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Exclusive Consolidated Memory Phases in *Drosophila*

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Two types of consolidated memory have been described in *Drosophila*, anesthesia-resistant memory (ARM), a shorter-lived form, and stabilized long-term memory (LTM). Until now, it has been thought that ARM and LTM coexist. On the contrary, we show that LTM formation leads to the extinction of ARM. Flies devoid of mushroom body vertical lobes cannot form LTM, but spaced conditioning can still erase their ARM, resulting in a remarkable situation: The more these flies are trained, the less they remember. We propose that ARM acts as a gating mechanism that ensures that LTM is formed only after repetitive and spaced training.

Memory is a complex and dynamic process, and the relations between the different memory phases continue to intrigue neuroscientists. Studies of cerebral pathologies or brain lesions show that one form of human memory can be impaired while others remain normal (*1*). In this context, the formation of long-lasting memory is of particular interest, because it is thought to involve sequential events sustained by metabolic pathways preserved throughout evolution (*2*–*5*).

In *Drosophila*, a single associative-learning trial (the short protocol) consisting of an odor accompanied by 12 pulses of electric shocks induces three temporally distinct phases of olfactory memory (*3*): short-term memory (STM) and middle-term memory (MTM), which are labile and rely on the adenosine 3′,5′-monophosphate (cAMP) pathway (*6*), and ARM, which is a form of consolidated memory. STM is impaired in the *dunce* (*dnc*) and *rutabaga* (*rut*) mutants. However, these mutants retain a significant level of early memory (*7*). MTM is affected in the *amnesiac* (*amn*) mutant, and ARM is affected in the *radish* (*rsh*) mutant (*8*, *9*). STM, MTM, and ARM are also observed after intensive conditioning in which stimuli are presented repeatedly without intervening rest periods (the massed protocol) (*10*). Another consolidated memory, LTM, appears after multiple spaced training sessions (the long protocol) and is protein synthesis–dependent (*10*). The current *Drosophila* model proposes that the short protocol and the massed protocol induce a sequential pathway that begins with learning, passes through STM and MTM, and terminates in ARM, whereas the long protocol generates an additional phase, LTM. ARM and LTM are thought to derive from MTM and to coexist 24 hours after spaced conditioning (*3*, *10*). However, *amn* mutants present near-normal ARM but defective MTM (*8*, *9*). Thus, the notion that ARM is derived from MTM is questionable. Many issues concerning *Drosophila* memory remain to be solved. Why are there two forms of consolidated memory? Are they spatially disconnected or do they rely on the same brain structures? And why does LTM form only after spaced conditioning and not after intensive massed conditioning?

The mushroom bodies (MBs) form a bilaterally symmetric structure in the central brain and are composed of different classes of intrinsic neurons that send their axons into vertical and median lobes. To identify the onset of the LTM phase, we studied a subpopulation of *alpha-lobe-absent* flies (the *ala* mutant) that lack MB α/α' vertical lobes. These flies learn normally but show no olfactory LTM 24 hours after spaced conditioning (*11*). *ala* memory was measured at several early time points after conditioning with the short or the long protocols (*12*). Thirty-minute memory performances were similar after both protocols (Fig. 1). However, spaced repetitions of the conditioning regime significantly decreased memory performance at 5 hours, in comparison with what was observed after the short protocol (Fig. 1). Thus, the more intensively flies lacking vertical lobes are trained, the less they seem to remember.

The main form of memory that persists 5 hours after conditioning with the short protocol is ARM (*3*, *10*). Why do flies without vertical lobes show almost no memory 5 hours after spaced conditioning? First, these flies do not display LTM because they lack the MB neuronal projections required to form LTM (*11*). Second, our results suggest that ARM is erased (or blocked) during a LTM-specific training protocol. Thus, in contrast to the assumptions of previous models (*3*), we find that ARM and LTM do not coexist. ARM is formed in *ala* flies after massed conditioning (*11*), which indicates that its absence is observed only after spaced conditioning. Those results suggest that, in wild-type flies, the LTM phase is promptly initiated after spaced conditioning and that LTM replaces ARM.

