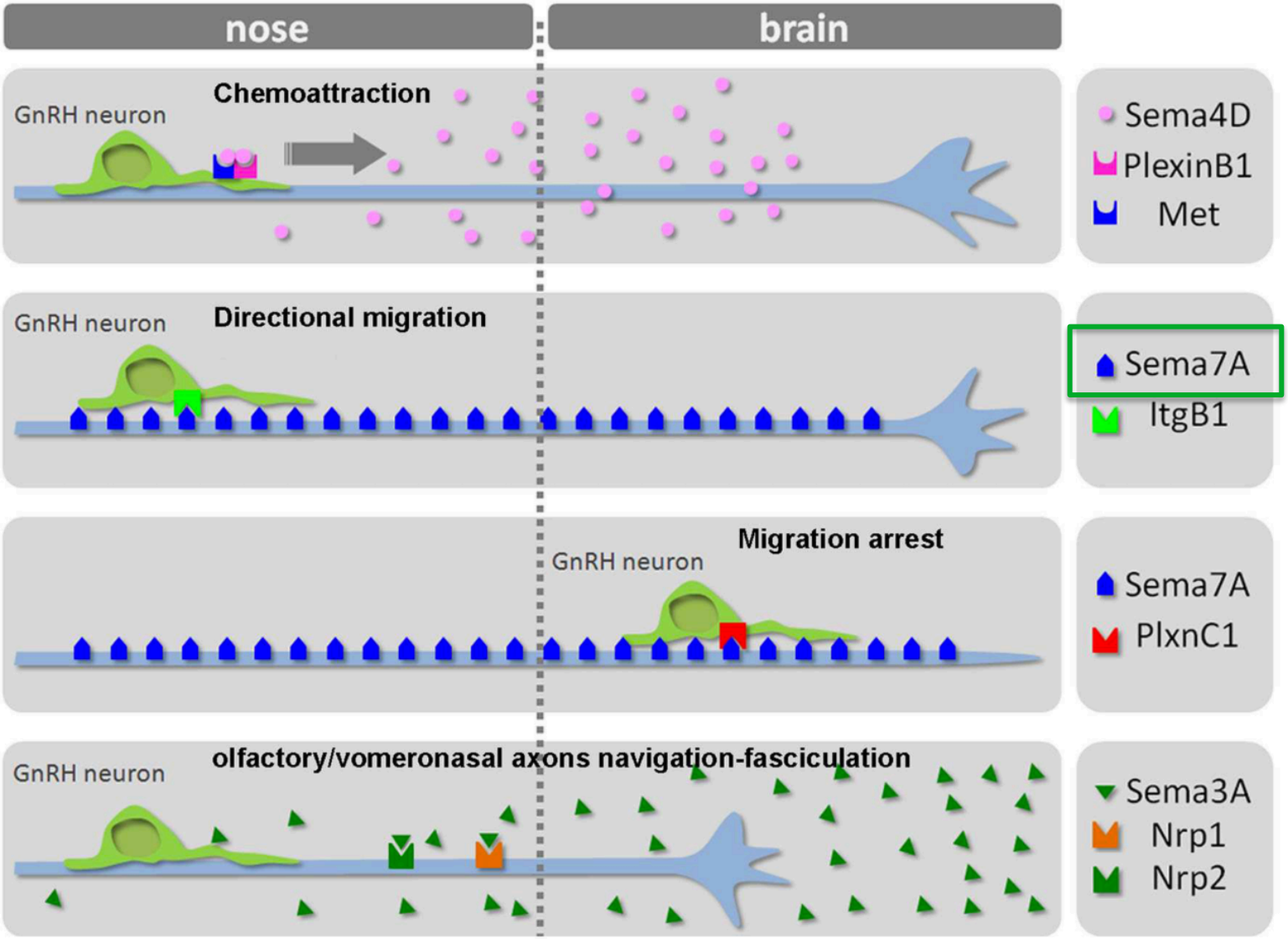


E 14.5

Messina and Giacobini, Neuroendocrinology 2013

1. Diffusible molecules:

Semaphorins in guidance of GnRH neuronal migration



Messina and Giacobini, Neuroendocrinology 2013

Mouse model with impaired GnRH function

Sema7A KO mice

Human Molecular Genetics, 2011, Vol. 20, No. 24 **4759–4774**
doi:10.1093/hmg/ddr403
Advance Access published on September 8, 2011

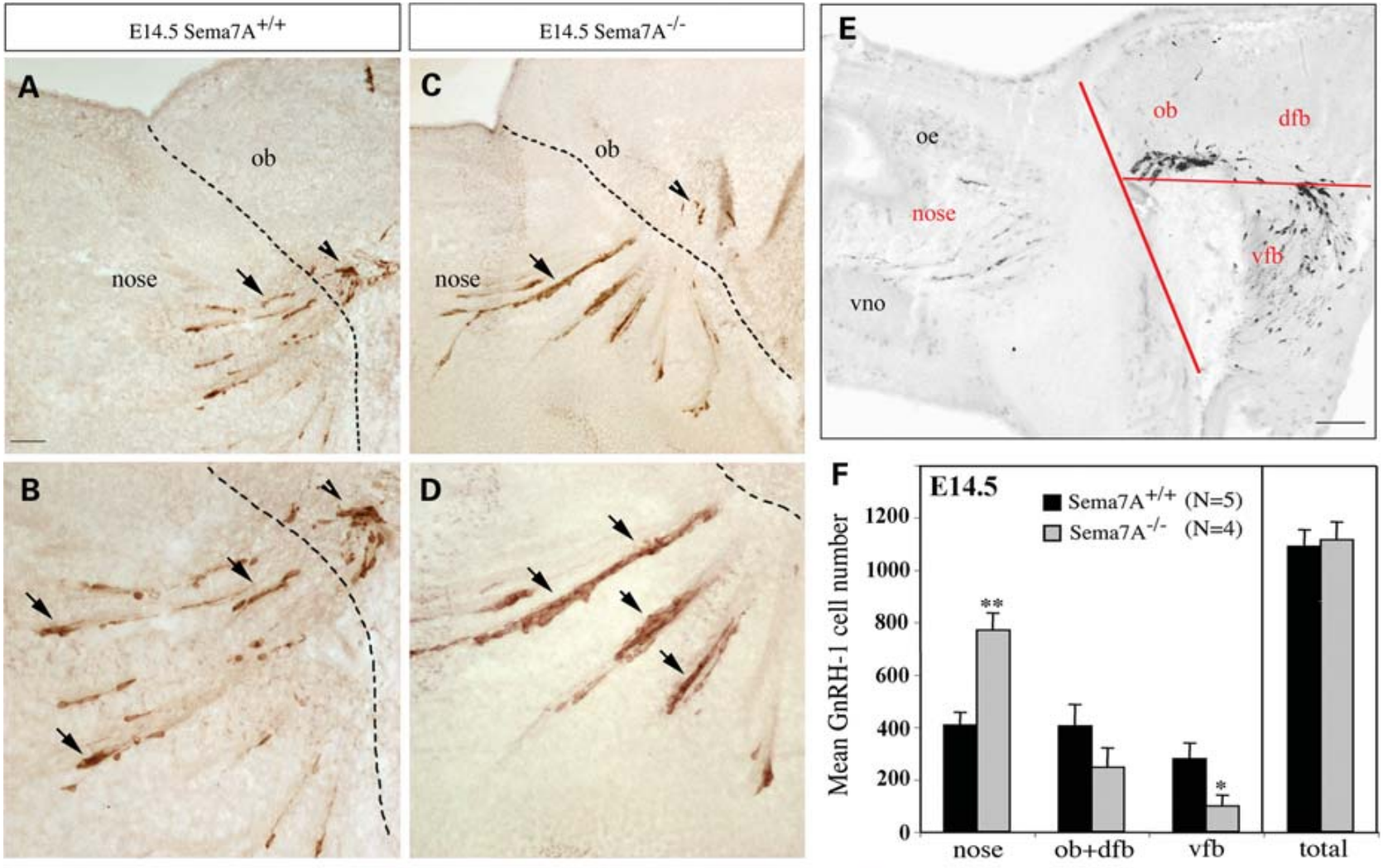
Dysregulation of Semaphorin7A/β1-integrin signaling leads to defective GnRH-1 cell migration, abnormal gonadal development and altered fertility

Andrea Messina[1,4], Nicoletta Ferraris[1], Susan Wray[2], Gabriella Cagnoni[1], Duncan E. Donohue[2], Filippo Casoni[2,4], Phillip R. Kramer[2], Alwin A. Derijck[3], Youri Adolfs[3], Aldo Fasolo[1], Ronald J. Pasterkamp[3] and Paolo Giacobini[1,2,4,*]

→ **Alteration in the GnRH system during development**

Sema7A KO mice

- Significant accumulation of GnRH cells in the nasal compartment
- Reduced gonadal size and altered fertility



