

**La trypanosomose humaine africaine:
sommeil, sécrétion hormonale et
monoxyde d'azote (NO)
Human African trypanosomiasis: sleep,
hormonal secretion, nitric oxide (NO)
Alain Buguet**

Collaborations

France: Institut de médecine tropicale du service de santé des armées, Marseille; Neurobiologie des états de vigilance, EA3734, Lyon; Institut de neurologie tropicale, EA3174, Limoges; Laboratoire de parasitologie, EA3677, Bordeaux; Centre de recherches du service de santé des armées, La Tronche;
Afrique: Centre international de recherches médicales de Franceville, Gabon; Projet de recherches cliniques sur la trypanosomiase, Daloa, Côte d'Ivoire; Laboratoire de physiologie, Abidjan, Côte d'Ivoire; Ministère de la santé, Brazzaville, Congo;
Instituto de Combate e Controlo das Tripanossomiasas, Luanda, Angola;
Canada: Defense Research Establishment Toronto.

- Mackensie, 1890 : by day as well as by night, patients sleep in short bouts alternating with short wake episodes; the patients do not sleep more than healthy persons;
- Lhermitte, 1910: narcolepsy of sleeping sickness; true sleep attacks;
- Manson, 1912: sleepy by day, restless by night.

Circadian rhythm disorder of the sleep-wake cycle?

1959

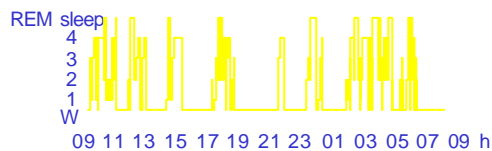
- Jouvet: polysomnography and paradoxical sleep;
 - Halberg: circadian rhythms .
- 1989**
- Buguet et al.: first 24-hour recording.

Polysomnography (PSG)

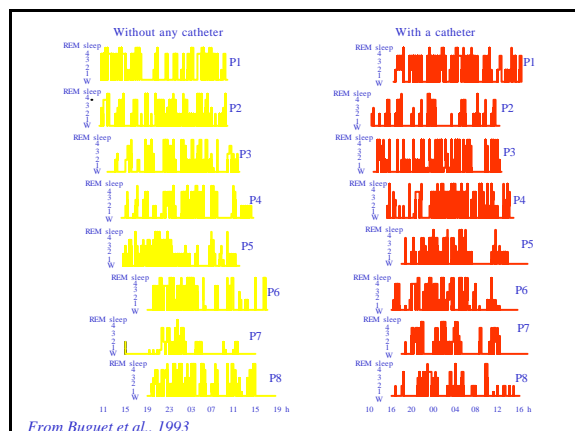
- Electroencephalogram
- Electro-oculogram
- Electromyogram
- Portable recorder

Healthy volunteer recorded in Angola: normal sleep patterns and distribution.

**First meningo-encephalitic patient
examined in Niamey, Niger**



From Buguet et al., 1989

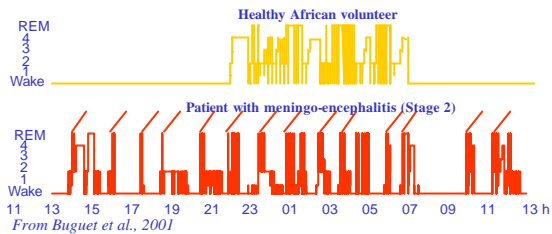


From Buguet et al., 1993

The PSG syndrome of sleeping sickness

The PSG syndrome of sleeping sickness was described by our team in Stage 2 patients. It includes:

- An alteration of the nychthemeral distribution of sleep and wakefulness, proportional to the severity of the symptoms.
- An alteration of the structure of sleep episodes, with the abnormal occurrence of SOREM.



The PSG syndrome is **pathological** and signs an **alteration of brain functioning**. The symptoms are also observed in a number of other pathological states, such as narcolepsy-cataplexy, depressive states, multiple sclerosis, Alzheimer's disease, Prader-Willi syndrome.

From Buguet et al., 2004

Is the PSG syndrome related to the stage of sleeping sickness?

Investigations in Angola

Recordings: Viana (passive) and Kuenza Norte (active field trial) in Angola.

