Robustness of adult neurogenesis maintains homeostasis and neutralises the early effects of intermittent fasting

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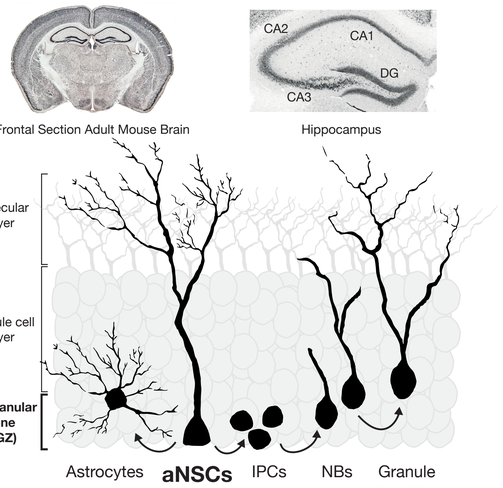
Introduction: Adult stem cells contribute to tissue maintenance and to its regeneration upon injury. With age, stem cell numbers and function decline, compromising homeostasis. Adult neural stem cells (aNSCs) reside only in restricted areas of the adult brain such as the dentate gyrus of the hippocampus. aNSCs transition between quiescent and active states and give rise to newly born neurons that integrate into the hippocampal circuit and modulate memory and emotions. Intermittent fasting (IF), known to extend life and healthspan, has been proposed to halt neural stem cell decline and increase neurogenesis, and therefore holds great potential as a strategy to improve cognitive ability and promote a healthier aging. We used lineage tracing and label retention to understand whether and at which stages IF regulates adult neurogenesis.

Does IF regulate aNSC behaviour and neurogenesis in the adult hippocampus?



The tools:

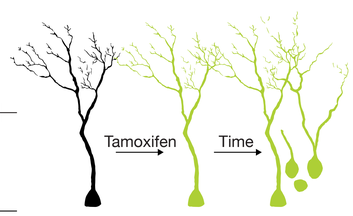
Lineage tracing

aNSC marker

Glast promoter — CreERT2

Reporter

Rosa26 promoter — STOP — YFP

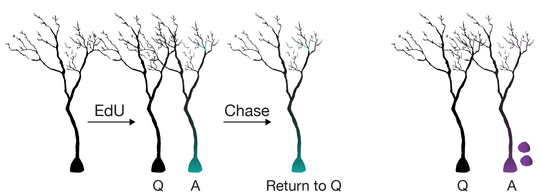


Label retention experiment

Thymidine analogue:
5-Ethynyl-2´-deoxyuridine (**EdU**)

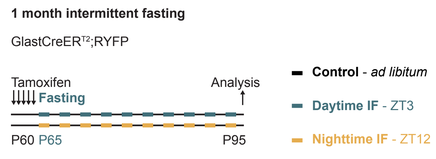
Proliferation

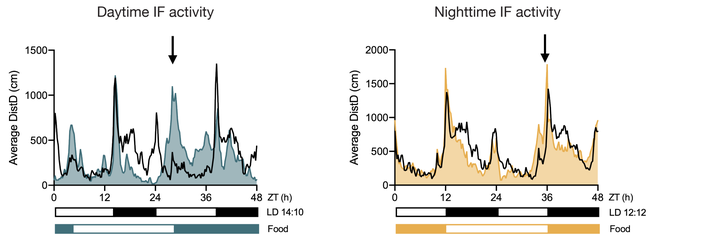
Ki67



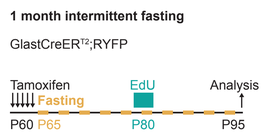
1. Morning refeeding time disrupts activity pattern

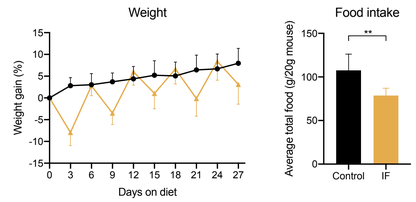
We fasted mice for 24h on alternate days for 1 month and used TSE Phenomaster metabolic cages to monitor their metabolic status and activity. Morning refeeding lead to a peak of activity during the light phase, when mice should be resting. To match the food-induced activity peak with the light/dark cues, we optimised our protocol and changed the time of refeeding to the evening, at the time when the lights went off.

