Excitatory inputs to spiny cells in layers 4 and 6 of cat striate cortex

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The principal target of lateral geniculate nucleus in the cat visual cortex is the stellate neurons of layer 4. In previously reported work with intracellular recording and extracellular stimulation in slices of visual cortex, three general classes of fast excitatory synaptic potentials (EPSPs) in layer 4a spiny stellate neurons were identified. One of these classes, characterized by large and relatively invariant amplitudes (mean 1.7 mV, average coefficient of variation (CV) 0.083) were attributed to the action of geniculate axons because, unlike the other two classes, they could not be matched by intracortical inputs, using paired recording. We have examined in detail the properties of this synaptic input in twelve examples, selecting for study those EPSPs where there was secure extracellular stimulation of the single fibre input to a pair of stimuli 50 ms apart. In our analysis, we conclude that the depression that these inputs show to the second stimulus is entirely postsynaptic, since the evidence strongly suggests that the probability of transmitter release at the synaptic site(s) remains 1.0 for both stimuli. We argue that the most plausible explanation for this postsynaptic depression is a reduction in the average probability of opening the synaptic channels. Using a simple biochemical analysis (c.f. Sigworth plot), it is then possible to calculate the number of synaptic channels and their probability of opening, for each of the 12 connections. The EPSPs had a mean amplitude of 1.91 mV (± 1.3 mV SD) and a mean CV of 0.067 (± 0.022). The calculated number of channels ranged from 20 to 158 (59.4 ± 48.7) and their probability of opening to the first EPSP had an average of 0.83 (± 0.09), with an average depression of the probability to 0.60 for the second EPSP.

Geniculate afferents also terminate in layer 6. Intracellular recordings were also made in the upper part of this layer and a total of 51 EPSPs were recorded from pyramidal cells of three principal types. Amongst this dataset we sought EPSPs with similar properties to those characterized in layer 4a. Three examples were found, which is a much lower percentage (6%) than the incidence of putative geniculate EPSPs found in layer 4a (42%).

Keywords: cat; EPSP; visual cortex; variance; conductance; spiny stellate

1. INTRODUCTION

The principal targets of lateral geniculate neurons in the cat visual cortex are the stellate neurons of layer 4 and the pyramidal neurons in layer 6. Within layer 4, spiny stellate neurons are by far the most abundant neuronal type. Their symmetrical dendritic tree is virtually entirely confined to layer 4 and therefore synaptic input can only come from axons that are forming boutons in this layer. Most spiny stellate neurons have simple receptive fields (Martin 1984; White 1989), and can be driven monosynaptically by either X or Y afferents of the lateral geniculate. In addition to input from geniculate afferents, spiny stellate neurons also receive excitatory input from other sources including other spiny stellate neurons and layer 6 pyramids, with geniculate and spiny stellate afferents targeting spines and layer 6 boutons dendritic shafts (Anderson *et al.* 1994). In previously reported work with intracellular recording and intracellular stimulation in visual cortex

slices, three general classes of fast EPSPs were identified in layer 4a spiny stellate neurons (Stratford *et al.* 1996). One of these classes, characterized by large and relatively invariant amplitudes (average amplitude of *ca.* 2.0 mV, average CV of *ca.* 8%) was attributed to the action of geniculate axons because, unlike the other two classes, they could not be matched by intracortical inputs using paired recording (Stratford *et al.* 1996; Tarczy-Hornoch *et al.* 1999). The purpose of the main part of this review is to present some of the structural and functional features of spiny stellate cells, including previously unpublished material. The excitatory synaptic inputs to these cells will then be described, with particular emphasis on the transmission characteristics of those connections, which have been identified as putative lateral geniculate EPSPs, and how they compare with the other inputs.

Geniculate afferents also terminate in layer 6 and intracellular recordings have also been made in this layer. Histological reconstruction of the cells revealed three principal types in agreement with Katz (1987). We also found that the firing properties of these cells were correlated with morphology, as has been reported for cortical cells in other visual cortex layers (Chagnac-Amitai *et al.* 1990; Larkman & Mason 1990; Mason & Larkman 1990) or other cortical areas (e.g. Kang & Kayano 1994). Using

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Table 1. Analysis of dendritic branching in spiny stellate neurons.

number of dendritic terminations per cell	38.3 ± 2.2 (n = 6)
number of dendritic trees per cell	6.3 ± 1.0 (n = 6)
number of terminations per dendritic tree	6.1 ± 2.6 (n = 38)
path length to terminations	163.9 ± 27.6 (n = 230)
intermediate segment length	19.9 ± 15.7 (n = 190)
terminal segment length	112.5 ± 33.8 (n = 230)
total dendritic length	4969 ± 257 (n = 6)
percentage of total length as terminal segments	86.9 ± 3.4 (n = 6)

Figure 1. (a) Reconstruction of a spiny stellate neuron made from *camera lucida* drawings, scale bar, 50 μm. (b) Dendrogram for neuron shown in (a), scale bar, 50 μm. (c) Amplitude frequency histogram showing, for six neurons, the range of number of terminations made per parent dendrite emerging from the cell body. (d) Significant correlation between the diameter of parent dendrite emerging from the soma and the number of terminal dendrites to which it gives rise. (e) Sholl plot for the neuron shown in (a). (f) Sholl plots for all of the six spiny stellate neurons reconstructed.

the same techniques as for layer 4, a total of 51 EPSPs were recorded from these three types of pyramidal cell. Unlike the data for layer 4, these did not fall into three clear-cut clusters, but among them we found EPSPs with similar properties to those characterized as of geniculate origin in layer 4.

2. MATERIAL AND METHODS

Recordings were made in visual cortex slices (area 17) taken from 12- to 16-week-old cats (1.0–1.6 kg). Details of slice prep-

aration and maintenance are given in Tarczy-Hornoch *et al.* (1999). Recording electrodes were filled with either 0.5 M (for all layer 6 and some of the spiny stellate neurons) or 2.0 M potassium methyl sulphate, as well as 5 mM potassium chloride and 2% biocytin (Sigma). Standard bridge-balance techniques were used. Resting potentials were measured relative to the potential recorded extracellularly prior to impalement. After an initial recovery period, neurons were usually held at membrane potentials of between –80 and –75 mV. Input resistance was estimated from the voltage response to small (0.1 nA) 100 ms current pulses. The responses to 1 nA, 0.5 ms depolarizing and

Figure 2. Reconstructions of the dendritic and axonal fields for three spiny stellate neurons made from *camera lucida* drawings. The histograms below each reconstructed cell show the actual numbers of *en passant* (black) and *terminaux* (white) boutons identified in each cortical layer. Bar, 200 μm .

hyperpolarizing current pulses, delivered at 1 Hz, were analysed to determine membrane time constant. Suprathreshold properties were investigated by using long (900 ms) depolarizing pulses delivered at 0.25 Hz. Action potential measurements were taken from the first spike evoked by just-threshold currents. After recording, slices were immediately fixed and processed (details given in Tarczy-Hornoch *et al.* (1999)). Neurons were reconstructed from *camera lucida* drawings made at $\times 1500$ magnification using a $\times 100$ oil immersion objective using methods adopted by Larkman (1991a,b).

3. RESULTS

(a) Spiny stellate neurons

(i) Morphology

All cells recorded were located in the upper half of layer 4 (4A) and appeared similar to the 4A cells described by Lund *et al.* (1979), in having approximate dendritic radial symmetry and axon collaterals projecting into layer 2/3. Detailed analysis was made of the dendritic arbour of six spiny stellate neurons, which were considered to be representative examples (table 1). One of these is shown in figure 1a. It has five dendrite stems emerging from the soma, which branch to produce a total of 37 terminal segments (figure 1b). The mean number of terminations per dendrite stem for this cell was *ca.* 7 (7.4 ± 4.1), with the degree of branching varying considerably between the stems (range of 3–14 terminations). The mean number of terminations per stem for all six neurons was 6.1 ± 2.6 ($n = 38$, figure 1c). The number of terminal segments a dendrite stem produced appeared correlated with its initial trunk diameter ($r^2 = 0.78$, $p < 0.01$, figure 1d). Although

the number of stems and terminations per stem varied somewhat, the total number of terminal dendrites per neuron was remarkably consistent (38.3 ± 2.2 , range 36–42).

Dendritic trees can be assessed quantitatively using a method devised by Sholl (1953). Figure 1e shows a Sholl plot for the neuron illustrated. The number of intersections rises rapidly to reach a peak at 90 μm when no further branching occurs. The number of dendrites then remains fairly constant until the number drops as the dendrites terminate, with the steepest region (between 160 and 170 μm) representing the mean path length to terminations (for this cell 164.3 ± 16.5). The Sholl plots for all the six neurons analysed are shown in figure 1f. It is clear from this that these neurons show remarkable homology in dendritic branching. A noticeable feature of the dendritic tree of all the neurons analysed is that most branching occurs relatively close to the soma. About 90% of the total branching in all of the dendritic trees occurs within a path length of *ca.* 60 μm from the soma. Owing to this, intermediate dendritic segments are short relative to the terminal segments (table 1), and as a result terminal segments constitute most of the total dendritic length ($86.9 \pm 3.4\%$). This dendritic branching pattern, where the terminal segments make up most of the total length of the cell is similar to that found in the basal trees of rat neocortical pyramidal cells (Larkman 1991a) and hippocampal CA1 pyramids (Bannister & Larkman 1995). The total combined dendritic length for the six cells ranged from 4770 to 5455 μm with a mean of 4943 ± 258 μm (table 1). Most spiny stellate dendritic segments bear conspicuous dendritic spines. Spines are absent, however, on the soma and some of the more proximal dendritic seg-

Table 2. Laminar distribution, by type, of boutons in three spiny stellate neurons.

	number of putative boutons	<i>en passant</i>	terminaux	boutons <i>en passant</i> (%)
layer 2/3	1188 ± 437 (46.8 ± 4.0%)	1030 ± 388	159 ± 52	86.3 ± 2.1
layer 4	1027 ± 395 (40.7 ± 5.1%)	869 ± 344	159 ± 51	84.3 ± 1.9
layer 5	220 ± 111 (9.1 ± 4.8%)	193 ± 103	21 ± 17	87.0 ± 2.7
layer 6	70 ± 62 (3.4 ± 3.8%)	59 ± 56	9 ± 8	87.0 ± 0.3

Table 3. Electrophysiological properties of spiny stellate neurons recorded with electrodes containing either 0.5 or 2.0 M potassium methyl sulphate.

	0.5 M	2.0 M
number of cells	18	11
holding potential (mV)	78.6 ± 2.0	76.4 ± 1.6
subthreshold membrane properties		
input resistance (mΩ)		
depolarizing pulses	103.1 ± 14.2	54.7 ± 14.7
hyperpolarizing pulses	101.0 ± 14.1	54.0 ± 14.5
membrane time constant (τ ₀)		
depolarizing pulses	22.1 ± 4.4	12.8 ± 2.3
hyperpolarizing pulses	21.7 ± 4.4	12.5 ± 2.1
current to bring cell to firing threshold (nA)	0.33 ± 0.10	0.79 ± 0.21
action potential characteristics		
amplitude of action potential (mV) ^a	72.1 ± 4.3	67.5 ± 5.8
rate of rise of action potential (V s ⁻¹)	346 ± 53.1	289 ± 53.5
rate of fall of action potential (V s ⁻¹)	85.9 ± 20.1	94.3 ± 17.2
half width of action potential (ms)	0.6 ± 0.1	0.6 ± 0.04
amplitude of AHP (mV) ^a	-19.3 ± 3.6	-18.9 ± 2.8
time to peak of AHP (ms) ^b	5.8 ± 2.4	2.1 ± 1.6
current frequency characteristics^c		
first interspike interval	140.1 ± 39.7 (0.99 ± 0.01)	108.3 ± 33.9 (0.97 ± 0.04)
second interspike interval	95.4 ± 31.1 (0.99 ± 0.01)	63.7 ± 13.7 (0.98 ± 0.03)
third interspike interval	84.5 ± 29.4 (0.98 ± 0.02)	52 ± 17 (0.96 ± 0.10)
steady state	± 12.2 (0.95 ± 0.04)	17.8 ± 7.5 (0.94 ± 0.06)

^a Relative to spike threshold.
^b From the time when spike downstroke crosses spike threshold.
^c Slopes given as spikes s⁻¹ nA⁻¹; figure in parentheses is the *r*² value for the regression.

ments up to a path length of *ca.* 30 μm (28 ± 6 μm, *n* = 74) from the soma. This has also been observed in adult cat spiny stellate neurons (Anderson *et al.* 1994). Given that *ca.* 90% of the total dendritic branching occurs within a path length of *ca.* 60 μm from the soma, most dendritic spines are located on terminal segments.

The axon arbours from three typical spiny stellate neurons are shown in figure 2. The axon in all cases emerges from the bottom (white matter side) of the soma. The main axon descends radially through layers 5 and 6 and in most cases can be traced into the white matter. Within layer 4, quite proximal to the soma, the axon trunk gives rise to a number of collaterals. Most of these ascend through layer 4 and into layer 2/3, where they branch extensively. Because of the nature of the orientation and branching of these ascending collaterals most of the local axon arbour of these neurons in layer 4 and 3 is restricted to a vertically orientated column, which had a diameter of about the same as the dendritic field of the parent neuron. Some of the axon collaterals, which emerge from the main descending axon, run horizontally and remain within layer 4 or descend obliquely into layer 5. Within layer 5 the

main axon trunk usually gives rise to several axon collaterals, which run horizontally within layer 5.

From the illustrations in figure 2 it can be seen that a large proportion of the local axon is contained within layers 2/3 and 4. We were able to identify two types of varicosity (putative bouton) on the axon collaterals; those that appeared as swellings (*en passant*) and those that were present on the end of spine-like appendages (*terminaux*). For the three neurons illustrated we identified and counted the numbers of both *en passant* and *terminaux* varicosities in different cortical layers (table 2; figure 2). Although the number of boutons varied considerably between different cortical layers the ratio of *en passant* to *terminaux* was remarkably similar (almost 6 : 1; table 2).

(ii) *Electrophysiological properties*

Recordings were made from a total of 29 spiny stellate neurons make a detailed assessment of their sub- and suprathereshold electrophysiological properties. Eighteen neurons were recorded using microelectrodes containing 0.5 M methyl sulphate and 11 with 2.0 M. It was found that the composition of filling solution had a significant

Figure 3. Firing properties of spiny stellate neurons. Examples of the two types of response observed. Most neurons exhibited firing characteristics typical of that shown in (a), in that they fired a continuous train of spikes with just suprathreshold depolarizing currents showing very little frequency adaptation. A smaller number of cells responded typically like the example shown in (b). These cells fired discrete volleys of spikes in response to small suprathreshold depolarizing current. With larger currents, however, they appeared to switch to a tonic mode of firing.

effect on the electrophysiological responses observed (table 3) and are therefore shown separately. Neurons recorded in 2.0 M filling solution had a significantly smaller input resistance and shorter membrane time constants than those recorded with 0.5 M electrodes. The values of R_N or τ_0 calculated from the depolarizing pulses were not significantly different from those derived from the hyperpolarizing pulses.

The action potentials elicited by long, just suprathreshold depolarizing current pulses, were asymmetric and were followed in all cases by an AHP, with no evidence of a DAP. In response to further modest depolarization, most neurons (20 out of 29) were able to fire a continuous train of spikes (figure 3a). The train of spikes elicited using small suprathreshold currents generally showed little frequency adaptation. However, when larger currents were used, frequency adaptation became more evident. The IFF-current slope for the first, second and third spike intervals, and steady state, which were all monophasic, were calculated for these neurons (table 3). The other nine neurons showed a somewhat different firing response (figure 3b). With just suprathreshold currents, instead of firing a more or less continuous train of spikes, these neurons tended to fire discrete volleys of spikes. The height of the first spike in each volley was usually lower than the rest. A prominent feature of the interval baseline was the presence of rhythmic oscillations. The frequency and the amplitude of these oscillations appeared to increase with an increase in the current. Only when much larger depolarizing currents were used were these neurons able to fire a continuous train of spikes, although the amplitude of spikes following the initial spike was somewhat attenuated.

(iii) Modelling

The neuron shown in figure 1a was selected for further, more detailed, morphological analysis to derive a passive electrotonic model. For each dendritic segment, in addition to the length, the diameter and spine density were measured and entered into a computer file. Using an in-house computer program the spines were then 'collapsed' into their dendritic shafts (Stratford *et al.* 1989). This transformation increases the length of each dendritic segment by a factor of $F^{2/3}$ and the diameter by a factor of $F^{1/3}$ (where F is the ratio, for that segment, of the surface area with spines to the surface area excluding spines). A single spine area of $1.7 \mu\text{m}^2$ was used for all spines (Larkman *et al.* 1992).

A more useful representation of dendritic length is morphotonic length (Larkman *et al.* 1992), or equivalent electrical length, which takes account of the differences in the diameter between different segments. The morphotonic transformation for this cell is shown in figure 4. Morphotonic length can be converted into electronic length by the division of a morphoelectric factor (Larkman *et al.* 1992; $= [R_m/R_i]^{1/2}$). We attempted to make estimates of R_m and R_i as follows. We constructed a detailed branching passive cable model of the cell by assuming uniform values for specific membrane capacitance (C_m) and resistance (R_m), and cytoplasmic or dendritic axial resistance (R_i). As the original recordings were performed with sharp electrodes, we allowed a shunt conductance (G_{sh}) at the soma. We used the recordings we had obtained from the real cell's voltage response to injected short current pulses (1 nA, 0.5 ms), to obtain optimum values for these electrical parameters by direct fitting (figure 4). The optimal fits obtained for both depolarizing and hyperpolarizing pulses are shown in figure 4. With brief depolarizing and hyperpolarizing pulses, the electrical parameters were similar, with mean values of $C_m = 0.95 \mu\text{F cm}^{-1}$; $R_m = 36\,000 \Omega \text{ cm}$, $R_i = 185 \Omega \text{ cm}$ and $R_{sh} = 316 \text{ M}\Omega$. The important implication arising from this passive modelling of the cell is that the dendritic length is electrotonically very short. Nine of the thirty-seven terminal dendrites had an electrotonic length to the soma of between 0.5 and 0.6λ , twenty-four between 0.4 and 0.5λ and four between 0.3 and 0.4λ . Expressing electrotonic attenuation in terms of loss of charge in propagating from the synaptic site to the soma, this result means that the maximum loss will be of the order of 15% (i.e. 85% of the charge reaches the soma) and more commonly the loss will be between 5 and 10%.

(iv) EPSPs

Table 4 summarizes the results presented in Stratford *et al.* (1996), showing the principal characteristics of the three classes of excitatory inputs, identified by cluster analysis of the EPSPs, resulting from minimal extracellular stimulation. Figure 5 gives an example of a class 1 EPSP, with figure 5a showing the criterion used for assuming that the stimulation recruits a single presynaptic axon. In this example, it was possible to increase the extracellular stimulation voltage well above the level at which there were no failures (*ca.* 4.3 V) without recruiting any further axons presynaptic to the cell. This was not true of the whole sample of class I EPSPs reported by Stratford *et al.* (1996) and so, as noted in that paper, a level of extracellular stimulation voltage had to be adopted for some

Figure 4. Obtaining an electrical model of a spiny stellate neuron. Using detailed morphological parameters for the neuron shown in figure 1*a* we simulated voltage recording subsequent to both a (*a*) hyperpolarizing and (*b*) depolarizing 1 nA current pulse of 0.5 ms duration. Optimum values for C_m , R_m , R_i and R_{sh} were then obtained by minimizing the mean square deviation away from the actual decay waveforms obtained from experimental recording, using a simplex routine. (*a*) $C_m = 0.95 \mu F cm^{-2}$; $R_m = 34\,000$; $R_i = 182 \Omega cm$; $R_{sh} = 340 M\Omega$. (*b*) $C_m = 0.95 \mu F cm^{-2}$; $R_m = 38\,000$; $R_i = 189 \Omega cm$; $R_{sh} = 291.55 M\Omega$. The fits were performed between 2 and 50 ms (after the start of the current pulse). The dots represent the recorded decay waveforms and the solid lines are the optimal fits with values for C_m , R_m , R_i and R_{sh} indicated. (*c*) The morphotonic and electrotonic representations of the neuron.

Table 4. Excitatory inputs to spiny stellate neurons.

	extracellular stimulation	paired recordings
<i>class 1</i>		
large amplitude	1.97 ± 1.4 mV	—
very small CV	0.083 ± 0.039	—
paired-pulse depression		
	$n = 20$ (42% of sample)	
<i>class 2</i>		
medium amplitude	1.04 ± 0.57 mV	1.07 ± 0.69 mV
small CV	0.122 ± 0.076	0.22 ± 0.10
no change on paired pulses		
	$n = 18$ (38% of sample)	$n = 14$
<i>class 3</i>		
small amplitude	0.33 ± 0.22 mV	0.23 ± 0.15 mV
large CV	0.47 ± 0.14	0.69 ± 0.28
paired-pulse facilitation		
	$n = 10$ (20% of sample)	$n = 7$

examples in which there was stimulation failure on either the first, or the second, of paired stimuli. To examine in more quantitative detail the behaviour of the EPSPs to paired stimulation we selected only those EPSPs, such as the one shown in figure 5, in which we could be confident that there was no stimulation failure to either the first or second stimuli. With some additional experimental data, there were 12 EPSPs, which showed reliable responses to paired stimulation. Figure 6*a–c* illustrates the experimental results for the EPSP shown in figure 5.

