Engagement of Rat Striatal Neurons by Cortical Epileptiform Activity Investigated With Paired Recordings

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Bracci, Enrico, Diego Centonze, Giorgio Bernardi, and Paolo Calabresi. Engagement of rat striatal neurons by cortical epileptiform activity investigated with paired recordings. J Neurophysiol 92: 2725–2737, 2004. First published July 7, 2004; 10.1152/jn.00585.2004. The striatum is thought to play an important role in the spreading of epilepsy from cortical areas to deeper brain structures, but this issue has not been addressed with intracellular techniques. Paired recordings were used to assess the impact of cortical epileptiform activity on striatal neurons in brain slices. Bath-application of 4-aminopyridine (100 μM) and bicuculline (20 μM) induced synchronized bursts in all pairs of cortical neurons (≤5 mm apart) in coronal, sagittal, and oblique slices (which preserve connections from the medial agranular cortex to the striatum). Under these conditions, striatal medium spiny neurons (MSs) displayed a strong increased spontaneous glutamatergic activity. This activity was not correlated to the cortical bursts and was asynchronous in pairs of MSs. Sporadic, large-amplitude synchronous depolarizations also occurred in MSs. These events were simultaneously detected in glial cells, suggesting that they were accompanied by considerable increases in extracellular potassium. In oblique slices, cortically driven bursts were also observed in MSs. These events were synchronized to cortical epileptiform bursts, dependent on non-N-methyl-D-aspartate (NMDA) glutamate receptors, and persisted in the cortex, but not in the striatum, after disconnection of the two structures. During these bursts, MS membrane potential shifted to a depolarized value (59 ± 4 mV) on which an irregular waveform, occasionally eliciting spikes, was superimposed. Thus synchronous activation of a limited set of cortico-striatal afferents can powerfully control MSs. Cholineric interneurons located <120 μm from simultaneously recorded MSs, did not display cortically driven bursts, suggesting that these cells are much less easily engaged by cortical epileptiform activity.

INTRODUCTION

The striatum is the major recipient of cortical afferents in the basal ganglia, and its neurons receive glutamatergic synaptic contacts from virtually every area of the cerebral cortex (Gerfen 1992; Goldman-Rakic 1983; Partha sarathy and Graybiel 1997). The striatum is therefore often regarded as a common input region of a neuronal loop that originates, and terminates, in the cortex, involving all the nuclei of the basal ganglia (some of which also receive a direct input from the cortex; Bevan et al. 2002) and parts of the thalamus (Bolam et al. 2000; Parent et al. 2000). Information processing along this neuronal loop is essential, or modulating epileptic activity originating in the cortex has been the object of a number of in vivo studies in animals and humans (Boda and Szente 1992; Bonhaus et al. 1986; Cassim et al. 2002; Depaulis et al. 1994; Gale 1992; Iadarola and Gale 1982; Vercueil and Hirsch 2002). These studies have provided important information, but the cellular details of the interaction between the cortex and the basal ganglia during seizures have not yet been elucidated. The aim of this study was to start to address this issue by investigating how the synaptic activity of the striatal neurons is affected by neurochemically induced paroxysmal activity in the cortex in brain slices. Paired whole cell recordings from cortical and/or striatal neurons were used to monitor the degree of synchronicity in the activity of these structures. Among several in vitro models of neurochemically induced epileptic activity (Gulyas-Kovacs et al. 2002; Traub and Jefferys 1994; Wong and Yamada 2001), we chose one that combines GABA<sub>A</sub> receptor antagonism and block of some potassium conductances, because this is known to generate robust cortical bursting and to strongly increase glutamate release in the striatum (Cepeda et al. 2001; Flores-Hernandez et al. 1994; Kita et al. 1985); this experimental condition also offers an interpretative advantage because it blocks GABA<sub>A</sub> receptor–mediated synaptic inhibition originating from the local striatal circuits (Kubota and Kawaguchi 2000; Tunstall et al. 2002).

METHODS

Male Wistar rats (postnatal day 20–35) were used as previously described (Calabresi et al. 2000c). All procedures used conformed with the guidelines of the European Union Council (86/609/EU) and the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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RESULTS

