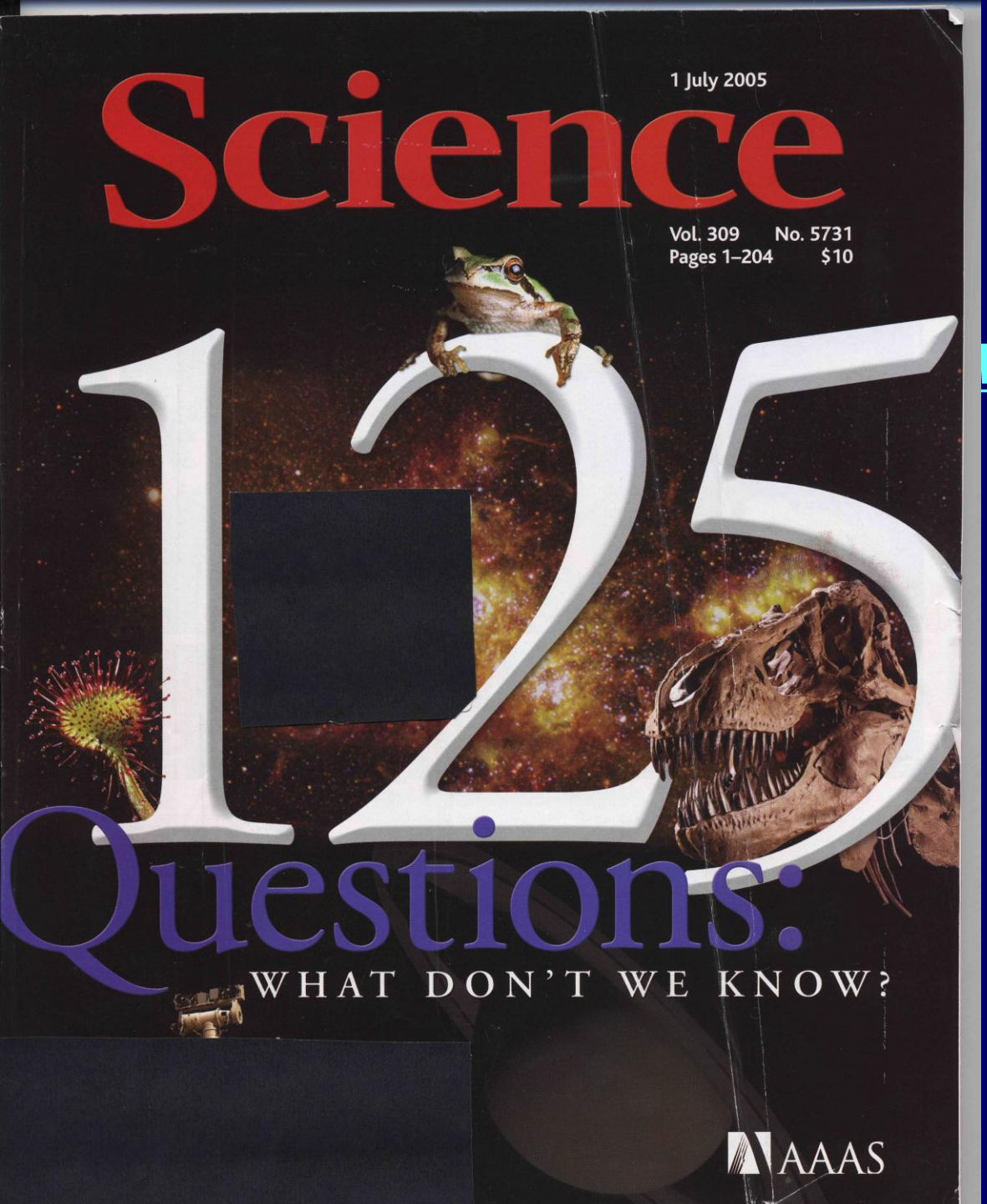
A 35 Year Experience in Translational Research Funded by NICHD

Use of Human Disease Models to Define the Genetic Control of Human Reproduction

William F. Crowley, Jr., M.D.
Daniel K. Podolsky Professor Medicine, Harvard Medical School
Director, Harvard Reproductive Endocrine Sciences Center of Excellence
NICHD U54 Specialized Cooperative Center of Excellence
Director of Clinical Research, MGH



Question #82:
What triggers puberty?

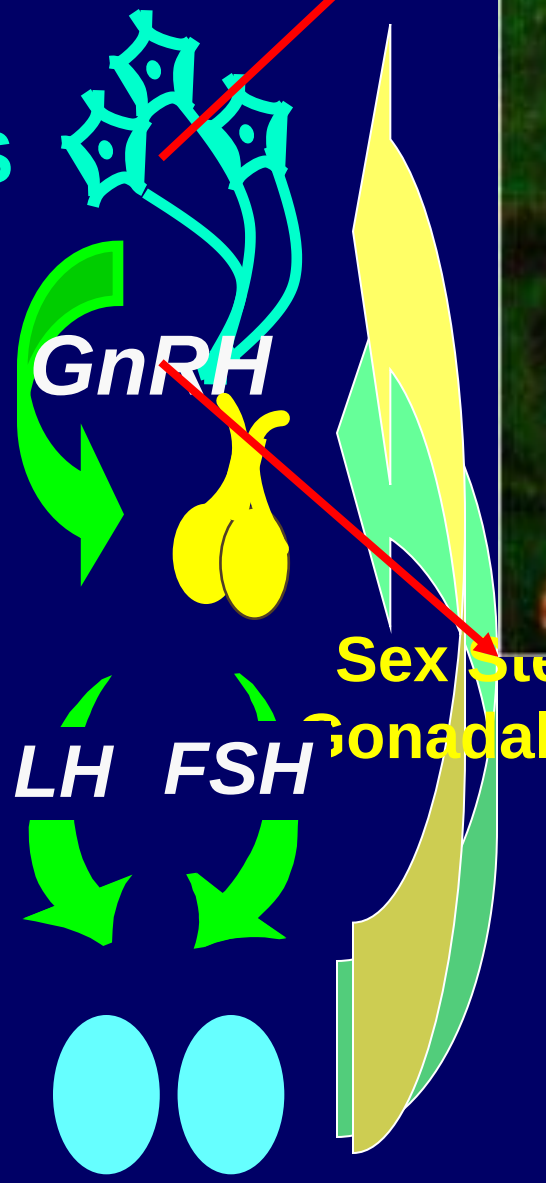
"…no one knows exactly what forces childhood to end."

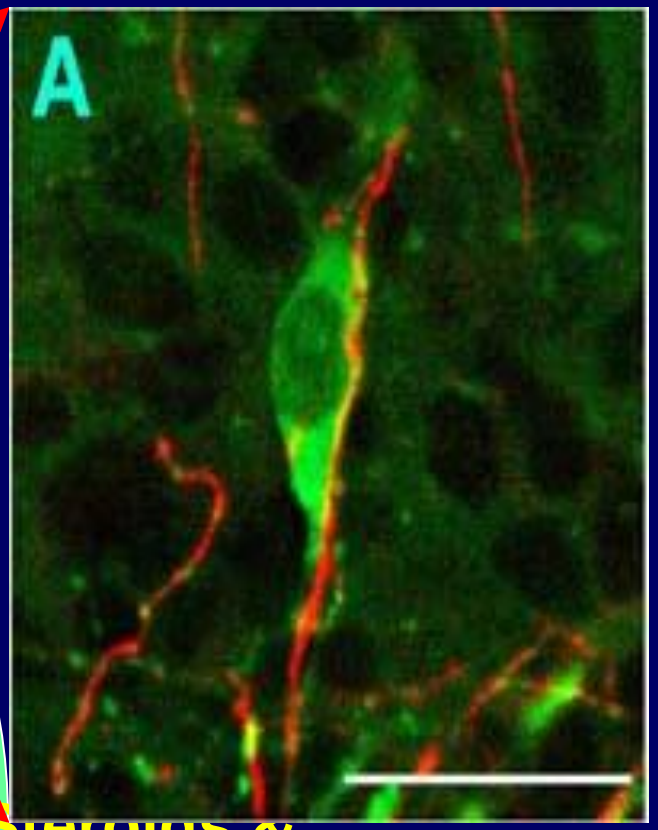
Normal HPG Axis

HYPOTHALAMUS

PITUITARY

GONADS

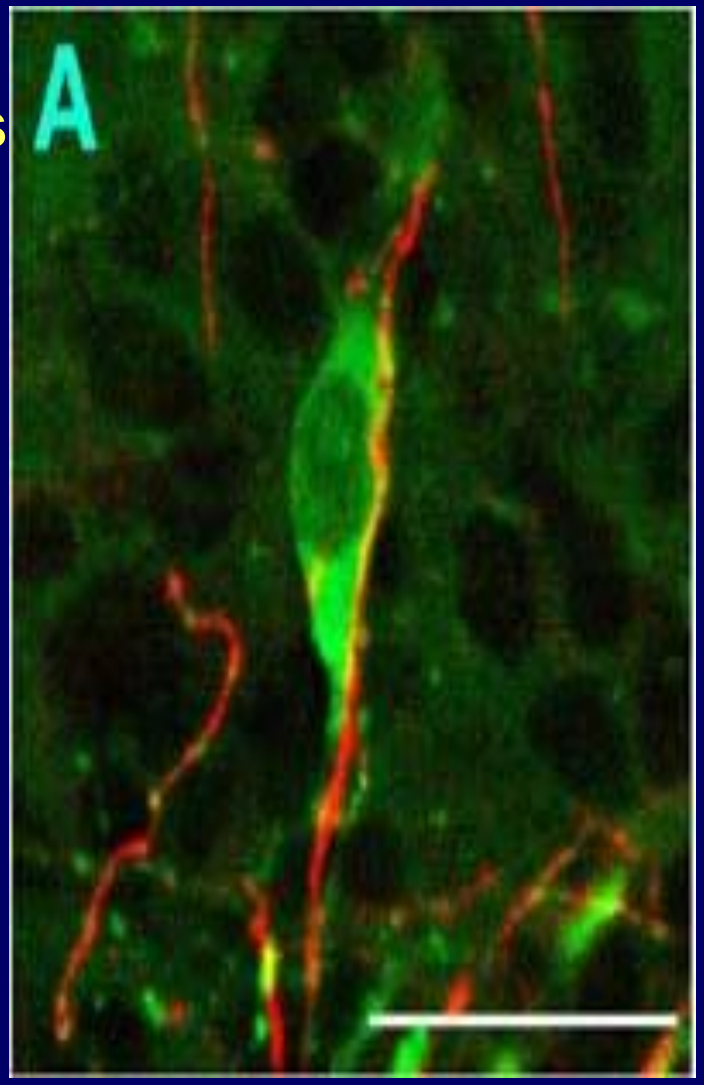




Sex Steroids &
Gonadal Proteins

Daunting Problems: Technical & Regulatory

- Small in size & # (~1,200 in humans
- Widely dispersed network
- High species variability
- GnRH not measurable
- Must measure LH, FAS q10’
- Research in children



Why Work on GnRH Neurons?

Big Opportunities!!:

Unique Biology in the Human

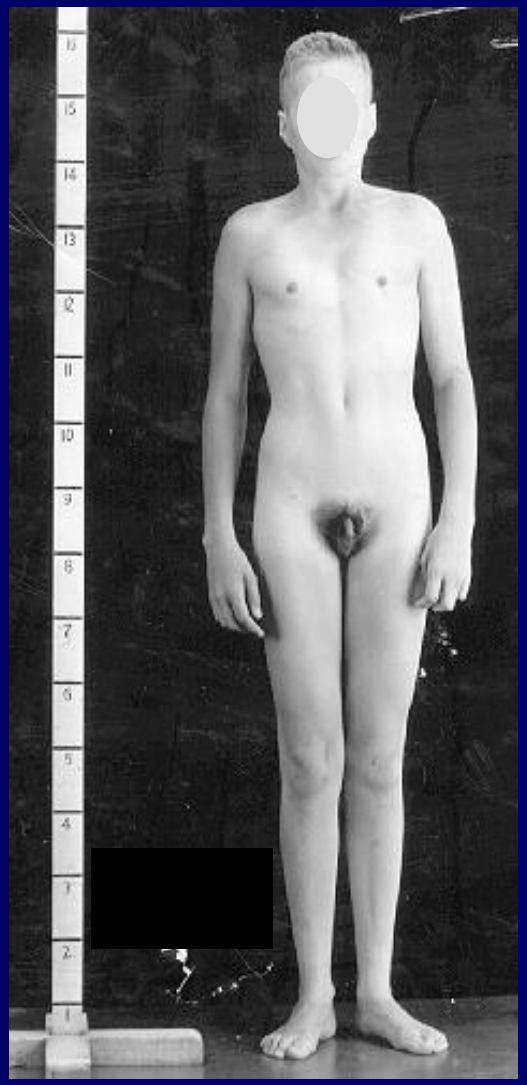
- ✓ **One of 'Big 3" mandates of hypothalamus**
  - **Nourishment => Growth (GHRH-IGF axis)**
  - **Deal with Stress => Adrenal (CRF-Cortisol axis)**
  - **Go forth & multiply => Reproduce (GnRH-Gonadal axis)**

- ✓ **Master regulator of all 'downstream' reproduction**
  - ***"Pilot Light of Reproduction"***

- ✓ **Critical role in evolution & survival of species**
  - **adapts Reproductive "Fitness" in response to environmental changes**
  - **e.g. malnutrition, exercise, circadian changes, olfaction, predators, fertility**

