Synaptic Conductance Estimates of the Connection Between Local Inhibitor Interneurons and Pyramidal Neurons in Layer 2/3 of a Cortical Column

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Abstract

Stimulation of a principal whisker yields sparse action potential (AP) spiking in layer 2/3 (L2/3) pyramidal neurons in a cortical column of rat barrel cortex. The low AP rates in pyramidal neurons could be explained by activation of interneurons in L2/3 providing inhibition onto L2/3 pyramidal neurons. L2/3 interneurons classified as local inhibitors based on their axonal projection in the same column were reported to receive strong excitatory input from spiny neurons in L4, which are also the main source of the excitatory input to L2/3 pyramidal neurons. Here, we investigated the remaining synaptic connection in this intracolumnar microcircuit. We found strong and reliable inhibitory synaptic transmission between intracolumnar L2/3 local-inhibitor-to-L2/3 pyramidal neuron pairs [inhibitory postsynaptic potential (IPSP) amplitude \(-0.88 \pm 0.67\) mV]. On average, 6.2 \pm 2 synaptic contacts were made by L2/3 local inhibitors onto L2/3 pyramidal neurons at 107 \pm 64 \, \mu m path distance from the pyramidal neuron soma, thus overlapping with the distribution of synaptic contacts from L4 spiny neurons onto L2/3 pyramidal neurons (67 \pm 34 \, \mu m). Finally, using compartmental simulations, we determined the synaptic conductance per synaptic contact to be 0.77 \pm 0.4 \, \text{nS}. We conclude that the synaptic circuit from L4 to L2/3 can provide efficient shunting inhibition that is temporally and spatially aligned with the excitatory input from L4 to L2/3.

Key words: GABAergic synapse, interneuron, modeling, sensitivity analysis, shunting inhibition

Introduction

Inhibitory connections contribute to postsynaptic non-linear dendritic computation and plasticity (Llinas et al. 1968; Miles et al. 1996; Larkum et al. 1999). The relevance of these connections for the shaping of sensory representations in neocortex has been demonstrated for several cortical areas including the visual, olfactory, and auditory system (Rose and Blakemore 1974;...
Sillito 1975; Pollak et al. 2002; Wu et al. 2008; Poo and Isaacson 2009; Liu et al. 2011; Adesnik et al. 2012; for a review, cf. Isaacson and Scanziani 2011). In the supragranular layers of the somatosensory cortex, action potential (AP) responses to sensory stimuli are especially sparse in L2/3 pyramidal neurons in vivo ("sparse" coding, Brecht et al. 2003; Celikel et al. 2004; de Kock et al. 2007; Kerr et al. 2007), despite the strong and reliable excitatory input L2/3 receives from L4 (Feldmeyer et al. 2002; Silver et al. 2003). It was found that GABAergic inhibitory neurons are numerous in supragranular layers in rat barrel cortex (Meyer et al. 2011). Therefore, it is critical to quantitatively understand how L2/3 interneurons contribute to the low AP spiking in L2/3 pyramidal neurons upon sensory stimuli.

The relevant L4-to-L2/3 microcircuit is composed of excitatory and inhibitory pathways that are activated by L4 spiny neurons following whisker deflection and that both converge onto L2/3 pyramidal neurons (Fig. 1D). The excitatory pathway of this microcircuit consists of L4 spiny neurons innervating L2/3 pyramidal neurons (Feldmeyer et al. 2002). The inhibitory pathway consists of L4 spiny neurons targeting L2/3 interneurons (Helmstaedter et al. 2008). These, in turn, target L2/3 pyramidal neurons. Previous reports showed that activation of subgroups of readily excitable L2/3 interneurons from L4 is very effective (Helmstaedter et al. 2008). These L2/3 interneurons can thus contribute substantially to the shaping of the precise temporal pattern of AP spiking in L2/3 pyramidal neurons.

In the present study, we therefore used in vitro paired recordings to characterize the connection between axonally defined locally projecting L2/3 interneurons ("L2/3 local inhibitors," Helmstaedter et al. 2009b) and L2/3 pyramidal neurons in the same column. While inhibitory synaptic connections within L2/3 have been studied (Thomson et al. 1996, 2002; Reyes et al. 1998; Gupta et al. 2000; Holmgren et al. 2003; Xu and Callaway 2009; Avermann et al. 2012; Pfeffer et al. 2013), knowledge on the absolute strength of synaptic conductance for precisely defined interneuron types is still missing. Dendritic computations were shown to be highly nonlinear and to critically depend on the precise location of synaptic contacts on the dendritic tree (Behabadi et al. 2012; Gidon and Segev 2012; Jadi et al. 2012). To obtain data for realistic quantitative models of the L4-to-L2/3 microcircuit, it was thus crucial to record not only synaptic transients, but also to determine the number and location of synaptic contacts per cell pair. This allowed us to obtain estimates of inhibitory synaptic conductance strength using an inverse compartmental modeling approach. With this we can provide the missing quantitative circuit data required for mechanistic models of the L4-to-L2/3 circuit within a cortical column.

Methods

Preparation, Solutions

All experimental procedures were performed according to the German Animal Welfare Act. Wistar rats (19–22 days old, both sexes) were anesthetized with isoflurane and decapitated. The brain was removed and slices of somatosensory cortex were cut in ice-cold extracellular solution containing 6–7 mM MgCl2/1 mM CaCl2 at 350 µm thickness in a thalamocortical plane [for a detailed description, see Agmon and Connors (1991)] with minor modifications (Feldmeyer et al. 1999; Helmstaedter et al. 2008) using a vibrating microslicer (Slicer HR-2; Sigmann Elektronik, Hueffenhardt, Germany). Slices were subsequently incubated for 30–45 min in extracellular solution at 22–24 °C. In some experiments (n = 6), brains were incubated at 35 °C for 45 min before measuring in vitro.

