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Forgetting Is Regulated through Rac Activity in *Drosophila*

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SUMMARY

Initially acquired memory dissipates rapidly if not consolidated. Such memory decay is thought to result either from the inherently labile nature of newly acquired memories or from interference by subsequently attained information. Here we report that a small G protein Rac-dependent forgetting mechanism contributes to both passive memory decay and interference-induced forgetting in *Drosophila*. Inhibition of Rac activity leads to slower decay of early memory, extending it from a few hours to more than one day, and to blockade of interference-induced forgetting. Conversely, elevated Rac activity in mushroom body neurons accelerates memory decay. This forgetting mechanism does not affect memory acquisition and is independent of Rutabaga adenylyl cyclase-mediated memory formation mechanisms. Endogenous Rac activation is evoked on different time scales during gradual memory loss in passive decay and during acute memory removal in reversal learning. We suggest that Rac's role in actin cytoskeleton remodeling may contribute to memory erasure.

INTRODUCTION

Initially acquired memory is vulnerable to forgetting. Traditionally, two psychological concepts, usually placed in opposition, have been raised to account for forgetting: decay and interference (Jonides et al., 2008; Wixted, 2004). The former holds that memory simply evaporates with time, whereas the later claims that forgetting principally arises from loading of irrelevant information. With the nature of the underlying process remaining unspecified, the decay and interference explanations of forgetting are under continuous debate (for recent debate, see Altmann, 2009; Lewandowsky et al., 2009). In recent years, molecular genetic approaches have led to the identification of a cohort of key memory molecules, inspiring theoretical explanations of numerous basic memory phenomena, such as coincidence detection (Bourne and Nicoll, 1993), consolidation (Kandel, 2001), memory allocation (Han et al., 2007), and spacing effect (Pagani et al., 2009). However, efforts to understand the molecular basis of early memory forgetting have long been overlooked, presumably due to the pervasive notion that early labile memory is dependent upon phosphorylation of pre-existing molecules by a variety of kinases (Kandel, 2001; Micheau and Riedel, 1999) and that such modification will be reversed passively by basal activities of cellular phosphatases (Genoux et al., 2002; Mansuy, 2003). Thus a dedicated mechanism for removing early memory may not exist.

However from a theoretical point of view it has long been speculated that there are adaptive benefits of a forgetting strategy that can respond to the environmental information (Anderson and Schooler, 1991; Bjork, 1989; Kraemer and Golding, 1997). For instance, when the biological significance of the acquired memory is decreased after an extended period of "disuse," or when the existing memory is inconsistent with current circumstances and thus might harm an individual's survival, the forgetting process may function to remove the unnecessary or inappropriate memory. On the basis of this notion, we launched an effort to identify *Drosophila* mutants of enhanced early memory with the expectation that such enhancement might result from a defect in forgetting. In analyzing these mutants (unpublished data), the effects of Rac-signaling relevant genes attracted our attention and prompted our study of Rac's role in forgetting.

Pavlovian olfactory aversive conditioning has been extensively characterized in *Drosophila* (Tully and Quinn, 1985). Single-session training yields a memory retention curve consisting of rapid forgetting of the labile early memory, including mainly short-term memory (STM) and mid-term memory (MTM), and a gradual appearance of a longer-lasting component, anesthesia-resistant memory (ARM). The early memory disappears within a few hours, leaving ARM the only memory component lasting over 1 day (DeZazzo and Tully, 1995). In addition to ARM, there exists another consolidated memory form, protein-synthesis-dependent long-term memory (LTM), which is elicited only with repetitive spaced training and lasts for at least a week (Tully et al., 1994). The present study focuses on one-session training-induced labile early memory and reveals that this component can be prolonged to more than 1 day by interfering with the functions of Rac.

Rac belongs to the Rho family GTPases. This family of small G proteins act as key regulators of cytoskeleton dynamics as well as other cellular processes by switching between GTP-bound active forms and GDP-bound inactive forms (Etienne-Manneville and Hall, 2002). They have been extensively studied in neuronal

development and activity-dependent structural plasticity where cytoskeleton remodeling is acutely required (Luo, 2000; Van Aelst and Cline, 2004). Their physiological roles in mature nervous systems, however, are much less well-defined. A major obstacle in approaching this question is attributed to the deleterious effects caused by perturbing their activities throughout development (Johndrow et al., 2004; Wang and Zheng, 2007). However conditional expression of dominant mutants can circumvent the developmental defects and thus serves as the preferred experimental strategy. With the genetic tools accessible to *Drosophila*, we demonstrate that Rac activity is critically involved in active regulation of early memory forgetting.

RESULTS

Two dominant Rac mutant proteins with amino acid substitution have been successfully used to characterize physiological functions of Rac in *Drosophila* (Luo et al., 1994). The dominant-negative N17 mutant (T17N) inhibits endogenous Rac activity by competing for an upstream activator, whereas the constitutively active V12 mutant (G12V) renders Rac persistently active as a consequence of its abolished intrinsic GTPase activity. Tissue-specific expression of transgenes encoding dominant mutants of *Drosophila* Rac1 (Drac1) was obtained through the Gal4/UAS binary system (Brand and Perrimon, 1993) whereas the temporal control of adult-onset expression was achieved by integration with *tubulin-Gal80^{ts}* (*Gal80^{ts}*), which encodes a ubiquitously expressed temperature-sensitive Gal80 protein that suppresses Gal4-induced expression at the permissive temperature (18°C) but not at the restrictive temperature (30°C) (McGuire et al., 2003). The specificity of expression was verified by coupling with a GFP reporter, which produced a pattern (Figure S1A available online) consistent with that reported previously (McGuire et al., 2003).

