

# Sleep and Serotonin: An Unfinished Story

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*Serotonin (5-HT) was first believed to be a true neuromodulator of sleep because the destruction of 5-HT neurons of the raphe system or the inhibition of 5-HT synthesis with p-chlorophenylalanine induced a severe insomnia which could be reversed by restoring 5-HT synthesis. However the demonstration that the electrical activity of 5-HT perikarya and the release of 5-HT are increased during waking and decreased during sleep was in direct contradiction to this hypothesis. More recent*

*experiments suggest that the release of 5-HT during waking may initiate a cascade of genomic events in some hypnogenic neurons located in the preoptic area. Thus, when 5-HT is released during waking, it leads to an homeostatic regulation of slow-wave sleep.*

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One may consider four epochs when looking back to the relationships between serotonin (5-hydroxytryptamine, 5-HT) and the sleep/waking cycle. Four epochs during 45 years may seem too many or possibly too few if one considers the quasi-exponential growth of the neurosciences. The relationships between 5-HT and sleep can be summarized like a “popular love story”. First, the encounter of a mysterious monoamine without a face, then the honeymoon, followed by a divorce and later by a reconciliation.

## THE ENCOUNTER BETWEEN SLEEP AND A MYSTERIOUS MONOAMINE (1955–1963)

In their seminal paper, Brodie et al. (1955) reported that reserpine could decrease cerebral 5-HT and induce se-

dition or a “sleep like state.” This was the first time that sleep and 5-HT met in the same paper. Even if the topography of 5-HT containing neurons was totally unknown at this time, further indirect evidence led the “hypnologists” to become interested in monoamines. The parenteral injection of L-5-hydroxytryptamine (L-5-HTP) was followed by cortical synchronization whereas injection of L-DOPA led to cortical desynchronization.

Then, by chance, it was discovered that administration of reserpine could trigger the continuous appearance of Ponto Geniculo Occipital (PGO) activity in the cat, even during waking (Delorme et al. 1965). For the first time, PGO activity which was believed to be “specific” to paradoxical sleep (PS) or REM sleep could be dissociated from this state. Last but not least, it was also discovered, during the same year, that administration of inhibitors of monoamine oxidase could selectively suppress PS in the cat for a long time (days or weeks) (Jouvet et al. 1965).

Thus, monoamines appear to be like some “Ariadne’s thread” which could lead the physiologist to the understanding of some biochemical mechanisms of the sleep/waking cycle. It should be recalled that, at this time, only acetylcholine was considered a genuine central neurotransmitter whose topography was indirectly known thanks to the histochemistry of cholinesterase.

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### THE HONEYMOON (1964–1973)

The publication by Dahlström and Fuxe (1964) opened the door. 5-HT containing perikarya were described in the raphe nuclei. Because the raphe system is relatively homogenous, it was not too difficult for neurophysiologists to destroy it by coagulating the midline of the ponto mesencephalon in the cat. This operation was followed by a dramatic and long-lasting insomnia (10–15 days). Then, it became possible to search for correlations between the intensity of insomnia for 10 days, the volume of the raphe lesion, and the amount of cerebral 5-HT in the telencephalon, which remained after degeneration of 5-HT terminals.

There were significant correlations between destruction of the rostral raphe (N. raphe dorsalis) and the decrease of both slow-wave sleep and telencephalic 5-HT, whereas the amount of PS was mostly correlated with the lesion of n. raphe pontis and magnus (Jouvet 1969). Of course these experiments could be easily criticized. The coagulation of the raphe could have destroyed not only 5-HT-containing neurons but also other perikarya whose neurotransmitters were and are still unknown. Moreover, those lesions did destroy ascending or descending axons crossing in the midline, and/or some small arteries perfusing more laterally situated structures (Mancia 1969).

Whatever these drawbacks might have been, it was nevertheless the first time that such dramatic and long-lasting insomnia was obtained which could be correlated with a specific biochemical cerebral alteration such as a decrease of cerebral 5-HT. This approach certainly helped the transition between the so-called “dry” neurophysiology (electrophysiology) to “wet” neurobiology (neurochemistry–neuropharmacology). Neuropharmacology, indeed, also contributed positively to the so-called “serotonergic hypothesis of sleep.” It was demonstrated by Koe and Weissman (1966) that p-chlorophenylalanine (PCPA) could inhibit tryptophan hydroxylase and impair 5-HT biosynthesis. It was subsequently shown that parenteral administration of PCPA in the cat was followed by a secondary total insomnia. This was not really the proof of a causal relationship between 5-HT and sleep.

However, parenteral (or intraventricular) injection of L-5-HTP could restore both SWS and PS during the insomnia produced by PCPA. These experiments were not unlike the “synthesis after fractionation” paradigm described by Claude Bernard, and led to a more causal approach between 5-HT and sleep. In fact, it was proven that  $[H^3]$ -5-HTP could be readily decarboxylated into  $[H^3]$ -5-HT after PCPA, whereas the cerebral levels of other amines (noradrenaline or dopamine) were not significantly altered. Serotonin (or somnotonin as it was called by Koella 1969) was thus believed to be the sleep neurotransmitter or “neurohormone” which

could produce sleep by inhibiting either the mesencephalic reticular formation or the locus coeruleus (at the time, the two putative waking centers). This theory (the monoaminergic theory of sleep and waking, Jouvet 1972) was a totalitarian one—5-HT for sleep, catecholamines for waking, one amine-one function! As for every totalitarianism, this dogma did not live very long!