In figure 6*a* the responses to the first stimulus are shown in red and to the second in black, with a 50 ms interval between the two stimuli. Note that for stimulus voltages

(for both stimuli) between 4.0 and 4.4 V there could be failures to either the first or the second stimulus (or both). Above 4.4 V there was a reliable response to both stimuli. The data used for analysis were collected at a stimulus strength of *ca.* 4.75 V, as indicated by the arrow labelled 4. Figure 6*b,c* illustrates the mean time courses and the amplitude histograms thus collected. The second of the paired EPSPs is, on average, considerably smaller than the first EPSP, showing a depression to 61%. A depression of this magnitude was relatively unusual among the dataset of 12 EPSPs, with an average paired-pulse depression at a 50 ms interval of 72.2%. Figure 6*d* illustrates, for another EPSP, a more typical paired-pulse depression and figure

Figure 5. Putative single fibre thalamic input to a spiny stellate neuron. (a) The response to increasing the stimulation voltage indicates that we are recording a single fibre input, which can be reliably stimulated with voltages above *ca.* 4.4 V; top, EPSP; bottom, failures. (b) Scatter plot showing the distribution of peak EPSP amplitudes (filled circles) and baseline noise (open circles) for 1100 consecutive trials recorded using a stimulation voltage of 4.8 V. (c) Overlay of the first three EPSPs recorded for this input illustrating how little trial-to-trial variance there is. (d) Amplitude frequency histogram showing the distribution of amplitudes for the EPSP (left to right) and noise (right to left) for the series of trials shown in (b). The distribution of EPSP amplitudes is normal with only little additional variance over that contributed by the noise ($\text{CV} = 7.1\%$).

6e–g shows the depression at two briefer intervals. At an interval of 10 ms, the depression is considerable, but temporal summation of the successive EPSPs leads to a larger peak depolarization associated with the second EPSP, in contrast to longer intervals.

The mechanism of paired-pulse depression can be either presynaptic, postsynaptic or both (see Zucker & Regehr 2002). Presynaptic depression is evidenced by a reduction in the release probability of transmitter release. If, as seems probable for the geniculate input, the initial release involves, on average, several quanta then depression will involve fewer quanta released by the second pulse. This should, therefore, be evident in the form of the amplitude histograms. The examples shown in figure 6c–f by contrast, are notable in two ways. The amplitude histogram of both the first and second pulse are Gaussian showing no skewness, and with a very low CV, after allowing for noise. There are two alternative explanations for this result. Zero skew can arise in an amplitude histogram either if the probability of release has a value of 0.5 or 1.0. The obvious possibility is that, however many quanta are released, the release probability is 1.0 on both trials, i.e. the same number of quanta are released on every trial. The residual CV would then be attributable to quantal variance. The less likely alternative is that the lack of skewness in the amplitude histograms is because the probability of release has a value of 0.5 and remains at that value for the second pulse. For this latter account to be correct, the low CV has to be explained by having a very large number of release sites (since the CV for simple binomial release is inversely proportional to the square root of m (the average number of quanta released)). Even

if the quantal variance was zero (i.e. receptors saturated by a transmitter, with a channel-open probability of 1.0), the average number of quanta released for a CV of 0.08 (see table 4) is 78, implying 156 release sites each with a release probability of 0.5. As there is no anatomical evidence that would lend plausibility to this suggestion, we favour the explanation that the release probability (at an unknown number of release sites) is 1.0 and the CV is explained by quantal variance. It should be noted that, on either general explanation, the evidence is against a change in release probability (because of the lack of significant change in skewness of the pair of histograms), thus pointing to a purely postsynaptic explanation for the paired-pulse depression. This depression is attributable to receptor desensitization (Hestrin 1992).

Jack *et al.* (1994) have reviewed the factors that may contribute to quantal variance. For more than one release site two types of quantal variance may contribute to an amplitude histogram. Their type II variance is attributable to variations in the average amplitude of the quantal effect at different release sites (i.e. intersite variance). This type of variance is not expressed in an amplitude histogram if all sites release on every trial (i.e. release probability of 1.0), so that, on the above interpretation, it can be ignored. Type I variance refers to the variability from trial to trial at each release site. A small CV value implies that the postsynaptic receptors are fully occupied since the quantal variance associated with variations in the transmitter content of individual vesicles has a CV of *ca.* 0.3 (e.g. Bekkers *et al.* 1990). Fully occupied receptors can still contribute to quantal variance, as documented for AMPA receptors (e.g. Jonas *et al.* 1993, see their fig. 13),

Figure 6. (a)–(c) EPSPs evoked with a 50 ms paired-pulse protocol (for the same input as that shown in figure 5). (a) Scatter plot showing the amplitude of the first (red) and second EPSP (black) in response to increasing stimulation voltage. Above *ca.* 4.4 V, EPSPs can be reliably evoked with both the first and second stimulation pulse. (b) Average voltage response obtained from 1100 trials. (c) Amplitude frequency histogram showing the distribution of amplitudes of the first (red; mean = 858 μm) and second EPSPs (grey; mean = 1396 μm) for all 1100 recorded trials. (d)–(g) Effect of shortening the time interval between paired pulses for another putative LGN input. Paired-pulse depression becomes larger with shorter time intervals between pulses. However, at very short intervals (10 ms) the two EPSPs temporally summate to produce a larger voltage depolarization. (d) 50 ms (77.8%), 300 trials; (e) 25 ms (71.4%), 400 trials; (f) 10 ms (55.8%), 200 trials.

because of the stochastic variation in the number of open channels due to channel gating.

Adopting the hypothesis that the CVs of the paired histograms are attributable to this sole cause, it is possible to make deductions from the data about the mechanism underlying the postsynaptic depression. Earlier studies by Jonas *et al.* (1993) and Raman & Trussell (1995) had shown, using glutamate application to membrane patches containing AMPA receptors, that the CV to a single pulse was well described by making the assumption of a simple binomial description of channel behaviour, that is, the individual AMPA channels are all independent and similar in behaviour, with the time course and peak open probability being identical (cf. Sigworth 1980). The following equation is then obeyed:

$$CV = \left[\frac{1 - P_o}{nP_o} \right]^{1/2}, \tag{3.1}$$

where P_o is the open probability and n is the number of

AMPA channels. Raman & Trussell (1995) also showed that their experimental data with paired-pulse depression could be modelled by a kinetic scheme in which the receptor desensitization was emulated by a change (reduction) in the maximum open probability of opening of the channels (see their fig. 7*B*). It is therefore possible to solve for the open probabilities for the first and second of the paired EPSPs using the following equations:

$$\frac{V_2}{V_1} = \frac{P_{o2}}{P_{o1}}, \tag{3.2}$$

$$\left[\frac{CV_2}{CV_1} \right]^2 = \frac{(1 - P_{o2})P_{o1}}{(1 - P_{o1})P_{o2}}, \tag{3.3}$$

where V refers to the peak response of the EPSP and the subscripts 1 and 2 indicate the first and second EPSP of the pair. Once the open probabilities are determined it is

Table 5. Detailed analysis of putative thalamic EPSPs. (P_{o1} denotes the channel-open probability to the first EPSP, n the deduced number of channels, Q the total charge associated with the EPSP and q the estimated charge per channel.)

