·Research Highlight·

Learning to memorize: shedding new light on prefrontal functions

Ning-long Xu

Institute of Neuroscience, State Key Laboratory of Neuroscience, CAS Center for Excellence in Brain Science, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China Corresponding author: Ning-long Xu. E-mail: xunl@ion.ac.cn

© Shanghai Institutes for Biological Sciences, CAS and Springer-Verlag Berlin Heidelberg 2015

Working memory is one of the essential higher cognitive functions that actively holds behaviorally-relevant information essential for guiding subsequent actions. It includes subsystems that store and manipulate singlemode or multi-modal sensory information, e.g., spatial information, visual images, auditory scenes, olfactory objects, or any combination of these. In addition to merely holding a certain amount of information for a short period of time, as is generally believed, the cognitive processes involved are far more complex, including the executive and attentional control of short-term memory, permitting interim integration, and the processing, disposal, and retrieval of information. Evolution-wise, working memory is essential for the behavioral flexibility that allows humans and animals to quickly adapt to rapidly changing environments.

A wealth of studies have been conducted in attempts to understand the neuronal process underlying working memory, and have identified a number of brain regions as crucial, including the prefrontal cortex (PFC), posterior parietal cortex, anterior cingulate, and parts of the basal ganglia. Among these regions, the PFC has drawn most attention due to the striking finding that individual neurons show persistent activity during the memory-retention period^[1-3] (termed the delay period, a hallmark of working memory tasks): elevated activity persists after the sensory stimuli have been removed until the holding period is over (from seconds to tens of seconds) and the behavioral choice has been made. This raises the immediate question of whether the persistent activity in the PFC during the delay period encodes the contents of working memory (memory storage). This has been under debate for the last two decades^[4]. Some studies find that PFC activity increases when the number of items to be memorized increases. This seems to support the hypothesis that the PFC plays an important role in memory storage, as a straightforward explanation would be that increasing the demands of storage would be expected to increase the activity level in a region where representations are being actively stored. However, an equally plausible explanation would be that if PFC activity reflects top-down signals to control more posterior regions where the actual representations are stored, maintaining higher loads of information might require increased PFC input in order for retained information to survive delay and distraction. Therefore, it is not yet clear that the PFC is the site where the representations are stored. The fact that the PFC has been found to play important roles in executive functions^[4] implies that its role in working memory might be controlling attention, selecting strategies, and manipulating information, rather than information storage^[2, 5].

To resolve this debate, it is therefore necessary to achieve a deeper understanding of the causal role of the PFC in working memory tasks. This would require temporally precise perturbation of neuronal activity in specific regions of the PFC during the delay period of a working memory task and monitoring its effect on task performance. Technically, such manipulation has not yet been achieved in primate and human subjects due to technical difficulties. In rodents, however, the temporally and spatially precise manipulation of neuronal activity has been exceedingly successful thanks to the recent development of new tools such as optogenetics and genetic manipulation techniques^[6]. Meanwhile, choicebased and precisely controlled behavioral paradigms in rodents have also been developed, allowing a high degree of stimulus control, accurate behavioral readout, and precise measurement of neuronal activity^[7-9]. On top of these behavioral paradigms, imposing an additional delay period before choice allows precisely timed, memorybased brain processes to be investigated using rodents^[10, 11]. However, imposing a delay immediately before choice could confound the memory content with motor planning components^[10]. In order to determine whether PFC activity during the delay period is responsible for memory storage, a more desirable paradigm would try to retrieve the same sensory information following a delay period, such that decision or behavioral choice can be made only after the memory retrieval is finished.

In a recent study^[12], using head-fixed mice, Liu et al. developed an olfactory delayed-nonmatch-to-sample paradigm (DNMS), a standard working memory task that had only been used in primates before (Fig. 1). In this task, head-fixed mice were presented briefly, at the beginning of each trial, with one of two odorants, and after a 4-5 s delay period, a second odorant was presented. The animal needed to decide whether the second odorant was the same as or different from the first one. If the two odorants differ, the animal should respond by licking ("go" response) a lickport, otherwise, the animal should withhold licking ("no-go" response). Therefore, the animal had to retain the information of the first odorant for the entire delay period in order to compare it with the second one: a typical requirement in working memory tasks. Mice can readily learn this memory-based decision task in as few as 5 days, and the learning process can be monitored.

