Interlaminar Differences of Intrinsic Properties of Pyramidal Neurons in the Auditory Cortex of Mice

Cortical information processing depends crucially upon intrinsic neuronal properties modulating a given synaptic input, in addition to integration of excitatory and inhibitory inputs. These intrinsic mechanisms are poorly understood in sensory cortex areas. We therefore investigated neuronal properties in slices of the auditory cortex (AC) of normal hearing mice using whole-cell patch-clamp recordings of pyramidal neurons in layers II/III, IV, V, and VI in the current- and voltage clamp mode. A total of 234 pyramidal neurons were included in the analysis revealing distinct laminar differences. Regular spiking (RS) neurons in layer II/III have significantly lower resting membrane potential, higher threshold for action potential generation, and larger $K_T$ and $I_h$ amplitudes compared with layer V and VI RS neurons. These currents could improve temporal resolution in the upper layers of the AC. Additionally, the presence of a T-type Ca$^{2+}$ current could be an important factor of RS neurons in these upper layers to amplify temporally closely correlated inputs. Compared with upper layers, lower layers (V and VI) exhibit a higher relative abundance of intrinsic bursting neurons. These neurons may provide layer-specific transfer functions for interlaminar, intercortical, and corticofugal information processing.

Keywords: auditory processing, cortical layers, $I_h$, interspike interval, patch-clamp, T-current

Introduction

The anatomical similarities between auditory and other sensory cortices have led to the hypothesis that fundamental principles of neocortical structure and connectivity are common to all sensory (and other) cortical areas (Rockel et al. 1980). However, there are several unusual and perhaps unique anatomical features of the auditory cortex (AC) that complicate attempts to define common rules for sensory neocortical organization. A major difference between the primary AC and other sensory cortices is the projection of many layer III neurons of the AC across the corpus callosum (Winer 1984, 1992). Moreover, small pyramidal neurons in lower layer III and layer IV appear to be the key neurons receiving thalamic afferents in the AC (Smith and Populin 2001), rather than spiny stellate neurons as in visual and somatosensory cortices (Jones 1975; Lund et al. 1979; Simons and Woolsey 1984; Linden and Schreiner 2003; Staiser et al. 2004).

The AC appears to perform sound analysis by combining spatially distributed coincident or time-correlated neuronal responses (Ehret 1997). Thus, temporal-coding is an essential task e.g. for the localization of sound sources and the processing of speech. Accordingly, the kinetics of inhibitory responses are faster in the AC (Buonomano 2000; Hefti and Smith 2002), and the AC may have a unique class of neurons that generate action potentials (APs) only transiently upon depolarization (Metherate and Aramakis 1999). Further unique features of the AC seem to be neurons of layer II/III that are connected to each other through 2 types of synaptic connections distinguished by release probability and synaptic strength (Atzori et al. 2001).

Interlaminar cortical information processing depends not only upon summation of excitatory and inhibitory inputs but also on intrinsic neuronal properties modulating these synaptic inputs (Metherate and Aramakis 1999). More recently, some neuronal properties were compared in layer II/III and layer V (Atzori et al. 2004). There were no obvious interlaminar differences in the passive properties, but neurons in layer II/III displayed a lower activation threshold compared with layer V neurons. Estimates suggested that the number of simultaneously active presynaptic neurons required to reach threshold must be twice as high in layer V neurons (Atzori et al. 2004).

Concerning the control of neuronal excitability, the hyperpolarization-activated inward current $(I_h)$ is of particular interest. This current contributes to several basic physiological processes including resting membrane conductance, stabilization of resting potential and the limitation of temporal summation of excitatory input (Magee 1998, 1999). Therefore, the $I_h$ is thought to represent a key modulator of neuronal excitability (Magee 1999; Berger et al. 2001, 2003). A further important factor of cortical excitability is the transient, low-voltage activated Ca$^{2+}$ current (T-current; Jahnsen and Linna 1984; Sutor and Ziegglansberger 1987), which transiently augments the excitability of neurons and boosts inputs during a very distinct time frame (Deisz et al. 1991).

Despite the studies mentioned above, the data concerning intrinsic properties of the AC are rather rare. Therefore, we investigated intrinsic neuronal properties in slices of the AC of mice using whole-cell patch-clamp recordings. We compared firing behavior during current injections and in particular properties of pyramidal neurons in layers II (including upper layer III), IV (including lower layer III), V, and VI. The data obtained indicate profound interlaminar differences in intrinsic neuronal properties and in the proportion of different neuron types. These characteristics are discussed in the context of potential filtering and amplification mechanisms for intercortical (columnar) processing.

Material and Methods

Preparation of Mouse AC Slices

Sixty-six NMRI and 10 Balb/C mice between days 25–70 after birth were used for the experiments. Mice possess adult-like hearing abilities approximately 18–20 days after birth (Song et al. 2006) and both strains do not have hearing impairments before the age of 3 months (Zheng et al. 2006).
The animals were anesthetized with ether and decapitated, as approved under the European Communities Council Directive and institutional animal care guidelines. The skull was opened dorsally and a tissue block, which contained the caudal half of the cerebrum, was removed. The specimens were incubated in ice-cold (about 4 °C) carbogenated artificial cerebrospinal fluid (ACSF) containing NaCl (124 mM), KCl (5 mM), NaH2PO4 (1.25 mM), MgSO4 (2 mM), d(+)-glucose (10 mM), NaHCO3 (26 mM), and CaCl2 (2 mM), carbonized with 95% O2/5% CO2; pH was 7.4 (Deisz 1999; Strauss et al. 2004). After 1-2 min, the block was cut in halves in the mid-sagittal plane and one half was mounted with cyanoacrylate glue on the bottom of a vibratome chamber. Frontal 300-μm slices of the area containing the AC were cut (Caviness and Frost 1980; Paxinos and Franklin 2001; Geisler and Ehret 2004) using a vibratome (TPI, St. Louis, MO). The second half was stored in ice-cold carbogenated ACSF and cut 10-15 min later. The slices were stored in beakers containing ACSF equilibrated continuously with carbogen at 32 °C for 1-2 h. Afterward, the beakers were stored at room temperature.

