Relationship between EEG Potentials and Intracortical Activity of Striatal and Cortico-striatal Neurons: an In Vivo Study under Different Anesthetics

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The functions of the basal ganglia are achieved through excitation of striatal output neurons (SONs) by converging cortical glutamergic afferents. We assessed the relationship between different patterns of activity in cortico-striatal (C-S) cells and the electrical behavior of SONs in vivo. Intracellular activities of rat C-S neurons in the orofacial motor cortex and of SONs, located in the projection field of this cortical region, were recorded under different anesthetics, which generate various temporal patterns of cortical activity. A surface electroencephalogram (EEG) of the orofacial motor cortex was simultaneously performed with intracellular recordings and EEG waves were used as correlates of a coherent synaptic activity in cortical neurons. Under barbiturate anesthesia C-S neurons showed rhythmic (5–7 Hz) supra-threshold depolarizations in phase with large amplitude EEG waves. The correlation activity of the SONs was characterized by large amplitude oscillation-like synaptic depolarizations that could trigger action potentials. Under ketamine–xylazine anesthesia C-S neurons exhibited a step-like behavior consisting of depolarizing plateaus (up states), leading to spike discharges, interrupted by hyperpolarizing periods (down states). The related activity of SONs was step-like membrane potential fluctuations with firing confined to the early part of the striatal up state. In C-S neurons and SONs up states coincided with slow recurrent EEG waves (~1 Hz). Finally, under neurolept-analgesia an apparently disorganized EEG activity was associated with a lack of rhythmic discharge in C-S neurons. This uncorrelated activity in C-S neurons resulted in an absence of spontaneous firing as well as of large amplitude synaptic depolarizations in SONs. In the present study we demonstrate that SONs shape their input–output relationship by filtering out uncorrelated synaptic activity and that a minimal synchronization in the cortico-striatal afferents is required to produce significant synaptic depolarization in SONs.

Introduction

The basal ganglia constitute a set of interconnected subcortical structures, including the striatum, pallidum, substantia nigra and subthalamic nucleus. They provide a major integrative system of the forebrain, which is critically involved in planning and execution of motor behaviors and in sensorimotor learning [reviewed by Graybiel (Graybiel, 1995)]. The basic process underlying the function of the striatum, the main input stage of the basal ganglia, is firing of GABAergic striatal output neurons (SONs), which leads to disinhibition of premotor networks such as the superior colliculus and thethalamic ventro-medial nucleus (Chevalier and Deniau, 1990). Excitation of SONs is mainly achieved through integration of glutameric monosynaptic inputs (Reubi and Cuenod, 1979; Herrling, 1985; Jiang and North, 1991; Kita, 1996). The subnuclei of the basal ganglia are separated into functional subdivisions of the cerebral cortex (McGeorge and Faull, 1989; Flaherty and Graybiel, 1991; Deniau et al., 1996). Despite this powerful excitatory, SONs recorded in anesthetized (Stern et al., 1998; Charpier et al., 1999a) as well as in awake animals (Wilson and Groves, 1981; DeLong et al., 1984) are characterized by their low rate of spontaneous firing. This hypo-excitability of SONs has been mainly attributed to a set of voltage-dependent potassium conductances acting as an inhibitory shunt on excitatory synaptic inputs (Nisenbaum and Wilson, 1995; Wilson, 1995) rather than to inhibitory synaptic interactions between SONs (Jaeger et al., 1994). However, blockade of GABAergic transmission in the striatum leads to an increase in spontaneous firing of SONs (Nisenbaum and Berger, 1992), which is probably due to disinhibition from fast spiking interneurons (Koos and Tepper, 1999).

The low excitability of SONs led to the assumption that they filter out weak, uncorrelated synaptic inputs and that significant synaptic depolarizations, i.e. those leading to action potential discharges, are driven by synchronized activity in many converging cortico-striatal afferents (Nisenbaum et al., 1994; Nisenbaum and Wilson, 1995; Wilson, 1995; Charpier et al., 1999a). A direct validation of this hypothesis requires experimental procedures by which intracellular activity of SONs can be recorded under different levels of synchrony in their cortico-striatal afferents. Up to now such a comparative study has not been performed. Therefore, we have here studied the relationship between the voltage behavior of SONs and the temporal structure of activity in their cortical afferents under different types of anesthesia (barbiturate, ketamine–xylazine and neurolept-analgesia), which generate different patterns of cortical activity (Steriade et al., 1993a; Contreras et al., 1997a; Steriade, 1997; Pinault et al., 1998; Charpier et al., 1999b). In separate experiments we performed intracellular recordings of cortico-striatal (C-S) neurons in the orofacial motor cortex and of SONs located in the projection field of this cortical area. To assess the link between different levels of cortical synchrony and the electrical events in SONs and C-S neurons, in each experiment intracellular activities were recorded simultaneously with surface electroencephalograms (EEG) of the orofacial motor cortex. Since an EEG is an averaging of cortical field potentials, which reflects correlated synaptic potentials in the related cortical cells (Klee et al., 1965; Creutzfeld et al., 1966; Contreras and Steriade, 1995), EEG waves were used as an indicator of synchronized activity in the cerebral cortex.

Materials and Methods

All animal experimentation was carried out in accordance with the European Community Council directive 86/609/EEC.

