Supplementary figures for

Reduced Excitatory Activity in the Developing mPFC Mediates a PV_H-to-PV_L Transition and Impaired Social Cognition in Autism Spectrum Disorders

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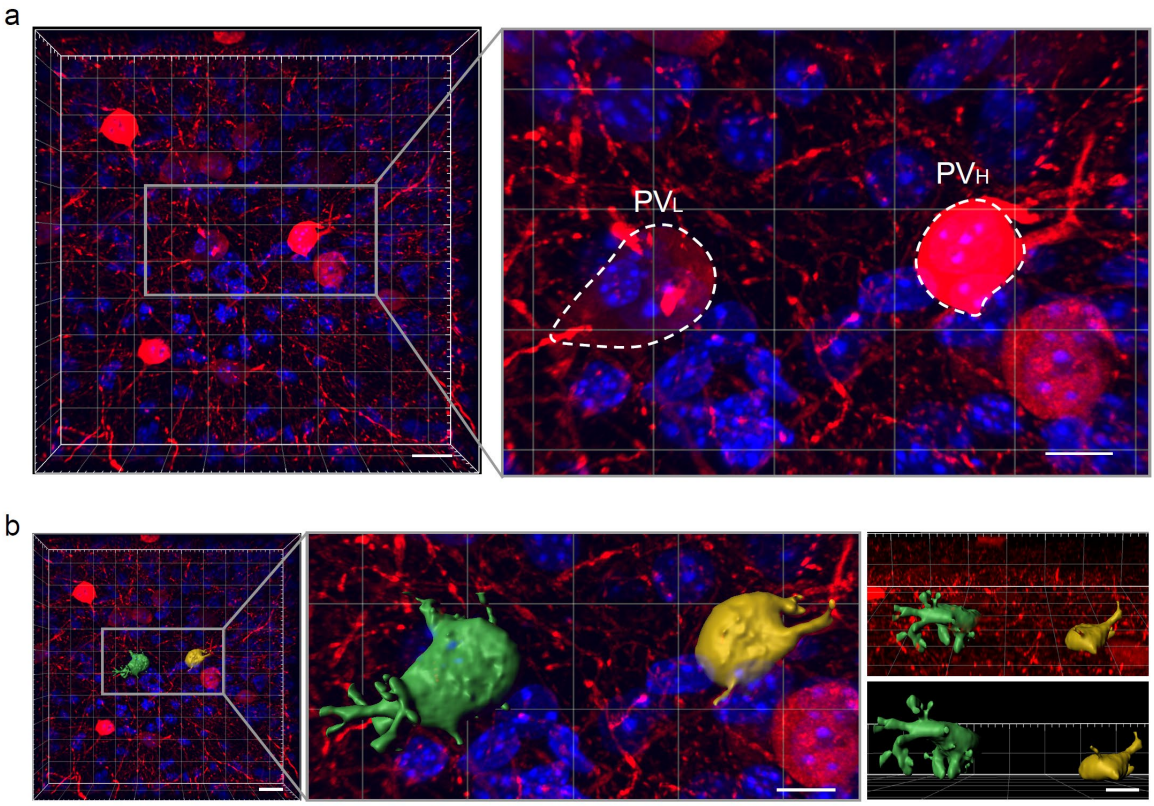
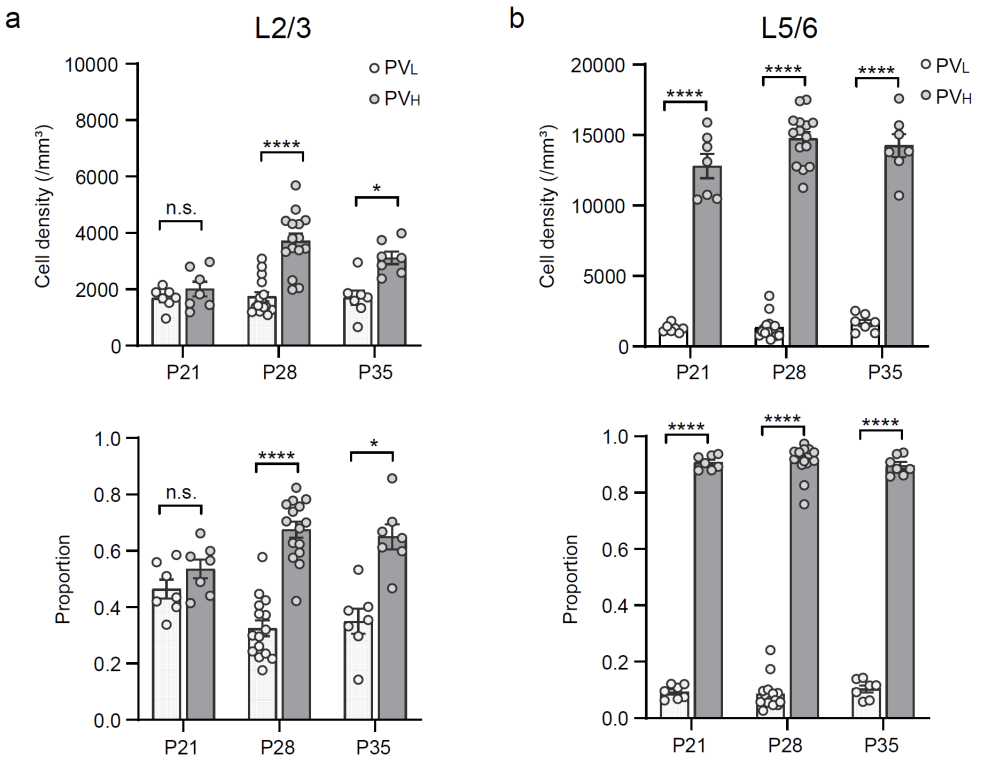
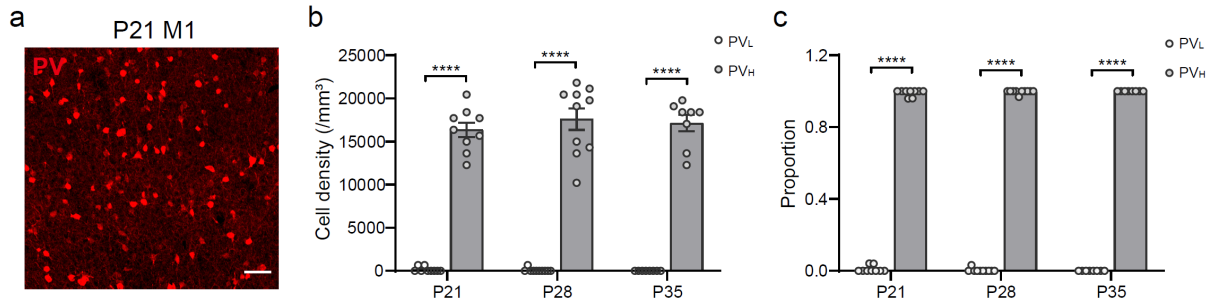


