Functional specificity of callosal connections in the cat visual cortex

(Caractéristiques fonctionnelles des connexions calleuses dans le cortex visuel du chat)

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II.3.1 Section of the optic chiasm ............................................................... 52
II.3.2 Visual stimulation ........................................................................... 54
II.3.3 Quantitative analyses of the angle maps ........................................ 54
II.3.4 Electrophysiological controls of the optical images and reference penetrations ...................................................................................... 55
II.3.5 Histological procedures ................................................................. 56
II.4 ANATOMICAL STUDY: FUNCTIONAL SELECTIVITY OF INDIVIDUAL CALLOSAL AXONS IN A17 AND A18 ....................................................................................................... 58
II.4.1 Extracellular neuronal tracer injections ........................................... 59
II.4.1.1 Choice of tracers ......................................................................... 59
II.4.1.2 Extracellular tracer injections and multi-unit recordings at the injection site ...................................................................................... 60
II.4.2 Reference penetrations .................................................................... 61
II.4.3 Histological procedures .................................................................. 61
II.4.4 Three-dimensional reconstructions .................................................. 63
II.4.5 Alignment of the reconstructed axons and injection sites with the optical maps (Figure 32) ........................................................................... 63
II.4.6 Quantitative calculations ................................................................. 64
III RESULTS AND DISCUSSIONS ............................................................ 65
III.1 CHAPTER 1. LAYOUT OF TRANSCALLOSAL ACTIVITY IN CAT VISUAL CORTEX REVEALED BY OPTICAL IMAGING ................................................................. 65
III.1.1 Summary ......................................................................................... 65
III.1.2 Introduction .................................................................................... 66
III.1.3 Results ............................................................................................ 68
III.1.3.1 Localization of the regions activated via the transcallosal pathway in visual cortical areas 17 and 18 ................................................................. 68
III.1.3.2 Characterization of the regions activated via the transcallosal pathway in A17 and A18 ........................................................................... 74
III.1.3.2.1 Layout of orientation domains activated via the transcallosal pathway in the TZ .............................................................................. 74
III.1.3.2.2 Transcallosal activity maps obtained by using A17 and A18 specific stimuli ...................................................................................... 74
III.1.3.3 Comparison of transcallosal and geniculo-cortical orientation maps 76
III.1.4 Discussion ....................................................................................... 81
III.1.4.1 Methodological considerations .................................................... 81
III.1.4.2 Localization of the regions activated via the transcallosal pathway .... 82
III.1.4.3 Layout of the transcallosally activated orientation domains .......... 84
III.1.4.4 Spatial distribution of the transcallosal domains activated by different spatial and temporal frequencies .................................................. 84
III.1.4.5 Matching the transcallosal and the geniculo-cortical maps ............. 85
III.2 REPRESENTATION OF THE IPSILATERAL HEMIFIELD AND RETINOTOPIC ORGANIZATION OF TRANSCALLOSAL ACTIVITY IN THE CAT VISUAL CORTEX ............. 87
III.2.1 Summary ....................................................................................... 87
III.2.2 Introduction .................................................................................... 88
III.2.3 Results ............................................................................................ 90
III.2.3.1 Stimulation of the central vertical meridian of the visual field ....... 90
III.2.3.2 Stimulation within the right visual hemifield ................................ 93
III.2.3.2.1 Localization of the regions activated via the transcallosal pathway in A17 and A18 ................................................................. 93
# Table of Contents

## III.2.3.2.2 Retinotopic organization of the regions activated via the transcallosal pathway in A17 and A18  
100

## III.2.3.2.3 Comparison of the patches of activity representing the ipsilateral visual field with the orientation maps  
103

## III.2.3.3 Stimulation within the left visual hemifield  
105

## III.2.3.4 Localization of the TZ, electrophysiological and histological controls  
107

### III.2.4 Discussion  
110

#### III.2.4.1 Methodological considerations  
110

#### III.2.4.2 Location of the transcallosal cortical maps  
111

#### III.2.4.3 Representation of the ipsilateral hemifield and retinotopic organization of callosal connections  
112

##### III.2.4.3.1 Patchy layout of representation of the ipsilateral hemifield and of the TC activity  
112

##### III.2.4.3.2 Extent of ipsilateral hemifield representation in mammals  
114

##### III.2.4.4 Contribution of each hemiretina to ipsilateral hemifield representation and activation of the transcallosal pathway  
115

## III.3 CHAPTER 3. FUNCTIONAL SPECIFICITY OF INDIVIDUAL CALLOSAL AXONS IN CAT VISUAL CORTEX  
117

### III.3.1 Summary  
117

### III.3.2 Introduction  
118

### III.3.3 Results  
119

#### III.3.3.1 Morphology of the reconstructed callosal axons  
119

#### III.3.3.2 Visuotopy of callosal axons  
122

#### III.3.3.3 Orientation selectivity of callosal neurons  
125

##### III.3.3.3.1 Injection sites (left hemispheres)  
125

##### III.3.3.3.2 Callosal axon terminals (right hemispheres)  
125

##### III.3.3.3.3 Retrogradely labeled somata of callosal neurons (right hemispheres)  
127

#### III.3.3.4 Patchy representation of the ipsilateral visual field and orientation selectivity of callosal terminals  
129

#### III.3.3.5 Direction selectivity of callosal neurons  
130

#### III.3.3.6 Ocular dominance selectivity of callosal neurons  
130

### III.3.4 Discussion  
133

#### III.3.4.1 Methodological considerations  
133

##### III.3.4.1.1 Labeling of callosal axons  
133

##### III.3.4.1.2 Branching pattern and laminar termination of callosal axons  
134

#### III.3.4.2 Visuotopic organization of callosal connections  
134

#### III.3.4.3 Orientation selectivity of callosal connections  
135

#### III.3.4.4 Direction and ocular dominance selectivity of callosal connections  
137

#### III.3.4.5 Comparison between interhemispheric callosal connections and intra-hemispheric long-range horizontal connections  
138

## IV GENERAL CONCLUSION  
139

### IV.1 SPECIFICITY OF TRANSCALLOSAL CONNECTIVITY BETWEEN CAT VISUAL AREAS 17 AND 18  
139

### IV.2 POSSIBLE FUNCTIONAL CORRELATES OF THE SPECIFICITY OF TRANSCALLOSAL CONNECTIONS  
141

#### IV.2.1 Corpus callosum and midline fusion  
141

#### IV.2.2 Corpus callosum and visual stimulus orientation  
142

### IV.3 DEVELOPMENT OF THE HIGHLY SPECIFIC INTERHEMISPHERIC CONNECTIVITY  
143

#### IV.3.1 Development and guidance of callosal axons before entry into the visual cortex  
143
IV.3.2 Development of callosal axons within the cortex ................................. 144
IV.3.3 Monocular and binocular mechanisms can drive interhemispheric correlated activity ........................................................................................................ 146
IV.4 OUTLOOK ........................................................................................................ 147

V REFERENCES ........................................................................................................ 148

LIST OF FIGURES ...................................................................................................... 170
CURRICULUM VITAE .................................................................................................. 173
Abstract

**Functional specificity of callosal connections in the cat visual cortex**

The work presented in this thesis aimed to increase our understanding of the anatomical and functional organization of the interhemispheric transfer of visual information through the main commissure of the brain: the corpus callosum. This interhemispheric activity was studied at the level of the cortical regions activated by callosal connections, as well as at the cellular level of individual callosal axons. The anatomical and functional organizations of callosal connections were investigated in the visual areas 17 and 18 (A17 and A18) of the adult cat, by combining optical imaging of intrinsic signals with callosal axon labeling.

The functional specificity of callosal connections was first studied at the level of the population of the neurons activated by these connections. In order to isolate the callosal inputs from the geniculo-cortical inputs, the split-chiasm preparation was used. The layout of the regions activated through the corpus callosum was characterized by using moving high contrast oriented gratings as visual stimuli. This approach revealed that the CC triggers the activation of orientation selective domains in the transition zone (TZ) between A17 and A18 and occasionally within portions of both of these areas. Transcallosally activated orientation domains were observed all along the TZ without any obvious interruption, and these domains were arranged around pinwheel centers. Interestingly, the TZ was divided in two parallel regions resembling A17 and A18 in their preferred temporal and spatial frequencies. The comparison of orientation maps evoked through the transcallosal and geniculo-cortical pathways revealed that these maps were similar within the TZ.

The retinotopic organization of the callosal connections was also investigated in this study, together with the representation of the ipsilateral hemifield in the visual cortex and the contribution of each hemiretina to this representation. The retinotopic maps obtained in split-chiasm cats revealed that the corpus callosum (CC) activates regions corresponding to the representation of the visual midline as well as part of the ipsilateral visual field. This representation of the visual field was highly compressed within the TZ and reaches an azimuth of up to +8°. The ipsilateral part of the visual field beyond +2° was represented within patches along the TZ. Similar retinotopic maps were obtained in intact animals. This similarity indicates that the representation of the ipsilateral portion of the visual field is transferred, predominantly, through the uncrossed projections of the temporal retina and the CC. The crossed projections from the nasal retina also trigger transcallosal connections, but only for the representation of up to 2-4° in the ipsilateral hemifield.

The functional specificity of callosal connections was furthermore studied at the level of individual callosal axons. The aim was to determine whether callosal connections link similar or different functional domains in the visual cortex of each hemisphere. The distribution of callosal axon terminals within functional maps (retinotopic, orientation,
direction and ocular dominance) of the visual cortex was analyzed using in vivo intrinsic signal optical imaging and extracellular neuronal tracer injections, in normal adult cats. The distributions of synaptic boutons of eight individual callosal axons were reconstructed in three-dimensions and compared with functional maps of the target zones as well as with the functional preference at the injection sites. These results confirmed the visuotopic organization of callosal connections and demonstrated that these connections mainly link cortical regions with the same orientation preference. The analysis of two callosal axons with respect to direction preference maps suggested that these callosal connections were less selective for direction than for orientation of visual stimuli. Finally, all callosal axons reconstructed in this study connected preferentially binocular regions.

These results reveal a highly specific organization of callosal connections between the cat visual areas 17 and 18 of each hemisphere. This connectivity suggests a specific functional role of the corpus callosum in visual information processing, in particular in the processing of orientation information of visual stimuli located in the central and ipsilateral parts of the visual field. These connections could be involved in the fusion of the two visual hemifields in a single visual field as well as in depth perception (stereopsis) in the central part of the visual field. The study of the anatomical and functional characteristics of callosal connections in adult animals also gives indications about the possible mechanisms involved in the development of this specific connectivity. Orientation selectivity may be a critical factor for the stabilization of callosal connections during development.
Résumé de la thèse de Doctorat

Caractéristiques fonctionnelles des connexions calleuses dans le cortex visuel du chat.

Le travail décrit dans cette thèse a pour but de mieux comprendre l’organisation anatomique et fonctionnelle du transfert inter hémisphérique des informations visuelles par la voie de la principale commissure cérébrale, le corps calleux. Ce dialogue inter hémisphérique est caractérisé dans cette étude au niveau des régions corticales activées par les connexions calleuses ainsi qu’au niveau d’axones calleux individuels, dans les aires visuelles 17 et 18 (A17 et A18) du chat adulte. Pour cela, la technique d’imagerie optique des signaux intrinsèques et des méthodes anatomiques de marquages neuronaux ont été utilisées.

La spécificité des connexions calleuses a été, dans un premier temps, étudiée au niveau de la population des neurones activés par ces connexions. Afin d’isoler l’activité calleuse de l’activité géniculo-corticale, le chiasma optique de chats adultes a été sectionné. Les régions activées par le corps calleux ont ainsi pu être localisées et caractérisées en utilisant comme stimuli visuels des réseaux de barres lumineuses orientées en mouvement. Cette approche a révélé que le corps calleux active des domaines d’orientation dans la zone de transition (TZ) entre les aires A17 et A18 et, parfois, dans certaines zones de chacune de ces deux aires. Des domaines d’orientation activés par les connexions calleuses ont été observés tout le long de TZ sans interruption apparente, ces domaines étant disposés autour de centres d’orientation (‘pinwheels’). Cette zone de transition est apparue divisée en deux régions parallèles, respectivement activées par des fréquences spatiales et temporelles caractéristiques des aires visuelles adjacentes. La comparaison des cartes d’orientation obtenues par l’activation de la voie transcalleuse, d’une part, avec celles obtenues par l’activation de la voie géniculo-corticale, d’autre part, a révélé que ces cartes étaient similaires dans TZ.

Pour compléter la caractérisation fonctionnelle des connexions calleuses, leur organisation rétinotopique a été analysée ainsi que la représentation de l’hémichamp ipsilatéral dans le cortex visuel et la contribution de chaque hémirétine à cette représentation. Les cartes rétinotopiques obtenues chez les chats chiasmotomisés ont révélé que le corps calleux activait des régions correspondant à la représentation de la partie centrale et de la partie ipsilatérale du champ visuel jusqu’à 8° d’azimuth. La partie ipsilatérale du champ visuel au delà de 2° était représentée dans des patches, situés le long de la TZ. Des cartes rétinotopiques présentant les mêmes caractéristiques ont été obtenues chez les animaux intacts. Cette similarité indique que la représentation de la portion ipsilatérale du champ visuel est transférée, principalement, par l’intermédiaire des projections non croisées de la rétine temporale et du corps calleux. Les projections croisées de la rétine nasale activent elles...
aussi les connexions calleuses, mais seulement pour les stimuli localisés jusqu’à 4° dans l’hémichamp ipsilatéral.

La spécificité fonctionnelle des connexions calleuses a également été étudiée au niveau d’axones calleux individuels, afin d’établir si ces connexions reliaient des domaines fonctionnels identiques ou différents. Les distributions des boutons synaptiques de huit axones calleux ont été reconstruites en trois dimensions. La distribution de ces boutons dans les cartes fonctionnelles (rétinotopie, orientation, direction et dominance oculaire) du cortex visuel a été comparée aux caractéristiques fonctionnelles des sites d’injection. Les résultats de cette étude confirment l’organisation visuotopique des connexions calleuses et démontrent que ces connexions relient préférentiellement des régions corticales de même préférence d’orientation. L’analyse de deux axones calleux au regard des cartes de préférence de direction suggère que les connexions calleuses sont moins sélectives pour la direction qu’elles ne le sont pour l’orientation des stimuli visuels. Enfin, tous les axones reconstruits dans cette étude connectaient principalement des régions binoculaires.

Ces résultats révèlent un haut niveau d’organisation des connexions calleuses entre les aires visuelles 17 et 18 de chaque hémisphère, chez le chat. Cette connectivité suggère un rôle fonctionnel spécifique du corps calleux dans le traitement des informations visuelles, en particulier dans le traitement des informations relatives à l’orientation des stimuli visuels situés dans les parties centrales et ipsilatérales du champ visuel. Ces connexions calleuses pourraient être impliquées dans la fusion des deux hémichamps visuels en un champ visuel unique, ainsi que dans la perception de la profondeur (stéréopsie) dans la partie centrale du champ visuel. L’étude des caractéristiques anatomiques et fonctionnelles des connexions calleuses chez des animaux adultes donne aussi des indications sur les éventuels mécanismes du développement de cette connectivité spécifique. Ainsi, la sélectivité d’orientation pourrait être un facteur déterminant dans la stabilisation de ces connexions au cours du développement.
Abbreviations

A17 and A18: visual cortical areas 17 and 18
CC: corpus callosum
GC: geniculo-cortical
LH: left hemisphere
OSI: orientation similarity index
RH: right hemisphere
ROI: region of interest
TC: transcallosal
TZ: transition zone between areas 17 and 18.
SCM: single-condition map
Publications

Articles

- Rochefort N., Buzás P., Kisvárday Z.F., Eysel U.T., Milleret C., Layout of transcallosal activity in cat visual cortex revealed by optical imaging (manuscript in revision for Neuroimage).

Abstracts and Scientific meetings

“...if the currently received statements are correct, the appearance of the corpus callosum in the placental mammals is the greatest and most sudden modification exhibited by the brain in the whole series of vertebrated animals...” T.H. Huxley (Huxley, 1863).
General Introduction

I. General Introduction

I.1 The corpus callosum, a bridge between two brains?

The animal kingdom contains a great diversity of forms, but a major unifying feature found in all arthropods and vertebrates, at least, is the pairwise organization of sensory and motor organs: one located on each side of the body’s longitudinal axis. In these animals, sensory abilities result from the correlated activity of pairs of sensory organs. Similarly, motor activity results from the coordination of pairs of motor organs. The brain’s physical structure, consisting of two hemispheres, elegantly reflects this functional organization.

In the 19th Century, correlations were established between sensory or motor deficits in human patients and the existence of cerebral lesions (observed post-mortem). The functional impairment in each patient was therefore associated with a specific region of the brain. In certain cases, functions stem from only one hemisphere, demonstrating the hemispheric asymmetry of the brain. In 1861, P. Broca discovered a striking example of this asymmetry in the domain of language (see translation of Broca’s report in Berker et al., 1986): in the vast majority of the population, only the left hemisphere is involved in speech generation (Zaidel, 1985). Subsequent studies confirmed the hemispheric functional specialization of the human brain. In general, the ‘dominant’ hemisphere (usually the left hemisphere for right-handers) is more committed to analytical, sequential, verbal, local, rational or objective processing, whereas the ‘minor’ hemisphere (usually the right hemisphere in right-handers) is more committed to operations requiring synthetic, global, spatial, intuitive or affective abilities. For example, the right hemisphere specializes in visuospatial processing, such as the processing of part-whole relations (Nebes, 1971), spatial relationships (Nebes, 1973), apparent motion detection (Forster et al., 2000), mental rotation (Corballis and Sergent, 1988), spatial matching (Corballis et al., 1999) and mirror image discrimination (Funnell et al., 1999).

The notion that each hemisphere is a separate entity, able to perform perceptual or mnemonic tasks on its own, requires communication between the two hemispheres. For example, as speech is generated exclusively from the left hemisphere, any information reaching the right hemisphere that is expressed verbally must first be transmitted to the left hemisphere. This information transfer occurs in the bundles of fibers linking the two hemispheres, and particularly in the largest of these bundles — the corpus callosum.

The corpus callosum (Latin for "tough body") is by far the largest bundle of nerve fibers in the entire nervous system of placental mammals. In adult humans, this structure has been estimated to contain 200 million axons; the true number is probably higher because this
estimate was based on light microscopy rather than on electron microscopy. For comparison, each optic nerve contains only 1.5 million axons and the auditory nerve contains only 32 thousand axons. Two smaller bundles of fibers connect the two cerebral hemispheres: the subcortical and anterior commissures.

Figure 1. The corpus callosum is a thick, curved plate of axons in the approximate center of the brain. A. Sagittal view. B. The brain is seen from above. The band of the corpus callosum fans out after crossing and joining the two hemispheres. (The front of the brain is at the top of the picture). From (Hubel, 1995); © Lennart Nilsson, Be-hold Man, Little, Brown and Company, Boston).

Before 1950, anatomists described the corpus callosum as a bundle of fibers linking “homofunctional” and “heterofunctional” cortical areas in the two hemispheres (for example, see Ramón y Cajal, 1955). However, its functions, other than connecting the two sides of the brain, remained a mystery. Rare cases of an absence of the corpus callosum at birth (callosal agenesis) were known to occur in humans. In other cases, the corpus callosum was completely or partially severed by neurosurgeons, either to treat epilepsy or to reach deep tumors, in the pituitary gland for example. However, neither neurologists nor psychiatrists had identified any cognitive or behavioral deficiency in such cases.

I.1.1 Initial discoveries concerning the functional role of the corpus callosum

In 1955, whilst working in the laboratory of R. Sperry, Ronald Myers revealed a functional role of the corpus callosum by training cats for a given task with only one of their eyes open and then testing these animals with only the other eye open. Normal cats performed the task successfully regardless of which eye was open. The same procedure was repeated in cats that
had undergone surgery in which the optic chiasm was cut such that the fibers crossing the chiasm were severed whereas those that did not cross the chiasm were left intact (Figure 2). These split-chiasm cats were tested and successfully performed the task. If these cats had learned the given task through the left eye open and were then asked to perform it correctly with the right eye open, then the required information must have crossed from the left hemisphere to the right hemisphere through the only known route: the corpus callosum. The experiment was repeated in cats in which both the chiasm and the corpus callosum had been surgically severed. These cats failed the test (Myers, 1956; Miner et al., 1956; Myers and Sperry, 1958). Thus, Myers established that the corpus callosum had at least one function in cats: the interocular transfer of monocularly learned tasks.

A few years later, in 1962, a function of the corpus callosum in the human brain was demonstrated by observations in a patient whose corpus callosum, anterior and hippocampal commissures had been sectioned to reduce severe epileptic seizures. After surgery, the patient could no longer verbally describe stimuli presented to his disconnected right hemisphere (Gazzaniga et al., 1962; Gazzaniga et al., 1965; Bogen et al., 1965). As explained above, a person’s ability to describe an object manipulated with their left hand depends on the activation of the cortical speech area located in the brain’s left hemisphere. However, the actual sensation of the object in the left hand is initially transmitted to the sensory area of the brain’s right hemisphere. This information must therefore be transferred from the right hemisphere to the left, through the corpus callosum. For this reason, the patient examined by Gazzaniga, Bogen and Sperry was unable to describe verbally the stimuli presented to his left hand or to his left visual field after callosotomy.

The effects of callosal disconnection in humans were not observed before 1962 for two main reasons. Callosotomy was initially not entirely effective at reducing seizures in patients with epilepsy. It was not until 1962 that two neurosurgeons, Vogel and Bogen, speculated that these operations were unsuccessful because the corpus callosum had not been fully sectioned. They decided to perform a complete commissurotomy on a former paratrooper who was experiencing severe and life-threatening seizures. The surgery was successful in controlling the patient’s seizures (cited by Gazzaniga, 2005). From this point on, treated patients could consequently be tested in order to reveal the effects of the commissurotomy. A second explanation results from the lack of accuracy in testing procedures. The studies of split-brain rats, cats and monkeys by Sperry and colleagues resulted in the development of sophisticated techniques for directly assessing the function of each hemisphere independently (Myers, 1956; Myers and Sperry, 1958). These techniques were used for the efficient testing of split-brain patients. This work provides a striking example of studies initially performed in animals enabling the human brain to be studied more accurately.

Since this first demonstration of the role of the corpus callosum, split-brain research (both in animals and in patients) has provided important insight into both hemispheric asymmetries [for example, in monkeys, (Hamilton, 1977; Hamilton and Vermeire, 1988) and
in humans, (Turk et al., 2002; Roser et al., 2005)] and the mechanisms through which the two hemispheres interact [in humans: review, (Gazzaniga, 2005); (Gazzaniga and Freedman, 1973; Ihori et al., 2000; Funnell et al., 2000; Aglioti et al., 2001; Tettamanti et al., 2002); in cats, (Berlucchi et al., 1978; Pito and Lepore, 1983; Mascetti, 1997)]. Severing the entire corpus callosum is now known to block the interhemispheric transfer of perceptual, sensory, motor and other forms of information. Studies of patients with partial lesions of the corpus callosum have provided information about the functional specificity and topographical organization of the corpus callosum (Gordon et al., 1971; Gazzaniga and Freedman, 1973; Peru et al., 2003; Berlucchi, 2004; Caille et al., 2005). Finally, the testing of acallosal patients (due to callosal agenesis) has also been fruitful in determining callosal functions (Lassonde et al., 1995). For example, midline visual deficits (Jeeves, 1991; Saint-Amour et al., 2004b), visuomotor integration deficits (Berlucchi et al., 1995) and the impairment of interhemispheric depth comparisons (Rivest et al., 1994) have been reported in acallosal patients.

Much research has been devoted to the callosal pathway and interhemispheric cooperation, but many aspects of the neuronal mechanisms of interhemispheric integration remain unclear. This is probably due to both the abundance and length of callosal fibers and to their manifold functions: there are callosal connections between sensory, motor, associative, frontal and limbic cortices, linking both heterologous and homologous areas.

I.1.2 Corpus callosum and vision, choice of the experimental model

The role of the corpus callosum can be studied in various sensory or motor systems. The advantage of the visual system for studying this structure is that, as recognized a century ago by Ramon y Cajal (Ramón y Cajal, 1898), at least one function of callosal connections can be inferred from the topographic organization of this system. In all mammals, visual information is highly lateralized, such that each hemifield projects to the contralateral hemisphere. In lower mammals — such as rodents and lagomorphs, with 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 (particularly predators such as cats and primates), axons arising from the nasal half of the retina cross the midline and project into the contralateral thalamus, whereas axons from the temporal retina remain on the ipsilateral side (Figure 2). If each hemisphere receives primary sensory inputs from only one half of the visual field, it follows that the visual world is represented in two physically discontinuous cortical maps split across the two hemispheres along the central vertical meridian (CVM) of the visual field. However, our perception of an object crossing or abutting the midline is neither disrupted nor disjointed. 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. Thus, the perceptual representation of the visual field must be integrated across the hemispheres. It has been suggested that the corpus callosum (CC) mediates the unification of
the two visual hemifields (Myers, 1956; Choudhury et al., 1965; Gazzaniga, 1966; Antonini et al., 1979; Berlucchi, 1981; Antonini et al., 1983).

Thus, mammals with frontal eyes appear to be a good experimental model for studying the function of the corpus callosum. The techniques used to investigate human brain function do not yet have a high enough spatial resolution for studying the activity of small neuronal populations and the topography of individual axons. Thus, in vivo studies in animals remain useful for investigating the functional organization of interhemispheric transfer. The development of stable anterograde tracers and imaging techniques has made it possible to investigate the precise layout of neuronal connections and their relationship to the functional organization of cortical areas in animals.

Since the pioneering studies of Hubel and Wiesel (Hubel and Wiesel, 1959), our knowledge of visual function has been obtained primarily from studies on higher mammals, notably the cat which has like humans, frontal binocular vision. Although homology between cortical territories in different species does not always exist, the organization of primary visual areas in cats has strong similarities to that in primates (review, Payne, 1993). This makes it possible to establish correlations with clinical observations, psychophysical and anatomical data obtained in humans. Reciprocally, fundamental studies in animals also increase our understanding of the etiology of human pathologies and facilitate the development of treatments or diagnostic tools – for example for amblyopia (Mitchell, 1989; Epelbaum et al., 1993; Sireteanu, 2000). The cat is also a particularly useful model for investigating the development of the visual system, because the postnatal maturation of this system takes place in the first postnatal months. This makes it possible to study the influence of visual experience on the development of visual pathways. Finally, the cat is the species in which visual callosal connections have been characterized in most detail, both in adults and during normal or pathological development.

For these reasons, the visual system of the cat was selected in this study in order to investigate the functional selectivity of callosal connections. Therefore, all the results presented relate to the callosal connections between visual areas 17 and 18 of the cat.

In the following sections, the functional architecture of the cat visual cortical areas 17 and 18 will be presented, followed by a review of anatomical and physiological data on callosal connectivity between these areas.
Figure 2. Schema of the cat visual system. Fibers originating from the temporal hemiretinae (T) project through the optic nerve (ON) and the ipsilateral optic tract (OT) to the magnocellular layer A1 and to the parvocellular layers (Cp) of the ipsilateral dorsal lateral geniculate nucleus (dLGN). Fibers coming from the nasal hemiretinae (N) cross the midline at the level of the optic chiasm (XO) and project onto the magnocellular layers A, Cm, and to the Cp of the contralateral dLGN. Optic radiations (OR) are composed of fibers originating from both eyes. Thus, at the level of the visual cortex (VCx), in the visual areas 17 and 18 (A17 and A18), each hemisphere contains a binocular representation of the contralateral visual hemifield. The neurons projecting an axon through the corpus callosum (CC) and the callosal terminals are mostly found in the transition zone (TZ) between A17 and A18, where the vertical meridian (VM) and its vicinity are represented. The double black arrow indicates the location of the section of the optic chiasm in the experimental model of split-chiasm animals (see chapter I.4). Colors indicate portions of the visual field projected in the different structures.
I.2 Functional architecture of the cat visual cortical areas

Around fifteen visual areas have been defined in the cat cerebral cortex, mainly on its occipital pole (Figure 3). The various visual regions are extensively interconnected via afferent and efferent fibers.

Numerous features distinguish areas 17 and 18 (A17 and A18) from other visual areas. The introduction of degeneration methods in the 1960s and of neuronal tracers in the 1970s allowed distinguishing the transgeniculate projections to A17 and A18 from those to area 19 (A19) and the middle suprasylvian visual cortex. Both A17 and A18 receive significant projections from the dorsal lateral geniculate nucleus (dLGN). The dominant input to A17 and A18 is derived from the functionally dominant α and β retinal ganglion cells, at the origin of the Y- and X- visual signals. Both α and β ganglion cell types, and their magnocellular A, A1
and C LGN target neurons generate strong and brisk responses (see review in Payne and Peters, 2002). In contrast, A19 and the middle suprasylvian visual cortex receive visual signals from $\alpha$ and $\gamma$ (including $\delta$, $\epsilon$, $\eta$) retinal ganglion cells. The $\delta$, $\epsilon$ and $\gamma$ ganglion cells and their target neurons in parvocellular layers C1 and C2 of the LGN respond sluggishly to visual stimuli and transmit action potentials rather slowly. The $\alpha$ cells contributing to this pathway have high activity and respond briskly, but appear to be only sparsely connected to A19 and middle suprasylvian visual cortex (see review in Payne and Peters, 2002). Areas 17 and 18 also differ from other areas in terms of their cytochrome oxidase activity. In frontal sections, high levels of cytochrome oxidase activity are observed in layer IV of both A17 and A18, whereas this prominent stripe is absent in either the paralimbic cortex or A19.

A17 and A18 are critical for processing the broadest band of fundamental visual attributes. They display very similar intra-laminar and inter-laminar flows of signals through the intrinsic circuitry and generate the emergent receptive field properties of orientation selectivity, movement direction selectivity, binocular interactions and spatial disparity. However, neurons of A17 and A18 display three major functional differences: their receptive field size, their spatial and their temporal sensitivities. Neurons in A17 tend to have smaller receptive fields and are sensitive to slower movements, whereas neurons in A18 tend to have larger receptive fields, lower spatial acuities and greater sensitivity to temporal transients (flickering or fast movements) (Orban, 1984a). These differences seem to arise from the afferent X- and Y- visual streams. Area 17 is innervated by both X- and Y-axons whereas A18 is innervated by Y-axons only (Figure 4). Both A17 and A18 also receive W-axon inputs from the parvocellular complex of the LGN.

I.2.1 Circuitry and functional processing in A17 and A18

The neurons in A17 and A18 receive inputs from widespread sources, including thalamo-cortical, cortico-cortical, inter-areal, interhemispheric and subcortical inputs.

As previously mentioned, A17 and A18 receive significant projections from the main divisions of the dorsal lateral geniculate nucleus (dLGN): laminae A, laminae C and the medial interlaminar nucleus (MIN) (Rosenquist et al., 1974; Bullier et al., 1984; Humphrey et al., 1985a; Humphrey et al., 1985b; Birnbacher and Albus, 1987) (Figure 4). Layer IV is the primary target of these thalamic afferents. The pyramidal cells in layer VI also receive direct input from X and Y type axons (as well as in their apical dendrites in layer IV). Additionally, the large pyramidal cells of the lower part of layer III receive significant innervations via Y and W pathways (Freund et al., 1985a; Freund et al., 1985b). Finally, the parvocellular layer C neurons in the LGN also terminate in the upper part of layer V and in the superficial half of layer I, in A17 (see review in Payne and Peters, 2002).
General introduction

Figure 4. Diagrams of the innervation of A18 (A) and A17 (B) by fibers from the LGN and the intrinsic circuitry of spiny neurons in A17 (C). Spiny cells are present in all layers except layer I. In layer IV, cells have a stellate morphology with radiating dendrites, whereas those in all other layers have a pyramidal morphology and are characterized by an ascending apical dendrite. Laminar terminations of local axon collaterals are indicated. Figure from (Payne and Peters, 2002).

The visual cortex presents a wide range of **cortico-cortical connections**. These connections can be either: excitatory or inhibitory, short-range or long-range, intra or inter-laminar, intra or inter-areal and finally intra or inter-hemispheric. Approximately 80% of the cortical neurons are excitatory (Lund et al., 1979; Martin and Whitteridge, 1984; McGuire et al., 1984; Gabbott et al., 1987). The axons of pyramidal cells, most of which terminating on dendritic spines, are considered to be the main sources of asymmetric synapses (84-87%), (Kisvárday et al., 1986). Thus, **intrinsic connections** consist mainly of an excitatory link between excitatory neurons (Salin and Bullier, 1995). There is morphological and functional evidence that cortico-cortical projections are also established by horizontally projecting GABA-ergic neurons. Inhibitory large basket cells in the supra and infragranular layers can send collaterals for up to 2 mm horizontally (Kisvárday and Eysel, 1993; Kisvárday et al., 1993; Buzás et al., 2001; Kisvárday et al., 2002), thus providing long-range lateral inhibition.

In addition to short and long-range intra-cortical lateral connections, the excitatory neurons in A17 and A18 send and receive significant inputs from **inter-areal and inter-hemispheric sources**. Indeed, feedback from higher to lower areas is essential in the cortical processing of visual information. For example, in the cat visual cortex, electrophysiological analysis and optical imaging have shown that deactivation (cooling) of the middle suprasylvian (MS) region of the visuoparietal cortex disrupts the representation of the direction of motion in A18 (Galuske et al., 2002). Direct projections from the MS cortex...
terminate in A17, A18 and A19 (Payne and Lomber, 2003). Feedback connections also play a role in modulating interhemispheric synchronization (Munk et al., 1995).

Interhemispheric inputs are provided by both homotopic and heterotopic areas. The cell bodies and axonal terminals of callosal neurons are densely packed along the transition zone between A17 and A18 (see below). The only contralateral cortical inputs from heterotopic areas reaching the transition zone (TZ) between A17 and A18 originate in areas 19 and 21a and in the postero-medial lateral suprasylvian (PMLS) and postero-lateral lateral suprasylvian (PLLS) regions (Segraves and Rosenquist, 1982b). Furthermore, callosal fibers exist between this transition zone and the contralateral visual claustrum (LeVay and Sherk, 1981). The visual claustrum projects back to the visual cortex, to the same areas from which it receives an input. The return projection is predominantly ipsilateral, but there is also a small crossed projection, terminating in a restricted zone around the transition zone between A17 and A18 (see Figure 18 in LeVay and Sherk, 1981). The claustrocortical axons terminate in all cortical layers but most heavily in layers IV and VI (LeVay and Sherk, 1981).

Finally, subcortical inputs to the visual cortex arise from several sources. Most of these inputs are related to the control of body motion or oculomotor function. In the cat, the pulvinar-lateral posterior (LP) complex transmits information from the middle suprasylvian region (MS) (Payne and Lomber, 2003) and the superior colliculus to the visual cortical areas (A17, A18, A19, PMLS and PLLS). Therefore, this pathway represents a secondary route from the retina to the cortex (Miller et al., 1980; Hughes, 1980). Diffuse projections also arise from the nucleus raphe dorsalis and the locus coeruleus to A17 and A18. These nuclei provide serotonergic and noradrenergic innervations, respectively (Leger et al., 1975; Tork et al., 1979). Finally, Horseradish peroxidase (HRP) injections into A17 and A18 labeled cholinergic afferents. These afferents originate from the diagonal band of Broca’s nucleus in the basal forebrain and from the lateral hypothalamus (Albus, 1981). The activities of these non-specific inputs are thought to relate to the activity state of the cortex.

I.2.2 Visual maps in A17 and A18

The detailed organization of visual field representations within A17 and A18 was elucidated in the 1960s through the use of microelectrode recording techniques (Hubel and Wiesel, 1962; Hubel and Wiesel, 1965). Visual maps were drawn on the cortical surface by relating the coordinates of the receptive field centers within the visual field to the individual recording sites. Figure 5 shows the isoazimuth and isoelevation lines of the visual field plotted on the cortical surface. The representation of the visual field is organized topographically. The contralateral hemifield is represented in the opposite cerebral cortex; the upper quadrant of the visual field is represented caudally whereas the lower quadrant is represented rostrally. Both A17 and A18 contain a representation of the contralateral visual hemifield. The transition zone between them, in which the callosal neurons and their terminals are densely packed, corresponds to the projection of the vertical meridian and its vicinity (Figure 5).
In the studies presented in this thesis, functional maps of the visual cortex were recorded by optical imaging. This method requires the imaged cortical surface to be as flat as possible. Considering the morphology of the visual cortex, mainly the rostral part of A17 and A18 was imaged in this study, therefore including the representation of the center and lower parts of the visual field.

