FLIES, GENES, AND LEARNING

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■ **Abstract**   Flies can learn. For the past 25 years, researchers have isolated mutants, engineered mutants with transgenes, and tested likely suspect mutants from other screens for learning ability. There have been notable surprises—conventional second messenger systems co-opted for intricate associative learning tasks, two entirely separate forms of long-term memory, a cell-adhesion molecule that is necessary for short-term memory. The most recent surprise is the mechanistic kinship revealed between learning and addictive drug response behaviors in flies. The flow of new insight is likely to quicken with the completion of the fly genome and the arrival of more selective methods of gene expression.

INTRODUCTION

When Benzer started his genetic dissection of behavior the conventional reaction was mixed, to put it politely. After a large seminar at Woods Hole an entrenched physiologist was heard to remark, "We have nothing to fear from this man." Nevertheless, other neurophysiologists, including some great ones, recognized a smart man with a genuinely new approach—one that could truly alter the field (Weiner 1999).

Thirty years later the sanguine and sourpuss viewpoints both have their merits. Forward-genetic studies of behavior—measuring a behavioral response in some genetically opportune animal, mutagenizing, selecting mutant individuals that perform the behavior aberrantly, mapping and cloning the affected genes—have provided simple, startling insights into learning and memory mechanisms. With mutants, with luck, one can leap in a single bound from a molecule to a memory process. However, the leap is perilous and insubstantial—it omits information about intermediate stages of cell biology, neurophysiology, and anatomy—and filling out the picture necessarily depends on inferences from other methods.

A principal advantage, and a concomitant disadvantage, of the forward-genetic approach is that it circumvents rational thought. Mutating, mapping, and cloning genes that affect a behavior such as learning can provide information totally foreign to current hypotheses. This can revolutionize our thinking, as the discovery

of homeobox gene complexes did to development (Gehring & Hiromi 1986). Nevertheless, the genetic approach, viewed from inside, often looks like mindless stamp collecting—a fishing expedition, in the words of study section reports.

Research with invertebrate systems, including insects, is similarly attractive and repulsive at the same glance. Invertebrates have simple genomes, simple nervous systems, and anthropomorphizable behaviors, such as recollection. They also often have neuronal circuitry that is reproducible from animal to animal, so one can compile a map of synaptic connections and a catalog of individual neuronal functions by assembling information from many animals. However, until recently, invertebrate animals were often taken as models of themselves rather than as simplified versions of us. This attitude has changed lately, with the demonstrated universality of genes and mechanisms and with the evident sequence similarity among the worm, fly, mouse, and human genomes (Rubin et al 2000).

Mutation-based studies of fruit fly learning started 30 years ago (Quinn et al 1974, Dudai et al 1976). This endeavour has been reviewed by Davis (1996) and Dubnau & Tully (1998). Comparisons with other invertebrate systems, particularly the marine snail *Aplysia californica* and honeybees, *Apis mellifera*, are covered in detail by Mayford & Kandel (1999), Menzel (1999), and Carew (2000). Here we concentrate on *Drosophila*, with particular attention to the relation between learning and memory and behavioral responses to drugs.

Learning in *Drosophila*

Fruit flies can learn a lot of things. Most impressively for their trainers, they can learn to run away from specific odors that they previously experienced with electric shock (Quinn et al 1974, Tully & Quinn 1985). In contrast, hungry flies can learn to run toward odors previously associated with sugar reward (Tempel et al 1983).

Flies can also learn visual, tactile, proprioceptive, and perhaps spatial cues (Menne & Spatz 1977, Folkers 1982, Guo et al 1996, Booker & Quinn 1981, Wustmann et al 1996, Wustmann & Heisenberg 1997). Not surprisingly, after introspection, male flies learn to attenuate their courtship behavior after experiencing rejection from females (Siegel & Hall 1979, Gailey et al 1984).

Memory after olfactory training persists for different periods depending on the specific training regime. Training to sugar reward appears to elicit longer memories than equivalent training to electric shock punishment, with surprising linear additivity of behavioral performance following the two reinforcements (Tempel et al 1983). Very strikingly, repetitive training with rest intervals interspersed (spaced training) can produce memory that lasts for several days (Tully et al 1994). This apparent requirement for spaced training parallels the protocol required for very-long-term memory formation in other species, including humans.

GETTING LEARNING MUTANTS

The genetic approach to learning is straightforward: (*a*) Mutagenize flies to produce individual mutant progeny; (*b*) breed these advantageously to generate progeny populations that are all affected at one gene; (*c*) test the mutant flies for their ability to learn or to remember; (*d*) assess them for behavioral, morphological, and developmental normalcy; (*e*) genetically map the affected gene; and (*f*) clone it if possible.

1) Mutagenesis

The methods used are critical. Chemical mutagenesis (using ethyl methane sulfonate or ethyl nitroso urea) works with very high efficiency, but changes DNA subtly, usually at a single nucleotide. Chemically induced mutants are therefore relatively easy to produce but hard to clone. In contrast, mutagenesis by mobilization of transposable elements (P-elements in *Drosophila*) can greatly facilitate cloning because the identified transposon leaves a molecular tag on the interrupted gene (Cooley et al 1988). On the downside, mutagenesis with transposons is between threefold and tenfold less efficient than mutagenesis with chemicals, depending how much faith and time one wishes to invest scoring transposition events. Furthermore, transposon hops tend to disrupt genes wholesale. This is in contrast to the single-nucleotide microsurgery that was critical to the behaviorally based isolation of the dnc^1 and rut^1 mutations as specific to learning. Benzer, Quinn, and their colleagues (Dudai et al 1976, Quinn et al 1979, Livingstone et al 1984, Folkers et al 1993) mutagenized (with chemicals), bred, and behaviorally tested about 5000 lines to obtain 4–6 good learning mutants. This effort took them about four person-years. With less efficient transposon mutagenesis such an effort would plausibly have entailed between 12 and 40 person-years—without the nucleotide microsurgery. Rapid clonability comes at a price, unless researchers use smart tricks. There are two established tricks: (*a*) selecting for genes that are preferentially expressed in anatomical learning centers and (*b*) selecting for genetic suppressors of female sterility caused by the mutant *dunce*. These are discussed later.

2) Fly Breeding Methods

Fly breeding methods can dramatically affect the scope of the screen and also the number of human lifetimes expended in getting mutants. About 20% of the fly genome is on the X-chromosome—one copy in males. Most new mutations are recessive (masked by a normal copy of the gene). Thus, finding new genes is much easier if they are X-linked. Special fly stocks (with attached X chromosomes) make such screening even easier—tenfold less labor than for other (autosomal) mutants. This buys time, but eventually the rest of the genome has to be dealt with.