Figure 1. Paired whole-cell recordings of L2/3 pyramidal neurons and L2/3 interneurons. (A) Bright field image of the barrel field prior to recording. Positions of recorded cells are depicted by black arrows. (B) High-magnification DIC images of the pre- (bottom picture) and postsynaptic (upper picture) neuron. (C) Computer-aided reconstruction of soma, dendrites, and axon of the presynaptic (L2/3 interneuron, red, red, and blue) and postsynaptic (L2/3 pyramidal neuron, white, white, and green) neurons. (D) Schematic drawing of the L4-to-L2/3 microcircuit. The connection between L2/3 interneurons (red) and pyramidal neurons (blue) was characterized in this study. Previous studies by Feldmeyer et al. (1999, 2002, 2006) and Helmstaedter et al. (2008) characterized the intracolumnar connections shown in gray.
30 min as in Frick et al. (2007). Inhibitory postsynaptic potential (IPSP) amplitude, AP–IPSP latency, 20–80% rise time, and decay time constant were not significantly different between both preparations (one-way ANOVA). During recording, slices were continuously superfused with an extracellular solution containing (in mM): 125 NaCl, 25 NaHCO3, 2.5 KCl, 1.25 Na2HPO4, 2 CaCl2, 1 MgCl2, and 25 glucose (equilibrated with 95% O2 and 5% CO2). The pipette (intracellular) solution contained (in mM): 135 K-gluconate, 10 HEPES, 10 phosphocreatine, 4 KCl, 4 ATP-Mg, and 0.3 GTP (adjusted to pH 7.2 with KOH). Biocytin (Sigma, Munich, Germany) at a concentration of 4–6 mg/mL was added to the pipette solution, and cells were filled during 1–2 h of recording.

Identification of Barrels

Slices were placed in the recording chamber under an upright microscope (Axioskop, Carl Zeiss, Göttingen, Germany) and inspected at low magnification ×5/0.15 NA using bright-field illumination. Barrels were identified as >2 light “hollows” in L4 separated by dark bands. The barrel field was photographed for later analysis.

Electrophysiological Recordings

Interneurons were searched in L2/3 above barrels using a water ×60/0.90 NA objective and infrared differential interference contrast (R-DIC) microscopy (Dodt and Zieglgansberger 1990; Stuart et al. 1993). The intrinsic electrical excitability of the interneuron was then recorded using rectangular current pulses of 500 ms length and varying amplitude [similar to Helmstaedter et al. (2009a); for details see below]. Pyramidal neurons in L2/3 were patched with a second pipette (Fig. 1A,B). APs were then elicited in the interneuron to test for synaptic connections. To capture small IPSPs, pyramidal neurons were depolarized to ~50 to ~60 mV during the connection test and the response was averaged over at least 18 sweeps. After recording, the pipettes were positioned above the recorded cells, and the barrel pattern was again photographed at low magnification using bright-field illumination. All recordings were performed in whole-cell configuration at 32–36 °C.

Histological Procedures

After recording, slices were fixed at 4 °C for at least 24 h in 100 mM PBS, pH 7.4, containing 4% paraformaldehyde. Slices containing biocytin-filled neurons were processed using a modified protocol described previously (Lübke et al. 2000). Slices were incubated in 0.1% Triton X-100 solution containing avidin-biotinylated horseradish peroxidase (ABC-Elite-Kit; Camon, Wiesbaden, Germany); subsequently, they were reacted using 3,3-diaminobenzidine as a chromogen under visual control until the dendritic and axonal Arborization was clearly visible. Slices were then mounted on slides, embedded in Mowiol (Kuraray Specialities Europe, Frankfurt am Main, Germany), and enclosed with a coverslip.

Reconstructions and Morphological Analysis

Three-dimensional reconstructions of biocytin-labeled pairs were made using the Neurolucida software (MicroBrightField, Colchester, VT, USA) and an Olympus Optical (Hamburg, Germany) BX51 microscope at a final magnification of ×1000 using a ×100, 1.25 NA objective (Fig. 1C). The reconstructions were used to construct average length–density maps of the presynaptic axons and dendrites. Pairs were then classified according to their axonal projection type of the presynaptic L2/3 interneuron with respect to the home barrel column (Fig. 2; cf. Results and Discussion; Helmstaedter et al. 2009b). A maximum value of 0.35 for axonal “laterality” was defined to separate “local” from “lateral” interneurons. This value corresponds to the intersection between the normal distributions of axonal laterality of the most similar local and lateral inhibitor group [local inhibitor group 1 and lateral inhibitor group 1; for a detailed description of interneuron groups, see Helmstaedter et al. (2009a)]. The same method was applied to calculate a maximum “verticality” of 0.26 (local inhibitor group 3 and transaminergic inhibitor group 1). Only pairs with complete staining of the dendrites and axons were chosen for identification of putative synaptic contacts. Potential contacts were identified as a close apposition of a presynaptic axon and the postsynaptic dendrite in the same focal plane, visualized at ×1000 magnification (cf. Feldmeyer et al. 2002).

Intrinsic Electrical Excitability

Rectangular current pulses of 500 ms duration and varying amplitude were injected into the soma of the interneuron (cf. Fig. 3). From these traces, 5 parameters were quantified as described previously (Helmstaedter et al. 2009a): (1) AP frequency
adaptation ratio (Fig. 3B), measured as offset between the initial and last frequency on a mono-exponential fit to the frequencies in the AP train shown in (A). (C) AP AHP minimum voltages in the AP train shown in (A). The offset between the first and the last AHP minimum voltage on a mono-exponential fit to the AHP minimum voltages was calculated as AHP minimum voltage adaptation. (F) AP half width of the first AP in the AP train shown in (A). (D) Membrane voltage responses of the same interneuron to subthreshold current injections at resting membrane potential. (E) I/V ratio corresponding to (D). The I/V ratio was used to constrain the somatic input resistance at resting membrane potential (cf. Methods).

Analysis of Postsynaptic Potentials

APs were elicited in the interneuron and the subsequent PSP changes in the pyramidal neuron were recorded. PSPs that were hyperpolarizing at the holding potential of −50 to −60 mV were classified as IPSPs. Unitary PSP properties were determined as described previously (Feldmeyer et al. 1999) with some modifications for the analysis of IPSPs. Briefly, a putative IPSP peak was determined within a “peak search window” of 6–18 ms after the presynaptic AP, and the IPSP amplitude was averaged over a 0.8-ms window (0.3 ms before and 0.5 ms after the determined peak time). Subsequently, a baseline potential measured in a 5-ms window just preceding the IPSP was subtracted. Failures were detected as events with amplitudes of <1.5 times the baseline noise within the baseline window or with latencies exceeding the average latency by >1.5 ms. To compensate for sweeps that were incorrectly classified as failures due to very small IPSP amplitudes, a corrected failure rate (frcorr) was calculated for each connection. First, all sweeps that were classified as failures (n failures) were averaged (averaged failure trace, aft) and analyzed as described above to determine the IPSP amplitude in that trace [IPSPamp(aft)]. Examples of failures, successful transmissions, an average failure trace, and an average trace of all sweeps for a weak connection (−0.23 mV IPSP amplitude) are given in Supplementary Figure 7. The ratio of the IPSP amplitude in the average failure trace [IPSPamp(aft)] to the IPSP amplitude in an average trace of all sweeps (IPSPamp) was assumed to approximate the ratio of incorrectly classified failures. Thus, the frcorr was calculated as

\[
\text{frcorr} = \frac{n_{\text{failures}} \times \left(1 - \frac{\text{IPSP}_{\text{amp(aft)}}}{\text{IPSP}_{\text{amp}}}\right)}{n_{\text{sweeps}}}
\]  

The time points at which the IPSP attained 20% and 80% of the total IPSP amplitude were determined on a parabolic fit to the initial and final IPSP rise phase, respectively. The difference between these time points was recorded as 20−80% rise time. The
decay was mono-exponentially fitted in a 50- to 70-ms window starting 5 ms after the IPSP peak.

