Horizontal Interactions in Cat Striate Cortex: II. A Current Source-Density Analysis

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Abstract

The current source-density (CSD) analysis was used to investigate the organization of tangential synaptic connections in primary visual cortex of normally reared (NR) kittens and of NR, binocularly deprived (BD) and dark-reared (DR) adult cats. Laminar profiles of field potentials, elicited by intracortical microstimulation were measured at various distances from the stimulating electrodes. To exclude contamination by axon collaterals of antidromically stimulated thalamo-cortical fibres, these were destroyed by injecting the cytotoxin N-methyl-D-aspartate (NMDA) into the lateral geniculate nucleus 13–27 days before recording. The CSD profiles revealed distinct layer-specific patterns of lateral spread of activity. Invariably, the most prominent, longest-lasting and far reaching responses were recorded in supragranular layers. Responses in layer IV were brief and confined to the vicinity of the stimulation site. Responses in infragranular layers spread as far as those in supragranular layers, but were of smaller amplitude. Latency considerations, the results of double shock stimulation, and the effects of translaminar cuts, suggest that these responses were monosynaptic and mediated by intracortical pathways with a conduction velocity of 0.3–5 m/s. The spatial spread of these responses changed substantially with age but was not influenced by visual deprivation. In NR adults, supragranular responses were recordable up to 2.5 mm from the stimulation site and layer IV responses up to 1 mm from the stimulation site. In kittens, the former responses spread up to 5 mm and the latter up to 2 mm from the site of stimulation. The amplitude of the responses decreased with distance from the stimulation site. This decrease was not always monotonic suggesting inhomogeneities in the tangential projections. The laminar distribution of current sinks and sources indicates that the pathways mediating tangential interactions form excitatory synapses on apical dendrites of pyramidal cells. It is concluded that the spatial spread of tangential excitatory interactions decreases with age, but that neither the laminar pattern nor the age-dependent reduction in the strength of tangential interactions are influenced by visual deprivation.

Introduction

There is a large body of evidence concerning tangential intrinsic connections in neocortical areas (for review see Gilbert, 1985; Gilbert and Wiesel, 1985; Martin, 1984; Swindale, 1982). However, comparatively little is known about the physiological properties of this axonal system. Using cross-correlation analysis to study tangential interactions Ts'io et al. (1986) found excitatory coupling between neurons with similar orientation specificity over distances of up to 3 mm and more recently Gray et al. (1989) and Gray and Singer (1989) demonstrated correlations in oscillatory responses between columns with similar orientation preference as far apart as 7 mm.

A physiological method that is complementary to single unit recording and particularly well adapted for functional visualization of axonal projection patterns in laminated structures is the current source-density (CSD) analysis of electrically evoked field potentials. This method has been applied in in vivo studies of the visual cortex (Mitzdorf and Singer, 1978) and allows determination of a number of important properties of neuronal connections: the conduction velocity of the activated pathways, the nature and laminar pattern of synaptic interactions, and to some extent also the location and geometry of the postsynaptic target cells (for review see Mitzdorf, 1985).

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<table>
<thead>
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<th>Animal</th>
<th>Age at LGN lesion [pnd]</th>
<th>Age at experiment [pnd]</th>
<th>Number of CSD profiles</th>
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<tr>
<td>NR-I</td>
<td>24</td>
<td>39</td>
<td>18</td>
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<tr>
<td>NR-II</td>
<td>36</td>
<td>49</td>
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<tr>
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<td>36</td>
<td>63</td>
<td>28</td>
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<tr>
<td>NR-IV</td>
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<td>adult + 13</td>
<td>21</td>
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<tr>
<td>NR-V</td>
<td>adult</td>
<td>adult + 15</td>
<td>18</td>
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<tr>
<td>NR-VI</td>
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<td>adult + 19</td>
<td>10</td>
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<tr>
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<tr>
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<td>adult + 13</td>
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In this study the authors have applied the CSD-technique to investigate the lateral spread of activity. This analysis has been extended to the developing visual cortex because of the evidence for extensive and experience-dependent reorganization of these connections during early postnatal life (Luhmann et al., 1986, 1990a,b).

Some of these results have been published in abstract form (Luhmann et al., 1987).

Materials and methods

Animals, rearing conditions, and LGN lesions

Ten cats, bred in the institute's cat colony and reared under various conditions were used in this study.

Six animals were reared normally (NR). Two animals were binocularly deprived (BD) by eyelid suture from the beginning of the second postnatal week. Another two cats were reared in a darkroom (DR) from birth until the experiment.

