**Metabolic and behavioral sex-related differences induced by conditional inactivation of Npy1r gene in mice**

# Metabolic and behavioral sex-related differences induced by conditional inactivation of Npy1r gene in mice

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Sex differences affect brain, behavior and metabolic functions, and contribute to vulnerability to disease; the mechanisms underlying the sex specificity of brain activity is, however, poorly known. Sex-biased effects of selective gene deletion have been reported in mice models relevant for emotional and stress-related behaviors and metabolic functions. Despite this evidence, the vast majority of rodent researchers continue to use exclusively males in their studies, and information on sex differences in these regards is sparse.

NPY is one of the most abundant neuropeptide within the CNS. In the hypothalamus, NPY is synthesized by ARC neurons, projecting to other nuclei of the circuit controlling food intake, including the PVN, VMH, and DMH nuclei. NPY is identified to date as the most potent orexigenic peptide and its chronic central administration produces hyperphagia, robust obesity and decreased thermogenesis. In addition, NPY plays a key role in the regulation of balance between reproductive functions and energy homeostasis via the activation of Y1Rs. Thus, in the hypothalamus NPY acts as integrator among feeding circuit, stress signals and neuroendocrine system.

The NPY-Y1R system is sexually dimorphic and sensitive to gonadal steroids.

We have previously generated a conditional knockout mouse model (Npy1r$^{rfb}$ mice), in which the Npy1r gene was specifically inactivated in forebrain principal neurons starting from juvenile age. Here we demonstrated that Npy1r$^{rfb}$ mice show a sex dimorphic phenotype, revealing the existence of NPY-Y1R neuronal subpopulations involved in sex-related differences of metabolic and behavioral functions.