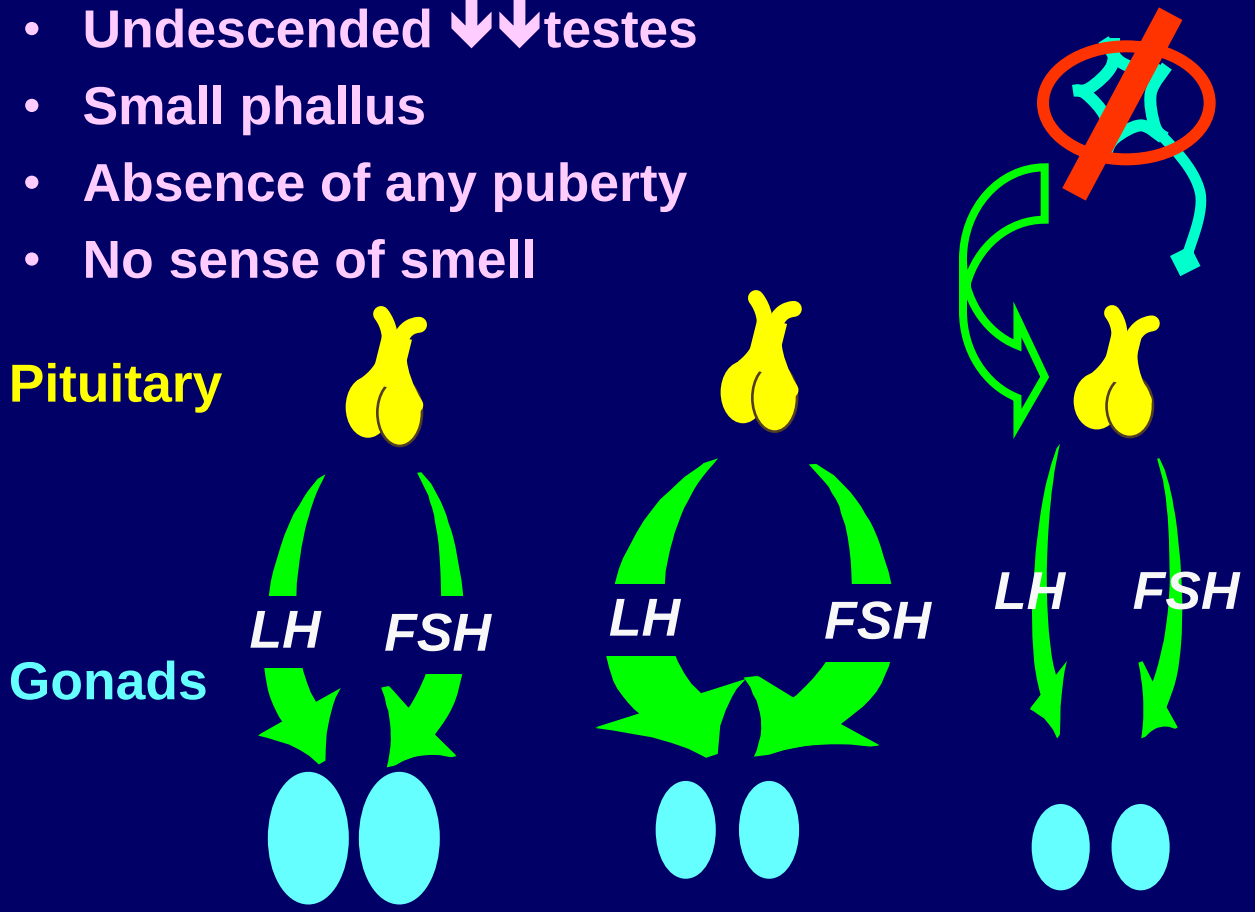
- ✓ **Human Genome Bioinformatics & New Genetic Tools:**
  - **Remarkable lack of redundancy (unlike GPCRS, Tx. factors, 2nd mess.)**
  - **? Implies its genetic control is above the hypothalamus & complex**
  - **Known genetic heterogeneity (X-linked; AR; AD)**

Hypogonadotropic Hypogonadism = UBO

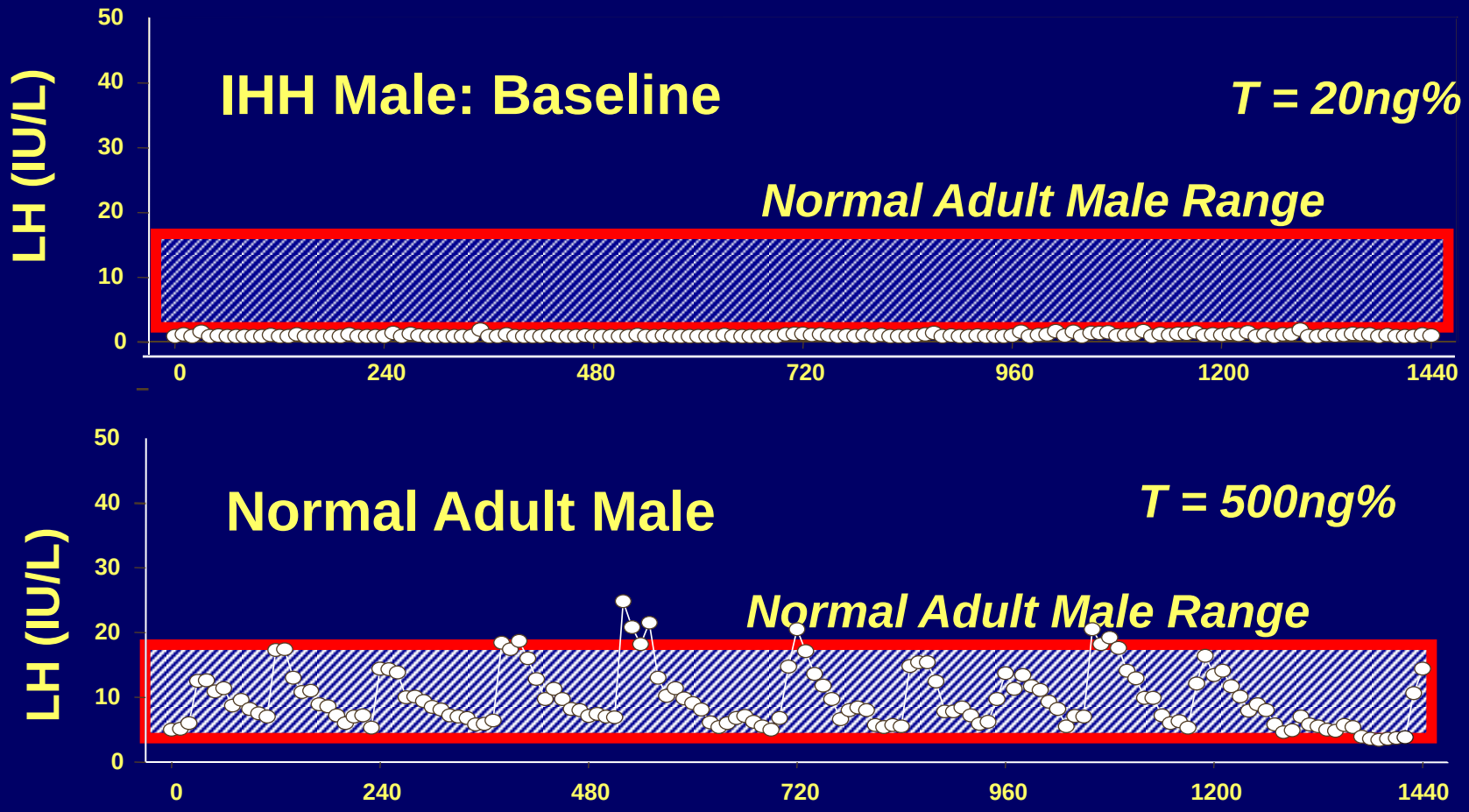


Key Findings in 1943

- Undescended ⬇⬇testes
- Small phallus
- Absence of any puberty
- No sense of smell



LH & FSH	Normal	Hyper ⬆⬆	Hypo ⬇⬇
Gonadal Fx	Normal	1º Failure	2º Failure



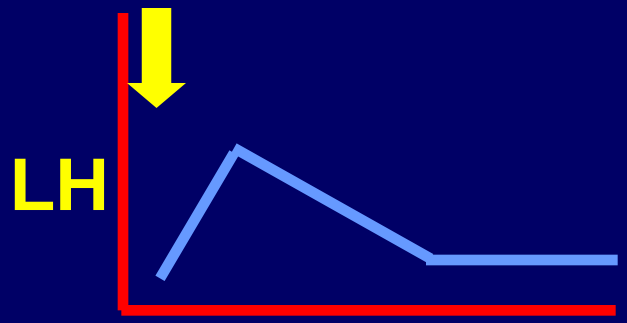
Hoffman & Crowley, NEJM 1980

GnRH Analogues = Agonists

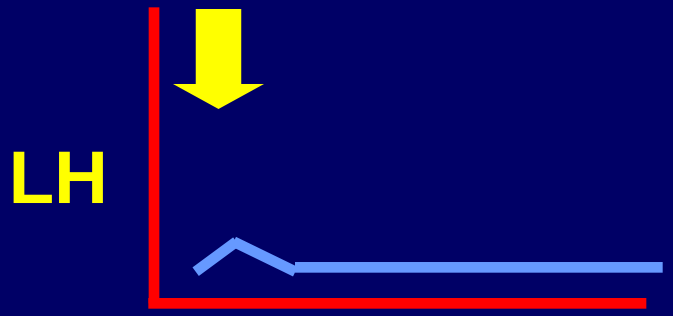
Amino Acid Sequence	In Vitro Potency	Name
1 2 3 4 5 6 7 8 9 10		
pGlu His Trp Ser Tyr Gly Leu Arg Pro Gly-NH2	**1**	**GnRH**
----------N -EtNH2	4	Fujino
---------- D-Ala----------	4	
----------D-Ala----------N –EtNH2	14	
----------D-Trp----------		Decapeptyl
----------D-Nal 2----------N -EtNH2		Nafarelin
----------D-Leu----------N -EtNH2	15	Leupron
----------D-Ser(tBu)----------N -EtNH2	20-40	Buserelin
----------D-Trp----------N -EtNH2	144	Deslorelin
----------D-His(imBzl)----------N -EtNH2	210	Histrelin

Attempted Replacement Rx in GnRH Deficiency with GnRHa:
Homologous Desensitization of GnRHR

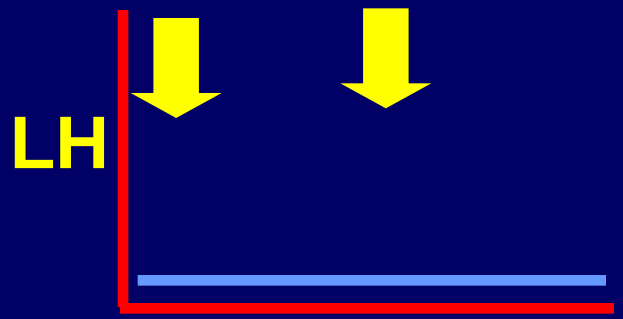
GnRHa 5 μg QD



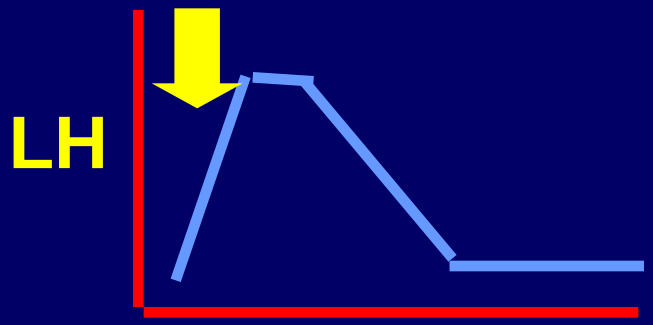
GnRHa 10 μg QD



GnRHa 10 μg BID



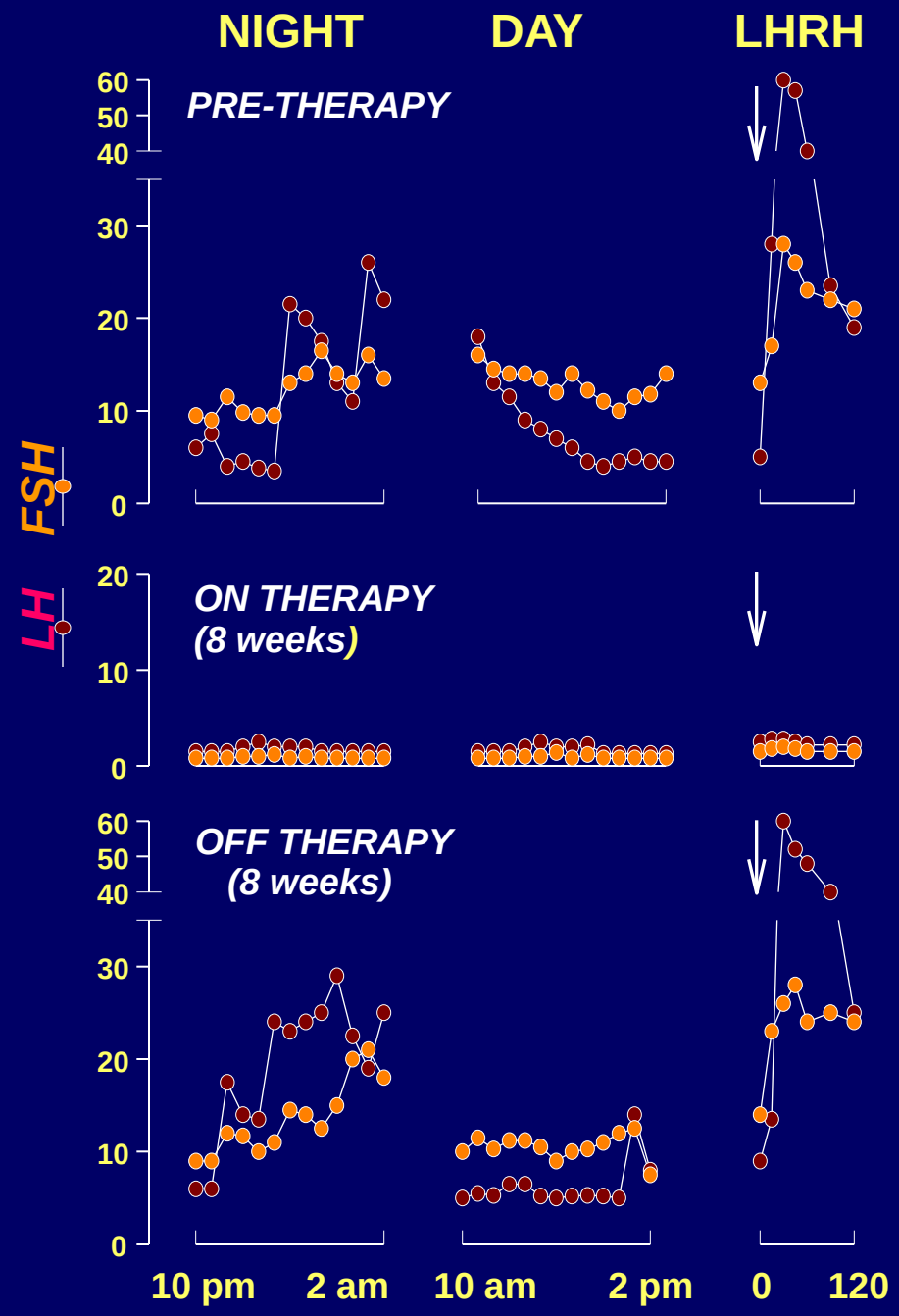
GnRHa 10 μg QOD



Crowley W, Vale W, Rivier J. NEJM, 1979

Potential Rx Applications of GnRHa-Induced Homologous Desensitization of GnRHR

Application	Alternative Rx.
• Prostate Cancer	Surgical Castration
• Endometriosis	Synthetic Progestins
• IVF Timing	Oral Contraceptives
• Precocious Puberty	None

