GnRH neurons network

Spatial and temporal aspects

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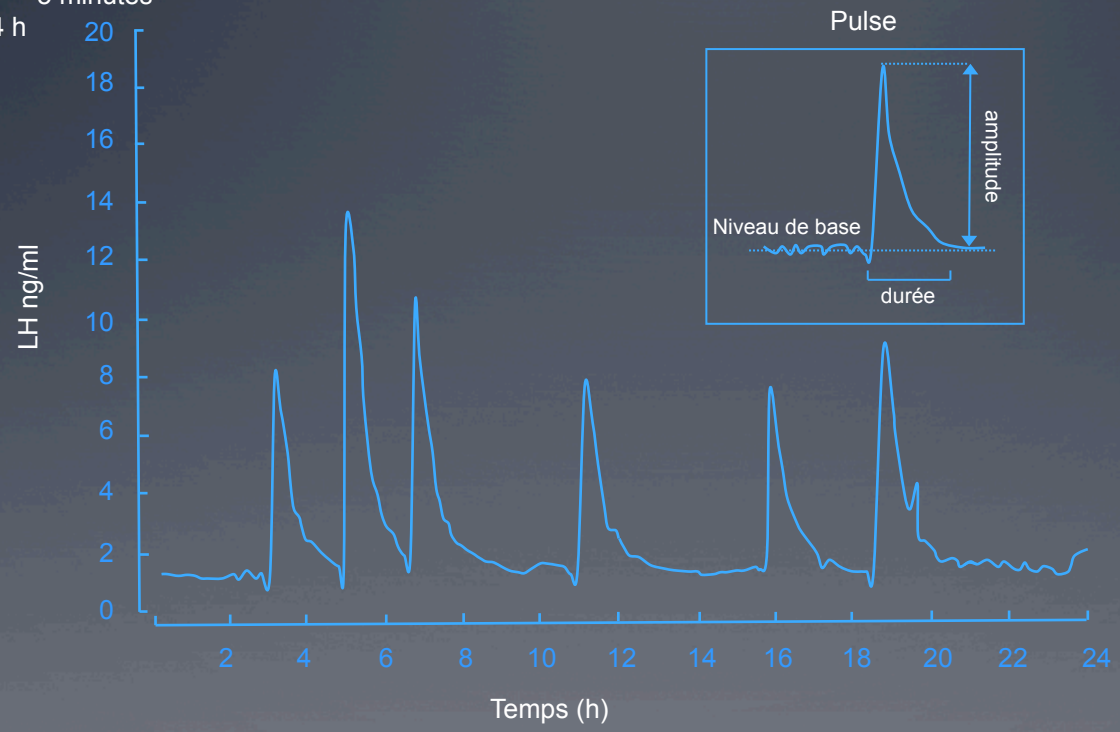
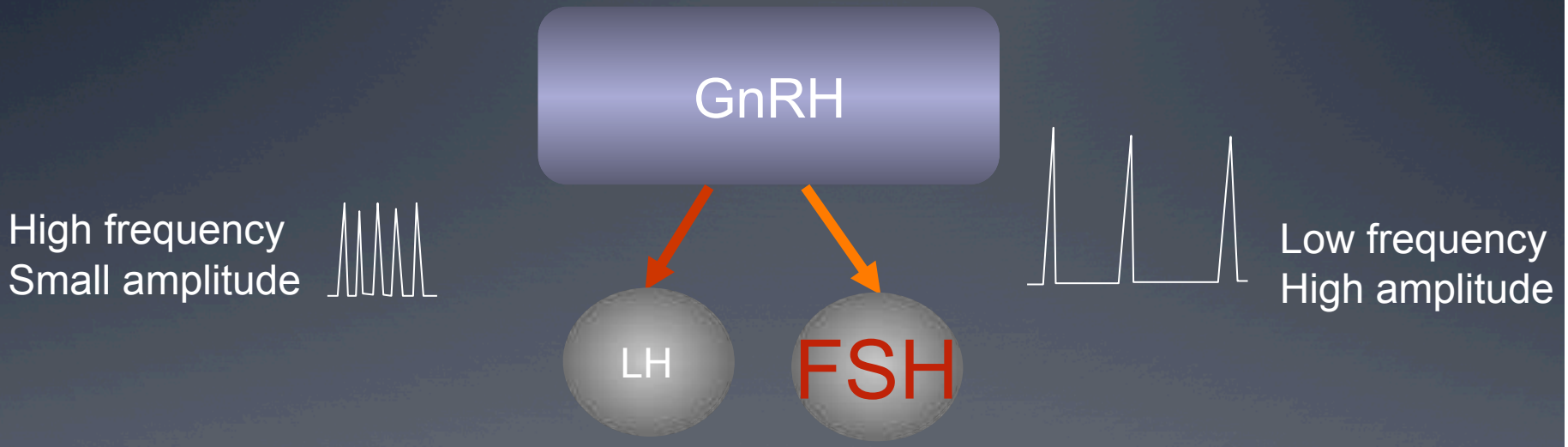
Pulsatility of hormone secretion

Cinétique de sécrétion de LH chez un bélier
Fréquence de prélèvement = 5 minutes
Durée de prélèvement = 24 h



Pulse = sudden and short lived secretory event

Frequency modulation

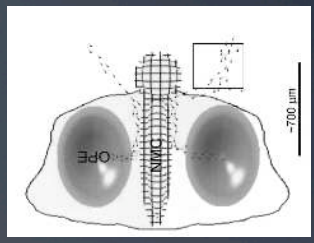


Methods

Pregnant mouse

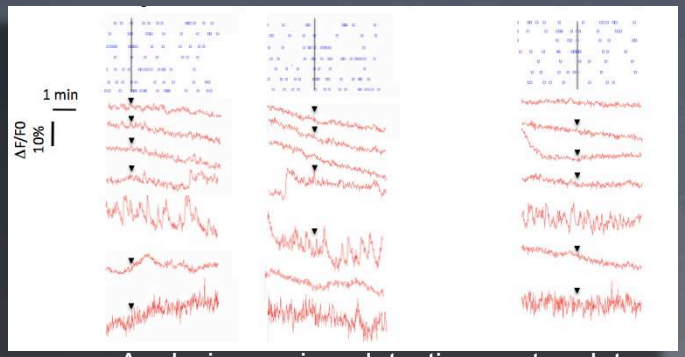
Embryos E11,5

Nasals placode dissection

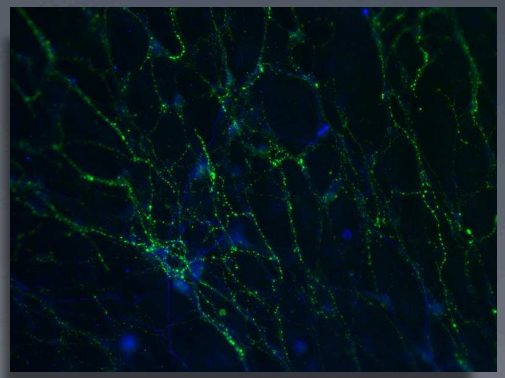


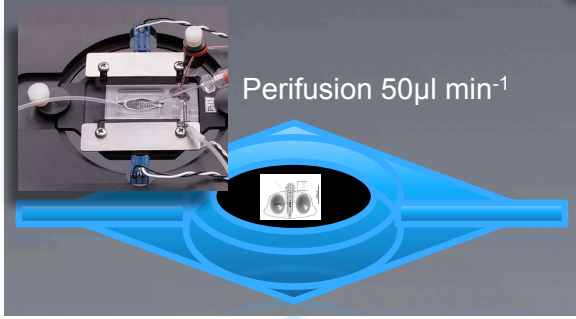
Culture 7-14 days in vitro

Calcium imaging



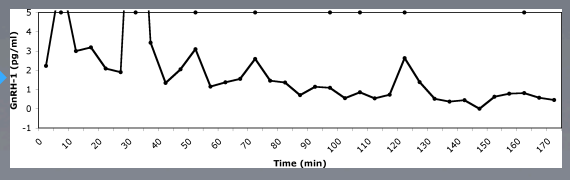
Analysis: maxima detection, raster plot and synchronisation





