Functional properties of GABA synaptic inputs onto GABA neurons in monkey prefrontal cortex

Diana C. Rotaru,1 Cameron Olezene,2 Takeaki Miyamae,2 Nadezhda V. Povyshева,3 Aleksey V. Zaitsev,4 David A. Lewis,2 and Guillermo Gonzalez-Burgos2

1Department of Neuroscience, Erasmus Medical Center, Rotterdam, The Netherlands; 2Translational Neuroscience Program, Department of Psychiatry, University of Pittsburgh, Pittsburgh, Pennsylvania; 3Department of Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania; and 4Scheunef Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

Submitted 9 October 2014; accepted in final form 23 December 2014

In cortical networks, GABA neurons are driven by glutamatergic excitatory inputs and critically regulate pyramidal cell activity via synaptic inhibition (Isaacson and Scanziani 2011). Recent studies have highlighted that in the rodent neocortex and hippocampus GABA neuron activity is also controlled by GABA_A receptor (GABA_A)-mediated synapses are a significant source of input onto GABA neurons, and the properties of these inputs vary among GABA neuron subtypes that differ in molecular markers and firing patterns. Some features of cortical interneurons are different between rodents and primates, but it is not known whether inhibition of GABA neurons is prominent in the primate cortex and, if so, whether these inputs show heterogeneity across GABA neuron subtypes. We thus studied GABA_A-R-mediated miniature synaptic events in GABAergic interneurons in layer 3 of monkey dorsolateral prefrontal cortex (DLPFC). Interneurons were identified on the basis of their firing pattern as fast spiking (FS), regular spiking (RS), burst spiking (BS), or irregular spiking (IS). Miniature synaptic events were common in all of the recorded interneurons, and the frequency of these events was highest in FS neurons. The amplitude and kinetics of miniature inhibitory postsynaptic potentials (mIPSPs) also differed between DLPFC interneuron subtypes in a manner correlated with their input resistance and membrane time constant. FS neurons had the fastest mIPSP decay times and the strongest effects of the GABA_A modulator zolpidem, suggesting that the distinctive properties of inhibitory synaptic inputs onto FS cells are in part conferred by GABA_ARs containing α1 subunits. Moreover, mIPSCs differed between FS and RS interneurons in a manner consistent with the mIPSP findings. These results show that in the monkey DLPFC GABA_A-R-mediated synaptic inputs are prominent in layer 3 interneurons and may differentially regulate the activity of different interneuron subtypes.

The macaque monkey DLPFC was identified by Brodmann in 1913 (Elston and Garey 2004) as a cortical region that lacks a clear analog or homolog in rodents or in many other groups of mammals (Preuss 1995). We previously identified at least eight different GABA neuron subtypes in layer 3 microcircuits of the monkey DLPFC (Gonzalez-Burgos et al. 2004, 2005; Krimser et al. 2005; Povyshева et al. 2007, 2008, 2013; Zaitsev et al. 2009). Monkey DLPFC layer 3 GABA neurons are divided into fast-spiking (FS) and non-FS groups (Krimser et al. 2005; Zaitsev et al. 2005, 2009). As in rodent cortex, in response to sustained excitatory currents FS interneurons of the monkey DLPFC layer 3 fire at a nearly constant frequency, whereas non-FS neurons exhibit spike frequency adaptation. The FS and non-FS groups of DLPFC interneurons are subdi-
vided into subgroups on the basis of their axonal morphology and expression of molecular markers (Zaitsev et al. 2005, 2009).

Interestingly, in rodents, the properties of inhibitory synaptic inputs onto cortical GABA neurons differ between interneuron classes distinguished by their firing patterns (Bacci et al. 2003; Pfeffer et al. 2013; Savanthrapadian et al. 2014). The relation between interneuron firing pattern and properties of inhibitory inputs, if conserved at least in part from rodents to primate neocortex, may be important for the roles that distinct GABA neuron subtypes are thought to play in shaping network activity in monkey DLPFC (Konstantoudaki et al. 2014; Wang et al. 2004). However, it is not known whether inhibition of GABA neurons is as prominent in the monkey DLPFC as in the cortex of other mammals and, if so, whether the features of inhibitory inputs vary between GABA neuron classes. Here we demonstrated that inhibitory inputs are prominent in interneurons from layer 3 of monkey DLPFC and characterized the properties of GABA<A> R-mediated synaptic responses in interneuron subtypes classified via their intrinsic membrane properties.

