Are fish Poisson? About the neurogenesis of the zebrafish using spatial point process analysis

Felix Cheysson
Joint work with Nicolas Dray, Laure Mancini (Institut Pasteur),
Udi Binshtok, David Sprinzak (Tel-Aviv University),
and more

Université Gustave Eiffel, CNRS, UMR 8050, **LAMA**.

Statistiques au sommet de Rochebrune
March 26th 2024

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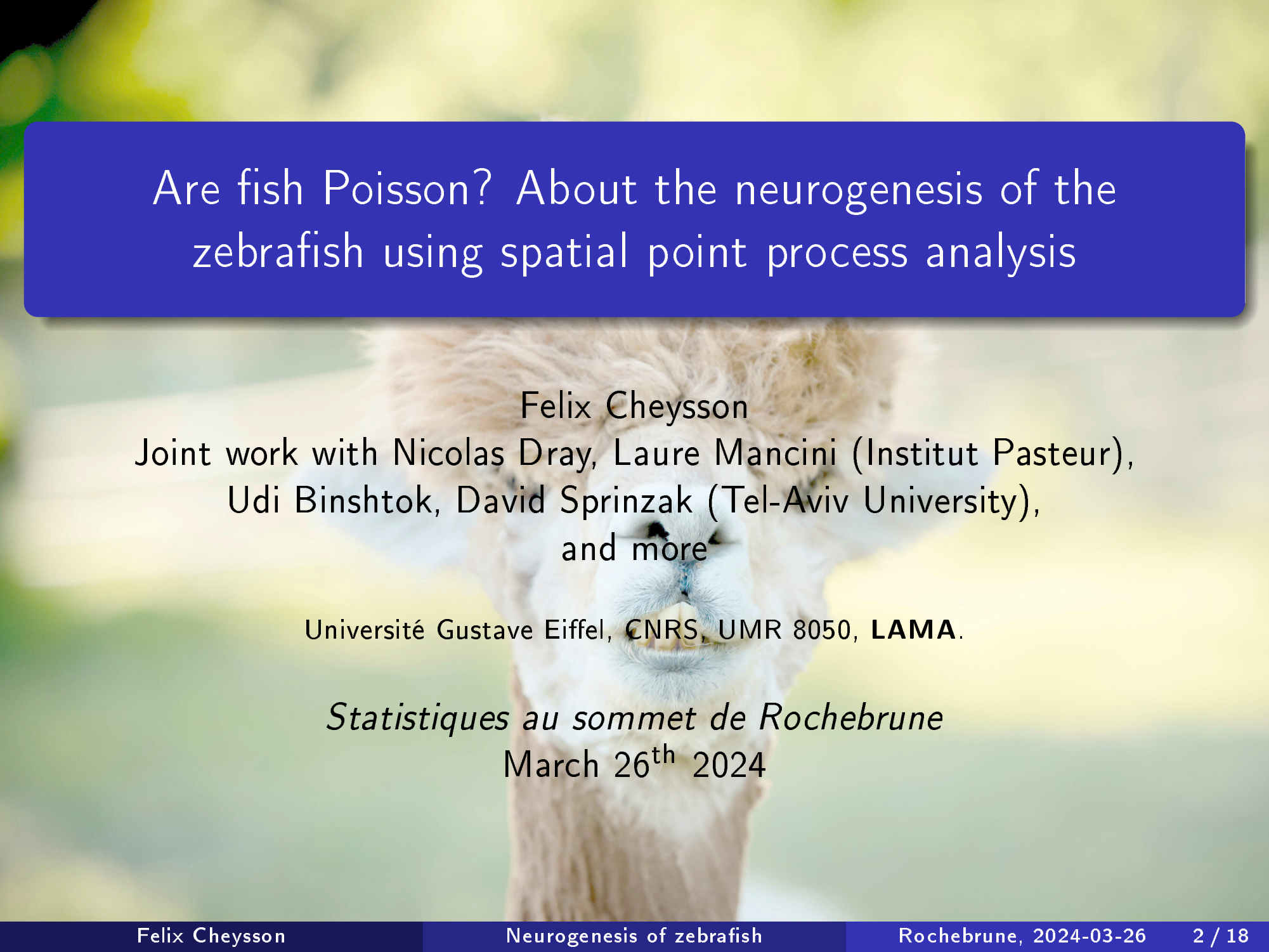

