Electrophysiological Properties and Input-Output Organization of Callosal Neurons in Cat Association Cortex

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The callosal projection plays a crucial role in sensory-motor integrative functions of the two hemispheres (Berlucchi et al. 1995; Innocenti 1994; Innocenti et al. 1995; Seymour et al. 1994) and the interhemispheric coherent activity of different oscillatory types in animals and humans, within fast-frequency range in brain-activated states of waking and rapid eye movement (REM) sleep (Engel et al. 1991; Kiper et al. 1999; Knyazeva et al. 1999; Nuñez et al. 1992) and within low-frequency range in different phases of slow-wave sleep (Bremer et al. 1956; Steriade et al., 1993a, 2001).

Morphological and physiological studies have reported different features of the callosal projection, such as the following: 1) the location of corpus callosum neurons mainly in cortical layers II/III but also in infragranular layers, among them layer V, in different neocortical areas (Barbas et al. 1989, 1994; Kasper et al., 1994; Matsubara et al. 1996; Milleret et al. 1994; Porter and White 1986; White and Czeiger 1991); 2) although the strict topography of this commissural pathway is usually emphasized, other studies indicate that the trajectories followed by callosal axons of each investigated area do not obey the rules of a strictly topographically ordered projection (Clarke et al. 1995), are extremely divergent (Clasca et al. 2000; Matsunami et al. 1994), and are not mirror-symmetric with respect to the midline (Olavarria 1996); 3) callosal neurons have significantly greater spine density and more complex apical and basal dendritic arbors than neurons with ipsilateral cortical projections (Soloway et al. 2002); also, callosal neurons have a different ultrastructure and synaptology compared with corticothalamic neurons (Farinas and DeFelipe 1991); 4) separate patches of thalamic and ipsilateral association axons are largely complementary on callosal neurons (Pandya and Rosene 1993); and 5) some properties of callosal neurons (Miller 1975; Swadlow 1985) and the suppressing effect of callosal volleys on the background firing of cortical neurons (Renaud et al. 1974) were studied with extracellular recordings, while intracellular studies of these neurons were used in experiments with conditioning procedures (Baranyi and Feher 1981).

In contrast to these numerous data related to the structural characteristics, connectivity, and some physiological properties of commissural neocortical cells, these neurons were not yet investigated with intracellular recordings to reveal differential types of responses to callosal volleys in electrophysiologically identified neuronal classes and their connectivity features in intact cortical and corticothalamic loops. The aims of the present study were to characterize excitatory and inhibitory postsynaptic potentials (EPSPs, IPSPs) in receiver and projection neurons of the callosal projection as a function of various neuronal types characterized by their responses to depolarizing current steps, to reveal converging callosal and thalamic inputs onto the same cortical neuron, and to test the presence of neurons implicated in the callosal-corticothalamic pathway of cat, as previously
shown with extracellular recordings in behaving macaque monkeys (Steriade et al. 1974).

**METHODS**

Experiments were conducted on 35 adult cats, under ketamine-xylazine anesthesia (10–15 and 2–3 mg/kg im, respectively) \((n = 19)\) or pentobarbital sodium (35 mg/kg, ip) \((n = 16)\). The animals were paralyzed with gallamine triethiodide after the electroencephalogram (EEG) showed typical signs of deep general anesthesia, consisting of a slow oscillation \((0.5–1\, \text{Hz})\) under ketamine-xylazine anesthesia or sequences of spindle waves \((7–14\, \text{Hz})\) under barbiturate anesthesia. Supplementary doses of anesthetics were administered at the slightest changes toward activated EEG patterns. The cats were ventilated artificially with the control of end-tidal \(\text{CO}_2\) at 3.5–3.7%. The body temperature was maintained at 37–38°C and the heart rate was approximately 90–100 beats/min. Stability of intracellular recordings was ensured by the drainage of cisterna magna, hip suspension, bilateral pneumothorax, and by filling the hole made for recordings with a solution of 4% agar.

