High cortical delta power correlates with aggravated allodynia by activating anterior cingulate cortex GABAergic neurons in neuropathic pain mice

Ya-Dong Li[1*], Jing Ge[1*], Yan-Jia Luo[1*], Wei Xu[1], Juan Wang[1], Michael Lazarus[2], Zong-Yuan Hong[3], Wei-Min Qu[1], Zhi-Li Huang[1]

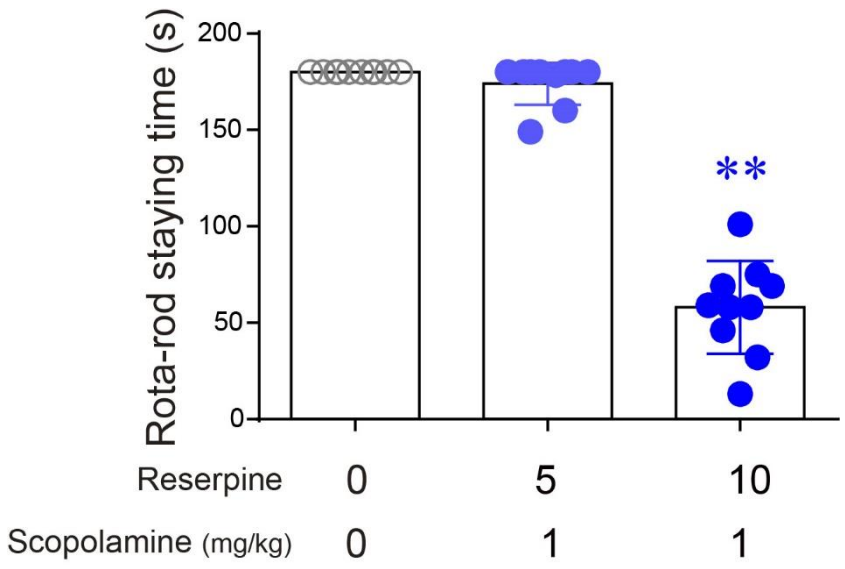
[1] Department of Pharmacology, School of Basic Medical Sciences; State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, and Institutes of Brain Science, Fudan University, Shanghai 200032, China

[2] International Institute for Integrative Sleep Medicine (WPI-IIIS), University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan.

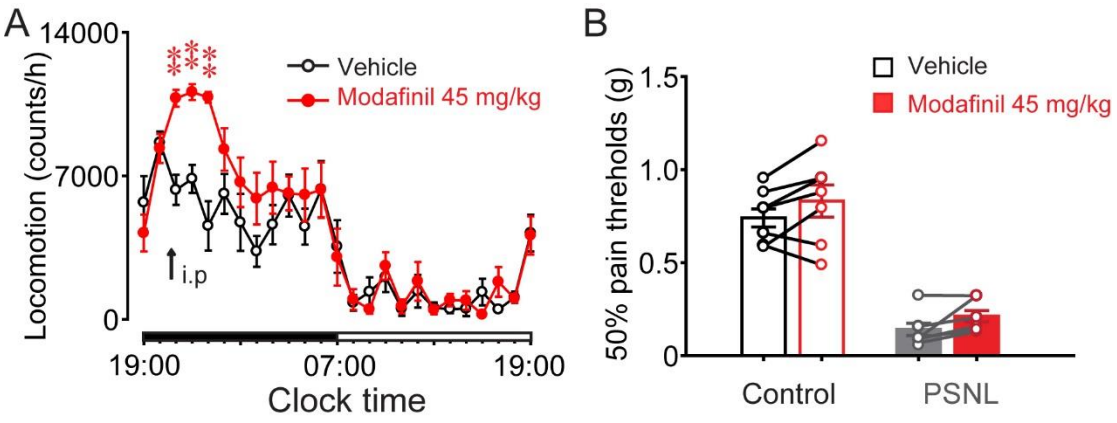
[3] Department of Pharmacology, Wannan Medical College, Wuhu, Anhui 241002, China

