Functional Properties of Fast Spiking Interneurons and Their Synaptic Connections With Pyramidal Cells in Primate Dorsolateral Prefrontal Cortex

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González-Burgos, Guillermo, Leonid S. Krimer, Nadya Povyshева, German Barrionuevo, and David A. Lewis. Functional properties of fast spiking interneurons and their synaptic connections with pyramidal cells in primate dorsolateral prefrontal cortex. J Neurophysiol 93: 942–953, 2005. First published September 22, 2004; doi:10.1152/jn.00787.2004. Recent studies suggest that fast-spiking (FS) interneurons of the monkey dorsolateral prefrontal cortex (DLPFC) exhibit task-related firing during working-memory tasks. To gain further understanding of the functional role of FS neurons in monkey DLPFC, we described the in vitro electrophysiological properties of FS interneurons and their synaptic connections with pyramidal cells in layers 2/3 of areas 9 and 46. Extracellular spike duration was found to distinguish FS cells from non-FS interneuron subtypes. However, a substantial overlap in extracellular spike duration between these populations would make classification of individual interneurons difficult. FS neurons could be divided into two main morphological groups, chandelier and basket neurons, with very similar electrophysiological properties but significantly different horizontal spread of the axonal arborization. In paired cell recordings, unitary inhibitory postsynaptic potentials (IPSPs) elicited by FS neurons in pyramidal cells had rapid time course, small amplitude at resting membrane potential, and were mediated by GABAA receptors. Repetitive FS neuron stimulation, partially mimicking the sustained firing of interneurons in vivo, produced short-term depression of the unitary IPSPs, present at connections made by both basket and chandelier neurons and due at least in part to presynaptic mechanisms. These results suggest that FS neurons and their synaptic connections with pyramidal cells have homogeneous physiological properties. Thus different functional roles of basket and chandelier neurons in the DLPFC in vivo must arise from the distinct properties of the interneuronal axonal arborization or from a different functional pattern of excitatory and inhibitory connections with other components of the DLPFC neuronal network.

INTRODUCTION

The primate dorsolateral prefrontal cortex (DLPFC) provides the neural substrate for active maintenance of information in working memory and thus is essential to control behaviors requiring integration of information across time (Miller and Cohen 2001). The neural substrate for active maintenance is believed to be the stimulus-specific persistent firing of DLPFC neurons during the delay period of working memory tasks (Fuster 1997; Goldman-Rakic 1987). Extracellular recordings of delay-related persistent firing in the DLPFC of monkeys typically represent the activity of pyramidal cells, which significantly outnumber GABA-containing interneurons (Schwartz et al. 1988). However, recordings of single units with fast spikes, thus thought to be interneurons, also display stimulus-selective persistent activity and other forms of task-related activity with specific temporal relations to the phases of the task and to pyramidal cell firing (Constantinidis et al. 2002; Rao et al. 1999, 2000; Wang et al. 2004). Therefore DLPFC GABA neurons appear to play a critical role in working-memory processes, an interpretation consistent with findings that GABA antagonists applied to DLPFC disrupt stimulus-selective pyramidal cell firing and impair performance on working memory tasks (Rao et al. 2000; Sawaguchi et al. 1988).

Based on the duration of their extracellular spikes, the putative GABA neurons displaying task-related firing in the monkey DLPFC in vivo (Constantinidis et al. 2002; Rao et al. 1999, 2000) were equated with the fast-spiking (FS) subpopulation of GABA neurons identified in vitro via intracellular recordings in the neocortex of rats (Connors and Gutnick 1990) and primates (González-Burgos et al. 2004). However, most GABA neuron subtypes, and not only FS cells, have action potentials (APs) with a faster time course than pyramidal cell spikes (Cauli et al. 1997, 2000; Kawaguchi 1993, 1995; Kawaguchi and Kubota 1993). Thus it is important to determine if the extracellular spike duration distinguishes FS cells not only from pyramidal neurons but also from other interneuron subtypes. Given the diversity of cortical interneuron subtypes, this knowledge is essential for understanding the function of DLPFC GABA neurons in vivo.

Identifying the role of GABA-mediated signaling in monkey DLPFC also requires characterization of the functional properties of the synaptic connections made by specific classes of GABA neurons. Because putative FS interneurons possess stimulus-selective persistent activity in the monkey DLPFC in vivo, it is important to understand how persistent firing affects synaptic strength at the connections made by FS interneurons. Although certain GABAergic synapses in rat neocortex are static [i.e., synaptic strength does not change significantly during repetitive presynaptic firing at some frequencies (Gupta et al. 2000; Wang et al. 2002)], most cortical synapses are dynamic, exhibiting short-term changes in strength, either synaptic depression (decreased strength) or facilitation (increased strength), in response to repetitive presynaptic firing (Zucker and Regehr 2002). Depressing synapses may transmit information on the fluctuations of the presynaptic firing rate and thus on the temporal structure of presynaptic spike trains (Abbott et al. 1997; Thomson 1997; Tsodyks and Markram...
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1997). On the other hand, facilitating synapses may enhance the sensitivity of postsynaptic neurons to the average firing rates of the presynaptic cells (Abbott et al. 1997; Thomson 1997; Tsodyks and Markram 1997). Thus depressing and facilitating synapses enrich the computational and information-processing capabilities of cortical circuits in significantly different ways. Characterizing the synaptic dynamics of connections formed by FS GABA neurons is therefore essential to understand the role of FS neurons in controlling pyramidal cell activity during persistent neuronal firing in the primate DLPFC. Furthermore, in rat neocortex, FS GABA neurons are composed of at least two different subpopulations that can be distinguished morphologically: basket cells, which provide inhibitory input to the soma, proximal dendrites and spines of pyramidal cells, and chandelier neurons, which target the initial segments of the pyramidal cell axon (Kawaguchi 1993, 1995; Kawaguchi and Kubota 1993). Whereas previous studies showed that in rat neocortex, FS neurons elicit IPSCs with short-term depression, typically the morphology of the FS neurons was not characterized (Galarreta and Hestrin 1998). Although chandelier and basket neurons have been previously described in the monkey DLPFC (Lund and Lewis 1993; Williams et al. 1992), it is not known whether they share the same functional properties.

