Laboratory of Neurobiology of Sleep-Wakefulness Cycle

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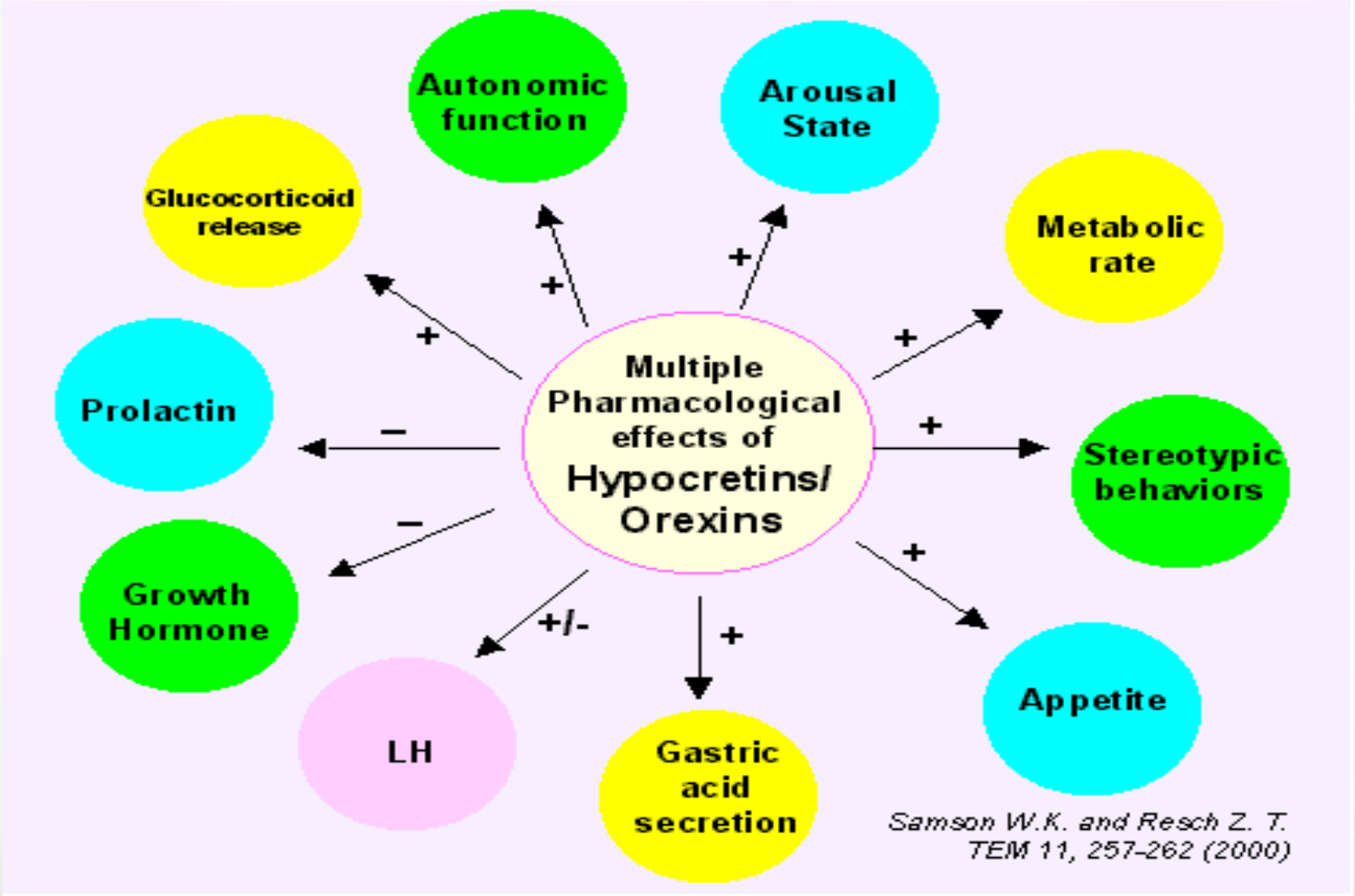
Serial electrical stimulations of Orexin (Hypocretin)-containing neurons in dorsomedial and lateral hypothalamus and recovery from some pathological states with sleep disorders

Orexins / Hypocretins



It is believed that the best-known function of Orexins is in promoting of arousal and waking. Orexins lengthen, and their disruption shorten the periods of wakefulness [*Alexandre C, Andermann M.L., Scammell T.E. (2013),Kilduff T. S, Peyron C. (2000) Sakurai T. at al (1998)].*

Genetic and pharmacological blockades of Orexin-mediated signaling impact arousal, and impaired Orexinergic signaling leads to narcolepsy [*Chemelli R.Met al (1999), Heifetz A., Morris G. B. et al (2012)].*

Projections from Orexin-producing neurons excite the most wake-promoting systems are :