**Electrophysiological Recordings**

The slices were transferred into a submerged-type recording chamber continuously perfused with 1-2 ml/min carbogenated ACSF at 32 °C (TC-102 Temperature Controller, Medical Systems, Greenvile, NY). The AC was visually localized within the slice according to the coordinates given in recent literature (Paxinos and Franklin 2001; Geisler and Ehret 2004). Due to variations in the angle of cutting in the frontal plane it is not possible to distinguish primary and secondary areas of the AC. Due to these inaccuracies we refer to the area of recording as AC but most neurons (probably between 90% and 95%) were recorded in the primary auditory area (Paxinos and Franklin 2001; Geisler and Ehret 2004).

Pyramidal shaped neurons of different layers were selected and approached under visual control using infrared video microscopy with a 40× magnification water-immersion objective (Olympus, Hamburg, Germany). For whole-cell whole-cell patch-clamp recordings a patch-clamp amplifier together with the corresponding PULSE 8.6 software (HEKA Elektronik, Lambrecht, Germany) was used. Patch-clamp pipettes were pulled from borosilicate glass pipettes with an outer diameter of 1.5 mm and inner diameter of 0.86 mm (Science Products, Hofheim, Germany) on a P 87 micropipette puller (Sutter Instruments, Novato, CA). The pipettes were filled with a solution containing: K+ gluconate (120 mM), NaCl (20 mM), MgCl2 (1 mM), CaCl2 (1 mM), N-(2-Hydroxyethyl)-piperazine-N’-2-ethanesulfonic acid (10 mM), ethylene glycol-bis(2-aminoethyll ether)-N,N,N’,N’-tetraethylic acid (11 mM), and Mg2+-ATP (2 mM); pH 7.2 adjusted with KOH (Strauss et al. 2004). All substances used were of analytical grade (Merck, Darmstadt, Germany). The resulting pipette resistance was in the range from 4 to 10 MΩ. A seal of 1 GΩ or more was obtained and recordings were carried out only if the resulting series resistance was less than 20% of the passive membrane resistance (\(R_{m0}\), see below). Recordings were accepted only if changes of the initial access resistance remained within 20%. In current-clamp mode 400 ms current pulses were applied in 25 pA steps starting at ~300 pA up to a maximum of 400 pA. During the voltage clamp experiments, the holding potential was set to ~60 mV. Hyperpolarizing command potential to ~140 mV for 2 s elicited inward rectifying currents. The selective antagonists for \(K_\text{Ca}^+\) (Ba2+) and \(I_\text{Na}^+\) (ZD7288; Cs2+ for both) were used to isolate individual currents. During these experiments, the slices were perfused at least 10 min with carbogenated ACSF including 4 mM CsCl, 1 mM BaCl2, or 0.05 mM ZD7288 (Tocris, Bristol, UK). For bath application of Ba2+ a modified ACSF was used containing no NaH2PO4 and an equimolar amount of MgCl2 instead of MgSO4. During recording, the shape of neurons and the position of pipettes were documented by taking digital images of the video signal (40× magnification, Fig. 1a) after completing recordings from a given neuron, the laminar position of the pipette (in relation to the rhinal fissure; Fig. 1b) was documented by taking digital images of the video signal (10× magnification water-immersion objective). Slices were then formalin-fixed and Nissl-stained to verify the topographical relations of recorded areas.

In some cases, 1% biocytin (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was added to the pipette solution. When biocytin containing pipette solutions were used, neurons were recorded for at least 10 min before the pipette was carefully withdrawn. Afterwards, the slices were individually stored for 1 h in carbogenated ACSF at room temperature before transfer to fixation solution (4% paraformaldehyde in 0.1 mM phosphate buffer). Fixed slices were stored at 4 °C, up to one week. For staining, slices were washed 2 h in 0.1 mM phosphate-buffered saline (PBS) and incubated 2 h in a mixture of 2 mg/ml bovine serum albumin in PBS (PBS-A), phencyclidine hydrochloride and 0.3% Triton X (1 ml/0.5 μl/30 μl, respectively). Afterwards, slices were washed in PBS (2 × 10 min, 1 × 20 min) and incubated with a horseradish peroxidase streptavidin complex (Perkin Elmer, Boston, MA) in PBS-A (1:200) at 4 °C over night. The next day slices were washed in PBS (2 × 15 min, 1 × 30 min) and preincubated for 15 min in a mixture of Tris(hydroxymethyl)amaminomethane (50 mM in H2O), 3,3’-diaminobenzidine tetrahydrochloride (50 mg/ml)/imidazol (1 M) (1 ml/10 μl/10 μl, respectively, pH = 7.6). Staining reaction was started by adding 50 μl of 3% (NH4)2SO3 and 25 μl of 0.03% H2O2. Fifteen minutes later the slices were washed in PBS (2 × 15 min), mounted on coated glass slides and dehydrated by a standard alcohol series for embedding in Entellan (Merck).

