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Extracellular glutamate diffusion determines the occupancy of glutamate receptors at CA1 synapses in the hippocampus

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Following exocytosis at excitatory synapses in the brain, glutamate binds to several subtypes of postsynaptic receptors. The degree of occupancy of AMPA and NMDA receptors at hippocampal synapses is, however, not known. One approach to estimate receptor occupancy is to examine quantal amplitude fluctuations of postsynaptic signals in hippocampal neurons studied *in vitro*. The results of such experiments suggest that NMDA receptors at CAl synapses are activated not only by glutamate released from the immediately apposed presynaptic terminals, but also by glutamate spillover from neighbouring terminals. Numerical simulations point to the extracellular diffusion coefficient as a critical parameter that determines the extent of activation of receptors positioned at different distances from the release site. We have shown that raising the viscosity of the extracellular medium can modulate the diffusion coefficient, providing an experimental tool to investigate the role of diffusion in activation of synaptic and extrasynaptic receptors. Whether intersynaptic cross-talk mediated by NMDA receptors occurs *in vivo* remains to be determined. The theoretical and experimental approaches described here also promise to shed light on the roles of metabotropic and kainate receptors, which often occur in an extrasynaptic distribution, and are therefore positioned to sense glutamate escaping from the synaptic cleft.

Keywords: glutamate spillover; diffusion; tortuosity; hippocampus

1. INTRODUCTION

In spite of major advances in understanding the mechanisms of neurotransmitter exocytosis, considerable uncertainty surrounds the spatial and temporal extent of the extracellular glutamate pulse evoked by presynaptic release at central synapses (Clements *et al*. 1992; Clements 1996; Barbour & Häusser 1997). This uncertainty reflects several outstanding questions about presynaptic vesicles. In particular, estimates of the glutamate contents are inexact (Burger *et al*. 1989; Bruns & Jahn 1995; Maycox *et al*. 1990), and it is unclear whether vesicles always discharge their entire contents upon fusion with the presynaptic membrane (Alvarez de Toledo *et al*. 1993). There is also some debate whether more than one vesicle can be released from a given release site in response to action potential invasion (Tong & Jahr 1994; Auger *et al*. 1998). Among other factors that preclude establishing a unique spatio-temporal glutamate concentration profile is the observation that synaptic geometries vary extensively, even within an anatomically defined pathway (e.g. Harris & Stevens 1989). Finally, glutamate transporters play a complex and incompletely understood role, not only in clearing the transmitter from the extraqcellular space, but also in buffering the diffusion of glutamate molecules away from the site of exocytosis (Diamond & Jahr 1997).

We have recently adopted a number of complementary approaches to gain a better understanding of the extracellular glutamate kinetics at CAl synapses in the rodent hippocampus *in vitro*.

2. QUANTAL ANALYSIS

First, we have examined the amplitude fluctuations of excitatory postsynaptic potentials or currents (EPSP/Cs) evoked in CAl pyramidal cells by minimal stimulation of afferent fibres. If EPSP/Cs showed tight clustering about integer multiples of a quantal amplitude, then one could conclude that the underlying quantum is relatively invariant. Taken together with estimates of the number of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors opening and of their channel kinetics, this approach could potentially shed light on the question of whether the glutamate concentration profile was sufficient to bind to the majority of receptors within the synaptic cleft. For example, if approximately 20 AMPA receptors open, and the quantal coefficient of variation (CV) is < 0.15, then the average probability of AMPA receptors being bound by glutamate and opening must be > 0.6 (Kullmann 1993).

In many cases, EPSP/C amplitude fluctuations show a broad distribution, without clearly discernible clustering at integer multiples of a quantal unit (Raastad *et al*. 1992). Although this finding is suggestive of a large quantal variability, which conceals the underlying probabilistic structure of transmission, there are also

many examples of clearly defined quantal peaks in histograms of evoked EPSC/P amplitudes (Kullmann & Nicoll 1992; Liao *et al*. 1992; Paulsen & Heggelund 1996; Larkman *et al*. 1991, 1997; Stricker *et al*. 1996). Some of these examples have been shown by Monte-Carlo methods to be highly unlikely to have arisen by chance (e.g. Kullmann 1993; Larkman *et al*. 1997).

