NeurEndoMaths2012
Tours, August 4th

Circadian control of the neuroendocrinology of growth and reproduction

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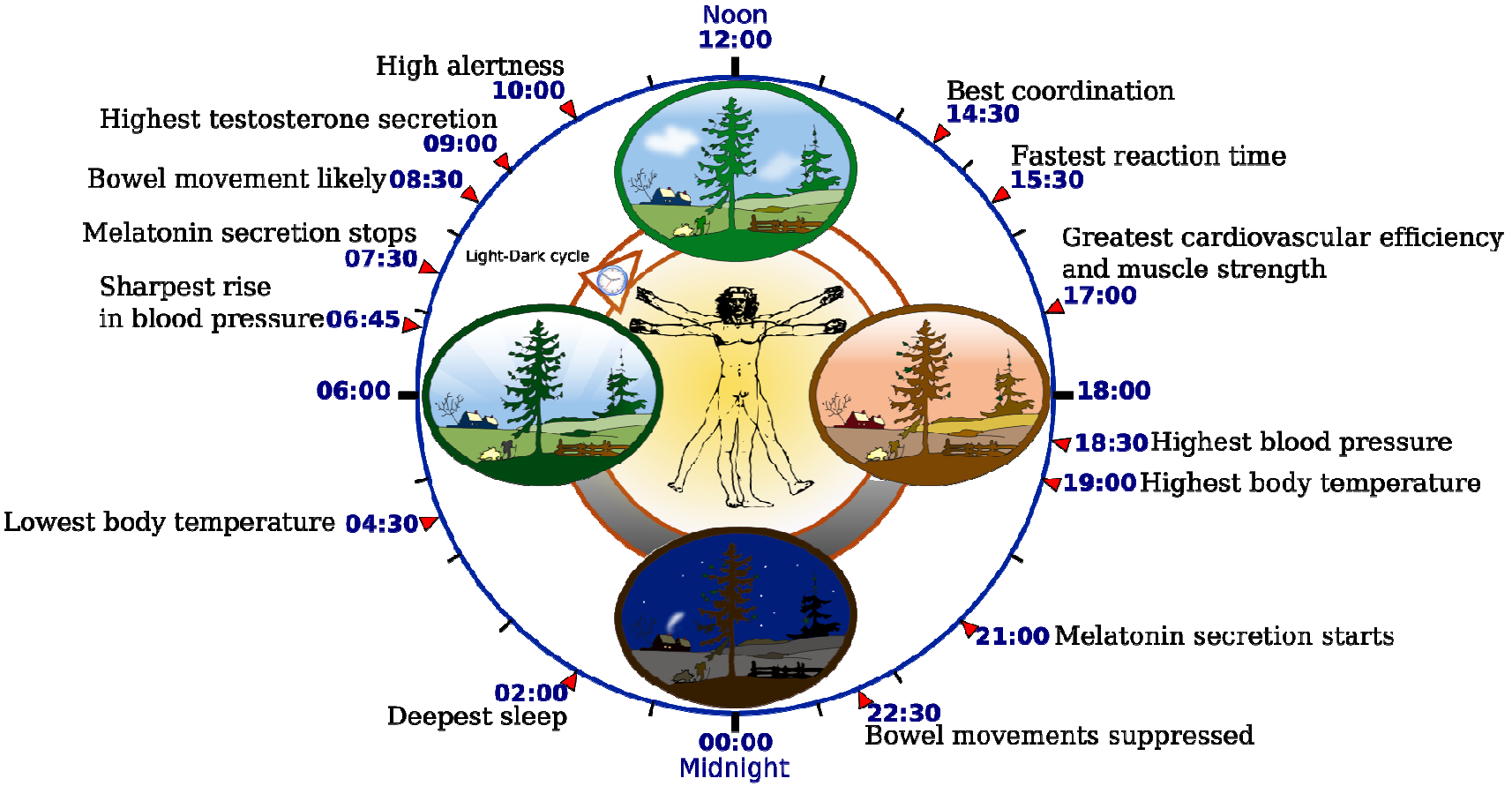
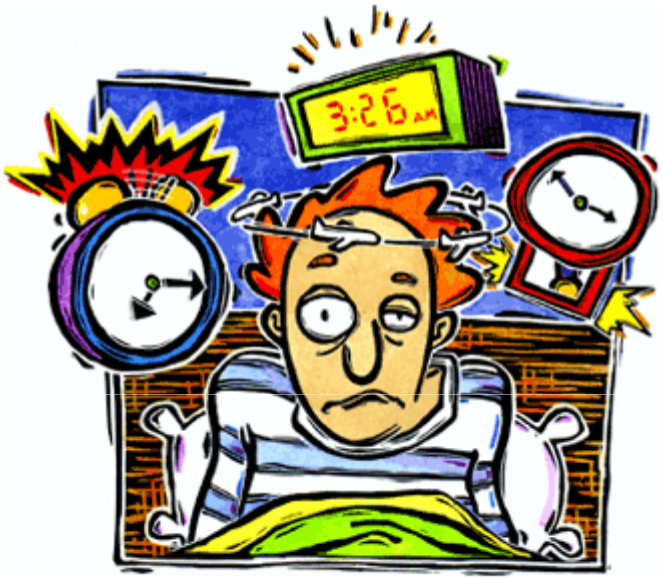
The daily rhythms of human physiology



Circadian misalignment and health



Jetlag

Desynchronosis after rapid transmeridian travels

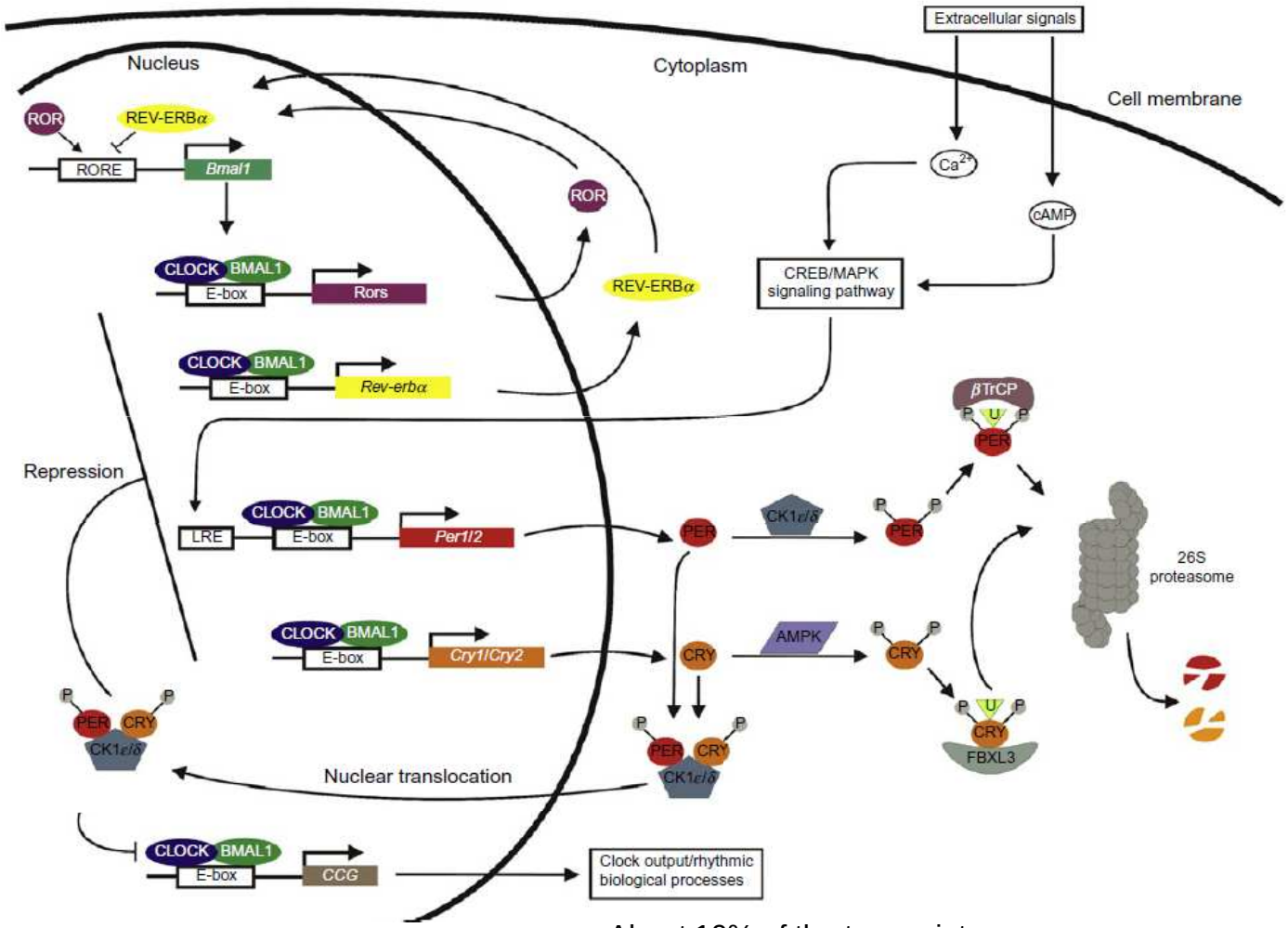
Nightshift working

Metabolic syndrom (diabetes, obesity)

Reproduction issues (dysmenorrhea, compromised pregnancy)

Increased risk of heart failure…

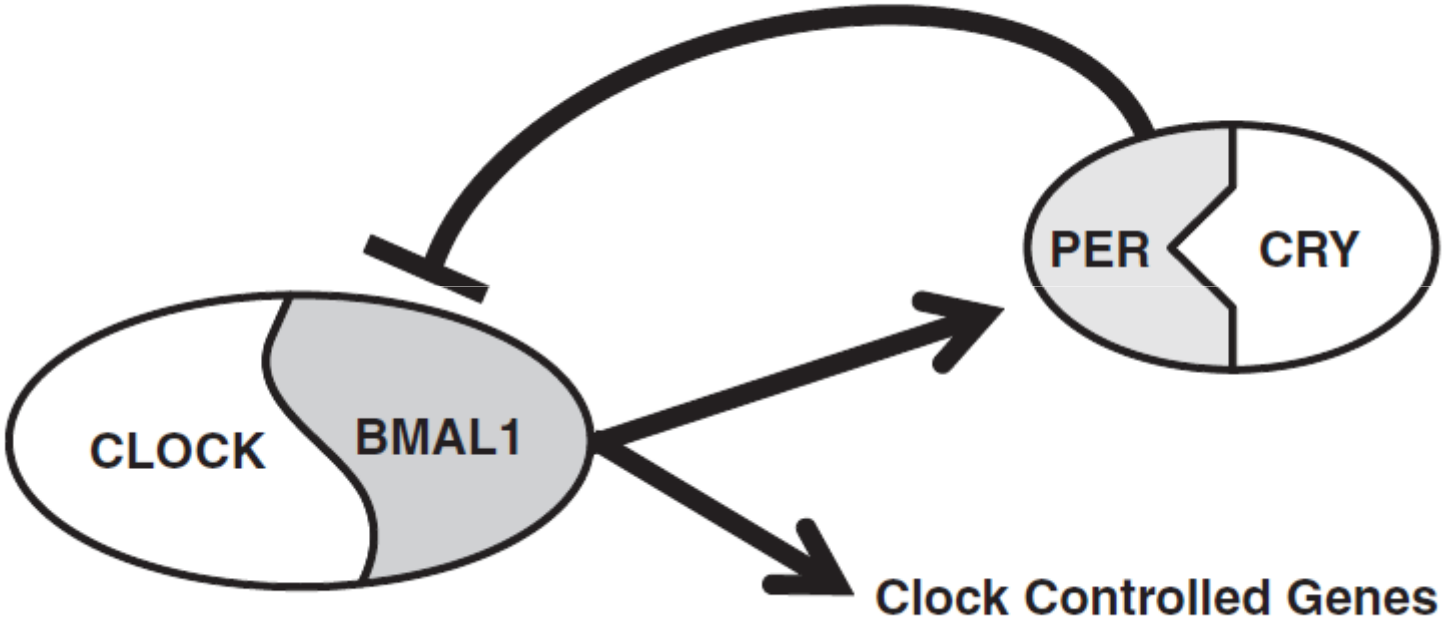
A molecular model of the mammalian circadian clock



About 10% of the transcriptome

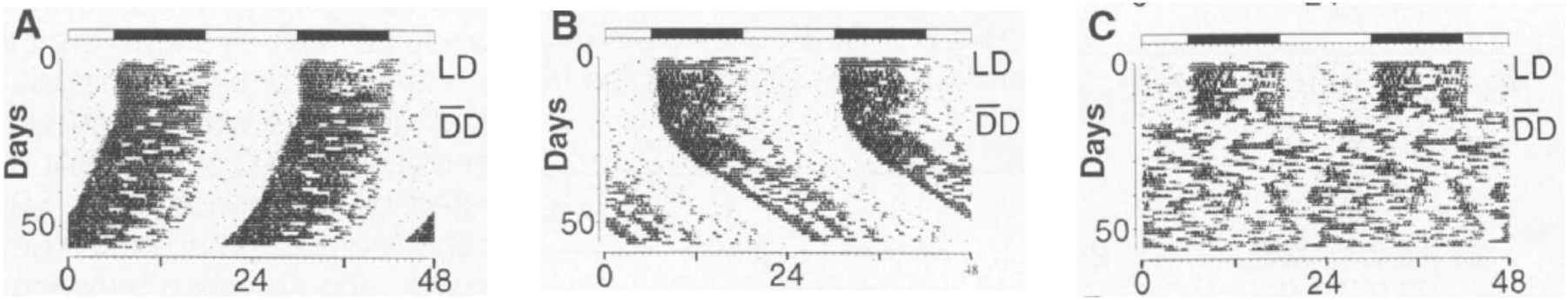
Lowrey PL, akahashi JS. Adv Genet. 2011

Simplified model : the "core" clock



Mouse genetics of the circadian clock

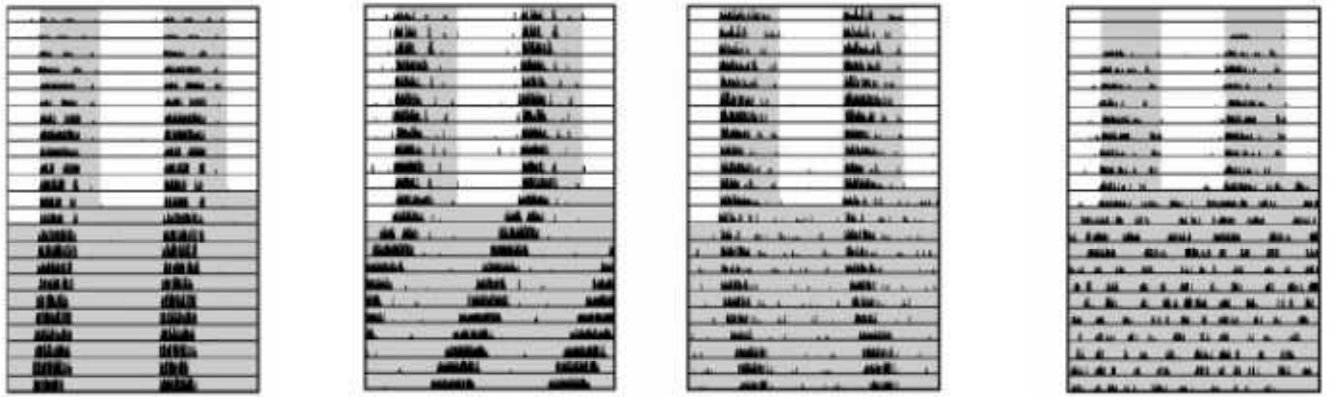
The Clock mutation : the first mouse circadian mutant



