Laboratório de Cronofarmacologia

Stop, stop, stop…

a necessary pause in the flow of time

INTERCONTINENTAL ACADEMIA

Regina P Markus
Lab of Chronopharmacology
IB USP
April - 2015

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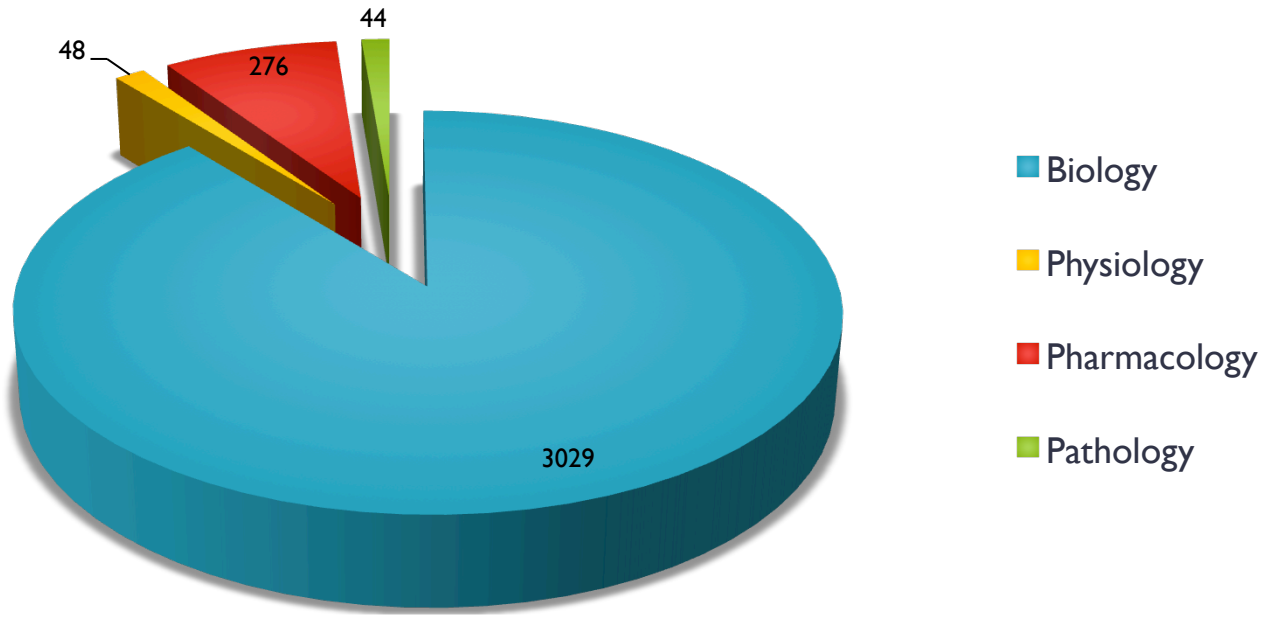
INTERCONTINENTAL ACADEMIA

CHRONO xxxxxx
citations in the PubMED



total - 3397

April 15th 2014

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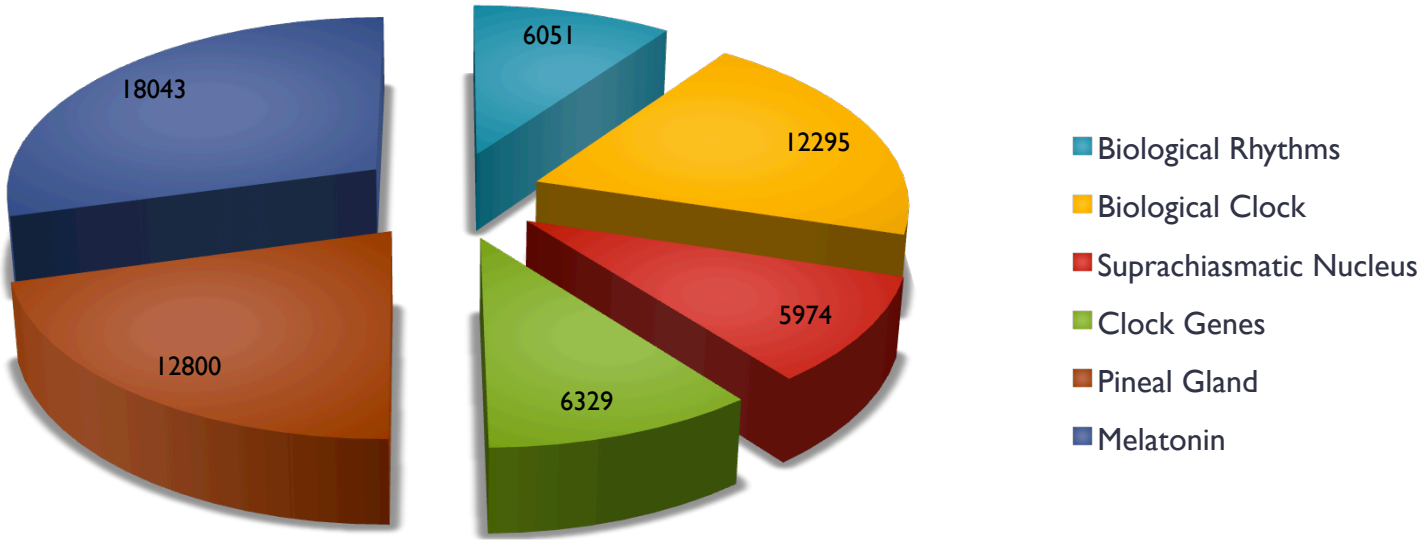
Uniterms

that indentifies the system



total - 61492

April 15th 2014

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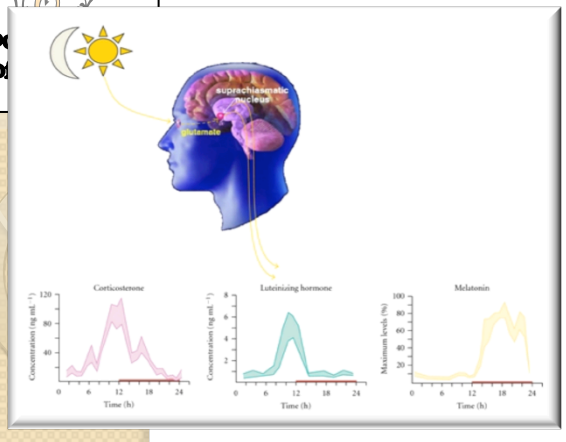
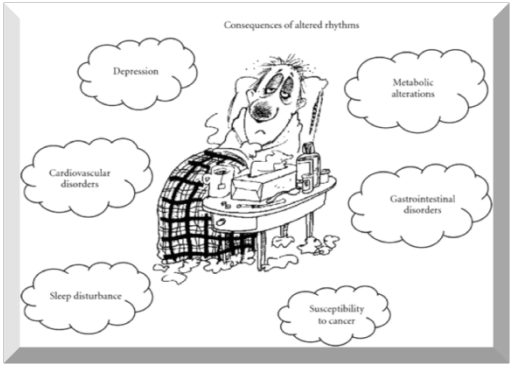

suprachiasmatic nuclei
pineal
Corticosterone
Luteinizing hormone
Melatonin
Time (h)


Arterial pression
respiration
Digestion
Endocrine function
Metabolism
Rest/activity
Memory consolidation
Brain Activity


Consequences of altered rhythms
Depression
Metabolic alterations
Cardiovascular disorders
Gastrointestinal disorders
Sleep disturbance
Susceptibility to cancer

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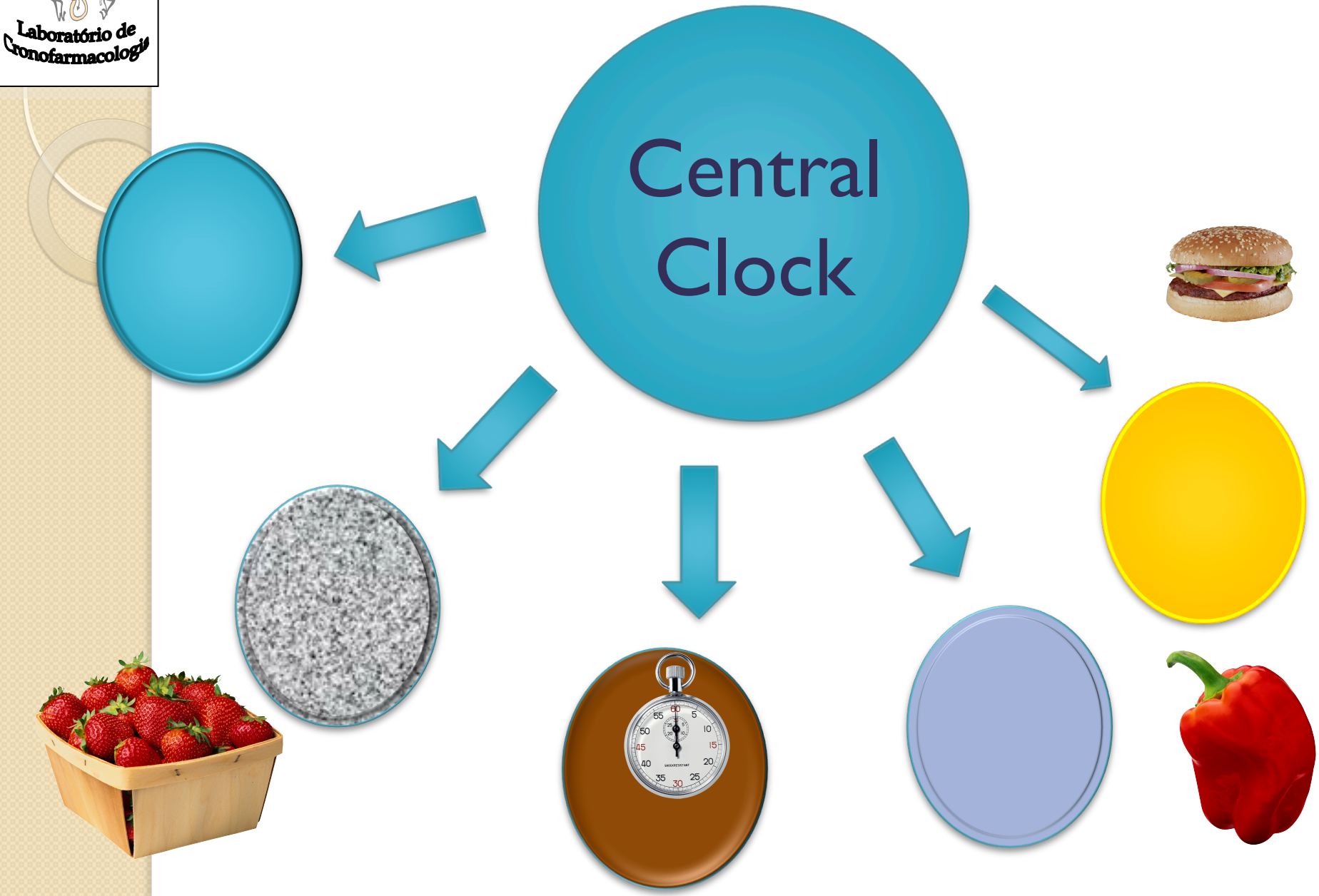

Haus E, Halberg F, Pauly JE, Cardoso S, Kuhl JF, et al. 1972. Increased tolerance of leukemic mice to arabinosyl cytosine with schedule adjusted to circadian system. **Science** 177:80–82

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Wire – directed command neurons -

Global Command –

TIME HORMONES

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Wire – directed command neurons -

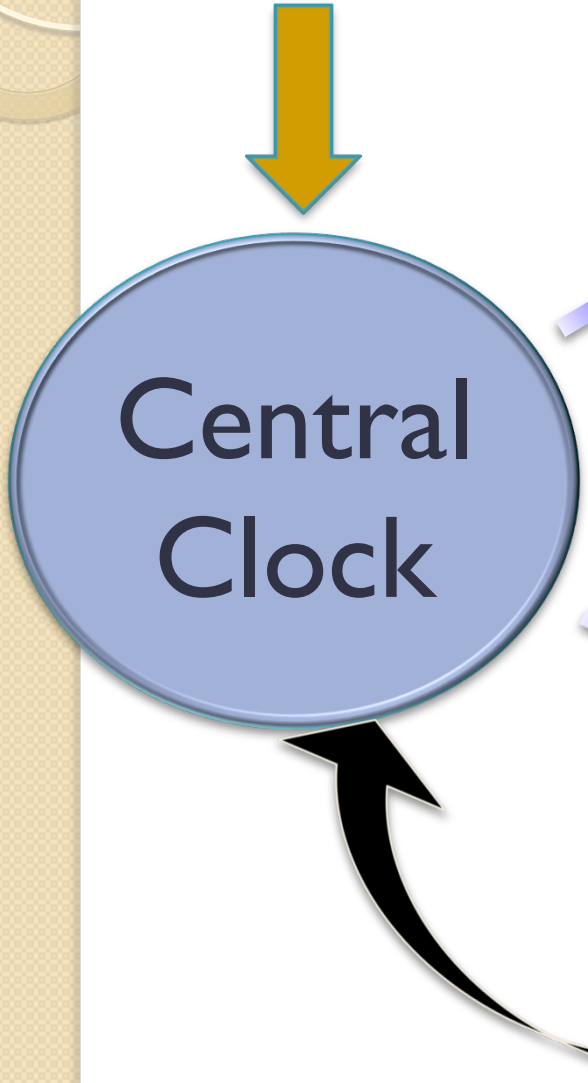
Global Command –

TIME HORMONES

Ooops..... What a mass...

Laboratóri
Cronofarma

Inputs to the clock



Wire – directed command neurons -

Global Command –

TIME HORMONES

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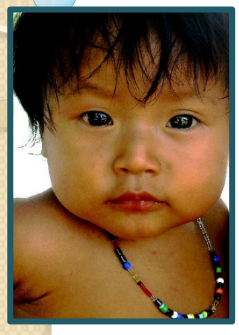
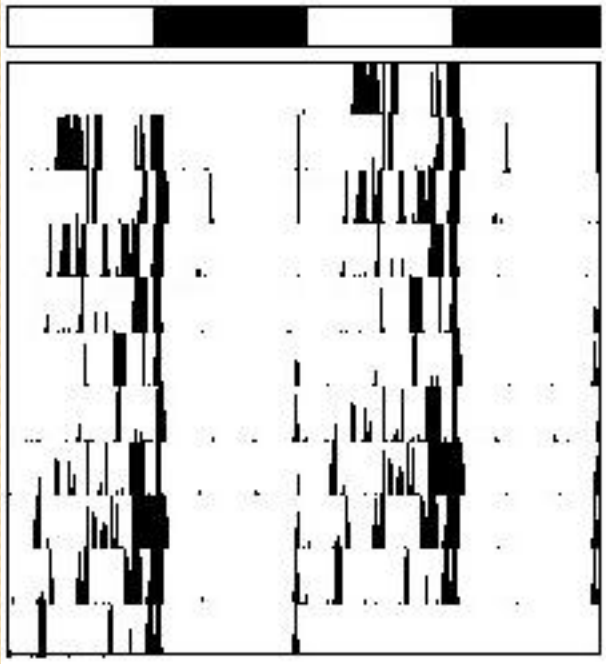
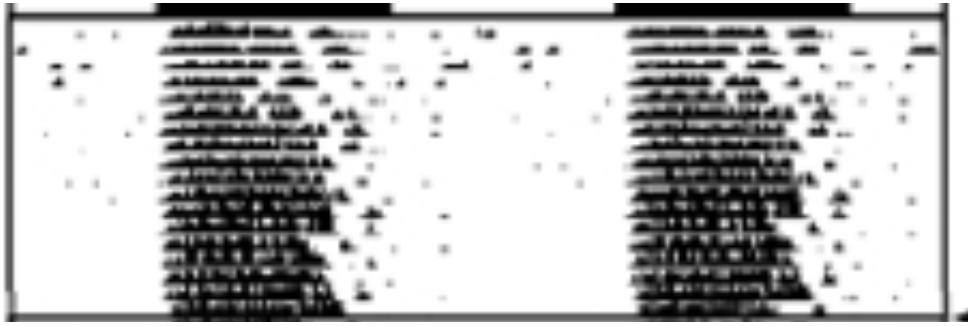


