the end of October or beginning of November, and was characterized by an increase in oestradiol-17β levels (Fig. 1, *c*, *g*).

The reproductive success was significantly lower in group 1 than in group 2 (Table 1; $p < 0.02$, Fisher's exact probability test). The hormone profiles of the non-pregnant animals in group 1 show that the effect occurs at a stage of the reproductive process around implantation; the follicular, luteal and post-implantation phases until the end of gestation are not affected. These findings corroborate the results from experiments with mink[18,19] where PCBs impaired reproduction: ovulation, mating and implantation occurred but were followed by early abortion or resorption. These results may be due to hormonal disturbance, to direct dominant-lethal action or to an embryo lethal effect caused by toxicants. Hormonal disturbance may be caused by disruption of the steroid synthetic pathway resulting in reduced circulating levels of hormones. PCBs are known to cause microsomal enzyme induction, accelerating hydroxylation of body steroids such as oestrogens[5]. To determine if pollutants suppress circulating levels of hormones during the implantation period, the four subgroups (pregnant and non-pregnant seals in groups 1 and 2) are compared. A statistical test revealed no difference between progesterone levels of pregnant animals in group 2 and non-pregnant animals in group 1. It is concluded that progesterone levels were not reduced in group 1. The rise in oestradiol levels of the non-pregnant seals in group 2—indicating follical growth—is lacking in non-pregnant seals of group 1, which suggests that the nature of non-pregnancy differs between the groups. Because there were only 2 non-pregnant animals in group 2, this could not be tested statistically. The results also demonstrate that the levels of oestradiol in all group 1 seals are lower than those in group 2 combined, ($p < 0.05$, Wilcoxon), although the initial rise in some of the animals of group 1 (Fig. 1*c*) was apparently sufficient to ensure reproductive success. The mechanisms behind the smaller increase in oestradiol levels and its consequences for the priming effect on the endometrium, as well as the maternal rejection response[20–22], will be discussed in detail elsewhere. Hypotheses about impaired steroid binding capacity by PCBs[23], a dominant-lethal action and an embryo lethal effect cannot be tested with the information available.

I conclude that the reproductive success of the seals receiving the diet with the highest level of pollutants was significantly decreased. No circumannual reduction in levels of circulating hormone levels was observed. The reproductive process is disrupted in the post-ovulation phase. The period around implantation seems to be the most sensitive stage, but no conclusions about the mechanism of action can be drawn. A similar experiment was simultaneously carried out with the American mink *Mustela vison*, a fish-eating mammal with a comparable reproductive physiology. It was designed to test whether pure PCBs had the same effect as the PCB-polluted fish. Three groups of 'Standard' type mink were fed either a basic diet of commercial mink cereal, or the same basic diet supplemented with livers from fish caught in the western part of the Wadden Sea or with Clopen A-60 or A-30. The results show that reproduction is inhibited at very low (25 μg per day) levels of PCB intake and that the effects of the pure PCB diet were identical to those of contaminated fish diet[18].

Irrespective of the precise mechanism involved, the results from this study show that the reproductive failure in common seals from the Dutch Wadden Sea is related to feeding on fish from that polluted area. The available epidemiological experimental data on effects and levels of PCBs in seals and mink fed on fish from this area[5,27] suggest that these organochlorines are the main cause of this failure.

I thank A. Grijzen, H. Jansens and G. van Weert of De Wever Hospital, Heerlen, for carrying out the radioimmunoassays; J. Schoonheden for the illustrations, M. C. Vleugel for care of the animals and J. Zegers for his assistance throughout the whole experiment.