Wild-type | *Clock/+* | *Clock/Clock*

Vitaterna et al, 1994

Knockout of Cryptochromes



Wild type
τ = 23.77 ± 0.07 h

cry1$^{-/-}$
τ = 22.51 ± 0.06 h
Immediate onset

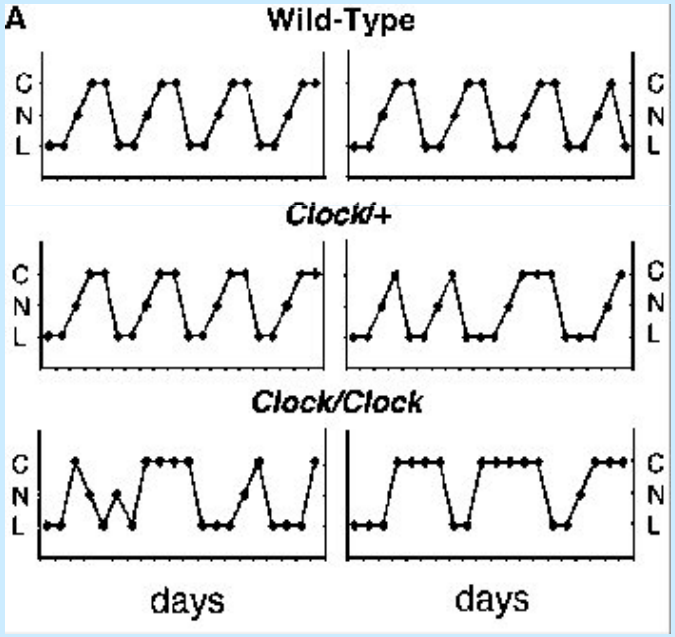
cry2$^{-/-}$
τ = 24.63 ± 0.06 h
Delayed onset

cry1$^{-/-}$*cry2*$^{-/-}$
Immediate arrhythmicity

van der Horst et al, 1999

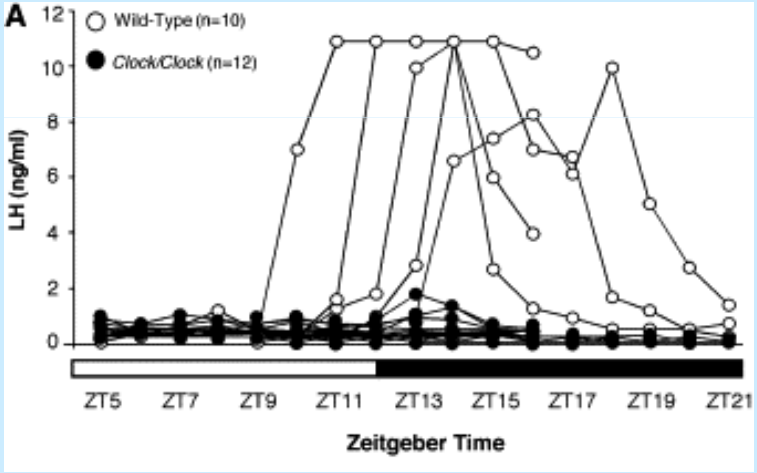
Impaired reproduction performance in Clock/Clock mutant females

Altered estrous cycles in Clock mutants, as assessed by vaginal smears



Miller et al., Curr Biol. 2004

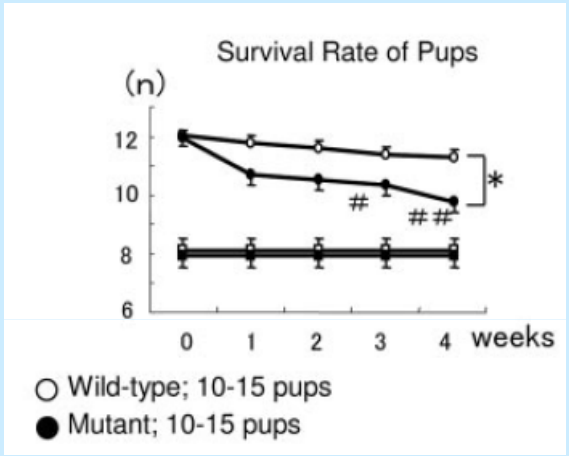
No LH peak in Clock mutants on the day of estrous

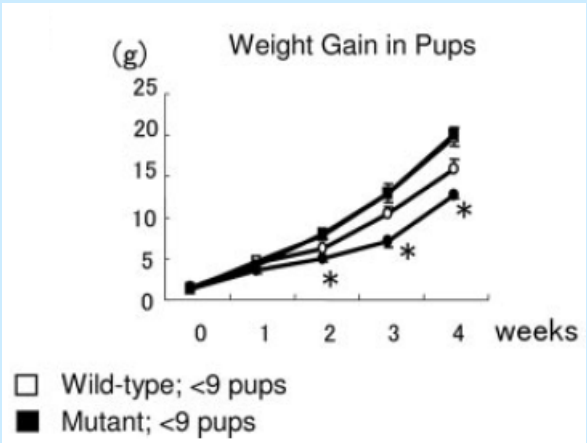


Miller et al., Curr Biol. 2004

Impaired lactation in Clock/Clock mutant dams

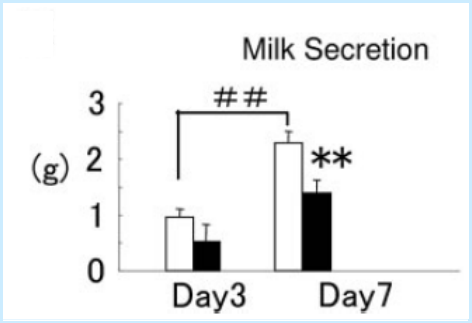
Decreased growth and survival of pups nursed by mutant dams

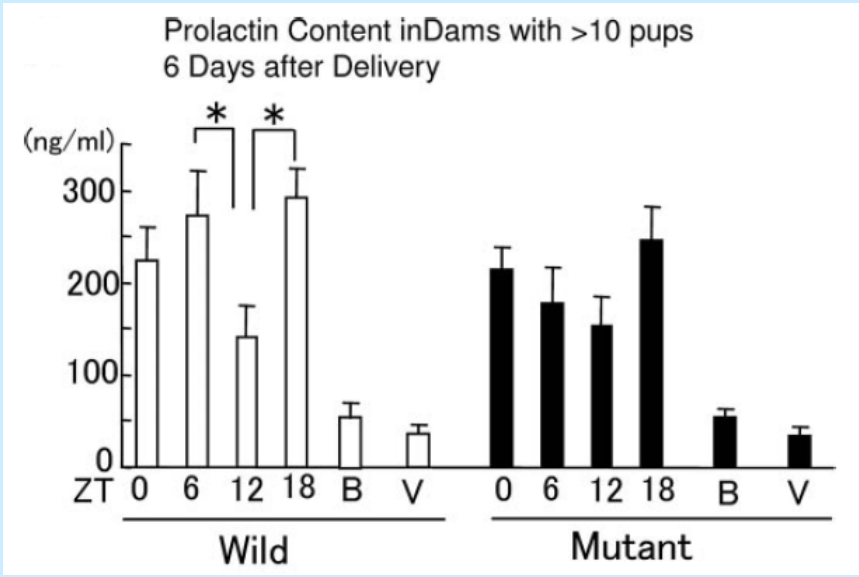




Hoshino et al.,Endocrinology, 2006

Alteration of the lactotroph axis of Clock mutant females



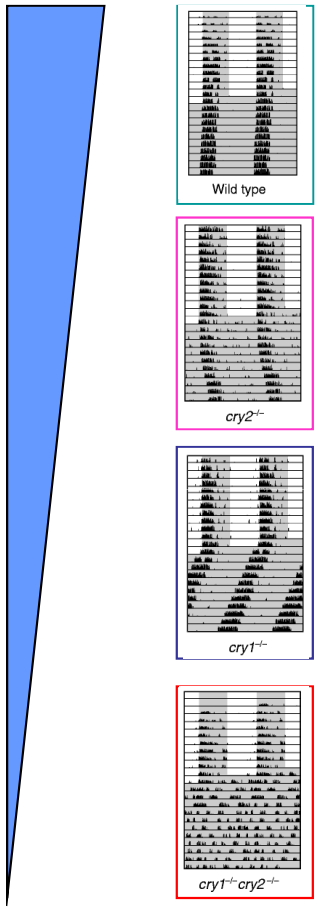


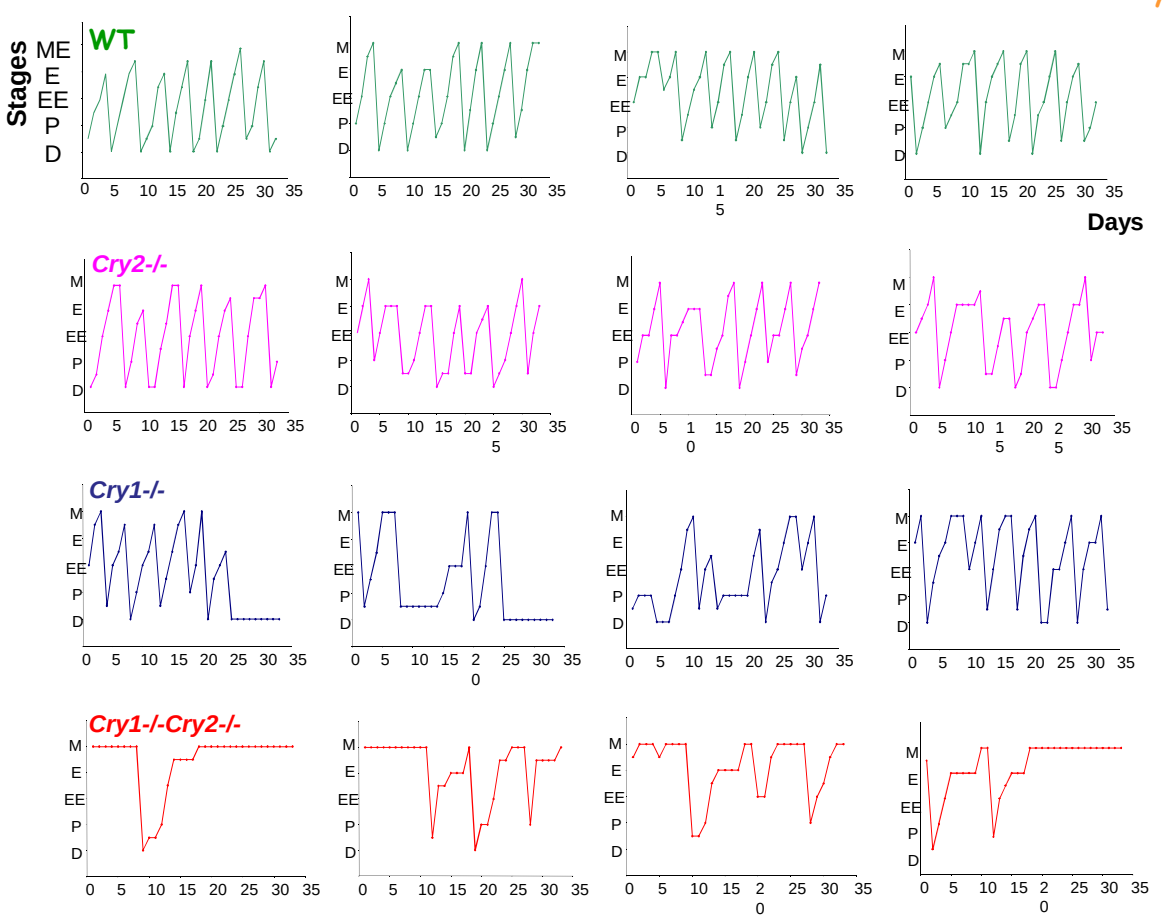
Hoshino et al.,Endocrinology, 2006

Vaginal smears in Cry mutant mice

Clock integrity

Cycle regularity

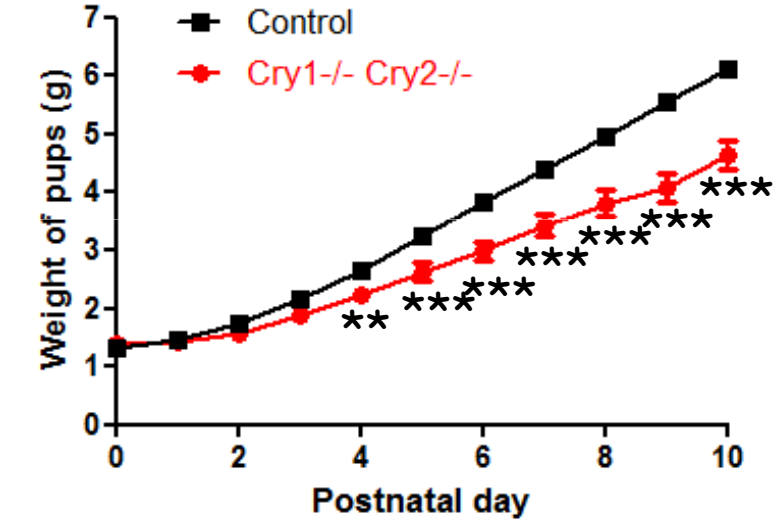




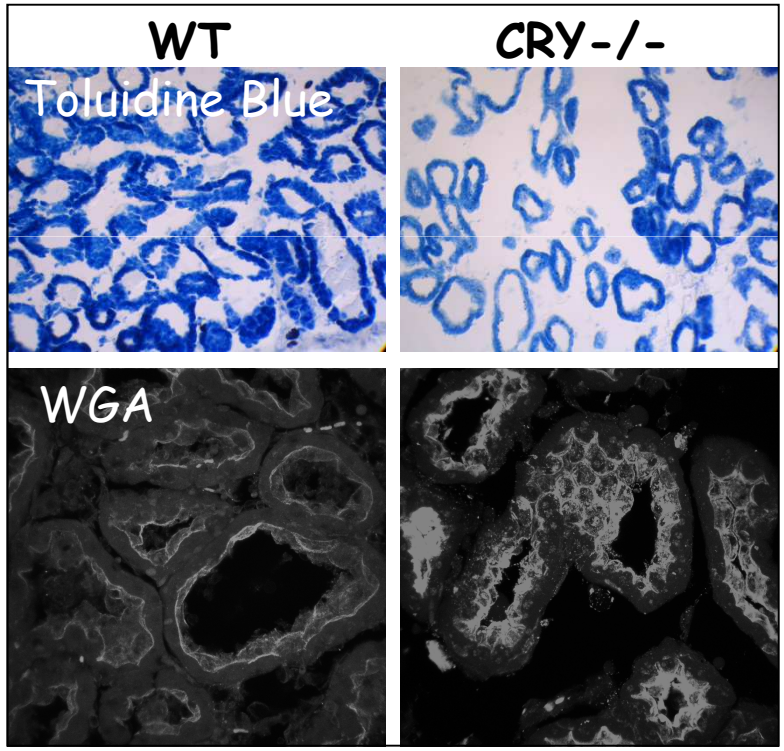
The alterations of the circadian clock and estrous cycles are correlated