Intracellular recordings from suprasylvian association areas 5 and 7 were performed using glass micropipettes filled with a solution of 3 M potassium acetate (KAc). A high-impedance amplifier with active bridge circuitry was used to record the membrane potential \((V_{\text{m}})\) and inject current into the neurons. Location of neurons was estimated by micromanipulator readings that differ by <15% from the position of Lucifer yellow-stained (Steriade et al. 1993a) or Neurobiotin-stained (Contreras and Steriade 1995) neurons. Field potentials were recorded in the vicinity of impaled neurons, using bipolar coaxial electrodes, with the ring (pial surface) and the tip (cortical depth) separated by 0.8–1 mm. Stimulating electrodes (similar to those used for field potential recordings) were inserted in homotopic points of the contralateral areas 5 and 7, as well as into thalamic nuclei that provide inputs to, and are targets of, cortical areas 5 and 7, namely lateroposterior (LP) and intralaminar centrolateral (CL) nuclei (see Steriade et al. 1997).

At the end of experiments, the cats were given a lethal dose of pentobarbital sodium. The experimental protocol was approved by the Committee for Animal Utilization of Laval University, permission nr. 2002-007.

**RESULTS**

**Database and neuronal identification**

We intracellularly recorded 545 cortical neurons. Of those, 98 were responsive to stimulation of homotopic sites in the contralateral cortex; 58 were responsive under ketamine-xylazine anesthesia, and 40 were responsive under barbiturate anesthesia. As no significant differences in postsynaptic potentials (PSPs) and/or antidromic responses were observed between these two groups of experiments, hereafter, we mention the anesthetic condition only in the legends of figures. Also, no notable difference was detected between area 5 and area 7 neurons.

Four classes of neurons responsive to callosal stimulation were identified electrophysiologically, according to their responses to depolarizing current pulses, in-line with previous in vitro (reviewed in Connors and Gutnick 1990) and in vivo (Gray and McCormick 1996; Núñez et al. 1993; Steriade et al. 1998b) studies. Regular-spiking (RS) neurons \((n = 74)\) displayed trains of single spikes that adapted quickly or slowly to maintained stimulation. Fast-rhythmic-bursting (FRB) neurons \((n = 10)\) gave rise to high-frequency \((300–600\, \text{Hz})\) spikebursts recurring at fast \((30–50\, \text{Hz})\) rates. Fast-spiking (FS) neurons \((n = 7)\) fired thin action potentials and sustained tonically high firing rates without frequency adaptation. Intrinsically bursting (IB) neurons \((n = 7)\) generated clusters of action potentials, with clear spike inactivation, followed by hyperpolarization and neuronal silence. Examples of neurons belonging to these categories are illustrated with their responses to direct depolarization in different figures. Neurons responsive to contralateral cortical stimulation were located at depths between 0.3 and 1.5 mm.

**Characteristics of EPSPs and IPSPs of callosal origin**

In 87 of 98 responsive neurons, stimuli applied to homotopic sites in the contralateral areas 5 or 7 were used to elicit EPSPs that were subthreshold for giving rise to action potentials. EPSPs’ latencies usually ranged between 1.3 and 4.5 ms. EPSPs as long as 19–20 ms \((n = 0.8\, \text{mm}\) in Fig. 7A) did not necessarily reflect polysynaptic pathways because antidromic response latencies in callosal neurons could be as long as 18.5 ms (see following text), indicating that some callosal neurons have very slow conduction velocities. At the resting membrane potentials \((V_{\text{m}})\) of \(-60\) to \(-80\, \text{mV}\), which were usually seen in our experiments, the amplitudes of EPSPs ranged from 1 to 4.3 mV (but much higher in FRB neurons; see following text) and they displayed simple \((\text{Fig. 1, A–B})\) or compound configurations; in the latter case, they consisted of successive depolarizations (Fig. 1D). In some instances, a small-amplitude negativity was detected before the EPSP, with onset latency at 0.5–0.7 ms \((\text{Fig. 1C, Fig. 2B})\), likely reflecting intracellularly the field presynaptic volley.

