Absence of a Prevalent Laminar Distribution of IPSPs in Association Cortical Neurons of Cat

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Contreras, Diego, Niklaus Dürmüller, and Mircea Steriade. Absence of a prevalent laminar distribution of IPSPs in association cortical neurons of cat. J. Neurophysiol. 78: 2742–2753, 1997. The depth distribution of inhibitory postsynaptic potentials (IPSPs) was studied in cat suprasylvian (association) cortex in vivo. Single and dual simultaneous intracellular recordings from cortical neurons were performed in the anterior part of suprasylvian gyrus (area 5). Synaptic responses were obtained by stimulating the suprasylvian cortex, 2–3 mm anterior to the recording site, as well as the thalamic lateral posterior (LP) nucleus. Neurons were recorded from layers 2 to 6 and were classified as regular spiking (RS, n = 132), intrinsically bursting (IB, n = 24), and fast spiking (FS, n = 4). Most IB cells were located in deep layers (below 0.7 mm, n = 19), but we also found some IB cells more superficially (between 0.2 and 0.5 mm, n = 5). Deeply lying corticothalamic neurons were identified by their antidromic invasion on thalamic stimulation. Neurons responded with a combination of excitatory postsynaptic potentials (EPSPs) and IPSPs to both cortical and thalamic stimulation. No consistent relation was found between cell type or cell depth and the amplitude or duration of the IPSPs. In response to thalamic stimulation, RS cells had IPSPs of 7.9 ± 0.9 (SE) mV amplitude and 88.9 ± 6.4 ms duration. In IB cells, IPSPs elicited by thalamic stimulation had 7.4 ± 1.3 mV amplitude and 84.7 ± 14.3 ms duration. The differences between the two (RS and IB) groups were not statistically significant. Compared with thalamically elicited inhibitory responses, cortical stimulation evoked IPSPs with higher amplitude (12.3 ± 1.7 mV) and longer duration (117 ± 17.3 ms) at all depths. Both cortically and thalamically evoked IPSPs were predominantly monophasic. Injections of Cl− fully reversed thalamically as well as cortically evoked IPSPs and revealed additional late synaptic components in response to cortical stimulation. These data show that the amount of feed forward and feedback inhibition to cat’s cortical association cells is not orderly distributed to distinct layers. Thus local cortical microcircuitry goes beyond the simplified structure determined by cortical layers.

METHODS

Experiments were carried out on adult cats (2.5–3.5 kg) of either sex, anesthetized with pentobarbital sodium (35 mg/kg). All wounds and pressure points were infiltrated with lidocaine. Animals were paralyzed with gallamine triethiodide and artificially ventilated. End-tidal CO2 (3.5–3.7%) and heart rate were continuously monitored. Body temperature was maintained at 37–39°C. The depth of the anesthetica was maintained by additional doses of the same anesthetic, to keep a constant picture of high-amplitude low-frequency waves in the electroencephalogram (EEG).

For intracellular recordings, the surface of the suprasylvian gyrus was exposed and bathed in mineral oil to prevent desiccation. The stability of the recordings was ensured by bilateral pneumothorax, drainage of the cisterna magna, hip suspension, and filling the holes made for recording with a solution of 4% agar. Glass micropipettes were inserted perpendicularly in the cortex. The pipettes were filled with a solution of potassium acetate (KAc, 3 M), or potassium chloride (KCl, 3 or 1.5 M), and had DC resistances on the order of 35–45 MΩ. The depth of the micropipettes was read on the display of the micromanipulator. A high-impedance amplifier (band-pass of 0–5 kHz) with active bridge circuitry was used to record and inject current into the cells. The signals were recorded on an eight-
channel tape with band-pass of 0–9 kHz and posteriorly digitized at 20 kHz for off-line computer analysis. The EEG was recorded focally, from the vicinity of intracellularly recorded neurons, by means of coaxial electrodes with exposed areas of 0.2 mm separated by 0.6 mm. For monopolar EEG recordings, the indifferent electrode was placed in the neck muscles.

