# Interactions between bone and the central nervous system

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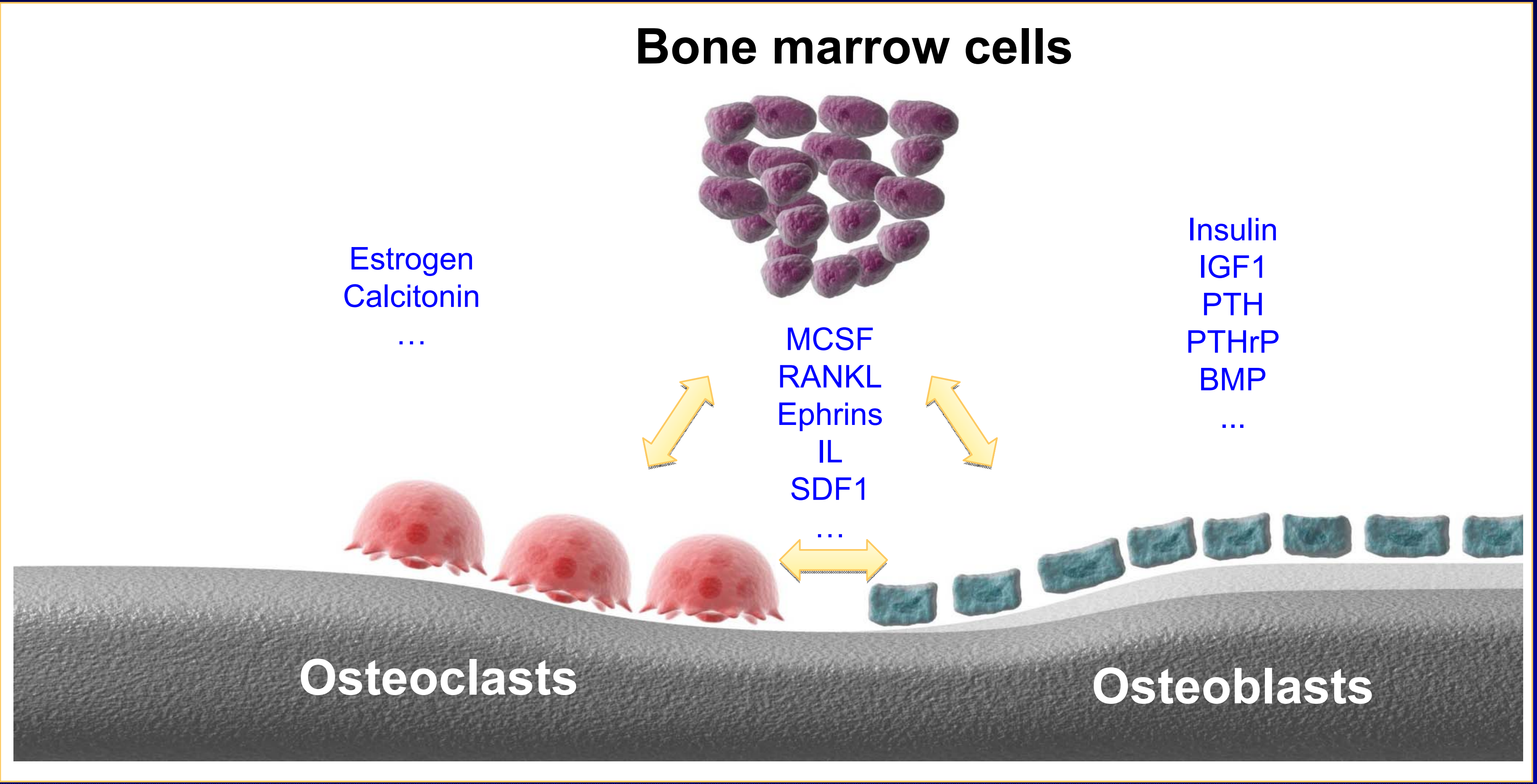
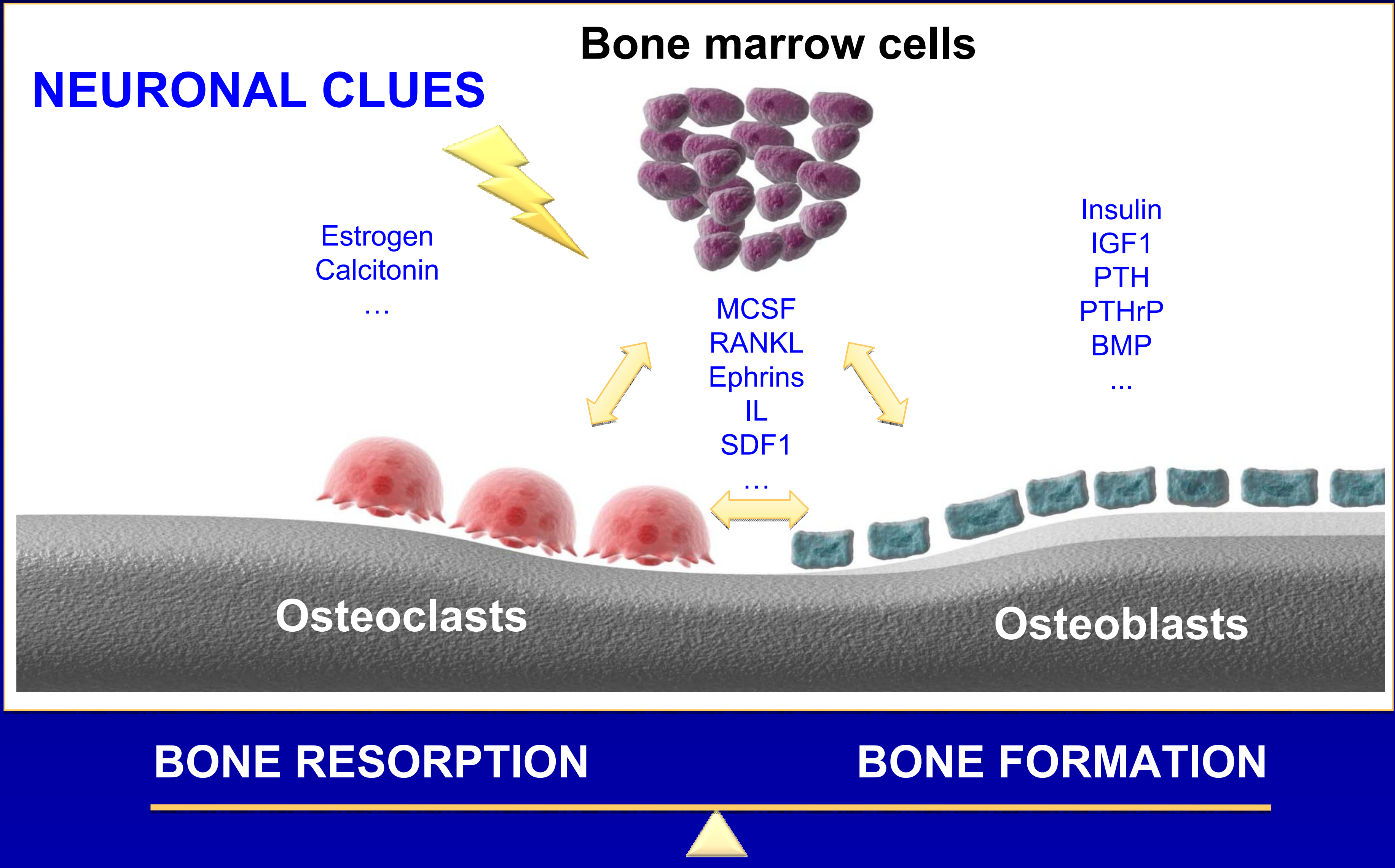
# BONE REMODELING




**BONE RESORPTION** **BONE FORMATION**

# BONE REMODELING



# THE BONE AND BRAIN CONNECTION

- Traumatic brain injury promotes ectopic bone formation and bone healing
- Bone formation and resorption markers display circadian patterns
- Bone are innervated
- Stroke, spinal injury and peripheral neuropathies provoke bone loss
- Obesity is associated higher BMD

# HYPOTHESIS

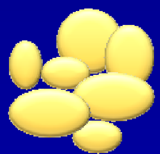
**BONE REMODELING IS AN HOMEOSTATIC PROCESS**

**REGULATED BY**

**THE CENTRAL NERVOUS SYSTEM**

# A model of hypothalamic dysfunction: The ob/ob mice

- Body weight
- Reproduction
- Immunity

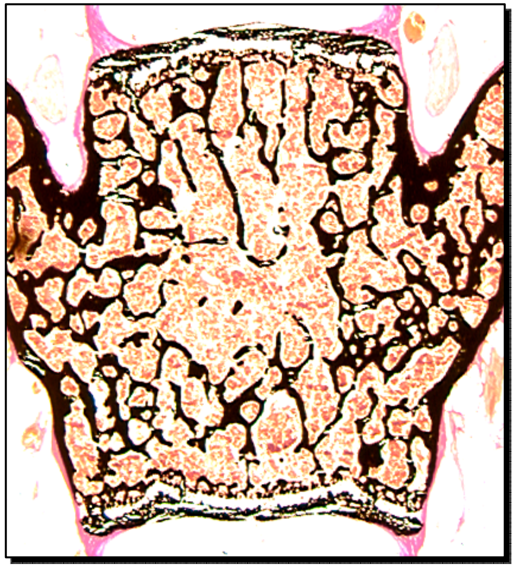


Leptin

Adipocytes

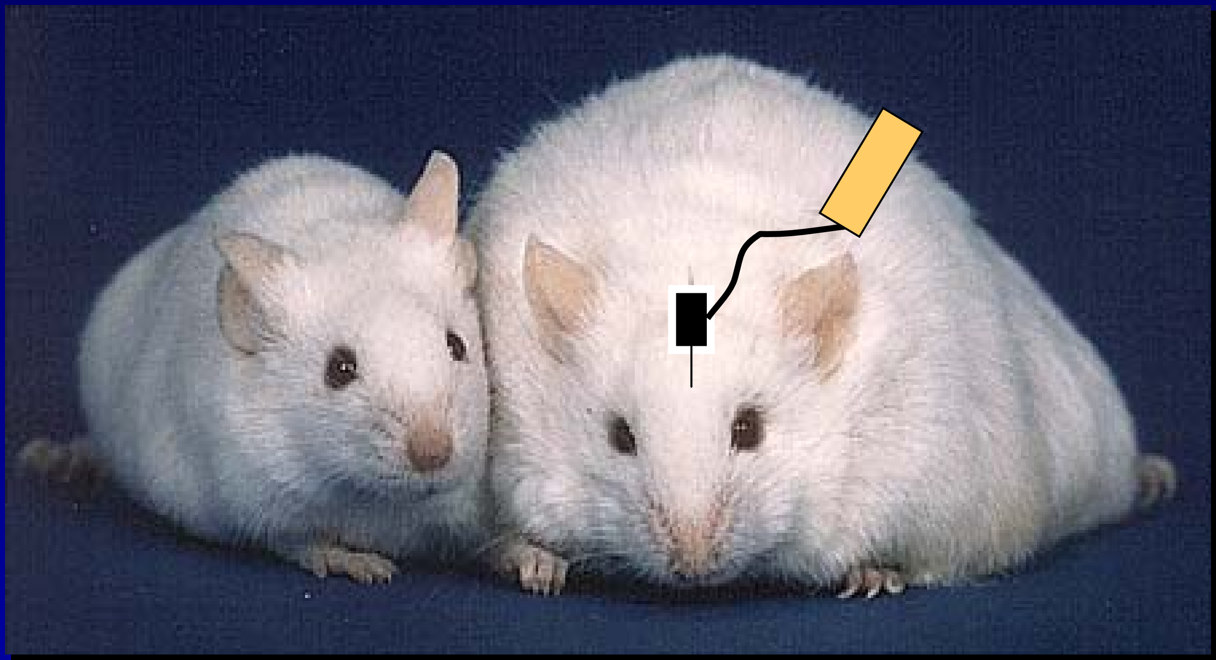
**WT** ***ob/ob***

# Leptin is an inhibitor of bone formation

| WT | *ob/ob* |
|---|---|
|  |  |

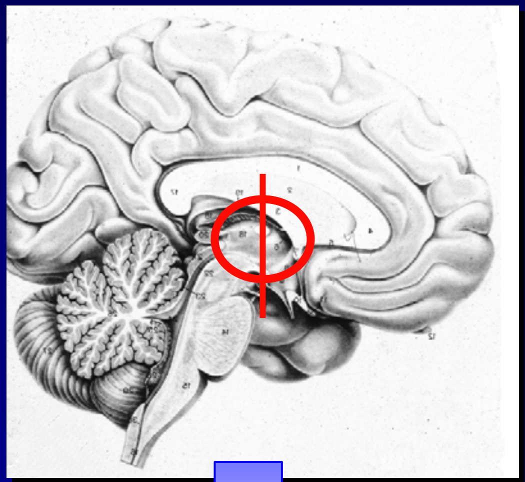
| | WT | *ob/ob* |
|---|---|---|
| **Bone Vol. (%)** | **14.0** ±0.4 | **19.4** ±0.6* |
| **BFR** | **64.6** ±18.0 | **110.3** ±15.2* |

