Reactivating hippocampus-mediated memories to disrupt the reconsolidation of fear

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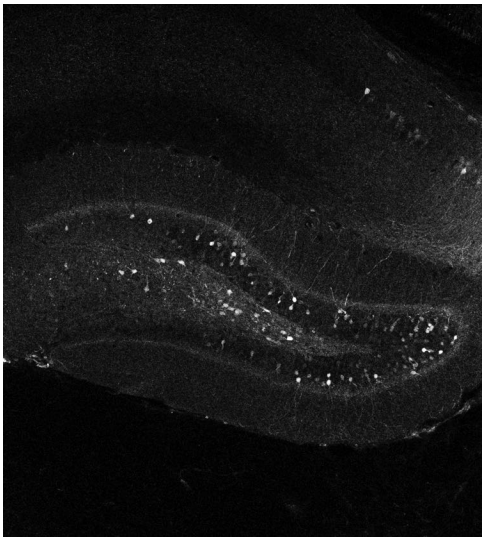
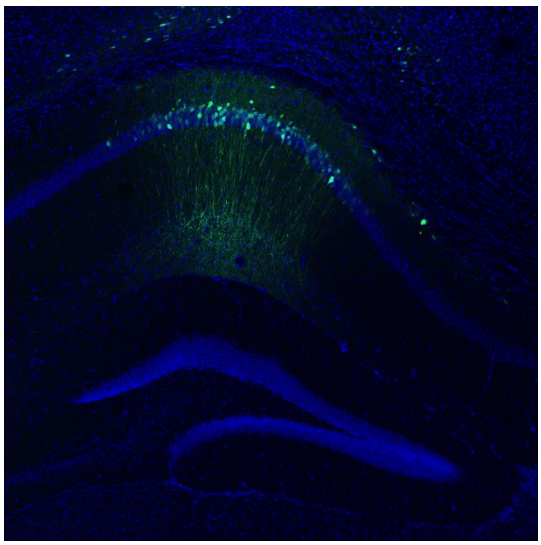
My project is centered around research questions related to how memories are formed and how they change over time. We view memories at a cellular level, in a brain region called the hippocampus. When memories are formed, a collection of neurons are recruited here. This collection of cells is referred to as an "engram".

Below: (Left) Dentate gyrus region of the hippocampus. Cells in white represent a "tagged memory". (Right) CA1 region of the hippocampus. Counterstain is DAPI (blue) which stains the nuclei of all cells. Cells in green (eYFP) represent positive memory (male and female mouse together for 1hr).

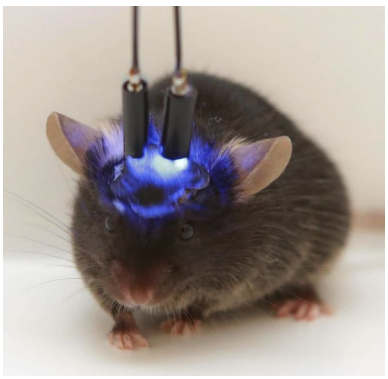


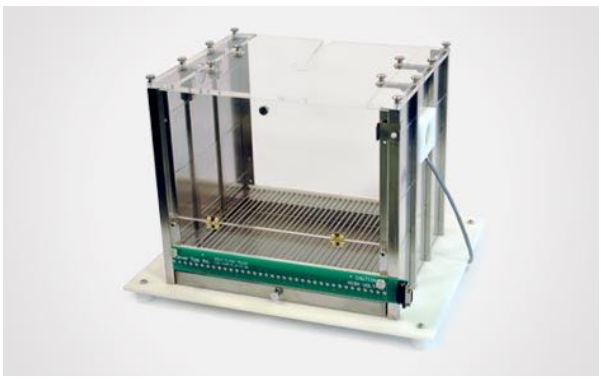


Within these neurons, genes are transcribed, proteins are made, and the connections between neurons are being strengthened. This *synaptic plasticity* referred to as "consolidation" allows for the reactivation of these same neurons when the memory is recalled.

Memory tagging: In mice, if we implant an optic fiber and inject an adeno-associated virus in a brain region called the "hippocampus" that is activity dependent, and doxycycline-regulated (see below), we can "tag" and label the cells involved in a memory and then later, using *optogenetics* we can artificially reactivate the memory by shining light into the brain with a laser.