**METHODS**

**Brain slice preparation.** Electrophysiological recordings were performed in brain slice tissue obtained from three adult macaque monkeys: one female (84 mo old) rhesus monkey (*Macaca mulatta*) and two male (42 and 60 mo old) long-tailed macaque monkeys (*Macaca fascicularis*) supplied by the University of Pittsburgh Primate Research Center. Housing and experimental procedures were conducted in accordance with US Department of Agriculture and National Institutes of Health guidelines and with approval of the University of Pittsburgh’s Institutional Animal Care and Use Committee. All animals were experimentally naïve at the time of entry into this study.

Tissue blocks containing portions of areas 9 and 46 were obtained from areas 9 and 46 of the DLPFC were studied from each hemisphere. For removal of the second block, the animals were deeply anesthetized and perfused transcardially with a cold artificial cerebrospinal fluid (ACSF) solution of the following composition (in mM): 210.0 sucrose, 10.0 NaCl, 1.9 KCl, 1.2 NaHPO<sub>4</sub>, 33.0 NaHCO<sub>3</sub>, 6.0 MgCl<sub>2</sub>, 1.0 CaCl<sub>2</sub>, 10.0 glucose, and 2.0 kynurenic acid, pH 7.3–7.4 when bubbled with 95% O<sub>2</sub>–5% CO<sub>2</sub>. Slices (300–350 μm thick) containing areas 9 and 46 of the DLPFC were cut in the coronal plane with a vibrating microtome (VT1000S, Leica Microsystems, Nussloch, Germany) in ice-cold ACSF. Immediately after cutting, slices were transferred to an incubation chamber maintained at room temperature and filled with a solution containing, in mM, 126.0 NaCl, 2.0 KCl, 1.2 NaHPO<sub>4</sub>, 10.0 glucose, 2.0 CaCl<sub>2</sub>, 1.0 MgCl<sub>2</sub>, 0.02 CNQX, and 0.1 D,L-AP5, bubbled with 95% O<sub>2</sub>–5% CO<sub>2</sub> and maintained at 30–32°C. Whole cell recordings were obtained from layer 3 nonpyramidal neurons visually identified with infrared differential interference contrast video microscopy (Olympus BX51 and BX61 or Zeiss FS Axioskop microscopes). Pipettes pulled from borosilicate glass had a resistance of 3–5 MΩ when filled with a solution containing, in mM, 120.0 KCl, 1.0 NaCl, 0.2 EGTA, 10.0 HEPES, 4.0 Mg<sub>2</sub>ATP, 0.3 Na<sub>2</sub>GTP, and 14.0 Na<sub>2</sub> phosphate, with 0.5% biocytin (pH adjusted to 7.2–7.3). Assuming an intracellular HCO<sub>3</sub><sup>-</sup> concentration of 15 mM (Farrant and Kaila 2007) and a permeability ratio PHCO<sub>3</sub><sup>-</sup>/ PCI ≈ 0.3 for GABA<sub>A</sub> channels (Farrant and Kaila 2007) and using the Goldman-Hodgkin-Katz equation, we estimated a GABA<sub>A</sub> reversal potential (E<sub>GABA</sub>) near zero (~0.66 mV). Miniature inhibitory postsynaptic potentials (mIPSPs) or currents (mIPSCs) were recorded at a potential of ~70 mV in the presence of tetrodotoxin (TTX, 1 μM), CNQX (20 μM), and D,L-AP5 (100 μM) applied to the bath solution. Recordings were performed with Multiclamp 200A or effulgentially 200B amplifiers (Axon Instruments, Union City, CA) operating in current-clamp (bridge) or voltage-clamp mode. Signals were low-pass filtered at 4 kHz, digitized at 10 or 20 kHz, and stored on disk for off-line analysis. Data acquisition was performed with Power 1401 data acquisition interface boards and Signal 3 software (Cambridge Electronic Design, Cambridge, UK). Throughout the experiments, the series resistance was monitored; if it exceeded 30 MΩ, recordings were excluded from data analysis.

To characterize the intrinsic membrane properties of neurons, rectangular hyper- and depolarizing current pulses of 500-ms duration were applied in 10-pA increments at 0.2 Hz with two repetitions. The membrane time constant was determined by fitting a single exponential to the average voltage response to hyperpolarizing current steps of –10 to –30 pA. The input resistance was estimated from the slope of the linear portion of voltage-current plots (usually between –50 and –10 pA), built measuring the amplitude of the voltage deflection near the end of the current step. Single action potential properties, including threshold, duration at half-maximal amplitude, and amplitude and size of the afterhyperpolarization (AHP), were measured from the response to current steps close to the threshold of firing for each individual cell, which usually elicited either one or a few action potentials. Spike frequency adaptation ratio was measured as the ratio between the last and the first interspike interval, measured with depolarizing current steps of 50–100 pA above the threshold of firing.