Paired-pulse ratios were determined from averaged traces. Recordings were made at different holding potentials, and a weighted linear fit for at least 3 holding potentials around the resting membrane potential was used to constrain the synaptic reversal potential. De- and hyperpolarizing rectangular current pulses of 500 ms length were used to estimate the membrane time constant ($\tau_m$). Subthreshold I-V curves were fitted assuming a quadratic relation between injected current and the resulting voltage deflection, enabling prediction of $R_m$ over a broad voltage range (Waters and Helmchen 2006). AP discharge patterns were quantitatively analyzed offline (for details, see Helmsätdeter et al. 2009b). Corrections for series resistance were done offline for all recordings.

Simulations

Ten pairs for which all previous analyses could be completed were chosen for simulation. Neuronal reconstructions were converted to a format readable by NEURON using custom-written procedures (cf. Helmsätdeter et al. 2009b). Modeling was performed using NEURON 5.8 (Hines and Carnevale 1997). Fitting was done with NEURON using the principal axis method (Brent 1973) for parameter searching. Parameters were determined for each neuron individually. $R_m$ was assumed to be 150 $\Omega$ cm based on previous studies (Trevelyan and Jack 2002; Sarid et al. 2007).

For this $R_m$, the membrane resistance at the holding potential ($R_m, E_{hold}$) and at the resting membrane potential ($R_m, E_{rest}$) were optimized to fit the experimental $R_m$ at the holding potential and resting membrane potential, respectively. $C_m$ was calculated as $R_m, E_{rest}/\text{experimental } \tau_m$. Spines were inserted into the dendrites by modifying the dimensions (diameter, $d$, and length, $l$) of each dendritic section as in Stratford et al. (1989):

$$F = (A_{sh} + A_sp)/A_{sh},$$

$$d' = d * F^{1/3},$$

$$l' = l * F^{1/3},$$

with $A_{sh}$ the area of the shafts and $A_sp$ the area of the spines. The area of each spine in $\mu m^2$ and spine density per $\mu m$ dendritic length were assumed to be 1 and 0.97, respectively (Lübke et al. 2003).

Inhibitory synaptic conductance $g_{syn}$ (nS) at the contact site was modeled as:

$$g_{syn}(t) = g_{syn\max} \times c_1 \left( c_2 \times \left( e^{-\frac{t}{\tau_{\text{decay}}}} - 1 \right) + \left( 1 - c_2 \right) \times e^{-\frac{t}{\tau_{\text{max}}}} \right),$$

with a dimensionless constant $c_1$ ranging from 0 to 1, and a constant $c_2$ defined so that the peak conductance equals $g_{syn\max}$ (cf. Supplementary Table 1 for further details on the implementation of the above equation in NEURON). The synaptic reversal potential was set to fit the experimentally observed reversal potential. Minimization of the mean squared error between the model output and the averaged experimentally recorded somatic IPSP in 2 fitting windows at the most depolarized potential served as target function to optimize the synaptic parameters:

$$L = \sum_{t=t_1}^{t_2} \left( g_{syn}(t) - g_{syn\exp}(t) \right)^2 \sum_{t=t_3}^{t_4} \left( g_{syn}(t) - g_{syn\exp}(t) \right)^2,$$

with $t_1$, $n_1$ and $t_2$, $n_2$, onset and duration of the first and second fitting window, respectively. Weighted fitting windows were chosen to obtain equally good fits to the early (first 15–20 ms) and late phase of the IPSP. On average, the double-weighted fitting window ($t_3$, $n_3$) to the postsynaptic IPSP began 3 ± 0.4 ms (range 3–4) after the presynaptic AP and had a duration of 15 ± 7 ms (range 10–37); the single-weighted window ($t_4$, $n_4$) began 19 ± 7 ms (range 15–40) after the AP and had a duration of 63 ± 14 ms (range 30–80).

For all data, mean ± SD are given.

Results

Whole-cell recordings from pairs of L2/3 interneurons and L2/3 pyramidal neurons were made in acute slices of rat barrel cortex (Fig. 1A, B). Barrel outlines in L4 were used to delineate the borders of putative cortical columns. Only cell pairs with both the presynaptic interneuron and the postsynaptic pyramidal neuron located in L2/3 of the same column were investigated. Figure 1C shows the reconstruction of the pair shown in Figure 1A, B. The L2/3 interneuron axon (shown in blue in Fig. 1C) was largely confined to the home column including the septa. The interneuron dendrites (shown in red in Fig. 1C) extended to L2/3 and the upper half of L4.

L2/3 interneurons were classified into axonal projection types with reference to cortical columns as previously reported (cf. Discussion; Helmsätdeter et al. 2009a, 2009b). In face of varying interneuron classifications (e.g., Karube et al. 2004; Markram et al. 2004; Ascoli et al. 2008; DeFelipe et al. 2013), we focused on the axonal projection with reference to functional cortical units as the key classification criterion since it directly predicts synaptic innervation within and across presumed cortical modules. One possible quantitative method to further subclassify interneurons based on morphological, intrinsic electrical, and functional anatomical properties was reported by Helmsätdeter et al. (2009a). The results of this subclassification are presented in Supplementary Methods, Results, and Discussion, Supplementary Figures 1–3, and Supplementary Tables 2–6.

