Role of NMDA Receptor Subtypes in Governing the Direction of Hippocampal Synaptic Plasticity

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Activation of *N*-methyl-D-aspartate subtype glutamate receptors (NMDARs) is required for long-term potentiation (LTP) and long-term depression (LTD) of excitatory synaptic transmission at hippocampal CA1 synapses, the proposed cellular substrates of learning and memory. However, little is known about how activation of NMDARs leads to these two opposing forms of synaptic plasticity. Using hippocampal slice preparations, we showed that selectively blocking NMDARs that contain the NR2B subunit abolishes the induction of LTD but not LTP. In contrast, preferential inhibition of NR2A-containing NMDARs prevents the induction of LTP without affecting LTD production. These results demonstrate that distinct NMDAR subunits are critical factors that determine the polarity of synaptic plasticity.

The molecular mechanisms underlying activity-dependent modification of synaptic strength have been under intensive investigation because of their fundamental importance in brain function and dysfunction (*1*, *2*). Homosynaptic long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission mediated by α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid subtype glutamate receptors (AMPARs) at Schaffer collateral–CA1 synapses of the hippocampus are by far the best-characterized cellular models of synaptic plasticity. Both LTP and LTD require *N*-methyl-D-aspartate subtype glutamate receptor (NMDAR) activation (*1*, *3*). However, the detailed mechanisms by which the activation of the same class of receptor can produce two opposing forms of synaptic modification remain unclear. A long-held belief has been that the degree of NMDAR activation, and hence the level of postsynaptic calcium elevation during the induction period, dictates the direction of NMDAR-dependent synaptic modification. The strongest evidence for this hypothesis comes from the conversion of LTP to LTD by a partial blockade of NMDARs with low concentrations of the NMDAR antagonist D,L-2-amino-5-phosphophonovaleric acid (APV) (*4*, *5*).

NMDARs are assembled from NMDAR subunit 1 (NR1) and at least one type of NR2 subunit (*6*). In the adult rat hippocampus, NR2A and NR2B are the predominant NR2 subunits (*7*, *8*). CA1 synapses contain multiple subtypes of NMDARs comprising NR1/NR2A, NR1/NR2B, or NR1/NR2A/NR2B subunits (*9*–*11*). Because the presence of different NR2 subunits may confer distinct gating and pharmacological properties to heteromeric NMDARs (*12*) and couple them to distinct intracellular signaling machineries (*13*, *14*), we hypothesized that the activation of distinct NMDAR subpopulations may determine the direction of CA1 synaptic plasticity that leads to LTP and LTD.

We first investigated the effect of blocking NR2B-containing NMDARs on LTP and LTD of CA1 field excitatory postsynaptic potentials (fEPSPs) induced by high- and low-frequency stimulation (HFS and LFS), respectively (*15*). The NR2B subunit–selective antagonist ifenprodil (3 μM) (*16*) completely abolished the induction of LTD by LFS, suggesting that LTD requires the activation of NR2B-containing NMDARs (Fig. 1A). Another NR2B-specific antagonist, Ro25-6981 (*17*) (0.5 μM) also abolished the induction of LTD (Fig. 1A). In the presence of either Ro25-6981 or ifenprodil, stronger LTD-producing protocols such as three episodes of LFS (Fig. 1B) or paired-pulse LFS (*18*, *19*) failed to induce LTD. Thus, the failure to produce LTD in the presence of NR2B antagonists may represent an absolute blockade of its induction, rather than an incremental increase in its induction threshold. Neither ifenprodil nor Ro25-6981 affected the induction of LTP by HFS (Fig. 1C).

It is unlikely that the selective blockade of LTD by NR2B-specific antagonists is due merely to a partial inhibition of NMDARs, because LTP rather than LTD is more sensitive to the partial NMDAR blockade produced by low concentrations of APV (*4*, *5*). To rule out such a possibility, we tested the effect of low-dose APV that generated a partial blockade of NMDAR-mediated excitatory postsynaptic currents (EPSCs) similar to that produced by NR2B antagonists (Fig. 2A). Ifenprodil and Ro25-6981, at concentrations that prevented LTD, suppressed NMDAR-mediated EPSCs by 39.4 ± 7.4% and 35.6 ± 5.0%, respectively. A similar degree of EPSC inhibition was obtained with

