chi-square analysis revealed this difference as significant ($\chi^2 = 9.06$, $P < .05$).

The data in both experiments indicate that early exposure to halothane in trace amounts causes apparently permanent learning deficits, since these deficits occurred in rats last exposed 75 to 90 days prior to the beginning of behavioral testing (group DU). Because exposure to halothane only after day 60 of age produced no behavioral deficits in either learning task (group UD), the critical exposure period appears to be that of early development. Correlative to the pattern of behavioral deficits, tissue samples from the superior parasagittal cerebral cortex of rats exposed to 10 ppm halothane from conception to parturition showed electronmicroscopic evidence of neuronal degeneration, as well as the permanent failure of formation of the synaptic web and postsynaptic membrane density in 30 percent of the postsynaptic membranes (Fig. 2); only slight neuronal damage was evident at the electronmicroscopic level in rats exposed to 10 ppm halothane as adults (*10*).

The above results reveal that exposure to very low concentrations of halothane throughout the period of major growth in the rat is sufficient to produce subsequent enduring deficits in learning tasks. The nature of the deficit is not revealed by these studies, but these findings raise the question as to whether or not pregnant women should avoid chronic halothane exposure even at trace levels of 10 ppm as a precaution against possible enduring damage to the brain of the fetus.

The present behavioral data provide no indication of damage in the adult rat exposed to 10 ppm halothane. However, the exposure period was only approximately 90 days. Assuming that our electromicroscopic data indicate a susceptibility of developing synapses, and assuming that the plasticity of adult brain in registering new learning involves synaptic changes, then longer exposure times, increased halothane concentration, or behavioral tasks more specific to memory registration might reveal deficits.

The results of this study provide an example wherein exposure to trace levels of a potential toxicant produced no gross evidence of physiological or behavioral damage. Even under light microscopy, various organs, including the brain, appeared normal. Only at the ultrastructural level did morphological damage become manifest. Furthermore, the evidence of synaptic and other neurological damage in the central nervous system may or may not have been accompanied by functional deficits, and only by behavioral testing could this be resolved. It is interesting that behavioral tests have proven here to be as sensitive as electronmicroscopy for detecting damage from exposure to a trace level of toxicant. In assessing the health burden of trace pollutants, at least those potentially affecting the central nervous system, it is clear that ultrastructural and behavioral data will need to be specifically sought as the most sensitive approach to determine exposure levels consistent with health and safety.

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6. Several 500-μl sample injections were made daily, with the use of a Varian Aerograph model 600-D gas chromatograph. The flow rate for the N_2 carrier gas was 25 ml/min. A flame ionization detector was employed. The 5-foot by ⅛-inch column (1.524 m by 0.32 cm), with squaline liquid phase coated to a 5 percent loading on VarAport 30 support, 80 to 100 mesh size (available from Varian), was maintained at 100°C.
7. The procedure was essentially identical to that of R. J. Barrett, N. J. Leith, and O. S. Ray [*Psychopharmacologia* **25**, 321 (1972)]. The maze was symmetrical, with arms 46.5 by 12.0 by 16.0 cm high. A 2.8-watt lamp mounted behind a green convex lens was centered on the end wall of each arm, facing a motorized, plexiglass door 15.5 cm away. The conditioned stimulus was produced by a 4.5-khz audio oscillator (Sonalert) pulsed at 10 per second. Intermittent shock (0.25 ma; 0.5 second on, 1.5 seconds off) was delivered through a grid floor and stainless steel plates that lined the inside walls of the maze. A 25-second intertrial interval was used, and trials consisted of switching the light cue in a predetermined random sequence to one of the previously dark arms. The subject could avoid (within 10 seconds) or escape the shock by interrupting the photocell beam in the lit goal box.
8. H. C. Fryer, in *Concepts and Methods of Experimental Statistics* (Allyn & Bacon, Boston, 1966), pp. 259–270. The significant pairwise comparisons by the LSD test were: UU versus DU ($P < .01$), UU versus DD ($P < .025$), DU versus UD ($P < .001$), UD versus DD ($P < .001$).
9. J. W. Davenport and T. P. Dorcey, *Horm. Behav.* **3**, 97 (1972); J. W. Davenport, W. W. Hagquist, G. R. Rankin, *Behav. Res. Methods Instrum.* **2**, 112 (1970). We used a constant 30-second intertrial interval. The problems, including T-6, consist of a symmetrical arrangement of barriers, requiring the subject to make the same sequence of left and right turns to get from one goal box to the other in a bidirectional shuttling procedure.
10. L. W. Chang, A. W. Dudley, J. Katz, in preparation. Evidence of neuronal degenerative changes in neonatal brain exposed to halothane included such observations as focal weakening and disruption of the nuclear envelope of cortical neurons, vacuolation and myelin-figure formation in many neuronal processes, focal accumulation of vesicular structures, and mitochondrial swelling in neuronal cytoplasm, neuronal necrosis, and edematous changes of the glial cells.
11. Supported by funds from both the University of Wisconsin Graduate School Research Committee and the Medical School Research Committee. We thank Ayerst Laboratories for donation of Fluothane (halothane).

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Interpreting the Failures to Confirm the Depression of Cerebellar Purkinje Cells by Cyclic AMP

Our proposal (*1, 2*) that 3′,5′-adenosine monophosphate (cyclic AMP) might mediate the action of norepinephrine (NE) on cerebellar Purkinje cells has been challenged by Lake and Jordan (*3*). Our proposal evolved from the reported increases in cyclic AMP synthesis produced by NE in cerebellar slices (*4*) and our observations that iontophoretic application of cyclic AMP would mimic the depressant effects of NE on the discharge rate and pattern of Purkinje cells (*1*) and that phosphodiesterase inhibitors would potentiate the depressant actions of NE and of cyclic AMP (*1, 2*). Subsequently, we showed that both NE and cyclic AMP hyperpolarized Purkinje cells (*5, 6*), and that identical hyperpolarizations were produced by stimulating the NE pathway arising in the nucleus locus coeruleus (*7, 8*). In addition, we showed that the proportion of Purkinje cells which reacted to an immunocytochemical method for the detection of cyclic AMP increased five- to ten-fold after stimulation of the locus coeruleus or topical application of NE (*9*).

Lake and Jordan challenged our hypothesis on three grounds. (i) They, like Godfraind and Pumain (*10*), were unable to depress the same frequency of Purkinje cells with cyclic AMP as we were. (ii) They (*3*) discount our

supportive pharmacology as "unspecific" and "untenable." (iii) They discount the immunocytochemical data as uninterpretable because cerebellar granule cells were maximally reactive in control samples (*9*).

The fundamental issue here concerns the number of tested Purkinje cells that respond to cyclic AMP and the significance of that number. Lake and Jordan (*3*) and Godfraind and Pumain (*10*) insist that cyclic AMP cannot be considered the second messenger for NE synapses on Purkinje cells unless cyclic AMP depresses as many Purkinje cells as NE does. Under the conditions and criteria of our experiments we have observed depressions in 156 out of 238 Purkinje cells tested with cyclic AMP in rats (*1, 2, 5, 7, 9, 11*), cats, pigeons, and frogs (*12*). We regard these observations as continued support for our proposals and experimental tests. In an effort to resolve the conflicts in evidence and interpretation, we offer the following comments.

Our procedure for testing cyclic AMP is to "warm up" the cyclic AMP barrel by passing high ejection currents (150 to 200 na) for 5 minutes or more into brain areas away from the test site in order to achieve reproducible trials later with Purkinje neurons (*1, 2, 5, 7, 9, 11, 12*) or other (*14*) test cells. In our studies, cells are recorded for several minutes without drug treatment in order to verify a stable control discharge pattern. We never test drugs during the excitatory rebound period which may follow the effects of depressant substances (*3*, figure 1). Our definition of a nonresponsive cell requires at least two tests with a 200-na ejection current that is fully balanced (that is, ejection current neutralized at the pipette tip by current of the same magnitude and opposite polarity passed through a saline barrel). We do not accept cells as tested which fail to meet these criteria. The anionic currents used to eject cyclic AMP may directly excite neurons, and even minor imbalances (between ejection and neutralizing currents) at high ejection currents may be sufficient to produce these artefactual excitatory responses, seen frequently with some cyclic AMP barrels.