*These authors contributed equally to this work

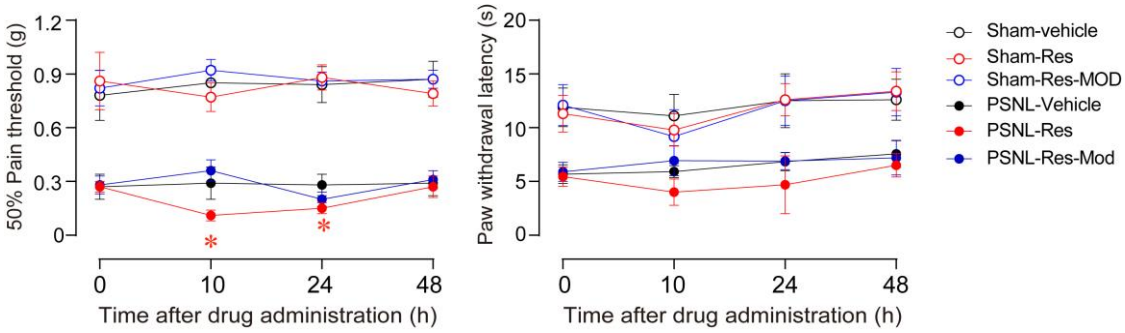
Supplementary materials



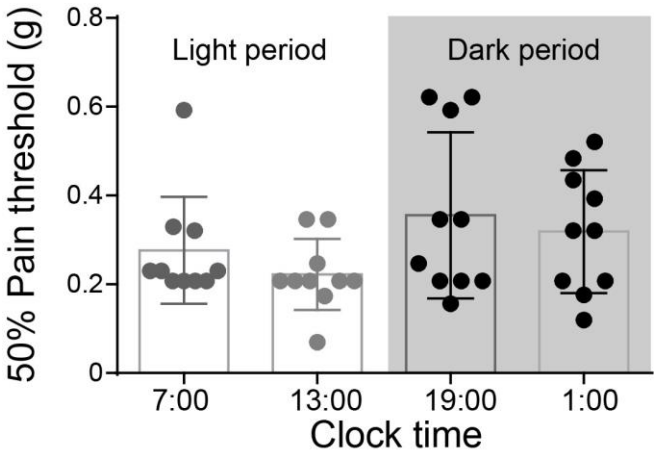
Supplementary Figure 1. The dosage of reserpine (5 mg/kg) and scopolamine (1 mg/kg) used in pain testing did not impair motor coordination of PSNL mice in the rota-rod test. Mice who could stay on the rota-rod for 180 s (cut-off time) were selected for testing. The average time mice stayed on the rota-rod was measured 30 min after scopolamine or vehicle administration (i.p.). Data are expressed as the means ± SEM (n = 10). ***P* < 0.01 vs. the vehicle group as assessed by one-way ANOVA followed by the PLSD post-hoc test.