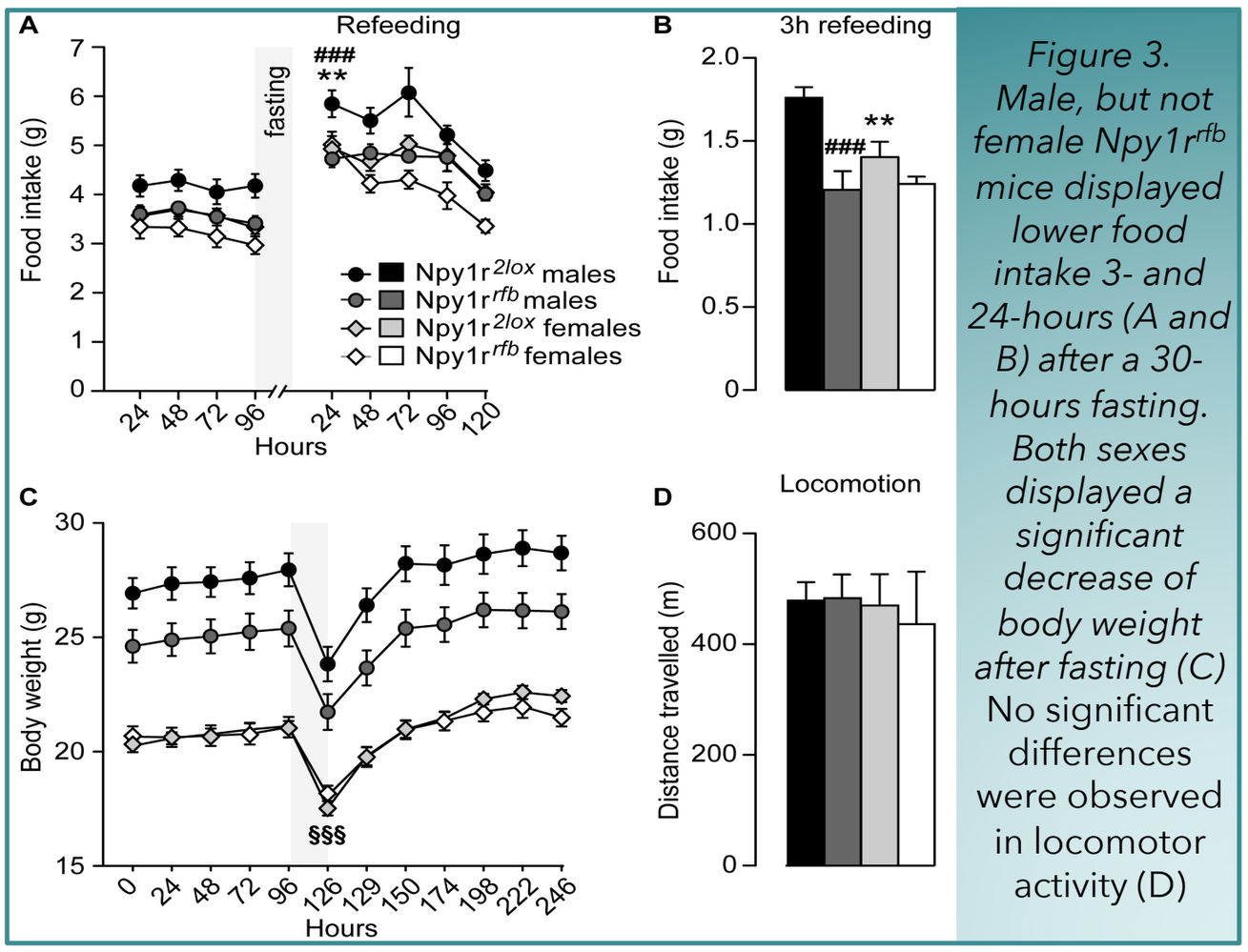


*Figure 3. Male, but not female Npy1r$^{rfb}$ mice displayed lower food intake 3- and 24-hours (A and B) after a 30-hours fasting. Both sexes displayed a significant decrease of body weight after fasting (C) No significant differences were observed in locomotor activity (D)*

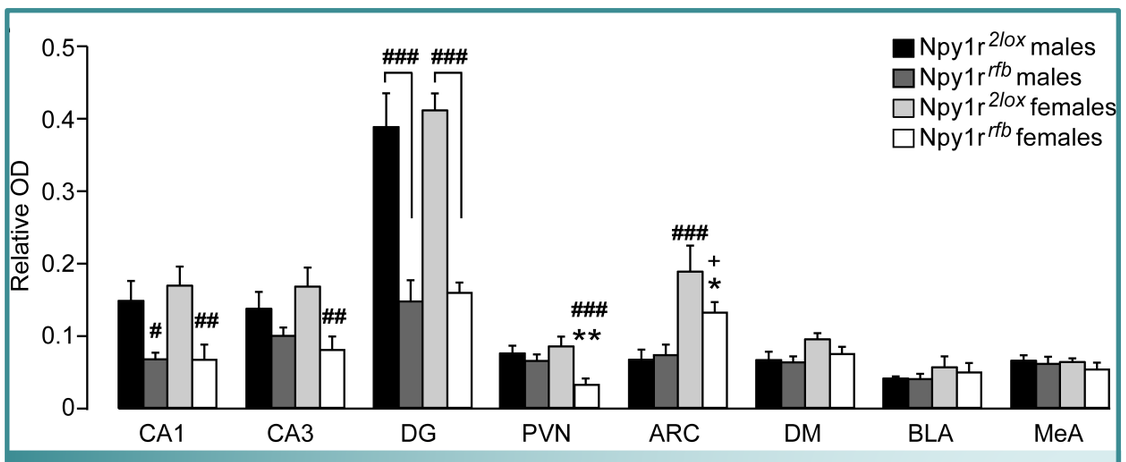


*Fig. 1 Sex-related differences in hypothalamic Npy1r gene inactivation pattern* by *in situ* hybridization