The quantal analysis approach thus gives mixed results: in some, but by no means all, cases a relatively low quantal CV (<0.2) is seen. This implies that, at some synapses, the glutamate concentration following exocytosis may be sufficiently high to cause a large proportion of postsynaptic AMPA receptors to open. In those cases where quantal clustering is not seen, implying a broad quantal variability, there are two possible explanations. First, the trial-to-trial amplitude variability at each individual release site ('intrasynaptic' variability) may be large: if the CV exceeds approximately 0.3, one does not actually expect to detect peaks in finitely sampled, multiquantal histograms of evoked response amplitudes. Second, the different release sites that contribute to the postsynaptic response may each have a low intrinsic amplitude variability, but if their means are different (and do not, fortuitously, coincide with a simple ratio), the resulting multiquantal amplitude distribution will again have shallower peaks and troughs than expected ('intersynaptic' variability). In order to choose between these possibilities, a potentially powerful method is to record from individual synapses, to determine their intrinsic variability. Indirect electrophysiological methods have been used to identify such 'single synapse' EPSCs (Dobrunz & Stevens 1997), suggesting a quantal CV of *ca*. 0.4. This is comparable to the variability seen in ultrastructurally identified inhibitory synapses with a single release site (Gulyás *et al*. 1993). Recording directly from individual excitatory synapses in cultured hippocampal neurons, Forti *et al*. (1997) estimated the quantal CV as *ca*. 0.3, which was lower than the overall variability of spontaneous quantal events originating throughout the neuron (CV ≈ 0.7). It may therefore be concluded that both intra- and intersynaptic variabilities contribute to conceal quantal peaks and troughs in evoked EPSP/C amplitude distributions, but the fact that they are sometimes seen strongly suggests that both types of variability can sometimes be low.

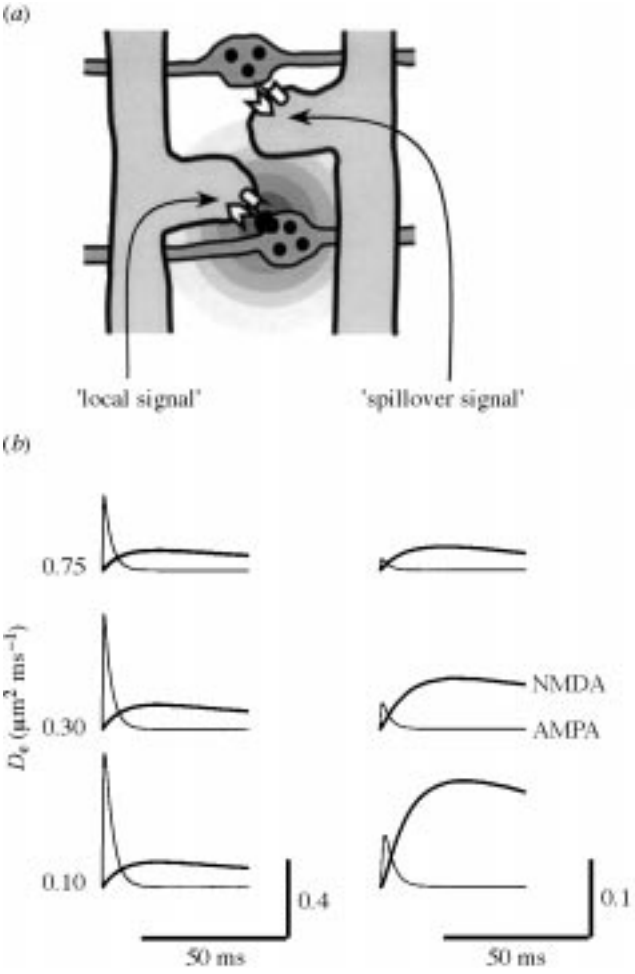

Figure 1. The glutamate spillover hypothesis. (*a*) Cartoon showing glutamate released at one synapse (bottom), where it activates the postsynaptic receptors ('local' response). If it also diffuses to the neighbouring synapse (top) it may activate receptors there too ('spillover' response). (*b*) Effect of varying the extracellular diffusion coefficient D_e on the activation of AMPA and NMDA receptors. The curves show the simulated time-course of the opening probability resulting from release of 5000 molecules of glutamate, with receptor kinetics given by Jonas *et al*. (1993) and Lester & Jahr (1992). A distance of 465 nm was assumed to separate one synapse from its nearest neighbour (see Rusakov & Kullmann (1998*a*) for details of the simulations). The local AMPA receptor-mediated signal and the spillover NMDA receptor-mediated signal both increase steeply as D_e is decreased.

