Dnmts and *Tet* target memory-associated genes after appetitive olfactory training in honey bees

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SUPPLEMENTAL MATERIALS

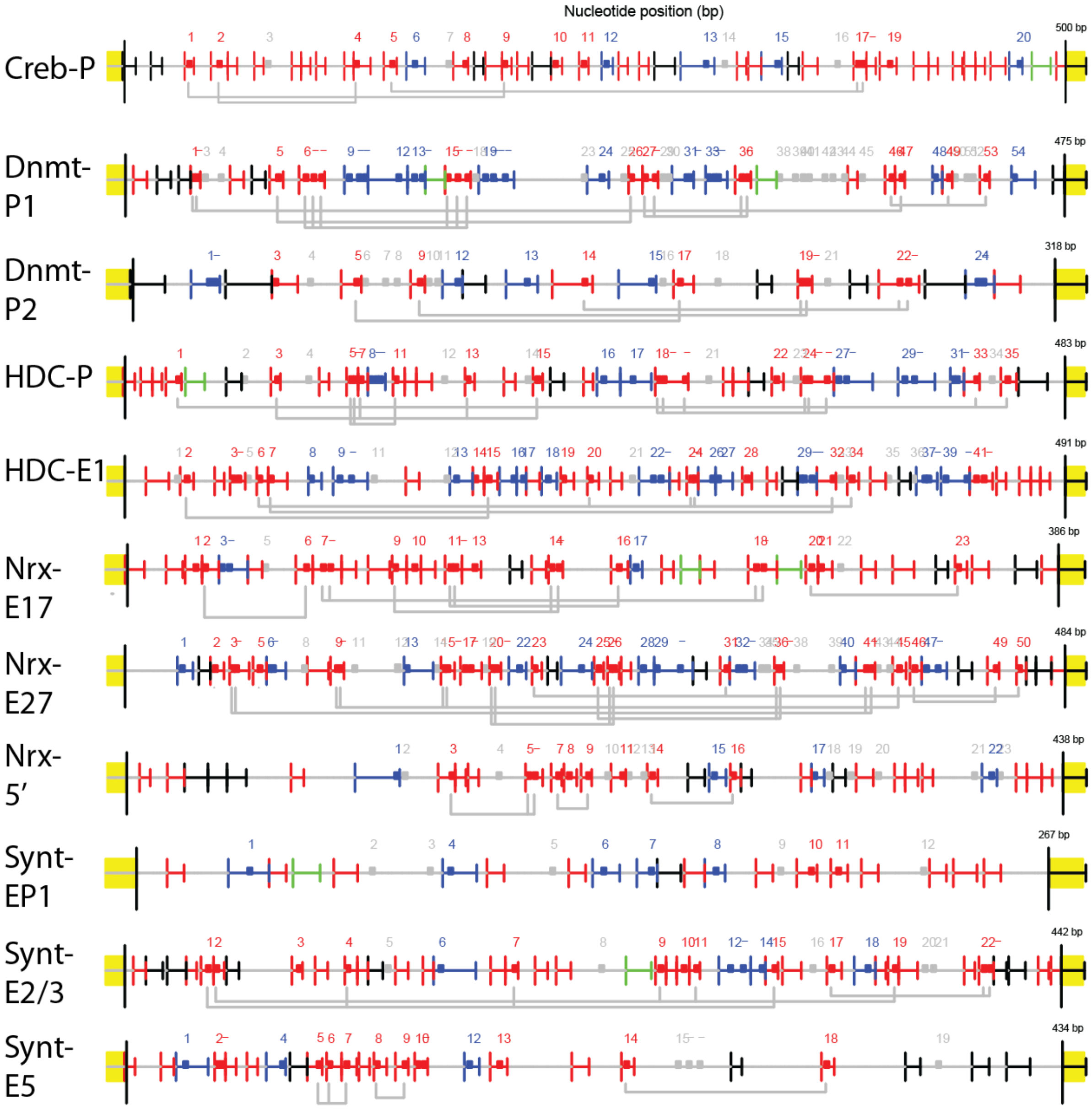

Figure S1 Amplicon prediction for Sequenome analysis. The amplicon prediction function of the MassArray R-pipeline was used (Thompson et al., 2009). Numbers represent CpG sites. Red indicates CpGs which cannot be analysed, because the respective fragment mass overlaps with another fragment. Grey CpGs are on fragments which are out of the measurable range. Blue CpGs can be analysed. Green parts are segments which were used for the conversion control analysis as they include non-CpG cytosines.

