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Absence of mitral cells in monolayer in monotremes

Variations in vertebrate olfactory bulbs

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Abstract. An invariant feature of the olfactory bulb in placental and marsupial mammals is the arrangement of the perikarya of mitral cells in a monolayer. Contrasting with this is the arrangement found in the olfactory bulbs of the monotremes, platypus and echidna, where the large perikarya are not only absent from the position of a monolayer (usually forming the external boundary of the internal plexiform layer) but occupy a region which would characterize them as tufted cells. In other classes of amniote vertebrates, reptiles and birds, the placement of large perikarya in the olfactory bulb ranges from a compact layer to a broad band. Such an overview among several vertebrate classes suggests that a monolayer of mitral cells may be a specialized subset of the tufted-mitral cell population. The accessory olfactory formation among mammals also exhibits variation in the compactness of the large perikarya: a broadband in most but a compact layer in a few others such as the chinchilla and the capybara. Such specialized alignment of perikarya (and, consequently, of their dendritic and axonal elements) may enable more refined signal processing than does random alignment of these elements. Such speculations can be tested using appropriate phylogenetic sampling, and monotremes provide particularly advantageous test cases.

Introduction

There is a great consistency in the organization of the olfactory bulbs of the anmiote vertebrates, but even within the mammals there are some striking variations in architecture.

The modern mammals fall into three great phylogenetic groupings. The first two groups, the placentals and marsupials, are believed to derive from common ancestors of the late Cretaceous period [*Lillegraven*, 1969], while the third group, the egg-laying monotremes, are of unknown origin and history. Current speculation favors the separation of monotreme ancestors from other mammal-like reptiles in Triassic times [*Hopson*, 1969], but contrary views do persist; for example, *Kühne* [1973] argues for a common Cretaceous origin for monotremes and marsupials *after* divergence from placentals.

The results of our examination of olfactory bulbs from these three major mammalian groups offer more evidence relating marsupials and placentals and separating them from the monotremes. They also led us to further consider the problem of variations in the structure of olfactory bulbs in mammals and in the other

amniote classes, particularly with regard to the arrangement of output cells into monolayers in some animals and not in others.

Materials and methods

We studied olfactory bulbs of the following animals. Placental mammals: capybara *Hydrochoerus hydrochaeris*, chinchilla *Chinchilla laniger*, kangaroo rat *Dipodomys spectabilis* and rat *Rattus norvegicus*; marsupial mammals: opossum *Didelphis virginiana*; monotreme mammals: duckbill platypus *Ornithorhynchus anatinus* and echidna (spiny anteater) *Tachyglossus aculeatus*; reptiles: python *Morelia spilotes*, copperhead *Ancistrodon contortrix*, Tegu lizard *Tupinambus nigropunctatus* and turtle *Pseudemys scripta*; and birds: chicken *Gallus domesticus*, emu *Dromaius novae-hollandiae*, English sparrow (weaver finch) *Passer domesticus* and dove *Zenaidura macroura*. These specimens are from the collections of the Department of Neurophysiology, University of Wisconsin, and the Laboratory of Comparative Neurology, Michigan State University.

All specimens had been prepared by perfusion with saline followed by formalin. The olfactory bulbs were then removed and embedded in celloidin, except for one platypus bulb which was embedded in paraffin. Sections were then cut and stained with thionine (Nissl method).

Results

Mammals: monotremes versus therians

In placental and marsupial mammals, one of the most distinct features of cell organization of the olfactory bulbs is the layer of mitral cells: a narrow lamina of large perikarya embedded in a thin basement layer of small granule cell bodies (fig. 1, M). As figure 1 shows, there is no such mitral cell layer, meeting *Ramón y Cajal*'s [1955, p. 7] criteria of regularity of position and alignment in the olfactory bulbs of either of the monotremes, platypus or echidna. In the place where one would expect the mitral cell layer, there is but a vague line formed by some granule cells. This line of granule cells forms the outer boundary of the internal plexiform layer. When appropriately stained, the internal plexiform layer can be distinguished from the external plexiform layer by the greater degree of myelination in the internal layer.

In the monotremes, the only large perikarya present are in the external plexiform layer, occupying a zone immediately deep to the external granule cells which surround the glomeruli. Such cells were designated as tufted cells by *Ramón y Cajal* [1955, p. 7], and the more internal their location the greater is the size of their perikarya. This characteristic is very evident in the echidna as well as in the opossum illustrated in figure 1.