Perifusion 50µl min^{-1}

GnRH assay

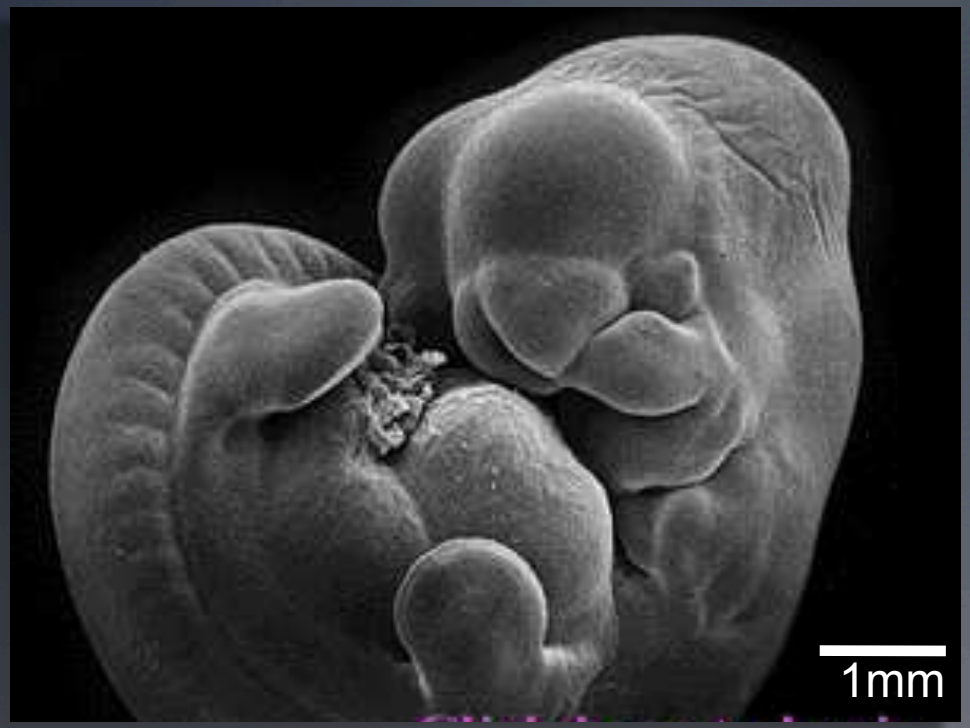


Cell identification

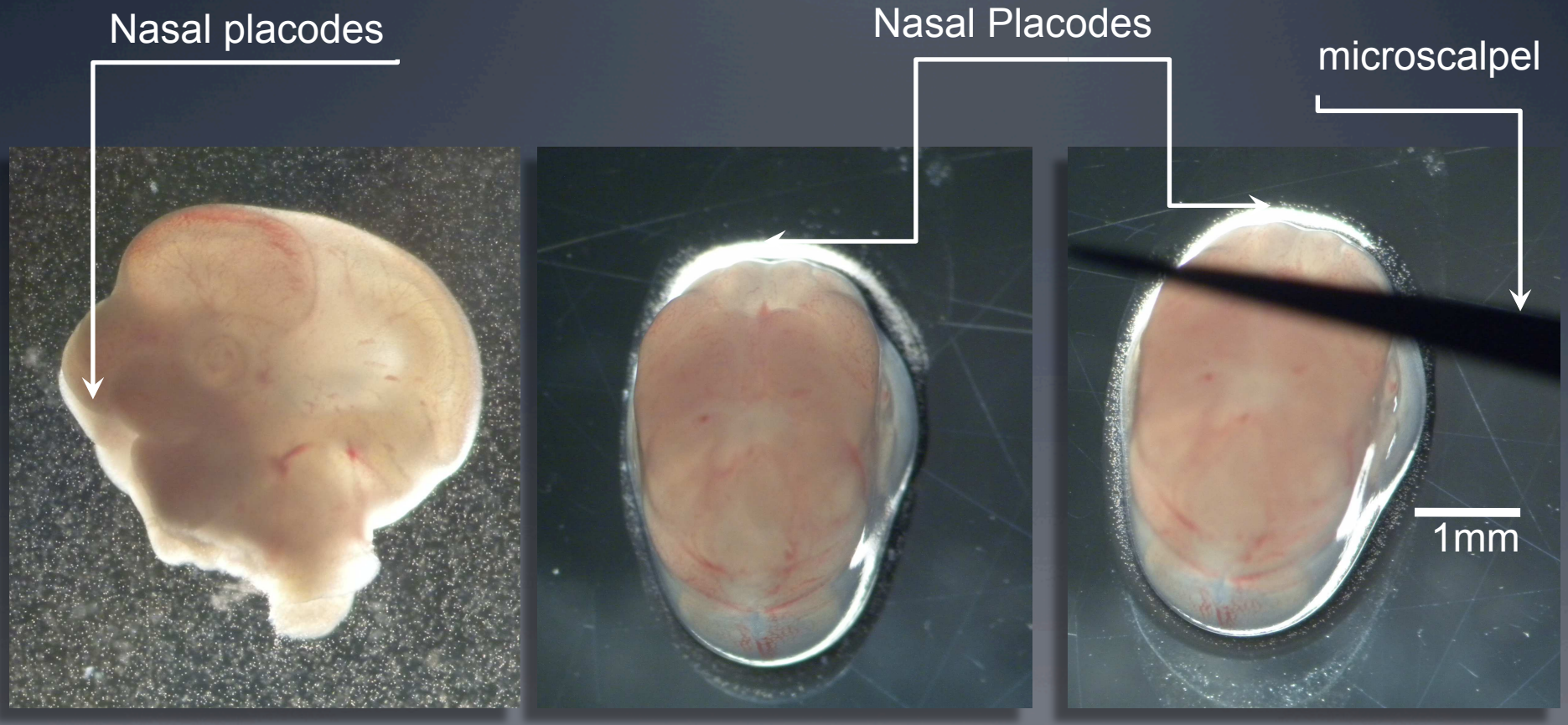
CaSciModOT

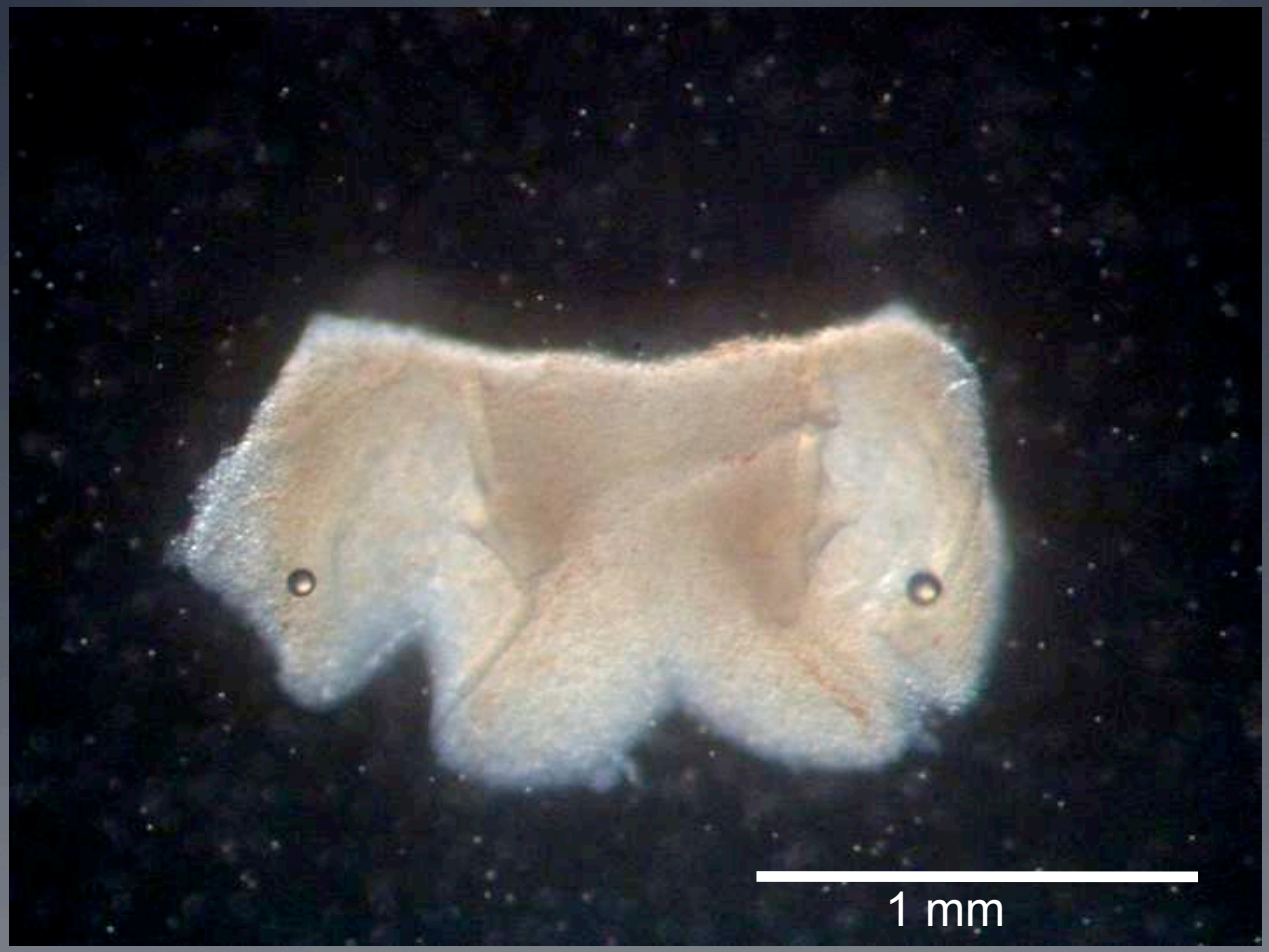
E11.5 embryo

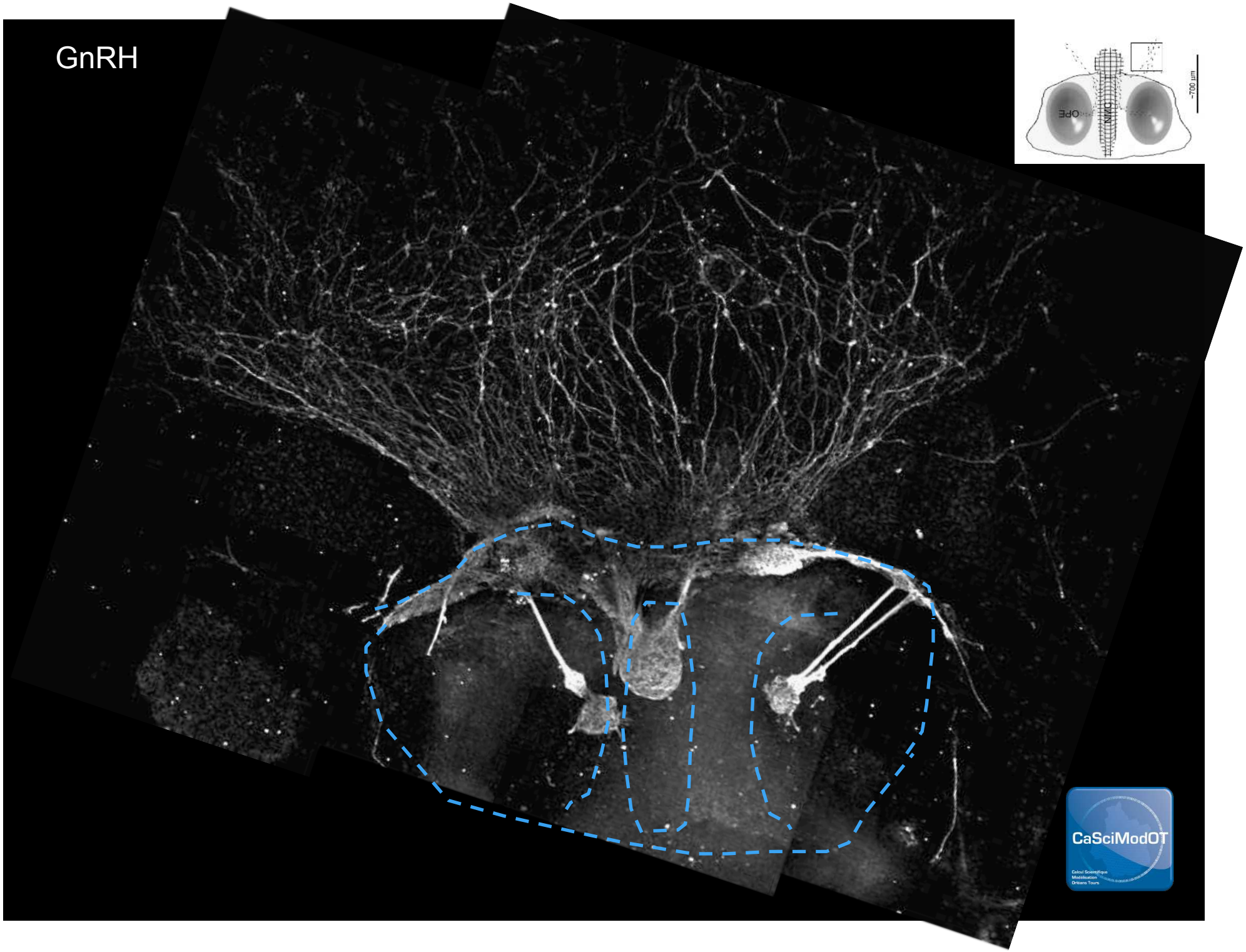