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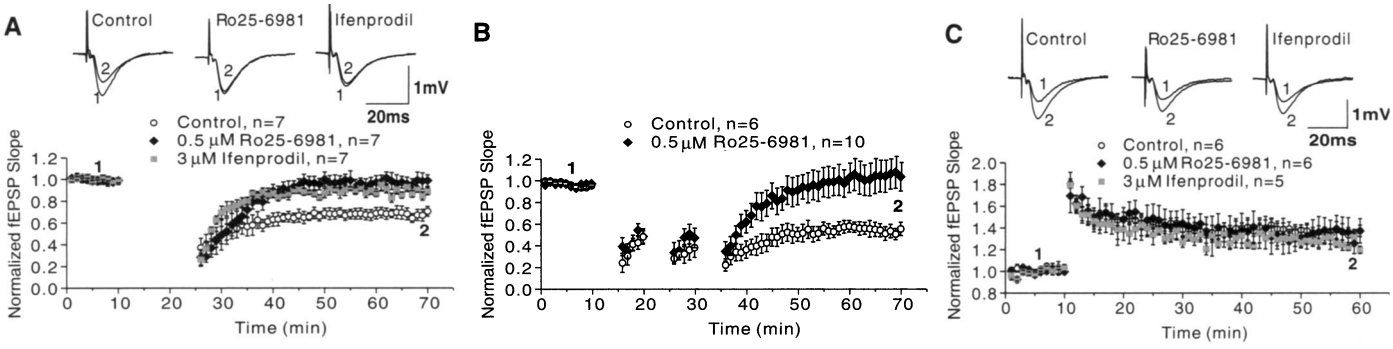

Fig. 1. Induction of homosynaptic LTD but not LTP in rat hippocampal slices requires the activation of NR2B-containing NMDARs. Representative fEPSPs averaged from 20 consecutive stimuli were taken before and after LTD or LTP induction at the time points (1 and 2) indicated in the graphs. (**A**) Continuous bath application of NR2B antagonists Ro25-6981 or ifenprodil prevented LTD produced by LFS. (**B**) Ro25-6981 also prevented the induction of LTD by three episodes of LFS. (**C**) HFS failed to produce LTP in the presence of NR2B antagonists.

0.5 μM APV (39.4 ± 4.5%). However, unlike NR2B antagonists, 0.5 μM APV preferentially prevented HFS-induced LTP without affecting LFS-induced LTD (Fig. 2, B and C). Thus, low-dose APV and NR2B antagonists have opposing effects on LTP and LTD.

One possible explanation for the selective effect of low-dose APV on LTP is that LTP induction requires the activation of NR2B-lacking NMDARs that are preferentially inhibited by this concentration of APV. Because NR2A-containing NMDARs are one of the major subpopulations of NMDARs in CA1 neurons (*7*, *8*) and because NR2A-containing NMDARs are more sensitive to APV blockade than NR2B-containing receptors (*20*), we hypothesized that the activation of this subpopulation of NMDARs is specifically involved in LTP induction. We examined the effect of an NR2A-specific antagonist, NVP-AAM077 (fig. S1), on LTP and LTD induction. In hippocampal slices, NVP-AAM077 (0.4 μM) blocked 52.5 ± 3.0% of the NMDAR-mediated EPSCs (fig. S1, B and C). The residual EPSCs were largely inhibited by ifenprodil. Thus, similar to results obtained in oocytes (fig. S1A), NVP-AAM077 at this concentration is also a specific antagonist for NR2A-containing NMDARs in CA1 neurons. NVP-AAM077 (0.4 μM) prevented both normal LTP induced by a single episode of HFS (Fig. 3A) and saturated LTP induced by multiple episodes of HFS (Fig. 3B). In contrast, NVP-AAM077 had little effect on LFS-induced LTD (Fig. 3C). These findings provide strong evidence that activation of NR2A- and NR2B-containing NMDARs is respectively required for the production of HFS-induced LTP and LFS-induced LTD.

