

- 13 L.J. Old, D.A. Clarke and M. Goldsmith, *Proc. Am. Ass. Cancer Res.* 3, 49 (1959).
- 14 Z.P. Pavelic, U. Boranic and K. Pavelic, *Exp. Path.* 15, 288 (1978).
- 15 W. Reid, in: *Endocrine Aspects of Breast Cancer*, p.231. Livingstone, Edinburgh 1958.
- 16 K. Stern and A. Duvelius, *Cancer Res.* 20, 587 (1960).
- 17 A.C. Stewart and R.W. Begg, *Cancer Res.* 13, 556 (1953).
- 18 S. Thunold, *Acta path. microbiol. scand.* 69, 521 (1967).
- 19 S. Thunold, *Acta path. microbiol. scand.* 72, 542 (1968).
- 20 M.F. Woodruff and M.O. Symes, *Br. J. Cancer* 16, 120 (1962).
- 21 R.W. Begg, *Cancer Res.* 11, 341 (1951).
- 22 F. Hamburger, *Science* 107, 648 (1948).
- 23 J.D. Meyer and J.A. Meyer, *J. nat. Cancer Inst.* 59, 1023 (1977).
- 24 W.O. Reinhardt, in: *Essays on Biology*, p.487. University of California Press, 1943.
- 25 K. Savard, *Science* 108, 381 (1948).
- 26 G. Simu, V. Toma and D. Nestor, *Oncology* 22, 36 (1968).
- 27 W. Dutz, E. Kohout, E. Rossipal and K. Vessar, *Path. A.* 11, 415 (1976).
- 28 G. Ceriotti, *J. biol. Chem.* 214, 59 (1955).
- 29 K.W. Giles and A. Meyers, *Nature* 206, 93 (1965).
- 30 I. Hrsak and T. Mariotti, *Eur. J. Cancer* 9, 717 (1973).
- 31 I. Hrsak and T. Mariotti, *J. nat. Cancer Inst.* 53, 113 (1974).
- 32 R.E. McCarty, *Cancer Res.* 24, 915 (1964).

## Cilia in stellate neurons of the rat cerebellum<sup>1</sup>

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**Summary.** Cilia in stellate neurons of the normal rat cerebellum are described. 8 cilia were observed in a total of 60 cells studied. An 8+1 pattern was found throughout their length. Furthermore, no arms, spokes or other accessory structures necessary for ciliary motion were seen. These findings make it possible to suggest that these cilia are probably without function and are related to the epithelial origin of these cells.

The presence of cilia in neurons was first described in the retinal rods and cones of kittens<sup>3</sup> and in the preoptic nucleus of the goldfish<sup>4</sup>. The existence of neuronal cilia was then related to cells performing either sensory or conducting functions<sup>3,5,6</sup>, as well as to those possessing secretory activities<sup>4,7,8</sup>. They were later reported in different regions of the nervous system: peripheral autonomic ganglia<sup>9,10</sup>, the spinal cord<sup>11</sup>, inner neuronal layers of the retina<sup>12</sup>, lateral geniculate nucleus<sup>13</sup>, cerebellum<sup>14</sup> and cerebral cortex<sup>15,16</sup>. Although Karlsson<sup>13</sup> observed 1 cilium in each of 2 neurons which he serially sectioned and three-dimensionally reconstructed, which led us to believe that these are a common feature among neurons, cilia have otherwise been presented as a rare and occasional finding. This work deals with the frequent occurrence and particular morphology of cilia in stellate cells of the cerebellar cortex of the rat.

**Material and methods.** The molecular layer of the cerebellar cortex of 4 adult male Wistar rats selected at random was studied. Small tissue fragments of the cerebellar vermis, Larsell lobules 4-6, were obtained under ether anaesthesia of the animals<sup>17</sup>. Blocks were fixed according to the Kanaseki and Kadota method<sup>18</sup>. Details of this procedure have been described in a previous study<sup>19</sup>.

