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Consolidated long-term memory in Drosophila melanogaster, from genetics to behaviour.

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The consolidation of long-term memory (LTM) implies sophisticated regulatory processes whose molecular bases are still to be fully elucidated. The fruit fly *Drosophila melanogaster* constitutes an excellent model in Neuroscience to study complex behaviour, such as sleep, learning and memory. Its fully characterized and numerically simple brain, combined with the variety of genetic tools available for manipulation, represents only a part of the numerous advantages that this insect offers for research compared with other models. This thesis explores the molecular basis underlying the consolidation of olfactory associative memory in fruit flies, presenting three different studies. An introductory excursus over the main concepts underlying this thesis is provided in the first chapter.

Chapter 2 is based on a study in which we assessed LTM-specific genetic expression, dissecting two phases of memory consolidation. Previous transcriptomics studies have identified genes involved in learning and memory processes. In our approach, we benefited from the well characterized CrebB transcription factor to access the genes regulated downstream of the LTM-induced signaling cascade. Applying the targeted DamID (TaDa) approach, we generated a CrebB-Dam methylase fusion protein and profiled, *in vivo,* CrebB-binding for the selection of candidate CrebB targets in the Mushroom Body (MB), the centre of olfactory memory formation. We conducted an RNAi screen of the extracted genes and selected *HERC2* and *cic*, as regulators for 24h memory and *unc-5* and *esn*, as regulators for 48h memory. These genes were further tested for their temporal and spatial specificity.

In chapter 3, we provided additional insights on the conserved function of *hacd1*, a gene previously identified as an LTM regulator. Knocking down this gene from MB neurons resulted in enhanced LTM. We generated a transgene line carrying a UAS-hHACD1 construct to induce the expression of the human homologue HACD1 in flies knocked down for the endogenous *hacd1*. The memory performance of the new transgenic line was comparable to control groups, suggesting that *hacd1* function is conserved in the human version. In addition, we showed that Hacd1 conserves the function of the yeast homologue, essential for fatty acids (FAs) metabolism. *D. melanogaster hacd1*, in fact, could complement the lethal mutation of *S. cerevisiae PHS1*. Profiling the level of FAs in pan-neuronal *hacd1* knockdown we finally evaluate the impact of this gene on their abundance.

Among the different regulations that occurs behind memory formation, sleep has been reported to play and active role during both learning and memory consolidation. In chapter 4, we implemented TaDa to identify new sleep-regulating genes. With the use of a Dam-Pol-II fusion protein, we profiled gene expression in the MB of sleep-deprived adult flies. Similar to the study presented in the first chapter, we extracted a list of candidate targets and performed an RNAi screen, this time testing sleep-related behaviour. From this screen we identified 5 genes (*alc*, *Gale*, *Prx5*, *CG5986* and *CG32806*) with significant altered sleep phenotypes.

Jury:

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