Unitary EPSCs of Corticogeniculate Fibers in the Rat Dorsal Lateral Geniculate Nucleus In Vitro

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Submitted 23 December 2002; accepted in final form 21 January 2003

Granseth, Björn and Sivert Lindström. Unitary EPSCs of corticogeniculate fibers in the rat dorsal lateral geniculate nucleus in vitro. J Neurophysiol 89: 2952–2960, 2003. First published January 29, 2003; 10.1152/jn.01160.2002. To investigate unitary corticogeniculate excitatory postsynaptic currents (EPSCs), whole cell patch-clamp recordings were obtained from 20 principal cells in slices of the dorsal lateral geniculate nucleus (dLGN) of DA-HAN rats. EPSCs, evoked by electrical stimulation of corticogeniculate axons, had size distributions with one or more quantal peaks. Gaussian curves fitted to such distributions gave a mean quantal size (q) of −5.0 ± 0.7 (SD) pA for the EPSCs. Paired-pulse ratio (EPSC2/EPSC1) was 3.3 ± 0.9 for stimuli separated by 40 ms. The mean quantal size was similar for facilitated EPSCs (−5.2 ± 0.8 pA), implying an increase in mean quantal content (m). Most corticogeniculate axons were capable of releasing only one or two quanta onto individual principal cells. Mean resting release probability (p) was low, 0.09 ± 0.04. Binomial models, with the same n but increased p, could account for both the basal and facilitated EPSC size distributions in 6/8 cells. It is suggested that the low resting efficacy of corticogeniculate synapses serves to stabilize this excitatory feedback system. The pronounced facilitation in conjunction with large convergence from many corticogeniculate cells would provide a transient, potent excitation of dLGN cells, compliant with the idea of a visually driven neuronal amplifier.

INTRODUCTION

The dorsal lateral geniculate nucleus (dLGN) relays visually evoked activity from the retina to the primary visual cortex. The projection by retinal ganglion cells onto principal cells in the dLGN has been investigated extensively (Hubel and Wiesel 1961; Usrey et al. 1999), including the quantal properties of the synaptic connections (Paulsen and Heggelund 1994, 1996). Neurons in the dLGN also receive feedback excitation from pyramidial cells in layer six of the primary visual cortex (Ferster and Lindström 1985a, b; Gilbert and Kelly 1975). Synapses formed by this projection have attracted much less attention although they constitute the most common glutamatergic terminals on principal cells (Erissir et al. 1997; Guillery 1969).

It is generally believed that the corticogeniculate pathway modulates the excitability of dLGN neurons in accordance with attentional demands (Guillery and Sherman 2002). One characteristic property of this feedback excitation is a pronounced facilitation with repetitive stimulation (Lindström and Wrobel 1990; McCormick and von Krosigk 1992; Turner and Salt 1998; von Krosigk et al. 1999). The facilitation is optimal at physiological extracellular calcium ion concentration and gives a three- to fourfold increase in synaptic efficacy as judged by paired-pulse stimulation in vitro (Granseth et al. 2002). Obviously, this facilitation should play an important role in the modulation of the dLGN relay. From the dynamics of the facilitation, it follows that the excitation per impulse increases with the firing frequency. The corticogeniculate feedback could thus function as a neuronal amplifier that regulates the gain of the dLGN transmission in the frequency domain (Ahlsten et al. 1985; Ferster and Lindström 1985b; Granseth et al. 2002; Lindström and Wrobel 1990).

Corticogeniculate excitatory responses in target principal cells may approach or even exceed the size of the retinal input when facilitated (Ahlsten et al. 1985; Granseth et al. 2002; Turner and Salt 1998). This impressive response can be finely graded in amplitude by changing the stimulation intensity, suggesting that dLGN principal cells receive weak excitation from many individual corticogeniculate axons. Our intention with the present study was to apply the tools of quantal analysis (Bennett and Kearns 2000; del Castillo and Katz 1954a, b) to investigate the synaptic properties of the corticogeniculate feedback excitation at the unitary level. Such analysis could identify important constraints for the operation of this neuronal pathway in vivo. It will be shown that the system is tailored to provide high resting stability combined with a transient, visually driven increase in the gain of signal transfer through the dLGN. The synaptic properties are well in line with the proposed function of the corticogeniculate feedback as a neural amplifier. Preliminary results have been presented in abstract form (Granseth et al. 1999).