How do memory phases relate to brain structures? MBs are implicated in the elaboration and retrieval of early olfactory memory phases (*13*–*18*), and MB vertical lobes are necessary for olfactory LTM (*11*). But it has not been directly shown that MB outputs are required for the retrieval of LTM, and ARM has not been formally linked to the MBs. In order to clarify several aspects of the MB/memory phase relationship, we studied flies expressing a thermosensitive version of the

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protein Shibire (*19*). Blocking synaptic transmission and possibly other endocytic processes (*20*) in subsets of MB cells projecting into all lobes, or only into the α/β lobes, led to a decrease in early memory (Fig. 2A) (*16*–*18*). We used a Gal4 enhancer-trap line (*21*) to drive UAS-*shi*^{ts} transgene expression in the γ lobes (fig. S1). With this Gal4 driver, we also observed a significant 2-hour decrease in memory, although odor perception and shock sensitivity were normal (table S2). To measure ARM, flies were trained with the short protocol and subjected to a cold shock 1 hour after conditioning to eliminate nonconsolidated memories (*9*). When all MB lobes were blocked during the experiment, ARM was erased (Fig. 2B). ARM was similarly decreased by blockage of the α/β lobes alone, but not significantly decreased by blockage of the γ lobes (Fig. 2B). Thus, ARM is supported by MBs and appears to rely more heavily on α/β neurons. Because ARM was found to be normal in the absence of either α or β lobes (*11*), we conclude that it can rely on either of those lobes.

To assess whether vertical lobes are required for LTM retrieval, MB output synapses were impaired during the test, 24 hours after spaced conditioning. When all lobes were inactivated, a strong decrease in performance was observed (Fig. 2C), indicating that MBs are indeed required for LTM retrieval. Inactivation of the α/β lobes generated a similar drop in perfor-

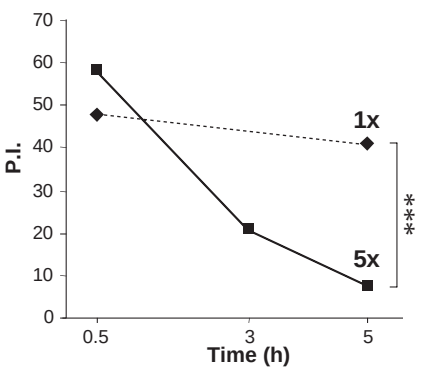

Fig. 1. Consolidated memory phases are mutually exclusive. The *ala* mutant was trained with one cycle (1×) (dashed line) or five spaced cycles (5×) (continuous line) and tested at various times after training. *ala* flies were processed as described (*11*). Data from flies without vertical lobes are presented here. PI, performance index. At 30 min, the 5× PI is not significantly different from the 1× PI (χ^2 P = 0.52). At 5 hours, the 5× PI is significantly lower than the 1× PI (χ^2 ***P < 0.0003). The numbers of flies lacking vertical lobes among the total *ala* flies were for 1×: 30 min, 140/1021; 5 hours: 134/1325; for 5×: 30 min, 229/1591; 3 hours, 158/1054; 5 hours, 106/902. After the long protocol, control *ala* flies with all MB lobes present did not display decreased memory at 5 hours (1×: 31.4, n = 257; 5×: 43.5, n = 183).

