SUPPLEMENTARY INFORMATION FOR:

DNA methylation regulates associative reward learning

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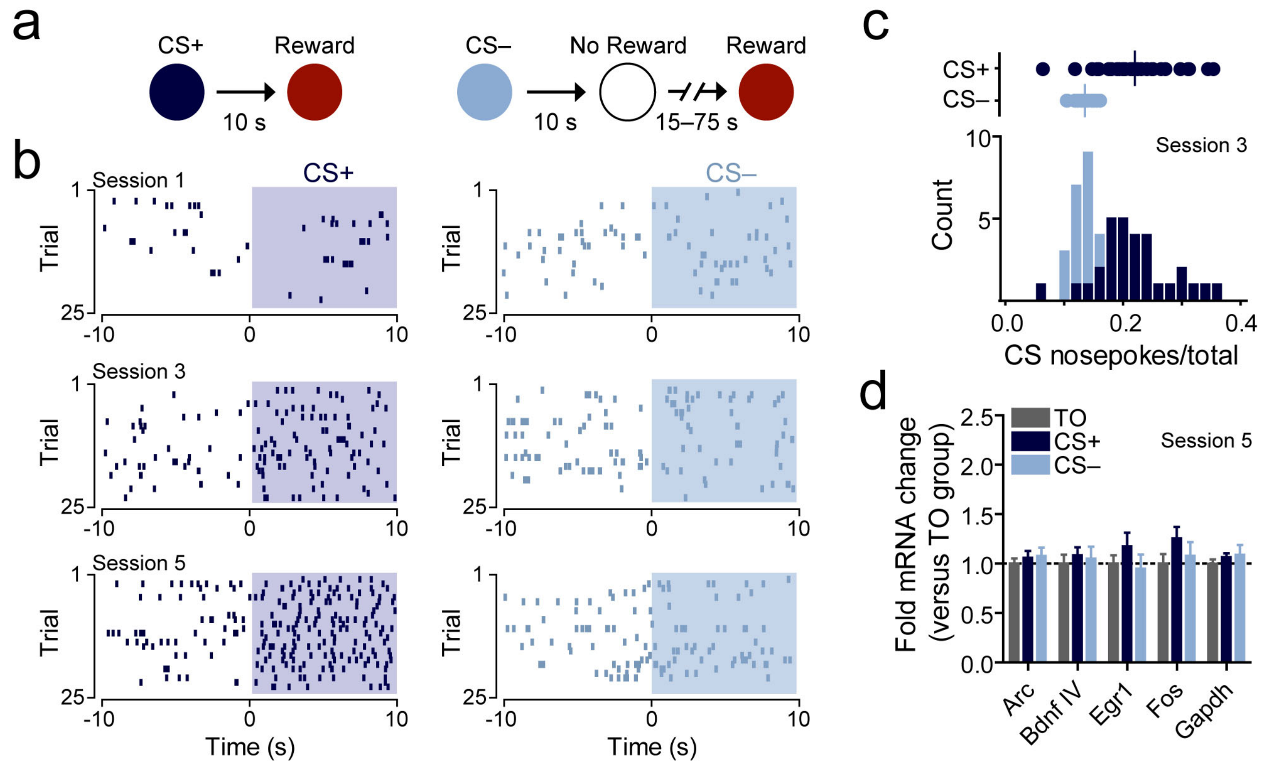
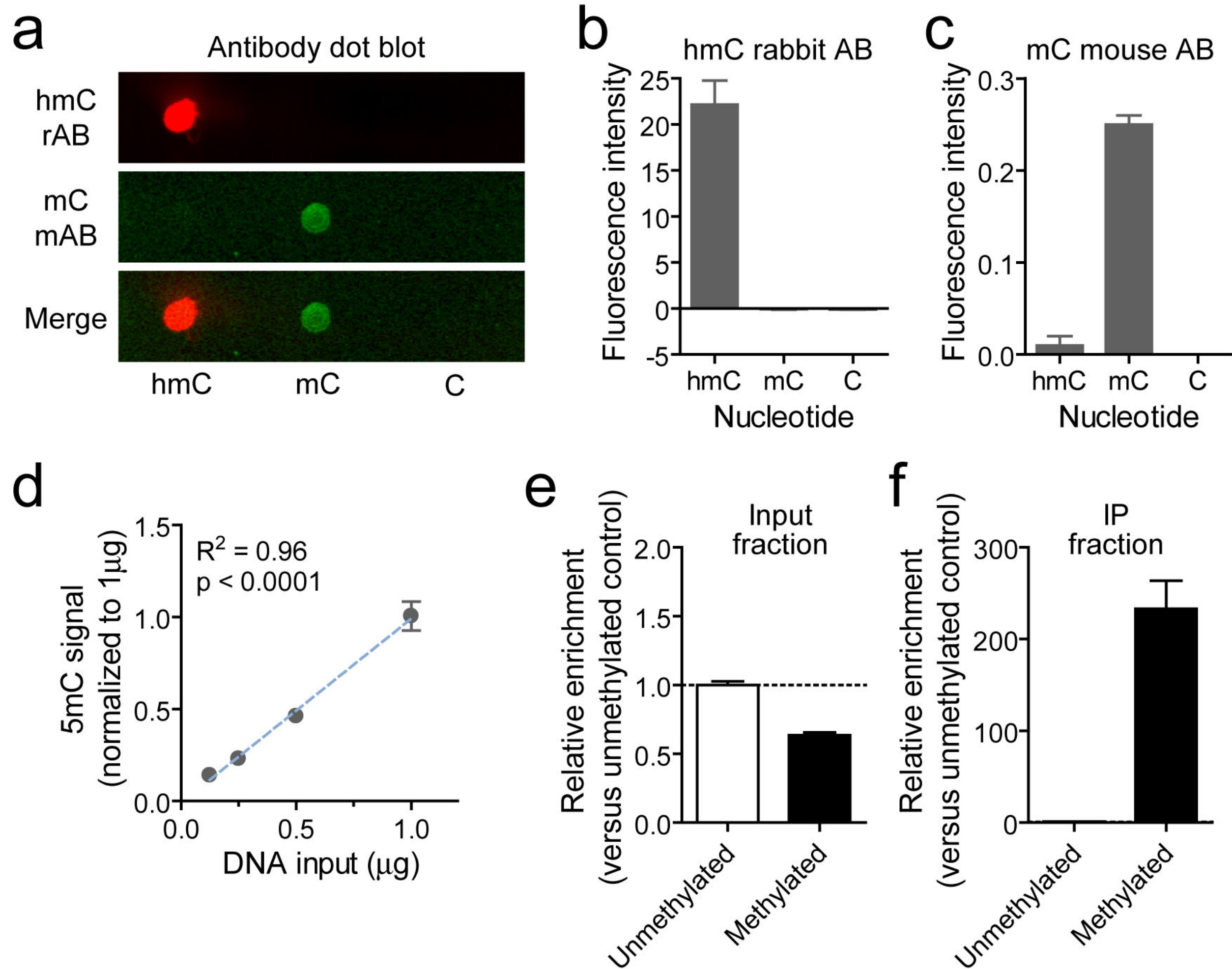
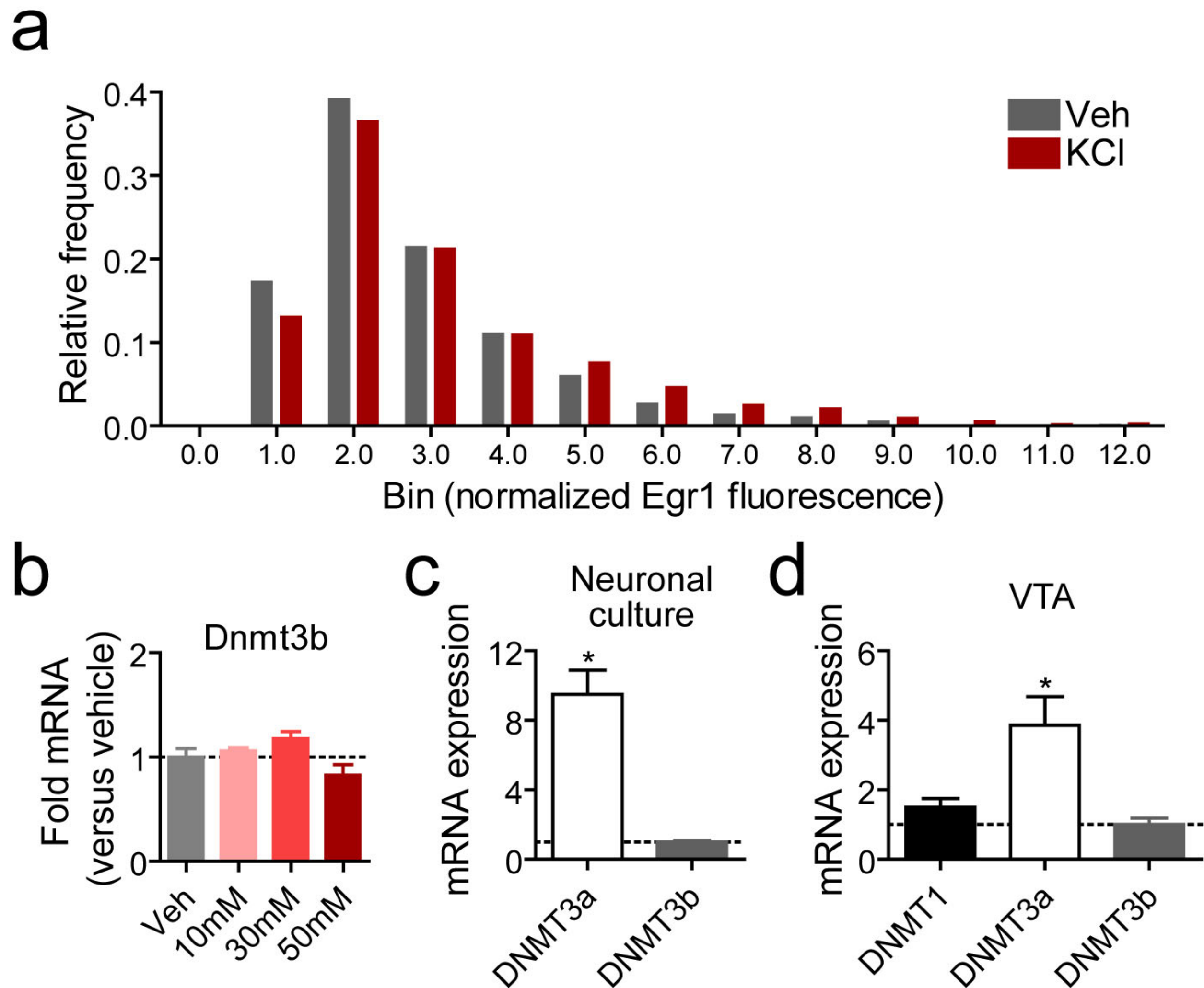