METHODS

Preparation of dLGN slices and recording procedures

Experiments were approved by the Committee for Ethics in Animal Research of Linköping in accordance with Swedish animal-welfare legislation. Pigmented DA-HAN rats (BK Universal, Sollentuna, Sweden) of both sexes, 22–37 days old, were anesthetized with halothane (ISC Chemicals, Avonmouth, UK) and decapitated. The brains were rapidly transferred to ice-cold Krebs medium containing (in mM) 248 sucrose, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, 3.0 KCl, 6.0 MgCl$_2$, 0.5 CaCl$_2$, 3.0 myo-inositol, 0.5 ascorbic acid, 4.0 lactic acid, and 10 glucose, equilibrated with 95% O$_2$ - 5% CO$_2$. Slices, 250–300 μm thick, containing the dLGN were cut on a vibroslicer (Campden Instruments, Leicester, UK). After at least 1-h incubation at 37°C, whole cell patch-clamp recordings were made with the HEKA EPC9.
amplifier (HEKA Elektronik, Lambrecht, Germany) with slices submerged in Krebs medium containing (in mM) 124 NaCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 3.0 KCl, 2.0 MgCl₂, 2.0 CaCl₂, and 10 glucose equilibrated with 95% O₂-5% CO₂ at 34°C. Picrotoxin (100 μM) and dl-APV (100 μM) were routinely included in the Krebs medium to block GABAₐ and N-methyl-D-aspartate (NMDA) receptors. Borosilicate glass microelectrodes (tip resistance, 3.5–6.0 MΩ) were filled with a cesium-gluconate-based buffer containing lidocaine N-ethyl bromide (QX-314) and TEA (Granseth et al. 2002). Principal cells in the dLGN were identified in an Axioskop FS microscope (Zeiss, Jena, Germany) with water-immersion objectives and infrared differential phase contrast optics and an infrared digital camera (C7500, Hamamatsu, Hamamatsu City, Japan). The neurons were voltage clamped at −70 mV, adjusted for a liquid junction potential of 8 mV, holding currents were 0 to −25 pA. Access resistance was less than 15 MΩ and not varying more than 10%. Input resistance was 0.3–0.8 GΩ, whole cell capacitance 120–200 pF.

Stimulation of axons

Amplitude graded voltage pulses of 0.2-ms duration, generated by Iso-flex stimulus isolators and a Master 8 pulse generator (AMPI, Jerusalem, Israel), were used with bipolar tungsten electrodes (World Precision Instruments, Sarasota, FL) to stimulate corticogeniculate axons rostroventrally and retinogeniculate axons caudoventrally to the dLGN (Granseth et al. 2002; Turner and Salt 1998). Pulse separation was 40 ms for paired and train stimulation with a repetition rate of 0.2–0.5 Hz. Stimulation intensities were adjusted to activate single or a few corticogeniculate axons. Lowest thresholds ranged from 1.2 to 4 V in different experiments. As the EPSC failure rate for single fiber stimulation was very high, more than one axon was often stimulated to obtain sufficient number of EPSCs for analysis. To avoid inconsistencies from stimulation of axons close to their threshold, intensities in the middle of a plateau in the recruitment curve was used. For retinogeniculate fibers, single fiber thresholds were readily determined, as EPSCs were large without failures. Thresholds were 6–10 V and unaltered when [Ca²⁺]₀, was lowered from 2.0 to 0.5 mM.

Data analysis

EPSC amplitudes were measured as the difference between the mean current over 1.5 ms at the peak of the EPSC and the preceding baseline using the PulseFit software (HEKA Elektronik), after digital baseline using the PulseFit software (HEKA Elektronik), after digital

mean current over 1.5 ms at the peak of the EPSC and the preceding

Statistics

Values are given as means ± SD unless otherwise specified. Data were statistically evaluated by paired or unpaired Student t-test, P < 0.05 was considered as significant. The Kolmogorov–Smirnov two-sample test was used for comparing cumulative distributions. Confidence intervals (CIs) were determined using the t-test distribution and significance level P < 0.05. Quantal models were evaluated by χ² test, using P > 0.3 to indicate an acceptable fit.

Chemicals

Picrotoxin, TTX (tetrodotoxin), dl-2-amino-5-phosphonovaleric acid (dl-APV), QX-314, TEA, 2,3,4,5,6-pentahydroxycaproic acid (gluconic acid), CsOH, EGTA, and adenosine 5′-triphosphate, magnesium salt (Mg-ATP), were purchased from Sigma, St. Louis, MO. All other chemicals were obtained from Merck, Darmstadt, Germany.