Different features of EPSPs, related to latencies, amplitudes, and slopes, have been detected in closely located neurons recorded in succession along the same micropipette track. Such variations in neurons separated by 50 \(\mu\)m or less were observed not only under ketamine-xylazine anesthesia, which produces active states associated with rich background activity, but also during the interspindle lulls of barbiturate anesthesia, during which the spontaneous field and cellular activity are negligible (Fig. 2).

Differences between EPSPs in the four electrophysiologically defined cellular classes were analyzed in a sample of 54 neurons that could be recorded for long enough periods of time to be characterized by depolarizing current pulses and by their responses to stimulation of homotopic sites in the contralateral association cortex (Fig. 3). FRB neurons exhibited callosally evoked EPSPs whose amplitudes were more than threefold as large \((\text{median, 11.3 mV})\) as RS, IB, and FS neurons \((\text{medians, 3–3.5 mV})\). These differences were statistically significant between FRB and RS \((P < 0.001, t\text{-test})\), FRB and IB \((P < 0.01)\), and FRB and FS \((P < 0.04)\) neurons. As the median latencies of EPSPs, measured at their onset, were much shorter in FRB cells \((\text{median, 1.35 ms})\) than in RS \((4\, \text{ms})\) and FS or IB neurons \((2.5\, \text{ms})\) neurons (Fig. 3). The differences in latencies were statistically significant between FRB and RS \((P < 0.002)\), FRB and IB \((P < 0.01)\), and FRB and FS \((P < 0.006)\) neurons. As to the differences in EPSPs’ latencies between FS and RS neurons and between IB and RS neurons, they were significant at \(P < 0.05\). In two of seven recorded IB neurons, the EPSPs exhibited a complex configuration that was initiated by a “spikelet”-like component, followed by a larger EPSP, occasionally leading to full action potentials (Fig. 4).
In all recorded neurons (n = 545), the specificity of excitatory or inhibitory inputs set into action by callosal volleys onto the somadendritic membrane of cortical neurons was tested by using two closely spaced (<1 mm) stimulating electrodes inserted into the depth of the contralateral cortex. For this analysis, we considered only those recordings (n = 268) in which at least one neuron within a given micropipette track was responding to stimulation of one of the two contralateral stimulating electrodes. Of those neurons, only 91 responded to contralateral stimulation with primary EPSPs to stimulation of one electrode. Three neurons responded with primary EPSPs to stimulation of one electrode and with IPSPs to the stimulation of the second contralateral electrode. In Fig. 5, the IPSP was maximal at −65 mV and fully reversed in polarity between −79 and −85 mV (Fig. 5, A2–A3), while the EPSP consisted of several depolarizing components that triggered action potentials around −60 mV (Fig. 5, B2–B3).

Convergence of callosal and thalamic EPSPs onto cortical cells, some of them corticothalamic

Twenty-eight neurons were driven at short latencies (<5 ms) from both homotopic sites in the contralateral cortex and one of the two stimulated thalamic nuclei (LP or CL) that are known to project to association areas 5 and 7 (Avendaño et al. 1988; Jones 1985). Such neurons are illustrated in Figs. 6 to 8. In 28 neurons, we could reveal EPSPs elicited by LP or CL thalamic stimuli, convergent with callosally evoked EPSPs, in antidromically identified corticothalamic neurons (see Fig. 6). This demonstrates complex patterns of callosal and thalamocortical pathways impinging on the same deeply lying corticothalamic.
neuron. The latencies of CL-evoked and callosally evoked EPSPs were examined in 18 neurons and the plot in Fig. 6 shows that the latencies of CL-evoked EPSPs were dispersed between approximately 1.5 and 4 ms, whereas close to 80% of EPSPs’ latencies evoked by callosal volleys were above 3 ms, up to approximately 8 ms.

