

Supplemental Information

Distinct Function of Estrogen Receptors in the Rodent Anterior Cingulate Cortex in Pain-Related Aversion

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Inventory of Supplemental Information

Figure S1. related to Figure 1 , Expression of estrogen receptors in the rostral anterior cingulate cortex (rACC).

Figure S2. related Figure 4, Formalin-induced conditioned place avoidance (F-CPA) in mice.

Figure S3. related to Figure 5, Expression of estrogen receptors in synaptosomal membrane fraction of the rostral anterior cingulate cortex (rACC).

Figure S4. related to Figure 5, Effects of estrogen receptor- α (ER α), estrogen

receptor- β (ER β) or G protein-coupled estrogen receptor-1 (GPER) agonist in the rostral anterior cingulate cortex (rACC) on mechanical and thermal sensitivity.

Supplemental Figures

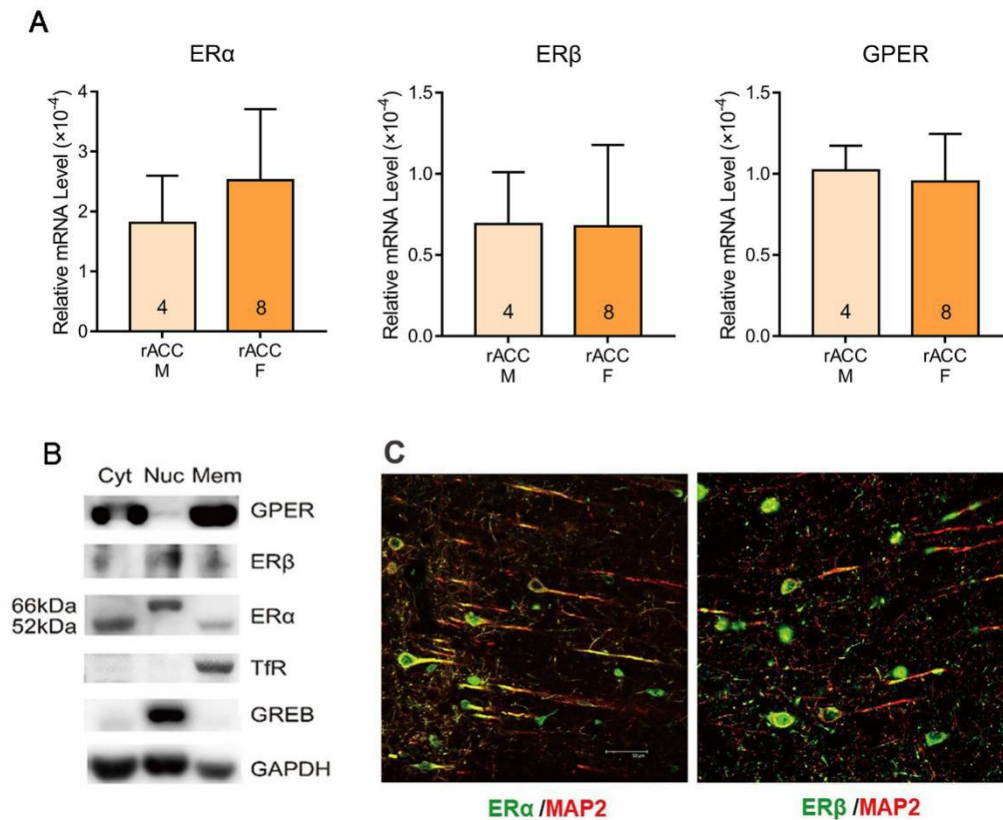


Figure S1. related to Figure 1, Expression of estrogen receptors in the rostral anterior cingulate cortex. **(A)** The relative mRNA expression levels of estrogen receptor- α (ER α), estrogen receptor- β (ER β) and G protein-coupled estrogen receptor-1 (GPER) in the rostral anterior cingulate cortex (rACC) from normal male (M) and female (F) rats. **(B)** ER α , ER β and GPER immunoreactive bands in isolation of cytoplasmic, membrane and nuclear protein extracts. Transferrin (TfR) and CREB are used as a marker of the membrane and nuclear protein respectively. **(C)** Double immunofluorescence reveals the colocalization of ER α and ER β (green) with neuronal dendrite marker microtubule-associated protein 2 (MAP-2, red).