35 patients classed following clinical and biological criteria:

- 24 at Stage 1;
- 11 at Stage 2.

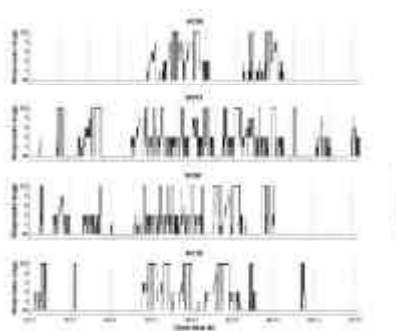
15 healthy volunteers.

Results

- All patients at Stage 2 exhibited the complete PSG syndrome (nychthemeral and structural);
- Among the 24 patients at Stage 1 and intermediary stage, 13 showed abnormal PSG signs;
- None of the 15 healthy volunteers did show any PSG abnormality.

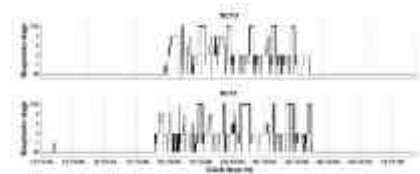
Stage 2 patients with the complete polysomnographic syndrome

In Angola



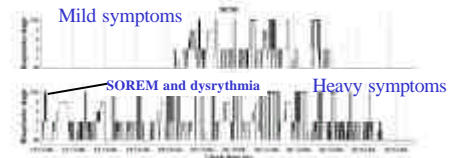
Stage 1 patients without any polysomnographic symptom

In Angola



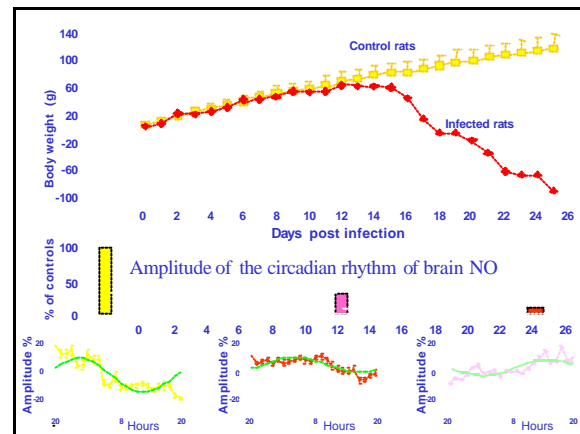
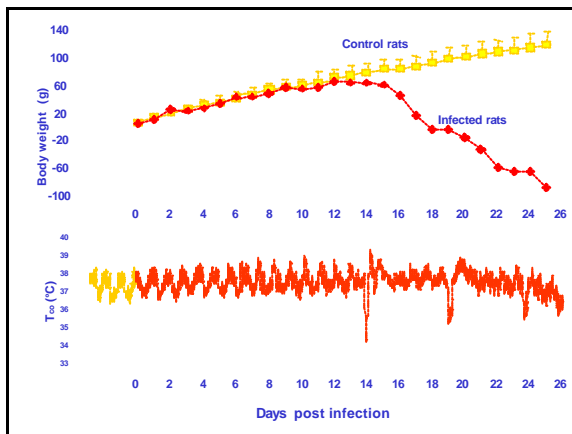
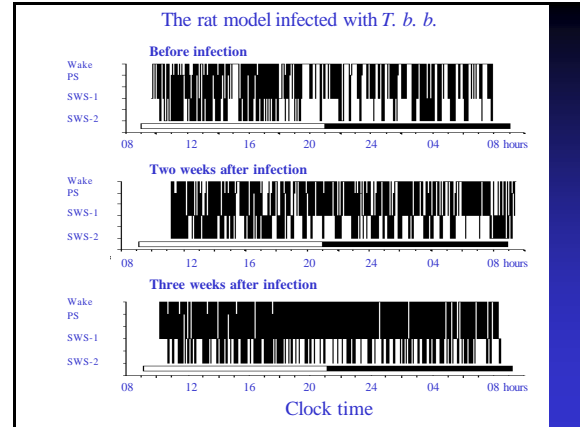
Stage 1 patients with polysomnographic symptoms

