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Supplemental Information

Epigenetic Status of *Gdnf* in the Ventral Striatum Determines Susceptibility and Adaptation to Daily Stressful Events

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Inventory of Supplemental Information

Supplemental Figure Legends

Figure S1. Schematic of the experimental design

A. Mice (BALB or B6) were subjected to 6 weeks of chronic ultra-mild stress (CUMS). Imipramine (IMI, 18 mg/kg/day) was dissolved in the drinking water and was given during the last 3 weeks of the CUMS session and during behavioral assays. Non-stressed (NS) mice were handled every day. At the end of the CUMS session, social interaction (SI), forced swim (FST), novelty-suppressed feeding (NSF), and sucrose preference (SPT) tests were performed, in this order.

B. Twenty-one days after the bilateral canulae implantation into the NAc of B6 mice, either PEI/*Gdnf* or PEI/*Egfp* complexes were injected. Two weeks later, SI and SPT were performed, in this order. Seven days after the bilateral canulae implantation into the NAc of BALB mice, the animals were exposed to CUMS for 4 weeks. Either PEI/*Gdnf* or PEI/*Egfp* complexes were injected on day 14 of the CUMS session. At the end of the CUMS session, SI and SPT were performed, in this order.

C. BALB mice were subjected to 6 weeks of CUMS. Suberoylanilide hydroxamic acid (SAHA), IMI, fluoxetine (FLX), or vehicle was systemically administered (25 mg/kg/day) for the last 5 days of the 6-week CUMS session and during the behavioral assays. At the end of the CUMS session, SI, FST, NSF, and SPT were performed, in this order.

D. Seven days after the bilateral injection of AAV vectors into the NAc, mice were subjected to CUMS for 4 weeks, and SI and/or SPT were performed.

E. Zebularine (ZEB) or RG108 was continuously delivered into the NAc of BALB mice by osmotic pumps. Five days after implantation of the osmotic pumps, mice were subjected to 4 weeks of CUMS. At the end of the CUMS session, SI, FST, NSF, and SPT were performed, in this order.

Figure S2. Effects of CUMS on depression-like behaviors in BALB mice

BALB mice were subjected to a 6-week CUMS session and were either given IMI (18 mg/kg/d) or water for the last 3 weeks of the CUMS session and during the behavioral assays. The sucrose preference test (A and B, n = 28-32 per group), forced swim test (C–E, n = 28-32 per group), novelty-suppressed feeding test (F–H, n = 28-32 per group),

and social interaction test (I and J, n = 23-26 per group) were performed. Stressed mice that were given water scored significantly lower on the sucrose preference test compared with non-stressed mice (NS), but this finding was reversed by continuous IMI treatment (A) with no observable difference in total fluid intake (B). The immobility times (C) and the first immobility bout (E) for stressed mice receiving water were significantly greater than those for NS-water mice, but this increase was reversed with continuous IMI treatment. Latencies to immobility were decreased by CUMS, but this reduction was also reversed by continuous IMI treatment (D). The latency to feed in the novelty-suppressed feeding test was significantly increased by CUMS, but this increase was reversed by continuous IMI treatment (F), with no observable difference in food consumption (G) or weight loss (H) among the groups. Social interaction times (I) and the number of initiations (J) in the social interaction test were significantly decreased by CUMS, but these reductions were reversed with continuous IMI treatment. *$p < 0.05$.

Figure S3. Effects of CUMS on depression-like behaviors in B6 mice

B6 mice were subjected to a 6-week CUMS session, after which the sucrose preference test (A and B, n = 24-28 for each group), forced swim test (C and D, n = 24-28 per group), novelty-suppressed feeding test (E and F, n = 24-28 per group), and social interaction test (G and H, n = 19-22 per group) were performed. CUMS had no effect on sucrose preference and forced swim tests (A–D). The latency to feed in the novelty-suppressed feeding test was significantly decreased by CUMS (E) with no observable differences in food consumption (F). Social interaction times (G), but not the number of interactions (H), were significantly greater for stressed mice than non-stressed (NS) mice. *$p < 0.05$.

Figure S4. Effects of CUMS on plasma corticosterone (CORT) levels

Plasma CORT levels in mice subjected to short-term (3 days) and long-term (38 days) stress were determined using an EIA assay. (A) In BALB mice, the plasma CORT levels were significantly greater than for the non-stressed (NS) condition at both timepoints (ANOVA, $F_{(2,17)} = 94.22$, $p < 0.001$; *post-hoc*, NS vs day 3, $p < 0.001$ and NS vs 38 day, $p < 0.001$). (B) Plasma CORT levels in B6 mice subjected long-term stress were comparable to those of animals in the NS condition (ANOVA, $F_{(2,18)} = 50.18$, $p < 0.001$; *post-hoc*, NS vs day 3, $p < 0.001$ and NS vs 38 day, $p > 0.05$). n = 6-8 per group. *$p <$

0.01 versus NS.

Figure S5. Effect of CUMS on mRNA expression for a variety of neurotrophic factors

mRNA expression levels of *Gdnf* (A), *Bdnf* (B), *Cdnf (C)*, *Vegf* (D), *Nt-3* (E), *Nt-4/5* (F), *Fgf2* (G), *Igf1* (H), and *Ngf* (I) in the striatum (STR; including both the dorsal and the ventral regions), hippocampus (HP), prefrontal cortex (PFC), amygdala (AMY), and hypothalamus (HYP) of BALB mice subjected to CUMS or the non-stress (NS) conditions with or without continuous imipramine (IMI) treatment (n = 6 per group). **p* < 0.05 versus NS mice receiving vehicle (normal water).

Figure S6. Effect of CUMS on HDAC2 occupancy at the *Bdnf* promoter

(A) ChIP assay revealed that HDAC2 levels at the *Bdnf* promoter II were not affected by CUMS and continuous IMI treatment in BALB mice. (B) Q-PCR revealed that the levels of *Bdnf exon2* mRNA were not affected by CUMS and continuous IMI treatment. n = 6-8 per group.

Figure S7. Effects of HDAC2 overexpression on depression-like behaviors and *Gdnf* expression in BALB mice

(A) Either AAV-HDAC2 or AAV-HDAC2 C262/274A was injected bilaterally into the NAc of non-stressed BALB mice. HA-HDAC2 and HA-HDAC2 C262/274A protein levels were detected with western blotting.

(B–D) AAV-mediated overexpression of HDAC2 or HDAC2 C262/274A in the NAc of non-stressed BALB mice did not affect (B) social interaction times (n = 9-11 per group), (C) sucrose preference (n = 11-12 per group), or (D) *Gdnf* mRNA expression (n = 6-8 per group).

Figure S8. Role of DNA methylation and MeCP2 on *Gdnf* transcription

A. Treating Neuro2a cells with 5-aza-2′-deoxycytidine (5aza-dC) decreased the levels of DNA methylation at the *Gdnf* gene promoter. **p* < 0.05.

B. Treating Neuro2a cells with 5aza-dC strongly induced *Gdnf* mRNA expression.

C. Luciferase activities of the CpG site 2-specific methylated or non-methylated reporter vectors in response to MeCP2 and HDAC2 overexpression or normal

expression in Neuro2a cells.

D. The *Gdnf* promoter sequence adjacent to CpG site 2 (shown in blue). The potential A/T motifs (shown in red) and the sequences of the mutant reporters are shown.

E. Non-methylated and methylated wild-type (wt), m1, m2, and m3 reporter activities were measured either with or without MeCP2 and HDAC2 overexpression. $*p < 0.05$.

F. Neuro2a cells were transfected with a CpG site 2-specific *Gdnf* reporter and HDAC2 expression vectors together with MBD1, MBD2, MBD3, or MeCP2 expression vectors. MeCP2 strongly repressed the activity of the site-specific methylated *Gdnf* reporter.

Supplemental Figures

Figure S1

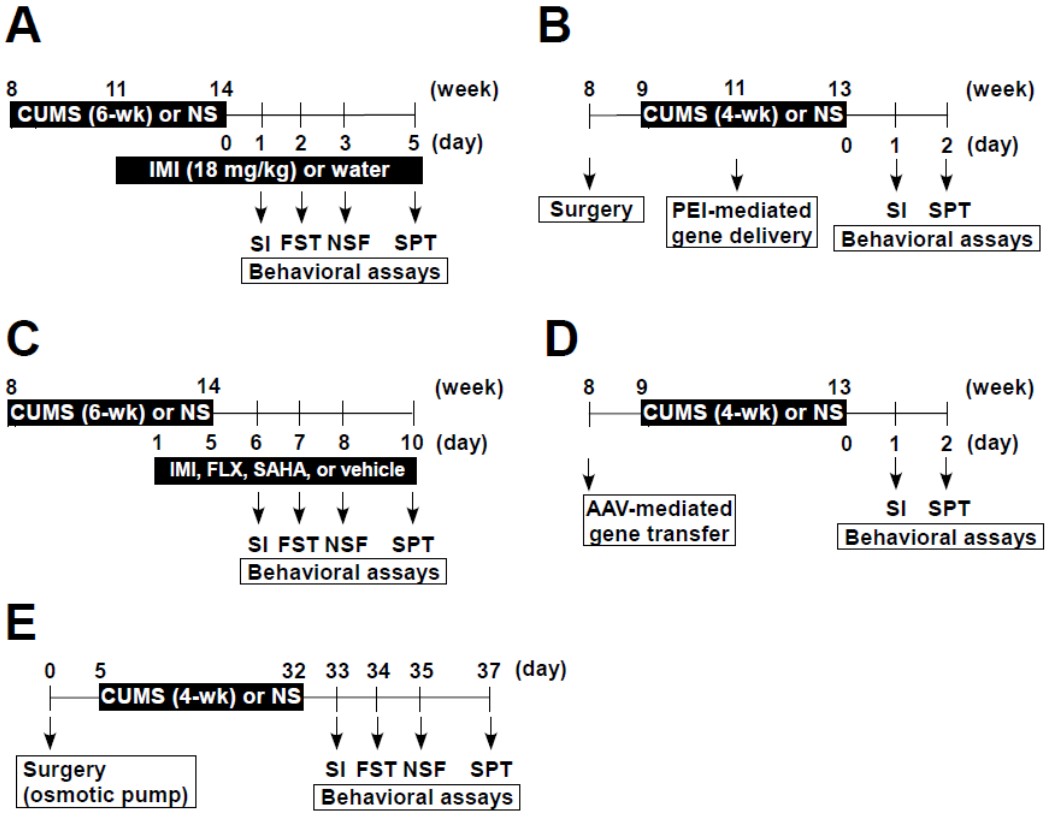
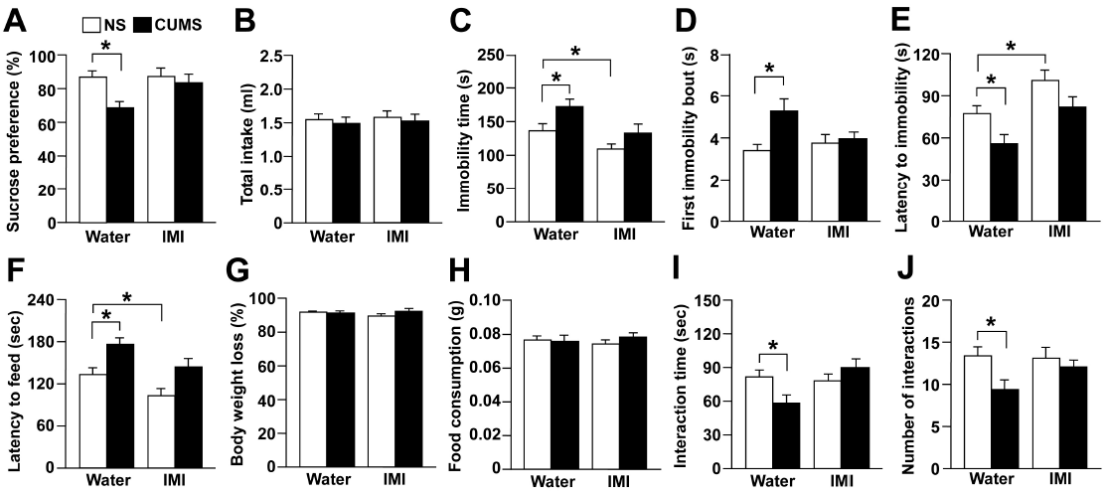


