Kisspeptin and RFRP neurons control breeding season but not induction of ovulation in the camel

Comparative anatomy unit- Department of biological and pharmaceuticals Veterinary sciences, Hassan II Agronomy and Veterinary Medicine Institute – Rabat- Morocco / Integrative and Cellular Neuroscience Institute – Strasbourg - France

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Animal Model : Dromadary camel (*Camelus dromedarius*)

Major socio-economic importance in Morocco

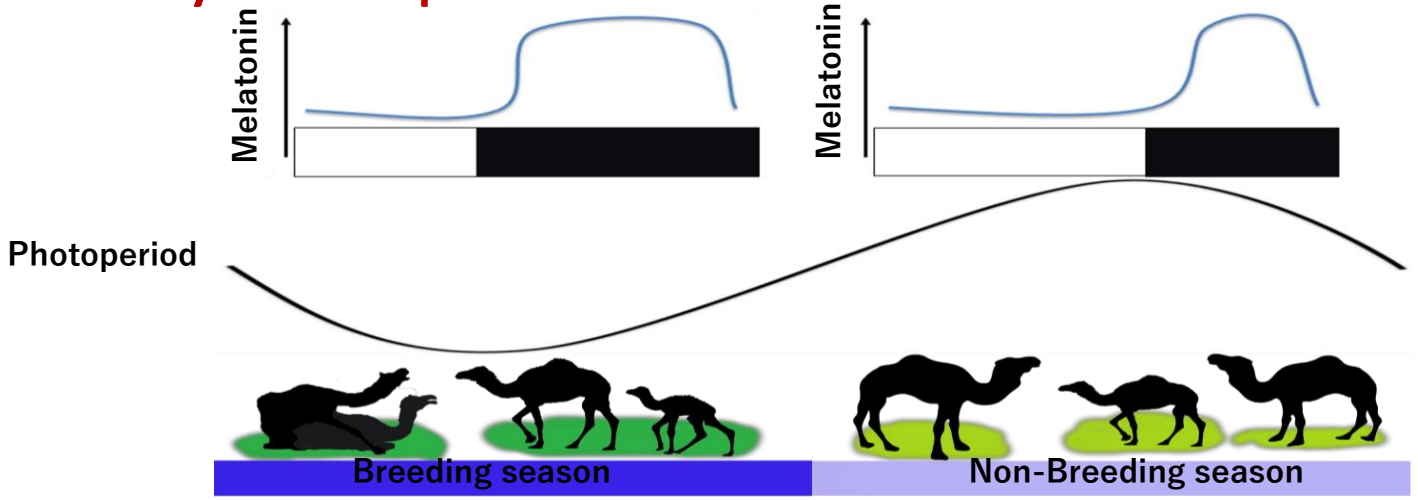
Environmental peculiarities of adaptation to harsh conditions

1) Adaptation to high temperature of the desert

2) Adaptation : dehydration / dryness

3) Adaptation to under nutrition

Seasonal rhythm of reproduction



El Allali et al., 2005, 2018

Ainani et al., 2017

Induction of ovulation

Camlin livestock faces several constraints that affect its productivity:

1) Low performance of reproduction

❖Camel calf (3-4 years)

2) Difficulties to control its sexual activity

Understanding the physiological and neuroanatomical processes which govern seasonality is necessary for properly controlling the reproductive activity of the dromedary camel.

Objectives

- Contribution of **Kisspeptin** and **RFRP-3** hypothalamic systems in the activation of the gonadotropic axis in dromedary camel
- Understand the mechanisms of the **Induction of ovulation** in this species

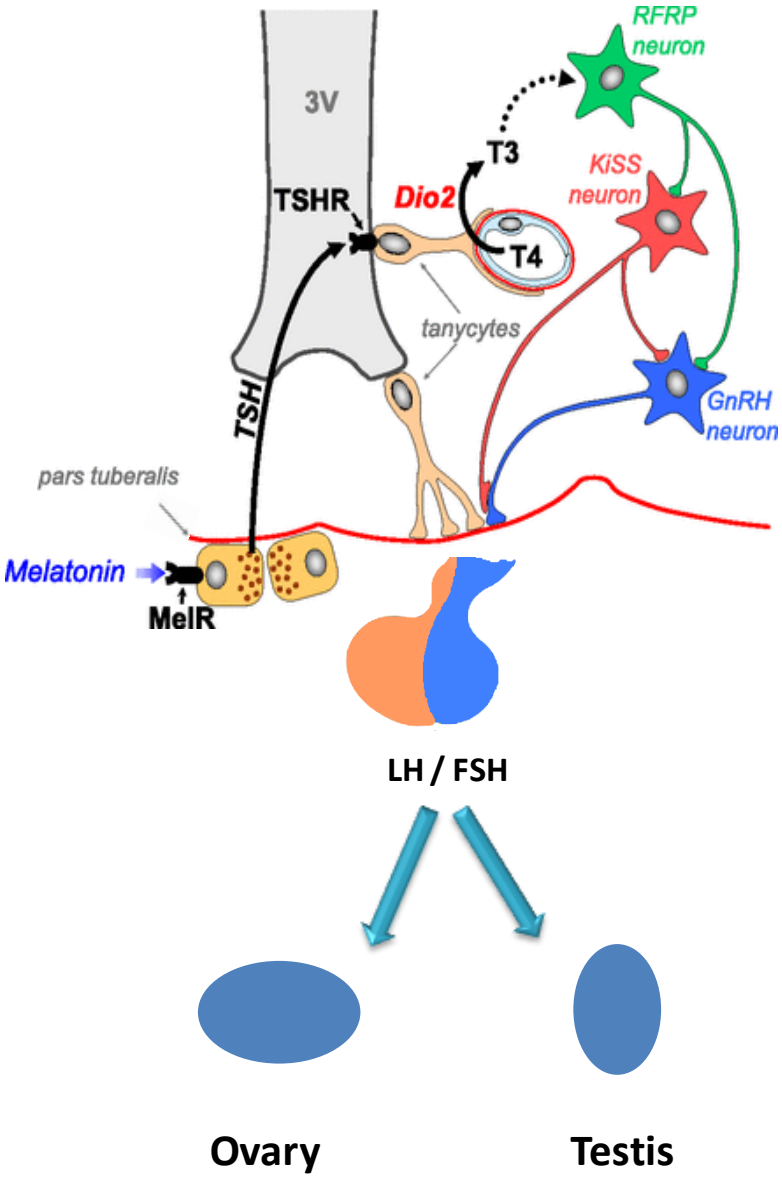
General plan

- **Part 1** : Mapping and seasonality of expression of Kisspeptin and RFRP-3 neurons in Dromedary camels

- **Part 2**: Central effect of β-NGF injection in Dromedary camels

- **Part 1** : Mapping and seasonality of expression of Kp and RFRP-3 neurons in Dromedary camels

Kisspeptin and RFRP-3 systems



Adapted from Klosen et al., 2013

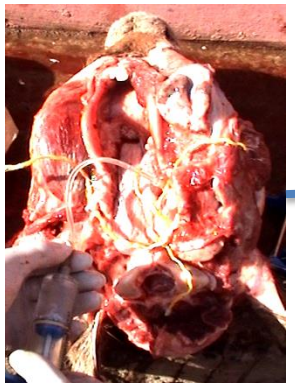
Kisspeptin System (Kp/Kiss1r):

- Discovered in 1996 as an inhibitor of metastases (Lee et al., 1996)
- Implication in reproduction in 2003 (de Roux et al., 2003, Seminara et al., 2003)
- A powerful activator of seasonal sexual activity (Simonneaux et al., 2009)
- GnRH neurons express Kiss1r
- Kp participates in the genesis of the pre-ovulatory peak of GnRH and LH.
- Kp is expressed at the level of the AVPV (POA) / Arc
- Kp neurons: mediators +/- feedback of steroids

