Published in final edited form as:

Nat Neurosci. 2015 September ; 18(9): 1194–1196. doi:10.1038/nn.4098.

GABA receptors in a state of fear

Andrew Holmes and

Laboratory of Behavioral and Genomic Neuroscience, National Institute on Alcohol Abuse and Alcoholism, US National Institutes of Health, Bethesda, Maryland, USA

Alon Chen

Ruhman Family Laboratory for Research on the Neurobiology of Stress, Department of Neurobiology, Weizmann Institute of Science, Rehovot, Israel, and the Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, Munich, Germany

Andrew Holmes: andrew.holmes@mail.nih.gov

Abstract

Our internal states can color our memories just as powerfully as the external environment. A study finds that hippocampal GABA_A receptors and associated microRNAs are important for generating state-dependent contextual fear memories.

What happens underwater stays underwater. Essentially, that was the conclusion of what became a classic study in psychology conducted some 40 years ago by Godden and Baddeley¹. The two researchers somehow persuaded a group of divers, who were otherwise enjoying a relaxing holiday in Scotland, to learn a long list of simple words either when they were in the water or on land, and then asked them to freely recall the words in one or the other environment. As Godden and Baddeley had suspected, more words were recalled when the divers were in the same environment, wet or dry, in which they had originally learned them in. This phenomenon is now well-known in psychology as state-dependent learning². It tells us that not only does the external environment direct what we learn and remember, but so too does the internal state of our body and mind. And this applies to all manner of memories. It might even apply to the kinds of memories that are indelibly linked to profoundly traumatic events that can spiral into an anxiety disorder or post-traumatic stress disorder. These disorders have become some of the most prevalent of all psychiatric conditions and place a heavy burden on individuals, their families and society at large. But, although we have been making appreciable progress in drawing an increasingly detailed schematic of the brain circuitry responsible for forming, storing and erasing fear memories^{3–5}, we still know surprisingly little about how the brain processes information about state to modulate fear memories.

We now have some important new insights into this question thanks to a study by Jovasevic *et al.*⁶ published in this issue of *Nature Neuroscience*. These authors, who have for some years studied the molecular and cellular machinery governing fear memories, were struck by

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

an apparent paradox. They noted that some drugs acting on GABA_A receptors disrupted memory, whereas others did not, and in fact could enhance state-dependent learning. One reason for these variable effects, they hypothesized, could lie in known differences in how certain drugs bind to specific subpopulations of GABA_A receptors. Specifically, they reasoned that those drugs that target and bind extrasynaptic GABA_A receptors might preferentially influence state-dependent fear learning.

The authors set about testing this idea in mice by first infusing gaboxadol, which activates extrasynaptic GABA_A receptors, into the hippocampus, a brain region central to the formation of contextual fear memories. One group of mice received drug infusion immediately before they were trained to associate a particular environment with foot shock, whereas another group of mice were infused before testing for retrieval of the fear memory the following day. Both of these groups showed less fear (freezing behavior) during the retrieval test, indicating an impairment in contextual fear memory. In contrast, a third group that was given gaboxadol infusions both before training and before testing had fear levels comparable to controls that had never received the drug. This pattern of results is consistent with hippocampal extrasynaptic GABA_A receptors mediating a state-dependent form of contextual fear (Fig. 1).

How does activating hippocampal GABA_A receptors change the intracellular signaling milieu to produce these behavioral effects? Jovasevic *et al.*⁶ went searching for some of the potentially critical molecular events acting downstream of the GABA_A receptor. Given prior hints that GABA_A receptors phosphorylate protein kinase C (PKC), the authors screened for changes in phosphorylation following gaboxadol administration and pinpointed an upregulation in phosphorylation of PKC β II on Ser660. This molecular change turned out to be a functionally relevant one, as inhibiting PKC β II interfered with the generation of state-dependent contextual fear memory by gaboxadol. Given the growing interest in PKC as a therapeutic target for memory deficits in various brain diseases⁷, it would be worthwhile following up on this finding to further define the role of PKC β II in state-dependent learning and potentially other forms of fear memory.

