Neural Substrates of Memory: From Synapse to System

Josh Dubnau,[1] Ann-Shyn Chiang,[2] Tim Tully[1]

[1] Cold Spring Harbor Laboratories, 1 Bungtown Rd., Cold Spring Harbor, New York 11724

[2] National Tsing Hua University, Department of Life Science, Hsinchu 30043, Taiwan

ABSTRACT: One of the fundamental challenges of modern neuroscience is to understand how memories are acquired, stored, and retrieved by the brain. In the broadest terms, attempts to dissect memory can be broken down into four experimental disciplines: (1) identification of molecular components, (2) *ex vivo* and *in vivo* cellular analysis of neuronal function, (3) theoretical modeling approaches of neural systems, and (4) organismal-level behavioral analyses. Our objective here is to offer a conceptually unifying perspective and to discuss this perspective in relation to an experiment analysis of memory in *Drosophila*. © 2003 Wiley Periodicals, Inc. J Neurobiol 54: 238–253, 2003

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INTRODUCTION

A central dogma of cellular neuroscience is that synaptic plasticity underlies behavioral plasticity. Or, in other words, that information is coded in some way by alteration of synaptic strength and/or connectivity in networks of neurons (Hebb, 1949; Kandel and Spencer, 1968; Martin et al., 2000). Evidence in support of this hypothesis derives from three types of observation. First is the obvious qualitative similarity between behavioral and synaptic plasticity. Both learning and synaptic plasticity involve long-lived alterations in responses to (paired) stimuli resulting from prior experience. Moreover, several forms of synaptic plasticity exhibit associative properties (McNaughton et al., 1978; Levy and Steward, 1979; Barrionuevo and Brown, 1983; Murphy and Glanzman, 1999; Weisskopf et al., 1999; Blair et al., 2001). This attribute of synaptic plasticity suggests a mechanism to explain behavioral phenomena where associative links appear to be forged between unrelated stimuli (the conditioned stimulus "CS" and the unconditioned, reinforcing stimulus "US"). Second, numerous *ex vivo* studies both in vertebrate and invertebrate model systems have established experimental connections between behavioral and neural plasticity (Martin et al., 2000). Work in *Aplysia* has established links between conditioning of the gill-withdrawal reflex and long-term facilitation (LTF), even in cocultured sensory motor neurons (Hawkins, 1984; Glanzman, 1995; Byrne and Kandel, 1996; Murphy and Glanzman, 1999). Analogous experiments in vertebrates have led to wide acceptance of the hypothesis that long-term potentiation (LTP) underlies several forms of behavioral plasticity (Bliss and Lomo, 1973; Bliss and Collingridge, 1993; Martin et al., 2000; Blair et al., 2001). Many of the biologic properties of LTP are similar to those of memory. Like early memory, LTP has a short-lived form that is resistant to inhibitors of protein synthesis and is manifested after relatively weak stimulation protocols. Like long-term memory, long-lasting LTP requires stronger, repetitive stimulation, and is sensitive to inhibitors of protein synthesis and to disruptions of CRE-mediated transcription (Bourtchuladze et al., 1994; Guzowski and McGaugh, 1997; Lamprecht et al., 1997; Josselyn et al., 2001; Kida et al., 2002; Pittenger et al., 2002).

Biochemical signaling pathways also are common to memory and synaptic plasticity. In particular, the cAMP signaling cascade appears to be involved in mammalian memory formation and long-lasting syn-

Correspondence to: J. Dubnau (dubnau@cshl.edu, tully@cshl.org).

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aptic plasticity (Bailey et al., 2000; Martin et al., 2000). Remarkably, many experimental interventions that disrupt these forms of synaptic plasticity *ex vivo* also appear to interfere *in vivo* with memory (Davis et al., 1992; Bourtchuladze et al., 1994; Huang et al., 1995; Lu et al., 1997; Nalbantoglu et al., 1997; Sutton et al., 2001). Moreover, the ability to detect LTP in behaving animals has strengthened the link between memory per se and mechanisms of synaptic plasticity first characterized *in vitro*. Growing evidence suggests that behavioral experience can induce LTP like phenomena *in vivo* (Morris et al., 1986; Morris, 1989; Mitsuno et al., 1994; McKernan and Shinnick-Gallagher, 1997; Rogan et al., 1997; Rioult-Pedotti et al., 2000; Tsvetkov et al., 2002).

Although most of the data linking synaptic plasticity with memory are correlative, these convergent observations from a variety of species and behavioral tasks constitute a strong body of evidence supporting the notion that activity-induced synaptic modulations, such as LTP, play a role in information storage in the brain. This idea, which has been referred to as the "synaptic plasticity and memory (SPM) hypothesis" (Martin et al., 2000), is the driving force behind the study of synaptic plasticity—and several plausible cellular mechanisms have been elucidated (Bear, 1999; Malenka and Nicoll, 1999; Malinow et al., 2000; Blair et al., 2001). Although a coherent explanation for complex forms of experience-dependent changes in behavior does not derive trivially from the SPM hypothesis, a simple model for elemental forms of associative learning has been proposed (Fig. 1) (Hawkins, 1984; Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Murphy and Glanzman, 1999). In this now popular hypothesis, sensory pathways for two unrelated environmental stimuli (CS and US) converge anatomically. Coincident stimulation by CS and US at the anatomical locus of convergence increases synaptic strength, while noncoincident stimulation weakens synaptic strength. The associative nature of synaptic plasticity mechanisms, such as NMDA receptor-dependent LTP, then yields modulation of synaptic efficacy between the CS sensory pathway and the follower neurons that ultimately drive motor output. Subsequent behavioral responses to the CS alone are thereby strengthened. Although this reductionist model has intuitive appeal and explains many of the findings from cellular neuroscience, it appears insufficient when viewed in the context of findings from whole animal behavior, from *in vivo* recordings and from theoretical modeling.

Computational modeling approaches have suggested that temporal and spatial patterns of activity in neuronal ensembles are a probable medium for storage and processing of information in the brain (Zipser et al., 1993; Durstewitz et al., 2000; Hinton, 2000; Aksay et al., 2001; Wang, 2001; Lever et al., 2002). Working memory, the ability to transiently hold information following the removal of environmental stimuli, is widely believed to derive from recurrent excitatory connections that result in a persistent activity pattern that can be maintained for brief periods. Experience-driven persistent changes in neural activity have been documented in the prefrontal cortex of primates, the oculomotor neural integrator of goldfish, the hippocampal place cell representation of rodents, the mushroom bodies (MB) or antennal lobes (AL) of insects and the lobulus parolfactorius of chick (Villa and Fuster, 1992; Gigg et al., 1994; Laurent and Naraghi, 1994; Fuster, 1995; Wehr and Laurent, 1996; Mizunami et al., 1998; Faber et al., 1999; Aksay et al., 2001; Menzel, 2001; Wang et al., 2001; Lei et al., 2002; Lever et al., 2002). These types of *in vivo* measurements of activity in behaving animals demonstrate that the pattern and firing rates of large groups of neurons can be altered by perceptual experience, can persist after the removal of environmental stimuli, and can be reenstated during retrieval. Although again correlative, these studies are consistent with the hypothesis that memories can be represented as recurrent patterns of neural activity. This view of memory, while not necessarily inconsistent with findings from cellular neuroscience, suggests that memory processing is a systems-level phenomenon in which firing patterns in neuronal ensembles play a role. Behavioral studies also yield a dynamic view of memory, suggesting that information after an initial associative event is sequentially processed through several distinct phases, each with unique underlying properties, in some cases relying on different anatomical loci and biochemical pathways (see below).