Lactation in Cry-/- females

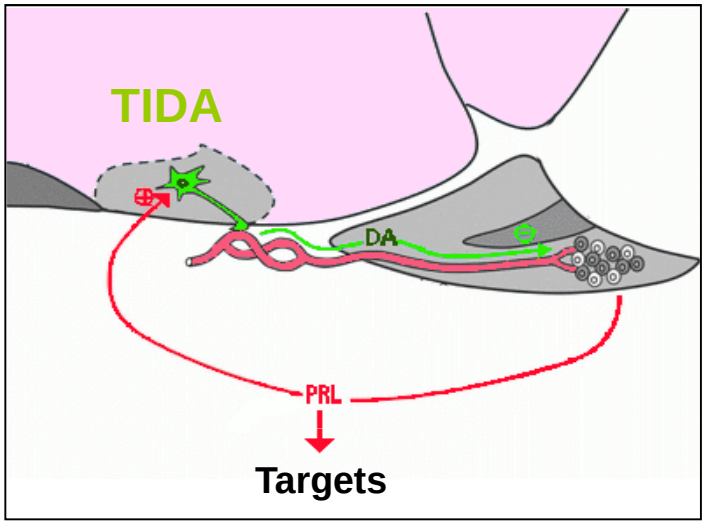
Growth of pups

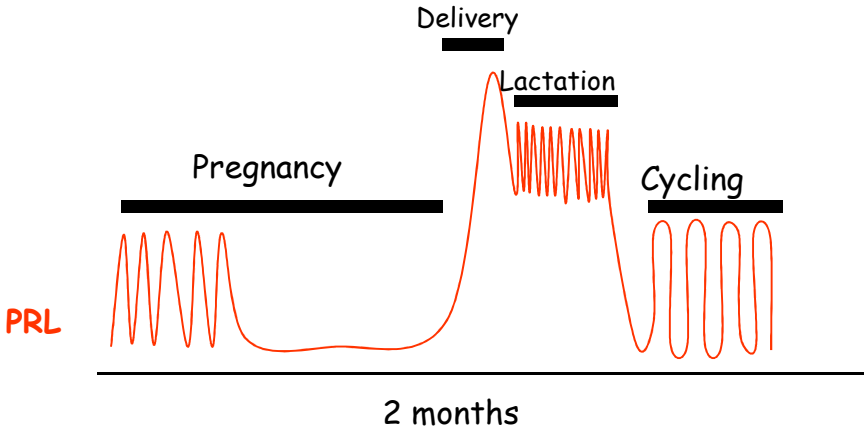


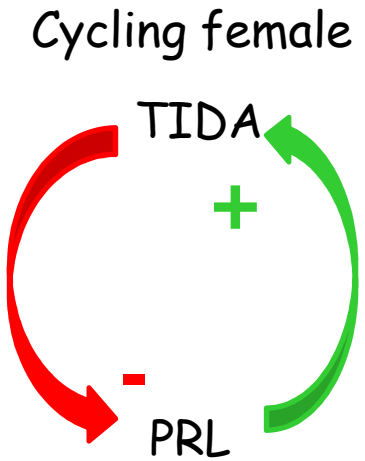
Mammary glands

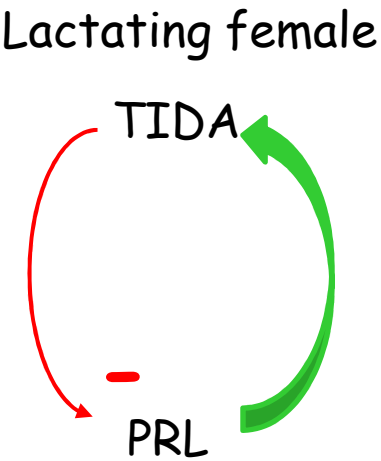


Plasticity of the feedback loop of the lactotroph axis

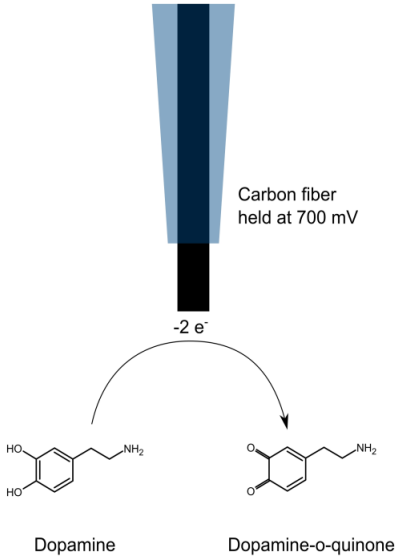


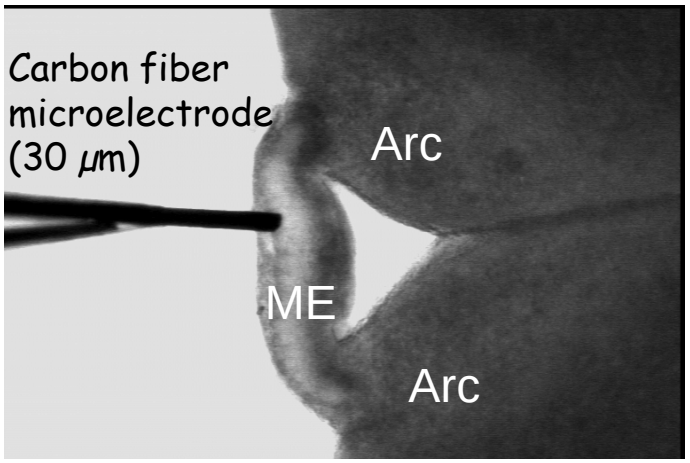


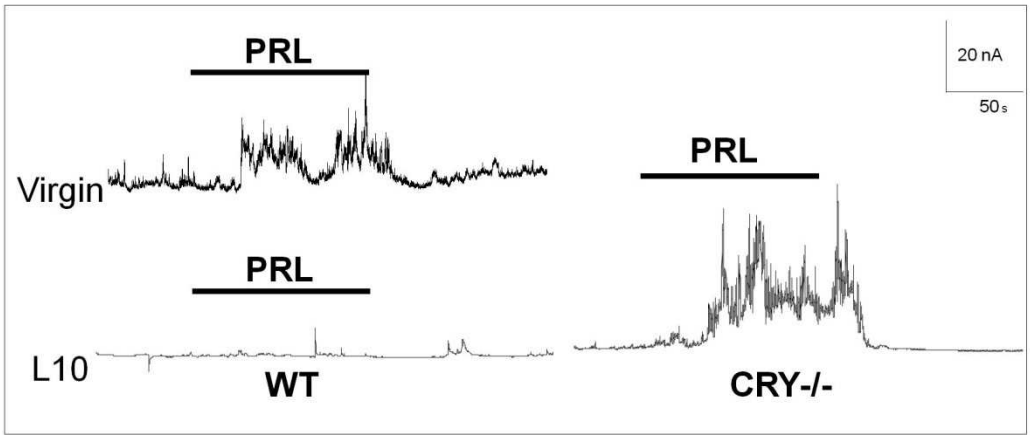




Amperometric detection of dopamine release in brain slices

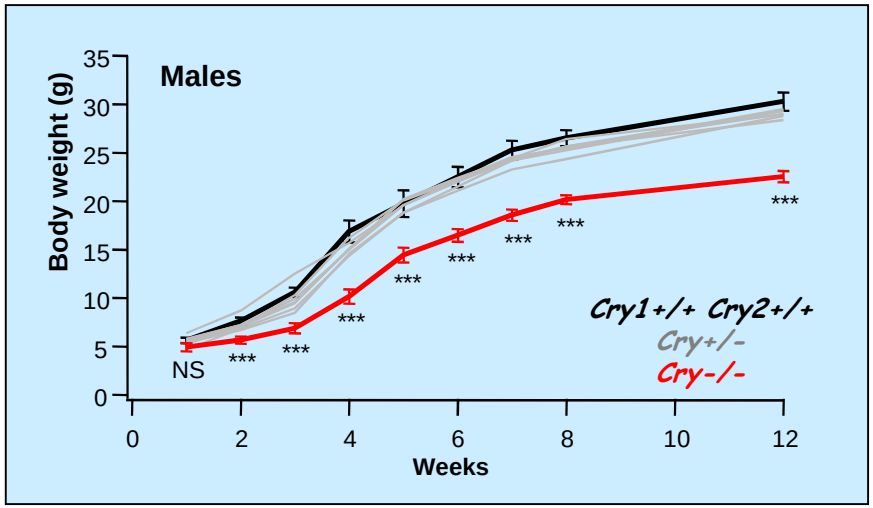


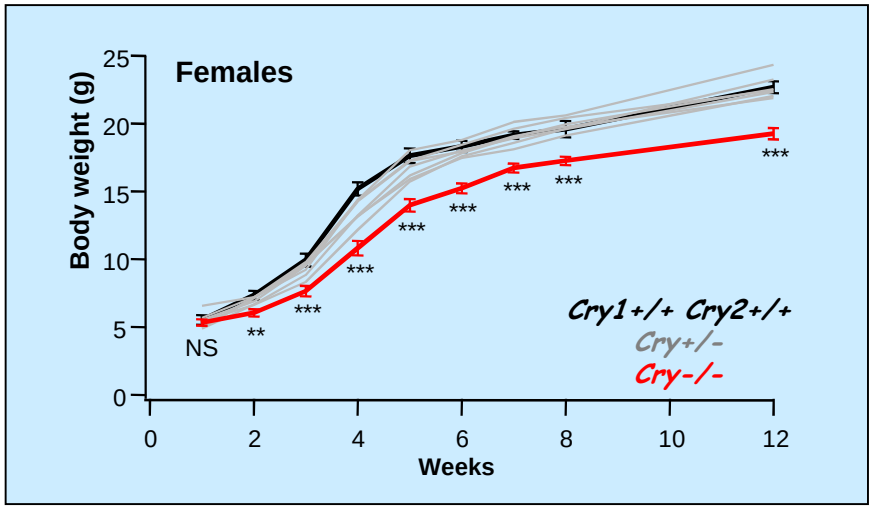




An inhibitory dopaminergic tonus persists in absence of Cryptochromes

Altered body growth in Cry mutant mice

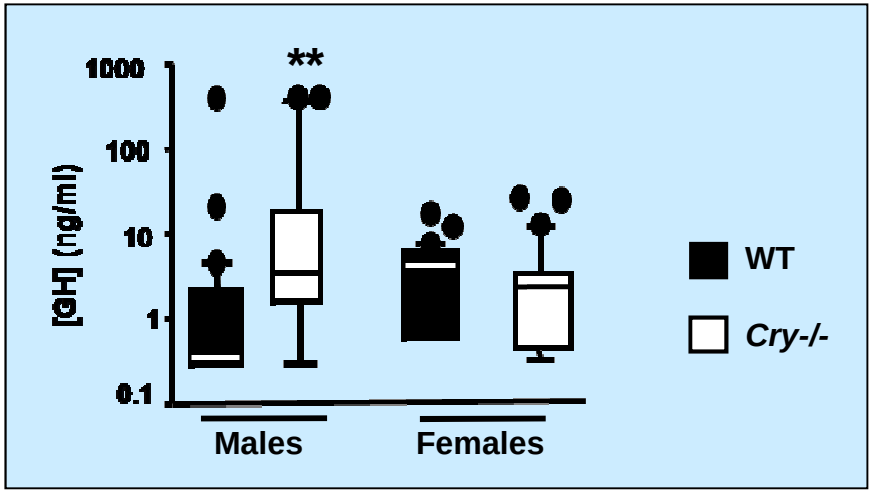




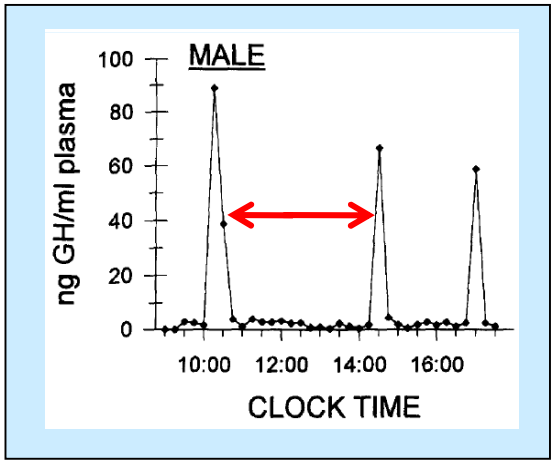
Bur IM, et al JBC 2009

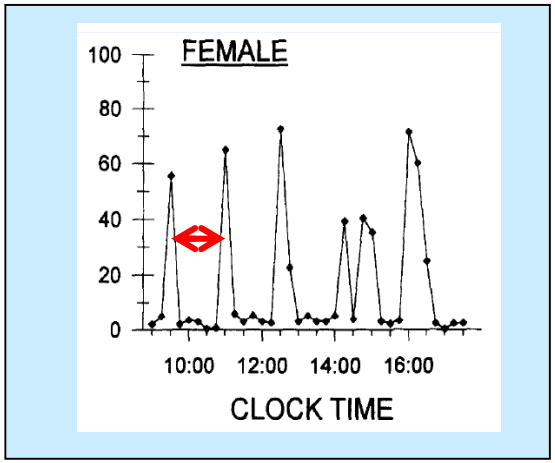
→ Is GH secretion altered in Cry-/- mice ?