Figure S2



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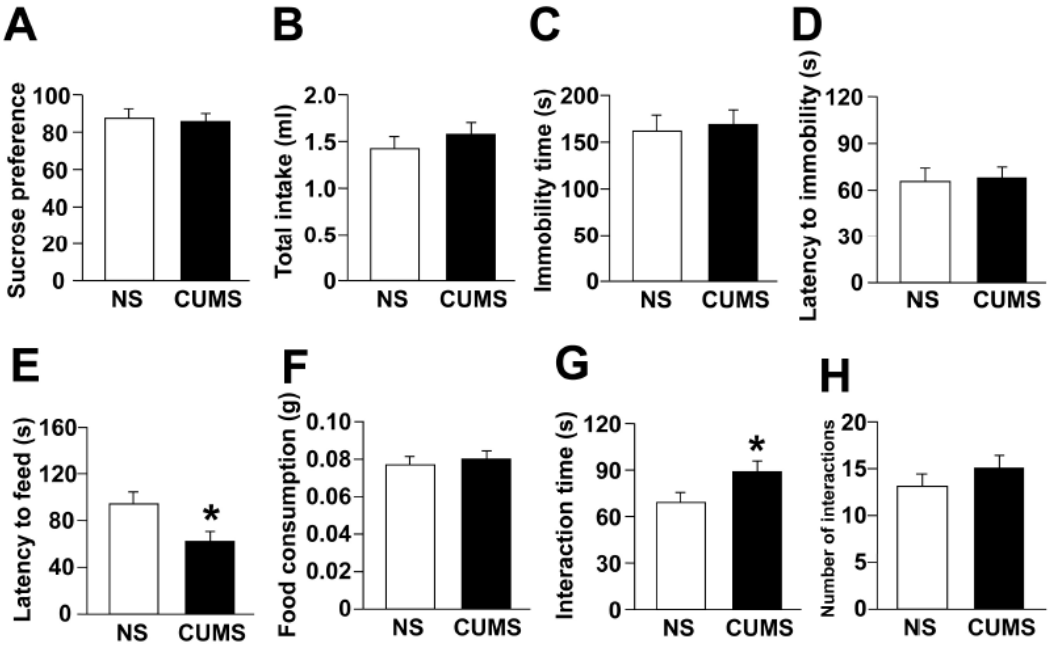

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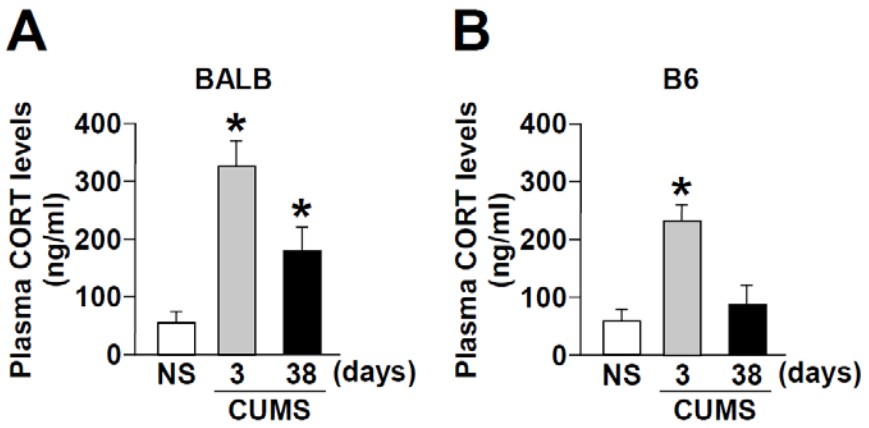
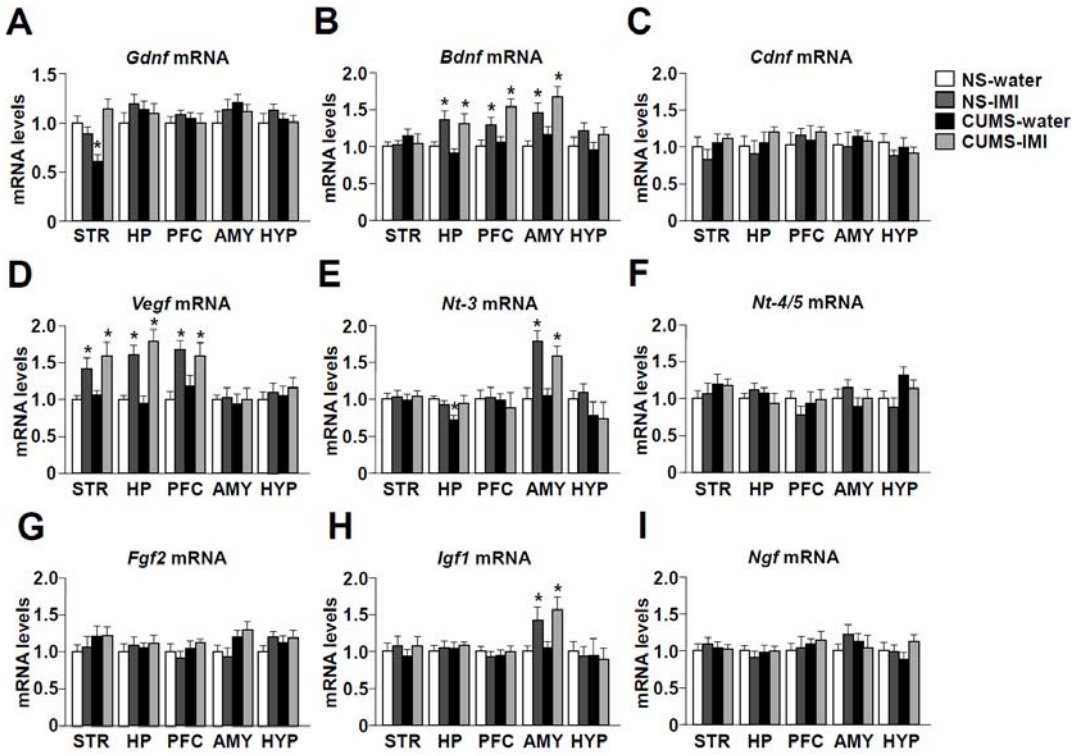


Figure S5



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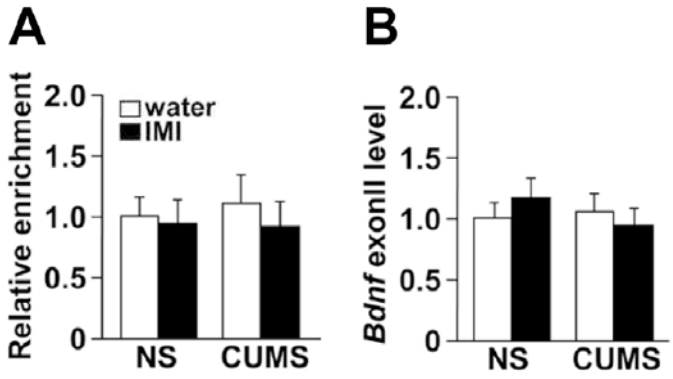