Homosynaptic, hippocampal CA1 LTP and LTD can also be produced by protocols other than HFS and LFS. We thus tested the effects of both NR2A and NR2B antagonists on CA1 LTP or LTD produced by pairing Schaffer collateral stimulation with postsynaptic depolarization under whole-cell recording conditions (*21*). Both ifenprodil and Ro25-6981 virtually abolished LTD production (Fig. 4A), whereas ifenprodil had no effect on LTP induction (Fig. 4B). Blockade of the NR2A-containing subpopulation by NVP-AAM077 has no effect on LTD (Fig. 4C). In the presence of the NR2A antagonist, the LTP-producing stimulus results in LTD instead (Fig. 4D). A similar conversion of CA1 LTP into LTD by a low concentration of APV has also been reported (*4*). The ability to convert the outcome of LTP-producing protocols to LTD after blockade of the NR2A subunits indicates that these protocols may be sufficient to activate both NR2A- and NR2B-containing NMDARs and that NR2B-mediated LTD was unmasked after the blockade of NR2A-containing receptors. Similar conversion of LTP into LTD cannot be induced by HFS in the presence of an NR2A antagonist (Fig. 3). A possible explanation is that NR2B-containing receptors are not activated by this short stimulating protocol. This is unlikely, however, because a clear Ro25-6981–sensitive component of NMDAR-mediated EPSCs could be recorded during HFS (fig. S2). Alternatively, a prolonged activation of NR2B-containing NMDARs may be required for LTD production (*22*, *23*). Consistent with this hypothesis, a low concentration of APV has previously been shown to convert LTP of fEPSPs into LTD when a longer LTP-induction protocol was used (*5*).

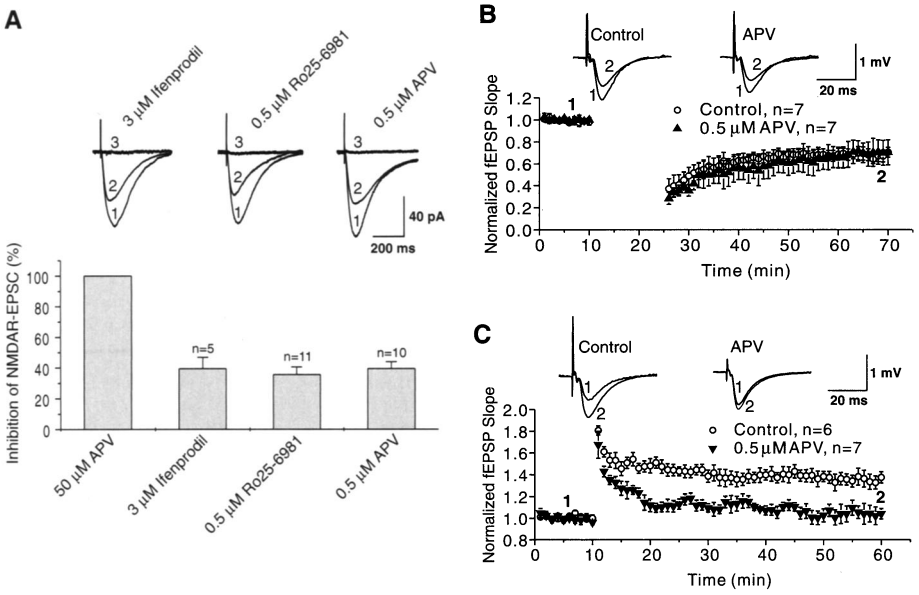

Fig. 2. APV at a concentration sufficient to reduce NMDAR-mediated EPSCs to a level similar to that observed with NR2B antagonists prevents the induction of LTP without affecting LTD. EPSCs were recorded under whole-cell voltage-clamp configuration, at a holding membrane potential of −60 mV, from CA1 neurons perfused with a Mg^{2+}-free solution that contained 6,7-dinitroquinoxaline-2,3-dione (DNQX) (20 μM) and bicuculline (10 μM). (**A**) Inhibition of NMDAR-mediated EPSCs by NR2B antagonists or by APV. Representative traces above the bar graph were taken before drug treatment (1); after either ifenprodil, Ro25-6981, or low-dose APV (0.5 μM) (2); and after a saturating dose of APV (50 μM) (3). The bar graph summarizes data from each group of neurons. These EPSCs were entirely mediated by NMDARs, because they were fully blocked by 50 μM APV. (**B** and **C**) Low-dose APV did not affect the ability of LFS to induce LTD of fEPSPs (B), but abolished HFS-induced LTP (C).