1. Reijnders, P. J. H. *Neth. J. Sea Res.* **10**, 223-235 (1976).
2. Reijnders, P. J. H. *Neth. J. Sea Res.* **12**, 164-179 (1978).
3. Reijnders, P. J. H., Drescher, H. E., van Haaften, J. L., Bøgebjerg Hansen, E. & Tougaard, S. in *Marine Mammals of the Wadden Sea* (eds Reijnders, P. J. H. & Wolff, W. J.) 19-32 (Balkema, Rotterdam, 1981).
4. Reijnders, P. J. H. *Z. Säugetierk.* **48**, 50-54 (1983).
5. Reijnders, P. J. H. *Neth. J. Sea Res.* **14**, 30-65 (1980).
6. Duinker, J. C. & Hillebrand, M. T. J. *Neth. J. Sea Res.* **13**, 256-281 (1979).
7. Duinker, J. C., Hillebrand, M. T. J., Nolting, R. F. & Wellershaus, S. *Neth. J. Sea Res.* **15**, 141-169 (1982).
8. Duinker, J. C., Hillebrand, M. T. J., Nolting, R. F. & Wellershaus, S. *Neth. J. Sea Res.* **15**, 170-195 (1982).
9. Duinker, J. C., Boon, J. P. & Hillebrand, M. T. J. *Neth. Inst. Sea Res. Publ. Ser.* **10**, 211-228 (1984).
10. Reijnders, P. J. H. in *Marine Mammals of the Wadden Sea* (eds Reijnders, P. J. H. & Wolff, W. J.) 38-47 (Balkema, Rotterdam, 1981).
11. Daniel, J. C., Jr. *J. Fish. Res. Bd Can.* **33**, 65-66 (1975).
12. Boyd, I. L. *J. Repr. Fert.* **69**, 157-164 (1983).
13. Raeside, J. I. & Ronald, K. *J. Reprod. Fert.* **61**, 135-139 (1981).
14. Amorsoso, E. C., Heap, A. B. & Renfree, M. B. in *Hormones and Evolution* (ed. Barrington, E. J. W.) 925-989 (1979).
15. Flint, A. P. F. *J. Repr. Fert. Suppl.* **29**, 215-227 (1981).
16. Renfree, M. B. & Calaby, J. H. *J. Reprod. Fert. Suppl.* **29**, 1-9 (1981).
17. Boshier, D. P. *J. Repr. Fert. Suppl.* **29**, 143-149 (1981).
18. Boer, M. den in *A. Rep. RIN* (ed. Rossum, T. van) 77-86 (Rijksinstituut voor Natuurbeheer, Leersum, 1983).
19. Jensen, S., Kilström, J. E., Olsson, M., Lundberg, C. & Orberg, J. *Ambio* **6**, 239 (1977).
20. Finn, R., St. Hill, C. A., Govan, A. J. Ralfs, I. G. & Gurney, F. J. *Br. Med. J.* **3**, 150-152 (1972).
21. Anderson, R. H. & Munroe, C. W. *Am. J. Obstet. Gynec.* **84**, 1096-1103 (1962).
22. Grossman, C. J. *Science* **227**, 257-261 (1985).
23. Bitman, J. & Cecil, H. C. *J. Agric. Fd Chem.* **18**, 1108-1112 (1970).
24. Osborn, D. B., Cooke, A. S. & Freestone, S. *Envir. Pollut.* **25**, 305-319 (1981).
25. Ronald, K., Foster, M. E. & Johnson, E. *Can. J. Zool.* **47**, 461-468 (1969).
26. Bouckaert, P. X. J. M. thesis (Univ. Nijmegen, Netherlands, 1984).
27. Reijnders, P. J. H. *Mar. Mamm. Sci.* (submitted).

Table 1 of this paper appears on page 418.

A common mammalian plan of accessory optic system organization revealed in all primates

H. M. Cooper & M. Magnin

Laboratoire de Neuropsychologie Expérimentale, INSERM U-94, 16 Avenue Doyen Lépine, 69500 Bron, France

The accessory optic system (AOS), which was described as early as 1870 by Gudden[1], constitutes a distinct midbrain visual pathway in all classes of vertebrates[2]. In non-primate mammals, retinal fibres of this system project to a set of three nuclei[3,4]: the dorsal (DTN), the lateral (LTN) and the medial (MTN) terminal nuclei. Whereas all AOS cells respond to the slow motion of large visual stimuli, the neurons are tuned to complementary directions of movement[5]: horizontal temporo-nasal direction for the DTN, vertical up and down for the LTN and vertical down for the MTN. It has thus been suggested that these nuclei establish a system of retinal coordinates for the detection of whole field motion[6]. As the AOS provides direct and indirect pathways to both oculomotor and vestibular structures[7,8], each of these nuclei is thought to be an essential link in the co-ordination of eye and head movements in relation to movement within the visual field. One problem for the generalization of this theory is that the medial terminal nucleus has never been found in primates. In this report we establish both the existence of this nucleus and its afferent input from the retina in all major groups of primates (prosimians, New and Old World monkeys and apes), indicating a common anatomical plan of organization of the AOS in mammals.

