Dynamic Properties of Corticothalamic Neurons and Local Cortical Interneurons Generating Fast Rhythmic (30–40 Hz) Spike Bursts

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Steriade, Mircea, Igor Timofeev, Niklaus Durmüller, and François Grenier. Dynamic properties of corticothalamic neurons and local cortical interneurons generating fast rhythmic (30–40 Hz) spike-bursts. J. Neurophysiol. 79: 483–490, 1998. Fast spontaneous oscillations (mainly 30–40 Hz) characterize cortical and thalamocortical neuronal networks during behavioral states of increased vigilance and depend on cell depolarization under the influence of ascending activating systems. We investigated, by means of intracellular recording and staining in vivo, the properties of fast-oscillating cortical neurons from cat’s motor and association areas, some projecting to the thalamus, others with locally arborizing axons. At a given level of depolarization, 28% of our neuronal sample discharged high-frequency spike bursts (300–600 Hz) that recur rhythmically between 20 and 50 Hz. Such fast rhythmic bursting neurons have been found in both superficial and deep cortical layers. Slight changes in membrane potential as well as synaptic activity in thalamocortical networks dramatically altered the discharge patterns, from single spikes to rhythmic spike-bursts, and eventually to fast tonic firing without frequency adaptation. Thus our data challenge the conventional idea that sharply defined, invariant features and distinct locations in certain cortical layers characterize some neocortical cell-classes. We demonstrate that the distinctions between intrinsic electrophysiological properties of neocortical neurons are much more labile than conventionally thought. The present results, which indicate that corticothalamic neurons discharge fast rhythmic spike bursts mainly at 30–40 Hz, suggest that this activity results in integrated fast oscillations within corticothalamic networks.

INTRODUCTION

Fast oscillations (mainly 30–40 Hz) of neocortical and thalamic electrical activity occur spontaneously in animals (Steriade et al. 1991, 1996a,b) and humans (Llínás and Ribary 1993). These oscillations have been implicated in various behavioral conditions of increased alertness (Bouyer et al. 1981; Murthy and Fetz 1996a,b) and in binding particular aspects of an object into a global image (reviewed in Singer and Gray 1995). The fast rhythms depend on neuronal depolarization and represent a signature of brain-activated states under the control of ascending activating modulatory systems (Steriade 1993). The neuronal substrates of cortical and thalamic fast rhythms have been revealed by intracellular recordings in vitro (Gutfreund et al. 1995; Llínás et al. 1991; Pedroarena and Llínás 1997; Plenz and Kitai 1996) and in vivo (Núñez et al. 1992; Steriade et al. 1991, 1996a,b). The impact exerted by fast-oscillating neurons on target brain structures is enhanced when, instead of single action potentials, neurons fire spike bursts at high frequencies. Such bursting cells have been described in the rostral part of thalamic intralaminar nuclei projecting to association cortex

METHODS

Adult cats were anesthetized with a mixture of ketamine and xylazine (10–15 and 2–3 mg/kg im, respectively) or with pentobarbital sodium (35 mg/kg ip), and the electroencephalogram (EEG) was monitored continuously during the experiments to ascertain the depth of the anesthesia. Additional doses of anesthetic were given at the slightest tendency toward an activated EEG pattern. The cats were paralyzed with gallamine triethiodide only after the EEG showed typical signs of deep general anesthesia and were ventilated artificially with the control of end-tidal CO₂ at 3.5–3.7%. The heart rate was 90–110 beats/min, and the body temperature was maintained at 37–38°C. Saline glucose was given as a fluid therapy during the experiments. At the end of experiments, which lasted for ~12 h, cats were given a lethal dose of Nembutal and perfused transcardially with physiological saline followed by 4% paraformaldehyde and 1% glutaraldehyde. The brain was removed and stored in 30% sucrose. This protocol is approved by the committee for animal care in our university and also conforms to guidelines recommended by the National Institutes of Health.