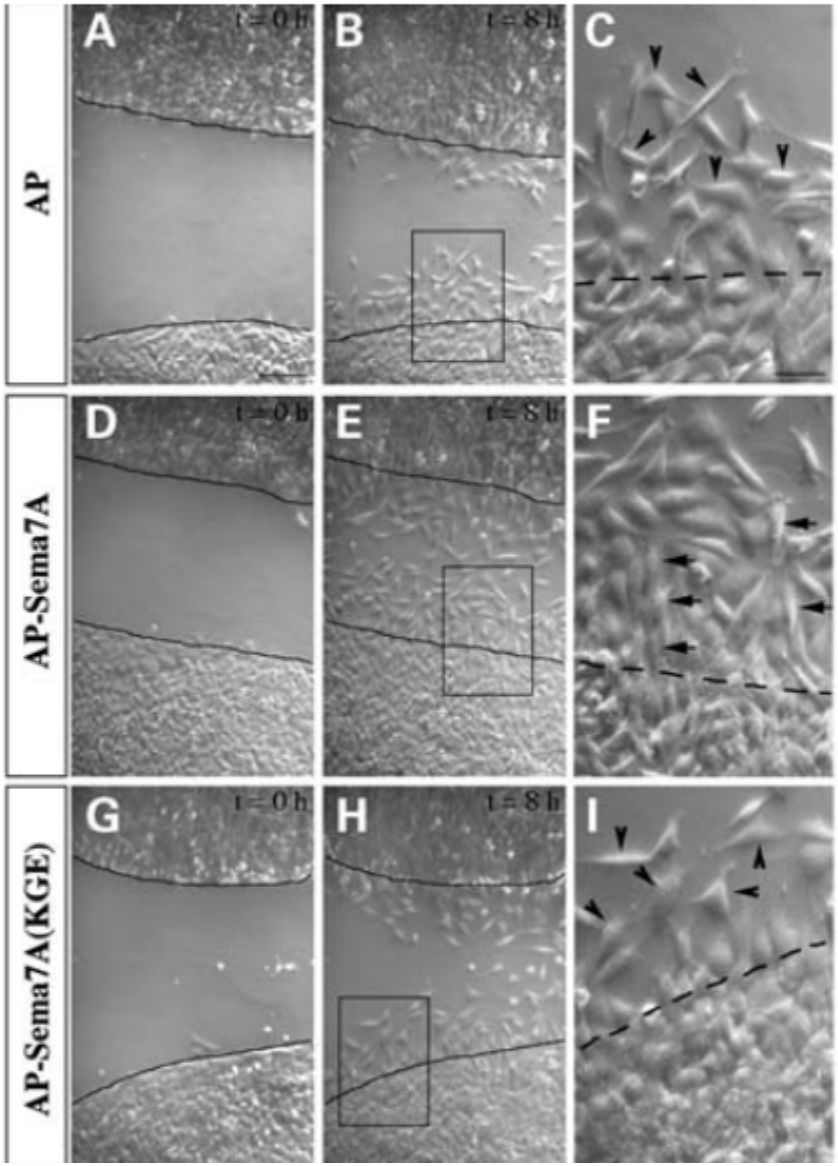
Messina et al. *Human Molecular Genetics.* 2011

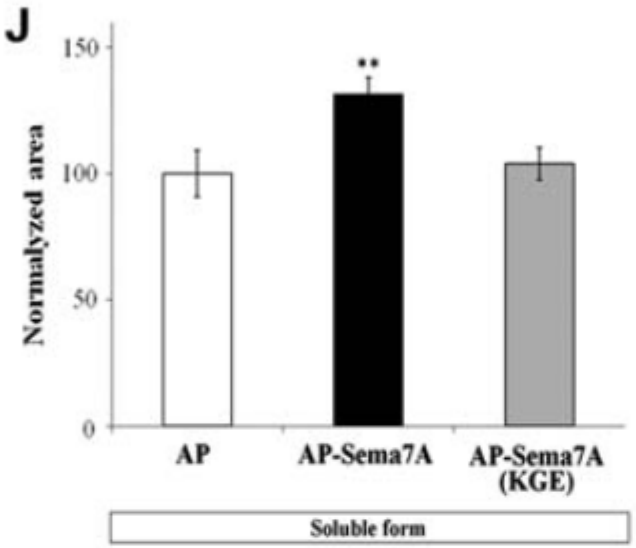
Sema7A KO mice

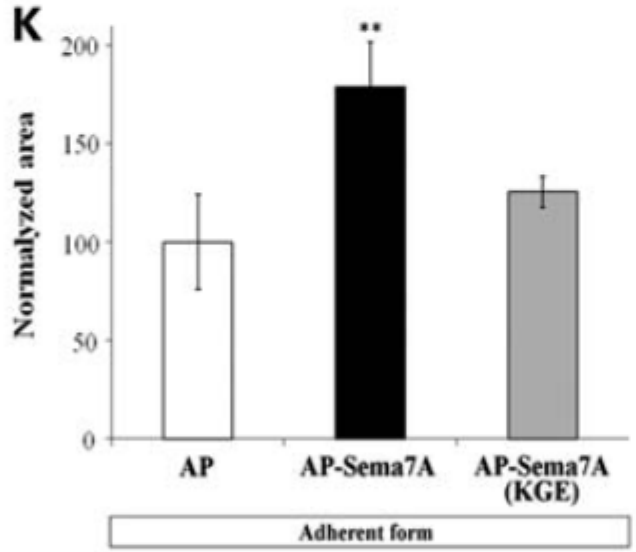
→ Role of Sema7A in cell migration

Wound-healing assay

GN11 cells







Insufficient semaphorin signaling contributes to some forms of reproductive disorders

PLEIOTROPIC NATURE OF SEMAPHORINS

Sema7A KO mice

SCIENTIFIC REPORTS

OPEN

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Opposite-sex attraction in male mice requires testosterone-dependent regulation of adult olfactory bulb neurogenesis

Roberta Schellino[1,2], Sara Trova[1,2], Irene Cimino[3,†], Alice Farinetti[4], Bart C. Jongbloets[5,‡], R. Jeroen Pasterkamp[5], Giancarlo Panzica[2,4,6], Paolo Giacobini[3,7], Silvia De Marchis[1,2,6,§] & Paolo Peretto[1,2,6,§]

Semaphorins mutations humans

Mutation screening of *SEMA3A* and *SEMA7A* in patients with congenital hypogonadotropic hypogonadism

Johanna Känsäkoski, Rainer Fagerholm, Eeva-Maria Laitinen, Kirsi Vaaralahti, Peter Hackman, Nelly Pitteloud, Taneli Raivio & Johanna Tommiska

Pediatric Research (2014) **75**, 641–644 doi:10.1038/pr.2014.23

GnRH deficiencies:

Hypogonadotropic Hypogonadism (HH)

Hypogonadotropic Hypogonadism (HH)