Proper characterization of the intrinsic properties and synaptic connections of GABA neurons exhibiting task-related firing in monkey DLPFC would require intracellular recordings combined with intracellular filling for physiological and morphological characterization. Although some studies reported intracellular recordings from interneurons in rat hippocampus and cortex in vivo (Henze et al. 2000a; Margrie et al. 2003; Nowak et al. 2003; Zhu and Connors 1999; Zhu and Zhu 2004), the feasibility of recording intracellularly from GABA neurons in the primate cortex in vivo is low, particularly for synaptically connected pairs of cells. Consequently, in this study, we used the controlled conditions provided by a living brain slice preparation to obtain whole cell voltage recordings from interneurons and pyramidal cells in layers 2/3 of the monkey DLPFC. To determine whether FS neurons can be distinguished from non-FS interneurons using extracellular spike recordings, we combined extracellular and intracellular recordings and morphological analysis of biocytin-filled cells. To determine the functional properties of the inhibitory postsynaptic potentials (IPSPs) elicited in pyramidal neurons by single, temporally isolated presynaptic spikes, we obtained simultaneous recordings from synaptically connected pairs of presynaptic FS interneurons and postsynaptic pyramidal cells. Finally, to determine whether the synaptic connections made by FS neurons onto pyramidal cells exhibit dynamic changes in strength during repetitive GABA release, we recorded IPSPs elicited by presynaptic spike trains in the FS neurons.

METHODS

Slice preparation

Brain slices were obtained from the DLPFC of young adult (3.5–6 kg; 4–5 yr old) cynomolgus monkeys (Macaca fascicularis) treated according to the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals as approved by the University of Pittsburgh Institutional Animal Care and Use Committee. The surgical procedure has been described in detail previously in studies using tissue slices from these animals (Gonzalez-Burgos et al. 2004; Urban et al. 2002). Briefly, the dura was removed in a location determined by stereotaxic coordinates and by the position of relevant sulcal landmarks, and a small block of tissue was excised containing portions of DLPFC areas 9 and 46 (Fig. 1A), including both the medial and lateral banks of the principal sulcus (Fig. 1B).

The tissue blocks were placed in an ice-cold solution containing (in mM) 210 sucrose, NaCl 10, 1.9 KCl, 1.2 NaHPO4, 33 NaHCO3, 6 MgCl2, 1 CaCl2, 10 glucose, and 2 kynurenic acid; pH 7.3–7.4 bubbled with 95% O2-5% CO2. Slices of 350- or 400-μm thickness were cut in the coronal plane and incubated at room temperature in a solution containing (in mM) 126 NaCl, 2 KCl, 1.2 NaHPO4, 10 glucose, 25 NaHCO3, 6.0 MgCl2, and 1.0 CaCl2. For recordings, slices were transferred to a submersion chamber and superfused with oxygenated ACSF [containing (in mM) 126 NaCl, 2.5 KCl, 1.2 NaHPO4, 25 NaHCO3, 2.0 CaCl2, 1.0 MgCl2, and 10 glucose] at 32–33°C.

Electrophysiology

Neurons were identified visually in layer 3 using infrared illumination and differential interference contrast optics (Fig. 1C). Tight seal (seal resistance; >5 GΩ) whole cell voltage recordings were obtained with Axoclamp-2A (Axon Instruments, Union City, CA) or BVC-700A (Dagan, Minneapolis, MN) amplifiers, operating in bridge mode and employing capacitance neutralization. Signals were low-pass filtered at 3 or 5 kHz, digitized at 10 or 20 kHz, and stored on disk for off-line analysis. Data acquisition and analysis were performed using custom-made programs written in LabView (National Instruments, Austin, TX). Patch pipettes (4–7 MΩ) were pulled from borosilicate capillary glass and filled with an internal solution containing (in mM) 120 K methylsulphate, 10 HEPES, 0.2 EGTA, 4.5 ATP, 0.3 GTP, 14 phosphocreatine, and 0.5% biocytin. The pH was adjusted to 7.2–7.3 using KOH. In nine of the experiments with synaptically connected pairs, the internal chloride concentration was increased.
close to the physiological value estimated in a recent study (Stuart 1999). The internal chloride concentration was 10 mM, with equal contributions from KCl and NaCl. In six experiments, the internal chloride concentration was 35 mM (30 mM KCl, 5 mM NaCl), and K methylsulphate was reduced to 100 mM to preserve the osmolarity at 300–310 mosM. The reversal potential of unitary IPSPs was estimated only in the connections in which the pyramidal neuron was recorded with 10 mM internal chloride. The equilibrium potential for chloride and potassium ions in these conditions were estimated to be ~66 and ~82 mV, respectively, using the Nernst equation calculated for a temperature of 32°C.

Intrinsic membrane properties were determined from the voltage responses elicited by injecting series of 500-ms current steps with amplitudes starting at ~100 pA and reaching values between 400 and 1000 pA in 10-pA increments. Input resistance was determined from the slope of a linear regression fit to the linear portion of the relation between injected current (usually between ~50 and ~10 pA) and the voltage deflection near the end of the 500-ms step. Membrane time constant was determined by fit of a single exponential to the on or off voltage response to hyperpolarizing current steps of ~10 pA. Properties of intracellular APs were measured using current steps close to threshold for each individual cell, which usually elicited either one or a few APs.