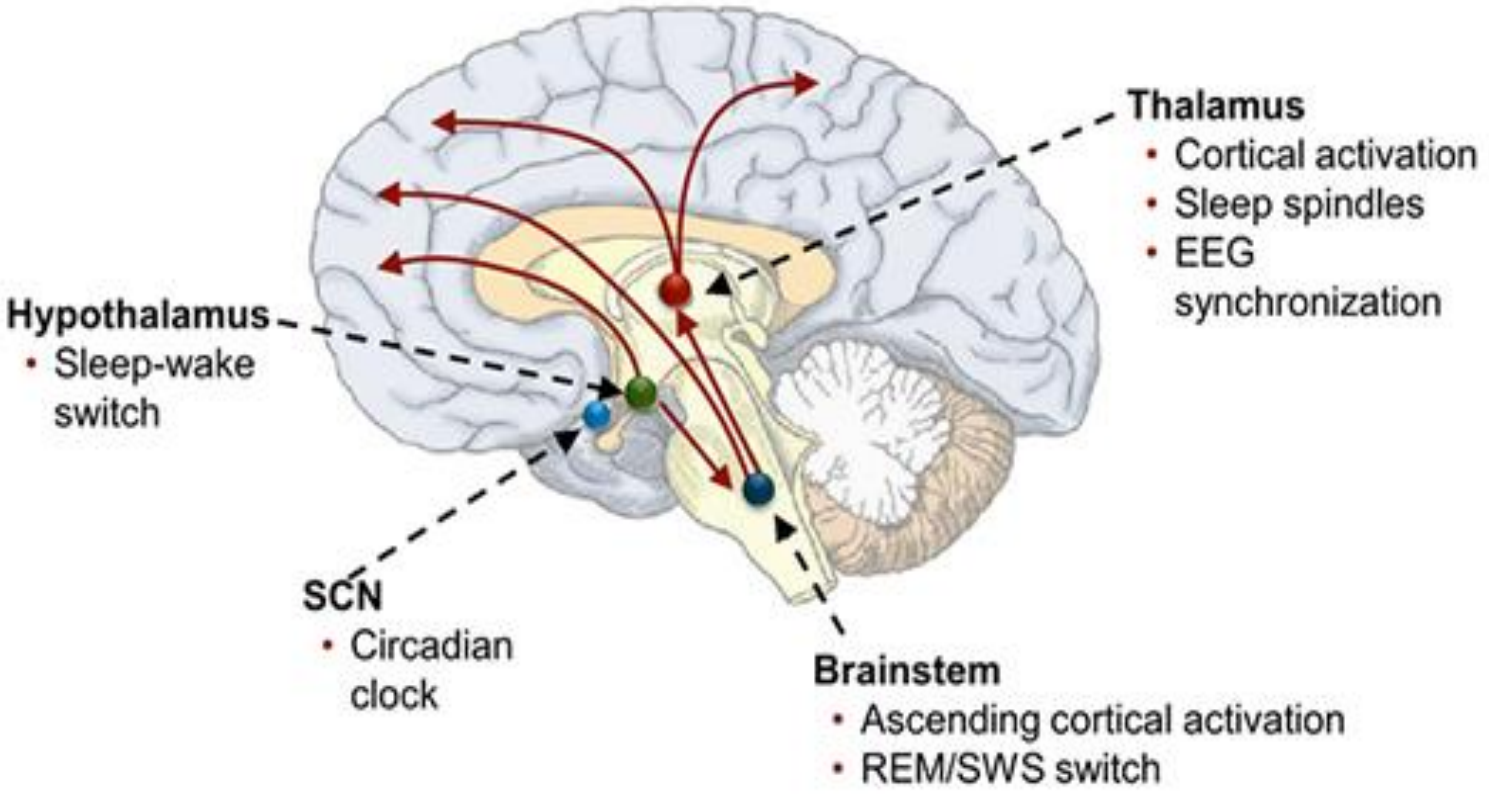
Locus Coeruleus *Norepinephrine*,

Dorsal Raphe *Serotonin,*

T**uberomammillary** *Histamine*, and

Basal Forebrain and Brainstem *Acetylcholine* neurons .

Wakefulness/Sleep: Neurophysiology



Saper, CB, et al. *Nature*. 2005;437:1257-1263.[1]

- In our Lab had shown that serial electrical stimulations of posterior hypothalamus can produce simultaneous activation of neocortex and hippocampus in cats during acute comatose state *[Nachkebia N., Nachkebia A., Chkhartishvili E., Oniani T., Gvilia I. (1999)].*

- Approximately at the same time it was shown that this region of hypothalamus consists of Orexin (Hypocretin) - containing neurons .

- Above mentioned facts gave us possibility for the suggestion that **Hypothalamic Orexin-containing neurons can take part in the acceleration of recovery from pathological state characterized by significant disorders of wakefulness and sleep**.

- Are Orexin-containing neurons of **DMH** and **LH,** and brain Orexinergic system in general, those cellular targets which can regulate sleep homeostasis and accelerate recovery of wakefulness and/or sleep phases from some pathological conditions – **experimental comatose state** and/or closer to it **barbiturate anesthesia-induced artificial sleep**?

Consideration of hypothalamic Orexinergic system as the neurophysiological substrate or cellular target necessary for the acceleration come out from **barbiturate anesthesia induced artificial sleep** and **acute barbiturate comatose state** is one of the main goals of the present investigation.

A total of 36 male wild albino rats, approximately 2.5-3 months of age, weight 220-250 g at the start of experimental researches served as subjects.

Modeling of acute comatose state by means of systemic injection of highest doses of **sodium ethaminal (90 mg/kg)** entirely disrupts **SWC** ultradian structure, eliminates normal cyclic alteration of wakefulness, slow wave and **REM** sleep and animals gradually fall into comatose state.

It results disturbances in normal functioning of various brain structures, there develops pathological pattern of electrical activity taking dominant position spontaneously in all parts of neocortex.

Come out from acute comatose state starts by recovery of EEG picture of normal deep slow wave sleep taking approximately 40 hours during spontaneous recovery in unstimulated controls (Fig.1.1 dark column).