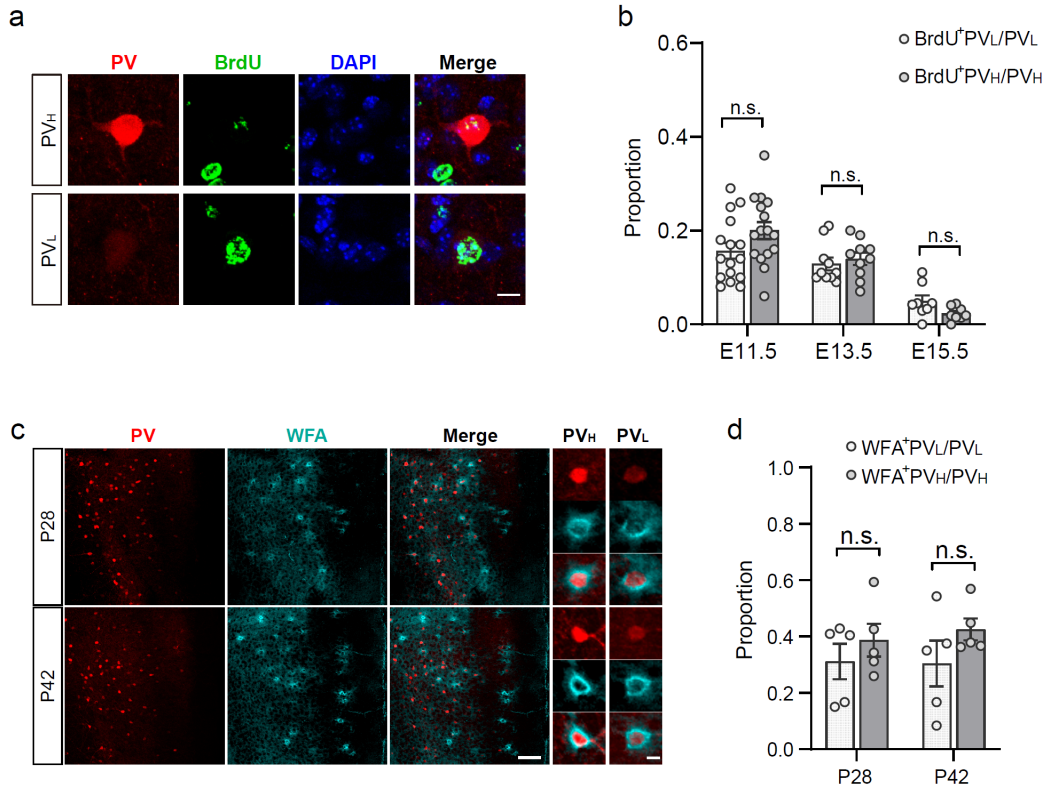
Supplementary Fig. 1. 3D-reconstruction of PV_H and PV_L neurons in the mPFC. (**a**) A representative z-stack confocal image showing PV_H (right) and PV_L (left) neurons in an mPFC brain section. Scale bars: 20 µm (left panel) and 10 µm (right panel). (**b**) 3D-reconstruction of the intact soma of the PV_L (green) and PV_H (yellow) neurons shown in (**a**). Scale bars: 20 µm (left panel), 10 µm (middle panel), and 10 µm (right panel).



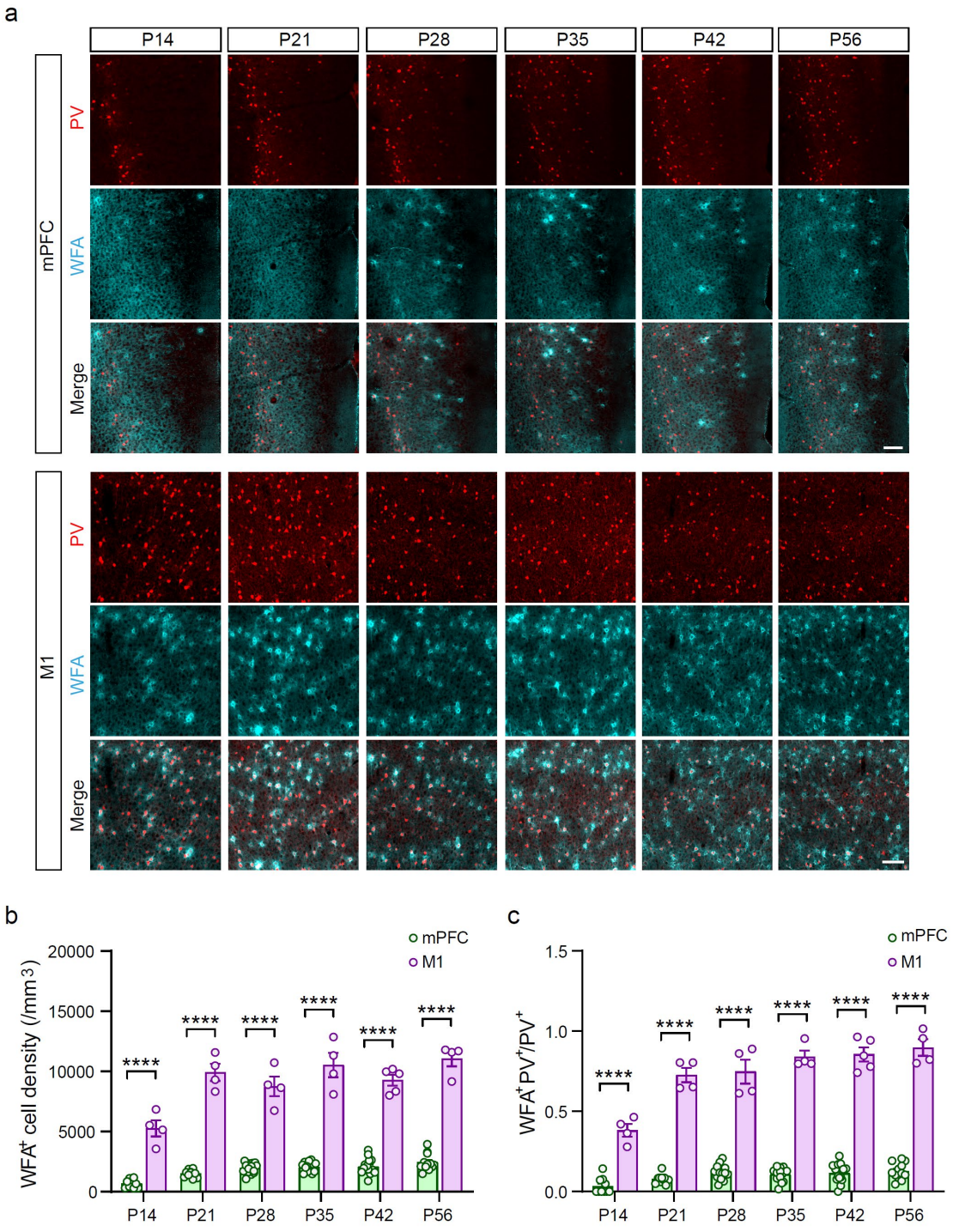
Supplementary Fig. 2. The distribution of PV_H and PV_L neurons in different layers of mPFC during postnatal development. (**a**) Top: The density of PV_H and PV_L neurons in L2/3 of the mPFC at P21, P28 and P35 (P21: n=7 mice, paired t test, t=1.233, df=6, P=0.2636; P28: n=15 mice, paired t test, t=5.679, df=14, ****P<0.0001; P35: n=7 mice, paired t test, t=3.295, df=6, *P=0.0165). Bottom: The proportion of PV_H and PV_L neurons in L2/3 of the mPFC at P21, P28 and P35 (P21: n=7 mice, paired t test, t=1.051, df=6, P=0.3339; P28: n=15 mice, paired t test, t=6.248, df=14, ****P<0.0001; P35: n=7 mice, paired t test, t=3.362, df=6, *P=0.0152). (**b**) Top: The density of PV_H and PV_L neurons in L5/6 of the mPFC at P21, P28 and P35 (P21: n=7 mice, paired t test, t=13.10, df=6, ****P<0.0001; P28: n=15 mice, paired t test, t=21.25, df=14, ****P<0.0001; P35: n=7 mice, paired t test, t=15.90, df=6, ****P<0.0001). Bottom: The proportion of PV_H and PV_L neurons in L5/6 of the mPFC at P21, P28 and P35 (P21: n=7 mice, paired t test, t=43.58, df=6, ****P<0.0001; P28: n=15 mice, paired t test, t=29.11, df=14, ****P<0.0001; P35: n=7 mice, paired t test, t=30.94, df=6, ****P<0.0001). Data are represented as mean ± SEM.



