


MICRO REPORT

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Neurexin-3 in the paraventricular nucleus of the hypothalamus regulates body weight and glucose homeostasis independently of food intake

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Abstract

Neurexin-3 (Nrxn3) has been genetically associated with obesity, but the underlying neural mechanisms remain poorly understood. This study aimed to investigate the role of Nrxn3 in the paraventricular nucleus of the hypothalamus (PVN) in regulating energy balance and glucose homeostasis. We found that Nrxn3 expression in the PVN was upregulated in response to metabolic stressors, including cold exposure and fasting. Using Cre-loxP technology, we selectively ablated Nrxn3 in CaMKII α -expressing neurons of the PVN in male mice. This genetic manipulation resulted in marked weight gain attributable to increased adiposity and impaired glucose tolerance, without affecting food intake. Our findings identify PVN CaMKII α -expressing neurons as a critical locus where Nrxn3 modulates energy balance by regulating adipogenesis and glucose metabolism, independently of appetite. These results reveal a novel neural mechanism potentially linking Nrxn3 dysfunction to obesity pathogenesis, suggesting that targeting PVN Nrxn3-dependent neural pathways may inform new therapeutic approaches for obesity prevention and treatment.

Keywords Neurexin-3, PVN, CaMKII α -expressing neuron, Adipogenesis, Obesity

Main text

The worldwide prevalence of obesity has escalated dramatically over recent decades, posing a major public health challenge. Genome-wide association studies have identified neurexin-3 (Nrxn3) as a novel obesity risk gene, with polymorphisms significantly associated with increased body mass index and waist circumference across diverse populations [1–4]. Neurexins are presynaptic cell-adhesion molecules that organize trans-synaptic signaling [5, 6]. However, there have been no direct reports on the role of neurexin-3 in regulating energy metabolism or obesity, and the neural circuits and mechanisms through which neurexin-3 influence energy

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balance remain largely undefined. The paraventricular nucleus of the hypothalamus (PVN) is a critical hub for central regulation of metabolism and body weight [7, 8], yet the role of PVN neurexins in energy homeostasis has not been explored. This study aims to bridge this knowledge gap by investigating the function of neurexin-3 in the PVN and its potential involvement in the neural control of energy balance and obesity development.

We first examined the expression of Nrnx3 in the PVN under different metabolic states. Western blotting revealed that PVN Nrnx3 levels (Anti-Nrnx3, #48004, Cell Signaling Technology) were significantly upregulated following exposure to either cold (4 °C for 3 h) (Fig. 1A) or fasting (36 h) (Fig. 1B), conditions known to activate PVN neurons and stimulate sympathetic outflow [7, 9]. To directly investigate the role of PVN Nrnx3 in regulating body weight, we generated PVN-specific Nrnx3 knock-out mice. This was achieved by injecting an adeno-associated virus expressing Cre recombinase under the control of the CaMKII α promoter (AAV9-CaMKII α -Cre-EGFP, PT-0137, BrainVTA Co., Ltd., China) into the PVN of Nrnx3-floxed mice (XM708997, Cas9-Nrnx3-CKO, Nanjing University-Nanjing Biomedical Research Institute) (Supplementary Fig. 1A, B and Supplementary Table). Immunofluorescent staining and Western blot analysis confirmed the virus's selective transduction of PVN neurons and the efficient knockdown of Nrnx3 protein levels in the PVN (Fig. 1C), a key brain region involved in regulating appetite and metabolism [7, 9–11]. Compared to AAV-CaMKII α -EGFP-injected male mice (hereafter, CTL group), AAV-CaMKII α -Cre-EGFP-injected male mice (hereafter, CKO group) exhibited progressive obesity, with a ~2-fold increase in body weight gain observed two months after AAV injection (Fig. 1D, E). Body composition analysis revealed a ~3-fold increase in fat mass, particularly in visceral fat (Fig. 1F, H). Although there was a trend towards increased weight in the spleen and kidney, these changes were not statistically significant (Supplementary Fig. 1C). Indirect calorimetry revealed no significant change in locomotor activity in Nrnx3 CKO mice (Fig. 1I). Importantly, the observed obesity was not associated with hyperphagia, as daily food intake was similar between Nrnx3 CKO and CTL mice (Fig. 1J). Furthermore, Nrnx3 CKO mice exhibited hyperglycemia and glucose intolerance (Fig. 1K), which are hallmarks of obesity-associated metabolic dysfunction. These findings suggest that the obesity in Nrnx3 CKO mice may be primarily due to increased fat formation, rather than hyperphagia or decreased locomotor activity.