RESULTS

Stimulation of corticogeniculate fibers

Unitary EPSCs, evoked by stimulation of individual corticogeniculate axons, were investigated in 17 dLGN principal cells. In seven of these were unitary EPSCs of retinogeniculate origin also evoked. The latter EPSCs were quite large in amplitude (~130 ± 35 pA) with distinct, easily determined thresholds (Paulsen and Heggelund 1994; Turner and Salt 1998). Compound corticogeniculate EPSCs of similar amplitudes were reliably evoked. They could be smoothly graded in amplitude over a 30- to 100-fold range (Granseth et al. 2002; Turner and Salt 1998). The apparent explanation is that dLGN principal cells receive convergent excitation from many corticogeniculate axons with small unitary EPSCs and high failure rates. The latter property made the identification of such unitary events quite difficult and time consuming.

The frequency of EPSC failures decreased with repetitive stimulation of corticogeniculate axons. Thus trains of five stimuli at 25 Hz were used to determine corticogeniculate fiber thresholds (Fig. 1). The overall EPSC failure rate for 20 trials at each stimulation intensity was used as critical parameter for axon recruitment. In the illustrated cell, discrete EPSCs were first encountered at a stimulation intensity of 1.2 V (Fig. 1A, *). The evoked EPSCs were similar in amplitude to spontaneous EPSCs (Fig. 1, A, top, and B, bottom, and C). The failure rate, averaged EPSC amplitude, and response potency (average amplitude of evoked EPSCs excluding failures) remained the same when the stimulation intensity was increased to 1.4 and 1.5 V (Fig. 1, D–F). With higher intensities, the failure rate decreased in two steps with corresponding changes in averaged EPSC amplitude and potency. These changes were accompanied by occasional evoked EPSCs about two and three times the original EPSC amplitude (Fig. 1, B and C). At least one EPSC component had a longer latency, causing an inflection of the rising phase of many compound EPSCs. The stepwise decrease in failure rate with corresponding change in average amplitude was taken to represent the recruitment of additional corticogeniculate axons. However, due to the high EPSC failure rate, it was not possible to exclude that small groups of axons with similar thresholds rather than single axons were recruited at these steps (see following text).

Mean quantal size of corticogeniculate EPSCs

The EPSCs evoked in principal cells by stimulation of single or a few corticogeniculate axons seemed to fluctuate in one or a few steps (Figs. 1–3). EPSC size histograms were constructed
for 17 cells to determine the unitary size of corticogeniculate EPSCs. To obtain sufficient numbers of evoked EPSCs for the analysis, more than one axon were regularly activated. For the cell illustrated in Fig. 2, the evoked EPSCs were obtained at a stimulation intensity that reliably recruited at least two corticogeniculate fibers (Fig. 2E). Even so, most stimuli (281/347; 81%) were followed by EPSC failures. The large majority of evoked EPSCs were of small amplitude, forming a peak in the EPSC size histogram (Fig. 2B). The mean size of these EPSCs was −4.9 pA, determined by the peak of a Gaussian curve fitted to the size distribution. A few large EPSCs could be multiples of this unit size (Fig. 2A, 3rd trace), although their number was too low to allow a reliable fit by any function (Fig. 2B). Because at least two axons were stimulated, contributing about equally to the evoked EPSCs (Fig. 2E), their unitary EPSCs were of similar small size (see also Fig. 6).

EPSCs of similar amplitudes occurred spontaneously in the same cell (Fig. 2, C and D). A Gaussian curve fit to the dominant peak of the size histogram gave the mean amplitude of −4.7 pA. The underlying distribution was not significantly different from that of the evoked corticogeniculate EPSCs (P = 0.43, Kolmogorov–Smirnov test). Because the slice preparation separated all excitatory corticogeniculate and retinogeniculate axons from their cell bodies, there cannot be any network activity and most spontaneous EPSCs can be assumed to represent single quantal events. To test for the possibility that occasional spikes in cut off axons affected the location of the peak of the distribution, TTX was added to a final concentration of 0.5 μM.

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**FIG. 1.** Unitary excitatory postsynaptic currents (EPSCs) evoked by stimulation of corticogeniculate fibers at different intensities. A–C: 3 consecutive traces of EPSCs evoked in a dorsal lateral geniculate nucleus (dLGN) principal cell by train stimulation of corticogeniculate fibers at denoted intensity, 5 stimuli at 25 Hz every 5 s. *, stimulus-evoked EPSCs; S, spontaneous EPSCs. Note that evoked EPSCs become larger and more frequent with increasing stimulation intensity. D–F: diagrams illustrating EPSC failure rate (number of stimuli without EPSC/total number of stimuli), average amplitude and potency (average amplitude of evoked EPSCs excluding failures) at different stimulus intensities. All data points from 20 traces with 5 stimuli. Note that failure rate and average amplitude change in 3 steps, taken to represent the recruitment of new corticogeniculate axons.

