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PERSPECTIVE

Forgetting and small G protein Rac

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It is far from understood why we forget things that are known to us seconds ago. Emerging evidence emphasizes that small G protein Rac could be a key to understanding this type of rapid early memory forgetting. This current perspective article will first review these studies and then discuss their implications for the internal processes underlying forgetting.

PSYCHOLOGY OF HUMAN FORGETTING

We are all familiar with what forgetting is, and also curious about how it happens. The first experimental study of forgetting is attributed to Hermann Ebbinghaus at the late nineteenth century (Ebbinghaus, 1885/1913). His famous study of his own retention curve (Fig. 1A) apparently shows a remarkable feature of forgetting that most memory loss occurs soon after learning. Passive decay and interference are two major psychological theories in debating for explaining this rapid forgetting. The passive decay theory contends that information in memory is intrinsically unstable and keeps getting lost if not used or consolidated; thereby forgetting primarily arises from passive rundown of the internal memory trace over time. On the other hand, the interference theory, largely based on memory experiments in psychological laboratories, assumes that forgetting per se is caused by competition from other memories or information acquired subsequently. The concepts of decay and interference still harbor at the heart of contemporary psychological models of human forgetting (Jonides et al., 2008); however, the underlying processes in the brain await to be identified.

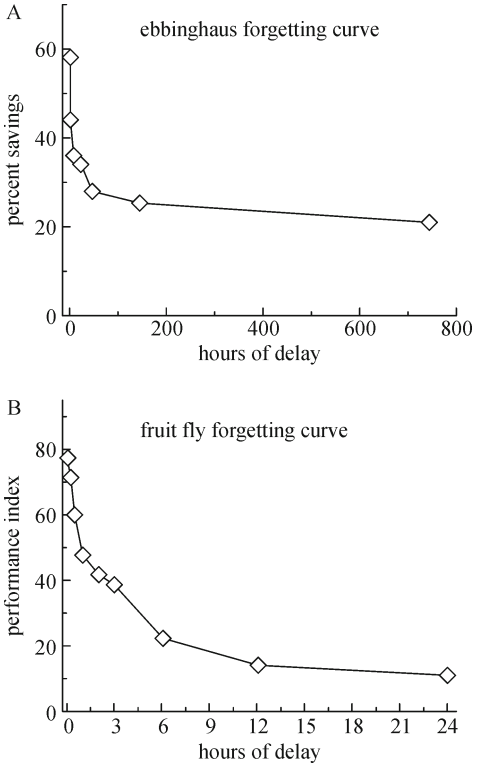

Figure 1. Forgetting curves of human and fruit fly. (A) Ebbinghaus tested himself as experimental subjects to learn a series of non-sense syllables. Memory retention was assayed as saving of time to relearn the same list after indicated intervals. Data from Ebbinghaus (1885/1913). (B) Retention curve of wild type fruit flies after one-session training of Pavlovian olfactory aversive conditioning. Performance index was calculated as the proportion of flies that avoided the punished odorant minus the proportion of flies that avoided the unpunished odor multiplied by 100. Part of the data has been published in Shuai et al. (2010).

FRUIT FLIES LEARN AND FORGET

The past twentieth century has witnessed the great success of using simple experimental animals to understand fundamental aspects of memory (Kandel, 2001). In recent years, *Drosophila melanogaster*, the fruit fly, is increasingly favored in terms of its relatively simple nervous system,

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considerable behavioral complexity, and accessibility to genetic manipulation (Vosshall, 2007). A behavioral assay of Pavlovian olfactory aversive conditioning is extensively used to determine the associative memory ability in fruit flies (Tully et al., 1994). In this assay, fruit flies are sequentially exposed to two odorants, one of which but not the other is paired with electric foot shock. When facing choice between the two odorants later, fruit flies are smart enough to avoid the punished one. This experience dependent avoidance response is initially close to saturated level, but dissipates over time just as we humans forget. A closer examination of the forgetting curve in fruit flies (Fig. 1B) can also find that memory decay is fast at the early stage and then drops to a plateau with much slower decline.

RAC REGULATES EARLY MEMORY FORGETTING IN FRUIT FLIES

The fast early memory decay in fruit flies has long been noticed and researches in the past three decades have even made it possible to isolate this rapidly decaying early memory component based on its sensitivity to cold amnesia treatment and its distinct molecular and neural substrates in formation (DeZazzo and Tully, 1995; Davis, 2005). However, what molecular mechanisms drive its fast decay is not known until a recent study suggests that Rac could be a key regulator (Shuai et al., 2010).

Rac belongs to Rho family small GTPases, which are best known for their roles in cytoskeleton regulation (Etienne-Manneville and Hall, 2002). The study took advantage of transgenic flies bearing dominant-negative or constitutively active forms of Rac to downregulate or upregulate Rac activity. To circumvent developmental abnormality, expression of dominant mutants was restricted in postdevelopmental nervous system through a spatiotemporally confined expression system available in fruit flies (McGuire et al., 2003). The results showed that these genetic manipulations of Rac activity are sufficient to change early memory decay rate in fruit flies. Rac inhibition preserves memory, while hyperactivation favors decay. In addition to passive decay, the same study went a step further to show that forgetting under situations of heightened interference such as irrelevant learning and reversal learning (Fig. 2) is also regulated by Rac, despite that they probably exploit different Rac activation kinetics. Thus, it is intriguing to see that the two major psychological explanations of forgetting eventually converge to the same molecular pathway.