Identification of L2/3 Interneuron Axonal Projection Type

The axonal projection type of the presynaptic interneuron was determined based on the distribution of axonal path length with reference to the layers and borders of the home column (Fig. 2, cf. Methods and Helmsätdeter et al. 2009b for details). Briefly, laterality of the axonal projection was quantified as the linear combination of the ratio of axonal path length beyond the lateral borders of the home column including the septa and the horizontal extent of axonal path length in units of width of the home column in L2/3 including half of the septa (Fig. 2A). Vertically was quantified as the ratio of axonal path length extending to regions below L2/3 (gray shaded area in Fig. 2B). Figure 2C shows the distribution of laterality and verticality for the 26 local inhibitors, defined as interneurons with laterality <0.35 and verticality <0.26 based on the classification previously made in an unbiased population of L2/3 interneurons (Fig. 2C, inset; cf. Methods; Helmsätdeter et al. 2009a).
Dendritic and Intrinsic Electrical Properties

The dendritic geometry of most local inhibitors would be qualitatively described as “multipolar” (Fig. 2). The local inhibitors shown in Figure 2A,B had 5 and 8 primary dendrites with a total length of 2.6 and 2.8 mm, respectively. On average, local inhibitors had 8.0 ± 2 dendrites with a total length of 3.1 ± 0.6 mm (n = 26). A gallery of all 26 local inhibitor morphologies is provided in Supplementary Figure 3. Figure 3A shows the AP discharge pattern of a local inhibitor upon somatic injection of a 500-ms current pulse. Figure 3D shows membrane voltage responses of the same interneuron to subthreshold current injections. These firing patterns would match the often-used description “fast spiking.”

Using these electrical recordings, 5 parameters were quantified as described previously (Helmstaedter et al. 2009a, cf. Methods): (1) AP frequency adaptation ratio (Fig. 3B), (2) AP AHP minimum voltage adaptation (Fig. 3C), (3) the AP half width of the first AP in the 100-Hz trace (Fig. 3F), (4) AP firing threshold, and (5) somatic input resistance at resting membrane potential, measured as i/V ratio (Fig. 3D,E). On average, the AP frequency adaptation ratio was −0.11 ± 0.21, the AHP minimum voltage adaptation was 1.9 ± 1.7 mV, the AP half width was 0.39 ± 0.2 ms, the AP firing threshold was −42.9 ± 5.7 mV, and the somatic input resistance was 73 ± 31 MΩ. Note, however, that the AP discharge properties of local inhibitors were very heterogeneous (range: −0.68 to 0.34, −1.2 to 6.2 mV, 0.25 to 0.83 ms, −54 to −24 mV, and 34 to 168 MΩ, for the 5 latter properties, respectively).

Synaptic Properties

After classification of L2/3 interneurons, the synaptic properties of the L2/3 local inhibitor-to-L2/3 pyramidal neuron connections were analyzed. Figure 4A-D shows data from a single connection. First, average unitary IPSP properties were determined at a depolarized postsynaptic potential (approximately −55 mV, Figure 4A, Figure 4. Properties of IPSPs in the local inhibitor-to-L2/3 pyramidal neuron connections. (A) Presynaptic AP and overlay of 20 successive postsynaptic IPSPs at a postsynaptic holding potential of −50 mV for the pair shown in Figure 6A. (B) IPSP unitary amplitude histogram for this connection. The unitary IPSP amplitudes (gray) of this pair were on average −1.75 ± 0.27 mV (n = 56 sweeps) and could be readily distinguished from noise (white). (C) AP–IPSP onset latency histogram for this connection. The AP–IPSP onset latency of this pair was on average 0.74 ± 0.17 ms. A Gaussian fit demonstrates the narrow distribution. (D) Train of 3 presynaptic APs at 10 Hz (top trace) and 8 consecutive sweeps at a hyperpolarized postsynaptic holding potential of −50 mV (middle trace). The PSPs were on average hyperpolarizing and depressing in amplitude (middle trace). Eight consecutive sweeps at a hyperpolarized postsynaptic holding potential of −90 mV (bottom traces). The PSPs were depolarizing on average (bottom trace). All recordings were performed using a “low chloride” internal solution (4 mM Cl−). (E and F) Histograms of IPSP amplitudes and AP–IPSP onset latencies for all 26 local inhibitor-to-L2/3 pyramidal neuron connections, respectively.)
bottom trace and Figure 4D, top traces); measurements were made using a “low chloride” internal solution (4 mM Cl−) to maintain assumedly physiological Cl− concentrations (cf. Methods). The presynaptic interneuron was stimulated at a frequency of 10 Hz. The IPSP amplitude of the pair shown in Fig. 4A-D was on average −1.75 ± 0.27 mV (n = 56 sweeps) and could be readily distinguished from noise (Fig. 4B). The AP–IPSP latency was 0.74 ± 0.17 ms (Fig. 4C). IPSP amplitudes were also measured at hyperpolarized holding potentials to extract the synaptic reversal potential (Fig. 4D, bottom traces; −90 mV holding potential).

Figure 4E shows a histogram of the IPSP amplitudes of the local inhibitory-to-L2/3-pyramidal-cell connections. The IPSP amplitudes of local inhibitors showed a broad distribution that could not be explained by the variability of postsynaptic baseline potentials among experiments (mean IPSP amplitude −0.88 ± 0.67 mV, average baseline potential −57 ± 4 mV, Pearson correlation 0.2, P = 0.4, n = 26, cf. Supplementary Fig. 4). The average 20–80% rise time and decay time constants were 3.5 ± 0.8 (n = 26) and 76 ± 53 ms (n = 26), respectively. Figure 4F shows a histogram of the AP–IPSP onset latencies in the local inhibitory-to-L2/3 pyramidal neuron connections. On average, AP–IPSP latencies were 1.1 ± 0.5 ms (n = 26), ranging from 0.6 to 2.8 ms for individual connections. Presynaptic APs reliably evoked IPSPs with a low average failure rate of 7.9 ± 14% (n = 26). This number represents the failure rate corrected for successful transmissions misclassified as failures (cf. Methods). The uncorrected failure rate was 10.7 ± 17% (n = 26). In 65% (17 of 26) of pairs, synaptic transmission never failed at all (18 to 56 sweeps per pair).

Intersoma distance was not significantly associated with the number of contacts or IPSP amplitudes in the present study (Spearman correlation coefficient = −0.117, P = 0.654, n = 17 and −0.127, P = 0.537, n = 26, respectively). Reciprocal connections were found in 16 of 22 cells tested (73%). EPSP amplitudes were 0.77 ± 0.8 mV on average and were not correlated to IPSP amplitudes (Spearman correlation coefficient = −0.08, P = 0.772, n = 16).