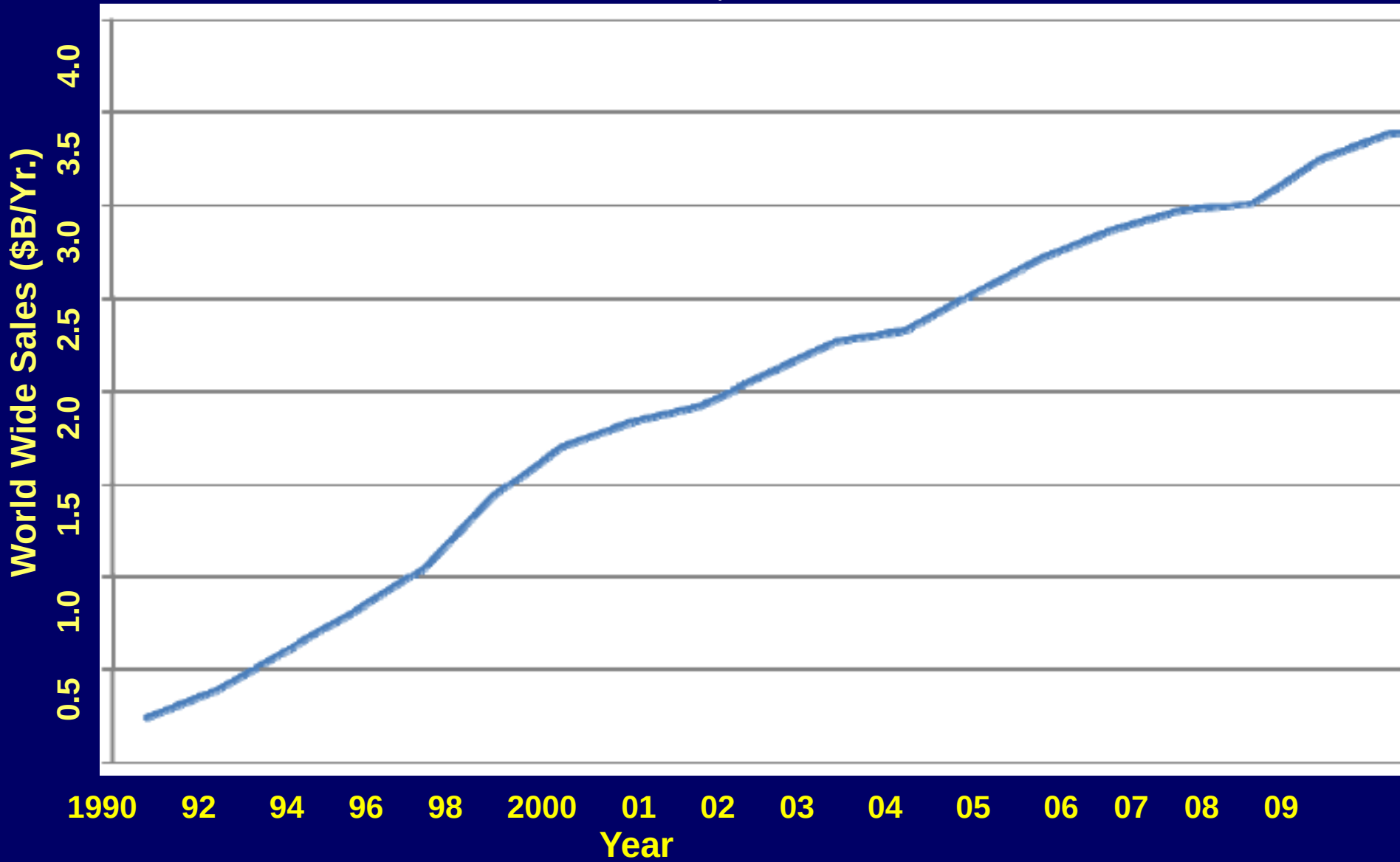


$GnRH_a$ => Reversible Biochemical Castration of Pituitary-Gonadal Axis in Children with Precocious Puberty

JCEM; NEJM, 1981

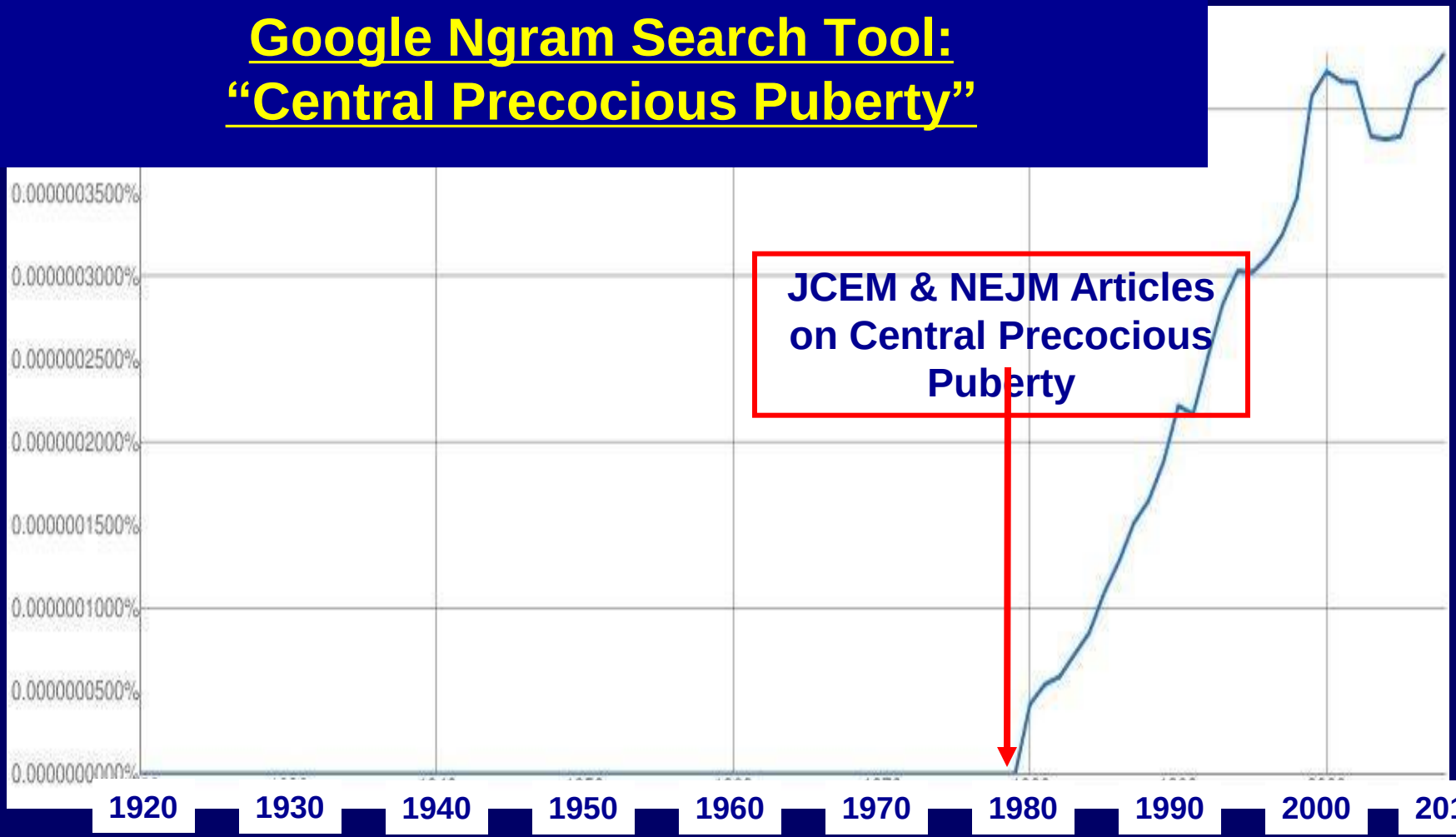
GnRHa Sales in $B/yr: 1990-2009

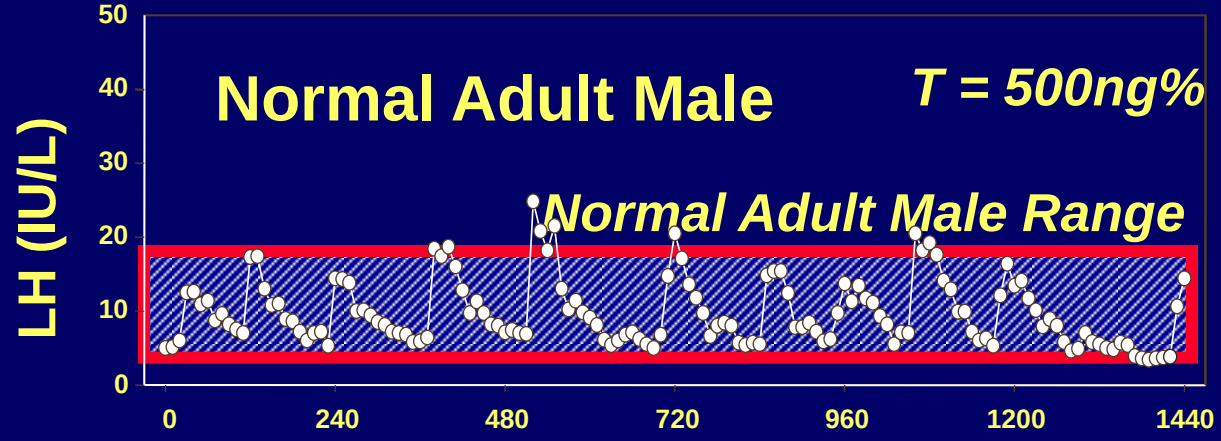
Source: www.evaluatepharma.com

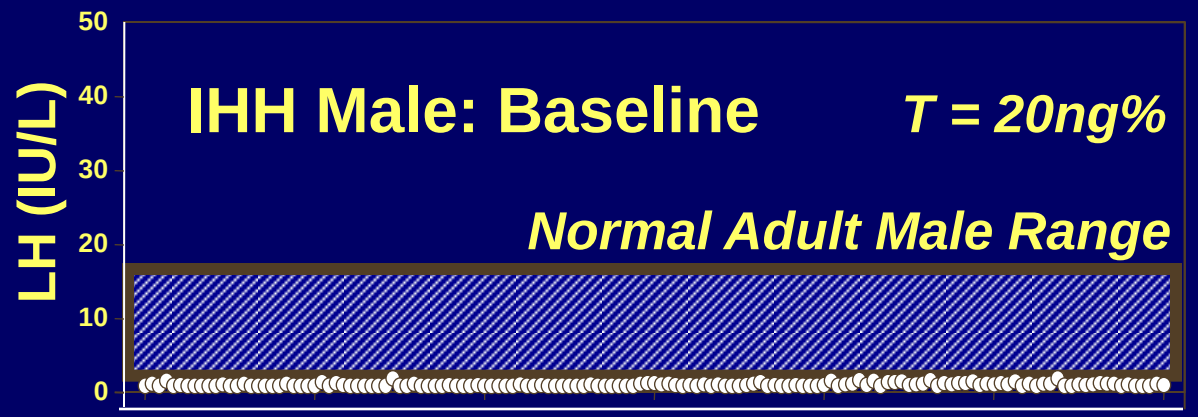


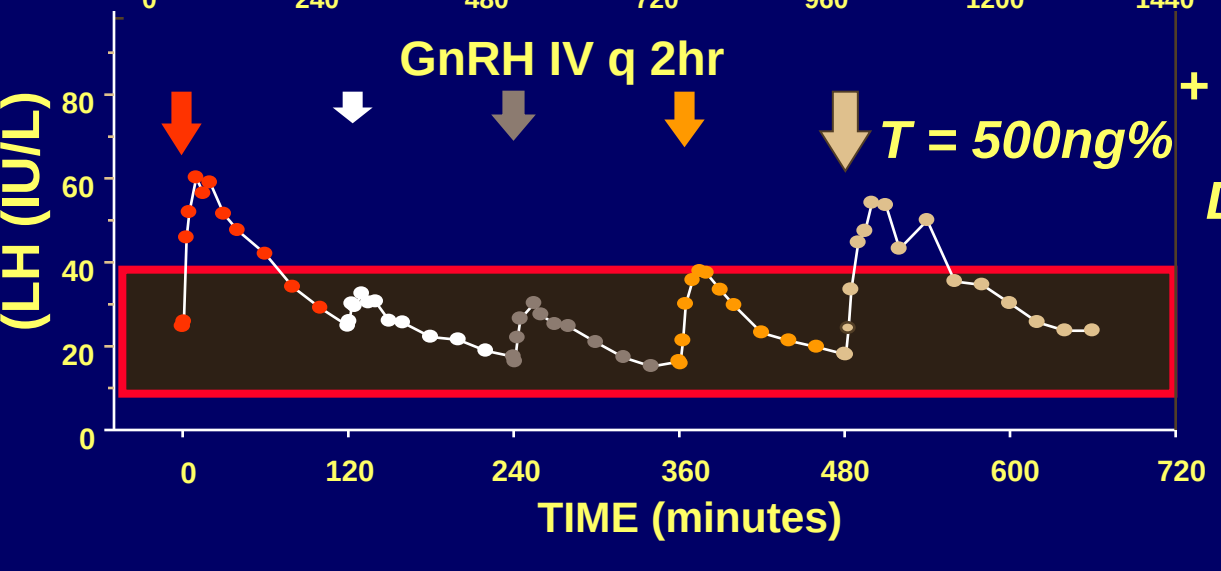
Literature Impact of NICHD Initiated Program

Google Ngram Search Tool: "Central Precocious Puberty"



