Archives Italiennes de Biologie, 142: 359-367, 2004.

SLOW-WAVE SLEEP: SEROTONIN, NEURONAL PLASTICITY, AND SEIZURES

M. STERIADE[1]

Laboratoire de Neurophysiologie, Faculté de Médecine, Université Laval, Québec, Canada G1K 7P4

JOUVET'S SEMINAL CONTRIBUTIONS TO SLEEP RESEARCH

This paper is dedicated to my friend Michel Jouvet, the man who performed the first animal experiments (13) to localize the brainstem and forebrain structures responsible for the physiological correlates of the behavioral state he called paradoxical sleep because the increased excitability of higher brain structures was associated with motoneuronal inhibition, an alert yet paralyzed brain. Nowadays, we still use muscular atonia, described by Jouvet and Michel (18), as the physiological sign that most reliably distinguishes paradoxical sleep.

Jouvet hypothesized (15) that paradoxical sleep is a guardian of psychological personality, a reprogramming of individuality during dreaming. He also wrote a novel, *Le Château des Songes* (16), with fabulous dreams and commentaries on them by a character of the 18th century. More recently, Jouvet traced the great moments of his life together with the achievements of other neurobiologists in the field of sleep (17).

Here, I would like to state that even those of Jouvet's ideas that were apparently disconfirmed by subsequent data are now less firmly challenged and some of them are confirmed by detailed analyses at the intracellular level. I specifically refer to the theory implicating neurons in the raphe nuclei and their major transmitter, serotonin (5-hydroxytryptamine, 5-HT), in the generation of slow-wave sleep. Most experimental data supporting this theory were accumulated during the 1960s and were reviewed in Jouvet (14). Subsequent data, mainly recordings from raphe neurons across the waking-sleep cycle, have refuted the role of serotonergic neurons in the genesis of slow-wave sleep since neurons in the dorsal and other nuclei of the raphe system decrease the rates of their regular discharges when the animal passes from waking to slow-wave sleep (25, 27, 57).

While Jouvet's original claims seemed unfounded in the light of subsequent experiments, more recent results may bring back on the scene the raphe and other 5-HT neuromodulatory actions as good candidates for inducing neuronal events related to slow-wave sleep. I shall briefly mention only four series of data.

[1] Corresponding Author: Prof. M. Steriade, M.D., D.Sc., Laboratory of Neurophysiology, Faculté de Médecine, Université Laval, Québec, Canada G1K 7P4 - Tel. 1 (418) 656 5547 - Fax 1 (418) 656 3236 - E-mail: mircea.steriade@phs.ulaval.ca

SLOW-WAVE SLEEP OSCILLATIONS AND THEIR INTEGRATION IN CORTICOTHALAMIC NETWORKS

To remain within the frame of slow-wave sleep, a state that, contrary to paradoxical sleep, has been regarded as associated with global inhibition of cortex and subcortical structures (33), therefore leading to "abject annihilation of consciousness" (8), let me present some evidence suggesting that these hypotheses became obsolete as they could not resist experimental testing and recent evidence from human studies.

Far from being inactive, intracellularly recorded neocortical neurons display during the depolarizing phase of the slow oscillation (0.5-1 Hz), which typically characterizes slow-wave sleep, firing rates that are as high as during natural states of waking and paradoxical sleep (51). The high activity of cortical neurons in slow-wave sleep leads to a coalescence of several types of brain rhythms during this behavioral state. Instead of analyzing distinct frequency bands of at least three or four distinct oscillations and even dissecting various sub-bands for each rhythm, it is more conceptually rewarding to understand the physiological basis of the grouping, by the slow oscillation, not only of other slow-wave sleep rhythms, such as spindles and delta, but also of fast (beta and gamma) rhythms that are conventionally assumed to only characterize the brain-activated states of waking and paradoxical sleep (40). Below, I discuss some of these grouped rhythms, due to the effect of the slow oscillation that is generated intra-cortically, as it was recorded in neocortical neurons in the absence of thalamus and brainstem (48). Following these initial intracellular recordings in anesthetized animals (47), the slow oscillation was also recorded in naturally sleeping animals (42) and humans (1, 4, 35).