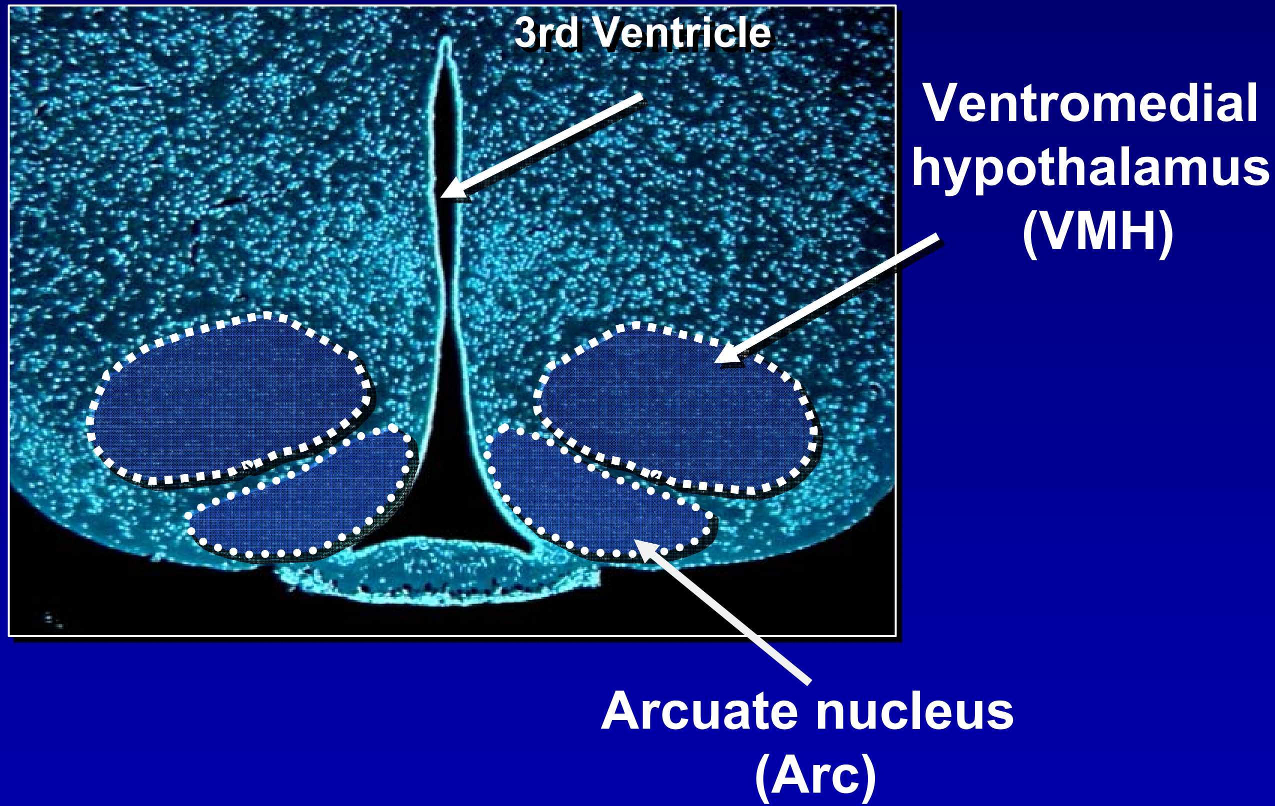
# Does leptin inhibits bone formation via a central relay?



**Leptin intracerebroventricular infusion (ICV)**
**(8 ng/h, 28 days treatment)**

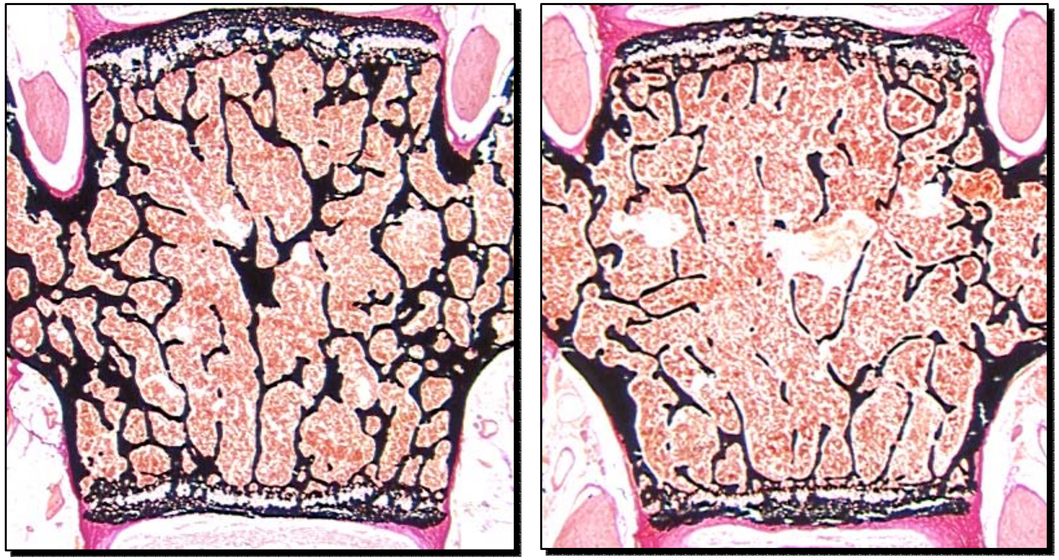
# Central versus peripheral?





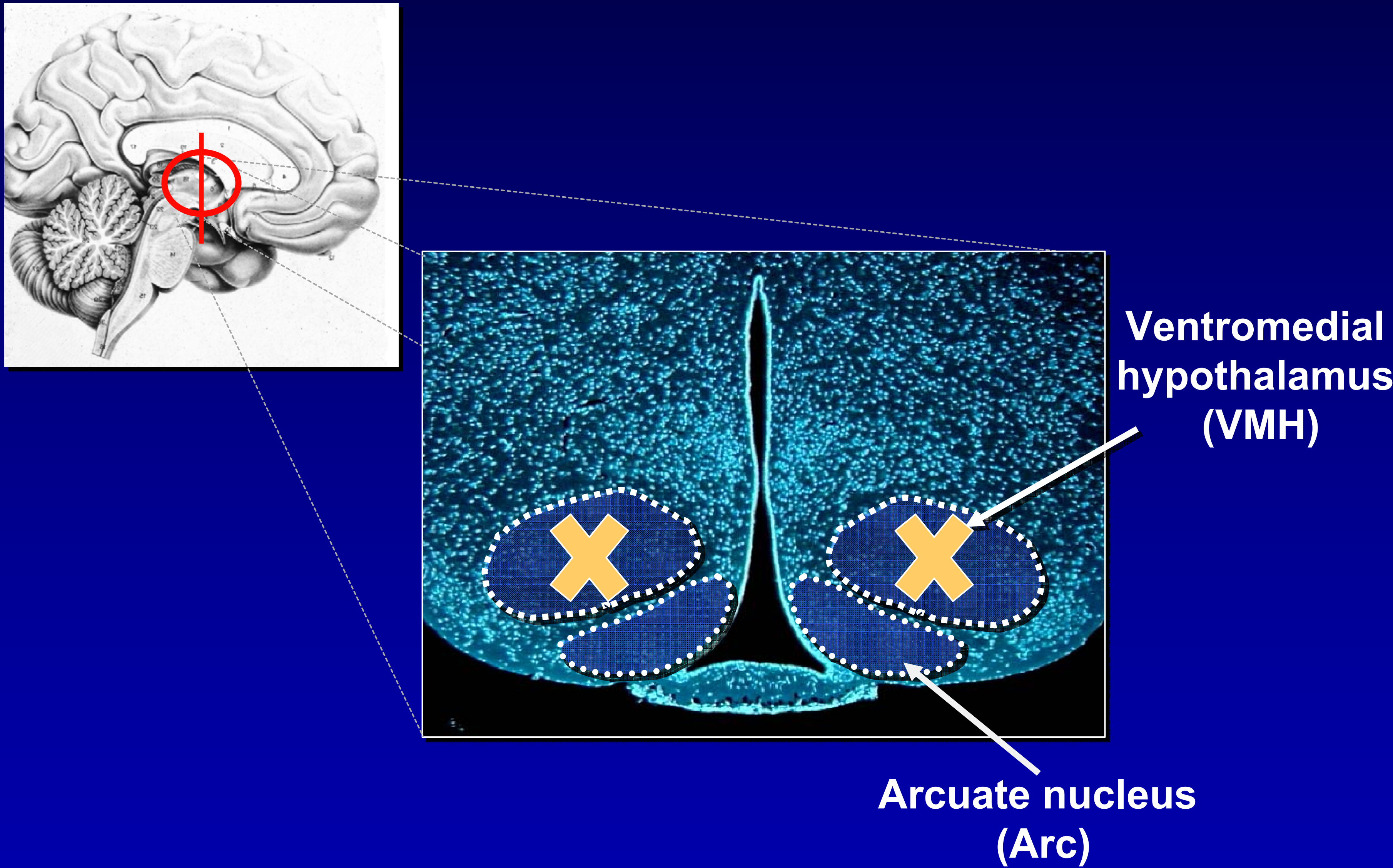
# Central leptin delivery corrects the bone phenotype of ob/ob mice

| | PBS | Leptin |
|---|---|---|

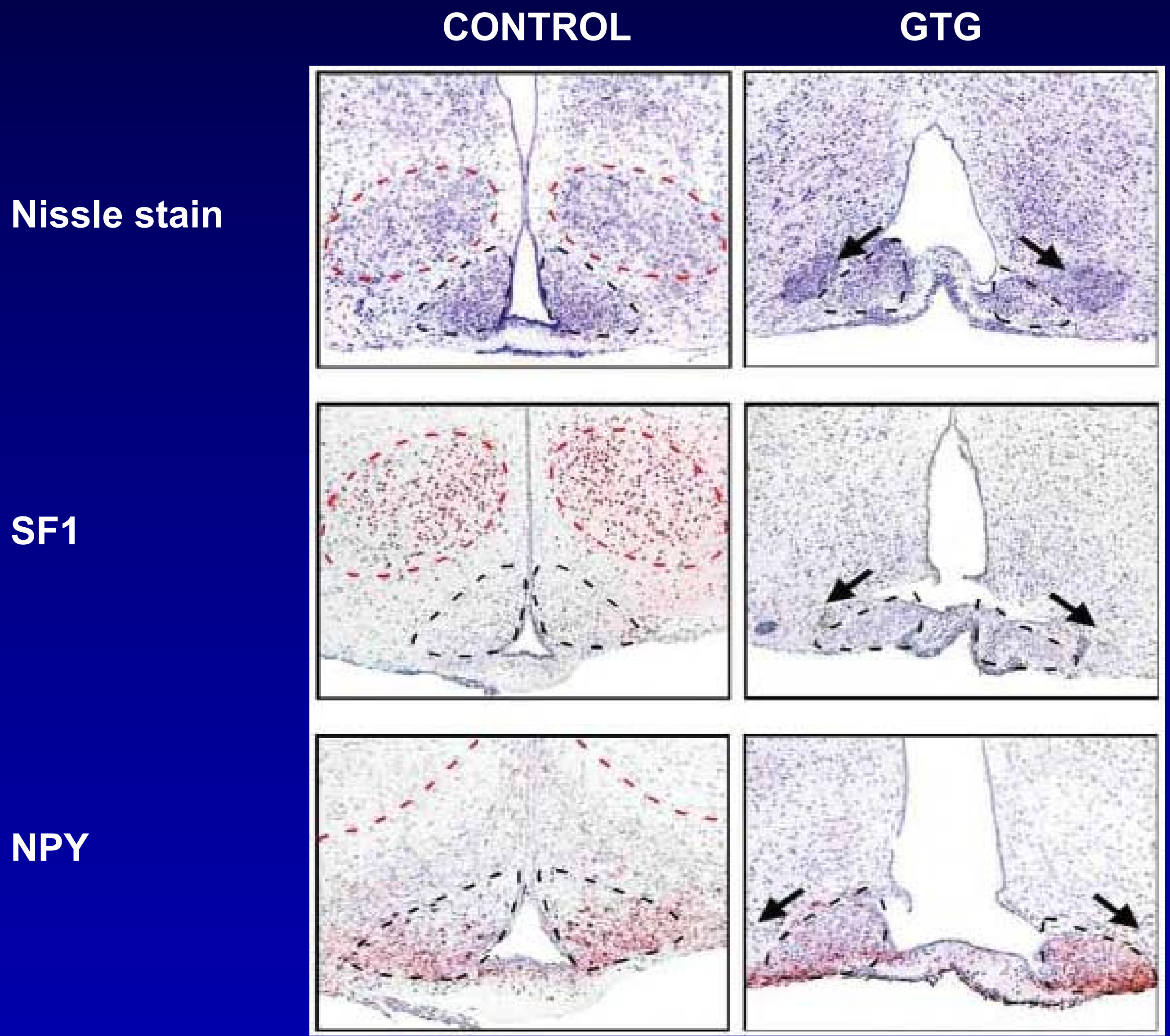


| | PBS | Leptin |
|---|---|---|
| Serum leptin (ng/ml) | 0 | 0 |
| Bone Vol. (%) | 18.3 ± 0.3 | 12.6 ± 0.7* |