**FIG. 2.** Size distributions of spontaneous and evoked corticogeniculate EPSCs. A: sample EPSCs evoked in a dLGN principal cell by stimulation of corticogeniculate fibers, stimulus repetition rate 0.5 Hz. Three evoked EPSCs (*) and one response failure are shown. B: size distribution of EPSCs evoked in 347 trials (281 failures) in the same cell. A Gaussian curve fitted to the distribution peaks at −4.9 pA. C: spontaneous EPSCs (*) occurring in the same cell. D: corresponding size distribution of 121 spontaneous EPSCs and distribution of baseline noise (shaded). A Gaussian curve fitted to the dominant distribution peaks at −4.7 pA. A 2nd peak was fitted with a Gaussian curve with peak at −7.7 pA. E: recruitment curve, based on failure rate for 5 stimuli at 25 Hz (details as in Fig. 1D). F: 10–90% rise time over EPSC size for spontaneous EPSCs. G: amplitude distribution of spontaneous EPSCs recorded after suprathreshold train stimulation of retinogeniculate axons. H and I: size distribution of spontaneous EPSCs before (H) and after (I) adding TTX to the extracellular buffer to a final concentration of 0.5 μM. Insets: corresponding sample records, same scale as in A, different cell from A to G. Further details in text.

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tration of 0.5 μM to block action potentials (5 cells). Because the modal EPSC amplitudes were the same before (−4.5 ± 0.8 pA) and after TTX (−4.5 ± 0.7 pA; \( P = 0.87 \), paired Student’s t-test), this possibility could be ruled out (Fig. 2, H and I). It follows that most evoked corticogeniculate EPSCs were single quantal events. Thus the mean quantal size \((q)\) in the illustrated cell was −4.9 pA.

The size distributions of evoked EPSCs for all 17 cells investigated were organized in one or more peaks that could be fitted by single or multiple Gaussian curves. The estimates of mean quantal size \((q)\) for individual cells ranged from −3.6 to −6.3 pA (mean: −5.0 ± 0.7 pA; Fig. 5A). Spontaneous EPSCs obtained in 11 cells revealed peaks at −3.5 to −5.6 pA (mean: −4.7 ± 0.6 pA) i.e., in the same range as for the evoked responses.

The size distributions of spontaneous EPSCs were skewed for all cells with a more or less pronounced shoulder or second peak at higher amplitudes (Fig. 2D). A Gaussian curve fitted to the second peak in the distribution gave a mean amplitude of −7.7 pA, a value rather small for a multiple of the dominant component (−4.7 pA). Another possibility is that larger single quantal events, possibly originating from retinogeniculate terminals, contributed to this second peak. Many EPSCs in this amplitude range had a fast rise time (Fig. 2F), more similar to evoked retinogeniculate EPSCs (0.76 ± 0.10 ms) than to corticogeniculate EPSCs (0.91 ± 0.23 ms). Also in support of this interpretation is the finding that the proportion of large EPSCs was increased in a 100-ms time period after repeated activation of retinogeniculate fibers (Fig. 2G). Such a procedure is likely to increase asynchronous release of transmitter from the stimulated retinogeniculate terminals. In two other cells, the quantal size of retinogeniculate EPSCs was estimated to −8.3 and −9.7 pA when \([\text{Ca}^{2+}]_0\) was lowered to 0.5 mM (data not shown). These figures are in accordance with the mean quantal size of retinogeniculate synapses in the guinea pig (Paulsen and Heggelund 1994 1996). Hence, the shoulder in the amplitude histograms for spontaneous EPSCs may partly be of retinogeniculate origin.

**Mean quantal size after paired-pulse facilitation**

As for population EPSCs, unitary corticogeniculate EPSCs displayed pronounced paired pulse facilitation. The paired pulse ratio (EPSC2/EPSC1) was 3.3 ± 0.9 at 40-ms stimulus separation, similar to compound corticogeniculate EPSCs (Granseth et al. 2002).

To investigate quantal events, EPSC size distributions were also plotted for facilitated responses in eight cells. The result for a cell with multiple quantal peaks is illustrated in Fig. 3. A stimulation intensity was selected in the middle of a well-defined plateau in the recruitment curve for both EPSCs (Fig. 3D, arrows) to ascertain that the same number of corticogeniculate fibers was activated. The EPSCs evoked by the first and second stimuli displayed quantal fluctuations as seen by the superimposed sample records and the EPSC size distributions (Fig. 3, A and B). The pattern of peaks was insensitive to changes in bin size (not shown), and dips and peaks were also visible in the corresponding autocorrelation functions (Fig. 3E). The mean separation of peaks was the same for the two EPSC size histograms (−5.6 ± 0.2 pA for each). Accordingly, a pronounced paired-pulse facilitation occurred without significant change in mean quantal size. The facilitation was instead accounted for by a substantial decrease in the EPSC failure rate (from 68 to 41%) with a corresponding increase in the number of events underlying all peaks in the EPSC size distribution.

