# The collapse of the BDNF/POMC system in the hypothalamus is responsible for the extreme obesity with hyperphagia observed in female heterozygous MeCP2 null mice

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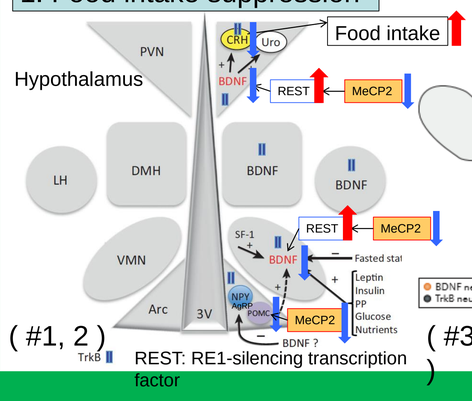
## OBJECTIVES

The objective was to elucidate the mechanism underlying the extreme obesity observed in female heterozygous MeCP2 null mice, generated a mouse model of Rett syndrome. Rett syndrome is an epigenetic X-linked neurodevelopmental disorder that affects girls due primally to mutations in the gene encoding methyl-CpG binding protein 2 ( MeCP2 ). Clinically, Zappella variant Rett syndrome patients have autism spectrum disorder and obesity from childhood.

### Hypothesis of the mechanism of obesity in the MeCP2$^{+/-}$ mice

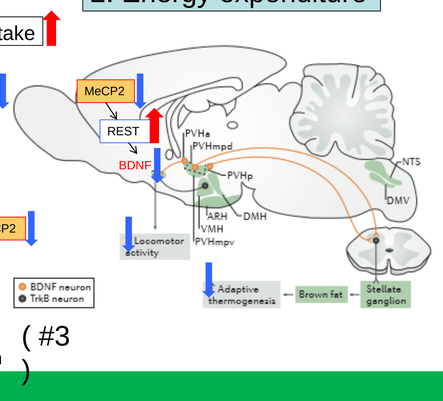
1. Food intake suppression




( #1, 2 )

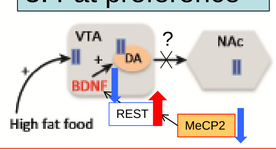
REST: RE1-silencing transcription factor

2. Energy expenditure




( #3 )

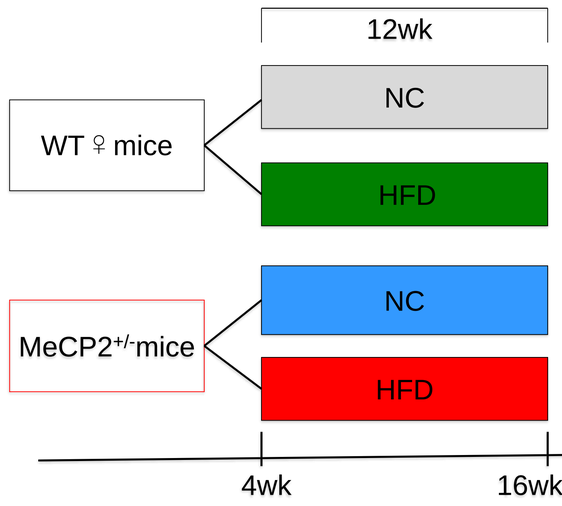
3. Fat preference




The sight-specific deletion of BDNF in the VTA of mice caused increased intake of palatable high-fat diet but not of standard-chow diet. ( #4 )

## METHODS

We examined the molecular biology and phisiology of female heterozygous MeCP2 null mice ( MeCP2 tm1.1 Bird/J, MeCP2$^{+/-}$ mice ) fed a normal-chow diet ( NC ) or a high-fat diet ( HFD ) for 12-weeks since 4-weeks of age using analytical tools. C57/BL6 mice were used as controls.




