Pattern of Development of the Callosal Transfer of Visual Information to Cortical Areas 17 and 18 in the Cat

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Abstract

The aim of this study was to investigate the development of visual callosal transfer in the normally reared cat. Two- to nine-week-old kittens and adults (used as controls) underwent section of the optic chiasm. Three days later, the animals were placed under anaesthesia and paralysed; unit activities were recorded from visual cortical areas 17 and 18 and from the white matter in one hemisphere. The units were tested for their responses to visual stimulation of each eye successively. Out of 1036 recorded neurons, 185 could be activated through the eye contralateral to the explored cortex via callosal transfer. Most of them could also be driven through the ipsilateral eye via the 'direct' geniculo-cortical pathway. For animals aged >2 weeks, virtually all of these units were located at the 17/18 border zone, with a majority in the supragranular layers. When activated through the corpus callosum, they displayed receptive fields located either on the central vertical meridian of the visual field or in the hemifield ipsilateral to the explored cortex. Such extension into the ipsilateral hemifield as well as receptive field disparities of binocular units decreased with age, while spontaneous activity, strength of response, orientation selectivity and ability to respond to slits moving at middle-range velocity increased. The main conclusion is that the transient callosal projections described by anatomists, which are present until 3 months of age, do not achieve supraliminar synaptic contacts with parts of areas 17 and 18 other than the 17/18 border zone, at least from 12 days after birth. However the visual callosal transfer in young animals displays some characteristics which disappear with age.

Introduction

An important principle in neural ontogeny of the visual system in mammals is that projections from one structure to another are widespread in early life ('exuberant' projections), and become restricted with maturation (for reviews see Stein *et al.,* 1985; Chalupa and Dreher, 1991; Innocenti, 1991). The few relevant physiological observations available from the cat visual system indicate that juvenile functional synapses with 'inappropriate' target regions are eliminated to give the adult connectivity pattern (Levay *et al.,* 1978; Shatz and Kirkwood, 1984; Luhmann *et al.,* 1990).

In this same species, histological data indicate that the callosal projections from the visual cortical areas 17 and 18 undergo this type of regressive process during development. Callosal axons are more numerous early in life that at adulthood (Berbel and Innocenti, 1988); correspondingly, the distribution of perikarya with callosal axons is more widespread in young animals than in adults (Innocenti *et al.,* 1977). The number of axons and perikarya is progressively reduced during the first 3 postnatal months (Innocenti *et al.,* 1977; Berbel and Innocenti, 1988). However, whether these supernumerary efferent callosal neurons establish effective synaptic contacts within the contralateral hemisphere has not been determined so far. Recent anatomical data from kittens do not clarify this issue since the various authors disagree upon the extent to which the exuberant callosal axons penetrate into the cortex (Innocenti, 1981; Kennedy *et al.,* 1987; Innocenti and Assal, 1991; Elberger, 1993).

We therefore tried to obtain direct answers to the following questions, using unit recordings: (1) Can a callosal transfer of visual information be detected soon after natural eye opening (at 8 – 10 days after birth)? (2) Do the exuberant callosal projections in young animals establish supraliminar functional synaptic contacts within the contralateral areas 17 and 18 despite the fact that callosal connections in the adult are almost completely restricted to the 17/18 border zone (Ebner and Myers, 1965; Berlucchi and Rizzolatti, 1968; Innocenti and Fiore, 1976; Payne *et al.,* 1991)? (3) Do the characteristics of the callosal transfer of visual messages change during development?

Some of the following results have previously been published in abstract form (Milleret *et al.,* 1990).

TABLE 1. Number of recorded units in each experimental group

	Age in weeks (days)					Adult	Total
	2 (12-17)	3 (19-24)	4 (28-29)	7 (49-50)	8-9 (57-66)		
Number of cats	6	5	2	2	2	8	25
Number of recorded cells							
Total	220	190	95	106	56	369	1036
C+ visual	28	20	4	42	28	63	185
C- visual	185	153	91	60	28	270	787
non-visual	7	17	0	4	0	36	64

Age is indicated in weeks and in corresponding day ranges. C+, callosal driven units; C-, cortical units not activated through the corpus callosum (including visually and non-visually activated units).

Materials and methods

Seventeen 9- to 63-day-old kittens, born in our colony and reared normally, were used. Eight adult cats served for reference. These 25 animals were divided into six groups (Table 1) according to their age at recording.

Surgery

Animals were anaesthetized with 1.2 ml/kg i.m. Saffan (Pitman-Moore, Switzerland) (10.8 mg/kg of Alfaxolone and 3.6 mg/kg of Alfadolone acetate) and underwent midsagittal section of their optic chiasm using a palatine approach. Xylocaine was injected into the tissues overlying the palate which was to be drilled to reach the optic chiasm. Antibiotics were then administered locally and i.m. Three days later, the animals were again anaesthetized with Saffan (1.2 mg/kg) and placed in the stereotaxic apparatus. The skull overlying the visual cortex was removed for future microelectrode penetrations and the opening was sealed with agar. The animals were then paralysed with Flaxedil (SPECIA, France), artificially ventilated and kept in optimal conditions (38°C, 4% CO_2) under continuous infusion of a mixture of Saffan (3.6 mg/kg/h), Flaxedil (10-15 mg/kg/h), Plasmagel and glucose. Their electrocardiogram was permanently monitored.

During these interventions, adequate measures were taken to minimize pain or discomfort of the animal, in accordance with institutional guidelines. Anaesthesia was always adjusted to the level at which the animal showed no sign of arousal or any increase in heart rate to mildly noxious stimulation such as pinching a paw.

Recording procedure

Unit activities were recorded with tungsten microelectrodes (l-2 MØ at 1000 Hz). Spikes were amplified, visualized, audio-monitored and if necessary selected through a window discriminator by standard techniques. Electrode penetrations were performed obliquely towards the midline (in a frontal plane, ~ 25° with respect to the sagittal plane) and covered a large extent of visual cortical areas 17 (A17) and 18 (A18) in the left hemisphere. To minimize the sampling bias, recordings were achieved along each track at regular intervals (100 μm) whenever possible, with a total penetration length usually between 2000 and 6000 μm, depending on the age of the animal and the location of the track. Two small electrolytic lesions (10 μA DC current during 15 s) were made, at about half-way along and at the end of each track.

