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Working Memory: It's a Gas, Gas, Gas

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The ellipsoid body, a doughnut-shaped part of the fly brain, is essential for visual working memory. Gaseous second messengers establish a functional ellipsoid body and act as a short-term aid in orientation behavior.

With all of our sensory and cognitive faculties, we can easily orient based on landmarks and move toward, for example, a nearby shelter. What is remarkable, however, is that with intermittent visible landmarks, say those that come from a transiently lit sky from lightning on a stormy night, a seconds-long working memory allows one to still orient and move toward that target. Such bits of visual feedback are sufficient to update a course based on self-derived orientation information. We can do this, sure, but so can many insects. In this issue of *Current Biology,* Roland Strauss and colleagues [1] report the mechanism underlying visual working memory in the fly *Drosophila melanogaster*. It turns out gas-based signaling events are critical in establishing this memory.

Flies can be tricked into revealing a visual working memory. If presented with two landmarks that cannot be reached in an otherwise empty landscape, a fly will walk back and forth between the landmarks for hours [2] (Figure 1A). In the simplest case, flies with their wings clipped are on an open platform (a diameter of about 10 cm) surrounded by a water moat to prevent them from escaping. The environment is well lit and two large vertical black bars provide the targets. Deviation from the straight path between the landmarks is minimal. In the 'disappearing landmark paradigm' [3], where one can measure the visual working memory, a new target is flashed on the wall of the arena, and a fly will orient toward that temporary target (Figure 1B). The trick is that all of the landmarks then disappear. No orientation cue! Being in a uniformly lit environment is as good as being in the dark. As long as a fly was distracted for less than four seconds, it will re-orient toward the original landmark and walk toward that now absent target (Figure 1C). How well flies re-orient after all of the landmarks disappear is a measure of visual working memory.

Clues about how a fly brain can support this type of memory comes from anatomical and physiological studies. The central complex of the fly brain has several readily recognizable structures. Important for visual working memory is the ellipsoid body, a doughnut-shaped structure that straddles the midline [4,5]. The ellipsoid body has four different types of 'ring neurons' (R1–4) that innervate 360 degrees of the structure in a tiled fashion. Other, large field neurons connect the ellipsoid body to the remaining structures in the central complex (i.e., fan-shaped body, protocerebral bridge, and noduli). Like a genetic spike through the head, mutations in genes, such as *ellipsoid body open* (*ebo*), that grossly alter the structure of the ellipsoid body provided the first evidence for a function of the ellipsoid body in a visual working memory [6]. Moreover, imaging of Ca^{2+} activity of large-field neurons that innervate the ellipsoid body showed that Ca^{2+} increases in a wedge of the ring correspond to the direction of walking in tethered flies [7]. Importantly, genetic analysis suggested that the function of the ring neurons depends on a competence factor for the ellipsoid body to support visual working memory [6]. That is, multiple redundant sets of ring neurons were sufficient for *ebo*-dependent action. These results suggest that a factor from the sets of ring neurons make the ellipsoid body functional. But how is this competence established?

With the idea that ellipsoid body competence might depend on a short-lived and readily diffusible factor, Strauss and colleagues [1] predicted that gaseous second messengers might be critical. Two gases were examined. The first is nitric oxide (NO), which has been

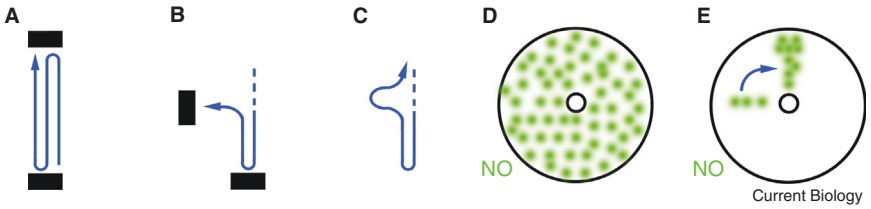

Figure 1. A visual distractor reveals mechanisms of visual working memory.
(A) An individual fly will walk back and forth between two landmarks (dark boxes). A walking trace is represented as the blue line. (B) A distracting landmark on the left attracts a fly, but when that and the original landmarks are removed (C) a fly will re-orient toward the now-absent original target. (D) The ellipsoid body is a doughnut shaped structure in the central brain. The model proposed by Strauss and colleagues is that the release of the gaseous second messenger NO promotes the competence of the ellipsoid body, which in turn is critical for establishing a working memory. (E) A second role for NO is to provide a short-term cue in a sector of the ellipsoid body that can be used by a fly to aid in re-orienting toward a previous target.

previously shown to act as a diffusible second messenger and has a role in behavioral plasticity [8]. The second is hydrogen sulfide (H_2S), which has been detected in vertebrate brains and can affect synaptic plasticity when exogenously applied [9].

Genetic and pharmacological intervention suggest that NO functions in visual working memory [1]. The enzyme responsible for making NO, nitric oxide synthase (NOS), is found in many places of the brain, including the R3 of ring neurons in the ellipsoid body and the fan-shaped body. Mutation of the NOS gene, or reduced expression of NOS in R3 neurons, led to flies that had little or no visual working memory. Interestingly, and getting back to the starting idea that the *ebo* gene plays a critical role in establishing the function of the ellipsoid body, over-expression of NOS in R3 (and R2) neurons improved the visual working memory deficit in *ebo*-mutants.

H_2S also aids in forming a visual working memory [1]. Cystathione beta-synthase (CBS) appears to be the rate limiting enzyme in generating H_2S in flies [10]. Mutation of the *CBS* gene leads to strongly reduced visual working memory, while transgenic expression of the *CBS* gene in R2 or R3 neurons rescued the *CBS* and *ebo* mutant phenotypes. Moreover, feeding flies N-acetyl-L-cysteine as a supplement in the food, and presumably increasing H_2S levels, also improved the *ebo*-mutant phenotype.