Stimulating coaxial electrodes were stereotaxically inserted in the thalamic lateroposterior (LP) nucleus and, under visual control, 2–3 mm anteriorly to the recording pipette in the suprasylvian cortical area 5 (Fig. 1). Stimulating electrodes were coaxial, with the ring at the surface and the tip at a depth of 0.8–1 mm, and they had the same characteristics as the recording ones (see above). Stimuli were applied with intensities ranging from 0.05 to 0.2 mA and a duration of 0.1 ms.

At the end of the experiments the animals were given a lethal doses of pentobarbital.

RESULTS

Database and cellular identification

Results are based on 160 cortical cells from the cortical suprasylvian area 5. Cells were classified as regular spiking (RS, \( n = 132 \)), intrinsically bursting (IB, \( n = 24 \)), or fast spiking (FS, \( n = 4 \)) according to their responses to depolarizing current pulses, following criteria established in vitro (Connors et al. 1982; McCormick et al. 1985) and in vivo (Núñez et al. 1993). The recordings that were used for the database and analysis had stable membrane potentials (\( V_{m} \)), means of coaxial electrodes with exposed areas of 0.2 mm separated by 0.6 mm. For monopolar EEG recordings, the indifferent electrode was placed in the neck muscles.

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Cells responded to thalamic and cortical stimulation with a combination of excitatory postsynaptic potential (EPSP) and IPSP, reflecting the activation of mono- and polysynaptic circuits. Some neurons were activated antidromically from the thalamus (\( n = 31 \)) or from the cortex (\( n = 18 \)). Among them, we found cells that were backfired from both the cortex and the thalamus (\( n = 10 \)), as shown in Fig. 1A depicting a neuron located at a depth of 1.1 mm. Cortical
volleys were delivered at 2–3 mm in front of the recorded cell. When applied at a depolarized $V_m$ ($-55$ mV, +1 nA DC), cortical stimuli elicited an antidromic spike (0.3-ms latency) followed by an EPSP (1.2-ms latency) that was suprathreshold for firing an action potential (Fig. 1A, Cx, stimulus marked by a triangle). The EPSP was cut short by an IPSP that had a peak amplitude (at 18 ms) of 17.5 mV (measured from $-60$ mV) and a duration of 95 ms (at half amplitude). In the same neuron (Fig. 1A), cortical stimulation failed to display antidromic invasion at a hyperpolarized $V_m$ ($-95$ mV, $-0.7$ nA DC) that increased the amplitude of the EPSP and reversed the IPSP. The composite but constant nature of Cx-evoked PSPs was shown by the stereotyped shape of the superimposed traces in Fig. 1A at both $V_m$s.

However, some aspects of IPSP’s shape may be ascribed to the postsynaptic nature of the activation and not necessarily to different GABAergic components. Thalamic stimulation (Fig. 1A, Th, stimulus marked by a filled circle) elicited in the same neuron a similar series of events, with antidromic invasion (0.3-ms latency) followed by orthodromic activation (1.7-ms latency). The Th-evoked IPSP had a peak amplitude of 13 mV (at 23 ms, measured from $-60$ mV) and a duration of 90 ms (at half amplitude). An example of an IPSP triggered by thalamic stimulation (Th, dot) is shown in Fig. 1B to illustrate how measurements were taken from the evoked IPSP: the dotted vertical line indicates the amplitude at the peak, selected by visual inspection, whereas the duration of the IPSP was taken at half amplitude (thick horizontal line). The IPSP depicted in Fig. 1B had a latency of 3.3 ms, the peak was at 16.6 ms, the duration was 67 ms, and it was not preceded by an EPSP. Maximum inhibitory responses were obtained by increasing the intensity of stimulation until reaching a value beyond which increasing intensity did not elicit a further increase in the amplitude of the response.

We found no statistically significant difference between the peak time ($P > 0.61$), amplitude ($P > 0.55$), and duration ($P > 0.57$) of IPSPs elicited in RS and IB cells (Mann-Whitney $U$ test). Therefore the values obtained for the cortically and thalamically elicited IPSPs in these two cellular classes are pooled and shown in Table 1.