24 hours 24 hours



24 hours 24 hours



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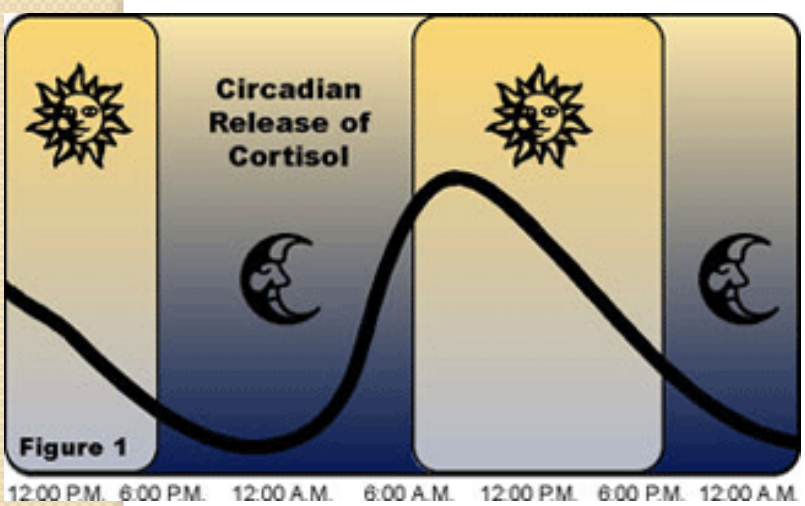
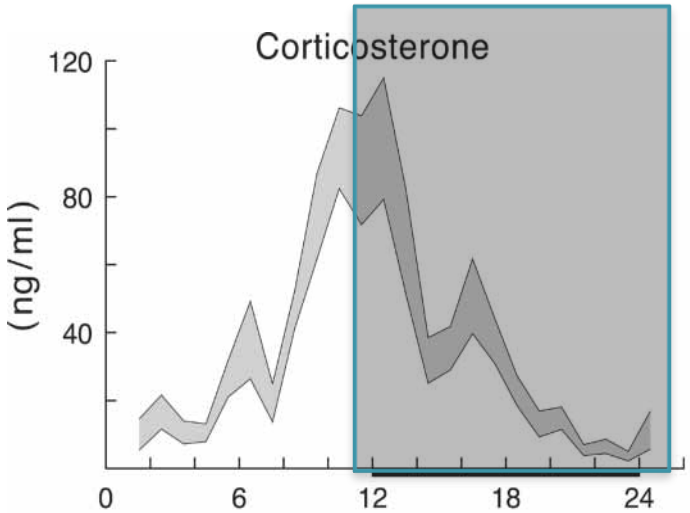
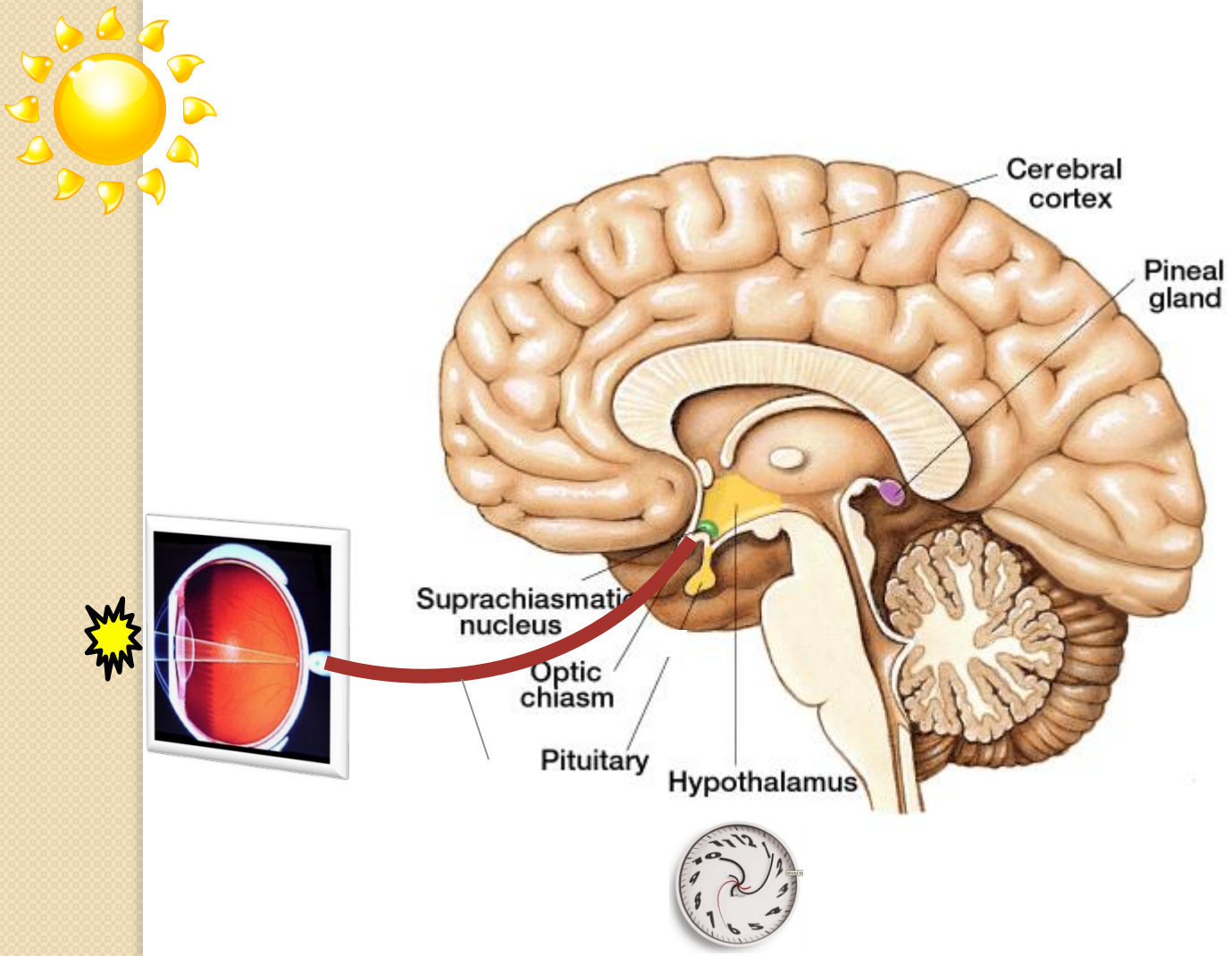
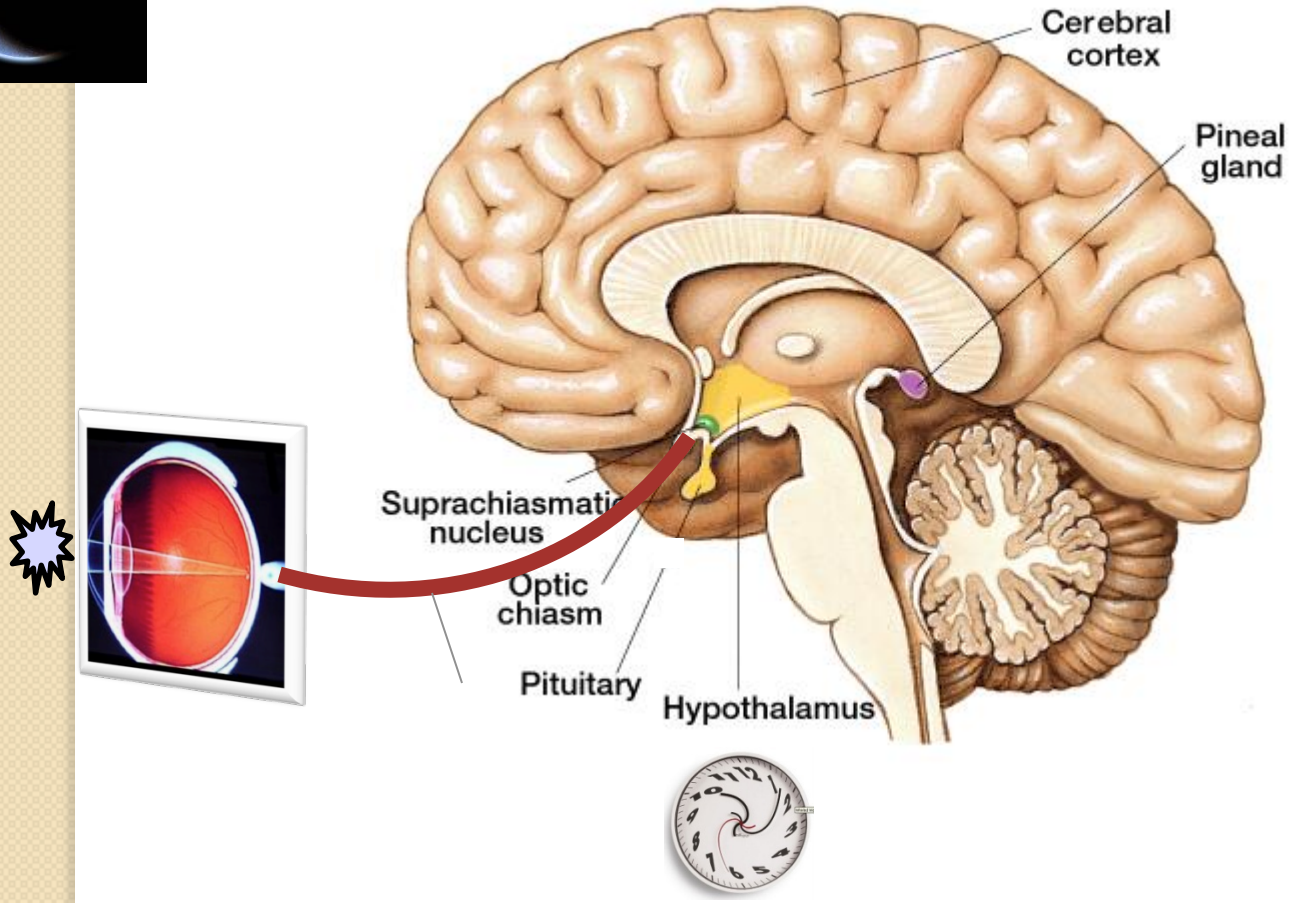






Modificado de http://history.wisc.edu/sommerville/351/351-19.htm



Modificado de http://history.wisc.edu/sommerville/351/351-19.htm

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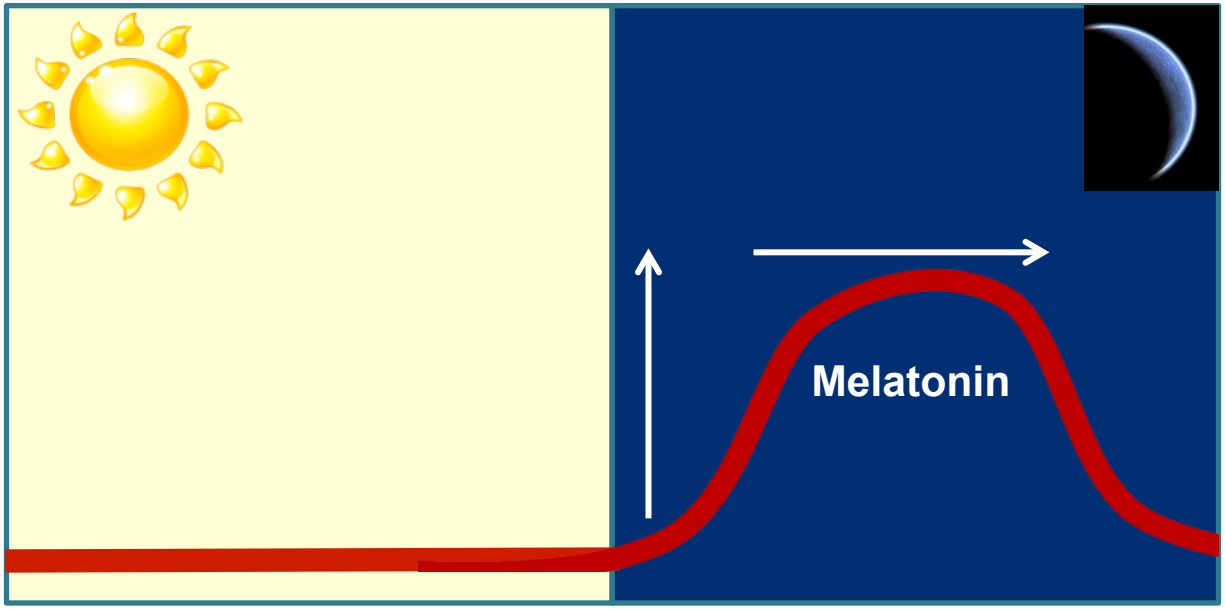
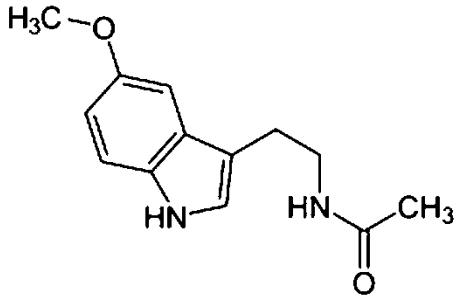
Melatonin – the darkness hormone

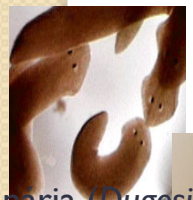




Melatonin the darkness hormone

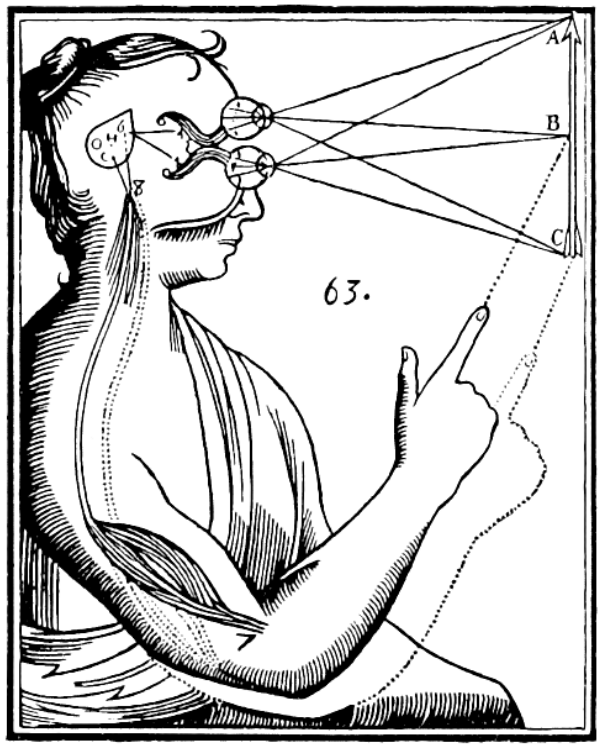
Dinoflagelados

algas verdes (*Euglena*)



planária (*Dugesia* sp.)

• c r u s t á c e o s (*Procambarus clarkii*).



René Descartes, dedicating much time to the study of the pineal gland, called it the **"principal seat of the soul"**.[36]

He believed it to be the point of connection between the intellect and the body.[37]

Descartes attached significance to the gland because he believed it to be the only section of the brain to exist as a **single part rather than one-half of a pair**.

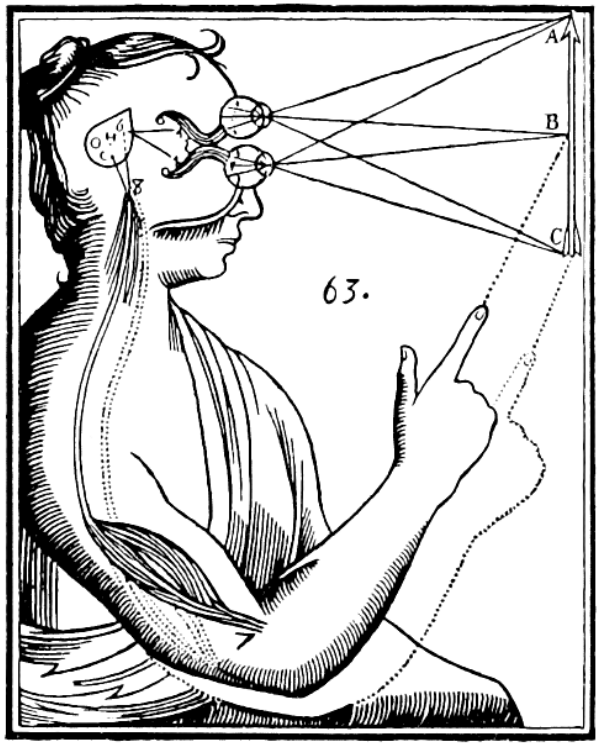
He argued that, because a person can never have "more than one thought at a time," external stimuli must be united within the brain before being considered by the soul, and he considered the pineal gland to be situated in "the most suitable possible place for this purpose," located centrally in the brain and surrounded by branches of the carotid arteries.[36]

http://en.wikipedia.org/wiki/Pineal_gland

Descartes and the Pineal Gland (Stanford Encyclopedia of Philosophy)

Jump up

^ Descartes R. "The Passions of the Soul" excerpted from "Philosophy of the Mind," Chalmers, D. New York: Oxford University Press, Inc.; 2002. ISBN 978-0-19-514581-6



Baruch de Spinoza criticized Descartes' viewpoint for neither following from self-evident premises nor being "clearly and distinctly perceived" (Descartes having previously asserted that he could not draw conclusions of this sort), and questioned what Descartes meant by talking of "the union of the mind and the body."[38]

http://en.wikipedia.org/wiki/Pineal_gland

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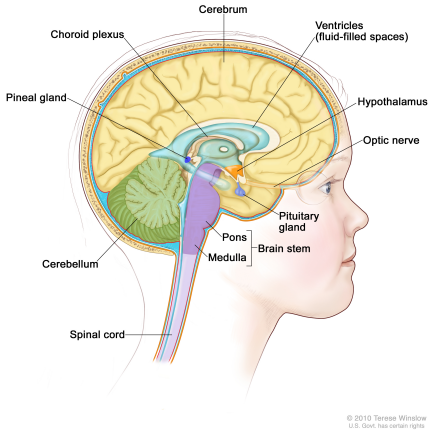
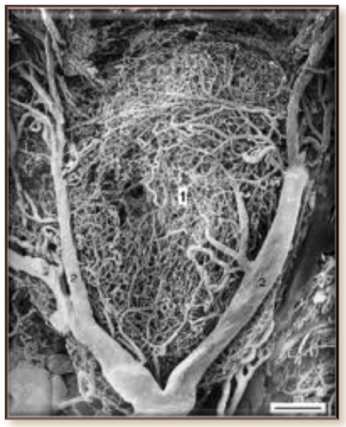
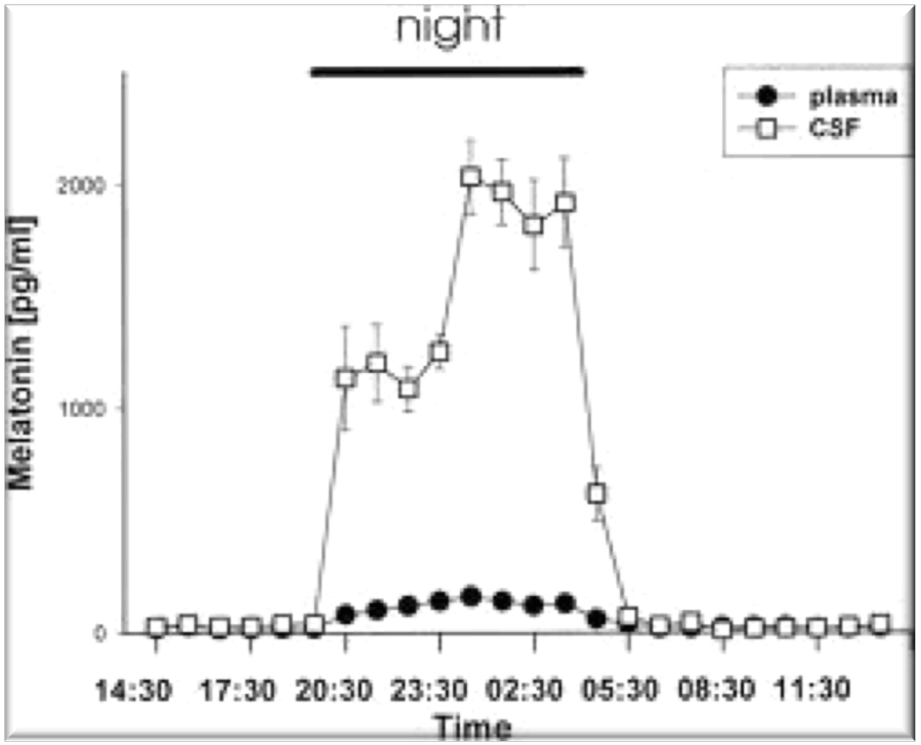
Melatonin to blood and CSF







Skinner and Malpaux Endocrinology **140**:4399–4405, 1999

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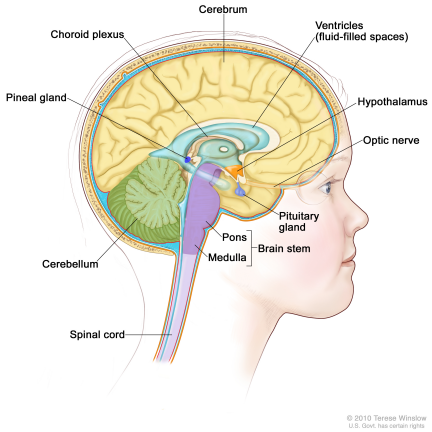
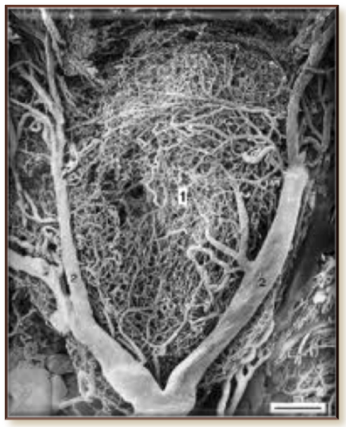
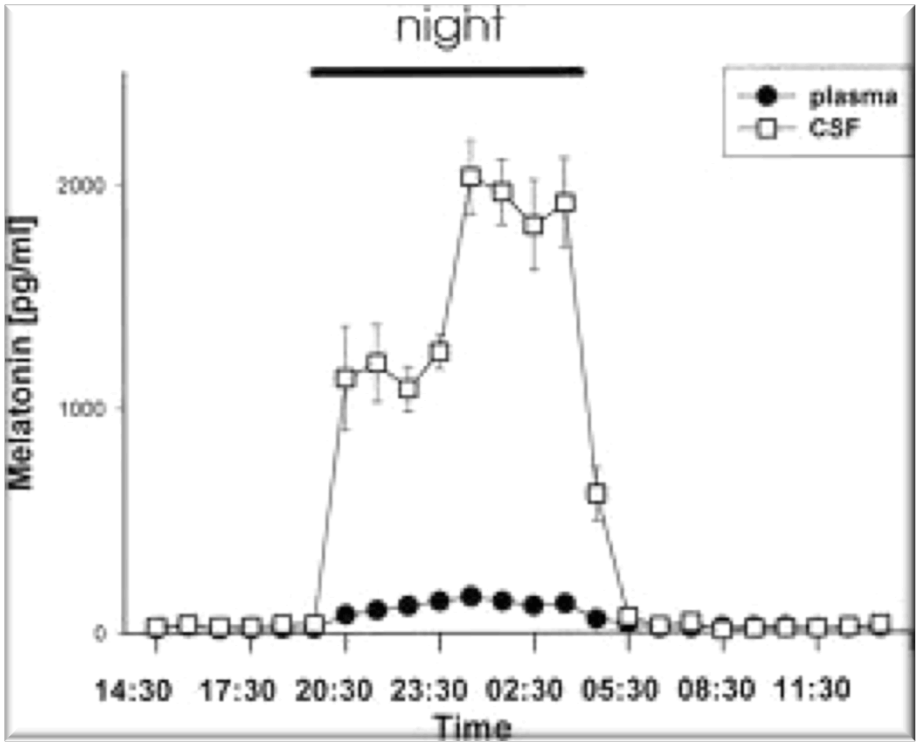
Melatonin to blood and CSF







Skinner and Malpaux Endocrinology **140**:4399–4405, 1999

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Melatonin Function

- **Temporal transduder** – darkness hormone
- **Cytoprotector** –
  - inhibit the pivotal transduction factor of inflammatory responses – NF-κB
  - antioxidant
- **Diseases**
  - Alzheimer's Disease
  - Cancer
  - Diabetes
  - autism
  - depression
  - sleep disturbance

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- Melatonin as an anti-inflammatory agent