**Analysis of miniature IPSPs and IPSCs.** For each neuron, we first assessed membrane properties in current clamp and then added TTX (1 μM) to study miniature synaptic events. In most neurons sampled [24 FS, 12 regular-spiking (RS), 6 burst-spiking (BS), and 6 irregular-spiking (IS) cells], we recorded mIPSPs. The effects of zolpidem on mIPSPs were tested in a subsample of these cells (14 FS, 7 RS, and 3 BS cells), as reported in Fig. 6. In a second neuron sample (10 FS and 12 RS cells), after addition of TTX the recording mode was switched to voltage clamp, and mIPSCs were studied. Table 1 reports the membrane properties of all neurons for which either mIPSPs or mIPSCs were recorded. We used Mini Analysis (Synaptosoft, Decatur, GA) to detect mIPSPs and mIPSCs. At least 200 nonoverlapping events were included to automatically generate an average event for each cell. The decay time was estimated by the weighted time constant obtained from fitting a double-exponential function to the 10–90% decay phase. The effects of zolpidem were analyzed during the last 5 min of a 15-min zolpidem application. For analysis of miniature event frequency, each data file was reanalyzed to include both nonoverlapping events.

**Morphological analysis.** Interneurons were filled with 0.5% biocytin during recording, and after recording the slices were fixed in 4% paraformaldehyde and stored in an antifreeze solution (1:1, glycerol–80°C until being

**J Neurophysiol** • doi:10.1152/jn.00799.2014 • www.jn.org

Downloaded from http://jn.physiology.org/ by 10.230.32.2 on October 25, 2016
Table 1. Summary of electrophysiological intrinsic membrane properties of subclasses of interneurons in layer 3 of monkey DLPFC

<table>
<thead>
<tr>
<th>Cell Class</th>
<th>Time Constant, ms</th>
<th>Input Resistance, MΩ</th>
<th>Spike Duration, ms</th>
<th>Adaptation Ratio (last ISI/first ISI)</th>
<th>Spike Threshold, mV</th>
<th>AHP Amplitude, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>FS (n = 34)</td>
<td>9.21 ± 0.62a</td>
<td>155 ± 16a</td>
<td>0.45 ± 0.01a</td>
<td>1.07 ± 0.02a</td>
<td>−50.5 ± 1.0a</td>
<td>18.9 ± 1.1a</td>
</tr>
<tr>
<td>RS (n = 24)</td>
<td>22.40 ± 1.48b</td>
<td>318 ± 32b</td>
<td>0.84 ± 0.05b</td>
<td>2.03 ± 0.13b</td>
<td>−51.8 ± 1.9b</td>
<td>12.0 ± 0.9b</td>
</tr>
<tr>
<td>BS (n = 6)</td>
<td>22.51 ± 5.49b</td>
<td>292 ± 26b</td>
<td>1.09 ± 0.11b</td>
<td>4.17 ± 0.87b</td>
<td>−55.6 ± 1.4b</td>
<td>5.7 ± 1.5b</td>
</tr>
<tr>
<td>IS (n = 6)</td>
<td>14.3 ± 1.6ab</td>
<td>288 ± 72b</td>
<td>0.66 ± 0.11b</td>
<td>3.23 ± 0.69b</td>
<td>−53.5 ± 1.6b</td>
<td>12.9 ± 2.4b</td>
</tr>
</tbody>
</table>

Except for spike threshold, all other electrophysiological properties showed significant differences between group means, as determined by single-factor ANOVA. In each column, groups labeled with a different letter are significantly different, as determined by Fisher least significant difference (LSD) comparisons (P < 0.05). DLPFC, dorsolateral prefrontal cortex; FS, fast spiking; RS, regular spiking; BS, burst spiking; IS, irregular spiking; ISI, interspike interval; AHP, afterhyperpolarization.

**RESULTS**

Electrophysiological properties of recorded interneurons.
To study GABA<sub>A</sub>R-mediated synaptic inputs onto layer 3 interneurons, the recorded cells were grouped on the basis of their intrinsic electrophysiological properties (Fig. 1A). We measured action potential duration and spike frequency adaptation via an adaptation ratio (Fig. 1B). Using cluster analysis of electrophysiological parameters, we previously divided the monkey DLPFC layer 3 interneurons into two main groups: FS and non-FS cells (Krimer et al. 2005; Zaitsev et al. 2005). Here, cells with spike duration < 0.6 ms and adaptation ratio < 1.25 (Fig. 1B) were classified as FS interneurons (n = 34). All other interneurons (n = 36) were classified as non-FS cells.