The authors next turned their attention to another recently reported regulator of GABA_A receptors⁸: microRNAs. miRNAs are group of small, noncoding RNAs with a widespread and profound effect on the expression of protein-coding genes⁹. miRNAs are attracting increasing interest, not only as regulators of brain function and behavior, but also as factors moderating risk for psychiatric illnesses¹⁰, and prior studies have already implicated miRNAs in fear memory formation and other stress-related behaviors¹⁰. Could they also influence state-dependent contextual fear? Beginning with the profiling of miRNAs that were differentially expressed in the hippocampus after fear conditioning, Jovasevic *et al.*⁶ whittled down the list of potential miRNA candidates to one, miR-33, which they postulated might target several mRNAs encoding GABA_A receptors and be decreased by a combination of fear training and gaboxadol.

The road of many studies in neuroscience leads ultimately to the question of causality. In this case, the question is whether miR-33 gates the capacity of extrasynaptic GABA_A receptors to produce state-dependent fear memories. Jovasevic *et al.*⁶ tackled this question

with viral constructs that, when infused into the hippocampus, allowed the researchers to either increase or decrease miR-33 locally. Using these tools, they found that overexpressing miR-33 was sufficient to disrupt the memory-impairing effects of single intrahippocampal gaboxadol infusions given before training or testing, but did not prevent the state dependency produced by drug infusion before both training and testing. They also found that virus-mediated miR-33 knockdown induced a state dependent–like fear memory when combined with a pre-training and pre-testing subthreshold dose of gaboxadol that, when administered alone, was insufficient to generate state dependency. On the basis of these data, the authors concluded that levels of miR-33 in the hippocampus set the bar for GABA_A receptor–mediated state-dependent memory, with low miR-33 enabling state dependency, but high miR-33 not sufficient to occlude it.

Although altering levels of hippocampal miR-33 clearly affected memory, miR-33 is not a specific regulator of GABA_A receptors, and the observed phenotype could well be mediated by changes in multiple miR-33 targets in the hippocampus. In this regard, it will be important to tie the various strands of evidence together and establish a plausible link between miR-33, memory and the GABA_A receptor. This question awaits resolution in future work, but Jovasevic *et al.*⁶ do provide initial hints by showing that virally altering levels of miR-33 changes the hippocampal expression of known regulators of the GABA_A receptor, such as synapsin-2.

Jovasevic *et al.*⁶ sought to add one final piece to their puzzle and asked how activating the GABA_A receptor in the hippocampus recruits a broader brain network to shape state-dependent fear. To do so, they quantified the expression of immediate early gene responses (c-Fos and early growth factor 1) in hippocampal projections areas after mice had been tested for gaboxadol-induced state-dependent contextual memory. They found an increase in neural activation in the dentate gyrus and lateral septum, but a decrease in the retrosplenial cortex. Although it is unclear whether this pattern of neural recruitment is unique to a GABA_A receptor–generated state-dependent memory, it would fit with the notion of a functional rebalancing of the extended hippocampal circuit in favor of subcortical regions at the expense of the retrosplenial cortex, as state-dependent fear memories are formed.

Jovasevic *et al.*⁶ further bolstered this idea by inactivating the retrosplenial cortex pharmacogenetically using DREADD (designer receptors exclusively activated by designer drugs). Inactivation before retrieval impaired contextual fear in undrugged controls, but appeared to further strengthen a gaboxadol-induced state-dependent contextual memory. Thus, engagement of this cortical area may have a dual role, acting to support the retrieval of contextual fear memories, but suppress fear memories that are state dependent—at least those driven by GABA_A receptor activation. This speaks to one of the key messages from the findings: state-dependent fear memories involve a set of neural systems and molecular genetic factors that, although overlapping, are distinct and dissociable from those subserving contextual fear that is not state bound. We hope that this will stimulate a renewed interest in studying this and other forms of state-dependent memory.

