Visual inter-hemispheric processing: Constraints and potentialities set by axonal morphology

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Abstract — The largest bundle of axonal fibers in the entire mammalian brain, namely the corpus callosum, is the pathway through which almost half a billion neurons scattered over all neocortical areas can exert an influence on their contralateral targets. These fibers are thus crucial participants in the numerous cortical functions requiring collaborative processing of information across the hemispheres. One of such operations is to combine the two partial cortical maps of the visual field into a single, coherent representation. This paper reviews recent anatomical, computational and electrophysiological studies on callosal connectivity in the cat visual system. We analyzed the morphology of individual callosal axons linking primary visual cortices using three-dimensional light-microscopic techniques. While only a minority of callosal axons seem to perform a strict ‘point-to-point’ mapping between retinotopically corresponding sites in both hemispheres, many others have widespread arbors and terminate into a handful of distant, radially oriented tufts. Therefore, the firing of a single callosal neuron might influence several cortical columns within the opposite hemisphere. Computer simulation was then applied to investigate how the intricate geometry of these axons might shape the spatio-temporal distribution of trans-callosal inputs. Based on the linear relation between diameter and conduction velocity of myelinated fibers, the theoretical delays required for a single action potential to reach all presynaptic boutons of a given arbor were derived from the caliber, \( g \)-ratio and length of successive axonal segments. This analysis suggests that the architecture of callosal axons is, in principle, suitable to promote the synchronous activation of multiple targets located across distant columns in the opposite hemisphere. Finally, electrophysiological recordings performed in several laboratories have shown the existence of stimulus-dependent synchronization of visual responses across the two hemispheres. Possible implications of these findings are discussed in the context of temporal tagging of neuronal assemblies. © 1999 Éditions scientifiques et médicales Elsevier SAS

1. Introduction

1.1. Two brains for one body?

Before investigating possible correlations between structure and function at the level of single axonal trees, let us briefly consider more generally how the macroscopic structure of organisms relates to their behavior and, in turn, to the structure of their brains. Despite the fascinating diversity of forms encountered in the animal kingdom, both sensory and motor organs of almost all species, at least all arthropods and vertebrates, come pairwise and are located on each side of their longitudinal axis. Only those organs involved in internal regulation fail to comply with this rule, suggesting that the symmetric layout applies manifestly to the apparatuses enabling organisms to relate with their environment. Indeed, our senses basically proceed by a balance between pairs of sensors, as our acts result from a dynamic equilibrium between pairs of effecters, and our decisions often follow judgments from contrasting points of view. If one considers, as modern cognitive neuroscience teaches us to do, that the organization of mental representations somehow reflects the structure of the brain, it is not surprising that our encephalon also comes in two halves. Yet, the essence of this bipartition remains far from being clear.

From early on, neurology and neuropsychology indicated a marked dichotomy between a ‘dominant’ hemisphere, usually the left one in right-handers, committed to analytical, sequential, verbal, local, rational or objective processing, and a ‘minor’ hemisphere concerned rather with operations requiring synthetic, global, spatial, more intuitive or affective abilities. Such a trend to conceive each hemisphere as an entity able to achieve a variety of perceptual or mnemonic tasks on its own was encouraged by famous observations in commissurotomized patients [6, 17,
66]. Nowadays, techniques allowing the monitoring of activity in the living brain demonstrate that well-defined tasks are accompanied by localized changes in activation levels. A marked asymmetry in the activation patterns is observed for most tasks, confirming the undeniable hemispheric functional specialization. However, even simple tasks do not involve exclusively one hemisphere; since in real life we keep swapping between left-dominated and right-dominated operations, the harmony of cerebral functions relies on a dynamical cooperation between both sides of the brain, and calls for a considerable traffic of information across the two hemispheres.

In addition to sub-cortical and anterior commissures, the major path for interhemispheric communication is the corpus callosum. Contrasting with the amount of research devoted to this pathway and the amazing popularity of the concept of interhemispheric cooperation, it is striking to realize how little we know about the neuronal mechanisms of interhemispheric integration (see [31]). This situation is probably due to the abundance of callosal fibers and to their manifold functions: callosal connections exist for sensory, motor, associative, frontal or limbic cortices, and they link heterologous as well as homologous areas. In order to study the role of the corpus callosum in interhemispheric integration, it seems wise to choose a system where straightforward questions can be addressed. In this respect, the primary visual cortex offers the advantage that, as recognized a century ago by Ramón y Cajal [57], at least one function of callosal connections might be inferred from the topographic organization of the visual system.

