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Characterization of Neocortical Principal Cells and Interneurons by Network Interactions and Extracellular Features

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Barthó, Peter, Hajime Hirase, Lenaïc Monconduit, Michael Zugaro, Kenneth D. Harris, and György Buzsáki. Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. *J Neurophysiol* 92: 600–608, 2004; 10.1152/jn.01170.2003. Most neuronal interactions in the cortex occur within local circuits. Because principal cells and GABAergic interneurons contribute differently to cortical operations, their experimental identification and separation is of utmost important. We used 64-site two-dimensional silicon probes for high-density recording of local neurons in layer 5 of the somatosensory and prefrontal cortices of the rat. Multiple-site monitoring of units allowed for the determination of their two-dimensional spatial position in the brain. Of the ~60,000 cell pairs recorded, 0.2% showed robust short-term interactions. Units with significant, short-latency (<3 ms) peaks following their action potentials in their cross-correlograms were characterized as putative excitatory (pyramidal) cells. Units with significant suppression of spiking of their partners were regarded as putative GABAergic interneurons. A portion of the putative interneurons was reciprocally connected with pyramidal cells. Neurons physiologically identified as inhibitory and excitatory cells were used as templates for classification of all recorded neurons. Of the several parameters tested, the duration of the unfiltered (1 Hz to 5 kHz) spike provided the most reliable clustering of the population. High-density parallel recordings of neuronal activity, determination of their physical location and their classification into pyramidal and interneuron classes provide the necessary tools for local circuit analysis.

INTRODUCTION

Transformation, transmission, and storage of information in the brain are achieved by cooperative action of neuronal ensembles. A main goal of neurophysiology is to understand the rules that determine the behavior of these flexible coalitions (Chicurel 2001; Deadwyler and Hampson 1995; Eichenbaum and Davis 1998). A first step in this process is the simultaneous recording from large numbers of neurons in the behaving animal. The recent advent of multisite, closely spaced extracellular recording techniques has improved single-unit sorting by taking advantage of the temporal coherence of spikes from closely spaced recording sites (Csicsvari et al. 1999, 2003b; Drake et al. 1988; Gray et al. 1995; McNaughton et al. 1983; O'Keefe and Recce 1993; Wilson and McNaughton 1993), allowing identification and classification of multiple extracellularly recorded neurons. In the neocortex, several classes of interneurons (Somogyi et al. 1998; Swadlow 2003) provide timing, oscillatory background, and other general contexts for the computations carried out by the principal cells (Buzsáki and Chrobak 1995). Therefore experimental separation of interneurons from pyramidal cells is of utmost importance in the understanding of the operation principles of the neocortex. The last, and perhaps most difficult, task is to identify subclasses of interneurons and principal cells from their extracellular signatures (Klausberger et al. 2003).

In the hippocampus, the combination of several extracellular features, such as spike duration, firing rate, and pattern and spike waveform, reliably separates pyramidal cells from interneurons (Csicsvari et al. 1999). The validity of neuron classification on the basis of extracellular features has been supported by in vivo intra- and juxtacellular labeling as well as simultaneous extra- and intracellular recordings from the same neurons (Henze et al. 2000; Klausberger et al. 2003; Sik et al. 1995). Similar classification criteria are not available in the neocortex. Mountcastle et al. (1969) have noted that the occasionally recorded "thin spikes" in the somatosensory cortex had different response properties than the majority of units and suspected that they were interneurons. Other observations indicated that fast-spiking neurons have receptive and evoked response properties different from the majority of slower, more regular firing cells (Constantinidis and Goldman-Rakic 2002; Simons 1978; Swadlow 2003; Swadlow and Gusev 2002; Swadlow and Lukatela 1996; Swadlow et al. 1998). Intracellular recordings and labeling in cortical slices showed numerous classes of interneurons on the basis of the firing rates, spike morphology, and spike dynamics (Connors et al. 1982; Gupta et al. 2000; Kawaguchi and Kubota 1993; Somogyi et al. 1998). However, the information gathered in intracellular experiments in vitro cannot be directly applied to extracellularly recorded spikes in the intact brain. Furthermore, intra- and juxtacellular studies carried out in vivo do not directly support the suggestion that fast firing neurons unequivocally identify interneurons (Azouz et al. 1997; Degenetais et al. 2002; Douglas et al. 1995; Gray and McCormick 1996; Steriade et al. 1998).

To provide more direct classification criteria for cell identification, we took advantage of large-scale parallel recording of unit activity in a small cortical volume (Csicsvari et al. 2003a). In a large data base, we found that ~0.25% of the recorded cell pairs had short-latency (<3 ms), putative monosynaptic interactions. Units exciting other units at monosynaptic latency were regarded as pyramidal cells, whereas units suppressing discharges of target partners were regarded as GABAergic inhibitory interneurons. These physiologically identified neurons helped us assess the physiological features of the extra-

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cellular spikes and classify the whole population into putative pyramidal and interneuron groups.

METHODS

Surgery and recording

For acute recordings, Sprague-Dawley rats (300-500 g; Hilltop Laboratories, Scottsdale, PA, or Zivic-Miller laboratories, Pittsburgh, PA) were anesthetized with urethan (1.5 g/kg) and placed in a stereotaxic frame. After preparing a 1 × 3-mm window in the skull, the dura was removed using a dissection microscope, and the probe was positioned so that the tips avoided blood vessels. The skull cavity was filled with a mixture of wax and paraffin, which decreased brain pulsation as well as provided lateral support for the probe shanks. The recording silicon probe was attached to a micromanipulator and moved gradually to its desired depth position. The probe consisted of eight shanks (200-μm shank separation), and each shank had eight recording sites (160 μm^2 each site; 1-3 MΩ impedance) with recordings sites staggered to provide a two-dimensional arrangement (20 μm vertical separation; see Fig. 3. For information on silicon probes manufactured at University of Michigan, see http://www.engin.umich.edu/center/cnct/). The middle shanks were centered at anterior-posterior, AP = −1.5 mm and mediolateral; ML = 3.5 mm position to record from the primary somatosensory cortex. After each acute experiment the probe was rinsed with de-ionized water. Probes were used repeatedly for several months without noticeable deterioration in performance. Extracellular signals were high-pass filtered (1 Hz) and amplified (1,000 times) using a 64-channel amplifier (Sensorium, Charlotte, VT). All data were digitized at 25 kHz (DataMax System, RC Electronics, Santa Barbara, CA) using 16-bit resolution and stored on a computer disk for later analysis.