Mild symptoms

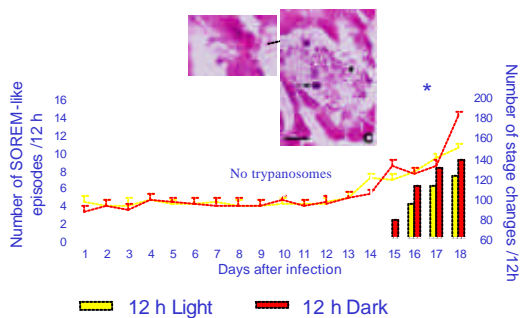


Conclusion

- Does the PSG syndrome sign the passage of the trypanosome in the central nervous system?
- To answer this question, an animal model of human African trypanosomiasis was developed.



From Darsaud et al., *Exp. Med. Biol.*, 2003



From Darsaud et al., *Sleep*, 2004

CONCLUSION

- In the rat infected with *T. b. brucei*, a complex hypothalamic syndrome occurs between the 10th and 14th days after infection.
- The hypothalamic syndrome is concomitant with the disturbances in biological rhythms (NO in the rat, hormones in man).
- The hypothalamic syndrome is concomitant with the discovery of trypanosomes inside the central nervous system.
- This syndrome is also concomitant with the apparition of the polysomnographic syndrome, especially the occurrence of SOREM.

- We therefore believe that the occurrence of SOREM, which is observed in man at stage 2 but also in stage 1 patients, is also concomitant to the penetration of trypanosomes in the central nervous system.
- We propose to pursue the research on polysomnography (PSG) in a follow-up study of patients at stage 1 after treatment with pentamidine.
- We expect to see a difference in treatment efficacy:
 - ✓ successful in Stage 1 patients without any PSG sign;
 - ✓ not efficient in Stage 1 patients with PSG signs.

Hormonal secretion in sleeping sickness

Hormonal secretion classification

Van Cauter and Refetoff, J. *Endocrinol. Invest.*, 1985; Van Cauter et al., *Serono Symposia*, 1990

The temporal organization of the 24-hour hormonal rhythms is either:

- controlled by an internal circadian clock: **cortisol** and **melatonin**;
- or strongly influenced by the sleep-wake cycle: type is represented by **prolactin** or **GH**;
- or reflecting both mechanisms: nocturnal oscillations of **renin**.

Blood samples in healthy volunteers

Hourly

Every 10 min



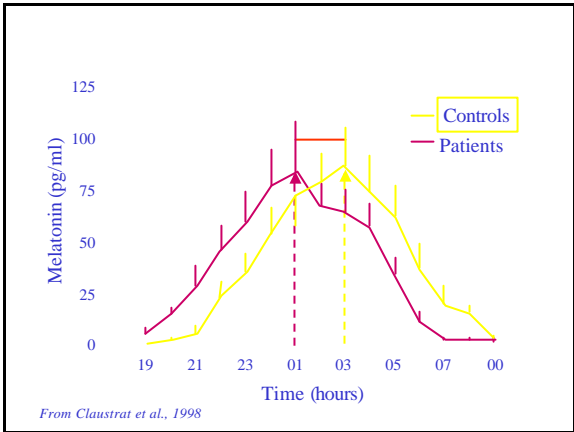
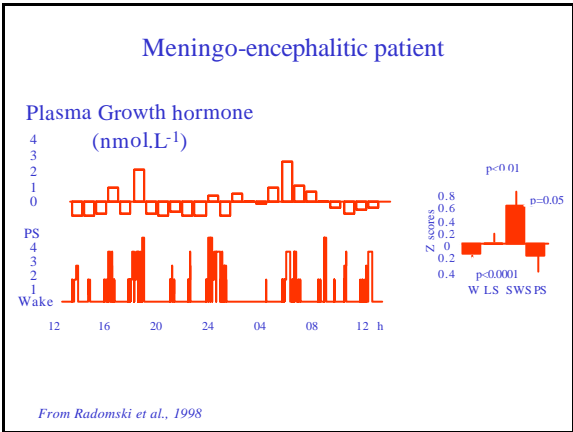
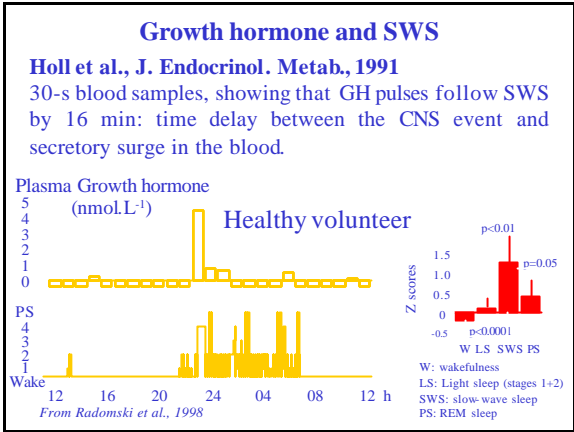
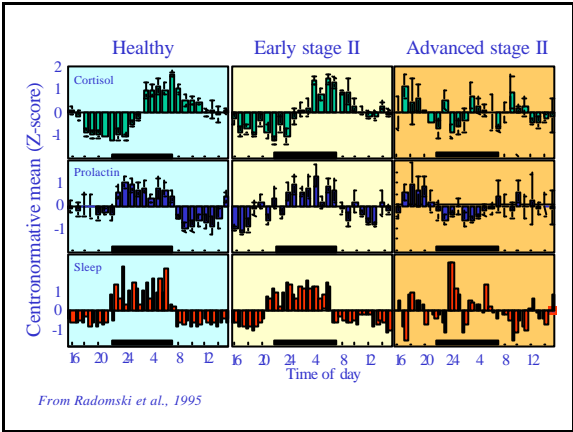
Blood samples in patients

