Intersynaptic diffusion of neurotransmitter

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According to many theories of brain function, the computational power of the brain depends upon the number of independent synapses it contains. A synapse will not be independent if its receptors are activated or modified by neurotransmitter released at neighbouring synapses. Recently, there have been several reports suggesting the occurrence of 'crosstalk' or 'spillover', and a large number of results consistent with crosstalk. However, the quantitative importance of this phenomenon remains uncertain. We estimate the significance of crosstalk using a simple model which predicts that, during concentrated synaptic activity, crosstalk between distinct synapses is likely to activate high-affinity receptors and may also desensitize certain receptors. Comparison of these predictions with the experimental data highlights the information that is required for a more detailed model of crosstalk.

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EFFICIENT, SYNAPTIC INFORMATION TRANSFER and memory storage is most simply accomplished if each synapse is both independent and independently modifiable. The question we address here is whether synapses are independent at the level of diffusion of neurotransmitter. In other words, can transmitter release from one synapse lead to significant activation of receptors at a neighbouring synapse? The answer to this question has potential significance for many issues related to synaptic transmission and is crucial to the full understanding of the mechanisms by which synaptic currents are generated. Crosstalk may be particularly relevant to phenomena such as long-term potentiation and long-term depression (LTP and LTD) in which associativity and cooperativity of synapses are of importance. It is also necessary to bear crosstalk in mind when interpreting experiments involving simultaneous activation of nearby synapses.

After a review of the experimental evidence for crosstalk between synapses, a simple three-dimensional (3D) model of glutamate diffusion is presented from which the levels of glutamate resulting from crosstalk can be estimated. Crosstalk may be limited by transmitter uptake and binding to receptors, thus increasing the independence of synapses. These limiting mechanisms are considered along with the potential benefits of crosstalk.

Experimental detection of crosstalk

Transmitter is thought to be released in the form of packets, quanta, that correspond to synaptic vesicles. Two or more vesicles may interact, that is, transmitter from each acts on common receptors, if the vesicles are released close together at similar times. Such interaction could occur on different distance scales. First, it may occur between vesicles released from separate boutons facing separate receptor clusters, which at excitatory synapses are associated with postsynaptic densities (PSDs) normally located on spines. Second, it may occur between vesicles released from distinct active zones of the same bouton onto separate postsynaptic receptor clusters. Both of these situations can be considered to represent crosstalk, thus defined as interaction between distinct synaptic contacts (themselves defined as the apposition of an active zone and a receptor cluster). Finally, vesicles released at a single active zone ('multivesicular release') could also interact, but, for the purposes of this article, this will not be considered to be crosstalk. Where a single presynaptic bouton is involved, it is difficult to distinguish between crosstalk and the effects of multivesicular release (see below). We will discuss examples of the different kinds of crosstalk and present a model for the separate bouton/separate receptor clusters type of interaction.

There are several ways to detect quantal interactions that reflect crosstalk, most of which are based on the detection of a non-linear summation of synaptic conductances with increasing number of contributing synaptic contacts. Receptor kinetics will influence how crosstalk affects the synaptic current waveform. Compound synaptic currents with a larger or smaller amplitude, slower decay or, under certain conditions, smaller variance than expected from linear combination of component currents could all indicate crosstalk. Several control experiments are required. In all cases, the non-linearities expected from voltage-clamp problems (which could affect the currents in a similar way to crosstalk) and the contribution of latency variations of the component currents should be assessed. The available experimental evidence primarly concerns crosstalk that leads to activation of ionotropic receptors and is thus detected electrophysiologically. Crosstalk can also affect presynaptic[1,2] and postsynaptic metabotropic receptors that may not have a direct electrical action.

The first evidence for non-linear interaction of transmitter quanta comes from work done on the neuromuscular junction in the 1970s. These studies showed that when acetylcholinesterase (AChE) is blocked, an increase in quantal content or iontophoretic application of ACh produces a slowing of the decay of the synaptic current[3,4] (Fig. 1); the effect is most prominent for spatially-restricted release of quanta. These findings were interpreted as resulting from overlap of transmitter from different quanta,

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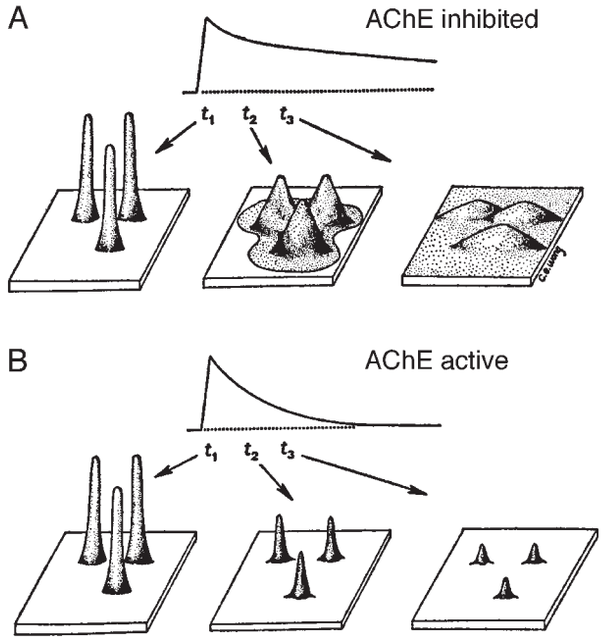