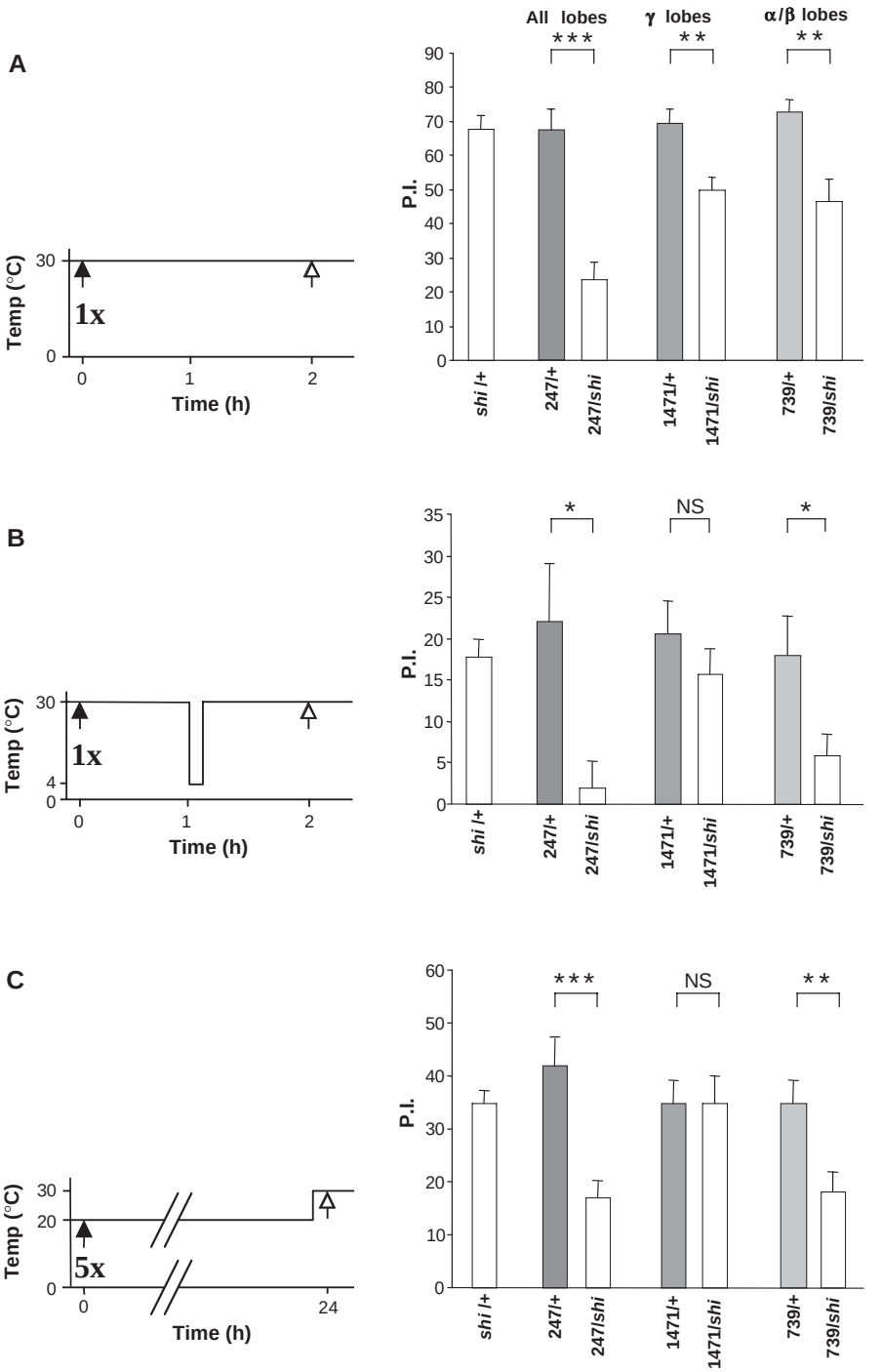

Fig. 2. Consolidated memories are localized in the MBs. Three Gal4 enhancer-trap lines were used to localize memories to the MBs: the *MB247* line (*247*), which shows expression in a subset of cells in all MBs lobes (*15*, *18*), the *Gal1471* line (*1471*), which shows expression in some γ neurons (supporting online material), and the *c739* line (*739*), which expresses Gal4 in some α/β neurons (*28*). The performances of *Gal4/UAS-shi*^{ts} (*Gal4/shi*) individuals were compared with those of appropriate genetic controls. The vertical black and white arrows represent time points for conditioning and testing, respectively. The values correspond to the means ± SEMs (n = 6 to 30). The P value shown corresponds to the t test comparison with appropriate genetic controls (*P < 0.05; **P < 0.01; ***P < 0.001; NS indicates no significant difference). (**A**) Two-hour memory relies on various MB neurons (n = 8 to 14). (**B**) ARM relies more heavily on α/β neurons. Flies in which neurons from all lobes (*247/shi*) or only α/β neurons (*739/shi*) were inactivated reveal a memory decrease in comparison to control flies. Inactivation of γ neurons (*1471*) does not affect ARM (n = 9 to 26). (**C**) LTM retrieval requires output from α/β neurons. Flies were trained at the permissive temperature and tested at the restrictive temperature 24 hours after five spaced cycles. Inhibition of neurons from all lobes (*247/shi*) or of only α/β neurons (*739/shi*) leads to a memory decrease. Inactivation of γ neurons (*1471*) does not affect LTM. *1471/+* versus *1471/shi* (P = 0.94); *shi /+* versus *1471/shi* (P = 0.98) (n = 8 to 30).

mance, whereas γ lobe inactivation did not affect LTM (Fig. 2C). Thus, α/β but not γ neurons are required for LTM retrieval. Because flies without β lobes show normal LTM (*11*), we conclude that the α lobes are the main center for the LTM retrieval. Altogether, those results suggest that ARM and LTM involve the same group of cells.