# Leptin binds to ObRb in the hypothalamus

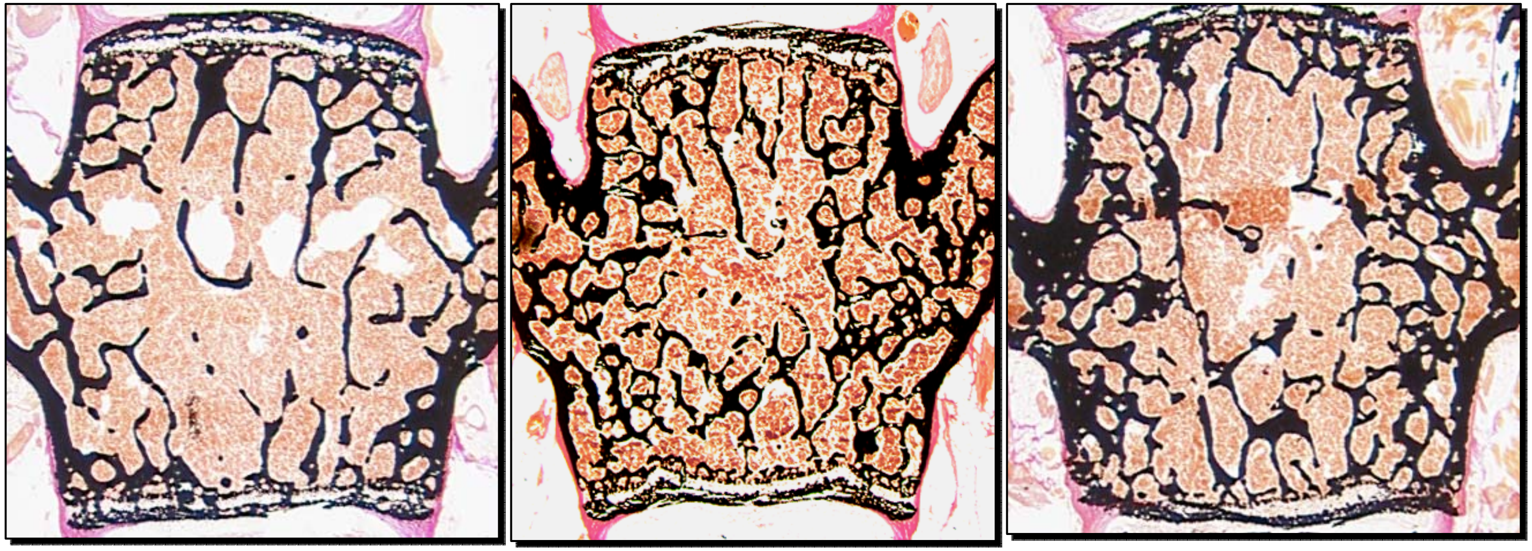


# Goldthioglucose destroys VMH neurons



# VMH neuron destruction increases bone mass in WT mice

**PBS** ***ob/ob*** **GTG**



| | PBS | *ob/ob* | GTG |
|---|---|---|---|
| **Bone Vol. (%)** | **14.0 ± 0.6** | **19.4 ±0.6*** | **19.3 ± 0.6*** |

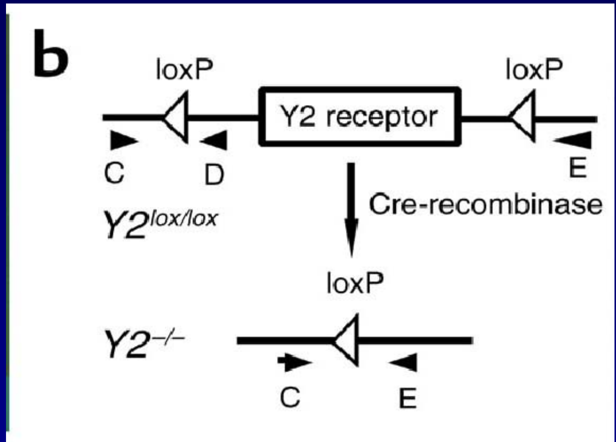
# Lack of ObRb in neurons, not osteoblasts, increases bone mass

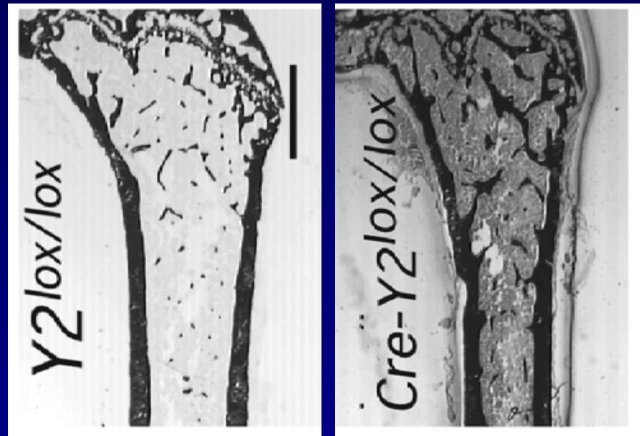
| | A: WT | A: $Lepr_{osb}$-/- | B: WT | B: $Lepr_{neuron}$-/- |
|---|---|---|---|---|
| BV/TV(%) | 16.7 ± 1.1 | 16.9 ± 1.3 | 15.4 ± 0.9 | 25.0 ± 1.0 * |
| BFR (µm³/µm²/yr) | 119.0 ± 29.2 | 112.2 ± 23.1 | 115.3 ± 4.8 | 188.7 ± 6.4 * |
| N.Ob/B.Pm | 21.2 ± 3.2 | 22.3 ± 5.2 | 22.5 ± 2.2 | 32.1 ± 2.2 * |
| Oc.S/BS | 10.4 ± 2.1 | 9.2 ± 1.8 | 15.3 ± 1.8 | 22.4 ± 1.7 * |

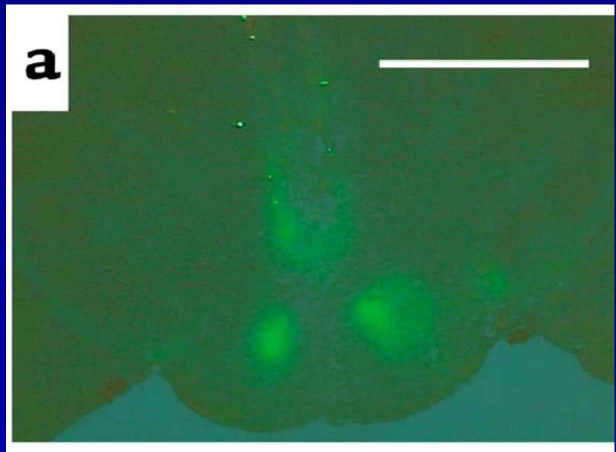
(Coll I-cre) (Synapsin-cre)

