INTRODUCTION

Postsynaptic receptor occupancy during synaptic transmission is an important variable in determining whether synaptic strength can be increased by the release of more transmitter molecules into the synaptic cleft, such as may, for instance, occur with the coordinated fusion of more than one vesicle at a single or at multiple neighboring release sites (Behrends and ten Bruggencate 1998; Edwards 1995; Frerking and Wilson 1996; Kirischuk et al. 1999; Lewis and Faber 1996; Perrais and Ropert 1999) or at least at a subset of contacts (DeFazio and Hablitz 1998; Nusser et al. 1997). Very recently, using the same approach, Nusser, Mody and coworkers have shown further evidence that the degree of receptor occupancy varies between different preparations, and that saturated and unsaturated synapses may occur on the same postsynaptic neuron (Nusser et al. 1999). Thus it appears that at some inhibitory CNS synapses at least, receptors are not saturated following the constitutive release of single quanta of GABA. However, the conclusion that these contacts are equally unsaturated during impulse-evoked transmission does not automatically follow. With evoked transmission, release of multiple vesicles (see above) or spillover from neighboring synapses (Destexhe and Sejnowski 1995; Isaacson et al. 1993; Rossi and Hamann 1998; Vogt and Nicoll 1999) may produce an enhancement of cleft transmitter concentration, driving peak receptor occupancy to saturation (Silver et al. 1996). In addition, it is possible that many spontaneous vesicular fusion events are rapidly reversible (Rahamimoff and Fernandez 1997), which would reduce the amount of transmitter released into the synaptic cleft (Clements 1996). For these reasons, when transmission is...
driven by a presynaptic impulse, receptor occupancy might well be higher than with spontaneous vesicle fusion.

While several studies have shown that benzodiazepines increase the peak amplitude of IPSCs evoked by selective activation of a single presynaptic inhibitory neuron or extracellular stimulation of inhibitory fibers (Roepstorff and Lambert 1994; Segal and Barker 1984; Vicini et al. 1986; Zhang et al. 1993; but see Williams et al. 1998), this does not necessarily indicate absence of saturation of postsynaptic GABA_A receptors at these synapses. Regardless of the absence or presence of an effect on peak amplitude, benzodiazepines have invariably been shown to prolong the decay phase of GABAergic mIPSCs (DeFazio and Hablitz 1998; De Koninck and Mody 1994; Frerking et al. 1995; Mellor and Randall 1997; Nusser et al. 1997; Perrais and Ropert 1999; Puia et al. 1994), in agreement with their effect on the decay phase of responses to short pulses of saturating concentrations of GABA applied to outside-out membrane patches (Mellor and Randall 1997; Perrais and Ropert 1999; but see Lavoie and Twyman 1996). Mody and coworkers have pointed out that, when quanta are not released in perfect synchrony, a prolongation by benzodiazepines of the decay phase of the quantal components will produce improved summation. The peak amplitude of the compound response will thus be enhanced without an increase in the peak synaptic responses generated at individual contact sites (De Koninck and Mody 1994; Mody et al. 1994).

The present study asks whether, despite this complication arising from quantal asynchrony and changes in quantal kinetics, information about receptor occupancy may still be obtained from the benzodiazepine effect on evoked IPSCs (i.e., compound, as opposed to quantal, responses). We use a relatively simple model calculation to show that, when asynchronous quantal currents are prolonged and therefore sum more effectively, the resulting enhancement in the peak amplitude of the compound IPSC is inevitably linked to an increase in its rise time, the magnitude of which can be estimated. We compare these predictions to the effect of the benzodiazepine flunitrazepam on IPSCs evoked in paired recordings from pre- and postsynaptic striatal neurons in cell culture. We find that the changes in rise times are far too small to explain the observed increases in IPSC peak amplitudes by improved summation of prolonged quanta, and therefore conclude that it must be largely due to an increase in quantal size at nonsaturated contact sites.

