The neurobiology of sleep

Dr Anna Zecharia
Biophysics Section
Imperial College London
anna.zecharia@imperial.ac.uk

What is sleep?



What is sleep?



- Natural, reversible state of 'behavioural quiescence'
- Species-specific stereotypic posture
- Reduced responsiveness to sensory stimuli
- Rebound after deprivation

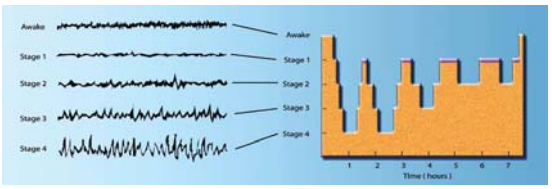
Why do we sleep?

- Plasticity?
  - Learning
  - Development
  - Synaptic 'downscaling' after wake-induced increase
- Neuronal protection?
  - Unfolded protein response?
- The Great British Sleep Survey (2012)
www.greatbritishsleepsurvey.com

Vassali & Dijk 2009, Eur J Neurosci.29(9):1830-41

How does the brain sleep?

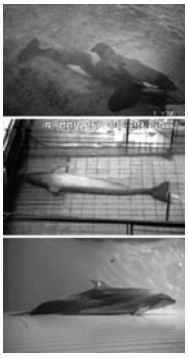
What does a sleeping brain look like?

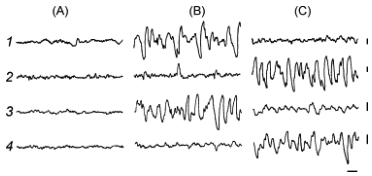


- 4 stages of non-rapid eye movement (NREM) sleep
- Followed by a period of rapid eye-movement (REM) sleep

- Architecture alters throughout the night

What does a sleeping brain look like?



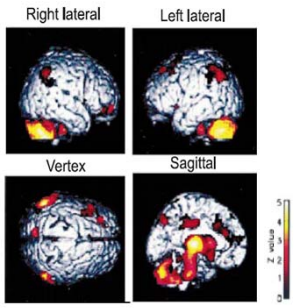


Cetacean sleep:

- Unihemispheric slow waves
- Contralateral eye open

Lyamin et al 2008 "Cetacean sleep: an unusual form of mammalian sleep"

Regional changes in blood flow

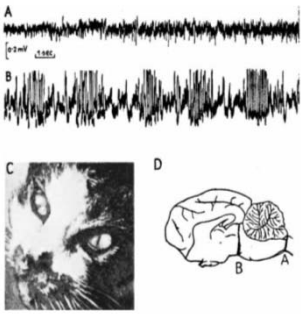


- *Relative* decreases in CBF:
  - Pons
  - Midbrain
  - Cerebellum
  - Thalamus
  - Cortical association areas

(deep SWS v wake, Kajimura *et al* 1999)
Relative rCBF – normalised for global flow

Sleep is an active process

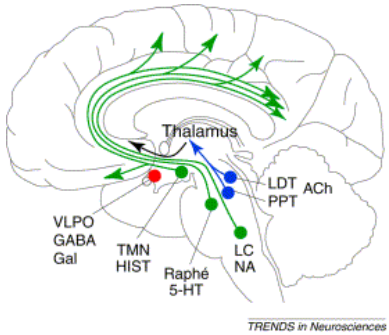
Sleep is an active process



Frederic Bremer (1937)

- Morruzi & Magoun (1949)
  Focal lesions - 'ascending reticular activating system' (ARAS)
- Morruzi & Magoun (1959)
  - Focal barbiturate application to the caudal brainstem induced wakefulness
  - The nucleus of the tractus solitarus (NTS) was identified as a brainstem 'hypnogenic centre'
- Sterman & Clemente (1962)
  - Electrical stimulation of the anterior hypothalamus (preoptic) induced sleep

The brain contains 'wake-active' and 'sleep-active' neurons



The sleep switch: hypothalamic control of sleep and wakefulness
(Saper *et al*, 2001)

'Sleep-active' neurons ***produce*** sleep

The preoptic hypothalamus

- Constantin von Economo (1923)

Persistent insomnia:

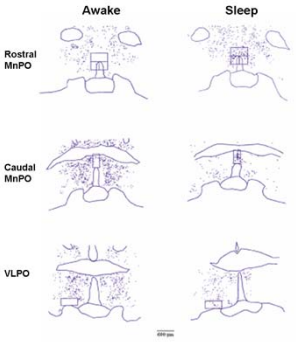
– **Their brains showed cell loss in the preoptic anterior hypothalamus/basal forebrain**

– **He concluded this was a hypnogenic region**

Encephalitis lethargica: excessive sleep

– Massive cell loss in the posterior hypothalamus and ARAS

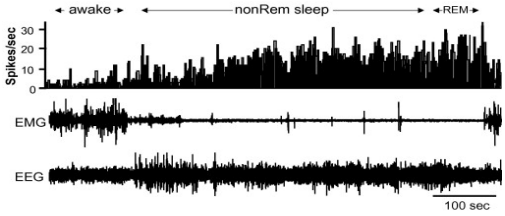
The preoptic hypothalamus – sleep active region



- *c-fos* – immediate early gene that is induced within a few minutes of neuronal activation
- The protein c-Fos can be measured for up to half an hour later

(Sherin et al, 1996; McGinty & Szymusiak, 2001)

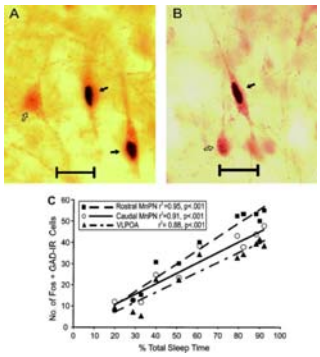
This c-Fos expression correlates with neuronal firing



- Extracellular VLPO neuron recording
- Shows sleep-related activity
- Firing progressively increases from light to deep NREM sleep

(Szymusiak et al, 2007 Review)

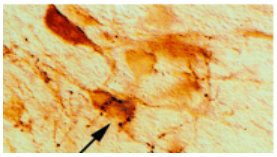
VLPO/MnPN neurons are GABAergic



- GABA – major inhibitory neurotransmitter
- $GABA_A$ (ion channel)
- $GABA_B$ (G protein)

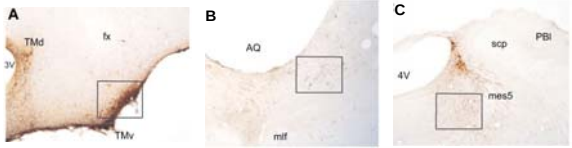
(Szymusiak et al, 2007 Review)

GABAergic sleep-active neurons project to arousal centres



(TMN; Sherin et al, 1996)

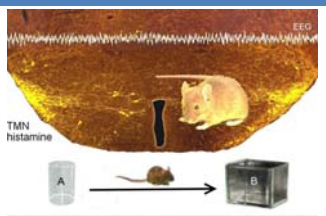
- Wake-active:
  - A –TMN (histamine)
  - B – LC (noradrenaline)
  - C – DRN (5-HT)



(Steininger et al, 2001)

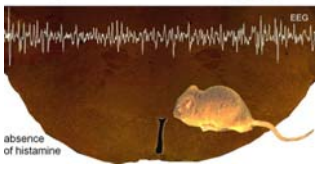
Arousal centres – histamine

Control



$HDC^{-/-}$

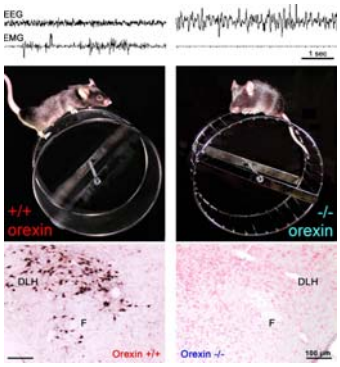
(histidine decarboxylase knockout)



Lin et al, 2011; Cell. Mol. Life Sci. 68:2499–2512

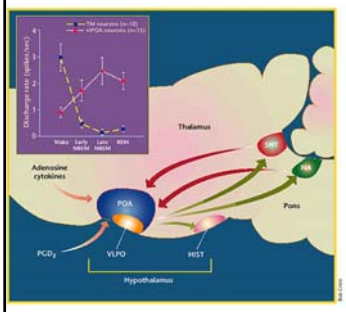
Arousal centres - orexin

Loss of orexin neurons leads to narcolepsy (modafinil)