![Figure 5](image-url)

Figure 5. Outline drawings of the cerebral hemispheres of the cat, to show the positions of areas 17 and 18 and the intervening transition zone, relative to the gyri and sulci. A: Posterior view of the left hemisphere. B: Medial view, including a marker of the Horsley-Clarke coronal planes. C: Dorsolateral view. In each view, the lines of elevation and azimuth in the visual-field maps of areas 17 and 18 are indicated as dashed and continuous lines, respectively. The hatched regions indicate the position and width of the transition zone. The asterisk in part A indicates the confluence of the transition zones between areas 17, 18, 20a and 20b. Cortical maps from Tusa et al., 1978, 1979. For abbreviations see Figure 3. s.c. superior colliculus; h.f.g. hippocampal fusiform gyrus. From (Payne, 1990b).
I.2.3 Functional architecture of A17 and A18

I.2.3.1 Orientation columns

In 1957, Mountcastle observed “deep” and “cutaneous” columns in the primary somatosensory cortex of the cat. Neurons below each surface domain had almost identical receptive fields and were responsive to the same modality (Mountcastle, 1957). A few years later, Hubel and Wiesel recognized a similar organization in the cat visual cortex. Single cells responded selectively to some attributes of a visual stimulus such as orientation. The cells sharing the same orientation preference formed vertical columns that extended across all cortical layers perpendicularly to the cortical surface (Hubel and Wiesel, 1962). The concept of the hypercolumn was subsequently introduced: a region of the cortex comprising the complete representation of all orientations (Hubel and Wiesel, 1974).

Studies of the functional organization of the visual cortex have been limited for some years by the available techniques: electrophysiological recordings are limited to sampling of visual neurons. Experimental methods for visualizing the functional organization of the cortical columns in large cortical regions therefore seem to be essential, to determine the mechanisms of information processing in these areas. Optical imaging is one such method and has been used to elucidate the organization of the functional columns on a large scale (Bonhoeffer and Grinvald, 1991; Bonhoeffer and Grinvald, 1993). This technique has revealed a characteristic feature of orientation domains: their radial arrangement in a pinwheel-like fashion around singularities — called “orientation” or “pinwheel” centers — either clockwise or counterclockwise (Bonhoeffer and Grinvald, 1993). Other functional maps have been recorded in the cat visual cortex including those for ocular dominance, direction of motion and spatial frequency (Shmuel and Grinvald, 1996; Crair et al., 1997; Kim et al., 1999; Issa et al., 2000; Kisvárday et al., 2001).

I.2.3.2 Ocular dominance columns

Another basic feature of A17 and A18 is the partial segregation of the geniculo-cortical terminals according to the eye of origin (Hubel and Wiesel, 1962; Hubel and Wiesel, 1965). Functionally, neurons can be preferentially activated by one eye than the other. This is especially encountered in layer IV, in which most neurons display a distinct preference for one eye over the other. Outside layer IV, most of the neurons can be excited by stimuli presented to either eye and extreme dominance by one eye is rare. This factor tends to obscure the ocular dominance columns, rendering them difficult to detect in the superficial and deep layers.
I.2.3.3 Direction preference columns

Electrophysiological studies have raised the question as to whether direction preference is also arranged in a columnar fashion (Tolhurst et al., 1981; Payne et al., 1981). The surface organization of direction selectivity has been characterized in cat A18 by multiple electrophysiological recordings (Swindale et al., 1987). It has also been shown that lines of discontinuity in direction preference run from one orientation singularity to another. Optical imaging has been used to obtain direction maps (Shmuel and Grinvald, 1996; Kisvárday et al., 2001) and the orthogonal relation between orientation and direction preferences has been confirmed.

I.2.3.4 Spatial frequency columns

The layout of neurons sharing the same spatial frequency (SF) preference has also been investigated. The organization of SF preferences has been described in cats as laminar (Maffei and Fiorentini, 1977), clustered (Tolhurst and Thompson, 1982), or columnar (Tootell et al., 1981; Bonhoeffer et al., 1995). These and other studies (Movshon et al., 1978b; Tolhurst and Thompson, 1981) suggest that, like ocular dominance, the SF preference of cortical cells varies both tangentially across the cortical surface and radially through the cortical laminae.

Intrinsic signal imaging has been used to characterize the tangential organization of SF preferences in the cat visual cortex (A17 and A18) and various models of organization have been proposed. A first set of experiments was interpreted as showing only regions of "high" and "low" SF preference (Bonhoeffer et al., 1995; Hubener et al., 1997; Shoham et al., 1997). The presence of high and low SF preferences in separate cortical domains is consistent with the proposed cortical segregation of X and Y inputs from the thalamus (Shoham et al., 1997). A second set of imaging experiments (Everson et al., 1998) supported a model in which the cat visual cortex (A17) contains multiple domains representing many different spatial frequencies. Furthermore, single-unit recordings in A17 have demonstrated a wide range of SF preferences and tuning bandwidths at single retinotopic loci (Movshon et al., 1978a; Tolhurst and Thompson, 1981; Robson et al., 1988). All these data suggest that SF preferences in the visual cortex are unlikely to result simply from differences between X and Y cell response characteristics. More recently, Issa et al. (Issa et al., 2000) showed that a wide range of SF preferences is mapped onto the visual cortex. This representation is mostly continuous, with SF preferences generally changing gradually and progressively in the tangential plane with occasional linear discontinuities.

The various functional domains differ in size, but these domains are generally smaller in A17 than in A18. Generally, the mosaic “tiles” of all the identified modular or columnar systems have linear dimensions of 0.4 to 0.7 mm, or some multiple thereof, in A17. In A18, the dimensions of the equivalent tiles are approximately twice those in A17: 1.0 to 1.2 m, or some multiple thereof (review in Payne and Peters, 2002).
I.2.3.5 General view of callosal connectivity between the cat visual areas 17 and 18

Retrograde and anterograde tracing techniques have demonstrated that, whereas most parts of A17 and A18 are free of interhemispheric projections, cell bodies and axonal terminals of callosal neurons are densely packed along the transition zone (TZ) between these areas (review Innocenti, 1986a). The portion of the visual field represented within the callosally connected zone has the shape of an hour-glass centered on the vertical meridian and spanning several degrees on either side (Payne, 1994).

The following chapters present a detailed description of the anatomical and physiological properties of callosal connections in the cat visual areas 17 and 18.

I.3 Anatomical characteristics of visual callosal connections in the cat visual areas 17 and 18

I.3.1 Neurons sending a callosal axon in A17 and A18

In A17 and A18, callosal axons arise from neurons in a strip of cortex centered on the transition zone (TZ) between A17 and A18 (Innocenti and Fiore, 1976; Sanides and Albus, 1980; Berlucchi, 1981; Segraves and Rosenquist, 1982b; Payne, 1986; Payne, 1991; Boire et al., 1995). The distribution of callosal neurons varies along the rostrocaudal axis of the TZ: their density is higher in posterior regions (Innocenti and Fiore, 1976; Boire et al., 1995), where their distribution can reach 3 mm in both A17 (Payne, 1991; Olavarria, 1996) and A18 (Houzel et al., 1994). This distribution shrinks in the anterior regions of the visual cortex and the density of callosal neurons decreases (Innocenti and Fiore, 1976; Boire et al., 1995) as shown in both Figure 6 and Figure 10. The extent of this distribution into A17 (3.5-4 mm) seems to vary little from one animal to another (Segraves and Rosenquist, 1982b; Payne, 1986; Payne, 1991). However, inter-individual variations have been described (Innocenti et al., 1985), mainly in A18. In this area, the distribution of callosal neurons appears variable at different rostro-caudal positions and is far from stereotyped from cat to cat (Sanides, 1978; Segraves and Rosenquist, 1982b; Payne, 1990b).

Cells projecting an axon through the callosal pathway are mainly located in the lower part of layer II/III, the upper part of layer IV and layer VI (Voigt et al., 1988; Boire et al., 1995). For homotopic connections between A17 and A18, the density of callosal neurons is higher in the supragranular layers (II/III). The density of these neurons in the infragranular layers gradually increases from A17 to A18 and on to higher visual areas: A19, PMLS and AMLS (Innocenti, 1980; Segraves and Rosenquist, 1982b; Segraves and Innocenti, 1985; Jouandet et al., 1985).
The vast majority of callosal neurons are large pyramidal cells as assessed by many studies based on the retrograde transport of horseradish-peroxidase (Jacobson and Trojanowski, 1974; Shatz, 1977b; Innocenti, 1980; Segraves and Rosenquist, 1982b; Payne, 1986). These cells are larger than the neighboring non-callosal cells (Innocenti, 1980). The callosal pyramidal cells are localized in layers II/III and VI (Voigt et al., 1988). In addition, non-pyramidal stellate cells, in layers II and IV and fusiform cells in layer VI also send a callosal axon in A17 and A18 (Voigt et al., 1988; Buhl and Singer, 1989). However, these cells represent only a small proportion of the callosal neuron population: around 20% with possible trans-synaptic contamination (Hughes and Peters, 1990; Peters et al., 1990). The callosal neuron population therefore consists principally of cells with the morphological characteristics of excitatory cells. This excitatory function has been confirmed electrophysiologically and histochemically (see below).

However, the proportion of non-large pyramidal cells suggests that some callosal neurons could use inhibitory transmitters. No GABA-callosal neurons have yet been detected.
by immunocytochemistry (Voigt et al., 1988) or selective uptake of radiolabeled transmitter (Elberger, 1989), but the existence of an inhibitory component remains compatible with the occasional observation of very rare symmetric callosal synapses (Voigt et al., 1988) and with the electrophysiological disclosure of short-latency transcallosal inhibition after pharmacological or cryogenic manipulation (Payne et al., 1991; Sun et al., 1994).

Figure 7. Left. Photomicrograph of callosal-projecting cells in the transition zone between A17 and A18, stained with HRP reaction product. Right. Drawing of five neurons shown in the left panel. The three neurons on the left are pyramidal cells with prominent apical dendrites that can extend up to 400 µm into layer I. Note also the profuse network of basal dendrites, extending laterally up to 300 µm from the cell somata. Many of the dendrites, both apical and basal, bear large numbers of spines. The two neurons on the right are stellate cells; their dendrites appear less well directed than the dendrites on the pyramidal cells. The dendrites of stellate cells rarely reach layer I and the distribution of the spines on these dendrites is relatively sparse. Descending axons to the white matter of all five neurons are marked by curved arrows. The axon collaterals of the left-hand pyramidal cell are indicated by filled and open, broad and narrow arrowheads. All these collaterals ascend toward the pial surface. Scale bar at bottom right, 100 µm. From (Payne, 1986).
General introduction

Figure 8. Diagram summarizing of the laminar distribution and morphology of cells, in the region of the transition zone between A17 and A18, that project to the contralateral hemisphere via the corpus callosum. Also shown is the laminar distribution of local axon collaterals (arrows). Callosal cells may have a pyramidal, stellate, or intermediate morphology and can be found in all layers except layer V. There are five times more The pyramidal cells in the superficial layers outnumber the cells in other layers and the cells with other morphologies by a factor of five to one (Payne, 1986).

I.3.2 Visual fibers in the corpus callosum

Electron microscopy studies have shown that approximately two-thirds of the visual callosal axons in cats are unmyelinated fibers with a diameter of 0.08 to 0.4 µm. The remaining fibers are myelinated axons of 0.25 to 4.0 µm in diameter (Berbel and Innocenti, 1988). This includes all axons in the posterior part of the corpus callosum, thus coming from all visual areas. The diameters of callosal axons reaching A17 and A18 range from 0.25 to 2.25 µm (Houzel et al., 1994). There are also probably thinner axons not visible under a light microscope. The heterogeneity in the diameter of these fibers was confirmed by electrophysiological studies, measuring the latency of antidromic activation of cells after electrical stimulation of the corpus callosum. The inferred conduction velocities ranged from 1.4 to 30 m/s (mean value 13 m/s) (Toyama et al., 1974; Harvey, 1980; McCourt et al., 1990).

In the splenium, the organization of callosal fibers linking A17 and A18 roughly respects the antero-posterior organization of the efferent neurons and thus preserves the topographic organization of the visual cortex (Innocenti, 1980; Payne and Siwek, 1991a). Then, axons from neurons in the caudal part of the visual cortex (sending information about the area centralis and the upper part of the visual field) are located in the caudal part of the splenium.
I.3.3  Callosal axon terminals in A17 and A18

The density of callosal fibers terminals is highest within a strip of cortex about 1-mm wide along the TZ (Fisken et al., 1975; Shatz, 1977b; Innocenti, 1980; Berlucchi, 1981; Segraves and Rosenquist, 1982a; Payne, 1986). This ribbon is more limited in extent than the callosal cell-zone (Ebner and Myers, 1965; Garey et al., 1968; Sanides, 1978; Segraves and Rosenquist, 1982a; Payne, 1986), but displays the same rostro-caudal gradient: it is wider in posterior regions and narrower in more anterior regions (see Figure 10 and Payne, 1986; Payne and Siwek, 1991b; Berman and Grant, 1992). A stereotyped disposition has been reported in A17, with callosal terminals present in the 1-2 mm along the TZ, (Payne, 1986; Payne and Siwek, 1991b) while the variability appeared mainly in A18 at the different rostro-caudal regions and in different animals (Sanides, 1978; Segraves and Rosenquist, 1982a; Payne, 1986; Payne and Siwek, 1991b). Studies with more sensitive methods have indicated that callosal terminals are also present in more distal zones of A17, up to 3 mm, at least in caudal regions (around P5) (Olavarría, 1996). Some rare clusters of callosal terminals have also been observed in more lateral parts of A18, i.e. beyond 3 mm (Sanides and Albus, 1980; Houzel et al., 1994). These clusters may correspond to the rare groups of cells with large receptive fields straddling the visual midline, which have been identified electrophysiologically in A18 with a strong inter-individual variability of presence and location (Sanides and Albus, 1980; Segraves and Rosenquist, 1982a). The overlap of the callosal sending zone with the callosal terminal zone suggests that some callosal neurons may also receive a callosal input. No such contact has been described in the visual cortex (Czeiger and White, 1993).
The callosal axons terminate in all layers, but their density is highest in layers II/III (Fisken et al., 1975; Shatz, 1977b; Innocenti, 1980; Payne and Siwek, 1991b), being somewhat lower in layer V and lowest in layer IV. In addition, the neurons in layers V and VI and some of the neurons in layer IV have apical dendrites ascending into layers II and III (Lund et al., 1979; Martin and Whitteridge, 1984). The callosal axons terminating in these layers may exert an influence on these apical dendrites.

These anatomical observations were confirmed by electrophysiological recordings with: 1) a section of the optic tract (Choudhury et al., 1965) or the optic chiasm (Berlucchi and Rizzolatti, 1968; Cynader et al., 1981; Leporé and Guillemot, 1982; Yinon et al., 1988;
Milleret et al., 1994), 2) electrical trans-synaptic activation of either the corpus callosum or the contralateral hemisphere (Harvey, 1980; Innocenti, 1980) and 3) cooling inactivation of the contralateral hemisphere (Payne et al., 1991). The results obtained indicated that most callosal neurons were located in the TZ, in supragranular layers.

Most callosal terminals form excitatory asymmetric synapses on the spines of apical and basal dendrites of pyramidal cells (in layers II/III and VI) and on varicose smooth dendrites shafts of non-pyramidal cells (in layer IV) (Fisken et al., 1975; Voigt et al., 1988). Then, a direct effect of interrupting transcallosal transmission would be the reduction of these pyramidal and non-pyramidal cells excitatory activities. Physiological observations in cooling experiments have provided evidence for such a decrease (Payne et al., 1991). These observations indicate that the main function of callosal axons is to activate excitatory cells (see also Naito et al., 1970). However, the description of inhibitory transcallosal effects with short latencies (Toyama and Matsunami, 1976; Harvey, 1980; Innocenti, 1980; Payne et al., 1991; Sun et al., 1994) suggests that some callosal terminals form excitatory synapses with inhibitory neurons. Indeed, some visual callosal terminals contact basket cells (known as inhibitory cells) in layers III-IV (Somogyi et al., 1983). A few symmetric callosal synapses have also been described, mainly on pyramidal cells (Voigt et al., 1988; Hughes and Peters, 1990).

Callosal-projecting neurons (pyramidal and stellate) also display “proximal” axonal arborization in their hemisphere of origin. As the contralateral branches, these terminations mainly connect supragranular layers (Innocenti et al., 1977; Voigt et al., 1988), form asymmetric synapses on dendritic spines and seem to avoid cells that send a callosal axon (Czeiger and White, 1993). Thus, callosal neurons seem to have the same selectivity in the choice of ipsi- and contra- lateral post-synaptic targets.

I.3.4  Topographic organization of visual callosal connections in A17 and A18

I.3.4.1  Visuotopic organization

Callosal connections link cortical sites in each hemisphere in a non mirror-symmetric pattern which suggests a visuotopic organization (Olavarria, 1996; Alekseenko et al., 2005). Callosal neurons with somata in A17 or A18 in one hemisphere project their axons to the contralateral hemisphere primarily within the TZ and not in the mirror-symmetric regions of A17 and A18 (Figure 11). Conversely, callosal neurons located in the TZ project their axons to the opposite hemisphere primarily within A17 and A18. The physiological explanation of this pattern supposes that: 1) callosal connections link sites that are in retinotopic correspondence, i.e. sites where the same part of the visual filed is represented, 2) the TZ contains two mirror-symmetrical representations of the ipsilateral visual hemifield and the CVM (see Figure 11, representation of the visual field locations A, B and C in the left hemisphere). The first point was confirmed by numerous electrophysiological studies which used, in particular, a section of the optic chiasm (see below). The second point is not clearly established but appears in
some figures from electrophysiological recordings across the TZ, in which the location of each recorded receptive field is indicated (see Tusa et al., 1978 and Figure 12 from Orban, 1984b).

Figure 11. Top. Diagram relating retinal projections, the visual field representation in the callosal zone of A17 and A18 (the portions of these areas sending or receiving callosal inputs) as well as the topography and ocular dominance of callosal connections. For simplification, the visual thalamus and the topographic callosal organization in A18 in the left hemisphere are not represented. From (Stryker and Antonini, 2001). Bottom. Schematic diagram of the mediolateral organization of callosal linkages in the TZ. Cortical loci in one hemisphere, marked with the letters A-E, are callosally connected with loci marked with the same letters in the opposite hemisphere. Thus, loci C-E outside the TZ in A17 of the left hemisphere connect with contralateral loci C-E located within the TZ. Symmetrically, loci A-C within the TZ of the left hemisphere connect with loci A-C outside the TZ in the contralateral A17. A similar projection pattern is represented in A18. Letters are represented closer in the TZ than outside the TZ to indicate that the TZ receives convergent input from contralateral regions outside the TZ and, conversely, that it gives rise to divergent input to contralateral regions outside the TZ. From (Olavarria, 1996).
I.3.4.2 Columnar organization

Almost forty years ago, the newly developed Nauta technique was applied to visualize degenerating fibers after section of the corpus callosum. It was already noted that the overall distribution of callosal terminals was uneven over the cortical surface (Heimer et al., 1967). Other studies of callosal projections of sensory, motor and association areas in several species described heterogeneous columnar patterns with “column” diameters ranging from 200 to 1000 µm [for review see (Innocenti, 1986a); striate cortex, (Berman and Payne, 1983; Voigt et al., 1988; Houzel et al., 1994); extrastriate cortex, (Segraves and Rosenquist, 1982a)].

Concerning the visual cortex, this columnar (or “banded”) organization was first observed in the monkey (Newsome and Allman, 1980; Van et al., 1982) and the rat (Cipolloni and Peters, 1979), but only rarely in the cat (Berman and Payne, 1983; Innocenti, 1986a). More sensitive methods suggested a tendency of cat callosal neurons and terminals to form patches along the TZ (Voigt et al., 1988; Boyd and Matsubara, 1994). The 3-D reconstructions of individual callosal axons indicated that, in adult animals, callosal terminals were non homogeneously distributed and formed a patchy pattern (Houzel et al., 1994). The earliest columnar organization of callosal terminals in the cortical plate was observed at about P12-P20, i.e. when the callosal axons just enter the cortex (Aggoun-Zouaoui et al., 1996). The somata of callosal-projecting neurons also display a patchy organization, which is less apparent than that of callosal terminals (Segraves and Rosenquist, 1982b; Voigt et al., 1988).

The functional significance of the columnar pattern of visual callosal projections remains unclear, although relations to orientation columns (Innocenti, 1986b; Voigt et al., 1988; Houzel et al., 1994), ocular dominance bands (Payne and Siwek, 1991b; Olavarria, 2001) and cytochrome oxidase-positive patches (Boyd and Matsubara, 1994) have been suggested. Indeed, both callosal neurons and terminals were described densely packed within cytochrome oxidase-dense domains of the visual cortex of cats (Boyd and Matsubara, 1994).
and macaques (Olavarria and Abel, 1996). More recently, it was shown that periodicities in the distribution of callosal neurons correlate with the pattern of ocular dominance columns (Olavarria, 2001): callosal neurons were found preferentially within ipsilateral cortical domains in A17 and A18 and within contralateral domains in the TZ (Figure 11). This suggests that callosal connections are not just specific for the same part of the visual world since they also specifically connect ocular dominance columns serving the same eye (Olavarria, 1996; Olavarria, 2001).

The distribution of the clustered callosal axon terminals with respect to the orientation preference map remains unknown. Two previous studies investigated the distribution of callosal neurons somata (retrogradely labeled) in orientation maps and they provided opposite results. In strabismic cats, callosal connections were found to link similar orientation domains (Schmidt et al., 1997). However, because the distribution and properties of callosal axons are strongly modified in strabismic cats, these results cannot be directly extended to normal cats (Innocenti and Frost, 1979; Berman and Payne, 1983; Elberger et al., 1983). Furthermore, in the visual cortex of the normal adult tree shrew, callosal connections were found to terminate without respect to the different orientation domains (Bosking et al., 2000).

I.4 Physiological characteristics of visual callosal connections in A17 and A18

The functional properties of the cells activated by transcallosal connections have been studied by different experimental approaches.

Forty years ago, Whitteridge and his collaborators observed that cortical responses to visual stimuli located at the center of the visual field (at or near the vertical midline) persisted after section of the ipsilateral optic tract and disappeared when the activity of the contralateral hemisphere was abolished by local cooling (Choudhury et al., 1965; Vesbaesya et al., 1967). Later, Hubel and Wiesel (Hubel and Wiesel, 1967) observed overlapping receptive fields of pairs of visual cells recorded simultaneously in both hemispheres of intact cats. The positions of these receptive fields indicated a likely involvement of the corpus callosum in the transfer of information about the center of the visual field. Then, the aforementioned authors as well as others recorded directly the activity of callosal fibers by placing an electrode in the splenium. They demonstrated that the recorded receptive fields were all located along the central vertical meridian of the visual field.

A beautiful neurophysiological demonstration of corpus callosum function came from the work of Giovanni Berlucchi and Giacomo Rizzolatti in Pisa in 1968 (Berlucchi and Rizzolatti, 1968). Berlucchi and Rizzolatti cut the optic chiasm along the midline and then made recordings from A17, close to the transition zone with A18, looking for cells that could be driven binocularly. In this protocol, any binocular cell in the visual cortex on the right hemisphere receive input from the right eye, directly via the ipsilateral retino-geniculo-cortical pathway and from the left eye, by way of the left hemisphere and the corpus callosum...
They observed that the corresponding pairs of receptive fields spanned the vertical midline and display similar orientation preference selectivity (see also below). The selective activation of the CC by the stimulation of the contralateral eye was confirmed electrophysiologically by combining the chiasmatomy with either a section of the CC (Berlucchi, 1981; Antonini et al., 1983; Milleret and Houzel, 2001), or an application of procaine in A17 and A18 in one hemisphere (Milleret and Buser, 1993). In both cases, responses of neurons to a visual stimulation of the contralateral eye disappeared in the visual cortex.

Figure 13. Split-chiasm preparation. The optic chiasm (XO) is sectioned midsagittally. Crossed fibers coming mainly from the nasal hemiretinae (N) are severed while uncrossed fibers originating mainly from the temporal hemiretinae (T) remain intact, projecting to the visual cortex via the optic nerve (ON), the ipsilateral optic tract (OT), the dorsal lateral geniculate nucleus (dLGN) and the optic radiations (OR). Split-chiasm cats can be stimulated monocularly in order to activate selectively either the transcallosal (TC) pathway or the geniculo-cortical (GC) pathway by stimuli presented on a video screen. For example, stimulation of the right (R) eye activated the right hemisphere (RH) only through the ipsilateral retino-geniculo-cortical pathway and the left hemisphere (LH) only through the corpus callosum (CC) (thick grey lines and arrows). Stimulation of the other eye, left (L) eye, induced the symmetric activation pattern. This allowed comparison of the transcallosal and geniculo-cortical activation patterns of the same region.

I.4.1 Proportion of visual cells activated by the transcallosal pathway

The split-chiasm preparation allows the evaluation of the proportion of visual cells activated by the transcallosal pathway in A17 and A18. However, a large disparity of results exists concerning this proportion: from 0% to 10% (Yinon et al., 1982), approximately 30% (Berlucchi and Rizzolatti, 1968; Leporé and Guillemot, 1982), 55% (Milleret and Buser, 1987; Milleret and Buser, 1993) or more than 75% (Cynader et al., 1986). This variability is likely due to the different delays after the surgery (from a few days to several years). Two studies (Milleret and Buser, 1987; Milleret and Buser, 1993) demonstrated that the amount of time between the chiasmatomy and the cortical exploration can modify the properties of the interhemispheric transfer. In particular, the distribution of the TC units and their functional characteristics (strength of the response, size of the receptive fields) can be subjected to significant modifications.
I.4.2  **Physiological properties of transcallosally activated units**

Given the retinotopic organization of the retino-geniculo-cortical pathway in the cat visual system, the transition zone between A17 and A18 comprises the representation of the central vertical meridian (CVM) of the visual field. As mentioned above, the receptive fields of callosally-projecting neurons (identified by antidromic activation, Harvey, 1980; Innocenti, 1980), of visual callosal fibers (located in the posterior part of the corpus callosum, (Hubel and Wiesel, 1967; Shatz, 1977a)) and of transcallosally activated neurons (Choudhury et al., 1965; Berlucchi et al., 1967; Berlucchi and Rizzolatti, 1968) are located on the CVM and in its vicinity. In 1991, Payne and his collaborators published a detailed study of the receptive fields of neurons that were included in the callosal sending zone and in the callosal recipient zone, defined respectively by retrograde and anterograde tracers. The portion of the visual field represented within the callosally connected zone bears the shape of an hour-glass, centered on the CVM and spanning several degrees apart from it on both sides (Payne and Siwek, 1991b), (Figure 14).

In the callosal sending zone, the representation of the visual field extends to only 4° or 5° into the contralateral field at the horizontal meridian and expands at progressively greater distances from that meridian to represent positions of up to 15° in the upper visual field and more than 20° in the lower visual field. In the callosal recipient zone, the extent of the representation of the ipsilateral visual field also increases both above and below the visual axis. These increases closely match those that occur in the transcallosal sending zone (Figure 14 and Figure 15). Indeed, in the callosal recipient zone, the centers of the receptive fields (RFs) reach to 2.5° (and the RFs extend to 3.6°) into the ipsilateral field, on the horizontal meridian whereas the RFs centers reach to more than 10° (and RFs extend to beyond 20°), towards the periphery of the visual field. The representation of the visual field is significantly reduced to approximately one-half to one-third of its normal extent, after either a callosotomy or a cryogenic inhibition of the efferent cortex (callosal sending zone) (Payne, 1990a).
The contribution of the transcallosal pathway to the representation of a portion of the ipsilateral visual hemifield was demonstrated by electrophysiological recordings of transcallosally activated units after a section of the optic chiasm (Milleret and Buser, 1984; Milleret et al., 1994; Milleret et al., 2005). All studies confirm that the receptive fields (RFs) of neurons activated by the transcallosal pathway are mainly centered on the vertical meridian or on its flanking regions. The lateral limits of these receptive fields reach 6° and 13° in the contra- and the ipsi-lateral hemifields, respectively (RFs centers position at 4° and 9°, respectively) (Milleret and Houzel, 2001; Milleret et al., 2005). The mean surface of these receptive fields is 16.4 ± 2.8 deg² (Figure 15). The position and the extent of these receptive fields also vary according to the elevation in the visual field (hourglass shape).

Single cortical units that are activated by callosal terminals are orientation selective (Berlucchi and Rizzolatti, 1968), (Figure 16). Altogether, these units encode all orientations equally (Leporé and Guillemot, 1982; Milleret et al., 1994).
I.4.3 Binocular interactions: ocular dominance and binocular disparity

Due to the spatial separation of the two ocular globes, each point of a tridimensional object is projected on non-corresponding regions of both eyes: the temporal part of one retina and the nasal part of the other. Visual cortical neurons receive this information from both eyes. The property of binocular integration of visual neurons in A17 and A18 results from the convergence of information through different pathways: 1) a contralateral pathway from the nasal hemiretina; 2) another contralateral pathway from a tiny portion of the temporal retina; 3) a pathway from the ipsilateral temporal hemiretina; 4) cortico-cortical inputs coming through the corpus callosum (Figure 2). Electrophysiological studies indicate that most cells activated by the transcallosal pathway are binocular (Berlucchi and Rizzolatti, 1968; Harvey, 1980; Innocenti, 1980; Leporé and Guillemot, 1982; Milleret et al., 1994; Milleret et al., 2005). However, the contribution of callosal connections to the binocular interactions in A17 and A18 remains unclear. Studies evaluated the proportion of binocularly-driven cells before and after the removal (or cooling) of the contralateral visual areas or the section of the corpus callosum. Conflicting results were obtained. Some studies described a strong reduction in the number of binocular cells within the TZ (Dreher and Cottee, 1975; Payne et al., 1980; Blakemore et al., 1983; Payne et al., 1984; Yinson et al., 1988). Other investigations reported only a slight reduction in the number of the binocular cells or no reduction at all (Leporé et al., 1983; Minciacchi and Antonini, 1984; Elberger and Smith, 1985; Gardner and Cynader, 1987; Berlucchi and Antonini, 1990). However, callosal connections may still intervene in some binocular processes such as binocular stereopsis.

After a section of the optic chiasm, the contralateral geniculo-cortical pathway is cut and can no longer contribute to the activation of cortical cells. Neurons in A17 and A18 can still be activated by the ipsilateral eye through the ipsilateral retino-geniculo-cortical pathway and by the contralateral eye through the corpus callosum (Figure 13). Binocular units are more strongly activated by the ipsilateral than by the contralateral (transcallosal) pathway, as shown in the distribution of ocular dominance (Figure 16). The corresponding receptive fields (RFs), plotted through the activation of each pathway, overlap. However, most pairs of RFs display position disparity of the crossed or uncrossed type with a mean of 5.4 ± 0.5° between their centers (Figure 16) (Cynader et al., 1986; Leporé et al., 1992; Milleret and Houzel, 2001; Milleret et al., 2005). As initially suggested by Blakemore (Blakemore, 1969; Mitchell and Blakemore, 1970) such position disparity may account for the involvement of the corpus callosum in “coarse” stereopsis along the visual midline, at various distances in front of or behind the fixation plane (e.g. Blakemore and Cooper, 1970; Timney et al., 1985; Leporé et al., 1986; Gardner and Cynader, 1987; Jeeves, 1991). More recent data indicate that the corpus callosum mainly contributes to stereopsis in front of the fixation plane (Milleret et al., 2005). The corpus callosum may also be involved in more subtle mechanisms of depth perception than stereopsis, such as relative motion or parallax, using the differences in relative motion of near and distant objects to judge depth (Rivest et al., 1994).
Figure 15. A. Experimental design. B-C. Distributions, within the visual field, of the transcallosal (TC, B) and the geniculo-cortical (GC, C) receptive fields of trancallosally activated units. El, elevation axis (which coincides with the central vertical meridian); Az, azimuth axis; i and c, positions of the ipsilateral (right) and contralateral (left) optic discs; n, number of receptive fields shown; m, mean receptive-field size ± SEM; in parentheses, extreme values. Receptive fields with centers at small (0 to -10 deg) and large (-10 to -40 deg) elevations were distinguished by taking the TC receptive fields elevation as a reference. D. Azimuths (in degrees) of medial limits, centers and lateral limits of the transcallosal (TC-RFs) and the geniculo-cortical (GC-RFs) receptive fields of trancallosally activated units, as shown in B and C. RFs with centers at small (0 to -10 deg) and large (-10 to -40 deg) elevations are distinguished. The extremes of each horizontal line correspond to the maximal values and are negative in the hemifield contralateral to the explored cortex and positive in the ipsilateral hemifield. Means are indicated by vertical bars and the SEM by a thick horizontal line. CVM, central vertical meridian. From (Milleret et al., 2005).
Figure 16. A. Orientation selectivity of the TC-GC binocular units. S, orientation selective units; NS, nonselective units; N, total number of units. Bottom, polar diagram showing the distribution of the preferred orientations encoded by orientation selective units. n, number of units. From (Milleret and Houzel, 2001). B. Ocular dominance of transcallosally activated units (TC units). Percentages of TC units were separated into five classes according to the relative strength of responses to contralateral and ipsilateral eye stimulation: contralateral activation only (c), preferential activation through the contralateral (c > i) or the ipsilateral eye (i > c), equal activation through both eyes (c = i). The two remaining classes represent the non TC units: those displaying only ipsilateral responses (“i” class) and the nonvisual cells (“nv” class). N, total number of units. C. Spatial distribution of pairs of receptive fields of binocular TC-GC units, after off-line realignment of the eyes, with open circles for GC-RF centers and filled circles for TC-RF centers. Lines connect corresponding centers of RFs observed for individual cells and indicate position disparity. El and Az, elevation and azimuth axes. N, number of units. m, mean position disparity value ± SEM (in deg). Extreme values are in parentheses. D. Distribution of position disparity values of receptive fields of the TC-GC binocular units. The analysis was performed with distinct elevation ranges (0 to -10 deg and -10 to -40 deg). N, number of units; m, mean ± SEM. From (Milleret et al., 2005).
1.5 Aims of the thesis

The work described in this thesis aimed to increase our understanding of the anatomical and functional organization of the interhemispheric transfer of visual information. In order to understand this complex network of reciprocal connections, more information is needed concerning the functional organization of the cortical regions activated by these connections, as well as, at the cellular level, the distribution of callosal axon terminals. The development of imaging techniques and stable anterograde neuronal tracers permit the investigation of both the precise topology of neuronal connections and their relationship to the functional organization of cortical areas. The results presented in this thesis concern exclusively the callosal connections between visual areas 17 and 18 (A17 and A18) of the adult cat.

In the first two chapters of this thesis, the functional specificity of callosal connections is investigated at the level of the population of the neurons activated by these connections. Split-chiasm preparations were used in order to isolate callosal activity from other inputs. Optical imaging of intrinsic signals revealed the layout of the regions activated through the transcallosal pathway, over large portions of A17 and A18.

The aim of the first chapter is to present the layout of this transcallosal activity, revealed by using moving oriented gratings as visual stimuli. The functional characteristics of the resulting “transcallosal maps” are described: the layout of the transcallosally activated orientation domains, their differential activation in response to specific spatio-temporal frequencies of the visual stimulus and, finally, their relation to the orientation domains activated through the direct geniculo-cortical pathway.

The second chapter analyzes the retinotopic organization of callosal connections and the layout of the ipsilateral visual field representation in the transition zone between A17 and A18. The results obtained in split-chiasm animals are compared with those obtained in intact animals. This comparison aims at revealing the contribution of each hemiretina to the cortical representation of the ipsilateral visual field, through the transcallosal pathway.

In the third chapter, the functional specificity of the callosal connections is investigated at the level of individual callosal axons. The aim was to determine whether callosal connections link similar functional domains in the visual cortex of each hemisphere. To answer this question, optical imaging of intrinsic signals was combined with extracellular neuronal tracer injections and anatomical methods. The distribution of callosal axon terminals within functional maps of the visual cortex (retinotopic, orientation, direction and ocular dominance maps) was compared with the functional properties at the injection sites.

Before the results of the aforementioned investigations are revealed, the chapter “Material and methods” presents the different experimental protocols.
II Material and Methods

II.1 Animals

Adult cats from the animal house of Ruhr-Universität were used in this study. These animals were reared under normal visual conditions, were in good health and had no apparent malformations or pathologies of the eyes or vision.

Seven cats (9 to 29 months old) were used to investigate the layout and the retinotopic organization of transcallosal activity in visual areas 17 and 18, after a section of the optic chiasm (chapter 1 and 2).

