



# Ghrelin and Circuit Formation

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Lee

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# Agenda

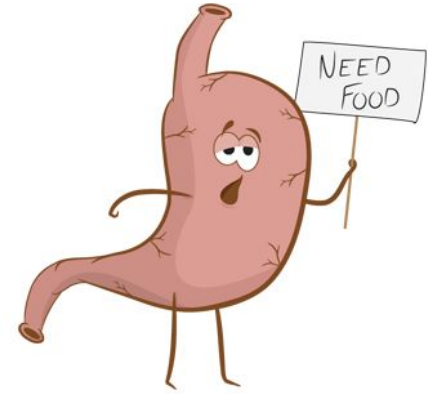
- Grelin: role, signaling
- Experiment 1: Does neonatal overnutrition cause changes in the ghrelin system?
  - Data
  - Results
- Experiment 2: What effect does a high fat diet have on ghrelin-expressing cells?
- Takeaway messages

# Themes

- Hypothalamus: where the nervous and endocrine systems meet
- Ghrelin system is meant to maintain an organism's metabolic homeostasis by regulating energy balance

# Ghrelin

- “Hunger hormone”
  - stimulates appetite and adiposity
- 28 amino acid peptide hormone
- Made predominantly by your stomach’s X/A like cells
- Synthesized from a polypeptide precursor (preproghrelin)
- Ghrelin levels in blood increase before meals, telling you to eat
- Ghrelin secretion drops as you eat



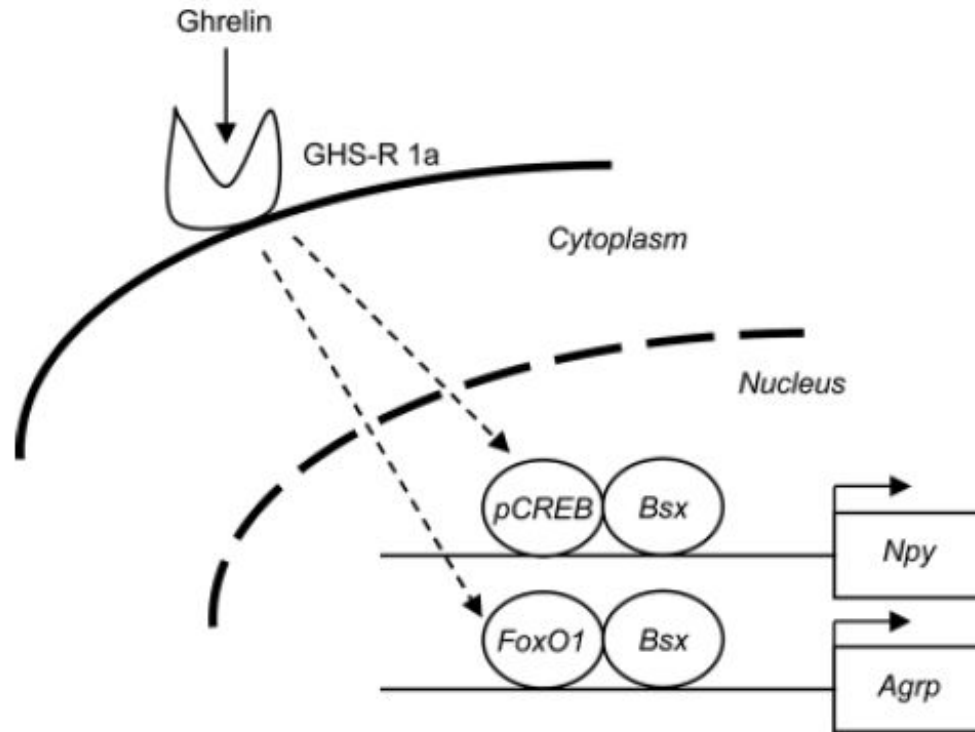
# Ghrelin System: some key players

- Ghrelin
- GHS-R1a – Growth Hormone Secretagogue Receptor 1a
  - Expressed in NPY/AgRP neurons in the ARC
- Hypothalamus
  - Tanycytes
- GOAT (Ghrelin O-acyl transferase)

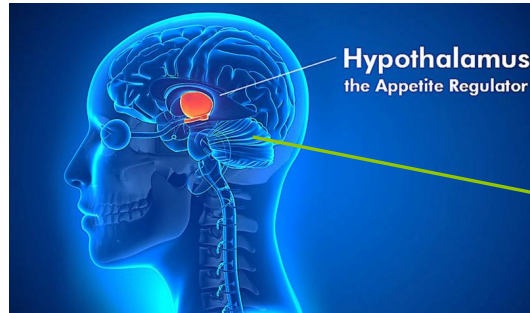
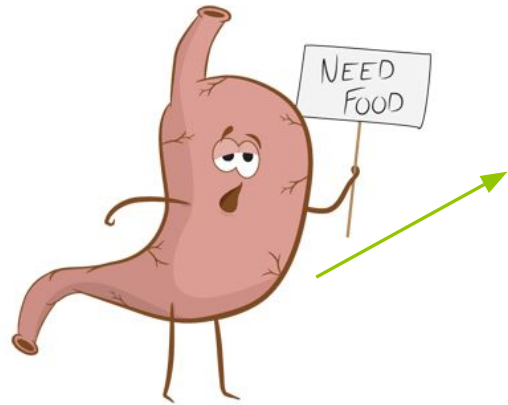


