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Cortical and thalamic cellular correlates of electroencephalographic burst-suppression

M. Steriade *, F. Amzica and D. Contreras

Laboratoire de Neurophysiologie, Faculté de Médecine, Université Laval, Quebec G1K 7P4 (Canada)

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Summary This experimental study on anesthetized cats used intracellular recordings of cortical, thalamocortical and reticular thalamic neurons (n = 54), as well as multi-site extracellular recordings (n = 36), to investigate the cellular correlates of EEG burst-suppression patterns, defined as alternating wave bursts and periods of electrical silence. Burst-suppression was elicited by the administration of the same or other anesthetic agents upon the background of an already synchronized EEG activity.

About 95% of cortical cells entered burst-suppression, in close time-relation with EEG activity, displaying sequences of phasic depolarizing events associated with bursts of EEG waves and an electrical silence of the neuronal membrane during flat EEG epochs. The membrane potential (V_m) hyperpolarized by ≈ 10 mV prior to any EEG change and the slow rhythms reflecting deep stages of anesthesia progressively disorganized with transition to burst-suppression. During flat EEG epochs, the apparent input resistance (tested through short hyperpolarizing current pulses) decreased (range 12–60%) and neuronal responsiveness to orthodromic volleys (tested by thalamic and cortical evoked excitatory postsynaptic potentials) was dramatically reduced. It is proposed that the decreased input resistance is mainly due to an increase in K^+ conductances.

At variance with cortical neurons, only 60–70% of thalamic cells ceased firing before overt EEG burst-suppression and were completely silent during flat periods of EEG activity. The remaining 30–40% of thalamic cells discharged rhythmic (1–4 Hz) spike bursts during periods of EEG silence. This rhythm, within the frequency range of delta waves, is generated in thalamic cells by the interplay between two of their intrinsic currents at critical levels of V_m hyperpolarization. However, with the deepening of burst-suppression, when silent EEG periods became longer than 30 sec, thalamic cells also ceased firing.

The assumption that full-blown burst-suppression is achieved through virtually complete disconnection in brain circuits implicated in the genesis of the EEG is corroborated by the revival of normal cellular and EEG activities after volleys setting into action thalamic and cortical networks.

Key words: Burst-suppression; NREM sleep; Spindles; Delta waves; Slow (< 1 Hz) rhythm; Intracellular recordings; Cortex; Thalamus

The electroencephalographic (EEG) burst-suppression consists of transient sequences of high-voltage slow waves intermingled with sharp waves, alternating with periods of depressed background activity or complete electrographic flatness. This aspect has been known since Derbyshire et al. (1936) have shown that wave bursts separated by periods of electrical silence may appear under different anesthetics. The term burst-suppression was introduced to describe the occurrence of alternating wave bursts and blackout sequences in deeply narcotized animals (Swank 1949), in the isolated cerebral cortex (Swank and Watson 1949; Henry and Scoville 1952), during coma with dissolution of cerebral functions (Bauer and Niedermeyer 1979), after trauma associated with cerebral anoxia (Stockard et al. 1975), and in the cortex infiltrated by tumoral tissue (Fischer-Williams 1963).

Despite the repeated description of this EEG aspect under a variety of clinical and experimental conditions, there are no available data as to the neuronal events associated with the burst-suppression pattern. This article reports, by means of intracellular recordings of cat's neocortical and thalamic neurons as well as multi-site simultaneous extracellular recordings, the evolution from the normally synchronized EEG activity to burst-suppression during various types of anesthesia. Our data shed light on the high degree of coherence between cerebral neurons during this EEG condition and on the differential propensity of cortical and thalamic cells to burst-suppression patterns.

Methods and material

Experiments were conducted on adult cats under different anesthetics: urethane (1.8 g/kg, i.p.); ketamine and xylazine (10–15 mg/kg and 2–3 mg/kg,

* Corresponding author. Tel.: (418) 656-5547; Fax: (418) 656-7898.

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i.m.); ketamine (40 mg/kg, i.m.) supplemented with nitrous oxide (N_2O); or sodium pentobarbital (35 mg/kg, i.p.). These anesthetics were repeated to maintain a permanent state of EEG synchronization. In addition to general anesthetics, pressure points and tissues to be incised during the surgical procedures were infiltrated with lidocaine. The animals were paralyzed with gallamine triethiodide and artificially ventilated by monitoring the end-tidal CO_2 concentration around 3.7–3.8%. Burst-suppression was achieved by the administration of the same or other anesthetics upon a background of an already synchronized EEG (see Results).

Intracellular recordings were performed with micropipettes (tip diameter 0.5 μm) filled with 3 M solution of K-acetate (DC resistance 25–40 MΩ), inserted into: (a) visual areas 17 and 18, suprasylvian association areas 5 and 7, and motor areas 4 and 6 of the neocortex; (b) dorso-lateral geniculate (dLG), lateroposterior (LP), ventroanterior-ventrolateral (VA-VL), and intralaminar centrolateral (CL) thalamic nuclei; and (c) rostrolateral and perigeniculate (PG) sectors of the reticular thalamic (RE) nuclear complex. For thalamic recordings, parts of the cortex and fornix overlying the thalamic nuclei where recordings were planned were removed to facilitate the passage of the micropipettes. The stability of intracellular recordings was insured by cisternal drainage, bilateral pneumothorax, hip suspension, and by covering the brain with 4% agar dissolved in saline. A high-impedance amplifier with active bridge circuitry was used to record and inject current inside the cells. The signals were digitally recorded on tape (bandpass: DC to 9 kHz) and data were fed into a computer with a sample rate of 20 kHz for off-line analysis. For extracellular recordings from multiple sites we used either coarser micropipettes or tungsten microwires (impedance: 2–8 MΩ). Extracellular data were filtered at 300 Hz and 10 kHz and sampled at 10 kHz for off-line spike discrimination and analysis.

The gross electrical activity was recorded from the cortical surface through stainless steel screws into the calvarium (EEG), from the depth of the cortex (electrocorticogram, ECoG), and from different thalamic nuclei (electrothalamogram, EThG). In some experiments, the mass electrical activity was also recorded in the pedunculo-pontine tegmental (PPT) region of the brain-stem.

Stimulating coaxial electrodes were inserted into appropriate thalamic nuclei and homotopic points in the contralateral neocortex (in the case of cortical recordings) and in the white matter or deep layers of the cerebral cortex (in the case of thalamic recordings). Testing volleys had durations of 0.05–0.3 msec and intensities ranging from 0.05 to 0.5 mA.

At the end of experiments, animals received a lethal dose of sodium pentobarbital and were perfused transcardially with saline followed by 10% paraformaldehyde. The location of recording and stimulating electrodes in the thalamus and PPT region was controlled on 40–75 μm sections stained with thionine.

Results

Data base, neuronal identification, and normal sleep oscillatory patterns in neocortical and thalamic neurons

The present results are based on 90 cells. Forty-two cortical, 33 thalamocortical, and 15 RE thalamic neurons entered the stage of burst-suppression by re-administration of the initial anesthetic, or by adding another anesthetic to the initial one, or by administering the muscarinic blocker scopolamine when the EEG was fully synchronized.

(a) Out of 42 neocortical cells, 30 were recorded intracellularly and 12 extracellularly. The resting membrane potential (V_m) of intracellularly recorded neurons was more negative than -60 mV and their input resistance ranged between 10 and 40 MΩ. All were regular-spiking, slow-adapting cells, as defined in vitro (Connors et al. 1982) and in vivo (Nuñez et al. 1993). Corticothalamic or callosal neurons were identified by means of antidromic invasion from appropriate thalamic nuclei or the contralateral cortical areas. (b) Thalamocortical cells (13 intracellularly and 20 extracellularly) were identified by their antidromic invasion from the respective cortical projection areas. Their activity included brief (5–15 msec), high-frequency (250–400 Hz) spike bursts, with progressively increased duration of interspike intervals (Domich et al. 1986). (c) RE thalamic neurons (11 recorded extracellularly, 4 intracellularly) were recognized by their long (> 50 msec) spike bursts, with a decreased duration followed by an increased duration of interspike intervals (Domich et al. 1986; Contreras et al. 1993). Spike bursts with a first and last long interval are illustrated in the expanded trace of Fig. 8B. The acceleration-deceleration pattern of spike bursts fired by RE thalamic cells is dissimilar to that of spike bursts in thalamocortical neurons (see above point b). Intracellularly recorded relay and RE thalamic neurons had V_ms more negative than -55 mV.