① Energy expenditure
- VO2
- Locomotor activity
- UCP1 and PGC1α in the brown adipose tissue (BAT) measured by real-time qPCR

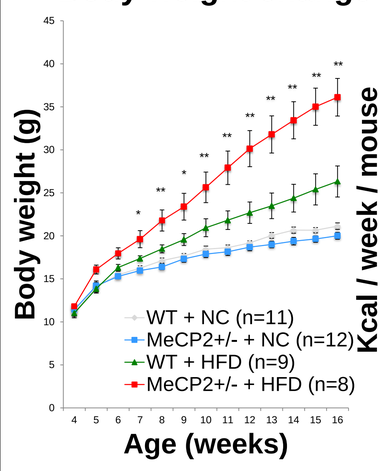
② Regulation of food intake
- Expression level of Leptin, NPY, AgRP, POMC, BDNF, CRH in thehypothalamus measured by real-time qPCR and Western blot analysis
- POMC and BDNF immunostaining

③ Preference test

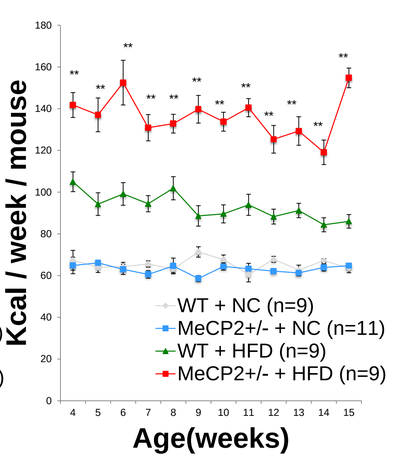
## RESULTS

### Figure1. Body weight gain in the MeCP2$^{+/-}$ mice

A Body weight change



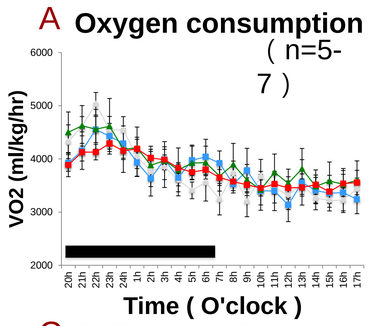

B Weekly food intake



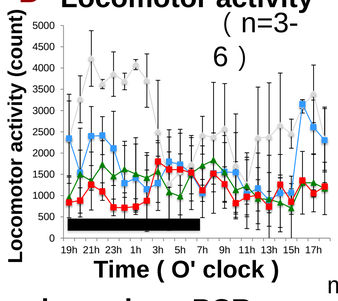

The data are shown as means ± SEM , ＊p < 0.05, vs WT mice fed High fat diet , ** p < 0.01 , vs WT mice fed High fat diet

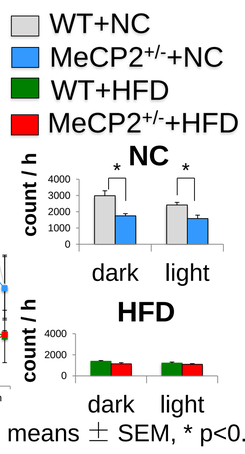
### Figure2. Thermogenesis in the BAT and locomotor activity

A Oxygen consumption ( n=5-7 )



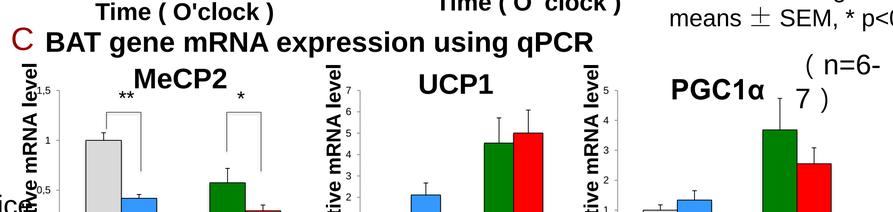

B Locomotor activity ( n=3-6 )




means ± SEM, * p<0.05

C BAT gene mRNA expression using qPCR ( n=6-7 )




means ± SEM, * p<0.05, ** p<0.01

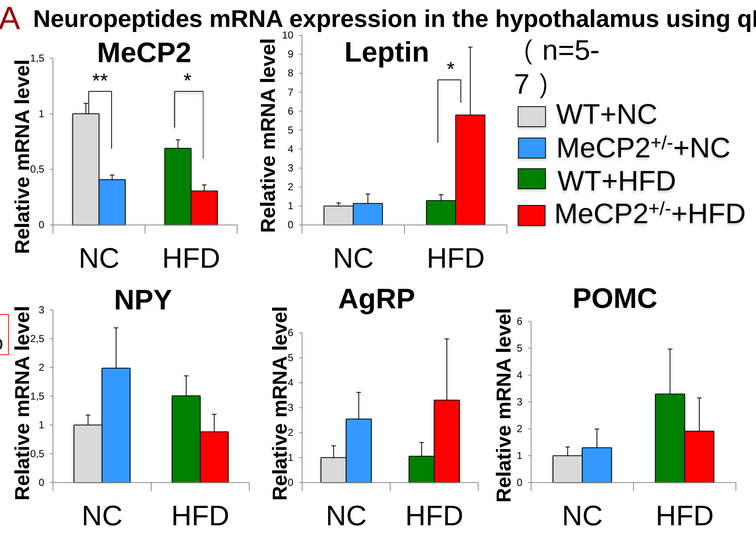
### Table1. Metabolic parameters in 16-week-old mice