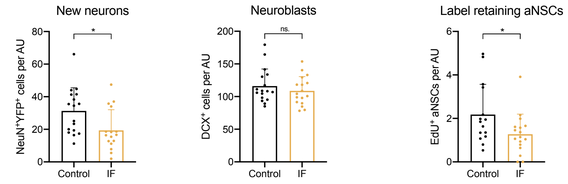




2. Short-term IF decreases the number of new neurons and alters aNSC behaviour





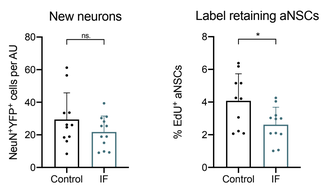


Proliferation?
Return to quiescence?

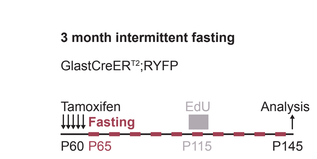
Analyse EdU incorporation

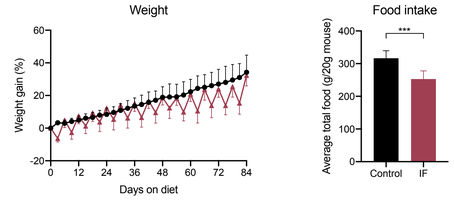
We used lineage tracing and a label retention protocol to characterise adult neurogenesis in response to IF and found that the number of new neurons produced after 1 month of IF was reduced compared to *ad libitum* fed mice. However, neuroblast production remained unafected at the time of analysis. We also found that fewer label retaining aNSCs were present at the end of the treatment. This could indicate lower proliferation or that fewer aNSCs returned to quiescence.

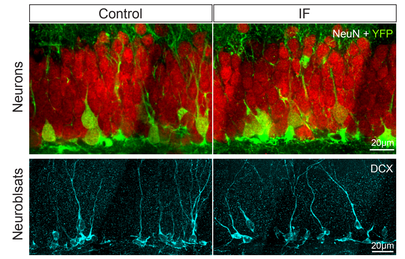
3. Neurogenesis responds similarly to daytime and nighttime IF

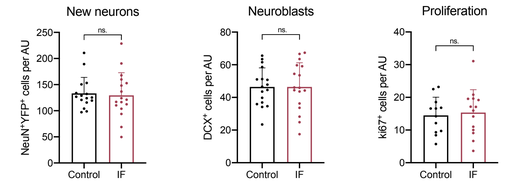


4. Adult neurogenesis is restored after 3 months of IF

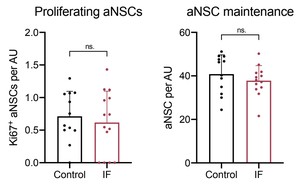


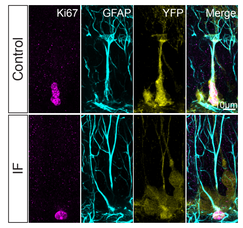




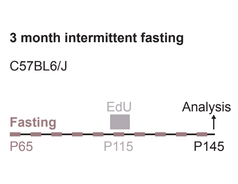


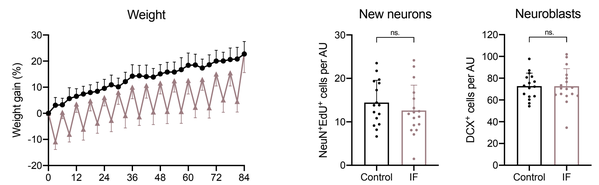
To our surprise, even though mice repeatedly lost and regained weight and underwent a mild caloric restriction, 3 months of IF did not increase adult neurogenesis. The total number of new neurons generated throughout the three months was the same in control and IF mice. Ongoing neurogenesis also remained unafected as seen by the number of neuroblasts and proliferation. The inicial change in aNSC behaviour did not have consequences on aNSC manintenance or activity.





5. Tamoxifen or strain don‘t interfere with the response to IF





We repeated the experiment in C57BL6/J mice and without tamoxifen to see if these variables affecte the response of adult neurogenesis to IF. Without lineage tracing, we evaluated neurogenesis by staining neuroblasts and identified new-born neurons as matture neurons (NeuN$^+$) that had incorporated EdU a month before the analysis. IF did not affect adult neurogeneis in this conditions either.

Conclusions and outlook

1. IF reeding time affects activity pattern but not neurogenesis.
2. aNSCs can sense and respond to IF.
3. They can adjust their behaviour and buffer inicial effects on neurogenesis.

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1. Characterise EdU incorporation to understand aNSC behaviour.
2. Investigate if longer IF affects adult neurogenesis.
3. Explore the consequences of refeeding periods after IF.

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Legend

aNSC: adult neural stem cell, IPC: intermediate progenitor cell, NB: neuroblast, IF: intermittent fasting
t-tests: ns.>0.05, * <0.05, **<0.01.