1.2. Need for interhemispheric cooperation in vision

In all mammals, each hemisphere handles visual information seen in the opposite hemifield. In inferior mammals, such as rodents or lagomorphs, which have lateral eyes and limited binocular vision, axons from almost all retinal ganglion cells cross the midline at the optic chiasm and terminate within the contralateral geniculate nucleus. In higher mammals with frontal eyes and a large binocular visual field, such as cats or primates, axons arising from ganglion cells located in the nasal half of the retina cross the midline and project to the contralateral thalamus, whereas axons from the temporal retinæ remain on the ipsilateral side. Since each hemisphere only gets primary sensory inputs from one half of the visual field (figure 1), it follows that the visual world is represented in two physically discontinuous cortical maps, split across the two hemispheres along the central vertical meridian of the visual field (CVM).

Despite this conspicuous gap in the cortical representation of the visual field, we do not perceive any fracture along the vertical midline. On the contrary, the main function of the elaborate circuitry responsible for gaze control is to capture relevant objects within the very center of the visual field where detailed analysis can be best achieved. As Ramón y Cajal stated in his chapter on ‘the necessity for the corpus callosum’ [58], this “suggests the postulate that correct mental perception of the visual space cannot be achieved without the existence of a bilateral perceptual brain center whose halves act in a concerted way with one another, in a way that unifies and places in the same orientation the two images that left and right retinas project”. In other words, the perceptual representation of the visual field has to be integrated across the hemispheres. In the following sections, we will briefly review classical anatomical and physiological data on callosal connectivity between primary visual cortical areas and summarize more recent data about single axon morphology and multiple recordings.

2. Basic layout of callosal connections between primary visual areas

In each hemisphere, one half of the visual field is represented in several different maps, each of them corresponding to a cytoarchitectonic area. Among those numerous maps, only the ‘primary’ ones (A17 or V1, and A18 or V2) cover the entire hemifield and respect a strict retinotopic organization. Furthermore, the projections in A17 and A18 are contiguous and arranged in a mirror-like fashion, both maps sharing a common representation of the CVM (cats: [70]; humans: [63]). As a consequence, the 17/18 border zone matches the CVM projection line, which is also the edge along which the cortical representation shifts from one hemisphere to the other (figure 1).

Remarkably, the 17/18 border also coincides with the narrow portion of primary visual areas where callosal connections are present. Indeed, retrograde and anterograde tracing techniques demonstrated that whereas most parts of areas 17 and 18 are free of interhemispheric projections, cell bodies and axonal terminals of callosal neurons are densely packed along their common border (reviewed in [28]). Seen from the cortical surface, the callosal zone forms a ribbon running along the CVM representation [40, 53]. The callosal ribbon is slightly wider in the representation of the area centralis, where the magnification factor is maximal, but as a whole, taking into consideration the smaller size of central receptive fields, the portion of visual field represented within the callosally connected zone has the shape of an hour-glass centered on the CVM and spanning several degrees apart from it on both sides [55]. In the cat, additional patches of callosal connections are found in more lateral parts of A18, but those regions were shown to be islands of
Mammals with frontal, binocular vision  
(Man, monkey, cat,..)  

representation  

Mammals with lateral, monocular vision  
(rabbit, rat,..)  

Figure 1. Visuotopic organization of the retino-geniculo-cortical pathway. Bottom. The central 150º of the visual field are represented as the arc of a circle, with its left and right halves in shades of green and purple respectively, the intensity of the color being proportional to eccentricity. The blue and orange arcs indicate the portions of the visual field seen through the left and right eye, respectively. The large black arrow represents a visual scene crossing the vertical meridian (dotted line). Center. Schematic of the visual pathways. Colors indicate the respective projections from the temporal retina of the left eye (light blue), the nasal retina of the left eye (dark blue), the nasal retina of the right eye (red) and the temporal retina of the right eye (yellow). Thin lines show the resting position of the optic axes. The head and the tail of the arrow represent the fragments of the visual scene projected at successive processing stages (retina, thalamus, cortical areas 17 and 18). Whatever the species, each hemisphere only receives direct information about the opposite half of the visual field. The cortical projection of the central vertical meridian (thick dotted line) coincides with the border between areas 17 and 18. Top. Theoretical perceptual representation of the visual field. Dense, reciprocal interhemispheric connections between the left and right 17/18 borders are supposed to link to the two partial maps and thereby allow coherent processing of visual scenes.

displaced cells representing fragments of the CVM [45, 60]. These fundamental observations corroborate the hypothesis that the primary function of callosal connections between A17 and A18 is to link the midline representations across the two hemispheres.
Accordingly, electrophysiological recordings showed that the receptive fields of trans-callosally activated neurons [10], as well as of single callosal fibers [3, 25] or of antidromically identified callosal efferent neurons [21, 27] are located on the CVM or in its immediate vicinity. Furthermore, the use of the split-chiasm preparation made it possible to demonstrate that trans-callosal and ipsilateral, geniculo-cortical inputs converging onto a given target neuron are precisely matched in their selectivity for stimulus orientation, direction and velocity, and that receptive fields plotted through both pathways are virtually superimposed [4, 47].