What, if any, are the potential clinical implications of these important insights from laboratory studies in mice? Drugs acting on the GABAergic system, such as diazepam

(Valium) and other benzodiazepines, have been mainstays of anti-anxiety medication for decades. However, more recent efforts to develop compounds with refined actions at subpopulations of GABA receptor subtypes did not pan out clinically¹¹. The new findings from Jovasevic *et al.*⁶ provide us with a more nuanced appreciation of how GABAergic drugs might affect the mnemonic aspects of anxiety. This is particularly pertinent to the question of improving the utility of cognitive-based strategies such as exposure therapy, often used to treat post-traumatic stress disorder. These strategies rely heavily on learning processes to produce a new, 'safe' memory for a traumatic event¹² and are tightly gated by external context¹³. Benzodiazepines are suspected of interfering with the efficacy of exposure therapy¹⁴. Could this be in part a result of the generation of a safety memory that is bound to a benzodiazepine-induced state and henceforth liable to failure when a patient is not on the drug? There is already evidence in rodents that this may well be the case¹⁵.

Looking beyond the GABAergic system, the other major finding is that state-dependent learning offers another in a growing list of behaviors that are linked to the actions of miRNAs. In other fields of medicine, such as in the treatment of cancer, miRNA-based therapeutics are gaining traction. Although this is still a nascent area for psychiatry, exploiting miRNAs to shape brain function and behavior will be an exciting avenue in the coming years.

References

1. Godden DR, Baddeley AD. *Br. J. Psychol.* 1975; 66:325–331.
2. Girden E, Culler E. *J. Comp. Psychol.* 1937; 23:261–274.
3. Milad MR, Quirk GJ. *Annu. Rev. Psychol.* 2012; 63:129–151. [PubMed: 22129456]
4. Lüthi A, Luscher C. *Nat. Neurosci.* 2014; 17:1635–1643. [PubMed: 25402855]
5. Maren S, Holmes A. *Neuropsychopharmacology*. 2015 Jul 29. advance online publication.
6. Jovasevic V, et al. *Nat. Neurosci.* 2015; 18:1265–1271. [PubMed: 26280760]
7. Sun MK, Nelson TJ, Alkon DL. *Trends Pharmacol. Sci.* 2015; 36:384–394. [PubMed: 25959522]
8. Zhao C, et al. *BMC Res. Notes*. 2012; 5:91. [PubMed: 22321448]
9. Bartel DP. *Cell*. 2009; 136:215–233. [PubMed: 19167326]
10. Issler O, Chen A. *Nat. Rev. Neurosci.* 2015; 16:201–212. [PubMed: 25790865]
11. Griebel G, Holmes A. *Nat. Rev. Drug Discov.* 2013; 12:667–687. [PubMed: 23989795]
12. Ressler KJ, Mayberg HS. *Nat. Neurosci.* 2007; 10:1116–1124. [PubMed: 17726478]
13. Bouton ME. *Biol. Psychiatry*. 2002; 52:976–986. [PubMed: 12437938]
14. Marks IM, et al. *Br. J. Psychiatry*. 1993; 162:776–787. [PubMed: 8101126]
15. Bouton ME, Kenney FA, Rosengard C. *Behav. Neurosci.* 1990; 104:44–55. [PubMed: 2317285]

Figure 1.

Activating hippocampal GABA_A receptors generates a state-dependent contextual fear memory. Under normal, drug-free circumstances, pairing a distinct context with foot shock (training) generates a robust fear memory for that context, as indicated by freezing on context reexposure (testing). When extrasynaptic GABA_A receptors are activated in the hippocampus by infusing gaboxadol either before training or before testing, freezing during the test is low, suggesting that the contextual fear memory has been disrupted, perhaps because its expression is dependent on the simultaneous engagement of hippocampal GABA_A receptors. Consistent with the generation of a state-dependent memory by GABA_A receptors, infusing gaboxadol into the hippocampus before both training and testing

produced robust expression of the contextual fear memory. Thus, hippocampal GABA_A receptors generate a neural state that gates context fear.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript