

Widely integrative properties of layer 5 pyramidal cells support a role for processing of extralaminar synaptic inputs in rat neocortex

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Abstract

We have compared the length, strength, conduction velocity and divergence of horizontal connections onto layers 2/3 and 5 neurons in slices of rat primary somatosensory neocortex. Slices were cut along laminar borders to eliminate most vertical connections, and excitatory postsynaptic potentials (EPSPs) were recorded from pyramidal cells in adjacent uncut tissue. When electric stimuli were delivered within the same layer as the recorded cell, EPSPs could be evoked up to 2000 μm away for both layers 2/3 and 5 pyramids. Estimates of horizontal axonal conduction velocities (~ 0.4 m/s) and the thresholds for activation also did not differ between layers. However, layers 2/3 cells rarely responded to stimuli delivered to isolated deeper layers, while layer 5 neurons were often excited by horizontal inputs from isolated layers 2/3 and 4.

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Other studies have been performed to elucidate the electrophysiology of long-range horizontal cortical connections in brain slices [6,10]; however, strong vertical connections in the cortex make it difficult to isolate horizontally-projecting axons without entraining the activity of a vertical column of cells. To isolate horizontal monosynaptic connections that could correspond to the long horizontally-projecting axons described in anatomical studies, we made horizontal cuts in slices effectively isolating the connections of individual layers into laminar strips. We then recorded synaptic potentials in layers 2/3 and 5 cells evoked from stimuli delivered in each of the laminar strips.

Sprague–Dawley rats aged 4–8 weeks were deeply anaesthetized with Nembutal and placed in an ice-water bath for 5 min. The rats were decapitated, their brains removed, and 400 μm coronal sections of somatosensory (SmI) cortex were made and bathed in cold physiological

solution. Slices were transferred immediately to a fluid/gas interface chamber, saturated with humidified 95% O_2 /5% CO_2 , maintained at $34 \pm 1^\circ\text{C}$, and superfused with artificial cerebral spinal fluid (ACSF) at a rate of 2–3 ml/min. The ACSF contained (in mM) 126.0 NaCl, 3.0 KCl, 2.0 MgSO_4 , 2.0 CaCl_2 , 26.0 NaHCO_3 , 1.25 NaH_2PO_4 , and 10.0 dextrose. A fragment of a razor blade bent to match the arched contour of the slice was used to cut, parallel to the pia, 500 μm laminar strips, each strip remaining attached to the flanking block of cortex. Some cortical lamination was visible and that along with the distance from the pia was used to determine the laminar borders for cutting. Four approximately 3 mm long strips of cortex containing layers 1 and 2/3, layer 4, layer 5, and layer 6 were cut into each slice. Intracellular recordings were then made in the intact flanking block of cortex in either layers 2/3 or layer 5.

Intracellular recordings were made with glass micropipettes (80–200 $\text{M}\Omega$) filled with 4 M potassium acetate. An acceptable intracellular recording was one that fulfilled the following general criteria: stable recording for > 10 min, resting membrane potential more negative than -60 mV, input resistance > 20 $\text{M}\Omega$, and an action potential amplitude of > 70 mV. Responses to intracellular current injection were used to classify pyramidal cells as adapting, nonadapting, and bursting. Adapting neurons fire single

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spikes and show frequency adaptation. Nonadapting neurons fire rhythmic trains of single spikes without frequency adaptation. Bursting neurons fire at least two action potentials at threshold and show prominent after-depolarizations.

A bipolar stimulating microelectrode controlled by a hydraulically driven micromanipulator was used to electrically stimulate (200 μ s, 10–100 μ A shocks at 0.2 Hz) horizontal connections isolated by laminar cuts. Postsynaptic potentials (PSPs) studied were evoked with only just suprathreshold stimulus current to avoid recruiting polysynaptic excitatory or inhibitory activity. Synaptic events were averaged over 64 trials to determine the latencies and shape data of the PSPs evoked from different laminar and lateral stimulus positions. Stimuli delivered in pairs at intervals between 3 and 5 ms were also used in an attempt to discriminate between mono- and polysynaptic excitatory postsynaptic potentials (EPSPs): failure of the second stimulus of a pair to elicit a synaptic response was taken as a polysynaptic connection [1]. EPSP latencies were used to estimate the axonal conduction times and contribute information to indicate whether neuron responses were mono- or polysynaptic. We presumed that a response was monosynaptic if it had smooth rising and falling phases, did not vary more than 0.1 ms in its onset time, responded to both paired stimuli, and fit to a linear velocity profile for EPSP latencies at different horizontal distances.

We recorded from 26 layer 5 cells and 13 layers 2/3 cells in which we were able to assess firing properties, input resistances, time constants, and the latencies of EPSPs evoked at different laminar and lateral positions. All impalement sites for layer 5 cell recordings were made 1400 μ m from the pia. Recordings in layers 2/3 were made between 400 and 550 μ m from the pia. Biocytin staining [5] was used in all cases to fill cells so their morphology could be determined. Background staining made it possible to confirm the laminar positions of horizontal cuts. Table 1 is a summary of the distribution of cell membrane and firing properties.

We evoked EPSPs in layers 2/3 and 5 cells from different laminar strips and at increasing horizontal distances. Fig. 1A shows an example of a layer 5 cell's responses to stimuli delivered in layer 5 at different lateral distances. Fast rising and slow, smooth falling EPSPs are seen with later onset

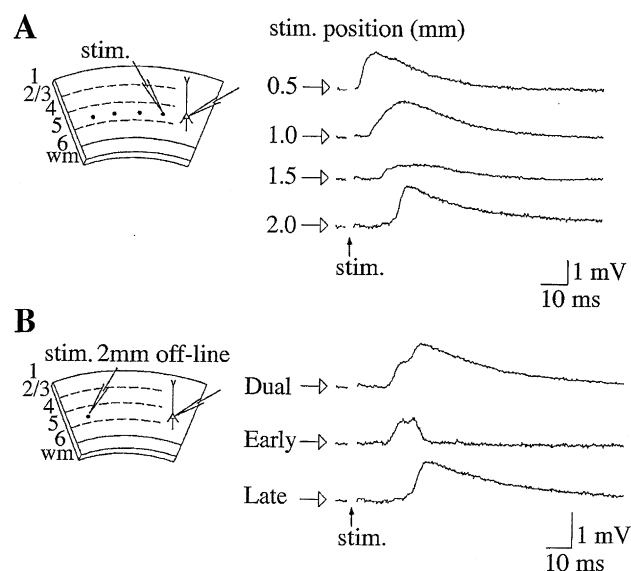


Fig. 1. A layer 5 neuron's responses to stimuli delivered in the same layer at different lateral distances. (A) Lateral stimulus positions were selected by moving the stimulus electrode horizontally at increments of 0.5 mm in layer 5. Dashed lines represent laminar cuts to isolate monosynaptic lateral connections; EPSPs with later onset times for the more distant placed stimulus positions are shown: no responses were recorded for stimuli placed greater than 2 mm laterally. (B) A rare example of presumed polysynaptic activity evoked in a layer 5 cell with a near threshold stimulus at a distant lateral position; episodes recorded of dual, early, and late EPSP activity are shown.

times for the more distant placed stimulus positions. Lateral stimulus positions were selected by moving the stimulus electrode horizontally at increments of 500 μ m. In no case could we evoke a monosynaptic response further than 2 mm from a cell. Fig. 1B shows an example of what we presume is polysynaptic activity evoked with a near-threshold stimulus at a distant lateral position. Episodes of the early and late components recorded alone are also shown. Another explanation for this kind of activity is that the stimulus activated two different axonal paths, one travelling a less direct route than the other or one synapsing further away from the soma than the other. In any case such activity was rare, so we were unable to test if it persisted when paired pulses were tested.

Distance profiles for EPSP response success are shown in Fig. 2A,B. Layer 5 neurons responded to stimuli delivered > 500 μ m away only for stimuli in layer 5 or 6. Layers 2/3

Table 1
Firing properties and membrane properties of layers 2/3 and 5 neurons

	Layer 5			Layers 2/3
Firing properties	Adapting	Nonadapting	Bursting	
Number of cells	13	9	4	13
Resting potential (mV)	75 \pm 5.1	74.0 \pm 6.3	76.0 \pm 1.0	88.7 \pm 10.3
Spike amplitude (mV)	86.1 \pm 9.1	83.7 \pm 8.2	86.6 \pm 6.6	80.0 \pm 10.7
Input resistance (M Ω)	36.4 \pm 6.3	23.1 \pm 5.3	20.6 \pm 0.8	27.5 \pm 8.8
Time constant (ms)	9.6 \pm 1.5	10.5 \pm 2.7	8.8 \pm 0.7	10.0 \pm 3.3

The firing properties and the characteristics of the membrane of the layers 2/3 and 5 cells studied are shown (\pm standard deviations).