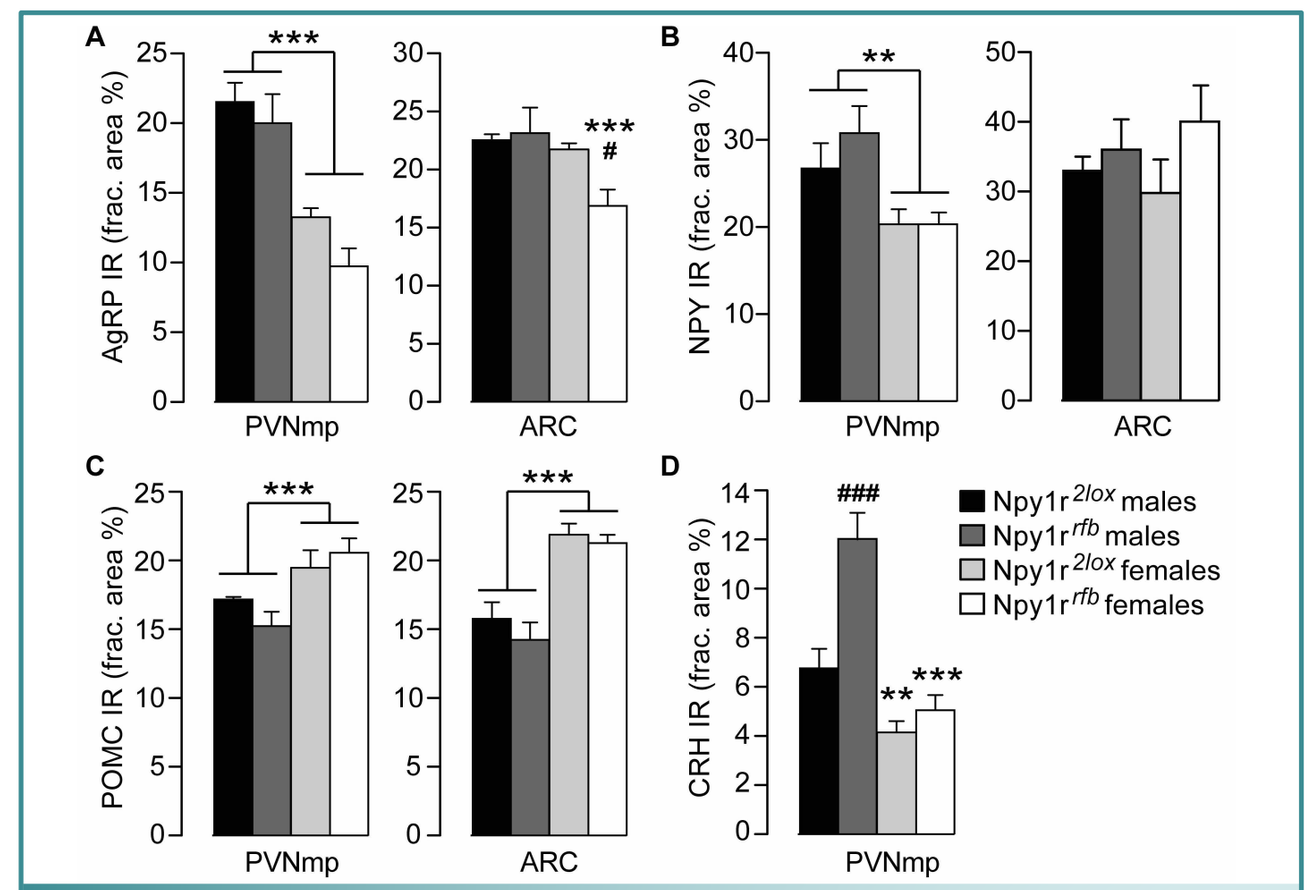


*Fig. 4 Female mice showed lower AgRP, NPY and CRH-IR signal in the PVNmp and higher POMC IR in PVNmp and ARC compared to males. Female, but not male, Npy1r$^{rfb}$ mice showed a significant decrease of AgRP-IR in the ARC. Male, but not female, Npy1r$^{rfb}$ mice displayed an increase in CRH-IR in the PVNmp compared with male Npy1r$^{2lox}$*