Similar results were seen in the remaining seven cells, three with multiple quantal peaks (as in Fig. 3) and four with a dominant first peak (as in Figs. 2 and 7). For all eight cells, the grand mean quantal size was −4.9 ± 0.9 pA for EPSC1 and −5.2 ± 0.8 pA for EPSC2 (Fig. 5A). Thus quantal content \((m)\) rather than quantal size \((q)\) was changed by the facilitation as expected for a presynaptic mechanism (del Castillo and Katz 1954b; Isaacson and Walmsley 1995; Stevens and Wang 1995).

A relatively long paired-pulse interval (40 ms) was chosen to avoid any residual threshold change for the second stimulus in the pair. Thus it seems unlikely that the large facilitation was caused by the recruitment of more corticogeniculate axons by the second stimulus. The similarity of the recruitment curves for the first and second stimulus (Fig. 3D) supports this interpretation. As a test of response independence, the averaged second EPSC was compared for trials where the first pulse evoked an EPSC or failed to do so. As seen in Fig. 4A, there was no difference in the amplitude of EPSC2 in the two situations. Similar results were found for the other seven cells.

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**FIG. 3.** Size distributions of EPSCs evoked by paired stimulation of corticogeniculate axons. A: size distribution of EPSCs evoked by 317 stimuli, bin size: 1 pA. Note high frequency of EPSC failures. Multiple Gaussian curves fitted to peaks in EPSC distributions are shown with thin lines, a sum of Gaussians by a thick line. Inset: superimposed individual traces. B: size distribution of EPSCs evoked by a second stimulus 40 ms after the first. Note that the paired-pulse facilitation was associated with a decreased number of failures and an increased number of events underlying peaks in the histogram. Gaussian curves overlap with, and are equidistant to those in A. Inset: superimposed individual traces. C: size distributions of spontaneous EPSCs and noise (shaded). D: recruitment curve with failure rates for EPSC1 (open circles) and EPSC2 (filled circles) vs. stimulation intensity. Each data point is the mean of 20 recordings. Arrows point to the used stimulation intensity (7.5 V). E: autocorrelation function for EPSC1 (open circles) and EPSC2 (filled circles), same bin size as for EPSC size distribution. Arrows mark peaks and dips.
explains its smaller average amplitude. A total response inde-
to the degree of facilitation irrespective of the transmitter release of $0.003$. Such independence is expected when evoked impulses in size regardless of EPSC 1 success.

The difference in amplitude of the paired EPSCs (top right) does not represent paired-pulse depression (Fig. 4A). This effect comes from the intentional sorting of responses so that all EPSC 1 failures were excluded from the average. For EPSC 2, some failures occurred, despite the facilitation, which explains its smaller average amplitude. A total response inde-

dependence was obvious when the size of EPSC 1 was plotted against EPSC 2 in each individual pair (Fig. 4B). There was no correlation between the points, not even when response failures to the first stimulus were excluded from the analysis ($R^2 = 0.003$). Such independence is expected when evoked impulses faithfully invade the presynaptic terminals and induce the same degree of facilitation irrespective of the transmitter release of the first impulse (Stevens and Wang 1995).

**Number of quanta released by single corticogeniculate axons**

In the CNS, small terminals seem to release only single quanta after a presynaptic action potential (Atwood and Karunanithi 2002; Dobrunz and Stevens 1997; Raastad et al. 1992; Stevens and Wang 1995). To determine the number of released quanta ($r_i$), the EPSC amplitude on the $ith$ trial is divided by the mean quantal size ($q$). If the release probability is large from facilitation during train stimulation the largest number of released quanta for a trial [max($r_i$)] is a good estimate for the putative number of available quantal release sites [$n = \text{max}(r_i)$]. When more than three putative release sites are involved, max($r_i$) is less likely to incorporate all potential quanta. For such situations the estimate should be increased by a correction factor, $+1$, [$n = \text{max}(r_i) + 1$] (see Bennett et al. 1977).