Visual stimulations

The nictitating membranes were retracted with 5% Neosynephrine (Laboratoire H. Faure, France) and the pupils were dilated with 1% atropine. Scleral contact lenses were used to protect the cornea from drying. The optic discs were plotted on a faintly illuminated (~ 13 cd m^{-2}) translucent tangent screen, 57 cm in front of the animal's eyes, several times during the course of the experiment.

The neuronal response properties and receptive field (RF) characteristics were analysed using moving and stationary light stimuli of different sizes and shapes (spots or slits). A hand-held stimulator was used for a preliminary analysis, then a back-projector was used for a more precise analysis and plotting (luminance ~ 50 cd mm^2). In some cases, when no response could be clearly established using the manual procedure, a computer-controlled optic bench was used for quantitative analysis.

Receptive field characteristics and analysis of neuronal response properties

Midsagittal section of the optic chiasm interrupts crossing fibres from the nasal hemiretinas but does not affect non-crossing fibres from the temporal hemiretinas. Thus, cortical units recorded on one side (here, the left hemisphere) may be excited either through the ipsilateral geniculo-cortical pathway or through the callosal route, depending on whether the ipsilateral (left) or the contralateral (right) eye is visually stimulated (Berlucchi and Rizzolatti, 1968).

The excitatory responses of the neurons to stimulation of each eye separately were compared and units were distributed into five classes of *ocular dominance:* units monocularly driven by the contralateral (1) or the ipsilateral (5) eye; units activated by both eyes with contralateral (2) or ipsilateral(4) dominance; binocular units activated equally by both eyes (3).

The routine procedure consisted of the analysis of the following neuronal response properties, as previously described (Milleret *et al.,* 1988a).
1. The *spontaneous activity* was rated in three classes: 1 (< 1 spike/s), 2 (l-2 spikes/s) and 3 (>2 spikes/s).
2. The *optimal stimulus parameters* were those eliciting the best responses (slit width and length, spot diameter, preferred orientation, direction of movement, if any, and velocity of the stimulus).
3. The *angle of selectivity* (width of orientation tuning) was the total range of orientations of an elongated stimulus which elicited a response.
4. By exploring the whole RF and its surroundings with stationary flashing stimuli, *on, off* and *on–off* zones were plotted whenever detected.
5. The *range of velocities* eliciting a response was tested using the optimal stimulus parameters (see 2 above). Strong or weak responses were noted using capital or small letters respectively: low (L, l: 0.2-10°/s); middle (M, m: 10- 100°/s); high (H, h: > 100°/s). Each neuron could then be assigned into one of the four types defined by Orban *et al.* (1981): low-pass units (L, Lm, Lmh), broad-band units
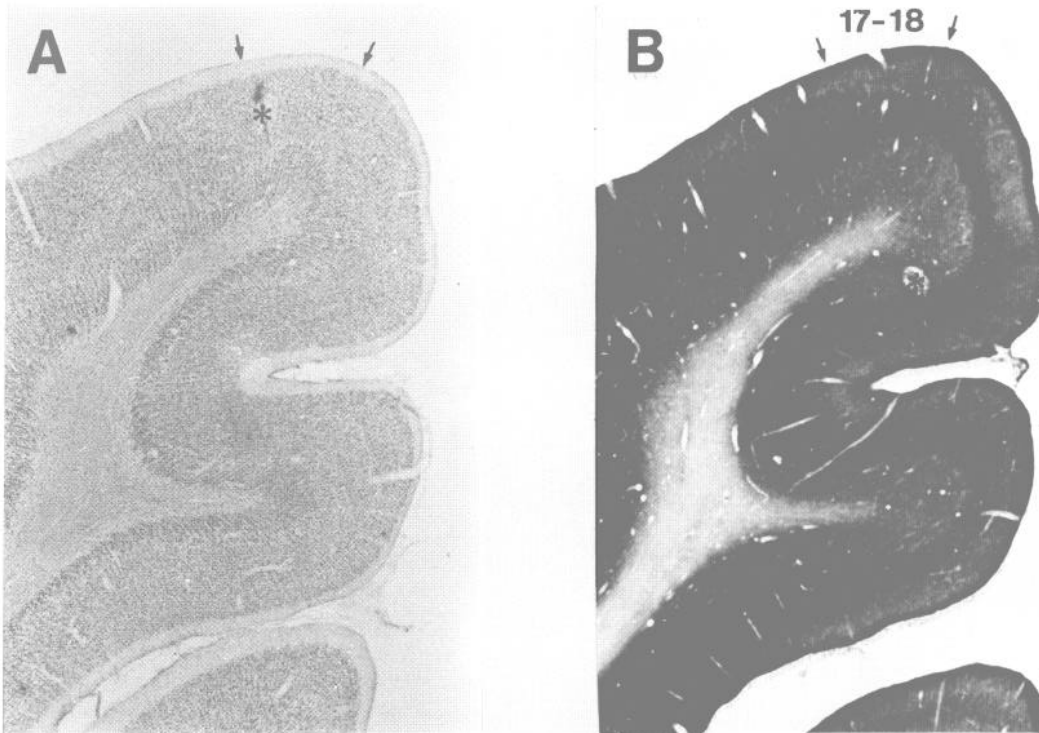
FIG. 1. Site of an electrode penetration passing through the 17/18 border and then area 17, in a 7-week-old kitten. Photomicrographs of two contiguous 50 µm thick sections are shown with (A) Nissl staining and (B) labelling for cytochrome oxidase. Two coagulations (indicated by asterisks) were performed during this penetration, one of which, visible in A, was at the 17/18 border zone and the second, in B, in area 17 (see reconstruction of this track in Fig. 2). The 17/18 border zone limits are indicated by arrows.

(LM, LMh, LMH), tuned units (lM, lMh, M, Mh), high-pass units (lmH, lMH, MH, mH, H).

