set of 3-mm-wide silver bars (separated by 1 mm) over a surface (4 by 4 cm) by passive thermal conduction. The skin-thermode interface temperature was used for analysis.
14. Response measures were normalized with (Grill-Warm)/(Cool-Warm). The cause of the selective decrease in COLD cell activity is presently undetermined but could reflect inhibition of COLD cells by the activity of warm afferent fibers.
15. The illustrated stimulus-response functions of the HPC and COLD lamina I STT cells studied with the grill show that, at low temperatures, the rate of firing of COLD cells approaches an asymptote and the rate of firing of HPC cells monotonically increases. Unpublished data obtained from other cells with colder probes indicate that HPC activity indeed continues to increase, whereas COLD activity does not, which is consistent with the behavior of C polymodal and cold-specific afferents, respectively (*1, 6*).
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18. The temperature of the grill thermode was uniformly set at 20°, 15°, 10°, 5°, or 0°C. Participants responded, using a 50-mm pain VAS and a word list, after placing a hand on the thermode for 50 s.
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Rearrangements of Synaptic Connections in Visual Cortex Revealed by Laser Photostimulation

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Assessing patterns of synaptic connections in the developing mammalian neocortex has relied primarily on anatomical studies. In a physiological approach described here, the patterns of synaptic connections in slices of developing ferret visual cortex were determined with scanning laser photostimulation. Functional synaptic inputs to pyramidal cells in cortical layers 2 and 3 originating from sites close to the neuronal cell body appeared at least 2 weeks before eye opening, prior to the formation of long-range horizontal connections. Extensive long-range horizontal connections appeared in the next 10 days of development. The number of local connections peaked at the time of eye opening; the number of these connections subsequently declined to the level found in the adult while the specificity of long-distance connections increased. Thus, the relative influence of local connections on the activity of layer 2 and layer 3 neurons declines as the cortex matures while the influence of longer range connections increases substantially.

Although neuronal activity participates in the development of circuitry in the visual system (*1*), most insights into the organization of local cortical circuits and their development have derived from anatomical approaches (*2–5*) rather than from the direct assessment of functioning synaptic connections (*6*). During development, axonal branches are unstable (*7*) and the locations of synapses are difficult to determine, even with electron microscopy (*8*). Thus, although the basic anatomical features of local circuits in developing visual cortex are well established (*4, 5*), the relation between these patterns of anatomical projections and the functional interactions among individual neurons remains speculative.

Our investigations focused on the development of horizontal connections in layer 2 and layer 3 of ferret visual cortex. In adult visual cortex, horizontal projections link regions with similar functional properties, forming clusters of axon collaterals in specific regions (*2, 9*). These horizontal connections originate primarily from pyramidal neurons, form excitatory synapses on other pyramidal neurons and interneurons (*10*), and extend several millimeters in the tangential plane of the cortical plate. Anatomical studies have demonstrated that the characteristic patchy patterns of horizontal projecting axons in visual cortex are not present initially but emerge gradually from a more diffuse state by activity-dependent mechanisms. These mechanisms involve the growth of long, unbranched axons; this is followed by the elaboration of collaterals in appropriate locations and the selective retraction of collaterals in inappropriate regions (*4, 11*). However, the locations of functional synapses, if any, along these collaterals are unknown. We developed scanning laser photostimulation to determine the number, position, and relative strength of functional horizontal connections at different stages of development.

Scanning laser photostimulation is based on highly localized laser photolysis of caged neurotransmitters (*12*) in brain slices (*13, 14*). We recorded from single neurons using whole cell, patch-clamp techniques in tangential cortical brain slices (350 μm thick), while the slices were continuously perfused with artificial cerebrospinal fluid containing "caged" glutamate, which is inactive until photolyzed by ultraviolet light (UV, 330 to 380 nm). The localized uncaging of glutamate at any x, y, z coordinate in the slice causes a small number of neurons in the region of the laser spot (≈15 μm in diameter) to generate action potentials; if any of these neurons form synapses with the recorded cell, a monosynaptic postsynaptic current (PSC) is generated (*15*). Photouncaging at a large array of locations throughout the brain slice (up to 1500 sites, 50 μm apart, covering approximately 3.8 mm^2) produces a map of the locations that generate PSCs in the recorded cell (Fig. 1) without contamination by fibers of passage.