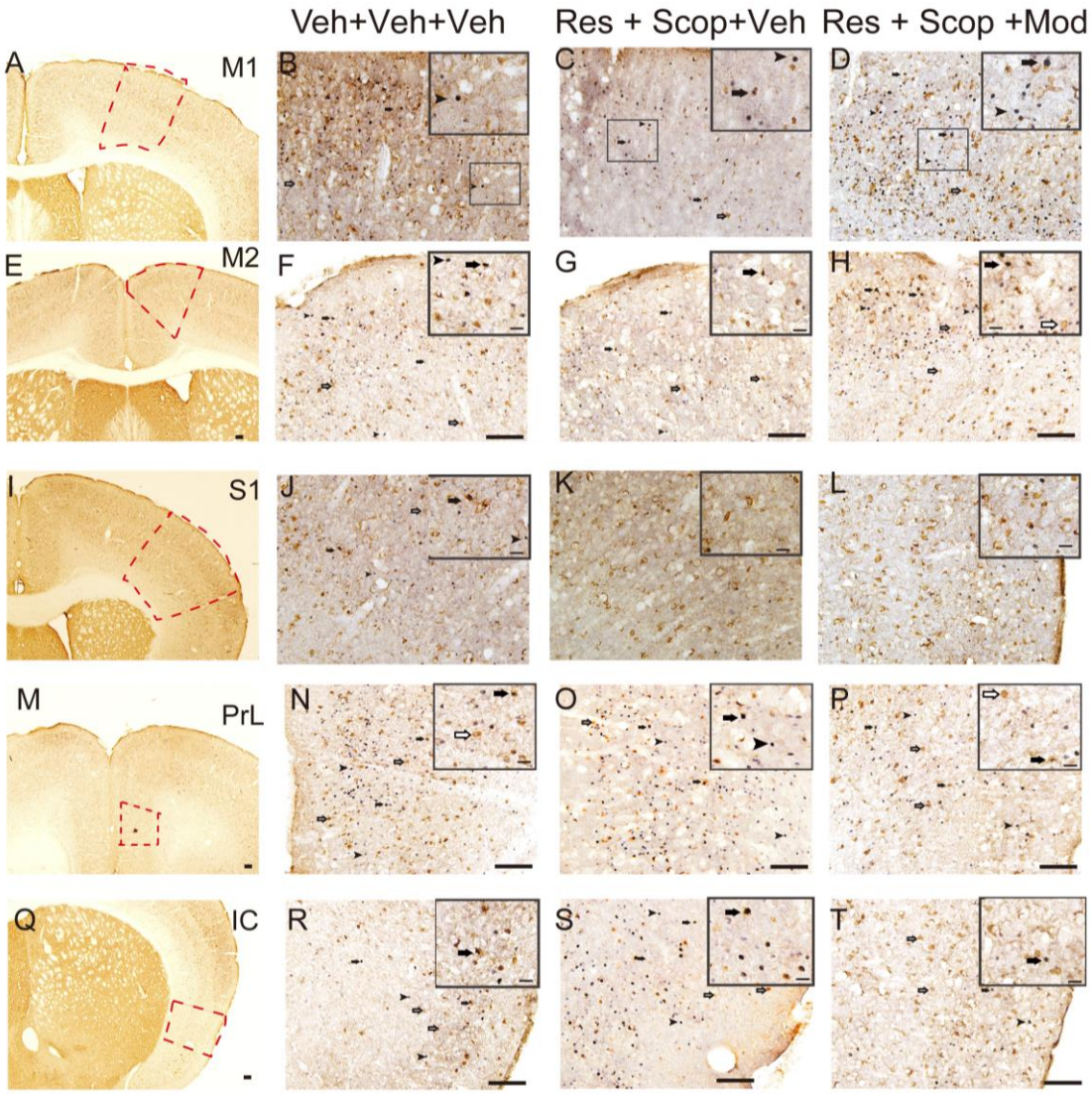
Supplementary Figure 2. Modafinil at 45 mg/kg increased locomotor activity of PSNL mice but did not affect mechanical pain thresholds in both naïve and PSNL mice. (A) Time-course changes in locomotion after administration of vehicle or modafinil (i.p. at 21:00) to PSNL mice. Data shown are the means ± SEM (n = 7), $^{**}P < 0.01$, using repeated-measures ANOVA followed by PLSD post-hoc test. (B) Mechanical pain thresholds in naïve and PSNL mice after administration of vehicle and modainil. Data are expressed as the means ± SEM (n = 7), $P > 0.05$, using paired *t*-test.