Short-Term Synaptic Dynamics

Figure 5A shows 3 consecutive postsynaptic IPSPs evoked by presynaptic APs at 10 Hz stimulation frequency. In the connection shown, the paired-pulse ratio was 0.68 for the second to first and 1 for the second to third IPSP at 10 Hz. Paired-pulse ratios at 10 and 40 Hz AP frequency for all L2/3 local inhibitors are shown in Figure 5B,C. Of 26 pairs, 23 showed on average short-term depression [average paired-pulse ratios: 0.78 ± 0.38 and 1.02 ± 0.53 (n = 26) for first to second AP and second to third AP at 10 Hz, respectively; 0.64 ± 0.29 and 0.85 ± 0.37 (n = 19) at 40 Hz]]. Paired-pulse ratios (10 and 40 Hz) of individual pairs were determined from an average of 27 (range 18–56) and 20 (range 15–20) sweeps, respectively.

Synaptic Contacts

Figure 6A shows a light microscopic image of a synapticily connected pair of a L2/3 local inhibitor and a L2/3 pyramidal neuron. Six putative synaptic contacts were found (indicated by black circles in Fig. 6A). Two of them were located on basal dendrites (contacts 1 and 6 in Fig. 6A) and 4 on apical oblique dendrites (contacts 2–4 and 5 in Fig. 6A; see Fig. 6B1–B6 for images at higher magnification). The presynaptic axon (indicated by black arrows in Fig. 6B1–B6) was found to intersect with the postsynaptic dendrite in the same foci plane. The average number of putative contacts was 6.2 ± 2 (range 3–10, n = 17 pairs). The average path distance of putative synaptic contacts to the soma of the postsynaptic pyramidal neuron was 107 ± 64 μm (range: 5–354, n = 106 contacts; see histogram in Fig. 9). 50% of putative contacts were located on basal dendrites and 47% were located on apical oblique dendrites. Only 3 putative contacts were located on the soma. The number of contacts was positively correlated with the absolute IPSP amplitude (Fig. 6C, correlation coefficient, 0.58, P = 0.016, n = 17 (two-sided Pearson correlation)).

Innervation Domains

To quantify the L2/3 local inhibitory-to-L2/3 pyramidal neuron innervation probability, 2D maps of axonal (L2/3 interneuron) and dendritic (L2/3 pyramidal neuron) length density were calculated (cf. Lübke et al. 2003; Helmsmaedter et al. 2009b). The axonal density map of local inhibitors was largely confined to the borders of the home column including septa (Fig. 6D1). Accordingly, single-cell morphologies demonstrate how axonal arborization of local interneurons largely respect home-column borders (cf. Supplementary Fig. 3, e.g., pair 2008-09-25-A and pair 2008-10-28-A).

Multiplication of the respective axonal and dendritic (Fig. 6D1–2) length densities yielded a predicted map of innervation probability (Fig. 6D3). The contour containing 90% of the predicted innervation probability (“innervation domain,” yellow outline in Fig. 6D3) can be interpreted as the area in which 90% of the contacts are made if the distribution of these contacts depended mainly on spatial proximity. It was strictly confined to the home column. The average path length of local inhibitor axon in the innervation domain was 15.5 mm with a bouton frequency of 0.3 ± 0.04 boutons per μm of axonal path length.

Synaptic Conductance Estimates

To constrain the unitary synaptic parameters underlying the observed experimental IPSPs, simulations using NEURON were
Figure 6. Light microscopic analysis of synaptic contacts and average axonal projection and innervation domain of local inhibitor-to-L2/3 pyramidal neuron connections. (A) Light microscopic image of a synaptically coupled local inhibitor-to-L2/3-pyramidal neuron pair at low magnification. Putative synaptic contacts made by the local inhibitor (lower, right neuron) are indicated by black circles and numbered from 1 to 6. (B1–B6) Images at higher magnification of the putative contacts 1–6 shown in (A). The presynaptic axon (indicated by black arrows) was found to intersect with the postsynaptic dendrite in a single focal plane. (C) The number of putative contacts identified using this method was significantly correlated with the IPSP amplitude of the respective connection (two-sided Pearson correlation). (D1–D3) The axonal length density map of local inhibitors was largely confined to the borders of the home column and L2/3. (D2) Dendritic length–density map and soma positions of the pyramidal neurons contacted by local inhibitors. (D3) Inhibitory innervation probability map of local inhibitors. Inhibitory innervation probability maps were obtained by multiplication of the respective axonal (D1) and dendritic length densities (D2). The yellow contour comprises 90% of the inhibitory innervation probability (i.e., "innervation domain"). The innervation domain can be interpreted as the area in which 90% of the contacts are made if the distribution of these contacts depended mainly on spatial proximity. The blue and white contours in (D1–D3) comprise 90% of respective length–density.
made for 10 synaptically connected local inhibitor-to-pyramidal neuron pairs. The unknown synaptic parameters were: synaptic reversal potential \(E_{\text{syn}}\), maximum synaptic conductance \(g_{\text{syn,max}}\), and synaptic time constants \(t_{\text{rise}}, t_{\text{decay1}}, \text{and} t_{\text{decay2}}\). For each connection, a detailed compartmental model of the postsynaptic pyramidal neuron was built using (1) the reconstruction of the postsynaptic pyramidal neuron soma and dendrites, (2) passive membrane properties (membrane capacitance \(C_m\), membrane resistance \(R_m\), and axial resistance \(R_a\)), calculated from the measured input resistance and membrane time constant (cf. Methods), (3) synapses, inserted at the light microscopically identified putative synaptic locations (number of synapses \(n_{\text{syn}}\); cf. Figs 6A and 7A), (4) synaptic reversal potentials \(E_{\text{syn}}\), and (5) synaptic conductances \(g_{\text{syn,max}}, t_{\text{rise}}, t_{\text{decay1}}, t_{\text{decay2}}, \text{and} c_i\), cf. Methods). The conductance at a synaptic contact was effectively modeled as a weighted linear combination of a bi- and a mono-exponential module (cf. Methods).

Figure 7A shows a 2D projection of the reconstruction of a pyramidal neuron (cf. Fig. 6A,B), with the 6 observed putative synapses indicated by black arrows (the morphologies of all reconstructed pyramidal neurons are provided in Supplementary Data 1). The measured somatic IPSP is shown in Figure 7B (gray line). Numerical simulations were then made using the model described above. Synaptic parameters at each synaptic contact in the model were optimized such that the simulated somatic IPSP best-fitted the average measured somatic IPSP (inverse modeling and principal component method, see Methods equation 6). The model somatic IPSP using the synaptic parameters that provided the best fit between the model output and the experimentally measured somatic IPSP is shown in Figure 7B as black trace (for details of the fitting procedure, cf. Methods; experimental IPSP traces and model output for all 10 simulated pairs are given in Supplementary Fig. 5). The underlying current and voltage deflections at the 6 synaptic sites are shown in Figure 7C. Synaptic parameters in the model were assumed to be homogeneous at the 6 synaptic sites. Differences in local voltage deflections thus possibly reflected local input resistance variations (e.g., contact 2 vs. contact 1 in Fig. 7A,C).