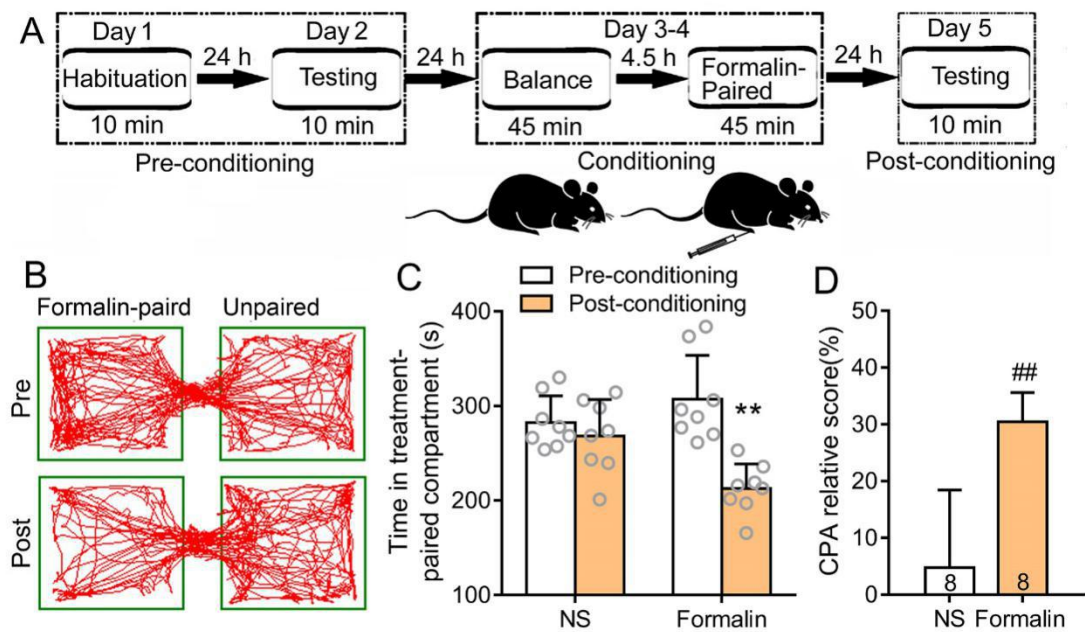


Figure S2. related Figure 4, Formalin-induced conditioned place avoidance in mice. (A) Schematic of the protocol for mouse formalin-induced conditioned place avoidance (F-CPA). (B-D) The successfully established F-CPA in mice, as demonstrated by the time spent in the treatment-paired compartment in pre-conditioning and post-conditioning tests (B and C), and relative CPA score (the percentage of the difference of time spent in the treatment-paired compartment between the preconditioning test and post-conditioning test, against the time spent in the treatment-paired compartment in the pre-conditioning tests, D). ** $p < 0.01$ versus pre-conditioning (two-tailed paired t -test), ## $p < 0.01$ versus NS (two-tailed Student's t -test), $n=8$.

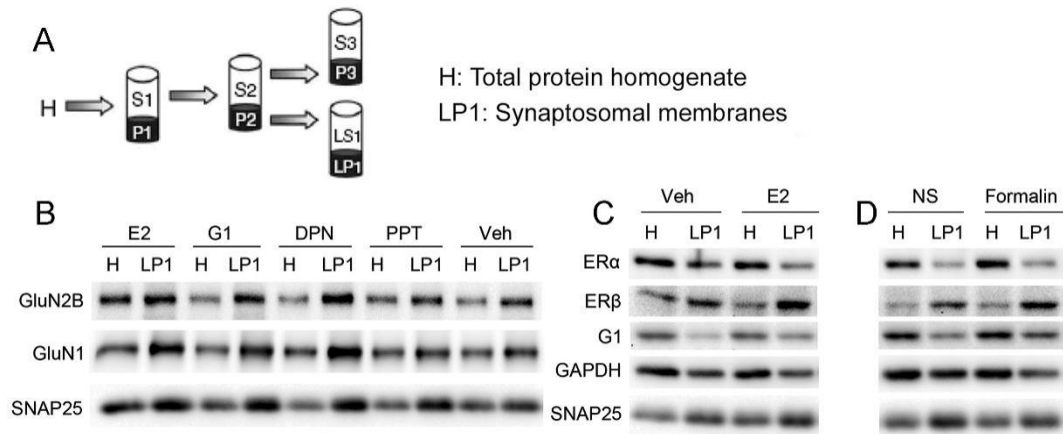


Figure S3. related to Figure 5, Expression of estrogen receptors in synaptosomal membrane fractionation of the rostral anterior cingulate cortex. (A) The extraction of synaptosomal membrane fractionation. Rostral anterior cingulate cortex (rACC) tissues from 4-6 rats were put together for synaptosomal membrane extraction. **(B)** estrogen receptor- β (ER β) agonist DPN, G protein-coupled estrogen receptor-1 (GPER) agonist G1 or 17 β -estradiol (E2) exposure increased GluN1 and GluN2B expression in synaptosomal membrane protein extracts from rACC slices. **(C)** E2 exposure increased synaptosomal membrane ER β and GPER but did not estrogen receptor- α (ER α) in rACC slices. **(D)** Intraplantar injection of formalin increased synaptosomal membrane ER β and GPER but did not ER α in the rACC.