**Data Analysis**

From current-clamp experiments, the following parameters were determined using the PULSE FIT 8.6 software (HEKA Elektronik): resting membrane potential (\(E_{\text{m0}}\)), membrane time constant (\(\tau_m\)), membrane resistance (\(R_m\)), firing behavior (initial bursting or regular spiking (RS)), AP threshold (\(E_{\text{thr}}\)), AP amplitude (\(A_{\text{pamp}}\)), and AP width (\(A_{\text{pwidth}}\)). \(R_m\) was measured at the ~100 pA current step. The \(\tau_m\) was determined by fitting an exponential function to the induced potentials at the ~100 pA current step. The \(E_{\text{thr}}\) was calculated by the statistical mean between the lowest potential where APs were elicited and the highest potential where spiking did not occur. The activation threshold (\(A_{\text{thr}}\)) was calculated by the difference of \(E_{\text{thr}}\) and \(E_{\text{m0}}\) \(A_{\text{pamp}}\) was measured from \(E_{\text{m0}}\) to the peak of the first AP in the sweep of the lowest potential where APs were elicited. \(A_{\text{pwidth}}\) was measured at 1/2 \(A_{\text{pamp}}\) of the same AP. The adaptation ratio of interspike intervals (\(I_{\text{ISI}}\)) was calculated by the difference of the lowest potential where APs were elicited and the highest potential where spiking did not occur.
measured in response to the lowest amplitude current pulse that resulted in firing of at least 6 APs (or an initial burst followed by at least 5 APs) throughout the 400 ms pulse. $I_{\text{SLim}}$ was calculated as the ratio of the first ISI and the last ISI. To further analyze $I_{\text{SLim}}$, in some cases, positive 1000-ms current pulses were applied in 10 pA steps. To calculate the surface area (SA) of neurons, the membrane capacity ($C_m$) was determined using $R_m$ and $\tau_m$ and divided by the specific capacity of 1 $\mu\text{F/cm}^2$ (Hille 1992).

Neurons included in this analysis met the following criteria: $E_{\text{m}}$ between −65 and −85 mV, $A_{\text{amp}} > 80$ mV, $A_{\text{width}} < 2.6$ ms, and firing patterns characteristic for pyramidial neurons (Agmon and Connors 1992; Metherate and Aramakis 1999; Rose and Metherate 2005).

Current traces (recorded during hyperpolarizations from −60 to −140 mV) were approximated by double-exponential fits (between −5 ms after the capacitance peak and at −10 ms before the end of the current trace) to original traces (without leakage subtraction). These double-exponential fits yielded the magnitudes of $K_h$ and $I_h$ (Fig. 2). The amplitude of the latter was determined by back-extrapolating the current to the start of the voltage step (Sciancalepore and Constanti 1998; Koch and Grothe 2003; Strauss et al. 2004; Fig. 2b). The difference between the total current and $I_h$ was taken as the magnitude of $K_h$. The validity of this approach was corroborated by pharmacological isolation of the 2 currents by Ba$^{2+}$ or ZD7288 application (Fig. 2a,c). As a further measure of $I_h$, the amplitude of the so-called "sag" during a hyperpolarizing current step from 0 to −300 pA was determined (Fig. 2d). Furthermore, the current densities $K_h/SA$ and $I_h/SA$ were determined.

To analyze the interlaminar differences of pyramidial neurons in the AC, we took the peculiarities of the AC into account and segregated the layers according to their thalamic and intracortical input (Linden and AC, we took the peculiarities of the AC into account and segregated the layers according to their thalamic and intracortical input (Linden and Schreiner 2003). Therefore, upper layer III was pooled with layer II (referred to layer II/III) and lower layer III with layer IV (referred to layer III/IV). Layers V and VI were handled separately.

Values in the text are given as means ± SD. Univariate $t$-tests and chi-squared tests were used (SPSS, SPSS Inc., Chicago, IL) to test for significance. $P$-values smaller than 0.01 were taken to indicate significant differences (otherwise mentioned in the text).

### Results

A total of 234 pyramidial neurons of the AC were analyzed. The measurements of all neurons (see below) did not show any correlation with the animal’s age or strain so that we pooled these data. Based on the firing pattern to positive current injections, 2 basic response types were distinguished: RS neurons ($n = 181, 77.4\%$) and intrinsically bursting (IB) neurons ($n = 53, 22.6\%$; Fig. 3). Nine neurons were biocytin-labeled during recording and revealed pyramidal shape and dendritic spines (Fig. 1c,d).

#### Comparison of RS and IB Neurons

In IB neurons, depolarizing current pulses at resting potential evoked bursts consisting of a series of 2–5 APs superimposed on a slow envelope of depolarization (Fig. 3a,b). The ISI of the first 2 APs within the burst was nearly constant (< ±0.3 ms) when the depolarizing current was increased (Figs 3a, 4a). After the initial burst, most of these neurons ($n = 48$) showed sustained firing of APs for the time of the current injection (Fig. 3a). We refer these neurons as "latent bursters" (IBL) because some of these neurons did not generate bursts on application of small depolarizing current pulses but a single first AP followed by a short pronounced after-depolarization (an equivalent after-depolarization is shown in the last burst of trace in Fig. 3b). With larger depolarization, a burst with 2–5 APs occurred. Five IB neurons showed multiple bursts during a 400-ms current injection near threshold (Fig. 3b) which we refer to as repetitive IB neurons (IBR). Four of these IBR neurons were found in layer V (11.5% of neurons measured in layer V; Fig. 4b) and one in layer IV (1.2% of neurons measured in layer III/IV; Fig. 4b). Injecting current pulses >75 pA above $E_{\text{thr}}$ evoked regular firing after the initial burst in these neurons (not shown in figures).
RS neurons responded to suprathreshold depolarizing current injection with a sustained and almost regular firing throughout the current duration (Fig. 3c,d), the AP frequency decreased slightly during a given constant current injection, though. Increasing the magnitude of injected current, increased the frequency of APs (Fig. 4a).