The synchronization of all types of neocortical neurons during the slow oscillation (2, 3), and especially those with thalamic projections, has a powerful impact on thalamic reticular and relay neurons, with the consequence of triggering spindle oscillations. The density of subunits of glutamate receptors is much higher, and the excitatory postsynaptic currents (EPSCs) evoked by corticofugal axons much ampler, in thalamic reticular, compared to thalamocortical, neurons (11). This explains why the synchronous activation of the glutamatergic corticothalamic pathway, as in slow-wave sleep, produces strong excitation of thalamic reticular neurons, as opposed to inhibitory postsynaptic potentials (IPSPs) in thalamocortical neurons (Fig. 1 in ref. 38). The excitation of thalamic reticular neurons and the rhythmic IPSPs leading to rebound spike-bursts in thalamocortical neurons represent the cellular basis of sleep spindles. Besides spindle generation by the slow oscillation, the cortex has an important role in spindle synchronization. Thus, spindles propagate in visual thalamic slices (22), whereas they are nearly simultaneous in widespread territories of the thalamus and cortex of both cats and humans (5, 6). After decortication, the thalamic synchronization of spindles is disorganized or completely lost and what remains is accounted for by the synchronizing power of the thalamic reticular nucleus (6).

Another oscillatory type that is grouped within the complex wave-sequence of the slow cortical oscillation is the fast activity in the frequency range of beta (~20-30

ing incoming thalamic inputs (19) and elaborate synaptic activities that are primarily dependent, but also outlasting, the spindle-related signals. This was shown with the experimental model of spindling, waxing-and-waning thalamocortical augmenting responses, during which the initial excitatory postsynaptic potential (EPSP) of cortical neurons follows by ~3 ms the first action potential in the spike-burst of simultaneously recorded thalamocortical neuron, but the augmentation phenomenon basically consists of a secondary EPSP that is cortical in origin (52). That neocortical neurons display augmenting responses even in the absence of thalamus was demonstrated by progressive depolarization and increased number of action potentials in their rhythmic spike-bursts evoked by repeated, rhythmic callosal volleys in thalamectomized animals (48).

Thus, neocortical neurons have the ability to progressively increase their responsiveness when activated within the frequency range of sleep spindles, even in the absence of spindle generators, thalamic neurons. In the intact brain, testing cortical excitability with repeated pulse-trains revealed that, first, the IPSP of the control response was progressively reduced in amplitude and replaced by an early depolarization and, second, single stimuli applied after the rhythmic pulse-trains elicited exclusively depolarizing responses, an enhancement that remained unchanged for up to 12-15 minutes (56).

The intriguing finding in our experiments was that neuronal plasticity, usually regarded as a beneficial phenomenon implicated in memory and learning, could develop during slow-wave sleep into self-sustained paroxysmal discharges, similar to spike-wave complexes at ~3 Hz seen during petit-mal epilepsy or to the more complex electrographic pattern seen during the Lennox-Gastaut syndrome. This unexpected transformation of a normal to a pathological event was first reported in limbic circuits, at which level rhythmic stimulation of basolateral amygdala at 5-6 Hz evoked short-latency hippocampal responses, which progressively increased in amplitude and developed into paroxysmal activity with self-sustained potentials whose shapes were virtually identical to responses evoked in the final stage of stimulation (36). Similarly, the enhanced responsiveness of cortical neurons driven by rhythmic callosal volleys in the frequency range of sleep spindles was followed by paroxysmal activity, which occurred within the same frequencies as in the preceding period of stimulation (48). The changes in neuronal responsiveness that lead to self-sustained oscillations of the paroxysmal type are already initiated during rhythmic stimulation with pulse-trains at 10 Hz and outlast the stimulation period with patterns of spike-wave epileptiform discharges at ~3 Hz. Such changes from normal (sleep-like) to pathological (epileptic-like) states have been reported in thalamic neurons driven by cortical volleys (37, 49) and in cortical neurons driven by cortical volleys (50). The behavioral significance of development from "memory" events in the electrical activity of corticothalamic reciprocal circuits to electrographic paroxysmal states that are antinomic to the notion of memory is not yet clearly understood. Let me just mention that slow-wave sleep, a state that is beneficial for the consolidation of memory traces (see above) is also the state during which spike-wave and Lennox-Gastaut seizures preferentially occur (41).