EPSP	no. of trials	V_1 (mV)	CV_1	V_2 (mV)	CV_2	P_{o1}	n	Q (pC)	q (fC)
1	200	1.317	0.0552	1.024	0.1268	0.93	25	0.392	16.9
2	100	3.722	0.0375	3.018	0.0655	0.89	91	1.140	14.1
3	100	5.218	0.0538	2.923	0.1007	0.69	158	0.784	7.2
4	200	0.981	0.0705	0.546	0.1731	0.84	38	0.224	7.0
5	1140	1.396	0.0710	0.858	0.2028	0.91	20	0.215	11.8
6	350	1.562	0.0960	1.325	0.1215	0.70	46	0.479	14.9
7	100	2.102	0.0913	1.610	0.1317	0.72	47	0.491	14.5
8	100	1.544	0.0870	1.310	0.1290	0.85	23	0.284	14.5
9	59	0.748	0.0963	0.581	0.1446	0.77	32	0.212	8.6
10	55	1.417	0.0620	1.143	0.1135	0.90	29	—	—
11	100	2.145	0.0392	1.649	0.0628	0.81	153	—	—
12	400	0.781	0.0439	0.578	0.0974	0.91	51	—	—
	mean	1.911	0.0669	1.380	0.1225	0.83	59.4	0.47	12.2
	s.d.	1.311	0.0218	0.833	0.040	0.09	48.7	0.31	3.7

then possible to calculate the number of AMPA receptors from equation (3.1). The results of these calculations are set out in table 5. The open probability of the channels for each EPSP shows a range from 0.70 to 0.93, with a mean value of 0.83. There is a wider range for the calculated total number of channels associated with a particular EPSP, from 20 to 158. This is not surprising given the equally wide range in their peak amplitude—from 0.748 to 5.218 mV. For nine of the 12 EPSPs it was possible to calculate the total charge associated with the EPSP, taking the time integral of the EPSP and the individual cell's input resistance. These values are listed in table 5 and allow, in turn, the calculation of the charge entering, on average, through each AMPA channel for the individual EPSPs. This value ranges from 7.0 to 16.9 fC, with an average value of 12.2 fC.

The values obtained for the AMPA channels may be compared with earlier results, obtained by direct methods, unlike the present calculations, which rely on a variety of assumptions. Values for the open probability of AMPA channels that have been reported range from 0.5 to 0.95 (Hestrin 1992; Jonas *et al.* 1993; Raman & Trussell 1995; Silver *et al.* 1996), so that the present calculations are in the appropriate range. The value for the charge flowing through a single channel is relatively high compared with those that can be derived from other data in the literature. A common value for the single-channel conductance is 10 pS (e.g. Hestrin 1992), but higher values, up to 25–30 pS have also been reported (Hestrin 1993; fig. 6a in Raman & Trussell 1995). Assuming an electrochemical driving force of 70 mV, an average open time of the channels can be calculated for both 10 and 30 pS conductances, so as to pass a charge of *ca.* 12 fC. The results are 17 and 5.7 ms, respectively. The former result is outside the range described for AMPA receptors (at room temperature or above) but 5.7 ms is within the appropriate range; for example, Yoshimura *et al.* (1999) found that the mean decay time constant for AMPA currents at 27 °C were 5.1 ms for rat visual cortex and 7.6 ms for kitten visual cortex (the decay time constant for a current is roughly the same as the mean open time for the channels).

Thus, the present analysis indicates that the putative

geniculate input to spiny stellate cells is mediated by a particular AMPA receptor with a relatively high conductance, consequently allowing relatively large charge entry while open. It is worth considering how secure this suggestion is, given the indirect method by which the estimate was made. There are two obvious criticisms of our data. The first is that we made our deductions from the CV of the peak voltage (of an EPSP), rather than from the CV of the peak current. Although the transformation from fluctuations in peak current to fluctuations in peak voltage is complex, modelling studies indicate that the CV is similar for the two measures (J. A. C. Read, unpublished data). A second qualification is that some of the variability in the peak voltage may have been attributable to other sources—for example, that not all AMPA receptors were fully occupied by the transmitter, with variations in the amount of transmitter released, due to differences in the vesicular content of the transmitter. As we have no information about the number of release sites, the number of AMPA receptors per release site and their aggregation density, this must remain a possibility. Additional contributors to the CV, such as this, will mean that the CV attributable to stochastic opening of the receptors will be less. As can be seen from equation (3.1), this will have the effect of lowering the estimates below their true value for both P_o and n . An underestimate of n would, in turn, lead to an overestimate of the amount of the charge entering per channel.

Among the class 2 EPSPs there was a sub-set of eight EPSPs that was comparable to the class 1 EPSPs in that no significant skewness in the amplitude histogram could be detected for either the first or second pulse (50 ms separation). Unlike the class 1 EPSPs, which showed a paired-pulse depression with a mean value of 72.2%, the mean change was to 97.7%. We interpret this subset as being comparable in presynaptic release characteristics to the class 1, i.e. on both first and second pulse the release probability was 1.0. Unfortunately, unlike the class 1 EPSPs, the absence of any significant postsynaptic depression means that an analysis such as that performed above cannot be made. It is not, therefore, possible to compare the properties of the AMPA channels for open

probability and channel charge, but it is possible to deduce that these AMPA channels differ in one respect: that there is no significant desensitization to paired pulses of 50 ms interval. This does not exclude the possibility of a briefer period of desensitization and it is well known that AMPA receptors of different types can vary considerably in the extent and the speed of recovery from desensitization (Colquhoun *et al.* 1992; Raman & Trussell 1995).

Other class 2 EPSPs did show significant paired-pulse depression at a 50 ms interval, but in those cases with adequate data the amplitude histogram showed one of two features: either it changed from zero skewness to a negatively skewed distribution ($n = 2$, mean depression 85.7%) compatible with the interpretation that the release probability had gone from 1.0 to 0.86, or the amplitude histograms went from negative to less negatively skewed histograms ($n = 3$, mean depression 81.8%) which is interpretable as a change from a high to a lower release probability (but still well above a release probability of 0.5). Thus, unlike the class 1 EPSPs, the class 2 EPSPs showed less depression and, when they did, it was explicable as a reduction in release probability. Further evidence in favour of this interpretation is given in Tarczy-Hornoch *et al.* (1999), using paired recordings.

Class 3 EPSPs show paired-pulse facilitation at all the tested intervals (see Stratford *et al.* 1996; Tarczy-Hornoch *et al.* 1999) and the evidence indicates that this is at least predominantly a presynaptic change, due to an increase in the probability of transmitter release in the second (and subsequent) pulses (Tarczy-Hornoch *et al.* 1999).

(b) Layer 6 pyramidal neurons

Sharp intracellular microelectrode recordings were made from a total of 28 neurons from layer 6 of cat area 17. In several cases biocytin was included in the recording electrode, enabling us to successfully reconstruct the entire dendritic tree and associated axon collaterals for nine neurons.

(i) Morphology

Qualitatively the identified pyramidal neurons appeared to fall into one of three morphological types described in the following paragraphs (figure 7). The three types appear similar to those described previously by Katz (1987).

Out of the nine neurons reconstructed, four resembled the claustrum-projecting neurons described by Katz (1987), characterized by a long tapering apical trunk, which in all cases reached the top of layer 2 (figure 7*a*). The apical trunk often gave rise to several apical oblique dendrites proximal to the soma, which largely remained within layers 5 and 6. Most neurons (three out of four) had somata located in the lower part of layer 6, close to the white matter border. The basal dendritic field of these cells was quite unusual in that in most cells there were several particularly long basal dendrites that often entered the white matter. Intra-cortical axon collaterals were restricted to layers 5 and 6 (figure 7*a*). The descending axon generally gave rise to several horizontal branches that ran within layer 6, parallel to the white matter. These branches gave rise to collaterals that branched within layers 5 and 6. The horizontal and more particularly the ascending axons were of small diameter, making tracing

Figure 7. *Camera lucida* drawings illustrating the three morphological types of layer 6 pyramidal neuron identified. From Katz (1987) it is probable that those shown in (a) project to the claustrum whereas those in (b) and (c) make projections to the LGN. Bar, 100 μ m.

of the more distal collaterals difficult. Both the horizontal and finer ascending axons bore *en passant boutons* and spine-like appendages terminating in boutons (*boutons terminaux*). For two cells we counted the number of both *en passant* and *terminaux* boutons present in both layers 5 and 6. Most boutons were located in layer 6 (mean 77% of total boutons). The ratio of *en passant* to *terminaux* boutons for layers 5 and 6 was very similar for both cells (88% : 12% and 82% : 18% for layer 6; 90% : 10% and 72% : 18% for layer 5).