In conclusion, our study identifies Nrnx3 in CaMKII α -expressing neurons of PVN as a novel regulator of energy balance and glucose homeostasis. Selective ablation of Nrnx3 in the PVN resulted in marked obesity and glucose intolerance, despite normal appetite and locomotor

activity. Furthermore, PVN Nrnx3 expression was upregulated by cold exposure and fasting, suggesting that Nrnx3 in these neurons may promote thermogenesis and/or lipid mobilization to maintain energy and glucose balance. These findings provide a neural basis for the genetic association between Nrnx3 and human obesity [1, 2].

The precise cellular mechanisms by which Nrnx3 modulates the activity of PVN CaMKII α -expressing neurons remain to be elucidated. As a synaptic organizer, Nrnx3 may regulate excitatory and/or inhibitory synaptic inputs to these neurons [5], influencing their responsiveness to metabolic cues. Alternatively, Nrnx3 may control the synaptic output of PVN CaMKII α -expressing neurons to downstream effector regions, such as the sympathetic outflow circuits in the brainstem and spinal cord [7, 9, 12]. Delineating the neural pathways and signaling cascades mediating the metabolic actions of PVN Nrnx3 warrants further investigation using cell type-specific circuit mapping and manipulation tools. Given the sex differences in obesity development and consequences [13], future studies should examine whether PVN Nrnx3 plays a similar role in regulating energy balance in females, since our current study focused on male mice. Furthermore, it will be important to explore the functions of Nrnx3 in other PVN neuronal subtypes and hypothalamic nuclei implicated in body weight control.

In conclusion, our findings reveal that Nrnx3 in PVN CaMKII α -expressing neurons plays a vital role in controlling body weight, adipogenesis, and glucose metabolism. This offers new understanding of how Nrnx3 dysfunction contributes to obesity, suggesting that targeting Nrnx3-dependent pathways in the PVN might lead to innovative treatments for obesity prevention and management.