10. GAIS, S., MÖLLE, M., HELMS, K. AND BORN, J. Learning-dependent increases in sleep density. *J. Neurosci.*, **22:** 6830-6834, 2002.
11. GOLSHANI, P., LIU, X.B. AND JONES, E.G. Differences in quantal amplitude reflect GluR4-subunit number at corticothalamic synapses on two populations of thalamic neurons. *Proc. Natl. Acad. Sci., USA* **98:** 4172-4177, 2001.
12. HOBSON, J.A., PACE-SCHOTT, E. AND STICKGOLD, R. Dreaming and the brain: toward a cognitive neuroscience of conscious states. *Brain Behav. Sci.*, **23:** 793-842, 2000.
13. JOUVET, M. Recherches sur les structures nerveuses et les mécanismes responsables des différentes phases du sommeil physiologique. *Arch. Ital. Biol.*, **100:** 125-206, 1962.
14. JOUVET, M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergebn. Physiol.*, **64:** 166-307, 1972.
15. JOUVET, M. Programmation génétique itérative et sommeil paradoxal. *Confront. Psychiat.*, **27:** 153-181, 1986.
16. JOUVET, M. *Le Château des Songes*. Paris, Odile Jacob, 1992.
17. JOUVET, M. *The Paradox of Sleep*. Cambridge (MA), The MIT Press, 1999.
18. JOUVET, M. AND MICHEL, F. Corrélations électromyographiques du sommeil chez le chat décortiqué et mésencéphalique chronique. *C. R. Soc. Biol. Paris*, **153:** 422-425, 1959.
19. KANDEL, A. AND BUZSÁKI, G. Cellular-synaptic generation of sleep spindles, spike-and-wave discharges, and evoked thalamocortical responses in the neocortex of rat. *J. Neurosci.*, **17:** 6783-6797, 1997.
20. KHATEB, A., FORT, P., ALONSO, A., JONES, B.E. AND MÜHLETHALER, M. Pharmacological and immunohistochemical evidence for serotonergic modulation of cholinergic nucleus basalis neurons. *Eur. J. Neurosci.*, **5:** 541-547, 1996.
21. KHATEB, A., SERAFIN, M. AND MÜHLETHALER, M. Midbrain reticular neurones in vitro are sensitive to amines and opiates. *Soc. Neurosci. Abstr.*, **15:** 451, 1989.
22. KIM, U., BAL, T. AND MCCORMICK, D.A. Spindle waves are propagating synchronized oscillations in the ferret LGNd *in vitro*. *J. Neurophysiol.*, **74:** 1301-1323, 1995.
23. LLINÁS, R., GRACE, A.A. AND YAROM, Y. In vitro neurons in mammalian cortical layer 4 exhibit intrinsic oscillatory activity in the 10- to 50-Hz frequency range. *Proc. Natl. Acad. Sci. USA*, **88:** 897-901, 1991.
24. LÜBKE, J.I., GREENE, R.W., SEMBA, K., KAMONDI, A., MCCARLEY, R.W. AND REINER, P.B. Serotonin hyperpolarizes cholinergic low-threshold burst neurons in the rat laterodorsal tegmental nucleus *in vitro*. *Proc. Natl. Acad. Sci. USA*, **89:** 743-747, 1992.
25. LYDIC, R., MCCARLEY, R.W. AND HOBSON, J.A. Serotonin neurons and sleep. II. Time course of dorsal raphe discharge frequency, PGO waves, and behavioral states. *Arch. Ital. Biol.*, **126:** 1-28, 1987.
26. MCCORMICK, D.A. AND WANG, Z. Serotonin and noradrenaline excite GABAergic neurones of the guinea-pig and cat nucleus reticularis thalami. *J. Physiol. Lond.*, **442:** 235-255, 1991.
27. MCGINTY, D.J. AND HARPER, R.M. Dorsal raphe neurons: depression of firing during sleep in cats. *Brain Res.*, **101:** 569-575, 1976.
28. MÖLLE, M., MARSHALL, L., GAIS, S. AND BORN, J. Grouping of spindle activity during slow oscillations in human non-REM sleep. *J. Neurosci.*, **22:** 10941-10947, 2002.
29. MONCKTON, J.E. AND MCCORMICK, D.A. Neuromodulatory role of serotonin in the ferret thalamus. *J. Neurophysiol.*, **87:** 2124-2136, 2002.
30. MONCKTON, J.E. AND MCCORMICK, D.A. Comparative physiological and serotoninergic properties of pulvinar neurons in the monkey, cat and ferret. *Thal. Rel. Syst.*, **2:** 239-252, 2003.
31. NIELSEN, T. Cognition in REM and NREM sleep. *Brain Behav. Sci.*, **23:** 851- 866, 2000.
32. NUÑEZ, A., AMZICA, F. AND STERIADE, M. Voltage-dependent fast (20-40 Hz) oscillations in long-axoned neocortical neurons. *Neuroscience*, **51:** 7-10, 1992.