Before entering burst-suppression, cortical and thalamic cells displayed 3 types of sleep oscillation (see Steriade 1993). (a) Spindles (7–14 Hz) were overwhelming under barbiturates, less pronounced under ketamine, and appeared only occasionally under urethane. During EEG spindle oscillations, GABAergic RE thalamic cells oscillated with rhythmic (7–14 Hz) spike barrages over a sustained depolarization, whereas thalamocortical cells exhibited a mirror image, with inhibitory postsynaptic potentials (IPSPs) within the same frequency range. The spindle sequences recurred

periodically, with a slow rhythm of 0.1–0.3 Hz. Both spindle rhythms (7–14 Hz and 0.1–0.3 Hz) have been described in the EEG and thalamic neuronal activity of cat (see Steriade et al. 1990) and they are also observed in the EEG of humans, during stage 2 sleep (Evans 1992). (b) Spike bursts within the delta frequency range

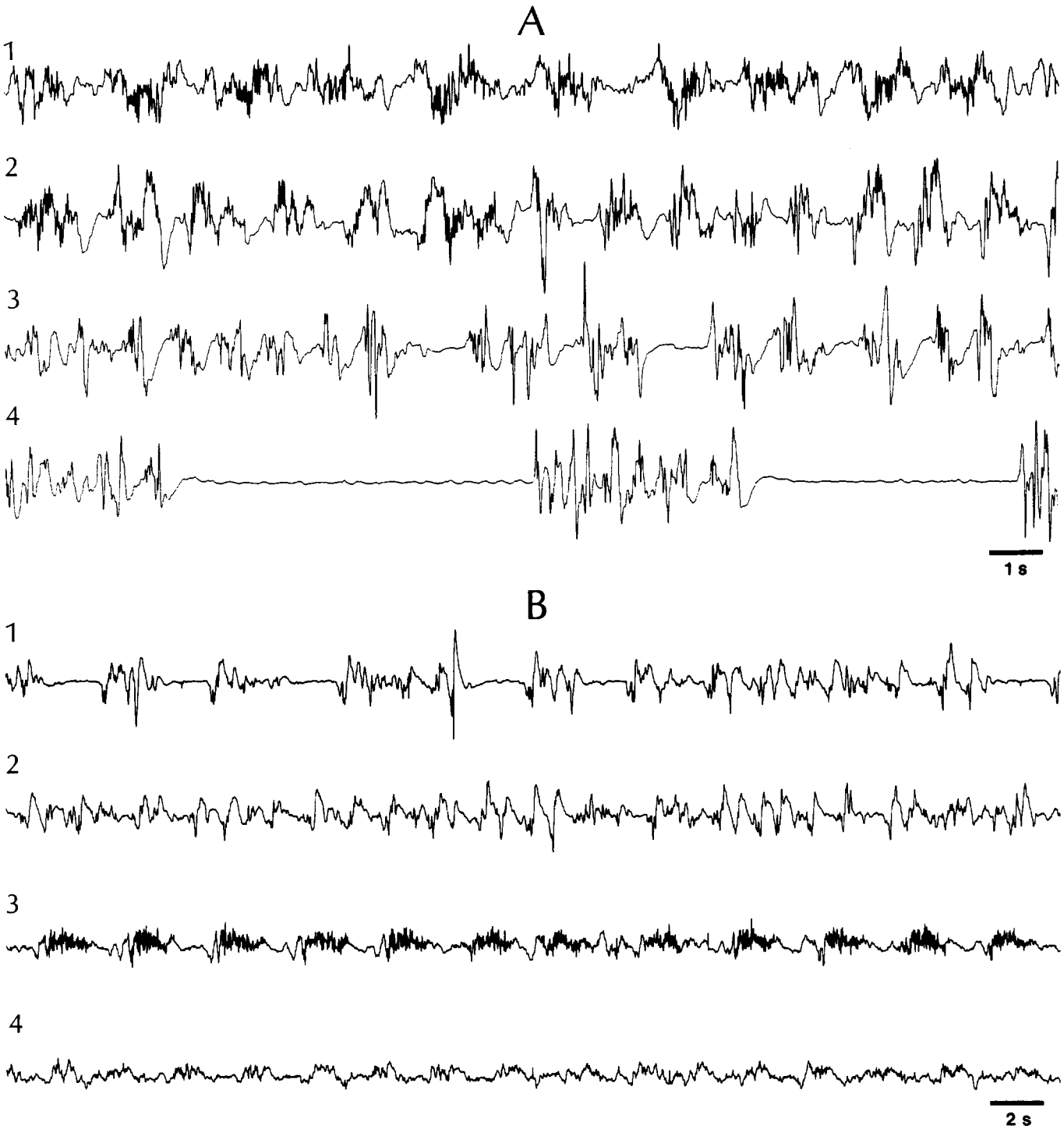


Fig. 1. EEG patterns of burst-suppression. Recordings by means of screws inserted into the calvarium overlying anterior suprasylvian (association) cortex. A: ketamine and xylazine anesthesia (1). On this control background, another dose of ketamine (5 mg/kg, i.v.) was administered (10 sec before onset of panel 2). Panels 3 and 4 are taken 2 min and 4 min after panel 2. Note, in 2–4, progressively increased amplitudes of sharp waves and appearance of flat periods with increasing durations. B_{1-4}: recovery from burst-suppression by removing N_2O (at 20 sec before onset of 1) that was previously added on a background anesthesia produced by urethane. The flat episodes were replaced by normal EEG activity (2) and thereafter by sequences of fast ($\approx$ 20 Hz) waves (3).

(1–4 Hz) occurred in thalamocortical cells during urethane or ketamine and xylazine anesthesia (see Figs. 9A and 10B). The thalamic origin of this clock-like, stereotyped, delta pattern was demonstrated both in vitro (McCormick and Pape 1990; Leresche et al. 1991) and in vivo (Steriade et al. 1991; Curró Dossi et al. 1992). Besides the single-cell firing at the delta frequency, focal waves within the same frequency range could also be seen, resulting from the synchronization of oscillatory thalamic neurons. (c) The slow rhythm (< 1 Hz) of cortical neurons appeared under urethane or ketamine and xylazine anesthesia. This oscillation (see Figs. 2–3) was recently described in a high proportion (88%) of cells recorded from sensory, association and motor areas, in either anesthetized or brain-stem-transected undrugged animals (Steriade et al. 1993c,d). The slow rhythm is cortical-generated, as it survives thalamectomy, and is reflected in both RE thalamic and thalamocortical neurons where it groups the other two sleep rhythms (spindles and delta) within complex wave sequences recurring every 2–5 sec (Steriade et al. 1993b). In ketamine and xylazine anesthetized animals, the slow cortical rhythm had higher frequencies (generally 0.5–0.9 Hz) than under urethane anesthesia (generally 0.2–0.5 Hz). Intracellular recordings showed that the slow oscillation of neocortical cells under urethane increases its frequency by administering ketamine (Steriade et al. 1993c). Besides, under ketamine EEG cortical waves became spiky and had quite high amplitudes (Fig. 1A).