Hourly

Every 10 min



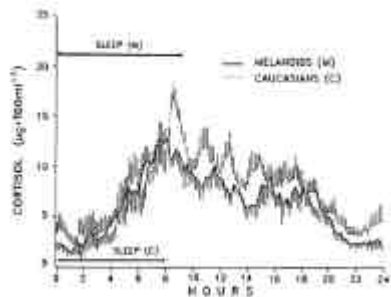
Hourly blood samples



Blood samples every 10 min

Brandenberger, J. Sleep Res., 1993
The 24-hour hormonal rhythms arise from a succession of secretory pulses of varying magnitude.
Therefore, influence of ultradian over circadian mechanisms : need to collect blood frequently.

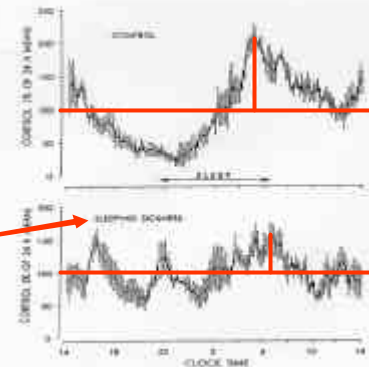
Comparison between Caucasians in Europe and Melanoids in Africa



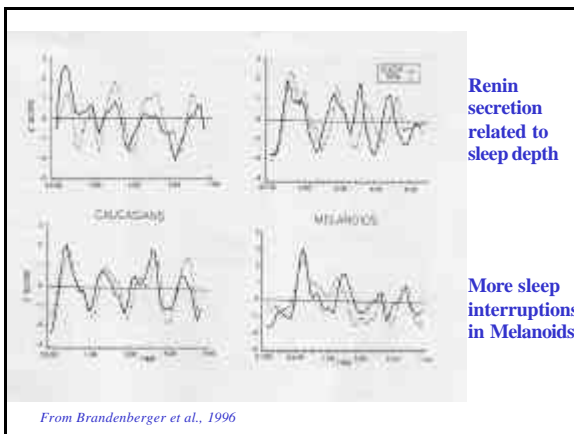
From Brandenberger et al., 1996

Healthy Africans vs African patients

Dysrhythmia



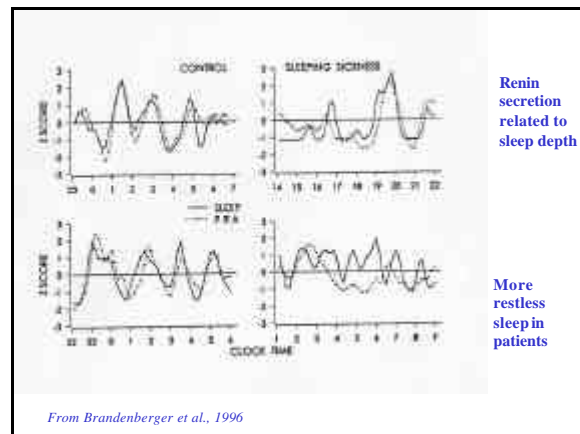
From Brandenberger et al., 1996



Renin secretion related to sleep depth

More sleep interruptions in Melanoids

From Brandenberger et al., 1996



Renin secretion related to sleep depth

More restless sleep in patients

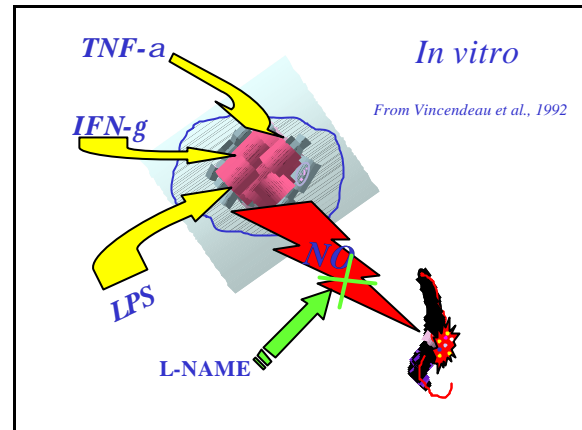
From Brandenberger et al., 1996

Conclusion

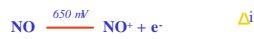