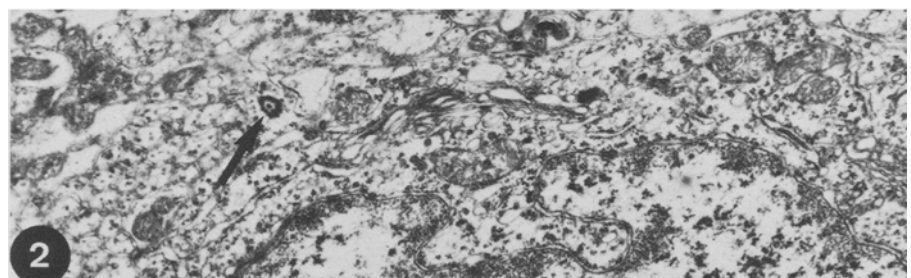
5 tissue blocks from each rat were selected at random. 1 silver ultra-thin section of the molecular layer (external half) was chosen at random from each block and stained with uranyl-acetate and lead citrate. The ultrastructural identification of stellate neurons was made according to Palay and Chan-Palay<sup>20</sup>. A total of 60 cellular profiles (15 from each animal) were studied.

**Results and discussion.** 8 cellular profiles each possessing 1 cilium were found in a total of 60 cells (13.3%). There was

Fig. 1. Cilium cut lengthwise enmeshed in the molecular layer neuropil (arrow).  $\times 14,400$ .



Fig. 2. The same cilium in a different section, cut perpendicularly to its axis (arrow). An 8+1 pattern is recognized.  $\times 14,400$ .



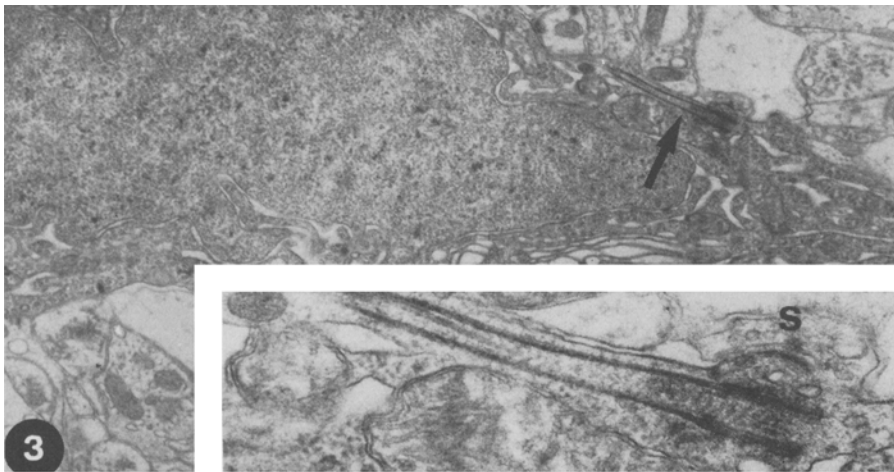


Fig. 3. Cilium cut lengthwise, running parallel to the plasmalemma of a stellate cell (arrow).  $\times 9600$  Inset. The same cilium seen at an higher magnification. S, Axosomatic synapse.  $\times 24,000$ .

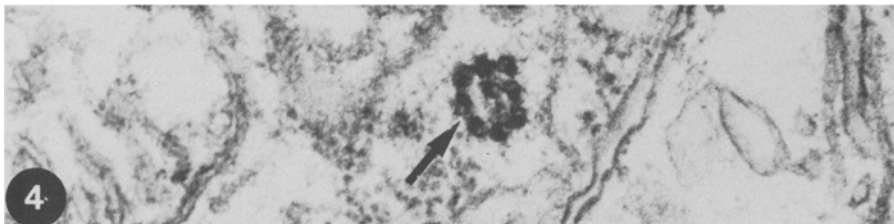


Fig. 4. Cross-section of a cilium at the level of its initial segment (arrow).  $\times 80,000$ .

not a significant interindividual variation: 2 rats had 2 ciliated cells, 1 rat had 3 ciliated cells and the 4th rat had 1 ciliated cell. The percentage of ciliated cells seems very high when compared with the results obtained in the only paper we know of which describes the presence of cilia in the cerebellum<sup>14</sup>. In that paper, however, the studies were made on immature rats, on rats intoxicated with diphenylhydantoin and on a few normal rats; cilia were found in meningeal fibroblasts, Bergmann glial cells and neuroblasts, but rarely in neurons.