Animal Preparation and Surgery

Experiments were performed in vivo on 29 adult male Sprague–Dawley rats (Charles River, France) weighing 240–300 g. In a first set of experiments (n = 13 rats) animals were anesthetized using sodium pentobarbital (66 mg/kg i.p.; Sanofi, Libourne, France). Barbiturate anesthesia was maintained throughout the experiment with additional doses of pentobarbital (20 mg/kg i.p.) every hour. In a second set of experiments (n = 6 rats) rats were first deeply
anesthetized with a mixture of ketamine (90 mg/kg i.p.; Imalgène; Rhône Mérieux, France) and xylazine (10 mg/kg i.p.; Sigma, France). Deep anesthesia was maintained by supplementary doses of ketamine (50 mg/kg i.m.) administered hourly.

In a last series of experiments (n = 10 rats) the animals were initially anesthetized with sodium pentobarbital (40 mg/kg i.p.) and ketamine (100 mg/kg i.m.). Once the surgical procedures had been completed (see below), neurolept-anesthesia (Flecknell, 1996) was obtained by injections of fentanyl (3 mg/kg i.p.; Janssen, France) and haloperidol (1 mg/kg i.p.; Haldol, Janssen, France), which were repeated every 20–30 min (Pinault et al., 1998; Chapier et al., 1999).

EEG was continuously monitored to assess the depth of anesthesia and additional doses of anesthetics were given to maintain a constant pattern of EEG waves that was characteristic of the type of anesthesia. In addition, the heart rate was monitored throughout the experiments.

After initial anesthesia a cannula was inserted into the trachea and the animal was placed in a stereotaxic frame. Wounds and pressure points of the heart were monitored throughout the experiments. The animal was placed in a stereotaxic frame. Wounds and pressure points were infiltrated with lignocaine (2%), repeated every 2 h. To obtain long-lasting stable intracellular recordings rats were immobilized with gallamine triethiodide (40 mg i.m. Flaxedil every 2 h; Specia, Paris, France) and artificially ventilated. Body temperature was maintained (36.5–37.5°C) with a homeothermic blanket. At the end of the experiments the animals were killed with an overdose of pentobarbital (200 mg/kg i.p.).

**Recordings and Stimulations**

**EEG Recordings**

Silver bipolar electrodes of low resistance (60 kΩ) were placed on the dura to record a focal EEG of the orofacial motor cortex (Neafsey Silver bipolar electrodes of low resistance (60 kΩ), 200 mg/kg i.p.). Cross-correlograms between sub-threshold intracellular activities and EEG waveforms (down-sampling at 300 Hz for both signals) were calculated using Spike 2. The amplitude of action potentials (APs) was calculated as the potential difference between their voltage threshold, evident as an abrupt increase in slope depolarization, and the peak of the spike waveform. Numerical values are given as means ± SD. Statistical significance was assessed by performing appropriate statistical tests, a one way analysis of variance (ANOVA), a Mann–Whitney rank sum test or a Levene median test. In some measurements the normality of distributions was tested using the Kolmogorov–Smirnov test and a Gaussian–Laplace fit was performed. Simple linear regression was done with a confidence interval of 95%.

**Results**

**Database**

Data were obtained from 35 C-S neurons and 22 SONs. In this neuronal sample both types of cells had a V_m < −55 mV, APs with a rise time < 700 µs and an amplitude > 50 mV.

**Electrophysiological Identification of SONs and C-S Neurons**

**(SONs)**

Whatever the anesthesia, neurons recorded within the striatum (Fig. 1A) could be identified as SONs using the distinctive electrophysiological features of these cells (Jiang and North, 1991; Nisenbaum et al., 1994; Nisenbaum and Wilson, 1995; Chapier et al., 1999a; Mahon et al., 2000): (i) a low V_m (≤72 mV) (Fig. 1B1–B3); (ii) a low apparent input resistance at rest (<40 MΩ); (iii) the presence of a slow ramp-like membrane depolarization in response to intracellular injection of a positive threshold current pulse (Fig. 1B1–B3). This classical delayed excitation, which leads to a long latency to spike discharge, is due to a voltage-dependent slowly inactivating potassium current available around –60 mV (Nisenbaum et al., 1994; Gabel and Nisenbaum, 1998). However, because in *vitro* intracellular recordings often exhibited continuous synaptic noise, the application of positive current pulses could coincide with spontaneous synaptic depolarizing potentials and consequently lead to earlier firing (Fig. 1B1, top trace). As shown by the pooled data presented in Table 1, basic electrical membrane properties of SONs (V_m, R_m, AP amplitude and rise time) were not significantly different under barbiturate, ketamine–xylazine and neuroleptic analgesia. In addition, the voltage-dependent intrinsic properties of SONs at potentials close to the firing threshold were not affected by the different anesthetics used (Fig. 1B1–B3).

**(C-S Neurons)**

C-S neurons were reliably identified by their antidromic activation from the contralateral striatum (Fig. 2A). Electrical stimulation of the contralateral striatum allowed us to avoid any antidromic activation of passing corticofugal axons (Wilson, 1987; Cowan and Wilson, 1994). The criteria used for identification of antidromic action potentials were: (i) the constant latency of the antidromic response despite imposed changes of membrane potential (Fig. 2B1); (ii) collision of the antidromic spikes with spontaneously occurring orthodromic APs (Fig. 2B2, top traces); (iii) the all or none property of the evoked spikes.
Figure 1. Scheme of in vivo experimental arrangement and electrophysiological identification of SONs. (A) Intracellular recording (Intra) of SONs (dark circle) was combined with a focal surface EEG of the contralateral orofacial motor cortex (see Materials and Methods for details). The recorded striatal cells were located in the projection field of this cortical area. Cx, cerebral cortex. (B) Voltage responses of SONs recorded under barbiturate (Barb, B1), ketamine–xylazine (K-X, B2) and fentanyl (Fent, B3) anesthesia to intracellular injection of positive (single trace) and negative current pulses (average response, n = 12). Under the three anesthetics the positive current pulse induced a slow ramp depolarization leading to a long latency for spike discharge (see the crossed arrow in B1). The apparent threshold of the ramp depolarization (dotted line) was similar under the three anesthetics. Note the inward rectification expressed as a decrease in the voltage response of the cell during a hyperpolarizing pulse compared to a depolarizing pulse of same intensity. In (B1) (upper trace) collision of a spontaneous depolarizing synaptic event with the current-induced response (arrow) produced a decrease in the latency of the spike discharge and an increase in the number of evoked action potentials. The resting membrane potential of the cells is indicated in the figure. The duration and intensity of the current pulses shown below the voltage responses apply to (B1)–(B3).