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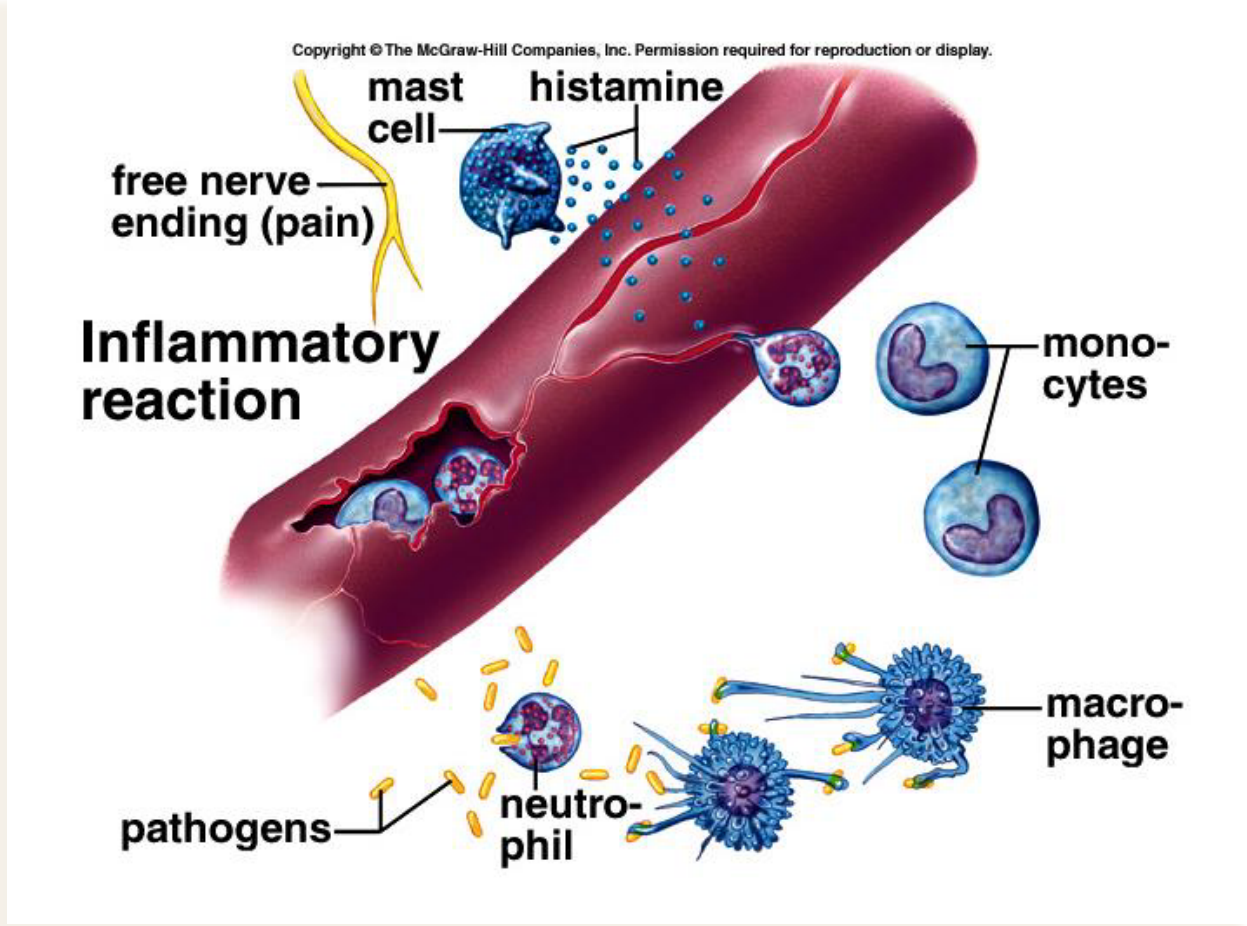
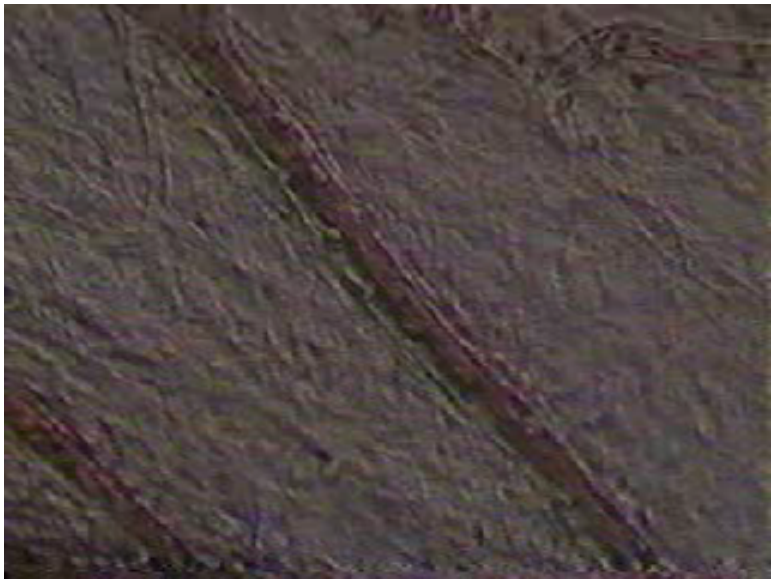
- Melatonin as an anti-inflammatory agent

Rolling, adhesion and transmigration of leukocytes



Intravital microscopy
LTB4 –induced leukocyte migration



Lotufo et al., Eur J Pharmacol. 2001

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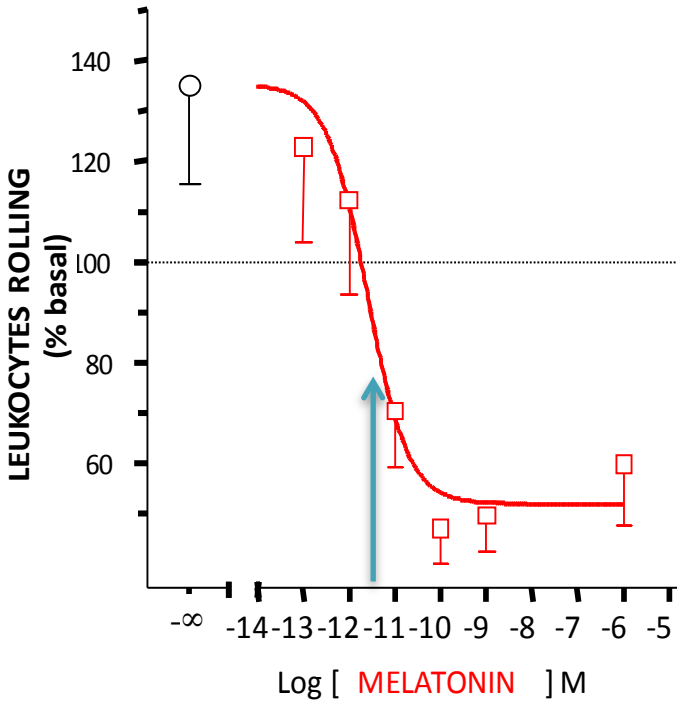
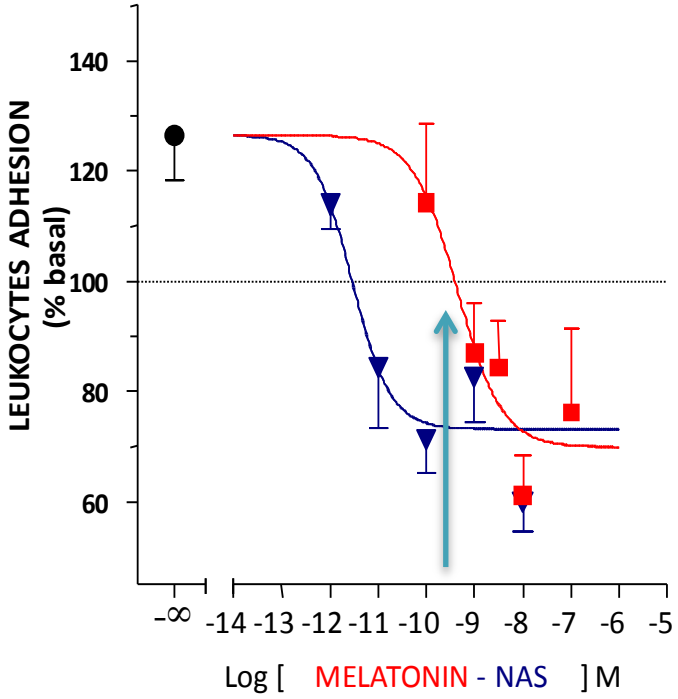
Effect of Melatonin on Rolling and Adhesion (LTB4) of Leukocytes



NAS no effect

MT2 R



MT3 R

Lotufo et al., 2001 –

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Big Challenge!!!!

Rats are nocturnal animals...

Why should the mounting of an inflammatory response be impaired at night?

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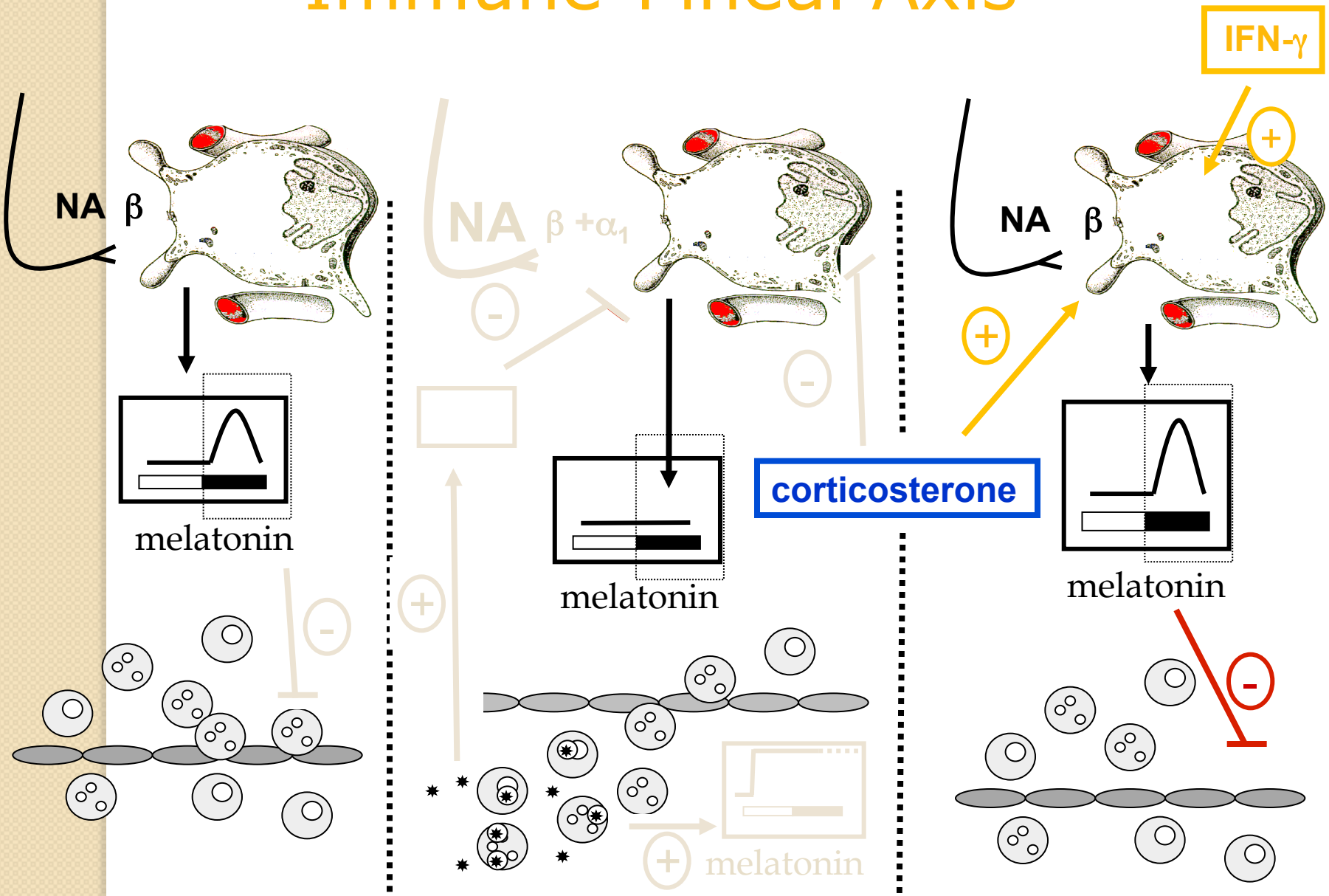
A basic characteristic of the inflammatory response is that it develops along the time in a very precise manner: **pro-inflammatory phase is followed by a recovery phase.**

The pro-inflammatory phase is designed to combat aggression, while the recovery phase provide the proper elements for healing the injured area.

Without a proper mounting of the inflammatory response, the recovery phase is also incomplete, favoring chronic inflammation, degenerative disease, or even cancer.

Immune-Pineal Axis



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Immune-Pineal Axis - Working Hypothesis

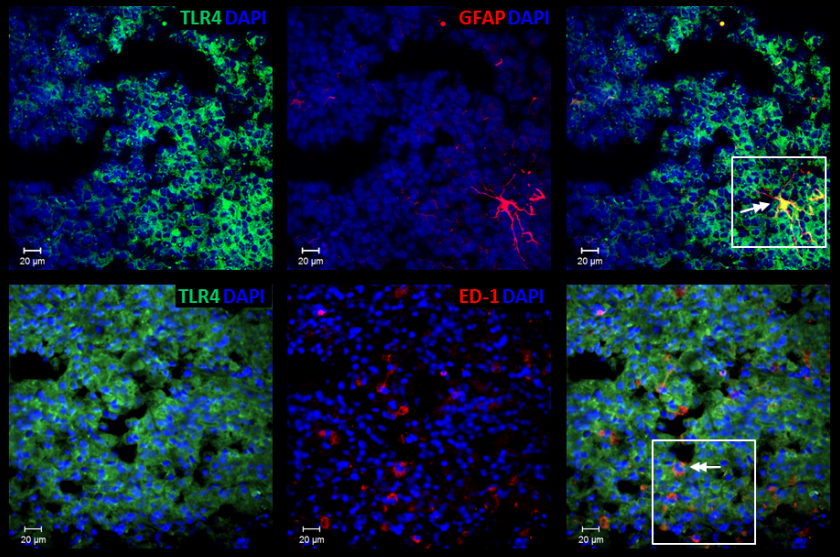
The pineal gland plays a role in:

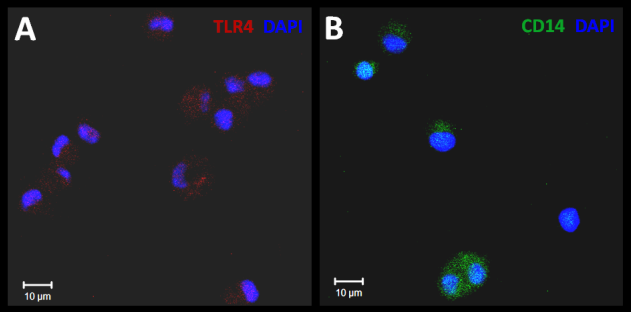
1 – surveillance against undesirable cell migration:
melatonin inhibits migration of blood cells to tissue under normal conditions

2 – mounting of an inflammatory response:
receptors for cytokines and pathogen associated molecular patterns located in pinealocytes → reduce or even suppress MEL synthesis

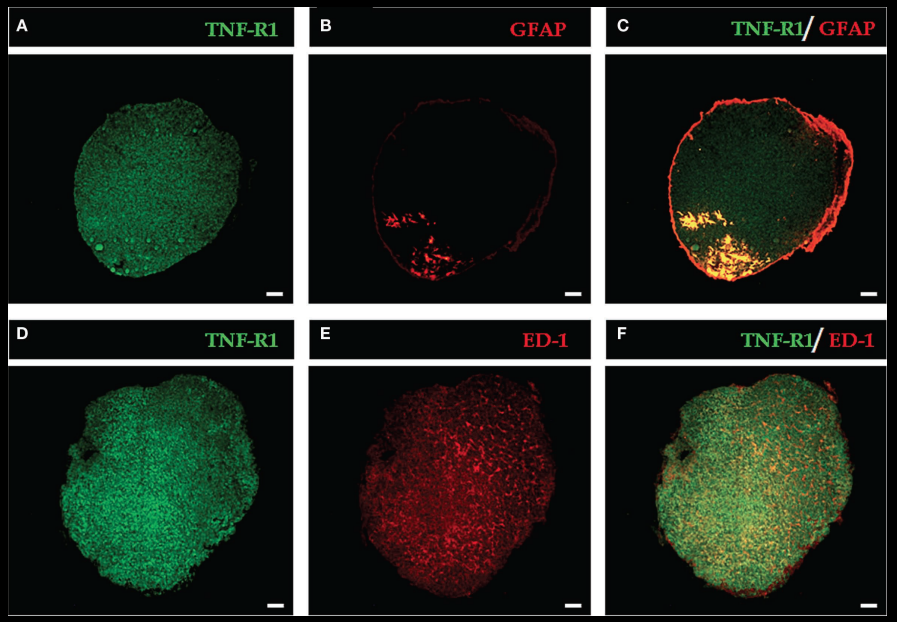
3 – activated immune-competent cells produce melatonin which will act locally favoring the recovery phase