Equivalent to 7 weeks human embryo


1mm

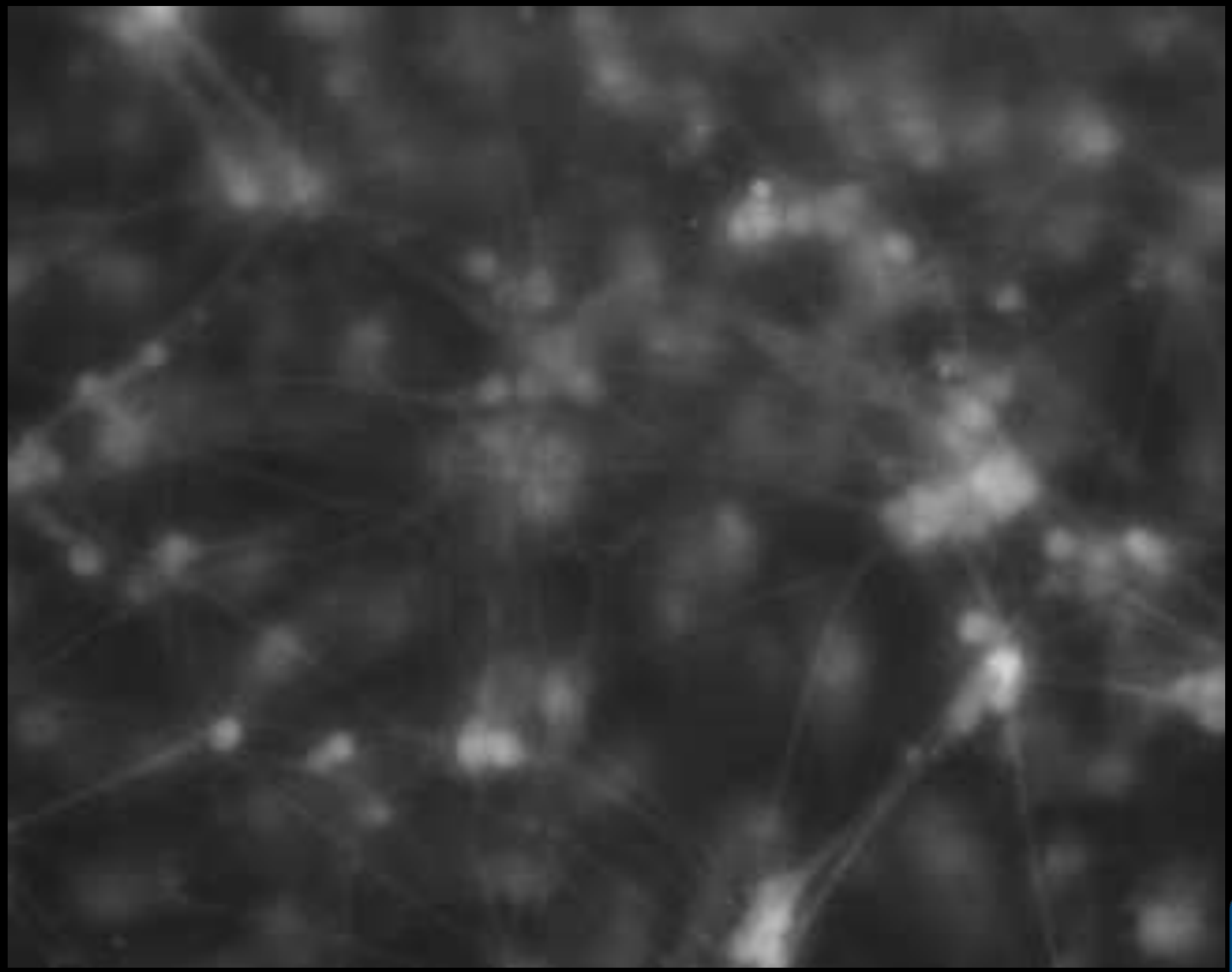
Dissection of nasal placodes



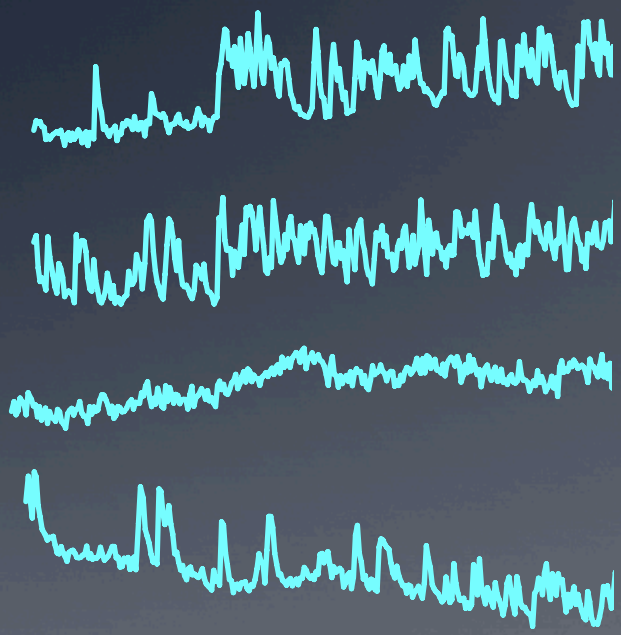

1 mm

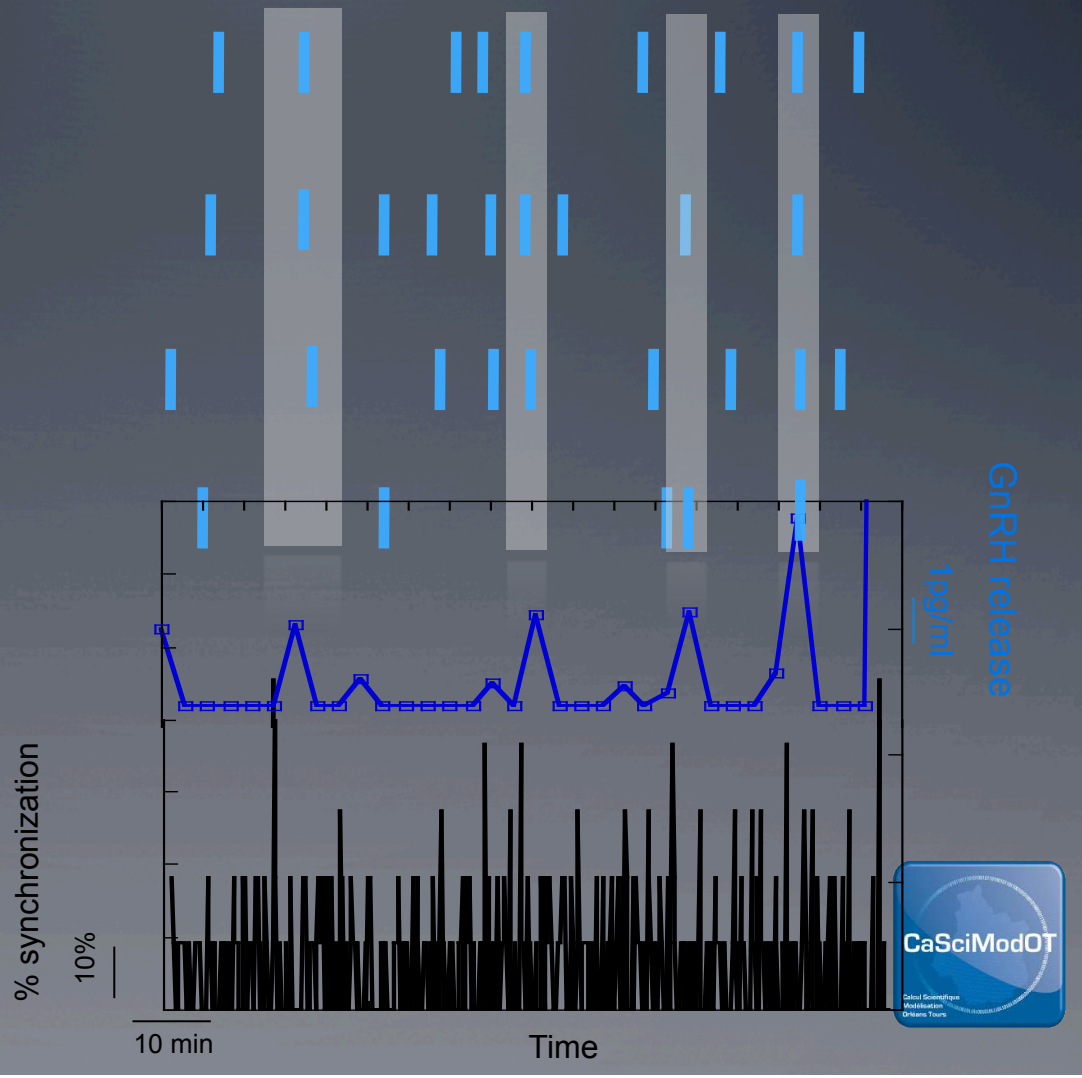

GnRH
OPE
~700 µm
CaSciModOT

Calcium green
Time lapse = 1 sec