We examined the development of local intracolumnar (≤0.5 mm from the electrode) synaptic connections and long-distance intercolumnar (>0.5 mm) synaptic connections in a sample of 27 neurons from tangential brain slices of layer 2 and layer 3 of ferret primary visual cortex. Animals ranged in age from postnatal day 17 (P17, birth = P0) to adult (>P55). Experiments were carried out in ferret brain slices from three age groups, on the basis of the developmental state of the visual system. The first group (P17 through P26) corresponded to the period before eye opening when some layer 2 and layer 3 cells were still migrating (n = 8 cells), the second group (P27 through P40) was from the period just before and shortly after eye opening (n = 12 cells), and the third group (mature, P41 through adult) consisted of slices obtained after eye opening and included adults (n = 7 cells).

In mature cells, stimulation at most sites (82.4 ± 3.6%) did not elicit PSCs in the postsynaptic cell (Fig. 1) (*16*). However, stimulation at several zones approximately 1 mm from the cell body evoked PSCs in the postsynaptic neuron. Because of their size, spacing, and location, these groups of functional synaptic inputs are likely to originate from the clustered axonal arbors of pyramidal cells that have been anatomically

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described (2, 4, 9). All mature cells had at least one cluster of synaptic inputs approximately 1 mm from the cell body (see Fig. 1B). Thus, the spatial maps of mature cells have features expected from the anatomy of individual neurons visualized with intracellular injection (2, 4).

The patterns of synaptic connections in the youngest animals that we examined (P17 through 26) were completely different from those in the adult (Fig. 2). Local responses to uncaged glutamate were dominated by a large, slow inward current (Fig. 2A). This current lasted several hundred milliseconds and was found in all cells from this age group but not in any other. The onset of the current during the laser pulse itself implies that it arose from direct activation of glutamate receptors on the recorded cell. That the slow component of this current was much reduced or absent in cells from older animals could be due to the presence of different subtypes of glutamate receptors present in young cells (17) or to a lower rate of glutamate uptake by the glia in the most immature brains (18). Superimposed on top of the large, direct current were a few small (3 to 15 pA), evoked synaptic currents.

Between P17 and P26, the fraction of locations generating PSCs in a given map was low (9.2 ± 1.1%) (Fig. 3), and almost all of the locations that provided synaptic inputs to the recorded cell were near the cell body (Fig. 3). In seven of eight pyramidal cells from P17 through P26 animals, stimulation at distances where clustered connections are found in the adult (>1.0 mm from the cell body) rarely evoked responses (2.5 ± 0.8% of the sites generated PSCs) (Fig. 3). Even near the cell body (<0.5 mm), few synaptic inputs were detected (Fig. 3). Morphologically, the axons of the recorded neurons from this age group were long (>0.5 mm), unbranched, and immature, in agreement with anatomical work (4, 19). Given the presence of extensive axons at these ages, the paucity of functional inputs onto cells from distant sites is surprising. Horizontal projections are reciprocal (20), thus suggesting that, at this age, the long, unbranched collaterals of pyramidal cells form few functional synapses at locations distant from the cell body of origin (21).

Shortly before and after eye opening (P27 through P40, $n = 12$), patterns of synaptic inputs to cells differed from those of younger and older animals. Synaptic inputs were most numerous during this time. In comparison to the P17 through P26 age group, the number of synaptic inputs in this age group increased both locally and at longer distances (Figs. 2B and 3). The percentage of stimulated sites evoking PSCs almost tripled, from 9.2 ± 1.1 to 25.6 ± 4.4%. Unlike maps from the youngest age group, for this age group numerous PSCs were generated by stimulation at distant sites, in addition to those in the immediate vicinity of the cell body (Fig. 3). The change from the youngest age to this age is best characterized as a constructive phase, with the addition, rather than the elimination, of synapses. Compared to mature maps, however, the patterns of synaptic inputs around the time of eye opening (Fig. 4B) were more evenly distributed and less organized than in the adult (Fig. 4C), although the fraction of sites stimulated at ≈1 mm from the cell body that resulted in PSCs was not statistically significantly different from that in the adult (Figs. 3 and 4).

Long-distance horizontal projecting axons were present at all ages examined. Therefore, early in development, when collaterals are first elaborated, projecting axons must fail to form functional synapses (22), form low numbers of synapses (21), or form synapses with a low probability of transmitter release (23). Thus, in the youngest animals, even when axonal arbors are extensive, cells receive few long-distance synaptic connections (Fig. 4A). These results imply that axonal projections in the developing ferret visual cortex do not form functional connections with equal probability along their entire length, especially during their initial formation. Thus, the influence of long-distance (>0.5 mm) synaptic interactions must be weak during this initial period of axonal outgrowth.

When the animals' eyes first open, the pattern of functional connections is reminiscent of the pattern of labeling found with retrograde tracing and intracellular filling methods at eye opening (2, 4, 19). The subsequent reorganization of long-distance synaptic connections between eye opening and maturity is also consistent with earlier anatomical experiments (2–5), supporting the idea that the functional connections undergo refinement during the period when anatomical rearrange-

