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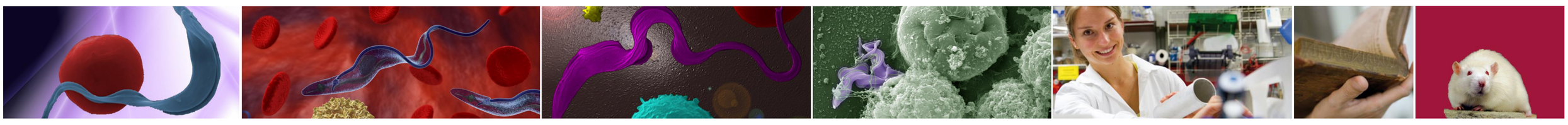

Cyclical appearance of African trypanosomes in the cerebrospinal fluid: new insights in how trypanosomes enter the CNS

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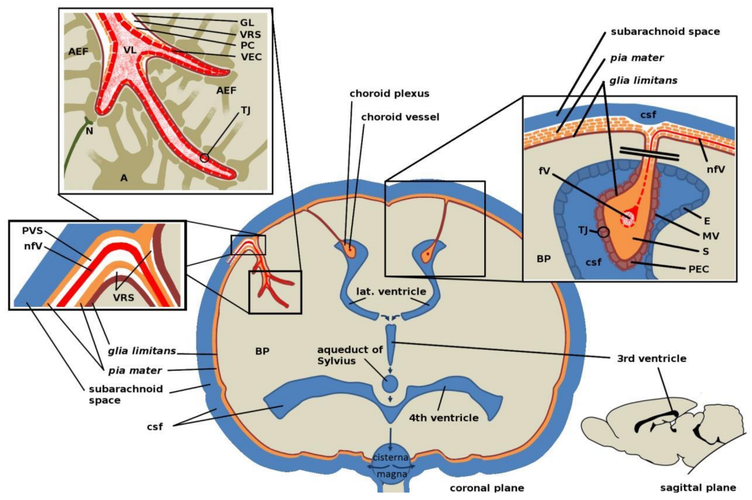
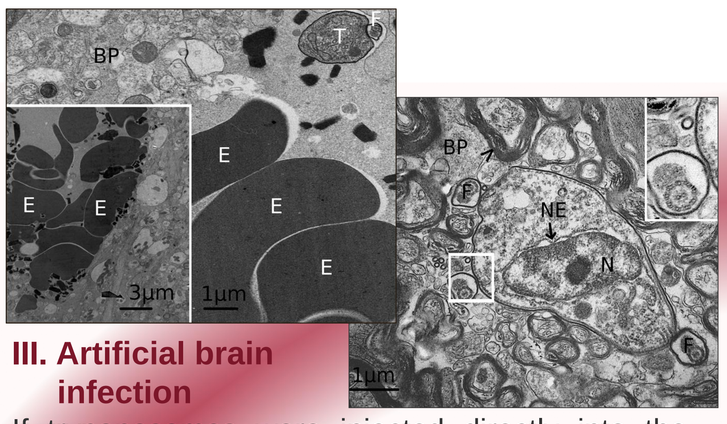
Abstract

Trypanosomes induce sleeping sickness. They are transmitted during a blood meal of the tsetse fly and appear primarily in the blood and lymphatic system, before they enter the central nervous system (CNS). During this 2nd stage, the parasite induces a deregulation of the sleep-wake-cycles. It was thus a logical assumption that trypanosomes cross the blood-brain barrier (BBB) and nestle somewhere between the brain cells. The brain, however, is completely covered by a dense barrier, the so-called glia limitans that surrounds the brain and covers the blood vessels, with the latter being the literal BBB. We show here that trypanosomes cannot develop inside the brain parenchyma. Indeed, when injected directly into the brain, this will not lead to an infection. This observation makes it necessary to reconsider the common opinion that the parasites cross the BBB and settle inside the brain, whereas factually crossing the blood-CSF barrier (BCB) seems much more important for pathogenesis. The BCB is formed by the choroid plexus, i.e. the part of the ventricles where CSF (cerebrospinal fluid) is formed. Anatomically, the ventricle system lies outside the brain and extends to the three meninges, which surround the brain outside the glia limitans. Our data show that trypanosomes infiltrate the pia mater, the innermost one of the meninges, and use this space as a refuge. In addition, we demonstrate cyclical infections of CSF. These results put new lights on the infection strategy and open new avenues for treatment and drug development. Effective medicals do not have to cross the BBB but the BCB or could be administered intrathecally into the meningeal space.