nIHH
normosmic **I**diopathic
Hypogonadotropic
Hypogonadism

KS
Kallmann **S**yndrome

Normal olfaction

Anosmia

Mouse model with impaired GnRH function

ARTICLES

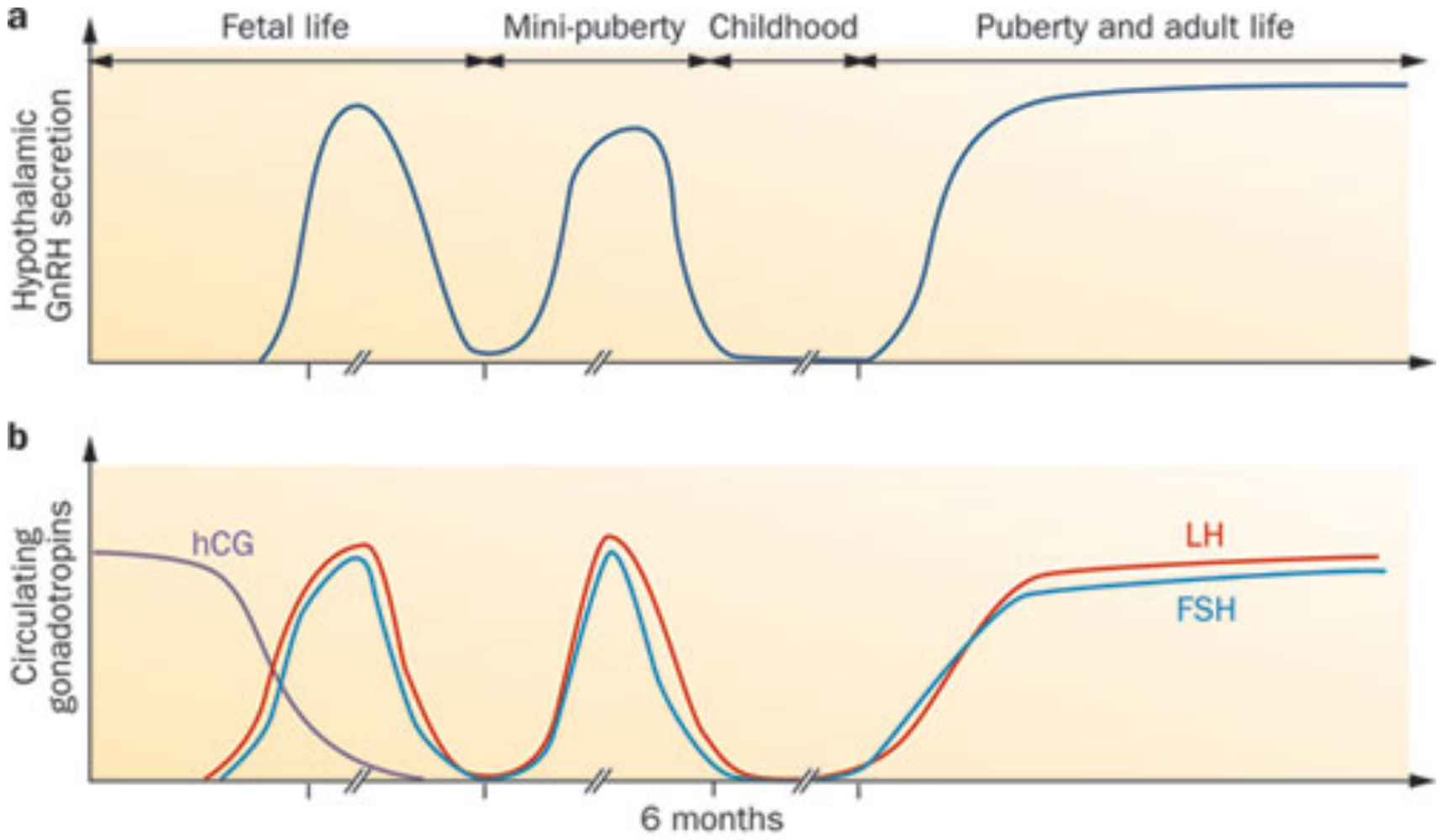
nature neuroscience

A microRNA switch regulates the rise in hypothalamic GnRH production before puberty

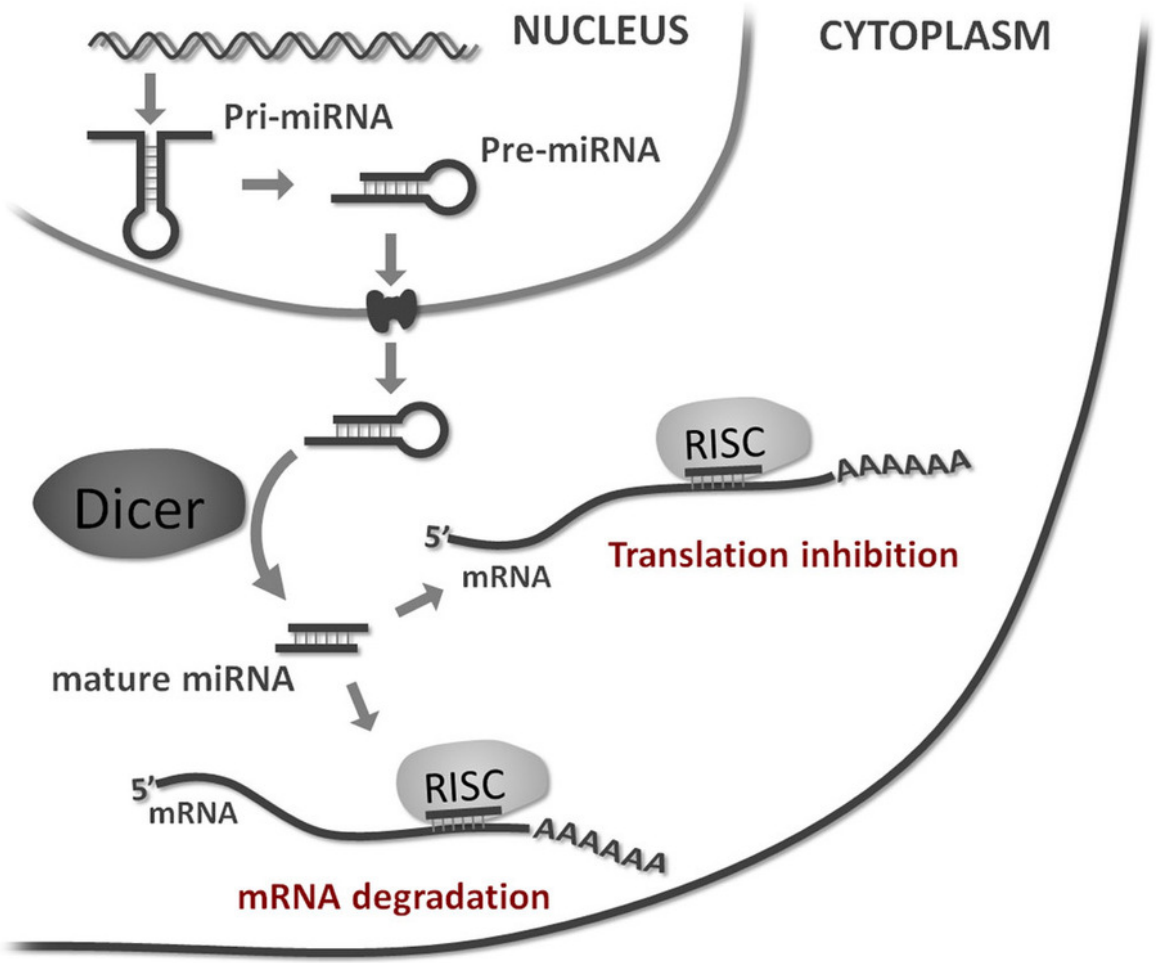
Andrea Messina[1,2,8], Fanny Langlet[1–3,9], Konstantina Chachlaki[1,2,9], Juan Roa[4–6,9], Sowmyalakshmi Rasika[7], Nathalie Jouy[1,2], Sarah Gallet[1,2], Francisco Gaytan[4–6], Jyoti Parkash[1,2,8], Manuel Tena-Sempere[4–6], Paolo Giacobini[1,2] & Vincent Prevot[1,2]

Published online 2 May 2016

- → **GnRH deficiency is not due to a developmental lack of GnRH neurons**
- → **Lack of GnRH expression is acquired during postnatal development**


a
Fetal life
Mini-puberty
Childhood
Puberty and adult life
Hypothalamic GnRH secretion
b
Circulating gonadotropins
hCG
LH
FSH
6 months

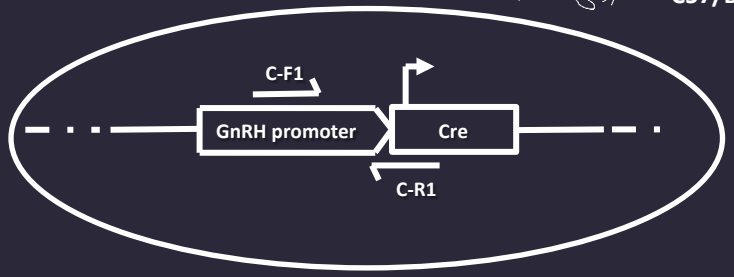
Role of miRNAs in the pubertal activation process of GnRH neurons



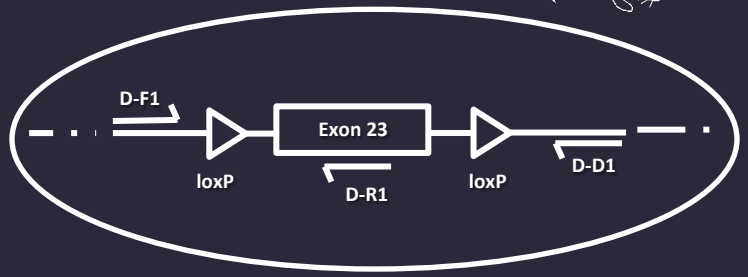
Role of miRNAs in the pubertal activation process of GnRH neurons

GnRH::Cre mice

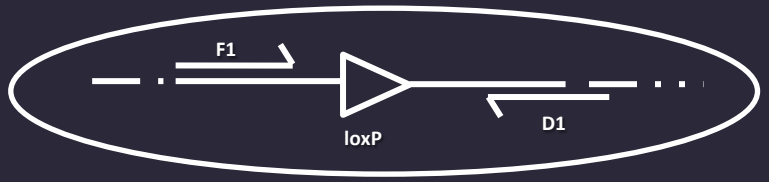
C57/Bl6



Dicer$^{loxP/loxP}$ mice



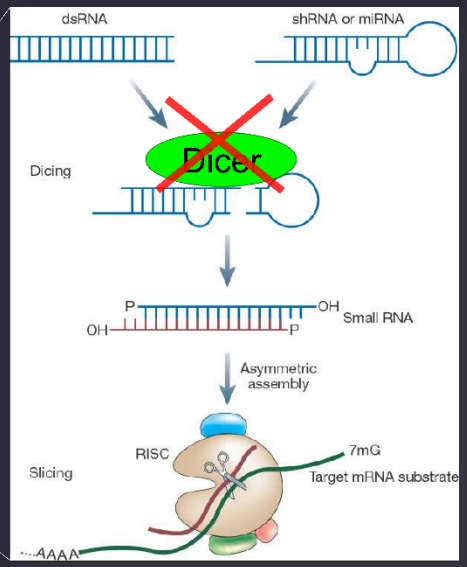
Cre-directed recombination



GnRH::Cre/Dicer$^{loxP/loxP}$ mice

GnRH neurons

Giacobini,P.; Prévot.,V;Messina,A.