### Table 1. Values of IPSPs evoked by cortical and thalamic stimulation in a sample of 60 cells from layers 2 to 6 in suprasylvian area 5

<table>
<thead>
<tr>
<th>Time-to-peak, ms</th>
<th>Cx</th>
<th>Th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude, mV</td>
<td>12.3</td>
<td>7.7</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>117</td>
<td>86.3</td>
</tr>
<tr>
<td>Reversal potential, mV</td>
<td>75.4</td>
<td>74.3</td>
</tr>
<tr>
<td>$R_m$ drop at peak, $M$</td>
<td>80%</td>
<td>60%</td>
</tr>
<tr>
<td>$R_m$ drop at 200 ms, $M$</td>
<td>20%</td>
<td>40%</td>
</tr>
</tbody>
</table>

Values are means ± SE. Both series of measurements were made in all 60 neurons. Time-to-peak was measured from the stimulation artifact.

### Distribution of inhibition according to depth

We used two different approaches to compare the inhibitory input among cells at different depths. In the first type of analyses, we compared cells at different depths recorded from successive tracks in the same area, whereas, in the second type of analyses, we compared cells located at the same depth and recorded simultaneously <2–3 mm apart in the anteroposterior axis.

An example of the distribution of amplitude and duration of Th-evoked IPSPs is shown in Fig. 2. Different symbols represent cells from different animals (see legend) that were connected by a trace to facilitate visualizing the variability within each experiment. The top plot represents the amplitude ($y$-axis) versus the cortical depth ($x$-axis) of each cell, whereas the bottom plot represents the duration of the IPSPs from the same cells. There was no preferential distribution of IPSP amplitude or duration according to depth.

Not only did cells at similar depths show different IPSP characteristics, but also cells located far apart in the vertical axis of cortex had similar features of IPSPs. This was a constant finding and is illustrated by the example of Fig. 3. Two RS cells were recorded along the same track, one at a depth of 0.3 mm (Fig. 3A), the other at 1.3 mm (Fig. 3B). Both the superficial and the deep cell displayed IPSPs in response to thalamic LP stimulation, with latencies of 5 and 8 ms, respectively, that were not preceded by a visible EPSP.

The IPSPs from both cells had similar amplitudes (measured...
at −65 mV) and durations (66 ms in the superficial cell, and 64 ms in the deeply lying cell). The degree of involvement of GABA_{A}^-activated conductances was inferred indirectly by the decrease in input resistance (R_{in}) during the peak of the IPSP, as compared with the R_{in} preceding the stimulation. The R_{in} was estimated from the slope of the regression line fitted to the voltage-current (V-I) plots (Fig. 3, left plots in A and B). Voltage values for the V-I plots were obtained from 10 ms before the stimulus (+), at the peak of IPSP (○), and 100 ms after the stimulus (●). At rest, the superficial cell (Fig. 3A) had a R_{in} of 21 MΩ, which decreased to 5 MΩ (72% drop) during the peak of the IPSP, and recovered to 20.5 MΩ at 100 ms. In the deep cell (Fig. 3B), the R_{in} at rest was 26 MΩ, at the peak of the IPSP it decreased to 9 MΩ (65% drop), and was 20.5 MΩ at 100 ms. By displacing the V_{m} with DC, the peak of the IPSP

FIG. 3. Superficial and deep cells in area 5 may display similar IPSPs in response to thalamic LP stimulation. Responses of 2 regular spiking (RS) cells at 0.3 mm (A) and 1.3 mm (B) depth to thalamic stimulation. V_{m} was displaced by DC, and 2 traces superimposed for each V_{m}. Left voltage-current (V-I) plots (in A and B) show the V_{m} at rest (●), at the peak of the IPSP (15 ms in A and 30 ms in B; ○) and at 100 ms after the stimulus (●) for the different currents. Values of V_{m} were fit by regression lines (rest, IPSP peak, IPSP at 100 ms). Right plots (in A and B) show amplitude of the IPSP with respect to V_{m} at the peak (○) and at 100 ms (●).
reversed at −66 mV in the superficial cell (Fig. 3A, right plot, ○), and it reversed at −81 mV in the deep cell (Fig. 3B, right plot, ●). The late phase of the IPSP (at 100 ms, ○) reversed at −73 mV in the superficial cell and at −87 mV in the deep cell.

