Synaptic Connections and Small Circuits Involving Excitatory and Inhibitory Neurons in Layers 2–5 of Adult Rat and Cat Neocortex: Triple Intracellular Recordings and Biocytin Labelling *In Vitro*

Alex M. Thomson, David C. West, Yun Wang[1] and A. Peter Bannister

Department of Physiology, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, UK
[1]Present address: Lilly Research Centre, Eli Lilly & Co. Ltd, Windlesham, Surrey GU20 6PH, UK

Dual and triple intracellular recordings with biocytin labelling in slices of adult neocortex explored small circuits of synaptically connected neurons. 679 paired recordings in rat and 319 in cat yielded 135 and 42 excitatory postsynaptic potentials (EPSPs) and 37 and 26 inhibitory postsynaptic potentials (IPSPs), respectively. Patterns of connectivity and synaptic properties were similar in the two species, although differences of scale and in the range of morphologies were observed. Excitatory 'forward' projections from layer 4 to 3, like those from layer 3 to 5, targeted pyramidal cells and a small proportion of interneurons, while excitatory 'back' projections from layer 3 to 4 selected interneurons, including parvalbumin immuno-positive basket cells. Layer 4 interneurons that inhibited layer 3 pyramidal cells included both basket cells and dendrite-targeting cells. Large interneurons, resembling cells previously described as large basket cells, in layers 4 and 3 (cat), with long myelinated horizontal axon collaterals received frequent excitatory inputs from both layers. A very high rate of connectivity was observed between pairs of interneurons, often with quite different morphologies, and the resultant IPSPs, like the EPSPs recorded in interneurons, were brief compared with those recorded in pyramidal and spiny stellate cells.

Introduction

In a simple model of local neocortical circuitry, ascending, thalamo-cortical input is perceived as entering layer 4 (and layer 6), being relayed from layer 4 to layer 3 and from layer 3 to layer 5 (and from 5 to 6) [see, for example (Gilbert, 1983; Gilbert and Wiesel, 1986; Bode-Greuel *et al.*, 1987)]. Modified or integrated versions of that information are sent to other cortical regions from pyramidal cells in all layers, particularly layer 3, and to subcortical regions from layers 5 and 6. In addition to dense synaptic connections between neurons within each layer, the basic anatomical substrates for inter-layer connections have been described. For example, the axons of layer 4 neurons make a dense, topographically precise projection to layer 3 (and to upper layer 5) in rat and cat (Valverde, 1976; Parnavelas *et al.*, 1977; Feldman and Peters, 1978; Gilbert, 1983; Burkhalter, 1989). Similarly, the axons of layer 3 pyramidal cells ramify densely, first in layer 3 and then in layer 5 in rat (Lorente de Nó, 1922; Burkhalter, 1989), cat (O'Leary, 1941; Gilbert and Wiesel, 1983; Kisvárday *et al.*, 1986) and primate (Spatz *et al.*, 1970; Lund *et al.*, 1993; Yoshioka *et al.*, 1994; Kritzer and Goldman-Rakic, 1995; Fujita and Fujita, 1996), typically passing through layer 4 without ramifying there. A dense axonal arbour in a given layer provides the substrate for a powerful synaptic input to that layer, but the class(es) of recipient cells and their somatic locations cannot always be ascertained from the location of the synapses.

To provide more detail about these local circuit connections, a number of studies have employed paired intracellular recordings to document the probabilities with which pairs of neocortical neurons make synaptic connections within the same layer and between layers and the properties of those synaptic connections. For example, connections between pairs of layer 5 pyramidal cells in the immature (Lübke *et al.*, 1996; Markram and Tsodyks, 1996; Markram *et al.*, 1997) and mature rat neocortex (Thomson *et al.*, 1993a; Thomson and Deuchars, 1997) are less frequent and typically involve pairs of neurons that are closer neighbours, but often generate EPSPs (excitatory postsynaptic potentials) of larger amplitude than are typical for pairs of pyramidal cells in layer 3 (Thomson and Deuchars, 1997; Reyes and Sakmann, 1999).

A very high probability input to large, tufted layer 5 pyramidal cells comes from layer 3 pyramidal cells whose somata are within 100 μm of the postsynaptic apical dendrite in adult (Thomson and Bannister, 1998) and immature rat neocortex (Reyes and Sakmann, 1999). In contrast, little (1:58, adult) or no (immature) input in the opposite direction, from layer 5 pyramids to layer 3 pyramids, was found. There was, however, a significant excitatory input from layer 5 to layer 2/3 interneurons with an adapting firing pattern, while fast spiking interneurons and pyramids in layers 2/3 received their strongest inputs from cells in the middle layers (Dantzker and Callaway, 2000). These data demonstrate the layer-specific selectivity with which pyramids choose their targets. The 'forward flow' of information (e.g. layer 3 to 5) involves a strong excitatory input to just one class of pyramids as well as to interneurons whose axonal arbours can ramify in both layers 5 and 3 (Thomson *et al.*, 1996). In contrast, the 'return' pathway (layer 5 to 3) appears to select layer 3 interneurons of a particular class, avoiding other interneuronal and pyramidal targets in that layer. An obvious question that arises is whether the forward pathway from layer 4 to layer 3 and any return connection from layer 3 to layer 4 neurons exhibit similar preferences.

Within single barrels in the barrel fields of the immature rat somatosensory cortex (Feldmeyer *et al.*, 1999a; Gibson *et al.*, 1999; Petersen *et al.*, 2000) and adult cat visual cortex (Stratford *et al.*, 1996) synaptic connections between layer 4 excitatory, spiny neurons (both spiny stellate and pyramidal cells) have been reported to occur in 11–30% of tested pairs of neurons. Probabilities of connections between inhibitory and excitatory neuron pairs in this layer were around 9% in adult cat visual cortex (Tarczy-Hornoch *et al.*, 1998), while in the immature rat 41% of regular spiking layer 4 neurons tested elicited EPSPs in simultaneously recorded interneurons and a very high probability of connectivity (both chemical and electrical) between pairs of interneurons was observed in immature layer 4, particularly where the two cells displayed similar electrophysiological characteristics (Galarreta and Hestrin, 1999; Gibson *et al.*, 1999).

The strong input from layer 4 to layer 3 that might be predicted from anatomical studies has not been as thoroughly documented with paired recordings to date. In the immature rat

neocortex the connectivity underlying the excitatory input from layer 4 to layer 3 pyramidal cells was reported to be remarkably high, although no ratios are given and the inputs were relatively weak, involving small EPSPs (Feldmeyer *et al.*, 1999b, 2000). The possibility exists, however, that this pathway develops its full potential only after thalamo-cortical inputs and layer 4 connectivity have matured. No descending excitatory connections from layer 3 to layer 4 were found and connections between these layers involving inhibitory interneurons were not observed. Since layer 3 pyramidal axons rarely ramify in layer 4, the lack of such a 'back projection' is precisely what might be expected for targets whose dendrites are confined to layer 4, i.e. small interneurons and spiny stellate cells. However, layer 4 also contains pyramidal cells and larger interneurons whose dendrites project into layer 3. Do layer 3 pyramidal axons therefore recognise layer 4 pyramids as inappropriate targets and distinct from layer 3 pyramids? One excitatory connection from a layer 3 pyramidal cell to a dendrite-targeting interneuron in layer 4 has been described in the adult cat (Buhl *et al.*, 1997), but the relative density of such connections was not determined.

The majority of paired recordings in neocortical slices have used rat and many more recent studies have used immature preparations in which the fine details of cortical circuitry may still be developing. However, a large body of *in vivo* work describing the response properties of and predicting functions for neocortical neurons and networks uses adult cats and primates. One question addressed in this study was, therefore, the degree to which some of the simplest building blocks of the local circuit in adult rat and cat can be considered comparable. For example, where a synaptic connection of a given type is observed to occur in either a high or a very low proportion of recorded pairs in one species, does it occur and with equivalent probability in the other and are the synaptic events elicited comparable? Paired and triple intracellular recordings were therefore made in slices of neocortex obtained from adult rats and cats. Synaptically connected neurons were filled with biocytin and cells identified after histological processing. Synaptic connections between layers 3 and 4 were of particular interest, but to allow comparison between species and with previous studies, the characteristics of other connections that have been more thoroughly documented in the rat were included.

Materials and Methods

Preparation

Young adult male Sprague–Dawley rats (120–160 g) were anaesthetized with inhaled Fluothane (AstraZeneka, Luton, UK) then 60 mg/kg i.p. sodium pentobarbitone (Sagatal; Rhone Merieux, Harlow, UK) and perfused transcardially with 50–100 ml ice-cold artificial cerebrospinal fluid (ACSF) with added sodium pentobarbitone (60 mg/l). This modified ACSF contained 248 mM sucrose, 25.5 mM $NaHCO_3$, 3.3 mM KCl, 1.2 mM KH_2PO_4, 1.0 mM $MgSO_4$, 2.5 mM $CaCl_2$ and 15 mM D-glucose equilibrated with 95% O_2/5% CO_2. The animals were decapitated, the brain removed and 450–500 μm coronal sections of brain including the neocortex cut (Vibroslice; Campden Instruments, UK). Slices were maintained at the interface between the sucrose-containing ACSF (without pentobarbitone) and warm, humidified 95% O_2/5% CO_2 at 35–36°C for 1 h. The sucrose-containing medium was then replaced with a standard ACSF in which 124 mM NaCl replaced the sucrose. This ACSF was used for all recordings, which commenced after another hour.

Slices of cat neocortex were obtained from animals anaesthetized for a different series of acute experiments reported elsewhere (Wang *et al.*, 2000, 2002). Young adult male cats (2.5–3.4 kg) were anaesthetized with a mixture of α-chloralose (70 mg/kg) and pentobarbitone sodium (6 mg/kg) injected i.v. The right carotid artery was cannulated and the skull overlying the occipital lobes removed. The animal was killed with an overdose of i.v. barbiturate (100 mg/kg), the left carotid artery tied off and the two jugular veins cut prior to perfusion through the right carotid with 200 ml of ice-cold modified ACSF (sucrose-containing). The dura overlying the visual cortex was cut and peeled back and a block including visual cortex removed. Slice cutting, maintenance and recording procedures were then identical to those used for the rat brain slices. All procedures complied with British Home Office regulations for animal use.

Electrophysiological Recordings

Paired and triple intracellular recordings were performed using conventional sharp electrodes (80–160 MΩ) containing 2 M potassium methylsulphate and 2% biocytin (w/v) under current clamp (AxoProbe and AxoClamp; Axon Instruments, CA). Sharp electrodes are more efficient for multiple recordings in thick slices of adult tissue which maintain the integrity of large neurons and local circuitry. Typically, a single stable intracellular recording was obtained in a previously unstudied region of the slice. A second electrode was then introduced and up to six other cells sampled until a synaptic connection was observed. Presynaptic firing was initiated by intracellular current injection at 1 pulse/3 s. Pulses were combinations of square wave and ramped currents. A third electrode was then introduced into a neighbouring area or layer, but one that had not previously been sampled. Again, neurons were sampled with this third electrode until a connection with one or both of the other neurons was found or until recordings deteriorated. In some experiments, after a triplet of connected cells had been recorded, one of the electrodes was withdrawn and a search made for other connected neurons. A map was drawn of the recorded region and the position of each sampled cell marked to aid subsequent identification of recorded neurons after histological processing. Continuous analogue recordings from presynaptic and postsynaptic neurons were made on analogue tape (Racal, Southampton, UK). Data from the longer and more detailed protocols in this series of experiments involving the frequency-dependent properties of the synaptic connections will be reported elsewhere.

Data Analysis

Data were digitized (5–10 kHz, voltage resolution 0.005–0.01 mV) and analysed off-line (Spike 2 data collection and in-house analysis software). Individual sweeps were observed and trigger points associated with the rising phase of each presynaptic action potential (AP) checked/edited. Sweeps including large spontaneous events or artifacts were rejected. Averaging of EPSPs and IPSPs was triggered by the rising phase of single presynaptic spikes for the average of the first EPSP or IPSP, the rising phase of the second presynaptic spike for the average second EPSP/IPSP in a train and so on. Some of the illustrated EPSP/IPSPs are therefore composite averages with single spike responses and second (third, ...) EPSP/IPSPs elicited at specific interspike intervals superimposed (individual averages in these composites include 20–200 sweeps). Average first or single spike EPSP/IPSP amplitudes were measured as the difference between an average of the voltage preceding the presynaptic spike and an average around the peak of the EPSP/IPSP. The 10–90% rise time (RT) and width at half amplitude (HW) were measured from averaged single spike EPSP/IPSPs.

Histological Processing

Recorded cells were filled with biocytin, either by passive diffusion from the recording electrodes or by passing positive current in a half duty cycle (0.5 nA, 500 ms, 1 pulse/s). Slices were fixed overnight in 0.1 M phosphate buffer containing 2.5% glutaraldehyde, 4% paraformaldehyde and 0.5% saturated picric acid, then washed in 0.1 M phosphate buffer. They were then embedded in gelatin, sectioned at 60 μm (Vibratome), cryoprotected in 30% sucrose plus 12% glycerol and permeabilized by freeze–thawing above liquid nitrogen. Biocytin was localized using the Elite ABC kit (Vector) overnight and visualized using 3′,3′-tetraminodiaminobenzidine (DAB) (Sigma) intensified with nickel chloride. Sections were then dehydrated and embedded in Durcupan resin (Fluka) on slides. Filled neurons were reconstructed using a drawing tube (100× objective, 1000× magnification).