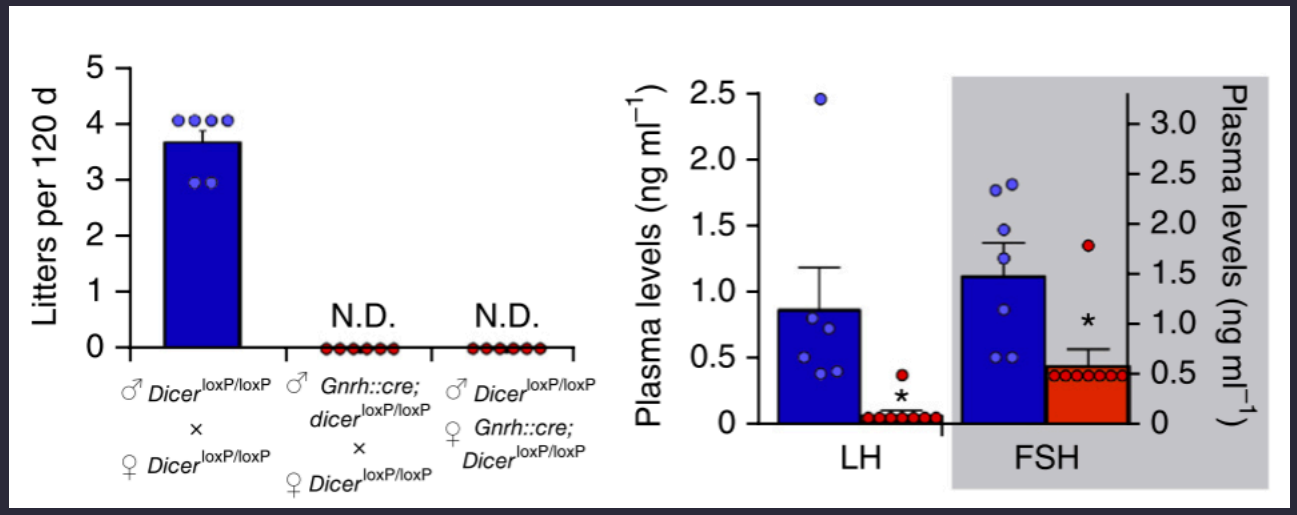


Dicer: RNAse III endonuclease essential for miRNA biogenesis

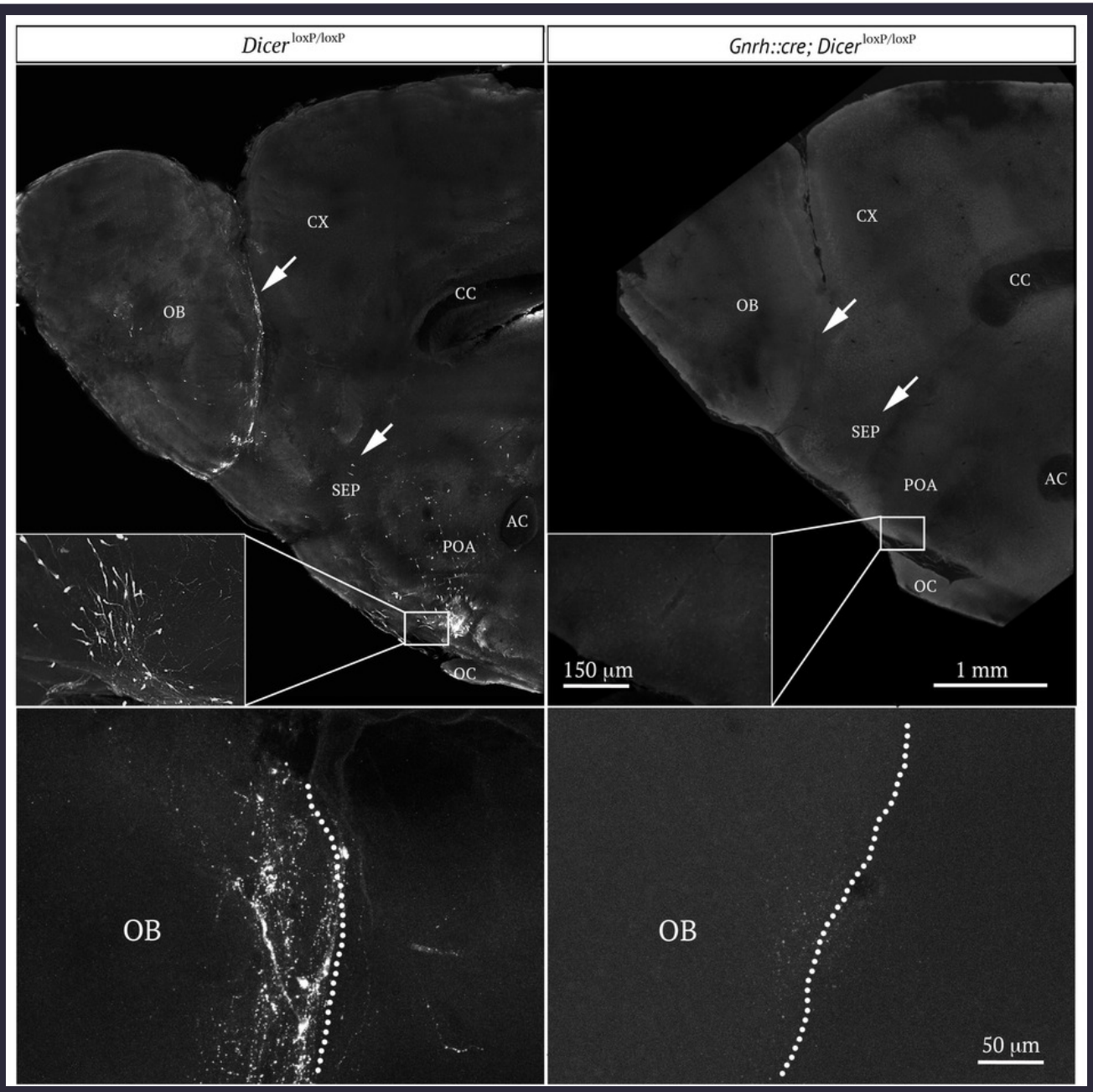
Mouse model with impaired GnRH function

GnRH::Cre/Dicer$^{loxP/loxP}$ mice ⚥

- Hypogonadism
- Sterility
- Hormonal levels are low

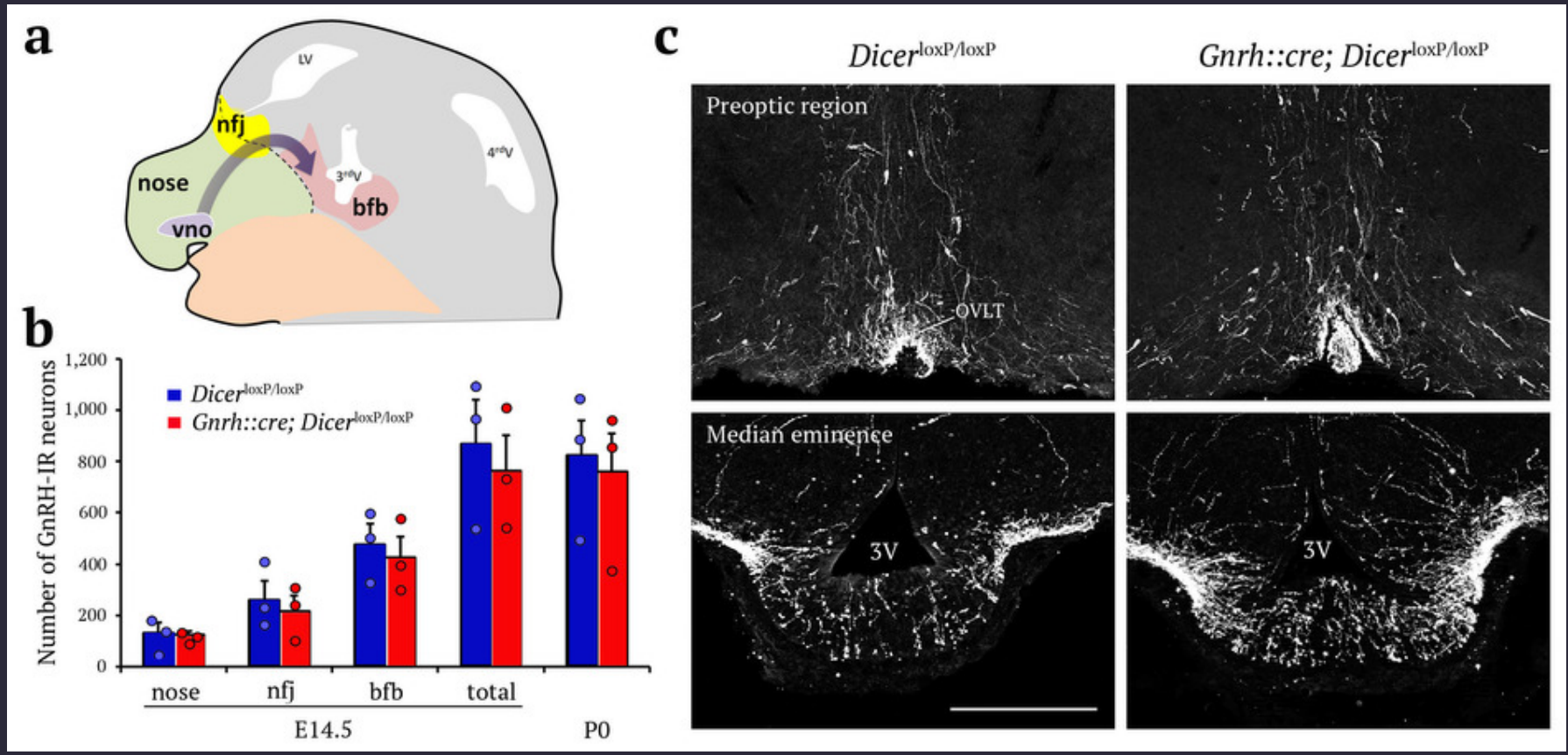


GnRH is lacking in the postnatal brain