Spontaneous and evoked cortical bursts in the presence of 4-AP and bicuculline

Co-application of the GABA_A receptor antagonist bicuculline (20 μM) and the potassium channel blocker 4-AP (100 μM) was used to elicit robust epileptiform activity in the cortex and enhance glutamate release in the striatum (Cepeda et al. 2001, 2003; Flores-Hernandez et al. 1994; Gulyas-Kovacs et al. 2002). To test the degree of synchrony of such epileptiform activity, paired current-clamp, whole cell recordings were obtained from cortical neurons, located in the layers II-V of different areas of the cortex. Cortical neurons were identified as regular-spiking, slowly adapting cells based on their response to hyperpolarizing and depolarizing current steps in control solution (Connors and Gutnick 1990; Schwinding et al. 1997). As shown in the example of Fig. 1A, when depolarized from resting membrane potential (at which no steady current was injected), these cells displayed regular firing and spike accommodation, while injection of negative current elicited a hyperpolarizing response followed by a slow depolarizing sag, typical of cells that possess hyperpolarization-activated cation conductances. The minimum steady firing frequency of these cells (observed with near-threshold positive current injection lasting > 0.5 s) was <15 Hz, while the maximal steady firing frequency was <75 Hz, a typical range for cortical pyramidal cells but not for inhibitory interneurons (Beierlein et al. 2003).

Some of the cells recorded also displayed a short burst of two to four spikes at the beginning of a depolarizing pulse (data not shown). Cortical cells with firing properties typical of fast-spiking interneurons (Bracci et al. 2002; Connors and Gutnick 1990) were also occasionally recorded, but their behavior was not investigated systematically, and they were not included in the analysis.

Paired recordings from cortical neurons revealed that co-application of 4-AP and bicuculline invariably elicited a widespread synchronized bursting activity in coronal, sagittal, and oblique slices. The features of the bursting activity elicited by 4-AP and bicuculline were consistent with previous reports of neurochemically induced epileptiform activity in cortical slices (Bruckner et al. 1999; Cepeda et al. 2001; Gulyas-Kovacs et al. 2002). Burstiform events were always absent in control solution. In coronal and parasagittal slices, 21 pairs of neurons located in the motor and/or somatosensory cortex at distances ±5 mm, all displayed spontaneous synchronized bursts. In oblique slices, similar synchronized bursts were observed in 13 pairs of neurons located in the medial agranular cortex (mAC) and/or in the adjacent somatosensory cortex (the distance between the neurons in these pairs was ±3 mm). No significant differences were found in the epileptiform activity expressed by the three slice types tested, in terms of burst duration, quiescent interburst intervals, and of number of action potentials per burst. In the three slice types, bursting activity induced by 4-AP and bicuculline was also characterized by a similar degree of variability. The interburst intervals varied between 10 and 110 s. In several cases, bursts appeared at regular intervals (Fig. 1B). The CV of the interburst interval, averaged across all the tested preparations, was 21 ± 6%. The pattern of action potentials generated during a burst by the two cortical neurons recorded simultaneously often differed substantially, as shown in the example in Fig. 1B. In all cases, however, the initial depolarizing shift of a burst was large enough to trigger an action potential.

While bursts appeared roughly synchronously in the two neurons recorded simultaneously, the onset time of the spontaneous burst (defined as the time when the membrane potential departed by >3 mV from the resting level, provided the final depolarization was >15 mV) was not identical in the two cells, as visible in the example in Fig. 1B. The difference between burst onset times was 8 ± 4 ms, with a maximum value of 15 ms. This difference was also variable across different bursts (CV, 43 ± 27%), and in 32% of the recorded pairs, one of the two cells was found to lead in some of the bursts but follow in the remaining ones. The time between the
onset of a burst and the peak of the first action potential was 18 ± 5 ms for spontaneous bursts.

In all cases, a single electrical stimulus delivered to the superficial cortical layers after >10 s from the end of a spontaneous burst was able to elicit in the cortical neurons a new synchronous burst, which was similar in amplitude and duration to those appearing spontaneously (Fig. 1C). These evoked bursts appeared in an all-or-none fashion when the stimulus amplitude was above a certain threshold, which was 120 ± 12% of the threshold for observing an evoked excitatory
postsynaptic potential (EPSP) in at least one of the cortical neurons in control solution. It was not possible to affect evoked burst amplitude or duration by increasing the stimulation intensity. The major difference observed between evoked and spontaneous bursts was that the evoked bursts had a much faster rate of depolarization (time from burst onset to the peak of the 1st spike was 2 ± 1 vs. 18 ± 5 ms of spontaneous bursts; \( P < 0.001 \)). In addition to this, the difference in burst onset in the two cells recorded simultaneously was shorter (4.0 ± 0.5 ms; \( P < 0.001 \)) and much less variable across different events (CV, 7 ± 2%).