Some of the slices in which interneurons of interest had been

Table 1
Paired intracellular recordings in rat and cat neocortex

Type of connection[a]	Connectivity ratio	Membrane potential (mV)	EPSP/IPSP amplitude (mV)	10–90% rise time (ms)	Width at half amplitude (ms)	Confirmed histology	Latency (ms)
L5 pyramid to L5 pyramid	1:11 (15:163)	–65 to –75	1.7 ± 1.46 (n = 15)	1.8 ± 1.4	16.2 ± 11.7	5	1.5 ± 0.15
L2/3 pyramid to L2/3 pyramid	1:4 (65:247)	–68 to –80	1.7 ± 1.3 (n = 38)	1.86 ± 0.8 (n = 20)	13.4 ± 5.2	18	1.5 ± 0.3
	1:10 (8:81)	**–68 to –70 [–70]**	**1.4 ± 0.5 (n = 6) [8.2]**	**2.5 ± 1.3 [2.0]**	**20.6 ± 15 [11.7]**	**8**	**1.7 ± 0.79**
L4 excitatory to L4 excitatory	**1:5.7 (4:23)**	**–68**	**1.1 (1)**	**2**	**17.6**	**2**	**0.6**
L3 pyramid to L5 pyramid	1:1.8 (16:29)	–70 to –72	1.4 ± 0.6 (n = 12)	2.0 ± 0.9	30 ± 4.1	8	1.4 ± .3
[Postsynaptic apical dendrite]			[3.8 ± 2.7] [n = 4]	[1.6 ± 0.8]	[11.1 ± 4.7]		
	1:1 (2:2)		**3.2, 1.3**	**2.0, 2.6**	**16, 9**	**2**	**2.8, 3**
L5 pyramid to L3 pyramid	1:29	–71	0.3			1	
L4 excitatory to L3 pyramid	1:3.6 (7:25)	–67, –70	3.3, 5.9	1.4, 2.6	26, 14	3	1.4, 1.4
(Presynaptic spiny stellates) (n = 4)	**1:10 (7:70)**	**–70 to –78**	**1.1 ± 0.4 (n = 4)**	**1.9 ± 0.4**	**15 ± 1.8**	**5**	**0.9 ± 0.24**
L5 pyramid to L5 interneuron	1:10.4 (7:73)	–76	0.9	0.6	4	2	1.4
L5 interneuron to L5 pyramid	*1:8 (9:73)*	*–52 to –60*	*1.23 ± 0.4 (n = 5)*	*4.2 ± 2.8*	*23 ± 11.8*	*5*	*1.1 ± 0.2*
L2/3 pyramid to L2/3 interneuron	1:5 (22:107)	–65 to –80	1.9 ± 1.6 (n = 11)	1.2 ± 0.9	8.1 ± 5.2	9	1.3 ± 0.3
	1:4 (6:25)	**–60 to –80**	**3.1 ± 1.4 (n = 5)**	**2.2 ± 1.4**	**8.2 ± 2.9**	**5**	**0.95 ± 0.2**
L2/3 interneuron to L2/3 pyramid	*1:6.3 (17:107)*	*–55 to –65*	*0.65 ± 0.44 (n = 5)*	*3.9 ± 1.5*	*21.5 ± 1.9*	*5*	*1.8 ± .8*
	1:3.6 (7:25)	***–55 to –60***	***<0.5 to 1 (7)***	***5.3 (1)***	***23.5***	***7***	***1.6 ± 0.7***
L4 excitatory to L4 interneuron	**1:5 (8:42)**	**–75**	**3.7 (1)**	**0.7**	**3.8**	**2**	**1.2**
L4 interneuron to L4 excitatory	***1:10 (4:42)***	***–63 to –65***	***0.6 to 1.1 (n = 3)***	***3.5 to 3.9***	***20 to 24***	***2***	***1.2 ± 0.3***
L4 exitatory cell to L3 interneuron	1:10 (1:10)	–70	<0.2			0	
	1:10 (3:31)	**–65 to –75**	**1.2 ± 0.24 (n = 3)**	**0.72 ± 0.04**	**5.6 ± 1.6**	f**3**	**0.6 ± 0.1**
L3 pyramid to L4 interneuron	1:12 (1:12)	–70	1.6	0.4	3.3	1	0.9
	1:5.3 (7:37)	**–70 to –75**	**1.0 ± 0.4 (n = 5)**	**0.85 ± 0.2**	**8.3 ± 5.64**	**7**	**1.0 ± 0.2**
L4 interneuron to L3 pyramid	*1:2 (6:12)*	*–55, 60*	*1.6, 1.9*	*2.8, 3*	*14, 34*	*3*	*1.1, 1.0*
	1:3.7 (10:37)	***–53 to –60***	***0.43 to 1.0 (4)***	***3.5 to 4.4***	***13 to 31***	***3***	***0.8 to 1.9***
L3 interneuron to L3 interneuron	*1:4 (2:8)*	–68, –50	2.0, 0.7	2.0, 2.4	8.0, 9.5	1	0.7, 1.5
	2:1	***–71, –52***	***0.8, 1.3***	***2.3, 3.9***	***7, 10***	***1***	***1.0, 0.8***
L4 interneuron to L4 interneuron	***1:2 (3:6)***	***–60 to –70***	***0.4 to 2.7 (n = 3)***	***2.7 to 2.9***	***11.0 to 11.7***	***3***	***0.8 to 1.1***
L5 interneuron to L5 interneuron	1:1.7 (3:5)						
L4 interneuron to L3 interneuron	***1:1***						

Connectivity ratios indicate the probability with which a synaptic connection of the type indicated was observed in the sample tested, obtained by dividing the number of connections of that type observed by the number of pairs tested. Absolute numbers of connections versus pairs tested are given in parentheses. All tested pairs and connections observed are included in these ratios. Those that were only briefly recorded and not stored for analysis are not, however, included in measurements of synaptic parameters, nor are the 'facilitating' EPSPs elicited in a small minority of interneurons. The postsynaptic membrane potential at which the EPSPs and IPSPs were recorded is given. The EPSP/IPSP amplitude, 10–90% rise time and width at half amplitude were measured from averaged events (20–200 events). The number of connections measured in this way is given in parentheses. For L2/3 pyramid–pyramid connections in cat, the pooled mean excludes one unusually large EPSP, whose parameters are given in brackets. The rat L3 to L5 pyramid–pyramid connections include two data sets, those recorded at the postsynaptic soma in L5 and those recorded from the postsynaptic apical dendrite in L3/4 (in brackets). Since it was not possible to confirm the morphology of all L4 cells involved in connections, layer 4 (L4) excitatory cells in the table include pyramidal and spiny stellate cells. The confirmed histology column indicates the number of paired recordings of each type for which both pre- and post-synaptic neurons could be adequately identified morphologically.

[a]Not bold, not italic, rat EPSP; bold, not italic, cat EPSP; not bold, italic, rat IPSP; bold, italic, cat IPSP.

recorded were fixed in a low gluteraldehyde solution (4% paraformaldehyde, 0.5% picric acid plus 0.025% gluteraldehyde) and first processed for immunofluorescent identification of cellular markers in biocytin-filled cells, before permanent peroxidase labelling of filled neurons, as above. Details of immunofluorescence protocols and the cocktails of antibodies used can be found in a previous publication (Hughes *et al.*, 2000). Typically, avidin labelled with 7-amino-4-methylcoumarin-3-acetic acid (AMCA) was used to visualize the biocytin. A fluorescein isothiocyanate (FITC)-labelled secondary antibody was used to identify a monoclonal mouse antibody and a Texas red-labelled secondary used to visualize a polyclonal rabbit antibody. Most commonly in this series, a monoclonal antibody directed against parvalbumin (clone PA-235; Sigma, Poole, UK) was used with a polyclonal antibody raised against another marker, such as calbindin (R8701 or R9501, K. Baimbridge). In some cases a mouse monoclonal antibody raised against gastrin/CCK (cholecystokinin) was used (no. 9303 CURE; UCLA). For these protocols to be successful, i.e. for the filled cells to be identifiable with AMCA fluorescence, strong labelling of recorded neurons with biocytin was required. For unambiguous identification of cell markers, full penetration of the tissue by the primary and the labelled secondary antibodies was also necessary, and in some sections this was not achieved. The monoclonal antibodies typically resulted in more complete penetration without additional permeabilization of the tissue. Cells are therefore reported as immuno-positive or immuno-negative for a given marker where the interneuron was identified with AMCA fluorescence and where FITC and/or Texas red fluorescence in that cell, and/or in adjacent cells at the same focal depth, demonstrated adequate penetration of the antibodies.

Results

In 23 experiments in rat and eight in cat neocortex, 998 paired recordings were made (rat, 679; cat, 319). These yielded 135 EPSPs and 37 IPSPs in rat and 42 EPSPs and 26 IPSPs in cat. All data from the cat were obtained from visual areas, predominantly area 17, and the majority of recordings were made in layers 2–4. In rat, layers 2–5 in somatosensory, motor and visual cortex were studied. Slices in which paired recordings were obtained, some of which also included search areas in which no connections were found, were processed histologically. In most examples it was possible to confirm pyramidal and/or interneuronal morphology and to identify the layer(s) from which recordings had been obtained from this material. However, some connected cells were recorded only briefly and were not well labelled, while in other cases, cells that had been tested, but had not contributed to recorded connections, were also labelled, making identification of precisely which neurons had contributed to recorded connections ambiguous. Prolonged viewing during the immunofluorescence stage of the protocols could also result in a weaker permanent reaction product in the sections so studied. Histological confirmation of connected pairs is listed in Table 1, therefore, only where both presynaptic and postsynaptic neurons were identifiable. In addition to their firing characteristics, inhibitory interneurons were identified during

recordings by their ability to elicit IPSPs in simultaneously recorded cells, while excitatory cells (pyramidal and spiny stellate cells) were identified by the EPSPs they elicited. Where one of a connected pair of cells was unstable or where the connection was of a type previously reported in detail, the presence of the connection and the amplitude of the resultant postsynaptic potential(s) was noted to provide connectivity data, but the EPSP/IPSP was not stored on tape and therefore not analysed.

Pyramid–Pyramid Connections in Layers 2/3 and 5 of Cat Visual Cortex

Of 81 pairs of layer 2/3 pyramidal cells tested in cat visual cortex (54 in area 17, 20 in area 19 and seven in area 18), seven were connected, two in area 19 and five in area 17, of which one was reciprocal (eight EPSPs, connectivity ratio 1:10, Figs 1 and 4). This includes two pairs in which the presynaptic pyramid was in layer 3 and the postsynaptic in layer 2 (Fig. 2). With the exception of one unusually large EPSP, the average amplitudes of the EPSPs recorded in cat layer 2/3 were similar to those recorded in the rat (Table 1). Mean EPSP duration was longer in cat, but as the range was broad, the difference was not significant ($P > 0.05$). The largest EPSP (8.2 mV) in this group is listed separately in Table 1 and involved two large layer 3 pyramids at the border with layer 4. In cat, as described previously in rat, pyramid–pyramid EPSPs typically exhibited paired pulse and frequency-dependent depression throughout trains of presynaptic spikes at high frequency (≥100 Hz) (Thomson, 1997) (Fig. 1). In some, modest facilitation of the second EPSP could occur at slightly longer interspike intervals, after recovery from the shortest interval depression (Fig. 2). However, even in these connections, third and subsequent EPSPs in trains typically exhibited depression throughout the range of interspike intervals studied (5–80 ms). More detailed analysis of responses to spike trains of different frequencies is reported elsewhere.

In the only two pairs tested in cat in which one pyramidal cell was recorded in layer 3 and one in layer 5, both yielded layer 3 to layer 5 connections (Fig. 1). In these two pairs the presynaptic somata were 37 and 45 m from the postsynaptic apical dendrite, respectively, and the postsynaptic pyramidal cells were large, burst firing cells. This is a very small sample, but is reminiscent of similar connections in rat cortex (below). No pairs of layer 5 pyramidal cells were tested in cat.

Pyramid–Pyramid Connections in Layers 2/3 and 5 of Rat Neocortex

The data obtained in rat (Table 1) confirm previous reports that the connectivity between neighbouring pyramidal cells in layers 2/3 is higher than in layer 5 and that the descending projection from layer 3 pyramids to large, tufted layer 5 pyramids is strong, largely unidirectional and tightly focused. The electrophysiological properties of layer 3 pyramid–pyramid EPSPs are similar in rat and cat. In the cat, however, a lower connectivity ratio was observed than in the rat (1:10 compared with 1:4). It should perhaps be noted, however, that the ratios observed here in adult rat layer 2/3 are considerably higher than our earliest studies in this species [e.g. 1:38 (Thomson and West, 1993)]. This probably reflects the development of more efficient search strategies over time and the use of thicker slices (450–500 compared with 400 μm) in our more recent studies. In the present study, synaptically connected layer 2/3 pyramidal cell pairs in rat were typically more widely separated in the horizontal plane (47 ± 37 μm) than were layer 5 pyramidal pairs (17 ± 12 μm), but in the cat, synaptically connected layer 2/3 pyramidal pairs were

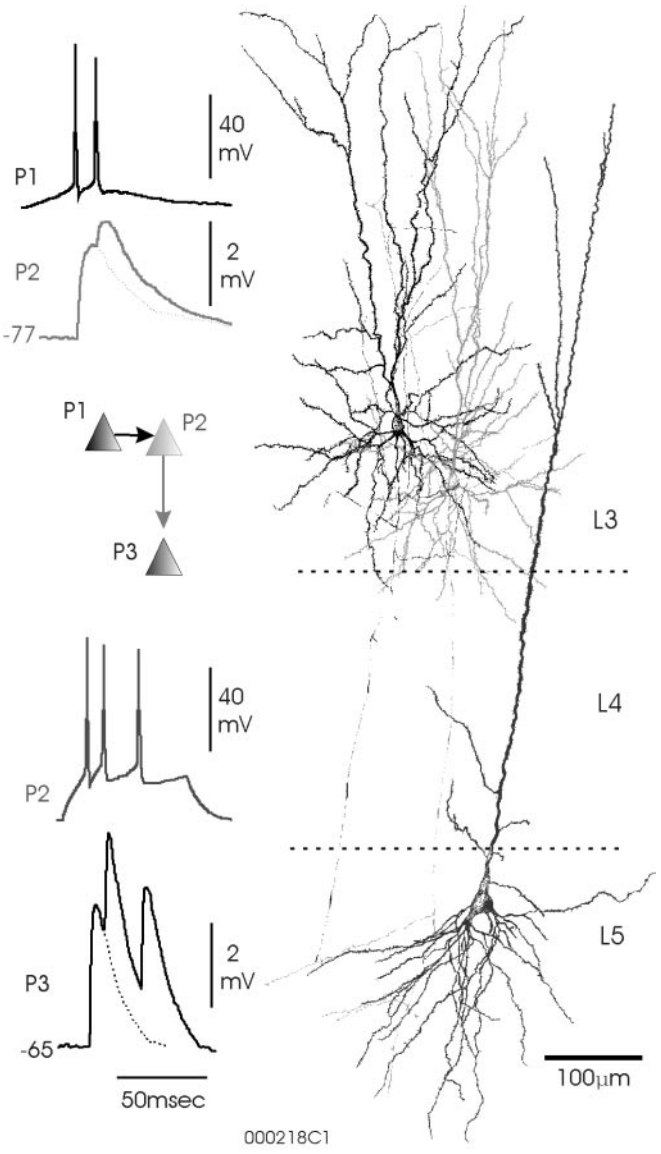

Figure 1. A presynaptic pyramidal cell in upper layer 3 (P1, black) elicited an EPSP in another layer 3 pyramidal cell (P2, grey) which in turn elicited an EPSP in a layer 5 pyramidal cell (P3, black) (000218C1). The part of the section containing the top of the layer 5 cell apical dendrite was lost in processing, but its large diameter in layer 2 indicates that it did not terminate in layer 2, but in layer 1. The cartoon inserts indicate the connections recorded. All postsynaptic responses illustrated in this and succeeding figures are averages (20–200 sweeps) triggered from the rising phase of the presynaptic AP. Averaged responses to trains of APs are composites of averages triggered by each of the APs in the train.

on average more widely separated again (69 ± 44 μm). The use of a similar search strategy in the two species may have introduced some bias in favour of finding connected pairs in the rat neocortex where neurons are, on average, smaller.