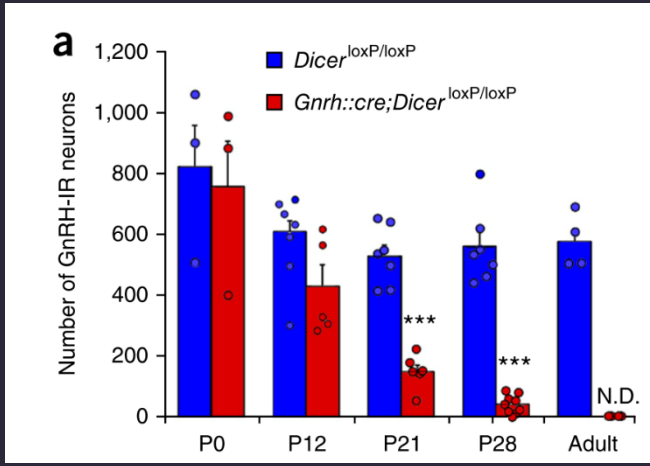
ADULT mice brain

GnRH cells migration is not affected



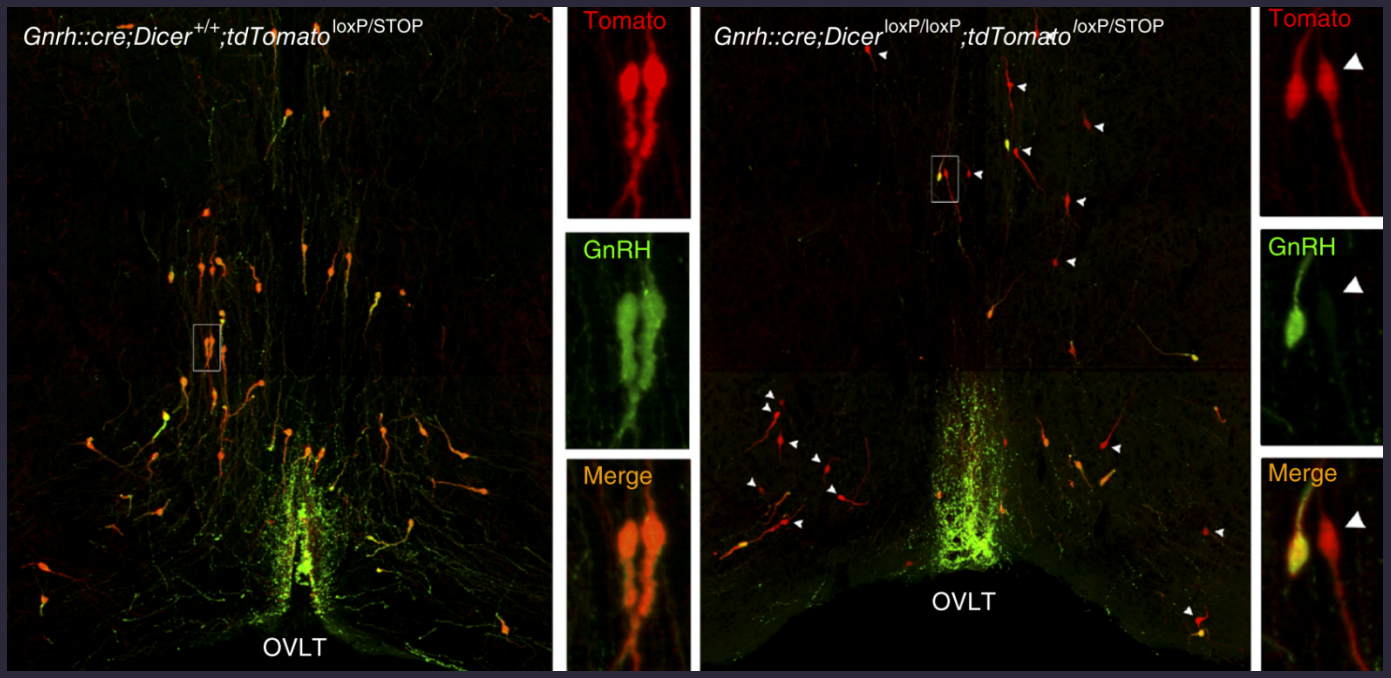
⟶ GnRH deficiency is acquired postnatally

GnRH disappears gradually during postnatal development



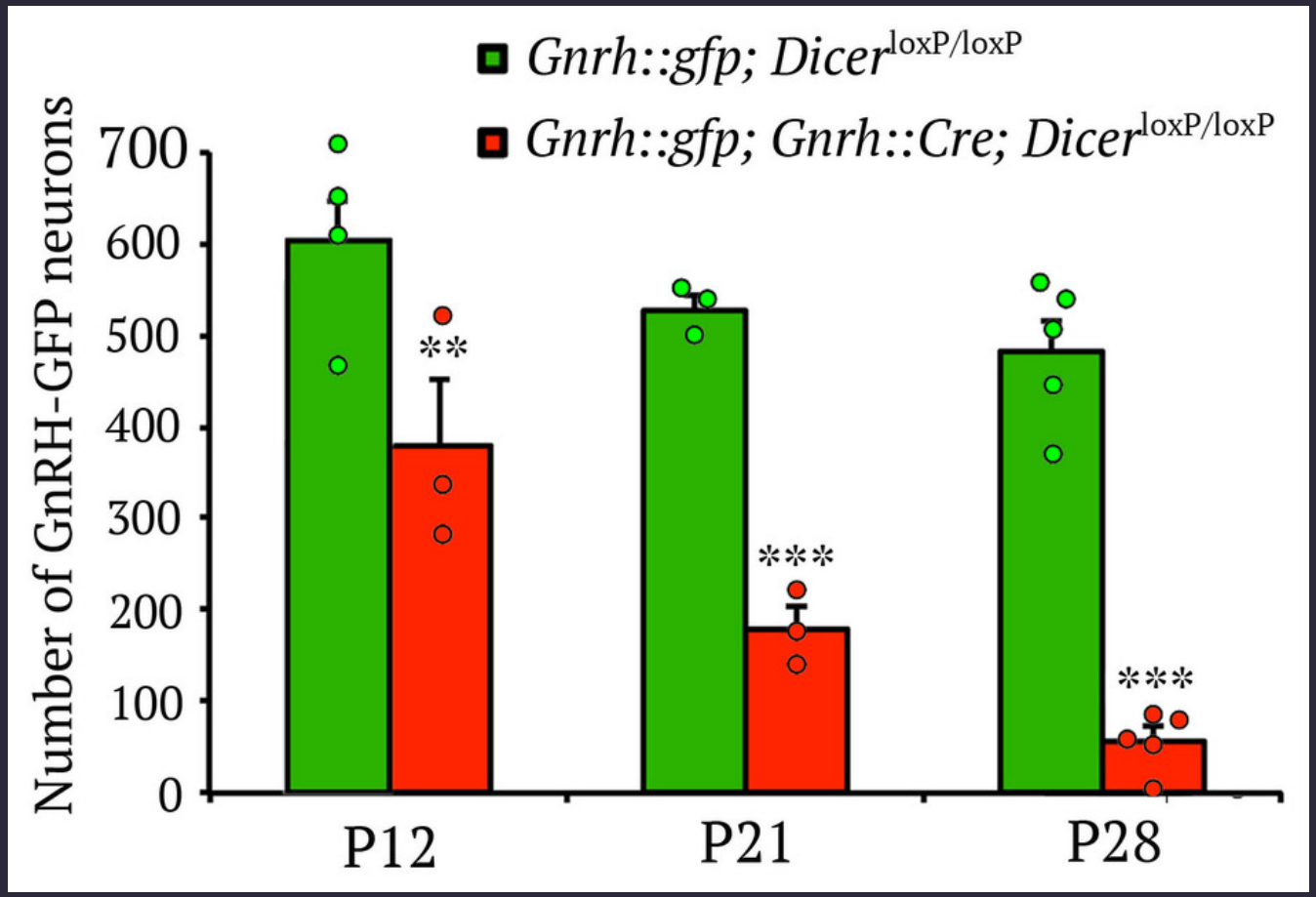
GnRH cells not die, but they simply loose GnRH expression in the absence of miRNAs