Below: (Left) c57BL/6 male mouse with fiber optic implant and laser patch cord attached expressing blue light. (Right) Standard fear conditioning chamber.





The mouse undergoes stereotaxic surgery where we inject the virus into the hippocampus; the virus takes about 10 days to become fully integrated within the cells. When the virus is activated it allows for the expression of two proteins: 1.) *channelrhodopsin-2* (ChR2), which is a light-sensitive protein that was originally discovered in green algae and 2.) *enhanced yellow fluorescent protein* (eYFP) which can be visualized following fluorescent immunohistochemistry and confocal microscopy. At the start of the experiment, the animals are fed a diet containing doxycycline (DOX), an antibiotic which prevents the virus from being active. This is achieved by blocking two other cellular components from binding. When we want to tag a specific experience, we then take the animals off DOX and since the virus is activity-dependent this means that any cell that expresses the immediate early gene *c-fos* will also express ChR2 and eYFP. Once the experience we want to tag is complete (e.g. 1hr paired with another mouse) we place the animals back on DOX and this closes the window of cellular tagging. Then any time later, since we have already fitted the animal with an optic fiber aimed at the hippocampus, we can artificially reactivate the memory using a blue laser beam (which is the wavelength of light that ChR2 responds to). This causes these protein channels to open changing the property of the cell such that an action potential occurs (the cell fires). Reactivation of these cells causes the animal to recall the original experience. So why would we want to do this? Good question. This brings us to the next topic…

<u>Memory modulation:</u> When a memory is recalled naturally, it becomes susceptible to modification for a brief time, a window of about 6 hours. During this time, anything that happens can interfere with how that memory is re-remembered or *"reconsolidated"*. The memory can be disrupted or enhanced. For instance, adrenaline or noradrenaline as it is called in the brain, can cause a memory to be remembered better. And in the case of memory disorders such as *post-traumatic stress disorder* (PTSD) this chemical can cause memories to be remembered so well that the individual cannot forget them even if they want to. So, some previous experiments have tried using drugs that block the noradrenaline system to reduce the strength of the memory and in some cases this has worked well. But that's exactly what it does, it reduces the strength of the memory but

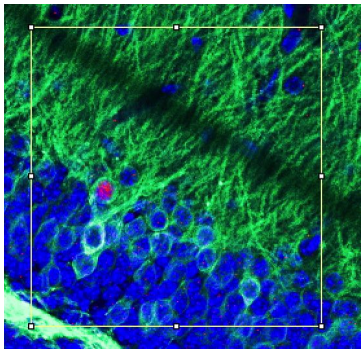
does not change the original memory, that memory is still there. People often complain about having a bad memory but in reality, it is extremely difficult to destroy memories and often the reason you forget things is due to a retrieval error and not because the memory itself is gone. So, what we're trying to do is take advantage of the reconsolidation process where memories can be altered for a brief period and our attempts are aimed at disrupting the original memory and not just the strength of it. We use animal models of PTSD where the animal experiences something negative - e.g. *fear conditioning* where an animal receives a brief foot shock in a context and then learns to pair the shock with that environment causing the animal to subsequently freeze (behaviour indicative of fear) when placed back in the context without the shock the following day during a recall session.

Below: Confocal microscopy and immunohistochemistry.

Prior to this, we have tagged a positive experience e.g. pairing a male mouse with a female mouse. During the recall session when the animal is naturally recalling the fear memory, when the animal would typically be freezing, and when the reconsolidation window is open, we artificially reactivate the positive memory to disrupt the original fear memory. We test the animal at later time points to assess its fear of the context compared to control animals. So far, we have had some interesting results. And theoretically, if we are meshing or intertwining the positive and negative memory in a way to reduce fear we should be able to detect this under the microscope as the tagged memory (positive) will show up as eYFP-labeled cells and the fear memory (negative) can

be detected with standard immunohistochemistry of immediate early genes if the brains are extracted within two hours after recall.

Below: Dentate gyrus region of the hippocampus. Counterstain is DAPI (blue) which stains the nuclei of all cells. Cells in green (eYFP) represent a positive memory (male and female mouse together for 1hr). Cell in pink represents a negative memory (fear conditioning recall).



While these techniques are not yet viable in humans, we hope this work will shed light on the basic neural underpinnings of how memory works and highlight the therapeutic value of memory modulation as a viable treatment for the suppression of fear responses, implicating the hippocampus as a specific node of future intervention.