Studies of the anatomy of callosal connections and their functional specificity (chapter 3) were carried out on four cats (6, 7, 14 and 22 months old).

All surgical procedures conformed to the guidelines of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 18.III, 1986).

II.2 Optical imaging of intrinsic signals

II.2.1 Advantages of the optical imaging of intrinsic signals over other imaging techniques

One of the aims of this work was to determine the layout and functional characteristics of the regions activated through the transcallosal pathway, over large portions of A17 and A18. Classical single- or multi-unit recording techniques are not very suitable for studies of neuronal populations of this type, because sampling is limited to a small part of the neuronal population.

Two general properties of the functional organization of neuronal populations can be studied: spatial distribution and temporal dynamics. Several imaging techniques have been developed for such studies but most have either a high spatial or a high temporal resolution, but not both. For example, electroencephalography and magnetoencephalography have been developed for studies of the dynamics of cortical processing in the intact human brain. However, the spatial resolution of these two methods is not yet high enough to resolve individual cortical columns. Positron emission tomography (PET) and functional magnetic resonance imaging (f-MRI) can be used for the three-dimensional localization of active regions in the functioning human brain, but their temporal and spatial (around 1 mm) resolutions are currently lower than those of other techniques used in animal models. For example, the 2-deoxyglucose method (2-DG) can be used for the postmortem visualization of
Material and methods

active brain regions (in animals), with a spatial resolution of around 50 µm. However, this technique has a time resolution of minutes or hours. Another disadvantage of the 2-DG method is that it is a one-at-a-time approach: only one stimulus condition can be tested in a single animal (although the two-isotope 2-DG method permits the mapping of activity resulting from two stimulus conditions).

Optical imaging based on intrinsic signals can be used to visualize active cortical columns at a high spatial resolution (50 µm) over large portions of the cortex in vivo (in animals). The number of stimulus conditions that can be tested is limited only by the physiological state of the animal. Only acute experiments were performed in the studies presented in this thesis. In these conditions, functional maps can be imaged over three or four days. However, optical imaging can also be used for long-term chronic experiments, such as studies of postnatal experience-dependent plasticity and development in the neocortex (Chapman et al., 1996; Gödecke et al., 1997; Crair et al., 1998).

Like the other methods described above, the temporal resolution of optical imaging of intrinsic signals is too low for studies of the dynamics of cortical processing. However, this is not the case for a more recent method of optical imaging based on voltage-sensitive dyes. The temporal (1 ms) and spatial (50-100 µm) resolutions of this technique are both high. However, this technique presents some difficulties to image, with a good spatial resolution, large cortical areas.

The optical imaging of intrinsic signals thus appears to be an appropriate technique for studies of the spatial organization of functional domains over large cortical regions. Because this method can be used to image large portions of cat visual areas 17 and 18, simultaneously in both hemispheres, it was chosen to study the interhemispheric transfer of visual information between these areas.

II.2.2 Sources of the intrinsic signals imaged optically

The optical imaging of intrinsic signals technique is based on slow intrinsic changes in the optical properties of active brain tissue (see review in Bonhoeffer and Grinvald, 1996). A strong coupling exists between neuronal activity, local metabolic activity and blood flow (Lassen and Ingvar, 1961; Sokoloff, 1977; Raichle et al., 1983; Fox et al., 1986). The main conclusion regarding the origin of the intrinsic signal is that following sensory stimulation, there is an initial increase in the concentration of deoxy-hemoglobin, due to an increase in the oxygen consumption of the active neurons. This increase causes a darkening of the cortex on the recording images at wavelengths between 600-700 nm, because deoxy-hemoglobin has a higher absorption spectra than oxy-hemoglobin at these wavelengths (Figure 17). It is followed by a larger decrease in the concentration of deoxy-hemoglobin, due to large but delayed changes in blood flow which supplies highly oxygenated blood to the activated cortical area. The third signal component originates from changes in blood volume. These changes probably result from local capillary recruitment or a rapid filling of capillaries and dilation of venules in an area containing electrically active neurons. These blood-related
components dominate the signal at wavelengths between approximately 400 and 600 nm. The light scattering component, which becomes a significant source of intrinsic signals above 630 nm, dominates the intrinsic signals in the near infra-red region above 800 nm.

The source of the intrinsic signal therefore depends on the illumination wavelength. At around 600 nm, oxygen consumption appears to dominate the signal. This oxymetry signal reflects both subthreshold and spiking activity, but it remains unclear whether it reflects mostly subthreshold synaptic potentials (Das and Gilbert, 1995; Das and Gilbert, 1997), or spiking activity (Toth et al., 1996).

The intrinsic signals that can be measured in the living brain are weak. In optimal conditions, neuronal activity results in a change in light intensity of about 0.1 to 0.2% of the total intensity of the reflected light (at 605 nm). Intrinsic signals must therefore be extracted from the cortical images with appropriate data acquisition and analysis procedures. Electrophysiological or histological confirmation is therefore necessary, whenever possible.

**II.2.3 The set-up**

Figure 18 and Figure 19 show the experimental set-up for optical imaging experiments *in vivo*. The head of the anaesthetized and paralyzed animal is rigidly attached in a Horsley-Clarke apparatus. The exposed cortex of the animal is sealed in an oil-filled chamber (Figure 20) and illuminated with an orange light (wavelength, 609 nm). Reflectance images are acquired with a camera while the cat is visually stimulated. Active regions appear darker.
Material and methods

Figure 18. Picture of the set-up for optical imaging experiments (Dr Milleret’s group, LPPA, Paris).

Figure 19. Set-up for optical imaging of functional maps in vivo. The exposed cortex of the animal is sealed in an oil-filled chamber and illuminated with light of 609 nm wavelength. The images are acquired with the camera, while the animal is visually stimulated with moving gratings on a computer screen. The acquired images are digitized by a computer controlling the entire experiment. The signal to noise ratio of the functional maps is improved by averaging several stimulus sessions. Functional maps are subsequently analyzed. To determine the quality of the maps during the imaging sessions, the data can be sent to a second computer for quasi on-line analysis. Figure modified from (Bonhoeffer and Grinvald, 1996).
Material and methods

II.2.4 Surgical procedures for optical imaging and electrophysiological recordings

The animals were prepared for recordings using standard procedures described previously (Buzás et al., 1998; Yousef et al., 1999). Anesthesia was induced with a mixture of ketamine (10 mg/kg; Ketavet; Pharmacia and Upjohn, Erlangen, Germany) and xylazine (1 mg/kg, i.m.; Rompun; Bayer Belgium, Sint-Truiden, Belgium). Neutral contact lenses with eye drops (1.5% saline) were used to protect the corneas from drying. The femoral artery was cannulated in order to monitor blood pressure (95-140 mm Hg) and to infuse a mixture of muscle relaxant (alcuronium chloride; 0.15 mg/kg/hour; Alloferin, ICN Pharmaceuticals, Frankfurt/Main, Germany) and glucose (24 mg/kg/hour; Glucosteril; Fresenius Kabi, Bad Homburg, Germany) in Ringer solution (Ringerlösung; Fresenius Kabi, Bad Homburg, Germany). A tracheal cannula was implanted for prolonged anesthesia (0.4-0.6% of halothane; Halothane; Halocarbon, NJ, USA) and artificial ventilation (1:2 mixture O₂ and N₂O). Expiratory CO₂ (3-4%), body temperature (38.5°C) and EEG were monitored continuously.

A craniotomy was performed on both hemispheres between stereotaxic coordinates (Horsley-Clarke) P7-A12 and L0.5-L6.5 in order to expose the cortical region corresponding to the representation of the central and lower parts of the visual field in both A17 and A18 (Tusa et al., 1978; Tusa et al., 1979). A round metal chamber (30 mm inner diameter) was mounted onto the skull using dental cement (Paladur; Heraeus Kulzer, Wehrheim, Germany).
Material and methods

and bone screws were placed in the frontal bone (Figure 20). Then, the dura mater was removed and the chamber was both filled with silicone oil (50 cSt viscosity; Aldrich, Milwaukee, WI, USA) and sealed with a round coverglass.

Before starting the imaging sessions, the nictitating membranes were retracted with 5% phenylephrinhidrochloride (Neosynephrin-POS; Ursapharm, Saarbrücken, Germany) and the pupils were dilated with 1% atropine sulfate (Atropin-POS; Ursapharm). Correction lenses for a viewing distance of 28.5 cm were applied on the basis of tapetal reflection.

II.2.5 Visual stimulation

Visual stimuli were generated with the stimulus generator VSG Series Three system (Cambridge Research Systems, Rochester, England) and presented on a video screen at 100 Hz in non-interlaced mode at a distance of 28.5 cm in front of the cat’s eyes. Animals were stimulated monocularly.

By using an ophtalmoscope, the locations of the optic disks and of the area centralis were projected onto the video screen in front of the animal. This video screen was centered on the vertical midline of the cat visual field and its height was adjusted in order to have the area centralis in the upper part of the screen, at a quarter of the screen’s length, from the top. This allowed mainly the lower part of the visual field to be stimulated, i.e. the region represented in the imaged area (rostral part of A17 and A18). The vertical midline was defined as the vertical line crossing the area centralis. The area centralis corresponded to the geometric center of the retinal area devoid of blood vessels. This position was confirmed by controlling the geometrical relation between the area centralis, the vertical meridian and the optic disk (Bishop et al., 1962).

For orientation maps, the stimuli consisted of full-field, high-contrast, square-wave luminance gratings that moved back and forth along the orthogonal axis of the orientation in either direction for half of the data acquisition period. A single stimulus trial consisted of gratings of four or eight equally spaced orientations (0, 45, 90, 135 deg and 0, 22.5, 45, 67.5, 90, 112.5, 135 deg, respectively) at spatial and temporal frequencies (generally, 0.15 cpd, 1.5 Hz i.e. 10 deg/s) that strongly activate the part of the visual cortex where most transcallosal connections are located (Figure 21).

The transition zone (TZ) between A17 and A18 was functionally localized in activity maps based on the different spatial and temporal frequency preferences between A17 and A18 neurons. These maps were obtained by using gratings of four equally spaced orientations (0, 45, 90 and 135 deg) at two different spatial and temporal frequencies (0.6 cpd, 1.5 Hz i.e. 2.5 deg/s to activate A17 and 0.15 cpd, 4.5 Hz i.e. 30 deg/s to activate A18) (Bonhoeffer et al., 1995).
Material and methods

For mapping horizontal eccentricity (azimuth), a narrow vertical bar (length, 53°; width 1°) containing a moving horizontal grating (0.15 cpd, 1.5 Hz i.e. 10 deg/s) was displayed at 14 different positions in the visual field along its horizontal axis (Azimuth axis, Az), by step of 1° to 4°, (degrees of azimuth: -16, -12, -8, -6, -4, -2, -1, 0, 1, 2, 4, 6, 8, 12), (Figure 22). For the retinotopic mapping of transcallosal activity (see chapter 2), this stimulus was presented up to +32°. In all positions, the grating was moving along a vertical axis, in one direction for half of the data acquisition period and then in the opposite direction. Negative and positive values corresponded to the left and the right hemifield respectively; 0° corresponded to the central vertical meridian (CVM). The presence of residual eye movements in paralyzed cats (Chow and Lindsley, 1968; Bishop et al., 1971) can modify the location of the vertical meridian. These movements were controlled in each experiment by regularly plotting the optic disks, the area centralis and easily identifiable intersections of blood vessels. In the present study, apparent shifts in eye positions appeared only during long recording sessions (approximately 5-6 hours). The magnitude of these movements was approximately 1-2°. In these cases, the results of the retinotopic mapping were corrected.

For mapping vertical eccentricity (elevation), images were acquired during the presentation of a narrow horizontal bar (width 1°) containing a moving vertical grating (0.15 cpd, 1.5 Hz i.e. 10 deg/s) at 14 different positions (degrees of elevation: +3, +1, -1, -3, -5, -7, -9, -11, -13, -15, -17, -19).

For ocular dominance maps, the stimulus trial consisted of full-field gratings of four equally spaced orientations (0, 45, 90 and 135 deg) at 0.15 cpd spatial and 1.5 Hz (10 deg/s) temporal frequencies that were presented monocularly using ferromagnetic liquid crystal eye shutters (Displaytech, Boulder, CO; or Vision Research Graphics, Inc.) controlled by the VDAQ software.

All stimuli were displayed in a pseudorandom sequence and were presented 50-225 times for orientation and spatial frequency maps and 450-1500 times for retinotopic and ocular dominance maps, depending on the experiment. Altogether, each eye was stimulated between 13 and 58 min per imaging session for orientation and spatial frequency maps and between 1 h. 56 min and 6 h. 28 min for retinotopic and ocular dominance maps, depending on the level of activation of the visual cortex during recordings.

II.2.6 Data acquisition
Optical imaging of intrinsic signals was carried out using the Imager 2001 imaging system (Optical Imaging, Inc., Mountainside, NJ, USA) and the data acquisition software VDAQ (NT version 1.0.1.0293, Optical Imaging, Mountainside, NJ, USA) (see a detailed description in Buzás et al., 1998; Yousef et al., 1999). Briefly, the cortex was illuminated with a circular
fiber optic slit lamp (Schott, Mainz, Germany) surrounding the camera optics (two SMC Pentax lenses, 1:1.2, f=50 mm, arranged in a tandem manner (Ratzlaff and Grinvald, 1991)). For simultaneous imaging of the two hemispheres, a converter (1:2, AF Telekonverter C/D7, Soligor, Leinfelden-Echterdingen, Germany) was added in front of the tandem lenses.

The vascular pattern of the cortical surface was imaged using 545±10 nm (green) light before and after each recording session. During data acquisition, the cortex was illuminated with 609±5 nm (orange) light and the camera was focused 700-750 µm (for optics of 1:1 magnification) or 900-1000 µm (for 1:2 magnification) below the cortical surface. During inter-stimulus intervals (10 sec), the animal viewed a stationary image of the next stimulus to be moved (Figure 23). Data acquisition commenced 1 sec after the stimulus grating began to drift. Camera frames were recorded for 4.5 sec at a rate of 25 Hz with a camera Teli CS8310C (Tokyo Electronic Industries, Tokyo, Japan), when the grating moved along the orthogonal axis of the orientation alternatively in one direction and the other for exactly the same duration. The camera frames were summed temporally into 10 data frames. The spatial resolution of the final images was 21.28 × 21.28 µm per pixel.

Figure 23. Stimulus sequence. Data acquisition period was preceded by an inter-stimulus interval of 10 seconds, while the animal viewed a stationary image of the next stimulus to be moved. At the end of this period, the stimulus grating started drifting and data acquisition commenced 1 sec later, for a total duration of 4.5 sec. During the acquisition period, gratings moved for exactly the same duration back and forth along the orthogonal axis of the orientation. All stimuli were displayed in a pseudorandom sequence.

II.2.7 Calculation of the functional maps

Single condition maps (SCMs) were calculated by summing the images associated with a particular attribute of the visual stimulus (orientation, direction, spatial frequency, azimuth position or stimulated eye) using the MIX software (Optical Imaging, Inc.). All SCMs were divided by the sum of images recorded for all stimulus conditions (cocktail blank) (see Bonhoeffer and Grinvald, 1993; Bonhoeffer and Grinvald, 1996). Cocktail blank (CB) was calculated by summing all data frames (DFi) collected during the presentation of an entire set of stimuli (i):

\[
CB = \sum_{i=1}^{n} DFi
\]
where \( n \) represents the number of stimulus grating conditions multiplied by the number of repetitions.

Single condition maps were calculated with:

\[
SCM(\alpha) = \frac{\sum_{i=1}^{n} DFi(\alpha)}{CB}
\]

where \( DFi(\alpha) \) represents the corresponding data frames for a particular stimulus condition \( \alpha \) (for example, one orientation of the stimulus) and \( n \) is the number of repetitions of this stimulus.

The gray value distribution of the image pixels of each SCM was clipped by discarding extreme values outside the range defined as \( \pm 2-3 \) times the mean absolute deviation around the mean. The SCMs were then scaled to gray values with a range between 0 and 255. Further analysis of the images was made using a custom-made software written in IDL (Research Systems, Boulder, CO, USA). The SCMs were filtered using a Laplace filter (high-pass, 50 pixels kernel) to remove low-frequency noise resulting from uneven illumination, followed by a boxcar filter (low-pass, 5-11 pixels kernel).

In the resulting images, low gray values (dark patches) corresponded to functional domains that were activated by a given stimulus (Bonhoeffer and Grinvald, 1996). Figure 24 shows four orientation maps from the visual cortex that were obtained in such a way. In the resulting maps, clear activity patches can be seen (dark regions). As expected, the patterns for orientations that are 90° apart are roughly complementary (A and C; B and D).

In some cases (for example, for orientation and direction maps), the full information contained in the data can best be displayed with a color code (Blasdel and Salama, 1986; Ts'o et al., 1990; Bonhoeffer and Grinvald, 1991). The responses for the four (or eight) different gratings are summed vectorially on a pixel by pixel basis (Bonhoeffer and Grinvald, 1996). For each pixel, four (or eight) vectors are summed: their lengths (\( r \)) correspond to the magnitude of the single condition responses and their angles (\( \varphi \)) correspond to the orientation of the grating that produced these responses:

\[
\begin{align*}
r &= \sqrt{\left(\sum_{\alpha} s(\alpha) \cos k\alpha\right)^2 + \left(\sum_{\alpha} s(\alpha) \sin k\alpha\right)^2} \\
\varphi &= \frac{1}{k} \arctg \left( \frac{\sum_{\alpha} s(\alpha) \cos k\alpha}{\sum_{\alpha} s(\alpha) \sin k\alpha} \right)
\end{align*}
\]

where \( s(\alpha) \) is the value at a given pixel of a single condition map. For orientation maps, the factor \( k \) is equal to 2 because orientation is cyclic over 180°, whereas for direction maps \( k=1 \) because direction is cyclic over 360°.
Figure 24. A-D, Single condition activity maps of cat visual cortex for four different orientations. Four images acquired during stimulation of a cat with moving gratings of four equally spaced orientations (0, 45, 90, 135 deg respectively). To correct the effect of uneven illumination of the cortex and non-specific vascular responses, all images were divided by the sum of the images obtained for all the different orientations (cocktail blank). Dark areas correspond to regions of stronger light absorption and hence of stronger activity. E, Blood vessel pattern of the imaged region. F, Corresponding angle map showing the orientation preference for every region of the imaged cortex. The activity maps obtained with different orientations were added vectorially on a pixel by pixel basis. The preferred angle of a region is color-coded (circle on the right).

In angle maps, the angle of the resulting vector, indicating the preferred orientation, was displayed as the hue of each pixel (Figure 24F). The color-code is presented in a circle on the right the angle map: red corresponds to regions responding best to moving gratings of horizontal orientation, regions preferring moving gratings of vertical orientation are coded in blue, etc…

In polar maps, the angle of the resulting vector was again displayed as the hue of each pixel while the vector length (indicating tuning strength) was additionally encoded as the brightness (i.e., intensity) of the color (Figure 25). Note that a vector with a low magnitude can be the result of either various stimulus orientations evoking the same strong response, or simply a weak response to all orientations.

Figure 25. Polar map corresponding to the vectorial summation of 8 single-condition maps: the preferred angle of a region is color-coded (circle on the right) and the brightness of the color indicates the strength of the tuning of the preferred orientation. The black dashed line shows the limit between the left hemisphere (above) and the right hemisphere (below). The black vertical short line at the top of the panel indicates stereotaxic coordinate AP0; Scale bar, 2 mm.
Direction preference angle maps were calculated using the vector maximum method (Kisvárday et al., 2001). According to this calculation, the direction tuning of each image pixel was determined and in contrast with the vector summation method, the maximum signal among each direction condition was associated with a given pixel.

Ocular dominance maps were obtained by subtracting the two monocular SCMs (left and right eye) from each other.

II.3 Optical imaging of intrinsic signals in split-chiasm cats.

II.3.1 Section of the optic chiasm

The optic chiasm was cut three days before optical imaging in order to permit good quality recordings in the visual cortex and comparison with results from previous electrophysiological studies (Milleret and Buser, 1993; Milleret et al., 1994; Milleret et al., 2005). For the retinotopic analysis of transcallosal activity (chapter 2), one cat was studied both before and after chiasmotomy. This animal was prepared for optical imaging (see below) and functional maps were imaged for one day. Then, the optic chiasm was sectioned. One or two days later, functional maps were imaged again.

For the section of the optic chiasm, animals were anesthetized with a mixture of ketamine (10 mg/kg; Ketavet; Pharmacia and Upjohn, Erlangen, Germany) and xylazine (1 mg/kg, intramuscular, i.m.; Rompun; Bayer Belgium, Sint-Truiden, Belgium) and were installed supine in a stereotaxic apparatus (Horsley-Clarke). The electrocardiogram and the rectal temperature were continuously monitored. The soft palate was cut along the midline and the exposed bony palate was treated with a local anesthetic (Xylocain; AstraZeneca, Rueil-Malmaison, France) and drilled to expose the dura. After incising the dura, the underlying optic chiasm was cut completely in the midsagittal plane. Finally, the soft palate was sewed. Antibiotics were applied both locally (Cébénicol, 0.4%; Chauvin, Montpellier, France) and i.m. (Extencilline; Aventis, Paris, France). Completeness of the optic chiasm section was verified post mortem using gold-chloride histochemistry on 50 µm frontal sections (Schmued, 1990), (Figure 26).
Material and methods

Figure 26. Histological control of the optic chiasm’s section. The completeness of each optic chiasm section was verified post mortem using gold-chloride histochemistry, on 50 µm frontal sections. The photomicrographs presented here illustrate some of these sections in two cats (Ca10 on the left and Ca16 on the right), from the most anterior to the most posterior portions of the optic chiasm (Horsley-Clarke coordinates are indicated on the top of each panel). The arrows indicate the location of the optic chiasm section. AP, Area preoptica; CA, Commissura anterior; CF, Columna fornicis; CH, Optic chiasm; CI, Capsula interna; FPM, Fasciculus prosencephali medialis; LD, Lemniscus diagonalis; ROI, Radiatio olfactoria interna; ST, Stria terminalis; VL, Ventriculus lateralis; VT, Ventriculus tertius. Scale bar, 1 mm.
II.3.2 Visual stimulation

Split-chiasm cats were stimulated monocularly in order to selectively activate the transcallosal (TC) pathway or the geniculo-cortical (GC) pathway (Figure 13). The crossed fibers coming mainly from the nasal hemiretinae were severed by the midsagittal section of the optic chiasm, while uncrossed fibers coming mainly from the temporal hemiretinae remained intact, projecting to the visual cortex. When the right eye was stimulated, the activation via the GC pathway was observed in the right hemisphere - the corresponding maps were called GC maps; the activation via the TC pathway was observed in the left hemisphere (TC maps). In order to compare the maps obtained through the activation of each pathway in the same hemisphere, we stimulated one eye for one recording session and then, using the same stimulus parameters, the other eye for another recording session.

The layout of transcallosal activity was investigated on orientation maps. A single stimulus trial consisted of gratings of four or eight equally spaced orientations (0, 45, 90, 135 deg and 0, 22.5, 45, 67.5, 90, 112.5, 135 deg, respectively) at spatial and temporal frequencies (0.15 cpd, 1.5 Hz i.e. 10 deg/s) that strongly activate the transcallosal pathway. In pilot measurements, we found that such a combination of stimulus parameters consistently gave the strongest TC activation. These parameters corresponded to intermediate values between the spatial and temporal frequencies known to optimally activate neurons of A17 and A18, i.e. 0.6 cpd, 1.5 Hz (2.5 deg/s) and 0.15 cpd, 4.5 Hz (30 deg/s), respectively (Movshon et al., 1978a; Orban, 1984a; Bonhoeffer et al., 1995). The same combination of stimulus parameters was applied for the moving grating inside the vertical bar used for the retinotopic mapping of TC activity (see Figure 22 and chapter 2).

II.3.3 Quantitative analyses of the angle maps

To compare quantitatively the orientation maps obtained in the same hemisphere by TC or GC activation, a cross-correlation analysis was performed from single condition maps (SCMs) obtained through the stimulation of either eye (TC and GC pathways) for each orientation (4 or 8) of the stimulus. As a control, cross correlation indices were calculated using the TC SCM from one animal and the GC SCM from another animal.

In addition, the angle maps obtained in the same hemisphere by TC or GC activation were compared quantitatively, by calculating an orientation similarity index (OSI) (Chapman et al., 1996; Gödecke et al., 1997). For the first analysis, we defined three regions of interest (ROI): the first one corresponded to the 1-mm-wide TZ where we observed most of the patches activated through the callosal pathway. The second and the third ROIs were defined, respectively, as the parts of A17 and of A18 visible on the maps, excluding the TZ. For a further analysis of the maps, the second and third ROIs were divided into 1-mm-wide bands parallel to the TZ.

For each pixel of a ROI, the angular distance between the orientation values from the two angle maps (obtained via the TC and the GC pathway, respectively) was calculated.
Material and methods

Angular distance is the absolute angle difference measured along the smallest arc. The OSI was calculated from the mean angular distance of the ROI ($\delta_{ROI}$) as follows:

$$\text{OSI} = 1 - \frac{\delta_{ROI}}{90}$$

Accordingly, for two identical maps the orientation similarity index is 1 and for two maps with orthogonal orientation preferences at each pixel, the similarity index is 0. Two maps with no correlation in angle preference would give a similarity index of 0.5. We calculated OSI for the angle maps (TC and GC maps) of the same region in each hemisphere where TC activated regions appeared (n=10 hemispheres of 7 cats). As a control, similarity indices were calculated using the TC angle map from one animal and the GC angle map from another animal.

II.3.4 Electrophysiological controls of the optical images and reference penetrations

After optical imaging, electrophysiological recordings were made in functional domains which were identified on the basis of SCMs (Figure 27A). Multi-unit activity was recorded at two or three cortical locations per cat, mainly in the center of a region (patch) activated via the callosal pathway. At each location, multi-units were recorded at two different depths (in layers II/III at approximately 300 µm and layers IV/V at approximately 1000 µm below the cortical surface). For each recorded unit, orientation preference and visuotopic location (receptive fields) were determined by stimulating successively the contralateral (TC pathway) and the ipsilateral eye (GC pathway) using a hand-held light stimulus. The spatial location of each receptive field within the visual field was inferred from the positions of the optic disk and area centralis of the stimulated eye, positions that were checked several times during each experiment (Vakkur et al., 1963). Position disparity between pairs of receptive fields of binocular units was measured by the angular distance between the centers of the two receptive fields, as previously reported (Milleret et al., 2005). For histological verification of the electrode penetrations, small electrolytic lesions (15 µA, 10 sec, tip negative) were made at the end of the recording sessions at two sites along each track.

In addition, reference penetrations were made at known stereotaxic coordinates along the TZ that was determined as described above. To this end, empty glass micropipettes were lowered 1000 µm deep into the cortex in parallel to the optical axis of the imaging camera and then removed. The exact locations of the entry points of the pipettes were marked on an enlarged printout of the image showing the vascular pattern of the cortical surface. Therefore, physical micro-lesions were made in the cortex and they could be localized in histological sections.
Figure 27. Electrophysiological and histological controls of the optical images. Example of electrophysiological recordings made in orientation domains identified on SCMs from cat Ca12. A. Transcallosal orientation SCM in the right hemisphere. The orientation of the grating is indicated on the lower right corner of the map. White arrowhead indicates the location of the electrophysiological recording site. Dashed lines show the limits of the TZ as identified by the optical imaging protocol based on the different spatial and temporal frequency preferences between A17 and A18 neurons (see paragraph II.2.5). Horsley-Clarke stereotaxic coordinate AP0 is indicated by the black vertical bar at the top of the panel. B. Spatial distribution of the receptive fields of the neurons recorded at the site indicated in A. These neurons were binocular so receptive fields of each recorded unit were plotted through the ipsilateral (i, right) and contralateral (c, left) eye. Since two neurons were recorded in this track, two pairs of receptive fields are shown. C and D. Photomicrographs of frontal histological sections with Nissl (C) and cytochrome oxidase (D) staining. Black arrowhead indicates the location of the electrode penetration in the cortex. White arrowhead identifies the location of electrolytic lesions performed at the end of cell recordings in this track. Dashed lines show the limits of the TZ as identified by histological criteria described in paragraph II.3.5. Horsley-Clarke stereotaxic coordinates (AP0) are indicated on the upper right corner of the picture. Scale bar, 1 mm. El, elevation; Az, azimuth; Lat, sulcus lateralis; Sspl, sulcus suprasplenialis.

II.3.5 Histological procedures

At the end of the experiment, animals received an overdose of anesthetics and were perfused transcardially with Tyrode’s solution followed by a mixture of 2% paraformaldehyde (Merck, Darmstadt, Germany) and 0.2% glutaraldehyde (Merck, Darmstadt, Germany) in 0.1M phosphate buffer solution (PB, pH=7.6). The brain was dissected, post-fixed in the same fixative for 2 hours and placed in 30% sucrose in phosphate-buffered saline solution for 2 days at 4°C. Afterwards, 40-µm-thick frozen sections of the visual cortex were cut in the frontal plane and alternate sections were processed to reveal Nissl substance or cytochrome oxidase activity. This allowed localization of the reference penetrations and the site of electrolytic lesions in A17, TZ or A18 (Figure 27C and D).

On these sections we also accurately identified the different cortical layers (Otsuka and Hassler, 1962; Price, 1985; Kageyama and Wong-Riley, 1986) and the relative thickness of these layers allowed to locate the transition zone (TZ) between A17 and A18. From A17 to
A18 in frontal sections, layers I and II/III thicken while layer VI becomes gradually thinner (see (Payne, 1990b; Milleret et al., 1994) for further details), (Figure 28). Thus, the TZ was not sharp but corresponded to a region with an average width of 1-1.5 mm at the cortical surface. In this study for the analysis of the TC maps we systematically defined a 1 mm-wide transition zone (Figure 29).

Figure 28. Frontal sections to show the transition zone between A17 and A18. Top. Photomicrograph of a section stained for Nissl substance, showing the cytoarchitecture of A17 (on the right) and A18 (on the left). Laminar borders are indicated. Note the increased thickness of layers II/III in A18 and that layers IV, V and VI lie deeper in the cortex. Bottom. Photomicrograph of a section reacted for the presence of cytochrome oxidase. The dense band in layer IV and lower layer III lies deeper in the cortex in A18 than in A17. Scale bar, 500 µm.

Figure 29. Photomicrographs of frontal histological sections at stereotaxic coordinates P2.5. Top, Nissl staining. Bottom, cytochrome oxidase staining. On these sections, different cortical layers were identified: layers I and VI on Nissl staining and layer IV on cytochrome oxidase staining. The relative thickness of these layers allowed locating the transition zone (TZ) between A17 and A18: from A17 to A18, layers I and II/III thicken while layer VI becomes gradually thinner. Dashed lines show the limits of the TZ. Scale bar, 1 mm. Lat, sulcus lateralis, WM, white matter.
II.4 Anatomical study: functional selectivity of individual callosal axons in A17 and A18

Figure 30 provides an overview of the experimental procedure used to investigate the functional selectivity of individual callosal axons in A17 and A18. Optical imaging of intrinsic signals was combined with the labeling and reconstruction of individual axons. Extracellular iontophoretic injections of Dextran tracers (Fluoro-Ruby and BDA, mainly anterograde) were performed in the left hemisphere. The animal was then allowed to recover for two weeks. Orientation, spatial frequency, ocular dominance and retinotopic maps of A17 and A18 were then recorded for each hemisphere. The imaged region of the visual cortex of each hemisphere was cut in horizontal sections and the tracers were revealed. It was then possible to reconstruct in three dimensions the injection site (in the left hemisphere) and the labeled callosal axons (in the right hemisphere) under a light microscope (x100). The overlay of 3D reconstructions on the functional maps allowed determining the number of synaptic boutons of these axons in each functional domain. This distribution was compared with the functional preference at the injection site in the contralateral hemisphere.

Figure 30. Experimental procedure used for analysis of the distribution of callosal terminals in functional maps.
II.4.1 Extracellular neuronal tracer injections

II.4.1.1 Choice of tracers

For studies of the functional selectivity of callosal terminals, an anterograde tracer able to label long-distance axons up to their synaptic boutons after small extracellular injections was required. Several anterograde neuronal tracers are currently available and each has advantages and disadvantages (Vercelli et al., 2000):

- **Carbocyanine dyes (DiI, DiA and DiO)** can label both alive and fixed cells, anterogradely as well as retrogradely. DiI has been used to trace long-projecting axons in fixed tissues (e.g., Vercelli et al., 1992; Elberger, 1994). A major limitation of DiI is that its application to regions such as the neocortex *in vivo* results in unsatisfactory diffusion, making difficult a study of ‘point-to-point’ connectivity. These dyes are rapidly internalized by endocytosis (Holmqvist et al., 1992), preventing a good labeling of the most distal axonal and dendritic branches. Finally, carbocyanine dyes labeling is incompatible with many histochemical techniques, including immunocytochemistry.

- **Horseradish peroxidase (HRP) or wheat germ agglutinin-coupled HRP (WGA-HRP)** are not appropriate to label individual axons over long distances after small extracellular injections. Furthermore, WGA-HRP can also label cells trans-synaptically (Apkarian and Hodge, 1989).

- **Phaseolus vulgaris leuco-agglutinin (Pha-L)** is a very good anterograde tracer, but has been reported to be unreliable in cats and monkeys (e.g., Brandt and Apkarian, 1992). Its use is more labor-intensive than other tracers (Gerfen and Sawchenko, 1984).

- **Biocytin and Neurobiotin** are effective only over short survival times — two to three days — because they are rapidly degraded (King et al., 1989; Izzo, 1991; Kita and Armstrong, 1991; Lapper and Bolam, 1991). For example, the quality of labeling with these tracers deteriorates after a 24 h survival time (Kita and Armstrong, 1991; Lapper and Bolam, 1991). Biocytin is mainly an anterograde tracer, whereas Neurobiotin also gives consistent retrograde labeling (Lapper and Bolam, 1991). Furthermore, this tracer can give transneuronal (Huang et al., 1992) and glial (McDonald, 1992) labeling.

- **Dextran tracers, biotinylated dextran-amine (BDA) or dextran tetramethylrhodamine (Fluoro-Ruby)**. Fluoro-Ruby (FR) undergoes both retrograde and anterograde transport but is more sensitive as an anterograde tracer (Gimlich and Braun, 1985; Schmued et al., 1990). FR probably binds to N-acetyl-glucosamine (like PHA-L) or, alternatively, lysine groups may facilitate its anterograde transport. Usually, the post-injection survival time required for effective labeling with FR and BDA ranged from 6–14 days: anterograde tracing is faster, whereas effective retrograde labeling takes 10–15 days. Retrograde BDA labeling is unreliable (Pare and Smith, 1996). FR is reported to be taken up by terminals and some transected fibers of passage (Bentivoglio et al., 1980; Schmued et al., 1990). BDA can also label broken fibers of passage. The anterograde transport of these dyes results in the homogeneous labeling of the entire axon and permits the complete
reconstruction of axonal arbors up to terminals. High-quality labeling is maintained over long periods of survival and no trans-synaptic labeling is observed (Brandt and Apkarian, 1992; Veenman et al., 1992).

In pilot experiments, efforts were made to image functional maps first and then to inject a tracer into a defined functional domain. The preparation of the animal and the imaging took two days. As animals can be subjected to acute imaging procedures for a maximum of four to five days, this left us with only two to three days for tracer migration. We found that this period was not long enough for the tracer to label callosal connections with small extracellular injections of biocytin, Pha-L and dextran tracers (in anesthetized and paralyzed animals). It was therefore necessary to inject the tracers before the imaging sessions. Dextran tracers were used, because, as previously reported, biocytin degrades after 48 hours and Pha-L staining is not always reliable in cats.