3. SPILLOVER

Second, we have obtained evidence that the glutamate transient at CA1 synapses may be sufficiently widespread to bind not only to receptors within the synaptic cleft where it is released, but also to high-affinity *N*-methyl-D-aspartic acid (NMDA) receptors at neighbouring synapses (Kullmann *et al*. 1996; Kullmann & Asztely 1998; figure 1*a*). This 'spillover' hypothesis is prompted in large part by comparing the trial-to-trial amplitude fluctuations of AMPA and NMDA receptor-mediated EPSCs in CA1 cells. The pharmacologically isolated NMDA component generally shows less variability than the AMPA component, as expected if more quanta were sensed by NMDA than by AMPA receptors. In keeping with this suggestion, minimal stimulation-evoked EPSCs show fewer failures of transmission when recorded under conditions where NMDA receptors contribute to the postsynaptic signal than when the signal is mediated exclusively by AMPA receptors (Isaac *et al*. 1995; Liao *et al*. 1995; Durand *et al*. 1996; Asztely *et al*. 1997; Min *et al*. 1998*a*). These findings can be explained by proposing that glutamate escaping from the synaptic cleft is able to activate high-affinity NMDA receptors but not low-affinity AMPA receptors at synapses in close proximity to releasing terminals. An alternative explanation, which does not rely on intersynaptic glutamate diffusion, is that AMPA receptors are non-functional or absent at a proportion of synapses, although they might be recruited by postsynaptic induction of long-term potentiation (Kullmann 1994; Isaac *et al*. 1995; Liao *et al*. 1995; Durand *et al*. 1996; Malenka & Nicoll 1997). This explanation has prompted the use of the term 'silent

synapse' to describe synaptic signals mediated exclusively by NMDA receptors. Although both phenomena may coexist, one observation that is difficult to reconcile with the 'latent AMPA receptor cluster' hypothesis is that, if the experiments are repeated at physiological temperature (as opposed to room temperature), the discrepancy in behaviour of the AMPA and NMDA receptor-mediated components is much smaller (Asztely *et al*. 1997; Min *et al*. 1998*a*). This observation, however, can be explained by enhanced extrasynaptic glutamate clearance, reducing the extent of spillover. In support of such a role for glutamate uptake, pharmacological blockade of transporters partially reverses the effect of raising the recording temperature (Asztely *et al*. 1997). Intersynaptic cross-talk may thus be, at least in part, an artefact of the subnormal recording temperature at which most *in vitro* experiments are carried out. This interpretation also calls for caution in extrapolating estimates of the spatio-temporal extent of glutamate diffusion following exocytosis obtained *in vitro* to the brain *in vivo*.

4. SIMULATIONS

The third approach that we have adopted is to reconstruct the elementary events surrounding glutamate diffusion by numerical simulation (Holmes 1995; Kleinle *et al*. 1996; Wahl *et al*. 1996; Barbour & Häusser 1997; Uteshev & Pennefather 1997; Rusakov & Kullmann 1998*a*). We computed the spatial and temporal glutamate concentration profile within and around the synaptic cleft, guided by a detailed ultrastructural analysis of the synaptic geometry and available estimates of the contents of a vesicle and of the distribution and kinetics of transporters (Rusakov & Kullmann 1998*a*). We obtained a reasonably general solution by treating the extracellular space surrounding the synaptic cleft as an isotropic porous medium (Nicholson & Syková 1998). Diffusion in such a medium can be described by Fick's second law:

$$\frac{\partial C}{\partial t} = D_{\mathrm{p}} \nabla^2 C,$$

where C is the glutamate concentration and D_{p} is the apparent diffusion coefficient. D_{p} is related to the diffusion coefficient in a free medium D_{f} by $D_{\mathrm{p}} = D_{\mathrm{f}}/\lambda_{\mathrm{g}}^2$, where λ_{g} is a 'tortuosity' factor. λ_{g} is effectively the porous-to-free ratio of the diffusion path length, which we estimated by applying principles of integral geometry to electron micrographs of the CA1 neuropil. Although the results of these simulations are contingent on the validity of the underlying parameter estimates, the physiologically plausible parameter domain can be extensively explored in an attempt to derive some robust conclusions, which can then be related to published kinetic schemes for glutamate receptors (Lester & Jahr 1992; Jonas *et al*. 1993). Among the conclusions thus obtained is the (perhaps counter-intuitive) finding that AMPA and NMDA receptors are more likely to be activated if the extracellular diffusion coefficient for glutamate D_{e} is low. (D_{e} describes the speed of diffusion within the extracellular matrix, and must be substituted for D_{f} in the expression above.) If glutamate diffuses away from the release site slowly, it persists at a sufficient concentration and for a sufficient length of time to allow a large proportion of the available receptors to open. If, on the other hand, glutamate diffuses rapidly, its concentration falls off too quickly for receptors to open with a high probability (figure 1*b*).