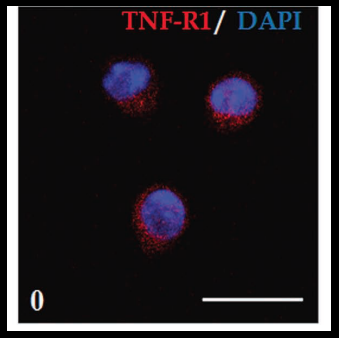
Binding Molecules to LPS





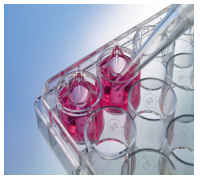
Receptor for TNF

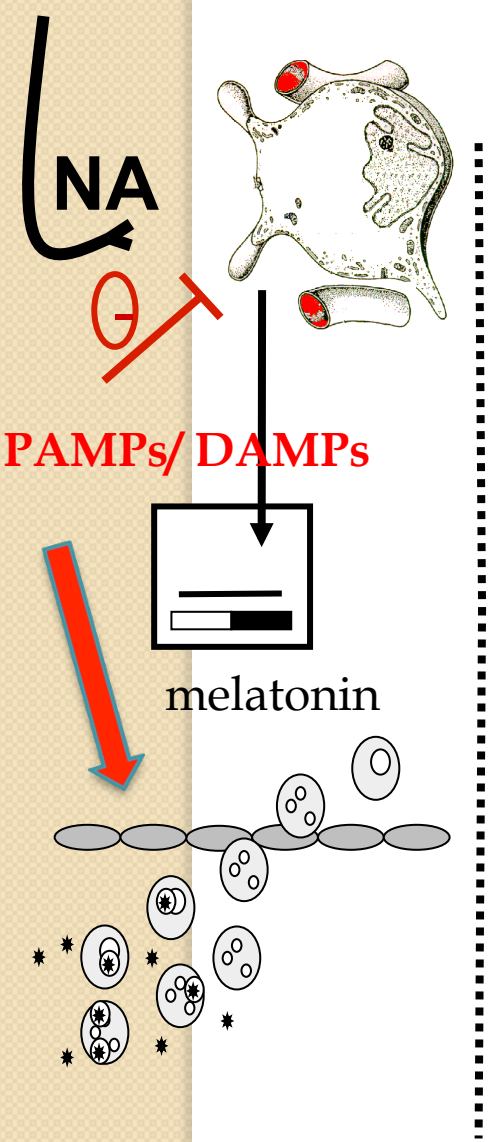


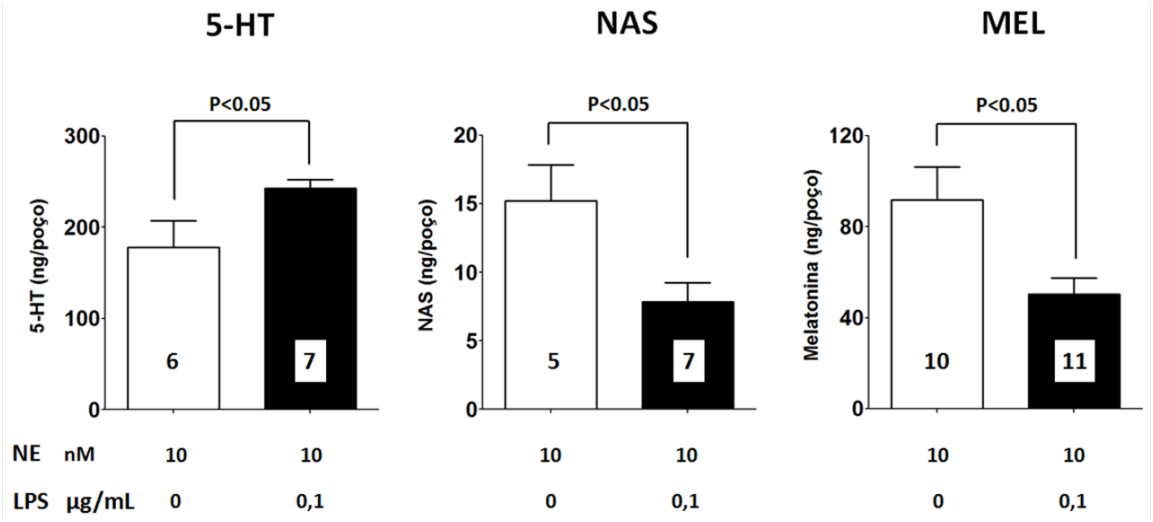


Cruz-Machado et al., 2010, Carvalho-Sousa et al., 2011

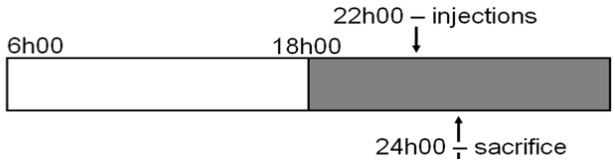
TRP → 5HTP → 5HT → NAS → MEL

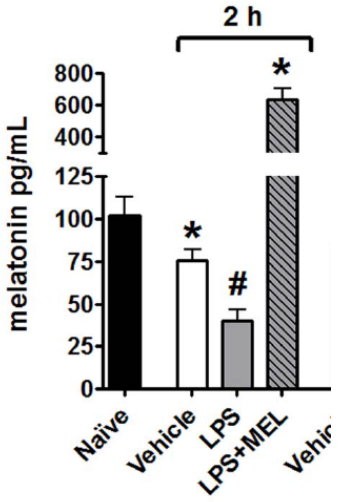






IV injection



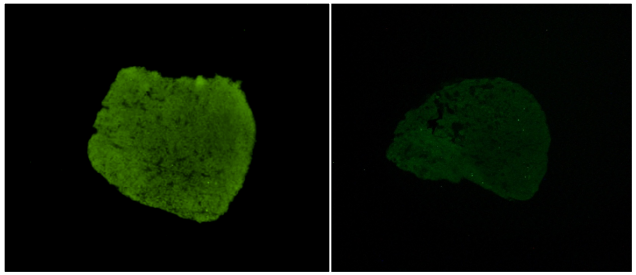


Rats LPS icv

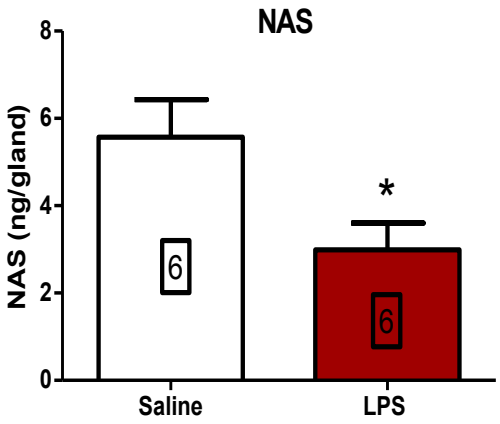
TRP → 5HTP → 5HT → NAS → MEL

LPS (3µg/5µl) icv
at ZT 6 and killed at ZT 18

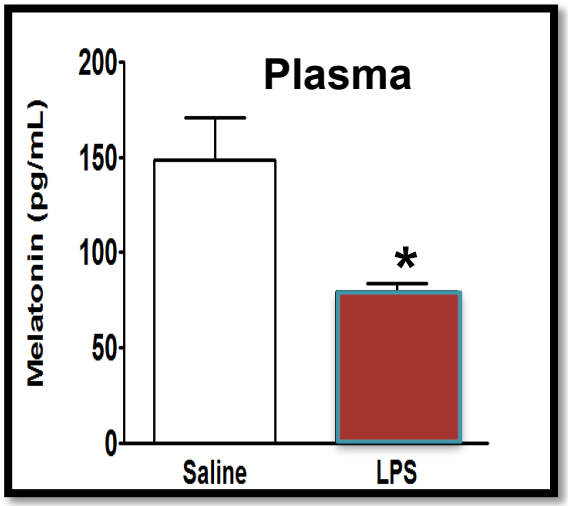
Pineal – AA-NAT



Pineal



Plasma



Pinato et al., Brain, Structure and Function, 2014

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P
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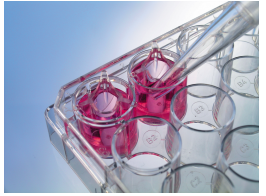
Stops Melatonin synthesis vitro and in vivo



Bacteria – (Tamura et al., 2010)
Funghi – (Pires-Lapa et al., 2012)
Cytokines – (Fernandes et al., 2006)
Hormones – (Fernandes et al., 2009)

Beta- amyloid peptide (Alzheimer) – (Cecon et al., 2015)

And…

Does not….. parasites

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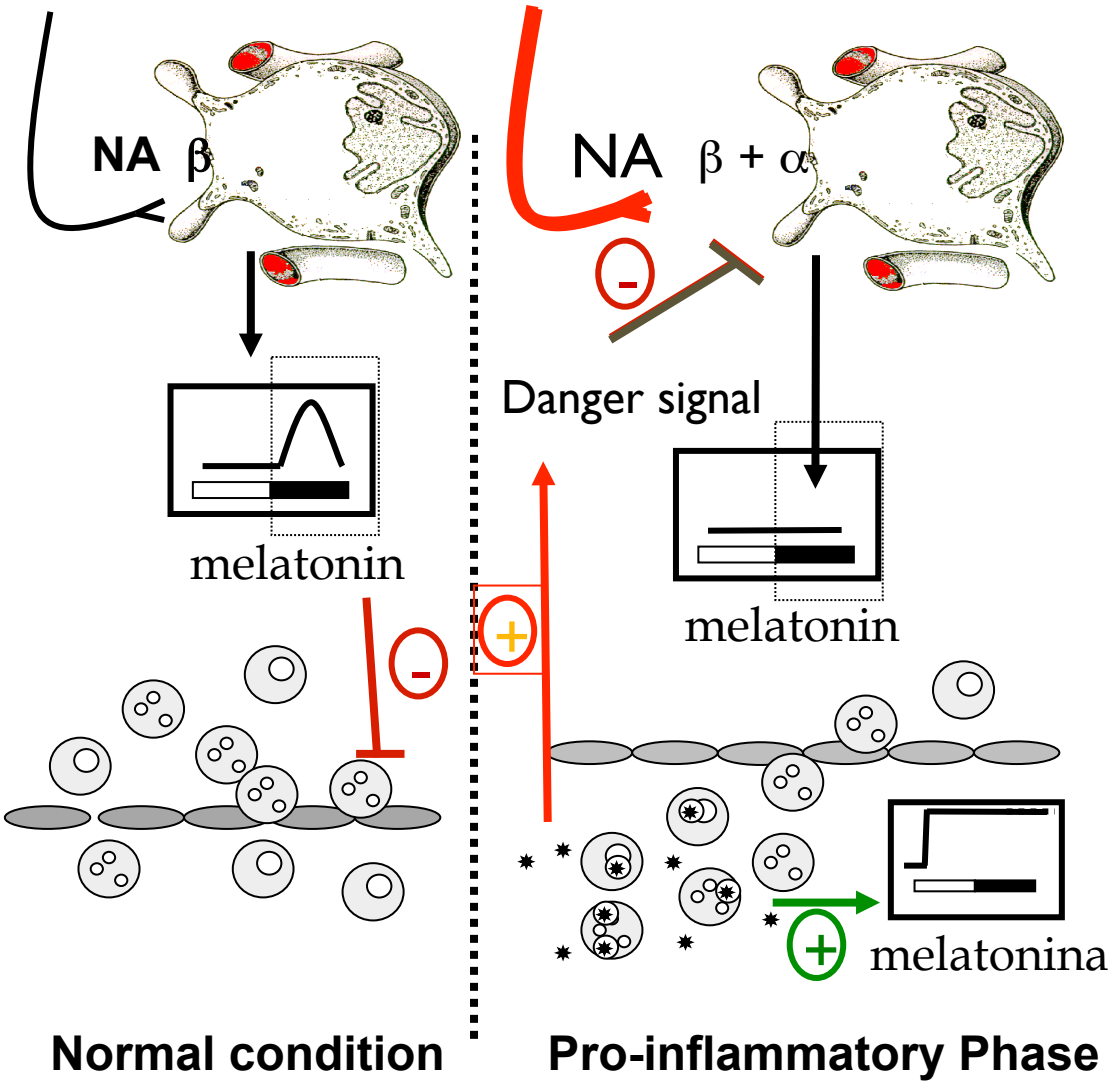
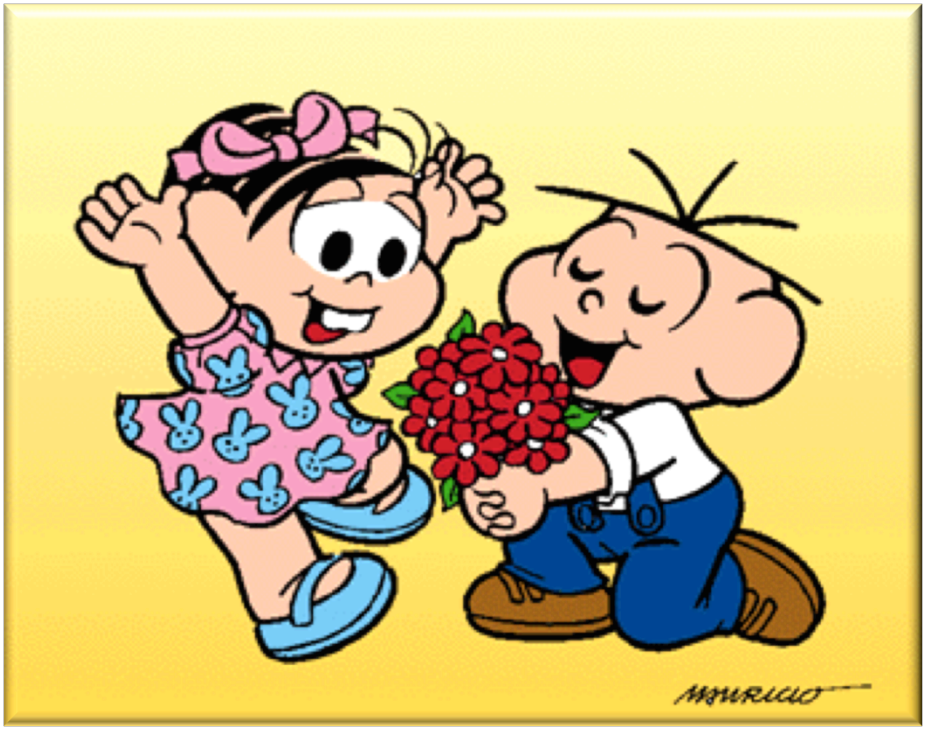
Immune-Pineal Axis




MAURICIO

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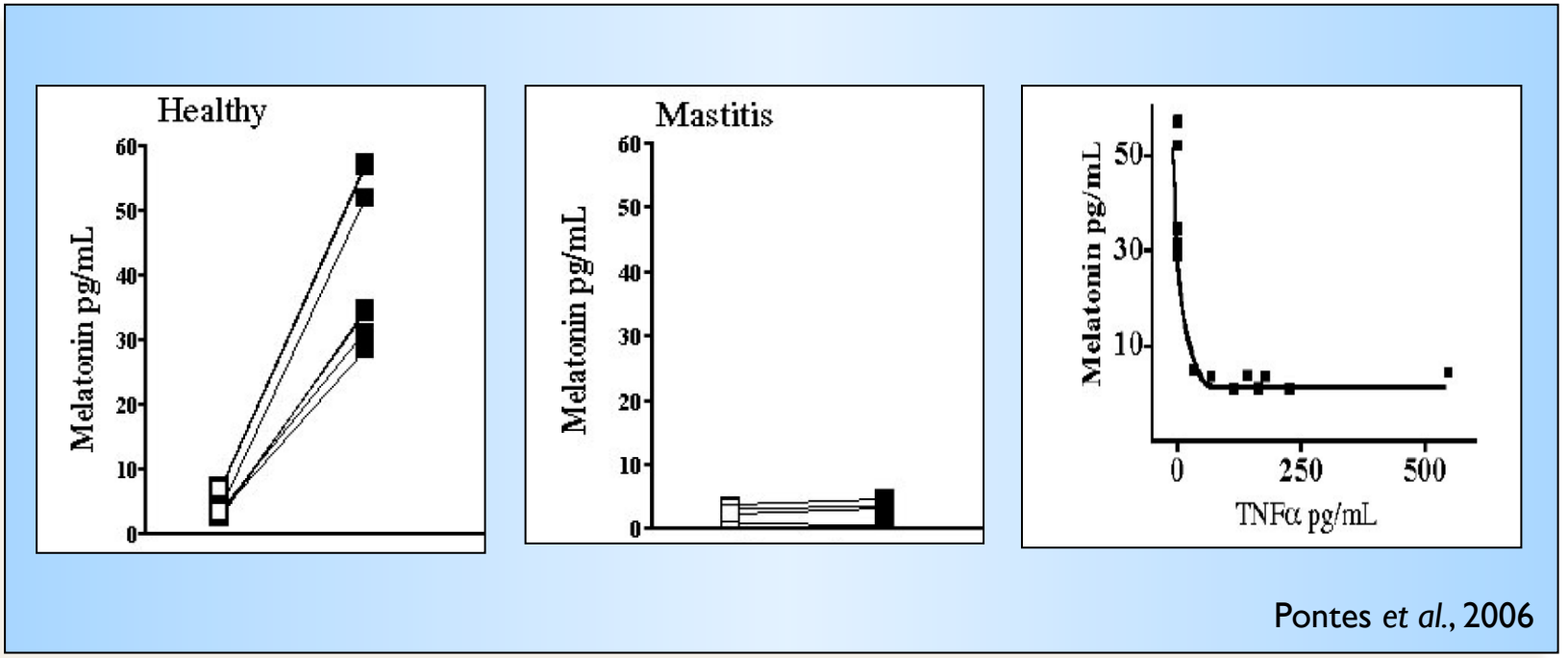
PINEAL

Mastitis → suppresses nocturnal MEL surge



Pontes *et al.*, 2006

Colostrum (milk of the first days after delivery; contains cells) → day 3

Maternity Unit at the Obstetric Clinics – USP, Br. The criteria for recently delivered mothers were: age (18–40), gestational age (37 weeks or more).
All the mothers had given birth to healthy term babies.-

PINEAL

NeuroImmunoModulation

Original Paper

Neuroimmunomodulation 2013;20:205–212
DOI: 10.1159/000347160

Received: July 9, 2012
Accepted after revision: January 9, 2013
Published online: May 8, 2013

The Concept of the Immune-Pineal Axis Tested in Patients Undergoing an Abdominal Hysterectomy

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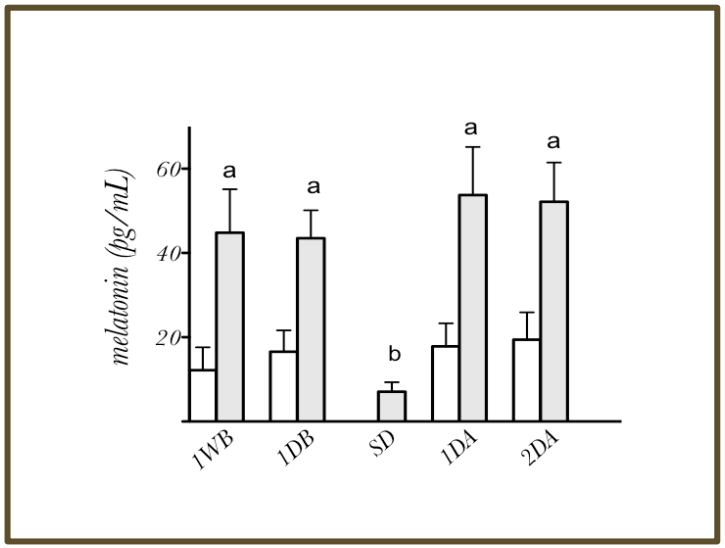
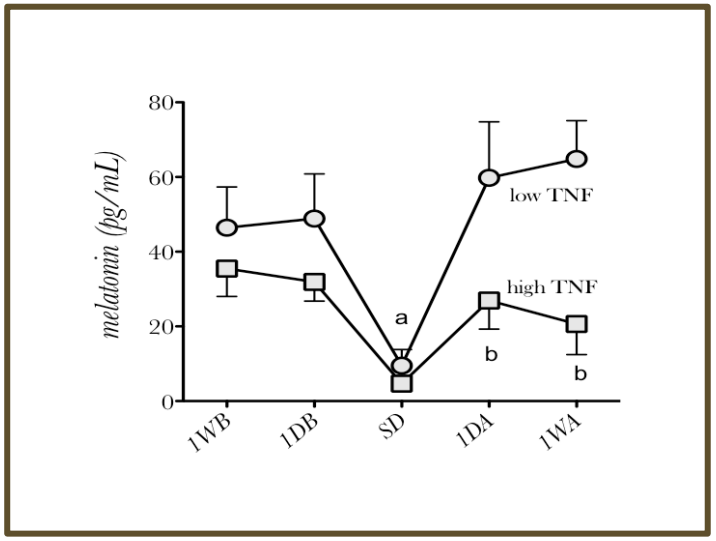
Pedro Augusto Carlos Magno Fernandes[g] Iraci L.S. Torres[a, b, d]
Maria Paz L. Hidalgo[c, f] Regina P. Markus[g] Wolnei Caumo[a, b, d, e]





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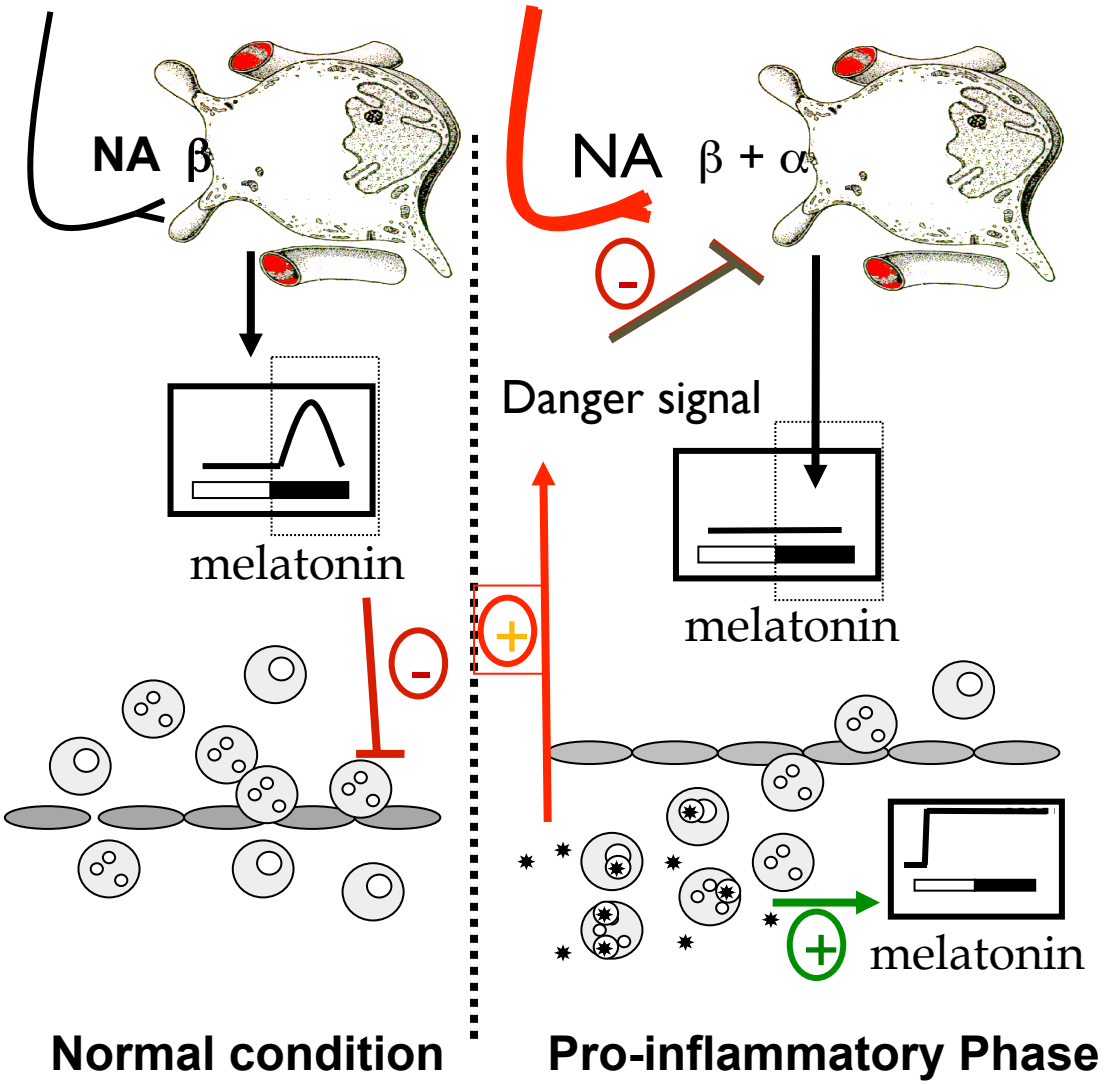
Immune-Pineal Axis