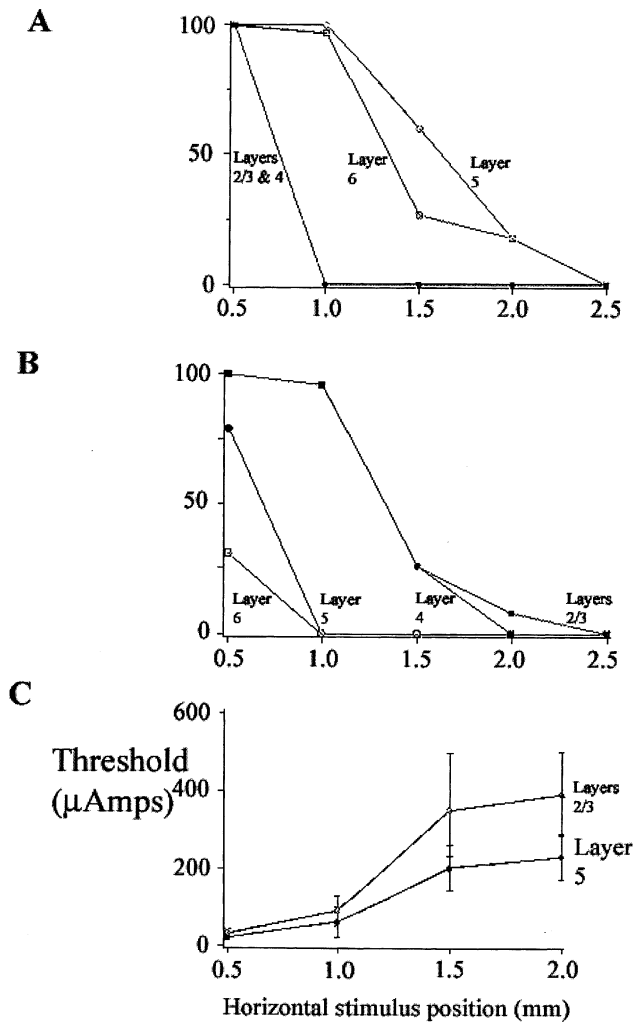


Fig. 2. (A) Distance profile of EPSP response success determined for 25 layer 5 neurons responding to stimuli at different horizontal and laminar positions in cut slices. Response success was best for stimuli delivered within 1 mm and fell off rapidly for more distant stimuli. Response success for stimuli delivered within 0.5 mm was 100% for every layer, but was better for stimuli delivered in layers 5 and 6 for more distant horizontal stimulus positions. (B) Distance profile of EPSP response success determined for 12 layer 2/3 neurons responding to stimuli delivered in different layers at different horizontal positions in cut slices. Like the result for layer 5 neurons, response success was best for stimuli delivered within 1 mm. But unlike the result for layer 5 neurons, layers 2/3 neuron response success was better for stimuli delivered in layers 2/3 and 4 and not 100% for stimuli in layers 5 and 6 even within 0.5 mm. Also, fewer layer 2/3 than 5 cells responded to stimuli positioned 2 mm away. (C) The threshold current amplitude to evoke a monosynaptic cell response plotted as a function of the horizontal stimulus position for layers 2/3 and layer 5 cell responses to stimuli delivered in the same layer; the current thresholds for layer 2/3 and 5 cell responses are very similar.

neurons responded to stimuli $>500 \mu\text{m}$ away only when stimuli were delivered in layers 2/3 or 4. Response success for both layers 5 and 2/3 neurons was best for stimuli delivered within $1000 \mu\text{m}$ and fell off rapidly for more distant stimuli. About 10% of the layers 2/3 and 5 cells responded to stimuli delivered at $2000 \mu\text{m}$ but only in the upper and lower layers, respectively. We did not find that

effective long horizontal axons are restricted to layer 5 or another layer. We did find that layer 5 neurons responded best to laterally distant stimuli delivered in the infragranular layers, and that layers 2/3 neurons responded best to laterally distant stimuli delivered in the granular and supragranular layers. Fig. 2C plots the threshold current as a function of the lateral stimulus position for layers 2/3 and 5 neuron responses to stimuli delivered in the respective layers. Neither layers 2/3 nor layer 5 stimuli could be distinguished as requiring less current to evoke a synaptic response at different horizontal distances.