The lateral geniculate nucleus (LGN) was lesioned bilaterally by injection of the excitotoxin N-methyl-D-aspartate (NMDA) 13–27 days before recording to exclude any contribution from antidromically activated thalamo-cortical axons to the tangential spread of activity in striate cortex. The animals were anaesthetized with 30 mg/kg ketamine hydrochloride and 15 mg/kg xylazine hydrochloride i.m. and then received intrageniculate injections of 2.0–3.5 μl 5% NMDA (Sigma). This resulted in complete destruction of the LGN and surrounding thalamic nuclei as evidenced by the massive gliosis in Nissl-stained sections (Fig. 1).

Surgical preparation, electrical stimulation, and recording of evoked potentials

After premedication with 0.01 mg atropine sulphate i.m., anaesthesia was induced by an i.m. injection of 30 mg/kg ketamine hydrochloride and 15 mg/kg xylazine hydrochloride. During recording anaesthesia was maintained by artificial ventilation with a mixture of N2O/O2 (70:30) and 0.2–0.4% halothane. The electrocardiogram and electroencephalogram were monitored continuously during the experiment and body temperature and end-tidal CO2 were kept at 38° C and 3.8%, respectively. Animals were paralysed with an i.v. infusion of 0.7 mg/kg×h hexacarbacholine bromide. To compensate for the renal and respiratory loss of fluids a solution of 5% glucose and saline (5:1) was administered at a rate of 3–6 ml/h through an orally inserted gastric catheter. After trepanation and removal of the dura over the dorsal crest of the lateral gyrus, two or three concentric stimulating electrodes were positioned stereotaxically at different AP-planes to a depth of approximately 1.5 mm into the primary visual cortex. Typically, when two stimulation electrodes were inserted, these were placed 6–8 mm apart at the rostral and caudal extremity of the trepanation on the dorsal crest of area 17 at AP planes A+2 and A-6, respectively. When a third electrode was used, it was inserted midway between the two and 1 mm lateral of the sagittal plane defined by the two electrodes. Field potentials were recorded with double-barrelled

![Fig. 1](image_url)
micropipettes with tip diameters of 3–4 μm. One barrel was filled with 1.5 M potassium-citrate (DCimpedance: 3–8 MΩ) for recording. The second barrel was filled with 5% horseradish peroxidase (HRP, Sigma type VI) in 0.1 M phosphate buffer (PB), pH 7.4, for marking the position of the electrode in the tissue. The recording electrode was inserted orthogonal to the cortical surface and the first penetration was started at a stereotaxically defined distance of 1 mm from the posterior stimulating electrode. After placement of all electrodes the cortex was covered with warm 4% agar in saline. Electrical stimuli of 50 μs duration were administered as double-shocks separated by 20 or 50 ms. Stimulus intensities were adjusted to saturation of the field potential and ranged from 3–10 μA. The recording electrode was connected to a FET equipped input stage (impedance 10^14 Ω) with conventional capacity compensation and the amplified signals were bandpass-filtered from 1 Hz to 3 kHz. Laminar profiles of field potentials were obtained by withdrawing the recording pipette in 50 μm steps from white matter to the pial surface. At each point, ten responses were averaged for each of the individual stimulating electrodes and stored digitally on a PDP 8. After completion of an electrode track, the electrode was advanced again into the cortex and HRP was deposited iontophoretically at a predetermined depth (+0.5–1.0 μA for 30 s; see Fig. 2). The recording electrode was then withdrawn, it was reinserted under microscopic control into the cortex 0.5–1.0 mm rostral of the previous recording site in the sagittal plane defined by the posterior and anterior stimulation electrode. This procedure was repeated until the recording electrode was within 1 mm of the anterior stimulation electrode. Recordings were made from both hemispheres with the exception of cat DR-II. The numbers of complete depth profiles obtained per animal are listed in Table 1.

Cortical transection
To control for the influence of volume conduction, and to verify that the tangential spread of activity was actually mediated by intracortical neuronal pathways, a vertical incision was made in one kitten (NR-I) between one of the stimulating electrodes and the recording electrode. After completion of a series of laminar profiles a fine scalpel was used to cut the cortex parallel to the frontal plane for a length of about 2.5 mm. Measurements were then repeated. Responses from the second stimulation electrode served as a control for the tissue preservation at the recording site. The depth of the cut was verified histologically.

