Lost and Found

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"To forget, or not to forget. What are the questions?" Hamlet's question was deeper - whether to live or to die - but as memory is so central to everyday life, retaining the capacity to remember is not so far from staying alive. A life without memory would be little life at all. So much has been investigated and written about memory – short and long-term, qualitatively different types of long-term memory, and the underlying brain areas and physiological mechanisms - that we are apt to ignore the potential importance of the 'flip-side' of memory, namely forgetting. Effective forgetting is also central to effective memory. Three new neurobiological papers have recently been published that highlight different aspects of forgetting (Madronal et al., 2016, Migues et al., 2016, Roy et al., 2016).

Forgetting may, in the simplest case, be a true loss of memory. The underlying memory 'trace' becomes somehow degraded to the point where it is impossible, with any accuracy, to reactivate even bits of the information that had previously been encoded in the brain. Madronal et al's and Migues et al's work speaks to this possibility. An alternative is that forgetting is sometimes a failure to access information that is still in some sense 'in there' but which cannot be reactivated. So called 'tip-of-the-tongue' experiences are of this kind, for they induce the curious mental dissociation of being unable to access a memory which we know that we have. Names of people are a case in point for, no sooner is the name given again, than one instantly recognises it as familiar amidst one's embarrassment of having forgotten it. Roy et al's paper zeroes in on the neurobiology of memory access – and they do so in the context of Alzheimer's Disease.

This background enables us to be a bit more precise about some of the diverse questions that a modern-day scientific Hamlet needs to consider as he or she seeks to track down the

underlying mechanisms of forgetting. First, are there specific areas of the brain where forgetting in the sense of true loss can be identified as happening – i.e. where a trace is lost following some treatment? Second, is forgetting a passive process happening in the background with the passage of time or is it an active process with determinants that can be identified or even altered? And third, if forgetting is sometimes due to a failure of access, are there ways in which the ostensibly inaccessible information could be re-activated?

In an elegant and exacting series of studies, Madronal et al (2016) devised a method of selectively inhibiting the granule cells of the dentate gyrus without affecting other parts of hippocampal circuitry, something impossible to do with traditional pharmacological techniques. They point out that *"…a major limitation of resolving the mechanisms of hippocampal function has been the lack of tools that allow for rapid, transient, efficient and specific inhibition of hippocampal cell-types…".* Optogenetic techniques may be one route, but they are not without issues of imprecision associated with the extent of light spread in a region (and virus spread in some cases). The technique the Sevilla lab deployed was systemic administration of the serotonin 1A receptor agonist, 8-OH-DPAT to transgenic mice expressing this receptor exclusively on dentate gyrus (DG) granule cells. This inhibited field-potentials recorded one synapse downstream in CA3, and thus communication through the DG-CA3-CA1 circuit. Importantly, however, it had no effect on the direct input to CA1 from the entorhinal cortex via the perforant path that by-passes DG and CA3. Using conditioned animals in the now well-established eye-blink paradigm of the Delgado-Garcia/Gruart laboratory, they also observed that this DG inhibition caused a loss of CS-US responding. This was apparently 'true forgetting' because the transient inhibition induced by 8-OH-DPAT resulted in long lasting loss of conditioned responding over several days, outlasting the transient inhibition of the dentate gyrus. Responding was not immediately restored by CS-US presentations serving as a reminder; learning had to occur again and a gradual re-learning curve revealed. Additionally, just giving the drug without pairing its presentation with CS-US presentations had no effect, suggesting that DG inhibition might be a necessary but not sufficient condition for forgetting, it requiring also a depotentiation process involving relevant stimuli to be successively presented. A series of further experiments focused on the role of the entorhinal cortex inputs, a possible role of adenosine at presynaptic terminals, and some intriguing work that targeted, with a specific peptide, an endogeneous, dentate gyrus-specific inhibitory receptor (neuropeptide Y1R). These studies collectively pointed to a model that incorporated an interesting new idea about maintaining memory traces in

hippocampal circuitry. Specifically, CA1 synaptic efficacy may be held in balance by the separate trisynaptic and direct pathway inputs. Interruption of the dentate gyrus mediated trisynaptic pathway activity results, when coupled with further CS-US presentations, in a rapid depotentiation of synaptic efficacy in the direct pathway from CA1 and consequential loss of memory.