\*Acyl group is a medium-chain fatty acid, usually n-octanoate

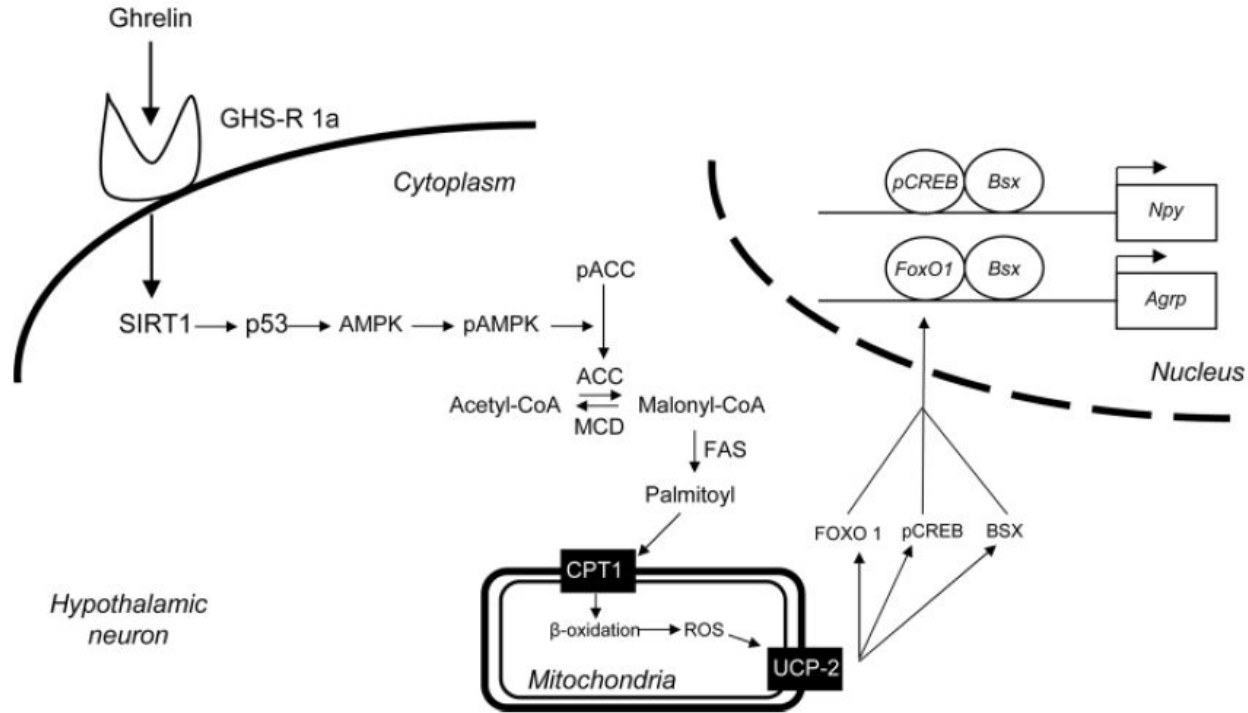
# Ghrelin's actions on the Hypothalamus



**BIG PICTURE:** Ghrelin upregulates AgRP and NPY expression in the ARC  
→Orexigenic effect



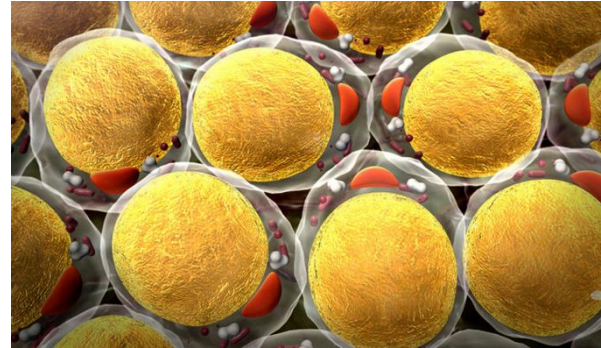
# Signaling

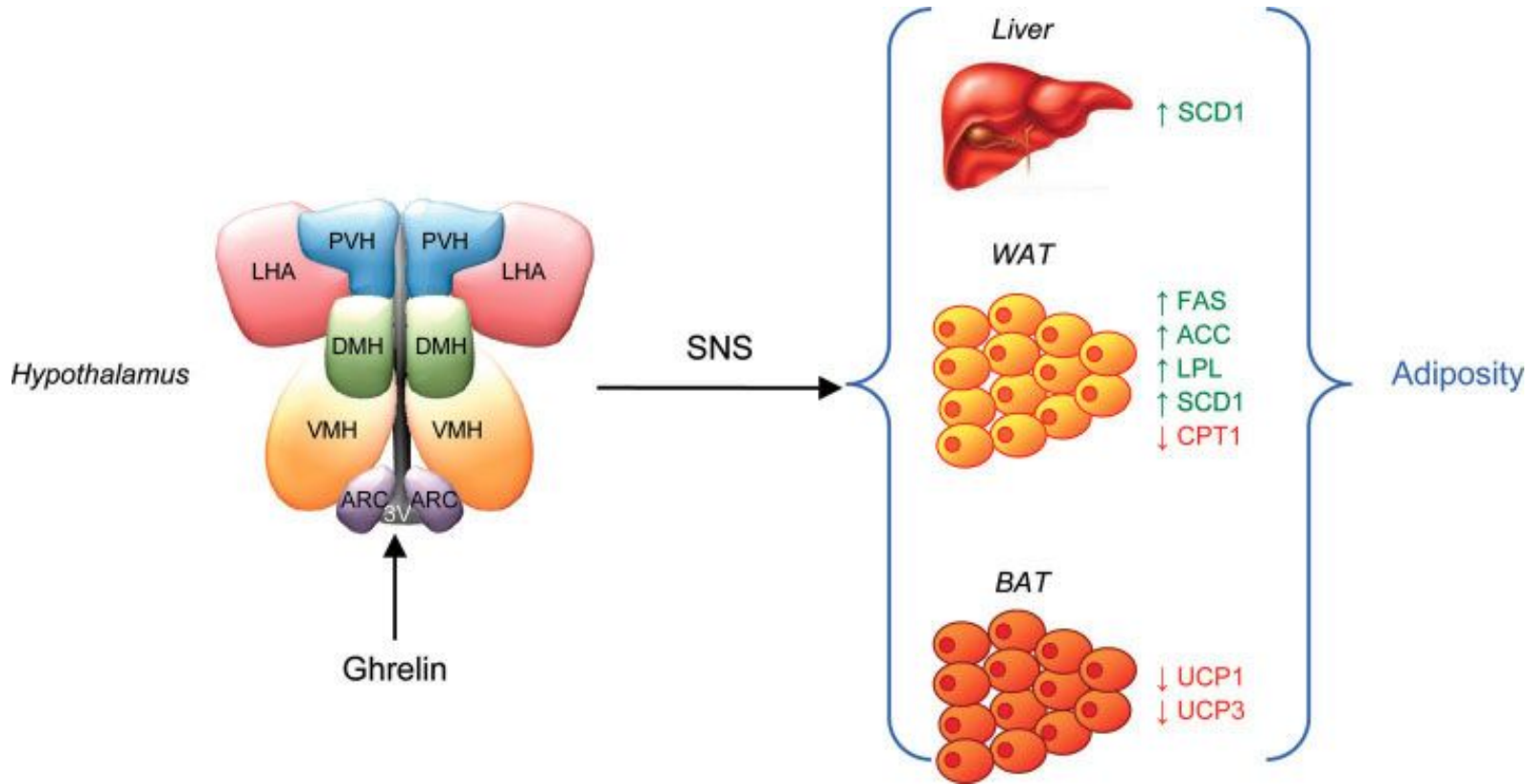




# Ghrelin: modulator of peripheral lipid metabolism

**BIG PICTURE:** Ghrelin promotes adiposity by stimulation of lipogenesis in white adipose tissue (WAT) & liver



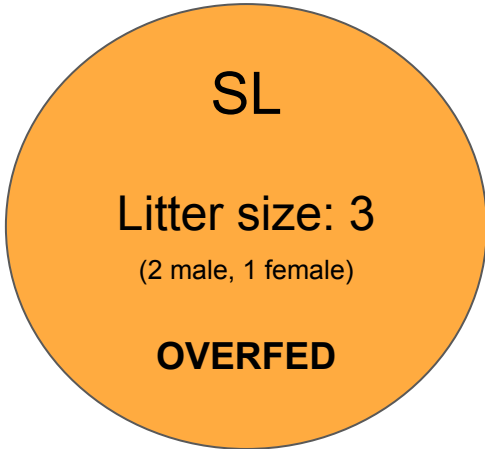


# Neonatal overnutrition causes early alterations in the central response to peripheral ghrelin

- Proven mouse model used to study postnatal overfeeding.
  - Small litter size = augmented growth before weaning; continuously overweight throughout life
  - Normal litter size = control group
- Variables
  - High fat, high sugar diet (HFHS)
  - Ghrelin administration (peripheral vs. central)
- Region of interest
  - Arcuate Nucleus of the Hypothalamus (ARH)

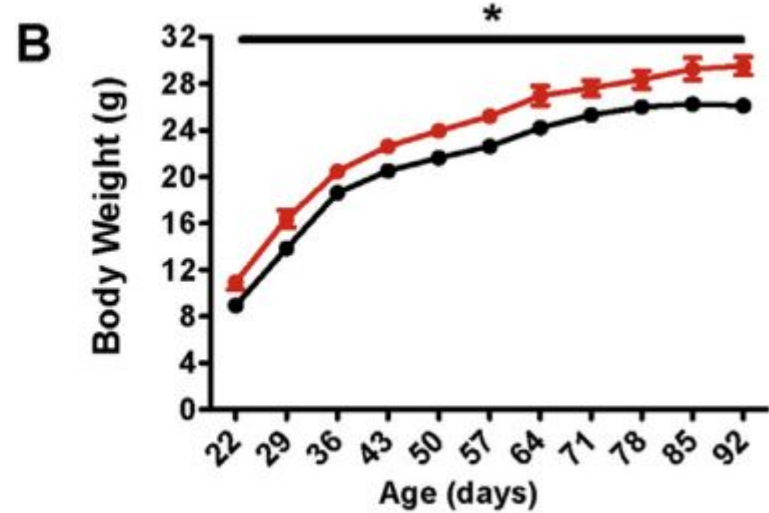
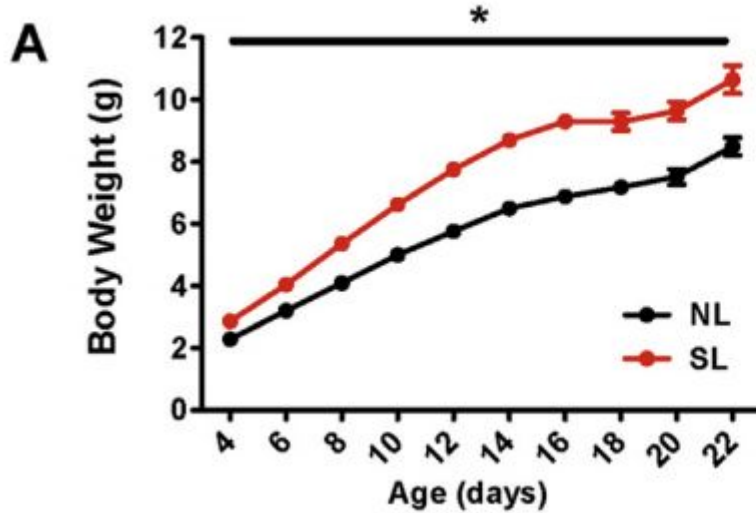
Postnatal overfeeding modifies ghrelin's capacity to influence the hypothalamus.

# Group differences



Both groups fed chow diet after weaning.  
All experiments used male only pups.  
Each experimental group used pups from three separate litters.