when the stimulation was just below threshold for antidromic activation (Fig. 2B2, lower trace). In most cases (25 of 35 C-S cells) antidromic activation was followed by a short latency mixed inhibitory synaptic potential (Fig. 2B2, top and bottom traces). The depth of intracellular recordings (1200–2600 μm from the cortical surface) indicated that the recorded C-S neurons were presumably located in the deep part of layer III and in layer V. This is consistent with previous findings obtained from intracellular labeling of crossed C-S neurons (Cowen and Wilson, 1994). Antidromic latencies, which ranged between 3.3 and 24 ms (mean = 7.3 ± 4.1 ms) with two preferred modes at 3–6 and 7–9 ms (Fig. 2C), were not related to the depth of recording (Fig. 2D). To assess the conduction velocity of C-S neurons we approximated the course of the axons as straight line segments from the soma to the striatum. The mean estimated conduction velocity was 1.48 ± 0.62 m/s (from 0.36 to 2.71 m/s, n = 35).

As for SONs, comparison of the basic membrane properties of the cortical neurons under the three conditions of anesthesia did not show any significant differences (Table 2). For instance, as demonstrated in Figure 3A, AP properties were not significantly modified (see Table 2). Moreover, C-S neurons displayed similar voltage changes in response to intracellular negative current pulses (Fig. 3B) and a post-inhibitory rebound of excitability, reminiscent of a low threshold activated calcium potential, was observed in all tested cells (n = 29 C-S neurons). An important result was that the firing pattern of C-S neurons was mainly driven by the background synaptic activity rather than by their intrinsic membrane properties. As illustrated by the C-S neuron shown in Figure 3C (barbiturate anesthesia), while the directly induced firing pattern was stereotypical during epochs of reduced synaptic activity (Fig. 3C1), it varied as the number of spontaneous synaptic events increased (Fig. 3C2).

**Relationship between EEG Waves and Intracellular Activities of SONs and C-S Neurons**

**Barbiturate Anesthesia**

In a first series of experiments intracellular activity of SONs (n = 10) and surface EEG were recorded under barbiturate anesthesia. As previously reported (van Luijtelaar, 1997; Charpier et al., 1999a), rat EEG under this anesthetic exhibited spontaneous recurrent spindles consisting of sequences of large amplitude waves (upper traces in Figs 4A1, B1 and 5A) that occurred with a frequency range of 5–7 Hz (Fig. 4A2). Simultaneously, SONs displayed grouped rhythmic depolarizing post-synaptic potentials clearly correlated with the negative EEG waves (Fig. 4A1, B1). Two main arguments attest that these membrane potential fluctuations represent excitatory post-synaptic potentials (EPSPs): (i) the frequency of oscillations was voltage-independent, indicating their synaptic origin (Charpier et al., 1999a); (ii) these spontaneous oscillations exhibited the same sensitivity to changes in membrane potential as glutamergic cortically evoked synaptic potentials (Kita, 1996; Charpier et al., 1999a). While these EPSPs remained mostly sub-threshold for spike discharge (Fig. 4A1), firing could occur with the largest EPSPs, which were systematically coincident with large amplitude EEG spindle waves (Fig. 4B1).

To examine the temporal relationship between cellular events in SONs and the corresponding cortical field potentials we used the peak of the negative cortical wave as the zero-time reference. The rationale here was that the peak of EEG waves indicated a relative synchrony of synaptic potentials in the cortical neurons involved in the EEG record (Klee et al., 1965; Creutzfeld et al., 1966; Contreras and Steriade, 1995). As illustrated in Figure 4A3, the onset of synaptic depolarization in SONs occurred during
ANOVA).

V sub-threshold EPSPs (arrow in Fig. 4A3) were delayed (+18.6 ± 14.1 ms, n = 5 cells) relative to the peak of cortical potential.

While the mean firing rate of SONs under barbiturate anesthesia was weak (<1 Hz) [see also Charpier and Deniau and Charpier et al. (Charpier and Deniau, 1997; Charpier et al., 1999a)], we were able to assess in five active SONs the timing of their spiking relative to the same zero-time reference as for sub-threshold events. As illustrated in Figure 4B1,B2, spike discharge in SONs mostly temporally followed the peak of the corresponding EEG wave. However, collision of the oscillation-like striatal depolarization with small synaptic events could lead to earlier AP firing (Fig. 4B2, arrow in inset). It is important to note that such a decrease in the firing latency of SONs was also observed following temporal summation of a small AMPSP with a depolarizing current pulse (see Fig. 1B, top trace).

AP latencies resulting from such complex synaptic potentials were not included in the Gaussian distribution (P = 0.8, Kolmogorov–Smirnov test) of SON firing probability (Fig. 4B3).

