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Supplementary Data

Orexin receptor type 2 agonism inhibits thermogenesis in brown adipose tissue by attenuating afferent innervation

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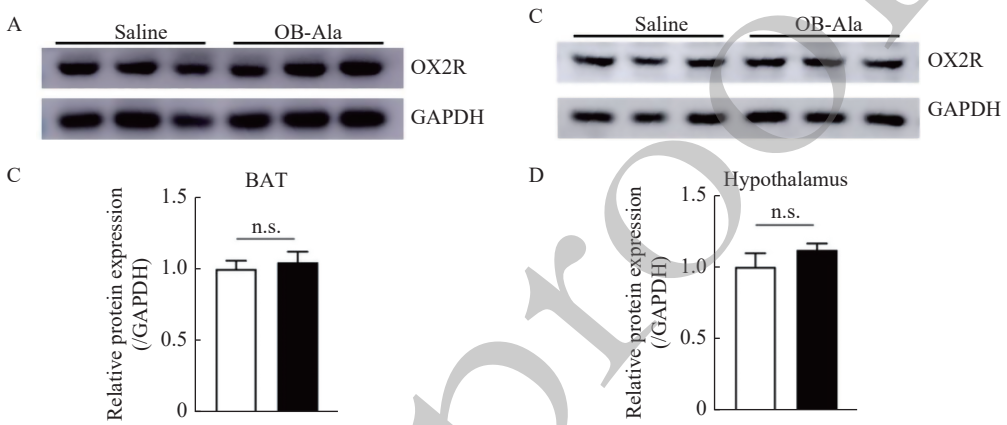

Fig. 1 **The effect of chronic infusion of OX2R agonist OB-Ala on OX2R protein expression in iBAT and hypothalamus.** Mice received daily i.p. injection of OX2R agonist OB-Ala (16 nmol/kg) or saline for 3 weeks. A: Protein levels of OX2R in iBAT analyzed by Western blotting. B: Quantitative analyses of (A). C: Protein levels of OX2R in hypothalamus analyzed by Western blotting. D: Quantitative analyses of (C). *n*=3 mice per group. OX2R: orexin receptor type 2; BAT: brown adipose tissue.

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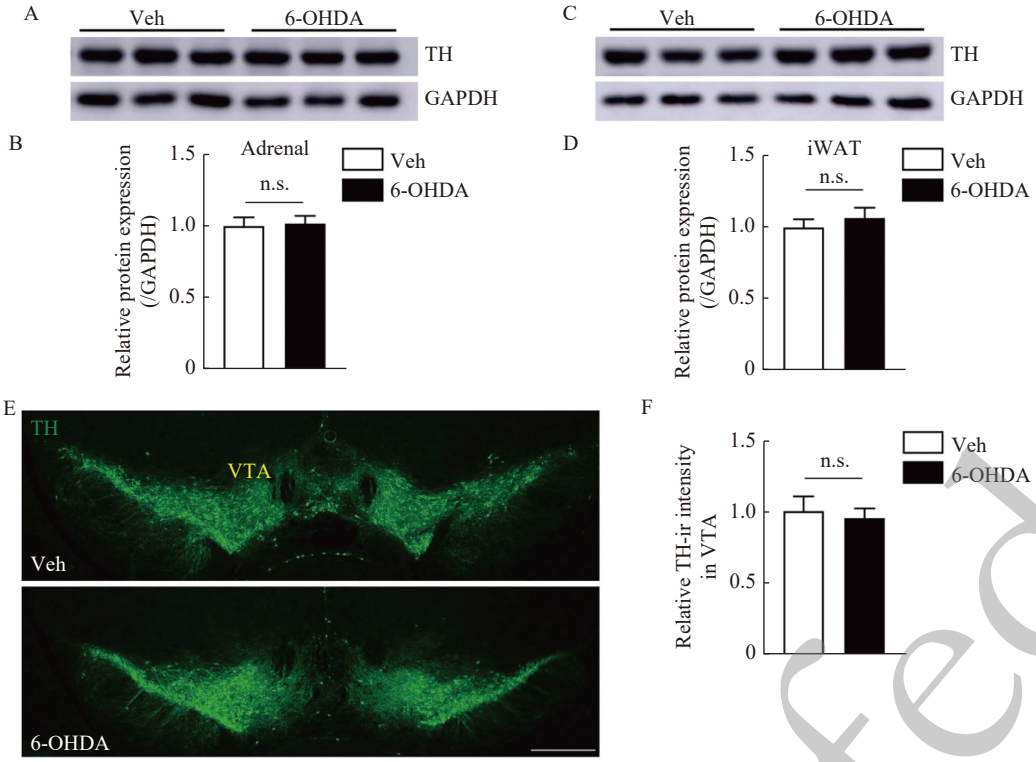