Paradoxical alteration of plasmatic GH content in Cry-/- males



Bur IM, et al JBC 2009





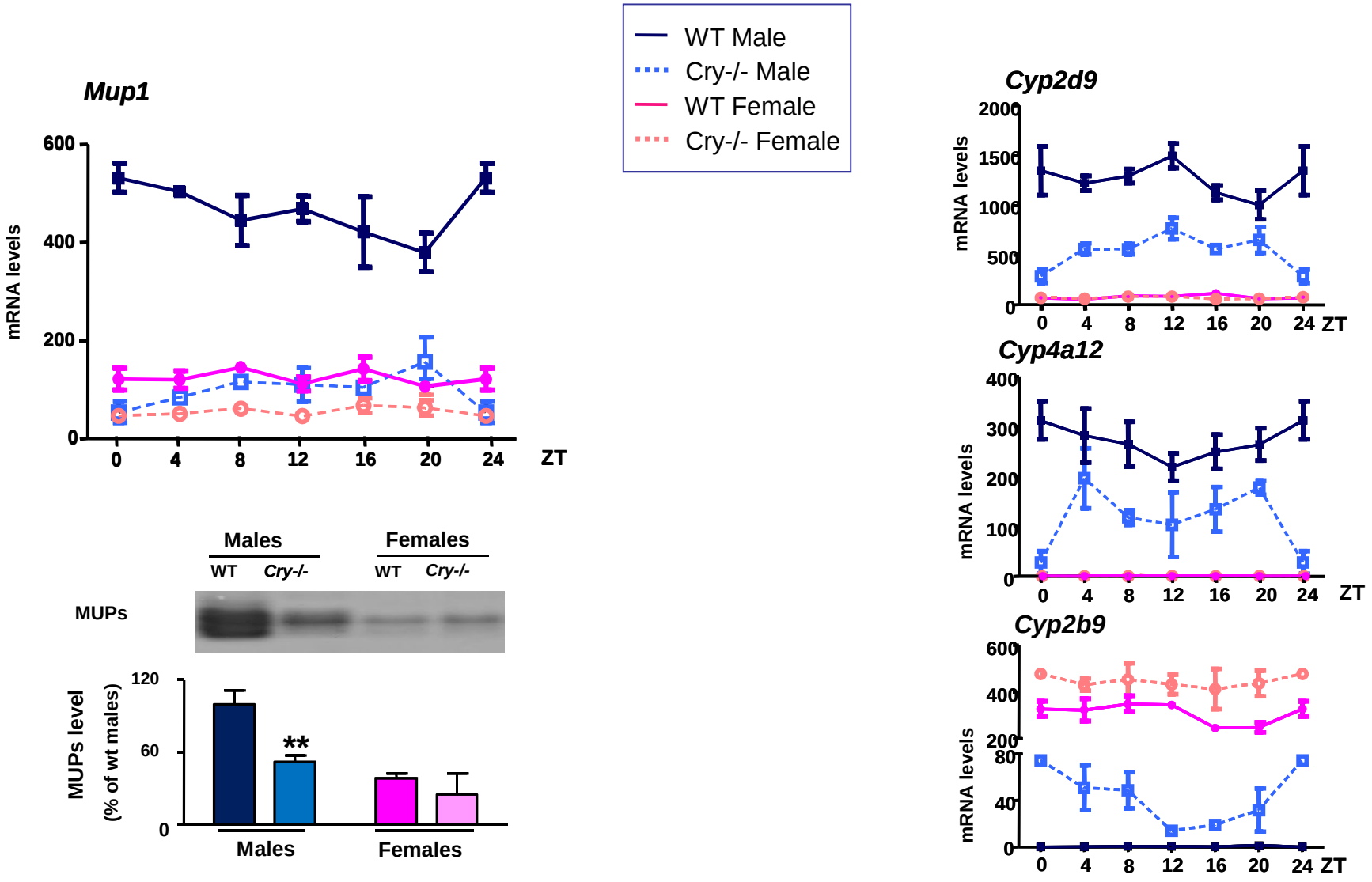
Shapiro BH et al, 1995

GH-dependent liver genes

High levels of Mups
Male-specific Cyp P450 …

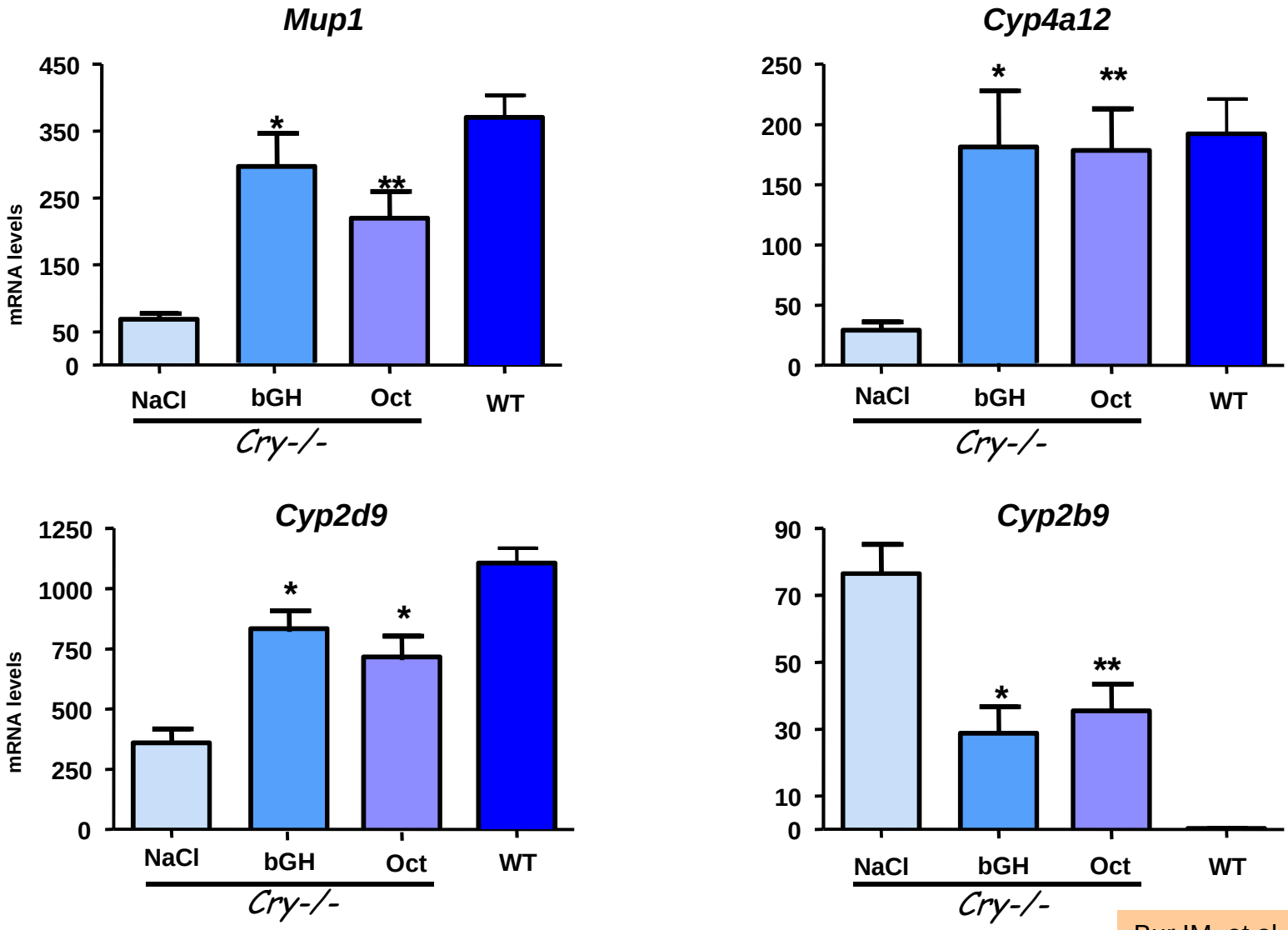
Low levels of Mups
Female-specific Cyp P450 …

The markers of GH pulsatility are altered in the liver of Cry-/- males



Bur IM, et al JBC 2009

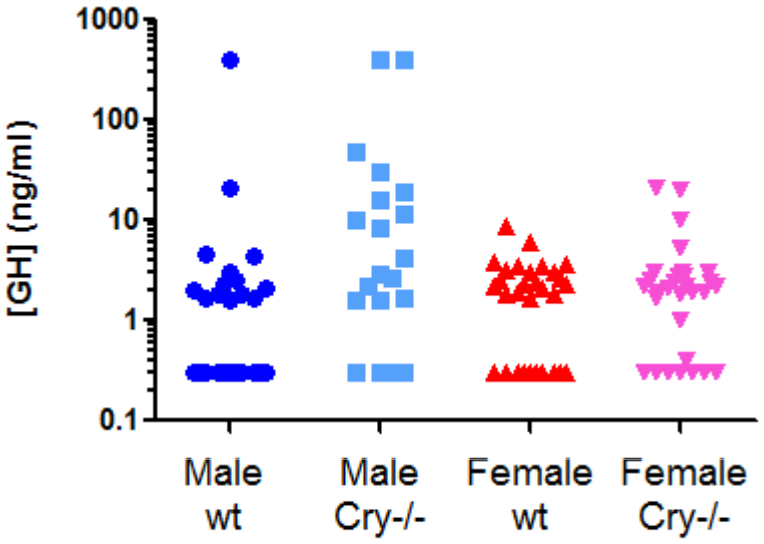
Restoring GH pulses reverts the liver feminization of Cry-/- males



Bur IM, et al JBC 2009

→ Cry genes are necessary to sustain GH pulsatility

A follow-up to the 2010 meeting held in Columbus



Analysis by Maurizio Tomaiuolo (FSU)

- The data collected from the WT and the cry -/- males do not seem to come from the same distribution WT males data are consistent with few (sharp?) synchronized peaks
- The cry -/- data are consistent with multiple unsynchronized peaks
- From the quantitative point of view the data of the cry -/- males do not seem to be more similar to the females data you sent, but qualitatively the cry -/- data seem to be consistent with the idea of GH being oscillatory and with multiple peaks (which is more representative of females GH profiles)

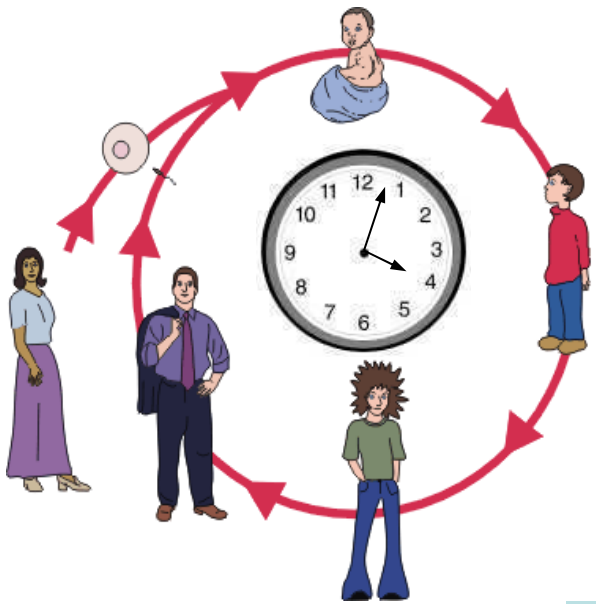
Sujet: Re: Data to analyze
De : Anmar Khadra <khadraa@niddk.nih.gov>
Date : 02/09/2010 23:41
Pour : Xavier Bonnefont <Xavier.Bonnefont@igf.cnrs.fr>

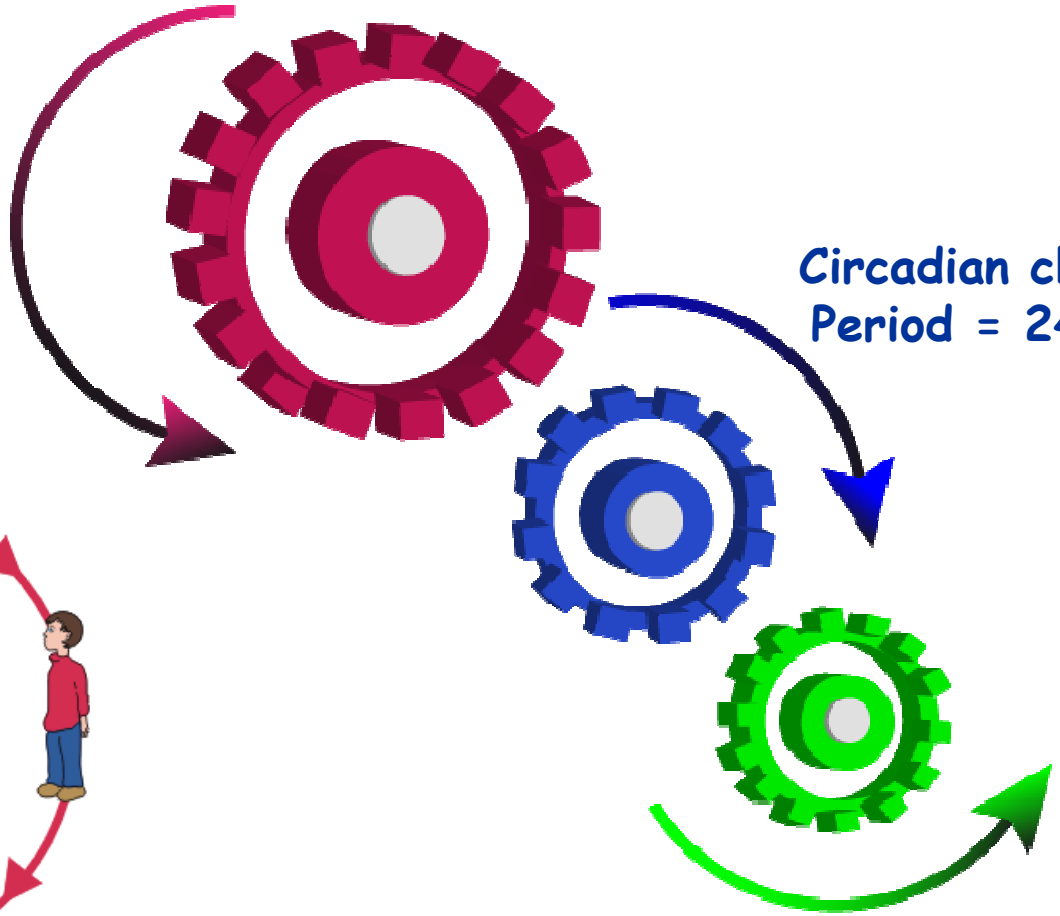
Hi Xavier,

I completely agree with you that it is more likely (and I would even go as far as saying most likely) the KO mice are pulsing at higher frequency than the WT, simply due to the fluctuations seen in the data points in your spread sheet file. The simulations I sent you confirms this, especially with the range of of data point distribution I obtained from my simulations of 100 random "model mice", which is consistent with your own distribution (unlike the abolished case).

