

# Immunochemical characterisation of the rodent brain neurons involved in the efferent control of the clitoris and the vagina

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***This study was supported by an unrestricted grant of Pfizer Global Research and Development.***

## Background

- the clitoris and vagina are the **main peripheral sexual organs** involved in female sexual response
- **spinal and supraspinal centers** controlling the clitoris and vagina have been identified in retrograde tracing studies conducted in female rats (*Marson & Murphy, 2006; Marson, 1995*) :
  - Spinal : lumbosacral segments (L6-S1)
  - Brain centers widely distributed
- **neurochemical phenotype** of the neurons belonging to these descending pathways remain unknown

## Background

- The female sexual response is controlled by numerous hormonal and neurochemical systems
- Critical role of the **melanocortin-4 receptors** (MC4-R) and **oxytocin** (OT)
- **Bremelanotide** (MC3- and MC4-R agonist) selectively increases sexual appetitive behaviors in female rats (*Pfaus et al., 2007,2004*) and positively affects sexual desire and genital arousal in pre- and postmenopausal women (*Safarinejad, 2008; Diamond et al., 2006*)
- pharmacological manipulation of **OT** at the central level modify both receptive and proceptive components of female sexual behavior (*Witt and Insel, 1991; Caldwell et al., 1994*).

## Objective

### **Aim :**

To examine if brain neurons involved in the efferent control of the rodent clitoris and vagina possess melanocortin-4 receptors (MC4-R) and/or contain oxytocin (OT).

### **General procedure :**

Double and triple immunocytochemical labeling against **pseudo-rabies virus** (PRV) injected in the clitoris and vagina, **MC4-R** and **OT** in brain sections from estrous females

## Materials and methods

**Day 0 :  
estrus**



PRV injection  
into the clitoris  
and vagina



**Day 5**

- deep anaesthesia
- perfusion
- brain collecting
- post-fixation
- cryoprotection
- slicing (30 $\mu$ m)