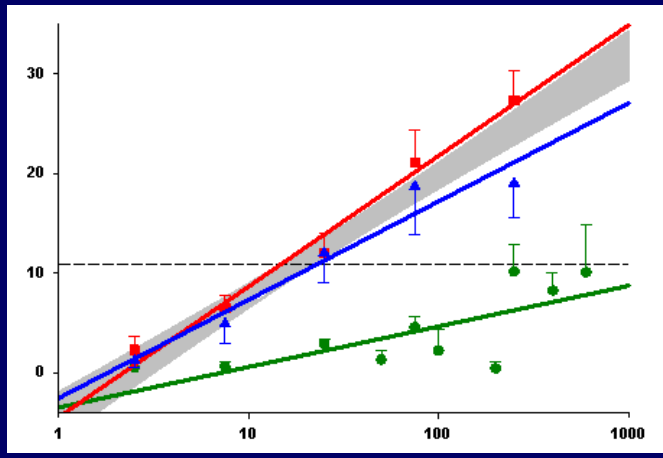






Pulsatile GnRH Rx: Re-Constitutes Normal HPG Axis in IHH

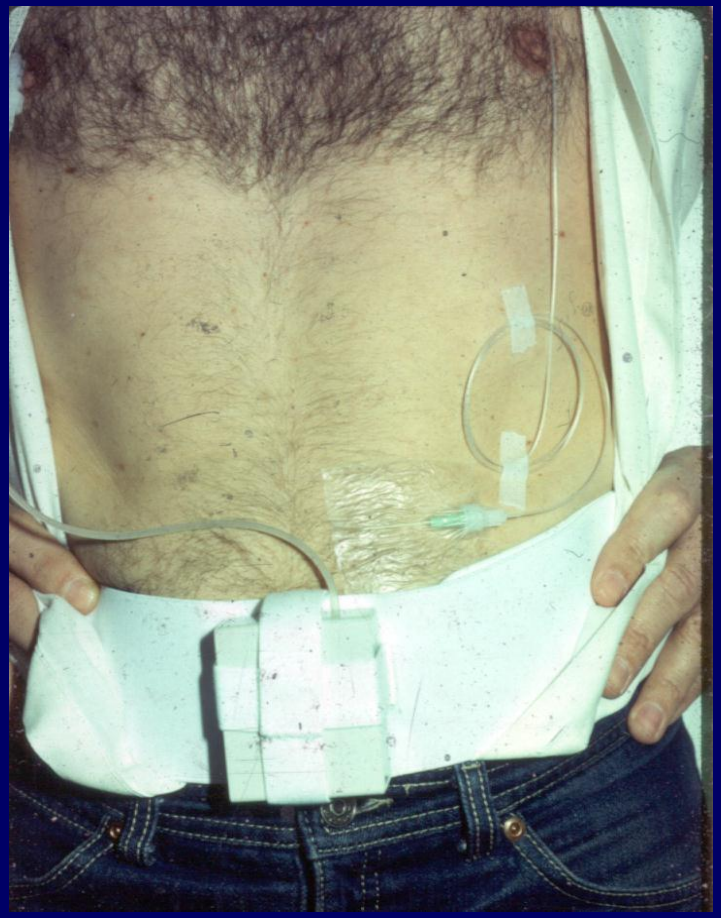
(Crowley et al, JCEM, 1980, Hoffman et, NEJM, 1980)



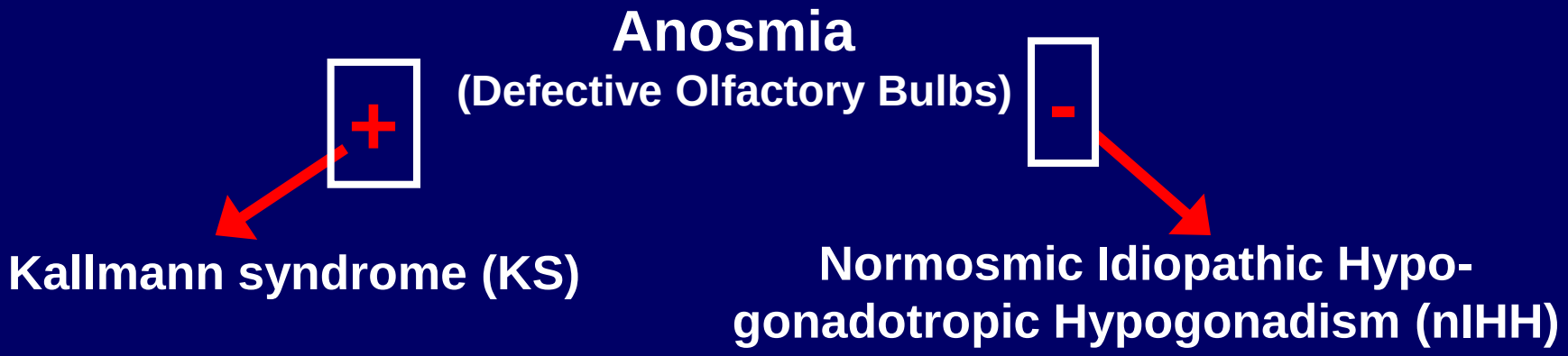
+ Generates *Experimental Opportunities: Dose-Response Curves*

(Spratt et al, JCEM)

Pulsatile GnRH Administration via Mini-Pump (Dean Kamen)



Hypogonadism with ➔🡻 Gonadotropins



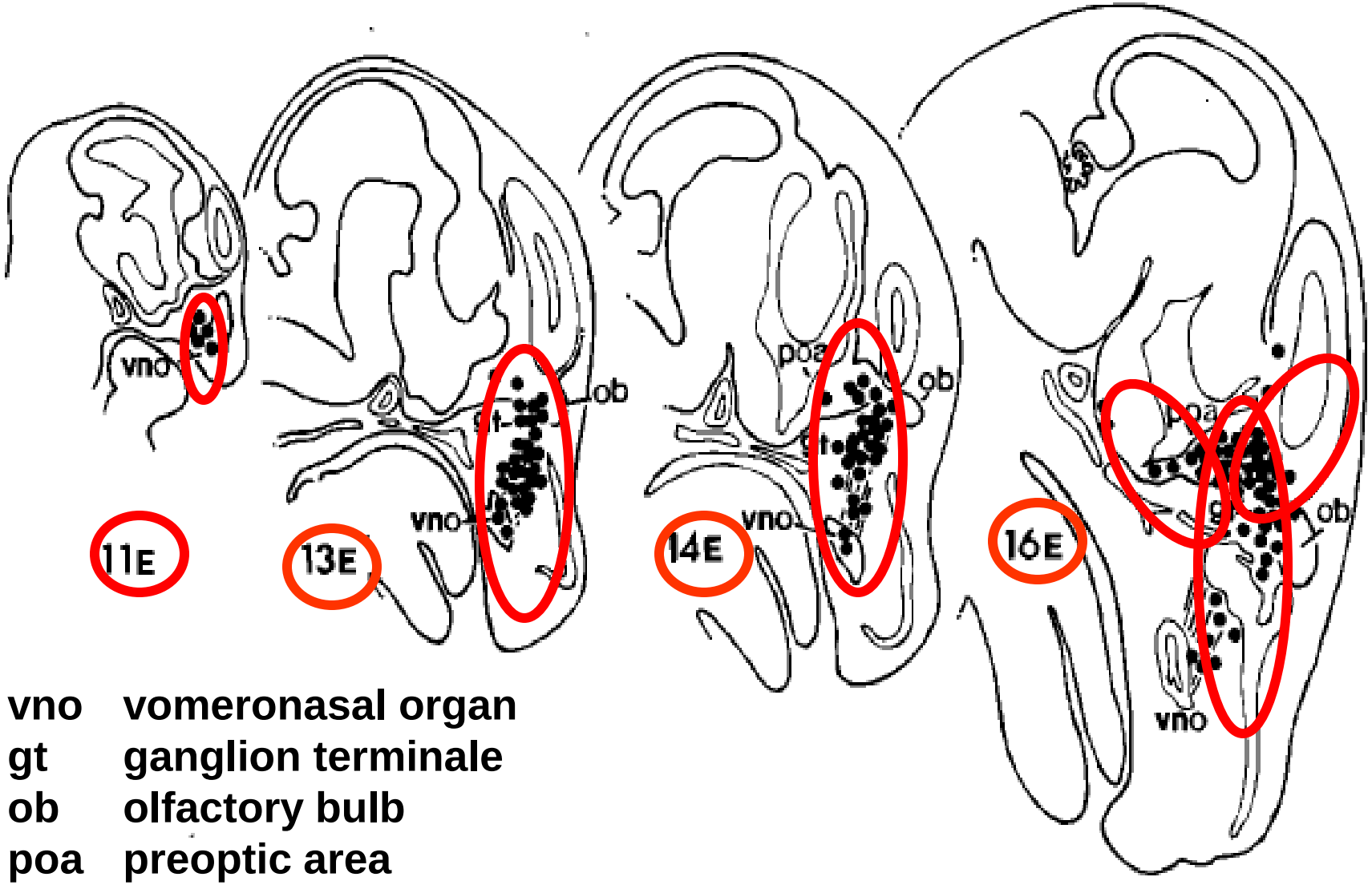
Isolated GnRH Deficiency

Developmental theme

Neuroendocrinetheme

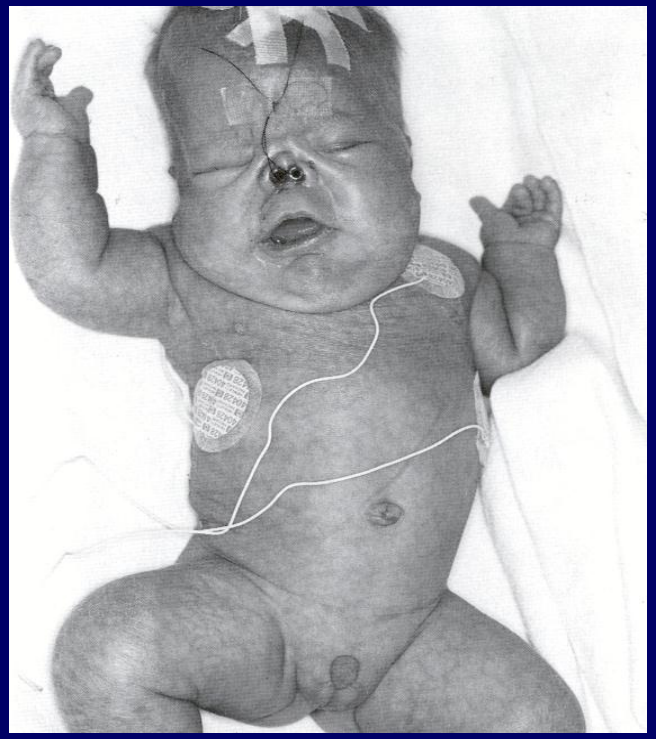
Critical Observation #1 (Mouse Development):
GnRH Cells of Origins are Extramural to CNS

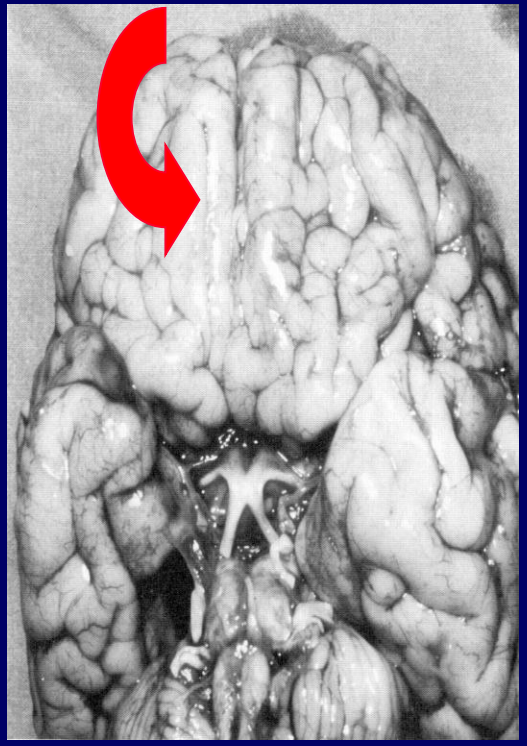
Schwanzel-Fukuda & Pfaff, *Nature*

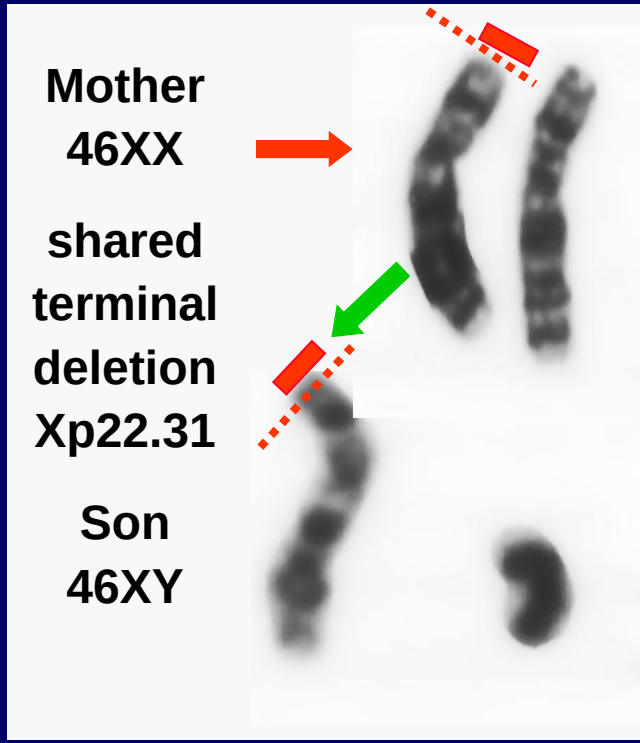


vno **vomeronasal organ**
gt **ganglion terminale**
ob **olfactory bulb**
poa **preoptic area**

Critical Observation #2: **Contiguous Gene Syndrome**
(Male) Del p22.31: KS, Ichthyosis, Ca^{++} **Disorder**





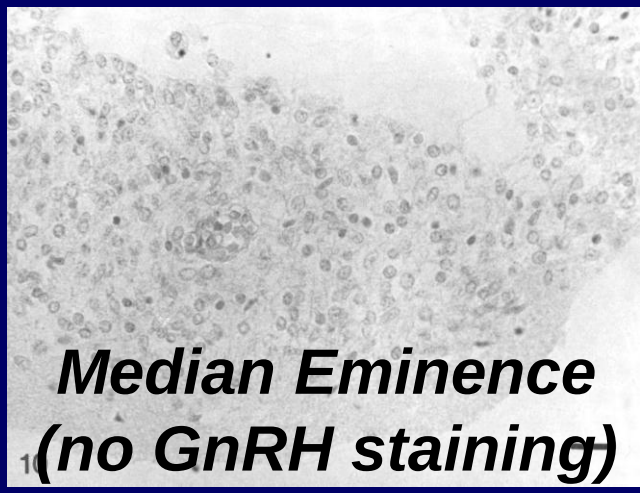


- ***↓LH, FSH, T during neonatal 'minipuberty' window***
- ***microphallus + cryptorchidism***
- ***absent olfactory bulbs & tracts***

