Efficacy and Stability of Quantal GABA Release at a Hippocampal Interneuron–Principal Neuron Synapse

Udo Kraushaar and Peter Jonas
Physiologisches Institut der Universität Freiburg, D-79104 Freiburg, Germany

We have examined factors that determine the strength and dynamics of GABAergic synapses between interneurons [dentate gyrus basket cells (BCs)] and principal neurons [dentate gyrus granule cells (GCs)] using paired recordings in rat hippocampal slices at 34°C. Unitary IPSCs recorded from BC–GC pairs in high intracellular Cl− concentration showed a fast rise and a biexponential decay, with mean time constants of 2 and 9 msec. The mean quantal conductance change, determined directly at reduced extracellular Ca2+/Mg2+ concentration ratios, was 1.7 nS. Quantal release at the BC–GC synapse occurred with short delay and was highly synchronized. Analysis of IPSC peak amplitudes and numbers of failures by multiple probability compound binomial analysis indicated that synaptic transmission at the BC–GC synapse involves three to seven release sites, each of which releases transmitter with high probability (>0.5 in 2 mM Ca2+/1 mM Mg2+). Unitary BC–GC IPSCs showed paired-pulse depression (PPD); maximal depression, measured for 10 msec intervals, was 37%, and recovery from depression occurred with a time constant of 2 sec. Paired-pulse depression was mainly presynaptic in origin but appeared to be independent of previous release. Synaptic transmission at the BC–GC synapse showed frequency-dependent depression, with half-maximal decrease at 5 Hz after a series of 1000 presynaptic action potentials. The relative stability of transmission at the BC–GC synapse is consistent with a model in which an activity-dependent gating mechanism reduces release probability and thereby prevents depletion of the releasable pool of synaptic vesicles. Thus several mechanisms converge on the generation of powerful and sustained transmission at interneuron–principal neuron synapses in hippocampal circuits. 

Key words: GABAergic interneurons; basket cells; dentate gyrus; unitary IPSCs; paired-pulse depression; release probability; functional release sites; vesicular pools; paired recording; multiple probability compound binomial analysis

GABAergic interneurons are the main presynaptic source of inhibitory synaptic transmission in the mammalian CNS (for review, see Freund and Buzsáki, 1996). Although interneurons numerically represent only ~10% of the neuronal population, they control the activity of the entire neuronal network. In the hippocampus, several interneuron types have been identified that form synapses on different domains of their postsynaptic target cells (Han et al., 1993; Freund and Buzsáki, 1996). Interneurons that innervate the perisomatic domain of principal neurons, referred to as basket cells, mediate a particularly powerful form of inhibition. Activation of a single basket-type interneuron can suppress repetitive discharge and delay spike initiation in principal neurons (Miles et al., 1996). Furthermore, activation of a single interneuron can entrain spiking of target neurons and synchronize the activity of large neuronal ensembles (Cobb et al., 1995). The impact of basket cell-mediated inhibition may be explained by the anatomical location of synaptic contacts in the perisomatic region of their postsynaptic target cells. Alternatively, the efficiency could be caused by specific functional properties of basket cell output synapses, such as the number of functional release sites (Edwards et al., 1990; Tamás et al., 1997), the number of GABA molecules released from a single vesicle (Frerking et al., 1995), the number of postsynaptic GABA_A receptors (Nusser et al., 1997, 1998), and the receptor occupancy after release (Edwards et al., 1990; Frerking et al., 1995).

Unlike principal neurons, basket-type interneurons are able to generate action potentials with high frequency during sustained current injection in vitro (Han et al., 1993; Martina et al., 1998) and in vivo (Penttonen et al., 1998; Csicsvari et al., 1999). This suggests that the dynamic properties of output synapses of interneurons are adapted to high-frequency activity. In the neocortex, GABA release from interneurons is more stable than glutamate release from principal cells during high-frequency stimulation (Galarreta and Hestrin, 1998; Varela et al., 1999); however, the mechanisms underlying this stability are unknown. Stability of inhibition during high-frequency stimulation could be generated by a larger readily releasable pool of synaptic vesicles in comparison with excitatory synapses (Stevens and Tsujimoto, 1995). Alternatively, stability of inhibition could be conferred by a reduction of release probability during repetitive stimulation (Betz, 1970; Wu and Borst, 1999) or activity-dependent replenishment of the releasable pool of synaptic vesicles (Kusano and Landau, 1975; Dittmann and Regen, 1998).

A rigorous analysis of the factors determining the strength and dynamics of GABAergic synapses requires the selective stimulation of identified presynaptic interneurons in the paired recording configuration (Miles and Ponce, 1996). We have therefore examined unitary IPSCs at the basket cell (BC)–granule cell (GC) synapse in the dentate gyrus of hippocampal brain slices. Our results indicate that transmission at the BC–GC synapse is very efficient, mainly because of a large quantal size and a high release probability. An activity-dependent gating mechanism that reduces release probability may help to preserve the releasable pool of synaptic vesicles.