Inhibition of Rac Activity Slows down Memory Decay

To probe the effects of Rac inhibition, dominant-negative Drac1(N17) was first expressed by a pan-neuronal *elav-Gal4* driver (Lin and Goodman, 1994) in combination with *Gal80^{ts}*. Crosses were reared at the permissive temperature (18°C). two- to four-day-old progeny were collected and exposed to 30°C for 3 days to induce the expression of Drac1(N17), which was verified by immunoblotting (Figure S1D). To evaluate behavioral effects, these Drac1(N17)-expressing adults were subjected to Pavlovian olfactory aversive conditioning (see Experimental Procedures) at 25°C along with similarly treated parental controls.

We compared retention curves at various time points after one-session training (Figure 1A). Drac1(N17)-expressing flies (*elav-Gal4/+; Gal80^{ts}/+; UAS-Drac1(N17)/+*) exhibited normal memory in the first 30 min after training (at 3, 15, and 30 min) but showed significantly slower memory decay at later time points from 2 hr up to 24 hr.

The normal performance in the first 30 min implies that the observed slower memory decay is not likely a result of strengthened acquisition of the initial memory. To further distinguish between a role of Rac in memory decay and in initial acquisition, we performed three additional experiments.

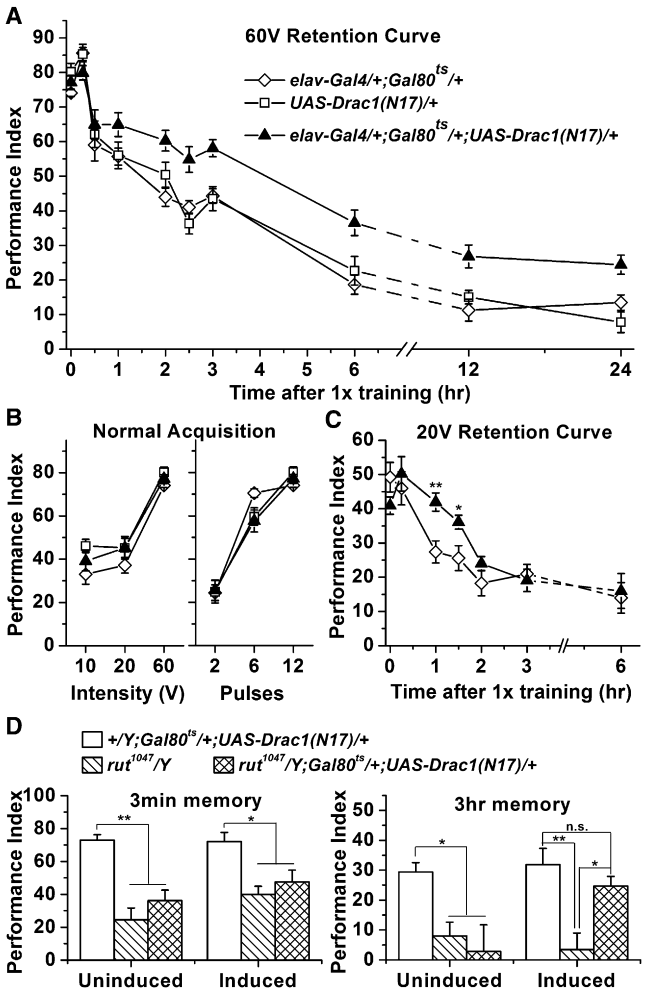

Figure 1. Normal Acquisition but Slower Memory Decay in Drac1(N17)-Expressing Flies

For induction of Drac1(N17) expression, flies received heat shock at 30°C for 3 days before Pavlovian conditioning.

(A) Retention curves were generated by testing conditioned odor avoidance at various time points after one-session training. Drac1(N17)-expressing flies (*elav-Gal4/+; Gal80^{ts}/+; UAS-Drac1(N17)/+*) displayed normal memory performance shortly after training (ANOVA, $p > 0.2$ for time points up to 1 hr) but slower memory decay thereafter (ANOVA, $p = 0.006$, 0.02, 0.002, 0.009, 0.002, 0.02 compared to *elav-Gal4/+; Gal80^{ts}/+*, 0.12, 0.002, 0.002, 0.046, 0.02, 0.0002 compared to *UAS-Drac1(N17)/+* for 2 hr, 2.5 hr, 3 hr, 6 hr, 12 hr, 24 hr, respectively). n = 6–16, means ± SEM.

(B) Immediate memory performance after one-session training with varied electric shock intensities (left) or number of electric shock pulses (right). n = 6–7, means ± SEM.

(C) Retention curves after weak training with 20 V electric shock (ANOVA, $p = 0.008$ for 1 hr, 0.02 for 1.5 hr). n = 5–10, means ± SEM.

(D) Induced expression of Drac1(N17) failed to reverse the immediate (3 min) memory defect of *rut^{1047}* mutant but significantly improved its 3 hr memory retention. Statistical significance (*$p < 0.05$; **$p < 0.01$) or nonsignificance (n.s.) is indicated. n = 6–12, means ± SEM.

See also Figure S1 and Table S1.

First, acquisition curves were examined for each genotype (Figure 1B) by plotting immediate (3 min) memory as a function of training intensity (the intensity of electric shock, 10 V, 20 V,