Other amniotes: reptiles and birds

Variations in the layering of large perikarya occur in the olfactory bulbs of the other two amniote classes of vertebrates, the reptiles and the birds.

Among reptiles, we observed the large perikarya in a loosely arranged layer, like that of the tufted cells, in the python (fig. 1) and the Tegu lizard, while in the turtle there is a layer like that of the mitral cells, broad but nonetheless distinct. Our observations in reptiles are confirmed by the more extensive and systematic studies of *Rudin* [1974]. He found a monolayer of mitral cells in the alligator *Caiman crocodilus;* a more scattered layer of mitral cells distinct from a cell-free outer plexiform layer in the turtle *Testudo denticulata;* a still more scattered layer of large perikarya in the two lizards *Chalcides ocellatus* and *Lacerta sicula;* and scattered perikarya not distinct from the outer plexiform layer in the snake *Natrix natrix* and the limbless lizard *Blancus cinereus*.

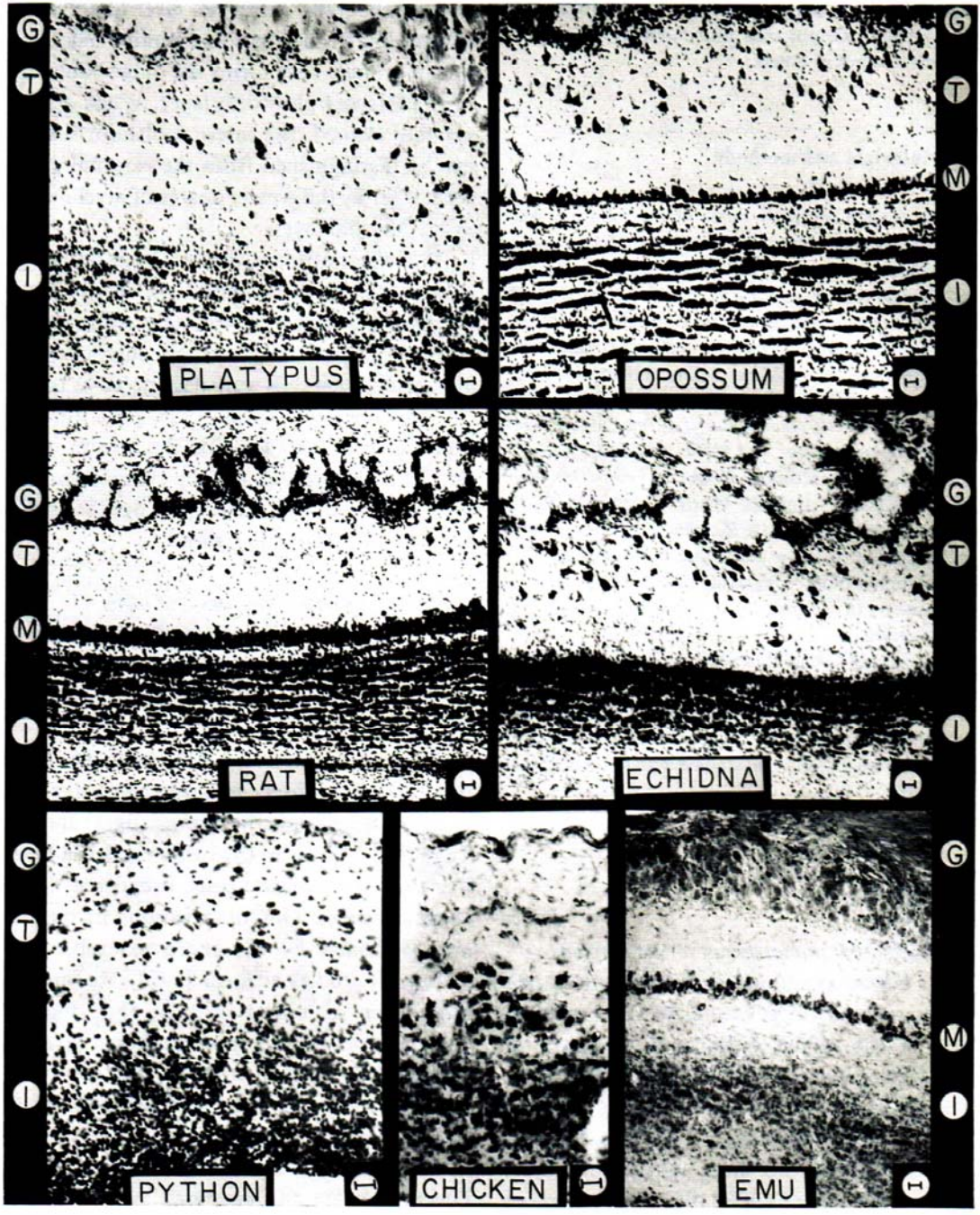

Fig. 1. Sections through layers of the olfactory bulb of selected vertebrates, in a plane perpendicular to the layers. Marsupial and placental animals, here represented respectively by opossum (*Didelphis virginiana*) and rat (*Rattus norvegicus*), show four distinctive layers of cell bodies going from the external sur-