Lin et al, 2011; Cell. Mol. Life Sci. 68:2499–2512

VLPO & arousal centres: reciprocal activity



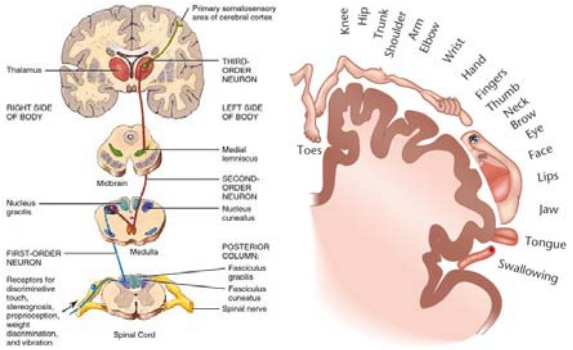
- VLPO begins firing just prior to sleep onset
- GABAergic inhibition of the TMN by VLPO
- Quietens the wake-related firing activity of e.g. TMN
- VLPO firing increases as sleep deepens

(McGinty & Szymusiak, 2001 Review)

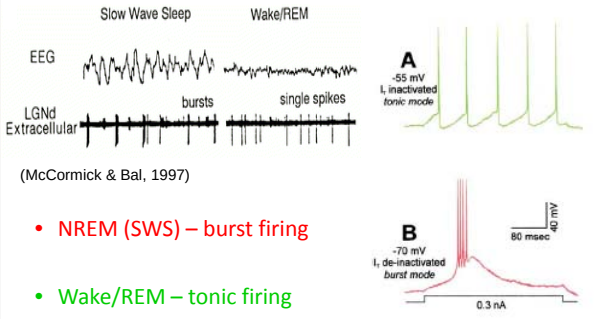
What is sleep? *An isolated brain?*

- Natural, reversible state of 'behavioural quiescence'
- Species-specific stereotypic posture
- Reduced responsiveness to sensory stimuli
- Rebound after deprivation

Sensory processing



Thalamocortical relay neurons have an **'awake'** and an **'asleep'** mode

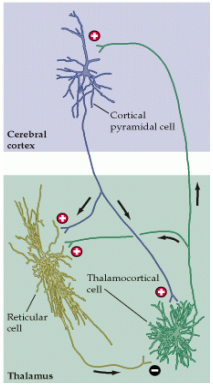


(McCormick & Bal, 1997)

- NREM (SWS) – burst firing
- Wake/REM – tonic firing

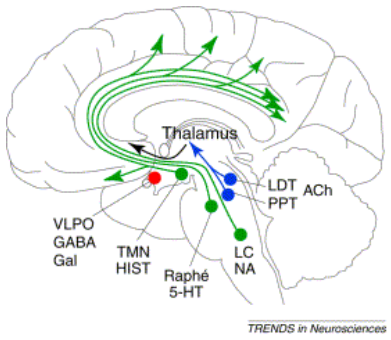
(Sherman & Guillery, 2002)

The thalamocortical system



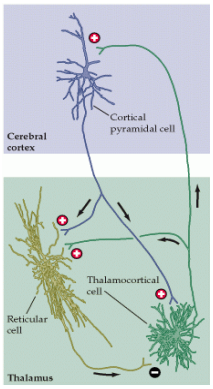
- During wakefulness:
  - 'the gateway to the cortex'
- During NREM sleep:
  - Sensory information becomes distorted

‘Wake-active’ neurons project to thalamus and cortex



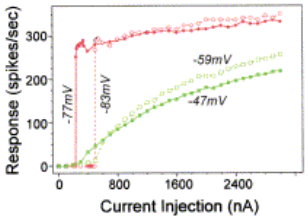
The sleep switch: hypothalamic control of sleep and wakefulness
(Saper *et al*, 2001)

The thalamocortical system



- During wakefulness:
  - ‘the gateway to the cortex’
- During NREM sleep:
  - Sensory information becomes distorted
- Due to the firing properties of thalamocortical neurons

