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LEARNING AND MEMORY

Conditions for fear

State-dependent memories are those that are most successfully retrieved when conditions at the time of recall are the same as they were at encoding, but the mechanisms that lead to state-dependent learning are not known. Jovasevic *et al.* now show that activation of extrasynaptic GABA type A receptors ($GABA_A$Rs) in the hippocampus immediately before training results in state-dependent fear conditioning, which is negatively regulated by microRNA-33 (miR-33).

Administration of gaboxadol (GBX; a $GABA_A$R agonist) to the hippocampus in mice just prior to fear-conditioning training (in which mice were placed in a novel context, and received a footshock) decreased freezing (a measure of the fear response) when the mice were tested 24 hours later in the same context. However, when GBX was administered twice — once before training, and once before the test — levels of freezing were similar to those of vehicle-treated mice. This suggests that the presence of GBX during training created a 'state' on which later retrieval of the fear memory was dependent.

Next, the authors investigated the molecular mechanisms by which GBX treatment created this state. Levels of phosphorylated protein kinase CβII (PKCβII) were increased in the hippocampus in mice that received GBX just before training and testing. Furthermore, hippocampal administration of a PKCβII antagonist just before testing reduced GBX-dependent freezing in these mice, suggesting that PKCβII activity is required for full retrieval of the memory that was made state-dependent by the presence of GBX during training.

Hippocampal expression of miR-33, which targets genes encoding proteins related to $GABA_A$R function, was decreased or increased following fear conditioning in the presence or absence of GBX, respectively. Lentivirus-mediated overexpression of miR-33 in the hippocampus blocked the effects of GBX treatment on fear conditioning in mice, and reduced expression of mRNAs encoding $GABA_A$R-related proteins in untrained mice. Furthermore, downregulation of hippocampal miR-33 expression by administration of a locked nucleic acid inhibitor reduced the GBX dose required immediately before training to create state-dependence in the fear-conditioning paradigm.

The authors also explored how GBX-state-dependency affected components of the extended hippocampal circuit that are normally required for fear conditioning. Relative to vehicle-treated fear-conditioned controls, GBX-treated fear-conditioned animals had more neurons labelled with early growth response protein 1 (EGR1; a marker of neural activation) in the dentate gyrus and lateral septum, and fewer EGR1-labelled neurons in the retrosplenial cortex (RSC). During retrieval tests, pharmacogenetic inactivation of neurons in the RSC resulted in decreased freezing behaviour in mice that were fear conditioned without GBX, but increased freezing in mice fear conditioned with GBX. Thus, RSC activation is required for 'normal' memory retrieval but not for the GBX-state-dependent fear conditioning.

Together, these findings demonstrate that hippocampal $GABA_A$R activation can induce a state on which fear memory is dependent. This state-dependency is mediated in part by PKCβII activity, is negatively regulated by miR-33 and is associated with enhanced subcortical and decreased cortical processing of the memory.

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ORIGINAL RESEARCH PAPER Jovasevic, V. *et al.* GABAergic mechanisms regulated by miR-33 encode state-dependent fear. *Nat. Neurosci.* **18**, 1265–1271 (2015)

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