# Resolving memory circuits with layer-dependent fMRI

**Jozien Goense**

School of Psychology and Institute of Neuroscience and Psychology, University of Glasgow, Glasgow G12 8QB, UK
Correspondence: Jozien.Goense@glasgow.ac.uk

**Memory encoding and retrieval requires directional exchange of information between different areas in the medial temporal lobe. In this issue of *Neuron*, Koster et al. (2018) use high-resolution fMRI combined with state-of-the-art data analysis methods to trace the information flow in memory circuits in hippocampus and entorhinal cortex.**

One of the promises of ultra-high field fMRI (≥7T) is that its increased spatial resolution allows us to resolve brain circuits and mechanisms. fMRI has been used for localization of brain functions for decades, but resolving circuits has been difficult until recently, because this requires showing causality and directionality, while typical fMRI is based on correlations. fMRI of cortical layers, with their segregated functions and connectivity, offers the potential to access mechanisms. However, fMRI depends on a hemodynamic response, and reflects a combination of neural activity, metabolism, vascular properties and MR-acquisition parameters (Logothetis, 2008). It has only recently been shown that fMRI-responses in cortical layers carry different information about stimuli (Goense et al., 2012), and that laminar functional connectivity can be used to probe directionality (Huber et al., 2017). In this study, Koster et al. (2018) take this a step further by applying laminar fMRI and sophisticated analysis tools (multivariate pattern analysis (MVPA) and informational connectivity (IC)) to resolve memory circuits.

Directionality of information flow within hippocampus and between different areas in the medial temporal lobe (MTL) is essential for the study of memory circuitry, as directionality is key to distinguish encoding, retrieval and association. The authors follow the information-flow through the circuit and provide evidence for the 'big-loop recurrence' model (figure 1) for inferring associations from memories. This model suggests a way to combine information from different memories, while not interfering with important memory functions such as pattern completion and pattern separation. An example of inferred associations is when one day you encounter a neighbour walking a dog, and next day you see the same dog being walked by a different person, you might think the two people live in the same household. This is an example of 'associate inference', the combination of information from separate memories, and the ability to link memories is an important cognitive function. However, there is an obvious conflict with the need to keep non-related memories separate (pattern separation, thought to reside in dentate gyrus (DG)), and the need to be able to reconstitute complete memories from partial information (pattern completion), such as when we recall an entire memory from a single clue. Pattern completion has been shown in animals, where place cells were found to retain their location information when landmarks are removed (Moser et al., 2008), and is thought to occur in the recurrent loops in CA3. How the brain keeps these processes separate and where exactly the association of different memories happens is not known, nor whether it occurs in the hippocampus or outside.

The big-loop model posits that the association of memories occurs in the layers of the entorhinal cortex (EC), by recirculating the output of the hippocampus that arrives in deep EC-layers back to the superficial EC input layers, from where it re-enters the hippocampus. As this recirculation happens outside the hippocampus it is thought to not interfere with the pattern separation and pattern completion that reside within the hippocampus. Other models propose a location of the mechanism to infer associations within the hippocampus or in the wider cortical networks (Preston and Eichenbaum, 2013).

The authors used a Paired Associate Inference task (PAI), which involves learning pairs AB and BC. The authors paired faces (A and C) with objects or scenes (B). In 'direct association trials' which test associative memory, recall of AB or BC is tested, and the task consists of showing one of the faces, and two objects or scenes, where the subject has to remember which belong together (Paired Association). In 'indirect association trials' only faces are shown and the subject has to infer association of the faces A and C through remembering the object or scene B, without ever having seen A and C together (Paired Associate Inference). Image B is not seen but remembered, and is the output of the hippocampus via the trisynaptic pathway through CA3 and CA1, which in the model is recirculated through the entorhinal cortex back to the hippocampus. Thus, this image should leave a trace in the output- and the input-layers of the entorhinal cortex.

Because the stimuli are designed such that A and C are always faces and B is either a scene or an object, the authors exploit the scene vs. object contrast to demonstrate recirculation. If a scene vs. object contrast is seen in EC in AC-trials it shows reactivation of the memory. the authors indeed found this contrast in EC. However, to provide evidence for the big-loop hypothesis, the signals in the superficial (input) and deep (output) layers of EC need to be distinct. This was shown by segmenting EC in superficial and deep voxels, and rerunning the analysis, where the authors found category information (scene vs. object) in both deep EC (dEC) and superficial EC (sEC). The presence of this information in the superficial layers suggests that the hippocampal output is recirculated to sEC. Following they used informational connectivity to determine the functional connectivity between layers and areas. Informational connectivity (IC) looks at the covariation of decoding accuracy between two regions, as opposed to covariation of signal amplitudes in regular functional connectivity. If trial-by-trial information (decoding accuracy) of two regions covaries, then the areas are thought to pass information between them. The authors found significant informational connectivity between sEC and dEC, while correcting for information in other MTL-regions. Next, they combined the prior two analyses to show that the reactivation strength in sEC depends on the degree of informational connectivity between sEC and dEC (laminar IC). sEC-reactivation and laminar IC were significantly correlated while dEC-activation and laminar IC were not. Furthermore, comparing laminar IC (within-EC) with IC between sEC and DG&CA3 suggests information flow from sEC back into the hippocampus. Another important control was incorporation of perirhinal (PRC) or parahippocampal cortex (PHC) which could alternatively be part of the big-loop, and performing the same analysis, but the authors did not find support for the longer cortical pathway. Finally, they correlated laminar IC-strength and sEC-reactivation with the subjects' performance on the PAI-task, and found both significantly correlated with task performance. These combined analyses and careful tracing of information flow and appearance of category information support the big-loop model.