Synaptic Connections Between Layer 4 Excitatory Cells in Cat Visual Cortex

Of 23 pairs of excitatory neurons in cat layer 4, four pairs yielded an EPSP, an average connectivity ratio of 1:5.7, which is similar to previous observations in immature rat (Feldmeyer *et al.*, 1999a,b; Gibson *et al.*, 1999) and adult cat (Stratford *et al.*, 1996). Two of these connections were confirmed histologically and involved spiny stellate to spiny stellate connections. In one

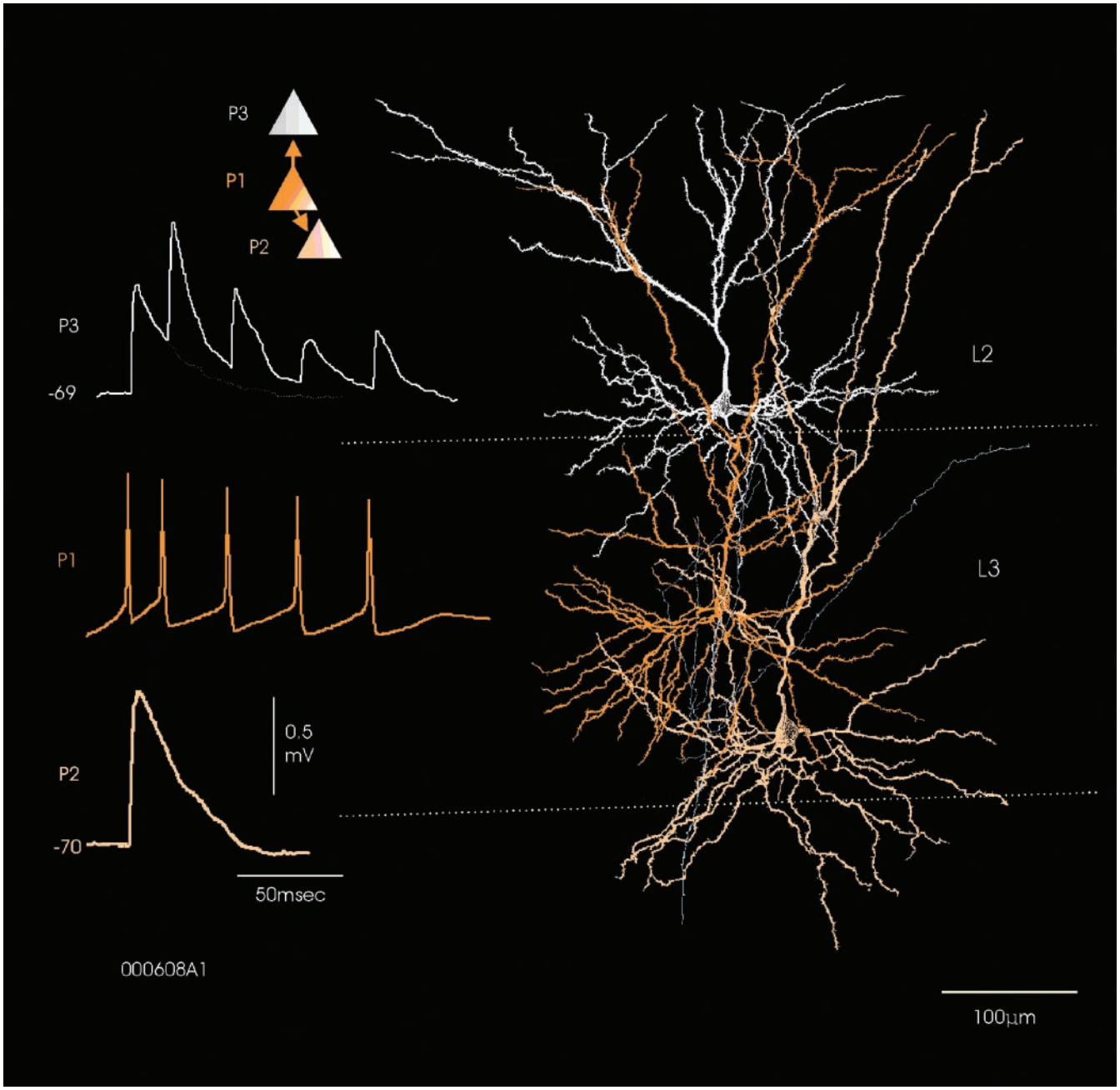

Figure 2. Synaptic connections between pyramidal cells in layers 2 and 3 of cat visual cortex. One layer 3 pyramidal cell (P1, orange soma/dendrites, blue axon) was found to be presynaptic to another pyramidal cell in deep layer 3 (P2, buff) and to one in layer 2 (P3, white). The cartoon insert indicates the connections recorded. The response of the layer 2 pyramidal cell to a brief train of five APs is shown. Moderate facilitation of the second EPSP is followed by depression of the third, fourth and fifth EPSPs in the train. The averaged response of the postsynaptic layer 3 pyramidal cell to a single presynaptic AP is shown below (000608A1).

Figure 3. Synaptic connections between spiny excitatory cells in layer 4 of cat visual (*A*) and rat visual (*B*) cortex. In (*A*) one layer 4 spiny stellate cell (SS1, orange) was presynaptic to another (SS2, buff) and both spiny stellate cells were presynaptic to a single layer 3 pyramidal cell (P,white). Close membrane appositions between the presynaptic spiny stellate axons (both in white) and the postsynaptic pyramidal cell were onto very distal portions of the pyramidal basal dendrites 000329B1. In (*B*) the presynaptic pyramidal cell (P1 soma/dendrites white, axon blue) innervated another, postsynaptic layer 4 pyramidal cell (soma/dendrites buff, axon pale yellow) 010711PB2.

Figure 4. Two pyramidal cells (P1, pale yellow with white axon, P2, orange with buff axon) in layer 3 of cat visual cortex were reciprocally connected. The EPSPs elicited by both connections exhibited brief train depression. The responses of one of these connections (P2 to P1) to two spike trains of different frequencies are illustrated and show that greater depression resulted from higher frequency presynaptic firing. Positions of close membrane appositions between the presynaptic axon and postsynaptic dendrites are indicated by circles (P2 to P1 in yellow, P1 to P2 in green). One of these layer 3 cells (P2) also received an input from a deep layer 4 pyramidal cell (P3) whose axon (grey) was too weakly stained to allow full reconstruction and identification of putative synaptic contacts. The EPSP elicited by this connection was slow and very small (the averaged response illustrated included total apparent failures of transmission) and is shown twice, once with the same gain used to illustrate the within layer 3 connections, for comparison, and once at higher gain (000317A1).

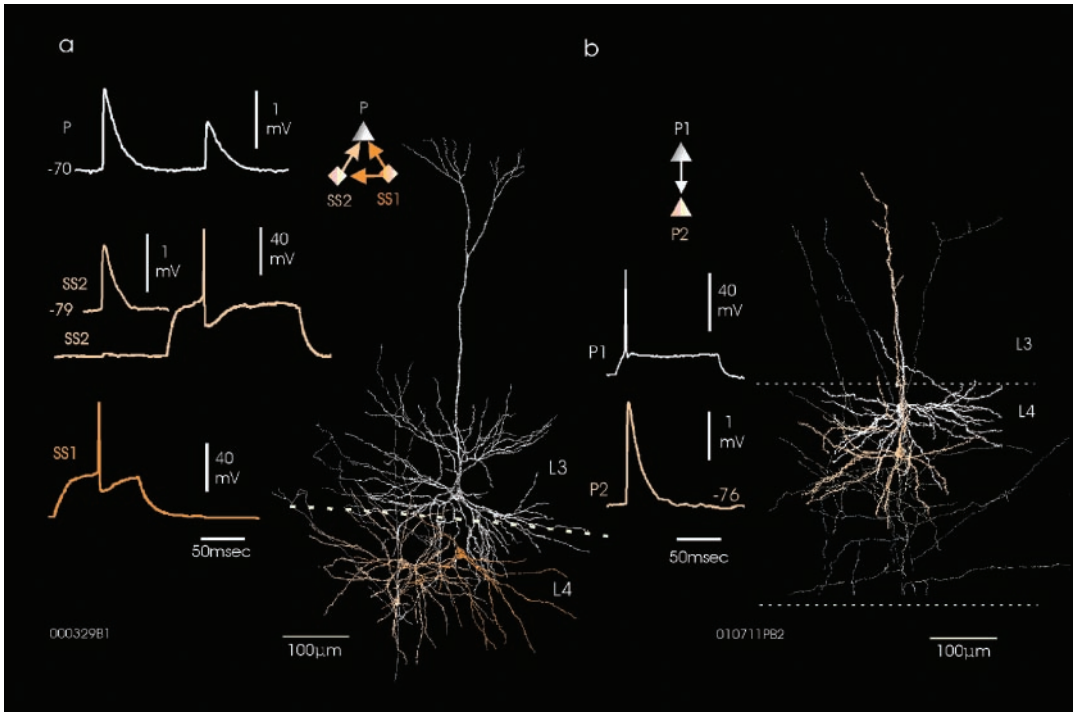

Figure 3

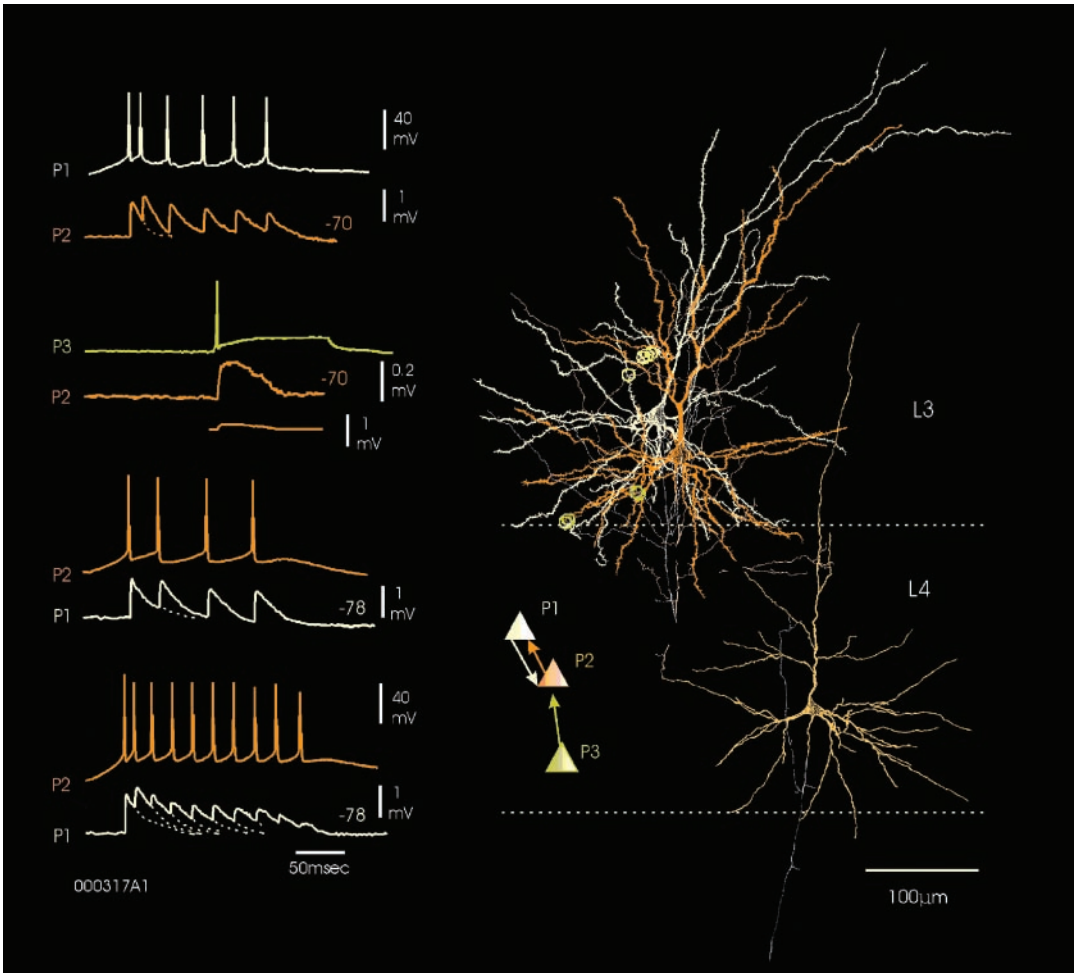

Figure 4

of these examples both layer 4 spiny stellate cells also activated EPSPs in a single layer 3 pyramidal cell (see Fig. 3*A*). Too few paired recordings were made in layer 4 of the rat neocortex to produce estimates of connectivity ratios. In the one connected pair confirmed histologically in rat, both layer 4 cells were pyramidal cells (Fig. 3*B*).

Excitatory Connections From Layer 4 to Pyramids in Layer 3 of Cat Visual Cortex

In cat, seven of 70 such pairs yielded an EPSP (1:10, all in area 17). Of the five pairs confirmed histologically, four involved presynaptic spiny stellate cells in middle to upper layer 4 (Fig. 3*B*). The EPSPs elicited by middle to upper layer 4 cells were similar in amplitude, but briefer on average than those elicited by other layer 3 pyramidal cells. Smaller, slower EPSPs were, however, elicited in layer 3 pyramidal cells by two deep layer 4 cells (one identified as a deep layer 4 pyramid). These EPSPs had average amplitudes of 0.2 and 0.15 mV, 10–90% rise times of 5 and 4 ms and widths at half amplitude of 53 and 50 ms at membrane potentials of –78 and –70 mV, respectively (Fig. 4). For a closer comparison with data obtained in the rat in which all presynaptic layer 4 cells were in middle to upper layer 4 the measurements of these two EPSPs are not included in the means in Table 1.

Excitatory Connections From Layer 4 to Pyramids in Layer 3 of Rat Neocortex

Of 25 such pairs tested in somatosensory and visual cortex, seven yielded an EPSP (1:3.6). All connections were ascending, i.e. from layer 4 to layer 3. Three pairs were confirmed histologically. In rat the majority of connected pairs appeared to involve layer 4 pyramidal cells as in this series no spiny stellate cells, but a large number of layer 4 pyramidal cells, were recovered histologically in rat neocortex. Only two of these EPSPs were stored and measured (Table 1). The others were brief recordings, but they were typically noted to be ≥2 mV in average amplitude and in rat larger than EPSPs elicited by other, simultaneously recorded presynaptic layer 3 pyramids.