Taken as a whole, classic anatomical and physiological data seem to indicate that interhemispheric connections are extremely precise and meet the requirements for achieving a point-to-point mapping between corresponding loci of the two hemispheres. However, detailed investigations using modern morphological techniques revealed that the organization of callosal connections is more complex than previously thought.

3. Diversity of cellular components within the callosal pathway

3.1. Callosal connections stem from neurons with different morpho-chemical phenotypes

During the last decade, the technique consisting of in vivo retrograde labeling followed by in vitro intracellular filling with lucifer yellow favored a more thorough analysis of cellular morphology than possible with horseradish-peroxidase techniques. Studies using this new method indicated that, as expected from their electrophysiologically and histochemically recognized excitatory function, the vast majority of callosal neurons are large pyramidal cells; however, as much as one-third of callosal cells have a different phenotype [8, 71]. Among those are spiny stellate, but also smooth stellate and fusiform cells, which suggests that some callosal neurons could use inhibitory transmitters. Despite the failure to identify GABA-containing callosal neurons, using either immunocytochemistry or selective uptake of radio-labeled transmitter ([13]; but see [11]), the hypothesis of an inhibitory component remains compatible with the occasional observation of symmetric callosal synapses as well as with the electrophysiological disclosure of short-latency transcortical inhibition after pharmacological or cryogenic manipulations [56, 67]. Therefore, given their various morphological and chemical phenotypes, callosal neurons do not constitute a homogenous population.

3.2. Visual callosal axons display a significant variability in their caliber

Electron microscopic studies in cats have shown that about two-thirds of visual callosal axons are unmyelinated fibers between 0.08 and 0.40 µm in diameter, whereas the remaining third consists of myelinated axons ranging from 0.25 to 4.0 µm [2]. This indicates that callosal axons display a considerable variability in caliber. However, these values were derived from samples of the posterior part of the corpus callosum, where axons from all visual areas are known to travel [36]. Since this caliber heterogeneity might have important functional implications (see below), it was interesting to investigate whether it also held for axons originating from well-defined portions of primary visual cortex.

The recent development of sensitive anterograde tracers allowed the inspection of individual callosal axons (see [24] for technical details and extensive description of the results). Briefly, small amounts of biocytin were injected extracellularly close to the area centralis projection along the 17118 border in adult cats; after adequate survival time, animals were killed and perfused with buffered aldehydes; the tracer was then revealed in serial sections, using a standard avidin-biotin-peroxidase histochemical procedure. The resulting material was highly contrasted and allowed the unambiguous distinction of stained axonal processes. Typically, a tracer injection yielding an uptake zone about 500 µm wide and spanning throughout the entire cortical thickness labeled callosal axons whose diameters were found to range between 0.25 and 2.25 µm, when measured at the midline (figure 2). Although this method obviously overlooks axonal segments falling below the resolution of the light-microscope (ca. 0.25 µm), it demonstrates that even within a restricted volume, corresponding roughly to the size of a functional cortical module, callosal neurons send axons with calibers differing by at least one order of magnitude.

Considering this wide range in axonal caliber and the diversity of cellular phenotypes, it is likely that the callosal pathway actually comprises several channels with diverse morphological and physiological features, playing distinct roles in the interhemispheric processing of visual information. Therefore, it seemed crucial to investigate the relations established by single callosal neurons. Since biocytin yields a complete staining of neurons, it allows one to follow all the branches stemming from a given neuron and, in principle, to reconstruct its entire axonal tree throughout a stack of consecutive sections. From a practical point of view, such an approach is surely exacting; providentially, techniques for computer-assisted microscopy underwent considerable progress during the last decade and
now enable one to examine the precise three-dimensional morphology of long-range projecting axons. Using the Neurolucida™ equipment [18], we performed such reconstructions for a sample of 20 callosal axons from cat visual cortex [24](Houzel and Milleret, unpublished results). Briefly, axons labeled by extracellular injections of the tracer were randomly selected at the midline and traced up to their distalmost endings in the target hemisphere, with a final magnification of 2000x, yielding a resolution of 0.23 µm. The acquisition program registered the spatial coordinates and geometrical properties of axonal segments, which could then be followed from one 75 µm-thick section to the next. Using a graded ocular under high magnification the diameter of each axon segment was recorded. Particular care was taken to register changes in diameter which occurred frequently at branching points, and to plot all differentiations characteristic of presynaptic structures, such as short-stalked boutons or en passant swellings. Tissue deformations resulting from the histological procedure were also estimated and corrected accordingly. Analysis of the spatial geometry of the reconstructed trees was done using ad hoc software (‘Maxsim’, see below).

