The multi-faceted role of ATP in control of melatonin synthesis – new insights for onset and progression of diseases.

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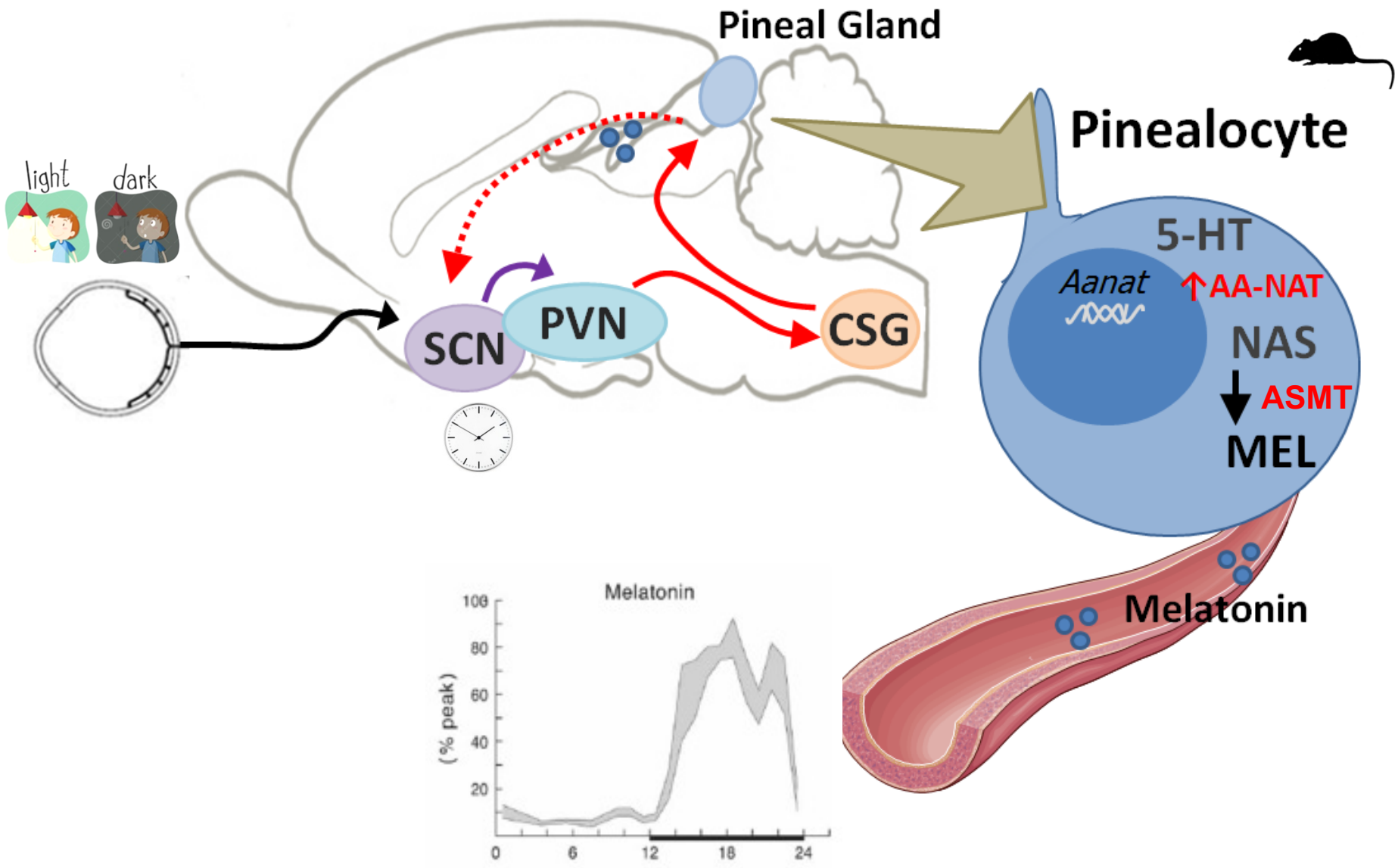
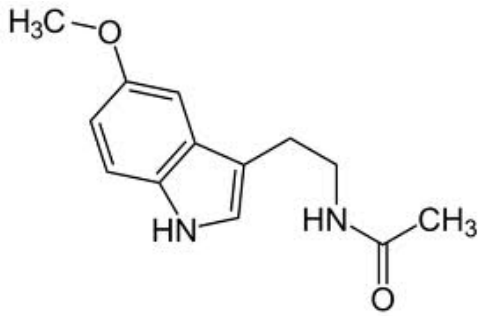
Melatonin: the chemical signal of darkness



da Silveira Cruz-Machado et al., *Sci Rep*, 2017

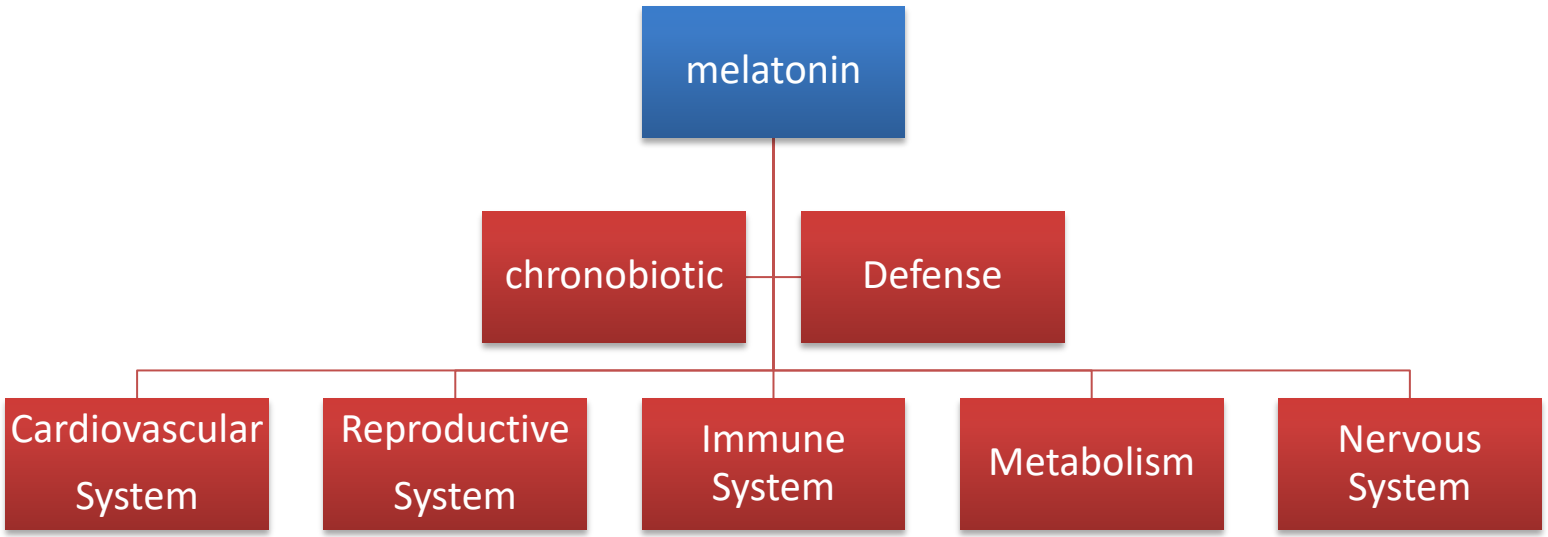


MELATONIN

oGPCRs:
- GPR50
- GPR61
- GPR62
- GPR135

MT1 **MT2**

free radical scavenger

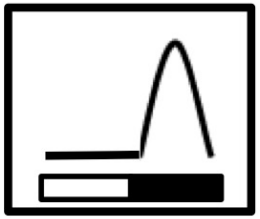


Jockers et al., *British Journal of Pharmacology*, 2017

Fine-tuning melatonin production is essential for regulating defense *response*

Physiological Conditions

Plasma melatonin



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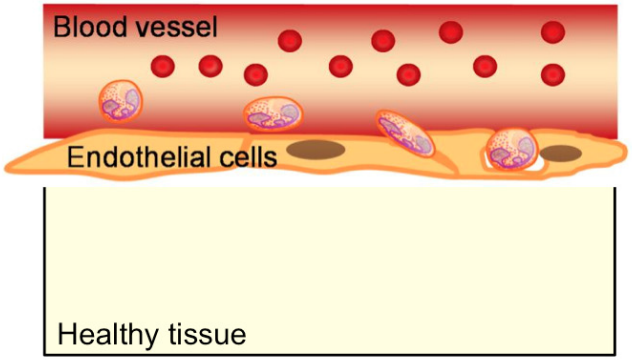
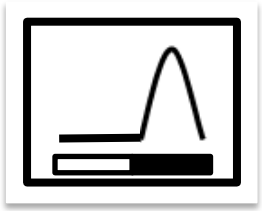
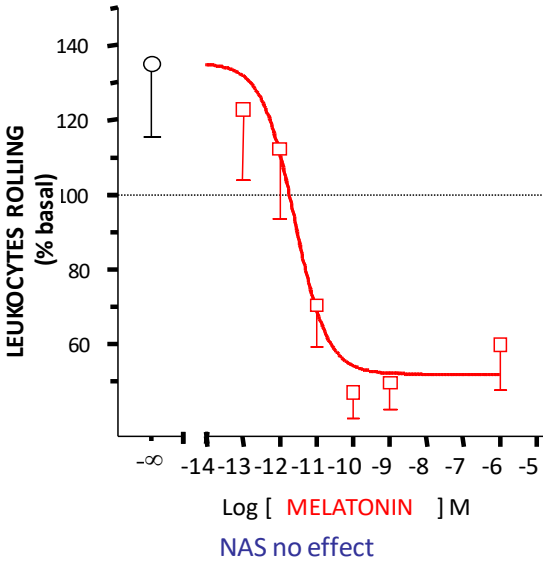
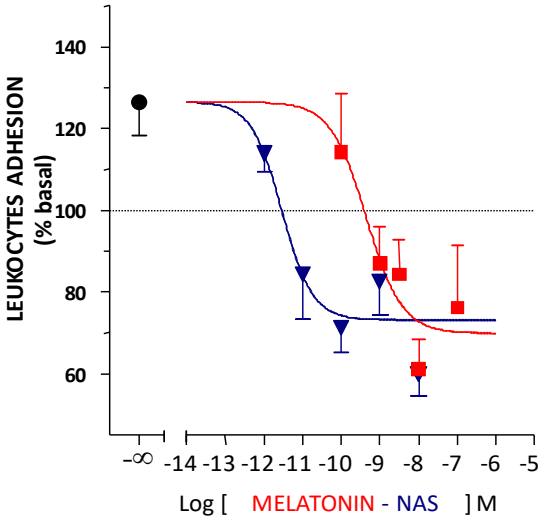




↓ rolling

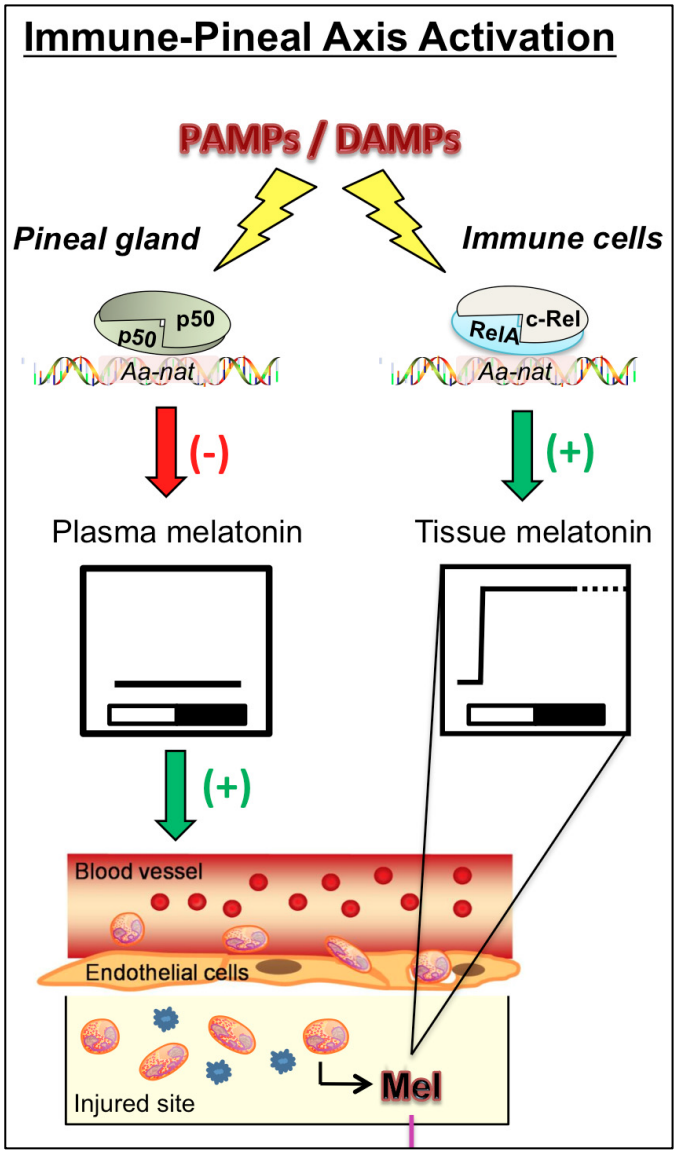


↓ adhesion



Lotufo et al., *Sci Rep*, 2001

Fine-tuning melatonin production is essential for regulating defense *response*



Infection
TNF
LPS
Zymosan
Pam3CSK4
heparan sulfate
Malaria
Leishimaniasis

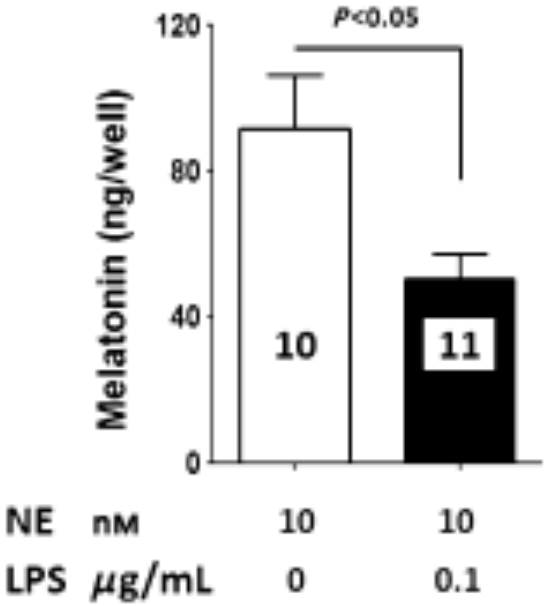
Models of disease
Alzheimer's (Aβ)