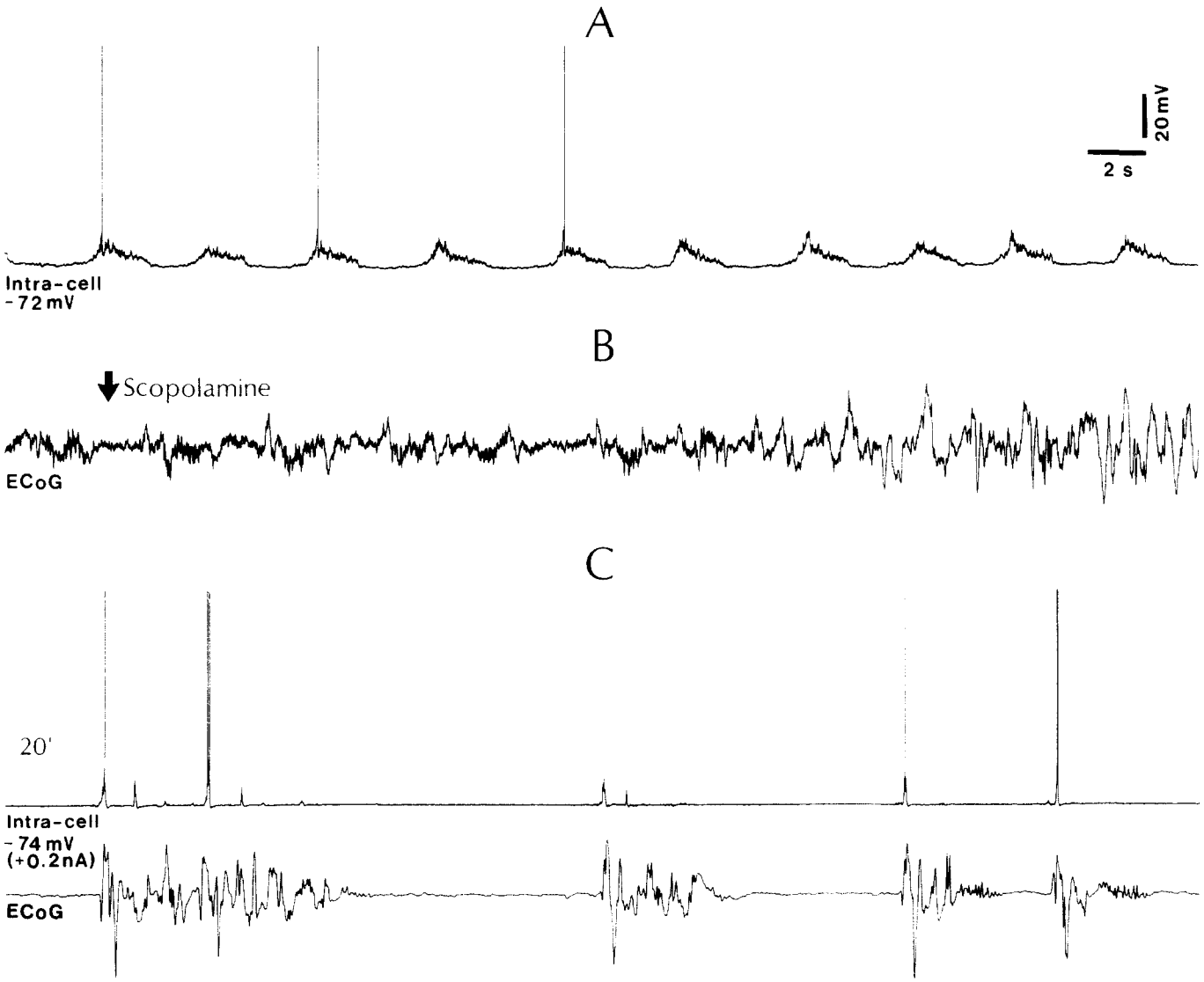


Fig. 2. Relation between ECoG and cellular activities during burst-suppression. Intracellular recording of corticothalamic neuron at a depth of 0.8 mm in area 7. A: slow (≈ 0.3 Hz) oscillation during control period (urethane anesthesia). B: effect of scopolamine administration (arrow; 0.5 mg/kg i.v.) on ECoG. C: 20 min later. Close-time relation between neuronal activity and ECoG during full-blown burst-suppression. In this and following figures depicting intracellular recordings, V_m is indicated.

The EEG pattern of burst-suppression

Although various anesthetics produced quite distinct EEG patterns, similar burst-suppression aspects resulted from additional doses of a given anesthetic or from the mixture of different anesthetics.

The EEG pattern of burst-suppression that followed administration of various anesthetics generally consisted of sharp waves with high amplitudes, at frequencies usually ranging between 2 and 7 Hz, separated by progressively longer periods of complete flatness. The electrical silence lasted for 1–2 sec during the initial stage of this pattern (Fig. $1A_3$), but reached 5–15 sec in later stages (Figs. $1A_4$ and 2C) and even 30 sec or more in some instances (see below, Figs. 7 and 9A). In most cases (85%), the normal EEG activity resumed after the burst-suppression episodes. During recovery, the flat periods became progressively shorter and were eventually replaced by grapho-elements characterizing the initial anesthesia (Fig. 1B). When this evolution had a tendency toward a desynchronized EEG (Fig. $1B_4$), we immediately administered a general anesthetic.

Cortical cellular correlates of EEG burst-suppression and changes in membrane conductance

Forty out of 42 cortical cells entered the burst-suppression pattern. The close relation, during burst-suppression, between the activity of an intracellularly recorded neuron in the suprasylvian area 7 and the focal waves recorded at the depth of the precruciate

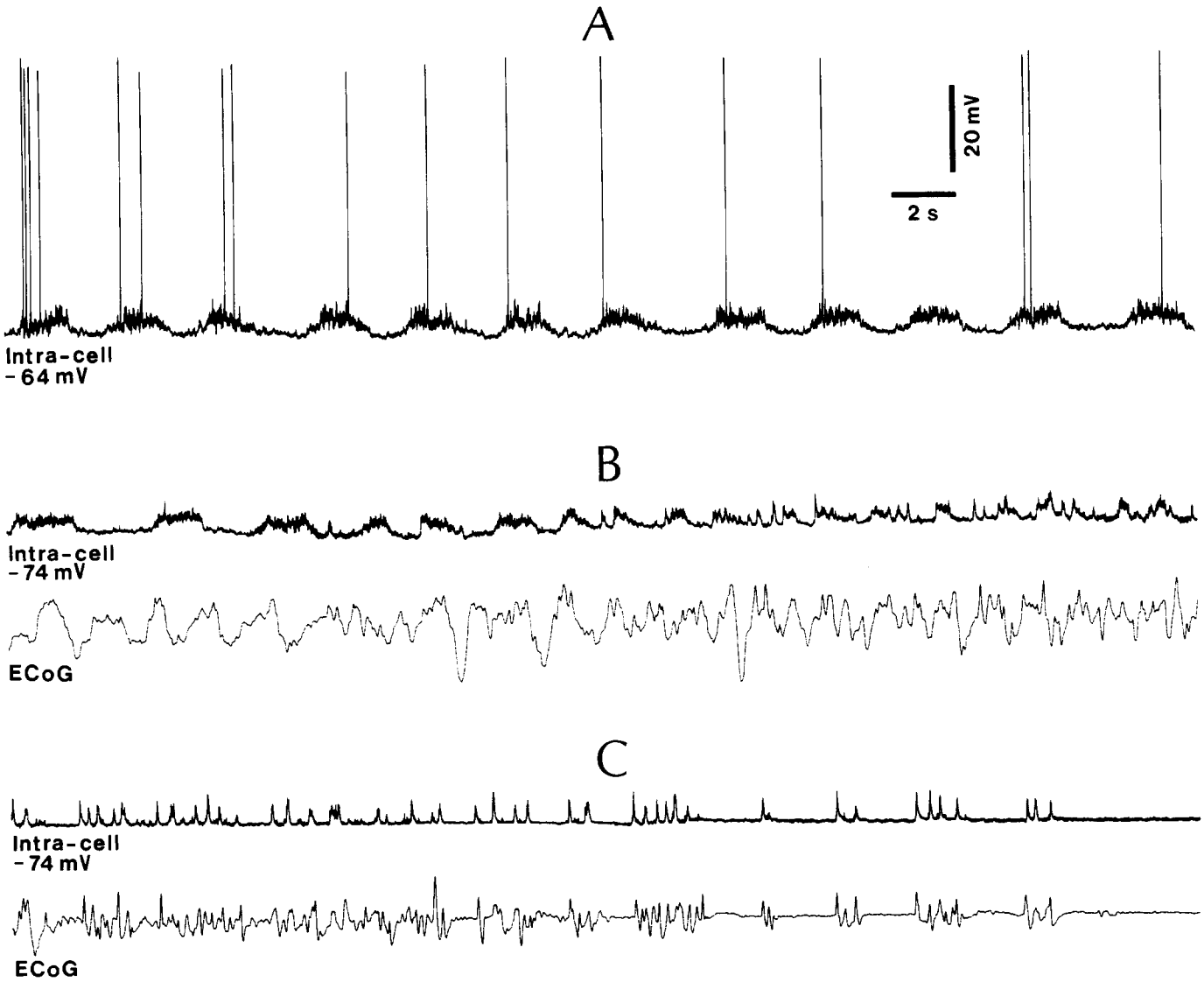


Fig. 3. Hyperpolarization of V_m and disorganization of slow cortical ($\approx$ 0.3 Hz) rhythm during burst-suppression. Area 5 neuron recorded intracellularly. A: slow oscillation ($\approx$ 0.3 Hz) under urethane anesthesia. B: on this background, administration of a barbiturate (5 mg/kg, i.v.) produced V_m hyperpolarization of 10 mV, abolished action potential generation, transformed the 0.3 Hz depolarizing envelopes into shorter depolarizing events, and disorganized the slow oscillation. Right part of panel C shows further V_m hyperpolarization and burst-suppression pattern reaching both cellular and ECoG activities, with periods of flatness lasting for 2–5 sec.