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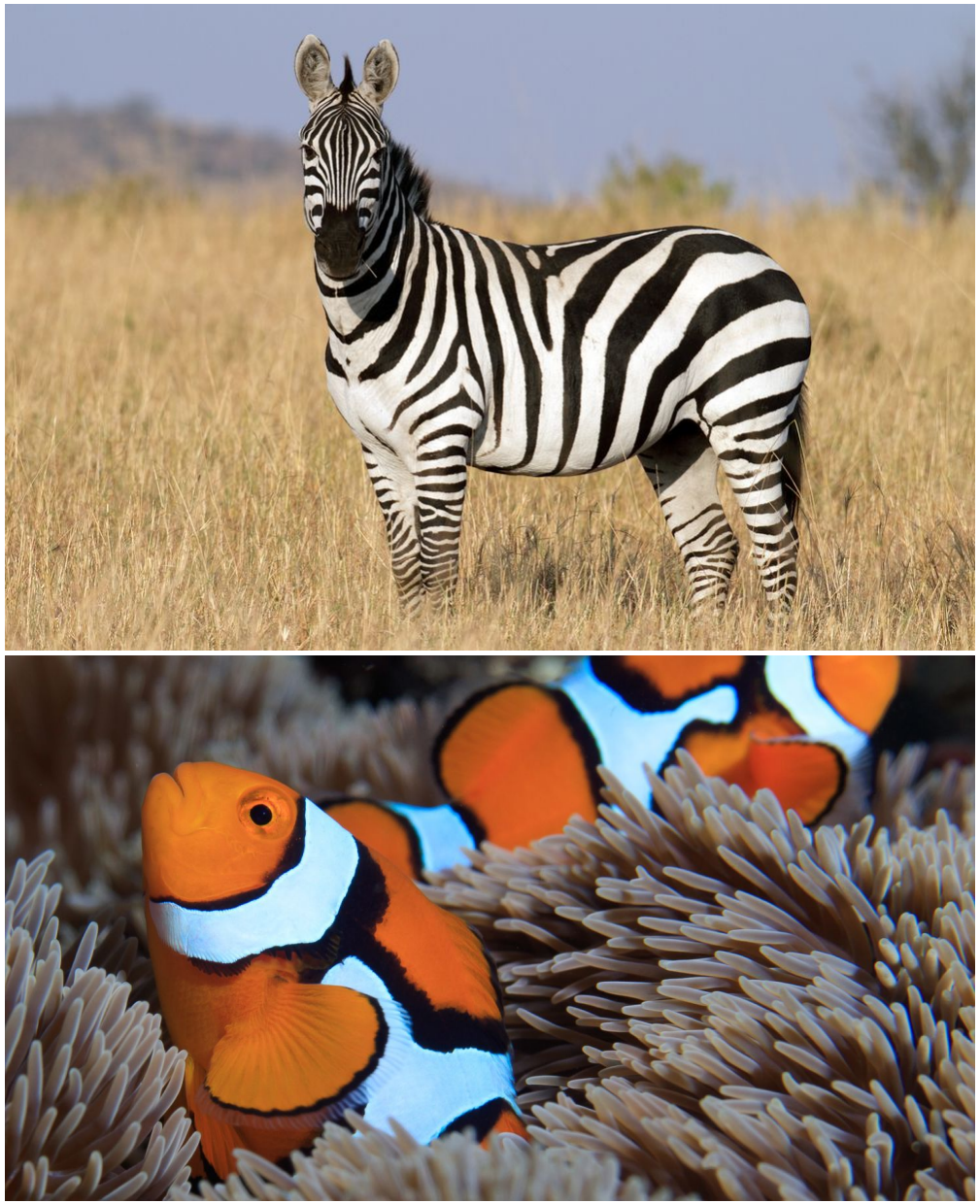
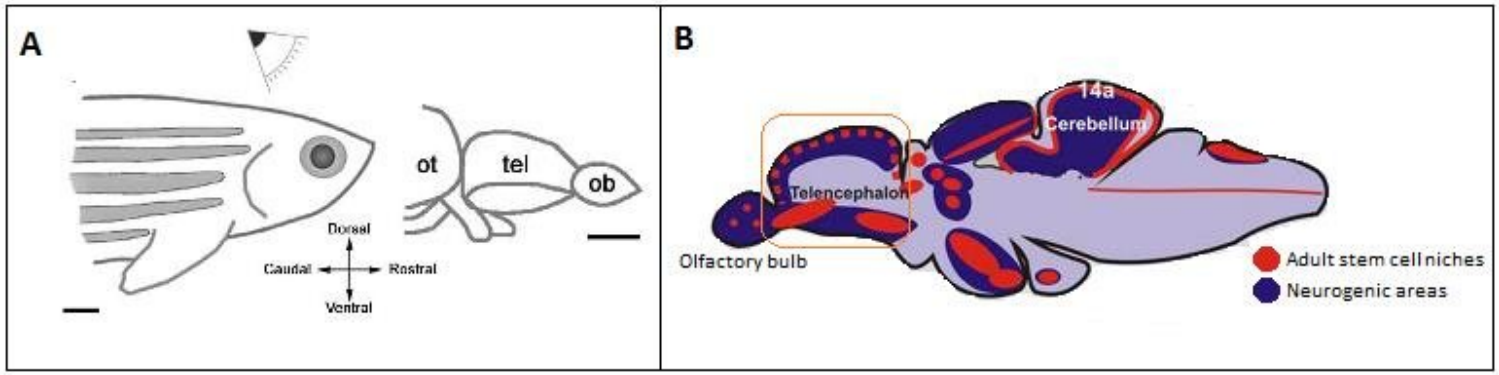
When a zebra loves a fish very much, then...