Gnrh::cre;Dicer$^{loxP/loxP}$;tdTomato$^{loxP/STOP}$ trigenic mice



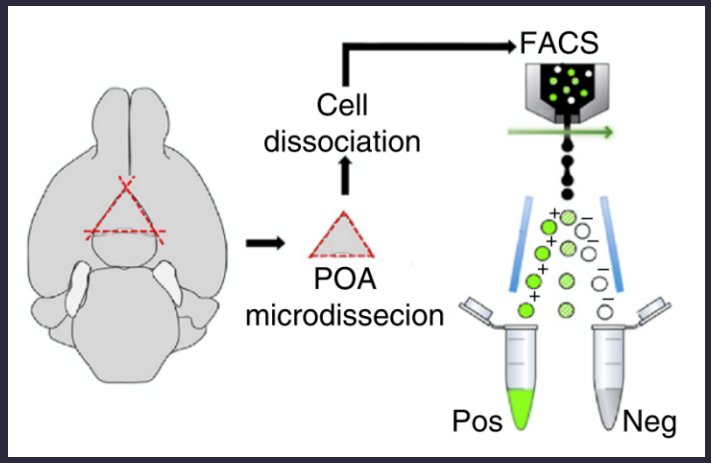
miRNAs regulate genes necessary for GnRH transcription: promoter modulators

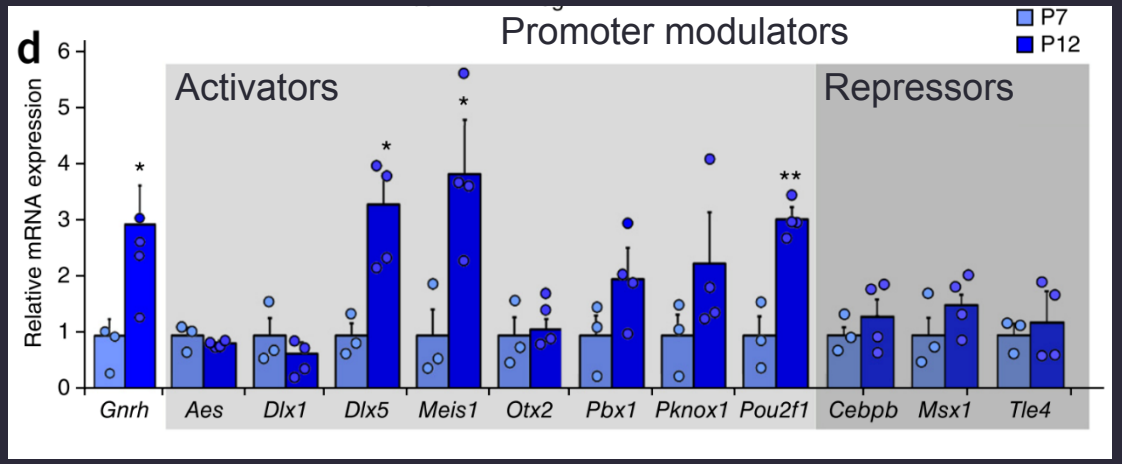
Gnrh::Gfp;Gnrh::cre;Dicer$^{loxP/loxP}$ mice

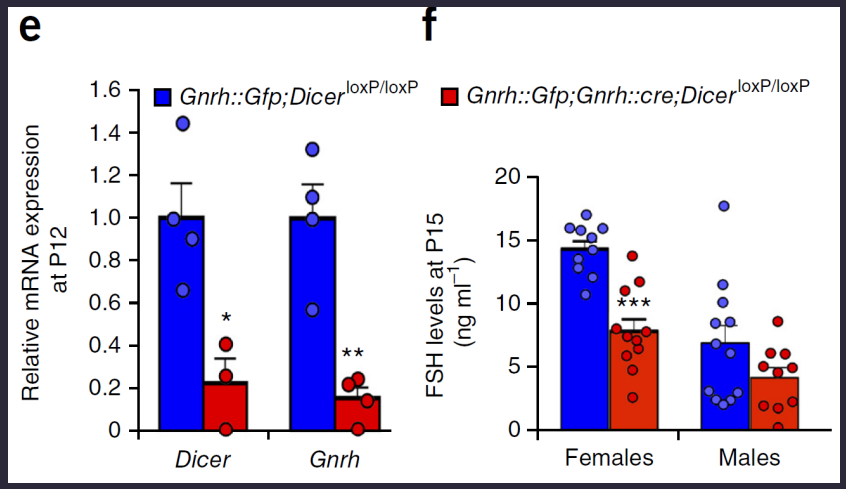


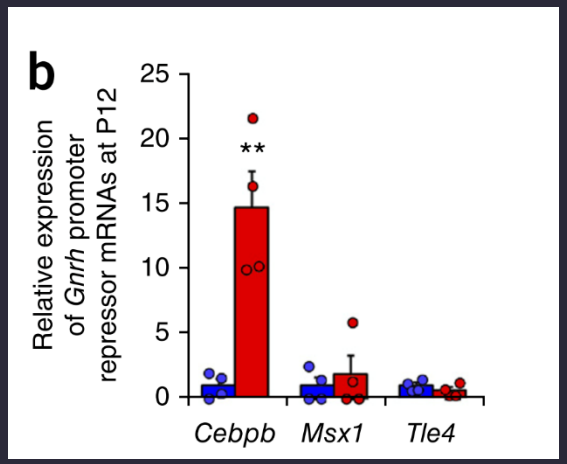
miRNAs control GnRH transcript levels indirectly by altering levels of GnRH promoter modulators

Gnrh::Gfp;Dicer$^{loxP/loxP}$ mice









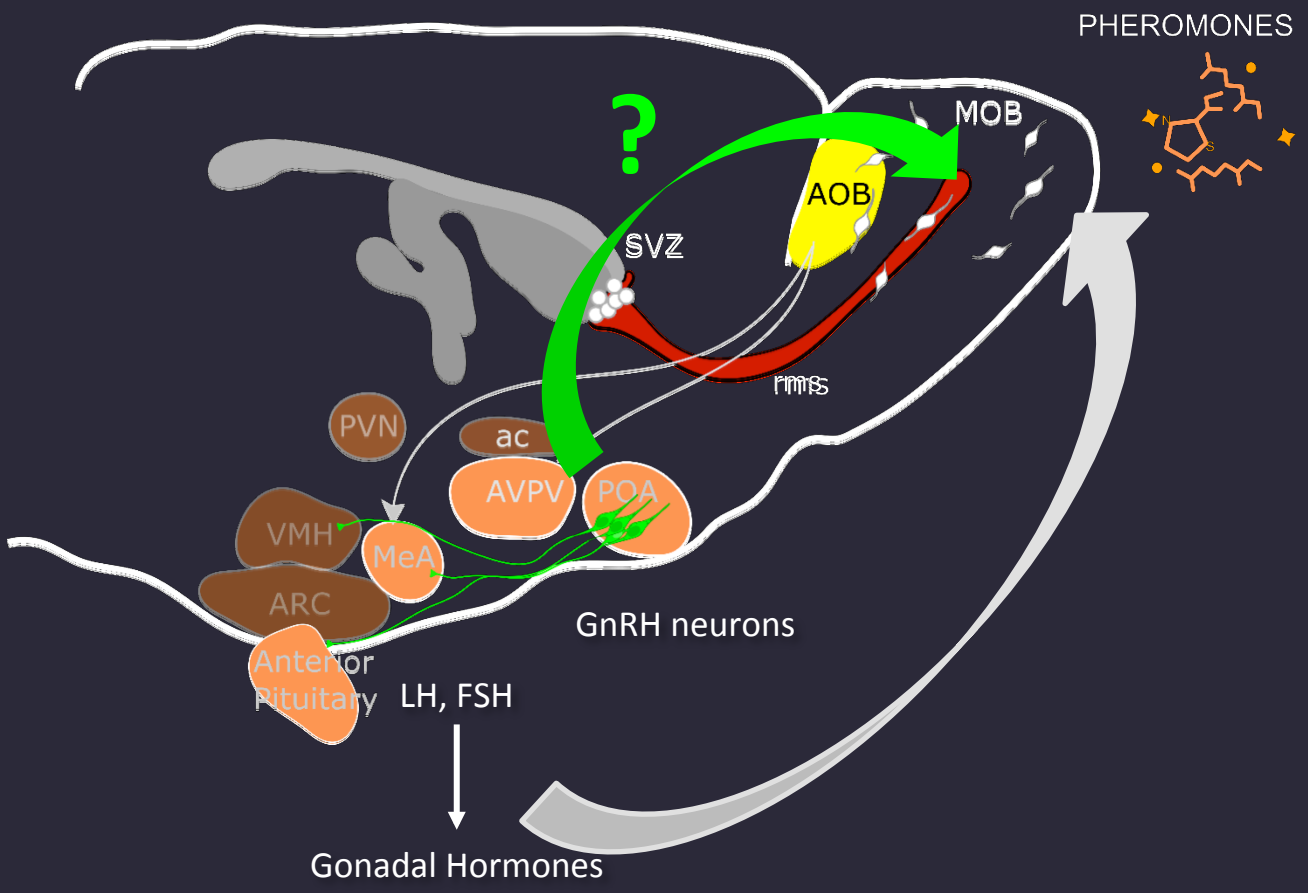
Is it possible that acquired deficiency on GnRH can cause secondary defects in odor perception?

