Reticular neurons were arrayed within a rhombomeric scaffold of eight segments in both species as illustrated in Figure 1A, C. Reticular neurons projected predominantly to the spinal cord (Fig. 1A, C), but a few neurons in each segment were labeled by midbrain dye applications (Fig. 1D). Notable among the ascending neurons were the group of "T reticular interneurons" (4), including a pair of "giant fiber" neurons (9), located in rhs 7 and 8 (Fig. 1B). Most of the ascending reticular neurons probably target midbrain premotor nuclei, rather than oculomotor nuclei, because intracellular dye injections of axons projecting to the oculomotor nuclei labeled very few reticular neurons (8).

The vestibular neurons were also largely segregated with respect to spinal and oculomotor targets (compare Fig. 1C with 1B, D). In agreement with the present results, intracellular analysis of vestibular neurons demonstrates that only those from the tangential complex in rhs 4–5 project in common to both oculomotor and spinal targets (8). The segmental relationships of the vestibular neurons were not as clearly definable as those of the reticular neurons (Fig. 1C, D). For example, the ipsilateral projections in the ascending pathways spanned rhs 1–3, and the neurons with contralateral axons were located in rhs 1–2 and 4–6 (Fig. 1B, D). Likewise, the descending vestibular projection originated across rhs 3–6 (Fig. 1A, B).

In summary, comparison of ascending and descending hindbrain projections in larval and juvenile fish leads to two conclusions. First, the premotor reticular and vestibular neurons responsible for postural control of the body and eyes are highly segregated with respect to both segmental location and targets. Second, only a very small subset of vestibular neurons, and possibly no reticular neurons, innervate both oculomotor and spinal motor nuclei. These observations also suggest that the segmented adult neuronal phenotype results directly from the retention of an embryonic rhombomeric blueprint.

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The Temporal Transfer Function of the *Limulus* Lateral Eye

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Extensive studies of the lateral eye of the horseshoe crab, *Limulus polyphemus*, have revealed important facets of visual physiology (1). Among these are the excitatory and inhibitory neural mechanisms that shape retinal responses to both spatial and temporal changes in light intensity.

Two studies of the temporal response properties of the eye (2, 3) show that the retina *in situ* is more sensitive to light flickering at higher temporal frequencies than previously reported for excised eyes (4), but the studies differ in their frequency of peak sensitivity. In brief, the temporal transfer function (TTF) measured by Brodie *et al.* (Ref. 2, solid line Fig. 1A) exhibits peak sensitivity in the range of 5–6 Hz with a gain of 10, whereas that measured by Batra and Barlow (Ref. 3, dashed line in Fig. 1A) peaks in the range of 3–4 Hz with a gain of only 5. Both studies measured TTFs of single ommatidia with light modulated by a sum-of-sinusoids technique (5). The former study, however, illuminated the entire retina, whereas the latter study limited the stimulus to a single ommatidium. Thus, the difference between the TTFs in Figure 1A (solid *vs.* dashed curves) may result from lateral inhibitory influences.

In our initial studies of the temporal properties of the eye, we found that the average TTF measured without background illumination (n = 5, Florida and Woods Hole horseshoe crabs) matched that measured by Batra and Barlow (3). In contrast, we found that the average TTF with background (n = 3, Florida horseshoe crabs) matched that measured by Brodie *et al.* (2). These results suggest that constant inhibition from surrounding receptors changed the temporal response properties of the recorded ommatidium. Note that all of these experiments were performed with different animals. If the change in TTFs in Figure 1A results from lateral inhibition, then we should be able to shift from one TTF to the other by turning the background illumination on and off in the same animal.

To carry out this experiment, we cut a 20-cm hole in the carapace anterior to one lateral eye and slipped a chamber with a built-in microsuction electrode around the optic nerve trunk. We teased away a nerve fiber of an ommatidium located at the center of the eye and pulled it into the microsuction electrode to record its response (6). We submerged the animal, which was stabilized on a platform, in a seawater tank containing a transparent glass window. We then aligned the optic axis of the recorded ommatidium with the center of a display monitor (Model 608 Tektronics) located on the other side of the glass window at a distance of 4.5 cm from the lateral eye containing the recorded ommatidium. We measured the TTF of the single ommatidium using a small spot (6° visual angle) modulated by a sum of sinusoids (Ref 5, Model 1010 Venus Visual Stimulator, Neuroscientific Corp.), with and without background illumination.