However, even with these procedures some Purkinje cells do not respond to cyclic AMP (*1, 6, 11, 12*). These nonresponses can be explained, in our view, by consideration of the iontophoretic release process and the nature of cyclic AMP responses. We observe that many iontophoretic pipettes do not pass cyclic AMP well even with a warm-up period. This conclusion is drawn from two different lines of evidence. First, when we reanalyzed data from 100 consecutively tested Purkinje cells and classified the data according to pipette, we found that the proportion of cells depressed by cyclic AMP ranged from a low of 28 percent (12 of 42 cells tested with 31 pipettes) to a high of 71 percent (41 of 58 cells tested with 9 pipettes). More than two-thirds of the depressed cells were obtained with less than one-fourth of the pipettes. A similar uneven distribution of results exists for pipettes used to test cyclic AMP on hippocampal pyramidal cells (*13*). Second, in tests of iontophoretic release in vitro with ^{3}H-labeled cyclic AMP (*15*), we found poor reproducibility between individual ejection trials with currents and durations similar to those used in vivo. With a large number of trials on several pipettes, a transfer constant for cyclic AMP was calculated (*16*) at the low value of 0.048 (standard error $= \pm$ 0.005).

To insist upon absolute agreement between the proportion of Purkinje cells depressed by both NE and cyclic AMP (*3*) implies that intracellular second messengers can be identified by the same procedures as intercellular synaptic transmitter candidates. In other cellular systems in which cyclic AMP is accepted as the intracellular second messenger for specific cellular events induced by hormones (that is, glycogenolysis, lipolysis), the threshold for this response is 100- to 10,000-fold lower for the hormone (that is, glucagon, ACTH) than for cyclic AMP (*17*). The enormously higher threshold for cyclic AMP could result from catalysis by phosphodiesterase or from membrane barriers around the intracellular cyclic AMP receptor [that is, protein kinase (*18*)]. In fact, iontophoresis of cyclic AMP intracellularly into cardiac Purkinje fibers reproduces all the effects of extracellular NE and cyclic AMP (*19*). Therefore, we would expect that higher extracellular concentrations would be required to elicit neuronal responses with cyclic AMP than with NE, and that these thresholds may not be reproducibly achieved by all iontophoretic pipettes.

However, our proposal is not based exclusively on the depressant effects of cyclic AMP on neuronal discharge. Neither the presence nor the absence of direct cyclic AMP actions would have meaning for the mediation hypothesis without the additional lines of pharmacological (*20*), electrophysiological, and cytochemical evidence which Lake and Jordan dismiss (*3*). Contrary to their demurral (*3*), changes in the immunocytochemical reactivity of granule cells for cyclic AMP are simply not relevant to the question of cyclic AMP as a second messenger for NE synapses on Purkinje cells.

In view of our data, the reported depressant effects of cyclic AMP on unidentified brainstem neurons (*21*), on hippocampal pyramidal cells (*13*), cells of the caudate nucleus (*14, 22*), and on cells presumed to receive a dopaminergic input in the limbic system (*22*), and sympathetic ganglia (*18*), the proposal that cyclic AMP might mediate the postsynaptic actions of catecholamines still seems reasonable to us.