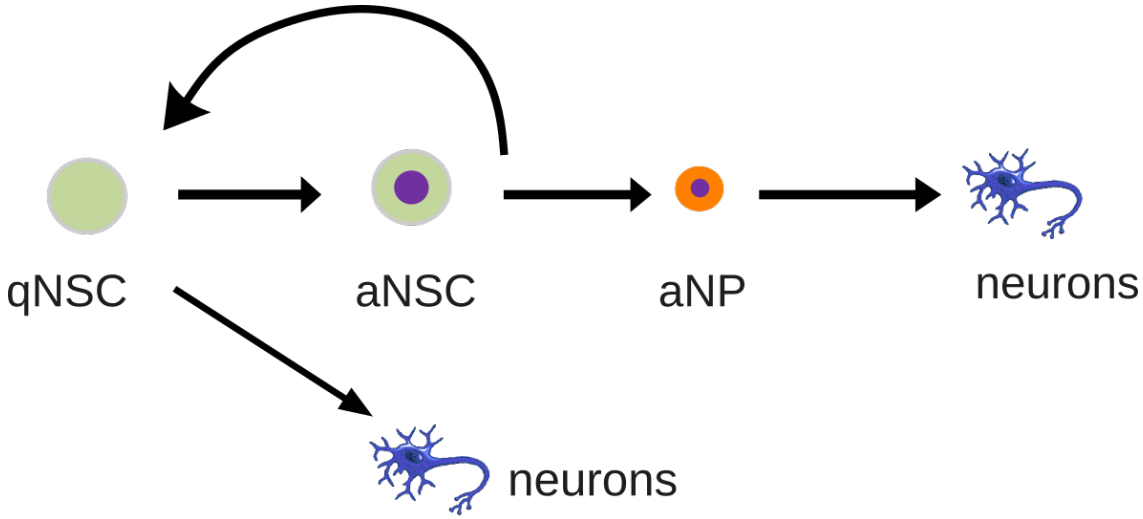
The zebrafish: a classic vertebrate model in biology

The zebrafish neurogenesis

- Development of Neural Stem Cells (NSCs) in the zebrafish dorsal pallium of the telencephalon.



- Homeostasis of Neural Stem Cells (NSCs) is maintained through renewal and differenciation mechanisms (Than-trong et al., 2020).

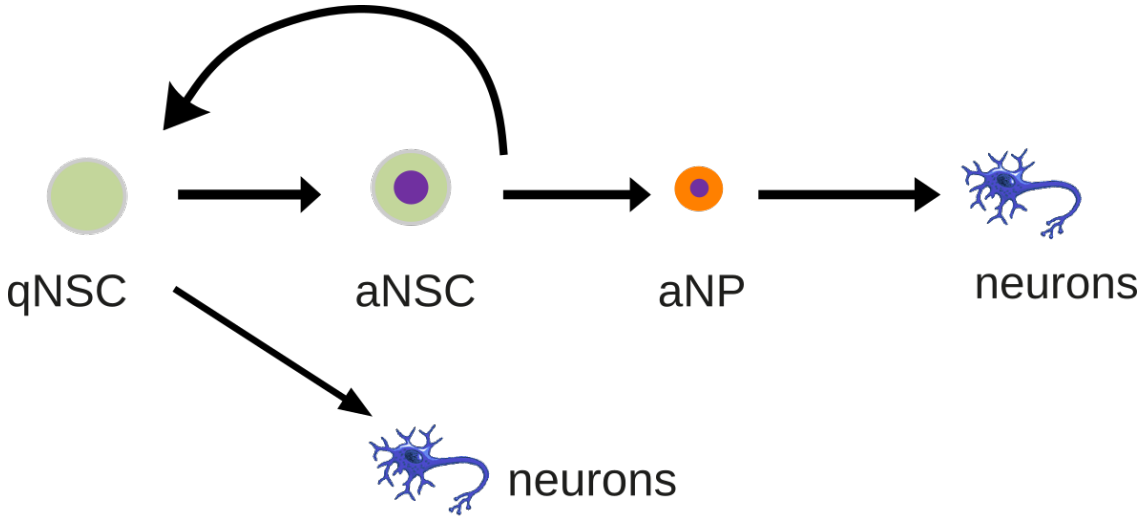


Live intravital imaging

- The Casper mutant (roy$^{-/-}$;nacre$^{-/-}$) (White et al., 2008).