Many birds have a layer like that of mitral cells; these include emus (fig. 1) and kiwis *Apteryx australis* [*Craigie*, 1930]. Others, such as chicken (fig. 1), have a layer of more moderate condensation. Still others, such as the 'English sparrow', have the diffuse broadband characteristic of tufted cells.

Thus, an almost complete range of variation is found among birds and among reptiles (fig. 2). Mammals, however, are sharply dichotomized, as illustrated in figure 2, into (1) prototherian monotremes who have only a broad zone of diffusely distributed large cell bodies and (2) therian marsupials and placentals, who have a condensed layer of mitral cell bodies, distinct from a zone of scattered perikarya of tufted cells.

Assessory olfactory bulb

Another instance where a distinct monolayer of perikarya occurs in one species, while it is absent in others, is found in mammals, in the accessory olfactory bulb. As shown in figure 3, this structure is in most mammals devoid of any semblance of distinct monolayer of mitral cell perikarya [cf. *Ramón y Cajal*, 1955, p. 120]. But in chinchillas and capybaras the large perikarya of the accessory olfactory

face inward: these are the glomerular layer (G), the layer of tufted cells (T), the monolayer of mitral cells (M) and the layer of internal granule cells (I). The mitral cell monolayer is not present in the monotreme mammals platypus *(Ornithorhynchus anatinus)* and echidna *(Tachyglossus aculeatus)*. Monotremes do show a distinction between the internal and external plexiform layers which are separated by the mitral cell layer in other mammals: the inner plexiform layer is more heavily myelinated and has a greater density of glial cell bodies, best seen here in the section from echidna. Both reptiles and birds show a range of variation in the disposition of large perikarya, ranging from a broad zone, as in the python *(Morelia spilotes)*, through a more condensed aggregation as in chicken *(Gallus domesticus)* to a near-monolayer as in emu *(Dromaius novae-hollandiae)*. Thionine (Nissl method). Scale bars each represent 20 μm.

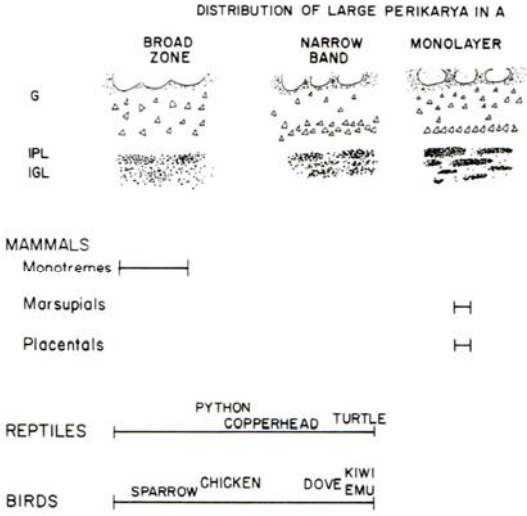

Fig. 2. Variability in the arrangement of large perikarya in the olfactory bulbs of three vertebrate classes. Diagram at top illustrates three grades of this arrangement: G = glomerular layer; IPL = internal plexiform layer; IGL = layer of internal granular cells. Large perikarya can be loosely distributed (as in platypus, echidna and python in figure 1), more condensed (as in chicken and emu in figure 1), or form a distinct monolayer of mitral cells along with a dispersion of tufted cells (as in opossum and rat in figure 1). Mammals can be sharply dichotomized based on this arrangement: prototherians (monotremes) have only the broad zone of dispersed perikarya; therians (marsupials and placentals) all have the dispersed tufted cells plus the mitral cell monolayer. Both reptiles and birds show examples from the range of possibilities as exemplified by sparrow *(Passer domesticus)*, chicken *(Gallus domesticus)*, dove *(Zenaidura macroura)*, [*Crosby and Humphrey*, 1939], kiwi *(Apteryx australis)*, and emu *(Dromaius novae-hollandiae)*; python *(Streptopelia risoria)*, copperhead *(Ancistrodon contortrix)* [*Crosby and Humphrey*, 1939] and turtle *(Pseudemys scripta)*.