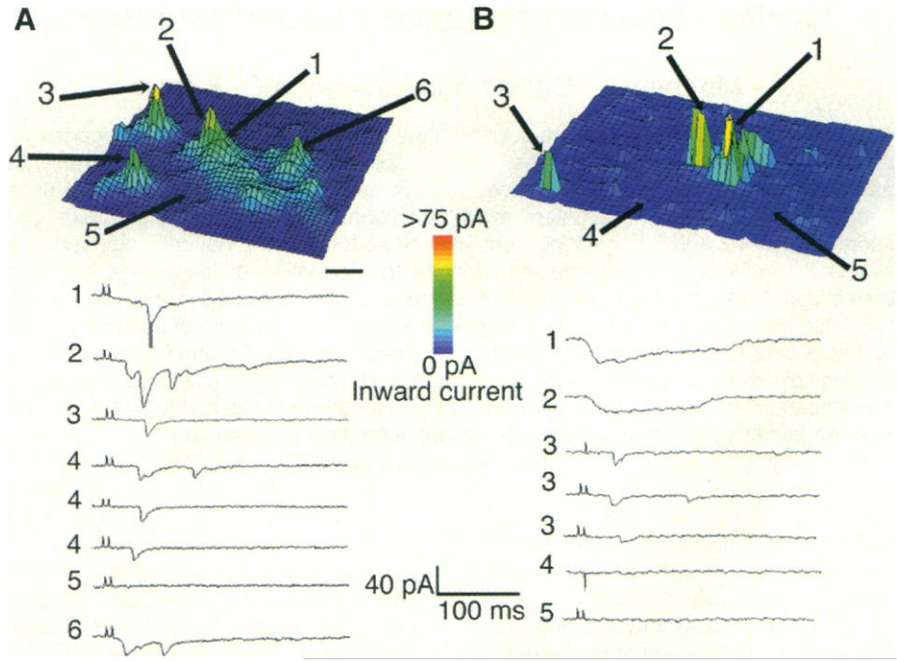


Fig. 1. Photostimulation-derived maps of the pattern of synaptic inputs to single mature neurons in tangential slices from ferret visual cortex. The magnitude of the synaptic response originating from each site stimulated is represented by the height and color of each pixel. (**A**) The pattern of synaptic inputs to a mature inhibitory neuron obtained from 966 sites covering an area of 2.4 mm². Numbered traces illustrate the electrophysiological responses in the recorded neuron after stimulation at the numbered sites. The vertical marks near the beginning of each trace indicate the opening and closing of the shutter. The cell body of the neuron is located at arrow 1; the neuron generated an action potential when stimulated at the cell body (trace 1). Arrow 2 shows the location of an input arising from stimulation near (<0.5 mm) the cell body. Clusters of inputs and recorded responses after stimulation at these sites are indicated by arrows 3, 4, and 6. Each trace is from stimulation at a different site. Most locations do not provide inputs to the cell (arrow 5). (**B**) Inputs to a mature pyramidal neuron derived from stimulation of 1474 sites covering an area of 3.7 mm². Stimulation of the neuronal dendritic tree produces direct evoked responses (arrows 1 and 2). Arrow 3 indicates a single cluster and the responses recorded after stimulation of these sites. Arrows 4 and 5 are unresponsive sites. For both maps, the scale bar is 250 μm.

ments also take place. Furthermore, in contrast to the youngest animals where PSCs were elicited near the cell body (Figs. 3 and 4A), in the mature animals many more PSCs were elicited about 1 mm from the cell body (Figs. 3 and 4C). Because clustered horizontal connections are found at about 1-mm intervals, the change (see Fig. 4) in the organization of inputs at various eccentricities probably reflects changes in the horizontal axonal collaterals, especially the addition of branches to form clusters.

In the developing cat cortex (whose development and organization are similar to those of the ferret), extensive anatomical evidence suggests that the long-distance, intercolumnar axonal projections undergo extensive rearrangements and remodeling (*4*, *11*). Our results suggest that an equally dramatic shift in the organization of local intracolumnar connections occurs during postnatal cortical development in the ferret. Local synaptic connections develop first and then increase in number, becoming at around the time of eye opening the most numerous connections observed at any age (*23*). The number of local connections then declines from the time of eye opening to the mature levels (*24*). The decline in intercolumnar synaptic connections indicates that these highly local connections undergo synaptic remodeling and reorganization as do longer range connections. Further-

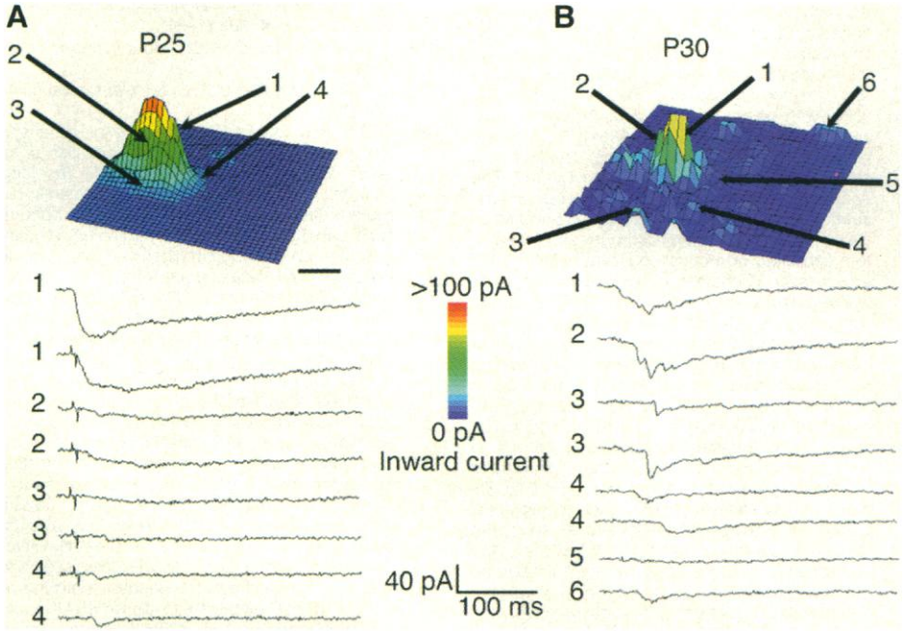
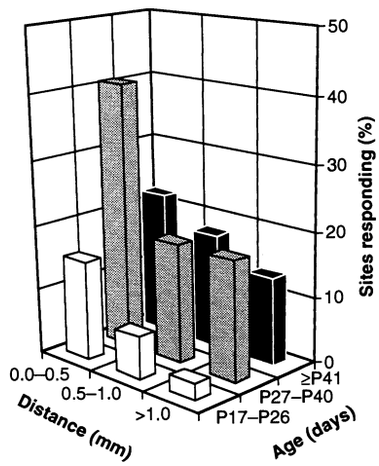