**Striatal activity in the presence of 4-AP and bicuculline**

To investigate how striatal neurons are affected by cortical epileptiform activity, we performed paired recordings from medium spiny neurons (MSs) in the striatum. MSs were identified in control solution based on their distinctive electrophysiological properties, which included very negative resting membrane potential (−80 to −90 mV), larger voltage deflections in response to positive than negative current injections, and delayed firing onset (Jiang and North 1991; Nisenbaum et al. 1994). We recorded 20 pairs of MSs located 50–300 μm apart, in coronal \( n \) = 7, sagittal \( n \) = 6, and oblique slices \( n \) = 7. As previously reported, synaptic potentials were not easily detected in one of the MS (at resting membrane potential) when action potentials were elicited in the other cell (Jaeger et al. 1994; Tunstall et al. 2002), as shown in Fig. 2A. Consistent with previous reports (Nisenbaum and Wilson 1995; Nisenbaum et al. 1994), co-application of 4-AP and bicuculline did not cause a significant change in membrane resting potential or input membrane resistance measured with small injections of negative current from resting membrane potential in MSs. However, the voltage deflections elicited by positive current steps increased in the presence of these agents, and (for suprathreshold steps) the latency to the first spike decreased, as previously described by Nisenbaum et al. (1994) and Nisenbaum and Wilson (1995), who showed that these effects are due to a selective effect of 100 μM 4-AP on a slowly inactivating \( I_A \) potassium current.

Co-application of 4-AP and bicuculline also elicited major changes in the MS spontaneous synaptic activity, which in control solution consisted of relatively sporadic synaptic potentials, as previously reported (Cepeda et al. 2001; Flores-Hernandez et al. 1994; Kita et al. 1985). The spontaneous activity in the presence of 4-AP and bicuculline included three different types of events: irregularly occurring EPSPs, slow depolarizations, and cortically driven bursts.

**Irregularly occurring EPSPS.** These consisted of frequent, irregular glutamatergic EPSPs, which were not correlated in the two simultaneously recorded MSs and were also not temporally related to the cortical bursts. A strong increase in the amplitude and frequency of these spontaneous events was invariably induced by 4-AP and bicuculline in all MSs recorded from sagittal, coronal, and oblique slices. An example of this type of activity in two MSs recorded simultaneously is shown in Fig. 2B, which also shows the spontaneous potentials previously recorded in control solution. These events were abolished by the non-\( N \)-methylaspartate (NMDA) receptor antagonist NBQX (10 μM). Numerical mean-removed cross-correlation analysis (see METHODS) applied to the paired recordings during this type of activity \( n \) = 12 failed to consistently reveal peaks in the correlograms for any particular time interval between −5 and 5 s, confirming that this activity was not significantly correlated in nearby MSs. Although the observed increase in MS resistance at depolarized potentials is expected to proportionally enhance EPSP amplitude, this phenomenon alone could not explain the increase in EPSP occurrence and size in the presence of 4-AP and bicuculline. In fact, the increase in amplitude of the large-amplitude EPSP detected (defined in each cell as those exceeding 90% of the maximal EPSP amplitude recorded in each pharmacological condition) was 1.120 ± 170%, significantly \( P < 0.001 \) larger than that in the increase in the input resistance measured with positive current steps \( =300 \) pA (which was \( <150\% \) in all cases). We limited this analysis to the large-amplitude EPSPs, because part of the smaller events could be undetectable under these conditions. B: in the same neurons in A, bath application of 4-AP and bicuculline resulted in a large increase in frequency and amplitude of spontaneous glutamatergic events with respect to control solution. These events were not synchronous or correlated in recorded MSs.
and were synchronized in the nearby MSs (Fig. 3A). These slow depolarizations were quite variable in shape and could be either subthreshold or suprathreshold (compare Figs. 3, A and B, and 4). The average duration of the slow depolarizations was 23 ± 12 s, and their average amplitude was 21 ± 7 mV (Fig. 3C). These events are similar to those observed by Cepeda et al. (2001) under the same pharmacological conditions, and, as described in that study, they were abolished by NBQX application (10 μM). This kind of activity was observed in 4/7 MS pairs in coronal slices, 4/6 pairs in sagittal slices, and 4/6 pairs in oblique slices. Simultaneous recordings from cortical and striatal neurons in all slice types (n = 17) revealed that there was no temporal correlation between the cortical bursts and the large striatal depolarizations, which never propagated back to the cortex (Fig. 3B). In this study, cortical neurons were not observed to generate slow depolarizations or spreading depres-
evoked glutamatergic potentials, and they were not significantly increased during bicuculline application, which also prompted a transient increase in extracellular potassium, promoting increased glutamate release, was an important factor underlying the synchronous striatal depolarizations (Somjen 2001) in addition to the epileptiform bursts described above.