The adaptation ratio of the ISI (ISIad) during constant current injection was between 0.2 and 1 in the RS neurons and thus significantly higher compared with IB neurons (ISIad 0.04 to 0.21; Table 1; Fig. 4c). As illustrated in Figure 4c, ISIad of most RS neurons clustered between 0.45 and 0.65. According to this distribution (Fig. 4c), we refer to RS neurons with ISIad values smaller than 0.45 as type I RS or adapting RS neurons (RS I; Figs 3c, 4c), as opposed to type II RS neurons (RS II), having ISIad greater than 0.45 (Figs 3d, 4c). This segregation of pyramidal neuron types becomes clearer when the frequency corresponding to the ISI between the first 2 APs at different current injections is compared. The frequency of the first 2 APs is nearly constant in IB neurons, whereas the following AP frequencies (\(f_{AP}\)) became faster when injected currents were increased (Fig. 4a; note that the second \(f_{AP}\) is the reciprocal value of the first ISI following the burst). In contrast, the first \(f_{AP}\) of RS I neurons increased significantly in relation to both the following AP frequencies and the first \(f_{AP}\) of a lower suprathreshold current injection (Fig. 4a). In RS II neurons the first \(f_{AP}\) increased only slightly relative to the following frequencies when currents were increased (Fig. 4a). It is important to note that RS I neurons could show a similar firing pattern as some IB neurons but the first ISI of RS I neurons decreased when depolarizing current was increased and the first AP of RS I neurons was followed by a less pronounced after-depolarization compared with IB neurons (Fig. 3c).

Considering the marked effects of \(I_h\) on neuronal excitability (e.g. Strauss et al. 2004), we routinely measured the amplitude of the sag as a possible index for the magnitude of \(I_h\) (Fig. 2d). The sag ranged from 0.17 to 4.79 mV (1.51 ± 1.05 mV, \(n = 64\)) in RS neurons and from 0.21 to 3.97 mV (1.62 ± 0.96 mV, \(n = 40\)) in IB neurons, the differences were not significant. However, the limitations of this estimate (cf. Day et al. 2005; Aponte et al. 2006) could be demonstrated by measuring the sag during application of Ba\(^{2+}\) to block \(K_{ir}\) channels. This revealed a significant increase of the sag in RS neurons by approximately 1260% (control: 1.1±0.79 mV, Ba\(^{2+}\) application: 13.87 ± 5.66 mV; \(n = 9\), \(P < 0.001\)). Therefore, a direct evaluation of the magnitude of the \(I_h\) was carried out in voltage clamp (Fig. 2), revealing a comparable \(I_h\) magnitude (control: -140.3 ± 61.5 mV, Ba\(^{2+}\) application: -156.3 ± 34.9 mV; \(n = 9\), \(P = 0.13\)).

Interestingly, even though \(I_h\) and \(K_{ir}\) amplitudes were on average not significantly different between RS and IB neurons (Table 1), the ratio of both currents (\(I_h/K_{ir}\)) was significantly higher in IB neurons (IB: 0.44 ± 0.26, RS: 0.37 ± 0.18, \(P < 0.0001\)). Moreover, the fast time constant of \(I_h\), \(\tau_{fast}\), was significantly higher in IB neurons (Table 1).

**Ionic Mechanisms of ISI Adaptation in RS Neurons**

The adaptation of the ISI of RS neurons may be due to activation of voltage-dependent K\(^+\)-currents (Schwindt et al. 1988; Lorenzon and Foehring 1993). We therefore tested the effects of K\(^+\)-current antagonists on ISIad (Fig. 4d; \(n = 18\) with Cs\(^+\), \(n = 9\) with Ba\(^{2+}\), and \(n = 7\) with ZD7288; Sciancalepore and Constanti 1998; Bal and Oertel 2000; Koch and Grothe 2003; Robinson and Siegelbaum 2003; Strauss et al. 2004). Accordingly, the ISIad values are approximately 33% higher with unspecifically blocked K\(^+\)-current by Cs\(^+\) application than in the control (Fig. 4d; 0.75 ± 0.12 vs. 0.5 ± 0.12, \(P < 0.001\), \(n = 18\)). This increase was caused by an increase of the first ISI, whereas subsequent ISIs (including the second ISI) during a trace of constant current application were essentially unaffected (Fig. 5a, c). Interestingly, the selective \(I_h\) antagonist ZD7288 had the same effect regarding ISIad as Cs\(^+\) application (Fig. 4d; 0.74 ± 0.08 vs. 0.5 ± 0.09, \(P < 0.01\), \(n = 5\)), but Ba\(^{2+}\) had no effect on ISI (Fig. 4d).
A considerable fraction of adaptation remained during K⁺-current block with both Cs⁺ and ZD7288 and ISIad was still below 1 (n = 2, not shown in figures). We therefore evaluated a possible contribution of T-currents (Jahnsen and Linnäs 1984; Sutor and Ziegglänzer 1987; Deisz et al. 1991) to the ISIad. To this end we applied the fairly selective T-current antagonist Ni²⁺ or depolarized the neurons by constant current injection (Em –65 mV) for steady-state inactivation of the T-current. Bath application of Ni²⁺ resulted in a marked increase of ISIad, from 0.48 ± 0.15 to 0.65 ± 0.16 (n = 20, P < 0.001). Constant current depolarization caused comparable alterations of ISIad from 0.58 ± 0.2 to 0.77 ± 0.05 (n = 33, P < 0.01). On average, the first ISI increased from 30.3 ± 10.5 ms to 40.4 ± 12 ms in Ni²⁺ (n = 19, P < 0.01) and from 31.1 ± 12 ms to 39.9±11.1 ms with depolarization (n = 38, P < 0.01). The ISI of the last APs was little affected under these conditions, for example, 67.7 ± 16.3 ms in control and 66.2 ± 13.7 ms in Ni²⁺, obvious from the nearly constant number of APs during the analyzed current pulses (Fig. 5). This indicates that the apparent ISIad of RS neurons consists of 2 distinct components, one with pharmacological properties indicative for h and the other with pharmacological and biophysical properties suggestive for the T-type Ca²⁺-current. Accordingly, it is likely that RS I neurons have a higher expression level of the channels to adjust ISI ad. These evaluations are far from a detailed biophysical characterization of the underlying mechanisms but provide at least a useful tool to distinguish between functional different sets of pyramidal neurons.