Synaptically connected pairs were identified during simultaneous recording from 2 to 4 neurons (Gonzalez-Burga et al. 2004; Urban et al. 2002). APs were evoked in the presynaptic interneurons by injecting short (3 ms) suprathreshold (1–3 nA) current steps that elicited APs with little trial-to-trial variability in latency. Once the presence of a synaptic connection was established, 50–200 single inhibitory postsynaptic potentials (IPSPs) were collected at a stimulation frequency of 0.1 or 0.05 Hz. Subsequently, trains of 10 presynaptic APs of different frequency (5, 10, 20, or 50 Hz) were evoked using identical stimulus parameters for each individual stimulus in the train and repeated between 10 and 20 times every 10 or 20 s. Timing and duration of the stimulation pulses were digitally controlled with a personal computer running custom-written Labview programs.

**Extracellular spike recording**

APs were recorded extracellularly with patch pipettes in loose cell-attached recording configuration. In these conditions, current transients recorded by the patch pipette resemble the first derivative of the membrane potential (Magee and Johnston 1995). Because neither interneurons nor pyramidal cells fire spontaneously in our experimental conditions, APs were evoked synthetically by focal extracellular stimulation applied using theta-glass pipettes with a tip diameter of 3–5 μm and filled with oxygenated extracellular solution. Chlorided silver wires were placed in the stimulation electrode to apply bipolar focal stimulation. The stimulation electrode was placed in layer 3 near the cells under study. Analysis of extracellular spike duration was performed on averages of 10–20 sweeps per cell. After averaging, extracellular spikes typically exhibited an initial downward deflection followed by a subsequent upward deflection relative to baseline (Fig. 2C). It was reported previously that the peak of the upward deflection in the extracellular spike approaches closely the return of the falling phase of the intracellular potential back to baseline (Henzl et al. 2000a). In our studies, this appeared to be true in some cases, whereas in others, the end of the positive deflection of the extracellular spike seemed to match more closely the intracellular spike duration at its onset. The end EC value (Fig. 2D) was calculated as the difference between spike end and spike onset values.

**Histological procedures**

In most experiments, biocytin (0.2–0.5%) was included in the intracellular pipette solution. After recordings were finished, slices were incubated for 5–20 min at 32–33°C and then fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline. The fixed slices were then transferred to 0.1 M Na-phosphate buffer, serially resected at 50 μm, and processed for visualization of the biotin label (Fig. 1D) using the Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA) and diaminobenzidine. Neurons were reconstructed employing the Neurolucida tracing system (MicroBrightField, Williston, VT). The Neurolucida system was employed also for the measurement of horizontal spread of the axonal arbors of chandelier and basket neurons. Putative sites of synaptic contacts were identified using an Axio Plan microscope (Zeiss, Oberkochen, Germany) equipped with differential interference contrast and a ×100 oil-immersion objective.

**IPSP data analysis**

The amplitude and kinetic parameters for single IPSPs evoked during low-frequency stimulation, were determined on traces obtained by averaging 30–50 consecutive responses, including failures. The amplitude of single IPSPs was estimated as the difference between membrane potential measured in a 2-ms window prior to the IPSP onset and that measured in a 2-ms window centered at the peak of the IPSP. IPSP decay time was estimated by fitting a monoeponential decay function.

The amplitude of summed IPSPs during repetitive stimulation was estimated, after averaging 30–50 consecutive responses including failures, as the difference between membrane potential measured in a 2-ms time window placed 5 ms before the first IPSP in the trains and the membrane potential at the peak of each IPSP in the train. To estimate the IPSP amplitude excluding IPSP summation, we measured the difference between membrane potential at the peak of each IPSP minus the potential measured immediately prior to the onset of each IPSP. This measure provides an accurate estimation of the amplitude for the first IPSP in each train. However, for subsequent IPSPs in the train, the IPSP amplitude is underestimated and therefore the magnitude of IPSP depression is overestimated. The magnitude of this error depends on the relation between IPSP rise time and decay: the faster the IPSP rise relative to decay, the smaller the error introduced. According to previous studies, this error is small and thus this measure provides a reasonable estimation of the IPSP amplitude (Gonzalez-Burgos et al. 2004; Markram 1997).

For single IPSPs, failures were defined as events in which the membrane potential measured at a 2-ms time window placed at the time of IPSP peak, yielded a value of <1.5 × the noise. Noise was defined as the value measured by a 2-ms time window prior to the IPSP onset. The failures detected in this way were confirmed by visual inspection of individual traces. The CV analysis was not performed if the amplitude of the first IPSP in the trains, which was averaged in blocks of six consecutive responses, changed >10%.

**Statistics**

The results are expressed as means ± SE, unless otherwise indicated. The statistical significance of differences between group means was tested using t-test, paired t-test and one- or two-way ANOVA followed by contrasts as stated in each case.
RESULTS

Electrophysiological and morphological properties of FS neurons

We showed recently that interneurons in the monkey DLPFC can be divided into at least two electrophysiological subclasses: FS and non-FS cells (Gonzalez-Burgos et al. 2004). FS cells are similar to the FS cell group of rat neocortex (Connors and Gutnick 1990), displaying brief APs with large afterhyperpolarization and weak or no spike frequency adaptation (Fig. 2, A and B). In contrast, non-FS cells have significantly longer AP duration and smaller afterhyperpolarization and show substantial spike frequency adaptation (Gonzalez-Burgos et al. 2004).

Putative GABA neurons recorded from the monkey DLPFC in vivo were suggested to correspond to the FS interneurons described in vitro (Rao et al. 1999). In vivo, FS cells were distinguished from pyramidal neurons based on differences in the extracellular AP duration and smaller afterhyperpolarization and show substantial spike frequency adaptation (Gonzalez-Burgos et al. 2004). However, in addition to the time course of the intracellular APs recorded at the soma, the time course of extracellular spikes is also affected by the neuronal morphology and distribution of active conductances (Henze et al. 2000a). To determine whether spike duration can distinguish FS interneuron subtypes in monkey DLPFC, we recorded spikes extracellularly from cells subsequently identified as FS or non-FS with intracellular recordings, as described previously (Gonzalez-Burgos et al. 2004). As shown in Fig. 2C, the time course of the extracellular spikes indeed resembled that of the first derivative of the intracellular APs. As reported previously (Henze et al. 2000a), quantitative analysis revealed differences between the time course of extracellular spikes and the first derivative (data not shown). Nevertheless, two different estimations of the AP duration at its base indicated that the extracellular spike duration is significantly shorter in FS cells than in non-FS cells (Fig. 2D).