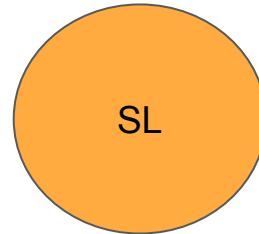
# Body weight from birth to adulthood



A) While nursing

B) After weaning to adulthood

*Not shown: SL has significantly more fat mass than NL. No significant difference in lean mass*

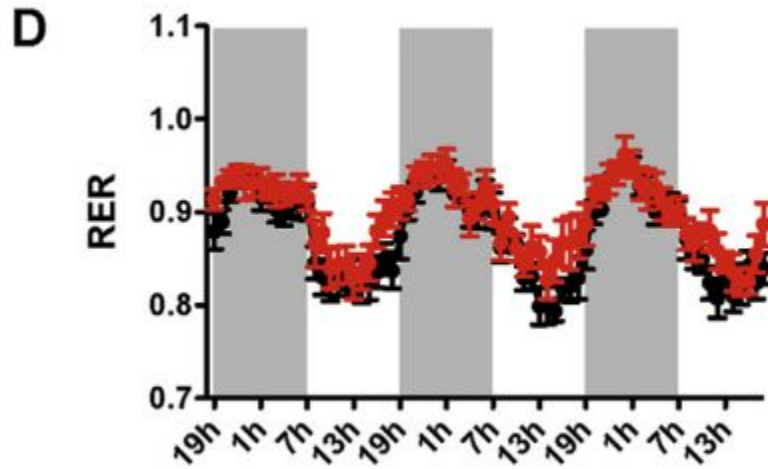
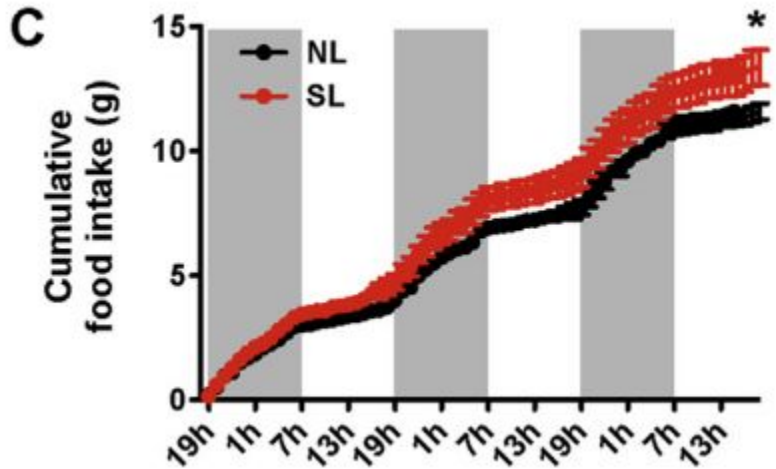


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**Overnutrition early in life causes higher body weight throughout life.**

# ADULT food intake & physical activity (at 90 days old)



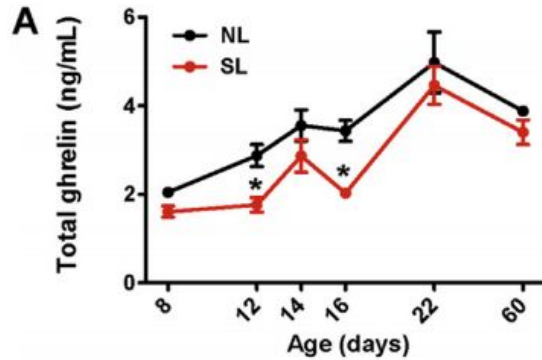
C) Food intake

D) Respiratory exchange ratio (muscles' oxidative capacity to get energy)

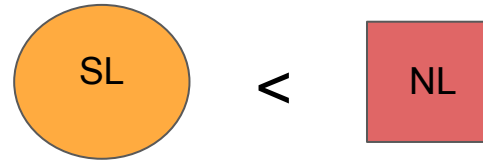


**Overnutrition early in life significantly increases food intake but not energy expenditure.**

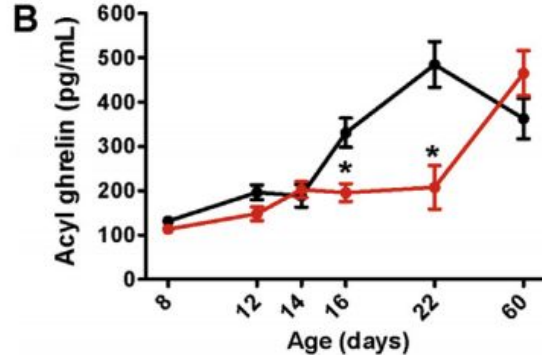
# Circulating ghrelin levels



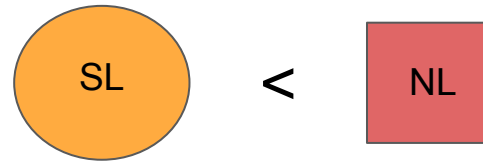
A) Total Ghrelin (Acyl and desacyl)



For NL mice ghrelin levels increased normally, reaching adult levels by day 60. For SL mice ghrelin levels were attenuated.



B) Acyl Ghrelin (aka active ghrelin)

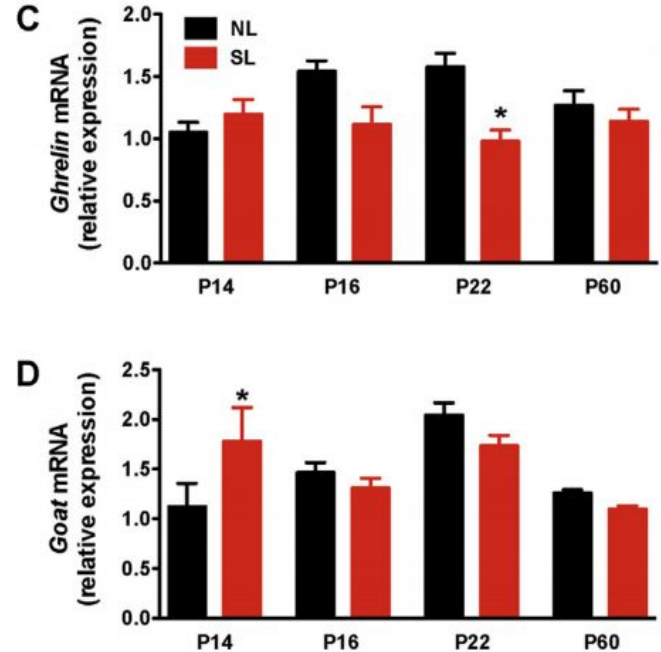


**Decreased ghrelin in the bloodstream during crucial development periods means less ghrelin uptake in the CNS. Could this lead to attenuation of receptors in the CNS? Ghrelin resistance?**

# Stomach levels of ghrelin mRNA and GOAT mRNA

*qPCR used to measure mRNA of Ghrelin & GOAT (an enzyme that activates ghrelin)*

Both Ghrelin **mRNA** and GOAT **mRNA** levels fluctuated in NL mice but, comparably, remained mostly unchanged in SL mice.