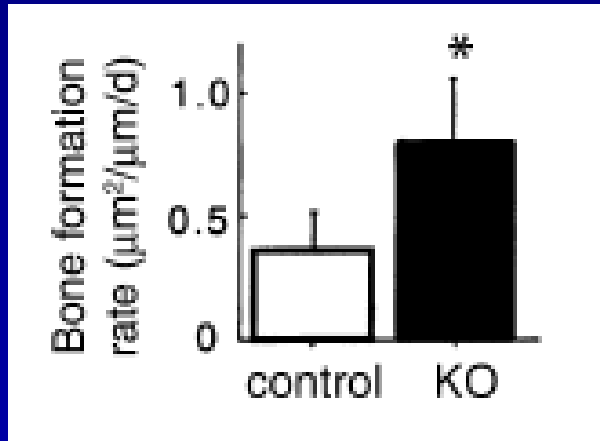
*Y. Shi and al, PNAS 2008*

# Hypothalamic Y2R inhibits bone formation



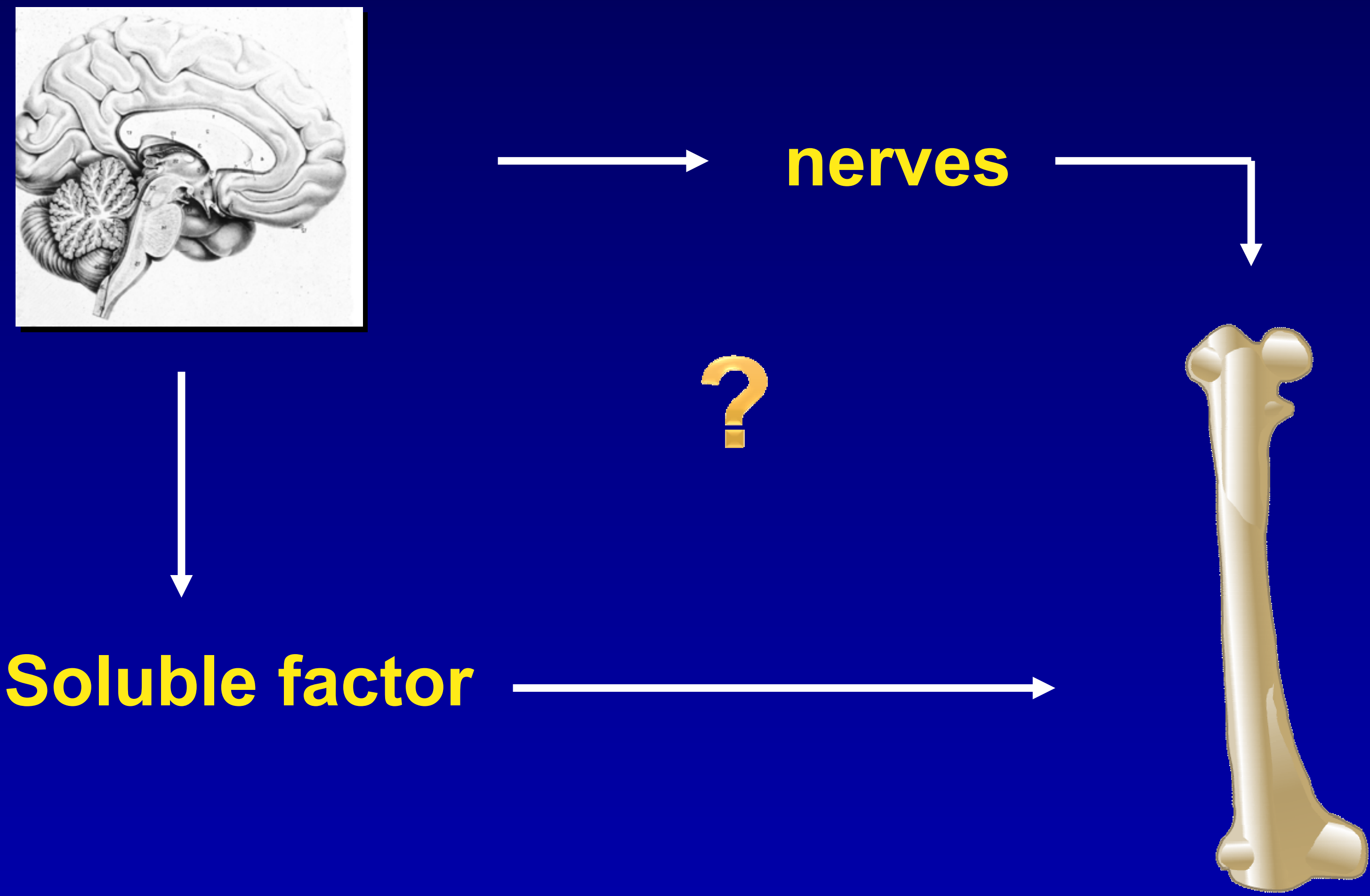



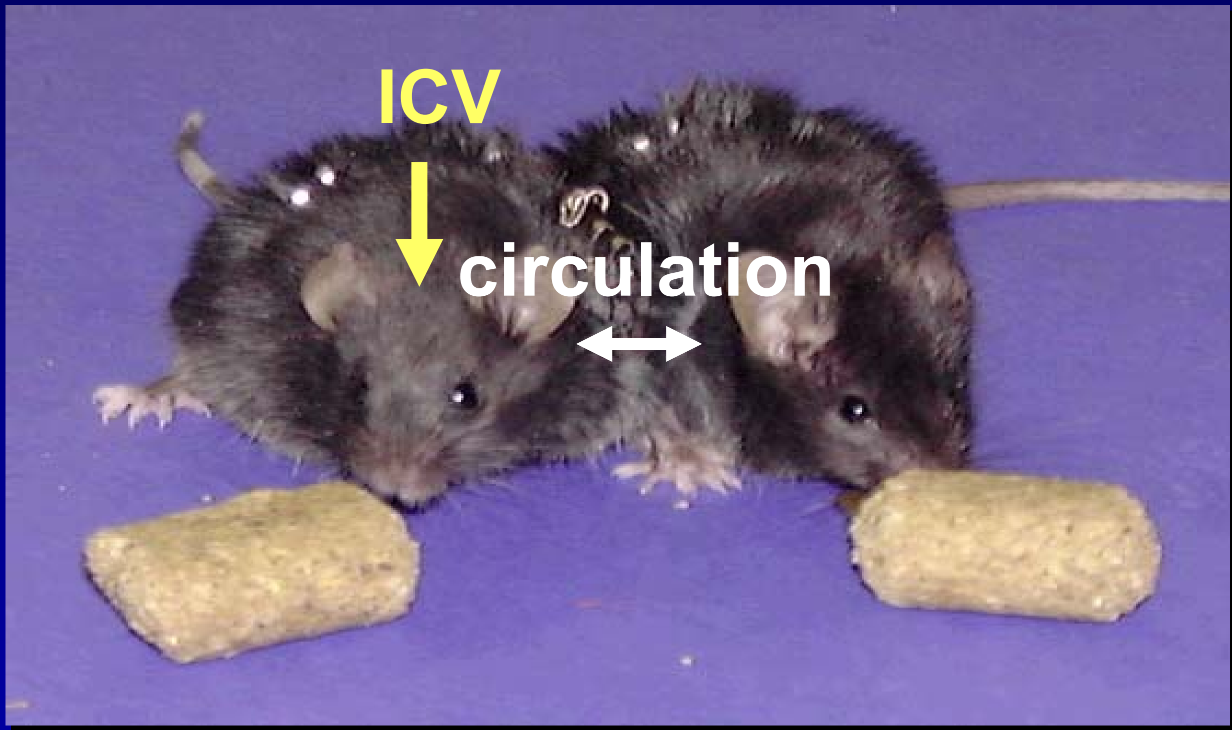




*P. Baldock and al, JCI 2002*

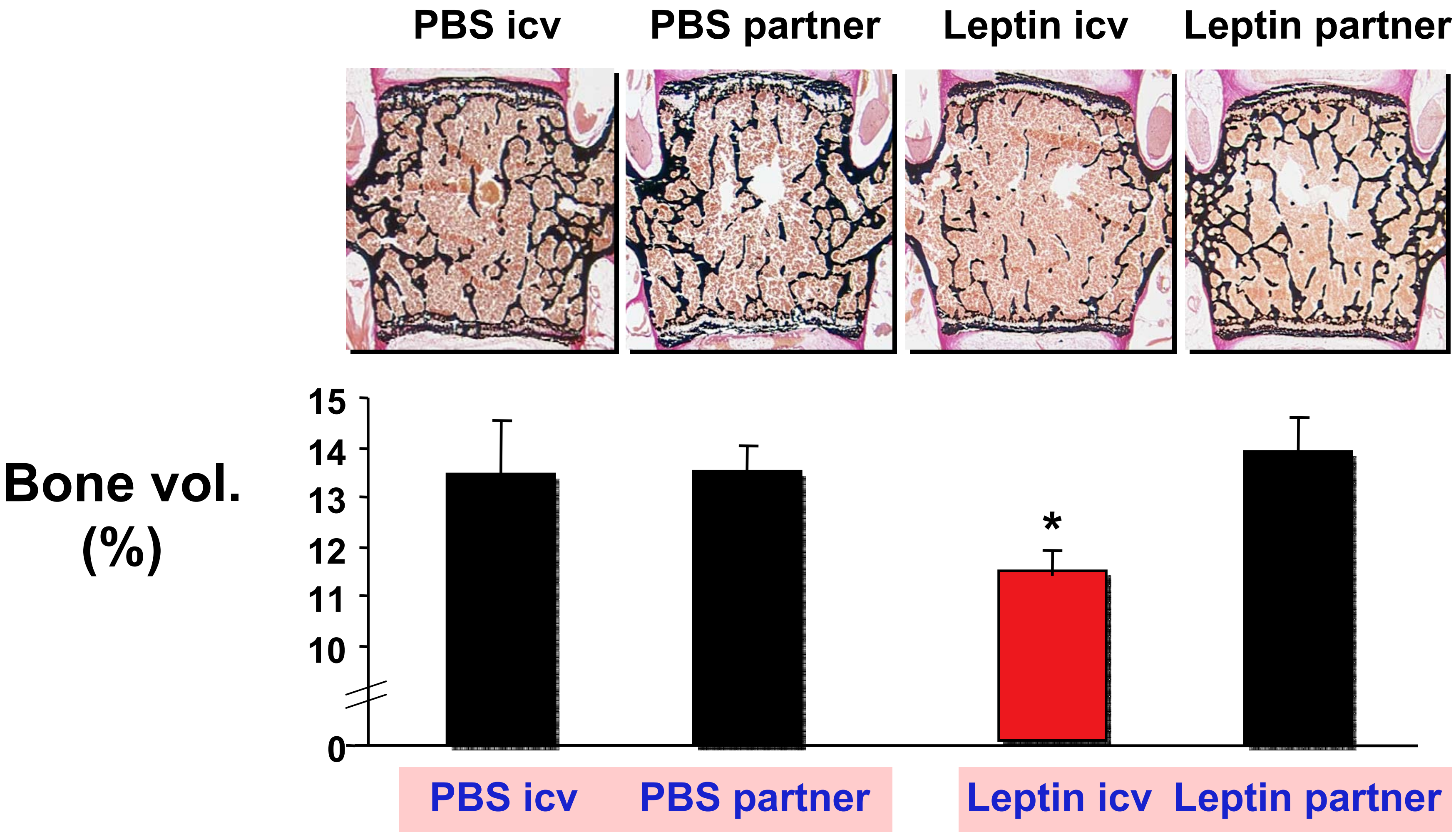
# How does the hypothalamus regulate osteoblast function?



# How does the hypothalamus regulate osteoblast function?

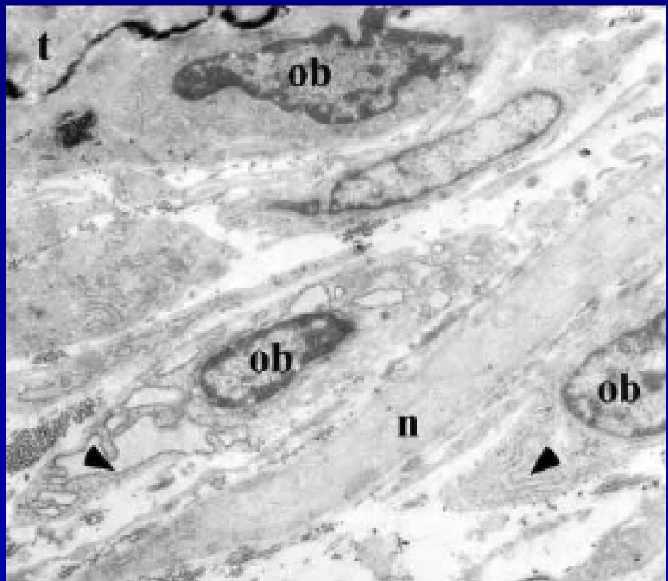


# Leptin does not use a humoral pathway to control bone mass



# BONES ARE INNERVATED

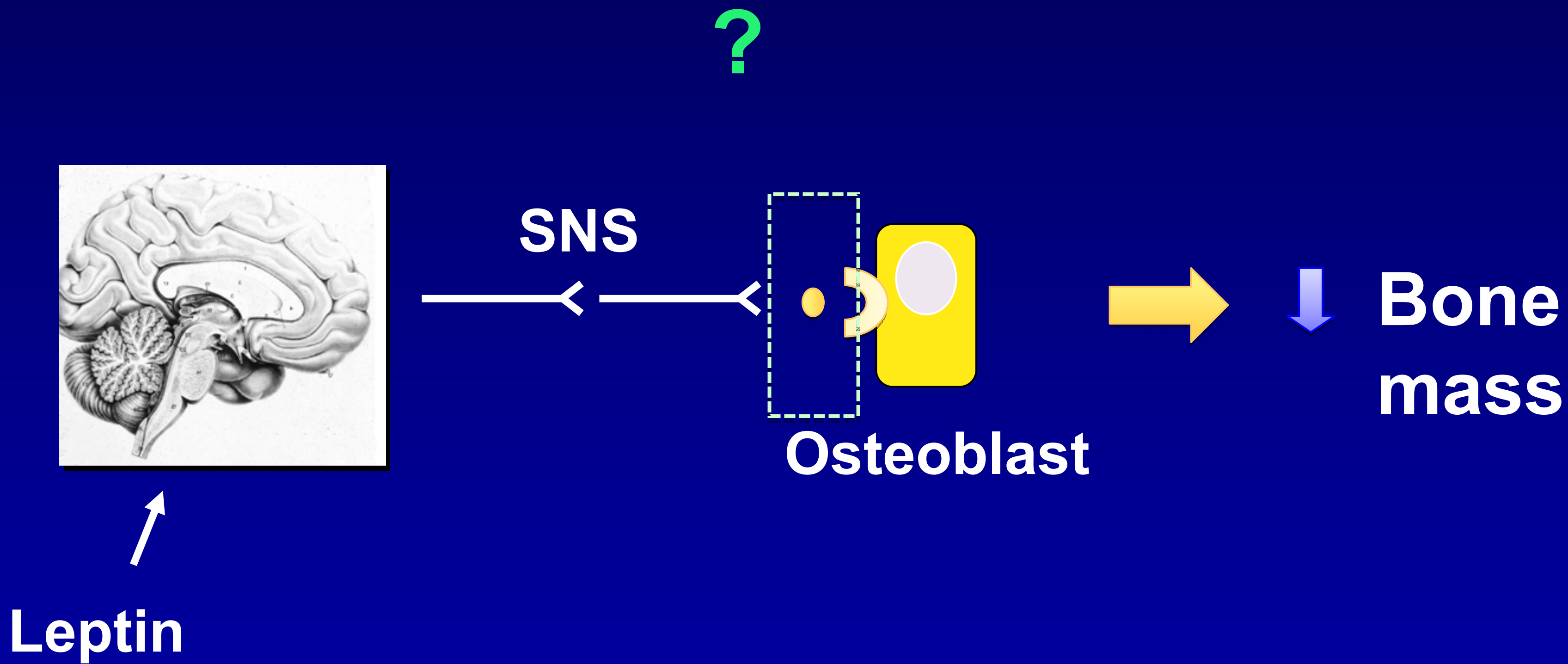
- Neurons are detected in the bone micro-environment
- Retrograde viruses injected in bone label hypothalamic neurons
- *Ob/ob* mice have a low sympathetic tone



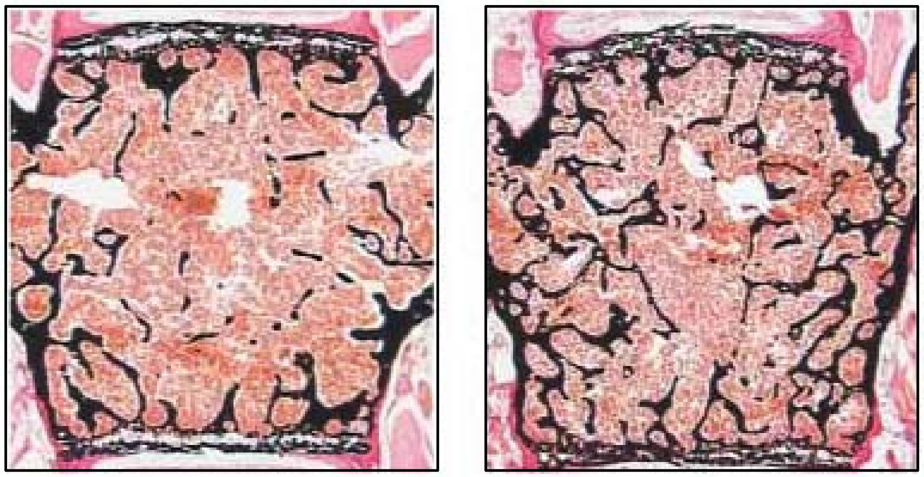

Neurofilament

TH

# THE NEURONAL MODEL

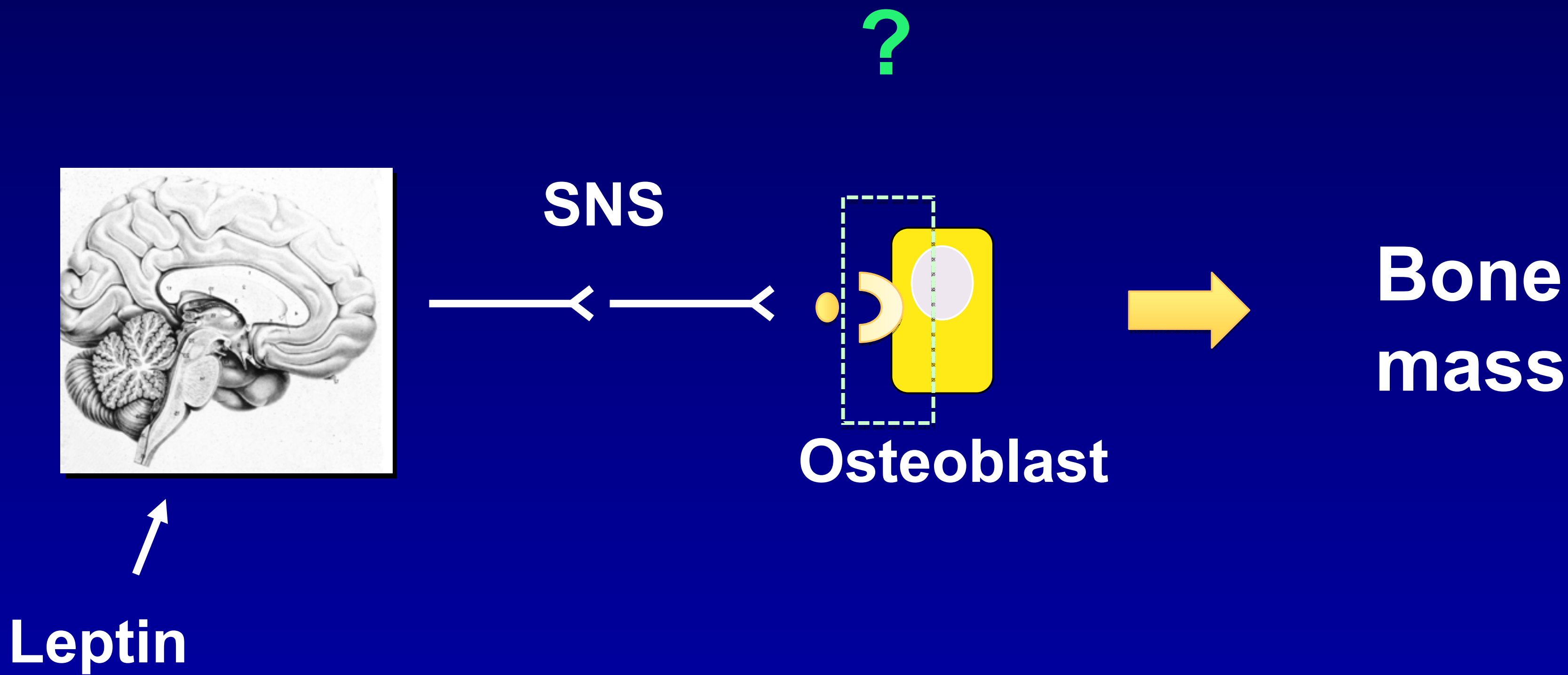


# Lack of catecholamines increases bone formation

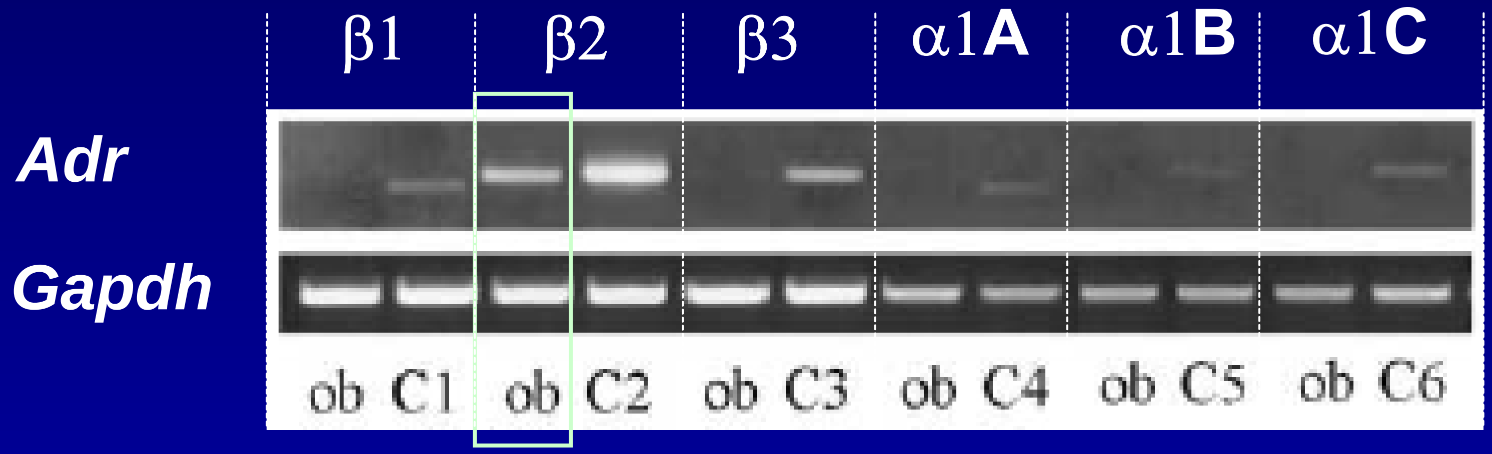



| | WT | *Dbh-/-* |
|---|---|---|
| **BV/TV (%)** | **10.2** ±0.3 | **13.2** ±0.8* |
| **BFR** | **82.3** ±4.5 | **105.4** ±5.8* |
| **ObNb/BPm** | **9.6** ±0.7 | **12.6** ±0.8* |