Supplementary Fig. 3. PV_H and PV_L neurons in M1 during postnatal development. (**a**) Representative image showing the immunoreactivity of PV in M1 at P21. Scale bar: 50 μm. (**b**) The density of PV_H and PV_L neurons in M1 at P21, P28 and P35 (P21: n=9 mice, paired t test, t=19.83, df=8, ****P<0.0001; P28: n=10 mice, paired t test, t=14.17, df=9, ****P<0.0001; P35: n=8 mice, paired t test, t=17.86, df=7, ****P<0.0001). (**c**) The proportion of PV_H and PV_L neurons in M1 at P21, P28 and P35 (P21: n=9 mice, paired t test, t=81.77, df=8, ****P<0.0001; P28: n=10 mice, paired t test, t=154.0, df=9, ****P<0.0001; P35: n=8 mice, paired t test, t=399.0, df=7, ****P<0.0001). Data are represented as mean ± SEM.

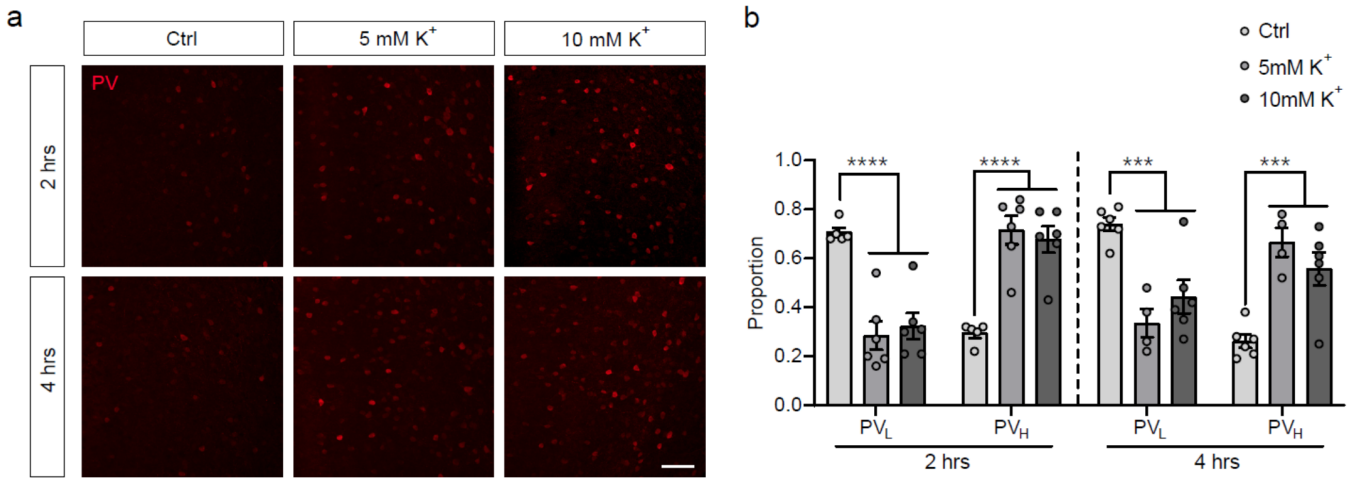


Supplementary Fig. 4. The expression of PV is independent of the age or the maturity of PV$^+$ neurons. (**a**) Representative images showing the colocalization of BrdU and PV$_H$ or PV$_L$ neurons in the mPFC at P21. Scale bars: 10 μm. (**b**) The proportion of the colocalization of PV$_L$ or PV$_H$ neurons with BrdU, which was injected at E11.5, E13.5 and E15.5 (E11.5: n=16 mice, paired t test, t=1.607, df=15, P=0.1288; E13.5: n=9 mice, paired t test, t=0.284, df=8, P=0.7834; E15.5: n=8 mice, paired t test, t=1.452, df=7, P=0.1898). (**c**) Representative images showing the colocalization of WFA and PV$_H$ or PV$_L$ neurons in the mPFC at P28 and P42. Scale bars: 100 μm and 10 μm (in Zoom-in images). (**d**) The proportion of the colocalization of WFA$^+$ with PV$_L$ or PV$_H$ neurons at P28 and P42 (P28: n=5 mice, paired t test, t=1.018, df=4, P=0.3661; P42: n=5 mice, paired t test, t=1.299, df=4, P=0.2639). Data are represented as mean ± SEM.