RFRP-3 System (RFRP/GPR147):

- Discovered in 2000 in birds as GnRH neuron inhibitor (GnIH)
- RFRP-3: Arg-Phe-amide Related Peptide
- Direct Projections of RFRP-3 Neurons on GnRH and Kp Neurons (Kriegsfeld et al., 2004, Rizwan et al., 2012)
- RFRP-3 neurons are localized in DMH / VMH
- These two neuronal populations are not regulated by steroids

Mapping of Kisspepin and RFRP-3 neurons in Dromedary camel



Wash (0.9% saline solution)
Fixation (PAF 4%)

Brain sampling

Post-fixing fixation (PAF 4% during 72h)

Cryoprotection in 20% sucrose (one week)

Freezing at -40 ° C

Cuting in cryostat

Detection technique

- Immunohistochemistry with fluorescence or enzymatic reaction (DAB)
- In situ hybridization

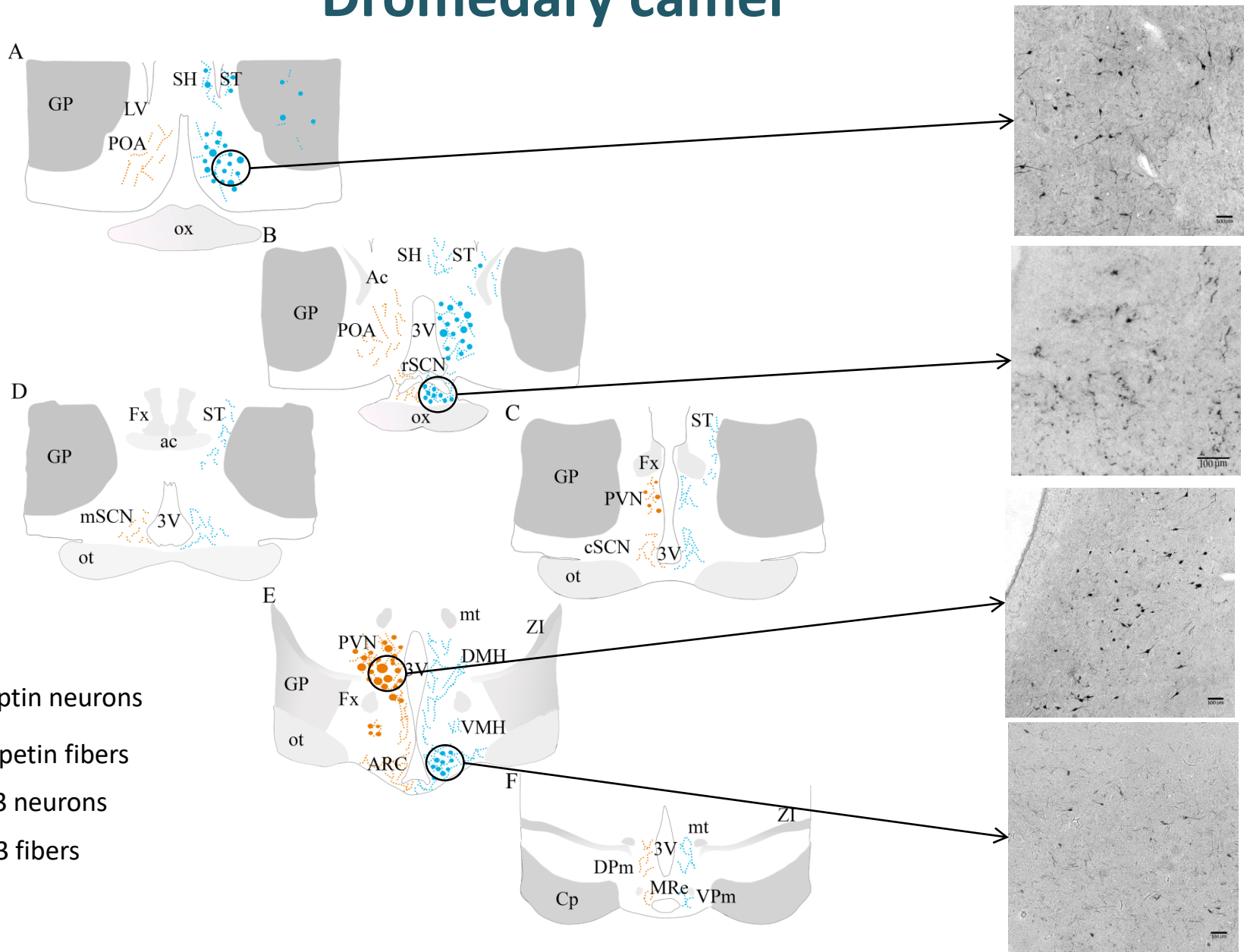
➢ Used antibodies

- JLV-1 for detection of Kp (1/500 in IF or 1/1500 in DAB)
- GA193 for the detection of the RFRP-3 (1/2000 in IF or 1/8000 in DAB)

➢ Used probes

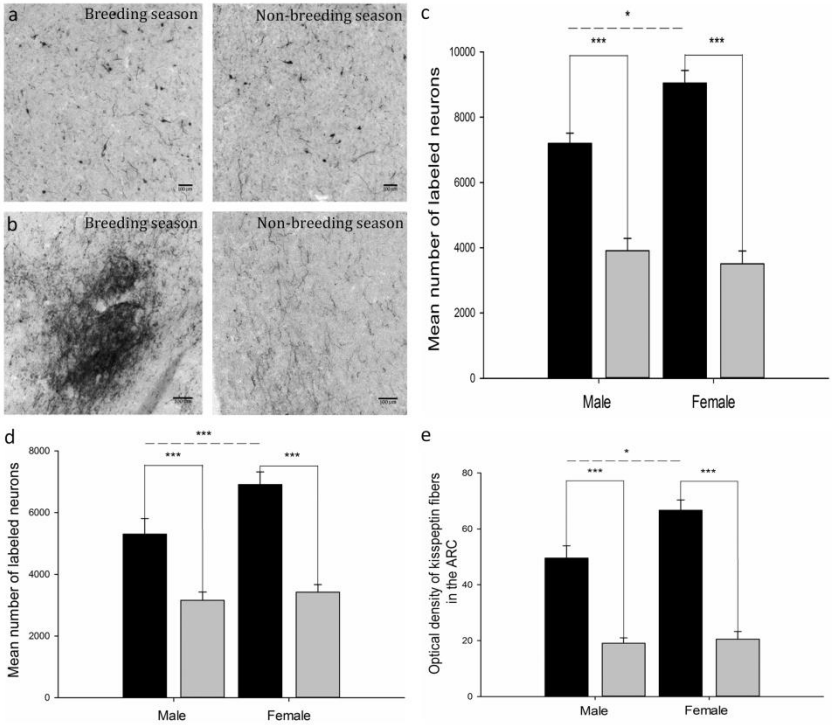
- Rat Kissepptin Probe for the detection of Kp mRNA
- Dromedary camel RFRP-3 Probe for the detection of RFPR-3 mRNA