6. The *quality of response* to the best stimulus parameters was subjectively rated in four classes: (1) just detectable and very irregular; (2) clear but weak; (3) clear, strong and reproducible; (4) very strong.

Since a particular emphasis was placed in this study on the RF *sizes* and on their positions in the visual field, the field limits for each eye were plotted separately using the optimal stimulus parameters to determine the 'minimum response fields'.

Histological and histochemical procedures

After the recording session, the animal was perfused with Ringer solution, then with a mixture of paraformaldehyde (3%), glutaraldehyde (0.5 %) and sucrose (4%) in phosphate buffer. The brain was removed, frozen and cut into 50 µm thick frontal sections which were alternately stained with cresyl violet or reacted to reveal cytochrome oxidase activity (Wong-Riley, 1979; Fig. 1). Both stainings facilitate identification of cortical layers and hence of the borders between visual areas (see below).

The completeness of the chiasmotomy was verified on sections stained according to the Weil or Schmued method (Schmued, 1990).

Identification of the explored visual areas

From the 4th week, electrophysiological criteria such as RF size, RF position and neuronal responsiveness to moving stimuli (Orban *et al.,* 1980; Milleret *et al.,* 1988a) roughly indicated the location of the recording site (A17, A18 or 17/18 border zone). More accurate cytoarchitectonic and histochemical criteria were used at all ages (including younger, 2- to 3-week-old kittens) based on Nissl (Otsuka and Hassler, 1962) and cytochrome oxidase activity staining (Price, 1985; Kageyama and Wong-Riley, 1986a,b; Payne, 1990b) of adjacent sections (Fig. 1). The boundaries of cortical layers were first examined in both A 17 and A 18. Layer I appeared as a cell-poor region with low cytochrome oxidase activity; its lower limit was marked by high cytochrome oxidase staining at -2 weeks of age. We did not differentiate layer II from layer III. The lower limit of layer III was identified by its typical pyramidal cells; cytochrome oxidase activity was not considered in this case because of disagreement in the literature (see references above), although we could confirm its presence in the deepest parts of layer III in both A17 and A18, as reported by Payne (1990b). Layer IV was characterized by high granular cell density and strong cytochrome oxidase activity. The latter was instead very weak in layer V, which contained sparse pyramidal cells. Finally, layer VI displayed a higher cell density and moderate, but significant cytochrome oxidase activity. The transition between A17 and A18 was then analysed, using variations in the relative thickness of some cortical layers. When moving medially from A18 towards A17, layers I and II/III get markedly thinner while layer VI gradually widens. In agreement with Payne's findings, the 17/18 border was not very sharp, but was a zone of transition (Figs 1 and 2A). Its average extent measured at the cortical surface was ~ 800 µm in the 2-week-old kittens and 1300 µm in adults.

Location of the recorded units versus spatial distribution of their receptive fields

After each experiment, all electrode tracks were reconstructed from the electrolytic lesions so that the areal location and laminar distribution of each recording site could be established (Fig. 2A). All RFs,
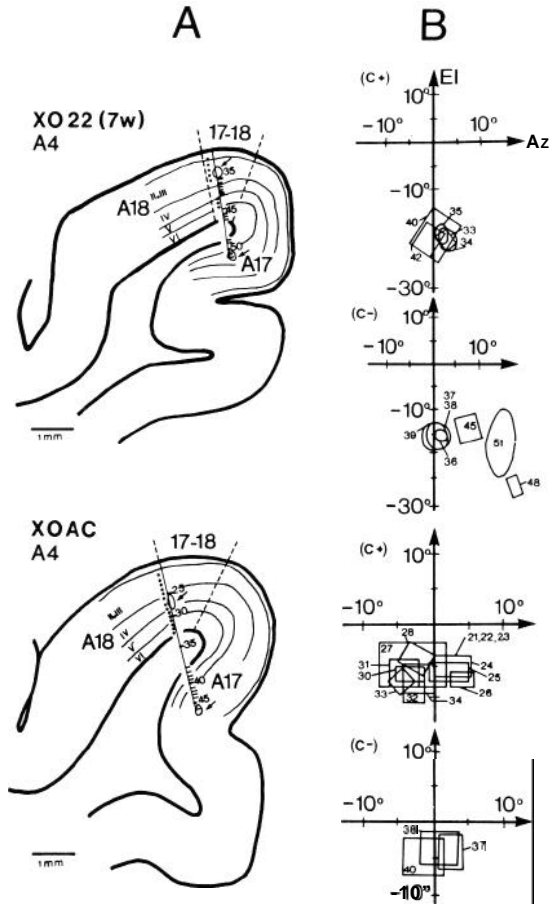
FIG. 2. Analysis of two typical penetrations through the 17/18 border and area 17 in a 7-week-old kitten (X022) and an adult (XOAC). (A) In both cases the electrode tracks were reconstructed with reference to two electrolytic lesions (arrows). Dots indicate units that could be activated through stimulation of the eye contralateral to the explored cortex via corpus callosum (C+); bars correspond to units that were only activated through the ipsilateral eye via the direct geniculo-cortical pathway (C –); numbers along the tracks designate every fifth unit recorded during the penetration. A17, A18 and 17- 18 designate area 17, area 18 and the 17/18 border respectively; II/III, IV, V and VI correspond to cortical layers. Notice that unit 35 in XOAC was located in the white matter, a few hundred micrometres away from the lower limit of layer VI. (B) Spatial distributions of the receptive fields corresponding to units in A. In each cat, the receptive fields of the C+ and C- units were mapped separately through visual stimulation of the contralateral and the ipsilateral eye respectively. Az, azimuth along the horizontal meridian; El, elevation along the central vertical meridian (in this figure and the following ones). Each rectangle or circle corresponds to the receptive field of a single unit. Rectangles designate orientation selective units and circles non-selective ones.
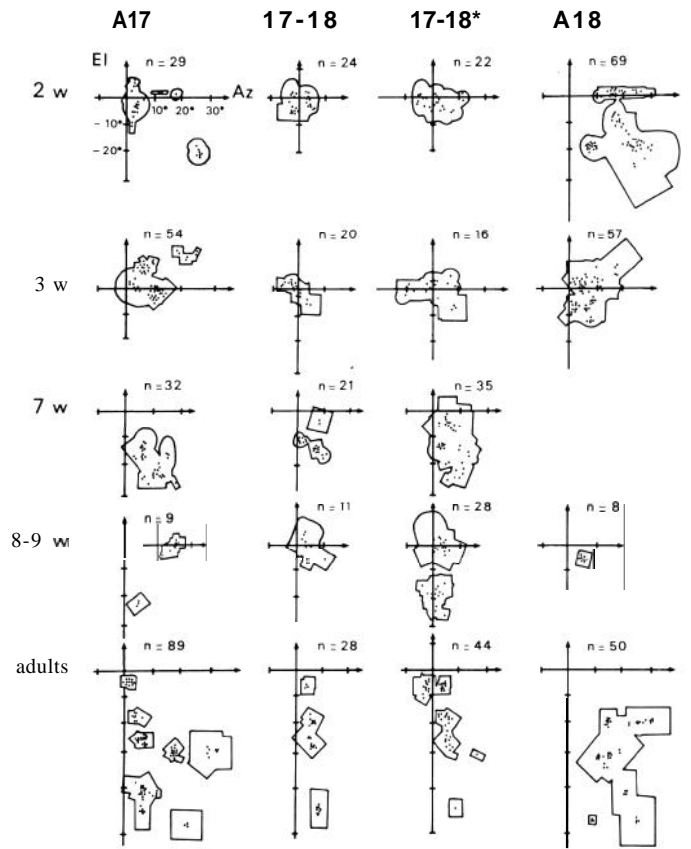