| ( n=7-8 ) | WT+NC | MeCP2$^{+/-}$ +NC | WT+HFD | MeCP2$^{+/-}$ +HFD |
|---|---|---|---|---|
| pWAT (g) | 0.12±0.02 | 0.46±0.13 | 0.64±0.2 | 1.74±0.33 a,b,c |
| sWAT (g) | 0.16±0.03 | 0.38±0.11 | 0.50±0.18 | 1.41±0.22 a,b,c |
| iWAT (g) | 0.05±0.01 | 0.19±0.04 | 0.31±0.12 | 0.64±0.15 aa,bb |
| BAT (g) | 0.067±0.004 | 0.061±0.004 | 0.109±0.013 | 0.089±0.012 |
| Liver (g) | 0.68±0.05 | 0.81±0.04 | 1.01±0.13 | 1.53±0.28 aa,b |
| T-cho ( mg/dl ) | 57.7±3.9 | 59.9±5.1 | 89.3±11.1 | 144.3±16.4 aa,bb,c |
| Glucose ( mg/dl ) | 79±6 | 121±20 | 183±19 aa | 185±21 aa |
| Insulin ( μIU/ml ) | 8.4±0.8 | 12.1±1.0 | 15.4±2.6 | 23.2±4.6 aa,b |
| HOMA-R | 1.6±0.2 | 3.8±0.9 | 7.8±2.0 a | 10.2±2.1 aa,b |
| Leptin ( ng/ml ) | 1.9±0.5 | 6.7±1.7 | 16.1±5.8 a | 18.6±9.4 aa,bb,c |

pWAT: perigonadal white adipose tissue, sWAT: subcutaneous white adipose tissue, iWAT: inguinal white adipose tissue, BAT: brown adipose tissue
a p<0.05, vs WT mice fed Normal chow diet aa p<0.01, WT mice fed Normal chow diet b p<0.05, vs MeCP2$^{+/-}$ mice fed Normal chow diet
bb P<0.01, vs MeCP2$^{+/-}$ mice fed Normal chow diet c p<0.05, vs WT mice fed High fat diet means ± SEM

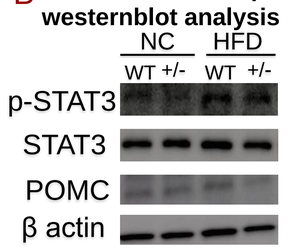
### Figure3. Regulation of food intake in the hypothalamus

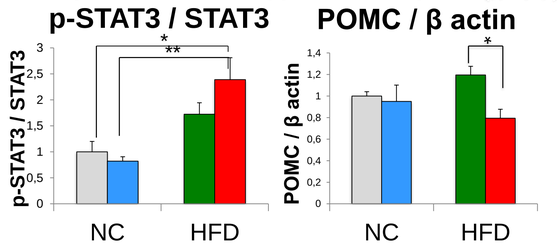
A Neuropeptides mRNA expression in the hypothalamus using qPCR



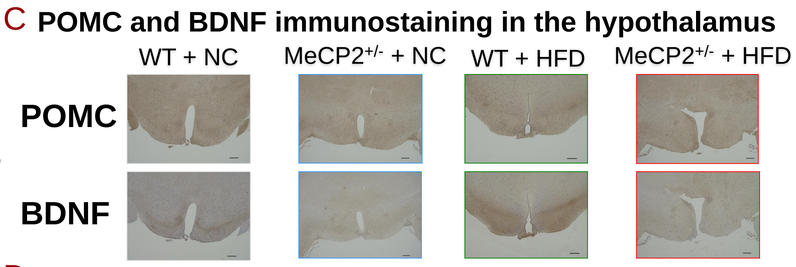

The data are shown as means ± SEM, * p<0.05, ** p<0.01 ( real-time qPCR in the hypothalamus)