Perfusion and histology
After two or three days of recording, animals received a lethal injection of sodium pentobarbitone (Nembutal®). They were perfused transcardially with saline, then with fixative containing 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer (PB) and finally with 20% sucrose in 0.1 M PB for cryo-protection. Tissue blocks containing the LGN and the visual cortex were infiltrated over night with 30% sucrose in 0.1 M PB at 4°C. A freezing microtome was used to cut 50–60 μm thick frontal sections of the LGN and parasagittal sections of the visual cortex. Sections from the LGN were Nissl-stained and cortical sections were either Nissl-stained or reacted with diaminobenzidine (DAB) for horseradish peroxidase (HRP) (Adams, 1977). Laminar boundaries of cortical sections were identified according to the criteria of Otuka and Hassler (1962), Garey (1971), and Innocenti et al. (1986). The relationship between cortical depth and the location of current sources and sinks was determined by comparing the corresponding DAB-reacted and Nissl-stained sections with the CSD profiles (Fig. 2).

CSD method
The authors applied the one-dimensional CSD analysis (Nicholson and Freeman, 1975), because it was previously established that the four prerequisites for its applicability are fulfilled in striate cortex: (i) the extracellular space has the properties of an Ohmic conductor; (ii) the electrical field is quasistatic; (iii) the conductivity in the extracellular space shows no significant changes with cortical depth; and (iv) the field potential is translationally invariant in the two directions parallel to the cortical layers (for a review of these conditions see Mitziolof, 1985). Under these assumptions, CSD profiles can be calculated according to the following formula:

\[
\frac{\delta^2 \phi}{\alpha^2} = \frac{\phi(z+\Delta z) - 2\phi(z) + \phi(z-\Delta z)}{(\Delta z)^2}
\]

where \( \phi \) is the extracellular field potential, \( z \) is the co-ordinate perpendicular to the layers, \( \Delta z \) is the distance between adjacent layers.
recording sites (50 \mu m in the present study), and n \cdot \Delta z is the differentiation grid (in the present study n was 3). In the following CSD profiles (Figs 3–8) current sinks are indicated by upward deflections (filled) and current sources by downward deflections (hatched).

Results

Thalamic lesions

In all animals in which the thalamic lesions were examined histologically (n = 6) light microscopic analysis of Nissl-stained frontal sections revealed massive gliosis in the thalamic region containing the LGN (Fig. 1B). No significant differences were detectable in the extent of gliosis between cats that were lesioned 13 days before recording (NR-II, NR-IV, DR-II) and those lesioned 19–20 days before the experiment (NR-III, NR-VI, DR-I).

Normally reared adult cats

We analysed 49 CSD depth profiles in striate cortex of three NR adult cats (Table 1). CSD profiles, computed from field potentials in the immediate vicinity (1 mm) of the stimulation electrode contained three distinct clusters of current sinks that differed in latency, duration, amplitude, and laminar position (Fig. 3B).

The earliest sinks occurred in upper layer IV and extended from 0.6–0.9 mm below the cortical surface. Their latency ranged from 0.8–1.0 ms and their duration was 3.5–4.5 ms. Their amplitudes were small and corresponding sources were not distinguishable. With a latency of 2.5–3.5 ms poststimulus, large sinks appeared in layer V at a depth of 1.2–1.4 mm. They lasted about 10–12 ms and had corresponding sources in layer VI at a depth of 1.6–1.7 mm. The most prominent and long-lasting sinks occurred in layers II and upper III at a depth of 0.05–0.4 mm. The onset latencies of these sinks increased towards the cortical surface and ranged from about 3 ms in upper layer III to 5.5 ms in upper layer II. This latency increase was paralleled by an increase in sink duration from 5 ms to more than 15 ms. The corresponding sources of these superficial sinks were located in lower layer III and upper layer IV at a depth of 0.45–1.1 mm. The CSD distribution elicited by the second of the two stimuli had the same spatio-temporal configuration, but all sinks and sources were larger in amplitude than after the first stimulus.

With increasing distance from the stimulation electrode the amplitudes of all sinks decreased. Responses recorded at a distance of 2.5 mm from the stimulation electrode are shown in Figure 3C. The short-latency layer IV input was no longer detectable and the sink in layer V was only faintly distinguishable and after only the second stimulus. The sinks in supragranular layers, however, were still prominent and were also readily evoked by the first stimulus. The latency of these sinks had increased to 7.5 ms which corresponds to a distance-dependent increase of about 3 ms/mm. The corresponding sources were again located in layer III and upper layer IV and responses to the second stimulus were larger than those to the first. Responses were no longer resolvable at recording distances of more than 3 mm.
Normally reared kittens

The authors obtained 77 CSD depth profiles from three NR kittens. The laminar pattern of the CSD profiles obtained from kitten striate cortex closely resembled that in the adult. However, in the kitten, responses were resolvable over much larger distances than in the adult. In the kitten, prominent sinks were consistently recorded 4 mm from the stimulation electrode and were common even at distances of 5 mm.

