“Saint Peter released from prision”
Filippino Lippi

Fisiopatología de la Narcolepsia

Dr. Pablo Torterolo
Profesor Agregado
Laboratorio de Neurobiología del Sueño
Departamento de Fisiología

Dr JEAN-BAPTISTE-ÉDOUARD GÉLINEAU

DE LA

NARCOLEPSIE

PAR LE

Dr GÉLINEAU

MEMBRE DE LA SOCIÉTÉ DES MÉDECINS, DU BUREAU DE BIENFAISANCE
DE LA SOCIÉTÉ FRANÇAISE D'HYGIÈNE
DE L'ACADÉMIE DE LA ROCHELLE, ETC., ETC.

J. TESSIER & Eugène TESSIER
IMPRIMERIE DE SURGÈRES
(Charente-Inférieure)

1881

Narcolepsia

"…es una neurosis rara o poco conocida caracterizada por una necesidad imperiosa de dormir, súbita y de corta duración que se reproduce a intervalos mas o menos largos…".

Gelineau, 1880.

“Por primera vez me di cuenta de que tenía un problema cuando **a los 14 años** me dormía en el liceo y era despertado por el profesor.

Fueron **nueve** los accidentes de auto que padecí entre los 16 y 20 años, todos por quedarme dormido mientras manejaba.

A los 21 años me diagnosticaron narcolepsia, pero entre los 21 y 25 años sufrí **seis** accidentes de transito, ya que no fui tratado correctamente.

A los 25 años me sentí normal y alerta por primera vez, **gracias a que el tratamiento fue efectivo**.”

Joseph Piscopo
Chairman of the
American Narcolepsy Association
(1986-1993)

Epidemiología

- 1 in 2000 (*¿1500 pacientes en Uruguay?).*

- Similar en ambos sexos.

- Edad de comienzo: 15 a 35 años.

Clínica

- Hipersomnia (síntoma inicial).
    - ataques de sueño.
    - somnolencia (con automatismos motores).

- Cataplejia (70%). Pérdida repentina del tono muscular.
    - parcial o completa (similar a la atonía del sueño REM)
    - desencadenada por emociones

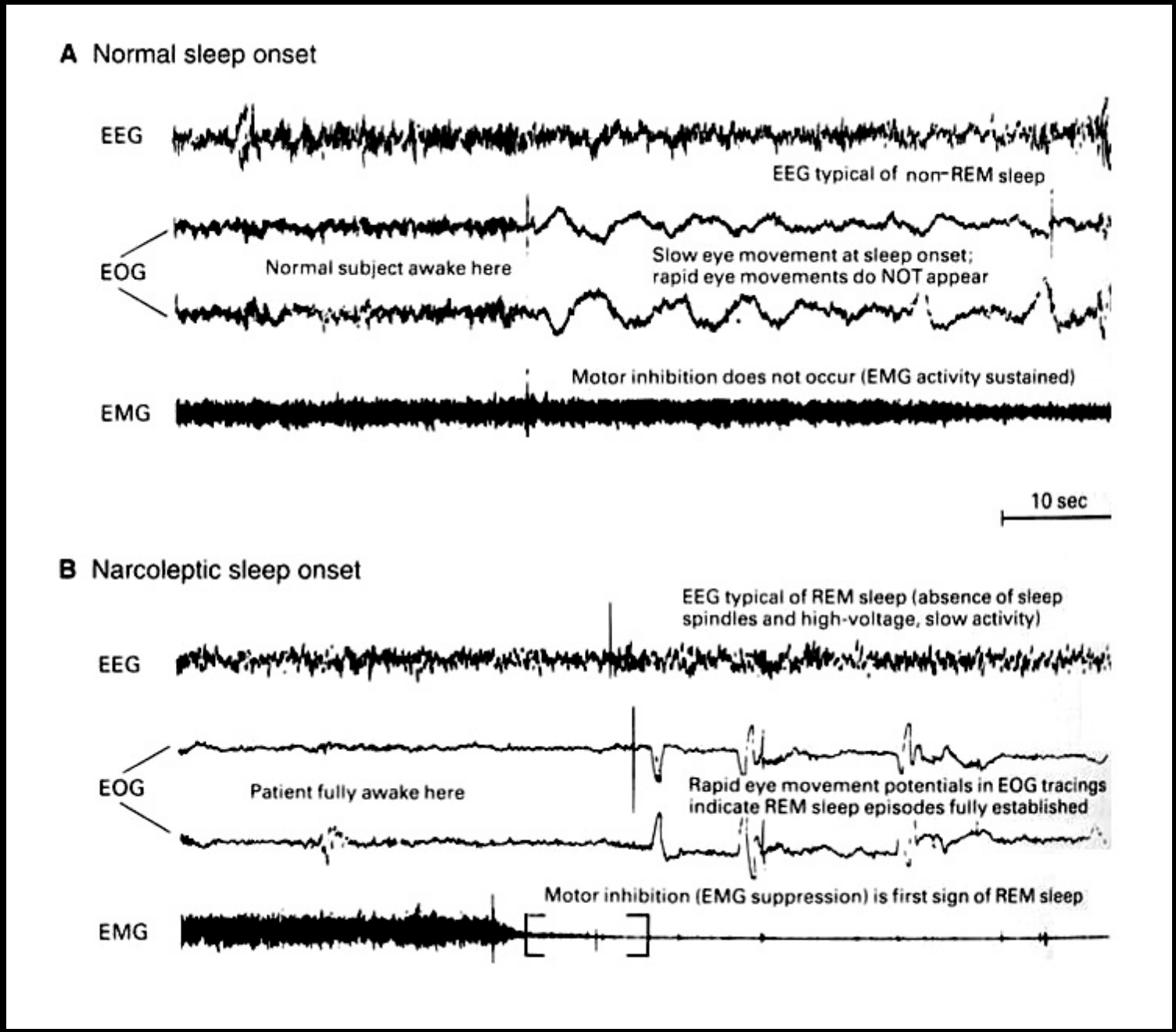
- Alucinaciones al comienzo del sueño (30%).

- Parálisis del sueño (25%).

Paraclínica

- Polisomnografía
    - sueño fragmentado
    - disminuye la latencia al sueño REM (“sleep onset REM”)

Sleep onset REM

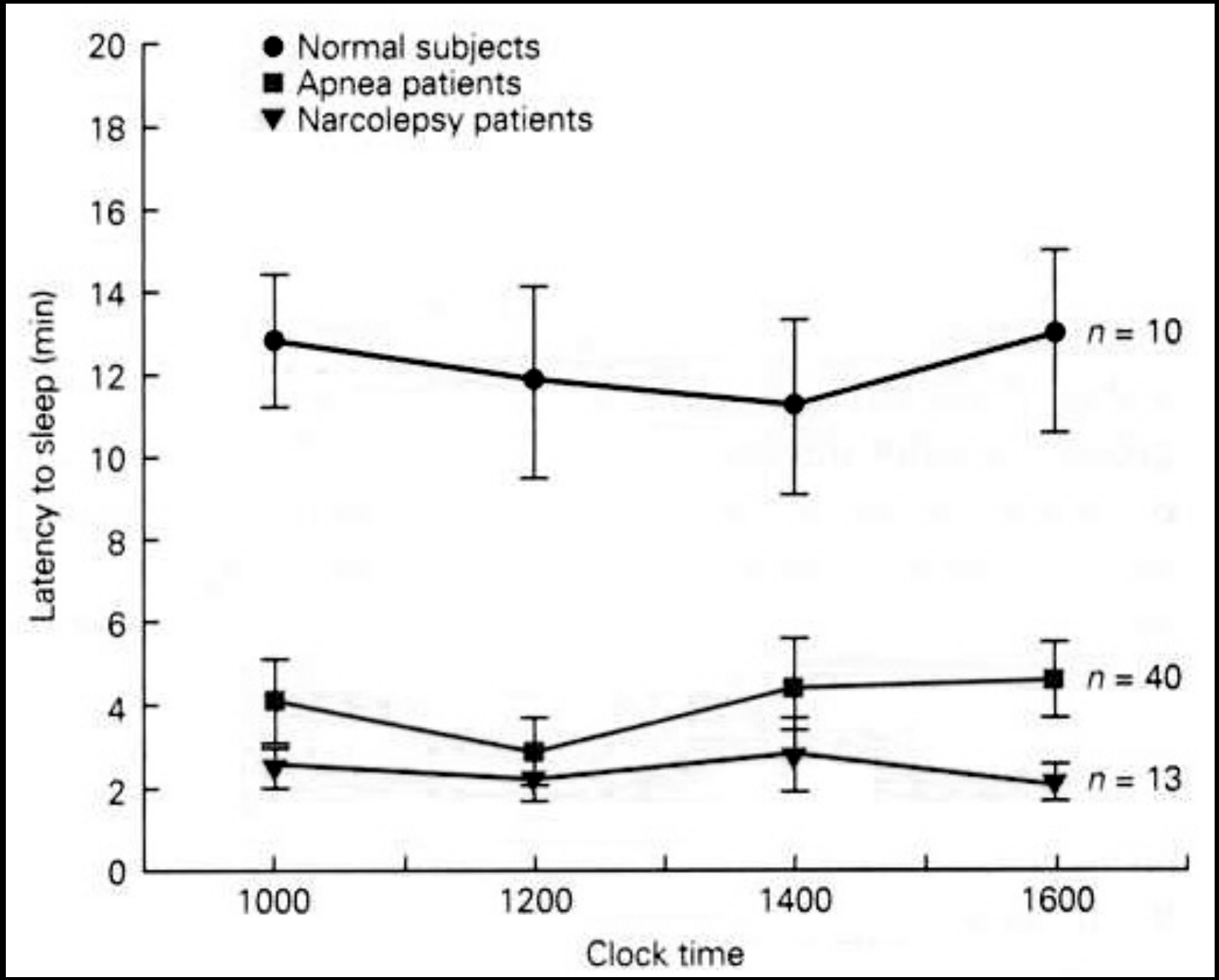


Kelly (1991). En: Principles of neural science

“Multiple sleep latency test”

- Latencia ≤ 8 minutos
- 2 o mas “Sleep Onset REM”

Disminuye la latencia al sueño en el “Multiple Sleep Latency Test”



Roth and Roehrs (2000). En: Principles of neural sciences

Fisiopatología

- **Trastorno en el mantenimiento de la vigilia** (Hipersomnia).

- **Intrusión del sueño REM (o sus signos) durante la vigilia** ("sleep onset REM", cataplejia, paralisis del sueño, alucinaciones)

Tratamiento farmacológico

- Sustancias promotoras de vigilia (Anfetaminas, metilfenidato, modafinilo, etc).

- Sustancias que inhiben el sueño REM (Antidepresivos).

Historia de las **hipocretinas y la Narcolepsia**: ejemplo de Neurociencia Traslacional (impacto de la investigación básica, para entender y tratar las enfermedades cerebrales)

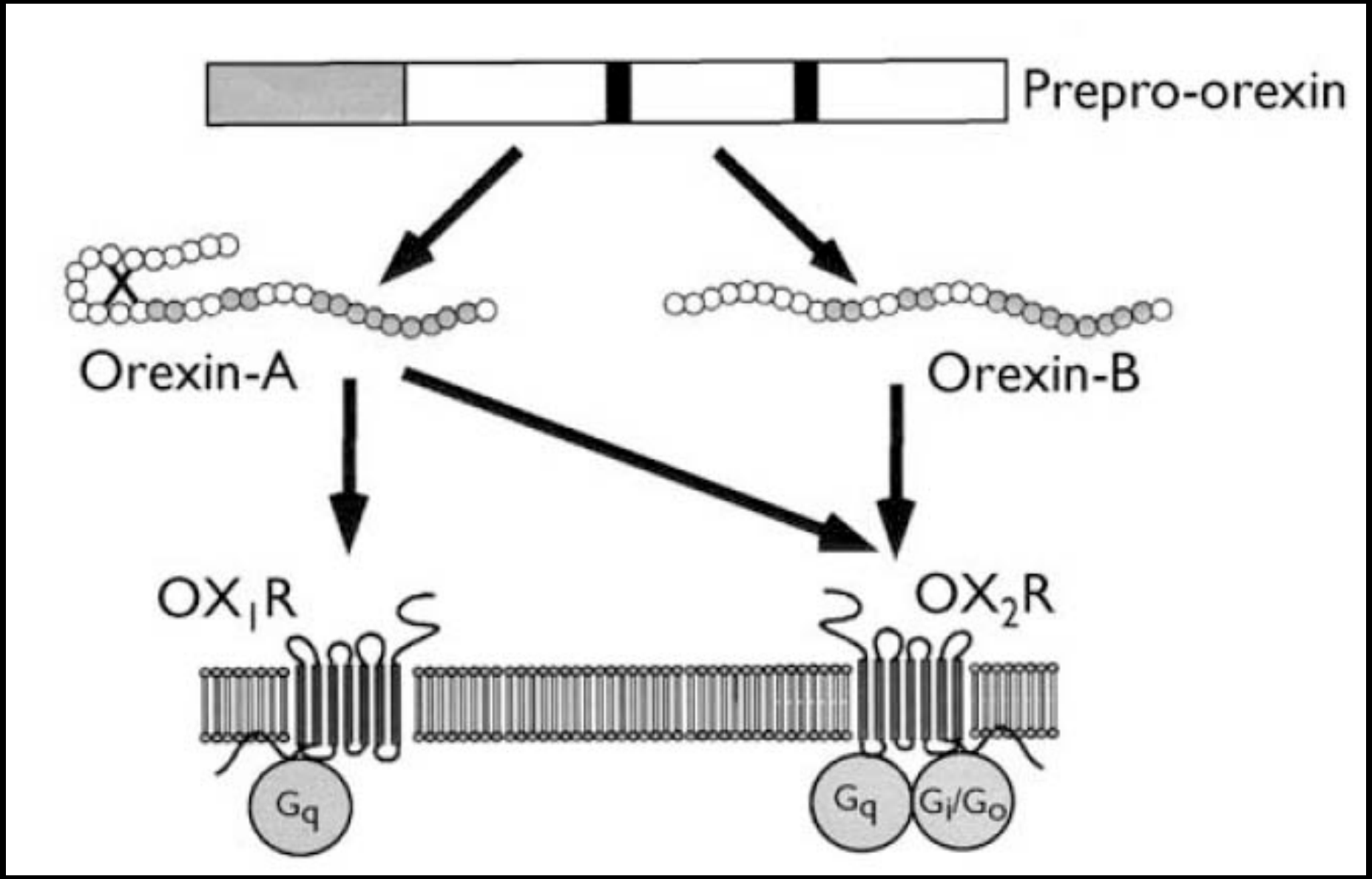
1998. - Descubrimiento de las hipocretinas (orexinas).
- Anatomía del sistema hipocretinérgico.

1999. - La Narcolepsia familiar canina: mutación en el gen del *receptor tipo 2 de la hipocretina*
- Ratones “knock-out” para las hipocretinas presentan un fenotipo narcoléptico.