The distribution of maximum synaptic conductances determined by this method was remarkably homogeneous \((0.77 \pm 0.35 \text{nS})\) synaptic conductance per synaptic site, range \(0.13–1.15, n=10, \text{Table 2}\), given the considerable variability in input parameters (cf. Table 1). On average, \(t_{\text{rise}} = 0.18 \pm 0.06 \text{ ms}\), and \(t_{\text{decay1}} \text{ and } t_{\text{decay2}} \) were \(2.97 \pm 1.85 \text{ and } 39.49 \pm 45.95 \text{ ms}\), respectively (further details of the modeling parameters including the weights of the individual modules are given in Supplementary Table 1).

Sensitivity Analysis

We then analyzed the sensitivity of our conductance estimates to errors in \(C_m, R_m, R_a, E_{\text{syn}}, \text{and } n_{\text{syn}}\) for 3 of the simulated cells. A single input parameter was varied at a time, all simulations were repeated, and the effect on the conductance estimate was recorded. Figure 8A shows that variation in \(C_m, R_m, R_a, E_{\text{syn}}, \text{and } n_{\text{syn}}\) resulted in \(g_{\text{syn,max}}\) errors of approximately \(20\%\). \(R_a\) and \(R_m\) variations of \(30\%\) each resulted in \(g_{\text{syn,max}}\) errors of \(<10\%\). Figure 8B shows that varying \(E_{\text{syn}}\) by 10 mV resulted in \(<40\%\) overestimation of \(g_{\text{syn,max}}\). Figure 8C shows the sensitivity of our simulations to the number of synapses. Each synapse was deactivated one at a time and the resulting error in \(g_{\text{syn,max}}\) was estimated. An underestimation of synaptic contacts by \(n = 1\) resulted in \(<45\%\) overestimation of \(g_{\text{syn,max}}\) (average error: 24%).

Discussion

The present study characterizes the connection between L2/3 local inhibitor interneurons and L2/3 pyramidal neurons in rat barrel cortex using physiological (“low chloride”) intracellular
Interneurons can carry a wide spectrum of morphological, molecular, and electrophysiological characteristics, so that numerous classifications have been proposed (Ascoli et al. 2008; DeFelipe et al. 2013). Since these classifications do not agree yet on a relevant set of classification parameters, we chose to relate to a quantitative classification of L2/3 interneurons based on their axonal projections with reference to cortical columns, since this measure provides direct prediction of synaptic innervation. Briefly, Helmstaedter et al. (2009b) identified 3 types of L2/3 interneurons as local, lateral, and transamin interneurons based on their axonal projection in relation to their home column and layer using a cluster analysis. Local inhibitors were predicted to innervate pyramidal neurons in L2/3 of the home column. Local inhibitors received a strong input from L4 spiny neurons (EPSP amplitude: 0.96 ± 0.93 mV) and may therefore be substantially activated by a whisker deflection.

**Table 1** Input parameters for the 10 simulated cells

|                | Average amplitude (mV) | Holding membrane potential (mV) | Apparent syn. rev. pot. (mV) | Number of synapses | Synapse to soma distance (µm) | Input resistance* (MΩ) | Membrane time constant (ms) |
|----------------|------------------------|--------------------------------|-------------------------------|-------------------|-----------------------------|------------------------|----------------------------|
| **Local inhibitors** |                        |                                 |                               |                   |                             |                        |                            |
| 20050804A       | −1.75                  | −55.5                           | −80.2                         | 6                 | 105.9                       | 138.8                  | 23.9                       |
| 20050829A       | −0.59                  | −63.4                           | −78.0                         | 4                 | 87.5                        | 59.5                   | 14.8                       |
| 20050912A       | −0.78                  | −63.6                           | −81.6                         | 5                 | 91.2                        | 51.0                   | 12.3                       |
| 20060927A       | −1.04                  | −55.7                           | −76.5                         | 5                 | 70.7                        | 52.4                   | 15.3                       |
| 20061018A       | −1.40                  | −60.1                           | −80.7                         | 7                 | 82.1                        | 102.0                  | 24.6                       |
| 20070714A       | −0.50                  | −57.4                           | −75.2                         | 7                 | 131.0                       | 41.3                   | 9.6                        |
| 20070809B       | −1.98                  | −56.3                           | −81.5                         | 10                | 94.4                        | 100.4                  | 22.3                       |
| 20080922A       | −0.57                  | −56.8                           | −90.1                         | 8                 | 113.5                       | 112.1                  | 13.4                       |
| 20080925A       | −0.81                  | −52.6                           | −82.3                         | 5                 | 206.1                       | 76.0                   | 12.1                       |
| 20081028A       | −1.61                  | −55.9                           | −82.6                         | 8                 | 98.0                        | 65.3                   | 11.6                       |
| Average (n = 10)| −1.10                  | −57.7                           | −80.9                         | 6.5               | 108.0                       | 79.9                   | 16.0                       |
| SD             | 0.54                   | 3.6                             | 4.1                           | 1.8               | 38.4                        | 31.9                   | 5.5                        |

*Note: IPSP, inhibitory postsynaptic potential; syn. rev. pot., synaptic reversal potential.

*Input resistance at holding potential.

**Table 2** Modeled synaptic parameters

|                | g_{syn,max} (nS) | \(\tau\)_{rise} (ms) | \(\tau\)_{decay1} (ms) | \(\tau\)_{decay2} (ms) |
|----------------|-----------------|----------------------|------------------------|------------------------|
| **Local inhibitors** |                 |                      |                        |                        |
| 20050804A       | 1.13            | 0.16                 | 2.4                    | 23.9                   |
| 20050829A       | 0.99            | 0.26                 | 2.1                    | 18.8                   |
| 20050912A       | 1.15            | 0.15                 | 2.0                    | 21.5                   |
| 20060927A       | 1.01            | 0.25                 | 4.0                    | 30.0                   |
| 20061018A       | 1.04            | 0.14                 | 2.8                    | 35.4                   |
| 20070714A       | 0.34            | 0.11                 | 7.7                    | 169.0                  |
| 20070809B       | 0.56            | 0.26                 | 1.8                    | 14.8                   |
| 20080922A       | 0.13            | 0.13                 | 3.2                    | 20.9                   |
| 20080925A       | 0.64            | 0.20                 | 1.0                    | 31.9                   |
| 20081028A       | 0.75            | 0.15                 | 2.7                    | 28.7                   |
| Average (n = 10)| 0.77            | 0.18                 | 3.0                    | 39.5                   |
| SD             | 0.35            | 0.06                 | 1.9                    | 46.0                   |