MATERIALS AND METHODS
Paired recording. Transverse hippocampal slices (300 μm thickness) were cut from brains of 18- to 25-d-old Wistar rats using a vibratome (DTK-1000, Dosaka). Animals were killed by decapitation, in agreement with national and institutional guidelines. Patch pipettes were pulled from thick-walled borosilicate glass tubing (2 mm outer diameter, 0.5 mm wall thickness); when filled with intracellular solution, the resistance was 3–5 MΩ for presynaptic recordings and 2–4 MΩ for postsynaptic recordings. Simultaneous recordings from synthetically connected BCs and GCs in the

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Correspondence should be addressed to Dr. P. Jonas, Physiologisches Institut, Universität Freiburg, Hermann-Herder-Strasse 7, D-79104 Freiburg, Germany. E-mail: jonas@uni-freiburg.de.
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dentate gyrus were obtained under visual control using infrared differential interference contrast videomicroscopy (Edwards et al., 1989; Stuart et al., 1993; Koh et al., 1995). A tight-seal (interference contrast videomicroscopy (Edwards et al., 1989; Stuart et al.,) dentate gyrus were obtained under visual control using infrared differential

Figure 1. Unitary IPSPs and IPSCs at the BC–GC synapse in the paired recording configuration. A, Schematic illustration of the BC–GC microcircuit in the dentate gyrus. High-frequency train of action potentials evoked in a putative BC by a current pulse (200 msec, 1.4 nA). C, Unitary IPSPs recorded from a BC–GC pair at –30 mV with 6 mM intracellular Cl– concentration. Single presynaptic action potential evoked by a depolarizing current pulse in the BC is shown on top, single IPSCs are shown superimposed in the center, and average IPSP is depicted at the bottom. D, Unitary IPSCs in a BC–GC pair with 6 mM intracellular Cl– concentration. Single presynaptic action potential is shown on top, single IPSCs at –100 and –50 mV are shown superimposed in the center, and average IPSCs at –100 to –50 mV (10 mV increment) are depicted at the bottom. E, Unitary IPSCs in a BC–GC pair with 140 mM intracellular Cl– concentration. Single presynaptic action potential is shown on top, single IPSCs at –100 and +30 mV are shown superimposed in the center, and average IPSCs at –70 to +30 mV (20 mV increment) are depicted at the bottom. F, Unitary IPSC peak amplitude plotted against recording time. Note that the amplitude was stationary (correlation coefficient $r = 0.05$, $p > 0.1$). Averages are from 30–100 single synaptic events. Data in C–F are from different pairs. Pair shown in F is pair #1 in Table 2.

### Solutions

The physiological extracellular solution contained (in mM): 125 NaCl, 25 NaHCO3, 25 glucose, 2.5 KCl, 1.25 Na2HPO4, 2 CaCl2, 1 MgCl2. In some experiments, the Ca2+ and Mg2+ concentrations were varied to alter the release probability. The intracellular solution contained (in mM): 145 KCl, 0.1 EGTA, 2 MgCl2, 2 Na2ATP, and 10 HEPES (K-methylsulfate intracellular solution, pH adjusted with KOH; see Fig. 1C,D) or a solution with (in mM): 145 CsCl, 0.1 EGTA, 2 MgCl2, 2 Na2ATP, and 10 HEPES (CsCl intracellular solution, pH adjusted with CsOH; see Fig. 1E) was used for the postsynaptic GC. For perforated-patch recordings, the intracellular solution contained (in mM): 78 KCl, 78 K-glucosinate, 0.1 EGTA, 2 MgCl2, 2 Na2ATP, 10 HEPES, and 18 μg/ml granicidin; for tip filling, the same solution without granicidin was used. Bicuculline methiodide was from Sigma (stock solution prepared in distilled water), CGP55845A was from Novartis
The mean frequency of spontaneous events was 8.2 events/msec, which is the maximum within a window of 2 msec duration after the presynaptic action potential. The peak current was determined as the difference between the voltage traces during stationary periods; the number of traces included was 20–50. Coefficients of variation (CV, SD/mean) of unitary IPSC peak amplitudes were calculated from at least 15 seizures for each condition. The quantal current generated at the i-th site was assumed to be the same, whereas the nonuniformity of individual release probabilities, \( q_i \), \( CV_{q_i} \), and \( CV_{q_i} \) were assumed to be the same, whereas \( \langle p \rangle \) was specified separately for each condition. The quantal current generated at the i-th site was denoted as \( p_i \) (the mean of all \( p_i \) values is \( \langle p \rangle \)), then the compound binomial model for three release sites would be stated as follows.

The probability to observe a failure is:

\[
P_{\text{fail}} = (1 - p_1)(1 - p_2)(1 - p_3).
\]
adopted. $\sigma_w$ was determined from the region of the baseline preceding the IPSC, with the same settings as those used for determining the peak current.

Estimates of the free parameters of the compound binomial model were obtained by fitting unbinned data by a maximum-likelihood method. The negative logarithm of the likelihood was minimized using a Simplex algorithm (Caceci and Cacheris, 1984) implemented in Pascal, running on 330–600 MHz Pentium PCs. Ranges for the parameters ($<q_r>: 30–500 \text{nA}$; $\sigma_p$, $\text{CV}$, and $\text{CV}_r$: 0.001–2; $<p>: 0.01–0.85; \sigma_a$: 0.01–100), and the negative log-likelihood was increased when these ranges were exceeded. The final fit results were within the defined parameter space, with the exception of $\sigma_w$, which was frequently at the upper border (100).

The criterion for convergence was a relative difference $<10^{-12}$ or $10^{-4}$ in the log-likelihood between best and worst vertex in 10 consecutive iterations (for two and four release conditions, respectively). Within a twofold range of starting values, the final results were relatively insensitive to the initial values. Independent fits were made for different numbers of functional release sites $N_f$ (range: 3–12). $N_f$ was accepted as the best-fit value when the corresponding log-likelihood was larger than that for $N_f-1$ and $N_f+1$, respectively.

Confidence intervals of parameter estimates (see Table 2) were obtained by bootstrap methods. To obtain balanced resampling (Davison et al., 1986; Efron and Tibshirani, 1998), 100 copies of the original data set (size $n$) were concatenated, and a random permutation of all 100$n$ elements was generated. Subsequently, 100 bootstrap replications were read off as successive blocks of length $n$ in the permuted data and were refitted, using the best-fit values for the original data set as initial values (Stricker et al., 1994, 1996). Errors were then estimated from percentile intervals (Efron and Tibshirani, 1998).