Fig. 2. (**A**) The pattern of synaptic inputs to a P25 pyramidal neuron resulting from stimulation of 997 sites covering an area of 2.5 mm^2. The same conventions as in Fig. 1 are used. The typical long slow response to direct stimulation with photostimulation in the youngest age group is shown at arrow 1. Arrows 2 and 3 indicate sites where stimulation generated both small PSCs and the direct current. Only a few PSCs were evoked more than 0.5 mm from the cell body (arrow 4). (**B**) The pattern of inputs to a P30 pyramidal neuron from the stimulation of 982 sites covering an area of 2.5 mm^2. Longer distance inputs are scattered in the tangential plane (arrows 3, 4, and 6), and many PSCs are seen near the cell body response (arrows 1 and 2). For both maps, the scale bar is 250 μm.

Fig. 3. Age-related changes in the origin of synaptic inputs with distance from the cell body. The percentage of stimulated sites that generated PSCs at various distances from the cell body in each map for each age group is shown. Within the P27 through P40 and the P17 through P26 age groups but not in the P41 through adult group, the percentages of PSCs found at 0.5 to 1.0 mm and >1.0 mm were significantly different from the percentages of PSCs at 0.0 to 0.5 mm ($P < 0.05$). At all distances from the cell body, the P27 through P40 group differed significantly from the P17 through P26 group ($P < 0.02$). The P41 through adult group differed significantly from the P17 through P26 group only at 0.5 to 1.0 mm and >1.0 mm ($P > 0.01$). Within 0.0 to 0.5 mm, the P27 through P40 and the P41 through adult groups were significantly different from each other at $P < 0.02$. All significance testing was done with the Student's *t* test.



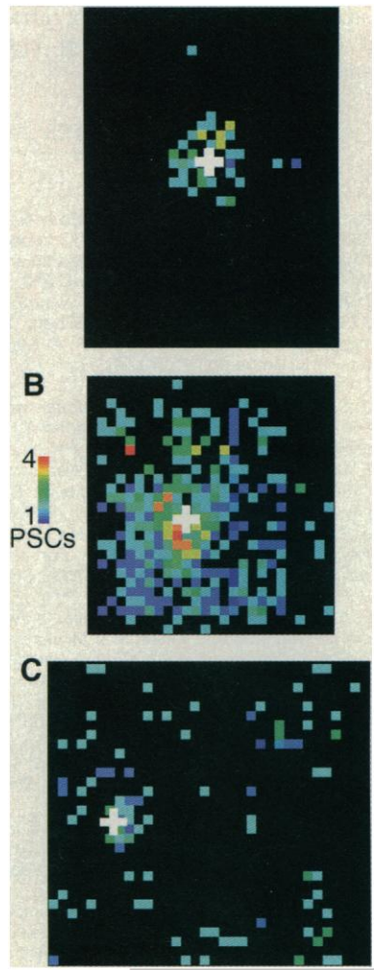


Fig. 4. Representative maps of the number of synaptic inputs to pyramidal cells at 50-μm resolution for each of the three age groups. The number of PSCs evoked at each location is represented by the colored squares; locations with no synaptic inputs are black. The location of the cell body is indicated by the white cross. (**A**) The location of PSCs evoked during the stimulation of 903 different sites covering an area of 2.3 mm^2 around a P20 pyramidal cell. Synaptic responses at P20 are confined to the region near the cell body response. (**B**) At P30, synaptic inputs are more numerous and loosely organized into groups, as in the upper right and in the bottom at the center of the map. Many synaptic inputs are located near the cell body. A total of 953 sites were stimulated, covering an area of 2.4 mm^2. (**C**) A mature pyramidal cell (P53) with synaptic inputs that form two discrete groups in the upper right and lower right corners. A total of 1268 sites were stimulated, covering an area of 3.2 mm^2. The scale bar is 250 μm.