**Interlaminar Comparisons**

Previous studies of rodent sensory cortex areas (e.g. Silva et al. 1991; Agmon and Connors 1992) showed a high number of IB neurons in deeper layers. We therefore compared the distribution of RS and IB neurons in different layers: layer II/III had significantly the lowest (8.3%), whereas layer VI had the highest percentage of IB neurons (35.5%; Fig. 4b). However, the latter proportion was not significantly different from that in layers III/IV and V (27.6% and 28.6%; Fig. 4b). The distribution of the 2 RS neuron types (cf. Fig. 4c) was not significantly different in layer V and VI but layer II/III had significantly more RS II neurons (69.1%) than deeper layers and layers II/III and III/IV had significantly fewer RS I neurons (22.6% and 19.5%) compared with deeper layers (25.7% and 25.8%; Fig. 4b).

**Interlaminar Comparison of RS Neurons**

To further analyze the characteristics of RS neurons in the different cortical layers, we compared the 15 standardized measurements and found significant differences in 10 of these measurements (Table 2). Because data of all layers were tested at once for differences of each parameter (and not pair wise for the sake of clarity) by using univariate f-statistics we will describe trends of significantly different measurements below.

![Figure 4](https://academic.oup.com/cercor/article-abstract/19/5/1008/296251/1012)

**Figure 4.** (a) Frequencies of AP generation (fAP) as function of the number of ISI (ISI#) of the 3 different pyramidal neuron types. The lower traces (solid squares) were current injections near threshold (25–50 pA above threshold), upper traces (open squares) were higher current injections (125–150 pA above threshold). The different neuron types can be distinguished by the change in frequency of the first ISI relative to subsequent intervals with increases of current injections. Note that the second ISI of IB neurons was the first interval following the burst. (b) Box plot of the proportions of RS and IB (latent bursting [IB] and repetitive bursting [IB]) neuron types in layers II/III to VI of the AC. Note the higher percentage of IB neurons in deeper layers and the high percentage of RS II neurons in layer II/III (P < 0.01, note that absolute numbers of neuron types were used for the chi-squared test). (c) Distribution of numbers of RS and IB neurons regarding ISIad. RS and IB neurons were clearly distinguished by ISIad (f-test, P < 0.001). For definitions of RS neuron types see main text. (d) Plot of ISIad changes in RS neurons from layer III and IV during the application of Cs⁺ (4 mM), ZD7288 (0.05 mM), during de polarization, and during the application of Ni²⁺ (200 μM) or Ba²⁺ (1 mM). Note the significant increase (f-test) of ISIad during depolarization and the application of Cs⁺, ZD7288, and Ni²⁺.
the complete list of means and standard deviations is given in Table 2. RS neurons of layers II/III and III/IV had lower $E_{\text{m}}$ ($-76.0 \ \text{mV}$ and $-75.7 \ \text{mV}$) than neurons in layers V and VI ($-73.8 \ \text{mV}$ and $-71.7 \ \text{mV}$). Correspondingly, the latter had the smallest $A_{\text{Pamp}}$ (99.5 pA; Table 2). $R_m$ had by far the smallest value in layer II/III RS neurons (55.1 MΩ) compared with other layers (Table 2). $E_{\text{m}}$ was least negative in RS neurons of layer II/III (–9.2 mV), intermediate in layer III/IV (–5.3 mV), and lowest in layers V and VI (–5.4 mV and –5.49 mV; Table 2). Because layer II/III had the most negative $E_{\text{m}}$ but the least negative $E_{\text{thr}}$, $A_{\text{thr}}$ was highest in these neurons (26.8 mV) and lowest in neurons of layers V and VI (19.6 mV and 16.8 mV; Fig. 6a; Table 2). $I_{\text{SA}}$ and $A_{\text{SAthm}}$ were not significantly different among layers in RS neurons. $\tau_m$ had largest values in layer V (10.45 ms) and other layers were not clearly different (3.2–7.4 ms; Table 2). $SA$ was largest in layer II/III (11.5 $\times 10^3 \ \mu\text{m}^2$) and smallest in layers III/IV and VI (8.8 $\times 10^3 \ \mu\text{m}^2$ and 8.9 $\times 10^3 \ \mu\text{m}^2$; Table 2). RS neurons of layer II/III had the largest amplitudes of $K_h$ and $I_h$ (–847.0 pA and –315.6 pA) and neurons in layer III/IV and V had the smallest amplitude of these currents (cf. Table 2). Interestingly, the densities of these currents ($K_h/SA$, $I_h/SA$) were not significantly different between RS neurons of different layers but showed the same trend as the current amplitudes (layer II/III neurons had the largest densities and neurons in layer III/IV and V the smallest) except the high values of current densities in layer VI (–93.1 pA/µm$^2$ and –28.5 pA/µm$^2$) due to the small SA of these neurons (cf. Table 2). In addition, RS neurons of layer V had the slowest time course of $I_h$ ($\tau_{\text{slow}}$ 21.9 ms), RS neurons of the other layers exhibited much faster and indistinguishable time courses (9–11 ms). The slow time constant of $I_h$ ($\tau_{\text{slow}}$) was comparable in all layers.

