Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens.

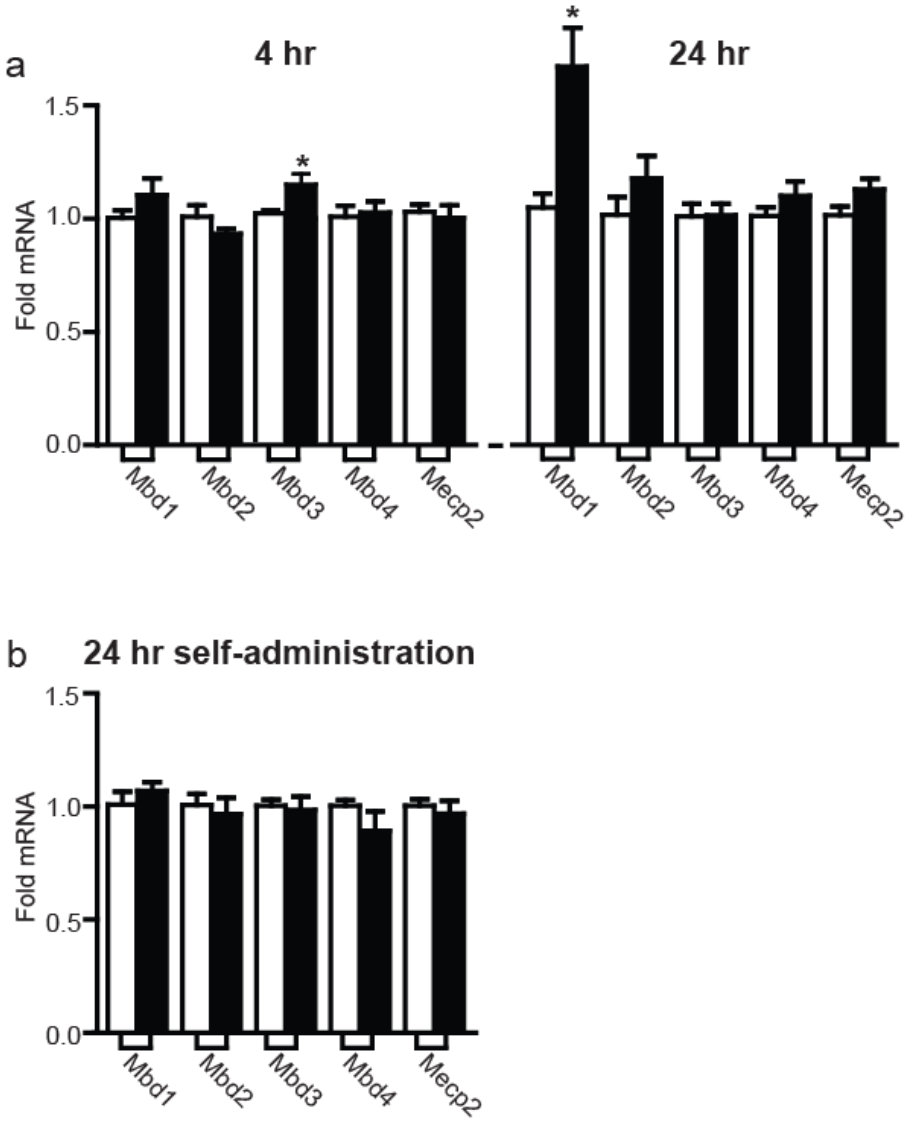
Quincey LaPlant, Vincent Vialou, Herbert E Covington III, Dani Dumitriu, Jian Feng, Brandon Warren, Ian Maze, David M Dietz, Emily L Watts, Sergio D Iñiguez, Ja Wook Koo, Ezekiell Mouzon, William Renthal, Fiona Hollis, Hui Wang, Michele A Noonan, Yanhua Ren, Amelia J Eisch, Carlos A Bolaños, Mohamed Kabbaj, Guanghua Xiao, Rachael L Neve, Yasmin L Hurd, Ronald S Oosting, Gouping Fan, John H Morrison, and Eric J Nestler

Supplementary Online Information

Supplementary Figures 1-4

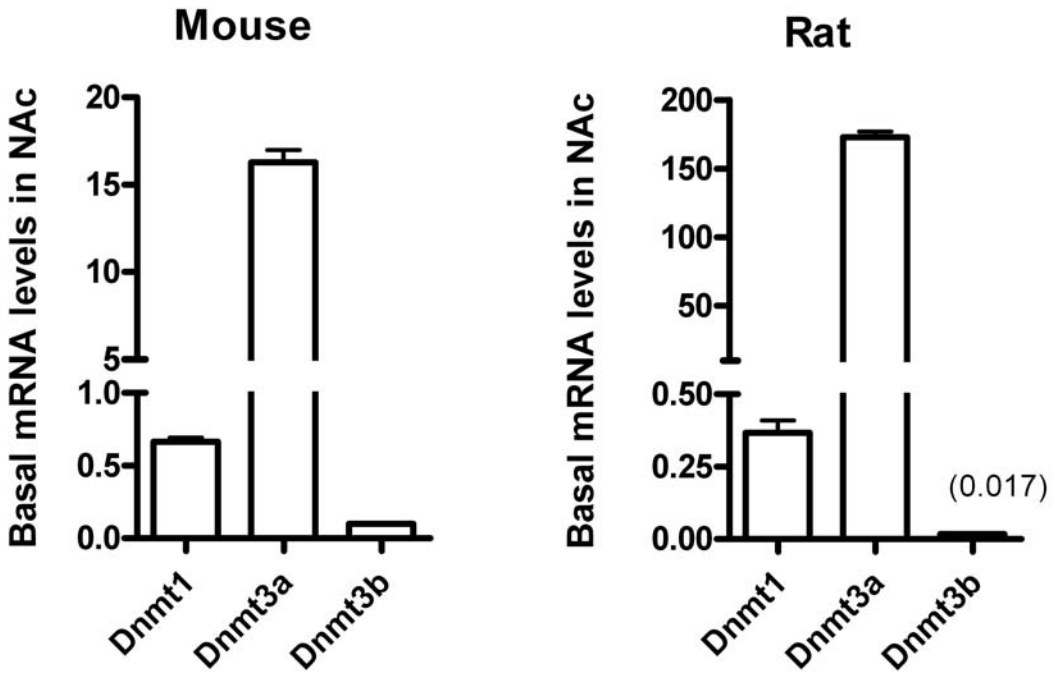
Supplementary Tables 1 and 2

Supplemental Figure S1



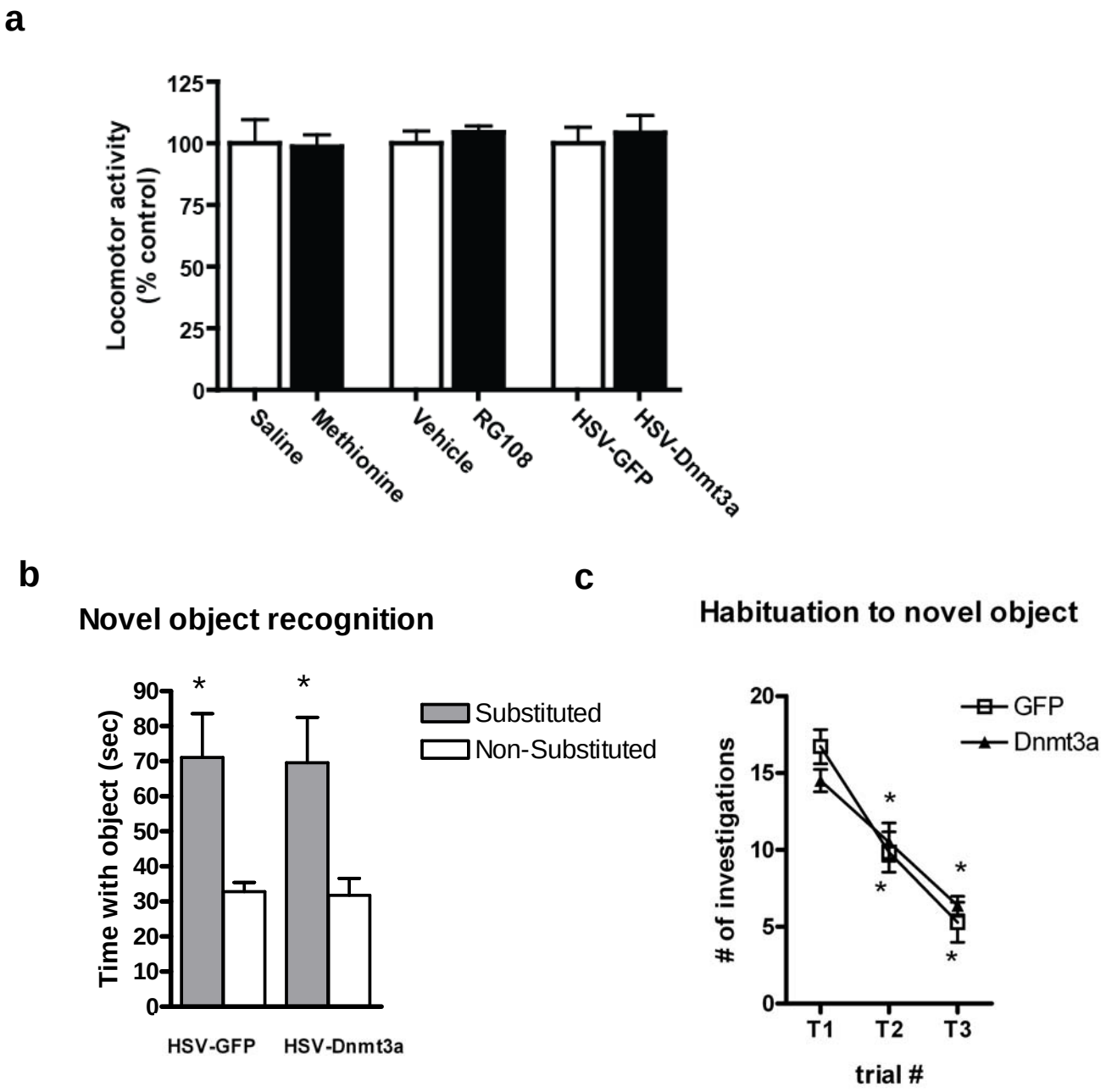
Supplemental Figure S1. **mRNA profiling of methyl-binding domain proteins (Mbd's) after chronic cocaine. (a)** qPCR analysis of NAc of chronic (7 days) cocaine treated mice indicated lack of regulation of Mbd's at 4 and 24 hrs, with the exception of a significant increase in Mbd3 at 4 hrs (**P*<0.05, n=7) and Mbd1 at 24 hrs (**P*<0.05, n=16). **(b)** However, Mbd1 and other Mbd's were unaltered in the NAc of self-administering rats examined 24 hours after the last cocaine dose.

Supplemental Figure S2



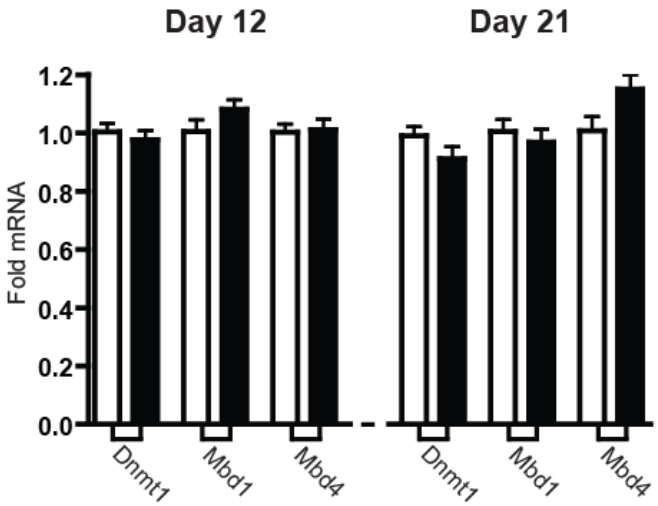
Supplemental Figure S2. **Relative Dnmt mRNA levels in NAc of mice and rats.** Data expressed as fold difference from the average level of all Dnmts from 20 control mice and 6 control rats. Dnmt3a shows the highest expression in the NAc. Dnmt expression was measure by qPCR.

Supplemental Figure S3



Supplemental Figure S3. **Experimental manipulation of DNA methylation does not alter locomotor activity or learning. (a)** Locomotor activity was measured by photobeam breaks in the CPP chamber during the first day of saline training. We found no significant locomotor effects of subcutaneous methionine, intra-NAc RG108, or intra-NAc HSV-Dnmt3a. **(b)** Mice given intra-NAc injections of HSV-GFP or HSV-Dnmt3a spend equally more time with a novel, substituted object compared to non-substituted objects. Mice were repeatedly (3 times, 5 min each) exposed to 3 objects with 3 min intervals between test sessions. On the final trial, one object is substituted with a novel object. **(c)** HSV-GFP and HSV-Dnmt3a treated mice become equally disinterested in a single novel object upon reexposure. Mice were repeatedly exposed to a single object for 5 min with 1 hr intervals between test sessions.