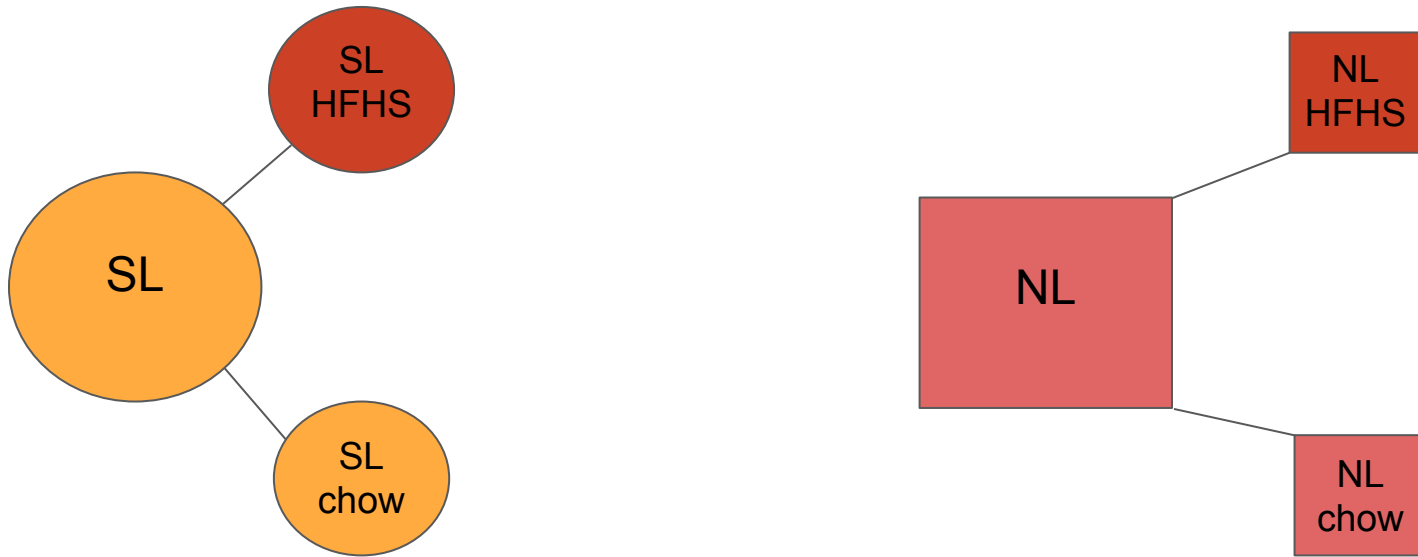


**The ghrelin systems of SL mice seem unaffected by changes during development.**



# High Fat High Sugar diet (HFHS)

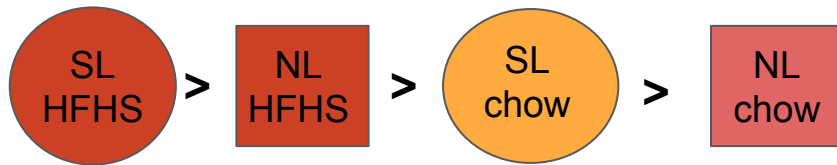
At 17 weeks of age both the SL and NL mice were split into two sub groups and fed different diets for 8 weeks. One subgroup from each group was given a HFHS diet.



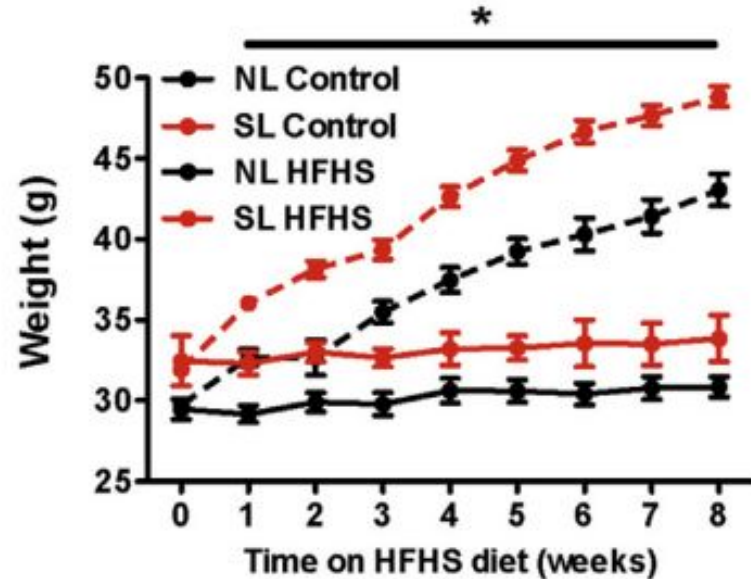
**How do adults, overfed early in life, compare with adults not overfed early in life, on a high fat high sugar diet?**

# Weight of adult SL & NL on HFHS diet

Mice overfed early in life gain MORE weight on HFHS diet than non-overfed mice!

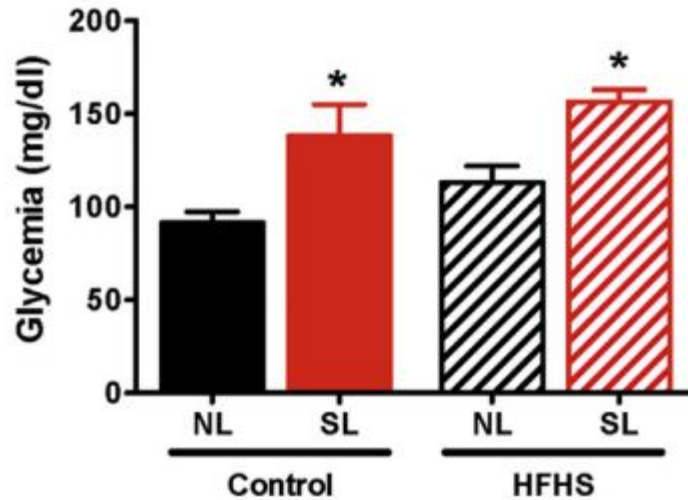


Fat mass numbers are similar to body weight numbers.

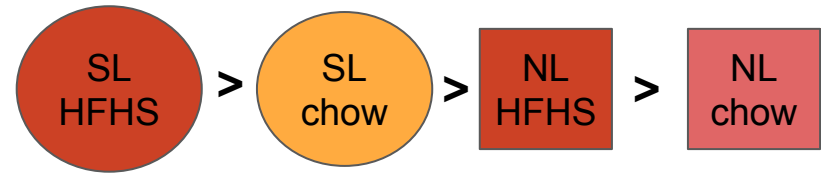


**Being overfed early in life causes higher rate of weight gain in adult life, even when eating the same foods as non-overfed individuals.**

# Glycemia



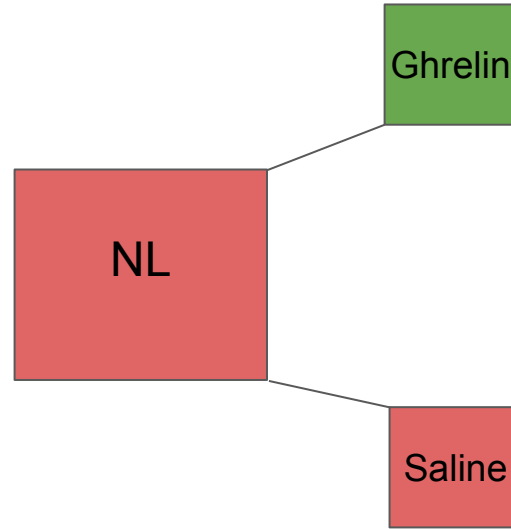
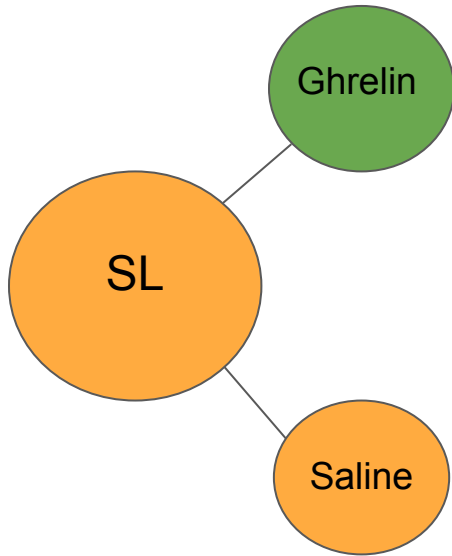
Even without the HFHS diet the SL group has higher blood glucose levels



**Overfed group got a head start on hyperglycemia.**

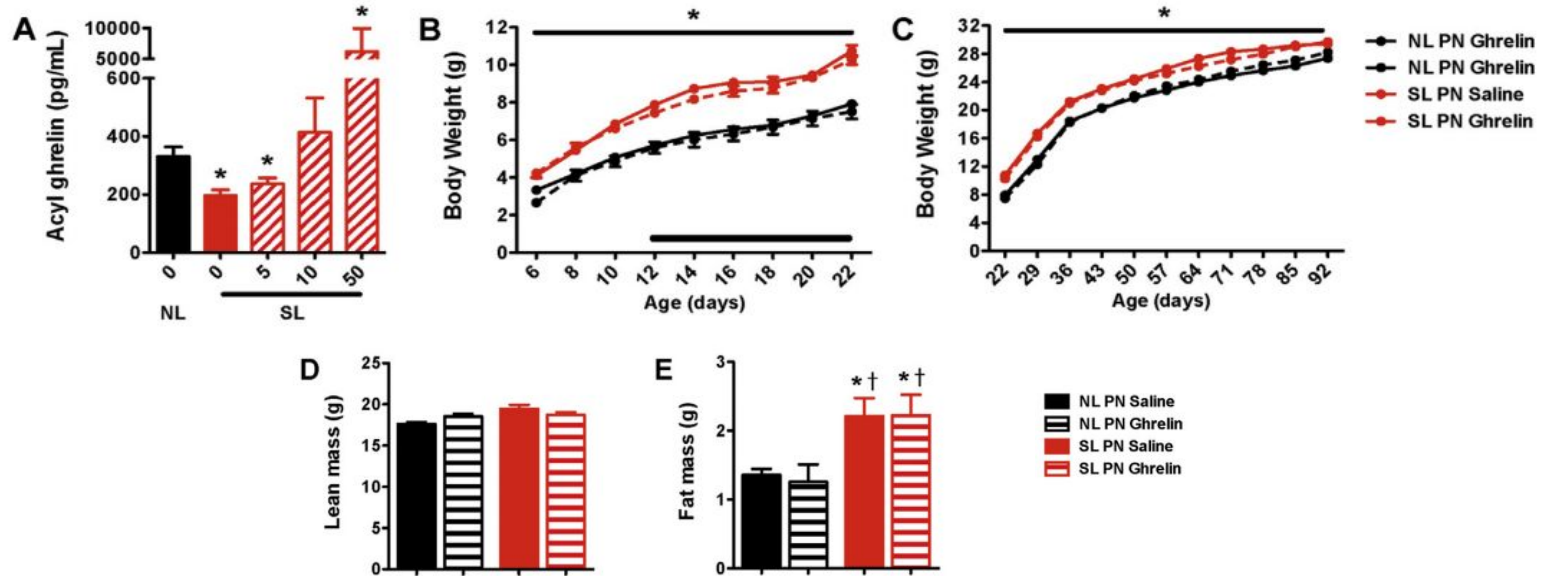
# Peripheral Ghrelin effect on hypothalamus