To determine the morphological properties of FS neurons, cells were filled with biocytin during electrophysiological recording. Based on specific characteristics of the axonal arbor,
FS interneurons could be divided into two main morphological classes, namely basket cells and chandelier neurons (Fig. 3). Cells were classified as chandelier (Fig. 3A) if the axon had multiple cartridges or vertical arrays of synaptic boutons, which are distinctive of this cell class and reflect the exclusive site of synaptic contact onto the proximal segment of the pyramidal cell axon. The axons of chandelier neurons typically did not reach layer 1 or the deep layers and had a total horizontal spread of 233 ± 19 μm (n = 12 chandelier cells). FS neurons that did not possess axon cartridges (Fig. 3B) had the synaptic boutons more evenly distributed across compartments of the axonal arbor. Nonchandelier FS neurons where characterized as basket cells because their axonal morphology resembled closely that of local-, medium- and wide-arbor basket cells described previously in monkey DLPFC (Lund and Lewis 1993). With a few exceptions (Ali et al. 2001; Angulo et al. 2003), neocortical basket cells, including those in monkey DLPFC (Lund and Lewis 1993), do not exhibit clear pericellular baskets of axonal boutons. Nevertheless, basket cell axons make synaptic contact onto the soma and proximal dendrites but not the axon of pyramidal cells, contrasting with chandelier neurons. The axons of FS basket cells recorded in this study terminated mainly within layers 2/3, although a minority of axon branches reached layer 1 or the deep layers. The lateral spread of the basket cell axon within layers 2/3 varied significantly from cell to cell (range from 205 to 1400 μm), but was on average (508 ± 48 μm, n = 20 basket neurons) significantly larger than that of the chandelier cell axon (P < 0.01, Student’s t-test). These results suggest that both chandelier and basket neurons innervate nearby postsynaptic targets and that, in addition, basket cells synapse onto postsynaptic neurons located significantly more distant in the horizontal domain within layers 2/3.

In this study, interneurons were classified as FS if they exhibited no significant spike frequency adaptation and spike duration of <0.5 ms, characteristics typical of FS neurons of the rat hippocampus and neocortex and of monkey DLPFC (Gonzalez-Burgos et al. 2004). To determine whether chandelier and basket neurons constitute distinguishable subpopulations of FS cells, we determined several other electrophysiological properties in addition to spike frequency adaptation and spike duration. The results shown in Table 1 indicate that basic physiological properties do not differ significantly between these cell types, suggesting that cells of these two subpopulations would respond similarly to excitatory or inhibitory inputs of similar amplitude and time course.

**FIG. 3. Morphological properties of layers 2/3 FS neurons in the monkey DLPFC.** A: 3-dimensional reconstruction of a chandelier FS cell filled with biocytin during recording. Note the presence of multiple vertically oriented axon branches, which correspond to cartridges of synaptic boutons. The cartridges of labeled boutons were frequently found located next to the axon initial segment of unlabeled pyramidal cell bodies (not shown). In both A and B, dendrites and axon are shown as thick and thin traces, respectively. B: 3-dimensional reconstruction of a basket cell labeled during recording. Contrasting to chandelier neurons, the axonal arborization of these FS cells branched in a multipolar manner, showed no vertically oriented cartridges and exhibited significantly larger horizontal spread. When found near unlabeled pyramidal cells bodies, the basket cell axonal branches did not show any consistent localization relative to the proximal segments of the pyramidal cell axon (not shown).

**TABLE 1. Intrinsic membrane properties of chandelier and basket fast spiking neurons in layers 2/3 of monkey DLPFC**

<table>
<thead>
<tr>
<th>Property</th>
<th>Chandelier Cells</th>
<th>Basket Cells</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Resting membrane potential, mV</td>
<td>-66.6 ± 1.0</td>
<td>-67.6 ± 1.2</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>184 ± 22</td>
<td>179 ± 13</td>
</tr>
<tr>
<td>Time constant (ms)</td>
<td>10.7 ± 1.1</td>
<td>9.5 ± 0.8</td>
</tr>
<tr>
<td>AHP amplitude, mV</td>
<td>28.4 ± 1.6</td>
<td>30.9 ± 2.0</td>
</tr>
<tr>
<td>Spike width at half amplitude, ms</td>
<td>0.35 ± 0.02</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>Spike width at base, ms</td>
<td>0.84 ± 0.06</td>
<td>0.76 ± 0.09</td>
</tr>
<tr>
<td>Spike amplitude, mV</td>
<td>78.7 ± 2.1</td>
<td>75.3 ± 2.7</td>
</tr>
<tr>
<td>Spike V threshold, mV</td>
<td>-39.6 ± 2.2</td>
<td>-36.6 ± 6.7</td>
</tr>
<tr>
<td>Spike I threshold, pA</td>
<td>100 ± 14</td>
<td>160 ± 23*</td>
</tr>
<tr>
<td>ISI ratio</td>
<td>1.10 ± 0.04</td>
<td>1.09 ± 0.05</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE. DLPFC, dorsolateral prefrontal cortex; AHP, afterhyperpolarization; spike V threshold, voltage threshold for spiking; spike I threshold, current threshold for spiking; ISI ratio: ratio between last and first interspike interval. *, statistically significant difference between means, P < 0.05. Student’s t-test.
The data reported in this study were obtained after recording from 15 synaptically connected pairs of presynaptic FS cells and postsynaptic pyramidal neurons. In 30 simultaneous recordings from FS interneurons and pyramidal cells, the probability of finding an inhibitory connection (0.40) was approximately four times higher than the probability to find an excitatory connection (0.10). As in our previous studies (Gonzalez-Burgos et al. 2004; Henze et al. 2000b; Urban et al. 2002), pyramidal cells of DLPFC layers 2/3 exhibited regular spiking but not burst spiking firing patterns. Thus all of the synaptic connections herein studied were between FS interneurons and regular spiking pyramidal cells.