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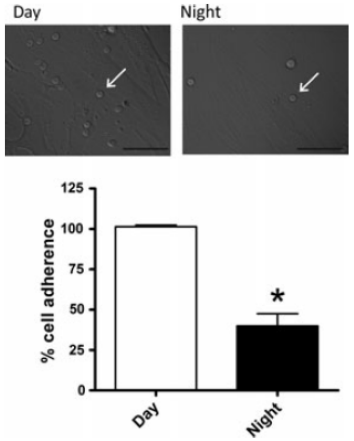
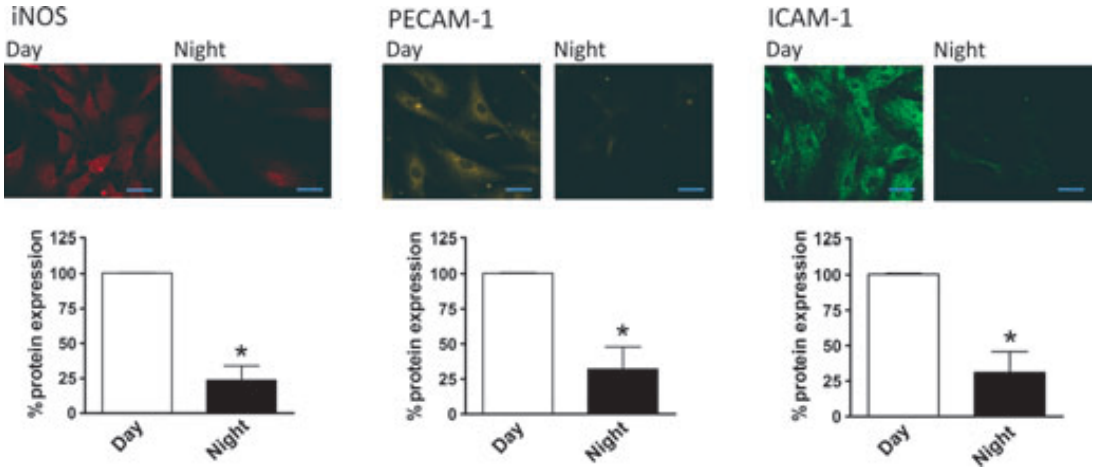
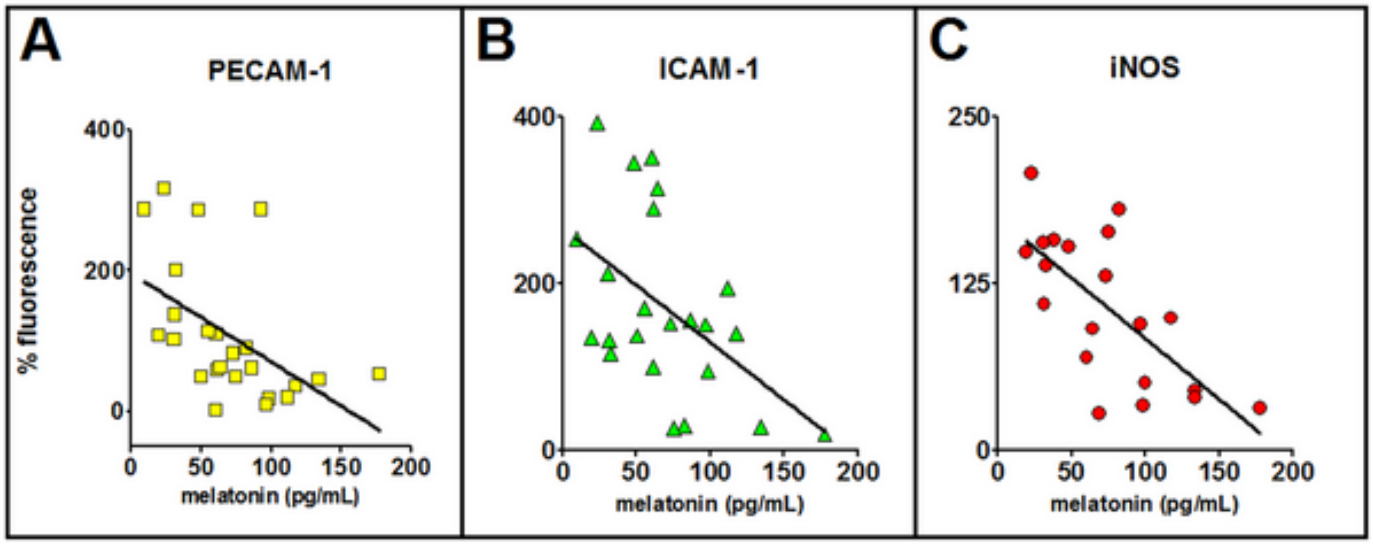
Melatonin PRIMES endothelial cells





Marçola et al., 2012

Melatonin PRIMES endothelial cells



Tamura et al., 2010

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INTERCONTINENTAL ACADEMIA

Questions????

What we raised??

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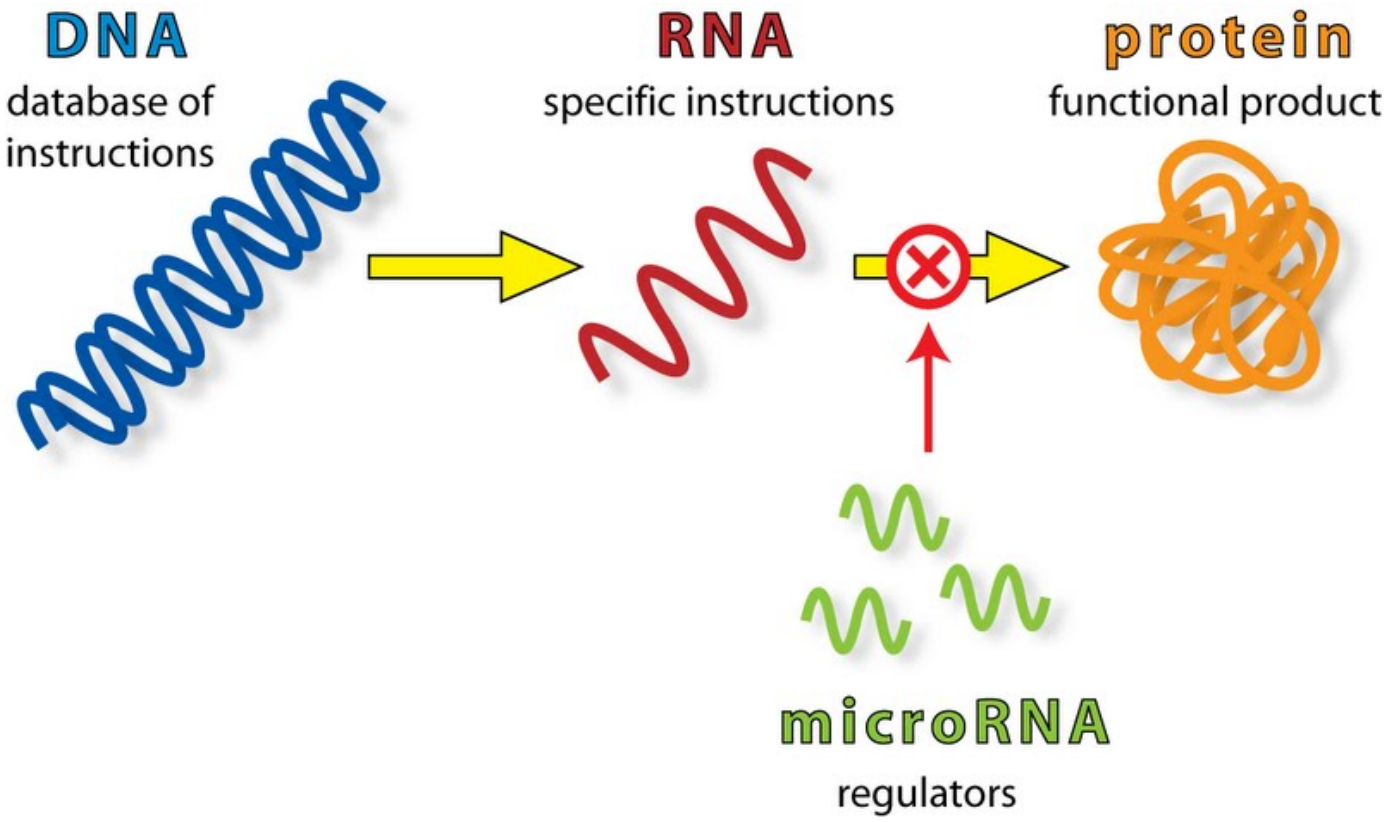
INTERCONTINENTAL ACADEMIA

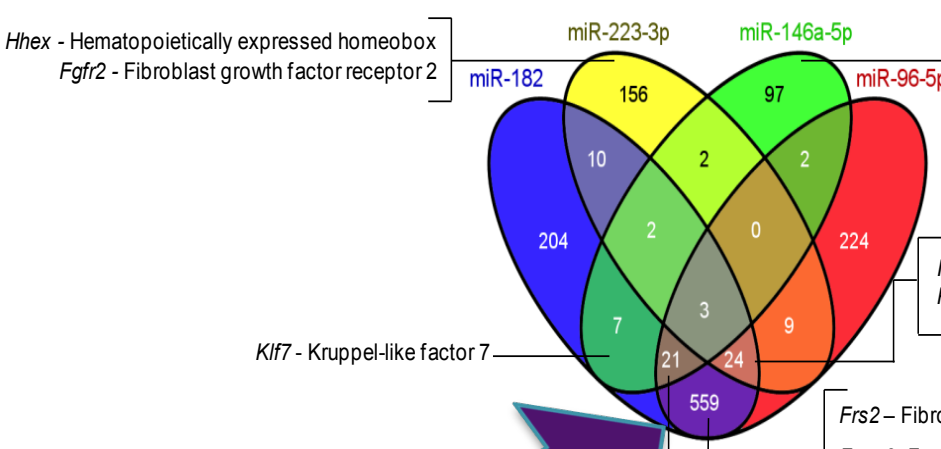
Questions????

How to control in a massive way the genomic output???

Via "junk" --- "trash" RNAs???


DNA
database of instructions
RNA
specific instructions
protein
functional product
microRNA
regulators

VENN diagram of the predicted targets of miRNAs more expressed in nighttime cells



Hhex - Hematopoietically expressed homeobox
Fgfr2 - Fibroblast growth factor receptor 2

Irak1 - Interleukin-1 receptor-associated kinase 1
Traf6 - TNF receptor-associated factor 6
Kfl4 – Kruppel-like factor 4

Igf1r - Insulin-like growth factor 1 receptor
Prtg - Protagenin

Klf7 - Kruppel-like factor 7

Sp8 - Sp8 transc

Frs2 – Fibroblast growth factor receptor substrate 2

Foxo3, Foxn3, Foxf2, Foxq1, Foxk2, Foxp2, Foxo1 – Forkhead Box Family

Cadm1/2 – Cell adhesion molecule 1 and 2

Lims1 , Lhx1, Lhx3 - Proteins associated to LIM domain

Creb - cAMP response element-binding protein

Efnb2 - Ephrin-B2

Fgf9 - Fibroblast growth factor 9 (glia-activating factor)

Myadm - Myeloid-associated differentiation marker

Grb2/10 - Growth factor receptor-bound protein 2 and 10

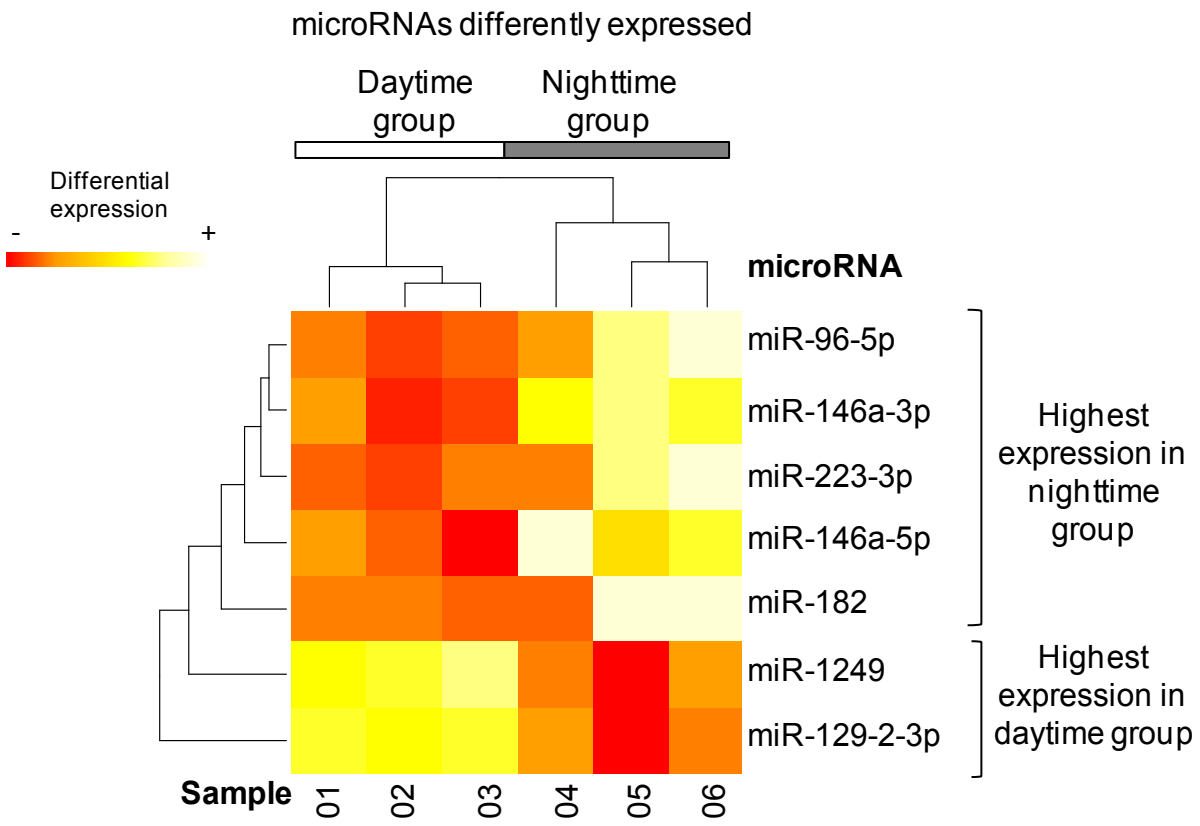
Flf1 -Vascular endothelial growth factor receptor