Given our limited understanding it perhaps is not surprising that these different levels of analysis have yielded somewhat different (albeit not necessarily contradictory) notions about information storage in the brain. A significant challenge for the field of neuroscience is an experimental synthesis of these ideas across levels of analysis (vertical integration) and model systems (horizontal integration; cf. Dubnau and Tully, 1998). With this ultimate goal in mind, we offer a behavior–genetic perspective on memory. Our discussion is biased towards a view from Pavlovian learning in *Drosophila* for two reasons. First, genetic analysis reveals (logical) links between behavioral responses and each of the underlying levels of organization (systems, cellular, and molecular), thereby supplying a unique experimental synthesis. Second, for technical and historic reasons cellular and

modeling approaches have been focused largely on hippocampal and amygdala-dependent tasks in a few vertebrate species. Although behavioral analyses have been applied broadly for numerous experience-dependent contexts in a variety of species, genetic dissection of behavioral plasticity has been most productive in *Drosophila*. The resulting vertical integration of function, in our view, has been lacking in these other model systems.

MEMORY: A BEHAVIOR

Experimental dissection of *Drosophila* memory at the organismal level has relied on behavioral, anatomical, pharmacologic, and genetic manipulations. These methods of intervention have revealed that memory is a dynamic biologic process involving a series of functionally distinct temporal phases and anatomical foci each with separate kinetic and mechanistic attributes (Fig. 2). In both flies and chicks, for instance, depolarizing drugs such as MSG or KCl, have little effect on learning but disrupt short-term memory (STM). Ouabain (an inhibitor of Na/K+ pump), on the other hand, blocks an intermediate form of memory (Allweis, 1991; Xia et al., 1997). Inhibitors of gene expression also are known to interfere with the appearance of long-lasting memory (Davis and Squire, 1984; Montarolo et al., 1986; Tully et al., 1994).

Anatomic lesion experiments also lend credence to this dynamic view of memory formation. Studies involving surgically, pharmacologically and genetically induced lesions have revealed distinct anatomical sites involved with different temporal phases of memory formation. Studies of patients with localized brain damage and of anatomically lesioned rodents have suggested that the hippocampal formation participates in acquisition and early retention of declarative (explicit) tasks but is not the site of their long-term storage (Milner, 1972; Squire, 1992; Chen et al., 1996; Frankland et al., 1998; Scoville and Milner, 2000). Analogous studies of taste aversion (passive avoidance) learning in chicks also suggest an anatomical dissection of initial acquisition from subsequent memory storage. Pretraining lesions of the intermediate medial hyperstriatum ventrale inhibit learning, whereas posttraining lesions do not (Patterson et al., 1990). In contrast, post- (but not pre-) training lesions of the lobus parolfactorius inhibit memory of this task (Gilbert et al., 1991). Analogous experiments in *Drosophila* and in bees also suggest a "memory transfer" from antennal lobe (AL) to mushroom body (MB) (Hammer and Menzel, 1998; McBride et al., 1999; Menzel, 2001). These and other studies support the idea that memory consolidation can involve the transfer of information between physically distinct anatomical foci.

This dissection of memory formation into temporally distinct phases, which are pharmacologically and anatomically separate, is convergent with, and extended by, findings from behavioral and genetic manipulations of olfactory memory in fruit flies. *Drosophila* have five distinct temporal phases of memory formation (Dubnau and Tully, 1998): acquisition (or learning; LRN), short-term memory (STM), middle-term memory (MTM), anesthesia-resistant memory (ARM) and long-term memory (LTM) [Fig. 2(B)]. Each of these memory phases can be modulated preferentially by different experimental manipulations. LTM, for example, appears within 24 h after spaced training (10 training sessions with a 15-min rest interval between each) but is not observed after massed training (10 training sessions with no rest interval between each; Tully et al., 1994). LTM also is blocked by inhibitors of protein synthesis and depends on proper function of the *Adf-1* and *CREB* transcription factors (Yin et al., 1994, 1995; DeZazzo et al., 2000). The requirement for CREB-dependent gene expression during long-term memory formation, in particular, appears highly conserved (Bourtchuladze

Figure 1 The cellular model of synaptic plasticity. Sensory pathways for two unrelated stimuli, CS (red) and US (black), converge anatomically. Coincident neural activity is detected at the locus of convergence (blue), resulting in altered synaptic responses to subsequent CS presentation and modification of motor output driven by follower neurons (Hawkins, 1984; Bliss and Collingridge, 1993; Murphy and Glanzman, 1999). Several cellular mechanisms for such associative synaptic plasticity have been suggested including both NMDA receptor- and VGCC-dependent mechanisms (discussed extensively in Bear, 1999; Malenka and Nicoll, 1999; Malinow et al., 2000; Blair et al., 2001). Most types of synaptic plasticity appear to involve mechanistically distinct short- and long-lived forms. Short-lived synaptic plasticity is induced by weak stimulation, and appears refractory to disruption by inhibitors of protein synthesis. In contrast, long-lasting synaptic modifications require stronger (repeated) stimulation, CREB-mediated gene expression and involve structural changes in synaptic connectivity (dashed lines; Dash et al., 1990; Bartsch et al., 1995).