3.3. **Callosal axons differ in the size of their terminal territories**

A puzzling result of our study was the diversity in the size of the terminal arbor of individual axons. Only a minority of them (three out of 20) terminated into a sparsely ramified tuft and distributed their boutons within a single conical volume (see arbor A in figure 3). The estimated cortical surface covered by such axons was found to be restricted to a few hundred µm². Interestingly, these narrow arbors were also restricted in their laminar distribution and occurred in anterior portions of the 17/18 border, i.e., in regions with relatively lower magnification factor than within the area centralis projection zone.

All other axons started to divide in the white matter, where they gave rise to a few branches which ramified extensively within the cortex. After all boutons of a given axon were projected radially onto the cortical surface, the smallest possible convex polygon encompassing all of them was usually larger than 0.25 mm² (12/17 axons), and could reach as much as 9 mm². Thus, callosal axons linking primary visual areas display a high variability in the extent of their terminal territory. Given the local magnification factors of visual maps in the origin and target zones, this implies that most callosal neurons do not establish a strict point-to-point mapping between topographically corresponding loci of the two hemispheres. As a rule, the heaviest site of termination is aimed at the homotopic contralateral site, but many axons form diverging, heterotopic connections between one cortical locus at the 17/18 border and several loci spread throughout the contralateral A 17, A 18 and 17/18 border (see examples C and D in figure 3). Conspicuous branches bearing numerous preterminal boutons (see micrograph in figure 3D) are thus aimed at cortical regions representing quite peripheral sectors of the visual field, and where physiological techniques failed to reveal any supraliminal influence from the contralateral visual cortex. This observation suggests that trans-callosal inputs do not merely contribute to the construction of the receptive fields of target neurons, but are likely to serve alternative or additional functions.

3.4. **Individual callosal neurons terminate within distinct columnar and laminar compartments**

Despite the diversity in the overall tangential extent of the terminal territory of these profuse arbors, their boutons were invariably clustered into several cortical domains which, having the shape of radially-oriented cylinders, will be referred to as ‘terminal columns’ although there is as yet no downright relationship
Figure 3. Architecture and topography of visual callosal axons. Four axons (A-D) of increasing projection territory are shown with different magnifications and viewing angles. Shaded band indicates layer IV, triangles point to the center of the 17/18 transition zone; ruler marks are placed every 500 µm. Left insets. Location of the respective tracer injection within the opposite hemisphere, labeled zone in black. Right columns. Schematic of the injection sites (inset) and of the modular clustering of callosal terminals. Boutons were projected onto the pial surface of a smoothed cortex for two-dimensional cluster analysis, and grouped according to their laminar position. The shading of each terminal sector is proportional to its contribution to the entire terminal territory of the axon (percentile of the total number of presynaptic boutons). **A.** Simple axon with restricted, homotopic termination. **B.** For this axon, two terminal columns, aligned along the rostro-caudal axis, appear upon changing the viewing angle. Rotation is indicated by the dorsal direction (arrowhead) and the standard Horsley-Clarke frontal plane (gray triangle) which was used for sectioning the brain. On the enlarged 3D views, arbor and boutons are plotted separately to ease identification of radial clusters. **C.** Axon with two terminal columns aligned along a medio-lateral direction; enlarged details from the front view show that the proximal and narrow column (in A18) is fed by a thinner branch than the more distal and larger bouquet (at the 17/18 border). **D.** This axon was labeled by the same injection than C; it terminates into five widely spaced columns. Details from the lateral-most column show branchinns patterns and preterminal structures (arrow in the microphotograph point to en passant boutons).
between them and the radially oriented functional modules characteristic of visual areas, such as orientation- or ocular dominance columns. Actually, callosal terminal columns are very irregular since, in our limited sample, their diameter varied from 100 to 800 µm, the spacing between two neighboring columns of an axon was found to range between 100 and 2000 µm and the number of terminal columns per axon ranged from 2 to 8. Furthermore, the boutons were not distributed homogeneously throughout the depth of terminal columns, but rather concentrated in certain layers. The majority of all columns (35 out of 50 for all the 20 axons) displayed a high density of boutons in supragranular layers, often associated with a secondary but substantial infragranular component (16/35). Other columns contained boutons spread throughout all layers (9/50), while fewer were restricted to layers IV (3/50) or V-VI (3/50). Axons usually terminated into several columns of different laminar distribution. The only clear trend in this intricate pattern was that all simple axons, terminating into a single column, were invariably restricted to the thalamo-recipient layer IV. To clarify possible hierarchical principles for this pathway, it will be necessary to correlate the spatial distribution of contralateral terminals of callosal neurons with the laminar location and the morphological phenotype of their cell bodies in the source hemisphere (see Conclusion).