Figure 4A illustrates unitary IPSPs recorded in pyramidal cells after eliciting APs in presynaptic FS neurons by somatic injection of suprathreshold current steps. The unitary IPSP latency was short (latency between presynaptic AP peak and 10% of IPSP peak was: 0.71 ± 0.09 ms; range: 0.21–1.24 ms) and had small variation across trials (Fig. 4B). The IPSP 10–90% rise time was rapid and displayed relatively small variability across synaptic connections (1.78 ± 0.26 ms, range: 0.57 to 3.62 ms). The IPSP decay phase could be well fit with a monoexponential decay function (time constant of decay at a resting potential of –68.8 ± 2.4 mV was 31.7 ± 8.4 ms, and exhibited a voltage-dependent increase at depolarized potentials, such that the depolarizing IPSPs recorded at hyperpolarized potentials decayed faster than the hyperpolarizing responses (the time constant of IPSP decay was 37.4 ± 11.7 ms for IPSPs recorded at –80 to –75 mV and 112.3 ± 17.2 ms for IPSPs recorded at –53 to –45 mV, n = 5; P < 0.05 Student’s t-test). In contrast to the decay time, the 10–90% rise time exhibited no significant changes with membrane potential (rise time was 1.86 ± 0.24 ms at –80 to –75 mV and 2.29 ± 0.28 ms, at –53 to –45 mV, n = 5; P = 0.55, Student’s t-test). This increase in the IPSP decay time with depolarization is probably due to the activation of voltage-dependent conductances located at or near the soma, which were previously shown to shape the time course of both IPSPs and EPSPs in somatosensory (Stuart 1999; Stuart and Sakmann 1995) and prefrontal cortex pyramidal neurons (Gonzalez-Burgos and Barrionuevo 2001; Gonzalez-Burgos et al. 2004).

The unitary IPSPs typically had small amplitudes when recorded at the pyramidal cell resting potential (–68.8 ± 2.4 mV) with intracellular concentrations of chloride near physiological values (Stuart 1999). Unitary IPSP amplitude varied significantly with the pyramidal cell membrane potential (–0.44 ± 0.08 mV at –53 to –45 mV and 0.38 ± 0.06 mV at –80 to –75 mV, n = 9), reversing from depolarizing to hyperpolarizing at potentials near the equilibrium potential for chloride, but distinct from the equilibrium potential for potassium (Fig. 4C). In three of three synaptic connections tested, the IPSPs were blocked after applying 20 μM of the GABA_A receptor antagonist bicuculline for 3 min (Fig. 4D). Together, the fast IPSP time course, the IPSP reversal near the equilibrium potential for chloride, and the pharmacological data suggest that the response of pyramidal neurons to GABA release from FS cells is mediated by activation of GABA_A receptor-gated chloride channels.

The low signal-to-noise ratio attained with tight seal whole cell recordings allowed the reliable distinction of failures from successes of transmission, in 11 of the 15 recorded synaptic connections. For APs evoked in the presynaptic FS cells at frequencies of 0.1–0.2 Hz, transmission between FS and regular spiking (RS) pyramidal neurons was highly reliable (Fig. 4B). The mean failure rate and the maximum rate of failures were both low (4.5 ± 1.4 and 8.7%, respectively), and 3 of the 11 connections (27%) exhibited a failure rate of 0%.

In 10 of the 15 synaptically connected pairs, the observed morphological features provided sufficient detail to identify the FS interneurons as basket (n = 8) or chandelier cells (n = 2). Examples of the morphological properties of neurons in synaptically coupled pairs of FS interneurons and RS pyramidal.
cells are shown in Fig. 5. In experiments in which both the pre- and the postsynaptic neurons were labeled, using light microscopy we identified putative appositions between synaptic boutons of the FS cell axon and the postsynaptic cell membrane. Putative appositions made by the axon of chandelier neurons were associated exclusively with the axon initial segment of the postsynaptic pyramidal cell, where a typical cartridge of presumed synaptic boutons was observed (Fig. 5A). In contrast, appositions between the varicosities of the basket cell axon and the pyramidal cell membrane were typically found at the soma or proximal dendrites of the postsynaptic neuron (Fig. 5B) and in no case close to the pyramidal cell axon initial segment. In all the connected pairs in which appositions could be identified, our observations suggested the presence of multiple synaptic contacts.

Effects of repetitive presynaptic firing at connections between FS cells and postsynaptic pyramidal neurons

To determine the effects of repetitive presynaptic firing on the properties of IPSPs, in 10 of the 15 synaptic connections IPSPs were recorded during stimulation of the presynaptic FS neuron with trains of suprathreshold current steps. As illustrated in Fig. 6A, repetitive presynaptic firing elicited depression of the unitary IPSPs in all of the connections tested. Stimulation at a frequency of 10 Hz produced significant IPSP depression, and the rate of depression increased with stimulation frequency. As illustrated in Fig. 6B, at a stimulation frequency >10 Hz, IPSP summation contributed significantly to the changes in postsynaptic membrane potential during the presynaptic spike trains. Nevertheless, all of the postsynaptic responses elicited by the summed IPSPs were significantly depressed compared with the first response in the trains at all stimulation frequencies tested (Fig. 6B). Thus temporal summation did not counteract significantly the effects of IPSP depression, contrasting with summation of EPSPs in DLPFC pyramidal cells (Gonzalez-Burgos et al. 2004).