We have found that repetitive electrical stimulations of Orexin-containing neurons in DNH and LH have similar effects.

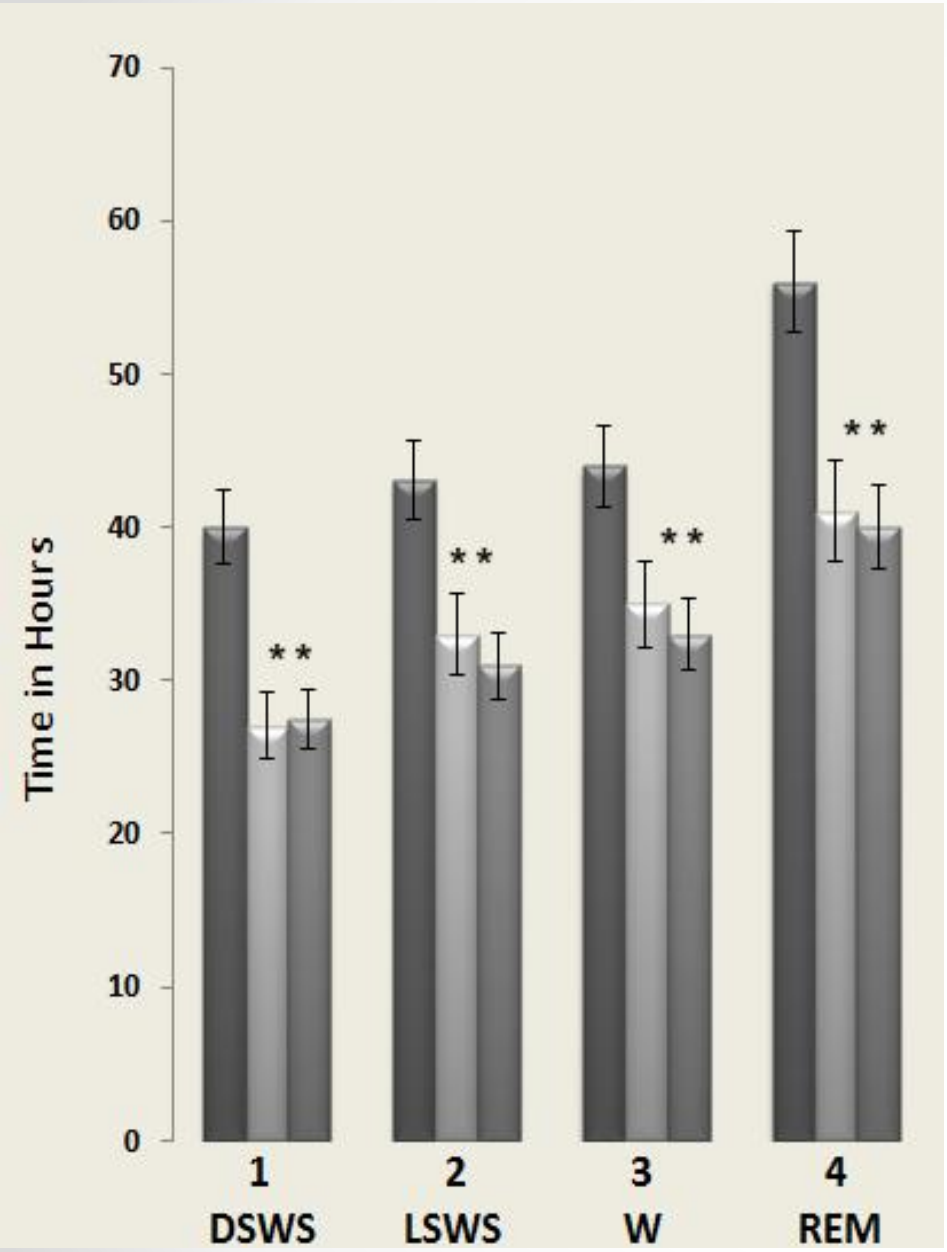


Fig.1

Influence of serial electrical stimulations of DMH and LH Orexin-containing neuronal regions on recovery rate of SWC phases from pharmacologically induced acute comatose state

On the ordinate – Time in min,

on the abscissa:

1 – latent period for deep slow wave sleep (DSWS) recovery;

2 – latent period for light slow wave sleep (LSWS) recovery;

3 – latent period for wakefulness (W) recovery;

4 - latent period for REM sleep recovery.

Black columns – spontaneous recovery SWC phases in unstimulated controls,

light gray columns – recovery of SWC phases under repetitive electrical stimulations of **DMH**, **dark gray columns** – recovery of SWC phases under repetitive electrical stimulations of **LH**

**=$p<0.01$, Data from Experiments I and II were compared to the data from unstimulated control.

- The dynamic of wakefulness recovery appeared especially important – it took place only after recovery of EEG picture of **deep** and **light slow wave sleep**, in both unstimulated controls and under repetitive stimulation of **DMH** and/or **LH** orexin-producing neuronal regions.

- Therefore repetitive electrical stimulation of **hypothalamic orexin-producing neuronal regions** accelerates wakefulness recovery by 9-11 h in comparison with unstimulated control .

Thus recovery from acute **barbiturate comatose state** starts with the restoration of EEG picture of normal deep slow wave sleep, continues with the reappearance of EEG picture of normal light slow wave sleep and ends with the recovery of wakefulness state.