Typical examples of responses recorded in a 63-day-old kitten at distances of 2, 3 and 4 mm from the stimulation site are shown in Figure 4. The short latency sink in layer IV was still present at a distance of 2 mm and had an onset latency of 2.0–2.5 ms. The amplitude of this sink was small and its duration short (Fig. 4A). The sinks in layer V began with a latency of about 2.5 ms, were still large at 2 mm, and their corresponding sources were in layer VI. Again the most prominent and long lasting sinks were in supragranular layers. As in the adult, their onset latencies increased towards the cortical surface and in this case ranged from 2.5–6.0 ms. The sources of these superficial sinks were located both, above in layer I/upper II at a depth of 0–0.3 mm, and below in layer III/upper IV at a depth of 0.5–0.7 mm. At a distance of 3 mm from the stimulation site, the sinks in layer IV were no longer resolvable, but those in supra- and infragranular layers were virtually unchanged (Fig. 4B). Only the onset latencies had increased by about 1 ms. A further increase of the lateral distance to 4 mm led to a consistent change in the laminar pattern. As exemplified in Figure 4C, the sink in layer V was no longer present. Instead, there were again prominent sinks in layer IV. These differed from the short latency sinks in layer IV because they were clearly associated with sources in layer VI and possibly also with sources in upper layer IV and layer III (see Discussion). The onset latency of these sinks was about 7 ms. The sinks in supragranular layers were still distinguishable but small, started after a delay of approximately 8 ms and lasted for up to 20 ms.

Beyond 4 mm, responses were restricted largely to supragranular layers. The example shown in Figure 5 is from the same kitten as that documented in Figure 4, but field potentials were recorded in the other hemisphere 5 mm from the stimulation electrode. In this case, large sinks occurred in layer II at a depth of 0.1–0.7 mm (Fig. 5B). These had an onset latency of about 10 ms and lasted up to 25 ms. The sinks in infragranular layers were no longer present and those in lamina IV had become very small. This pattern of lateral interactions was basically the same in all young NR kittens, but there were some age-dependent variations.

Responses in the two youngest kittens NR-I (39 days) and NR-II (49 days), differed from those obtained in the older kitten NR-III (63 days). As expected, the responses in younger kittens had longer latencies and the distance-dependent latency increase was more pronounced than in the older kitten and adults. Thus, as exemplified in Figure 6, onset latencies of characteristic supragranular sinks increased from 2–3 ms at a distance of 1 mm (Fig. 6A) to about 8 ms at 2 mm distance (Fig. 6B). Surprisingly, however, onset latencies of the supragranular sinks did not increase proportionately as recording distance was increased beyond 3 mm, but rather remained in the same range of about 10 ms (Fig. 6C). Another difference between the younger and older kitten was that in the younger animals very large and delayed sinks occurred in layer IV at short recording distances (Fig. 6A). These were clearly distinguished from the small early sinks because they had prominent sources both in supra- and infragranular layers. The onset latency of these delayed sinks ranged from 4–5 ms and their duration from 15–20 ms. As in older animals, the sinks of layer V had a tendency to move up towards layer IV as recording...
Fig. 5. Averaged field potentials (A) and corresponding current source-densities (B) in area 17 of a 63-day-old kitten reared normally (NR-III) evoked by double shocks (50 ms interstimulus interval). The recording electrode was positioned 5 mm caudal from the stimulating electrode.

Fig. 6. Current source-density profiles in the striate cortex of a 7-week-old kitten reared normally (NR-III). Double-shocks of 20 ms (A–C) or 50 ms (D) intervals were applied at various distances from the recording electrode, as indicated by the number in brackets above each profile. In the recording track D measurements have been stopped inadvertently about 100 μm below the cortical surface. Amplitude calibration in A and B, 32 mV/mm²; in C and D, 16 mV/mm². Note the different time scales in A-C and D.

distance increased. However, the corresponding sources remained in layer VI (Fig. 6B).