Whereas FS neurons had homogeneous firing properties (Fig. 1), non-FS cells had heterogeneous electrophysiological properties and were divided into three subtypes (Fig. 1): regular spiking (RS), with progressive spike frequency adaptation; burst spiking (BS), with a burst of high-frequency spikes at the onset of stimulation followed by spike frequency adaptation; and irregular spiking cells (IS), with variable firing frequency and overall spike frequency adaptation. Most non-FS interneurons were RS (24/36, 66.6%), whereas BS cells (6/36; 16.7%) and IS cells (6/36; 16.7%) constituted smaller subpopulations. Table 1 summarizes some intrinsic electrophysiological properties of FS, BS, RS, and IS interneurons.

Consistent with the findings from our previous studies, FS interneurons of monkey DLPFC were either basket cells or chandelier neurons (Fig. 2) and non-FS cells had heterogeneous morphology (Fig. 2). Staining of the biocytin-filled recorded neurons confirmed that all layer 3 neurons included in this study had nonpyramidal morphology, including small rounded or ovoid-shaped cell body and lack of apical dendrite or high dendritic spine density. However, for most cells the morphological properties recovered were incomplete, precluding their classification into particular morphological subtypes. Importantly, in mouse neocortex inhibitory synaptic inputs showed different properties between interneurons classified by their firing properties into FS, RS, IS, and BS groups very similar to those studied here and independent of morphological subclass (Bacci et al. 2003; Dumitriu et al. 2007; Pfeffer et al. 2013).

**Properties of miniature synaptic responses in FS and non-FS interneurons.** In each interneuron characterized by firing properties, GABA<sub>A</sub>R-mediated mIPSPs were recorded in the presence of TTX (1 μM), CNQX (20 μM), and D,L-AP5 (100 μM). To increase the driving force for the synaptic currents and improve event detection, the pipette solution contained a high Cl<sup>−</sup> concentration, yielding largely depolar-
different interneuron dendritic tree properties or different synapse properties are at least in part due to cell type-specific properties of the synapses underlying the mIPSPs. Therefore, the differences in mIPSP properties between interneurons of a given subclass (Fig. 4) may be determined, at least in part, by the longer dendrite (Spruston et al. 1993; Williams and Mitchell 2008). The properties of mIPSCs recorded with electrodes at the soma may be altered compared with mIPSCs at the synaptic sites, because the somatic voltage clamp does not uniformly control voltage throughout the somatodendritic membrane (Spruston et al. 1993; Williams and Mitchell 2008). The magnitude of such distortion is dependent on dendritic tree properties and synapse location, and thus if those features differ between FS and RS cells then differences in mIPSC properties may be influenced by a different magnitude of space-clamp error in FS versus RS neurons. IPSCs produced by more distal GABA synapses are subject to larger space-clamp errors and have slower rise time and smaller amplitude at the soma (Spruston et al. 1993; Williams and Mitchell 2008). We found that mIPSCs recorded from individual FS or RS neurons had highly variable amplitude and 10–90% rise time (ranging from 0.2 to 3 ms; Fig. 9A), consistent with different degrees of voltage-clamp error. To obtain an estimate of mIPSC properties relatively independent of space-clamp
Errors, we selected from each FS or RS neuron the mIPSCs with faster rise time (10–90% rise time < 1 ms), presumably originated from proximal synapses and thus recorded with smaller space-clamp error. Consistent with more proximal synapse locations, as shown in Fig. 9B, fast-rising mIPSCs had significantly larger mean amplitude than slow-rising mIPSCs in each cell type. Moreover, the mean amplitude of fast-rising mIPSCs did not differ between FS and RS interneurons (P = 0.636), whereas the fast-rising mIPSCs had faster decay in FS neurons (Fig. 9C). Therefore, the differences in mIPSC properties between cell types appear to be relatively independent of differences in space-clamp error and to at least partly reflect different synaptic properties, in accordance with the effects of zolpidem on mIPSPs (Fig. 6).