The general surgical procedures for chronic recordings have been described (Csicsvari et al. 2003b). In short, rats of the Sprague-Dawley strain (400-900 g) were anesthetized with a mixture (4 ml/kg) of ketamine (25 mg/ml), xylazine (1.3 mg/ml), and acepromazine (0.25 mg/ml) and placed in the stereotaxic apparatus. Instead of silicon probes, wire tetrodes were attached to a custom-prepared microdrive. The tetrodes were implanted the same way as in the acute experiments. During implantation the tips of the tetrodes were lowered ~400 μm below the brain surface. After recovery from surgery, the probes were moved gradually, and recordings were made at several depth locations. In both acute and chronic experiments, location of layer V neurons was assessed by recording delta waves and unit activity. Delta waves reverse in polarity below layer IV, and units are silenced during positive delta waves (Buzsáki et al. 1988). The physiological data were collected during sleep and spontaneous exploration in the home cage. All procedures conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

SPIKE SORTING AND CONNECTION ANALYSIS. The continuously recorded wide-band signals were high-pass filtered (0.8-5 kHz) digitally. Units were identified and isolated by a semiautomatic "cluster cutting" algorithm ("KlustaKwik"; available at: http://osiris.rutgers.edu/buzsaki/software) followed by manual clustering (Csicsvari et al. 1998). Auto- and cross-correlations were calculated to verify the clustering procedure. The quality of spike clusters was estimated by measuring the Mahalanobis distance ("isolation distance") from the cluster center within which as many points belong to other clusters as belong to the specified cluster (Harris et al. 2001). Center of mass was calculated as the mean *x-y* position of the recording sites weighted by the spike amplitude at each site. For the identification of excitatory connections, short-latency and -duration sharp peaks in the cross-correlograms were used as described by Csicsvari et al. (1998). Significant peaks (1-ms bin width) within 3 ms of the center bin were considered as putative monosynaptic connections. A peak in a cross-correlogram was defined significant when at least one of the bins exceeded 99.9th percentile of the mean. Similarly, short-latency troughs were considered to be due to inhibition when at least two neighboring 1-ms bins were <0.1th percentile of the mean (Constantinidis et al. 2002). The mean (control number of spikes) was calculated between –50 and –10 ms to control for a potential low-frequency fluctuation of firing rate. For cell pairs recorded from the same electrode, the 0- to 1-ms bin was not considered because our clustering program cannot resolve superimposed spikes. This artificial trough was excluded from the analysis.

Histological procedures

After completion of the experiments, the rats were killed and perfused through the heart first with 0.9% saline solution followed by consisting of 4% paraformaldehyde, 15% picric acid, and 0.05% glutaradehyde in 0.1 M phosphate buffer. The brains were sectioned by a Vibroslice at 60 μm in the coronal plane, washed extensively in phosphate buffer, and treated with 0.5% Triton to enhance penetration. Sections were mounted on slides, Nissl-stained, and coverslipped. The tracks of the silicon probe shanks were reconstructed from multiple sections.

RESULTS

Unit recording and clustering

Figure 1 illustrates recordings with the 64-site probe from layer V of the somatosensory cortex of the urethan-anesthetized rat. Most of the 64 recording sites yielded unit activity in every experiment ($n = 16$), and the same units were recorded by several recording sites of the same shank. The voltage profile of spikes across the recording sites provided an approximate location of the cell body of the recorded neuron. The spike amplitude from presumed perisomatic locations often exceeded 0.3 mV. The spike amplitude and waveform variability across recording sites were used for separating single units (Gray et al. 1995; Wise and Angell 1975). The eight recording sites in each shank provided a total of 28 two-dimensional views of unit clusters for each shank (Fig. 1). The reliability of spike cluster separation was quantitatively determined by the isolation distance measure of unit quality and the spike contamination of the refractory period in the auto-correlograms (Harris et al. 2000). Silicon shanks placed in layer V pyramidal layer yielded 12-18 high-quality units per shank. A total of 1,414 units were recorded from the primary somatosensory area and the prefrontal cortex (anesthetized $n = 16$ rats, freely moving $n = 7$ rats).

Monosynaptic neuronal interactions in layer V neurons

Monosynaptic connections were assessed by examining the short-latency interactions between neurons pairs as described in the hippocampus (Csicsvari et al. 1998). Paired recordings from layer V neurons in vitro showed that short onset latency monosynaptic excitatory postsynaptic potential (EPSP) responses corresponded to monosynaptic connections between pyramidal cell-interneuron pairs (Angulo et al. 2003; Gupta et al. 2000; Somogyi et al. 1998; Thomson and Deuchars 1997; Thomson et al. 1995) and pyramidal-pyramidal cells (Deuchars et al. 1994; Holmgren et al. 2003; Markram 1997). Short-latency inhibitory postsynaptic potentials (IPSPs) were also evoked by single interneuron spikes in both pyramidal and interneuron targets (Buhl et al. 1997; Maccaferri et al. 2000;