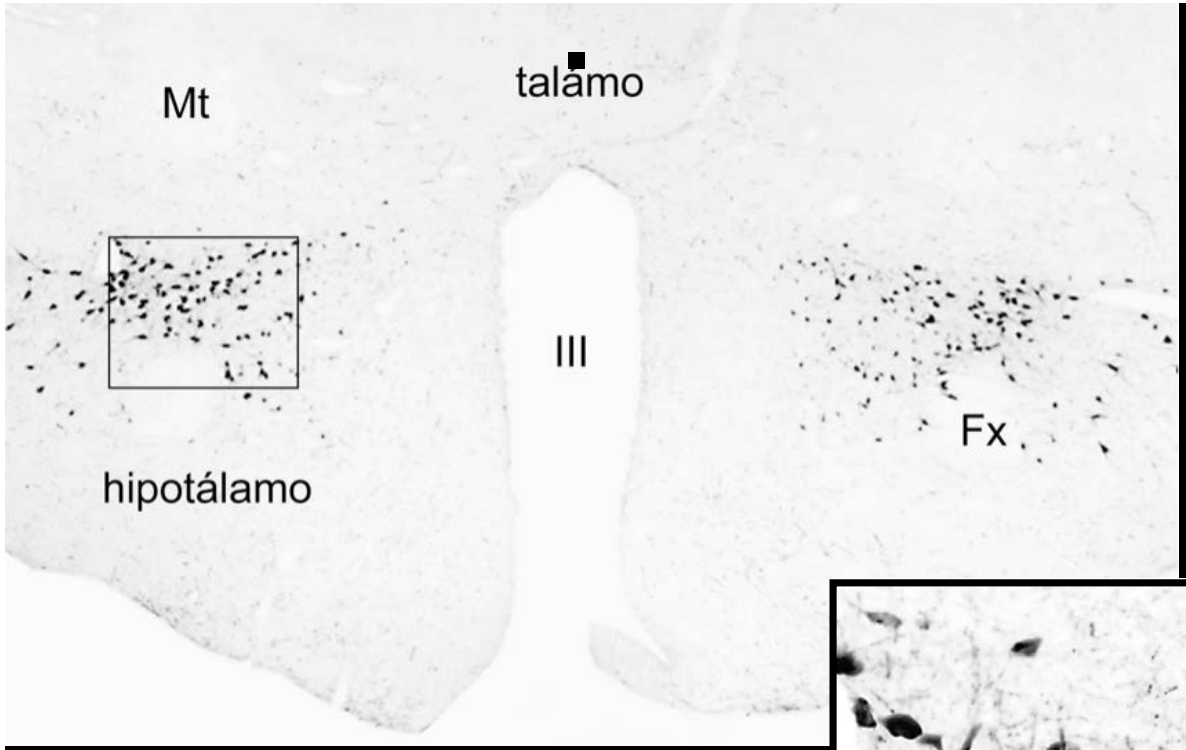
2000. - La narcolepsia humana muestra una degeneración de las neuronas hipocretinérgicas.

2001.
- Titulación de hipocretinas en el LCR como método diagnóstico de Narcolepsia.
- Tratamiento de sustitución en modelos animales de Narcolepsia.
- Tratamiento de sustitución en pacientes Narcolépticos.

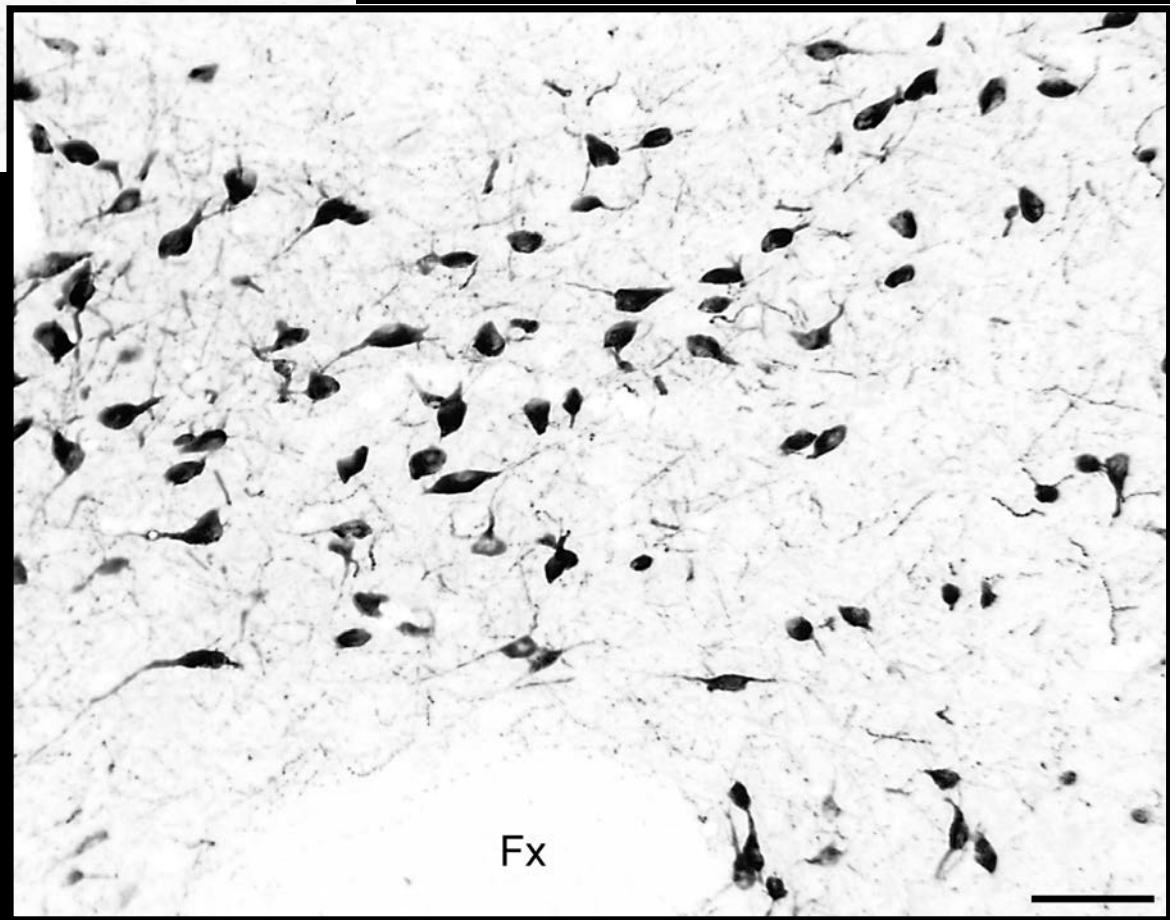
Las hipocretinas/orexinas son neuromoduladores peptídicos



Sakurai (2002). Neuroreport, 13: 987-995

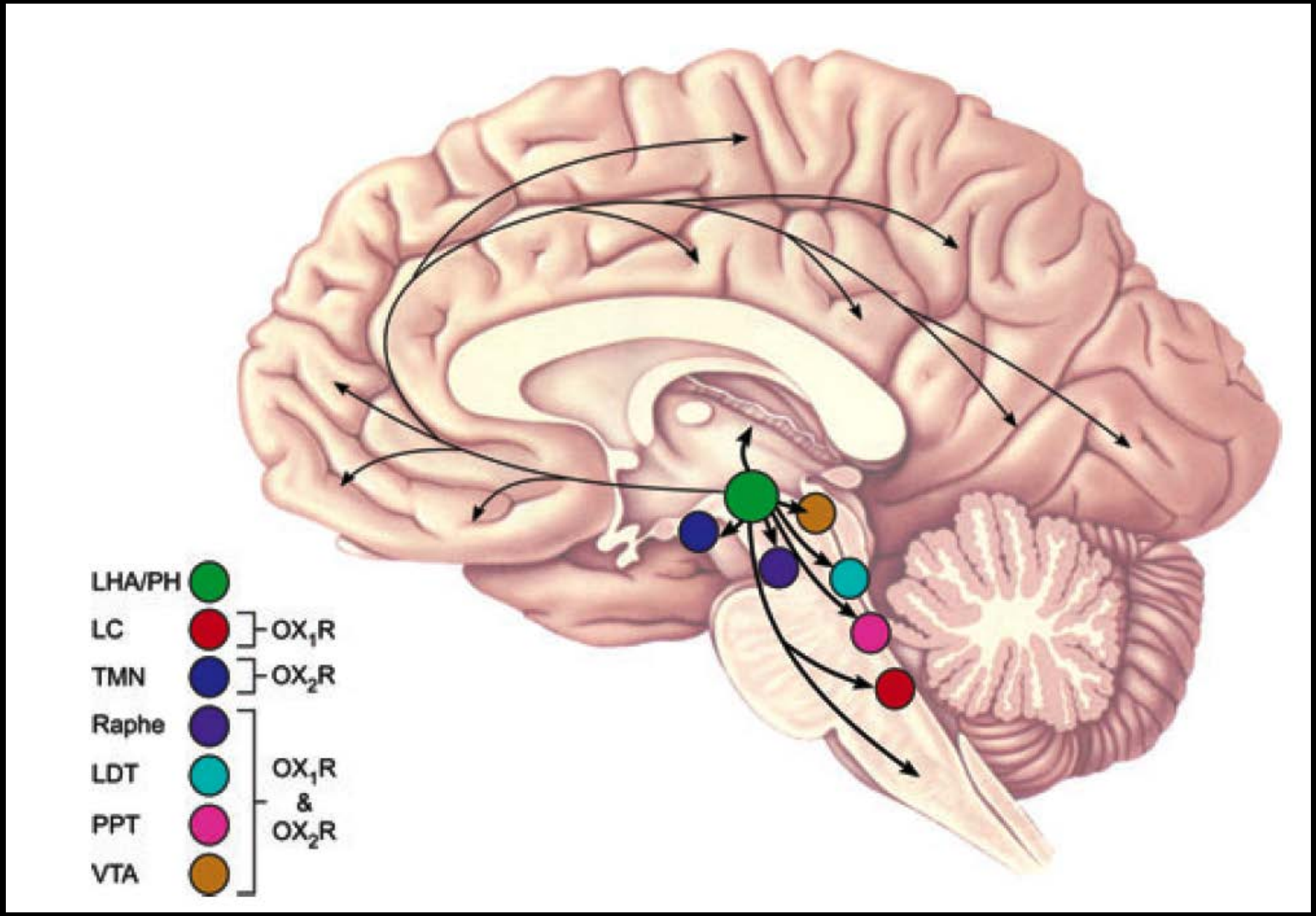


Las neuronas hipocretinérgicas se encuentran en la región perifornical de hipotálamo