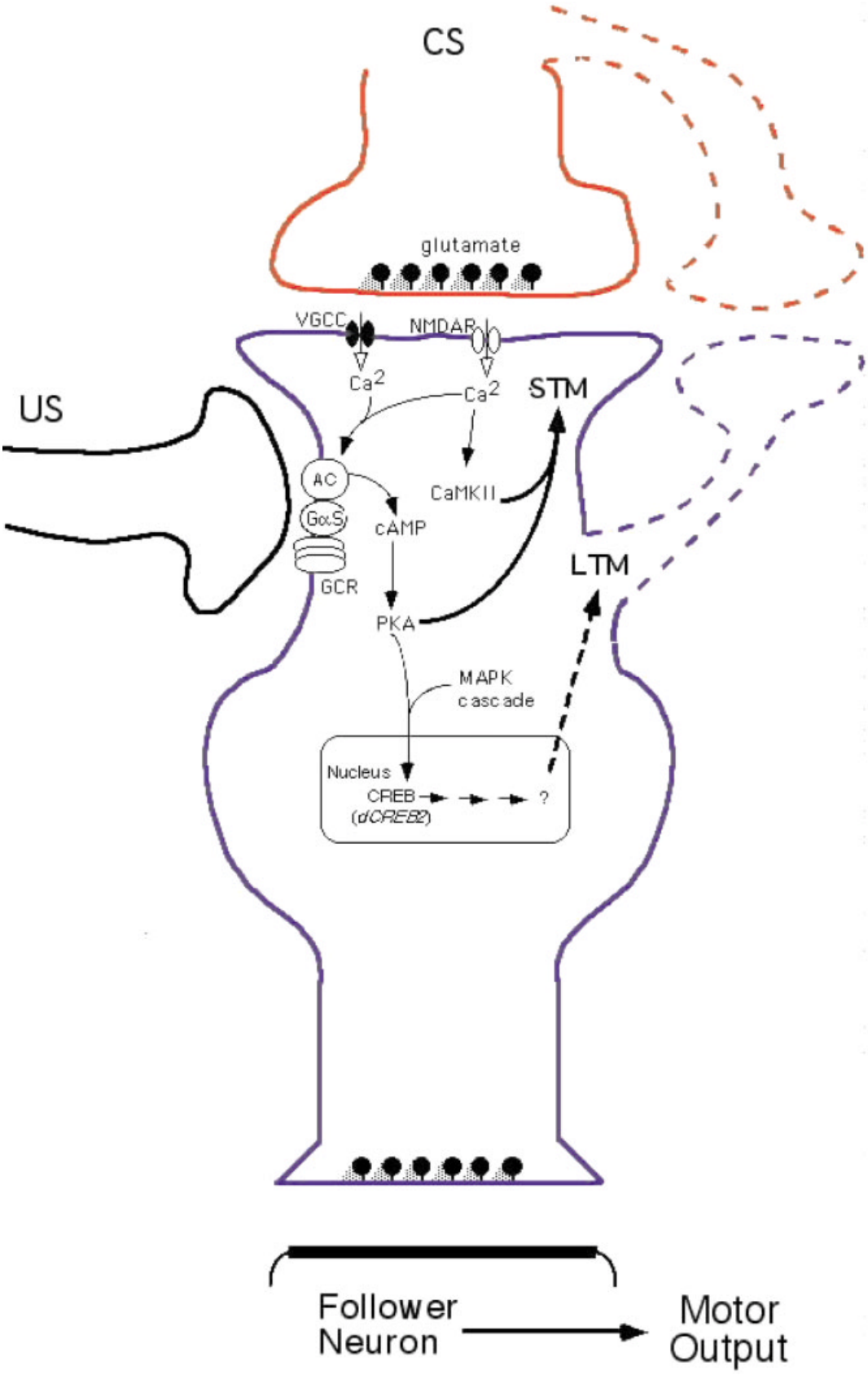
CS
glutamate
VGCC
NMDAR
Ca2
Ca2
STM
US
AC
CaMKII
GαS
cAMP
GCR
LTM
PKA
MAPK
cascade
Nucleus
CREB
(dCREB2)
?
Follower
Neuron
Motor
Output