# THE NEURONAL MODEL

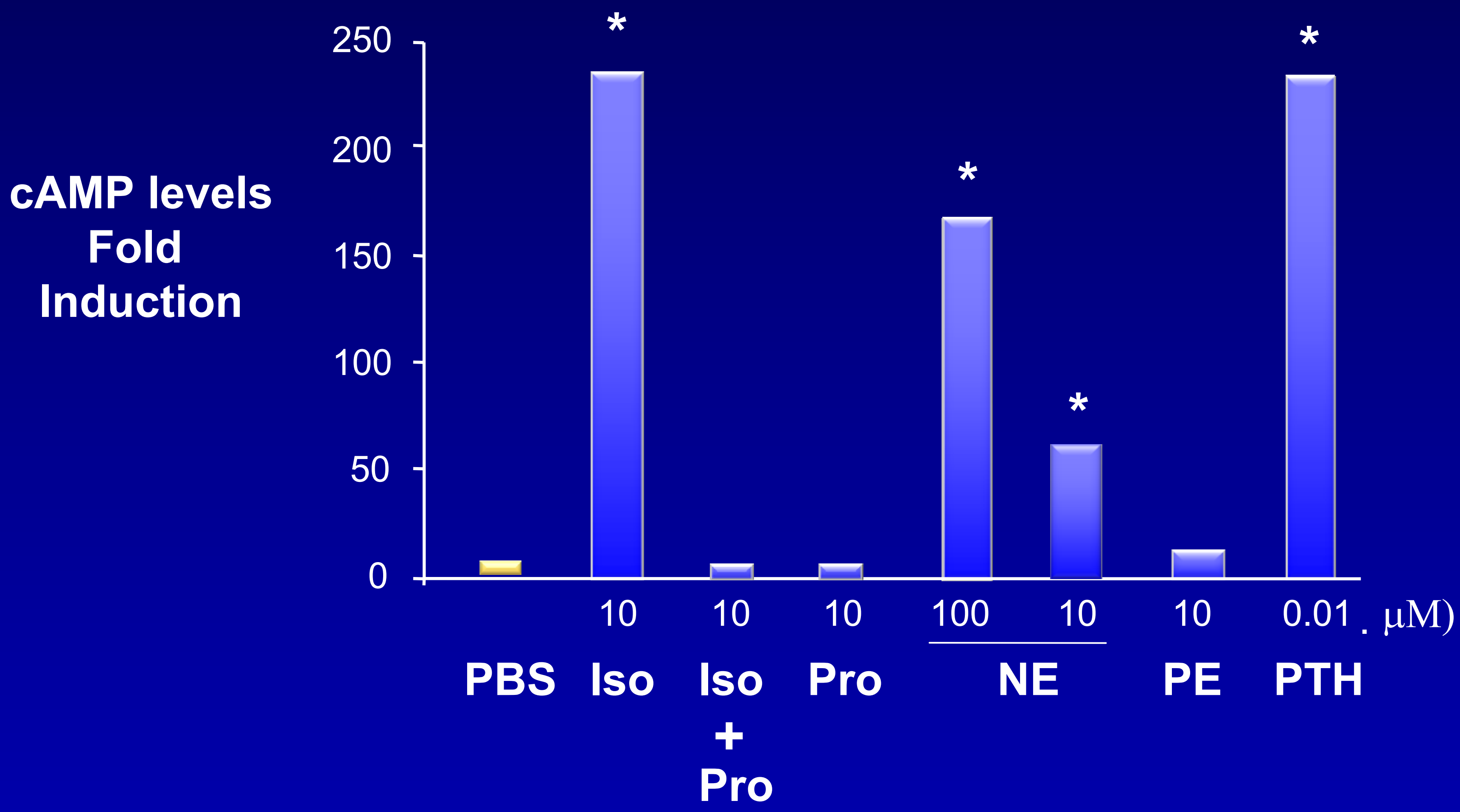


# The β2-Adrenergic receptors is expressed by osteoblasts




**Ob: Primary osteoblasts**
**C: Positive control**

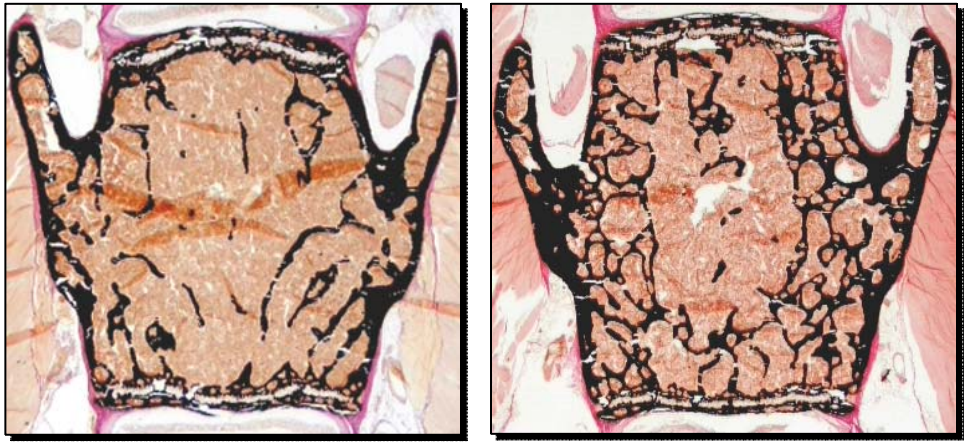
# Induction of cAMP production by βAR agonists in osteoblasts



# β-agonists decrease bone mass

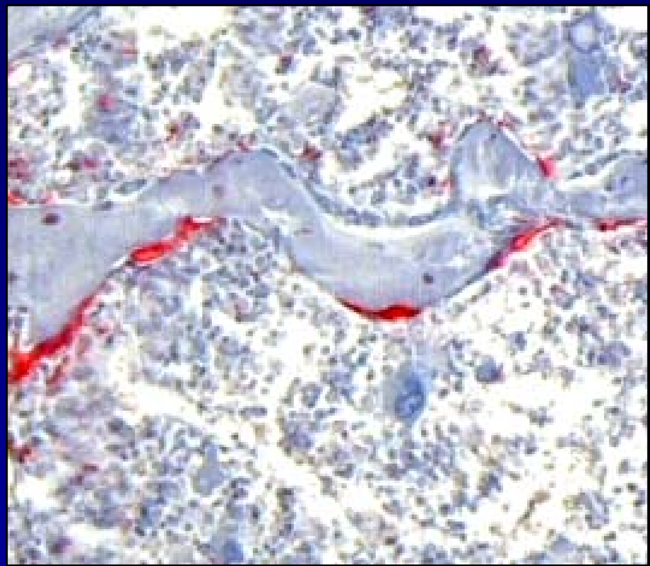
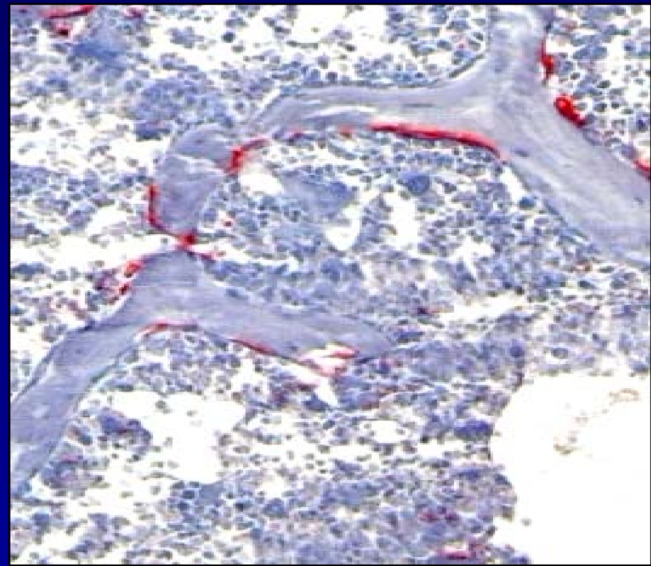
| | PBS | Isoproterenol |
|---|---|---|
| |  |  |
| Bone Vol. (%) | 17.8 ±0.6 | 11.7 ±0.6 * |
| BFR/BS | 143.4 ± 7.7 | 109 ± 11.1* |
| ObNb/BPm | 18.2 ± 0.7 | 12.2 ± 2.2* |

# Lack of β2AR increases bone formation

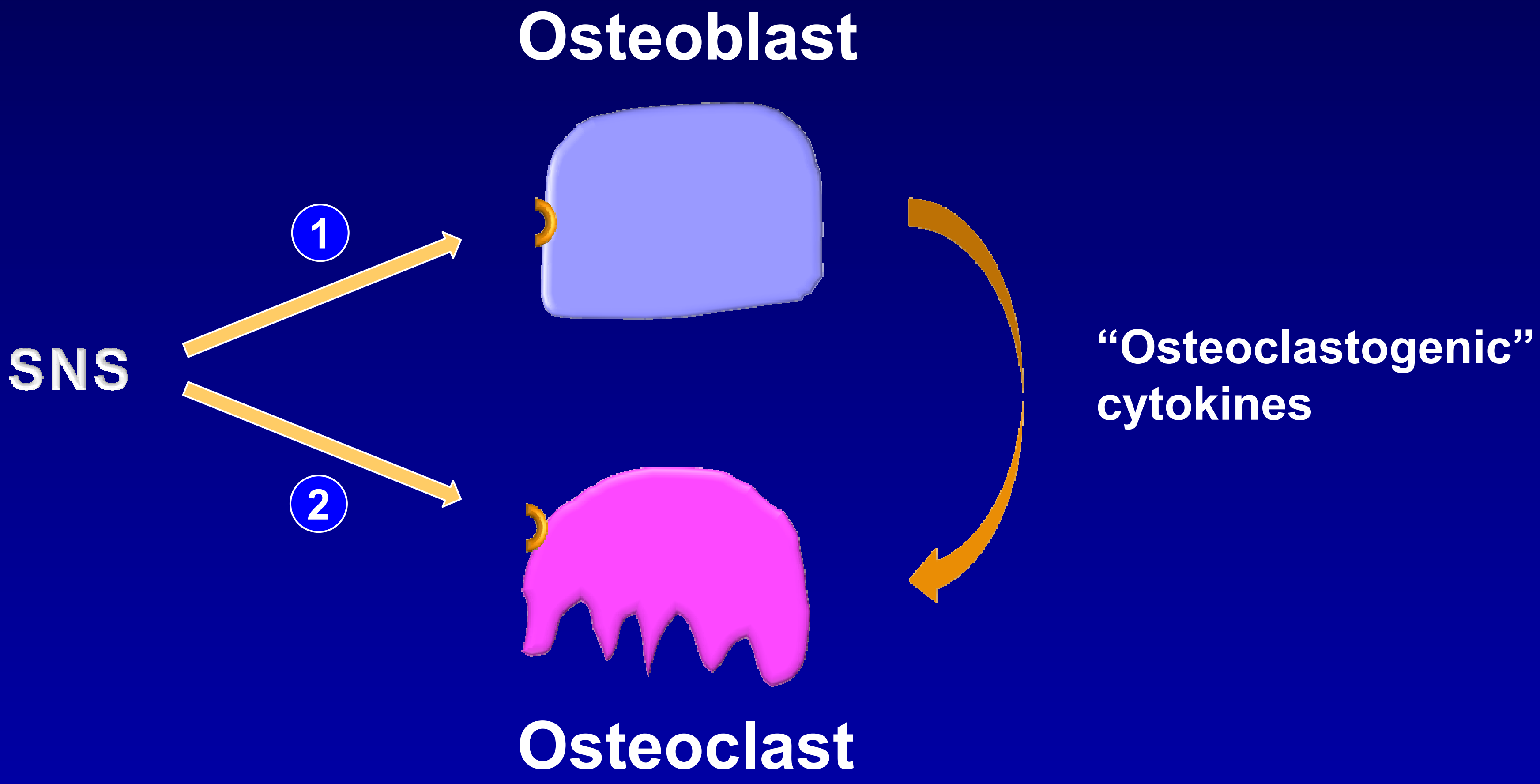


| | WT | β2AR-/- |
|---|---|---|
| BV/TV (%) | **11.9** ±0.9 | **17.9** ±1.1* |
| BFR | **253.8** ±20.3 | **354.1** ±1.1* |
| ObNb/BPm | **13.6** ±0.8 | **20.5** ±1.1* |
| Body weight | normal | normal |
| Insulin/leptin | normal | normal |