It has been proposed that increased glutamate release can trigger a positive feedback mechanism resulting in large, transient increases in extracellular potassium (Anderson and Andrew 2002; Kager et al. 2002). To test whether a similar phenomenon could underlie the slow striatal depolarizations, we performed paired recordings from MSs and glial cells, because glial cells act as extracellular potassium sensors (Croning et al. 1995). Glial cells were identified in control solution based on the absence of action potentials and displayed a resting membrane potential of $74 \pm 6$ mV ($n = 13$) and an input resistance of $>50$ MΩ. Although glial cells can express glutamate receptors (Verkhratsky and Steinhauser 2000), in the striatum, these cells did not display any detectable spontaneous or evoked glutamatergic potentials, and they were not significantly depolarized by bath application of glutamate (100 μM for 3 min; $n = 4$). Nevertheless, in the presence of 4-AP and bicuculline, the slow large depolarizations observed in the MSs were accompanied by simultaneous depolarizations with a similar time course in glial cells ($n = 9$) in all slice types, as shown in the example in Fig. 4. The amplitude of glial depolarization was $15 \pm 4\%$ of that observed in simultaneously recorded MSs. We concluded that a transient increase in extracellular potassium, promoted by increased glutamate release, was an important factor underlying the synchronous striatal depolarizations.

To confirm that the irregularly occurring EPSPs and the slow depolarizations did not depend on cortical activity, we carried out MS paired recordings ($n = 13$) in the striatum after surgical removal of the cortex in four sagittal, four coronal, and five oblique slices. In these isolated preparations, the fast spontaneous activity observed in the presence of 4-AP and bicuculline did not significantly differ in amplitude or frequency from that recorded in the intact slices. Similarly, the slow depolarizations were still present, and they did not significantly differ in amplitude or frequency from those observed in the intact slices.

CORTICALLY DRIVEN BURSTS. Consistent with the observation that most corticostriatal fibers are severed in sagittal and coronal slices (Kawaguchi et al. 1989), in these preparations, it was not possible to observe any striatal correlate of the cortical epileptiform bursts. Conversely, oblique slices (described in METHODS and shown in Fig. 5, A and B) preserve connections from the medial agranular cortex (mAC) to the striatum (Kawaguchi et al. 1989; Wickens et al. 1996). In these preparations, local stimulation of the superficial layers of the mAC in control solution elicited glutamatergic potentials in 19/33 MSs located in the dorsal portion of the striatum (Fig. 5C). The maximal amplitude of this evoked event was 3–9 mV, and their latency was $>8$ ms, consistent with a previous study involving long-latency EPSPs, presumably due to progressive disinhibition of the cortex. Simultaneous recordings from a cortical neuron revealed a progressive increase in the amplitude of the evoked EPSP during bicuculline application, accompanied by the appearance of a large, slow inhibitory postsynaptic potential (IPSP), presumably mediated by GABA<sub>A</sub> receptors. In the striatal MS, a long-latency–evoked EPSP (completely absent in control ACSF, arrow) appeared after 2 min of bicuculline application. After 4 min in bicuculline, amplitude of this evoked event is increased and its latency reduced. Evoked events in the MS are indicated by arrows.
using this preparation (Kawaguchi et al. 1989) and confirming that they were solely due to local activation of the cortical layers (Kawaguchi et al. 1989). Interestingly, in 5/9 MSs in which it was not possible to evoke EPSPs with local stimulation of the mAC in control solution, bath-application of bicuculline progressively induced the appearance of a longer-latency–evoked EPSP. This phenomenon is shown by the experiment in Fig. 5D, where a striatal MS and a pyramidal neuron located in the superficial layers of the mAC were recorded simultaneously. This suggests that disinhibition of the cortex by bicuculline allowed the stimulus to polysynaptically excite a larger set of cortical neurons, a portion of which were synaptically connected to the recorded MS.

Electrical stimulation of the dorsolateral striatum evoked antidromic spikes in 4/11 neurons located in the central layers of the mAC (data not shown), further confirming that part of the corticostratial fibers were intact in oblique slices.

Consistent with these findings, in the presence of 4-AP and bicuculline, cortical bursts elicited synchronized depolarizing synaptic responses in 17/29 MS tested in oblique slices. These cortically driven responses co-existed with the irregular EPSPs and the slow depolarizations described above and were large enough to elicit action potentials in 5/17 MSs, while they remained subthreshold in the remaining 12. An example of a suprathreshold response is shown in Fig. 6A. In this case, in concomitance with a burst in a simultaneously recorded cortical neuron, a MS displays a large initial depolarizing shift, on which a rather irregular waveform is superimposed, occasionally reaching firing threshold. This depolarizing event is terminated by a slower decay to the resting membrane level. On average, the latency between the onset of the cortical bursts and that of the corresponding striatal bursts assessed with paired recordings was 12 ± 5 ms (n = 9). Stimulation of the mAC during quiescent interbursts intervals invariably elicited a burst similar to those occurring spontaneously; these evoked events in turn elicited striatal responses that were similar to those elicited by spontaneous cortical bursts. An example is shown in Fig. 7B.