3) Testing for Learning

To date, all the mutants isolated have been identified in tests for deficiencies in olfactory learning. However, flies demonstrably learn to many cues (Quinn et al 1974, Tully & Quinn 1985, Tempel et al 1983, Menne & Spatz 1977, Guo et al 1996, Booker & Quinn 1981, Wustmann et al 1996, Wustmann & Heisenberg 1997). It is a matter of time and youth until new researchers isolate new mutants using other tasks.

Also to date, a disproportionate number of the informative mutants (*dunce*, *rutabaga*, *amnesiac*) have been isolated with a relatively crude, behaviorally fragile test—that of Quinn et al (1974). Some new mutants, including one really interesting one (*volado*, see below), were isolated with a behaviorally more robust test. However, it is possible that the original screen is not as benighted as it looked 10 years ago. Seemingly less effective "sensitized" screens may be the best way to get informative mutants.

In the first behavioral screen (Quinn et al 1974, 1979; Dudai et al 1976; Livingstone et al 1984), about 4000 fly stocks carrying random, chemically induced mutations in single genes were tested for their ability to learn in the olfactory conditioning test. The first olfactory learning mutant identified in this paradigm, by Byers in Benzer's lab, was *dunce* (Dudai et al 1976). *dunce* flies learn very poorly in the olfactory paradigm, although they can sense odorants and shock and they appear normal in other behaviors. Continuing this approach, several additional mutants [*rutabaga* (Livingstone et al 1984), *turnip* (Choi et al 1991), *cabbage* (Aceves-Pina & Quinn 1979), *amnesiac* (Quinn et al 1979), and *radish* (Folkers et al 1993)] were identified—most by Sziber in Quinn's lab.

A variant olfactory test (Tully & Quinn 1985) has now been used as a primary screen (more than 2000 stocks tested for 3-h memory) to isolate 3 new mutants, *latheo* (Boynton & Tully 1992), *linotte* (Dura et al 1993), and *nalyot* (DeZazzo et al 2000) and as a secondary screen to confirm 4 candidate learning mutants, *leonardo*, (Skoulakis & Davis 1996), dPKA-RI (Goodwin et al 1997), *volado* (Grotewiel et al 1998), and *NF1* (Guo et al 2000).

4a) Measurements of Sensory Acuity

With a mutant in hand, one has to measure the ability of mutant flies to perceive the cues (conditioned stimulus) and reinforcement (unconditioned stimulus) and to carry out the behavior. For olfactory learning, this means ability to smell the odors, sense the electric shock, and run with apparent coordination in an appropriate direction.

4b) General Assays for Normalcy

In evaluating a mutant, if one is honest, one has to decide whether the fly looks and behaves normally and if not, whether the abnormality is related to the lack of

learning. Not all sick mutants are irrelevant. Severe *dunce* mutations confer partial lethality and nearly total female sterility. Nevertheless, the original behaviorally isolated *dunce* mutants (involving very partial loss of enzymatic function) live and behave normally and breed almost normally, but have learning deficiencies comparable to the severest *dunce* gene mutants. Homozygous *turnip* flies can barely get off the ground; however, heterozygous *turnip* flies live, walk, fly, and learn fine, but they forget very rapidly (Quinn et al 1979, Tully & Quinn 1985, Mihalek et al 1997). Deletion of the complete *volado* gene is lethal (compromising other, more subtle, behavioral assays). However, removal of either one of the two major splice forms of the gene transcript yields a healthy but learning-deficient fly (Grotewiel et al 1998).

5) Genetic Mapping

There is no rocket science here, but hard work sometimes. Traditional recombination mapping using a behavioral assay is arduous. It involves recombining the chromosome containing the behavioral mutation with a homologous chromosome containing several evenly spaced morphological marker mutations, then generations of back-crosses, crosses to generate populations, and behavioral assays of the populations to assess each recombinant chromosome (See Dudai et al 1976). When necessary, mutations can be more finely mapped by using the extensive collection of chromosomal deletions available in *Drosophila* (Byers et al 1981, Folkers et al 1993). This allows positional cloning of otherwise inaccessible genes (see below).

All these steps are circumvented if the mutation is transposon generated. In this case, one simply takes mutant larvae from the population, squashes their salivary glands, hybridizes cloned DNA from the transposon to the polytene chromosome squashes, and notes the location to which transposon DNA has hybridized (see Feany & Quinn 1995).

The availability of the entire fly genome sequence makes cloning and localizing P-element–generated mutations even simpler. Identified DNA sequence from that flanking the P-element can be used to search the fly genome database. The precise insertion location of the transposon is then apparent in minutes.

6) Cloning the Gene

This is the payoff. In some cases [*dunce* = cAMP phosphodiesterase (Byers et al 1981, Davis & Kiger 1981, Chen et al 1986) and rutabaga = adenylyl cyclase (Livingstone et al 1984, Levin et al 1992)] it consolidates lucky enzymatic guesses as to learning machinery into engraved metabolic truth. In other cases [*amnesiac* = PACAP-like neuropeptide (Quinn et al 1979, Feany & Quinn 1995) and *volado* = α-integrin (Grotewiel et al 1998)], the forward-genetic approach can provide genuinely new intellectual entrees into the learning process.

6a) Selective Expression of Cloned Genes

Constructing transgenic flies with an extra, cloned gene is straightforward nowadays (Spradling 1986). The question is where, when, and how to express it. The two promoters most frequently used to drive fly transgenes are the hsp70 promoter and the GAL4 upstream activating sequence (GAL4-UAS).

The heat shock–inducible hsp70 promoter allows temporal control of transgene expression, but promiscuously in all tissues. Transgenes inserted downstream from this promoter can be induced before, during, or after a learning experience to assess the role of a particular gene in learning or memory. Dominant-negative (blocking) transgenes can be used to interrupt learning at defined times (Drain et al 1991, Yin et al 1994). Alternatively, the wild-type gene can be reintroduced and acutely expressed to restore normal retention to a mutant fly stock (Grotewiel et al 1998, Guo et al 2000). Such restoration (called "transformation rescue" by fly people) can provide definitive evidence that the correct gene has been identified from the mutant, and it allows one to ask when and where that gene product might act. On the downside, Hsp70-driven transformation rescue does not always work. Failure to rescue with an acutely expressed, Hsp70-driven, transgene may occur because (*a*) misguided researchers have identified the wrong gene, (*b*) the gene is required developmentally rather than acutely at the time of learning, (*c*) promiscuous expression of the gene in all tissues destroys the anatomical specificity required for learning a specific task, or (*d*) the transgene happens not to be expressed at the critical level required for appropriate cell signaling.