54. STICKGOLD, R., JAMES, L. AND HOBSON, J.A. Visual discrimination learning requires sleep after training. *Nat. Neurosci.*, **3:** 1237-1238, 2000.
55. STICKGOLD, R., WHITBEE, D., SCHIRMER, B., PATEL, V. AND HOBSON, J.A. Visual discrimination improvement. A multi-step process occurring during sleep. *J. Cogn. Neurosci.*, **12:** 246-254, 2000.
56. TIMOFEEV, I., GRENIER, F., BAZHENOV, M., HOUWELING, A., SEJNOWSKI, T.J. AND STERIADE, M. Short- and medium-term plasticity associated with augmenting responses in cortical slabs and spindles in intact cortex of cats *in vivo*. *J. Physiol., Lond.*, **542:** 583-598, 2002.
57. TRULSON, M.E. AND JACOBS, B.L. Raphe unit activity in freely moving cats: correlation with level of behavioral arousal. *Brain Res.*, **163:** 135-150, 1979.
58. VON KROSIGK, M., BAL, T. AND MCCORMICK, D.A. Cellular mechanisms of a synchronized oscillation in the thalamus. *Science*, **261:** 361-364, 1993.

33. PAVLOV, I.P. "Innere Hemmung" der bedingten Reflexe und der Schlaf - ein und derselbe Prozess. *Skand. Arch. Physiol.*, **44:** 42-58, 1923.
34. SAKAI, K. AND CROCHET, S. Differentiation of presumed serotonergic dorsal raphe neurons in relation to behavior and wake-sleep states. *Neuroscience*, **104:** 1141-1155, 2001.
35. SIMON, N.R., MANDSHANDEN, I. AND LOPES DA SILVA, F.H. A MEG study of sleep. *Brain Res.*, **860:** 64-76, 2000.
36. STERIADE, M. Development of evoked responses into self-sustained activity within amygdalo-hippocampal circuits. *Electroencephalogr. Clin. Neurophysiol.*, **16:** 221-236, 1964.
37. STERIADE, M. Alertness, quiet sleep, dreaming. Pp. 279-357. In: PETERS, A. AND JONES, E.G. (Eds.) *Cerebral Cortex*, (Vol. 9, *Normal and Altered States of Function*), New York, Plenum, 1991.
38. STERIADE, M. Corticothalamic resonance, states of vigilance, and mentation. *Neuroscience*, **101:** 243-276, 2000.
39. STERIADE, M. *The Intact and Sliced Brain*. Cambridge (MA), The MIT Press, 2001.
40. STERIADE, M. Impact of network activities on neuronal properties in corticothalamic systems. *J. Neurophysiol.*, **86:** 1-39, 2001.
41. STERIADE, M. *The Neuronal Substrates of Sleep and Epilepsy*. Cambridge (UK), Cambridge University Press, 2003.
42. STERIADE, M., AMZICA, F. AND CONTRERAS, D. Synchronization of fast (30-40 Hz) spontaneous cortical rhythms during brain activation. *J. Neurosci.*, **16:** 392-417, 1996.
43. STERIADE, M., CONTRERAS, D., CURRÓ DOSSI, R. AND NUÑEZ, A. The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. *J. Neurosci.*, **13:** 3284-3299, 1993.
44. STERIADE, M., CURRÓ DOSSI, R., PARÉ, D. AND OAKSON, G. Fast oscillations (20-40 Hz) in thalamocortical systems and their potentiation by mesopontine cholinergic nuclei in the cat. *Proc. Natl. Acad. Sci. USA*, **88:** 4396-4400, 1991.
45. STERIADE, M., DESCHÊNES, M., WYZINSKI, P. AND HALLÉ, J.P. Input-output organization of the motor cortex during sleep and waking. Pp. 144-200. In: PETRE-QUADENS, O. AND SCHLAG, J. (Eds.) *Basic Sleep Mechanisms*. New York: Academic, 1974.