Inflammation in Humans
Caesarean
Mastitis
Hysterectomy

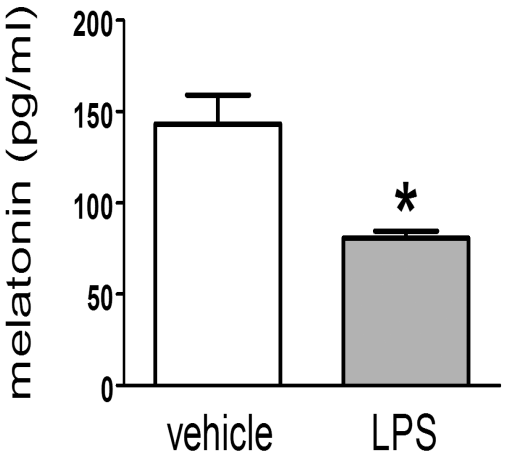
Environmental danger signals
Air pollution (São Paulo city)

Inflammatory mediators regulating pineal melatonin synthesis

- **LPS – *in vitro***

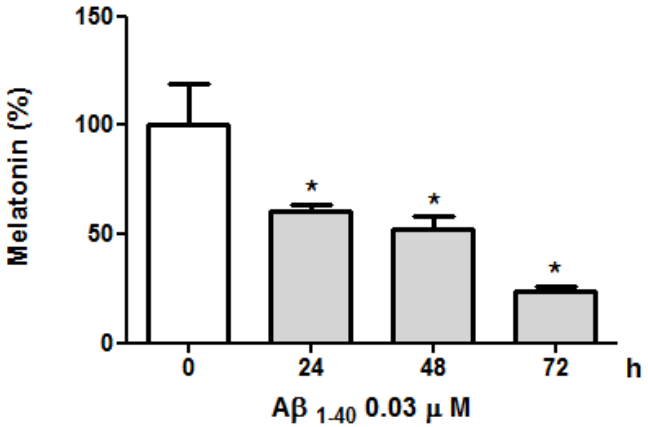


- **LPS – *in vivo*, icv**



Pinato et al., *2015*

- **Amyloid β peptide**

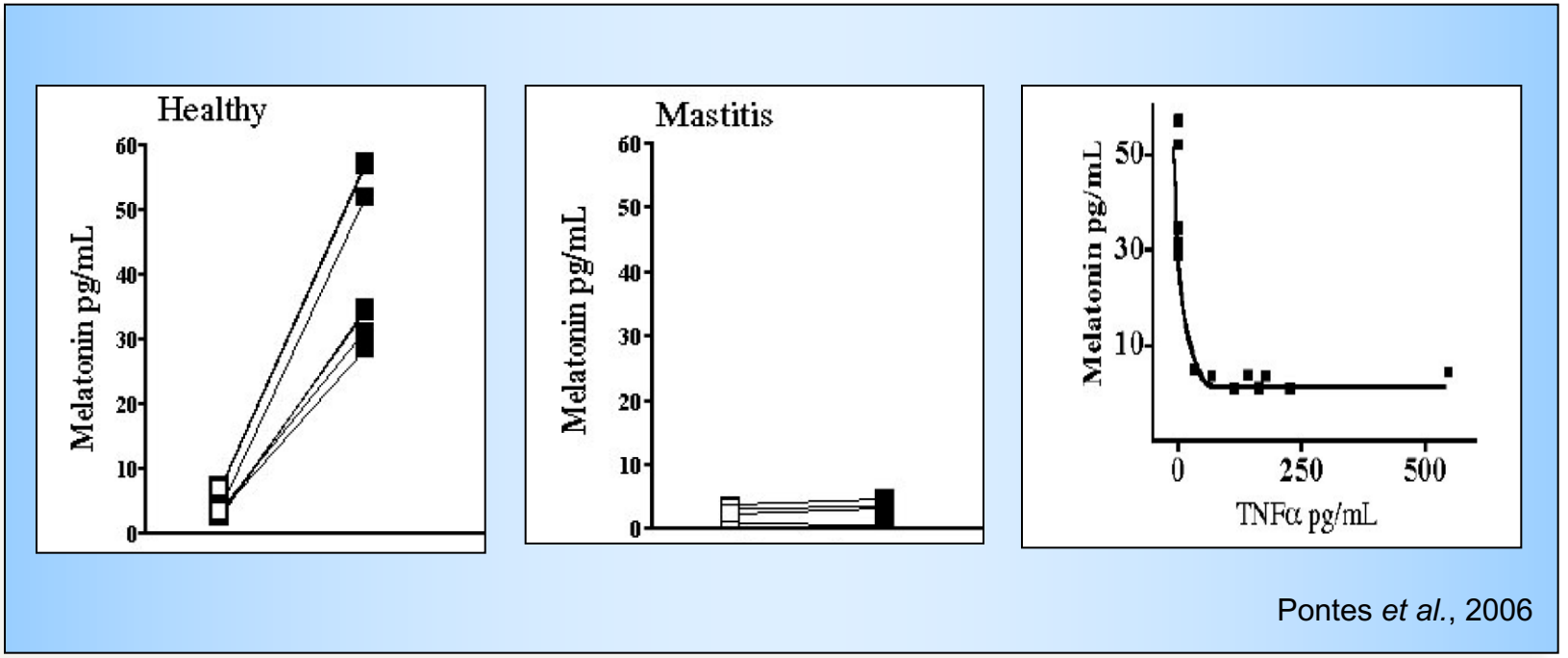


Cecon et al., 2015

Da Silveira Cruz-Machado et al., J Pineal Res, 2010

TNF – controlling the nocturnal melatonin surge in humans

Mastitis → suppresses nocturnal MEL surge

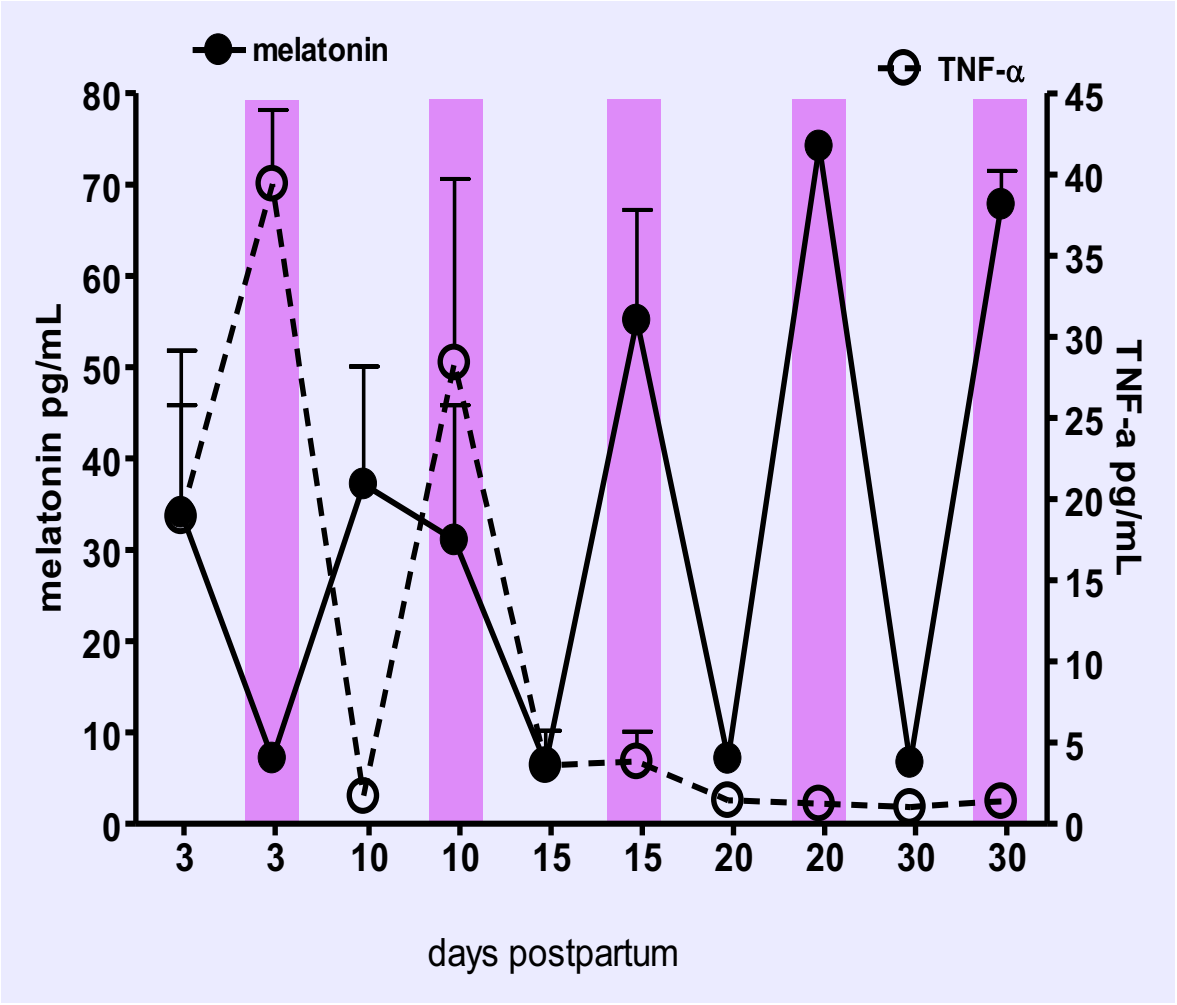


Pontes *et al.*, 2006

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.

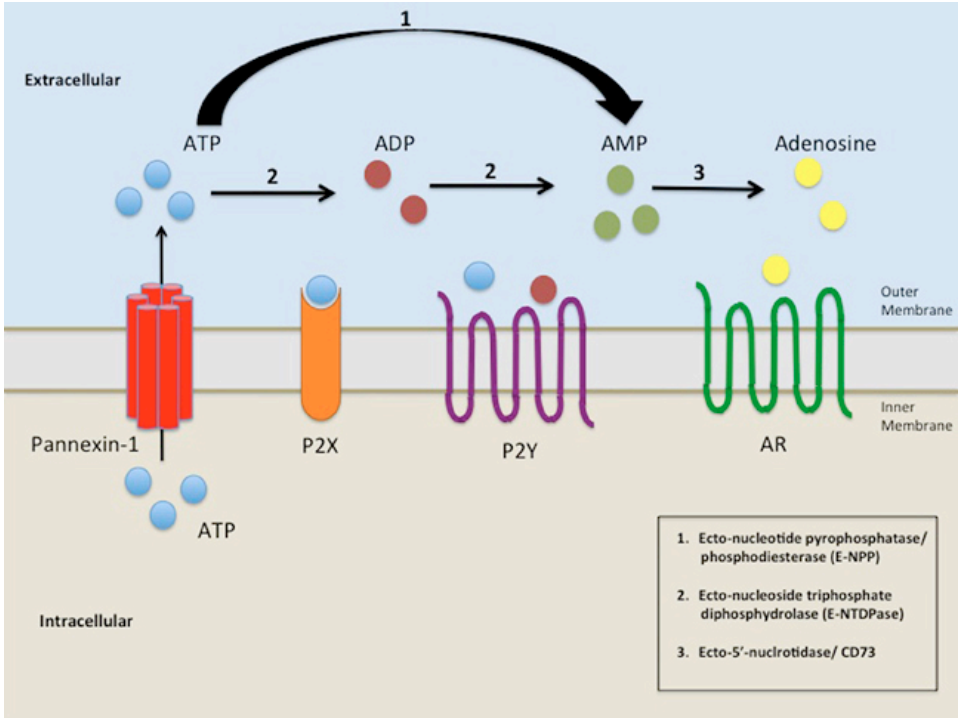
Restoration of daily rhythm of melatonin



obtained after a great reduction in the levels of TNF-α.

Pontes et al., 2006

Contribution of the purinergic system in the modulation of the pineal output



✓ ATP acts as a cotransmitter in the pineal gland

Mortani-Barbosa, Ferreira & Markus. Eur. J. Pharmacol. 401:59, 2000

✓ Ectonucleotidases are expressed in the pineal

Ornelas et al., in preparation

✓ ATP triggers P2Y1 and P2X7 receptors

Ferreira & Markus. Eur. J. Pharmacol. 415:151, 2001.
Souza-Teodoro et al., J. Pineal Res 2016

✓ Purinergic stimulus is translated by increasing activity of PLC and $[Ca^{2+}]_i$

Ferreira et al., Pharmacology 69:33-37, 2003

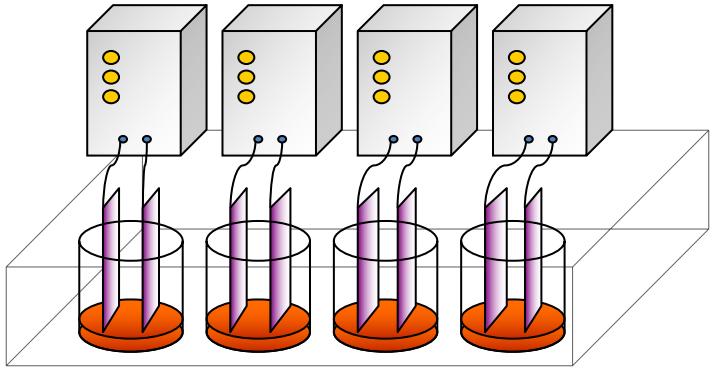
Cecon et al., unpublished

➢ Functional role of purinergic signaling in the pineal physiology and the immune-pineal axis

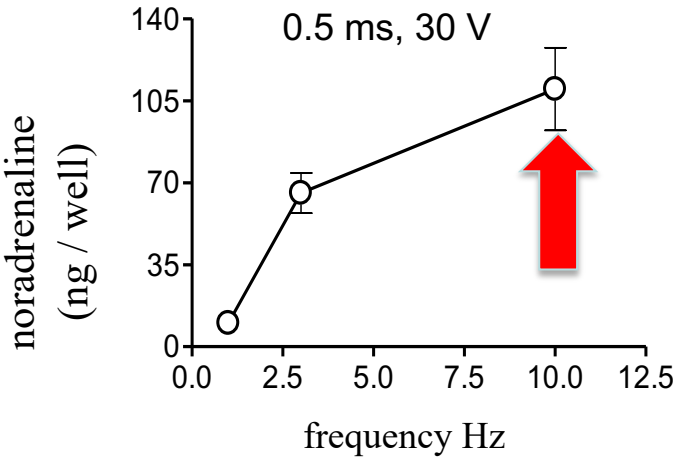
Dargenio-Garcia et al., submited

The sympathetic co-transmission in the rat pineal gland

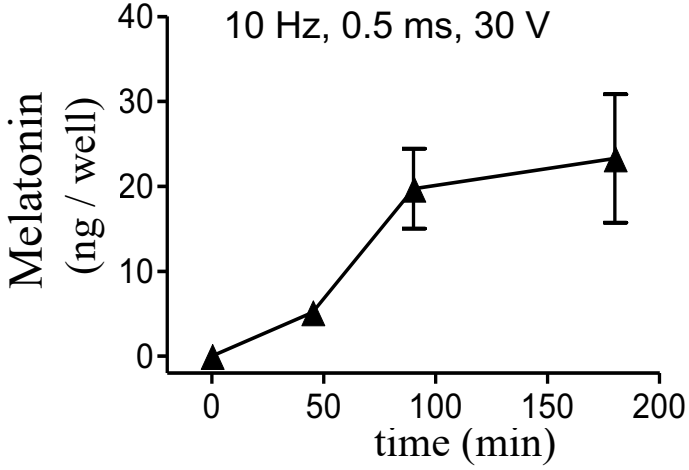
Transmural electrical field stimulation of conari nerve terminals