Models of vesicular pool dynamics. Synaptic depression during trains of pulses was described by models with two pools (releaseable/available and unavailable pool) of synaptic vesicles (Liu and Tsien, 1995a,b; Weis et al., 1999; Matveev and Wang, 2000).

$$p_k(N_f) = \frac{p_k}{(N_f)} = \frac{p_k}{(N_f)}$$

(6)

$$k$$

(7)

where $p_k$ is the release probability, $k$ is the rate of refilling of the releasable pool, $f$ is the stimulation frequency, and $N_f$ is the number of vesicles in the releasable pool at any point in time, with initial value and upper limit $N_i$ (the “capacity” of the releasable pool). The stochastic implementation of the model assumed a univesicular release constraint in response to a presynaptic action potential (Korn et al., 1982). The release probability was $p_k(N_f) = 1 - \exp(-\alpha N_f)$, where $\alpha$ denotes the time-integrated fusion rate for a single vesicle (Dobrunz and Stevens, 1997; Matveev and Wang, 2000). The probability of refilling of each vacancy in the releasable pool in the time interval $\Delta t = 1/2$ between two consecutive stimulations was $p_{\text{refill}} = 1 - \exp(-k \Delta t)$ (Matveev and Wang, 2000). $p_k$ during and after high-frequency stimulation was computed by averaging the results from 1000 Monte-Carlo simulations, using programs written in Pascal.

Activity-dependent decrease of release probability and increase of refilling rate were modeled as the activation of a two-state modification process:

$$a(t) = a_0 - (a_1 - a_0)\exp(-t/\tau),$$

with $a_0$ and $a_1$ indicating initial and final values of $a(t)$ during the stimulation and recovery period. Activity-dependent decreases of release probability and increases in refilling rate were implemented as:

$$p_k = p_k[1 - a_{\text{act}}(t)],$$

with $a_{\text{act}} \leq a_{\text{max}} = 0,$ and

$$k' = k[k + a_{\text{act}}(t)],$$

with $a_{\text{max}} \leq 0,$

(9)

where $a_{\text{act}}$ indicates the maximal modification. Thus the activity-dependent pool models had the following free parameters: pool capacity $N_{0,i}$, initial release probability $p_{0,i}$, refilling rate $k$, maximal modification $a_{\text{max}}$, and rates of activity-dependent modification $\alpha$ and $\beta$. Parameters of the pool model were specified arbitrarily (see Fig. 10). Alternatively, estimates of the parameters of the pool model were obtained using a least-squares method, minimizing the sum of squares of differences between experimental observations and model predictions with a Simplex
Unitary IPSPs and IPSCs at the BC–GC synapse

We examined inhibitory synaptic transmission between pairs of monosynaptically connected BCs and GCs in slices from mature rats (18–25 d old) at physiological temperatures (34°C). This synapse has several technical advantages (Fig. 1). First, interneurons and principal neurons in this circuit can be distinguished by morphological criteria (Fig. 1) and by the ability of the presynaptic interneuron to generate high-frequency trains of action potentials during sustained current injection (Fig. 1B). Second, paired recordings from synaptically connected interneurons and principal neurons can be obtained with relatively high probability, because the axonal arborization of BCs is extensive (Fig. 1A) (Freund and Buzsáki, 1996; Geiger et al., 1997). Third, although unitary IPSPs and IPSCs have small amplitudes at physiological intracellular Cl\(^-\) concentrations (Fig. 1C,D), the amplitude of unitary IPSCs with high intracellular Cl\(^-\) concentration (149 mM) is substantially larger, with excellent signal-to-noise ratio under these conditions (Fig. 1E). Fourth, the voltage-clamp conditions of the unitary IPSCs are ideal, because of the mainly perisomatic location of synaptic contacts and the favorable electrotonic properties of postsynaptic granule cells. Finally, synaptic transmission in the paired recording configuration is stationary over long periods of time, with a stimulation frequency of ≤ 0.25 Hz (Fig. 1F).

Figure 2 shows the properties of unitary IPSCs in high intracellular Cl\(^-\) in an individual pair, and Figure 3 summarizes the results from 78 BC–GC pairs. The mean synaptic latency, measured from the steepest point in the rising phase of the presynaptic action potential to the onset of the IPSC, was 1.1 msec. The rise of the synaptic events was almost instantaneous (average 20–80% rise time 0.26 msec). The mean unitary IPSC peak amplitude (including failures of transmission) was 504 ± 54 pA (110–2650) (78). Train-induced depression, critical frequency\(^a\) was 5.0 Hz (23). The mean reversal potential of unitary IPSCs is +4.2 mV (Table 1), this corresponds to a peak conductance change of 6.8 nS. The decay of the unitary IPSCs was better fitted with the sum of two exponentials than with a single exponential in the majority of pairs; the average values of the time constants were 1.9 msec (38% amplitude contribution) and 9.4 msec, respectively. Synaptic transmission at the BC–GC synapse was very reliable; the mean percentage of failures was 6.5%. Thus BC-mediated inhibition shows short latency, rapid onset, large peak-conductance change, long duration, and high reliability (Table 1).

