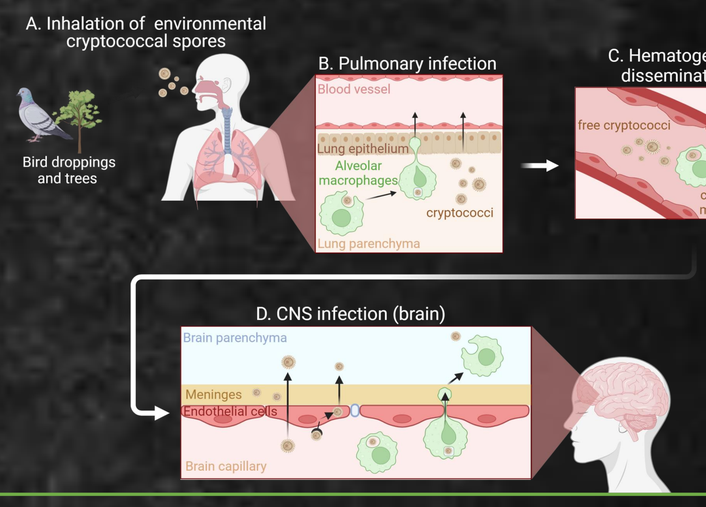
Proof-of-concept longitudinal follow up of cryptococcosis in mice: Assessment of host-pathogen interaction by using magnetic resonance imaging and multi-photon microscopy

L. Roosen[1], D. Maes[1], L. Musetta[1], M. Nobis[2], U. Himmelreich[1]

[1] *Biomedical MRI unit, Department Imaging and Pathology, KU Leuven, Leuven, Belgium*
[2] *Intravital Imaging Expertise Center, VIB-KU Leuven Center for Cancer Biology, Leuven, Belgium*

1. Introduction

Cryptococcus neoformans (**CN**) and *Cryptococcus gattii* are harmful to humans, causing potentially life-threatening disease (1). Infection occurs when the pathogen is inhaled, leading to **lung disease**. Following this, cryptococci can then **spread to the central nervous system**, causing meningoencephalitis and/or focal lesions (1,2,3).



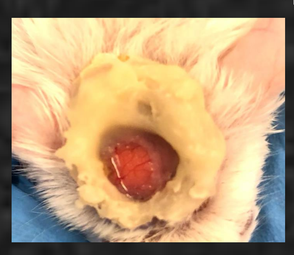
2. Problem definition and aim

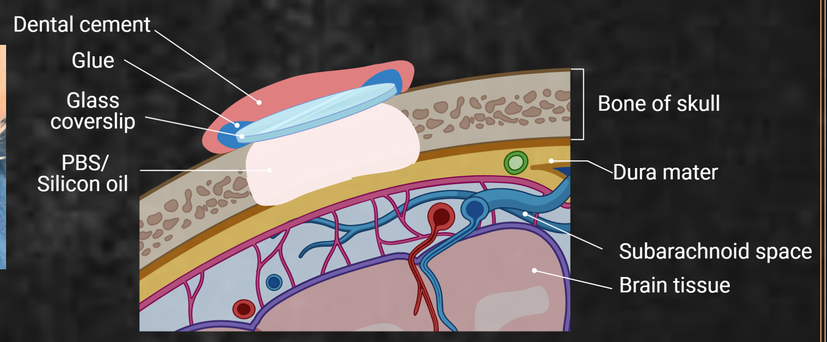
The exact mechanism of how the fungus **crosses the blood-brain barrier** remains **unclear**, encompassing three hypotheses:

- Paracytosis
- Transcytosis
- Trojan Horse (circulating macrophages as carrier cells)

Our study employs Magnetic Resonance Imaging (**MRI**) and Two-Photon Intravital Microscopy (**2P-IVM**) with optical cranial window surgery to track disease progression and brain dissemination. Using a red fluorescent CN KN99α E2-Crimson strain, we **optimize** cranial window surgery to establish lesion formation underneath.