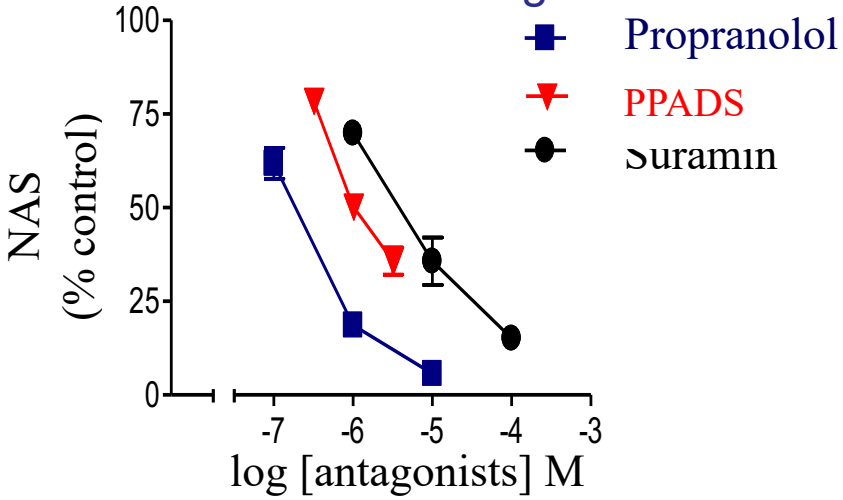
Frequency-response curve



Time-response curve

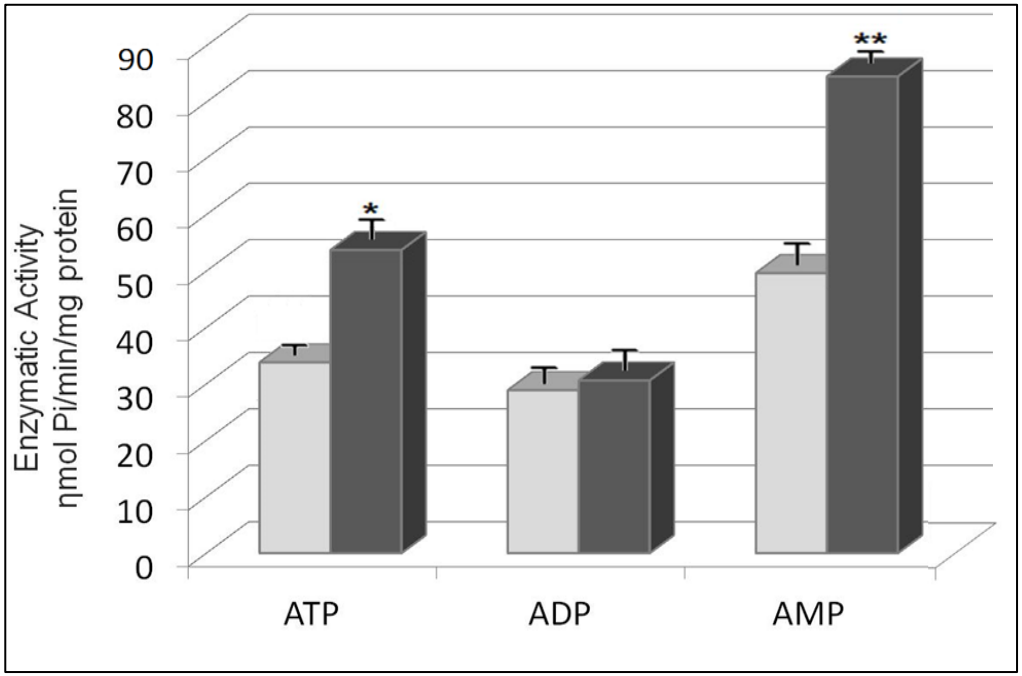


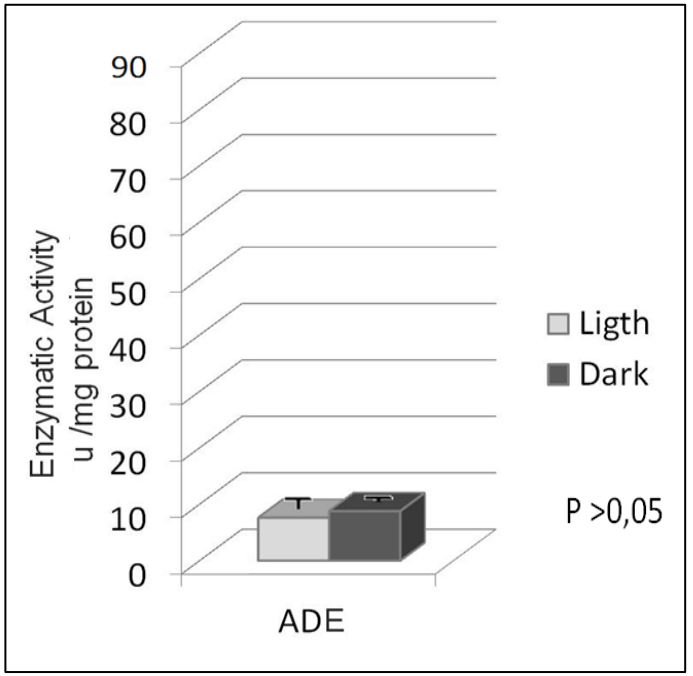
Effect of antagonists



Mortani-Barbosa et al., 2000

Expression and daily variation of nucleotide and nucleoside hydrolysis



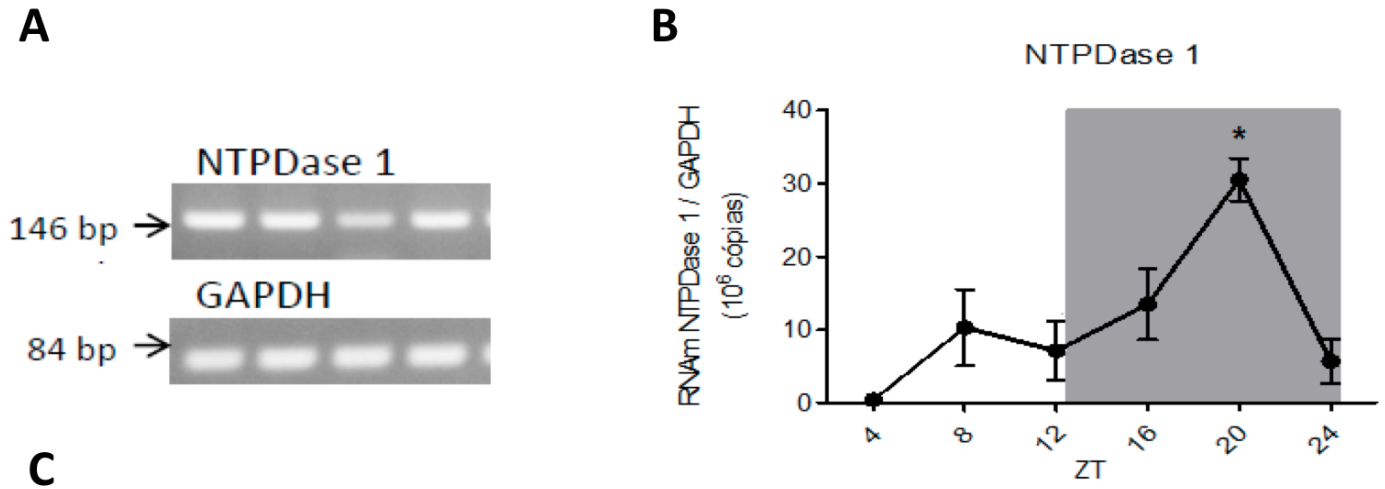


✓ The ectonucleotidase activity showed a significantly increase in ATP and AMP hydrolysis in the dark phase (* $p<0.05$).

Therefore, the purinergic system presents a daily adaptation for regulating physiological pineal gland activity