A minimal number of activated axons can be derived from discrete steps in the recruitment curves. For the cell in Fig. 1, the recruitment curve showed three plateau levels indicating the activation of at least three corticogeniculate axons. For these plateau levels, $\text{max}(r_i)$ were 1.2, 2.8, and 4.2. Assuming that single axons were recruited at each step (see preceding text), the likely number quanta contributed by these axons was 1, 2, and 2 (the last figure obtained by adding the correction factor $+1$). A similar analysis identified 35 plateau levels in 15 dLGN cells. The number of quanta added by each new level ranged from 1 to 6 [mean: $2.0 \pm 1.1$; max($r_i$) $\geq 4$ adjusted by $+1$ as in preceding text]. The largest values were found for the highest plateau levels when recruitment of multiple axons with near identical thresholds is more likely. Therefore the preceding value might be an overestimate. When the analysis was restricted to the first plateau level (axons with lowest thresh-
olds), the number of quanta ranged from 1 to 3 (mean: 1.6 $\pm$ 0.6, 15 cells). Hence, it is likely that single corticogeniculate axons form rather few active synaptic contacts with individual dLGN neurons.

**Quantal release probability**

When a train of stimuli activates only one putative release site, quantal content is a direct measure of release probability ($m = p$). Four cells were studied in this situation, all with clear facilitation as evident from averaged recordings (Fig. 6, top). For the illustrated cell, the first to fifth stimulus in the train evoked 3, 8, 9, 16, and 15 EPSCs in 60 trials. Averages of evoked EPSCs excluding failures (potency) revealed constant response amplitudes ($-4.1$ to $-4.9$ pA), similar to spontaneous events ($-4.7$ pA). Thus the corresponding release probabilities were 0.05, 0.13, 0.15, 0.27, and 0.25. In all four cells, mean release probability increased from $0.09 \pm 0.03$ for the first response to $0.28 \pm 0.07$ at maximal facilitation (25 Hz).

In cases with multiple quanta (Fig. 3), an estimate of the mean release probability can be found by dividing the mean

![Image](http://jn.physiology.org/doi/10.1152/jn.00403.2002.supp/10.1152/jn.00403.2002.supp)
quantal content with the putative number of release sites ($p = \frac{m}{n}$) (Bennett et al. 1977). Putative release sites were calculated from $\text{max}(r_i)$ as in the preceding text. For $\text{EPSC}_1$ in the 17 cells, the mean release probability ranged from 0.03 to 0.20 (mean: 0.09 ± 0.04; Fig. 5B). Paired-pulse facilitation caused a two- to fourfold increase in release probability, the facilitated $p$ ranged from 0.10 to 0.38 (mean: 0.25 ± 0.10, 8 cells). Putative release sites were <10 in these calculations which, if anything, could have been underestimated despite correction (+1). It follows that the obtained values for $p$ provides an upper limit for the mean release probability at the involved synapses.

In an attempt to extend our analysis, we tried to fit the EPSC size distributions to binomial models of transmitter release. The binomial parameter $n$ was constrained to be less or equal to 10 in all cases. In 12 of the 17 cells the distributions were well accounted for by a simple binomial model ($P > 0.7$, $\chi^2$ test; Fig. 5E). Two cells had weaker fits ($P > 0.3$, $\chi^2$ test). The binomial models seemed to be more adequate to account for recordings where few release sites were active (Fig. 5E). In the cells where paired-pulse facilitation was investigated, the model had a constant number of putative release sites for the first and facilitated responses. The average binomial value for $p$ was 0.10 ± 0.05 for $\text{EPSC}_1$, not significantly different to the estimate from $\text{max}(r_i)$ ($P = 0.90$, paired Student’s $t$-test). For $\text{EPSC}_2$, it was 0.27 ± 0.18, not significantly different to the value from $\text{max}(r_i)$ ($P = 0.97$, paired Student’s $t$-test).

In one cell (Fig. 7), a nonuniform binomial model with two putative release sites with different release probabilities were required to account for the EPSC size distributions. For $\text{EPSC}_1$, release probabilities 0.04 and 0.22 gave an excellent fit as did 0.15 and 0.57 for the facilitated $\text{EPSC}_2$ ($P = 0.99$ for both; $\chi^2$ test). The average EPSC amplitudes were similar for the third to fifth stimuli so the EPSC size distributions were pooled. Again, a nonuniform binomial model with $P$ values of 0.17 and 0.63 was applicable ($P = 0.94$; $\chi^2$ test). Indeed, the shapes of the EPSCs revealed two components with slightly different delays (Figs. 1B and 7A). The remaining two cells where simple binomial models did not suffice had EPSCs involving at least eight release sites. No attempts were made to apply nonuniform binomial models to these cells.

In all 15 cells where binomial values for $n$ and $p$ were obtained, the values agreed well with estimates based on $\text{max}(r_i)$ (Fig. 5, C and D). Thus both methods confirm that corticogeniculate synapses have low basal release probability, which is substantially increased by facilitation.