One important issue is the functional meaning of the discrete, columnar layout of callosal projections. Thirty years ago, applying the newly developed Nauta technique to visualize fibers degenerating after section of the entire corpus callosum, Heimer et al. [22] already noted that the overall distribution of callosal terminals was uneven over the cortical surface. Closer inspection with progressively more sensitive methods suggested a tendency of callosal neurons and terminals to form patches along the VI/V2 border [7,71], but the significance of this pattern remained elusive. Recently, Olavarria and Abel [52] showed that callosal connections link preferentially cortical domains characterized by strong cytochrome-oxidase activity which, at least in primates, are undoubtedly related to segregated processing streams [9]. Although this correlation provides a first hint that the patchiness of interhemispheric linkage might relate to the functional compartmentalization of visual areas, it is not known how far this might hold for the relations established by single axons. This aspect could be investigated further by combining anatomical experiments with the functional determination of cortical domains (see Conclusion). For the time being, the careful observation of purely morphological data might provide additional insights into the consequence of this columnar pattern for information processing.

### 3.5. Callosal axons differ in their architecture

Axonal bifurcations were usually accompanied by the shrinkage of daughter branches, whose respective diameters could be reduced by the same factor or not. Detailed inspection of such branching points was carried out for entire trees, and yielded a comparison of the relative caliber of ramifications supplying their distinct terminal columns. Since many arbors were exhaustively described in a previous paper [24], we will simply use the three representative examples illustrated in figure 3 to underline the main conclusions.

The axon shown in figure 3B forms two radial clusters of boutons which, as can be easily perceived from the top and side views, are supplied by two independent branches that parted from the main trunk within the white matter. Since those branches had very similar calibers, and ran almost side by side for a considerable distance (ca. 2 mm), the architecture of such axons could be qualified as ‘parallel’.

In contrast, axon C, which also terminated into two columns, was characterized by a tangentially running trunk from which arose a set of radially ascending branches of different calibers. As can be seen in the enlargement of the front view (‘main branchings’; figure 3C), the proximal branch supplying a narrow column within A18 was much thinner (0.26 µm) than the trunk, which maintained its original caliber (1.12 µm) for several millimeters, until it ramified into a second, more distal tuft at the 17/18 border. In opposition with the previous type, the architecture of such axons was qualified as ‘serial’.

Many axons that terminated into more than two columns displayed more complex branching patterns. One such tree is shown in figure 3D. It terminated into five columns spread throughout areas 17 and 18. The detailed front view reveals that the most proximal bouquet (in A18) was supplied by 2-3 offshoots forming a typical ‘serial’ column. On the other hand, the top view indicates that the remaining part of this tree was characteristic of what was defined as a ‘parallel’ architecture, since the two broad columns (on the 17/18 border) are fed by branches with comparable calibers, traveling in a similar direction over a considerable distance. Finally, one can distinguish an additional branch running between these two columns, with a rostro-caudal direction, i.e., exactly along the 17/18 border. This suggests some degree of reconvergence and the existence of cross-talks between spatially distant terminal columns (see also figure 4D).

Therefore, callosal axons display one more variable feature, namely their architecture; in other words, they differ in the geometrical properties of the branches supplying their different columns. Since conduction...
Figure 4. Simulation of the spatio-temporal activation profiles for individual callosal axons. A. Quantitative values returned by our simulation or measured physiologically by different authors (shaded columns). From left to right: diameter of the axonal trunk of the reconstructed arbors (as measured at the midline); raw conduction velocity, derived from the linear equation of Waxmann and Bennet [73]; conduction velocity after correction for tissue shrinkage; conduction velocity measured in vivo by Harvey [21]; latency required for a simulated action potential to travel from the midline to the first bouton of the arbor; latency estimated after antidromic activation by McCourt et al. [43]. B. Activation profile of a ‘parallel’ tree (see figure 3B for its morphology). In the front view as well as in the progressively enlarged side views, axonal segments and boutons are colored according to their time of activation (µs), considering that the spike crossed the midline (arrow) at time zero. Histograms (100 µs binwidth) indicate the distribution of activation times for the entire arbor (top) and for individual columns (a and b). C. Activation profile of a ‘serial’ axon (morphology in figure 3C). Different side views corresponding to successive time points (t1-t7) are shown. The portion of the arbor (t1-t3) or the fraction of boutons (t4-t7) activated during a temporal window of 100 µs is shown in red, while the rest of the tree is in gray. D. Activation of a complex axon (morphology in figure 3D). Front view and lower top view: segments are color-coded according to their activation time, as in B. In the upper top view, colors indicate the primary level of bifurcation to ease the recognition of the converging branch (see text).
velocity is tightly related to axonal caliber, these geometrical properties have direct consequences on the timing of trans-callosal impacts.