Three of the five remaining neurons showed a similarity to most of the LGN-projecting neurons described by Katz (1987), which he termed type I LGN projecting neurons. The axo-dendritic morphology of these cells (figure 7*b*) was quite different to that of the putative claustral projecting neurons described above. The main apical dendrite that often reached, but never extended beyond the layer 3/4 border, gave rise to apical oblique dendrites along most of its length, with some of the apical oblique dendrites extending into layer 4. All of the neurons had somata located in a central portion of layer 6. A distinctive feature of the axon arbour (figure 7*b*) was the presence of conspicuous, large diameter axon collaterals that emerged from the descending axon and ascended to layer 4 where

Figure 8. Suprathreshold properties of layer 6 neurons. Representative examples of the three firing types identified, showing responses to just threshold and increasing suprathreshold depolarization. (a) Type 1 threshold, (b) type 1 0.1 nA above threshold, (c) type 1 0.5 nA above threshold, (d) type 2 threshold, (e) type 2 0.1 nA above threshold, (f) type 2 0.5 nA above threshold; (g) type 3 threshold, (h) type 3 0.1 nA above threshold, (i) type 3 0.5 nA above threshold.

Table 6. Action potential characteristics for the three firing types of layer 6 neuron identified.

	type 1 ($n = 11$)	type 2 ($n = 13$)	type 3 ($n = 4$)
peak amplitude of action potential (mV) ^a	79.1 ± 3.3	74.1 ± 5.9	68.0 ± 5.3
slope of rising phase of spike ($V s^{-1}$)	330 ± 76	262 ± 70	205 ± 26
slope of falling phase of spike ($V s^{-1}$)	65 ± 9	71 ± 13	95 ± 9
ratio of rise to fall of spike	5.1 ± 0.9	3.8 ± 1.0	2.2 ± 0.5
amplitude of spike undershoot or fAHP (mV) ^a	-8.6 ± 2.7	-12.6 ± 2.7	-15.7 ± 1.0
time to peak of spike undershoot (ms) ^b	3.1 ± 0.5	3.2 ± 0.6	2.3 ± 0.4
amplitude of sAHP (mV) ^a	-15.7 ± 3.6	-15.8 ± 1.9	—
time to peak of sAHP (ms) ^b	78.2 ± 14.5	41.4 ± 13.3	—
amplitude of DAP (mV) ^a	-5.7 ± 3.3	—	-13.0 ± 0.9
time to peak of DAP (ms) ^b	12.5 ± 3.7	—	5.9 ± 1.5

^a Relative to the spike threshold.

^b From the time when the spike upstroke crosses the threshold.

they branched profusely with some of the finer branches entering layer 3. The ascending axons were considerably thicker than those of claustral projecting neurons. Some of the ascending axons, rather than climbing vertically, rose obliquely before branching in layer 4, some horizontal distance away (up to 0.5 mm) from the neuron's dendritic field. The ascending collaterals bore several *en passant* boutons and *boutons terminaux*. Qualitatively, the density of boutons, particularly *boutons terminaux*, appeared to be higher in layer 4 compared with layers 5 and 6. This was confirmed quantitatively in bouton counts made in three neurons, where boutons in layer 4 represented 70% of the total number of boutons (layer 5, 11% and layer 6, 19%). The ratio of *en passant*: *terminaux* boutons in layer 4 was also significantly lower to that found in layers 5 and 6 (mean for layer 4, $51 \pm 3\%$ *terminaux*, mean for layers 5 and 6 pooled, $34 \pm 9\%$). These data may be relevant to the recent suggestion of Anderson & Martin (2001) that *boutons terminaux* may show more presynaptic facilitation than *en passant* boutons.

The other two reconstructed layer 6 pyramidal neurons had apical dendritic systems that were somewhat different to those of the LGN-projecting neurons described above (figure 7c). The main apical dendrite branched extensively just proximal to the soma, forming a terminal arbor, with no clear central apical dendrite visible (as in the other two types described above). Most of the apical dendritic tree was confined to layers 5 and 6. In only one of the neurons reconstructed was the axon reasonably well filled (figure 7c). These cells could be analogous to the type II LGN projecting neurons that were described by Katz (1987).

(ii) Electrophysiology

From the inspection of the suprathreshold responses we were able to divide neurons into three firing types based on the qualitative differences observed for the shape of the after-potentials, which followed the action potentials elicited by just threshold currents (figure 8). The measurements of the after-potential characteristics and the spike shape for the three groups are given in table 6.

The action potentials of firing type 1 neurons (figure 8*a*) were followed by a distinctive DAP. As the initial repolarizing undershoot was reasonably small (-8.6 mV) and significantly smaller than for the other two types, the DAP was almost large enough to bring the membrane potential to the spike threshold. Following the DAP was a sAHP, peaking some 80 ms after the spike-firing threshold. The initial repolarizing undershoot following action potentials of firing type 2 neurons (figure 8*b*) was significantly deeper than type 1 neurons (table 6) and was followed by a sAHP which was identical in amplitude to that observed in type 1 neurons but peaking significantly earlier (41 versus 78 ms). The action potentials in firing type 3 neurons were followed by a small brief DAP (peaking significantly sooner than the DAP of type 1 neurons), with no evidence of a sAHP. The DAP appeared to be due to an initial rebound of the membrane potential following the initial spike repolarization, which was significantly deeper (-15.7 mV) than in both firing type 1 and 2 neurons. As well as, or as a result of, differences in after-potentials observed there were also differences in the spike shape between the firing types (see table 6).

A characteristic feature of firing type 1 neurons (not observed in the other two firing types) was the ability to fire a doublet of spikes in response to just suprathreshold current (figure 8*a*). This pattern of firing appeared to be as a result of the prominent DAP following the relatively small repolarizing AHP. As a result, only a relatively small amount of additional depolarization was necessary to generate a second spike at the peak of the DAP. As the level of depolarization was increased, neurons responded by increasing the number of spikes, with strong frequency adaptation evident over at least half of the 900 ms current pulse. In response to just suprathreshold depolarizing current pulses firing type 2 neurons responded by firing a series of widely spread spikes, in contrast to type 1 neurons (figure 8*b*). In some of the neurons the first interspike interval with small suprathreshold currents was often larger than the steady-state intervals. With larger currents frequency adaptation was only evident over the first few spikes. Firing type 3 neurons, in common with type 2, were able to fire tonically with very little frequency adaptation evident (figure 8*c*).