Circadian timekeeping contributes to multiple timescale neuroendocrine rhythms

Infradian rhythms (> 24 h)
ex: estrus cycle, PRL pulses





Circadian clock
Period = 24 h

Ultradian rhythms (< 24 h)
ex: GH pulsatility

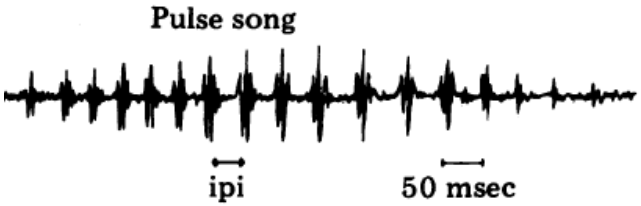
Bonnefont , J. Neuroendocrinol. 2010

Proc. Natl. Acad. Sci. USA
Vol. 77, No. 11, pp. 6729–6733, November 1980
Genetics

Circadian rhythm mutations in *Drosophila melanogaster* affect short-term fluctuations in the male's courtship song

(interpulse interval/*per* mutations/diplo-X transformed males)

C. P. KYRIACOU AND JEFFREY C. HALL

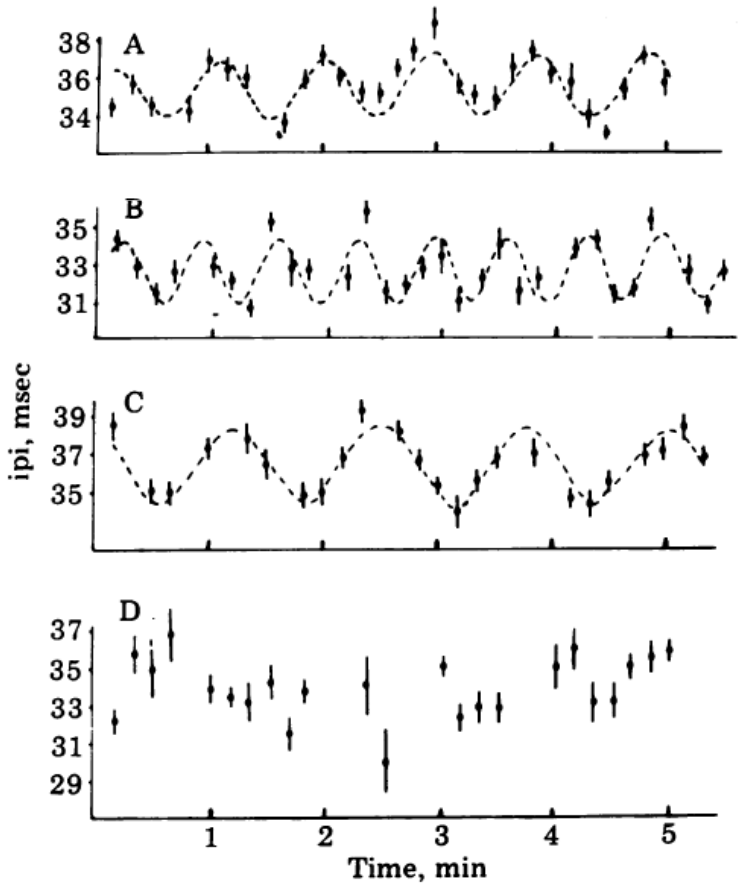


per^{+} T=24 hr

per^{s} T=19 hr

per^{l} T=28 hr

per^{0}



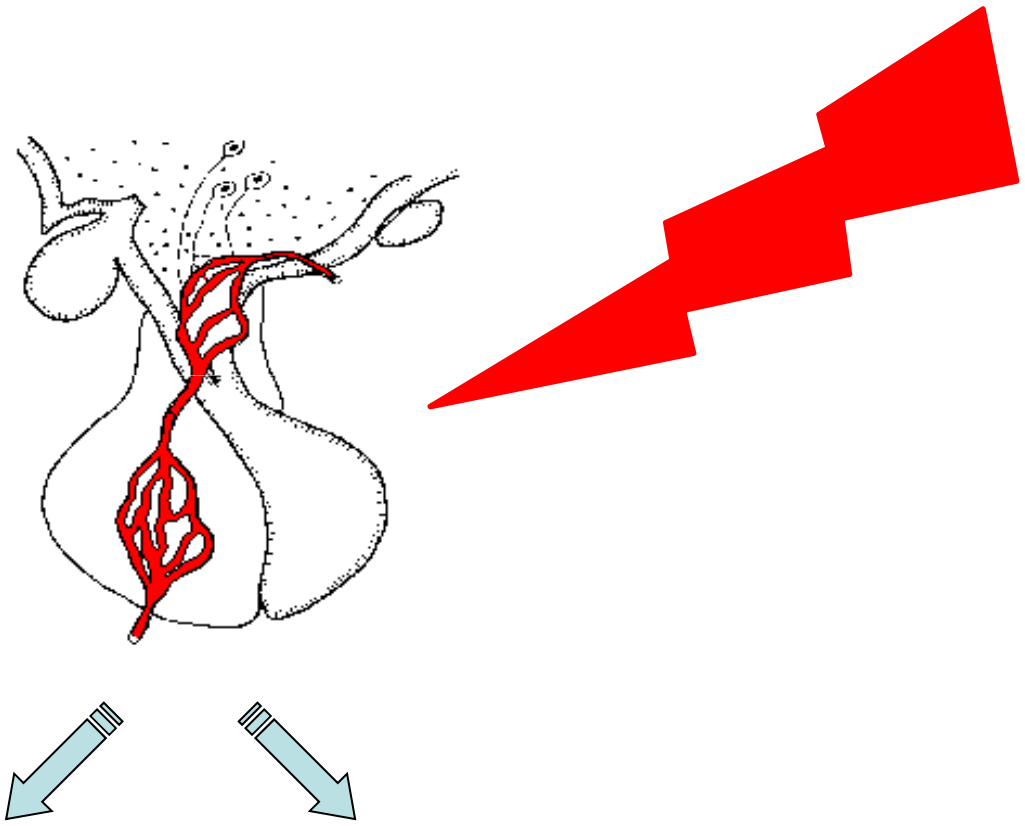
p= 56 sec

p= 40 sec

p= 76 sec

Rhythms and pulsatilities in the hypothalamus and pituitary

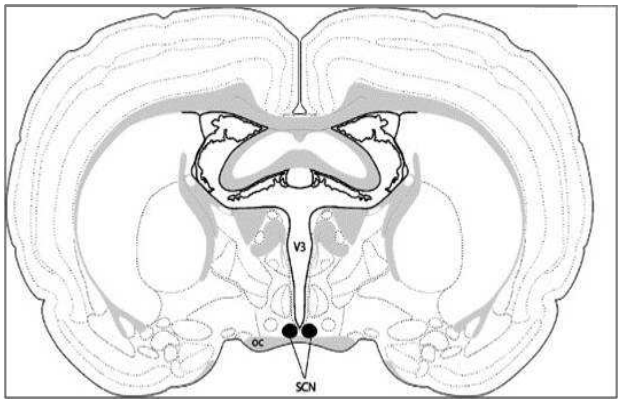
Circadian system

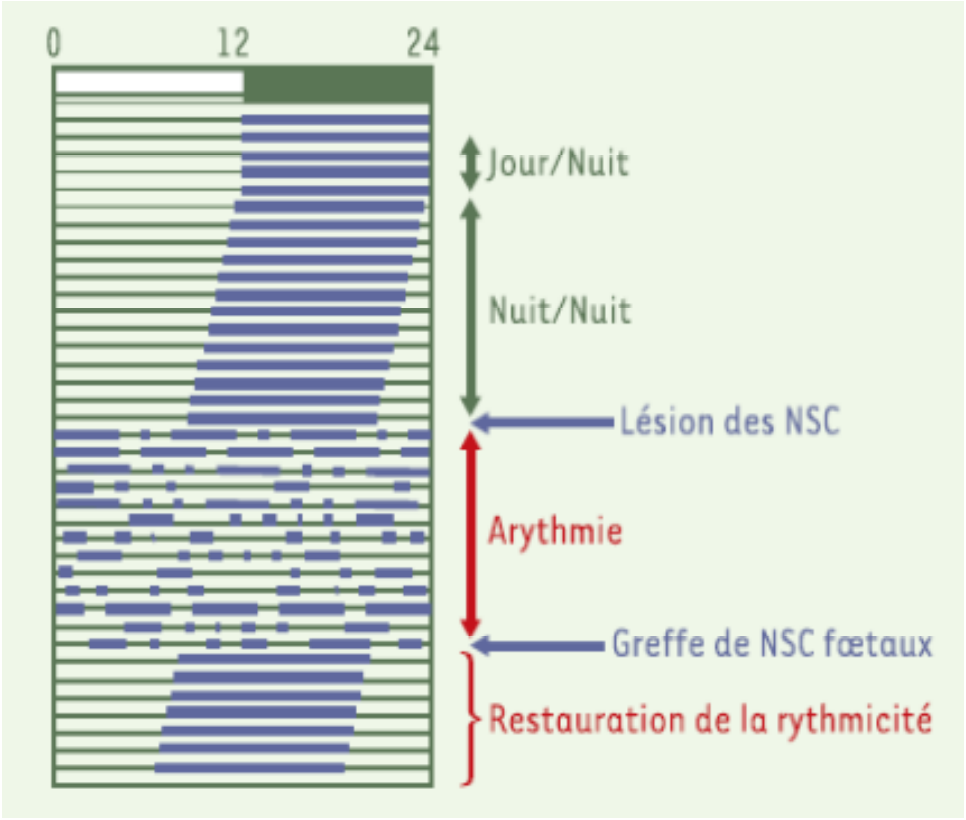


Metabolism
ultradian GH

Reproduction
LH, PRL cycles

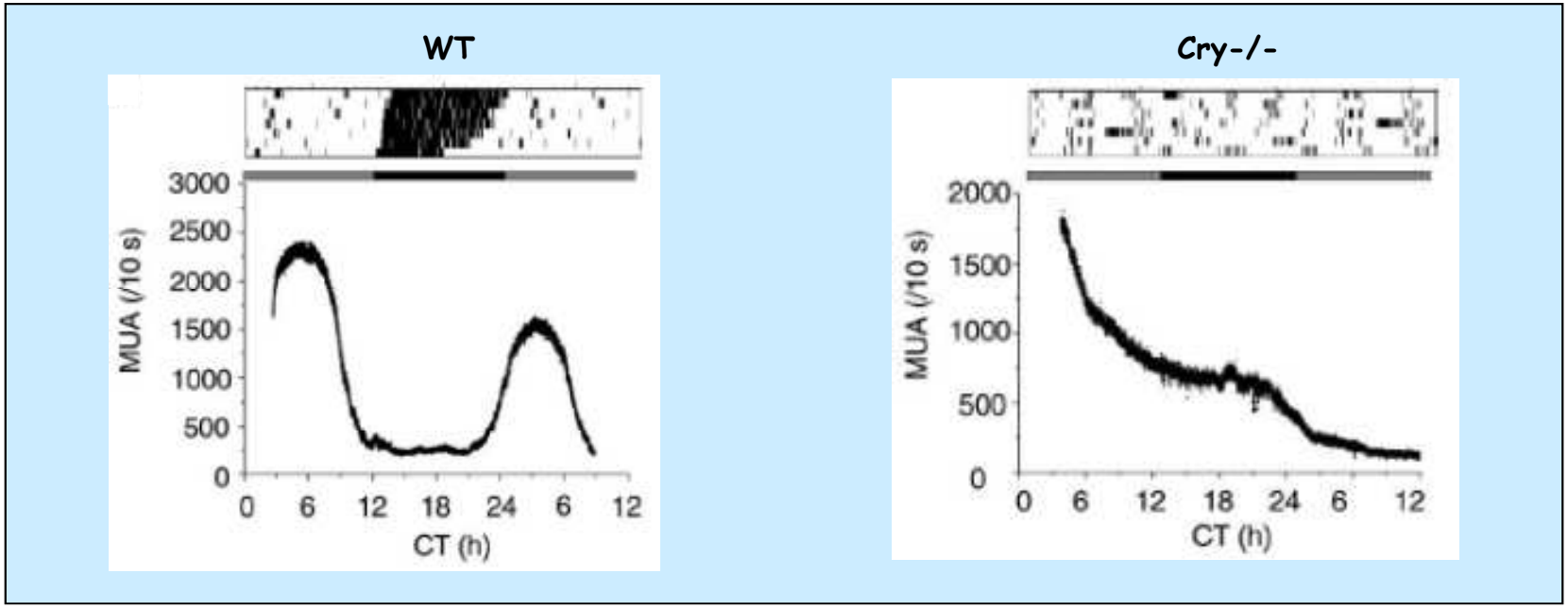
The central circadian clock is located in the suprachiasmatic nuclei





Cryptochromes regulate the circadian outputs of the SCN

Extracellular firing from SCN slices

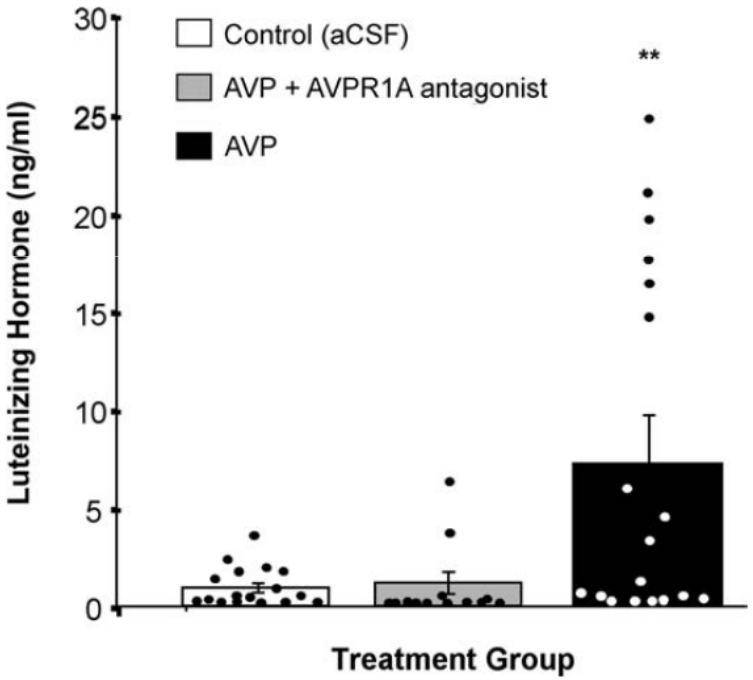


Albus, Bonnefont et al, Curr Biol 2002

The circadian molecular clockwork paces the firing of action potentials in the SCN

