the bovine and *Drosophila* opsin sequences, and we have found no homologies other than to opsin genes. We did not detect SP1116 hybridization to DNA from the eubacteria *Klebsiella pneumoniae* and *Escherichia coli*, the fungus *Blastocladiella emersoni*, and the plant *Zea maize* (lanes 3 and 4 in Fig. 3b). Thus we are able to detect homologies only in organisms for which there is independent evidence for the existence of photosensory pigments. Finally, additional Southern blot results suggest that many of the genomic fragments that hybridize to SP1116 are also homologous to a *Drosophila* opsin DNA probe (Table 1).

On the basis of our observations that opsin-like genes can be identified in a wide variety of vertebrate, invertebrate, and unicellular species, we speculate that a primitive photopigment gene first evolved in unicellular organisms and was passed on, with some degree of sequence conservation, to a wide variety of present-day species. Because it is possible to identify more than one class of opsin-like genes in some animals, it seems likely that the progenitor gene was amplified during evolution, giving rise to a family of related products. Some of these products are probably themselves visual pigments, since many organisms have more than one type. Recently, Nathans and Hogness (*6*) found that the human cone pigment genes have weak sequence homology to the opsin gene and similar intron positions, supporting the idea that they arose from a common precursor (*17*). Perhaps in other cases a duplicated photopigment gene became adapted to a different function. This could have occurred during the evolution of *Halobacterium*, resulting, for example, in one class of photopigment that is involved in ion pumping and another that subserves a sensory function.

The bovine opsin probe has excellent potential for identifying genes encoding photosensory pigments in a wide variety of species. Comparisons of the structure of photosensory receptors should reveal the extent to which vertebrate, invertebrate, and unicellular organisms employ the same functional mechanisms for triggering a sensory response.