Therefore barbiturate acute Coma time consists from the latencies of DSWS, LSWS and wakefulness recovery.

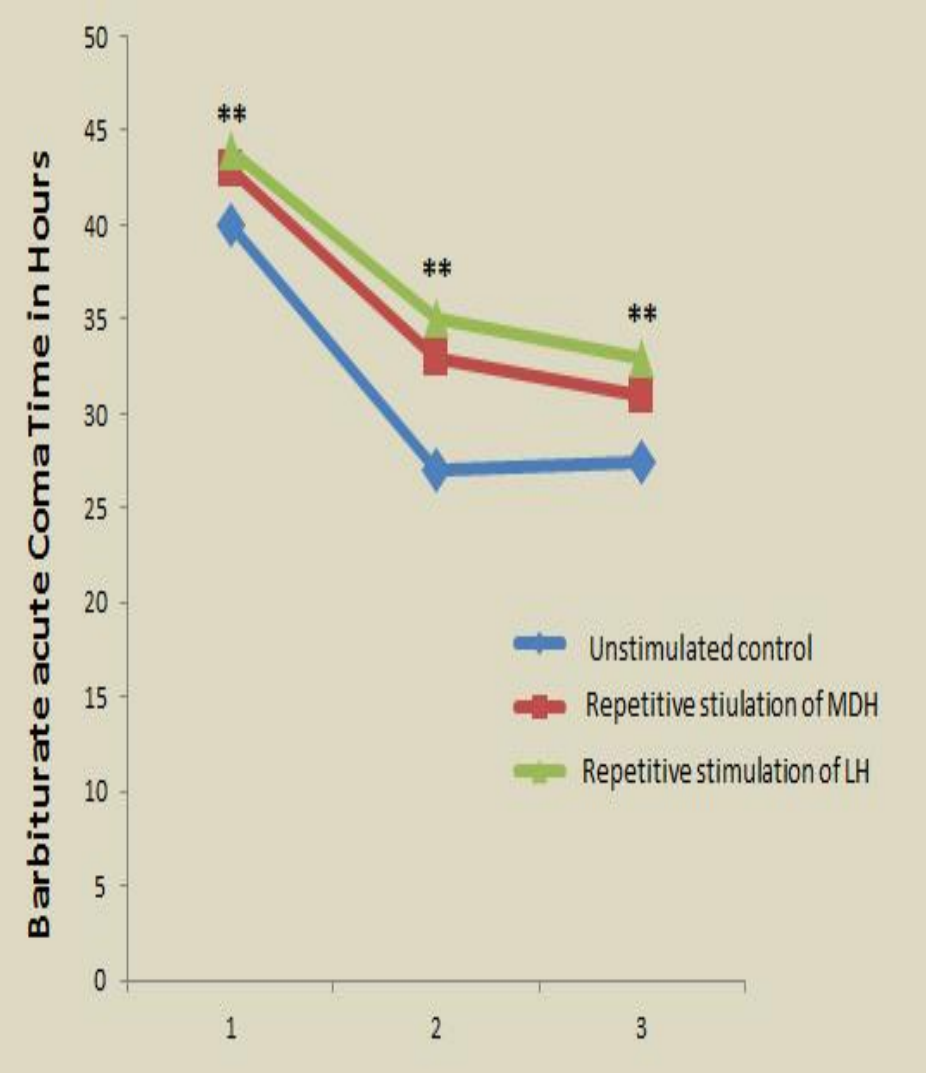


Fig.2

Changes in barbiturate acute Coma time under repetitive electrical stimulations of DMH and LH

1 – Latency of DSWS recovery from barbiturate acute comatose state,

2 - Latency of LSWS recovery from barbiturate acute comatose state,

3 - Latency of wakefulness recovery from barbiturate acute comatose state.

**=$p<0.01$, Data from Experiments I and II were compared to the data from unstimulated control.

- Indeed these facts indicate to the significant acceleration of regulation of sleep homeostasis disturbed during pharmacologically induced experimental comatose state.

- Therefore artificial activation of hypothalamic Orexin-containing neuronal regions can have therapeutic implication.