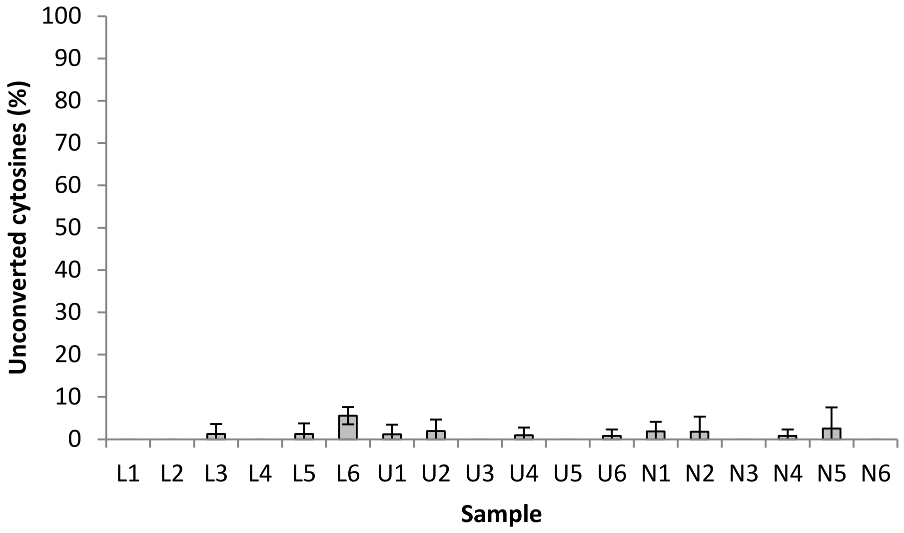

Figure S2 Bisulfite conversion control for Sequenome analysis. The conversion control pipeline of the MassArray R-pipeline was used (Thompson et al., 2009). For all amplicons conversion of non-CpG C's was assessed and pooled per sample. Mean and 95% confidence interval are presented. L= learner group; U= unpaired group; N = naïve group

Table S1 Fluidigm qPCR primer list

Gene candidate	GB number (Assembly 4.0)	Forward primer	Reverse primer
Actin	17681	TTCCCATCTATCGTCGGAAG	CTCTCTTTGATTGGGCTTCG
Aldoreductase	18109	TAGTCCCCTTGGATCACCTG	TTGGGTCATCTGGTTTAGCC
Asparaginesynthetase (ASNS)	13219	TGGAATTTGGGCTCTTTTTG	TTCTGGACCACGGTGTGTAA
Histone acetyltransferase p300 (HAT p300)	10171	ACCAAGTGGAGGTCAACCTG	ATATTGTGGGTGGGCAAGAA
cAMP response element binding protein (CREB)	11585	AATTGCAACCCAAGGTGAAG	TCAGTATGCACAAGGCCAAG
Cueball (cue)	17323	CCAAAAGACGGGAAAAATGA	ACGCGTTAAAATCCCACTTG
DNA methyltransferase 1a (DNMT1a)	19865	TGATCCAAAAACAGATGAGGA A	TACAGCACCATTCGGATGAC
DNA methyltransferase 1b (DNMT1b)	15130	GAAATTACATGGGTGGGAGAA	GTCACTGCCTCTTCGAAACC
DNA methyltransferase 2 (DNMT2)	10767	TGAGTCCTCCATGTCAACCTT	GCCAAATTGACAAGGGCTTA
DNA methyltransferase 3 (DNMT3)	14232	CCTCCAACTGGACTTTGGAC	ACGTTCGGATTGTCCTTCAG
Ether a go-go (EAG)	15597	GATGACCAAGGGCCTAGACA	ATGCTCGTTGAACACCTTCC
Glyceraldehyde-3-phosphate	14798	GATGCACCCATGTTTGTTTG	TTTGCAGAAGGTGCATCAAC