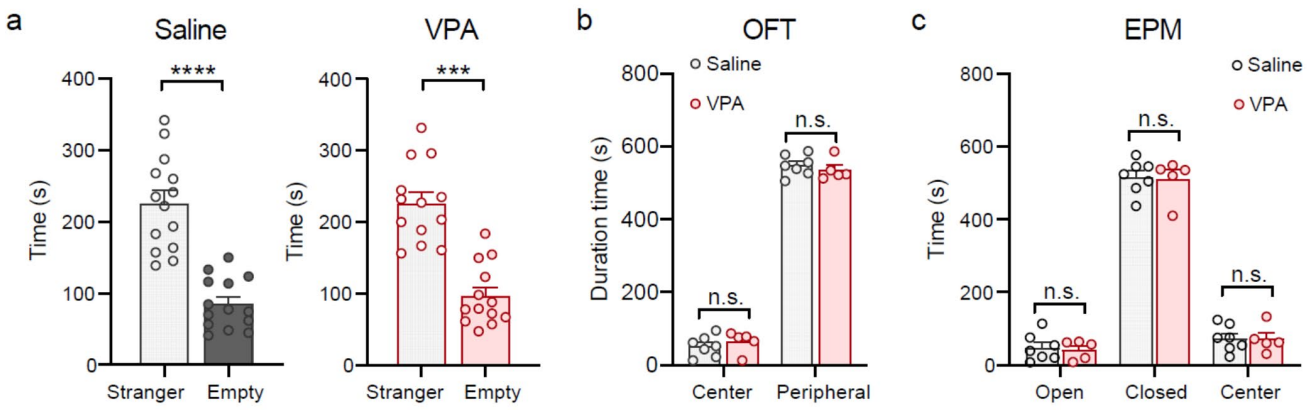


Supplementary Fig. 5. The maturation of M1 is earlier than that of the mPFC. (**a**) Representative images showing the immunoreactivity of PV and WFA in the mPFC and M1 from P14 to P56. Scale bars: 100 μm. (**b**) The density of WFA^+ neurons in the mPFC and M1 from P14 to P56 (P14: mPFC n=10 mice, M1 n=4 mice, t=10.46, df=12, ****P<0.0001; P21: mPFC n=10 mice, M1 n=4 mice, t=18.92, df=12,

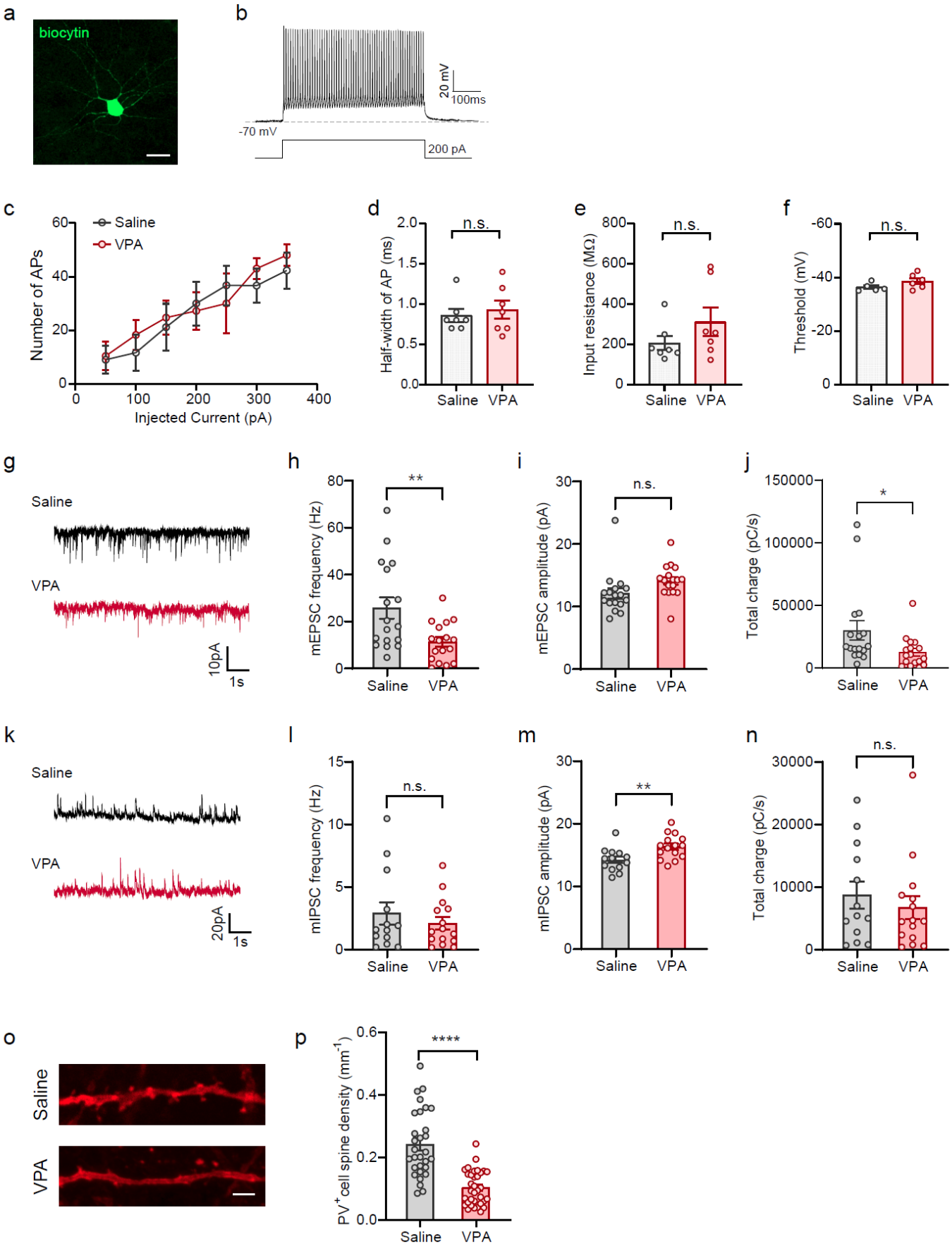
****P<0.0001; P28: mPFC n=15 mice, M1 n=4 mice, t=15.35, df=17, ****P<0.0001; P35: mPFC n=13 mice, M1 n=4 mice, t=15.05, df=15, ****P<0.0001; P42: mPFC n=14 mice, M1 n=5 mice, t=17.97, df=17, ****P<0.0001; P56: mPFC n=12 mice, M1 n=4 mice, t=17.56, df=14, ****P<0.0001). (**c**) The proportion of WFA^+PV^+ neurons in PV^+ neurons in the mPFC and M1 from P14 to P56 (P14: mPFC n=10 mice, M1 n=4 mice, t=10.04, df=12, ****P<0.0001; P21: mPFC n=10 mice, M1 n=4 mice, t=21.32, df=12, ****P<0.0001; P28: mPFC n=15 mice, M1 n=4 mice, t=15.00, df=17, ****P<0.0001; P35: mPFC n=13 mice, M1 n=4 mice, t=24.87, df=15, ****P<0.0001; P42: mPFC n=14 mice, M1 n=5 mice, t=20.08, df=17, ****P<0.0001; P56: mPFC n=12 mice, M1 n=4 mice, t=20.19, df=14, ****P<0.0001). Data are represented as mean ± SEM.