At two weeks of age both the SL and NL mice were split into two sub groups. One subgroup from each group was administered ghrelin and while the control groups were given a saline solution.



# Ghrelin injection has no impact

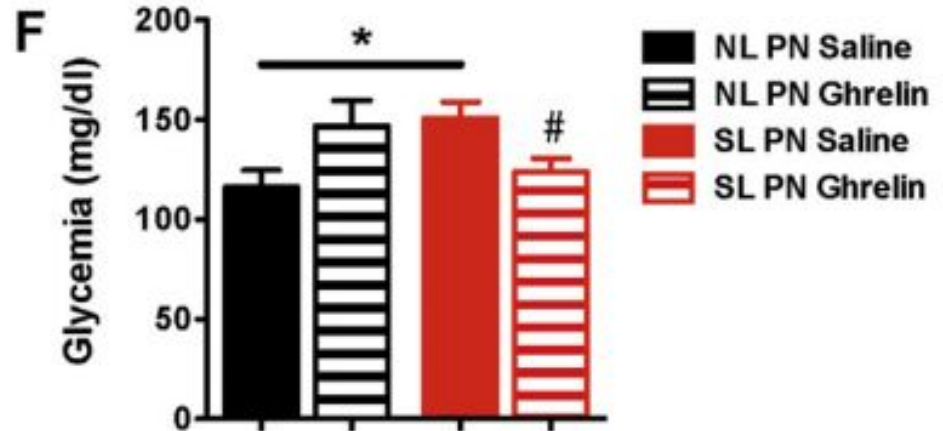
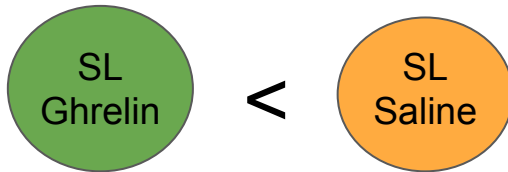
It takes about 10ug/kg ghrelin administration to bring SL mice to a normal ghrelin level. However, regular ghrelin injections did not have an effect on body mass/composition.



No significant effects with ghrelin injection on weight or fat mass.

# Ghrelin injections lowered GLYCEMIA levels!!

There was a significant difference in glycemia levels between SL mice treated with saline and SL mice treated with ghrelin.



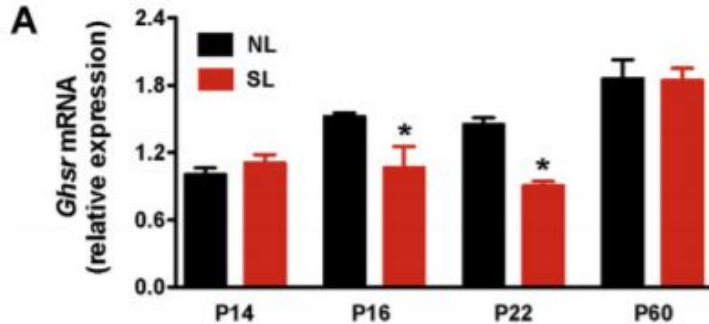
Could ghrelin administration play a role in treating hyperglycemia?

# Immunohistochemistry- cFOS mRNA Analysis

**Upregulation of cFos immunoreactive cells indicates recent neuron activity.**

# In the ARH- mRNA analyses after peripheral Ghrelin administration

(without ghrelin injections:) mRNA expression for the ghrelin receptor is lower when nursing but returns to normal reaching adulthood

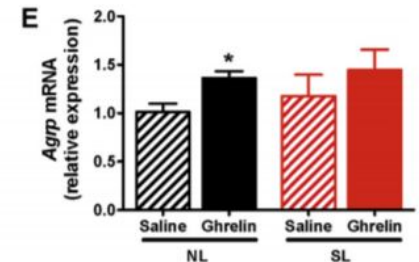
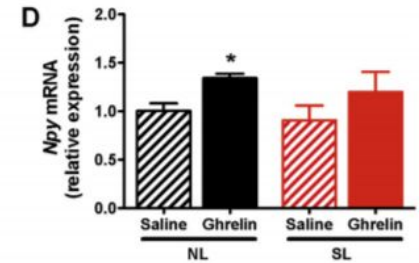
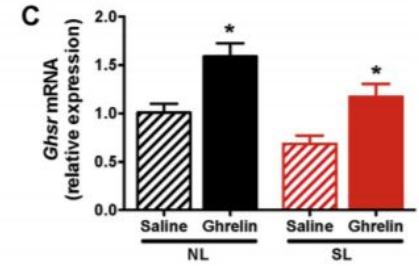


Peripheral ghrelin administration does not have the same effect on orexigenic modulators in overfed mice as in controls.

This makes sense, if the body is being overfed it needs less signaling to eat.

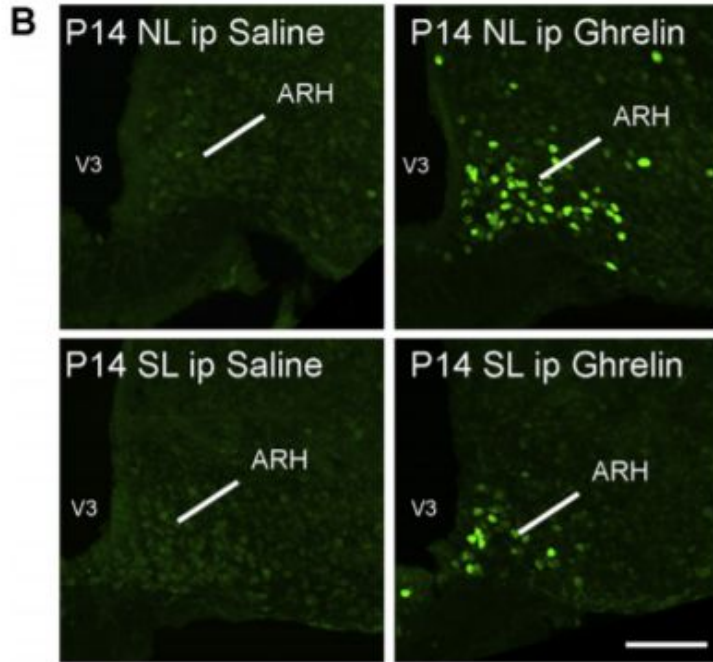
(POMC neurons were not affected.)

**Remember: Npy and AgRP neurons increase appetite**





# cFos positive cells in ARH after peripheral Ghrelin administration



We can clearly see that there are fewer cFos immunoreactive cells fluorescing in these images taken on P14.

Again, by P60 there was **no significant difference** in cFos immunoreactive cells between the SL and NL groups.

This same study was done on non-ARH areas of the hypothalamus but showed no significant differences. The researchers believe this is because the ARH is specifically important to the ghrelin system during early development.

**Changes during early development are key in the ghrelin systems in the ARH**

# PAY ATTENTION

This is where it gets exciting!!