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13. M. Segal and F. E. Bloom, *ibid.* **72**, 79 (1974); of 44 pyramidal cells tested, 57 percent were depressed by cyclic AMP; of these, three-fourths of the responsive cells were obtained in five of ten experiments.
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The Tuned-Receptor Paradigm

Wasserman (*1*) presents an excellent systematic review of the spectral sensitivities of invertebrate photoreceptors, but he has built out of them a straw man in his attempt to show that "the tuned-receptor paradigm is not universally valid." His demonstration arises from his own distortion of the classical color vision paradigm. This paradigm, as he states clearly enough, is that "color discrimination must involve a comparison of the relative responses in a set of receptors. An array of paradigmatic receptors tuned to different portions of the spectrum will . . . produce different relative receptor responses to lights of different wavelengths." Since "this is also true of a contraparadigmatic system," it is not clear in what sense a narrowly tuned receptor is paradigmatic while a broadly tuned one is contraparadigmatic. The essence of the Palmer-Young [*1*, references 2 and 3] paradigm is surely the necessity for the intercomparison of the responses of receptors with different tuning curves, and has nothing to do with the shape or narrowness of these curves. I know of no claim to the contrary appearing in the literature. The existence of a limited continuum of receptor types, arbitrarily classified into α and β groups, is no evidence for qualitatively different color vision mechanisms. The paradigm-contraparadigm formulation therefore appears to me a false dichotomy.

In this context, I fail to understand the author's statement that the tuning notion is "distinct from" the concepts that Palmer's and Young's "particles" correspond to light sensitive pigments and that "information about color is not extracted from the response of one receptor but by comparing the relative responses of receptors which differ in their sensitivities to different spectral stimuli." The tuning notion, as I understand it, clearly embraces these concepts.

Finally, I am surprised at the suggestion that most authors who failed to find selective bleaching of one of the two peaks of the β-receptors did so for lack of adaptation intensity. The intensities required for substantial pigment bleaching are well known and accessible to most visual researchers. Rapid regeneration of the pigment or pigments (*2*) appears a more likely explanation. I also note, in addition to the explanations offered, that the two spectral peaks may represent two interconvertible states of a single pigment rather than two independent pigments (*3*).

There is no doubt that scientific investigators sometimes "adhere to a paradigm even when there is evidence that is incompatible with the paradigm," but Wasserman presents no such evidence.

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Evidence incompatible with an established concept is frequently ignored or rejected at first. Later, if this new evidence becomes compelling, it is often said, unfortunately, that the evidence really conforms with that which was always known. My purpose in writing my review of invertebrate receptors (*1*) was to draw attention to new evidence that I thought had become compelling; I had hoped that a review of this new evidence would stimulate a serious consideration of its implications for receptor function. Hillman's response (*2*) to my review is that I misrepresented the prior belief structure and that a proper portrayal of these prior beliefs would be consistent with the evidence presented in my review. Hillman's criticism was consensually validated by three distinguished referees and has to be taken as a statement of widely held views. Obviously, if we are to make future substantive progress in this area, we must analyze Hillman's position carefully. Unfortunately, Hillman has not made the clearest possible case for his position; his response exhibits a number of errors, including the logical error of equivocation. A careful analysis is therefore doubly required.

From the title to the end of my review, I explicitly distinguished between the subordinate concept of the tuned-receptor paradigm, which referred solely to the shape of the receptor spectral sensitivity function, and the superordinate concept of color vision in general, which subsumes auxiliary subordinate concepts (such as the concept that color vision involves a comparison between receptors). In my review, the term "paradigm" never referred to anything other than the shape of the receptor function.

The assertion that I created a "straw man" in my review of invertebrate photoreceptors derives from the confusion created by Hillman's equivocation between the tuned-receptor paradigm described by me and the "classical color vision paradigm" described by him. The equivocation is to be found in Hillman's third sentence which represents me as "clearly stating" something that I never did say. The further statement that ". . . it is not clear in what sense a narrowly tuned receptor is paradigmatic . . ." rests on this earlier equivocation: Clarity depends on keeping the terms of the discussion consistent.

Hillman's subsequent complaint that he ". . . fail[s] to understand . . ." me is a quite understandable result of this equivocation-dependent lack of clarity, which has made it difficult for him accurately to read my review. For example, he asserts that I argued for ". . . *qualitatively* [italics mine] different color vision mechanisms." I actually said the opposite, namely, that β ". . . receptors undoubtly would involve a color vision system that is *quantitatively* [italics added] different . . ." (*1*, p. 269). I never said nor did I ever imply that the data under review disconfirmed any concept of color theory other than