**DISCUSSION**

Stimulation of single or a few corticogeniculate axons evoked EPSCs in principal cells of the dLGN with apparent quantal characteristics. The quanta size was small (about 5 pA) and, at low rates of stimulation, the release probability was low (~0.1). Single corticogeniculate axons released only a few quanta per target neuron (typically 1–2). As shown before for compound EPSCs (Granseth et al. 2002), unitary corticogeniculate synapses displayed pronounced paired-pulse facilitation. Mean quanta size was essentially unchanged by the facilitation, which instead could be accounted for by an increase in transmitter release probability. These findings are consistent with a presynaptic origin of the facilitation, as
shown at many other mammalian central synapses (Atluri and Regehr 1996; Dobrunz and Stevens 1997; Stevens and Wang 1995; Thomson et al. 1995).

Quantal size of corticogeniculate EPSCs

The mean quantal size ($q$) for corticogeniculate EPSCs was about $5 \text{pA}$, i.e., somewhat smaller than the estimates for retinogeniculate EPSCs (Paulsen and Heggelund 1994, 1996). This dissimilarity in size may be related to differences in the number of postsynaptic receptors, the amounts of transmitter released per quanta, dendritic filtering, or simply be a reflection of imperfect space clamp. Corticogeniculate axons are known to terminate at distal dendrites of principal cells, whereas retinogeniculate synapses terminate close to the soma (Erisir et al. 1997; Guillery 1969; Sefton and Dreher 1995). To improve the space clamp, the patch-clamp electrodes were filled with cesium-gluconate buffer containing TEA and QX-314 to block various ion conductances. Even so, the recorded rise time of corticogeniculate EPSCs was significantly slower than that of retinogeniculate EPSCs ($P < 0.001$, Student’s $t$-test) suggesting that a certain degree of dendritic filtering affected the measurements. A quantal size in the $5 \text{pA}$ range is not exceptionally low, however. Similar values have been reported for synapses in the hippocampus (Raastad et al. 1992; Stevens and Wang 1995) and ventral posterior thalamic nucleus (Golshani et al. 2001).

The quantal size of evoked and spontaneous EPSCs were surprisingly similar even though the spontaneous size histograms were in all likelihood assembled from events at hundreds of corticogeniculate synapses (Erisir et al. 1997; Guillery 1969), whereas the evoked EPSCs originated from only a small fraction of these. This finding suggests that the quantal size of corticogeniculate EPSCs is remarkably homogenous, which in turn may explain why quantal peaks were evident in multi-quantal responses (see Fig. 3). Such stereotype EPSCs are in good agreement with a hard-wired organization of the adult early visual pathway. In more flexible systems such as the hippocampus, with multiple forms of long-term plasticity, quantal size may vary considerably between synapses resulting in less visible peaks in EPSC size histograms (Raastad et al. 1992; Stevens and Wang 1995).

Unitary EPSCs evoked by corticogeniculate axons

Retinogeniculate axons are known to evoke a few large unitary responses in dLGN principal cells (Paulsen and Heggelund 1994; Turner and Salt 1998). Unitary EPSCs involved the release of $>10$ quanta. Such synaptic connections are suitable for a faithful relay of incoming signals from the retina (Hubel and Wiesel 1961). For a feedback system like the corticogeniculate pathway, it seems more adequate with small unitary EPSCs that allows for a wider spectrum of modulation in the frequency domain.

It may be argued that pruning of corticogeniculate terminal branches during the preparation of slices is responsible for the observed small size of unitary EPSCs. Such pruning could occur but was probably not a major factor. A nice tracer study by Bourassa and Deschênes (1994) showed that single corticogeniculate axons terminate in rod like zones in the rat dLGN with approximately the same line of orientation as our dLGN slices. As the cross section of the 0.8-mm long rods were $100 \times 150 \mu m$, they could be fully contained in a 250- to 300-$\mu m$-thick slice.

A rough estimate indicates that the soma of $100-200$ principal cells are contained within the terminal field of individual corticogeniculate axons (Bourassa and Deschênes 1994; Sefton and Dreher 1995). Because each axon has $400$ synaptic boutons, there would be two to four synapses per target cell. The number will be considerably lower if dendrites from surrounding neurons are considered. Such dendrites penetrate the terminal field and are likely targets because corticogeniculate axons are known to terminate at distal dendrites (Erisir et al. 1997; Guillery 1969; Sefton and Dreher 1995). Given the length of principal cell dendrites (Parnavelas et al. 1977) 5 to 10 times more principal cells might be contacted. Even if corticogeniculate axons would terminate selectively onto on- and off-center cells (Hubel and Wiesel 1961), the number of terminals per potential target neuron would be less than two. Such considerations make it unlikely that our low number of putative release sites from single axons onto individual dLGN cells is a substantial underestimate.