The GAL4 system (Brand & Perrimon 1993), when used astutely, allows introduced genes to be expressed selectively in chosen tissues or subsets of cells, but usually without temporal control. The GAL4 system is binary, involving two separate transgene constructs in flies. The yeast transcription factor GAL4 lacks a functional homolog in flies. It can therefore be used to drive expression of other transgenes that are introduced in separate constructs downstream from a GAL4-responsive promoter, GAL4-UAS. Briefly, one transforms *Drosophila* with a desired transgene, downstream from a GAL4-UAS. Normally, this transgene will not be expressed. One then makes, or sends away for, another fly stock containing a second P-element transgene—this time consisting of the gene for the GAL4 transcription factor on an intrinsically weak promoter. In this case, experience shows, the introduced GAL4 gene will usually respond to the collection of enhancers in its neighborhood and will frequently be expressed in the same subset of cells or tissues as its nearest-neighbor gene. There are several ways, using stainable reporter genes, to identify the set of tissues expressing GAL4 protein in a particular fly stock.

Next one simply crosses the two fly stocks containing the two separate transgene constructs—one with the regionally expressed GAL4 transcription factor, the other with the GAL4-UAS promoter–driven, learning-related gene of choice. Double-transgenic progeny from this cross will express the learning-related protein with the regional specificity of the GAL4 line used. This technique is universally important

in *Drosophila* because a large number of region-restricted GAL4 driver lines have been generated and catalogued that express GAL4 in different subregions of the adult fly brain (Armstrong et al 1995, Yang et al 1995). Consequently, given a cloned gene that might be related to learning (be it wild type, hyperactive, or engineered for dominant-negative blocking), one can, by simple crosses, arrange to express the gene in a number of brain structures of interest and generally to see what genes influence what memories in what tissues.

Tissue-specific expression of transgenes can also be used to rescue learning and memory of mutant flies. With this method one can identify those brain structures where expression is sufficient for learning (Zars et al 2000, Waddell et al 2000). In one defining instance, appropriate tissue-specific expression of a wild-type transgene has rescued learning and memory performance in a mutant when acute and developmentally sustained expression of that gene with the hsp70 promoter has worked poorly (DeZazzo et al 1999, Waddell et al 2000).

6b) Analysis of Cloned Gene Product(s)

This is often conceptually mundane, but it can yield momentous results. For example, Davis and colleagues (Davis & Kiger 1981, Chen et al 1986, Levin et al 1992, Nighorn et al 1991, Han et al 1992) cloned or helped clone the *dunce* and *rutabaga* genes, found that both genes were highly expressed in anatomical structures (the mushroom bodies) that were known to be involved in olfactory learning, and thereafter undertook a successful search for other genes that were expressed with similar regional specificity (Skoulakis & Davis 1996, Grotewiel et al 1998), with important findings.

INFORMATION WE HAVE GAINED FROM THE MUTANTS

Chemical Mutagenesis

A fragile, seemingly primitive olfactory learning paradigm was used, along with chemical mutagenisis and brute labor, to isolate the mutants *dunce*, *rutabaga*, *amnesiac*, *radish*, and *turnip*.

dunce   The first *dunce* mutant (Dudai et al 1976) was isolated by its deficient olfactory learning in the assay of Quinn et al (1974). Other mutations in this gene came from a screen for female sterility mutants (Mohler 1977). The *dnc* locus is very complex, stretched over at least 148 kilobases (Davis & Davidson 1984, 1986; Chen et al 1986, 1987; Qui et al 1991). The *dnc* gene encodes at least 10 alternative RNA splice-forms and presumably many variant DNC proteins.

rutabaga   The original *rut* mutation (Livingstone et al 1984) was isolated, like *dunce*, because of its learning deficiency in the assay of Quinn et al (1974). Other

P-element-induced alleles of *rut* were isolated because they suppressed the female sterility of *dnc* mutants (Bellen et al 1987, Levin et al 1992).

Molecular cloning of both the *dnc* and *rut* genes was aided, first by the directed identification in these mutant stocks of biochemical defects (Byers et al 1981, Davis & Kiger 1981, Livingstone et al 1984). The correspondence of metabolic function of the *dnc* and *rut* gene products was astonishing. The two enzymes lay in the same biochemical pathway, a pathway used throughout the animal kingdom for cellular responses to outside messengers. The *rut* gene encodes a Ca^{2+}/Calmodulin-stimulated (type I) adenylate cyclase, AC (Levin et al 1992); the *dnc* gene encodes cAMP phosphodiesterase (Chen et al 1986, Qiu et al 1991). Therefore, RUT makes cAMP and DNC degrades it.

amnesiac The first *amnesiac* mutation was identified in a deliberate screen for mutants that affected memory (Quinn et al 1979). However, the *amnesiac* gene was cloned by a trick from a P-element-induced allele (Feany & Quinn 1995) and has been repeatedly cloned since (e.g. Moore et al 1998, Toba et al 1999). The *amnesiac* gene encodes a protein that has sequence features of a pre-pro-neuropeptide and that has limited homology to the mammalian neuropeptide/hormone pituitary adenylyl cyclase–activating peptide (PACAP) (Vaudry et al 2000). This finding makes sense because neuropeptides often act in parallel with conventional monoamine neurotransmitters to provide reinforcement. The homology to PACAP, together with supporting genetic and biochemical evidence, indicates that the AMN peptide stimulates cAMP synthesis. The piece of evidence that led to a clonable *amn* allele (Feany & Quinn 1995) is this: Severe alleles of the learning mutant *dnc* are learning defective and female sterile. A P-element insertion in the *amn* gene rescues the female fertility phenotype of mutant *dnc* females. Therefore, mutations in the *amn* gene act to counter the effect of *dunce* mutations (i.e. too much cAMP). Furthermore, *amn* mutants are hypersensitive to ethanol (Moore et al 1998) as well as being forgetful (discussed below). Feeding *amn* flies forskolin (an activator of adenylate cyclases) or increasing PKA activity reverts ethanol sensitivity to wild-type levels (Moore et al 1998).

No one knows whether the AMN peptide is a true homolog of mammalian PACAP. Nevertheless, artificial application of mammalian PACAP38 induces changes in synaptic signaling at the fly larval neuromuscular junction (NMJ) (Zhong & Pena 1995). These PACAP-induced changes in flies are mediated by the cAMP cascade—as with PACAP in mammals and, evidently, the AMN peptide(s) in flies (Feany & Quinn 1995, Moore et al 1998). The PACAP38 peptide, applied to the fly NMJ, elicits a slow inward current lasting tens of seconds, followed by an enhanced outward K^+ current. These responses to PACAP are absent in *rut* mutants (Zhong 1995), indicating that RUT adenylyl cyclase activation, and hence cAMP signaling, is required for PACAP action. This NMJ response actually requires the simultaneous activation of both the Ras and cAMP pathways; activation of either pathway alone is insufficient for synaptic change (Zhong 1995). Analysis of the PACAP response in flies mutant for the Ras GTPase-activating protein, NF1,

which negatively regulates ras in mammals, indicates that the signal tranduction events downstream from PACAP are complex. The PACAP response is abolished in *NF1* mutants, but it can be restored by pharmacological stimulation of the cAMP cascade (Guo et al 1997).

radish, Anesthesia-Resistant Memory, and Long-Term Memory   Long-term memory has been historically defined as (*a*) memory that persists for a long time; (*b*) memory that is dependent on spaced training; (*c*) memory that is resistant to disruption by anesthesia, electroconvulsive shock, cooling, concussion, or other agents that interfere with patterned neural activity; (*d*) memory that is dependent on new protein synthesis; and (*e*) memory that requires the cAMP response-element binding protein (CREB) transcription factor (Yin et al 1994, Tully et al 1994). In brief, *Drosophila* mutants seem to further resolve the previous definitions of long-term memory into two separate parts.