FIG. 3. Overall extents of the explored parts of the visual field based on unit responses to the eye ipsilateral to the explored cortex in A17, at the 17/18 border zone and in A18, according to age groups (in weeks, w). In each panel the extent is delimited as the envelope of the receptive field limits. Each individual receptive field is only identified by its centre marked by a dot. n, number of units in each group. Column 17/18 refers to C- units and 17/18* to C+ units.

through the ipsilateral geniculo-cortical pathway or were visually unresponsive (C - units, Table 1). Most data reported herein concern the C+ units.

C + units could be identified as early as the 12th day (Table 1), with section of the optic chiasm performed at 9 days. We did not try to identify any callosal transfer before that age because visual cortical responses are known to be very poor and sluggish even in the intact animal (Buisseret and Imbert, 1976).

Cortical location of the C+ units

Already at 12 days after birth, the C+ units were found almost exclusively at the 17/18 border zone (Table 2), as in the adult. Most C+ units were located in layers II/III, whatever the age (Fig. 4). More C + units were found in deeper layers at 2 - 3 weeks than at 7 or at 8-9 weeks or in the adults (x^2 test, P <0.05). These distributions also hold for comparisons between individual animals. A few C + units were also found in A17 (Table 2), in layers IV and V (Fig. 1), but their number did not differ significantly from that observed in the adult (x^2 test with Yates's correction, P <0.05). No C+ units were detected in A18 at any age. Three C + units were identified in the white matter of the youngest animals, beneath either the 17/ 18 border or A 17, at 100-300 µm below the lower limit of layer VI. Units of the C- type were recorded from the white matter at all ages, between 100 and 600 µm at 2-3 weeks, 100 and 300 µm at 7 weeks and from 100 to 500 µm in the adults (e.g. unit 35 in Fig. 2A).

subdivided according to the stimulated eye (Fig. 2B) and the recording site, were redrawn into the visual field with respect to the corresponding optic disc projection. Axes of the visual field [central vertical meridian (CVM) and horizontal meridian] were then positioned taking into account age-dependent changes (Milleret *et al.,* 1988b). The spatial distributions of the RFs of the units recorded in the different areas and mapped through the eye ipsilateral (Fig. 3) or contralateral (Fig. 5) to the explored cortex could thereby be established for each age group. Note that in all cases, a large extent of the visual field was explored.

Results

Out of the 1036 recorded units, 185 were visually activated through the corpus callosum (C + units, Table 1). Others were either only driven

TABLE 2. Number of cortical units as a function of the age group (in weeks) and recording site

Age (weeks)	17 C+	17 C-	17118 C+	17118 C-	A18 C+	A18 C-	WM C+	WM C-	Non loc. C+	Non loc. C-	N
2	1	28	22	29	0	72	3	8	2	55	220
3	4	56	16	25	0	70	0	9	0	10	190
4	-	-	-	-	-	-	-	-	4	91	95
7	4	36	38	22	0	0	0	6	0	0	106
8 - 9	0	9	28	11	0	8	0	0	0	0	56
Adult	1	124	44	34	0	51	0	13	18	84	369

A17, area 17; 17118, 17118 border; A18, area 18; WM, white matter; non lot., non-localized) C+ and C-, as m Table 1 N, numbers of umts per expernnental group. Total number of cells = 1036
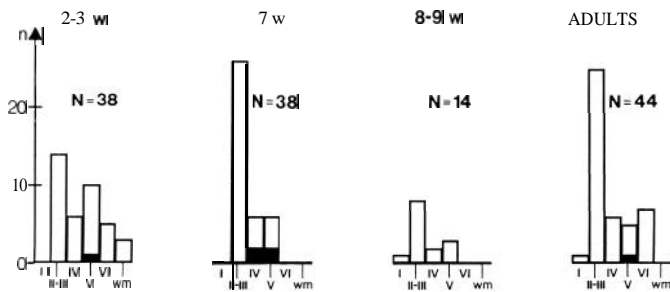