We previously found that the first surgical procedure did not alter the physiological or anatomical properties of the pyramidal neurons and local circuits studied in slices from the non-homotopic tissue blocks obtained from the contralateral hemisphere during a subsequent surgical procedure (Gonzalez-Burgos et al. 2000; Henze et al. 2000). To determine the effect of prior surgical procedure on inhibitory synaptic inputs onto interneurons, we analyzed the mIPSP and mIPSC data (Figs. 3, 4, 7, and 8) by two-way ANOVA using cell type and surgical procedure (first vs. second block) as the main factors. We found that the mIPSP frequency differed between cell types $[F_{(3,40)} = 4.26, P = 0.010]$ but was not affected by surgical procedure $[F_{(1,40)} = 0.003, P = 0.954]$, nor was there a significant interaction between factors $[F_{(3,40)} = 1.99, P = 0.131]$. Similar findings were obtained for mIPSP amplitude [cell type: $F_{(3,40)} = 10.9, P = 0.0002$; surgical procedure: $F_{(1,40)} = 0.663, P = 0.420$; interaction: $F_{(3,40)} = 0.294, P = 0.829$] and mIPSP decay time constant [cell type: $F_{(3,40)} = 17.121, P = 0.00001$; surgical procedure: $F_{(1,40)} = 0.896, P = 0.349$; interaction: $F_{(3,40)} = 0.691, P = 0.562$]. Importantly, whereas the mean mIPSP amplitude and decay time constant values were essentially identical in FS, RS, BS, or IS neurons recorded from slices of the first versus second tissue blocks (data not shown), the mean mIPSP frequency showed a tendency to differ between the first and second blocks, the difference being significant only in FS cells ($n_{FS} = 1.1$ Hz ($n = 11$) vs. $3.6 \pm 0.4$ Hz ($n = 13$), $P = 0.016$; RS: $3.0 \pm 0.7$ Hz ($n = 4$) vs. $2.0 \pm 0.3$ Hz ($n = 8$), $P = 0.468$; BS: $1.4 \pm 0.8$ Hz ($n = 2$) vs. $2.0 \pm 0.9$ Hz ($n = 4$), $P = 0.274$; IS: $2.9 \pm 0.2$ Hz ($n = 4$) vs. $5.4 \pm 1.4$ Hz ($n = 2$), $P = 0.202$; Fisher LSD comparisons]. Two-way ANOVA analysis of the mIPSC data (Figs. 7, 8) similarly showed a significant effect of cell type without significant effect of surgical procedure or interaction on mIPSC amplitude [cell type: $F_{(1,18)} = 5.988, P = 0.024$; surgical procedure: $F_{(1,18)} = 0.387, P = 0.542$; interaction: $F_{(1,18)} = 1.401, P = 0.252$] or mIPSC decay time constant [cell
effects of prior surgery. However, consistent with the tendency observed for mIPSP frequency in FS and RS neurons, the mIPSC frequency was significantly affected by the prior surgical procedure \[F(1,18) = 10.6, P = 0.0044\] but without interaction \[F(1,18) = 0.284, P = 0.600\] with the significant effect of cell type \[F(1,18) = 4.771, P = 0.042\]. The surgical procedure indeed decreased mIPSC frequency in both FS and RS neurons, such decrease being significant only in FS cells \([FS: 4.9 \pm 0.58 \text{ Hz} (n = 6) \text{ vs. } 2.4 \pm 0.53 \text{ Hz} (n = 4), P = 0.020; RS: 3.11 \pm 0.90 \text{ Hz} (n = 6) \text{ vs. } 1.32 \pm 0.33 \text{ Hz} (n = 6), P = 0.055]\). Overall, the two-way ANOVA analysis indicated that most mIPSP and mIPSC parameters were not affected by the first surgical procedure. Whereas the prior surgery seemed to decrease the mIPSP and mIPSC frequency in FS neurons, such effect does not, however, change the conclusions of our study, as the significant differences between cell type were independent of the effects of prior surgery.

**DISCUSSION**

In this study we found that, as in rodent neocortex, in layer 3 of monkey DLPFC GABA\(_A\)-mediated synapses are a prominent source of synaptic input onto GABAergic interneurons, since mIPSPs and mIPSCs of significant amplitude and frequency were detected in each recorded interneuron, irrespective of subtype. We also observed that some properties of the GABA\(_A\)-mediated synaptic inputs differ between GABA neuron subtypes, suggesting that in monkey DLPFC regulation of GABA neuron activity by inhibition is GABA neuron subtype specific.

**GABA\(_A\)-mediated synaptic inputs differ between DLPFC interneuron subtypes.** We divided the recorded interneurons into FS and non-FS groups that had many subthreshold and firing properties similar to those of the FS and non-FS classes described in our previous studies (Gonzalez-Burgos et al. 2004; Krimer et al. 2005; Zaitsev et al. 2005, 2009). The non-FS interneurons studied here comprised RS, BS, and IS subtypes showing differences in their inhibitory inputs, as found previously for similar electrophysiological classes of cortical interneurons in rodents (Bacci et al. 2003; Dumitriu et al. 2007; Pfeffer et al. 2013; Savanthrapadian et al. 2014). We found a significantly faster IPSP and IPSC decay in layer 3 FS cells, in a manner consistent with data from other cortical regions and layers in rodents (Bacci et al. 2003; Cossart et al. 2006; Dumitriu et al. 2007), suggesting that faster synaptic inhibition onto FS neurons is highly conserved across layers, areas, and species. The faster decay of inhibition onto FS...
neurons may be important for generation of synchronized oscillatory activity of FS neurons at high-frequency bands (Bartos et al. 2007).