The diffusion coefficient for glutamate in the extracellular space D_{e} has not been measured experimentally, but is likely to be less than the diffusion coefficient in free aqueous solution D_{f}, which is generally given as *ca*. $0.75\,\mu\mathrm{m}^2\,\mathrm{ms}^{-1}$ (see below). Over a plausible range (0.1–$0.75\,\mu\mathrm{m}^2\,\mathrm{ms}^{-1}$), changing D_{e} has markedly different effects on the occupancy of AMPA and NMDA receptors, as well as on the extent of extrasynaptic signalling by the two classes of receptors. We computed the receptor occupancy by adopting the following parameter estimates:

(i) number of glutamate molecules released: 5000 (Bruns & Jahn 1995);
(ii) resting extracellular glutamate concentration: $0.6\,\mu\mathrm{M}$ (Bouvier *et al*. 1992);
(iii) density of transporters in the extracellular space: $0.1\,\mathrm{mM}$ (Takahashi *et al*. 1996);
(iv) transporter binding, unbinding and translocation rate constants: $5\times10^6\,\mathrm{M}^{-1}\mathrm{s}^{-1}$, $100\,\mathrm{s}^{-1}$, $20\,\mathrm{s}^{-1}$, respectively (Diamond & Jahr 1997; Wadiche *et al*. 1995).

Within the synaptic cleft, our simulations suggest that the average opening probability of AMPA receptors at the peak of the response is *ca*. 0.38 when $D_{\mathrm{e}} = 0.75\,\mu\mathrm{m}^2\,\mathrm{ms}^{-1}$, but that this increases to 0.58 when $D_{\mathrm{e}} = 0.3\,\mu\mathrm{m}^2\,\mathrm{ms}^{-1}$ and 0.78 when $D_{\mathrm{e}} = 0.1\,\mu\mathrm{m}^2\,\mathrm{ms}^{-1}$. In contrast, the opening probability of NMDA receptors only changes from 0.10 to 0.13 over the same range of D_{e}. This difference reflects the much higher affinity of NMDA than AMPA receptors for glutamate (Patneau & Mayer 1990): NMDA receptors are close to saturation even when D_{e} approaches D_{f}, while the occupancy of AMPA receptors is incomplete even with the smallest values of D_{e}. The lower absolute opening probabilities of NMDA receptors result in part from their kinetics in response to a brief pulse of glutamate, but also from greater steady-state desensitization.

Although varying D_{e} has a much bigger impact on AMPA than on NMDA receptor function within the synaptic cleft, it has an opposite effect on extrasynaptic signalling by the two receptor classes. Since extrasynaptic NMDA receptors are generally only found in a very low concentration, an important question raised by the spillover hypothesis is whether glutamate diffusing out of one synapse can reach the receptors at another synapse. We have estimated the distance separating one hippocampal synapse (in CA1 or in the dentate gyrus) from its nearest neighbour to be *ca*. $0.5\,\mu\mathrm{m}$, taking into account tissue shrinkage during fixation (Rusakov & Kullmann 1998*a*; Rusakov *et al*. 1998). At this distance, if 5000 molecules are released, and if the extracellular diffusion coefficient D_{e} is the same as in free solution ($D_{\mathrm{e}} = D_{\mathrm{f}} \approx 0.75\,\mu\mathrm{m}^2\,\mathrm{ms}^{-1}$), our numerical simulations predict that the opening probability of NMDA receptors at the neighbouring synapse is approximately 15% of that of the receptors at the 'donor' synapse. If, however,