Fig. 1. ***Schematic representation of the interaction between quanta at the neuromuscular junction.*** *The trace at the top of each panel represents the synaptic response (end-plate current) while the diagrams below represent the concentration profiles of ACh from three vesicles at three times during the response.* **(A)** *With AChE inhibited, transmitter from nearby vesicles interacts to produce a longer-lasting synaptic conductance.* **(B)** *With the esterase active, vesicles are independent. This review asks whether a similar interaction can occur between synaptic contacts in the CNS, and also whether it can be prevented by an analogue of the esterase, such as neurotransmitter uptake carriers. Reproduced, with permission, from Ref. 3.*

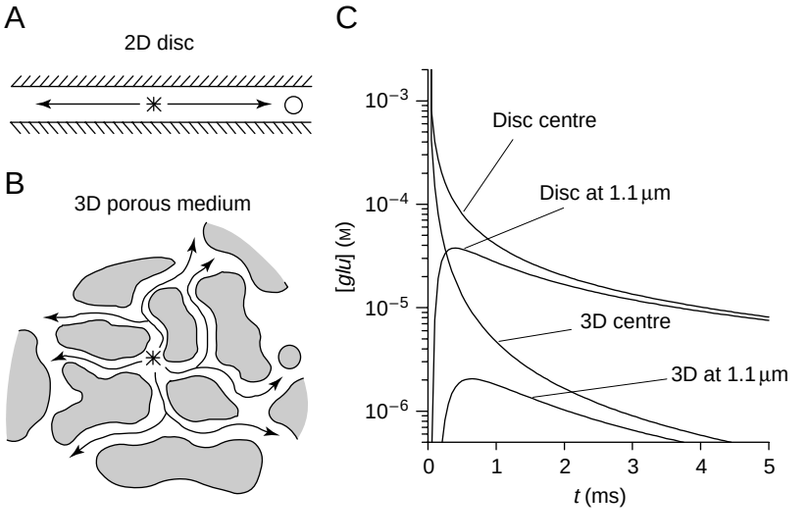

Fig. 2. ***Dilution of transmitter is more rapid in a three-dimensional structure than in a two-dimensional structure.*** **(A)** *Cross-section of a two-dimensional disc of the sort often used for modelling diffusion within the synaptic cleft.* **(B)** *Diagram of a porous medium containing obstacles to diffusion. In both these panels the star represents the point of release and the circle the point of detection of transmitter.* **(C)** *Analytical solutions[38] for diffusion in a disc and in a porous medium (without binding or uptake). The equation for the disc is*

$$[glu] = \frac{M}{4h\pi Dt}\exp\left(\frac{-r^2}{4Dt}\right)$$

*where [*glu*] represents the transmitter (glutamate) concentration;* M *is the quantity of transmitter released (7.8 × 10⁻²¹ moles = 4700 molecules);* h *denotes the height of the disc (20 nm);* D *is the diffusion coefficient (for glutamine; see Table 1);* r *is the distance from the instantaneous line source of transmitter and* t *is the time after release. One curve shows the concentration time course at the point of release ('Disc centre') and another the concentration at a distance of 1.1 μm ('Disc at 1.1 μm'). Also shown are analogous curves for diffusion in a restricted but three-dimensional space (see Box 1). The equation is*

$$[glu] = \frac{M}{8\alpha\left\{\pi(D/\lambda^2)t\right\}^{3/2}}\exp\left\{\frac{-r^2}{4(D/\lambda^2)t}\right\}$$

in which the only new symbols are α and λ, which are parameters introduced in Box 1 and whose values are given in Table 1. The concentration timecourses after instantaneous release of transmitter from a point source are shown for the point of release ('3D centre') and a point 1.1 μm distant ('3D at 1.1 μm').

which would normally be prevented by AChE; this hypothesis was later supported by models of the neuromuscular junction[5,6]. At glycinergic synapses on the goldfish Mauthner cell, supralinear summation of separate inputs and non-linear interaction of synaptic responses with iontophoretic glycine applications were demonstrated[7]. The results were shown, using a model of glycine diffusion and receptor occupancy, to be consistent with crosstalk between synapses.