A faster decay in FS neurons could be due to synapse morphology, which shapes the cleft GABA concentration transient (Farrant and Kaila 2007; Nusser et al. 2001; Szabadics et al. 2007), GABA_A,R phosphorylation (Kittler and Moss 2003; Nusser et al. 1999), and GABA_A,R clustering (Christie et al. 2002). In addition, GABA_A,Rs with α1 subunits have faster IPSC current decay (Farrant and Kaila 2007; Lavoie et al. 1997), and we found stronger effects of the α1 subunit-prefering positive modulator zolpidem on mIPSPs in FS neurons, consistent with a greater contribution of α1-GABA_A,Rs in FS cells. In rodents, GABA synapses onto FS cells have the highest density of α1-GABA_A,Rs (Klausberger et al. 2002) and IPSCs in FS cells have high sensitivity to zolpidem (Bacci et al. 2003). A greater contribution of α1-GABA_A,Rs in FS cells suggests that α1-GABA_A,R-preffering pharmacological modulators may more strongly or selectively enhance inhibition onto FS cells. In monkey DLPFC, pyramidal neurons are significantly innervated by FS cells (Gonzalez-Burgos et al. 2005) and have GABA synapses with a substantial zolpidem sensitivity (Gonzalez-Burgos et al. 2009). Thus future studies are needed to test the relative effects of α1-GABA_A,R subunit-prefering modulators on pyramidal cell inhibition versus FS cell-mediated disinhibition in monkey DLPFC microcircuits.

Relation to interneuron diversity. Although here we classified the interneurons by their intrinsic membrane properties, our findings may be interpreted in the context of the interneuron diversity described in monkey DLPFC. In previous studies, we combined the analysis of electrophysiology, morphology, and molecular markers to characterize the interneurons of monkey DLPFC layer 3 (Zaitsev et al. 2005, 2009). We found that, as in rodents (Hu et al. 2014), monkey DLPFC interneurons with FS firing pattern uniquely express the calcium-binding protein parvalbumin (PV) (Zaitsev et al. 2005, 2009). PV-negative interneurons of monkey neocortex express one of two other calcium-binding proteins, calretinin or calbindin, and show non-FS firing patterns (Conde et al. 1994; DeFelipe 1997; DeFelipe et al. 1999; Jakab et al. 1997; Meskenaite et al. 2004). Comparison of our present data with results of our previous studies suggests that the RS interneurons belong to the calretinin-positive subclass, whereas BS cells may be calbindin-positive interneurons that also express somatostatin (Zaitsev et al. 2005, 2009). IS interneurons, which were relatively rare in our previous studies, may correspond to the cholecystokinin-positive interneurons that also express cannabinoid receptors (Eggen et al. 2010; Galarreta et al. 2004).

In the DLPFC and other areas of monkey neocortex, PV is found in symmetric GABA synapses (Blumcke et al. 1991; Williams et al. 1992), originated from chandelier and basket cells (Blumcke et al. 1991; Conde et al. 1994; DeFelipe et al. 1999; Melchitzky et al. 1999; Williams et al. 1992; Zaitsev et al. 2005, 2009). Only PV-positive basket cells, however, may provide significant inhibition onto other GABA neurons, since in monkey DLPFC, as in other cortical areas of monkey and

---

**Fig. 5.** Interneuron membrane properties partially shape mIPSP amplitude and time course. A: a significant correlation was observed between mIPSP amplitude and the cells’ input resistance. B: a significant correlation was observed between mIPSP decay time constant and the cells’ membrane time constant.

