Letter: The Indusium Griseum: Anatomic Study with Potential Application to Callosotomy

To the Editor:

We read with great interest the paper by Tubbs et al\(^1\) describing the human indusium griseum (IG) to be a glial membrane above the corpus callosum (CC) without neuronal cells nor connections with the hippocampus. The study, carried out by magnetic resonance imaging and histological and immunohistochemical (IHC) techniques, led the Authors to conclude that transection of this membrane, unavoidable when performing callosotomy (ie, to relieve drug-resistant epilepsy), should not be the reason for the postoperative memory deficit observed in some patients.

However, our current preliminary results seem to indicate that IG contains neurons, some of them immunopositive to the neuronal nitric oxide synthase (nNOS), the enzyme responsible for the synthesis of the nitric oxide (NO).

In our study, the CC and the overlying IG were collected from 13 adult human autopic brains without evident pathologies. The work was approved by the Research Ethics Committee of our University and conducted in line with the principles of the Helsinki Declaration of 1975, as revised in 2008. After the specimens were fixed in formalin, paraffin, or frozen

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**FIGURE.** Sagittal sections of IG. A, B. Staining of IG by Luxol fast blue A and eosin B. The identification of IG is possible thanks to the orientation of its fibers which travel perpendicularly to those of the CC. C. nNOS-positive cells (arrows) are present within the IG, often at the border with CC. Several IG areas contain nNOS-positive nerve fibers (arrowheads). D. NEUN-positive cells are distributed throughout the entire IG (arrows). Scale bars, 100 μm.
sections were cut according to a sagittal plane along the genu of CC and overlying IG. The identification of IG in tissue sections was obtained in light microscopy by Luxol fast blue and eosin staining: both techniques mark the fibers of the IG travelling sagittally, allowing to distinguish them from those of the CC, running coronally (Figure A and B). Immunohistochemical procedure to detect nNOS and neuronal marker (NeuN) showed the presence of nNOS-positive neuronal-like cells (Figure C), and of NeuN-positive cells (Figure D). Neuronal NOS-positive neuronal-like cells displayed bipolar fusiform or rectangular morphologies, were located along rostro-caudal and medio-lateral directions, and often they were found at the boundary between IG and CC (Figure C). The lack of neurons claimed by Tubbs et al was probably due to the fact they did not use neuronal markers for their ihc analyses, being their study based on synaptophysin, neurofilament and Luxol fast blue/periodic acid-Schiff staining of IG. Our observations are in accordance with a previous Golgi study performed in rats and with a very recent paper by Rasonja et al demonstrating that IG in human adult brains is immunopositive to neuronal and synaptic markers. Thus, the presence of neurons and of nNOS positive neuronal-like cells in IG suggest that it is not a merely rudimentary tissue, but likely plays a functional role in the adult brain. As evidenced by a recent immunohistochemical paper the rat IG contains neurons expressing neurokinin-1 receptor, sending fibers to the underlying CC and to the cingulate cortex (Cg). Altogether, these observations suggest an intercommunication among IG, CC and Cg, leading to the hypothesis that CC lesioning could provoke functional detrimental effects.

Disclosures

UNIVPM (PSA 2016) to Dr Fabri. Fellowship to Dr Lorenzi was supported (60%) by PSA 2016. The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.