In cat the EPSPs elicited by cells in middle to upper layer 4 were on average similar in duration but smaller than those observed in rat and the connectivity ratios were higher in rat (Table 1). However, the probabilities of a layer 3 pyramidal cell receiving an input from another layer 3 pyramidal cell and from a layer 4 excitatory cell were approximately equal in each of the two species (1:4 compared with 1:3.6 in rat and 1:10 compared with 1:10 in cat). All connections were ascending, i.e. no EPSP was elicited in a layer 4 excitatory neuron by APs in a layer 3 pyramidal cell in these 94 pairs. Connections between layer 4 excitatory cells (ratio 1:5.7, cat) can in principle be in either direction, while the excitatory connection between layer 4 and layer 3 is essentially unidirectional. The probability of any single layer 4 excitatory cell innervating another layer 4 excitatory cell and innervating a deep layer 3 pyramidal cell would therefore appear to be similar.

Morphology of Labelled Interneurons

Forty seven interneurons were stained sufficiently well in cat visual cortex for identification, two in layer 2, 16 in layer 3, 27 in layer 4 and two in layer 5. Small to medium sized multipolar interneurons whose dendrites and often dense axonal arbours were confined to the layer of origin were found in layers 2–5 (one in layer 2, six in layer 3, six in layer 4 and one in layer 5). Some of these cells resembled cells described previously as small to medium sized basket cells, but no ultrastructural analysis of the synapses they formed was done to confirm this and their dendritic and axonal arbours indicated a non-homogeneous population. Five of these cells were immuno-positive for parvalbumin.

Six neurogliaform cells were recovered (one in layer 2, one in layer 3 and four in layer 4), but no connections involving these cells could be unambiguously confirmed. Other interneurons whose processes were largely confined to the layer of origin included six cells with 'flask-shaped' somata (one in layer 3 and five in layer 4) from which the axon and all the major dendrites exited one pole in a tuft with only a few very short dendrites issuing from other parts of the soma. In deep layer 4 (three cells) the main dendrites of these cells exited the more superficial pole and extended through the depth of the layer. In middle and upper layer 4 (two cells) and in upper layer 3 the main dendrites exited the deeper pole and extended to the lower border of the layer. The axons of two of these 'flask-shaped' cells formed long almost straight collaterals, radiating vertically and diagonally out from the soma and terminating in discrete arbours 100–200 μm away (one such arbour originating in layer 4 innervated lower layer 3), while the axon of another such cell formed a dense, vertically oriented arbour that spanned layer 4, with a single major branch ascending into lower layer 3, looping and descending again to ramify in layer 4 (Fig. 11). Finally, a small multipolar neuron in middle to upper layer 4 that targeted more distal dendrites of its single identified postsynaptic neuron was recovered.

Eight layer 3 interneurons with widely varying morphologies had dendrites that extended into upper layer 4. These included a large multipolar, parvalbumin immuno-negative deep layer 3 cell with long myelinated horizontal axonal projections in layer 3 and a single myelinated collateral that descended through layer 4 without branching and formed a discrete arbour in layer 5 (Fig. 6). This cell resembled those described previously as large basket cells (Somogyi *et al.*, 1983; Kisvárday, 1992), but again no ultrastructural analysis was performed to confirm this. Two layer 3 bi-tufted/bipolar double bouquet cells had dendrites that extended into layer 4. The dendrites of a third layer 3 double bouquet cell were too weakly labelled for reconstruction, but the axon was well labelled. All three double bouquet cells had dense local axonal abours spanning most of layer 3 and the upper half of layer 4 in addition to the bundles of unmyelinated descending axon collaterals bearing *en passant* boutons ('horse tails') that reached the deep layers and identify this cell class. A Martinotti-like cell with bi-tufted, sparsely spiny dendrites generated many fine, ascending axon collaterals which made small dense axonal abours throughout upper layer 3, layer 2 and layer 1 and which made synaptic contact with the dendrites of a layer 2 pyramidal cell (Fig. 7). Two of the deep layer 3 interneurons whose dendrites extended into layer 4 resembled small basket cells. One was a small horizontally oriented interneuron with a small discrete axonal arbour spanning upper layer 4 and lower layer 3. The axon of the other was largely ascending and confined to layer 3. Finally, the axon of a pyramidal-shaped interneuron at the border between layers 3 and 4 descended into layer 4. Of the 16 layer 3 interneurons recovered, therefore, half were confined entirely to the superficial layers and half had dendrites that extended into layer 4, but of these only the double bouquet cells and one small multipolar cell had a significant axonal ramification in layer 4.

Ten layer 4 interneurons with a range of morphologies had both dendritic and axonal arbours that extended into layer 3. These included four large multipolar cells that resembled large basket cells, one of which was cholecystokinin (CCK) immuno-

positive (Fig. 10) and one CCK immuno-negative (Fig. 11). Two smaller multipolar cells with dense local axonal arbours, one of which was parvalbumin immuno-positive, resembled small to medium sized basket cells. A deep layer 4 pyramidal shaped cell with a single long 'apical' dendrite and a single axon collateral that both ascended into layer 3 (in addition to a local axonal arbour spanning layer 4) was also parvalbumin immuno-positive. In addition to the CCK immuno-positive cell which innervated fourth and fifth order dendrites of its one identified postsynaptic target pyramid, one other layer 4 cell extending into layer 3 that innervated dendritic pyramidal targets was identified. This was a small bipolar, proximal dendrite-targeting cell in upper layer 4 (Fig. 9). Finally, one bi-tufted interneuron with dendrites spanning the depth of layer 4 and an elongated axonal arbour in upper layer 4 and lower layer 3 and a medium sized 'flask shaped' neuron in upper layer 4 whose major dendrites and several discrete axonal arbours extended into layer 3 were also recovered. It therefore appears that a significant proportion of layer 4 interneurons (10:27 in this sample) displaying a wide range of morphological features innervate layer 4 and lower layer 3. However, no layer 4 interneuron whose axon extended beyond layer 3 and into layer 2 was recovered.

The two layer 5 interneurons filled in the cat in this study were a large bipolar cell with dendrites extending into deep layer 6 and middle layer 4 and a medium sized multipolar interneuron whose axon was confined to layer 5.

The interneurons filled in the rat cortex in this study did not display such a wide range of sizes or morphological characteristics, although a range of morphologies similar to that observed here in cat has been reported in rat (Kawaguchi and Kubota, 1997; Gupta *et al.*, 2000), including several subclasses of basket cells (Wang *et al.*, 2002) and VIP immunoreactive bipolar cells with vertically oriented axonal arbours (Rozov *et al.*, 2001). Most of the recovered cells in the present study were multipolar and had axons and dendrites confined to the layer of origin (or to layers 2 and 3). Four of the layer 3 interneurons recovered are illustrated in Figure 5, with the connections recorded.

In both species the majority (~80%) of identified inter-

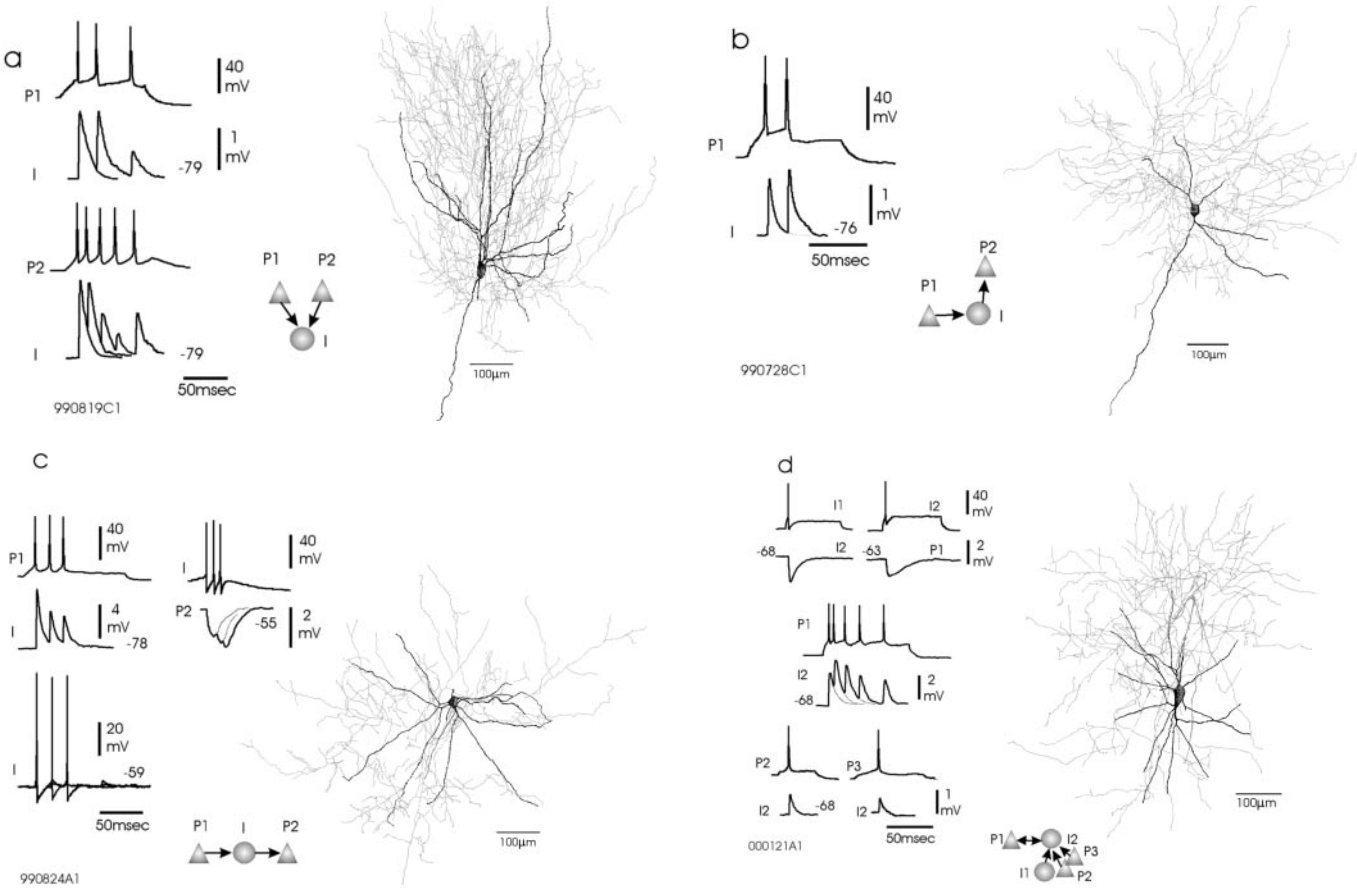

Figure 5. Interneurons in layer 3 of the rat neocortex (soma/dendrites in black, axons in grey) synaptically connected to layer 3 pyramidal cells. A classical fast spiking interneuron in (*A*) received two inputs from layer 3 pyramidal cells. The EPSPs depressed during trains of APs (990819C1). The classical fast spiking interneuron in (*B*) received an excitatory input from a layer 3 pyramidal cell and inhibited a layer 2 pyramidal cell making close membrane appositions with its proximal dendrites. The brief recording of the IPSP is not illustrated (990728C1). The classical fast spiking, parvalbumin immuno-positive interneuron in (*C*) also received an excitatory input from one pyramidal cell in layer 3 and inhibited another, its axon making close membrane appositions with third and fourth order dendrites. Responses to brief trains of three APs are illustrated as a composite average of responses recorded at –78 mV and as three superimposed single sweep responses recorded closer to the firing threshold, at –59 mV. This large EPSP readily elicited postsynaptic APs from –59 mV after a period of rest. However, the EPSP was depressed during trains and only rarely did all presynaptic APs in a high frequency train elicit postsynaptic APs. In the three single sweeps illustrated here only one train of three presynaptic APs elicited three APs in the postsynaptic interneuron. In the other two sweeps, only the first EPSP elicited an AP.Postsynaptic APs are truncated. The IPSPs elicited in the other pyramidal cell (P2) by brief trains of three APs in the interneuron are shown (990824A1). In (*D*) a classical fast spiking, parvalbumin immuno-positive interneuron (I2) is reconstructed. Its axon made multiple close membrane appositions with the soma of its postsynaptic target (P1). It received an IPSP from another interneuron (I1, not recovered histologically), was reciprocally connected with a layer 3 pyramid (P1) and received EPSPs from another two layer 3 pyramids (P2 and P3, averaged single spike responses). The EPSP from P1 was depressed during trains of five presynaptic APs. The IPSP elicited in this interneuron (I1 to I2) was larger and faster than the IPSP that I2 elicited in P1 (000121A1).

neurons, including basket cells and double bouquet cells, as well as flask-shaped and multipolar proximal dendrite-targeting cells, displayed fast spiking behaviour, with non-accommodating firing patterns. Some of these exhibited sub-threshold mem- brane potential oscillations that could give rise to an interrupted or stuttering firing pattern. This pattern was most commonly observed in parvalbumin immuno-positive interneurons, in both species, but was not exclusive to this group. Regular and burst firing interneurons were also recorded in both species and three of these cells (two in rat) received strongly facilitating EPSPs from pyramidal cells. These were typically small interneurons, but most were incompletely recovered.

Within Layer Connections Between Pyramidal Cells and Interneurons in Layer 2/3 of the Cat Visual Cortex

In layers 2/3 of the cat visual cortex, 25 such pairs were recorded (12 interneurons). Six of these pairs yielded EPSPs (five interneurons, 1:4) and seven yielded IPSPs (1:3.6) (three of which were reciprocal connections). All but one of the EPSPs recorded in cat interneurons were 'depressing', i.e. the EPSPs declined in average amplitude with successive APs in trains (Figs 6 and 11). More detailed analysis of these events will be published elsewhere.

The layer of origin and interneuronal soma/dendritic morphology were confirmed for all 12 interneurons, but some yielded only partial axonal recoveries. The postsynaptic interneuron for the one 'facilitating' EPSP recorded in cat cortex was, for example, only partially recovered. The dendritic and axonal arbours of three of the well-labelled presynaptic layer 3 interneurons were restricted to the superficial layers (two were reciprocally connected with layer 3 pyramidal cells). Two of these, a multipolar and a 'flask-shaped' internerneuron innervated both layer 3 and layer 2. The third, which resembled a small basket cell, appeared only to innervate layer 3. A fourth interneuron that was reciprocally connected with a layer 3 pyramidal cell had a sparsely spiny, bi-tufted dendritic tree extending into layer 4. All its axon ascended, with many fine, vertical collaterals coursing to layer 1, forming small clusters of boutons *en route* in all three layers and forming close membrane appositions with the distal dendrites of the pyramidal cell (a Martinotti-like cell, Fig. 7). The reciprocal connection involving this cell was, however, only briefly recorded and the recording is not illustrated.