Fig. 2 **The effect of iBAT 6-OHDA microinjection on TH expression in adrenal, iWAT and VTA.** Mice received single microinjection of 6-OHDA (10 mg/mL) into iBAT and tissues were analyzed two weeks after injection. A: TH protein levels in adrenal analyzed by Western blotting. B: Quantification of (A). C: TH protein levels in iWAT analyzed by Western blotting. D: Quantification of (C). E: TH-ir positive fibers in VTA. F: Quantification of (E). *n*=3 per group. Scale bar = 500 μm in (E). 6-OHDA: 6-hydroxydopamine; iBAT: intrascapular brown adipose tissue; VTA: ventral tegmental area.

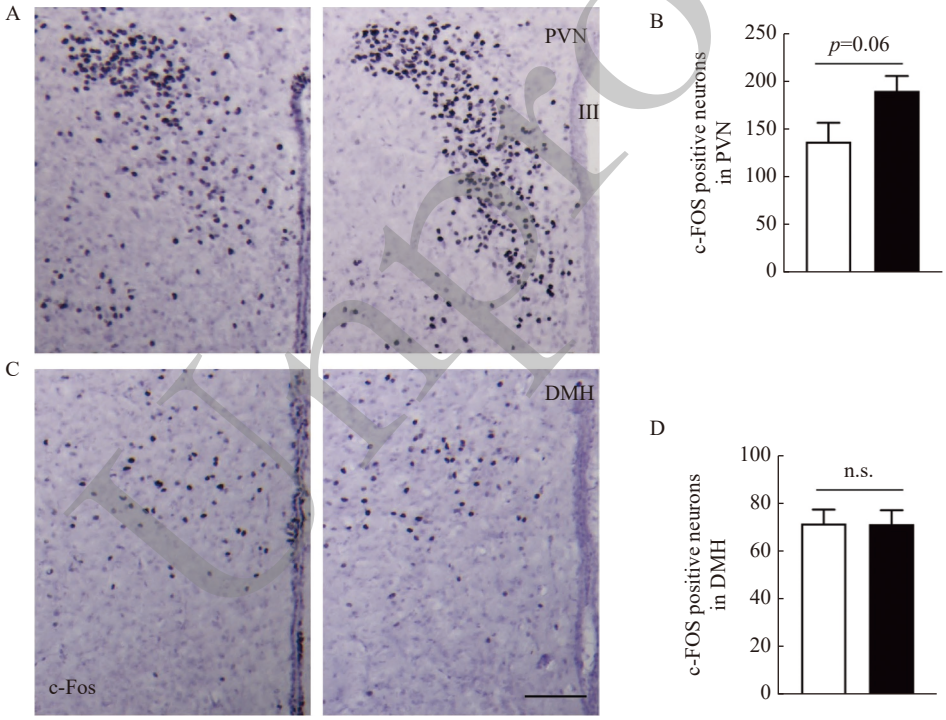

Fig. 3 **c-Fos level in PVN and DMH after i.c.v. injection of OX2R agonist OB-Ala.** Mice received i.c.v. injection of 0.3nmol OB-Ala or saline, and tissues were analyzed 90 minutes after injection. A and B: c-Fos levels in PVN by immunohistochemistry staining and quantification. C and D: c-Fos levels in DMH by immunohistochemistry and quantification. *n*=3 for saline; *n*=4 for OB-Ala. Scale bar = 50 μm in (C). i.c.v: intra-cerebroventricular; III: third ventricle; PVN: paraventricular nucleus; DMH: dorsal medial hypothalamus.