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Intracortical connectivity revealed by spike-triggered averaging in slice preparations of cat visual cortex

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Intracortical connectivity was studied in slice preparations of cat's visual cortex by spike-triggered averaging. The experiments documented the unitary postsynaptic potentials underlying the inhibitory and excitatory connections from layer III–IV border cells to supragranular cells, as demonstrated previously by cross-correlation studies. In addition the analysis demonstrated the existence of two excitatory connections, between supragranular and layer V cells, that were not detectable in previous cross-correlation studies.

Visual cortical circuitry underlying the selectivity of neuronal responsiveness to visual stimuli has been studied by cross-correlating the activity of cortical cells[11,13–16]. However, such cross-correlations have demonstrated only a fraction of the synaptic connections suggested by axonal and dendritic morphology of cortical and geniculate cells[3,5,9,12]. Cross-correlation analysis has limited capability of detecting synaptic connections with small, slowly rising synaptic potentials[2,7]. Moreover, cross-correlations between cortical cells obtained in vivo typically include a large central peak from common synaptic input that may obscure components due to direct connections between the cells.

The present study was designed to document the neuronal connections between specific layers which remained undetected by cross-correlation analysis, by using spike-triggered averaging of intracellular potentials[6,10]; this provides more direct and sensitive measures of the strength and polarity of the synaptic connections[2,7]. The experiments were performed in slice preparations of the visual cortex, which provides stable intracellular recording and allows visual control of experimental procedures, such as insertion of recording microelectrodes into particular cortical layers.

Coronal slices (thickness, 500 μm) were dissected from visual cortex (area 17) of 9 adult cats[8] and placed in a recording chamber of the interface type[4]. Intracellular and extracellular recordings were made simultaneously from pairs of cortical cells in the same column. A glass microelectrode filled with 2 M potassium methylsulfate (electrical resistance, 50–150 MΩ) was used for intracellular recording of cortical cells. The other recording electrode was triple barreled; one barrel contained a carbon fiber electrode[1] for extracellular recording of cortical cells. Another barrel contained 200 mM sodium glutamate to chemically activate the cortical cells. The remaining barrel was filled with saline containing 2% Pontamine sky blue, to mark the recording sites. The two recording electrodes were each mounted on a 3-dimensional oil-driven micromanipulator (Narishige MO-103), whose axes were aligned with the columnar and laminar structures of the cortical slice. One electrode was advanced orthogonal to the cut-sur-

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face of the slice and the other electrode obliquely at angle of 75° to the cut-surface. Pairs of cortical cells were sampled at various separations (0.05–1.1 mm) in layers II–V aligned along the columnar axis.

Five to 27 neuronal pairs were sampled in a single slice, and the site of the last extracellular recording was marked by iontophoretic ejection (5 μA for 5 min) of Pontamine sky blue. A second dye mark was placed at a distance of 0.5–1 mm from the first, to calibrate the shrinkage of histological sections. The cortical location of the marked sites was determined on histological sections (50 μm in thickness and stained with Cresyl violet) of the slice. Shrinkage (2–15%) was determined as a ratio of the distance of the spots to the distance measured from the micromanipulator readings. The locations of the other cells, recorded extracellularly and intracellularly, were determined by reference to the micromanipulator readings, after correcting for the shrinkage of histological sections.

The intra- and extracellular responses of the neuronal pairs during glutamate stimulation were amplified, displayed on an oscilloscope, and recorded on magnetic tape by means of a PCM data recorder (NF circuit; frequency band, DC-10 kHz). The recorded responses were processed off-line by a PDP 11/23 computer. Pulses of a single cortical cell were generated by a time-amplitude window discriminator (BAK, DIS-1) and used to average the intracellular potentials. The spike-triggered averages included 50–500 events.

Synaptic connectivity was studied in 139 neuronal pairs, consisting of 108 target and 60 source cells, recorded intra- and extracellularly, respectively. Cells with stable resting membrane potentials exceeding −50 mV were selected for analysis. Synaptic connections were demonstrable in 21 pairs by the spike-triggered averaging.