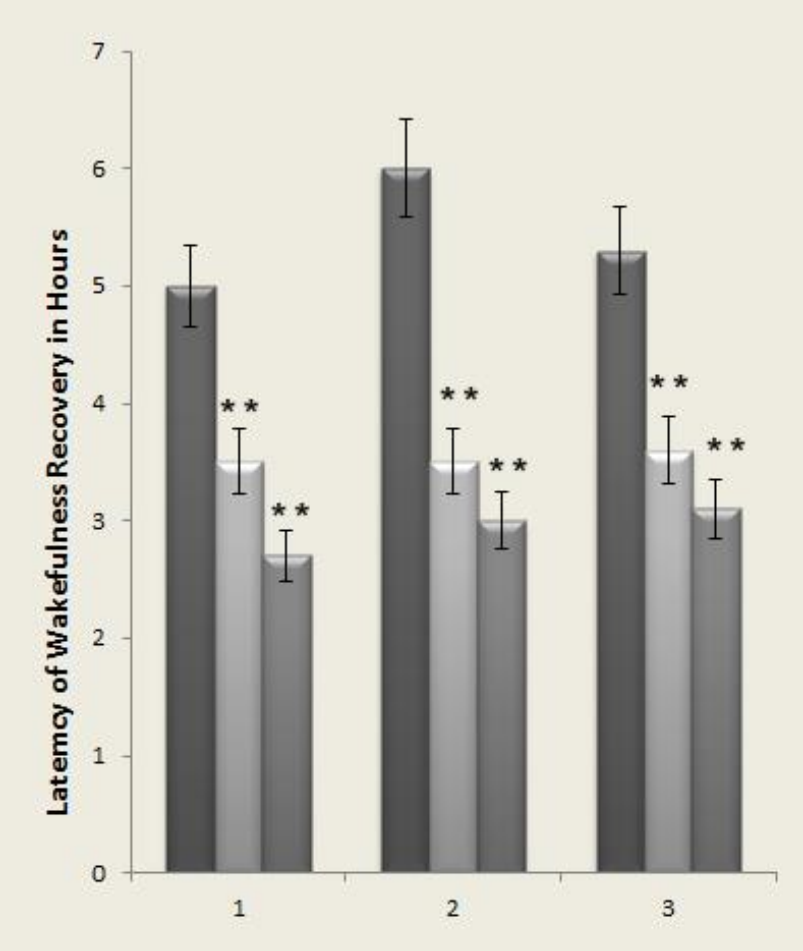


Fig.3 **Influence of serial electrical stimulations of DMH and LH Orexin-producing neuronal regions on recovery rate of wakefulness from different depth of barbiturate anesthesia-induced artificial sleep**

On the ordinate – Time in hours, on the abscissa:
1 - wakefulness recovery from barbiturate anesthesia (50 mg/kg of Sodium Ethaminal);
2 - wakefulness recovery from barbiturate anesthesia (60 mg/kg of Sodium Ethaminal);
3 - wakefulness recovery from barbiturate anesthesia (70 mg/kg of sodium ethaminal);

Black columns – spontaneous recovery of wakefulness in unstimulated control,
Light gray columns – wakefulness recovery under serial electrical stimulations of DMH,
Dark gray columns – wakefulness recovery under serial electrical stimulations of LH.
** = $p<0.01$. Data from Experiments III, IV, V, VI, VII and VIII were compared to the data from corresponding unstimulated controls.

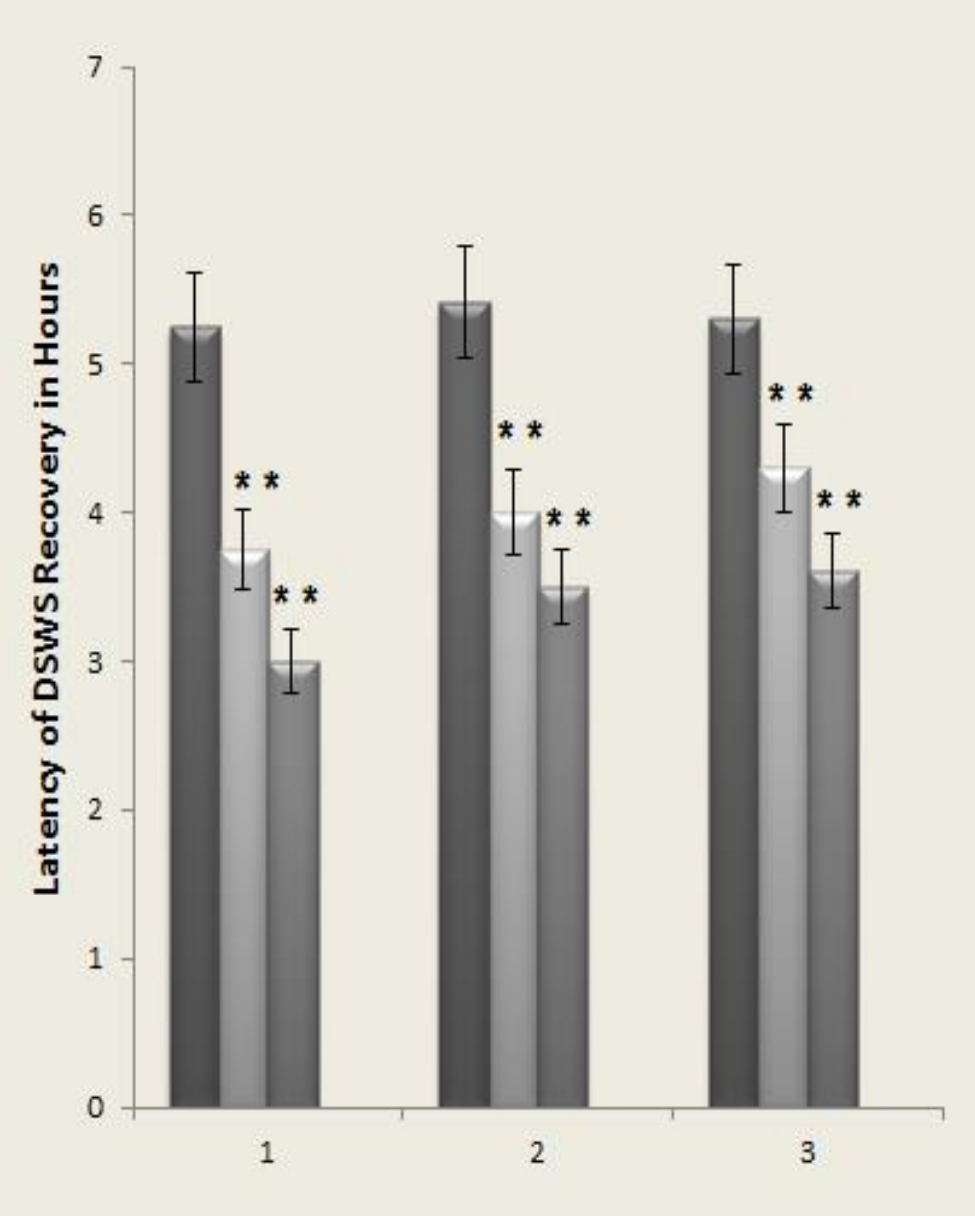


Fig.4 **Influence of serial electrical stimulations of DMH and LH Orexin-containing neuronal regions on the recovery rate of normal slow wave sleep from different depth of barbiturate anesthesia-induced artificial sleep**