At mammalian central synapses, despite a large body of data consistent with crosstalk (see below), there are only a few situations where there is good evidence for its existence. For hippocampal inhibitory synaptic currents, inhibition of GABA uptake slowed the decay of large (compound) but not small IPSCs[1,8]. Inhibiting uptake hardly altered the IPSC amplitude, suggesting that voltage-clamp problems were not an issue. The interpretation that this observation results from crosstalk is very strongly supported by the demonstration of synaptically-induced $GABA_B$ receptor-mediated inhibition of excitatory transmission (also enhanced by uptake block)[1]. The effect of uptake inhibition suggests a role for transmitter uptake in preventing crosstalk. In the cerebellum, compound parallel fibre (PF)–Purkinje cell (PC) excitatory synaptic currents decay surprisingly slowly[9,10]; voltage-clamp problems and latency variations cannot alone account for the timecourse[11,12]. Since small evoked PF–PC currents decay more rapidly than the larger compound currents[12], it is likely that crosstalk involving AMPA receptors occurs at these synapses and is responsible in part for the slowness of decay. A recent study of hippocampal LTP (Ref. 13) has shown that tetanic induction of LTP in neighbouring cells, while induction of AMPA receptor-expressed LTP in the recorded cell is prevented, nevertheless results in an augmented NMDA receptor-mediated EPSC component in the recorded cell. This result is most simply explained by assuming that LTP in the neighbouring connections results in an increased transmitter release that stimulates the recorded cell's NMDA receptors via crosstalk.

Certain synaptic geometries would be expected to favour interactions between quanta. One example is the mossy-fibre–unipolar brush cell synapse[14,15], where several release sites are clustered within <1 μm of each other and the synaptic current is very long-lasting. Another is calyceal synapses, where a long-lasting component of the synaptic current has been attributed to interactions between quanta, based in part on modelling studies[16]. At both of these synapses, however, it is difficult to discriminate between interactions resulting from quanta released at a given active zone and crosstalk of quanta released from separate active zones onto distinct receptor clusters. Other

Box 1. What influences transmitter diffusion in brain tissue?

Volume fraction and tortuosity

Brain tissue is filled with obstacles to diffusion: cells and their processes. A diffusion equation modified to describe diffusion in porous media can be applied to brain tissue and yield useful approximations over a wide range of conditions[a]. In this formulation two parameters are introduced that describe the average texture of the substance. The derivation is based upon two assumptions: (1) the diffusing substance is restricted to a fraction, α, of the total volume, called the 'volume fraction' which in the brain corresponds to the extracellular volume; (2) the average diffusional path between two points is longer by a factor, λ, than it would have been for unrestricted diffusion. This is called the 'tortuosity'. For unrestricted diffusion, these parameters both take a value of 1. For restricted diffusion, $\alpha \leq 1$ and $\lambda \geq 1$. These parameters are used to correct the diffusion coefficient and concentration in an otherwise unchanged diffusion equation: D is replaced by D/λ^2 and, since one is generally interested in the concentration of diffusate within its restricted compartment, the resulting concentration is increased by a factor of $1/\alpha$ (Fig. 2 legend, p. 378).

The modified equation only becomes applicable once diffusion around obstacles begins to occur. It is only considered to be accurate over distances that are large compared with the typical dimensions of the obstacles to diffusion. Diffusion from one synapse to another (imagine them to be on different spines) would normally involve diffusion around some of the characteristic obstacles to diffusion, but the use of the diffusion equation for porous media on this scale can only give approximate solutions (see Box 2).

The parameters α and λ have been determined experimentally by a number of workers for various parts of the brain. For example, Nicholson and Phillips[a] used iontophoresis and ion-selective electrodes to determine values of $\alpha = 0.21$ and $\lambda = 1.55$ for various cations and anions in the cerebellum. These values are typical of other brain regions, although some regional variations[b] and deviations from isotropy[c] have been reported. As pointed out by Gardner-Medwin[d], such values for the tortuosity could plausibly be predicted for brain tissue on a geometrical basis, indicating that within the restricted extracellular space small molecules diffuse as in aqueous solution and hence arguing against the notion of non-specific binding of such molecules. One report arguing in favour of non-specific binding, of dopamine and some analogues[e], is now interpreted in terms of a specific uptake mechanism[f].

Measurements of brain volume fraction and tortuosity cannot be used to infer the properties of diffusion within synaptic clefts because: (1) clefts are smaller than the obstacles to diffusion, rendering the modified equation inapplicable; (2) clefts occupy only a small fraction of the brain's extracellular volume and can thus have only a minor influence on the measures of the diffusion parameters.

Receptor binding sites

Any binding of transmitter molecules will modify their diffusive behaviour. Although the available evidence suggests the absence of significant 'non-specific' binding of small diffusing molecules, the multiplicity of receptors for neurotransmitters must be considered: for glutamate these are AMPA, metabotropic and NMDA receptors. Their affinities for glutamate (derived from physiological determinations) and concentrations (derived from binding studies) are listed in Table 1. Unfortunately, one of the most important receptor parameters for the calculations that follow is the association rate constant for binding of transmitter, a parameter that for the various receptors represented in the model has only been approximately determined at best. The concentration of metabotropic receptors is likely to be inaccurate, since no high-affinity ligands are currently available for precise binding studies.

An interesting glutamate receptor whose properties are also listed is the kainate-binding protein (KBP)[g], present at rather high concentrations in the cerebella of some species, notably birds. We shall examine the possibility that this receptor is able to buffer extracellular glutamate to a significant extent.

