

PROFESSOR JEAN-BERNARD FORTILLAN

**Public announce of the discovery  
of the Sleep-Wake system  
at french Academy of Pharmacy  
(Wednesday December 2, 2015)**

# The pineal gland and the Sleep-Wake system

Therapeutic applications



PHOTO DE MARIANNE FOURTILLAN

Chemical engineer and pharmacist by training, Jean-Bernard Fourtillan taught medicinal chemistry as a professor at the University of Poitiers School of Medicine and Pharmacy from 1972 to 2008. In addition, he was hospital pharmacist, and created, in 1981, with his wife Marianne, pharmacist, the company CEMAF. A Biomedical Research Center equipped with a clinic and a bioanalysis laboratory, CEMAF was a global leader in the assaying of drugs, in biological fluids, by coupling gas or liquid chromatography with mass spectrometry. CEMAF carried out pharmacokinetic studies of drugs on behalf of pharmaceutical laboratories. It is in this Biomedical Research Center that Jean-Bernard Fourtillan discovered the Sleep-Wake system.

## Public announce of the discovery of the Sleep-Wake system at french Academy of Pharmacy (Wednesday December 2, 2015)

### **The pineal gland and the Sleep-Wake system Therapeutic applications.**

Jean-Bernard Fourtillan, *Pharmacist, Honorary Professor of Medicinal Chemistry*

In higher vertebrates, the perception of ambient light is processed by the suprachiasmatic nuclei of the hypothalamus, which orchestrates circadian regulation of the body by adapting it to the organism's lifestyle and its environment. Within the photoneuroendocrine system, the pineal gland plays a transduction role, converting the rhythm imposed by the suprachiasmatic nuclei into an endocrine hormone message, via secretion of three hormones constituting the sleep-wake system:

- melatonin (MLT), discovered by A.B. Lerner, in 1958;
- Valentonin (VLT), N-acetylated  $\beta$ -carboline, the sleep hormone discovered by J.B. Fourtillan (1994); and
- 6-methoxy-harmalan (6-MH), the waking hormone discovered by W.M. Mc Isaac, in 1961.

They are secreted together by the pineal gland between 10 p.m. and 6 a.m., throughout the year, and correspond to the three acetylations of serotonin.

The Sleep-Wake system ensures the regulation of the body's psychic and autonomic states during the 24 hours of the nycthemeron, in the following way:

- **between 10 p.m. and 6 a.m.**, the pineal gland secretes the three hormones. MLT, a neuroprotective hormone, "traps" free radicals. For pharmacokinetic reasons, the concentrations of VLT in the blood and the brain are higher than those of 6-MH. This

prevalence enables VLT to produce sleep by specific allosteric modulation of 5-HT<sub>2C</sub> serotonergic receptors, during this eight-hour period. Simultaneously, and in the same way, VLT lowers blood pressure and heart rate by activating central noradrenergic  $\alpha_2$  receptors and induces muscle relaxation by activating dopaminergic D<sub>1</sub> and D<sub>2</sub> receptors of the extrapyramidal system.

- **from 6 a.m.**, pineal secretions are seen to stop. 6-MH, which is eliminated much slower than VLT, becomes prevalent in the brain, causing—by specific blocking of the above-mentioned receptors—waking, higher blood pressure and heart rate, and muscle contraction, **until 10 p.m.**

Structure-activity correlations of the three hormones made it possible to specify their modes of action and to synthesize 569 VLT substitutes; all produce restorative physiological sleep, with a prevalence of slow-wave sleep and REM sleep.

Dysfunctions of the Sleep-Wake system provide an explanation of the mechanisms of various neurologic disorders. A quantitative deficit of these hormones is responsible for sleep disorders, depression, and neurodegenerative disorders like Parkinson's and Alzheimer's, while excess secretion is the cause of psychotic states.

Several therapeutic applications follow from this discovery. For insufficient pineal secretion, for example, it is necessary to administer, at night, on the one hand, oral MLT and, on the other hand, transdermal VLT and 6-MH in the form of a patch to be removed the following morning upon rising.

1

Mister President,  
ladies and gentlemen,

today, it's a great honour for me to announce, at the board of National  
academy of Pharmacy, the discovery of the Sleep-Wake system.

→ National academy of Pharmacy - Paris - 2 December 2015



# The pineal gland and the Sleep-Wake system

## Therapeutic applications

Jean-Bernard Fourtillan, Pharmacist,  
Honorary Professor of Medicinal Chemistry

**SISTER JOSEFA MENÉNDEZ FUND**

# 2

Sleep-Wake system is constituted by three hormones :

- melatonin: discovered by Lerner in 1958 ;
- 6-methoxy-harmalan, identified, in the pineal gland, by Mc Isaac in 1961 ;
- and Valentonin that I discovered, in 1994, in the pineal gland.

I discovered Valentonin, the true sleep hormone, under unique conditions.

→ The 3 hormones of the Sleep-Wake system



A.B. Lerner discovers  
**melatonin**  
the first hormone identified  
in the pineal gland



W.M. Mc Isaac and al. discover  
6-methoxy-harmalan  
the second hormone identified  
in the pineal gland



J-B. Fourtillan discovers  
**Valentonin**  
the true sleep hormone,  
in the pineal gland

## Valentonin: Key to the Sleep-Wake system

# 3

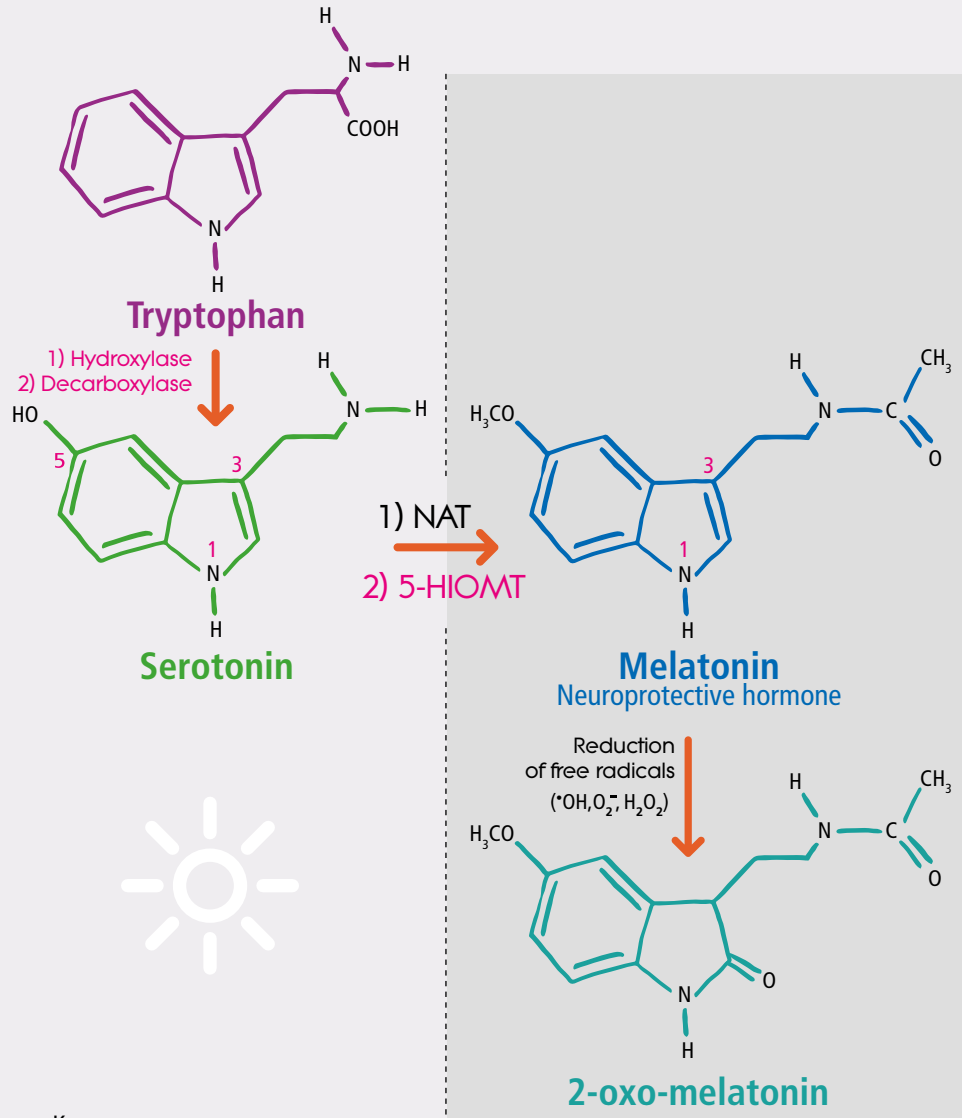
Contrary to what is commonly believed, melatonin is not the sleep hormone.

Melatonin is a neuroprotective hormone. It reduces free radicals which destroy neurons. Insofar MLT prevents neuronal degeneration.

*In vitro*, REITER demonstrated that melatonin is the most powerful known reducer of free radicals in biological fluids.

*In vivo*, I have confirmed this property by showing the presence of large amounts of 2-oxo-melatonin in the brain, during the night, which is produced by the reduction of free radicals by MLT (redox reaction).

→ Role of melatonin:  
neuroprotective hormone by « trapping » free radicals



Key :

**NAT** : enzyme N-acetyltransferase;

**5-HIOMT** : enzyme 5-hydroxyindole-O-methyltransferase

# 4

At the beginning of the 1990s, many researchers were interested in melatonin.

But nobody was able to measure correctly endogenous melatonin, when secreted in blood stream by pineal body, due to melatonin concentrations as low as some picograms per milliliter of plasma.

It should be noted that 1 picogram of melatonin per milliliter of plasma, represented as 1 pg/ml, corresponds to a concentration equal to 1 billionth of a milligram of melatonin per milliliter of plasma.

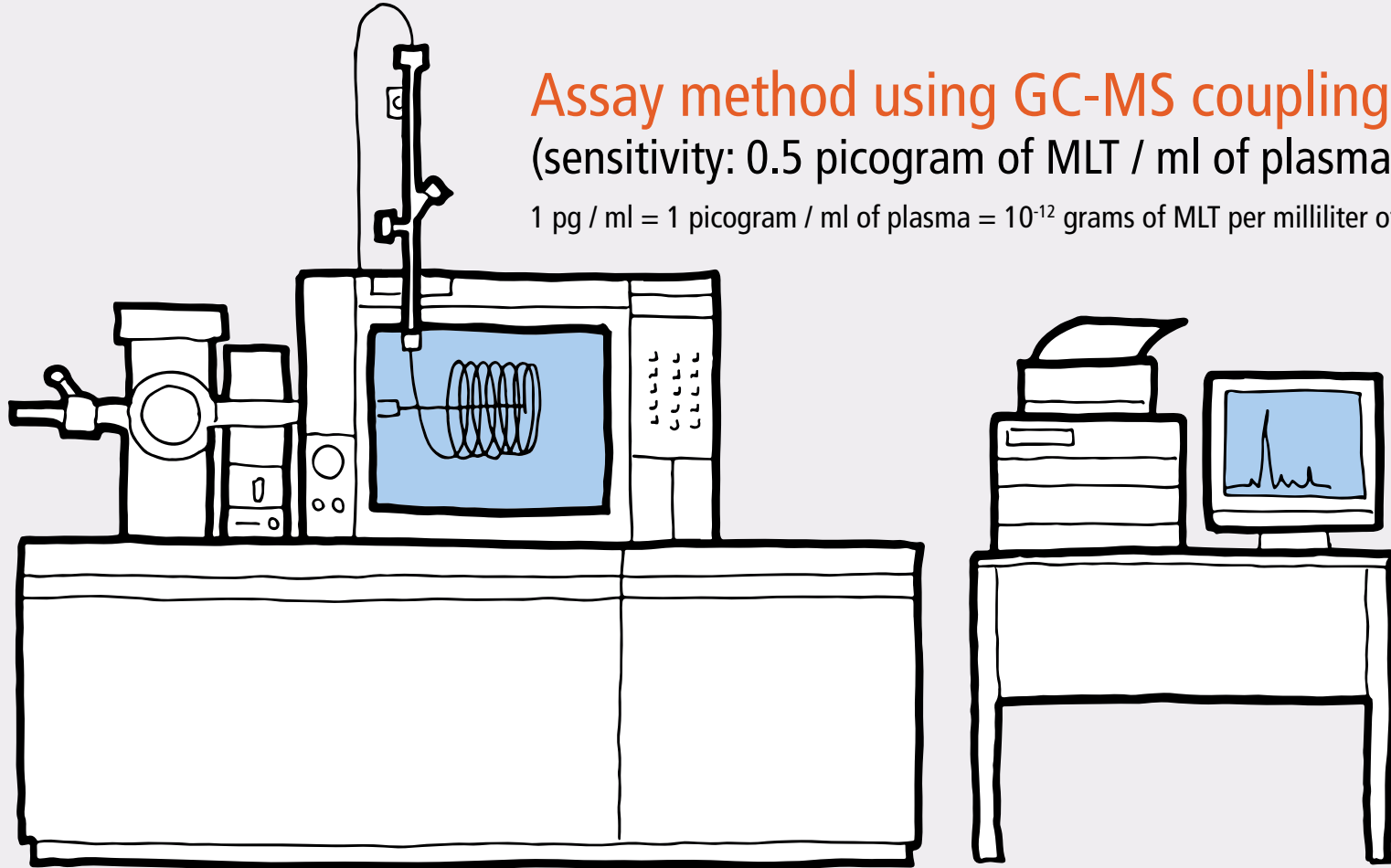
In 1994, we developed, at the CEMAF Biomedical Research Center, a specific and highly sensitive method for assaying melatonin. It brings into play a coupling of gas chromatography to mass spectrometry. Its high sensitivity enables us to assay, with accuracy and precision, plasma melatonin concentrations as low as 0.5 picogram of melatonin per ml of plasma.

→ Study of melatonin secretion by the pineal gland  
Assaying melatonin in the plasma



Assay method using GC-MS coupling  
(sensitivity: 0.5 picogram of MLT / ml of plasma)

1 pg / ml = 1 picogram / ml of plasma =  $10^{-12}$  grams of MLT per milliliter of plasma



*Reference: Fourtillan, J.B., Gobin, P., Faye, B. & Girault, J. A highly sensitive assay of melatonin at the femtogram level in human plasma by gas chromatography / negative ion chemical ionization mass spectrometry. *Biol. Mass Spectrom.*, 23, 499-509 (1994).*

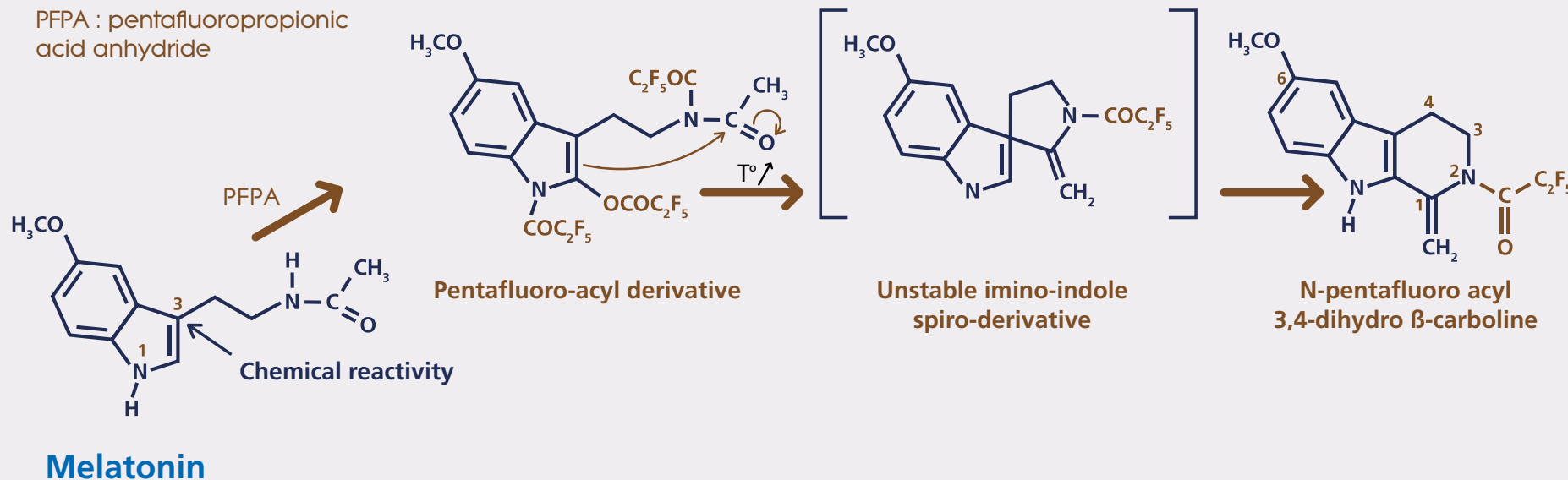
# 5

In this assay method, it is necessary to transform melatonin into a pentafluoro-propionyl derivative, in order to make it volatile, and allow gas chromatography to be carried out.

This tricyclic perfluoro acyl derivative is a  $\beta$ -carboline.

On april 1994, when I was thinking about the mechanism of derivatization's reaction, I understood in a few seconds how was synthesized the true sleep hormone in the pineal gland.