Several layer 3 interneurons innervated both layer 3 and layer 5. The axon of one large (parvalbumin immuno-negative) layer 3 interneuron, in addition to generating a dense local arbour and major myelinated axonal branches that extended horizontally for more than 1 mm, had a single myelinated branch descending through layer 4 to layer 5, where it generated a second, much narrower arbour (Fig. 6). This interneuron was reciprocally connected with a smaller, layer 2 interneuron and received EPSPs from a layer 3 pyramid and from three layer 4 excitatory cells (partial recoveries). The dendrites of another presynaptic layer 3 interneuron were too weakly stained for reconstruction, but the axon was well labelled. It also generated a dense local arbour as well as long horizontal branches and sent four vertical axon collaterals, in a narrow bundle, down through layer 4 to layer 5. This axon made six close membrane appositions with primary and secondary dendrites of a layer 3 pyramid in which it elicited an IPSP 1 mV in amplitude (at –63 mV). It thus resembled the double bouquet cells described previously (see for example Tamás *et al.*, 1997). Another small incompletely recovered layer 3 interneuron, with a more accommodating firing pattern than a classical fast spiking cell, was reciprocally connected with two layer 3 pyramidal cells and generated an IPSP in a layer 5 cell. Thus, while some layer 3 interneuronal axons are restricted to layer 3, others inhibit both layers 3 and 5 (see also Buhl *et al.*, 1997). In this series, no layer 3 interneuron in the rat that demonstrably innervated layers 3 and 5 was recovered, but double bouquet cells have also been described in this species (Kawaguchi and Kubota, 1997).

Within Layer Connections Between Excitatory and Inhibitory Neurons in Cat Layer 4

In 42 paired recordings in layer 4, one cell was an excitatory neuron (a pyramidal or spiny stellate cell) and the other an inhibitory interneuron (17 interneurons). Eight of these pairs yielded excitatory connections (five interneurons, including one reciprocal connection), giving a ratio of 1:5, similar to that observed in layer 3. The five postsynaptic interneurons were all multipolar cells, three with dendrites confined to layer 4. In one of these examples a spiny stellate cell innervated a parvalbumin immuno-positive interneuron (whose axon could not be reconstructed) as well as another spiny stellate cell (Fig. 8). Two of the postsynaptic interneurons had dendrites extending into layer 3 and also received excitatory input from layer 3 pyramidal cells. One of these was a large cell (CCK immuno-negative) which generated a very wide axonal arbour spanning the border between the two layers (Fig. 11). The axon of the other cell, a smaller multipolar interneuron, descended through the depth of layer 4 and into layer 5.

In four of these 42 pairs in cat, inhibitory connections were observed (1:10, one reciprocal). The presynaptic interneurons were variously multipolar, bipolar (Fig. 9) and 'flask-shaped', all with an axon and three with dendrites that also extended into layer 3. Very few equivalent pairs were recorded in rat. In the one such pair confirmed histologically, the axon of a small deep layer 4 interneuron made close membrane appositions with a very proximal basal dendrite of a labelled target cell. The axonal arbour of this interneuron was narrow (100 m in the horizonal plane) and spanned the depth of layer 4. It appeared to make basket-like contacts (Ramón y Cajal, 1891) with the somata of unlabelled layer 4 neurons, thus resembling a clutch cell (Kisvárday *et al.*, 1985).

Within Layer Connections Between Pyramidal Cells and Interneurons in Layers 2/3 of the Rat Neocortex

In layers 2/3 of the rat neocortex, 107 pairs in which one cell was a pyramidal cell and the other an interneuron (25 interneurons) were recorded. Of these, 22 pairs yielded an excitatory (1:5) and 17 an inhibitory connection (1:6.3) (including three reciprocal connections). Four of the EPSPs were strongly facilitating, i.e. the first AP of a train resulted in a small EPSP (or no discernable postsynaptic response), but successive APs elicited EPSPs of steadily increasing amplitude. Three of these facilitating EPSPs were recorded in a single interneuron and one in another (these EPSPs are excluded from the means in Table 1). The remaining layer 3 pyramid to interneuron connections resulted in 'depressing' EPSPs (Fig. 5*A*,*C*,*D*). With any one postsynaptic interneuron the frequency-dependent effects were consistent, i.e. if the input from one pyramidal cell was strongly facilitating, the inputs from any other presynaptic excitatory cells studied were also strongly facilitating, and likewise for 'depressing' EPSPs (see for example Fig. 5*A*). Four of the interneurons receiving depressing EPSPs were immuno-positive for parvalbumin (Fig. 5*C*,*D*), two were immuno-negative for parvalbumin and three were not successfully tested by immunofluorescence (Fig. 5*A*).

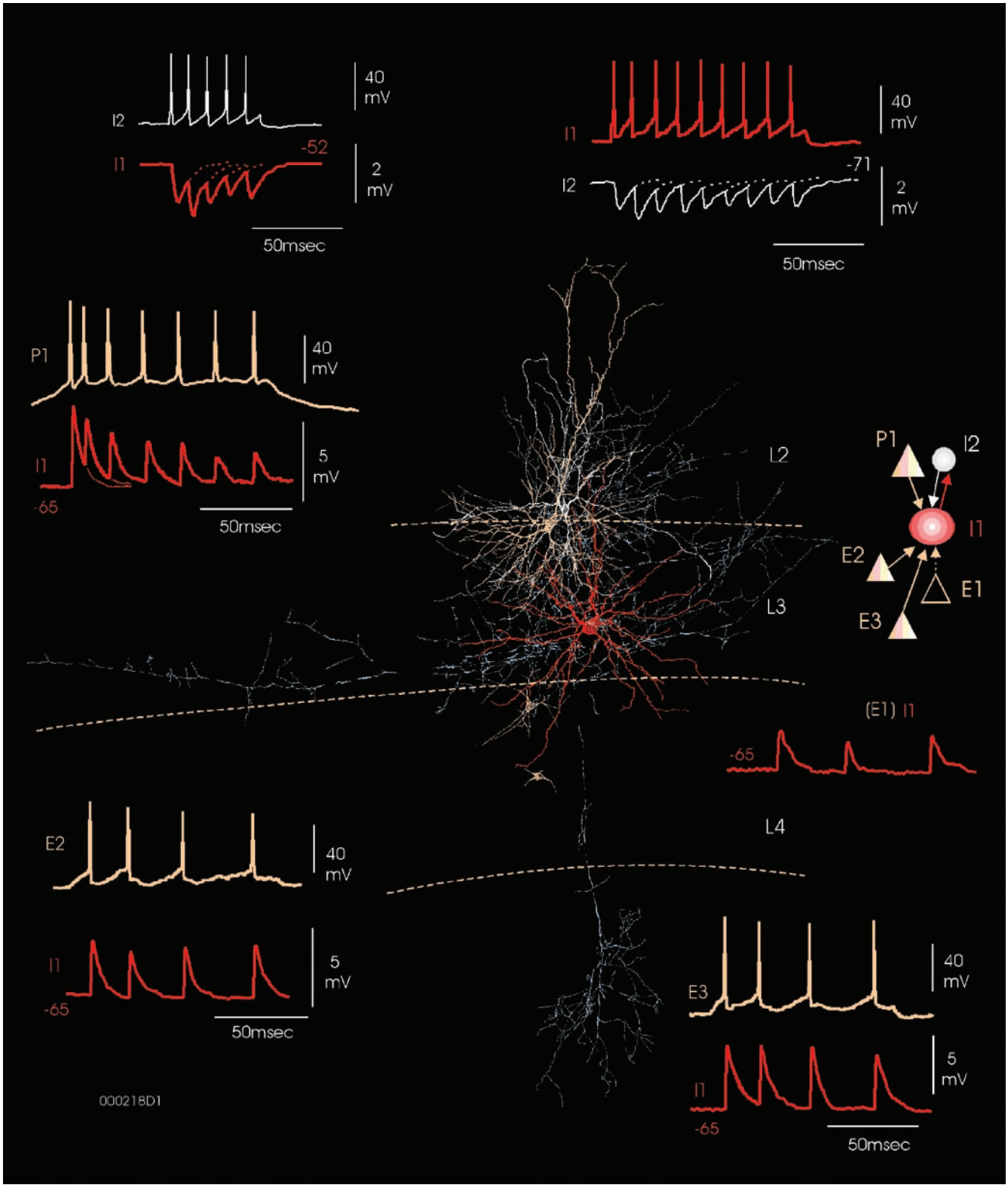

Figure 6. Synaptic connections made and received by a large parvalbumin immuno-negative, multipolar layer 3 interneuron in cat visual cortex (I1, soma/dendrites in red, axon in blue). This interneuron was reciprocally connected to a smaller layer 2 interneuron (I2, soma/dendrites white, axon grey). The IPSPs elicited by these connections in response to trains of presynaptic APs are shown. This large interneuron (I1) also received depressing EPSPs from a layer 3 pyramidal cell (P1, buff) and from three (two poorly stained, one unidentifiable) layer 4 excitatory cells (E1–E3, buff) (000218D1).

Comparing the two species, the probability, within these samples, of a layer 3 pyramidal cell innervating a neighbouring layer 3 interneuron was similar in rat (1:5) and cat (1:4), while the probability of a layer 3 interneuron inhibiting a neighbouring layer 3 pyramidal cell was higher in cat than in rat (1:3.6 compared with 1:6.3).

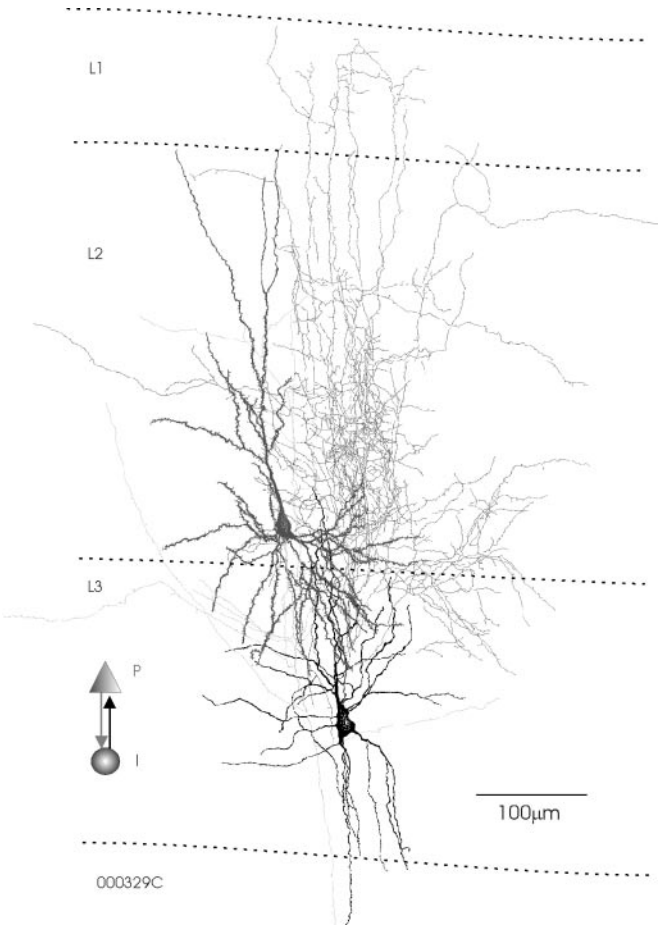

Figure 7. A layer 3 interneuron in cat visual cortex (soma/dendrites black, axon dark grey) with ascending axon that innervated layers 3, 2 and 1 was reciprocally connected to a layer 2 pyramidal cell (soma/dendrites dark grey, axon pale grey). The interneuron's axon made three close membrane appositions with the fifth order dendrites of the postsynaptic pyramidal cell. The recordings were brief and are not illustrated (000329C).

Within Layer Connections Between Pyramidal Cells and Interneurons in Layer 5 of the Rat Neocortex

In layer 5 of the rat neocortex, 73 pairs in which one neuron was a pyramidal cell and the other an inhibitory interneuron were recorded. Of these, seven exhibited an excitatory connection (1:10.4) and nine an inhibitory connection (1:8). These connectivity ratios are similar to those described for equivalent connections in previous studies. The EPSPs were largely depressing, although one showed modest facilitation (30%) of the second EPSP but depression of succeeding EPSPs in the train. The small circuits studied with triple recordings that were confirmed histologically involved one sparsely spiny interneuron that received an EPSP from a layer 5 pyramid and elicited IPSPs in three other L5 pyramids and one smooth parvalbumin immuno-positive interneuron that received one EPSP from a L5 pyramid, an IPSP from another (unidentified) L5 interneuron, with which it was reciprocally connected and elicited IPSPs in two other L5 pyramids.

Excitatory Inputs From Layer 4 to Layer 3 Interneurons

In 31 pairs recorded in cat, one neuron was an excitatory cell in layer 4 and the other an inhibitory interneuron in layer 3 (nine interneurons). In these 31 pairs three EPSPs were recorded, which would give a ratio of 1:10. All three EPSPs were, however, recorded from a single interneuron. This was the largest layer 3 interneuron recovered, the large parvalbumin immuno-negative interneuron (described above) whose dendrites extended into layer 4 and which generated a wide axonal arbour in layer 3 and a narrow arbour in layer 5 (Fig. 6). The three EPSPs elicited in this interneuron by the three presynaptic layer 4 cells were similar (amplitude 0.9–1.5 mV, RT 0.7–0.8 ms, HW 5–7.5 ms) and smaller than the one EPSP elicited in this interneuron by a layer 3 pyramidal cell (3.9 mV, RT 0.8 ms, HW 4.7 ms). These data suggest that only a minority of layer 3 interneurons, perhaps only the largest, are activated by layer 4 cells, but that those that are activated receive a strong ascending drive. This is, however, too small a sample from which to extrapolate far. In rat, 10 similar pairs were studied (five interneurons), only one of which yielded a small EPSP (<0.2 mV average amplitude). None of the 41 pairs tested resulted in an inhibitory connection from a layer 3 interneuron to a layer 4 excitatory cell.

Inhibitory Connections From Layer 4 Interneurons to Layer 3 Pyramidal Cells

Of 37 pairs in cat visual cortex in which a layer 4 interneuron was recorded simultaneously with a layer 3 pyramidal cell (15 interneurons), 10 yielded inhibitory connections. This gives a connectivity ratio (1:3.7) similar to inhibitory to excitatory cell connections within layer 3 in cat (1:3.6). This is higher than inhibitory to spiny cell connections within layer 4 (1:10) in this and a previous study in adult cat (Stratford *et al.*, 1996; Tarczy-Hornoch *et al.*, 1998), but lower than in studies of intra-laminar connections in immature rat layer 4 (Galarreta and Hestrin, 1999; Gibson *et al.*, 1999).