REFERENCES AND NOTES

1. G. Wald, *Nature (London)* **219**, 800 (1968); E. A. Dratz and P. A. Hargrave, *Trends Biochem. Sci.* **8**, 128 (1983).
2. E. Southern, *J. Mol. Biol.* **98**, 503 (1975).
3. D. W. Cleveland *et al.*, *Cell* **20**, 95 (1980); V. Wilson *et al.*, *J. Mol. Biol.* **166**, 457 (1983); W. McGinnis, R. L. Garber, J. Wirz, A. Kuroiwa, W. J. Gehring, *Cell* **37**, 403 (1984).
4. Hybridization conditions were varied to allow the probe to reanneal with genomic sequences containing different degrees of homology. Stringent hybridizations (allowing ~10% mismatch) were performed at 68°C and blots were washed in 0.2× standard saline citrate (SSC). Moderate (~20% mismatch) and low-stringency (~35% mismatch) hybridizations were performed at 58°C and washed in 0.2× or 2× SSC, respectively. The mismatch estimates assume that (i) a perfectly matched DNA duplex has a melting temperature (T_m) of about 87°C in 1× SSC, (ii) changing the salt concentration (N) of the hybridization solution influences T_m by the relation $\Delta T_m = 16.6 \log \Delta N$, and (iii) the T_m of a DNA duplex decreases by about 1°C for every 1% base-pair mismatch. In addition, the degree of hybridization to a fixed amount of DNA depends on the relative genome size of the organism. For a more detailed discussion, see T. Maniatis, E. F. Fritsch, J. Sambrook, Eds., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982), pp. 324 and 388; G. A. Beltz, K. A. Jacobs, T. H. Eickbush, P. T. Cherbas, F. C. Kafatos, *Methods Enzymol.* **100**, 266 (1983); J. Meinkoth and G. Wahl, *Anal. Biochem.* **138**, 267 (1984).
5. Genomic DNA was isolated by a modification of the method of N. Blin and D. W. Stafford [*Nucleic Acids Res.* **3**, 2303 (1976)]. The DNA was further purified by CsCl–ethidium bromide density gradient centrifugation. An aliquot was compared electrophoretically with λ DNA to ensure that fragment sizes exceeded 50 kilobase pairs.
6. J. Nathans and D. S. Hogness, *Cell* **34**, 807 (1983); *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4851 (1984).
7. H. Rees and R. N. Jones, *Int. Rev. Cytol.* **32**, 53 (1972).
8. G. L. Walls, *Am. J. Ophthalmol.* **17**, 892 (1934); F. Crescitelli, in *Biochemical Evolution*, H. Gutfreund, Ed. (Cambridge Univ. Press, Cambridge, 1981), p. 301; in *Handbook of Sensory Physiology*, F. Crescitelli, Ed. (Springer-Verlag, Berlin, 1977), vol. VII/5, p. 391.
9. F. Crescitelli, in *Handbook of Sensory Physiology*, H. J. A. Dartnall, Ed. (Springer-Verlag, Berlin, 1972), vol. VII/1, p. 245; L. Yu and R. S. Fager, *Invest. Ophthalmol. Visual Sci.* **22** (suppl.), 43 (1982).
10. A. Oksche, *Ophthalmic Res.* **16**, 88 (1984); H. W. Korf and I. Vigh-Teichmann, *ibid.*, p. 96; R. G. Foster, B. K. Follett, J. N. Lythgoe, *Nature (London)* **313**, 50 (1985); L. Z. Bito and D. G. Turansky, *Comp. Biochem. Physiol.* **50A**, 407 (1975); J. N. Lythgoe, *Vis. Res.* **24**, 1539 (1984).
11. T. Ebrey, M. Tsuda, G. Sassenrath, J. L. West, W. H. Waddell, *FEBS Lett.* **116**, 217 (1980); H. R. Saibil and M. Michel-Villaz, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5111 (1984).
12. W. Baehr *et al.*, *Invest. Ophthalmol. Visual Sci.* **26** (suppl.), 43 (1985); J. E. O'Tousa *et al.*, *Cell* **40**, 839 (1985); C. S. Zuker, A. F. Cowman, G. M. Rubin, *ibid.*, p. 851.
13. K. W. Foster *et al.*, *Nature (London)* **311**, 756 (1984).
14. R. Henderson, *Annu. Rev. Biophys. Bioeng.* **6**, 87 (1977).
15. Y. A. Ovchinnikov, *FEBS Lett.* **148**, 179 (1982).
16. J. L. Spudich and R. A. Bogomolni, *Nature (London)* **312**, 509 (1984).
17. L. Stryer, *ibid.*, p. 498.
18. W. Baehr and M. Applebury, *Biophys. J.* **41**, 340a (1983); W. Baehr, R. Martin, M. L. Applebury, *Invest. Ophthalmol. Visual Sci.* **25** (suppl.), 155 (1984).
19. D. S. Papermaster, B. G. Schneider, M. A. Zorn, J. P. Kraehenbuhl, *J. Cell Biol.* **77**, 196 (1978); S. A. H. Al-Mahdawi, C. Converse, A. Cooper, *Biochem. Soc. Trans.* **9**, 319 (1981).

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Technical Comments

Human Brain Receptor Distribution

D. F. Wong *et al.* (*1*) describe the use of ^{11}C-labeled 3-*N*-methylspiperone and positron emission tomography for the measurement of regional alterations in dopamine and serotonin receptors in the living human brain and demonstrate the feasibility of routine imaging of receptor ligand distributions by emission tomographic methods. The ultimate goal of this and earlier studies (*2*) is the development of a quantitative procedure for measurement of regional neurotransmitter receptor density in vivo in the human brain. Such methodology would then allow direct testing of hypotheses implicating receptor alterations in a variety of neurologic and psychiatric disorders. While the experiments presented by Wong and his colleagues are encouraging with respect to eventual achievement of this goal, we have reservations about their data analysis and interpretation.

Wong *et al.* present a three-compartment model describing tracer distribution in brain tissue similar to that in use in a number of laboratories, including ours (*3, 4*):

$$C_p \underset{K_2}{\overset{K_1}{\rightleftharpoons}} C_e \underset{K_4}{\overset{K_3}{\rightleftharpoons}} C_r \qquad (1)$$

where C_p is the content of tracer in the plasma, C_e is the content of exchangeable (free plus nonspecifically bound) tracer in tissue, and C_r is the content of receptor-bound tracer in tissue. The descriptions of the intercompartmental transfer coefficients in the model above require clarification. First, K_1 and K_2, describing diffusion of ligand between capillary plasma (C_p) and extracellular space (C_e) require redefinition in order to account for potential influences of cerebral blood flow on ligand delivery to the brain.