To get additional evidence that epileptiform bursts appeared synchronously in large populations of cortical and striatal neurons, we recorded field potentials in the mAC (layer V) and the dorsal striatum with extracellular electrodes. As shown in Fig. 6B, these experiments revealed that the epileptiform bursts in both structures could easily be detected extracellularly, and that the temporal relationship between cortical and striatal FPs was similar to that observed with intracellular recordings. Extracellular recordings also showed that, in control solution, repeated tetanic stimulation (50–200 stimuli at 100 Hz delivered every 5 min) applied to layers III–VI of the mAC elicited post-tetanic bursts comprising several population spikes in the mAC (Bracci et al. 1999), although in this case, the spatial extent of such bursts was limited to 1–2 mm from the stimulator tips. In 3/7 oblique slices tested with paired extracellular recordings, these tetanically evoked cortical bursts were able to propagate to the striatum, where they elicited field EPSPs and population spikes (data not shown).

Recordings from striatal glial cells (n = 4) in oblique slices failed to reveal significant dendritic depolarizations in these cells concomitant with the bursts occurring simultaneously in cortical and striatal neurons, suggesting that, unlike the slow depolarizations, these bursts were not accompanied by a large increase in extracellular potassium in the striatum.

Cortical and striatal synchronized bursts were fully abolished by NBQX (10 μM), and surgical separation of the two structures resulted in the loss of striatal bursts, while cortical bursts persisted without significant difference in burst amplitude, duration, and frequency (n = 4), confirming that striatal bursts resulted from the synchronous activation of a large number of corticostratial afferents.

Furthermore, when 4-AP was applied without bicuculline in coronal (n = 4), sagittal (n = 4), or oblique slices in which the striatum had been surgically disconnected from the cortex (n = 3), a strong increase in the irregularly occurring spontaneous synaptic events of MSs (n = 16) was invariably observed, but no epileptiform bursts were recorded, confirming that these events depend on cortical inputs even when GABAergic transmission is intact.

Cortically induced striatal bursts were reminiscent of the spontaneous, periodic depolarizations that are observed in MSs in vivo and are referred to as “up-states” (Stern et al. 1997, 1998; Wickens and Wilson 1998; Wilson 1993; Wilson and Kawaguchi 1996). To compare the features of the present striatal bursts with those of the up-states, we analyzed the distribution of the time spent by MSs at different membrane potentials during 4-min episodes of suprathreshold bursting (n = 5). As shown in Fig. 6C, a bimodal distribution was apparent in all cases. As a consequence of the long quiescent interburst periods observed under the conditions of this study, a much larger proportion of time was spent at the more hyperpolarized resting value here than in the in vivo studies mentioned above. The part of the distribution centered around the more positive peak value was (separately) fitted by a single Gaussian curve (see METHODS). Such fitting curves peaked at 59 ± 4 mV and had an average SD of 6 ± 2 mV. These values are comparable with those observed in vivo for the up-states (Wickens and Wilson 1998). An example of such a distribution and of its Gaussian best fit is presented in Fig. 6D.

Striatal large aspiny cholinergic interneurons (LAs) receive a prominent glutamatergic input from the thalamus, but a synaptic input to these cells also originate from a small fraction of cortical terminals (Lapper and Bolam 1992; Thomas et al. 2000). To study whether the epileptiform cortical bursts could synaptically engage the LAs, we performed simultaneous recordings from pairs comprising of a MS and a LA located closely apart (50–120 μm) in oblique slices in the presence of 4-AP and bicuculline (n = 7). LAs were identified visually, based on their large soma and proximal dendrites, and electrophysiologically, based on their distinctive responses to negative and positive current injections in control solution, including a relatively positive resting membrane potential (~58 to ~65 mV) and a large depolarizing sag in response to negative current injections (Bennett et al. 2000; Kawaguchi 1993). An example of these properties is shown in the inset of Fig. 7. Of seven recorded LAs, four generated spontaneous action potentials at resting membrane potential (no current injected), consistent with previous reports (Bennett et al. 2000). In these cells, a small steady negative current was injected to hyperpolarize the neuron just below threshold and prevent spike generation that would have made the analysis of synaptic activity less straightforward.
In control solution, stimulation of mAC, which evoked EPSPs in 4/7 MSs, failed to evoke detectable responses in 7/7 LAs. In the presence of 4-AP and bicuculline, epileptiform bursts in the cortex elicited synchronous bursts in MSs. In the example shown here, striatal bursts were accompanied by action potential generation. B: simultaneous field potential (FP) recorded in layer V of the mAC and the dorsal striatum confirmed that cortical bursts were accompanied by nearly simultaneous striatal bursts. In this particular example, cortical and striatal bursts occurred in doublets of temporally close events separated by longer quiescent intervals. C: distribution of the time spent at different membrane potential levels during a 240-s continuous recording from a MS in an oblique slice during bursting activity induced by 4-AP and bicuculline. A strongly asymmetrical, bimodal distribution is apparent. D: enlargement of the smaller, more depolarized part of the distribution shown in B. This part of the distribution was best-fitted with a single Gaussian curve, which peaked at $-61.3 \pm 4.4$ mV.