Figure 6b demonstrates the dependency of $R_m$ on the sum of $K_h$ and $I_h$ amplitudes: higher $K_h$ and $I_h$ amplitudes were correlated with lower $R_m$ values and vice versa.

The function of the fit is $R_m = 164.12 \ e^{0.0008 (K+h)}$ and its correlation coefficient $r^2 = 0.54$. Furthermore, this plot reveals that the significantly lower $R_m$ values in layer II/III (crosses in Fig. 6b) were correlated with their high $K_h$ and $I_h$ amplitudes. This relationship is further substantiated by the effects of Cs+ on $R_m$ in layer II/III RS neurons, Cs+ increased $R_m$ by 633% ± 597% ($n = 16$), in layer III/IV by 437% ± 288% ($n = 14$; $P < 0.05$; layer V and VI neurons have not been tested with Cs+ so far). This indicates that the low values of $R_m$ in layer II/III RS neurons were mediated by Cs+-sensitive currents.

15 of the 234 neurons (6.8%) measured had $I_h$ amplitudes smaller than 50 pA. These neurons were excluded from our statistics regarding differences of $I_h$ parameters. However, eleven of these 15 neurons were found in layers III/IV and V, which had the lowest $I_h$ amplitudes compared with layers

Table 1

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<th>Intrinsic properties of RS and IB neurons in layers II/III to VI in the AC (*$F$-test, $P &lt; 0.01$)</th>
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Figure 5. Voltage traces during a 1000 ms suprathreshold current injection of a RS neuron in upper layer III of the AC. The scale bars apply to traces (a)–(d). The amplitudes of APs in (a)–(d) have been truncated. (a) Control condition ($E_{\text{m}} = -77.1 \ \text{mV}$, current amplitude 230 pA). (b) Depolarized condition to $E_{\text{m}} = -66.7 \ \text{mV}$ (current amplitude 235 pA). (c) Bath application of 4 mM CsCl ($E_{\text{m}} = -75.7 \ \text{mV}$, current amplitude 210 pA). (d) Bath application of 4 mM CsCl and 0.2 mM NiCl$_2$ ($E_{\text{m}} = -75.7 \ \text{mV}$, current amplitude 130 pA). Note the increase of $ISL_{\text{m}}$ in the depolarized condition, in the presence of Cs$,^+$, and the additional increase in the presence of Ni$^{2+}$.
II/III and VI (Table 2). Accordingly, it is likely that the neurons with these low \( I_h \) amplitudes are not mere artifacts but may be examples of the smallest range of \( I_h \) amplitudes in the AC.

**Interlaminar Comparison of IB Neurons**

In IB neurons, interlaminar differences were less pronounced than in RS neurons. Only \( E_{\text{thr}} \) was significantly different (Table 2). \( E_{\text{thr}} \) was lowest in layer V neurons (~59.7 mV), intermediate in layers III/IV and VI (~55.7 and ~54.4 mV) and highest in layer II/III (~48.0 mV).

**Discussion**

**Methodological Considerations**

Although RS neurons were formerly categorized as adapting or nonadapting (Agmon and Connors 1992), we found a continuum of \( IS_{\text{ad}} \) values (between 0.2 and 1) in these neurons which is similar to recent results (Metherate and Aramakis 1999; Rose and Metherate 2005). However, most of the neurons analyzed in our experiments were nonadapting (Agmon and Connors 1992), we found a continuum of \( IS_{\text{ad}} \) values (between 0.2 and 1) in these neurons which is similar to recent results (Metherate and Aramakis 1999; Rose and Metherate 2005).

For some (approximately 25% of RS neurons) whole-cell recordings in this study, \( IS_{\text{ad}} \) decreased over time (up to 13% in >10 min). An earlier paper (Metherate and Aramakis 1999) reported a similar decrease in some of their neurons and hypothesized that this finding probably reflects washout of an intracellular constituent, for example, Ca\(^{2+}\) buffer or free Ca\(^{2+}\) because \( IS_{\text{ad}} \) is thought to involve spike-induced activation of Ca\(^{2+}\)-dependent K\(^+\)-currents (Schwindt et al. 1988; Lorenzon and Foehring 1993). Nevertheless, in our experiments, current-clamp measurements to analyze \( IS_{\text{ad}} \) were conducted during the first 3 min after rupture of the cell-attached patch so that a possible washout should have been minimal. Moreover, the loss of intracellular constituents due to diffusion into the pipette might contribute to a time-dependent depression of the \( I_h \) referred to as “ rundown” (Zhou and Lipsius 1993). To obtain a near physiological view, we measured the \( I_h \) amplitudes during the first minutes (<3 min) after rupture of the patch, when loss should have been minimal. The loss of intracellular constituents may also contribute to the effects observed during the application of some of the antagonists 10–20 min after control measurements. However, the reversibility of Cs\(^+\) effects and the insignificant effect of Ba\(^{2+}\) on \( IS_{\text{ad}} \) (Fig. 4d) for instance indicates that the effects were due mostly to the established effects of the drugs. Moreover, experiments decreasing the T-current by DC depolarization immediately after rupture of the patch are consistent with perfusion results (Fig. 4d).