An interesting observation in all three kittens was that the amplitudes of responses did not always decrease monotonically with increasing recording distance. An example is shown in Figure 6. At a recording distance of 3 mm sinks had disappeared from layers IV and V and were
confined to layers II/III. At 3.5 mm, however, prominent sinks were again distinguishable in layer IV and small sinks were present in layer V. The former were associated with large sources in supragranular layers as were the layer IV sinks recorded close to the stimulation electrode. The sinks in supragranular layers were expressed well and were displaced towards the cortical surface. However, this is partly due to interference with the simultaneous sources of the delayed layer IV responses, which probably mask some of the deeper supragranular sinks. Except for the layer V sink, which at greater recording distances moved up to lower layer IV, all sinks again tended to be larger after the second of the double shocks.

**Visually deprived cats**

The effect of visual deprivation by BD or DR was studied in 73 CSD depth profiles from one 82-day-old cat and 3 adults (Table 1). When compared with NR cats of the same age neither BD nor DR had any significant effect on the laminar distribution or horizontal spread of activity.

The CSD profile recorded from the striate cortex of an adult BD cat (BD-I) at a distance of 2.5 mm from the stimulating electrode (Fig. 7B) shows basically the same pattern as the corresponding profile of a NR adult (Fig. 3C). Long-lasting current sinks were prominent in supragranular layers and in layer V. The corresponding sources of the supragranular sinks were located above and below in layers II and III/IV, those of the laminar V sinks were located mainly in layer VI. A similar spatio-temporal pattern of sinks and sources was found in the second BD animal (BD-II) and in the DR adult (DR-II). In all three visually deprived cats, tangential interactions were restricted in their lateral extent to approximately 2.5 mm and at this distance were confined to supra- and infragranular layers.

In the younger DR cat (DR-I), which was 82 days old at the time of recording, there were, however, indications for a retardation of developmental changes in the CSD profiles (not shown). The laminar pattern and the spatial extent of the tangential interactions more closely resembled those of the younger NR kittens NR-I and NR-II, which were 39 and 49 days old, than those of the 63-day-old kitten NR-III. As in the younger kittens, large delayed sinks occurred in layer IV. For distances up to 1.5 mm these were preceded by the short duration layer IV sinks, that were observed in all animals. At greater distances they occurred in isolation, decreased in amplitude but remained distinguishable over distances of up to 4 mm. The corresponding sources were located in layer V and probably also in lower layer II. In addition to these layer IV responses, sinks were again present both in supra- and infragranular layers. They underwent similar distance-dependent changes in amplitude, latency and laminar distribution as in NR kittens and were still readily elicitable at a distance of 3.5 mm. At 4 mm, the infragranular sinks had disappeared, but the supragranular and layer IV responses were still present.

**Effect of cortical transection**

In order to test whether the observed tangential interactions were actually mediated by intracortical connections, a 2 mm deep and 2.5 mm-long cut was made parallel to the frontal plane between the caudal stimulating electrode and the more rostral recording site in kitten
NR-I. This cut transected all cortical layers up to a depth of about 2 mm.

Figure 8A shows the typical CSD profile 3 mm rostral to the stimulating electrode, obtained before severing the intracortical connections. After the cut, measurements were repeated along the same recording tract. As described in Figure 8B, the cut completely abolished all responses. To exclude that this effect was due to damage of cortical tissue in the vicinity of the recording electrode, we also measured responses in the same track from a second stimulating electrode positioned 0.5 mm rostral to the recording site. The corresponding CSD profile is shown in Figure 8C. It is characteristic of short recording distances with prominent short latency sinks in layers IV–VI and lower III. As is typical for recordings in close proximity of the stimulating electrode, there were no sinks in layers II and upper III. These appear only at greater recording distances.