**METHODS**

**Cell culture and solutions**

Culture methods closely followed those of Gottmann et al. (1994). Following deep ether anesthesia, pregnant Wistar rats were decapitated and the uterus dissected out and placed on an ice-cold glass dish. Embryos (embryonic day 17) were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium). Embryos were removed and their brains placed into ice-cold culture medium (Eagle’s minimal essential medium).
which leads to an expression for the peak amplitude of the modeled multiquantal PSC

$$I(t_{\text{pp}}) = M_\text{max} \cdot \left( \frac{g}{m} \right) \left( \frac{m}{m-n} \right) \left( e^{-m} - e^{-m} \right)$$  \hspace{1cm} (4)

*Equations 3 and 4* allow an analytic determination of the dependence of time-to-peak and peak amplitude of the compound response on the quantal decay rate and the time course of release.

However, in the above calculation, the fact that quantal events themselves have a finite rise time is disregarded. In a second approach, therefore multiquantal responses were modeled by a difference of two exponentials

$$M^*(t) = A \cdot (e^{-t} - e^{-m})$$  \hspace{1cm} (5)

where $n$ is the rate constant of rise of the quantal response.

Calculating the maximum of the above function leads to an expression for the quantal rise time $t_{\text{pp}}$

$$t_{\text{pp}} = \frac{\ln n - \ln m}{m-n}$$  \hspace{1cm} (6)

Let $M_{\text{max}}$ be the peak amplitude of a quantal event; then $M_{\text{max}} = M^*(t_{\text{pp}})$. Inserting the expression for the rise time into Eq. 5 provides an alternative expression for the factor $A$ and finally results in an equation that describes the quantal current in terms of its peak amplitude and two rate constants representing the time course of the rising and the decay phases, respectively

$$M^*(t) = M_{\text{max}} \cdot \left( \frac{n}{m} \right) \left( \frac{m}{m-n} \right) \left( e^{-m} - e^{-m} \right)$$  \hspace{1cm} (7)

As discussed above, the occurrence of the quantal events in our model is governed by a single exponential decay of the form $G(t) = g \cdot e^{-\lambda t}$. Convolution leads to an expression for the time course of the compound IPSC

$$I(t) = \int_0^t M^*(t-r) \cdot G(r) \cdot dr$$

$$= M_{\text{max}} \cdot \left( \frac{n}{m} \right) \left( \frac{m}{m-n} \right) \times \left[ \frac{g}{n-g} (e^{-g} - e^{-g}) - \frac{g}{m-g} (e^{-g} - e^{-g}) \right]$$  \hspace{1cm} (8)

This expression is too complex to allow one to solve for the time-to-peak or peak amplitude of the compound PSC. However, these values can be easily determined graphically from the computed PSC waveforms $I(t)$.

**RESULTS**

**Modeling multiquantal IPSCs: an increase in peak amplitude due to a reduced quantal decay rate is coupled to a prolongation of time-to-peak**

We used a simple convolution model, as described in (METHODS), to calculate the dependence of rise time and peak amplitude on the quantal decay rate $m$. This value was chosen to be 0.05 ms$^{-1}$. This corresponds to a decay time constant of 20 ms, close to the mean value previously determined in this preparation (Behrends and ten Bruggencate 1998). The parameter $g$ represents the decay rate of the release function and therefore the degree of quantal synchrony. To produce PSC waveforms with rise times in the experimentally observed range (4.5 ± 0.3 ms, mean ± SE; see RESULTS) (see also Rumpel and Behrends 1999), $g = 0.5$ ms$^{-1}$ and $g = 1$ ms$^{-1}$ were chosen as boundary values for a first working estimate, resulting in time-to-peak of 3.15 and 5.12 ms, respectively.

Figure 1 provides a summary of the modeling results. Analytic results from Eqs. 3 and 4, disregarding the rise time of the quantal responses (see METHODS), are shown by continuous lines, whereas symbols represent the values obtained graphically from PSC waveforms calculated according to Eq. 8. Here, a rise time of the quantal events of 2 ms was assumed (Behrends and ten Bruggencate 1998). As shown in Fig. 1A, the time-to-peak of a compound IPSC increases with decreasing quantal decay rate. This confirms the intuition that with longer duration of quantal events, more later quanta can overlap with the earliest responses and add to the rising phase of the compound PSC. With rapidly decaying quantal events, only the very first quanta will contribute to the peak amplitude, thus keeping the rise time low.