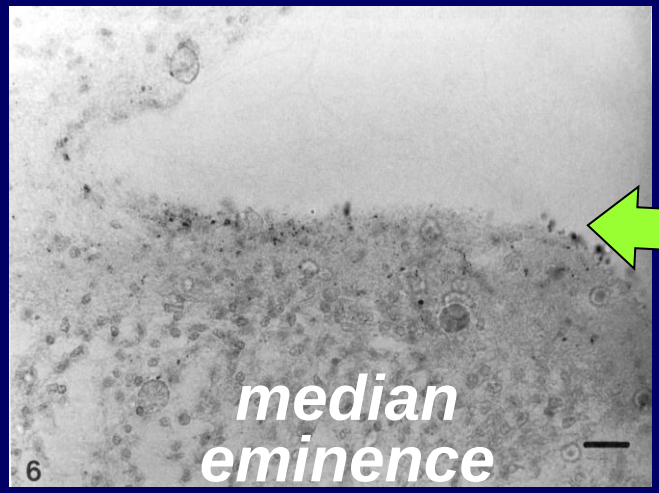
Bick, AJ Med Gen 1989

GnRH Immunostaining of Human Fetal Brain at 19 Weeks Gestation

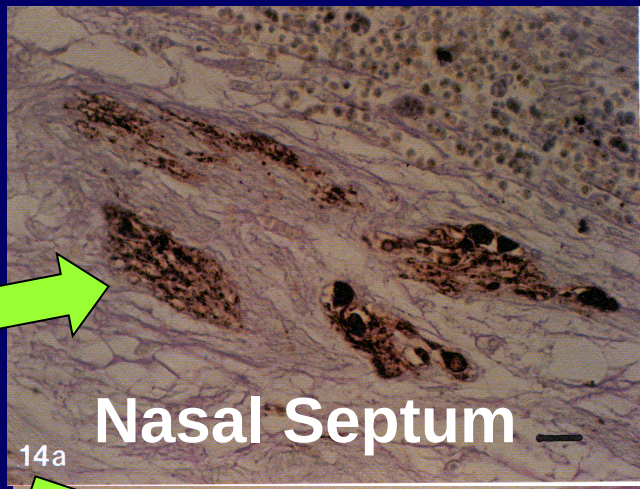
KS Fetus:
term del of Xp22.31

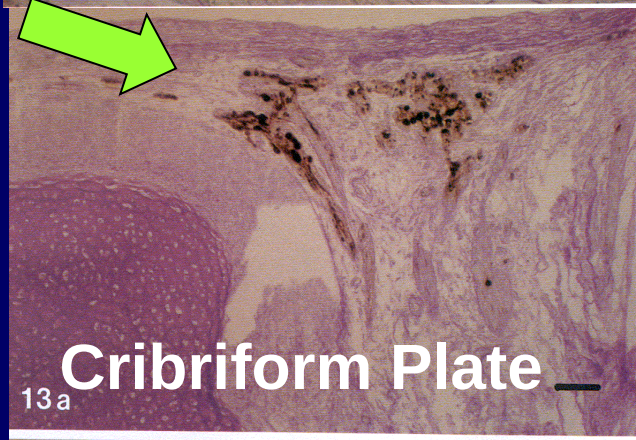


Normal Fetus



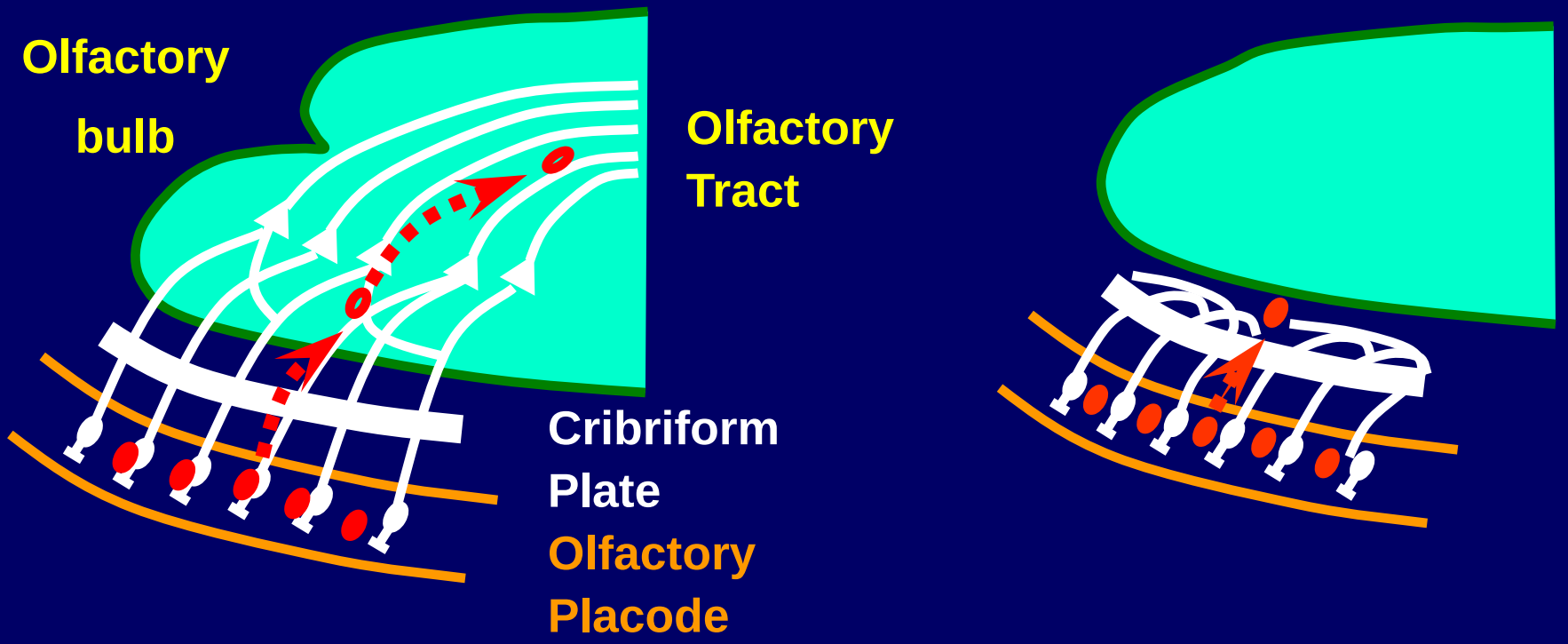
GnRH Neurons

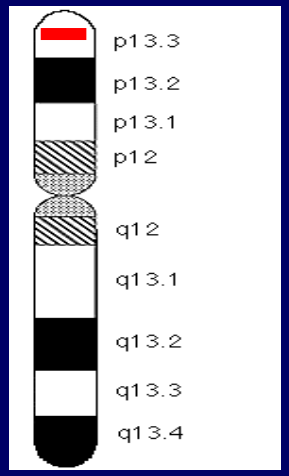


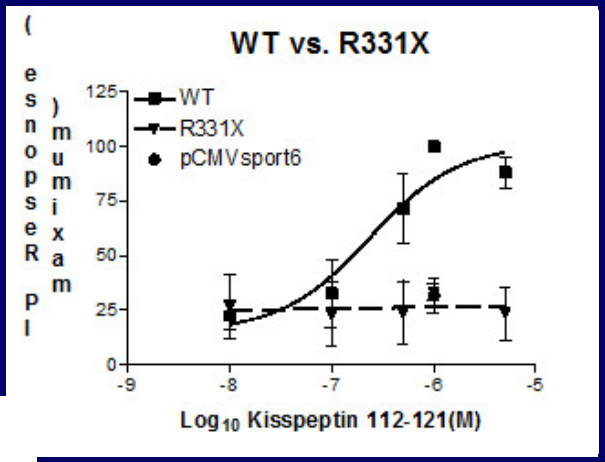


Schwanzel-Fukada et al.
Molec Brain Res 1989

KAL1= Gene for GnRH Neural Migration: Establishes Neurodevelopmental Theme





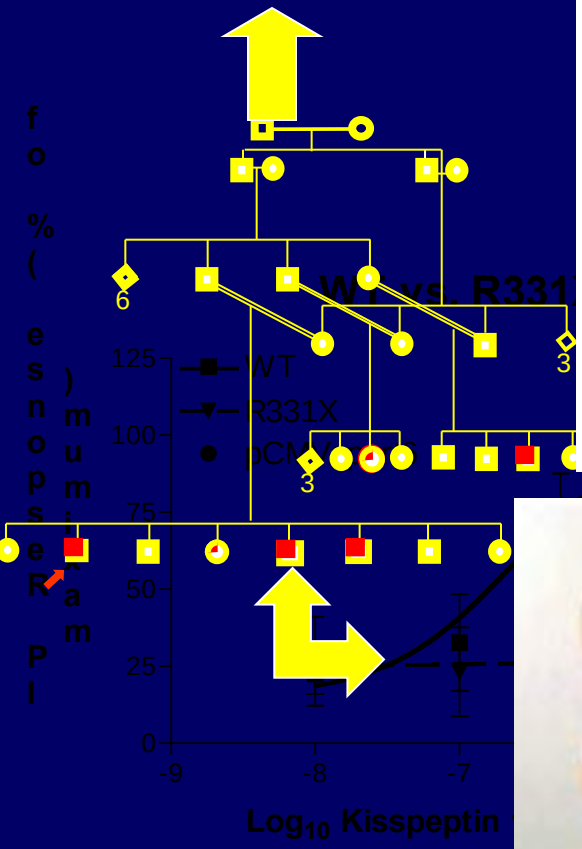


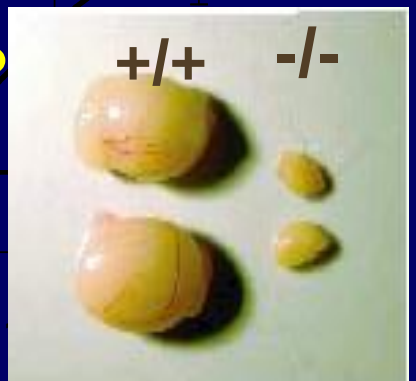
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

The GPR54 Gene as a Regulator of Puberty

Stephanie B. Seminara, M.D., Sophie Messager, Ph.D., Emmanouella E. Chatzidaki, B.Sc., Rosemary R. Thresher, Ph.D., James S. Acierno, Jr., B.S., Jenna K. Shagoury, B.S., Yousef Bo-Abbas, M.D., Wendy Kuohung, M.D., Kristine M. Schwinof, M.A., Alan G. Hendrick, Ph.D., Dirk Zahn, Ph.D., John Dixon, B.A., Ursula B. Kaiser, M.D., Susan A. Slaugenhaupt, Ph.D., James F. Gusella, Ph.D., Stephen O'Rahilly, M.D., Mark B.L. Carlton, Ph.D., William F. Crowley, Jr., M.D., Samuel A.J.R. Aparicio, B.M., B.Ch., Ph.D., and William H. Colledge, Ph.D.