Mapping of Kisspepin and RFRP-3 neurons in Dromedary camel

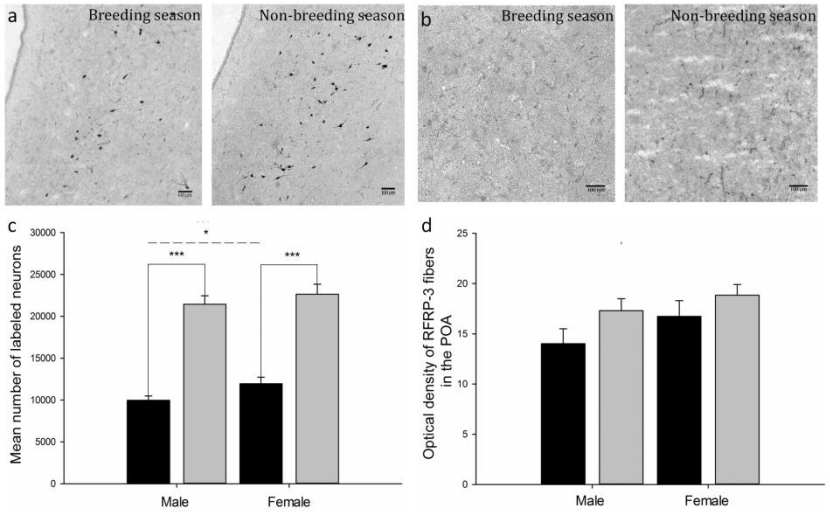


Seasonality of expression of Kisspeptin and RFRP-3 neurons

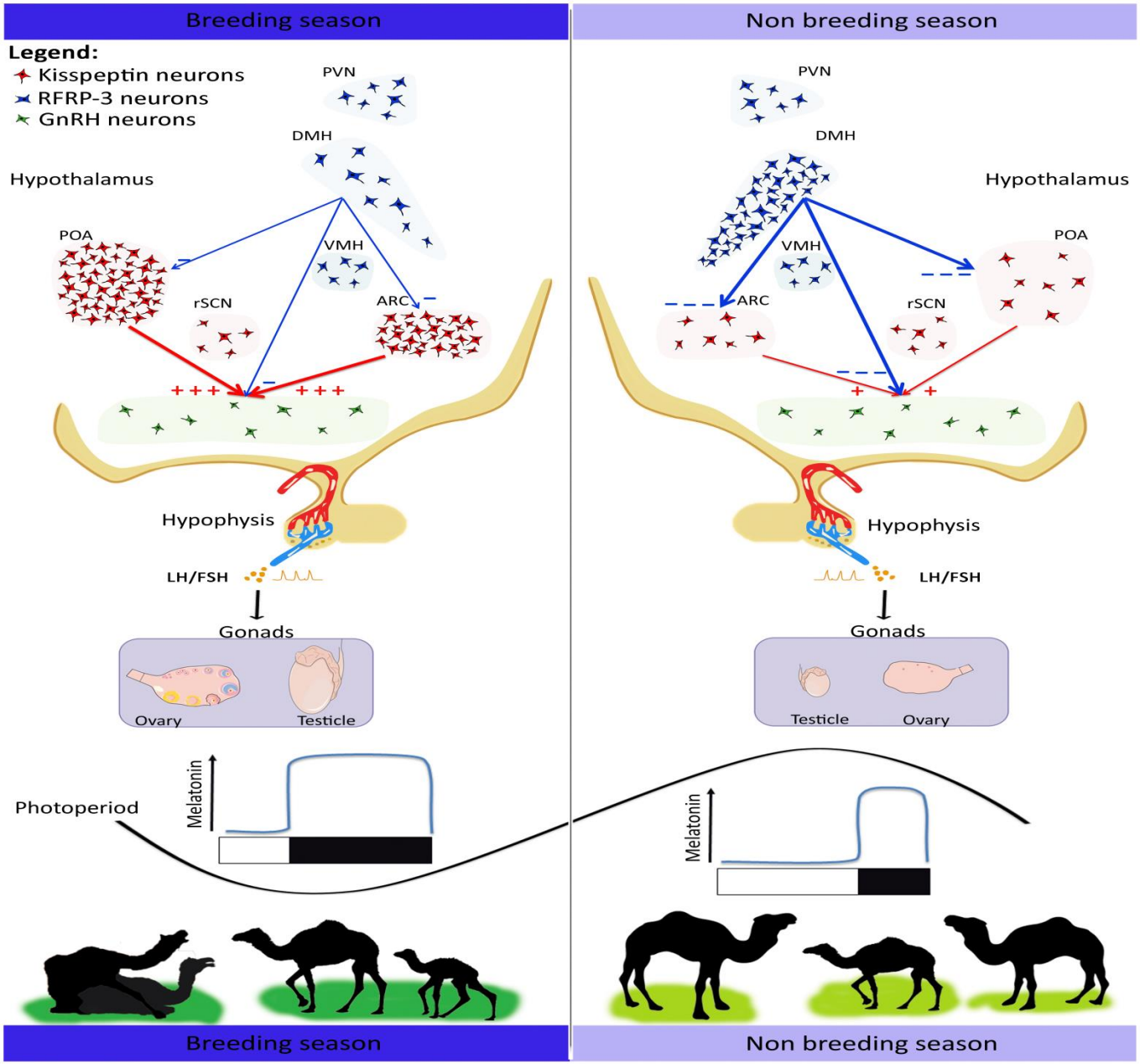
Kp neurons and fibers



RFRP-3 neurons and fibers



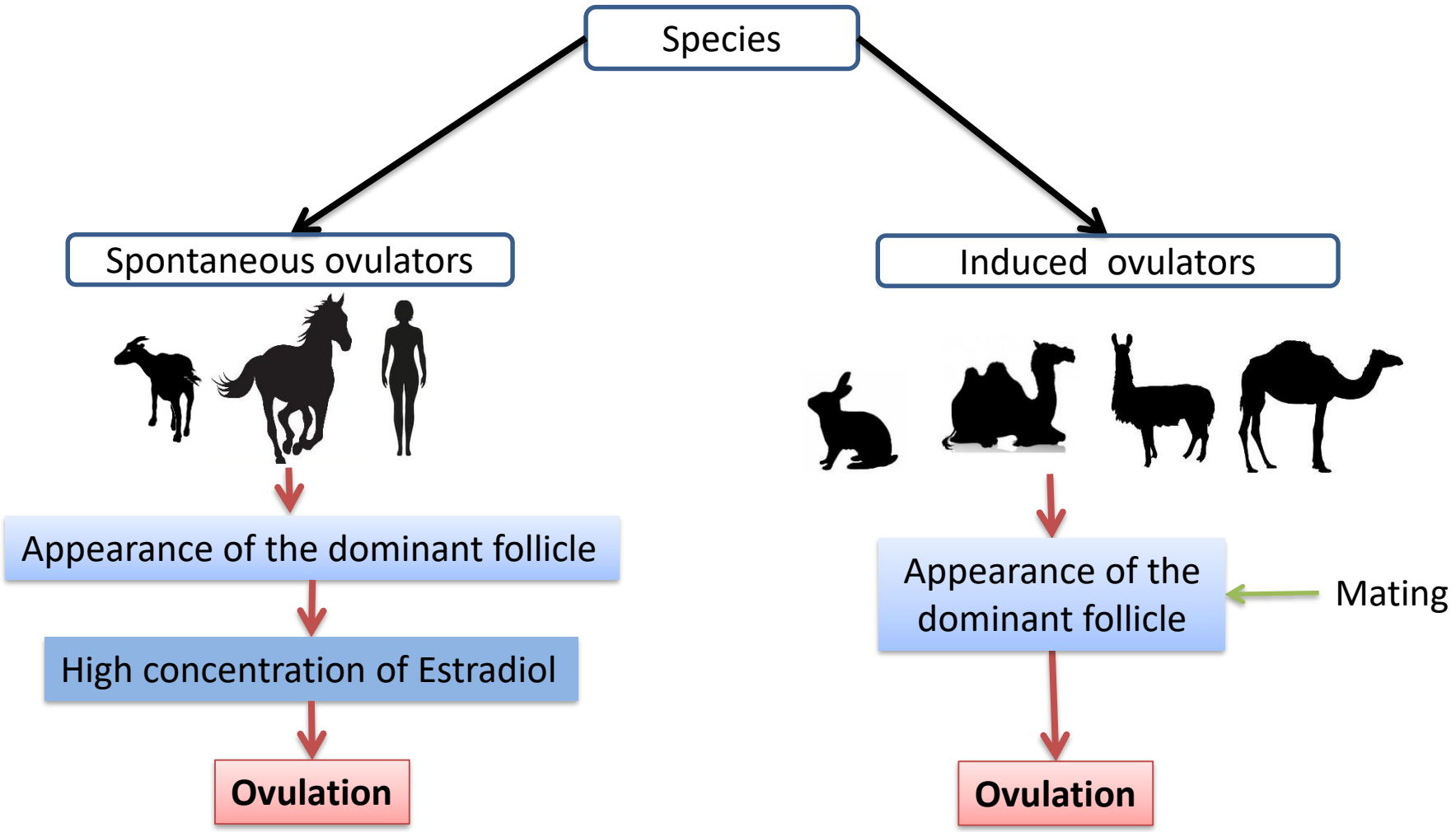
Conclusion 1



Ainani et al, 2019. J Comp Neurol

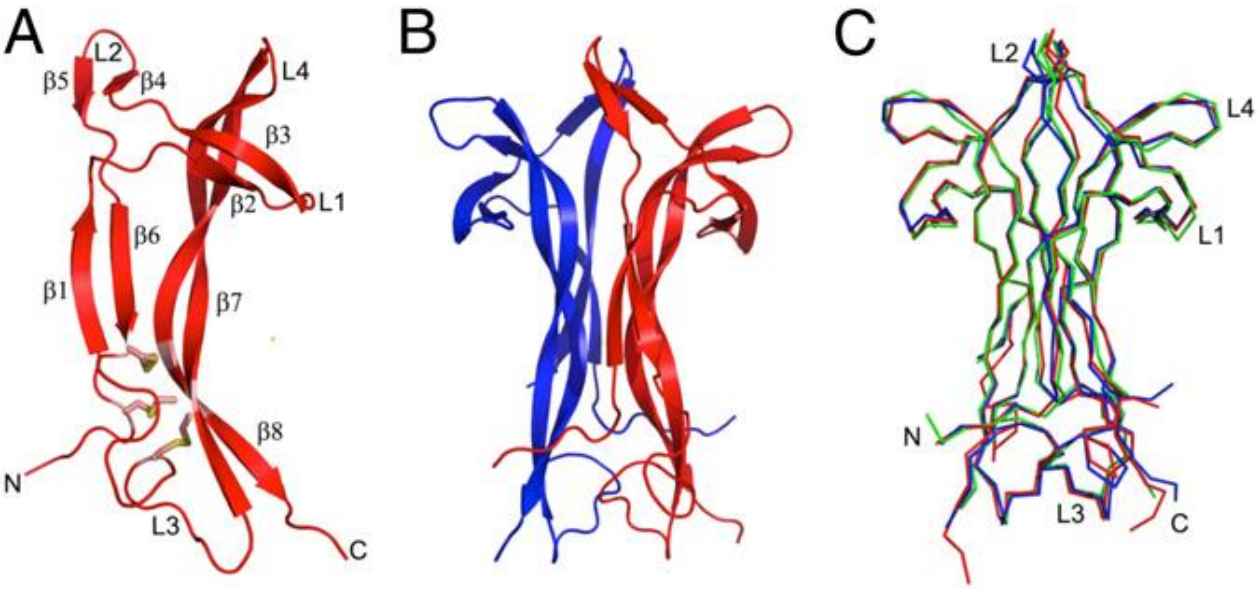
- **Part 2**: Central effect of β-NGF injection in Dromedary camels

Dromedary camel : Induced ovulator species



Nerve growth factor beta (NGF-beta) as Ovulation Inducting Factor

Ovulation Inducting Factor (OIF) is presence in the seminal plasma (SP) of the males (Xu et al., 1985, Zhao et al 2001, Ratto et al., 2010, Fatnassi et al., 2017)



Ratto MH et al., 2012

OIF (30% of SP proteins)

=

Nerve growth factor beta (NGF-beta)

What is the central target of β-NGF from the seminal plasma?

Hypothesis

β-NGF from the seminal plasma induces the activation of the kisspeptin neurons in the hypothalamus

frontiers in Veterinary Science

REVIEW
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Check for updates

Effect of the Camelid's Seminal Plasma Ovulation-Inducing Factor/β-NGF: A Kisspeptin Target Hypothesis