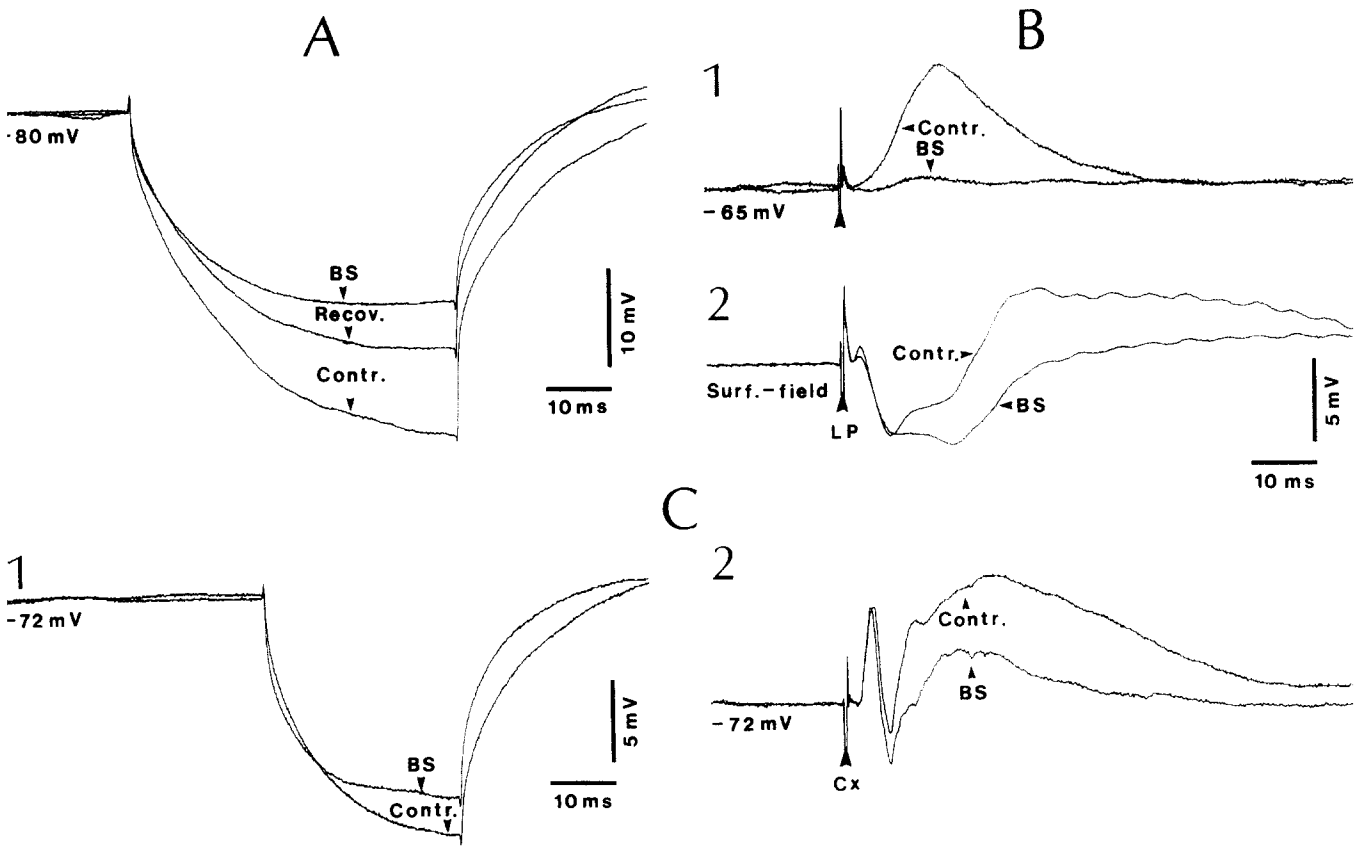
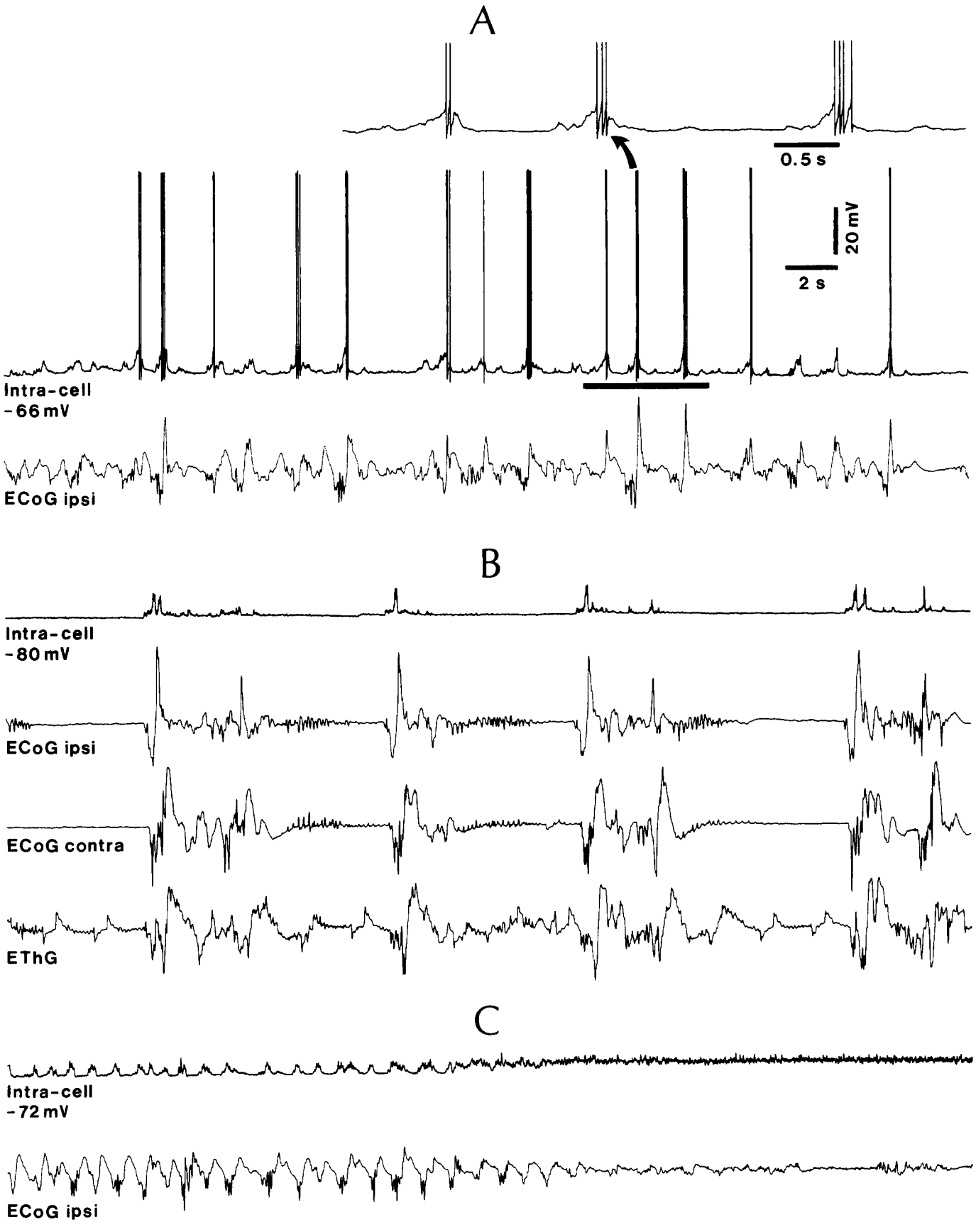


Fig. 4. Changes in membrane conductance and synaptic responsiveness during burst-suppression. A: neuron recorded at a depth of 0.8 mm in area 5. Input resistance measured through hyperpolarizing current pulses (1 nA, 50 msec) during a control period (Contr.), flat epochs of burst-suppression (BS), and initial period of recovery (Recov.). B: neuron recorded at a depth of 0.3 mm in area 7, simultaneously with the field potential at the cortical surface. Responses to stimulus applied to the LP thalamic nucleus during a control period (Contr.) and flat epochs of BS. C: neuron at a depth of 0.9 mm in area 5. In 1, hyperpolarizing current pulses (1 nA, 30 msec) during Contr. and BS. In 2, biphasic EPSP of the same cell, elicited by stimulation of the homotopic cortical focus in area 5 of the contralateral hemisphere. In all cases, averaged responses (12 in A, 10 in B, and 16 in C).

area 6, 12 mm apart, is depicted in Fig. 2. In this case, the hypersynchronized EEG state was elicited by administration of scopolamine, a muscarinic antagonist. The EEG synchronization induced by muscarinic blockers (see Fig. 2B), previously described by Wilker (1952), is explained by the fact that muscarinic receptors antagonize the low-frequency rhythms of cortical cells, transforming them into tonically activated firing (Steriade et al. 1993a; see Discussion). During the initial urethane anesthesia, the cellular slow ($\approx$ 0.3 Hz) oscillation consisted of prolonged depolarizing envelopes that occasionally led to action potentials, separated by long-lasting hyperpolarizations (Fig. 2A). During burst-suppression elicited by scopolamine administration, the V_m hyperpolarized by 8 mV (the record in Fig. 2C is under steady depolarizing current, + 0.2 nA, to elicit some action potentials), the slow rhythm disappeared, and the neuron displayed sequences of phasic depolarizing events appearing exclusively during the bursts of EEG waves. The periods of flatness in EEG recordings were associated with a complete electrical silence of the neuron.