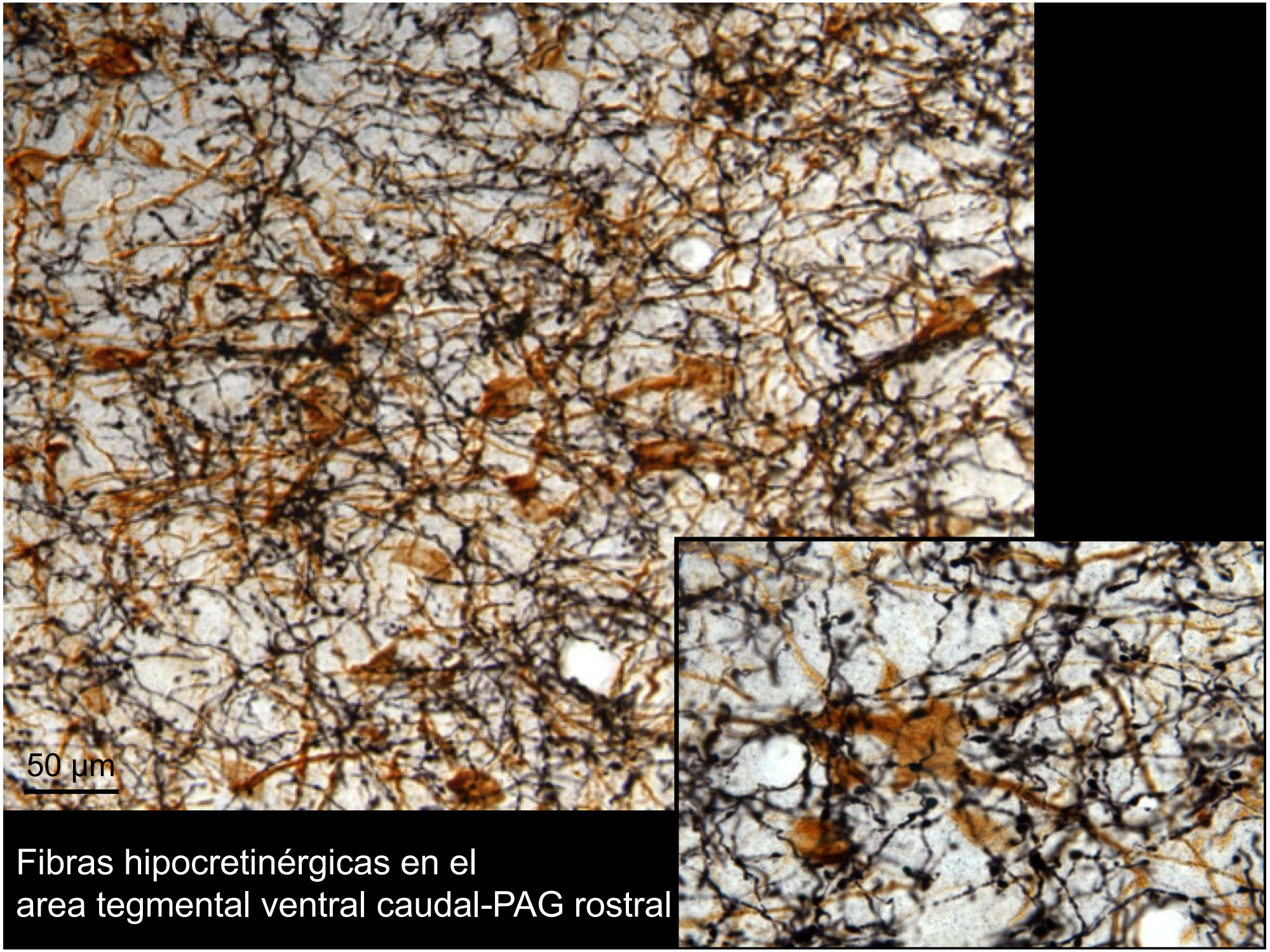


Torterolo y Vanini (2003). RMU, 19:27

Las neuronas hipocretinérgicas proyectan a amplias regiones del SNC

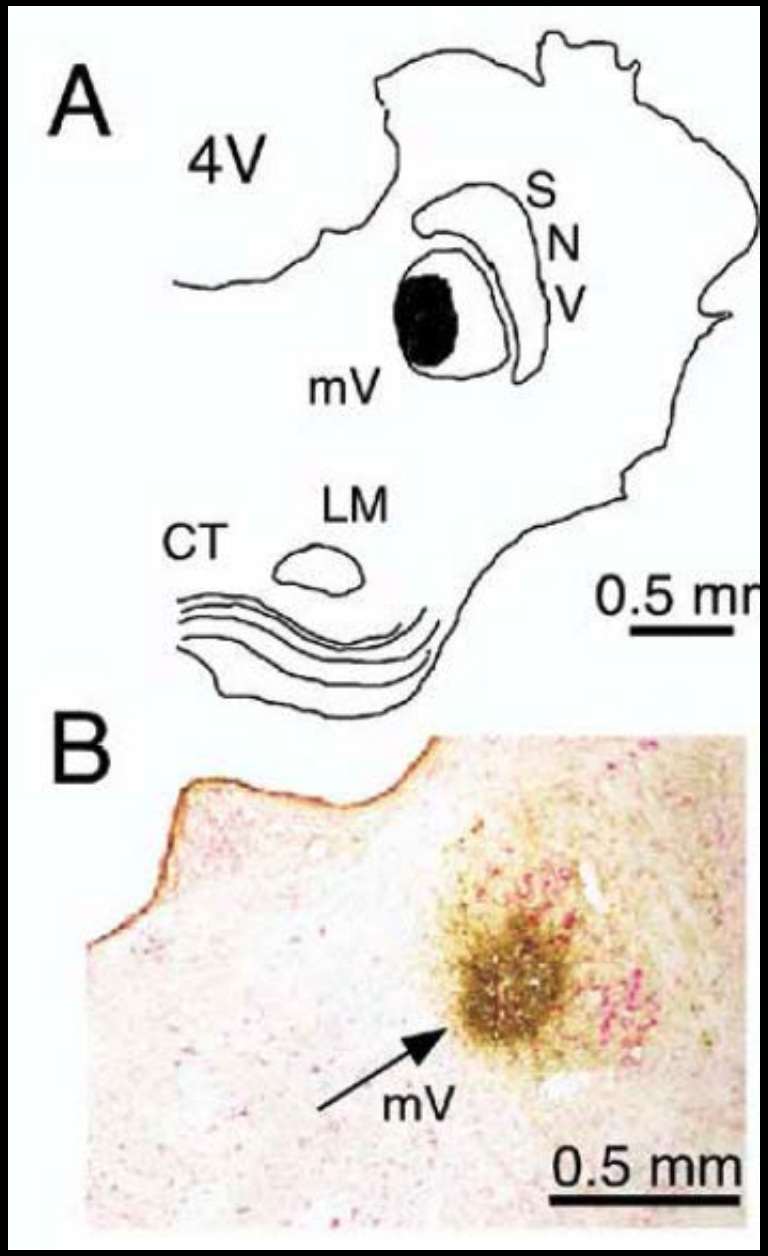


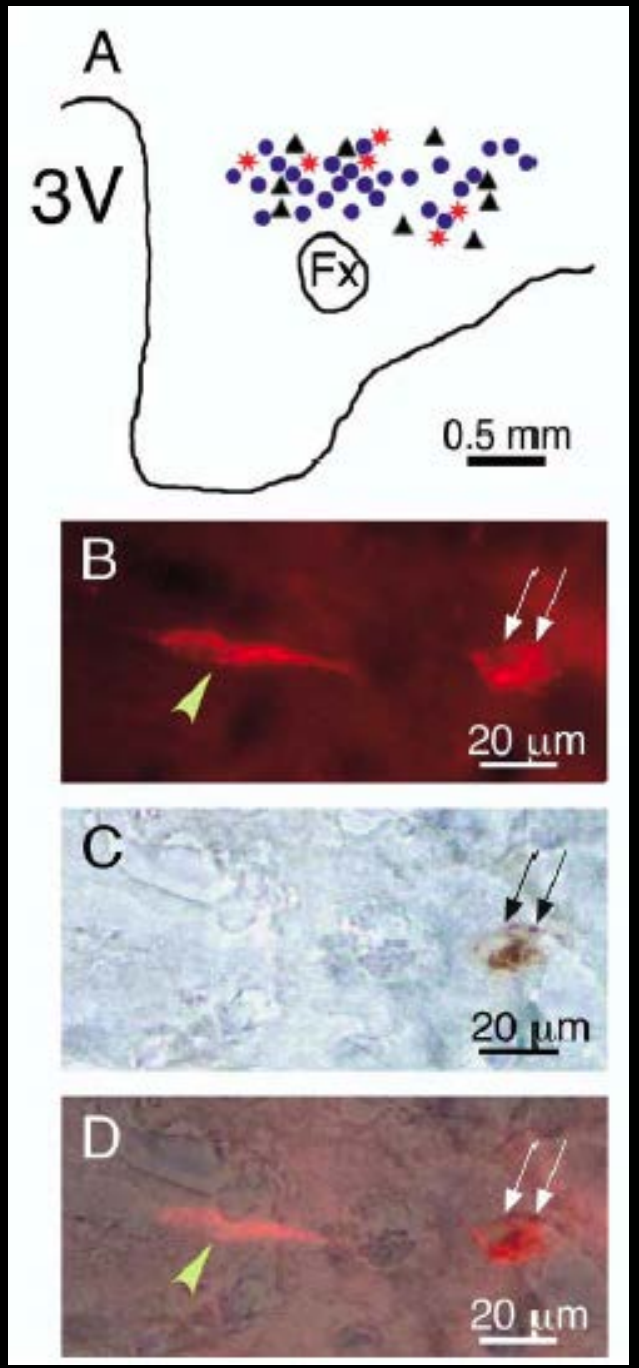
Sakurai (2010). Ann NY Acad Sci, 1200: 149



Fibras hipocretinérgicas en el
area tegmental ventral caudal-PAG rostral

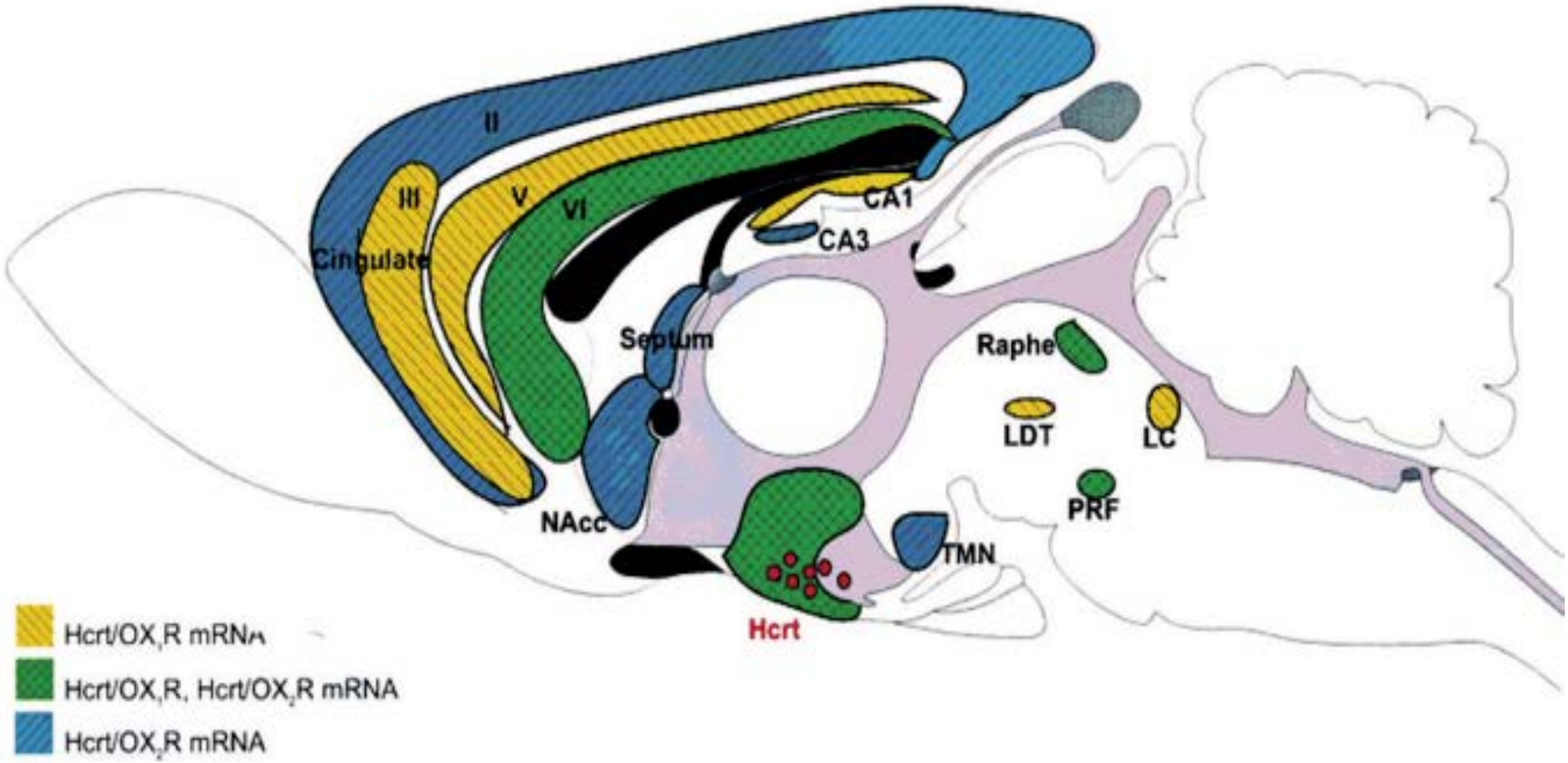
Neuronas hipocretinergicas proyectan a los nucleos motores





McGregor, Damian, Fabbiani, Torterolo, et al. (2005).
Neuroscience, 136:1073.

Distribución de receptores hipocretinérgicos tipo 1 y tipo 2



Kilduff y de Lecea (2001). J. Comp. Neurol., 435: 1-5

Narcolepsia canina:

- Cataplejia desencadenada por emociones
- Hipersomnia
- Sueño fragmentado
- “Sleep onset REM”
- Responden a los mismos fármacos que la narcolepsia humana.

Narcolepsia canina

Mignot et al. Center for Narcolepsy- Stanford University

Narcolepsia canina familiar:

- Mutación en el gen del **receptor tipo 2 de la hipocretina** (Lin et al. 1999).

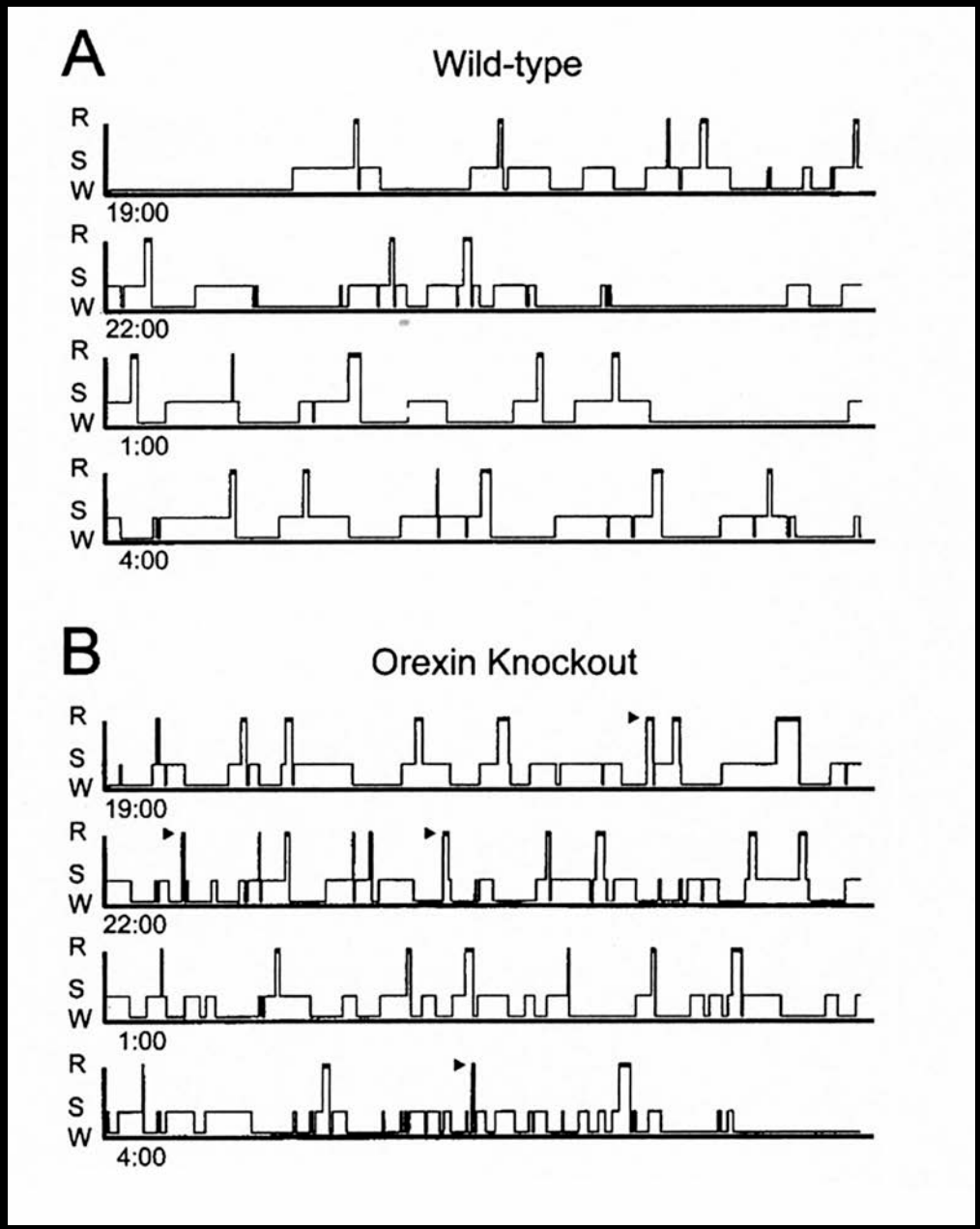
Narcolepsia canina espontánea:

- Niveles de hipocretinas no detectables en el LCR (Ripley et al. 2001).

Ratones “knock-out” para la preprohipocretina

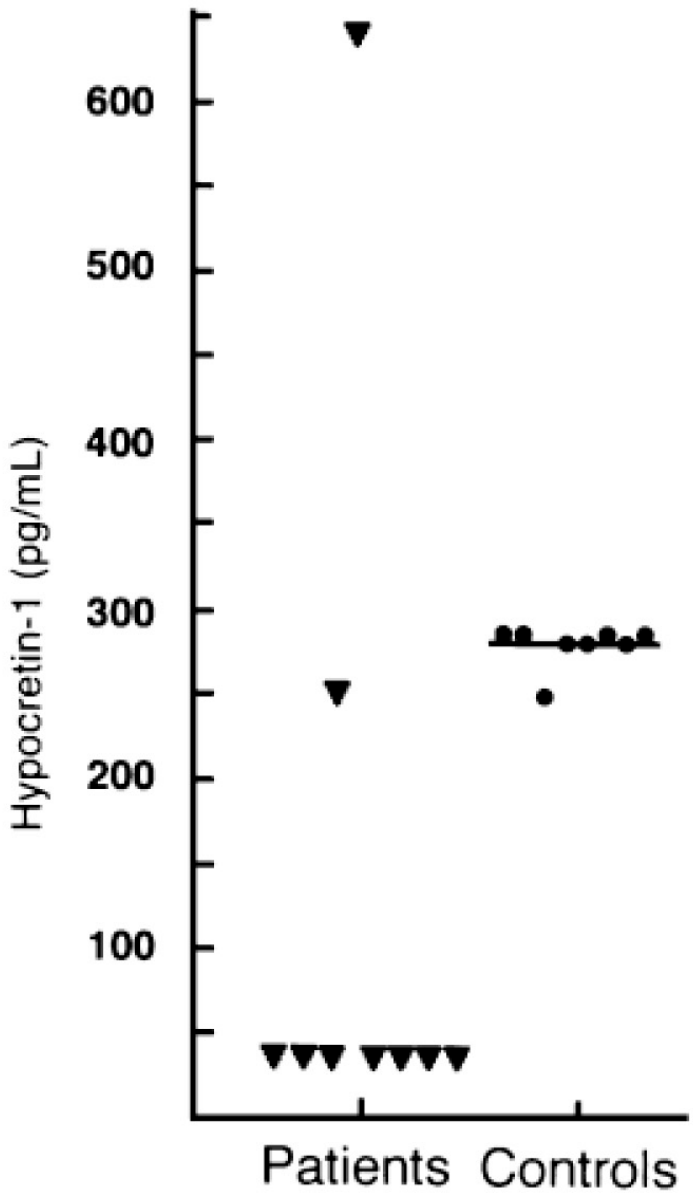
Chemelli et al., (1999). Cell, 98:437-451

Los ratones “knock-out” para la preprohipocretina entran al sueño REM directamente desde vigilia