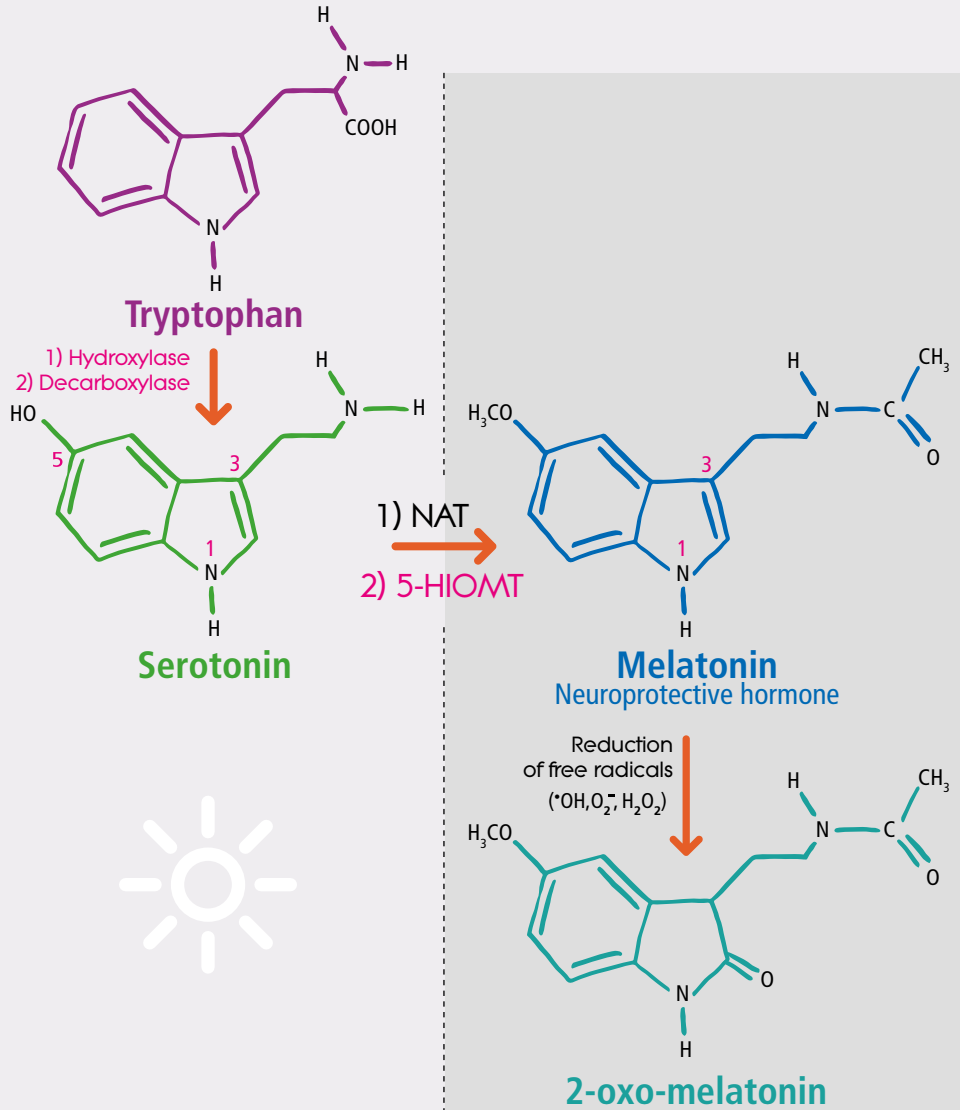
→ The discovery of Valentonin : Derivatization of melatonin for assaying by Gas Chromatography/Mass Spectrometry





I had in mind that melatonin is synthesized by an enzymatic acetylation of serotonin under the action of the N-acetyltransferase (NAT) enzyme, in the pineal gland.

→ Biosynthesis of melatonin from serotonin  
Enzymatic acetylation by N-acetyltransferase (NAT)



Key :

**NAT** : enzyme N-acetyltransferase;

**5-HIOMT** : enzyme 5-hydroxyindole-O-methyltransferase

# 7

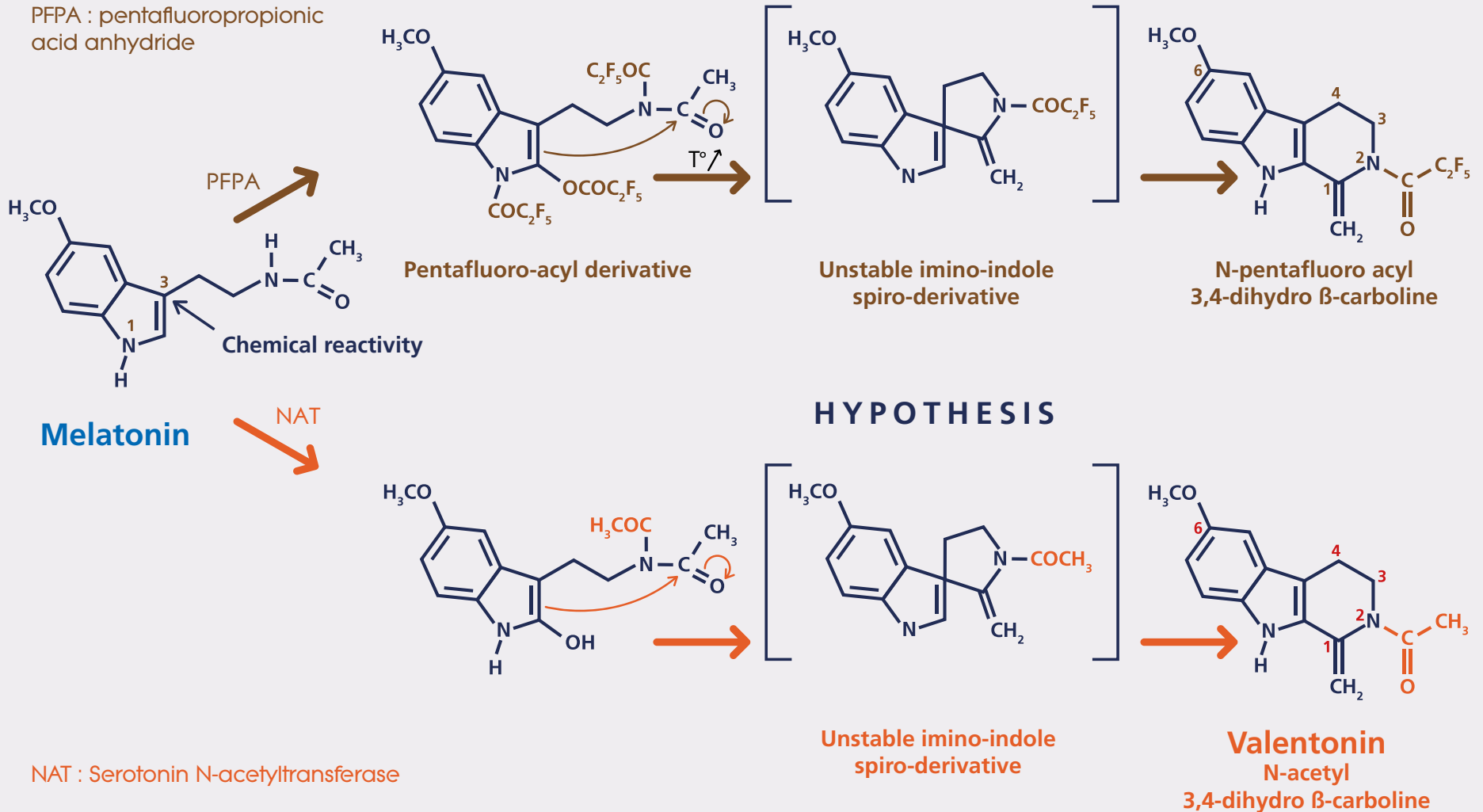
I made the hypothesis that, in the pineal gland, like serotonin, melatonin undergoes, as of its formation, a new acetylation under the action of the N-acetyltransferase (NAT) enzyme, which leads to a pentafluoro-acyl  $\beta$ -carboline on the nitrogen (N) atom at the 2 position. The chemical structure of this compound being identical to that of the derivative used to enable chromatography of melatonin by GC-MS coupling to be carried out, in which the perfluoro-propionyl group ( $-\text{CO}-\text{C}_2\text{F}_5$ ) is replaced with an acetyl group ( $-\text{CO}-\text{CH}_3$ ).

This hypothesis very quickly proved consistent with the reality.

I had before my eyes the chemical structure of the sleep hormone, which I named « Valentonin ».

A few days after this discovery, after having synthesized the Valentonin, I put into evidence its hypnotic properties.

# → Hypothesis of a melatonin enzymatic acetylation by serotonin N-acetyltransferase (NAT) enzyme

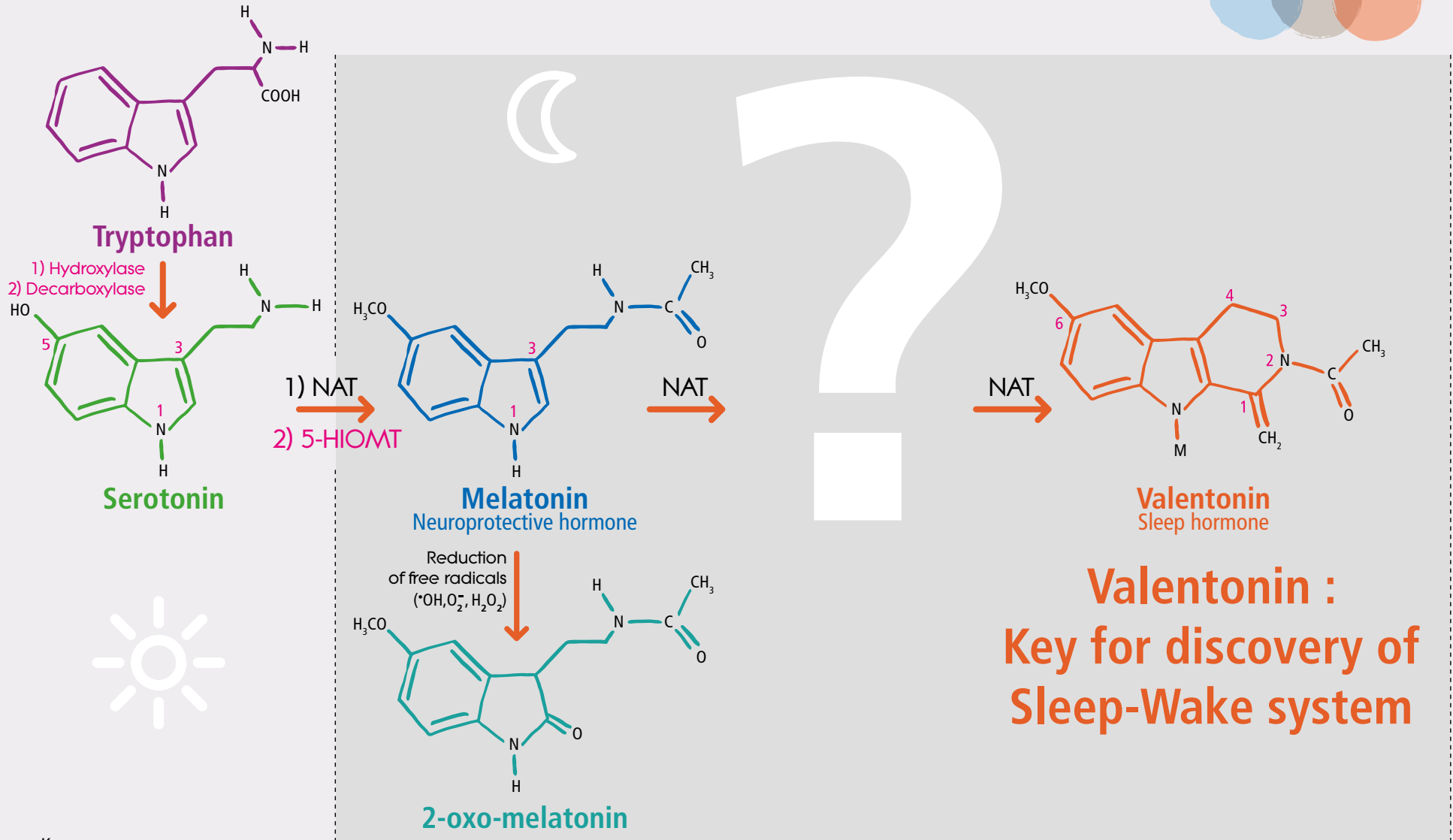




Finding Valentonin, the true sleep hormone, was the key for the discovery of the Sleep-Wake system.

Indeed, one had to imagine its biosynthesis for discovering it, since Valentonin, as secreted in the blood stream by the pineal gland, cannot be detected for pharmacokinetic reasons.

→ The discovery of Valentonin : the sleep hormone  
 The key for the discovery of the Sleep-Wake system.



Key :

**NAT** : enzyme N-acetyltransferase;

**5-HIOMT** : enzyme 5-hydroxyindole-O-methyltransferase

In dogs, Valerian produces physiological sleep, which is refreshing for the body, at the opposite of benzodiazepinic compounds, which give non refreshing « anesthetic » sleep.

By decreasing wakefulness, with loss of consciousness, Valerian leads to sleep.

Moreover Valerian :

- lowers blood pressure and heart rate ;
- and gives muscle relaxation.

These three pharmacological properties are features of physiological sleep. They are observed during polysomnographic studies.

Therefore, during the nocturnal rest period, Valerian regulate both psychic and autonomic lives.

One can name it as nighttime hormone.





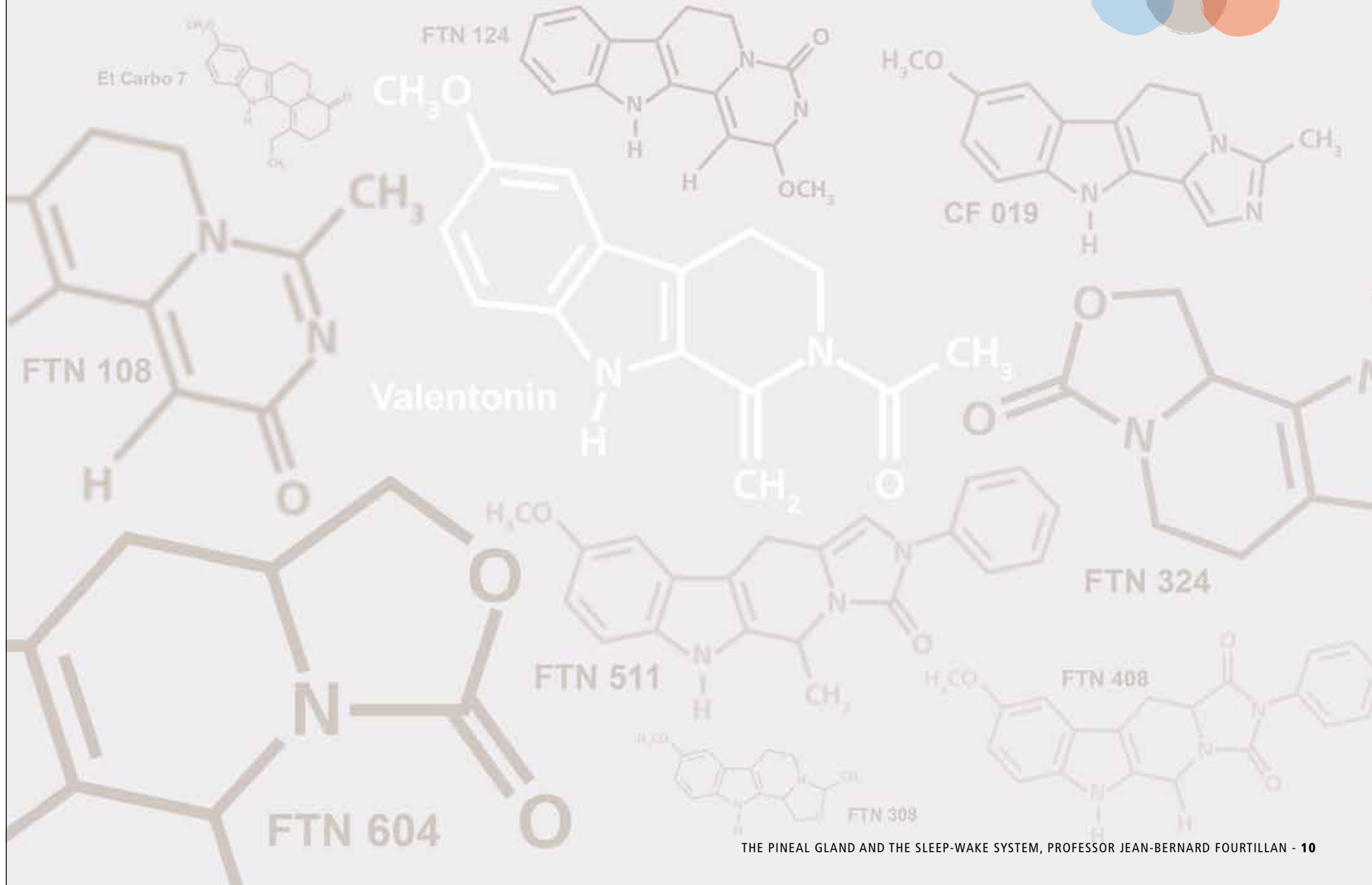
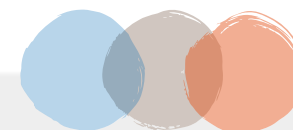
# 10

Valentonin cannot be administered orally because it is broken down completely in gastrointestinal fluids.

Having perfectly determined the chemical pharmacophore characteristics in correlation with the pharmacological activity of Valentonin, we synthesized, between 1994 and 2007, 569 orally-active synthetic substitutes, which I named as valentenergics.

The identification of such pharmacophore characteristics was so precise that, without any exception, each one of the 569 new synthetic valentenergics was hypnotic, giving physiological sleep.