Similar to the activity of SONs, C-S neurons (n = 19) under barbiturate anesthesia exhibited large spontaneous membrane depolarizations that occurred in phase with the EEG spindle waves (Fig. 5A). However, in contrast to SONs, most C-S neurons (14 of 19 cells), which had a more depolarized resting potential (Table 1 versus Table 2), were spontaneously active with a mean firing frequency of 1.6 ± 1.26 Hz (0.26–4.6 Hz, n = 14). APs in C-S neurons were triggered at the peak of the largest synaptic depolarizations, which were systematically associated with large amplitude negative EEG waves (Fig. 5A). To assess the temporal relationship between firing of C-S neurons and epochs of cortical synchronization, we measured, as for SONs, the timing of individual AP in C-S neurons relative to the reliable and consistent zero-time reference, i.e. the peak of negative EEG waves (Fig. 5B). As illustrated by the typical example of firing probability density shown in Figure 5C, C-S neuron spikes mostly preceded the peak of the corresponding cortical waves. For the studied C-S neurons (n = 6) and SONs (n = 5) we found that the probability density of firing in both cell types could be fitted to a normal function. As shown in Figure 6, the mean value of the pooled distributions was −11.4 ± 15.3 ms (empty bars) for C-S neurons and +13.9 ± 12.2 ms (filled bars) for SONs. This temporal dispersion of cortical and striatal firing was statistically different (P < 0.01, Levene median test). Therefore, the mean spike timing of SONs was delayed by ~25 ms compared with that of C-S neurons. We also noticed that the slope of membrane depolarization leading to firing was slower in SONs than in C-S neurons (Fig. 6, inset).

**Ketamine-Xylazine Anesthesia**

SONs (n = 7) recorded under ketamine-xylazine anesthesia exhibited a spontaneous activity different to that observed under barbiturate anesthesia. They displayed step-like membrane potential fluctuations consisting of recurrent sustained depolarizing plateaus (up states) interrupted by hyperpolarizing periods (down states) (Fig. 7A, lower trace). This type of activity is classically observed in rats anesthetized with a combination of urethane and ketamine-xylazine (Wilson and Kawaguchi, 1996; Stern et al., 1997, 1998). As in the experiments under barbiturate anesthesia, SONs were mostly silent, while one or two APs could be triggered during periods of membrane depolarization (Fig. 7A,C,E). We found that the alternation of up and down states was correlated with a slow rhythm of EEG potentials (Fig. 7A). This cortical oscillation had a frequency near 1 Hz (Fig. 7B) and exhibited faster waves in each cycle. This is similar to the slow cortical oscillations present in cats under ketamine-xylazine anesthesia (Steriade, 1995a,b; Contreras and Steriade, 1995).

As in the previous set of experiments conducted under barbiturate anesthesia, we aimed to assess the relationship between EEG waves and synaptic activity in SONs. Although EEG waves were less stereotypical and more complex than under barbiturate anesthesia, the onset of striatal up states, defined as the beginning of the stable depolarizing state (Fig. 7E), was always associated with an early positive deflection in the slow cortical field potentials. However, since the EEG waves varied in shape (Fig. 7C), we used reliable onset of up states as the zero-time reference. Pooling the data obtained under ketamine-xylazine anesthesia we found variability in the striatal up state durations, which ranged between 82.5 and 677 ms (mean = 287 ± 104.5 ms, n = 128 up states). An example of such fluctuations in up state duration in a single SON is illustrated in Figure 7C,D. Examination of simultaneous records of EEG and intracellular activity in SONs revealed that up states and the related slow EEG waves had roughly the same duration (see Fig. 7A,C,E). The temporal properties of firing of SONs were assessed by measuring the time of arrival of APs on the up state. We measured spike latency (Δt) with respect to onset of the up state (Fig. 7E) from spontaneously active SONs (four of the seven recorded cells). Δt for the first and second APs were reported as a function of up state duration (Fig. 7F) and we found (n = 4 cells) that the spike discharge was weighted heavily toward latencies distributed between 0 and 200 ms (Δt = 105 ± 85.5 ms, n = 71 APs).

In C-S neurons (n = 6) we observed a step-like behavior of membrane potential similar to that observed in SONs and most of the up states were supra-threshold for spike discharge (Fig. 8A). As shown by the superimposed records in Figure 8D, the onset of C-S neuron up states was precisely timed with the early positive wave of the slow EEG potentials (see also Fig. 8D). The up state duration in C-S neurons (Fig. 8C) ranged between 120...
1097 ms, with a mean value of 436 ± 199.5 ms (n = 144 up states), and was significantly longer (P < 0.0001, Mann–Whitney rank sum test) than that calculated for SONs (Fig. 7D). As for SONs, we measured the latency of AP discharges in C-S neurons with respect to onset of the up state (Fig. 8D). We found that the probability of AP discharge in C-S cells was greatest during the first 200 ms of the up state (AP 1, Δt = 86 ± 107 ms, from 0 to 536 ms, n = 131 APs), then the probability of firing decreased progressively throughout the up state (AP 2, Δt = 183 ± 158 ms, from 16 to 776 ms, n = 79 APs). The later APs spread out at the end of the up state (APs 3–6, Δt = 339 ± 216 ms, from 51 to 938.5 ms, n = 85 APs). An example of the temporal distribution of spikes during successive up states in a single C-S cell is shown in Figure 8E.