As expected, inclusion of the rise time of quanta shifts the curves toward larger times-to-peak (symbols). However, the point of reference given by the experimentally determined mean time-to-peak of the compound IPSCs (4.5 ms) with an assumed quantal decay rate of 0.05 ms$^{-1}$ is straddled by the estimates using $g = 1$ ms$^{-1}$ and $g = 0.5$ ms$^{-1}$, irrespective of whether or not the quantal rise time is taken into account.

Similarly, Fig. 1B shows the change in PSC peak amplitude resulting from a slowing of quantal decay. PSC peak amplitude is given as a fraction of the maximal amplitude attained when quanta are released in perfect synchrony ($g \to \infty$) or become step functions with $m \to 0$. An isolated decrease in quantal decay rate, as expected when receptors are saturated at peak (see INTRODUCTION), does indeed result in an enhancement of PSC peak amplitude. The slope of the relationship obtained from Eq. 4 (continuous lines) depends on $g$, such that with more synchronous release, peak amplitude is affected less by changes in quantal decay. This is still obvious when accounting for the quantal rise time, as shown by symbols. However, quanta with a finite rise time superimpose more effectively than when they rise instantaneously. This effect shifts the relation toward larger relative peak amplitudes and decreases its slope. Therefore the effect on peak PSC amplitude of a change in quantal decay rate depends both on the synchrony of release and on the quantal rise time.

Figure 1C plots the increase in time-to-peak against the increase in peak amplitude of the compound response, both as a consequence of decreasing quantal decay rate. Depending on the degree of quantal asynchrony ($g$), the analytic results (continuous curves) predict that time-to-peak increases by between 1.8 and 2.3 ms, for an increase in peak amplitude by 15%. Note that, for this to occur, the quantal decay rate must decrease from 0.05 ms$^{-1}$ to 0.01644 ms$^{-1}$ ($g = 0.5$ ms$^{-1}$) or 0.00305 ms$^{-1}$ ($g = 1$ ms$^{-1}$), corresponding to a change from a quantal $\tau_{\text{decay}}$ of 20 ms to 61 or 328 ms. For amplitude increases >15%, the slope of the curves increases rapidly, leading to improbably high values for the increase in time-to-peak.

When the rise time of quanta is accounted for, this relationship is shifted to the left, and the steep increase in time-to-peak occurs even earlier (symbols). From our calculations, therefore, we can conclude that, with a pure change in quantal decay rate, for an increase in peak amplitude >10–15%, time-to-peak must increase by at least >1–2 ms.
Benzodiazepine agonist flunitrazepam causes a robust increase in IPSC decay time and a variable enhancement of peak amplitude

Application of 500 nM flunitrazepam typically resulted in an increase in duration as well as in amplitude of IPSCs (Fig. 2A). The IPSC decay was fitted by a bi-exponential function: \( I(t) = A_1 e^{(-t/\tau_1)} + A_2 e^{(-t/\tau_2)} \). In 22 experiments, 500 nM flunitrazepam increased the fast decay time \( \tau_1 \) (26.7 ± 2.5 ms in control, 40.7 ± 5.8 ms in flunitrazepam, \( P < 0.01 \)) as well as the slow decay time \( \tau_2 \) (97 ± 10 ms in control, 137 ± 13 ms in flunitrazepam, \( P < 0.01 \)). For a more comprehensive measure we calculated a weighted global time constant of IPSC decay (Rumpel and Behrends 1999). Control \( \tau_{\text{glob}} \) was 55.8 ± 4.1 ms and increased to 83.0 ± 4.9 ms in flunitrazepam (paired \( t \)-test: \( P < 0.0001 \)).