→ 569 valentenergics, synthetic substitutes of Valentonin  
Results of structure-activity relationships



# 11

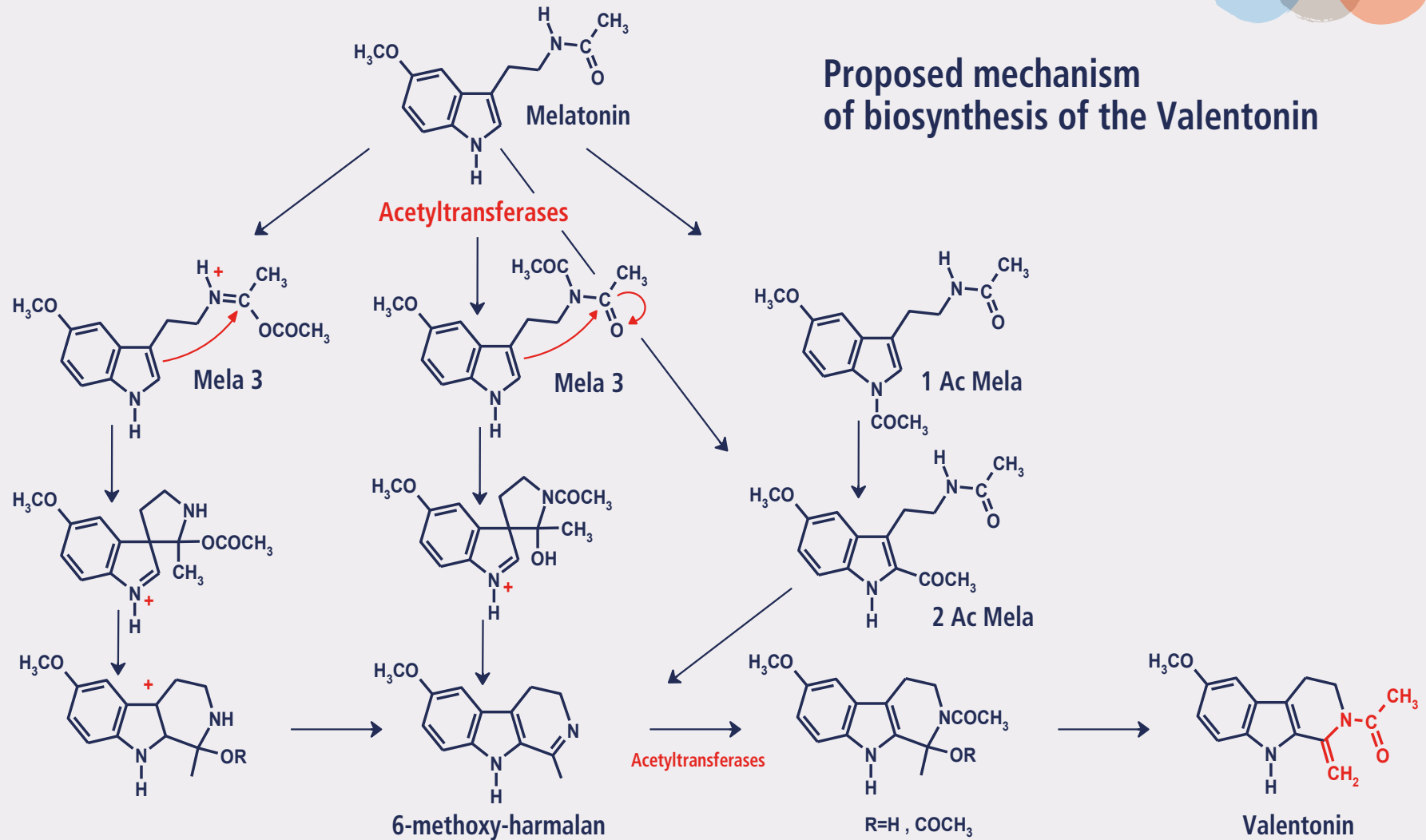
After my discovery of Valentonin in 1994, we sought, with my chemist colleagues, the mechanism of Valentonin biosynthesis by acetylation of melatonin, catalyzed by the pineal enzyme N-acetyltransferase (NAT).

We listed four possible pathways. All these biosynthesis pathways share a step of formation of 6-methoxy-harmalan.

A bibliographic search in the scientific literature, however, taught me that the presence of 6-methoxy-harmalan in the pineal gland of cattle slaughtered at night had been demonstrated in 1961 by W.M.Mc Isaac

After having formally identified 6-methoxy-harmalan in the pineal gland of cattle, Mc Isaac *et al.* carried out a study of its pharmacodynamic properties.

→ The discovery of the waking hormone : 6-methoxy-harmalan



Mc Isaac, W.M., Khairallah, P.A. & Page, I.H. 10-Methoxyharmalan, a potent serotonin antagonist which affects conditioned behavior. *Science*, 134, 674-675 (1961)

# 12

6-MH has the same pharmacodynamic properties as LSD. Compared to Valentonin, the pharmacodynamic properties are inverted, which coincides with the disappearance of the acetyl group on the nitrogen atom (N) of Valentonin.

The psychostimulant activity of 6-MH is very high, as intense as LSD; the increase in alertness that it induces maintains the waking state during the activity period.

Moreover, 6-MH:

- increases blood pressure and heart rate;
- and causes muscle contraction.

Thus, during the activity period, 6-methoxy-harmalan ensures, in a direction opposite that of Valentonin, the regulation of the psychic and autonomic states of the body.

It can be described as the daytime hormone.

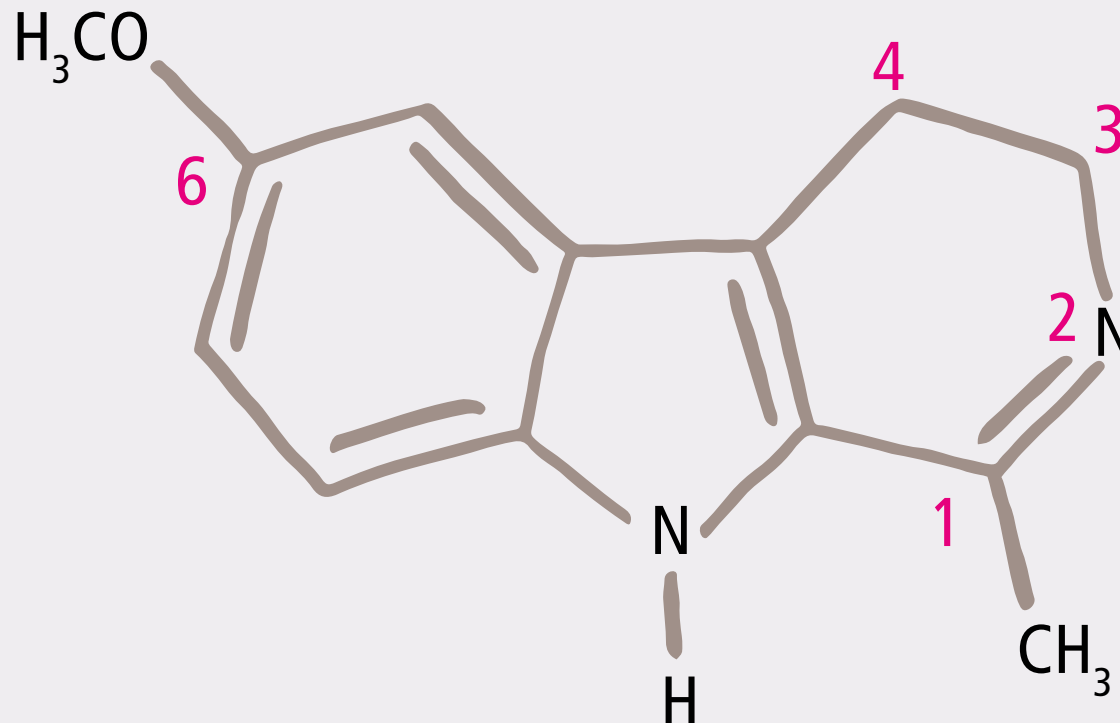
It is the hormone of cognition, awareness and thought, a role wrongly attributed to dopamine.

Furthermore, Mclsaac put forth the hypothesis that this compound could be the agent responsible for psychotic states. This we have subsequently confirmed, making it possible to abandon the dopaminergic hypothesis, reduced to rubble many years ago.

→ 6-Methoxy-harmalan (6-MH): **the daytime hormone**



## 6-methoxy-harmalan (6-MH)



**Increases** wakefulness, blood pressure, heart rate and causes muscle contraction

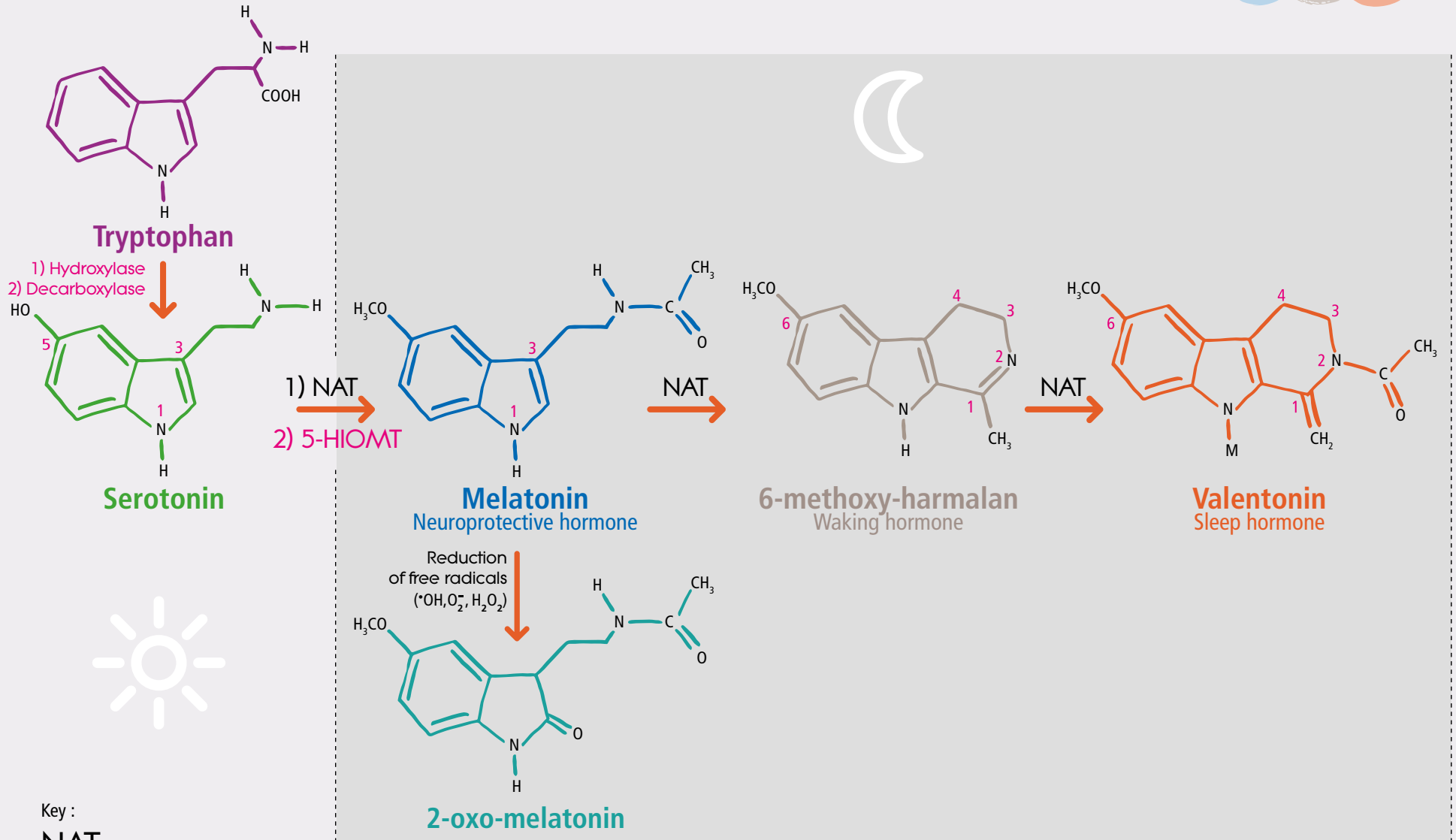
# 13

Now, as a puzzle, we have completed the Sleep-Wake system.

It is constituted by 3 hormones synthesized, in the pineal gland, from serotonin, via three successive enzymatic acetylations, between 22:00 and 6:00, during the rest period.

Insofar serotonin is the precursor of this biochemical cascade, which is the endocrine function of the pineal gland.

→ The Sleep-Wake system: 3 pineal hormones  
 biosynthesized by 3 successive acetylations of serotonin



# 14

Before speaking about the modes of action of pineal hormones, it seems essential to specify the roles of serotonin, their precursor.

Serotonin intervenes, on two levels, as :

- neurotransmitter in serotonergic neurons ;
- and precursor of the three hormones of the Sleep-Wake system in the pineal gland.



## The two functions of serotonin

---

Neurotransmitter  
in serotonergic neurons



Precursor of the 3 hormones  
of the Sleep-Wake system in the pineal gland

---

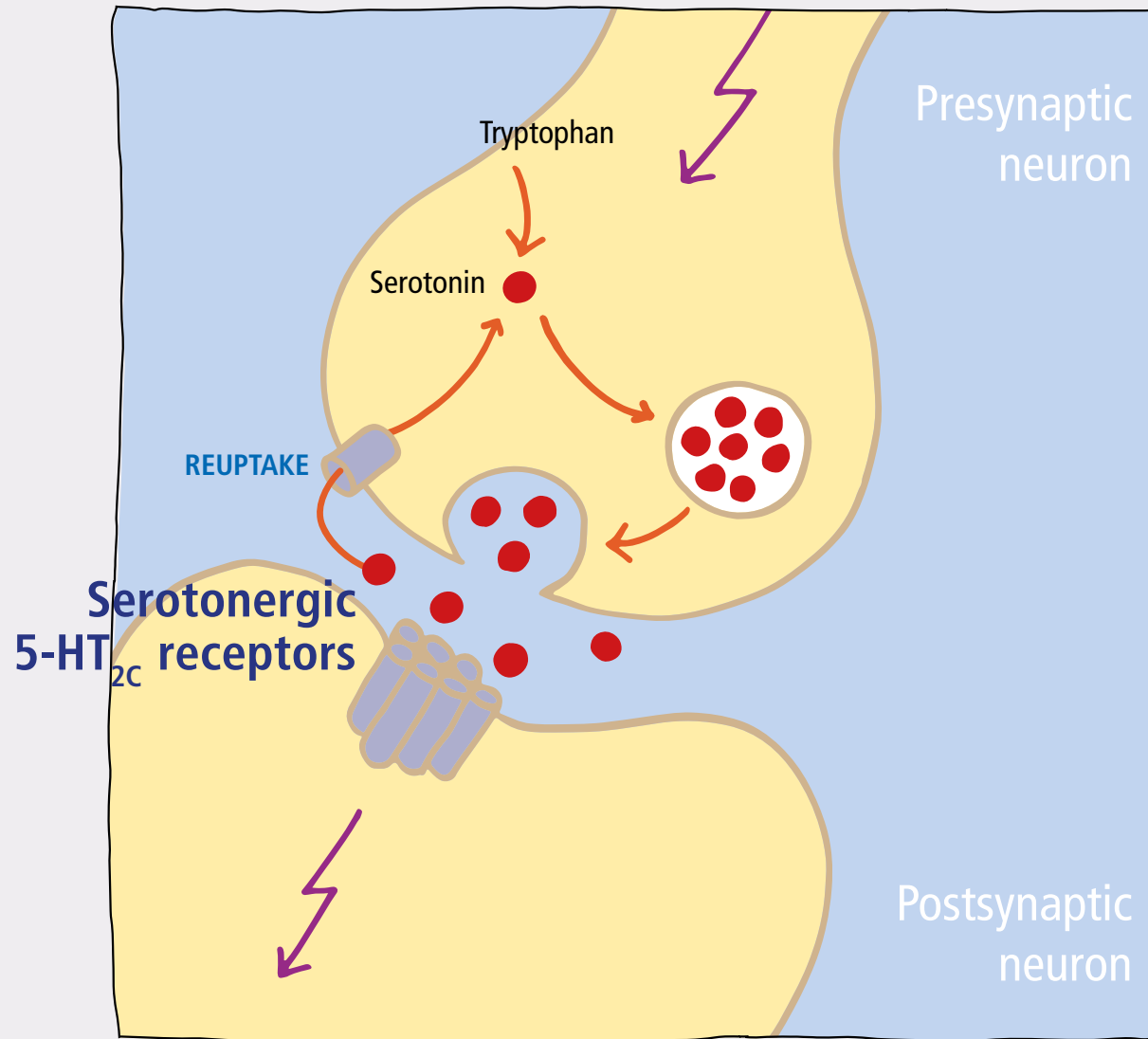
# 15

In this diagram I have represented two serotonergic neurons which relay information via a synapse.

The postsynaptic sides of these neurons, neuro-inhibitors, are equipped with serotonergic 5-HT<sub>2c</sub> receptors, which allow to transmit neuronal signal of vigilance's reduction.

The activation of 5-HT<sub>2c</sub> receptors by serotonin is expressed as decreased vigilance, which can lead to sleep.

→ Serotonin : neurotransmitter of information  
in serotonergic neurons which regulate vigilance



**Serotonergic synapse**

# 16

How do the two daytime and nighttime hormones act?

This figure shows that the stereochemical configurations of 6-MH, VLT and the three neurotransmitters are identical with regard to molecular dimensions, in particular the distances (**d**) between the nitrogen poles (**N**) and the other ends of these molecules.

In these three neurotransmitters, the free 360° rotation of the ethylamine chains around the bonds connecting them to the aromatic supports enables them to take the steric configurations of the various receptors with which they bind.