Supplementary Fig. 6. Enhancing neuronal activity increased PV expression. (**a**) Immunostaining of PV 2 and 4 hrs after incubation of acute brain slices in ACSF with normal K^+ concentration (Ctrl) or high K^+ concentration (5 mM or 10 mM). Scale bars: 100 μm. (**b**) High K^+ concentration decreased the proportion of PV_L and increased that of PV_H neurons (2 hrs-PV_L: Ctrl vs. 5mM K^+, Ctrl n=5 mice, 5mM K^+ n=6 mice, q=5.726, df=54, ****P<0.0001; Ctrl vs. 10mM K^+, Ctrl n=5 mice, 10mM K^+ n=6 mice, q=5.204, df=54, ****P<0.0001; 2 hrs-PV_H: Ctrl vs. 5mM K^+, Ctrl n=5 mice, 5mM K^+ n=6 mice, q=5.726, df=54, ****P<0.0001; Ctrl vs. 10mM K^+, Ctrl n=5 mice, 10mM K^+ n=6 mice, q=5.204, df=54, ****P<0.0001; 4 hrs-PV_L: Ctrl vs. 5mM K^+, Ctrl n=6 mice, 5mM K^+ n=4 mice, q=5.146, df=54, ****P<0.0001; Ctrl vs. 10mM K^+, Ctrl n=6 mice, 10mM K^+ n=6 mice, q=4.208, df=54, ***P=0.0002; 4 hrs-PV_H: Ctrl vs. 5mM K^+, Ctrl n=6 mice, 5mM K^+ n=4 mice, q=5.146, df=54, ****P<0.0001; Ctrl vs. 10mM K^+, Ctrl n=6 mice, 10mM K^+ n=6 mice, q=4.208, df=54, ***P=0.0002, 2way ANOVA Multiple comparisons). Data are represented as mean ± SEM.



Supplementary Fig. 7. VPA mice showed normal social ability and anxiety. (**a**) Both control (Saline) and VPA mice spent significantly more time in contacting with a mouse than with an empty cup in the social ability test (Saline: n=14 mice, paired t test, t=6.031, df=13, ****P<0.0001; VPA: n=13 mice, paired t test, t=5.378, df=12, ***P=0.0002). (**b**) The time that Saline or VPA mice spent in the center zone or the peripheral zone in the OF test (Saline n=7 mice, VPA n=5 mice; Center: t=0.7362, df=10, P=0.4785; Peripheral: t=0.7362, df=10, P=0.4785). (**c**) The time that Saline or VPA mice spent in the open arms (Open), the closed arms (Closed), and the center zone (Center) in EPM test (Saline n=7 mice, VPA n=5 mice; Open: t=0.0709, df=10, P=0.9449; Closed: t=0.2239, df=10, P=0.8274; Center: t=0.2790, df=10, P=0.7860). Data are represented as mean ± SEM.