FIG. 4. Number (n) of cortical units activated through the corpus callosum (C + units) recorded in cortical layers I, II/III, N, V, VI and in the white matter (wm) in the different experimental groups. Since there were few such units in the 2-, 3-, 8- and 9-week-old kittens, these cases were grouped two by two (2−3 w; 8−9 w). Open bars indicate cell counts at the 17/18 border zone and black bars those in A17 (histograms not stacked but superimposed). N, total number of units.
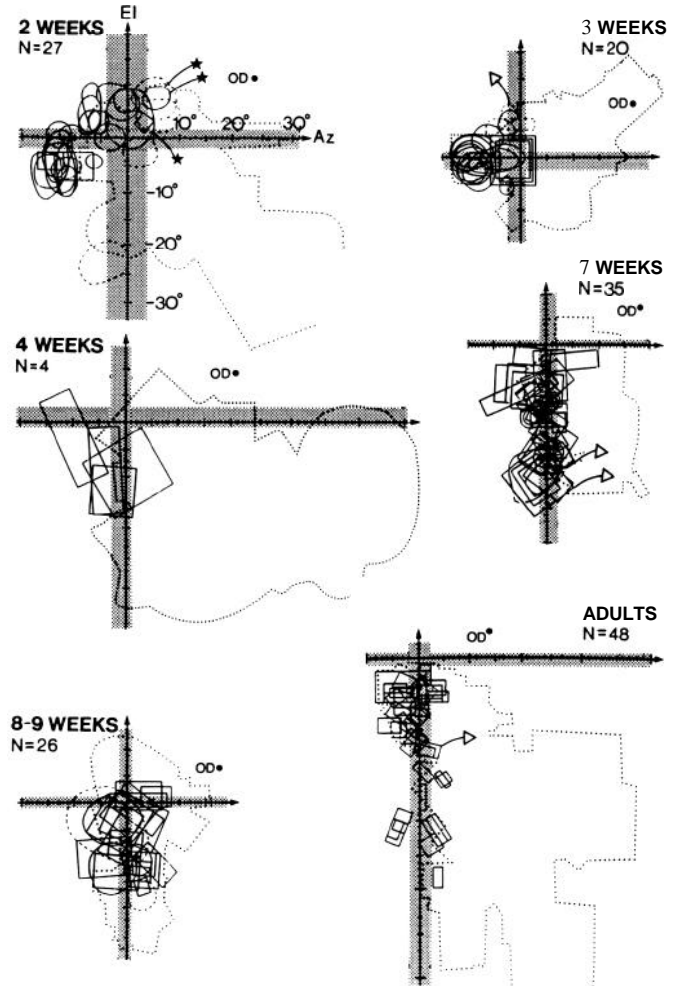

FIG. 5. Spatial distribution of the receptive fields of the C + units when activated through the corpus callosum in 2- to 9-week-old normally reared kittens and in adult cats. Shaded areas along visual axes Az and El indicate the limits of variations in position of these axes at a given age, as previously described (Milleret et al., 1988b). OD, optic disc of the right eye, stimulated to activate the callosal transfer to the left cortex. Rectangles and circles as in Figure 2. Dashed lines, receptive fields with uncertain limits. Stars and triangles, receptive fields of units recorded in the white matter and in area 17 respectively; all others were recorded from units located at the 17/18 border. N, number of mapped receptive fields of C+ units. Dotted line, extent of the explored visual field drawn as the envelope of the receptive field limits of the neurons activated through the eye ipsilateral to the explored, left cortex (C + and C- units).

Evolution of the receptive field characteristics of the C + units

Since most C + units were binocularly activated (see below), their RF characteristics were determined through stimulation of each eye separately.

Spatial distribution of the C+ unit receptive fields

When C + units recorded at the 17/18 border zone were stimulated through the eye contralateral to the explored cortex, their RF contours ('callosal RFs') either included the CVM or were markedly displaced towards the ipsilateral hemifield. Their maximal extension into the ipsilateral hemifield could reach as much as 19 deg from the CVM in the youngest animals (Fig. 5; 2 weeks) and decreased to ~8 deg in the adult. In both Al7 and the white matter, all callosal RFs included the CVM and their lateral extension was very limited (Fig. 5; 2, 3 and 7 weeks and adults). When 17/18 units were stimulated through the ipsilateral eye, their RF contours ('geniculo-cortical RFs') also generally included the CVM (Fig. 3; 17/18*) and displayed some lateral extension: up to 13 deg in the youngest animals but ~8 deg in the adults (at least in the central part of the visual field). This indicates that the visual angle subtended by the callosal recipient zone decreases during development, despite the increase in width of the architectonically defined 17/18 border zone (see Materials and methods).

Size of the C+ unit receptive fields

Callosal RFs never increased in size with elevation from the area centralis projection along the CVM , even at − 30 deg (Fig. 5; 7 weeks) or -40 deg (Fig. 5; adults). However, their mean size decreased with age from 29.7 ± 5.5 deg^2 (n = 46) at 2-3 weeks to 10.6 ± 2.8 deg^2 (n = 48) in the adult (Kolmogorov-Smimov test at 5%). At 7-9 weeks, large RFs still persisted so that the mean RF size (37.4 ± 7.5 deg^2, n = 61) did not differ significantly from that observed at 2 − 3 weeks. The same was true for geniculo-cortical RFs. Their size was generally not significantly different from that of callosal RFs (Kolmogorov-Smirnov test at 5%). fluwever there were some individual exceptions (e.g. Fig. 6).

Disparity of callosal and geniculo-cortical RFs

The two RFs of each binocular cell (callosal and geniculo-cortical RFs) were mapped. Disparity in RF position became apparent (Fig. 6). It was especially clear and frequent at 2 weeks, with 21 out of 26 binocular C+ units showing angular distances between their RF centres reaching

up to 25 deg (mean distance, 16 deg). Older animals also contained units displaying disparity. However they were less frequent the older the animal, with 12 of 18 binocular units at 3 weeks, 21 of 3 1 at 7 weeks, 8 of 26 at 8-9 weeks and 10 of 32 at adulthood (significant

difference between 2 weeks and adults: x^2 test, P < 0.05). Similarly, the mean and maximal angular distances between RF centres decreased with age: they were respectively 14 and 18 deg at 3 weeks, 12 and 1.5 deg at 7 weeks, 5.5 and 14 deg at 8-9 weeks and 1.6 and 5 deg in the adults. Interestingly, the angular distance between RF centres and their relative position varied from unit to unit in the course of a single penetration (Fig. 6; 3 and 7 weeks, adults).