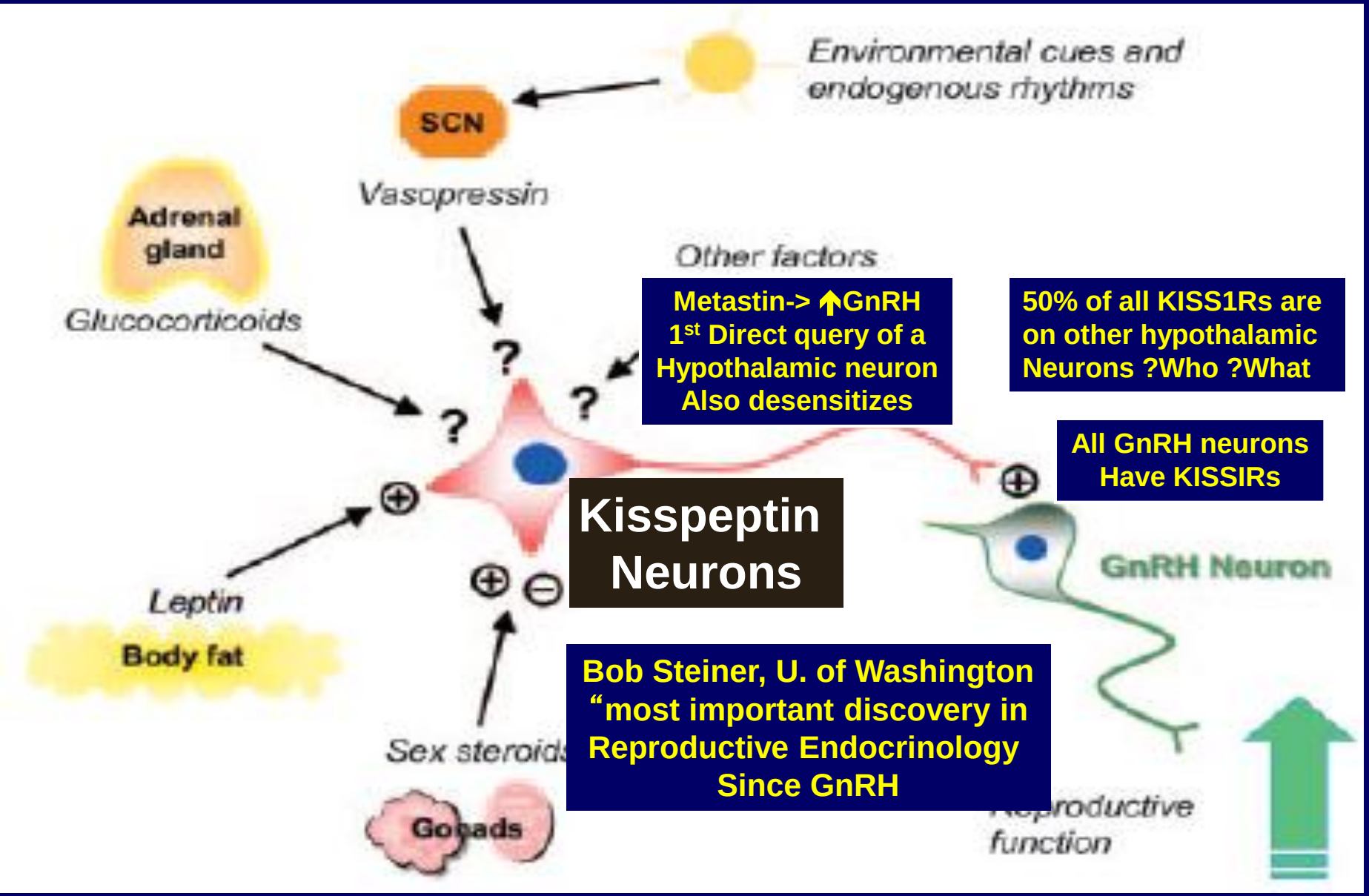




Potter

Seminara NEJM, 2003

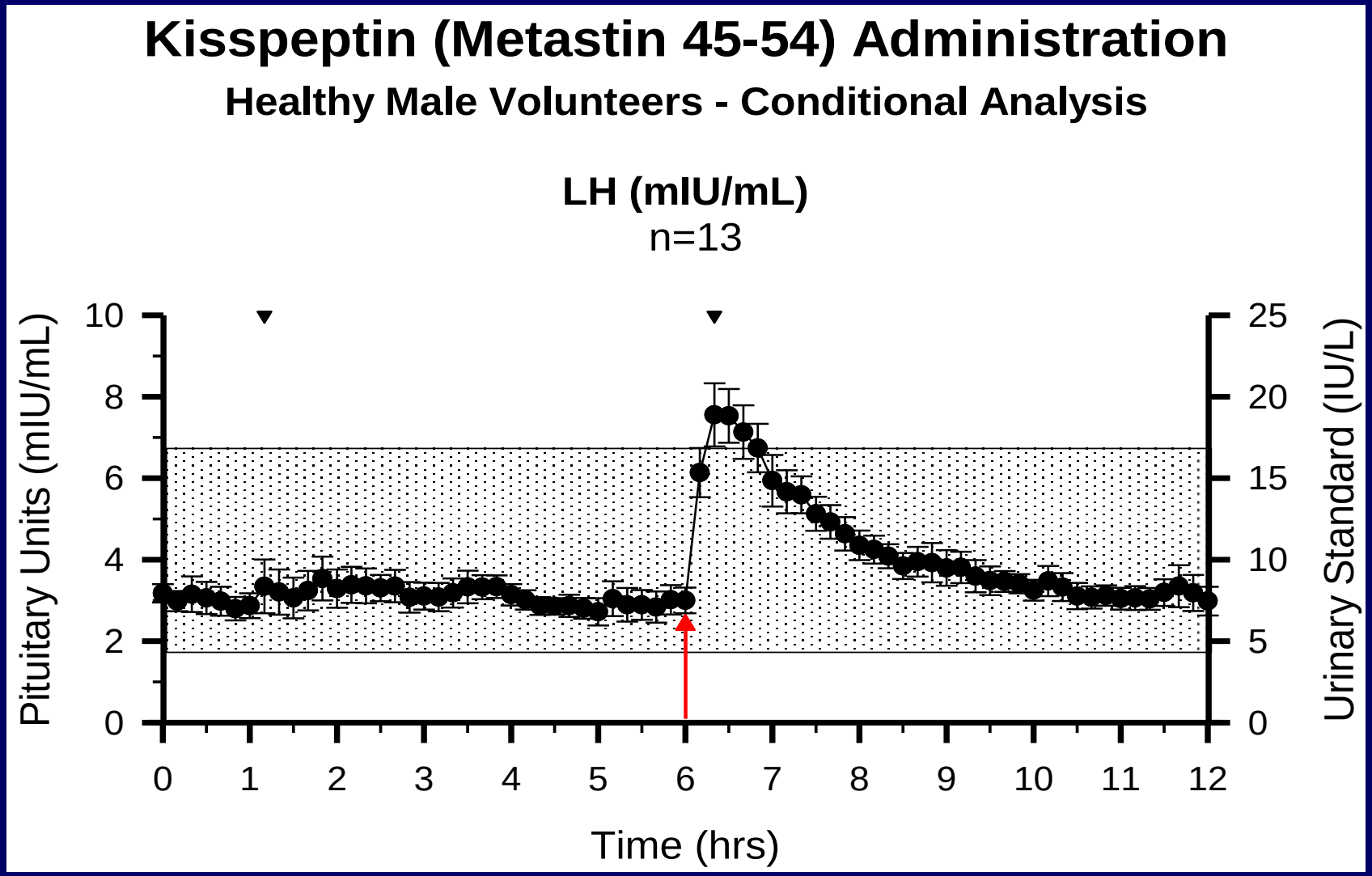
Kisspeptin Signaling System: Neuroendocrine Theme



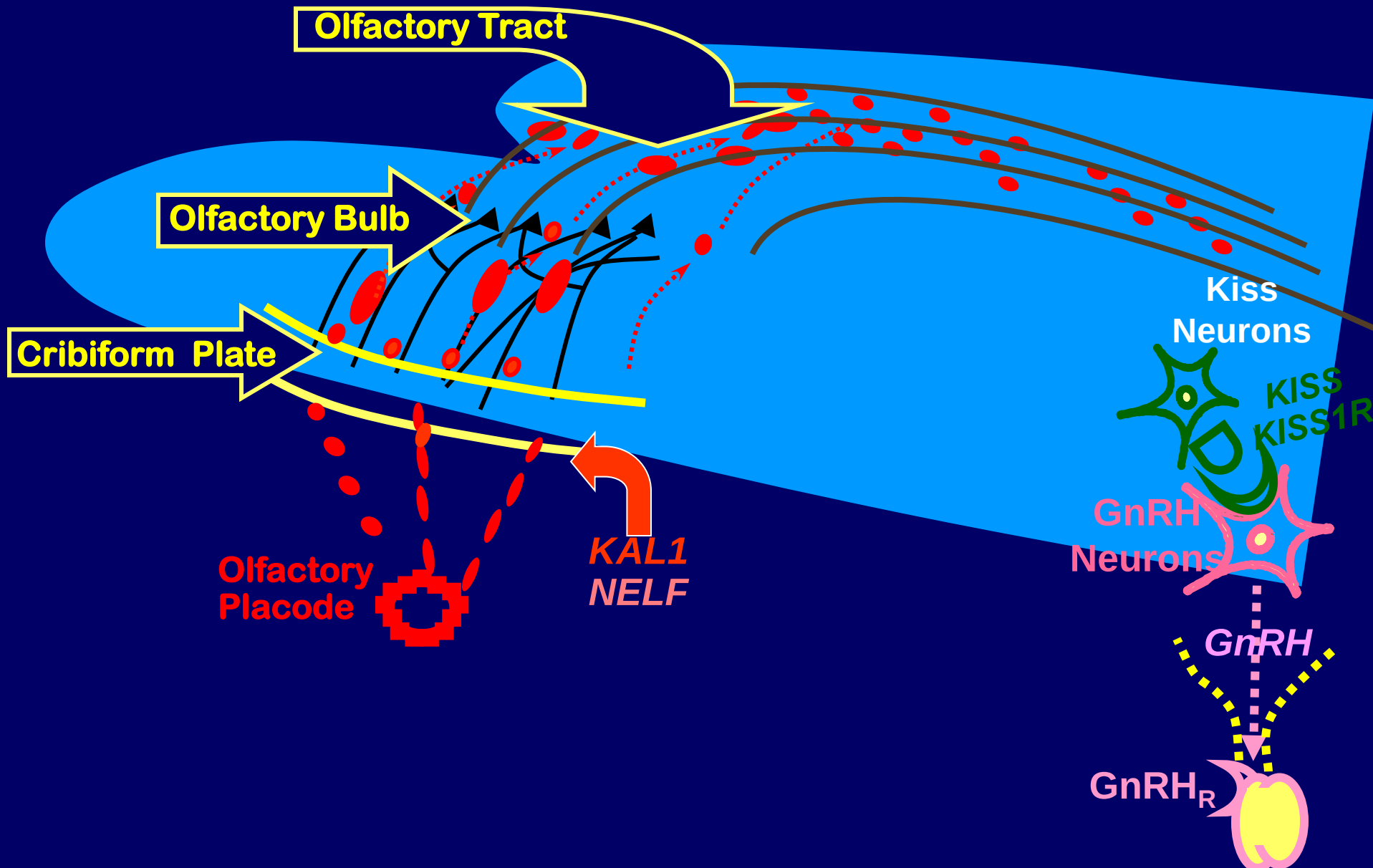
Dungan et al, Endocrinology 147(3): 1154, 2006

Kisspeptin Administration to Normal Volunteers

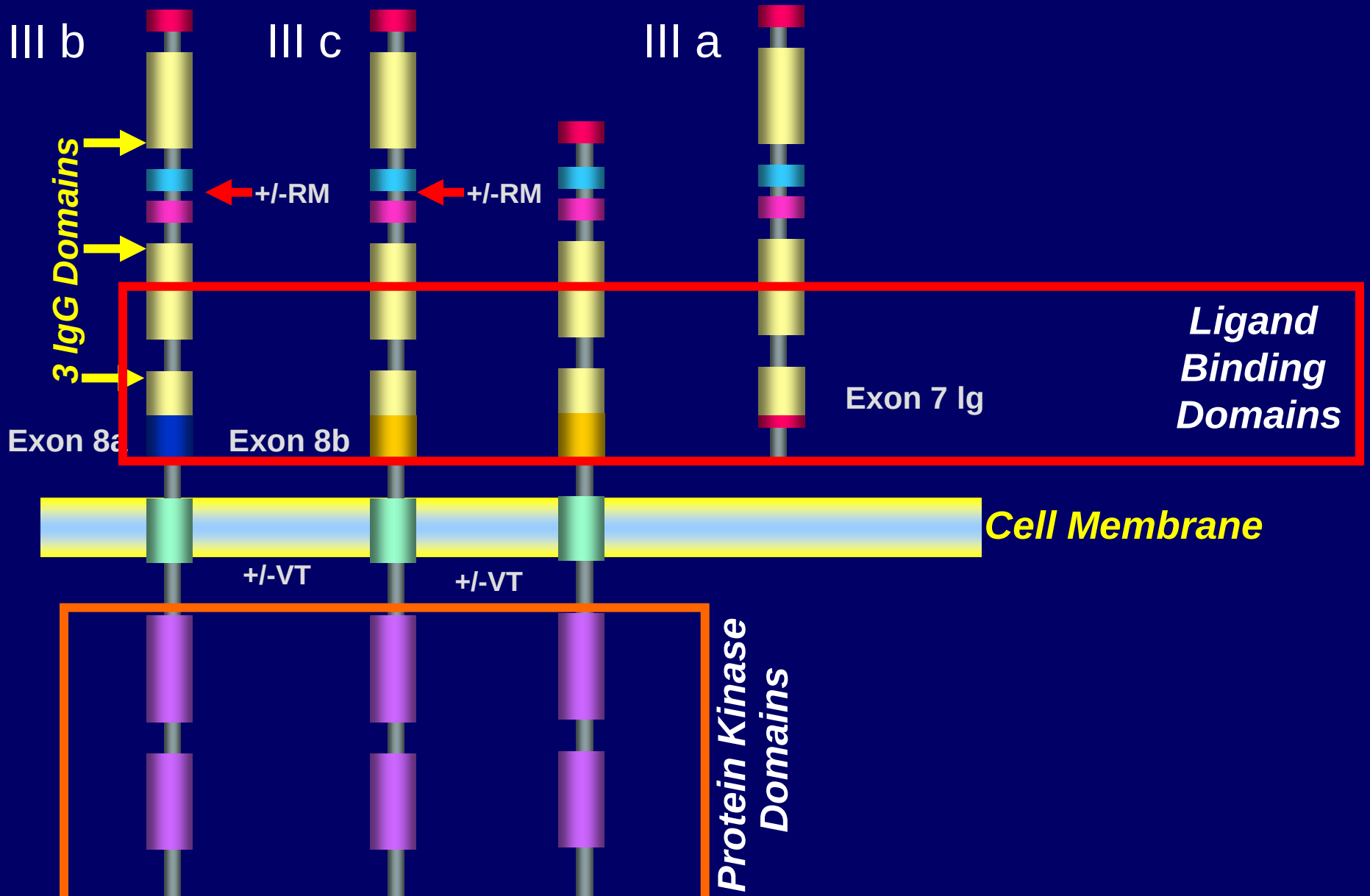
(Chan & Seminara)



GnRH Neuronal Ontogeny: A Story Told by Patients with GnRH Deficiency



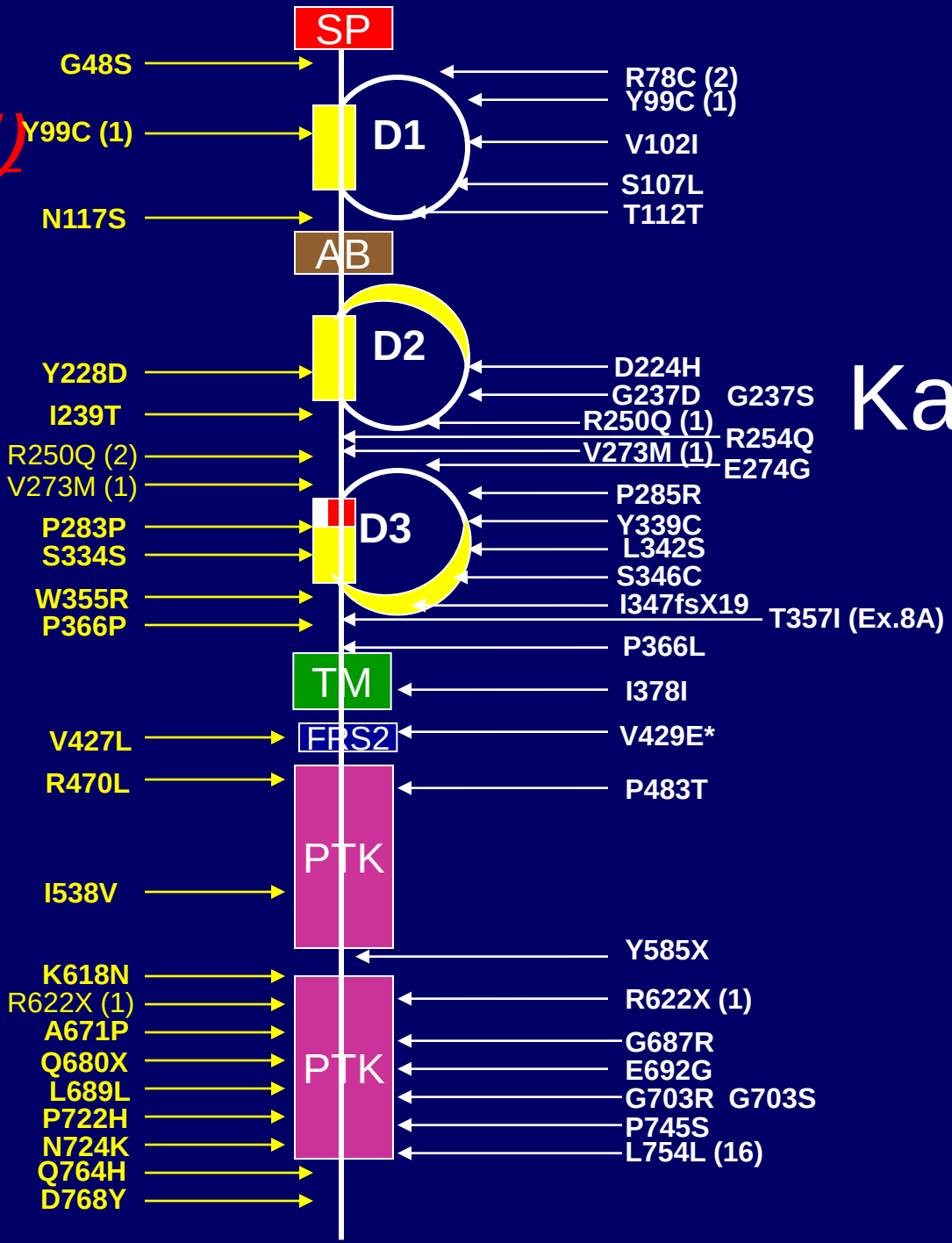
FGFR System Problem: 4 Receptors + 23 Ligands ???