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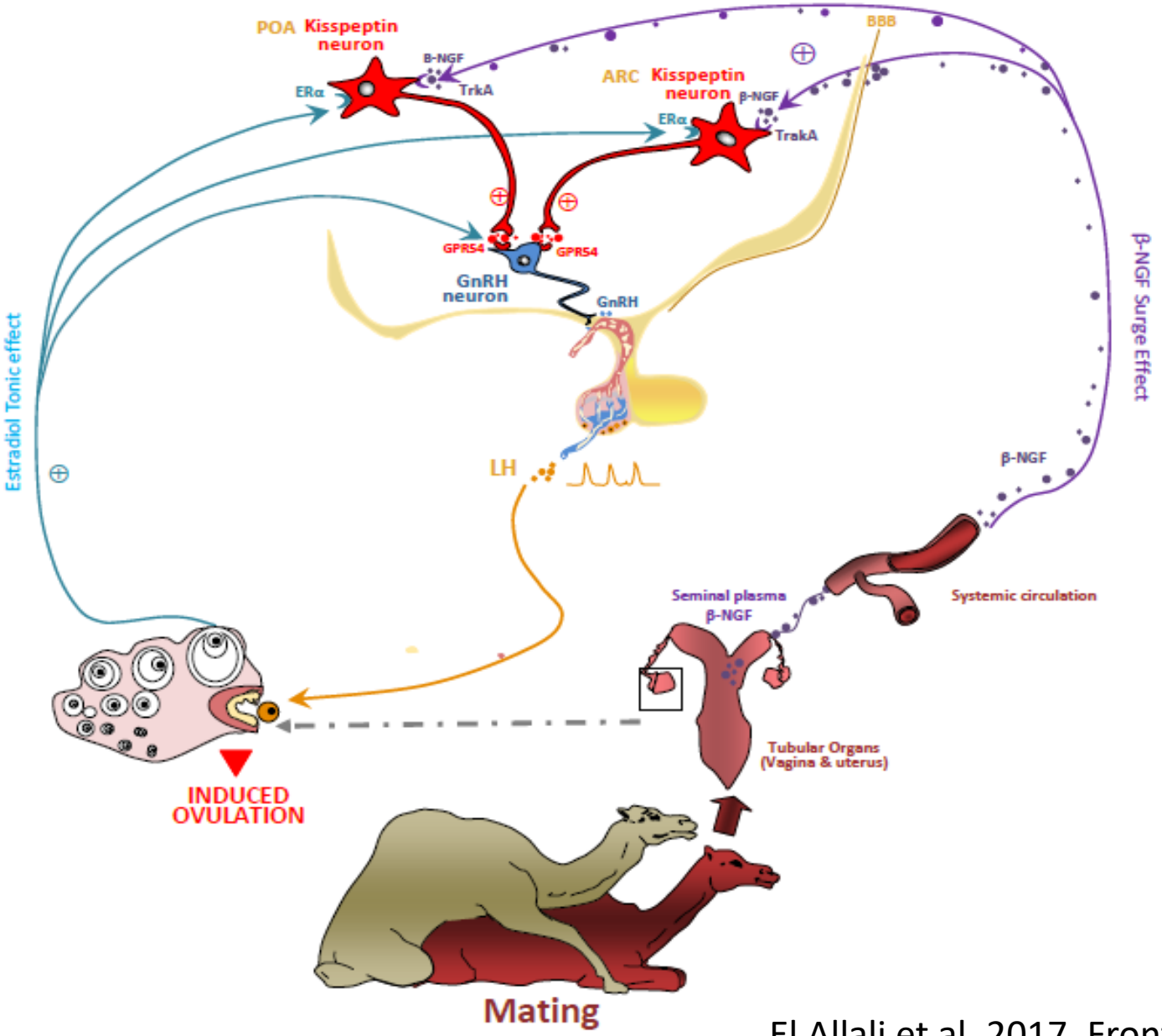
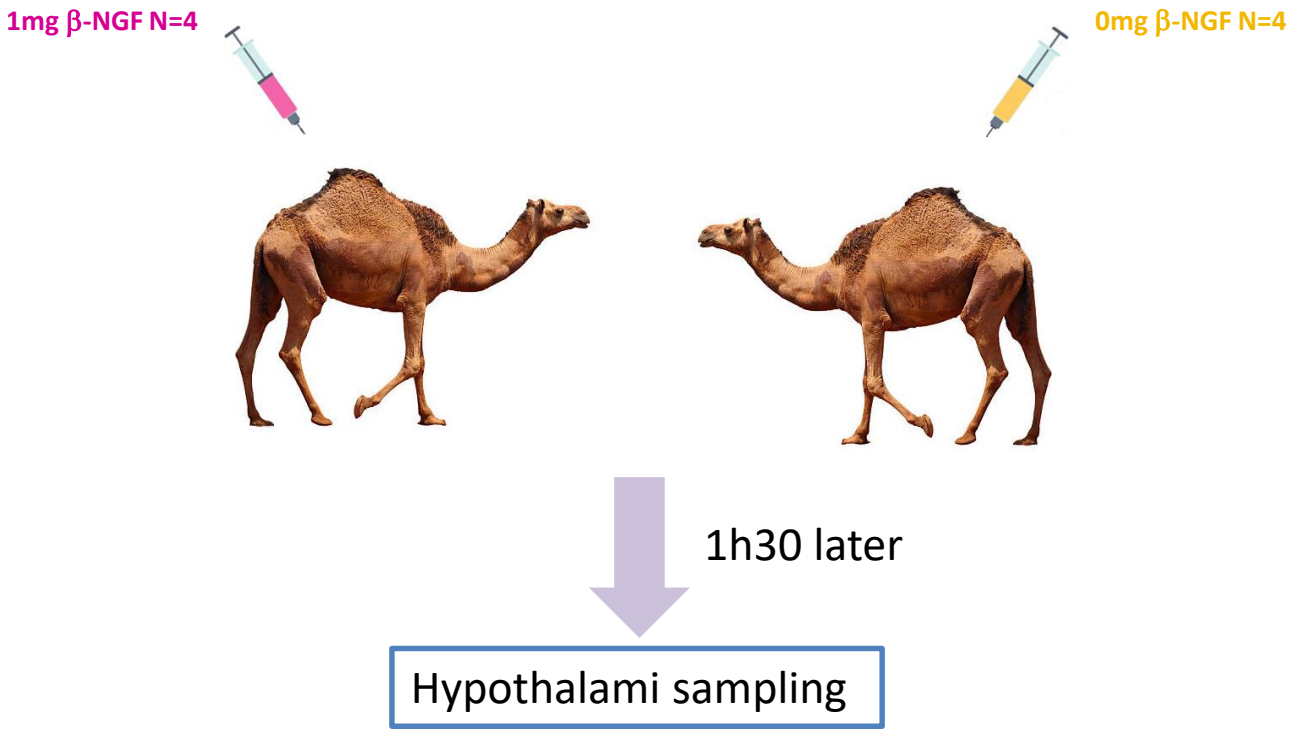
Hypothetical model



El Allali et al, 2017. Front Vet Science

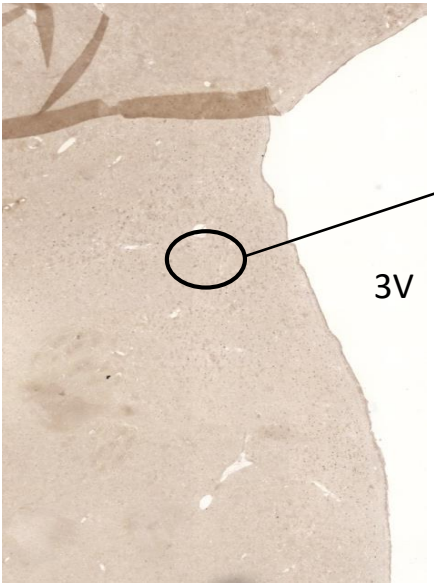
Experimental protocol



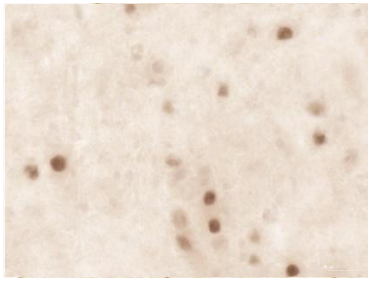
- Immunocytochemestry to visualize
  - c-Fos Expression
- Double immunocytochemestry to phynotype the neurons that express c-Fos

Central effect of the injection of β-NGF

1mg of β-NGF



A



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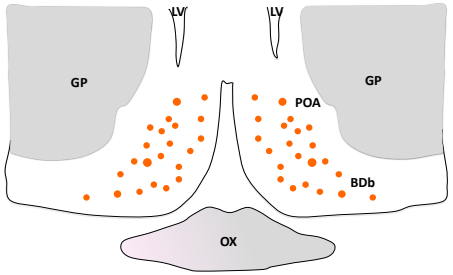

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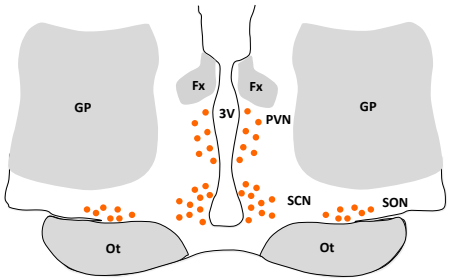
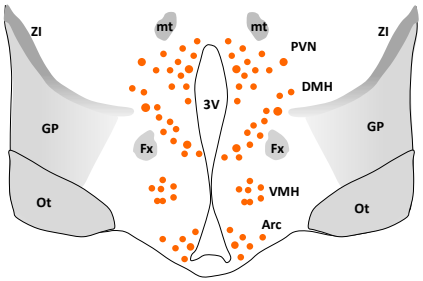
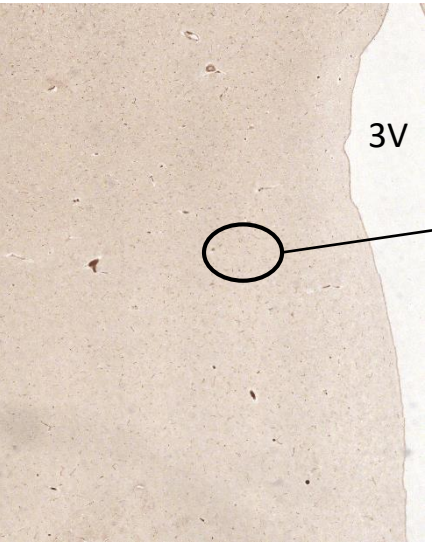