Fig. 1A represents an example of a pair of cortical cells with an inhibitory synaptic connection. The source cell was located in the juxtagranular (JG) area (border between layer III and IV) and the inhibited target cell in the supragranular (SG) layer (0.45 and 0.27 mm from the cortical surface). During glutamate stimulation, the extracellular electrode recorded impulse discharges of different amplitudes from several JG cells (upper trace). The postsynaptic effect of the cortical cells was sought in intracellular

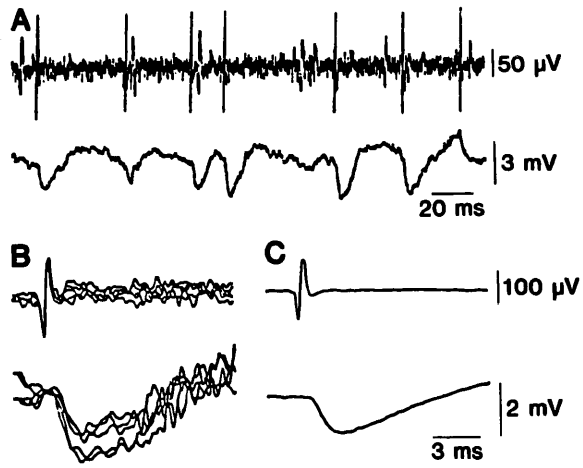

Fig. 1. Simultaneous recording from neighboring cortical cells and spike-triggered averaging. A: extra- (upper trace) and intracellular recordings from cortical cells (lower trace). Impulse discharges were recorded extracellularly from cortical cells in the JG area during iontophoretic ejection of glutamate (current intensity, 40 nA), while postsynaptic potentials were recorded intracellularly from the SG cell layer. B and C: superposed and averaged traces of the largest impulses (upper traces) and postsynaptic potentials (lower traces) triggered by those impulses. Spike-triggered averaging was made for 481 impulses. All traces were obtained by AC recording (time constant, 0.08 s), and upward deflection in both recordings represents positivity. Voltage and time calibration are common to B and C.

recording from the partner cell (lower trace). In this case, the membrane potential of the partner cell was depolarized by passing currents to enhance the inhibitory postsynaptic potentials (IPSPs). The hyperpolarizing postsynaptic potentials occurred in the partner cell in synchrony with the largest extracellular action potentials, but not with the small impulses. The hyperpolarizing potentials were probably IPSPs, since they were about 200 μV in amplitudes without current injection (Fig. 2B), became very large (about 2 mV) by a depolarization of 20 mV, and were almost nulled by a hyperpolarization of 10 mV. The correspondence of the IPSPs with the largest impulses was further confirmed by superposing the intracellular traces triggered by the largest impulses (Fig. 1B). The spike-triggered average (Fig. 1C) demonstrates that the onsets of the IPSPs began 0.7 ms after the negative peak of the impulses, consistent with a monosynaptic delay. This suggests that the JG cell exhibiting the largest extracellular impulses had an inhibitory connection with the partner SG cell.

Similar inhibitory connections were demonstrated by spike-triggered averaging in 7 pairs of cortical

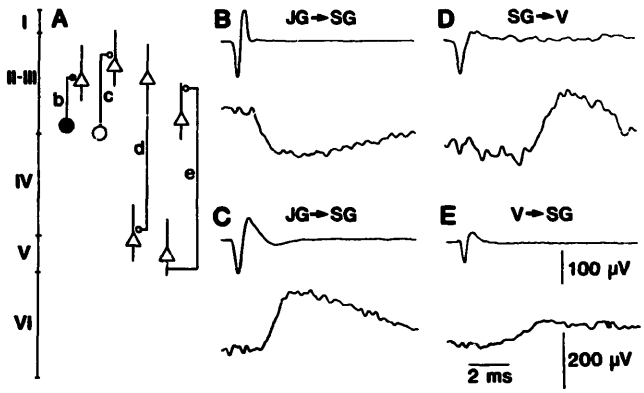

Fig. 2. Unitary postsynaptic potentials demonstrated by spike-triggered averaging. A: schematic diagram representing laminar location of source and target cells. Neuronal connections b–e were identified by the presence of unitary postsynaptic potentials shown in B–E, respectively. Open circles and triangles represent excitatory cells and filled circles represent an inhibitory cell. B: unitary IPSP evoked in an SG cell from a JG cell. C–E: unitary EPSPs produced in an SG cell from a JG cell, in a layer V cell from an SG cell and in an SG cell from a layer V cell, respectively. Spike-triggered averaging was made for 360, 424, 55 and 157 impulses in B, C, D and E, respectively. Voltage and time calibration are common to all traces. All traces were obtained by AC recording (time constant, 0.08 s), but were corrected for the distortion of the postsynaptic potentials due to the AC recording.

cells. Three of the 7 pairs exhibited divergent inhibitory connections from a single source cell to multiple neighboring target cells (within 200 μm). The source and target cells were all located in the JG area (depth, 0.35–0.5 mm), and the SG layer (0.15–0.35 mm), respectively, and the intracortical separation of the connected cells was relatively small (150 ± 60 μm). The unitary IPSPs all exhibited delays in the monosynaptic range (0.6 ± 0.1 ms) and had small amplitudes (90 ± 50 μV) without current injection (resting membrane potential, between −50 and −70 mV). However, they seemed capable of strong shunting action, since all the IPSPs were substantially potentiated (up to a few millivolts) by depolarization (about 20 mV) of the membrane potential.