Direct recording of quantal IPSCs at the BC–GC synapse

Rigorous analysis of the transmitter release process requires direct recording of quantal currents (Katz, 1969; Isaacson and Walmsley, 1995). We therefore examined quantal BC–GC IPSCs in conditions of low release probability with a reduced Ca\(^{2+}/Mg\(^2+\) concentration ratio in the bath solution (Fig. 4). Figure 4, A and B, shows unitary IPSCs in a pair at two different Ca\(^{2+}/Mg\(^2+\) concentrations. When the Ca\(^{2+}/Mg\(^2+\) concentration ratio was reduced, the amplitude of the unitary IPSC decreased, and the number of failures increased substantially, to >80% with Ca\(^2+\) concentrations <0.5 mM (Fig. 4C). Under these conditions, the amplitude of the successful unitary IPSCs excluding failures reached an asymptotic value corresponding to the quantal size (Fig. 4D). The mean peak amplitude of these putative quantal IPSCs was 129 ± 19 pA at –70 mV. With the mean reversal potential of unitary IPSCs of +4.2 mV, this corresponds to an apparent quantal
conductance change of 1.7 ± 0.3 nS. The mean CV of the putative quantal IPSC in conditions of low release probability, including both intrasite and intersite components, was 33.7 ± 1.0% (five pairs at Ca2+ concentrations of 0.1–0.3 mM, corrected for baseline noise). Thus direct recording of quantal IPSCs at the BC–GC synapse reveals a large quantal size and a moderate variability of the quantal amplitude.

**The time course of quantal release**

Asynchrony of transmitter release can provide a significant contribution to the time course of the average postsynaptic conductance change at various synapses (Diamond and Jahr, 1995; Isaacson and Walmsley, 1995; Geiger et al., 1997). To determine the time course of quantal release at the BC–GC synapse, we used the approach of first latency measurements (Barrett and Stevens, 1972) (Fig. 5). The time course of quantal release (open bars), determined from the distribution of first latencies (filled bars) in conditions of reduced Ca2+/Mg2+ concentration ratio, rose and decayed within a time window of ~1 msec, indicating that GABA release was highly synchronized (Fig. 5A). To test the validity of the first latency approach, single quantal events in low Ca2+/Mg2+ concentration ratio were aligned to their onset, averaged, and reconvolved with the time course of quantal release in the same pair. The time course of the reconvolved IPSC was almost indistinguishable from that of the averaged unitary IPSC (four pairs), suggesting that quantal contributions superimposed independently (Fig. 5B). The logarithmic plot of the mean time course of release from four pairs illustrates that the release period decayed approximately exponentially, with a time constant of 0.23 msec (Fig. 5C). Thus quantal release at the BC–GC synapse showed high synchrony, comparable to that of fast excitatory synapses (Isaacson and Walmsley, 1995; Geiger et al., 1997).

To further examine the possibility of cross talk (Barbour and Häusser, 1997) or multivesicular release (Auger et al., 1998) at higher release probabilities, we tested whether the decay time course of average unitary IPSCs became slower as the Ca2+/Mg2+ concentration ratio was increased. IPSCs in conditions of normal and reduced Ca2+/Mg2+ concentration ratio, normalized to their respective peak current amplitudes, showed almost identical time courses (Fig. 5D). The mean decay time constant was very similar for Ca2+ concentrations between 0.1 and 2 mM, with only a slight prolongation at higher concentrations (Fig. 5E; 2–12 pairs per Ca2+ concentration). Furthermore, as indicated by the scatter plot from individual evoked IPSCs, the decay time constant was independent of the IPSCs amplitude (Fig. 5F; four pairs). Thus the analysis of the decay time course of IPSCs provides no evidence for cross talk or increase in the number of fused vesicles with increasing Ca2+ concentration at this synapse.

**Number of functional release sites and release probability**

Because a decrease of the Ca2+/Mg2+ concentration ratio reduced the amplitude of the IPSCs excluding failures (Fig. 4), it appeared likely that the BC–GC synapse comprises multiple functional release sites. To determine the number of functional release sites and the probability of release at individual sites quantitatively, we fitted IPSC peak amplitude distributions at different Ca2+/Mg2+ concentration ratios with compound binomial models of release (MP-CBA; see Materials and Methods) (Fig. 6) (Redman, 1990). Failures were also included in the fit because (1) they could be unequivocally distinguished from successful IPSCs because of the favorable recording conditions and (2) they appeared to be entirely failures of synaptic transmission (stimulation failures were not present in the paired recording configuration, and conduction failures appeared unlikely; see Discussion).

Two examples are shown in Figure 6, with amplitude distributions in 2 mM Ca2+/1 mM Mg2+ on top and in 0.5 mM Ca2+/2.5 mM Mg2+ at the bottom. The estimated number of functional release sites was seven and five, and the mean release probability in 2 mM Ca2+/1 mM Mg2+ was 0.61 and 0.57, respectively. Bootstrap analysis further indicated that the errors in the estimates of number of functional release sites and release probability were small for both pairs (Fig. 6C, Table 2).

In five pairs in which IPSC amplitudes, rise times, and series resistance were stationary and the total number of traces was >700, the number of functional release sites estimated by MP-CBA ranged from three to seven, and the mean release probability in physiological divalent concentrations was between 0.41 and 0.63 (Table 2). In all pairs both intrasite (Liu and Tsien, 1995a) and intersite (Nusser et al., 1997) components of quantal variability appeared to be present. Although the compound binomial model accounted for inhomogeneity of release probability in principle,
the high value of the shape parameter $\alpha_{0}$ ($10^{1.4} - 10^{2}$) (Table 2) indicated that the release probability was relatively uniform among sites (Silver et al., 1998).

MP-CBA further allowed us to investigate the dependence of the estimated mean release probability on extracellular Ca$^{2+}$ concentration, as shown in Figure 6D. Fitting the data with a Hill equation revealed a half-maximal release probability at a Ca$^{2+}$ concentration of 1.5 mM, an apparent Hill coefficient of 2.4, and an extrapolated maximal release probability of 0.79. Fitting the data at the low-concentration limit ($\leq$1 mM) in double logarithmic representation gave similar results (apparent Hill coefficient 2.5; fit results not illustrated). In conclusion, single action potentials in the presynaptic BC trigger powerful inhibition of the postsynaptic GC, attributable to a large quantal size, a high release probability at physiological Ca$^{2+}$ concentrations, and the presence of multiple functional release sites.