**Neurolept-analgesia**

A striking feature of SONs recorded under neurolept-analgesia (fentanyl–haloperidol) (n = 5) was the presence of small amplitude, temporally disorganized synaptic potentials (Fig. 9A, bottom trace) and the absence of spontaneous APs. This intracellular activity was associated with an apparently irregular EEG, composed of a mixture of waves (Fig. 9A, top trace). In contrast to the two previous anesthesias, the EEG did not show a clear rhythmicity while a predominant frequency around 2 Hz was revealed by the spectral analysis (data not shown). Given the noisy-like activity in the EEG and SONs, we calculated cross-correlograms of simultaneously recorded cortical potentials and striatal depolarizations to test whether temporal correlations could be detected between the two types of activity. No significant peak was found in the cross-correlograms (Fig. 9B), indicating the absence of a temporal correlation between the EEG waves and fluctuations of the membrane potential of SONs. However, because of the sporadic occurrence of relatively large amplitude EEG waves (asterisk in Fig. 9A), we examined the
intracellular activity in SONs associated with these larger cortical potentials. We selected periods of paired recordings where such EEG waves were observed (Fig. 9C1). The corresponding EEG wave-trigger average did not reveal any correlated synaptic depolarization in SONs (Fig. 9C2). It is important to stress that the largest EEG potentials under neurolept-analgesia had a

Table 2

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<th>Barbirurate anesthesia (n = 19)</th>
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<th>Neurolept-analgesia (n = 10)</th>
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<td>$R_n$ (MΩ)</td>
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<td>26.7 ± 2.71 22 to 30</td>
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<td>AP (mV)</td>
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<td>64.3 ± 6.84 69 to 78</td>
<td>65.6 ± 5.14 59 to 72</td>
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<td>AM peak (µs)</td>
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<td>447.5 ± 89.8 310 to 525</td>
<td>474.5 ± 94 295 to 625</td>
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<td>$\tau$ (ms)</td>
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<td>10.33 ± 1.35 9 to 11.7</td>
<td>8.32 ± 2.42 5 to 11</td>
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$\tau$ = time constant of the membrane. The slight differences observed under the three anesthetics are not significant ($P > 0.3$, ANOVA).

Figure 3. Basic electrophysiological features of C-S neurons. (A and B) Intrinsic membrane properties of C-S neurons were not affected by the different anesthetics, barbiturate (Barb), ketamine-xyalazine (K-X) and fentanyl (Fent). (A) Action potential characteristics. The DC superimposition of spikes clearly shows the constancy of their amplitude, duration and voltage threshold (horizontal line). (B) I/V relationship. Superimposition ($n \geq 3$) of single voltage responses of three C-S neurons (top traces), recorded under the different anesthetics, to intracellular injection of hyperpolarizing current pulses of increasing intensity (lowest traces). Neurons were slightly depolarized (idc, +0.3–0.4 nA) from rest to obtain an identical membrane potential. Note the rebound spike discharge produced after the hyperpolarizing pulses of high intensity. The apparent $R_n$ measured at rest by averaging of successive responses to current pulses of weak intensity (see Materials and Methods), was similar in the three cells ($R_n = 25–27$ MΩ). (C) The background synaptic activity affects the current-induced firing pattern of C-S neurons. Response of a C-S neuron (barbiturate anesthesia) to three successive depolarizing current pulses (lowest traces in C2) in the absence (C1) or presence of spontaneous activity (C2). The superimposed expanded responses at right are from the corresponding records shown at left. Note the stereotypical firing of the cell in the absence of synaptic noise while the coincidence with synaptic potentials led to an apparently haphazard firing pattern.
weak amplitude and duration compared with the standard EEG waves recorded under barbiturate or ketamine–xylazine anesthesia.

Most of the C-S neurons (nine of 10 cells) recorded under neurolept-analgesia were spontaneously active at rest, with a mean frequency of 8.2 ± 8 Hz. The main characteristic of this firing activity was that spikes were not triggered by large rhythmic synaptic depolarizations (Fig. 10A, lower traces). This was consistent with the absence of recurrent large amplitude EEG waves (Fig. 10A, top trace), which probably indicates that cortical cells were not engaged in a sustained synchronized oscillatory activity. As shown by the instantaneous frequency of firing during different epochs of recording (Fig. 10B), no recurrent firing pattern was found in C-S cells. However, short periods of rhythmic firing (around 5 Hz) could exceptionally be observed (Fig. 10Aa, Ba, horizontal bar). To confirm the apparent absence of regular firing patterns in C-S neurons we calculated the coefficient of variation (CV = SD/mean) of spike intervals. For the cell illustrated in Figure 10 the CV, calculated over a period of 20 s, was of 0.93 (with a mean ± SD of 183 ± 170.5 ms). Since a CV of 1 would be expected for random spiking activity (Stern et al., 1997), our results strengthen the hypothesis that C-S neurons under neurolept-analgesia did not display regular spiking. Altogether, our results indicate that synaptic activity within the cortex under this condition of anesthesia was not sufficiently correlated to produce powerful