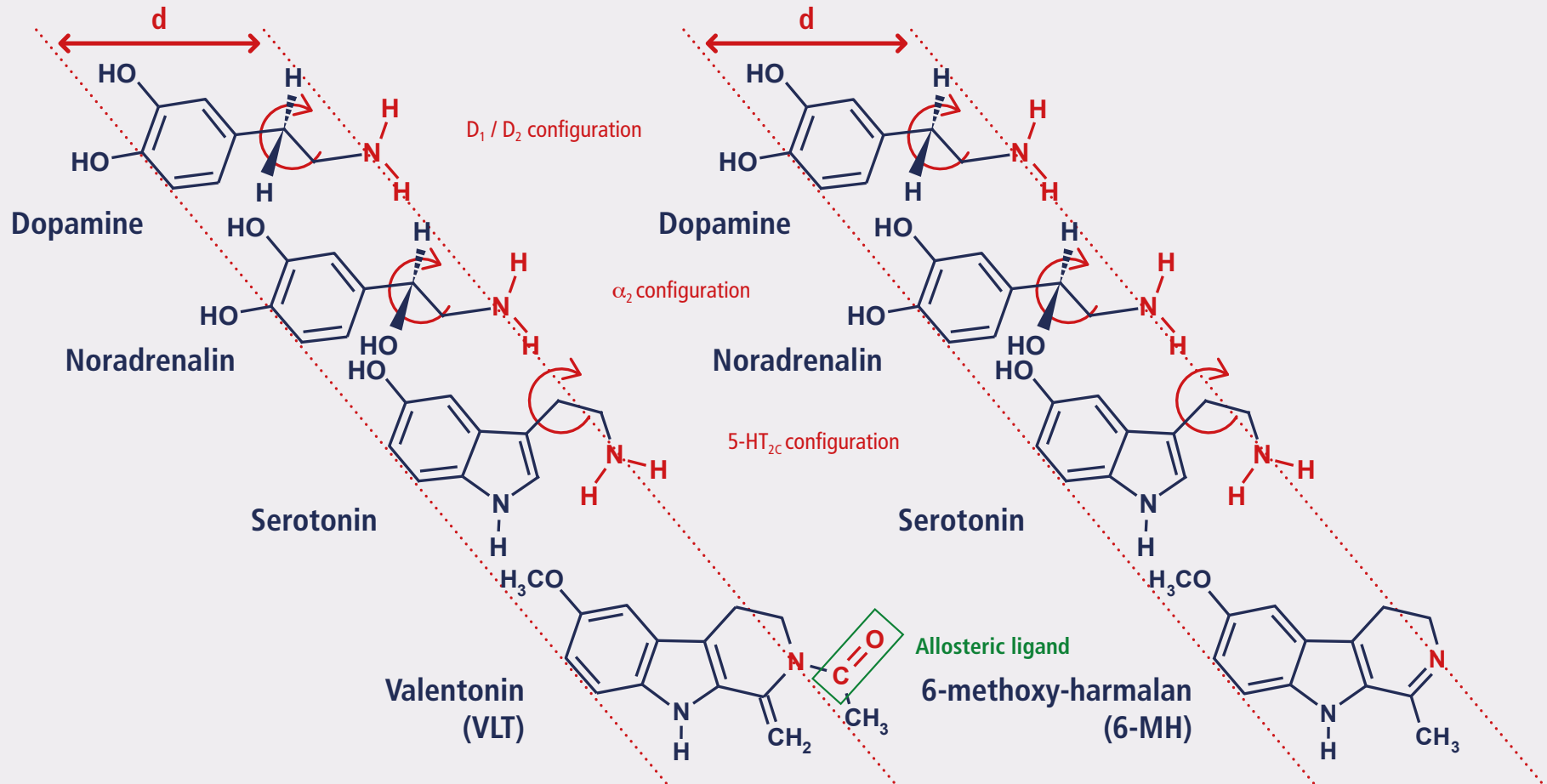
The rigid structures of Valentonin and 6-methoxy-harmalan, which result from cyclization of the melatonin chain, give these hormones the molecular dimensions and steric configurations suited to their binding with 5-HT<sub>2C</sub>,  $\alpha_2$  and D<sub>1</sub>/D<sub>2</sub> receptors.

The difference in chemical structure between VLT and 6-MH resides, only, in the presence - in the VLT molecule - of an acetyl group (-CO-CH<sub>3</sub>), which is responsible for the inversion of the pharmacodynamic activities of 6-methoxy-harmalan. As we will see, the carbonyl group (C=O) bound on the nitrogen atom (**N**) constitutes an allosteric ligand by virtue of which Valentonin activates 5-HT<sub>2C</sub>,  $\alpha_2$ , and D<sub>1</sub>/D<sub>2</sub> receptors by allosteric modulation.

→ Valentonin and 6-methoxy-harmalan:  
modulators of 5-HT<sub>2C</sub>, α<sub>2</sub>, and D<sub>1</sub> / D<sub>2</sub> receptors



Stereochemical configurations and molecular dimensions  
of three neurotransmitters, Valentonin, and 6-methoxy-harmalan



# 17

What is activation by allosteric modulation?

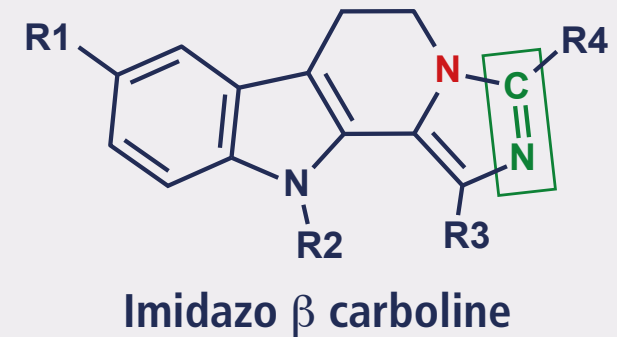
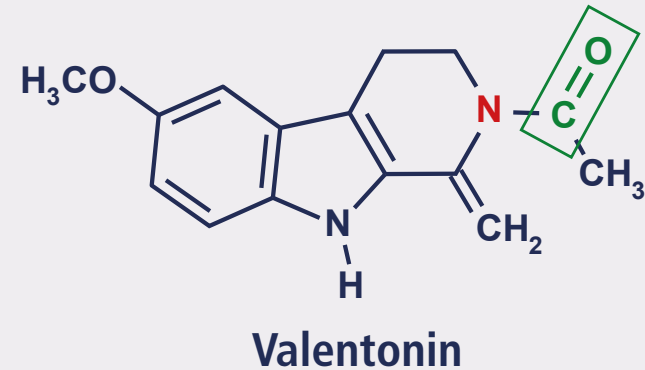
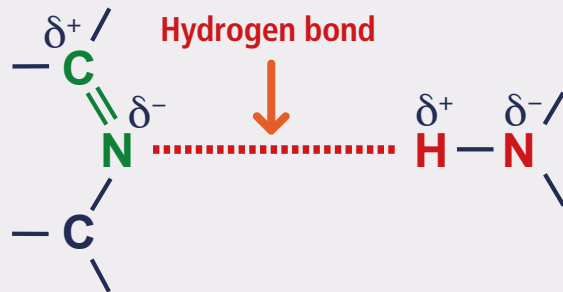
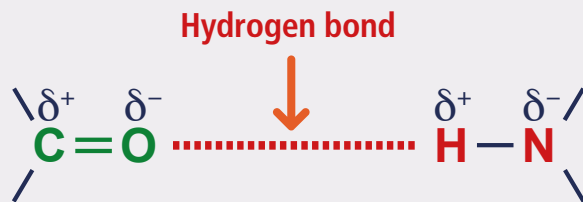
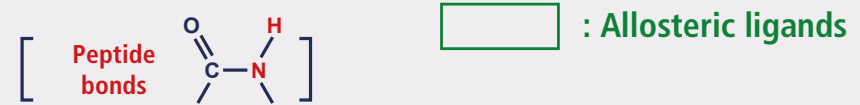
The establishment of hydrogen bonds between, on the one hand, the oxygen atom (O) of the carbonyl (C=O) of Valentonin and, on the other hand, the amino group (N-H) of the peptide bonds of protein recognition sites of receptors, causes 5-HT<sub>2C</sub>, α<sub>2</sub> and D<sub>1</sub>/ D<sub>2</sub> receptors to deform, making them more sensitive to their respective neurotransmitters.

→ Allosteric modulation of 5-HT<sub>2C</sub>, α<sub>2</sub>, and D<sub>1</sub> / D<sub>2</sub> receptors by Valentonin and valentonergics



Allosteric ligands

Complementary ligands of receptor



# 18

Application to the modulation of 5-HT<sub>2C</sub> receptors by VLT and 6-MH.

Due to the similarity of its stereochemical configuration to the 5-HT<sub>2C</sub> configuration of serotonin, Valentonin will take a position near the receptor and deform it by establishing hydrogen bonds. This allosteric deformation makes the 5-HT<sub>2C</sub> receptor more sensitive to serotonin. Activation of the receptor leads to reduced alertness, which will lead to sleep.

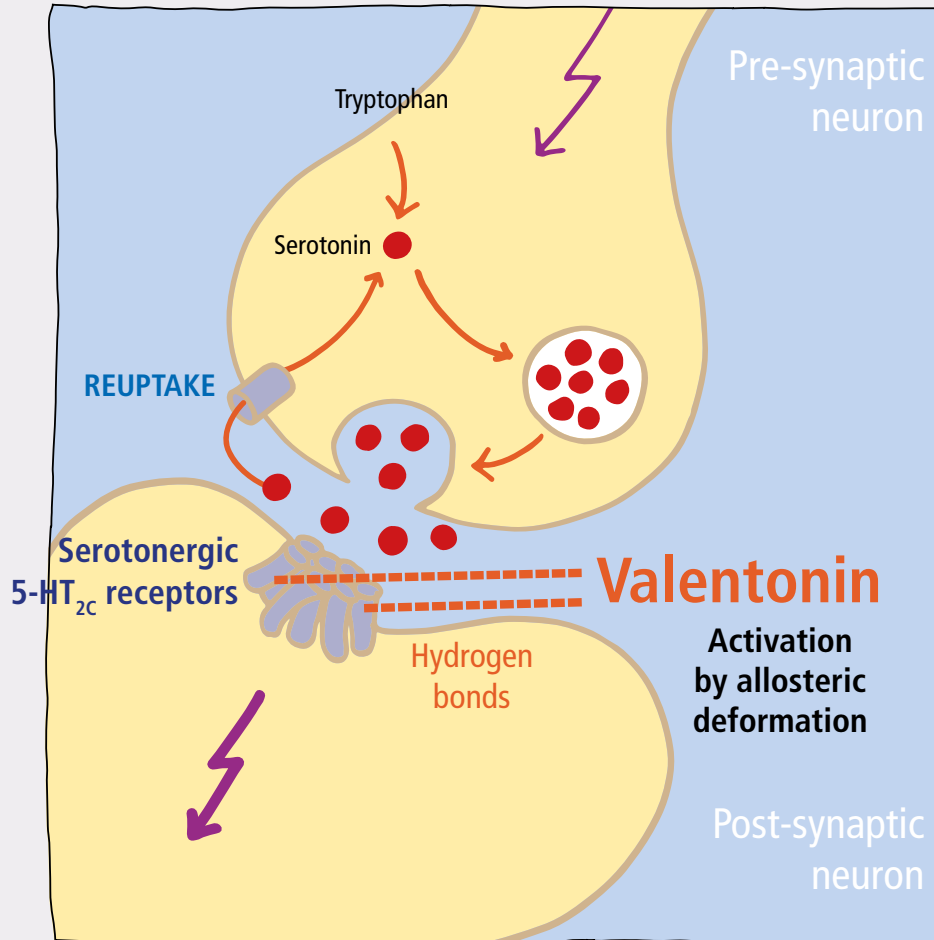
On the other hand, in the absence of allosteric ligand, 6-methoxyharmalan will prevent part of the serotonin molecules from reaching 5-HT<sub>2C</sub> receptors. There is a reduction in signal transmission and an increase in alertness.

What must be remembered is that these modulations of VLT and 6-MH are carried on this three receptor types specifically, without influencing the many other receptors for these neurotransmitters.

→ Modes of action of VLT and 6-MH on alertness by modulation of 5-HT<sub>2C</sub> serotonin receptors

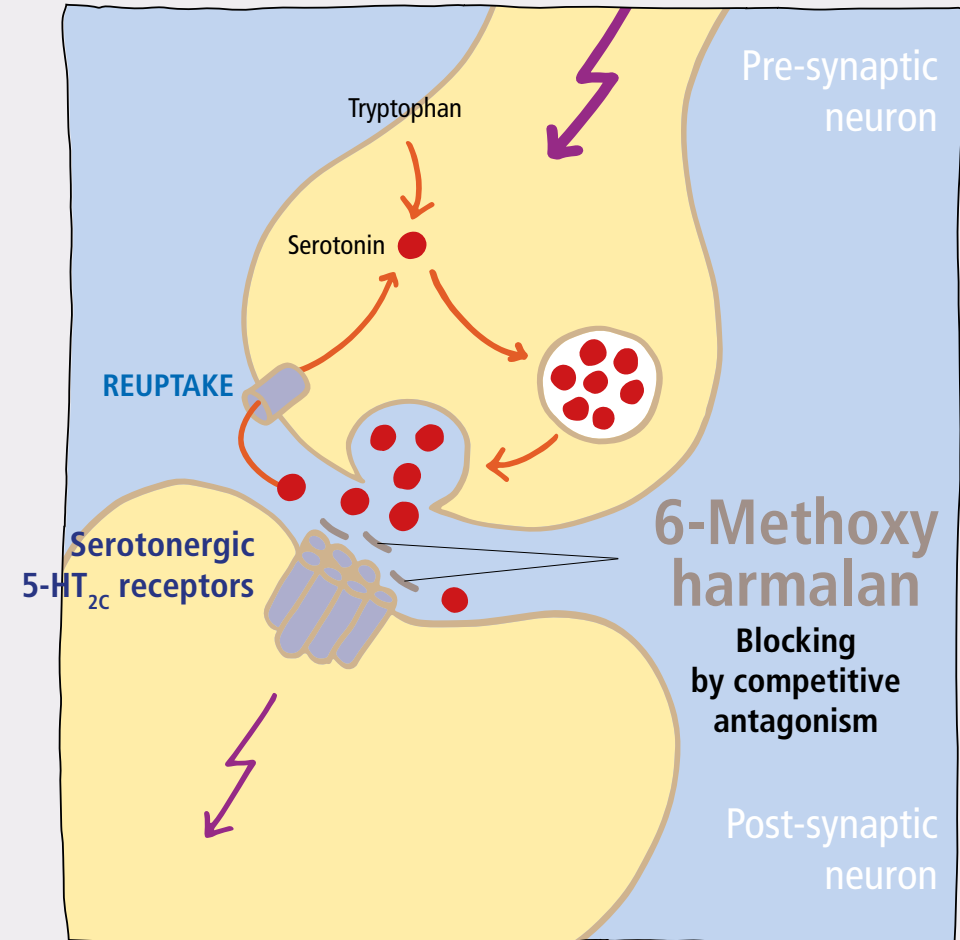


How does Valentonin **decrease** wakefulness ?  
By allosteric activation of serotonergic 5-HT<sub>2C</sub> receptors



Serotonergic synapse

How does 6-methoxy-harmalan **increase** wakefulness ?  
By blocking serotonergic 5-HT<sub>2C</sub> receptors



Serotonergic synapse

19

To understand the functioning of the Sleep-Wake system, it is necessary to know the modes of secretion of the three pineal hormones.

Because melatonin is the only one of the three hormones secreted by the pineal gland whose endogenous plasma levels can be measured, my investigations were limited to study of the secretion of melatonin by the pineal gland.

→ Modes of secretion of the 3 hormones  
of the Sleep-Wake system by the pineal gland



How are the 3 pineal  
hormones secreted  
during the 24 hours  
of the nycthemeron ?

Only concentrations of MLT secreted  
in the plasma, by the pineal gland,  
can be measured.

20

Thus, in 1994, we were able to establish curves of the changes in concentrations of melatonin, as secreted by the pineal gland in the plasma, over 24 hours, in 24 healthy young and elderly volunteer subjects, at two times of the year :

- end of May-beginning of June, in 12 young subjects ;
- and in October, in 12 elderly subjects.



## Curves of changes in plasma concentrations of MLT secreted by the pineal gland over 24 hours:

in 12 healthy  
young adult subjects  
• May-June 1994 •

in 12 healthy  
elderly subjects  
• October 1994 •

Reference: **Fourtillan, J.B., Brisson, A. M., Gobin, P., Fourtillan, M., Ingrand, I., Decourt, J.Ph. & Girault, J.** Melatonin secretion occurs at a constant rate in both young and older men and women. *Am. J. Physiol. Endocrinol. Metab.*, **280**, E11-E22 (2001).

# 21

Here are the results for the 12 young subjects.

The shapes of the curves correspond to those of a constant-rate release of melatonin in the bloodstream by the pineal gland.

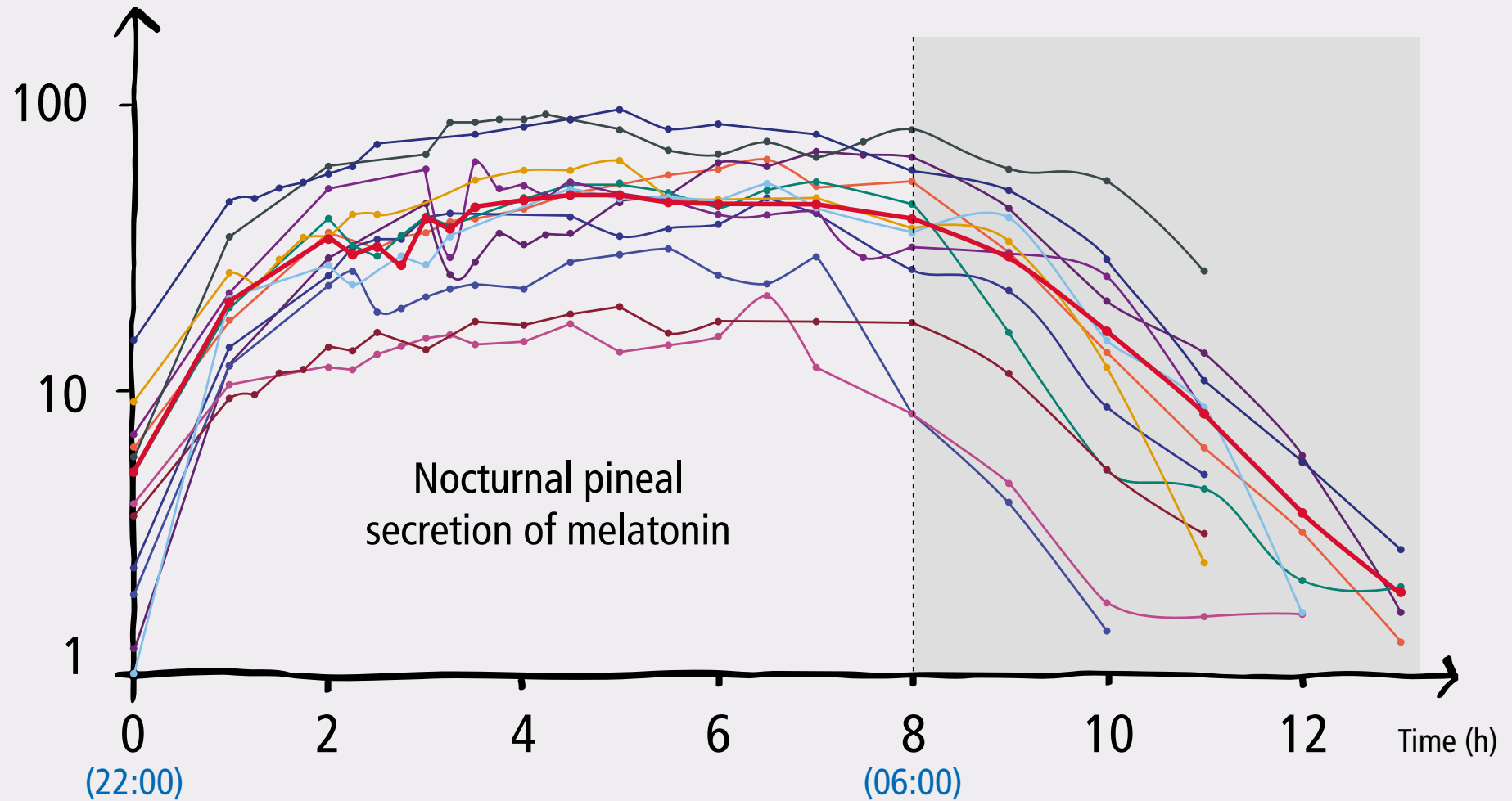
For all the subjects, the secretions begin around 22:00 and stop around 6:00 in the morning.