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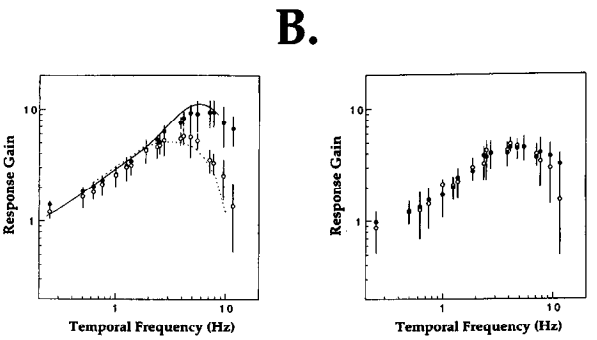

Figure 1. *(A) Temporal transfer functions measured by Batra and Barlow (dashed line), Brodie et al. (solid line), and in our preliminary experiments with (filled circles) and without (unfilled circles) background illumination. (B) TTF from three single ommatidia in as many eyes. Filled circles denote responses recorded with constant background illumination and unfilled circles those recorded without background. Error bars denote standard deviation.*

Figure 1B shows the average TTF measured with (filled circles) and without (unfilled circles) background illumination from three ommatidia in as many eyes. Note that background illumination did not change the shape of the TTFs, indicating that constant lateral inhibitory inputs do not influence the temporal response properties of an ommatidia. However, past studies show that modulated lateral inhibitory inputs generated by flickering backgrounds can indeed change temporal response properties (6). The close correspondence of the TTFs measured with and without background in Fig. 1B strongly suggests that the different shaped TTFs in Fig. 1A do not result from the effects of lateral inhibition. The cause of the differences is not known but may result from differences among horseshoe crabs. We are also exploring the possibility that the ambient temperature of a crab's seawater environment before surgery may influence temporal response properties of its eyes.

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Lead Affects Learning by *Hermissenda crassicornis*

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Research on lead has a long scientific history, the metal having first been identified as a neurotoxin over 200 years ago. In humans, average blood levels of lead have dropped since 1978, from over 20 to less than 3 μg/dl (30 ppb) (1). Because lead has multiple sites of action, the mechanisms underlying its toxicity are complex, and its effects on human health and development are multifarious, ranging from alterations in intracellular metabolic pathways and heme synthesis to lowered intelligence scores to increased aggressive behavior (2).

Blood levels of lead above 10 μg/dl cause demonstrable intelligence or behavioral deficits in children (3). Because many physiological properties underlying learning and memory are known for the nudibranch mollusc *Hermissenda,* we have been investigating whether this animal can effectively serve as a model for lead toxicity studies (4,5). In this paper, we report the first effects of lead on learning in *Hermissenda.*

Upon arrival from Sea Life Supply (Sand City, CA), the animals were adapted to lab conditions for three days. They were initially tested and grouped for similar robust behavioral responses. Subsequently the nudibranchs were placed in either natural seawater (NSW; control animals) or NSW containing 4.76 mg/l lead acetate (experimental animals); all animals were fed a daily diet of the cnidarian hydroid *Tubularia sp.* After three undisturbed days of lead exposure, all animals underwent three days of associative (Pavlovian) conditioning (6), consisting of 50 trials of light (condition stimulus; CS) paired with agitation (unconditioned stimulus; UCS). The animals were tested the following day for evidence of behavioral conditioning by measuring foot contraction in response to light alone (7). The animals' responses were scored as follows: contraction, no-response, or foot extension.

In all three experiments reported here, lead significantly reduced the ability of *Hermissenda* to undergo associative conditioning (Fig. 1A–C). Of the animals in the three control groups (n = 56), 40–70% (12/32, 11/17, 5/7 respectively) exhibited the positive foot contraction response (Fig. 1B), while an average of only 9.3% (4/43; n = 43) of those exposed to lead contracted (Fig. 1C). Most animals exposed to lead either did not respond to the condition stimulus (28/43), or their foot actually extended in normal locomotory behavior (11/43; Fig. 1A). The combined experimental data were subjected to Chi-Square analysis for binned data between lead and no-lead, yielding a Chi-Square value of 20.190 (df = 2), with a probability of <0.0001 (Fig. 1C). Clearly, lead significantly affected the ability of *Hermissenda* to acquire the associative learning, behavioral change of foot contraction elicited by the CS, light.

The lead exposure levels used in these experiments were higher than those acceptable for human circulating blood levels (1,3). However, they are within the range typically used to challenge and raise vertebrate lead levels, and equal to those used in other studies on neurologic effects in invertebrates (8,2).

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