RAC SIGNALING CASCADE AND ITS HOMOLOG IN VERTEBRATE

Rac orchestrates actin cytoskeleton remodeling through a number of downstream signaling pathways. In one of them, Rac activity triggers sequential activation of two kinases PAK and LIMK. Upon its activation, LIMK will then phosphorylate and inhibit Cofilin, a potent actin depolymerization factor. This Rac/PAK/LIMK/Cofilin signaling cascade has been extensively investigated over the past few years in a wide range of areas from cultured cell morphogenesis to neuronal development (Arber et al., 1998; Yang et al., 1998; Edwards et al., 1999; Ng and Luo, 2004). Intriguingly, overactivation of fruit fly homolog of Cofilin also slows down early memory decay, which mimics the effect of Rac inhibition. Conversely, abolishment of the Rac's binding site with PAK efficiently blocks its function in forgetting (Shuai et al., 2010). These results raise the possibility that the same Rac/PAK/LIMK/Cofilin pathways might be opted for regulation of forgetting.

Does the Rac-dependent forgetting discussed above in fruit flies also apply to vertebrate? In mice, there are three *Rac* genes. Two of them, *Rac1* and *Rac3*, have expression in the

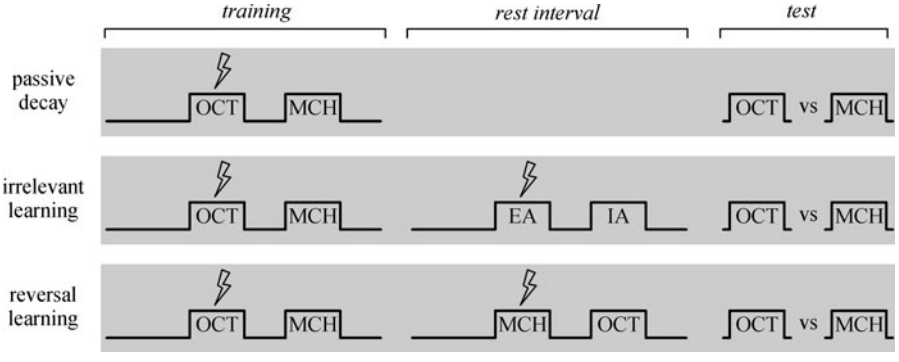

Figure 2. Forgetting-related behavioral paradigms in fruit flies. Fruit flies are first subjected to training with one of the odorant OCT paired with electric shock punishment, but the other odorant MCH not. For "passive decay", flies were thereafter left undisturbed in food vials until test. For "irrelevant learning", flies were exposed to new learning with a novel pair of odorants EA and IA at the rest interval. For "reversal learning", flies were subjected to training again but with the original odorant-punishment contingency reversed, i.e., MCH but not OCT was paired with punishment this time. Retention of the initial learning was tested for all three paradigms.

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nervous system, but only *Rac3* knock out animals can survive to adulthood. A recent behavioral study reports that these *Rac3* knockout mice display a range of behavioral abnormalities including reduced behavioral flexibility in reversal learning (Corbetta et al., 2008), which resembles what has been observed in fruit flies resulted from Rac inhibition. Additionally, mice lacking LIMK1, one of the Rac downstream factors, are compromised in reversal learning of water maze, while show enhanced memory after fear conditioning (Meng et al., 2002). These fragmented observations appear to be in agreement with a conserved role of Rac signaling cascade in forgetting. However, one shall also bear in mind that these mice studies used knock out animals which may bear developmental abnormality. Thus, it is of necessity to further validate the Rac-forgetting pathway proposed here by analysis of conditional knockout animals in the future study.

"OXIDATION PROCESSES" LEADING TO RUST IN MEMORY

The Rac-forgetting signaling cascade discussed above leads to Cofilin inhibition, which in turn exerts multiple effects on actin cytoskeleton, e.g., tilting the actin dynamics from disassembly to assembly, slowing down actin turnover (Bamburg, 1999). The results thus give the most interesting implication that actin remodeling could be the cellular machinery contributing to forgetting.

Actin cytoskeleton takes part in a wide range of neuronal functions, among which governing neuronal morphogenesis is the most appreciated one. It is believed that actin reorganization serves as the driving force to achieve stable neuronal structural changes sustaining long-lasting memory (Lamprecht and LeDoux, 2004). Part of the supporting evidence comes from observations that actin polymerization, particular at the postsynaptic spines, is favored during the consolidation of long-term potentiation (Fukazawa et al., 2003; Okamoto et al., 2004; Lynch et al., 2008; but see also Ouyang et al., 2005). Thus, it may seem paradoxical that actin polymerization contributes to both forgetting and long-term memory maintenance. However, there exists the possibility that different actin subpopulations or various actin dynamics are engaged in these two antagonistic functions. There is emerging evidence that actin cytoskeleton can modulate synaptic efficiency in a number of ways without causing visible morphological change of synapses, such as organizing the presynaptic vesicle scaffold and supporting traffic of synaptic machinery (Cingolani and Goda, 2008). It is thereby of considerable interest to investigate whether this category of synaptic modulation by neuronal actin could be related to early memory forgetting.

Decay theory was once forcefully criticized for not looking at the process by which forgetting could occur. One of the criticisms frequently cited was from McGeoch (1932): "Rust does not occur because of time itself, but rather from oxidation processes that occur with time." However, with the rapid advance of molecular and cellular neuroscience, we may be able to reveal the oxidation process lead to rust in early memory one day.

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