46. STERIADE, M., DOMICH, L., OAKSON, G. AND DESCHÊNES, M. The deafferented reticularis thalami nucleus generates spindle rhythmicity. *J. Neurophysiol.*, **57:** 260-273, 1987.
47. STERIADE, M., NUÑEZ, A. AND AMZICA, F. A novel slow (< 1 Hz) oscillation of neocortical neurons *in vivo*: depolarizing and hyperpolarizing components. *J. Neurosci.*, **13:** 3252-3265, 1993.
48. STERIADE, M., NUÑEZ, A. AND AMZICA, F. Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms. *J. Neurosci.*, **13:** 3266-3283, 1993.
49. STERIADE, M., OAKSON, G. AND DIALLO, A. Cortically elicited spike-wave afterdischarges in thalamic neurons. *Electroencephalogr. Clin. Neurophysiol.*, **41:** 641-644, 1976.
50. STERIADE, M. AND TIMOFEEV, I. Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. *Neuron.*, **37:** 563-576, 2003.
51. STERIADE, M., TIMOFEEV, I. AND GRENIER, F. Natural waking and sleep states: a view from inside neocortical neurons. *J. Neurophysiol.*, **85:** 1969-1985, 2001.
52. STERIADE, M., TIMOFEEV, I., GRENIER, F. AND DÜRMÜLLER, N. Role of thalamic and cortical neurons in augmenting responses: dual intracellular recordings *in vivo*. *J. Neurosci.*, **18:** 6425-6443, 1998.
53. STEVENS, D.R., GREENE, R.W. AND MCCARLEY, R.W. Serotonin 1 and serotonin2 receptors hyperpolarize and depolarize separate populations of medial pontine reticular formation neurons in vitro. *Neuroscience*, **47:** 545-553, 1992.

SUMMARY

This article starts by enumerating several data that vindicate Michel Jouvet's hypothesis regarding serotonin as a factor promoting slow-wave sleep. The core of the article is devoted to the description of neuronal bases underlying sleep oscillations, with emphasis on the cortically generated slow oscillation (0.5-1 Hz) that groups both low-frequency (spindles and delta) and high-frequency (beta/gamma) rhythms. The low-frequency rhythms are generated by the synchronous firing of cortical neurons during the depolarizing phase of the slow oscillation, which impacts on the thalamic circuitry to generate spindles and clock-like delta potentials. The fast activity is voltage-dependent and occurs during the depolarization component of the slow cortical oscillation. This coalescence of brain rhythms, discovered in intracellular studies of neocortical and thalamic neurons, is now supported by EEG studies during human sleep. The rich spontaneous activity of neocortical neurons during slow-wave sleep is associated with neuronal plasticity that may play a role in consolidating memory traces acquired during the state of waking. Surprisingly, neuronal plasticity, usually regarded as a beneficial phenomenon implicated in memory and learning, could develop during slow-wave sleep into self-sustained paroxysmal discharges, similar to spike-wave complexes that appear in some epileptic syndromes.

Acknowledgements. - Personal studies were supported by grants from the Canadian Institutes for Health Research (MT-3689 and MOP-36545), Human Frontier Research Program (RG-0131), U.S. National Institute of Health (NS-40522) and Natural Sciences and Engineering Council of Canada (170538).