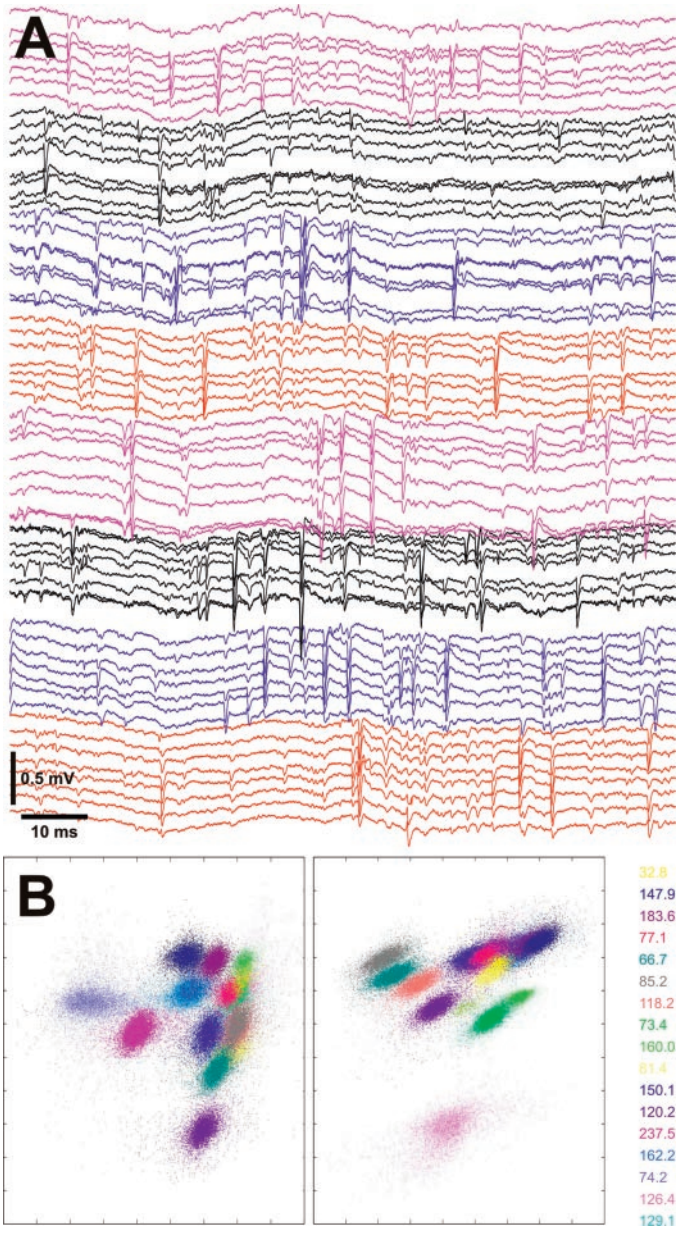

FIG. 1. Parallel recording of unit activity in layer V of the somatosensory cortex by an 8-shank probe. Each shank had 8 recording sites (for tip details Fig. 3). A short segment of wide-band signal (field and unit activity) is displayed. Recordings from the 8 parallel shanks are color-coded and plotted below each other. Note temporally coherent spikes on several sites of a given shank and lack of "cross-talk" across shanks. *Bottom*: 2 separate 2-dimensional views of unit clusters from 1 shank. The 8 recording sites provided a total of 24 2-dimensional views of unit clusters for each shank. Clusters are color-coded. Quality of clusters ("isolation distance") was estimated by measuring the Mahalanobis distance (color coded figures) from the cluster center within which as many points belong to other clusters as belong to the specified cluster (Harris et al. 2001).

Tamas et al. 1997; Thomson and Bannister 2003). Neurons with <1-Hz firing rates (n = 281) were excluded from these analyses. Of the 56,845 cross-correlograms (n = 6,754 from prefrontal cortex recordings) 107 (0.2%) had short-latency (<5 ms onset) significant peaks or troughs (Fig. 2). Of these, 58 had large and narrow peaks (Fig. 2*A*), indicating that the presynaptic neuron was an excitatory pyramidal cell. The precise short-latency drive could often be visualized by superpositioning of the spike trains (Fig. 2*B*). Another 21 pairs had significant short-latency suppression (Fig. 2*C*), indicating that the presynaptic neuron was an inhibitory interneuron. To quantitatively assess the time course of monosynaptic inhibition, an exponential curve was fit for the spike bins after the presynaptic spike (the inhibitory trough on the cross-correlogram). The mean time constant of spike suppression was 5.17 ± 3.12 ms, although with substantial variability as indicated by the large SD. Finally, 28 pairs showed both short-latency peaks and troughs, suggesting that these pairs were mutually connected pyramidal-interneuron pairs (Fig. 2*D*). As expected from anatomical connectivity, the detected physiological connections were not distributed homogenously among the population. The majority of monosynaptically connected pairs were detected from the neuronal pool recorded by the same shank (excitatory connections = 0.87%; inhibitory connections = 0.55%), although pairs were also identified between shanks as well (excitatory connections = 0.15%; inhibitory connections = 0.09%). Often a single neuron was connected to several postsynaptic targets. More than a quarter (29%) of the physiologically identified interneurons suppressed activity of two to seven target cells. Convergence of excitation is illustrated by the finding that among the excitatory connections 40% of the target cells were excited by 2–12 presynaptic neurons. Most neurons that were excited were shown to be putative inhibitory interneurons by their spike suppression effects of their targets (58%). Neurons that were classified as excitatory in one or more connections never suppressed activity of any target. Conversely, putative interneurons were never found to excite other partners. Figure 3 illustrates the circuit analysis of multiple uni- and bidirectionally connected pairs in a small neuronal pool. The largest "hub" involved interneuron 3, which was excited by 5 of the 15 neurons recorded by the same shank and 7 of the 37 neurons recorded by the surrounding shanks. In turn, it inhibited 4 of the 15 cells from the same shank and 3 of the 37 neurons recorded by the other shanks. Five connections were bidirectional. These simultaneous recordings also indicated that the magnitude and time course of the monosynaptic effects were quite heterogeneous on the postsynaptic targets.

The preceding findings in urethan-anesthetized animals were confirmed by wire tetrode recordings from the somatosensory area in drug-free, behaving rats. Of the 3,814 unit pairs, 9 putative excitatory and 2 putative inhibitory connections were identified. Reciprocal interaction was detected in one pair. The similar proportions of putative monosynaptic connections in the drug-free and anesthetized preparations indicated that urethan anesthesia did not affect the fast monosynaptic connections dramatically.