Six of these layer 4 interneuron to layer 3 pyramid pairs (involving five interneurons) were confirmed histologically. These interneurons had axonal arbours that innervated both layer 3 and layer 4. A bipolar interneuron in upper layer 4 inhibited a spiny stellate cell and another interneuron in layer 4 as well as a deep layer 3 pyramidal cell, making close membrane appositions with second order spiny postsynaptic dendrites (Fig. 9). A large CCK immuno-positive interneuron in layer 4, with a dense local axonal arbour in upper layer 4 and layer 3, long horizontal projections and a narrow descending projection to layer 5, innervated a layer 3 pyramid, making close membrane appositions with its fourth and fifth order dendrites (Fig. 10). Two of the layer 4 interneuron to layer 3 pyramid IPSPs originated from a single large CCK immuno-negative basket cell, which innervated lower layer 3 and layer 4 and had long horizontal axonal projections. It made contact with the somata and very proximal dendrites of its postsynaptic layer 3 targets. This cell also inhibited a small 'flask-shaped' deep layer 4 interneuron and received excitatory inputs from another layer 3 pyramid and from several unstable layer 4 excitatory cells, as well as an inhibitory input from another small 'flask-shaped' interneuron in deep layer 4 (Fig. 11). Thus interneurons whose somata are in layer 4 but which also inhibit layer 3 pyramidal cells include both basket cells that target very proximal portions of pyramidal cells and interneurons that innervate pyramidal dendrites.

Of 12 pairs in rat neocortex in which a layer 4 interneuron was recorded simultaneously with a layer 3 pyramidal cell (five interneurons), six yielded inhibitory connections. This also yields a high connectivity ratio (1:2), suggesting that while layer 3 interneurons rarely inhibit layer 4 spiny cells in either species, with the exception perhaps of layer 3 double bouquet cells, layer 4 interneurons of a variety of subclasses provide significant inhibition to lower layer 3 pyramidal cells in both species.

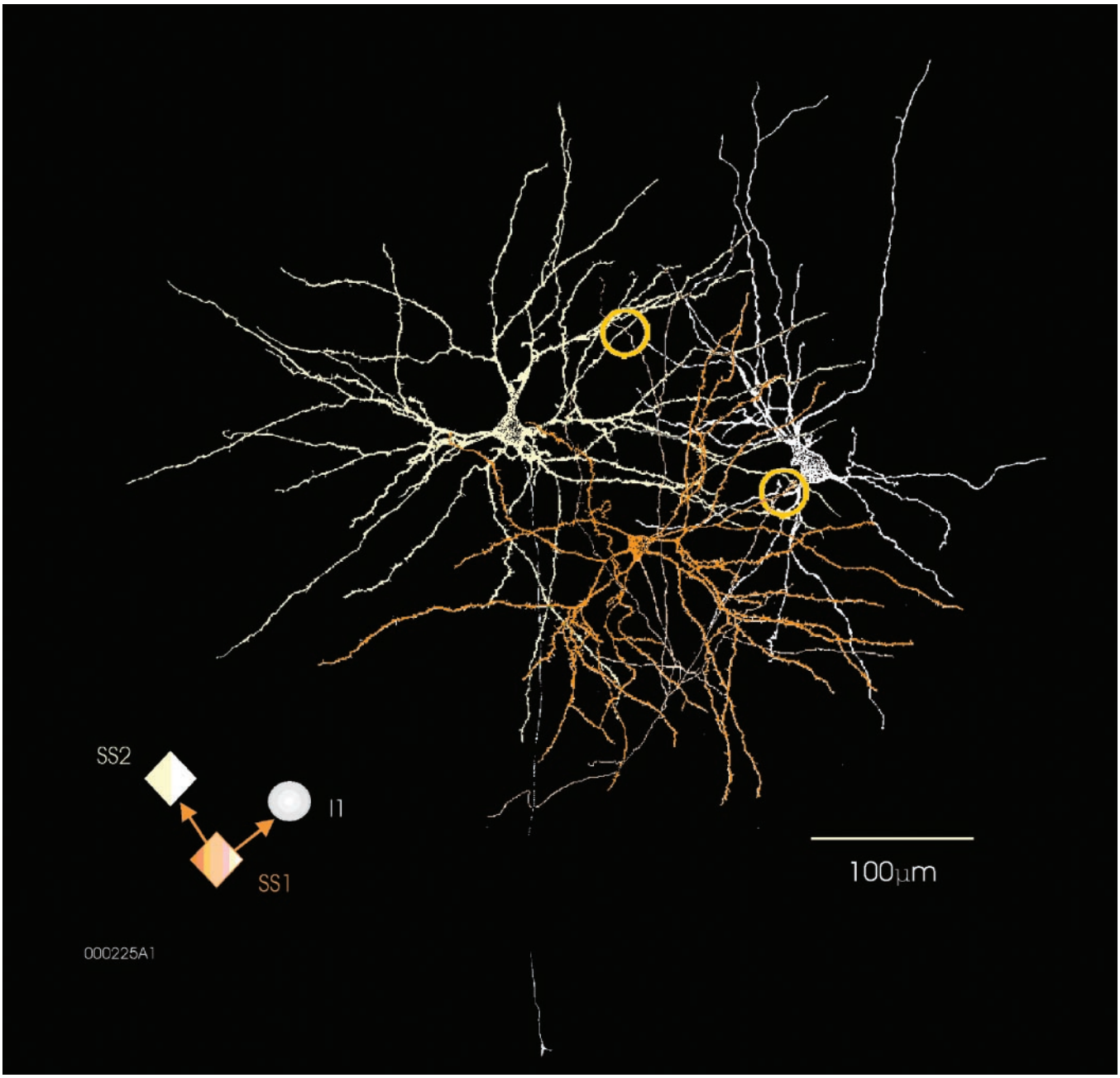

Figure 8. A multipolar parvalbumin immuno-positive interneuron (I1, soma/dendrites white, axon too weakly stained for reconstruction) in layer 4 of the cat visual cortex received excitatory inputs from a spiny stellate cell (SS1, soma/dendrites orange, axon buff) that also innervated another spiny stellate (SS2, soma/dendrites pale yellow, axon pale grey). The recordings were brief and are not illustrated. The circles indicate the positions of close membrane appositions between the axon of SS1 and its postsynaptic targets (upper onto SS2, lower onto I1) (000225A1).

Excitatory Inputs from Layer 3 Pyramidal Cells to Layer 4 Interneurons in Cat Visual Cortex

Of the 37 such pairs recorded (15 interneurons), seven yielded an EPSP (1:5.3). The postsynaptic interneurons were in the upper half of layer 4 and most had both dendrites and axons that ascended into layer 3. Four of these cells that were successfully tested with immunofluorescence for parvalbumin were found to be parvalbumin immuno-positive and (like the CCK immuno-negative cell) were either classical fast spiking or late spiking, fast spiking cells, with an interrupted or stuttering firing pattern. One of these interneurons was a basket cell with an intermediate sized axonal arbour that projected upwards and ramified predominantly in layer 3 where it was reciprocally connected with a pyramidal cell, while another (whose axon could only be partially reconstructed) sent a horizontally oriented axon away from its soma in layer 4 and had a major vertical branch that descended to layer 5. The CCK immuno-negative layer 4 basket cell that was postsynaptic to a layer 3 pyramidal cell (and presynaptic to two others) had a broad axonal arbour, innervating upper layer 4 and lower layer 3 (Fig. 11).

The resultant EPSPs recorded in the parvalbumin immuno-positive cells were the briefest recorded (RT 0.7–0.8 ms, HW 4.4–6.4 ms, amplitudes 0.7–1.7 mV). The EPSP in the CCK

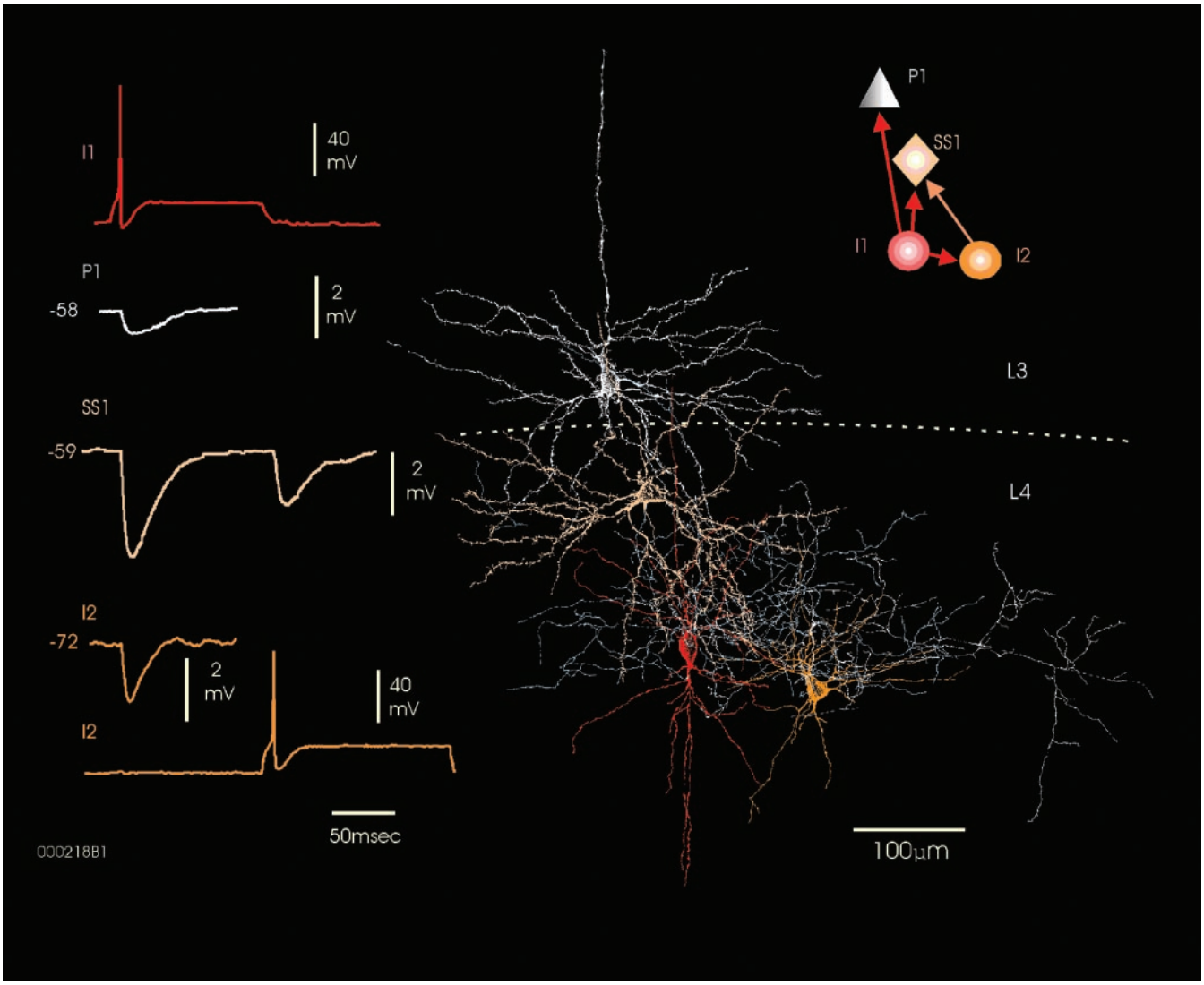

Figure 9. A bipolar interneuron in layer 4 (I1, soma/dendrites red, axon blue) of cat visual cortex elicited IPSPs in another, multipolar layer 4 interneuron (I2, soma/dendrites orange, axon white), in a spiny stellate cell (SS1, buff) and in a deep layer 3 pyramidal cell (P1, white). The axon of this cell made close membrane appositions with the secondary dendrites of its postsynaptic targets. The multipolar interneuron (I2) also inhibited the spiny stellate cell (SS1), making close membrane appositions with third and fourth order dendrites (000218B1).

immuno-negative cell was of slightly longer duration (RT 0.7 ms, HW 9 ms, amplitude 1.23 mV). All these EPSPs were 'depressing' (Fig. 11). Since the connectivity ratio (1:5.3) was similar to that for pyramid to interneuron connections within layer 3 in the cat (1:4), layer 3 pyramidal axons do not appear to distinguish between interneuronal targets whose somata are in layer 3 and those whose somata are in mid to upper layer 4, although they appear to avoid making contact with the apical dendrites of layer 4 pyramidal cells. In the rat 12 such pairs were recorded, one of which yielded an EPSP. Clearly this is too small a sample to allow a comparison, but demonstrates that a similar connection exists in both species.

Interneuron to Interneuron Connections

In eight paired recordings in rat layer 3 both cells were interneurons. These pairs yielded two IPSPs (ratio 1:4). In one example, a parvalbumin immuno-positive interneuron received, from an unidentified interneuron, an IPSP that was 2 mV average amplitude at –68 mV (RT 2 ms, HW 8 ms). This postsynaptic interneuron was also reciprocally connected with a layer 3 pyramidal cell in which it generated a slower IPSP. Of five interneuron–interneuron pairs tested in rat layer 5, two were connected (one reciprocal, three IPSPs, ratio 1:1.7).

In the only interneuron–interneuron pair recorded in layer 2/3 in the cat, a small layer 2 interneuron was reciprocally connected with a large (parvalbumin immuno-negative) layer 3 interneuron (amplitudes 0.8 and 1.3 mV, RT 2.3 and 3.9 ms, HW 7 and 10 ms at –71 and –52 mV for L3 to L2 and L2 to L3 IPSPs, respectively) (Fig. 6). In six layer 4 interneuron pairs in cat, three pairs (involving five interneurons) yielded IPSPs (Figs 9 and 11). Each of the three pairs involved interneurons with very different morphologies. The large CCK immuno-negative interneuron was presynaptic to one small 'flask-shaped' interneuron in deep layer 4 and postsynaptic to another (Fig. 11). A bipolar interneuron in upper layer 4 was presynaptic to a small multipolar interneuron also in upper layer 4 (Fig. 9). In addition, in the only such test performed, an interneuron in upper layer 4 inhibited an interneuron in layer 3. These data suggest that the

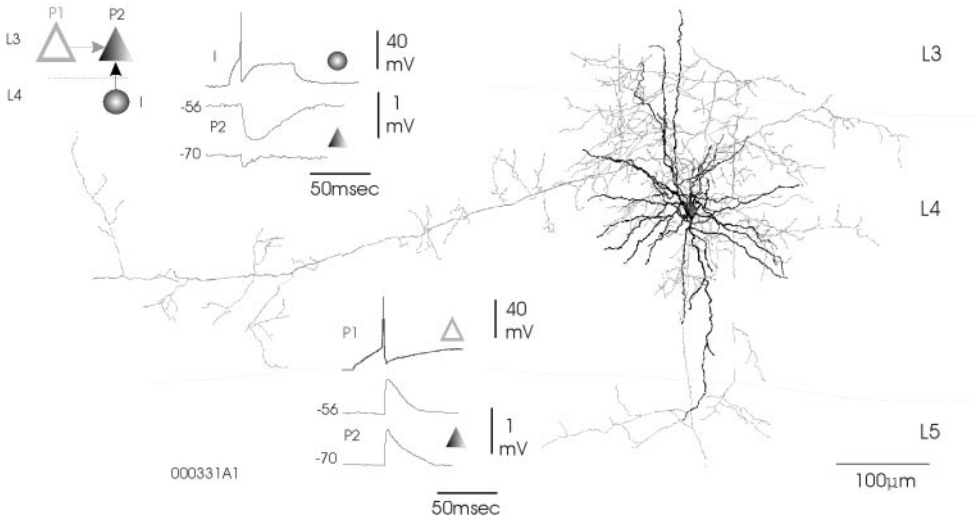

Figure 10. A large, CCK immuno-positive interneuron in layer 4 of the cat visual cortex inhibited a layer 3 pyramidal cell (P2), which also received an excitatory input from another layer 3 pyramidal cell (P1, the pyramidal cells are not illustrated). Both inputs were onto branches of the same primary dendrite of the postsynaptic pyramid, with the inhibitory input located more distally (fourth and fifth order dendrites) than the excitatory input (fourth order dendrites) and the two inputs summed linearly (not illustrated). Responses to these two inputs were recorded at two postsynaptic membrane potentials (000331A1).

connectivity between interneurons in all layers and even between adjacent layers and in both species is high (average ratio 1:1.9), as reported previously in immature rat neocortex (Gibson *et al.*, 1999) and can involve pairs of cells with very different morphologies (Tamás *et al.*, 1998). Pooling data from all layers for each of the two species, both the EPSPs and the IPSPs recorded in postsynaptic interneurons were briefer than those recorded in postsynaptic excitatory cells (Table 2), as described previously for EPSPs in adult cat (Tarczy-Hornoch *et al.*, 1998) and rat (Thomson *et al.*, 1993a,b, 1995) and IPSPs in adult cat (Tamás *et al.*, 1997) neocortical interneurons.