In passing, one will note the large variability, from 1 to 10, between the extremes of melatonin secretions.

→ Study in 12 healthy young adult subjects  
(6 men and 6 women), conducted out in May-June 1994



Plasma concentrations  
of melatonin (pg/ml)



22

End of May-beginning of June, for young subjects, pineal secretions begin between 22:00 and 22:30, and stop between 6:05 and 6:20 in the morning.

Thus, pineal secretion of melatonin lasted 8 hours, on average, whereas the length of night was 8 hours and 20 minutes.

→ Melatonin is secreted between 22:00 and 06:00 in the morning



22:00



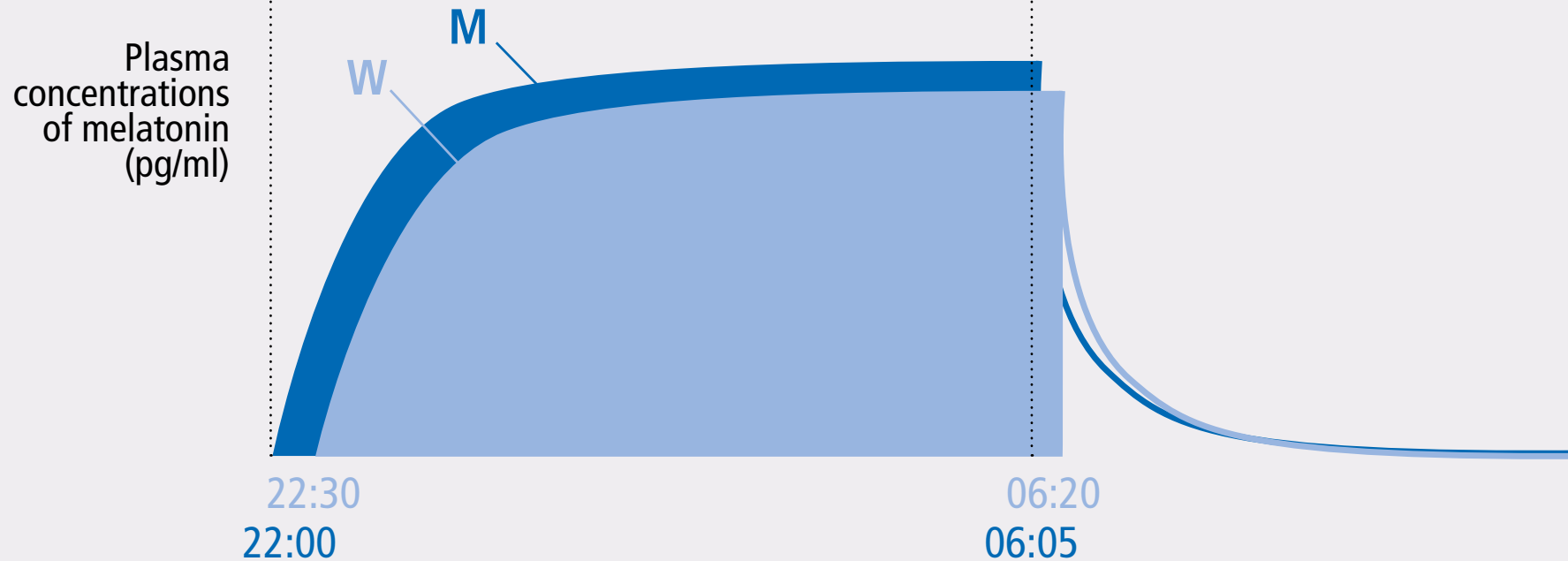
06:00



21:40

May / June (young subjects)

06:00

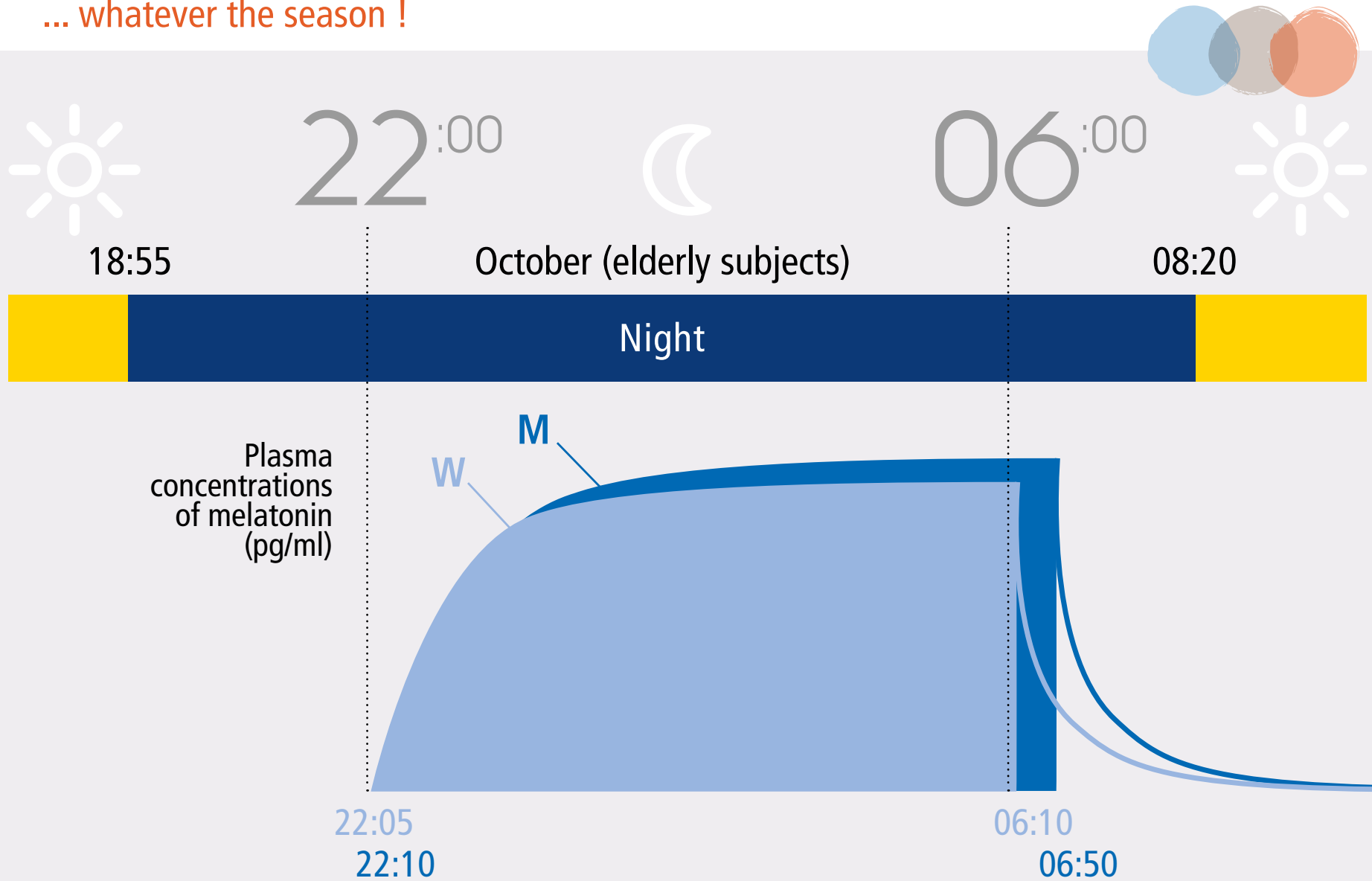


23

Identical results were observed in elderly subjects studied during the month of October, whereas the length of the night increased to 13 hours and 25 minutes.

Thus, contrary to what is commonly reported in the scientific literature, pineal secretion of melatonin occurs between 22:00 and 6:00 in the morning, whatever the season.

→ Melatonin is secreted between 22:00 and 06:00 in the morning  
... whatever the season !

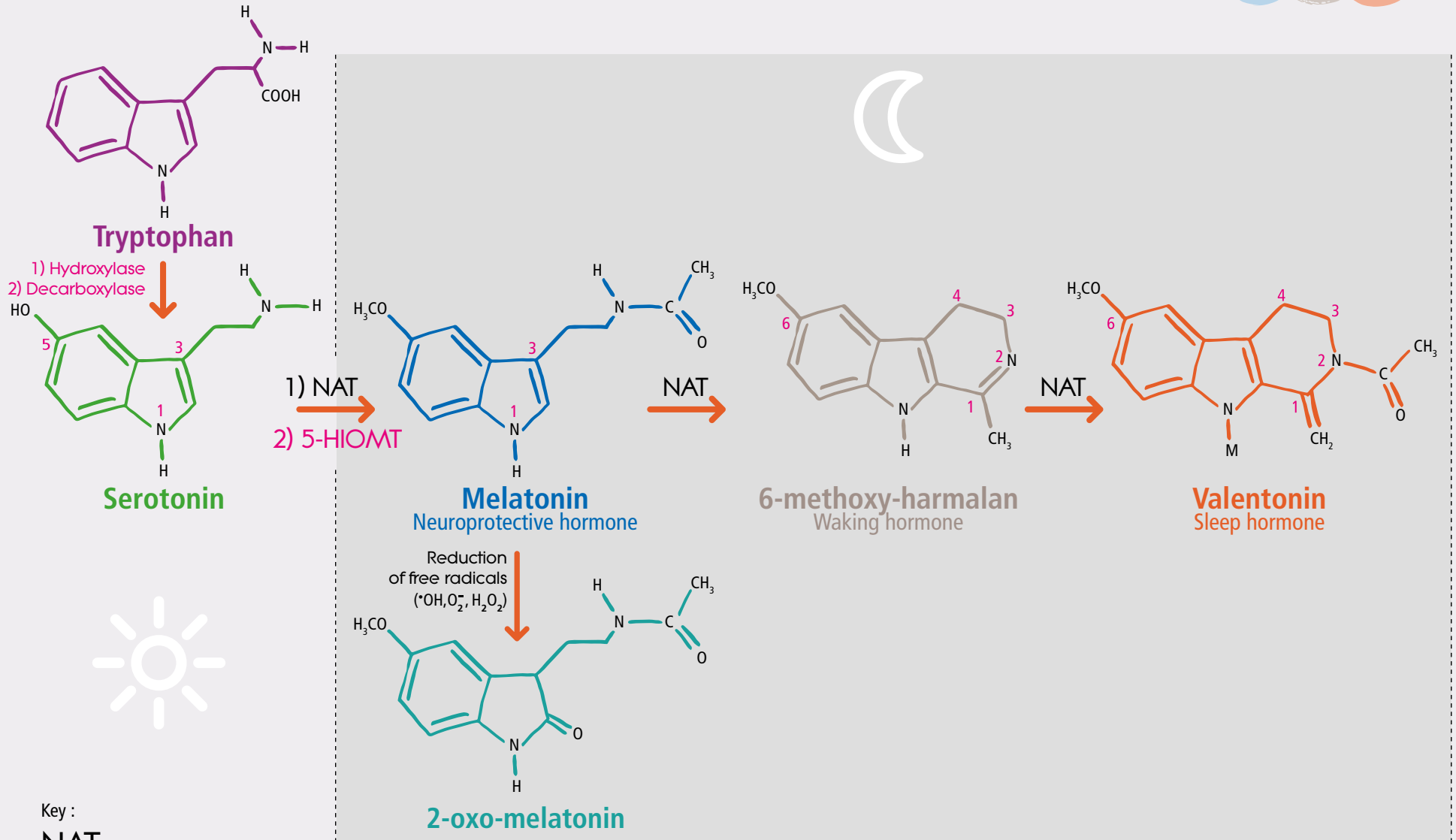


24

The knowledge of the simultaneous biosynthesis of pineal hormones, according to successive three steps of enzymatic acetylation from serotonin, allowed me to claim the following postulate :

The only condition to ensure, in a harmonious fashion, alternating periods of rest, between 22:00 and 6:00 in the morning, and of activity, between 6:00 in the morning and 22:00, is that the pharmacokinetic properties of Valentonin and 6-methoxy-harmalan are strictly identical in all mammals with nocturnal sleep, for example in humans and in dogs.

→ The Sleep-Wake system: 3 pineal hormones  
 biosynthesized by 3 successive acetylations of serotonin



Key :

**NAT** : enzyme N-acetyltransferase;

**5-HIOMT** : enzyme 5-hydroxyindole-O-methyltransferase

# 25

It is based on this thought that I decided, in 2006, to determine the pharmacokinetics of the three pineal hormones, after intravenous administration, in dogs.

Results are presented in this table.

Volume of distribution ( $V_d$ ) values of VLT and 6-MH are respectively 10 times and 36 times greater than that of melatonin. Which means that, for a given quantity secreted by the pineal gland, the plasma concentrations of VLT and 6-MH would be respectively 10 times and 36 times lower than those observed with melatonin, and therefore impossible to measure.

→ The pharmacokinetics of Valentonin and 6-methoxy-harmalan are identical in all mammals with nocturnal sleep



## The pharmacokinetics of Valentonin and 6-methoxy-harmalan are identical in humans and dogs



	MLT	VLT	6-MH
$T_{1/2Z}$	1 hour	0.70 hour	2.27 hours
$V_d$	1 liter / kg	10 liters / kg	36 liters / kg

For a given quantity secreted by the pineal gland, the plasma concentrations of VLT and 6-MH are respectively 10 times and 36 times lower than those of MLT

# 26

The results obtained in dogs, identical to those that would be obtained in humans, are extremely interesting.

After having set the doses of VLT and 6-MH in a ratio of 4 to 1, respectively, I was able to simulate, using suitable software, the curves of changes in plasma concentrations of the three hormones over 24 hours.

Nature designs things well!!

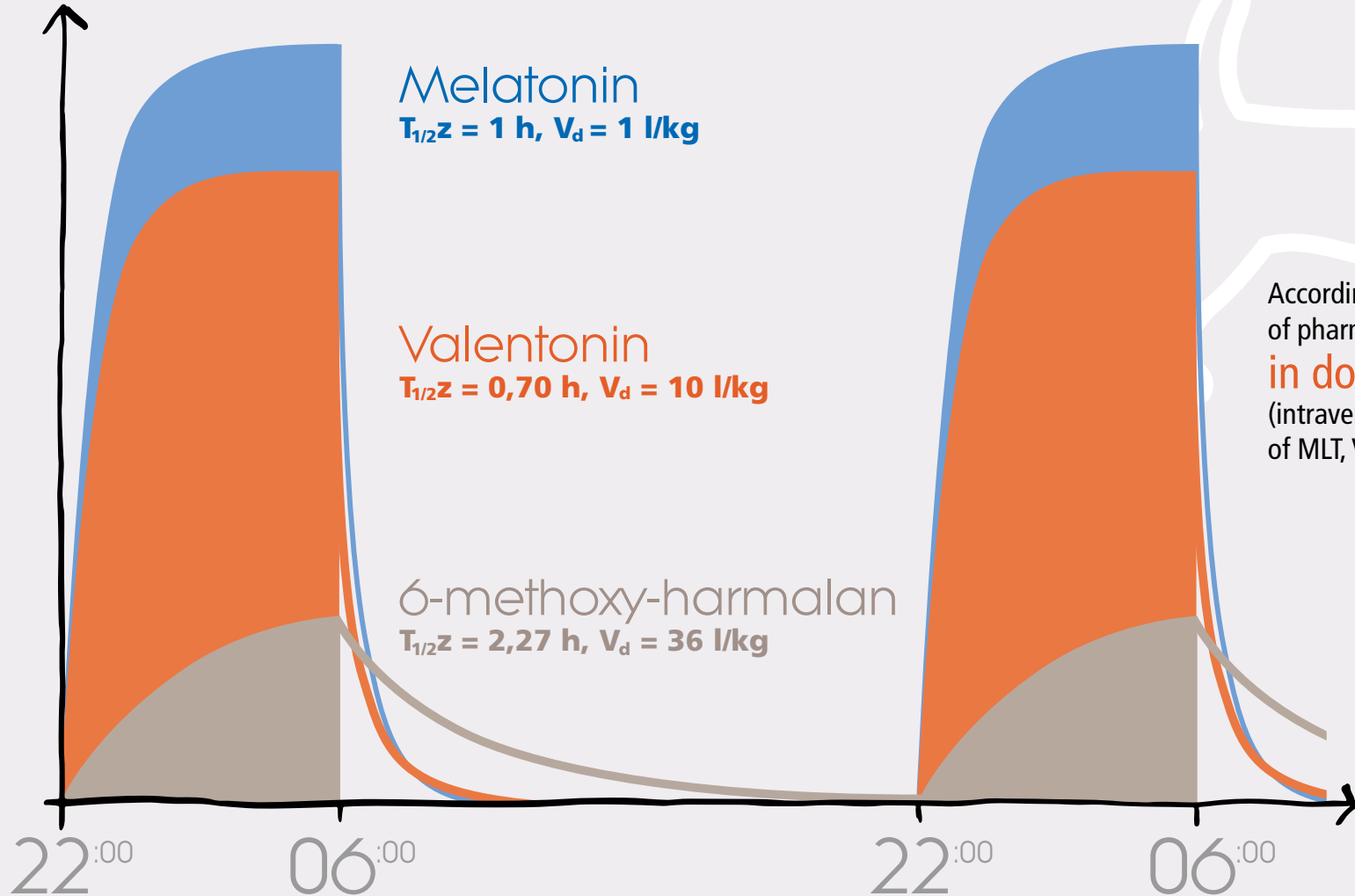
Indeed, one sees that the VLT concentrations - in the blood and the extravascular fluids, including the brain - are higher than those of 6-MH during the rest period between 10:00 p.m. and 6:00 a.m.