On the ordinate – Time in hours,
on the abscissa:
1 – slow wave sleep recovery from barbiturate anesthesia (50 mg/kg of sodium ethaminal);
2 - slow wave sleep recovery from barbiturate anesthesia (60 mg/kg of sodium ethaminal);
3 - slow wave sleep recovery from barbiturate anesthesia (70 mg/kg of sodium ethaminal);

Black columns – spontaneous recovery of slow wave sleep in unstimulated control,
Light gray columns – slow wave sleep recovery under serial electrical stimulations of DMH,
Dark gray columns – slow wave sleep recovery under serial electrical stimulations of LH.

** = $p<0.01$. Data from Experiments III, IV, V, VI, VII and VIII were compared to the data from corresponding unstimulated controls.

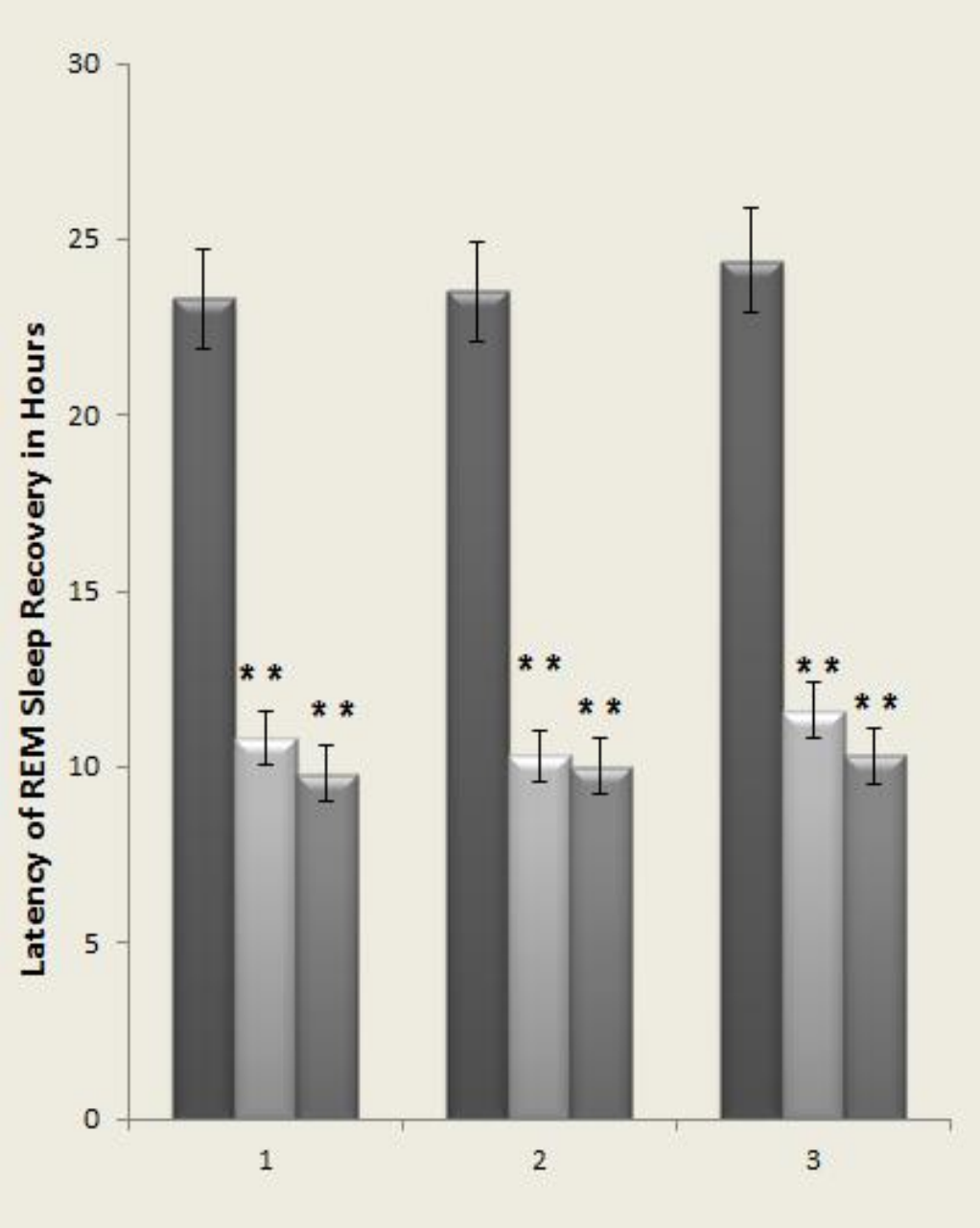


Fig.5 Influence of repetitive electrical stimulations of DMH and LH Orexin-containing neuronal regions on the recovery rate of REM sleep from different depth of barbiturate anesthesia-induced artificial sleep

On the ordinate – Time in min,
on the abscissa:
1 – REM sleep recovery from barbiturate anesthesia (50 mg/kg of sodium ethaminal);
2 - REM sleep recovery from barbiturate anesthesia (60 mg/kg of sodium ethaminal);
3 - REM sleep recovery from barbiturate anesthesia (70 mg/kg of sodium ethaminal);

Black columns – spontaneous recovery of REM sleep in unstimulated control,
Light gray columns – REM sleep recovery under repetitive electrical stimulations of DMH,
Dark gray columns – REM sleep recovery under serial electrical stimulations of LH.