Chemelli (1999). Cell 98:437-451

Los pacientes narcolépticos carecen de hipocretinas en el LCR

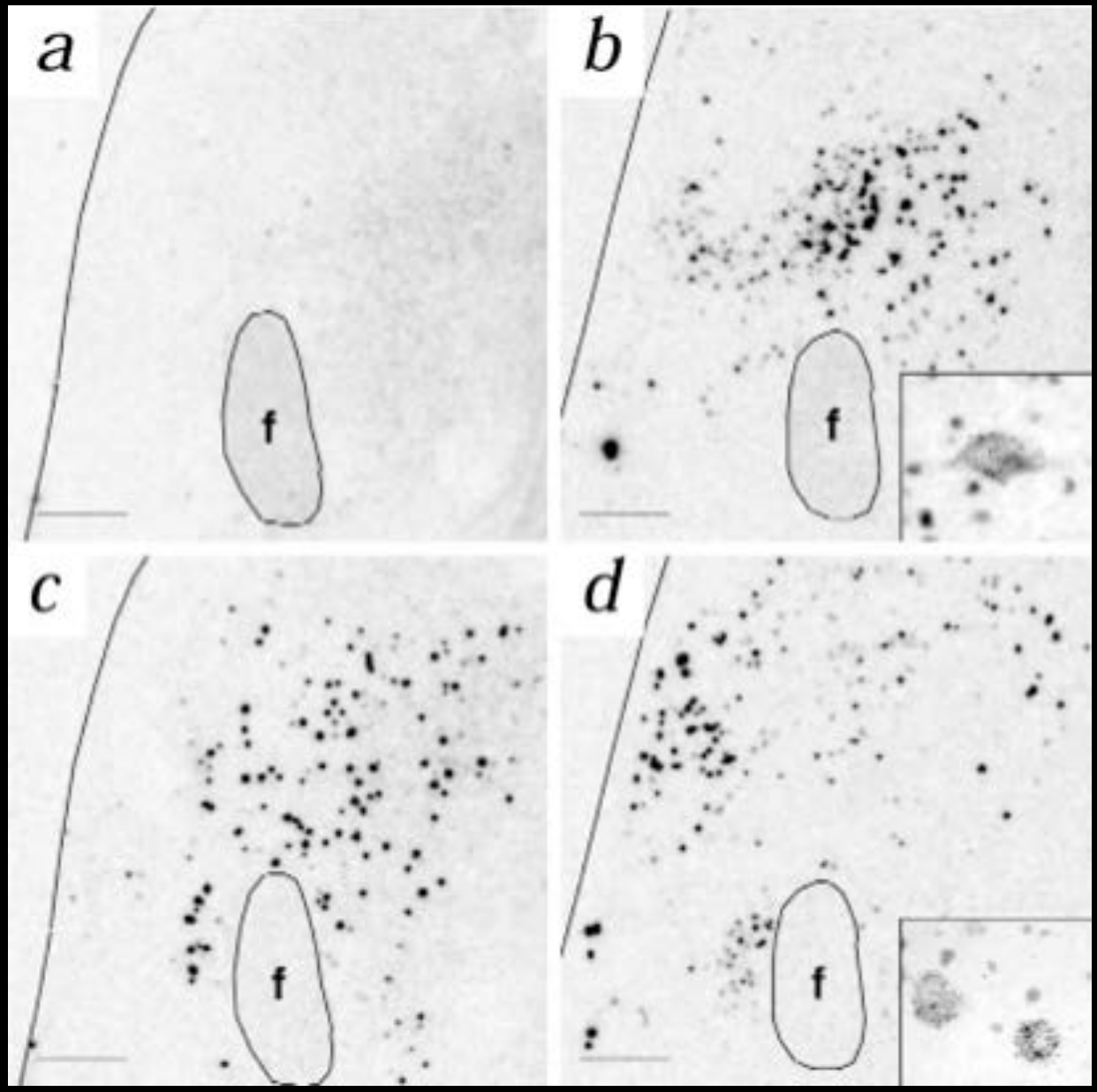


Nishino et al., (2000). Lancet: 355:39

Los pacientes narcolépticos presentan una degeneración de las neuronas hipocretinérgicas

Hipocretinas

MCH



Peyron (2000). Nat. Med. 6:991-997

Etiología

Sospecha de etiología autoinmune, muy localizada, dirigida a algún antígeno presente en las neuronas hipocretinérgicas.

¿Cómo es la actividad de las neuronas hipocretinérgicas durante la vigilia y el sueño?

Michael Chase
Department of Physiology, UCLA School of Medicine

Sleep Research Online 4(1): 25-32, 2001
http://www.sro.org/2001/Torterolo/25/

1096-214X

Hypothalamic Neurons that Contain Hypocretin (Orexin) Express *c-fos* During Active Wakefulness and Carbachol-induced Active Sleep

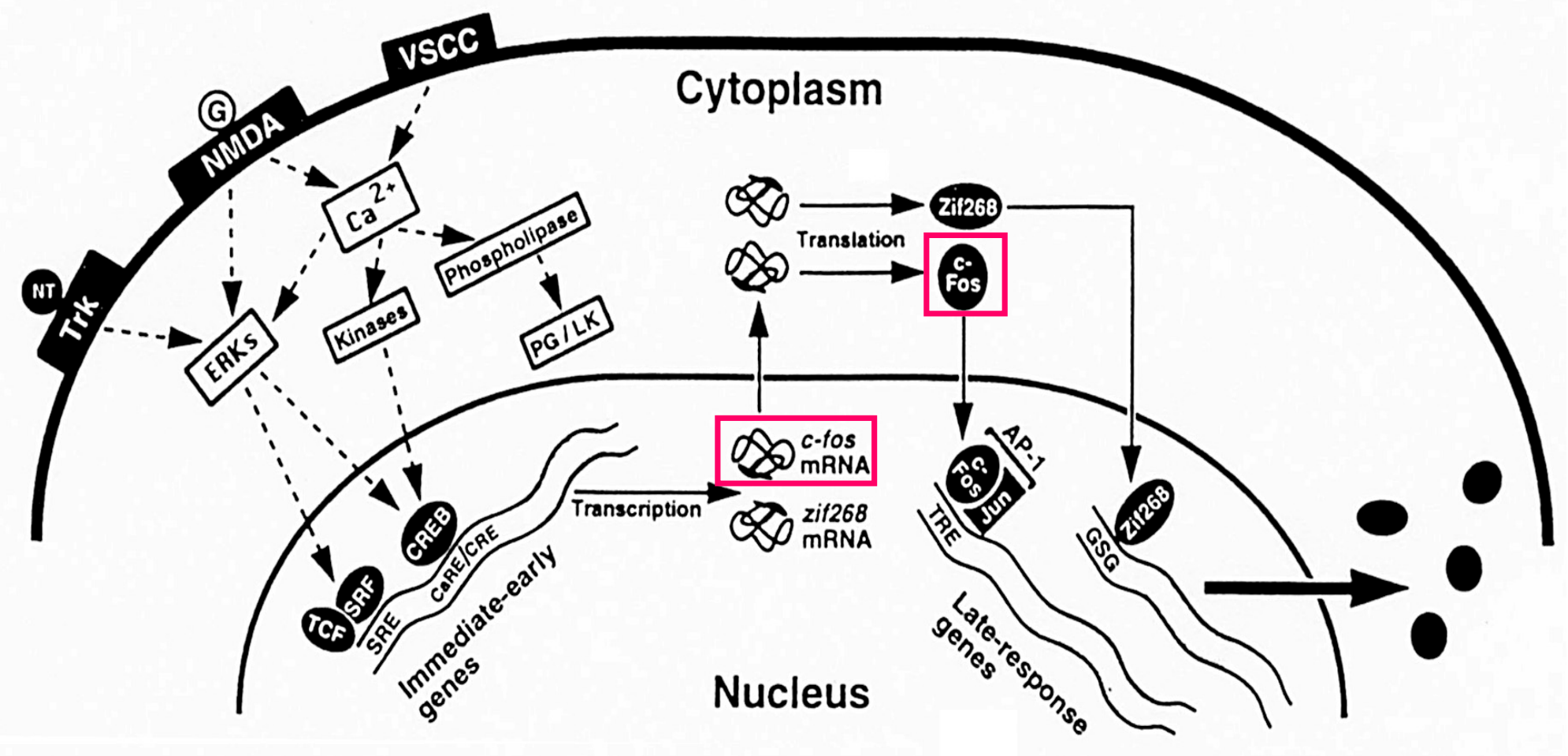
Pablo Torterolo, Jack Yamuy, Sharon Sampogna,
Francisco R. Morales and Michael H. Chase

Department of Physiology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90095

Hypothalamic hypocretinergic neurons have been implicated in the control of a variety of behavioral states. However, the activity of these neurons during sleep and wakefulness has not been described. Consequently, we examined Fos immunoreactivity (as a marker of neuronal activity) in hypocretinergic neurons during the following behavioral states: a) quiet wakefulness (QW), wherein the cats were awake and restrained in a head-holder device; b) active wakefulness (AW), wherein the cats were freely exploring a new environment and exhibited almost continuous locomotor activity; and c) carbachol-induced active sleep (AS-carbachol), wherein prolonged periods of active sleep were induced by the microinjection of carbachol into the nucleus pontis oralis. The majority of hypocretinergic neurons expressed *c-fos* during AW (184.7±19.6, 79%), whereas only a small number of these cells expressed *c-fos* during QW (3.0±1.9, 2%). Surprisingly, a large portion of the hypocretinergic neurons were Fos immunoreactive during AS-carbachol (60.3±11.0, 34%). The significant difference ($p<0.0001$) between QW and AW in the number of hypocretinergic neurons that expressed *c-fos* indicates that these neurons are not involved in the maintenance of wakefulness *per se*, but more likely to play a role in arousal-type behaviors that accompany locomotor-exploratory activity. In addition, the intriguing finding that a larger number of hypocretinergic neurons express *c-fos* during AS-carbachol than during quiet wakefulness suggests that these neurons play a role in active sleep-related processes.

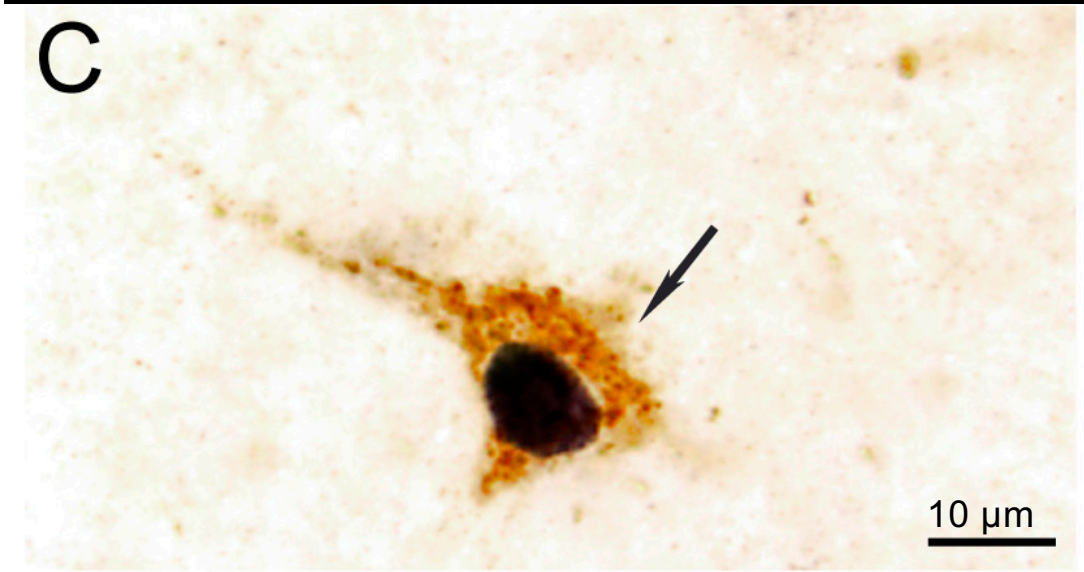
CURRENT CLAIM: Hypocretinergic neurons are active during arousal accompanied by locomotor explorative activity and during carbachol-induced active sleep.

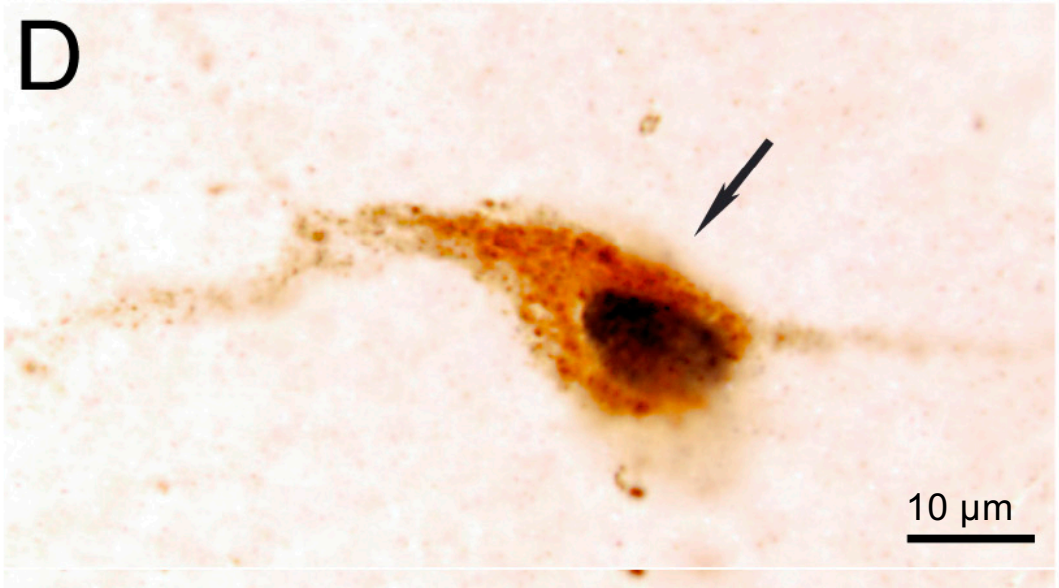
Proteína Fos como índice de actividad neuronal