The slow intracellular rhythm was progressively disorganized with transition to burst-suppression (Fig. 3A and B). In this cell the V_m hyperpolarized by 10 mV and abolition of action potentials took place prior to

Fig. 5. Cortical and thalamic activities during burst-suppression. Intracellular recording of neuron in area 7, and focal waves recorded at the depth of ipsilateral and contralateral cortical areas 5 (ECoG ipsi and ECoG contra, respectively) as well as in the ipsilateral LP thalamic nucleus (EThG). A: control period during ketamine and xylazine anesthesia, showing slow oscillation ($\approx$ 0.6 Hz) of neuron, related with ECoG wave complexes of similar frequency. Part marked by horizontal bar on intracellular recording is expanded above to depict details of rhythmic depolarizing envelopes and action potentials (spikes truncated). B: burst-suppression pattern elicited by adding N_2O upon the already synchronized EEG. Note: V_m hyperpolarization by 14 mV; flat periods of 5–7 sec in cellular trace; close correspondence between cellular activity and ECoG; EThG did not display complete flatness during silent cortical periods. C: recovery from burst-suppression; ketamine and xylazine were administered immediately after the period depicted in C.


A
0.5 s
20 mV
2 s
Intra-cell
-66 mV
ECoG ipsi
B
Intra-cell
-80 mV
ECoG ipsi
ECoG contra
EThG
C
Intra-cell
-72 mV
ECoG ipsi

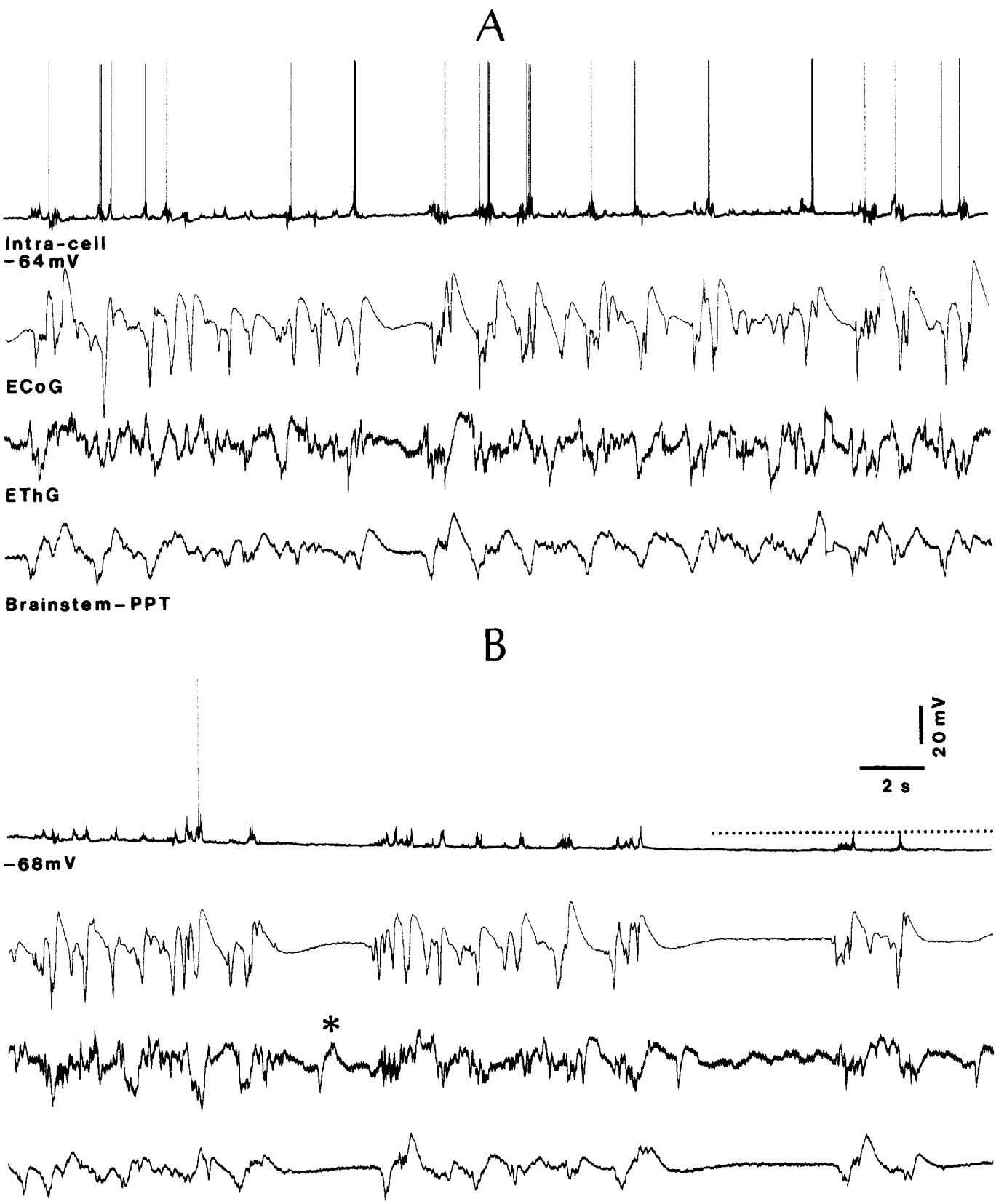


Fig. 6. Relations between cortical, thalamic, and brain-stem activities during burst-suppression. Initial anesthesia with ketamine and xylazine. Intracellular recording of area 5 callosal neuron and simultaneous recordings of focal waves from ipsilateral area 5 (ECoG, 2 mm rostral to intracellular recording), LP thalamic nucleus (EThG), and pedunculopontine (PPT) area. A: 30 sec after adding N_2O. The slow oscillation (≈ 0.7 Hz) was synchronous in all traces; note, in the middle of this panel, the first flat period lasted for ≈ 2 sec. B: 2 min later. V_m progressively hyperpolarized, reaching -78 mV, as compared to -64 mV in A (dotted line at right corresponds to -68 mV, as at left). Three flat periods are observed in all cellular and EEG traces, with the exception of EThG (see wave marked by asterisk).

any change in the EEG activity (Fig. 3B). The prolonged depolarizing envelopes of the 0.3 Hz rhythm (Fig. 3A) became shorter and less rhythmic, developing into transient depolarizations (Fig. 3B) that, eventually, became grouped and separated by periods of electrical silence lasting for 2–5 sec (right part in Fig. 3C). Those silent periods corresponded to the flat EEG epochs defining burst-suppression.

We estimated the changes in membrane conductance in cortical neurons that could be recorded during control and burst-suppression epochs (n = 6). Conductance changes were measured by using hyperpolarizing pulses of 50 msec or shorter, with an intensity of 1 nA. The increase in membrane conductance of cortical neurons during flat EEG periods of burst-suppression largely varied in this sample, ranging from 60% to 12%. These two extreme values are shown in Fig. 4A and C_1. The area 5 cell in Fig. 4A displayed an apparent input resistance of 32 MΩ during the control period (before burst-suppression), of 19 MΩ during flat periods of burst-suppression, and of 24 MΩ during the initial period of recovery from burst suppression. The neuron in Fig. $4C_1$, tested with a shorter (30 msec) hyperpolarizing pulse, displayed a much less evident decrease in the input resistance, from 16 MΩ to 14 MΩ. As the neurons hyperpolarized by $\approx$ 10 mV dur-