Intrinsically bursting cells

In several descents through area 5, IB cells were mostly encountered deeper than 0.7 mm (n = 19), but a smaller number of IB cells (n = 5) was located between 0.2 and 0.5 mm depth. The responses to thalamic stimulation of two superficial IB cells are illustrated in Fig. 4. The superficial cell in Fig. 4A was located at a depth of 0.2 mm and discharged typical spike bursts during spontaneous spindle oscillations (Fig. 4A2), similarly to the spindle-related bursts in deeply lying (1–1.5 mm) IB cells of area 5 (see Fig. 7 in Steriade et al. 1993b). Thalamic stimulation (Fig. 4A1) triggered an EPSP, with a latency of 3 ms, giving rise to an orthodromic spike that was followed by an IPSP with an amplitude of 6 mV (at a Vm of −60 mV) and a duration of 68 ms at half amplitude. Interestingly, spontaneous activity related to spindling generated strong bursting (Fig. 4A2), whereas thalamic electrical stimulation gave rise to single spikes followed by inhibition. This difference is possibly due to the fact that, in contrast to spontaneous spindle-related inputs, electrical stimuli to the thalamus readily recruit local cortical inhibition by backfiring corticothalamic axons. That cortical stimulation entrains stronger inhibition than thalamic stimulation is shown by IPSPs with higher amplitudes and longer durations triggered by cortical volleys (see Table 1).

The other superficial IB cell fired spike bursts in response to depolarizing current pulses of increasing amplitude (Fig. 4B2). This cell was located at a depth of 0.25 mm and responded to thalamic stimulation with an EPSP at a latency of 4 ms, which was cut short by an IPSP of 8-mV amplitude (at −60 mV) and lasting for 70 ms. The postinhibitory burst in Fig. 4B may well originate from a low-threshold Ca2+ spike (LTS) but, at least in guinea pig frontal cortex, such LTSs were only found in deep layers and were absent from superficial layers (de la Peña and Geijo-Barrientos 1996). In addition to intrinsic factors, such as LTSs, synchronous excitatory inputs at the return of evoked hyperpolarizations may be taken in consideration, as was previously shown for the spontaneously occurring long-lasting hyperpolarizations of the slow oscillation (Contreras and Steriade 1995; Steriade et al. 1993a).

Dual impalements

To directly compare the amplitude of the inhibitory input to different cells, triggered by the same stimuli, we recorded pairs of cortical cells simultaneously and studied their responses to thalamic and cortical stimulation. In the example of Fig. 5, two cells were recorded simultaneously at a similar depth, ~1.1 mm. Cell 1 was 2 mm anterior to cell 2 and therefore closer to the cortical stimulating electrode (see scheme in Fig. 1). Both cells responded to cortical and thalamic stimulation under different backgrounds of current injection, as indicated by the pulse protocols in Fig. 5. And both were corticothalamic cells because they were antidromically invaded from the LP nucleus with latencies of 0.6 ms for cell 1 and 0.5 ms for cell 2 (see insets at bottom). Antidromic invasion after thalamic stimulation was followed by an EPSP at 2.5 ms latency in cell 1, and 2.8 ms latency in cell 2 (Fig. 5, bottom panel, expanded detail indicated by arrow) that was crowned by action potentials and followed by a prominent IPSP in both cells. Cortical stimulation (top panels, Cx stim) triggered in both cells a longer lasting IPSP with a higher decrease in Rm, as compared with thalamic stimulation (bottom panels, Thal. stim). Noteworthy, cell 2 generated bursts of two to four action potentials at 230 Hz on depolarizing pulses applied at hyperpolarizing Vm (Fig. 5, Cx stim, cell 2, detail at right) and riding on a humplike
FIG. 5. Simultaneously recorded neurons at similar depths may display dissimilar IPSPs. Cell 1 (left column) and cell 2 (right column) were 2 mm apart and were recorded simultaneously at a depth of 1.1 mm in area 5. Thalamic (bottom panel) and cortical (top panel) stimulation was applied on a background of different current injections (values are indicated). Both cells were antidromically activated from the thalamus and orthodromically from an adjacent focus in area 5 (expanded details indicated by arrow). Inhibitory responses in cell 1 had smaller amplitude and duration than in cell 2. Further details in text. In this and the following figures, small deflections that are concomitant with action potentials in the simultaneously recorded neuron are due to capacitive coupling.