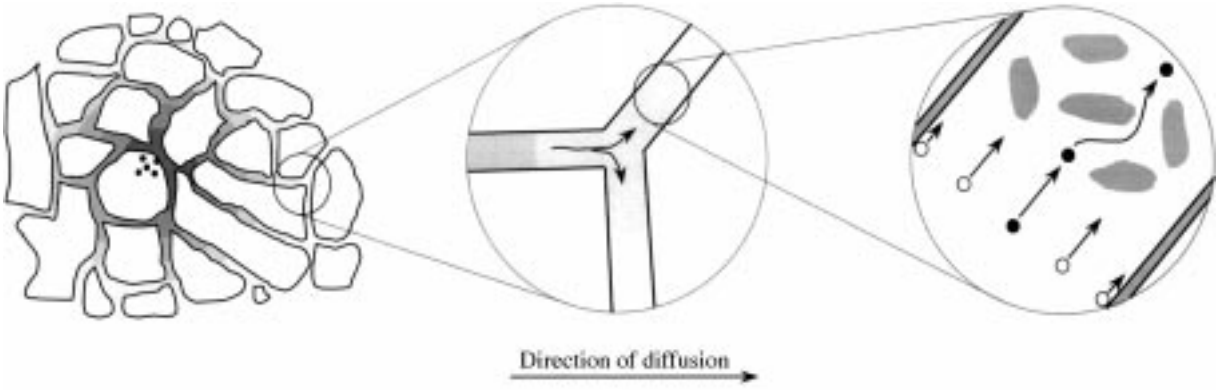

Figure 2. Geometric and viscous components of tortuosity. The panel at the left illustrates the diffusion of a substance (e.g. neurotransmitter) from a focal source. Diffusion is retarded by cellular obstacles (expanded section in centre), a process that can be accounted for by geometric tortuosity. In addition, two processes impede the movement of diffusing particles in the extracellular space (expanded section at right): macromolecules act as obstacles and cell walls exert a drag effect. Other factors that affect the spread of glutamate (in particular, active uptake and local diffusion barriers) are not shown.

$D_e < D_f$, the relative opening probability at the neighbouring synapse increases steeply: to 26% when $D_e = 0.3\,\mu m^2\,ms^{-1}$ and to 52% when $D_e = 0.1\,\mu m^2\,ms^{-1}$. AMPA receptors, in contrast, do not respond appreciably to glutamate escaping the synaptic cleft because their affinity is much lower: expressed as a fraction of the opening probability within the donor synaptic cleft, the opening probability at the neighbouring synapse is 2% when $D_e = 0.75\,\mu m^2\,ms^{-1}$, only increasing to 5% when $D_e = 0.1\,\mu m^2\,ms^{-1}$ (figure 1*b*).

5. EXTRACELLULAR DIFFUSION COEFFICIENT

The opening probability of glutamate receptors at hippocampal synapses depends on many parameters, some of which are known with insufficient precision to treat the above estimates with a high level of confidence. Nevertheless, our simulations illustrate clearly the mechanism by which the extracellular diffusion coefficient for glutamate plays a critical role in determining the extent of activation of glutamate receptors, both within the synapse where release occurs and at neighbouring synapses. Although glutamate diffusion is likely to be affected by several phenomena, and D_e may not have a unique value throughout the brain, we have begun to address the phenomena that determine it with a theoretical approach, which leads to experimentally testable predictions. The apparent diffusion coefficient for small molecules confined to the extracellular space has been estimated from the movement of inorganic ions, such as tetramethylammonium (TMA^+), injected iontophoretically and detected with ion-selective electrodes (Nicholson & Syková 1998). When compared to the diffusion coefficient in free solution, this approach has yielded estimates of the tortuosity of the extracellular space. A consistent observation is that the tortuosity varies with the volume fraction occupied by cells. That is, under conditions of cellular oedema, such as during experimental models of ischaemia, or when extracellular osmolarity is manipulated directly, the tortuosity factor increases from a typical value of *ca.* 1.6 to values approaching 2.2 (Nicholson & Syková 1998). A distinction must, however, be made between the tortuosity caused by obstacles that can be seen on electron micrographs ('geometric' tortuosity λ_g) and that caused by interactions with extracellular macromolecules ('viscosity' component of tortuosity λ_v) (figure 2). Indeed, we have recently argued that λ_g for any random assembly of space-filling obstacles actually has a unique value, approximately 1.4 for radial diffusion, as long as the surfaces have no preferred orientation (Rusakov & Kullmann 1998*b*). The major effect of altering the cell volume fraction must therefore be accounted for in some way by changes in λ_v.

If macromolecules are anchored to the cell walls, their effective concentration in the extracellular space will increase when extracellular water shifts into the intracellular compartment, while the overall increase in path length imposed by cell processes may not change. This means that the contribution of viscosity to the overall tortuosity will increase. In other words, the dependence of the overall tortuosity λ_{tot} on the volume fraction occupied by cells can be explained by changes in λ_v (Rusakov & Kullmann 1998*b*). In support of this account, perfusing tissue with high molecular weight dextran increases λ measured for TMA^+ ions (Prokopová *et al.* 1996). We have developed a model to explain the relationship between λ_v and the fraction of the extracellular space occupied by macromolecules, and have shown consistency with the experimental data on TMA^+ diffusion. What do these results say about the activation of AMPA and NMDA receptors?