Pdgfr - Platelet-derived growth factor receptor alpha

Gdnf - Glial cell derived neurotrophic factor

Igf2bp1 - Insulin-like growth factor 2 mRNA binding protein 1

HOUR OF THE DAY – miRNAs endothelial progenitor cells

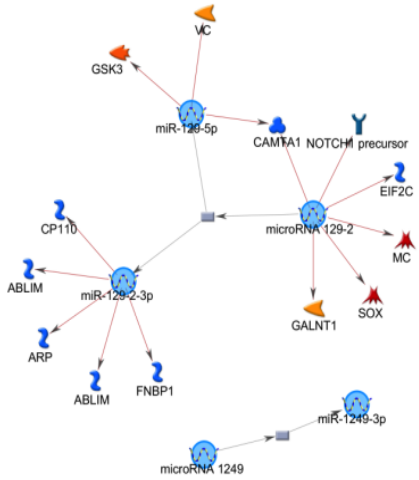


Marçola et al. on going collaboration – IEP H. Sirio-Libanês

METACORE® → nighttime

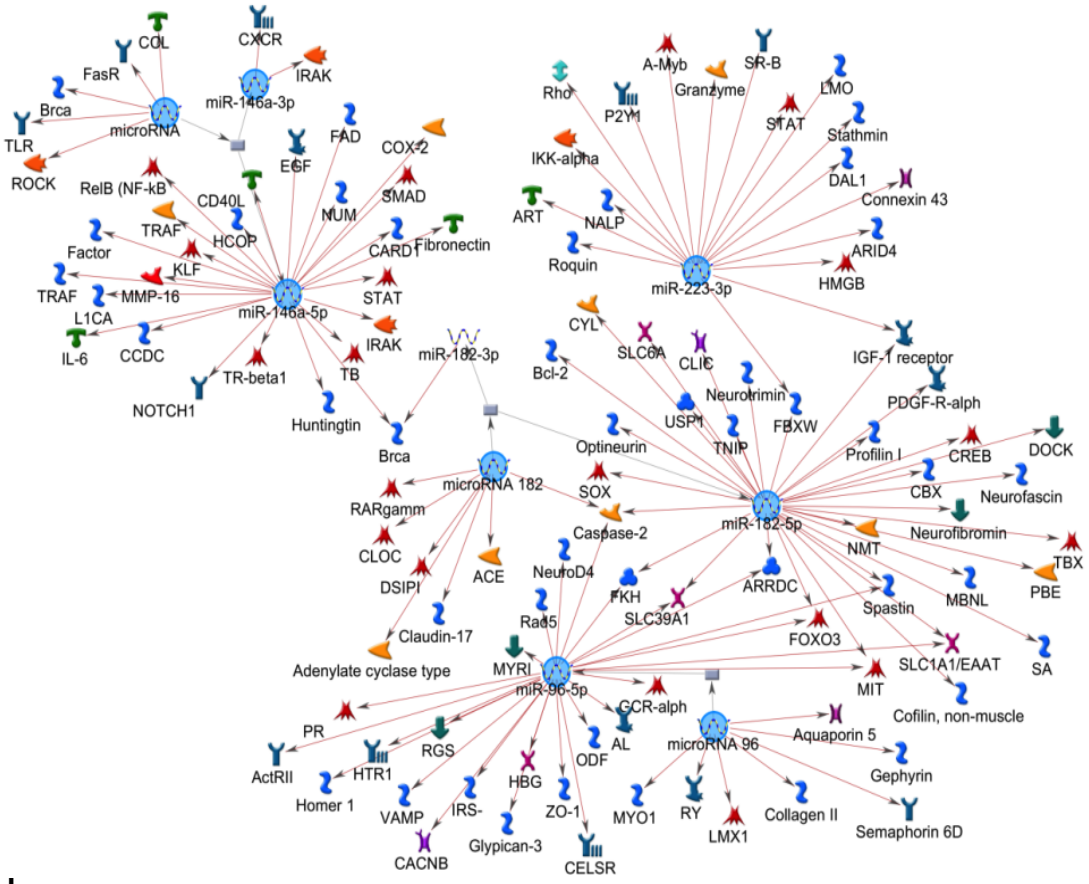
A

Network of miRNAs interactions in daytime cells



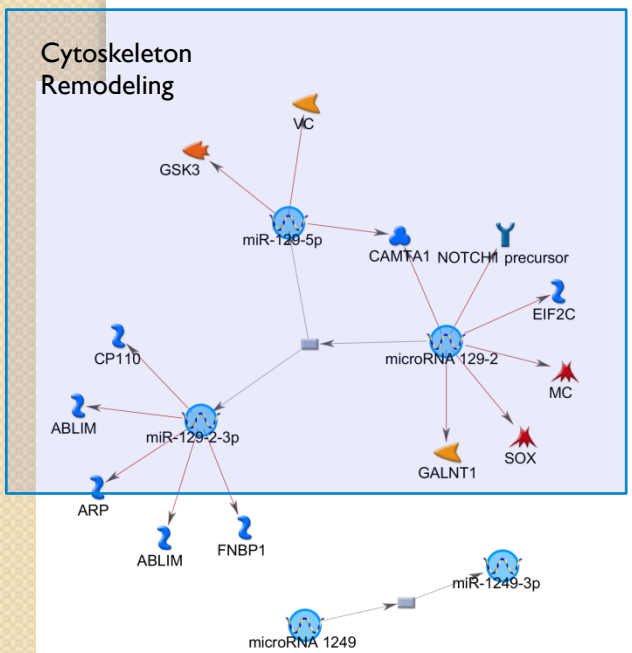
B

Network of miRNAs interactions in nighttime cells

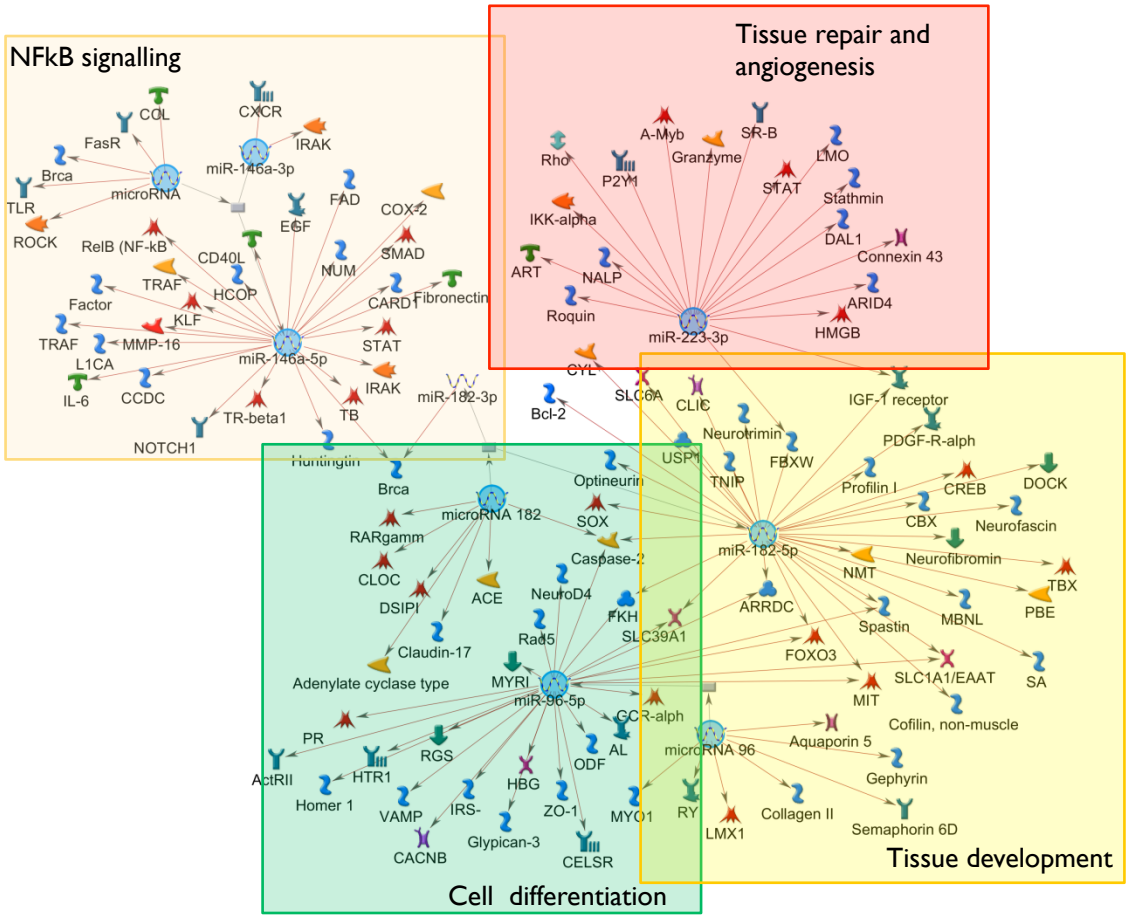


Marçola - 2015 - submitted

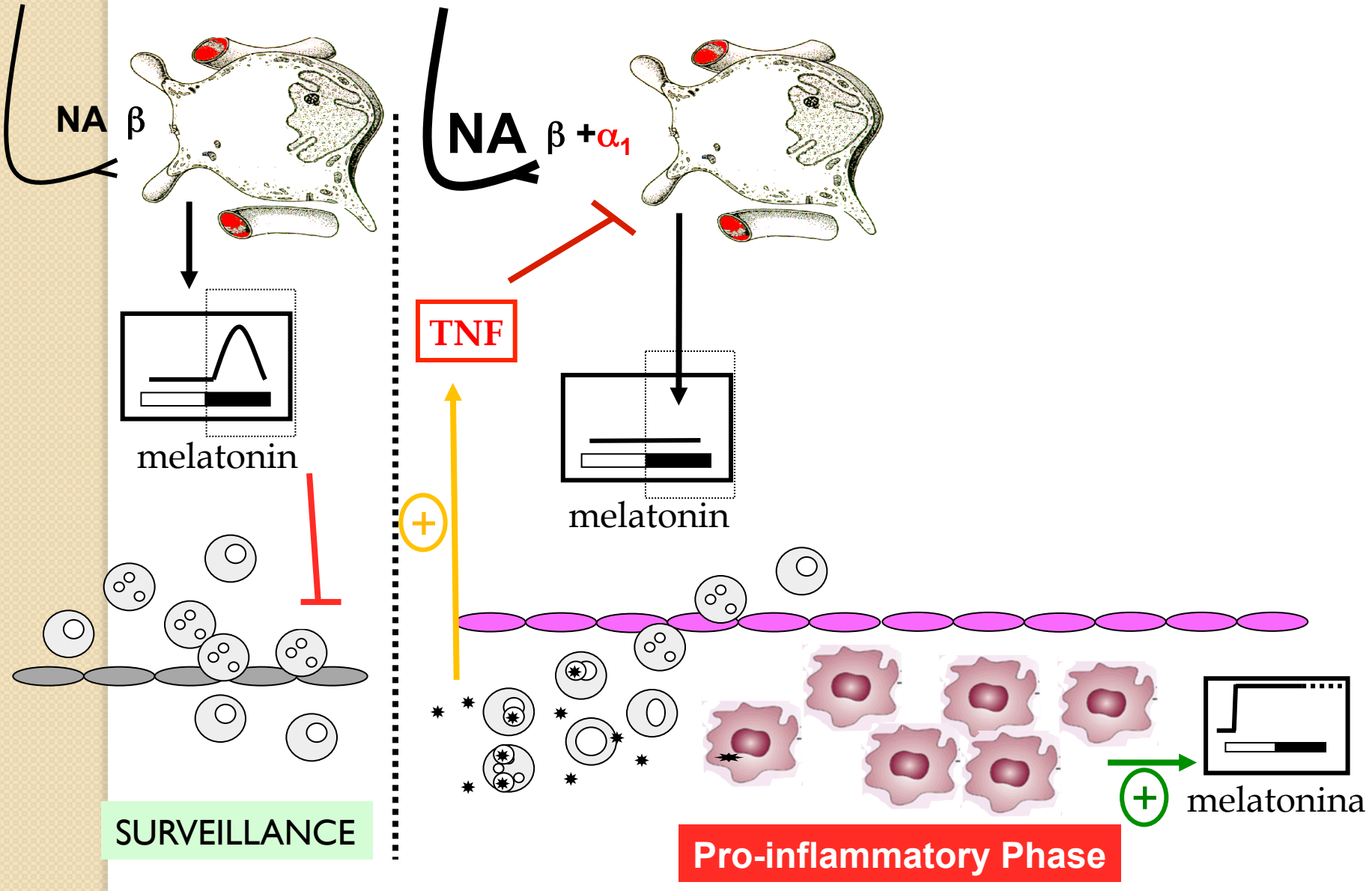
Network of miRNAs interactions in daytime cells



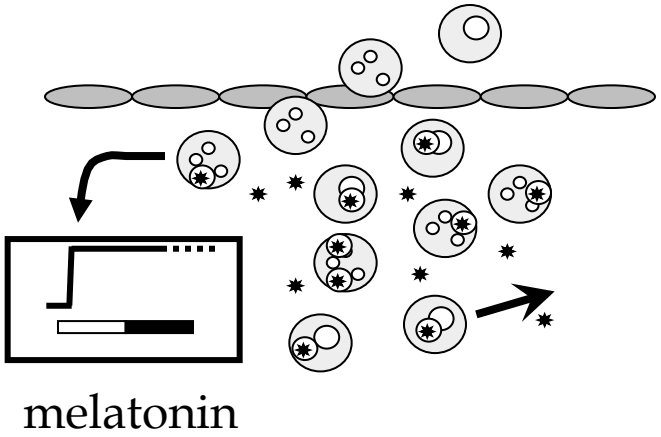
Network of miRNAs interactions in nighttime cells



Immune-Pineal Axis



Activation of the NF-kB pathway in MN and PMN triggers melatonin synthesis



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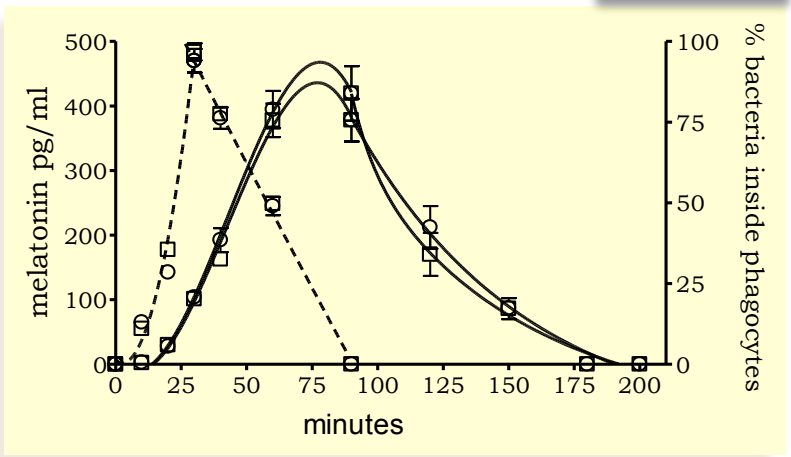
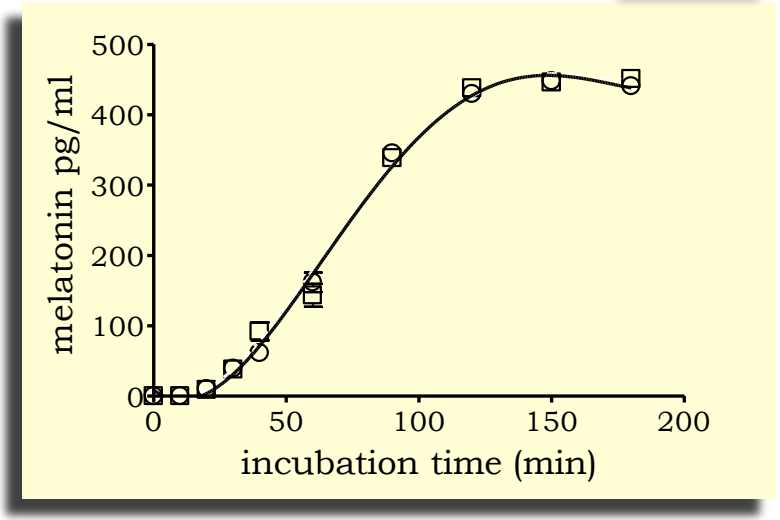
PMN and MN cells
Paracrine Signal

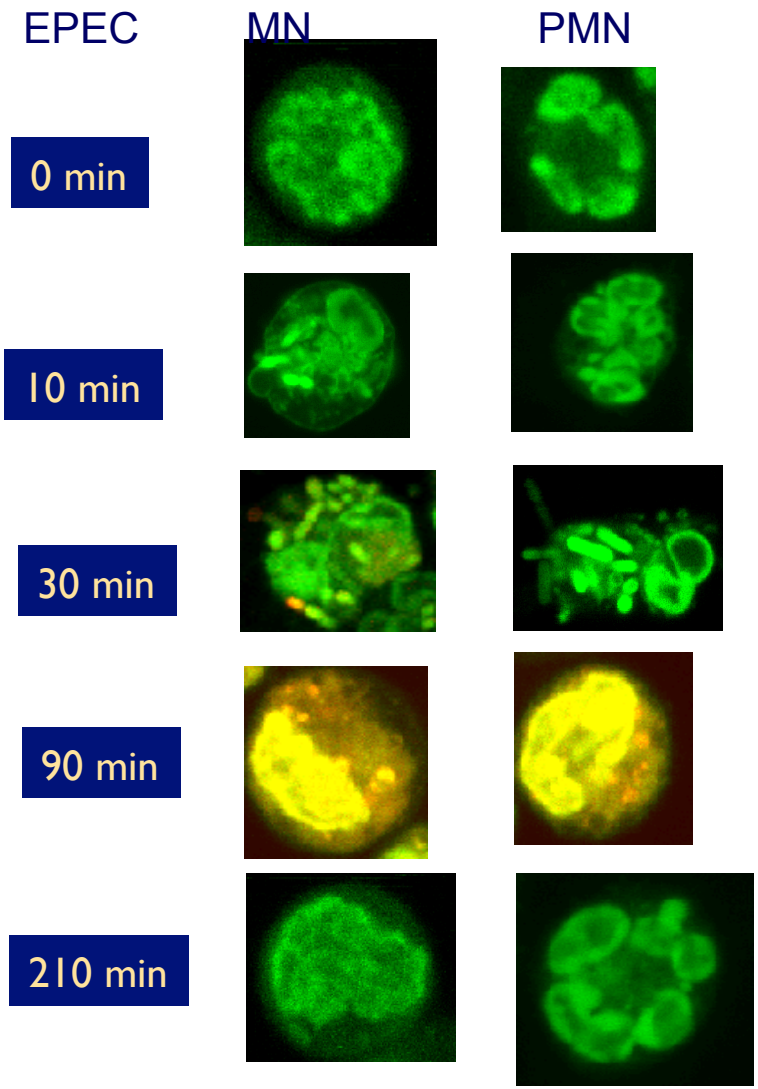
MACROPHAGES

EPEC



ZYMOSAN





Pontes et al. 2006

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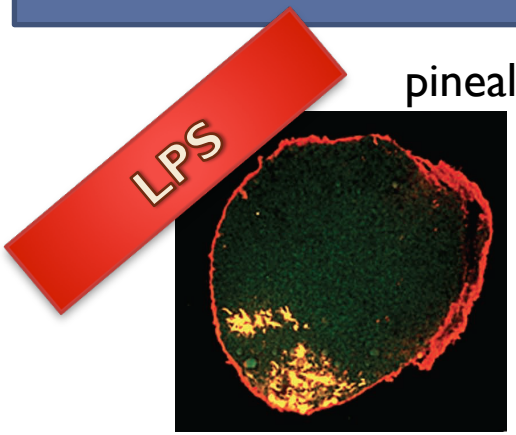
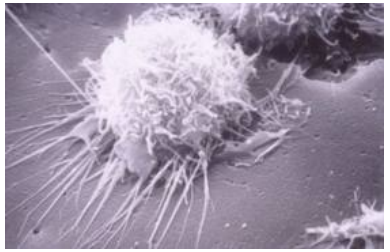
integration

One transcription factor – two different cell millieu



pineal

LPS
Zymosan



p50/p50

p50/Rel A

c Rel /Rel A

Melatonin

Pro-Inflamt cytokines
iNOS
adhesion molecules

Melatonin

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MELATONIN – synthesized by immune-competent cells

1988	→	PBMcells	→ Finocchiaro et al.
2000	→	bone marrow cells	→ Conti et al.
2004	→	macrophages	→ Martins et al.
2004	→	lymphocytes	→ Carrillo-Vico et al.
2006	→	colostral MN and PMN	→ Pontes et al
2009	→	splenocytes	→ Lahiri et al.,
2010	→	mastocytes	→ Maldonado et al.,
2015	→	microglia	→ Adriessa Santos master thesis

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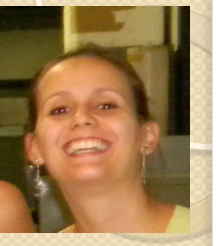
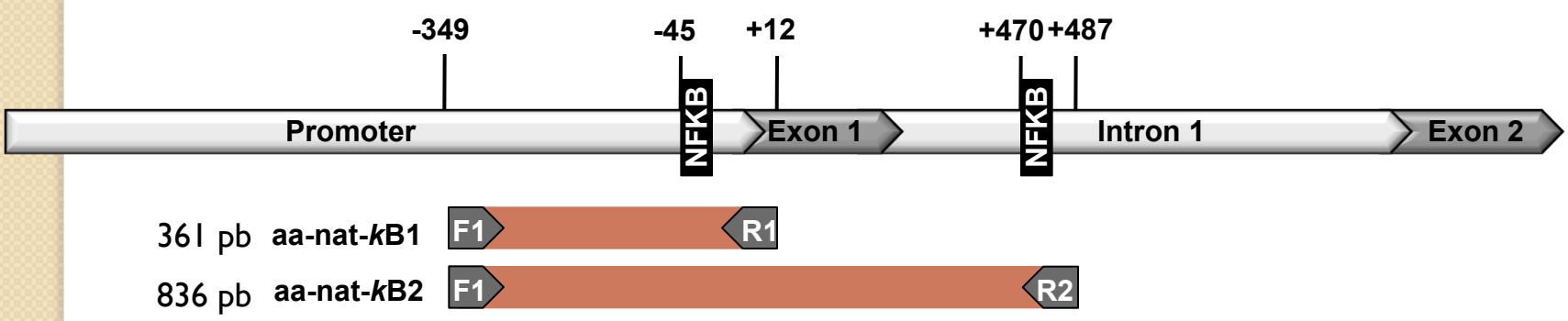


κB-elements
in the promoter of *aa-nat*



Expression of NFκB promoter linked to a Red fluorescence reporter in macrophages → positive responses induced by LPS and TNF

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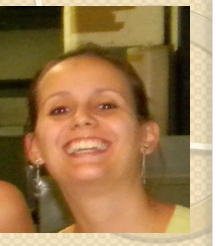
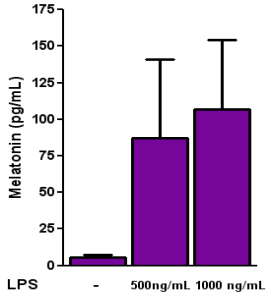
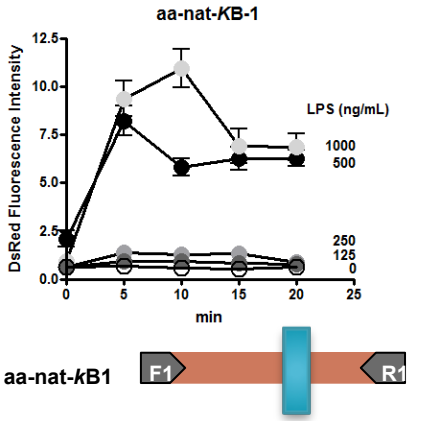
Expression of the reporter