Previous studies of synaptic connections in rat neocortex have shown that short spike trains elicit short-term changes in postsynaptic response amplitude. In monkey DLPFC, the IPSP depression elicited by stimulus trains in the presynaptic FS cells was also short-termed, reversing completely between consecutive applications of the trains (data not shown). Additional experiments in which single stimuli were delivered to the FS cells at different intervals after a spike train revealed that IPSPs recovered from depression within <1.5 s (Fig. 6C). These data show that there was no interaction, and thus no accumulation of depression, between stimulus trains.

FIG. 5. Morphological properties of synaptically connected pairs of FS interneurons and pyramidal cells. A, left: photomicrograph of a synaptically connected pair of a presynaptic chandelier cell and a postsynaptic pyramidal neuron. The cells were filled with biocytin during the electrophysiological recording. The pyramidal cell and interneuronal axons are marked by the red and black arrows, respectively. Right: partial reconstruction of the same cell pair. Dendrites and axons are shown in thick and thin traces, respectively. The pyramidal cell is shown in red and the chandelier neuron in black. Note that the chandelier cell axon is apposed to the pyramidal cell axon initial segment and appears to establish multiple synaptic contacts in this region. In both figures, the location of the putative synaptic contacts, as identified by differential interference contrast microscopy, is shown by the small arrows and circles. No putative appositions were observed at the proximal dendrites or soma of the pyramidal cell. The putative synapses formed a vertical arrangement or cartridge, typical of chandelier neuron axons. B, left: photomicrograph of a synaptically connected pair of a presynaptic basket cell and a postsynaptic pyramidal neuron filled with biocytin during recording. Note that the basket cell axon (black arrow) was found in close proximity to the pyramidal cell axon initial segment (red arrow), but no putative appositions were observed in this region. Right: 3-dimensional reconstruction of the same cell pair. Dendrites and axons are shown in thick and thin traces, respectively. The pyramidal cell is shown in red and the basket neuron in black. One putative synaptic contact, indicated by the small arrow and circle, was observed at a proximal basal dendrite. Other putative synaptic contacts (not shown) were identified by light microscopy, but none of them was located at the pyramidal cell axon initial segment. Despite close proximity of the two axons, no evidence putative appositions was found.
Several lines of evidence suggested that, similarly to single IPSPs, the pyramidal cell response during presynaptic FS cell spike trains was primarily mediated by GABA<sub>A</sub> receptor channels. First, IPSP trains reverted from depolarizing to hyperpolarizing at membrane potentials depolarized to the equilibrium potential for chloride (Fig. 6D). Second, in contrast to the purely GABA<sub>A</sub>-mediated single IPSPs, repetitive GABA release during spike trains could produce significant activation of GABA<sub>B</sub> receptors during late portions of the trains. Given a potassium equilibrium potential of about −82 mV, GABA<sub>B</sub> receptor-activated K<sup>+</sup> currents were predicted to be hyperpolarizing at both the depolarized (−50.4 ± 1.4 mV) and hyperpolarized (−70.8 ± 2.8 mV) somatic membrane potentials at which IPSP trains were recorded. Thus any hyperpolarization contributed by GABA<sub>B</sub> receptors would affect the hyperpolarizing and depolarizing IPSPs in opposite manners, yielding different apparent levels of depression after summation of the GABA<sub>A</sub>- and GABA<sub>B</sub>-mediated potentials by the end of the trains. In contrast to this prediction, we observed a similar degree of depression for hyperpolarizing and depolarizing IPSPs [the ratio IPSP (10)/IPSP (1) in 20 Hz trains was 0.34 ± 0.04 and 0.38 ± 0.11, n = 3, for depolarizing and hyperpolarizing IPSPs, respectively]. Finally, in 2/2 connections tested, bath application of the GABA<sub>A</sub> antagonist bicuculline (20 μM) during repetitive FS cell stimulation abolished completely not only the first IPSP but also the remaining IPSPs during trains (Fig. 6E).

If IPSP depression involves presynaptic mechanisms, then it may be associated with an increase in the IPSP failure rate. We tested this possibility in five connected pairs (3 presynaptic basket cells, 1 presynaptic chandelier neuron, and 1 unidentified FS cell) in which failures were reliably distinguished from success of transmission during 20-Hz trains (Fig. 7, A and B). This analysis revealed that the failure rate increased between the first and second IPSPs in the train (Fig. 7C) in a manner consistent with a contribution of presynaptic mechanisms to IPSP depression. Further evidence for a presynaptic contribution to IPSP depression was obtained from an analysis of the coefficient of variation (CV) of IPSPs. We assumed that, at synapses made by FS neurons, GABA release is quantal and follows a binomial distribution with a single release site per synaptic contact. An analysis of the changes in the coefficient of variation and in IPSP amplitude during trains revealed a greater proportional change in CV than in mean IPSP (Fig. 7D), suggesting a significant contribution of presynaptic mechanisms to IPSP depression (Clements 1990; Faber and Korn 1991) as reported also for GABAergic synapses in hippocampus and neocortex (Ali et al. 2001; Hefft et al. 2002; Kraushaar and Jonas 2000). In addition, we determined whether or not the properties of the IPSPs were consistent with the case of synaptic connections that involve multiple synaptic contacts, as suggested by our qualitative morphological assessment (Fig. 5). If these connections would involve a single release site, then IPSP depression during the trains should be associated with an increase in failure rate but with no change in the average success amplitude. In contrast to this prediction, the average success amplitude was significantly smaller for the second than for the first IPSP in the trains (Fig. 7E), displaying a paired pulse ratio [IPSP (2)/IPSP (1)] of 0.64 ± 0.10.

We identified the morphological subclass of FS neuron in 8 of the 10 connected pairs tested with repetitive stimulation of the presynaptic interneuron and found that connections made by both chandelier (n = 2) and basket neurons (n = 6) exhibited IPSP depression. Table 2 summarizes the functional properties of the synaptic connections received by pyramidal cells from basket neurons, chandelier neurons, or FS interneurons for which the morphological subclass could not be correctly identified. The data suggest that synaptic connections
made by FS neurons have qualitatively similar properties, independently of the morphological subtype.