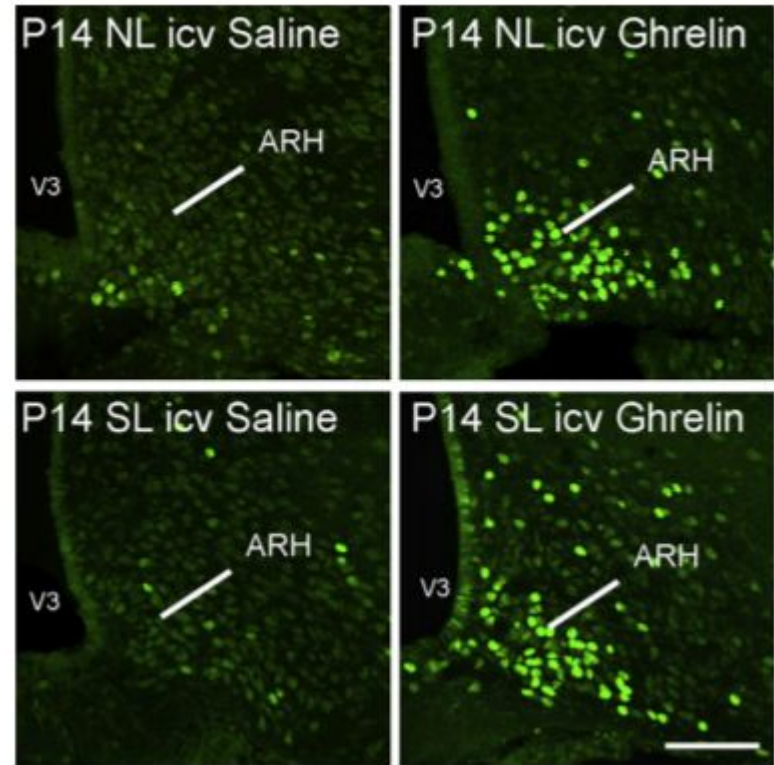


# CNS response to intracerebroventricular ghrelin injection

We have a transportation problem!!

It looks like ARH neurons in SL mice CAN respond to ghrelin similar to NL mice. The only difference from that last slide is the ghrelin was injected peripherally. Suggesting that the problem is not at the receptor level (GHS-R), but in transportation from the periphery to the brain.

**Not a receptor problem!!**



# Assessment of Ghrelin Uptake

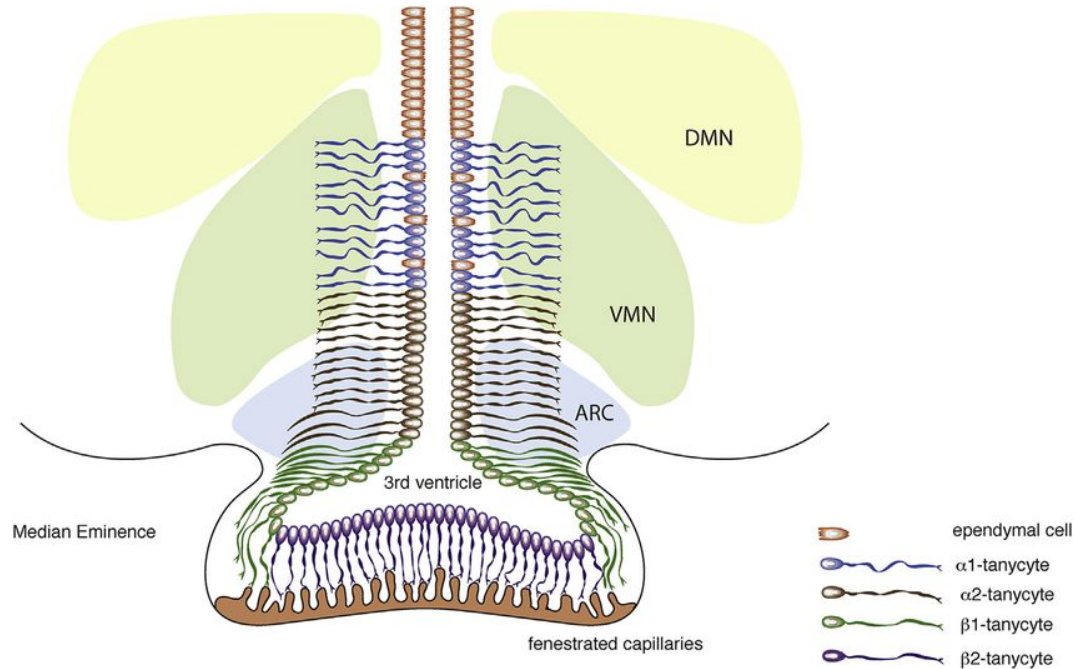
Fluorescent bioactive ghrelin injected IV and mice sacrificed 5 min later to assess tanyctic ghrelin uptake by fluorescence microscopy.

# Remember tanycytes of the Median Eminence?

Tanycytes are glia cells specific to the Hypothalamus which aid in the transportation of hormones from the periphery across the blood brain barrier.

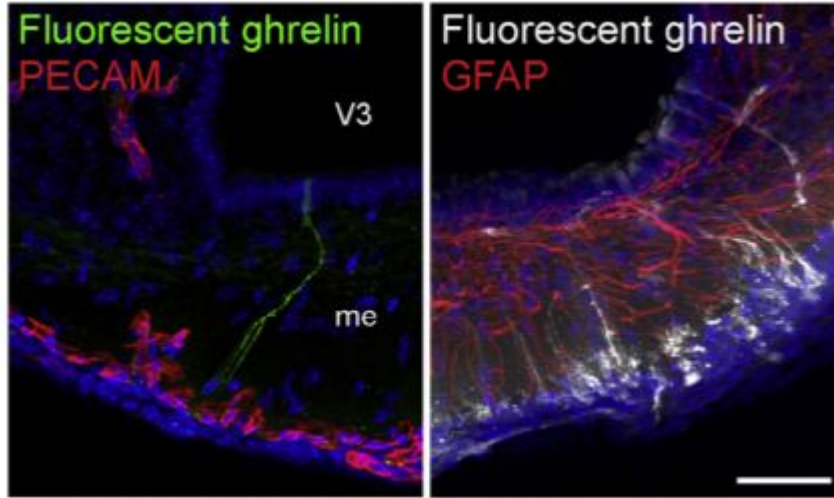
**The researchers used fluorescent ghrelin (intravenously in wild type mice) to establish the role a tanycytes in ghrelin uptake. Sure enough 5 minutes after administration ghrelin was SOLELY present in ME tanycytes!**

They also observed ghrelin transportation to the brain, from the periphery, via tanycytes in vitro.

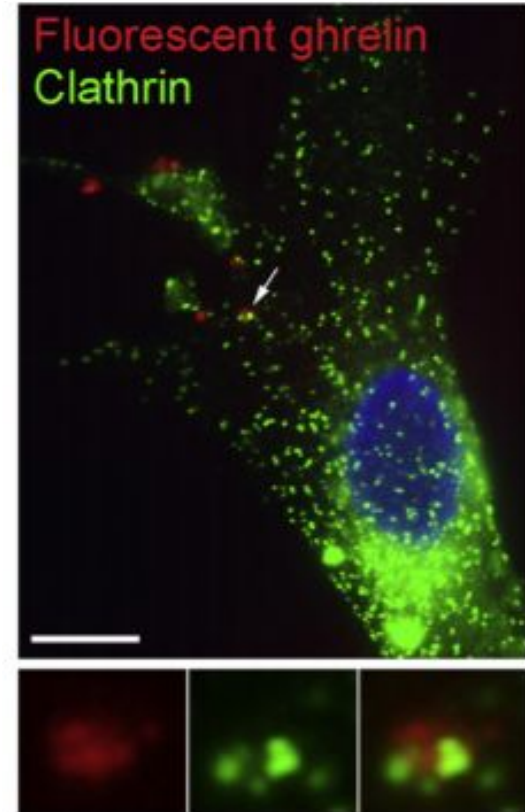


**Let's see!!**

# Tanycyte transportation



Tanycyte cell process transporting ghrelin (green on left, and white on right) 5 minutes after intravenous injection.



Fluorescent ghrelin being encapsulated in clathrin vesicles

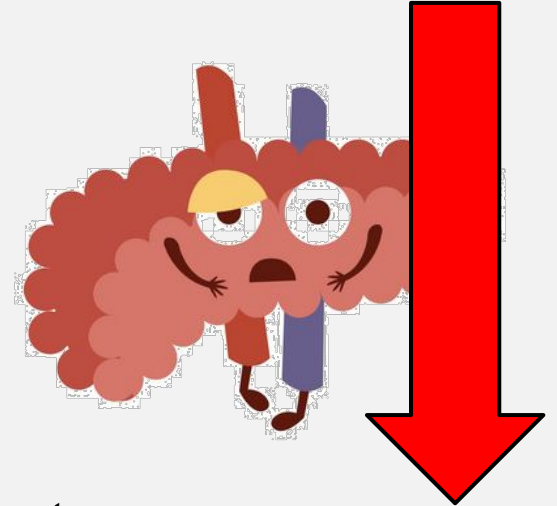
# Neonatal

## Neonatal ghrelin

- Lifelong role in energy balance regulation

## Ghrelin exposure for 7-14 days

- Newborn rats saw reduction in...
  - pancreatic weight
  - pancreatic DNA synthesis
  - DNA content
- Ghrelin may **directly act** on pancreas development



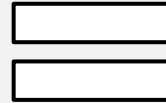
# Neonatal overnutrition

How is ghrelin affected?