Uptake carriers

Uptake carriers could affect transmitter diffusion both by binding and by sequestering transmitter[h]. Several glutamate carriers have now been cloned and their properties are similar to those previously determined in various preparations[i], with apparent affinities (K_ms) for glutamate of around 10 μM. It is difficult to measure the uptake capacity of brain tissue, because, in intact preparations (such as slices), tortuosity and uptake itself create severe problems of access, which are likely to give rise artefactually to apparently low-affinity uptake mechanisms. We use in the model of Box 2 the highest value of uptake capacity for an intact preparation (see Table 1), including the apparently low-affinity component. To derive a carrier concentration from the maximum uptake rate, we used a recent estimate of glutamate transporter turnover rate, 14 s^{-1} (Ref. j). Little information is available concerning the association rate constant of glutamate for the transporter binding site, except that the above turnover rate imposes a lower limit of $1.4 \times 10^6\ M^{-1}\ s^{-1}$, for the simple kinetic model of uptake that we employ (see Box 2).

Resting glutamate concentration

Evidence of tonic NMDA receptor activation has been reported, suggesting that the resting glutamate concentration is sufficient to activate at least some NMDA receptors[k]. In agreement with this notion, measurements of the extracellular glutamate concentration using *in vivo* microdialysis consistently yield figures of 1–3 μM (Refs l, m), but doubts remain over the influence of neighbouring damaged tissue. The probable stoichiometry of uptake would permit a lower concentration to be attained[n,o].

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Box 2. Calculating glutamate diffusion

We wish to calculate how much glutamate will reach an inactive synapse when one or more neighbouring synapses are activated. Here we are interested in quite distinct synapses, such as those found on different spines and involving different presynaptic cells. This requires a model of diffusion in brain tissue taking receptor binding and uptake into account. The modified diffusion equation introduced in Fig. 2 (p.378) and Box 1 will be used (an active synapse and its inactive neighbour can be imagined to be at the star and open circle, respectively, of the diagram in Fig. 2B). We shall employ the simplest methods possible. A related problem has recently been addressed by Nicholson[a].

In order to keep the calculations manageable, we assume that the diffusion problem has spherical symmetry; this means that the diffusion equation has only one spatial variable (the radius, r). The relevant diffusion equation (with the modifications for porous media) is thus:

$$\frac{\partial[glu]}{\partial t} = \frac{D}{\lambda^2}\left\{\frac{\partial^2[glu]}{\partial r^2} + \frac{2}{r}\frac{\partial[glu]}{\partial r}\right\} - (\textit{binding to receptors}) - (\textit{binding to transporters}) \quad (1)$$

where $[glu]$ is the concentration of glutamate in the extracellular space; D the diffusion coefficient; and t, time. λ is the tortuosity defined in Box 1, with the value given in Table 1. Binding to receptors and transporters will be defined below. We define B_{max} and U_{max} to be the total concentrations of receptor binding sites (only one type at a time is modelled) and uptake carriers, respectively, while B and U represent their free (unliganded) concentrations. All these concentrations are uniform concentrations in the extracellular space (i.e. already corrected for α). The model uses the following reaction schemes:

$$\textit{binding} \qquad B + glu \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B - glu \quad (2)$$

$$\textit{uptake} \qquad U + glu_o \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} U - glu \xrightarrow{k_3} glu_i + U \quad (3)$$

where glu_o represents extracellular glutamate and glu_i sequestered glutamate, giving the equations

$$\textit{binding to receptors} = -\frac{dB}{dt} = k_1 B[glu] - k_{-1}(B_{max} - B) \quad (4)$$

$$\textit{binding to transporters} = k_2 U[glu] - k_{-2}(U_{max} - U) \quad (5)$$

with

$$\frac{dU}{dt} = (k_{-2} + k_3)(U_{max} - U) - k_2 U[glu] \quad (6)$$

We implicitly assume that the lamellar structure of the extracellular space enables rapid equilibration orthogonal to the lamellae. The resting extracellular glutamate concentration is zero. Binding to receptor sites is assumed to be independent (that is, without cooperativity). The parameters used in the model are listed in Table 1.

The inclusion of binding and uptake equations complicates the solution of this problem and a numerical method was thus used to calculate an approximate solution for the system of equations at points (in reality, spherical shells) separated by 20 nm in space and by 10 ns in time. We chose a very simple approach (space discretization using central difference approximations and time discretization using Euler's method). The initial conditions represented the instant of release of one vesicle of transmitter per active synapse. In order to preserve

examples might include mossy fibre–CA3 pyramidal cell boutons, climbing fibre–Purkinje cell varicosities and cerebellar mossy fibre–granule cell synapses.