Thalamic stimulation led to a drop in $R_{in}$ of cell 1 from 44.1 to 14 MΩ (68% drop) at the peak of the IPSP, and $R_{in}$ was back to 37 MΩ at 150 ms. In cell 2, thalamic stimulation brought $R_{in}$ from 22.8 down to 6 MΩ (71% drop) at the peak, but the $R_{in}$ was still decreased (15 MΩ) at 200 ms.

In summary, the results presented in Figs. 5 and 6 show that two cells recorded at the same depth, 2 mm apart in the anteroposterior axis of the suprasylvian cortex, had different amounts of inhibition associated with cortical or thalamic stimuli. Cell 2 was the target of more powerful inhibition to both stimuli than cell 1, as seen from the longer duration of the IPSP and the percentage drop in $R_{in}$ during the peak of the IPSP. The $R_{in}$ decrease was higher in cell 2 than in cell 1, both after cortical (90% compared with 70%) and thalamic (71% compared with 68%) stimulation.

Another example of two cells recorded simultaneously...
thalamic (17 mV, 90 ms) stimulation. Reversal was similar after cortical (−80 mV) and thalamic (−78 mV) stimulation.

Effect of stimulation intensity

Varying the intensity of stimulation revealed further differences in the local inhibitory circuit activated by cortical or thalamic volleys. By increasing the stimulation strength, the inhibitory input increased in all investigated cells (n = 12). Figure 8 depicts a pair of cells that were recorded simultaneously at depths of 1.2 mm (cell 1) and 1 mm (cell 2). Both cells had prominent and apparently similar IPSPs in response to cortical and thalamic stimulation. However, by changing the intensity of stimulation in a stepwise manner, some differences were revealed between the two cells (right column in Fig. 8 depicts expanded details). At the lowest intensity, cortical stimulation triggered a clear-cut biphasic IPSP in cell 1 (10 mV, total duration of 50 ms) but only a brief arrest of firing, without an apparent IPSP, in cell 2. Increasing the intensity of cortical stimulation revealed more steps in IPSP amplitude for cell 2 than for cell 1. In the case of thalamic stimulation, the smallest intensity did not evoke an IPSP but reduced firing frequency in both cells; thereafter, four steps of increased intensity revealed four different degrees of inhibition in cell 1, but a ceiling effect was observed in cell 2 after the second step.

Effect of intracellular Cl⁻ infusion

The IPSPs recorded after both types of stimulation had complex shapes due to the activation of mono- and polysynaptic pathways. An attempt was made to dissociate the Cl⁻-dependent GABAₐ component from the K⁺-dependent GABAₐ component (n = 13), as described in vitro (Connors et al. 1988).

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**FIG. 6.** Voltage-current V-I plots from simultaneously recorded cells depicted in Fig. 5. Values of Vₘ were obtained from 10 to 30 ms before stimulation (+), 30 ms after stimulation, coincident with the peak of the IPSP (○), and 150–200 ms after stimulation (●). Vₘ values were plotted against DC current, and Rₘ was read from the slope of regression lines fitted to the data.

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**FIG. 7.** Two deeply lying cells recorded simultaneously are characterized by different amounts of inhibitory input. Cell 1 (left column) and cell 2 (right column) were at depths of 1.2 and 1 mm, respectively. Cell 1 showed a smaller amplitude and duration IPSP to both cortical and thalamic stimulation. Stimuli applied during 2 different current levels, as indicated.

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Intracellular injection of Cl\textsuperscript{-} reversed the evoked IPSPs and revealed additional differences between cortical and thalamic stimulation. The cell depicted in Fig. 9 was located at 1.2 mm depth and was recorded with a 3-M KCl-filled pipette. Cortical stimulation applied after several minutes of impalement gave rise to an antidromic spike at 0.6-ms latency (see inset) followed by a strong depolarizing response at 4-ms latency, which triggered burst firing with spike inactivation. Bursting was not intrinsic but due to synaptic input, as indicated by the one-to-one correspondence between spikes and the individual components of the input, revealed by the hyperpolarizing pulse (detail in top panel). The initial response to cortical stimulation was followed, 80 ms later, by a secondary and similarly strong depolarizing response that is normally hidden by the IPSP. Thalamic stimulation (Fig. 9, bottom panel) gave rise to a single depolarizing event at a latency of 2.5 ms. Hyperpolarization blocked the occurrence of the spike and increased the amplitude of the thalamically evoked depolarizing synaptic response. To sum up, cortical stimulation set into action polysynaptic chains, whereas thalamic stimulation seemed to activate more circumscribed cortical zones.