Along the same micropipette track, different impaled neurons revealed a great variety of responses to thalamic or callosal volley. Figure 7A shows that, of four neurons recorded from layer III to layer V, three of them exhibited thalamically evoked short-latency (1–3 ms) EPSPs, while the deeply lying neuron was antidromically invaded from the thalamic rostral intralaminar CL nucleus; only one of those neurons (depth 0.8 mm) also responded with a long-latency EPSP to callosal stimulation. Similar differences between successively recorded neurons along the same track are illustrated in Fig. 7B, showing convergent EPSPs from both thalamocortical and callosal pathways (neuron at 1.5 mm), while another neuron was antidromically identified from the intralaminar thalamus.

Generally, in contrast to responses evoked by thalamocortical volleys that were observed in most neurons within a cortical column, stimuli applied to homotopic sites in the contralateral cortex activated only some neurons, at restricted cortical depths.

Callosally projecting neurons

Nineteen neurons were identified antidromically as projecting through corpus callosum. Of those, 14 neurons were located in layers II–III and the upper part of IV, and 5 neurons were located more deeply in layer IV and V. Criteria for antidromic identification (see Lipski 1981) were fixed-latency, take-off of action potentials directly from the baseline, collision with spontaneously occurring action potentials at proper

FIG. 2. Different amplitudes of callosal EPSPs in 2 closely spaced (approximately 50 μm) neurons. Barbiturate anesthesia. A and B, two area 5 neurons recorded in immediate succession, at depths of 516 and 564 μm. Stimuli were applied to contralateral area 5 during spindles (see simultaneously recorded depth electroencephalogram (EEG) from the area where intracellular recordings were made) as well as during interspindle lulls. Responses during interspindle lulls in both A and B are marked by horizontal bars and expanded below (arrows) in A2 and B2 (superimposed traces) and A3 and B3 (averages of 10 responses).
time intervals (Fig. 8A2) and, as a subsidiary criterion, faithful following of stimuli at or over 100 Hz. In 16 neurons, latencies of antidromic responses were between 1.3 ms (see neuron at 0.96 mm in Fig. 7) and 3.1 ms (neuron in Fig. 8B). The remaining three neurons had much longer latencies, ≈18.5 ms (Fig. 8A). It is possible that, in the latter case, fine collaterals of the parent callosal axon were stimulated. All antidromically identified neurons were RS.

DISCUSSION

The major findings of these experiments are as follows. 1) Various classes of cortical neurons from association areas 5 and 7, as identified electrophysiologically by depolarizing current pulses, exhibit statistically significant differences in amplitudes and latencies of EPSPs evoked by contralateral cortical stimuli, with FRB neurons displaying largest EPSPs, with shortest latencies. 2) Neurons receiving callosal excitatory inputs are located not only in layers II/III but also in infragranular layers. 3) Callosal inputs converge with thalamic inputs onto the same cortical neuron and the latencies of callosally evoked EPSPs are longer than those of thalamically evoked EPSPs. 4) Callosal synaptic inputs activate antidromically identified corticothalamic neurons.

The fact that FRB neurons, firing high-frequency bursts at fast frequencies, exhibited EPSPs whose amplitudes were threefold larger, and latencies two- or threefold shorter, than those found in the three other cellular classes may have functional consequences. Indeed, FRB neurons have ipsilateral cortical connections with neurons of a similar type, as suggested by fast, subthreshold depolarizing events occurring within the same frequency range as the high-frequency spike-bursts of other FRB neurons, and long horizontal axons (Gray and McCormick 1996; Steriade et al. 1998b). Also, FRB neurons located in deep layers have been identified as projecting to thalamic nuclei, among them to the rostral intralaminar CL nucleus (Steriade et al. 1998b) that has widespread cortical projections (Jones 1985; Steriade et al. 1997). These connectivity features allow callosally elicited activities to be spread, via FRB neurons, across contralateral cortical and corticothalamic networks. It is known that FRB neurons are implicated in the generation of fast (gamma, 30–60 Hz) oscillations because of the high frequencies of their spike-bursts. They are the best candidates to transfer and synchronize through interhemispheric pathways spontaneous and evoked gamma rhythms (Engel et al. 1991; Kiper et al. 1999; Knyazeva et al. 1999; Nuñez et al. 1992). This is also the case with the slow sleep
oscillation that is generated intracortically (Steriade et al. 1993a,b) and can be transferred to the contralateral cortex, as demonstrated by dual intracellular recordings from right and left cortices (Contreras and Steriade 1995). Moreover, focal paroxysms of spike-wave type are generated intracortically in FRB neurons (see Figs. 7–8 in Steriade et al. 1998a) and can therefore be preferential targets for synchronizing epileptiform processes between the two hemispheres, with subsequent spread to the thalamus.