Under baseline conditions, $\lambda_{tot} \approx 1.6$ (Nicholson & Syková 1998). If $\lambda_g = 1.4$ (Rusakov & Kullmann 1998*b*), setting $\lambda_{tot} = \lambda_v \lambda_g$ implies $\lambda_v \approx 1.14$. This suggests an upper limit for the diffusion coefficient for glutamate in the extracellular space: $D_e = D_f/\lambda_v^2 \approx 0.57\,\mu m^2\,ms^{-1}$. This is an upper limit because it does not consider other phenomena that can retard the movement of glutamate molecules. At least two processes may contribute to reduce D_e further. First, local diffusion bottlenecks could cause a deviation in the spatiotemporal glutamate profile from that predicted by adopting this value of D_e. For instance, we have observed a decrease of *ca.* 40% in the intermembrane distance at the perimeter of synaptic clefts in rat CA1 (Rusakov &

Kullmann 1998*a*). If extracellular macromolecules are more densely packed in this region, they could represent a significant barrier to the movement of glutamate and locally reduce the apparent diffusion coefficient to *ca.* $0.15\,\mu m^2\,ms^{-1}$ (Rusakov & Kullmann 1998*b*). Second, interactions between glutamate and binding sites will further retard its movement away from its site of release: if a molecule binds reversibly to a site it is effectively unavailable for diffusion down the concentration gradient during the time spent in the bound state. Under conditions of relatively low glutamate level this form of buffered diffusion can be characterized by the following formula for the diffusion coefficient in the presence of binding sites D_e':

$$D_e' = \frac{D_e}{1 + [B]/K_d^*},$$

where $[B]$ is the total concentration of binding sites and K_d^* is an apparent dissociation coefficient for the binding sites (Zador & Koch 1994; Wagner & Keizer 1994; Rusakov & Kullmann 1998*a*). This implies that diffusion can be significantly slowed by the presence of a high concentration of binding sites with a relatively low affinity for glutamate. However, when the glutamate level is relatively high, the binding sites are likely to soak up a large part of the vesicle contents if they are unoccupied prior to exocytosis. It remains to be determined whether binding sites with the appropriate properties are present in or around the synaptic cleft, and whether they are normally partly occupied by glutamate.

6. EXPERIMENTAL MANIPULATION OF VISCOSITY

Prompted by the theoretical analysis described above, we have recently tested the hypothesis that retarding diffusion should alter the occupancy of glutamate receptors. Adding high molecular weight dextran (40 kDa) to artificial cerebrospinal fluid in a 5% concentration (and corrected for changes in osmolarity) more than doubles its viscosity. This is an inert polysaccharide that penetrates the neuropil (Nicholson & Tao 1993) and so offers the possibility of altering the speed of glutamate diffusion following exocytosis. Our calculations suggest that this concentration of dextran should almost halve the extracellular diffusion coefficient: ignoring diffusion bottlenecks and effects of binding sites, D_e should decrease from $0.57\,\mu m^2\,ms^{-1}$ to $0.30\,\mu m^2\,ms^{-1}$. This change should significantly enhance the occupancy of AMPA receptors within the synaptic cleft where glutamate is released. Our simulations, however, suggest that it should have a much smaller effect on the occupancy of NMDA receptors at the same site, since they are almost saturated even with the fastest diffusion coefficients.

We monitored the effect of perfusing guinea pig hippocampal slices with 5% dextran, either by recording evoked CA1 EPSCs in whole-cell mode or by monitoring extracellular field EPSPs (Min *et al.* 1998*b*; figure 3). Overall, dextran perfusion reversibly enhanced the amplitude of AMPA receptor-mediated EPSP/Cs by $16 \pm 8\%$ (mean $\pm$ s.e.m.). NMDA receptor-mediated EPSP/Cs were enhanced by $20 \pm 6\%$. The increase in