Discussion

Methodological considerations

Previously the CDS method has been used to analyse the spatio-temporal pattern of synaptic activity in the cerebellum, the LGN, the superior colliculus and the in vivo and in vitro visual cortex (for review see Mitzdorf, 1985). In the visual cortex the one-dimensional CSD analysis of electrically evoked field potentials has been shown to resolve the principal excitatory connections (Mitzdorf and Singer, 1978). Current sinks were attributed to inward currents that are associated with activation of excitatory synapses and current sources to loop-closing passive outward currents that leave the postsynaptic profiles. When applying the one-dimensional version of the CSD method to analyse the tangential spread of activity, it is crucial to estimate the contribution of volume-conducted tangential currents to the CSD profiles. Several arguments suggest, that tangential current spread is small in relation to the large distance over which responses were observed: first, increasing the recording distance by as little as 1 mm often led to disappearance of layer IV sinks that had been prominent in the penetration closer to the stimulation electrode. Second, response latencies changed when the distance between stimulation and recording electrode was increased by 1 mm. Third, when stimulation and recording sites were 3 mm apart no responses were left after a transcranial cut midway between the sites. These results indicate that the recorded responses reflect only synaptic currents that are generated within a distance of maximally 1 mm from the tip of the recording electrode. Thus, all sinks recorded beyond a distance of 1 mm from the tip of the stimulation electrode must result from activity that is conveyed by neuronal connections. Several pathways may be
considered: first, antidromically activated afferents from white matter that bifurcate and have branches terminating at some distance. Second, subcortical and cortical afferents that pass tangentially through cortex before terminating, and third, intracortical connections. The authors attempted to exclude the first possibility by lesioning the LGN, the major source of subcortical input to striate cortex, and by allowing sufficient time for the axons to degenerate. The authors are confident that this procedure was successful because the CSD profiles recorded at distances beyond 1.5 mm from the site of stimulation provided no indication of activity conveyed by LGN afferents. If sinks were present in layers IV and VI, neither their latency nor their source distributions were compatible with synaptic activity mediated by bifurcating LGN afferents (for comparison see Mitzdorf and Singer, 1978). The authors also consider it unlikely that other subcortical projections contributed to any substantial extent to the tangential spread of activity. It is known from previous CSD studies that chiasmal stimulation evokes CSD profiles which closely resemble those evoked by stimulation of the optic radiation (Mitzdorf and Singer, 1978) or of the white matter underlying area 17 (Bode-Greuel et al., 1987). This indicates that the nonthalamic input to striate cortex, which is activated by radiation and white matter stimulation, does not generate any prominent sinks in addition to those induced by specific visual afferents. Furthermore, recent in vitro data by Chagnac-Amitai and Connors (1989) and observations in rat visual and somatosensory cortex (Luhmann and Prince, unpublished observations) indicate that responses to orthodromic stimulation of infragranular layers or white matter are restricted to the activated vertical column and under normal conditions do not spread laterally over several millimeters. The most likely assumption is, therefore, that the CSD profiles reflect responses that are mediated by intrinsic tangential connections which originate or pass in the vicinity of the stimulation electrode. This assumption is supported by the sink/source distribution evoked by remote stimulation. Both in adult (Fig. 3C) and in kitten cortex (Fig. 5B) long latency sinks are located in supragranular and infragranular layers with their corresponding sources situated below.

Another methodological restriction deserving consideration is the interference between sinks and sources. If sources coincide with sinks both spatially and temporally these summate linearly. Thus, sinks may be delayed or truncated by preceding or following sources or may be reduced in amplitude if sources are superimposed. Despite this methodological restriction, the CSD analysis is an adequate tool to study afferent and intrinsic projections in cat visual cortex and confirmed the main excitatory connections (Mitzdorf and Singer, 1978), that have been demonstrated also with anatomical and other electrophysiological techniques.

**Tangential interactions in adult striate cortex**

In the striate cortex of adult cats the CSD profiles revealed three major routes for the lateral spread of activity, which are in agreement with our anatomical data. One pathway generates small short latency sinks in layer IV and does not extend further than 1.5 mm from the stimulation site. The related sources were also small and, if distinguishable, located both above and below the respective sinks. This suggests that the targets of this pathway the dendrites of cells located within and below layer IV. The amplitude of the layer IV response was small, indicating that the corresponding connections are sparse and generate only weak synaptic currents or that the geometry of the target cells allows for only small vertically oriented dipole moments. The short latency of these responses and their resistance to paired pulse attenuation suggests that they are monosynaptic. Assuming a synaptic delay of 0.8 ms (Tsumoto and Suda, 1982), the authors calculated a conduction velocity of maximally 5 m/s for this pathway. However, as it is likely that neuronal profiles are activated directly by current spread at some distance from the stimulation electrode this value is probably an overestimation.

A second projection conveys activity to layer II and upper layer III and extends up to 2.5 mm from the point of stimulation. The corresponding source distributions indicate that this pathway terminates on apical dendrites of neurons located in supragranular laminae and upper layer IV. With increasing recording distance these sinks moved towards more superficial locations which suggests that remote interactions preferentially involve more distal segments of the apical dendrites and/or more superficially located neurons. Whether these long-range projections to supragranular targets are mono- or polysynaptic cannot be decided from the CSD patterns alone. The monotonic increase of sink latency with increasing distance, the marked paired pulse facilitation of the response to the second stimulus, and anatomical evidence, (see discussion below and preceding paper) are compatible with monosynaptic connections. In this case the conduction velocity of the tangential projections would be in the order of 0.3–0.6 m/s and thus characteristic of thin, nonmyelinated axons. These supragranular responses persisted at distances from the stimulation electrode at which sinks had disappeared in deeper layers. Therefore it seems unlikely that these superficial responses result from activity ascending vertically within cortical columns. The present data thus support the conjecture derived from previous CSD analyses (Mitzdorf and Singer, 1978) that the delayed and long-lasting inward currents at apical dendrites of supragranular pyramidal cells result from long-range, tangential projections.