Development of C + unit properties

Comparing neuronal response properties across the experimental groups clearly indicated that the interhemispheric transfer of visual information was subject to a classical maturational process (Milleret *et al.,* 1988a). These properties changed similarly whichever eye was stimulated, except for some differences in strength of the responses.

Spontaneous activity

The mean spontaneous activity of most C + units (97 %) was very low (< 1 spike/s) before 4 weeks. It then progressively increased, with -50% of the unit sample reaching up to 1-2 spikes/s at 8-9 weeks. Only very few units discharged at > 2 spikes/s, even in the adult.

Unit types

Using stationary flashed stimuli, the C+ units responded most often with uniform on/off responses, and hence, were 'complex' according to Hubel and Wiesel's criteria (1962). In developmental studies of the visual cortex, two unit types were previously defined based on their responses to moving oriented stimuli (Imbert and Buisseret, 1975): orientation-non-selective (NS) and orientation-selective (S) units. The same distinction could be made in our C+ unit sample whatever the recording site (cortex or white matter), with an increase in the relative proportion of S units with age (Fig. 7A). The difference between the proportions of S and NS units was statistically significant between 2- to 3-week-old animals and adults and between 7- to 9-week-old animals and adults (x^2 test with Yates' correction, P <0.05 in both cases).

In summary, the 17/18 border zone units are not fully mature at 7-9 weeks as assessed from orientation selectivity, RF size or position disparity.

Distribution of the preferred orientations of the selective C+ units

The distribution of the preferred orientations encoded by the selective C+ units (Fig. 8) showed that in adults all orientations were equally represented. In the 7-week-old animals the distribution tended to be
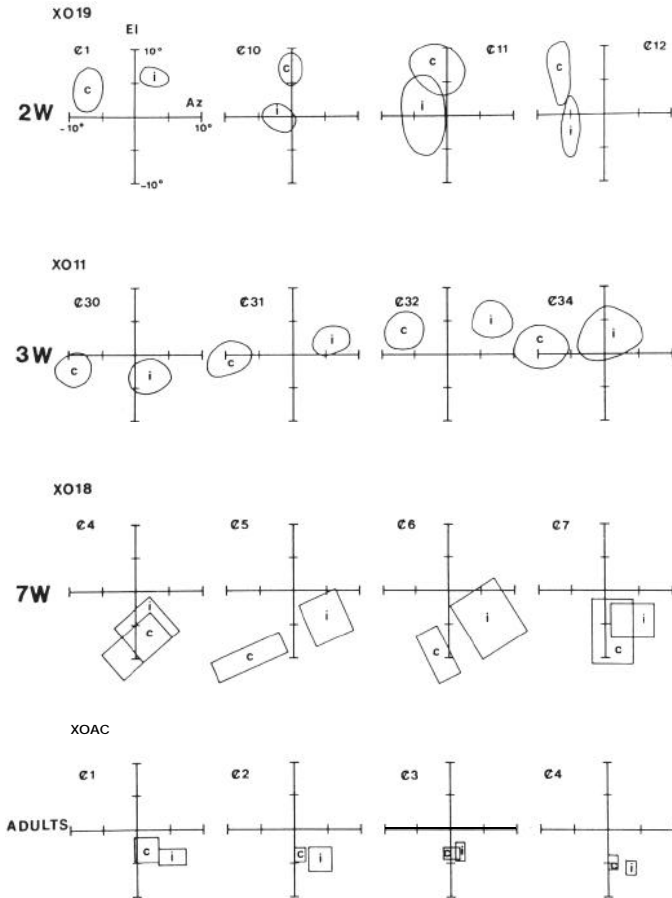

FIG. 6. Spatial location of the ipsilateral (i) and contralateral (c) receptive fields of selected binocular cortical units recorded at the 17/18 border in four cats (X019, X011, X018 and XOAC). Four different units (Ø) for each group are presented, in four panels from the most superficial to the deepest in the cortex, from left to right. 2W, 3W, 7W indicate the age of kittens in weeks.
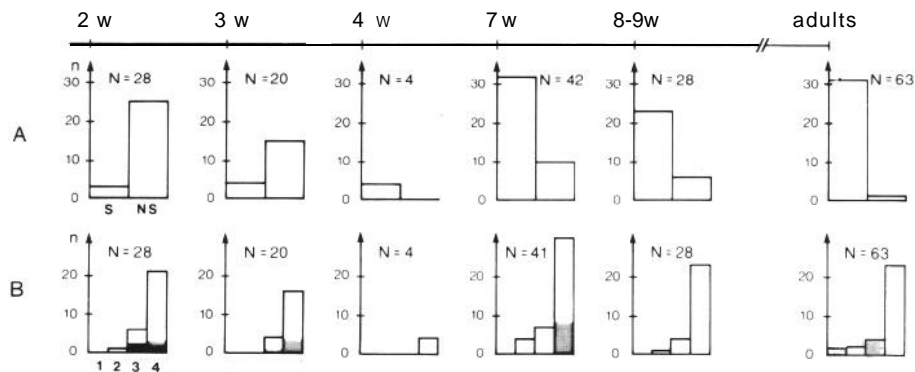