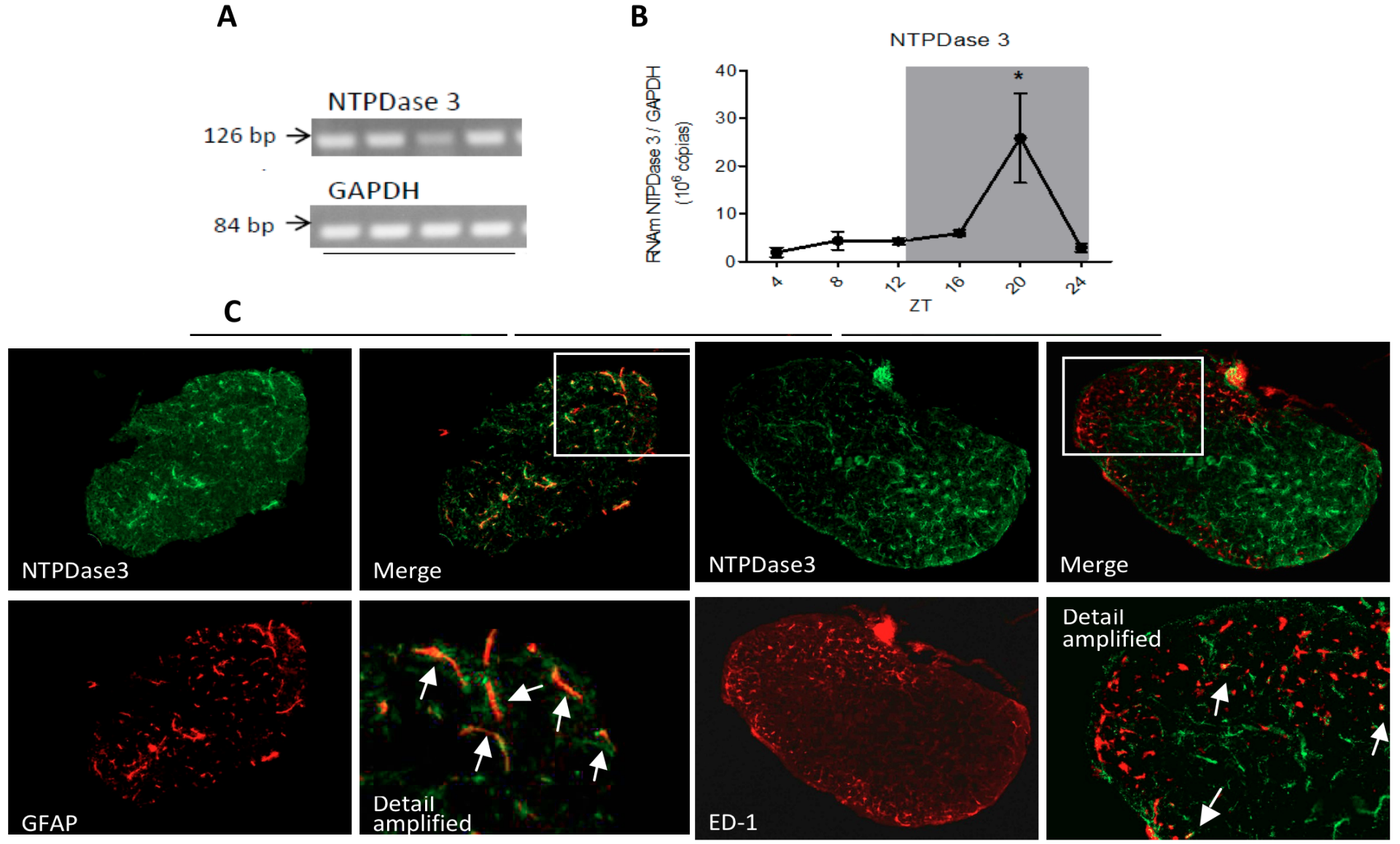
Ornelas et al., in preparation 2016

NTPDase1 expression in rat pineal gland



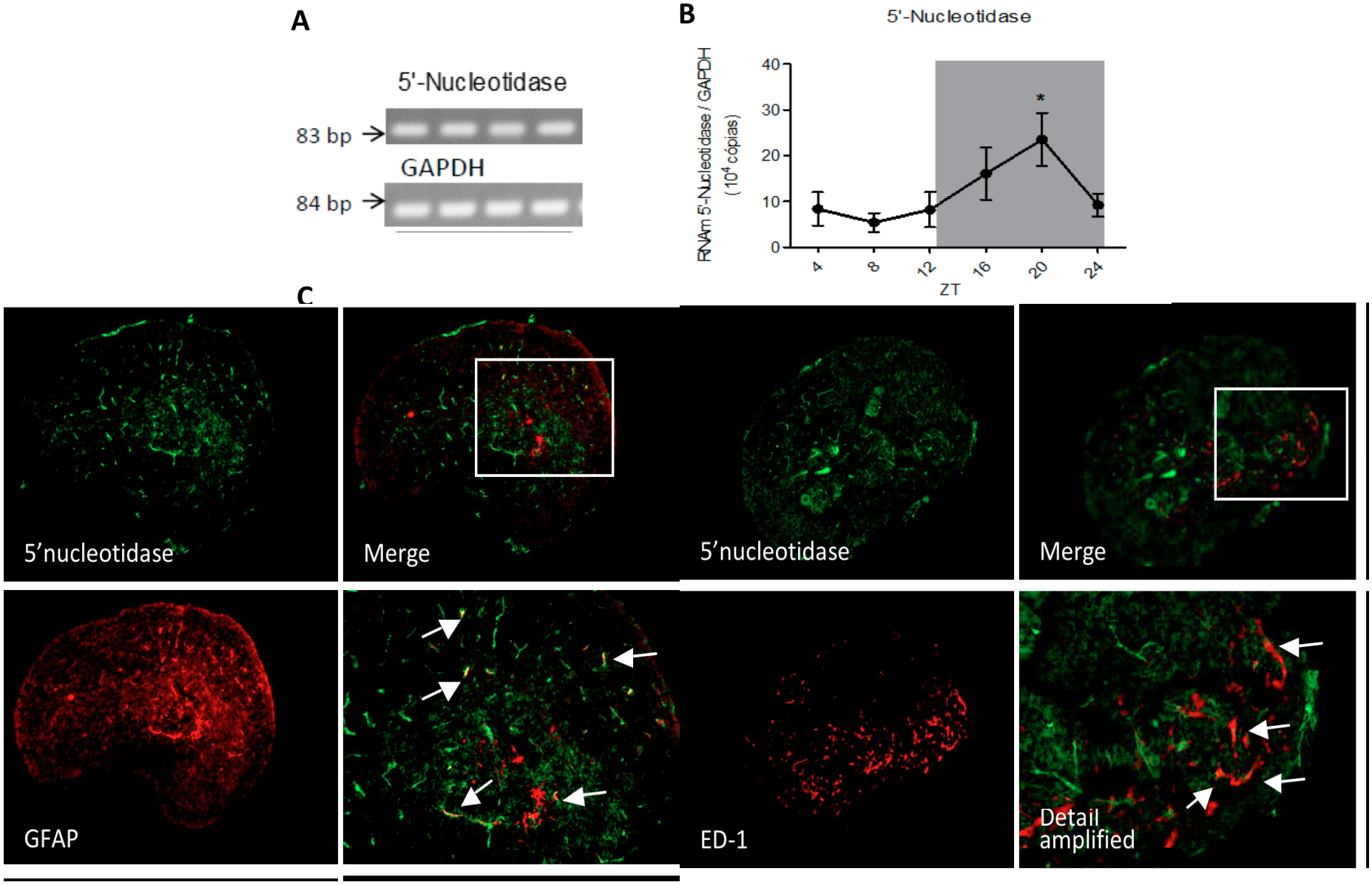
Ornelas et al., in preparation 2016

NTPDase3 expression in rat pineal gland



Ornelas et al., in preparation

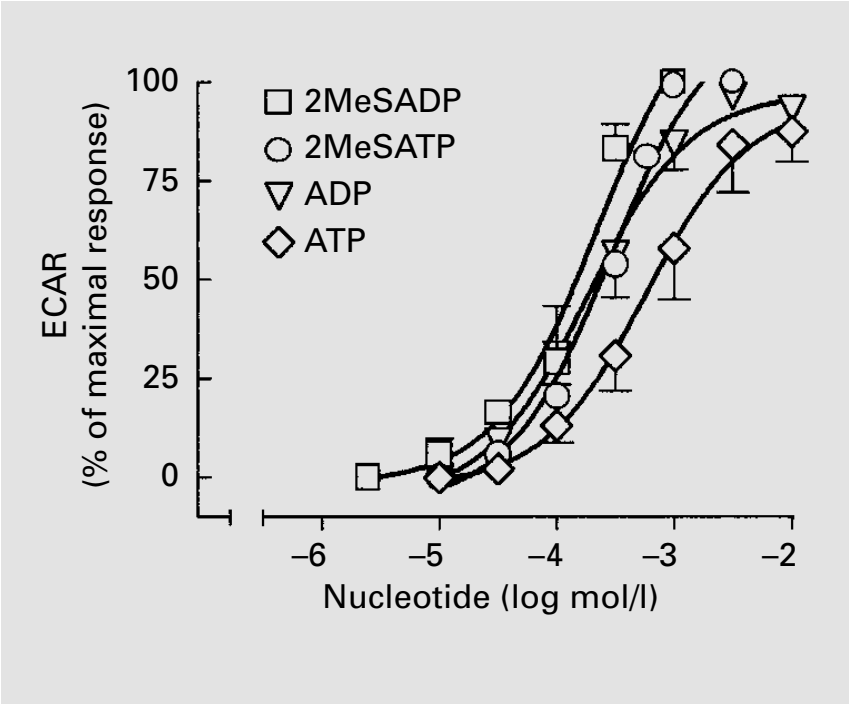
5’nucleotidase expression in rat pineal gland