Although questions remain whether the hemodynamic response is sufficiently specific to allow separation of cortical layers, the authors controlled for this by regressing out the signal in adjacent layers, and by comparing the signal in superficial- and deep layers in distant regions of EC, which do not share the same penetrating veins. However, how the anatomy of the vascular bed affects the laminar profile of fMRI-responses has almost exclusively been studied in early sensory areas, and although it is likely that the microvascular architecture in EC resembles that of sensory cortex, much less is known about how the vascular anatomy of the hippocampus affects its high-resolution fMRI-signals.

An even more complicated issue is the relation between fMRI- and neural signals. It is implicitly assumed here that fMRI-activation equals neural activity. But we still do not exactly know what fMRI-signals represent, especially not at the laminar level. Moreover, most studies about the neural processes that drive the fMRI- and functional connectivity signals have been done in primary sensory areas, but little is known about areas higher in the

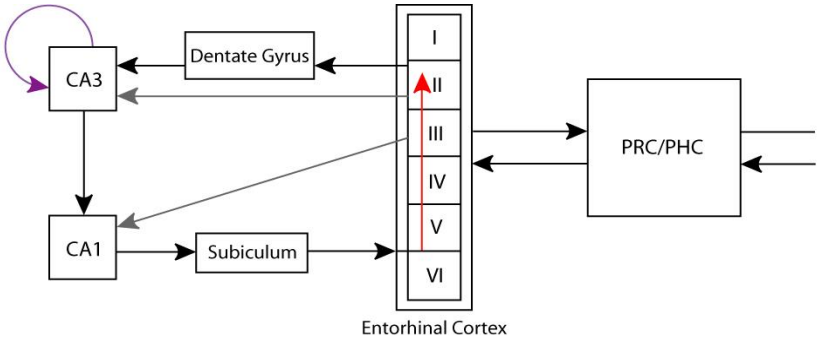
cortical hierarchy such as MTL. This is particularly pertinent in the entorhinal-hippocampus memory system which strongly relies on both rate-codes and phase-codes. Phase-codes such as phase-coupled theta- and gamma-oscillations or spike-LFP coupling, where place cells fire preferentially during a certain phase of the theta-wave are important coding mechanisms in the entorhinal-hippocampus system (Buzsaki and Moser, 2013). However, it is unknown how such phase- or timing codes are reflected in fMRI-responses, or even whether they contribute in any substantial manner to fMRI-activation or functional connectivity. This question argues for further study using electrophysiology, for instance with laminar recording, which has been used to show neural responses indicating feedforward and feedback in the layers of macaque perirhinal cortex during learning and recall (Takeuchi et al., 2011).

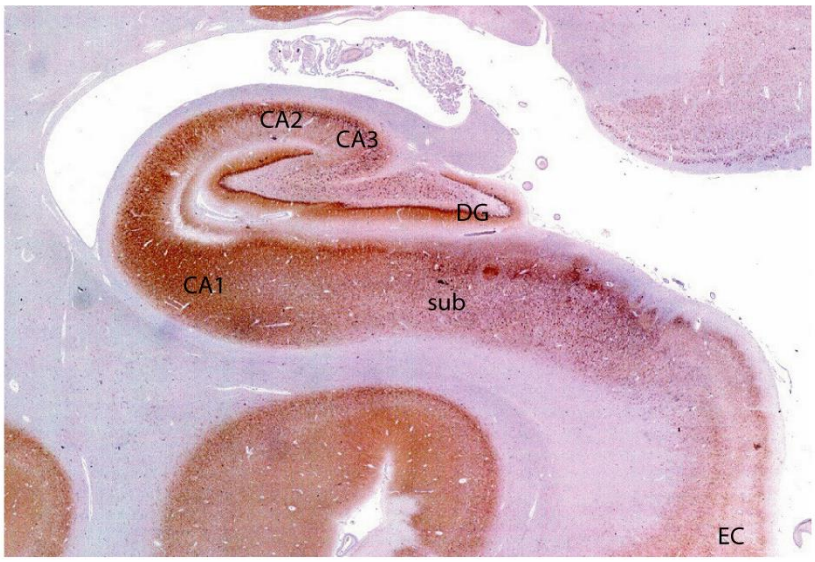
Although the data supports the big-loop model, the data are not inconsistent with other models. It does not rule out the monosynaptic pathway through CA1 (which connects EC and CA1), or pathways through the wider cortex beyond PRC/PHC, for instance via prefrontal cortex (Preston and Eichenbaum, 2013; Schapiro et al., 2017). As the authors mentioned, given the inherent limitations of fMRI connectivity analyses, one cannot definitely rule out that the observed laminar connectivity is not mediated by longer indirect pathways through the cortex instead of by the direct pathway from dEC to sEC. Some of these limitations are due the inherent correlational nature of fMRI, and it would not be realistic to test all alternate circuits. Ideally, future studies prove causality, and this may require experiments using optogenetics, chemogenetics or targeted lesions in animals, for instance in monkeys or rodents (Del Ferraro et al., 2018). Animal studies would also be important to address the neurovascular coupling questions raised above. As fMRI moves towards circuit-based questions, these questions will take on greater importance.

To summarize, this work elegantly shows how laminar fMRI combined with state-of-the-art data analysis can be used to resolve cortical circuits, and sets a standard for future work. The results support the big-loop hypothesis to allow association of different memory traces in MTL. The work also opens up many exciting questions for future research, and contributes to a better understanding of the mechanisms of memory encoding and retrieval in MTL.

**Figure**

Anatomy of the human hippocampus (MAP2-stain), and hippocampal-entorhinal circuit with big-loop connection in red. Anatomical image from the Teaching Website on Neuropathology and Neuroimaging of Unicamp University, Campinas, Brazil http://anatpat.unicamp.br, with permission.

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