II.4.1.2 Extracellular tracer injections and multi-unit recordings at the injection site

Tracer injections were made 13-19 days prior to optical imaging recordings in order to allow for interhemispheric transport and labeling of callosal projections. For surgery, anaesthesia was induced with a mixture of ketamine (10 mg/kg; Ketavet; Pharmacia and Upjohn, Erlangen, Germany) and xylazine (1 mg/kg, i.m.; Rompun; Bayer Belgium, Sint-Truiden, Belgium). The anaesthesia was prolonged with halothane (0.4-0.6% of halothane; Halothane; Halocarbon, NJ, USA) using tracheal intubation and artificial ventilation (1:2 mixture O₂ and N₂O). Vital parameters such as expiratory CO₂ (3-4%) and body temperature were monitored continuously. After shaving the cat’s head, the exposed skin was scrubbed with the antiseptic tincture (betadine) and the head was installed in a stereotaxic apparatus. A craniotomy (2x3 mm) was made in the left hemisphere along the region of the transition zone between A17 and A18, centered on Horsley-Clarke coordinates A0.5/L3. Extracellular iontophoretic injections were made at two locations spaced 1mm apart along the antero-posterior axis. The tracer filled glass micropipettes (GB100F-10; Science products, Frankfurt/Main, Germany; tip diameter 10μm) were advanced through a small slit on the dura mater 300-400 μm below the cortical surface. At one site, 5% of biotinylated dextran-amine (BDA, 10,000 MW; Molecular Probes, Leiden, Netherlands) and at the other site 5% of dextran tetramethylrhodamine (Fluoro-Ruby, 10,000 MW; Molecular Probes, Leiden, Netherlands) in 0.1M phosphate buffer saline (PBS; pH=7.6) was injected by passing positive 2 μA (500 ms ON/500 ms OFF duty cycle, square-wave) for 20 minutes.

In addition, multi-unit recordings were made via the same glass pipettes. The visuotopic location of the receptive fields and the preferred orientations were determined using hand-held visual stimuli. At the end of the experiment, the piece of bone from the craniotomy that was kept in Ringer solution was put back, fixed with bone wax and the skin of the skull was sewed. Antiseptic ointment (Betaisodona; Mundipharma, Germany) was applied locally and antibiotics (0.5ml/5Kg; Tardomyocel; Bayer Vital, Leverkusen, Germany) were
injected i.m. Intubation was removed and the animal recovered in the animal house until optical imaging recordings. For prevention, an anti-fever agent was given i.m. (1 ml/10 Kg, Tolfedine 4%; Vétoquinol, Goch, Germany).

II.4.2 Reference penetrations

After optical imaging, reference penetrations were made for aligning the optical images with the sections containing labeling. To this end, empty glass micropipettes (10-15 µm tip diameter) were lowered 1000 µm deep into the cortex parallel to the optical axis of the imaging camera and then withdrawn. The exact locations of the entry points of the pipettes were marked on an enlarged printout of an image of the cortical surface. In this way, the microlesions caused by the pipettes in the cortical tissue could be localized in histological sections. In each hemisphere, 5 to 10 reference penetrations were made, using 500-1500 µm inter-penetration distances.

II.4.3 Histological procedures

At the end of the experiment, animals received an overdose of anesthetics and were perfused transcardially with Tyrode’s solution followed by a mixture of 4% paraformaldehyde (Merck, Darmstadt, Germany) and 0.1% glutaraldehyde (Merck, Darmstadt, Germany) in 0.1M phosphate buffer solution (PB, pH=7.6). Blocks of cortex containing the optically imaged regions were dissected and cut in 60 µm-thick horizontal sections using a vibratome. The corpus callosum of all animals was also dissected and placed in sucrose solutions (10%, 20% and 30%) until it sank. Thereafter, 72 µm-thick frozen sections were cut parallel to the sagittal plane, using a cryotome. For distinguishing the labeling by the two tracers, all sections from left and right cortex were double-stained to BDA (in light-colored brown) and Fluoro-Ruby (in bluish black) (Figure 31). For sections of the corpus callosum, BDA and Fluoro-Ruby labeling was revealed, respectively, in alternate sections.

BDA labeling was revealed using the avidin-biotin-complexed horseradish peroxidase (ABC; Vector Laboratories, Burlingame, CA, USA) method. The sections were washed for 2x20 minutes in 0.1M PB and incubated in ABC 1:200 in 0.1 M Tris-buffered saline solution (TBS; pH=7.6) at 4°C, overnight. Enzymatic reaction was revealed with 0.05% 3,3’-diaminobenzidine-4-HCl (DAB; Sigma-Aldrich, Deisenhofen, Germany) in TRIS (pH=7.6) for 20 minutes and completed in the presence of 0.0025% H2O2 for 1-3 minutes.

Subsequent Fluoro-Ruby labeling was revealed using a rabbit antitetramethylrhodamine antiserum (1:5000; Molecular Probes, Leiden, Netherlands) diluted in 0.1 M Phosphate-buffered saline (PBS; pH=7.6) containing 2 % Normal Goat Serum at 4°C, overnight. Sections were washed two times in PBS for 10 minutes and incubated with the secondary antiserum (1:200, peroxidase anti-rabbit; Vector Laboratories, Burlingame, CA, USA) in PBS at 4°C, overnight. Enzymatic reaction (0.05% DAB in TRIS) was supplemented with 0.005% CoCl2 or 0.6% Nickel-sulfate intensification (Adams, 1981; Hancock, 1984) for
20 minutes and completed in the presence of 0.0025% H₂O₂ for 1-3 minutes. All reagents and solutions contained 0.2% Triton X-100.

After light microscopic inspection of the wet sections of both hemispheres the top 20 (left hemisphere) and 40 (right hemisphere) sections were processed for resin embedding. Accordingly, they were rinsed in TRIS for 2x15 minutes and PB for 2x15 minutes, postfixed in 0.5% OsO₄ in 0.1M PB for 10-20 minutes and dehydrated in an ascending series of ethanol followed by 2x15 minutes in propyleneoxide. Finally, sections were embedded in Durcupan ACM (Fluka, Neu-Ulm, Germany) and mounted on microscopic slides (Somogyi and Freund, 1989). The remaining sections of both hemispheres including sections of the corpus callosum were not osmicated. Instead, they were dry-mounted on chrome-gelatine-coated slides, rinsed 2x10 minutes in Xylene and coverslipped in DePeX (ERVA Finebiochemica GmbH KG, Heidelberg, Germany). These cortical sections (not treated with osmium) served to determine the origin of labeled callosal axons according to their color (Figure 31D and E): the injection site of BDA (light-colored brown) or of Fluoro-Ruby (bluish black) (Figure 31A and C).

![Figure 31. Light microscopic images of BDA and Fluoro-ruby (FR) labeled neurons. Each tracer was revealed in one color: black for FR and light-colored brown for BDA. A, B, C. Two injection sites in cat Ca09 (left hemisphere). The two colors can be clearly distinguished in A and in higher magnifications in B (for the site of FR) and C (for the site of BDA). D, E. Labeled callosal axons in sections not treated with osmium, in the right hemisphere of cat Ca09. The deep cortical sections (below the 40th section) were not treated with osmium in order to determine the origin of labeled callosal axons according to their color: the injection site of Fluoro-Ruby (black) or of BDA (light-colored brown). F, G, H. Labeled callosal axons in superficial sections treated with osmium. Cat Ca15 (F); cat Ca09 (G, H). Scale bars, 250 µm (A); 25 µm (B-H).](image)
II.4.4 Three-dimensional reconstructions

Eight labeled callosal axons of the right hemispheres (contralateral to the injection sites) were reconstructed in three-dimensions using a light microscope (Leica DMRB) at ×1000 magnification and the neuron reconstruction system, Neurolucida (MicroBrightField, Colchester, VT, USA). The axons and their synaptic boutons (club-like and en-passant) were traced in the entire depth of the cortex and partly in the white matter using the most superficial 30-40 sections. Neighbouring sections were aligned with the help of corresponding cut ends of labeled axonal processes and small blood vessels (< 20 µm diameter) providing a 5-50 µm matching accuracy (Kisvárday et al., 1997).

In order to match the anatomical reconstructions with the optical images, the reference penetrations and the layout of surface blood vessels were also reconstructed. Finally, the borders of cortical layers were determined on the basis of relative density of neuronal cell bodies and fibers, soma size and the presence of layer-specific cell types such as large pyramidal cells in lower layer III upper IV and giant pyramidal cells in layer Vb.

II.4.5 Alignment of the reconstructed axons and injection sites with the optical maps (Figure 32)

The 3D-reconstructions were first corrected for optical shrinkage (Buzás et al., 1998). For this, Z-values (corresponding to the depth of the reconstruction) were multiplied by a correction factor ($f_0$):

$$f_0 = \frac{n_{\text{resin}}}{n_{\text{oil}}}$$

where $n_{\text{resin}} = 1.549$ is the index of refraction of embedding resin and $n_{\text{oil}} = 1.518$ is the index of refraction of the immersion oil.

Then, the reconstructions were tilted and rotated into the plane of the optical images using reference penetrations that were made in parallel to the optical axis of the imaging camera. Through the histological procedure, sections underwent some tissue shrinkage, mainly due to dehydration. Therefore, the reconstructions were also corrected for this shrinkage. It was then possible to match the anatomical reconstructions with the optical images with the help of the entry points of reference penetrations and the layout of the surface blood vessels (Buzás et al., 1998). This aligning method has an estimated error inferior to 50 µm (Yousef et al., 1999).

Finally, the reconstructions had to be scaled to the same size and resolution as the ones of the cortical image. The pixel spatial resolution of the optically recorded maps was 21.28 x 21.28 µm² and the 3D reconstructions were converted into the same pixel format. The reconstructions were overlaid with a grid of the same size as that of optical images and the number of axon terminals was counted in every pixel. The resulting color-coded bouton density maps permitted a direct comparison between the distribution of synaptic boutons and the functional maps on a pixel-by-pixel basis.
Material and methods

II.4.6 Quantitative calculations

Each reconstructed axon was analyzed with Neurolucida and Neuroexplorer software (MicroBrightField, Colchester, VT, USA) in order to determine the main morphometric parameters such as the total length of axons, their antero-posterior and medio-lateral extent and the number of synaptic boutons in layers II/III, IV and V/VI.

In addition, the functional topography of individual callosal axons was determined using a custom-made software written in IDL (Research Systems, Boulder, CO). First, as described above, the anatomical data were converted into the same pixel format as that of the functional maps. A bouton density map was generated and compared with the functional maps on a pixel-by-pixel basis. The number of synaptic boutons in each functional domain (orientation, direction or ocular dominance) was obtained. We used a resolution (bin size) of 22.5° for orientation maps, of 45° for direction maps and the ocular dominance was divided into seven categories as introduced by Hubel and Wiesel (Hubel and Wiesel, 1962). For the analysis of the distribution of the synaptic boutons in orientation domains, the data were normalized in order to make a direct comparison between individual cases. First, orientation preferences were expressed relative to that of the injection sites using 22.5° resolution (bin size). The resulting values ranged between –90° and +90°. Second, the number of boutons was expressed as a percentage of the total number of boutons for each axon. The distributions of the synaptic boutons in terms of orientation preference were divided according to iso- (± 30°), oblique- (± 30-60°) and cross- (± 60-90°) orientation categories with respect to the orientation preferences of the injection sites.
III Results and Discussions

III.1 Chapter 1. Layout of transcallosal activity in cat visual cortex revealed by optical imaging

III.1.1 Summary

In this chapter, the contribution of interhemispheric connections to orientation maps in cat visual cortex is investigated using optical imaging of intrinsic signals. In order to isolate the functional inputs arriving via the corpus callosum (CC) from other inputs, the split-chiasm preparation was used. The regions activated through the CC in visual areas 17 (A17) and 18 (A18) were localized and characterized by using moving high contrast oriented gratings as visual stimuli. This approach revealed that the CC triggers the activation of orientation selective domains in the transition zone (TZ) between A17 and A18 and occasionally within portions of both of these areas. Transcallosally activated orientation domains were observed all along the TZ without any obvious interruption, and these domains were arranged around pinwheel centers. Interestingly, the TZ was divided in two parallel regions resembling A17 and A18 in their preferred temporal and spatial frequencies. Finally, the comparison of orientation maps evoked through the transcallosal and geniculo-cortical pathways revealed that these maps were similar within the TZ. This similarity decreased progressively with increasing distance from the TZ toward A17 and A18.

Article
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Poster Abstracts
III.1.2 Introduction

The functional maps of the visual cortex are shaped by the retino-geniculo-cortical pathway, the intra-cortical connections (intra-areal, inter-areal or feedback connections), and the interhemispheric pathway via the corpus callosum (CC). To analyze the relation of these pathways with these maps, several studies using the optical imaging technique have correlated the anatomy of intra-cortical connections with functional maps of the visual cortex (Kisvárday et al., 1994; Bosking et al., 1997; Buzás et al., 1998; Buzás et al., 2001; Chisum and Fitzpatrick, 2004). Others attempted to determine the functional contribution of intra-areal connections (Das and Gilbert, 1995; Toth et al., 1997) and of feedback pathways (Galuske et al., 2002) to orientation maps. However, the contribution of the interhemispheric callosal connections to orientation maps is poorly understood mainly because of the difficulty to isolate the transcallosal activity from other inputs. Two anatomical studies have investigated the distribution of callosal connections in orientation maps but they provided contradictory results. In strabismic cats, these connections preferentially link similar orientation domains (Schmidt et al., 1997) whereas they link different orientation domains in the normal tree shrew (Bosking et al., 2000). In the present study, the aim was to optically image interhemispheric activity only (namely orientation domains activated through the CC) in A17 and A18 of normally-reared adult cats.

Because of the nature of the experimental protocols, previous anatomical and electrophysiological studies of the transcallosal activity in cat visual cortex were inherently limited to sampling from only a small part of the neuronal population. Hence, the global organization of the regions activated via transcallosal connections could not be determined. Here this is made possible by using optical imaging of intrinsic signals. The distribution of transcallosal activity in the cat visual cortex was determined over large portions of both A17 and A18 after section of the optic chiasm. By changing the eye stimulated, it was furthermore possible to distinguish and compare the maps obtained via the activation of the GC pathway (GC maps) with those obtained via the activation of the TC pathway (TC maps) (see Figure 13).

The first point considered in this study was the localization of the regions activated via the TC pathway both in A17 and A18. Until now, it has remained unclear whether the TZ is activated by the TC pathway homogeneously all along the antero-posterior axis or if there are some interruptions. In addition, the extent of TC activation in A17 and A18 (outside of the TZ) remained to be elucidated.

Furthermore, the functional characteristics of the transcallosally activated orientation domains were investigated. Single cortical units that are activated by callosal terminals are orientation selective (Berlucchi and Rizzolatti, 1968). Altogether, these units encode all orientations equally (Leporé and Guillemot, 1982; Milleret et al., 1994). However, the spatial distribution of these orientation preferences has not been characterized specifically. A number
of studies focused on the functional architecture of TZ (Diao et al., 1990; Payne, 1990b; Bonhoeffer et al., 1995; Ohki et al., 2000). But none of them distinguished the inputs of the GC pathway from those of the TC pathway, while both coexist in this region. Thus, in the TC orientation maps, the shape and the layout of the TC orientation domains were characterized.

The distribution of these TC orientation domains was compared for different spatio-temporal frequencies of oriented gratings stimuli. Based on electrophysiological and behavioral studies, it is generally accepted that only visual stimuli at low spatial frequencies (0.1-0.2 cpd) and moderate temporal frequencies (4-10 deg/s) are effective for the interhemispheric transfer of visual information (Berardi et al., 1987; Berardi et al., 1988). In the present study, oriented gratings of low and high spatio-temporal frequencies were used. This allowed determining whether callosal activation can be induced by both types of stimuli, and, if so, whether the sites activated by these different spatio-temporal frequencies have different spatial arrangements.

Finally, the TC and GC angle maps of the same regions were compared in order to determine the respective contribution of each pathway to the orientation maps in TZ, A17 and A18. Most transcallosally activated units are binocular: in split-chiasm animals, they can be activated both by the contralateral eye through the CC, and by the ipsilateral eye via the direct GC pathway (Berlucchi and Rizzolatti, 1968; Harvey, 1980; Innocenti, 1980; Leporé and Guillemot, 1982; Milleret et al., 1994; Milleret et al., 2005). The corresponding pairs of receptive fields display similar orientation preference selectivity. This study tested if this matching also occurs at the level of functional orientation maps. Indeed, it is not known whether oriented stimuli evoke the same activity pattern of iso-orientation patches via the TC pathway as they do through the GC pathway.

The data presented in this study demonstrate for the first time that the CC can evoke a patchy, orientation specific activity pattern in the TZ as well as within portions of A17 of A18. The TZ always appeared as the main transcallosally activated region. The transcallosally activated orientation domains were present all along the TZ and these domains were organized around pinwheel centers. Furthermore, the layout of TC patches differed for high and low spatio-temporal frequencies of oriented gratings. Finally, a strong similarity of TC and GC orientation maps was established within the TZ and this similarity diminished progressively within A17 and A18 with increasing distance from the TZ.
III.1.3 Results

Transcallosal activity maps were visualized in vivo in cat visual cortex (n=10 hemispheres in 7 cats), using optical imaging of intrinsic signals. The spatial distribution of transcallosally activated orientation domains was characterized both in A17 and A18, and was then compared to that of geniculo-cortical orientation domains in the same cortical regions.

III.1.3.1 Localization of the regions activated via the transcallosal pathway in visual cortical areas 17 and 18

The first aim was to localize the regions activated by oriented stimuli via the callosal pathway both in A17 and A18. Figure 33 (A-D) shows single-condition orientation maps recorded simultaneously in both hemispheres of cat Ca10, whose optic chiasm section is illustrated in Figure 26. Since only the right eye was stimulated, the dark patches observed in the right hemisphere (RH) corresponded to the regions activated via the geniculo-cortical pathway (GC maps), while those observed in the left hemisphere were activated via the transcallosal pathway (TC maps) (see Figure 13).

As expected when using full-field gratings, a wide portion of the right hemisphere was activated via the GC pathway as indicated by the presence of dark patches in the most part of the exposed cortical region and for all orientations tested (Figure 33A-D). According to stereotaxic coordinates, this activated region encompassed parts of A17 and A18 as well as the transition zone between A17 and A18 (TZ) (Tusa et al., 1978; Tusa et al., 1979). It is known from previous anatomical studies that this activated region comprises somata of neurons projecting an axon to the opposite hemisphere through the CC (Innocenti and Fiore, 1976; Olavarria, 1996). In the left hemisphere, dark patches also appeared for stimuli of each orientation (Figure 33A-D). These were however mainly localized along an approximately 1-mm-wide band running from the postero-lateral (stereotaxic coordinates P6L4) to the antero-medial (A6L1.5) part of the lateral gyrus. Indeed, orientation polar maps calculated for the two hemispheres clearly showed that the TC orientation specific response (Figure 33E, LH) was strongest along a narrow band whereas the GC map (Figure 33E, RH) comprised almost the entire exposed region. The observation that the TC activated region extended to the posterior border of the imaged area suggests that the posterior limit of TC activation was likely located even more caudally. Similarly, the anterior limit of TC activation was most likely obscured within the interhemispheric fissure.
Figure 33. Layout of the transcallosal activity in visual areas A17 and A18 revealed by optical imaging of intrinsic signals (cat Ca10) in the split-chiasm preparation (see schema at the top of the figure). The right eye of the cat was stimulated with gratings of 4 different orientations (indicated in the lower right corner of panels A-D). A-D. Single-condition orientation maps showing the pattern of cortical activity evoked by each stimulus. In these and subsequent gray scale optical images, darker gray indicates stronger activation. Since the right eye was stimulated, dark patches observed in the right hemisphere (RH) correspond to the regions activated via the ipsilateral geniculo-cortical pathway; the left hemisphere (LH) was activated only via the callosal pathway. Patches activated via the callosal pathway appeared in LH for all the orientations tested. They were mainly localized along a band running from the postero-lateral to the antero-medial part of the lateral gyrus. In this example, some patches (white arrowheads) also appeared in A18 (around Horsley-Clarke stereotaxic coordinates A3.7, L4.0), far from this transition zone. E. Polar map corresponding to the vectorial summation of the 4 single-condition maps in A-D. The preferred orientation angle of each pixel is color-coded (as indicated on the circle) and the brightness of the color indicates the relative strength of the tuning of the preferred orientation. F. Blood vessel pattern of the imaged region. In each panel, the white dashed line delineates the border of the craniotomy. Thin black dashed lines show the limits of the transition zone between A17 and A18, as defined in Figure 34. Short vertical line at the top of each panel shows stereotaxic coordinate AP0. Thick dashed lines show the stereotaxic midline. Scale bar 2 mm. A, anterior; P, posterior.
Altogether, this 1-mm-wide region activated via the TC pathway appeared in 10 out of 13 hemispheres tested in 7 split-chiasm cats. In two cases, TC activity patches appeared after 5 stimulus trials (1 trial corresponds to 1 presentation of each stimulus orientation), in four cases they appeared after 10 trials and in four cases they were observed after 25 trials. In the three remaining cases, no TC activation was observed in the exposed cortical region although the cortex could be activated by the GC pathway.

The main location of the transcallosally activated region corresponded to the stereotaxically determined location of the TZ between A17 and A18 (Tusa et al., 1978; Payne, 1990b). To confirm this, the spatial (SF) and the temporal frequency (TF) of the stimulus were changed in order to preferentially activate either A17 or A18. Orientation SCMs were recorded at low SF and high TF (“A18 condition”, 0.15 cpd, 4.5 Hz) and at high SF and low TF (“A17 condition”, 0.6 cpd, 1.5 Hz), stimulating the eye ipsilateral to the hemisphere studied. Such maps were recorded systematically in the 10 hemispheres where the TC activation could be detected. For each spatio-temporal frequency condition, the sum of the 4 orientation SCMs was normalized to the “cocktail-blank”, i.e. the sum of images recorded for all stimulus conditions (see Material and Methods; A18, Figure 34A and E; A17, Figure 34B and F). Consistent with earlier results (Bonhoeffer et al., 1995), it was found that the antero-lateral part of the lateral gyrus, corresponding to A18, could be activated more strongly by low SF and high TF than by high SF and low TF. On the contrary, the posterior-medial region corresponding to A17 was activated predominantly by high SF and low TF. The overlay with TC single-condition orientation maps confirmed that the patches activated via the callosal pathway were mainly distributed along the transition zone between the regions of A17- and A18-specific activation (Figure 34C and G).

The location of the TZ was also confirmed by electrophysiological recordings in three animals. Figure 35 shows three recording sites from where multi-unit activity was recorded. These sites were located in the center of TC patches observed in TC orientation SCMs (white arrowheads in Figure 35A and D, see also Figure 27 in Material and methods). For each recording site, receptive fields were plotted at two different depths. Visual stimulation was first carried out through the contralateral eye in order to ascertain callosal activation, and then through the ipsilateral eye to determine the response properties of the same cell activated through the geniculo-cortical pathway (Figure 35B and E). The receptive fields of cells activated by the contralateral eye were found along the vertical meridian or within the ipsilateral visual field, which is characteristic of the TZ and of TC units (Payne, 1990b; Milleret et al., 1994). A mean position disparity of 4.5 degrees was found between TC and GC pairs of receptive fields, confirming earlier measurements of transcallosally activated neurons recorded in the TZ (Milleret et al., 2005). Furthermore, the orientation selectivity was essentially the same whether the units were activated through the GC pathway (ipsilateral eye stimulated) or the TC pathway (contralateral eye), and they were similar to the orientation of the grating used to obtain the SCM (Figure 35A-B and D-E and Figure 27A-B).
Finally, this functional delineation of the TZ was corroborated by histological controls. Cytoarchitectonic criteria were used to delimit the TZ in frontal sections (see Material and Methods and Otsuka and Hassler, 1962; Payne, 1990b; Milleret et al., 1994). The recording sites (corresponding to the centers of TC patches) were located within the TZ (Figure 35C) or within its 1-mm-wide flanking regions (see also below and Figure 35F). Similar histological controls were performed in three other animals in order to localize reference penetrations that were made along the TZ, which was defined using the area-specific functional maps described above (see Figure 34). These reference penetrations were found in the histologically defined TZ (data not shown).

Figure 34. Localization of the transition zone (TZ) between A17 and A18 (cat Ca10). A-D. Localization of the TZ in the left hemisphere (LH) by activating the geniculo-cortical pathway (stimulation of the left, ipsilateral eye) with stimuli optimized for each cortical area (A17 and A18). A. Localization of A18 (dark region). The sum of all orientation SCMs corresponding to A18 stimuli (0.15 cpd, 4.5 Hz) was divided by the “cocktail blank” (see text for further details). B. Localization of A17 (black region). The sum of all orientation SCMs acquired with A17 stimuli (0.6 cpd, 1.5 Hz) was divided by the “cocktail blank”. In both maps, the location of the TZ was defined as the 1-mm-wide zone delineated by the two dashed lines. These lines were copied at the same cortical location on panels C and D. C. Transcallosal single-condition orientation map obtained with a vertical grating (stimulation of the right, contralateral eye). D. Blood vessel pattern of the imaged region in A, B and C. E-H. Localization of the TZ in the right hemisphere (RH) of the same animal. E, F. Area-specific geniculo-cortical activation patterns (right, ipsilateral eye stimulated) using the same stimuli as in A and B respectively. G. Transcallosal single-condition orientation map obtained with a horizontal grating (left, contralateral eye stimulated). H. Blood vessel pattern of the imaged region in E, F and G. M, medial. Scale bar 2 mm. Other conventions as in Figure 33.
Figure 35. Electrophysiological and histological controls of the localization of the transition zone and of the transcallosally activated regions from case Ca12 (A-C) and case Ca10 (D-F). A and D. Transcallosal single-condition orientation maps in the right hemisphere (RH; stimulation of the left, contralateral eye). The orientation of the grating is indicated on the lower right corner of each map. White arrowheads indicate the location of each electrophysiological recording site (one in A and two in D) positioned in the center of transcallosal patches. Dashed lines show the limits of the TZ as identified by the optical imaging protocol described in Figure 34. Horsley-Clarke stereotaxic coordinate AP0 is indicated by the black vertical bar at the top of each picture. B and E. Spatial distribution of the receptive fields of the neurons recorded at each site indicated in A and D. These neurons were binocular so receptive fields of each recorded unit were plotted through the ipsilateral (i, right) and contralateral (c, left) eye. Since two neurons were recorded in each of the three tracks, two pairs of receptive fields are shown for each track. In E, recording site numbers are indicated as numbers before the decimal points. C and F. Photomicrographs of frontal histological sections (Nissl staining) showing the location of each electrode track. Black arrowheads indicate the location of the electrode penetration in the cortex. White arrowheads identify the location of electrolytic lesions performed at the end of cell recordings in each track. Dashed lines show the limits of the TZ as identified by histological criteria described in the Methods. Horsley-Clarke stereotaxic coordinates are indicated on the upper right corner of each picture. Scale bar, 1 mm. El, elevation; Az, azimuth; Lat, sulcus lateralis; Spl, sulcus suprasplenialis. Other conventions as in Figure 33.
In some cases, patches activated by the stimulation of the contralateral eye were also found in regions that appeared to be outside the TZ. These patches were observed in each recording session in which TC activation appeared. These recording sessions were separated by a minimum of two hours and a maximum of one day. The location and contrast of these patches were, nevertheless, variable from animal to another.

In three hemispheres, some patches were observed in the 1 mm-wide flanking regions of A17 (3 cases, examples in Figure 34G and Figure 35D) and A18 (1 case, Figure 34G) along the TZ. Interestingly, these patches were observed only in posterior regions (at stereotaxic coordinates P6 to P0.2). In two other cases, no broadening of the activated zone was observed beyond the TZ (see for example Figure 33). In the five remaining hemispheres, the caudal parts of A17 and A18 (posterior to P2) could not be imaged because of the limited extent of the craniotomy or the presence of large blood vessels.

In one case (Ca10, left hemisphere) in which TC patches appeared after only five stimulus trials, additional patches were observed within A18, further away from the TZ. The most visible ones were located at stereotaxic coordinates around A3.7 L4 (Figure 33, white arrowhead). These patches showed a lower gray-level (i.e. lighter patches) than the ones located within the TZ, indicating weaker (or less orientation selective) input from the opposite hemisphere. This was observed in two sessions separated by 2 hours and in an additional one, the following day.

Finally, in two cases (Ca10, LH and RH), an even weaker pattern of activity patches was observed outside the zones of activation described above in TC orientation single condition maps. This could extend as far as 2-3 mm from the TZ. The orientation vectors resulting from the vectorial summation of the SCMs, nevertheless, showed an organized pattern of orientation domains and pinwheels centers in these regions, only with diminished orientation vector magnitudes (see polar maps in the left hemisphere, A18, Figure 33E). Again, this was observed in different recording sessions separated by a maximum of one day.

In summary, the main region activated through the CC was the TZ. Occasionally, additional portions of both A17 and A18 also appeared activated.
III.1.3.2 Characterization of the regions activated via the transcallosal pathway in A17 and A18

The following sections further characterize the functional domains activated via the TC pathway.

III.1.3.2.1 Layout of orientation domains activated via the transcallosal pathway in the TZ

The TZ is known to display a specific arrangement of functional domains, which is thought to be due to its intermediate position between two different areas, A17 and A18. Orientation domains (Singer, 1981; Löwel et al., 1987; Diao et al., 1990; Ohki et al., 2000; Shmuel and Grinvald, 2000) and ocular dominance columns (Löwel and Singer, 1987; Löwel et al., 1988) have been found to take on an elongated shape and to be orthogonal to the TZ. A similar arrangement was observed in the GC orientation SCMs, i.e. when the visual cortex was activated by the direct GC pathway (Figure 33, RH). In the TZ, the spacing between these GC iso-orientation bands was fairly constant, with a mean value of $1.16 \pm 0.3$ mm.

As revealed by the TC orientation SCMs, TC activated regions appeared with a patchy pattern (Figure 33A-D, Figure 34C and G). These patches had a diameter of approximately 500 µm and, in some SCMs, they were found aligned with the TZ (see for example Figure 33C and Figure 34C) with a mean inter-patch distance of $1.1 \pm 0.2$ (center-to-center). In other SCMs, the patches were not systematically lined up with the TZ (see examples in posterior parts of Figure 33A and in Figure 34G) although the distance between them (nearest neighbor) was similar and reasonably constant (1.1-1.2 mm). The corresponding transcallosal orientation angle maps (example in Figure 33E, Figure 38A1 and A2) were observed along the whole antero-posterior extent of the TZ without any obvious interruption. They showed all the essential features of orientation maps that are usually obtained in the visual cortex of normal cats, including a pinwheel-like organization of iso-orientation domains around point singularities (Bonhoeffer and Grinvald, 1993).

III.1.3.2.2 Transcallosal activity maps obtained by using A17 and A18 specific stimuli

The interhemispheric transfer of visual signals depends on the characteristics of the callosal projecting neurons. These neurons have been known to reside in both A17 and A18, which show major differences between the functional properties of their cells. It was therefore interesting to determine whether TC activity can be evoked by stimuli optimized to activate A17 and A18. The spatial arrangement of such callosal activation was also investigated.
Figure 36. Transcallosal maps obtained using different spatial (SF) and temporal (TF) frequencies as visual stimuli. **Top** (A-E): Cat Ca16. A, B. Single-condition orientation maps elicited in both hemispheres by the visual stimulation of the left eye with vertical gratings of low SF and high TF (“A18 stimulus”, 0.15 cpd, 4.5 Hz in A) or of high SF and low TF (“A17 stimulus”, 0.6 cpd, 1.5 Hz). Because of the section of the optic chiasm, the activation of the geniculo-cortical pathway appeared in the left hemisphere (LH) and the activation of the callosal pathway in the right hemisphere. In each panel, the oriented grating is illustrated on the left, near the midline (thick broken line). The patches activated via the callosal pathway in the RH were located along the TZ (dashed line), but they were closer to A18 for low SF and high TF and closer to A17 for high SF and low TF. C, D. Differential orientation maps: the sum of all orientation SCMs corresponding to A18 stimuli (C) or to A17 stimuli (D), was divided by the “cocktail blank”. Notice that “A18 stimuli” activated A18 in LH via the geniculo-cortical pathway and the TZ, on the side of A18, in RH via the transcallosal pathway; in contrast, A17 stimuli activated A17 in LH via the geniculo-cortical pathway and the TZ, on the side of A17, in RH via the transcallosal pathway. E. Blood vessel pattern of the imaged region in panels A-D. **Bottom** (F-J): cat Ca10. Same legend as above except that the right eye was stimulated. Thus, in this case, the geniculo-cortical activation is illustrated in the right hemisphere (RH), while the transcallosal one appears in the left hemisphere (LH). To compare with precision the positions of the transcallosal patches in the LH, arrowheads were drawn on the “A18 stimulus” map and were copied to the same place on the “A17 stimulus” map. The black rectangle in J indicates the imaged region in panels F and G. Scale bar 2 mm. Other conventions as in Figure 33.
In most cases, both the A17 stimulus (0.6 cpd, 1.5 Hz) and the A18 one (0.15 cpd, 4.5 Hz) were effective in evoking TC activity patches mostly in the TZ (Figure 36). A direct comparison of the locations of these patches was made in 3 hemispheres. Interestingly, the patches were found in different locations for the two stimulus conditions. For example, Figure 36 shows two pairs of transcallosal SCMs recorded during the presentation of gratings of the same orientation but at different spatio-temporal frequencies (Figure 36A-B and F-G). Dark patches were all located within and along the TZ but mainly on the side of A18 for low SF and high TF (Figure 36A and F) while mainly on the side of A17 for high SF and low TF (Figure 36B and G).

The extent of spatio-temporal frequency dependent activity in the TZ is well illustrated by maps where the sum of all orientation SCMs corresponding to A18 (or A17) stimuli was divided by the sum of all orientation SCMs acquired with both A17 and A18 stimuli (“cocktail blank”). These maps demonstrate that the differential activation of A18 and A17 subdivided the TZ in the right hemisphere via the GC pathway as well as in the left hemisphere via the TC pathway (Figure 36C-D and H-I).

These data indicate that the CC transfers activity that is driven by high SF at low TF as well as low SF at high TF stimuli. The differential activation of A17 and A18 by these stimuli is reflected within the TC activated region, suggesting a functional subdivision of the TZ.

**III.1.3.3 Comparison of transcallosal and geniculo-cortical orientation maps**

As demonstrated above, functional maps can be evoked in the TZ via the callosal pathway as well as through geniculo-cortical inputs. Taking advantage of the split-chiasm model, it was possible to compare the functional maps evoked by each of these two input streams.

Figure 37 shows orientation SCMs recorded while stimulating either the left or the right eye in successive recording sessions. When the left eye was stimulated, the GC activity map was observed in the left hemisphere (LH) and TC activity patches were observed in the right hemisphere (RH). The opposite arrangement was seen when the right eye was stimulated. The arrowheads in Figure 37A and B mark the locations of the patches activated via the TC pathway (Figure 37A RH and Figure 37B LH), facilitating comparison with the GC maps (Figure 37A LH and Figure 37B RH). Clearly, the TC activation patches most often overlapped considerably with the GC activity patches, indicating that these patches were binocularly driven.

A cross-correlation analysis was performed from TC and GC single condition maps for each orientation (4 or 8) of the stimulus. For example, the cross-correlation coefficient calculated in the TZ of the RH of cat Ca10 (maps shown in Figure 37) was equal to 0.75. The mean cross-correlation coefficient between TC and GC SCMs within the TZ of all animals (n=10 hemispheres) was equal to 0.6±0.14. Control values were obtained from the comparison of TC SCM from one animal with GC SCM from another animal. The cross-correlation coefficient was then equal to –0.05 ±0.15 in average, and was significantly
different from the one calculated with the SCMs of the same animal (t-test, \( p=7 \times 10^{-3} \)). These data provide further evidence for the spatial overlap between the TC and the corresponding GC orientation maps. Since the two hemispheres were imaged together and thus, processed identically, the gray level differences between them indicate differences in the strength of the evoked optical signal. Comparison of the GC and the TC activated hemispheres in Figure 37A and Figure 37B shows that the majority of co-localized patches were darker in the GC maps than in the TC maps. This suggests that the GC pathway (ipsilateral eye activation) was generally more powerful in evoking the optical response than the TC pathway (contralateral eye activation).

Figure 37. Comparison of the transcallosal and the geniculo-cortical orientation maps (case Ca10). A, B. Single-condition orientation maps obtained in the same animal and in the same conditions (horizontal grating) except for the eye stimulated (A, left eye; B, right eye). Arrowheads indicate the same locations in A, B and C. C. Blood vessel pattern of the imaged region. Scale bar 2 mm. See Figure 33 for conventions.
Figure 38. See legend on the next page
To complete the analysis on the relationship between TC and GC activity maps, the distribution of preferred orientations was also compared in angle maps. Figure 38 shows examples of transcallosal (A) and geniculo-cortical (B) angle maps from the same hemisphere. In all cases, a strong similarity was observed between the TC and the GC angle maps along the TZ. For comparison, the correspondence between pinwheel center positions was tested since these centers represent readily comparable locations (Figure 38A1-B1 and A2-B2). Here, arrowheads mark the location of some pinwheel centers of the TC angle maps. These were then overlaid onto identical positions in the GC angle maps. As expected, not all pinwheel centers matched precisely since their exact location can depend on various factors such as-stimulus parameters and they are prone to minor changes within the SCMs (Kisvárday et al., 2000). Nevertheless, the position of some pinwheel centers matched exactly (with a precision of 50µm): within the TZ, 40 ± 13 % of them matched and, outside the TZ, only 20 ± 5 % also did.