Although only nine of the twenty-seven neurons recorded from were characterized morphologically, an interesting observation was that each of the three morphological types appeared to fall into one of the three firing types described. All four of the putative claustral-projecting neurons displayed type 1 firing properties, the three putative type I LGN-projecting neurons displayed type 2 firing properties, while the two putative type II LGN-projecting neurons displayed type 3 firing properties. Relationships between morphology and electrophysiological properties have been reported previously for cortical cells in other layers of visual cortex (Chagnac-Amitai *et al.* 1990; Larkman & Mason 1990; Mason & Larkman 1990) and other cortical areas (e.g. Kang & Kayano 1994). The projections of layer 6 neurons to the LGN and visual claustrum are both thought to play an indirect role in the shaping of the visual field properties of layer 4 and more superficial layers. This study indicates that claustral projecting cells may have a characteristically different firing behaviour (phasic) than those projecting to

the LGN (tonic), indicating a possible relationship between firing pattern and location within the cortical circuitry.

(c) EPSPs

Fifty-one EPSPs, arising from the extracellular stimulation of putative single axons were collected from the various layer 6 pyramidal cells. There was no obvious difference in the features of the EPSPs recorded in the three types of cell, so the data are treated as a single set. There was a wide range of peak amplitudes (0.174–5.89 mV) with a modal amplitude of 1.1 mV. The CV of the peak amplitude also showed a wide range (0.049–0.643) with a modal value of 0.155. Thirty-one of the EPSPs gave reliable paired-pulse data (at 50 ms interval). Subjecting these 31 EPSPs to the same cluster analysis as had been applied to the inputs to spiny stellate cells did not yield any clear separation into two or more classes. The great bulk of 31 EPSPs showed paired-pulse depression to less than 90%. These 25 EPSPs had a mean amplitude of 1.69 mV with a mean CV of 0.169. The four EPSPs with no significant change on the second pulse (between 90 and 110%) had a mean amplitude of 1.66 mV and CV of 0.20. The two EPSPs, which showed paired-pulse facilitation ($>110\%$), had a mean amplitude of 0.81 mV and CV of 0.23. Thus, if there are distinct classes of excitatory inputs, the sample is not large enough for this to be revealed by cluster analysis.

As an alternative, we looked through the data from the 31 EPSPs, seeking EPSPs that showed the same characteristics as the class 1 inputs to spiny stellate cells, including the feature of zero skewness in both of the amplitude histograms and significant paired-pulse depression ($>15\%$ reduction). We found three examples, one of which is illustrated in figure 9. When we performed the same analysis as that documented in table 5, the results for the three EPSPs were quite similar with channel-open probabilities for the first pulse of 0.71, 0.72 and 0.82. The number of channels, and the associated peak amplitudes were 174 to 3.56 mV, 41 and 2.10 mV, and 20 and 0.93 mV. The paired-pulse depression of these EPSPs were 73, 71 and 78%, respectively. Thus, these three EPSPs are strongly reminiscent of class 1 spiny stellate EPSPs and, by analogy, might be assumed to be geniculate inputs. Although the sample is small, they represent less than 10% of the total EPSP sample (with reliable paired-pulse data) and this may be compared with the apparently greater frequency (42%) of presumed geniculate inputs on to spiny stellate cells. The lower frequency, of 10%, may, however, be compared with the anatomical data that both types of geniculate axon (X and Y) have, on average 10 and 14% of their boutons in layer 6 with the great bulk of the remaining boutons in layer 4 (Humphrey *et al.* 1985). It would therefore be expected that geniculate innervation of layer 6 cells was sparser than for the cells of layer 4.

4. CONCLUSIONS

In this review we have provided information about the combined morphological and electrophysiological properties of the two main classes of spiny cell, which receive a lateral geniculate input. In addition, we have used circum-

Figure 9. Putative geniculate input to a layer 6 pyramidal neuron. (a) Voltage traces for the first four recorded trials (from a total of 200) shows little trial-to-trial variance. (b) Amplitude frequency histogram for all 200 trials recorded. Paired-pulse depression (at an interval of 50 ms) is 78%. Hatched bars, noise; shaded bars, second EPSP; white bars, first EPSP.

Table 7. Synaptic properties of putative thalamic EPSPs.

EPSP	1	2	3	4	5	6	7	8	9	10	11	12	mean
no. of AMPA receptors	25	91	158	38	20	46	47	23	32	29	153	51	59.4
possible no. of release sites	1	4	6	2	1	2	2	1	1	1	6	2	2.4
average no. of receptors/release sites	25	22.75	26.3	19	20	23	23.5	23	32	29	25.5	25.5	24.6
possible quantal size (mV)	1.32	0.93	0.87	0.49	1.40	0.78	1.05	1.54	0.75	1.42	0.36	0.39	0.94
possible quantal charge (pC)	0.39	0.29	0.13	0.11	0.22	0.24	0.25	0.28	0.21	—	—	—	0.24

stantial methods to identify, among the cells' excitatory inputs, the EPSPs that are likely to arise from a lateral geniculate input. The striking result that emerges is that transmitter release from the putative geniculate axons is very reliable, with the probability of release at all the sites on a single axon having a value of 1.0, not just to a single action potential but also to a subsequent one (at an interval of 50 ms). This unusual feature of their release characteristics allowed us to gain some tentative insight into the properties of their AMPA receptors. If our calculations are correct, then the AMPA receptors at geniculocortical synapses have a relatively high open probability, high unit conductance, an average mean open time and a slow recovery from desensitization. As a general rule a high open probability and high unit conductance are characteristic of the response of some AMPA receptor sub-types when they are exposed to a relatively high concentration ($\gg 1$ mM) of glutamate (e.g. Raman & Trussell 1995; Smith & Howe 2000). This would be achieved if the individual release sites for a geniculate axon on the cell were each made up postsynaptically of a few closely aggregated AMPA receptors giving a small cleft volume and hence higher average glutamate concentrations above the receptors. This raises the question of how many release sites are associated with each geniculate axon. One striking feature of the estimated number of AMPA receptors for each axon, as listed in table 5, is that the estimates are roughly multiples of 25. Making the assumption that each release

site has approximately that number of AMPA receptors leads to the analysis displayed in table 7.

There are two notable features of this analysis. The first is that, in this limited sample, the commonest geniculocortical connection (to the spiny stellate cells) has either one or two release sites, but a minority of axons have larger numbers. The second feature is that the average deduced quantal size is nearly 1 mV and four quanta, on average, would inject 1 pC of charge. These values are two to five times larger than comparable estimates for other excitatory inputs on to spiny cells in the upper layers of the cortex. For example, Tarczy-Hornoch *et al.* (1999) used values of 0.1–0.3 mV for the range of quantal sizes in cat layer 4, and observed a value of 0.2 mV for a layer 6 to spiny stellate connection. Similarly, Yoshimura *et al.* (1999) gave estimates for pyramidal-to-pyramidal cell connections in the superficial layers of kitten cortex that amounted to *ca.* 0.1 pC per quanta (calculated from their peak current and average time course). It therefore seems likely that the geniculocortical synapses are relatively more powerful per release site, than the other major excitatory inputs on to the spiny stellate cell. This tentative conclusion goes some way to explaining the otherwise apparent paradox that the geniculocortical synapses may represent only 5% of the total excitatory input, as measured by number of synapses (Peters & Payne 1993; Ahmed *et al.* 1994), yet appear to be the dominant input in setting the cells' functional behaviour (Reid & Alonso