Figure 4. Temporal relationship between intracellular activity of S0Ns and the related EEG activity under barbiturate anesthesia. (A1 and B1) Spontaneous intracellular activity of a SDN (lower traces) simultaneously recorded with the focal EEG (upper traces) of the contralateral orofacial motor cortex. The resting potential (horizontal arrows) and the peak potential of synaptic depolarizations (oblique arrow) are indicated in the figure. (A1) Period of rhythmic membrane depolarizations mostly sub-threshold for spike discharge. (A2) Fast Fourier transforms performed on the EEG period shown in (A1). (Inset) Expansion of the band frequency 0–20 Hz showing a strong periodicity at 6 Hz. (A3) Superimposition of n = 12 sub-threshold striatal depolarizations and of the corresponding EEG waves (upper traces). The negative peak of the EEG spindle wave was taken as the 0 time reference (dashed line) for alignment of all traces. Note that the peak synaptic depolarization (arrow) was delayed relative to this time reference. (B1) Period of supra-threshold activity. (B2) Superimposition of n = 12 supra-threshold synaptic depolarizations (lower traces) and of the corresponding EEG waves (upper traces) using the same temporal reference as in (A3). The timing of action potentials was calculated with respect to the 0 time reference (vertical dashed line). (Inset) Examples of recordings showing the temporal relationship between action potential firing and the corresponding EEG wave. Collision of a small spontaneous synaptic event (arrow) with the large amplitude oscillation-like depolarization led to an earlier spike discharge (asterisk). (B3) Histogram of striatal spike arrival time distribution using the 0 time reference as indicated in (B2). Removing extreme values (see Results for explanation), the probability density of firing was fitted with a Gaussian curve (mean = 15.8 ms, σ = 6.2 ms). Results presented in the different panels are from the same cell.
cortical field potentials and to induce temporally coherent firing in cortical cells.

**Discussion**

SONs are characterized by a low level of spontaneous firing and it is assumed that this low excitability is due to their non-linear electrical properties, which produce a shunting effect on synaptic potentials (Wilson, 1992, 1995; Nisenbaum and Wilson, 1995). To determine the ‘natural’ temporal structure of the ongoing cortical activity able to produce SON firing, data should be obtained in awake, behaving animals. However, the experimental constraints inherent in in vivo intracellular recordings, which were essential for the present investigation, require the use of anesthetized animals. Using three different anesthetics that induced different patterns of cortical activity we provide experimental evidence that a certain degree of synchrony in C-S neuron activity is required for significant depolarization in SONs.

**EEGs and Anesthetic-dependent Activity of C-S Neurons**

It is well established that activity in cortical cells is critically dependent on the type of anesthesia, different anesthetics inducing distinctive cortical rhythms mimicking different sleep stages (Steriade, 1997). This was obvious in the present study where the three different anesthetics we used induced EEG waves that were associated with dissimilar patterns of activity in C-S neurons. In the first series of experiments we used a barbiturate, an agonist of the γ-aminobutyric acid (GABA<sub>A</sub>) receptor that potentiates chloride-dependent synaptic activity (Barker and McBurney, 1979). This compound induced an EEG activity dominated by the occurrence of spindles that consisted of rhythmic, large amplitude EEG waves recurring with a strong periodicity in the 5–7 Hz range. This cortical spindling has been classically described in barbiturate-anesthetized rats (van Luijtelaar, 1997; Charpier et al., 1999a). Barbiturate EEG waves, which were mainly negative, symmetrical in shape and short in duration (<50 ms), had a systematic clear-cut temporal relation with large amplitude sub- or supra-threshold EPSPs in C-S neurons. This is consistent with results obtained in barbiturate-anesthetized cats, where cortical cells were found to display
rhythmic depolarization at ~6 Hz during EEG spindles sequences (Steriade, 1997). Our data are also consistent with the widely assumed correlation between excitatory synaptic events in single cortical cells and negative surface cortical waves and support the assumption that EEG potentials are an averaging of synchronized synaptic potentials from a large number of cortical neurons (Klee et al., 1965; Creutzfeld et al., 1966; Contreras and Steriade, 1995). Therefore, it is likely that barbiturate anesthesia produced synchronized oscillations in cortical networks (Contreras et al., 1997a) leading to coherent firing in C-S neurons.

Use of the ketamine–xylazine mixture produced another state of cortical synchrony. Ketamine is known to block the N-methyl-D-aspartate (NMDA) receptor (Anis et al., 1983) while xylazine is an adrenergic α2 mimetic (Nicoll et al., 1990). EEG records under ketamine–xylazine exhibit a slow rhythm at ~1 Hz. These slow cortical potentials are similar to the slow oscillations present throughout resting sleep in mammals and in urethane or ketamine–xylazine-anesthetized cats (see Steriade, 1993; Steriade et al., 1993a,b; Contreras and Steriade, 1995). They had an asymmetric shape and consisted of an early sharp deflection of high amplitude followed by a slow envelope of smaller amplitude (see Fig. 8A,D). The corresponding intracellular activity of C-S neurons was characterized by step-like membrane potential shifts, the so-called up and down states, which have been widely described in C-S neurons under urethane- and ketamine–xylazine anesthesia (Cowan and Wilson, 1994; Stern et al., 1997). However, the present study provides the first EEG correlates of the up and down states in C-S neurons. Here we have shown that transition to the up state in C-S cells is closely related to the initial high amplitude positivity of the EEG.
potential. Moreover, individual up states had approximately the same duration and time course as the corresponding cortical field potential. Altogether, these observations suggest that entry into the up state in the C-S neuron population is synchronized and that the dwell time in the up state of individual C-S neurons would be similar.

Finally, in the last series of experiments anesthesia was achieved by the use of fentanyl, a synthetic opioid acting mainly on the opioid receptor Mu (Inoue et al., 1994), combined with haloperidol, a D2 dopaminergic antagonist (Meshul and Allen, 2000). This neurolept-analgesia induced slow cortical waves that were often hidden by a higher frequency background activity and no regular rhythmic activity could be clearly detected. However, some EEG waves of relatively high amplitude could be observed, but they were noticeably smaller than those obtained under barbiturate and ketamine–xylazine anesthesia. This arhythmic EEG was associated with a temporally disorganized firing activity in C-S neurons, probably resulting in a lack of synchronized firing among C-S neurons.