4. Possible implications of axonal architectures for temporal processing

4.1. Investigation of the computational properties of axonal trees

As already mentioned by Segev and Schneidman [61], a decisive step in modeling the electrical properties of axons was the work of Goldstein and Rall [19], indicating how branching points or abrupt changes in diameter modify the impedance of conducting segments and thereby affect the shape as well as the velocity of traveling action potentials. With computer-simulation of compartmental Hodgkin-Huxley models, the theoretical consequences of geometrical irregularities on spike propagation and bouton activation were re-investigated in great detail [38, 39]. Particularly elegant, dynamic implementation of such models recently allowed the examination of more complex, and hence more realistic axonal trees [41]. These approaches suggest that minor irregularities, such as varicosities or branching points, are likely to delay or accelerate the propagation of a spike. However, they also indicate that most of the time required to activate an axonal tree results from the conduction along its long, homogenous branches: “in other words, the pure delays from the axonal cables (placing the branches end to end and neglecting the intervening branch points and varicosities) account for most (67-78%) of the delay” [42]. Therefore, despite the lack of information about many parameters important for accurate modeling, such as the local density of ionic channels at branching points and at nodes of Ranvier [5, 74], it seemed reasonable to use currently available data to derive average conduction delays. Furthermore, all callosal axons we reconstructed had a trunk diameter greater than 0.25 µm, and were thus presumably myelinated. Since the conduction velocity of myelinated axons increases linearly with fiber diameter, our task was greatly simplified.

Rather than simulating the successive bioelectrical events responsible for spike propagation, we chose a simple approach consisting of the following steps: First, electron microscopic examination of some samples (Aggoun-Zouaoui et al., unpublished observations) indicated that biocytin specifically stained the axoplasm; that all labeled callosal axons above 0.25 µm diameter were indeed myelinated; and that the ratio of axoplasm diameter to fiber diameter (including the myelin sheath) was systematically close to 0.7, agreeing with previous measurements in rabbits and cats [2, 75]. Second, based on the linear relation derived from visual callosal axons by Waxmann and Bennet [73] \( (V_c = 5.5 \times D_f, \text{where } D_f \text{ is the fiber diameter, i.e., axoplasm diameter/0.7}) \), we calculated the conduction velocity \( (V_c) \) of each axonal segment. Third, we summed these conduction delays, segment per segment, and computed the resulting activation time for every single bouton of the reconstructed arbors, considering that an action potential crossed the midline at time zero. Finally, activation times were recorded and color-coded in the 3D graphical representation of each axonal tree, which could be enlarged and rotated as required. These steps were performed using a program specifically developed for this purpose, whose implementation was described elsewhere (Maxsim [68]).

4.2. Spatio-temporal profiles suggest that callosal axons promote the synchronous activation of their terminal columns

This simulation paradigm allowed the investigation of theoretical spatio-temporal activation profile of several reconstructed axons. Since an exhaustive report of the results was published elsewhere [30], we will recapitulate only the main findings, using the four representative axons whose morphology was described above.

For the simple axon of mid-range caliber (1.4 µm) described in figure 3A, the conduction velocity was 11.2 m/s. Its proximal-most bouton was located 13 mm away from the midline and was activated 1840 µs after the spike crossed this point; the distal-most bouton, only a few hundred pm apart, was reached 316 µs later, indicating that the narrow terminal territory of this axon was entirely activated within a very short time interval. In contrast, the activation of the boutons of axons with more widespread arbors spanned as much as 2700 µs. The table in figure 4A gives the ranges and mean values returned by the simulation for all axons; they are compatible with experimentally measured conduction velocities and interhemispheric latencies.

The activation profile of a typical ‘parallel’ axon is illustrated in figure 4B, by enlarged side views of the arbor and the boutons. Not surprisingly, this architecture leads to quasi-simultaneous invasion of both terminal columns, all boutons being activated within 400 µs. A quantitative estimation of the synchronicity is given by the distribution of activation times, indicating a substantial overlap in the activation of the columns. The paired coactivation index, expressed by the fraction of boutons of the axon which were active while boutons of the other column were also active, was 60%.

The next panel of figure 4 shows the activation profile for an axon with ‘serial’ architecture (whose
morphology is illustrated in figure 3C). The distribution of activation times indicates a fair degree of temporal overlap between the two terminals columns, despite their significant spatial separation. A sequence of the successive simulation frames shows that the coactivation is due to the marked reduction in diameter of the branch supplying the proximal column, which introduces a supplementary conduction delay and leads to the simultaneous invasion of both columns. Moreover, since the distal column contained about four times as many boutons than the proximal one, the coactivation index was very high (85%).

As mentioned earlier, the architecture of many complex axons was characterized by both serial and parallel components. In addition, we noted the occurrence of cross-talks between several columns. Figure 4D emphasizes one such case, described previously in figure 3D: in the top panel, branches are color-coded according to the level of the bifurcation they stem from. One can easily recognize a branch of high caliber emerging from the posterior column (d) and running along the rostro-caudal axis for about 1.5 mm before converging onto the anterior column (c) where it finally ramified into a terminal tuft. However, if the axonal segments are color-coded according to their respective activation time (lower panel), one cannot distinguish anymore the boutons supplied by this converging branch from those belonging originally to the anterior column, because they are all activated simultaneously. In addition, these two columns show a considerable degree of coactivation with the more proximal column (a), which was supplied by typical serial branches. As a result, the coactivation index for this axon was 95%.