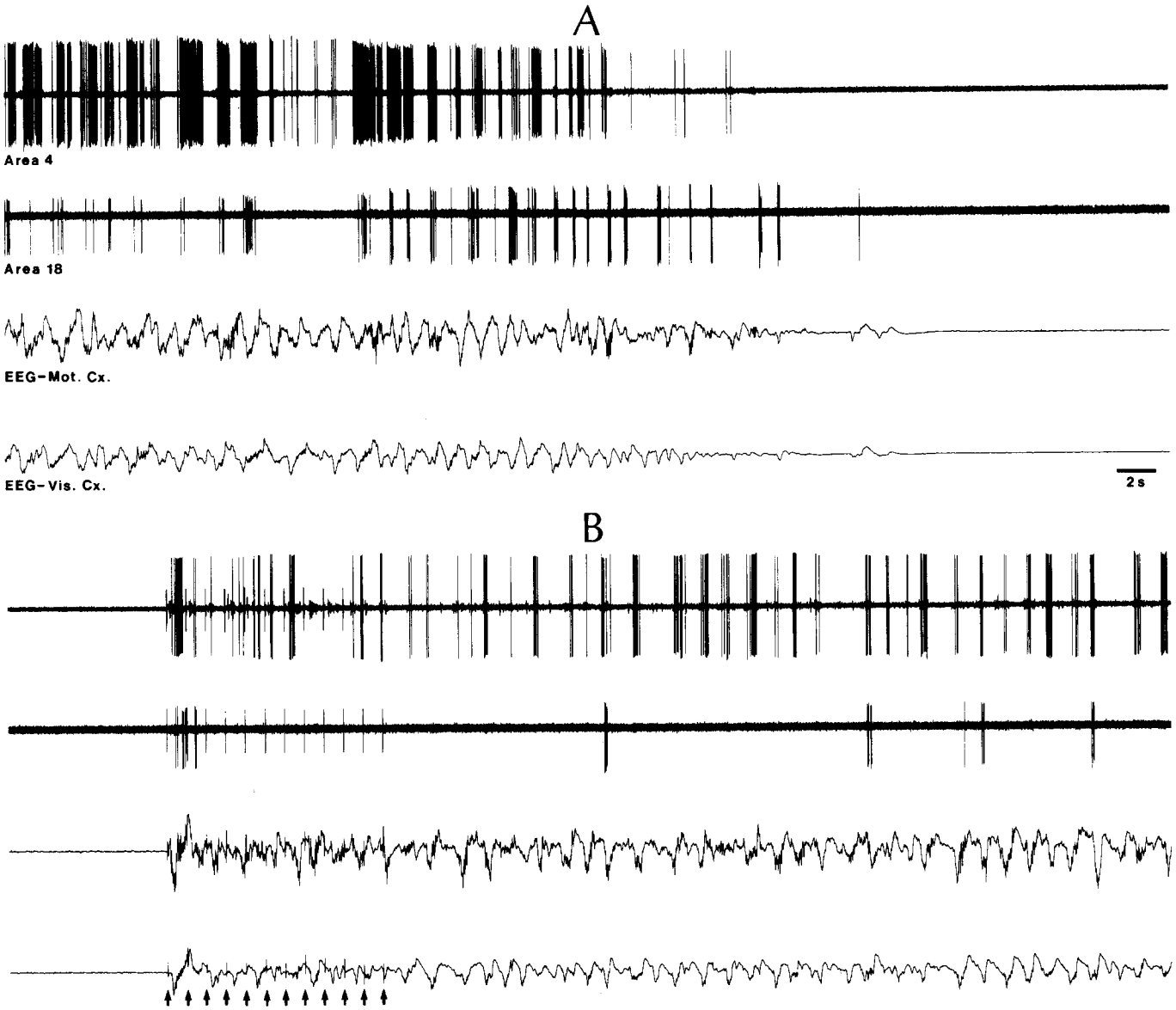


Fig. 7. Recovery from cortical burst-suppression by thalamocortical volleys. Simultaneous activity of extracellularly recorded neurons in cortical areas 4 and 18 (two top traces) and surface EEG from motor and visual areas. Initial anesthesia with ketamine and xylazine. Additional administration of urethane (0.2 g/kg, i.v.) led to burst-suppression, as seen in right part of A. Complete silence in cellular and EEG activities lasted for 32 sec (a period of 8 sec separates A from B) until 12 stimuli were applied to the CL intralaminar thalamic nucleus (arrows). Stimuli were followed by self-sustained recovery from burst-suppression.

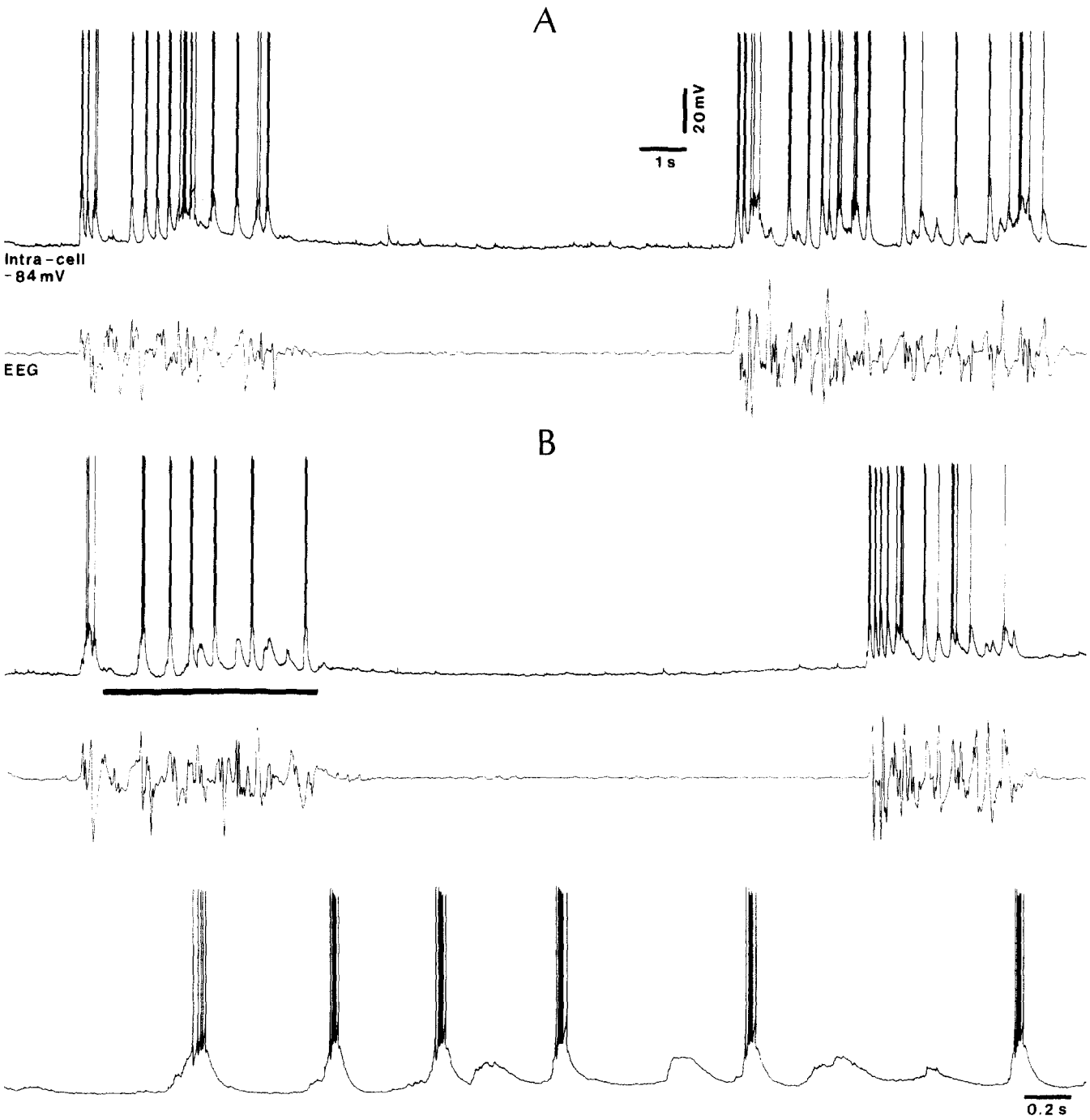
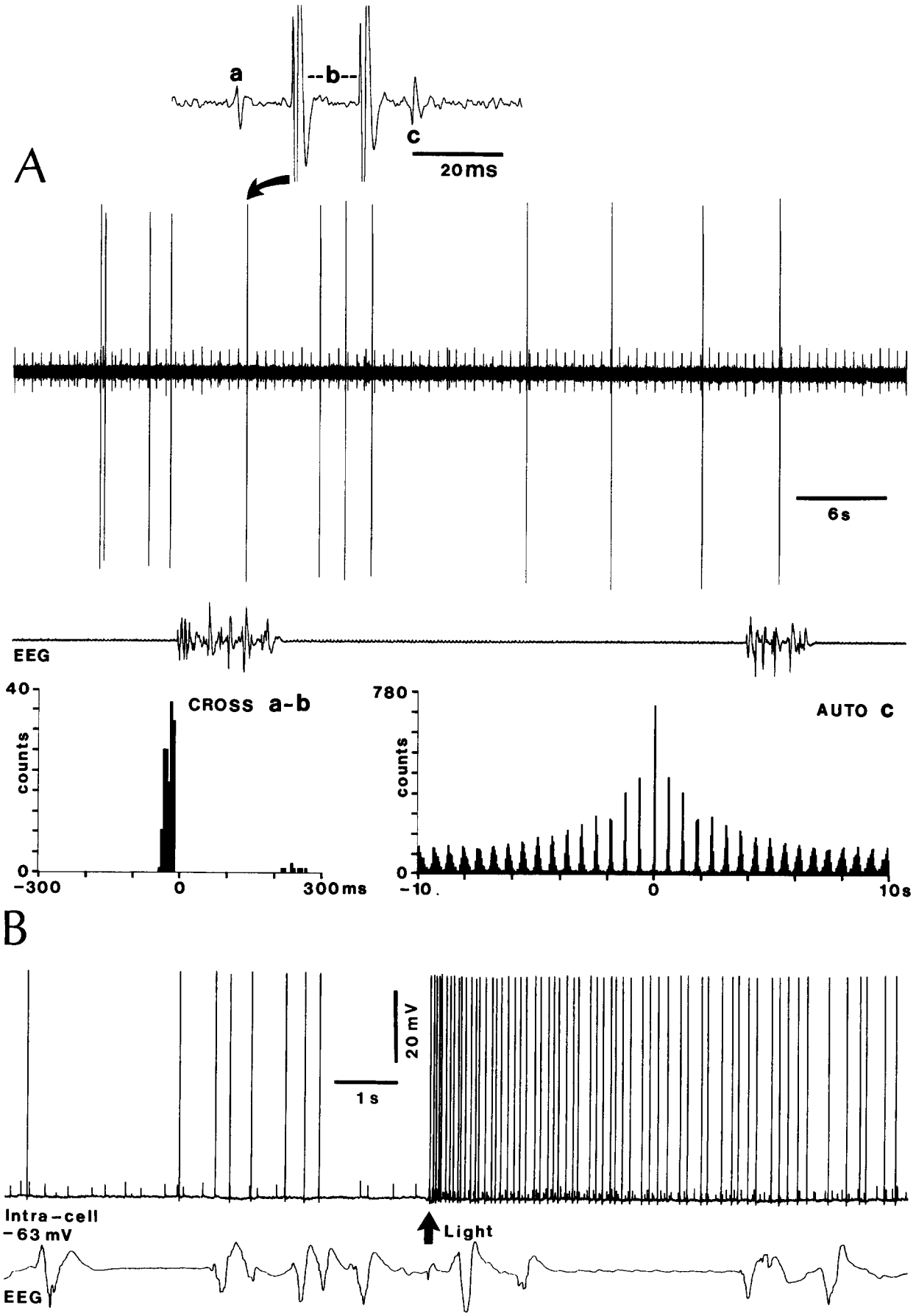