The *radish* mutation selectively eliminates anesthesia-resistant memory (ARM) (Folkers et al 1993), leaving protein synthesis–dependent memory intact (Tully et al 1994). ARM occurs after ordinary training and lasts at least 3 days (Tully et al 1994). Therefore, ARM is a legitimate form of long-term memory.

Transgenic flies with an inhibitory form of a fly CREB transcription factor, dCREBb, have normal ARM (see above). However, they are completely devoid of protein synthesis–dependent long-term memory (Yin et al 1994, Tully et al 1994). This form of long-term memory lasts for at least 7 days and requires spaced training, protein synthesis, and the transcription factor CREB. We call it long lasting long-term memory (LLTM) to distinguish it from ARM. Additionally, startling results have been reported (Yin et al 1995a) that indicate that transgenic flies with a superabundance of active CREB have "flashbulb memory"—memory that persists for days after a (normally ephemeral) single training trial. These experiments have not been pursued as expected.

The *radish* mutant provides a unique handle on ARM (Folkers et al 1993). Twenty-four hours after spaced training, memory is composed of two experimentally separable portions: Half the 24-hour memory can be abolished by inhibiting protein synthesis, and the other half can be abolished by the *rsh* mutation (Tully et al 1994, Yin et al 1994). Flies that are fed the protein synthesis inhibitor cyclo-heximide, or flies that express an inhibitory CREB transgene, display only half the normal 24-hour memory. Flies with both the drug inhibitor and the transgene still have half this memory, indicating that blocking CREB transcription and blocking most of protein synthesis affect the same process to the same extent. Mutant *rsh* flies also show only half-normal 24-hour memory. However, introducing the inhibitory CREB transgene into the *rsh* mutant eliminates all 24-hour memory, indicating that *rsh* and CREB affect entirely separate memory processes. There are two interpretations of these experiments: (*a*) Protein synthesis–dependent memory is exactly equivalent to CREB-dependent memory. The CREB transcription factor lies at or near the top of the gene regulation cascade that leads to LLTM and is a bottleneck for that cascade; all transcription-dependent memory goes through it. (*b*) A second, completely separable, form of long-term memory requires a normal

rsh gene (Folkers et al 1993). Mutant *rsh* flies are entirely lacking in consolidated ARM (Folkers et al 1993, Tully et al 1994).

The *rsh* mutation has been localized to a 180-kb region of the X-chromosome by genetic mapping. DNA spanning the interval has been cloned and sequenced in its entirety by the genome project. Several interesting candidate genes within the interval are currently being tested for a *rsh*-specific mutation.

turnip   This odd mutant implicates the protein kinase C (PKC) pathway (Choi et al 1991). Homozygous *tur* mutant flies are sluggish and have a reduced response to electric shock (Mihalek et al 1997). However, heterozygous *tur/+* flies are healthy and behaviorally responsive. They learn normally, but memory decays rapidly (Quinn et al 1979, Tully & Quinn 1985, Choi et al 1991, Mihalek et al 1997). The *tur* mutation comaps, in a dose-dependent manner, with low PKC activity. However, the mutation does not lie in or near any of the identified fly genes encoding PKC family members (Choi et al 1991).

P-Element Screening

Tully and colleagues have performed a P-element-based behavioral screen for learning and memory mutants. The genes affected in P-element-induced mutations are readily clonable. They have reported three new mutants, *latheo*, *linotte*, and *nalyot* (Boynton & Tully 1992, Dura et al 1993, DeZazzo et al 2000).

latheo   The *latheo* gene encodes a component of the origin recognition complex (Pinto et al 1999). Complete loss-of-function *lat* mutations are lethal. Partial loss-of-function mutants learn poorly but lack mushroom bodies, a finding that bodes ill for learning specificity of the gene. Intriguingly, however, LAT protein is detectable in presynaptic boutons, and the NMJ of *lat* mutants has abnormal synaptic properties (Rohrbough et al 1999). Therefore, it is conceivable that *lat* encodes a multifunctional protein involved in both DNA replication and synaptic plasticity. This is an example of the unique strength of a forward-genetic approach that assumes nothing besides the fact that single gene mutations can impact learning performance. It has provided completely unexpected novel information.

linotte   The identity of the gene affected in the *linotte* mutant (Dura et al 1993) is disputed. The *lio* gene either encodes a novel protein (Bolwig et al 1995) or it is an allele of the *derailed* receptor tyrosine kinase (Dura et al 1995). Regardless, *lio* mutants have structural brain defects that extend to the mushroom bodies and central complex (Moreau-Fauvarque et al 1998, Simon et al 1998). Whether these defects are responsible for the retarded learning performance, however, is undetermined.

nalyot   *nalyot* is an allele of the myb-related Adf1 transcription factor (DeZazzo et al 2000). *Adf*1 is an essential gene, but partial loss-of-function mutants are

viable. Such a partial mutation, nal^{P1}, has a mild effect on learning and a pronounced effect on long-term memory. The nal^{P1} mutation also causes a modest reduction in the number of synaptic boutons at the larval NMJ. On the contrary, increased *nal* expression appears to cause a modest increase in the number of boutons. The authors propose (*a*) that the Adf1 transcription factor is directly involved in regulating the structural aspect of synaptic plasticity in concert with a *Drosophila* CREB that regulates functional plasticity (see below) and (*b*) that the lack of structural plasticity measured in the mutant NMJ may underlie the long-term memory deficit observed in *nal* mutant flies after spaced training. However, the experiments published do not directly address these interpretations. Although they restored initial learning of nal^{P1} flies to wild-type levels with *nal* transgenes, they did not report rescue of the longer-term (1 or 7 day) memory defects. Therefore, conclusive evidence that the long-term memory defect depends directly on the NAL gene product remains to be provided. By the authors' own assessment, "the level of performance at earlier memory phases is not a reliable predictor of performance at later memory stages"(p155).

Accurate *nal* expression is critical. Ectopic expression of *nal* under the control of several neural-specific or glial-specific GAL4 promoters is lethal. Furthermore, ubiquitous expression of NAL, driven at high levels by the heat-shock promoter, is actually deleterious to olfactory memory.