- Multicolor fluorescence and harmonics multiphoton microscopy (Dray et al., 2015).
  - Fluorescence: to detect NSC markers, and markers for activation events.
  - Harmonics: provide persistent landmarks for longitudinal imaging.
  - Video.

The BBQ: Big Biological Question

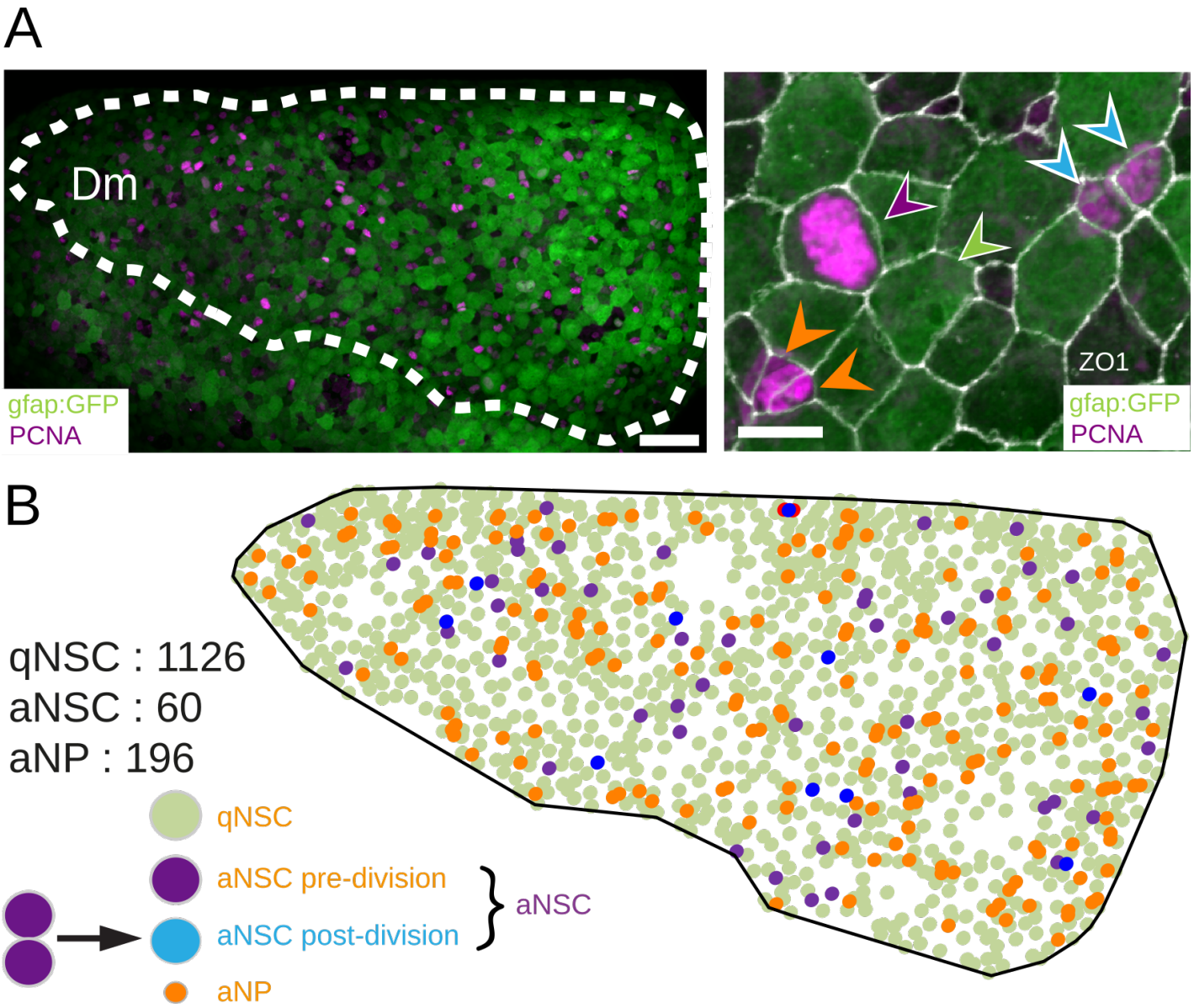


What spatial and temporal regulation mechanisms explain the homeostasis of the cell population and spatial organisation of the pallium?

Our approach:

- Spatial statistics to study the dependance between cell divisions.
- Biological experiments to (in-)validate the hypothetised signalling pathways.