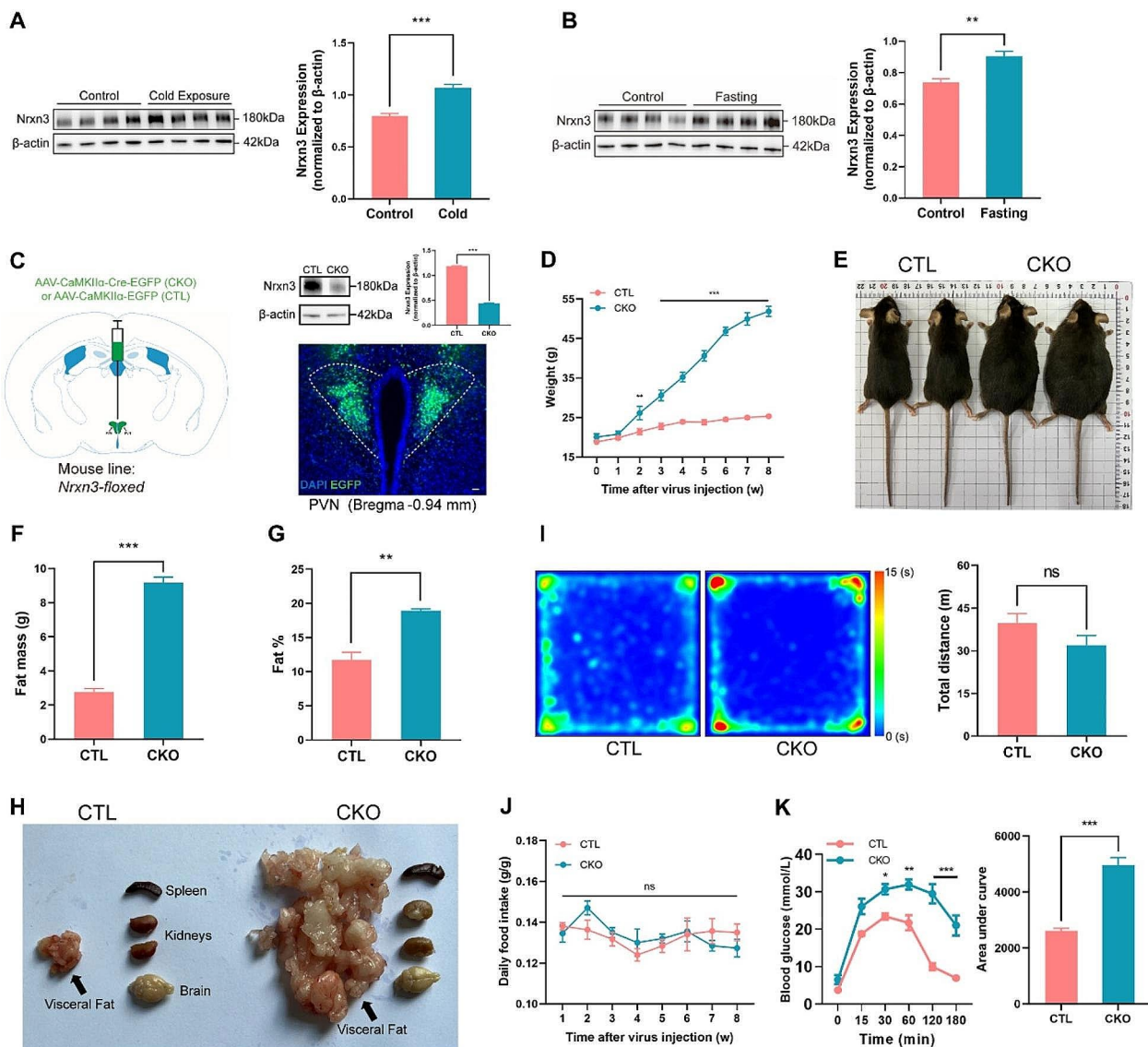


Fig. 1 Deletion of Neurexin-3 in PVN CaMKIIα-expressing neurons regulates body weight, adiposity, and glucose homeostasis. **(A–B)** Western blot analysis indicates upregulated Nrnx3 protein levels in the PVN following cold exposure (4 °C, 3 h, **A**) or after 36 h of fasting (**B**), compared to control conditions (*n* = 4 mice per group). **(C)** Left: Schematic diagram of viral injection. Top right: Western blot analysis of Nrnx3 protein levels in PVN. Bottom right: Representative immunofluorescence staining images demonstrating selective transduction of PVN neurons by AAV9-CaMKIIα-Cre-EGFP injection in Nrnx3-floxed mice (*n* = 4 per group). **(D)** Nrnx3 CKO mice show a progressive increase in body weight gain compared to controls (*n* = 5 per group). **(E)** Representative images of the mice two months post-virus injection. **(F–G)** Body composition analysis two months after virus injection reveals increased fat mass **(F)** and total fat percentage **(G)** in Nrnx3 CKO mice (*n* = 5 per group). **(H)** Representative images of visceral fat and other tissues in CTL and CKO mice. **(I)** ANY-MAZE software (Stoelting Co.) records the total distance traveled in a 50 cm (L) x 50 cm (W) x 34.5 cm (H) open field arena, which shows no significant change in Nrnx3 CKO mice during the open field test (*n* = 5–6 per group, total duration: 10 min). **(J)** Daily food intake does not differ significantly between Nrnx3 CKO and CTL mice (*n* = 5 per group). **(K)** Blood glucose levels were measured at 0, 15, 30, 60, 120, and 180 min after glucose injection. Mice were fasted for 12 h before the 0-minute measurement (*n* = 5 per group). Data are presented as mean ± SEM. ns, not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.001, as determined by Student’s *t*-test

Abbreviations

PVN	paraventricular nucleus of the hypothalamus
Nrnx3	Neurexin-3
CaMKIIα	Ca2+/calmodulin-dependent protein kinase IIα
CKO	conditional knockout
AAV	adeno-associated virus

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13041-024-01124-3>.

- Supplementary Material 1
- Supplementary Material 2

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Author contributions

M.M. and H.S. conceived the study. M.M., H.S., Z.Z. and S.G. performed experiments. M.M., H.S., T.X., C.S., S.M., M.L. and A.L. analyzed data. M.M., Z.Y. and W.X. supervised the study. M.M. wrote the manuscript with input from all co-authors. All authors read and approved the final manuscript.

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Data availability

All study data, including analyses, are in this article.

Declarations

Ethics approval and consent to participate

Animal experiments were approved by Southeast University's Animal Care and Use Committee.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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