FIG. 6. Cortical epileptiform activity engages striatal MSs in the oblique slice. A: in oblique slices, paired recordings from a mAC neuron and a striatal MS revealed that, in the presence of 4-AP and bicuculline, epileptiform bursts in the cortex elicited synchronous bursts in MSs. In the example shown here, striatal bursts were accompanied by action potential generation. B: simultaneous field potential (FP) recorded in layer V of the mAC and the dorsal striatum confirmed that cortical bursts were accompanied by nearly simultaneous striatal bursts. In this particular example, cortical and striatal bursts occurred in doublets of temporally close events separated by longer quiescent intervals. C: distribution of the time spent at different membrane potential levels during a 240-s continuous recording from a MS in an oblique slice during bursting activity induced by 4-AP and bicuculline. A strongly asymmetrical, bimodal distribution is apparent. D: enlargement of the smaller, more depolarized part of the distribution shown in B. This part of the distribution was best-fitted with a single Gaussian curve, which peaked at $-61.3 \pm 4.4$ mV.

In control solution, stimulation of mAC, which evoked EPSPs in 4/7 MSs, failed to evoke detectable responses in 7/7 LAs. In the presence of 4-AP and bicuculline, LAs displayed a strong increase in the irregular spontaneous synaptic activity, qualitatively similar to that observed in MSs in coronal and sagittal slices and in oblique slices during the interburst intervals. This activity was not correlated to that recorded in nearby MSs and was fully blocked by co-application of the non-NMDA glutamate receptor antagonists NBQX (10 $\mu$M) and the NMDA glutamate receptor antagonist AP5 (20 $\mu$M). A comparative analysis (see METHODS) of the spontaneous EPSPs in MSs and LAs revealed that these events were significantly ($P < 0.05$) rarer in LAs than in MSs in control solution, but increased proportionally more in these cells (770% of control frequency) than in MSs (407% of control) in the presence of 4-AP and bicuculline ($P < 0.001$). Spontaneous EPSP amplitude was not significantly different in LAs and MSs; the increase in amplitude induced by 4-AP and bicuculline was larger in MSs, but this phenomenon was not statistically significant. These results are shown in Fig. 7C. A similar
FIG. 7. Different response of striatal MSs and cholinergic interneurons to cortical epileptiform activity in the oblique slice. In an oblique slice, paired recordings from a striatal MS and a striatal cholinergic interneurons (LA) located 50 μm apart, revealed that, in the presence of bicuculline and 4-AP, the LAs was not synaptically engaged either by spontaneous (A) or electrically evoked (B, arrows) cortical bursts. The LA was identified based on its large size and its distinctive electrophysiological responses to negative and positive steps of current, including a prominent depolarizing sag during hyperpolarizing steps (inset). C. Plots showing the increase in frequency (left) and amplitude of spontaneous EPSPs in LAs (n = 7) and MSs (n = 15) induced by co-application of 4-AP and bicuculline with respect to control solution, in oblique slices.
increase in the frequency and amplitude of the spontaneous EPSPs was also induced by 4-AP and bicuculline in LAs of four isolated striatal slices (n = 5). This shows that this phenomenon did not depend on the presence of intact corticostriatal connections and was presumably due to spontaneous release of glutamate from cortical and/or thalamic afferents severed from their cellular bodies. As in the intact slices, these spontaneous EPSPs were fully abolished by co-application of NBQX and AP5.

In the intact oblique slices, in sharp contrast to 5/7 nearby located MSs, none of the seven LAs recorded displayed significant synaptic responses driven by the cortical bursts. This is shown in Fig. 7A, where a MS and a LA located 50 μm apart were recorded simultaneously. While the MS generated large rhythmic depolarizing responses, the LA did not display any activity correlated to these events. Similarly, when an electrical stimulus was delivered to the mAC, this elicited a burst similar to those occurring spontaneously in the MS, but not in the LA (Fig. 7B). We conclude that the connections from the mAC to the LAs in the oblique slice are much less efficient and/or numerous than those impinging on nearby MSs.