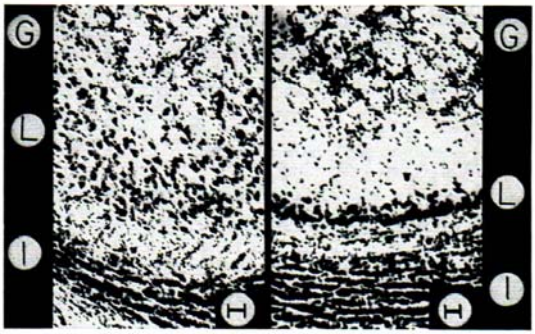

Fig. 3. Contrasting arrangement of large perikarya in the accessory olfactory bulbs of two mammals, both placental rodents. At left, the large perikarya in the kangaroo rat *(Dipodomys spectabilis)* are arranged in a loose band as is the case in most mammals; some show near-monolayered arrangement as in this example from chinchilla *(Chinchilla laniger)* at right. G = Glomeruli; L = large perikarya; I = internal granule cells. Scale bars represent 20 μm.

formation are arranged into a compact layer much like that of the mitral cells of the main olfactory bulb.

Discussion

Combining the observations by *Andres* [1970] of cyclostomes, elasmobranchs and amphibians with those by *Rudin* [1974] of reptiles and our own of reptiles, birds, and mammals reveals one common feature: the largest of the large perikarya are found deepest in the external plexiform layer, more superficial large perikarya are progressively smaller as they lie more externally in the bulb. Two characteristics mark the most deeply situated, largest, perikarya: their horizontal dendrites are greater in length than are those of smaller perikarya, and in many instances the perikarya become aligned, in extreme cases into a monolayer sheet of mitral cells. Since all the animals we have considered have the diffusely distributed large perikarya which may be considered as tufted cells, and only certain ones have the mitral cell monolayer, we suggest that the mitral cells are a specialized, layered subset of the tufted cells. Since both mitral and tufted cells contribute axons to the major efferent pathway from the olfactory bulb, the lateral olfactory tract [*Switzer*, 1973], they together make up the class of principal output neurons in the terminology of [*Shepherd* [1974]. The distinctive feature of the subset of mitral cells is the laminar alignment of their perikarya.

Why lamination? Other systems

Although little is yet clear as to how functional significance is related to laminar arrangements of neuron perikarya, there have been several attempts to correlate this structural order with specialization of function.

(1) The alignment of the perikarya of Purkinje cells in the cerebellum into a monolayer is found in animals with complex organization of bodily movement, while less complicated animals exhibit the perikarya of Purkinje cells in bands several cells thick. It is tempting to suggest that the monolayering of Purkinje cells parallels the attainment of refined motor control. *Braitenberg and Atwood* [1958] suggested that this laminar arrangement serves for converting arrays in a temporal dimension into spatial arrays and vice versa, and *Shepherd* [1974] shows how this can be a general property of several laminated regions of vertebrate brains. If it is characteristic of precisely arranged perikarya that their dendritic elements are isodimensional, then the alignment of the perikarya on a surface implies mutual alignment of similar dendritic elements among neighboring perikarya. Identical dimensions among homologous elements within a population of Purkinje cells may have meaning in

terms of equal electronic length or equal time course of activation. Activation of a particular population of Purkinje cells for a precise motor act must occur within a very narrow time spectrum, that is, it must be an isochronic event.

(2) *Parks and Rubel* [1975] have made significant progress in correlating function with structural specialization. In the auditory nuclei of chicken the monolayer of perikarya of nucleus laminaris is in a precise geometric relationship to the nucleus magnocellularis. Their evidence suggests that the graded difference in length of the axons projecting, in a point-to-point mapping, from nucleus magnocellularis to nucleus laminaris, allows graded transmission or propagation delays which play a role in sound localization.

(3) The visual systems of vertebrates, and particularly those of mammals, abound in laminated structures: retinas, optic tecta, lateral geniculate nuclei and visual neocortices all show elaborate degrees of precise layerings of cell bodies. The preservation of spatial relationships among neural activities evoked by visual stimuli might be regarded as a functional advantage conferred by the laminar arrangements; however, similar topological fidelity is maintained in the somatic sensory pathways in the absence of pronounced monolayering. A clear delineation of the advantages of lamination in visual neural structures remains elusive. It may well be that temporospatial interrelationships in neural activity are more important in visual perception than in other sensory modalities, and the temporal aspect should receive more attention.