*Note: \(g_{syn,max}\), maximum synaptic conductance per synaptic site; \(\tau\)_{rise}, rise time constant; \(\tau\)_{decay1} and \(\tau\)_{decay2}, decay time constants 1 and 2.

solutions. Our main findings are: (1) L2/3 pyramidal neurons receive an on average strong and reliable intracolumnar, intralaminal input by L2/3 local inhibitors (IPSP amplitude = −0.88 ± 0.7 mV); (2) using inverse modeling, we found an estimate of synaptic peak conductance per synaptic site of 0.77 ± 0.4 nS; (3) synaptic contacts were located at 107 ± 64 µm distance from the L2/3 pyramidal neuron soma which is within the range of the sites of excitatory synapses established by L4 spiny neurons and L2/3 pyramidal neurons.

**Interneuron Classification**

Interneurons can carry a wide spectrum of morphological, molecular, and electrophysiological characteristics, so that numerous classifications have been proposed (Ascoli et al. 2008; DeFelipe et al. 2013). Since these classifications do not agree yet on a relevant set of classification parameters, we chose to relate to a quantitative classification of L2/3 interneurons based on their axonal projections with reference to cortical columns, since this measure provides direct prediction of synaptic innervation. Briefly, Helmstaedter et al. (2009b) identified 3 types of L2/3 interneurons as local, lateral, and transamin interneurons based on their axonal projection in relation to their home column and layer using a cluster analysis. Local inhibitors were predicted to innervate pyramidal neurons in L2/3 of the home column. Local inhibitors received a strong input from L4 spiny neurons (EPSP amplitude: 0.96 ± 0.93 mV) and may therefore be substantially activated by a whisker deflection.

**IPSP Properties**

In many previous studies of the rat primary sensory areas, inhibitory connections were measured with “high chloride” intracellular solutions in the postsynaptic cell to facilitate the measurement of postsynaptic currents (e.g., Nicoll et al. 1996; Reyes et al. 1998; Gupta et al. 2000; Holmgren et al. 2003; Otsuka and Kawaguchi 2009; Xu and Callaway 2009) or per voltage clamp only (Packer and Yuste 2011; Pfeffer et al. 2013), which hampers a comparison with the IPSP amplitudes reported here. Other reports described intralaminar IPSP amplitudes recorded (1) by potassium methyl chloride filled sharp electrodes and elicited by unclassified L2/3 interneurons (−0.65 ± 0.44 mV, in rat somatosensory and motor cortex pyramidal neurons; Thomson et al. 2002) or by L4 and L5 fast-spiking interneurons (−1.22 ± 0.71 mV, in rat somatosensory, visual, and motor cortex pyramidal neurons; Thomson et al. 1996), and (2) by whole-cell patch clamp and elicited by L2/3 fast- and non–fast-spiking interneurons (−0.52 ± 0.11 and −0.49 ± 0.11 mV, respectively, in unclassified excitatory cells of GAD67-GFP knock-in mouse somatosensory cortex; Avermann et al. 2012). These values are within the range of the IPSP amplitudes reported here (−0.88 ± 0.67 mV).

The average 20–80% rise time and decay time constant of IPSPs were 3.5 and 76 ms for local inhibitors. This was considerably longer than previously reported for excitatory connections made onto L2/3 pyramidal neurons by L4 spiny neurons (20–80% rise time: 0.8 ms, decay time constant: 12.7 ms; Feldmeyer et al. 2002) and by other L2/3 pyramidal neurons (20–80% rise time: 0.7 ms, decay time constant: 15.7 ms; Feldmeyer et al. 2006). Slow kinetics of IPSPs were previously reported in studies using various techniques (e.g., Thomson et al. 1996, 2002; Holmgren et al. 2003).
AP half width and input resistance of interneurons were negatively correlated with the absolute IPSP amplitude in the present study (cf. Supplementary Table 7 and Supplementary Fig. 6). This is generally in line with results from a recent study by Pfeffer et al. (2013), showing that parvalbumin-positive interneurons of the mouse visual cortex, which were described to show features of fast-spiking interneurons, exhibited stronger inhibition onto pyramidal neurons than somatostatin and vasoactive intestinal peptide-positive interneurons.

Number and Positions of Contacts

Some studies reported high numbers of putative synaptic contacts in basket cell-to-pyramidal neuron connections in various layers of the somatosensory rat cortex using light microscopy (17.2; average of basket cells, Markram et al. 2004). Estimates using electron microscopy in the same cortical area, however, were considerably smaller (2.5, Thomson et al. 1996). Our data lie between these previous measurements (6.2 ± 2 contacts per connection, range 3–10, n = 17 pairs). The path distance of the contacts to the soma was on average 107 ± 64 µm. This soma-contact distance is larger but overlapping with the distance reported for basket cells (79 µm; average of basket cells, Markram et al. 2004), and not predominantly perisomatic. The criteria for basket cell definition often include a large somatic or perisomatic distribution of synaptic contacts [for a review of interneuron classifications, see Markram et al. (2004)]. In our study, however, only 3 somatic contacts could be identified at the light microscopic level (out of 106 total contacts). Synaptic contacts established using light microscopy are generally overestimated on dendrites and underestimated on the soma (Tamas et al. 1997), as some somatic contacts may not be visible due to the limited resolution in the vicinity of a heavily stained soma. However, even if some somatic contacts had to be considered, the distribution of putative synaptic contacts would remain largely dendritic. It is therefore possible that the number of somatic contacts formed by locally projecting basket cells in L2/3 of a barrel column is indeed smaller than previously estimated (Chattopadhyaya et al. 2004; quantitative estimates of perisomatic boutons of basket cells are as low as 30% including proximal dendrites).