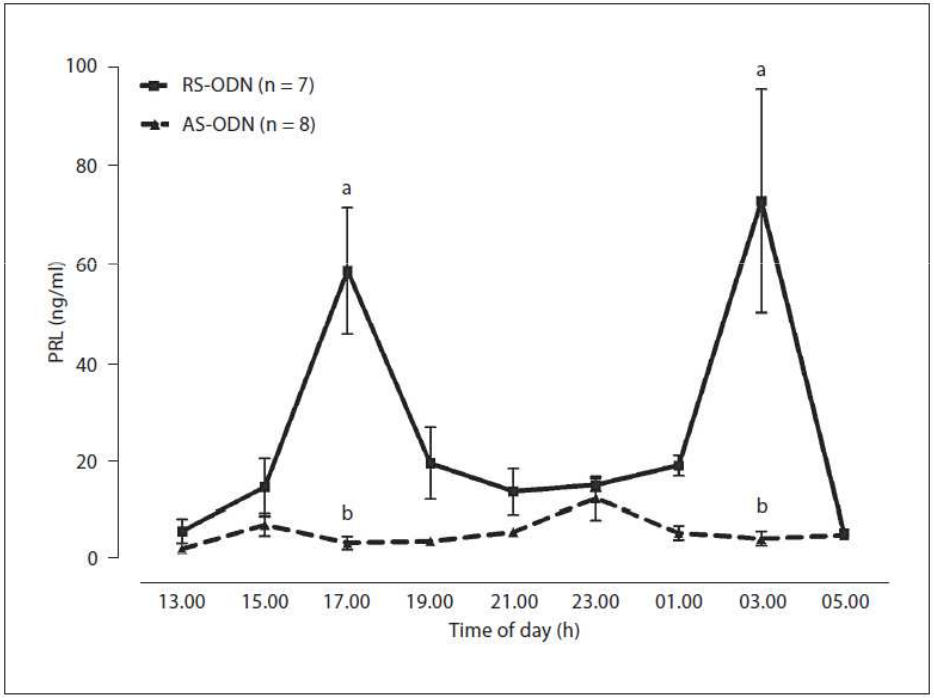
The suprachiasmatic nuclei are involved in the regulation of female reproduction

AVP treatment restores a LH surge in *Clock/Clock* mice



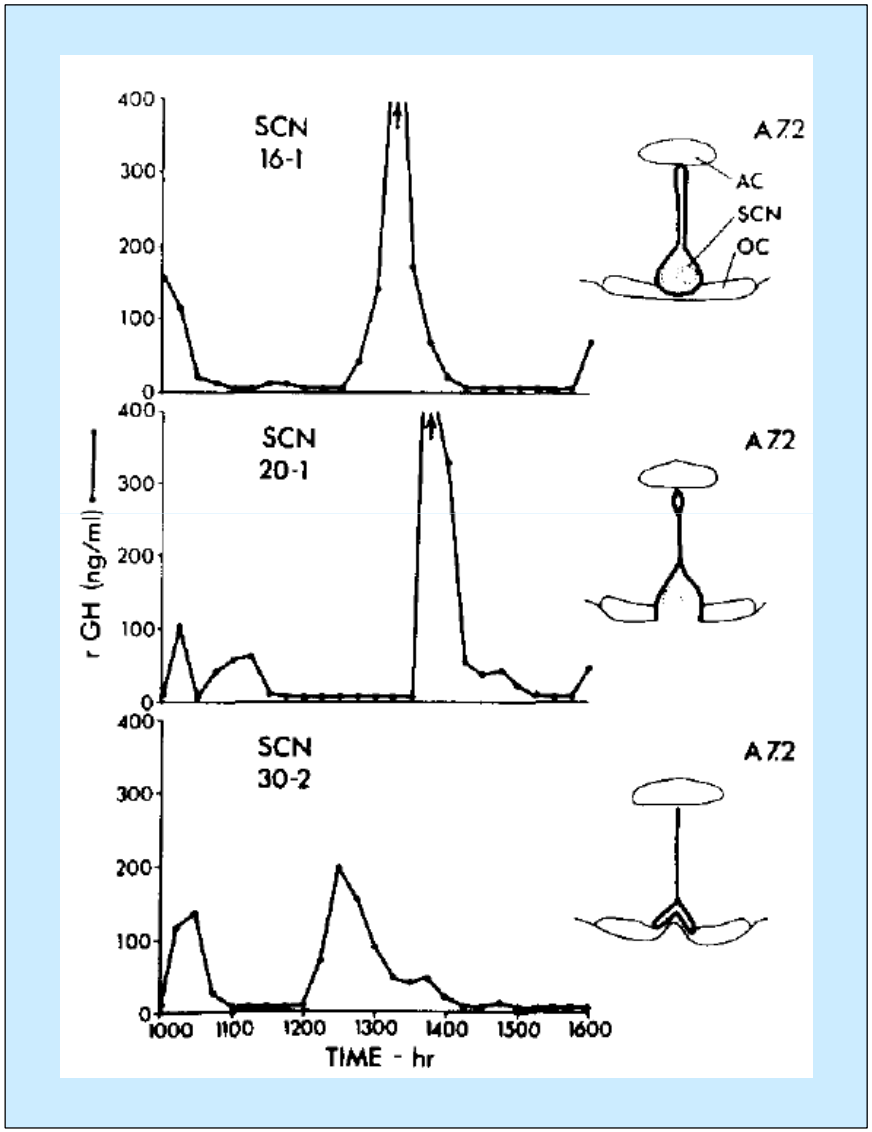
Miller et al., Biol Reprod, 2006

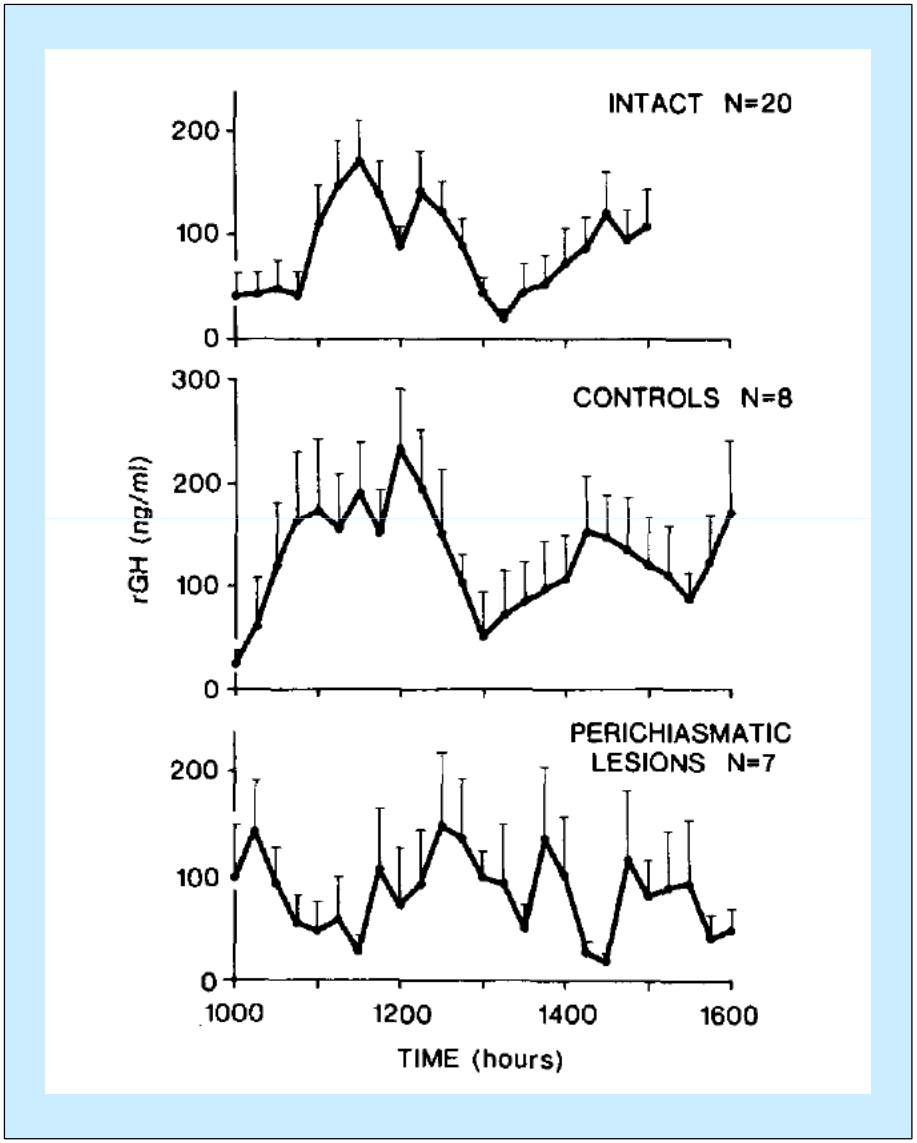
The knockdown of circadian clock genes suppresses the cervically-stimulated PRL surges



Poletini et al., Neuroendocrinology, 2010

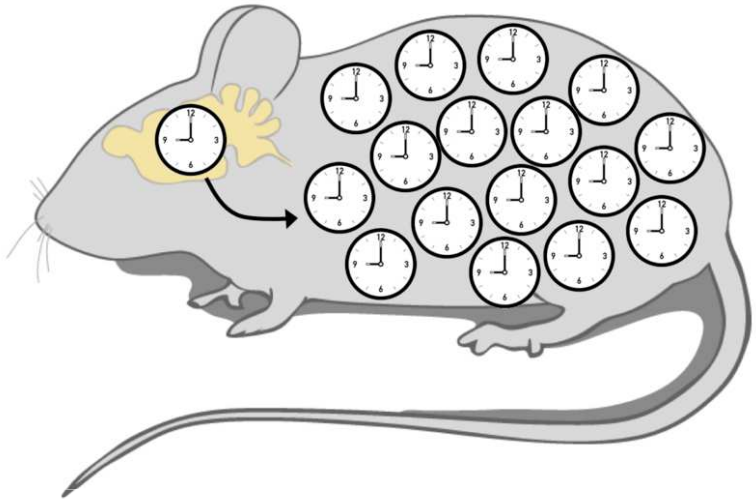
Ultradian pulsatilitity of GH secretion in rats with SCN lesions



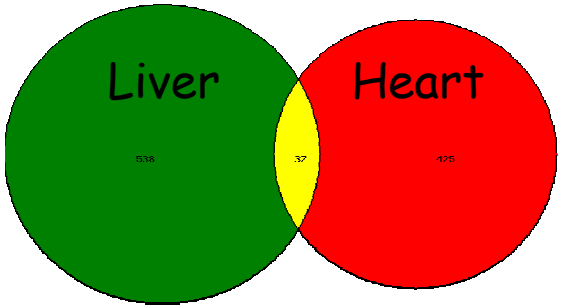


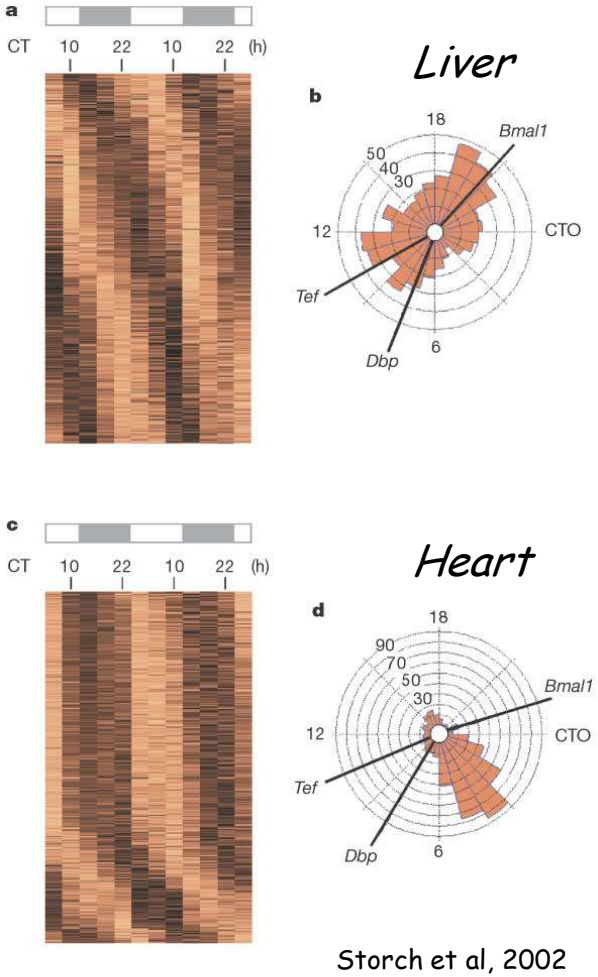
Willoughby and Martin, Brain Res, 1978

The clockwork web



About 10% of the genes expressed in each tissue are under circadian regulation

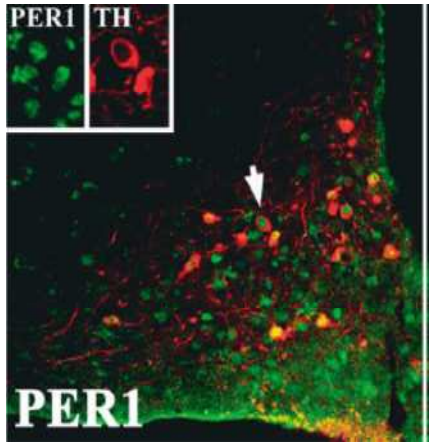




Storch et al, 2002

Circadian clock genes in hypothalamic endocrine neurons

Dopaminergic neurons of the arcuate nucleus



Kriegsfeld et al, 2003; Sellix et al, 2006

11202 • The Journal of Neuroscience, December 3, 2003 • 23(35):11202–11213

Cellular/Molecular

Circadian Gene Expression Regulates Pulsatile Gonadotropin-Releasing Hormone (GnRH) Secretory Patterns in the Hypothalamic GnRH-Secreting GT1–7 Cell Line