Several studies, in which the number of active synapses was changed by altering release probability, have produced data consistent with the involvement of crosstalk. For example, reducing the release probability can accelerate the decay of synaptic currents at both GABAergic[17] and glutamatergic synapses[12,18–24], although the decay of some glutamatergic EPSCs remains unchanged with this manipulation[22,23] (rapid desensitization could, however, obscure the effect of crosstalk on the decay). Analogously, the time course of miniature events has been shown to be more rapid than multi-quantal events at many synapses[12,18,19,21–24] (although some of this difference may be attributable to asynchrony of release[22,23]). Desensitization of receptors has been suggested to be more complete when more quanta are released[18]. Finally, changing the release probability has been shown to change the effective concentration of glutamate acting on NMDA receptors[2,25]. However, all these observations are also consistent with changes in the number of vesicles released at individual synaptic contacts[25]. It is currently unknown whether an action potential can release more than one vesicle per synaptic contact.

There are several experimental observations that could be interpreted in terms of crosstalk. It has proved difficult to demonstrate activation of $GABA_B$ receptors following stimulation of single GABAergic neurons, and stimulation of many fibres or synchronous activity in multiple interneurons is usually necessary to produce a clear $GABA_B$ response[26]. One reason for this could be that postsynaptic $GABA_B$ receptors are located extrasynaptically and accumulation of GABA resulting from crosstalk between several neighbouring active synapses is necessary to activate them. A two-dimensional model incorporating crosstalk between neighbouring synapses has lent support to this proposal[27]. Similarly, at hippocampal mossy fibre synapses[2], increasing release or blocking uptake appears to be necessary to activate presynaptic metabotropic glutamate receptors, which are thought to be located extrasynaptically. Recently, evidence has been presented from the hippocampus suggesting that some synaptic stimuli result in activation of NMDA receptors but not AMPA receptors. This has been interpreted as showing the existence of synapses that express NMDA receptors but not AMPA receptors and are therefore normally 'silent'[28–31]. An alternative interpretation is that NMDA and AMPA receptors are normally co-localized at synapses but that glutamate diffusing from a neighbouring synapse (on a cell different to that recorded from) is only sufficient to activate the high-affinity NMDA receptors[13,32]. Interactions between quanta caused by crosstalk could also compromise quantal analysis, which assumes the independence of quanta.

the spherical symmetry when more than one synapse was active, the released glutamate was distributed throughout a spherical shell at the appropriate radius. For a single vesicle, the calculated effects of uptake or binding (reduction of peak glutamate concentration reached at the test synapse) differed by less than 12% between release from a shell and release from a point. A first shell at $r = 1.1$ μm represented up to 12 synapses (the coordination number for hexagonal close packing). The second shell at $r = 2.2$ μm contained up to 74 synapses: the number of synapses expected within a radius of 2.75 μm (assuming 1 synapse μm^{-3}; Refs b–e) that were not represented in the first shell. A third shell at 3.3 μm brought the total number of active synapses to 238.

As pointed out in Box 1, the application of this diffusion equation for intersynaptic diffusion is an approximation. In addition, there is an obvious difference between our model and reality in that the spherical symmetry of our model precludes the presence of a disc (synaptic cleft) around the point of release. Examination of electron micrographs suggests that the synaptic cleft often 'branches' near the edge of the postsynaptic density (PSD), which in many structures typically has a radius of 100–200 nm (Refs b–e). The volume of the disc-like synaptic cleft [$V_{disc} = \pi(r_{disc})^2 h$; h = cleft height = 20 nm] is about one-fiftieth the volume of extracellular space contained within the radius at which neighbouring synapses are placed in the model [$V_{sphere} = {}^4/_3\alpha\pi(r_{neighbour})^3$], suggesting that the influence of the disc-like region *per se* on crosstalk at the neighbouring synapses is unlikely to be very important, unless it retains transmitter significantly.

Probably the most important factor determining the peak transmitter concentration that can be generated by crosstalk is the volume of extracellular space accessible from the point of release at early times into which transmitter must be diluted in order to reach the neighbour. If the local volume fraction is lower than in the model, the concentration will be correspondingly higher. To give an illustration, a somatic synapse would sense a volume fraction reduced by a factor of about 2; a smaller effect will also be experienced by synapses on large dendrites. Ultimately, three-dimensional electron-microscopic reconstruction of the extracellular space around synapses may be necessary to quantify local deviations from the bulk volume fraction. Average volume fractions can also differ between regions of the brain. For instance, in the CA1 region of the hippocampus, the volume fraction is 0.12 (Ref. f) rather than 0.21, so there the concentrations should all be nearly double those reported here.

Of the receptor parameters appearing in the model, the most critical and probably the least certain are the association rate constants for the transmitter binding sites. Faster association rates than used in the model for uptake transporters and KBP would make them more effective at reducing crosstalk; more rapid binding to NMDA and AMPA receptors would lead to greater activation of these receptors. Diffusion-limited association rate constants for molecules with diffusion coefficients similar to that of glutamate can attain 10^9 M^{-1} s^{-1} (see Ref. g, for acetylcholine analogues binding to acetylcholine esterase).