---

**Fig. 6.** Effects of the positive modulator zolpidem on mIPSPs in FS and RS interneurons. A: mIPSPs recorded in control conditions (left) and after zolpidem application (right) from FS neurons (top) and RS neurons (bottom). Shown are representative individual mIPSPs and average mIPSP waveforms. B: bar graph summarizing the % change in mIPSP area produced by 1 μM zolpidem application. The difference between group means was significant (P < 0.05, single-factor ANOVA).
rodent neocortex, chandelier cells mostly innervate the pyramidal cell axon initial segment (Melchitzky et al. 1999; Williams et al. 1992). The interneuron targets of PV basket cell synapses in monkey DLPFC are not fully characterized, but experiments in rodents suggest that, among interneuron targets, PV basket neurons mainly innervate other PV-positive cells (Pfeffer et al. 2013). PV-negative calretinin-positive interneurons are a main source of inhibitory inputs onto GABA neurons in monkey DLPFC, since the axon terminals from these cells target preferentially, although not exclusively, other GABA

FIG. 7. Properties of miniature inhibitory postsynaptic currents (mIPSCs) recorded from FS and RS interneurons. A: representative examples of mIPSCs recorded from FS and RS neurons. B, top: examples of average mIPSC waveforms recorded from FS (gray trace) and RS (black trace) neurons. Bottom: the average waveforms normalized to the same amplitude illustrate the differences in decay time course. C, left: cumulative probability distribution histograms of mIPSC decay time constant. The distributions differed significantly between groups. Right: bar graph summarizing mIPSC decay time constant. The group means were significantly different (Student’s t-test). D, left: cumulative probability distribution histograms of mIPSC amplitude. The distributions did not differ significantly between groups. Right: bar graph summarizing mIPSC amplitude. The group means did not differ significantly (Student’s t-test).

Fig. 7. Properties of miniature inhibitory postsynaptic currents (mIPSCs) recorded from FS and RS interneurons. A: representative examples of mIPSCs recorded from FS and RS neurons. B, top: examples of average mIPSC waveforms recorded from FS (gray trace) and RS (black trace) neurons. Bottom: the average waveforms normalized to the same amplitude illustrate the differences in decay time course. C, left: cumulative probability distribution histograms of mIPSC decay time constant. The distributions differed significantly between groups. Right: bar graph summarizing mIPSC decay time constant. The group means were significantly different (Student’s t-test). D, left: cumulative probability distribution histograms of mIPSC amplitude. The distributions did not differ significantly between groups. Right: bar graph summarizing mIPSC amplitude. The group means did not differ significantly (Student’s t-test).

FIG. 8. Analysis of mIPSC frequency. A: cumulative probability distributions of interevent intervals in FS and RS interneurons. Gray curves are the distributions obtained for each neuron. Black curve is the distribution obtained for the total mIPSP sample in each cell type. B: interevent interval distributions differ significantly between FS and RS cells. The difference between distributions was assessed by Kolmogorov-Smirnov test. C: bar graph summarizing mean mIPSC frequency. The difference between group means was assessed with Student’s t-test.

Fig. 8. Analysis of mIPSC frequency. A: cumulative probability distributions of interevent intervals in FS and RS interneurons. Gray curves are the distributions obtained for each neuron. Black curve is the distribution obtained for the total mIPSP sample in each cell type. B: interevent interval distributions differ significantly between FS and RS cells. The difference between distributions was assessed by Kolmogorov-Smirnov test. C: bar graph summarizing mean mIPSC frequency. The difference between group means was assessed with Student’s t-test.
neurons via symmetric synapses (Melchitzky et al. 2005; Melchitzky and Lewis 2008). In contrast, inputs from calbin-
din-positive, somatostatin-containing interneurons in monkey
DLPFC are much less likely to target other GABA neurons
than calretinin-containing inputs (Melchitzky and Lewis 2008).
Integration of the present data with the results of previous
studies of monkey neocortical circuits thus suggests that in-
hibitory inputs are present in most, if not all, GABA neuron
subtypes, and therefore that interneuron activity in primate
splits of monkey DLPFC, some
IPSCs are produced via spontaneous interneuron firing (Gon-
zalez-Burgos et al. 2014). However, mIPSPs and mIPSCs are
studied with firing blocked by TTX; thus the higher frequency
of mIPSPs and mIPSCs in FS neurons is unrelated to firing
activity in inputs onto these cells. If the probability of action
potential-independent GABA release were similar at inputs
onto FS and non-FS neurons, then the higher mIPSC frequency
might indicate that FS cells have a higher density of inhibitory
inputs. However, interneurons receive GABAergic input ori-
ginating from multiple sources (Pfeffer et al. 2013), and mIPSCs
originating from different sources may display different release
probability (Goswami et al. 2012), suggesting that mIPSC
frequency may not directly indicate input density.

Action potential-independent miniature synaptic events are
observed in vivo (Destexhe and Pare 1999; Pare et al. 1997)
and not exclusively in brain slice conditions. Miniature synap-
tic events may mediate maintenance of synaptic function, by
regulating synaptic receptor density and clustering or acting as
a trophic factor that prevents synapse loss or elimination
(Kaeser and Regehr 2014). If so, then the higher miniature
event frequency would make GABA synapses in FS neurons
more stable and less susceptible to factors that enhance GABA
synapse elimination or turnover. Importantly, action potential-
dependent and -independent forms of GABA release originate
from the same vesicle pool (Hua et al. 2010; Wilhelm et al.
2010), suggesting that the synaptic properties conferred by a
high mIPSC frequency similarly affect action potential-evoked
IPSCs.