### THE DIVORCE (1975–1985)

According to the serotonergic theory of sleep, 5-HT was a sleep neuromodulator. Thus, the electrical activity of 5-HT neurons of the raphe should increase at sleep onset at the same time that the liberation of 5-HT should increase somewhere in the thalamus or the cortex. Those two predictions were not confirmed. On the one hand, it was demonstrated that the unitary electrical activity of n. raphe dorsalis increased during waking, then decreased during slow-wave sleep to become totally silent during PS (McGinty and Harper 1976). On the other hand, voltammetry demonstrated that the 5-hydroxyindolacetic acid (5-HIAA) electrical signal (an indirect measure of 5-HT release) also increased during waking at the cortical or thalamic level and decreased subsequently during slow-wave sleep (Cespuglio et al. 1984).

For these reasons, any relationship between 5-HT liberation and sleep was totally impossible. Moreover, during this period, the physiologists did not discover the postsynaptic target responsible for the hypnogenic effect of local injection of L-5-HTP during the PCPA induced insomnia in the cat.

Whereas intraventricular injection of L-5-HTP could restore sleep, microinjections of L-5-HTP in any structures of the brain stem (area postrema, medulla, pons, raphe system, mesencephalic reticular formation) did not restore sleep.

Then, without any cerebral hypnogenic target to explain the restoration of sleep following 5-HT intracerebral injection, the PCPA-5-HTP paradigm was dismissed and the 5-HT theory of sleep went to the graveyard of the forgotten theories of sleep. It was replaced by the more sophisticated (but still erroneous) theory of hypnogenic peptides (Borbely and Tobler 1989) heralded by the so-called “delta sleep inducing peptide.”

### THE RECONCILIATION (1985–PRESENT)

Two different paths open a possible reconciliation between cerebral 5-HT and sleep. First, as shown in the preceding section, the relationship between 5-HT liberation and sleep onset was totally disproven. However, a possible “diachronistic” relation is still possible. In

deed, according to the homeostatic regulation of sleep (Borbely et al. 1989) there is a correlation between the length of previous waking and the length or the intensity of subsequent sleep. It has been hypothesized that a process "S" increases during waking and exponentially decreases during sleep. This factor is responsible for the intensity of slow-wave sleep (which is indirectly estimated by the delta power spectrum of the sleep EEG). If some systems of 5-HT neurons belong to this process "S," then they should be active during waking and inactive during sleep. Moreover, since sleep deprivation by the so-called swimming-pool technique is followed by increased delta-wave sleep, then the impairment of 5-HT biosynthesis, during the sleep deprivation process, should suppress the subsequent rebound of slow-wave sleep. This hypothesis has been fully confirmed. PCPA administration during sleep deprivation totally suppresses slow-wave sleep during the rebound whereas PS still occurs (Sallanon et al. 1983). Thus it may be postulated that some 5-HT neurons (n. raphe dorsalis) which fire regularly in a clock-like fashion during waking may be participating in the process "S" by measuring both the duration and the intensity of waking. The liberation of 5-HT during waking in a strategic location of the anterior hypothalamus might herald a cascade of postsynaptic genomic events which will trigger sleep onset.

Second, is the postsynaptic target of 5-HT. It has become evident that total insomnia follows only after lesions located either in the raphe system or in the preoptic area (McGinty and Serman 1968) and it is likely that, in some way, 5-HT and the preoptic area participate in sleep mechanisms. In fact, microinjections of very small doses of L-5-HTP (0.2–0.5 µg) in the preoptic area may restore long periods of physiological sleep in a cat made totally insomniac by injection of PCPA (Denoyer et al. 1989). The delay between the injection of 5-HTP and sleep onset is around 40 minutes. This relatively long delay suggests that 5-HT has triggered a cascade of events in the preoptic area and possibly the neighboring suprachiasmatic nucleus. The use of immunohistochemistry of 5-HT has served to localize the smallest hypnogenic area in which microinjections of 5-HTP could restore sleep.

A lesion of the perikarya of this region made by *in situ* injections of ibotenic acid in a normal cat is followed by a total suppression of deep slow-wave sleep and PS for several weeks. This finding is in accordance with the hypothesis that the postsynaptic target of 5-HT is a true hypnogenic area (Sallanon et al. 1989).

How the preoptic area may be responsible for sleep onset is not yet fully understood. However, in a cat which is totally insomniac after cellular lesion of the lateral preoptic area, it is possible to restore sleep for several hours by injecting small doses of muscimol (a GABA agonist) at the level of the posterior hypothalamus, an area rich in histaminergic containing neurons

which play a determining role in waking. A likely hypothesis is that a GABAergic system originating in the lateral preoptic area and descending upon the posterior hypothalamus might inhibit the waking neurons which are located in this area (Kitahama et al. 1989).