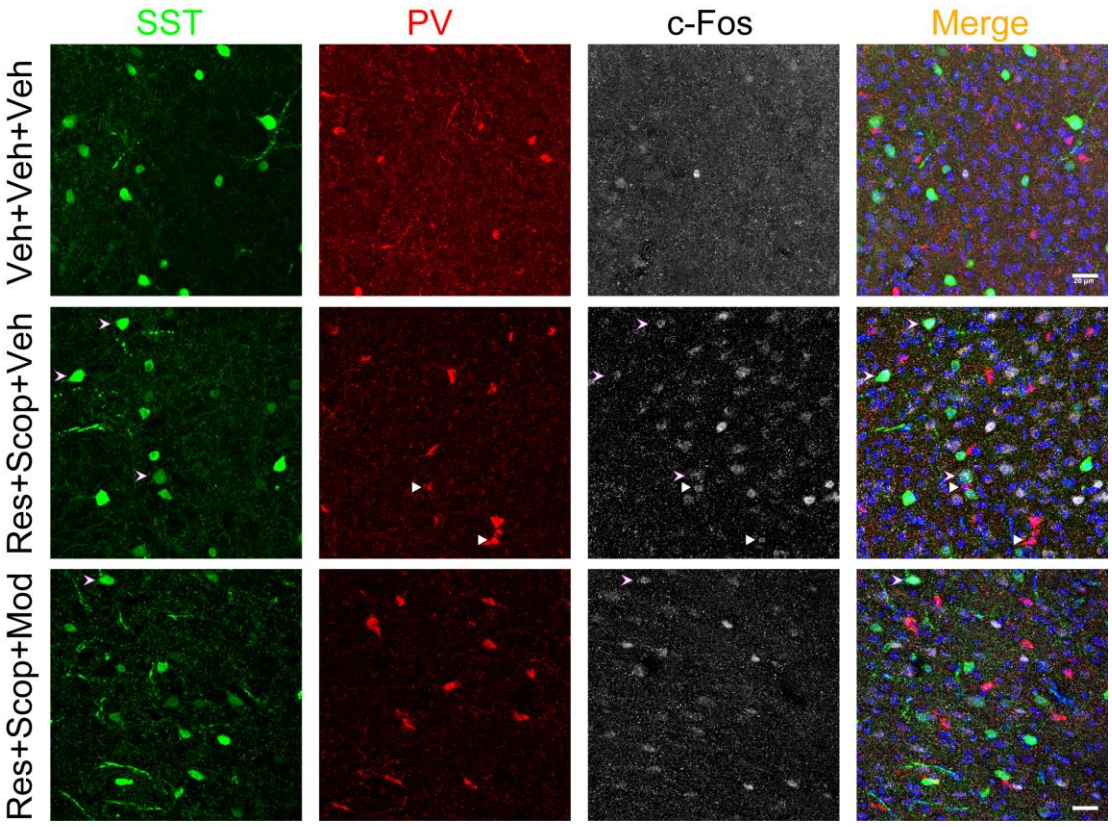
Supplementary Figure 3. Time course changes in mechanical and thermal pain sensitization after modafinil administration in reserpine-treated sham and PSNL mice. (A) Mechanical pain thresholds decreased for 24 hours in PSNL mice treated with reserpine (5 mg/kg, i.p. at 11:00) and scopolamine (1 mg/kg, i.p. at 20:30) but not in sham mice. Modafinil (45 mg/kg, i.p. at 20:45) reversed the low mechanical pain thresholds at 10 h after reserpine treatment in PSNL mice. (B) Paw withdrawal latency did not change in reserpine-treated sham and PSNL mice. Data shown are the means ± SEM (n = 8-10), $^{*}P < 0.05$ compared to vehicle control, using repeated-measures ANOVA followed by PLSD post-hoc test .