A third group of sinks occurred in layer V. Because the corresponding sources were prominent in layer VI and small in lamina IV, the targets of this layer V input are most likely to be the apical dendrites of layer VI pyramidal cells. The latency of these deep sinks increased with increasing recording distance and this increment was in a range similar to that of the supragranular sinks. It is unlikely, however, that these deep responses arose from vertically descending axons of cells in supragranular layers, because they preceded supragranular responses and even occurred in isolation. Thus, these deep responses seem to be mediated by a separate slowly conducting tangential projection that extends nearly as far as the supragranular connections. The same arguments that support a monosynaptic origin of the superficial sinks also hold for the infragranular responses.

Like the supragranular responses, the deep sinks also moved upwards with increasing recording distance. This suggests that perhaps also in infragranular layers more input synapses on more distal parts of the apical dendrites of the respective target cells. Such a continuous shift in the laminar position of terminals could be a effective mechanism for a distance-dependent weighting of coupling strength.

The pattern of tangential connections emerging from the CSD analyses is consistent with our anatomical data (Luhmann et al., 1990a), which indicate that lateral projections in adult striate cortex extend for 2.5–3.0 mm in supra- and infragranular layers, but are confined to a range of less than 2 mm in lamina IV. Our CSD analyses extend these morphological data in that they demonstrate that the far reaching tangential connections are excitatory and form synapses on particular segments of apical dendrites of pyramidal cells. In this respect our data are complementary to observations of Ts'o et al. (1986), who demonstrated by cross-correlation analysis that lateral excitatory interactions between pyramidal neurons in layers II/III of cat striate...
cortex extend for up to 3 mm. Furthermore, the present data agree well with the finding that neurons with large fields and multiple discharge zones (Singer and Tretter, 1976a; b; Luhmann et al., 1990b) and very elongated receptive fields (Gilbert, 1977) occur preferentially in supragranular laminae and layer VI, corroborating the hypothesis that the receptive field properties result from far-reaching tangential excitatory connections in these layers (for review see Gilbert and Wiesel, 1985; Singer, 1985a; b; Wiesel and Gilbert, 1986).

**Tangential connections in kitten striate cortex**

The three tangential pathways that were identified in striate cortex of adult cats were also present in kittens. As suggested by the similar sink/source distributions, the mode of termination of these projections is similar to that of the adult and the conduction velocities are also in a similar range. However, in the kitten, these horizontal connections are more extensive. The supragranular and infragranular projections still produced sinks at distances of up to 5 mm and the layer IV pathway conveyed activity over at least 2 mm. This agrees with our anatomical data which showed longer and denser lateral connections in kittens than in adult cats (Luhmann et al., 1986, 1990a). However, the CSD data seem to underestimate the maximal extent of tangential projections since they revealed no interactions beyond 6 mm. In contrast the morphological results and the receptive field analyses in the two accompanying papers suggest interactions over distances of up to 10 mm. A possible explanation for this discrepancy may be the relatively low sensitivity of the CSD method. The most far-reaching connections were sparse and hence the resulting synaptic currents may have been too small and not synchronous enough to be resolvable with the CSD method. As in adult cats, long latency sinks occurred in layer IV at larger recording distances. These sinks had their sources located in layer VI and thus most likely result from the infragranular projections that shift from layer V to layer IV as recording distance increases (see above).

In kittens, more often than in adults, the authors also observed that response amplitudes did not decrease monotonously with recording distance. This presumably reflects the patchy organization of lateral projections within kitten striate cortex (Luhmann et al., 1986, 1990a). However, tangential connections are also patchy in the adult (Gilbert and Wiesel, 1983; Martin and Whitteridge, 1984). Therefore the more frequent occurrence of nonmonotonous CSD profiles in the kitten probably arises from the longer range of interactions, which makes homogeneities more easily detectable. Chervin et al. (1988) demonstrated in vitro that there were also distinct variations in horizontal excitatory interactions in the visual cortex of adult cats. In disinhibited neocortical slices, they found a spatially periodic pattern in the propagation of paroxysmal field potentials with a spatial frequency of 1/mm, suggesting a close relation to the columnar systems.