In our study, 2 cilia were cut lengthwise. The axis of one of them lay nearly perpendicular to the neuronal surface and was bounded by an extension of the plasma membrane; it had no associated basal body and projected between the elements of molecular layer neuropil for about  $2.0\ \mu\text{m}$  (figure 1). A cross-section of this cilium showed 8 peripheral doublets and a flattening of the circular arrangement of filaments (figure 2).

The other cilium possessed a basal body and lay along the cell, immediately beneath the plasma membrane, and only its tip protruded from the neuronal surface (figure 3). Moreover, an axosomatic synapse closely related to the base of the cilium was seen (figure 3, inset). This observation does not fit in with the generally accepted idea that not only neuronal cilia, but also their perikaryal implantation zone, are completely devoid of specialized contacts<sup>21</sup>.

The other 6 cilia, cross-sectioned at the level of their initial segments, always exhibited the same appearance (figure 4). They were located in the cytoplasm, near the cellular membrane, and presented the flattened morphology and 8+1 pattern mentioned above. We stress, however, that this configuration is classically described as being localized at some distance from the perikaryon in the shaft of the cilium; this being a modification of the 9+0 pattern which characterizes the proximal segment of neuronal cilia<sup>12,15</sup>.

The lack of arms, spokes or other accessory structures which are fundamental for ciliary motion<sup>22,23,25</sup> as well as the morphological alteration we observed, support the assumption that neuronal cilia are vestigial and functionless

structures which owe their existence to the epithelial origin of the parent cells<sup>16</sup>. The different kinds within the 9+0 (or modified 8+1) pattern could, in fact, correspond to degenerate forms, whereas the normal 9+2 pattern may be maintained by selection because it functions most efficiently in motility<sup>25</sup>.

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- 3 E. De Robertis, J. biophys. biochem. Cytol. 2, 319 (1956).
- 4 S.L. Palay, Anat. Rec. 139, 262 (1961).
- 5 J. Wersäll, Acta otolar. suppl. 126, 1 (1956).
- 6 A.J. De Lorenzo, Ann. Otol. Rhinol. Lar. 69, 410 (1960).
- 7 B.G. Barnes, J. Ultrastruct. Res. 5, 453 (1961).
- 8 B. Ziegler, Z. Zellforsch. mikrosk. Anat. 59, 486 (1963).
- 9 A. Lasansky and E. De Robertis, J. biophys. biochem. Cytol. 7, 679 (1960).
- 10 J. Taxi, C. r. Soc. Biol. 155, 1860 (1961).
- 11 D. Duncan, V. Williams and R. Morales, Tex. Rep. Biol. Med. 21, 185 (1963).
- 12 R.A. Allen, J. Ultrastruct. Res. 12, 730 (1965).
- 13 U. Karlsson, J. Ultrastruct. Res. 16, 429 (1966).
- 14 M.P. Del Cerro and R.S. Snider, J. Microscopie 6, 515 (1967).
- 15 H.A. Dahl, Z. Zellforsch. mikrosk. Anat. 60, 369 (1963).
- 16 A. Peters, S.L. Palay and H.F. Webster, in: The Fine Structure of the Nervous System: The Neurons and Supporting cells. Saunders, Philadelphia 1976.
- 17 O. Larsell, J. comp. Neurol. 97, 281 (1952).
- 18 T. Kanaseki and K. Kadota, J. Cell Biol. 42, 202 (1969).
- 19 M.M. Paula-Barbosa and E.G. Gray, J. Neurocytol. 3, 471 (1974).
- 20 S.L. Palay and V. Chan-Palay, in: Cerebellar Cortex Cytology and Organization. Springer, Berlin 1974.
- 21 U. Karlsson, J. Ultrastruct. Res. 16, 482 (1966).
- 22 J. André, J. Ultrastruct. Res. 5, 86 (1961).
- 23 P. Satir, J. Cell Biol. 26, 805 (1965).
- 24 E. De Robertis, W.W. Nowinski and F.A. Saez, in: Cell Biology. Saunders, Philadelphia 1970.
- 25 P. Satir, J. Cell Biol. 12, 181 (1962).