With the limited sample of myelinated axons examined arising from areas 17 and 18, our study is hardly representative of the diversity of the callosal pathway. Given this restriction, one may nevertheless conclude that many visual callosal axons are likely to convey information with delays comparable to those observed for intrahemispheric connections [50]. However large the terminal territory of a callosal axon, its architecture is such that action potentials can invade it within no more than a couple a milliseconds. Moreover, this architecture appears well suited to lead to the simultaneous activation of targets distributed across segregated terminal columns. Synchronization might be achieved through equalization of conduction times in parallel arbors, or through appropriate modulation of calibers in serial axons; moreover, the degree of coactivation might be enhanced, or adjusted, by converging branches with fitting geometrical properties. Given the theoretical and practical limitations of our approach, it is impossible to provide an accurate estimation of the actual time scale for this coactivation. Time bins of 10 and 100 µs were chosen to calculate coactivation indices and to represent the distribution of activation times, respectively. Therefore, the synchronization is likely to operate at the millisecond range, and probably below. This issue is nevertheless complicated by the absence of a consensus regarding the temporal precision relevant for neuronal processing (for example, see [69]).

Another critical issue is whether one might be able to validate experimentally the results of such simulations. It is probable that with the further development of voltage-sensitive dyes and fast optical systems (see [44]), these methods will become applicable to thin axons and could thus be used for in vitro preparations [37]. This might be of great help to investigate structure-function relationships in simple axons. However, it is unlikely that, in a foreseeable future, any tool will render possible the direct visualization of spatio-temporal patterns of activation in complex, long-range projecting axons in situ, i.e., within their physiological environment including the network of afferent and efferent connections.

4.3. Functional significance of the properties of callosal axons

In the eventuality of a genuine coactivation of callosal terminals within the millisecond range as predicted in our study, how could it participate in the interhemispheric integration of visual scenes? Ten years ago, while recording simultaneously the activity from several columns of the cat visual cortex, Gray et al. [20] observed that neuronal responses to light stimuli were often oscillatory, and could be precisely synchronized, on a millisecond time scale, provided that the neurons were driven by a coherent stimulus. Stimulus-dependent synchronization was later observed for numerous areas and various species and is thought to provide a general code for the dynamic grouping of neurons into functionally coherent assemblies required to establish relations among the various components of sensory patterns. The synchronous discharges of a group of neurons would be effectively summed up by target cells, thereby increasing the saliency of the responses of the assembly for subsequent joint processing. Such a flexible temporal code could operate at a fast time scale and provide a solution to the ‘binding’ problem (reviewed in [65]).

Since callosal axons are capable of coactivating target neurons located in several columns of the recipient hemisphere, they might well contribute to the precise synchronization of distributed neuronal responses. Moreover, callosal connections fulfill all three postulated conditions for serving stimulus-dependent synchronization [64]: i) in contrast to the feed-forward connections responsible for the generation of neurons with feature-selective receptive-fields,
interhemispheric projections are basically reciprocal (see Introduction); ii) since they develop rather late [26, 47], callosal connections are highly susceptible to experience-dependent modification. Not only their global layout [29], but also their functional characteristics [46] and their fine architecture [23] can be profoundly altered in response to early manipulation of visual inputs. Hence, they are capable of reflecting frequently occurring feature constellations, as required for cortico-cortical networks involved in perceptual grouping; iii) finally, in order to account for new feature configurations, assembly-forming connections should retain some degree of flexibility throughout adulthood. Indeed, interhemispheric connections were shown to be susceptible not only to deafferentation, but also to sensory experience in adult cats [45, 48].

4.4. Callosal axons and interhemispheric synchronization

Precise temporal coupling of oscillatory visual responses was found to occur not only between distant columns of the same hemisphere but also across hemispheres, between neurons located close the 17/18 border zones. In both cases, the degree of synchronization reflected global stimulus properties such as continuity, collinearity or common fate [14]. A recent, careful examination of the temporal patterning of visual responses revealed that interhemispheric synchronization might occur with different time scales [51]. In addition to the narrow peaks typical of millisecond-range coupling, cross-correlations computed over large peristimulus periods revealed the existence of broader peaks, ranging between 30-100 or 100-1000 ms. After section of the posterior corpus callosum, precise synchronization was abolished, intermediate-range peaks were markedly reduced, but some looser coupling was still present. In contrast, extensive lesions of extrastriate cortex did not affect the sharp synchronization, whereas they strongly reduced the occurrence of intermediate and loose coupling [49]. This indicates that the loose coupling of visual responses involves polysynaptic loops and feedback projections from heterotopic areas, whereas the precise interhemispheric coupling, presumed to serve the dynamic formation of assemblies, is mediated by reciprocal callosal connections between primary visual areas.