Figure S7

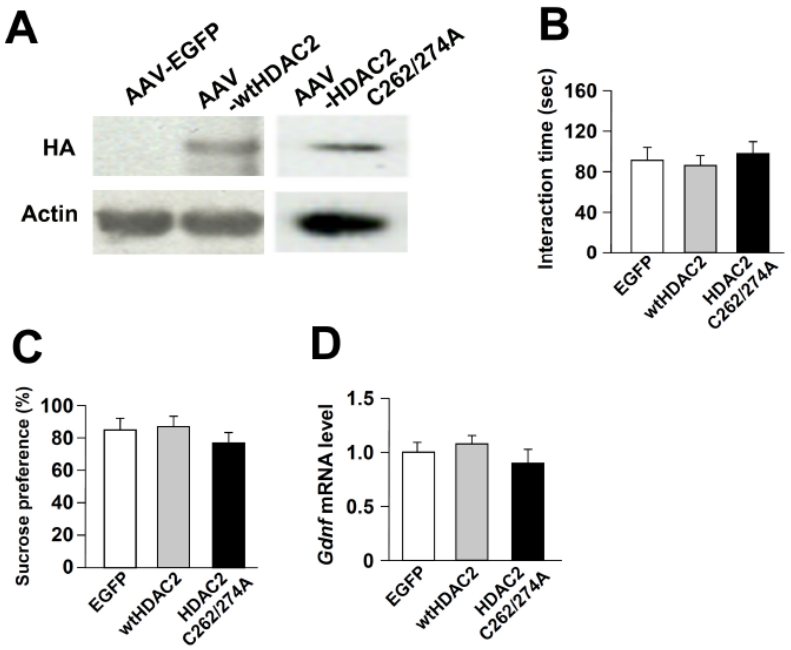

Figure S8

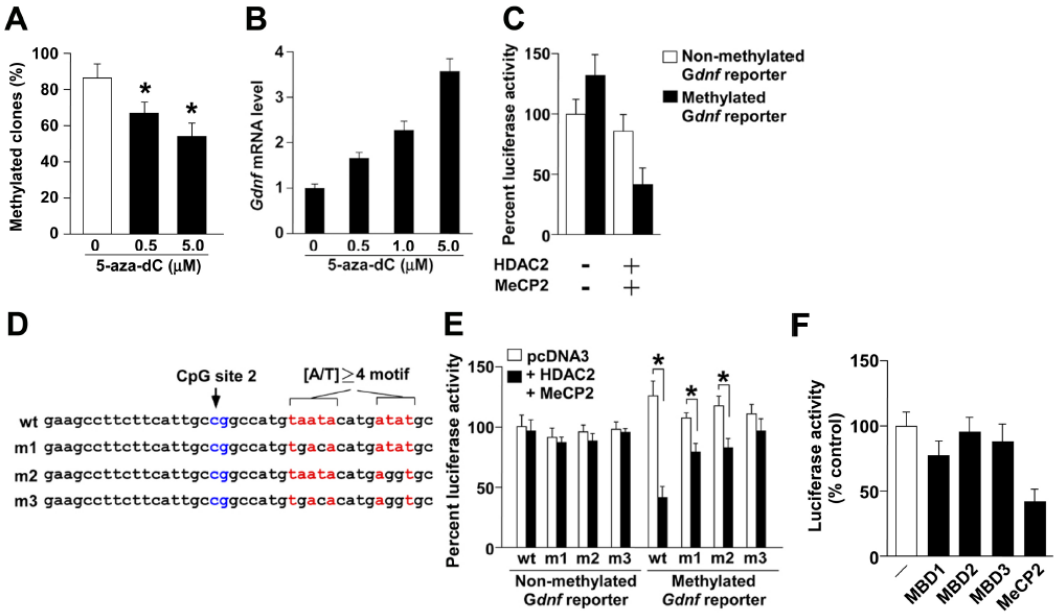

Table S1: Complete statistical summary analysis of behavioral data.

Behavioral Paradigm	Strain	Measurement	Statistical Test	Comparison	Statistics	P value	Fig.
Sucrose preference test	BALB	Sucrose preference	2-way ANOVA	Factor 1: Stress	F(1,116)=14.1	<0.01	S2A
				Factor 2: Drug	F(1,116)=5.5	<0.05	
				Interaction (F1 x F2)	F(1,116)=10.4	<0.05	
			Post-hoc test	NS-water vs CUMS-water		<0.01	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.01	
		Total intake (water + sucrose)	2-way ANOVA	Factor 1: Stress	F(1,116)= 0.2	>0.05	S2B
				Factor 2: Drug	F(1,116)= 0.2	>0.05	
				Interaction (F1 x F2)	F(1,116)= 0.02	>0.05	
Forced swim test	BALB	Immobility time	2-way ANOVA	Factor 1: Stress	F(1,116)=15.3	<0.01	S2C
				Factor 2: Drug	F(1,116)=10.7	<0.01	
				Interaction (F1 x F2)	F(1,116)=1.72	>0.05	
			Post-hoc test	NS-water vs CUMS-water		<0.001	
				NS-water vs NS-IMI		<0.05	
				CUMS-water vs CUMS-IMI		<0.01	
		Latency to immobility	2-way ANOVA	Factor 1: Stress	F(1,116)=10.8	<0.01	S2E
				Factor 2: Drug	F(1,116)=18.4	<0.01	
				Interaction (F1 x F2)	F(1,116)=2.1	>0.05	
			Post-hoc test	NS-water vs		<0.01	

				CUMS-water			
				NS-water vs NS-IMI		<0.05	
				CUMS-water vs CUMS-IMI		<0.05	
		First immobility bout	2-way ANOVA	Factor 1: Stress	F(1,116)=9.9	<0.01	S2D
				Factor 2: Drug	F(1,116)=6.7	<0.02	
				Interaction (F1 x F2)	F(1,116)=4.8	<0.03	
			Post-hoc test	NS-water vs CUMS-water		<0.01	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.05	
Novelty-suppressed feeding test	BALB	Latency to feed	2-way ANOVA	Factor 1: Stress	F(1,116)=12.9	<0.01	S2F
				Factor 2: Drug	F(1,116)=13.7	<0.01	
				Interaction (F1 x F2)	F(1,116)=0.7	>0.05	
			Post-hoc test	NS-water vs CUMS-water		<0.01	
				NS-water vs NS-IMI		<0.05	
				CUMS-water vs CUMS-IMI		<0.01	
		Food intake	2-way ANOVA	Factor 1: Stress	F(1,116)=1.2	>0.05	S2H
				Factor 2: Drug	F(1,116)=1.8	>0.05	
				Interaction (F1 x F2)	F(1,116)=0.5	>0.05	
		Body weight loss	2-way ANOVA	Factor 1: Stress	F(1,116)=0.004	>0.05	S2G
				Factor 2: Drug	F(1,116)=0.6	>0.05	
				Interaction	F(1,116)=0.1	>0.05	

				(F1 x F2)			
Social interaction test	BALB	Social interaction time	2-way ANOVA	Factor 1: Stress	F(1,94)=0.8	>0.05	S2I
				Factor 2: Drug	F(1,94)=4.8	<0.05	
				Interaction (F1 x F2)	F(1,94)=11.8	<0.001	
			Post-hoc test	NS-water vs CUMS-water		<0.05	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.001	
		Total numbers of interactions	2-way ANOVA	Factor 1: Stress	F(1,94)=21.9	<0.001	S2J
				Factor 2: Drug	F(1,94)=7.3	<0.01	
				Interaction (F1 x F2)	F(1,94)=4.4	<0.05	
			Post-hoc test	NS-water vs CUMS-water		<0.001	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.01	
Sucrose preference	B6	Sucrose preference	Unpaired t test		t = 1.02	>0.05	S3A
		Total intake (water + sucrose)	Unpaired t test		t = -0.75	>0.05	S3B
Forced swim test	B6	Immobility time	Unpaired t test		t = -0.09	>0.05	S3C
		Latency to immobility	Unpaired t test		t = -0.11	>0.05	S3D
Novelty-suppressed feeding test	B6	Latency to feed	Unpaired t test		t = 5.13	<0.001	S3E
		Food intake	Unpaired t test		t = -0.28	>0.05	S3F

Social interaction test	B6	Interaction time	Unpaired t test		t = -2.32	<0.05	S3G
		The number of interactions	Unpaired t test		t = -0.38	>0.05	S3H
Social interaction test	BALB-CUMS	Social interaction time	Unpaired t test		t = -2.38	<0.01	1J
	B6-NS	Social interaction time	Unpaired t test		t = -3.55	<0.01	
Sucrose preference test	BALB-CUMS	Sucrose preference	Unpaired t test		t = -2.12	<0.05	IK
	B6-NS	Sucrose preference	Unpaired t test		t = 0.94	>0.05	
Social interaction test	BALB-NS	Social interaction time	One-way ANOVA		F(3,66)=2.068	>0.05	3A
	BALB-CUMS	Social interaction time	One-way ANOVA		F(3,69)=8.99	<0.001	
			Post-hoc test	vehicle vs IMI		>0.05	
				vehicle vs FLX		>0.05	
				vehicle vs SAHA		<0.001	
Forced swim test	BALB-NS	Immobility time	One-way ANOVA		F(3,70)=11.47	<0.001	3D
			Post-hoc test	vehicle vs IMI		>0.05	
				vehicle vs FLX		>0.05	
				vehicle vs SAHA		<0.001	
	BALB-CUMS	Immobility time	One-way ANOVA		F(3,74)=8.06	<0.001	
			Post-hoc test	vehicle vs IMI		>0.05	
				vehicle vs FLX		>0.05	
				vehicle vs SAHA		<0.001	
Novelty-suppressed feeding test	BALB-NS	Latency to feed	One-way ANOVA		F(3,70)=3.89	<0.05	3C
			Post-hoc test	vehicle vs IMI		>0.05	
				vehicle vs FLX		>0.05	

				vehicle vs SAHA		<0.05	
	BALB-CUMS	Latency to feed	One-way ANOVA		F(3,74)=5.56	<0.001	
			Post-hoc test	vehicle vs IMI		>0.05	
				vehicle vs FLX		>0.05	
				vehicle vs SAHA		<0.01	
Sucrose preference test	BALB-NS	Sucrose preference	One-way ANOVA		F(3,70)=0.62	>0.01	3B
	BALB-CUMS	Sucrose preference	One-way ANOVA		F(3,74)=8.28	<0.001	
			Post-hoc test	vehicle vs IMI		>0.05	
				vehicle vs FLX		>0.05	
				vehicle vs SAHA		<0.001	
Social interaction test	BALB	Social interaction time	Unpaired *t* test	AAV-EGFP vs AAV-dnHDAC2	t = -3.21	<0.01	3H
Sucrose preference test	BALB	Sucrose preference	Unpaired *t* test	AAV-EGFP vs AAV-dnHDAC2	t = -2.51	<0.05	3I
Social interaction test	B6-NS	Social interaction time	Unpaired *t* test		t = 1.48	>0.05	3K
	B6-CUMS	Social interaction time	One-way ANOVA		F(2,41)=5.53	<0.01	
			Post-hoc test	EGFP vs wtHDAC2		>0.05	
				EGFP vs HDAC2 C262/274A		<0.01	
Social interaction test	BALB	Social interaction time	One-way ANOVA		F(2,27)=1.12	>0.05	S7B