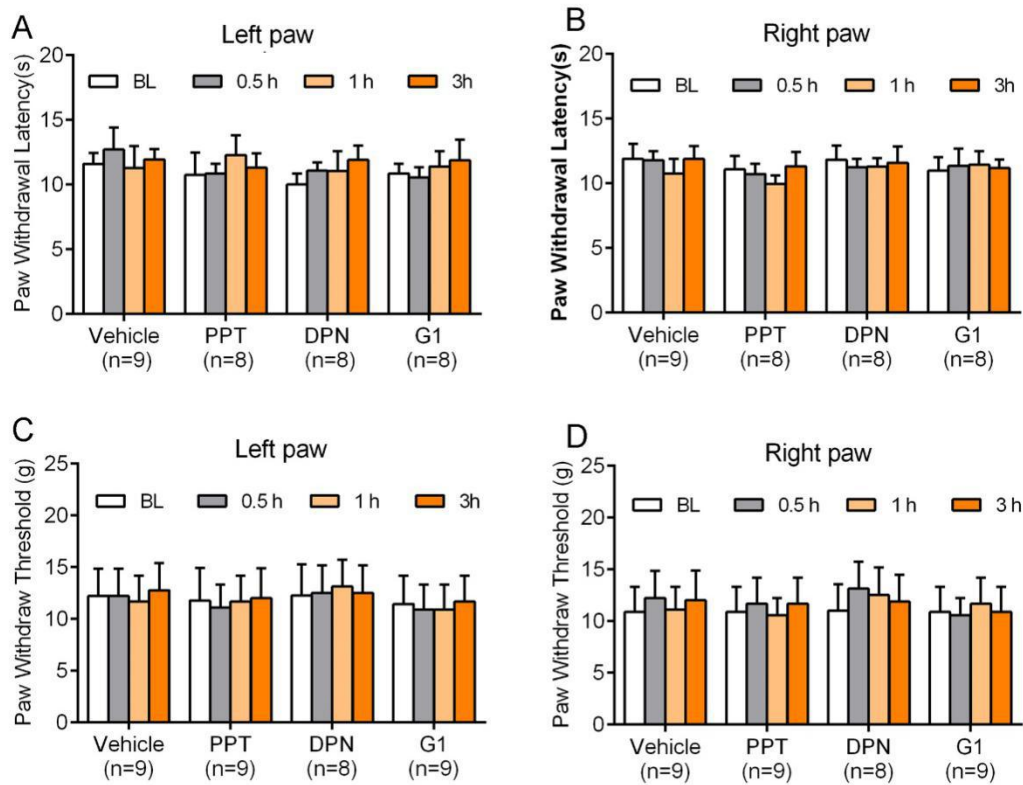


Figure S4. related to Figure 5, Effects of estrogen receptor- α , estrogen receptor- β or G protein-coupled estrogen receptor-1 agonist in the rostral anterior cingulate cortex on mechanical and thermal sensitivity. (A,B) Intra-rostral anterior cingulate cortex (Intra-rACC) microinjection of vehicle, estrogen receptor- α (ER α) agonist PPT (2 pg/ μ l, 0.5 μ l/hemisphere), estrogen receptor- β (ER β) agonist DPN (0.02 ng/ μ l, 0.5 μ l/hemisphere) or G protein-coupled estrogen receptor-1 (GPER) agonist G1 (0.2 μ g/ μ l, 0.5 μ l/hemisphere) did not affect the paw withdrawal latency in response to radiant heat stimulation. **(C,D)** Intra-rACC microinjection of vehicle, ER α agonist PPT (2 pg/ μ l, 0.5 μ l/hemisphere), ER β agonist DPN (0.02 ng/ μ l, 0.5 μ l/hemisphere) or GPER agonist G1 (0.2 μ g/ μ l, 0.5 μ l/hemisphere) did not alter paw withdrawal threshold in response to *von* Frey hairs.