dehydrogenase (GAPDH)			
GB18684	18684	TATTGTTGCTTCGGATCGTG	CGGTGTTCTTTTTCCGATCT
Histone 1B	12700	GCTAAGAAGCCAGCAACACC	TACCTTCGATGCGCTCTTTT
Headcase (HDC)	11140	ATCCGGGAAGAGGAAGTGAT	TGTTCTCCATGGTGTCGTGT
Histone 1.2	12218	GCCAATCCAACAAAGAAAGC	ATTGGTGACCGTCGTGATTT
Heatshockprotein 90 (HSP90)	14758	GGCTGCCAAGAAGCATTTAG	AGCTTCAGCTTTTTGCCTCA
cytoplasmic aconitate hydratase (Irp1)	16072	TATCGGAAAAGCTGGACCAC	TATCGGAAAAGCTGGACCAC
Molybdenum cofactor sulfurase (mal)	18250	CATCCTTGCTAATGCTTCACC	GCAACAATTCCTCCTTGCAT
molybdenum cofactor biosynthesis protein 1 (Mocs1)	11214	TTGTATGCCAGCAGAAGGAA	TTGGTTCACCACCAGTTAAGC
Mitochondrial ribosomal protein L35 (Mrpl35)	14476	AACTTTTGCTGGCCGTCAT	AACTTTTGCTGGCCGTCAT
Neprilysin 2(Nep2)	16619	TTGAAGAACAGCACGAAACG	ATAATCCAAGAGCGGCTTGA
Neurexin I	13455	CGGAGAACGCTGCCTTAATA	TCAGAACAATGGCGATCAAC
N-acetylneuraminate lyase(NPL)	17289	TAGTGAGGCAGCTCCAAACA	CGAGGAATTCTCCCATGTGT
Pyridoxamine 5'-phosphate oxidase (PPO)	13619	TGTGGAGGCAGAGCCTTAGT	TACACCGTCGCAAAAACTGA
RNA-polymerase (Rpb8)	10191	TATCCTGATGGAGGGGAGTG	GGGTTCATTGCTTGCTTCAT
Ribosomal protein L32 (RPL32)	10903	CGTAACCTTGCACTGGCATT	TTGCTCATGGTGTGAGCAGT
Regulatory particle non-ATPase 9 (RPN9)	16614	CAAGCTTTCAATGCTGGTGA	CTTTGCTCCATTGTGGCTTT
Sec61beta	13924	CCCAGCAAAGCTATAGCACCT	TTCATCAGAACCAGCTCCTGT
Stress sensitive B (sesB)	17499	TGATGTAGGCAAAGCTGGTG	GGCAGCACGATAGATGATGA
Synaptotagmin 1 (Synt)	20036	CCAAACACGATCAGATCGGTG	CTTCAGATCCGTACGTGAAGG
Ten-eleven translocation methylcytosine dioxygenase (Tet)	13880	TCACGAGCAAAAGACACCTGG	ACATGTTTTCCGGCTTATCG

Table S2 Sequenome primer list

Primer name	Forward	Reverse	Size (bp)
CREB Promotor	aggaagagagTTGGTAAGTATTTAGTTGGAGAATAA	cagtaatacgactcactatagggagaaggctAACATCATCAAAACAAATAATATC	500
Dnmt3 Promotor 1	aggaagagagGAAATTTGGGAAGAAAGTGGG	cagtaatacgactcactatagggagaaggctAAAAATTCTCTTCATCAAAAAAAAC	475
Dnmt3 Promotor 2	aggaagagagGTTTTTTTTGATGAAGAGAATTTTT	cagtaatacgactcactatagggagaaggctAAATTCTTACCTAACCACCATCCC	318