What is the link between ARM and the earlier phases of memory? We propose that ARM is not mainly derived from MTM because the *amn* mutant, which is defective for MTM, has normal ARM (*8*, *9*). The *amn* gene encodes a neuropeptide that might stimulate the cAMP pathway (*22*, *23*) via Rut-adenylyl cyclase activation. ARM therefore might be at least partially independent of cAMP regulation. To strengthen this hypothesis, ARM was measured in the *rut* mutant and found to be normal (Fig. 3), which confirms a previous study of other *rut* alleles (*24*). Thus the ARM pathway is at least partially independent of the STM/MTM pathway.

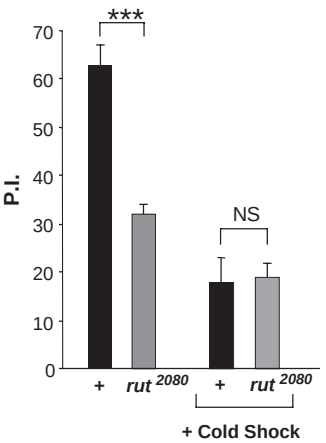

Fig. 3. The *rutabaga* mutant displays normal ARM. Wild-type *CS* (+) and *rutabaga*^{2080} (*rut*^{2080}) flies were subjected to 2 min of cold-shock anesthesia 1 hour after single-trial training. The 2-hour memory scores are not statistically different (n = 8) (t test, P = 0.85).

In mammals, competing memory systems have been described that involve different anatomical structures (*25*). We now reveal a competition between two types of consolidated memory, ARM and LTM, within the same structure, the MBs. Several observations suggest that cAMP-independent learning and memory occur, as originally suggested (*24*). First, significant levels of ARM are found in the *amn* and *rut* mutants, although their STM and MTM are strongly affected. Second, mutants with defects in the catalytic subunit (DCO) of PKA—the kinase normally activated by cAMP—display weak but stable memory for several hours after a single trial conditioning (*26*), which could be consolidated ARM. Third, null mutations affecting enzymes of the cAMP pathway do not abolish immediate memory (*7*). This cAMP-independent learning mode must rely on the MBs, because their ablation abolishes immediate olfactory memory (*13*). We propose that cAMP-independent learning could later give rise to *rsh*-dependent ARM (Fig. 4B).