$$C_a \underset{K_2^*}{\overset{K_1^*}{\rightleftharpoons}} C_e \underset{K_4}{\overset{K_3}{\rightleftharpoons}} C_r \qquad (2)$$

Introduction of C_a, the arterial tracer concentration, replacing C_p, allows direct quantitative comparison of different brain regions from the same animal. K_1^* and K_2^* are then functions of both the blood-brain barrier permeability surface area product (K_1) and cerebral blood flow (F), as described previously (*5*).

$$K_1^* = F(1 - e^{-K_1/F}) \qquad (3)$$

$$K_2^* = K_2 K_1^*/K_1 \qquad (4)$$

Further, the transfer coefficient K_3, describing the association of free ligand with the receptor sites of interest, is not a simple first-order kinetic rate constant, but represents the product of a bimolecular rate constant and the receptor concentration (*4*).

$$K_3 = K_a R \quad (5)$$

where K_a is the association rate constant for the ligand binding reaction and R represents the free receptor concentration available for binding. Only under conditions of trace receptor occupancy will K_3 become a pseudo first-order rate constant proportional to free receptor density (*4*). Under such conditions, ligand binding will reflect free rather than total receptor densities and may be modified by synaptic levels of endogenous neurotransmitter in addition to alterations in total receptor number (*6*). The maximum drug dose (200 μg) used by Wong *et al.* may be a pharmacologically significant dose in a 70-kg individual (*7*), and the assumptions regarding K_3 thus may be invalid.

The derivation of the analytic expression used by Wong *et al.* (equation 10 in (*1*)] appears to be based on several assumptions with regard to the transfer coefficients discussed above (*8*). Specifically, use of equation 10 requires that $K_1, K_2 >> K_3 >> K_4$. In the absence of direct experimental verification of these relationships, one cannot conclude that equation 10 accurately describes the experimental results.

A further issue concerns the chemical identity of the measured radiotracer in the tissue regions of interest. Wong *et al.* invoke the presence of labeled metabolites of the injected ligand within the systemic circulation, but offer no information about the presence or absence of such products within brain tissue.

While one can simplify the requisite data for in vivo receptor binding by deleting arterial blood, tissue time-activity curves, and cerebral blood flow determinations from the calculations, it has not been demonstrated that one may safely do so. We therefore urge that a more conservative approach be applied before age-related changes in ligand uptake are interpreted as reflections of altered neurotransmitter receptor density.

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REFERENCES AND NOTES

1. D. F. Wong *et al.*, *Science* **226**, 1393 (1984).
2. D. Comar *et al.*, *Nature (London)* **280**, 329 (1979); W. C. Eckelman *et al.*, *J. Nucl. Med.* **20**, 350 (1979); M. E. Raichle, *Brain Res. Rev.* **1**, 47 (1979); T. J. Tewson *et al.*, *Brain Res.* **192**, 291 (1980); H. K. Kulmala *et al.*, *Life Sci.* **28**, 1911 (1981); B. Drayer *et al.*, *J. Comp. Asst. Tomog.* **6**, 536 (1982); C. D. Arnett *et al.*, *J. Neurochem.* **40**, 455 (1983); E. S. Garnett *et al.*, *Nature (London)* **305**, 137 (1983); H. Scholl *et al.*, *J. Nucl. Med.* **24**, 417 (1983); T. J. Tewson, *ibid.*, p. 442; P. B. Zanzonico *et al.*, *ibid.*, p. 408; W. C. Eckelman *et al.*, *Science* **223**, 291 (1984).
3. D. C. Perry *et al.*, *Brain Res.* **199**, 49 (1980); D. C. Chugani *et al.*, *J. Nucl. Med.* **25**, P72 (1984).
4. M. A. Mintun *et al.*, *Ann. Neurol.* **15**, 217 (1984); K. A. Frey *et al.*, *Soc. Neurosci. Abstr.* **10**, 556 (1984).
5. C. S. Patlak and J. D. Fenstermacher, *Am. J. Physiol.* **229**, 877 (1975); S. I. Rapoport *et al.*, *Brain Res.* **172**, 354 (1979); S. I. Rapoport *et al.*, *Am. J. Physiol.* **242**, R339 (1982).
6. T. F. Seeger *et al.*, *Brain Res.* **305**, 303 (1984); K. A. Frey *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
7. S. M. Larson *et al.*, *J. Nucl. Med.* **25**, P57 (1984); S. R. Burns *et al.*, *Ann. Neurol.* **18**, 117 (1985).
8. It is further assumed that the derivation of equation 10 was via substitution of equations 7 and 8 in equation 6 [not 5, as stated in (*1*)].