How could two gaseous second messengers both impinge on *ebo*-dependent visual working memory? In NO and H_2S signaling, regulation of cGMP and cGMP-dependent protein kinase (PKG) seem to be important [1,11]. Tests of genetic interactions in flies mutant for the *ebo* gene and key components of cGMP/PKG function show that PKG signaling is a common downstream pathway for visual working memory. Moreover, manipulation of the transcription activator CREB in multiple ways implicates CREB-dependent regulation of gene expression in establishing a normal ellipsoid body and normal behavioral function [1,11].

The best explanation for how the multiple different genes outlined above can influence a four-seconds-long memory is that they help to establish an ellipsoid body that is capable of the plastic changes needed for this behavior. This brings us back to the idea of a competence factor that is needed to establish a functional ellipsoid body. Strauss and colleagues concentrated [1] on the *homer* gene, which is downstream of CREB control, preferentially expressed in dendrites, and critical for synaptic plasticity [1]. Tests of *homer* function point to a critical role of *homer* in visual orientation memory. Thus, the *homer* gene product, thought to function as part of a scaffold for proteins at the synapse, seems to be acting as a critical factor in establishing a competent ellipsoid body.

Finally, a gaseous second messenger could also act in a short-term fashion for the seconds-long memory [1]. Flies were fed a NOS inhibitor, and a few minutes later visual working memory was altered. Moreover, manipulation of ion channels that depend on cGMP, as well as NOS itself, led to flies with an altered visual working memory. This suggests that local NO-dependent signaling might be used in the ellipsoid body to help flies re-orient toward the initial target in the vanishing landmark paradigm.

These results point toward a two-part model for the function of the gaseous second messengers in visual working memory [1]. The first is to establish a competent ellipsoid body, one that is capable of updating connections to influence moment-to-moment decisions on orientation and taxis (Figure 1D). The second, which has less experimental support, but is nevertheless attractive, is that transient elevation of NO or H_2S in parts of the ellipsoid body is used to aid in re-orientation behaviors (Figure 1E). Being able to image either gas, or the effects of a gas, in behaving flies would obviously help test the latter model.

In a broader context, a general feature of learning and memory genes is emerging, namely that they can act at different time scales depending on the behavioral task. For example, genes that act in olfactory memory, which can last for hours, have a role in place memory, which lasts tens of minutes, and in visual working memory in the range of seconds [1,11–14]. Regardless of the open questions — such as the range of functions for the gaseous second messengers in learning and memory across paradigms, time scales, and developmental stages — the discovery that the NO and H_2S-based gaseous second messengers influence visual working memory will change our understanding of working memory mechanisms and the organizational features of brain structures.

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Regeneration: Organizing the Blastema in Planarians

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The processes that trigger regeneration after injury and link new and old tissue are not fully understood. New findings indicate that, after decapitation, planarians build an organizing center from stem cells at the old midline that directs head patterning and outgrowth.

An essential difference between embryogenesis and adult regeneration is their initiation. Whereas development begins with fertilization and, in most cases, uses maternal or yolk-derived cues for symmetry breaking, adult regeneration begins with injury and must be capable of acting upon a potentially wide variety of altered tissue compositions to restore form. Therefore, mechanisms to enable the robust recovery of tissue pattern are likely fundamental to regenerative abilities.

Planarians have emerged as a powerful model to uncover mechanisms of regenerative pattern control because of the ease of whole-animal adult RNAi and whole-mount histology, the extreme regenerative abilities of these animals, and the ability to recover viable mutants showing excess, duplicated or absent tissue regionalization [1]. Planarian head regeneration begins with early injury-induced expression of the secreted Wnt inhibitor *notum* at anterior-facing wound sites [2] in pre-existing muscle cells [3]. Subsequently, transcription factors *foxD* and *zic1/zicA* are expressed within a subpopulation of neoblast stem cells to specify the differentiation of a focus of cells at the midline of the extreme anterior, termed the anterior pole [4–6]. The anterior pole expresses secreted molecules that modulate Wnt and activin signaling (*notum* [2] and *follistatin* [7,8], respectively) and are required for head patterning and outgrowth, suggesting this region likely constitutes a crucial signaling center in regeneration. Inhibition of canonical Wnt signaling could be the primary molecular function of the anterior pole because the phenotype resulting from *zic-1* RNAi — the failure to regenerate a head and the absence of anterior *notum* expression — could be rescued by simultaneous inhibition of *beta-catenin-1* [4]. However, it has remained unclear how the anterior pole forms spatially in the blastema and whether it truly represents an organizing center akin to its embryonic equivalents with sufficient and instructive abilities. Now, in a paper published in this issue of *Current Biology*, Oderberg *et al.* [9] present an extensive analysis of anterior pole formation and function using transplantation, surgery, and a new method for mounting and imaging planarian blastemas *en face*.

First, these authors used transplantation experiments to directly demonstrate the existence of an organizer activity present within the anterior pole. Juxtaposing tissue from disparate regions is known to cause outgrowths and duplicated tissue in planarians (reviewed in [10]) through a process that has been termed ‘positional conflict’ [3] or ‘intercalary regeneration’ [11]. However, Oderberg *et al.* [9] showed that transplantations of small tissue fragments containing anterior pole tissue, and not neighboring lateral anterior tissue, could trigger the outgrowth of well-patterned and symmetrical head tissue that contained eyes. Furthermore,