The enhancement of IPSC peak amplitudes was highly variable. Whereas in 18 of 22 experiments flunitrazepam evoked an increase in peak amplitude by up to 77%, in 4 experiments a small decrease was observed (−4.8 ± 0.2%). Averages of 10–20 traces immediately before and after flunitrazepam application revealed a mean increase in peak amplitude of 20.4 ± 5.1% (\( n = 22, P < 0.001 \), paired \( t \)-test). Because the effects of flunitrazepam on IPSC amplitude and decay were not readily reversible, a new dish of cells was used for each experiment.

In cells where flunitrazepam enhanced IPSC amplitudes (\( n = 18 \)), the mean increase in peak amplitude was by 26.0 ± 5.3%. As illustrated in Fig. 2B, the relative increases in peak amplitude and in global decay time correlate significantly (\( P = 0.012, R = 0.52, \text{correlation} \ z\text{-test}, \ n = 22 \)).

Enhancement by flunitrazepam of IPSC peak amplitudes is not accompanied by the expected increases in time-to-peak

To test the prediction of our model calculations, all experiments where flunitrazepam enhanced the IPSC peak amplitude (\( n = 18 \)) were assessed for changes in time-to-peak. IPSCs showed a mean rise time (0–100%) of 4.5 ± 0.3 ms in control and of 4.9 ± 0.4 ms in 500 nM flunitrazepam. The mean change in time-to-peak was small (+0.38 ± 0.11 ms; range: −0.8 to +1.3 ms) but significant (\( P < 0.01 \), paired \( t \)-test). Figure 3 plots the increase in time-to-peak against the fractional increase in peak IPSC amplitude. The experimental data and the theoretical results from Fig. 1C are shown superimposed. It is clear that most of the data points lie outside our predictions, i.e., the fractional increase in amplitude is far too large to be solely accounted for by enhanced superposition of quantal events because of a prolonged falling phase.

Flunitrazepam increases both peak amplitude and decay time of mIPSCs

mIPSCs were detected in the presence of TTX (1 \( \mu \)M). KCl (15 mM) was applied to increase their frequency. More than or equal to 100 events per cell were aligned by their rising phase and averaged. In control, the mean peak amplitude of mIPSCs was 28.57 ± 3.25 pA and the mean decay rate 0.0447 ± 0.0050 ms\(^{-1} \) (\( n = 5 \)). In 500 nM flunitrazepam, mIPSC peak amplitudes increased to a mean of 36.23 ± 3.11 pA. In all cases, the amplitude distributions before and after flunitrazepam exposure were significantly different with \( P < 0.001 \) (Kolmogorov-Smirnov test), and the mean decay rate of mIPSCs was reduced to 0.0354 ± 0.0034 ms\(^{-1} \) (\( P < 0.05 \), paired \( t \)-test). The mean increase in peak amplitude was by 26.8 ±

\( \Delta I_{\text{max}} / I_{\text{max}(m=0.05 \text{ms})} \)

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FIG. 1. Modeling results: slowing of quantal decays leads to a coupled increase in time-to-peak and amplitude of the compound response. Throughout this figure, continuous lines show predictions calculated from Eqs. 3 and 4 (METHODS), assuming instantaneously rising quanta, while the discrete values marked by symbols give results obtained from compound responses modeled according to Eq. 8 (METHODS), accounting for a quantal rise time of 2 ms. Also, results are given using 2 different values for \( g \), the decay rate of release: 1 ms\(^{-1} \) (thin lines, open triangles) and 0.5 ms\(^{-1} \) (thick lines, closed circles). A: time-to-peak of the compound response plotted against quantal decay rate. Note that the mean time-to-peak of 4.5 ms of inhibitory postsynaptic currents (IPSCs) with a decay rate of −0.05 ms\(^{-1} \) of miniature IPSCs (mIPSCs) in this preparation gives a data point (X) that lies between the results for the 2 values of \( g \), confirming that they are realistic boundary estimates for our preparation. B: peak amplitude of the compound response plotted against quantal decay rate. Peak amplitude is expressed as a fraction of the maximal amplitude that will be reached with fully synchronous release or with nondecaying quantal events. C: time-to-peak plotted against relative increase in peak amplitude of the compound response. See text for details.
6.6%, and the decay time constant increased by 26.3 ± 7.3%. The times-to-peak of mIPSCs before and after application of flunitrazepam were not significantly different (1.96 ± 0.25 vs. 2.05 ± 0.26 ms). Experimental results from one cell are shown in Fig. 4.