Sucrose preference test	BALB	Sucrose preference	One-way ANOVA		F(2,31)=2.12	>0.05	S7C
Social interaction test	BALB-NS	Social interaction time	One-way ANOVA		F(2,28)=0.32	>0.05	6A
	BALB-CUMS	Social interaction time	One-way ANOVA		F(2,31)=6.16	<0.01	
			Post-hoc test	Vehicle vs ZEB (10μM)		>0.05	
				Vehicle vs ZEB (100μM)		<0.01	
Sucrose preference test	BALB-NS	Sucrose preference	One-way ANOVA		F(2,31)=0.83	>0.05	6B
	BALB-CUMS	Social interaction time	One-way ANOVA		F(2,31)=10.25	<0.001	
			Post-hoc test	Vehicle vs ZEB (10μM)		>0.05	
				Vehicle vs ZEB (100μM)		<0.01	
Novelty-suppressed feeding test	BALB-NS	Latency to feed	One-way ANOVA		F(2,31)=2.41	>0.05	6C
	BALB-CUMS	Latency to feed	One-way ANOVA		F(2,31)=5.45	<0.01	
			Post-hoc test	Vehicle vs ZEB (10μM)		>0.05	
				Vehicle vs ZEB (100μM)		<0.05	
Forced swim test	BALB-NS	Immobility time	One-way ANOVA		F(2,31)=13.18	<0.01	6D
			Post-hoc test	Vehicle vs ZEB (10μM)		>0.05	
				Vehicle vs ZEB (100μM)		<0.01	
	BALB-CUMS	Immobility time	One-way ANOVA		F(2,31)=7.99	<0.01	

			Post-hoc test	Vehicle vs ZEB (10μM)		>0.05	
				Vehicle vs ZEB (100μM)		<0.01	
Social interaction time	BALB-CUMS	Social Interaction time	One-way ANOVA		F(2,29)=3.52	<0.05	6G
			Post-hoc test	Vehicle vs RG108 (100 μM)		<0.05	
Sucrose preference test	BALB-CUMS	Sucrose preference	One-way ANOVA		F(2,35)=4.95	<0.05	6H
			Post-hoc test	Vehicle vs RG108 (100 μM)		<0.05	

Abbreviations: NS; non-stress, CUMS; chronic ultra-mild stress, IMI; imipramine, FLX; fluoxetine, SAHA; suberoylanilide hydroxamic acid, ZEB; zebularine, BALB; BALB/c, B6; C57BL/6.

Table S2: Complete statistical summary analysis of expression data.

Target (Method)	Strain	Region	Statistical Test	Comparison	Statistics	P value	Fig.
Gdnf mRNA (Q-PCR)	BALB	STR	2-way ANOVA	Factor 1: Stress	F(1,20)=6.2	<0.05	S5A
				Factor 2: Drug	F(1,20)=33.7	<0.001	
				Interaction (F1 x F2)	F(1,20)=25.5	<0.001	
			Post-hoc test	NS-water vs CUMS-water		<0.001	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.001	
		HP	2-way ANOVA	Factor 1: Stress	F(1,20)=0.02	>0.05	
				Factor 2: Drug	F(1,20)=0.06	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.01	>0.05	
		PFC	2-way ANOVA	Factor 1: Stress	F(1,20)=0.13	>0.05	
				Factor 2: Drug	F(1,20)=0.58	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.004	>0.05	
		AMY	2-way ANOVA	Factor 1: Stress	F(1,20)=0.26	>0.05	
				Factor 2: Drug	F(1,20)=0.0001	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.34	>0.05	
		HYP	2-way ANOVA	Factor 1: Stress	F(1,20)=0.46	>0.05	
				Factor 2: Drug	F(1,20)=0.21	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.02	>0.05	
Bdnf mRNA (Q-PCR)	BALB	STR	2-way ANOVA	Factor 1: Stress	F(1,20)=0.07	>0.05	S5B
				Factor 2: Drug	F(1,20)=0.16	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.03	>0.05	
		HP	2-way ANOVA	Factor 1: Stress	F(1,20)=0.06	>0.05	
				Factor 2: Drug	F(1,20)=19.4	<0.05	
				Interaction	F(1,20)=0.06	>0.05	

			Post-hoc test	(F1 x F2)			
				NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.05	
				CUMS-water vs CUMS-IMI		<0.05	
		PFC	2-way ANOVA	Factor 1: stress	F(1,20)=3.37	>0.05	
				Factor 2: Drug	F(1,20)=29.09	<0.001	
				Interaction (F1 x F2)	F(1,20)=0.11	>0.05	
			Post-hoc test	NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.05	
				CUMS-water vs CUMS-IMI		<0.01	
		AMY	2-way ANOVA	Factor 1: stress	F(1,20)=0.09	>0.05	
				Factor 2: Drug	F(1,20)=20.45	<0.001	
				Interaction (F1 x F2)	F(1,20)=1.12	>0.05	
			Post-hoc test	NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.05	
				CUMS-water vs CUMS-IMI		<0.01	
		HYP	2-way ANOVA	Factor 1: stress	F(1,20)=0.73	>0.05	
				Factor 2: Drug	F(1,20)=0.09	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.69	>0.05	
Cdnf mRNA (Q-PCR)	BALB	STR	2-way ANOVA	Factor 1: stress	F(1,20)=0.002	>0.05	S5C
				Factor 2: Drug	F(1,20)=0.06	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.22	>0.05	

		HP	2-way ANOVA	Factor 1: stress	F(1,20)=0.06	>0.05	
				Factor 2: Drug	F(1,20)=0.19	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.45	>0.05	
		PFC	2-way ANOVA	Factor 1: stress	F(1,20)=0.31	>0.05	
				Factor 2: Drug	F(1,20)=0.06	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.02	>0.05	
		AMY	2-way ANOVA	Factor 1: stress	F(1,20)=0.02	>0.05	
				Factor 2: Drug	F(1,20)=1.09	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.63	>0.05	
		HYP	2-way ANOVA	Factor 1: stress	F(1,20)=2.61	>0.05	
				Factor 2: Drug	F(1,20)=0.32	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.03	>0.05	
Vegf mRNA (Q-PCR)	BALB	STR	2-way ANOVA	Factor 1: stress	F(1,20)=0.03	>0.05	S5D
				Factor 2: Drug	F(1,20)=25.98	<0.001	
				Interaction (F1 x F2)	F(1,20)=0.41	>0.05	
			Post-hoc test	NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.05	
				CUMS-water vs CUMS-IMI		<0.001	
		HP	2-way ANOVA	Factor 1: stress	F(1,20)=2.17	>0.05	
				Factor 2: Drug	F(1,20)=37.83	<0.001	
				Interaction (F1 x F2)	F(1,20)=0.21	>0.05	
			Post-hoc test	NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.01	
				CUMS-water vs CUMS-IMI		<0.01	

		PFC	2-way ANOVA	Factor 1: stress	F(1,20)=0.02	>0.05	
				Factor 2: Drug	F(1,20)=27.78	<0.001	
				Interaction (F1 x F2)	F(1,20)=0.31	<0.05	
			Post-hoc test	NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.01	
				CUMS-water vs CUMS-IMI		<0.05	
		AMY	2-way ANOVA	Factor 1: stress	F(1,20)=0.08	>0.05	
				Factor 2: Drug	F(1,20)=0.01	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.01	>0.05	
		HYP	2-way ANOVA	Factor 1: stress	F(1,20)=1.84	>0.05	
				Factor 2: Drug	F(1,20)=0.1	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.05	>0.05	
Nt-3 mRNA (Q-PCR)	BALB	STR	2-way ANOVA	Factor 1: stress	F(1,20)=0.02	>0.05	S5E
				Factor 2: Drug	F(1,20)=0.25	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.001	>0.05	
		HP	2-way ANOVA	Factor 1: stress	F(1,20)=7.14	<0.05	
				Factor 2: Drug	F(1,20)=4.47	<0.05	
				Interaction (F1 x F2)	F(1,20)=5.04	<0.05	
			Post-hoc test	NS-water vs CUMS-water		<0.05	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.05	
		PFC	2-way ANOVA	Factor 1: stress	F(1,20)=1.02	>0.05	
				Factor 2: Drug	F(1,20)=0.04	>0.05	

				Interaction (F1 x F2)	F(1,20)=0.04	>0.05	
		AMY	2-way ANOVA	Factor 1: stress	F(1,20)=0.68	>0.05	
				Factor 2: Drug	F(1,20)=19.6	<0.001	
				Interaction (F1 x F2)	F(1,20)=0.008	<0.05	
			Post-hoc test	NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.05	
				CUMS-water vs CUMS-IMI		<0.05	
		HYP	2-way ANOVA	Factor 1: stress	F(1,20)=1.00	>0.05	
				Factor 2: Drug	F(1,20)=0.67	>0.05	
				Interaction (F1 x F2)	F(1,20)=1.38	>0.05	
Nt-4/5 mRNA (Q-PCR)	BALB	STR	2-way ANOVA	Factor 1: stress	F(1,20)=0.09	>0.05	S5F
				Factor 2: Drug	F(1,20)=0.02	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.002	>0.05	
		HP	2-way ANOVA	Factor 1: stress	F(1,20)=0.03	>0.05	
				Factor 2: Drug	F(1,20)=0.22	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.001	>0.05	
		PFC	2-way ANOVA	Factor 1: stress	F(1,20)=0.03	>0.05	
				Factor 2: Drug	F(1,20)=0.02	>0.05	
				Interaction (F1 x F2)	F(1,20)=1.58	>0.05	
		AMY	2-way ANOVA	Factor 1: stress	F(1,20)=1.99	>0.05	
				Factor 2: Drug	F(1,20)=0.001	>0.05	
				Interaction (F1 x F2)	F(1,20)0.26	>0.05	
		HYP	2-way ANOVA	Factor 1: stress	F(1,20)=0.27	>0.05	
				Factor 2: Drug	F(1,20)=0.14	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.9	>0.05	