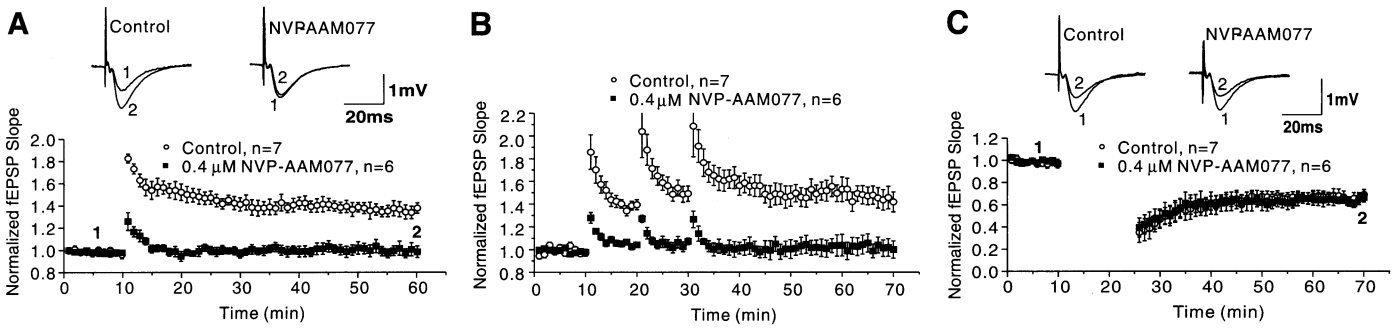

Fig. 3. NR2A-containing NMDARs are required for HFS-induced LTP but not LFS-induced LTD in hippocampal slices. (**A** and **B**) Selective inhibition of NR2A-containing NMDARs by NVP-AAM077 abolished the LTP of fEPSPs induced by either (A) one or (B) three HFS episodes, indicating an absolute requirement of NR2A-containing NMDAR activation in LTP induction. (**C**) NVP-AAM077 failed to prevent LFS-induced LTD, suggesting NR2A-containing NMDAR activation is not required for LTD.

Our results demonstrate that NR2A and NR2B subunits, in addition to postsynaptic intracellular calcium concentration (*4*, *5*) and correlated pre- and postsynaptic activation (*24*–*26*), are critical factors in determining the polarity of synaptic plasticity in the hippocampal CA1 area. Our findings are consistent with the coincidence between a gradual, postnatal, developmental increase in the NR2A/NR2B ratio of synaptic NMDARs in the hippocampus (*11*, *27*) and the increasing difficulty of inducing LTD at these synapses (*19*, *28*). The absolute requirement for activation of NR2A-containing NMDARs in LTP production is in agreement with a dominant role of the NR2A subunit in LTP, as demonstrated by studies of genetically altered mice that lack NR2A (*29*) or its carboxyl tail (*14*). However, the necessity of NR2B activation for LTD is at odds with some previous studies (*30*–*32*). Transgenic overexpression of NR2B in mouse forebrain has been reported to increase LTP but not LTD (*30*). How overexpression of NR2B leads to enhanced LTP in these mice remains unclear. We speculate that the enhanced LTP may have resulted at least in part from an increase in total functional NMDARs, as a result of overexpression of NR2B subunits. The unaltered LTD in these mice may imply that the endogenous NR2B-containing NMDARs are already sufficient, i.e., not a limiting factor, for LTD production. Another inconsistency with our current results is the enhancement of LTD by ifenprodil reported by Hendricson *et al.* (*31*). These conflicting data may have arisen at least in part from the different drug concentrations and experimental conditions used in the two studies. Here, we additionally confirmed ifenprodil's blockade of LTD by using another NR2B antagonist, Ro25-6981. Our finding that both antagonists prevent LTD produced by a number of different protocols, without affecting LTP, provides evidence that NR2B NMDAR activation is required for hippocampal CA1 LTD in 3- to 4-week-old rats. This is further corroborated by the specific prevention of LTP induction and the conversion of LTP into LTD with NR2A receptor antagonism.

The differential requirements for distinct NMDAR subpopulations may not be limited to the adult hippocampal CA1 synapse. In the barrel cortex, a reduction of NR2B-containing receptor proportion, but not in the amplitude or kinetics of NMDAR-mediated EPSCs, is responsible for the critical period of LTP induction (*33*). Similarly, in the visual cortex, a change in the ratio of NR2A/NR2B-containing NMDARs has been shown to be related to the experience-dependent modulation of synaptic plasticity (*34*). Moreover, reduction of synaptic NR2B-containing NMDARs correlates with the loss of ocular dominance plasticity in the developing visual cortex (*35*). NR2B antagonists block the LTP of EPSCs, whereas low-dose APV inhibits the LTD of inhibitory postsynaptic currents in the visual cortex (*36*). Thus, the roles of NR2A and NR2B subunits in the formation of different forms of synaptic plasticity may vary depending on developmental stages and brain areas.

