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Flexible memory controls sperm competition responses in male *Drosophila melanogaster*

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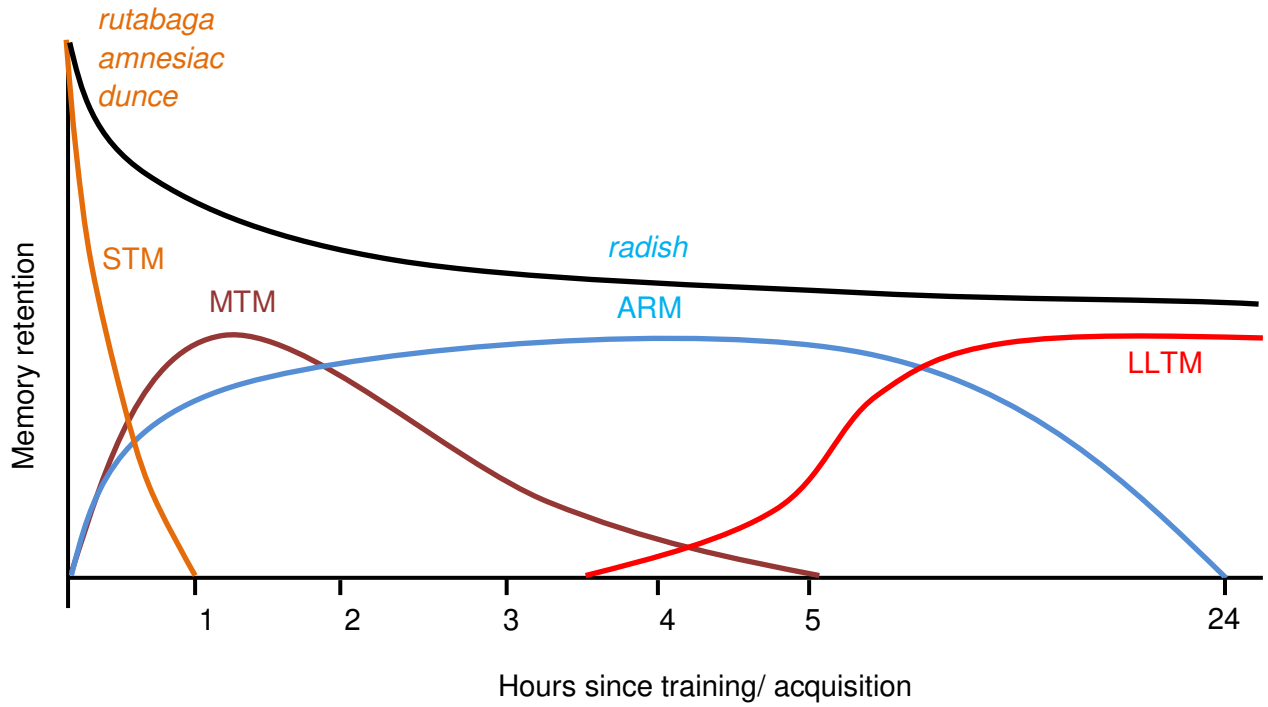
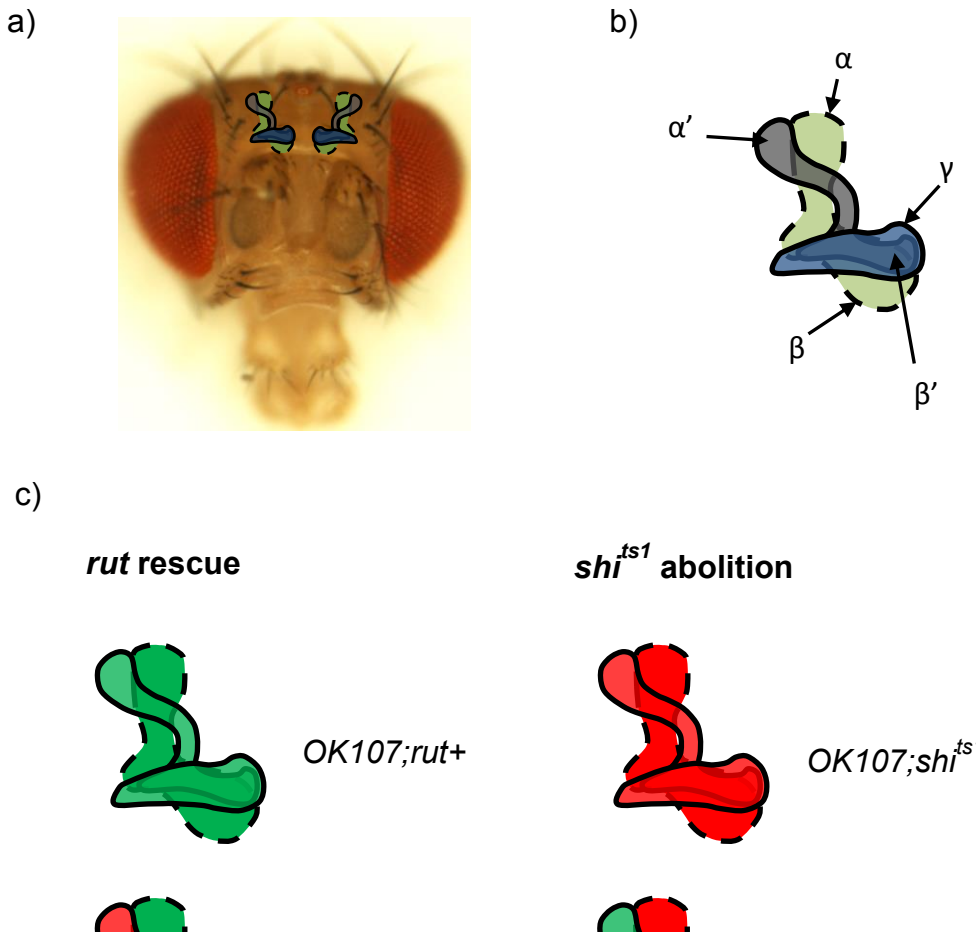
Supplementary Material