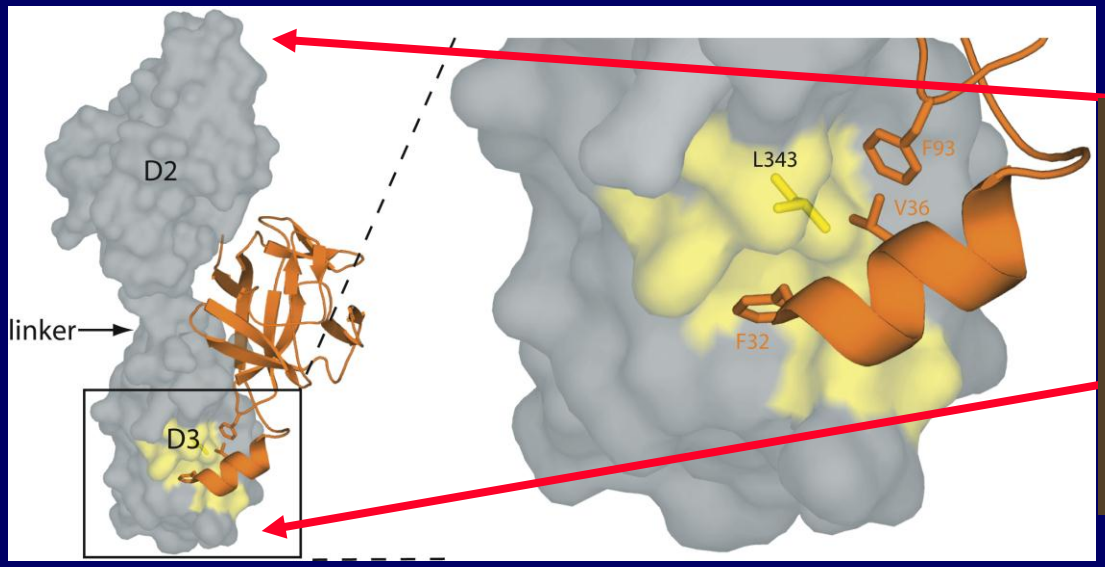


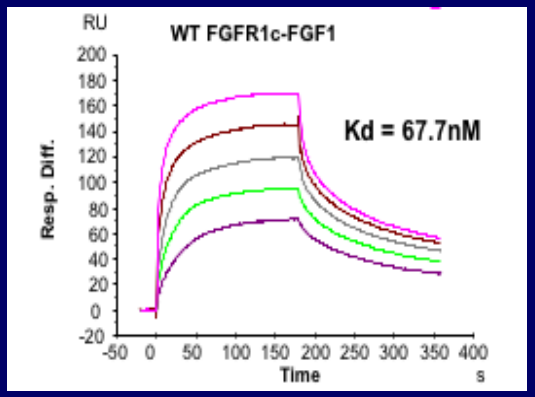
Mutations in *KAL2 (FGFR1)*

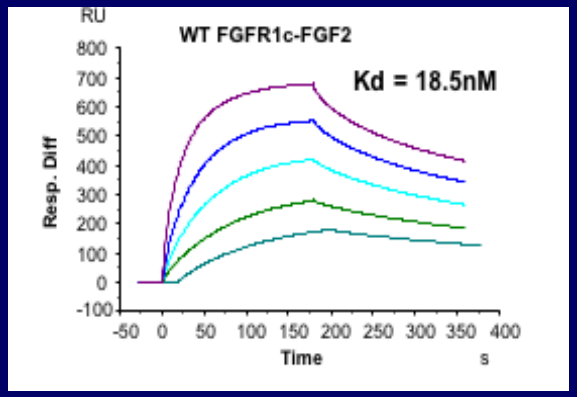
nIHH

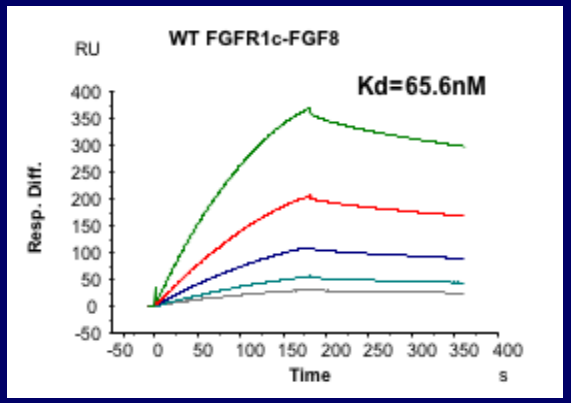
Kallmann

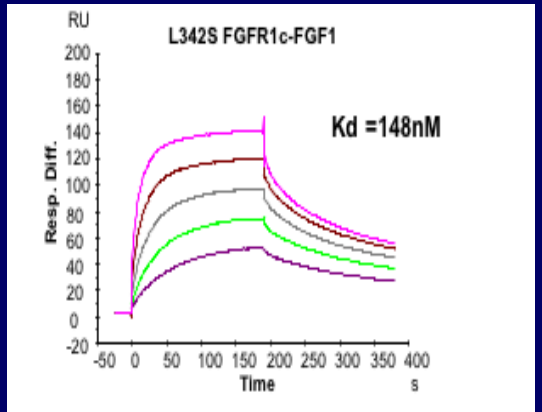


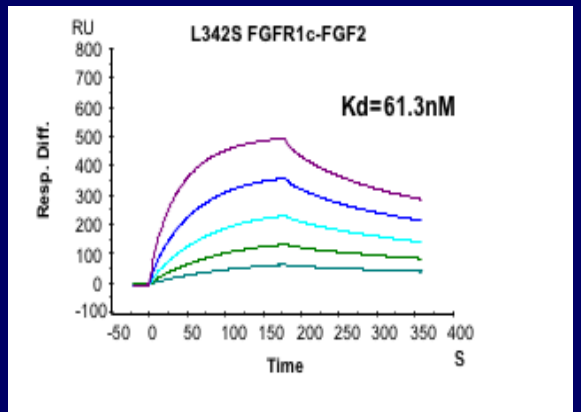

D2
linker
D3
L343
F93
V36
F32

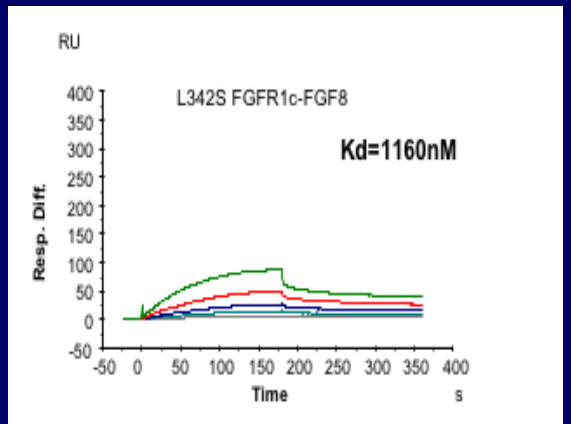

WT FGFR1c-FGF1
RU
Resp. Diff.
Kd = 67.7nM
Time
s


WT FGFR1c-FGF2
RU
Resp. Diff
Kd = 18.5nM
Time
s

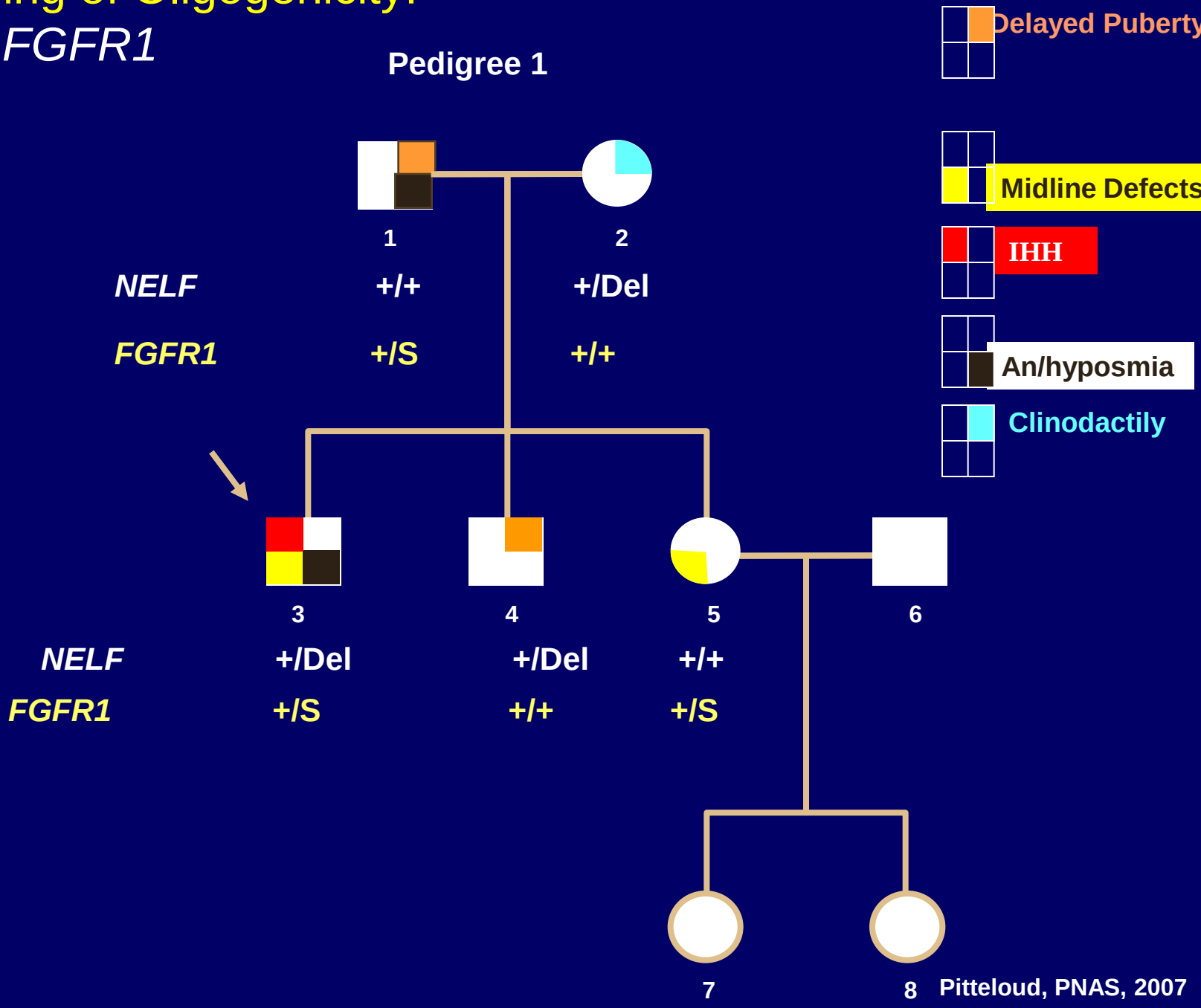

WT FGFR1c-FGF8
RU
Resp. Diff.
Kd=65.6nM
Time
s


L342S FGFR1c-FGF1
RU
Resp. Diff.
Kd =148nM
Time
s


L342S FGFR1c-FGF2
RU
Resp. Diff.
Kd=61.3nM
Time
S

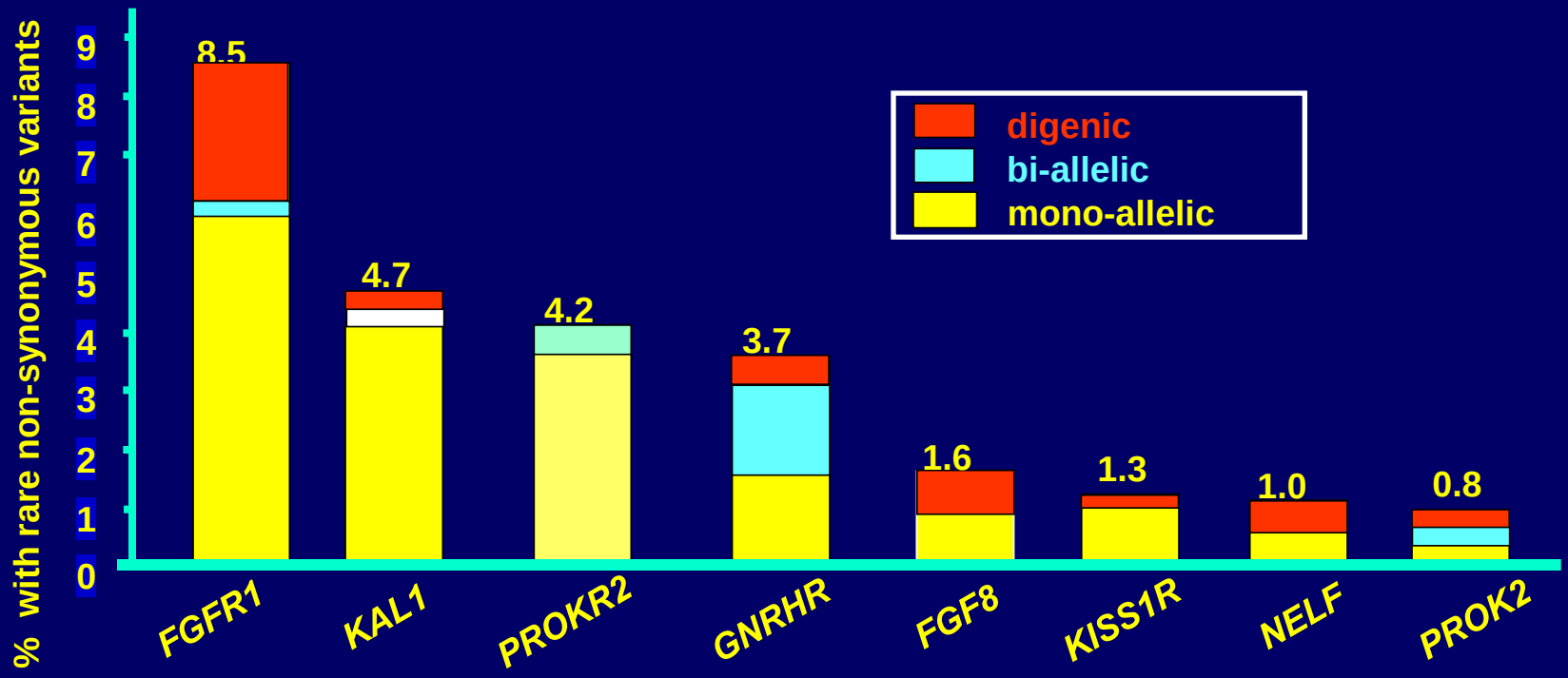

L342S FGFR1c-FGF8
RU
Resp. Diff.
Kd=1160nM
Time
s

First Inkling of Oligogenicity:
NELF & FGFR1



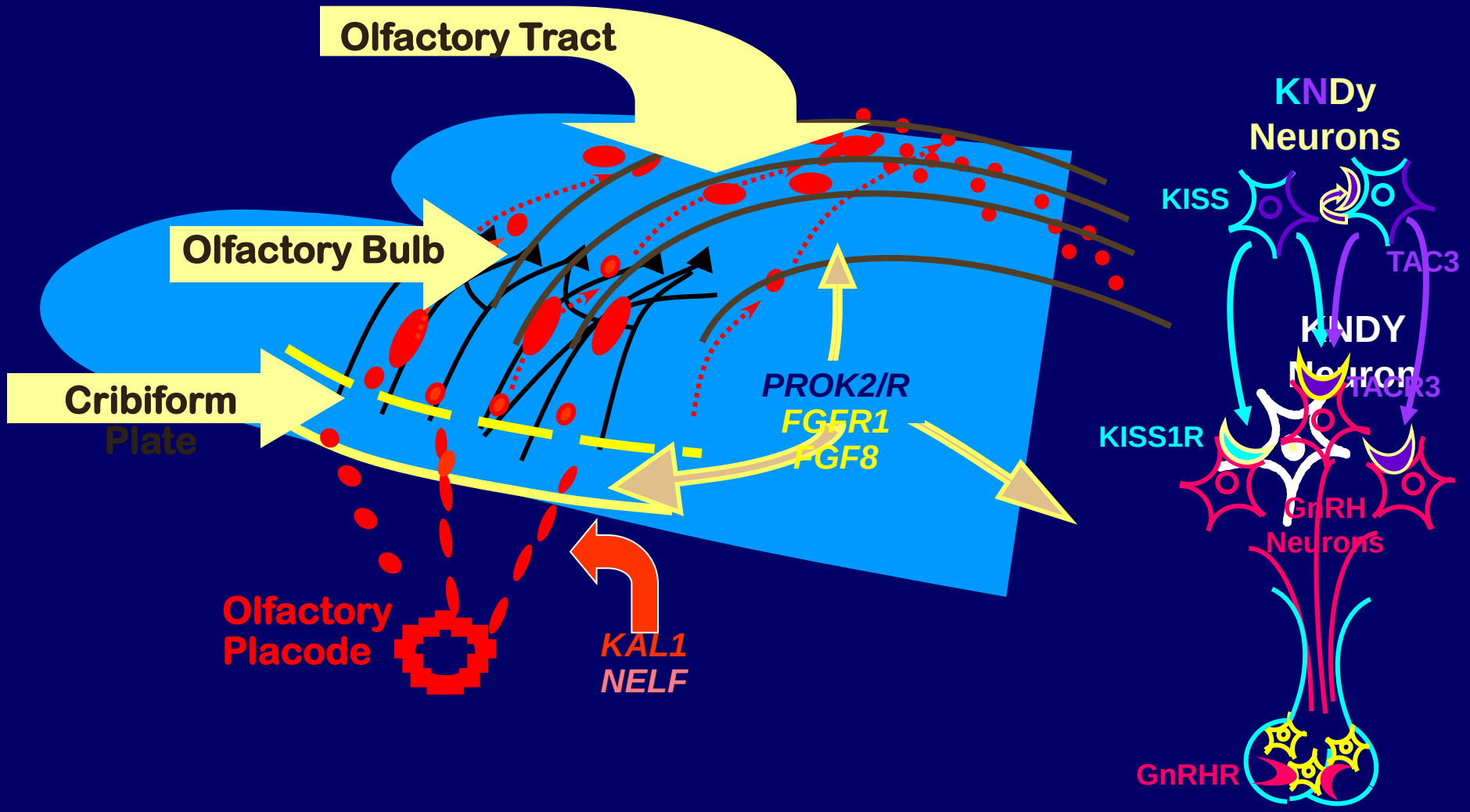
Pitteloud, PNAS, 2007

Oligogenicity in 376 GnRH Deficienct Probands (11%)



# of alleles with non-synonymous rare variants	GnRH Def. patients (n=397)	controls (n=179)
0	78%	90%
1	17%	10%
2: same gene	2.5%	0%
≥2: different genes	2.5%	0%

GnRH Neuronal Ontogeny: A Story Told By a Human Disease Model: GnRH Deficiency

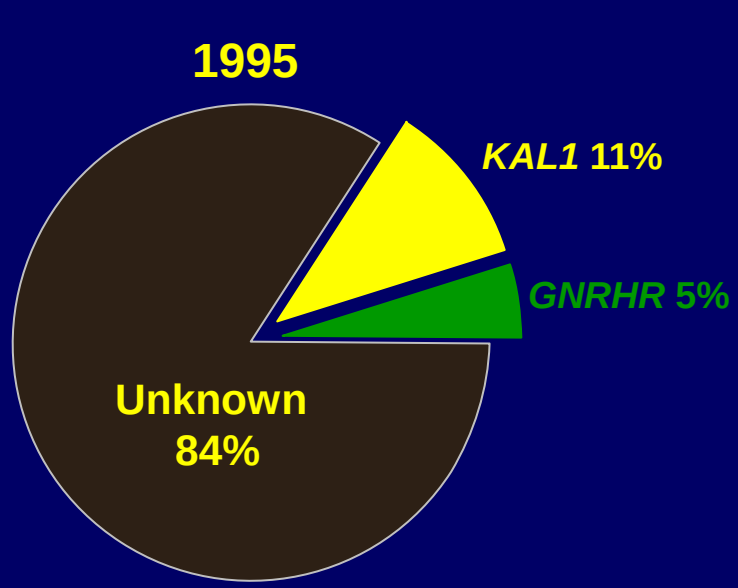


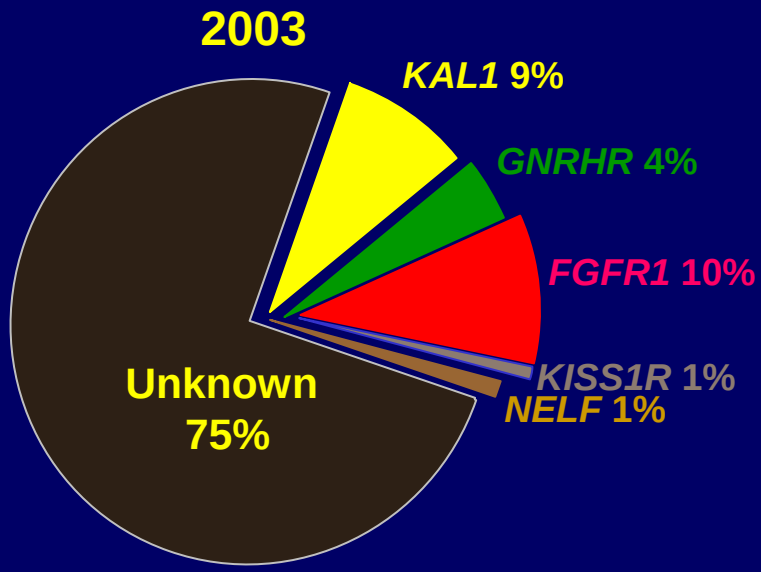
Neuro-Developmental Genes