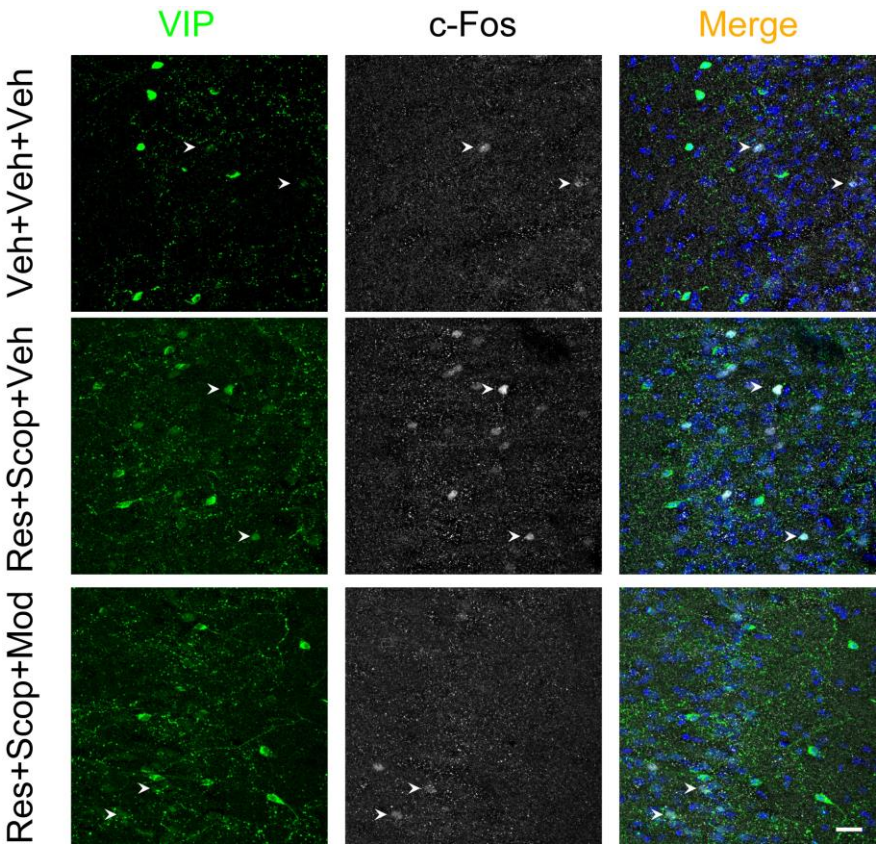
Supplementary Figure 4. Mechanical pain thresholds fluctuated in the light and dark period. Mechanical pain thresholds of PSNL mice were tested at 7:00, 13:00, 19:00 and 1:00, respectively. The pain thresholds had a higher trend in the dark period when the mice were mainly arousal, but lower in the light period without statistically significant difference. Data were shown as means ± SD (n=10), one-way AVOVA followed by PLSD post-hoc test.



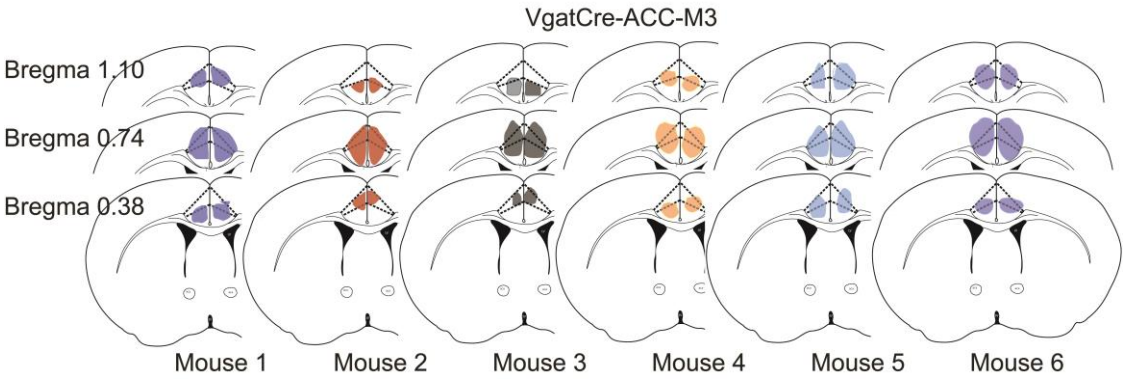
Supplementary Figure 5. Immunohistochemical double-staining for c-Fos and GFP in GAD67-GFP PSNL mice. Mice were sacrificed at 22:30, 1.5 hours after vehicle, reserpine, or modafinil injection, respectively. The locations of the cortical regions M1, M2, S1, PrL, and IC in the coronal sections are shown in panel A, E, I, M, and Q. Brain sections of GAD67-GFP PSNL mice stained for c-Fos (black) and GFP (brown) after injections of vehicle+vehicle+vehicle (panel B, F, J, N, and R), 5 mg/kg reserpine+1 mg/kg scopolamine+vehicle (panel C, G, K, O, and S), or 45 mg/kg modafinil after 5 mg/kg reserpine+1 mg/kg scopolamine (panel D, H, L, P, and T). Scale bar = 100 μm.



Supplementary Figure 6. C-fos expression in SST+ and PV+ GABAergic neurons in the ACC after drug admistration in PSNL mice. Immunofluorescence staining for SST, PV and c-Fos showing that c-Fos expression (white) is increased in both SST (green) and PV (red) positive neurons in the ACC in PSNL mice following reserpine+scopolamine injections and decreased after modafinil injection. White arrows indicate SST+c-Fos+ cells, pink arrows indicate PV+c-Fos+ cells. Scale bar, 20 μm.