The characterization of \( I_h \) in our experiments relied on 2 established pharmacological methods, first an isolation of \( I_h \) by application of Ba\(^{2+}\) ions to obtain a reliable magnitude of \( I_h \) without contamination by \( K_r \), and second by the well-established \( I_h \) antagonist ZD7288. This together with estimates of \( I_h \) from voltage clamp as well as current-clamp experiments provides evidence for the presence of \( I_h \) throughout all layers of the AC, which is in line with results on the sensorimotor cortex (Strauss et al. 2004).
significant differences comparing the $I_h$ amplitudes in control conditions and during Ba2+ application, which is in line with previous reports (Day et al. 2005; Aponte et al. 2006). Aponte et al. (2006) for instance provided strong evidence for the presence of $I_h$ despite no obvious sag in interneurons of the rat hippocampus.

**Distribution of Neuron Types**

In vitro studies of the AC (Metherate and Aramakis 1999; Hefti and Smith 2000, 2002) and the present experiments have identified at least 2 classes of pyramidal neurons, RS and IB neurons. The IB neurons have also been found in other cortical areas not only in layers IV and V (Connors et al. 1982; McCormick et al. 1985; Chagnac-Amitai and Connors 1989; McCormick and Pape 1990; Silva et al. 1991; Agmon and Connors 1992) but also in layer II/III of the sensorimotor cortex of guinea pigs (Gutnick et al. 1982; Deizs 1996).

Despite this consensus concerning the presence of IB neurons in the neocortex, the percentage of these neurons is less clear. In the AC of the rat, a recent study found 10% IB neurons in layer V and none in layer II/III (Atzori et al. 2004). This percentage of IB neurons in layer V is similar to our results if only repetitive IB neurons in layer V are counted (IBr; 11.5%, Fig. 4b). In contrast, an earlier study (Hefti and Smith 2000) found 36.8% IB neurons in layer V of the AC in rats, which is similar to our percentages of all IB (IBr and IBc) neurons in layer V (Fig. 4b). The considerable variability regarding the number of IB neurons between our experiments and previous studies is probably due to different definitions and hence inclusion criteria to count as IB or RS neurons. For instance, Atzori et al. (2004) included only those neurons as IB that we refer to as IBr, whereas Hefti and Smith (2000) probably had also included "latent bursting neurons" (IBc). These IBr share a constant ISI between the first 2 APs with IBr neurons, but full bursts may be precluded due to a variable control of burst generation by other conductances (e.g., $I_h$; Strauss et al. 2004). In any case, RS and IB neurons in the AC are not only distinguished by their firing pattern (burst recruitment, ISAd) but also by the relation of $I_h$ and $K_{ir}$. The significantly higher ratio of $I_h$ versus $K_{ir}$ in IB neurons may denote weaker shunt currents in these neurons supporting burst activation (Day et al. 2005). Our finding, that $I_h$ is not significantly different between RS and IB neurons, differs from studies in rat subiculum where bursters have larger $I_h$ magnitudes and different temporal summation properties than RS neurons (Van Welie et al. 2006). It supports, however, the suggestion that the $I_h$ is not responsible for the distinct firing pattern of pyramidal neurons (Van Welie et al. 2006).

The strong effect of subtle increases of depolarization on the ISI in RS I neurons is probably due to the marked voltage-dependent kinetic of the T-current becoming faster with larger depolarizations. However, a fraction of adaptation remained during T-current and K+ current block with both Ni2+ and Cs+ (Fig. 5d). Here, likely candidates contributing to the low ISAd may be M-currents.

Neurons that generate APs only early in response to current pulses and then cease firing (Lorenzon and Foehring 1993; Metherate and Aramakis 1999) were not found. Perhaps these phasic neurons are characteristic for the developing cortex because they were observed in rats during postnatal weeks 2 and 3 (on average, mice used for this study were 6 weeks old). Onset neurons, which fire a single AP at the beginning of

**Figure 6.** (a) Plot (means and one-sided standard deviations) of $E_{ir}$ (lower bars) and $E_{ir}$ (upper bars) of RS neurons in different layers of the AC. Note the larger difference between $E_{ir}$ and $E_{ir}$ in layer II/III vs. deeper layers ($P < 0.001$, Table 2). (b) Scatter plot of the sum of $K_{ir}$ and $I_h$ amplitudes as a function of $R_{inv}$ in IB and RS neurons. Note that higher $K_{ir}$ and $I_h$ amplitudes are correlated with lower $R_{inv}$ values indicating that $K_{ir}$ and $I_h$ contribute to the low $R_{inv}$ values in layer II/III.
a suprathreshold current pulse, are probably also characteristic for the developing AC (Metherate and Aramakis 1999; Kotak and others 2005) because we did not find this neuron type in our study.