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Response: Since we described the imaging of dopamine and serotonin receptors in the living human brain (*1*), we have continued to observe that the ratio of the tracer activity in the caudate nucleus and putamen to that in the cerebellum is a linear function of time for at least 120 minutes after injection of the tracer 3-*N*-[^{11}C]methylspiperone ([^{11}C]NMSP).

This observation led us to propose the ratio index for the estimation of relative receptor density (*2*). We subsequently derived a method for the measurement of absolute receptor density (*3*). The ratio index evolved from the labeling in vivo of neuroreceptors in rodents (*4*). A three-compartment model helps explain why the caudate-cerebellar ratio as a function of time can be an index of the rate of ligand binding, k_3 (*2*).

Frey *et al.* raise the issue of capillary versus arterial plasma for the model. The definitions of the constants K_1^* and K_2^* suggested by them are essentially the same as our K_1 and k_2 (*5*): K_1 is a clearance related both to blood (or plasma) flow (F) and to the permeability surface (PS) area product in the expression:

$$K_1 = F\,(1 - \exp\{-PS/F\})$$

and k_2 is the fractional clearance of the ligand from brain, defined as:

$$k_2 = K_1/\lambda$$

where λ is the distribution volume or partition coefficient of the ligand between brain and blood. The complex relation between PS product, clearance K_1, and arterial and mean capillary concentration has been previously derived. K_1 and k_2 operate on the arterial concentration at all times, from influx to steady state; hence, the reason for our choice of such constants (*5*).

We agree that $k_3 = k_{on} B'_{max}$, where B'_{max} is the quantity of receptors available for binding and k_{on} is the association rate constant (referred to as R and K_a, respectively, by Frey *et al.*) (*3, 7*). The bimolecular association constant k_{on} may change with age, but we hypothesize that a decrease in the number of available receptors is more likely. In this regard, animal and human autopsy studies have not revealed changes in affinity with age, but only changes in receptor number (*6*). Hence we attribute changes of k_3 to changes of B'_{max}. An observed change of B'_{max} can reflect a reduction of the total receptor number or a change of occupancy by endogenous or exogenous ligands, but an effect of endogenous ligands is not likely with such high-affinity ligands as NMSP.

The amount of injected [^{11}C]NMSP did not affect the rate of binding of the radioligand to the caudate or putamen with doses even greater than the tracer range of [^{11}C]NMSP. There is no evidence from [^{11}C]NMSP studies thus far that pharmacologic doses of the radioligand were used. Even in experiments with prior administration of pharmacologic blocking doses of unlabeled haloperidol, the binding of [^{11}C]NMSP was irreversible during the period of observation. Thus, there was no departure from a pseudo–first-order reaction. No significant dissociation from the receptor has ever been observed by us.

We have also employed a complete three-compartmental model that allows separate estimation of the values of K_1, k_2, and k_3 of [^{11}C]NMSP binding, as well as B_{max} (the absolute density of receptors) for [^{11}C]NMSP and the K_I (inhibitory constant) of haloperidol, a dopamine receptor blocking agent (*3, 7*). We have found that k_3 is not negligible compared to k_2. Using arterial or arterialized plasma concentrations of [^{11}C]NMSP corrected for metabolites, and two PET scans per subject (one of which was preceded by blocking doses of haloperidol), we found that human caudate B_{max} averaged 14 pmol/g, and K_I for haloperidol was 2.0 nM in young normal volunteers. These values are similar to those obtained in autopsy studies of human caudate nuclei with [^{3}H]NMSP and [^{3}H]spiperone (*3, 7, 8*).

Frey *et al.* also raise the issue of tissue