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GABA in locus coeruleus in REMS regulation

Presentation for
J. Sleep Dis. Therapy

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SLEEP AND WAKING ARE INSTINCT BEHAVIORS AND THEY REPRESENT STATES OF CONSCIOUSNESS

SLEEP IS A REVERSIBLE STATE WHERE CONSCIOUSNESS REMAINS IN A SUBDUED STATE
BASED ON EEG AND EMG SLEEP-WAKING WAS OBJECTIVELY CLASSIFIED INTO AWAKE AND SLEEP

ADDITIONAL PARAMETER, CLASSICALLY AT LEAST EOG, HELPED IDENTIFICATION OF REM SLEEP (REMS)

(PGO AND HIPPOCAMPAL WAVES ALSO HAVE BEEN USED)
SLEEP-WAKING STAGES IN CAT

Thankachan & Mallick
SLEEP-WAKING STAGES IN RAT

Active Wakefulness

Quiet Wakefulness

Slow Wave Sleep 1

Slow Wave Sleep 2

REM Sleep

Kaur & Mallick
HOW IS REM SLEEP (REMS) REGULATED BY THE BRAIN?
(Neural Regulation of REMS)

Depending on the firing rate of neurons during REMS, in the brain there are

• REM-ON neurons (those increasing firing) and

• REM-OFF neurons (those decreasing firing/silent)
REM-ON NEURON

Mallick et al., Sleep Res. Online, 1998
REM-OFF NEURON

Mallick et al., Sleep Res. Online, 1998
Based on recording of REM-ON and REM-OFF neuronal activities from isolated, independent studies it was proposed that for REMS

i) activity of REM-ON neurons inhibit REM-OFF neurons;

ii) acetylcholine from REM-ON neurons inhibit the Noradrenergic (NA-ergic) REM-OFF neurons

Hobson and McCarley, 1974; Sakai, 1980
QUESTIONS WE RAISED/ASKED

• Is cessation of NA-ergic REM-OFF neuronal activities in locus coeruleus (LC) a pre-requisite/pre-condition for REMS generation or it is an associated phenomenon

• Activation of REM-OFF neurons in LC should not allow REMS to happen

• Does acetylcholine inhibit the REM-OFF neurons
REM-OFF NEURON

HYPOTHESIS

IF LC REM-OFF NEURONS MUST STOP FIRING FOR GENERATION OF REMS

THEIR ACTIVATION IN AN ATTEMPT NOT TO ALLOW THEM TO CEASE FIRING

WOULD CAUSE REMS LOSS OR AT LEAST WOULD SIGNIFICANTLY REDUCE REMS
Mild sustained stimulation of LC reduced REMS

Cessation of activities of LC-NA-ergic REM-OFF neurons is a pre-requisite for the generation of REMS

Previously, activities of REM-ON and REM-OFF neurons were recorded independently in separate animals, in isolated experiments on different days.

For confirmation and to understand their temporal correlation, we recorded in freely moving normally behaving, surgically prepared chronic animals, both REM-OFF and REM-ON neuronal activities simultaneously along with electrophysiological (EEG, EMG, EOG, PGO) waking-sleep-REMS patterns.
Temporal correlation between REM-ON and REM-OFF neuronal firing

**Mallick et al., Sleep Res. Online, 1998**
in vitro studies showed that Acetylcholine depolarized LC neurons i.e. not inhibition (Egan and North, 1985)

REM-OFF NEURONS MUST STOP FIRING FOR REMS GENERATION

BUT HOW?

Hobson and McCarley, 1974; Sakai, 1980
Therefore, it was hypothesized

In LC, excitatory cholinergic input from REM-ON neurons was translated into an inhibition on REM-OFF neurons through GABA interneuron for REMS generation.

Challenge was to simultaneously gather information on Neuro-Micro-Anatomo-Pharmacolo-Physiologic-Behaviral aspects.