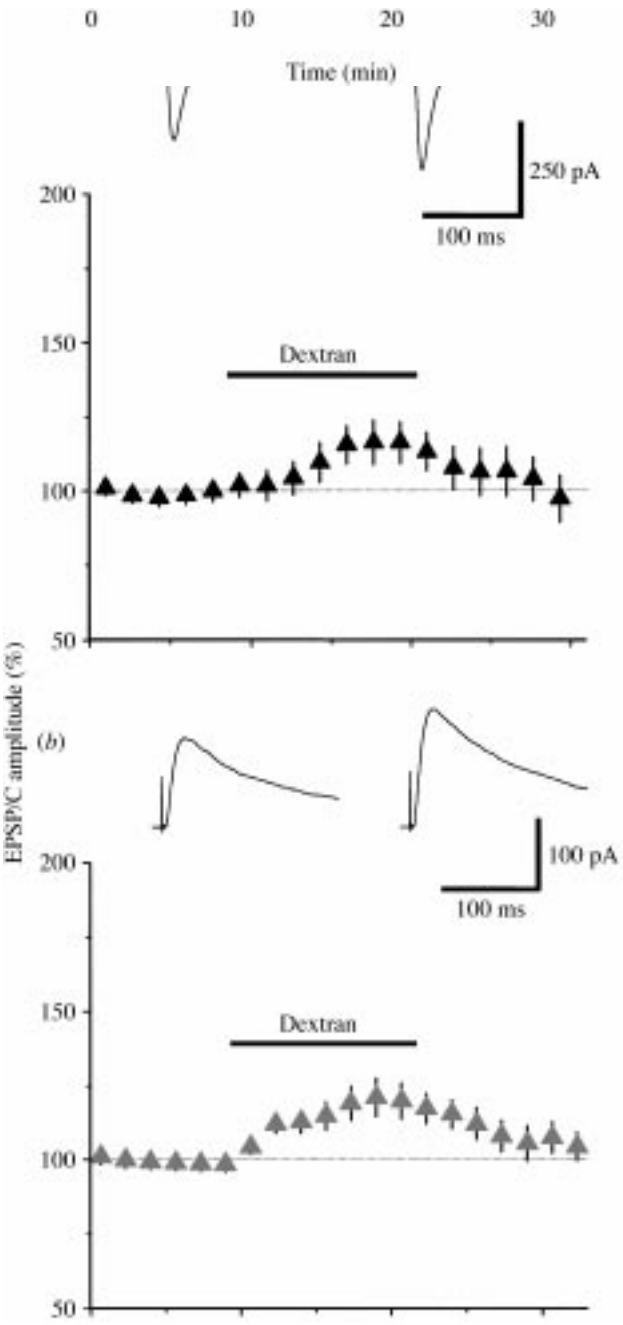

Figure 3. Effect of increasing the medium viscosity on AMPA and NMDA receptor-mediated transmission at Schaffer collateral synapses. (*a*) Effect of dextran perfusion on AMPA receptor-mediated Schaffer collateral signals recorded in CA1 pyramidal cells. Similar results were obtained whether extracellular EPSPs or whole-cell EPSCs were recorded. (*b*) Effect of dextran perfusion on NMDA receptor-mediated fEPSPs and EPSCs. Insets: EPSC time-course before (left) and during (right) dextran perfusion. Data from Min *et al.* (1998*b*).

the AMPA receptor-mediated component can be explained by an increase in occupancy of synaptic receptors. The fact that the NMDA receptor-mediated EPSP/Cs increased at least as much as the AMPA component, however, points to enhanced activation of receptors at a distance from the release site, because

receptors within the synaptic cleft where glutamate is released should be close to saturation.

How do these effects of dextran perfusion compare quantitatively with the expected effect of manipulating the glutamate diffusion coefficient? This question can be addressed by using the model and parameter estimates adopted by Rusakov & Kullmann (1998*a*) and asking what proportion of AMPA and NMDA receptors open at the synapse where release takes place and at its nearest neighbour. Adding together the response elicited at the two sites should give an estimate of the receptor activation in the face of different extracellular diffusion coefficients. Decreasing D_e from $0.57\,\mu m^2\,ms^{-1}$ to $0.30\,\mu m^2\,ms^{-1}$ was associated with an approximate 40% increase in the overall opening probability of the AMPA receptors, while the opening probability of the NMDA receptors increased by *ca.* 19%. That is, the increase in the simulated NMDA component agrees well with that observed experimentally. The predicted increase in the AMPA component was, however, larger than observed. The agreement can be improved by adjusting one or more of the parameters in the simulation. These include the number of glutamate molecules released, the density and kinetics of transporters, and the resting level of glutamate, which determines the degree of steady-state receptor desensitization. Briefly, if the extracellular diffusion coefficient is lower under baseline conditions (prior to adding dextran), the occupancy of synaptic AMPA receptors within the cleft where release takes place will approach 100%. Under these conditions, dextran will have less effect on the AMPA component, but should still enhance the spillover component of the NMDA component. For instance, if D_e is *ca.* $0.25\,\mu m^2\,ms^{-1}$ under baseline conditions, and decreases to *ca.* $0.13\,\mu m^2\,ms^{-1}$ with dextran, then the overall opening probability of both AMPA and NMDA receptors, estimated at the synapse where release takes place and at the nearest neighbour, increases by *ca.* 20%, in agreement with the experimental data. Alternatively, a similar improvement in agreement between the simulations and experimental results can be obtained by increasing the number of glutamate molecules released: this again has the effect of enhancing receptor occupancy, leaving less room for slowing of diffusion to increase the size of the postsynaptic response.

