Response to Boban et al: computer-assisted 3D reconstruction of the nucleus basalis complex, including the nucleus subputaminalis (Ayala’s nucleus)

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We appreciate the suggestions of Boban et al. (2006) in clarifying the designation of a circumscribed basophilic nuclear complex as depicted in Fig. 2 (Ch4 p?) of our recent publication (Teipel et al., 2005). The basophilia of the neurons forming this cluster was the main reason why we decided to include this cluster into a Ch4al–Ch4p continuum. Furthermore, Hedreen et al. (1984) preferred the Mesulam et al. (1983) terminology as a thorough and very useful description which may prove to be definitive. At the time of our joint MRI-cytoarchitectonic study of the NBC we had no gapless serial sections from the single case investigated because every second section of this series was either stored in formalin or used for paraffin embedding and neuropathological diagnosis (cf. Fig. 1A in Teipel et al., 2005).

Meanwhile, we could cut and stain complete 400 μm thick serial sections [details on the method are given in Heinsen et al. (2000)] from two control cases, a 50-year-old female who died due to aspiration and a 43-year-old-male who died due to embolism of the pulmonary artery.

Our 3D reconstructions (Fig. 1) agree with previous results of Kostovic (1986) and Simic et al. (1999) on the development and architectonics of the nucleus basalis complex. The authors stress the fact that the medial and lateral parts of Ayala’s (1915) nucleus form a continuum. However, this is easily recognized only in the developing brain, whereas in adults the cell clusters forming this nucleus are separated by wide cell-free gaps (Fig. 2). This separation may be resulting from the growth of the vascular system or of myelin-containing fiber tracts (Fig. 3) or, alternatively, from neuronal death. Perhaps all three of these factors contribute to the separation between cell clusters. The wide distribution of individual clusters (Fig. 2, white arrows) can explain why these dissipated parts of Ayala’s nucleus are often neglected or erroneously assigned to Mesulam’s et al. (1983) Ch4p compartment. In the male control case (Fig. 2B) we found a close topographical relationship between the rostral lateral part of Ayala’s nucleus (black arrow in Fig. 2B) and spindle-shaped and less basophilic neurons at the Ch4al–Ch4p transition. Based on simple topographic relationships one would assume that these neurons represent a ramification or transition of the Ch4i cluster into lateral and medial Ch4p compartments on corresponding sides of posterior parts of the anterior commissure. However, the majority of neurons in the rostral subputaminal nucleus were spindle-shaped whereas the majority of Ch4p neurons were multipolar. Both neuron classes have in common a marked basophilia. In the most lateral part of the anterior commissure, neuronal size, shape and basophilia of either the periputaminal or the Ch4p neurons were nearly indistinguishable and cluster size, cluster distribution and lower neuron density in periputaminal clusters served to distinguish Ch4p neuron clusters from periputaminal clusters in our cases.

Our 3D reconstructions supplement Fig. 9 of Halliday et al. (1993). In addition, individual differences in size and shape of the rostral extension of the periputaminal part of Ayala’s nucleus can be seen. Our reconstruction differs from that of Ulfig (1989). We could not identify the dorsal finger-like extension. Ulfig used 800 μm thick sections and conventional profiles of styropor slices for 3D reconstruction. This technique may blur finer details such as nerve-cell clustering in the NBC.

Hedreen et al. (1984) described neurons at the level of the crossing of the anterior commissure that closely resemble NbM neurons. At closer inspection basophilic fusiform to
Fig. 1 Computer-assisted 3D reconstruction of the left nucleus basalis complex (NBC) and neighboring structures of a 50-year-old non-demented woman. (A) view from the occipital pole, (B) ventral view from the anterior perforate substance. In (A) the first of a total of 44 gallocyanin-stained 400 μm thick sections used for the 3D reconstruction was left in situ. Putamen, nucl. accumbens, globus pallidus and anterior commissure are displayed in a transparent mode.
elongated neurons could be found surrounding the anterior commissure at various loci. We have included these clusters in our reconstruction (Figs 1 and 2, magenta). With the exception of the clusters at the level of the anterior commissure cell-density in these clusters is low.

Simic et al. (1999) found the Ch2 cluster to continue into the Ch3 cluster at the basal tip of the paraterminal gyrus. This is in contrast to the observations in our two cases. At the tip of the paraterminal gyrus cellular strands of Ch4am, Ch3 and Ch2 run in parallel for a short distance (Figs 1A and 3B). Ch3 appears to be a prominent component of the NBC (Figs 1 and 2, yellow). However, cell density in this cluster is low and only a high section thickness allows a fairly reproducible delineation. Widely dissipated polygonal, smaller and less basophilic nerve cells in Ch3 distinguish this cluster from Ch4am and Ch2. Consequently, the total cell number (cell density multiplied with the volume of the nuclear complex) will be rather low.

It is possible to manually delineate even highly irregular cell clusters on the surface of serial sections. A section thickness of 400 μm proved to be optimal for investigation of telencephalic hemispheres of adult humans free from section artefacts including wrinkles and fissures. However, this high section thickness absorbs the outlines of delicate neuronal clusters positioned in the depth of a thick section. The full complexity of the human CNS can only be perceived after the microscopic investigation using a stereo microscope. Computer-assisted 3D reconstruction (Amira®) was achieved by combining the individual profiles of nerve cell clusters and by assigning a surface to the 2D profiles. It is impossible to control each step of computation. Therefore, the resultant 3D mathematical/computer model only partially reflects the true size and shape of delicate components (cf. the outlines of Ch2 in Fig. 2) and a 3D reconstruction is not free of artefacts. Therefore, a more comprehensive understanding of structure and function of the intact and diseased nervous system should be based on a multidimensional approach comprising in vivo and postmortem tools of investigation. Quantitative unbiased stereological investigations as proposed by Heinsen et al. (2000) appears to be a useful methodological approach to assess inter-individual and disease-related variability of the neuron number of the NBC. Special designs will be necessary to estimate the neuron number in the widely dissipated nerve cell clusters of Ayala’s nucleus.

In summary, we agree with Boban et al. (2006) that Ch4 p? of Fig. 2 of our recent paper (Teipel et al., 2005) represents NSP. Unequivocal neuroanatomical terminology is an indispensable basis to elucidate the pathogenesis of complex neurodegenerative diseases and to transfer findings of animal models to the highly specialized human brain.
References

Fig. 3 (A) Darkfield exposure of a serial section indicated by the line in Fig. 2A; (B) same section in brightfield. The medial (subputaminal) parts of Ayala’s nucleus are associated either with fibre tracts in this region or with perforating branches of the central anterolateral arteries. This topographic association could explain the arrangement and dispersion of cell clusters of the medial part of Ayala’s nucleus.