a
biocytin
b
20 mV
100ms
-70 mV
200 pA
c
Saline
VPA
Number of APs
Injected Current (pA)
d
Half-width of AP (ms)
n.s.
Saline
VPA
e
Input resistance (MΩ)
n.s.
f
Threshold (mV)
n.s.
g
Saline
VPA
10pA
1s
h
mEPSC frequency (Hz)
**
i
mEPSC amplitude (pA)
n.s.
j
Total charge (pC/s)
*
k
Saline
VPA
20pA
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l
mIPSC frequency (Hz)
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m
mIPSC amplitude (pA)
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n
Total charge (pC/s)
n.s.
o
Saline
VPA
p
PV+cell spine density (mm-1)
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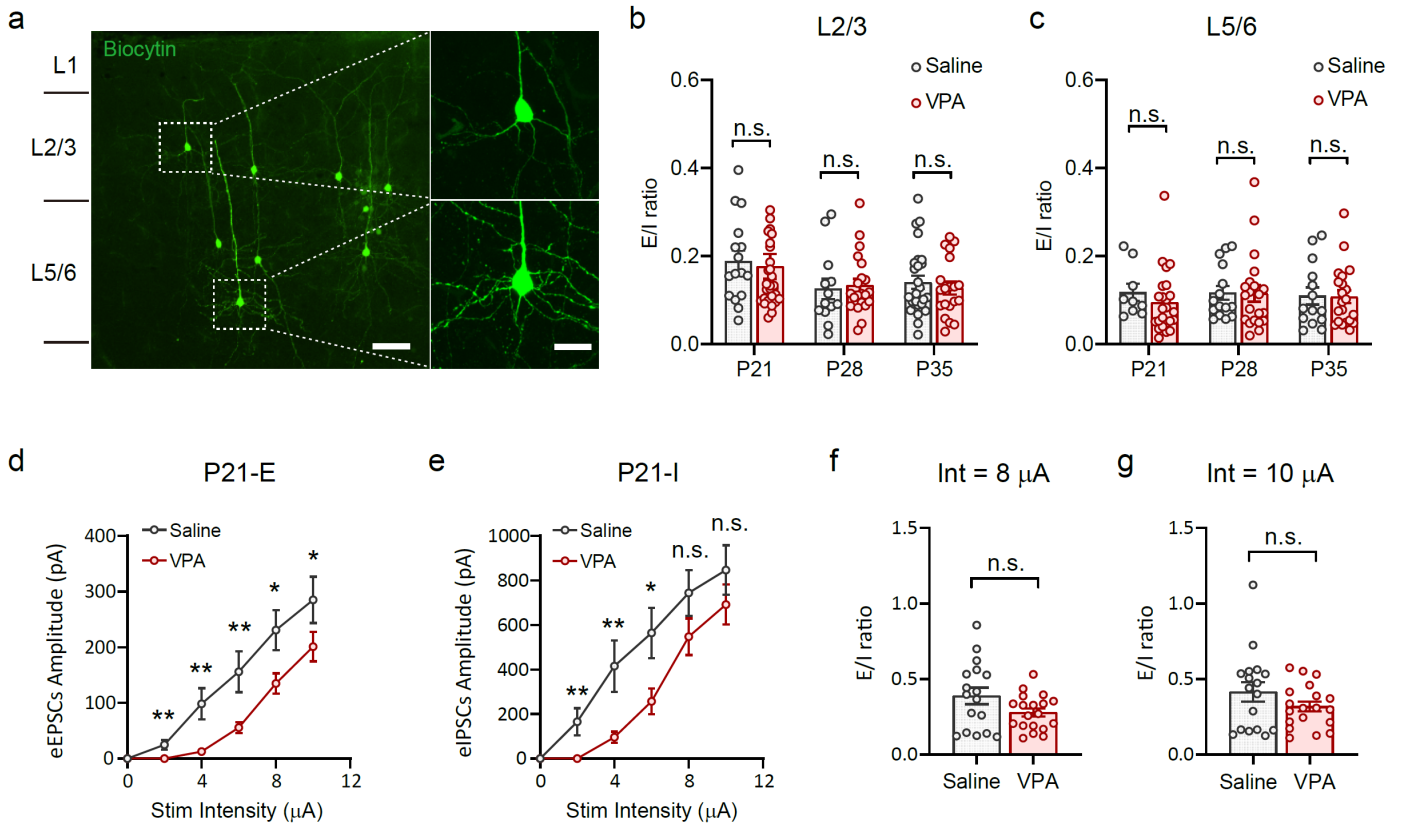
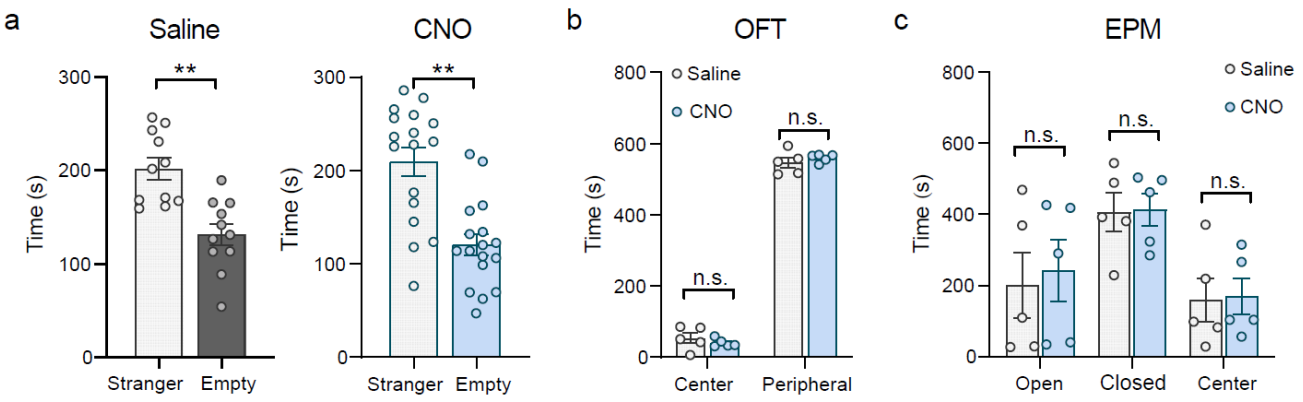
Supplementary Fig. 8. PV^+ neurons in the mPFC of VPA mice showed unaltered intrinsic firing properties, but decreased excitatory synaptic inputs. (**a**) Representative images showing biocytin-backfilled PV^+ neurons in the mPFC. Scale bars: 20 μm. (**b**) A representative trace showing typical firing of PV^+ neurons upon current injection. (**c**) I-V curve of PV^+ neurons in the mPFC of Saline and VPA mice (Saline n=6 cells, VPA n=7 cells, 50pA: t=0.1926, df=11, P=0.8508; 100pA: t=0.7705, df=11, P=0.4572; 150pA: t=0.3305, df=10, P=0.7479; 200pA: t=0.2580, df=8, P=0.8029; 250pA: t=0.5265, df=5, P=0.6210; 300pA: t=0.7224, df=3, P=0.5222; 350pA: t=0.6201, df=3, P=0.5791). (**d**) The AP half-width of PV^+ neurons in the mPFC of Saline and VPA mice (Saline: n=7 cells, VPA: n=7 cells, t=0.5270, df=12, P=0.6078). (**e**) The input resistance of PV^+ neurons in the mPFC of Saline and VPA mice (Saline: n=7 cells, VPA: n=7 cells, t=1.326, df=12, P=0.2096). (**f**) The AP threshold of PV^+ neurons in the mPFC of Saline and VPA mice (Saline: n=5 cells, VPA: n=6 cells, t=1.645, df=9, P=0.1344). (**g**) Representative mEPSCs traces recorded in mPFC PV^+ neurons from control (Saline) and VPA mice. (**h**) PV^+ neurons in the mPFC of VPA mice showed decreased mEPSC frequency (Saline n=17 cells, VPA n=17 cells, t=2.92, df=32, **p=0.0063). (**i**) PV^+ neurons in the mPFC of VPA mice showed unaltered mEPSC amplitude (Saline n=17 cells, VPA n=17 cells, t=1.97, df=32, p= 0.0576). (**j**) PV^+ neurons in the mPFC of VPA mice showed decreased mEPSC total charge (Saline n=17 cells, VPA n=17 cells, t=2.14, df=32, *p=0.0400). (**k**) Representative mIPSCs traces recorded in mPFC PV^+ neurons from control (Saline) and VPA mice. (**l**) PV^+ neurons in the mPFC of VPA mice showed unaltered mIPSC frequency (Saline n=13 cells, VPA n=15 cells, t=0.81, df=26, p= 0.4274). (**m**) PV^+ neurons in the mPFC of VPA mice showed increased mIPSC amplitude (Saline n=13 cells, VPA n=15 cells, t=2.95, df=26, p= 0.0067). (**n**) PV^+ neurons in the mPFC of VPA mice showed unaltered mIPSC total charge (Saline n=13 cells, VPA n=15 cells, t=0.72, df=26, p=0.4765). (**o**) Representative images of dendritic segments of PV^+ neurons in the mPFC of control and VPA mice. Scale bar: 2.5 μm. (**p**) PV^+ neurons in the mPFC of VPA mice showed decreased spine density (Saline N=4 mice/ n=30 images VPA N=4 mice/ n=34 images, t=6.72, df=62, ****p＜0.0001). Data are represented as mean ± SEM.