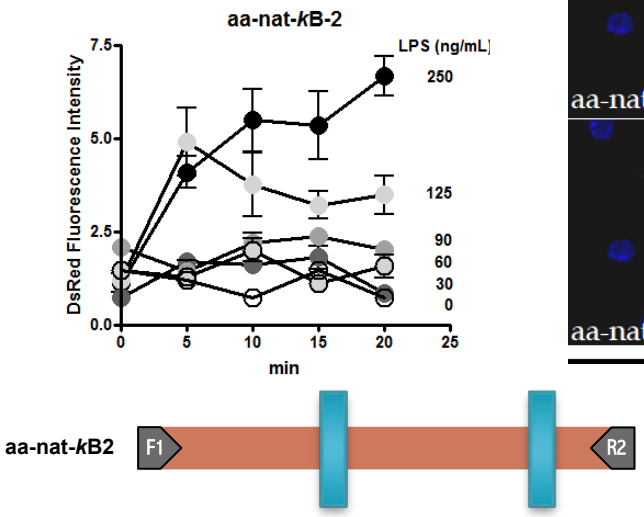


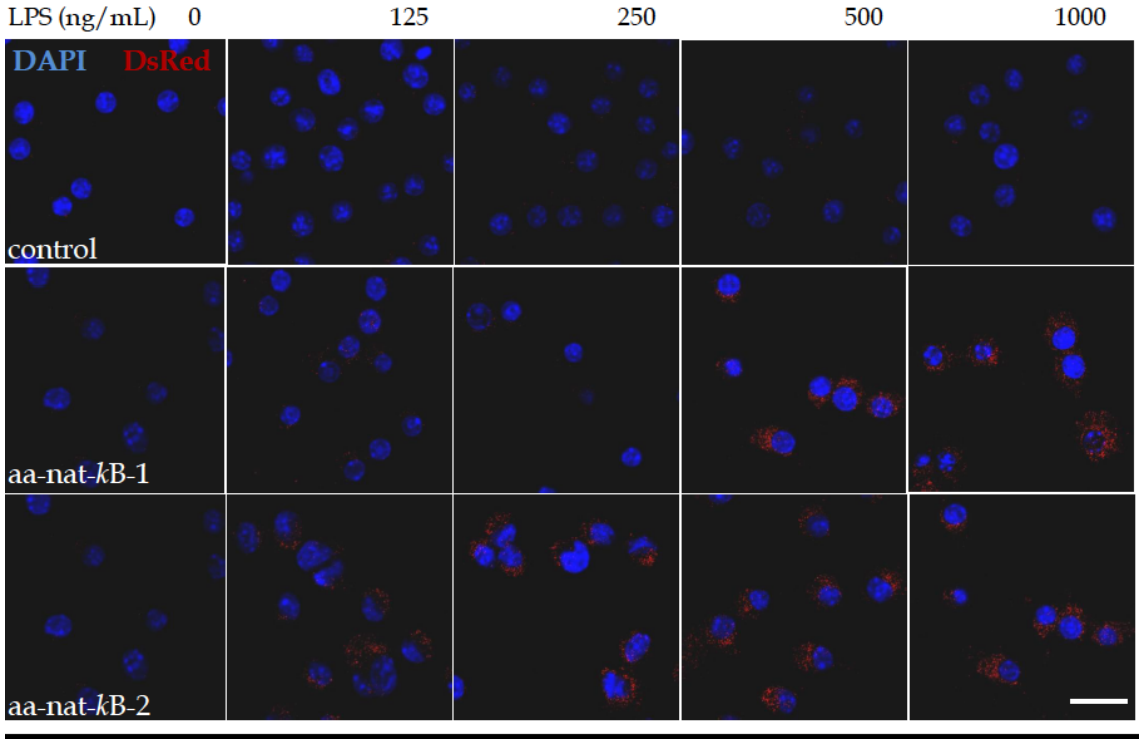
LPS

RAW 264.7 macrophages









Muxel et al., 2012

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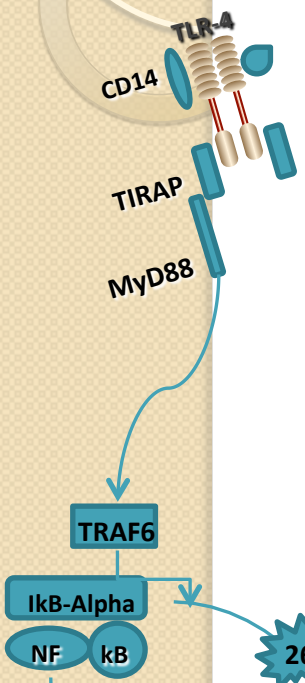
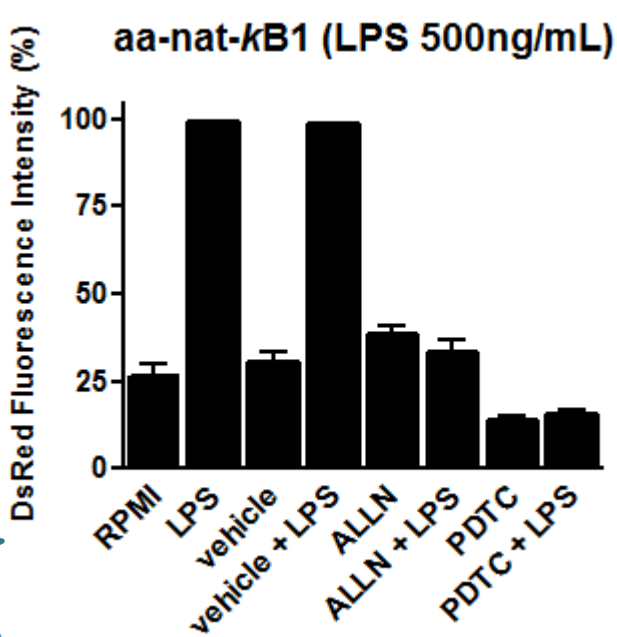
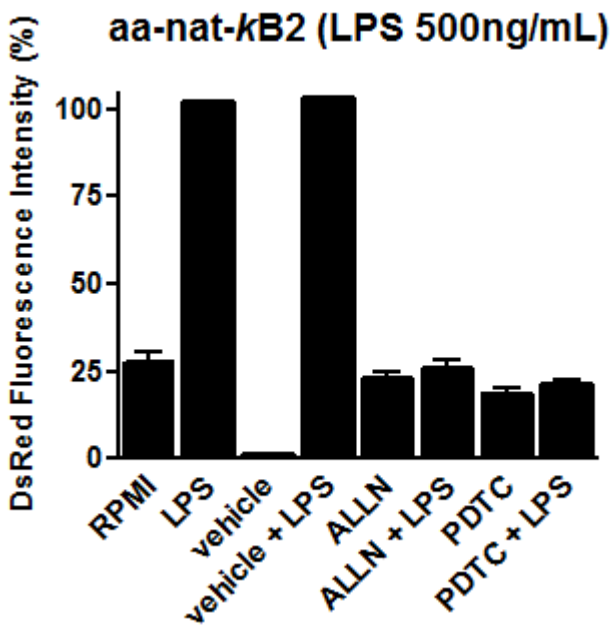
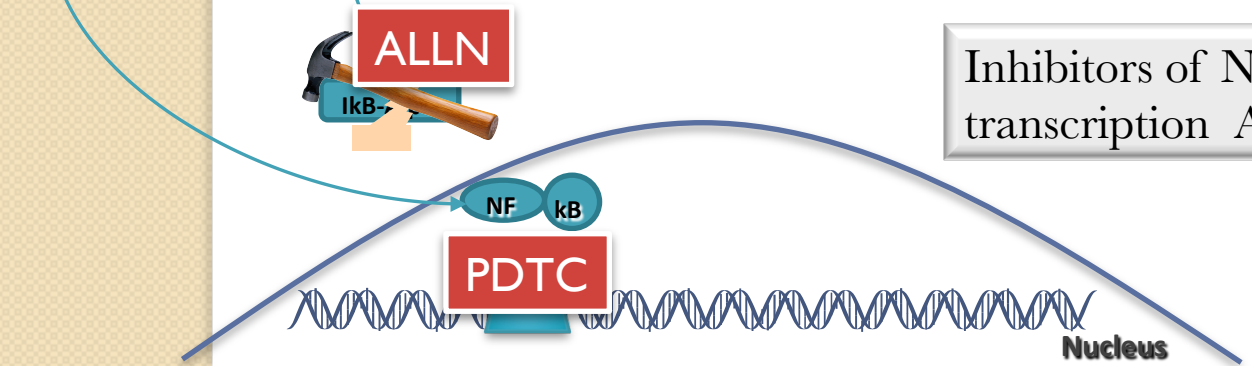
aa-nat-κB element mediates gene transcription







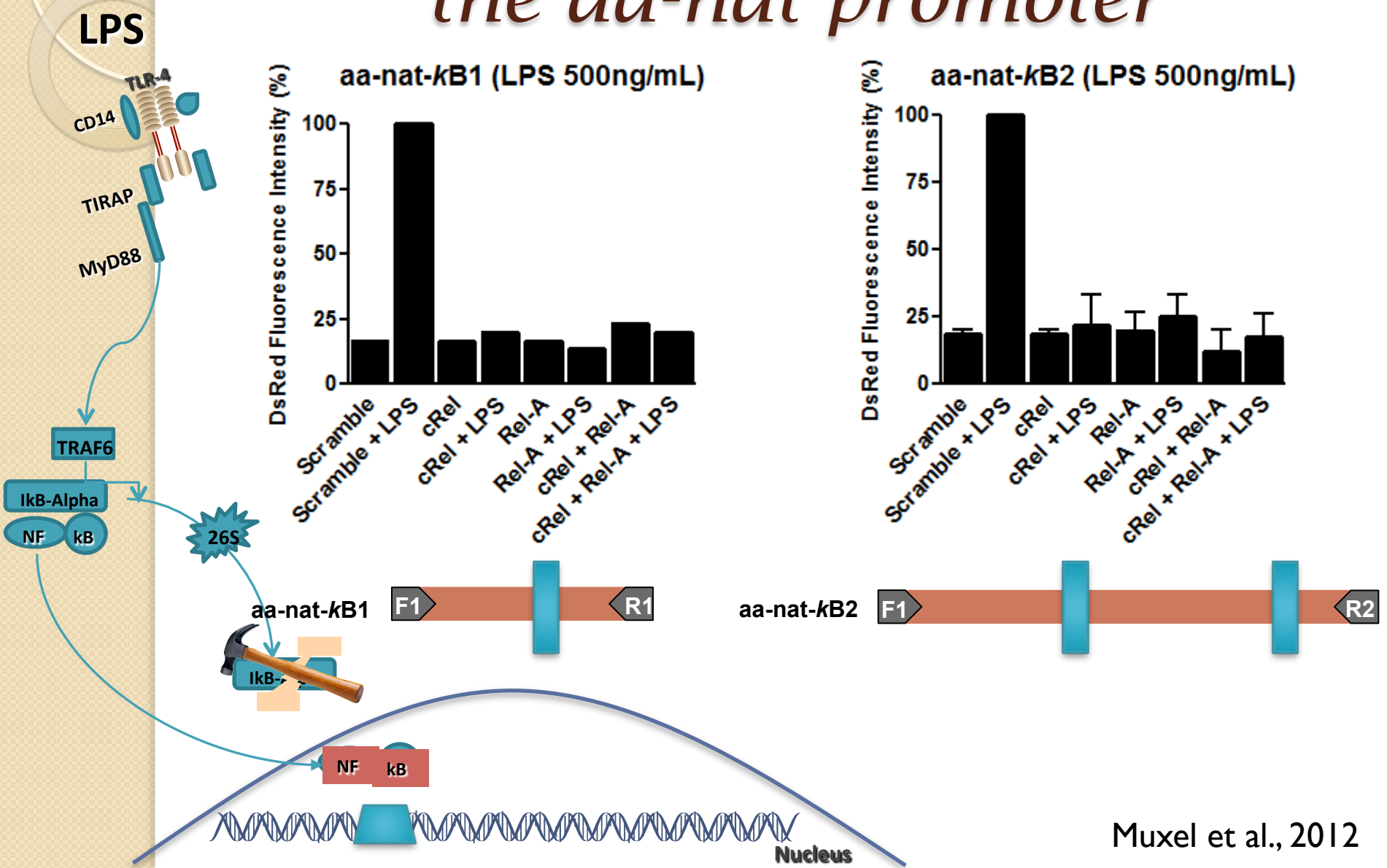


Inhibitors of NFkB blocks LPS-induced gene transcription ALLN – 50 mM; PDTC 25 mM

Muxel et al., 2012

Laboratório de Cronofarmacologia

The dimer RelA/cRel activates the aa-nat promoter



Muxel et al., 2012

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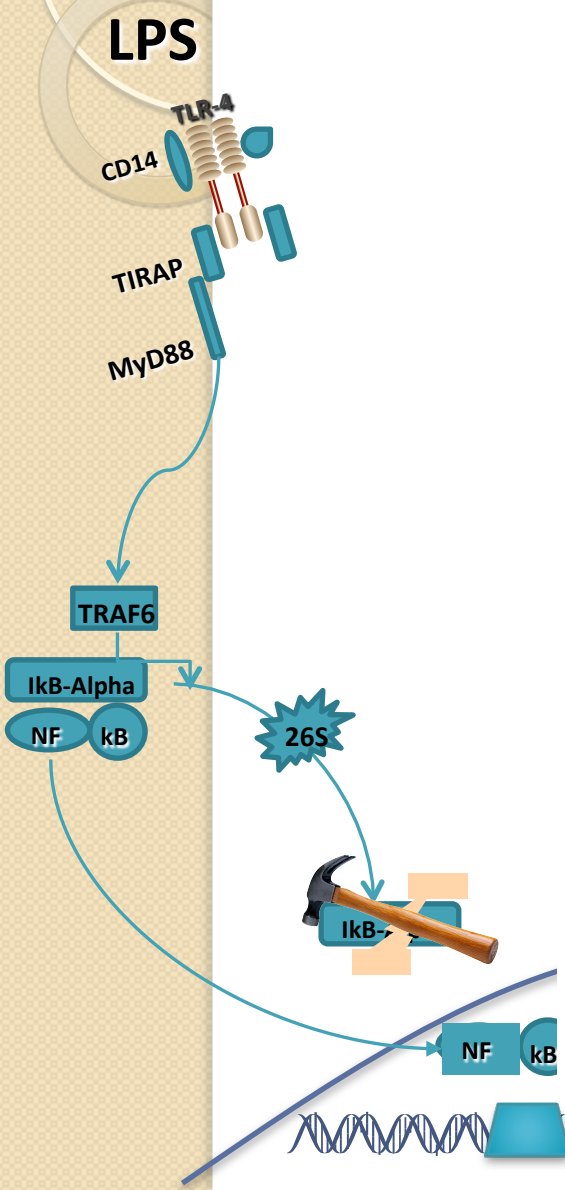
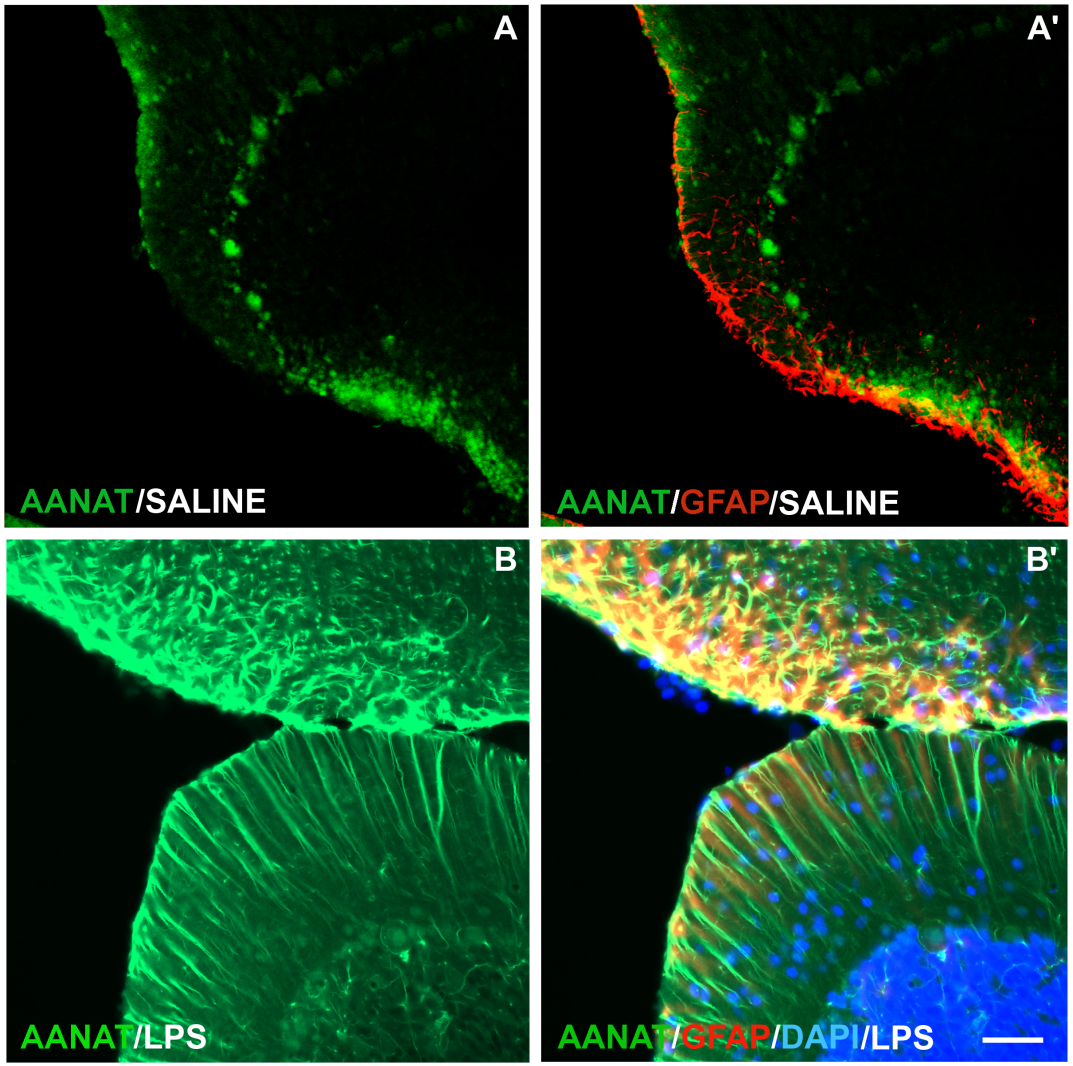
And the Brain





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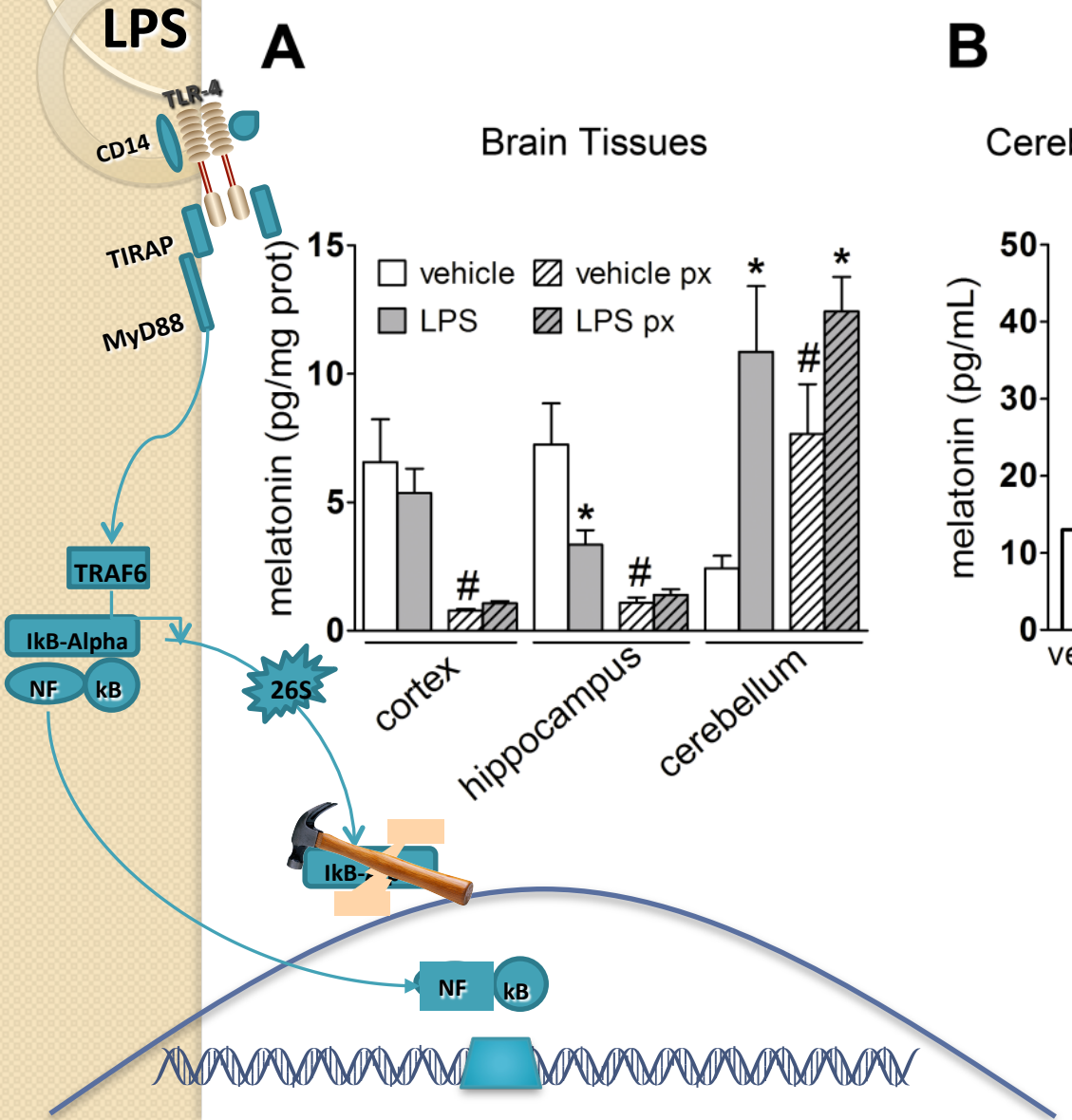
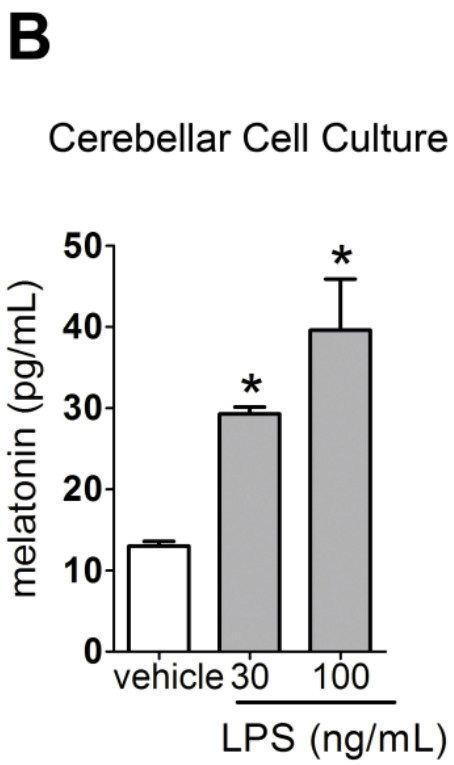
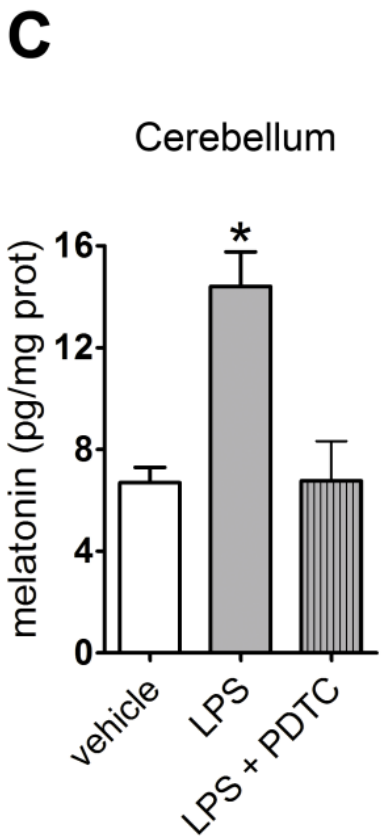
The cerebellum synthesizes melatonin