Intracellular recordings and staining were performed in cat’s motor areas 4 and 6 of pericruciate gyri, and association areas 5, 7 and 21 of suprasylvian gyrus, by means of glass micropipettes.
filled with a solution of 2 M potassium acetate and 2% neurobiotin (dC resistances of 35–50 MΩ). The stability of intracellular recordings was improved by the drainage of cisterna magna, hip suspension and bilateral pneumothorax, and by filling the hole made for recording with a solution of 4% agar. A high-impedance amplifier with active bridge circuitry was used to record the membrane potential and inject current into the cells. Intracellular activities were recorded, together with field potentials from the depth and cortical surface, on an eight-channel tape with a band-pass of 0–9 kHz, digitized at 20 kHz for off-line computer analysis. Only stable intracellular recordings, with membrane potentials more negative than −55 mV, overshooting action potentials, and input resistance >20 MΩ were retained for analysis. In addition to impalements, field potentials were recorded from different neocortical areas. For intracellularly stained neurons, the brain was sectioned at 80 μm, processed with the avidin-biotin ABC standard kit, mounted on gel-dipped slides, and cover-slipped. Well-filled neurons were scanned from three to five consecutive slices and digitally reconstructed. The estimation of neuronal depth by reading the microdrive position display had an error of <15% when compared with the depth location of intracellularly stained neurons (see also Contreras and Steriade 1995; Steriade et al. 1993d).

RESULTS

During ketamine-xylazine anesthesia, the EEG displayed a slow (<1 Hz) oscillation associated with spindle (7–14 Hz) oscillations, resembling the EEG patterns of the slow oscillation during natural slow-wave sleep in chronically implanted animals (Steriade et al. 1996a,b). Under barbiturate anesthesia, the EEG was dominated by spindles. Although the EEG was dissimilar under these two types of anesthesia, similar responses to depolarizing current pulses, elicit fast spike-bursts, were obtained.

Of 122 cortical neurons in which depolarizing currents were injected intracellularly, 54% were regular spiking (RS), 15% were intrinsically bursting (IB), and 3% were conventional fast spiking (FS). These three types of cortical neurons have been described in previous in vitro and in vivo studies (see Connors and Gutnick 1990; Gutnick and Mody 1995; Kawaguchi 1993; McCormick et al. 1985; Núñez et al. 1993; Thomson et al. 1996).

The remaining 28% (n = 35) of the total neuronal sample fired high-frequency bursts upon direct depolarization, with an intraburst frequency of 300–600 Hz. These bursts were different from those of IB cells because of two features. 1) The interspike intervals were distributed regularly within the bursts, thus lacking the first long interspike interval, typical of IB neurons. 2) Spikes were not inactivated during the bursts and displayed clear-cut and fast afterhyperpolarizing potentials.

The majority of those 35 bursting cells (n = 23), which we call here fast-rhythmic bursting (FRB) neurons, discharged high-frequency spike-bursts recurring rhythmically at a rate of 20–50 Hz, mainly 30–40 Hz. FRB neurons were found in pericruciate motor areas 4 and 6 (n = 9) and in suprasylvian association areas 5, 7 and 21 (n = 14). Of those 23 FRB cells, 8 were recorded from layers II and III, and 15 were recorded within layers V and VI. Nine FRB cells were stained intracellularly; six neurons were found to have a pyramidal shape (4 of them were also identified electrophysiologically as corticothalamic), whereas three neurons had locally arborizing axons (see Fig. 2). In addition to the four FRB cells that were identified as corticothalamic both electrophysiologically and morphologically, six other FRB cells were identified as corticothalamic by their antidromic responses to stimulation of the appropriate thalamic nucleus (see Fig. 1A). Criteria for antidromic responses were: takeoff directly from the baseline, fixed latency, and collision with spontaneously occurring action potentials at proper time intervals.

In the sample of 23 FRB cells, the mean intraburst frequency was 412.5 ± 19.4 (SD) Hz (major mode of interspike intervals at 2.5 ± 0.1 ms) and the frequency of rhythmic bursts (resulting from interburst intervals) increased from 33.1 ± 3 Hz (slightly suprathreshold current pulses) to 47.1 ± 5.2 Hz (higher intensities of currents). Further increases in the current intensity led to tonic firing without frequency adaptation (see Figs. 2 and 3). During the tonic firing mode, the mean frequency was 385.1 ± 20.5 Hz (single mode of interspike intervals at 2.6 ± 0.1 ms). There was no statistically significant difference (P > 0.5) between the discharge frequency in rhythmic spike bursts and that during tonic firing.