** = $p<0.01$. Data from Experiments III, IV, V, VI, VII and VIII were compared to the data from corresponding unstimulated controls.

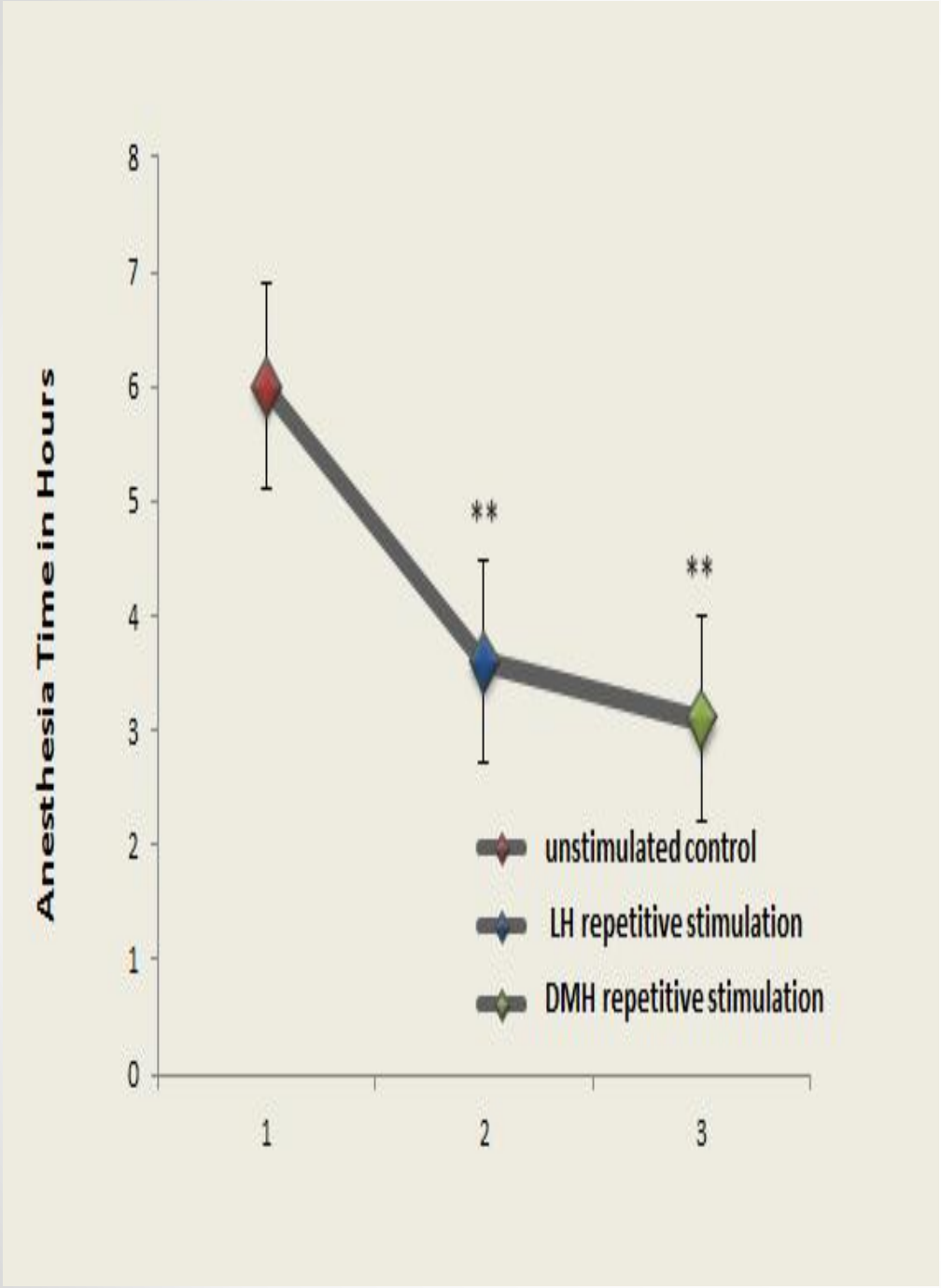


Fig. **6 Changes in barbiturate anesthesia time (70 mg/kg of sodium ethaminal) under the influence of repetitive stimulation of DMH and LH orexin-producing neuronal regions.**

On the abscissa:
1 – Data from unstimulated control animals,
2 – data from animals with repetitive electrical stimulation of DMH,
3 - data from animals with repetitive stimulation of LH.

On the ordinate – time in hours.