**Immunocytochemistry**

**Antibodies against :**

- PRV
- MC4-R
- OT

- Double labelling :  
PRV/MC4-R and PRV/OT
- Triple labelling :  
PRV/MC4-R/OT



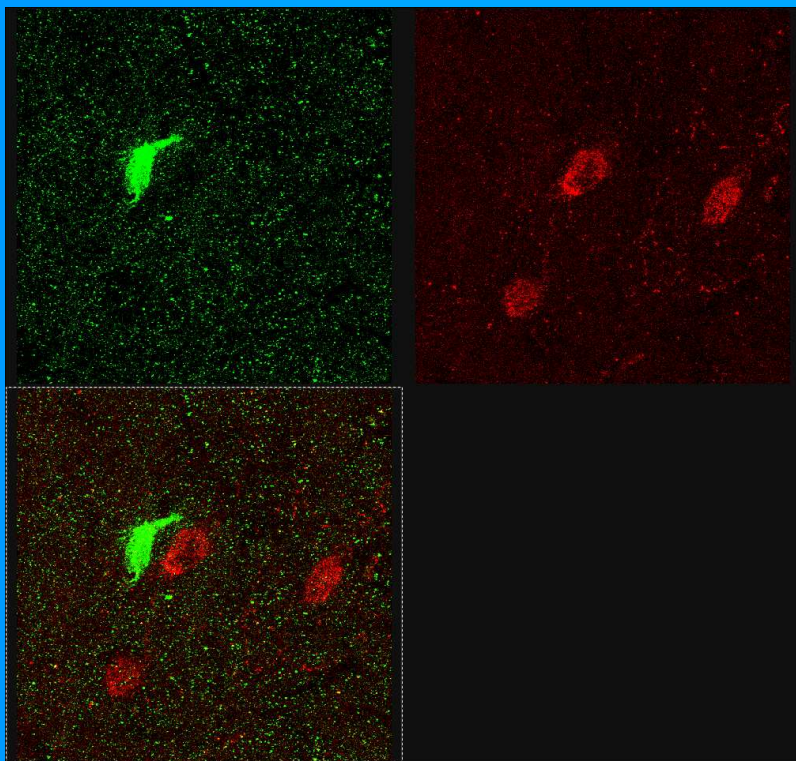
**Confocal laser  
scanning analysis**



## Results : double labelling PRV/MC4-R in the PVN – confocal analysis

PRV

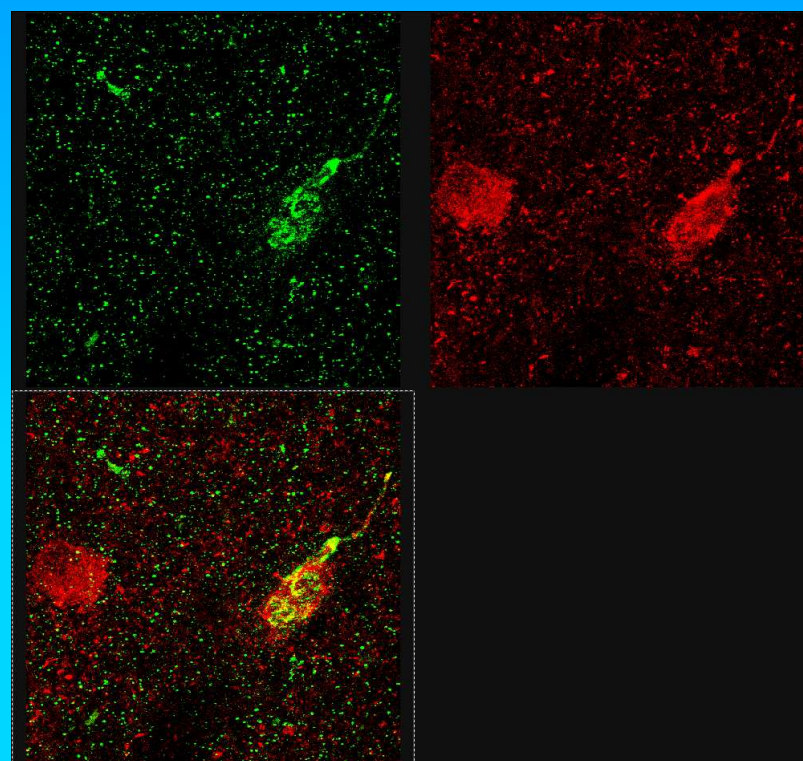
MC4-R



No colocalization