The different patterns of cortical activity described here did not result from an anesthetic-dependent alteration in intrinsic membrane properties of cortical neurons. Indeed, we did not notice any significant difference in the basic electrical features of C-S cells recorded under the different anesthetics. Therefore, it is likely that the various temporal patterns of C-S firing mainly result from modulation of synaptic activity within the cortical networks and/or in the cortico-thalamic loops, due to the specific actions of the different anesthetics.

**Figure 8.** Temporal properties of spike discharge in a C-S neuron under ketamine–xylazine anesthesia. (A) A C-S neuron displaying rhythmic depolarizations (lower trace) associated with EEG waves (upper trace). The membrane potential fluctuations were similar to those observed in striatal cells (see Fig. 7). Up states in C-S neurons were correlated in time with the slow cortical field potentials. (B) Superimposition (n = 33) of C-S depolarizations (lower part) and the corresponding EEG waves (upper part). (C) Histogram of the distribution of up state durations (mean = 425.6 ± 206.2 ms). (D) A single C-S depolarization and its associated cortical field potential. Up state duration and latency of spike discharge (Δt) were measured as indicated. (E) Δt relative to the first (Δt = 88.5 ± 110.1 ms), second (Δt = 199 ± 158.9 ms) and following action potentials (third to sixth, Δt = 440.3 ± 235.8 ms) as a function of up state duration. Data presented in this figure are from the same cell.
cortical waves, it is likely that large synaptic depolarizations in EEG waves. Since C-S neuron firing was in phase with these ∼6 Hz waves systematically associated with large amplitude spontaneous large amplitude oscillation-like depolarizations at reliable model of C-S neuron synchronization, SONs displayed properties.

anesthetic-dependent alteration in their intrinsic membrane depolarizations in SONs described here result from an the different patterns of sub- and supra-threshold synaptic responses. Since the current-induced voltage responses of SONs were similar under the three anesthetics, it is very unlikely that activity of the afferent cerebral cortex. In addition, the mean timing of striatal firing was delayed by ~25 ms with respect to the cortical discharge. This temporal shift can be explained by the time required for integration of cortico-striatal information. This includes the conduction time of C-S spikes from the cerebral cortex to the contralateral striatum (mean = 7 ms), the synaptic delay [~0.3 ms (Eccles, 1964)] and the rise time of synaptic potentials due to the membrane time constant of SONs [~8 ms (Kawaguchi et al., 1989)]. The time lag between spontaneous firing of SONs and C-S neurons is also consistent with the time-to-peak of EPSPs evoked by electrical stimulation of the contralateral cortex [~22 ms (Wilson, 1986)].

A recent computer model (Marsalek et al., 1997; Diesmann et al., 1999) of propagation of synchronous activity in neural networks similar to the cortico-striatal pathway, i.e. a large number of active neurons converging onto target neurons, predicted that if the firing probability density of afferent neurons has a Gaussian distribution, the firing probability density of the target neurons would also have a Gaussian fit, but with a smaller standard deviation and a decrease in the number of active cells. This theoretical study is in accordance with our experimental data obtained under barbiturate anesthesia, which therefore strongly suggest a causal link between cortical synchrony and firing of striatal neurons.

Ketamine-xylazine anesthesia provided another model of

Figure 9. Striatal activity under neurolept-analgesia. (A) Intracellular recording of a SON (bottom) and the corresponding EEG (top). Note the absence of large amplitude EEG waves associated with a lack of large amplitude synaptic depolarizations in striatal cells. The asterisk indicates one of the largest EEG waves, shown in (C1). (B) Cross-correlation between the EEG and the intracellular record. Note the absence of high rhythmic and high correlation between the two. Ordinate, correlation coefficients. (C) Lack of significant synaptic activity in SON under neurolept-analgesia. (C1) Superimposition (n = 10) of the largest EEG waves and the corresponding intracellular striatal synaptic activity over a 20 s period of simultaneous recordings. (C2) Average of the EEG waves shown in (C1) and the corresponding mean striatal synaptic event. Note the absence of EEG wave-triggered synaptic events in the SON.

links connecting C-S neurons to SONs. Previous morphological studies (Cowan and Wilson, 1994; Kincaid et al., 1998) have shown that axons of C-S neurons form extended arborizations within the striatum making glutamergic synapses onto numerous SONs. In turn, each SON receives excitatory inputs from a large number of converging C-S neurons. While it is widely assumed that this cortical pathway constitutes the main excitatory input to the striatum, a possible involvement of glutamergic thalamo-striatal inputs (Wilson et al., 1983) cannot be excluded. However, several arguments suggest a relatively minor role of the thalamus in the electrical activity of the SONs recorded in this study. First, we previously showed (Charpier et al., 1999a) that local cortical cooling suppressed the corresponding EEG waves as well as synaptic potentials in the related striatal neurons, while barbiturate-induced spindles in the thalamus are still present following decerebration (Contreras et al., 1997b). Second, morphological studies have shown that thalamic and cortical terminals contact distinct SONs (Dubé et al., 1988). Finally, extracellular recording of identified thalamo-striatal neurons shows that spontaneous firing of these cells is not correlated with the rhythmic striatal depolarizations (unpublished observation). Therefore, we can assume that the striatal synaptic depolarizations described in the present study mainly result from activity of the afferent cerebral cortex. In addition, since the current-induced voltage responses of SONs were similar under the three anesthetics, it is very unlikely that the different patterns of sub- and supra-threshold synaptic depolarizations in SONs described here result from an anesthetic-dependent alteration in their intrinsic membrane properties.

