

Influence of sulfur metabolism genes on processes related to learning and memory in *Drosophila melanogaster*

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The gas-transmitter hydrogen sulfide (H₂S) is one of the most important biological mediators produced by the transsulfuration pathway (TSP) and is involved in many physiological and pathological processes related to oxidative stress, reproduction, learning, and memory. The transsulfuration pathway (TSP) converts homocysteine to cysteine after the breakdown of methionine. In *Drosophila melanogaster* and other eukaryotes, the enzymes cystathionine- β -synthase (CBS) and cystathionine- γ -lyase (CSE), play a central role in H₂S formation and metabolism. We obtained fly lines with sulfur metabolism gene deletions using the CRISPR/Cas9 technique. We also obtained lines with double deletion of CBS and CSE genes. In this study, we investigated the role of H₂S in learning and memory processes using the courtship paradigm with mating rejection. It was shown that in fly lines with double gene deletion and CBS gene deletion, short- and long-term memory formation was completely impaired. In the fly line with CSE gene deletion, only impaired memory consolidation processes were observed. We obtained and analyzed transcriptome libraries from the heads of naive males with gene deletions. Sequencing was performed on the Illumina NextSeq 500 platform. Sequencing yielded approximately 15-20 million reads for each library. Statistical analysis showed that there were approximately 4 times as many down-regulated genes as up-regulated genes. Of particular interest is that the maximum number of genes that significantly changed the level of gene expression was found in lines with single deletion of the CBS gene (295 genes). At the same time, lines with double deletion showed a significantly lower number with altered gene expression levels (190 genes).

Transcriptome analysis revealed multiple changes by Gene Ontology terms. We found significant down-regulation of genes that are involved in learning and memory processes in all lines with CBS gene deletion and especially in lines with double deletion of sulfur metabolism genes.