Bursting closes the gateway

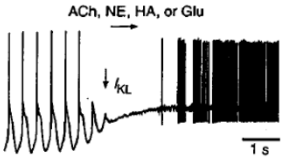


(Sherman & Guillery, 2002)

- Tonic
  - Linear spike response to increasing current injections
  - Faithful representation of sensory information
- Bursting
  - Non-linear response
  - Distorted information flow

Acetylcholine, noradrenaline, histamine and glutamate:

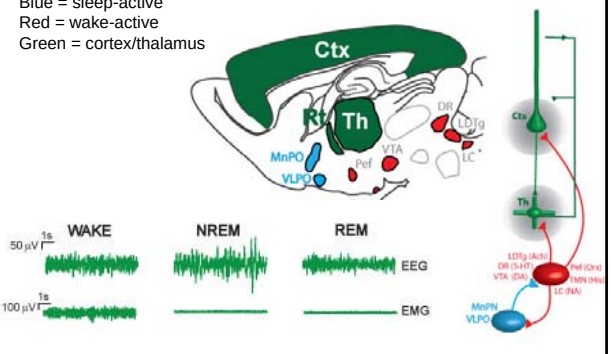
- Can switch TC firing mode from burst to tonic
- Due to a direct depolarising effect on TC cells:



(McCormick, 1992)

Hypothesis: drugs may hijack the sleep-wake circuitry

Blue = sleep-active
Red = wake-active
Green = cortex/thalamus



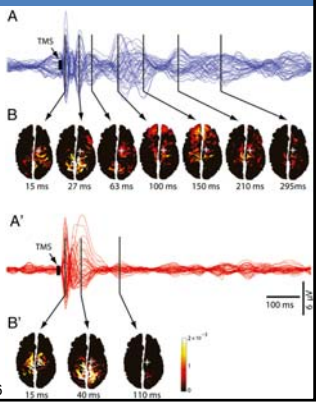
Franks & Zecharia 2011; Canadian Journal of Anesthesia 58(2):139-48

Cortical connectivity

NREM sleep is also associated with reduced cortical connectivity
(Massimini et al, 2007; PNAS)

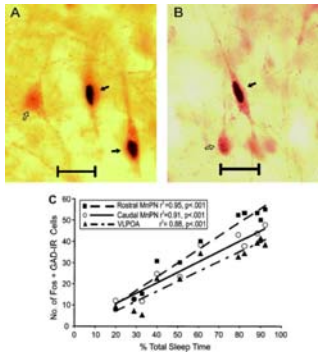
Awake

Midazolam



Ferrarelli et al 2010; PNAS 9;107(6):2681-6

Sleep-active neurons are GABAergic



- GABA – major inhibitory neurotransmitter
- $GABA_A$ (ion channel)
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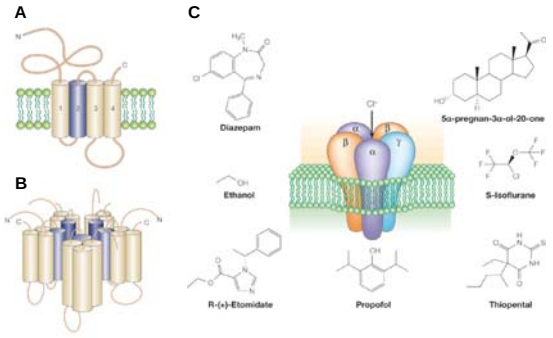
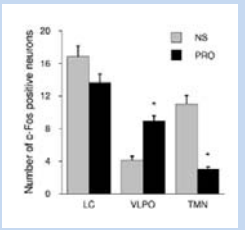
(Szymusiak et al, 2007 Review)

GABA? Interesting…..



Propofol can influence arousal circuitry: GABAergic gateway?