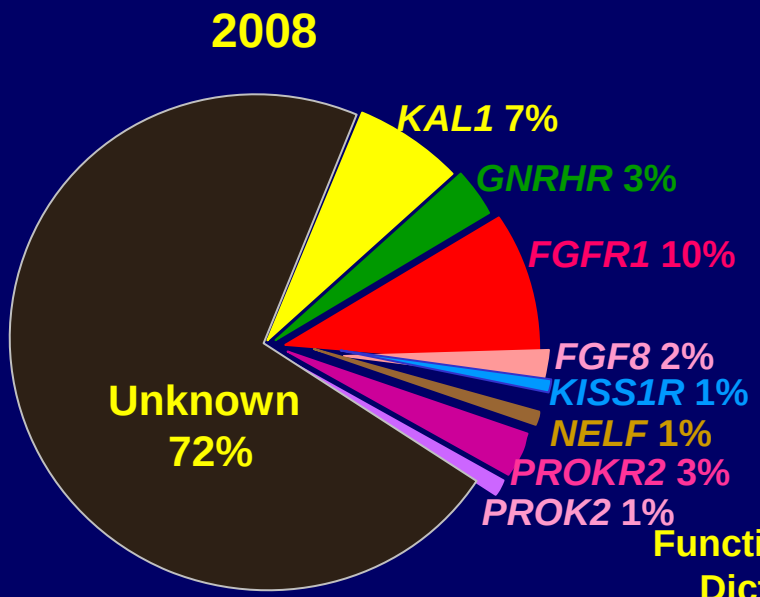
Overlap???

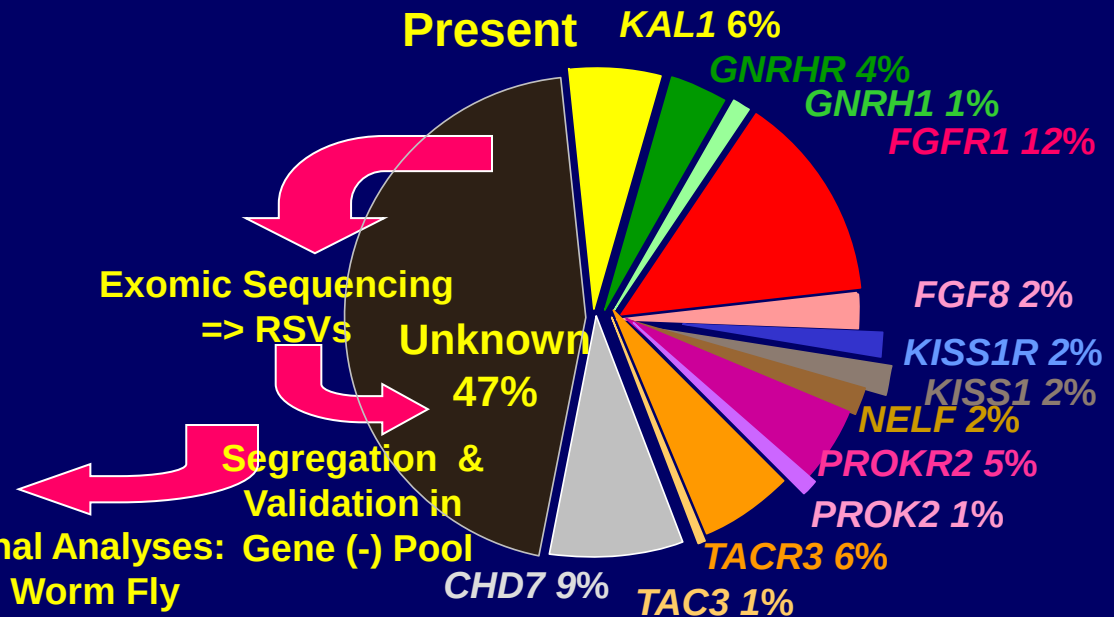
Neuro-Endocrine Regulatory Genes

Complexity of Genetic Architecture of GnRH Neuronal Network over 15 years

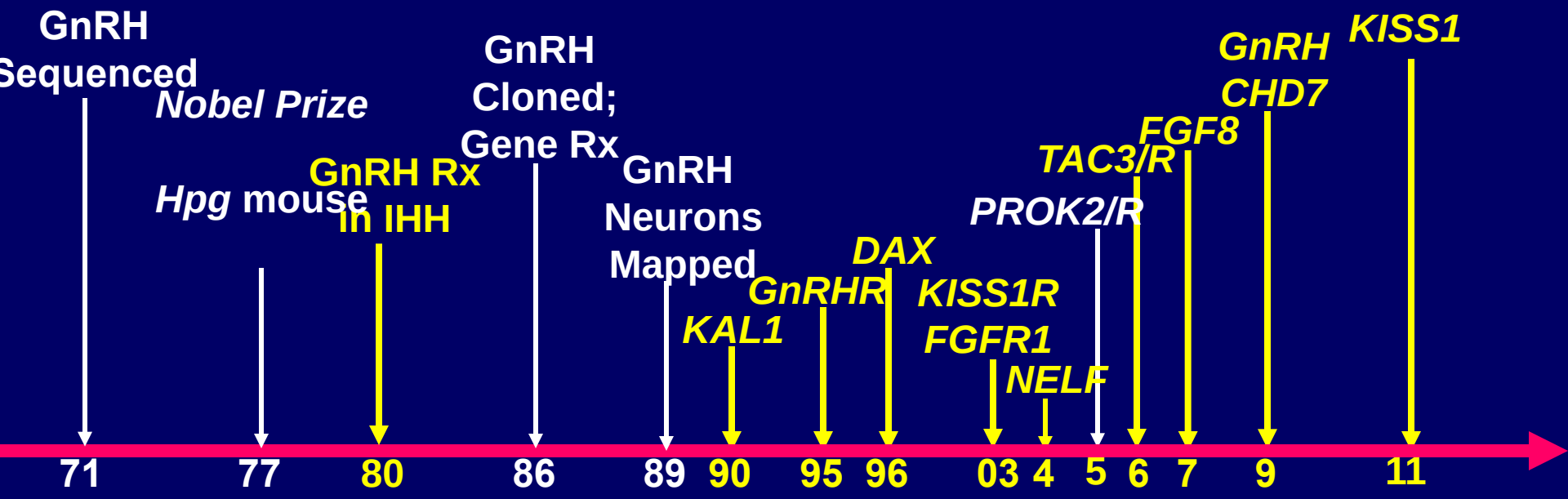








GnRH: Timeline of Landmark Discoveries



Human

Non-Human

Translational Research: It takes a Village


Stephanie Seminara, M.D.


Nelly Pitteloud, M.D.


Ravikumar Balasubramanian, M.D., Ph.D.


Janet Hall, M.D.


Corrine Welt, M.D.


Ursula Kaiser, M.D.

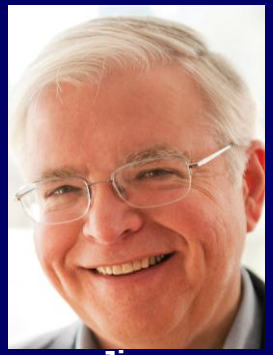

Gerry Sykiotis, M.D., Ph.D.


Yee-Ming Chan, M.D., Ph.D.


Jenn Kaina, N.P.


Andrew Dwyer, R.N., N.P.


Margaret Au, G.C.


Jim Gusella, Ph.D


Lacey Plummer, M.S.


Ginny Hughes, M.S.


Ning Jiang, M.D.


Cassie Buck, G.C.

MGH Reproductive Endocrine Unit Training Program (T32) = 80

- >60 % = Women
- 80% in Academics = peer-reviewed funding)
- 50% >10 years = Prof.
- Other 50% = Assoc. Prof.
- 3 Presidents of Endocrine Society
- 10 Members of Endo Society leadership

NICHD: Keys to Our Success

Programs

R01 Grant Program

U54 Specialized Center

T32 Training Grant

Visionary Leadership

Marvin Karten

Michael McClure

Lou DePaolo

Yvonne Maddox

Susan Taymans

Translational Research is Expensive