Depression induced by paired-pulse stimulation

In the intact hippocampal network, BCs can generate high-frequency trains of action potentials (Penttonen et al., 1998; Csigvary et al., 1999). This raises the question of whether dynamic changes of transmission occur at BC–GC synapses during repetitive stimulation. We first examined paired-pulse modulation (Fig. 7). When two action potentials were elicited in the BC, separated by intervals of variable duration, the amplitude of the second IPSC was smaller than that of the first (Fig. 7A). The maximal paired-pulse depression, measured for 10 msec interpulse intervals, was $37 \pm 6\%$. Recovery from PPD was complete after 5 sec; when fitted with a single exponential function, the time constant of recovery was 1.97 sec (Fig. 7B) (3–11 pairs).

To determine whether the depression was presynaptic or postsynaptic in origin, we examined the variation of peak current amplitudes during the first and second IPSC for interpulse intervals of $\geq$100 msec. Figure 7C shows a summary plot of the inverse of the square of the CV ($\text{CV}^{-2}$) against the mean amplitudes of the second IPSC (nine pairs); both $\text{CV}^{-2}$ and mean were normalized to the values of the first IPSC (Malinow and Tsien, 1990). The data points were superimposed with the predictions of a model with variable presynaptic and postsynaptic contributions to PPD, using Equation 8 of Silver et al. (1998) and mean values for $N_{p}$, <p>, CV$_{1}$, and CV$_{2}$ as obtained by MP-CBA (Table 2). A comparison of data points and model curves suggests that the results were more consistent with a reduction in $<p>$ than a reduction in $<q>$, implying that PPD was mainly presynaptic in origin.

If PPD was caused by the activation of presynaptic GABA$_{B}$ receptors (Deisz and Prince, 1989; Lambert and Wilson, 1994) after release of GABA from a single BC, it should be blocked by the selective, high-affinity GABA$_{B}$ receptor antagonist CGP55845A (Kaupmann et al., 1997); however, PPD for 100 msec intervals was not significantly different in the absence and presence, respectively, of 5 $\mu$m CGP55845A (Fig. 7D) ($p > 0.2$), indicating that PPD is independent of presynaptic GABA$_{B}$ receptor activation.

If PPD was caused by depletion of the releasable pool of synaptic vesicles (Stevens and Tsujimoto, 1995; Debanne et al., 1996; Dobrunz and Stevens, 1997), its extent should be dependent on the average release probability. In contrast to this prediction, the extent of PPD at the BC–GC synapse was not significantly different in various Ca$^{2+}$/Mg$^{2+}$ concentration ratios (Fig. 8A) ($p > 0.2$).
Furthermore, if PPD was caused by depletion, the peak amplitude of the second IPSC evoked by paired stimulation should be inversely related to that of the first (Debanne et al., 1996); however, amplitudes of IPSCs evoked by paired stimuli were not significantly correlated at the BC–GC synapse (Fig. 8B). Thus PPD at the BC–GC synapse was presynaptic in origin, but unexpectedly appeared to be independent of both extracellular Ca$^{2+}$ concentration and previous release.

**Depression induced by multiple-pulse stimulation**

We then examined dynamic changes of transmission at the BC–GC synapse during high-frequency stimulation, as reported previously for inhibitory synapses in the neocortex (Galarreta and Hestrin, 1998; Varela et al., 1999). The onset of depression was biexponential, with time constants of 61 msec and 17.6 sec for a stimulation frequency of 20 Hz (Fig. 9C) (23 pairs). The steady-state depression increased with the frequency of stimulation; half-maximal depression occurred at 5.0 Hz (Fig. 9D). After a 20 Hz train of action potentials, recovery from depression was biexponential, with time constants of 1.3 and 31.7 sec (Fig. 9E) (23 pairs).

To determine whether the depression was presynaptic or postsynaptic in origin, CV$^{-2}$ was plotted against the mean unitary IPSC in steady-state conditions at a given frequency; both CV$^{-2}$ and mean were normalized to the control values at 0.25 Hz (Fig. 9F). The data points were superimposed with the predictions of a model with variable presynaptic and postsynaptic contributions to depression (Fig. 7C). Similar to PPD, comparison of data and predictions suggests that multiple-pulse depression was mainly presynaptic in origin. In the presence of 5 μM CGP55845A, the time course of depression was qualitatively similar, but the steady-state IPSC amplitude was significantly larger (33 ± 6% vs 21 ± 2%, p < 0.01) (Fig. 9G) (seven pairs), suggesting a small contribution of GABA<sub>B</sub> receptors to multiple-pulse depression.

Frequency-dependent depression is often interpreted as a depletion of the releasable pool of synaptic vesicles (Liley and North, 1953; Stevens and Tsujimoto, 1995). If this were the case, its extent would depend on the average release probability (Dittman and Regehr, 1998), and amplitudes of consecutive IPSCs in the train may be correlated (Matveev and Wang, 2000). We therefore examined depression during 20 Hz trains at different Ca$^{2+}$/Mg$^{2+}$ concentration ratios (Fig. 9H). In conditions of reduced release probability (0.5 mM Ca$^{2+}$), the fast component of depression was comparable, but the slow component showed a reduced amplitude and a slower time course (Fig. 9H, open symbols). The ratio of failures at the end of the train to those before the train was significantly larger in 0.5 mM Ca$^{2+}$ (0.55 ± 0.08) than in 2 mM Ca$^{2+}$ (0.27 ± 0.03; p < 0.001). Furthermore, amplitudes of subsequent IPSCs in the second portion of the train were negatively correlated (Fig. 9I). These results are consistent with the hypothesis that the slow component of depression is caused by depletion of the vesicular pool (Matveev and Wang, 2000).