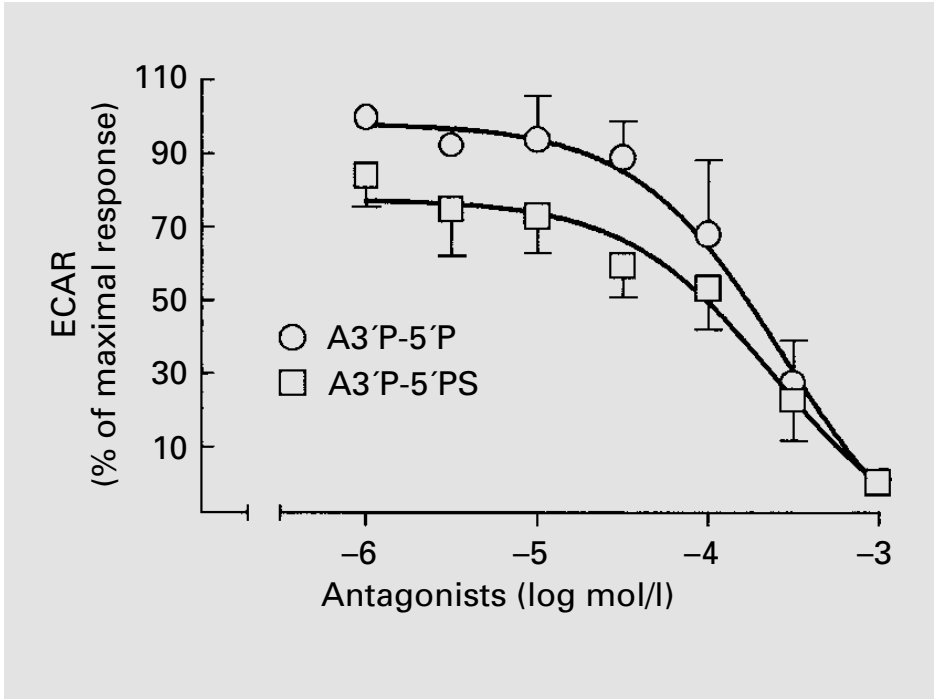
Ornelas et al., in preparation

Pharmacological profile – P2Y1 and P2X7 receptor

Agonists Effect



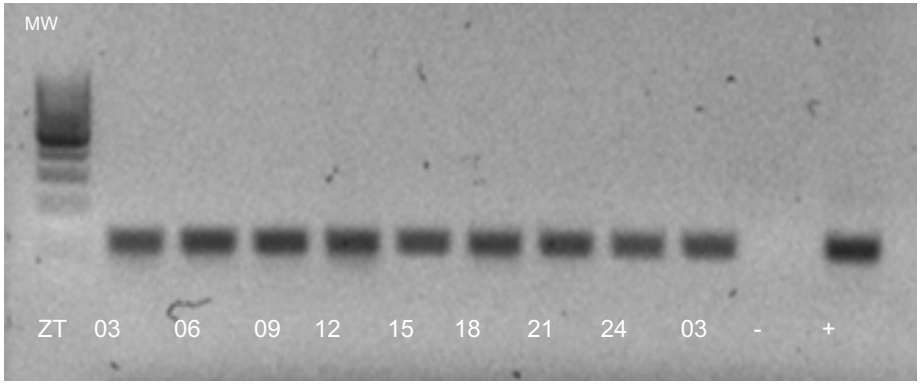
Antagonists Effect



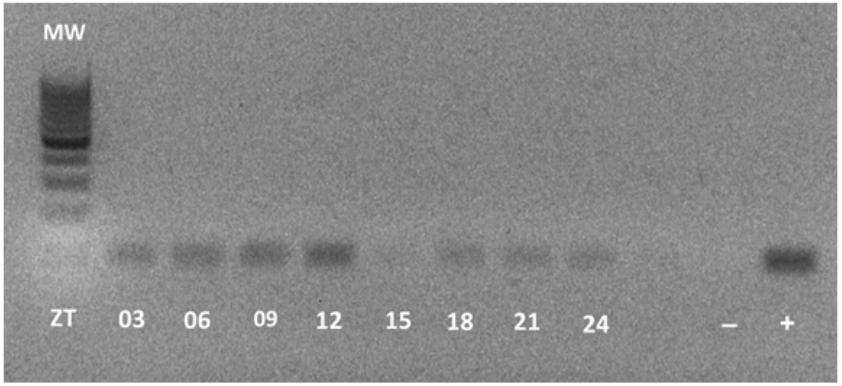
Ferreira et al., 2003

Purinergic receptors in the pineal gland

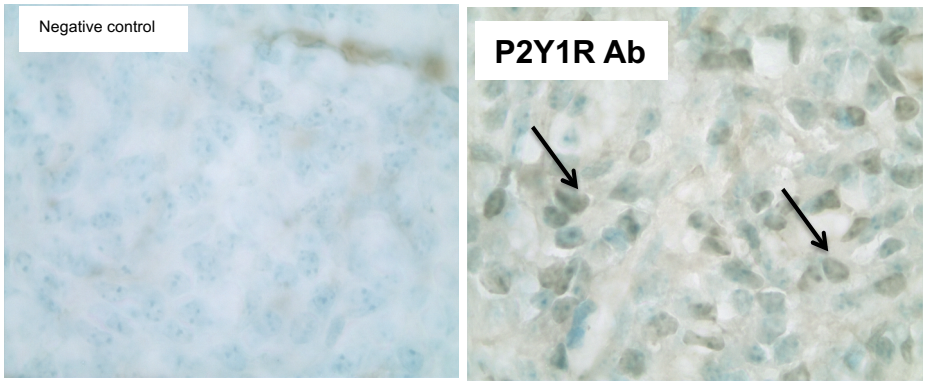
P2Y1 mRNA

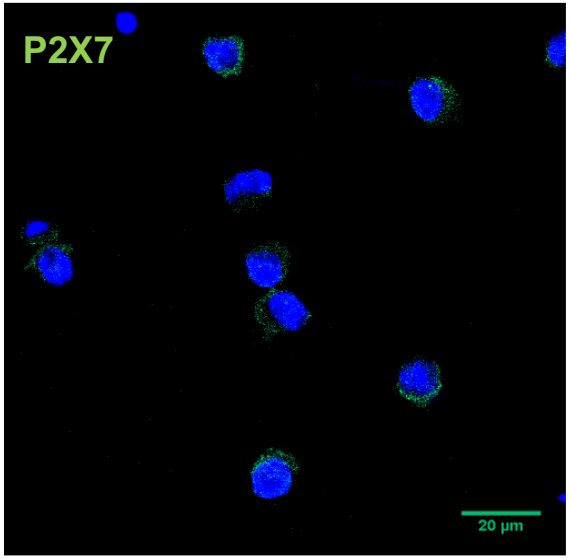


P2X7 mRNA



Immunohystochemistry

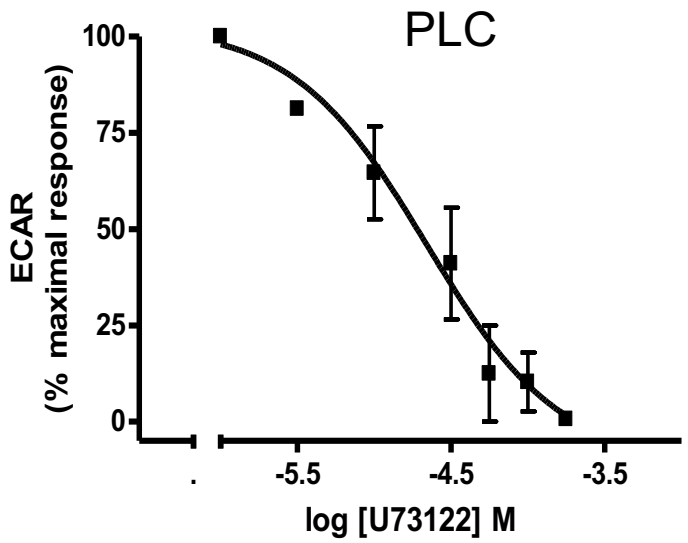


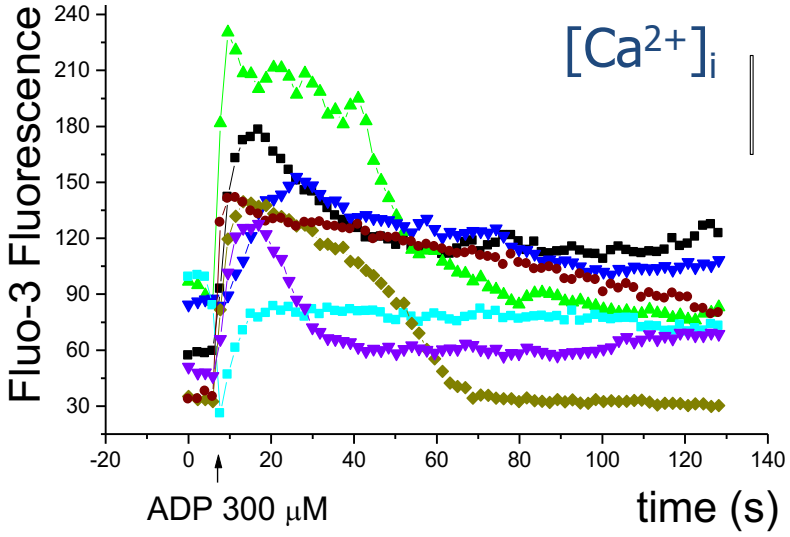


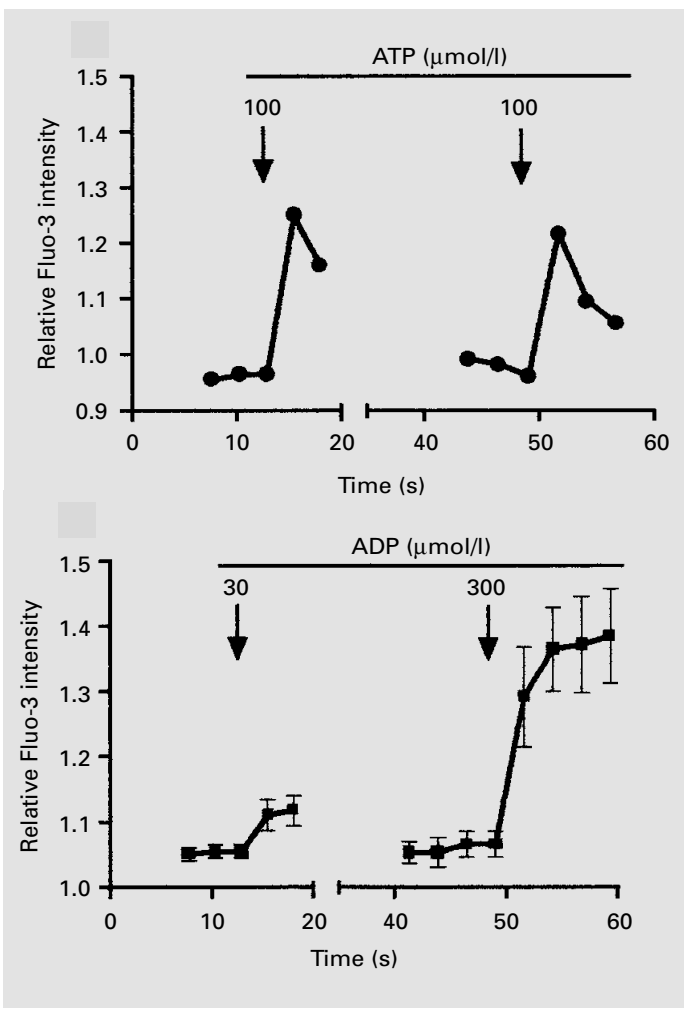
Dargenio-Garcia et al., unpublished

Purinergic signaling in the pineal gland

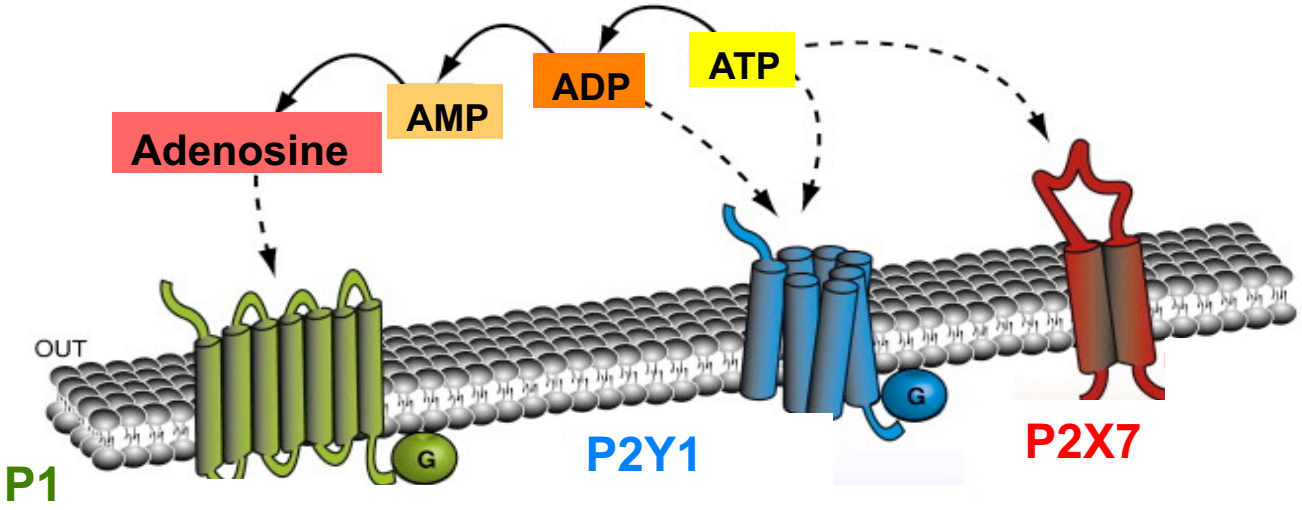
✓ ATP-stimulus is translated by increasing the activity of PLC, and $[Ca^{2+}]_i$,