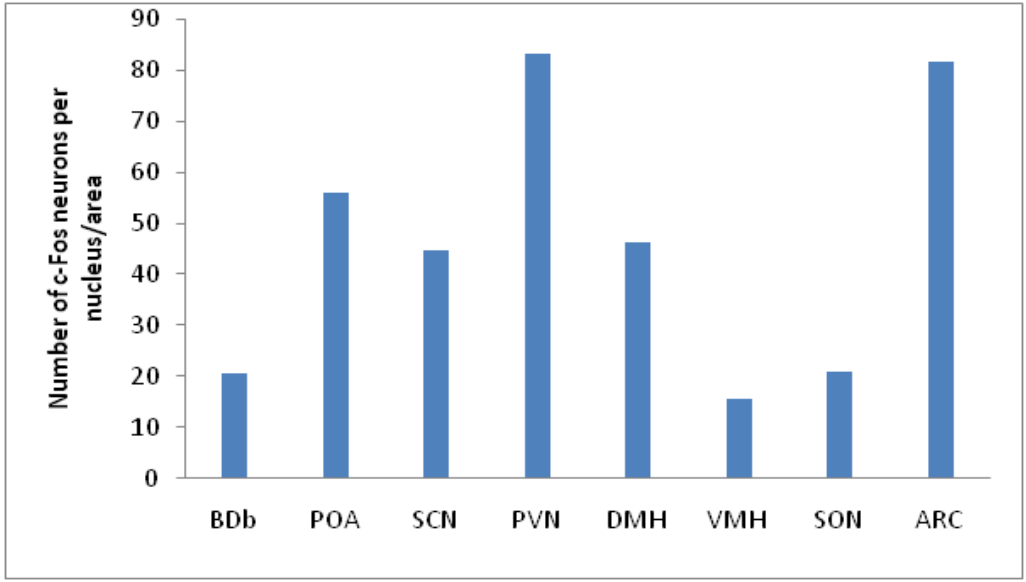
D



POA: Pre-optic area;
BDb : Bande diagonal de broca ;
SCN: suprachiasmatic nucleus;
GP: Globus palidus,
Fx: Fornix,
OX: Optic Chiasm ;
PVN : Paraventricular nucleus;
DMH : Dorsomedial nucleus;
VMH : Ventromedial nucleus;
SON : Supraoptic nucleus;
ARC: Arcuate nucleus;
Ot: Optic truct;
LV : Lateral Ventricle ;
V: 3rd ventricle.