Although the anatomical data indicate that the larger lateral spread of activity observed in kitten visual cortex results from transiently expressed long-range horizontal connections, the authors cannot exclude the possibility that an immature GABAergic system promotes this effect considerably. Recent in vitro studies in rat visual and somatosensory cortex have shown that GABAergic mechanisms are indeed significantly less pronounced in immature than in adult animals (Luhman and Prince, 1988), but biochemical data demonstrate that in 5.5- to 8-week-old kittens, as used in the present study, the number of GABA receptors (Shaw et al., 1985) and glutamate decarboxylase activity (Fosse et al., 1989) already reached adult levels. In conclusion, the CSD profiles recorded in kittens indicate that the spatial extent of tangential excitatory interactions gets reduced substantially during the first two postnatal months, whereby the basic laminar pattern of lateral interactions is preserved. Because the CSD analysis reveals only bulk activity this does not exclude the persistence of far-reaching collaterals in adults. Our data imply, however, that the density of connections reaching further than 2 mm decreases markedly with age. This is in agreement with our morphological data.

**The effect of visual deprivation**

In contrast to the anatomical data (Luhmann et al., 1986, 1990a), the CSD analysis failed to reveal any influence of visual deprivation on the developmental changes of tangential interactions in older kittens (DR-I) and adult cats (BD-I, BD-II, and DR-II). The anatomical data suggested that deprivation accentuates the pruning of intrinsic connections by reducing the number and density of patches below the normal adult level. In the CSD data this should have led to a decrease of response amplitude and hence also to a reduction in the distance over which responses were measurable, but this was not the case. The authors offer an unequivocal explanation for this discrepancy. As discussed in the anatomical paper it is possible that deprivation leads to changes in the intrinsic connections which reduce the uptake and transport of label, but leave their functions intact. It is also conceivable that deprivation actually reduces the number of intrinsic connections below the normal level, but at the same time causes additional changes which counteract the expected weakening of excitatory interactions. The authors favour the latter hypothesis. Deprivation leads to a marked loss of response selectivity in cortical neurons and it has been suggested that this reflects a down regulation of inhibitory interactions (Leventhal and Hirsch, 1977; Pettigrew, 1974; Watkins et al., 1978; for review see Frégona and Imbert, 1984). Reduction in inhibition, however, can very effectively counterbalance a concomitant weakening of excitatory interactions even if these are monosynaptic. Therefore, a reduction in the number of excitatory synapses could be very effectively compensated for by a concomitant reduction of inhibition. The CSD data from the deprived animals thus do not necessarily contradict the morphological results.

**Conclusions**

The CSD analysis of developmental changes in tangential intrinsic connections confirmed and extended our anatomical findings (Luhmann et al., 1990a). It provided independent support for the hypothesis that these projections are initially exuberant and are pruned during the first two postnatal months. It also confirmed that far-reaching interactions are mediated by two distinct pathways that project to supra- and infragranular layers, respectively. In addition, it indicated that these two pathways form excitatory synapses with apical dendrites of pyramidal cells, a finding that is in agreement with recent histochemical (LeVay, 1988) and ultrastructural data (Kisvárday et al., 1986). These combined results suggest that pyramidal cells are selectively and reciprocally coupled over long distances through tangential excitatory connections. These projections, in particular the very far reaching ones, terminate remote from the cell soma as indicated by the large spatial separation between sinks and sources. Therefore one would predict that horizontal interactions have a modulatory function as assumed for most feedback loops. The authors have recently discovered that cells within functional columns engage in highly synchronous oscillatory activity in the 40 Hz range when activated by an appropriately oriented light stimulus (Gray and Singer, 1989). When columns encoding the same stimulus orientation are activated simultaneously their oscillatory activities can synchronize even if these are several millimeters apart (Gray et al., 1989). The authors propose that this synchronization is
mediated by tangential intrinsic connections; thus these could serve to generate coherent activation patterns in spatially distributed but functionally related cell assemblies.

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Abbreviations

BD    binocularly deprived
CSD  current source-density
DAB  diaminobenzidine
DR  dark-reared
FET  field effect transistor
GABA  γ-aminobutyric acid
HRP  horseradish peroxidase
LGN  lateral geniculate nucleus
NMDA  N-methyl-D-aspartate
NR  normally reared
PB  phosphate buffer

References