- The 24-hour distribution of hormonal secretion is disturbed in cortisol, prolactin, GH and also renin.
- But the internal relationship between hormonal secretion and the sleep-wake cycle is maintained.
- Melatonin rhythm is affected through a phase advance, but not a dysrhythmic alteration.

- Therefore, the clock itself is not directly affected: no disturbance in the 24-hour rhythmicity of melatonin.
- The PSG syndrome may be related to a dysfunction of serotonergic raphé neurones: this is supported by the occurrence of SOREM episodes and that of a phase advance in the melatonin acrophase.
- **However, the starter has to be a rapidly active messenger: why not NO?**

Implication of NO in HAT



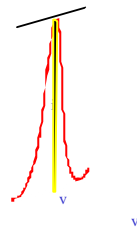
Voltammetric measurement of NO



Measurement of the oxidation current between 400 and 1400 mV.

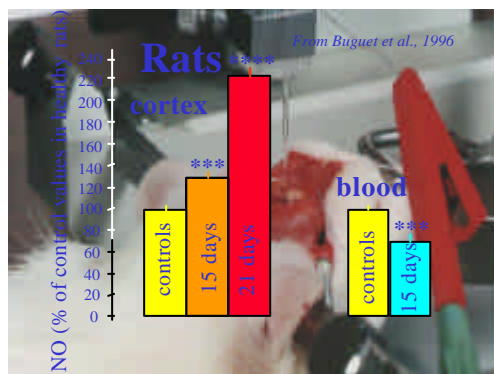
V: applied potential.

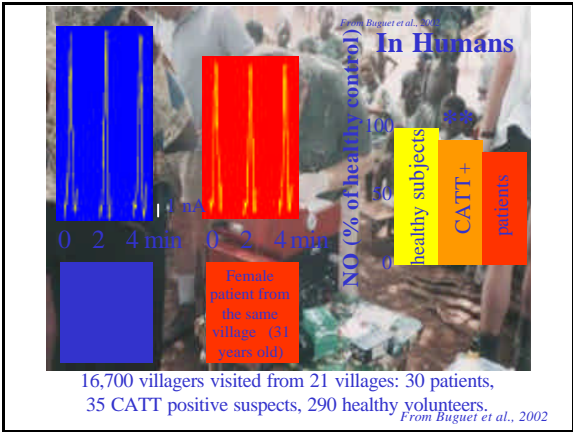
i: measured intensity of the current, which is proportional to NO concentration.