Durée du film = 400 sec



Max detection





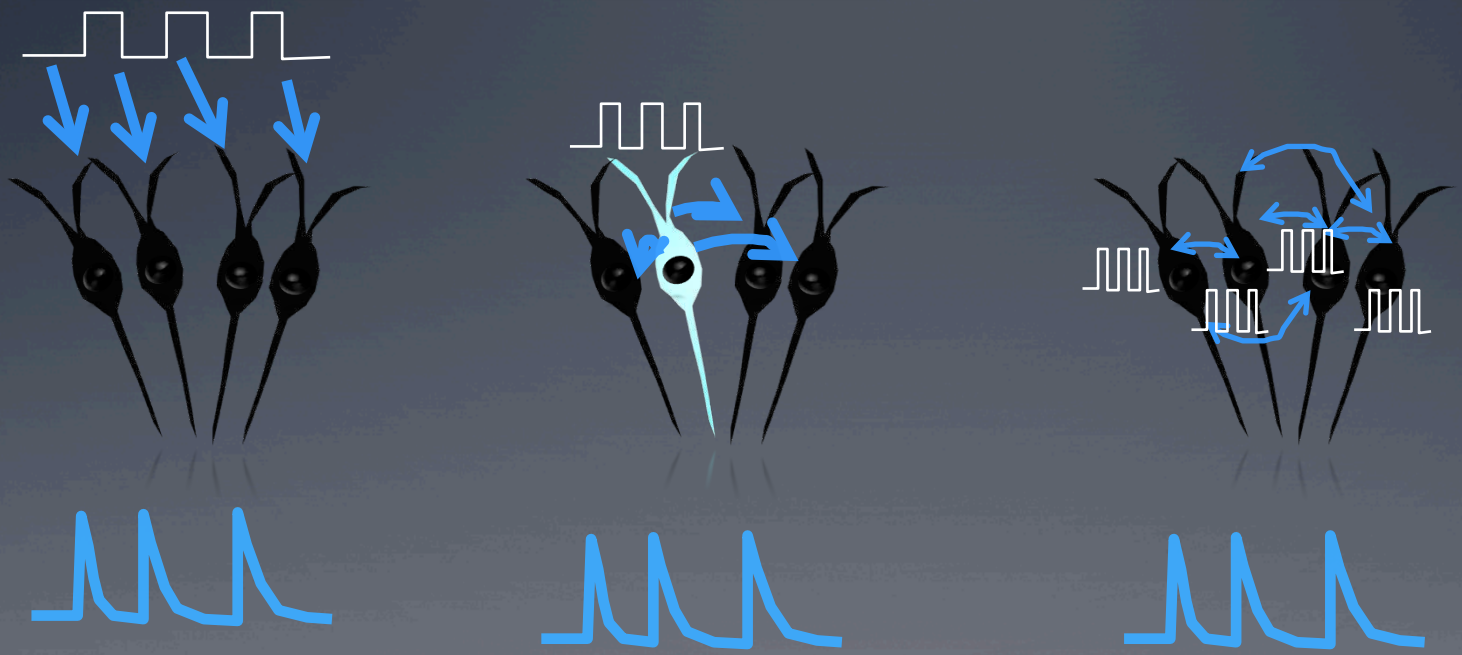
Constantin, S., Caraty, A., Wray, S., & Duittoz, A. H. (2009). Development of gonadotropin-releasing hormone-1 secretion in mouse nasal explants. Endocrinology, 150(7), 3221–3227.