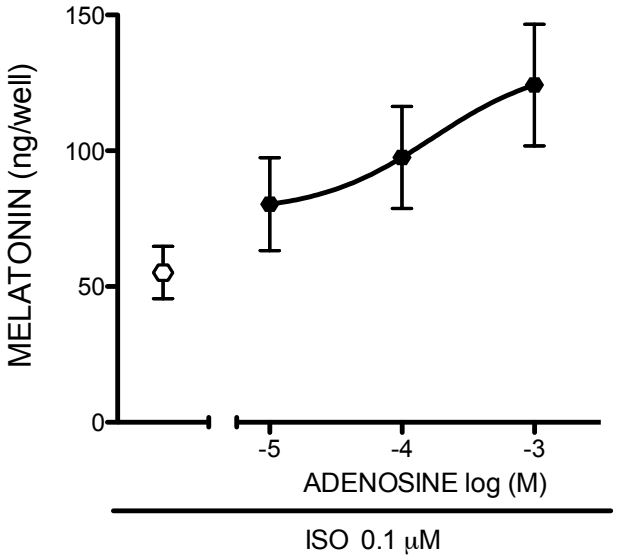


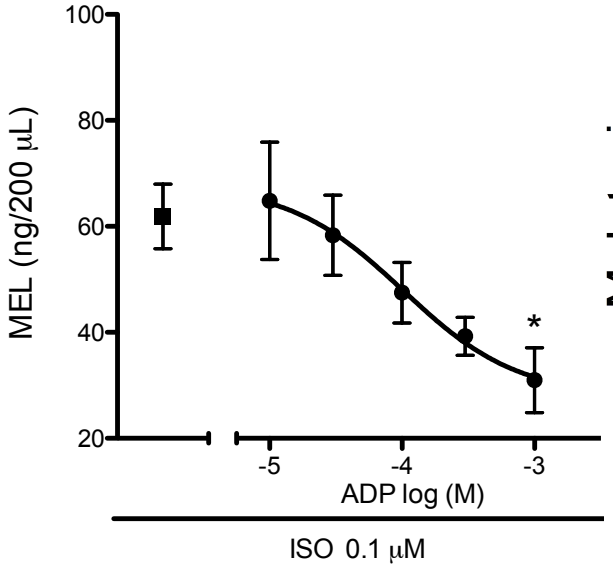


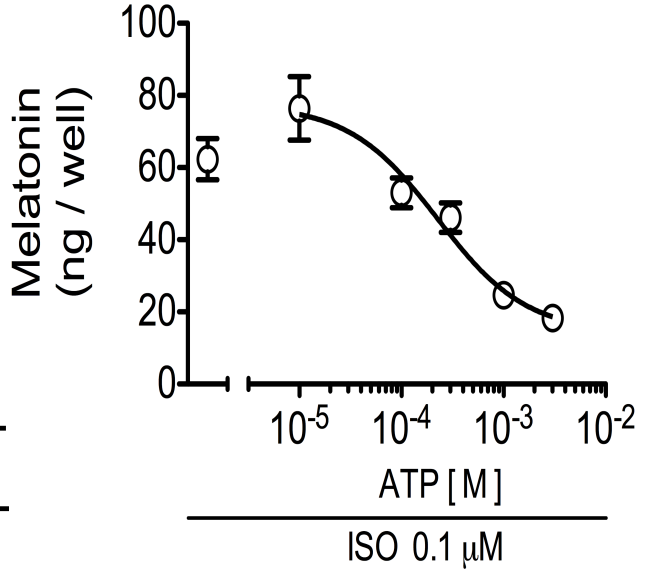


Functional role of purinergic signalling in the pineal







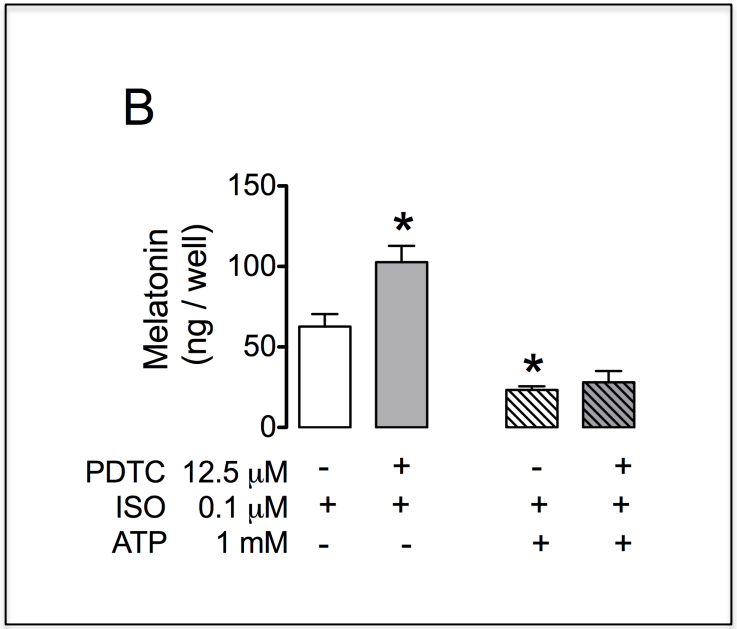


Sarda et al., 1989; Nonaka et al., 1991

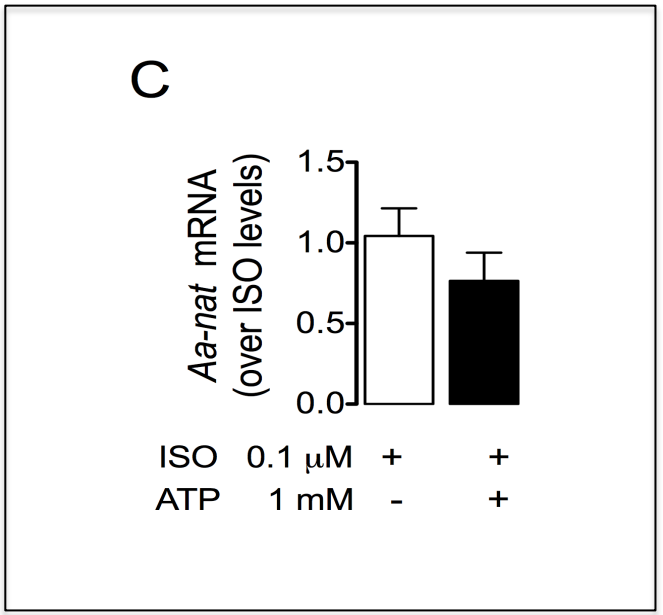
MELATONIN

Control of AA-NAT by ATP

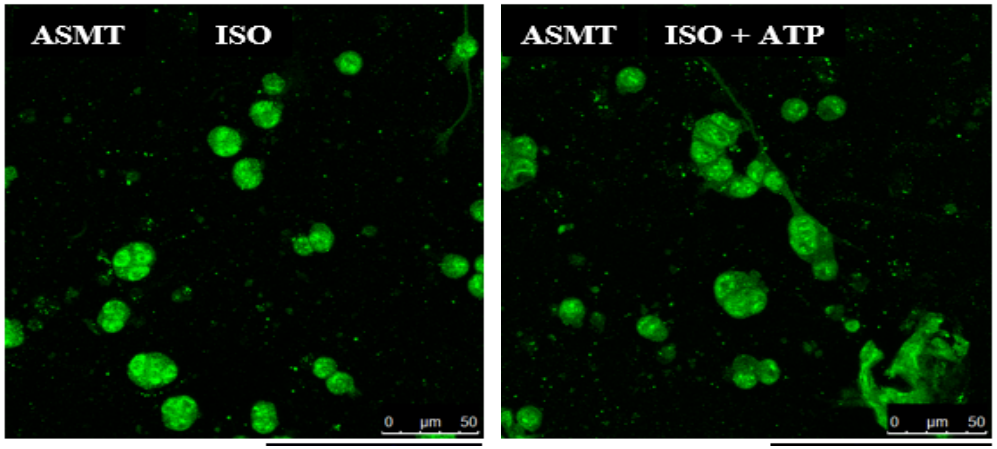
Inhibition of NF-kB

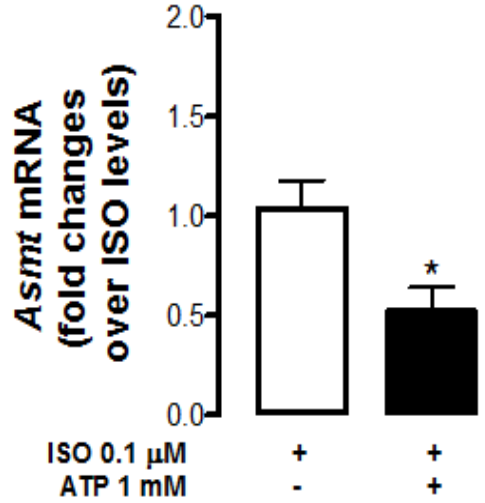


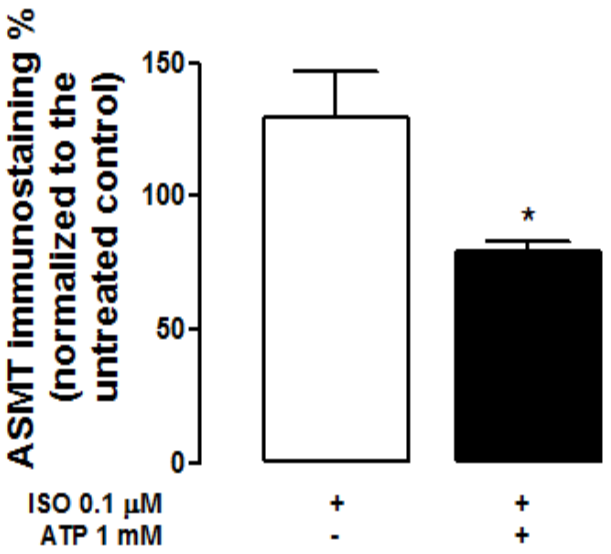
Aa-nat mRNA



Souza-Teodoro et al., J. Pineal Res., 2016



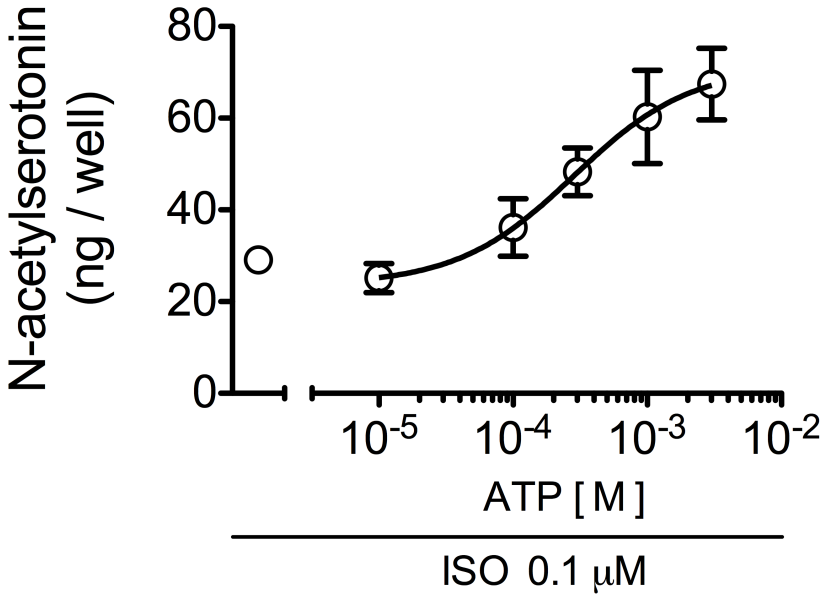


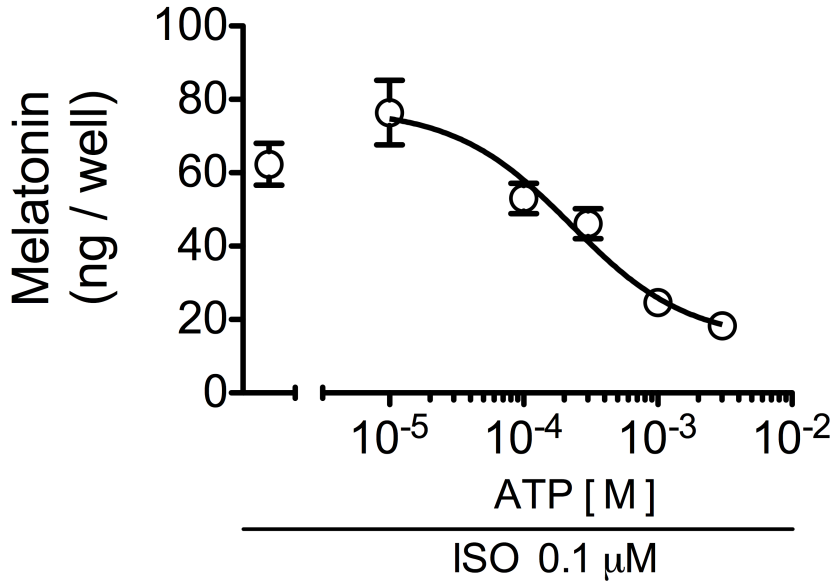


Souza-Teodoro et al., J. Pineal Res., 2016

Dual ATP/ADP effect
opposite directions on pineal melatonergic system

N-acetylserotonin ↑ **melatonin** ↓

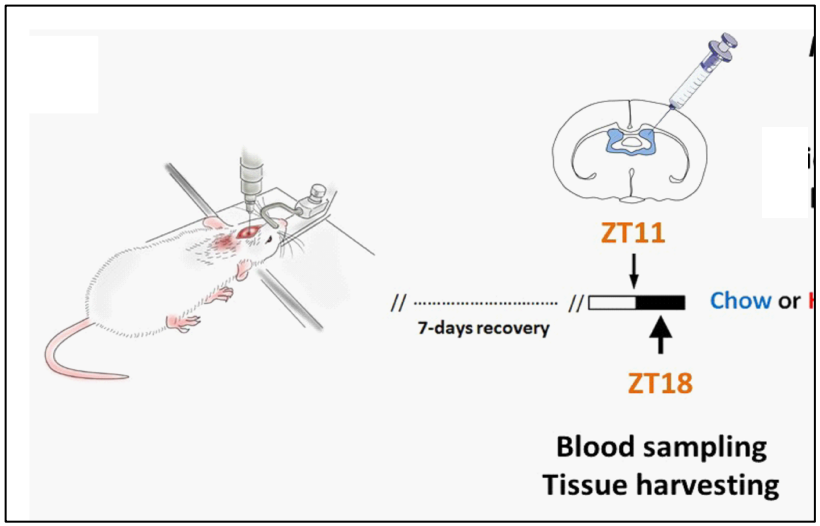


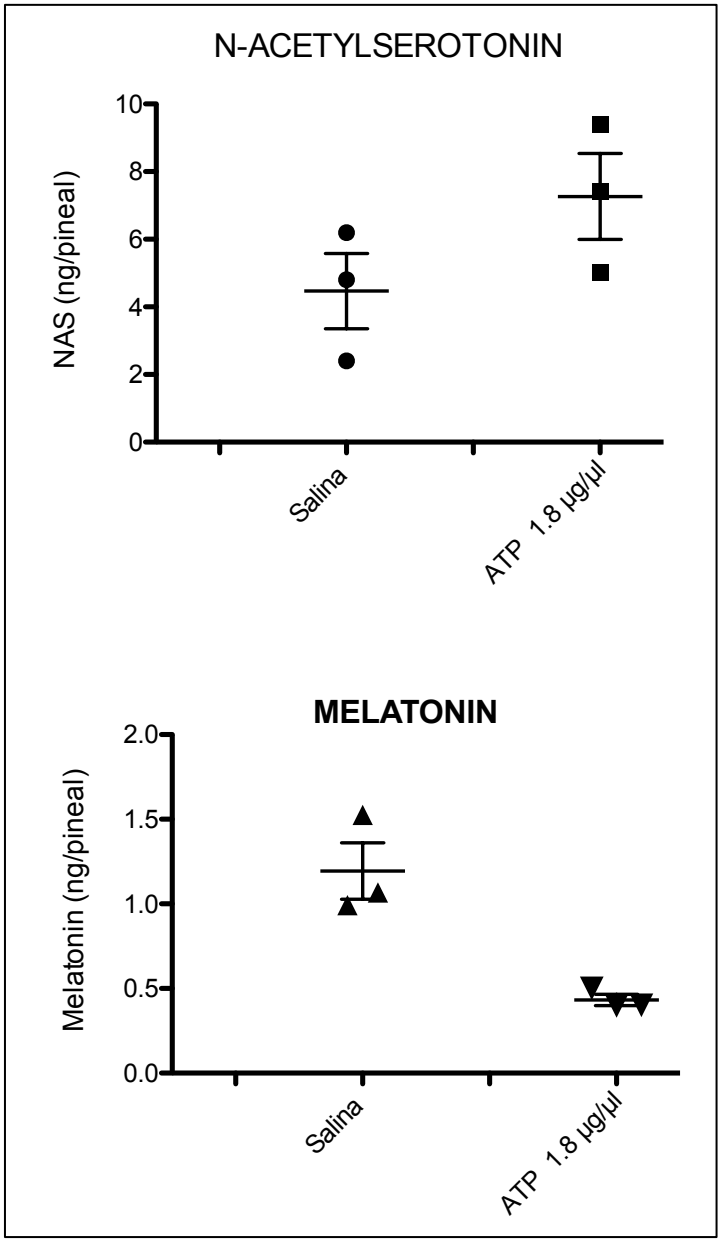


Therefore: an independent functional role of melatonin and its precursor

Souza-Teodoro et al.,J. Pineal Res. 2016

Dual effect of ATP *in vivo*



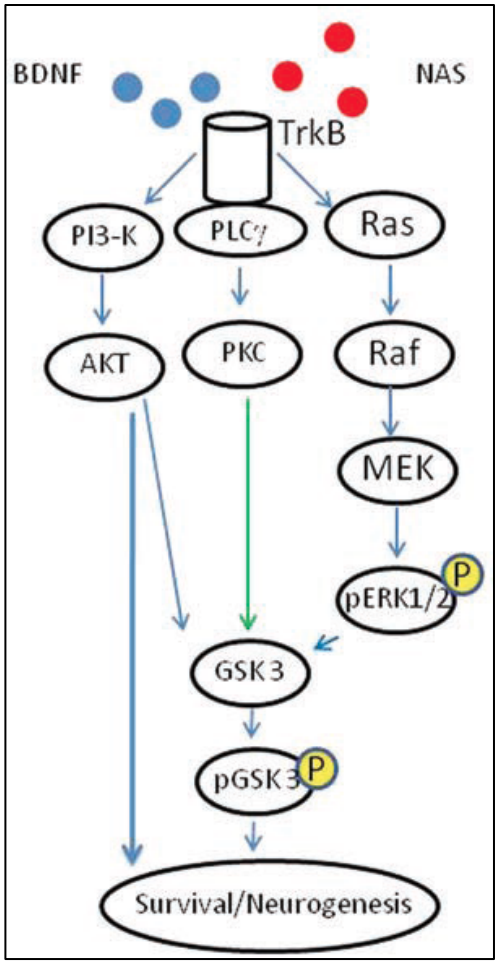


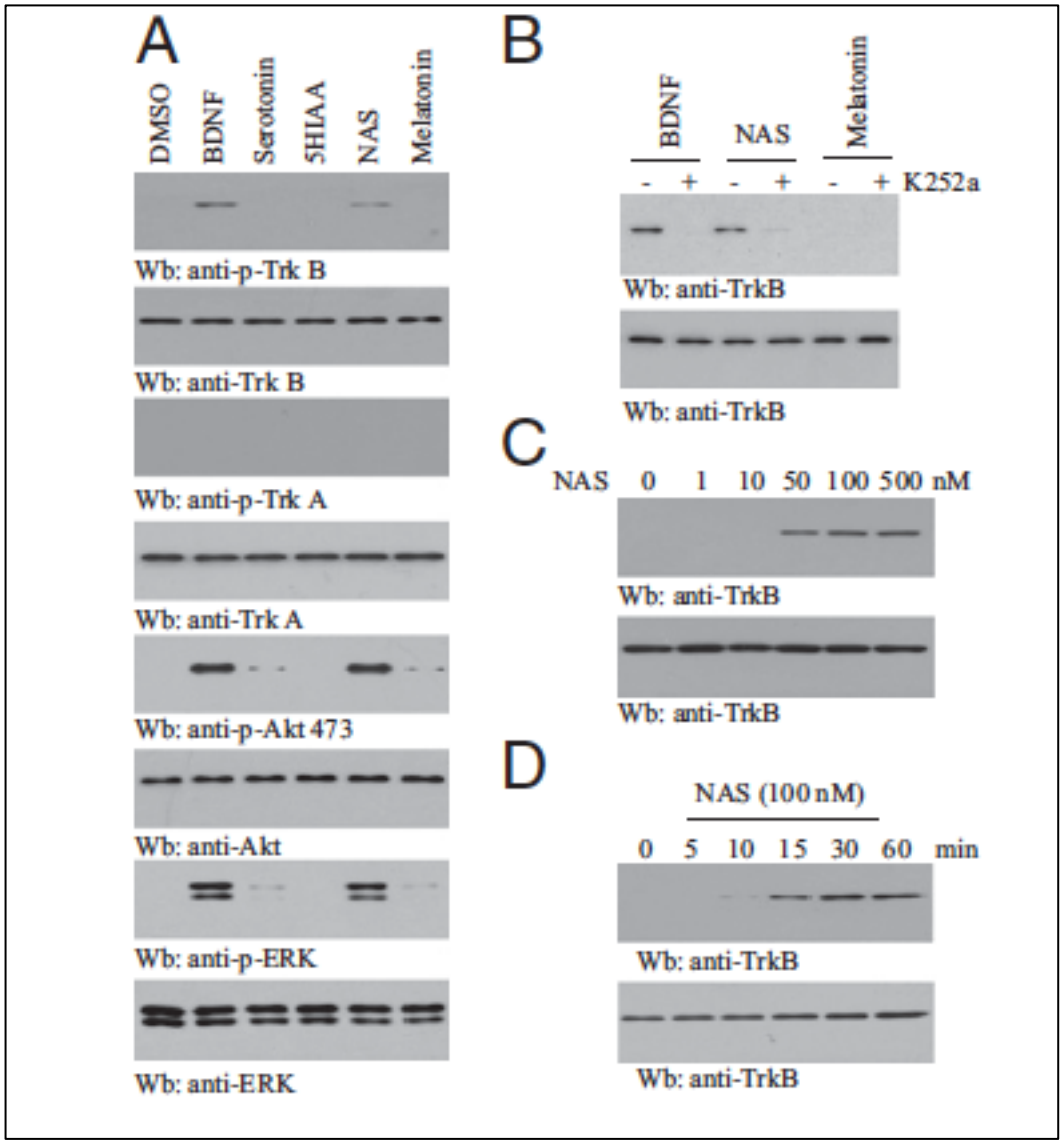
Therefore:
an independent functional role of melatonin and its precursor

Souza et al.,on going

N-acetylserotonin: unexpected roles in neuronal cell biology

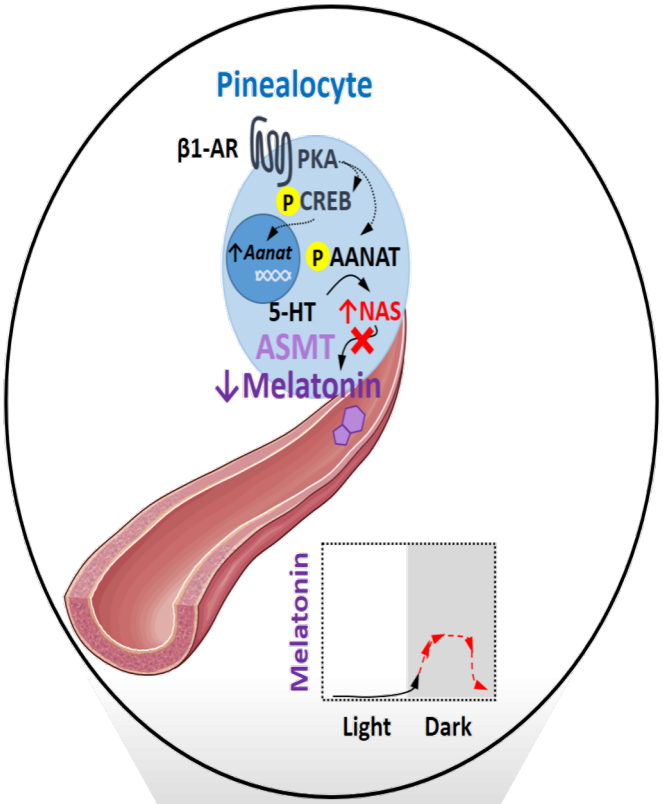
neuroprotective actions by activating TrkB/CREB/BDNF pathway





Jang et al., PNAS, 2010; You et al., Redox Biology, 2017

ATP



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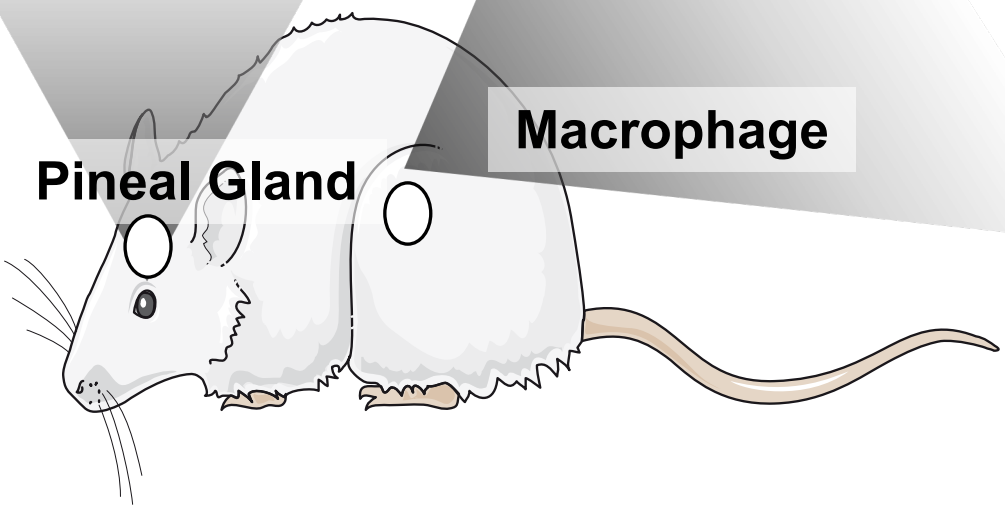
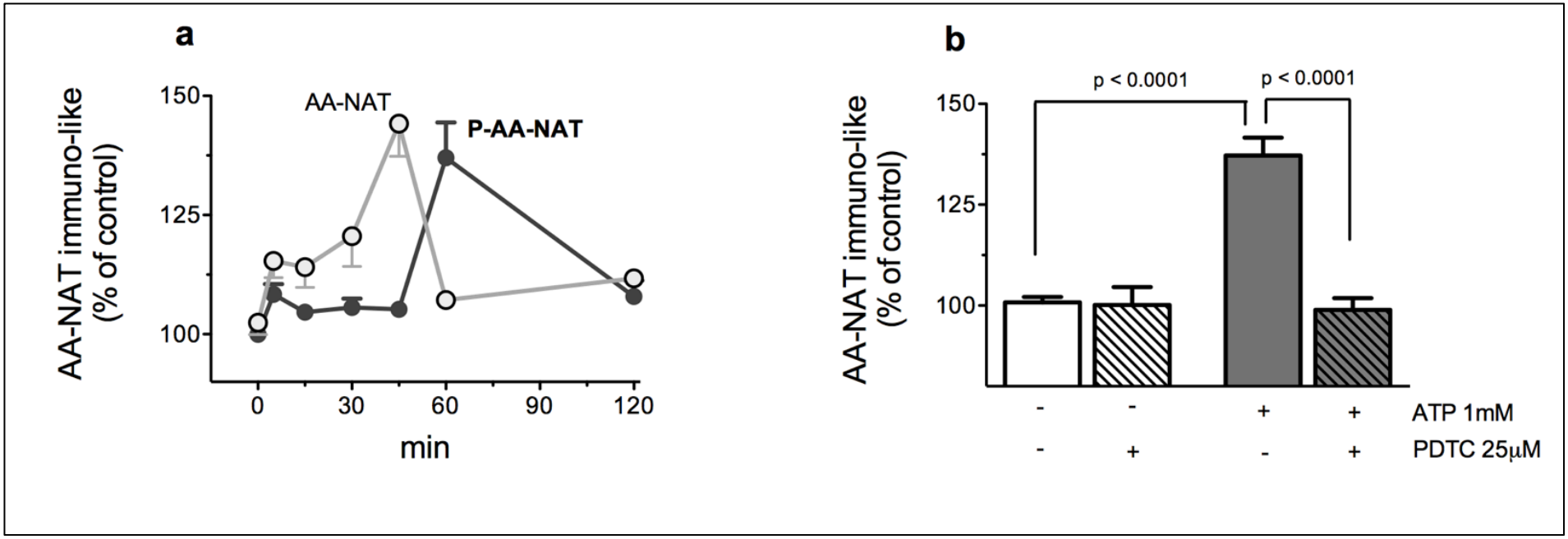
Macrophage

