Supplementary Information

P2X2 receptors in pyramidal neurons are critical for regulating vulnerability to chronic stress

Xiao-Jing Kuang1,*, Can-Yuan Zhang1,*, Bing-Yi Yan1, Wei-Zhong Cai1, Cheng-Lin Lu1, Li-Jia Xie1, Shu-Ji Li1, Peng-Li Kong1, Jun Fan1, Shu-Min Pan1, Ting Guo1 and Xiong Cao.1,2 *

1. Key Laboratory of Mental Health of the Ministry of Education, Guangdong-Hong Kong-Macao Greater Bay Area Center for Brain Science and Brain-Inspired Intelligence, Guangdong Province
Key Laboratory of Psychiatric Disorders, Department of Neurobiology, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, P. R. China.

2. Microbiome Medicine Center, Department of Laboratory Medicine, Zhujiang Hospital,
Southern Medical University, Guangzhou, Guangdong 510515, P. R. China.

*These authors contributed equally to this work.

*Correspondence should be addressed to caoxiong@smu.edu.cn
Figure. S1

Figure. S1. Social interaction test. (A) Representative heatmaps of normalized time spent during SI test of C57BL/6J mice. (B) Statistical comparison of SI ratio after CSDS (n = 8 -12, n = 8 -12, \(*p = 0.042\), one-way ANOVA). The data are shown as mean ± SEM. \(*p < 0.05\), \(**p < 0.01\), \(***p < 0.001\).

Related to Figure 1.
Figure. S2. P2X2 expression of cultured PFC neurons treated with ATP₆S or ATPase treatment. (A-B) Statistic analysis of P2X2 mRNA expression in the PFC cultured neurons after ATP₆S (A) (n = 6-7, t(11) = 3.203, p = 0.008, unpaired t test) or ATPase treatment (B) (n = 5-6, t(9) = 8.968, p < 0.001, unpaired t test). (C-D) Western blots representation (top) and quantification (bottom) of P2X2 protein level in the PFC cultured neurons after ATP₆S (C) (n = 6-7, t(11) = 3.734, p = 0.003, unpaired t test) or ATPase treatment (D) (n = 6, t(10) = 2.520, p = 0.030, unpaired t test). The data are shown as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.
Figure. S3 Generation of P2rx2-cKO mice. (A-B) Representative images (A) and body weight (B) (n = 12, t(22) = 0.209, p = 0.840; unpaired t test) of adult P2rx2-cKO and control mice. (C-D) Gross appearance (C) and H&E-stained coronal sections (mPFC or hippocampus, Scale bar, 500 μm) of the brain (D) of P2rx2-cKO or littermate control mice. (E) Immunofluorescence for NeuN (red) and GFAP (green) in the mPFC or hippocampus of P2rx2-cKO and littermate control mice. Scale bar, 50 μm. The data are shown as mean ± SEM. Related to Figure. 2.
Figure. S4

Figure. S4. Relative behaviors of P2rx2 conditional knockout mice. (A) SI ratio before and after CSDS of P2rx2-cKO and control mice (n = 12-14, p = 0.042, interaction effect, matching two-way ANOVA). (B-C) Statistics analysis of P2rx2-cKO and control mice in NSF (B, latency to food: t(30) = 0.579, p = 0.570; food consumption: t(30) = 0.960, p = 0.340; n = 15-17, unpaired t test) and OFT (C, number of standing: n = 13-16, t(27) = 1.288, p = 0.210, unpaired t test). (D) Social interaction ratio before and after CSDS of CamkIIα-CreERT; P2rx2lox/lox and control mice (n = 8-13, p = 0.030, interaction effect, matching two-way ANOVA). (E-F) Statistics analysis of CamkIIα-CreERT; P2rx2lox/lox and control mice in NSF (E) (latency to food: t(19) = 0.018, p = 0.986; food consumption: t(19) = 0.491, p = 0.629; n = 9-12, unpaired t test) and OFT (F) (number of standing: n = 14-16, t(31) = 0.421, p = 0.680, unpaired t test). The data are shown as mean ± SEM. *p < 0.05.