Fig. 8. Burst-suppression pattern in intracellularly recorded RE thalamic neuron (rostrolateral district). Urethane anesthesia supplemented by pentobarbital. A and B: 2 epochs showing close relation between the activity of RE thalamic cell and EEG during burst-suppression. Part marked by horizontal bar in B is expanded below.

Fig. 9. Thalamocortical neurons may remain active during EEG burst-suppression. Neurons recorded extracellularly (A) and intracellularly (B) from the dLG nucleus. A: 3 cells (a–c) simultaneously recorded by the same microelectrode. EEG burst-suppression elicited by ketamine (5 mg/kg, i.v.) over a background of urethane anesthesia. Note that during long-lasting (≈ 30 sec) flat EEG periods, all 3 dLG neurons remained active. The cross-correlogram shows that neurons a (upward, initially positive spike; see above expanded trace) and b (large spike) fired in synchrony, the action potentials of a preceding those of b by ≈ 10–20 msec. Neuron c (initially negative spike) discharged rhythmically at delta frequency (≈ 1.6 Hz); its autocorrelogram computed over 10 sec illustrates the delta oscillation. B: persistence of spontaneous and light-evoked activity in dLG cell during flat EEG episodes.


A
a
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c
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CROSS a-b
counts
0
-300
0
300ms
780
AUTO C
counts
0
-10
0
10s
B
20 mV
1 s
Intra-cell
-63 mV
Light
EEG

ing burst-suppression (see above), we measured these changes in membrane conductance by manually clamping the V_m at the control value.

In contrast with the variable or small changes in input resistance estimated from the voltage deflections elicited by injecting current pulses into the soma, we observed dramatic changes in neuronal responsiveness tested by using synaptic volleys. The thalamic evoked EPSP was drastically diminished or suppressed during burst-suppression (Fig. $4B_1$) and, in some instances, we observed a selective diminution of the late component of the callosal evoked EPSP (Fig. $4C_2$).

The relations between the intracellular events of cortical neurons and burst-suppression episodes in global activities recorded from cortical, thalamic, and brain-stem tegmental fields are illustrated in Figs. 5 and 6. The main point arising from these simultaneous recordings is that, while cortical areas of both hemispheres as well as the PPT area at the meso-pontine junction showed periods of alternating wave bursts and flatness matching the activity of intracellularly recorded cortical neurons, the thalamic recording displayed signs of activity during the periods of electrical silence in the cortex and brain-stem. The neuron in Fig. 5 displayed a complete silence 3–4 sec before the flatness in the ipsi- and contralateral ECoG. The wave bursts of the EThG corresponded to those in the ECoG; however, in no instance became the EThG totally silent (Fig. 5B). Similarly, the silent periods of the callosal neuron depicted in Fig. 6 corresponded to flat epochs of the same duration in the ECoG and brain-stem PPT recordings; during those epochs, however, the EThG exhibited smooth or sharp waves (asterisk in Fig. 6B). The persistence of electrical activity in the thalamus during silent periods in cortical cells and ECoG corroborates our results from recordings of thalamic neurons (see next section).

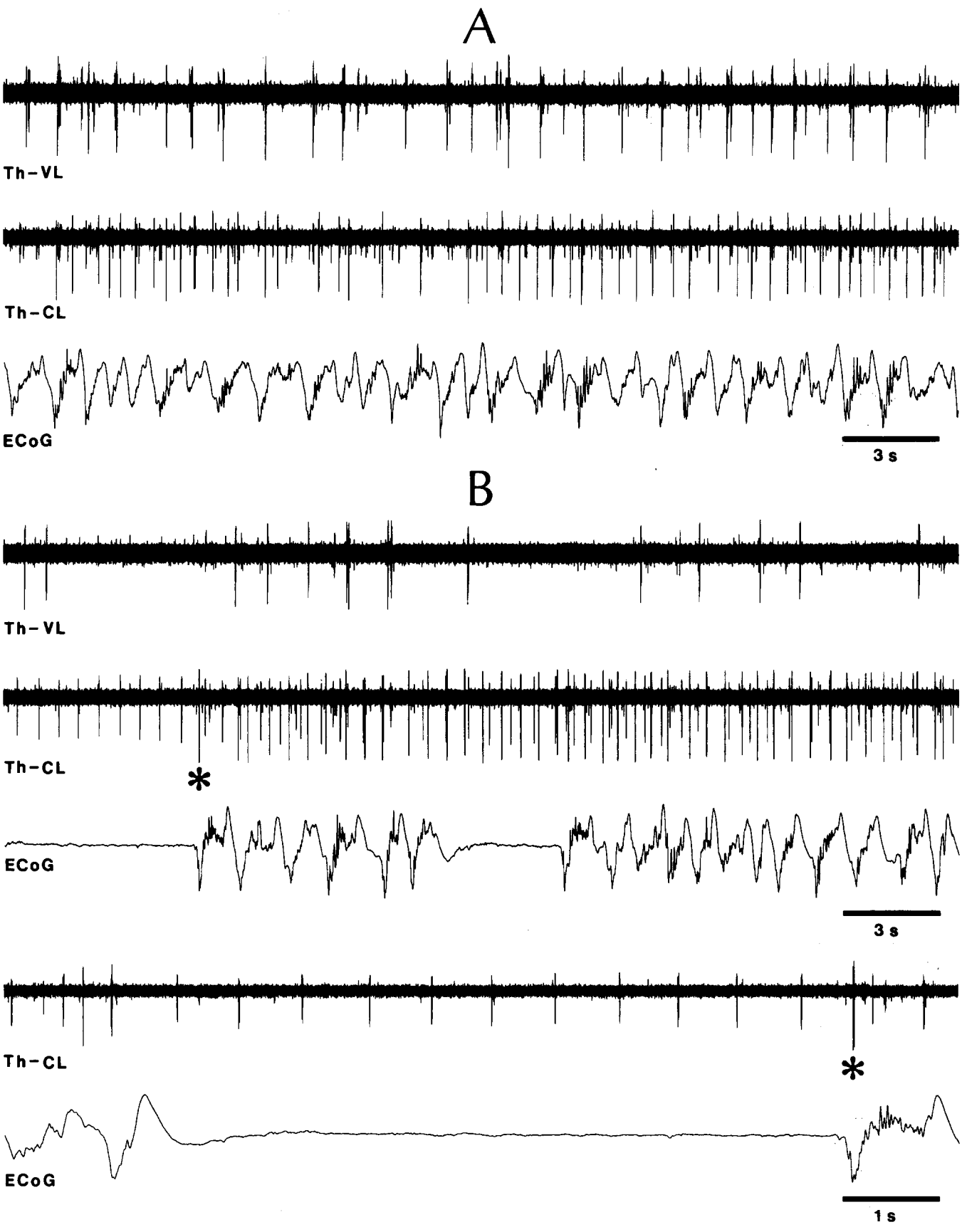
While the complete flatness of the cell membrane might have suggested that virtually all cortical neurons were unresponsive during this state, in some cortical neurons (n = 18) thalamic volleys delivered during the epochs of electrical silence were able to elicit neuronal firing or subthreshold depolarizing potentials as well as the revival of EEG activity. Moreover, the restoration of EEG and cellular discharges continued as self-sustained activities, for variable periods of time (10–50 sec), after one or a few pulses to thalamocortical pathways (Fig. 7). This simultaneous recording of neurons and EEG from motor and visual cortices shows that setting into action the thalamocortical projections from the CL intralaminar nucleus to both those areas resulted in the reappearance of EEG and single-cell activities after more than 30 sec of electrical silence. Similar results were obtained by stimulating various cortical fields, the brain-stem PPT area, or prethalamic specific relays such as the bulbar nuclei giving rise to medial lemniscal pathways (data not shown).