REFERENCES

1. ACHERMANN, P. AND BORBÉLY, A. Low-frequency (< 1 Hz) oscillations in the human sleep EEG. *Neuroscience,* **81:** 213-222, 1997.
2. AMZICA, F. AND STERIADE, M. Short- and long-range neuronal synchronization of the slow (< 1 Hz) cortical oscillation. *J. Neurophysiol.,* **75:** 20-38, 1995.
3. AMZICA, F. AND STERIADE, M. Disconnection of intracortical synaptic linkages disrupts synchronization of a slow oscillation. *J. Neurosci.,* **15:** 4658-4677, 1995.
4. AMZICA, F. AND STERIADE, M. The K-complex: its slow (< 1 Hz) rhythmicity and relation to delta waves. *Neurology,* **49:** 952-959, 1997.
5. CONTRERAS, D., DESTEXHE, A., SEJNOWSKI, T.J. AND STERIADE, M. Control of spatiotemporal coherence of a thalamic oscillation by corticothalamic feedback. *Science,* **274:** 771-774, 1996.
6. CONTRERAS, D., DESTEXHE, A., SEJNOWSKI, T.J. AND STERIADE, M. Spatiotemporal patterns of spindle oscillations in cortex and thalamus. *J. Neurosci.,* **17:** 1179-1196, 1997.
7. DESTEXHE, A., CONTRERAS, D., SEJNOWSKI, T.J. AND STERIADE, M. Modeling the control of reticular thalamic oscillations by neuromodulators. *Neuro Report,* **5:** 2217-2220, 1994.
8. ECCLES, J.C. Chairman's opening remarks. Pp. 1-3. In: WOLSTENHOLME, G.E.W. AND O'CONNOR, M. (Eds) *The Nature of Sleep.* London, Churchill, 1961.
9. GAIS, S., PLIHAL, W., WAGNER, U. AND BORN, J. Early sleep triggers memory for early visual discrimination skills. *Nat. Neurosci.,* **3:** 1335-1339, 2000.

Hz) and gamma (~30-60 Hz) rhythms. I consider these two rhythms together because a neuron can pass from beta to gamma frequency in a period as short as 0.5-1 second, due to a slight depolarization (42). The presence of fast activity during slow-wave sleep was surprising to those who consider these oscillations as reflecting high cognitive processes and conscious events during waking and REM sleep. However, the fact that beta/gamma activity is voltage (depolarization) dependent (23, 32, 44) explains the presence of fast activity during the depolarizing phase of the slow sleep oscillation. The grouping of not only spindles, but also relatively fast (beta) activity, by the cortical slow oscillation was also reported during natural slow-wave sleep in humans (28).

To sum up, the slow oscillation has the virtue of coalescing low-frequency as well as fast rhythms due to the properties of neocortical neurons and their intra-cortical and corticothalamic connections.

NEURONAL PLASTICITY AND SEIZURES DURING SLOW-WAVE SLEEP

With the rich (higher than 10 Hz) spontaneous firing of neocortical neurons during slow-wave sleep (51), there is no surprise that these neurons have better to do than to be in a "quiet", "resting" state, as some terms usually applied to slow-wave sleep would suggest. Actually, monkey's motor cortical responses to callosal volleys are not diminished and may even be enhanced during slow-wave sleep, compared to waking (45). This finding indicates that, during slow-wave sleep, at least some cortical neurons maintain an internal dialogue that may explain various forms of mental activities during this state in which there is a complete absence of information from external signals because of synaptic inhibition at the thalamic level (40).

Human studies have also shown that, far from being a period of complete inactivity, slow-wave sleep is implicated in mental processes since dreaming mentation is not confined to paradoxical sleep but also appears, closer to real life events, during slow-wave sleep (12). The recall rate of dreaming mentation in slow-wave sleep is high (31). Also, the idea that slow-wave sleep is implicated in the consolidation of memory traces acquired during the waking state (43) is supported by human studies demonstrating that the overnight improvement of discrimination tasks requires several steps, some of them depending on the early slow-wave stages (54, 55). The enhancement of visual discrimination skills by early stages of sleep led to the conclusion that procedural memory formation may be associated with slow-wave oscillations (9) and, after training on a declarative learning task, the density of human sleep spindles is significantly higher, compared to the non-learning control task (10).

That neuronal plasticity results from spontaneously occurring slow-wave sleep oscillations has been demonstrated using intracellular recordings from thalamic and cortical neurons. Here, I shall focus on cortical neurons. During stages 2-3 of sleep, cortical neurons are targets of thalamic inputs during spindles, but not passive receivers of these rhythmic volleys. Cortical neurons play an active role in amplify-