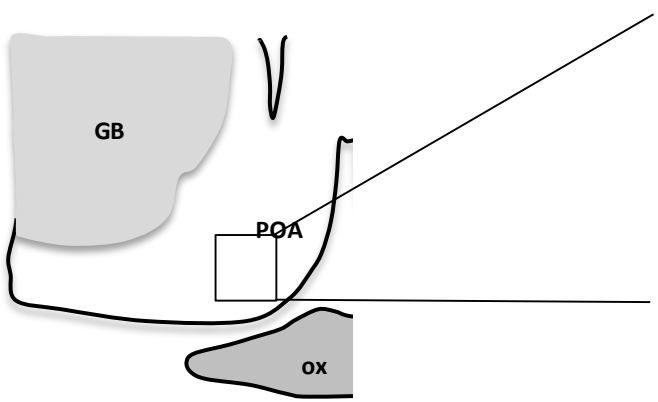
0mg of β-NGF

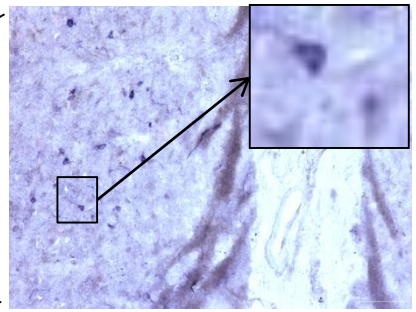


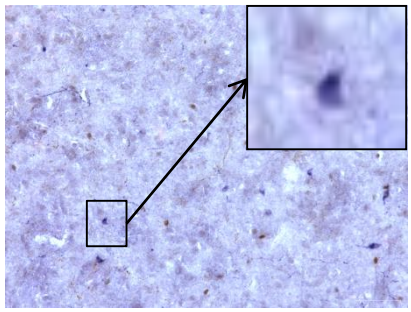


Phynotyping neurons that express c-Fos

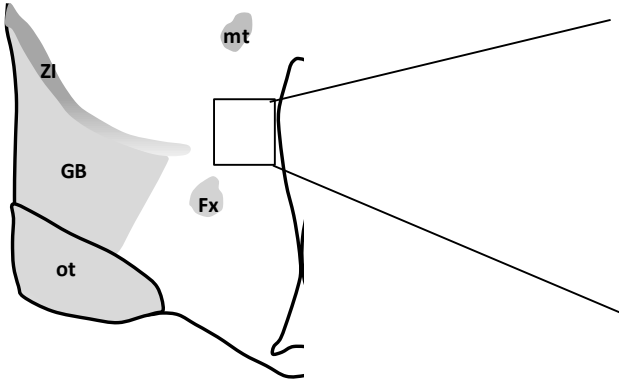
c-Fos/Kisspeptin

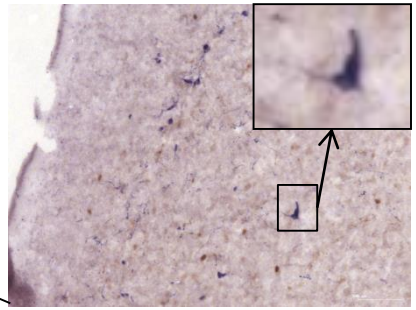


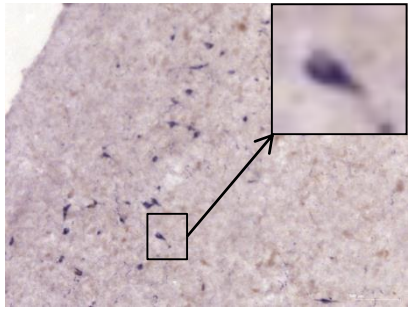




c-Fos/RFRP-3

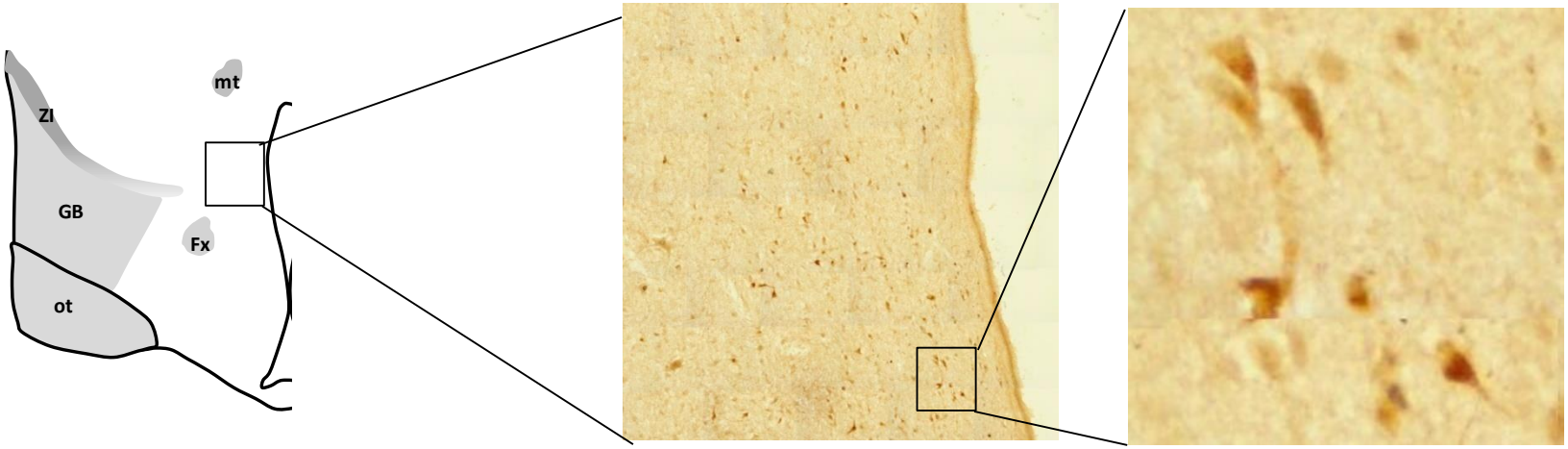


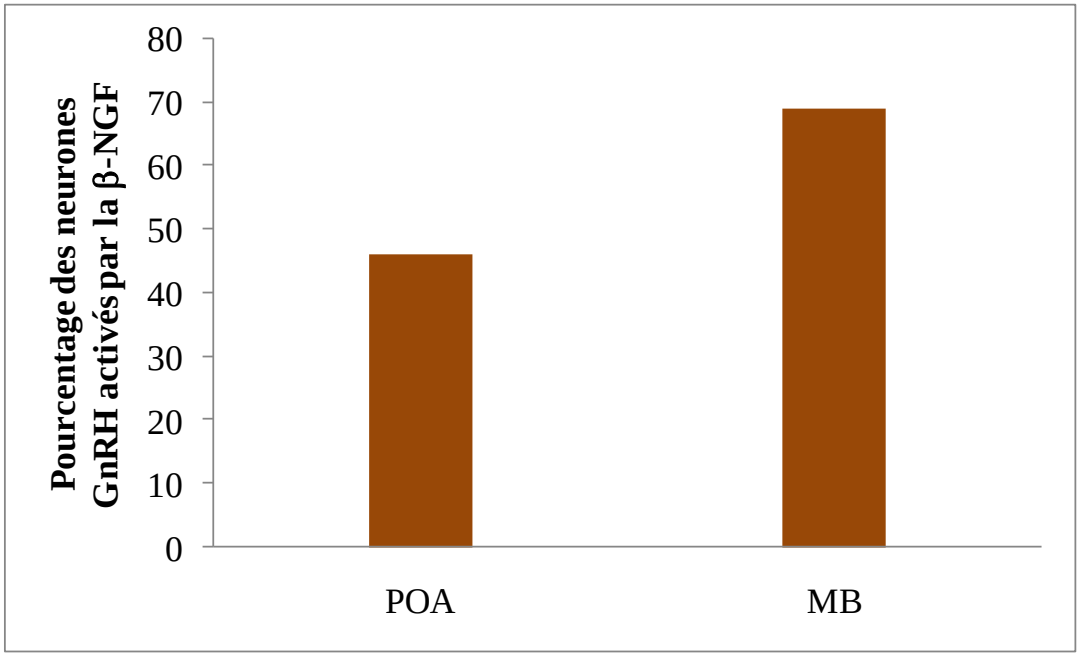




Phynotyping neurons that express c-Fos

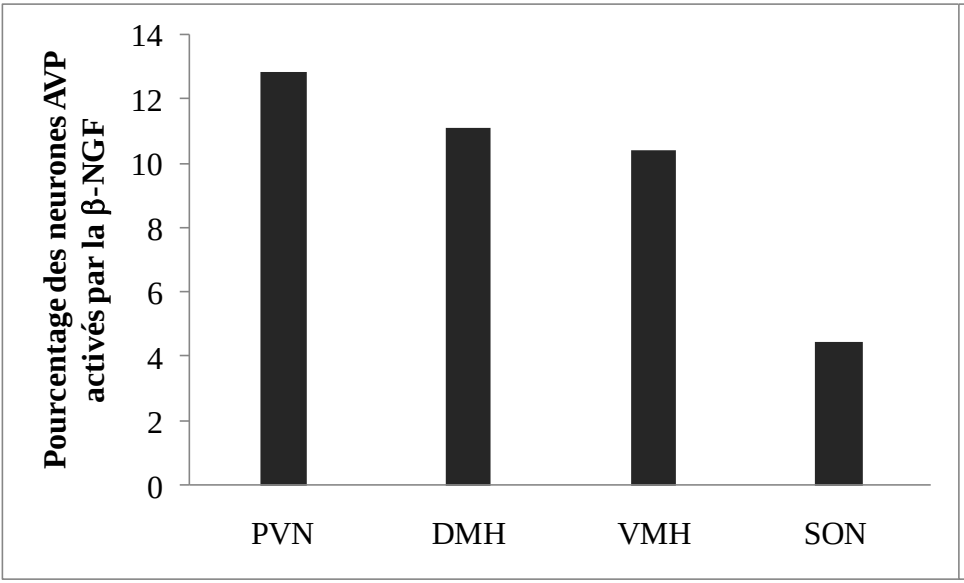
c-Fos/GnRH



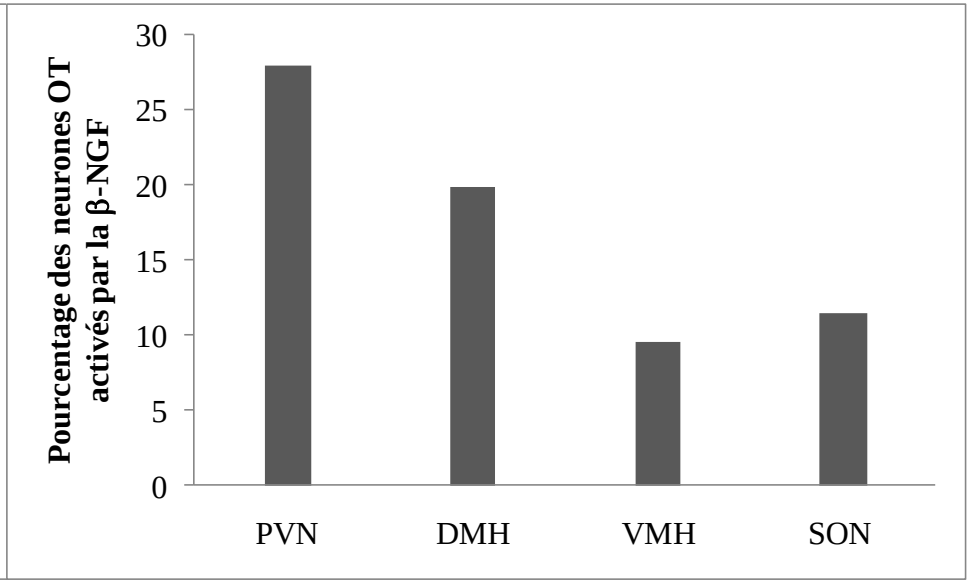


Phynotyping neurons that express c-Fos