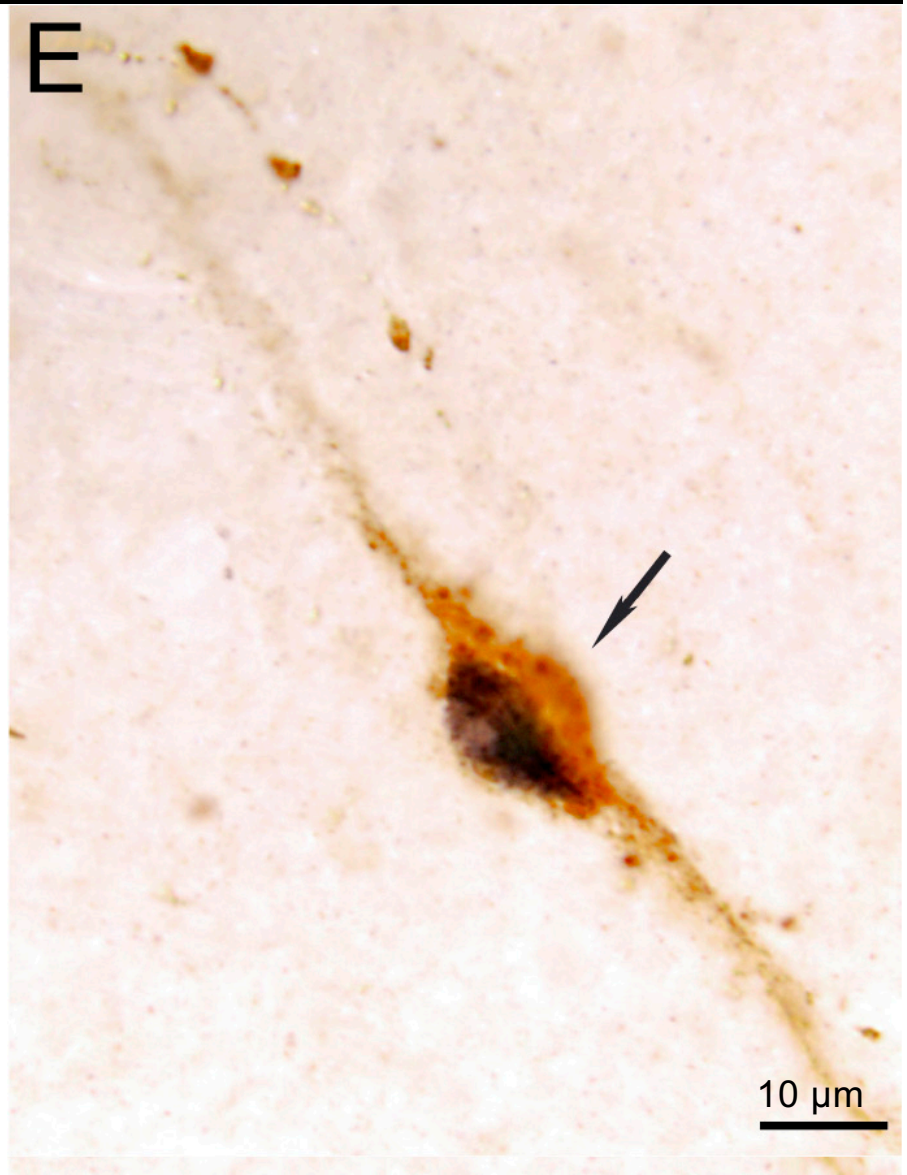


Chaudhuri (1997). Neuroreport, 8:13

Doble inmunohistoquímica para hipocretina-2 y Fos

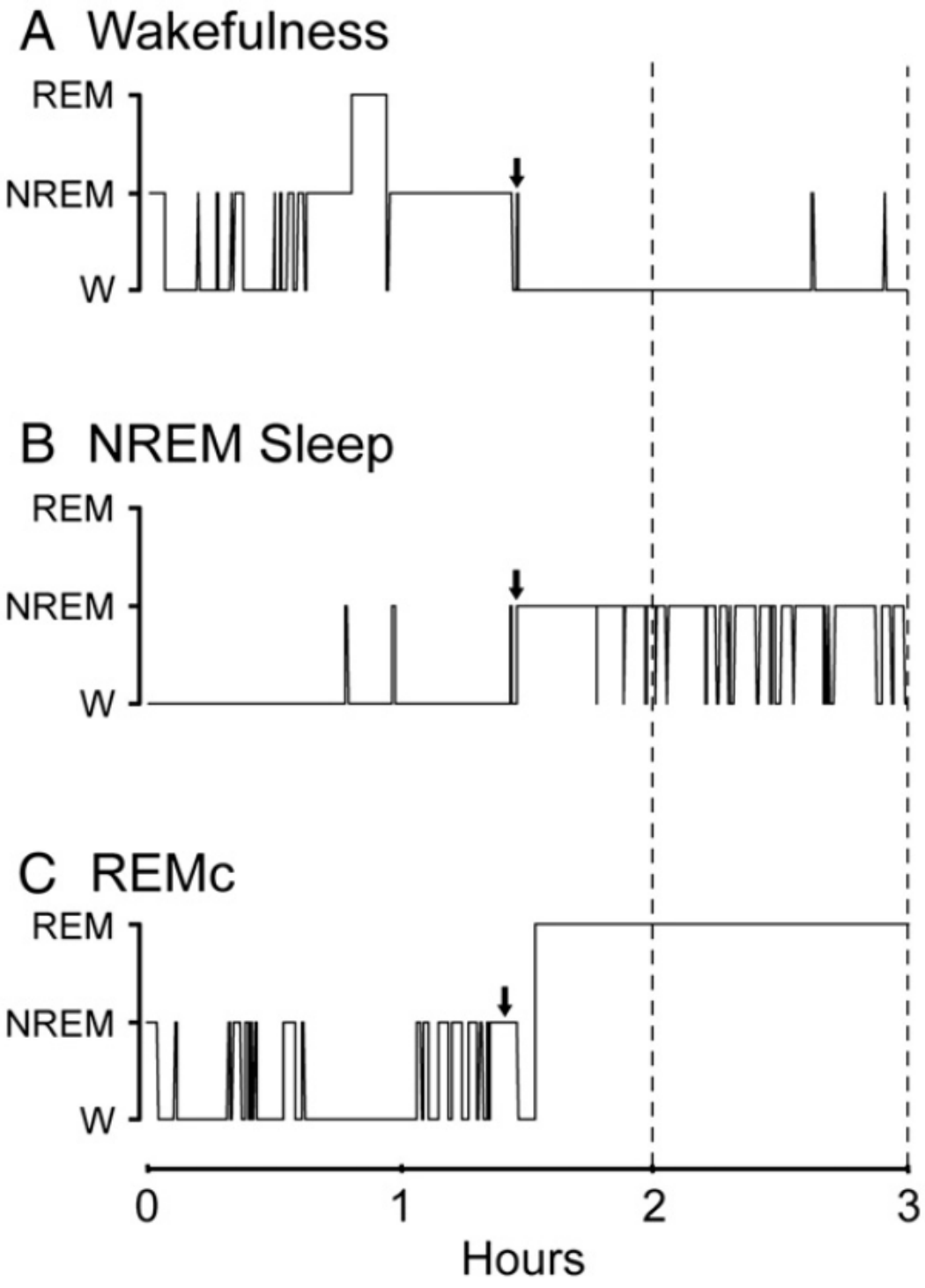




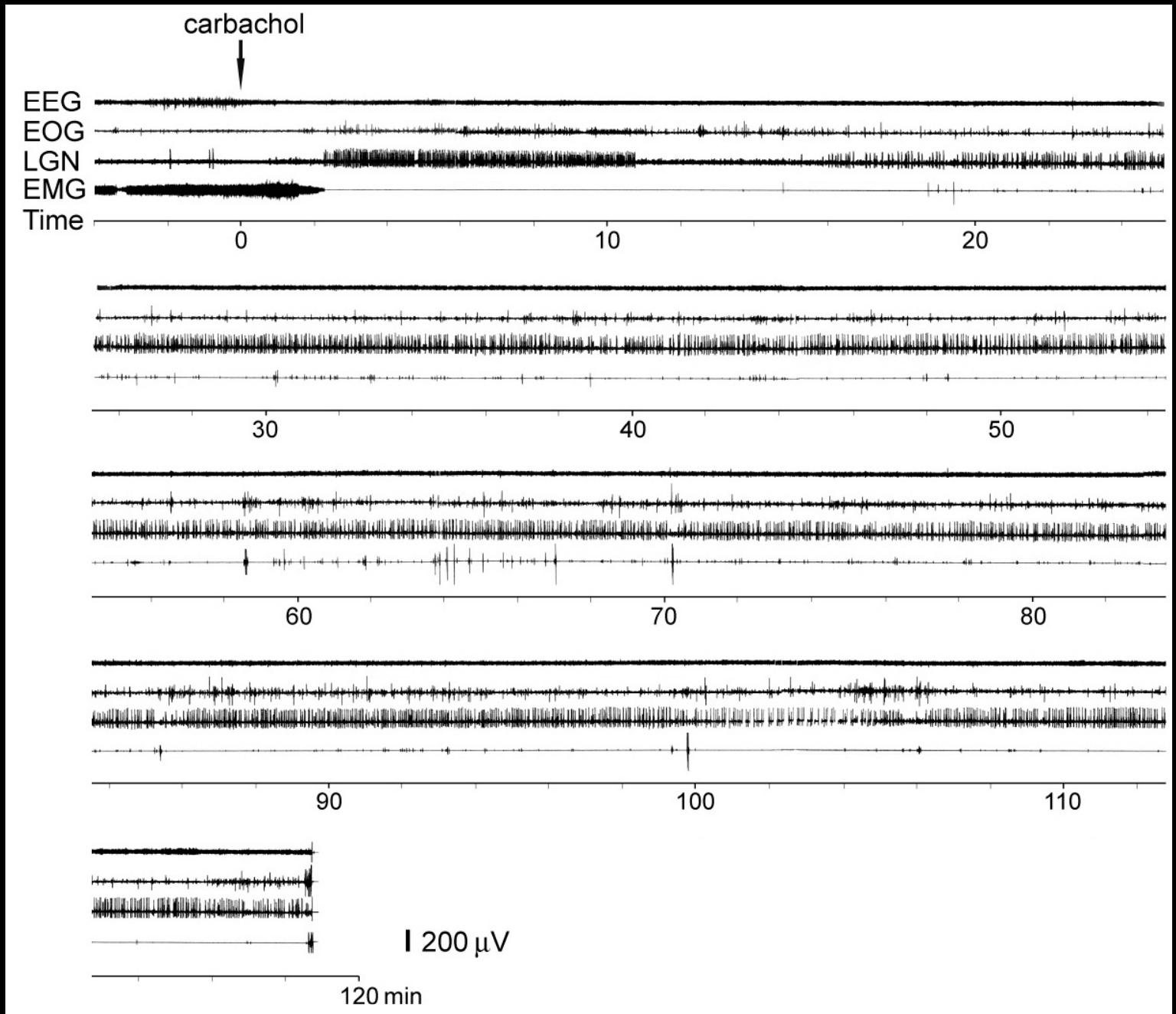


1. Vigilia tranquila
2. Vigilia alerta
3. Vigilia activa con actividad motora-exploratoria
4. Sueño lento
5. Sueño REM (Inducido por microinyecciones de carbacol en el NPO)