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TABLE 1. Diffusion model parameters

System	k_1 (M^{-1} s^{-1})	k_{-1} (s^{-1})	B_{max} (M l^{-1})	k_2 (M^{-1} s^{-1})	k_{-2} (s^{-1})	k_3 (s^{-1})	U_{max} (M l^{-1})	Vesicular content (moles)	Refs
AMPA	10^7	2×10^3 (EC_{50} = 0.5 mM, K_d = 200 μM)	$3 \times 10^{-7}/\alpha$	–	–	–	–	–	33,41,42
KBP	10^7 (estimate)	300 (K_d = 30 μM)	$4 \times 10^{-6}/\alpha$	–	–	–	–	–	43
NMDA	5×10^6	5 (EC_{50} = 2.3 μM, K_d = 1 μM)	$3 \times 10^{-7}/\alpha$	–	–	–	–	–	44–46
mGluR	10^7 (estimate)	50 (EC_{50} = 10–20 μM)	$10^{-7}/\alpha$	–	–	–	–	–	47,48
Uptake	–	–	–	10^7 (5×10^7; estimates)	86 (486)	14	$1 \times 10^{-6}/\alpha$ (V_{max} = 15 mmol l^{-1} s^{-1})	7.8×10^{-21} (4700 molecules)	49–51

The diffusion parameters used to derive the values in the table are defined in Boxes 1 and 2, and these are $\alpha = 0.21$ (dimensionless)[39], $\lambda = 1.55$ (dimensionless)[39] and $D = 7.6 \times 10^{-6}$ cm^2 d^{-1} (Ref. 40). Receptor binding site K_d values were calculated from the equation $K_d = (\sqrt{2} - 1)\ EC_{50}$, that is, by assuming that the binding of two transmitter molecules to two independent, identical sites is required for receptor activation. The association rate constants (k_1) were judged to be important receptor parameters, so they were fixed, according to the available information, and the dissociation rate constants (k_{-1}) were calculated from $k_{-1} = k_1 K_d$. For the uptake kinetic scheme of Box 2, it can be shown that $k_{-2} = k_2 K_m - k_3$, where $K_m = 10$ μM (see Box 1). The bracketed value for k_2 is the faster binding rate constant used in Fig. 3A; the appropriate value for k_{-2} is also given. When necessary, we assumed 100 mg total protein/ml brain tissue. The temperature in the model is 25°C. The affinity of KBP for glutamate is based on the K_i for inhibition of kainate binding to KBP by glutamate (V. Teichberg, pers. commun.). Binding maxima (B_{max}) values represent double the receptor concentration, because receptors are each assumed to possess two binding sites.

