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Knockdown of CaMKII impairs long-term-memory in honeybees

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Honeybees possess a highly flexible repertoire of cognitive abilities and are able to perform complex learning tasks. The neuronal and molecular bases underlying these behaviors are key to understanding higher brain functions and social organization, but are still not fully understood. The calcium/calmodulin-dependent protein kinase II (CaMKII) is a highly abundant protein in the brain of vertebrates and initiates biochemical cascades associated with memory formation. In adult honeybees CaMKII preferentially occurs in the mushroom bodies (Kamikouchi et al., 2000 JCN 417:501; Pasch et al., 2011 JCN 519:3700), centers for sensory integration and memory processes. To close the gap between the molecular level and honeybee behavioral plasticity, knockdown of CaMKII in the mushroom bodies was induced and followed by classical olfactory conditioning. siRNA against CaMKII (siCaMKII) or GFP (control) was injected via the occelar tract to cause RNA interference and create a loss of function phenotype. Furthermore, pharmacological inhibition was used as a parallel approach (KN93: active inhibitor of CaMKII, KN92: ineffective analog). Injections of Cy-3-labeled siRNA visualized the distribution in the mushroom bodies. Quantitative RT-PCR confirmed the reduction of CaMKII mRNA 2 hours after the injection, and quantitative western blots showed successful downregulation of the protein 8 hours after injection. Based on these results honeybees were subjected to olfactory conditioning using the proboscis extension response 8 hours after siRNA injection (18 hours after injection of pharmaca). All bees showed normal performances during memory acquisition and during memory retention 1 hour later. However, memory retention 24 hours and 72 hours after learning was significantly impaired in bees injected with siCaMKII and KN93 compared to the control groups indicating that CaMKII is an important mediator for the formation of long term memory in the honeybee brain. Funded by the Graduate School of Life Sciences (GSLS), University of Würzburg.