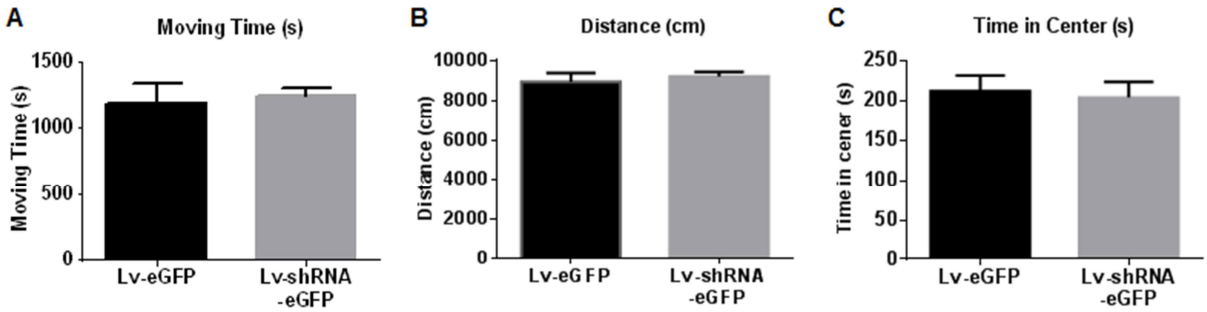
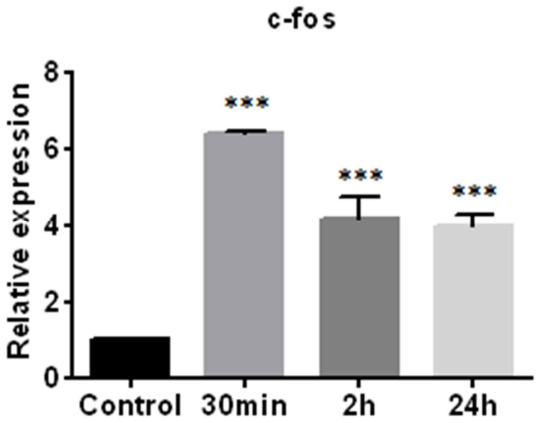
Supporting Information

Supplementary Figures



Supplementary Figure 1. RGS4 knock-down in the NAc did not alter locomotor behavior. Prior to the morphine CPP test, the basal locomotor activity levels of the mice that received accumbal injections of Lv-shRNA-eGFP and Lv-eGFP were examined in the open field test. Each group exhibited similar (A) total moving times, (B) total distances, and (C) times spent in the center. All data are expressed as a mean ± standard error of the mean (SEM; n = 6 for each group).



Supplementary Figure 2. Chronic morphine treatment induced changes in the temporal mRNA profiles of immediate early gene (c-fos) activity in the NAc. To examine the expression of c-fos in the NAc after chronic morphine treatment, mice were injected with morphine (15 mg/kg) for 5 consecutive days. Mice were sacrificed at 30 min, 2 h, and 24 h after the last morphine injection, and NAc tissue samples were subjected to a real-time PCR analysis. All data are expressed as means ± standard error of the mean (SEM; n = 6 for each group) and were analyzed with a one-way ANOVA followed by a Fisher's LSD post hoc test. * $p < 0.05$, ***$p < 0.001$ vs. vehicle-treated control.

Materials and Methods

Open field test

An open field analysis was used to measure the post-treatment activities of mice in a novel environment. The measured parameters included number of ambulatory movements, total moving distance, time of ambulatory movement, and resting time; these were determined using the TruScan Photo Beam Activity System (Coulbourn Instruments; Whitehall, PA, USA)

Real-time polymerase chain reaction

RNA extraction and real-time PCR procedures were performed as previously described by our research group [1,2]. Total RNA was isolated from NAc tissue using an RNAeasy® Lipid Tissue Mini kit (Qiagen; Valencia, CA, USA) according to the manufacturer's instructions, and the concentrations of the RNA samples were determined via measurements of optical density using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc). Next, first-strand complementary DNA (cDNA) was prepared using random primers (Takara Bio; Tokyo, Japan) with SuperscriptTM II reverse transcriptase (Invitrogen; Carlsbad, CA, USA) according to the manufacturer's instructions, and the cDNA was diluted to 8 ng/μL with RNase-free water. The primer sequences for c-fos (NM_010234.2) were as follows: forward sequence (5'-3'), GGGCTGCACTACTTACACGT and reverse sequence (5'-3'), TGCCTTGCCTTCTCTGACTG.

Real-time PCR amplifications were performed using TOPreal™ qPCR 2X PreMIX (Enzynomics; Daejeon, Korea) with a Stratagene MX3000P (Agilent Technologies; Santa Clara, CA, USA), according to the manufacturer's instructions. The thermal cycling profile consisted of a pre-incubation step at 94°C for 10 min, followed by 45 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 30 s, and elongation at 72°C for 20 s. Additionally, a melting program was used to verify that only one product was amplified. The amplification curves from each real-time PCR reaction were generated within the software, and the threshold cycle values were determined; β-actin was used as a housekeeping gene for normalization to an internal control for each sample. All results are expressed as a mean fold change using the $2^{-\Delta\Delta CT}$ method [3].

References

1. Kim J, Yang M, Son Y, Jang H, Kim D, Kim JC, Kim SH, Kang MJ, Im HI, Shin T, Moon C (2014) Glial activation with concurrent up-regulation of inflammatory mediators in trimethyltin-induced neurotoxicity in mice. Acta Histochem 116 (8):1490-1500. doi:10.1016/j.acthis.2014.09.003

2. Son Y, Kang S, Kim J, Lee S, Kim JC, Kim SH, Kim JS, Jo SK, Jung U, Youn B, Shin T, Yang M, Moon C (2016) Possible involvement of hippocampal immediate-early genes in contextual fear memory deficit induced by cranial irradiation. Neurobiol Learn Mem 133:19-29. doi:10.1016/j.nlm.2016.05.012

3. Livak KJ, Schmittgen TD (2001) Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25 (4):402-408