We found no correlation between a layer 5 neuron's firing pattern and its ability to respond to a distant laterally placed stimulus. Averages (\pm standard deviation) of the longest lateral responses for each class of layer 5 neuron were as follows: adapting = $1.39 \pm 0.39 \text{ mm}$, nonadapting = $1.50 \pm 0.38 \text{ mm}$, and bursting = $1.33 \pm 0.24 \text{ mm}$. It is remarkable that of the 25 layer 5 neurons tested, 100% responded to stimuli delivered $500 \mu\text{m}$ away in every layer. Neither a particular class of layer 5 neuron nor layer 5 neurons in general distinguished themselves by responding to distant laterally placed stimuli, but layer 5 neurons were significantly superior at responding to a wider range of extralaminar inputs, which is in agreement with other published findings indicating that layer 5 neurons can associate with inputs arriving at different cortical layers [7,9].

The latencies of EPSPs recorded in layers 2/3 and 5 neurons to stimuli delivered in layers 2/3 and 5, respectively, were used to estimate and compare conduction velocities for the horizontally-projecting presynaptic axons. A graph of the EPSP onset latencies versus the horizontal stimulus positions for both layers is shown in Fig. 3. Estimates of axonal conduction velocities were made by calculating the inverse of the slope of each line fit by regression analysis. The estimates for conduction velocities

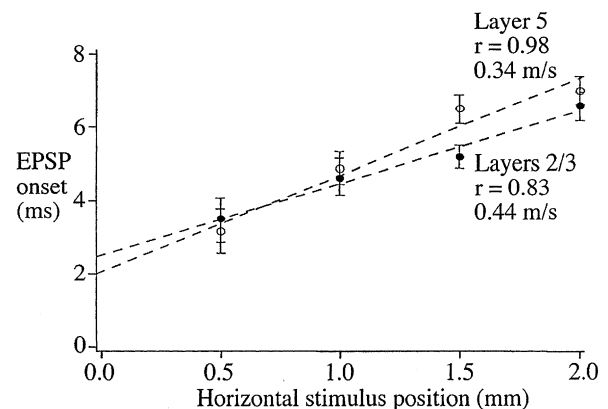


Fig. 3. The latencies of EPSPs recorded in layers 2/3 and 5 neurons to stimuli delivered in layers 2/3 and 5, respectively, were used to estimate and compare axonal conduction velocities. EPSP onset latencies are graphed as a function of the horizontal stimulus positions. Estimates for axonal conduction velocities were between 0.35 and 0.45 m/s for axons in both layers with overlap in the calculated variances.

were between 0.34 and 0.44 m/s for axons in both layers with overlap in the calculated variances. These estimates fall in the range of values for the axon conduction velocities reported in cat visual cortex (3.5 ± 1.2 mm/ms) by Hirsch and Gilbert [4] and in rat visual cortex (0.15–0.55 mm/ms) by Murakoshi et al. [12], but were about three times slower than one estimate [13] for the conduction velocity of an axon collateral in CA3 (1.3 m/s). Our estimate of a conduction velocity assumes axons do not deviate from a direct line between the stimulating and the recording electrodes, which is unlikely but probably does not affect our estimate enough to explain the difference between our measurement and the value from the hippocampus.

Some data on the functional length of horizontal connections in the cortex have been available from recordings in pairs of neurons [11] and from cross-correlation analysis [3], but indicate that horizontal functional interactions exist mainly in a range of up to 400 μ m, well below the range of 2000 μ m we found for approximately 25% of layer 5 neuron connections. Evidence to support our findings is from cortical anatomy, reports of long-range horizontal connections observed projecting for up to 6 mm in the cortex [2,8], and physiology, cell pairs varying in horizontal separations of up to 2 mm showing correlated firing in the visual cortex in one study [14]. The fact that we did not see functional connections beyond 2000 μ m could be due to the slice technique which reduces the probability of including large numbers of long axons in any one slice, so we can not claim based on our data that even longer functional horizontal connections do not exist.

Completely neglected in this study were the possible roles that synaptic inhibition and intrinsic neuronal excitability could play in accounting for our results and how they could contribute to the complexity of the pattern of horizontal connections in the cortex. We did not record from interneurons or make dual impalement recordings that would have provided valuable information on the pattern of connections. We did find that in neocortical slices a proportion of layers 2/3 and 5 neurons are able to respond to monosynaptic inputs originating from as far as 2 mm away, but only for inputs originating from their same layers. Layers 2/3 and layer 5 axon conduction velocities were not significantly different and were estimated to be approximately 0.4 m/s. Every layer 5 neuron tested responded to electrical stimuli delivered in supragranular, granular and infragranular layers.

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