LTM conditioning leads to the disappearance of ARM, and our data suggest that ARM and LTM involve the same group of neurons. The distinction between the two consolidated memories could thus rely on antagonistic molecular mechanisms within the same cells. Alternatively, different subsets of competing MB neurons might support ARM and LTM. Our results could explain the observation that a truncated and persistently active isoform of an atypical protein kinase C (PKMζ) enhances 4-day memory after massed, but not after spaced, training (*27*), because ARM is absent after spaced training.

Why are there two forms of consolidated memory, and why are they mutually exclusive? We propose that ARM acts as a gating mechanism for LTM formation (Fig. 4B). After massed training, ARM is formed in α/β neurons (Fig. 4C) and prevents LTM formation in the α lobes. After spaced training, ARM is erased, which releases the constraint on the LTM pathway. Such a mechanism would ensure that only information that has been encountered on independent occasions, and which, therefore, has a high predictive value, is stored in LTM. In contrast, a stimulus encountered only once for a short time (single trial) or for a longer time (massed training) would generate a semistabilized memory (ARM) that does not involve a heavy cascade of gene expression.

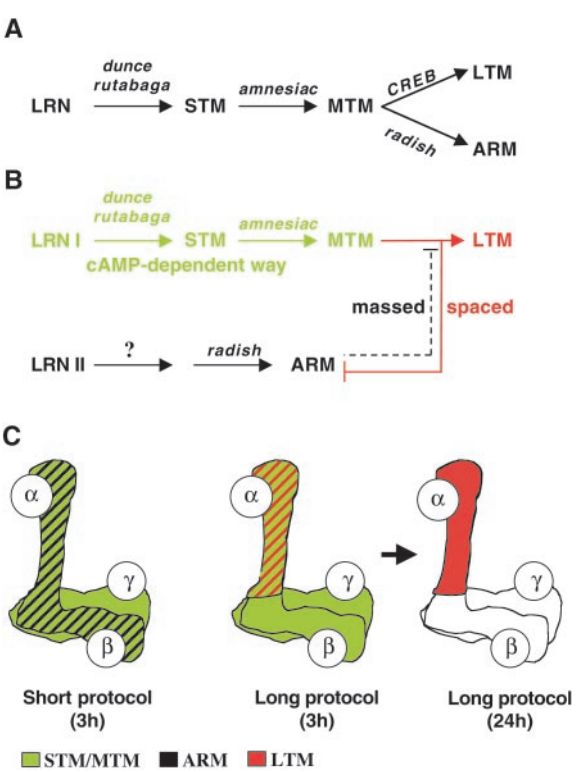

Fig. 4. Model of competitive memory phases. (**A**) A former model that holds that ARM and LTM coexist after spaced conditioning (*3*, *10*). (**B**) A new model that postulates mutually exclusive consolidated phases. LTM and ARM are supported by parallel pathways: the former is cAMP-dependent, and the latter is *rsh*-dependent. After massed conditioning ARM is hypothesized to prevent the formation of LTM. After spaced conditioning, LTM replaces ARM. The dashed line represents a hypothetical pathway. The possibility that unidentified molecular interactions connect the two learning pathways cannot be excluded. (**C**) Anatomical representation of memory phases. STM and MTM are supported by the α/β and γ lobes, and ARM is supported by the α/β lobes. Three hours after spaced conditioning, ARM is erased and LTM forms in the α lobes.

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Scale Errors Offer Evidence for a Perception-Action Dissociation Early in Life

Judy S. DeLoache,[1]* David H. Uttal,[2] Karl S. Rosengren[3]

We report a perception-action dissociation in the behavior of normally developing young children. In adults and older children, the perception of an object and the organization of actions on it are seamlessly integrated. However, as documented here, 18- to 30-month-old children sometimes fail to use information about object size and make serious attempts to perform impossible actions on miniature objects. They try, for example, to sit in a dollhouse chair or to get into a small toy car. We interpret scale errors as reflecting problems with inhibitory control and with the integration of visual information for perception and action.