Figure 1

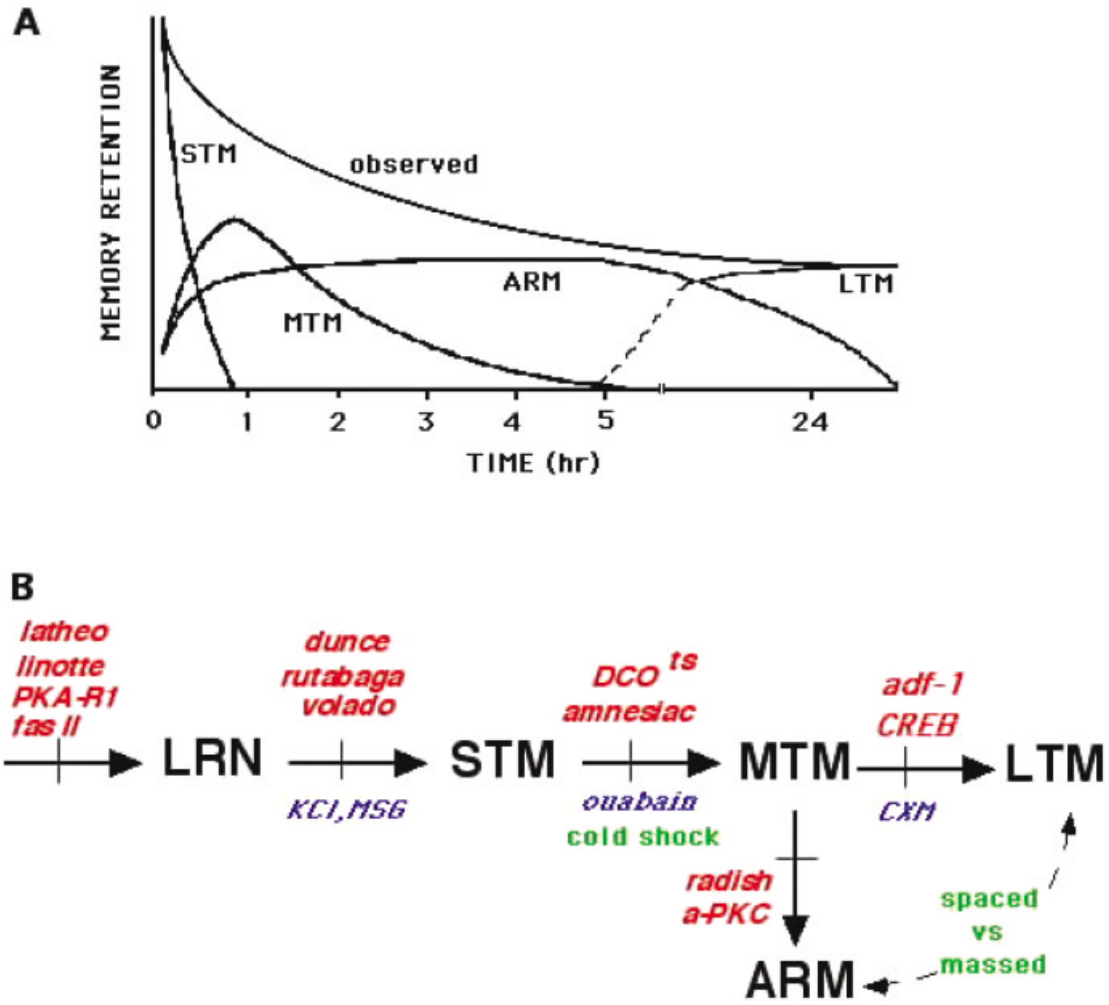

Figure 2 The behavioral model of memory formation. (A) The decay of memory observed over time appears relatively seamless. Experimental disruptions, however, reveal several temporally, mechanistically, and anatomically distinct memory phases underlying memory retention—including short-term (STM), middle-term (MTM), anesthesia resistant (ARM), and long-term (LTM) memory (Milner, 1972; Quinn and Dudai, 1976; Davis and Squire, 1984; Allweis, 1991; Squire, 1992; Folkers et al., 1993; Tully et al., 1994; Chen et al., 1996; Xia et al., 1997; Frankland et al., 1998). (B) In flies, behavioral, pharmacologic, and genetic interventions have confirmed and extended this dynamic view of memory processing. Single-gene mutations (red), pharmacologic interventions (blue), and behavioral manipulations (green) preferentially affect specific memory phases. CREB-dependent LTM, for instance, forms only after spaced training and is disrupted by inhibitors of gene expression (Tully et al., 1994). ARM, in contrast, is induced after a single training session and is resistant to inhibitors of gene expression. Unlike LTM, ARM is independent of CREB but is disrupted in *rsh* mutants (Quinn and Dudai, 1976; Folkers et al., 1993; Tully et al., 1994). Thus, ARM and LTM are mechanistically distinct forms of consolidated memory that can exist in parallel (Tully et al., 1994).

et al., 1994; Guzowski and McGaugh, 1997; Lamprecht et al., 1997; Josselyn et al., 2001; Kida et al., 2002; Pittenger et al., 2002).

Anesthesia-resistant memory (ARM) also is a long-lasting form of memory that has been observed in many species. In flies, memory immediately after training can be disrupted with a variety of anesthetic agents (DeZazzo and Tully, 1995; Xia et al., 1997). Within several hours, however, memories become resistant to these same treatments. Although the appearance of anesthesia-resistant forms of memory has been described in marine invertebrates (Yamada et al., 1992), several insects species (Erber, 1976; Quinn and Dudai, 1976; Folkers et al., 1993; Tully et al., 1994;

Xia et al., 1999), chicks (Allweis, 1991), rodents (Galluscio, 1971), and humans (Weissman, 1967), a genetic dissection of ARM from CREB-dependent LTM has been demonstrated only in *Drosophila*. In flies, ARM appears to be mechanistically distinct from *Adf1*- and *CREB*-dependent LTM. Unlike LTM, ARM decays away within 4 days and is not blocked by inhibitors of protein synthesis. Multiple training sessions produce increasingly higher levels of ARM, but such levels are similar after 10 massed versus 10 spaced training sessions (Tully et al., 1994; T. Tully unpublished data). ARM is not disrupted by perturbations of the CREB gene (Yin et al., 1994, 1995) but is disrupted in *rsh* mutants (Quinn and Dudai, 1976; Folkers et al., 1993; Tully et al., 1994), and may require activation of atypical PKC (Drier et al., 2002; see also Zhao et al., 1994). In contrast, LTM in *rsh* mutants is normal. Thus, ARM and LTM are genetically and functionally independent forms of long-lasting memory that exist in parallel for several days after spaced training. ARM, which forms more rapidly and with less training than LTM, also appears to coexist temporally with a third memory phase, MTM.

The "early memory" that is resistant to protein synthesis inhibitors also can be dissected into separate components. Three hours after training, when ARM is maximal, approximately 50% of observed memory still is cold shock sensitive. This disruptable form of memory is referred to as MTM. Mutations either in *amnesiac* (*amn*) or in the catalytic subunit of PKA (DC0) disrupt MTM preferentially (W. Li et al., 1996; Tully et al., 1996). In *amn* mutants, for example, initial learning and 7-h retention are near-normal but memory retention at intermediate time points is greatly reduced.

Evidence for MTM in wild-type flies emerged from reversal learning experiments (Tully et al., 1996). During the first training session, odor A (the CS+) is paired with a foot shock (US) and odor B (the CS−) is not. After a given retention interval, a second (reversal) training session then is administered; odor B becomes the CS+ and odor A becomes the CS−. Different retention intervals are used for different groups of animals to quantify the disruptive effects of "reversal" throughout the memory consolidation process. This reversal learning procedure preferentially disrupts the same stage of memory (MTM) as that missing in *amn* mutants. Consequently, the "reversal retention" curves of wild-type and *amn* flies appear similar. In contrast to the effect of reversal learning on MTM, STM (which precedes MTM kinetically) and ARM (which appears as MTM fades) both appear resistant to the these disruptive effects. STM seems genetically distinct, as well, because it appears preferentially disrupted in *dunce*, *rutabaga*, and *volado* mutants (Tully et al., 1996; Dubnau and Tully, 1998; Grotewiel et al., 1998).

Acquisition also appears to be a genetically distinct biologic process. Mutations either in *latheo*, *linotte*, *PKA-RI*, or *fas II* each result in reduced performance measured immediately after Pavlovian olfactory conditioning, but the rate of memory decay thereafter appears normal (Dubnau and Tully, 1998; Cheng et al., 2001). A striking generalization from these behavior–genetic studies is that most single-gene disruptions are reasonably phase specific—though secondary effects on temporally "downstream" memory stages often result from "upstream" disruptions. Hence, information processing in *Drosophila* consists of both sequential and parallel stages, the relative contributions from which to the behavioral manifestation of memory retention change with time.

Biochemical complexity is superimposed on this temporally dynamic view of memory formation. Behavioral screens initially identified *dunce* (PDEII) and *rutabaga* (AC) mutants, which then were discovered to carry lesions in components of the cAMP signaling cascade. Subsequent reverse-genetic disruptions of other known genetic components of this pathway [$G\alpha_s$ (the stimulatory alpha subunit of G protein), *DC0* (catalytic subunit of cAMP-dependent protein kinase, PKA), *PKA-RI* (regulatory subunit of PKA), and *dCREB2* (cAMP-response element binding protein)] all yielded olfactory memory defects (Dubnau and Tully, 1998; Waddell and Quinn, 2001). Molecular identifications of several other genes involved with olfactory memory have suggested additional biochemistries apparently distinct from cAMP signaling. *Volado* encodes an integrin (Grotewiel et al., 1998), and *fasII* encodes a fly homolog of NCAM (Cheng et al., 2001), thereby suggesting a role for cell adhesion. *Leonardo* encodes the fly homolog of *14-3-3* (Skoulakis and Davis, 1996; Philip et al., 2001), a cytoplasmic modulator of several cellular functions including MAP kinase signaling. *Nalyot* encodes the ADF1 transcription factor, which is involved in aspects of developmental plasticity that are distinct from those regulated by CREB (DeZazzo et al., 2000). Most surprisingly, perhaps, is the discovery that *latheo* encodes an integral member of the Origin Recognition Complex (*ORC3*; involved in DNA replication). LAT nevertheless is expressed in presynaptic terminals at the larval neuromuscular junction where it appears to modulate calcium-dependent transmitter release (Pinto et al., 1999; Rohrbough et al., 1999). Further genetic complexity underlying this form of Pavlovian learning is suggested by a more recent behavioral

screen for single-gene mutations disrupting one-day memory after spaced training.