more, our observations imply that the relative influence of very local synaptic connections on cell activity is probably greatest during the period before eye opening and declines in the adult. Theoretical models of self-organizing systems have proposed that local synaptic interactions are central to the emergence of functional architecture in cortex (*25*); understanding the actual strength and extent of intracortical connections should allow critical testing of such models.

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14. Ferret brain slices were prepared and maintained as described [L. C. Katz, *J. Neurosci.* **7**, 1223 (1987)], except that slices were initially placed in sucrose artificial cerebral spinal fluid [sucrose-ACSF: 248 mM sucrose, 5 mM KCl, 5.3 mM KH_2PO_4, 1.3 mM $MgSO_4$, 3.2 mM $CaCl_2$, 10 mM dextrose, 25 mM $NaHCO_3$, pH = 7.4; K. G. Aghajanian and K. Rasmussen, *Synapse* **3**, 331 (1989)], which was replaced after 45 min with normal ACSF (125 mM NaCl, 5 mM KCl, 5.3 mM KH_2PO_4, 1.3 mM $MgSO_4$, 3.2 mM $CaCl_2$, 10 mM dextrose, 25 mM $NaHCO_3$, pH = 7.4). The tangential slices (350 μm) of area 17 and area 18 were removed from a heated (33°C) interface holding chamber and submerged in a small room-temperature perfusion chamber in ACSF containing approximately 1 mM caged glutamate [L-glutamic acid, α (4,5-dimethoxy-2-nitrobenzyl ester) hydrochloride; Molecular Probes (*12*)]. In the electrophysiological recording, we used standard "blind" whole cell, patch-clamp recording techniques. Electrodes (5 to 9 megohms) were filled with a standard internal solution (110 mM CsOH, 110 mM D-gluconic acid, 10 mM CsCl, 11 mM EGTA, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM Hepes, 3 mM adenosine triphosphate, 1.8 mM guanosine triphosphate; pH = 7.2) containing approximately 0.2 to 0.5% biocytin. Because of the high level of chloride ions in the internal solution (14 mM), we did not distinguish between excitatory and inhibitory inputs. [In other experiments, we observed that 16.9 ± 2.3% of local inputs were inhibitory (n = 9 cells)]. After recording, slices were fixed with 4% paraformaldehyde, cryoprotected, and resectioned at 60 μm on a freezing microtome. Biocytin-labeled cells were visualized by standard immunocytochemical staining techniques (Vectastain, antibody dilution of 1:1000 to 1:2000) intensified with heavy metals [J. C. Adams, *J. Histochem. Cytochem.* **29**, 775 (1981)]. To facilitate reconstruction of labeled neurons, usually only a single cell was recorded from each slice. The scanning laser photostimulation system is described in detail elsewhere (L. C. Katz and M. B. Dalva, *J. Neurosci. Methods*, in press). The system consisted of a continuous-wave, 50-mW UV argon laser (Coherent Enterprise model 622), whose beam was directed into a 40× oil immersion objective (Nikon 1.3 NA Fluor). We scanned by moving the objective, nose piece, and laser beam with a computer-controlled motion control system (Newport, Klinger Motion Master 2000) and custom written software. On average, a map consisted of 738 locations (range, 403 to 1516), spaced at 50-μm intervals, stimulated with a 4- to 8-ms pulse at one site every 2 s, corresponding to an average area of 1.7 mm^2 (range, 1.0 to 3.8 mm^2). A few stimulation sites (one to five) produced the largest, fastest response resulting from the direct activation of glutamate receptors on the recorded neuron; these sites were assumed to be the location of the cell body. Because all the patch recording electrodes contained biocytin, neuronal cell type could be determined and in some cases the neuron's axonal arbor could be superimposed on the map of the synaptic inputs to the cell. At each site, electrical responses were digitized (TL1 interface, Axon Instruments) and the x,y,z position of the stimulation site was recorded. Each location was usually stimulated once; thus, the total number of inputs may have been underestimated (*17*). The peak amplitude and number of evoked PSCs in each trace were measured in the first 80 ms after the stimulus. We counted the number of PSCs in each trace using a computer program that calculated the instantaneous derivative for each point in the first 80 ms after the stimulus. The derivative was then examined for zero crossings and slope. Post-synaptic currents were defined by two zero crossings and a slope of more than 2 SD larger than the average derivative during the last 150 ms of the trace. Each trace and derivative were also examined by an investigator to prevent errors. Using this method, we found that distinguishing direct activation of the recorded cells' glutamate receptors from evoked synaptic responses was straightforward.