Conversely, when pineal secretions stop at 6:00 a.m., within 15 to 30 minutes 6-methoxyharmalan concentrations become and remain higher than those of Valentonin throughout the body, until 10:00 p.m.

What miraculous concordances!

→ Prediction of VLT and 6-MH secretion curves in dogs, humans and mammals with nocturnal sleep

Plasma concentrations



According to the results of pharmacokinetics in dogs (intravenous administrations of MLT, VLT and 6-MH)

# 27

In all mammals, the endocrine function of the pineal gland, which corresponds to the secretion of the three hormones, is exerted between 22:00 and 6:00 in the morning.

Thus, at 6:00 in the morning, the pineal gland has finished its job !

In this figure we have shown the secretion curves in mammals with nocturnal sleep.



# 28

How does trigger the endocrine function of the pineal gland ?

In humans and mammals, retinal cells perceive ambient light. This information is processed by the suprachiasmatic nucleus, located just above the optic chiasma.

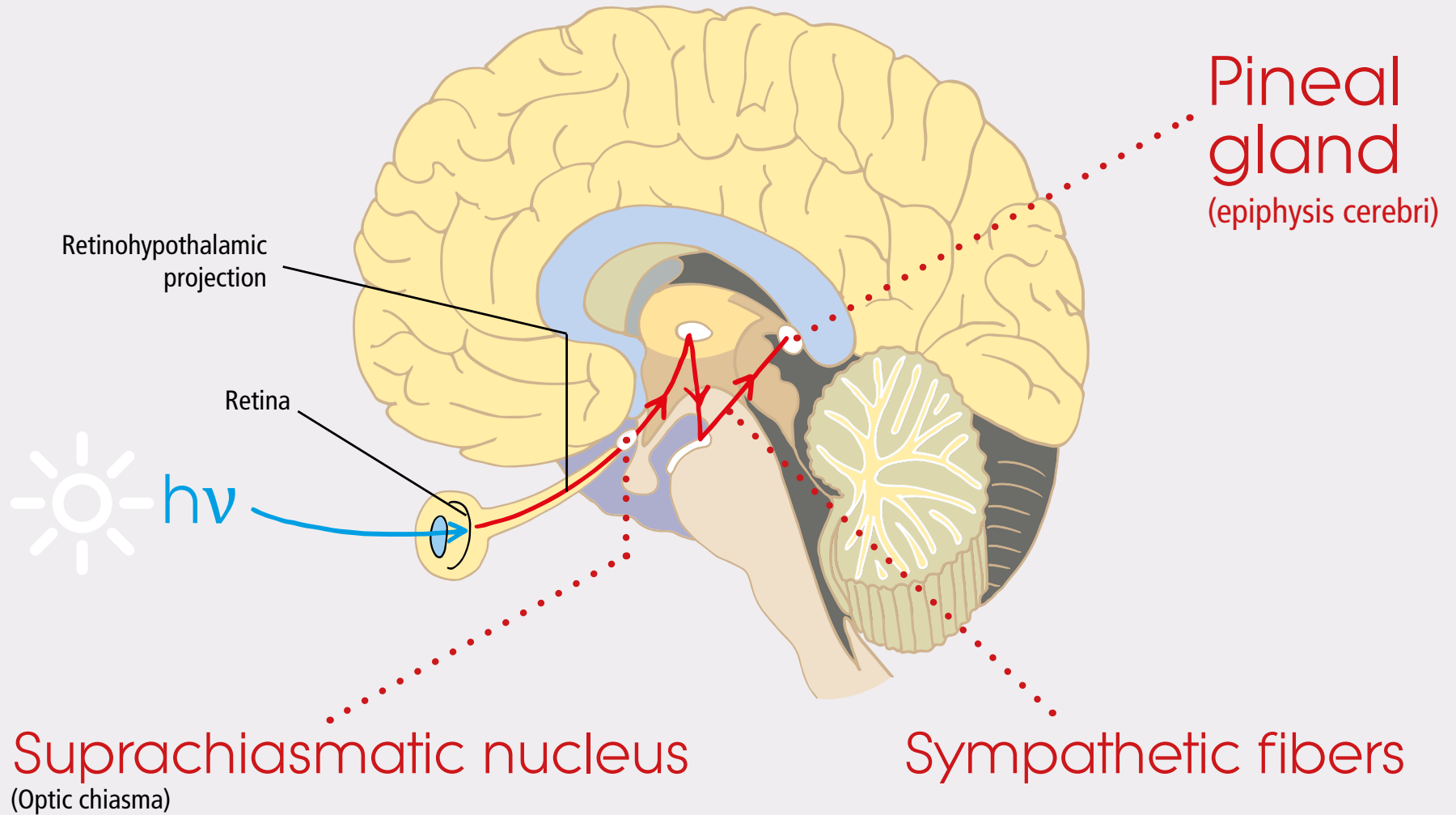
The suprachiasmatic nucleus is the true « biological clock ». It keeps the life of the organism in rhythm, in 24-hours sections, by adapting it to its lifestyle and its environment.

How does that occur ?

The pineal gland plays a transduction role : it converts the rhythm set by the suprachiasmatic nucleus into an endocrine message, via secretion of the three hormones.

→ Triggering of endocrine function of the pineal gland

The photoneuroendocrine transduction, from 22:00 to 6:00 in the morning



29

Let's go back over sleep.

How do we pass from the waking state to the sleep state ?

The sleep state, with loss of consciousness, appears when wakefulness falls below a certain level that can be described as the « sleep barrier ».

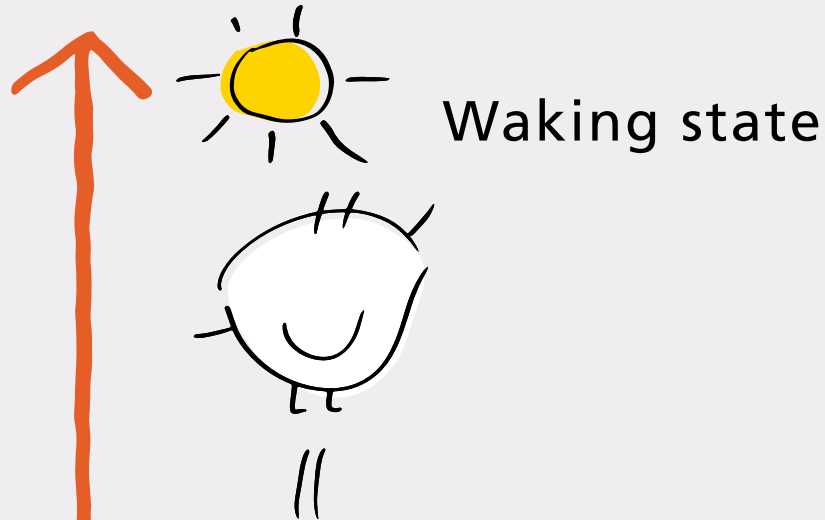
In normal subjects, during the period of rest between 22:00 and 6:00 in the morning, the prevalence of VLT concentrations triggers the sleep state, and maintains it, if the VLT concentrations at serotonergic 5-HT<sub>2C</sub> receptors are sufficient to lower and maintain the level of wakefulness below the sleep barrier.

In depressive patients the stress responsible of the depression increase the level of wakefulness. The Sleep-Wake system should be strengthened, in order to maintain the level of wakefulness below the sleep barrier.

→ The sleep barrier:  
triggering of the waking and sleep states



Wakefulness



6:00–22:00

Prevalence of 6-MH

**SLEEP BARRIER**



22:00–6:00

Prevalence of VLT

# 30

We can now explain what occurs between 22:00 and 6:00 in the morning, in three areas of the brain, at :

- noradrenergic  $\alpha_2$  receptors ;
- serotonergic 5-HT<sub>2c</sub> receptors ;
- and dopaminergic D<sub>1</sub>/D<sub>2</sub> receptors.

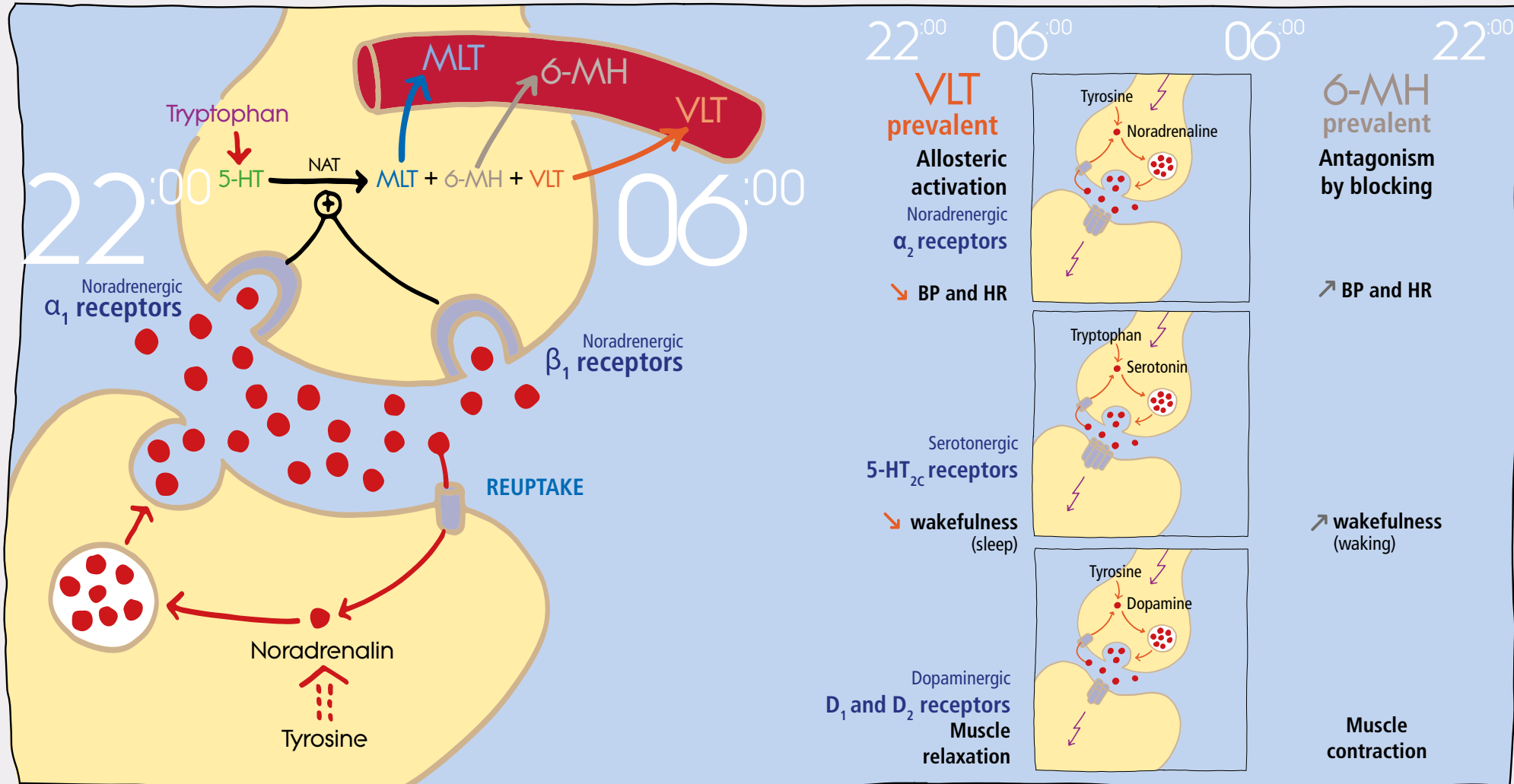
From 22:00, and until 6:00 in the morning, once we are away from daylight, photoneuroendocrine transduction ends in noradrenergic stimulation at pinealocytes. Which triggers the pineal secretion of the three hormones in the bloodstream.

During this rest period, for pharmacokinetic reasons mentioned before, VLT is prevalent, because its concentrations at these receptors are higher than those of 6-MH. At the conclusion of competitive binding of the two hormones, the action of VLT becomes predominant. The result is an allosteric activation of the three receptors with :

- decreased wakefulness, and induction of physiological sleep ;
- lower BP and HR ;
- and muscle relaxation.

As soon as pineal secretion shuts down, at 6:00 in the morning, and until 22:00, 6-MH becomes prevalent in the brain. Consequently, there is a reversal of the preceding actions, together with increase in wakefulness which causes and maintains waking until 22:00 at night.

→ How does the Sleep-Wake system function during 24 hours from 22:00 (« *primum movens* ») ?



# 31

Most of the neurological disorders result from a quantitative deficit of the three hormones, reflecting an insufficiency of the endocrine function of the pineal gland. Which can be demonstrated by too-low plasma levels of melatonin, as a marker, when measured from 1:00 in the morning.

In cases of too-low values of the marker, it is necessary to prescribe hormone replacement therapy, as a combination of Valentonin and 6-methoxy-harmalan in a dose ratio of 4 to 1, transdermally, using dual reservoir patches. The patches are applied at bedtime and removed in the morning upon waking.

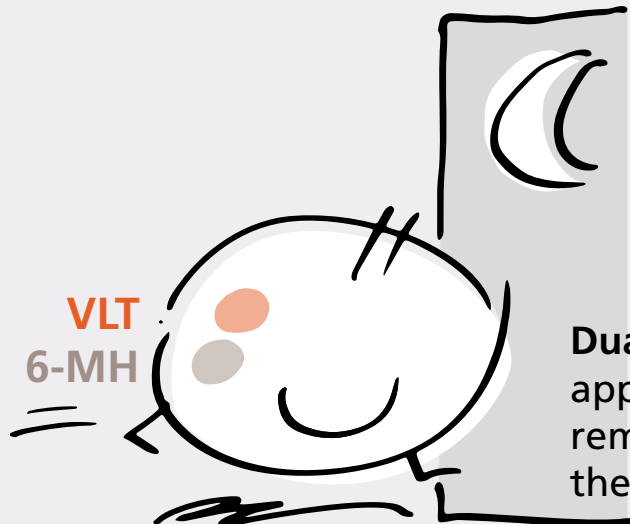


# MELT

Melatonin is the **MARKER** of synthesis of the 3 hormones of the Sleep-Wake system.

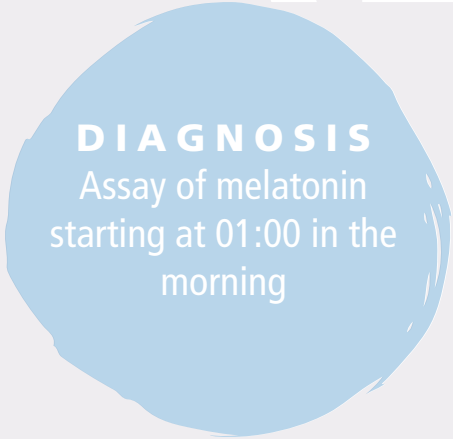


**Insufficient secretion**



**VLT**  
**6-MH**

**Dual-reservoir PATCH**  
applied at bedtime and removed upon waking in the morning



**DIAGNOSIS**  
Assay of melatonin starting at 01:00 in the morning



**Excessive secretion**



**VLT**

**Single-reservoir PATCH**  
applied upon waking in the morning and removed at bedtime at night

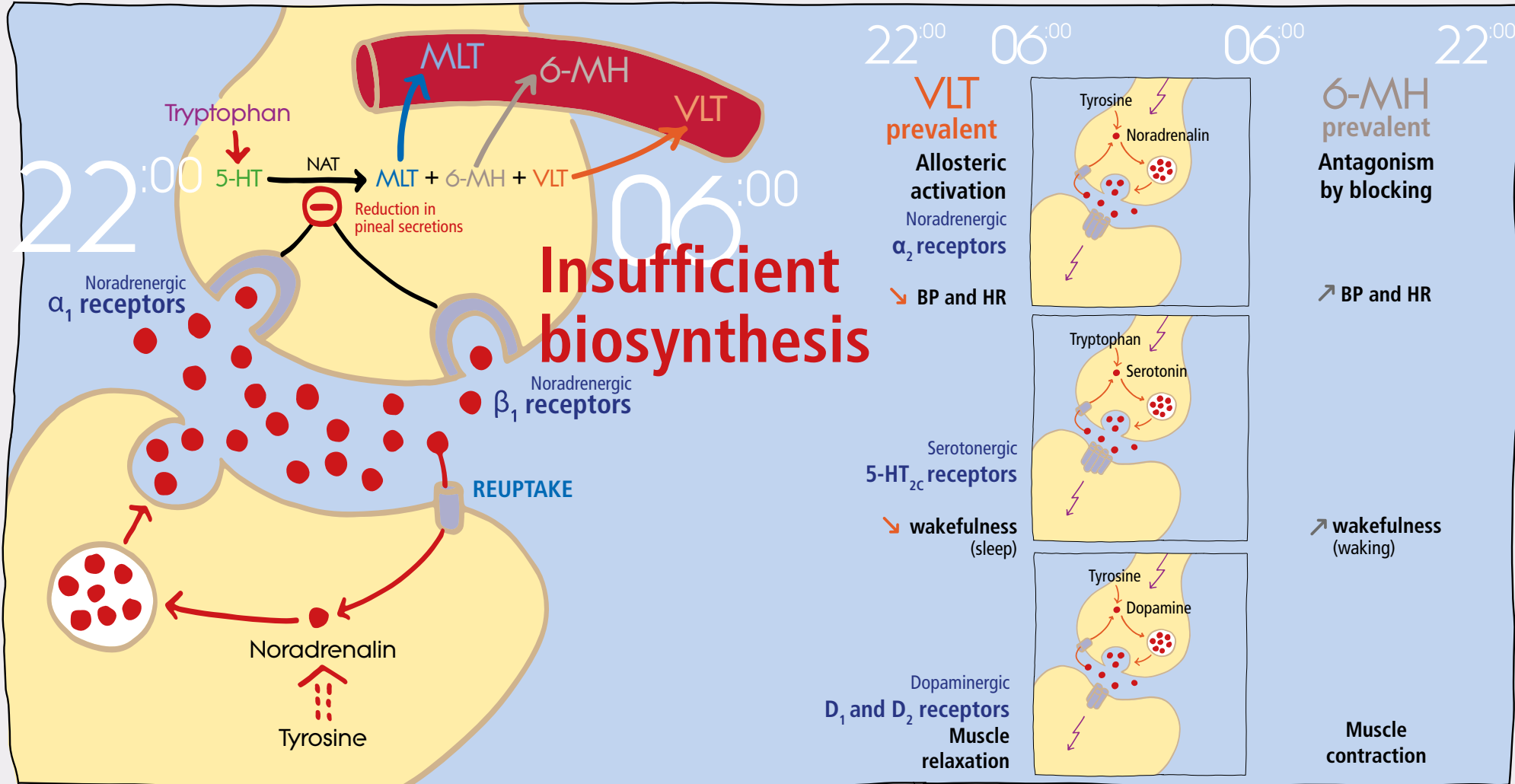
# 32

As we have just seen, sleep disorders are caused by an insufficiency of pineal production not only of Melatonin, but also of the two other pineal hormones.