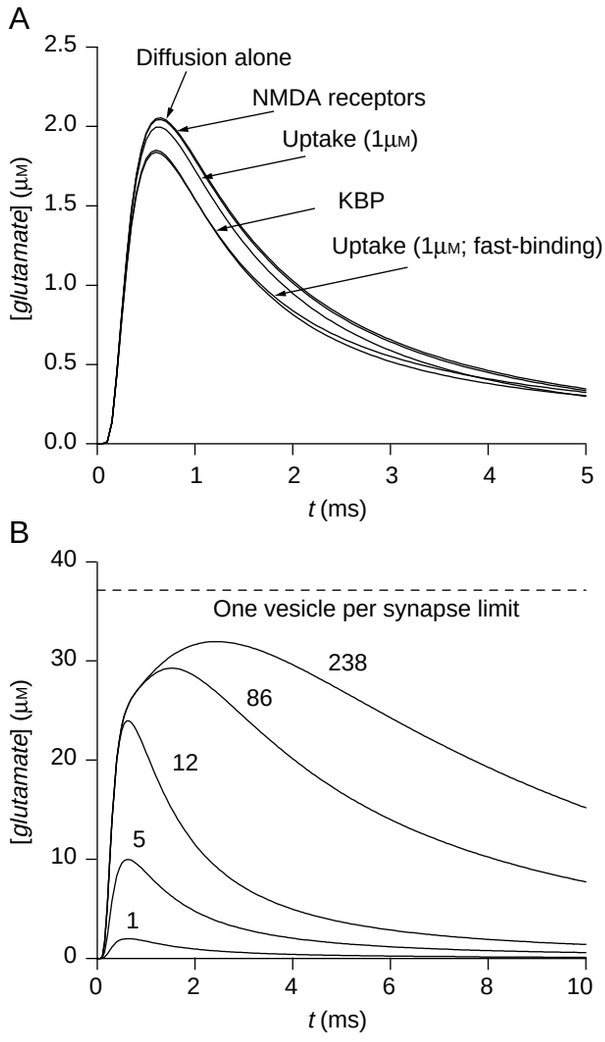

Fig. 3. ***Estimations of glutamate concentrations generated by crosstalk.*** **(A)** *The concentration reached at a distance of 1.1 μm from the point of release of one vesicle containing 4700 glutamate molecules, calculated using the model of Box 2, in the presence of various types of receptor or uptake. The effects of AMPA and metabotropic receptors (neither is shown), like those of NMDA receptors, were slight. KBP buffers glutamate, reducing the peak concentration. Uptake can significantly reduce the peak concentration. The effectiveness of all these proteins at reducing crosstalk depends on their rates of transmitter binding. The point is illustrated for uptake, where increasing the binding rate five times leads to a greater reduction of the crosstalk concentration of glutamate.* **(B)** *Similar simulations, with uptake carriers present, in which the number of vesicles released nearby was varied. The contents of 1, 5 or 12 vesicles were liberated in a spherical shell of radius 1.1 μm centred on the point whose concentration is plotted. For the 86 vesicle curve, the contents of an additional 74 vesicles were released in a shell at* r = *2.2 μm and for the 238 vesicle curve a third shell at 3.3 μm contained the contents of a further 152 vesicles. The broken line represents the equilibrium concentration reached if one vesicle per synapse is liberated throughout a structure containing 1 synapse* μm^{-3} *and having a volume fraction* α = *0.21 (neglecting uptake and binding). This value is independent of the diffusion model, depending only on the vesicular content and the two parameters given above.*

In summary, good evidence for crosstalk has been obtained at only a few types of central synaptic connection, but a large body of data is consistent with the phenomenon and several important experimental results are open to alternative interpretation if crosstalk is significant. Since it has proved difficult to determine experimentally the importance of crosstalk, it would be useful to know how much effect to expect. The varied synaptic geometries and spacings in the brain suggest that the extent of crosstalk will depend upon the type of synapse considered. We now estimate the concentration of glutamate reaching neighbouring but distinct synapses to enable us to assess the likely impact of crosstalk.

Estimating the extent of crosstalk for glutamatergic synapses

For models of diffusion within a synaptic cleft or between synaptic contacts that basically share the same synaptic cleft (such as the mossy fibre–unipolar brush cell synapse mentioned above), the geometry of choice has been a disc[33–37]. For some problems, however, such 2D models are only useful over relatively small distances and short times, because transmitter dilution is more rapid in 3D structures (Fig. 2). In order to model diffusion of glutamate between separate spine synapses, for example, it is necessary to take the complex 3D structure of brain tissue into account in some way.

How this was done with an approximate model without making the problem too complex is explained in Boxes 1 and 2, with the parameter values listed in Table 1. The model is intended to represent (loosely) diffusion of glutamate between spiny synapses such as those found in the cerebral cortex, hippocampus and molecular layer of the cerebellar cortex. Mean synapse densities are probably in the range 0.8–1 synapses μm^{-3} in the above regions[52–55]. Assuming synapses to be arranged in a hexagonal close packing arrangement with a density of 1 synapse μm^{-3}, the mean distance between neighbouring synapses will be about 1.1 μm and each synapse will have 12 nearest neighbours. In other, less dense packing arrangements, synapses would have fewer but closer neighbours. We calculated the expected concentration of glutamate arriving at a 'test synapse' from various numbers of active neighbours and examined the effect of uptake transporters and binding to receptors. The effects of the glutamate concentration at the (inactive) test synapse were judged by estimating the occupancy of AMPA and NMDA receptors.

Key results from these computations are presented in Figs 3 and 4. For diffusion alone, in the absence of binding or uptake, a vesicle of 4700 molecules of glutamate will generate a concentration of about 2 μM at 1.1 μm from the point of release. Obviously, the peak concentration will depend on the distance (see equations in Fig. 2 legend). The presence of AMPA receptors (150 nM w.r.t. total brain tissue; see Table 1), metabotropic receptors (50 nM) or NMDA receptors (150 nM) produced only slight reductions in the peak concentration at this distance (<1%), NMDA receptors having the largest effect (Fig. 3A); all these receptor types were thus excluded from further calculations. Kainate-binding protein (KBP), which is present at high concentrations in the cerebella of several species[56], was able to reduce crosstalk somewhat in our simulation, suggesting that this may be a role it plays physiologically. Glutamate uptake had a similar but lesser effect.

The effect of the various binding sites would be significantly increased if the binding rate constants were greater than those used in the model, since the brief duration and low concentration of the transmitter

transient do not allow binding to approach equilibrium; this point is illustrated by including a calculation of the effect of the same concentration of uptake transporters, but with five-times faster binding of glutamate ($k_2 = 5 \times 10^7$ M^{-1} s^{-1}; see Table 1). Unfortunately, the association rate constant is a rather uncertain parameter for most of the binding sites represented. The effect of uptake (with the 'standard' binding rates) on the peak concentration is almost exclusively due to binding (>99% of glutamate neutralized by uptake) rather than sequestration. This is a direct consequence of the uptake turnover rate used in the calculation[45]; a faster turnover rate would increase the influence of sequestration on the peak concentration.