Data showing convergent callosal and thalamic LP excitatory inputs onto the same cortical neuron, sometime identified as corticothalamic (see Fig. 6), can be related to an electron
microscopic study showing that axon terminals from another thalamic nucleus, the mediodorsal (MD) one, make asymmetrical synaptic contacts with dendritic spines of layer III callosal cells (Kuroda et al. 1995). These findings, together with the previous (Steriade et al. 1974) and present demonstration of a bisynaptic callosal-corticothalamic pathway (see Fig. 6), demonstrate the complexity of converging thalamocortical and callosal excitatory inputs acting on the feedback corticothalamic neurons. Our data, together with previous findings reporting orthodromic responses evoked by contralateral cortical stimuli in corticostriatal neurons (Wilson 1987), indicate the effectiveness of callosal volleys in driving corticofugal neurons projecting to either thalamus or caudate nucleus.

Differences between cortical responses to thalamic and callosal volleys have been previously described at the extracellular level in association areas 5 and 7 (Kitsikis and Steriade 1975). Among these differences, one of the most striking is the powerful postinhibitory rebound that follows the early excitation elicited by stimulating appropriate thalamic nuclei, as opposed to the absence or small amplitude of the rebound after callosal stimulation, although an inhibitory wave followed the early callosally evoked excitation (see Fig. 2 in Kitsikis and Steriade 1975). It suggested that the postinhibitory rebound does not evolve as a mere consequence of preceding inhibition, but may also "require a source of excitatory synaptic drives which are brought into action by afferent thalamic, and not by transcortical stimulation." This assumption has more recently been supported by dual intracellular recordings of cortical and thalamic neurons, showing the leading role of spike-bursts fired by...
thalamocortical neurons in the generation of cortical postinhibitory rebound (Grenier et al. 1998).

Concerning the IPSPs in response to callosal stimulation, the relatively short latency (approximately 4.5 ms) revealed in Fig. 5, compared with the longer latency (approximately 6.5 ms) of the EPSP elicited in the same neuron from a closely spaced stimulating electrode in the contralateral cortex, may suggest a monosynaptic inhibitory pathway. Indeed, several lines of evidence indicate that, besides the known excitatory callosal neurons, inhibitory cells may also course through the corpus callosum: 1) nonpyramidal, bitufted cells and aspiny neurons from layers II/III and V were retrogradely labeled from callosal projection in rat visual cortex (Martinez-Garcia et al. 1994); and 2) GABA-immunoreactive neurons, located in both superficial and deep layers, were found to give rise to callosal pathways between somatosensory cortices of rats (Gonchar et al. 1995). However, the bisynaptic inhibitory effect (through prior excitation of a local-circuit GABAergic neuron at the site