The properties of callosal axons described in this paper are, alone, not sufficient to account for the occurrence of precise, zero-phase lag synchronization between the two hemispheres. They indicate only that callosal neurons located within one hemisphere are capable of providing synchronized inputs to several columns of the opposite hemisphere. As noted above, these inputs are not limited to regions where supra-threshold contralateral influences can be recorded. Interestingly, recent in vitro work suggests that precisely timed, subthreshold oscillations of membrane potential affect the spatio-temporal integration of additional converging inputs, and thereby facilitate the subsequent synchronization of neuronal discharges [72]. One can hypothesize that heterotopic callosal connections establish temporal relations essential to the integrate representation of sensory features, while homotopic connections allow accurate topographic correspondence between left and right maps and thereby contribute to the generation of feature-selective receptive fields, such as in disparity-sensitive neurons. We have shown that neurons with narrow contralateral arbors link topographically corresponding loci, and that contralateral and ipsilateral inputs converging onto callosal-recipient neurons are precisely matched in their functional characteristics, including retinotopic location. It remains to be established whether neurons with larger terminal territories, often consisting of both homotopic and heterotopic components, contribute to one or both tasks. However, the diversity of the cellular components participating in interhemispheric networks favors the hypothesis of several trans-callosal channels.

There is no unique strategy to produce zero-phase lag synchronization between remote groups of neurons. Such precise synchronization might be achieved through common inputs, through reciprocal connections and/or through local excitatory and inhibitory interconnections within more intricate networks. Computer simulations suggest that any of these three non-mutually exclusive mechanisms could result in the temporal patterns observed experimentally [32, 33]. Whatever the solution(s) actually used by interhemispheric connections, it is essential to keep in mind that ‘callosal projecting’ neurons do not only project to the contralateral hemisphere, but also have local axonal collaterals providing inputs to remote targets within the hemisphere of origin (see below). If, as one might infer from data on cortico-cortical connections, the conduction delays are equalized for both termination sites, such bi-hemispheric projecting neurons could be responsible for inter-hemispheric synchronization. They would be crucial participants in the integration of sensory features across the two hemispheres, and thereby in forming a unified cortical representation of the visual world, across the central vertical meridian.

5. Conclusion: directions for future research

As suggested in the previous section, information about the relations between the contralateral and the ipsilateral axonal arbors of callosal neurons are required for understanding the neuronal mechanisms of interhemispheric cooperation. Evidence for ipsilateral
axonal collateralization come from double-labeling experiments [1] or antidromic activation studies [15] revealing that some callosal neurons in the rat frontal cortex also project to ipsilateral cortical or subcortical structures. These findings are compatible with the recent observation that callosal neurons survive the transection of their callosal axon [54]. For visual areas, it was shown that a few extrastriate callosal neurons of cats projecting to the contralateral 17/18 border also project to the homologous ipsilateral region [62]. The existence of ipsilateral terminals of callosal neurons in primary visual cortex is supported by the observation of the initial segments of axonal collaterals directed to the ipsilateral hemisphere in cats [27] and of local synapses labeled within the cortex contralateral to peroxidase injections in mice [12]. However, due to technical limitations, our current knowledge of the topology of the ipsilateral axonal arbors of callosal neurons is extremely limited. It could now be complemented by single neuron tracing work.

It will also be mandatory to investigate to what extent the organizing principles observed in cats might hold for other species as well, particularly in primates, whose visual cortex laminar and modular compartmentalization is much better defined, and in other well-studied inferior mammals such as the rat. Remarkably, callosal linkages between primary visual areas of rodents are not strictly limited to the 17/18 border; numerous layer V neurons located within the peripheral visual field representation of A17 have a contralateral projection. Based on targeted retrograde tracer injections, it was recently proposed that these connections do not link retinotopically corresponding loci but rather mirror-symmetric points from both hemispheres [34]. In this respect, such projections would resemble the interhemispheric connections between cat or primate extrastriate areas and might play a role in the computation of optic flow and/or in symmetry detection. It is likely that the axons responsible for these projections have distinctive architectures.

Further, the possible relations between callosal terminals columns and functional domains of visual areas should be clarified. Straightforward correlations could be obtained by combining single neuron tracing with optical recording of intrinsic cortical signals. This technique could be applied simultaneously for both hemispheres, and would provide answers to both previous questions.

Ideally, further experiments should be conducted in species with less convoluted brains. This is essential in order to obtain direct correlations in each individual animal without having to rely on complex methods for tissue flattening or deconvolution algorithms, which inevitably introduce deformations. Small-sized brains would offer the additional advantage of reducing the time needed for reconstruction. In addition to the rat, the marmoset monkey seems to be an ideal preparation in this respect. Moreover, studies would benefit of recent electrophysiological work which provided the most complete and accurate topographic maps of visual areas ever published for a primate [16, 35, 59].

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