Which mechanisms for synchronization ?

External neuronal network

Internal pace-maker neuron

Integrated model



The two last hypothesis imply the existence of cell to cell communication process within the network

Experiment definition

Pour chaque expérience :

1. On a M_E neurones (N_k) qui sont disposés dans le plan ,
2. A chaque instant $t_i = i\Delta t$, pour chaque neurone N_k, on mesure le taux de fluorescence X_i^k,
   $\Delta t \sim 20s^*$ (ne pas "manquer" de pics)
   $X_i^k \sim KC_i^k$ où C_i^k quantité de Calcium libérée par le neurone N_k,
3. Toutes les $T_g = k_G \Delta t$, on mesure le taux moyen de GnRH dans la solution baignant les neurones :

*ou 1s

$$G = \sum_{k=1}^{M_E} G_k$$

Ca event: definition

Pour le Neurone N_k, ce sont les instants T_i^k tels que

$$X_i^k = \max_{j \in \{i-nl,\ldots,i+nr\}} X_j^k$$

tels que

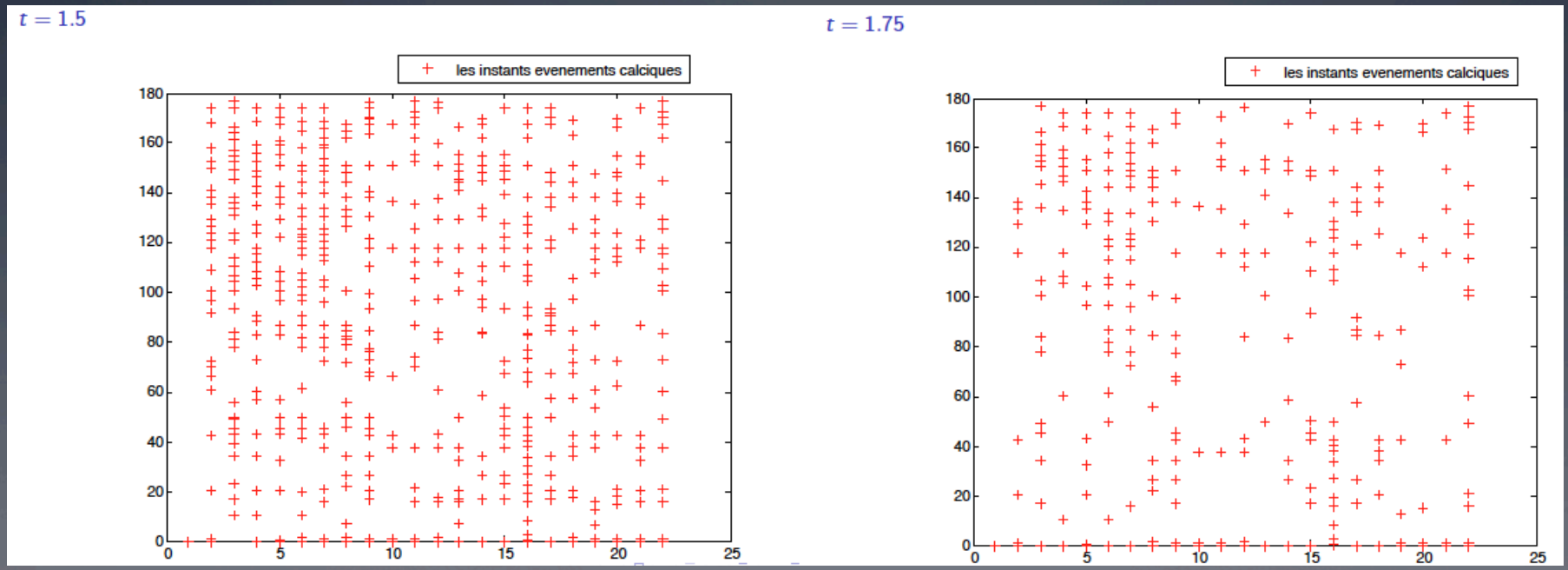
$$X_i^k \geq m_i^k + t * \sigma_i^k$$

où

m_i^k :moyenne des $(X_j^k)_{j \in \{i-nl,\ldots,i+nr\}}$ aux instants voisins de T_i^k

σ_i^k :écart-type des des $(X_j^k)_{j \in \{i-nl,\ldots,i+nr\}}$ aux instants voisins de T_i^k.

t dependency

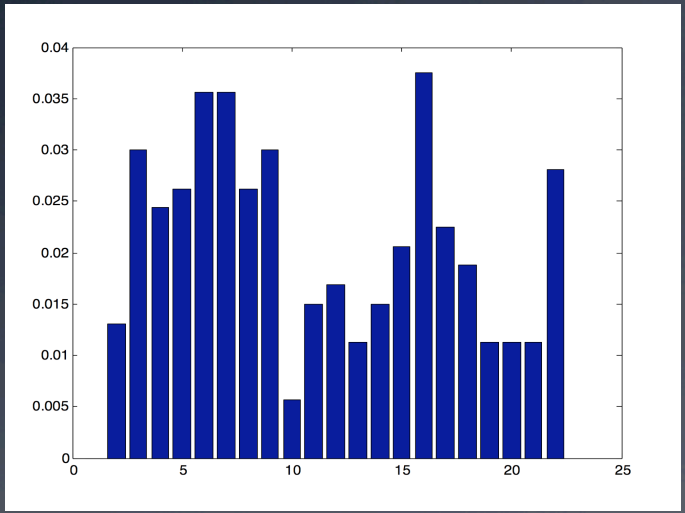


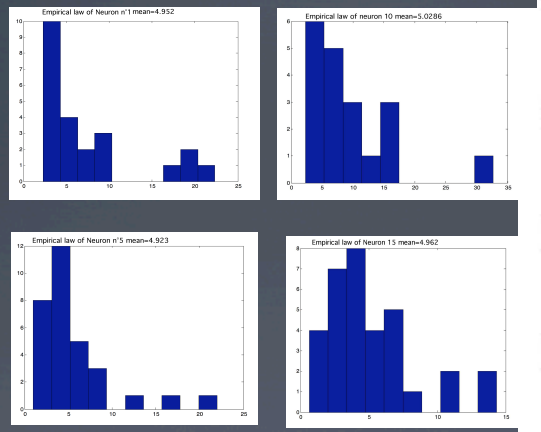
How modelling can help ?