PRV

MC4-R



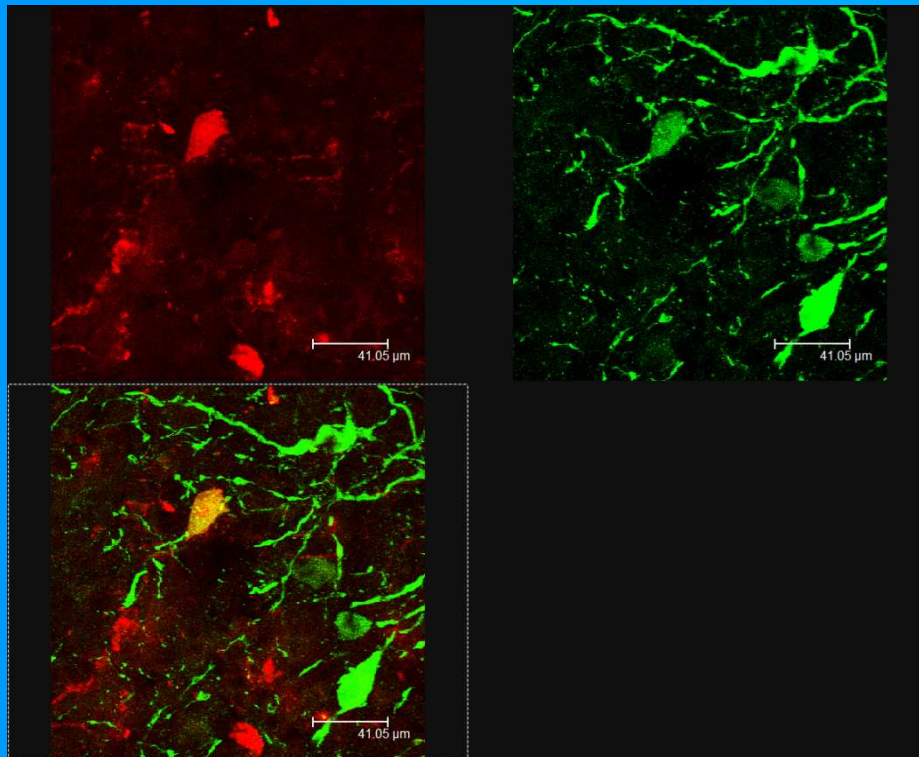
1 double labeled  
PRV/MC4-R neuron



## Results : double labelling PRV/OT in the PVN – confocal analysis

OT

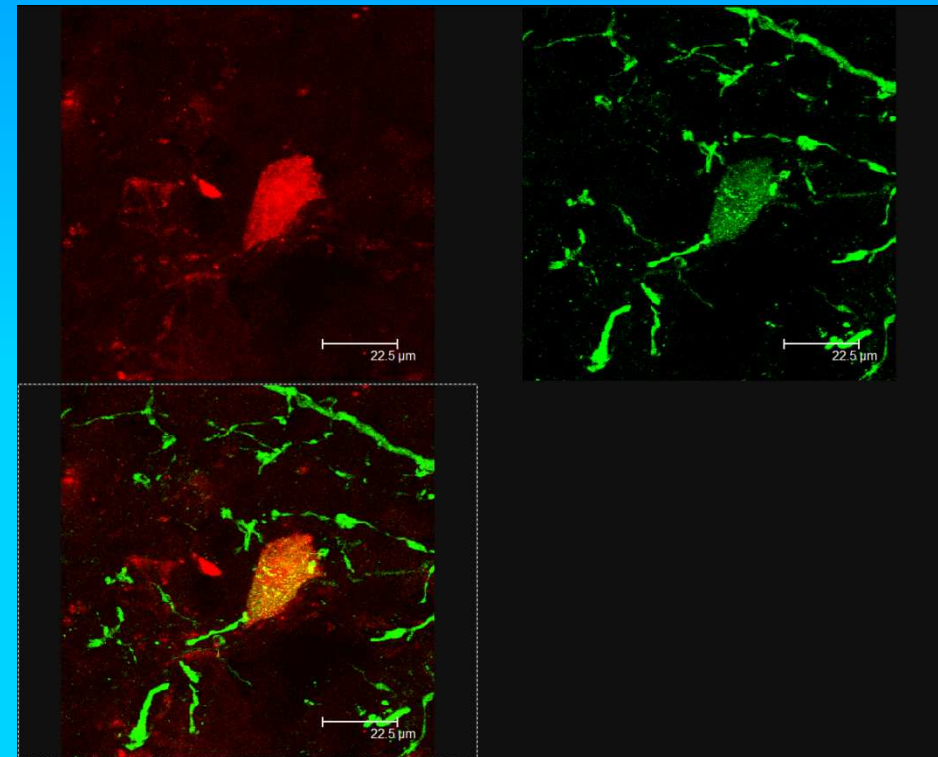
PRV



Colocalization

OT

PRV



Double labeled PRV/OT neuron  
(magnification)

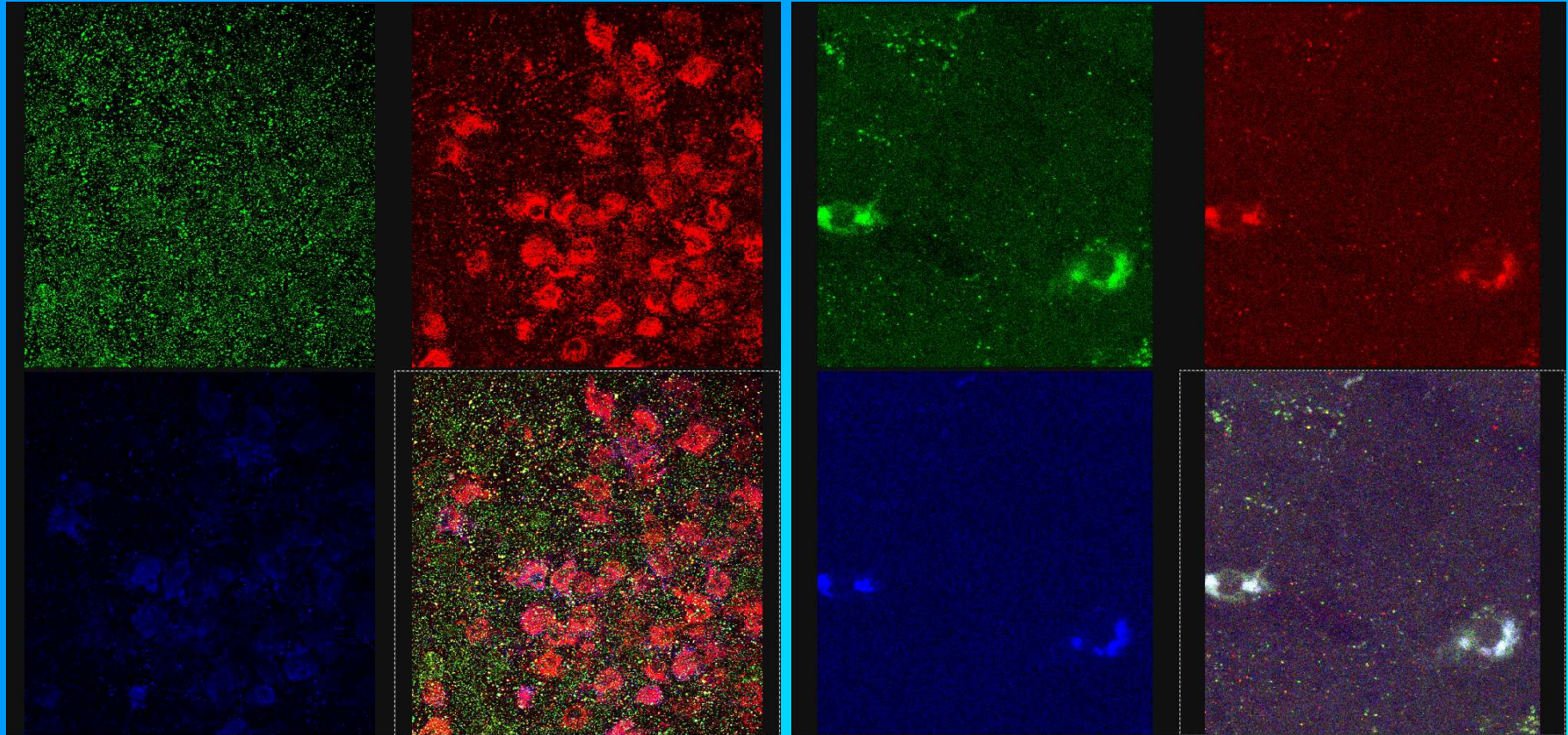
# Results : triple labelling PRV/MC4-R/OT in the PVN