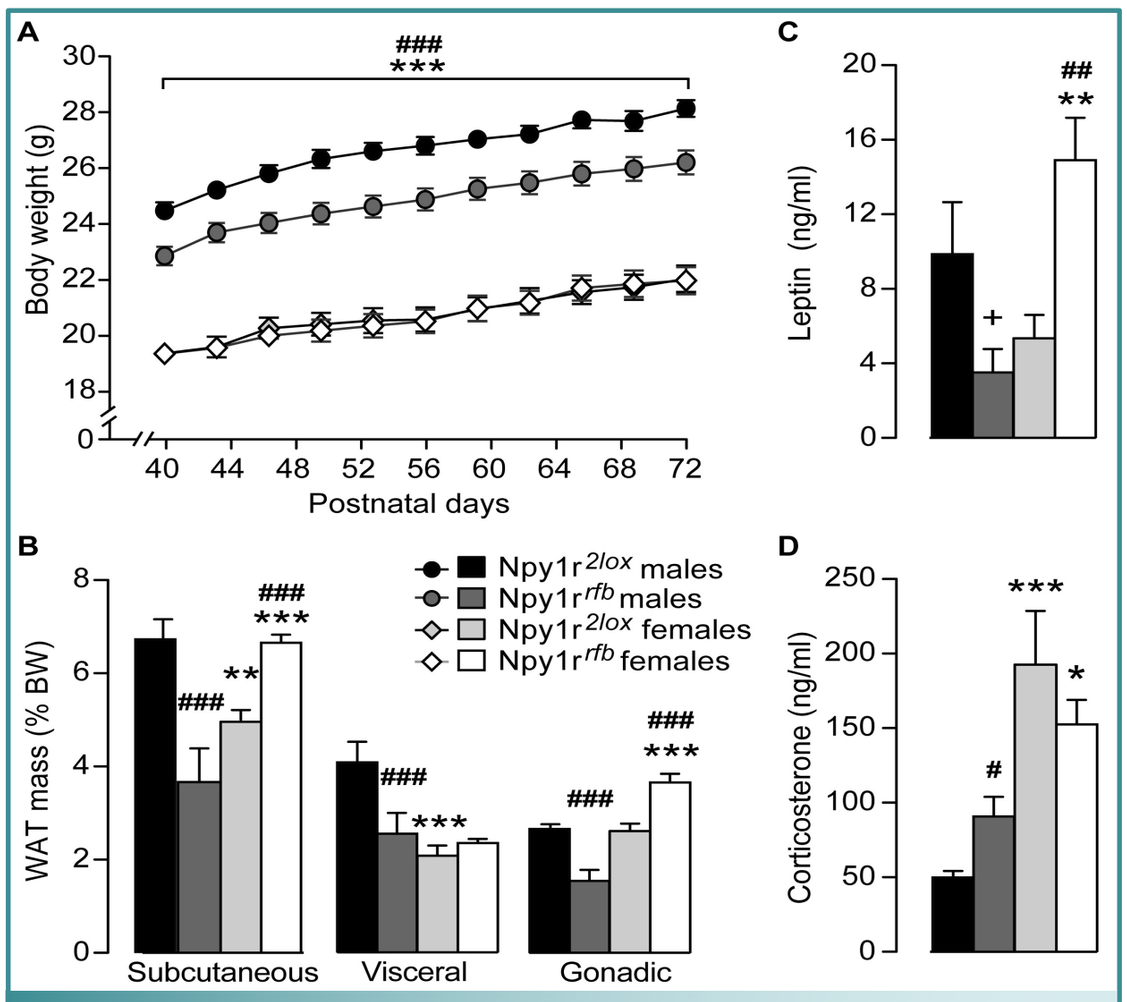


*Fig. 2 Conditional Npy1r gene inactivation diversely affected body weight growth, WAT weight and hormone serum levels in male and female mice*

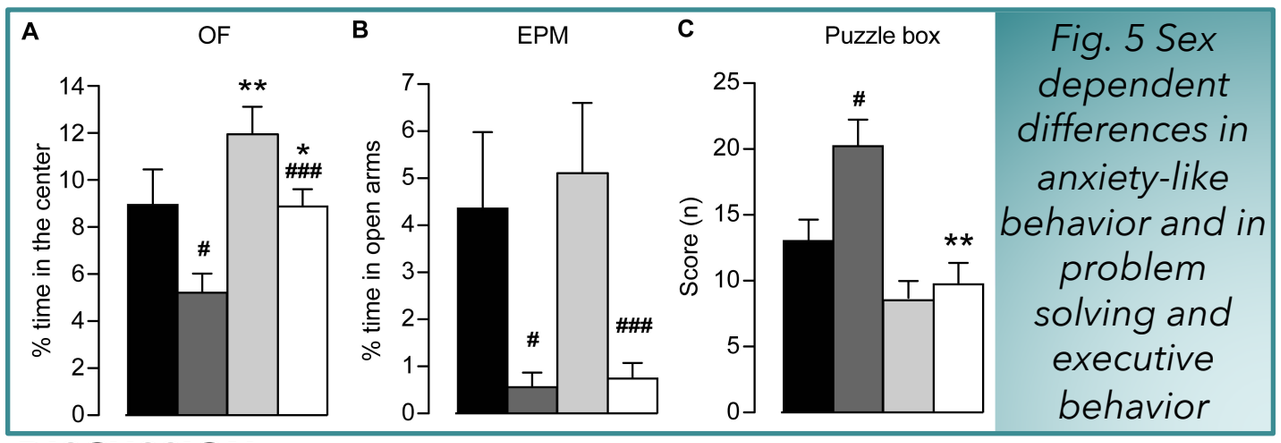


*Fig. 5 Sex dependent differences in anxiety-like behavior and in problem solving and executive behavior*

## DISCUSSION

Here we demonstrated that the conditional knockout of *Npy1r* gene differentially affected male and female phenotype, with males showing metabolic, hormonal and behavioral effects and females being only marginally impacted. Our results indicate that female mice are resilient to the effects of limbic *Npy1r* gene inactivation on hormone and metabolic functions, suggesting the presence of an estrogen-dependent relay necessary to ensure the maintenance of the homeostasis also in case of Y1R malfunctioning.

**Sources:** This work was supported by Cariplo Foundation, Compagnia di San Paolo, Torino and Fondazione CRT, Torino

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