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Micro-RNA regulation of olfactory learning and memory in honeybees

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Honeybees forage in an ever-changing olfactory environment, which requires constant learning and re-learning of floral odours associated with food sources. Plastic olfactory memories hence are crucial to a honeybee's foraging success, however the molecular mechanisms regulating olfactory learning and memory are not well understood. Here, we investigated how learning and long-term memory formation affects gene expression in the honeybee brain using an olfactory conditioning paradigm (proboscis extension reflex, PER). A microarray gene expression analysis comparing groups of conditioned and control bees found 77 genes differentially expressed between these two groups. Most of these genes were down regulated in trained bees, with only a few non-coding RNAs upregulated. Many of the down-regulated genes were enriched with binding sites for microRNAs (miRNAs), that putatively dysregulate these genes during memory formation. Indeed, qRT-PCR analysis validated that seven miRNAs were upregulated in trained bees. Of these seven miRNAs, we further investigated miR-210, which is associated with foraging, and miR-932, which is embedded in a key neurological gene: the synaptic adhesion molecule neuroligin 2 (Nlg2). We suggest that modules of miRNAs may regulate synapse development during learning and memory processes. To test this hypothesis we used small interference RNAs to inhibit miR210 and miR932. Feeding cholesterol-conjugated antisense RNA to bees resulted in the inhibition of miR-210 and of miR-932. Loss of miR-932 impaired long-term memory formation but not memory acquisition. Functional analyses showed miR-932 interacts with Act5C, providing first evidence how a miRNA directly targets actin. An activity-dependent increase in miR-932 expression may therefore control actin-related plasticity mechanisms and affect memory formation in the brain.