** = $p<0.01$ experimental versus unstimulated control.

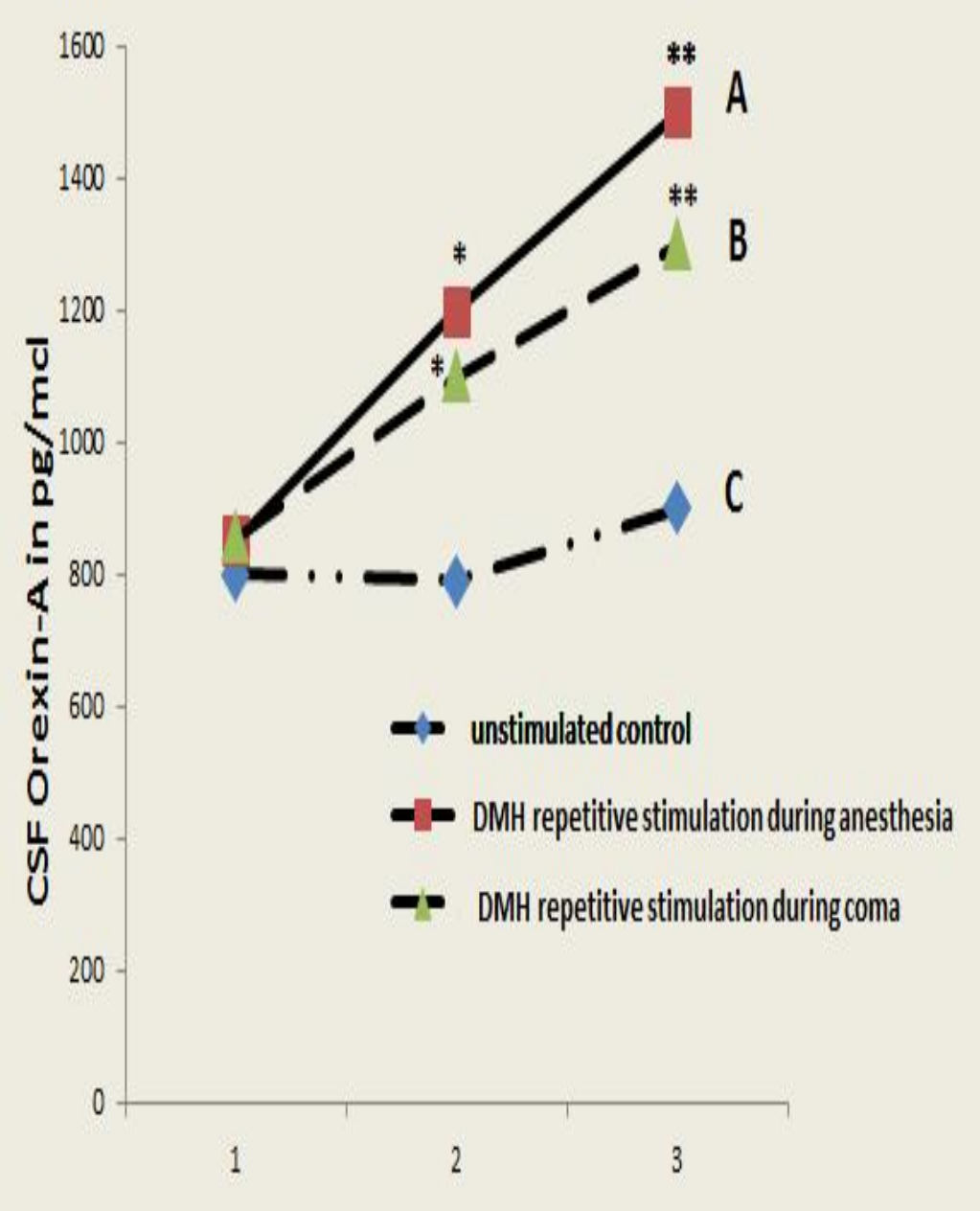


Fig. 7 **Changes in the content of CSF Orexin-A during comatose state and barbiturate anesthesia induced artificial sleep**

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On the abscissa:
A – Barbiturate anesthesia 70 mg/kg;
B – Comatose state;
C–unstimulated control;

1 – data of Orexin-A measurement in pre-treatment period;
2 – data of Orexin-A measurement 1.5 h after starting of barbiturate anesthesia and comatose state;
3 - data of Orexin-A measurement at the period when recovery was started.

* =$p<0.5$, ** =$p<0.01$, experimental versus unstimulated control.

- Indeed, despite significant similarities between sleep disorders during these two pathological states mechanisms for their development are very different.

- **Comatose state** is linked to the longer isolation of forebrain structures from midbrain and medullar ascending signaling and recovery of SWC phases from this state can be realized on the basis of forced put in action of forebrain neuroanatomical and neurochemical linkages.

- In the case of **anesthesia-induced artificial sleep** neuroanatomical and neurochemical signaling between forebrain and midbrain/medullar regions is switched off for a shorter period of time and with coming out from general narcosis normal functioning of these functional linkages gradually restores. Coming out becomes easier under artificial activation of hypothalamic Orexinergic system.

Special interest deserve findings obtained by us **for the first time showing** that repetitive electrical activations of **DMH** and **LH** significantly elevated the content of **Orexin A** in CSF after **1.5 h** from starting of these pathological conditions and at the period when come out is starting.

CONCLUSION

Repetitive electrical stimulations of DMH and LH Orexin-producing neuronal regions significantly elevates the content of endogenous Orexin A in CSF, shortens anesthesia and coma time and promotes come out from acute comatose state and deep barbiturate anesthesia, through the acceleration of normal sleep-wakefulness cycle recovery.

Gratitude to members of laboratory of Neurobiology of Sleep-Wakefulness Cycle

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