Excitatory synaptic connections were demonstrated in similar spike-triggered averages in 14 neuronal pairs. Three groups of excitatory connections were distinguished (Fig. 2C–E, Table I): (1) from the JG area to the SG layer (depth, 0.3–0.5 mm and 0.15–0.35 mm) for 7 pairs, (2) from the SG layer to layer V (0.2–0.3 mm and 0.9–1.05 mm) for 3 pairs, and (3) vice versa (0.9–1.05 mm and 0.2–0.45 mm) for 4 pairs. The 7 JG-SG connections included 2 cases of convergence onto a single target cell from several neighboring source cells and one case of divergence onto two target cells from a single source cell.

The unitary excitatory postsynaptic potentials (EPSPs) for the first two groups of excitatory connections had comparable amplitude, rise time and half width; in contrast, the layer V-SG connection had EPSPs significantly smaller in amplitude, slower in rise time and longer in half width (cf. Fig. 2C–E and Table I). This suggests that the excitatory synapses of the layer V-SG connection were located more distal to the somata and would be less effective in activating their targets than those of the other two connections[2]. The 3 groups of excitatory connections showed a considerable range in delays of the EPSP onsets (cf. Fig. 2C–E, Table I). The delays were clearly within the monosynaptic range for the EPSPs of the JG-SG connections, while those for the other two connections were substantially longer. However,

TABLE I

Neuronal connections and unitary postsynaptic potentials demonstrated by spike-triggered averaging

The numerators in column 'number' represent number of neuronal pairs with synaptic connections and the denominators represent total number of neuronal pairs examined. JG-SG, SG-V and V-SG represent juxtagranular-supragranular, supragranular-layer V and layer V-supragranular connections, respectively.Parameters of unitary postsynaptic potentials were measured under membrane potentials between −50 and −70 mV without current injection.

	Number	*Intercellular distance (μm)*	*Delay (ms)*	*Amplitude (μV)*	*Rise time (ms)*	*Half width (ms)*
IPSP						
JG-SG	7/89	150 ± 60	0.6 ± 0.1	90 ± 50	1.6 ± 0.8	7.5 ± 2.7
EPSP						
JG-SG	7/89	230 ± 70	0.7 ± 0.3	140 ± 90	1.7 ± 0.6	6.3 ± 1.8
SG-V	3/27	720 ± 130	2.8 ± 1.4	210 ± 70	1.7 ± 0.1	5.0 ± 0.9
V-SG	4/23	660 ± 80	2.2 ± 0.3	60 ± 30	2.8 ± 0.9	12.8 ± 5.0

the latter also probably reflect monosynaptic pathways; target cells were never excited to generate impulses by the EPSPs produced by the source cells activated by glutamate stimulation in the slice, so disynaptic impulse transmission seems very unlikely under the present experimental conditions. Consequently, the longer delay of the EPSPs for the SG-layer V and layer V-SG connections may be explained by longer conduction distances or slower velocities of impulse conduction along the axon of source cells. In fact the ratio of distance to delay was comparable for all four groups. The delay for the layer V-SG connections was slightly shorter than the reverse connections, possibly due to faster conduction velocities of layer V cell axons.

The amplitudes and rise time of unitary EPSPs of the JG-SG and SG-layer V connections were characteristic of somatic EPSPs, but remarkably, they were not significantly changed by membrane hyperpolarization (about 20 mV) via current injection, in contrast to the considerable effect of membrane depolarization on the unitary IPSPs. This suggests that the conductances of unitary EPSPs may be significantly smaller than those for the unitary IPSPs.

In summary, spike-triggered averaging in cortical slices has demonstrated one inhibitory and 3 excitatory connections in visual cortex. Two of these connections, the inhibitory and excitatory JG-SG connections, have already been found in cross-correlation analysis[11,14,15]; the remaining two, the excitatory SG-layer V and layer V-SG connections were newly demonstrated by the present study. The latter connections remained undetected by cross-correlation analysis, probably because they were weaker or more diffuse than the JG-SG connections. The layer V-SG EPSPs were smaller and rose more slowly than the JG-SG EPSPs; both factors would reduce the size of the correlogram[2]. Moreover, there was convergence and divergence in the JG-SG connections, but these were never seen in SG-layer V or layer V-SG connections.

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