The relation between visual experience and action is a classic and fundamental problem in psychology and neuroscience. We report here the initial investigation and documentation of a new phenomenon—dramatic failures by very young children to use visual information about size when interacting with familiar kinds of objects. The original impetus for this research came from informal observations in our labs and homes of young children attempting to perform actions on objects that were impossible owing to extreme differences between the relative sizes of the child and the object. Examples include children seriously trying to sit in dollhouse chairs, get inside small toy cars, and put doll shoes on their own feet. These errors of scale indicate that the usual integration of perception and action sometimes breaks down in normally developing young children. We propose that scale errors reflect a combination of immaturity in inhibitory control and in the integration of visual information processed by two neurally and functionally distinct systems (*1*–*4*).

To systematically investigate the occurrence of scale errors in a controlled setting, we gave 18- to 30-month-old children experience with large objects, followed by exposure to miniature replicas that were identical to their larger counterparts except for size (*5*). We assumed that very recent experience with the larger objects and very high similarity between the large and small ones would increase the likelihood that scale errors would occur.

Each child was observed in a laboratory play room containing three large play objects—an indoor slide that they could walk up and slide down, a child-sized chair that they could sit in, and a toy car that they could get inside and propel around the room with their feet. The room also contained several other play items (including a doll and doll-related items, books, etc.). The children were allowed to play naturally with whatever they wanted, except that the experimenter made sure that they interacted at least twice with each of the three large target objects. Next, the child was escorted from the room, and the large target objects were replaced with the miniature replicas. The child then returned to the room; if he or she did not spontaneously interact with the replica objects, the experimenter drew the child's attention to them without commenting on their size.

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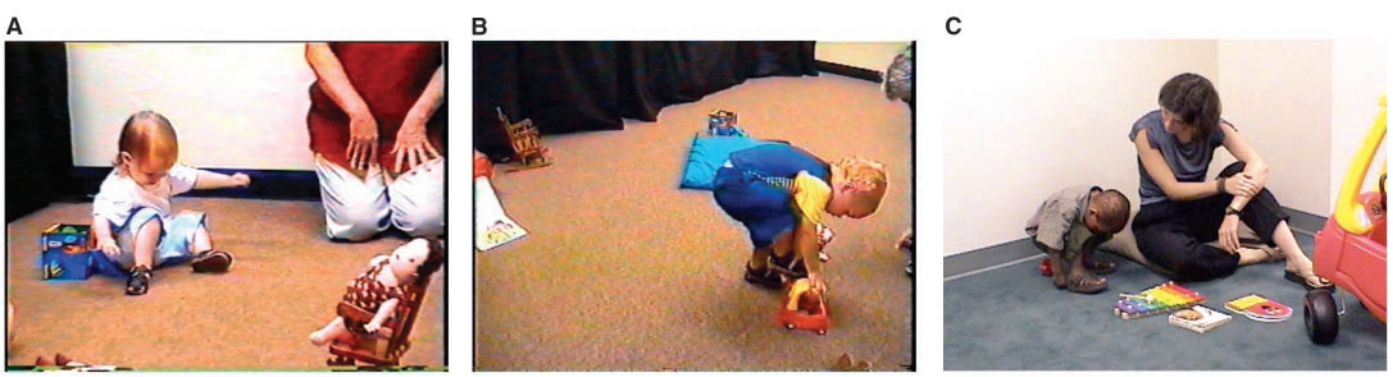

Fig. 1. Three examples of scale errors. (**A**) This 21-month-old child has committed a scale error by attempting to slide down a miniature slide; she has fallen off in this serious effort to carry out an impossible act. (**B**) This 24-month-old child has opened the door to the miniature car and is repeatedly trying to force his foot inside the car. (**C**) This 28-month-old child is looking between his legs to precisely locate the miniature chair that he is in the process of sitting on.