Cells as a marked point process



Point process formalism

- Consider a point process $\mathbf{X}$ as a locally finite random set on $\mathbb{R}^2$ with density ρ.
- Second-order density $\rho^{(2)}$: for any f measurable,

$$\mathbb{E}\left[\sum_{x\in\mathbf{X}}\sum_{y\in\mathbf{X},y\neq x} f(x,y)\right] = \int\int f(x,y)\rho^{(2)}(\mathrm{d}x,\mathrm{d}y).$$

- Define $g(x,y) = \rho^{(2)}(x,y)/\rho(x)\rho(y)$.
- Assuming stationarity of $\mathbf{X}$, $g(x,y) = g(x-y)$, its reduced second moment measure is

$$\mathcal{K}(A) = \int_A g(x)\mathrm{d}x, \qquad \text{for } A \text{ Borel set.}$$

Statistics for second order moments

- Assuming isotropy of $\mathbf{X}$, Ripley's K-function:

$$K(r) = \mathcal{K}\big(b(x,r)\big) \quad \left(= \frac{1}{\beta}\mathbb{E}\Big[|\mathbf{X} \cap b(x,r) \setminus \{x\}|\Big]\right).$$

- Example: for the Poisson process, then $g(x) = 1$ and $K(r) = \pi r^2$.
- Estimator for the K-function from a window W:

$$\widehat{K}(r) = \widehat{\beta}^{-1} \sum_{\substack{x \in \mathbf{X} \cap W \\ y \in \mathbf{X} \cap W, x \neq y}} \sum w(x,y)^{-1} \frac{\mathbb{1}\{|x-y| \leq r\}}{|\mathbf{X} \cap W|},$$

  where $w(x_i, x_j)$ provides an *edge correction*.
- L-function $\widehat{L}(r) = \big(\widehat{K}(r)/\pi\big)^{1/2}$ stabilises the variance.
- Generally, explicit formulas for the mean and variance of $\widehat{K}(r)$ unavailable (unless Poisson, see Lang and Marcon, 2013).

In the marked temporal case

- Assume the processes $\mathbf{X}_j^t = (\mathbf{X}_j^1, \ldots, \mathbf{X}_j^T)$ are stationary (in time and space), and isotropic.
- Study interactions between types of cells through Ripley's function:

$$\widehat{K}_{ij}^t(r) = \left(\widehat{\beta}_i \widehat{\beta}_j |W|\right)^{-1} \sum_s \sum_{\substack{x \in \mathbf{X}_i^s \cap W \\ y \in \mathbf{X}_j^{s+t} \cap W, x \neq y}} \sum w(x,y)^{-1} \mathbb{1}\{|x-y| \leq r\}.$$

- Under *random labelling*, each process $\mathbf{X}_i^t$ can be seen as a random thinning of the marked process $\mathbf{X}^t$, so that $K_{ij}^t(r) = K^t(r) = K(r)$ for any i, j.
- Inference usually achieved through Monte Carlo tests, or normal approximations when available.

A test of independence between point processes

- We want to test the dependence between NSCs in their different states (quiescent, qNSCs; activated, aNSCs; progenitors, aNP).
- Due to cellular constraints, Poisson hypothesis is wrong: need to test dependence between processes under another null hypothesis.
- Simulation envelopes (= fluctuation envelopes) can be computed under random labelling:

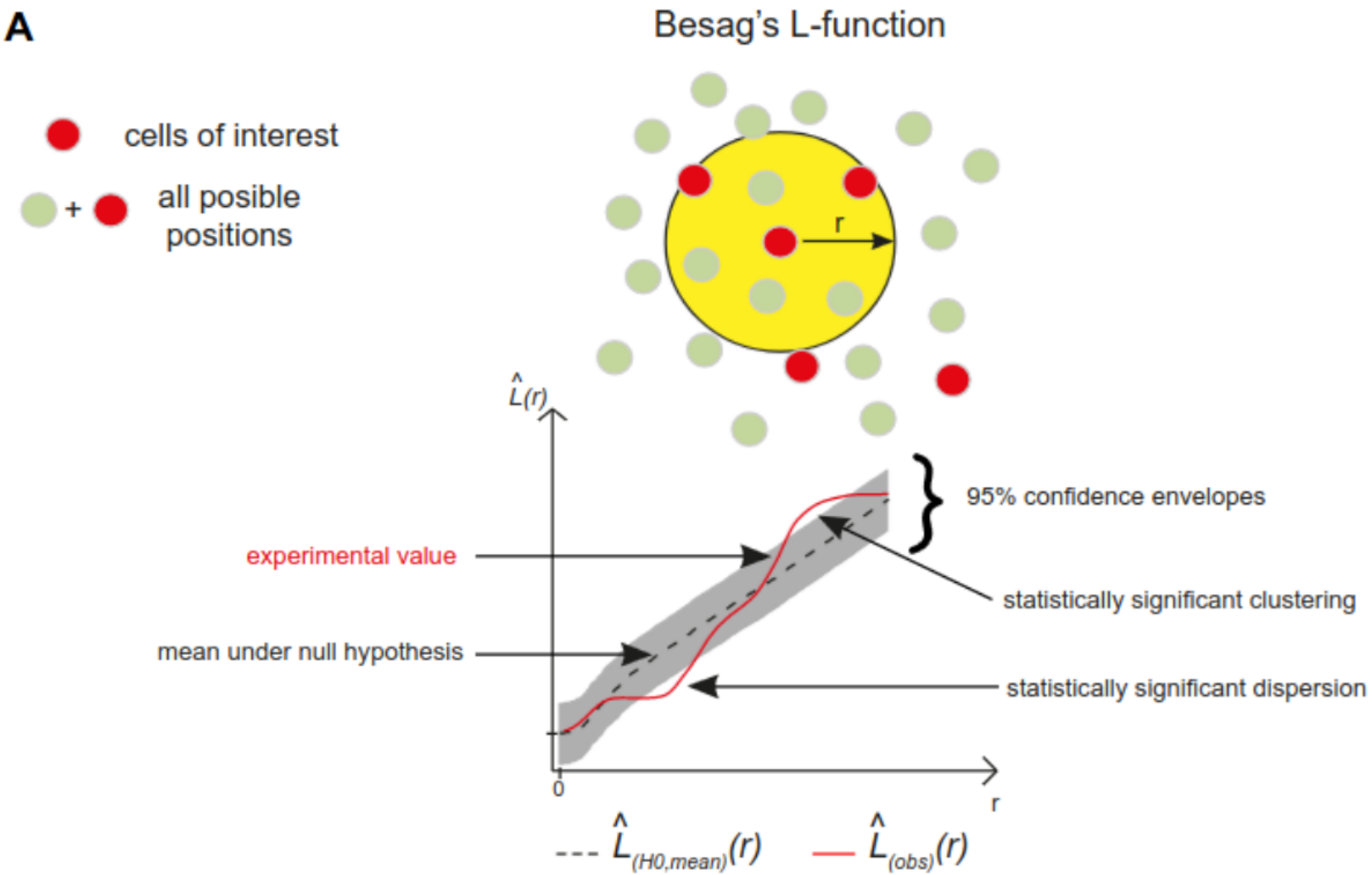
$$\mathcal{H}_0 : \forall i, \mathbf{X}_i^t \text{ is an independent random thinning of } \mathbf{X}^t.$$

- Idea: permutation test.
  - Simulate samples $\left(\widetilde{\mathbf{X}}_{(1)}^t, \ldots, \widetilde{\mathbf{X}}_{(m)}^t\right)$ under random labelling.
  - Under $\mathcal{H}_0$, $\mathbf{X}^t$ has the same distribution as any $\widetilde{\mathbf{X}}_{(k)}^t$.
  - Conclude via any test statistic $f(\mathbf{X}^t)$ (e.g. Ripley or L-function):