Pineal Gland



In RAW 264.7 macrophages ATP stimulation …

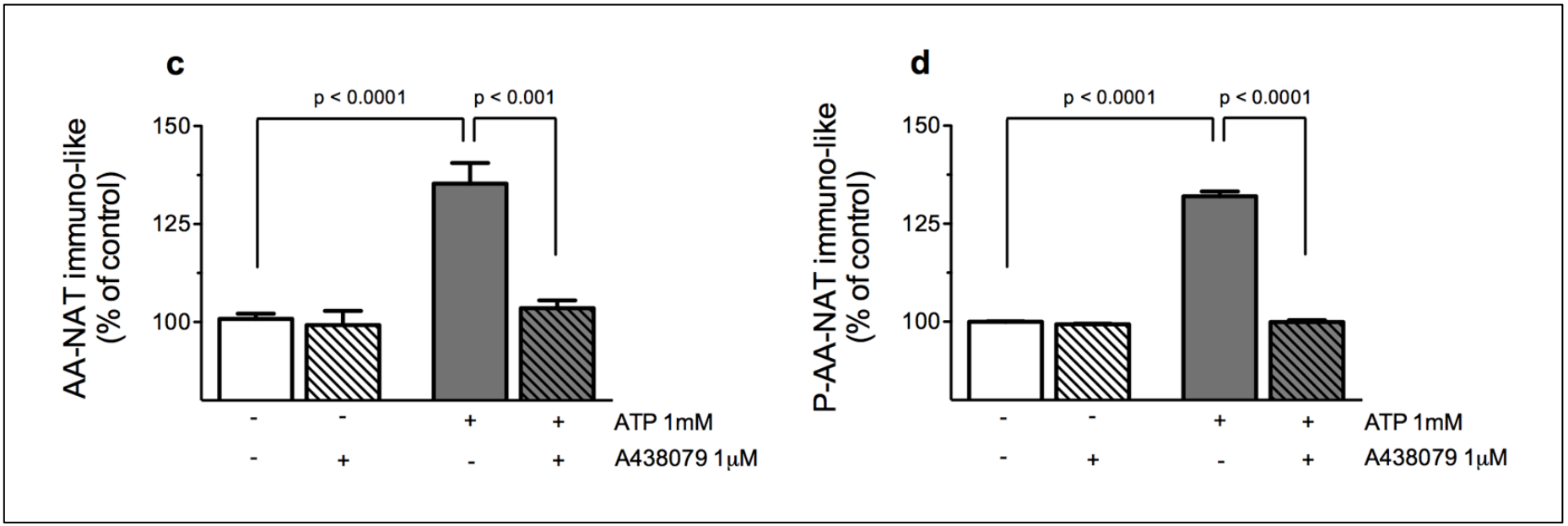
- induces the increase in AA-NAT/P-AANAT protein content via the NFκB pathway



Dargenio-Garcia et al., submitted

In RAW 264.7 macrophages ATP stimulation …

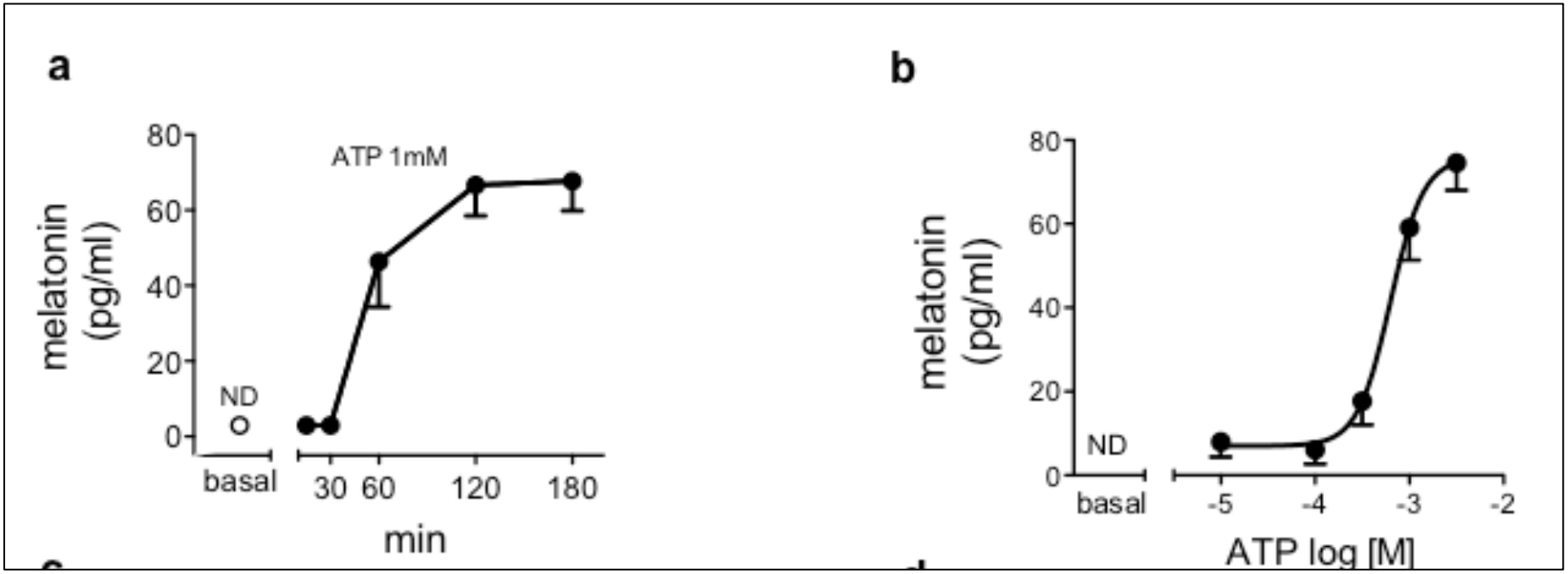
- through P2X7 receptor stimulation



Dargenio-Garcia et al., submitted

In RAW 264.7 macrophages ATP stimulation …

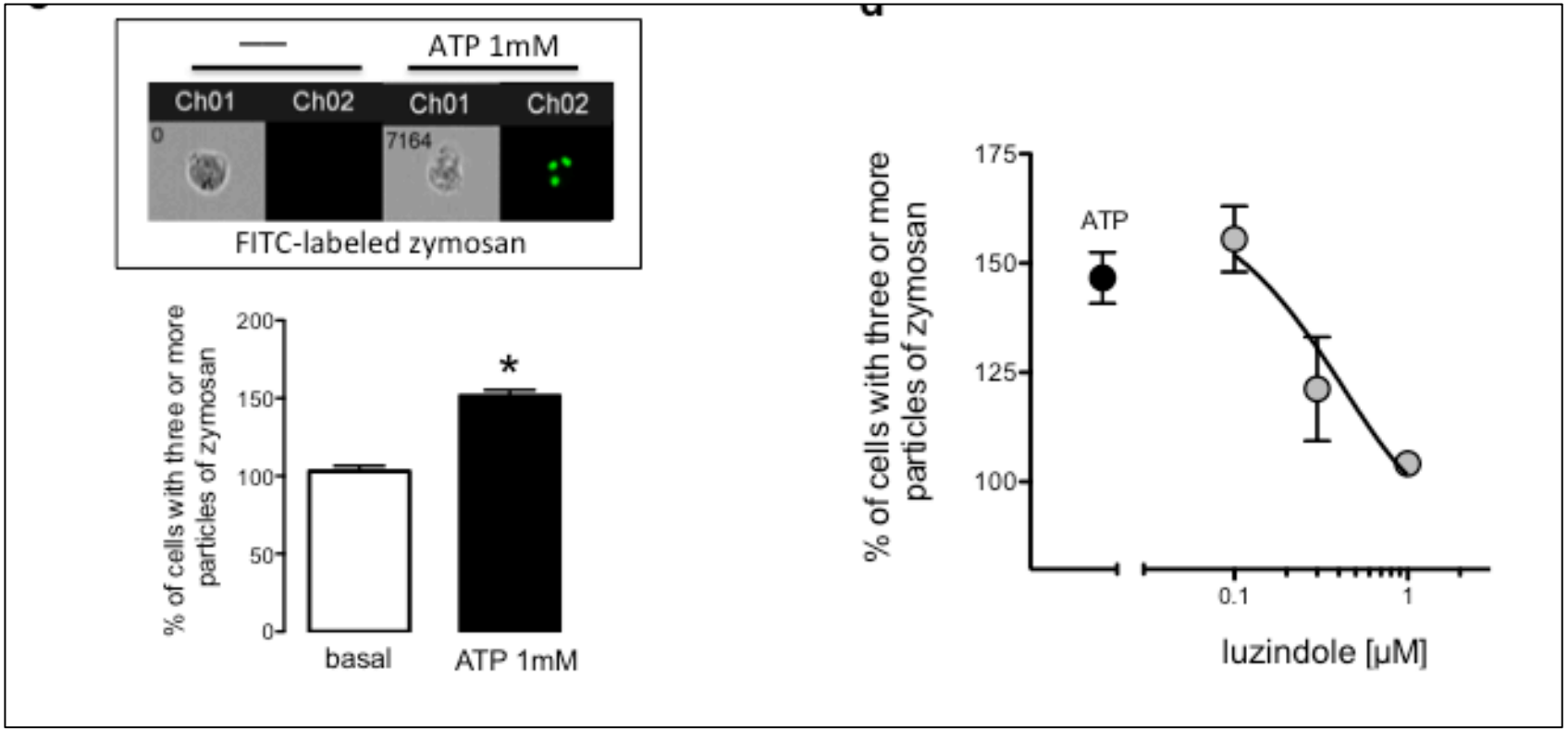
- induces melatonin synthesis



Dargenio-Garcia et al., submitted

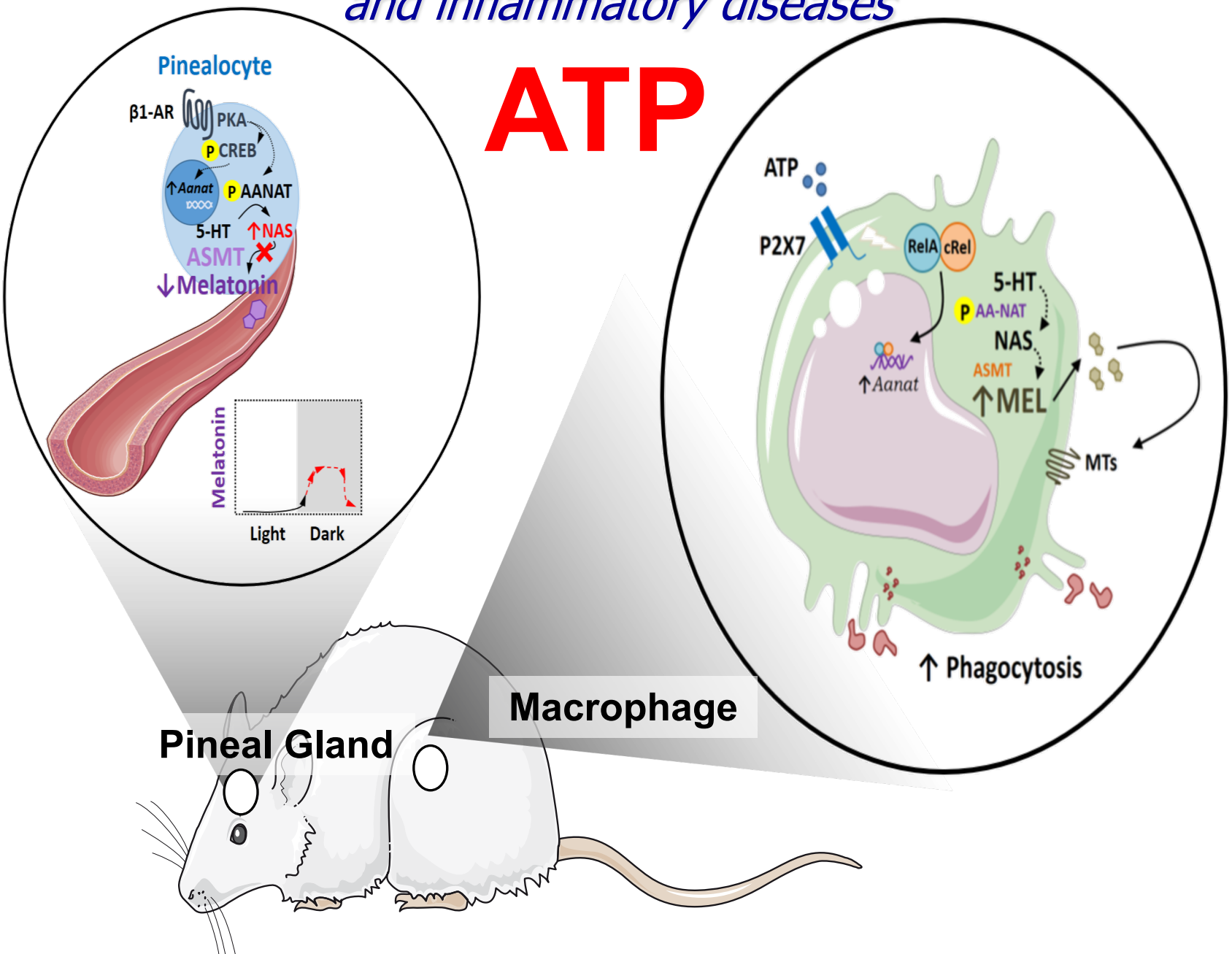
In RAW 264.7 macrophages ATP stimulation …