Figure 38 (previous page). Quantitative comparison of the transcallosal and the geniculo-cortical angle maps. A, B. Transcallosal (A) and geniculo-cortical (B) angle maps from the same hemisphere (1, cat Ca10; 2, cat Ca01). White arrowheads were placed on pinwheel centers of the transcallosal angle map and then copied at the same position onto the geniculo-cortical map. Thick dashed lines show the location of the TZ. C. Blood vessel patterns of the imaged regions in A and B. D, E. Transcallosal and geniculo-cortical angle maps from the same hemisphere of Ca10 (but at lower magnification than in A1 and B1). Dotted lines within A18 delimit three 1-mm-wide regions (bands 1 to 3), parallel to the transition zone (TZ), that were used for the quantitative analysis presented in the histogram K. F. Blood vessel pattern of the imaged regions in D and E. Scale bar, 2 mm. G. Map of orientation similarity indices (OSI) calculated for each pixel from the angle maps presented in D and E (Cat Ca10). White pixels correspond to regions with the same orientation preference on TC and GC maps (thus with an OSI equal to 1). Black pixels correspond to regions with orthogonal orientation preference on both maps and thus with an OSI equal to 0. Dark grey pixels correspond to regions with no correlation in angle preference in both maps and thus with an OSI equal to 0.5. H, I. OSI maps calculated for each pixel from the angle maps of two more cats (Ca16 and Ca12), respectively. The TZ appears clearly whiter than the other regions indicating that the similarity between TC and GC angle maps decreased progressively with increasing distance from the TZ toward A17 and A18. Scale bar, 2 mm. J. Mean “orientation similarity index” calculated in 10 hemispheres (from 7 cats), for the 1-mm-wide transition zone (TZ) between A17 and A18 as well as for the regions of A17 and A18 visible on the maps (excluding the TZ). The grey bars represent the mean of the orientation similarity indexes calculated with the transcallosal and geniculo-cortical maps of the same hemisphere of the same animal. The white bars represent the controls where the transcallosal map from one animal was compared with the geniculo-cortical map from another animal (white bars). Both groups (same hemisphere vs. control) appeared significantly different for each region (Mann-Whitney test; ** p<0.0001; * p<0.01). The difference between the TZ and A17 was also significant, as well as the one between the TZ and A18 (Mann-Whitney test; p<0.0001). The difference between A17 and A18 was not significant. Error bars indicate the standard deviation. K. The orientation similarity index was calculated in the TZ, in A17 and in A18, for each 1-mm-wide region being parallel to the TZ (bands 1, 2, and 3 as indicated on map D). In all cases (n=6), the index is negatively correlated with distance from the TZ (mean correlation coefficient = -0.97±0.03). The name of each animal and the analyzed hemisphere are indicated in the abscissa. See Figure 33 for similar conventions.
To confirm the above results obtained by visual inspection, the similarity between the overlapping TC and GC angle maps was quantitatively evaluated by calculating an orientation similarity index (OSI, see Material and Methods; Chapman et al., 1996; Gödecke et al., 1997). This index expressed the average similarity of optically imaged orientation preference values within a region of interest on a scale between 0 and 1. First, three regions of interest were defined for each map: the 1-mm-wide transition zone between A17 and A18, and the regions of A17 and A18 (outside the TZ) that were visible on the maps. The orientation similarity indices were then calculated for each of these divisions (n=10 hemispheres; TZ, A17, A18 in Figure 38G, gray bars). Control OSI values were also calculated by comparing TC maps to GC maps of another, randomly chosen, animal (Figure 38G, white bars). Values around 0.5 in the controls suggest that the maps of different animals were uncorrelated. The similarity indices were the highest in the TZ (OSI=0.72±0.04), and it was significantly lower both within A17 and 18 (OSI=0.59±0.05 and 0.56±0.06, respectively, p<0.0001, Mann-Whitney test). For each division, the OSI was significantly higher than in the controls (TZ, p<0.0001; A17, p<0.01; A18, p<0.015, Mann-Whitney test). This quantitative analysis confirmed the strong similarity between the TC and the GC angle maps along the TZ. It also indicates a degree of similarity of these maps in A17 and A18.

To further analyze the results concerning A17 and A18, these regions were divided, wherever possible, into 1-mm-wide bands parallel to the TZ and calculated the orientation similarity index for each of these bands (dotted lines in Figure 38D, E and F). The global width of the regions of A17 and A18 that could be analyzed varied from case to case: in A17, it was a 1-mm-wide region in all cases except one (2 mm wide) whereas in A18 it was 1 mm (n=4), 2 mm (n=4) or 3 mm wide (n=2). Figure 38H shows that, in all cases (n=6), the index is negatively correlated with distance from the TZ (mean correlation coefficient = -0.97±0.03).

These data confirm that, within the region where the TC patches were most evident (i.e. in the TZ and in its flanking regions), the orientation specific activities evoked by the GC and TC pathways are in close match. As a function of distance from the TZ, this similarity decreases both in A17 and A18.
III.1.4 Discussion

In this study, optical imaging of intrinsic signals revealed that the corpus callosum (CC) can mediate the activation of orientation selective domains, systematically in the transition zone (TZ) between A17 and A18 and occasionally within portions of both of these areas. Orientation domains appeared all along the TZ, without any obvious interruption, and these domains were arranged around pinwheel centers. Interestingly, these orientation patches were organized as separate “rows” for high and low spatio-temporal frequencies along the TZ. Finally, a strong similarity was found between TC and GC orientation maps within the TZ, and this similarity decreased progressively with increasing distance from the TZ toward A17 and A18.

III.1.4.1 Methodological considerations

Optical imaging of intrinsic signals was chosen because it allows viewing of large portions of cat visual cortex simultaneously in both hemispheres. Thus, both the activation of the GC pathway (including the soma of callosal neurons) as well as that of the callosal terminals were visible on the same maps. This method also provides reliable information about the topographical organization of the supragranular layers (Grinvald et al., 1988; Frostig et al., 1990) where most callosal neurons and their axon terminals are located (Fisken et al., 1975; Innocenti, 1980; Leporé and Guillemot, 1982; Milleret et al., 1994; Houzel et al., 1994). Furthermore, intrinsic signals recorded by optical imaging reflect both spiking and subthreshold activities (Das and Gilbert, 1995; Bonhoeffer and Grinvald, 1996; Toth et al., 1996). Thus, both types of activities could be revealed simultaneously in the TC recipient zone, which has not been previously possible with extracellular recordings.

The split-chiasm preparation offers several advantages to study transcallosal activity in cat visual cortex. First, it allows selective activation of the CC by visual stimulation of the eye contralateral to the explored cortex (see General introduction, Figure 13). It thus permits the dissociation of the TC and GC activation pathways by visually stimulating one eye or the other. Second, after chiasmotomy, the temporal retina of the eye contralateral to the explored cortex is the main source of activation of the CC. In intact animals, this pathway is a major route (if not the main one) to activate the callosal connections linking visual areas in both hemispheres. This point is demonstrated in detail in chapter 2. Finally, this experimental model allows direct comparison of the data to those of previous electrophysiological studies using the split-chiasm preparation (Berlucchi and Rizzolatti, 1968; Leporé and Guillemot, 1982; Milleret and Buser, 1993; Milleret et al., 1994; Milleret et al., 2005). Although this surgical approach presents many advantages as listed above, a few unavoidable limitations are also related to this technique. The chiasmotomy decreases the general excitability of the visual cortex by silencing the crossed retinal fibers coming mostly from the nasal retinae.
However, previous electrophysiological experiments indicate that this decrease occurs immediately after the section and is followed by a substantial recovery within the following 2-3 days (Milleret and Buser, 1993). In the present study, preliminary experiments revealed that the visually evoked optical responses could be greatly improved when the animal was allowed to recover after surgery with normal visual experience (i.e. awake) for 2-3 days. On the contrary, continuous anesthesia prevented or delayed the recovery of visual excitability of the cortex. Therefore, in this study, after sectioning the optic chiasm, the animals were allowed to recover in the animal facility for 3 days prior to the optical imaging recording sessions. Another potential disadvantage concerns the possible post-traumatic reorganization of the network including visual cortex that could, consequently, affect the intrinsic signal imaging. However, electrophysiological data indicate that this reorganization does not significantly affect supra-threshold activities since, after recovery, both orientation and spatial frequency selectivities are apparently unchanged with respect to those observed under normal conditions (Berlucchi and Rizzolatti, 1968; Leporé and Guillermot, 1982; Berardi et al., 1987; Milleret et al., 1994). Nevertheless, post-traumatic reorganization could affect sub-threshold activity and/or population activity. The data presented in this study do indicate that the main features of the functional maps are preserved in spite of the surgery. For example, as in the intact animal, GC orientation domains in the TZ displayed an elongated shape close to orthogonal to the TZ. The spacing between bands of the same orientation preference was fairly constant, and similar to that observed in normal animals. Furthermore, the TC orientation angle maps showed all the essential features of orientation maps that are obtained in the visual cortex of normal cats. Finally, the TC maps and GC maps were highly similar along their entire visible extent in the TZ.

### III.1.4.2 Localization of the regions activated via the transcallosal pathway

The main location of TC activity revealed here by optical imaging corresponds to the TZ. This is consistent with previous anatomical and electrophysiological studies that show the densest terminations of callosal axons in a 1-mm wide band-like zone at the transition zone between A17 and A18 (Berlucchi and Rizzolatti, 1968; Harvey, 1980; Leporé and Guillemot, 1982; Payne and Siwek, 1991b; Milleret et al., 1994; Boire et al., 1995).

Transcallosally activated patches were also observed in flanking regions of A17 and A18 along the TZ. But, in contrast to the TZ, this was not systematic and occurred strictly in posterior regions of the lateral gyrus, up to 1 mm from the TZ. This is in agreement with anatomical data showing that the density of callosal terminals near the TZ falls off significantly over the adjacent 1 mm of cortex in both A17 and A18 (see for example Payne and Siwek, 1991a; Payne and Siwek, 1991b; Houzel et al., 1994). Our data indicate that such activity is visible only caudally. This is also supported by anatomical data showing that the density of callosal terminals (and that of callosal neurons) in A17 and A18 near the TZ is the highest caudally, i.e. in the region where the area centralis is represented; then, it decreases progressively when moving rostrally (Innocenti and Fiore, 1976; Innocenti et al., 1985; Payne
and Siwek, 1991b). Electrophysiological data also show that TC units in A17 and A18 are more numerous in the posterior portions of the lateral gyrus than in its anterior parts (see for example Harvey, 1980; Milleret et al., 1994; Milleret et al., 2005).

Surprisingly, in two cases, an organized pattern of activity was observed in the TC maps throughout A18 (and A17 when it was visible). But this activity displayed a lower signal magnitude than found in the TZ as well as within the portions of A17 and A18 flanking the TZ caudally. Since very few TC unit were recorded electrophysiologically within these regions under similar experimental conditions (Milleret and Buser, 1993; Milleret et al., 1994; Milleret and Houzel, 2001), the present observations indicate that most of this activity is likely to be related to sub-threshold rather than supra-threshold inputs. One possibility is that some of these inputs originate directly from collateral branches of callosal axon endings. Indeed, anatomical data indicate that callosal terminals with decreasing density are present within the flanking regions of the TZ (Shatz, 1977b; Payne and Siwek, 1991a; Payne and Siwek, 1991b; Houzel et al., 1994). This may account for the weak activity found in those regions flanking the TZ. In addition, some rare clusters of callosal terminals have also been observed within the anterior part of A18, far from the TZ, i.e. beyond 2 mm (Sanides and Albus, 1980; Houzel et al., 1994). This may account for the few TC patches that were observed in a lateral region of A18 in only one case. They could correspond to the rare clusters of cells with large receptive fields straddling the visual midline that have been identified electrophysiologically in A18 with a strong inter-individual variability of presence and location (Sanides and Albus, 1980; Segraves and Rosenquist, 1982a). Alternatively, this TC activity could be transmitted indirectly through intra-hemispheric horizontal connections originating in the TZ (where the TC activation is strongest) and projecting into both A17 and A18. Finally, a last possibility is that a part of such sub-threshold widespread activation is driven through interhemispheric feedback or subcortical pathways. Contralateral cortical inputs coming from heterotopic areas to the transition zone (TZ) between A17 and A18 originate in areas 19, 21a, postero-medial lateral suprasylvian (PMLS) and postero-lateral lateral suprasylvian (PLLS) (Segraves and Rosenquist, 1982a). Furthermore, callosal fibers exist between this transition zone and the contralateral visual claustrum (LeVay and Sherk, 1981), see General Introduction § I.2.1). The mesencephalic reticular formation (MRF) might be one of the subcortical structures activated by visual stimulation of the contralateral eye and inducing a general activation of the visual cortex. Ascending projections from the MRF do not directly project to the cortex but send fibers to the basal forebrain, which is the major cholinergic source of the entire neocortex (Semba and Fibiger, 1989) including cat visual areas 17 and 18 (Albus, 1981). Visually induced gamma oscillations and response synchronization can be facilitated by electrical stimulation of the MRF (see for example, Singer et al., 1976; Lewandowski et al., 1993; Munk et al., 1996; Rodriguez et al., 2004). However, no study has demonstrated that stimulating such “non specific” subcortical structures could activate orientation preference domains throughout extensive portions of the visual cortex.
III.1.4.3 Layout of the transcallosally activated orientation domains

This study shows the distribution of orientation selective domains that are transcallosally activated over large portions of visual cortex. To this author’s knowledge, this has not been imaged before.

In orientation SCMs, transcallosally activated orientation domains were observed for all eight orientations tested. This is consistent with previous electrophysiological recordings showing orientation selective TC activity in cat visual cortex and clustering of TC units with similar orientation preferences, extending across all cortical layers (Berlucchi and Rizzolatti, 1968; Leporé and Guillemot, 1982; Milleret et al., 1994).

Considering all orientations tested, TC activation was observed along the whole antero-posterior extent of the TZ without obvious interruption (within the imaged region between stereotaxic coordinates P6-A6; Figure 33E, Figure 38). This continuous activation along the TZ could not be predicted from single unit recordings. For example, one previous study using closely spaced electrode penetrations showed the presence in the TZ of patches of cortex in which the ipsilateral visual field was represented (Diao et al., 1990). These patches, which were likely to be activated via the CC (see for example Payne, 1990b), were widely spaced (2-4 mm) along the TZ. This point is analyzed in chapter 2.

III.1.4.4 Spatial distribution of the transcallosal domains activated by different spatial and temporal frequencies

In orientation maps, the strongest TC activation was obtained with spatio-temporal frequencies (0.15 cpd, 1.5 Hz) of oriented gratings that were intermediate between the ones known to optimally activate neurons of A17 and A18 (0.6 cpd, 1.5 Hz and 0.15 cpd, 4.5 Hz respectively). This optimal combination of spatio-temporal frequencies is similar to the one used in a previous study to activate monocularly the transition zone between A17 and A18 in normal animals (Saint-Amour et al., 2004a). In the same study, it was noticed that the average optimal spatial frequency found in this transition zone was more related to A18 than to A17 (Saint-Amour et al., 2004a), as in the present study.

To localize the TZ, the visual cortex was activated through the GC pathway by oriented gratings of different spatial (SF) and temporal (TF) frequencies. Simultaneously, it was possible to observe in the opposite hemisphere the layout of the TC domains activated through the CC. Interestingly, for high SF and low TF (0.6 cpd, 1.5 Hz), TC domains appeared in the TZ closer to A17 whereas, for low SF and high TF (0.15 cpd, 4.5 Hz), they appeared in the TZ but proximal to A18. This suggests a topographic organization of the TC activated regions according to the spatial frequency of the stimulus. More detailed experiments would be necessary to define this topography more precisely (Issa et al., 2000). Concerning the temporal frequencies, electrophysiological studies indicated that the optimal
velocities of stimuli increase progressively when passing the TZ in a medio-lateral direction, from A17 to A18 (Orban et al., 1980; Orban, 1984a).

In agreement with the present data, previous electrophysiological and behavioral studies showed that interhemispheric communication through the CC in the TZ is strong for visual stimuli at low SF (0.2 cpd) and moderate TF (2 Hz) (Berardi et al., 1987; Berardi et al., 1988). This led some authors to suggest that TC neurons recorded within the TZ “resemble cells recorded within A18” or “behave as driven by Y inputs” (Blakemore et al., 1983; Berardi et al., 1987; McCourt et al., 1990). The present study indicates that TC activation by high SF and low TF is also effective. According to functional differences between the major input channels to the visual cortex (Sherman and Spear, 1982; Sherman, 1985), it is suggested that TC responses evoked by high SFs and low TFs may be signaled through the X-type channel. In fact, a recent study showed that the receptive fields of the TC units in the region of A17 flanking the TZ were markedly smaller and less numerous than those inside the TZ (see Fig. 7 in Milleret et al., 2005). This supports the hypothesis that with regard to preferred spatial frequency, the parts of the TZ are dominated by the properties of the visual area to which they are adjacent, i.e. A17 and A18.

### III.1.4.5 Matching the transcallosal and the geniculo-cortical maps

Most activated patches showed a higher activation level (lower gray value) in the GC orientation maps than in the TC maps, for the same number of stimuli. This can be explained by the more “powerful” nature of the thalamo-cortical input (Stratford et al., 1996). It also corroborates electrophysiological studies performed on split-chiasm cats revealing that callosal inputs are generally weaker, less brisk and more fatigable than thalamo-cortical inputs (Berlucchi and Rizzolatti, 1968; Leporé and Guillemot, 1982; Berardi et al., 1987; Milleret et al., 1994).

When the topographic organization of these maps was compared, in the TZ, a strong similarity of the TC and GC maps was observed both in the orientation SCMs and the angle maps. These results are compatible with electrophysiological data indicating that TC units are mostly binocular and display similar orientation preferences regardless of whether they are activated through one pathway or the other (Berlucchi and Rizzolatti, 1968; Milleret et al., 1994; Milleret et al., 2005). A gradual decrease of similarity between the TC and GC angle maps also appeared with increasing distance from the TZ. The TZ is the region where spike responses occur to TC activation. In other regions (less or not at all activated transcallosally), the orientation similarity index decreased progressively with distance from the TZ and the optical responses were less and less sharply tuned to orientation. In these regions, TC activation may have a sub-threshold influence on the target cells (see also above).

Altogether, the data described here contribute to a better understanding of the relationship between the callosal input and the functional organization of the target region, predominantly the transition zone between A17 and A18 where the visual midline and its
vicinity are represented. The findings imply that callosal connections can contribute to several functional attributes of visual cortical cells which are essential for normal perception. For example, the continuous representation of stimulus orientation at high and low spatio-temporal frequencies can allow the integration of a broad range of stimuli.
III.2 Representation of the ipsilateral hemifield and retinotopic organization of transcallosal activity in the cat visual cortex

III.2.1 Summary

The split-chiasm preparation was used to analyze the retinotopic organization of transcallosal connections in cat visual cortex. The representation of the ipsilateral hemifield was also investigated, together with the contribution of each hemiretina to this representation. Optical imaging of intrinsic signals was carried out in order to obtain retinotopic maps in both hemispheres of intact and split-chiasm adult cats.

The maps obtained in split-chiasm cats revealed that the corpus callosum (CC) activates regions corresponding to the representation of the visual midline as well as part of the ipsilateral visual field. This representation is highly compressed within the TZ and reaches an azimuth of up to 8° in the ipsilateral visual field. The ipsilateral part of the visual field beyond +2° was represented within patches along the TZ. Similar retinotopic maps were obtained in intact animals. This similarity indicates that the ipsilateral visual field representation is transferred principally through the uncrossed projections of the temporal retina and the CC. The crossed projections from the nasal retina also trigger transcallosal connections, but only for the representation of up to 2-4° in the ipsilateral hemifield.
III.2.2 Introduction

Unlike other regions of visual space that are represented only in the contralateral hemisphere, regions near the midline are represented in the visual cortex of both hemispheres. Callosal connections, which link sites in the visual cortex of the two hemispheres, are thought to play an important role in processing information from this central vertical part of the visual space. Compared to other systems of cortical connections, however, there is still considerable uncertainty about the distribution of this interhemispheric activity with respect to the topographic cortical map of visual space and the contribution of each hemiretina to transcallosal activation.

As described in the general introduction (§3.4), previous anatomical and physiological data have shown that in cat visual areas 17 (A17) and 18 (A18), callosal connections are retinotopically organized, displaying a non mirror-symmetric organization between the hemispheres (Olavarria, 1996; Alekseenko et al., 2005). Thus, the callosal neurons, whose cell bodies are located in rather large portions of A17 and A18 within one hemisphere, project their axon in the contralateral hemisphere, essentially within the transition zone (TZ) between both areas. Reciprocally, the callosal neurons whose cell bodies are located within the TZ project their axons into the other hemisphere mainly within the regions of A17 and A18 flanking the TZ. This has been demonstrated caudally, in the projection zone of the area centralis where the magnification factor of the central visual field representation is higher than in the rostral part of A17 and A18. In more rostral regions, many studies indicated that transcallosal fibers terminate more strictly in the TZ (e.g. see Innocenti, 1986a for review; see also Shatz, 1977b; Payne et al., 1991; Payne and Siwek, 1991b; Houzel et al., 1994). Therefore, as demonstrated in chapter 1, most of the neurons activated via the callosal afferents are located in the TZ (Choudhury et al., 1965; Berlucchi and Rizzolatti, 1968; Harvey, 1980; Innocenti, 1980; Leporé and Guillemot, 1982; McCourt et al., 1990; Payne and Siwek, 1991b; Milleret and Buser, 1993; Milleret et al., 1994; Milleret et al., 2001; Milleret et al., 2005). The retinotopic organization of these transcallosally activated neurons along the antero-posterior extent of the TZ, remains unknown.

Previous electrophysiological studies have demonstrated that the transcallosal receptive fields (TC-RFs) of these neurons are distributed along the CVM, with a shift toward the ipsilateral hemifield (see General introduction §4.2). The spatial distribution of these TC-RFs after chiasmotomy was recently analyzed in detail (Milleret et al., 2005): their global envelope may reach as far as 6° within the hemifield contralateral to the explored cortex and 13° within the ipsilateral one. This visual field representation in the TZ has also been investigated in intact adult cats by electrophysiological recordings after stimulation of the eye contralateral to the explored cortex (Payne, 1990b). The results showed that the TZ contains a significant ipsilateral visual field representation whose extent varies according to the elevation in the visual field. The shape of this representation resembles an hourglass: it extends to 3° on
the horizontal meridian and becomes progressively wider (up to 20°) at more positive and negative elevations.

The distribution of interhemispheric activity with respect to the topographic map of visual space remains unclear, as does the contribution of each eye and each hemiretina to the triggering of TC activation. This is mainly because of the difficulty in both isolating the TC activity from the GC one and visualizing this activity in large portions of the visual cortex. Only one study has analyzed the distribution of callosal connections with respect to the map of visual space using optical imaging of intrinsic signals combined with injections of neuronal tracers in the normal tree shrew (Bosking et al., 2000). This study demonstrated that callosal connections extend in regions representing the ipsilateral visual field for up to 15°. These connections are arranged in a topographic fashion that links visuotopically corresponding sites in the two hemispheres. However, neither the distribution of the transcallosally activated regions representing the ipsilateral visual field nor the contribution of each hemiretina to this representation has been investigated.

A correlation between periodicities in the distribution of callosal neurons and the pattern of ocular dominance columns suggested that transcallosal connections would be stabilized by the correlated activity of crossed and uncrossed projections from the same temporal retina (Olavarria, 2001). Callosal cells were found to correlate preferentially with ipsilateral eye columns in regions of A17 and A18 located outside the TZ, and with contralateral eye columns within the TZ (see General introduction, §3.4). Thus, callosal fibers preferentially link cortical loci that are under the influence of the same eye. This suggests that callosal fibers would be mainly activated by the same eye through the crossed and uncrossed projections of the temporal retina (Olavarria, 2002). However, numerous previous studies indicate that callosal connectivity is shaped by binocular interactions (Shatz, 1977b; Lund et al., 1978; Innocenti and Frost, 1979; Milleret, 1994; Milleret and Houzel, 2001; Milleret et al., 2005).

In the study reported here, optical imaging of intrinsic signals was used both to map the distribution and the retinotopic organization of TC activity, and to determine the contribution of each hemiretina to the triggering of TC activity in the cat visual cortex. Optical imaging of intrinsic signals was carried out in order to obtain retinotopic maps in both hemispheres of intact and split-chiasm adult cats. The maps obtained in split-chiasm cats revealed that the corpus callosum (CC) activated regions corresponding to the representation of the visual midline as well as part of the ipsilateral visual field, highly compressed within the TZ and reaching an azimuth of up to 8°. The ipsilateral part of the visual field (beyond +2°) was represented within patches along the TZ. Similar retinotopic maps were obtained in intact animals. These results demonstrate the contribution of each hemiretina and of the transcallosal pathway to the ipsilateral visual field representation in the TZ.
III.2.3 Results

Eleven hemispheres from six intact adult cats and six hemispheres from four split-chiasm animals were analyzed and the activities obtained in each experimental condition were compared. Because the TC activation is directly identifiable in split-chiasm animals (see General introduction, §4), data from split-chiasm animals are systematically presented first as a reference. Depending on the animal or the experiment, either eye was visually stimulated. To facilitate comparisons, all data are presented as if the left eye was stimulated (see Figure 39); thus, mirror symmetric images of the activities recorded with stimulation of the right eye are presented. The results are presented according to the part of the visual field stimulated: the central vertical midline, the right hemifield and the left hemifield.

III.2.3.1 Stimulation of the central vertical meridian of the visual field

The representation of the central vertical part of the visual field includes the representation of the area centralis (Tusa et al., 1978; 1979). In terms of azimuthal coordinates, the following data have been obtained when the vertical bar was presented at 0°, -1° or +1°.

In the split-chiasm animals (Figure 40, on the right), the GC activation in the LH was generally quite robust, particularly in the posterior parts of the lateral gyrus where the area centralis and its surroundings are represented. In all cases, this activation included the TZ and a part of A17; in two cases (cats Ca01 and cat Ca16), it also included the part of A18 bordering the TZ. Within the RH, the TC activation generally displayed a similar distribution, but in most cases, it was weaker than the GC activation in the other hemisphere (see for example Ca16). As stated in chapter 1 (§ III.1.3.1 ), for each hemisphere studied, the location of the TZ was defined from GC orientation maps obtained with two different spatio-temporal frequencies of the stimulus, known to activate preferentially either A17 or A18 (“A18 condition”, 0.15 cpd, 4.5 Hz) and at high SF and low TF (“A17 condition”, 0.6 cpd, 1.5 Hz) (see § III.2.3.4 ). This location corresponded to the stereotaxically determined location of the TZ (Tusa et al., 1978; Payne, 1990b) and was controlled by electrophysiology and/or, post-mortem, by histology (see § III.2.3.4 ).

In the intact animals, an activity was observed but the distinction between the GC and the TC activations could not be made. Indeed, it is well known that the largest density of ganglion cells in the cat is situated along a “vertical median strip” of the retina, approximately 0.2 mm wide, including the area centralis (Stone, 1966; Stone, 1978). Whatever the ganglion cell type (X, Y, W), such a region includes the largest density of retinal fibers projecting bilaterally (Kirk et al., 1976a; Kirk et al., 1976b). As in split-chiasm animals, a bilateral activation was observed (Figure 40, on the left), but the activation within the RH was generally the strongest one. This is likely related to the highest proportion of crossed retinal fibers compared to uncrossed ones (Stone, 1966).
Figure 39. Experimental protocols. Optical imaging of intrinsic signals was performed in A17 and A18, simultaneously in the two hemispheres, both in intact (A) and split-chiasm (B) adult cats. All animals were stimulated monocularly (here, through the left eye). Top, left. Visual stimulus used for retinotopic maps; a narrow vertical bar (length, 53°; width 1°) containing a moving horizontal grating was presented at different positions along the horizontal axis. Negative and positive values corresponded to the left (L) and the right (R) hemifield respectively and 0° to the central vertical meridian. A. In intact animals, both the crossed fibers coming mainly from the nasal hemiretinae (N) and the uncrossed fibers originating mainly from the temporal hemiretinae (T) remained intact, projecting to the visual cortex via the optic nerve (ON), the ipsilateral optic tract (OT), the dorsal lateral geniculate nucleus (dLGN) and the optic radiations (OR). When stimulated monocularly, the geniculo-cortical (GC) pathway and the TC pathways could be activated from both the temporal and the nasal retina of each eye, in one or the other hemisphere (thick lines and arrows). A1-A4. In intact cats, four pathways could induce, at least in principle, a TC activation from: A1, the temporal retina, projecting ipsilaterally through the GC pathway and then contralaterally through the CC; A2, the temporal retina, projecting contralaterally through the GC pathway and then ipsilaterally through the CC; A3, the nasal retina, projecting ipsilaterally (GC pathway) and then contralaterally through the CC; A4, the nasal retina, projecting contralaterally (GC pathway) and then ipsilaterally through the CC. In each panel, dashed lines indicate the stimulated visual hemifield. B. The optic chiasm (XO) was sectioned in the midsagittal plane. Crossed fibers coming mainly from the nasal hemiretinae were severed (dashed lines). A monocular stimulation activated selectively either the transcallosal (TC) pathway or the GC pathway. For example, stimulation of the left (L) eye activated the left hemisphere (LH) only through the ipsilateral GC pathway and the right hemisphere (RH) only through the CC (thick lines and arrows). The TC activity could be triggered by the stimulation of: B1, the temporal retina, projecting ipsilaterally (GC pathway) and then contralaterally through the CC; B2, the nasal retina, projecting ipsilaterally (GC pathway) and then contralaterally through the CC.
Figure 40. Representation of the central vertical meridian in the right (RH) and left (LH) hemispheres of normal (on the left) and split-chiasm (on the right) adult cats. In both experimental groups, the left eye was stimulated with the vertical bar (containing a horizontal moving grating) located at the central vertical meridian (or at $+1^\circ$/$-1^\circ$) of the visual field of each animal. A schema of the experimental protocol for each group is shown on the top of the figure. For each cat, the blood vessel pattern of the imaged region is shown on the left and the single-condition map corresponding to the representation of the vertical meridian is shown on the right. In these and subsequent gray scale optical images, darker gray indicates stronger activation. In split-chiasm animals, since the left eye was stimulated, dark regions observed in the left hemisphere (LH) correspond to the regions activated via the ipsilateral geniculo-cortical pathway; the right hemisphere (RH) was activated only via the callosal pathway. The anterior part of the cortex is on top of each panel. In each panel, the thick dashed line shows the stereotaxic midline; thin dashed lines show the limits of the transition zone between A17 and A18. Short horizontal line on the left of each panel shows stereotaxic coordinate AP0. The name of each adult cat is indicated on the left of the corresponding blood vessel pattern of the imaged region. Az, azimuth. Scale bar, 2 mm.
III.2.3.2 Stimulation within the right visual hemifield

The following maps were obtained when the vertical bar was presented to the temporal retina of the left eye, from azimuthal position +2° and beyond. Thus, configurations A1, A2 and B1 presented in Figure 39 were tested. The TC maps imaged in split-chiasm cats were compared with the maps obtained in intact animals. Surprisingly, they clearly display common characteristics.

III.2.3.2.1 Localization of the regions activated via the transcallosal pathway in A17 and A18

The cat Ca01 is presented first because it was investigated both before and after section of the optic chiasm and thus allowed direct comparisons of the maps obtained in each condition. Figure 41 shows single-condition maps corresponding to the representation, in both hemispheres, of the azimuthal positions 0°, +4° and +8°, for each condition.

After chiasmotomy (Figure 41, bottom), since only the left eye was stimulated, the dark regions observed in the left hemisphere (LH) corresponded to the regions activated via the retino-geniculo-cortical pathway (GC maps), while those observed in the right hemisphere (RH) were activated via the transcallosal pathway (TC maps) (see Figure 39B). During the course of the experiment, the long narrow vertical bar was presented from -16° to +16° along the horizontal axis of the visual field by steps of 4°. Bilateral activations (thus a TC activation) were elicited by the visual stimulus at 0°, +4° and +8° in the right hemifield. When the visual stimulus was presented centrally in the visual field (at 0°), as indicated above, a rather symmetric pattern appeared in both hemispheres. When the visual stimulus was presented at +4° within the right hemifield, a different pattern of activity was observed. In the LH, the GC map appeared as a high contrasted band of activity at a location in agreement with the established retinotopic organization of cat visual cortex (Tusa et al., 1978; Tusa et al., 1979). In the RH, the TC maps appeared as a row of three patches within the TZ, from the postero-lateral (stereotaxic coordinates P4L4) to the antero-medial (A2L1.5) part of the lateral gyrus. The contrast of these patches was lower than that of the activity in the other hemisphere, and it decreased from the posterior to the anterior regions of the lateral gyrus. At +8°, the GC maps still appeared in the LH as a high contrasted “band” of activity, in a more lateral position than at +4°. In the RH, only the most posterior TC patch was still visible.

Data obtained in the same animal (Ca01) before the section of the optic chiasm were nearly identical to those obtained after the section (Figure 41, top). Both GC and TC activities displayed the same arrangement in both experimental conditions, indicating that the activity observed in the right hemisphere of the intact animal is likely coming from the TC pathway.
Maps with similar characteristics to those observed in cat Ca01 were obtained in all the other cats, whether they were intact or split-chiasm animals. Three examples are presented showing single-condition maps recorded in another split-chiasm adult cat (Ca10, Figure 42) and two other intact cats (Ca02, Figure 43 and Ca15, Figure 44).

In the case of split-chiasm cat Ca10 (Figure 42), the vertical bar was presented from -13° to +13° along the horizontal axis of the visual field. From -1° to -13° (left hemifield), no activated regions appeared in either hemisphere. At 0°, the activated region in the LH included both the TZ and the posterior part of A17, while the TC map in the RH included the TZ as well as some portions of the flanking regions of both A17 and A18. From positions of the stimulus at +1° to +13° (right hemifield), the GC activity in the LH was progressively located more laterally in A18. Bilateral activations were observed for positions of the bar between 0° and +7°. In the RH, a transcallosal (TC) activity appeared for each stimulus, mainly within the 1-mm-wide TZ and from stereotaxic coordinates P6L4 to A6L1.5. The observation that the TC activated region extended to the posterior border of the imaged area
suggests that the posterior limit of TC activation was most likely located even more caudally. Similarly, the anterior limit of TC activation was most likely obscured within the interhemispheric fissure. From +2° to +7°, this TC activity was patchy with three TC patches that could be distinguished (most visible at +5°, see black arrows in Figure 42). Here, their contrast did not change from posterior to anterior parts of the lateral gyrus, but it decreased progressively with eccentricity so that only one patch remained visible at +7°. Interestingly, the GC activated region on the left hemisphere also appeared patchy for some positions of the stimulus (+2°, +3°, +5°) although the size of these patches was bigger than that of the TC patches.

In the case of the intact animal Ca02 (Figure 43), the vertical bar was presented from -8° to +20° along the horizontal axis of the visual field. From -1° to -8° (left hemifield), the activated regions in the RH included successively more and more lateral parts of the lateral gyrus, first in A17 and the TZ, and then in A18. Bilateral activations were observed for positions of the bar between -1° and +8°. At –1°, a weak activity appeared within the TZ in the LH. At 0°, a rather symmetric pattern of activity was observed in both hemispheres, within the TZ and a part of A17. Between +0° and +12°, the activated regions in the LH included successively more and more lateral parts of the lateral gyrus: first in A17 and the TZ, and then in A18. For stimuli between +2° and +8°, the activated region in the RH again appeared mainly within the TZ, from stereotaxic coordinates P2.5 to A5.5. This activity was patchy with four patches that could be distinguished (see black arrows in Figure 43). Their contrast decreased with eccentricity within the visual field so that they were barely visible at +8°.