Reduced

- Ghrelin circulating levels
- Ghrelin uptake from periphery to brain
- Ghrelin receptor expression

Harder for peripheral ghrelin to **activate arcuate neurons**



**Silenced  
ghrelin  
system**



# Arcuate Nucleus

## Arcuate Nucleus of the Hypothalamus (ARH/ARC)

- Some neurons have NPY, which influences hunger
  - When **activated**, food intake **increases** and can result in obesity

## ARH neurons

- Of SL mice can respond to ghrelin but...
  - Peripheral ghrelin struggle to reach neurons
- Of NL mice
  - Peripheral ghrelin injections increase ghrelin content
    - SL mice do not display this! Unless intracerebroventricular injection

# Neonatal ghrelin

## Neonatal ghrelin blockade

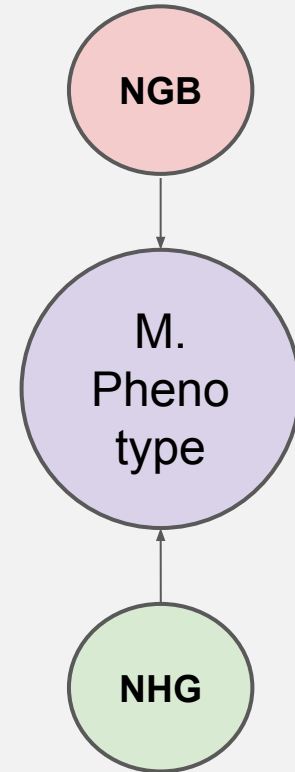
- Enhanced densities of ARH projections

## Neonatal hyperghrelinemia

- Weakened ARH projection development

However, both link to the **same metabolic phenotype**

- Elevated body weight
- Hyperglycemia



# Neonatal ghrelin, continued

## SL mice

- Normalizing ghrelin levels showed no significant changes in metabolic outcomes

## Hypoghrelinemic SL mice

- Had **reduced** central responses to peripheral ghrelin

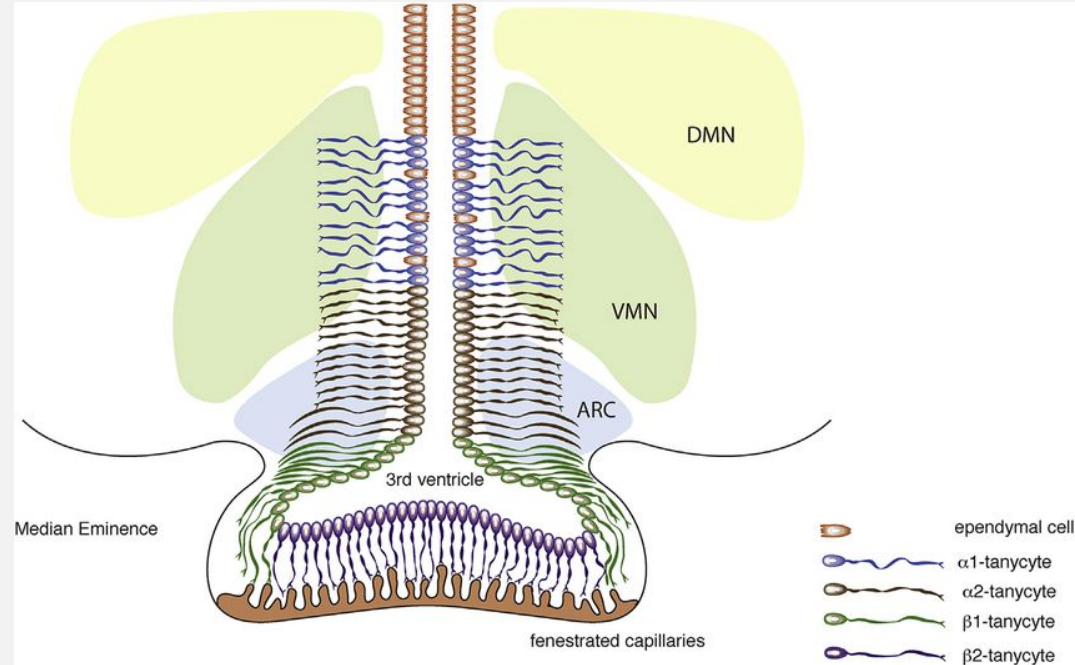
# Tanycytes

- Critical regulators of **hormone transport** into the brain

- Also into the ARH

Diet-induced obesity in adults

- Alters the tanycytes' ability to transport hormones



# Caloric restriction

Obese adults show lower ghrelin levels

- Neonatal overfeeding affects circulating ghrelin levels during vital development periods
- **Negative correlation** between circulating ghrelin and body weight

In adult rats, caloric-restrictive weight loss can **reverse** central ghrelin resistance

For metabolic programming, the restriction's effect is heavily dependent on **timing**

- Beneficial if done **early** in development
- Detrimental if done **late** (after weaning)

# Prader-Willi Syndrome: the exception

Complex genetic condition

Infancy

- Weak muscle tone (**hypotonia**)
- Feeding difficulties
- Poor growth and development

Childhood and on

- Severe overeating (**hyperphagia**) leads to morbid obesity

Linked to **elevated ghrelin** levels, including early life

- **Positive correlation** between circulating ghrelin and body weight



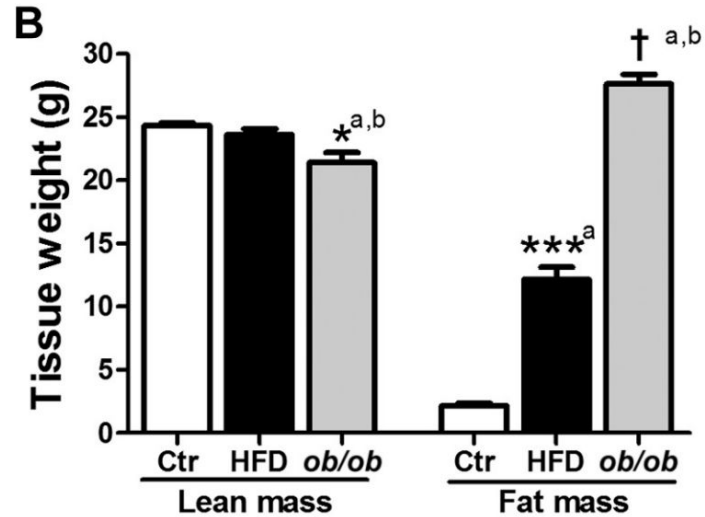
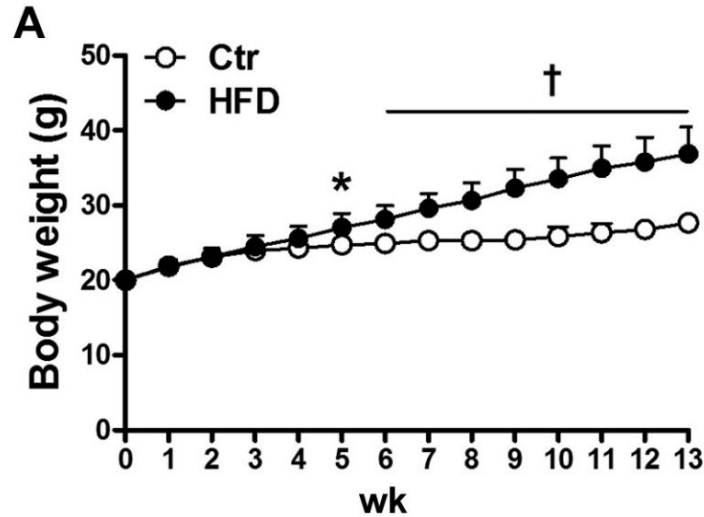
# High-fat diet increases ghrelin-expressing cells in stomach, contributing to obesity

- Experiment Cohorts
  - Standard chow-fed control group (Ctr)
  - High-fat diet (HFD)
  - Genetically Obese (*ob/ob*)
- Analyzing across the board
  - Body weight and composition
  - Distributions of preproghrelin mRNA-expressing cells
  - Plasma ghrelin
  - Ghrelin-reactive IgG

HFD in mice induced: obesogenic changes, number of preproghrelin mRNA-expressing cells in stomach, increased affinity of plasmatic IgG for ghrelin