The FRB cells were characterized by a series of features. 1) An overwhelming majority (18 of 23) had very brief action potentials, ranging from 0.2 to 0.5 ms at half-amplitude (mean = 0.37 ± 0.03 ms). This feature also characterized long-axonated excitatory corticothalamic neurons (Fig. 1A), which is surprising because such thin spikes are conventionally ascribed to FS local-circuit inhibitory neurons (see Gutnick and Mody 1995). 2) In response to suprathreshold depolarizing pulses, they discharged rhythmic (20–50 Hz), high-frequency (300–600 Hz) spike bursts (Fig. 1B). The number of action potentials within a burst, as well as their frequency, increased by raising the intensity of testing pulses, thus leading to an increase in burst duration (see Fig. 1B, right). The increased number of action potentials stemmed from depolarizing afterpotentials (DAPs; Fig. 1B). This is a common feature of various cell classes, including RS and IB (Chagnac-Amitai and Connors 1989) as well as rhythmically bursting neurons from the visual cortex (Gray and McCormick 1996) that, in some respects, are similar to the neurons described in the present study. And 3) the fact that corticothalamic FRB cells, responding with high-frequency and rhythmic spike bursts to depolarizing current pulses, also displayed spontaneously occurring, compound excitatory postsynaptic potentials, consisting of fast (300–400 Hz) depolarizing events (Fig. 1C), suggests that FRB cells are interconnected.

Importantly, the intrinsic discharge pattern of fast and rhythmic spike bursts was not an invariant stigma of FRB neurons. Indeed, after a passive response or single spikes in response to a subthreshold or a slightly suprathreshold depolarizing current pulse, corticothalamic FRB neurons discharged rhythmic spike bursts with an increased number of spikes per burst when the stimulus intensity was raised; eventually, with a further increase in stimulus intensity, the neurons reached the pattern of tonic firing (300–600 Hz) without frequency adaptation (Fig. 2A). As is also the case with the very brief duration of their action potentials, the fast tonic firing without adaptation of corticothalamic cells generally is regarded as exclusively characterizing FS inhibitory interneurons. The progressive changes in discharge patterns, from
FIG. 1. Fast-rhythmic–bursting (FRB) corticothalamic neurons. Cats under ketamine-xylazine anesthesia. A: physiological identification of a corticothalamic cell from layer VI in area 5. Stimulus (arrowhead) to thalamic lateral posterior (LP) nucleus elicited an antidromic (a) spike followed by orthodromic (o) response (top, resting membrane potential -55 mV). At a hyperpolarized level (bottom), the antidromic response failed but the orthodromic response survived. This neuron is an example of a cell interposed in a corticothalamocortical loop. B: fast rhythmic bursts in an identified corticothalamic neuron from area 5, elicited by direct depolarization of the cell. Responses to 3 depolarizing steps (0.4, 0.8, and 1.2 nA) are illustrated. Initial part of each response is expanded (right; oblique arrow, depolarizing afterpotentials, DAPs). Note progressive increase in the number of action potentials within bursts (≤500 Hz) and in the number of rhythmic bursts (from 20 to 30 Hz) by increasing the direct depolarization. C: a corticothalamic neuron fired high-frequency spike bursts in response to a depolarizing current pulse (0.8 nA) and also displayed spontaneously occurring, compound excitatory postsynaptic potentials consisting of fast (~300–400 Hz) depolarizing wavelets (see 1 at left, and 4 individual traces at right). This suggests that this FRB neuron is the target of another excitatory FRB cell.

Single action potentials or spike doublets to fully developed rhythmic spike bursts and finally to tonic firing without adaptation, was not only found in corticothalamic neurons, but also in intracellularly stained, sparsely spiny local-circuit neurons (Fig. 2, B and C). The similarity between the firing patterns of corticothalamic and local-circuit neurons also was demonstrated by quantitative analyses showing the increase in the number of action potentials within bursts and in the number of repetitive spike bursts by increasing the depolarizing current, as well as the replacement of spike bursts by tonic firing with further increases in intensities (Fig. 3). Finally, the FRB neurons described here were located at all.
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within the superficial layers II and III as well as in the deep layers V and VI where cortical cells projecting to the thalamus are found (Jones 1985).