To confirm that the cholinergic interneurons did not contribute significantly to cortically induced striatal bursting, we bath-applied the muscarinic antagonist atropine (10 μM) and the nicotinic receptor antagonist mecamylamine hydrochloride (1 μM) in the presence of 4-AP and bicuculline in oblique slices (n = 4). These antagonists did not significantly affect the amplitude, duration, and frequency of cortical epileptiform bursts and also failed to significantly affect the associated MS striatal bursts. This confirms that the striatal LAs were not significantly engaged by the cortical bursts, because their activation would be expected to affect MSs through a variety of mechanisms (Calabresi et al. 2000b).

DISCUSSION

These results show for the first time that epileptiform activity in the cortex can evoke synchronized depolarizing bursts accompanied by action potentials in striatal projection neurons, while simultaneously recorded cholinergic interneurons are hardly affected by such a paroxysmal cortical activity. In addition to this phenomenon, even when the striatum was disconnected from the cortex, application of epileptogenic agents that interfere with GABAergic transmission and potassium channels triggered slow depolarizations that were synchronous in MSs and seem to be promoted by a transient increase in extracellular potassium.

We elicited epileptiform activity with the GABA<sub>A</sub> receptor antagonist bicuculline and the potassium channel blocker 4-AP in coronal, sagittal, and oblique slices. While the cortical activity recorded intracellularly was similar in these three preparations, only in oblique slices were we able to observe synaptic responses in striatal neurons that were correlated to cortical activity. This extends the previous observations that electrical stimulation of the cortex fails to elicit responses in striatal neurons in coronal and sagittal slices, while it evokes subthreshold responses in oblique slices (Kawaguchi et al. 1989; Wickens et al. 1996). Coronal and sagittal slices provide a viable tool to study the physiology of the corticostriatal synapses, which can be activated by stimulation of the peristriatal white matter (Calabresi et al. 2000b), showing that severed corticostriatal axons survive and maintain their physiological properties in vitro. These surviving axons, depolarized by 4-AP, were presumably responsible for the irregularly occurring EPSPs observed in all slices. In the oblique slices, in which a part of the connections from the mAC to the striatum is intact (Kawaguchi et al. 1989; Wickens et al. 1996), focal electrical stimulation of the mAC in control solution elicited long-latency, presumably monosynaptic subthreshold EPSPs in MSs, as previously described (Kawaguchi et al. 1989). This stimulation was ineffective in a subset of MSs, but progressive disinhibition of the cortex often resulted in the generation of a polysynaptic-evoked EPSP in these cells, a phenomenon never observed in the other slices. When the cortex was fully disinhibited, MSs received a strong, suprathreshold synaptic drive, showing that the residual connections from the mAC can still exert a powerful influence on MS if simultaneously activated.

The observation that, in the oblique slice, the MS cortically driven bursts were blocked by the non-NMDA ionotropic glutamate receptor antagonist NBQX, and, unlike the slower striatal depolarizations, were not accompanied by glial cell depolarizations, suggest that these events were mainly shaped by glutamatergic EPSPs. Since GABA<sub>A</sub> receptors were blocked in our experiments, we can exclude a contribution of these receptors to the observed MS bursts. This is significant, since GABA<sub>A</sub> receptor reversal potential is more positive than resting membrane potential (Calabresi et al. 2000a; Kita 1996; Koos and Tepper 1999; Tunstall et al. 2002), and MSs receive GABAergic input from other MSs as well as from interneurons (Bennett and Bolam 1994; Bolam et al. 2000; Koos and Tepper 1999; Tunstall et al. 2002).

In vivo intracellular recordings from anesthetized rats have revealed that MSs undergo simultaneous depolarizations (up-states), characterized by persistence of the membrane potential around a plateau level and generated by an interplay of intrinsic membrane conductances and cortical synaptic inputs (Stern et al. 1997, 1998; Wickens and Wilson 1998). MSs receive GABAergic contacts from other MSs and striatal interneurons, which receive direct innervation from the cortex (Bracci et al. 2002, 2003; Kawaguchi 1993; Koos and Tepper 1999; Kubota and Kawaguchi 2000; Ramanathan et al. 2002; Tunstall et al. 2002). It is possible that GABAergic potentials of local striatal origin play a significant role in these up-states. The bursts observed here were similar to the up-states in their membrane potential distribution (although not in the proportion of time spent in the depolarized state). This shows that, even when slowly inactivating A-conductances and GABA<sub>A</sub> receptors are blocked by 100 μM 4-AP (Nisenbaum and Wilson 1995; Nisenbaum et al. 1994) and bicuculline, respectively, MSs can still undergo a transition to a depolarized level similar to that of the up-states. Under the conditions of this study, the firing rate of the cortical neurons was larger than the one recorded in anesthetized rats (Stern et al. 1997). This apparently compensated for the smaller number of corticostriatal fibers present in the slices, because the glutamatergic input to MS was large enough to cause MS depolarizations comparable with those observed in vivo.