Discussion

The data presented here confirm a number of observations made previously in rat neocortex, extend these to include connections between layers 3 and 4 in the adult rat and compare these directly with parallel studies in adult cat visual cortex. Using triple recordings and sampling several putative presynaptic and postsynaptic neurons it was also possible to determine whether individual neurons received inputs from more than one layer and/or innervated other neurons in more than one layer, allowing the position of these neurons in the local circuit to be more completely identified. Although connectivity ratios for some connections differed between the two species, the overall pattern was similar, as were the properties of the synapses, indicating that some of the fundamental building blocks of the cortical circuit are common amongst mammals.

The major differences observed between the two species were differences of scale, with a wider range of neuronal dimensions and morphologies, broader interneuronal axonal and dendritic arbours and more widely separated connected pairs in cat than in rat. These differences in scale may account for some of the differences in connectivity ratios in a slice preparation, since even in the rat neocortex, which contains relatively small neurons, increasing slice thickness from 400 to 500 μm appeared to increase these ratios in our earlier studies. Although cells were typically recorded in the middle 200 μm of the 500 μm slice and very few of the histologically recovered cells had dendrites that had been severed in the preparation of the slices, the axons of many of the pyramidal cells and larger interneurons reached (and presumably had originally extended beyond) the cut surfaces of the slice. It should therefore be remembered that slice preparations lead to an underestimate of connectivity, possibly of the functional strength of connections and of the extent of large axonal abours.

Excitatory 'Forward' Projections

Connections that might be classified as 'forward' projections, e.g. from layer 4 to layer 3 and from layer 3 to layer 5, share some properties and differ from those that might be described as 'back' projections (e.g. from layers 5 to 3 and 3 to 4). Both types of 'forward' projection involve excitatory inputs to both excitatory and inhibitory neurons and in this might appear to be relatively indiscriminate. There is, however, selectivity in the pyramidal cells targeted by these pathways. In layer 5 the 'forward' projections from layer 3 almost exclusively target the larger pyramidal cells in upper layer 5 with apical dendritic tufts in layer 1 (Fig. 1) (Thomson and Bannister, 1998) and in the present study most of the identified layer 3 pyramidal targets of layer 4 spiny cells were larger pyramidal cells in deep layer 3 (Fig. 3). In addition, only one cat layer 3 interneuron (of nine interneurons tested with 31 layer 4 cells) demonstrably excited by layer 4 cells was identified in this study (Fig. 6). This was an unusually large, parvalbumin immuno-positive interneuron which received inputs from three of three layer 4 cells tested. Similarly, only a handful of interneurons in layer 5 demonstrably innervated by layer 3 pyramidal cells have been described previously (Thomson *et al.*, 1996). Larger samples are required to determine whether the interneuronal targets of these forward projections do indeed comprise specific sub-classes.

Excitatory 'Back' Projections

No EPSPs were elicited in layer 4 excitatory cells by layer 3 pyramidal cells in this (94 tested pairs) or a previous study (Feldmeyer *et al.*, 1999b, 2002). Similarly, only very rarely was a layer 3 pyramidal cell found to be excited by a layer 5 pyramidal cell (Thomson and Bannister, 1998; Reyes and Sakmann, 1999). The few 'back' projections involving excitatory cells that have

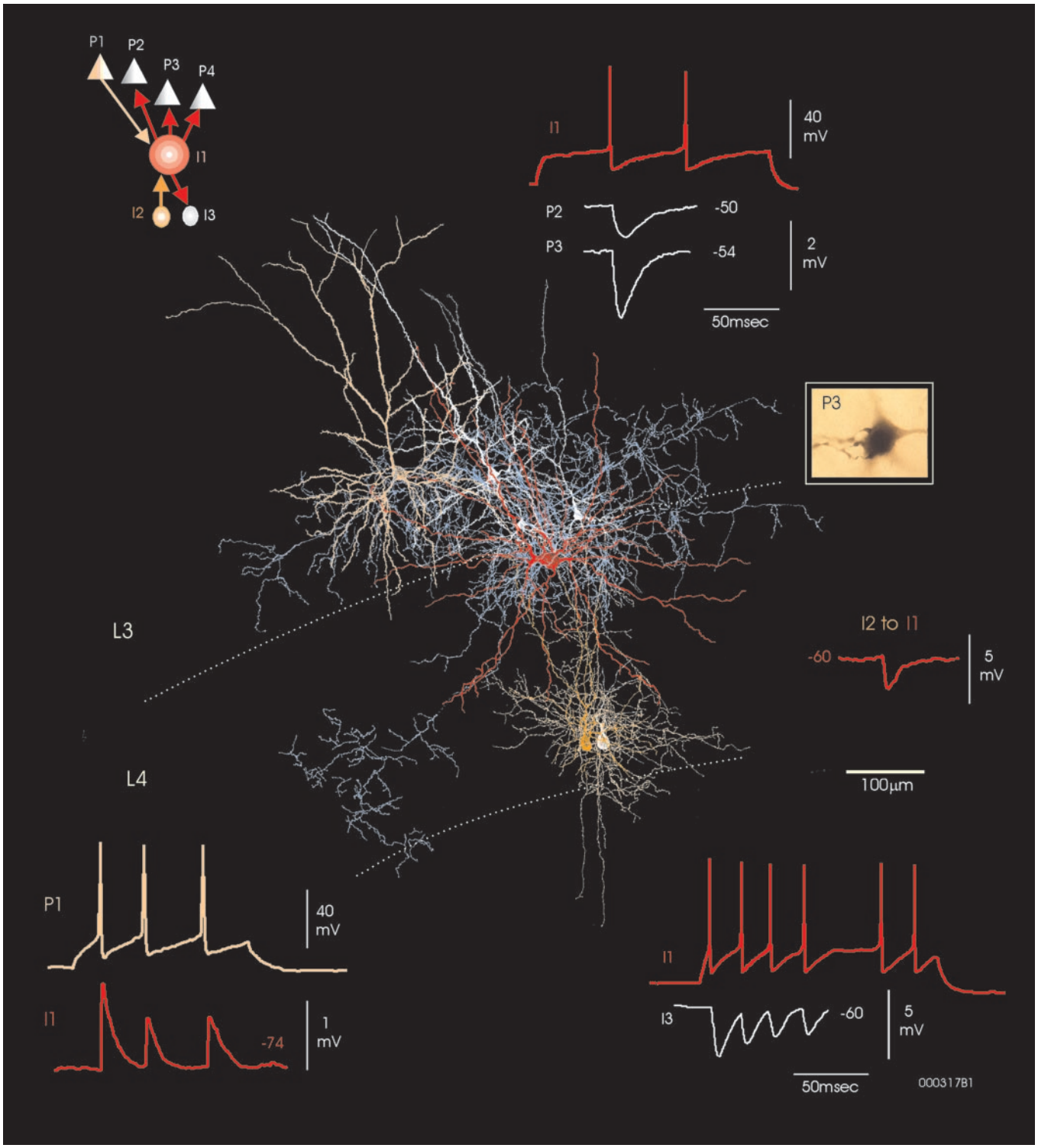

Figure 11. A large, late fast spiking, stuttering multipolar (CCK immuno-negative) interneuron (I1, soma/dendrites red, axon blue) in upper layer 4 of the cat visual cortex inhibited three small layer 3 pyramidal cells (P2–P4, white), with which it made very proximal contacts. A photomicrograph of one example, P3, is illustrated in the insert, in which three putative contacts onto the soma and a very proximal dendrite can be seen. The two IPSPs illustrated were recorded from P2 and P3 (P4 was recorded only briefly). I1 also inhibited a deep layer 4 'flask-shaped' interneuron (I3, white, axon not reconstructed). The proximal dendrites of this large interneuron (I1) received excitatory input from another layer 3 pyramidal cell (P1, buff) and inhibitory input from another small deep layer 4 'flask-shaped' interneuron (I2, soma/dendrites orange, axon buff) (000317B1).

been recorded (layer 5 to layer 3) involved very small EPSPs and cell pairs separated further in the horizontal plane than is typical for the very tightly focused 'forward' layer 3 to layer 5 projection. Reverberating excitation between layers 3 and 5 and between layers 4 and 3 is therefore extremely unlikely to occur. The possibility of a significant ascending layer 5 to layer 3 or descending layer 3 to layer 4 'back' projection that involves excitatory cells in distant columns cannot be discounted, but the targets of the long horizontal collaterals of these pyramidal axons have yet to be fully identified.

Table 2

Both EPSPs and IPSPs elicited in postsynaptic interneurons are, on average, similar in amplitude, but briefer, than those elicited in excitatory cells (pyramidal and spiny stellate cells) in both rat and cat neocortex

		EPSPs				IPSPs		
		Amplitude (mV)	Rise time (ms)	Width at half amplitude (ms)		Amplitude (mV)	Rise time (ms)	Width at half amplitude (ms)
Interneurons	Rat ($n = 20$)	1.6 ± 1.5 (0.0–6.8)	1.0 ± 0.7 (0.4–3.7)	8.31 ± 3.9 (3.3–18.5)	Rat ($n = 5$)	1.1 ± 0.7 (0.2–2.0)	2.2 ± 0.2 (2.0–2.4)	8.6 ± 0.7 (8.0–9.5)
	Cat ($n = 13$)	2.0 ± 1.3 (0.7–4.4)	0.9 ± 0.3 (0.7–2.0)	7.2 ± 4.1 (3.8–11.7)	Cat ($n = 5$)	1.2 ± 0.8 (0.4–2.7)	2.9 ± 0.5 (2.9–3.9)	10.1 ± 1.7 (7.0–11.7)
Excitatory cells	Rat ($n = 49$)	1.9 ± 1.4 (0.2–5.9)	1.9 ± 0.9 (0.6–4.6)	15.9 ± 8.2 (6.0–39)	Rat ($n = 13$)	1.2 ± 0.5 (0.2–1.9)	3.6 ± 2.1 (1.5–8.0)	22.4 ± 8.8 (10–39)
	Cat ($n = 15$)	1.78 ± 1.85 (0.2–8.2)	2.4 ± 1.1 (1.5–2.0)	19.4 ± 13.2 (9.5–53.0)	Cat ($n = 13$)	0.73 ± 0.3 (0.2–1.1)	4.1 ± 0.6 (3.0–5.3)	20.3 ± 6.4 (12.0–23)

The mean widths at half amplitude of both EPSPs and IPSPs were almost twice as long in excitatory cells. The durations of the synaptic potentials are, however, similar in the two species. Data from all layers are pooled and means, standard deviations and ranges (in parentheses) are given. Synaptic potential amplitude, 10–90% rise time (RT) and width at half amplitude (HW) were measured from averaged events. Two EPSPs included with average amplitudes of 0 mV (rat, EPSPs in interneurons) were strongly facilitating inputs that failed to elicit any postsynaptic response to the first presynaptic spike, but elicited increasing responses to later spikes in the train. Later EPSPs in the trains were used for measurement of rise times and half widths.

The scarcity or absence of intra-columnar 'back' projections to excitatory cells does not arise, simply because the potential presynaptic axons do not innervate the region(s) occupied by the postsynaptic dendrites. Layer 5 pyramidal axons project to layer 3 where they innervate interneurons (Dantzker and Callaway, 2000) and the apical dendrites of other layer 5 pyramids (Deuchars *et al.*, 1994). Similarly, although layer 3 pyramidal axons do not typically ramify in layer 4 and spiny stellate cell dendrites remain largely confined to this layer, the pyramidal cells in layer 4 do send their spiny apical dendrites into layer 3, where they would be readily accessible to layer 3 pyramidal axons (for examples see Figs 3*A* and 4). Moreover, the ascending dendrites of upper layer 4 interneurons are commonly targeted by layer 3 pyramidal axons in layer 3. Excitatory 'back' projections therefore exhibit specificity in the targets they select, powerfully exciting interneurons but avoiding excitatory cells. Although the layer 4 interneurons excited by layer 3 pyramidal cells displayed a range of morphological features, all four that were successfully tested for parvalbumin immunoreacitivity were positive and two (one of which was parvalbumin immuno-positive) were seen to make close membrane appositions with very proximal portions of their pyramidal targets, suggesting, perhaps that this 'back projection' favours proximally targeting interneurons.

Inter-laminar Inhibitory Projections

With the exception of dendrite-targeting double bouquet cells, the axonal arbours of layer 3 interneurons were either confined to the superficial layers or included descending projections to layer 5 and, in previous studies, to layer 6 (Tamás *et al.*, 1997, 1998), with little or no ramification in layer 4. Indeed, no inhibitory connections from layer 3 interneurons to layer 4 excitatory cells were observed in this study (31 tested pairs in cat, 10 in rat), suggesting that the axons of many layer 3 interneurons, like those of layer 3 pyramidal cells, may avoid spiny layer 4 targets. The major difference in the parallel descending excitatory and inhibitory pathways from layer 3 to layer 5 was the relatively narrower arbours of the interneuronal axons of all types in the deep layers. This may not, however, represent selection of a different target population. In both cases, the somata of the layer 5 pyramidal targets of layer 3 axons lie within a very narrow field, since the interneurons often target proximal, while the pyramidal cells target more distal, dendritic sites. These interneurons and those in layer 5 whose axons ramify extensively in layers 5 and 3 could act to coordinate activity in these two layers, relatively independently of layer 4.