Potential Intrinsic Filter Mechanisms

The proportion of adapting RS (RS1) neurons in layers V and VI is significantly higher than in layer II/III and III/IV (Fig. 4b). Because the T-current seems to be a considerable factor for low ISLad values in these neurons (Fig. 4d) it is likely that temporally closely correlated inputs might recruit the “boosting” of EPSP by the T-current (Deisz and others 1991), whereas later events of temporally dispersed input might be attenuated by the activity of Ca2+-dependent K+ currents (see above). Moreover, temporally closely correlated excitatory inputs may preferentially be strengthened by temporal summation limited by $l_h$ (Magee 1998) because this current was an additional factor for low ISLad (Fig. 4d). Regarding the transfer function of such RS I neurons this firing behavior would be particularly suited to augment brief transient inputs, whereas tonic input would be less effectively conveyed, that is, such neurons might improve transfer of transients, so that synchronous excitatory input from upstream neurons would be improved as shown before by an in vivo study (DeWeese and Zador 2006).

The high $A_{thr}$ found in layer II/III compared with layer V RS neurons appears to be at variance with data of Atzori and others (2004) who found the opposite differences in the AC of developing rats (postnatal weeks 3 and 4). The reason for this discrepancy is unclear but it should be noted that the resting membrane potential (e.g. $E_{rest} \approx 58.9$ mV, Atzori and others 2004 vs. $-76.0$ mV in this study) differs considerably. In any case, we envisage that the high $A_{thr}$ in layer II/III RS neurons would cause a preferential transfer of high amplitude input and therefore improve the signal to noise ratio. Hence, these neurons might provide a higher probability of columnar transfer as opposed to a presumably smaller input from corticocortical and commissural fibers. In contrast, the low $A_{thr}$ in layer V and VI neurons might favor cortical (e.g. commissural) and corticofugal spread of the processed and filtered signal.

The large $K_h$ and $l_h$ amplitudes in layer II/III may effectively prevent temporal summation (Magee 1999; Koch and Grothe 2003; Day and others 2005; Carr and Surmeier 2007), which, together with the higher $A_{thr}$, would provide improved temporal resolution (Buonomano 2000; Hefti and Smith 2002). The low $R_m$ in RS neurons of layer II/III allows synaptic potentials to be rapid and brief and thus contributes to the improvement of temporal resolution mentioned above (Golding and others 1995; Bal and Oertel 2000). Moreover, the low $R_m$ attenuates spread of single inputs and favors spread of temporally closely correlated inputs in the upper layers (Magee 1998; Golding and others 1995; Bal and Oertel 2000; Ferragamo and Oertel 2002). Because $R_m$ is Cs+-sensitive it corresponds to higher $K_h$ and $l_h$ amplitudes in layer II/III RS neurons (Fig. 6b, compare Day and others 2005). But whether $K_h$ and $l_h$ are the only candidates influencing the low $R_m$ in layer II/III is not clear and should be tested in future experiments.

RS neurons in layer II/III possess the largest values of SA. It remains to be seen by further morphological characterization of the neurons whether the SAs are indeed different between the layers or whether the capacitance measurements reflect only part of the neuronal surface, that is, the perisomatic area accessible with the clamp. Nevertheless, this result is in line with anatomical findings where pyramidal neurons of layers II and III in the AC of cats have larger dendritic fields (but smaller soma sizes) than those of layer V (Winer 1984, 1985, 1992; Winer and Prieto 2001). Therefore, it is likely that the size of dendritic fields and the number and length of dendrites, respectively, correlate with larger SAs of RS neurons in layer II/III rather than with somatic size. This would suggest that the capacitance measurements indeed detect a large proportion of the neuronal surface. In turn, assuming a comparable total resting conductance per area (cf. Table 2), this might account additionally for the low input resistance in layer II/III. Interestingly, layer II/III neurons have on average also the lowest resistance per area, that is, the highest conductance per area ($K_h/SA$ and $l_h/SA$). This feature would cause very brief synaptic potentials per se, which, with a high density of fast $l_h$, would synergistically contribute to little temporal summation. Conversely, the particularly slow $l_h$ in layer V neurons would tend to facilitate temporal summation (Magee 1998). Considering the crucial dependence of the time course of $l_h$ on subunit composition of channels the time constant in layer V might suggest a relative low expression of HCN 1 subunits which exhibit fast $l_h$ activation in comparison to subunits HCN 2 and 4 (Strauss and others 2004; Stieber and others 2005).

The functional implications of the significant differences between the parameters of neurons in layers V and VI (Table 2) demands further research because the connectivity of these layers is similar (Linden and Schreiner 2003). It would be interesting to correlate the connectivity of a given neuron with its intrinsic parameters. The laminar differences of intrinsic properties of IB neurons in the AC of mice are less pronounced than in RS neurons (Table 2). Nevertheless, the differences between IB of different layers may be functionally relevant. The high $E_{th}$ of IB neurons in layer II/III would tend to suppress subthreshold activity. The lower $E_{th}$ of IB neurons in layer V as well as their higher percentage in layers V and VI (Fig. 4b) may facilitate cortico-cortical and corticofugal output of a computed and synchronized signal (Chagnac-Amitai and Connors 1989).

Conclusions

The differences of intrinsic neuronal properties in the AC of mice govern the neuronal transfer functions of synaptic inputs pertinent to information processing, for example, suppression of signals below threshold, bandpass amplification and coincidence detection. Perhaps these layer-specific neuronal properties found here for the AC may be a more general feature present also in other (sensory) cortical areas.

Notes

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