7. CONCLUSIONS

The results from the different approaches described above can be summarized as follows. First, the finding that EPSC amplitudes at CA1 synapses are sometimes (but not always) clustered, with a relatively low quantal variability, implies that AMPA receptors may have a reasonably high probability of opening in response to glutamate release. Second, there is circumstantial evidence that (at least at room temperature) glutamate can diffuse from one synapse, where it is released, to a neighbouring synapse where it activates NMDA receptors. Third, the results of simulations of glutamate release, diffusion, uptake and receptor activation are compatible with appreciable intersynaptic cross-talk and show that it is more pronounced when the extracellular diffusion coefficient is low. Finally, prompted by a theoretical analysis of the factors that retard the movement of molecules in the extracellular space, we have found that increasing extracellular viscosity enhances receptor activation. These approaches point to the following conclusion: presynaptic activity can result in high AMPA receptor occupancy within the CA1 synapse where release takes place, and near-saturation of NMDA receptors. In addition, NMDA (but not AMPA) receptors at nearby synapses are also activated by glutamate escaping the synaptic cleft. Taken together, this work underlines the critical role that diffusion plays in synaptic signalling and in intersynaptic cross-talk.

An important project for the future is to estimate receptor occupancy and the spatial extent of receptor activation under more physiological conditions. Intersynaptic cross-talk would compromise the specificity of synaptic communication, so it would be surprising if glutamate spillover occurred in the living brain. Indeed, we have observed a smaller discrepancy in quantal content mediated by AMPA and NMDA receptors at physiological temperature than at room temperature. Another important issue is the degree to which the principles that apply at CA1 synapses can be extrapolated to other synapses, whether glutamatergic or mediated by other transmitters. Scanziani *et al.* (1997) have suggested that extrasynaptic group 2 metabotropic receptors at mossy fibre synapses in the hippocampus are activated by synaptically released glutamate (see also Min *et al.* 1998*b*). Since the candidate receptors are found in the preterminal membrane (Yokoi *et al.* 1996), glutamate must diffuse a considerable distance to reach them. Mossy fibre synapses also present further challenges for the understanding of the role of diffusion in activation of synaptic receptors, since, unlike CA1 synapses, they contain multiple active zones and postsynaptic densities (Chicurel & Harris 1992). In this system, as well as in other calyceal synapses, such as in the auditory pathway in the brainstem, glutamate diffuses within the cleft and may spread from one release site to receptors located opposite other release sites (Trussell *et al.* 1993). We have recently found that dextran perfusion causes a much larger enhancement of AMPA receptor-mediated EPSPs at mossy fibre synapses than at CA1 synapses (Min *et al.* 1998). This observation can be explained by slowing glutamate diffusion, which enhances the extent of cross-talk between neighbouring clusters of AMPA receptors within the synapse. In contrast to CA1, where glutamate only affects AMPA receptors at the (usually) solitary postsynaptic density (Harris & Stevens 1989), glutamate within mossy fibre synapses may affect receptors at several closely spaced densities.

Finally, a large body of work has shown that exogenous application of agonists at the kainate subtype of glutamate receptors profoundly depresses GABA release, in the apparent absence of axo-axonic synapses (Clarke *et al.* 1997). Kainate and metabotropic receptors are frequently found at a distance from glutamate release sites, leading to the suggestion that they are activated by glutamate escaping from the synaptic cleft (Baude *et al.* 1993; Lerma 1997). Whether synaptically released glutamate is able to activate them under physiological conditions remains to be determined.

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