HDC Promotor	aggaagagagGTTTAAAATTGTTATTTGAAAGTGT	cagtaatacgactcactatagggagaaggctCTATATTATCACACACACACCCATT	483
HDC Exon 1	aggaagagagGAAAAGTATAAGGGTGTGTTTGATA	cagtaatacgactcactatagggagaaggctTTCCTAATAACATCCAATCCAAATC	491
Neurexin 1 exon/intron 17	aggaagagagTTTGGTAAATAGAGTATTTTGGAATTT	cagtaatacgactcactatagggagaaggctAAAACATATCCAAACAATTTTCCCTAT	386
Neurexin 1 exon/intron 26	aggaagagagGAAATTGAAAAAGGAAAGTAATTAATAG	cagtaatacgactcactatagggagaaggctAAAAACTAACAAACTCACCTAAC	484
Neurexin 1 5'end	aggaagagagGAGAAATTTGATTGAGGAATGTGTT	cagtaatacgactcactatagggagaaggctTTCCTAACCTCAAACCTCAAATATC	438
Synaptotagmin 1 Promotor/exon 1	aggaagagagATTTAAAGATGGGAAGAAAGGATTG	cagtaatacgactcactatagggagaaggctTTTTACCTTATATTAAAATTTCCCA	267
Synaptotagmin 1 exon 2/3	aggaagagagGGAATTGTTAGTTTTGGATATGGGT	cagtaatacgactcactatagggagaaggctCCAATTCAAATTTACCTAACCACCT	442
Synaptotagmin 1 exon 5	aggaagagagGGTATATTAGAATTTATTGGGAAGG	cagtaatacgactcactatagggagaaggctATTCTTTTCTCTCATAAAAAAAACCTA	434

Table S3 p-values methylation site analysis

LEARNING STIMULATION

Amplicon	CpG	L~U	L~N	U~N	Group	Age	Season
Creb Promotor	All	0.969	0.740	0.675	0.883	0.436	0.082
	6	0.432	0.136	0.397	0.111	**0.010**	0.696
	8	0.754	0.425	0.593	0.733	0.423	0.800
	10	0.244	**0.018**	***0.086***	**0.006**	0.754	0.059
	11	0.919	0.338	0.352	0.535	0.320	0.612
	12	0.337	0.951	0.332	0.525	0.463	0.429
	13	0.587	**0.017**	**0.023**	**<0.001**	***0.072***	0.920
	15	0.348	0.820	0.22	0.126	0.101	**0.004**
	19	0.863	0.311	0.358	0.241	**0.014**	***0.058***
	20	0.321	0.503	0.735	0.628	0.629	0.587
Dnmt 3 Promotor 1	All	0.106	0.608	***0.054***	0.115	0.705	0.982
	9.10.11	0.744	0.828	0.608	0.882	0.530	0.526
	12	***0.071***	0.396	0.309	0.184	0.659	0.708
	13.14	0.265	0.207	**0.037**	**0.015**	**0.046**	0.102
	19.20.21.22	0.252	0.614	0.131	0.193	0.203	0.178
	24	0.222	0.298	**0.046**	0.119	0.762	0.782
	31.32	0.757	**0.042**	**0.031**	**0.031**	0.328	0.867
	33.34.35	0.692	0.321	0.559	0.569	0.253	0.323
	54	0.143	0.143	1	0.193	0.364	0.454
Dnmt 3 Promotor 2	All	**0.045**	***0.093***	0.606	***0.077***	0.524	0.694
	3	0.249	0.731	0.380	0.477	0.707	0.225
	11.12	***0.092***	0.618	0.161	0.1850	0.457	0.787