Supplemental Figure S4



Supplemental Figure S4. **NAc mRNA profiling of Dnmt1, Mbd1, and Mbd4 following social defeat. (a)** qPCR analysis of NAc of mice subjected to chronic (10 days) social defeat taken 1 or 10 days after social interaction tests (Day 12 and 21) indicated lack of significant regulation of Dnmt1, Mbd1, and Mbd4 ($P>0.05$).

Supplemental Table 1

Fig. 3	Treatment	Mouse	Cocaine dose (mg/kg)	Pref score (sec)	SEM
a	METHIONINE	Wt	7.5	63.48	51.67
	SALINE	Wt	7.5	257.92	61.35
c	RG108	Wt	10	304.58	26.05
	VEHICLE	Wt	10	85.17	39.78
	RG108	Wt	15	286.35	39.32
	VEHICLE	Wt	15	214.58	55.91
g	HSV-DNMT3a	Wt	7.5	47.27	37.28
	HSV-GFP	Wt	7.5	170.17	71.69
	HSV-DNMT3a	Wt	10	130.97	40.06
	HSV-GFP	Wt	10	246.85	35.14
h	AAV-Cre	floxed-Dnmt3a	7.5	428.48	46.77
	AAV-GFP	floxed-Dnmt3a	7.5	243.64	70.11
	AAV-Cre	floxed-Dnmt1	7.5	275.51	61.70
	AAV-GFP	floxed-Dnmt1	7.5	251.91	36.11

Pref, preference; wt, wildtype.

Supplemental Table 2

Mouse Primers	
Dnmt1 fwd	atcctgtgaaagagaaccctgt
Dnmt1 rev	ccgatgcgatagggctctg
Dnmt3a-1 fwd	gagggaactgagaccccac
Dnmt3a-1 rev	ctggaaggtgagtcttggca
Dnmt3b_fwd	gttaatgggaacttcagtgaccaa
Dnmt3b_rev	ctgcgtgtaattcagaaggct
mbd2	GACGAGACCCTTCTGTCTGC
mbd2_R	TGGACTCGCTCTTCCTGTTT
mbd3	AGCCACAACTGGCACGTTAC
mbd3_R	GCTGGCGACTCTTATTCATCTTG
mbd4	GGACAACAGAGTCCGTGGAG
mbd4_R	ATCACCAGGTCCTTTCCATCT
mecp2	CAAACAGAGAGGAGCCTGTGGACAG
mecp2_R	TTTATTTCAGTTAATCGGGAAGCTTTG
ChIP primers (mouse)	
mPro_dnmt3a-500_fwd	gcgtttggtagagctcaagg
mPro_dnmt3a-500 _rev	gaccggcaccctactgataa
mPro_dnmt3a-2000_fwd	CTAATTCCCAGCTCGCTTTG
mPro_dnmt3a-2000 _rev	ACATGCCAGGCTATTGGAAC

Rat Primers	
Rn_DNMT1_fwd	CTCGTGGTCTCCTTCCTCAG
Rn_DNMT1_rev	AGGGGAAGAGAGATGGCATT
Rn_DNMT3a_fwd	ACGCCAAAGAAGTGTCTGCT
Rn_DNMT3a_rev	CTTTGCCCTGCTTTATGGAG
RN_DNMT3b_fwd	CATAAGTCGAAGGTGCGTCGT
RN_DNMT3b_rev	ACTTTTGTTCTCGCGTCTCCT
RN_mecp2_fwd	GGACGCGAAAGCTTAAACAG
RN_mecp2_rev	CTGGAGCTTTGGGAGATTTG
RN_mbd4_fwd	ATCTCCGTGCAAAAACCATC
RN_mbd4_rev	TGATTTTCCCAAAGCCAGTC
RN_mbd3_predicted_fwd	AACACTGCACTGCCTGTACG
RN_mbd3_predicted_rev	ACAGCAGCGTCTCATCTGTG
RN_mbd2_fwd	ACCTGGGAAATGCTGTTGAC
RN_mbd2_rev	TTGCTTGAAAATGGATGCAG
RN_mbd1_fwd	GCCTGGTGAAAGAAGACTGC
RN_mbd1_rev	AGTTCTGTGGGCTCTGGATG