A small number of synaptic contacts per axon is consistent with anatomical (Murphy and Sillito 1996) and in vivo physiological findings in adult cats (Lindström and Wróbel 1990). In this system, the compound corticogeniculate EPSP can be finely graded in amplitude over more than a 10-fold range without resolvable unitary components. Such a finding implies the convergence of large number of axons with small unitary components onto the target principal cell. A similar pattern of convergence can be inferred from recordings in rat dLGN slices (Granseth et al. 2002; Turner and Salt 1998). Compound corticogeniculate EPSC may exceed $400 \text{pA}$, implying convergence of $>40$ axons. Together with anatomical data of a large number of synapses of cortical origin on dLGN neurons (Erisir et al. 1997; Guillery 1969; Sefton and Dreher 1995), the picture emerges of a feedback system with large convergence of small unitary events. With coordinated activation, such inputs may have considerable excitatory potency.

Release probability

The mean release probability of the corticogeniculate synapses at low stimulus repetition rates was $0.1$. The values were similar whether derived from $max(r_i)$ or binomial models. This figure is, if anything an overestimate, because the number of putative release sites may have been underestimated. It should be noted that simple binomial models tended to account less well for situations where larger numbers of release sites were involved. Release probability might accordingly be rather heterogeneous among corticogeniculate synapses and better described by nonuniform binomial models. However, the release probability is consistently low ($<0.22$) throughout this investigation. A low resting release probability is generally associated with facilitation (Atwood and Karunanithi 2002; del Castillo and Katz 1954b; Dobrunz and Stevens 1997; Isaacson and Walmsley 1995; Thomson et al. 1995), so also for the corticogeniculate synapse. The magnitude of paired-pulse facilitation was similar for unitary EPSCs as for compound EPSCs (Granseth et al. 2002) and accounted for by a proportional increase in release probability. What makes the corticogeniculate system remarkable is the overall dominance of
strongly facilitating synapses. This property underlies our contention that facilitation has an important functional role for this feedback pathway.

Function of corticogeniculate system

The corticogeniculate cells in layer six of primary visual cortex form the recurrent limb of a positive feedback/feedback system. The neurons receive monosynaptic excitation from dLGN principal cells and project back to principal cells with monosynaptic excitatory connections (Ahlström et al. 1982; Ferster and Lindström 1983; Lindström and Wrobel 1990). In addition, the cells have intracortical axon collaterals that terminate on simple cells in layer four of the primary visual cortex (Ferster and Lindström 1985a,b). This arrangement of excitatory connections has been suggested to function as a neuronal amplifier that might boost the transfer of visual signals, both at the level the dLGN and at layer four, the main entry to the cortex (Ahlström et al. 1985; Ferster and Lindström 1985b; Granström et al. 2002; Lindström and Wrobel 1990). The properties of corticogeniculate EPSCs on principle cells in the dLGN, as revealed in this study, are fully compatible with the proposed function.

Positive feedback circuits are useful as neuronal amplifiers but has the drawback that they might easily become destabilized. The low basal release probability of corticogeniculate synapses makes this particular system quite resistant to disturbances caused by spontaneous impulse activity. The limited number of quanta released by individual corticogeniculate axons works in the same direction by providing a small cell-to-cell excitation. Even with pronounced facilitation, the excitation provided by single corticogeniculate cells would be insufficient to fire the target principal cells in the dLGN. Obviously, the simultaneous activation of a group of converging layer six cells would be required for a substantial feedback excitation. Under physiological conditions, such a constellation of cells would readily be activated only by adequate visual stimuli.

Because the facilitation is presynaptic, there is a marked independence between synapses from different axons converging onto the same principal cell. Only synapses from activated corticogeniculate cells would be facilitated, and the effect would rapidly evaporate once the relevant visual stimulus is terminated. This type of facilitation provides for an elegant, stimulus specific increase in gain of the transfer of visual signals through the dLGN. Because intracortical synapses of corticogeniculate cells show similar facilitation (Ferster and Lindström 1985b; Tarczy-Hornoch et al. 1999), there is in a fact a two-stage gain increase for the transfer of visual signals to supragranular neurons. Such gain increases would emphasize behaviorally relevant visual signals, enhance feature linking (Ferster and Lindström 1985b; Sillito et al. 1994) and possibly be the neuronal substrate for an increase in visual attention (Ahlström et al. 1985; Granström et al. 2002; Lindström and Wrobel 1990).

We thank E. Ahlström for helpful assistance in early experiments. This work was supported by a grant from the Swedish Medical Research Council (Proj. nr. 4767). B. Granström is a PhD student at Forum Scientum supported by the Swedish foundation for strategic research.

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