Using Neuroanatomy to Screen for Learning Genes

The products of the *dunce* and *rutabaga* genes are expressed at high levels in the fly mushroom bodies (MBs) (Nighorn et al 1991, Han et al 1992), structures that are central to olfactory learning (discussed below). In addition, the PKA catalytic subunit encoded by the *DC0* gene is also more abundant in MBs (Skoulakis et al 1993). However, reduced *dnc* expression in the MBs does not necessarily correlate with learning deficiency (Qiu & Davis 1993). Furthermore, MB-enhanced expression has been argued to be due to the unusually high cell density and parallel organization of the MB (Ito et al 1998). Notably though, not all genes appear to be preferentially expressed in MBs, and Davis and coworkers have identified the new learning mutants *leonardo* (Skoulakis & Davis 1996) and *volado* (Grotewiel 1998) using P-element-based enhancer trapping to visualize enriched expression in the MBs.

volado Studies with this mutant implicate alteration of cell adhesion in the process of short-term memory (Grotewiel et al 1998). Although a mechanistic understanding is currently lacking, we believe that studies of this gene product will eventually provide key insight into the molecular events of synaptic remodeling that are believed to underlie learning and memory.

The *vol* gene was isolated because it is preferentially expressed in MBs. The gene encodes two splice variants of an α-integrin. Integrins are cell-surface

receptors that mediate cell adhesion and signal transduction (Hynes 1992). Mutant *vol* flies are markedly reduced in short-term olfactory memory (Grotewiel et al 1998). However, memory in *vol* mutants can be fully rescued by heat-shock induction of the short *vol* cDNA transcript in adult flies, just prior to olfactory training. Strikingly, the ability to rescue memory decays with the same kinetics as *vol* RNA expression, a result strongly suggesting that the VOL integrin is acutely needed for memory.

Rohrbough et al (2000) have reported a synaptic role for VOL, and other *Drosophila* integrins also influence synaptic morphology and function (Beumer et al 1999). It remains to be determined whether the memory deficit of *vol* mutants is due to chronic alteration in synaptic structure (caused by changess in cell adhesion) that prevents modulation, or whether it is acute VOL signaling that is critical for memory formation. It is also not known if cAMP signaling regulates VOL-mediated cell adhesion.

leonardo   The *leonardo* gene was isolated, like *volado*, because it is preferentially expressed in MBs (Skoulakis & Davis 1996). *leo* mutant flies are defective in olfactory learning and short-term memory. The *leo* mutation affects the zeta isoform of the mundanely named protein 14-3-3.

Proteins of the 14-3-3 family are involved in several intracellular signaling pathways. They can activate and repress protein-kinase-C (PKC) activity (Aitken et al 1995, Xiao et al 1995), activate tyrosine hydroxylase and tryptophan hydroxylase, the rate-limiting enzymes in catecholamine and serotonin biosynthesis (Ichimura et al 1995), and interact with several signal-transduction cascades, including RAF-1 in the mitogen-activated protein kinase (MAPK) pathway (Fantl et al 1994, Freed et al 1994, Irie et al 1994, Li et al 1995).

The LEO protein is enriched in presynaptic termini, and *leo* mutants have reduced synaptic transmission at the larval NMJ, especially under stress conditions (Broadie et al 1997). The LEO gene product has been proposed as a candidate to mediate voltage-dependent Ca^{2+} influx and presynaptic vesicle exocytosis.

LEO does in fact demonstrably interact with the presynaptically located *Drosophila* calcium-dependent potassium (K_{Ca}) channel Slowpoke (dSlo) via the slowpoke-binding-protein Slob (Zhou et al 1999). Through this interaction LEO regulates the voltage sensitivity of the dSlo channel (DiChiara & Reinhart 1995, Cui et al 1997). Whether LEO also exerts an effect on synaptic efficacy via activation of PKA and/or the RAS/RAF mitogen-activated protein kinase cascade is unknown.

Testing Available Mutants for Learning Deficiency

A shortcut approach to studying learning is to behaviorally assess ready-made mutant stocks, in the hope that they have defects in learning or memory that are relatively specific. Sometimes there are quick payoffs.

Ddc   *Ddc* mutations lie in the structural gene for aromatic-amino-acid-decarboxylase, a necessary enzyme on the pathways to the major monoamine neurotransmitters—serotonin, dopamine, and octopamine in invertebrates. Constitutive *Ddc* mutations are lethal, but temperature-sensitive mutant stocks can be acutely blocked in adulthood and are viable. Livingstone & Tempel (1983) and Tempel et al (1984) temperature-shifted such mutants and tested their biochemistry and learning behavior. *Ddc* mutant flies have lower serotonin and dopamine levels after a few days at the restrictive temperature. In these flies learning was reduced in accordance with the reduction in DDC enzyme activity (Tempel et al 1984). Both our lab and others have had difficulty in repeating this work—in getting temperature-shifted flies that were healthy and learning-deficient at the same time.

NF1   The inferred role of NF1 (a GTPase-activating protein for *ras*), discussed above with *amnesiac*, prompted direct learning measurements of *Drosophila NF1* mutant stocks. The mutant flies are in fact defective in olfactory learning (Guo et al 2000). This defect can be rescued in adult flies by heat-shock induction of either an *NF1* transgene or a PKA transgene. Therefore, the cell-signaling pathway (identified in studies of the larval neuromuscular junction) leading from PACAP-binding to synaptic enhancement also appears to mediate olfactory learning in the adult. In humans the *NF1* gene is linked to the human disease neurofibromatosis (Shen et al 1996). Some neurofibromatosis patients have learning disabilities; however, it is not known whether the learning impairment is due to an acute role for NF1 in synaptic signaling or to brain-developmental consequences of the mutation. Results from flies provide intriguing information bearing on this issue. *NF1* flies are learning defective and small in size (Guo et al 2000, The et al 1997). Their small size can be rescued by induction either of an NF1 or a PKA transgene during development but not in adulthood (The et al 1997). In contrast, the learning deficiency of "small" *NF1* mutant flies can be rescued by induction either of an NF1 or a PKA transgene in adults (Guo et al 2000)

Genetically Engineered Alterations of Learning and Memory

Generation of transgenic flies is straightforward and quick. Genes can be made transiently inducible by cloning them downstream from the heat-shock-inducible hsp70 promoter. Obtaining direct gene knockouts in flies is now possible (Rong & Golic 2000) although it is methodologically tricky at present, but making and introducing dominant-negative gene products is easy. This approach has been useful in studies of learning and memory.

cAMP-Dependent Protein Kinase   Knowing that levels of cAMP were central to learning, Drain et al (1991) directed their attention to the obvious downstream target of the cAMP signal, cAMP-dependent protein kinase (PKA). Induction of inhibitory fragments of PKA before training blocked olfactory learning. This was the first demonstration of transgenic alteration of learning. The role of PKA in

learning was later confirmed by direct studies with mutants (Skoulakis et al 1993, Li et al 1996, Goodwin et al 1997).

dCREB2 Similar experiments with the *Drosophila* gene for the cAMP-response-element-binding protein (CREB) (Yin et al 1995b) were more consequential. They showed that CREB-dependent gene expression is required for long-lasting long-term memory (LLTM) after associative learning (discussed above in connection with *radish*). Expression of an inhibitory (blocking) CREB gene abbreviates such memory (Yin et al 1994). This is consistent with work in other species and suggests that CREB-dependent transcription is critical for long-term memory formation. Expression of an activating CREB has been reported to induce hypertrophied long-term memory after one short training session (Yin et al 1995a). This result is potentially revolutionary to memory studies However, it has not been followed up in the manner one would have expected.