3. Methodology





Groups parameter optimization implantation optical cranial window:

(A)

	Meninges	No meninges
Right window + Silicon oil	N=2	N=2

(B)

	Silicon oil	PBS
Left window	N=3	N=3
Right window	N=4	N=3

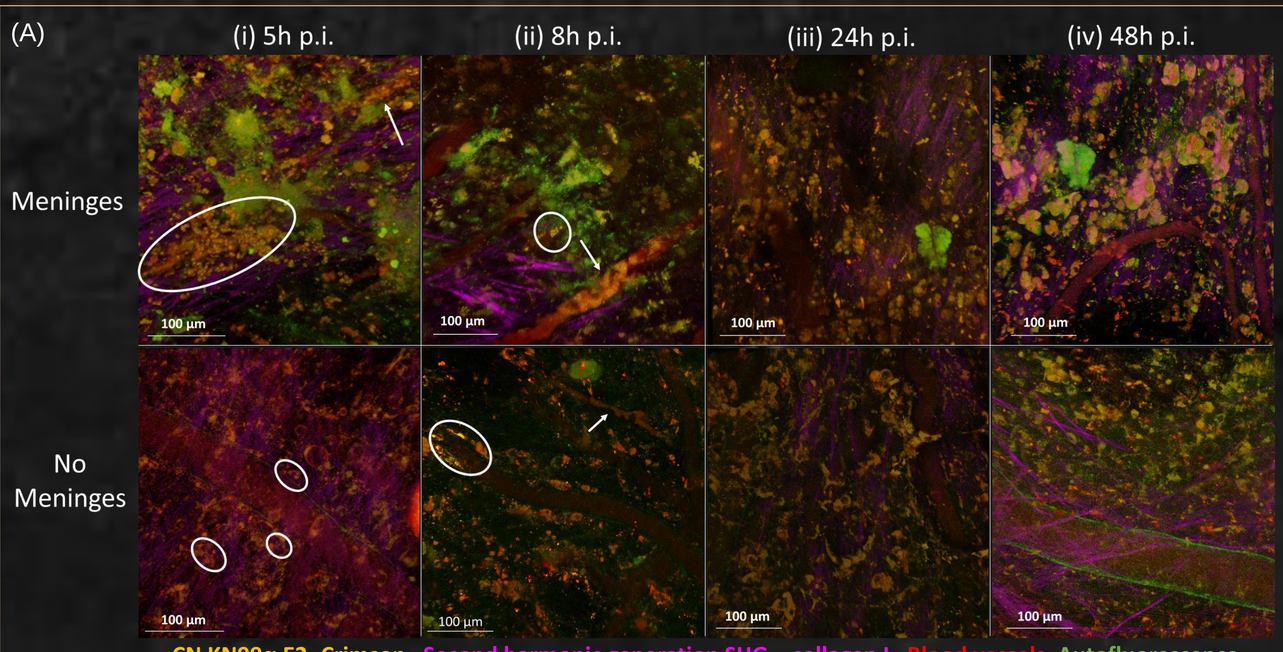
Proof-of-principle experiments:
Surgical implantation of MRI-compatible cranial optical imaging windows in female BALB/c mice

→ i.v. injection CN KN99α E2-Crimson (50 000 cells/100µL) 14 days post-surgery:

(1) 2P-IVM up to 8h or 24/48h: cellular level of early and late seeding in the brain

(2) MR images at day 4 and day 7 post infection to confirm lesion formation at cortex, underneath the skull