To provide harmonious regulation of the body by the Sleep-Wake system, it is necessary to administer, transdermally, the combination (VLT/6-MH) (4/1).

Dual reservoir patches should be applied at bedtime, at night, and removed upon waking in the morning.

→ Sleep disorders are due to insufficient pineal secretions  
 Treatments with a combination (VLT / 6-MH) (4/1), transdermally



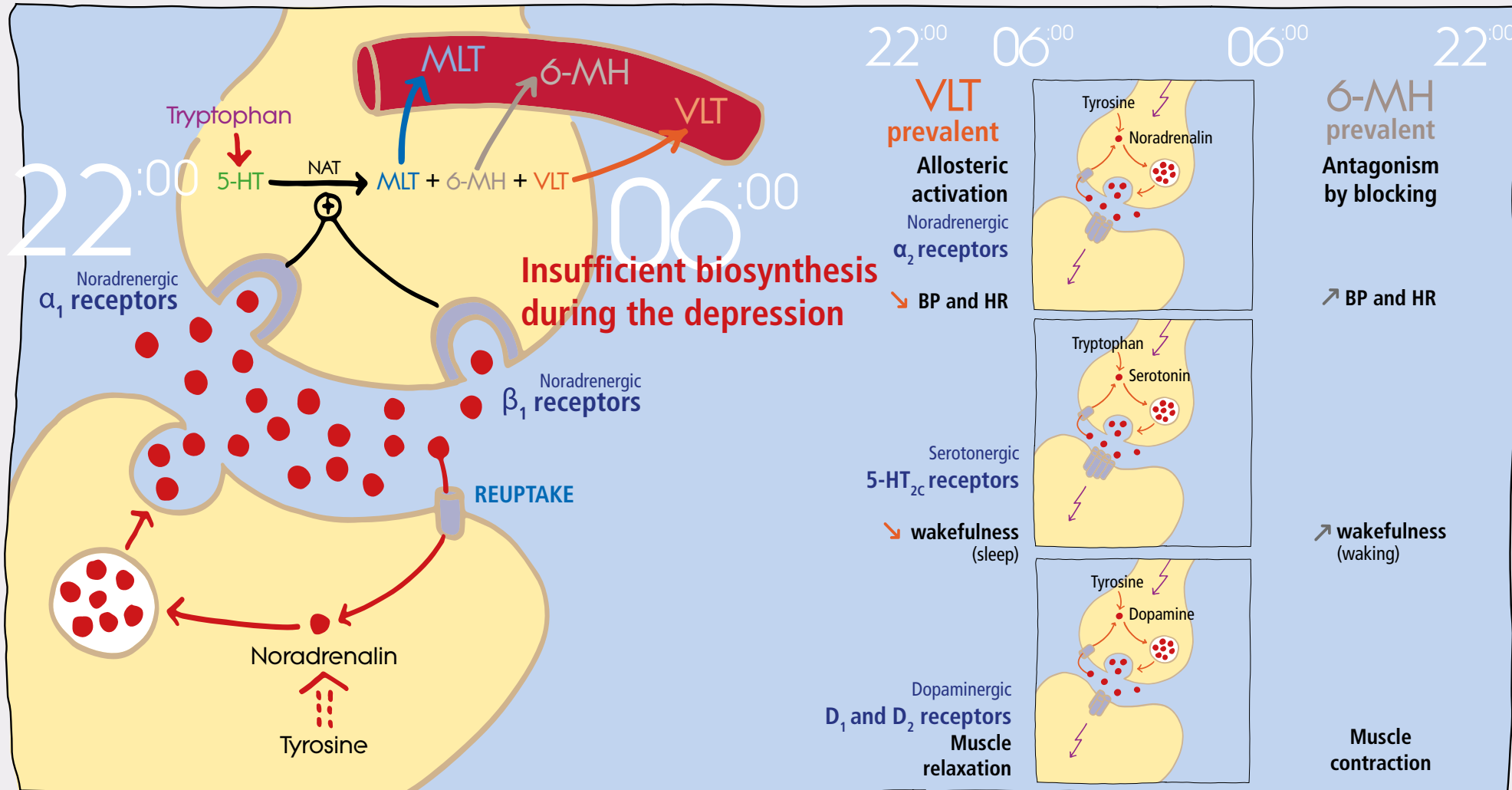
## Transdermal hormone replacement therapy

33

The discovery of the Sleep-Wake system shows that depressive disorders are transient sleep disorders.

They must therefore be treated in the same way as sleep disorders, by administering the combination (VLT/6-MH) (4/1), only during the depressive episode.

→ In depressive disorders, the Sleep-Wake system should be strengthened by administering the combination (VLT / 6-MH) (4/1), during the depression



## Transdermal hormone replacement therapy

# 34

Setting aside benzodiazepines and related drugs, it is seen that the therapeutic activities of all psychotropic medicines, ergoline-derived antiparkinsonians, as well as antihypertensives of the Clonidine family (centrally-acting  $\alpha_2$  agonists), come from their action on the Sleep-Wake system.

This is true of antidepressants.

The current treatment for depression consists in administering Clomipramine (Anafranil®)-type noradrenalin reuptake inhibitors (NRIs), and Fluoxetine (Prozac®)-type serotonin reuptake inhibitors (SRIs).

In both cases, the efficacy of the antidepressants results from the activation of the same 5-HT<sub>2C</sub> serotonin receptors as Valentonin, either:

- directly, with SRI antidepressants, by stimulation of 5-HT<sub>2C</sub> receptors; or
- indirectly, with NRI antidepressants, by increasing secretion of the pineal hormones and, in particular, of Valentonin, which will specifically activate, by allosteric stimulation, 5-HT<sub>2C</sub> receptors and intensify sleep.

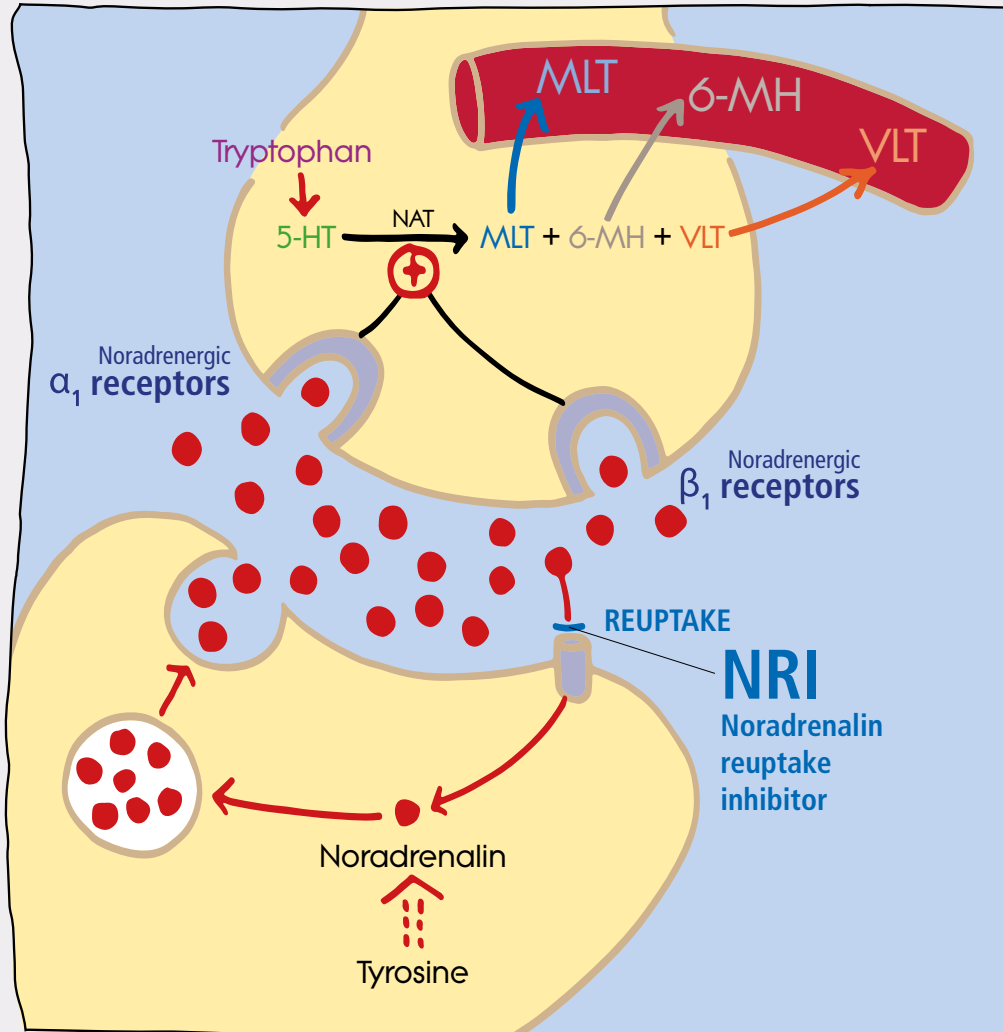
But in both cases, with many undesirable effects:

- in part because reuptake inhibition affects all the body's noradrenergic and serotonergic receptors; and
- in part because it goes on 24 hours a day, while it should take place only between 10:00 p.m. and 6:00 a.m.

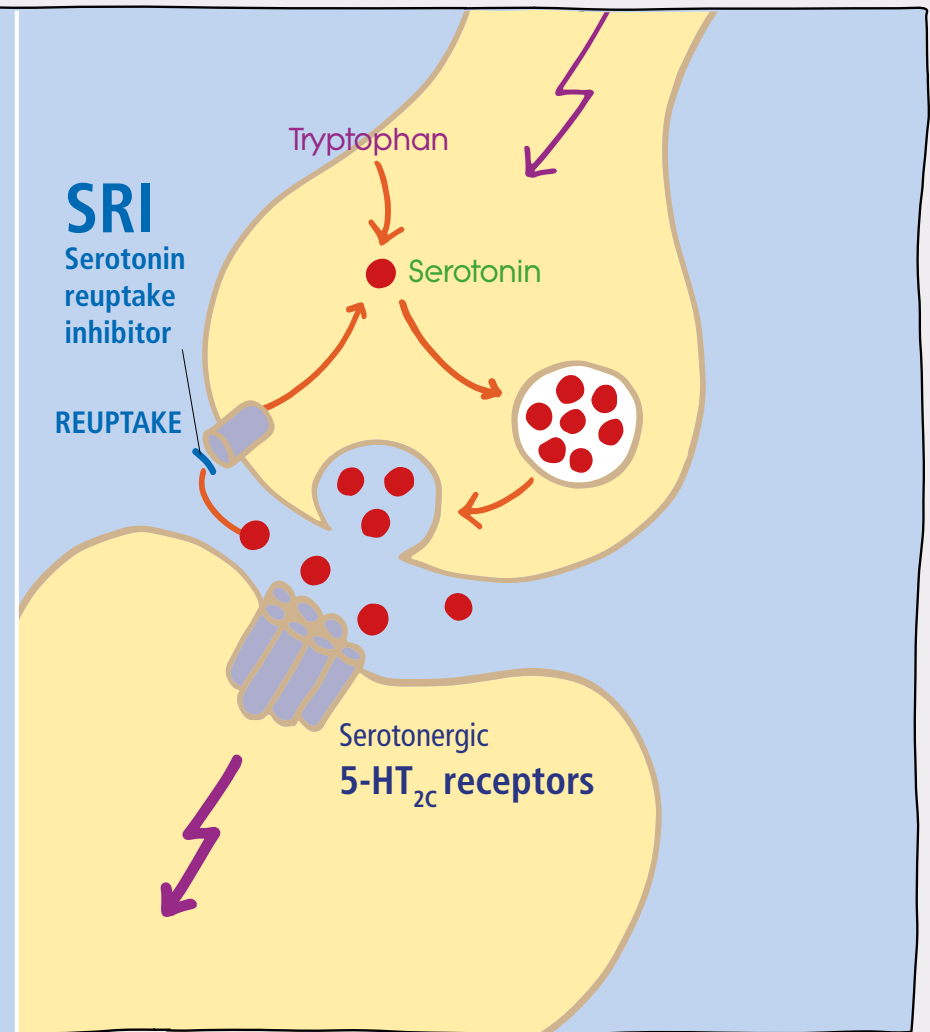
→ Depressive disorders  
Treatments with NRI and SRI antidepressants



Inhibition of noradrenalin reuptake at presynaptic membrane of noradrenergic neurons of pinealocytes



Inhibition of serotonin reuptake at presynaptic membrane of serotonergic neurons



35

Thus, future treatments for all neurological disorders caused by deficiency of the endocrine function of the pineal gland will consist in the transdermal administration of a combination (Valentonin + 6-methoxy-harmalan) in a dose ratio of 4 to 1.

This is the only way to treat these disorders without disturbing the regulation of the psychological and autonomic states of the body.

→ Future treatments for neurological disorders caused by pineal insufficiency by transdermal administration of the combination (VLT/6-MH) (4/1)



# Neurological disorders

Sleep disorders

Parkinson's disease  
and parkinsonian syndromes

Alzheimer's disease

Depressive disorders

Psychotic disorders

Neurodegenerative disorders

Thank you for attention!

END OF PRESENTATION AnP OF 02/12/2015



Complementary information

# 36

The same stereochemical configurations and molecular dimensions (distances **d**) of 6-methoxy-harmalan (6-MH) and Lysergide (LSD) are in correlation with their analogous pharmacodynamic profiles.

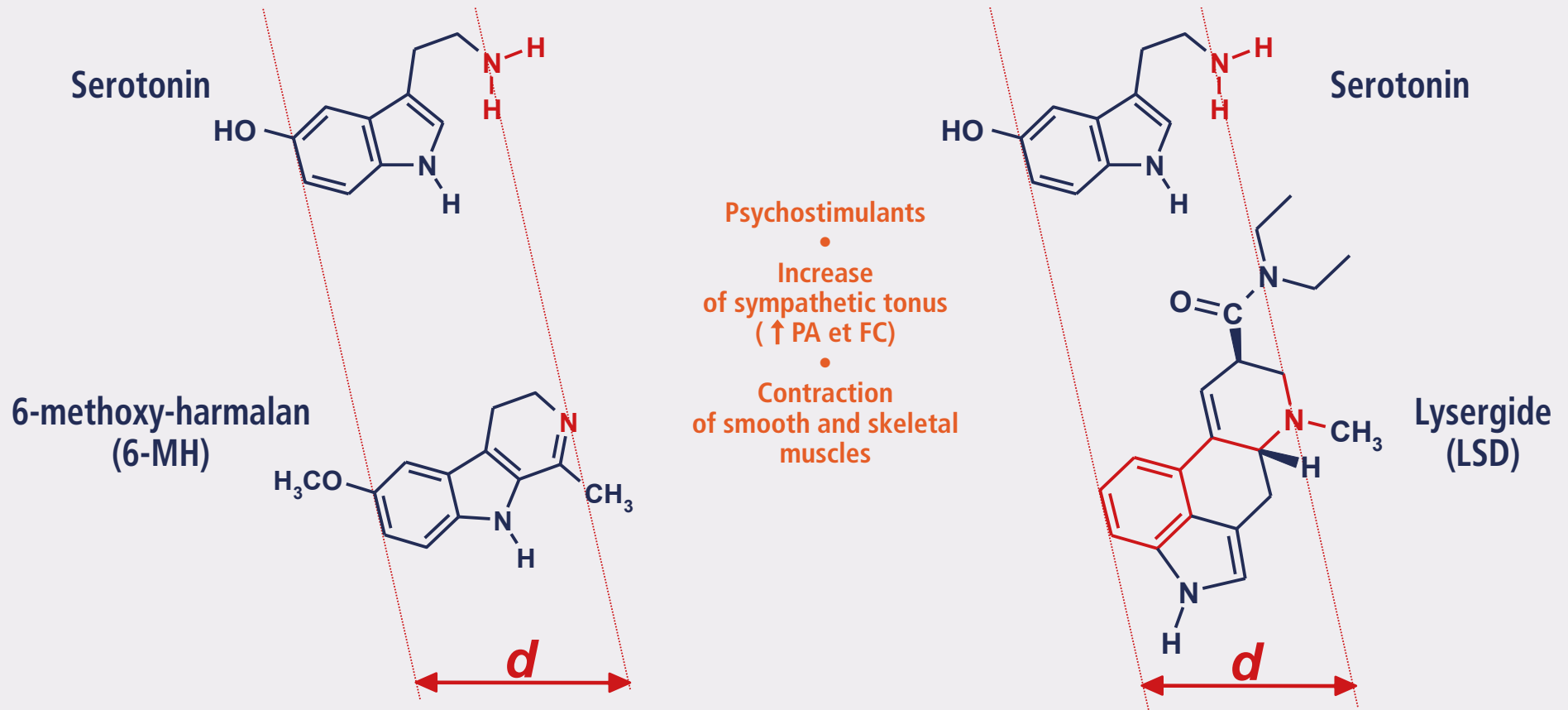
Insofar these two compounds exhibit the following pharmacodynamic properties :

- potent pharmacostimulants ; their activity levels are in the same order of magnitude ;
- they raise blood pressure and heart rate, by increasing sympathetic tonus, as a consequence of their antagonist action on noradrenergic  $\alpha_2$  receptors ;
- they cause smooth and skeletal muscles to contract, consecutively to their antagonist action on dopaminergic  $D_1/D_2$  receptors.