Fgf2 mRNA (Q-PCR)	BALB	STR	2-way ANOVA	Factor 1: stress	F(1,20)=1.35	>0.05	S5G
				Factor 2: Drug	F(1,20)=0.01	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.02	>0.05	
		HP	2-way ANOVA	Factor 1: stress	F(1,20)=0.13	>0.05	
				Factor 2: Drug	F(1,20)=2.37	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.92	>0.05	
		PFC	2-way ANOVA	Factor 1: stress	F(1,20)=0.09	>0.05	
				Factor 2: Drug	F(1,20)=0.69	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.7	>0.05	
		AMY	2-way ANOVA	Factor 1: stress	F(1,20)=0.05	>0.05	
				Factor 2: Drug	F(1,20)=0.17	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.38	>0.05	
		HYP	2-way ANOVA	Factor 1: stress	F(1,20)=0.101	>0.05	
				Factor 2: Drug	F(1,20)=0.007	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.42	>0.05	
Igf1 mRNA (Q-PCR)	BALB	STR	2-way ANOVA	Factor 1: stress	F(1,20)=1.24	>0.05	S5H
				Factor 2: Drug	F(1,20)=0.03	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.04	>0.05	
		HP	2-way ANOVA	Factor 1: stress	F(1,20)=0.04	>0.05	
				Factor 2: Drug	F(1,20)=1.13	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.07	>0.05	
		PFC	2-way ANOVA	Factor 1: stress	F(1,20)=0.01	>0.05	
				Factor 2: Drug	F(1,20)=0.25	>0.05	
				Interaction (F1 x F2)	F(1,20)=1.04	>0.05	
		AMY	2-way ANOVA	Factor 1: stress	F(1,20)=0.09	>0.05	
				Factor 2: Drug	F(1,20)=32.63	<0.001	

				Interaction (F1 x F2)	F(1,20)=0.17	>0.05	
			Post-hoc test	NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.01	
				CUMS-water vs CUMS-IMI		<0.01	
		HYP	2-way ANOVA	Factor 1: stress	F(1,20)=0.05	>0.05	
				Factor 2: Drug	F(1,20)=0.29	>0.05	
				Interaction (F1 x F2)	F(1,20)=1.70	>0.05	
Ngf mRNA (Q-PCR)	BALB	STR	2-way ANOVA	Factor 1: stress	F(1,20)=0.05	>0.05	S5I
				Factor 2: Drug	F(1,20)=0.54	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.02	>0.05	
		HP	2-way ANOVA	Factor 1: stress	F(1,20)=0.08	>0.05	
				Factor 2: Drug	F(1,20)=1.8	>0.05	
				Interaction (F1 x F2)	F(1,20)=1.59	>0.05	
		PFC	2-way ANOVA	Factor 1: stress	F(1,20)=0.22	>0.05	
				Factor 2: Drug	F(1,20)=0.22	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.03	>0.05	
		AMY	2-way ANOVA	Factor 1: stress	F(1,20)=0.26	>0.05	
				Factor 2: Drug	F(1,20)=0.29	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.03	>0.05	
		HYP	2-way ANOVA	Factor 1: stress	F(1,20)=0.16	>0.05	
				Factor 2: Drug	F(1,20)=0.002	>0.05	
				Interaction (F1 x F2)	F(1,20)=1.84	>0.05	
Gdnf mRNA (Q-PCR)	BALB	dSTR	2-way ANOVA	Factor 1: stress	F(1,20)=2.89	>0.05	1A
				Factor 2: Drug	F(1,20)=20.82	<0.001	
				Interaction (F1 x F2)	F(1,20)=14.79	<0.01	

			Post-hoc test	NS-water vs CUMS-water		<0.01	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.001	
		vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=7.03	<0.05	
				Factor 2: Drug	F(1,20)=14.23	<0.01	
				Interaction (F1 x F2)	F(1,20)=17.67	<0.001	
			Post-hoc test	NS-water vs CUMS-water		<0.001	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.001	
	B6	dSTR	Unpaired t test	NS vs CUMS	t = -0.80	>0.05	1B
		vSTR	Unpaired t test	NS vs CUMS	t = -3.84	<0.01	
GDNF protein (ELISA)	BALB	dSTR	2-way ANOVA	Factor 1: Stress	F(1,36)=22.27	<0.001	1C
				Factor 2: Drug	F(1,36)=28.16	<0.001	
				Interaction (F1 x F2)	F(1,36)=40.57	<0.001	
			Post-hoc test	NS-water vs CUMS-water		<0.001	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.001	
		vSTR	2-way ANOVA	Factor 1: Stress	F(1,36)=16.7	<0.001	
				Factor 2: Drug	F(1,36)=21.95	<0.001	
				Interaction (F1 x F2)	F(1,36)=18.69	<0.001	
			Post-hoc test	NS-water vs CUMS-water		<0.001	
				NS-water vs		>0.05	

				NS-IMI			
				CUMS-water vs CUMS-IMI		<0.001	
	B6	dSTR	Unpaired t test	NS vs CUMS	t = -0.55	>0.05	
		vSTR	Unpaired t test	NS vs CUMS	t = -3.55	<0.01	
Hdac1 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=2.97	>0.05	2E
				Factor 2: Drug	F(1,20)=2.53	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.53	>0.05	
Hdac2 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=55.59	<0.001	
				Factor 2: Drug	F(1,20)=38.25	<0.001	
				Interaction (F1 x F2)	F(1,20)=12.16	<0.01	
			Post-hoc test	NS-water vs CUMS-water		<0.001	
				NS-water vs NS-IMI		>0.05	
				CUMS-water vs CUMS-IMI		<0.001	
Hdac3 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=2.01	>0.05	
				Factor 2: Drug	F(1,20)=0.007	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.71	>0.05	
Hdac4 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	NS-water vs CUMS-water	F(1,20)=2.01	>0.05	
				NS-water vs NS-IMI	F(1,20)=35.08	<0.001	
				CUMS-water vs CUMS-IMI	F(1,20)=0.07	>0.05	
			Post-hoc test	NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.01	
				CUMS-water vs		<0.01	

				CUMS-IMI		
Hdac5 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=0.01	>0.05
				Factor 2: Drug	F(1,20)=44.86	<0.001
				Interaction (F1 x F2)	F(1,20)=0.35	>0.05
			Post-hoc test	NS-water vs CUMS-water		>0.05
				NS-water vs NS-IMI		<0.01
				CUMS-water vs CUMS-IMI		<0.001
Hdac6 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=0.004	>0.05
				Factor 2: Drug	F(1,20)=33.84	<0.001
				Interaction (F1 x F2)	F(1,20)=0.051	>0.05
			Post-hoc test	NS-water vs CUMS-water		>0.05
				NS-water vs NS-IMI		<0.01
				CUMS-water vs CUMS-IMI		<0.01
Hdac7 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=1.01	>0.05
				Factor 2: Drug	F(1,20)=69.13	<0.001
				Interaction (F1 x F2)	F(1,20)=0.42	>0.05
			Post-hoc test	NS-water vs CUMS-water		>0.05
				NS-water vs NS-IMI		<0.001
				CUMS-water vs CUMS-IMI		<0.001
Hdac8 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=0.17	>0.05
				Factor 2: Drug	F(1,20)=2.22	>0.05
				Interaction (F1 x F2)	F(1,20)=0.17	>0.05
Hdac9	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=1.47	>0.05

mRNA (Q-PCR)				Factor 2: Drug	F(1,20)=0.16	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.61	>0.05	
Hdac10 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=0.01	>0.05	
				Factor 2: Drug	F(1,20)=29.97	<0.001	
				Interaction (F1 x F2)	F(1,20)=0.47	>0.05	
			Post-hoc test	NS-water vs CUMS-water		>0.05	
				NS-water vs NS-IMI		<0.01	
				CUMS-water vs CUMS-IMI		<0.05	
Hdac11 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=0.09	>0.05	
				Factor 2: Drug	F(1,20)=0.41	>0.05	
				Interaction (F1 x F2)	F(1,20)=1.23	>0.05	
HDAC2 (Western blotting)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,26)=11.41	<0.01	2F
				Factor 2: Drug	F(1,26)=3.48	>0.05	
				Interaction (F1 x F2)	F(1,26)=6.49	<0.05	
			Post-hoc test	NS-water vs CUMS-water		<0.01	
				NS-water vs NS-IMI		>0.05	
				NS-water vs NS-IMI		<0.05	
		HP	2-way ANOVA	Factor 1: Stress	F(1,26)=0.49	>0.05	2G
				Factor 2: Drug	F(1,26)=1.89	>0.05	
				Interaction (F1 x F2)	F(1,26)=0.06	>0.05	
Hdac2 mRNA (Q-PCR)	B6	vSTR	Unpaired t test		t = 0.81	>0.05	2H

HDAC2 (Western blotting)	B6	vSTR	Unpaired t test		t = 0.08	>0.05	
Bdnf exon2 mRNA (Q-PCR)	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=0.06	>0.05	S6B
				Factor 2: Drug	F(1,20)=0.07	>0.05	
				Interaction (F1 x F2)	F(1,20)=0.009	>0.05	
Gdnf mRNA (Q-PCR)	BALB-NS	vSTR	One-way ANOVA		F(3,26)=0.77	>0.05	3E
	BALB-CUMS	vSTR	One-way ANOVA		F(3,28)=8.56	<0.001	
			Post-hoc test	vehicle vs IMI		>0.05	
				vehicle vs FLX		>0.05	
				vehicle vs SAHA		<0.001	
	BALB	vSTR	Unpaired t test	AAV-EGFP vs AAV-dnHDAC2	t = -5.62	<0.001	3J
	B6-NS	vSTR	Unpaired t test		t = 1.19	>0.05	3L
	B6-CUMS	vSTR	One-way ANOVA		F(2,21)=4.65	<0.05	
			Post-hoc test	EGFP vs wtHDAC2		>0.05	
				EGFP vs HDAC2 C262/274A		<0.05	
	BALB	vSTR	One-way ANOVA		F(2,19)=2.46	>0.05	S7D
	BALB-NS	vSTR	One-way ANOVA		F(2,18)=1.24	>0.05	6E
	BALB-CUMS	vSTR	One-way ANOVA		F(2,18)=4.31	<0.05	
			Post-hoc test	vehicle vs ZEB (10 μM)		> 0.05	
				vehicle vs ZEB (100 μM)		<0.05	
Dnmt1 mRNA	BALB	vSTR	One-way ANOVA		F(3,25)=5.12	<0.01	6I
			Post-hoc test	NS-vehicle vs CUMS-vehicle		<0.01	

				CUMS-vehicle vs CUMS-ZEB		<0.01	
				CUMS-vehicle vs CUMS-RG108		<0.01	
Dnmt3a mRNA	BALB	vSTR	One-way ANOVA		F(3,25)=6.78	<0.01	
			Post-hoc test	NS-vehicle vs CUMS-vehicle		<0.05	
				CUMS-vehicle vs CUMS-ZEB		<0.05	
				CUMS-vehicle vs CUMS-RG108		<0.05	
Dnmt3b mRNA	BALB	vSTR	One-way ANOVA		F(3,25)=0.25	>0.05	

Abbreviations: Q-PCR; quantitative real-time PCR, NS; non-stress, CUMS; chronic ultra-mild stress, IMI; imipramine, FLX; fluoxetine, SAHA; suberoylanilide hydroxamic acid, ZEB; zebularine, BALB; BALB/c, B6; C57BL/6, HP; hippocampus, vSTR; ventral striatum, dSTR; dorsal striatum, AMY; amygdala, HYP; hypothalamus, PFC; prefrontal cortex.

Table S3: Complete statistical summary analysis of ChIP data.

Target	Ab.	Strain	Region	Statistical Test	Comparison	Statistics	P value	Fig.
Gdnf promoter	AcH3	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=7.61	<0.05	2A
					Factor 2: Drug	F(1,20)=29.48	<0.001	
					Interaction (F1 x F2)	F(1,20)=24.09	<0.001	
				Post-hoc test	NS-water vs CUMS-water		<0.001	
					NS-water vs NS-IMI		>0.05	
					CUMS-water vs CUMS-IMI		<0.001	
		B6	vSTR	Unpaired t test	NS vs CUMS	t = -5.22	<0.001	
	AcH4	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=0.27	>0.05	2B
					Factor 2: Drug	F(1,20)=2.23	>0.05	
					Interaction (F1 x F2)	F(1,20)=1.79	>0.05	
		B6	vSTR	Unpaired t test	NS vs CUMS	t = -1.26	>0.05	
	H3K27 me3	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=0.02	>0.05	2C
					Factor 2: Drug	F(1,20)=3.03	>0.05	
					Interaction (F1 x F2)	F(1,20)=0.13	>0.05	
		B6	vSTR	Unpaired t test	NS vs CUMS	t = 2.75	<0.05	
	H3K4 me3	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,20)=5.97	<0.05	2D
					Factor 2: Drug	F(1,20)=2.98	>0.05	
					Interaction (F1 x F2)	F(1,20)=0.79	>0.05	
				Post-hoc	NS-water vs		< 0.05	

				test	CUMS-water			
		B6	vSTR	Unpaired t test	NS vs CUMS	t = 3.11	<0.05	
	HDAC 2	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,27)=5.38	<0.05	2I
					Factor 2: Drug	F(1,27)=3.93	>0.05	
					Interaction (F1 x F2)	F(1,27)=4.45	<0.05	
				Post-hoc test	NS-water vs CUMS-water		<0.01	
					NS-water vs NS-IMI		>0.05	
					CUMS-water vs CUMS-IMI		>0.05	
		B6	vSTR	Unpaired t test	NS vs CUMS	t = -1.11	>0.05	2J
Bdnf promoter 2	HDAC 2	BALB	vSTR	2-way ANOVA	Factor 1: Stress	F(1,27)=0.33	>0.05	S6A
					Factor 2: Drug	F(1,27)=0.08	>0.05	
					Interaction (F1 x F2)	F(1,27)=0.004	>0.05	
Gdnf promoter	MeCP2	B6	HP and vSTR	Unpaired t test	HP vs vSTR	t= 3.61	<0.05	4H
		BALB	vSTR	One-way ANOVA		F(2,20)=5.73	<0.05	4I
				Post-hoc test	NS-water vs CUMS-water		<0.05	
					CUMS-water vs CUMS-IMI		>0.05	
		B6	vSTR	One-way ANOVA		t = -3.08	<0.01	
Bdnf promoter 2	MeCP2	BALB	vSTR	One-way ANOVA		F(2,20)=0.06	>0.05	
Gdnf promoter	Re-ChIP	BALB	vSTR	One-way ANOVA		F(2,15)=7.25	<0.01	5C

				Post-hoc test	NS-water vs CUMS-water		<0.01	
					CUMS-water vs CUMS-IMI		<0.05	
		B6	vSTR	Unpaired t test	NS vs CUMS	t = 0.09	>0.05	
Bdnf promoter	Re-ChIP	BALB	vSTR	One-way ANOVA		F(2,15)=0.34	>0.05	
Gdnf promoter	Re-ChIP	B6 and BALB	vSTR	Two-way ANOVA	Factor 1: Stress	F(1,15)=7.78	<0.01	7D
					Factor 2: Strain	F(1,15)=9.67	<0.05	
					Interaction (F1 x F2)	F(1,15)=11.34	<0.01	
				Post-hoc test	B6-NS vs B6-CUMS		<0.01	

Abbreviations: NS; non-stress, CUMS; chronic ultra-mild stress, IMI; imipramine, BALB; BALB/c, B6; C57BL/6, vSTR; ventral striatum; HP, hippocampus.

Table S4: List of all primer sequences used in Q-PCR and ChIP assay.

Experiment	Gene	Sequence (5' to 3')
Q-PCR	*Gdnf*	F: GGATGGGATTCGGGCCACT
		R: AGCCACGACATCCCATAACTTC
	Bdnf	F: GAGGGCTCCTGCTTCTCAA
		R: GCCTTCATGCAACCGAAGT
	Cdnf	F: TGCCGTGAAGATTTGTGAGA
		R: TCTGCCACTCTCATCTTCCA
	Vegf	F: GAGGATGTCCTCACTCGGATG
		R: GTCGTGTTTCTGGAAGTGAGCAA
	Nt-3	F: CCGGTGGTAGCCAATAGAACC
		R: GCTGAGGACTTGTCGGTCAC
	Nt-4/5	F: GAGGCACTGGCTCTCAGAAT
		R: CGAATCCAGCGCCAGC
	Ngf	F: CAGAACCGTACACAGATAGC
		R: CTGTGTCTATCCGGTGAAC
	Igf1	F: GTGTGGACCGAGGGGCTTTT
		R: GCTTCAGTGGGGCACAGTAC
	Fgf2	F: CCAACCGGTACCTTGCTA TG
		R: TATGGCCTTCTGTCCAGGTC
	Hdac1	F: TGCGTGGAAAGAAAACAACC
		R: ACCCAGACCCCTCCTAAATG
	Hdac2	F: GGGACAGGCTTGGTTGTTTC
		R: GAGCATCAGCAATGGCAAGT
	Hdac3	F: AGAGAGGTCCCGAGGAGAAC
		R: ACTCTTGGGGACACAGCATC
	Hdac4	F: CAATCCCACAGTCTCCGTGT
		R: CAGCACCCCACTAAGGTTCA
	Hdac5	F: TGTCACCGCCAGATGTTTTG
		R: TGAGCAGAGCCGAGACACAG
	Hdac6	F: TCCTCAGCTGTGTTGACCTG
		R: TGTCCTCCCCAAACTTGTTC
	Hdac7	F: GGTGGACCCCCTTTCAGAAG
		R: TGGGTAGCCAGGAGTCTGGA
	Hdac8	F: AGCCATCAACTGGTCTGGAG
		R: CCAGGACAGCATCATTGAGA

	Hdac9	F: GCGAGACACAGATGCTCAGAC
		R: TGGGTTTTCCTTCCATTGCT
	Hdac10	F: CCACTCCAGAGGAGATCCAG
		R: GCGACTGGCAATCACTGTTA
	Hdac11	F: TCATGGGTGACAAGCGAGTA
		R: CTCATCTTCTGTGCCCCACT
	Bdnf exon2	F: CTAGCCACCGGGGTGGTGTAA
		R: AGGATGGTCATCACTCTTCTC
	Dnmt1	F: CCATTGGCCTGGAGATTAAG
		R: GGCTCTGGGTGAGAGCACTA
	Dnmt3a	F: GAGGGAACTGAGACCCAC
		R: CTGGAAGGTGAGTCTTGGCA
	Dnmt3b	F: GCCCATGCAATGATCTCTCT
		R: CCAGAAGAATGGACGGTTGT
	Gapdh	F: AGGTCGGTGTGAACGGATTTG
		R: TGTAGACCATGTAGTTGAGGTCA
	β-actin	F: AAGATGACCCAGATCATGTTTGAGAC
		R: CTGCTTGCTGATCCACATCTGCTGG
ChIP and Re-ChIP assay	*Gdnf* gene (for H3ac, H4ac, H3-K27met3, and H3-K4met3)	F: CACGTCACGCAGTGAGAGCT
		R: AGAAGACAAGCAGCCTGCAC
	Gdnf gene (for HDAC2 and MeCP2)	F: CAGCATGGAAATGAAGCCTA
		R: TAGTTTAGTCCCCAGGCTAG
	Gdnf exon3	F: GATATTGCAGCGGTTCCTGT
		R: AACATGCCTGGCCTACTTTG
	Bdnf promoter 2	F: CCGTCTTGTATTCCATCCTTTG
		R: CCCAACTCCACCACTATCCTC

Supplemental Experimental Procedures

Behavioral procedures

Sucrose preference test. Mice were habituated to drink water from two bottles for 7 days. Mice were then submitted to 48 h of forced exposure to 1% sucrose solution to avoid neophobia. Mice were submitted to water deprivation for 16 h prior to performing the sucrose preference test. Then, two preweighted bottles (one containing tap water and another containing 1% sucrose solution) were presented to each mouse for 4 h. The positions of the water and sucrose bottles (left or right) were switched every 2 h. The bottles were weighed again and the weight difference was considered to be the mouse intake from each bottle. The sum of water and sucrose intake was defined as the total intake, and sucrose preference was expressed as the percentage of sucrose intake from the total intake.

Forced swim test. Mice were placed in a water tank (25 cm high × 15 cm in diameter filled with water at 23 °C to a depth of 15 cm) for 5 min and the duration of floating (i.e., the time during which the mouse made only small movements necessary to keep its head above water), latency period until the first episode of immobility, and duration of first immobility (first immobility bout) were scored.

Novelty-suppressed feeding test. Mice were singly housed, and food pellets were removed from their cages on the day before testing. Twenty-four hours after food removal, the percent loss of body weight of mice was estimated, and mice were transferred to a clean holding cage in the testing room. The testing apparatus consisted of square open field (25 cm long × 20 cm wide × 20 cm high). A piece of chow was placed in the center of the testing apparatus. Each mouse was placed in the testing apparatus and the time until the first feeding episode was recorded for up to 5 min. After termination of the test, the mouse was returned to its home cage with food pellets, and the amount of food consumed was measured for 30 min.

Social interaction test. Each mouse was placed in a measuring cage for 120 min. A male juvenile (4–5 weeks old) was then introduced into the cage and the amount of time spent in social interaction (e.g., grooming, licking, sniffing, or crawling over or under the other mouse) with the testing adult mouse was recorded during a 3 min session.

Drugs

For continuous treatments, IMI was dissolved in tap water at a concentration of 160 mg/l. This concentration was estimated to achieve a final dose of 18 mg/kg/day based on the average amount of water consumed and the average weight of the mice used in this study. IMI was administered for the last 3 weeks of each CUMS session and during behavioral experiments. Drug solutions were protected from light in opaque water bottles and were replaced every other day. Vehicle-treated animals received regular (tap) drinking water.

For subchronic IMI, FLX, and SAHA treatments, the drugs were dissolved in saline at a concentration of 5 mg/ml. SAHA was dissolved in DMSO at a concentration of 50 mg/ml as a stock solution and diluted with saline just before injection. Mice received daily intraperitoneal injections of IMI, FLX, or SAHA (all 25 mg/kg/day) or saline. SAHA stock solutions were diluted 1:9 with saline (5 mg/ml final concentration) and administered immediately (Guan et al., 2009). These drugs were administered on the last 5 days of each CUMS session and during behavioral experiments.

For the osmotic delivery of ZEB or RG108, anesthetized mice were surgically implanted with two subcutaneous Alzet minipumps (model 1004) and bilateral guide cannulae (Plastics One) targeting the NAc. The minipumps were activated the evening before surgery by incubating them in 37 °C water to initiate a continuous delivery at 0.11 µl/h over 28 d. Bilateral cannulae were delivered into the NAc at +1.5 mm AP, ±1.0 mm ML, and −4.0 mm DV from bregma (Paxinos and Franklin, 1997). Mice were allowed to recover from surgery for 5 days before beginning the CUMS session.

Corticosterone assay

Mice were sacrificed by decapitation after a 60-min period of confinement stress (see "CUMS procedure") on day 3 or day 38 of the CUMS session. Non-stressed controls were rapidly removed from their cages and decapitated. Trunk blood was collected in heparinized tubes, and the plasma was separated by centrifugation and stored at -80ºC for corticosterone measurements. The corticosterone concentration was determined using a commercial enzyme immunoassay kit (Cayman Chemical).

Quantitative real-time PCR

Total RNA from dissected tissues or cells was extracted using the TRIzol Reagent

(Invitrogen) and treated with DNase (DNA-free; Ambion). The quality of RNA was determined based on the A_{260}/A_{280} ratio, which was 1.8–2.0 for all RNA preparations. 1 μg total RNA was used for cDNA synthesis using the QuantiTect Reverse Transcription Kit (Qiagen). cDNA was stored at −80 °C until use. Real-time PCR was performed using the Applied Biosystems 7300 Fast Real-Time PCR System with SYBR green PCR Master Mix (Applied Biosystems) according to the manufacturer's protocol. PCR conditions were 15 min at 95 °C, followed by 45 cycles for 15 s at 95 °C and 30 s at 60 °C. Primer sequences are shown in Table S4. Amplification of a single PCR product was confirmed by monitoring the dissociation curve and electrophoresis on 1.2% agarose gels stained with ethidium bromide. Amplification curves were visually inspected to set a suitable baseline range and threshold level. The relative quantification method was employed for the quantification of target molecules according to the manufacturer's protocol, where the ratio between the amount of each target molecule and a reference molecule within the same sample was calculated. All measurements were performed in triplicate. *Gapdh* and *β-actin* were used to normalize the relative expression levels of each target mRNA. All data shown are normalized to *Gapdh*. *β-actin*-normalized data were very similar to *Gapdh* data.

ELISA

Dissected tissues of HP, dSTR, the frontal cortex, and the pooled dissected tissues of vSTR, the amygdala, or the hypothalamus of 3−4 mice were placed in a buffer (pH 7.6) containing 20 mM Tris-HCl, 150 mM NaCl, 0.05% (vol/vol) NP-40, a protease inhibitor cocktail (Sigma) and sonicated. Samples were placed in a microcentrifuge and centrifuged at 4 °C for 10 min at maximum speed. The supernatant was then removed and frozen. Protein concentration was determined using a BCA Protein Assay Kit (Pierce), and 100 μg of protein was used in the ELISA assay (GDNF Emax Immunoassay System; Promega) according to the manufacturer's manual.

Western blotting

Dissected tissues from the HP or pooled dissected tissues from vSTR of 2–3 mice were used. Twenty or fifty micrograms of proteins were separated on 7% or 12% Tris-acetate gels, respectively, and transblotted onto polyvinylidene difluoride membranes (GE Healthcare Bio-Sciences). The membranes were incubated with antibodies directed

against HDAC2 (1:1000; Abcam), HA (1:5000; Abcam), GDNF (1:1000; Santa Cruz Biotechnology), or actin (1:2000; Sigma). After incubation with appropriate horseradish peroxidase-conjugated secondary antibodies (Cell Signaling), the blots were developed using the ECL-Plus detection Kit (GE Healthcare Bio-Sciences). Densitometric analysis was performed using Inquiry software (Neuroscience Inc.).

Chromatin immunoprecipitation

ChIP analyses were performed as described previously (Kumar et al., 2005; Tsankova et al., 2006; Guan et al., 2009) with minor modifications. Dissected tissues of HP or vSTR were minced into 1-mm pieces that were immediately frozen on dry ice and stored at −80 °C until further use. The pooled dissected samples of vSTR from 3–4 mice were used for ChIP assays. To crosslink the protein-DNA complexes, tissues were placed in 1% formaldehyde for 15 min at room temperature. Fixation was quenched by adding glycine at a final concentration of 0.125 M. The tissue was washed three times with ice-cold PBS containing protease inhibitors (Complete Tab, Roche Diagnostics) and homogenized with 12 strokes in 10 mM Tris, 10 mM NaCl, and 0.2% NP-40. The homogenate was centrifuged at 4500 $\times g$ for 5 min. The supernatant was removed and the cell pellet was then homogenized 2 more times using a nuclear lysis buffer (Upstate) with protease inhibitors. Each sample was sonicated on ice resulting in the formation of genomic DNA fragments (size, 200–1000 bp). Nuclear lysates were centrifuged at 20,000 $\times g$ for 20 min to remove insoluble material. The resulting lysates were precleared for 2 h at 4 °C using Protein A/G PLUS agarose beads (Santa Cruz Biotechnology) and immunoprecipitated overnight at 4 °C using 5 µg of antibodies directed against acetylated H3 (Millipore), acetylated H4 (Millipore), trimethylated H3K27 (Millipore), trimethylated H3K4 (Millipore), HDAC2 (Abcam), or MeCP2 (Abcam). All assays included normal rabbit IgG (Santa Cruz Biotechnology) and no-antibody immunoprecipitations to control the specificity of each antibody used. Chromatin-antibody complexes were collected with Protein A/G PLUS agarose beads and sequentially washed with low salt, high salt, LiCl, and TE (twice) buffers for anti-acetylated H3, anti-acetylated H4, anti-trimethylated H3K27, and anti-trimethylated H3K4 ChIP. For anti-HDAC2 and anti-MeCP2 ChIP, complexes were washed 5 times with RIPA buffer and once with TE buffer containing 50 mM NaCl. Chromatin was eluted with $NaHCO_3$/SDS buffer. ChIP, input (reserved from the precleared step), and

negative control samples were incubated in high salt conditions at 65 °C overnight for crosslink reversal. DNA fragments were then purified by treatment with RNaseA, proteinase K, and multiple extractions with phenol/chloroform/3-methylbutan-1-ol.

For the re-ChIP assays, the complexes were eluted from primary immunoprecipitation with anti-MeCP2 antibodies (Abcam) by incubation with 10 mM DTT at 37 °C for 30 min and diluted 1:40 in a buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH 8.1), followed by re-immunoprecipitation with anti-HDAC2 antibodies (Abcam) or anti-CREB antibodies (Millipore). Subsequent steps for ChIP re-immunoprecipitations were the same as those for initial immunoprecipitations.

Purified DNA samples were subjected to semiquantitative PCR analysis and quantitative real-time PCR analyses (Applied Biosystems 7300 Fast Real-Time PCR System). Primer sequences are shown in Table S4. Real-time PCR ChIP data were analyzed using the ΔΔCt method and normalized to input as described previously (Kumar et al., 2005; Tsankova et al., 2006). The relative ratios of modified histones, HDAC2, and MeCP2 on the genomic regions of the genes of interest between experimental groups are indicated in the figures. Consistent with previous reports (Tsankova et al., 2004, 2006), the active histone markers for acetylated histone 3 and acetylated histone 4 on the *Gapdh* promoter were in large numbers, whereas these markers were in very low numbers on the *Globin* promoter (data not shown). In contrast, repressive marker histone 27 dimethylated on lysine was enriched on the *Globin* promoter (data not shown). These results validated the ChIP protocol used.

Immunoprecipitation

Immunoprecipitation experiments were performed as described previously with minor modifications (Guan et al., 2009). To prepare the nuclei, freshly dissected tissues were washed with ice-cold PBS; homogenized in a buffer containing 0.32 M sucrose, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 1 mM $NaHCO_3$; and then centrifuged at 710 ×*g* for 10 min at 4 °C to obtain nuclear pellets. The nuclear envelope was removed by the addition of 1% Triton X-100 and extracted with a lysis buffer containing 1 mM EDTA, 0.5 mM EGTA, 10 mM Tris-HCl (pH 8), 150 mM NaCl, 0.1% sodium deoxycholate, and 0.5% *N*-lauroylsarcosine. All buffers included protease inhibitors. The pooled dissected tissues from the vSTR of 3–4 mice were used for the immunoprecipitation experiments.