	13	0.259	0.455	0.763	0.465	0.696	0.188
	15	***0.086***	**0.006**	0.163	**0.002**	0.387	0.281
	24.25	**0.022**	**0.046**	0.6059	**0.003**	0.283	**0.019**
HDC Promotor	All	0.841	0.346	0.454	0.595	0.340	0.343
	8.9.10	1	1	1	1	0.464	0.534
	16	**0.031**	0.109	0.506	***0.071***	0.696	1
	17	0.835	0.533	0.677	0.828	0.511	0.908
	22	0.831	0.114	0.164	0.213	0.807	0.359
	27.28	0.169	0.770	0.113	0.165	0.821	**0.048**
	29.30	0.121	***0.093***	0.946	0.169	0.805	0.654
	31.32	0.868	0.679	0.804	0.918	0.298	0.704
HDC exon 1	All	0.732	0.827	0.901	0.932	**0.046**	0.921
	3.4	0.692	0.432	0.692	0.554	1	**<0.001**
	8	1	1	1	1	1	1
	9.10	0.897	0.146	0.132	0.226	0.333	0.629
	13	**0.018**	**0.004**	0.428	**0.002**	0.420	0.494
	14	0.143	0.292	0.772	0.328	0.926	0.957
	16	0.502	**0.029**	0.106	***0.060***	0.720	0.448
	18	**0.003**	***0.195***	***0.051***	**0.003**	0.831	0.711
	19	***0.079***	0.935	***0.079***	***0.100***	0.240	0.683
	22.23	**0.024**	0.342	0.119	**0.049**	0.315	0.845
	26	**<0.001**	**<0.001**	0.218	**<0.001**	0.146	0.769
	27	0.503	0.503	1	0.715	0.885	***0.082***
	28	0.800	0.529	0.384	0.499	**0.009**	0.720
	29.30	***0.064***	***0.088***	0.797	***0.099***	0.882	0.376
	37.38	0.189	0.189	1	0.221	**0.041**	0.385
	39.40	0.833	0.521	0.686	0.794	0.117	0.831
	41.42	0.141	***0.073***	0.694	0.151	0.501	0.528
Neurexin 1 exon/ intron 17	All	**0.038**	0.104	0.687	**0.004**	0.484	**<0.001**
	1	0.399	0.343	0.870	0.610	0.794	0.523
	3.4	0.431	0.164	0.431	0.401	0.898	1
	10	0.124	0.112	0.734	***0.053***	0.120	**0.031**
	13	0.241	1	0.241	0.325	0.920	0.131
	21	**0.050**	***0.098***	0.826	**0.002**	0.654	**<0.001**
Neurexin 1 exon/ intron 26	All	0.246	**0.017**	0.124	**0.014**	0.584	***0.060***
	1	0.457	0.379	0.121	0.305	0.636	0.969
	6.7	0.125	1	0.147	**0.016**	1	**<0.001**
	12	0.151	0.114	0.810	0.176	0.682	0.158
	13	0.721	**0.039**	**0.050**	**0.031**	0.419	0.209
	17.18	0.558	0.126	**0.034**	***0.060***	0.681	0.225
	22	0.718	0.441	0.652	0.634	0.542	**0.007**
	24	0.467	**0.015**	**0.039**	**0.006**	0.888	0.204
	28	0.823	0.301	0.379	0.554	0.502	0.609
	29.30	0.120	0.503	0.323	0.169	0.443	***0.088***
	32.33	0.115	0.510	0.328	0.137	**0.048**	0.121
	40	0.307	0.070	0.268	0.175	0.560	0.956
	47.48	0.971	0.773	0.708	0.927	0.838	0.346
Neurexin 1 5' end	All	0.194	0.160	0.845	0.199	0.182	0.219
	1	0.340	0.267	0.827	0.282	**0.007**	0.306
	8	0.594	0.155	0.352	0.289	0.254	0.215
	11	**0.027**	0.124	0.508	**0.040**	0.471	0.339
	15	0.328	0.709	0.591	0.601	0.693	0.213
	17	0.171	0.516	0.484	0.213	**0.011**	0.531
	22	0.370	0.535	0.751	0.639	0.331	0.703

Synaptotagmin 1 exon/ intron 2 and 3	All	0.141	0.517	0.388	0.270	***0.092***	0.574
	3	0.391	0.280	0.061	0.155	0.740	0.612
	6	0.479	**0.031**	***0.093***	**0.004**	**0.028**	**0.030**
	10	0.131	0.131	1	0.235	0.612	0.570
	12.13	0.233	0.170	0.774	0.374	0.947	0.582
	14	0.292	0.547	0.676	0.513	0.114	0.332
	18	0.232	0.283	0.924	0.278	***0.051***	***0.079***
Synaptotagmin 1 Exon/ intron 5	All	0.690	0.705	0.998	0.903	0.261	0.815
	1	0.718	0.404	0.250	0.507	0.577	0.387
	2.3	0.116	0.320	0.584	0.299	0.972	0.777
	4	0.401	0.174	0.554	0.387	0.457	0.738
	10.11	0.134	0.766	0.357	0.292	0.519	0.964
	12	0.923	0.658	0.555	0.812	0.234	0.293
	13	1	0.111	0.181	**0.045**	0.686	**0.017**
Synaptotagmin 1 Promotor and exon 1	All	0.687	0.345	0.167	0.348	0.260	0.887
	1	**0.023**	0.112	0.389	**0.032**	0.444	0.165
	4	0.204	0.103	0.630	0.184	0.132	0.921
	6	0.689	0.689	1	0.895	1	0.104
	7	0.295	0.126	0.594	0.305	0.737	0.796
	8	0.177	1	0.152	0.172	0.123	0.253
	10	0.640	0.369	0.162	0.334	0.246	0.563