Similar patterns of activity were observed in the other intact animals. For example, in the case of Ca15 (Figure 44), the vertical bar was presented from -14° to +10° along the horizontal axis of the visual field. From 0° to -14° (left hemifield), the activated regions in the RH included successively more and more lateral parts of the lateral gyrus. Bilateral activations were observed for positions of the bar between -2° and +4°. At –1° and –2°, a patchy activity appeared in the left hemisphere and was slightly visible at –3°. Between +0° and +10° (right hemifield), the activated regions in the LH were successively in more and more lateral parts of the lateral gyrus. For stimuli between +2° and +4°, the activated region in the RH again appeared within the TZ, from stereotaxic coordinates P2.5 to A3, and three patches could be distinguished (see black arrows in Figure 44). At +6°, they were no longer visible.
Figure 42. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between $-13^\circ$ and $+13^\circ$, in both hemispheres of the split-chiasm cat Ca10. The blood vessel pattern of the imaged region is shown on the higher left corner. The left eye was stimulated. The schemas at the top of the figure indicate the three possible pathways that could elicit a TC activity. Dark patches observed in the right hemisphere (RH) correspond to the regions activated via the transcallosal pathway. Other conventions as in Figure 40. Scale bar, 2 mm.
Figure 43. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between −8° and +20°, in both hemispheres of the intact cat Ca02. The blood vessel pattern of the imaged region is shown on the higher left corner. The left eye was stimulated. The schemas at the top of the figure indicate the possible pathways that could elicit a TC activity. Dark patches observed in the right hemisphere (RH), for positions of the stimulus beyond +2°, correspond likely to regions activated via the transcallosal pathway. Other conventions as in Figure 40. Scale bar, 2 mm.
Figure 44. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between $-12^\circ$ and $+12^\circ$, in both hemispheres of the intact cat Ca15. The blood vessel pattern of the imaged region is shown on the higher left corner. The left eye was stimulated. The schemas at the top of the figure indicate the possible pathways that could elicit a TC activity. Dark patches observed in the right hemisphere (RH), for positions of the stimulus beyond $+2^\circ$, correspond likely to regions activated via the transcallosal pathway. Other conventions as in Figure 40. Scale bar, 2 mm.
Altogether, the regions activated in the right hemisphere for stimuli located within the right hemifield (ipsilateral hemifield) systematically appeared within the 1-mm-wide TZ. For a position of the stimulus beyond +2° and up to 8°, these regions displayed a patchy pattern in most cases: in 4 out of the 6 hemispheres tested in the split-chiasm cats (Figure 45) and in 6 out of the 6 hemispheres tested in the intact animals (Figure 46). Depending on the animal, the number of patches could vary as well as their size, their location along the TZ, and their contrast. After chiasmotomy, a maximum of 3 patches was observed: 0.6 to 2.2 mm in length (antero-posterior axis) and 0.7 to 2.2 mm in width (medio-lateral axis), separated by 1.8 to 5.8 mm along the TZ (Table 1). These patches were observed between stereotaxic coordinates P4.3 and A5.7. In all cases the number of visible patches decreased with increasing eccentricity of the stimulus and no patch could be observed beyond +8°. In the intact animals, 3 to 6 patches could be observed: 0.4 to 2.7 mm in length (antero-posterior axis) and 0.4 to 1.4 mm in width (medio-lateral axis), separated (from center to center) by 0.8 to 3.9 mm along the TZ (Table 1). These patches were observed between stereotaxic coordinates P2.4 and A8.1. In all cases, the number of visible patches also decreased with increasing eccentricity of the stimulus and no patch could be observed beyond +8°.

In one hemisphere of an intact cat (Ca07), a patchy activity (3 patches) also appeared within the TZ for these stimuli. Because the imaged region was half smaller as that of the other cases and the activity was weak, these data are not shown. In the two remaining hemispheres tested in one split-chiasm cat (Ca16), only a weak diffuse TC activation was seen within the TZ, although the contralateral cortex could be activated by the GC pathway (data not shown).

<table>
<thead>
<tr>
<th>CAT</th>
<th>Number of patches (max)</th>
<th>Length (AP) min - max</th>
<th>Width (ML) min - max</th>
<th>Inter-patch distance min-max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA07</td>
<td>3</td>
<td>0.4 - 2.1</td>
<td>0.4 - 0.8</td>
<td>1.8 - 3.9</td>
</tr>
<tr>
<td>CA08</td>
<td>6</td>
<td>0.3 - 1.3</td>
<td>0.3 - 1.0</td>
<td>0.8 - 2.7</td>
</tr>
<tr>
<td>CA09</td>
<td>4</td>
<td>0.5 - 1.3</td>
<td>0.7 - 1.0</td>
<td>1.7 - 3.9</td>
</tr>
<tr>
<td>CA15</td>
<td>3</td>
<td>1.1 - 2.7</td>
<td>0.9 - 1.1</td>
<td>1.4 - 3.4</td>
</tr>
<tr>
<td>Split-chiasm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA01</td>
<td>3</td>
<td>1.0 - 1.8</td>
<td>0.8 - 1.0</td>
<td>2.2 - 2.6</td>
</tr>
<tr>
<td>CA10 LH</td>
<td>3</td>
<td>0.6 - 1.9</td>
<td>0.7 - 1.4</td>
<td>1.8 - 3.2</td>
</tr>
<tr>
<td>CA10 RH</td>
<td>3</td>
<td>1.1 - 2.2</td>
<td>1.3 - 2.2</td>
<td>3.2 - 5.8</td>
</tr>
<tr>
<td>CA17</td>
<td>3</td>
<td>0.6 - 1.8</td>
<td>0.9 - 1.4</td>
<td>2.3 - 2.6</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of the activated regions in the right hemispheres for stimuli located within the right (ipsilateral) hemifield beyond +2° and up to 8°, in intact and split-chiasm animals. These regions systematically appeared within the 1-mm-wide TZ and displayed a patchy pattern. The inter-patch distance (in mm) indicates the center-to-center distance separating the patches. The length and width of the patches are expressed in mm. AP, antero-posterior axis; ML, medio-lateral axis; min, minimum; max, maximum.
III.2.3.2.2 **Retinotopic organization of the regions activated via the transcallosal pathway in A17 and A18**

After chiasmotomy, in 4 out of 6 hemispheres studied (see above), patchy TC responses were systematically obtained for positions of the stimulus between +2° and +7° or +8° (Figure 45). The TC activity was quite strong at +2°, but decreased with eccentricity, as assessed by the number of visible patches, so that it was almost invisible at +7° or +8°. This portion of the ipsilateral visual field was represented as one single row of patches located within the TZ for all eccentricities. Thus, with this method’s spatial resolution, no fine retinotopic organization was observed within the TZ or within the patches; for example, in the case of Ca10 (RE), the regions within the TZ that were activated with the stimulus at +2° appeared to be located at the same place as the ones obtained with the stimulus at +3° or +5°. Then, at +7° all patches except one disappeared. Beyond +8° of azimuth, no TC activation has been observed in any animal. Thus, no new patch appeared with increasing eccentricity of the stimulus. Only patches already present at +2° remained and disappeared progressively.

In intact animals, similar characteristics of the maps were observed. The regions activated for stimuli located within the ipsilateral hemifield (right hemifield) systematically appeared as a row of patches within the TZ from +2° to +7° or +8° (Figure 46). This activity was strongest at +2°, and then decreased with eccentricity after +5°, so that it was almost invisible at +7° or +8°. Beyond +8° of azimuth, no such activity has been observed in any animal.
Figure 45. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between +2° and +8° (right hemifield), in both hemispheres of split-chiasm animals. For each case, the name of the animal and the blood vessel pattern of the imaged region are shown on the left. The left eye was stimulated so dark patches observed in the right hemisphere (RH) correspond to the regions activated via the transcallosal pathway. In one case (Ca10), the right eye (RE) was stimulated so TC activity was observed on the left hemisphere. To facilitate comparisons with the other cases, these maps are presented as if the left eye was stimulated; thus, mirror symmetric images of the recorded maps are presented. Arrows indicate the location of the TC patches for the most median position of the stimulus i.e. +2°, +3° or +4°. For comparison, these arrows are copied at the same position for the more lateral eccentricities of the stimulus. Other conventions as in Figure 40. Scale bar, 2 mm.
Figure 46. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between $+2^\circ$ and $+8^\circ$ (right hemifield), in both hemispheres of intact animals. For each case, the name of the animal and the blood vessel pattern of the imaged region are shown on the left. The left eye was stimulated. The schemas at the top of the figure indicate the two possible pathways that could elicit a TC activity in these intact animals. Dark patches observed in the right hemisphere (RH) correspond likely to regions activated via the transcallosal pathway. Black arrows indicate the location of the TC patches for the most median position of the stimulus i.e. $+2^\circ$, $+3^\circ$ or $+4^\circ$. For comparison, these arrows are copied at the same position for the more lateral eccentricities of the stimulus. Other conventions as in Figure 40. Scale bar, 2 mm.
Comparison of the patches of activity representing the ipsilateral visual field with the orientation maps

In three hemispheres of both three intact animals and two split-chiasm animals, orientation maps have been recorded in the region of the visual cortex where patches of activity appeared for an ipsilateral visual stimulation. To explain this patchy organization, these patches were compared with orientation maps of the same region (Figure 47). The proportion of each orientation domain within these patches is shown in the graphs of Figure 48. In one split-chiasm cat (Ca10 RH), no single orientation domain was significantly more represented than the others: the distribution of the proportion of each orientation domain was uniformed [V-test (modified Rayleigh test), V=0.42; p>0.1]. In all other cases (split chiasm and intact animals), the distributions of these proportions were not uniformed and they had a significant tendency to be centered on the vertical orientation (90°) (V-test, V>2.32; p<0.01) This significant overrepresentation of the vertical orientation domains was, however, quite limited: in most cases, the distributions were close to what would be expected for a random distribution (dotted line in Figure 48). Thus, within the patches of activity representing the ipsilateral hemifield, a small bias was shown in all cases except one toward the representation of vertical orientation preference.

Ocular dominance maps were recorded in only two intact animals (Ca09, Ca08) in the region of patches of activity representing the ipsilateral visual field. The overlap of the patches with these maps did not reveal any specific link between the patches and the ocular dominance domains (data not shown). Indeed, in the region of the TZ (where these patches were located), the ocular dominance domains did not clearly appear, indicating that this region was mainly binocular.

To summarize, the stimulation, through the left eye (temporal retina), of the right visual hemifield beyond +2° and up to +8° induced a patchy transcallosal activation in the TZ of the right hemisphere of split-chiasm cats. Uncrossed projections from the temporal retina are therefore effective in triggering the interhemispheric transfer of visual information though the CC (Figure 39, A1, B1). Maps with similar characteristics were observed for the same stimuli in intact animals. This similarity suggests that, in intact animals, ipsilateral visual field representation is probably triggered predominantly by the transcallosal pathway (Figure 39, A1, B1) and not, or only weakly, by the crossed pathway from the temporal retina (Figure 39, A2).
Figure 47. Comparison of the patches of activity representing the ipsilateral visual field with the orientation maps of the same region, in two intact (Ca02 and Ca08) and one split-chiasm (Ca01) animals. For each animal, three maps are presented: on the left, the retinotopic map obtained with the presentation of the stimulus in the ipsilateral visual field at an azimuth indicated above the map; in the middle, the same retinotopic map with patches of activity indicated by white ellipses; on the right, the orientation map of the same region with the white ellipses representing the “ipsilateral visual field” patches. For comparison, black arrows are at the same location in the three maps. The anterior part of the cortex is on top of each panel. Other conventions as in Figure 40. Scale bar, 2 mm.

Figure 48. Orientation domains represented within the patches of activity observed in split-chiasm animals (A) and in intact ones (B) for stimuli presented in the ipsilateral hemifield. Each graph indicates the percentage of pixels, within these patches, preferentially activated for an orientation of the stimulus (0°, 22.5°, 45°, 67.5°, 90°, 112.5°, 135°, 157.5°). For each animal, the analysis was performed for the eccentricity of the stimulus at which the patches were the most visible. This eccentricity is indicated in the legend after the name of each cat. The dotted line indicates the percentage of pixels that would be expected in each bin for an even distribution (12.5%).
III.2.3.3 Stimulation within the left visual hemifield

The following data have been obtained when the visual stimulus (vertical bar) was presented in the left hemifield stimulating the nasal retina of the left eye from -1° and beyond. Thus, configurations A3, A4 and B2 described in Figure 39 were investigated.

After chiasmotomy, the stimulation of the left visual hemifield through the left nasal hemiretina did not evoke any visible activation within both hemispheres: no TC activity was visible in this case (see for example Figure 42). This indicates that configuration B2 (Figure 39) provides either no TC activation, or a weak one that cannot be seen through this experimental approach.

In contrast, in the intact animals, such stimulation always evoked a strong activity in the right hemisphere, likely through the crossing GC pathway (Figure 39, A4). In accordance with the retinotopic organization of the visual cortex, this activity was progressively located more laterally in A18 for positions of the stimuli from -1° to -8°. Some activity could also be evoked in the left hemisphere (Figure 49). Patches of activity appeared when the visual stimulus was presented at -1° (cats Ca02, Ca09 and Ca15), at -2° (cats Ca08 and Ca15) and presumably at -4° (cat Ca15, Ca01), (Figure 49, black arrows). Because this activity was absent after chiasmotomy and since it was observed only within the TZ, such activation may have a transcallosal origin. Thus a second pathway could trigger ipsilateral field representation, i.e. through the crossing GC pathway and the transcallosal one (Figure 39, A4). Beyond -4° of azimuth, no such activity has been observed in any animal.

Thus, when the left hemifield was stimulated through the left eye, an activity appeared in the TZ of the left (ipsilateral) hemisphere. Crossed projections from the nasal hemiretina (Figure 39, A4) may therefore trigger some TC activation. This pathway transfers the representation of a smaller part of the ipsilateral hemifield (up to 2-4°) than uncrossed projections from the temporal hemiretina (up to 8°) (Figure 39, A1). By contrast, uncrossed projections from the nasal retina (Figure 39, A3, B2) provide either no activation, or a weak one that cannot be detected with this experimental approach.
Figure 49. Single-condition maps obtained with the presentation of the visual stimulus (vertical bar) at azimuthal positions between -1° and -8° (left hemifield), in both hemispheres of intact animals. For each case, the name of the animal and the blood vessel pattern of the imaged region are shown on the left. The left eye was stimulated. The schemas at the top of the figure indicate the two possible pathways that could elicit a TC activity. Patches of activity observed in the left hemisphere (LH) are indicated by black arrows. Other conventions as in Figure 40. Scale bar, 2 mm.
III.2.3.4 Localization of the TZ, electrophysiological and histological controls

The location of the transcallosally activated region observed in split-chiasm animals (Figure 45) corresponded to the stereotaxically determined location of the transition zone (TZ) between A17 and A18 (Tusa et al., 1978; Payne, 1990b). The same region was activated for stimuli presented in the hemifield ipsilateral to the explored hemisphere in intact animals (Figure 46). To confirm the location of the TZ, GC orientation maps were systematically recorded with different spatial (SF) and temporal frequencies (TF) of the stimulus in order to preferentially activate either A17 (Figure 50C, H and Figure 51A) or A18 (Figure 50B, G and Figure 51B), as described in chapter 1 (§ III.1.3.1). These maps confirmed that the regions activated via the callosal pathway were distributed mainly along the transition zone between the regions of A17- and A18-specific activation (Figure 50D, E, I, J and Figure 51C).

The location of the TZ was also confirmed by electrophysiological recordings in one animal. Figure 50 shows two recording sites from where multi-unit activity was recorded in the right hemisphere of split-chiasm cat Ca10. These sites were located in the regions activated by a stimulation of the left (contralateral) eye at +3° (transcallosal activity) and by a stimulation of the right (ipsilateral) eye at −2° (geniculo-cortical activity) (see white arrowheads in Figure 50H, I). The receptive fields of cells activated by the contralateral eye were found along the vertical meridian or within the ipsilateral visual field (Figure 50J), which is characteristic of the TZ and of transcallosally activated units (Payne, 1990b; Milleret et al., 1994).

Finally, this functional delineation of the TZ was corroborated by histological controls. Cytoarchitectonic criteria were used to delimit the TZ in frontal sections (see Material and Methods and Otsuka and Hassler, 1962; Payne, 1990b; Milleret et al., 1994). The recording sites (corresponding to TC activated regions) were located within the TZ or within its 1-mm-wide flanking regions (Figure 50K). Similar histological controls were performed in seven other animals (split-chiasm animals: Ca01, Ca16, Ca17; intact animals Ca07, Ca08, Ca09, Ca15) in order to localize reference penetrations that were made along the TZ defined using the area-specific functional maps described above (see Ca01, Figure 51).
Figure 50. Localization of the transition zone (TZ) between A17 and A18 in the left (LH) and right (RH) hemisphere of split-chiasm cat Ca10. A, E. Blood vessel pattern of the imaged region in LH and RH. B, C. Localization of the TZ in the left hemisphere (LH) by activating the geniculo-cortical pathway (stimulation of the left, ipsilateral eye) with stimuli optimized for A17 and A18. B. Localization of A18 (dark region). The sum of all orientation SCMs corresponding to A18 stimuli was divided by the “cocktail blank” (see text for further details). C. Localization of A17 (black region). The sum of all orientation SCMs acquired with A17 stimuli was divided by the “cocktail blank”. In both maps, the location of the TZ was defined as the 1-mm-wide zone delineated by the two dashed lines. These lines were copied at the same cortical location on panel D. D. Transcallosal single-condition map obtained with the visual stimulus for retinotopic mapping (vertical bar) at -5° in the ipsilateral visual field (stimulation of the right, contralateral eye). F-G. Localization of the TZ in the right hemisphere (RH) of the same animal. Area-specific geniculo-cortical activation patterns (right, ipsilateral eye stimulated) using the same stimuli as in B and C respectively. H. Transcallosal single-condition map obtained with the visual stimulus (vertical bar) at +3° in the ipsilateral visual field (stimulation of the left, contralateral eye). I. Geniculo-cortical single-condition map obtained with the visual stimulus at -2° (stimulation of the right, ipsilateral eye). Other conventions as in Figure 40. Scale bar, 2 mm. J, K. Electrophysiological and histological controls from the RH, cat Ca10. J. Spatial distribution of the receptive fields of the neurons recorded at each site indicated in H and I by white arrowheads. Same legend as Figure 35 E-F. Scale bar, 1 mm.
Figure 51. Localization of the transition zone (TZ) between A17 and A18 in the right (RH) hemisphere of cat Ca01, studied before and after chiasmotomy. A, B. Localization of the TZ in the right hemisphere (RH) by activating the geniculo-cortical pathway (stimulation of the right, ipsilateral eye) with stimuli optimized for A17 and A18, see Figure 50. The lines delineated the TZ were copied at the same cortical location on panels C and D. C. Transcallosal single-condition map obtained with the stimulus for retinotopic mapping (vertical bar) at +4° in the ipsilateral visual field (stimulation of the left, contralateral eye), after chiasmotomy. D. Blood vessel pattern of the imaged region in A-C. Short horizontal line on the top of each panel shows stereotaxic coordinate AP0. A, anterior; M, medial. Scale bar, 2 mm. E. Histological controls. Photomicrographs of frontal sections (Nissl staining) showing the location of each reference penetration indicated by white arrowheads in panel C. Other conventions as in Figure 50. Scale bar, 1 mm.
III.2.4 Discussion

In this study, retinotopic maps obtained by optical imaging of intrinsic signals in split-chiasm cats revealed that the corpus callosum (CC) activates regions corresponding to the representation of the visual midline and part of the ipsilateral visual field (up to 8°), highly compressed within the TZ. The ipsilateral part of the visual field (beyond +2°) was represented within patches along the TZ. The similarity of the results obtained in split-chiasm and normal animals indicates that the representation of the ipsilateral hemifield is triggered predominately by the transcallosal pathway in intact animals, through uncrossed projections from the temporal retina. These results also show that the nasal hemiretina can contribute to the representation of the ipsilateral hemifield by triggering TC activation, but to a lesser extent (up to 2-4°) than the temporal retina (up to 8°).

III.2.4.1 Methodological considerations

Retinotopic maps over large regions of the visual cortex were obtained in both hemispheres by optical imaging of intrinsic signals. Intact and split-chiasm animals were compared in order to determine the contribution of the transcallosal pathway to the representation of the ipsilateral hemifield in intact animals. Three cats were investigated, in acute experiments, before and after the section of their optic chiasm, so that they were continuously anaesthetized and paralyzed. In such conditions, transcallosal maps appeared in only one animal after the optic chiasm section (Ca01). As indicated in chapter 1 (§4.1), visually evoked optical responses were improved greatly if animals were allowed to recover after surgery with normal visual experience (i.e. awake) for 2-3 days. Therefore, in this study, all other split-chiasm animals were studied after a post-surgery recovery period of 3 days in the animal facility.

Monocular visual stimulation was preferred to binocular stimulation in order to define the contribution of each hemiretina to transcallosal pathway activation. The visual stimulus that was used (vertical bar, 53° in length and 1° in width) can be considered as a rather restricted stimulus. However, the corresponding cortical activation via the direct retinogeniculo-cortical pathway was larger than expected given the width of the stimulus. This is directly related to the point spread function of the visual cortex. The large activated area recorded by optical imaging of intrinsic signals in response to “point-like” stimuli reflects both spiking and subthreshold activity (Das and Gilbert, 1995): as assessed by electrophysiological recordings, the spiking activation would account for only 5% of the activated area, the remaining 95% being generated by subthreshold activation through horizontal connections radiating from the locus of spiking activity. A study (Toth et al., 1996) has shown that optical imaging signals in A17 in response to a restricted stimulus generate activity which extends for several millimeters across the cortical surface. Similar observations have also been reported in the tree shrew (Bosking et al., 2002), in which a line stimulus, thinner than the average V1 receptive field, evoked a broad strip of neural activity of almost constant size for all stimulus locations tested within the central ten degrees of visual space.
Small changes in stimulus position produced orderly changes in the location of the peak population response. In the present study, the proportions of spiking and subthreshold activities are likely similar in transcallosal and geniculo-cortical maps. Since the patches observed in the callosal terminal zone were rather small (between 0.4 and 2.7 mm in length, and 0.4 and 2.2 mm in width), the region of spiking activity was probably even smaller.

Two main factors can induce errors in retinotopic maps: incorrect positioning of the vertical midline and the presence of residual eye movements in paralyzed cats. The vertical midline was defined as the vertical line crossing the area centralis. The area centralis was defined, with an ophtalmoscope, as the geometric center of the retinal area devoid of blood vessels. This position was confirmed by controlling the geometric relation between the area centralis, the vertical meridian and the optic disk (Bishop et al., 1962). The fact that retinotopic maps with the same characteristics were obtained in all animals studied (n=9) as well as in the different imaging sessions of each cat, suggests that the vertical meridian was well positioned. The presence of residual eye movements (Chow and Lindsley, 1968; Bishop et al., 1971) was controlled by plotting regularly the optic disks, the area centralis and readily identifiable intersections of blood vessels. In this study, the magnitude of these movements, when they occurred, was around 1-2° and the results of the retinotopic mapping were corrected accordingly.

III.2.4.2 Location of the transcallosal cortical maps

In split-chiasm cats, transcallosal activity was localized within the TZ, except in the posterior regions where it also appeared in the regions of A17 and A18 flanking the TZ. In intact animals, the representation of the ipsilateral visual field was also mainly localized within the TZ. These results are consistent with the transcallosal orientation maps presented in chapter 1.

The convergent/divergent pattern displayed by callosal connections in previous studies (Olavarria, 1996), see Figure 11 in General introduction) was not observed in the retinotopic maps obtained in this study. One explanation of the results presented here, i.e. the absence of activated regions outside the TZ for a stimulation of the ipsilateral hemifield, is that these terminals trigger an activity that is too weak or too sparse for detection by optical imaging of intrinsic signals. Another explanation is that the divergent pattern of callosal connections previously reported (Olavarria, 1996) is wider in the posterior part of the visual cortex and more compressed in the anterior part (within the TZ). Indeed, this pattern has been described at an antero-posterior level around stereotaxic coordinate P5 (Olavarria, 1996). In the study reported here, mainly anterior regions of the visual cortex were imaged (from stereotaxic levels P3 to A7 in most cases). In one case (Ca10), the imaged region included more posterior parts of the visual cortex (to P6) and wider transcallosal activity was observed in the posterior region (see Figure 42).
III.2.4.3 Representation of the ipsilateral hemifield and retinotopic organization of callosal connections

The results presented in this study demonstrate the extent of the ipsilateral hemifield representation and of the callosal visual field - the part of the visual field that activates the transcallosal pathway - in the cat visual cortex. Ipsilateral hemifield representation extended to 8° when the contralateral eye was stimulated, in both intact and split-chiasm animals (including one animal studied before and after chiasmotomy). Moreover, in both conditions, the ipsilateral representation appeared as a row of patches of activity along the TZ. This demonstrates that the representation of the ipsilateral visual field is predominantly triggered by transcallosal connections. The similarity of the maps obtained in normal and split-chiasm animals also indicates that the split-chiasm preparation is relevant for analysis of the interhemispheric integration of visual information in cat visual cortex.

III.2.4.3.1 Patchy layout of representation of the ipsilateral hemifield and of the TC activity

The patchy distribution of ipsilateral hemifield representation between +2° and +8°, mainly transferred through the TC pathway, suggests different explanations. A first hypothesis would be that these patches reflect the orientation of the stimulus and would be therefore mainly localized in vertical (vertical bar) and horizontal (horizontal moving grating) orientation domains. Quantitative analysis of the representation of each orientation domain within these patches showed only a small bias toward vertical orientation domains. Furthermore, the variability of the size of these patches cannot be explained by the orientation properties of the stimulus. Finally, with the exception of one cat (Ca10 RH), the geniculo-cortical activity obtained with the same stimulus was not patchy. Variability of the size and of the location of these patches could not be accounted for by the ocular dominance either. Ocular dominance maps were obtained in two animals and no specific relation was observed between the patches and ocular dominance domains.

Alternatively, the ipsilateral hemifield may be represented within patches in the TZ. This hypothesis is supported by electrophysiological and anatomical data. In particular, a previous electrophysiological study performed in intact adult cats described patches along the TZ in which the cells had their receptive field centers located in the ipsilateral visual field (Diao et al., 1990). Small retinotopic maps (around 6 x 4 mm in size) were obtained from recordings made in about 150 closely spaced electrode penetrations in the region of the TZ in intact animals. The characteristics of these patches are strikingly similar to those observed here: the most ipsilateral receptive field centers were positioned at –7.2° from the vertical midline, the linear size of the ipsilateral-field patches varied from about a few micrometers to 1 mm, their inter-distance also varied and ranged from 1.5 to 4 mm (Figure 52). In addition, the cortical coordinates of the ipsilateral field zones, and the location of the visual field represented, varied between animals. Finally, the cells recorded in these patches were strongly dominated by the contralateral eye (particularly for eccentricities at an azimuth beyond +3°). These results and those presented here (on a larger scale) strongly suggest that the
representation of the ipsilateral hemifield is not continuous along the TZ but displays a patchy layout. The size and the location of these patches along the TZ differ between animals. This organization in concentric patches explains the fact that the position of the patches in the optically imaged retinotopic maps did not change according to the azimuth of the stimulus in the ipsilateral visual field: only patches already present at +2° disappeared progressively for increasing eccentricities (see Figure 45 and 46).

Figure 52. Retinotopic maps obtained from recordings made in almost 150 closely spaced electrode penetrations in the TZ, in normal adult animals. Different gray levels were used to show regions of constant receptive field (RF) azimuth. Zero represents the vertical meridian and negative values represent the ipsilateral hemifield. Bottom. Three examples of the distribution of ipsilateral visual field patches in the TZ. Each black patch includes the representation of the ipsilateral hemifield beyond +1° (up to 7.2°). Dashed lines represent iso-azimuthal contours. The anterior part of the brain is on the right and the medial part on the top. Scale bar, 1mm. Modified from Fig. 1 and 4 in (Diao et al., 1990). The characteristics of these patches strikingly resemble those observed in the present study (compare with Figure 45 and Figure 46).

The similarity of the layout of ipsilateral hemifield representation in split-chiasm and intact animals indicates that this representation is mainly transferred by transcallosal connections. This suggests that terminals of callosal connections transferring ipsilateral hemifield representation are clustered in patches within the TZ. A patchy distribution of callosal connections within the TZ has been reported in several anatomical studies: at the level of individual axons displaying terminal clusters with diameters of 300-600 µm (Houzel et al., 1994; Aggoun-Zouaoui et al., 1996) and at the level of the population of callosal terminals with patches of various size comprised between 500 µm and 1.5 mm (Berman and Payne, 1983; Innocenti, 1986a; Voigt et al., 1988; Boyd and Matsubara, 1994). The results presented in this study provide a functional explanation for this specific distribution: the callosal connections involved in ipsilateral hemifield representation are clustered. This does not exclude the possibility that callosal connection terminals are also organized with respect to other functional properties of the visual cortex (e.g. orientation or ocular dominance.
domains). Indeed, the population of callosal terminals may be clustered in rather large patches corresponding to the representation of the ipsilateral hemifield and, within these patches, the terminals of single-callosal axons may also display a patchy pattern corresponding to ocular dominance or orientation domains. This point is analyzed in the third chapter, at the level of individual callosal axons.

Finally, it should be noted that the patchy layout described above concerns only the transcallosal activity involved in ipsilateral hemifield representation beyond +2°. Transcallosal activity was also observed throughout the TZ, with a uniform or only slightly patchy distribution when the visual stimulus was located in the central vertical meridian and at +1° (see Figure 40). This finding is consistent with the continuous orientation maps of transcallosal activity obtained in Chapter 1 in which TC activation was observed along the whole antero-posterior extent of the TZ with no obvious interruption. Indeed, the visual stimuli used to obtain such maps were full-field oriented gratings stimulating a large part of the visual field of the animal including the central meridian and at least 15° of the ipsilateral hemifield.

III.2.4.3.2 Extent of ipsilateral hemifield representation in mammals

The results presented here indicate that a portion of the ipsilateral hemifield (8°) is represented in the TZ of the cat visual cortex, in agreement with previous electrophysiological data (Whitteridge and Clarke, 1982; Pettigrew and Dreher, 1987; Diao et al., 1990; Payne, 1990b; Milleret et al., 1994; Milleret et al., 2005). One of these studies described patches of cortex in which cells had receptive field centers located up to 7.2° into the ipsilateral visual field, in normal adult cats (Diao et al., 1990). In split-chiasm cats, the centers of the receptive fields of transcallosally activated units reached an azimuth of 9° into the ipsilateral hemifield (RF lateral limits at 13°) (Milleret and Houzel, 2001; Milleret et al., 2005).

The extent of the ipsilateral hemifield representation observed in the study reported here is therefore similar to the extent of receptive fields recorded in the TZ at similar stereotaxic levels in previous studies (Payne, 1990b). The centers of the corresponding receptive fields reach to –2.5° (RFs extend to –3.6°) on the 0° horizontal meridian, represented around stereotaxic level P5-P6, and they reach to more than 10° (RFs extend to beyond 20°) towards the periphery of the visual field, around stereotaxic level A12 (Payne, 1990b). According to the hourglass shape of the ipsilateral representation described above, the representation of the most lateral eccentricities should have appeared on retinotopic maps in anterior regions of the visual cortex (where the lower part of the visual field is represented). However, the part of the TZ that could be imaged was in most cases restricted to stereotaxic levels P5-A6 because beyond stereotaxic level A6, the TZ was obscured in the interhemispheric fissure. Nevertheless, in three cases (Ca15, Ca10 (RE), Ca17), the representation of the most lateral eccentricities evoking an activity (+6°, +7°) was located mainly in anterior regions (around AP0) of the area activated by ipsilateral stimuli (the most anterior patches).
Ipsilateral visual hemifield representation has frequently been associated with albino or pigment-deficient cats (Hubel and Wiesel, 1971; Shatz, 1977b; Shatz and LeVay, 1979; Ault et al., 1995), but it has also been found in the visual cortex of other species: mouse (10°, Drager, 1975; Wagor et al., 1980), hamster (10°, Tiao and Blakemore, 1976), guinea pig (10-15°, Choudhury, 1978), sheep (at least 15°, Clarke and Whitteridge, 1976; Pettigrew et al., 1984), opossum (6-8°, Volchan et al., 1988), tree-shrew (15° Bosking et al., 2000), ferret (15°, Law et al., 1988; White et al., 1999), marmoset monkey (8°, Fritsches and Rosa, 1996), baboon (+2°, Kennedy et al., 1985) and rhesus monkey (1-2°, Van et al., 1982).

### III.2.4.4 Contribution of each hemiretina to ipsilateral hemifield representation and activation of the transcallosal pathway

This study investigated the contribution of each hemiretina to transcallosal pathway activation. The similarity of the maps obtained for split-chiasm and intact animals with the stimulation of the ipsilateral hemifield through the contralateral eye indicates that this ipsilateral representation is triggered predominantly by the transcallosal pathway. In split-chiasm animals the only possible pathway that triggers the interhemispheric transfer of ipsilateral representations is that involving uncrossed projections from the temporal retina of the contralateral eye (Figure 39, B1). Comparisons with intact animals suggest that the same pathway is activated under normal conditions (Figure 39, A1). Crossed projections from the temporal retina therefore seem to play a limited role in representation of the ipsilateral field (Figure 39, A2, right hemisphere).

These results are consistent with the demonstrated transcallosal contribution of the contralateral eye to the representation of ipsilateral visual fields in the TZ (reviewed in Payne, 1990b; see also Berlucchi and Rizzolatti, 1968; Leporé and Guillemot, 1982; Milleret et al., 1994; Milleret et al., 2005). They are also in accordance with the observation that, within the TZ, callosal neurons are preferentially correlated with contralateral ocular dominance domains (Olavarria, 2001). However, these data do not corroborate the hypothesis that crossed projections from the temporal retina play a substantial role in ipsilateral hemifield representation (Whitteridge and Clarke, 1982; Payne, 1990b; Olavarria, 2001; Olavarria, 2002). If this were the case, then optic chiasm sectioning would strongly affect ipsilateral field representation in the hemisphere contralateral to the stimulated eye (the right hemisphere in this study). Instead, the maps obtained in split-chiasm animals displayed similar characteristics to those obtained in intact animals. It is possible that this crossed pathway from the temporal retina triggers activity too weak to be distinguished from TC activity with this method of investigation. It also remains possible that ipsilateral field representation in intact animals is mediated principally through this crossed pathway (from the temporal retina) and that, in certain circumstances, such as lesions of the optic chiasm, the transcallosal pathway is activated to compensate for the effect of the lesion. Finally, if these crossed projections from the temporal retina of the stimulated eye would be involved in ipsilateral field representation, they could, in principle, trigger TC activation in the opposite hemisphere (Figure 39, A2, left hemisphere). In the study reported here, this potential activity would overlap with the direct
GC activity from the temporal retina, and therefore could not be detected. One way to reveal this activity would be to cut the ipsilateral optic tract and to observe activity in the ipsilateral hemisphere in response to visual stimulation in the contralateral hemifield.

The results of this study also indicate that stimulation of the ipsilateral hemifield through the ipsilateral eye leads to weak activity in the TZ in intact animals only. Thus, crossed projections from the nasal hemiretina (Figure 39, A4) can trigger some TC activation. This pathway transfers the representation of a smaller part of the ipsilateral hemifield (up to 2-4°) than the uncrossed projections from the temporal hemiretina (up to 8°) (Figure 39, A1). Indeed, callosal neurons within the TZ are preferentially correlated with contralateral ocular dominance domains indicating a limited transcallosal contribution of the ipsilateral eye to ipsilateral visual field representation in the TZ (Olavarria, 2001). The same contralateral dominance appeared in electrophysiological recordings of cells within patches of ipsilateral field representation. These cells were dominated by the contralateral eye and this dominance was more pronounced for eccentricities of the stimulus beyond 3° (Diao et al., 1990). Thus, in accordance with the present data, the ipsilateral eye can trigger the representation of a part of the ipsilateral visual field up to 3-4°.

Unlike the crossed projections from the nasal hemiretina, uncrossed projections from the same nasal retina (Figure 39, A3, B2) provide either no activation or activation too weak to be detected by optical imaging of intrinsic signals. This can be explained by the observation that the nasal retina of the ipsilateral eye contains only a few cells that project to the ipsilateral side of the brain (Leventhal, 1982; Murakami et al., 1982; Jacobs et al., 1984). However, electrophysiological studies in split-chiasm cats have shown that transcallosal connections can activate cells with receptive fields extending up to 6° into the contralateral hemifield (RF centers at 4°) (Milleret and Houzel, 2001; Milleret et al., 2005). Thus, uncrossed projections from the nasal retina may participate in ipsilateral hemifield representation in the ipsilateral hemisphere, providing a weak activity undetectable with this imaging technique.