To understand the interactions between fast and slow depression, we examined the quantitative predictions of pool models with different forms of activity dependence (Betz, 1970; Kusano and Landau, 1975). Figure 10 illustrates the predictions of a two-pool model with constant release probability and replenishment (Fig. 10, model a) and two alternative models with either activity-dependent reduction in release probability (model b) or activity-dependent increase in replenishment (model c). In the absence of evidence for multivesicular release (Fig. 5), we implemented a univesicular release constraint (Korn et al., 1982; Matveev and Wang, 2000).
Table 2. Number of release sites and release probability at the BC–GC synapse determined by MP-CBA

<table>
<thead>
<tr>
<th>Pair</th>
<th>Ca^2+/Mg^2+ (mm)</th>
<th>Events</th>
<th>Failures</th>
<th>Nq&lt;sup&gt;a&lt;/sup&gt;</th>
<th>(p)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>(q)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>CV&lt;sub&gt;1&lt;/sub&gt;</th>
<th>CV&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Log&lt;sub&gt;10&lt;/sub&gt;[α]&lt;sup&gt;d&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>#1</td>
<td>2/1</td>
<td>468</td>
<td>1 (1)</td>
<td>7 ± 1</td>
<td>0.61 ± 0.07</td>
<td>149 ± 7</td>
<td>0.29 ± 0.07</td>
<td>0.11 ± 0.11</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>0.5/2.5</td>
<td>322</td>
<td>430 (429)</td>
<td></td>
<td>0.08 ± 0.01</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>#2</td>
<td>2/1</td>
<td>612</td>
<td>34 (33)</td>
<td>3 ± 0</td>
<td>0.63 ± 0.02</td>
<td>248 ± 4</td>
<td>0.22 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.5/2.5</td>
<td>227</td>
<td>909 (908)</td>
<td></td>
<td>0.07 ± 0.004</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>#3</td>
<td>2/1</td>
<td>533</td>
<td>13 (8)</td>
<td>5 ± 0</td>
<td>0.57 ± 0.01</td>
<td>227 ± 5</td>
<td>0.14 ± 0.02</td>
<td>0.36 ± 0.01</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.5/2.5</td>
<td>246</td>
<td>731 (729)</td>
<td></td>
<td>0.06 ± 0.004</td>
<td></td>
<td></td>
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<tr>
<td>#4</td>
<td>3/3</td>
<td>197</td>
<td>0 (0)</td>
<td>6 ± 0.5</td>
<td>0.67 ± 0.03</td>
<td>164 ± 14</td>
<td>0.10 ± 0.02</td>
<td>0.49 ± 0.02</td>
<td>1.6 ± 0.3</td>
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<tr>
<td></td>
<td>2/3</td>
<td>351</td>
<td>10 (14)</td>
<td></td>
<td>0.42 ± 0.02</td>
<td></td>
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<tr>
<td></td>
<td>1/3</td>
<td>115</td>
<td>93 (70)</td>
<td></td>
<td>0.17 ± 0.02</td>
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<tr>
<td></td>
<td>0.3/3</td>
<td>29</td>
<td>431 (428)</td>
<td></td>
<td>0.01 ± 0.002</td>
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<td>4/0.5</td>
<td>111</td>
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<td>7 ± 1</td>
<td>0.72 ± 0.07</td>
<td>129 ± 6</td>
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<td>0.37 ± 0.03</td>
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<td>2/1</td>
<td>118</td>
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<td>0.41 ± 0.04</td>
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<td>1/2</td>
<td>165</td>
<td>27 (30)</td>
<td></td>
<td>0.23 ± 0.03</td>
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<tr>
<td></td>
<td>0.5/2.5</td>
<td>55</td>
<td>282 (280)</td>
<td></td>
<td>0.03 ± 0.003</td>
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*p*Series resistance compensation (85–90%) was used in all postsynaptic recordings. Series resistance before compensation in the postsynaptic granule cells was 10.5 MΩ at the beginning and 12.3 MΩ at the end of the entire recording period (mean values). Analysis for stationariness of IPSC amplitudes indicated that the mean relative difference between first and second portion of each data set was ~7% (range ~25% to 34%). Runs analysis showed that failures in conditions of low release probability were distributed randomly in all cells except pair #5 (p > 0.05).

*β*Failures represent measured values; predicted values are given in parentheses.

*γ*Consistent with the estimate of (p), the skewness of IPSC amplitude distributions (excluding failures) changed from positive to negative values as the Ca<sup>2+</sup> concentration was increased; skewness was 0.84 for 1 mM Ca<sup>2+</sup> (two pairs), 0.12 for 2 mM Ca<sup>2+</sup> (five pairs), and ~0.56 and ~0.28 for 3 and 4 mM Ca<sup>2+</sup> (one pair in each condition).

*α*Amplitude histograms in physiological Ca<sup>2+</sup> concentrations were also analyzed by fitting unbinned data with sums of Gaussian functions without constraints (Edwards et al., 1996; Jonas et al., 1993; Nusser et al., 1998). In all five pairs, a fit with four or five approximately equally spaced Gaussian functions was possible. Using this approach, the mean number of sites determined from the spacing of the Gaussians was determined as 170 pA, similar to that obtained by MP-CBA (183 pA).