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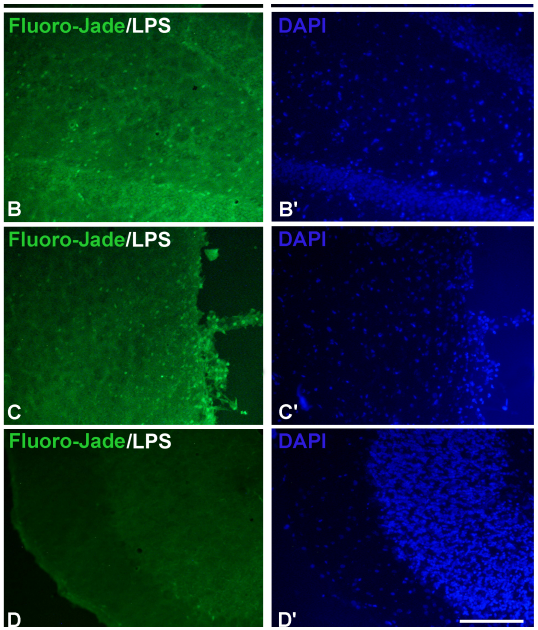
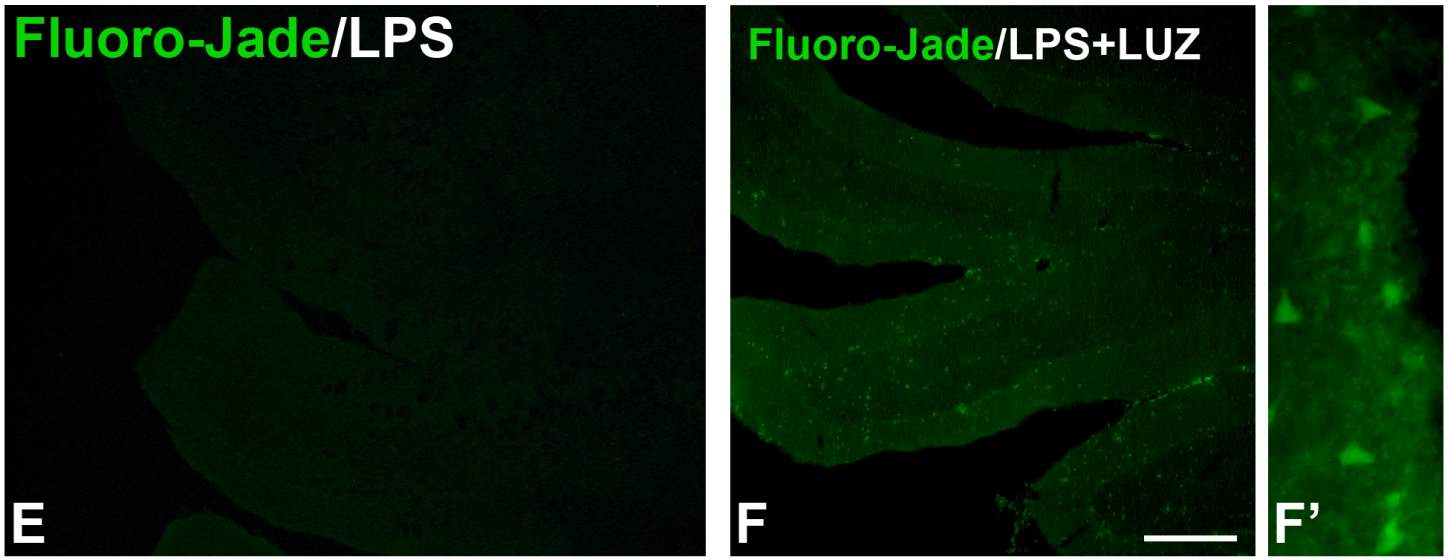
role of cerebellar melatonin

HIPOCAMPPUS

CORTEX

CEREBELLUM





Pinato et al.

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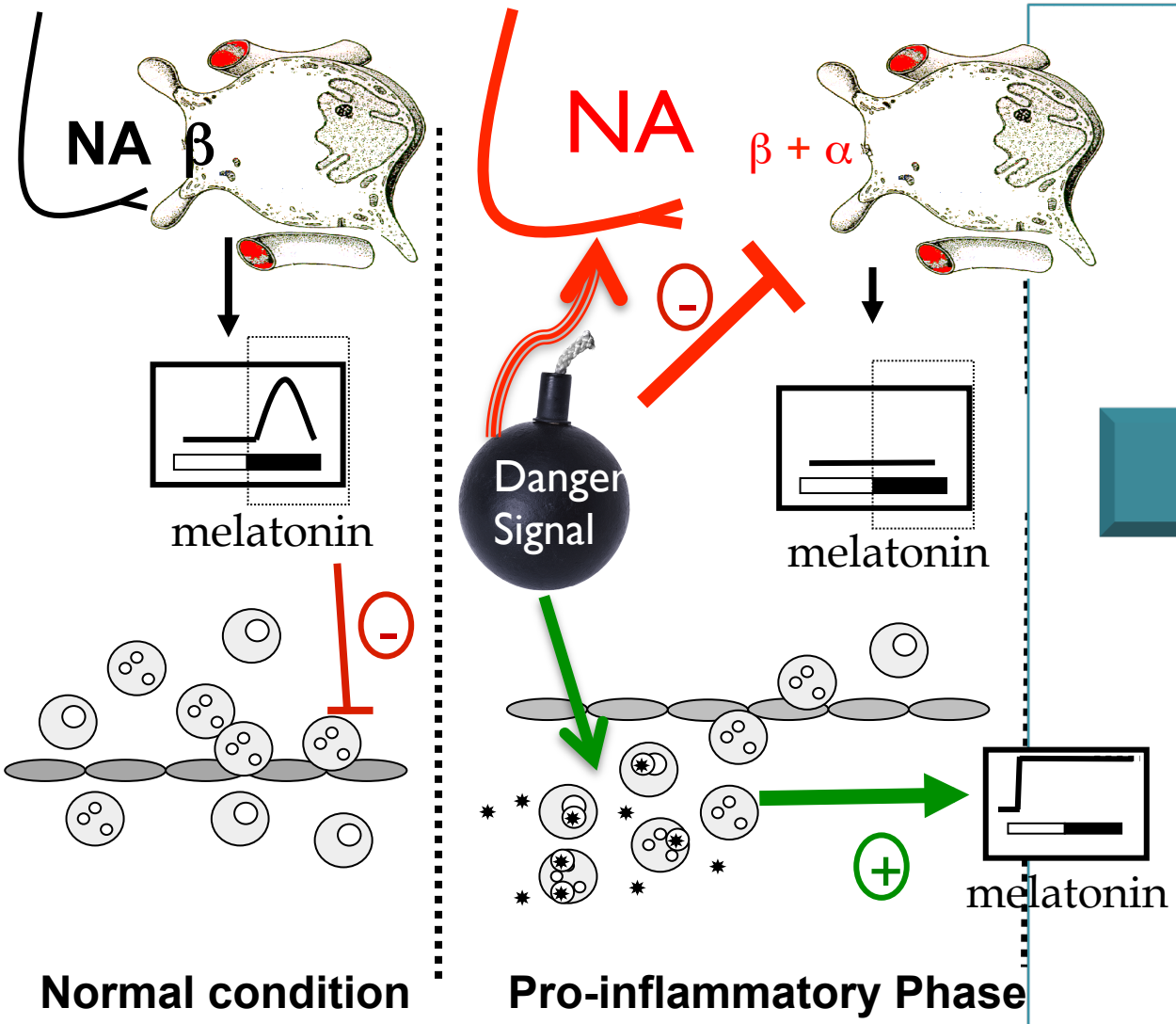
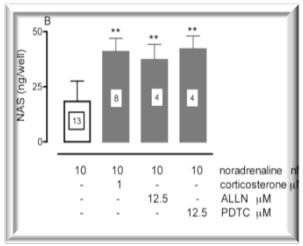
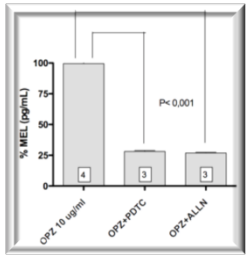
Immune-Pineal Axis





Ferreira et al., 2005

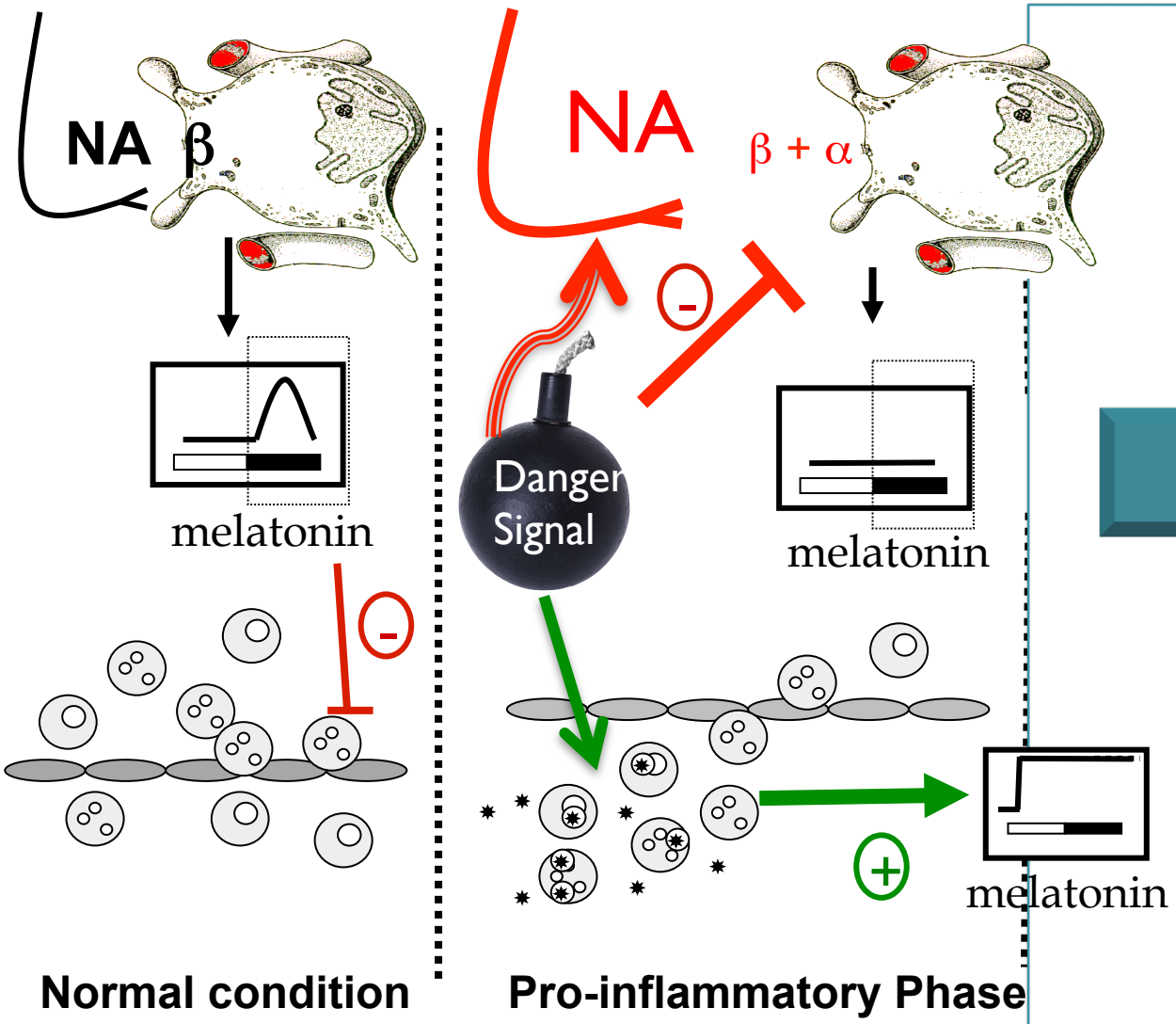
NFκB

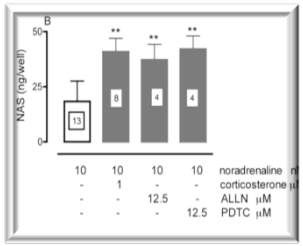


Lapa – on going

Laboratório de Cronofarmacologia

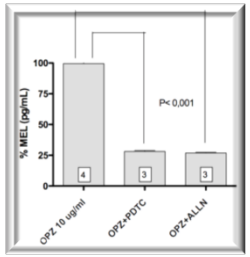
Immune-Pineal Axis





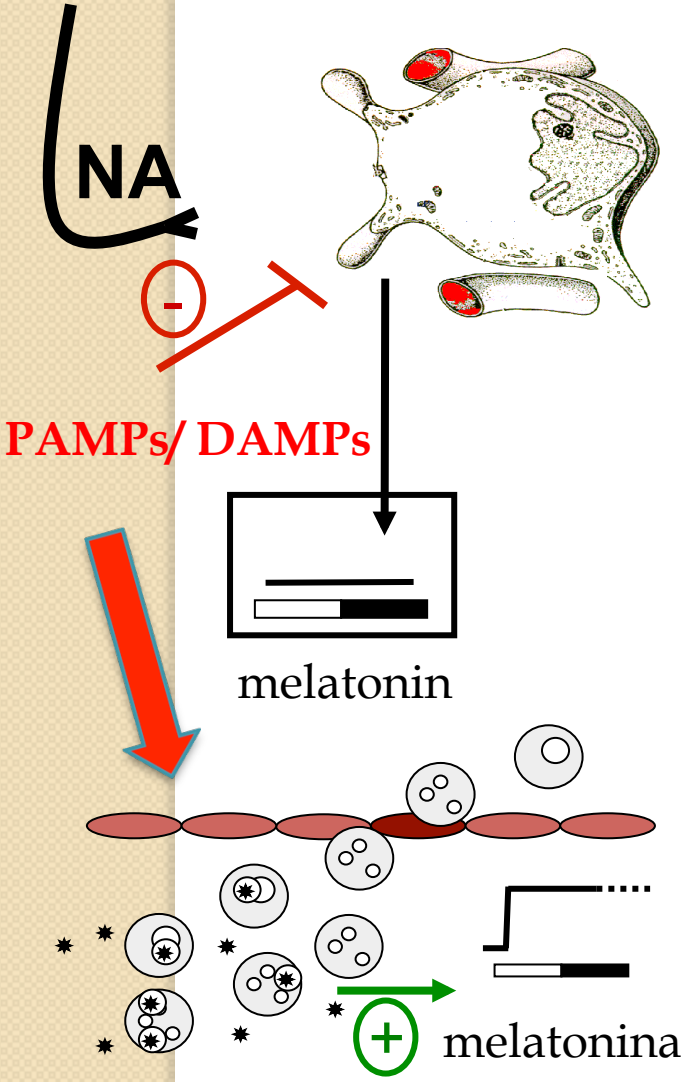
Ferreira et al., 2005

NFκB



Lapa – on going

Immune-Pineal Axis



1. PAMPs and DAMPs supress noctunral MEL surge
    1. Bacteria
    2. Fungi
    3. Air pollution
    4. Peptides, GAGs,
2. Endothelial cells are activated in the absence of MEL
3. Macrophages/ microglia – synthesize melatonin

FAPESP

CNPq
Conselho Nacional de Desenvolvimento Científico e Tecnológico

CAPES

LCF

Zulma S Ferreira – docente
Pedro ACM Fernandes – docente

Eduardo K Tamura – pós-doc
Maria Fernanda Laranjeira da Silva – pós-doc

Daiane G Franco – doutoranda
Marco Lapa – doutorando
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Claudia Emanuele Sousa – doutoranda
Kelly D A Lima – mestranda
Camilla Petrilli – mestranda
Marina Marçola – doutoranda
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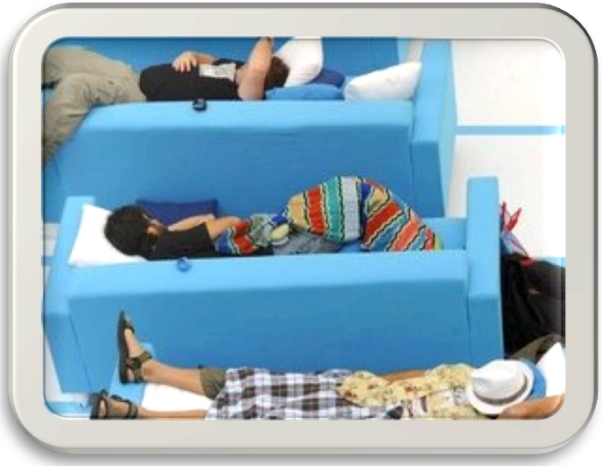
Claudia Lúcia M Silva **UFRJ**
Luciana Pinato – UNESP
Wolnei Caumo – UFRGS
Maria Paz Hidalgo - UFRGS

USA .....
Margarita L. Dubocovich

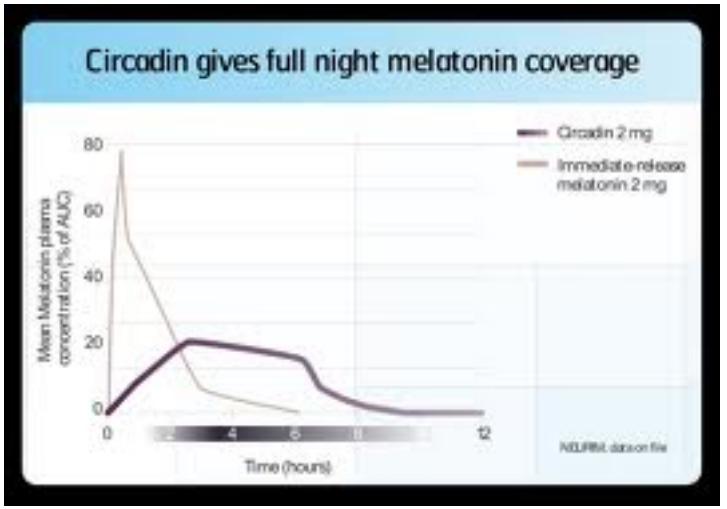
FRANCE .....
Valérie Simonneaux
Béatrice Bothorel


NATROL
Timed Release
Melatonin
3 mg
Helps Establish
Normal Sleep Pattern*
Dietary Supplement / 60 TABLETS




circadin 2 mg


enlyten
MELATONIN
COOL MINT 14 STRIPS
slide


Circadin gives full night melatonin coverage
Circadin 2 mg
Immediate-release melatonin 2 mg
Mean Melatonin plasma concentration (% of AUC)
80
60
40
20
0
0
4
8
12
Time (hours)