Analysis of the larval neuromuscular junction (NMJ) in learning mutants and in flies carrying the inhibitory and activated CREB transgenes has indicated that the cAMP cascade and CREB are directly involved in synaptic plasticity. Increased neuronal activity (elevated via *ether-a-go-go* and *Shaker* mutants) and increased cAMP concentration (elevated by the *dnc* mutant) both induce exuberant presynaptic growth and increased synaptic transmission (measured physiologically at the NMJ). The structural synaptic growth is accompanied by a reduction in levels of the neural cell adhesion molecule (N-CAM) homolog *FasII* (Schuster et al 1996). Indeed, a concomitant reduction in *FasII*-mediated cell adhesion is critical to allow synaptic growth. Mutant larvae with low levels of *FasII* show exuberant synaptic arborization at the NMJ. In contrast, larvae with higher levels of *FasII* show reduced arborization. Structure does not necessarily reflect function. In *FasII* larvae the average synaptic output is reduced, suggesting the normal aliquot of synaptic release machinery is shared among an increased number of synapses.

NMJs of *dnc* mutant larvae are different. They have increased arborization and an increased average output per bouton (Budnik et al 1990, Zhong et al 1992). These results suggest that cAMP levels affect both structural and functional synaptic change, whereas *FasII* affects only structure.

cAMP alters functional change, among other ways, via the CREB transcription factor. Expression of the inhibitory CREB transgene in *dnc* mutant larvae blocks functional but not structural plasticity (Davis et al 1996). In contrast, an activated CREB transgene increases presynaptic transmitter release. These results suggest that cAMP regulates functional synaptic plasticity via CREB and also structural plasticity via a *FasII*-dependent pathway.

Adenylate Cyclase-Stimulatory G Protein Unregulated Gs signaling apparently blocks learning. Disruption of Gs_α adenylate cyclase-stimulatory G protein mediated signaling in the MBs (done with GAL4 drivers), but not in the central complex, absolutely abolished olfactory learning (Connolly et al 1996). These experiments confirmed the idea that signaling through the cAMP second-messenger

system—in the MBs—is essential for olfactory learning. These results are surprising in that the extent of the learning defect (they do not learn at all) greatly exceeds that of flies that either have grossly disorganized or reduced MBs (Heisenberg et al 1985) or that lack MBs altogether as a result of chemical treatment that ablates them (de Belle & Heisenberg 1994).

Ca^{2+}/Calmodulin-Dependent Protein Kinase   Calcium is an important factor in neuronal signaling. A major intracellular respondent of Ca^{2+} is type II Ca^{2+}/calmodulin-dependent protein kinase (CAMKII). A potential role for this enzyme in associative and nonassociative behavioral plasticity was assessed by expressing an inhibitory transgene (Griffith et al 1993, Jin et al 1998) under the control of the heat-shock promoter in a similar manner to that employed for PKA by Drain et al (1991). Induction of a CAMKII inhibitory transgene inhibits associative learning measured in the courtship conditioning paradigm (Griffith et al 1993). The performance of these flies in olfactory associative conditioning has not been reported.

The *Drosophila* CAMKII gene gives rise to multiply spliced mRNAs. Eight different isoforms have been identified that differ at the junction of the regulatory and association domains of the kinase (Griffith & Greenspan 1993). This alternative splicing produces CAMKII enzymes with altered substrate specificity and differing sensitivity to Ca^{2+}/calmodulin binding (GuptaRoy et al 2000). *Drosophila* CAMKII has many reported targets, most interestingly for the purposes of this review, the eag K^+ channel subunit, the *leonardo*-associated protein Slob (Zhou et al 1999), the Adf1 transcription factor encoded by the *nalyot* gene (GuptaRoy et al 2000), and Discs large protein (a PDZ family protein), which regulates the clustering of synaptic molecules (Koh et al 1999).

ANATOMY OF *DROSOPHILA* LEARNING

Studies in many insects have indicated the importance of mushroom bodies (MBs) in learning (Strausfeld et al 1998, Zars 2000). Insightful analysis of the MBs in *Drosophila* has confirmed a role for these structures in olfactory learning and memory. Mutant flies that were identified based on their defective MB anatomy do not learn olfactory tasks (Heisenberg et al 1985). Similarly, chemical ablation of MBs abolishes olfactory learning (de Belle & Heisenberg 1994).

The MBs are only two synapses away from olfactory reception. Information from olfactory receptors on the antennae and maxillary palps travels via the antennal lobes to the MB calyces. These calyces contain dendrites of the intrinsic MB neurons, the Kenyon cells. Axons from these Kenyon cells project from the calyx down the stalk-like pedunculus. Toward the front of the central brain the pedunculus splits into five lobes (α, α', β, β', and γ) and the spur. The lobes are assumed to be the synaptic output region of the MB, although input also comes into the lobes.

The function of the MBs is not exclusively olfactory (Heisenberg 1998, Strausfeld et al 1998, Zars 2000). Although the *Drosophila* MBs are dispensable for several types of learning, including visual, tactile, and motor (Wolf et al 1998), they are believed to receive multimodal sensory information. In fact, visual deprivation reduces MB calycal volume (Barth & Heisenberg 1997). This structural plasticity is absent in the learning mutants dnc^1 and amn^1 (see below) and is therefore believed to reflect functional adaptation and long-term memory. Whether MBs are required for consolidated memory of visual stimuli or any other nonolfactory task has not been tested.

A stunning study (Liu et al 1999) has demonstrated that the MBs are required for context generalization in visual learning, a basic cognitive process. Flies can learn to associate visual patterns with the presence or absence of heat punishment (Guo et al 1996). Following learning, they fly toward the pattern predicted to avoid the heat. Wild-type flies can associate visual patterns with heat and are unaffected by changes in the illumination conditions between training and testing trials—for example, a change from monochromatic color to white light, or from intermittent to steady light (Liu et al 1999). In contrast, flies that lack MBs—as a result of chemical ablation—are unable to learn and remember the visual task if illumination conditions are changed between training and testing. Nevertheless, these MB-less flies are able to learn if the light conditions are kept constant. These results suggest the MBs are essential for the fly to be able to extract relevant information from multiply variable visual stimuli.