Characterization of pyramidal cells and interneurons by extracellular features

The monosynaptic interactions were used as criteria for the classification of the remaining, nonidentified units into pyramidal cell and interneuron classes. Neurons with short-latency, narrow peaks were regarded as putative excitatory (pyramidal) cells, whereas neurons with short-latency suppression of activity as putative inhibitory interneurons. In addition, units that were excited by a presynaptic pyramidal cell were also distinguished. In principle, these neurons could be other pyramidal

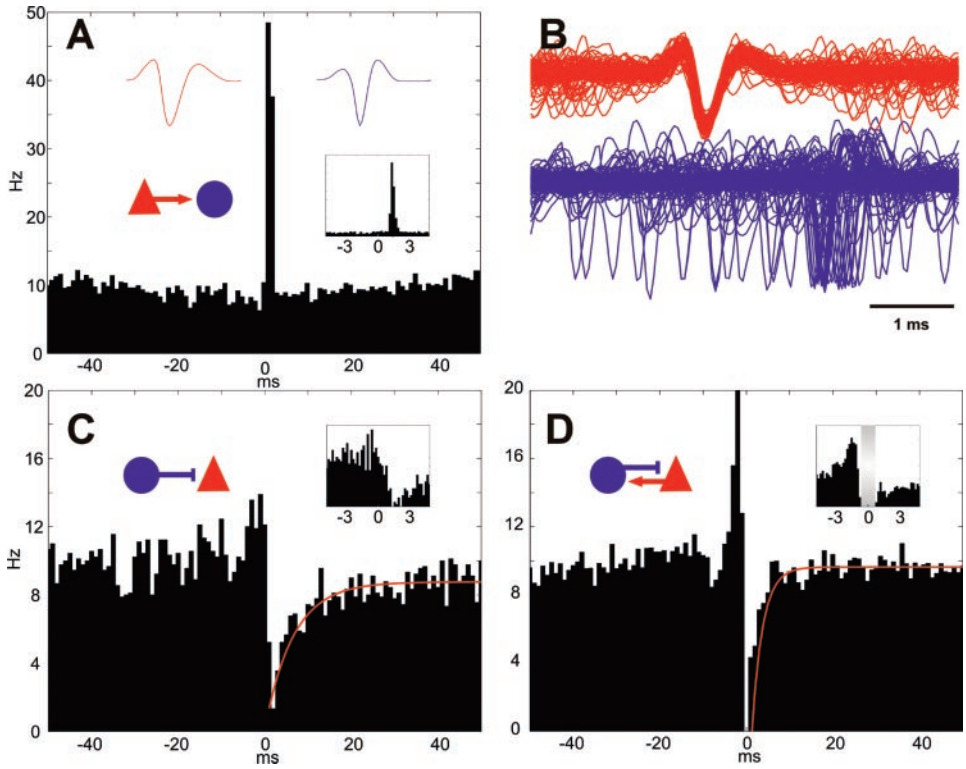

FIG. 2. Short-latency, monosynaptic interactions between neuron pairs. *A*: excitatory drive by a putative pyramidal cell (red triangle). Note large, sharp peak at ~2 ms in the cross-correlogram. Reference event is the spike of the putative pyramidal neuron (*time 0*). *Inset*: higher temporal resolution of the histogram. Averaged waveforms of the units (filtered: 600 Hz to 5 kHz) are also shown. On the bases of spike duration, the target cell was classified as a putative interneuron (blue circle; see text). *B*: superimposed traces of the neuron pair from 2 recording sites with the largest amplitude for each spike. Arrow, monosynaptically driven spikes. *C*: inhibitory suppression. Reference event: spike of the putative interneuron (blue circle). Note strong and immediate suppression of target spikes. The 2 neurons were recorded from different shanks (200-μm lateral separation). Red line indicates exponential fit of suppression time course. *D*: reciprocal monosynaptic interactions of neurons recorded from the same shank. Reference event: spike of the putative interneuron (blue circle). Note excitation of the putative interneuron and strong suppression of the pyramidal cell (red triangle) spikes by the interneuron. Shading indicates the blank period of spike sampling (see METHODS).

cells. However, single-spike discharge in the presynaptic neuron has not been shown to bring the postsynaptic pyramidal cell to threshold (Markram et al. 1997). On the other hand, several studies reported reliable spike transmission between pyramidal-interneuron pairs (Holmgren et al. 2003; Marshall et al. 2002; Swadlow 2003). Using these criteria, several features of the extracellularly recorded units, including discharge frequency, spike duration, spike asymmetry, and amplitude ratio of the negative and positive peaks, as well as features of the auto-correlogram, such as center of mass, time from zero to peak, and burstiness ratio, were examined together with the whole recorded population (Fig. 4). Of these criteria, spike duration provided the best separation. Spike duration was measured between the trough and the following peak of the unfiltered trace because these time points reflect the maximum rate of rise and the maximum rate of decay of the intracellularly recorded action potential (Henze et al. 2000). This criterion was consistently reliable for providing a bimodal distribution of units in somatosensory cortex, prefrontal cortex and in the drug-free animal (Fig. 4; 7.42 vs. 92.57%). The spike duration of putative interneurons was significantly ($P < 0.0001$; *t*-test) more narrow (0.43 ± 0.27 ms) than that of the putative pyramidal cells (0.86 ± 0.17 ms). Table 1 summarizes the distribution of the various interactions in the two groups. Approximately 40% of narrow spike neurons either inhibited target cells or were excited by a presynaptic partner. None of them were classified as excitatory. We also noted that 8 of 54 unconnected narrow spike neurons had bursty auto-correlograms (see following text), whereas none of the 40 neurons with significant interactions did. Excitatory forward connections were detected only in the wide spike group (6.9%) and nearly all of the inhibited neurons belonged to this group (Table 1).