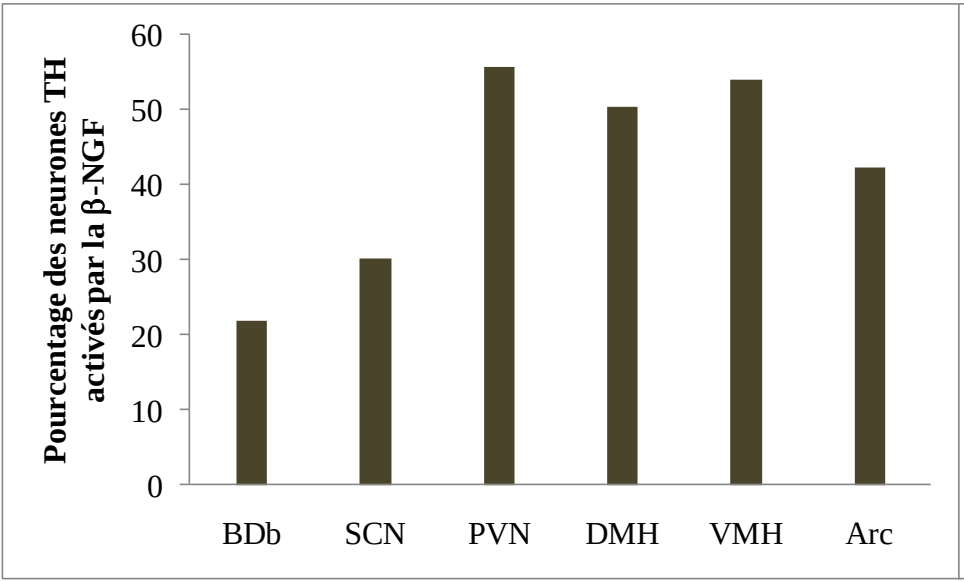
c-Fos/Vasopressin



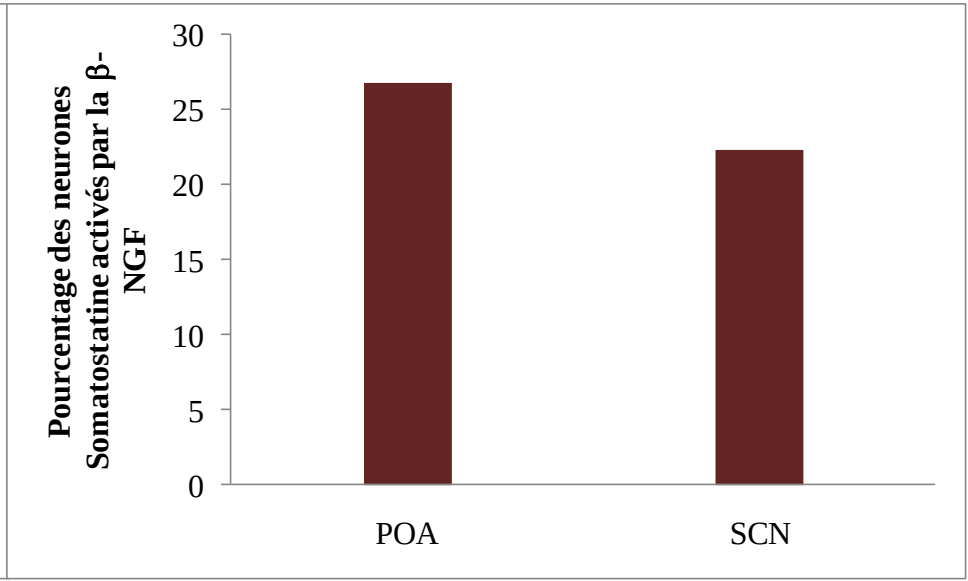
c-Fos/Oxytocin



c-Fos/Tyrosine Hydroxylase

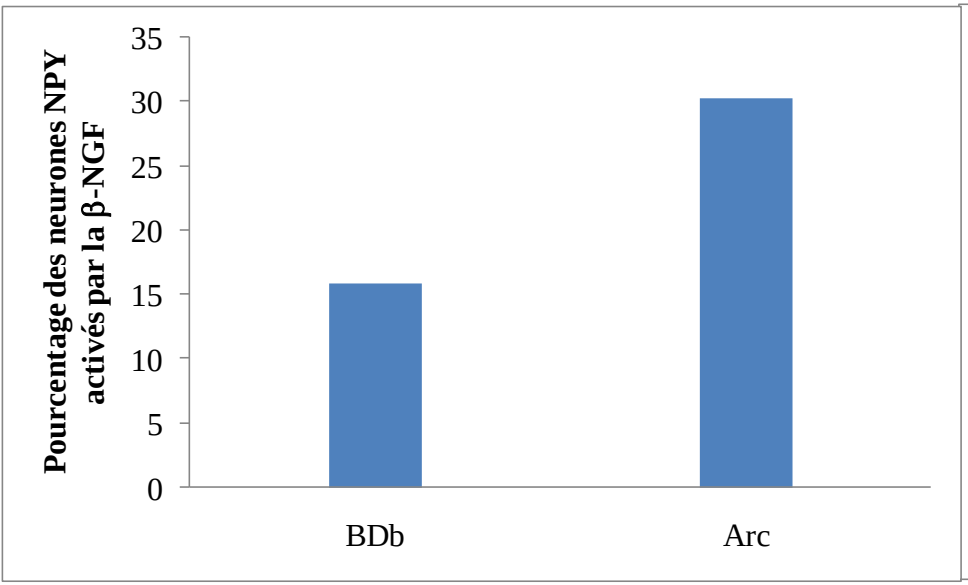


c-Fos/Somatostatin

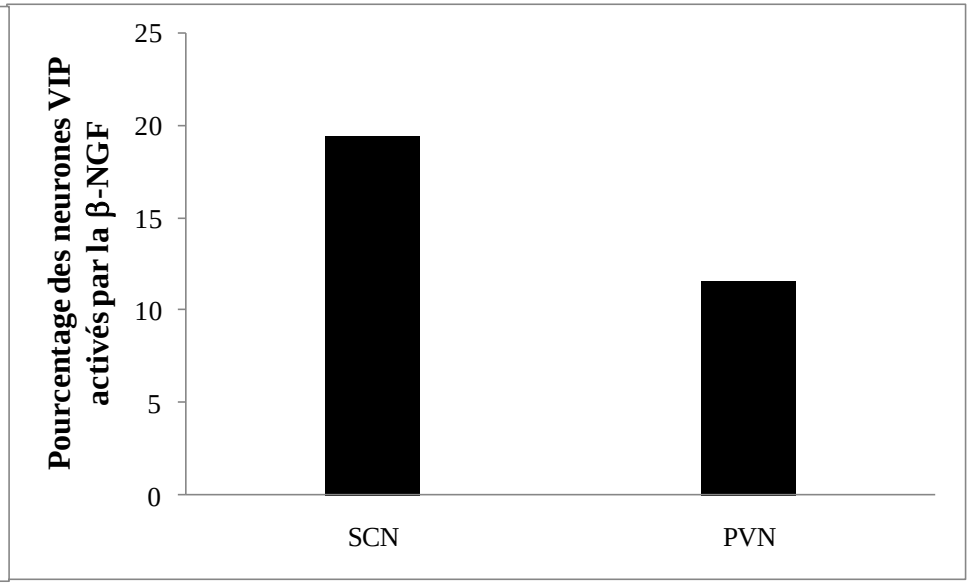


Phynotyping neurons that express c-Fos

c-Fos/NPY



c-Fos/VIP



Conclusion 2

- β-NGF injection induces a neuronal activation (c-Fos expression) in the hypothalamus of dromedary camel.
- The double immunostaining shows that the neurons activated by the β-NGF are neither of Kp nor RFRP-3.
- The β-NGF induces an activation of GnRH neurons
- The β-NGF induces an activation of Vasopressin, Oxytocin, Tyrosine hydroxylase, Somatostatin, VIP and Neuropeptide Y neurons
- These results indicate that the ovulatory effect of β-NGF in dromedary camel may be directly mediated by GnRH.
- The activation of the other different neurons may indicate that β-NGF induces other effects different than inducing ovulation.

Work in progress

- Finishing the phenotyping of neurons expressing c-Fos
- Study the mapping of the β-NGF receptors (TrkA and P75)
- Study of other factor in the signaling pathways of β-NGF :
  - p-CREB
  - P-AKT

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