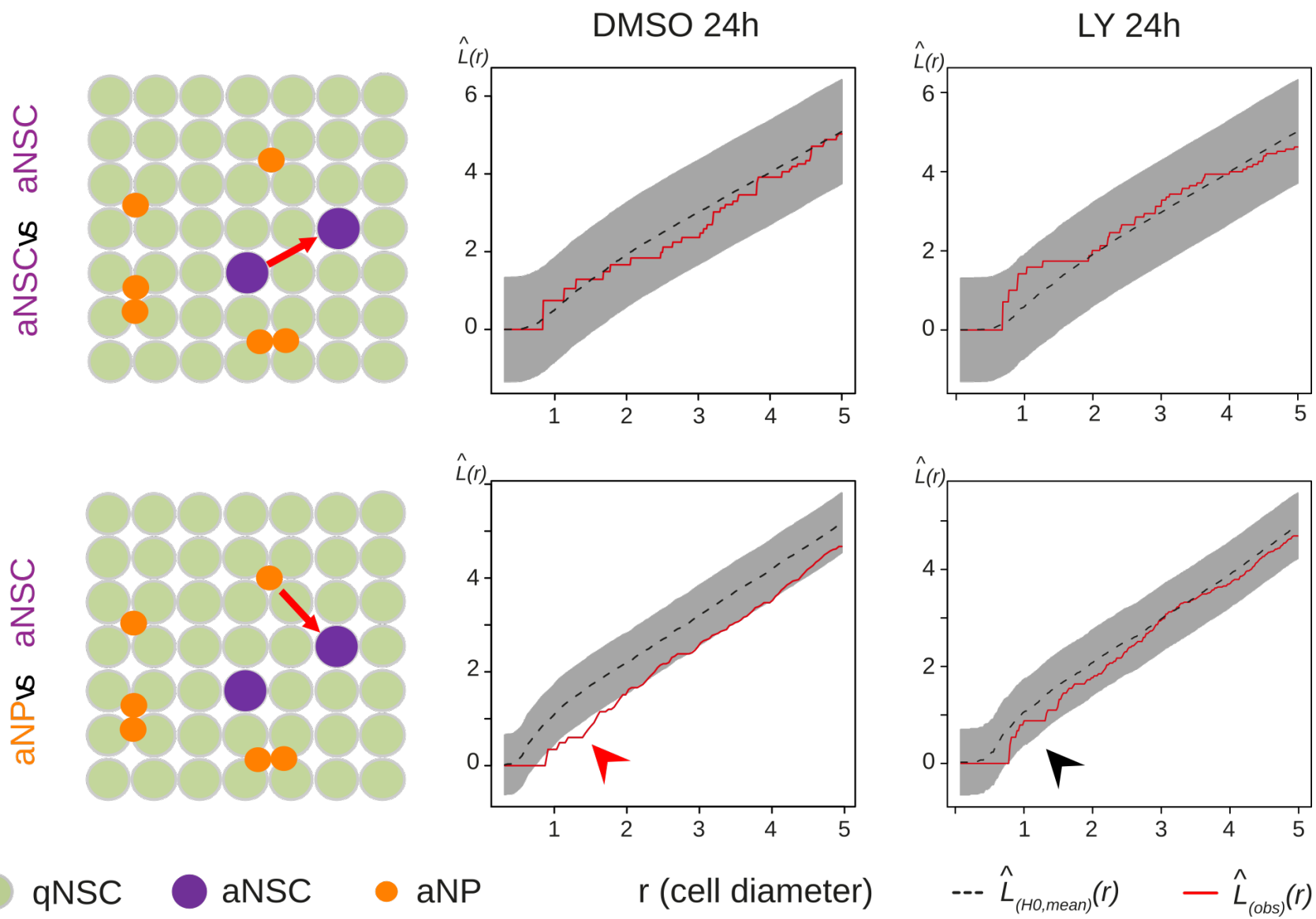
$$\mathbb{P}_{\mathcal{H}_0}\left(f(\mathbf{X}^t) > f_{(k)}\right) = 1 - \frac{k}{m+1},$$

where $f_{(k)}$ denotes the k-th largest of the simulated values $f\left(\mathbf{X}_{(k)}^t\right)$.

Pictures are worth a thousand words



Are fish Poisson?

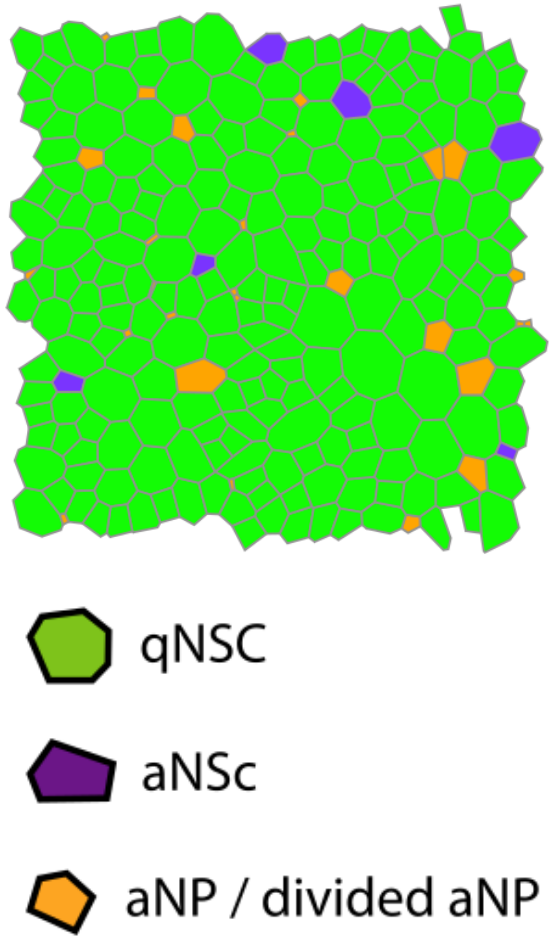


An experimental testing of inhibitive local feedback

- aNP-mediated feedback inhibition on aNSCs?
- Notch3 signaling (= a pathway using the receptors Notch3 on qNSC) promotes NSC quiescence, and aNPs express the Notch ligand DeltaA.
- Experiment in zebrafish:
  - Decrease Notch signaling through a short treatment.
  - Abolition of the local inhibition verified by testing (cf. LY).
  - qNSCs for fish under the treatment rapidly undergo rapid division.