In conclusion, this study indicates that representation of the ipsilateral portion of the visual field (up to 8°) is transferred mainly through the CC, activated by uncrossed projections from the temporal retina. Crossed projections from the nasal retina can also activate the CC, thereby participating in the ipsilateral hemifield representation, but to a lesser extent (to 2-4°). Furthermore, these results show that this ipsilateral part of the visual field (beyond +2°) is represented within patches along the transition zone between A17 and A18.
III.3 Chapter 3. Functional specificity of individual callosal axons in cat visual cortex

III.3.1 Summary

The functional selectivity of intrahemispheric cortico-cortical connections within A17 and A18 has been investigated in numerous studies. By contrast, the functional selectivity of interhemispheric connections remains poorly understood. This functional selectivity was investigated in this study at the level of individual callosal axons. The distribution of callosal axon terminals was studied on functional maps (retinotopic, orientation, direction and ocular dominance) of the visual cortex, by using optical imaging of intrinsic signals and extracellular neuronal tracer injections in normal adult cats. The distributions of the synaptic boutons of eight individual callosal axons were reconstructed in three-dimensions and compared with functional maps of the target zones as well as with the functional preferences at the injection sites. All callosal axons displayed a clustered termination pattern, in the lower half of layer III and the upper part of layer IV, although some collaterals were also found in layers V/VI.

These results confirmed the visuotopic organization of callosal connections and demonstrated that these connections mainly link cortical regions with the same orientation preference. The analysis of two callosal axons with respect to direction preference maps suggested that these callosal connections were less selective for direction than for orientation. All callosal axons reconstructed in this study preferentially connected binocular regions. These results support the hypothesis that callosal connections are part of the functional network involved in the processing of orientation information of visual stimuli in the central part of the visual space. This orientation selectivity, in addition to corresponding visuotopy, may be a source of interhemispheric correlated activity that would influence the stabilization of callosal connections during development.
### III.3.2 Introduction

Callosal connections are not homogeneously distributed in cat visual cortical areas 17 and 18, and instead form a patchy pattern in adult animals (see General introduction §3.4.2). This patchy distribution has been described at the level of individual axons (Houzel et al., 1994; Aggoun-Zouaoui et al., 1996) and at the level of the population of callosal terminals (Berman and Payne, 1983; Innocenti, 1986a; Voigt et al., 1988; Boyd and Matsubara, 1994). To obtain a better understanding of the functional role of the visual callosal pathway, it is useful to determine how the observed clusters relate to the functional organization of the visual cortex. Do callosal connections link similar functional domains? In this regard, it has been observed that both callosal neurons and callosal terminals are densely packed within cytochrome oxidase-dense domains of the visual cortex of cats (Boyd and Matsubara, 1994) and macaques (Olavarria and Abel, 1996). Furthermore, periodicities in the distribution of callosal neurons are correlated with the pattern of ocular dominance columns (Olavarria, 2001). The results presented in the second chapter of this thesis suggest an additional functional explanation for this specific distribution: these clusters (or patches) may correspond to the target zone of callosal connections involved in the representation of the ipsilateral hemifield. This explanation does not rule out the possibility that callosal connection terminals are also organized with respect to other functional properties of the visual cortex, at the level of single-callosal axons.

The distribution of callosal axons with respect to orientation preference maps remains unknown. So far, two studies have investigated the distribution of callosal neuron somata (retrogradely labeled) and have yielded opposite results. In the study of Schmidt et al. (Schmidt et al., 1997), callosal connections were found to link similar orientation domains in strabismic cats. However, a major drawback of this study is that the results cannot be directly extended to normal cats because the distribution and properties of the callosal axons of strabismic animals may differ considerably from those of normal cats (Innocenti and Frost, 1979; Berman and Payne, 1983; Elberger et al., 1983; Milleret and Houzel, 2001). In the other study, callosal connection terminals were not correlated with particular orientation domains in the visual cortex of the normal adult tree shrew (Bosking et al., 2000). Instead, these connections appeared to link visuotopically corresponding sites, suggesting that visuotopy is the primary factor constraining their distribution.

In the study described here, the distribution of the synaptic boutons of individual callosal axons was investigated on the orientation map of the visual cortex of normal adult cats. Optical imaging of intrinsic signals was combined with small extracellular injections of neuronal tracers (mainly anterograde). The orientation preference of the injection sites was compared with the distribution of the callosal axon terminals in orientation maps of the target zones. All callosal axons studied preferentially connected domains with the same orientation preference as that of the injection site. The relations of these axon terminal distributions to visuotopy and ocular dominance maps were also analyzed.
### III.3.3 Results

Eight callosal axons from four adult cats were reconstructed in three dimensions. The distribution of their synaptic boutons was analyzed with regard to orientation maps and was compared with the orientation preference at the injection site, in the opposite hemisphere.

#### III.3.3.1 Morphology of the reconstructed callosal axons

The morphological characteristics of the eight callosal axons are summarized in Table 2. These axons were selected in the superficial histological sections (horizontal plane) where most terminals are located, in order to select ones with well-labeled synaptic boutons (see for example Figure 31 in Material and methods). These axons were then followed down to 317-722 µm within the white matter and then reconstructed with all of their branches from the white matter to the cortical surface. Their total length (including all branches) varied from 11.2 to 31.2 mm. The total number of synaptic boutons ranged between 307 and 766. Most of the synaptic boutons of the eight callosal axons were systematically located in the lower part of layers II/III (Table 2, Figure 53, Figure 54) i.e. in layer III. In three axons, approximately one third of the boutons was located in layer IV. A few boutons were also found in layer V/VI.

<table>
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<tr>
<th>CAT</th>
<th>Age (months)</th>
<th>Days of tracers transport</th>
<th>Axon number</th>
<th>Synaptic boutons</th>
<th>Total length µm</th>
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<td>8</td>
<td>674</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 2. Experimental parameters and morphological characteristics of the eight reconstructed callosal axons. RH, right hemisphere; A17, area 17; A18, area 18; A, anterior; P, posterior; M, medial; L, lateral.

In a plane parallel to the cortical surface (horizontal plane), the eight axons reconstructed in this study presented two or three clusters of synaptic boutons (Figure 53, Figure 54). These clusters were in most cases sharply separated except in axon 8 where they were adjacent. Typically, a single callosal axon formed two clusters (average diameter 494±146 µm) showing an average lateral spacing of 978±327 µm in the horizontal plane.
Figure 53. Three-dimensional reconstructions of callosal axons (in right hemispheres). A, B, C. Frontal views of axons 1 and 2 (cat Ca06), axon 3 (cat Ca07) and axon 8 (cat Ca09). The limits of cortical layers II/III, IV, V/VI and of the white matter (WM) are indicated. The arrows on the top of each panel point the location of the 1-mm-wide transition zone (TZ) between A17 (on the right) and A18 (on the left). These limits (cortical layers and TZ) are those observed in one frontal section at an antero-posterior level corresponding approximately to the center of the callosal axon’s extent in the horizontal plane (axons 1, 2: P2; axon 3: A1; axon 8: A3). D, E, F. Horizontal views (in a plane parallel to the cortical surface) of the same axons. A, anterior; D, dorsal; M, medial. Scale bar 500 µm.
Figure 54. Three-dimensional reconstructions of callosal axons. A, B, C. Horizontal, frontal and sagittal views of axons 4, 5, 6 and 7 of cat Ca09. Same legend as in Figure 53. Scale bar 500 µm.
In a frontal plane, these axons presented different types of architecture. These types correspond to the three main architectures of callosal axons that have previously been described in the cat visual cortex (Houzel et al., 1994). According to this classification, one axon displayed a parallel architecture (axon 3), characterized by the formation, usually in the white matter, of branches of comparable length that run parallel to each other in the frontal plane and supply different clusters. One axon displayed a serial architecture (axon 1) characterized in the frontal plane by a tangentially running trunk with radial collaterals to the cortex. The last six axons displayed a mixed parallel-and-serial architecture (axons 2, 4, 5, 6, 7, 8).

III.3.3.2 Visuotopy of callosal axons

Small extracellular injections of neuronal tracers were performed in the left hemisphere of four animals in cortical regions around stereotaxic coordinates A0-A1.2 and L1.7-L3 (Table 3). These sites were thus located in regions where the central lower part of the visual field was represented (Tusa et al., 1978; Tusa et al., 1979): azimuth between 0° and +5° and elevation between −5° and −15° (Table 3). These values were confirmed in three cases (Ca07, Ca09, Ca15) by the receptive fields location of the units recorded before the injection, via the same glass pipettes containing the tracers (Figure 55). In addition, another confirmation came in two cases (Ca09, Ca15) from the position of the injection sites in optically imaged retinotopic maps (Figure 56).

<table>
<thead>
<tr>
<th>CAT</th>
<th>visual area</th>
<th>Stereotaxic coordinates</th>
<th>azimuth</th>
<th>elevation</th>
<th>OD</th>
<th>Axon number</th>
<th>Terminates area</th>
<th>Stereotaxic coordinates</th>
<th>azimuth</th>
<th>elevation</th>
<th>OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA06</td>
<td>TZ</td>
<td>A0-L3</td>
<td>-5°/0°</td>
<td>0°/5°</td>
<td>bino/ipsi</td>
<td>1</td>
<td>A17</td>
<td>P3/P5-L1.5/L3</td>
<td>-5°/0°</td>
<td>0°/5°</td>
<td>bino/ipsi</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>A17</td>
<td>P1/P2-L0/L2</td>
<td>-5°/0°</td>
<td>0°/5°</td>
<td>bino</td>
</tr>
<tr>
<td>CA07</td>
<td>TZ</td>
<td>A0-L3</td>
<td>0°/+2°</td>
<td>-2°/−5°</td>
<td>no map (bino)</td>
<td>3</td>
<td>T3</td>
<td>A1.5/A2-L2/L3</td>
<td>0°/+2°</td>
<td>-1.4°</td>
<td>bino/ipsi</td>
</tr>
<tr>
<td>CA09</td>
<td>TZ</td>
<td>A2-L2</td>
<td>-1°/+1°</td>
<td>-14°/-16°</td>
<td>bino</td>
<td>4</td>
<td>T3</td>
<td>A1.5/2.5-L2/L3,3.5</td>
<td>-1°/+3°</td>
<td>-16°</td>
<td>bino</td>
</tr>
<tr>
<td></td>
<td>TZ/A17</td>
<td>A0.5-L2</td>
<td>+1°/+3°</td>
<td>-11°/-14°</td>
<td>bino/ipsi</td>
<td>5</td>
<td>T3/A17</td>
<td>A1/2.5-L2/L2.5</td>
<td>-1°/+1°</td>
<td>-16°/-17°</td>
<td>bino</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>6</td>
<td>T3</td>
<td>A0/A1.5-L2/L3,5.5</td>
<td>-1°/+1°</td>
<td>-14°</td>
<td>bino</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>T3/A17</td>
<td>A0.5/A2.5-L2/2.5</td>
<td>-2°/+4°</td>
<td>-14°/-16°</td>
<td>bino/ipsi</td>
</tr>
</tbody>
</table>

Table 3. Visuotopy and ocular dominance (OD) of callosal connections. For OD analysis, the ocular dominance was divided into seven categories as it was introduced by Hubel and Wiesel (Hubel and Wiesel, 1962). The abbreviation “bino” (binocular) indicates that most of the labeled cell somata at the injection sites or of the callosal synaptic boutons in the target zone were located in the forth category. In some cases, these distributions showed another smaller peak either in the third category or in the fifth category, indicated by the abbreviations “/contra” and “/ipsi” respectively. In two cats (Ca07 and Ca15), no ocular dominance maps were recorded in the region of the injection sites (“no map”). However, electrophysiological recordings indicated that both eyes could activate neurons at these sites (“bino”). The dominance of either eye was not quantified. LH, left hemisphere, RH, right hemisphere; A17, area 17; A18, area 18; TZ, transition zone between areas 17 and 18; A, anterior; L, lateral, P, posterior.
In the opposite hemisphere, the visuotopic locations of the reconstructed callosal axons were also determined. The callosal terminals of each axon were located in regions corresponding to the representation of the same part of the visual field as that represented at the corresponding injection site (azimuth between 0° and +6°; elevation between 0° and –15°) (Table 3). These values were determined from the stereotaxic coordinates of the callosal axons and, in all but one case (Ca06), from the location of these axons in optically imaged retinotopic maps (Figure 56 and Figure 59). Only stereotaxic coordinates were available for Ca06 because no retinotopic maps were recorded.

Another argument supporting the visuotopic organization of callosal connections comes from the experiment (Ca09) in which two different tracers were injected at nearby locations (see Figure 29 in Material and methods). In the opposite hemisphere, labeled axons coming from each injection site overlapped but were precisely shifted in the same direction and with the same distance that separated their respective injection sites (Figure 56A).

The respective locations of injection sites and callosal axons within areas 17 and 18 and the transition zone (TZ) were also compared (Table 3). As in the preceding chapters, the TZ was localized functionally in optically imaged activity maps based on the different spatial and temporal frequency preferences of A17 and A18 neurons. In addition, the location of the TZ was confirmed in all cases except one (Ca06) by imaging the representation of the ipsilateral visual field (see for example Figure 59).

For two injection sites, the non-mirror symmetric pattern of callosal connections described in a previous study (Olavarria, 1996) was observed. In one cat (Ca06), the tracer injection was made in the TZ and resulted in labeled axons in the contralateral A17.
another cat (Ca15), the injection in A18 gave rise to labeled callosal axons in the TZ. In accordance with this divergent/convergent pattern, the axon with the broadest terminal arbors (antero-posterior and medio-lateral extent, Table 2) was one located in A17 (axon 2, Ca06) whereas the most compact axon (axon 8, Ca15), with closely neighboring clusters, was located inside the TZ (Table 3). However, in the two other animals (Ca07, Ca09), the non-mirror symmetric pattern did not clearly appear since injections within the TZ labeled axons located within the TZ in the opposite hemisphere.

Figure 56. Visuotopy of callosal axons, cat Ca09. A. Blood vessel pattern of the imaged region of cat Ca09 for retinotopic maps. Two different tracers were injected at nearby locations (red and blue circles) in the left hemisphere (LH). In the opposite hemisphere (right hemisphere, RH), labeled axons coming from each injection site overlapped but were precisely shifted in the same direction and with the same distance that separated their respective injection sites. B. C. Overlap of callosal axons with single condition retinotopic maps for horizontal eccentricity (azimuth). The azimuth of the visual stimulus (vertical bar) is indicated on the right (0° for LH and +1° for RH). D. Overlap of callosal axons with single condition retinotopic maps for vertical eccentricity (elevation). The elevation of the visual stimulus (horizontal bar) is indicated on the side of each map (from −12° to −22°). The anterior part of the cortex is on the right. The thick horizontal dashed line shows the stereotaxic midline; thin dashed lines show the limits of the transition zone between A17 and A18. Short horizontal line on the bottom of each panel shows stereotaxic coordinate AP0. LH, left hemisphere; RH, right hemisphere; A, anterior; D, dorsal; M, medial, P, posterior. Scale bar 2mm.
III.3.3.3 Orientation selectivity of callosal neurons

In each case, the orientation preference and distribution of the labeled somata at the injection sites (left hemispheres) were compared to the orientation preference and distribution of axon terminals of the corresponding labeled callosal axons (right hemispheres).

III.3.3.3.1 Injection sites (left hemispheres)

The diameter of the injection cores varied from 230 to 393 µm (Figure 57B, 3G). In 4 of 5 cases the orientation preference of the injection site was evaluated and confirmed as follows. First, it was determined from the distributions in the different orientation domains of image pixels within the regions of the injection cores (white circle in Figure 57B, 3G), using 22.5° binning (Figure 57D, 3H). The orientation preference of the domain containing the majority of the pixels was defined as the orientation preference of the injection site and was confirmed by the two following approaches. Second, the orientation preference distributions of the labeled neuronal somata (Figure 57C, 3F) were determined. For two injection sites (Ca06, Ca09a) more than 48% of the labeled somata were located in one type of orientation domain and less than 17% of the other somata were in other orientation domains. For two other sites (Ca09b, Ca15), more than 51% of the boutons were observed in two domains with close orientations (± 22.5°) and less than 10% of the other somata were in other orientation domains. These orientation preference distributions were in all cases very close to that of the pixels within the injection cores (Figure 57).

Finally, the orientation preference was also confirmed by multi-unit electrophysiological recordings via the same glass pipette as the one used for iontophoretic injection of the neuronal tracers. The orientation preference corresponded to the one determined by the two previous methods, with ± 12°. In the last case (Ca07), a necrosis of the cortical surface prevented us for imaging the orientation map of the injected hemisphere. The orientation preference was therefore established only on the basis of the electrophysiological recordings.

III.3.3.3.2 Callosal axon terminals (right hemispheres)

The distribution of the callosal axon terminals in terms of orientation preference showed that these synaptic boutons were mainly located in orientation domains with the same orientation preference as that of the corresponding injection site. Figure 58 shows two examples of axons (with two clusters) connecting similar orientation domains (0° for Ca06, 112.5° for Ca09). To compare the eight reconstructed axons, orientation preferences were expressed relatively to that of the injection sites using 22.5° resolution. The number of synaptic boutons was expressed as a percentage of the total number of boutons for each axon (Figure 58B). The eight distributions were significantly centered on 0° orientation difference [V-test (modified Rayleigh test), V>2.32; p<0.01]. In all axons, no significant difference was observed between the distributions of club-like and en-passant boutons.
Figure 57. Analysis of the orientation preference at the injection sites (left hemispheres) in cat Ca06 (A-D) and Ca15 (E-H). A and E. Light microscopic image of the injection site (BDA). B and F. Distribution of the labeled neuronal somata (in black) superimposed on orientation preference map (the preferred angle of a region is color-coded as indicated in the half circle). The injection core is indicated by a white circle. C and G. Number of labeled neuronal somata in the orientation domains. D and H. Number of pixels in the orientation domains within the injection core. A, anterior; L, lateral. Scale bar, 1mm (B and F); 500µm (A and E).
The distribution of the synaptic boutons regarding orientation preference was also divided according to iso- (± 30°), oblique- (± 30-60°) and cross- (± 60-90°) orientation categories with respect to the orientation preference of the injection sites. The eight callosal axons connected preferentially iso-orientation domains with, in average, 72 % (standard deviation=17) of their synaptic boutons (Figure 58C). The percentages of synaptic boutons of the eight callosal axons in iso-, oblique- and cross-orientation domains are indicated in Table 4. All of them had more than 50% of their synaptic boutons in iso-orientation domains but some appeared much more selective (axons 1, 3, 7 with more than 83%) than others (axons 2 and 5 with 50 and 51%). This difference was explained neither by a specific morphology of the axons, their size, nor the location of the corresponding injection site (relatively to pinwheel centers). However, the two axons with the lowest percentages also have the lowest number of synaptic boutons. Although the quality of their labeling was not different from that of the other axons, it is possible that some branches were not labeled or were not reconstructed because they were located too deep in the white matter.

III.3.3.3.3 Retrogradely labeled somata of callosal neurons (right hemispheres)

Retrogradely labeled neuronal somata of callosal axons were observed in the hemisphere contralateral to the injection sites. Their number ranged between 3 and 24. In the case of Ca15, only three labeled somata were observed: each one located in each of the three orientation preference categories (iso-, oblique-, cross-). In the other three cases (Ca06, Ca07 and Ca09), the labeled somata were mainly located in iso-orientation domains (68.5% in mean, standard deviation=29) (Figure 58E, Table 4). In the case of the highest number of labeled somata (Ca07, n=24), all of them (100%) were in iso-orientation domains (Figure 58D and E, Table 4): the distribution of the labeled somata presented a significant bias toward 0° orientation difference [V-test (modified Rayleigh test), V>2.31; p<0.01]. The other two distributions (Ca06 and Ca09) did not reach statistical significance (V-test, V=0.25 and 0.63; p>0.1), most likely because of the small number of labeled neurons.

<table>
<thead>
<tr>
<th>CAT</th>
<th>Injection site (LH)</th>
<th>Orientation selectivity of the callosal synaptic boutons (RH)</th>
<th>Orientation selectivity of the retrogradely labeled somata of callosal neurons (RH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>orientation preference</td>
<td>Axon Number</td>
<td>Iso- (%)</td>
</tr>
<tr>
<td>CA06</td>
<td>0°</td>
<td>1</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>90°</td>
<td>3</td>
<td>91</td>
</tr>
<tr>
<td>CA07</td>
<td>90°</td>
<td>4</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>51</td>
<td>18</td>
</tr>
<tr>
<td>CA09</td>
<td>6</td>
<td>61</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>92</td>
<td>2</td>
</tr>
<tr>
<td>CA15</td>
<td>112.5°</td>
<td>8</td>
<td>77</td>
</tr>
</tbody>
</table>

Table 4. Orientation selectivity of callosal connections. Percentages of the eight callosal axon terminals and of the retrogradely labeled somata of callosal neurons in iso- (± 30°), oblique- (± 30-60°) and cross- (± 60-90°) orientation domains with respect to the orientation preference of the injection sites.
Figure 58. Orientation selectivity of callosal connections. A. Orientation preference maps with the location of the injection core (white circle) in the left hemisphere and the distribution of the synaptic boutons (in black) of a corresponding callosal axon in the right hemisphere (axon 1 in cat Ca06, axon 7 in cat Ca09). B. Distribution of the synaptic boutons of the callosal axons in the orientation domains. Orientation preferences were expressed relative to that of the injection sites using 22.5° resolution (0° corresponds to an orientation preference similar to that of the injection site; 90° corresponds to an orientation preference orthogonal to that of the injection site). The number of boutons was expressed as a percentage of the total number of boutons for each axon. The eight distributions have their pick at 0 ± 22.5°. The mean distribution is indicated by a black dashed line. The thin horizontal dotted line indicates the percentage of boutons that would be expected for an even distribution. C. Percentages of synaptic boutons of callosal axons in iso- (± 30°), oblique- (± 30-60°) and cross- (± 60-90°) orientation domains with respect to the orientation preference of the injection sites. The eight callosal axons connected preferentially iso-orientation domains with, in mean, 72 % (standard deviation=17) of their synaptic boutons. Error bars indicate standard deviation. D. Distribution of the retrogradely labeled somata of callosal neurons (in black) superimposed on orientation preference maps of the right hemisphere of cats Ca07 and Ca06. E. Number of retrogradely labeled somata of callosal neurons in the orientation domains. Orientation preferences were expressed relative to that of the injection sites. The mean distribution is indicated by a black dashed line. Scale 500µm. A, anterior; M, medial; L, lateral.
III.3.3.4 Patchy representation of the ipsilateral visual field and orientation selectivity of callosal terminals

We have seen in the second chapter of this thesis that a part of the ipsilateral visual field (up to 8°) was represented within patches along the TZ. One axon reconstructed in this study ended in one of these patches (axon 8, cat Ca15). As all the other axons, the synaptic boutons of this axon were furthermore mainly located in orientation domains with the same orientation preference as that of the corresponding injection site (in this case, 67.5°). Figure 59 shows how this axon relates to both the “ipsilateral visual field” patch and the specific orientation domains. Within the patch activated for the visual stimulus at 4° in the ipsilateral visual field, the axon’s terminals are located in a specific orientation domain.

Figure 59. Visuotopy and orientation selectivity of axon 8 (cat Ca15). The distribution of the synaptic boutons (black or white dots) of axon 8 was superimposed on three single-condition retinotopic maps and one orientation map of the same cortical region. For each retinotopic map, the azimuth of the visual stimulus (vertical bar) is indicated on the right (+1°, +2°, +4°). Black arrows point patches of activity at +2°. The anterior part of the cortex is on the right and the stereotaxic midline on the top of each panel. Thin dashed lines show the limits of the transition zone between A17 and A18. Short horizontal line on the top of each panel shows stereotaxic coordinate AP0. Scale bar 1mm.
III.3.3.5 Direction selectivity of callosal neurons

Direction preference maps were imaged in both hemispheres of one cat (Ca15) and in the right hemisphere of a second cat (Ca07) (Figure 60). For Ca15, the injection site was located in a domain with a direction preference of 315° (Figure 60A). For Ca07, no direction and orientation maps could be recorded but the orientation preference, determined by electrophysiology, was 90° at the injection site.

These values were compared with the distribution of the callosal axon terminals in the direction domains in the opposite hemisphere. These distributions showed either three or four peaks: at 135°, 270°, 315° for axon 8 (Ca15) and at 0°, 180°, 225°, 315° for axon 3 (Ca07) (Figure 60B, 5C). This suggests that callosal axons do not connect the direction preference domains with the same specificity as that for orientation domains. A majority of synaptic boutons had a direction preference orthogonal to the preferred orientation of each axon (67.5° for axon 8 and 90° for axon 3, Table 4). For Ca15, most of the synaptic boutons were located in a domain with a direction preference of 135° - the opposite direction preference to that at the injection site (315°).

III.3.3.6 Ocular dominance selectivity of callosal neurons

Ocular dominance maps were imaged in the left hemispheres of two cats (Ca06 and Ca09) and in the right hemispheres of all cats studied. Concerning the injection sites region of the two cats (Ca07 and Ca15) in which no ocular dominance maps were recorded, electrophysiological recordings indicated that both eyes could activate neurons at these sites. The dominance of either eye was not quantified. In the two other animals, injection sites were located either at the periphery of an ipsilateral eye domain (Ca06) or in a binocular one (Ca09) (Figure 61A and C). For the analysis, the ocular dominance was divided into seven categories as introduced by Hubel and Wiesel (Hubel and Wiesel, 1962). Most of the labeled cell somata at the injection sites were located in the forth and fifth category for Ca06 and only in the forth category for Ca09 (Figure 61A and C, Table 3). The tracers were injected blindly with regard to orientation or ocular dominance domains. Because the orientation selectivity of callosal axons was the main aim of this work, the reconstructed axons were labeled by injection sites that were clearly located within an orientation domain. In these selected cases, it appears that injection sites were not within the middle of an ocular dominance domain but rather in binocular regions.

Most of the synaptic boutons of all eight axons were also in the forth category (Figure 61B and D, Table 3) indicating that these axons connect preferentially binocular cortical regions. Some of the distributions of the synaptic boutons showed another smaller peak either in the third category or in the fifth category (axons 1, 3 and 8). In axon 1, these two peaks showed the same ocular dominance preference (binocular/left eye) as that of the injection site (Figure 61A-B). In case of the other two axons (axons 3 and 8), no ocular dominance maps
were recorded in the region of the injection sites but electrophysiological recordings indicated that both eyes could activate neurons at these sites.

Figure 60. Direction selectivity of callosal connections. A. Distribution of the labeled neuronal somata (in black) superimposed on direction preference map in the left hemisphere of cat Ca15. The preferred direction of a region is color-coded as indicated in the circle, on the right of the panel. The injection core is indicated by a white circle. The graph shows the number of labeled neuronal somata in the direction domains. B. Distribution of the synaptic boutons (in black) of the corresponding callosal axon in the direction map of the right hemisphere (axon 8, cat Ca15). The graph shows the number of synaptic boutons in the direction domains. C. Distribution of the synaptic boutons (in black) of the callosal axon 3 (cat Ca07) in the direction map of the right hemisphere. The graph shows the number of synaptic boutons in the direction domains. Scale 1 mm. A, anterior; M, medial; L, lateral.
Figure 61. Ocular dominance selectivity of callosal connections. A. Ocular dominance (OD) map with the location of the injection core (white circle) in the left hemisphere of cat Ca06. In all OD maps of the figure, darker regions correspond to domains preferentially activated by the left eye (LE). The graph indicates the number of pixels, within the injection core, in the classical seven categories of ocular dominance. This injection labeled the axon shown in panel B. B. Distribution of the synaptic boutons (in black) of callosal axon 1 in the OD map of the right hemisphere of cat Ca06. The graph on the right indicates the number of synaptic boutons in the seven OD domains. C. Ocular dominance map with the location of the injection cores (white circles) in the left hemisphere of cat Ca09. Top. The graph indicates the number of pixels, within the injection core indicated by the arrow, in the OD domains. This injection labeled the callosal axons 4 and 5. Bottom. The graph indicates the number of pixels, within the injection core indicated by the arrow, in the OD domains. This injection labeled the callosal axons 6 and 7. D. Distribution of the synaptic boutons (in black) of callosal axons 4, 5, 6, 7 in the OD map of the right hemisphere (cat Ca09). The graphs on the right indicate the number of synaptic boutons in the seven OD domains. A, anterior; M, medial; ipsi, eye ipsilateral to the imaged hemisphere; L, lateral; RE, right eye. Scale 1 mm.
III.3.4 Discussion

This study investigated the functional selectivity of eight axons connecting visual areas 17 and 18 of each cerebral hemisphere through the corpus callosum. All these axons presented two or three clusters of synaptic boutons in a plane parallel to the cortical surface. The main finding was that callosal axons connect regions of similar orientation preference, with a visuotopic correspondence between both injection sites and axon projection zones. An analysis of two callosal axons with respect to direction preference maps indicated that these callosal connections were less selective for direction than for orientation. Finally, all callosal axons reconstructed in this study preferentially connected binocular regions.

III.3.4.1 Methodological considerations

III.3.4.1.1 Labeling of callosal axons

The quality and specificity of callosal axon labeling were crucial in this study. Dextran tracers (BDA and Fluoro-ruby) after a migration time of approximately 15 days in awake animals (see Material and methods, chapter 4.1.1) strongly labeled callosal axons and their synaptic boutons: all reconstructed branches ended with bulges and were therefore probably filled to their extremities. A critical factor was the time of migration of the tracers. For a migration time shorter than 6 days, a labeling was observed in the corpus callosum but not (or very sparsely) in the opposite hemisphere of the injection. For a migration time of 8-10 days in awake animals, only small numbers of retrogradely labeled neuronal somata were observed in the opposite hemisphere of the injection site. Thus, under these experimental conditions, the anterograde transport of dextran tracers was more effective than the retrograde transport from one hemisphere to the other.

The choice of the axons being reconstructed induces a bias in an already restricted selection. The well-labeled axons could be the largest myelinated ones, and the thinnest callosal axons or branches likely fall below light-microscopic resolution. The dextran tracers may also label some transected fibers of passage (Bentivoglio et al., 1980; Schmued et al., 1990) or may selectively avoid certain callosal neuron populations. However, the eight Dextran labeled axons reconstructed in this study showed all the major characteristics of previously described callosal axons that were labeled with another tracer (biocytin) (Houzel et al., 1994). An indicator of the high accuracy of the three-dimensional reconstructions presented here is the total number of synaptic boutons for each axon, ranging from 307 to 766. These numbers are larger than those previously reported for reconstructed callosal axons (labeled with biocytin) in the cat visual cortex (Houzel et al., 1994). In this previous study, nine callosal axons (among seventeen) had less than 307 boutons and only four had more than 500 boutons. Furthermore, the reconstructions of the eight callosal axons in the study reported here displayed, in most cases, more complex arbors and denser clusters of terminals than reported for those labeled with biocytin (Houzel et al., 1994). This observation and the fact that the quality of biocytin labeling deteriorates after a 24 h survival time (Kita and
Armstrong, 1991; Lapper and Bolam, 1991) suggest that dextran tracers are more reliable than biocytin for callosal axon labeling.

The technical limitations of this type of anatomical study are now mostly related to the time-consuming reconstruction of these axons. Although Neurolucida software offers the advantage of working in three-dimensions, the reconstruction process is still completely manual (taking about one month for a single callosal axon). This limits the number of reconstructed axons that can be studied. However, there is hope for future work: at the last SFN meeting, MicroBrightField representatives mentioned that they were developing an automated system for three-dimensional reconstructions from serial sections.

**III.3.4.1.2 Branching pattern and laminar termination of callosal axons**

The distribution pattern of the eight callosal axons was determined from serial sections comprising the entire cortical depth and the adjoining white matter, 317-722 µm below layer VI. It is nonetheless possible that a few axonal branches were given off at even deeper locations in the white matter. However, such deep collaterals would probably not have significantly changed the projection pattern of the reconstructed axons because the inter-cluster zones did not contain labeled axons. Furthermore, the eight axons reconstructed in this study represented the three major morphological types identified in previous reconstructions of callosal axons (Houzel et al., 1994): “parallel type” (the main axon branches into secondary collaterals in the white matter), “serial-type” (the main axon branches in the cortical layer of termination) and a third type representing a combination of the two other types. Another important feature of callosal axons is that they terminate mainly in the lower part of layer III. Only a few collaterals were encountered in deep layers, and layer I was almost completely avoided. This laminar distribution confirms previous findings (Fisken et al., 1975; Shatz, 1977b; Innocenti, 1980; Leporé and Guillemot, 1982; Milleret et al., 1994; Houzel et al., 1994; Payne, 1994). Finally, all the axons reconstructed in this study displayed clusters of synaptic boutons in a plane parallel to the cortical surface, in accordance with previous observations (Houzel et al., 1994; Aggoun-Zouaoui et al., 1996). The term “cluster” is commonly used to describe groups of labeled terminals or neurons that are separated from each other by more than 500 µm. If this precise definition is used, all but one of the axons presented two or three clusters, the remaining axon presenting adjoining “clusters” of terminals (axon 8).

**III.3.4.2 Visuotopic organization of callosal connections**

Previous findings (Olavarria, 1996) have described a non-mirror symmetric pattern of callosal connections: axons coming from the transition zone between A17 and 18 (TZ) project into contralateral regions outside the TZ whereas axons coming from regions outside the TZ project into the contralateral TZ (see Figure 11). This anatomical pattern strongly suggested a visuotopic organization of callosal connections. However, in these experiments, no physiological measures were performed. As a result, the precise relationship between the
distribution of retrogradely labeled neuronal somata and the visual field representation could not be determined. In the present study, although optically recorded retinotopic maps did not permit one-degree resolution, callosal connections appeared to link sites in the two hemispheres responding to the same region of visual space. Rather than displaying point-to-point connectivity, the distributions of axon terminals seem to be centered around the visuotopic corresponding sites. These results confirm the visuotopic organization of callosal connections.

For two injection sites (Ca06, Ca15), the non-mirror symmetric pattern was observed. It has been suggested that this convergent/divergent pattern of projections should be reflected in the morphology of individual callosal axons, with axons projecting to regions outside the TZ having broader terminal arbors than axons projecting within the TZ (Olavarria, 1996). Consistent with this prediction, the axon with the broadest terminal arbors was located in A17 (axon 2, Ca06) whereas the most compact axon (axon 8, Ca15), with less sharply separated clusters, was located within the TZ. This can be explained by the compression of ipsilateral visual field representation along the horizontal axis, within the TZ. However, in the other two animals (Ca07, Ca09), the non-mirror symmetric pattern did not clearly appear since injections within the TZ labeled axons located within the TZ in the opposite hemisphere. One possibility is that the TZ was not sharply defined enough to distinguish this pattern. Another hypothesis considers that the injection sites in cat Ca09 were at more anterior positions than the other ones. So it can be also suggested that this non-mirror symmetric pattern is more clear-cut in the posterior part of the visual cortex (where the area centralis is represented) than in the anterior part.

In addition to this visuotopic organization, all axons analyzed in this study presented clusters of terminals (2 or 3) suggesting a relationship between callosal connections and functional domains of the visual cortex. The distribution of these callosal axon terminals was therefore analyzed with respect to the orientation, direction and ocular dominance domains of the visual cortex.

### III.3.4.3 Orientation selectivity of callosal connections

All callosal axons analyzed in this study connected regions of similar orientation preference (±30°) with a mean of 72% of their synaptic boutons (Table 4). These results are consistent with those of electrophysiological studies in split-chiasm cats showing that thalamo-cortical and callosal inputs converging on a given neuron convey information about overlapping receptive fields with the same orientation tuning (Berlucchi and Rizzolatti, 1968; Leporé and Guillemot, 1982; Blakemore et al., 1983; for review see Houzel and Milleret, 1999). The high specificity of the anatomy of callosal terminals with respect to orientation domains indicates that callosal connections probably transfer information about the orientation of the visual stimulus and contribute to the orientation selectivity of the neurons they activate. It should be noted that the orientation selectivity of a neuron does not result from only one excitatory input (in this case, the callosal input). Instead, it is modulated by the
excitatory and inhibitory network (Vidyasagar et al., 1996). Consequently, callosal axons terminating in different orientation domains (and not only iso-orientation domains) could, in theory, also trigger the activation of orientation selective neurons. The influence of callosal axons on the orientation selectivity of visual neurons may be enhanced by the specific connectivity revealed in this study.

