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"MEMORY" IN THE MAMMALIAN BRAIN

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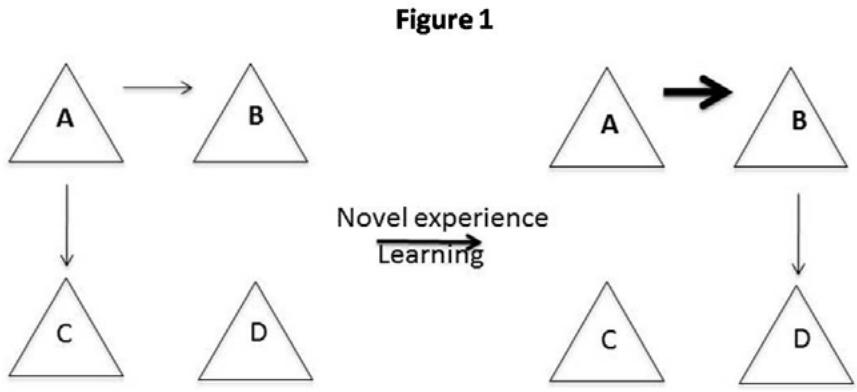
Abstract ▶ Understanding the nature of memory storage is one of the holy grails of modern neuroscience. It has long been recognized that memory storage would involve structural changes in the brain. The development of fluorescence labeling and in vivo imaging techniques have shed unprecedented light on how sub-cellular structures in the brain are modified in an experience dependent manner. Here, I review some of the recent findings on the nature of memory traces in the mammalian brain.

Key Words: Memory, Neuroscience, Synaptic imaging, Synapse.

Introduction

The ability to remember and adapt to the environment is critical for survival. A part of this ability comes from the genetic and epigenetic information that we inherit. They reflect "memories" of our ancestors' past that provided them with a survival advantage. The brain provides an additional substrate to store relatively more "real time" information that is relevant to an individual's experience. Information in the brain is stored in interconnected population of neurons. Within this network of neurons, the ability or the strength of individual connections (synapses) to influence the activity of the connected neurons varies widely. During a novel experience, the new information can be stored by changing either the pattern of synaptic connectivity between neurons or the strength of existing synapses (Figure 1)[1]. Donald Hebb's cell assembly theory provides a framework for understanding how neuronal activity can shape synaptic connectivity patterns or strengths. He posited that synapses are selectively strengthened between neurons that are coactive in response to the encoded information[2]. The "Hebbian" school of thought has arguably been the most important guiding framework for much of the neuroscience research on information storage in the brain. In this review, I focus on the recent progress, enabled by *in vivo* imaging, in our understanding of experience dependent changes on synaptic plasticity and its relevance to information storage in the mammalian brain. Experience plays a profound role in sculpting neuronal connectivity during development. In the developing mammalian brain, Hubel and co-workers elegantly demonstrated how visual experience modifies the structural and functional properties of the visual cortex. They found that, in the feline and primate binocular visual cortices, neurons are selectively activated by inputs to one eye or the other. Neurons that are responsive to each eye are organized as alternating columns, also referred to as ocular dominance columns[3,4]. When one of the eyes was deprived of visual experience by suturing the eye-lid (monocular deprivation or MD) for several weeks, the neurons that previously responded to inputs to this eye switched their response to the open eye inputs[3]. To test if such experience dependent functional alteration is accompanied by structural changes, Hubel and co-workers injected a radiolabeled amino acid tracer in one of the eyes of the animals and subsequently, performed autoradiograms of the tangentially sectioned visual cortex.

Figure 1: *Experience dependent circuit remodeling. A simplified schematic of a neural circuit consisting of four neurons (A-D) in which A, B and C are part of a network (left). The arrow indicates synaptic connections and the width of the arrow represents synaptic strength. Learning or exposure to new experience rearranges the connectivity pattern and strength resulting in a network comprising of A, B and D.*



Consistent with the functional data, they found alternating patches of labeled and unlabeled axons corresponding to the inputs from labeled and unlabeled eyes, respectively. MD, followed by dye injection in the open eye, showed an expansion of the area occupied by labeled inputs carrying information from the open eye with a concomitant reduction in the area occupied by deprived eye inputs. They noted that such alterations are restricted to a period in development, termed as critical period, beyond which changes to experience would not result in structural changes[3].