- potentiates the macrophage phagocytic ability through melatonin receptors



Dargenio-Garcia et al., submitted

New insights into development and progression of neurological and inflammatory diseases



L'heure, c'est l'heure ;
avant l'heure, c'est pas l'heure ;
apres l'heure, c'est plus l'heure

Jules Jouy

"Time is the hour; before the hour, it's not time; after the hour, it's more time"

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- ✓ ATP acts as a cotransmitter in the pineal gland;

Mortani-Barbosat al., Eur. J. Pharmacol. 401:59, 2000

- ✓ Ectonucleotidases enzymes present daily variation;

Ornelas et al., in preparation

- ✓ ATP triggers P2Y1 and P2X7 receptors potentiating N-acetylserotonin and inhibiting melatonin synthesis;

Ferreira & Markus. Eur. J. Pharmacol. 415:151, 2001

Souza-Teodoro et al., J. Pineal Res., 2016

- ✓ $[Ca^{2+}]_i$ mediate a PLC-induced enhance in N-acetylserotonin synthesis, while melatonin reduction is related to an ASMT inhibition in the pineal;

Ferreira et al., Pharmacology 69:33-37, 2003

Souza-Teodoro et al., J. Pineal Res., 2016

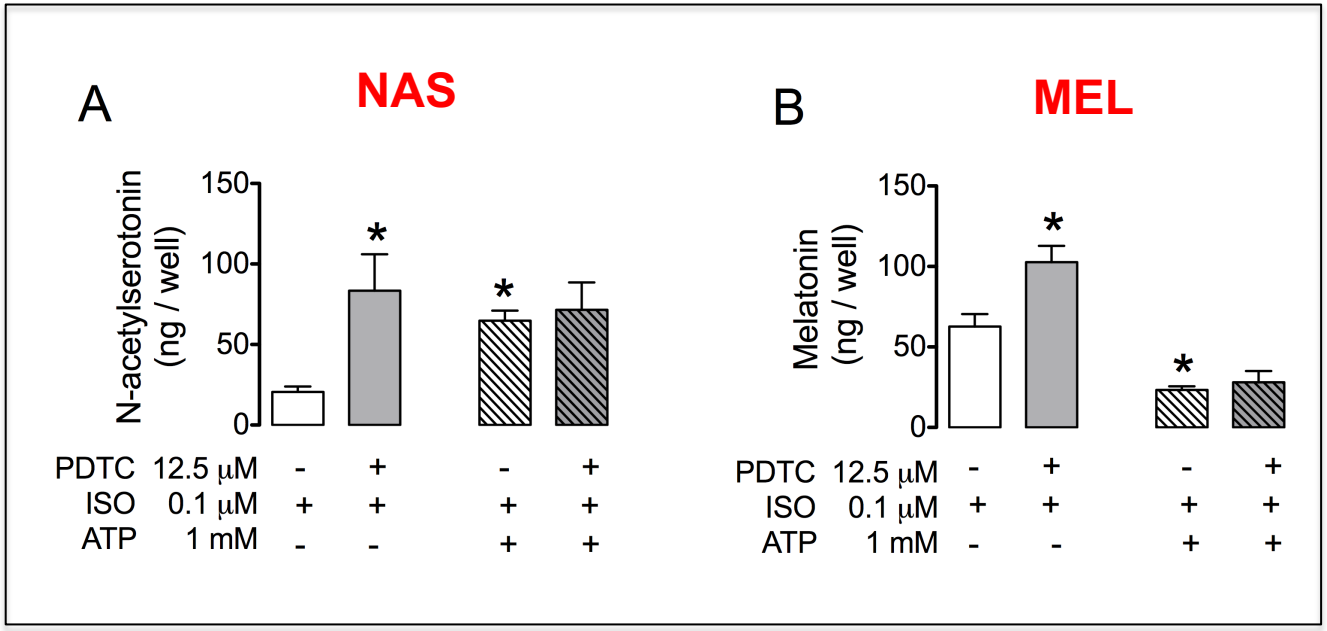
- ✓ In RAW 264.7 cells ATP increases melatonin production through P2X7 receptors, NFκB pathway, AA-NAT/P-AANAT, which further potentiates phagocytosis.

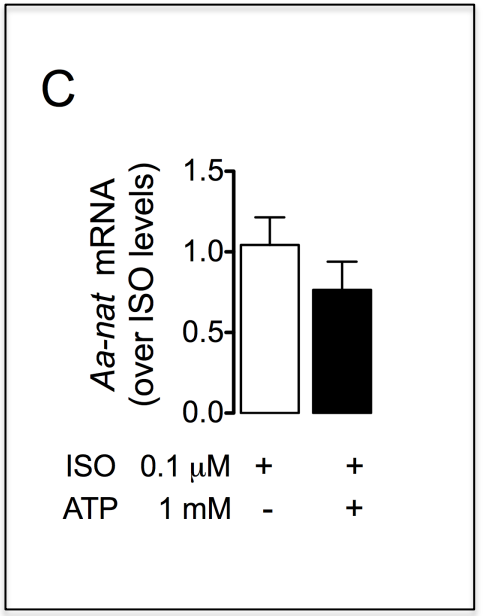
Dargenio-Garcia et al., submitted

Putative control of Aa-nat by ATP

Inhibition of NF-kB

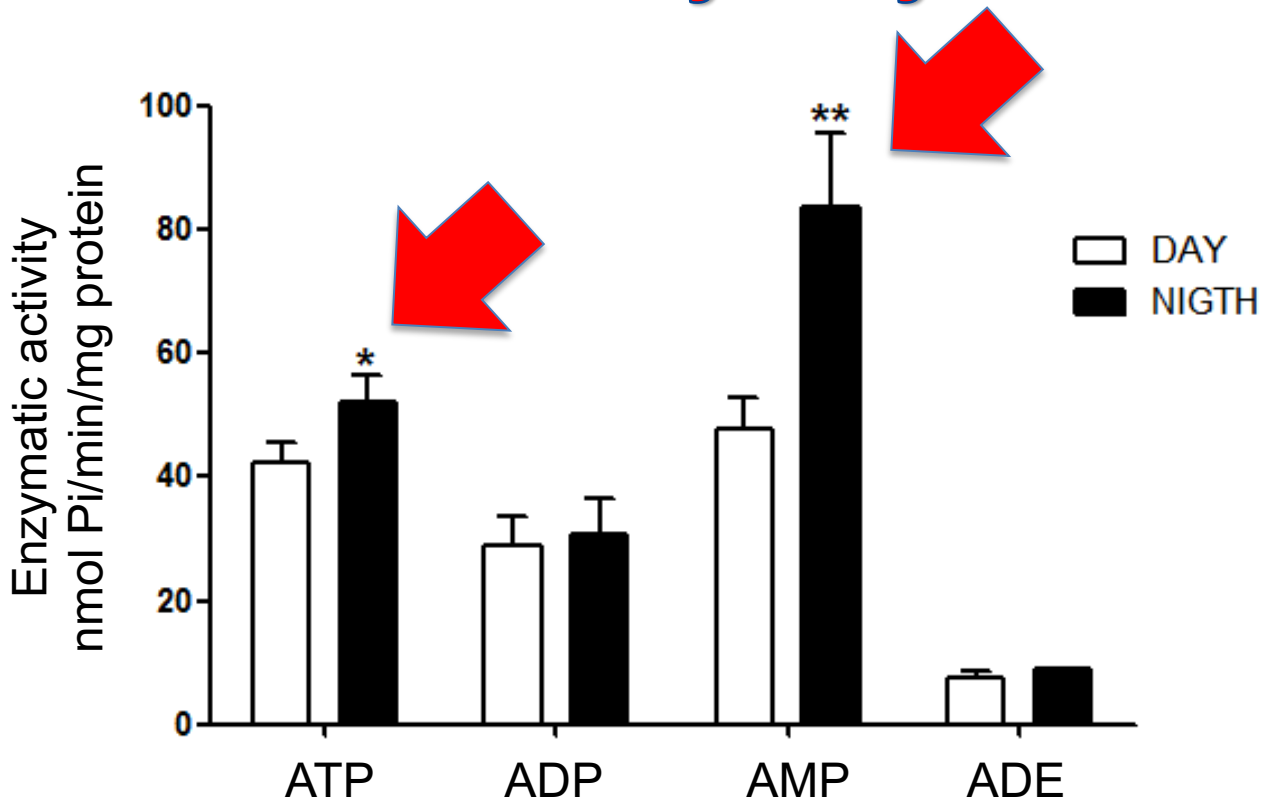
Aa-nat mRNA





Souza-Teodoro et al., J. Pineal Res., 2016

Daily variation of nucleotide and nucleoside hydrolysis

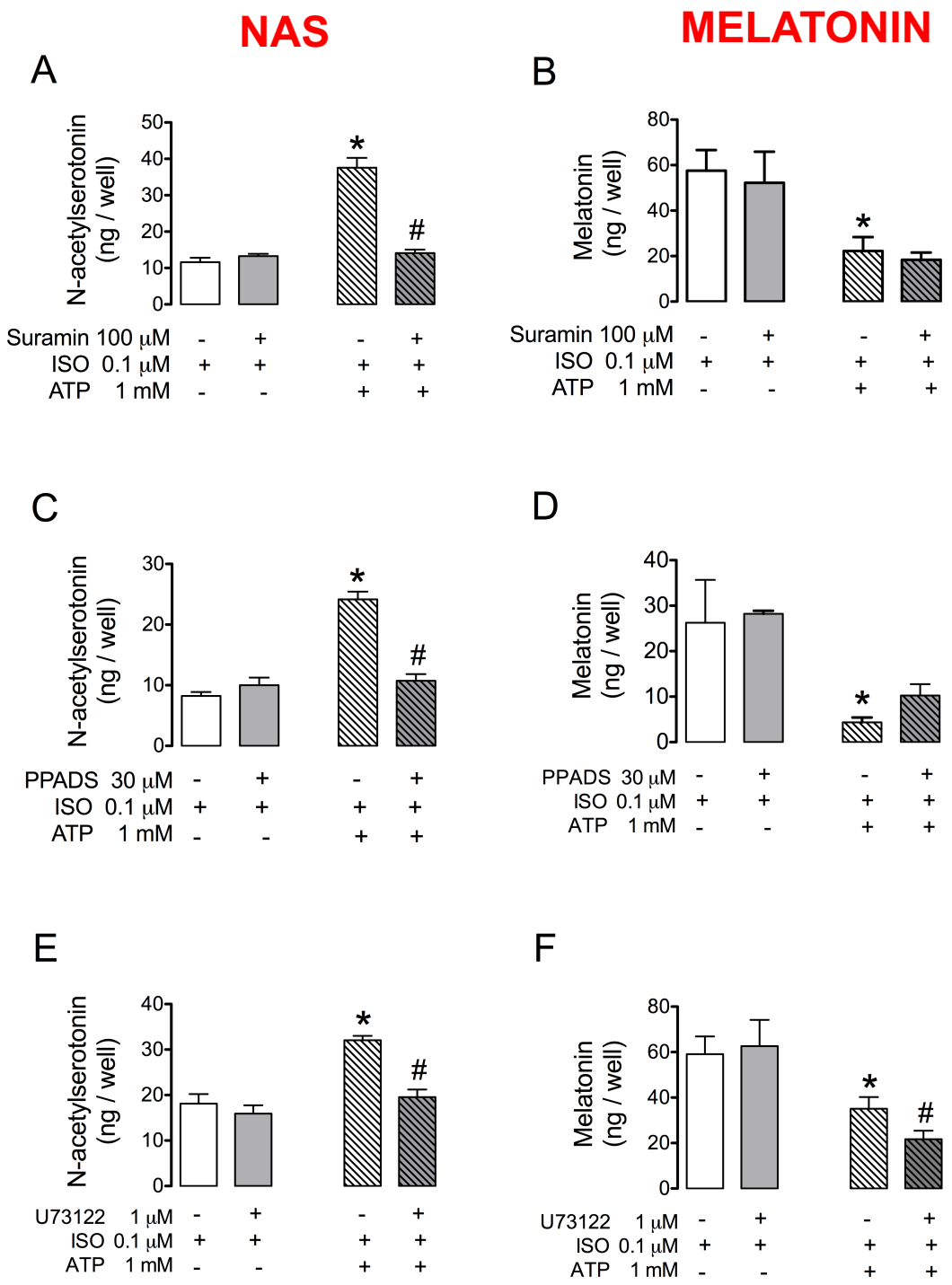


✓ The ectonucleotidase activity showed a significantly increase in ATP and AMP hydrolysis in the dark phase (* $p<0.05$).

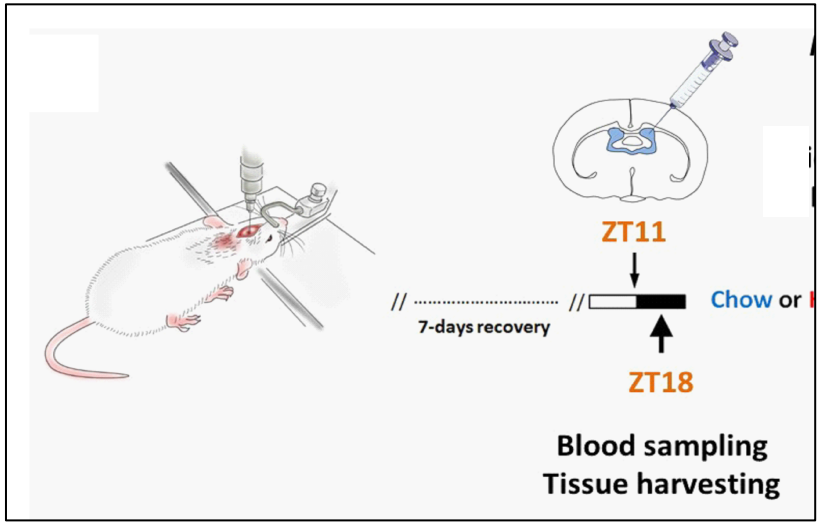
Therefore, the purinergic system presents a daily adaptation for regulating physiological pineal gland activity

Ornelas et al., in preparation 2016

Purinergic receptor involved



Souza-Teodoro et al., J. Pineal Res, 2016


ZT11
7-days recovery
Chow or
ZT18
Blood sampling
Tissue harvesting

NAS

NAS may be an endogenous neuroprotectant.