The idea that forgetting may be an active process is also the key theme of the work of Migues et al (2016). Adopting a more biochemical perspective linked in part to the work of Todd Sacktor in New York, e.g. (Pastalkova et al., 2006), they suggest that the retention of long-term memory depends on maintaining GluA2 containing AMPA receptors at post-synaptic sites, a sub-unit that like others cycles into and out of the post-synaptic density. Key pieces of evidence for this idea are that the strength of long-term memory correlates positively with the level of GluA2 expression, that active removal of GluA2-AMPA receptors from post-synaptic sites leads to memory erasure, and that blocking their internalization can prevent it. The new study, using object-place recognition memory, tested whether blocking post-synaptic GluA2-AMPA removal can prevent forgetting over time. They observed that blocking GluA2-AMPA receptor internalization in the hippocampus of rats after learning prevented forgetting of object location memory over days. Contrary to the predictions of interference views of forgetting, the same treatment potentiated new location learning, rather than impairing it. Parallel electrophysiological studies implicated depotentiation as a likely contributor to forgetting, just as Madronal et al (2016) suggest in their work.

In the history of neuropsychology, loss of memory in global amnesia has generally been ascribed to a failure to store new information, or to transfer it from short- to long-term memory, as in the initial account offered of patient H.M. (Scoville and Milner, 1957). Henry Mollaison could apparently remember a lot of things from prior to his bilateral surgical resection of the medial temporal lobe for the relief of epilepsy, including language and pretty much all the ‘semantic’ information he had acquired earlier in life, but he was unable to keep track of ongoing events nor intentionally learn new things (Corkin, 2002). It came therefore as something of a surprise to the neuropsychological community when (Warrington and Weizkrantz, 1968, Warrington and Weiskrantz, 1974) raised the possibility that at least some aspects of amnesia could be due to retrieval failure. They tested a number of different amnesic patients, some with mammillary body lesions coupled to damage to the anterior

thalamus, and showed using both word-stem cues and Gollin's fragmented pictures, that their subjects could sometimes recall information they had previously been unable to remember. While the debate was never fully resolved, it later transpired that they had stumbled across the phenomenon later to be called 'priming'. This refers to a process whereby the earlier presentation of information creates a temporary state in the brain whereby the whole information can be successfully recovered later when fragments of that information are presented as an implicit retrieval cue (such as a word-stem). That this occurs when there is brain damage to circuits of the medial temporal lobe and mid-line thalamic structures connecting it to the prefrontal lobe raises the intriguing possibility that it may also occur in degenerative conditions such as Alzheimer's Disease (AD). The possibility that some aspects of the forgetfulness in AD are due to retrieval failure has both diagnostic and therapeutic implications.

Roy et al (2016) studied context fear conditioning in transgenic mice expressing the delta exon 9 variant of presenilin (PS1) in combination with the Swedish mutation of human amyloid precursor protein (hAPP). Such 'AD mice' may hold onto information for a short time, but rarely for long. In this case, overnight forgetting of context fear was observed. Interestingly, this was seen in younger mice before deposition of amyloid plaques as well as in older mice with plaques – yet another example of now widely recognised need to focus on early events in AD. The next step was to examine whether this forgetting was a true loss of memory traces or a failure of access. Earlier work from the Tonegawa group had shown that tagging granule cells in the dentate gyrus with channelrhodopsin during the acquisition of context fear memory can enable memory retrieval later when the tagged neurons are optogenetically activated by blue light. They therefore did the same experiment in this murine model of Alzheimer's Disease that displays overnight forgetting and found, when tested in a different context in which freezing is not normally observed, that light activation of the tagged neuronal ensemble resulted in expression of context fear. There is an important detail with respect to what is happening here: the light will be illuminating many cells in the DG but only a subset will have been tagged with ChR2 during acquisition; only the tagged subset will be re-activated. Further experiments in the study examined both functional connectivity in AD mice and controls, and the capacity for selective LTP induction to restore access, following the idea being developed by this group to the effect that persistent cellular connectivity between multiple engram cell ensembles is critical for effective retrieval. A key aspect of this is 'selectivity' – indicating that the appropriate pathways of a connected

ensemble have to be activated and/or re-potentiated for access to memory to be observed and for it to be sustained. There remain some obstacles to using optogenetics to address all the issues of selectivity (Shrestha and Klann, 2016), but this exciting technique is shedding new light on the neurobiological questions in a manner that goes beyond the already extensive clinical literature on memory loss in AD patients.

It is a long way from the testing of memory retrieval in amnesic patients with fragmented pictures and word-stems 40 years ago to the selective optogenetic activation of a subset of granule cells in the dentate gyrus today. But the conceptual link is the idea that memory retrieval requires the selective activation of appropriate links, whether by natural stimuli or artificially such as with a drug. However, a drug is unlikely 'to do it on its own' without appropriate stimulation. In the same vein, Madronal et al (2016) and Migues et al (2016) emphasise that the 'devil-is-in-the-detail' with a respect to understanding activity-dependent forgetting. It is likely some way off, but these neurobiological studies raise, in different ways, the intriguing idea of rescuing long-term memory deficits, with a peptide or another engram-based approach, in a manner that takes account of this neuronal activity and selectivity.

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