Supplementary Figure 1. Behavioral training and IEG expression after task acquisition. **a-b,** Representative examples of learned increases in anticipatory nosepoke responses during the reward predictive CS+, as compared to an animal trained in the CS- task in which rewards and audio cues were delivered in an unpaired fashion. Data are shown as peri-event raster plots, with trials stacked on the y axis and time relative to cue presentation on the x axis. Each tick mark represents an individual nosepoke response. **c**, Variability in learned cue-evoked nosepokes in behavioral session 3. Top panel, distribution of individual animals in terms of cue-specific approach responding (total nosepokes during cue period divided by total nosepokes in the entire behavioral session). Bottom panel, frequency distribution of cue-related nosepokes. Although some animals exhibited significant anticipatory nosepoke responding during the CS+ presentation, anticipatory responding in other CS+ animals overlapped with CS- animals, indicating poor memory formation. Only animals that contributed mRNA data in VTA experiments were included. **d**, After task acquisition, experience with reward-paired cues does not alter IEG expression in the VTA. Experience with a reward-predictive CS+ did not change expression of any gene (n = 9-11 per group; one-way ANOVA: main effect of training, $p > 0.3$ for all comparisons). Animals were sacrificed 1hr after the 5th conditioning session.



Supplementary Figure 2. Validation of methylated DNA immunoprecipitation to detect changes in DNA methylation. **a**, Antibody dot blots confirmed specific binding of hydroxymethylcytosine antibodies to hmC and methylcytosine antibodies to mC, with little non-specific binding and unmodified cytosines (C). **b-c**, Quantification of antibody dot blots. **b**, hmC-specific antibody generated significantly more hmC binding signal, as compared to mC and unmodified cytosine signal (n = 2 per group; one-way ANOVA: main effect base modification on fluorescence intensity, $F_{(2,5)} = 74.05$, $P < 0.0028$). **c**, mC-specific antibody generated significantly more mC binding signal, as compared to hmC and unmodified cytosine signal (n = 2 per group; one-way ANOVA: main effect base modification on fluorescence intensity, $F_{(2,5)} = 300.5$, $P < 0.0004$). **d**, Methylated DNA enrichment, as determined by qPCR at the *Fos* promoter locus (Figure 1i), exhibited significant dependence on input DNA concentration in response to a serial dilution. **e-f**, Methylated DNA enrichment, as determined by qPCR for a synthetic DNA duplex consisting of either completely unmethylated or completely methylated cytosine bases. Although less methylated DNA was detected in the input fraction (**e**), ~200 fold more methylated DNA was detected in the IP fraction following immunoprecipitation with an mC antibody. Error bars represent s.e.m.