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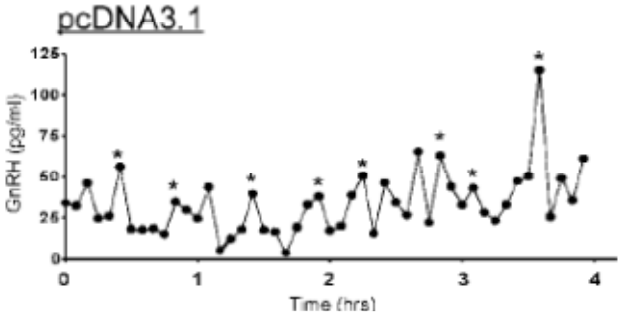
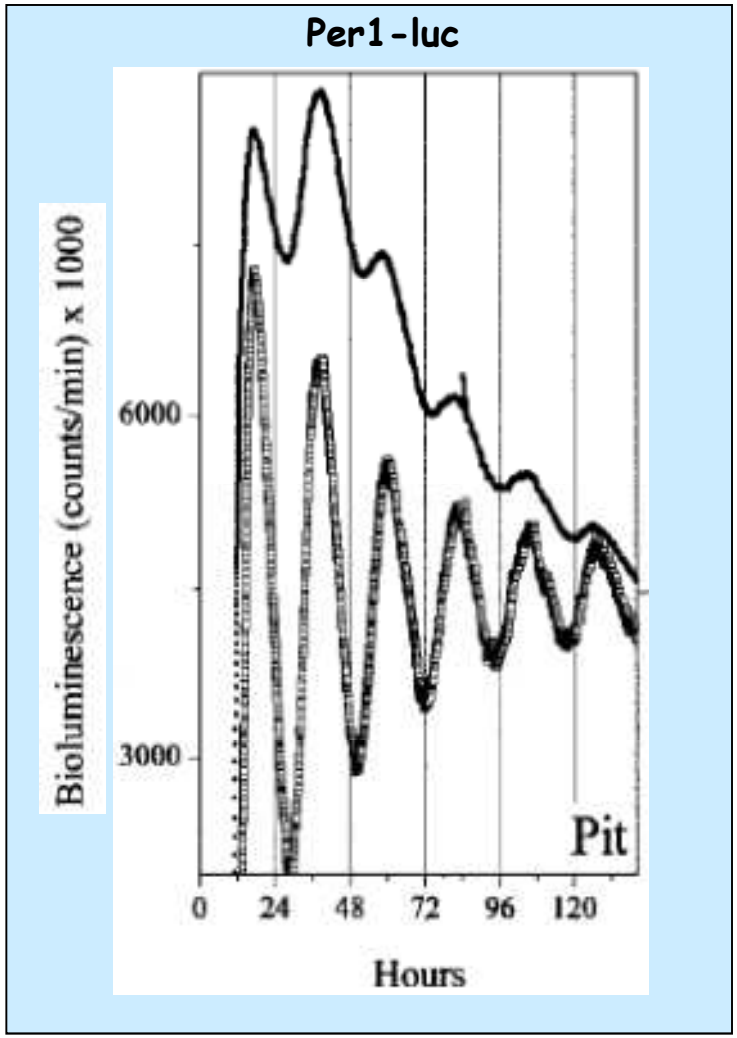
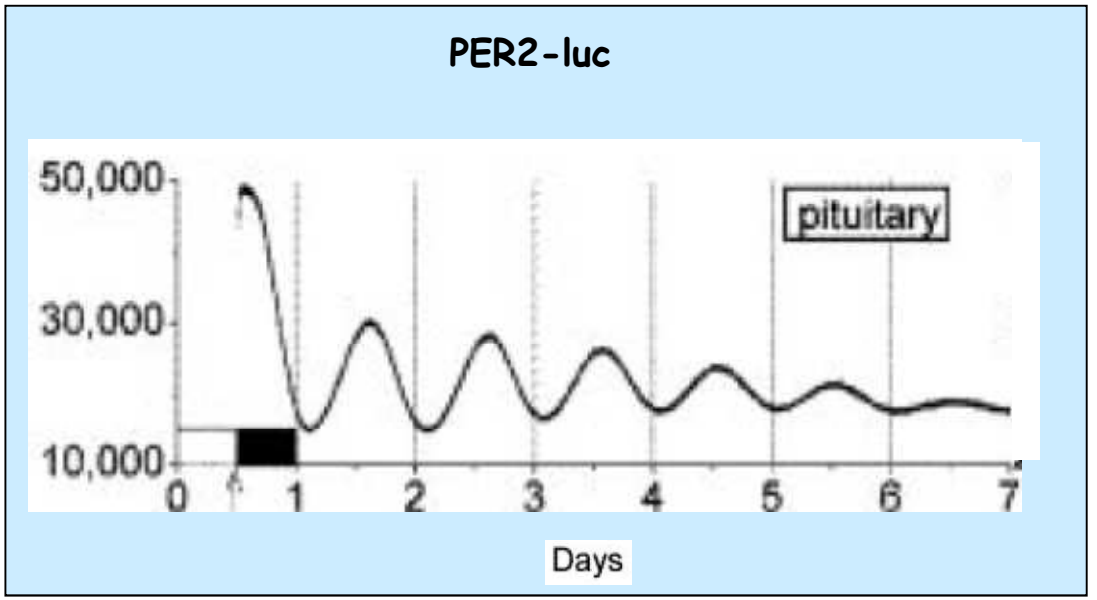
Department of Reproductive Medicine, University of California, San Diego, School of Medicine, La Jolla, California 92093-0674



Circadian oscillations of luciferase-based reporters in pituitary explants



Abe et al, 2002

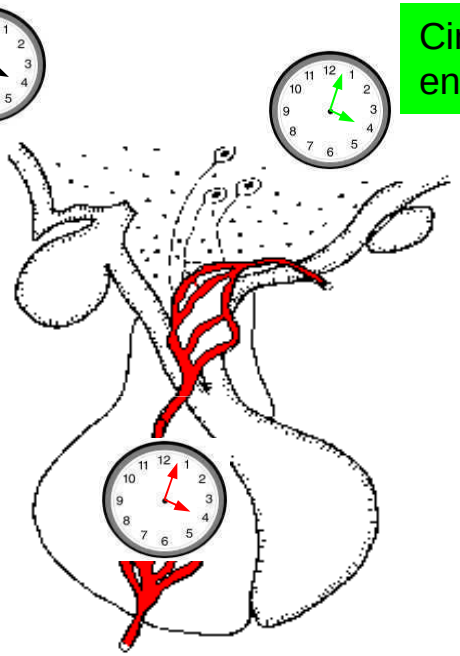


Yoo et al, PNAS 2004

Circadian clock genes along the neuroendocrine axis

Central clock :
Suprachiasmatic nuclei

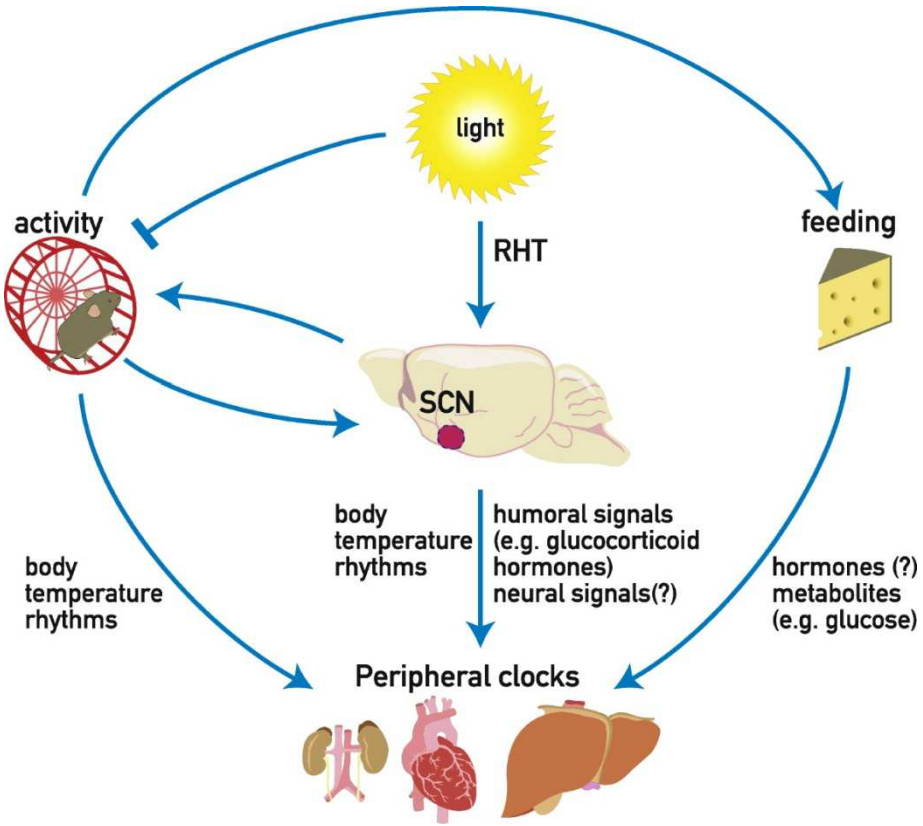
Circadian clock genes in endocrine neurons

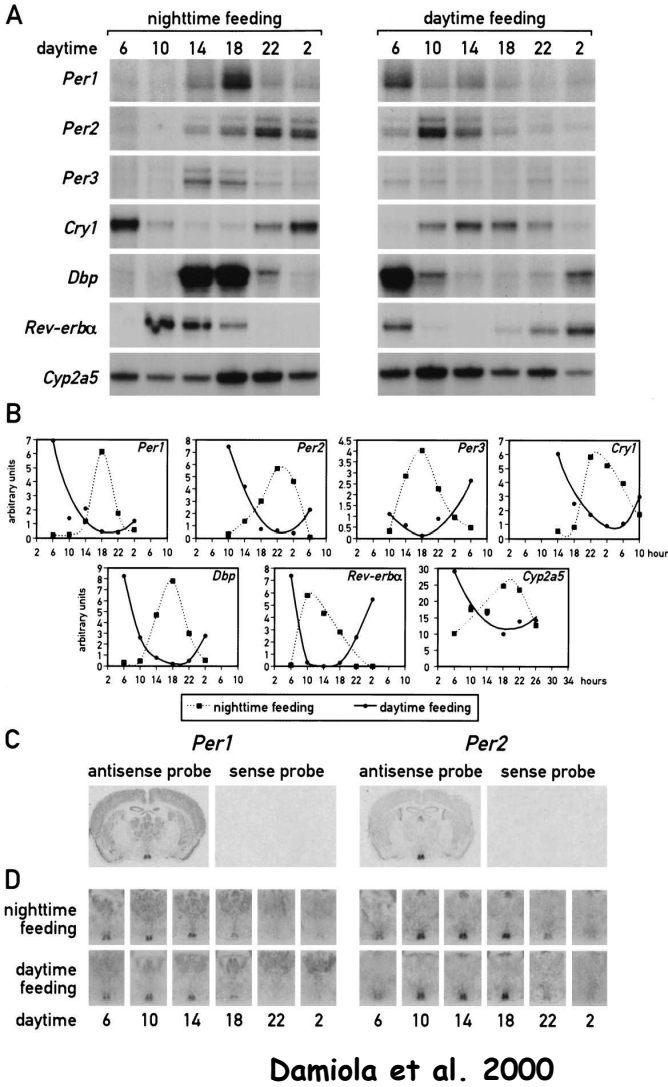


Circadian clock genes in the pituitary ?

Hormonal pulses

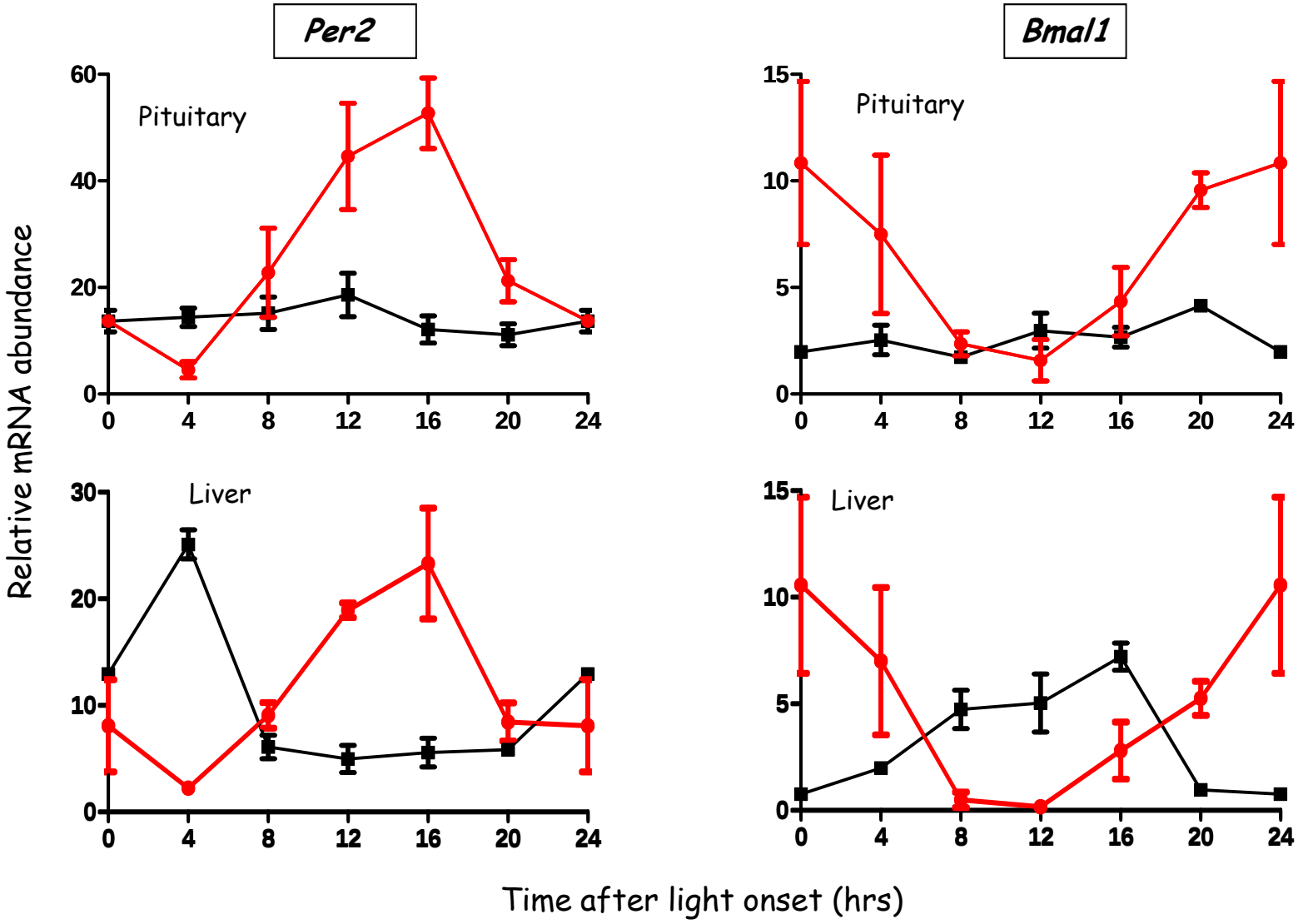
Central and peripheral circadian oscillators are entrained by different cues