*δ*In pairs #1, #2, #4, and #5, a compound binomial with a positive correlation between q and p values gave slightly better fits than a model with a negative correlation, whereas in pair #3 a negative correlation gave better results. In either case, the results reported correspond to the model that gave the best fit. Errors given in the table were determined as (16–84%–percentile intervals)/2 of the parameter estimates from 100 bootstrap replications of the original data set, which would correspond to an SEM for a normal distribution.

Figure 7. Properties of PPD of IPSCs at the BC–GC synapse. A, IPSCs evoked by pairs of action potentials in the presynaptic BC, separated by intervals of variable duration. Traces shown are averages of 30 unitary IPSCs and were normalized to the same amplitude for the first average IPSC (absolute values of A<sub>f</sub> were 1393, 1180, and 1216 pA, respectively). B, Time course of recovery from PPD. The ratio of amplitudes of the second (A<sub>s</sub>) and the first (A<sub>f</sub>) unitary IPSC, both measured from their respective baselines as indicated in A, was plotted against the interpulse interval. The curve represents a fitted exponential function with a time constant of 1.97 sec. Number of pairs is indicated in parentheses above the data points. C, Coefficient of variation analysis suggests a presynaptic locus of PPD. The inverse of the square of the coefficient of variation of A<sub>r</sub> (CV<sup>–2</sup>) was plotted against the mean peak amplitude; data were normalized by the mean and mean, respectively, of A<sub>f</sub>. Data are from 10 pairs. Intervals between presynaptic action potentials were 100 msec ( ), 300 msec ( ), 1 sec ( ), 2 sec ( ), and 3 sec ( ). Curve a represents the prediction of Equation 8 of Silver et al. (1998) for a pure change in release probability p superimposed on the data points (number of release sites = 5, release probability = 0.53, CV<sub>1</sub> = 0.18, CV<sub>2</sub> = 0.34, no variation in p). Curve e represents the prediction of a pure change in quantal size q, and curves b–d show predictions for mixed changes (75, 50, and 25% contribution of changes in p, with p = r<sup>x</sup> and q = r<sup>y</sup>, where r is the fractional contribution of the change in p and x is the normalized mean). D, PPD appears to be independent of presynaptic GABA<sub>B</sub> receptor activation. Left, Average IPSCs in control conditions (top) and in the presence of 5 μM CGP55845A in the bath solution (center) are depicted, together with a superposition of both traces (bottom). Right, Summary bar graph of mean A<sub>f</sub>/A<sub>r</sub> in control conditions and in the presence of CGP55845A. Extracellular Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations were 2 and 1 mM, respectively. Interpulse interval, 100 msec. Failures included in all averages. Number of pairs indicated in parentheses above the bars.

Among the alternatives tested, the model with the activity-dependent reduction in release probability would be most consistent with the experimental observations. Unlike the other models, it predicts the biexponential time course of multiple-pulse depression (Fig. 10B) very closely. Furthermore, the model predicts the approximate extent of PPD and the independence of PPD on release probability (Fig. 10, legend). If this model is used to fit the depression data in Figure 9, C–E and H, with release probabilities...
Quantal size at the BC–GC synapse

The estimated quantal conductance at the BC–GC synapse in high intracellular Cl⁻ concentration was 1.7 nS (Fig. 4D). If the single-channel conductance of postsynaptic GABAₐ receptors is assumed to be 30 pS at 34°C (Brickley et al., 1999; Perrais and Ropert, 1999), this would imply the opening of 57 GABAₐRs channels at the peak of a quantal IPSC. If the current flow through GABAₐRs expressed in GCs follows the Goldman-Hodgkin-Katz equation, the quantal conductance in physiological Cl⁻ concentrations would be predicted to be ~0.5 nS [chord conductance at +10 mV calculated using the Goldman-Hodgkin-Katz current equation for 6 mM intracellular Cl⁻ (Hille, 1992; also see Bormann et al., 1987)]. Considering that the resting input conductance of a GC is approximately 2 nS (Spruston and Johnston, 1992), this suggests that a quantal event has considerable shunting effects under physiological conditions.

Our values of the quantal conductance at the BC–GC synapse are larger than previous estimates for inhibitory synapses on hippocampal granule cells [0.2–0.4 nS, high intracellular Cl⁻ (Edwards et al., 1990); 0.4 nS, low intracellular Cl⁻ (Nusser et al., 1998)]. Differences in recording temperature (34°C in this paper vs 21–23°C in the other studies) certainly contribute to this apparent difference but are unlikely to explain it entirely. It is possible that different presynaptic interneuron subtypes were activated in the different studies. Presynaptic interneurons in the present experiments were selected according to fast spiking during sustained current injection, which will bias the sample toward parvalbumin-positive basket cells (Freund and Buzsáki, 1996; Cauli et al., 1997; Lübke et al., 1998). In contrast, interneurons activated in the other studies by minimal extracellular axonal stimulation may also include CCK/VP-positive basket cells or HICAP cells with axons co-aligned with the commissural-associational pathway (Freund and Buzsáki, 1996; Cauli et al., 1997; Lübke et al., 1998).

Release probability and Ca²⁺ dependence

The mean release probability at the BC–GC synapse in physiological divalent concentrations, determined by compound binomial analysis, was ~0.4–0.6 (Fig. 6). The dependence of release probability on extracellular Ca²⁺ concentration at the BC–GC synapse is consistent with the cooperative binding of >2 Ca²⁺ ions to the putative Ca²⁺ sensor, probably synaptotagmin (Südhof, 1995). The apparent Hill coefficient (2.4) is lower, however, than that at the frog neuromuscular junction (~3.8) (Dodge and Rahamimoff, 1967) or at glutamatergic autapses (3.3) (Reid et al., 1998). This may reflect a difference between inhibitory or excitatory terminals (e.g., in the synaptotagmin isoforms present). Alternatively, the lower Hill coefficient of inhibitory synapses may be attributable to intersite differences in the EC₅₀ values among terminals of the same connection, which will result in reduction of the apparent Hill coefficient.