FIG. 6. Convergence of callosal and thalamic excitatory inputs onto corticothalamic neuron. Ketamine-xylazine anesthesia. RS neuron located in layer V of area 5 was antidromically invaded from thalamic centrolateral (CL) nucleus at 2.4-ms latency (see C2–3), synaptically excited from thalamic lateroposterior (LP) nucleus (3 mm apart from CL nucleus) at 4.8 ms (see A2), and synaptically excited from the contralateral area 5 at 3.7 ms (see B2). Top: the same type of responses, superimposed at a lower speed to also show hyperpolarizations following the initial responses (A, LP stimulation; B, callosal stimulation; and C, CL stimulation; in all cases, second range of responses are expanded and at higher speed). Diagram shows callosally (CC) and thalamically (LP) evoked excitation in corticothalamic neuron antidromically activated from CL nucleus. Plot depicts latencies of EPSPs evoked by thalamic (CL nucleus) and callosal volleys in a sample of 18 neurons.
of recording) may seem more reasonable. Some FRB neurons that receive callosal inputs at short latencies (see Fig. 3) have been formally identified by intracellular staining as basket aspiny or sparsely spiny cells (Steriade et al. 1998b) and FS (presumably GABAergic) neurons are also the target of callosal afferents.

It had been traditionally assumed that neurons receiving inputs from, and projecting to, callosal pathways are located in

**FIG. 7.** Variety of responses to thalamic CL nucleus and callosal stimulation in 4 neurons recorded along the same micropipette tracks in area 5. A and B: 2 tracks in 2 different animals. A: ketamine-xylazine anesthesia. All illustrated responses were recorded during the depth-positive phase of the field slow oscillation reflecting cell hyperpolarization. Top (averaged responses, n = 10): represent at left (A1) responses to thalamic CL stimuli, and at right (A2) responses of the same neurons to stimuli applied to contralateral area 5. First 2 traces are FS neurons recorded at 0.57 and 0.8 mm; the bottom 2 traces are RS neurons recorded at 1.3 and 1.32 mm. V_m indicated in each case. Bottom panels: expanded early responses (single sweeps; same organization as top). Note antidromic response to the thalamic CL stimulus in the deeply lying (1.32 mm, 4th) neuron; CL-evoked EPSPs at different latencies (from 1st to 3rd neuron: 2.2 ms, 0.9 and 4.3 ms). Callosal stimulation evoked long-latency EPSP in only the 3rd neuron. B: barbiturate anesthesia. Differential responses to thalamic CL and callosal stimulation in 3 neurons recorded at different cortical depths. All responses were recorded during inter spindle lulls. Responses to single stimuli applied to thalamic CL nucleus (spike truncated) and to contralateral area 5. Note convergent EPSPs from CL and callosal pathway in cell 3, and antidromic response to CL stimulation in cell 2 (latency 1.3 ms).
superficial layers II/III and that callosal synaptic linkages are mirror-symmetric. However, many data, including ours, challenge this conventional view. Tract-tracing studies showed that primary and association visual areas contain callosally connected neurons that originate in layers II/III but also VI (Innocenti et al. 2002). Excitatory contacts have been demonstrated between layer V pyramidal neurons in callosally connected slices, and EPSCs were mediated by both AMPA and NMDA receptors (Kumar and Huguenard 2001). Also, in the present experiments callosally elicited EPSPs were found in neurons recorded not only in layers II/III but also below 0.8 mm and in deep layers (Figs. 6–8). As to the previous view of callosally connected symmetric foci, more recent results revealed nonsymmetric sites with respect to the midline in cat visual cortex (Olavarria 1996). Other data similarly showed that, although the strongest callosal projections arise from
homotopic areas in the parabelt auditory cortex, weaker callosal inputs originate from superior temporal gyrus (Hackett et al. 1999).

Activities in callosal pathways are implicated in plastic changes. Stimulation of homotopic sites in the contralateral cortex, at 10 Hz (mimicking sleep spindles) or 40 Hz (similar to the prevalent oscillation during waking and REM sleep), induces long-lasting potentiation of control responses (Cissé et al. 2002; Steriade et al. 2002). This potentiation occurs at a depolarized level and may lead to self-sustained paroxysmal events in thalamically lesioned animals, thus emphasizing the role played by the callosal pathway in plasticity, even in the absence of thalamus (see Fig. 14 in Steriade et al. 1993b).

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