

## **Minireview**

# **Mushroom Body Subdomains in *Drosophila*: Innate or Learned Representations of Odor Preference and Sexual Orientation?**

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Behavioral plasticity is not an exclusive characteristic of higher organisms. Worms, mollusks, insects and other invertebrates demonstrate an astonishing degree of flexibility in their daily activities. To the neurobiologist, one distinct advantage of studying these creatures is that they have relatively “simple” nervous systems which are capable of “higher” functions commonly attributed to vertebrates. For example, the fruit fly *Drosophila* can learn through a variety of sensory modalities (Heisenberg, 1989; Tully, 1991) and has a consolidated memory persisting for more than a week ( $>1/4$  of its life span) (Tully et al., 1994).

In insect brains, the mushroom bodies (MBs) are structures which have drawn considerable attention for their participation in memory formation and other complex functions (Erber et al., 1987; Heisenberg, 1994; Menzel et al., 1994). Within the bewildering networks of the protocerebrum, this symmetrically paired neuropil is highly ordered and invitingly simple. Consisting primarily of parallel Kenyon cell arrays, MBs receive a variety of sensory inputs and forward outputs to many areas of the brain. Their size, unusual shapes and connectivity are remarkably variable throughout the insect subclass. The social hymenoptera represent the zenith of MB evolution, endorsing the notion that these structures serve as centers of higher function (Menzel et al., 1994; Strausfeld et al., 1995). In this minireview I will concentrate on MB structure and function in *Drosophila melanogaster*.

### **Associative Odor Learning in *Drosophila***

Although the coincidence detection of associative learning probably occurs in single cells (Hawkins et al., 1993), animals likely require the computational power of neuronal assemblies for the temporal integration of complex environmental stimuli (Dudai et al., 1987). In *Drosophila*, evidence for MB participation in odor learning is convincing, even though flies are not especially well endowed [ $\approx 2500$  Kenyon cells/MB compared with  $\approx 170,000$  in the honeybee (Heisenberg, 1994; Menzel et al., 1994)]. Primary input is delivered to the MB calyx from the antennal lobe via side-branching fibers of the antennal-glomerular tract (Heisenberg, 1980, 1994). Chemical ablation of the MBs abolishes conditioned odor avoidance while leaving olfactory acuity, visual learning, and other aspects of behavior intact (de Belle and Heisenberg, 1994; Wittig et al., 1995). Genetic variants with MB structural defects have similar odor-specific learning deficits (Heisenberg, 1989; de Belle and Heisenberg, 1995). These results suggest that MBs function as signal convergence detectors in associative odor (but not visual) learning in flies. Furthermore, optic lobe input to the MBs has not been identified (Heisenberg, 1994) [unlike in the honeybee, for instance (Menzel et al., 1994)] indicating that visual information is likely processed elsewhere in the brain. At the cellular level, gene products of *dunce* (*dnc*), *rutabaga* (*rut*) and the catalytic subunit (*DCO*) of protein kinase A all participate in the cAMP cascade (Dudai, 1988), affect olfactory conditioning (Tully, 1991; Davis, 1993) and are preferentially expressed in the MBs (Davis, 1993). In the rat, expression of *dnc* homologues in brain structures known to function in

memory consolidation (Engels et al., 1995) emphasizes the importance of *Drosophila* as a model organism.

### ***Mushroom Body Plasticity in Structure and Function***

Neuronal plasticity during development and in memory consolidation are activity-dependent processes which rely on shared cellular mechanisms (Bailey and Kandel, 1993). In flies, MB development and structural plasticity during adult life are strongly influenced by genotype, environment and experience (Heisenberg, 1989, 1994; Heisenberg et al., 1995). The lack of MB plasticity in *dne* and *rut* (Heisenberg, 1989) further implies a connection between experience and MB-mediated memory formation.