1995; Ferster *et al.* 1996; Alonso *et al.* 2001). This is achieved by as few as 30 geniculate cells supplying a total of 100–125 synapses (Peters & Payne 1993; Alonso *et al.* 2001). These geniculate axons are thus convincing examples of ‘drivers’ rather than ‘modulators’ in the qualitative distinction that has been made concerning synaptic inputs (see Sherman & Guillery 1998). The question then arises whether the other principal excitatory inputs to the spiny stellate cell can also be regarded as ‘drivers’, or are they ‘modulators’? Sherman & Guillery (1998) observed that it was not known whether any corticocortical pathway represented a driver or a modulator input. One reason for this uncertainty is that it is not possible to use the criterion that a driver is responsible for the receptive-field properties in a sensory system when the receptive field is not available. For this reason, Sherman & Guillery suggested the use of the cross correlogram, with driver inputs inducing sharp peaks, and they illustrated the distinction by comparing the cross correlograms for both a retinal and a layer 6 cortical input to geniculate neurons. A recent publication by London *et al.* (2002) offers a quantitative measure (the SIE) that appears to capture what Sherman & Guillery have in mind. Early work on cross correlograms (Kirkwood 1979; Fetz & Gustafsson 1983) has indicated that the major factor contributing to a sharp cross correlogram is the rise time and peak amplitude of the EPSP, with the time course and amplitude of the cross correlogram being fairly closely linked to the derivative of the rising phase of the EPSP (see London *et al.* 2002). Comparison of the three principal excitatory inputs to the spiny stellate cell is made relatively easy by the fact that all three inputs have similar 10–90% rise times (of 1.1 ± 0.5 , 1.4 ± 0.3 and 1.4 ± 0.5 ms, respectively, for geniculate, layer 4 and layer 6 inputs). Thus, the ‘sharpness’ of the cross correlogram should be primarily influenced by the peak amplitude of the respective EPSPs. (The recent study of London *et al.* (2002) indicates the smaller amplitude inputs will have a smaller SIE than that expected from their peak amplitude.) Table 4 summarizes their relative peak amplitudes, but this will give a misleading impression of the extent to which their cross correlograms will be sharp, since it does not take account of the changes in amplitude with repetitive activity. Figure 10 summarizes the changes in the amplitude histograms of the three classes of input. Even with a paired pulse at 50 ms (≈ 20 Hz) the changes are dramatic and with repetitive activity (see Tarczy-Hornoch *et al.* 1999) they can be greater, for example, layer 6 inputs can double in peak amplitude. Similarly geniculocortical EPSPs can be halved, whereas layer 4 inputs may show some depression, but it is usually relatively small (see tables 2 and 3 and fig. 4 of Tarczy-Hornoch *et al.* 1999). Thus, in conditions of repetitive activity, the different classes of EPSP will all have both similar rise times and similar peak amplitudes (cf. table 4) and hence they can all be regarded as possible drivers. The extent to which any one of these three inputs could be the dominant driver will then depend on both their relative firing frequencies in physiological conditions and, equally importantly, whether the firing between different input neurons of one class is correlated. Correlated firing between geniculate neurons converging on the same cortical cell is documented (Alonso *et al.* 1996), but is not known for the other inputs.

The second notable feature of this analysis is that the paired-pulse depression displayed by the putative geniculocortical synapses is attributed entirely to a post-synaptic cause—AMPA receptor desensitization. The basis for this conclusion is that the form of the amplitude histograms to both first and second pulse are only interpretable on the assumption that there is no change in the probability of transmitter release and the more plausible of the two possibilities is a probability of 1.0. On this assumption we then reached the further, more tentative, conclusion that the AMPA receptor behaviour and the quantal size might be different at the geniculocortical synapses from the other principal excitatory synapses on the spiny stellate cell. These two deductions are at odds with the results reported by Gil *et al.* (1997, 1999). They showed that muscarinic and nicotinic agonists had opposite effects of depression and facilitation on thalamocortical synapses of rodent somatosensory cortex and that these modulatory effects were likely to be the result of changes in transmitter release. Furthermore, in their second paper (Gil *et al.* 1999) they presented evidence that the probability of transmitter release for thalamocortical synapses was high, but less than 1.0: this conclusion is a corollary of their conclusion that nicotine enhances transmitter release. There may be two reasons for this apparent discrepancy in the accounts of the probability of transmitter release. Our results were obtained in slices from 12- to 16-week-old cats whereas their studies were in slices from 2- to 3-week-old rodents. The difference might therefore be related to the stage of cortical development. An alternative, or additional, explanation is technical. The experiments of Gil *et al.* (1999) were performed in 2 mM calcium, 2 mM magnesium at 27–28 °C, whereas our experiments were in 2.5 mM calcium, 1 mM magnesium at 34–36 °C. The differences, both in ionic composition and temperature, would favour a higher probability of release in our experiments. The results of Volgushev *et al.* (1995) on ‘all or none’ EPSPs in rat visual cortex are in line with this suggestion. They found, in 4–6 week old rats that some EPSPs recorded in supragranular neurons were large with extremely low variance in control conditions (2 mM calcium, 1.5 mM magnesium), but the EPSPs could be reduced in amplitude by paired-pulse stimulation or by raising the magnesium concentration. The reduced EPSPs could either continue to behave as all-or-none responses or they could fluctuate between several amplitude levels—in the latter case, clear evidence that they were generated by the activity of several release sites, as they point out. By analogy it is therefore possible that at a more physiological concentration of 1.5 mM calcium we would have found in the cat visual cortex that the probability of release was lower and hence susceptible to change either by modulatory agents or the mechanism of presynaptic depression, as observed by Gil *et al.* (1997). This suggestion seems likely, given the increasing evidence that activity-dependent depression of thalamocortical synapses plays a very important role in sensory processing, including different behavioural states (Chung *et al.* 2002; Castro-Alamancos & Oldford 2002).

The other discrepancy between our conclusions and that of Gil *et al.* (1999) is that the quantal size of thalamocortical synapses may be larger than that of intracortical synapses. As already discussed, this deduction by us is

Figure 10. Properties of the three classes of inputs into spiny stellate neurons. (a)–(c) Examples of amplitude frequency histograms from each class of input. (a) All class 1 EPSPs that had reliable responses to both the first and second stimuli (50 ms apart) showed paired-pulse depression, which was considered to be essentially postsynaptic in origin. (b) All class 2 inputs that were considered to have a release probability of close to 1 for both the first and second release showed no paired-pulse depression. (c). Class 3 inputs showed large paired-pulse facilitation. Filled circles, first EPSP; open circles, second EPSP; triangles, noise distribution. (d) Summary graph for the three input types: type 1, $n = 12$; type 2, $n = 8$; type 3, $n = 7$.

much more tentative; it relies on the assumption that the CV of the amplitude histograms is entirely attributable to quantal variance and that that variance is in turn attributable to the open probability of the AMPA channels. Gil *et al.* (1999) have presented convincing experimental evidence that the quantal size of both thalamocortical and intracortical synapses are similar and it is therefore possible that our result is a consequence of oversimplistic assumptions. Nevertheless it is also possible that there is a genuine difference, reflected also in the higher probability of transmitter release. If the thalamocortical synapses achieved a higher concentration of glutamate in the synaptic cleft, perhaps as a result of multivesicular release (Oertner *et al.* 2002) or because they are organized as multiple release site synapses (cf. Silver *et al.* 1996), the tentative conclusion we have reached would not be unexpected. This unresolved issue does indicate the importance of further structural and functional studies of thalamocortical synapses.

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GLOSSARY

- AHP: after-hyperpolarization
 AMPA: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
 CV: coefficient of variation
 DAP: depolarizing after-potential
 EPSP: excitatory postsynaptic potential
 IFF: instantaneous firing frequency
 LGN: lateral geniculate nucleus
 sAHP: slow long lasting after-hyperpolarizing potential
 SIE: synaptic information efficacy