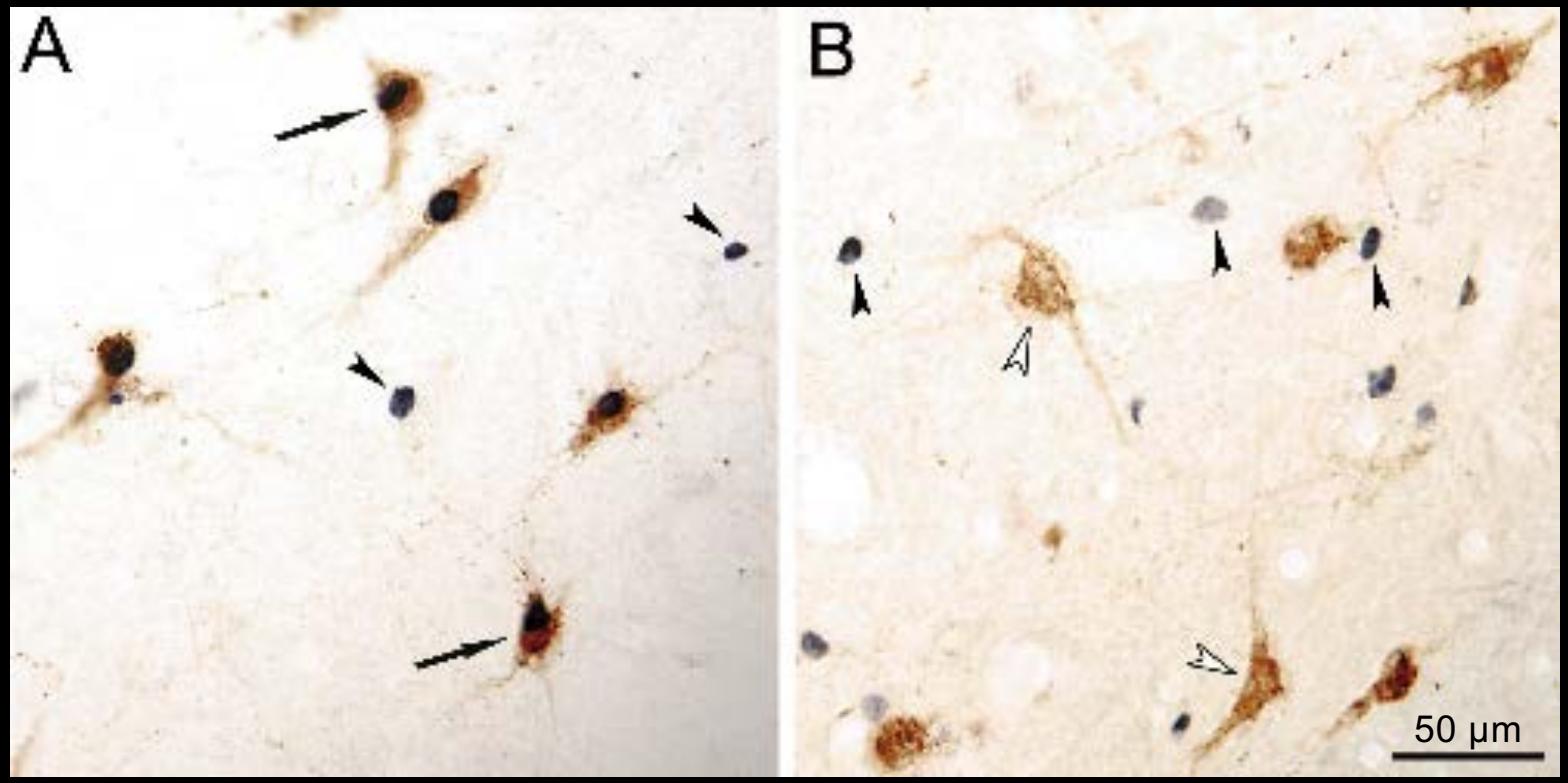
- Locomoción


A Wakefulness
REM
NREM
W
B NREM Sleep
REM
NREM
W
C REMc
REM
NREM
W
0
1
2
3
Hours

Sueño REM inducido por microinyecciones de carbacol en el NPO

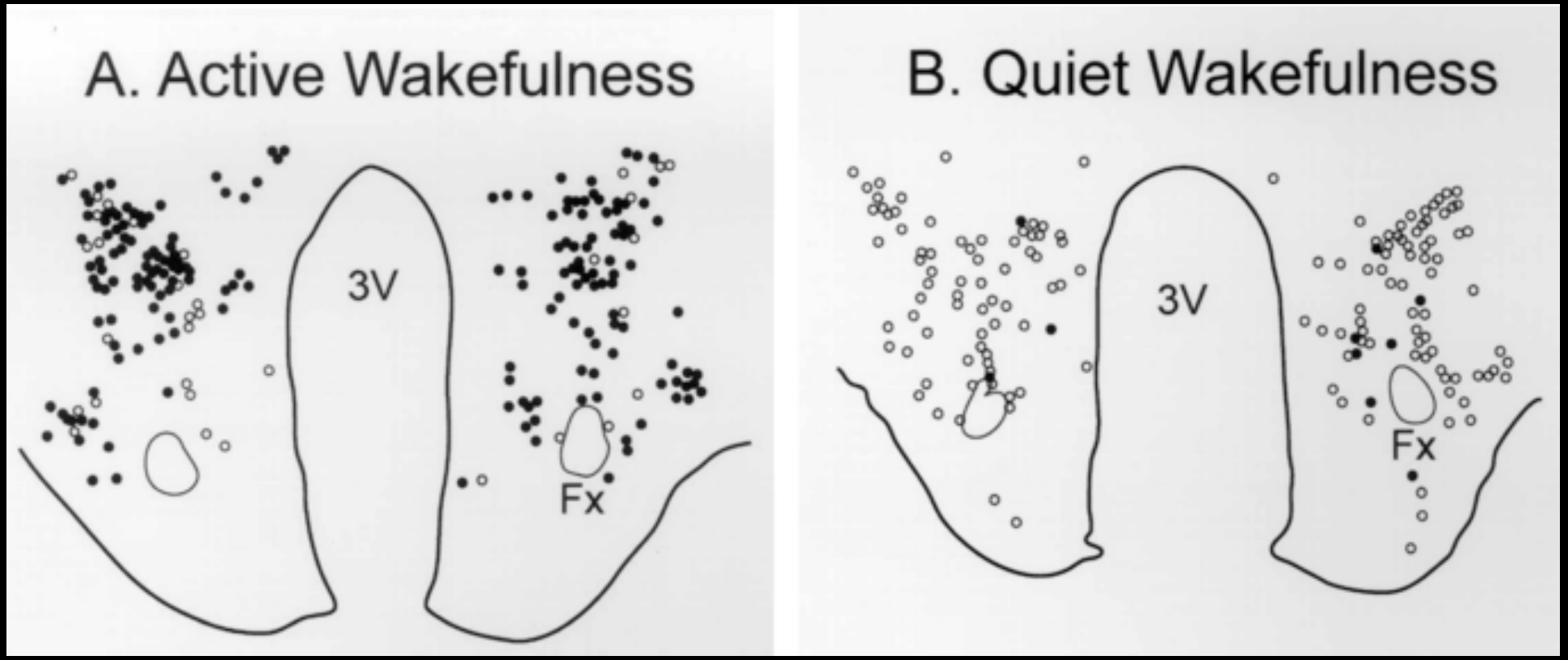


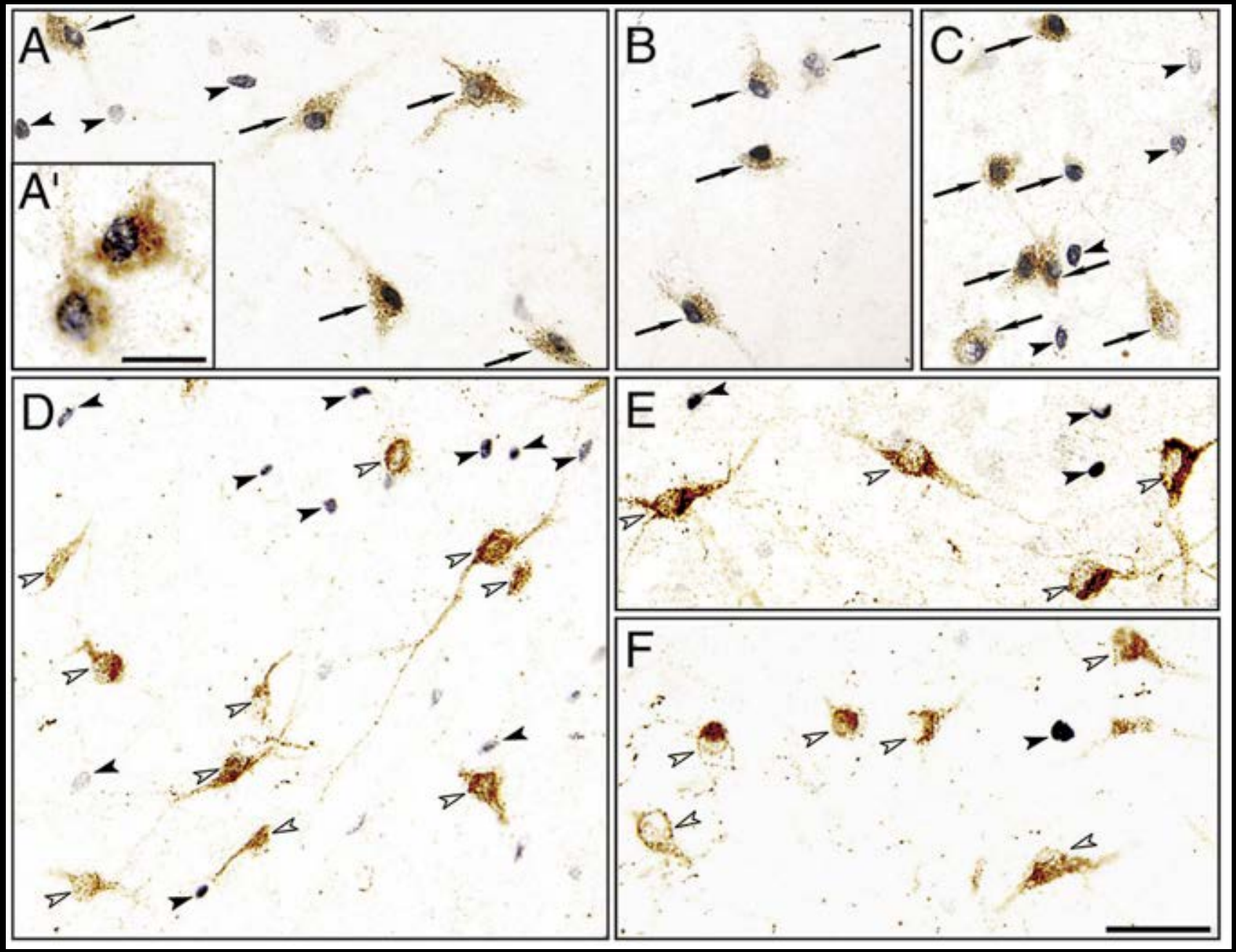
Las neuronas hipocretinérgicas estan activas durante la vigilia con actividad motora exploratoria



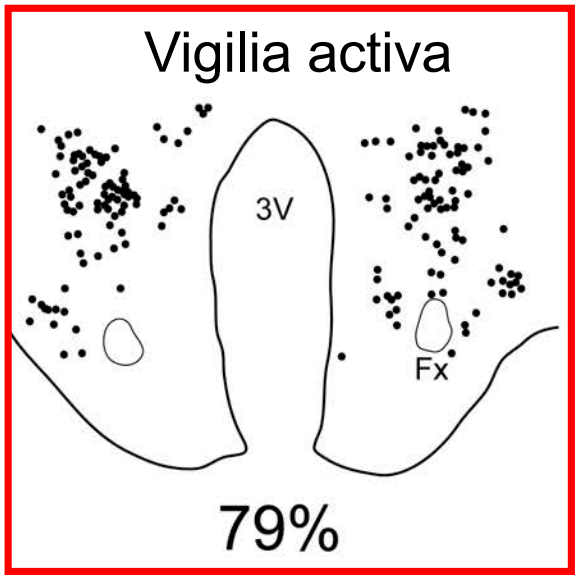
Vigilia con actividad-motora exploratoria

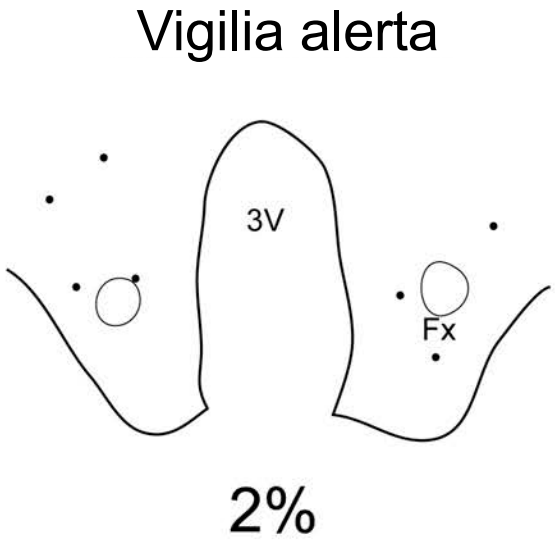
Vigilia tranquila

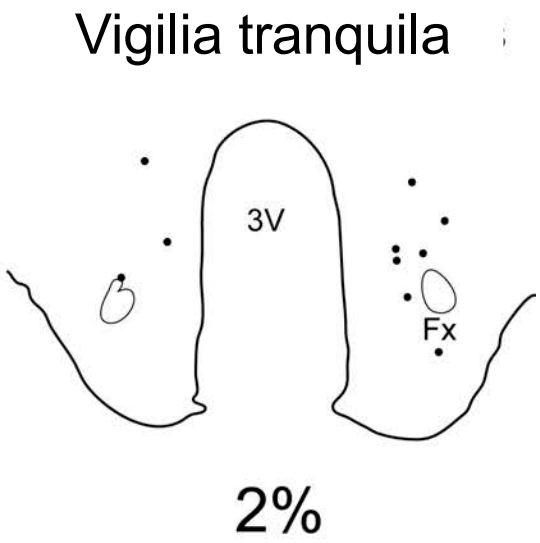

A. Active Wakefulness
3V
Fx
B. Quiet Wakefulness
3V
Fx

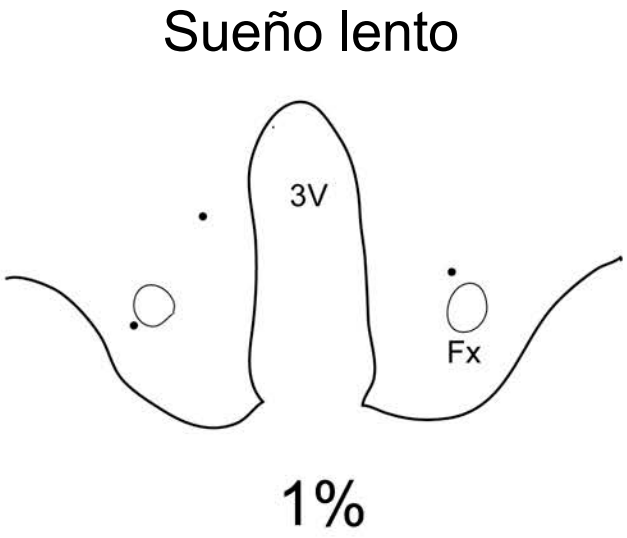

A
A'
B
C
D
E
F

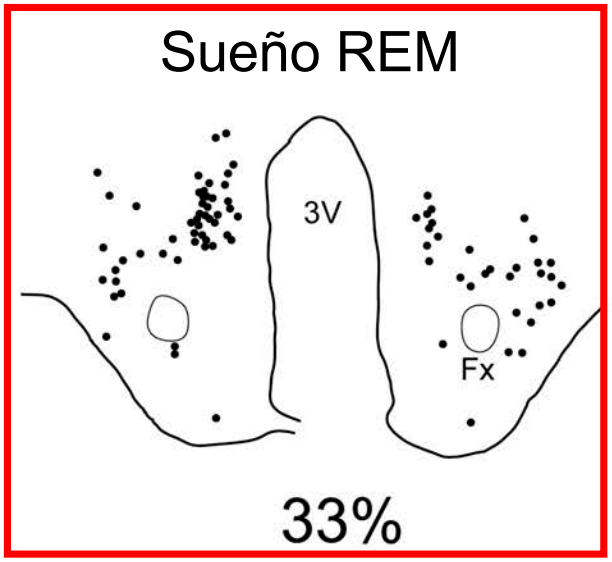
Las neuronas hipocretinérgicas expresan Fos en vigilia con actividad motora y en sueño REM