The nuclear lysates were precleared with Protein A/G PLUS agarose beads (Santa Cruz Biotechnology) for 1 h at 4 °C and incubated for 16 h at 4 °C with 5 μg of anti-HDAC2 (Abcam) or anti-CREB (Millipore) antibodies and normal rabbit IgG antibodies (Santa Cruz Biotechnology) as a negative control, followed by incubation for 4 h at 4 °C with Protein A/G PLUS agarose beads. The beads were washed 5 times with RIPA buffer. Proteins were eluted with a sample buffer containing 1% SDS and 2% 2-mercaptoethanol. Western blot analyses of the immunoprecipitated anti-HDAC2, immunoprecipitated anti-CREB, input (reserved from the precleared step), and negative control (rabbit IgG) samples were performed with anti-MeCP2 (Abcam) antibodies as described above.

DNA methylation assay

CpG islands were defined by the following criteria: CG >55%, observed CpG/expected CpG >0.65, and length >300 bp. Genomic DNA from the HP and vSTR of mice and Neuro2a cells were isolated using the DNeasy Blood and Tissue Kit (Qiagen). Bisulfite treatment of DNA was carried out using the EpiTect Bisulfite Kit (Qiagen) according to the manufacturer's protocol. The resulting converted DNA was amplified by PCR (forward primer, ATGGAAATGAAGTTTAGGTTTG; reverse primer, AAAACTATCCTTCCTCCTCC). The PCR conditions were as follows: 94 °C for 2 min; followed by 35 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min; and finally 10 min at 72 °C. After PCR amplification, the PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and cloned into the pGEM-T Easy vector (Promega) according to the manufacturer's instructions. At least 12 independent recombinant clones containing an insert of the correct size from each PCR sample were analyzed on an Applied Biosystems model 377 DNA sequence using T7 primers.

DNA constructs

The expression vector pcDNA3-GDNF was constructed by PCR amplification of the open reading frame of mouse GDNF (accession no., NM_010275; forward primer, ATGGGATTCGGGCCACTTGGA; reverse primer, TCAGATACATCCACACCGTTT) with the Kozak sequence followed by an initiation codon and inserted into the pcDNA3 vector (Invitrogen). The expression vector pcDNA3-HDAC2 was constructed by PCR amplification of the open reading frame of mouse HDAC2 (accession no., NM_008229;

forward primer, ATGGCGTACAGTCAAGGAGG; reverse primer, TCAAGGGTTGCTGAGTTGTT) with the Kozak sequence and hemagglutinin (HA)-tagged sequences followed by an initiation codon and inserted into the pcDNA3 vector. The expression vectors for dominant-negative HDAC2 (a replacement of His141, which is located in the catalytic domain, by Ala) and HDAC2 C262/274A were constructed by site-directed mutagenesis using the KOD-Plus-Mutagenesis Kit (Toyobo). The expression vector pcDNA3-EGFP was constructed by inserting the open reading frame of EGFP (pEGFP-C1 vector, Clontech) with a Kozak sequence followed by an initiation codon into the pcDNA3 vector. For the MBD1, MBD2, MBD3, and MeCP2 expression vectors, the full-length cDNA for mouse MBD1 (accession no., NM_013594; forward primer, ATGGCTGAGTCCTGGCAGGA; reverse primer, CTACAAAACTTCTTCTTTCA), MBD2 (accession no., NM_010773; forward primer, ATGCGCGCGCACCCGGGGGG; reverse primer, TTACGCCTCATCTCCACTGT), MBD3 (accession no., NM_013595; forward primer, ATGGAGCGGAAGAGGTGGGA; reverse primer, CTACACTCGCTCTGGCTCCG), and MeCP2 (accession no., NM_001081979; forward primer, ATGGCCGCCGCTGCCGCCAC; reverse primer, TCAGCTAACTCTCTCGGTCA) were amplified by PCR with a Kozak sequence followed by an initiation codon and inserted in the pcDNA3.1 vector (Invitrogen). For the reporter construct, a 245-bp DNA fragment of the *Gdnf* promoter and a part of the first exon containing CpG sites 2–8 were amplified by PCR (accession no., D88352; forward primer, CCAACCTCAGAAGCCTTCTT; reverse primer, GAACAGCCGAGAGAGGAGAA) and inserted into the pGL3 vector (Promega). All constructs were verified by DNA sequencing.

In vitro methylation of reporter plasmids

Reporter vectors were incubated with *Hpa*II DNA methyltransferase (New England Biolabs) in a buffer containing *S*-adenosylmethionine. This procedure was repeated until full protection from *Hpa*II digestion was achieved. For CpG site 2-specific methylation, a 245-bp fragment containing CpG sites 2–8 on the *Gdnf* reporter was excised from the methylated reporter plasmids by *Kpn*I and *Xho*I digestion. The resulting fragment was ligated into the non-methylated pGL3 reporter vector that was digested with *Kpn*I and *Xho*I. The sequences adjacent to CpG site 2 were solely CCGG,

but those of other CpG sites within the used reporter construct were DCGH (D denotes adenine, guanine, or thymine; H denotes adenine, cytosine, or thymine), so that the CpG site 2 was solely methylated by *Hpa*II DNA methyltransferase. The concentration of each ligation product was calculated by analyzing fractionation on a 1.5% agarose gel and comparing band intensity of the expected ligation product size against a standard curve of known DNA concentration to directly transfect an equal amount of correctly ligated plasmids into Neuro2a cells. The completeness of *in vitro* methylation and maintenance (until cell harvesting) was confirmed by bisulfite sequencing.

PEI-mediated gene delivery

pcDNA3-GDNF or pcDNA3-EGFP plasmid DNA (10 μg) was diluted in a sterile solution of 5% glucose to a final volume of 18.4 μl and complexed with 1.6 μl of linear PEI. Mice were deeply anesthetized intraperitonealy with sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame (Narishige). The skull was exposed, and a small portion of the skull over NAc was removed bilaterally with a dental drill. Stainless steel guide cannulae (26 gauge, Plastics One) were implanted into NAc (+1.5 mm AP, ±1.0 mm ML, −4.0 mm DV; Paxinos and Franklin, 1997). Seven days after surgery, mice were subjected to CUMS, and on day 14 of the CUMS session, PEI-plasmid complexes were injected (0.5 μl/hemisphere) at a rate of 0.1 μl/min. Accuracy of the coordinates were determined in pilot experiments where methylene blue dye was infused instead of the PEI/DNA complex, and brains were sliced to display the site of the dye. Fourteen days after surgery, successful transduction of the target genes to NAc was confirmed by immunohistochemistry and Western blotting for EGFP (rabbit anti-GFP antibody; Invitrogen) and HA (rabbit anti-HA antibody; Abcam), respectively.

AAV-mediated gene transfer

Plasmid DNA pAAV-MCS (CMV promoter, Stratagene) carrying HA-HDAC2, HA-HDAC2 C262/274A, or HA-dnHDAC2 cDNA were constructed from pcDNA3-HA-HDAC2, pcDNA3-HA-HDAC2 C262/274A, and pcDNA3-HA-dnHDAC2 plasmids. The plasmid DNA pAAV-EGFP was constructed by insertion of the EGFP cDNA fragment from the pEGFP-C1 plasmid (Clonetech) into the pAAV-MCS vector. Plasmid DNAs pAAV-EGFP, pAAV-HA-HDAC2,

pAAV-HA-dnHDAC2, or pAAV-HA-HDAC2 C262/274A were cotransfected with plasmids pHelper and pAAV-RC to HEK293 cells using the standard calcium phosphate method. After 60 h, the cells were harvested and crude AAV vector solutions were obtained by repeated freeze/thaw cycles. Virus particles were purified and concentrated using a ViraBind AAV Purification Kit (Cell Biolabs Inc.), according to the manufacturer's protocol. For virus injections, mice were anesthetized intraperitonealy with sodium pentobarbital (50 mg/kg) and placed in a stereotaxic frame. The skull was exposed, and a small portion of the skull over NAc was removed bilaterally with a dental drill. Subsequently, AAV vectors were dissolved in physiological saline (0.5 μl) and injected bilaterally into NAc (+1.5 mm AP, ±1.0 mm ML, −4.5 mm DV; Paxinos and Franklin, 1997) at rate of 0.1 μl/ min. The needle was slowly withdrawn after 5 min. Mice were allowed to recover for 1 week after surgery and then subjected to CUMS for 4 weeks. Successful transduction of NAc region was confirmed histologically by immunolabeling with EGFP and HA.

Immunohistochemistry

Animals were deeply intraperitonealy anesthetized with sodium pentobarbital (50 mg/kg) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were post-fixed for 3 days and 30-μm sections were obtained using a cryostat. Double immunohistochemistry was performed on the free-floating sections. Antibodies for anti-HDAC2 (Abcam), anti-CREB (Millipore), and anti-MeCP2 (Abcam) were used. Images were acquired using an LSM 510 META laser confocal microscope with multichannel excitation and detection options using the optimal factory recommended filter configurations to minimize spectral bleed-through (Zeiss). The images were analyzed using Zen2008 software (Zeiss).

Cell culture, transfection, and assays

Neuro2a cell lines were obtained from Health Science Research Resources Bank (HSRRB, Osaka, Japan) and maintained in DMEM supplemented with 10% fetal bovine serum, 1% sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine at 37 °C in 5% CO_2. To examine the effect of 5-aza-2′-deoxycytidine on *Gdnf* mRNA levels, Neuro2a cells (10^5 cells) were treated for 4 consecutive days with different concentrations of 5-aza-2′-deoxycytidine, which was replaced with fresh

growth medium everyday. For the reporter assay, Neuro2a cells were transiently cotransfected in 24-well plates using Lipofectamine 2000 (Invitrogen) with pGL3 or methylated or non-methylated pGL3-*Gdnf* reporter vectors (0.2 μg/well), together with the HDAC2 (0.2 μg/ well), MeCP2, MBD1, MBD2, or MBD3 expression vectors (0.2 μg/well), and/or an empty vector (pcDNA3). The pCMV-β-galactosidase vector (0.1 μg/well) was also cotransfected as a control for transfection efficiency. In all cases, the total amount of transfected plasmid DNA per well was matched with the empty plasmids (e.g., pGL3 or pcDNA3). Twenty-four hours after transfection, luciferase and β-galactosidase activities were measured using a Luciferase assay system (Promega) and a β-galactosidase assay system (Promega), respectively. Luciferase activity was normalized to β-galactosidase activity. All reporter assays were performed in triplicate, and at least three independent experiments were performed.

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