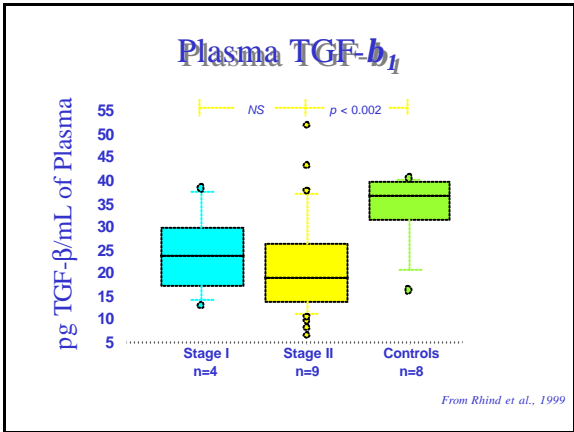
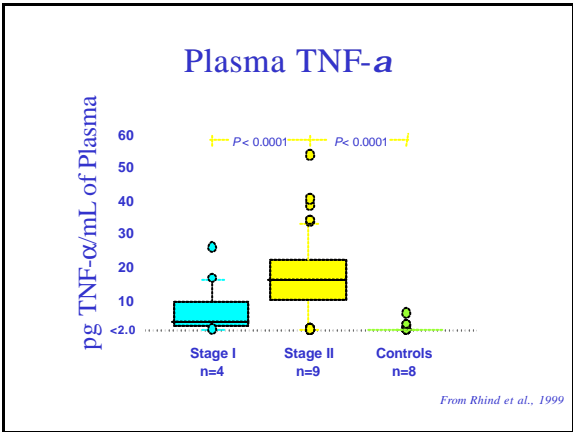
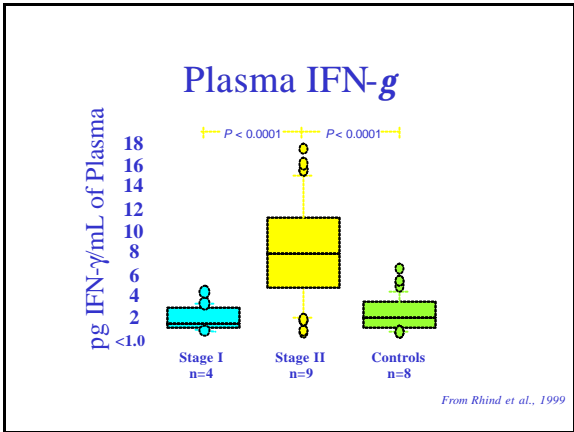
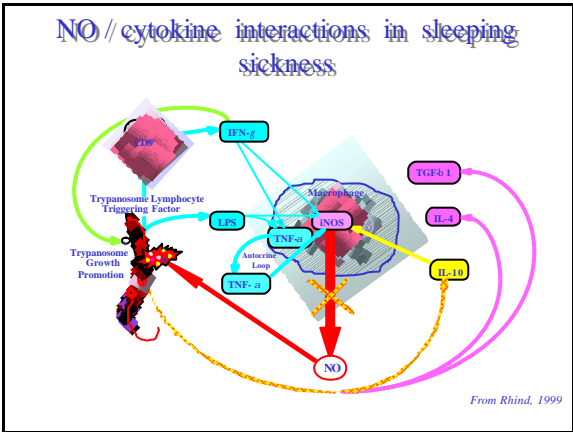
From Cesputio et al., 1996

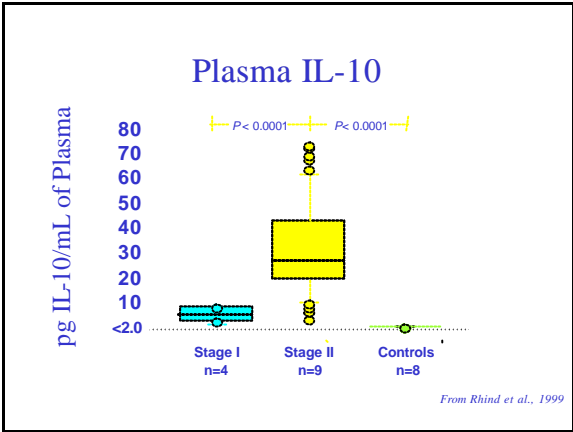
In trypanosomiasis, studies were conducted in the brain and blood of animals and in the blood of humans