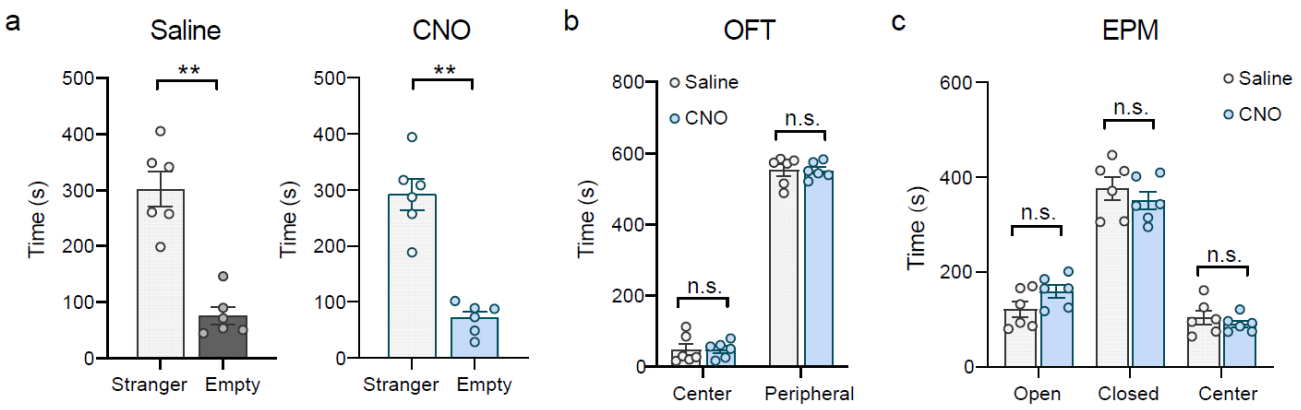


Supplementary Fig. 9. VPA mice showed unchanged E/I ratio in mPFC pyramidal neurons. (**a**) Representative images showing biocytin-backfilled pyramidal neurons in L2/3 and L5/6 after recording. Scale bars: 100 μm and 30 μm (in Zoom-in images). (**b**) The E/I ratio recorded in L2/3 pyramidal neurons in the mPFC of Saline and VPA mice at P21, P28, and P35 (P21: Saline n=16 cells, VPA n=30 cells, t=0.2836, df=44, P=0.7780; P28: Saline n=13 cells, VPA n=21 cells, t=0.3190, df=32, P=0.7581; P35: Saline n=27 cells, VPA n=20 cells, t=0.6031, df=45, P=0.5495). (**c**) The E/I ratio recorded in L5/6 pyramidal neurons in the mPFC of Saline and VPA mice at P21, P28, and P35 (P21: Saline n=9 cells, VPA n=24 cells, t=0.8386, df=31, P=0.4081; P28: Saline n=15 cells, VPA n=21 cells, t=0.0564, df=34, P=0.9554; P35: Saline n=14 cells, VPA n=21 cells, t=0.0817, df=33, P=0.9354). (**d**) Evoked EPSCs amplitude at different stim intensity recorded in L2/3 pyramidal neurons in the mPFC of Saline and VPA mice (2μA：Saline n=21 cells, VPA n=22 cells, t=2.829, df=41, **P=0.0072; 4μA：Saline n=20 cells, VPA n=22 cells, t=3.169, df=40, **P=0.0029; 6μA：Saline n=20 cells, VPA n=22 cells, t=2.752, df=40, **P=0.0089; 8μA：Saline n=20 cells, VPA n=21 cells, t=2.398, df=39, *P=0.0214; 10μA：Saline n=17 cells, VPA n=21 cells, t=2.475,

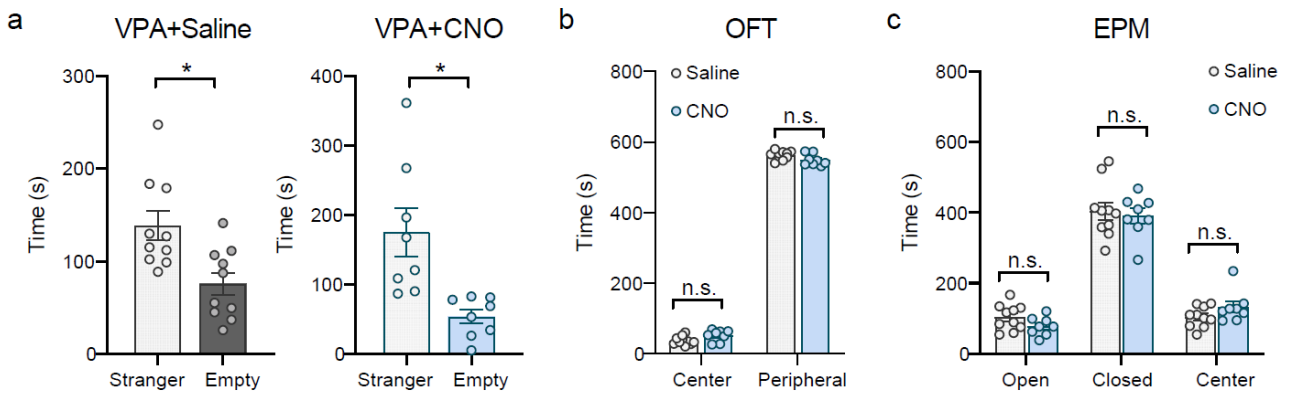
df=36, *P=0.0181;). (**e**) Evoked IPSCs amplitude at different stim intensity recorded in L2/3 pyramidal neurons in the mPFC of Saline and VPA mice (2μA：Saline n=18 cells, VPA n=21 cells, t=2.958, df=37, **P=0.0054; 4μA：Saline n=18 cells, VPA n=21 cells, t=2.901, df=37, **P=0.0062; 6μA：Saline n=18 cells, VPA n=21 cells, t=2.526, df=37, *P=0.016; 8μA：Saline n=18 cells, VPA n=20 cells, t=1.505, df=36, P=0.1411; 10μA：Saline n=18 cells, VPA n=20 cells, t=1.094, df=36, P=0.2811;). (**f**) The E/I ratio recorded in L2/3 pyramidal neurons in the mPFC of Saline and VPA mice at the stim intensity of 8 μA (Saline: n=17 cells; VPA: n=19 cells; t=1.879, df=34, P=0.0688). (**g**) The E/I ratio recorded in L2/3 pyramidal neurons in the mPFC of Saline and VPA mice at the stim intensity of 10 μA (Saline: n=17 cells; VPA: n=19 cells; t=1.376, df=34, P=0.1779). Data are represented as mean ± SEM.