How the MBs actually function remains essentially mysterious (Heisenberg 1998). Their intricate anatomical organization, with lobes projecting in three orthogonal directions in the fly brain, is conceptually intriguing (Strausfeld 1976, Strausfeld et al 1998, Crittenden et al 1998). The only simple MB feature that we can see is a functional parallel with another system that is critical to learning, the hippocampal–entorhinal cortex system in mammals. Both systems show elegantly regular, only slightly scrutable anatomical organization and appear suited to deal with complex, multimodal assemblies of information.

It is now clear that even among the intrinsic cells of the MB, the Kenyon cells, there is great diversity. Multiple subpopulations of Kenyon cells with different projection patterns are distinguishable by different gene expression (Yang et al 1995, Ito et al 1997). Localization of learning-related gene products has indicated the MBs as a critical site of cAMP cascade action in olfactory learning (see Figure 1). The products of the *dnc*, *rut*, and *DC0* genes are all preferentially expressed in the MB Kenyon cells (Nighorn et al 1991, Han et al 1992, Skoulakis et al 1993). In fact, expression of *rut* in the MBs (using the GAL4 system) is sufficient for olfactory learning (Zars et al 2000).

We, working with others, have also used the GAL4 method to confirm the importance in memory of two large Dorsal Paired Medial (DPM) cells that express the *amn* gene product (Waddell et al 2000). Restoration of *amn* gene expression to these cells reestablishes normal olfactory memory. AMN neuropeptide is provided to the MB lobes by these large DPM neurons.
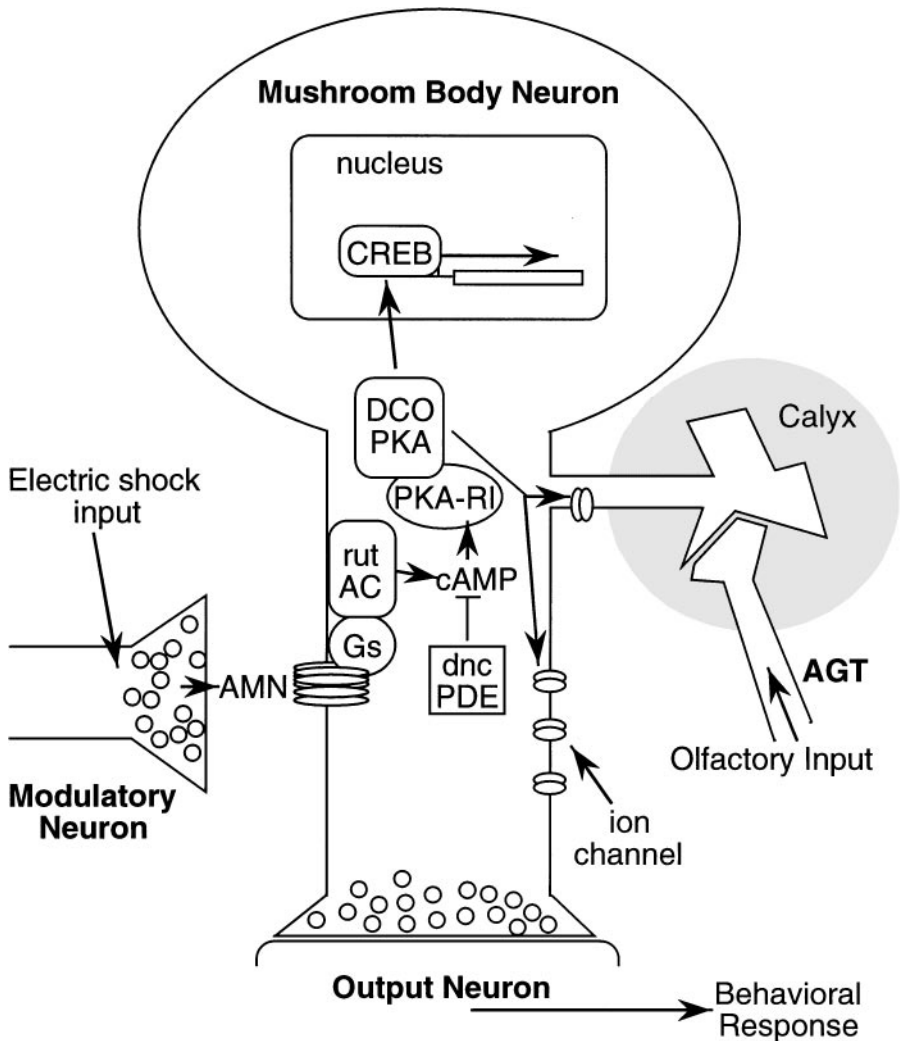
Figure 1 A model for olfactory learning in *Drosophila*. A mushroom body (MB) neuron receives convergent sensory input from olfactory presentation [via the antennoglomerular tract (AGT) interneurons that synapse in the MB calyx] and electric shock (via modulatory neurons that release the AMN neuropeptide, perhaps with a monoamine). Coincident activity of these two input paths triggers a synergistic stimulation of the RUT adenylate cyclase and subsequently, elevation of cAMP levels. Depending on the training paradigm, the cAMP elevation results either in a short-lived modification of MB neuron excitability (short-term memory) or a long-lasting functional and structural change (long-term memory). The duration of protein kinase (PKA) activation is a critical factor (Muller 2000). Persistent PKA activation supports long-term memory in part through activated cAMP response-element binding protein (CREB)-dependent transcription.

A caveat of studies that use the GAL4 method is that regional redundancy of expression cannot be easily discounted. Few GAL4 lines express only in the desirable brain region. It is conceivable that expression in the noncommon tissue or the background expression is sufficient for the rescue. Using several different GAL4 driver-lines that have overlapping expression patterns can reduce this problem.

In a screen for learning mutants it is reasonable that one will obtain flies that are grossly affected in structural brain anatomy and circuitry, as well as more subtle mutations that leave the brain largely intact. This has been the case. Both *latheo* and *linotte* have notable brain defects in the mushroom bodies (Moreau-Fauvarque et al 1998, Simon et al 1998, Pinto et al 1999).

FLIES ON DRUGS

Genes that affect learning are also involved in the response of flies and mammals to drugs of human abuse (Berke & Hyman 2000). The first findings on this in flies came from work with the inebriometer (Cohan & Hoffman 1986, Weber 1988, Moore et al 1998). In this device flies on precarious, slanted perches inside a glass cylinder are exposed to ethanol vapor in an air current. As the alcohol they breathe increases, they (understandably) turn about, stagger, fall off their perches, and finally tumble down through the inebriometer tube into a fraction collector. Wild-type flies "elute" from the column with a peak at about 20 minutes. Various sensitive or resistant mutants elute at different peak times. This is drug behavior reduced to the methods of conventional chemistry.

The first ethanol-hypersensitive mutant characterized, *cheapdate*, turned out to be a P-element allele of the memory mutant *amnesiac*. Following up on this finding, Moore et al (1998) found that other learning mutations in the cAMP pathway— *rutabaga* and *DC0*—also altered the flies' susceptibility to ethanol. Apparently, the signaling pathway first identified in memory formation in flies influences their ability to handle ethanol.