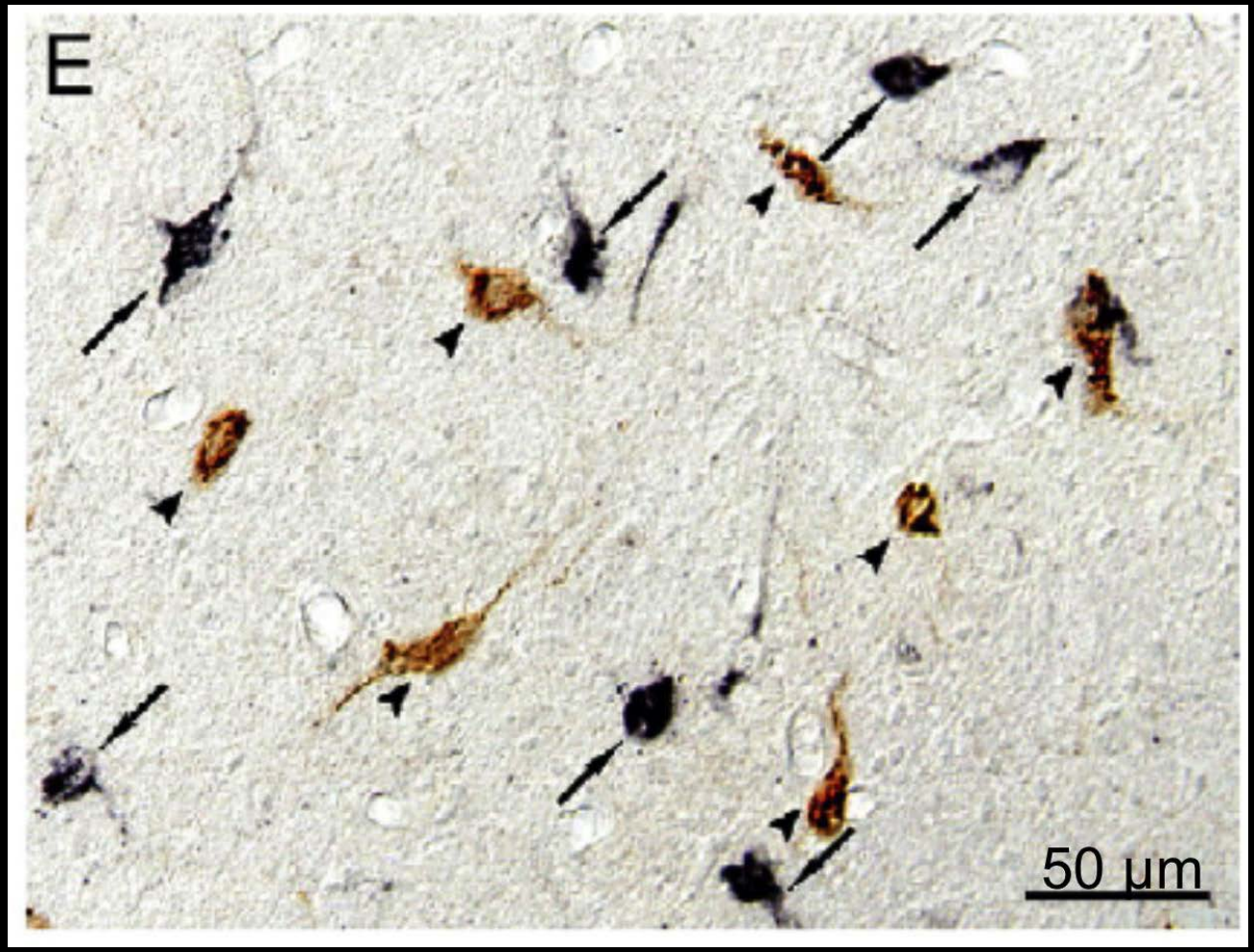




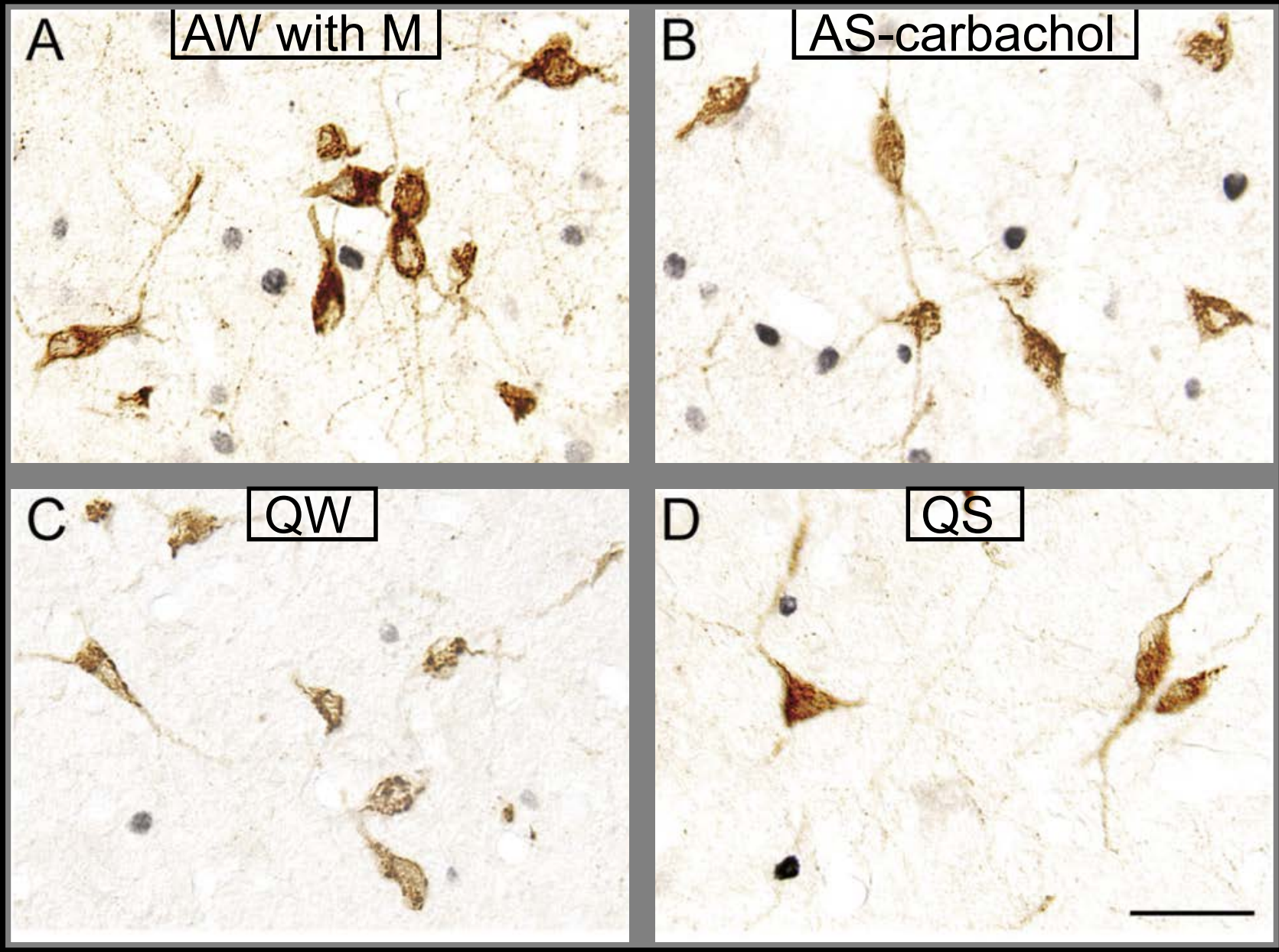




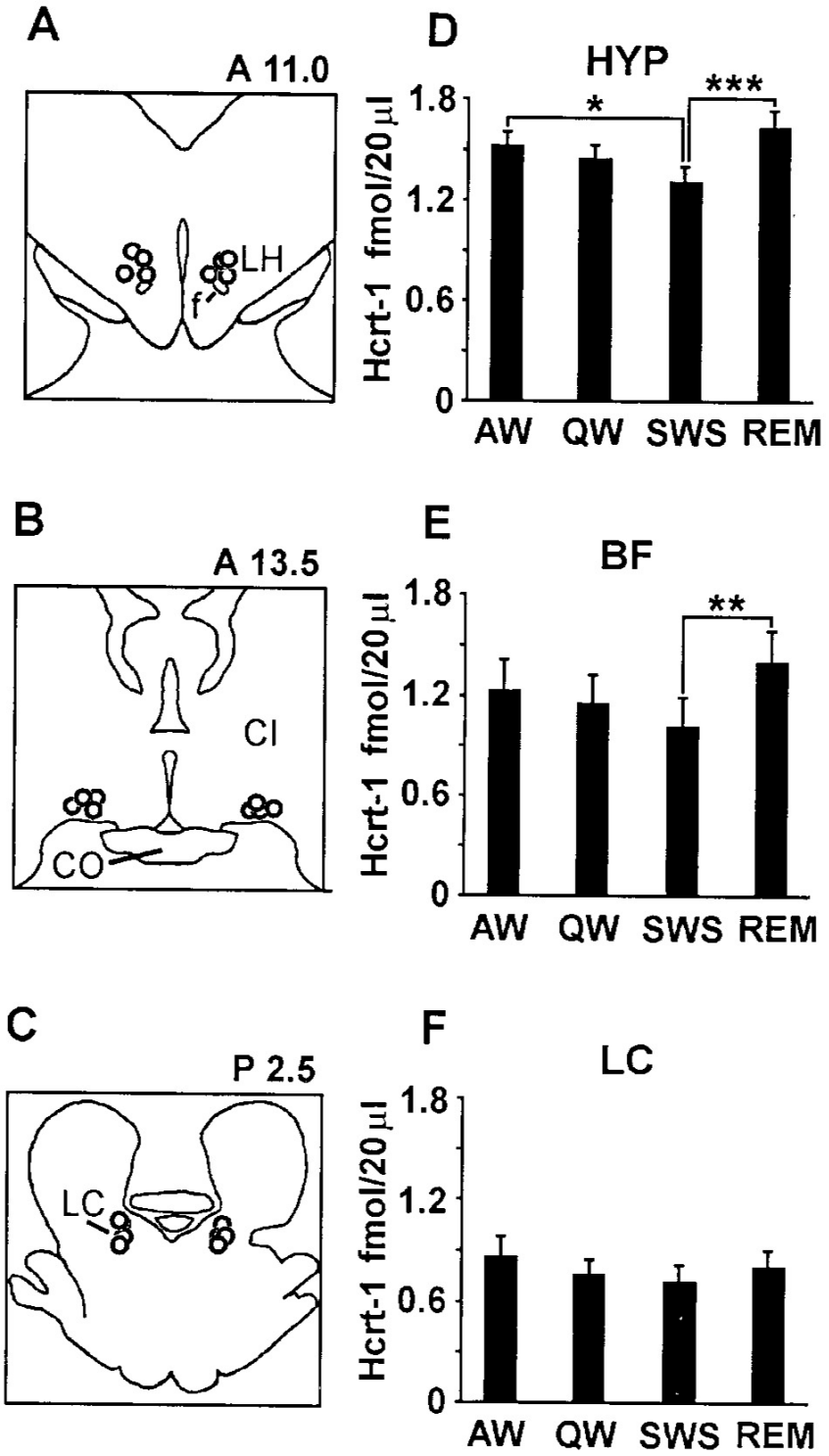
Las neuronas MCHérgicas e hipocretinérgicas están localizadas en la misma región del hipotálamo

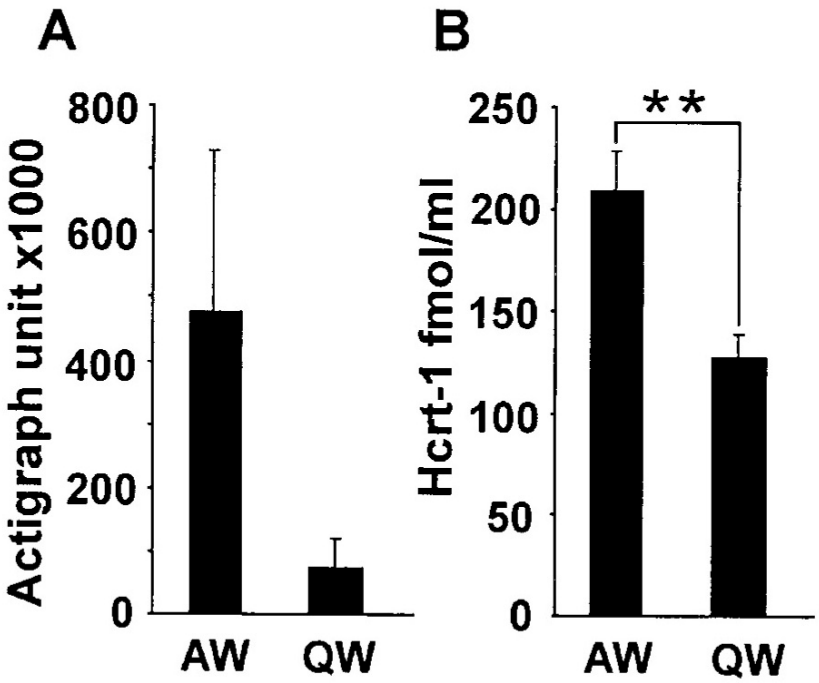


Las neuronas MCHérgicas no expresan Fos en los estados comportamentales analizados

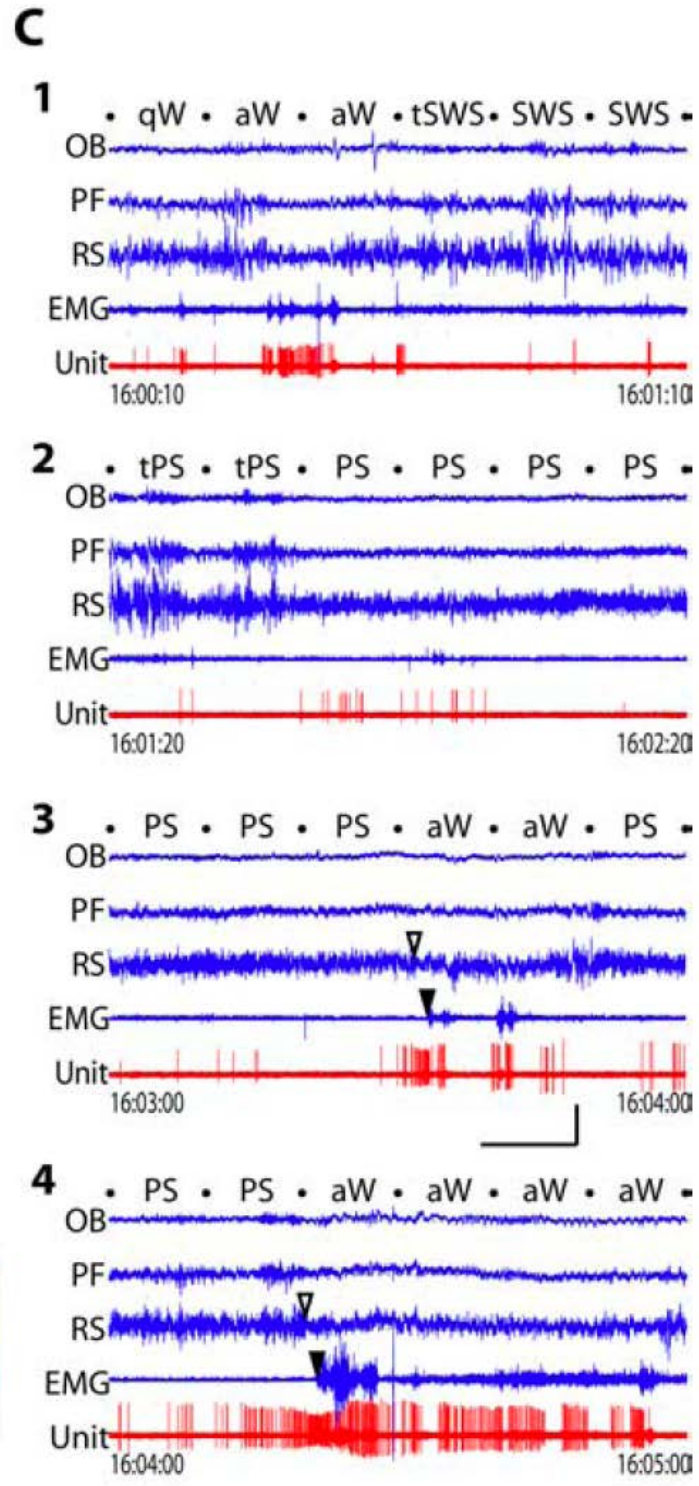


La liberación de hipocretinas aumenta en vigilia activa y sueño REM





Kiyashchenko et al. (2002). J. Neurosci., 22:5282-5286



Neuronas hipocretinérgicas durante el sueño REM:

- Inhibición durante el Sueño REM-Tónico
- Activación durante el Sueño REM-Fásico

Lee et al. (2005). J. Neurosci., 25:6716

Sleep Science 7 (2014) 19–29

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The hypocretins (orexins) mediate the "phasic" components of REM sleep: A new hypothesis

Pablo Torterolo[a,*], *Michael H. Chase*[b,c]

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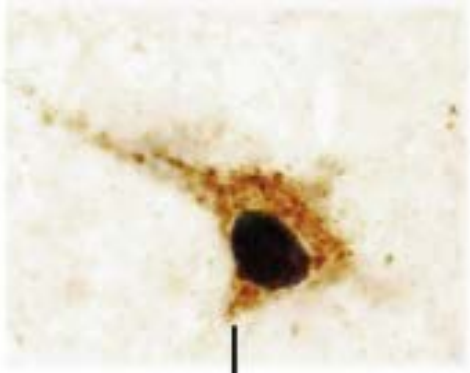
[b]*WebSciences International, Los Angeles, USA*
[c]*UCLA School of Medicine, Los Angeles, USA*



The activity of the hypocretinergic neurons increases in correlation with the "phasic" components of REM sleep

premotor/motor nuclei
preautonomic/autonomic nuclei
respiratory neuronal network

Hypocretinergic neurons project and activate areas involved in the generation of the "phasic" components of REM sleep.

Hypocretinergic neurons induce the "phasic" components of REM sleep

Torterolo y Chase (2014). Sleep Science, 7:19-29

nature Vol 450|15 November 2007|doi:10.1038/nature06310

LETTERS

Neural substrates of awakening probed with optogenetic control of hypocretin neurons