4. Results



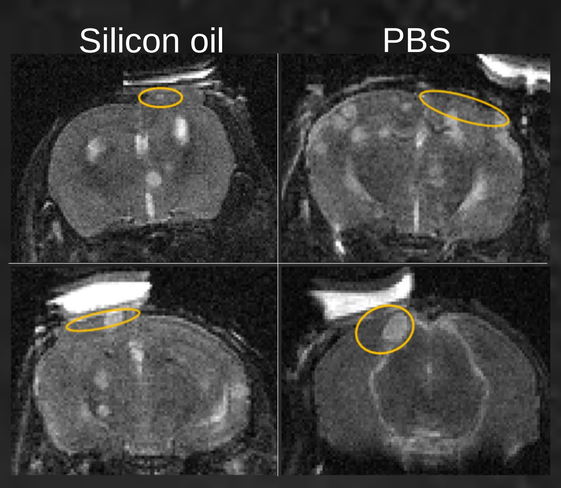
2P-IVM images capture cryptococcal infection progression in representative mice, with retained meninges or surgically removed meninges, up to (i) 5 hours, (ii) 8 hours, (ii) 24 hours, and (iv) 48 hours post infection (p.i.) of CN KN99α E2-Crimson

(i) 5 hours p.i.: In the mouse with intact meninges, a dense cluster of cryptococcal cells (white circle) is visible, with blood vessels occluded by cryptococci (white arrow). In the mouse without meninges, smaller cryptococcal cell clusters (white circle) are noted.

(ii) 8 hours p.i.: Both cohorts of mice (with and without meninges) show smaller cryptococcal clusters (white circles) and blood vessel occlusions (white arrows).

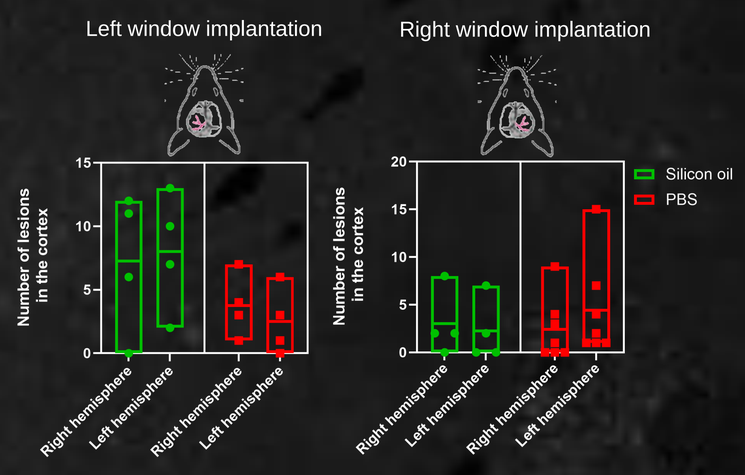
(iii) 24 hours p.i.: Numerous cryptococcal clusters are observed in both mice, with individual cryptococcal cells appearing larger.

(iv) 48 hours p.i.: Cryptococcal cells increase in size, forming giant Titan cells (≥10 µm) and producing smaller daughter cells (~4 µm) in both groups.



Axial T_2-weighted MRI scans showing representative images from four groups of mice infected with CN KN99α E2-Crimson

Examples highlight lesion formation in the region of interest (yellow circles) observed at 7 days post injection.



Analysis of cortical lesion formation in four experimental groups was conducted to optimize conditions (silicon oil vs. PBS) for optical cranial window implantation on the left vs. right parietal plate

No consistent trend in lesion formation was observed between the left and right hemispheres with a left window or right window implantation.

Additionally, no significant differences were detected between silicon oil vs. PBS.

5. Conclusion

Development of a multimodal imaging platform to further investigate neurological cryptococcal dissemination and immune-related interactions within the central nervous systems at: cellular level (2P-IVM) and macroscopic level (MRI).

➔ Differentiate between a chance occurrence (e.g.: single cryptococcus encapsulated in a macrophage) vs. an event that triggers pathological processes (lesion formation).

Optimal parameters of the surgical optical cranial window implantation:
(A) Preserving meninges provides a more natural disease model
(B) Lesion formation is heterogeneous and unaffected by the window placement, or the compound used

Overall, this multimodal imaging platform provides a promising tool to deepen our understanding of cryptococcosis pathogenesis and offers a valuable preclinical framework for evaluating therapeutic strategies.

6. References

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