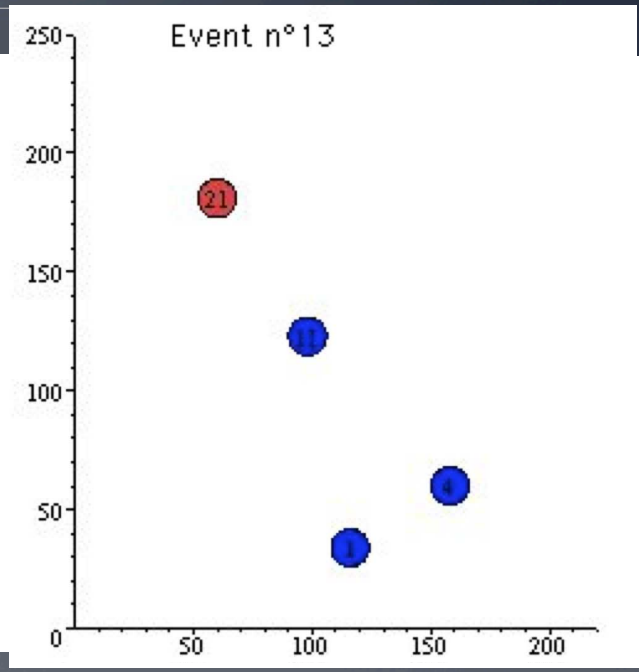
- Is the max detection method correct to analyse the data ?
- How to define t without the biologist (bad) eye ?
- Is synchronization detection dependent on t
- Understanding mechanisms involved in synchronization of GnRH neurons
- Understanding the link between synchronization and pulsatile secretion

Spatial analysis

Analysis of Ca events distribution





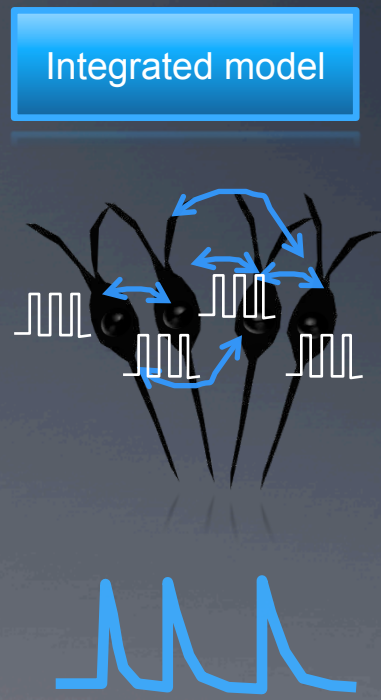


$$p(k) = P(X = k) = \mathrm{e}^{-\lambda}\frac{\lambda^k}{k!}$$

For most neurons, the distribution of Ca_i events intervals follows a Poisson law, each neurone having its own law.
No evidence of any stable association between neurones during synchronization events.

Conclusion

- Synchronization of calcium activity in GnRH neuron does not involve a leader neuron or a group of defined cells.
- Synchronization among GnRH cells : no recurrent associations during synchrony events
- GnRH neurons network is acting like an integrated model



Reconstructing Ca signalling

shot noise analysis

Definition

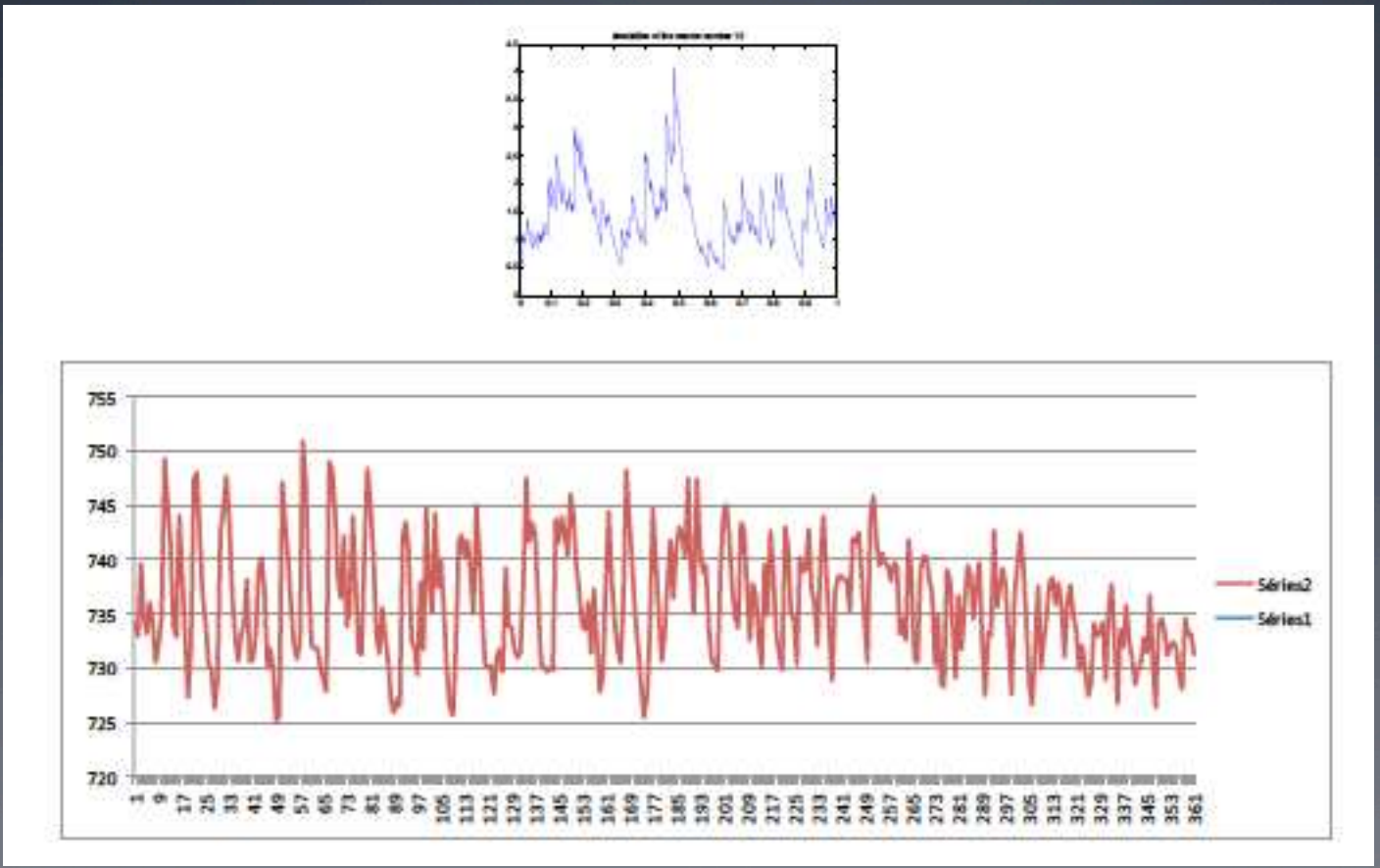
Un shot noise plus adapté, pour la réponse calcique chaque neurone N_k :

$$X^k(t) = \sum_i \beta_i^k g((t - \tau_i^k)\theta_k)$$

le paramètre θ_k est à ajuster pour chaque neurone N_k en fonction de de sa vitesse d'élimination du Calcium •
A insérer dans le Toy model ?

avec t

Shot noise simulation



Stochastic Toy model of GnRH neuron network

Background

Measurable

1g GnRH = 6 10^{23} molecules
1 vesicle = 6 10^{4} molécules

100-200 neurones = 100 pg GnRH

Estimation

1 vesicle = 100 ag GnRH
1000 vesicles = 100fg GnRH

1 neuron = 5000-10000 vesicles

Volume total libre (Vt) = Volume cell body (Vc) + volume dendrite (Vd) + volume axon (Va) – volume nucleus (Vn)