15. The recorded responses were likely monosynaptic, on the basis of several observations. Neither in the current- nor in the voltage-clamp mode did synaptic inputs alone elicit an action potential in any recorded neuron within the first 150 ms after the stimulus ($n \approx$ 200 cells). Furthermore, the region excited by the uncaging of glutamate (10 to 15 μm in diameter) was only the size of a few neuronal cell bodies. Because cortical circuits are highly divergent, only a few synapses on any given cell were likely activated by a single stimulus and thus were unlikely to lead to polysynaptic activation. The patterns of sites that generate PSCs in postsynaptic cells (that is, clear clusters) were most consistent with monosynaptic activation. Finally, extracellular recordings failed to detect any evidence of polysynaptic activation (L. C. Katz and M. B. Dalva, *J. Neurosci. Methods*, in press).
16. On the basis of its dendritic and axonal morphology, the neuron in Fig. 1A was identified as an inhibitory interneuron, probably a large basket cell; all other cells shown are pyramidal neurons [E. G. Jones and S. H. C. Hendry, in *Cerebral Cortex: Cellular Components of the Cerebral Cortex*, A. Peters and E. G. Jones, Eds. (Plenum, New York, 1984), vol. 1, chap. 8]. About 20% of the cells we recorded from were later identified as inhibitory neurons.
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21. There are at least three possible explanations for the sparse inputs. First, in our preparation of the slices, some connections, especially the long-distance ones, may have been inadvertently disrupted. Given the distances that axons from the filled cells traverse (several millimeters in some cases) and the labeling observed with biocytin injections into slices, many of the connections within a few millimeters of the cell were probably intact. Furthermore, we would expect to have disrupted connections more in the older animals than in the younger ones, in which horizontal connections are less extensive. Yet, we routinely observed extensive long-distance synaptic inputs in the mature cases but not in the young ages. In addition, local connections (those near the cell body) should be far less susceptible to truncation, but in the mature cases we observed fewer of these connections. Second, it is possible that the resolution of the map of connections was too coarse and that the number of sites stimulated was insufficient to excite all the cells in contact with the recorded neuron. Because both the region of "direct" glutamate response and the area that produced an action potential were larger in the younger animals than in the adult, the maps from these younger age groups were effectively less coarse than in the adult. However, we found the fewest connections at the youngest ages. Although we might be underestimating the actual number of connections in the mature cases, in the younger animals the accuracy of our maps is likely higher. Thus, we believe it most likely that not all axon collaterals form functional synapses along their entire length early in development.
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24. Anatomical methods have also suggested that local intracolumnar axonal projections are far more dense in young animals than in mature animals (*2*, *4*), but the degree to which this reflects genuine functional connections, rather than labeling artifacts, has not been determined.
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