Mechanisms involved in the blood depression in NO





Gobert et al., 2000: L-arginine modulates parasite killing by NO

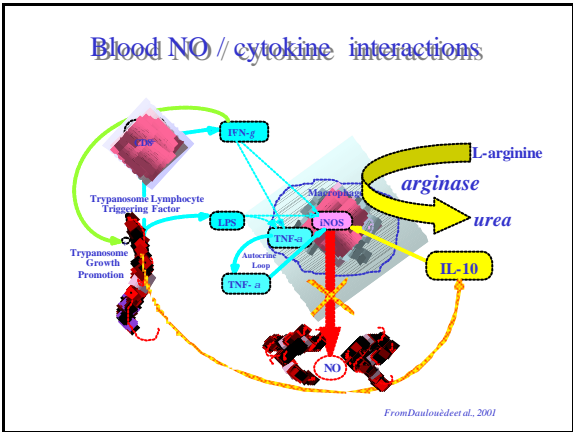
In infected mice: plasma L-arginine is decreased

In the host, L-arginine is the substrate of NOS and arginase

L-arginine is used by trypanosomes (polyamine, DNA and trypanothione synthesis)

In infected mice: L-arginine i.p. restores NO Production and trypanolysis

From Vincendeau et al., 2004




Mechanisms involved in the brain increase in NO


NOS activity is increased in the brain of infected mice

From Keita et al., 2000

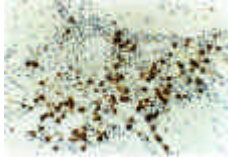
NADPH-diaphorase activity in the brain parenchyma of a mouse infected by *T. b. brucei* since 11 months.



Where in the brain?



Nitrotyrosine immunostaining (×200) of inflammatory cells in perivascular infiltrates and in the cerebral parenchyma of a mouse infected for 11 months.



Nitrotyrosine immunostaining (×200) of inflammatory cells in the meninges of a mouse infected since 2 months.

From Keita et al., 2000

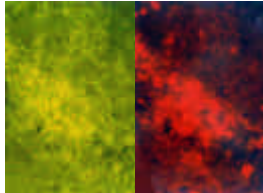
From Keita et al., 2000

Increased NOS activity is due to iNOS



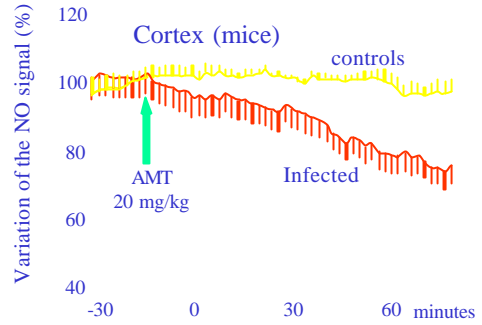
Immunohisto-chemistry of iNOS ($\times 200$) in the meninges and the brain of a mouse infected since 5 weeks.

Fluorescein(FITC)-immunostaining of macrophagic cells (yellow-green)...

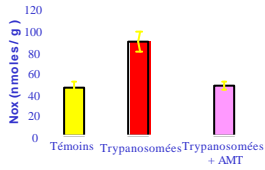


... and rhodamine-immunostaining of iNOS positive cells (red) in the same field of a CNS section from a *T. b. brucei*-infected mouse (5 weeks post-infection).

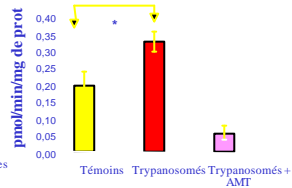
Pharmacology



Cortical Nox concentration after AMT



Cortical iNOS activity after AMT



In conclusion

The duality of action of NO in the blood and in the brain is due to changes in the activity of iNOS.

These changes may explain:

- why trypanosomes can escape the host's nitridergic reactions and multiply in the bloodstream;
- and how the accumulation of NO in the brain may lead to damages of the blood-brain barrier and neurons.