In the barbiturate-anesthetized preparation, which provides a reliable model of C-S neuron synchronization, SONs displayed spontaneous large amplitude oscillation-like depolarizations at ~6 Hz that were systematically associated with large amplitude EEG waves. Since C-S neuron firing was in phase with these cortical waves, it is likely that large synaptic depolarizations in SONs resulted from synchronized discharges in a large population of cortical afferents. This rhythmic behavior of SONs (Charpier and Deniau, 1997; Charpier et al., 1999a) is similar to that described for extracellular single-unit recordings of striatal neurons in barbiturate-anesthetized cats (Sedgwick and Williams, 1967; Katayama, 1978; Katayama et al., 1980). Assuming a uniform behavior within the population of C-S neurons and that of SONs under barbiturate anesthesia, we found that C-S cells had a higher level of spontaneous firing compared to SONs and that the temporal dispersion of cortical firing on synaptic depolarization was significantly wider than that of striatal cells. In addition, the mean timing of striatal firing was delayed by ~25 ms with respect to the cortical discharge. This temporal shift can be explained by the time required for integration of cortico-striatal information. This includes the conduction time of C-S spikes from the cerebral cortex to the contralateral striatum (mean = 7 ms), the synaptic delay [~0.3 ms (Eccles, 1964)] and the rise time of synaptic potentials due to the membrane time constant of SONs [~8 ms (Kawaguchi et al., 1989)]. The time lag between spontaneous firing of SONs and C-S neurons is also consistent with the time-to-peak of EPSPs evoked by electrical stimulation of the contralateral cortex [~22 ms (Wilson, 1986)].

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Ketamine-xylazine anesthesia provided another model of
cortical synchronization. Under these anesthetics C-S neurons displayed step-like membrane potential fluctuations. Similarly, SONs exhibited rhythmic plateau-like depolarizations that could trigger APs. This alternation of up and down states in striatal cells has been extensively studied by Wilson’s group (Wilson and Kawaguchi, 1996; Stern et al., 1997, 1998). As discussed above, the relationship between EEG waves and C-S neuron up states suggests that transition into the up state occurs simultaneously among C-S cells. Moreover, the firing of C-S neurons was mainly clustered during the first 200 ms of the up state, the probability of firing decreasing progressively on membrane depolarization. Therefore, it is likely that synchronization of entry into the up state among the C-S neuron population leads to coherent firing of these cells, at least during the earliest part of the up state. This will produce highly correlated excitatory synaptic potentials in SONs leading to a sharp depolarization that causes transition to the up state and spike firing. How do we explain the fact that the striatal up state often lasted >200 ms in spite of the decreasing firing probability in C-S neurons? It has been proposed by Wilson and Kawaguchi that the level of membrane depolarization (≥–60 mV) reached during the striatal up state leads to deactivation of the potassium current responsible for inward rectification in the down state (Wilson and Kawaguchi, 1996). This would result in an increase in membrane resistance, allowing sustained membrane depolarization during the striatal up state, while the weight of excitatory synaptic inputs is weakened due to the decrease in the probability of firing in C-S neurons. During the later part of the up state in C-S neurons the lowering and temporal spreading of firing probably results in a lack of synchrony of spike discharges among C-S neurons. Therefore, the uncorrelated excitatory synaptic events in SONs will become ineffective in maintaining membrane depolarization and will act in synergy with activation of the inwardly rectifying potassium current, leading striatal cells to return to the down state (Wilson and Kawaguchi, 1996). Altogether, these mechanisms could explain, first, the significantly shorter mean duration of striatal up states compared with that of cortical up states and, second, that striatal up states could last >200 ms.

Under neurolept-analgesia C-S cells and SONs displayed arhythmic synaptic depolarizations that were associated with a temporally disorganized EEG. While the activity of C-S neurons could be supra-threshold for spike discharge, their firing did not present any predominant frequency and any obvious recurrent rhythmic patterns. This uncorrelated activity in C-S cells resulted in an absence of large amplitude synaptic potentials and consequently a lack of firing in SONs.

Concluding Remarks
Our findings under barbiturate and ketamine–xylazine anesthesia indicate that supra-threshold depolarizations in SONs are associated with epochs of synchronization in their cortical inputs. Moreover, under neurolept-analgesia the temporally disorganized activity in C-S neurons is not able to produce significant depolarization in SONs. Altogether, these results

Figure 10. C-S neurons recorded under neurolept-analgesia. (A) Three epochs (a–c) of firing activity in a C-S neuron. The EEG recording (top trace) corresponds to the period of C-S neuron activity shown in (a). (B) Plots of the instantaneous frequency from the three recording periods, indicated by letters as in (A).
demonstrate that striatal neurons shape their input-output relationship by filtering out uncorrelated synaptic inputs and that a minimal synchronization in the cortico-striatal afferents is needed to reach their firing threshold. This is consistent with the short time constant of SONs (Kawaguchi et al., 1989) and their voltage-dependent outward potassium currents, which act as an inhibitory shunt on synaptic depolarization (Wilson, 1992, 1995). Furthermore, we found that during periods of robust cortical synchronization spike timing variability at the striatal level was relatively less than that at the cortical level, indicating that the flow of cortical information will be transmitted within the basal ganglia, via firing of SONs, with high reliability.

Notes
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