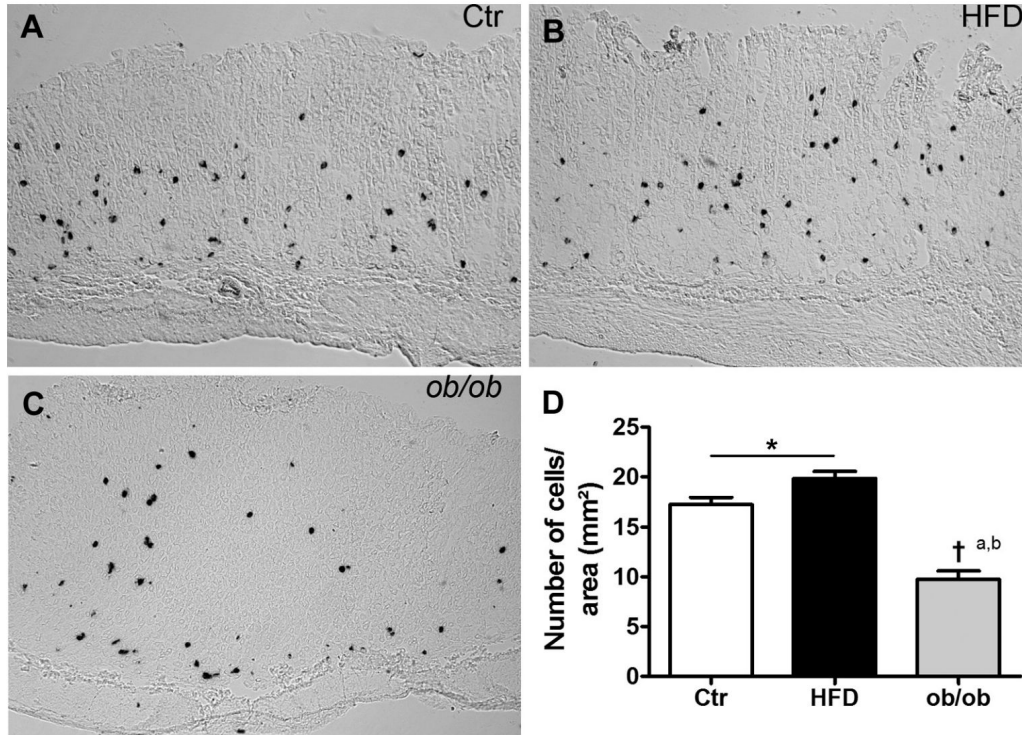
François et. al 2015

# HFD effects on body weight and body composition

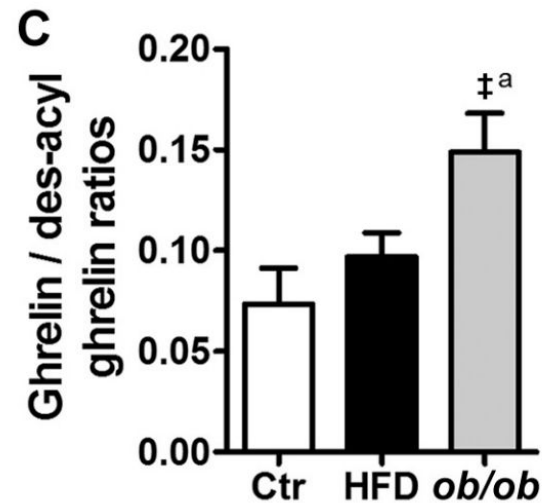
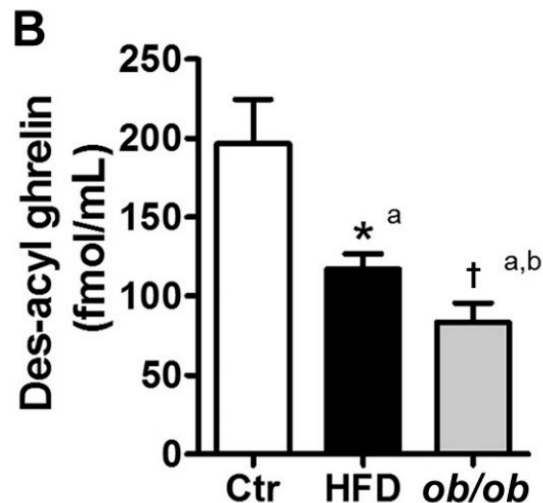
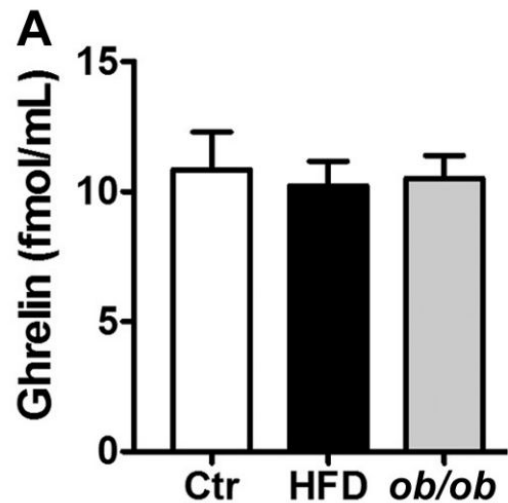




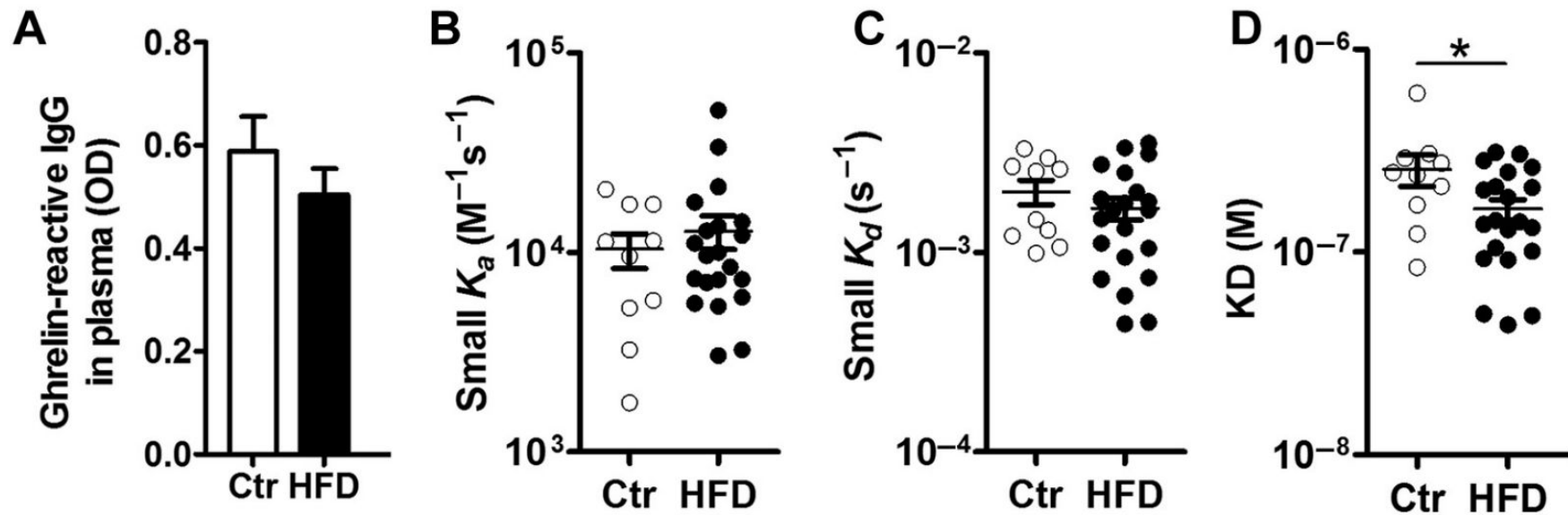
# Preprogrelin mRNA expression by in situ hybridization



# Plasma ghrelin



# Ghrelin-reactive IgG



# Experiment Takeaways

- Experiment 1: Neonatal ghrelin plays an important role in hypothalamic development and lifelong metabolic regulation
- Experiment 2: High Fat Diet increased number of ghrelin-expressing cells in the stomach of adult mice

# Themes

- Hypothalamus: where the nervous and endocrine systems meet
  - Metabolic hormones transmit signals to your brain, which influences the brain's development

- Ghrelin system is meant to maintain an organism's metabolic homeostasis by regulating energy balance
  - Intended to keep you at a neutral energy state

# Why is looking at Ghrelin important?

- Link between nutrition and the hormonal signals regulating food intake
- Obesity is a major health problem



**Ghrelin**

Thank you: Dr. Boyle,  
TAs/IAs, Cogs 163  
classmates!!