Subclasses of cortical neurons

Following classification of the neurons on the basis of functional connectivity into putative pyramidal and interneuron classes, we searched for potential subgroups within the populations. The auto-correlograms of putative pyramidal cells showed two extreme behaviors. "Bursting" cells had large peaks at 3-6 ms followed by an exponential decay (Fig. 5*A*). Cells were classified as bursting, if the maximum peak on the auto-correlogram between 3 and 6 ms was ≥50% of the maximum bin value of the first 50 ms. The criterion for "regular spiking" (nonbursting) cells was that the mode of interspike-interval histogram was >35 ms. Regular spiking neurons rarely discharged in bursts, and the auto-correlogram showed an exponential rise from *time 0* to tens of milliseconds (Fig. 5*A*). Cells that did not match either of these criteria were labeled "unclassified." Of the putative pyramidal cell population, 53.3% were assigned to regular spiking, 18.24% bursting, and 28.45% belonged to the unclassified or intermediate group. The average firing rate was similar in these three groups (5.62 ± 3.27, 4.02 ± 3.46, and 3.42 ± 3.06 Hz, respectively). The spike duration of the wide-band recorded signal was also similar across these putative groups (0.79 ± 0.15, 0.85 ± 0.13, and 0.88 ± 0.15 ms, respectively). Neurons in each of these groups excited postsynaptic targets. Of the identified excitatory cells, 68.42% was regular spiking, 17.54% bursting, and 14.04 unclassified. Similarly, within the inhibited cell group 65.85% were regular spiking, 14.63% bursting, and 19.51% unclassified (Fig. 5*B*).

As discussed in the preceding text, neurons that were excited at monosynaptic latency could, in principle, include pyramidal cells and interneurons. This group was heterogeneous by firing frequency, spike duration, and other criteria. Nevertheless, the auto-correlograms in this group were more similar to the pu-

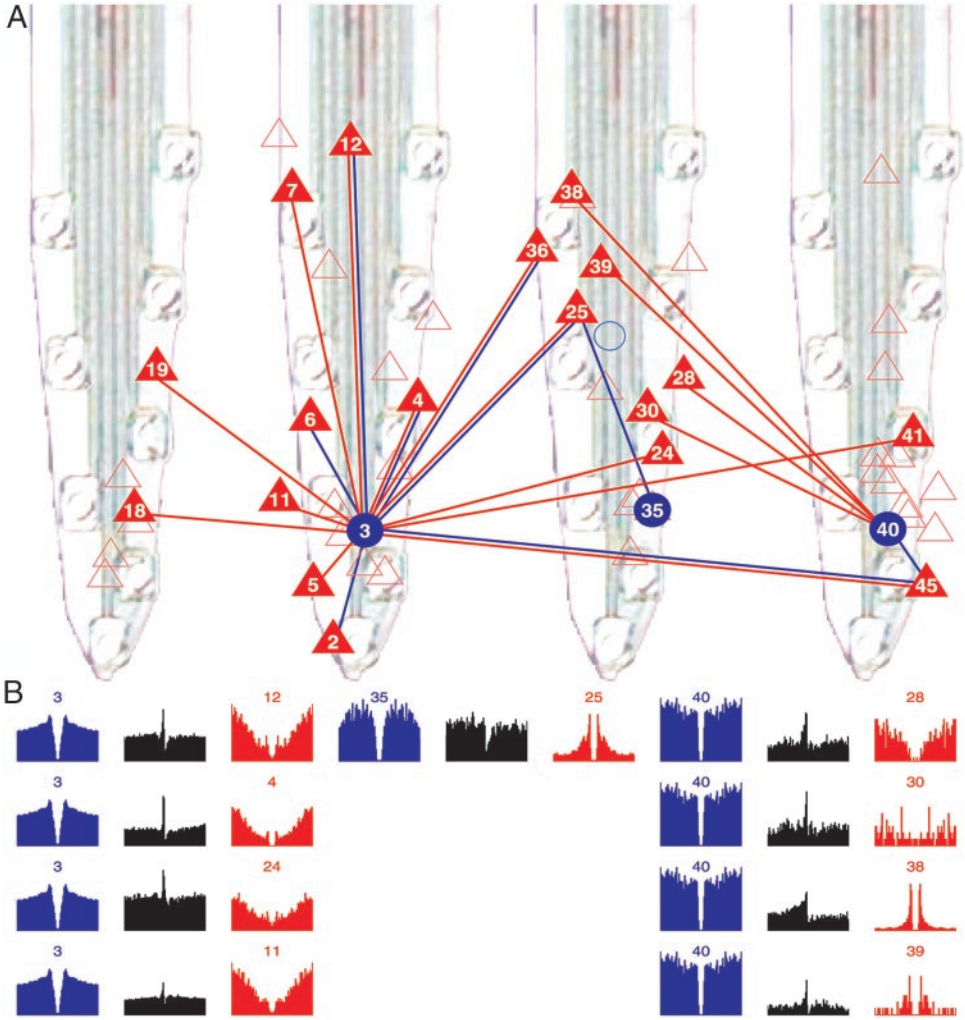

FIG. 3. Tracing of functional connections in local networks. *A*: units recorded by only 4 shanks are shown. Filled symbols, participating pyramidal cells (red triangles) and interneurons (blue circles). Empty symbols, neurons not connected functionally. Red line, monosynaptic excitation; blue line, monosynaptic inhibition. Note that a single interneuron (e.g., 3 and 40) is activated by large number pyramidal cells, and an interneuron inhibits several local and distant pyramidal cells. The relative position of the neurons was determined by calculating the "center of mass" on the basis of the spike amplitude recorded from multiple sites. Sites are spaced 20 μm vertically. The shanks were 200 μm apart but for illustration purposes they are placed closer to each other here. *B*: auto-correlograms (blue and red) and cross-correlograms of some of the pairs shown in *A*. Note short-latency interactions.

tative interneuron group, identified by their inhibitory effects and narrow spikes than to those of the pyramidal cells. No unit in this group had either a bursting or regular spiking type auto-correlogram, typical of pyramidal cells.