We used both autoradiographic and histochemical anterograde tracing techniques to study the retinal projection to the AOS in the two primate suborders[9] Strepsirhini (prosimians: lemurs and lorises) and Haplorhini (simians: New and Old World monkeys, apes and man). For the Strepsirhines we studied five mouse lemurs (*Microcebus murinus*) and one bushbaby (*Galago demidovii*). Haplorhine species included two marmosets (*Callithrix jacchus*), three macaques (*Macaca fascicularis*) and two gibbons (*Hylobates concolor*). Most animals were injected with a radioactive amino acid mixture (500-

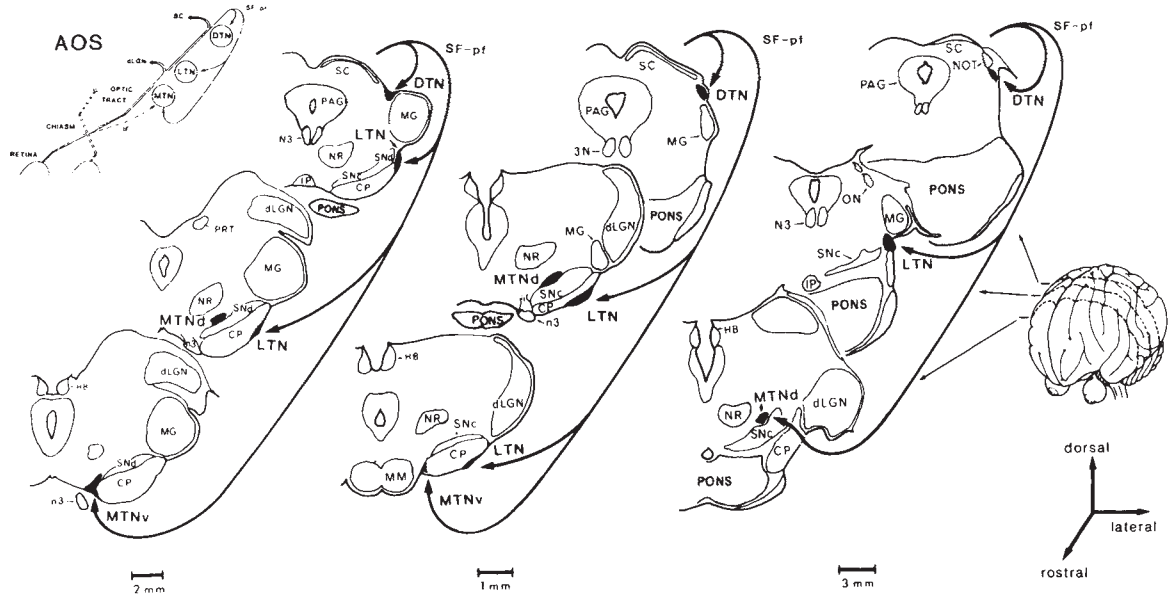

Fig. 1 Organization of the accessory optic system compared in a non-primate (cat), a strepsirhine primate (mouse lemur) and a haplorhine primate (gibbon). Animals received an intraocular injection of radioactive amino acids to reveal the distribution of retinal fibres and terminals. Drawings, scaled to the same size, are from coronal sections (lower right: approximate section levels shown on brain of gibbon) showing the relative positions of the three terminal nuclei (MTN, LTN, DTN, dark shading) of the AOS. Arrows, course of the superior fasciculus (SF-pf) (the main component of the AOT). As shown in the simplified schema in the upper left, this fasciculus separates from the main optic tract in the region of the brachium of the superior colliculus and then courses rostrally. The AOS in strepsirhine primates is similar to that of other mammals, but the MTNv is reduced in size. In haplorhine primates a retinal projection to the MTNv is lacking, and AOS fibres show a shortened trajectory through the cerebral peduncle to the MTNd. The extensive rostral development of the pons and pyramidal tracts in primates suggests that the reduction of the MTNv in Strepsirhini and its absence in Haplorhini is the consequence of a resulting morphological reorganization in the anterior region of the midbrain. AOS, accessory optic system; CP, cerebral peduncle; dLGN, dorsal part of the lateral geniculate nucleus; DTN, dorsal terminal nucleus of the AOS; HB, habenular nucleus; IF, inferior fasciculus; IP, interpeduncular nucleus: LTN, lateral terminal nucleus of the AOS; MG, medial geniculate nucleus; MM, mamillary nucleus; MTNd, dorsal division of the medial terminal nucleus of the AOS; MTNv, ventral division of the medial terminal nucleus of the AOS; n3, oculomotor nerve; N3, oculomotor nucleus; NOT, nucleus of the optic tract; NR, red nucleus; ON, olivary pretectal nucleus; PAG, periaqueductal gray; PRT, pretectum; SC, superior colliculus; SF-pf, posterior fibre branch of the superior fasciculus; SNc, substantia nigra, pars compacta; SNd, substantia nigra, pars diffusa.