We can use these data to predict the enhancement of peak amplitude of the compound IPSC by the kinetic and amplitude changes observed in the miniature responses. Figure 5A shows a number of PSC waveforms (thin lines) calculated according to Eq. 8 for various values of quantal decay rate, m: 0.1, 0.0666 ..., 0.05, 0.0333 ..., 0.02, 0.01 ms⁻¹; corresponding to quantal decay time constants of 10, 15, 20, 30, 50, and 100 ms. The decay rate of release, g, was fixed at 1 ms⁻¹ and the quantal amplitude was kept constant. The two dashed lines show the two boundary conditions that will give the maximal PSC peak amplitude (here set to 1) for a given quantal size: g → ∞ and m → 0. The two bold lines are PSC waveforms calculated using the mean values for m observed before and after application of flunitrazepam but neglecting the change in quantal size. The time-to-peak of the calculated PSC changed from 4.03 to 4.24 ms (by 0.21 ms) while the amplitude increased from 0.940 to 0.949 (1%). Even a decrease of m to 0.01 ms⁻¹ (τdecay of 100 ms) results only in a 4% enhancement of peak amplitude. At the same time, the time-to-peak would have to increase by 1.43–5.46 ms.

With more asynchronous release (g = 0.5 ms⁻¹) the measured flunitrazepam-induced prolongation of quantal decay alone would produce a 2.1% increase in peak amplitude with an increase in time-to-peak by 0.38 ms (not shown).

In contrast, as shown in Fig. 5B, including the observed increase in quantal size by multiplying with a factor of 1.27 results in an increase in the peak response by 28% at g = 1. At g = 0.5 this increase would be by 30%.

We can therefore conclude that modeling the compound response with the values obtained from mIPSC recordings slightly overestimates the increase in peak amplitude.

**DISCUSSION**

Because of their action to increase agonist binding affinity at the GABAₐ receptor, benzodiazepines are a unique pharmacological tool for probing receptor occupancy at GABAₐ-ergic synapses. While from the first such experiments receptor saturation was concluded (De Koninck and Mody 1994; Mody et al. 1994), newer studies suggest that, at some synapses at least, postsynaptic receptors are not invariably saturated following quantal transmitter release (DeFazio and Hlabitz 1998; Frerking and Wilson 1996; Frerking et al. 1995; Mellor and Randall 1997; Nusser et al. 1997, 1999).
A recent report suggests that benzodiazepines might also increase the single channel conductance of the GABA_A receptor (Eghbali et al. 1997), which would invalidate any conclusions regarding receptor occupancy derived from their effects. However, in agreement with most other studies, flunitrazepam increased the open probability without a change in unitary conductance of GABA_A receptor–gated Cl^- channels in outside-out patches activated by 1–3 μM GABA in our preparation (data not shown). Therefore the fact that benzodiazepines potentiating mIPSC amplitude indicates a lack of receptor saturation.

As outlined in the INTRODUCTION, however, it is not self-evident that spontaneous quantal transmission is a good estimate for what happens at a single contact during impulse-evoked transmission. In the present work, therefore we attempted to adapt this kind of experiment to multiquantal or compound (as opposed to quantal) IPSCs. The problem, here, was that an increase in compound IPSC amplitude may be produced without a change in the number of channels open at the peak of quantal responses, but can be solely due to more efficient temporal summation because of a slower decay of quantal currents (De Koninck and Mody 1994; Mody et al. 1994). By mathematically predicting the increase in time-to-peak that will result from enhanced summation, we have shown here that it is possible to distinguish between this effect and a true increase in quantal size underlying an increased peak amplitude of the compound response. Comparison of these predictions with our experimental results obtained from striatal neurons in cell culture (cf. Fig. 3) indicates that an increase in quantal size must have occurred and therefore suggests that receptors were not saturated at individual contact sites during evoked IPSCs.