B STAT3 and POMC protein expression in the hypothalaus by westernblot analysis （n=4-7）

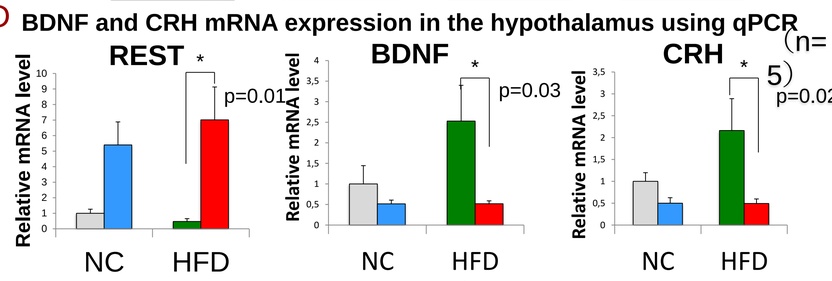



C POMC and BDNF immunostaining in the hypothalamus



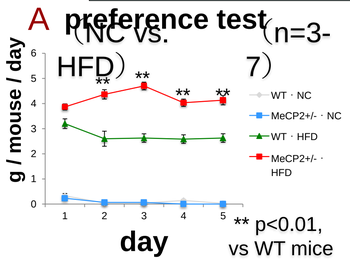

D BDNF and CRH mRNA expression in the hypothalamus using qPCR （n=5）




The data are shown as means ± SEM ＊p<0.05, ** p<0.01

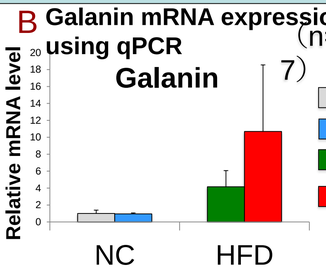
### Figure4. Preference and reward system

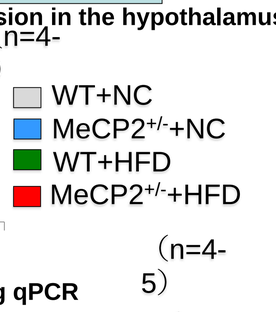
A preference test (NC vs. HFD) （n=3-7）




** p<0.01, vs WT mice

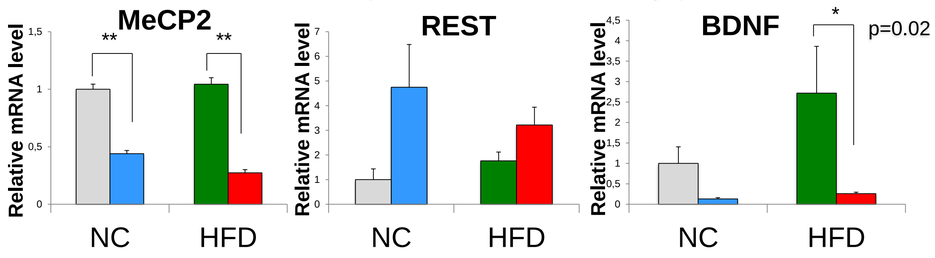
B Galanin mRNA expression in the hypothalamus using qPCR （n=4-7）




（n=4-5）

C REST and BDNF mRNA expression in the VTA using qPCR




The data are shown as means ± SEM, * p<0.05, ** p<0.01

## CONCLUSIONS

1. Our experiments showed that the MeCP2$^{+/-}$ mice fed with a HFD expressed extreme obesity with hyperphagia.
2. Oxygen consumption and locomotor activity were not different between the MeCP2$^{+/-}$ mice fed with a HFD and the controls fed with a HFD. A dietary preference test revealed that the MeCP2$^{+/-}$ mice fed with a HFD greatly preferred to HFD.
3. A decrease of POMC expression in the hypothalamus and decreased expressions of BDNF in the hypothalamus and the VTA could account for central leptin resistance, which might lead to hyperphagia on the MeCP2 $^{+/-}$ mice fed with a HFD.
4. We think that epigenetic pathogenesis rather than disturbances in the leptin receptor / Stat3 signaling could be one of the mechanisms underlying the hyperphagia with leptin resistance observed in the MeCP2$^{+/-}$ mice fed with a HFD.

## References

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