Some callosal axons appeared much more orientation selective (axons 1, 3, 7 with more than 83% of their synaptic boutons in iso-orientation domains) than others (axons 2 and 5 with only 50 and 51% of their synaptic boutons in such domains). This heterogeneity was not explained by a specific morphology of the axons or the location of the corresponding injection site (relative to pinwheel centers). However, the two axons with the lowest percentages had the smallest numbers of synaptic boutons, although the quality of labeling was not different from that of the other axons. One possible explanation is that some branches were not labeled or were not reconstructed because their branching point was located too deep in the white matter. Alternatively, the population of callosal axons may be heterogeneous in terms of orientation selectivity and sub-populations of these axons may serve different functions. In this regard, the axons selected for analysis may belong to a sub-population better labeled with dextran tracers than other sub-populations. It is also possible that the orientation specificity of callosal connections varies with the laminar location of the callosal neuron somata (in layer II/III or in layer IV). Indeed, such variation has been observed in long-range intra-hemispheric connections: layer II/III lateral connections preferentially link similar orientation domains whereas layer IV lateral connections link all orientation domains in a rather balanced manner (Yousef et al., 1999). This hypothesis could not be tested in the study reported here because extracellular neuronal tracer injections performed in the supragranular layers also labeled neurons in layer IV and it was not possible to identify the neuronal somata corresponding to the callosal axons analyzed in the opposite hemisphere.

The orientation selectivity of callosal connections has been investigated in only two previous studies on different species (normal tree-shrew, (Bosking et al., 2000) and strabismic cats, (Schmidt et al., 1997)). These two studies resulted in opposite conclusions. In the visual cortex of the normal adult tree shrew, callosal connections display only a very small bias toward the linking of sites with the same orientation preference (the significance of this bias was not quantified) (Bosking et al., 2000). In adult strabismic cats, however, this bias was shown to be significant (Schmidt et al., 1997). Both these previous studies were based on the retrograde labeling of callosal neurons. This method indicates the localization of callosal neuron somata projecting at least one axonal branch into the cortical region of the injection site. The approach used in the present study (anterograde labeling and reconstruction of callosal axons) provides additional information: the distribution of all terminal clusters or branches of single-callosal axons. This methodological difference may in part explain the result obtained in the tree-shrew. Alternatively, the visual cortices of the two species may differ in interhemispheric connectivity, as it has already been shown for intra-hemispheric connections (Fitzpatrick, 1996).
The fact that callosal connections link sites with similar orientation preferences suggests that orientation selectivity strongly influences the development and/or the stabilization of callosal connections. This hypothesis is strengthened by the results obtained in strabismic cats (Schmidt et al., 1997). Although the distribution and the properties of callosal axons of strabismic cats strongly differ from those of normal cats (Innocenti and Frost, 1979; Berman and Payne, 1983; Elberger et al., 1983), callosal connections link in both cases cortical regions with similar orientation preferences. A possible explanation is that callosal connections would be stabilized between neurons displaying correlated activities. In that case, similar orientation selectivity, together with corresponding visuotopy, would be a source of interhemispheric correlated activity stabilizing callosal connections during development.

III.3.4.4 Direction and ocular dominance selectivity of callosal connections

In this study, direction preference maps were recorded in only two animals. Two callosal axons were analyzed (axons 3 and 8) and the distribution of their synaptic boutons revealed no selectivity for a single direction. In accordance with their orientation specificity, most of the synaptic boutons of these axons were located in both domains with a direction preference orthogonal to their preferred orientation. For axon 3, this absence of direction selectivity may be because this axon arose from a neuron that was not direction selective, since the direction preference at the injection site was not known in this case. Conversely, for axon 8, the distribution of the labeled neurons at the injection site showed a clear direction preference. This suggests that at least some callosal connections are orientation but not direction selective. Studies of larger numbers of axons are required to generalize this observation. Indeed, to this author’s knowledge no previous electrophysiological evidence has been published indicating that callosal connections link cells with matching direction preferences in the cat visual cortex.

The ocular dominance (OD) selectivity of callosal axons was not the main aim of this study. In the cats selected for the analysis of orientation selectivity, injection sites were in binocular regions rather than in the middle of an OD domain. In the opposite hemisphere, most of the synaptic boutons of all eight callosal axons were also located in binocular regions (forth category in OD diagrams). These results are in accordance with the hypothesis that callosal axons preferentially connect cells with the same ocular dominance (Olavarria, 2001). However, they also suggest that these connections link mainly binocular cortical regions rather than regions preferentially activated by the same eye. A previous study (Olavarria, 2001) indicated that the distribution of callosal cells was significantly biased toward domains that were eye-specific. This bias was observed in OD patterns revealed by intraocular injections of a transneuronal tracer. However, in the examples shown in the article, many callosal neurons were located at the borders between OD columns, where binocular responses are more common (see Figs 2-4 in Olavarria, 2001). Furthermore, the results presented here are in accordance with electrophysiological studies indicating that most transcallosally activated units are binocular in normal cats (Leporé and Guillemot, 1982; Blakemore et al., 1983; Milleret et al., 1994; Milleret et al., 2005). This binocularity may account for the lack of
bias of callosal neurons for eye-specific domains in a normal cat and the clear bias in strabismic cats reported in a previous study based on 2-deoxyglucose autoradiography (Schmidt et al., 1997).

Finally, it should be noted that the orientation selectivity of callosal connections revealed in this study does not contradict the hypothesis that these connections link regions with similar ocular dominance (Olavarria, 2001): callosal connections within an OD domain (or a binocular region) can also terminate in specific orientation domains.

III.3.4.5 Comparison between interhemispheric callosal connections and intra-hemispheric long-range horizontal connections

The orientation selectivity of callosal connections suggests similar organizational principles and function of both callosal and intra-hemispheric long-range horizontal connections in cat visual cortex. Both types of long-range connection originate from and terminate on similar cell types in supragranular and infragranular layers (Innocenti, 1986b), have a patchy termination pattern and preferentially link similar orientation domains (Gilbert and Wiesel, 1989; Houzel et al., 1994; Schmidt et al., 1997; Kísvárday et al., 1997; Yousef et al., 1999). However, whereas callosal axons preferentially connect ocular dominance columns serving the same eye ((Olavarria, 2001) but see (Schmidt et al., 1997) and results presented here), intra-hemispheric horizontal connections show no significant preference for ocular dominance territories (Matsubara et al., 1987; Löwel and Singer, 1992; Schmidt et al., 1997). Furthermore, callosal connections link visuotopically corresponding sites whereas intra-hemispheric long-range connections link sites whose receptive fields are widely displaced. Callosal and intra-hemispheric long-range horizontal connections may therefore have different functions in the cat visual cortex. Intra-hemispheric long-range connections appear to be well suited for enhancing the response to collinear contour elements along distances beyond the classical receptive fields of neurons in A17 and A18. By linking cortical sites with the same functional properties in both hemispheres, callosal connections may be involved in the fusion of the two visual hemifields into a single visual field as well as in depth perception (binocular stereopsis).
IV General conclusion

The results presented in this study characterize the interhemispheric dialogue between the visual areas of each hemisphere. The anatomical and functional organizations of callosal connections were investigated by combining optical imaging of intrinsic signals with callosal neuron labeling and anatomical methods. Split-chiasm preparation was used to isolate the callosal inputs from the geniculo-cortical activity. These results revealed a highly specific organization of transcallosal connectivity between the visual areas 17 and 18 of each hemisphere, in the adult cat. This connectivity suggests specific functional roles of the corpus callosum in visual information processing. Finally, the study of the anatomical and functional characteristics of callosal connections in adult animals also provides clues about the possible mechanisms involved in the development of this specific connectivity.

IV.1 Specificity of transcallosal connectivity between cat visual areas 17 and 18

As pointed out by Nobel prize-winning neurophysiologist David H. Hubel, “callosal connections provide a vivid example of the remarkable specificity of neural connections. Every callosally connected cell in the visual cortex must get its input from cells in the opposite hemisphere with exactly matching properties. This is a beautiful direct evidence for selective connectivity in the nervous system” (Hubel, 1995).

The results presented in this thesis and those of previous studies support this view. Evidence for the selective organization of callosal connections is provided by:

1) The specific nature and localization of pre and post-synaptic elements.

2) The correspondence between the site of origin and the target zone of callosal axons. These axons link sites in visuotopic correspondence, which respond preferentially to the same orientation of the visual stimulus and to stimulation of the same eye (or of both eyes in most cases).

3) The convergence of interhemispheric and geniculo-cortical information on target neurons.

Variability in the diameter and architecture of callosal axons has been reported (Houzel et al., 1994). In the present study, slight heterogeneity was also observed in both the architecture and degree of orientation selectivity of the eight reconstructed axons. However, striking similarities also appeared, as all the axons presented clusters of terminals and linked cortical sites representing the same part of the visual field and the same orientation of the
visual stimulus. The variability previously reported may be partly accounted for by the choice of neuronal tracer, resulting in weak labeling in some cases (Biocytin) (Houzel et al., 1994).

The results presented in this thesis demonstrate that the selectivity of callosal connections is reflected in the distribution of callosal axon terminals within the functional maps of the visual cortex. At the level of the population of transcallosal connections, the results presented in chapter 1 indicate that these connections mainly activate the transition zone (TZ) between A17 and A18, along its whole antero-posterior extent, with no obvious interruption (at least within the imaged region between stereotaxic coordinates P6-A6). Based on the retinotopic maps analyzed in chapter 2, two subpopulations of these connections can be distinguished. The first transfers the representation of the vertical meridian and activates the entire TZ. The second transfers the representation of the ipsilateral hemifield (between $+2^\circ$ and $+8^\circ$) and is clustered within patches along the TZ. In addition, the results presented in chapter 3 show that the terminals of individual callosal axons also appear to be clustered. These clusters were located mainly in similar orientation domains.

The functional selectivity of callosal connections thus arises from different levels of cluster organization (see Figure 59). Within the TZ, a population of callosal axons clusters in patches (mean diameter, $1.2\pm0.3$ µm) corresponding to the representation of the ipsilateral hemifield. Furthermore, the terminals of single callosal axons are also clustered (mean diameter of the clusters, $494\pm146$ µm) in orientation domains. This does not rule out the possibility that these connections also link regions with similar ocular dominance (binocular regions in particular) (Olavarria, 2001). Callosal connections within an ocular dominance domain may also terminate in specific orientation domains.

In addition to their callosal axon, callosal neurons also have a proximal (ipsilateral) branch. The functional selectivity of these proximal branches remains unknown. If these branches preferentially link cells with similar functional properties, then callosal neurons could influence the activity of several neurons coding the same orientation, in both hemispheres. The demonstration of this point requires the identification of proximal branches of callosal axons. This identification could be achieved by labeling these branches through the retrograde labeling of the corresponding callosal neurons somata. By reconstructing these branches and overlaying them on the functional map of their target zone, it would be possible to demonstrate their functional selectivity. In the experiments presented in chapter 3, some callosal neurons were labeled retrogradely (see Figure 58). In 20 cases (of 46), a well-labeled proximal branch was observed. The three-dimensional reconstruction of these branches and the analysis of their distribution in functional domains are currently underway.
IV.2 Possible functional correlates of the specificity of transcallosal connections

IV.2.1 Corpus callosum and midline fusion

The results presented in chapter 2 indicate that callosal connections transfer the representation of an ipsilateral portion of the visual field (up to 8°). They also show that the ipsilateral part of the visual field is represented within patches along the transition zone between A17 and A18. The origin or function of this patchy representation is unclear, but it seems to be suitable for a highly compressed representation in the 1-mm-wide transition zone. The developmental constraints responsible for this specific organization remain to be determined.

Ramon y Cajal already postulated in his Teoria general de los entrecruzamientos de las vías nerviosas (Ramón y Cajal, 1898) 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” (Ramón y Cajal, 1898; Ramón y Cajal, 1955). Ever since the first studies of the corpus callosum, it has been proposed that the transcallosal pathway may mediate the unification of the two visual hemifields (Myers, 1956; Choudhury et al., 1965; Gazzaniga, 1966; Antonini et al., 1979; Berlucchi, 1981; Antonini et al., 1983). The representation of the same portion of the visual field in both hemispheres could be involved in this fusion. The portion represented twice would be the zone of overlap of the two hemifields. The linking of sites with similar functional properties (visuotopic correspondence and orientation preference) by transcallosal connections provides support to this hypothesis. It should be noted that the portion of the visual field representation that receives and sends callosal projections gradually increases from A17, A18 and A19 to the more lateral suprasylvian visual areas (Segraves and Rosenquist, 1982a). The receptive fields in the lateral suprasylvian areas are considerably larger than those in A17 and A18: callosal representation of the visual field can reach 50° or more in the ipsilateral hemifield (Antonini et al., 1983; Berlucchi and Antonini, 1990). These large receptive fields are probably involved in the cross-integration of visual information from the two hemifields (Berlucchi and Antonini, 1990).

This midline function of the corpus callosum is probably common to highly lateralized sensory systems, since it has also been demonstrated in the somatosensory system [cat: (Manzoni et al., 1980; Guillemot et al., 1988); monkey: (Guillemot et al., 1987); human: (Schiavetto et al., 1993)]. In the somatosensory cortex, S1, and the motor cortex, M1, cells projecting a callosal axon have been reported to be restricted to a narrow band representing the body midline (Innocenti, 1986a). However, more recent findings indicate that in S1 of Japanese monkeys (Macaca fuscata), a substantial number of neurons have bilateral receptive
fields in the representations of the hand/fingers, shoulders and arms which may be involved in integrating the information required for the cooperative actions of the two hands (Iwamura, 2000). These bilateral cells are probably connected to the other hemisphere via the corpus callosum. As delicate and precise manual operations are generally performed with both hands in front of the individual and in the axis of gaze, the presence of distal homotopic connections is consistent with the midline function of the CC.

It has been proposed that the evolutionary origin of the corpus callosum in placental mammals lies in the mechanism of midline fusion in the sensory cortices. Commissural fibers may initially have been involved in binding mechanisms related to midline fusion and bimanual coordination, abilities that are observed in many so-called primitive mammals (Aboitiz and Montiel, 2003).

IV.2.2 Corpus callosum and visual stimulus orientation

In chapter 3, it was demonstrated that callosal connections preferentially link similar orientation domains. This suggests that transcallosal connections transfer information related to the orientation of a visual stimulus and are thus part of the functional network involved in the processing of this information. Callosal connections are therefore probably involved in the mechanisms underlying the generation of orientation-tuned responses, along the whole antero-posterior extent of the TZ, as demonstrated in chapter 1 (at least in the imaged zone, between P6 and A6). The similarity between transcallosal and geniculo-cortical orientation maps in this region indicates that both pathways can mediate neuronal orientation selectivity. A precise match of orientations between TC and GC pathways could be essential for detecting position disparity and could be thus involved in depth perception (stereopsis).

The specific connectivity of transcallosal connections provides an anatomical basis for the interhemispheric synchronization observed between the neurons in the TZ of each hemisphere (Engel et al., 1991). These neurons, connected through the corpus callosum, displayed oscillatory activity in the “gamma” band (40-60 Hz) and synchronized their discharge when stimulated by a coherent stimulus. Three types of synchronization have been identified, based on the coherence of the visual stimulus (Nowak et al., 1995). The most precise synchronization (T for Tower) occurs almost exclusively between neurons with overlapping receptive fields (at least a partial overlap) and, in most cases, between neurons with similar optimal orientation. After a section of the corpus callosum, this coupling was totally abolished. Interestingly, bilateral lesions in extrastriate cortical regions had little effect on T synchronization, except that neurons with different orientation preferences were less often synchronized in animals with such lesions than in normal animals. Extrastriate cortical areas therefore contribute to a greater range of orientation mismatch through transcallosal connections. These feedback pathways may modulate the activity of neurons that are activated by “non-specific” transcallosal connections linking neurons with different orientation preferences. Indeed, in chapter 3, it was shown that callosal axons terminate preferentially in
iso-orientation domains (72% of their synaptic boutons, on average), but that they also terminate in other orientation domains (oblique- and cross-orientation domains) with in mean 28% of their synaptic boutons. The synaptology of callosal connections remains unknown. The combination of neuronal tracers with ultrastructural microscopy could give information about the distribution and nature of the various inputs (geniculate, intra- and inter-hemispheric connections) converging on the neurons sending or receiving callosal inputs.

IV.3 Development of the highly specific interhemispheric connectivity

Many studies have suggested that callosal connections are maintained during development because the callosal and thalamocortical pathways are activated in a synchronous and congruent manner for a given specificity of a visual stimulus (Hubel and Wiesel, 1967; Berlucchi and Rizzolatti, 1968; Léporé and Guillemot, 1982; Innocenti, 1986b; Payne, 1990b; Payne and Siwek, 1991b; Houzel and Milleret, 1999). Anatomical studies have indicated that matched retinotopy between callosal and intrahemispheric projections could be the driving force for the stabilization of callosal connections (Olavarria, 1996; Bosking et al., 2000). However, this hypothesis did not explain the fact that callosal connections were not homogeneously distributed but formed a patchy pattern in the visual areas of the adult brain (Houzel et al., 1994). For this reason, several attempts have been made to find a match between callosal connections and one or more of the functional domains of the visual cortex. Both callosal neurons and terminals are densely packed within cytochrome oxidase-dense domains of the visual cortex of cats (Boyd and Matsubara, 1994) and macaques (Olavarria and Abel, 1996). However, the functional significance of these domains remains unclear. Furthermore, periodicities in the distribution of callosal neurons correlate with the pattern of ocular dominance columns (Olavarria, 2001). In this thesis, it has been shown that callosal axons preferentially link binocular regions, displaying the same orientation preference. These results suggest that orientation similarity, like corresponding visuotopy and ocular dominance preference, is a source of correlated interhemispheric activity that could influence the stabilization of callosal connections during visual cortex development. The arguments in favor of this hypothesis are presented below, after a review of anatomical and electrophysiological data concerning the development of these connections.

IV.3.1 Development and guidance of callosal axons before entry into the visual cortex

The projection of a callosal axon, as opposed to another type of axon, by a neuron seems to depend mainly on intrinsic factors acting very early during the embryonic period, even before neuron migration has been completed (Shoukimas and Hinds, 1978; Schwartz et al., 1991; Auladell et al., 1995). The future position of the neurons in the cortical plaque is also
determined at this early stage (Rakic, 1974; Rakic, 1975). Then, the growth cones of callosal axons must be guided over a long distance to the specific destination in the opposite hemisphere. A recently described transient neuronal population in the developing corpus callosum may be involved in axonal guidance, but this role remains to be defined (Riederer et al., 2004). The observation of the main branching points of callosal axons in adult cats or young kittens suggests that two regions in the white matter are critical in this pathway. The first one is located at the exit of the corpus callosum and the second is located just below cortical areas 17 and 18 (Houzel et al., 1994). For the first region, transient clusters of microglial cells and astrocytes have been observed during the first postnatal month, in the ventral part of the white matter below visual cortical areas 17 and 18, i.e. on the path of the growing callosal axons (Innocenti et al., 1983; Rochefort et al., 2002; Rochefort et al., 2005). These cells may participate in the selection and the guidance of growing axons. Indeed, microglial cells do not only display a macrophagic function, they can also secrete neurotoxic factors (Thery et al., 1994) and growth factors (Chamak et al., 1994). In the mouse visual cortex, three midline structures - the glial wedge, glia within the indusium griseum, and the glial sling - have been shown to be involved in the development of the corpus callosum (Silver et al., 1982; Shu and Richards, 2001; Shu et al., 2003). The interactions between growing callosal axons and glial clusters remain to be characterized in the cat.

Among all callosal axons reaching the opposite hemisphere, only some of these axons will enter the cortex, in the vicinity of the TZ (see review Innocenti, 1995). The other branches stop just below the cortex, indicating the probable occurrence of local interactions in this second critical region (Innocenti and Price, 2005). These interactions may involve subplate neurons (Friauf et al., 1990; McConnell et al., 1994), which are present in the young kitten at the time at which the callosal axons enter the cortex (Chun and Shatz, 1988). The branches that do not enter the cortex are eliminated. Transient populations of macrophages described above may be involved in clearing the debris of the eliminated axons in the white matter (Innocenti et al., 1983; Rochefort et al., 2002; Rochefort et al., 2005).

### IV.3.2 Development of callosal axons within the cortex

Callosal axons were found to enter the cat visual cortex during the first postnatal week, i.e. before eye opening that occurs around postnatal days 7 and 10 (P7-P10). As early as P6, the branches of some callosal axons were found to be distributed in a discrete, disjunctive manner reminiscent of the terminal columns found in older animals (Aggoun-Zouaoui et al., 1996). Synaptic boutons appear from P12, in the cortical plate, and also display a columnar organization at about P12-P20 (Aggoun-Zouaoui et al., 1996). Electrophysiological studies have shown that these synaptic boutons are already functional, since transcallosally activated units were recorded from P12 (in the TZ) (Milleret et al., 1994). These findings suggest that callosal axons may recognize functional columns as early as when they grow into the cortex, at P6 in the earliest cases, or at least when they establish the first synapses at about P12-P20.
Geniculo-cortical axons arrive in layer IV (in the cortical plate) of the cat visual cortex at approximately embryonic day 60 (Ghosh and Shatz, 1992), almost 10 days earlier than callosal axons. Anatomical evidence suggests that ocular dominance column formation begins between P7 and P14 (Crair et al., 2001). This is after the earliest disjunctive growth of callosal axons into the grey matter (P6-8), but corresponds to the formation of the first synapses (P12-20). Orientation-specific responses can be recorded in the cat visual cortex from the end of the first postnatal week (Hubel and Wiesel, 1963; Albus and Wolf, 1984; Frégnac and Imbert, 1984; Braastad and Heggelund, 1985) (see for review, Frégnac and Imbert, 1984). Thus, the template for the orientation preference columns appears to be established before that for ocular dominance specificity or at least before responses to the ipsilateral eye become strong. This has been confirmed by an optical imaging study, showing that orientation maps can already be recorded at the end of the second postnatal week, whereas only a weak pattern of alternating eye preference, strongly dominated by the contralateral eye, can be observed at this time point (Crair et al., 1998). Callosal axons may recognize the template for the orientation preference columns and develop in accordance with it. Thereafter, both ocular dominance and orientation selectivity may influence the development of callosal connections, since maturation of the callosal pathway is not complete until about the 5th postnatal month (Aggoun-Zouaoui et al., 1996).

These observations do not rule out the possibility that the initial layout of connections is specified by experience-independent factors, such as molecular cues or self-generated, patterned activity (see for review Katz and Shatz, 1996, Price et al., 2006). Visual experience would then support the further refinement of this connectivity. The influence of visual experience in the development of the orientation selectivity of callosal connections could be revealed by studies in which visual experience is altered. Previous studies have indicated that the development of callosal connections involves the stabilization of some of these connections as well as the elimination of axonal exuberances. After an early asymmetric visual deprivation (monocular occlusion or strabismus), the callosal axon exuberances that are normally eliminated, are stabilized (Shatz, 1977b; Lund et al., 1978; Milleret, 1994; Milleret and Houzel, 2001; Milleret et al., 2005). In addition to this expansion of the callosal axons projection zone, the physiological properties of the transcallosally activated units are modified: their receptive fields are abnormally large (Milleret and Houzel, 2001), the disparity between TC and GC activated receptive fields is modified (Milleret et al., 2005) and after a monocular deprivation, only half of these units are orientation selective (compared to more than 90% in normal conditions). However, the orientation selective units are still preferentially activated by the same orientation through the TC and the GC pathways. During the normal development, this matching of orientation preferences appears as soon as the neurons display an orientation selectivity. Therefore, many physiological properties are modified by an asymmetric visual deprivation, but not the matching between the orientation preferences of callosally connected neurons. It would be interesting to confirm this point by studying the distribution of callosal connections in the functional maps after an early
monocular visual deprivation, using the protocol described in chapter 3. The same approach could be used to study callosal connections distribution after a reverse-suturing experiment in which kittens see alternatively with one eye or the other but never with both eyes at the same time. It was shown that this protocol leads to the development of identical orientation maps for both eyes (Gödecke and Bonhoeffer, 1996). Finally, the distribution of these connections could be studied in cases in which the two eyes never see the same orientation. This could be achieved by fixing goggles in front of kitten eyes, before eye opening: these goggles would be fitted with plano-convex cylindrical lenses, through which the animals would see elongated images of their environment (Tanaka et al., 2006). The orientation of this elongation (vertical, horizontal or oblique) would be different for each eye. The last two experimental approaches would also determine whether the development of callosal connections depends on the activity of one or both eyes.

**IV.3.3 Monocular and binocular mechanisms can drive interhemispheric correlated activity**

The results presented in chapter 2 indicate that the transcallosal pathway can be activated by the ipsilateral temporal retina and the contralateral nasal retina. The pathway through the uncrossed projections of the temporal retina and the CC (pathway 1) transfers a representation of up to $8^\circ$ of the ipsilateral portion of the visual field, whereas the pathway through the crossed projections from the nasal retina and the CC (pathway 2) participates in a lesser extent in the representation of the ipsilateral hemifield (up to $2-4^\circ$).

If correlated activity between transcallosal (TC) and geniculo-cortical (GC) pathways is considered to induce the stabilization of callosal connections, either one or both eyes could be involved in this process. For the pathway through the uncrossed projections of the temporal retina of one eye and the CC (pathway 1, Figure 39, A1), the correlated GC activity may come from the uncrossed GC pathway from the temporal retina of the other eye (as in the split-chiasm preparation) or the crossed GC pathway (from the nasal retina of the same eye). Either of these GC pathways may trigger the activity correlated with that of pathway 2 (Figure 39, A2). Thus, the results presented in this study show that both monocular and binocular mechanisms can drive interhemispheric correlated activity. The existence of crossed projections activating the TC pathway (pathway 2) and the possibility of correlated activity through the crossed GC pathway may account for the observation that neonatal sectioning of the optic chiasm leads to a loss of callosal connections in the striate cortex (Boire et al., 1995).

A previous study revealed a correlation between periodicities in the distribution of callosal neurons and the pattern of ocular dominance columns (Olavarria, 2001). Based on this observation, it was predicted that callosal connections would be stabilized by correlated activity driven by ganglion cells from the same temporal retina (“uniocular” process).
projecting to both hemispheres (Olavarria, 2001; Olavarria, 2002). The data presented in this thesis do not support the hypothesis that the crossed projections from the temporal retina play a substantial role in the activation of the transcallosal pathway and in the representation of the ipsilateral hemifield in adult cats (see chapter 2). However, it remains possible that this crossed pathway from the temporal retina is involved in the development of callosal connections and that, after this period, it triggers only weak activity that cannot be distinguished from TC activity using optical imaging of intrinsic signals.

The results presented in chapter 3 of this thesis indicate that all eight callosal axons analyzed linked binocular domains. Although this sample is limited and may therefore be biased, these results suggest that callosal connections link mainly binocular cortical regions, rather than regions preferentially activated by the same eye (Olavarria, 2001). This is consistent with electrophysiological studies, which have shown that most transcallosally activated units are binocular in normal cats (Berlucchi and Rizzolatti, 1968; Leporé and Guillemet, 1982; Blakemore et al., 1983; Milleret et al., 1994; Milleret et al., 2005). These observations and the many studies indicating that an asymmetric perturbation of visual experience strongly modifies the distribution and the properties of callosal connections (Shatz, 1977b; Lund et al., 1978; Milleret, 1994; Milleret and Houzel, 2001; Milleret et al., 2005), suggest that binocular interactions are involved in the development of interhemispheric connections.

**IV.4 Outlook**

Callosal connections constitute a useful model for studies of sensory processing. They have many characteristics in common with other cortico-cortical connections and display, in addition, several advantages for experimental purposes: the “sending” and the “receiving” zones are clearly separated, the fibers linking these zones are accessible and it is possible to distinguish the different input pathways (for example by sectioning the optic chiasm and the optic tract). The split-chiasm preparation is particularly suitable for studies of the organization of the callosal visual input to cortical areas. Indeed, the similarity of the retinotopic maps obtained in normal and split-chiasm animals (chapter 2) demonstrates that transcallosal connections are predominantly triggered by the uncrossed pathway from the temporal retina.

The “dialog” between the two hemispheres is far from completely elucidated. The results presented in this thesis provide new insights into the anatomical and functional organization of callosal connections between the cat visual areas (17 and 18), at the level of the cortical regions activated by these connections and at the level of individual callosal axons. These results, obtained in normal adult cats, could be used as a reference for identifying plastic changes in callosal cortical maps during normal development and in response to altered visual experience both during development and in adulthood. The combination of optical imaging of intrinsic signals with anatomical methods appears to be a fruitful approach for this type of investigation.


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References


List of figures

Figure 1. The corpus callosum is a thick, curved plate of axons in the approximate center of the brain ................................................................. 13
Figure 2. Schema of the cat visual system ................................................................................................................................. 17
Figure 3. Cat visual cortex. A. Outline drawings of dorsolateral (left) and medial (right) views of the left hemisphere to show cortical gyri and sulci of visually responsive cortex and related structures .............................................................. 18
Figure 4. Diagrams of the innervation of A18 (A) and A17 (B) by fibers from the LGN and the intrinsic circuitry of spiny neurons in A17 (C) .................................................................................................................. 20
Figure 5. Outline drawings of the cerebral hemispheres of the cat, to show the positions of areas 17 and 18 and the intervening transition zone, relative to the gyri and sulci ........ 22
Figure 6. Callosal sending zone ........................................................................................................................................ 26
Figure 7. Left. Photomicrograph of callosal-projecting cells in the transition zone between A17 and A18, stained with HRP reaction product. Right. Drawing of five neurons shown in the left panel .................................................................................................................................. 27
Figure 8. Diagram summarizing of the laminar distribution and morphology of cells, in the region of the transition zone between A17 and A18, that project to the contralateral hemisphere via the corpus callosum .......................................................................................................................... 28
Figure 9. Line drawing of a median view of the bisected corpus callosum, showing the map of visual field elevation for the fibers connecting A17 and A18 in the two hemispheres .... 29
Figure 10. Demonstration of the extent of the callosal cell zone (A-D) and callosal terminal zone (E-H) in A17 and A18 after 40 injections of a mixture of HRP and tritiated proline into all visual areas in the contralateral hemisphere ... 30
Figure 11. Top. Diagram relating retinal projections, the visual field representation in the callosal zone of A17 and A18 (the portions of these areas sending or receiving callosal inputs) as well as the topography and ocular dominance of callosal connections .......................................................... 32
Figure 12. Changes in RF azimuth in penetrations crossing the TZ ............................................................ 33
Figure 13. Split-chiasm preparation ........................................................................................................................................... 35
Figure 14. A. Composite drawing of the receptive fields plotted for more than 700 sites studied in the callosal sending zone, in the left hemisphere .................................................. 37
Figure 15. A. Experimental design. B-C. Distributions, within the visual field, of the transcallosal (TC, B) and the geniculo-cortical (GC, C) receptive fields of trancallosally activated units ..... 39
Figure 16. A. Orientation selectivity of the TC-GC binocular units. B. Ocular dominance of transcallosally activated units (TC units). C. Spatial distribution of pairs of receptive fields of binocular TC-GC units .................................................................................................................. 40
Figure 17. Left. Hemoglobin absorption spectra and absorption coefficient ratios of oxy- (HbO₂) and deoxy- (Hb) hemoglobin ........................................................................................................... 44
Figure 18. Picture of the set-up for optical imaging experiments (Dr Milleret’s group, LPPA, Paris) ........................................................................................................................................ 45
Figure 19. Set-up for optical imaging of functional maps in vivo ......................................................................................... 45
Figure 20. Chamber for optical imaging experiments ............................................................................................................. 46
Figure 21. Full-field gratings of four equally spaced orientations (0, 45, 90, 135 deg) .................. 47
Figure 22. Visual stimulus used for retinotopic mapping of horizontal eccentricity ............... 48
Figure 23. Stimulus sequence ...................................................................................................................................................... 49
Figure 24: A-D. Single condition activity maps of cat visual cortex for four different orientations .... 51
Figure 25. Polar map corresponding to the vectorial summation of 8 single-condition maps: the preferred angle of a region is color-coded (circle on the right) and the brightness of the color indicates the strength of the tuning of the preferred orientation ........................................ 51
Figure 26. Histological control of the optic chiasm’s section .............................................. 53
Figure 27. Electrophysiological and histological controls of the optical images .................. 56
Figure 28. Frontal sections to show the transition zone between A17 and A18 ................... 57
Figure 29. Photomicrographs of frontal histological sections at stereotaxic coordinates P2.5. Top, Nissl staining. Bottom, cytochrome oxidase staining ......................................................... 57
Figure 30. Experimental procedure used for analysis of the distribution of callosal terminals in functional maps .................................................................................................................... 58
Figure 31. Light microscopic images of BDA and Fluoro-ruby (FR) labeled neurons .......... 62
Figure 32. Alignment of 3D-reconstructions and functional maps........................................ 64
Figure 33. Layout of the transcallosal activity in visual areas A17 and A18 revealed by optical imaging of intrinsic signals (cat Ca10) in the split-chiasm preparation (see schema at the top of the figure) ................................................................................................................. 69
Figure 34. Localization of the transition zone (TZ) between A17 and A18 (cat Ca10) ...... 71
Figure 35. Electrophysiological and histological controls of the localization of the transition zone and of the transcallosally activated regions from case Ca12 (A-C) and case Ca10 (D-F). A and D ..................................................................................................................................... 72
Figure 36. Transcallosal maps obtained using different spatial (SF) and temporal (TF) frequencies as visual stimuli ........................................................................................................... 75
Figure 37. Comparison of the transcallosal and the geniculo-cortical orientation maps (case Ca10). A, B. Single-condition orientation maps obtained in the same animal and in the same conditions (horizontal grating) except for the eye stimulated (A, left eye; B, right eye) ........ 77
Figure 38. Comparison of TC and GC angle maps ............................................................... 78
Figure 39. Experimental protocols ..................................................................................... 91
Figure 40. Representation of the central vertical meridian in the right (RH) and left (LH) hemispheres of normal (on the left) and split-chiasm (on the right) adult cats ......................... 92
Figure 41. Single-condition maps obtained with the presentation of the visual stimulus (vertical bar) at 0°, +4° and +8°, before (top) and after (bottom) the section of the optic chiasm of cat Ca01 .......................................................... 94
Figure 42. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between −13° and +13°, in both hemispheres of the split-chiasm cat Ca10 .......................................................... 96
Figure 43. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between −8° and +20°, in both hemispheres of the intact cat Ca02 .......... 97
Figure 44. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between −12° and +12°, in both hemispheres of the intact cat Ca15 .... 98
Figure 45. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between +2° and +8° (right hemifield), in both hemispheres of split-chiasm animals .................................................................................................................. 101
Figure 46. Single-condition maps obtained with the presentation of the vertical bar at azimuthal positions between +2° and +8° (right hemifield), in both hemispheres of intact animals ................................................................................................................................. 102
Figure 47. Comparison of the patches of activity representing the ipsilateral visual field with the orientation maps of the same region, in two intact (Ca02 and Ca08) and one split-chiasm (Ca01) animals .................................................................................................................. 104
Figure 48. Orientation domains represented within the patches of activity observed in split-chiasm (A) and in intact animals (B) for stimuli presented in the ipsilateral hemifield .......... 104
Figure 49. Single-condition maps obtained with the presentation of the visual stimulus (vertical bar) at azimuthal positions between -1° and -8° (left hemifield), in both hemispheres of intact animals ................................................................................................................................................... 106
Figure 50. Localization of the transition zone (TZ) between A17 and A18 in the left (LH) and right (RH) hemisphere of split-chiasm cat Ca10 ............................................................................................................. 108
Figure 51. Localization of the transition zone (TZ) between A17 and A18 in the right (RH) hemisphere of cat Ca01, studied before and after chiasmotomy ................................................................. 109
Figure 52. Retinotopic maps obtained from recordings made in almost 150 closely spaced electrode penetrations in the TZ, in normal adult animals .......................................................... 113
Figure 53. Three-dimensional reconstructions of callosal axons (in right hemispheres) .... 120
Figure 54. Three-dimensional reconstructions of callosal axons ........................................ 121
Figure 55. Receptive fields location and preferred orientation of the units recorded at the injection sites (left hemispheres) in cats Ca07, Ca09 and Ca15 ................................................................. 123
Figure 56. Visuotopy of callosal axons, cat Ca09 .................................................................. 124
Figure 57. Analysis of the orientation preference at the injection sites (left hemispheres) in cat Ca06 (A-D) and Ca15 (E-H) .................................................................................................................. 126
Figure 58. Orientation selectivity of callosal connections ....................................................... 128
Figure 59. Visuotopy and orientation selectivity of axon 8 (cat Ca15) .................................. 129
Figure 60. Direction selectivity of callosal connections ...................................................... 131
Figure 61. Ocular dominance selectivity of callosal connections ........................................ 132
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