Our current knowledge of cellular mechanisms is unlikely to explain MB-specific plasticity and other potential emergent properties of neuronal assemblies (Hawkins et al., 1993). Are MB Kenyon cells functionally equivalent isomorphic arrays? Until recently, evidence to the contrary has not been very helpful in piecing together the MB puzzle. Kenyon cells in *Drosophila* arise from four neuroblasts which divide continuously from late embryonic development through to eclosion (Ito and Hotta, 1992). If nothing else, these cells are at least different ages. “Earlier” cells may serve in a pathfinding role for “later” ones. During metamorphosis, most (but not all) Kenyon cells regenerate their projections, possibly to facilitate the exchange of larval circuitry with synaptic connections meaningful to the requirements of adults (Heisenberg, 1989, 1994). If Kenyon cells are not functionally equivalent, do they acquire distinct attributes through experience (“learning”) or are differences genetically “pre-programmed”? MBs are sexually dimorphic in wild type flies (Heisenberg et al., 1995), which may have something to do with pre-programming of courtship-related odor preferences (Hall, 1994). A more extreme sexual dimorphism is seen in *mushroom-body-miniature* (*mbm*) mutants (Heisenberg, 1989; de Belle and Heisenberg, 1995). Regional differences in fiber diameter and density (Heisenberg, 1980) and the curious staining pattern of an antibody (fb45) that identifies four continuous parallel fiber bundles (Bicker et al., 1993) are other observed but unexplained MB internal heterogeneities.

### ***The P[GAL4] Enhancer-Trap System***

Two papers appearing in a recent issue of *Neuron* provide an exciting breakthrough in our understanding of MB organization and function in *Drosophila*. The force behind both studies is the P[GAL4] enhancer-trap system, a powerful genetic tool for identifying development-, tissue- and cell-specific patterns of gene expression (Brand and Perrimon, 1993). This versatile system warrants description here. A transposable element engineered with the yeast GAL4 transcription factor gene is pseudo-randomly inserted into the genome. In a few (of “many”) flies, endogenous genetic elements will “enhance” GAL4 expression. A second transposable element containing a GAL4-specific UAS<sub>G</sub> promoter-driven *lacZ* “reporter” gene is inserted into a separate strain of flies. The specificity of a particular GAL4 enhancer can be

visualized as *lacZ* ( $\beta$ -galactosidase) expression patterns in the progeny of crosses between P[GAL4] and P[UAS<sub>G</sub>-*lacZ*] flies. Similarly, the specificity of a particular GAL4 enhancer can be harnessed to target expression of any desired cloned gene fused with the UAS<sub>G</sub> promoter.

### ***Mushroom Body Subdivision***

Yang et al. (1995) have used the P[GAL4] enhancer-trap system to reveal a previously unknown structural complexity within the MBs. GAL4 lines identify specific subsets of Kenyon cells reflecting differential patterns of gene expression. Thus, MB intrinsic elements are likely not functionally equivalent isomorphic arrays. In general, the MBs seem to be arranged in concentric rings of longitudinal subdivisions. Several GAL4 lines are reminiscent of previously observed MB internal structure (Heisenberg, 1980; Bicker et al., 1993). What is the significance of these patterns?

If Kenyon cells are genetically pre-programmed, patterns of GAL4 expression might represent temporally invariant parallel channels of information flow (Yang et al., 1995). Odor meaning could be inherited rather than shaped by experience (Heisenberg, 1989). For example, larvae and adults live in distinct olfactory worlds and have opposing responses to some odors (Heisenberg, 1989). Perhaps this would be mirrored in the MBs as different GAL4 expression patterns. It is also tempting to speculate that separate phases of memory consolidation described in flies (Tully et al., 1994) are spatially represented in the MBs as partitioned cellular processes. For instance, *rut* is probably involved in short-term memory but not acquisition (Tully et al., 1994). This may reflect the observation that *rut* adenylate cyclase is more prominently expressed in the MB peduncle and lobes (Kenyon cell axons) than in the dendrites of the calyces (Davis, 1993; Heisenberg, 1994), where olfactory and other signals probably converge (Heisenberg, 1980, 1989; Yang, et al. 1995). It would be interesting to look for variation in GAL4 expression patterns in the backgrounds of mutant genes known to affect different phases of memory consolidation [e.g., *latheo* (*lat*), *linotte* (*lio*), *dnc*, *rut*, *amnesiac* (*amn*), cAMP-responsive element-binding protein (*CREB*) and *radish* (*rsh*) (Tully et al., 1994)]. GAL4 enhancers are themselves candidate participants in memory formation. Discrete MB subdivisions could also correspond with input from a variety of sensory modalities [as in the honeybee (Menzel et al., 1994)] or may be involved in a wider range of higher functions in addition to odor learning, such as courtship (Erber et al., 1987; Hall, 1994; Ferveur et al., 1995).

Alternatively, if Kenyon cells acquire different meaning through experience (Heisenberg, 1989), we might expect to see variability in GAL4 expression patterns throughout the course of development. One of the eight lines described by Yang et al. (1995) fits this description. It would be especially interesting to grow flies in contrasting environments to explicitly test this “experience” hypothesis (e.g., Heisenberg et al., 1995). As

suggested by Yang et al. (1995), Kenyon cells might be isomorphic within functionally distinct MB subdivisions, thus fulfilling any or all of the above predictions.