Plasticity and Function of the Olfactory System

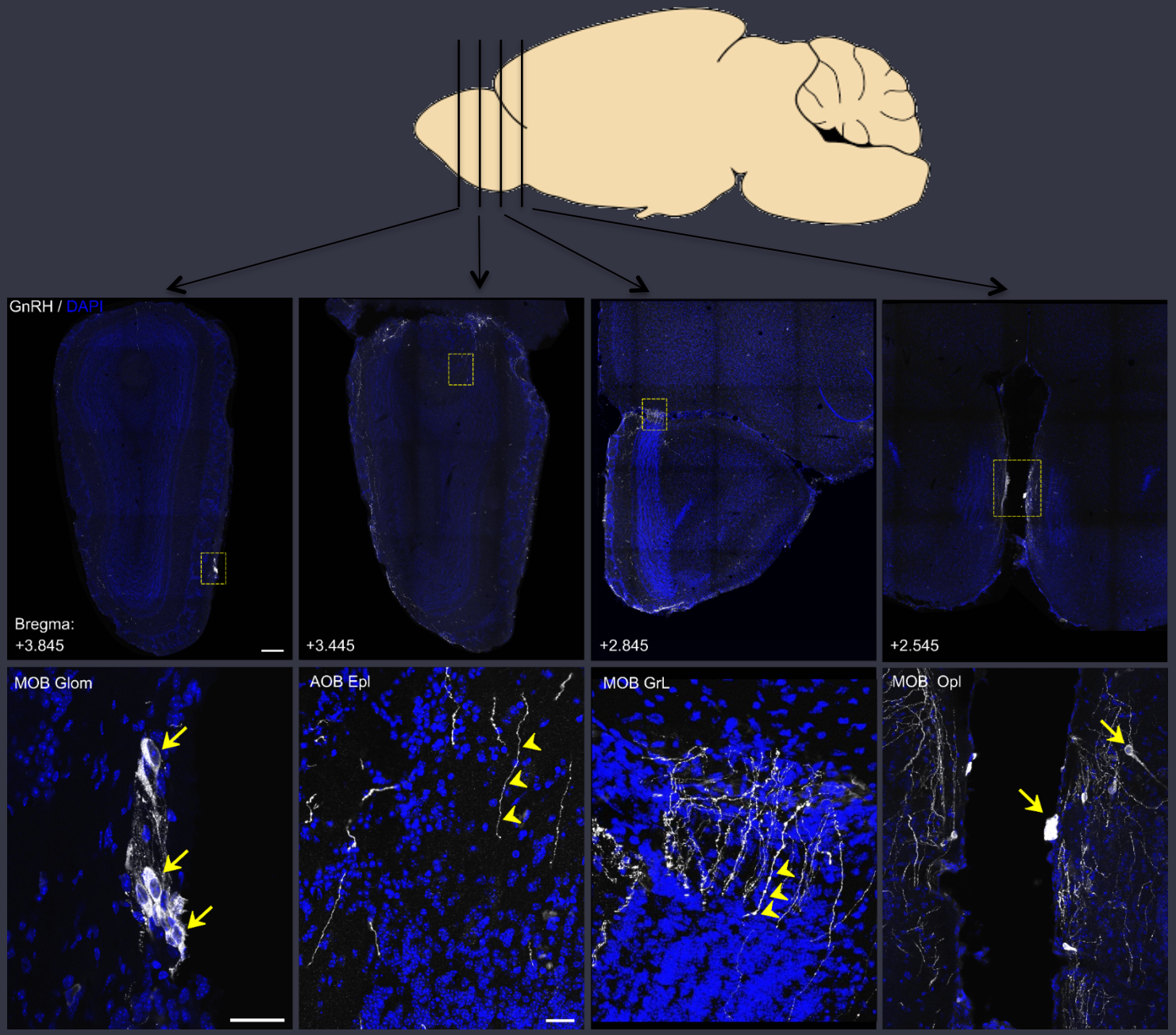
Adult Neurogenesis

HPG axis

Sexual behavior



GnRH-IR fibers and cell bodies surround the OB



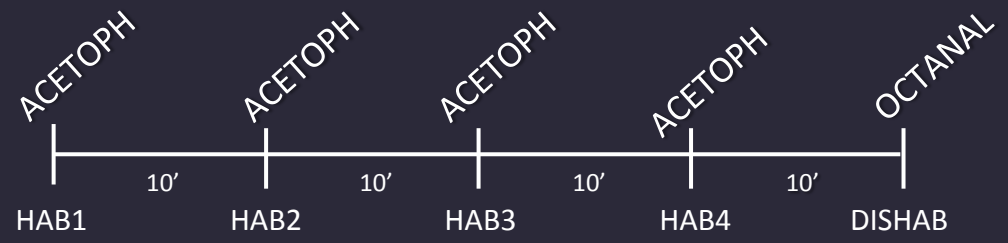
3D-reconstruction analysis of GnRH-fibers into the OB

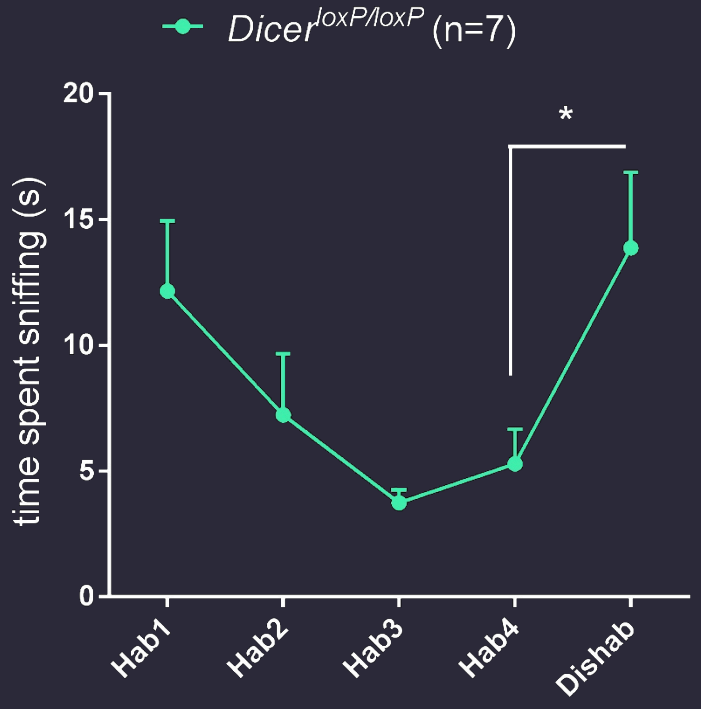
Impaired non-social odor discrimination in GnRH::Cre/Dicer$^{loxP/loxP}$ male mice