![Graph A](image1)

**GRAPH A.** Flunitrazepam increases the amplitude and decay time constant of mIPSCs without affecting their rise time. A: superimposed, averaged (n = 100) waveforms of mIPSCs before and after application of flunitrazepam (ftz, 500 nM). The mean mIPSC amplitude increased by 23.7% in this cell. Inset a: superimposed peak-scaled decay phases of mIPSCs. Note the slower decay after flunitrazepam application. B: cumulative amplitude distributions from this experiment. Note the shift toward larger amplitudes after application of flunitrazepam (P = 0.0014, Kolmogorov-Smirnov test). C: cumulative distributions of mIPSC rise times (0–100%). The thin lines are Gaussian fits. No significant change was detected (P = 0.356). Similar results were obtained in 4 other cells (see text). Inset c: superimposed, differentiated traces of average mIPSC waveforms before and after (*) application of flunitrazepam. Note the increase in the rate of current rise due to the increase in peak amplitude without a change in rise time.

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**GRAPH B.** Flunitrazepam increases the amplitude and decay time constant of mIPSCs without affecting their rise time. A: superimposed, averaged (n = 100) waveforms of mIPSCs before and after application of flunitrazepam (ftz, 500 nM). The mean mIPSC amplitude increased by 23.7% in this cell. Inset a: superimposed peak-scaled decay phases of mIPSCs. Note the slower decay after flunitrazepam application. B: cumulative amplitude distributions from this experiment. Note the shift toward larger amplitudes after application of flunitrazepam (P = 0.0014, Kolmogorov-Smirnov test). C: cumulative distributions of mIPSC rise times (0–100%). The thin lines are Gaussian fits. No significant change was detected (P = 0.356). Similar results were obtained in 4 other cells (see text). Inset c: superimposed, differentiated traces of average mIPSC waveforms before and after (*) application of flunitrazepam. Note the increase in the rate of current rise due to the increase in peak amplitude without a change in rise time.
Deconvolution of a compound PSC waveform with that of a miniature synaptic response has often been used to extract the time course of transmitter release (Dempster 1986; Diamond and Jahr 1995; Van der Kooi 1988a,b; Van der Kooi and Molgo 1994). Conversely, convolution may be used to estimate the waveform of a compound PSC given the average underlying quantal waveform and the release function. In the present context, it was our aim to understand the effect of changes in quantal decay rate on the peak amplitude and the time-to-peak of the compound response. By extracting, from the simplest form of this convolution, equations that can be solved for these variables, we were able to obtain a full, continuous description of this effect, including its dependence on the degree of asynchrony of release (cf. Fig. 1). To account for quantal rise time, values of peak amplitude and time-to-peak had to be obtained graphically from PSC waveforms calculated from a more complex expression (Eq. 8). This second approach results in an in even faster increase of time-to-peak with peak amplitude (Fig. 1C). This can be explained by the additional time available for summation.

It should be noted that, for all of these calculations, the release function was assumed to rise instantaneously followed by an exponential decline. For the problem under discussion, it is the effective duration of the phasic release function that is important, not its precise shape. This effective duration defines quantal synchrony and the time-to-peak of the compound response (given a constant quantal decay rate \( m \)). The values chosen for the decay rate of the release function (\( g \)) resulted in realistic times to peak and were therefore reasonable estimates of the effective duration of phasic release. Using a function of different shape, such as the gamma-function (Barrett and Stevens 1972; Geiger et al. 1997; Isaacson and Walmsley 1995), will not affect our results, provided that its parameters are chosen to give the same control time-to-peak of the compound PSC and therefore the same degree of quantal synchrony.

The release function can, in suitable preparations, be measured directly from the latency of single quanta at a low probability of release (Barrett and Stevens 1972; Isaacson and Walmsley 1995). However, in our preparation as in many others, release sites are distributed at various terminals of branching axons rather than at a single, large ending as at the neuromuscular junction or the endbulb of Held. Therefore the quantal latency distribution will be strongly dependent on axonal conduction, which has been shown to be affected by the interventions necessary to lower release probability, such as very low Ca\(^{2+}\) concentrations or application of Cd\(^{2+}\) (Lüscher et al. 1994). Therefore it is doubtful whether, in this situation, release kinetics can be inferred by this method.