Related to Figure 2.
Figure. S5

(A) Representative heatmaps of normalized time spent during SI test before (left) and after (right) CSDS of \( P2rx2^{loxp/loxp} \) mice injected with AAV-CamkIIα-EGFP-Cre or control virus. (B) SI ratio (n = 10-11, p = 0.010, interaction effect, matching two-way ANOVA) of \( P2rx2^{loxp/loxp} \) mice injected with AAV-CamkIIα-EGFP-Cre or control virus. (C) Representative heatmaps of normalized time spent during SI test before (left) and after (right) CSDS of CamkIIa-Cre mice injected with AAV-DIO-P2X2 or control virus. (D-E) SI ratio (D) (n = 7-9, p = 0.035, interaction effect, matching two-way ANOVA) and NSF (E) (n = 11-12, t_{21} = 0.384, p = 0.700; unpaired t-test).
test) of CamkIIa-Cre mice injected with AAV-DIO-P2X2 or control virus. (F) Representative heatmaps of normalized time spent during SI test before (left) and after (right) CSDS of P2rx2-cKO mice injected with AAV- DIO-P2X2 or control virus. (G) SI ratio (n = 6-7, p = 0.025, interaction effect, matching two-way ANOVA) of P2rx2-cKO mice injected with AAV- DIO-P2X2 or control virus. The data are shown as mean ± SEM. *p < 0.05.

Related to Fig. 3.
Figure S6. CSDS results of mice applying AAV-DIO-GCaMP6s virus. (A, B) Representative heatmaps (A) and analysis of social interaction (B) (n = 6-7, time in the interaction zone with target: p = 0.017; SI ratio: p = 0.747; interaction effect, matching two-way ANOVA) of P2rx2-cKO and CamkIIa-cre control mice injected with AAV-DIO-GCaMP6s virus in CSDS paradigm. (C, D) Representative heatmaps (C) and analysis of social interaction (D) (n = 6-7, time in the interaction zone with target: *p = 0.035; SI ratio: p = 0.060; interaction effect, matching two-way ANOVA) of P2X2 overexpression and control mice injected with AAV-DIO-GCaMP6s virus. The data are shown as mean ± SEM. *p < 0.05, **p < 0.01.

Related to Figure 4.
| Gene     | Former primer               | Reverse primer               |
|----------|-----------------------------|------------------------------|
| P2rx1    | CCG AAG CCT TGC TGA GAA     | GGT TTG CAG TGC CGT ACA T    |
| P2rx2    | CAG AAC TGG CAC ACA AGG G   | CAG TCA CAC AGA AAG GAG CC   |
| P2rx3    | GGT GGC TGC CTT CAC TTC    | TCA GCC CCT TTG AGG AAA      |
| P2rx4    | CCA ACA CTT CTC AGC TTG GAT| TGG TCA TGA TGA AGA GGG AGT  |
| P2rx5    | CAC AGT CAT CAA CAT TGG    | AGG TAG ATA AGT ACC AGG TCA  |
|          | TTC C                       | CAG AAG                      |
| P2rx6    | TGT CCC CAG TAC TCC TTC CA | CAC CAG TGA TTG GCT GTC C    |
| P2rx7    | GGG GGT TTA CCC CTA CTG TAA| GCT CGT CGA CAA AGG ACA C    |
| Synaptophysin | TCT TTG TCA CCG TGG CTG TGT T | TCC CTC AGT TCC TTG CAT GTG T |
| SNAP 25  | CTG GCA TCA GGA CTT TGG TT | ATT ATT GCC CCA GGC TTT TT   |
| Synaslin 1 | CCA GCT CAA CAA ATC CCA GT | GGT GTC AGT CGG AGA AGA GG   |
| PSD 95   | TCT GTG CGA GAG GTA GCA GA | AAG CAC TCC GTG AAC TCC TG   |
| Rab4b    | ACT ATT GGC GTG GAG TTT GG  | CAC AGA GGA TGA CCA CGA TG   |
| Tubb4    | GGG GAC CTC AAC CAC CTA GT | ATC CTG GCA TGA AGA AGT GG   |
| MAP 2    | TCA GGA GAC AGG GAG GAG AA  | GTG TGG AGG TGC CAC TTT TT   |
| VGLUT2   | GCT CAC CTC CAC CTT CAA TAT| CCA CTT GCT CCA TAT CCC ATG  |
|          | G                                      |
|----------|----------------------------------------|
| VGAT     | ACG ACA AAC CCA AGA TCA CG             |
|          | AAG ATG ATG AGG AAC AAC CCC             |
| β-actin  | CCA CCA TGT ACC CAG GCA TT             |
|          | CGG ACT CAT CGT ACT CCT GC              |

**Supplementary Table 1. qPCR primers.**