PRV

MC4-R

PRV

MC4-R



OT

No colocalization

2 triple labeled PRV/MC4-R/OT neurons

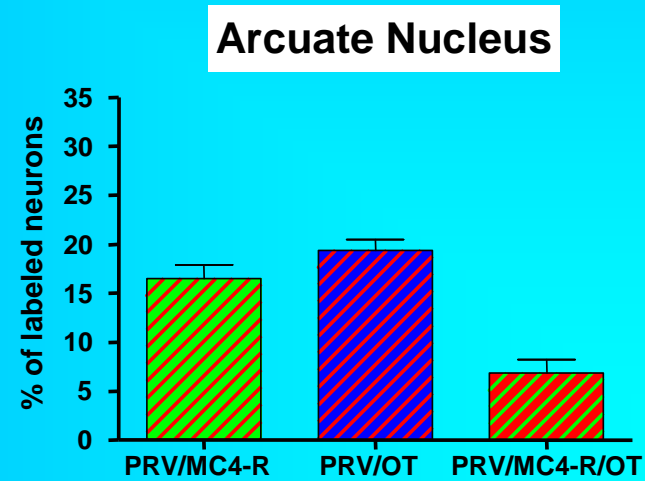
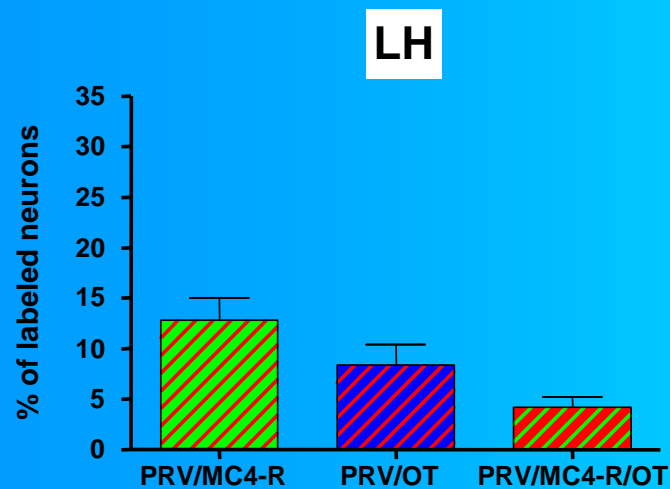
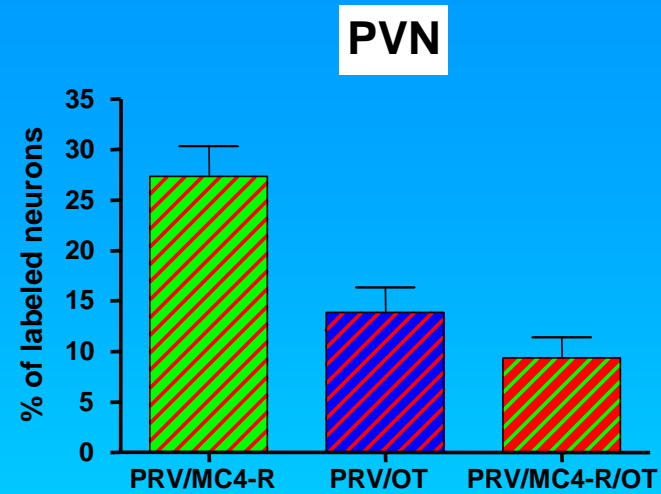
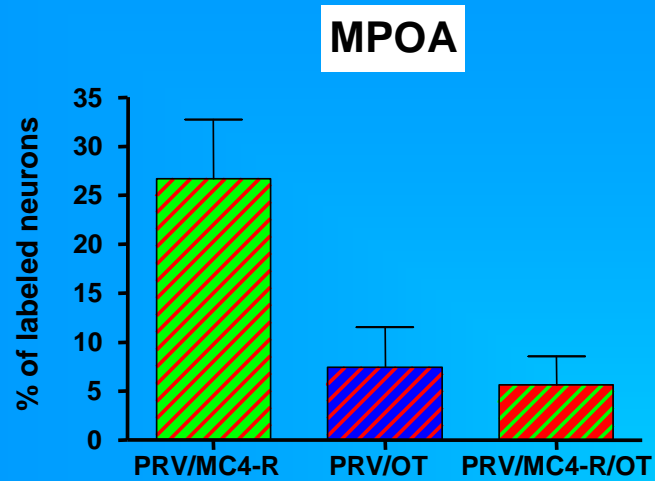