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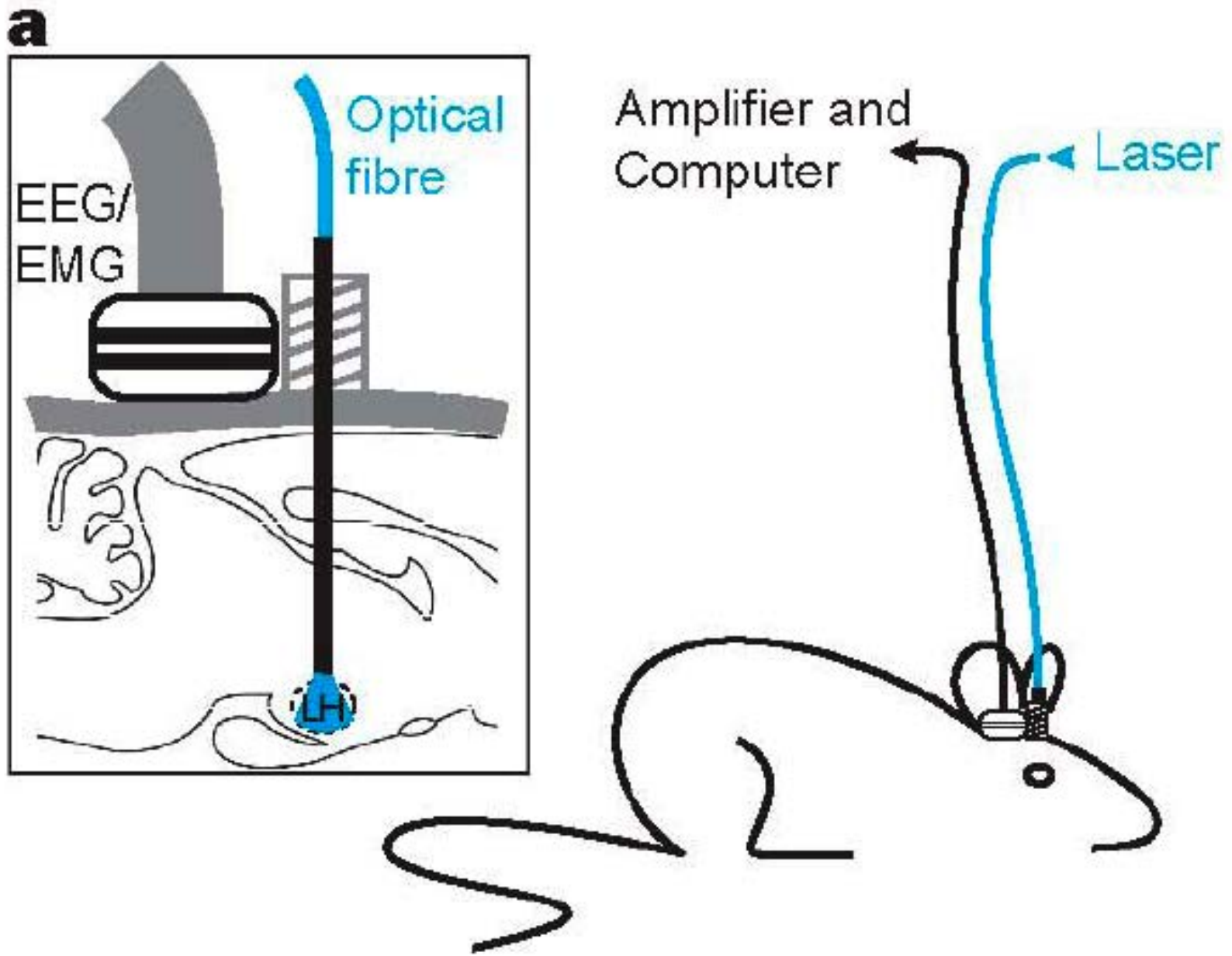
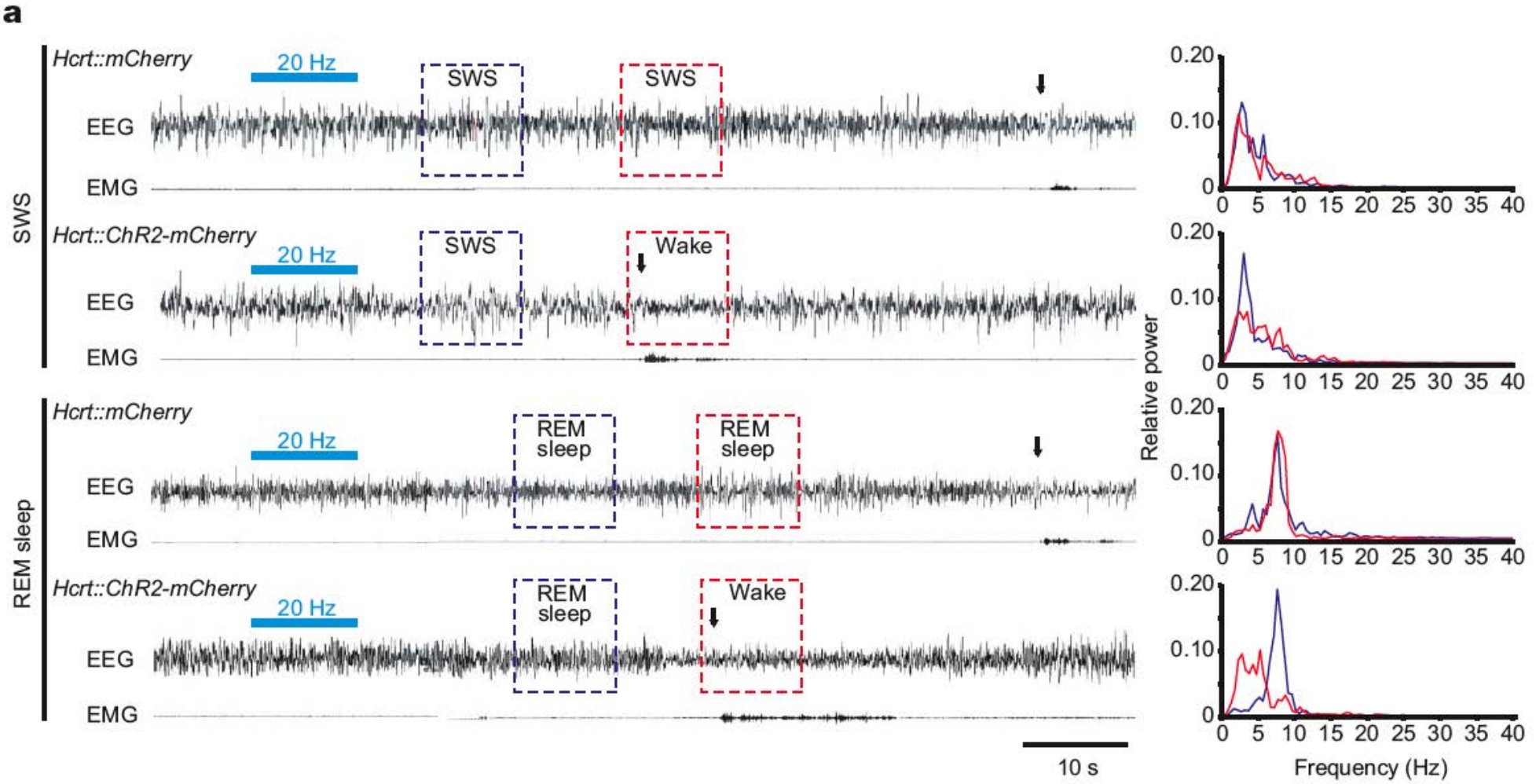
The neural underpinnings of sleep involve interactions between sleep-promoting areas such as the anterior hypothalamus, and arousal systems located in the posterior hypothalamus, the basal forebrain and the brainstem[1,2]. Hypocretin[3] (Hcrt, also known as orexin[4])-producing neurons in the lateral hypothalamus[5] are important for arousal stability[2], and loss of Hcrt function has been linked to narcolepsy[6–9]. However, it is unknown whether electrical activity arising from Hcrt neurons is sufficient to drive awakening from sleep states or is simply correlated with it. Here we directly probed the impact of Hcrt neuron activity on sleep state transitions with *in vivo* neural photostimulation[10–18], genetically targeting channelrhodopsin-2 to Hcrt cells and using an optical fibre to deliver light deep in the brain, directly into the lateral hypothalamus, of freely moving mice. We found that direct, selective, optogenetic photostimulation of Hcrt neurons increased the probability of transition to wakefulness from either slow wave sleep or rapid eye movement sleep. Notably, photostimulation using 5–30 Hz light pulse trains reduced latency to wakefulness, whereas 1 Hz trains did not. This study establishes a causal relationship between frequency-dependent activity of a genetically defined neural cell type and a specific mammalian behaviour central to clinical conditions and neurobehavioural physiology.

mCherry, continuous light illumination for 1 s evoked robust action potential trains under current-clamp conditions (Fig. 1e) with firing frequencies ranging from 8 to 25 Hz. Moreover, as in hippocampal neurons[12], 15-ms blue-light pulses were able to drive reliable action potential trains from 1 to 50 Hz under whole-cell current-clamp conditions (Fig. 1f), although reliability decreased slightly with increasing frequency (Fig. 1g). At 20 Hz, 100% of light pulses gave rise to action potentials, whereas at 50 Hz, 84 ± 14% of light pulses gave rise to action potentials ($n = 6$ cells; Fig. 1g). We found that basal properties including input resistance (366 ± 43 MΩ, $n = 6$)[21] and resting membrane potential were unaltered by ChR2–mCherry expression ($P > 0.05$; Fig. 1h). Together, these data show that lentivirus-mediated expression of ChR2–mCherry in Hcrt neurons is well tolerated, specific and sufficient to drive precise and reliable action potential firing across a range of frequencies.

We next studied the effect of photostimulation of *Hcrt::ChR2-mCherry* neurons on the sleep–wake cycle of freely moving mice. *Hcrt::ChR2-mCherry* or *Hcrt::mCherry* (control) lentiviruses were stereotactically delivered to the lateral hypothalamus; to determine the behavioural consequences of Hcrt neuron activation, we quantitatively analysed sleep recordings of mice chronically implanted with electroencephalographic (EEG) and electromyographic (EMG) elec-


a
Optical
fibre
EEG/
EMG
LH
Amplifier and
Computer
Laser

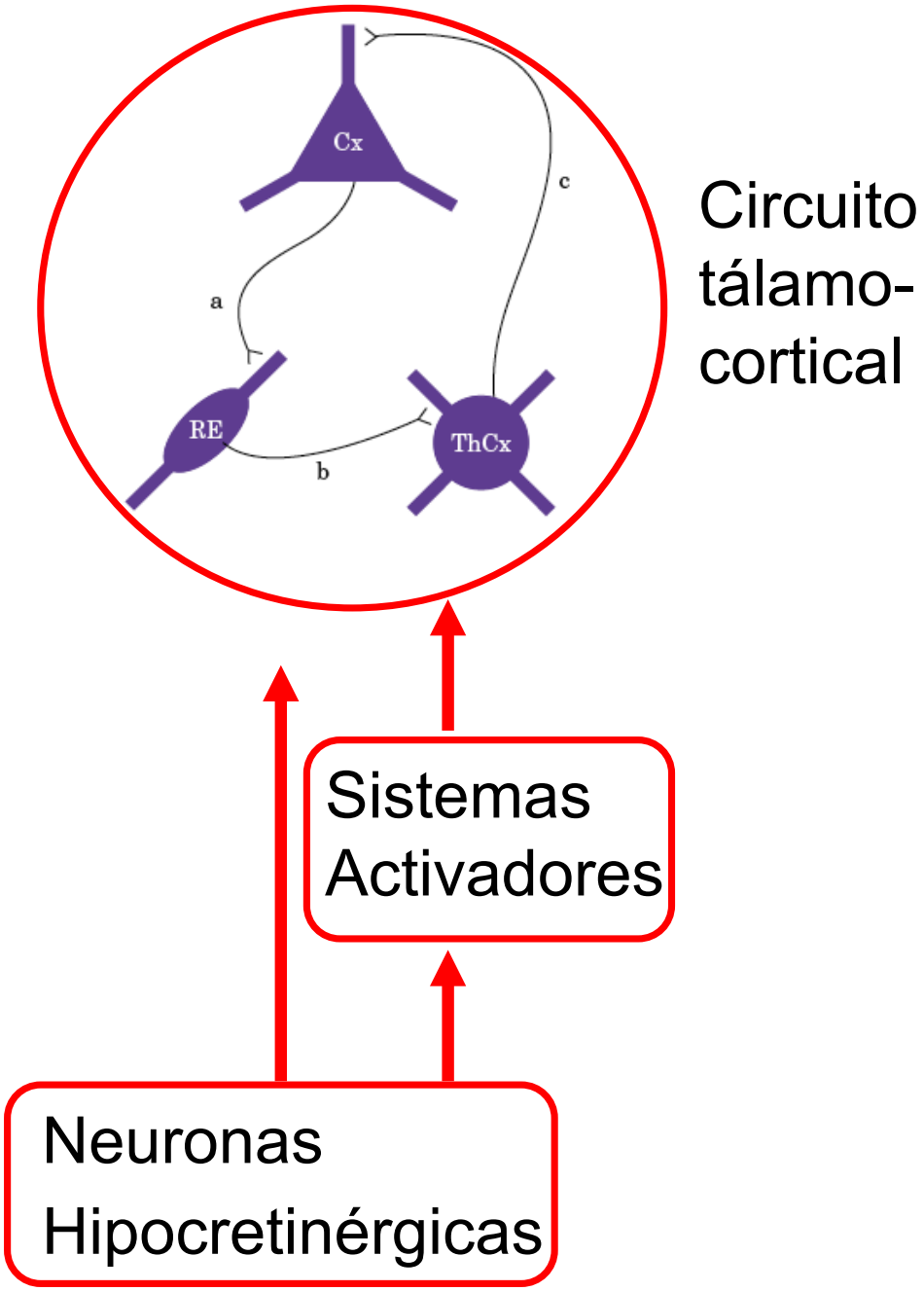
La activación de las neuronas hipocretinérgicas aumenta las transiciones a la vigilia.

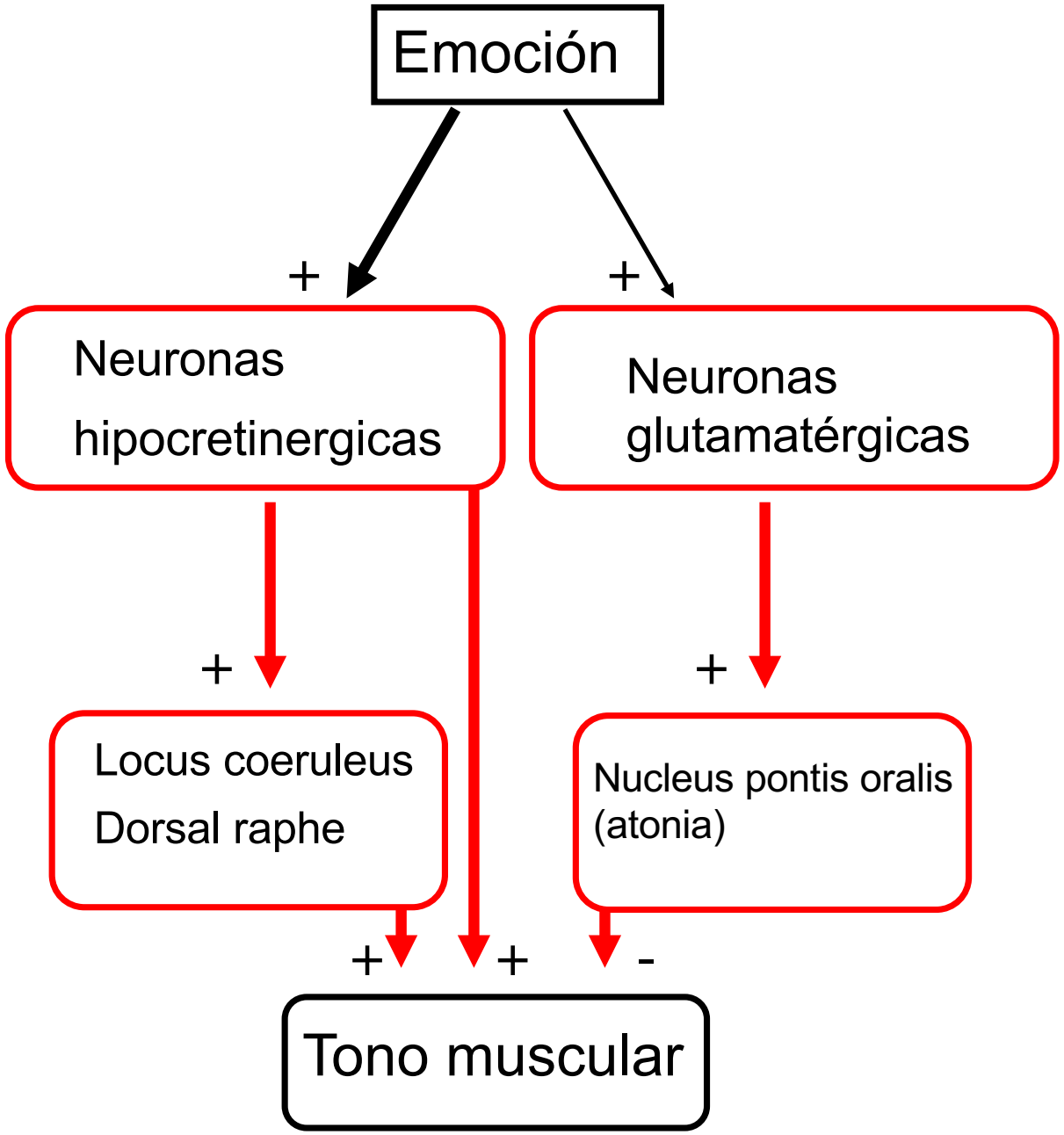


Las neuronas hipocretinergicas se activan durante la vigilia activa con actividad motora-exploratoria pero no durante vigilia tranquila, locomocion forzada o sueño lento.

Las neuronas hipocretinergicas se activan durante los componentes fásicos del sueño REM.

Neuronas hipocretinérgicas facilitan el estado de vigilia actuando directamente sobre el circuito tálamo-cortical y a través de otros sistemas activadores




Emoción
+
+
Neuronas hipocretinergicas
Neuronas glutamatérgicas
+
+
Locus coeruleus
Dorsal raphe
Nucleus pontis oralis (atonia)
+
+
-
Tono muscular

Conclusiones

- Las neuronas hipocretinérgicas promueven la vigilia.

- Un déficit en el sistema hipocretinérgico es la base patogénica de la Narcolepsia.

“El sueño del escolar”. Jean-Baptiste Greuze