This new paradigm in mice has opened up a playground for a range of manipulation and recording techniques such as optogenetic tools and multi-electrode recording for dissecting the functional role of the PFC in working memory. By expressing channelrhodopsin-2 (ChR2) in inhibitory interneurons or Natronomonas halorhodopsin in excitatory pyramidal neurons in the medial prefrontal cortex (mPFC), Liu et al. silenced the mPFC using light stimulation only during the delay period and examined the DNMS task performance. The findings are rather striking: the activity in the mPFC during the delay period is only required during the learning phase, typically from day 1 to day 5, but not for well-trained animals. This can shed light on the role of the mPFC in memory storage during the working memory task, if one considers the difference between the underlying processes in different learning stages: during the learning phase, many novel, attention-demanding components could occur during the delay period in order for the subject to accomplish the task, while after becoming well-trained, memory storage becomes the major if not the only factor that matters during the delay period. It is therefore suggested that the delay-period mPFC activity is necessary only for a novel, attention-demanding working memory task, but not for the simple short-term storage of olfactory information in the well-trained stage. This provides new clues to the lasting debate on whether the mPFC is the location for memory storage or rather for conducting executive functions such as controlling attention, selecting strategies, and manipulating information.

Another question concerns the specificity of the persistent activity in the mPFC during the delay period, i.e., whether it requires a specific subpopulation of neurons in the mPFC to be activated, or a general elevation of



Fig. 1. Behavioral paradigm. A. Apparatus for head-fixed go/no-go paradigm using olfactory cues, compatible with optogenetic stimulation. B. Task structure for olfactory DNMS paradigm. Adapted from the reference^[12] with permission.

mPFC activity is sufficient. Instead of silencing it, Liu *et al.* activated the mPFC during the delay-period by expressing ChR2 in excitatory neurons and delivered blue light only during the delay period, which led to a general elevation of mPFC activity. Interestingly, this manipulation impaired, rather than improved, the task performance during learning stages, but not in the well-trained stage. Therefore, the working memory task during the learning stages requires the activation of a rather specific subpopulation of mPFC neurons, although their exact specificity requires further investigation.

An important question regarding the persistent activity in the mPFC is whether and how it evolves with learning, which was rarely addressed in earlier studies. In the study by Liu *et al.*, the authors monitored population activity in the mPFC throughout the course of learning a working memory task. Indeed, the population dynamics in the mPFC evolves with learning: the delay-period activity is more prominent and distinguishes the two odorant stimuli in the learning stages, but this diminishes in the well-trained stage, consistent with the optogenetic manipulation results. This provides another dimension of evidence that the mPFC is involved in the attention-demanding learning phase, rather than in a simple memory-storage process in the welltrained stage.

Liu *et al.* developed a standard working memory assay in mice, and combined it with temporally precise neuronal perturbation and recording techniques, from which the authors provided new evidence that could help resolve the long-standing debate over the functional role of the persistent prefrontal delay-period activity in working memory. It seems that at least part of the prefrontal region, the mPFC, is crucial for animals to accomplish novel, attention-demanding, and memory-based tasks, but is not required for memory storage *per se.* This suggests that future investigations should focus more on additional brain regions in memory storage in the working memory task; this storage may be distributed, involving multiple brain regions in the hierarchy, including the sensory areas of relevant modalities.

Received date: 2015-01-07; Accepted date: 2015-01-26

REFERENCES

- Funahashi S, Bruce CJ, Goldman-Rakic PS. Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J Neurophysiol 1989, 61: 331–349.
- [2] Curtis CE, D'Esposito M. Persistent activity in the prefrontal cortex during working memory. Trends Cogn Sci 2003, 7: 415–423.
- [3] Romo R, Brody CD, Hernandez A, Lemus L. Neuronal correlates of parametric working memory in the prefrontal cortex. Nature 1999, 399: 470–473.
- [4] Kane MJ, Engle RW. The role of prefrontal cortex in workingmemory capacity, executive attention, and general fluid intelligence: an individual-differences perspective. Psychon Bull Rev 2002, 9: 637–671.
- [5] Postle BR. Working memory as an emergent property of the mind and brain. Neuroscience 2006, 139: 23–38.
- [6] Fenno L, Yizhar O, Deisseroth K. The development and application of optogenetics. Annu Rev Neurosci 2011, 34: 389–412.
- [7] Uchida N, Mainen ZF. Speed and accuracy of olfactory discrimination in the rat. Nat Neurosci 2003, 6: 1224–1229.
- [8] Komiyama T, Sato TR, O'Connor DH, Zhang YX, Huber D, Hooks BM, et al. Learning-related fine-scale specificity imaged in motor cortex circuits of behaving mice. Nature 2010, 464: 1182–1186.
- [9] Xu NL, Harnett MT, Williams SR, Huber D, O'Connor DH, Svoboda K, et al. Nonlinear dendritic integration of sensory and motor input during an active sensing task. Nature 2012, 492: 247–251.
- [10] Erlich JC, Bialek M, Brody CD. A cortical substrate for memory-guided orienting in the rat. Neuron 2011, 72: 330– 343.
- [11] Guo ZV, Li N, Huber D, Ophir E, Gutnisky D, Ting JT, et al. Flow of cortical activity underlying a tactile decision in mice. Neuron 2014, 81: 179–194.
- [12] Liu D, Gu X, Zhu J, Zhang X, Han Z, Yan W, et al. Medial prefrontal activity during delay period contributes to learning of a working memory task. Science 2014, 346: 458–463.