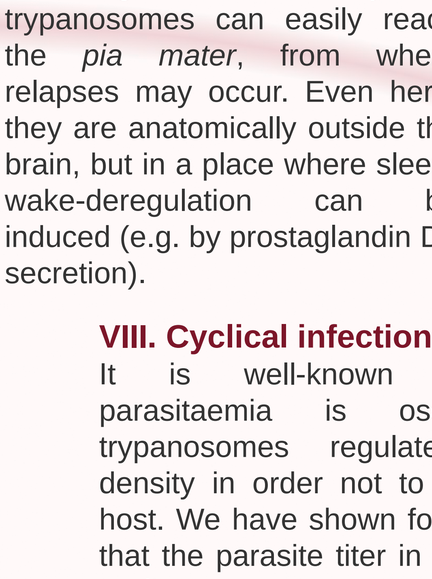
Several ways into the brain

Blood and brain are separated by a tight barrier of endothelial cells, a basal membrane and astrocyte endfeet (left). The blood-CSF barrier consists of fenestrated vessel endothelium and choroid plexus epithelial cells (right).



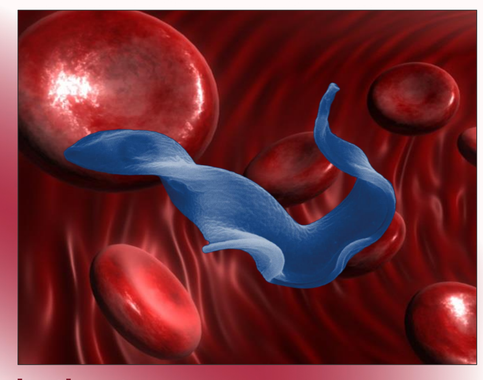
III. Artificial brain infection

If trypanosomes were injected directly into the brain parenchyma (e.g. striatum, left), they could not develop and disintegrated within 3 days. In contrast, parasites injected into the ventricle lead to a blood infection. That means, that –even if trypanosomes *could* cross the BBB- this would not lead to an brain infection.



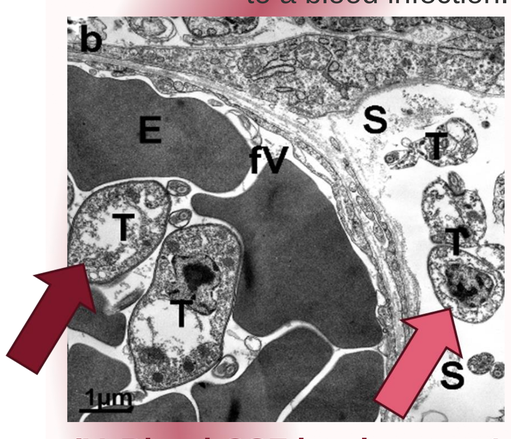
II. Blood-brain barrier

The parasites can be easily found within blood vessels, but we could never locate them within grey matter brain parenchyma in our model system (Wistar Rats and *T. brucei brucei).*



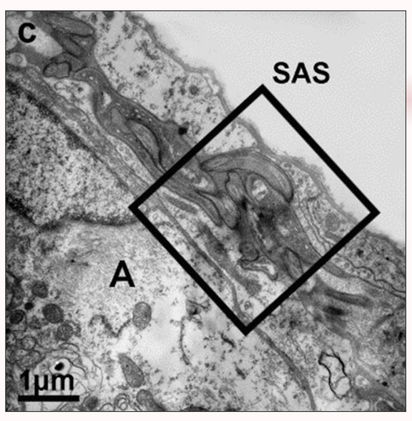
I. Haemolymphatic stage

Trypanosomes are transmitted during a blood meal of the tsetse fly to the mammalian host. At this time they appear primarily in the blood and lymphatic system.