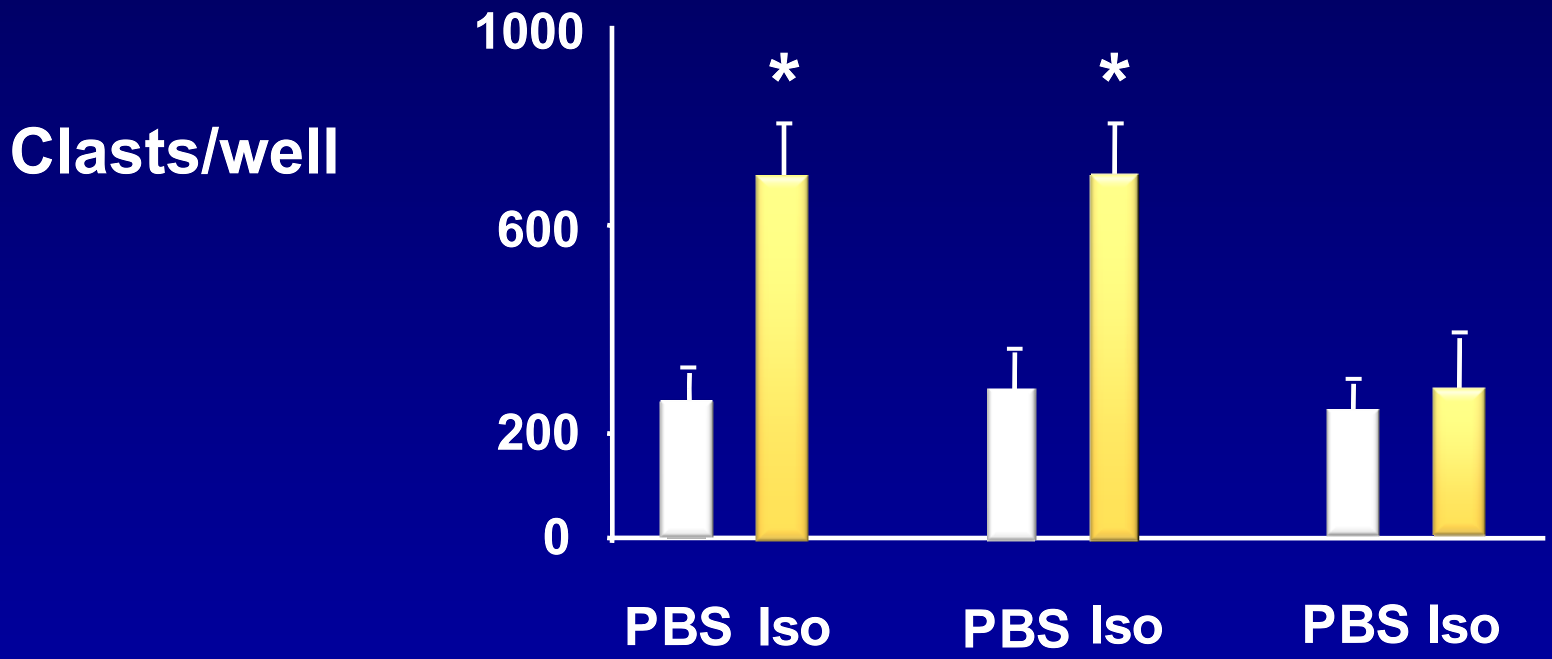
# The SNS regulates osteoclastogenesis

| | WT | *Adrβ2-/-* |
|---|---|---|
| |  |  |
| OcN/BPm | 9.0 ±0.2 | 6.0 ±0.2* |
| OcS/BS | 28.0 ±0.8 | 23.6 ±1.5* |
| Dpd | 21.1 ±1.3 | 15.0 ±1.3 |

# How does β2AR signaling stimulate osteoclast differentiation?

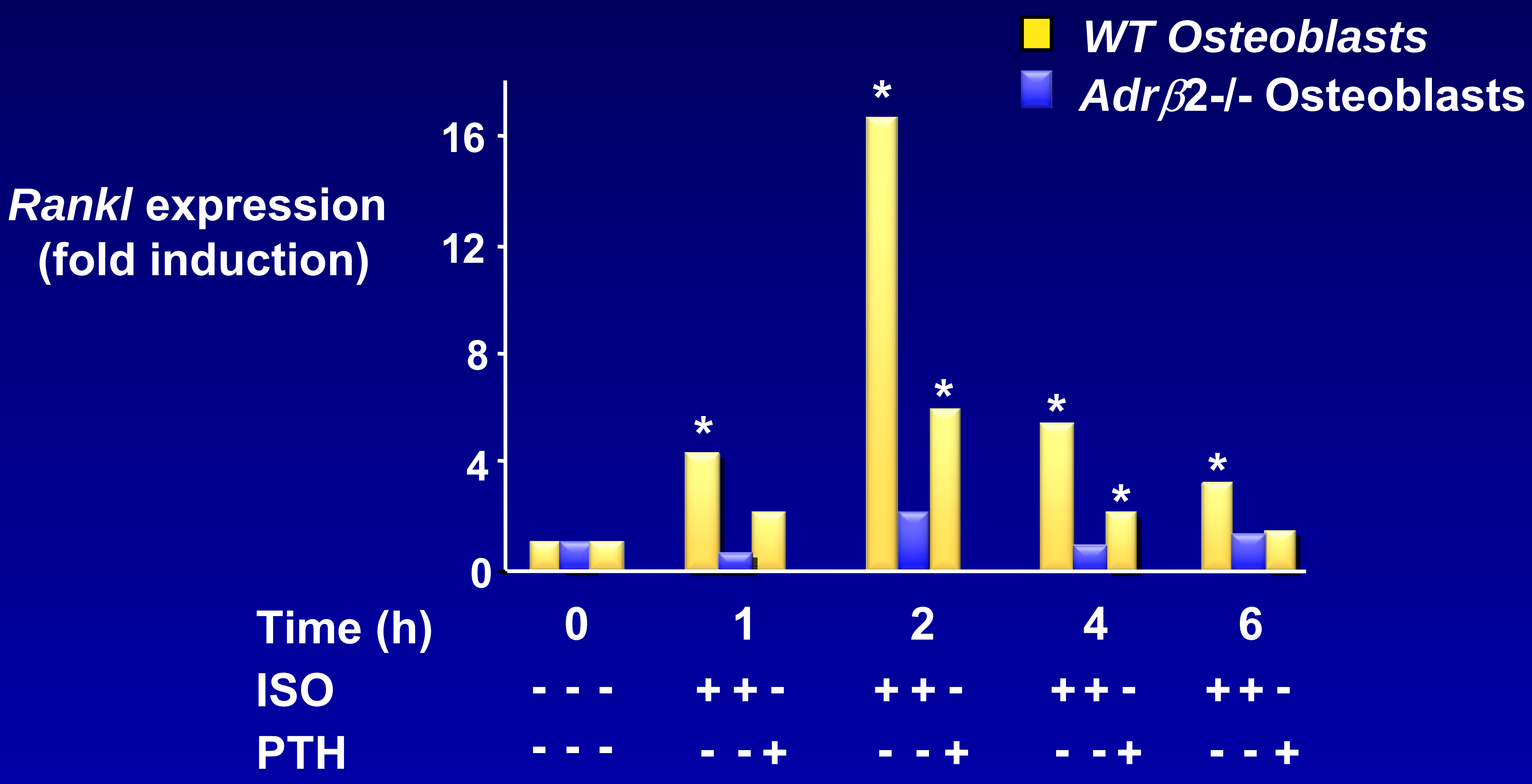


# Adrenergicsignaling in osteoblasts
# -not osteoclasts-
# favors osteoclastogenesis

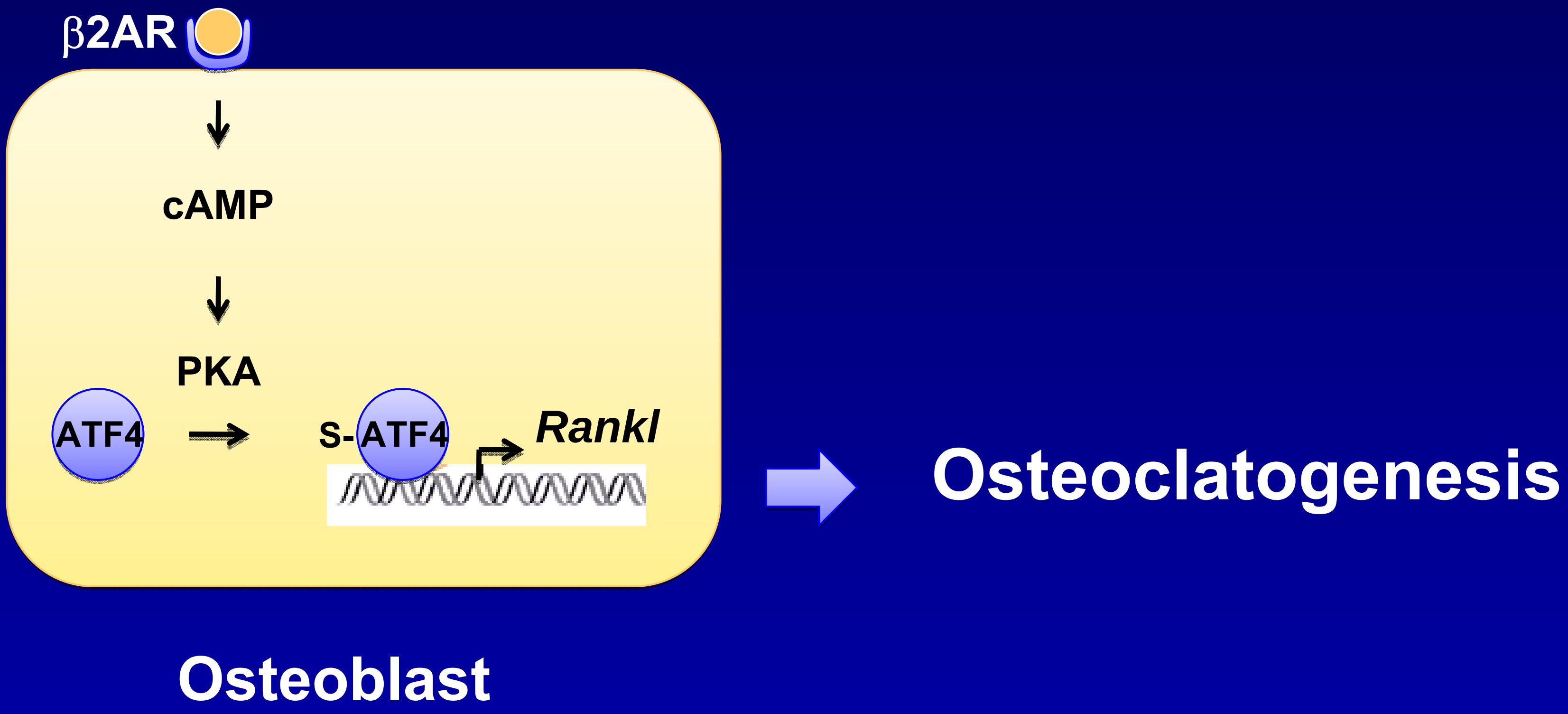



| | | | | | | |
|---|---|---|---|---|---|---|
| Osteoblasts: | wt | wt | wt | wt | -/- | -/- |
| Osteoclast progenitors: | wt | wt | -/- | - /- | wt | wt |