The mechanisms by which distinct subpopulations of NMDARs that contain different NR2 subunits determine the polarity of synaptic plasticity remain to be determined. In addition to distinct kinetics of calcium influx gated through NR2A- and NR2B-containing NMDARs (*11*, *37*), different NR2 subunits may couple to different postsynaptic signaling pathways (*13*, *38*). As such, the activation of NR2A-containing NMDARs could trigger different signaling events from the activation of their NR2B-containing counterparts, and such subunit-specific signaling outcomes could determine the direction of synaptic changes. Whatever the precise mechanism, our study establishes that NMDARs of different subunit composition have critical roles in determining the polarity of synaptic plasticity in hippocampal CA1 synapses of adult rats. Although the activation of NR2A-containing NMDARs leads to LTP formation, the activation of NR2B-containing NMDARs produces LTD. The present study will help to unravel the detailed molecular mechanisms underlying hippocampal CA1 synaptic plasticity, the proposed cellular model of learning and memory (*1*–*3*).

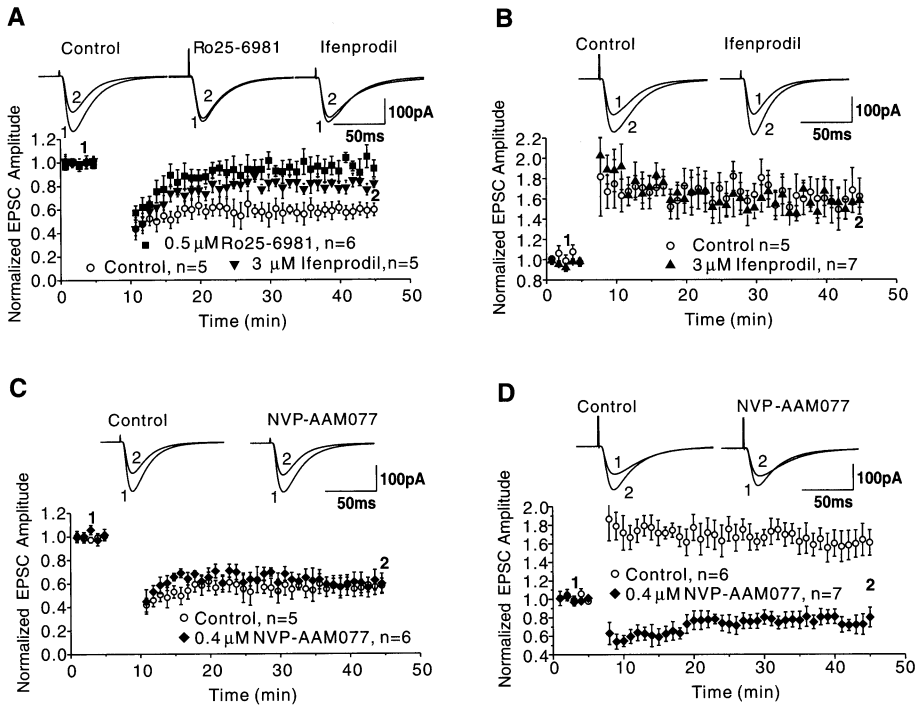

Fig. 4. CA1 LTP and LTD produced by pairing presynaptic stimulation with postsynaptic depolarization differentially require activation of NR2A- and NR2B-containing NMDARs. EPSCs of CA1 neurons were recorded under whole-cell mode at a holding membrane potential of −60 mV before and after induction protocols. (**A** and **B**) Blocking NR2B-containing NMDARs with either ifenprodil or Ro25-6981 prevented the production of (A) LTD, but not (B) LTP. (**C** and **D**) Blockade of the NR2A component by NVP-AAM077, on the other hand, failed to prevent LTD (C), but abolished LTP induction and converted it into LTD (D).

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Supporting Online Material
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Materials and Methods
Figs. S1 and S2
References and Notes

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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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