The state of inhibitory responses was monitored by simultaneously recording one cell with KCl-filled pipette and a reference cortical cell with a KAc-filled pipette ($n = 7$). In the example shown in Fig. 10, cell 1 was recorded at 1.3
mm depth with KAc and responded to thalamic stimulation, applied during depolarizing current pulses, with a robust EPSP-IPSP sequence. The EPSP had a latency of 3.5 ms, and the IPSP had an amplitude of 13 mV and a duration of 130 ms (measured at −60 mV) and was reversed by hyperpolarizing pulses at −70 mV. $R_m$ was evaluated from the slope of $V-I$ plots (not shown) and dropped from 18.5 MΩ at rest to 6 MΩ during the peak of the IPSP, and was 13 MΩ at 150 ms after the stimulus. Cell 2 was recorded simultaneously at a depth of 1.2 mm, with a KCl-filled pipette, and responded to the same thalamic stimuli, after several minutes of impalement, with an antidromic spike (0.5-ms latency, see detail in Fig. 10, bottom) followed by a high-amplitude and long-duration (70 ms) depolarizing potential (see detail in Fig. 10, top) that triggered burst firing. Thalamic stimulation applied during hyperpolarizing pulses failed to invade antidromically the cell and triggered a depolarizing synaptic response of increased amplitude.

Thalamically evoked IPSPs were progressively reversed by intracellular Cl$^-$ injections. This is shown for a cell located at a depth of 0.8 mm, recorded with a 1.5-M KCl-filled pipette (Fig. 11). The top and bottom panels in Fig. 11 show two depolarizing current pulse of different amplitudes (values are indicated), during which thalamic stimuli were delivered. Superimposed traces show the evoked IPSP immediately after impalement and ~15 min after impalement. Before the IPSP reversal by the leak of Cl$^-$ inside the cell, the IPSP had and amplitude of 4.2 mV (peak at 30 ms) and 130 ms in duration, measured from −60 mV. Its reversal was at −70 mV. The $R_m$ of the cell was 26 MΩ, dropped to 12 MΩ at the peak of the IPSP, and was 16 MΩ at 120 ms. After several minutes of recording, hyperpolarizing IPSPs were no longer evoked, and stimulation triggered a burst of action potentials. The burst was followed by a pause, whose duration depended on the amount of current being applied (40 ms in top panel, with a pulse of +0.5 nA, and 160 ms in the bottom panel, with a +0.2-nA pulse). Thereafter the firing resumed due to the application of depolarizing current.

**DISCUSSION**

The three major findings of this study are as follows. 1) Cortically as well as thalamically evoked IPSPs in neurons recorded from cortical association area 5 showed local differences in amplitude and duration that did not correspond to their localization in various layers. 2) IPSPs elicited by thalamic or cortical stimulation were almost completely reversed after intracellular injection of Cl$^-$. 3) IB cells from
FIG. 11. Progressive reversal of thalamically evoked IPSPs with Cl\(^-\). Cell recorded with a 1.5-M KCl-filled pipette showed a progressive reversal of the IPSPs. Top and bottom panels show, for different background current levels (depolarizing pulses of +0.5 and +0.2 nA), superimposed responses to thalamic stimulation applied immediately after impalement and a few minutes after impalement.

all layers showed robust inhibitory responses, not different from those recorded in RS cells.

Some methodological issues

The rather short latency of thalamically evoked antidromic responses in cortical neurons reported in this study (0.3–0.6 ms) may be surprising because layer VI cells have a small size and, presumably, a slow conduction velocity. However, several data from the recent and older literature explain that such short latencies are compatible with the conduction velocities and morphological characteristics of corticothalamic axons. 1) Axons originating in cells of cat’s suprasylvian area 5 (where our experiments have been conducted) give rise to collaterals distributing to the striatum as well as the LP thalamic nucleus; the latter are thick and end in clusters of large boutons within the LP nucleus (Paré and Smith 1996). It is plausible that the thick corticothalamic axons described in this study conduct at high velocities and explain the short latencies found in our paper. 2) Another, nonexclusive possibility is that our stimulus spread to the centrum medianum (CM) nucleus that is located at no more than 1–1.5 mm medial to LP (or the stimulus activated cortico-CM passing axons). In a previous study, it was found that the cortical cells in areas 5 and 7, projecting to CM, have high conduction velocities, up to 33 m/s, much faster than in other corticothalamic systems (Steriade et al. 1978).