Sensory experience dependent structural plasticity in adults

Animals continue to learn and remember in adulthood and whether it involved structural changes, as envisioned by Hebb, remained unknown. Visualization of synaptic structure in the mammalian brain slices through light and electron microscopy was consistent with the idea of new synapse formation with learning. With the advent of fluorescence labeling technology and two-photon imaging, it became possible to study synaptic changes associated with experience in the brain of living mammals. Two photon imaging, through a glass cranial window or thinned skull, of excitatory neurons sparsely labeled with green or yellow fluorescence protein (GFP or YFP) in the adult mouse brain revealed that dendritic spines, tiny protrusions on the membrane that harbor synapses, are largely stable but a fraction of them turn over(appear and disappear) over a period of days (Figure 2)[5,6]. Altering animals' experience by trimming their whiskers in a checkerboard pattern resulted in an enhancement of spine turnover in the barrel cortex, presumably enabling adaptive remodeling of neural circuits (Figure 3)[6]. Such experience dependent changes in spine turnover are not limited to whisker sensation but has since been found to be ubiquitous for all senses. In the visual cortex, MD causes an increase in spine formation in deep cortical neurons but not in the superficial neurons[7]. The spines formed during MD persisted even after restoring binocular vision. Moreover, repeating MD did not further increase new spine formation. These results revealed that structural changes of dendritic spines in excitatory neurons could serve as memory traces in adulthood[7].

Figure 2: *In vivo imaging of synapses. A. (Left) Cranial window (5mm). (Middle)Visual cortex identified by visual evoked hemodynamic response. (Right) Z projection of the entire volume of layer 2/3 dendrites. Scale bar – 50 mm. Dendrite in white box is zoomed in B (Scale bar – 10 mm). C. Same dendritic segments imaged repeatedly over a period of many weeks (Scale bar – 8 mm). The solid white triangle represents a stable spine. The solid and open yellow triangles indicate gain and loss of dendritic spines, respectively.*

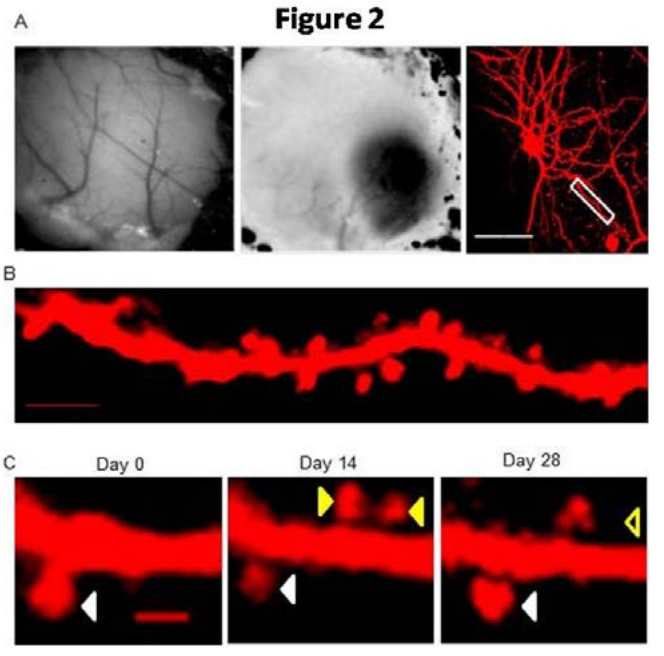
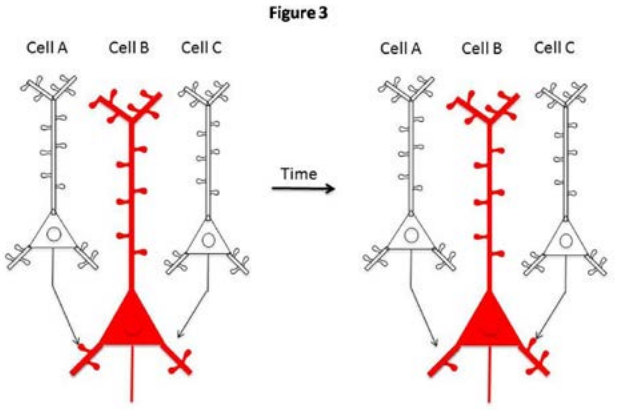


Figure 3: *Spine dynamics and circuit remodeling. An illustration of a simple circuit consisting of three neurons. The neuron, indicated in red, loses and gains spines at different locations. Spine loss and gain in the 'red' neuron alters its connectivity with cells A and B.*



In contrast to excitatory neurons, most inhibitory neurons lack dendritic spines and the synapses are located in the dendritic shaft. Earlier observations on excitatory neurons revealed that, though dendritic spines are dynamic in adulthood, the dendrites that harbor them are quite stable[6]. In contrast, the dendrites of inhibitory neurons are dynamic even in adults[8]. Interestingly, of all the layers in the visual cortex, dendritic plasticity of interneurons is limited to the superficial layer 2/3[9]. These interneurons exhibit branch extensions and retractions resulting in synapse formation and elimination, respectively. Visual deprivation enhances branch retractions, resulting in increased synapse loss[10]. Also, in a subset of inhibitory neurons that possess dendritic spines, removal of visual input caused rapid reduction in their spine density[11]. Deprivation induced synapse loss onto inhibitory neurons could reduce the inhibitory tone which then can create a permissive environment for plasticity in excitatory neurons. Thus, both excitatory and inhibitory neurons in the cortex exhibit plasticity to adapt to changes in sensory experience.