Together, these behavior–genetic studies indicate that even this simple associative task in a relatively uncomplicated invertebrate brain depends on a substantially complex molecular cascade operating in both sequential and parallel steps during the processing of information from acquisition to long-term memory storage. Indeed, a behavioral view of memory suggests that multiple cellular mechanisms are required to process information during the seconds, minutes, hours, days and years after a new experience initially is perceived. How is the behavioral phenomenology of memory reconciled with the SPM hypothesis? Can the phenomena of multiple memory phases, parallel processing of information and anatomic transfer all be explained by biochemical changes in synaptic strength? Some insight can be derived from an anatomical dissection of insect olfactory memory.

MEMORY: AN ANATOMY

Convergent studies from several insect species have revealed a role for the mushroom body (MB) in olfactory memory formation. MB neurons are believed to integrate multimodal information, including olfactory stimuli, and to modulate behavioral responses via motor output (Strausfeld, 1976; Y. Li and Strausfeld, 1997; Rybak and Menzel, 1998). In adult *Drosophila*, one hemisegment of the MB consists of approximately 2500 kenyon cells, the primary afferents of which convey olfactory input via the antennal-glomerular tract (AGT; see Fig. 5). The AGT projects from the antennal lobe (AL; Strausfeld, 1976; Strausfeld and Li, 1999; Jefferis et al., 2002; Marin et al., 2002; Wong et al., 2002), which itself receives olfactory input from sensory neurons in the antennae (Vosshall et al., 1999; Kim et al., 2000; Vosshall et al., 2000; de Bruyne et al., 2001), to the MB and separately to (presumed) motor output regions in the protocerebrum. MB efferents also project to these protocerebral regions (Strauss and Heisenberg, 1993; Ito et al., 1998; Rybak and Menzel, 1998). Organized in this fashion, MB appear to be a modulatory input to a more direct reflex pathway—not unlike vertebrate structures (vestibulo-ocular system, rabbit eyeblink, amygdala-dependent fear conditioning).

Consistent with this anatomic view, either chemical ablation of MB neurons or genetic perturbation of cAMP signaling within them completely abolishes olfactory memory in flies, with no effects on the "task-relevant" sensorimotor responses (olfactory acuity and shock reactivity; de Belle and Heisenberg, 1994; Connolly et al., 1996). Strikingly, MB neurons appear to be the sole relevant site of cAMP signaling for this type of learning, because expression of a *rutabaga+* adenyl cyclase transgene in MB is sufficient to "rescue" the learning deficits of *rutabaga*^{1} mutants (Zars et al., 2000a, 2000b). Thus, to the extent that cAMP-dependent synaptic plasticity is relevant to olfactory learning, these data strongly implicate MB neurons themselves as the anatomical site of synaptic modification. This hypothesis is consistent with the finding that multiple components of the cAMP signaling cascade (AC, PDE, PKA catalytic subunit, and RI regulatory subunit) are expressed at high levels (although not exclusively) in MBs (Han et al., 1996; Dubnau and Tully, 1998; Waddell and Quinn, 2001). A more recent finding, that mutants with structural defects of the vertical (alpha) lobes of the MB are deficient in long-term memory, further implicates MB neurons and suggests the presence of functional compartmentalization within this neuroanatomic structure (Pascual and Preat, 2001).

With this as the milieu, imagine the surprise of Waddell et al. (2000) when they raised an antibody against a putative neuropeptide product of the *amnesiac* gene and found expression not in the MB but rather in a pair of large, "dorsal paired medial" (DPM) neurons elsewhere in the protocerebrum. They determined, however, that these *amnesiac*-expressing DPM cells send massive arborizations onto MB lobes, which contain the axonal outputs from MB (Fig. 5). Transgenic expression of the *amnesiac* cDNA in DPM cells throughout development is sufficient to restore normal memory to *amnesiac* mutants (DeZazzo et al., 1999; Waddell et al., 2000). It is not yet certain, however, if the *amnesiac* peptide functions acutely as a neurotransmitter or, instead, promotes developmental fidelity of the DPM-MB neuronal connections; Waddell et al. did not look for structural defects of DPM cells in *amnesiac* mutants. Importantly, DPM cells seem to participate in behavioral plasticity, because disrupting synaptic transmission in these cells using a dominant-negative temperature-sensitive dynamin transgene (Kitamoto, 2001) produces 1-h memory deficits (Waddell et al., 2001). In fact, this reversible disruption of dynamin function most likely does not inhibit directly the dense-core vescicle-mediated release of neuropeptides, such as *amnesiac*. Thus, the action in DPM cells of another, dynamin-sensitive neurotransmitter seems more likely. Nonetheless, this conceptual link between neuroanatomy and gene function suggests that *amnesiac*, or at least the cells expressing *amnesiac*, exerts a presynaptic effect on mushroom body axons.

A simple cellular model of olfactory learning has

been proposed based on these findings (Waddell and Quinn, 2001). Coincidence detection of the unconditioned stimulus (mediated by DPM cells) and conditioned stimulus (mediated by AGT neurons) occurs in mushroom body *axons*, yielding a presynaptic modification of mushroom body output (Waddell et al., 2000). In this model, phenomenologically distinct memory "phases" would result from the kinetic attributes of distinct biochemical mechanisms all acting in intrinsic MB neurons (DeZazzo and Tully, 1995; Dubnau and Tully, 1998; Waddell and Quinn, 2001). Several observatioins suggest, however, more complex model. First, *amnesiac* mutants exhibit a deficiency in MTM, but learn relatively normally (Quinn and Dudai, 1976; Tully et al., 1990; Feany and Quinn, 1995; Tully et al., 1996). This is consistent with the observation (Waddell et al., 2000) that dynamin-mediated disruption of synaptic transmission in DPM cells abolishes 60-min memory but NOT acquisition. Thus, DPM cells cannot be the solemediators of the unconditioned stimulus. More likely, *amnesiac*, and the *amnesiac*-expressing DPM cells, participate post-acquisition in later phases of memory processing—perhaps as a persistent neural activity (see below).