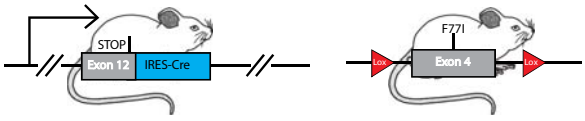
- TMN – reduced expression
- VLPO – increased expression
- LC - unaffected

(Nelson *et al*, 2002)

Testing the TMN: The Cre/Lox system

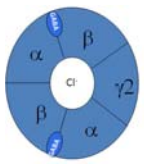
Cre recombinase x 'floxed' allele = deletion

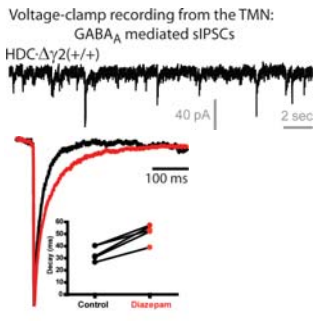
HDC-Cre mouse X Floxed gamma-2 mouse



Zecharia et al 2012; J Neurosci 19;32(38):13062-75

$GABA_A$ receptors in the TMN contain the γ_2 subunit

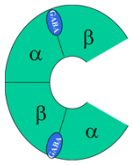


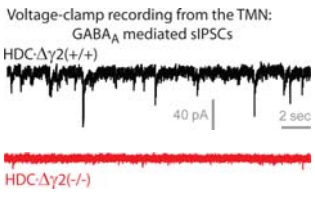


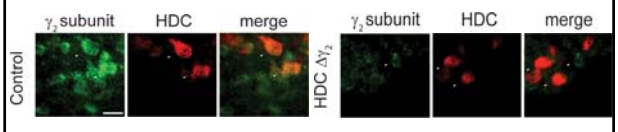
Tuberomammillary nucleus (TMN)

(Zecharia et al, 2012)

Successful deletion of γ_2 in the TMN

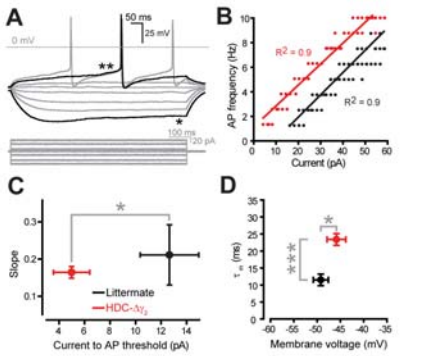






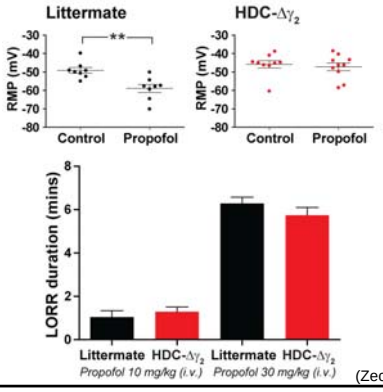
(Zecharia et al, 2012)

γ_2 removal increases the excitability of histaminergic neurons



(Zecharia et al, 2012)

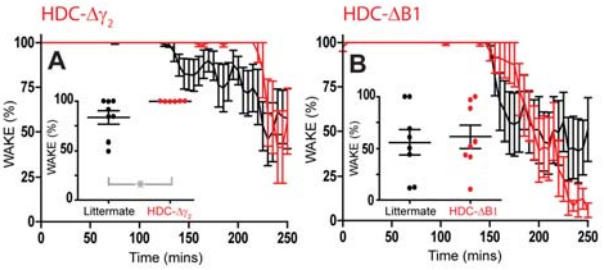
γ_2 removal abolishes the effects of propofol on histaminergic neurons, but **not** anesthesia



(Zecharia et al, 2012)

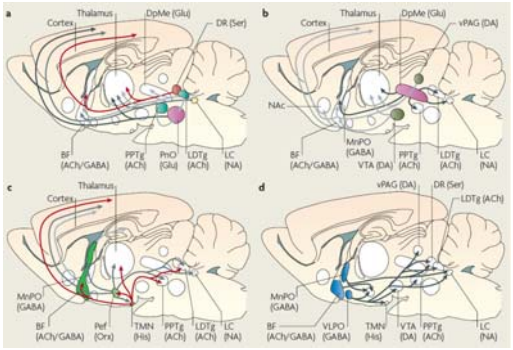
But - HDC-$\Delta\gamma_2$ mice take longer to habituate

Novel environment = new cage & bedding



(Zecharia et al, 2012)

Neural pathways of sleep and arousal



(Franks, 2008)

Understanding sleep neurobiology is a challenge!

'Can affect' is not the same as 'is important for'
We need cell-type selective approaches