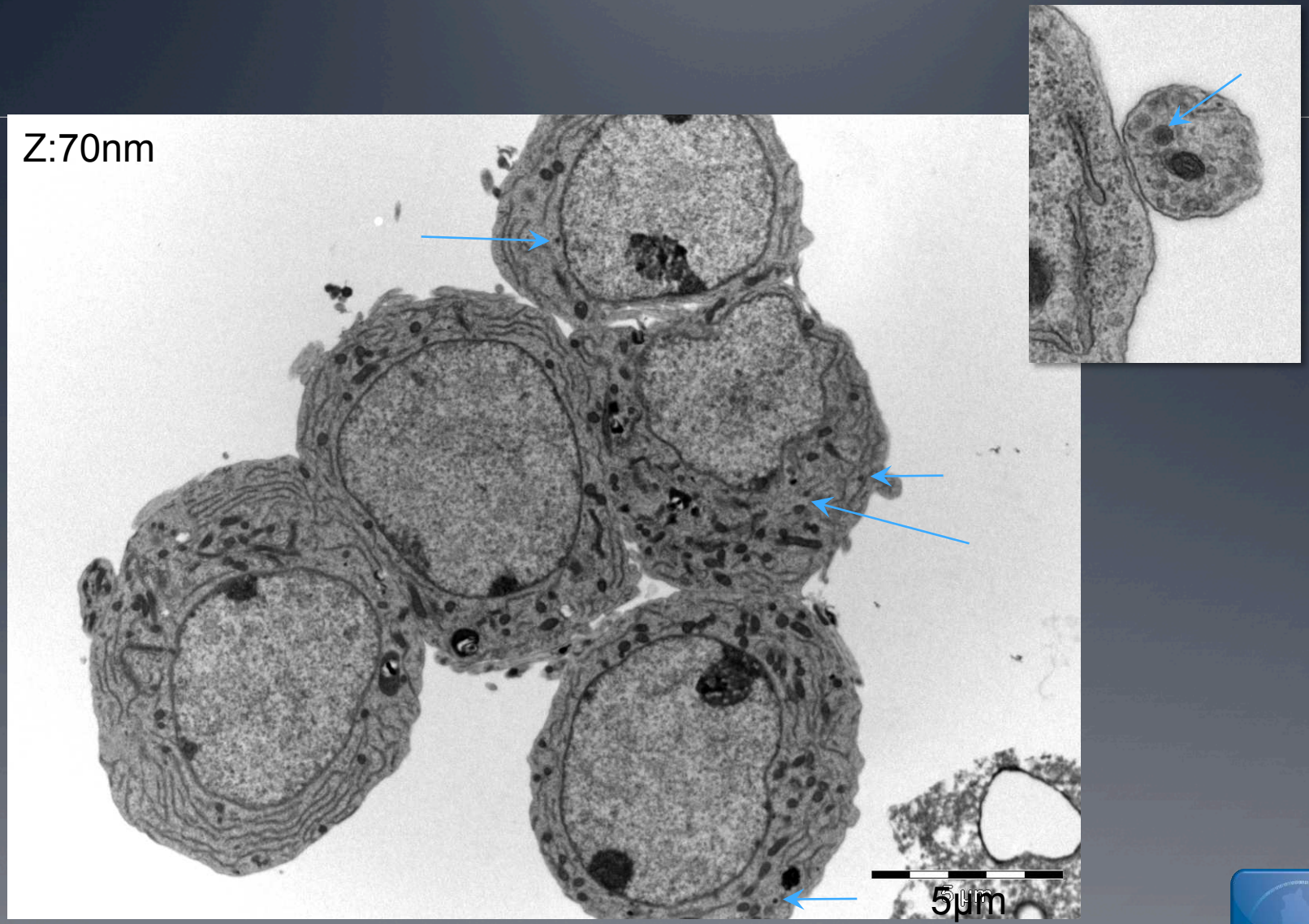
Vc = 4/3 π 8x8x12 μm^3
Vd = 2x(4/3 π 2x2x2000) μm^3
Va =4/3 π 2x2x1000 μm^3
Vn = 4/3 π 5x5x8 μm^3

Σ Vv = 10000 x(4/3 π 0.1^3) = 42 μm^3

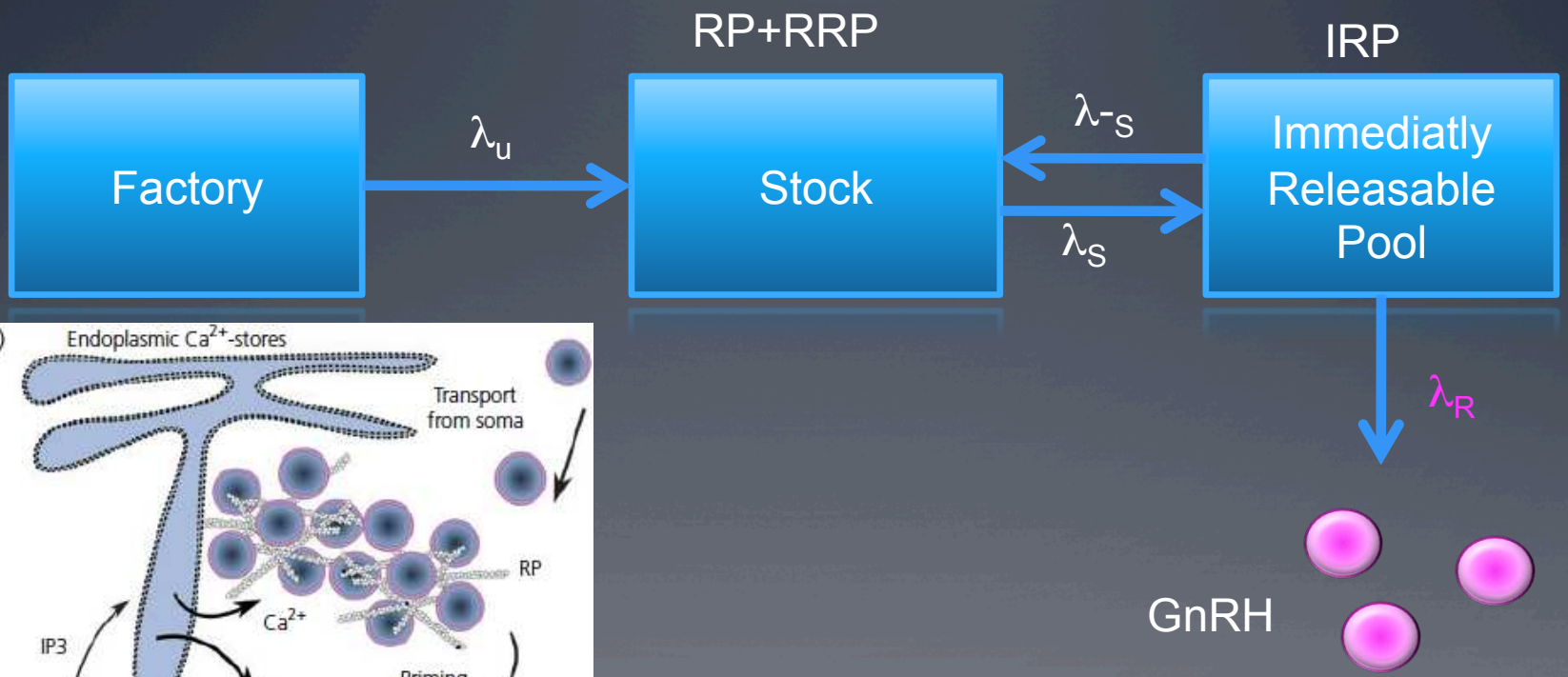
Vt # 70000 μm^3

1 vesicle = 0.000006 % Vt

CaSciModOT


Z:70nm
5µm

Vesicles pools



Bergquist, F., & Ludwig, M. (2008). Dendritic transmitter release: a comparison of two model systems. Journal of Neuroendocrinology, 20(6), 677–686.

Toy model's ingredients

Processus de Poisson de paramètre $\lambda_R(t)$

-Concentration GNRH faible, relargage d'un nombre Ni (petit)

$\lambda_R(t)$ petit

-Si elle dépasse un certain seuil , relargage d'un nombre NR (grand)

$\lambda_R(t)$ GRAND

PAr exemple

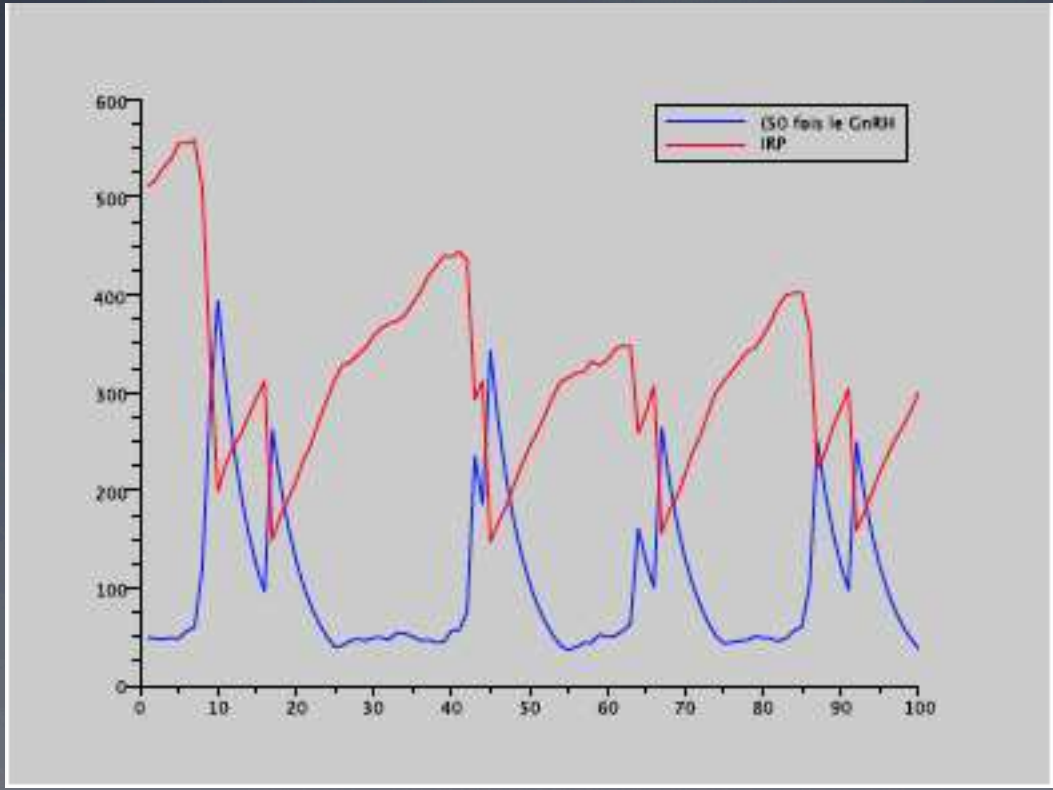
$$\lambda_R(t) = N_i + a\tanh((G - sG) * \varepsilon)$$