# Isoproterenol stimulates *Rank*l expression

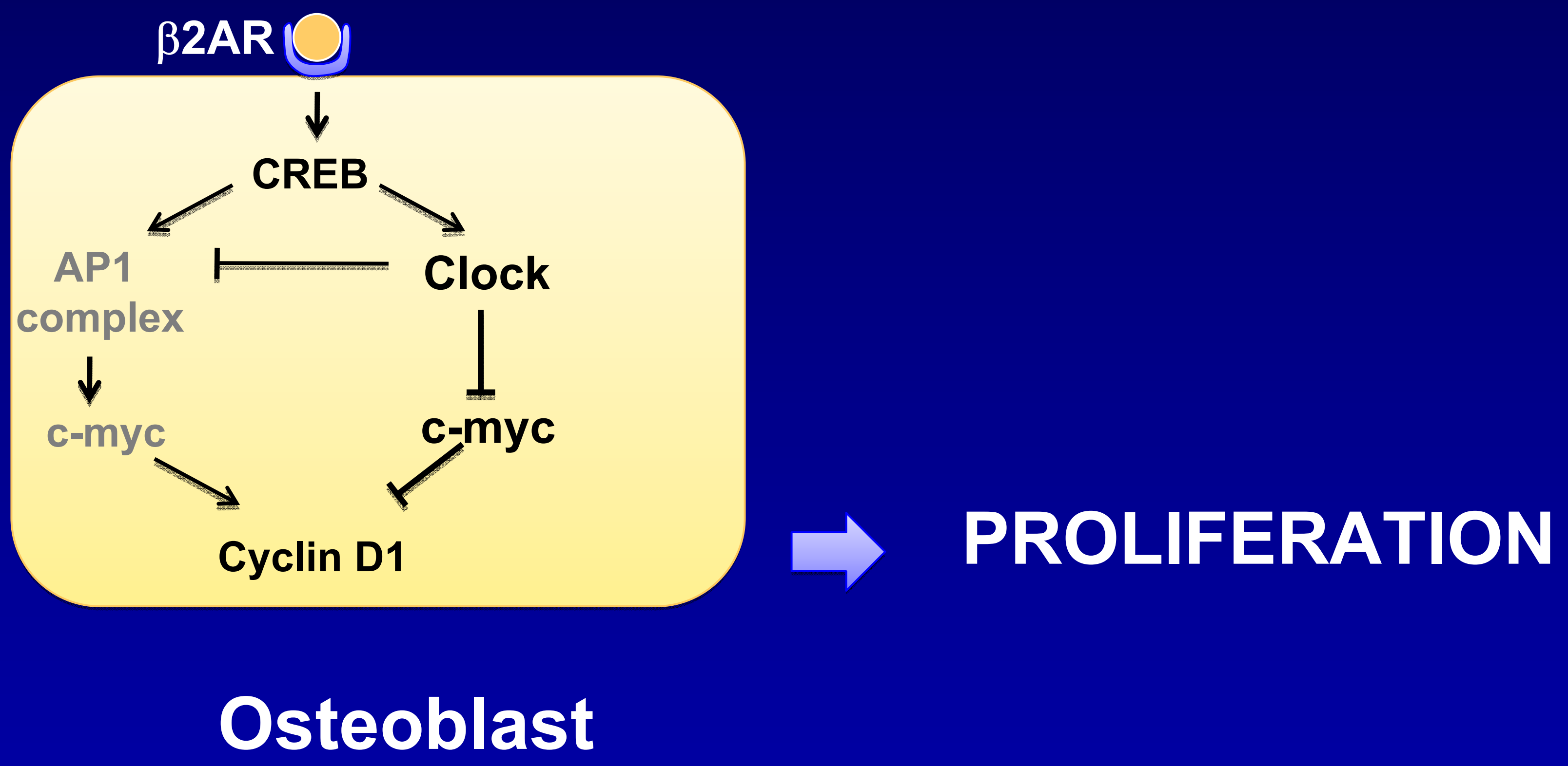


# β2AR signaling in osteoblasts regulates *Rankl* expression via ATF4



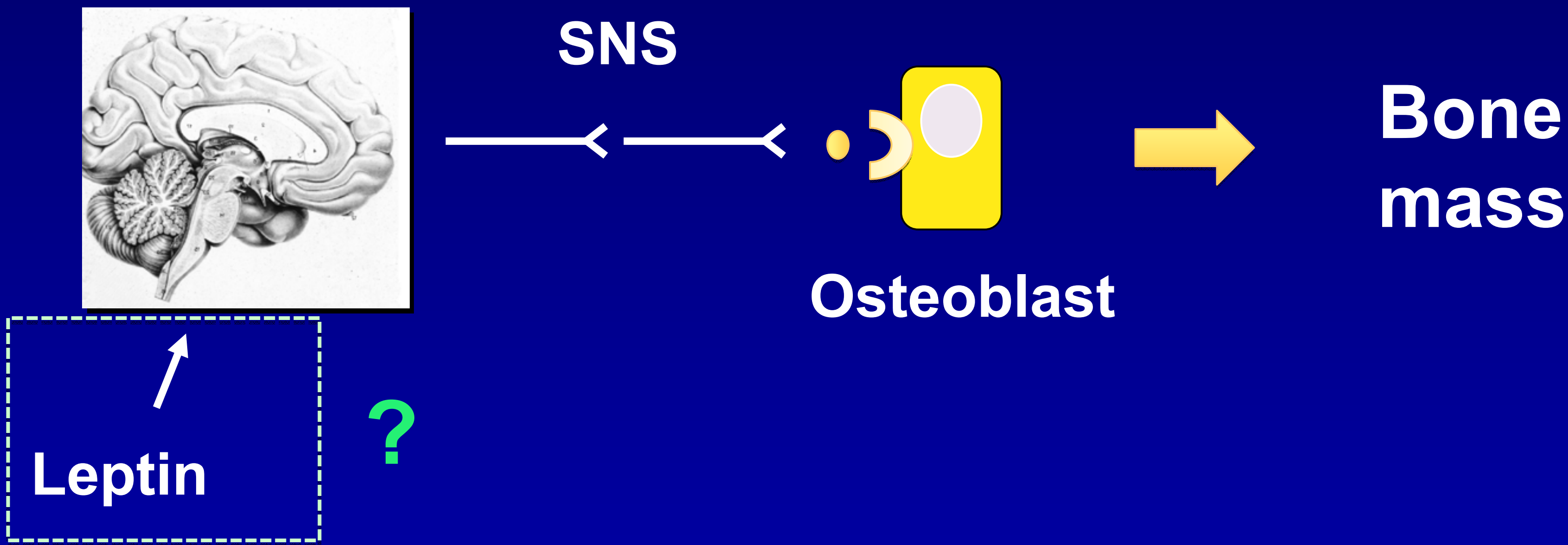

*Elefteriou & al., Nature 2006*

# $\beta$2AR signaling in osteoblasts regulates osteoblast proliferation via clock genes

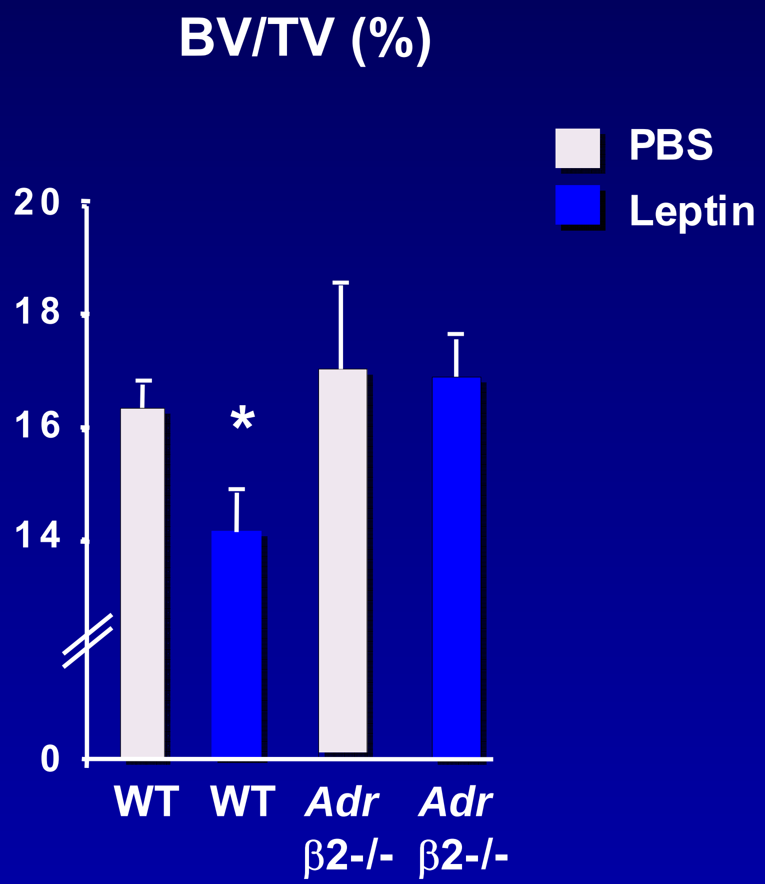


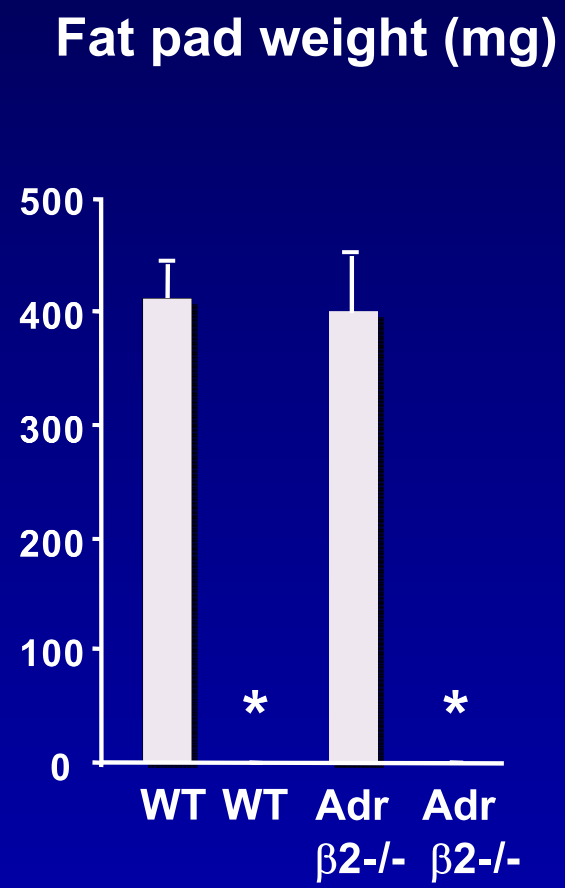

*Fu & al., Cell 2005*

# THE NEURONAL MODEL

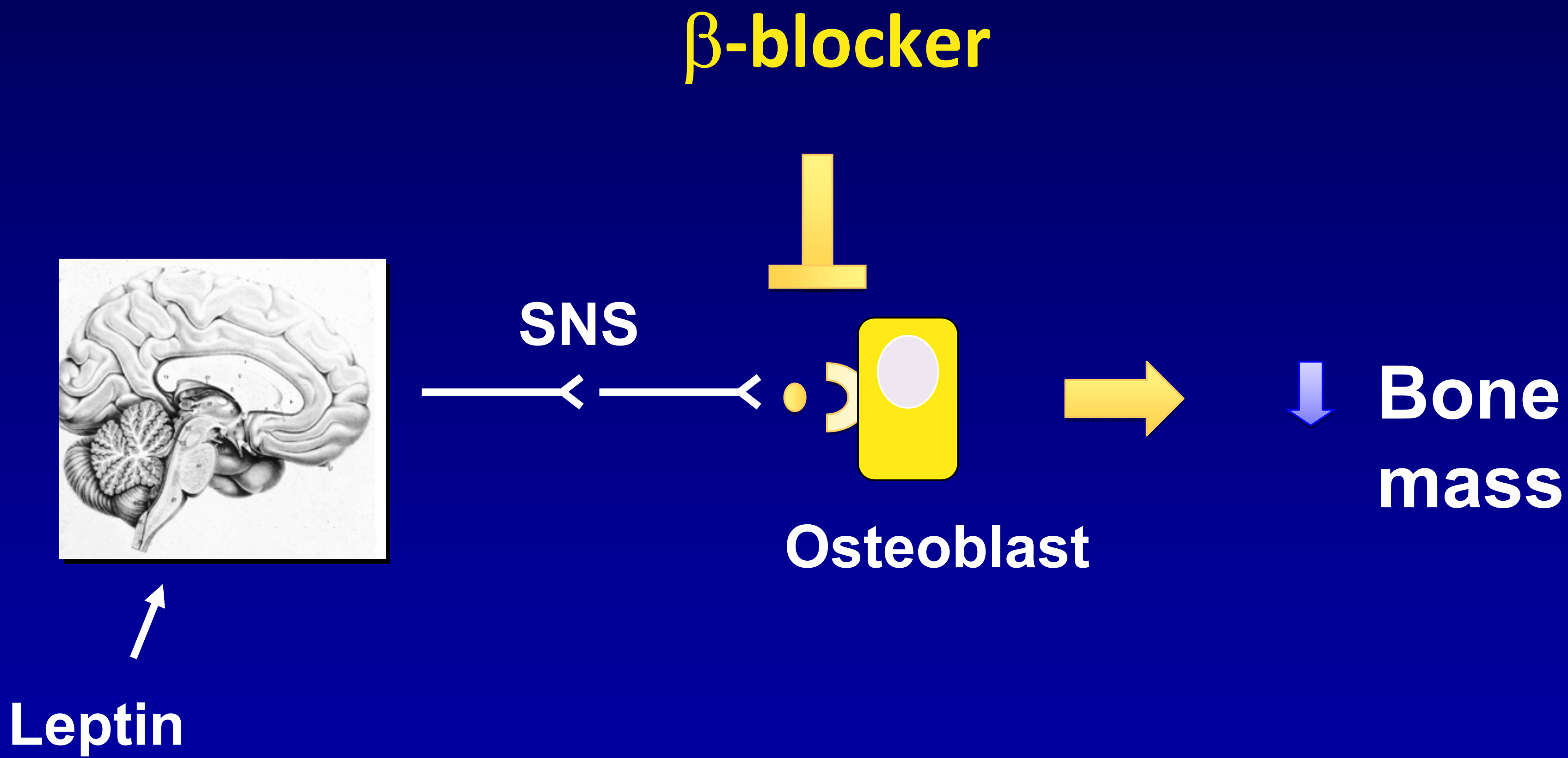


# Adrβ2 is required for leptin anti-osteogenic function



# Are β-blockers good for bones?

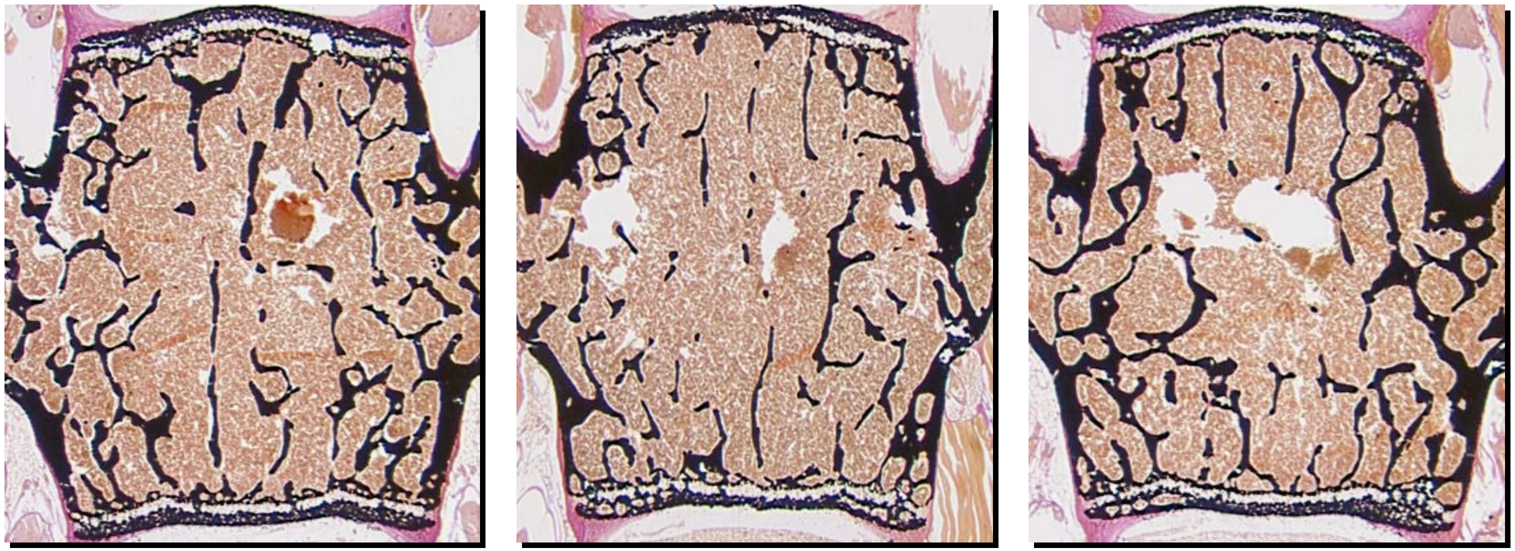


# β-blockers increase bone mass

| | PBS | Propranolol |
|---|---|---|
| |  |  |
| Bone Vol. (%) | 13.8 ±0.3 | 16.2 ±0.8* |
| BFR | 116.8 ± 6.3 | 169.3 ± 8.6* |
| NbOb/BPm | 19.0 ± 1.4 | 26.7 ± 2.4* |