The finding that synaptic transmission from mushroom body neurons is not required during acquisition (Dubnau et al., 2001; Dubnau and Tully, 2001; McGuire et al., 2001) further refines this model. By using the same temperature-dependent dynamin transgene (Kitamoto, 2001) to disrupt synaptic transmission reversibly in mushroom bodies, Dubnau et al. (2001) were able to dissociate the temporal requirements for neuronal activity during acquisition, storage, and retrieval of olfactory memory. In response to these transient disruptions of MB output, acquisition and storage appear normal, but memory retrieval specifically is abolished—a result confirmed by McGuire et al. (2001). Because disrupted dynamin blocks neurotransmitter release from, but not to, MB neurons, these results argue that coincidence detection of olfactory associative learning must occur anatomically "upstream" of synaptic output from MB neurons. When combined with the finding that cAMP signaling in MB is required for acquisition (Connolly et al., 1996; Zars et al., 2000a, 2000b), these data further suggest that the biochemistry of synaptic plasticity occurs in MB neurons (Dubnau et al., 2001; Dubnau and Tully, 2001). Resulting alterations in synaptic strength then would modulate MB output in response to subsequent exposure to the CS+ (odor) alone during memory retrieval. This notion does not exclude the possibility that Hebbian processes upstream of MBs also contributes to this form of olfactory learning. The early involvement of AL, in fact, is suggested by findings from honey bee, where memory first appears to be established in AL and then is transferred to MB during the first few minutes after training (Hammer and Menzel, 1998; McBride et al., 1999).

Further anatomical complexity underlying olfactory memory is suggested from new gene discovery. A recent large-scale behavioral screen has identified 60 new mutants with defective 1-day memory after spaced training in the olfactory task (Dubnau et al., 2002). These new mutants were generated with enhancer-trap transposons that drive expression of reporter genes (green flourescent protein or beta galactosidase) in various patterns in the adult brain. A majority of these enhancer-trap mutants show reporter gene expression in well-characterized anatomies such as MB and AL. Several, however, do not. The *murashka* mutant, for example, is defective in 1-day memory after spaced training [Fig. 3(A)] and drives reporter-gene expression in several neurons that appear to send projections into mushroom body calyces and lateral horn [Fig. 3(B) and data not shown]. This observation raises the notion that "*murashka*" neurons might represent a novel "input" to MBs. This idea, of course, now is testable by using the dominant-negative dynamin transgene. Thus, additional anatomies, perhaps associated with different aspects of memory formation, may be added to the "olfactory memory circuit" as was the case for DPM cells.

Viewed from the perspective of the SPM hypothesis, these various behavioral intricacies seem confusing: Transient disruption of neuronal activity in MB neurons during training leaves acquisition intact (Dubnau et al., 2001; Dubnau and Tully, 2001; McGuire et al., 2001), suggesting that the synaptic modifications resulting from olfactory experiences occur anatomically upstream of neurotransmitter release from MB. Yet a temporally subsequent phase of memory involves dynamin-dependent neural activity in *amnesia*-expressing DPM cells, which synapse onto MBs (axons). These data are difficult to explain solely by a mechanism involving experience-driven synaptic plasticity because they invoke a requirement for neuromodulation that is anatomically downstream of acquisition and temporally subsequent to the acquisition of a new experience. One possible solution to this apparent contradiction is a recurrent activity loop (Gronenberg, 1987). MB structure, in fact, suggests the presence of intrinsic and extrinsic feedback (Strauss and Heisenberg, 1993; Ito et al., 1998; Rybak and Menzel, 1998), raising the possibility that reverberating neural activity within a circuit that includes MB is involved in memory consolidation. Perhaps therein lies a role for *amnesiac*?

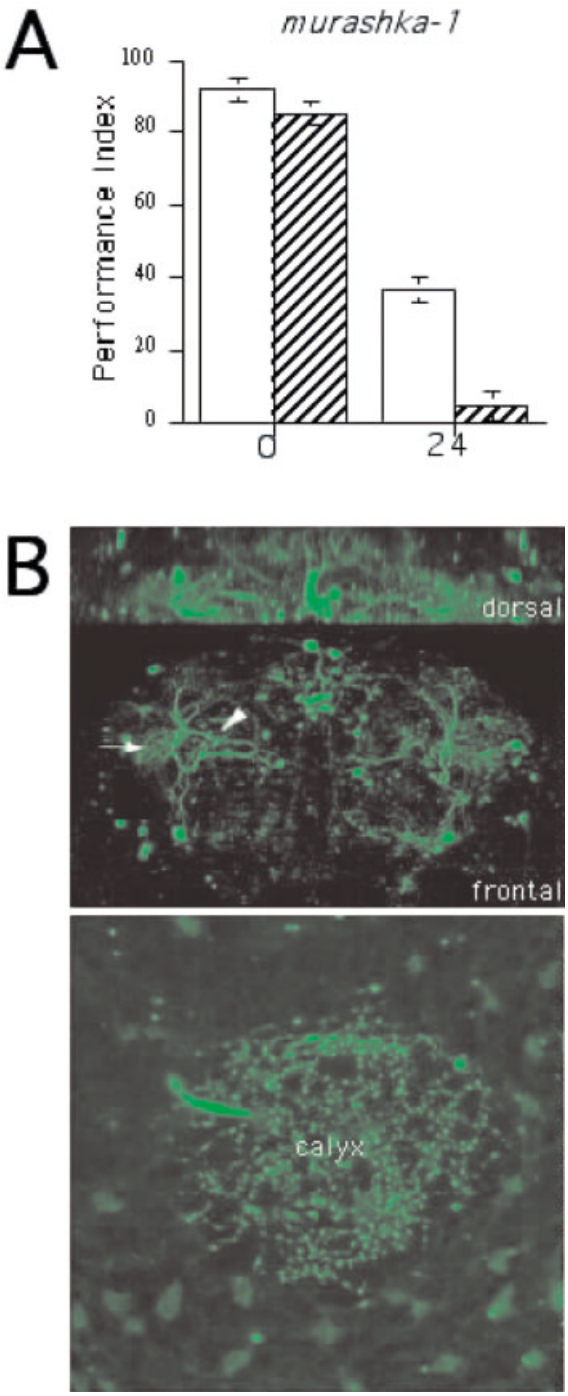

Figure 3 New anatomies for *Drosophila* memory. The *murashka-1* mutant carries an enhancer-trap transposon in a novel gene, which might function in ubiquitin-dependent proteolysis. (A) *murashka*-1 mutants display relatively normal levels of short-term memory (0) but exhibit a severe disruption in memory measured 24 h after spaced training (24). (B) Upper panel: GFP reporter-gene expression driven by the *murashka-1* Gal4 transactivator is not detected in intrinsic mushroom body neurons but rather appears in a few neurons ventral and lateral to the mushroom bodies. These neurons nevertheless send projections into mushroom body calyces (arrowhead) and the lateral horn (arrow). Lower panel: higher magnification (rear view) reveals *murashka1*-expressing neurons terminating in the calyx neurpillar region.