Deconvolution estimates of the decay rate of the release function at other mammalian CNS synapses range from 3 ms\(^{-1}\) (Geiger et al. 1997; Williams et al. 1998) in slice preparations at temperatures >30°C, to 0.26 ms\(^{-1}\) (Diamond and Jahr 1995) in cell culture at 20–22°C. The boundary values we estimated from the control time-to-peak (1 and 0.5 ms\(^{-1}\)) are in good agreement with those findings. More precisely, the mean control time-to-peak of IPSCs in this study was 4.5 ± 0.3 ms, which, according to Eq. 8, with \( m = 0.0447\) ms\(^{-1}\) would correspond to a \( g \) of 0.81 ms\(^{-1}\).

However, measurements of time-to-peak are susceptible to error, particularly in the presence of noise, when filtering is used and when uncompensated series resistance and electrotonic distance slows the response of the voltage clamp. All three conditions must be considered for our recordings, and therefore our estimate of \( g \) probably represents a lower limit. Furthermore, the changes in time-to-peak we observed following flunitrazepam application were almost certainly too small to be accurately measured, despite the fact that they reached statistical significance. Nonetheless, the changes in time-to-peak predicted if the enhancement of IPSC amplitude was exclusively due to slower quantal decays should have been detectable (cf. Fig. 3): in 13 of 18 cases, IPSC amplitude was increased by ≥10%, which would have necessitated an increase in time-to-peak of at least 1 ms.

Voltage-clamp errors may also have affected our measurements of changes in IPSC amplitude, but will lead to an underestimation rather than an overestimation of amplitude increase. Therefore our main conclusion, that the observed increases in peak amplitude must be due to larger quantal size would be unaffected.

Finally, although such an effect has not been described, a strong synchronization of release by flunitrazepam might explain some of the increase in IPSC peak amplitude. However, as shown in Fig. 5A, even total synchronization (i.e., \( g \rightarrow \infty \)) would only increase peak amplitude by <10%. Also, such an effect would strongly reduce the time-to-peak of the IPSC, which we did not observe.

Conditions under which slower quantal decays can increase compound PSC amplitude

Which are the conditions that would enable an isolated decrease in quantal decay rate to produce significant changes in the peak amplitude of compound responses? In their original demonstration of this effect, De Koninck and Mody (1994) used summation of mIPSCs recorded from dentate granule cells before and after application of the BZ\(_1\)-selective agonist zolpidem. These mIPSCs were unchanged in amplitude, but their decay time constant was strongly slowed from a control value of about 5 ms. Quanta were summed with delays between their occurrence governed by an exponential function that had a time constant of 5 ms. This provides an exact match of our model of the release function, the time constant corresponding to \( g = 0.2\) ms\(^{-1}\). Equation 8 predicts that, indeed, with this relatively asynchronous release, compound peak amplitude is very sensitive to changes in quantal decay constant alone, changing by 28.3 and 56.0% for an increase in quantal decay time constant from 5 to 10 and 20 ms, respectively. At the same time, the time-to-peak of the compound responses increases by 1.93 and 4.24 ms, respectively. In this case, the assumption of asynchronous release (but see Williams et al. 1998) together with a rapid decay of control quanta explains the vigorous effect on peak amplitude. A small but significant contribution also results from the rapid rising phase of mIPSCs in dentate granules (<1 ms), which was accounted for in the above calculations. Thus strong increases in the peak amplitude of compound responses can occur as a result of changes in quantal decay rate when the decay time constant of the release function is not fast with respect to the kinetics of the quantal components. However, under these conditions as well, the
time-to-peak of the compound response increases in a predictable fashion, making it possible to separate effects due to changes in quantal kinetics from a possible additional change in quantal sizes.