Dendritic spines possess the postsynaptic components of a synapse. The presynaptic components are contained within the axonal boutons. The axonal boutons are also dynamic in an experience and cell type dependent manner. In the layer 1 of mouse somatosensory cortex, the axonal afferents from the thalamus are more stable than the ones from layer 6[12]. However, the relevance of such differential remodeling of different synapse types to animal's experience is still unknown. Interestingly, changing the visual experience by introducing lesions focally in the retina led to massive restructuring of axons in the visual cortex that received information from the lesioned area[13]. Thus, both pre and postsynaptic sites remodel in response to changes in animals' experience.

Learning and memory associated structural plasticity

Experience dependent appearance and disappearance of synapses are not limited to sensory modalities. Mice that learned to perform anew motor task showed an increase in spine formation in the neurons of motor cortex. This was followed by elimination of some of the spines that existed before the training. Practicing the same task did not further increase spine formation whereas learning another new motor task promoted addition of new spines. Further, the number of new spines formed correlated with the extent of task acquisition, thus revealing a direct link between spine formation and learning[14].

Spine formation and elimination have also been shown to correlate with acquisition of fear. In a paradigm, referred to as fear conditioning, mice are exposed to a tone followed by a brief electric foot shock. Subsequently, mice respond by freezing upon exposure to the same tone. In the auditory cortex, pairing of a tone with a shock significantly increased formation of new spines and these spines persisted for long periods of time, presumably serving as a memory trail[15]. Interestingly, in the frontal association cortices, fear conditioning led to elimination of spines and the level of freezing correlated with the percentage of spines eliminated. In contrast, extinction of fear, achieved by repeated safe exposure to the same tone used for fear conditioning, resulted in new spine formation and these spines are specifically removed upon reconditioning of fear with the same stimulus[16].

The above studies make a strong case for spine remodeling as structural correlates of memory. However, a causal link between spine changes and memory storage was not made in these studies. If new spine formation stores new memories then specifically removing those spines after memory formation should erase the associated memory. Recently, a novel optical probe, named AS-PaRac1, was developed to address this question. This probe localizes to activated synapses and contains photoactivatable Rac1, a small GTPase whose activation causes spine shrinkage. Consistent with a causal role for new spines in storing memories, optical activation of AS-PaRac1selectively eliminated spines that were formed following motor learning and consequently, resulted in loss of the relevant motor memory[17].

Multi-color synaptic imaging *in vivo*

Though dendritic spines are good surrogates for excitatory synapses, they do not represent the synapse themselves. Some spines may not have synapses or may have synapse with different levels of maturity. Further, they only represent excitatory synapses. A significant fraction of synapses made on excitatory neurons are inhibitory and they lack a structural surrogate. Recently, direct visualization of synapses was achieved by fluorescence labeling of proteins residing in excitatory (PSD95) and inhibitory synapses (gephyrin)[18]. Simultaneous expression of YFP, PSD95-mCherry and Teal-Gephyrin enabled visualization of dendritic spines, mature excitatory synapses and inhibitory synapses, respectively. A vast majority of the dendritic spines contained PSD95 but a significant fraction (~20%) were devoid of it. Post-hoc electron microscopy revealed that these spines carry synapses that might be immature. Surprisingly, an equally large fraction of dendritic spines (~20%) had both PSD95 and gephyrin. These spines are dually innervated by both excitatory and inhibitory synapses (DIS).Such a heterogeneous spine population also exhibited differential remodeling properties. For instance, PSD95 lacking spines are highly dynamic whereas the DIS are extremely stable structures. Interestingly, within the DIS, the excitatory synapses are more stable but the inhibitory synapses appear and disappear on a day-to-day basis. Overall, in over a period of a week, the excitatory synapses tend to appear and disappear at different locations of a neuron, reflecting circuit rearrangement, whereas inhibitory synapses are formed and removed at the same sites, suggesting a role in functional gating of activity at these sites[18].

Direct visualization of spine and synaptic dynamics can provide a better understanding of etiology of many diseases associated with the nervous system. In a mouse model of Huntington's disease, visualization of dendritic spine dynamics over a period of 6 weeks revealed an increase in spine formation, but the newly formed spines could not persist as stable spines. Such abnormal remodeling of spines preceded the onset of motor symptoms and therefore, could be causal to symptoms in Huntington's disease[19]. More recently, labeling of synapses in various mouse models of autism revealed a common core phenotype, an increase in the dynamics of spines containing PSD95 but lacking gephyrin, presumably carrying intracortical synapses[20].

Conclusion

Recent developments in synaptic imaging described above allow examination of both excitatory and inhibitory synapse remodeling with unprecedented resolution. It has also revealed heterogeneity in dendritic spines based on their synaptic content. However, we are still oblivious to how synaptic distribution and dynamics of a neuron relates to the source of their afferent inputs. The stage is now set to integrate information from multiple levels, such as molecules, circuits and experience, and study their influence on neurons at the resolution of single synapses.

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