# Propranolol prevents bone loss following ovariectomy

| sham | OVX | OVX +β-blocker |
|---|---|---|



| | | | |
|---|---|---|---|
| **Bone Vol. (%)** | **14.2 ± 0.8** | **12.1 ± 0.4*** | **14.1 ± 0.7** |

# HUMAN CLINICAL DATA

- **2006 Meta-analysis: 28.5% reduction in hip fracture risk** (*Wiens et al, 2006*)

- **Some studies reporting no effects**
  - **Confounding factors**
  - **# of patients**
  - **Long-term vs short-term**
  - **βAR selectivity/dose**
  - **Skeletal sites**

**Prospective studies are needed ($$$)**

# What is the role of βAR signaling in bone pathophysiology and diseases?

SNS outflow → βAR ← Drugs

**βAR**

OSTEOBLASTS

signaling

**BONE RESORPTION**
*Elefteriou and al, Nature 2005*

**HSC TRAFFICKING**
*Katayama and al, Cell 2006*

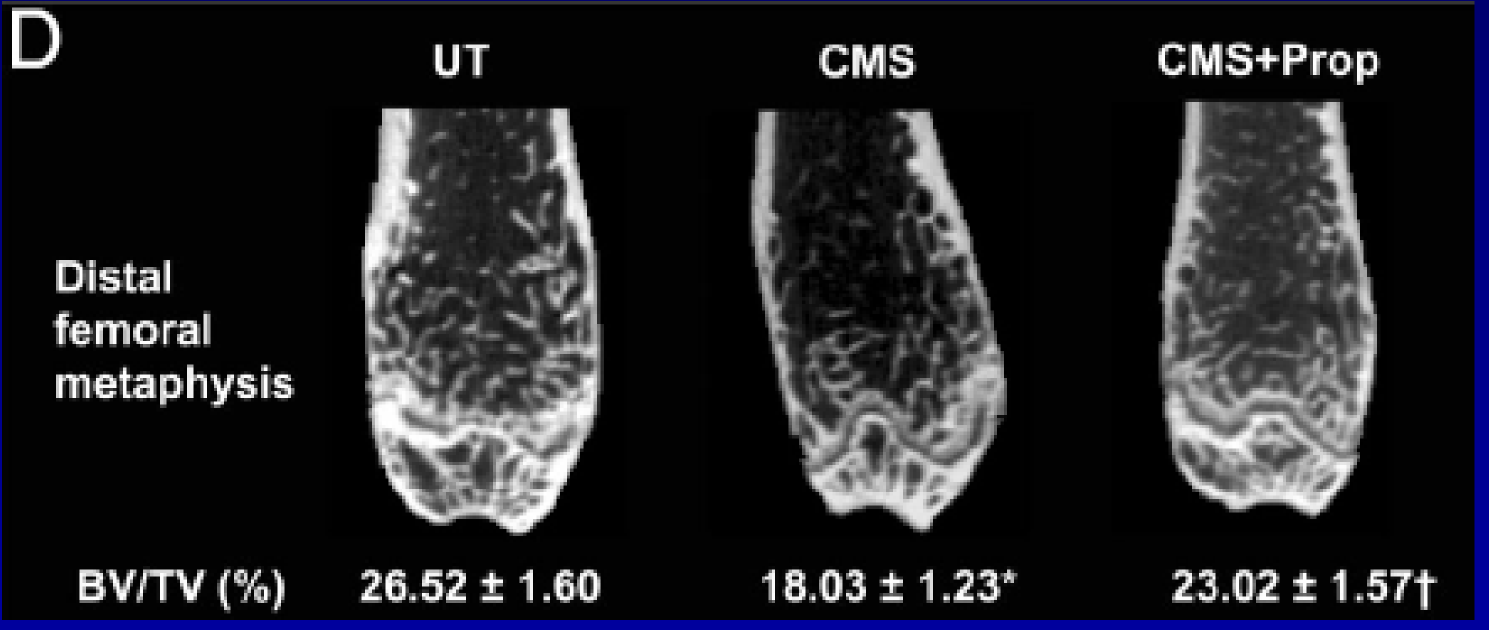
**BONE FORMATION**
*Elefteriou and al, Nature 2005*

**INSULIN SECRETION**
*Hinoi and al, JCB 2009*

# PRE-SYNAPTIC EFFECT:
# Depression and Bone Loss

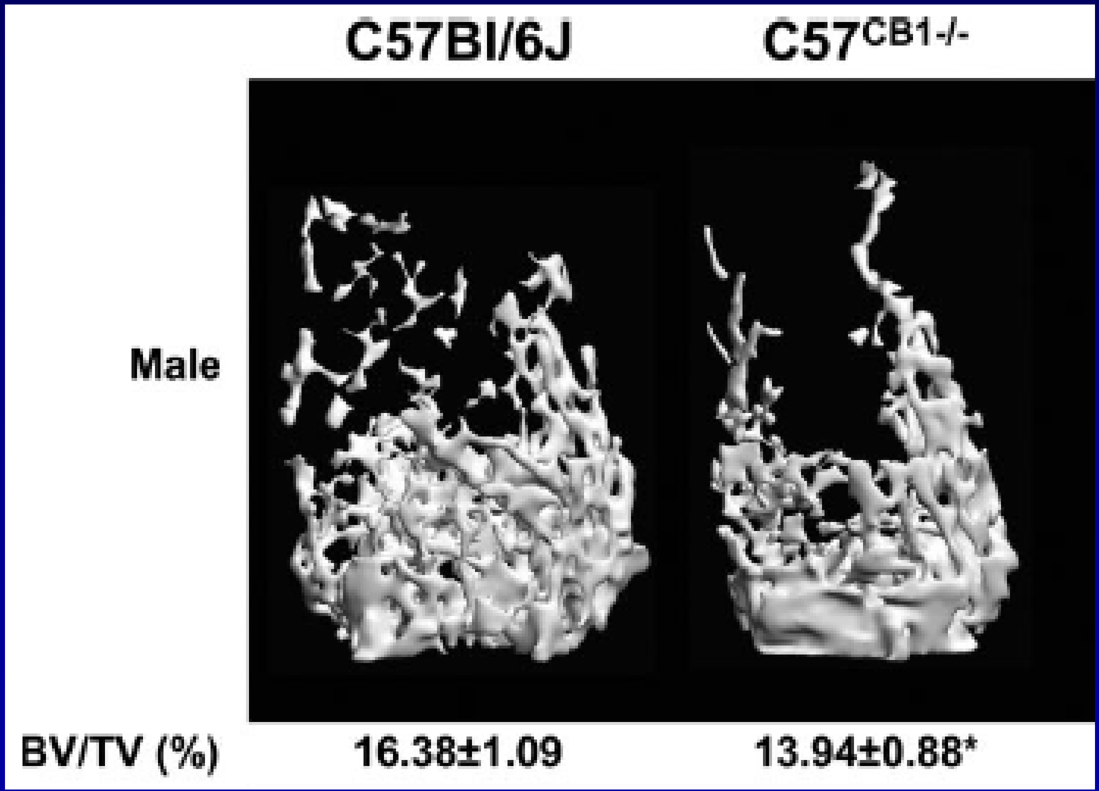
- Major depression is associated with bone loss and increased fracture risk
- Associated with hypercorticolism and SNS activation

# Stress/depression decreases bone mass through SNS activation



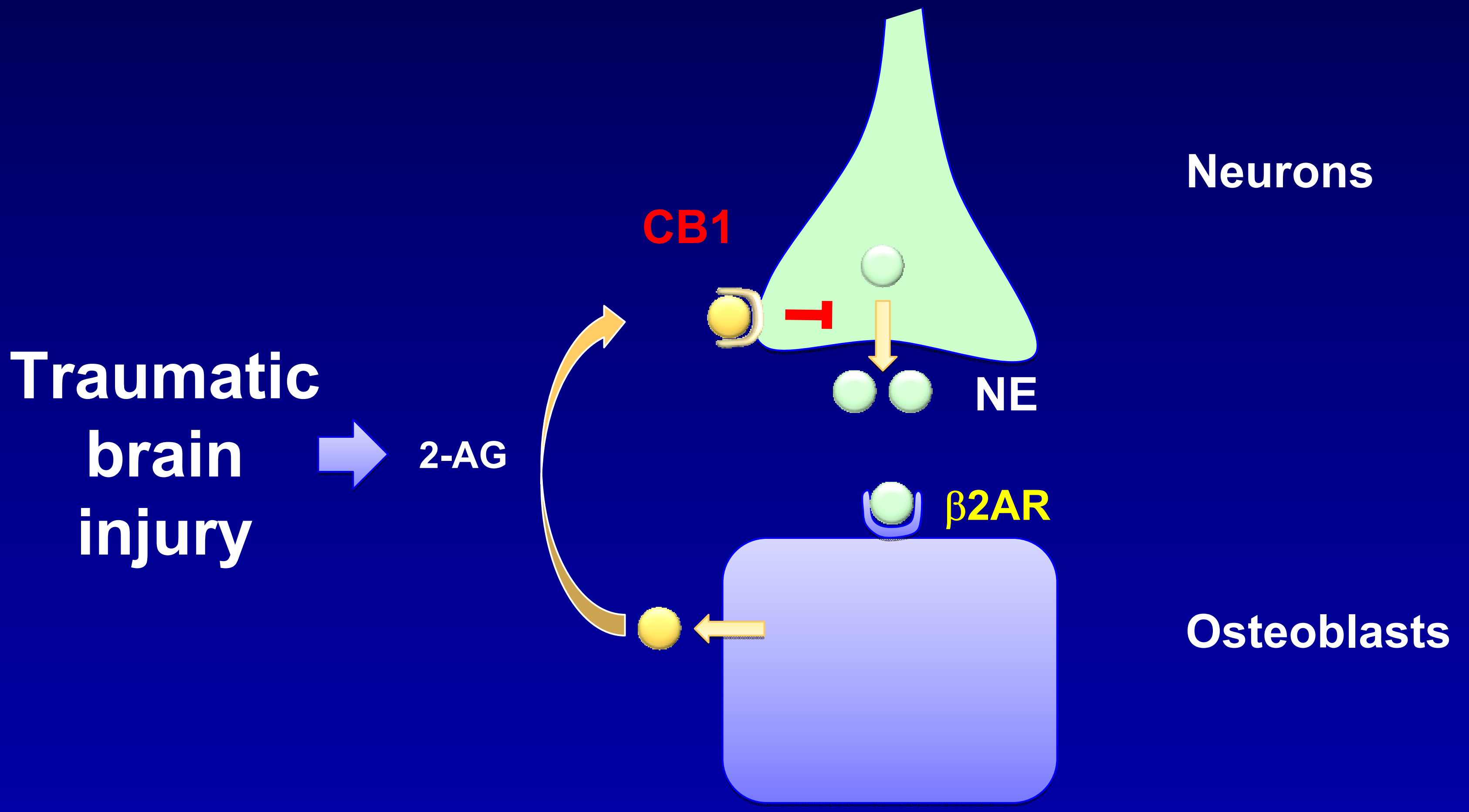

*Yirmiya and al, PNAS 2006*

# POST-SYNAPTIC EFFECT:
# The cannabinoid system




*Tam and al, Mol Pharm 2006*

# CB1 STIMULATION IN PRE-SYNAPTIC NEURONS INHIBITS NE RELEASE




*Tam and al., FASEB J., 2007*
2-AG: 2-arachidonoyl glycerol

# SUMMARY

✓ Diseases and clinical observations are great opportunities to unravel novel mechanisms

✓ Multiple genetic mouse models are critical to address hypotheses raised by such clinical observations and to demonstrate mechanisms

✓ Bone remodeling is regulated by the CNS and SNS

✓ Drugs/diseases affecting SNS outflow or β2AR signaling in osteoblasts alter bone remodeling and bone mass

*Post-doctoral and student positions are open in the lab...*

PROJECTS:

- SNS and bone
- Skeletal dysplasia in NF1
- Calorie restriction, sirtuins and bone
- Mechanism of cancer bone metastasis

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**Center for Bone Biology**
**VANDERBILT UNIVERSITY**
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