Some indirect support of this idea comes from measurements of endogenous Ca++ oscillations in MB neurons (Rosay et al., 2001). Although no behavioral effect of these oscillations has been established, it is intriguing to note that *amnesiac* mutants exhibit altered oscillation amplitudes. This finding implicates *amnesiac*, which is expressed in DPM cells, in the modulation of ongoing Ca++ oscillations in MBs. More direct evidence for the involvement of such an ongoing modulatory influence on memory derives from spatially restricted disruptions of dynamin-dependent synaptic transmission. When transmission is blocked in DPM cells during memory formation and NOT during memory retrieval, 3-h memory retention is partially disrupted (Fig. 4). This finding is consistent with the notion that ongoing neural activity in DPM cells is required to modulate activity in MB neurons during memory consolidation.

This behavioral view of olfactory memory challenges the notion that simple forms of memory rely solely on cellular mechanisms that alter synaptic strength in response to neural activity at a singular locus of coincidence. Such a model dictates that different memory phases must result from overlayed biochemical mechanisms with different kinetic properties all within the same neuron(s) (Schacher et al., 1990; Chain et al., 1995; Grunbaum and Muller, 1998; Muller and Carew, 1998; Muller, 1999, 2000; Sutton et al., 2001). To this end, short-lived modifications of preexisting synaptic proteins, for instance, have been proposed to underlie STM, whereas LTM has been suggested to involve reenforcement of these early modifications via gene expression-dependent growth of new synaptic connections (see Fig. 1). More integrative studies that attempt to correlate genetic, pharmacologic and anatomic disruptions with experience-dependent changes in behavior strongly suggest a more complicated model.

MEMORY: A SYNTHESIS

Memories are sequentially processed through several different phases that appear mechanistically and in some cases anatomically distinct. The sustained alterations in neural activity observed *in vivo* after behav-

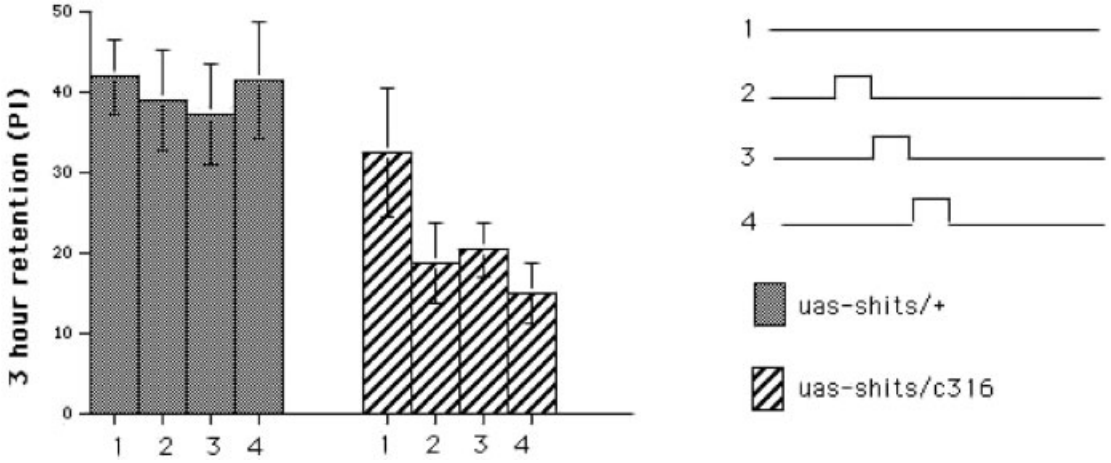

Figure 4 Disruption of neurotransmission in DPM cells after training blocks memory formation. shi^{ts1}/C316 animals, expressing a dominant-negative temperature-sensitive dynamin transgene in DPM cells (Waddell et al., 2000), were tested 3 h after Pavlovian olfactory conditioning (Tully and Quinn, 1985). shi^{ts1}/+ (control) and shi^{ts1}/C316 flies were trained and tested at the permissive temperature (20^0C). Animals either were stored at permissive temperature for the entire 3-h retention interval (1) or were transiently shifted to restrictive temperature for 30 min starting immediately (2), 30 min (3), or 60 min (4) after training. For training, groups of about 100 flies received one training session, during which they were exposed sequentially to one odor (CS+) paired with footshock and then a second odor (CS−) without foot shock. In all cases, conditioned odor avoidance was tested 3 h after training. During the test trial, flies were exposed simultaneously to the CS+ and CS− in a T-maze. After 2 min, flies were trapped in either T-maze arm, anesthetized, and counted. From this distribution, a performance index (PI) was calculated, so that a 50:50 distribution (no memory) yielded a PI of zero and a 0:100 distribution away from the CS+ yielded a PI of 100. A single PI is the average of two reciprocal experiments. In the first, octanol (OCT) is the CS+ and methyl-cyclohexanol (MCH) is the CS−; in the second, MCH is the CS+ and OCT is the CS−. Each experimental group includes an $N = 8$ PIs.

ioral experience (Villa and Fuster, 1992; Gigg et al., 1994; Laurent and Naraghi, 1994; Fuster, 1995; Wehr and Laurent, 1996; Mizunami et al., 1998; Faber et al., 1999; Aksay et al., 2001; Menzel, 2001; Lei et al., 2002; Lever et al., 2002; Perez-Orive et al., 2002) and the disruptive effects of interfering with ongoing activity (Fig. 4; Dubnau et al., 2002) are consistent with the hypothesis that memory soon after training relies on maintenance of an activity "trace"—likely in more that one anatomic region. Similarly, the observation that memories can be transferred over time between interconnected anatomical foci (Milner, 1972; Patterson et al., 1990; Gilbert et al., 1991; Squire, 1992; Hammer and Menzel, 1998; McBride et al., 1999; Scoville and Milner, 2000; Menzel, 2001) supports a systems-level interpretation of memory consolidation, wherein structural change (long-term memory) may not occur in all the anatomical foci participating in early memory. Genetic and anatomic dissection of olfactory memory in flies are consistent with this view and demonstrate, moreover, that even elemental forms of learning in a tiny insect brain involve ongoing dynamic processing of information in large networks of neurons.