The relatively long latency of orthodromic excitatory responses after thalamic stimulation, >1–1.5 ms in most instances, may be due to the hyperpolarization of thalamocortical (TC) cells during anesthesia. A depolarizing event triggered on a background of membrane hyperpolarization leads to a LTS and a spike burst instead of a single spike; the LTS has a rising time of a few milliseconds, which delays the firing of the TC cell after the stimulation (Deschênes et al. 1984; Jahnsen and Llina 1984). We cannot rule out the possibility that collaterals of corticothalamic axons were activated when stimulating the thalamus. However, this is unlikely to cause a major contamination because antidromic pyramidal stimulation has been shown to be much less effective in evoking inhibition than local synaptic activation (Krnjević et al. 1966; Renaud et al. 1974). Besides, this was not a major concern, because the objective of this paper was not to differentiate between corticocortical and thalamocortical pathways, but to determine whether there is a distinct laminar distribution of inhibitory inputs. Two differences were evident, however, between cortically and thalamically evoked IPSPs: 1) cortically elicited IPSPs were of higher amplitude, longer duration, and associated to larger increases in conductance than those evoked by thalamic stimulation; and 2) only thalamic stimulation was capable of eliciting IPSPs in isolation.

It is possible that synaptic potentials were contaminated by afterhyperpolarizations when stimulation led to firing of action potentials. Although most of the inhibition evoked by cortical or thalamic stimulation reversed with Cl\(^-\) injections (see Figs. 10 and 11), it is likely that intrinsic currents may participate in shaping the synaptic responses. No attempt was made to dissociate the intrinsic components in the synaptic responses investigated here.
Absence of prevalent laminar distribution

In motor cortex in vitro (van Brederode and Spain 1995), strong inhibitory responses to local stimulation consisted of GABA$_{A,B}$ components, were present in layers 2 and 3, and were weak or absent in layer 5. This difference was attributed, at least in part, to the strong $I_h$ in layer V neurons. In visual cortex in vivo (Douglas and Martin 1991), both types of GABAergic responses were present in all layers, but GABA$_A$ IPSPs were more pronounced in deep layers, whereas IPSPs in superficial layers developed more slowly and lasted longer. These data led to the proposal of a “canon-ical” circuit for the visual cortex, despite the rich interconnectivity between layers (Gilbert and Wiesel 1979; Kisvárday et al. 1987; Martin and Whitteridge 1984; Somogyi et al. 1983). Our data suggest that the microcircuitry in the association cortex is more complicated and that the amount of inhibition associated to thalamic or cortical inputs is determined by the local microcircuitry in a manner that remained beyond the methods of exploration used here.

A concern when comparing inhibitory responses between cells at different depths relates to the homogeneity of the excitatory drive reaching the local inhibitory interneurons that impinge on different cells. In other words, are differences in IPSPs’ amplitudes and durations artificially caused by differences in the excitatory drive to interneurons, due to the particular placement of stimulating electrodes? In this study, synaptic responses were evoked by strong, synchronous stimulation of the thalamus or the cortex. Such responses constitute an inseparable combination of EPSPs and IPSPs resulting from the activation of polysynaptic neuronal chains. In such conditions, evoked responses in any particular animal might be biased toward a certain laminar distribution of IPSPs, due to the placement of stimulating electrodes. However, no trend was seen in any particular experiment (see Fig. 2). In addition, to avoid this problem, we recorded pairs of cells simultaneously, at distances between 2 and 5 mm.