→ Structure-activity relationships for 6-MH and LSD  
6-MH and LSD have the same pharmacodynamic properties



Stereochemical configurations and molecular sizes of 6-methoxy-harmalan, serotonin, and Lysergide



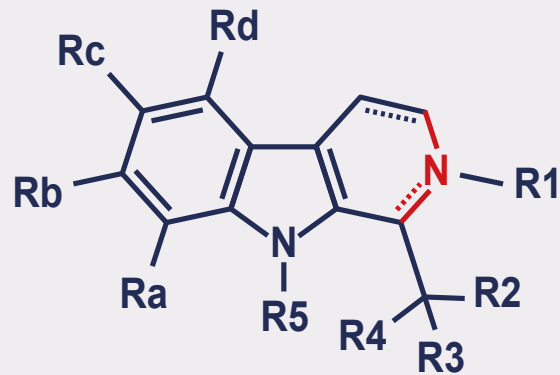
# 37

It has been demonstrated, experimentally, that LSD is a serotonergic 5-HT<sub>2C</sub> receptor antagonist.

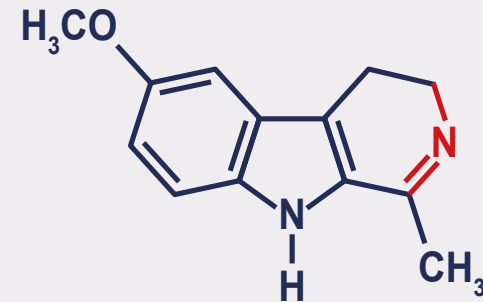
The serotonergic 5-HT<sub>2C</sub> receptor blockade is responsible, via an increase in vigilance, of the psychostimulant properties of LSD.

It has been shown experimentally by S. Goldstein et al., that 117  $\beta$ -carboline derivatives, whose chemical structures have the same general formula as 6-methoxy-harmalan, are serotonin antagonists with respect to serotonergic 5-HT<sub>2C</sub> receptors. The 5-HT<sub>2C</sub> receptor antagonist activity of 6-MH is directly related to its chemical structure, which corresponds exactly with the pharmacophore skeleton of the compounds described in the patent number FR 2 797 644 of ADIR.

→ Lysergide (LSD) and 6-methoxy-harmalan (6-MH) are serotonergic 5-HT<sub>2C</sub> receptors antagonists



General formula of the 5-HT<sub>2C</sub> antagonists of the patent FR 2 797 644 (ADIR & Company)



6-methoxy-harmalan

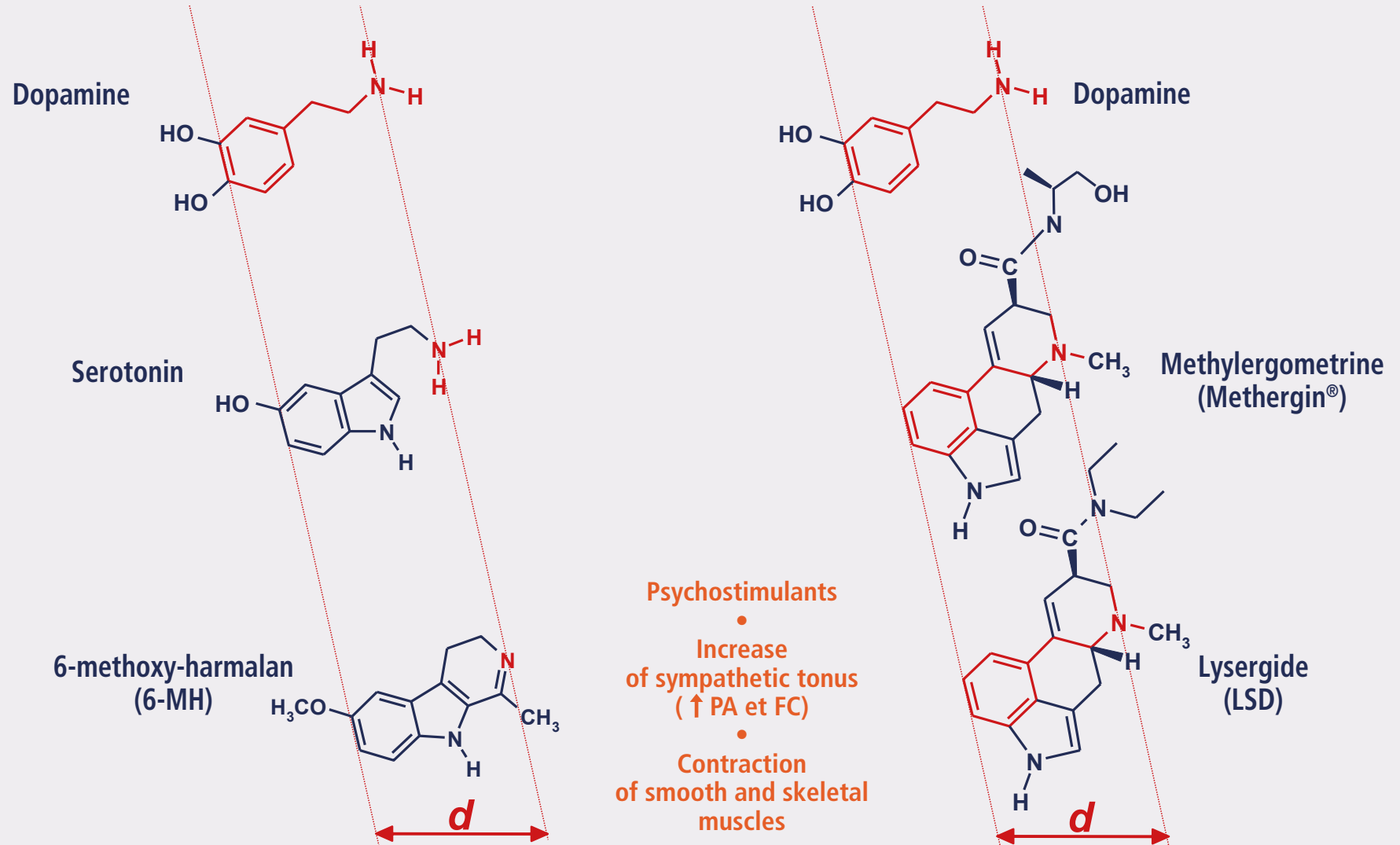
38

Methylergometrine is a hemisynthetic derivative of lysergic acid, like Lysergide. These two compounds have the same pharmacodynamic properties. Their oxytocic properties, in correlation with their antagonistic effects on dopaminergic receptors, have a medical use in obstetrics.

→ The oxytocic properties of LSD and 6-MH  
find a medical use in obstetrics with Methylergometrine



Stereochemical configurations and molecular sizes of 6-methoxy-harmalan, serotonin, dopamine, Lysergide and Methylergometrine



# 39

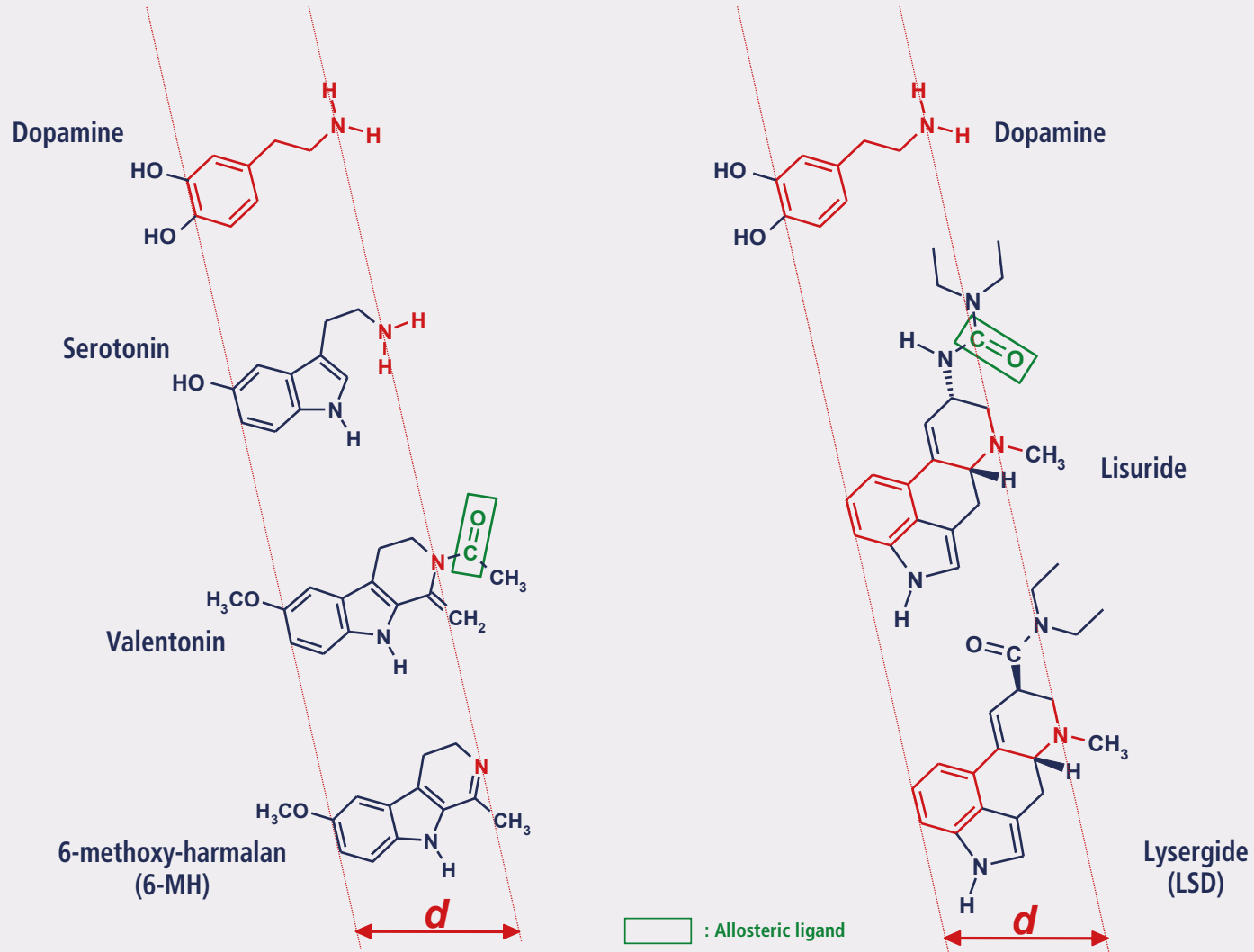
As seen before, the addition of an acetyl group (-CO-CH<sub>3</sub>), on the nitrogen (N) atom of 6-MH, has for consequence an inversion of its pharmacodynamic activities, as shown with Valentonin. In this hormone, the carbonyl (C=O) of the acetyl group bound on the nitrogen atom (N) constitutes an allosteric ligand, by virtue of which Valentonin activates 5-HT<sub>2C</sub>, α<sub>2</sub>, and D<sub>1</sub>/D<sub>2</sub> receptors by allosteric modulation.

In the same way, when passing from Lysergide (LSD) to Lisuride, whose lysergic acid carbonyl group (C=O) has a favorable position and orientation for allosteric stimulation of dopaminergic D<sub>1</sub> and D<sub>2</sub> receptors, we get an antiparkinsonian and antiprolactin medication.

→ From Lysergide to antiparkinsonian ergoline derivatives  
 addition of an allosteric ligand in favorable position



Stereochemical configurations and molecular sizes of Valentonin, dopamine, and ergoline-derived antiparkinsonians



# 40

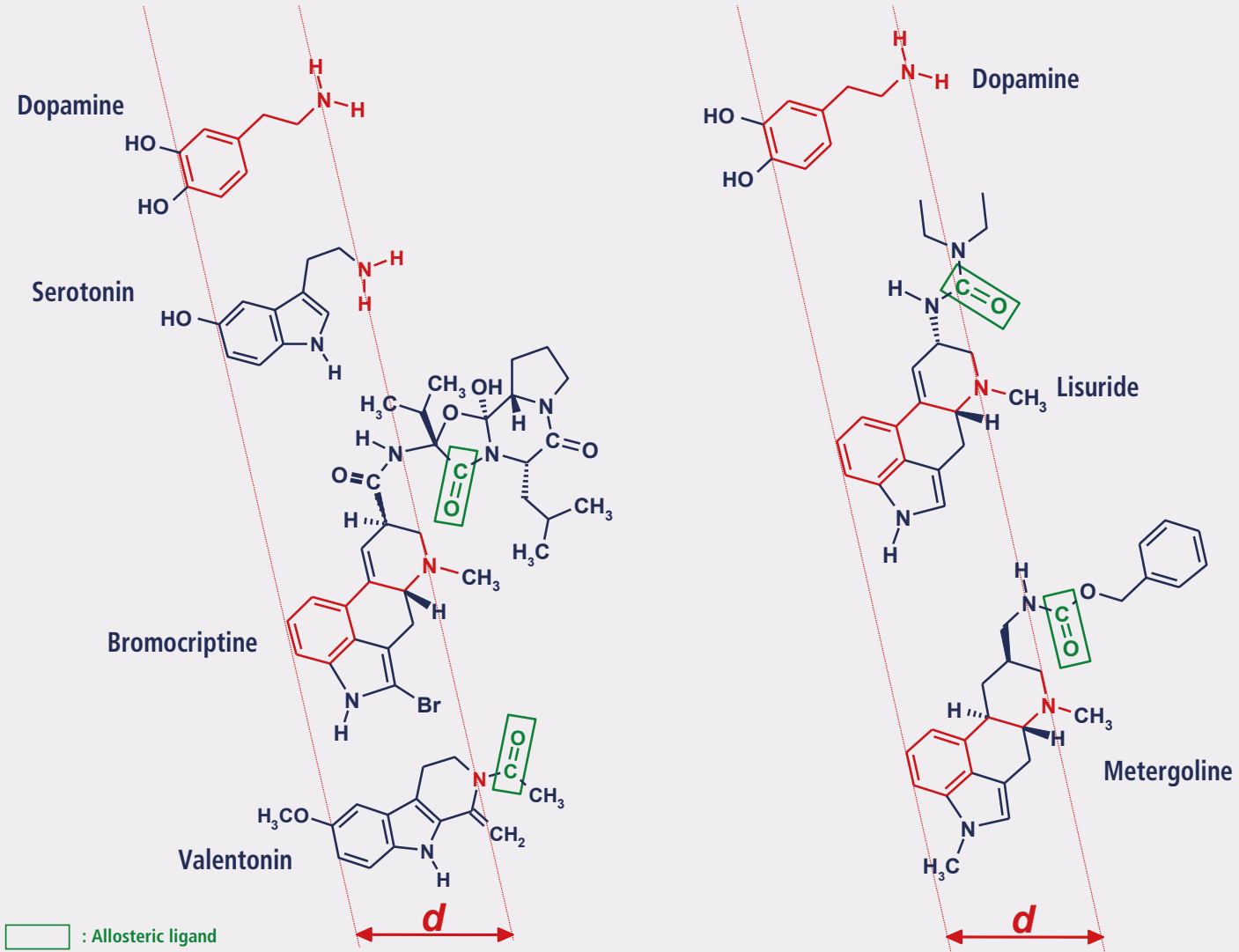
The ergoline-derived  $D_1/D_2$  dopaminergic agonists (Lisuride, Metergoline, Bromocriptine), used in the treatment of Parkinson's disease, owe their dopaminergic activities to their valentnergic properties, in correlation with their chemical structures. Because they have in their chemical structure the pharmacophore characteristics of Valentonin.

Their side effects include drowsiness and hypotension, which are valentnergic properties.

→ The Valentonin and ergoline derived antiparkinsonians:  
allosteric activators at  $D_1/D_2$ ,  $\alpha_2$ , and  $5-HT_{2C}$  receptors



Stereochemical configurations and molecular sizes of ergoline-derived antiparkinsonians



# 41

Clonidine and its analogues (antihypertensive noradrenergic  $\alpha_2$  receptor agonists) are valentnergics

Valentnergic's pharmacological actions provide an explanation not only of the heretofore unexplained action of Clonidine and its analogues on sympathetic tonus (lower BP and HR) used in the treatment of hypertension (noradrenergic  $\alpha_2$  agonist with CNS action) but also the drowsiness side effects of this class of medications (serotonergic 5-HT<sub>2C</sub> agonists).

→ Valentonin, 6-methoxy-harmalan, Clonidine and its analogues:  
allosteric activators of central noradrenergic  $\alpha_2$  receptors



Stereochemical configurations  
and molecular sizes of Clonidine,  
its analogues, and Valentonin