IV. Blood-CSF barrier, part 1

We could follow the actual route of trypanosomes by electron microscopy. Dark red arrow: Parasites within a blood vessel of the choroid plexus. Light red arrow: Trypanosomes that have crossed the fenestrated endothelium and are located inside the connective tissue of the choroid plexus stroma.

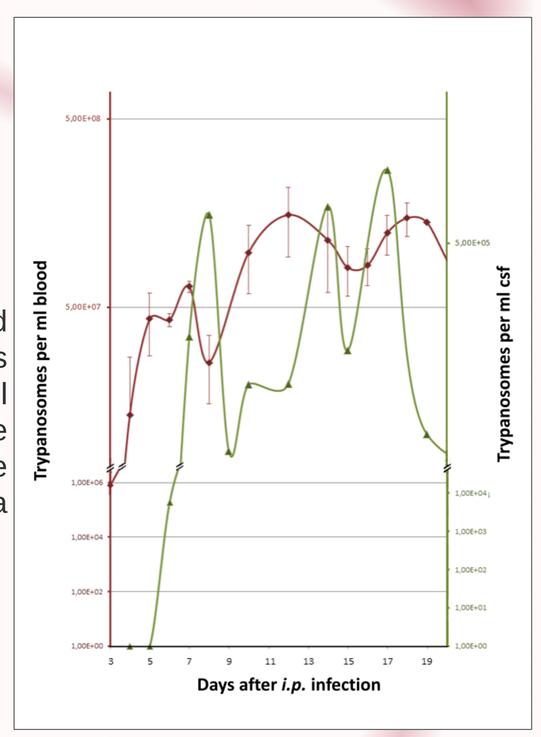


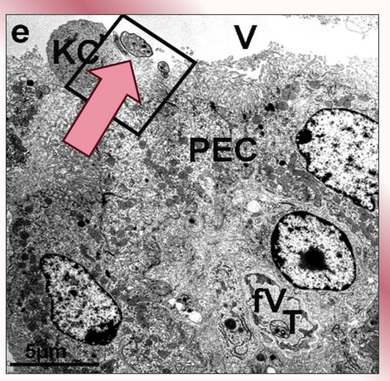
IX. Settling in the *pia mater*

Following the ventricle system, trypanosomes can easily reach the *pia mater*, from where relapses may occur. Even here, they are anatomically outside the brain, but in a place where sleep-wake-deregulation can be induced (e.g. by prostaglandin D2 secretion).

VIII. Cyclical infection of CSF

It is well-known that blood parasitaemia is oscillating, as trypanosomes regulate their cell density in order not to overgrow the host. We have shown for the first time that the parasite titer in CSF is also a cyclic process.



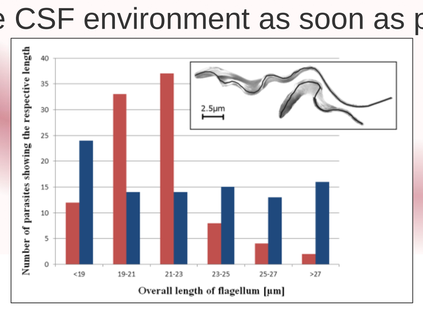


V. Blood-CSF barrier, part 2

The actual blood-CSF barrier is formed by plexus epithelial cells which are interconnected by tight junctions. Arrow: Trypanosome that has overcome this cell layer to reach the ventricle system.

VI. Length of trypanosomes in blood and brain

Trypanosomes that had been isolated from the brain (35 days *post-infectionem*, blue bars) are much longer than those isolated from blood (4 days *p.i.*, red bars). Furthermore, they are extremely agile and swim in a directed way, what could be an essential feature in order to leave the hostile CSF environment as soon as possible.



VII. CSF, a hostile environment

Trypanosomes will die within CSF (human or rat) within 30 hours, compared to 60 hours in HMI-9 medium. This is not due to a lack of nutrients, but indicates that there are toxic substances (e.g. neuropeptides).