



**Figure 3 - Supplement 1: cFOS-OXT-AVP staining and CNO validation**

(A) Experimental design: WT mice were isolated between P28 and P35 or kept in group. After isolation, mice were perfused and brain slices were immunostained with cFOS, OXT and AVP antibody. (B) PVN cFOS<sup>+</sup> cells (absolute value, Unpaired sample t-test,  $t_{(22)}=2.723$ ,  $p=0.0124$ ). (C) PVN OXT<sup>+</sup> cells (absolute value, Mann-Whitney sample test,  $U=35.5$ ,  $p=0.0344$ ). (D) Representative confocal images of PVN stained with cFOS, OXT and AVP. (E) PVN AVP<sup>+</sup> cells (normalized on DAPI, Mann-Whitney test,  $U=45$ ,  $p=0.1277$ ). (F) PVN AVP<sup>+</sup> cells (absolute value, Unpaired sample t-test,  $t_{(22)}=1.569$ ,  $p=0.131$ ). (G) PVN AVP<sup>+</sup>/cFOS<sup>+</sup> cells (Unpaired sample t-test,  $t_{(22)}=1.811$ ,  $p=0.0838$ ). (H) PVN OXT<sup>+</sup>/AVP<sup>+</sup> cells (Mann-Whitney test,  $U=61$ ,  $p=0.7859$ ). (I) Representative confocal images of PVN for OXT-hM4Di expression. (J) Left: experimental paradigm, OXT neurons from OTX-hM4Di mice were subjected at multiple depolarizing current steps in absence or presence of CNO 10 $\mu$ M in the recording chamber. Right: example traces from 30pA depolarizing current injection. (K) Number of action potentials (APs) across increasing depolarizing current steps (Two-way RM ANOVA followed by uncorrected Fisher's LSD post-hoc analysis, CNO main effect  $F(1, 8)=90.15$ ,  $p<0.001$ , Current step main effect  $F(5, 40)=11.99$ ,  $p<0.001$ , CNO x Current step  $F(5, 40)=7.556$ ,  $p<0.001$ ). (L) Resting membrane potential of recorded cells (Unpaired samples t-test,  $t(8)=1.502$ ,  $p=0.1715$ ,  $n=5$  from 1 mouse). Data are represented as mean $\pm$ SEM.