Data from in vitro experiments have shown a lower threshold for excitation than for inhibition on cortical stimulation (Gil and Amitai 1996). In our study IPSPs were never evoked alone on cortical stimulation, but they could be triggered in isolation after thalamic stimulation (see Figs. 1B and 3). This result may suggest a lower threshold for inhibition than for excitation in thalamocortical pathways, compared with corticocortical ones. However, at high-intensity stimulation, cortically elicited IPSPs were consistently of higher amplitude and longer duration and were accompanied by larger increments in membrane conductance (see dual intracellular recordings in Figs. 5 and 7). In those cases in which IPSPs were observed in isolation, the latency was longer than 3 ms, which is compatible with at least a bisynaptic pathway, therefore involving intermediary intracortical steps.

The IPSPs presented in this study were mostly monophasic, as has recently been found in vivo (Contreras et al. 1996) and in vitro (Gil and Amitai 1996). As well, the IPSPs were reversed almost completely by Cl$^-$. This result is at variance with the expected biphasic (GABA$_A$- and GABA$_B$-mediated) IPSP that has been described in vitro (Connors et al. 1988; van Brederode and Spain 1995). One possible explanation that has recently been advanced (Contreras et al. 1996) is that the evoked GABA$_A$ IPSP shuts off cellular activity in a large proportion of cells, thus leading to a generalized phenomenon of disfacilitation during which K$^+$ currents, responsible for the resting $V_m$, dominate the membrane behavior. It was then hypothesized that the apparent absence of GABA$_B$ components is due to the overwhelming effect of disfacilitation. Such a scenario may only be observable in a situation, such as in vivo, with important spontaneous activity. In the experiments presented here, under barbiturate anesthesia, background activity is smaller than under ketamine-xylazine, and the additional explanation may be offered that GABA$_A$ IPSPs are masked by the decreased $R_m$ of a long-lasting GABA$_A$ IPSP. It has been shown in vitro (Thompson and Gähwiler 1992) that pentobarbital sodium increases the decay time constant of GABA$_A$-mediated currents, consequently prolonging GABA$_A$-mediated IPSPs, as well as that long-lasting GABA$_A$ IPSPs may be due to polysynaptic activity. Long-lasting (0.1–0.4 s) monophasic IPSPs, with peak amplitudes at ~30 ms, have been described in vivo (Pollen and Lux 1966; Renaud et al. 1974); in those studies the percentage decrease in $R_m$ at the peak of the IPSP varied considerably, from <50 to 100% (see also Krnjević et al. 1966) (up to 60%). This variation, including our own results (60–90% according to the stimulated pathway), probably reflects the number and location of synapses activated, which in turn depends on the intensity and location of the stimulation. The time course and amplitude of the IPSPs presented in the present study is in full agreement with those earlier studies.

An important step in the evaluation of the excitatory-inhibitory balance in a given region of the cortex is to gain knowledge of underlying anatomic circuits. Given the remarkable heterogeneity of the laminar distribution of thalamocortical axons in different cortical areas (Jones 1985), some basic questions are as follows. 1) Is there a laminar arrangement of inputs that predicts a laminar organization of excitatory and/or inhibitory responses? 2) What is the distribution of symmetric and asymmetric synapses over the membrane of cells located at different depths? And 3) do thalamic or cortical inputs contact preferentially a specific cell type? A classical description of laminar distribution of thalamic inputs (Lorente de Nó 1938) separated a “specific” system with terminal arborizations densely packed in layers 3 and 4 and an “unspecific” system terminating sparsely in layers 1 and 6. Thalamic inputs to primary sensory areas, such as the visual cortex, show characteristic laminar differences in the distribution of EPSPs and IPSPs (Ferster and Lindström 1983). The LP-pulvinar nuclear complex projects to layers 4 and 1 of area 5 in the cat (Roberson 1978). Anatomic data indicate that there is a widely distributed layer 1 projecting system in the thalamus, interspersed among neurons projecting to deeper layers (Avendano et al. 1990; Steriade and Deschénes 1984). Despite the diversity of inhibitory inputs to cortical cells, the large decreases in $R_m$ associated with the IPSPs indicate that a large proportion of the inhibitory action occurs at or near the cell soma. Therefore neither anatomic nor the present physiological data indicate any preferential distribution of inhibitory inputs to distinct layers in the association cortex. In contrast to successful studies in primary cortices (mainly...
motor and visual), association areas defy more global approaches and require for its understanding combined physiological and anatomic studies at the single-cell level.

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REFERENCES