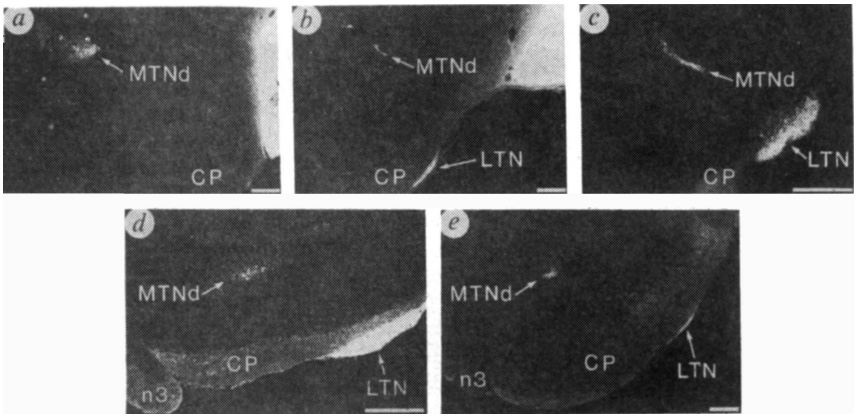

Fig. 2 Distribution of anterograde label transported from retinal ganglion cells to the MTNd (and the LTN) in: *a*, gibbon; *b*, macaque; *c*, marmoset; *d*, lemur; *e*, cat. The microphotographs of these coronal sections are viewed under dark-field illumination. Note the similar topographic location of the MTNd medial to the cerebral peduncle and the substantia nigra (towards upper left) in all species. Scale, 500 μm. CP, cerebral peduncle; LTN, lateral terminal nucleus of the accessory optic system; MTNd, dorsal division of the medial terminal nucleus; n3, oculomotor nerve.

1,000 μCi of ^{3}H-proline and ^{3}H-leucine) in the posterior vitreal chamber of the eye. After survival times of 24–60 hours the brains were processed according to the usual autoradiographic procedures for frozen or paraffin sections. In other primates, the eye was injected with 10–20 μl of a 40% solution of the anterograde tracer horseradish peroxidase (HRP; Sigma type VI) dissolved in sterile saline with 2% DMSO. Survival time was 36–60 hours. Frozen sections of the brains from these animals were treated with the tetramethylbenzidine method[10]. Brains from each series were sectioned in either frontal, sagittal, or horizontal planes and stained with cresyl violet to study cytoarchitecture. Retinal projections to the AOS were compared in species from eight other orders of mammals: Carnivora, Rodentia, Chiroptera, Dermoptera, Hyracoidea, Pholidota, Marsupiala and Edentata.

In all mammalian species studied, fibre tracts and the three terminal nuclei of the AOS were labelled by anterograde transport of the neuronal tracer from retinal ganglion cells. The majority of retinal fibres that form the accessory optic tract (AOT) cross in the optic chiasma, although a small component remains uncrossed. The AOT diverges from the main optic tract at the level of the brachium of the superior colliculus. The tract becomes visible on the superficial aspect of the brainstem as it retraces a rostral pathway to the anterior base of the midbrain[11], terminating in each of the AOS nuclei along its course (Fig. 1).

