Supporting Information

Stratinaki et al. 10.1073/pnas.1214696110

SI Methods

Studies on Human Postmortem NAc. ***Characteristics of subjects.*** Human brain specimens were obtained from the Dallas Brain Collection (1). Briefly, after obtaining next of kin permission, brain tissue was collected from cases at the Dallas County Medical Examiners Office and Transplant Service Center at the University of Texas (UT) Southwestern Medical Center. Subjects with known history of neurological disorders or head injury were excluded. Clinical records and collateral information from telephone interviews with a primary caregiver were obtained for each case. Two board certified psychiatrists carried out an extensive review of the clinical information and made independent diagnoses followed by a consensus diagnosis using Diagnostic and Statistical Manual of Mental Disorders IV criteria. Demographic characteristics associated with the tissue are presented in Table S1. The collection of human brain specimens was approved by the Institutional Review Board of UT Southwestern Medical Center.

Tissue preparation. Cerebral hemispheres were cut coronally into 1- to 2-cm blocks, and the nucleus accumbens (NAc) was dissected and immediately placed in a mixture of dry ice and isopentane (1:1, vol:vol). The frozen tissue was then pulverized on dry ice and stored at −80 °C.

RNA isolation, RIN determination, and PCR. The RNA analysis of human postmortem NAc was accomplished as described (1). RNA integrity number determination was performed by isolating total RNA using TRIzol (Invitrogen), followed by analysis with an Agilent 2100 Bioanalyzer. *Regulator of G protein signaling* (*RGS*) *4* mRNA was measured by using quantitative PCR with the following primers: Fwd 5′ CCAGGCAACCAAAGAGGTGA 3′and Reverse 5′ GGCCTCATCAAAGCAGGTTATT 3′, which recognizes splice variants *RGS4-1*, *RGS4-2*, *RGS4-3*, and *RGS4-5* variants (2, 3). *RGS4* mRNA levels were normalized to *GAPDH* mRNA levels, which were not affected by antidepressant treatment.

1. Stan AD, et al. (2006) Human postmortem tissue: What quality markers matter? *Brain Res* 1123(1):1–11.
2. Ding L, Mychaleckyj JC, Hegde AN (2007) Full length cloning and expression analysis of splice variants of regulator of G-protein signaling RGS4 in human and murine brain. *Gene* 401(1-2):46–60.
3. Ho AM, MacKay RK, Dodd PR, Lewohl JM (2010) Association of polymorphisms in RGS4 and expression of RGS transcripts in the brains of human alcoholics. *Brain Res* 1340:1–9.

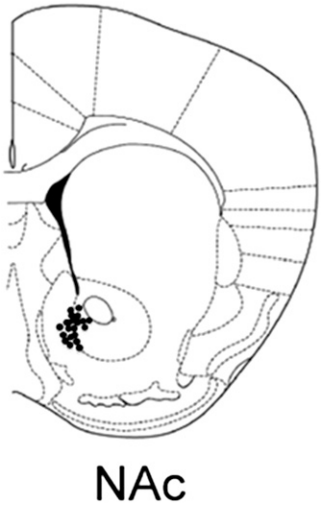

Fig. S1. Injection sites in the nucleus accumbens. Placements were circumscribed within the dorsomedial part of the NAc, including parts of both shell and core at A/P coordinates between +1.5 to +1.7 from bregma. All animals with a misplacement of the needle tract (~5% of all animals used) were excluded from the study.