Thus the same second-messenger pathway is implicated in fly learning and fly ethanol behavior. Moreover, a handful of neurotransmitters known to stimulate this pathway—the AMN neuropeptide and a number of monamime transmitters—also appear as central in genetic studies of both behaviors.

Flies, acutely exposed to ethanol, stagger and fall over, as we do. Chronically exposed flies become less sensitive to the sedative effects of the drug—also like us (Scholz et al 2000). This functional drug tolerance is plausibly believed to reflect adaptive neuronal changes resembling learning (Cunningham et al 1983, Fadda & Rossetti 1998). Flies that have reduced octopamine levels (because of a mutation in the tyramine β-hydroxylase enzyme that synthesizes it) are impaired in their ability to develop tolerance to ethanol (Scholz et al 2000). Octopamine is plausibly involved in fly learning (Dudai et al 1987), and in the honeybee it substitutes for positive reinforcement (sugar reward) in classical conditioning of the proboscis extension response (Hammer & Menzel 1998).

Cocaine is another drug of human abuse. Volatilized cocaine, administered to flies, induces several odd behaviors (McClung & Hirsh 1998, Bainton et al 2000). The easiest such behavior to measure is this: Following mechanical agitation that shakes flies to the bottom of a tube, normal flies race to the top of the tube—negative geotaxis. Cocaine decreases this upward mobility in a quantifiable, dose-dependent manner (Bainton et al 2000).

Flies develop a behavioral sensitization to cocaine (McClung & Hirsh 1998, 1999). An enhanced behavioral response is observed following subsequent cocaine exposure even after a single dose. Sensitization is thought to be a contributing factor to addiction. As with ethanol-induced behaviors, the usual suspect molecular components shown to affect learning influence cocaine-induced behaviors: monoamines, G-protein-coupled receptor signaling, and cAMP cascade regulation.

The monoamine tyramine appears to be required for this sensitization (McClung & Hirsh 1999). *inactive* mutant flies have low tyramine levels and do not show behavioral sensitization, although their initial response to cocaine is similar to wild-type flies. Tyramine may compete with octopamine for receptor binding or it may potentiate the effects of the other monoamines, dopamine and serotonin, by inhibiting their uptake. In most cases, changes in cAMP synthesis will be central to the downstream effects. In fact, type II cAMP-dependent protein kinase regulatory subunit mutant flies have decreased sensitivity to cocaine (and ethanol) and fail to sensitize to repeated cocaine exposure (Park et al 2000).

Dopamine modulates the response of *Drosophila* to cocaine, ethanol, and nicotine (Bainton et al 2000, Li et al 2000). Therefore, although the cellular targets of these drugs are likely different, they all engage the fly dopaminergic system, and hence the cAMP cascade, perhaps in a reward-based manner.

FUTURE PROSPECTS

Lucky for us fly teachers: New molecular genetic tools arrive quickly at our door, often from fly researchers who have no interest in flies' learning. A tool that looks to be required for continued rapid progress, and one that seems imminent, is a spatially selective method of regulating transgene expression with the addition of temporal control.

The GAL4 method (Brand & Perrimon 1993) for tissue-selective transgene expression brought new meaning to our little fly lives. Nevertheless—especially for learning researchers—the method is retarded by a lack of ability to turn the critical genes on and off at appropriate times.

It is quite likely that a given GAL4 driver, with a desired expression in the adult brain, expresses in other, often unrelated tissue during development. Such idiosyncratic expression of a transgene might well be developmentally lethal or affect the behavior of the mature fly. In a time-dependent field like learning that deals with acquisition and recollection, storage and retrieval, long-term and short-term, time control is essential to dissecting processes. Fly learning researchers

face a quandary. However, a solution beckons. It should be possible to temporally block expression from GAL4 lines by combining the GAL4/UAS system with the GAL80 protein that represses the GAL4 transcription factor (Lee & Luo 1999). If a temperature-sensitive GAL80 can be developed it should be possible to keep the developmentally expressed GAL4 (and any transgene under GAL4/UAS control) inactive until adulthood simply by manipulating the temperature. In this manner we would be able to add temporal control to the entire complement of brain region–specific GAL4 lines that are already available.

Genetic dissection of neuroanatomy with the GAL4 system (e.g. Armstrong et al 1995, Yang et al 1995, Ito et al 1998) is helping us map the neural networks in the fly brain and is concurrently providing us with tools to explore gene function in distinct neurons. Understanding the functionality of the circuits is critical if we hope to understand and model their properties. Despite plentiful analyses of the larval NMJ and dissociated neuronal preparations (Wu et al 1998, Lee & O'Dowd 2000), current technology is limiting physiological analyses of the intact adult *Drosophila* brain. However, circuit physiology is currently being productively studied in the MBs and connected neuropil of several larger insects—primarily cockroaches (Mizunami et al 1998), locusts (Stopfer & Laurent 1999), and honeybees (Faber et al 1999). Honeybees actually have smaller neurons on average than do flies. It is likely that the methodology can be adapted to flies, given a scientist with sufficient courage and dexterity. The combination of genetics and functional recording would change the field.

Tully and colleagues are using current DNA chip technology to search for learning-related genes. Finding the memory-relevant CREB target genes is of great interest. The most exciting use of such genes is the possibility of histological reporter systems for learning-related gene expression, or stainable antibodies for proteins that are selectively upregulated or modified following learning. Such a "tag" would allow one to identify the neurons that are modified in a functional circuit underlying a particular learning behavior. This would promote *Drosophila* from one of the anatomically least tractable animals, with respect to functional anatomy, to one of the most informative.

Interesting mutants come from all directions: anatomy screens, neurochemistry screens, and fertility screens. Most recently, scientists have been isolating new fly mutants that show an altered response to drugs (Moore et al 1998, Singh & Heberlein 2000). In view of the demonstrated commonality of the genes involved in the drug response and learning, some new molecular players will be relevant to the learning process. The supply of candidate mutants should increase dramatically.

The availability of the entire fly genome sequence has already quickened research. More interestingly, the Berkeley *Drosophila* Genome Project has generated and catalogued a large number of P-element insertion lines (Spradling et al 1999). If you are a lucky researcher, both a DNA sequence and a mutant fly stock for your favorite gene are already available by mail. This availability should stir the hearts of fly investigators to test their favorite mutants for learning defects, because the approach is very easy and potentially very interesting.

Nevertheless, in the end there will be no substitute for the head-butting forward-genetic approach. As ever, this amounts to mutagenizing flies, selecting mutants that have aberrant behavior, and cloning the affected genes. At heart this amounts to walking the beaches and peeking into bottles, with the hope that nature might have left a message inside. Some such messages have enlightened us about pattern formation in development. With luck, they will enlighten us about our capacity to remember.

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CONTENTS