Problème : estimer les paramètres a et ε !

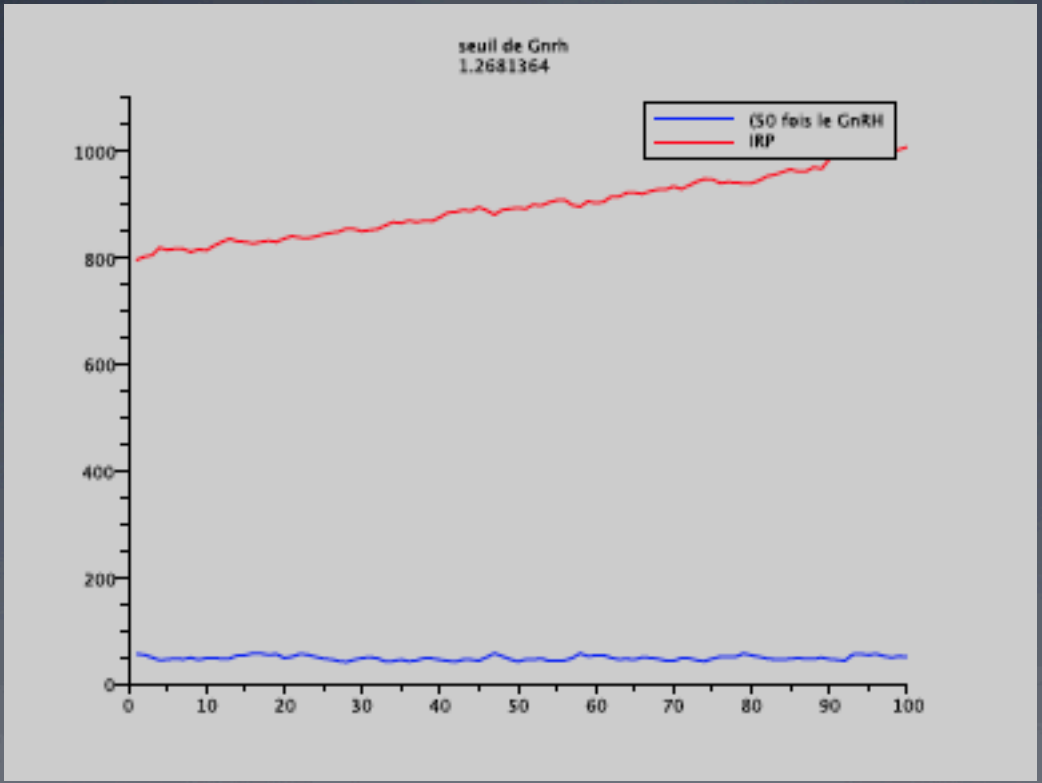
Evolution du modèle :

confier le mécanisme de relargage aux substances qui inhibent ou provoquent la sécrétion du GnRh.

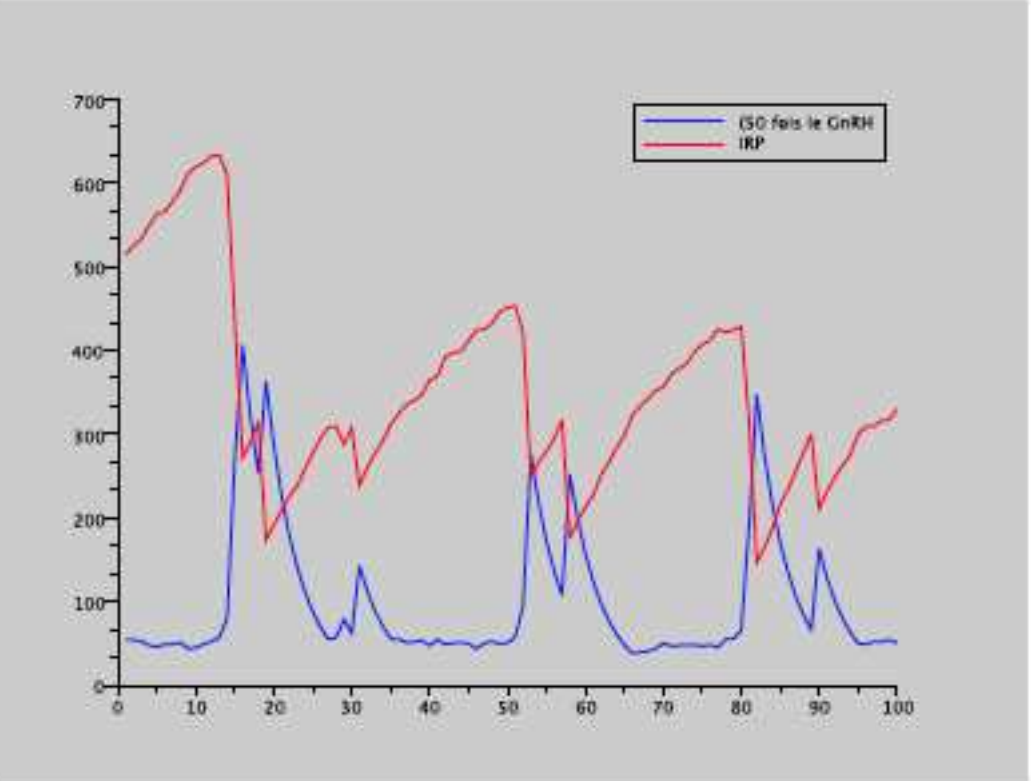
Cruising mode



Blocking mode: Sg increased



Blockade released



General conclusion

Modelling helped to understand the network behaviour and oriented biological experiments

Ca signal reconstruction has to be finished and will allow to see whether synchronization is an endogenous behaviour or an external imposed phenomenon

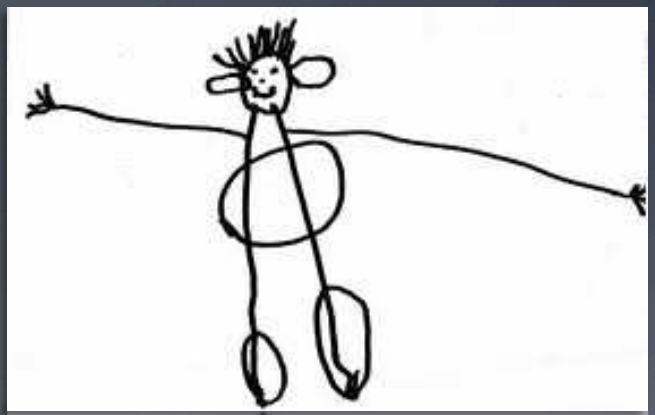
Toy model has to be completed with Ca signal input and with other putative regulators

Biologist x Mathematician =

Reality

Experiment (s)

(Toy) Model



Many many many thanks to

MiDynNet Team

Sarah Geller
Monique Ottogalli
Didier Lomet
Isabelle Franceschini
Yves Tillet
Pascal Vaudin
….

LMPT Team

Guy Barles
Hervé Choplin

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