### ***Mushroom Bodies and Courtship***

MB participation in courtship behavior has been suggested for various insect species including *Drosophila* (Erber et al., 1987; Hall, 1994; Ferveur et al., 1995). In an elegant study, O'Dell et al. (1995) have taken advantage of the specificity of enhancer elements identified by Yang et al. (1995) to further investigate the MB role in male courtship. Instead of activating the *lacZ* reporter construct, GAL4 was used to express the sex-determining gene *transformer* (*tra*). Defined subsets of Kenyon cells were “feminized” in the male progeny of crosses between P[GAL4] and P[UAS<sub>G</sub>-*tra*] flies. Expression of *tra* in some MB domains resulted in “bisexual” courtship by these partially feminized males. This suggests that mate discrimination is dependent on a subset of MB intrinsic elements and highlights the notion that Kenyon cells are not functionally isomorphic arrays. Are courtship preferences based on odor representations in the MBs?

Both male and female flies rely on olfactory cues for heterosexual courtship (Hall, 1994) and a variety of olfactory pathway components have been implicated as centers for mate discrimination (Ferveur et al., 1995; O'Dell et al., 1995). Nondiscriminatory courtship by certain partially feminized males implies the existence of gender-specific mate discrimination centers in the brain (O'Dell et al., 1995). If we suppose a MB focus for male detection of male anti-aphrodisiac pheromonal cues (Hall, 1994), feminization would lead to a loss of function. However, *mbm tra2* “masculinized” females ( $\approx$ MB-less pseudo-males) display male courtship toward female targets (Heisenberg, 1994). It is therefore more likely that the MBs are a focus for female detection of male aphrodisiac pheromonal cues (Hall, 1994), with feminization leading to a gain of function. This idea receives confirmation by the finding that MB structural mutant females are unreceptive to male courtship (Heisenberg, 1994). We might also expect MB ablation (de Belle and Heisenberg, 1994) to revert the sexual preference of partially feminized bisexual males toward females.

### ***Future Prospects***

Making sense out of nervous system organization in terms of function is a daunting task. Even though MBs are possibly the least complicated structures in the comparatively simple brains of insects, “*we have only just begun to understand ‘MB structure and function’ and a lot more work needs to be done*”. These are exasperatingly familiar truisms. However, recent interesting results in *Drosophila* neurogenetics and neuroethology indicate that we are approaching (or have just arrived in) an important period of synthesis.

Genetic dissection of memory consolidation and (Heisenberg, 1989; Tully, 1991; Tully et al., 1994), courtship (Hall, 1994) and other brain functions in *Drosophila* is becoming increasingly more profitable. Armed with exciting new techniques we can now examine the

structures generating behavior in exquisite detail. The GAL4 enhancer-trap system provides a means to create transgenic flies in which foreign genes can be induced according to endogenous development-, tissue- and cell-specific patterns of gene expression (Brand and Perrimon, 1993). Applying this system to the MBs of flies has exposed previously unknown genetically-specified substructure (Yang et al., 1995). A first step toward interpreting meaning from this novel diversity revealed functional differences in mate preferences during courtship (O'Dell et al., 1995).

“Next step” options now seem endless. Behavioral defects in some MB structural mutants (Heisenberg, 1989; de Belle and Heisenberg, 1995) may be related to loss or altered identity of specific Kenyon cell subsets. In combination with these mutants, GAL4 expression patterns make ideal internal markers and have the potential for vastly improving structure–function mapping resolution within the MBs. In addition, P[GAL4] inserts provide convenient molecular access to genetic elements possibly involved in MB structural plasticity during development and/or memory formation (Yang et al., 1995). By far the most exciting and promising application of GAL4 technology to “MB questions” involves expressing UAS<sub>G</sub>–toxin gene constructs to kill or “switch off” specific subsets of Kenyon cells. This approach has been successfully used in other parts of the fly nervous system (e.g., Sweeney et al., 1995). Tetanus seems to be the toxin of choice, since it abolishes synaptic transmission in a reversible fashion, but is not lethal to the cell. The behavioral effects of toxin expression in various Kenyon cell domains will most certainly provide fascinating insights into MB function. This direction of research receives my vote for “The Most Important Contribution to the Decade of the Brain Award” in *Drosophila*.

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