Importantly, since we used whole cell recording conditions that
modify the intracellular Cl\(^-\) concentration, we could not
determine the physiological value of \(E_{\text{GABA}}\), the reversal potential of the GABA\(_\text{A}R\) current, which is mainly a Cl\(^-\) current.
In rodent interneurons, during early development \(E_{\text{GABA}}\) is
depolarized above the neurons’ spike threshold and thus
GABA\(_\text{A}R\)-mediated inputs are excitatory (Sambandan et al.
2010). In mature FS and non-FS interneurons, \(E_{\text{GABA}}\) remains
depolarized (Banke and McBain 2006; Hollrigel et al. 1998;
Sambandan et al. 2010; Vida et al. 2006), but the depolarizing
IPSCs in mature interneurons produce shunting inhibition
(Banke and McBain 2006; Hollrigel et al. 1998; Sambandan et
al. 2010; Vida et al. 2006). Inhibitory mIPSCs and miniature
excitatory synaptic currents (mEPSCs) could regulate interneu-
ron activity in vivo, when firing activity in the monkey DLPFC
network is low. In FS neurons of monkey DLPFC, the mEPSC
frequency is \(~20\text{ Hz}\) (Povyyshev et al. 2006), thus significantly
higher than mIPSP and mIPSC frequency (\(~4\text{ Hz}\); see Figs. 4
and 8). A high mEPSC-to-mIPSC ratio could make monkey
DLPFC FS neurons readily responsive to external inputs ar-
iving when the local network is in a low-firing activity state.
Interestingly, FS chandelier neurons are thought to have an
excitatory effect on pyramidal cells under quiescent network
conditions (Woodruff et al. 2011), whereas FS basket cells are
generally inhibitory (Szabadics et al. 2006; Woodruff et al.
2009). Thus external inputs arriving onto chandelier versus
basket cells could have opposite effects on the transition of the
cortical network from quiescent to active states. Whether
chandelier and basket FS neurons in monkey DLPFC have a
similar mEPSC-to-mIPSC ratio remains to be determined.

A common active network state in monkey DLPFC is the
persistent firing during the delay period of working memory
tasks (Funahashi et al. 1989; Fuster 1973; Miller et al. 1996),
thought to be mediated by recurrent excitation and inhibitory
feedback control (Wang et al. 2004). Such inhibitory feedback
may be provided by monkey DLPFC interneurons displaying
delay-related firing (Constantinidis et al. 2002; Constantinidis
and Goldman-Rakic 2002; Rao et al. 1999; Wilson et al. 1994),
possibly driven by sustained synaptic input from nearby pyra-
midal cells (Gonzalez-Burgos et al. 2004). Pronounced spike
frequency adaptation such as that displayed by monkey
DLPFC layer 3 pyramidal neurons (Amatrudo et al. 2012;
Henze et al. 2000; Zaitsev et al. 2012) decreases pyramidal cell
excitability during persistent excitation (Carter and Wang

Delay-related firing in pyramidal cells with spike frequency adaptation is thus optimized by some level of disinhibition such as that produced by endocannabinoid-mediated suppression of inhibition (Carter and Wang 2007), which is prominent in monkey DL-PFC (Gonzalez-Burgos et al. 2014). Disinhibition could also be mediated by the inhibitory inputs onto GABA neurons described here. Understanding the role of pyramidal cell disinhibition in shaping delay-related firing in the monkey DL-PFC requires additional studies to characterize the patterns of inhibitory connections between monkey DL-PFC interneurons and to identify the interneuron subtypes that exhibit delay-related activity during working memory tasks (Constantinidis et al. 2002; Constantinidis and Goldman-Rakic 2002; Rao et al. 1999; Wilson et al. 1994).

ACKNOWLEDGMENTS

We thank Olga Krimer for her excellent assistance with histochemical methods and neuronal reconstruction.

GRANTS

This work was funded by National Institute of Mental Health Grant R01 MH-051234.

DISCLOSURES

D. A. Lewis currently receives investigator-initiated research support from Bristol-Myers Squibb and Pfizer and in 2012–2014 served as a consultant in the areas of target identification and validation and new compound development to Autifony, Bristol-Myers Squibb, Concert Pharmaceuticals, and Sunovion.

AUTHOR CONTRIBUTIONS


REFERENCES