The MTN is the most conspicuous of the three AOS nuclei in non-primates and can be divided into a ventral division (MTNv) located at the ventromedial base of the cerebral peduncle, and a dorsal division (MTNd) extending into the region of the substantia nigra[3,12]. In primates, a retinal projection

to the ventral division was observed only in Strepsirhines but the dorsal division receives retinal terminals in all species studied.

The projection to the MTNd is remarkably similar in primates and non-primates (Fig. 2). It is located in a similar position in all species (medial to the substantia nigra pars compacta and ventro-lateral to the medial lemniscus and the red nucleus). The distribution of terminal label (silver grains or HRP granules) in the MTNd is often reticular in appearance, as synaptic contacts of retinal axons are mainly situated on dendrites rather than on the cell bodies[13]. Fibres of the AOT course to the nucleus by penetrating directly through the cerebral peduncle, forming a characteristic obliquely-oriented fibre bundle in the region of the substantia nigra.

In some primates (*Macaca, Callithrix*) the MTNd is rather small, whereas in the gibbon (*Hylobates*) the nucleus extends rostrocaudally in the midbrain for >2.0 mm. The MTNd is partially embedded within the substantia nigra and in some instances the cells of these two structures actually intermingle. Several cytological features allowed us to distinguish the cells of each nucleus, however. The MTNd neurons are typically pale stained, and ovoid or fusiform in shape. In contrast, nigral cells are comparatively large sized, polygonal shaped, and intensely stained with cresyl violet; they also have a large clear cell nucleus and a dense compact nucleolus. Depending on the species, the two populations of cells are also distinguished by additional features. For example in the gibbon, as in other apes and man, the cytoplasm of nigral cells is densely packed with black granules of melanin[14]. These cells show an endogenous red fluorescence under dark-field illumination due to the presence of melanin whereas the MTNd cells, which lack pigment, do not fluoresce. Anterograde label was distributed only over the cells of the MTNd.

In the strepsirhine primates *Microcebus*[15], *Galago*[15] and *Nycticebus*[16], there is also a small but distinct ventral division of the MTN, which receives retinal afferents. As in other mammals, such as the cat, the MTNv is located at the base of the cerebral peduncle, immediately rostral to the emergence of the oculomotor nerve. The cells in this part of the MTN are densely stained, small and round in shape (average diameter 14 μm), and thus differ from those of the dorsal division, which are highly elongated (typically 8 μm × 40 μm). The cytoarchitecture and pattern of retinal projections to the two subdivisions of the MTN in *Microcebus* closely resemble those of the cat. In the cat the ventral and dorsal subdivisions clearly form a topographically continuous nucleus, whereas in *Microcebus* these two parts are interconnected by a few fine fibres. In haplorhine primates we could not identify either terminal label or a cell group typical of the MTNv.

The absence of a distinct ventral portion of MTN in haplorhine primates has been interpreted as the result of a simple regression of the nucleus during primate evolution[17,18]. However, we suggest that the neurons of the MTNv have migrated in position to a more dorsal location and form a single constituent nucleus with the MTNd. Such a dorsal shift of the MTNv in primates could be a consequence of the extensive development of the pyramidal tract[19] (related to precise motor control of the limbs), resulting in a rostral extension of the pons below the cerebral peduncle which influences the topographical relations between the nuclear groups in this region. Since in other mammals the neurones of the ventral and dorsal divisions of the MTN differ in cytoarchitecture[12], efferent connections[7,8] and histochemical properties[12], the hypothesis of a dorsal topological shift of the MTNv in primates could be experimentally verified if the same distinctions exist in this single constituent of the MTN.

Our results unequivocally demonstrate that in primates at least one subdivision of the MTN is present and receives a monosynaptic retinal projection. This conclusion is supported by the presence of retinal terminals to a well defined cellular aggregate located in a homologous region of the midbrain tegmentum and by the typical course of accessory optic fibre tracts to the nucleus. Preliminary results[20] also provide evidence that, as in the rat[7,8], the rabbit[7,8] and the cat[21], the MTN in *Macaca* also sends an efferent projection to the vestibular nuclei. Thus the intrinsic organization of the AOS and its relations with other sensory systems are constant features in all mammalian species.