Damiola et al. 2000

The pituitary oscillator(s): at the crossroad between peripheral and central clocks



Bur IM, et al PLoS One 2010

Chronobiology International, 29(2): 103–130, (2012)

DNA Microarray Analysis and Functional Profile of Pituitary Transcriptome Under Core-Clock Protein BMAL1 Control

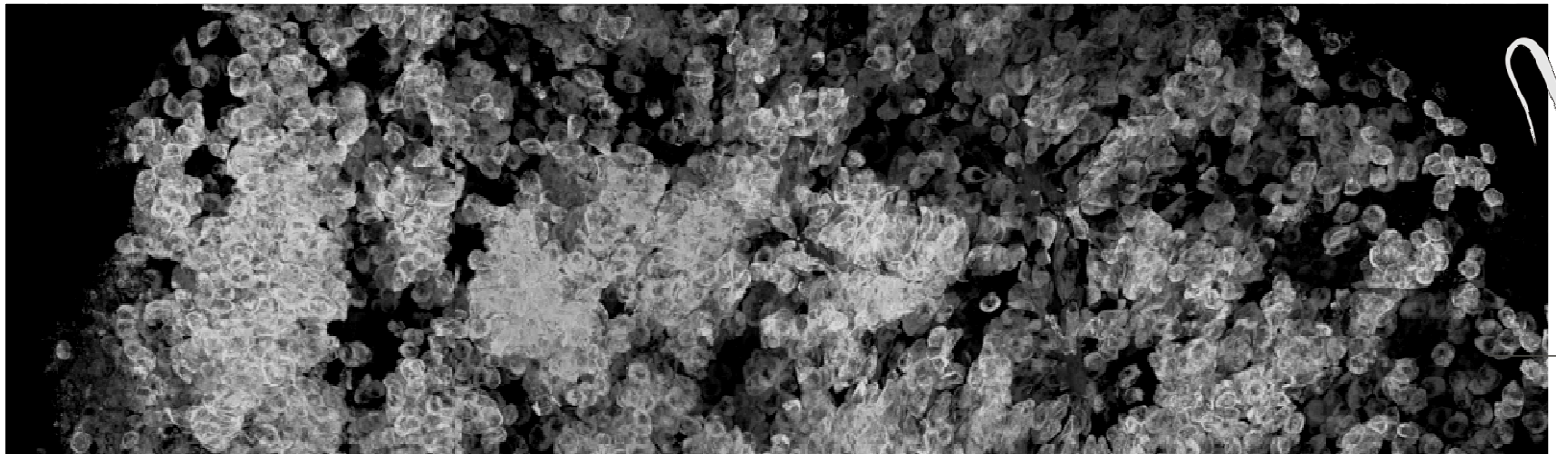
F. Guillaumond,[1] D. Becquet,[2] B. Boyer,[2] O. Bosler,[2] F. Delaunay,[3] J. L. Franc,[2] and A. M. François-Bellan[2]

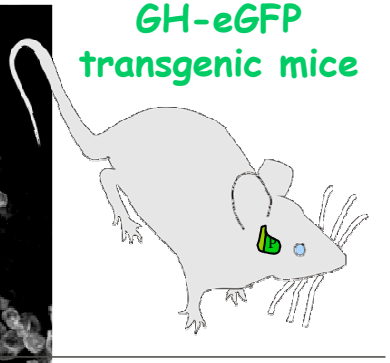
TABLE 3. Biological processes associated with genes whose expression was altered in *Bmal1*$^{-/-}$ mouse pituitary as determined by Gene Ontology-term enrichment

GO ID	Term	Modified genes	*p* value	Fold enrichment	Genes
GO:0048511	Rhythmic process	11	1.056E-06	8.020	*Ccrn4l, Arntl, Npas2, Nr1d1, Dbp, Tef, Per3, Nfil3, Cry1, Ncor1, Clock*
GO:0001558	Regulation of cell growth	6	.0092	4.66	*Nov, Wisp1, Ctgf, Mapt, Esm1, Igfbp3*
GO:0042445	Hormone metabolic process	5	.0369	3.97	*Aldhia2, Rbp4, Dio2, Adh7, Nr3c1*
GO:0009725	Response to hormone stimulus	7	.0279	3.031	*Me1, Rbp4, Hmgcs2, LOC677317, Mgp, Gal, Timp3, Cd24a*
GO:0006629	Lipid metabolic process	23	.0002	2.361	*Pgap2, Rbp4, Scd3, Pik3c2a, Alox12e, Enpp2, Ugt8a, Sorl1, Acot1, Adh7, Nr3c1, Pip5k1a, Gdp3, Aldh1a2, Dgat2, Hmgcs2, Smpdl3a, Sult1a1, LOC100047936, Plcg2, Mboat1, Pla2g7, Hsd11b1, Acsl6*
GO:0051254	Positive regulation of RNA metabolic process	13	.0144	2.217	*Eny2, Cebpb, Thra, Nr4a2, Sox4, Klf15, Arntl, Plagl1, Npas2, Tef, Neurod1, Jak3, Clock*
GO:0007155	Cell adhesion	16	.0119	2.038	*Pvr, Thra, Ptprm, Npnt, Cpxm2, Edil3, Neo1, Cx3cl1, Cd24a, Mia1, Wisp1, Col7a1, Ctgf, Nptn, Cntn1, Spp1*
GO:0010941	Regulation of cell death	15	.0260	1.904	*Pgap2, Cebpb, Nr4a2, Nr3c1, Pea15a, Cx3cl1, Gal, Cd24a, Dapk1, Serinc3, Plcg2, Tgm2, Neurod1, Igfbp3, Spp1*
GO:0006357	Regulation of transcription from RNA polymerase II promoter	16	.0254	1.856	*Cebpb, Thra, Trim27, Nr4a2, Sox4, Arntl, Plagl1, Npas2, Id2, Id1, Tef, Neurod1, Jak3, Per3, Ncor1, Clock*
GO:0048731	System development	44	.0027	1.551	*Rbp4, Insl3, Thra, Npnt, Sox4, Rorc, Cx3cl1, Elk3, Nr3c1, Grem1, Cd24a, Timp3, Ckb, Aldh1a2, Ank1, Serpine2, Ctgf, Mapt, Tgm2, Tgfa, Acsl6, Spp1, Wnt10a, Cebpb, Ugt8a, Nr4a2, Mgp, Gal, Ywhae, Mycn, Id2, Id1, Plcg2, Nptn, Hsd11b1, Ush1c, Neurod1, Id4, Jak3, Fabp7, Igfbp3, Tnfaip2, Ncor1, Bmp6*

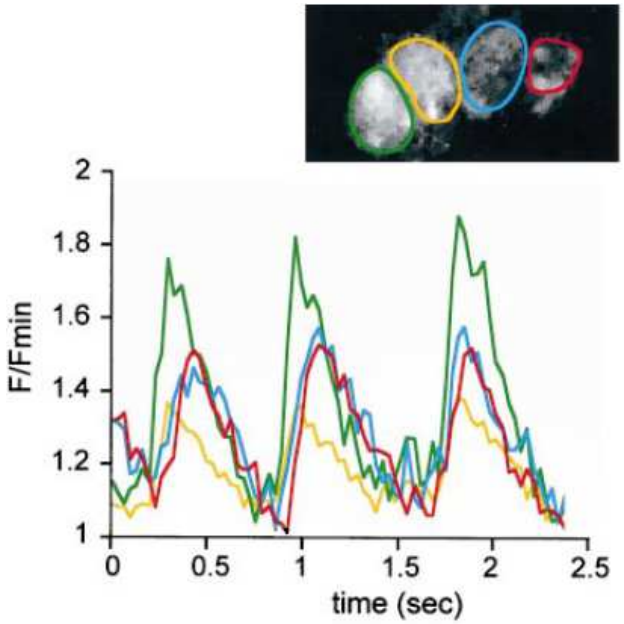
Spatial organization of endocrine pituitary cells

Topological arrangement of endocrine cells in the pituitary gland





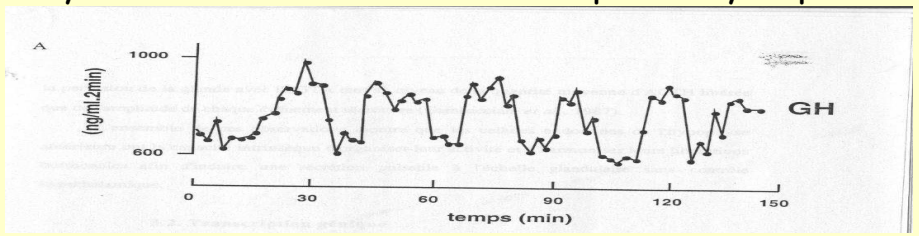
Bonnefont et al. PNAS 2005, Sanchez-Cardenas et al. PNAS 2010



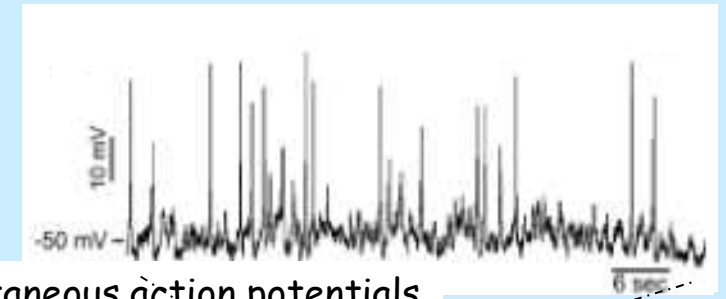
Guérineau, Bonnefont et al. JBC 1998

Intra-pituitary pulsatile activities

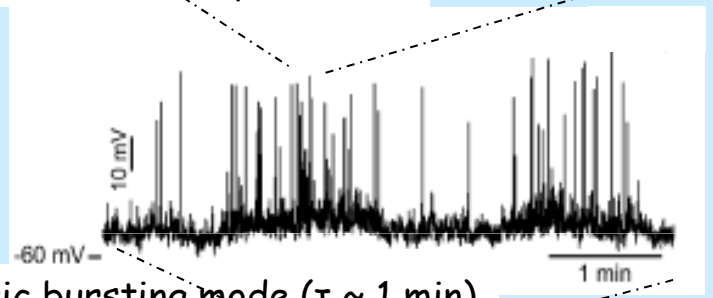
Rhythmic hormonal release from pituitary explants



After Stewart et al, 1985



Spontaneous action potentials



Intrinsic bursting mode (τ ~ 1 min)

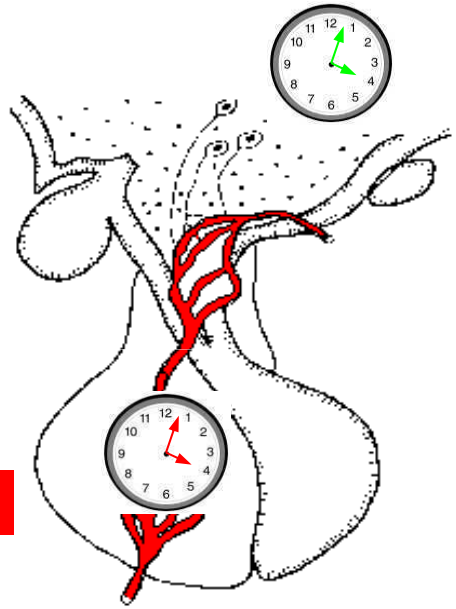
Slow oscillations (τ ~ 10 - 30 min)

Bonnefont and Mollard, FEBS Lett 2003

Are circadian clock genes involved in shaping pituitary rhythms?

Perspective : targeted inactivation of circadian clock genes

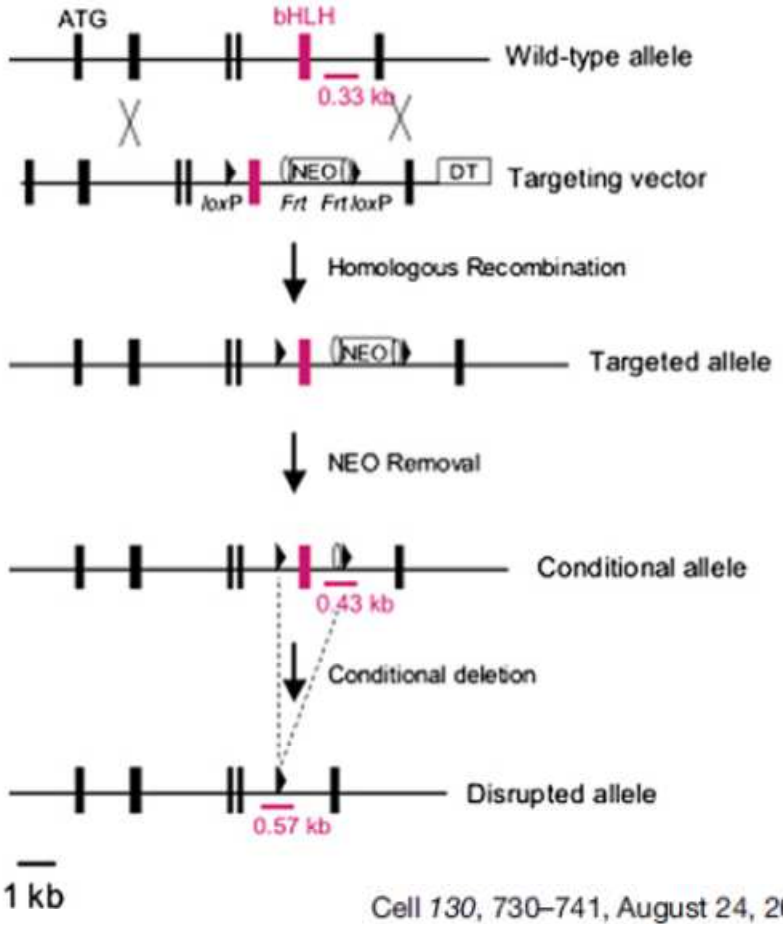
Endocrine neurons of the hypothalamus



Pituitary cells

Hormonal pulses?

Bmal1 conditional mutation targeting strategy



Cell *130*, 730–741, August 24, 2007

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