It was overcome by microinjection of agonist/antagonist of one or more types of receptors in LC in various sequence/combinations.
Recording in behaving rats by modulating LC neurons electrically or chemically

**Following methods used/studies were carried out**

- Bilateral Chemitrode/Electrode implantation by Stereotaxic surgery

- *in vivo* studies in freely behaving surgically prepared chronic rats

  - Stimulation of LC neurons

- Single or combination of agonist and/or antagonist microinjection into LC with/ without simultaneous stimulation of PrH
Effect of local microinjection of cholinergic antagonist (scopolamine) and agonist (carbachol) bilaterally into the LC on REMS

Mallick et al., Neuroscience, 2001

REMS duration per episode was not affected, however, frequency of REMS generation was increased
Effect of local microinjection of GABA-ergic antagonist (picrotoxin) and GABA bilaterally into the LC on REMS

Mallick et al., Neuroscience, 2001

REMS duration per episode was significantly affected, however, frequency of REMS generation remained unaffected
Effect of local microinjection of cholinergic and GABA-ergic antagonist/agonist in various combinations/sequences into the LC on REMS

Mallick et al., Neuroscience, 2001

REMS duration per episode was reduced in (A); it was increased in (B), while in (C) REMS did not continue.

REMS could not be sustained.
Comparison of the effect PrH Stimulation and microinjection of GABA and Picrotoxin in LC on REM Sleep

Kaur et al., Synapse, 2001
Mallick et al., Neuroscience, 2001; and Kaur et al., Synapse, 2001
PHYSIOLOGICAL VALIDITY?

HYPOTHESIS

IF THE PROPOSED NEURAL CONNECTIONS ARE EXPERIMENTALLY DISTURBED SO THAT THE NA-ergic LC-REM-OFF NEURONS REMAIN ACTIVE,

REMS SHOULD BE SIGNIFICANTLY REDUCED

AND SIMULTANEOUSLY

REMS LOSS ASSOCIATED SYMPTOMS SHOULD BE EXPRESSED/OBSERVED DUE TO ELEVATED LEVELS OF NA EVEN IN NORMAL ANIMALS (Contd…)
PHYSIOLOGICAL VALIDITY?

HYPOTHESIS

We had already shown that REMSD increased Na-K ATPase activity and it was due to elevated level of noradrenalin (NA) in the brain (Gulyani & Mallick, Neuroscience, 1995; Mallick et al., J. Neurochem., 2000).

Therefore, we proposed that

i) GABA-antagonist, picrotoxin, into LC should not allow REM-OFF neurons to cease activity resulting in increased Na-K ATPase activity;

ii) Modulation of Na-K ATPase activity in LC should alter REM-OFF neuronal activity and REMS
Picrotoxin (every 6h) bilaterally into the LC for 48h reduced REMS (A) and increased Na-K ATPase activity (B) significantly.

A.

Kaur et al., Behav Brain Res. 2003

B.

Kaur et al., Behav Brain Res. 2003
Comparison of Na-K ATPase activity in rat brain

A. Picrotoxin every 6h into LC

B. REMSD

We had confirmed that the REMS loss associated increase in Na-K ATPase activity is actually mediated by elevated level of NA (Gulyani and Mallick, 1995; Mallick et al., J. Neurochem, 2000)
Anti-endobain-antibody into LC and simultaneous recording of waking-sleep-REMS

Normal condition: Endobains in optimum level

Anti-endobain-antibody will activate LC-REM-OFF neurons

Na-K ATPase
Endobains
Increased NA release

LC REM-OFF NA-ergic neuron

Jaiswal et al., J. Sleep Res., 2009
Effect of anti-ouabain antibody into LC on sleep-waking-REMS

(A) Percentage (mean ± SEM)

(B) Frequency (mean ± SEM)

(C) Time (sec/episode) (mean ± SEM)

Baseline | IgG | Anti-ouabain antibody

Jaiswal et al., J. Sleep Res., 2009
CONCLUSION: TAKE HOME MESSAGE

NA-ergic LC REM-OFF NEURONS ARE ACTIVE IN ALL STAGES EXCEPT DURING REMS.

THEIR CESSION BY GABA IS PRE-REQUISITE FOR REMS.

NON-CESSION/CONTINUOUS ACTIVATION OF REM-OFF NEURONS INDUCES REMS-LOSS AND ELEVATION OF BRAIN NA LEVEL, WHICH IN TURN CAUSES REMS-LOSS ASSOCIATED SYMPTOMS.

THUS, UPON REMS LOSS AT LEAST ELEVATED NA IS A PRIMARY CAUSE FOR INDUCING REMSD ASSOCIATED SYMPTOMS.
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