The axons and dendrites of some of the layer 4 interneurons studied here were also largely confined to their layer of origin. However, 10 of 27 layer 4 interneurons recovered had axons and dendrites that extended into layer 3. These ascending projections ranged from single axonal and dendritic branches to very wide axonal arbours involving many collaterals that innervated both layers. Some of the interneurons, situated in middle to upper layer 4, that demonstrably inhibited layer 3 cells also received excitatory input from layer 3. They differ significantly from layer 3 interneurons, however, in that the majority of their dendrites are located in a major thalamo-recipient layer. They are well positioned, therefore, to integrate the inputs they receive from layer 4 and layer 3 excitatory cells with direct thalamic input, particularly those that are parvalbumin immuno-positive, parvalbumin-containing interneurons being a major thalamo-recipient class of interneurons in layer 4 (Staiger *et al.*, 1996). Indeed, the four layer 4 interneurons innervating both layers and excited by layer 3 pyramidal cells that were so tested were all found to be parvalbumin immuno-positive. The large CCK immuno-negative basket cell with a fast, late spiking, stuttering firing pattern may also have been parvalbumin-containing. By generating IPSPs in cell populations in both layers, these cells could synchronize activity and/or provide a temporal framework within which efficient and meaningful information transfer between the layers could occur.

The long, myelinated, horizontal axon collaterals of some of the larger, upper layer 4 interneurons generated well-separated, tightly focused clusters of boutons hundreds of micrometres from the soma. Some of these may be similar to the large basket cells described previously (Somogyi *et al.*, 1983; Kisvárday, 1992), which are reported to be densely interconnected (Kisvárday *et al.*, 1993). Interestingly, the only layer 3 interneuron (of nine tested) in the cat to receive excitatory input from layer 4 had similar long, myelinated, horizontal axon collaterals. These long collaterals could therefore provide patchy inhibition (Kisvárday and Eysel, 1992) that was controlled by correlated activity in layers 3 and 4 and direct thalamo-cortical input. No equivalently long, patchy horizontal interneuronal projections were found in the rat in this study, but one such layer 4 cell was reported previously (Thomson *et al.*, 1996).

Time Course of Synaptic Events in Excitatory and Inhibitory Cells

This survey of synaptic connections within and between cortical layers and in two species also allowed the properties of the synaptic events at different synapses to be compared. The properties of any given class of synapse were similar to those described previously for adult rat and were also found to be very similar when similar connections in rat and cat were compared. Synaptic events recorded in inhibitory interneurons were on average much briefer than those recorded in excitatory cells in both species, as reported previously for EPSPs in fast spiking interneurons in rat neocortex that display only a modest *N*-methyl-D-aspartate receptor-mediated component (Angulo *et al.*, 1999). These brief time courses and the very high connectivity ratios (>1:2) found when pairs of interneurons were tested support the proposed role for interneuronal circuits in the generation/maintenance of the fast gamma rhythms associated with attention and arousal (Buzsáki and Chrobak, 1995; Traub *et al.*, 1996). Whether these interneurons are as commonly interconnected via electrical junctions as they are in the developing rat neocortex (Galarreta and Hestrin, 1999; Gibson *et al.*, 1999; Támas *et al.*, 2000) was not determined in the present study. No events indicative of such connections were apparent. It is, however, possible that our recording conditions biased existing gap junctions towards the closed state and that under appropriate conditions *in vivo*, perhaps under the influence of ascending neuro-modulatory systems, these circuits involve both electrical and chemical junctions. The longer duration of IPSPs in pyramidal and spiny stellate cells, in addition to their slower and more powerfully accommodating firing properties, help to explain why networks of excitatory cells do not themselves generate these faster rhythms, firing at best perhaps only on alternate cycles. Their firing is, however, phase locked to these faster oscillations generated by interneuronal circuits. The phase locking will facilitate synchronous activity within selected sub-populations of spiny cells.

Notes

This work was supported by the Medical Research Council, Novartis Pharma and the Wellcome Trust. Monoclonal antibody 9303 raised against gastrin/CCK was provided by CURE/Gastroenteric Biology Centre, Antibody/RAI Core, NIH grant DK41301. Polyclonal antibody R301 to parvalbumin was a kind gift from Professor K. Baimbridge, University of British Columbia, Canada.

Address correspondence to Alex M. Thomson, Department of Physiology, Royal Free and University College Medical School, Rowland Hill Street, London NW3 2PF, UK. Email: alext@ucl.ac.uk.

References

Angulo MC, Rossier J, Audinat E (1999) Postsynaptic glutamate receptors and integrative properties of fast-spiking interneurons in the rat neocortex. J Neurophysiol 82:1295–1302.

Bode-Greuel KM, Singer W, Aldenhoff JB (1987) A current source density analysis of field potentials evoked in slices of visual cortex. Exp Brain Res 69:213–219.

Buhl EH, Tamás G, Szilagyi T, Stricker C, Paulsen O, Somogyi P (1997) Effect, number and location of synapses made by single pyramidal cells onto aspiny interneurones of cat visual cortex. J Physiol 500: 689–713.

Burkhalter A. (1989) Intrinsic connections of rat primary visual cortex: laminar organization of axonal projections. J Comp Neurol 279: 171–186.

Buzsáki G, Chrobak JJ (1995) Temporal structure in spatially organized neuronal ensembles: a role for interneuronal networks. Curr Opin Neurobiol 5:504–510.

Dantzker JL, Callaway EM (2000) Laminar sources of synaptic input to cortical inhibitory interneurons and pyramidal neurons. Nature Neurosci 3:701–707.

Deuchars J, West DC, Thomson AM (1994) Relationships between morphology and physiology of pyramid-pyramid single axon connections in rat neocortex *in vitro*. J Physiol 478:423–435.

Feldman ML, Peters A (1978) The forms of non-pyramidal cells in the visual cortex of the rat. J Comp Neurol 179:761–794.

Feldmeyer D, Egger V, Lübke, J, Sakmann B (1999a) Reliable synaptic connections between pairs of excitatory neurones within a single barrel of developing rat somatosensory cortex. J Physiol 521:169–190.

Feldmeyer D, Silver RA, Lübke J, Sakmann B (1999b) Synaptic connections between spiny layer 4 neurones and layer 2/3 pyramidal cells of rat barrel cortex. J Physiol 518P:141–142.

Feldmeyer D, Lubke J, Silver RA, Sakmann B. (2002) Synaptic connections between layer 4 spiny neurone-layer 2/3 pyramidal cell pairs in juvenile rat barrel cortex: physiology and anatomy of interlaminar signalling within a cortical column. J Physiol 538:803–822.

Fujita I, Fujita T (1996) Intrinsic connections in the macaque inferior temporal cortex. J Comp Neurol 368:467–486.

Galarreta M, Hestrin S (1999) A network of fast-spiking cells in the neocortex connected by electrical synapses. Nature 402:72–75.

Gibson JR, Beierlein M, Connors BW (1999) Two networks of electrically coupled inhibitory neurons in neocortex. Nature 402:75–79.

Gilbert CD (1983) Microcircuitry of the visual cortex. Annu Rev Neurosci 6:217–247.

Gilbert CD, Wiesel TN (1983) Clustered intrinsic connections in cat visual cortex. J Neurosci 3:1116–1133

Gilbert CD, Wiesel TN (1986) Intrinsic connectivity and receptive field properties in visual cortex. Vision Res 25:365–374.

Gupta A, Wang Y, Markram H (2000) Organizing principles for a diversity of GABAergic interneurones and synapses in the neocortex. Science 287:273–278.

Hughes DI, Bannister AP, Pawelzik HP, Thomson AM (2000) Double immunofluorescence, peroxidase labelling and ultrastructural analysis of interneurones following prolonged electrophysiological recordings *in vitro*. J Neurosci Methods 101:107–116.

Kawaguchi Y, Kubota Y (1997) GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb Cortex 7:476–486.

Kisvárday ZF (1992) GABAergic networks of basket cells in the visual cortex. Prog Brain Res 90:385–405.

Kisvárday ZF, Eysel UT (1992) Cellular organization of reciprocal patchy networks in layer III of cat visual cortex (Area 17). Neuroscience 46:275–286.

Kisvárday ZF, Martin KA, Whitteridge D, Somogyi P (1985) Synaptic connections of intracellularly filled clutch cells: a type of small basket cell in the visual cortex of the cat. J Comp Neurol 241:111–137.

Kisvárday ZF, Martin KAC, Freund TF, Maglóczky Zs, Whitteridge D, Somogyi P (1986) Synaptic targets of HRP-filled layer III pyramidal cells in the cat striate cortex. Exp Brain Res 64:541–552.

Kisvárday ZF, Beaulieu C, Eysel UT (1993) Network of GABAergic large basket cells in cat visual cortex (area 18): implication for lateral disinhibition. J Comp Neurol 327:398–415.

Kritzer MF, Goldman-Rakic PS (1995) Intrinsic circuit organization of the major layers and sublayers of the dorsolateral prefontal cortex in the rhesus monkey. J Comp Neurol 359:131–143.

Lorente de Nó R (1922) La corteza cerebral del raton Trab. Lab Invest Biol Madrid 20:41–78.

Lübke J, Markram H, Frotscher M, Sakmann B (1996) Frequency and dendritic distribution of autapses established by layer 5 pyramidal neurons in the developing rat neocortex: comparison with synaptic innervation of adjacent neurons of the same class. J Neurosci 16:3209–3218.

Lund JS, Yoshioka T, Levitt JB (1993) Comparison of intrinsic connectivity in different areas of macaque monkey cerebral cortex. Cereb Cortex 3:148–162.

Markram H, Tsodyks M (1996) Redistribution of synaptic efficacy between neocortical pyramidal cells. Nature 382:807–810.

Markram H, Lübke J, Frotscher M, Roth A, Sakmann B (1997) Physiology and anatomy of synaptic connections between thick tufted pyramidal neurones in the developing rat neocortex. J Physiol 500:409–440.

O'Leary JL (1941) Structure of the area striate in the cat. J Comp Neurol 75:131–164.

Parnavelas JG, Lieberman AR, Webster KE (1977) Organization of neurons in the visual cortex, area 17, of the rat. J Anat 124:305–322.

Petersen CH, Sakmann B (2000) The excitatory neuronal network of rat layer 4 barrel cortex. J Neurosci 20:7579–7586.

Ramón y Cajal S (1891) Sur la structure de l'écorce cérébrale de quelques mammifères. Cellule 7:3–54.

Reyes A, Sakmann B (1999) Developmental switch in the short-term modification of unitary EPSPs evoked in layer 2/3 and layer 5 pyramidal neurons of rat neocortex. J Neurosci 19:3827–3835.

Rozov A, Jerecic J, Sakmann B, Burnashev N. (2001) AMPA receptor channels with long-lasting desensitization in bipolar interneurons contribute to synaptic depression in a novel feedback circuit in layer 2/3 of rat neocortex. J Neurosci 21:8062–8071.

Somogyi P, Kisvarday ZF, Martin KA, Whitteridge D. (1983) Synaptic connections of morphologically identified and physiologically characterized large basket cells in the striate cortex of cat. Neuroscience 10:261–294.

Spatz WB, Tigges J, Tigges M (1970) Subcortical projections, cortical associations and some intrinsic interlaminar connections of the striate cortex in the squirrel monkey (*Saimiri*). J Comp Neurol 140:155–174.

Staiger JF, Zilles K, Freund TF (1996) Distribution of GABAergic elements postsynaptic to ventroposteromedia thalamic projections in layer IV of rat barrel cortex. Eur J Neurosci 8:2273–2285.

Stratford KJ, Tarczy-Hornoch K, Martin KAC, Bannister NJ, Jack JJB (1996) Excitatory synaptic inputs to spiny stellate cells in cat visual cortex. Nature 382:258–261.

Tamás G, Buhl EH, Somogyi P (1997) Fast IPSPs elicited via mutliple synaptic release sites by different types of GABAergic neurone in the cat visual cortex. J Physiol 500:715–738.

Tamás G, Somogyi P, Buhl EH (1998) Differentially interconnected networks of GABAergic interneurones in the visual cortex of the cat. J Neurosci 18:4255–4270.

Tarczy-Hornoch K, Martin KAC, Jack JJB, Stratford KJ (1998) Synaptic interactions between smooth and spiny neurones in layer 4 of cat visual cortex *in vitro*. J Physiol 508:351–363.

Thomson AM (1997) Activity-dependent properties of synaptic transmission at two classes of connections made by neocortical pyramidal axons *in vitro*. J Physiol 502:131–147.

Thomson AM, Bannister AP (1998) Postsynaptic target selection by descending layer III pyramidal axons: dual intracellular recordings and biocytin filling in slices of rat neocortex. Neuroscience 84:669–683.

Thomson AM, Deuchars J (1997) Synaptic interactions in neocortical local circuits: dual intracellular recordings *in vitro*. Cereb Cortex 7:510–522.

Thomson AM, West DC (1993) Fluctuations in pyramid–pyramid EPSPs modified by presynaptic firing pattern and postsynaptic membrane potential using paired intracellular recordings in rat neocortex. Neuroscience 54:329–346.

Thomson AM, Deuchars J, West DC (1993a) Large, deep layer pyramid–pyramid single axon EPSPs in slices of rat motor cortex display paired pulse and frequency-dependent depression, mediated presynaptically and self-facilitation, mediated postsynaptically. J Neurophysiol 70: 2354–2369.

Thomson AM, Deuchars J, West DC (1993b) Single axon excitatory postsynaptic potentials in neocortical interneurons exhibit pronounced paired pulse facilitation. Neuroscience 54:347–360.

Thomson AM, Deuchars J, West DC (1995) Properties of single axon excitatory postsynaptic potentials elicited in spiny interneurons by action potentials in pyramidal neurons in slices of rat neocortex. Neuroscience 3:727–738.

Thomson AM, West DC, Hahn J, Deuchars J (1996) Single axon IPSPs elicited in pyramidal cells by three classes of interneurones in slices of rat neocortex. J Physiol 496:81–102.

Traub RD, Whittington MA, Stanford IM, Jefferys JGR (1996) A mechanism for the generation of long-range synchronous fast oscillations in the cortex. Nature 383:621–624.

Valverde F (1976) Aspects of cortical organization related to the geometry of neurons with intra-cortical axons. J Neurocytol 5:509–529.

Wang, Y, Jones, JFX, Jeggo RD, de Burgh Daly M, Jordan D, Ramage, AG (2000) Effect of pulmonary C-fibre afferent stimulation on cardiac vagal neurones in the nucleus ambiguous in anaesthetized cats. J Physiol, 526, 157–165.

Wang Y, Gupta A, Toledo-Rodriguez M, Wu CZ, Markram H. (2002) Anatomical, physiological, molecular and circuit properties of nest basket cells in the developing somatosensory cortex. Cereb Cortex 12:395–410.

Yoshioka T, Levitt JB, Lund JS (1994) Independence and merger of thalamocortical channels within macaque monkey primary visual cortex: anatomy of interlaminar projections. Vis Neurosci 11:1–23.