We thank C. Blakemore for helpful criticism of the text and N. Boyer, P. Giroud, M. Rouvière and S. Bello for technical assistance. This work was supported by grants from INSERM, the DGRST and the Ministere de l'Education Nationale.

Received 24 January; accepted 6 October 1986.

1. Gudden, B. *Arch. Psychiat.* **2**, 364-366 (1870).
2. Fite, K. V., Brecha, N., Karten, H. J. & Hunt, S. P. *J. comp. Neurol.* **195**, 279-288 (1981).
3. Hayhow, W. R. *J. comp. Neurol.* **113**, 281-314 (1959).
4. Hayhow, W. R. *J. comp. Neurol.* **126**, 653-672 (1966).
5. Simpson, J. I., Soodack, R. E. & Hess, R. in *Reflex Control of Posture and Movement* (eds Granit, R. & Pompeiano, O.) 715-724 (Elsevier, Amsterdam, 1979).
6. Simpson, J. I. *A. Rev. Neurosci.* **7**, 13-41 (1984).
7. Giolli, R. A., Blanks, R. H. I. & Torigoe, Y. *J. comp. Neurol.* **227**, 228-251 (1984).
8. Giolli, R. A., Blanks, R. H. I., Torigoe, Y. & Williams, D. D. *J. comp. Neurol.* **232**, 99-116 (1985).
9. Pocock, R. I. *Proc. zool. Soc. Lond.* 19-55 (1918).
10. Mesulam, M. M. *J. Histochem. Cytochem.* **26**, 106-117 (1978).
11. Retzius, G. *Biol. Unters. Neue Folge* **8**, 65-74 (1898).
12. Gregory, K. M. & Giolli, R. A. *Expl Brain Res.* **60**, 501-508 (1985).
13. Lenn, N. J. *Brain Res.* **43**, 622-628 (1972).
14. Schwyn, R. C. & Fox, C. A. *J. Hirnforsch.* **15**, 95-126 (1974).
15. Cooper, H. M. *J. comp. Neurol.* **249**, 28-47 (1986).
16. Weber, J. T. & Giolli, R. A. *Brain Res.* **365**, 164-168 (1986).
17. Giolli, R. A. *J. comp. Neurol.* **121**, 89-108 (1963).
18. Lin, H. & Giolli, R. A. *Expl Brain Res.* **63**, 163-176 (1979).
19. Heffner, R. & Masterton, B. *Brain Behav. Evol.* **12**, 161-200 (1975).
20. Magnin, M. *et al.*, manuscript in preparation.
21. Magnin, M., Courjon, J. H. & Flandrin, J. M. *Expl Brain Res.* **51**, 298-303 (1983).

A cell surface molecule distributed in a dorsoventral gradient in the perinatal rat retina

Martha Constantine-Paton, Andrew S. Blum*, Rosalia Mendez-Otero† & Colin J. Barnstable*

Department of Biology, Yale University, Kline Biology Tower, P.O. Box 6666, New Haven, Connecticut 06511, USA
* Laboratory of Neurobiology, The Rockefeller University, New York, New York 10021, USA

Brain topography may have its earliest expression as spatial gradients of molecules controlling the deposition of neurones and neuronal processes[1,2]. In the vertebrate visual system there is evidence that the stereotyped alignment of central retinal projections relies on an initial spatially organized distribution of molecules in both the retina and its central target nuclei[3–6]. We used an immunological approach to look for molecules that are so organized and produced a monoclonal antibody (JONES) which shows a pronounced dorsal to ventral gradient of binding in the rat retina throughout the period when retinal ganglion cell axons are forming topographically organized projections within the central nervous system (CNS). Binding is present throughout the radial thickness of the retinal epithelium in regions where post-mitotic neurones are generated but is not associated with any consistent histological characteristic of the tissue. The antibody was shown to bind on the cell surface of freshly dissociated retinal cells, and dorsal retinal quadrants were found *in vitro* to have nearly twice as much antigen as ventral retinal quadrants. Initial biochemical characterization of the target epitope reveals that it is a lipid present in chloroform/methanol extracts from perinatal retina and is sensitive to neuraminidase digestion.

† Permanent address: Universidade do Estado do Rio de Janeiro, Brazil.