DISCUSSION

The main finding of the present experiments is a physiological method for the identification and separation of inhibitory interneurons and principal cells in cortical networks. Although our work was carried out in layer V of the rat, the method based on short-latency cross-correlation of spike dynamics can be used in any other layers and networks regardless of the species. The identified minority then can be used as a template for classifying the recorded population into at least two major classes (putative principal cells and interneurons) and estimate the error associated with the classification. Parallel recording of neuronal activity in the two-dimensional space also allows for the identification of anatomically functionally connected assemblies.

Identification of neuron types on the basis of synaptic interactions

The main hypothesis of the present work was that neurons with robust short-term cross-correlation reliably identify the nature of the presynaptic neuron. A prerequisite of this approach is a reliable separation of individual neurons. To date, the most widely used method for the separation of multiple neuronal activity, recorded in a small piece of tissue, is based on the temporal correlation of spikes (Drake et al. 1988; McNaughton et al. 1983; O'Keefe and Recce 1993). This can be achieved by monitoring unit activity with closely spaced multiple sensors, such as tetrodes and silicon probes (Csicsvari et al. 1999, 2003a; Gray et al. 1995; Harris et al. 2000; Quirk and Wilson 1999; Wilson and McNaughton 1993). Silicon probes with multiple, staggered recording sites used in the present study provided distinct neuronal clusters in layer 5 of the neocortex. Although the reliability of single neuron separation cannot be objectively determined without information about the intracellularly recorded action potentials (Henze et al. 2000), the Mahalanobis distance between spike clusters is a reliable indicator for the "cleanness" of the clusters (Harris et al. 2001). The geometrically precise distribution of the eight recording sites also allowed for the determination of the "center of mass", i.e., the approximate two-dimensional position of cell bodies of the putative single neurons.

One of ~400 unit pairs showed robust short-latency interactions, indicative of monosynaptic connections. Large, narrow peaks in the cross-correlograms within 3 ms were taken as

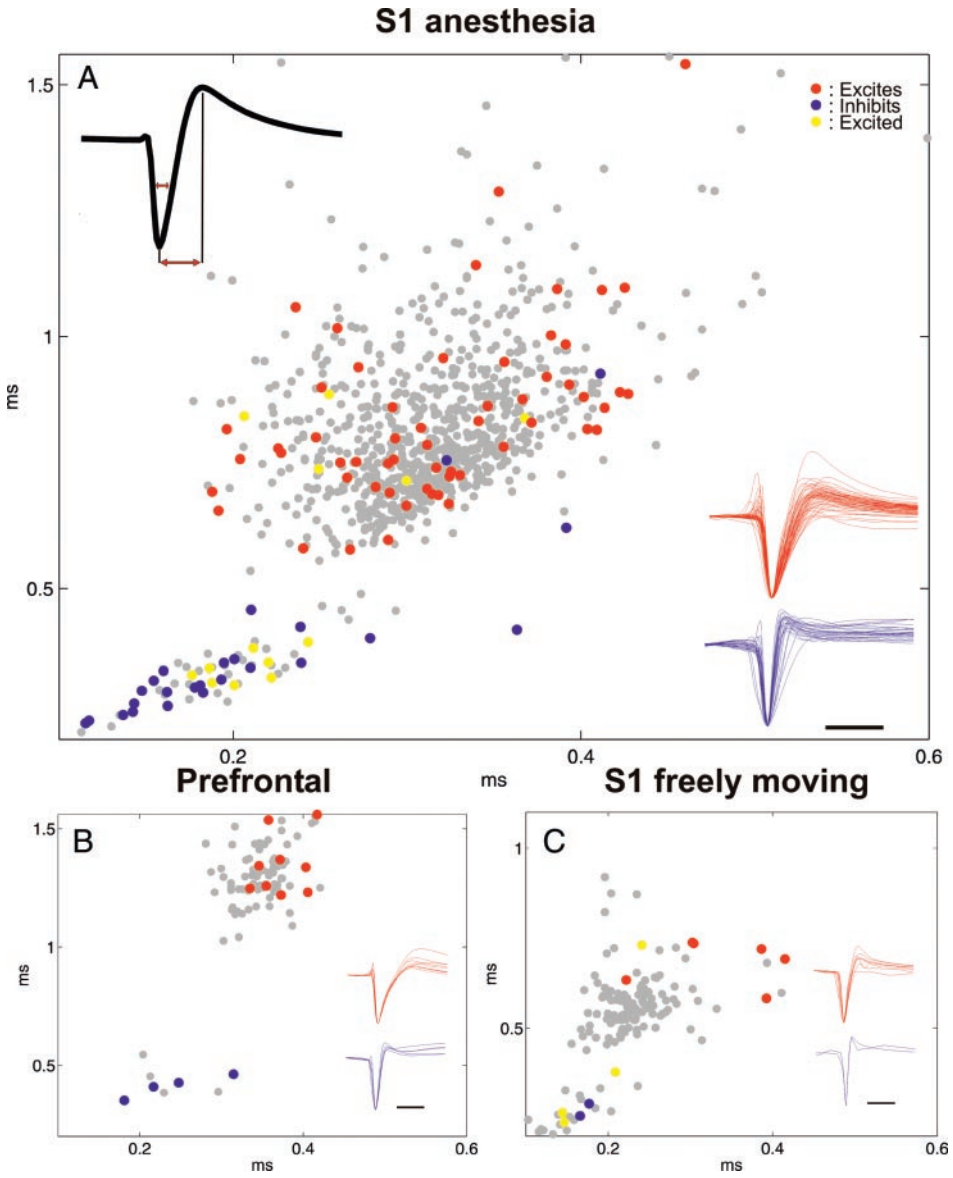

FIG. 4. Separation of putative neocortical interneurons and pyramidal cells. *A*: black trace, temporal parameters used in the clustering process (half-amplitude duration, *x* axis; trough to peak time, *y* axis). Red and blue dots: excitatory and inhibitory cells identified by monosynaptic interactions in the somatosensory cortex (S1). Green dots: neurons excited monosynaptically. Gray dots: unidentified units. *Inset*: average waveforms of identified excitatory and inhibitory units (1 Hz to 5 kHz) normalized for peak-to-peak amplitude. *B*: same as *A* but for units recorded in the prefrontal cortex (PFC). *C*: same as *A* but for units recorded by wire tetrodes in freely moving rats. Note clear separation of unit clouds in each case.