Mitral cells

Since we have little understanding of the kind of processing that takes place in the olfactory bulb it is difficult to speculate on what the advantages are of monolayer arrangement of perikarya of mitral cells. If the action of the olfactory bulb is that of a coincidence detector, a generalized frequency filter and signal amplifier, then precision through isodimensional elements would seem crucial.

A more specific advantage of the monolayering of mitral cells in particular may reside in the properties of dendritic bundling as set forth by *Scheibel and Scheibel* [1975]. They describe the horizontal dendrities of mitral cells in rats as forming bundles similar to those seen in other regions of the central nervous system. Such bundles can serve, in their view, as a system of interacting membranes which make up the repository of 'central programs' which shape regular patterns of neural output. Alignment of mitral perikarya would facilitate the formation of these bundles of horizontal dendrites (as, indeed, the bundling of dendrites would facilitate the alignment of the perikarya). This conjecture concerning the relationship of bundling and alignment can be checked by comparing (1) the extent of dendritic bundling in those birds or reptiles displaying a narrow band of mitral cells with those exhibiting a broad band and (2) the accessory olfactory formation of animals displaying a compact layering of perikarya (chincilla and capybara) with that of almost any other mammal, for the degree of dendritic bundling.

The olfactory formations of either reptiles or birds may be explored to determine what electrophysiological differences exist among animals whose mitral perikarya exhibit degrees of lamination ranging from loose to compact. Among mammals, the accessory olfactory bulb may provide material for a similar analysis. In either case one may test a hypothesis in each extreme and remain within the same animal class.

Finally, the main olfactory bulb of mammals may provide the most critical tests of the functional implication of the monolayered structural specialization. The mammals are clearly dichotomized into therians, with a pronounced mitral monolayer, and monotremes, with no monolayer of large perikarya. What can the therians do with their dramatic and precise lamina of mitral cell bodies that the monotremes cannot?

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References

Andres, K.H.: Anatomy and ultrastructure of the olfactory bulb in fish, amphibia, reptiles, birds and mammals; in Wolstenholme and Knight, Taste and smell in vertebrates. A Ciba Foundation symposium, pp. 177–196 (Churchill, London 1970).

Braitenberg, V. and Atwood, R.P.: Morphological observations on the cerebellar cortex. J. Comp. Neurol. *109:* 1–27 (1958).

Craigie, E.H.: Studies on the brain of the kiwi *(Apteryx australis)*. J. Comp. Neurol. *49:* 223–357 (1930).

Crosby, E.C. and Humphrey, T.: Studies of the vertebrate telencephalon. I. The nuclear configuration of the olfactorius anterior of certain reptiles, birds and mammals. J. Comp. Neurol. *71:* 121–213 (1939).

Hopson, J.A.: The origin and adaptive radiation of mammal-like reptiles and nontherian mammals. Ann. N.Y. Acad. Sci. *167:* 199–216 (1969).

Kühne, W.G.: Systematic position of monotremes reconsidered (Mammalia). Z. Morph. Ökol. Tiere *75:* 59–64 (1973).

Lillegraven, J.A.: Latest Cretaceous mammals of upper part of Edmonton formation of Alberta, Canada, and review of marsupial-placental dichotomy in mammalian evolution. Paleont. Contr. Univ. Kans. *50:* (1969).

Parks, T.N. and Rubel, E.W.: Organization and development of brain stem auditory nuclei of the chicken: organization of projections from n. magnocellularis to n. laminaris. J. Comp. Neurol. *164:* 435–448 (1975).

Ramón y Cajal, S.: Studies on the cerebral cortex (limbic structures) (Lloyd-Luke, London 1955).

Rudin, W.: Untersuchungen am olfaktorischen System der Reptilien. III. Differenzierungsformen einiger olfaktorischen Zentren bei Reptilien. Acta anat. *89:* 481–515 (1974).

Scheibel, M.E. and Scheibel, A.B.: Dendrite bundles, central programs, and the olfactory bulb. Brain Res. *95:* 407–421 (1975).

Shepherd, G.M.: The synaptic organization of the brain. An introduction (Oxford University Press, London 1974).

Switzer, R.C.: Relationships of the tufted cells of the olfactory bulb to the lateral olfactory tract; PhD thesis East Lansing (1973).

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