## Results : double labelling

Brain areas	PRV	OT	PRV/OT	%PRV/OT
Medial preoptic area	9,7 ±3,3	6,8 ±2,0	0,2 ±0,1	1,7 ±1,2
Paraventricular nucleus	15,5 ±4,1	40,6 ±3,6	1,3 ±0,4	8,4 ±1,9
Supraoptic nucleus	0,0	30,1 ±6,5	0,0	0,0
Lateral hypothalamus	6,5 ±2,1	10,8 ±1,4	0,2 ±0,2	3,8 ±3,4
Lateral hypothalamic area	1,8 ±1,8	7,3 ±6,8	0,0	0,0

Brain areas	PRV	MC4-R	PRV/MC4-R	% PRV/MC4-R
<b>LIMBIC SYSTEM</b>				
Bed nucleus of stria terminalis	1,5 ± 0,9	3,2 ± 0,6	0, 8 ± 0,7	14,7 ±11,4
Lateral septum	1,9 ±0,5	1,3 ±0,2	0,7 ± 0,4	20,5 ±11,9
<b>HYPOTHALAMUS</b>				
Medial preoptic area	8,2 ±3,4	9,2 ±3,5	1,0 ±0,3	14,5 ±2,3
Paraventricular nucleus	10,7 ±2,8	22,5 ±4,7	2,9 ± 0,8	23,2 ±4,0
Supraoptic nucleus	0,0	32,0 ±1,5	0,0	0,0
Lateral hypothalamus	1,6 ±0,3	8,8 ±2,9	0,3 ±0,1	11,6 ±3,0
Ventromedial nucleus	0,5	0,0	0,0	0,0
Arcuate nucleus	13,4 ±6,2	9,0 ±0,3	3,2 ±1,8	16,8 ±2,1
<b>MIDBRAIN</b>				
Periaqueductal gray	6,1 ±2,6	2,1 ±1,1	0,9 ±0,7	25,6 ±8,9
Red nucleus	27,8 ±5,0	24,4 ±4,6	9,3 ±1,2	30,9 ±2,5

## Results : double and triple labelling



Percentages of PRV-labeled neurons immunoreactive for MC4-R, for OT, and for both MC4-R and OT.

## Summary

- The majority of double PRV/MC4-R and PRV/OT was located in the paraventricular nucleus, medial preoptic area, lateral hypothalamus and arcuate nucleus.
- PRV positive neurons were more likely to be immunoreactive for MC4-R than for OT.
- Scattered triple labelled PRV/MC4-R/OT neurons were detected in the medial preoptic area and the paraventricular nucleus.

## Conclusion

These data strongly suggest that **MC4-R, and to a less extent OT,** are involved in the **efferent control** of the clitoris and vagina, and consequently facilitate our understanding of how the melanocortineric pathways regulates female sexual function.