evidence that the presynaptic neuron was excitatory. Potential interactions recorded by the same recording sites within 1 ms could not be studied with the present methods because activity was ignored in this time bin by our spike-detection program. Previous studies in the hippocampus in vivo revealed high spike-transmission probabilities between pyramidal cells and interneurons either by cross-correlation or by intracellular stimulation of the presynaptic pyramidal neuron (Csicsvari et al. 1998, 1999; Marshall et al. 2002). Similarly, cross-correlation between thalamocortical and putative neocortical interneurons identified monosynaptic excitatory connections (Swadlow and Gusev 2001). Paired recordings from neocortical pyramidal cells and target interneurons also showed high reliability of synaptic transmission (Holmgren et al. 2003; Somogyi et al. 1998; Thomson and Bannister 2003), although EPSPs rather than spike transmission probabilities have been studied in those in vitro studies. It should be noted here that the cross-correlation method used here creates a bias in favor of active neurons.

TABLE 1. *Physiological interactions of neurons with wide and narrow action potentials*

Type	*n*	Exciting	Inhibiting	Excited Only	Inhibited Only	Excited and Inhibiting	Unconnected
Wide	1039	72 (6.9)	5 (0.5)	6 (0.6)	20 (1.9)	2 (0.2)*	936 (90.1)
Narrow	94	0 (0.0)	28 (29.8)	11 (11.7)	1 (1.1)	20 (21.3)*	54 (57.4)

Parentheses enclose percentages. Exciting, neurons with significant short-latency peaks in cross-correlogram (reference neuron). Inhibiting, neurons with significant short-latency suppression in cross-correlogram (reference neuron). Excited only, neurons with significant short-latency peaks in cross-correlogram (target neuron). Inhibited only, neurons with significant short-latency suppression in cross-correlogram (target neuron). Excited and inhibiting, neurons which were both excited by a reference neuron and inhibiting a target neuron. *These percentage values are excluded from the total (100%) because they reflect subgroups with dual effect.

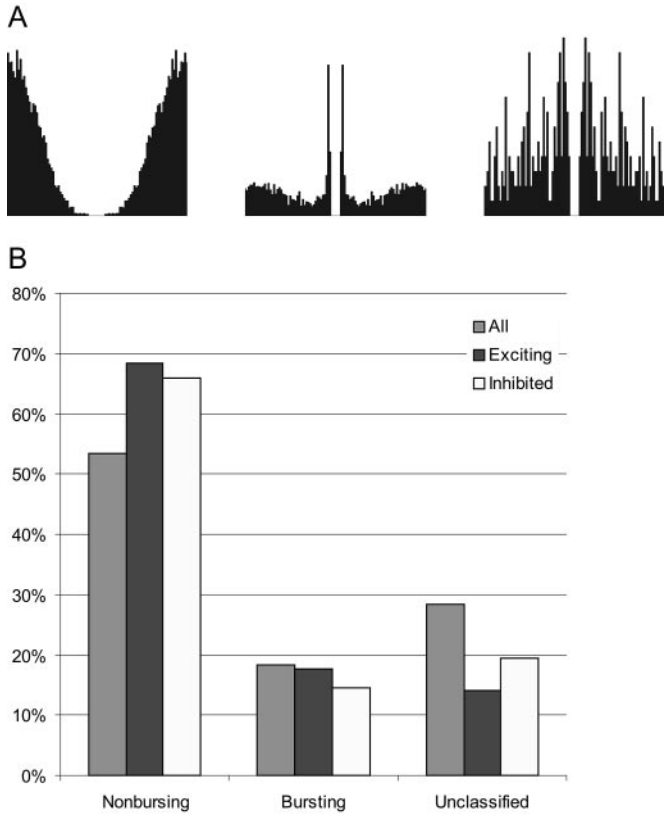

FIG. 5. Classification of putative pyramidal neurons on the basis of spike dynamics. *A*: putative pyramidal cells were classified into "nonbursting" (regular spiking; *left*), "bursting" (*middle*), and unclassified (*right*) groups on the basis of their auto-correlograms. Three examples are shown. *B*: distribution of the 3 groups. Note that the probability of monosynaptic excitation or being inhibited by an interneuron was similar in the groups.

Connected pairs with very low firing rates may have remained undetected.

Suppression of unit activity in the cross-correlogram by identified inhibitory neurons is a novel observation, although short-latency suppression in cross-correlograms has been reported earlier (Constantinidis et al. 2002). Several pairs, recorded by either the same or different shanks, displayed strong, short-latency suppression after the presynaptic spike. We regard these interactions as support for the inhibitory nature of the presynaptic neuron. In principle, a third-party bias, involving direct excitation of neuron 1 and feed-forward inhibition of neuron 2 by a third, unrecorded neuron, could also result in suppressed activity in the cross-correlogram between neurons 1 and 2. This scenario is not very likely though for the following reasons. First, in several cases we have seen strong and complete cessation of spiking in the putative postsynaptic neuron. For third-party excitation-feed-forward inhibition explanation, an additional assumption should be made that whenever neuron 1 fired, it was always discharged by the third party and not by other neurons in the network. This is unlikely given the low threshold and large convergence of excitatory inputs onto inhibitory interneurons (Somogyi et al. 1998; Swadlow 2003). Second, the spike waveform of most putative inhibitory interneurons was significantly shorter than that of the putative postsynaptic cells, indicating that they belonged to different neuron types. In support of our interpretation, paired recordings from interneurons and neocortical pyramidal cells in vitro revealed that IPSP transmission is highly reliable and effective in suppressing spiking activity brought about either by synaptic activity or intracellular depolarizing pulses (Gupta et al. 2000; Somogyi et al. 1998; Thomson and Bannister 2003). Although direct comparison of in vitro and in vivo situations is not straightforward, the temporal dynamics of in vitro measured IPSPs (Tamas et al. 1997; Thomson et al. 1996) and the time constant of spike suppression in our study (~5 ms) were similar.