**Variability of flunitrazepam action**

One of the most prominent features of flunitrazepam action in the present study was the high variability of changes in IPSC peak amplitude. The fact that the amplitude changes produced by flunitrazepam covary positively and significantly with the changes in decay time constant (Fig. 2B) might be taken to suggest that some of this variance is due to a heterogeneity in the flunitrazepam sensitivity of GABA_\text{A} receptors involved in different unitary connections. We do not have direct information about the subunit composition of the GABA_\text{A} receptors subserving inhibitory transmission in the cultured neurons studied here. It is possible that striatal neurons express several combinations of GABA_\text{A} receptor subunits, which may differ in their response to benzodiazepines. Flunitrazepam, unlike zolpidem, does not discriminate between BZ_1 and BZ_2 binding sites, and therefore the response to the agonist used here should be independent on the subtype of the \(\alpha\)-subunit expressed, which, in rat striatum is primarily of the \(\alpha_2\) variant (Fritschy and Mohler 1995; Fujiyama et al. 2000). On the other hand, it is not impossible that combinations of subunits exist at these contacts that make benzodiazepine-insensitive receptors, such as those containing the \(\delta\)- or \(\epsilon\)-subunits instead of the \(\gamma_2\)-subunit. The \(\delta\)-subunit is known to be expressed at moderate levels in normal rat striatum, while the \(\gamma_2\)-subunit is expressed abundantly (Fritschy and Mohler 1995).

An alternative explanation for the variability of flunitrazepam action is that the less benzodiazepine-responsive IPSCs are primarily mediated by saturated contacts, whereas unsaturated synapses contribute more to those where flunitrazepam has a strong effect. Higher receptor occupancy at a subset of contacts may result from lower numbers of postsynaptic receptors (Auger and Marty 1997; Nusser et al. 1997) or from the presence of subunit combinations with particularly high affinity for GABA (Auger and Marty 1997). Alternatively, a high peak cleft concentration of the transmitter may also increase receptor occupancy. Both high transmitter concentrations and high receptor affinity might be associated with longer IPSC decays because of longer presence of GABA or slower unbinding. In both cases, IPSCs with longer decay times in control would show less relative change in peak amplitude with flunitrazepam application. A significant negative correlation between these values is, indeed, present in our dataset (\(n = 22\), \(R = 0.541\), \(P < 0.01\), correlation z-test).

In 4 of 22 experiments, we observed a small decrease in peak amplitude after application of flunitrazepam. The most likely reason for this decrease is tonic GABA_\text{A} receptor desensitization by subliminal concentrations of tonically released GABA (Brickey et al. 1996; Gaspary et al. 1998; Overstreet et al. 1999; Wall and Usowicz 1997). This effect would be enhanced when the binding affinity of the GABA_\text{A} receptors is increased by flunitrazepam. At synapses where tonic release of GABA is strong, it may outweigh the positive effect on responses to phasic GABA release. However, we cannot exclude the possibility that some GABA_\text{A} receptors can display an inverse response to flunitrazepam.

The increase in peak amplitude of the compound response calculated from the flunitrazepam-induced change in peak amplitude and kinetics observed with mIPSCs slightly overestimated the experimentally observed changes in compound IPSC amplitude, suggesting that, on average, more contacts are saturated with evoked transmission than with spontaneous release. However, in 3 of 22 cases, the increase in compound IPSC amplitude was several times larger than the mean increase in mIPSC size (cf. Fig. 3). In these cases, it is possible that some contacts were involved in generating these IPSCs that had extremely low receptor occupancy. Responses arising at such contacts might be too small under control conditions to be detected in mIPSC recordings. The strong change in amplitude likely to arise from flunitrazepam treatment at such contacts would then not contribute to the change in mIPSC amplitude distribution, because they did not contribute to the control sample. In fact, following flunitrazepam application, they would probably still be smaller than the control average, in effect subtracting from the measured change in mean mIPSC amplitude.

In summary, we have used modeling of compound synaptic responses to distinguish between the contribution of a change in quantal size and quantal kinetics to benzodiazepine-induced increases in the peak amplitude of IPSCs in rat striatal neurons. In our preparation, we conclude that only an increase in quantal size can explain our experimental findings, strongly suggesting that during impulse-evoked transmission postsynaptic receptors are not uniformly saturated. The approach used here may be useful for similar studies at other synapses where different conditions may prevail.

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