DISCUSSION

Characterization of interneuron subtypes based on extracellular and intracellular recordings

Recent studies suggest that FS interneurons in the monkey DLPFC display task-related firing during delayed response tasks (Constantinidis et al. 2002; Rao et al. 1999, 2000; Wang et al. 2004; Wilson et al. 1994). The putative interneurons recorded in vivo were classified as FS by virtue of having spikes with shorter duration than pyramidal cells. However, we previously showed that intracellularly recorded spikes are shorter in either FS or non-FS cells, compared with pyramidal neurons (Gonzalez-Burgos et al. 2004). Here, by combining extracellular and intracellular recordings from single neurons, we found that extracellular recordings can distinguish, as a population, FS cells from non-FS interneurons. The extracellular spike duration measured between spike onset and the peak of the upward deflection (see Fig. 2) was 0.50 ± 0.11 ms, 0.84 ± 0.06 ms and 1.12 ± 0.14 ms for FS, non-FS and pyramidal neurons, respectively. These data suggest that the duration of extracellular spikes recorded in vivo would be distributed in at least three cell populations with short, intermediate and long values, respectively. However, there is a substantial overlap between the distributions of spike width in FS and non-FS interneurons (this study, and see Gonzalez-Burgos et al. 2004). In addition, the baseline firing rate can distinguish interneurons from pyramidal cells, but not interneuron subclasses (Wang et al. 2004). Therefore we suggest that with the types of extracellular recordings utilized in vivo, the proper classification of many individual neurons would be difficult.

Previous morphological studies had identified chandelier and basket-like interneurons in the monkey DLPFC and shown that they contain the calcium-binding protein parvalbumin (Lund and Lewis 1993; Williams et al. 1992), but their physiological membrane properties had not previously been compared. In this study, we found that the electrophysiological properties of chandelier and basket-like interneurons were very similar and typical of parvalbumin-positive FS cells. In particular, because AP duration did not differ between chandelier and basket neurons (Table 1), our data clearly indicate that these two cell types cannot be distinguished based on the duration of extracellular spikes recorded in vivo. In the rat hippocampus, basket cells and chandelier neurons also have similar FS properties in vitro but display cell-type specific firing patterns in vivo (Klausberger et al. 2003). These results suggest that chandelier and basket cells may have different patterns of functional connectivity with other elements of the neuronal network.

In addition to the basket-like FS interneurons described here, basket neuron subtypes with physiological properties different from those of chandelier cells may also exist in the DLPFC of primates. For example, in both rat neocortex and hippocampus, a subpopulation of parvalbumin-immunoreactive basket cells, which display spike-frequency adaptation and contain cholecystokinin, has been described (Dantzker and Callaway 2000; Karube et al. 2004; Kawaguchi and Kondo 2002; Pawelzik et al. 2002). Cholecystokinin-positive basket-like neurons also are present in monkey DLPFC (Lund and Lewis 1993), but their electrophysiological properties currently are not known.

<table>
<thead>
<tr>
<th>hIPSP Amplitude, mV</th>
<th>dIPSP Amplitude, mV</th>
<th>Latency, ms</th>
<th>Rise time, ms</th>
<th>hIPSP decay constant, ms</th>
<th>dIPSP decay constant, ms</th>
<th>Failure rate</th>
<th>CV</th>
<th>IPS Decay%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50 ± 0.15</td>
<td>0.39 ± 0.10</td>
<td>0.66 ± 0.09</td>
<td>1.83 ± 0.52</td>
<td>42.9 ± 19.1</td>
<td>103.5 ± 17.8</td>
<td>6.9 ± 2.2</td>
<td>0.60 ± 0.08</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>0.38*</td>
<td>0.63*</td>
<td>0.88 ± 0.36</td>
<td>0.95 ± 0.23</td>
<td>31*</td>
<td>167*</td>
<td>0†</td>
<td>0.41 ± 0.10</td>
<td>0.29 ± 0.06</td>
</tr>
<tr>
<td>0.34 ± 0.05</td>
<td>0.30 ± 0.05</td>
<td>0.68 ± 0.15</td>
<td>1.97 ± 0.33</td>
<td>24.3 ± 13.1</td>
<td>3.7 ± 1.9</td>
<td>0.55 ± 0.12</td>
<td>0.24 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>

Data are shown as means ± SE. hIPSP, hyperpolarizing IPSP; dIPSP, depolarizing IPSP; CV, coefficient of variation. *: for these individual neurons, only hIPSPs or dIPSPs were recorded. †: no failures were detected in chandelier neuron connections. #: Depression was measured as the ratio between IPSP(10) and IPSP(1) amplitudes in 20 Hz trains.
Functional properties of IPSPs in synaptic connections between FS cells and pyramidal neurons

We found that IPSPs mediated by FS neurons had characteristics typical of responses primarily mediated by perisomatic synapses and GABA_A receptors. Although chandelier and basket-like neurons are morphologically distinct classes of FS cells, the functional properties of single IPSPs were qualitatively and quantitatively similar between these cell types. The small amplitude of the IPSPs at potentials near rest prevented us from accurately measuring and comparing the magnitude of the GABA_A conductance activated by the different cell types. Independent of the magnitude of the GABA conductance, chandelier cell inputs are thought to be more powerful to inhibit pyramidal cell firing than basket cell inputs because axon cartridges make contact near the AP initiation site. However, this hypothesis must be specifically tested because the AP initiation site and the soma may be electrically and biochemically well-coupled, thus acting as a single functional compartment.