The changes in discharge patterns of FRB cells, as investigated by progressively increasing the intensity of direct depolarization (see Fig. 2), also were observed by comparing, in the same neuron, the depolarization-dependent activity during periods with and without synaptic activity in barbuturate anesthetized animals (n = 8) displaying spindle oscillations at 7–14 Hz, a rhythm generated in the thalamus and characterizing early stages of sleep (Steriade et al. 1993c). Without exception, suprathreshold current pulses elicited fast rhythmic (30–40 Hz), high-frequency (300–600 Hz) spike bursts when applied during interspindle lulls; by contrast, pulses with identical parameters triggered tonic firing at high rates (300–500 Hz), virtually without frequency adaptation, when applied during sequences of spindle waves, which are accompanied by powerful synaptic activity transferred along thalamocortical pathways. In privileged conditions (n = 5), we could make the comparison between interspindle lulls and spindle sequences by applying a series of similar current pulses, at different intensities, in the same corticothalamic neuron (Fig. 4). These two conditions simulated the poor spontaneous synaptic activity in cortical slices (as during interspindle lulls) and the presence of synaptic bombardment in vivo (as during spindles). Invariably, the transition from single spikes to fast rhythmic bursts and to tonic firing, which typically was observed by applying depolarizing pulses during periods of relative silence, changed into the disruption of rhythmic spike bursts and their tendency to be transformed into fast tonic firing during epochs rich in synaptic activity (Fig. 4).

DISCUSSION

Data presented here show that fast-oscillating, rhythmically bursting neurons are located in both superficial (II–III) and deep (V–VI) cortical layers and that deeply lying, formally identified corticothalamic neurons are part of these cells. The fact that the same fast-oscillating bursting cortical neuron displayed different firing patterns as a function both of slight modifications in membrane potential and of activity in incoming pathways challenges the view that sharply defined, invariant features, and distinct somatic locations in certain cortical layers characterize cortical cell classes with specific firing patterns. Instead, the present data demonstrate the dynamic electrophysiological properties of neocortical neurons, changing their firing from RS to FRB and, further, to FS patterns (see Figs. 2 and 4). Other transformations, from IB to RS patterns, have been reported in studies in which the IB-cell type developed into a RS type during arousal elicited by stimulating the brain stem reticular forma-

FIG. 3. Similarieties between corticothalamic and local-circuit neurons with respect to their changes in firing patterns by increasing the intensity of depolarizing current pulses (200 ms in duration). Two antidromically identified corticothalamic neurons (triangles and diamonds) and a local-circuit neuron (circles). Different points represent the means. A: increase in the number of action potentials within spike-bursts (ordinate) by increasing the depolarizing current (abscissa). B: numbers of spike bursts are almost constant over a broad range of direct currents. Note that, at higher stimulation intensities (above ~1.2 nA), spike bursts coalesced into tonic firing (black symbols).