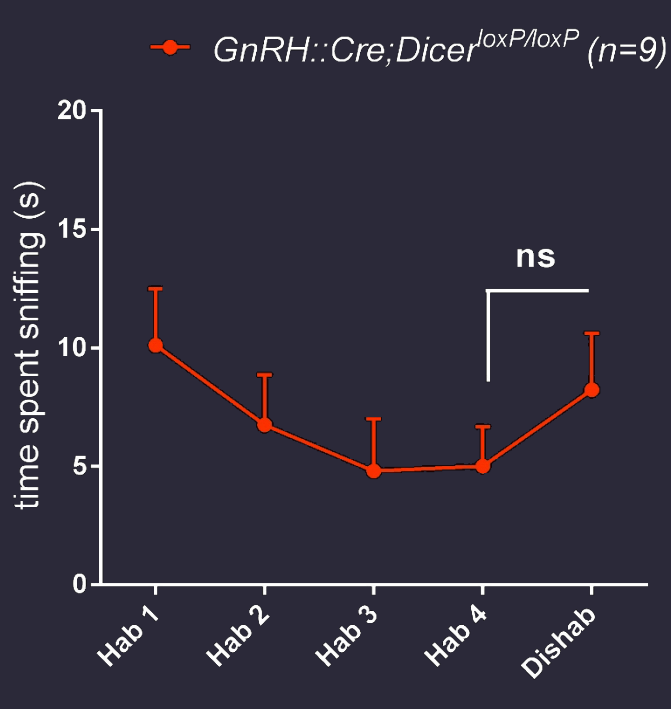
OLFACTORY ASSAYS FOR NON-SOCIAL ODORS

males

Habituation-Dishabituation Test





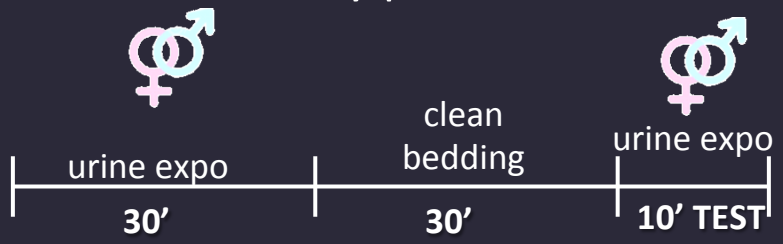


Altered opposite social odors preference in GnRH::Cre/Dicer$^{loxP/loxP}$ male mice

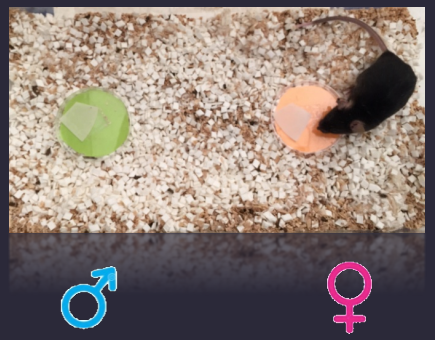
OLFACTORY ASSAYS FOR SOCIAL ODORS

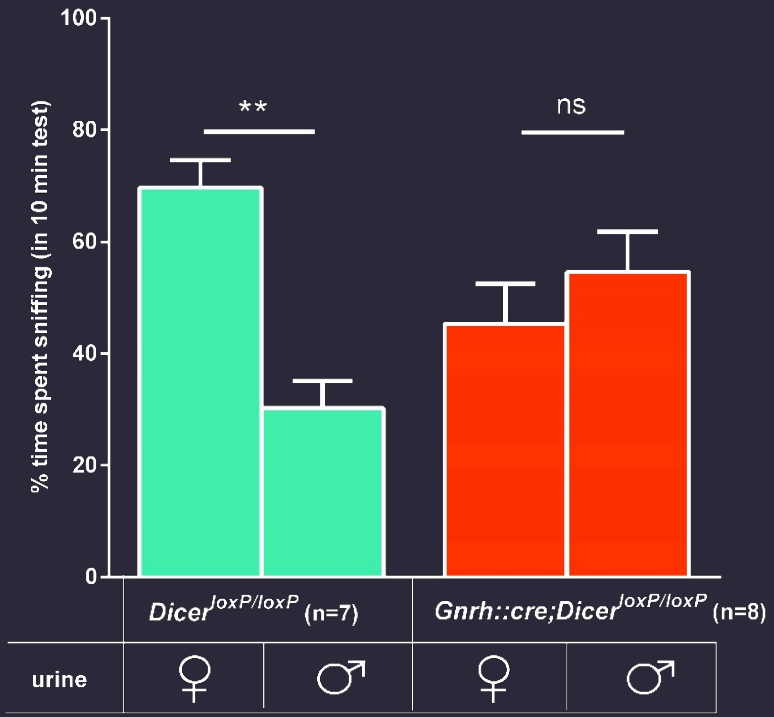
males

Olfactory preference Test



contact-contact

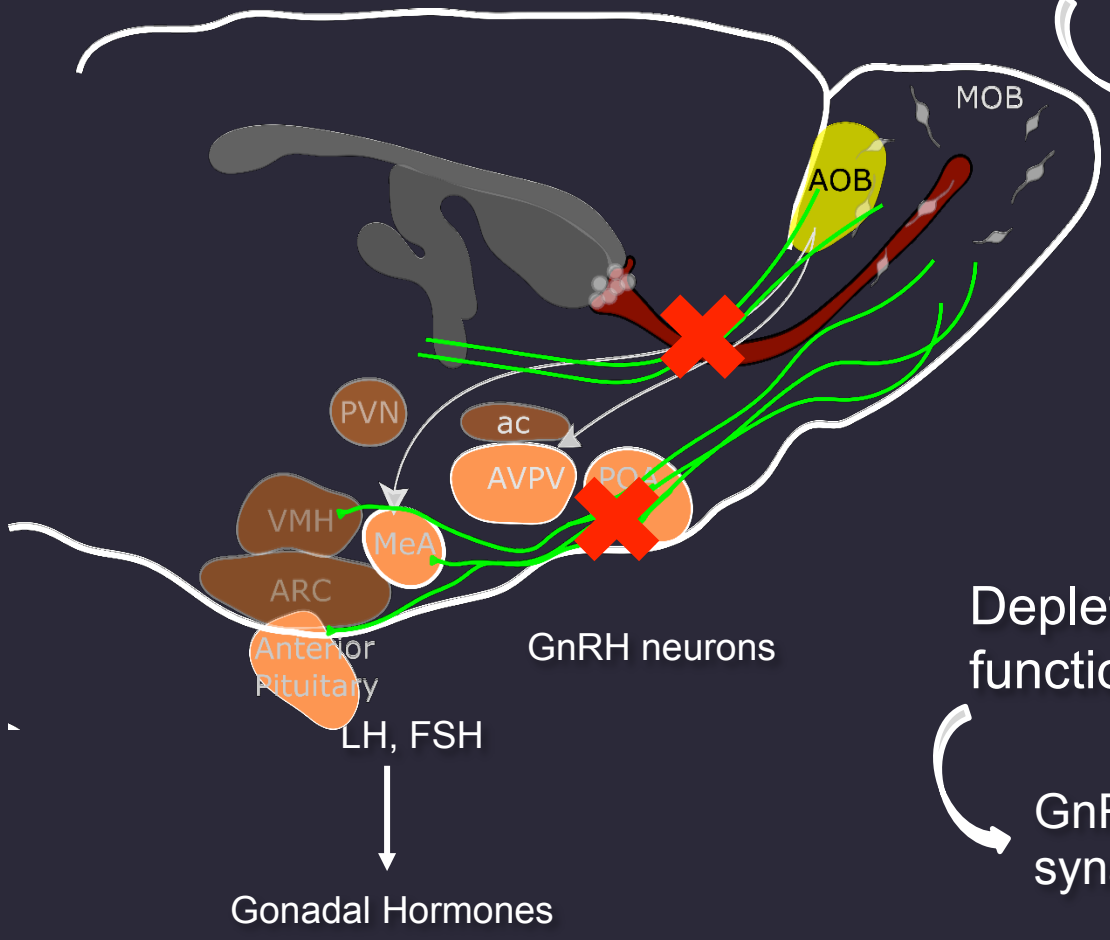




⬇ Impaired ability to discriminate both non-social and social odours

HYPOTHESIS

The OLFACTORY SYSTEM…



Contains GnRH-IR fibers

Where they come from?

From a population different from the hypothalamic resident GnRH neurons?

OP or NC embriological origin?

Depleted Olfactory function

GnRH as neuromodulator at synaptic level in the OB?

AKNOWLEDGMENTS

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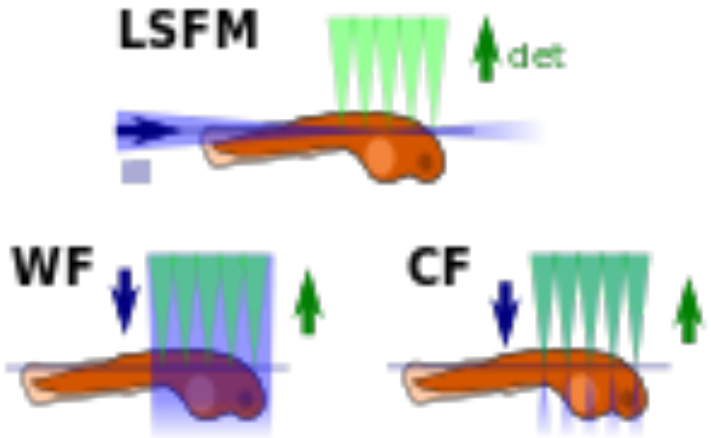
Livio Oboti
(National Institute of Health, WDC)

Light-sheet laser scanning microscopy; 3DISCO (optical clearing technique)

humans



LSFM : Light Sheet Fluorescence Microscopy
CF: Confocal Microscopy