The maximal release probability that is approached at high Ca²⁺ concentrations (extrapolated value ~0.8) (Fig. 6D) indicates a high efficacy of coupling of Ca²⁺ influx to exocytosis at the BC–GC synapse. Furthermore, the high maximal release probability suggests that axonal conduction of a single spike is reliable, despite the extensive arborization of the BC axon (Freund and Buzsáki, 1996). The proportion of conduction failures in physiological divalent concentrations is probably much less than 0.2 (1 - maximal release probability), because an increase in Ca²⁺ concentration is likely to reduce the safety factor of transmission, for example, caused by screening of surface charges (Frankenhaeuser and Hodgkin, 1957).

The BC–GC synapse is a multi-site connection

The present results indicate that synaptic events at the BC–GC synapse are generated at three to seven functional release sites (Fig. 6, Table 2). This value appears to be in approximate agreement with the estimated number of anatomical release sites at the BC–GC synapse.
inhibitory synapses in the hippocampus determined by electron microscopic analysis (10–12 inhibitory contacts between basket cells and pyramidal neurons in the CA1 subfield; eight inhibitory contacts between axo-axonic cells and GCs (Buhl et al., 1994); two to six inhibitory contacts between perisomatic inhibitory cells and pyramidal neurons in the CA3 region (Miles et al., 1996); two to four inhibitory contacts between BCs and GCs (Geiger et al., 1997; J. Lübke, personal communication). It remains to be determined whether BC–GC synaptic contacts comprise a single release site or multiple release sites, as reported previously for dendrite-targeting interneurons in the neocortex (Tamás et al., 1997).
Desensitization of postsynaptic GABA<sub>A</sub> receptors is not the major factor underlying PPD, because CV analysis suggested that the primary locus of PPD is presynaptic. Presynaptic receptors do not appear to be involved, because PPD at the BC–GC synapse was unaffected by the high-affinity GABA<sub>A</sub> receptor antagonist CGP55845A (Kaumann et al., 1997). Synaptic refractoriness is not expected to contribute to PPD, because recovery from refractoriness is thought to be complete within ~10 msec (Stevens and Wang, 1995). Finally, pool depletion was unlikely, because the extent of PPD was independent of extracellular Ca<sup>2+</sup> concentration, and a negative correlation between the amplitudes of first and second IPSCs was not observed (Fig. 9C, Table 2). The best-fit parameters were as follows: capacity of the releasable pool <i>N</i><sub>0</sub> = 51 vesicles per site, refilling rate <i>k</i> = 0.059 sec<sup>-1</sup>, maximal activity-dependent modification <i>a</i><sub>max</sub> = 0.59, and rates of <i>a</i> = 0.36 and <i>b</i> = 3.9 sec<sup>-1</sup>, yielding a half-maximal inhibitory frequency of 10.7 Hz and a maximal time constant of 0.26 sec of the activity-dependent process. For details, see Materials and Methods.

**The slow component of multiple-pulse depression: depletion of the releasable pool?**

During a train of presynaptic action potentials, unitary IPSC peak amplitudes at the BC–GC synapse decline biexponentially (Fig. 8). The independence of PPD on extracellular Ca<sup>2+</sup> concentration, and a negative correlation between the amplitudes of consecutive IPSCs (Borst and Sakmann, 1985) suggests a mainly postsynaptic origin. Presynaptic autoreceptors provide a small contribution to slow depression, as indicated by the slight reduction of multiple-pulse depression by CGP55845A (Jensen et al., 1999; this paper); however, depletion of the releasable pool of synaptic vesicles (Liley and North, 1953) is the most likely mechanism for this slow depression. This is supported by the dependence of slow depression on release probability (Fig. 9F) and the negative correlation between amplitudes of consecutive IPSCs (Fig. 9F) (Matveev and Wang, 2000).

Assuming that the two phases of multiple-pulse depression are shaped by a fast depression mechanism upstream of the release machinery and a slow depression mechanism corresponding to depletion of the readily releasable pool, we estimate that the capacity of the readily releasable pool at the BC–GC synapse is ~50 per release site. It is not clear whether this pool corresponds to the “readily releasable pool” (Stevens and Tsujimoto, 1995) or comprises both a readily releasable and a “reserve” pool (Liu and Tsien, 1995b). If our pool corresponds to the readily releasable pool, it would be larger than that at excitatory synapses in cultured neurons (~20) (Stevens and Tsujimoto, 1995).
Activity-dependent gating of release may stabilize inhibition

The possible coexistence of a fast depression generated by a gating mechanism upstream of the exocytotic event and a slow depression caused by depletion of the releasable pool raises the question of how these two forms of depression interact during high-frequency activity. One possibility is that activity-dependent reduction in release probability, somewhat counter-intuitively, protects against depletion of the releasable pool of synaptic vesicles, thus conferring stability of transmission (Betz, 1970; Brenchow et al., 1998). As shown in Figure 10B, the predicted IPSC amplitudes during a high-frequency train for the model with constant rates and the model with activity-dependent reduction of release probability show crossover, implying that vesicles saved in the early phase can be released in the late phase of the train (shaded area). Because GABAergic interneurons in the hippocampus in vivo generate action potentials over a wide range of frequencies with mean values of 10–20 Hz (Penttonen et al., 1998; Csicsvari et al., 1999), redistribution of synaptic efficacy within a train may contribute to the stability of transmission at GABAergic synapses in the hippocampal network of the behaving rat.

REFERENCES


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