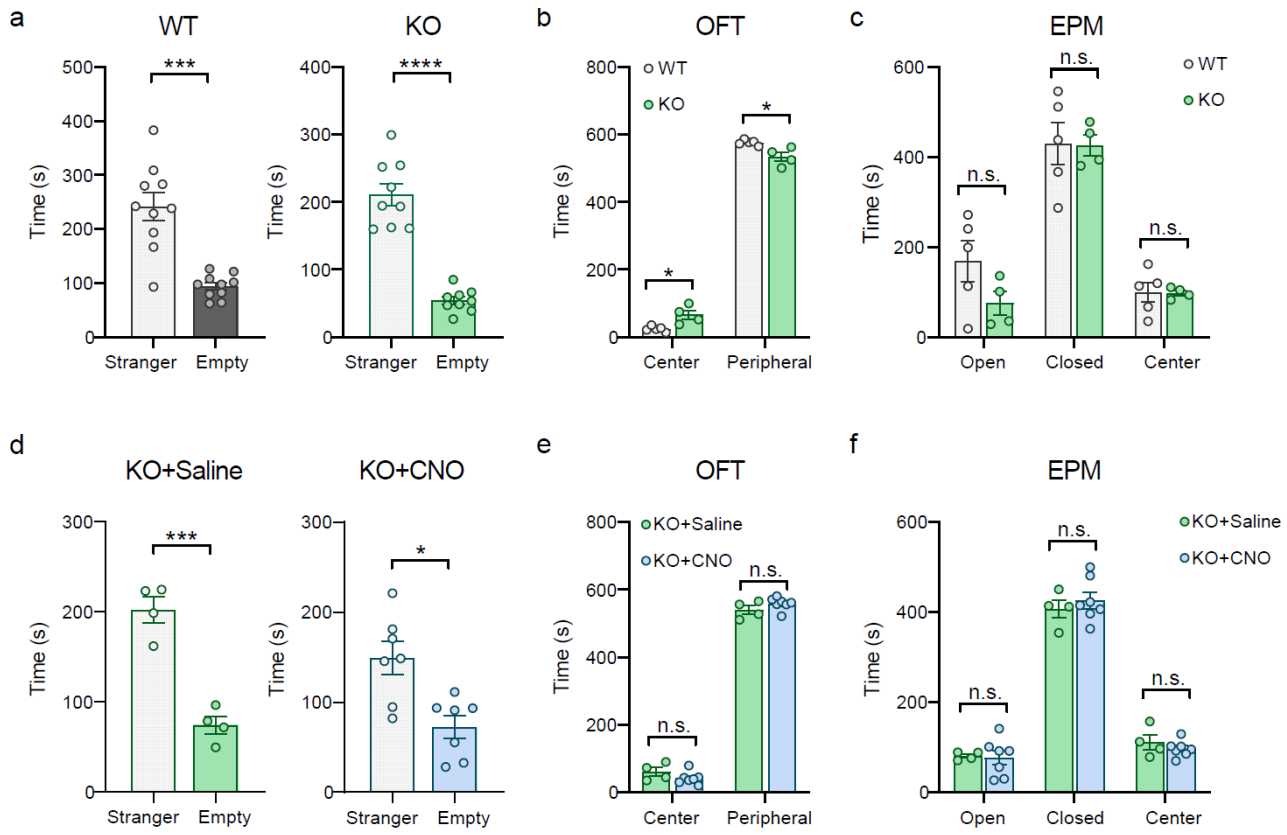
Supplementary Fig. 10. Selectively suppressing excitatory activity in the developing mPFC did not change the social ability or anxiety level of wild-type mice. (**a**) Both Saline- and CNO-treated mice spent significantly more time in contacting with a mouse than with an empty cup in the social ability test (Saline: n=6 mice, paired t test, t=5.010, df=5, **P=0.0041; CNO: n=6 mice, paired t test, t=6.432, df=5, **P=0.0013). (**b**) The time that Saline- or CNO-treated mice spent in the center zone or the peripheral zone in OF test (Saline n=6 mice, CNO n=6 mice; Center: t=0.0026, df=10, P=0.9979; Peripheral: t=0.0026, df=10, P=0.9979). (**c**) The time that Saline- or CNO-treat mice spent in the open arms, the closed arms, and the center zone in EPM test (Saline n=6 mice, CNO n=6 mice; Open: t=1.813, df=10, P=0.0999; Closed: t=0.8372, df=10, P=0.4221; Center: t=0.8011, df=10, P=0.4417). Data are represented as mean ± SEM.



Supplementary Fig. 11. Selectively suppressing inhibitory activity in the developing mPFC did not change the social ability or anxiety level of wild-type mice. (**a**) Both Saline- and CNO-treated mice spent significantly more time in contacting with a mouse than with an empty cup in the social ability test (Saline: n=11 mice, paired t test, t=3.654, df=10, **P=0.0044; CNO: n=17 mice, paired t test, t=3.902, df=16, **P=0.0013). (**b**) The time that Saline- or CNO-treated mice spent in the center zone or the peripheral zone in OF test (Saline n=5 mice, CNO n=5 mice; Center: t=0.8397, df=8, P=0.4255; Peripheral: t=0.8397, df=8, P=0.4255). (**c**) The time that Saline- or CNO-treated mice spent in the open arms, the closed arms, and the center zone in EPM test (Saline n=5 mice, CNO n=5 mice; Open: t=0.3261, df=8 P=0.7527; Closed: t=0.1017, df=8, P=0.9215; Center: t=0.1140, df=8, P=0.9121). Data are represented as mean ± SEM.



Supplementary Fig. 12. Selectively enhancing the excitatory activity in the developing mPFC did not affect the social ability or anxiety level of VPA mice. (**a**) Both Saline- and CNO-treated VPA mice spent significantly more time in contacting with a mouse than with an empty cup in the social ability test (Saline: n=10 mice, paired t test, t=2.772, df=9, *P=0.0217; CNO: n=8 mice, paired t test, t=2.809, df=7, **P=0.0262). (**b**) The time that Saline- or CNO-treated VPA mice spent in the center zone or the peripheral zone in OF test (Saline n=10 mice, CNO n=8 mice; Center: t=2.085, df=16, P=0.0535; Peripheral: t=2.085, df=16, P=0.0535). (**c**) The time that Saline- or CNO-treat VPA mice spent in the open arms, the closed arms, and the center zone in EPM test (Saline n=10 mice, CNO n=8 mice; Open: t=1.669, df=16, P=0.1146; Closed: t=0.4425, df=16, P=0.6640; Center: t=1.582, df=16, P=0.1332). Data are represented as mean ± SEM.



Supplementary Fig. 13. Selectively enhancing the excitatory activity in the developing mPFC rescued the anxiety-like behavior of FMR1 KO mice in OF test. (**a**) Both WT and KO mice spent significantly more time in contacting with a mouse than with an empty cup in the social ability test (WT: n=10 mice, paired t test, t=5.114, df=9, ***P=0.0006; KO: n=9 mice, paired t test, t=9.413, df=8, ****P<0.0001). (**b**) The time that WT or KO mice spent in the center zone or the peripheral zone in OF test (WT n=5 mice, KO n=4 mice; Center: t=3.329, df=7, *P=0.0126; Peripheral: t=3.329, df=7, *P=0.0126). (**c**) The time that WT or KO mice spent in the open arms, the closed arms, and the center zone in EPM test (WT n=5 mice, KO n=4 mice; Open: t=1.637, df=7, P=0.1456; Closed: t=0.0592, df=7, P=0.9545; Center: t=0.0652, df=7, P=0.9499). (**d**) Both Saline- and CNO-treated KO mice spent significantly more time in contacting with a mouse than with an empty cup in the social ability test (Saline: n=4 mice, paired t test, t=10.51, df=3, **P=0.0018; CNO: n=7 mice, paired t test, t=3.569, df=7, *P=0.0118). (**e**) The time that Saline- or CNO-treat KO mice spent in the center zone or the peripheral zone in OF test (Saline n=4 mice, CNO n=7 mice; Center: t=1.438, df=9, P=0.1842; Peripheral: t=1.438, df=9, P=0.1842). (**f**) The time that Saline- or CNO-treat KO mice spent in the

open arms, the closed arms, and the center zone in EPM test (Saline n=4 mice, CNO n=7 mice; Open: t=0.1918, df=9, P=0.8522; Closed: t=0.6564, df=9, P=0.5280; Center: t=0.9454, df=9, P=0.3691). Data are represented as mean ± SEM.