FIG. 2. Changes in discharge patterns of FRB neurons by increasing the intensity of direct depolarization (200-ms pulses in both A and B neurons). Ketamine-xylazine anesthesia. A: identified corticothalamic neuron in area 7 that, upon subthreshold depolarization (0.4 nA), displayed a passive response; pulses of 0.8, 1, and 1.2 nA elicited high-frequency spike bursts with increasing repetition rates (from 30 to 40 Hz) and number of action potentials within each burst; and, finally, fired tonically at 450 Hz, without frequency adaptation (1.4 nA). Intracellular staining showed its pyramidal shape and location in layer VI. B: similar transformation, from single spikes to rhythmic spike bursts (~40 Hz) and finally to tonic firing by increasing the intensity of direct depolarization in a morphologically identified local-circuit, sparsely spiny neuron located in layer 3 of area 7. Spontaneous action potentials showed their very brief duration (0.3 ms at half-amplitude; not depicted). Oblique arrow points to subthreshold depolarization. C: camera-lucida reconstruction of a local-circuit cell and a photomicrograph of the same neuron (same as in B).
FIG. 4. Changes in responses of a corticothalamic neuron from area 21 (antidromically identified from the LP nucleus) to depolarizing current pulses with different intensities during periods poor and rich in synaptic activity. Barbiturate anesthesia. Field potentials were simultaneously recorded from the related thalamic LP nucleus and from the depth of cortical areas 5, 7, and 21 (the latter in the immediate vicinity of the impaled neuron). Depolarizing current pulses (duration, 200 ms) with 4 intensities (0.4, 0.8, 1, and 1.2 nA) were applied during interspindle lulls, with negligible or absence of synaptic activity, and during spindle sequences, rich in synaptic activity generated by thalamocortical volleys. Note the transformation from rhythmic (35 Hz) spike bursts into tonic firing (450 Hz) without frequency adaptation during neuronal silence and disruption of intrinsically generated rhythmic spike bursts by network synaptic activity.
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To a certain extent, the FRB neurons reported in this study are similar to the bursting cells described recently by Gray and McCormick (1996). However, distinct from those neurons, which have been found only in superficial layers of visual cortex, our FRB neurons were located in all investigated neocortical regions (motor areas 4–6 and association areas 5–7–21) and at all depths from 0.25 to 1.5 mm. More importantly, the FRB cells described here were capable to produce either single spikes or rhythmic spike bursts or tonic firing (300–600 Hz) without frequency adaptation, depending on the strength of depolarizing current and the synaptic activity in the network.

Thus the present data indicate that the distinctions between the intrinsic electrophysiological properties of neocortical cell classes are much more labile than conventionally thought. For the most part, this is due to synaptic noise that allows cortical neurons to transform synaptic inputs into sequences of action potentials, with precisely timed firing patterns (Mainen and Sejnowski 1995). On the basis of some observations on layer VI cells (see Fig. 3 in Kang and Kayano 1994), where corticothalamic neurons are located, we hypothesize that the same network, submitted to repetitive inputs leading to cells’ depolarization, would favor the transformation of RS into FRB cells. Thus a progressive increase in the proportion of FRB neurons would result from dynamic neuronal properties under physiological conditions of increased vigilance, associated with depolarization of cortical neurons.

The mechanisms of short- and long-range synchronization among fast-oscillating neurons implicate both long-axoned excitatory (Steriade et al. 1996a,b) and local-circuit inhibitory (Buzsáki and Chrobak 1995; Llinás et al. 1991; Lytton and Sejnowski 1991; Traub et al. 1996a,b; Tsodyks et al. 1997) neurons. Magnetoencephalographic data (Llinás and Ribary 1993) and intracellular recordings in conjunction with extracellular unit and field potential from multiple sites in the thalamus and cortex (Steriade et al. 1993b, 1996a,b) have suggested that intralaminar and specific thalamic nuclei play an important role in the synchronization of fast oscillations in reciprocal corticothalamic loops. The present data support the hypothesis that corticothalamic neurons are among the best candidates in the synchronizing process in view of their propensity to develop fast rhythmic bursts of action potentials. The very high frequencies of spikes within bursts may lead to temporal summation and produce fast oscillations in thalamic targets, and, after intrathalamic synchronization (Steriade et al. 1996b), the activity is reflected back through thalamocortical projections. The role of corticothalamic pathways in the spatiotemporal maps and the stimulus-dependent synchronization of thalamic neurons has been demonstrated (Nicolelis et al. 1993; Silitto et al. 1994).

Although the present results were obtained by applying depolarizing current pulses, the same changes in membrane potential of cortical neurons may occur during natural shifts in vigilance, with transition from functionally deafferented to brain-active states. This has been shown experimentally by driving ascending activating systems (Munk et al. 1996; Steriade et al. 1996a,b, 1997). What could be the role of spontaneously occurring fast oscillations? It was shown that brief stimuli to brain stem cholinergic nuclei, which simulate ponto-geniculo-occipital waves during the dream state, reset and enhance the synchronization of spontaneously occurring fast oscillations (Steriade and Amzica 1996; Steriade et al. 1996a). Similar effects may be obtained by relevant signals during wakeful, attentive states.

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