We suggest an hypothesis for olfactory memory in *Drosophila* incorporating data from behavioral, cellular, and theoretical approaches developed from numerous model systems. Patterns of activity in large neural ensembles represent a given perceptual experience (context). These experience-driven activity patterns serve two related functions. First, they maintain a neural representation (working memory) of the recent perceptual experience for some short period thereafter. Second, they drive synaptic plasticity in a subset of connections within the network. In this model, the role of synaptic modification is to alter network connectivity to favor the reinstatement of a similar neural representation during subsequent exposure to a related context (memory retrieval). A second role of the initial experience-driven synaptic modifications might be to favor ongoing activity patterns that, in turn, drive further synaptic plasticity at additional (downstream) anatomical loci [Fig. 5(A)]. Unlike the transient and easily disruptable nature of

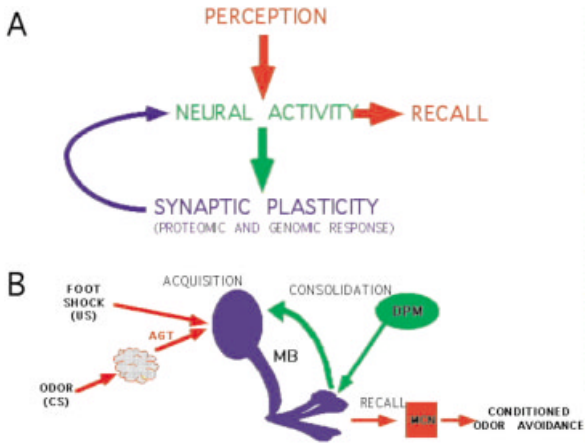

Figure 5 A systems view of olfactory memory in *Drosophila*. (A) Spatial and temporal patterns of NEURAL ACTIVITY in large networks are the neural representation of a behavioral experience (context). This neural activity serves two purposes. First is to maintain a labile memory "trace" of the PERCEPTION. Second is to drive a proteomic and genomic response resulting in SYNAPTIC PLASTICITY. In this model, the role of SYNAPTIC PLASTICITY again is twofold. First is to favor short-term persistence of the activity "trace," which drives memory consolidation into structural change. Second is to favor reinstatement of this NEURAL ACTIVITY during RECALL. (B) Olfactory information, represented by temporal and spatial patterns of neural activity in AL (Laurent and Naraghi, 1994; Wehr and Laurent, 1996; Stopfer and Laurent, 1999; Lei et al., 2002), is conveyed via AGT to MB (Strausfeld, 1976; Strausfeld and Li, 1999; Chiang et al., 2001) where it is "compared" with multimodal information (likely including footshock?) representing "context" in MB. Short-term memory of this "CS-US association" is stored in the form of short-lived biochemical modifications of synaptic strength that favor reinstatement of a neural representation of the "US context" in response to subsequent exposure to the CS. We suggest that short-term memory involves cAMP-dependent synaptic plasticity in MB (Connolly et al., 1996; Zars et al., 2000) (Zars et al., 2000) and perhaps cAMP independent mechanisms in AL (McBride et al., 1999; Muller, 1999; Muller, 2000; Menzel, 2001). Initial synaptic modifications driven by the behavioral experience are short lived, but are maintained by a reverberating pattern of activity that persists for up to several hours (Fig. 3) during which long-lived (CREB transcription-dependent) synaptic plasticity is established (CONSOLIDATION). In this model, synaptic transmission is not required from MB neurons for AQUISITION of olfactory associative learning (Dubnau et al., 2001; McGuire et al., 2001) but is required for a "readout" during RECALL and to drive CREB-dependent synaptic plasticity during CONSOLDATION.

activity patterns, synaptic modifications can be stable and lasting. Thus, new experiences are available for retrieval immediately after learning and during the minutes, hours, and days during which long-term memory is consolidated fully. The persistence of neural activity observed in many species after training may have evolved to bridge the temporal gap between short-lived (but relatively rapidly formed) biochemical modifications of synaptic efficacy and the more stable gene expression-dependent modifications that likely require persistent neuronal activity.

In the case of olfactory memory in insects, information is represented in the AL in the form of temporal and spatial activity traces generated by the perception of specific odors in antennal sensory neurons (Strausfeld, 1976; Wehr and Laurent, 1996; Vosshall et al., 1999; Kim et al., 2000; Vosshall et al., 2000; de Bruyne et al., 2001; Lei et al., 2002). Data from locust, bee, and moth indicate that olfactory experience leads to persistent, synchronous oscillatory activity both in local AL interneurons and in the projection neurons (PN) that convey olfactory inputs (via the AGT) to MB cells (Laurent and Naraghi, 1994; Wehr and Laurent, 1996; Stopfer and Laurent, 1999; Lei et al., 2002) [Fig. 5(B)]. This synchronized pattern of odor-evoked activity in PNs drives 20–30 Hz local field potentials in MBs. Consistent with these observations are the odor-induced Ca++ oscillations in *Drosophila* MB (Rosay et al., 2001; Wang et al., 2001). A recent study, moreover, provides evidence that inhibitory feedback from protocerebrum onto MB calyx serves to refine the specificity of odor representations recorded in MB neurons (Perez-Orive et al., 2002). This neural representation of olfactory experience is likely merged with multimodal information in MBs (Strausfeld, 1976; Li and Strausfeld, 1997; Rybak and Menzel, 1998). Recent behavioral studies on visual learning in *Drosophila* lend support to the idea that MB neurons are involved in behavioral discriminations based on a comparison of "present" with "past" environmental contexts (Y. Liu et al., 1999; Tang and Guo, 2001). Mizunami et al. (1998), in fact, claim that MB neurons of the roach exhibit many of the defining characteristics of hippocampal "place cells."

We hypothesize that these various experience-dependent neural activities ultimately drive lasting synaptic modifications in MB neurons (Dubnau et al., 2001; McGuire et al., 2001), which then participate in memory retrieval. During the first hours after training, ongoing synaptic activity within DPM cells (Fig. 4), MB, and perhaps as yet unidentified neurons serves two purposes. First is to maintain an endogenous memory "trace" after exogenous CS-US presentations cease. This memory trace then drives the activity-dependent biochemistries required for more lasting changes in synaptic strength and structure (Tully et

al., 1994; Yin et al., 1994, 1995; DeZazzo et al., 2000; Dubnau et al., 2002). Although this model currently is centered around MB for *Drosophila* olfactory learning, studies of the expression patterns of new "memory" genes are beginning to reveal much greater anatomical complexity (Ito et al., 1998; Waddell et al., 2000; Dubnau et al., 2002; Fig. 3). Biochemical study of these new memory genes also reveals a greater biochemical complexity (Skoulakis and Davis, 1996; Connolly and Tully, 1998; Grotewiel et al., 1998; Cheng et al., 2001; Philip et al., 2001).

Conceptually, our model of *Drosophila* olfactory memory formation is not new. Rather, it attempts to incorporate older, more systemic notions of memory formation into a model system that provides the necessary behavior–genetic tools to unravel the anatomical and biochemical complexities that translate simple synaptic plasticity into experience-dependent behavior responses. Tools for high resolution mapping of gene expression in whole brains (Chiang et al., 2001; Jefferis et al., 2002; Marin et al., 2002; Rein et al., 2002; Wong et al., 2002), *in vivo* imaging and recordings of neural activity (Laurent and Naraghi, 1994; Wehr and Laurent, 1996; Rosay et al., 2001; Wang et al., 2001; Lei et al., 2002) and genetic disruption of neuronal transmission in defined anatomical foci (Dubnau et al., 2001; Kitamoto, 2001; McGuire et al., 2001) render this more wholistic model imminently testable. As more anatomical and biochemical detail is described, computational models surely will follow, eventually yielding a sophisticated, model-driven approach to link gene function to behavior.

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