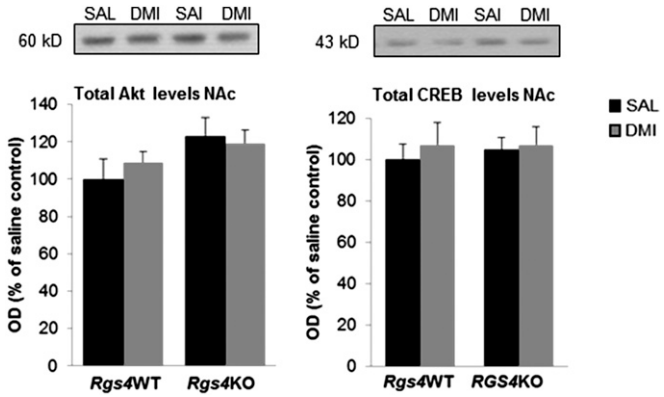

Fig. S2. Akt and cAMP response element binding protein (CREB) levels following chronic desipramine (DMI) treatment. Western blot analysis shows no change in total Akt levels (*Left*) or total CREB levels (*Right*) in NAc following chronic DMI (10 mg/kg) or saline (SAL) treatment (i.p. twice a day for 2 wk). No differences in baseline Akt or CREB levels were observed between genotypes. Mice were tested in the hyponeophagia paradigm, and NAc tissue was dissected 2 h after the test and 24 h after the last DMI injection (Fig. 3 *A*–*C*). $n = 5$ per group.

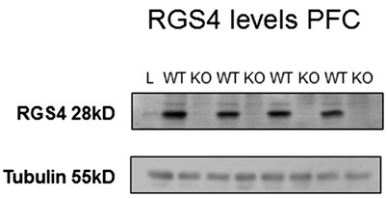

Fig. S3. Specificity of the anti-Rgs4 antibody (U1079) used in this study. Western blot analysis of mouse prefrontal cortex samples, using the U1079 anti-Rgs4 antibody, shows that the 28-kDa band corresponding to Rgs4 is absent uniquely in tissue from *Rgs4*KO mice.

Table S1. Characteristics of subjects included in human postmortem NAc analysis

Treatment group	Sex	Age	Race	PMI, h	RIN	Depression	Medication	Drug
Control								
13	F	83	C	30	8.5	—	—	—
66	M	31	C	16	8.1	—	—	—
70	M	63	C	14	7.7	—	—	—
81	M	19	C	20	9.0	—	—	—
82	M	48	C	15	9.5	—	—	—
84	M	20	C	21	8.2	—	—	—
93	M	60	C	20	8.5	—	—	—
96	M	43	C	15	6.1	—	—	—
103	M	60	AA	11	9.3	—	—	—
108	M	63	C	12	4.9	—	—	—
115	M	34	C	21	9.7	—	—	—
120	M	48	C	17	8.6	—	—	—
122	F	50	C	10	9.0	—	—	—
131	M	54	C	11	8.8	—	—	—
136	M	77	C	13	8.9	—	—	—
143	M	47	C	22	8.6	—	—	—
145	M	60	C	27	8.5	—	—	—
Depression								
12	F	81	C	7	3.9	12	Off	—
15	F	46	C	11	8.3	15	Off	—
21	M	54	C	6	8.9	21	On	SSRI, SNRI
21	M	33	C	18	8.5	24	On	SSRI
51	M	42	C	17	9.1	51	Off	—
52	M	24	C	18	6.9	52	Off	—
63	M	18	C	24	n.a.	63	Off	—
65	M	40	C	21	7.3	65	On	SSRI, TCA
80	M	35	C	9	7.4	80	On	SSRI
89	M	61	C	21	9.1	89	On	SNRI
92	M	50	C	21	9.4	92	On	SNRI
94	F	57	C	9	7	94	On	SSRI
101	F	59	C	20	7.8	101	Off	—
106	M	61	C	19	7.5	106	Off	—
119	F	41	AA	13	n.a.	119	Off	—
139	F	26	C	19	8.1	139	On	SSRI

Data for each of the patients and control subjects from which postmortem NAc tissue was obtained from the real-time PCR studies. AA, African-American; C, Caucasian; F, female; M, male; n.a., not applicable; PMI, postmortem interval; RIN, RNA integrity number; SNRA, serotonin/norepinephrine reuptake inhibitor; SSRI, serotonin selective reuptake inhibitor; TCA, tricyclic antidepressant.