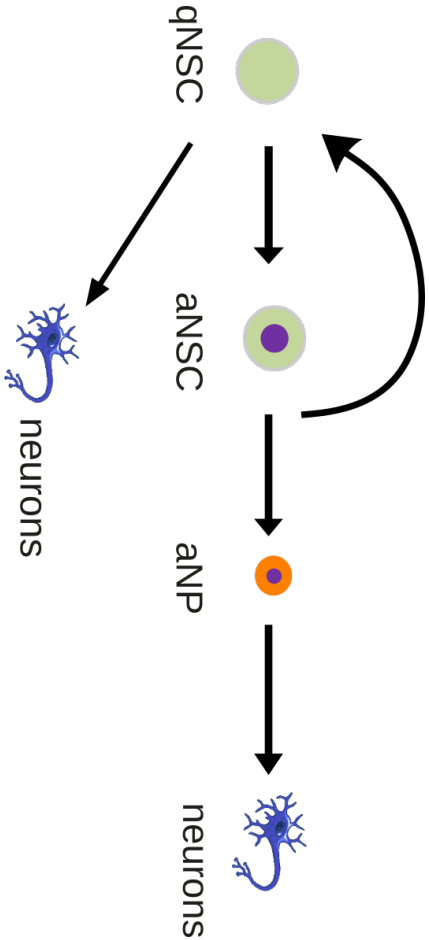
A lattice model for neurogenesis

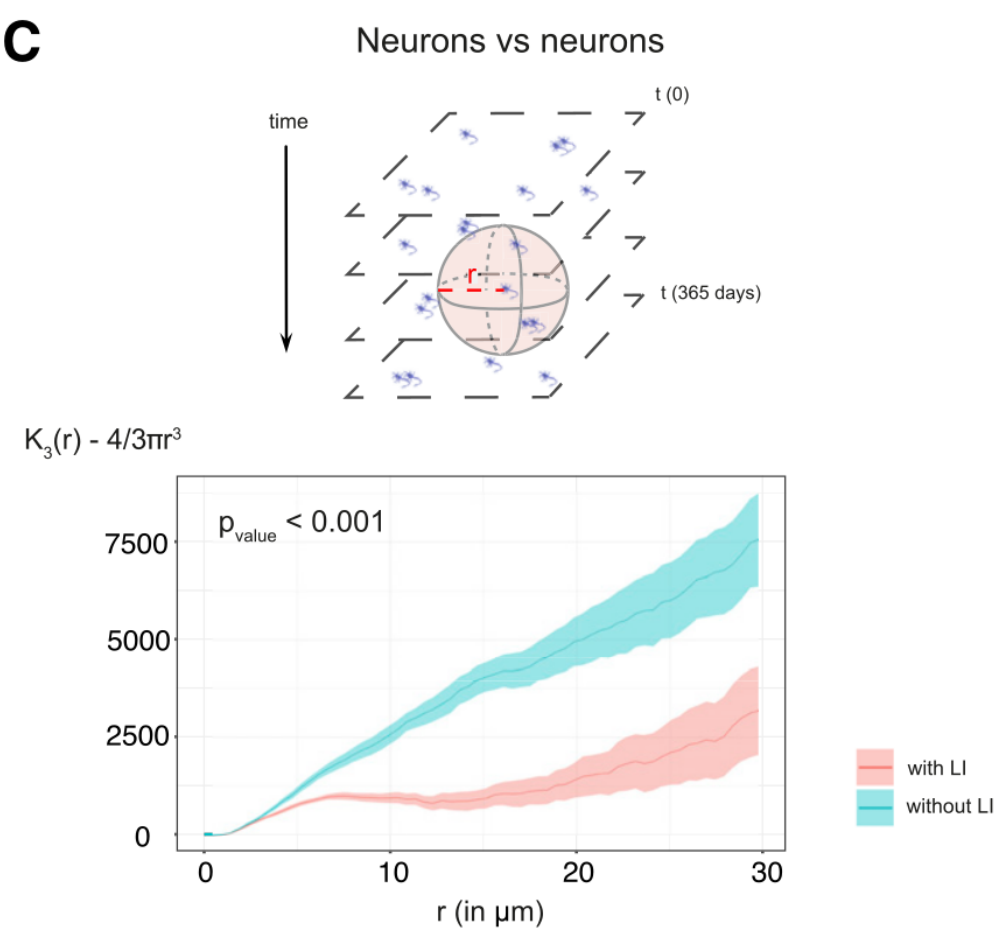
- Construction of a lattice model (system of EDPs + rules for lattice construction) to simulate and explore different mechanisms of interaction in NSCs (Video).
- Driven by data, and verified showing that the empirical statistical behaviour of the model is similar to that of the data.
- Neurons cannot be seen on live imaging of the pallium: explore spatial distribution of neurons on the simulations.



Lateral inhibition homogenises neurons in the pallium

- Comparison through two-sample permutation tests between simulations with and without lateral inhibition.
- Lateral inhibition supports more homogeneous neurogenesis output.





Thank you for your attention.






For Further Reading I

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