A third consideration of the short-latency interactions is the nature of the postsynaptic neuron in pairs with large, short-latency peaks. Pyramidal cells excite both pyramidal cells and interneurons. Paired recordings between some layer 5 pyramidal neuron pairs by Markram et al. (1997) showed 5–15 putative release sites and relatively low probability of EPSP failures. However, spikes were never evoked in the postsynaptic neuron even by presynaptic spike bursts. Our observations indicate that at least some of the postsynaptic neurons in our pairs were interneurons. First, in the hippocampal CA1 region with very sparse local collaterals, monosynaptic peaks between neuronal pairs always involved postsynaptic interneurons (Csicsvari et al. 1998, 1999). Second, several units with a short-latency peak in our study had narrow spikes, indicating that they were interneurons (see following text). Third, some pairs had bidirectional interactions, identifying the excited postsynaptic neurons as an inhibitory cell. Fourth, none of the units with short-latency excitation had auto-correlograms that would unequivocally identified them as bursting or regular spiking, features that characterized many identified pyramidal cells. However, in several cases, the postsynaptic nature of the monosynaptically excited neuron could not be identified by the preceding criteria. Their spike width and waveform were indistinguishable from those of the pyramidal cells. It is notable though that some interneuron types in both hippocampus and neocortex have action potentials and firing rates quite similar to those of pyramidal cells (Degenetais et al. 2003; Freund and Buzsáki 1996).

Identification of small groups of neurons in the recorded population by their short-time correlations allowed us to use them as templates for the classification of the whole population. Of the several parameters tested, the duration of the wide-band recorded spike proved to be most efficient, confirming Mountcastle's original suggestion that "thin" spikes correspond to inhibitory interneurons (Mountcastle et al. 1969), at least in layer 5 of the neocortex. In the hippocampus, combination of parameters (spike duration, shape, firing rate, spike dynamics) proved more efficient for the separation of pyramidal cells and interneurons than using just a single parameter (Csicsvari et al. 1999). Previous investigators used discharge rate differences elicited by afferent stimulation to distinguish these two major categories (cf. Swadlow 2003). In our experiments, firing-rate differences did not provide reliable separation, although many inhibitory neurons and unidentified short-duration spike neurons fired at high frequency. The auto-correlograms of physiologically identified and putative interneurons were never as fast decaying or slow rising as those of pyramidal neurons. Nevertheless, there was an overlap with putative pyramidal cells in the unclassified group.

Unit classification based on spike duration resulted in a clear bimodal distribution with ~7% of units belonging to the interneuron group. Approximately 15-20% of neocortical neu-

rons are believed to be GABAergic and are equally distributed in the cell body and dendritic layers of the cortex (Beaulieu and Colonnier 1983). Although there are no separate percentage estimates for interneurons in layer 5 only, the 7% figure appears low. One potential explanation for this low number is that not all inhibitory interneurons have short-duration action potentials (Freund and Buzsáki 1996). Indeed, a minority of neurons that inhibited their target partner(s) but had wider spikes than the designated interneuron group generated most of the classification errors in the identified cell groups. Furthermore, a portion of the units that were excited at a monosynaptic latency had wide spikes. Some or all of these excited neurons with wide spikes could belong to the GABAergic interneuron group. In the hippocampus, most interneurons with cell bodies in the pyramidal layer have short-duration spikes (putative basket and chandelier cells), whereas spike duration of stratum oriens/alveus interneurons is quite heterogeneous (Csicsvari et al. 1999). A large portion of all narrow spikes cells (40%) was either inhibiting other neurons or excited by a presynaptic partner. This proportion is exceptionally high compared with very low incidence of short-latency interactions in the population as a whole (1 in 400 pairs). This finding indicates that most spike-spike interactions occur among pyramidal-interneuron pairs rather than among pyramidal-pyramidal cell pairs.

Although physiological identification of neuronal interactions support the general validity of spike duration-based classification of neurons, it involves both commission and omission errors. For example, a few units in the narrow spike group without significant interactions had bursty auto-correlograms. Because no such spike dynamics was observed in the physiologically identified group, it is possible that this subgroup corresponds to the "chattering" or fast bursting neurons described earlier (Azouz et al. 1997; Steriade et al. 1998). The ambiguity of a small portion wide spike units has been discussed in the preceding text. Another obvious caveat is that most of our results were carried out under anesthesia, which may have influenced the firing patterns of neurons. We expect therefore that firing rate differences and spike dynamics features may prove additionally useful parameters in unit classification in drug-free behaving animals (Constantinidis and Goldman-Rakic 2002). Neuron identification on the basis of short-term cross-correlations can serve as a reliable tool for further improvement of interneuron and principal cell separation.

Our identified and putative pyramidal cells showed two extreme spike dynamics: bursting with large peaks at 3-10 ms in the auto-correlogram and regular spiking corresponding to a slow slope of the auto-correlogram. In vitro investigations also described these two modes of firing patterns and attributed burst propensity to distinct dendritic morphology (Kim and Connors 1993; Mainen and Sejnowski 1996). Nevertheless, the relationship between the in vitro and in vivo groups remains to be investigated. We found no differences in their firing rates, ability to excite target neurons, or probability of being inhibited by interneurons.

Circuit analysis

Most axonal wiring in the neocortex is local (Douglas et al. 1995) and most neuronal interactions occur locally. Analysis of these local interactions requires high-density recording of unit activity. The two-dimensional silicon probes used here appear ideal for such a task because of their ability to record from large number of units and because multiple recording sites allow for the determination of the spatial layout of the recorded units (Csicsvari et al. 2003a). After identification of neuronal types, as described here, functional connectivity in local circuits can be studied (Holmgren et al. 2003) at least between pyramidal cells and interneurons. Further work in behaving animals will reveal how such local interactions are modified by state changes and experience (Constantinidis et al. 2002; Csicsvari et al. 1998).

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