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DNA methylation and demethylation in honeybee long-term memory formation

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It has been shown that changes in DNA methylation are crucial for long-term memory formation, however the specific role and dynamics of DNA methylation and its targets are still unknown. DNA methyltransferases (Dnmts) and ten-eleven-translocation enzym (Tet) are catalysing methylation and demethylation processes respectively. Here, we first examined the role of Dnmts during long-term memory formation using the honeybee as a model. We trained bees to associate a specific odour with a sugar reward and inhibited Dnmts using two functionally different drugs (Zebularine and RG108). During the memory retention test bees were presented with the learned and a new odour to test for odour-specific memory, which is the ability of bees to form a memory of a specific odour and not generalize to other odours. We showed that associative odour-specific long-term memory is impaired if Dnmts were inhibited. We therefore next investigated the expression of memory related target genes 24 hours after training, and found several of them upregulated after Dnmt inhibition in trained bees. Interestingly, Dnmt3 was upregulated in response to Dnmt inhibition after training, suggesting that Dnmt3 is auto-regulated. Using Sequencing and Sequenome mass spectrometry, we identified differentially methylated regions (DMRs) after long-term memory formation. Because memory formation and its regulation is a temporally dynamic process, we also examined the expression of Dnmts over time for the first 5 hours and at 24 hours after training. Dnmt1a and Tet (ten-eleven-translocation gene involved in demethylation) were upregulated at 1 hour and downregulated 24 hours after training. In contrast Dnmt3 was upregulated 5 hours after training returning to baseline level 24 hours after. Our study provides the first molecular evidence for how DNA methylation regulates long-term memory formation and which genes are targeted by Dnmts and Tet.