Thalamic neuronal correlates of EEG burst-suppression

The behavior of thalamic neurons was less globally correlated with EEG burst-suppression patterns than was the case of cortical neurons. Only 60–70% of them (11 RE thalamic and 20 thalamocortical cells) ceased firing before the occurrence of burst-suppression; thereafter, those cells discharged during EEG wave bursts and were completely silent during flat periods of the EEG. An example of such an RE thalamic cell, whose activity was closely related to EEG burst-suppression patterns, is illustrated in Fig. 8. During flat EEG epochs lasting for $\approx$ 10 sec, the membrane repolarized from its activity during preceding wave bursts, but signs of synaptic activity could still be visible (Fig. 8A). Interestingly, the frequency of cyclic depolarizing events during periods of activity closely corresponded to the frequency of EEG spiky waves (see $\approx$ 2 Hz and $\approx$ 7 Hz in both cellular and EEG activities during the first and second burst episodes in Fig. 8B).

The thalamic neurons deviating from the close EEG-cell correspondence during burst-suppression were mainly recorded from dLG, VA-VL and CL nuclei. Basically, during flat EEG periods some of those cells displayed rhythmic spike bursts within the delta frequency range (cell c in Fig. 9A) or they only decreased the firing rates, whereas during active EEG epochs they exhibited an increase in their background noise and their rhythmic burst firing became less regular, with intermingled single spikes. All 3 simultaneously recorded dLG neurons depicted in Fig. 9A discharged during flat EEG periods: two of them fired action potentials in good time-relation (cell a preceding cell b by $\approx$ 10–20 msec), while cell c displayed a spectacular rhythmicity within the delta frequency range. Another, intracellularly recorded, dLG neuron was typically driven by light stimulation, its activated firing continuing regardless of the occurrence of flat EEG epochs (Fig. 9B). Similar persistence of rhythmic bursting activity within the delta frequency range was observed in the VA-VL and CL thalamic nuclei (Fig. 10). However, with the deepening of the burst-suppression pattern, when the periods of silent epochs in the

Fig. 10. Delta activity in thalamocortical neurons, surviving during burst-suppression. Simultaneous recording of VL and CL thalamocortical neurons and of ECoG from pericruciate area 4. Ketamine and xylazine anesthesia (A) supplemented by urethane (0.3 g/kg, i.v.) (B). Part of B, marked by asterisk marking the spike burst of larger action potential, is expanded below to illustrate the clock-like delta activity of neuron with smaller action potential during EEG flat period.


A
Th-VL
Th-CL
ECoG
3 s
B
Th-VL
Th-CL
*
ECoG
3 s
Th-CL
*
ECoG
1 s

EEG activity became longer than 30 sec, all thalamic cells ceased firing.

Discussion

This study provides the first cellular data on EEG burst-suppression patterns. Our results demonstrate a close correspondence between neocortical as well as thalamic activities at the single-cell level and mass electrical events recorded from the cortex, thalamus and upper brain-stem. As discussed below, the distinct preservation of thalamic spike bursts during periods of electrical silence in the neocortex is in keeping with the oscillatory properties of thalamocortical cells at appropriate levels of V_m hyperpolarization produced by either pharmacological or morphological disconnection procedures.

A major and consistent finding was that the V_m hyperpolarization of cortical neurons reliably preceded the overt EEG aspects of burst-suppression (see Fig. 3B). This precursor cellular sign is attributable to an increase in GABAergic inhibitory processes at both thalamic and cortical synapses, together with a disfacilitation avalanche in excitatory brain-stem-thalamocortical circuits. Although the actions exerted by various anesthetics are diverse, there is general consensus that synaptic transmission is particularly susceptible to depression by anesthetic agents. Various data point to a common ability of different anesthetics (among them urethane, ketamine, N_2O, and barbiturates, as used in the present experiments) to potentiate the effects of GABA receptor stimulation (Nicoll and Madison 1982; Gage and Robertson 1985; see also reviews by Roth and Miller 1986; and Keane and Biziere 1987). Synaptic inhibition would consequently lead to disconnection throughout thalamocortical systems.

In addition to these linked processes of synaptic inhibition and disfacilitation, the hyperpolarization of thalamic and cortical cells at increasing levels of anesthesia is ascribable to: (a) the inactivation of inward Ca^{2+} currents (Krnjevic and Puil 1988; Takenoshita and Steinbach 1991) as well as the depression of a mixed Na^+-K^+ current (I_h) (Tokimasa et al. 1980) that contributes to the depolarization of neurons; and (b) an increase in K^+ conductance (Nicoll and Madison 1982; Berg-Johnsen and Langmoen 1987; Sugiyama et al. 1992). As to the powerful effect exerted by scopolamine in promoting burst-suppression patterns (see Fig. 2), it is also probably due to an increase in K^+ currents, as activation of muscarinic receptors diminishes or suppresses the voltage-dependent K^+ current I_M, the Ca^{2+}-dependent K^+ I_{AHP}, as well as an Na^+-activated K^+ current of cortical pyramidal neurons (Halliwell 1986; McCormick and Williamson 1989; Schwindt et al. 1989). These actions explain why the increase in release of acetylcholine from the cerebral cortex is associated with states of EEG activation (Celesia and Jasper 1966).

It is then likely that the increase in g_K plays the major role in the induction of burst-suppression episodes. This proposal is based on two cellular correlates of this EEG pattern: the V_m hyperpolarization and the increase in membrane conductance (see Fig. 4). The discrepancy between a wide range of decreased input resistance (from 12% to 60%) in various neurons, as estimated from direct current injection and a constant and marked decrease up to suppression of EPSPs elicited by thalamic or cortical orthodromic volleys, is postulated to result from the site of conductance increase, probably far away from the somatic impalement site.

The persistence of rhythmic spike bursts within delta frequency (1–4 Hz) in thalamocortical cells during periods of complete EEG flatness (Figs. 9 and 10), at a time when the overwhelming majority of cortical neurons were silent, reflects the pacemaking properties of thalamocortical neurons at critical levels of hyperpolarization, namely, ≈ 10 mV more negative than the resting V_m (McCormick and Pape 1990; Leresche et al. 1991; Steriade et al. 1991; Curró Dossi et al. 1992). At such V_m levels (around -70 mV), the delta oscillation of thalamic cells results from the interplay between the hyperpolarization-activated cation current I_h and the low-threshold Ca^{2+} current I_t (McCormick and Pape 1990; Soltesz et al. 1991) that generates high-frequency bursts of fast Na^+ action potentials in thalamic cells (Deschênes et al. 1984; Jahnsen and Llinás 1984). While the I_h may also be present in neocortical neurons, the intrinsic delta oscillation is less likely to be generated in these neurons because only a small subpopulation displays the LTS and the de-inactivation of this conductance requires unusually large amounts of hyperpolarization, -100 to -120 mV (Nuñez et al. 1993), that are not reached in states of quiescent sleep or anesthesia.

The revival of normal EEG and cellular activities from burst-suppression by volleys applied to prethalamic, thalamocortical, or corticothalamic pathways, corroborates the assumption that full-blown burst-suppression is achieved through complete disconnection within these brain circuits and indicates that, in some instances, a few repetitive stimuli or even a single volley may be enough to produce recovery from the dramatic blackout during burst-suppression. In this line of thinking, the spontaneous recurrence of cyclic EEG wave bursts may be seen as triggered by remnant activities in different parts of the affected circuitry, mainly in the dorsothalamic–RE thalamic network in which a significant proportion of neurons remained active during burst-suppression. As yet, however, it is unclear why this recovery is transient and whether or

not there is a real periodicity in the re-appearance of electrical activity. According to the state of the whole system, the wave bursts may fade and be replaced by electrical silence or may recover toward a normal pattern. The elucidation of this point could shed light on the mechanisms underlying the alternating pattern between sequences of wave bursts and complete flatness in the EEG and cellular activities. Another type of EEG activity occurring in the normal EEG-synchronized sleep, termed the cyclic alternating pattern (Evans 1975; Terzano et al. 1983, 1988) that is organized in sequences of two or more decasecond cycles, may be viewed as a periodic oscillation of surviving arousal systems within sleep or coma.

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