Independent of the biophysical properties of the synaptic connections, important functional differences between subclasses of FS cells may arise from the spatial innervation pattern determined by morphological properties of the axonal arbor (Karube et al. 2004). In monkey DLPFC, we found that the basket cell axons had a significantly larger horizontal spread, suggesting that these cells are better fit to mediate lateral inhibition than chandelier neurons. Overall, basket cells and chandelier neurons have different target selectivity because chandelier neurons target only pyramidal cells (Peters 1984), whereas FS basket cells synapse both onto pyramidal cells and other FS neurons. In rat neocortex, FS basket neurons are highly interconnected by chemical and electrical synapses (Whittington and Traub 2003). This network of interconnected FS cells is involved in the generation and maintenance of synchronous rhythmic activity for which both the electrical and chemical connections between FS neurons are strictly necessary (Whittington and Traub 2003). Therefore the wider spread of the basket cell axon may be related to the role of these neurons in the generation and maintenance of synchronous rhythmic activity across a wide cortical field via long-distance connections with large numbers of other FS neurons.

Our finding that IPSPs elicited by FS interneurons in nearby pyramidal cells are mediated by GABA_A receptor-gated chloride channels indicates that the behavioral (Sawaguchi and Iba 2001; Sawaguchi et al. 1988) and electrophysiological (Rao et al. 2000; Sawaguchi 2001) effects of pharmacological manipulations of GABA_A receptors in monkey DLPFC in vivo are mediated at least in part by actions on FS cell-mediated GABAergic transmission. During recordings with near physiological intracellular concentrations of chloride (Stuart 1999), IPSPs elicited by FS cells reversed from depolarizing to hyperpolarizing at potentials near the equilibrium for chloride (−66 mV) and thus had small depolarizing amplitude at the average resting potential (−68.8 ± 2.4 mV) of DLPFC pyramidal cells in vitro. Fluctuations in pyramidal cell membrane potential in vivo would increase the computational complexity within the PFC network because IPSP polarity may change during prolonged FS cell spike trains. In vivo, the membrane potential of pyramidal neurons of the rat PFC and other cortical regions, periodically shifts between a depolarized (up) and a hyperpolarized (down) state, at a rate of ~1 Hz (Lewis and O’Donnell 2000). Membrane potentials at the down and up states have typical mean values of −80 to −70 and −60 to −55 mV, respectively (Lewis and O’Donnell 2000; Peters et al. 2004; Petersen et al. 2003). Thus FS-to-pyramidal cell connections would be hyperpolarizing during the up states and slightly depolarizing during the down state. Independent of IPSP polarity, activation of perisomatic GABA_A receptors causes inhibition during the duration of the increase in GABA_A conductance. Outside this time window, depolarizing IPSPs are excitatory and hyperpolarizing IPSPs are inhibitory (Gulledge and Stuart 2003). Our data thus suggest that IPSPs elicited by FS cells may have an inhibitory effect during cortical up states, whereas the effects during the down states may depend on the particular temporal relation with glutamatergic inputs (Gulledge and Stuart 2003). Up and down states occur in the neocortex of both anesthetized and awake rats (Petersen et al. 2003). Interestingly, the up states appear to result from the combined activation of inhibitory and excitatory conductance (McCormick et al. 2003). Moreover, blockade of GABA_A receptors transforms the up states into paroxysmal discharges, suggesting that GABA_A-mediated IPSPs are critical for the maintenance of stable up states (Sanchez-Vives and McCormick 2000). Whether these state transitions are found in primate neocortex and, if so, whether they contribute to natural firing patterns during working memory, remains to be investigated.

Synaptic dynamics of fast spiking interneuron inputs onto pyramidal cells

Based on the properties of temporal summation of EPSPs, we proposed previously that recruitment of FS neurons in DLPFC circuits could provide feedback inhibition to the pyramidal cell network during persistent delay-related firing (Gonzalez-Burgos et al. 2004). The significant synaptic depression at FS to pyramidal cell connections suggests that the efficacy of FS cell-mediated IPSPs may decrease progressively after several seconds of persistent firing. However, due to the irregular nature of task-related firing of DLPFC neurons in vivo and to the rapid recovery from synaptic depression, facilitatory synapses that show significant depression during constant frequency stimulus trains still can sustain transmission throughout the delay period of working-memory tasks (Gonzalez-Burgos et al. 2004). Whether FS cell-mediated transmission is also sustained during presynaptic stimulation with natural spike trains remains to be determined. Nevertheless, it seems likely that feedback inhibition is mediated by non-FS interneuron subtypes eliciting peridendritic IPSPs with frequency-dependent facilitation (Beierlein et al. 2003; Pouille and Scanziani 2004). Facilitating IPSPs may be more efficient to balance the local excitation and thus control the average firing rate of pyramidal cells during persistent delay-related firing.

The finding of synaptic depression at connections made by FS neurons in monkey DLPFC suggests that these synapses may be more likely to signal information on the temporal structure of spike trains than on the average firing rate of FS neurons (Abbott et al. 1997; Thomson 1997; Tsodyks and Markram 1997). In DLPFC, for both pyramidal cells and interneurons the variability of interspike intervals increases
during the mnemonic period of delayed-response tasks (Compte et al. 2003). Such an increase in spiking variability is consistent with a model in which the temporal structure of spike trains conveys information about task-related events, and this information is efficiently coded by depressing synapses. Thus a further understanding of the physiological role of synaptic depression in FC cell-mediated IPSPs would require the identification of the excitatory inputs that drive FC neuron spike trains during working-memory tasks. In a previous study, we found that unitary EPSPs elicited in FC neurons by nearby pyramidal cells display frequency-dependent depression (Gonzalez-Burgos et al. 2004). Moreover, general activation of monosynaptic excitatory inputs by focal extracellular stimulation also elicited depressing EPSPs in FC cells (Gonzalez-Burgos et al. 2004). The presence of synaptic depression at both synaptic inputs and output of FC neurons suggests that in the DLPFC, FS cells may be able to transform a temporal code conveyed by excitatory inputs into a GABA-mediated inhibitory signal with equally precise temporal characteristics.

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