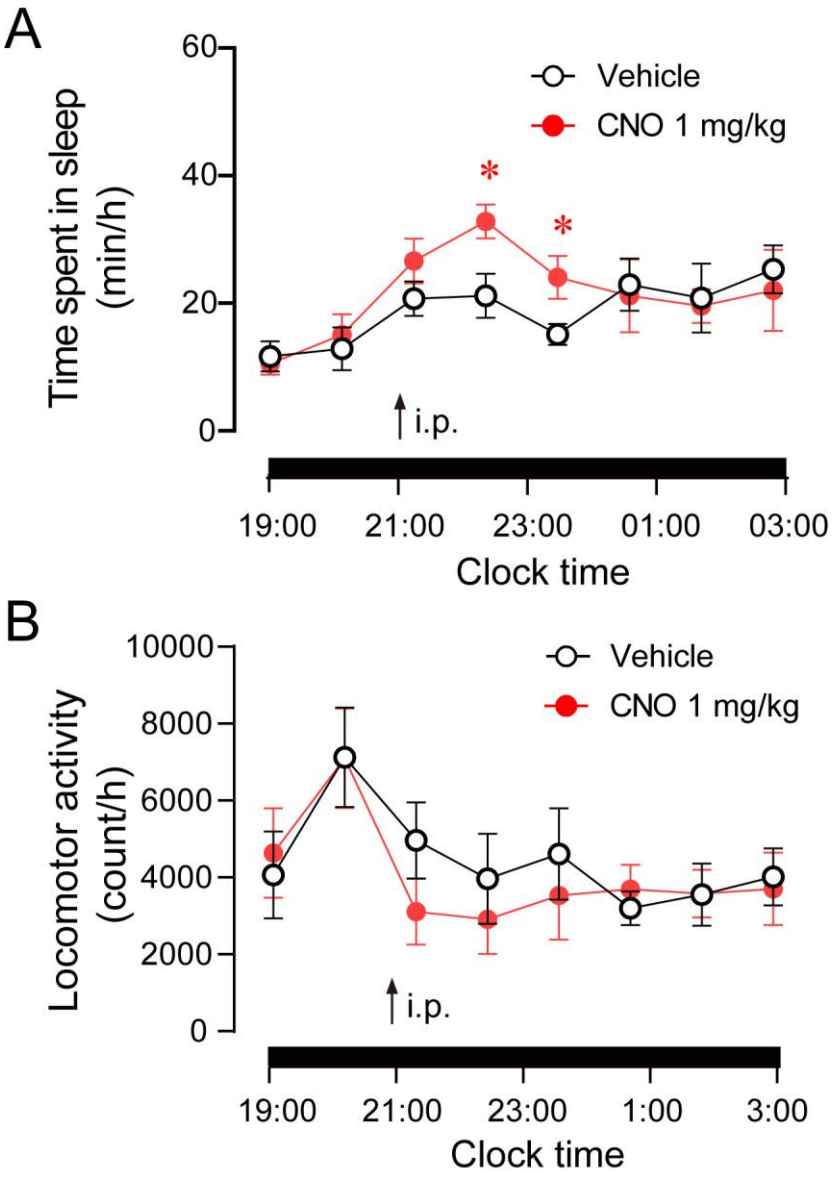


Supplementary Figure 7. C-fos expression in the ACC VIP+ GABAergic neurons after drug admistration in PSNL mice. Examples of c-Fos immunofluorescence in coronal brain slices of vehicle+vehicle+vehicle, reserpine+scopolamine+vehicle and reserpine+ scopolamine+modafinil mice. No obvious changes of c-Fos immunoreactive (white) in VIP positive neurons (green) were seen after reserpine+scopolamine or reserpine + scopolamine +modafinil injections. White arrows indicate VIP+c-Fos+ cells Scale bar, 20 μm.