Calcium conductances play a prominent role in ictal depolarizations of cortical neurons (Schiller 2002); it is possible that calcium conductances also contributed to the cortically driven bursts in striatal MSs. Investigating this important issue will require calcium imaging techniques and local application of...
calcium channel antagonists, because it will be essential not to affect the underlying cortical bursts to preserve the glutamatergic synaptic input into striatal neuron.

Striatal cholinergic interneurons, which are thought to play an essential role in the modulation of the striatal function (Apicella 2002; Calabresi et al. 2000b; Zhou et al. 2002), receive numerous glutamatergic synaptic contacts. These contacts mainly originate from thalamic afferents, but a fraction of corticostriatal terminals also synapse on cholinergic interneurons (Lapper and Bolam 1992; Thomas et al. 2000; Wilson et al. 1990). The striking absence of glutamatergic potentials correlated with cortical bursts in LAs, in sharp contrast to those observed in MSs located nearby, shows that these neurons receive a much smaller input (if any) from the mAC and supports the view that they either receive little functional input from the cortex, or alternatively, receive connections only from specific cortical regions (Bennet and Wilson 2000; Thomas et al. 2000). The observation that the frequency of spontaneous EPSPs in LAs was lower in control solution than in MSs, but increased proportionally more in the presence of 4-AP and bicuculline, is also consistent with the notion that the glutamatergic afferents impinging on LAs may have a different origin from those impinging on MSs.

In all slice types used, co-application of 4-AP and bicuculline resulted in the appearance of sporadic, slow depolarizing events. These events are similar to those observed by Cepeda et al. (2001) in the same pharmacological conditions and were likewise blocked by non-NMDA ionotropic glutamate receptor antagonists. The use of paired recordings has allowed us to show that 1) the slow depolarizations take place synchronously in striatal neurons located nearby; 2) they bear no temporal correlation to epileptiform cortical bursts; and 3) they are unable to propagate to the cortex in the brain slices tested. The observation that striatal glial cells were not depolarized by glutamate but underwent slow depolarization that was simultaneous to the ones observed in spiny neurons, shows that these events were associated with a significant increase in extracellular potassium concentration, because glial cell membrane potential is sensitive to variations in potassium reversal potential (Croning et al. 1995). In striatal MSs, this increase in potassium, summated to the effects of large spontaneous glutamate release, is likely to trigger a depolarization, which is then amplified by the inward rectification properties of these cells (Nisenbaum and Wilson 1995). This process may become autoregenerative, because glutamatergic terminals would be depolarized by transient potassium accumulation, thus promoting action potential generation in MSs, leading in turn to more potassium release. Computational models have indeed shown that neurons tonically exposed to glutamate can generate, in response to a phasic increase in glutamate or external potassium, an all-or-none slow depolarizing process characterized by autoregenerative accumulation of extracellular potassium (Kager et al. 2002; Somjen 2001). This mechanism has been proposed to underlie spreading depression in the cortex. We propose that a similar phenomenon may underlie the generation of the slow depolarizations observed in the striatum in the presence of 4-AP and bicuculline. Under these conditions, the glutamatergic terminals impinging on MSs are presumably reached by extremely frequent action potentials generated ectopically in the axons (Traub et al. 2001), and therefore provide an incessant, but variable release of glutamate. In this situation, the temporal coincidence of a number of such synaptic events in a restricted region may trigger a self-sustaining mechanism similar to spreading depression, giving rise to a slow depolarization. The fact that the amplitude of such striatal events was smaller than cortical and hippocampal spreading depression may be tentatively explained as a consequence of the more anisotropic orientation of MS processes (Kawaguchi et al. 1989), and of a different balance between their inward and outward voltage-activated conductances (Nisenbaum and Wilson 1995); these factors would limit in the striatum the extent of the positive-feedback interplay between extracellular accumulation of potassium and voltage-activation of net inward currents that is believed to underlie cortical spreading depression (Somjen 2001). The ability of the striatal tissue to generate similar depolarizing events in response to increased excitatory inputs is expected to be larger in the intact brain, where potassium accumulation is favored by the higher compactness of the tissue. It will therefore be of interest to investigate if a similar phenomenon takes place in vivo under physiological or pathological conditions.

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REFERENCES


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