Supplementary figure S1: Memory phases during associative memory in *Drosophila melanogaster* with genes targeted in this study and known to be involved in each stage. The three phases of anaesthesia sensitive memory (ASM) are shown in orange (short term memory), dark red (medium term memory) and red (long-lasting term memory). Anaesthesia resistant memory is shown in blue. The black line is the behavioural degradation of memory shown phenotypically. All genes targeted in this study are above the black line and coloured as to the memory phase they affect. Reproduced with amendments from [1].

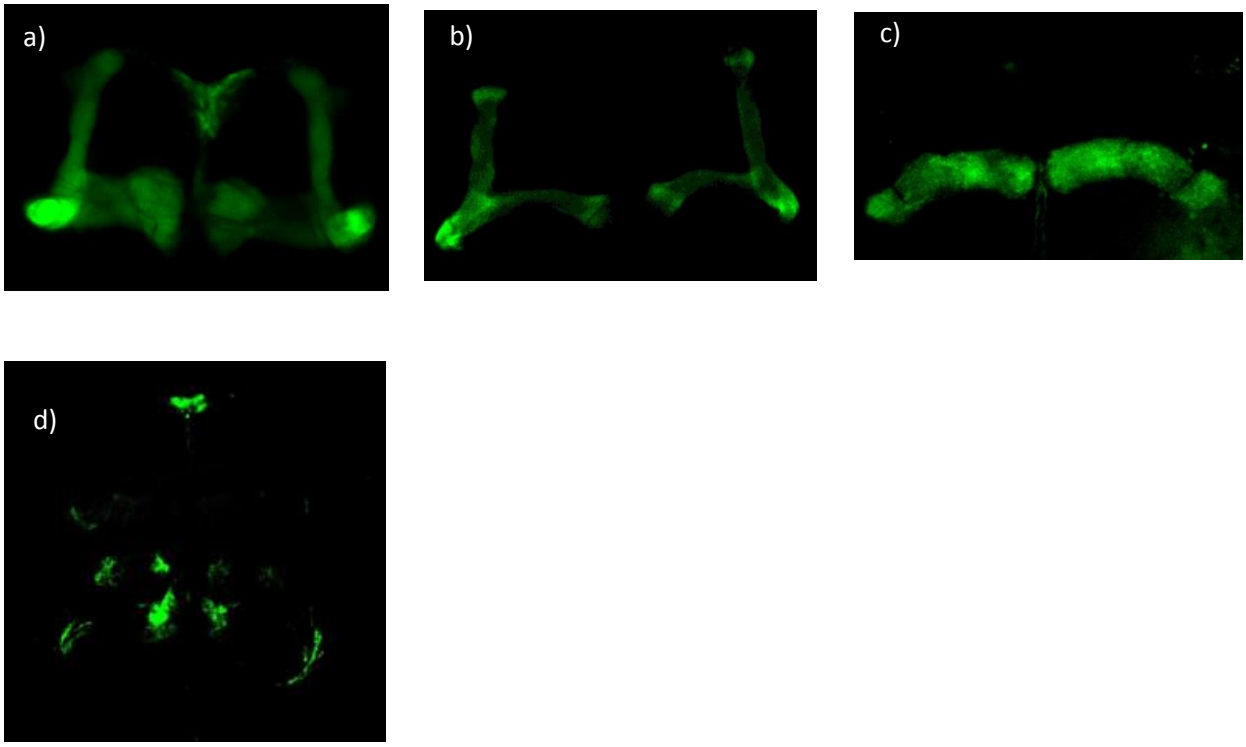


Supplementary figure S2: Genetic manipulations applied to various mushroom body lobes to disentangle neural mechanisms behind extended mating duration. a) Mushroom body location in the fly head. b) Representation of all MB lobes. c) MB lobes activated with expression of wildtype *rut* or inhibited with expression of shi^{ts1}. Each MB diagram represents

the activation or inactivation of particular lobes depending on genotype. Gal4 drivers crossed to *rut* are displayed on the left hand side, whereas those same Gal4 drivers crossed to *shi*^{ts1} are displayed on the right hand side. Lobes shaded in green represent active lobes, lobes shaded in red are inactive, and lobes shaded in orange represent inactivation of part of the MB lobe.

Confirmation of driver tissue specificity

Females from *OK107-GAL4, NP3061-GAL4, NP1131-GAL4* and *1471-GAL4* lines were crossed to UAS-mCD8::GFP males. Brains of male progeny were dissected and fixed in 4% paraformaldehyde (in PBS) for 30 minutes, and then rinsed once in 0.3% PBST. Brains were mounted in 80% glycerol (in PBS) and imaged on a Zeiss LSM 700 confocal microscope. Z-stack, 20 x lens, 1µm spacing in the Z axis. Pictures were processed in ImageJ [2, 3].



Supplementary figure S3: Expression pattern of a) *OK107;GFP*, expressed in all the lobes of the mushroom body b) *NP3061;GFP*, expressed in the α/β lobes of the mushroom bodies

only c) *NP1131;GFP*, expressed in the γ and α'/β' lobes of the mushroom bodies and d)

1471;GFP, expressed in the γ lobes of the mushroom body only.

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