FIG. 7. Development of orientation selectivity and ocular dominance of the C + units from 2 weeks (2 w) of age to adulthood (adults). N, total number of units; n, number of units per class. (A) Number of each unit type. S, orientation-selective units; NS, non-selective units. (B) Distribution of the C+ units into the four possible classes of ocular dominance: 1, units only driven through the contralateral eye via the corpus callosum; 2 and 4, units activated through both eyes, with dominance of the contralateral and ipsilateral eye respectively; 3, binocular units equally activated through both eyes. Open, grey and black areas indicate the units recorded at the 17/18 border, in Al7 and in the white matter respectively. All cell numbers are counted from the abscissa (as in Fig. 4).
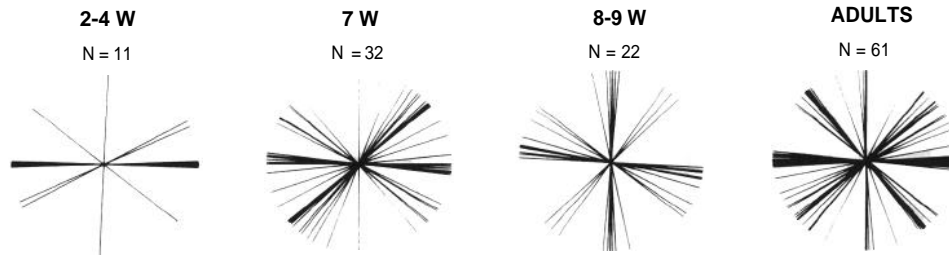
FIG. 8. Distribution of the preferred orientations encoded by the orientation-selective C+ units at various ages (2 -4 weeks, 7 weeks, 8-9 weeks and adults). This distribution is represented as a polar diagram, with the orientation encoded by each unit being indicated by a bar. N, number of units per group.

similar to that in the adults. In younger animals the S unit sample was too small to draw any clear conclusion.

Range of effective velocities of the moving stimulus

Globally, the optimal stimulus velocities progressively increased with age. In the youngest animals, most of the C+ units were of the low-pass type; in older animals, most were either low-pass or broad-band. Only a few tuned or high-pass units were found even in the adults.

Quality of response

In the youngest kittens, one third of the neurons behaved almost as adult neurons when callosally activated (class 2 as defined in Materials and methods) while others responded sluggishly (class 1). Intervals of up to 1 min between stimulus presentations were sometimes necessary to elicit reliable responses and in some cases their existence could only be ascertained through quantitative analysis (Fig. 9). On average, the response quality increased with age to become adult-like at -8-9 weeks. No particularly vigorous responses (class 4), as observed in A18 of intact animals (Milleret *et al.,* 1988a), were ever obtained in the chiasmotomized animals at any age. Most C+ units responded better to ipsilateral than to contralateral stimuli.

Ocular dominance properties of the C+ units

Contrasting with other neuronal response properties, the ocular dominance distribution of the C+ units was similar in animals with different ages. Activation was often more efficient through the ipsilateral ('direct' geniculo-cortical pathway) than through the contralateral eye (callosal transfer; see above), for both the cortical units and the few units recorded within the white matter (Fig. 7B; 2 weeks).

Discussion

In summary, we show that supraliminar callosal transfer of visual information is almost exclusively limited to the histologically determined 17/18 border zone, as early as the 12th day as in the adult cat, however with some immature characteristics.

Comparison with previous data from adults

Most of our data obtained in the adult confirms previous findings (Choudhury *et al.,* 1965; Vesbaesya *et al.,* 1967; Berlucchi and Rizzolatti, 1968; Harvey, 1980; Innocenti, 1980; Cynader *et al.,* 1981; LeporC and Guillemot, 1982; Milleret and Buser, 1990; Payne, 1990a,b; Payne *et al.,* 1991). Visual cortical units activated through the corpus callosum (C+ units) are well known to be mainly located in the supragranular layers of the 17/18 border zone and to be complex neurons with RFs located on or close to the CVM. These units are orientation-selective, with all orientations being equally represented. After chiasmotomy, they can be activated from both eyes with a preference for the ipsilateral one through the 'direct' geniculo-cortical pathway. We cannot compare the mean RF sizes of the C+ units to
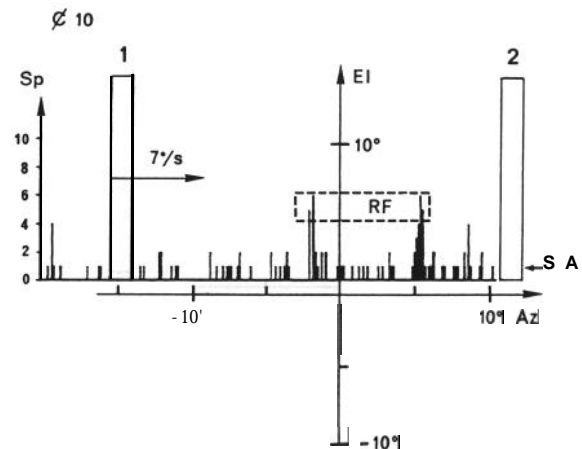

FIG. 9. Example of a quantitative analysis of a transcallosal response that could not be identified through the manual stimulation procedure. The unit was recorded from Al7 in a 3-week-old kitten (X01 1). A vertical slit was moved horizontally in the visual field from position 1 to position 2 (velocity, 7 deg/s) across the presumed receptive field (dashed contour, RF). The neuronal activity was collected as a peristimulus time histogram made up of 50 stimulus presentations. Sp, number of spikes; SA, mean spontaneous activity level. Bin width, 10 ms.

previous data since the delays between sectioning the optic chiasm and the recording session differ between protocols. We know from our own observations (Milleret and Buser, 1993) that some progressive reorganizations occur during these delays.

We observed that C+ unit RFs did not generally increase in size with eccentricity along the CVM, contrasting with the other C- visual cortical neurons (Hubel and Wiesel, 1965). This particular feature presumably contributes to fine-grain perception along the vertical central part of the visual field. Moreover, binocular C+ units displayed disparity in the position of their RPs (Fig. 6), which could be related to the role of callosal connections in depth perception along the CVM (see below).

Synaptic connections into the cortex

The transient callosal projecting neurons which are present until 3 months after birth do not appear to make supraliminar contacts within the contralateral areas 17 and 18 outside their common boundary. This does not exclude developmental changes within the 17/18 border zone itself, such as a selective stabilization of some developing synapses (Changeux and Danchin, 1976) or a regression of terminal boutons (Sur *et al.,* 1984) and/or of callosal terminal arbors. The changes in C + neuron properties and the decrease of their laminar extension with age support such possibilities.

The absence of callosal transfer in both Al8 and Al7 during the

first postnatal month cannot be due to the suppression of the crossed retinal inputs through chiasmotomy, which suppresses the callosal output to the contralateral side, because even in the youngest animals neurons of both areas could be activated through the ipsilateral route (Milleret et al., 1988a).