The following problems should be solved before clear pictures of the role of 5-HT in sleep mechanisms can be drawn. First, on what kinds of receptors does 5-HT or 5-HTP act in the preoptic area? Which 5-HT agonist (1A, 1B, 1C, 1D, 2, 3, or 4) may restore sleep when injected into the preoptic area after PCPA? It should be recalled that 5-HT microinjections induced cortical synchronization when injected in the nucleus basalis (Cape and Jones 1998).

Second, what is the significance of a latency of about 40 minutes between injection of 5-HTP and sleep onset? Does this correspond to a cascade of genomic events? 5-HT has been shown to control VIP synthesis in the suprachiasmatic nucleus (Kawakami et al. 1985). VIP containing neurons are also found in the lateral preoptic area, and VIP is also a hypnogenic peptide (Riou et al. 1982).

We have summarized most of the evidence for and against the role of 5-HT-dependent hypnogenic mechanisms in the preoptic area. Most of the negative data against a role of 5-HT in sleep have received some explanations. The link between indoleamine liberation in the preoptic area, and a possible cascade of events including possibly VIP and GABAergic mechanisms need more verification.

The story of the relation between 5-HT and sleep is not yet finished!

## REFERENCES

- Borbely AA, Tobler I (1989): Endogenous sleep-promoting substances and sleep regulation. *Physiol Rev* 69:605–670
- Borbely AA, Achermann P, Trachsel L, Tobler I (1989): Sleep initiation and initial sleep intensity: Interactions of homeostatic and circadian mechanism. *J Biol Rhythms* 4:149–160
- Brodie BB, Pletsher A, Shore A (1955): Evidence that serotonin has a role in brain function. *Science* 122:968
- Cape EG, Jones BE (1998): Differential modulation of high-frequency  $\gamma$  electroencephalogram activity and sleep-wake state by noradrenaline and serotonin microinjections into the region of cholinergic basalis neurons. *J Neurosci* 18:2653–2666
- Cespuglio R, Faradji H, Guidon G, Jouviet M (1984): Voltammetric detection of brain 5-hydroxyindolamines: A new technology applied to sleep research. *Exp Brain Res* 8:95–105
- Dahlström A, Fuxe K (1964): Evidence for the existence of monoamine neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand* 62:(Suppl.)232

- Delorme F, Jeannerod M, Jouvet M (1965): Effects remarquables de la réserpine sur l'activité EEG phasique pontogéniculo-occipitale. *CR Soc Biol (Paris)* 159:900–903
- Denoyer M, Sallanon M, Kitahama K, Aubert C, Jouvet M (1989): Reversibility of parachlorophenylalanine induced insomnia by intrahypothalamic microinjection of L-5-hydroxytryptophan. *Neuroscience* 28:83–94
- Jouvet M, Vimont P, Delorme F (1965): Suppression élective du sommeil paradoxal chez le chat par les inhibiteurs de la mono-amino-oxydase. *C R Soc Biol* 159:1595–1599
- Jouvet M (1969): Biogenic amines and the states of sleep. *Science* 163:32–41
- Jouvet M (1972): The role of monoamines and acetylcholine containing neurons in the regulation of the sleep waking cycle. *Ergebnisse der Physiologie* 64:166–307
- Kawakami F, Okamura H, Fukui K, Yanaihara C, Yanaihara N, Nakajima T, Ibata Y (1985): The influence of serotonergic inputs on peptide neurons in the rat suprachiasmatic nucleus: An immunocytochemical study. *Neurosci Lett* 61:273–277
- Kitahama K, Sallanon M, Okamura H, Geffard M, Jouvet M (1989): Cellules présentant une immunoréactivité au GABA dans l'hypothalamus du chat. *C R Acad Sci (Paris)* 308:507–511
- Koe BK, Weissman A (1966): P-chlorophenylalanine, a specific depletor of brain serotonin. *J Pharmacol Exp Ther* 154:499–516
- Koella WP (1969): Serotonin and sleep. *Exp Med Surg* 27:157–169
- McGinty DJ, Sterman MB (1968): Sleep suppression after basal forebrain lesion in the cat. *Science* 160:1253–1255
- McGinty DJ, Harper RM (1976): Dorsal raphe neurons: Depression of firing during sleep in cats. *Brain Res* 101:569–575
- Mancia M (1969): EEG and behavioural changes owing to splitting of the brain stem in cats. *Electroencephalogr Clin Neurophysiol* 27:487–503
- Riou R, Cespuglio R, Jouvet M (1982): Endogenous peptides and sleep in the rat. III. The hypnogenic properties of vasoactive intestinal peptide. *Neuropeptides* 2:265–277
- Sallanon M, Buda C, Janin M, Jouvet M (1983): Serotonergic mechanisms and sleep rebound. *Brain Res* 268:95–104
- Sallanon M, Denoyer M, Kitahama K, Aubert C, Gay N, Jouvet M (1989): Long lasting insomnia induced by pre-optic neuron lesions and its transient reversal by muscimol injection into the posterior hypothalamus in the cat. *Neuroscience* 32:669–683