Supplementary Figure 3. Activity-induced changes in EGR1 protein and comparisons between *Dnmt* isoforms in neuronal culture and in the brain. **a**, Relative frequency distribution of cellular EGR1 levels following vehicle and KCl treatment. KCl specifically increased the proportion of cells with high EGR1 protein levels. **b**, 1hr KCl stimulation did not alter *Dnmt3b* levels (n=3 per group; one-way ANOVA: main effect of treatment, $F_{(3,11)} = 3.91$, $P = 0.0544$). c, Differential expression of *Dnmt* mRNA in neuronal cultures at 10 days in vitro. *Dnmt3a* expression was significantly higher than *Dnmt3b* expression (n = 3 per group; Student's *t*-test $t_4 = 6.112$, $*P = 0.0036$). **d**, Differential expression of *Dnmt* isoforms in adult rat VTA tissue. Again, *Dnmt3a* expression was significantly elevated as compared to *Dnmt3b* and *Dnmt1* (n = 5 per group; one-way ANOVA: main effect of isoform, $F_{(2,14)} = 8.97$, $P = 0.0041$; Tukey post-hoc tests revealed significantly higher mRNA for *Dnmt3a* vs *Dnmt3b* and *Dnmt1*, $*P < 0.05$).

Supplementary Table 1

mRNA RT-qPCR primers

Gene	Forward sequence	Reverse sequence
Arc	GCTGAAGCAGCAGACCTGA	TTCACTGGTATGAATCACTGCTG
BDNF exon IV	CTGCCTAGATCAAATGGAGCTTCT	GGAAATTGCATGGCGGAGGTAA
Dnmt1	GTGTGCGGGAATGTGCTCGCT	CAGTGGTGGTGGCACAGCGT
Dnmt3a	AGCAAAGTGAGGACCATTACCACCA	TGTGTAGTGGACAGGGAAGCCA
Dnmt3b	TGGCAAGGATGACGTTCTGTGGT	CTGGCACACTCCAGGACCTTCC
Egr1	TCCTCAAGGGGAGCCGAGCG	GGTGATGGGAGGCAACCGGG
Fos	CAGCCTTTCCTACTACCATTCC	ACAGATCTGCGCAAAAGTCC
FosB	TGCAGCTAAATGCAGAAACC	CTCTTCGAGCTGATCCGTTT
ΔFosB	AGGCAGAGCTGGAGTCGGAGAT	GCCGAGGACTTGAACTTCACTCG
Gapdh	ACCTTTGATGCTGGGGCTGGC	GGGCTGAGTTGGGATGGGGACT

Genomic DNA PCR primers (MeDIP and ChIP experiments)

Gene	Forward sequence	Reverse sequence	Gene location/nomenclature
Egr1	CACTGCGTCTAAGGCTCTCC	AGGTCTCGGGTTGTATTCCG	Promoter (E1)
Egr1	CGGTGACACCTGGAAAGTGA	GAGTCAGGCCGGGGTTCTA	Promoter (E2)
Egr1	CCTTTCCTCACTCACCCACC	CGGCTCCCCTTGAGGATTG	Promoter/TSS spanning (E3)
Egr1	CAACTCATCAAACCCAGCCG	GATTCGACACTGGAAGGGCT	Intragenic (E4)
Fos	GGTGCGAATGTTCGCTCGCCT	GGACCGGCCGTGGAAACCTG	Promoter (F1)
Fos	GAGCAGTCAGAGAAGGCAGG	TAGGTCTACGGGAACCCCTC	Intragenic (F2)
Gapdh	GGTCGGAGCCCACACGCTTG	TCCCGCTCGGCTCATCCAGT	Promoter (G1)