It is unclear whether areas 17 and/or 18 receive infraliminar contralateral influences, undetectable in our experimental conditions since retino-geniculate, geniculo-cortical and intracortical pathways form transient functional (infra- and/or supraliminar) synapses with inappropriate targets during development (Levay et al., 1978; Shatz and Kirkwood, 1984; Luhmann et al., 1990). Neither our results nor recent anatomical findings solve this problem (see Introduction).

Contrasting with the above data, callosal transfer was observed in both A17 and A18 in addition to the 17/18 border zone in the same experimental conditions, except that the animals were subjected to early induced strabismus or monocular deprivation (Milleret and Houzel, 1991; Houzel et al., 1992).

Are the subplate neurons temporary targets for ingrowing callosal axons?

Our recordings from the white matter suggest that some transient callosal axons may establish synaptic contacts within the contralateral subplate, below the 17/18 border zone and areas 17 and 18 (Table 2). This is consistent with the observation of numerous subplate neurons in young kittens (Chun and Shatz, 1988). The units recorded from the white matter in our series also exhibited a clear and dominant input from the ipsilateral geniculo-cortical pathway (Fig. 7B), making them unlikely to be fibres. Thus, subplate neurons which have been shown to be transient synaptic links between the growing geniculo-cortical axons and their ultimate target neurons in the cortex (Friauf et al., 1990) could also be a transient relay for callosal axons.

Since subplate neurons are almost absent from the adult cat (Chun and Shatz, 1988), the C– units recorded from the white matter in this group could be either geniculo-cortical or cortico-geniculate or intrahemispheric cortico-cortical fibers (Ramón y Cajal, 1911) or else basal dendrites of layer VI neurons (Katz, 1987; Vercelli and Innocenti, personal communication). The latter possibility cannot be discarded since some C– units were recorded 100 – 300 μm below the VIth layer, where pericarya do not occur.

Corpus callosum and representation of the ipsilateral visual field during development

Our results confirm recent data (Milleret and Buser, 1990; Payne, 1990a, b; Milleret and Buser, 1993) showing that the corpus callosum of the adult cat is involved in the representation of the ipsilateral visual field (Pig. 5). We further demonstrate that this ipsilateral representation is present as early as the 12th day and is even larger than in the adult.

Inferring as we did the position of the area centralis projection from that of the optic disc (see Materials and methods) might have introduced errors in the mapping of the central vertical meridian, in turn affecting the accurate determination of the callosal RF's extension into the ipsilateral hemifield, especially in the youngest animals (see shaded area along CVM in Fig. 5). However this possibility can be ruled out. First, very lateral RFs were observed in several of the youngest kittens. Second, such a large ipsilateral extension was also observed in slightly older animals which displayed intraocular media already clear enough to localize their area centralis by the conventional method (Bonds and Freeman, 1978). These animals showed very limited inter-individual variations in their retinal landmarks (Fig. 5; see also Fig. 2 in Milleret et al., 1988b). Further, the ipsilateral extension progressively decreased with age. Finally, the callosal RFs were always localized either close to the CVM or in the ipsilateral hemifield (Fig. 5), never in the contralateral hemifield.

The reduction in the ipsilateral callosal RFs with age can be explained in at least three ways. Due to the growth of the eye, a given retinal area sees a larger portion of the visual space in the kitten than in the adult. This induces a progressive decrease in the angular distance between area centralis projection and optic disc projection (Milleret et al., 1988a, b) and could also explain the change in the ipsilateral extension.

A spatial restriction of the terminal field of the callosal fibres could also result from the visual angle represented in the callosal recipient zone (17/18 border) decreasing during development as shown in Figure 3 (17/18*). The decrease in extension of the ipsilateral visual field would in this case result from a restriction of the portion of the visual field allocated to the callosal pathway.

Another possible explanation, namely the reduction in size of the callosal RFs with age, does not hold since no significant decrease in the mean RF size was found between 2 – 3 and 7 – 9 weeks. The most likely explanation is the elimination of the RFs located in the ipsilateral visual field. As suggested by previous anatomical data, the development of the callosal system requires cortical neurons located at some distance from the CVM representation to withdraw their callosal axons (Innocenti and Clarke, 1984). The temporary extension could thus be explained by assuming that some temporary exuberant callosal projecting pericarya in A17 and/or in A18 converge onto the contralateral 17/18 border zone in kittens. Thus, some heterotopic interhemispheric connections would be functional early in life.

Corpus callosum and development of depth perception along the central vertical meridian

The geniculo-cortical and callosal RFs of the binocular C+ units did not coincide in many cases (Fig. 6). This was unlikely to be due to errors in mapping the area centralis projection because the absence of correlation was consistently found in animals of all ages; the mapped RFs were always located in the expected region of the visual space. Moreover, the disparity in RF position tended to decrease with age and could differ between successive units in a given penetration.

The disparity values observed in the adult match those previously reported at the 17/18 border zone either in the intact animal (Pettigrew and Dreher, 1987) or after chiasmotomy (Leopore et al., 1992). They are, however, larger than those previously reported for A17 of intact kittens (Pettigrew, 1974; Freeman and Ohzawa, 1992), probably because of the difference in cell populations and the possibility that, in our case, the callosal route involves transient heterotopic connections.

Given the now classical views on neuronal mechanisms for binocular depth perception (Barlow et al., 1967), with involvement of the corpus callosum (see Jeeves, 1991 for review), the decrease in disparity with age may be related to the development of stereoscopic perception along the CVM: both phenomena follow the same time course (Graves et al., 1987).

Acknowledgements

Abbreviations

A17   visual cortical area 17
A18   visual cortical area 18

C+	units that could be activated through stimulation of the eye contralateral to the explored cortex via the corpus callosum
C-	units that were only activated through the ipsilateral eye via the direct geniculo-cortical pathway
CVM	central vertical meridian of the visual field
NS	orientation-non-selective cell
RF	receptive field
S	orientation-selective cell

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