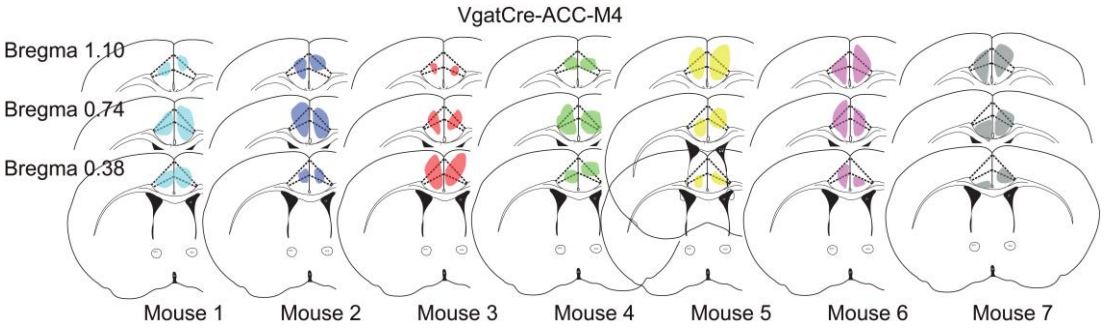


Supplementary Figure 8. Mapping of the AAV-DIO-hM3Dq-mCherry-expressed regions in the ACC. Coronal brain atlas diagram showing approximate distribution of 6 hM3Dq-mCherry infected cells (between 1.10 and 0.38 mm from bregma) for each individual mouse indicated by mouse ID# and associated color outline. Whole-brain sections were imaged by a 10× or 20× objective on the VS120 virtual

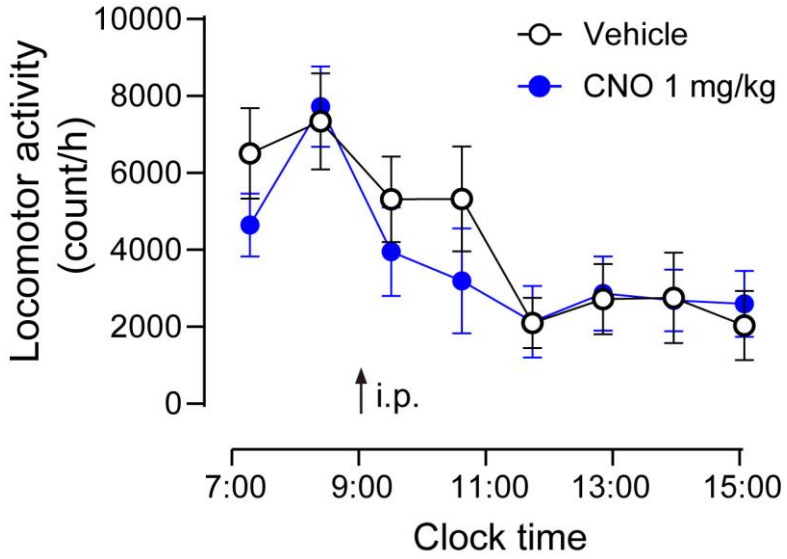
microscopy slide scanning system (Olympus). For cell mapping of neurons, neural bodies were quantified semi-automatically using ImageJ software, then the boundary of mCherry cell region to the appropriate brain regions was manually drawn based on the mouse brain atlas.



Supplementary Figure 9. Activation of ACC GABAergic neurons slightly increased sleep amount in naïve mice. (A) Time spent in sleep was increased after CNO injection in naïve Vgat-Cre mice transduced with hM3Dq in the ACC during the dark period. (B) Locomotor activity did not change after CNO injection. The balck bar means dark period. Data are expressed as the means ± SEM (n = 6), *P < 0.05, using two-way ANOVA followed by PLSD post-hoc test.



Supplementary Figure 10. Mapping of the AAV-DIO-hM4Di-mCherry-expressed regions in the ACC. Coronal brain atlas diagram showing approximate distribution of 7 hM4Di-mCherry infected cells (between 1.10 and 0.38 mm from bregma) for each individual mouse indicated by mouse ID# and associated color outline.



Supplementary Figure 11. Inhibition of ACC GABAergic neurons did not affect locomotor activity in PSNL Vgat-Cre mice transduced with hM4Di after CNO injection during the light period. Data are expressed as the means ± SEM (n = 7), using two-way ANOVA followed by PLSD post-hoc test.