Shunting Inhibition Versus Inhibitory Summation

The same inhibitory synaptic input can cause (1) PSP deflections toward its respective synaptic reversal potential and (2) a reduction in the postsynaptic membrane resistance, thereby creating an electric short circuit (i.e., shunting inhibition). In theory, the latter effect prevails if the synaptic reversal potential is close to the membrane resting potential and, following Ohm’s law, can lead to a divisive effect on other synaptic input in spatiotemporal proximity, thereby providing regional control of dendritic computation (Rall 1964; for a review, cf. Koch 1999). To determine the precise role of shunting inhibition in a given network like the barrel cortex, it is therefore necessary to know the spatiotemporal distribution of excitatory and inhibitory inputs on the postsynaptic cell (for a review, cf. London and Hausser 2005). In the present study, the number and distribution of putative synaptic contacts made onto L2/3 pyramidal neurons by local inhibitors was more distal than but highly overlapping with those formed onto L2/3 pyramidal neurons by other L2/3 pyramidal neurons (2.8, 91 ± 47 µm; Feldmeyer et al. 2006) and by L4 spiny neurons (4.5, 67 ± 34 µm; Feldmeyer et al. 2002; cf. Fig. 9). Given

Figure 8. Sensitivity of conductance estimates to variations in other model parameters. (A) Sensitivity of the synaptic conductance estimates ($g_{syn,max}$) to variations in the membrane capacitance ($C_{m}$, top), membrane resistance ($R_{m}$, middle), and axial resistance ($R_{a}$, bottom). (B) Sensitivity of $g_{syn,max}$ to variations in the synaptic reversal potential ($E_{syn}$). (C) Sensitivity of $g_{syn,max}$ to the variations in the number of synapses ($n_{syn}$). Each synapse was deactivated one at a time and the resulting error in $g_{syn,max}$ was estimated.
The number of L2/3 interneurons converging onto a L2/3 pyramidal neuron is thus considerably smaller than that of L4 spiny neurons (~300–400, Lübke et al. 2003) and L2/3 pyramidal neurons (~270, Feldmeyer et al. 2006) converging onto a L2/3 pyramidal neuron. The present data on the location of inhibitory contacts suggest that approximately 125–560 (20–90 * 6.2) inhibitory contacts from L2/3 local inhibitors are located on a L2/3 pyramidal neuron in close proximity to the approximately 1350–1800 (300–400) * 4.5 (Feldmeyer et al. 2002; Lübke et al. 2003) and approximately 750 (270 * 2.8) (Feldmeyer et al. 2006) excitatory boutons from L4 spiny neurons and L2/3 pyramidal neurons, respectively. However, L2/3 interneurons have a more compact geometry than L2/3 pyramidal neurons and receive more and stronger synaptic inputs by L4 spiny neurons upon whisker deflection (L2/3 interneurons vs. L2/3 pyramidal neurons: 3-fold higher anatomical convergence and 1.8-fold higher EPSP amplitudes, Feldmeyer et al. 2002; Helmstaedter et al. 2008). Furthermore, L2/3 interneurons received a strong reciprocal input from L2/3 pyramidal neurons in the present study. It is thus likely that L2/3 interneurons are efficiently activated by L4 excitatory neurons (cf. Helmstaedter et al. 2008) and provide inhibition at the sites of excitatory input to L2/3 pyramidal neurons with only a short delay of a few milliseconds (1.1 ± 0.5 ms, this study). The peak of the inhibitory conductance transient could therefore occur in the early phase of the voltage transient in L2/3 pyramidal neurons following whisker deflection. An early activation of inhibitory connections onto L2/3 pyramidal neurons is supported by a reduction of spiking upon pre-depolarization in the early, but not in the late, phase following whisker deflection (Brecht et al. 2003).

An essential step in further unraveling inhibitory contributions is therefore the numerical simulation of the L4-to-L2/3 intracortical microcircuits. To enable such a simulation, it is crucial (1) to characterize the conductances underlying the measured potential deflections and (2) to identify the in vivo spiking pattern of L2/3 interneurons.

**Modeling of Synaptic Conductances**

We constrained the synaptic properties of L2/3 local inhibitor-to-L2/3 pyramidal neuron connections using realistic multicompartamental models. The average maximum conductance per synaptic site was found to be approximately 0.8 nS. If the single channel conductance is assumed to be approximately 20–30 pS (Brickley et al. 1999; Perrais and Ropert 1999), roughly 25–40 GABA receptors per synaptic site are open at the peak of the conductance transient. Previously, peak conductances have been calculated from the relation between IPSP amplitudes and holding potentials under the assumption of negligible electrotonic distance between the synaptic sites and the somatic recording site (for details, see Benardo 1994). Subsequent division by the number of inhibitory contacts yielded an estimate for the peak synaptic conductance per synaptic site. An upper conductance estimate using this approach on our simulated cells yields (1.1 mV/23 mV/80 MΩ = 0.1 nS conductance per synaptic site. The underestimate using the analytical approach can be attributed to the significant filtering effects of fast synaptic conductance transients when measured at the soma.

The peak conductance estimates and the predicted time constants of synaptic conductance change using the inverse modeling approach presented here were remarkably similar between the two analyzed pairs, while the IPSP amplitudes, numbers of putative synaptic contacts, and postsynaptic electrophysiological (input resistance and membrane time constant) and
morphological properties varied substantially among the connections. Of note, the model estimates for excitatory NMDAR and AMPAR conductances are in the same range as our estimates for inhibitory synaptic conductances (0.52 ± 0.24 and 0.40 ± 0.34 nS conductance/site for AMPAR and NMDAR, respectively, Sarid et al. 2013).

The validity of inverse modeling results depends critically on the validity of the model assumptions. We therefore made a sensitivity analysis to quantify the dependence of the obtained conductance estimates to errors made in assumptions about model parameters (cf. Fig. 8). Errors in assumptions on membrane conductance, synaptic reversal potential, and number of synaptic contacts were found to have the largest impact on model predictions. Overall, however, this analysis demonstrated that the conductance estimates were rather robust with perturbations in model parameters ($C_{	ext{m}}, R_{	ext{m}}, E_{	ext{syn}},$ and $n_{	ext{syn}}$). This supports inverse modeling as a powerful tool to recover parameters that defy direct measurement (cf. also Schaefer et al. 2003, 2007, for an application of inverse modeling to conductance density estimation; and Keren et al. 2005; Druckmann et al. 2011 for inverse modeling of AP trains).

Outlook

The reliable connection between L2/3 local inhibitors and L2/3 pyramidal neurons as found here allows direct predictions about the strength, spatial extent, and temporal characteristics of inhibition on L2/3 pyramidal neurons in the L4-to-L2/3 microcircuit in rat barrel cortex, following a whisker stimulus. To investigate the effect of stimulus-evoked activity in the entire L4-to-L2/3 circuit, however, numerical models of this microcircuit will be required that implement the experimentally found constraints on anatomical connectivity, electrical excitability, and synaptic properties (Helmstaedter et al. 2007; Sarid et al. 2007). We suggest that such an in silico approach—when based on solid experimental data—may help to gain a mechanistic understanding of the activation of L4 and L2/3 neurons in a cortical column following a sensory stimulus.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org.

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Notes

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