If several neighbouring synapses release glutamate, the concentrations reached at the test synapse are naturally higher (Fig. 3B). There is, however, an upper limit to the concentration that can be attained. This is reached when every synapse (except the central one) releases a vesicle of transmitter. This limiting concentration is about 37 μM (in the absence of receptors or uptake; Fig. 3B, broken line). This limit is only exceeded if more than one vesicle per synapse is released or if one or more are released very close to (or at) the test synapse.

What effect will these concentrations of glutamate have on postsynaptic receptors at the inactive test synapse? We assumed that each receptor possesses two identical, independent binding sites that must both be occupied by agonist molecules for the receptor to be activated. We then estimated receptor activation by computing the fraction of receptors that would be 'doubly-occupied' (Fig. 4 legend). The results of these calculations are shown in Fig. 4 (A and B). Essentially, only concentrated release from many neighbouring synapses is sufficient to activate NMDA receptors significantly, while even such concentrated release has little effect on AMPA receptors. The postsynaptic effect of crosstalk is also sensitive to the values of the binding rate constants of the NMDA and AMPA receptors: faster binding rates would allow a closer approach to equilibrium binding and thus a greater postsynaptic effect.

It is thought that singly-bound AMPA receptors have a significant chance of desensitizing[33,57–59], so we also plot the fraction of AMPA receptors in our model that bind at least one transmitter molecule (Fig. 4C). This suggests that desensitization may be significant, especially with the larger and longer-lasting glutamate transients, for which the cumulative fraction of receptors that bind a transmitter molecule at some time during the transient would reach high levels.

All of the above discussion concerns the postsynaptic effect of crosstalk at an inactive test synapse. It is also of interest to know whether crosstalk will significantly modify the response of an active test synapse. Answering this question requires knowledge of the timecourse of transmitter in the synaptic cleft, something that is beyond the scope of this article[36]. However, it is probable that for low-affinity receptors that are far from being saturated when crosstalk peaks, the absolute effect of crosstalk will be somewhat increased in comparison with that at an inactive synapse. This would be a consequence of the nonlinear activation of receptors. The situation would be reversed for high-affinity receptors that are saturated, since additional agonist would have little further effect.

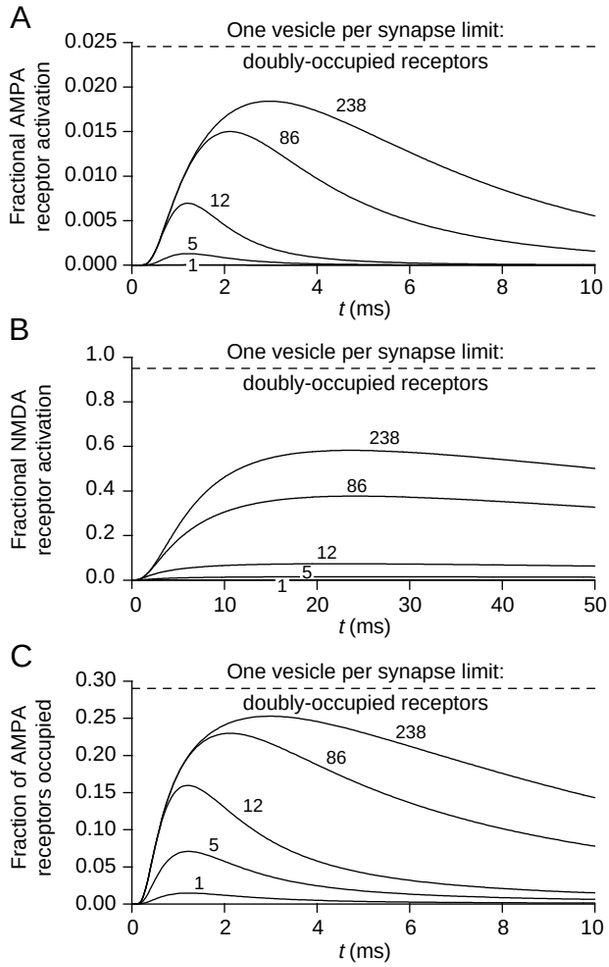

Fig. 4. ***The effect of crosstalk on AMPA*** **(A)** ***and NMDA*** **(B)** ***receptor activation.*** *An estimate of the effect of the crosstalk glutamate concentrations shown in Fig. 3B on these glutamate receptor types can be obtained by assuming that receptor activation requires ligand binding to two identical, independent sites (and ignoring more complex receptor kinetics and desensitization) and hence calculating the fraction of receptors that are 'doubly-occupied':*

$$fractional\ \ activation\ = \left(\frac{B_{\max} - B}{B_{\max}}\right)^2$$

where $(B_{max}–B)/B_{max}$ *represents the fraction of receptor binding sites occupied by transmitter. The* B_{max} *values are given in Table 1 and* B *was calculated according to the methods of Box 2. The dashed lines represent the equilibrium activation expected from the concentration at the 'crosstalk limit' (naturally also ignoring desensitization).* **(C)** *This indicates the fraction of AMPA receptors with at least one (of two) binding sites occupied. This is given by*

$$fractional\ \ occupation\ = 2\left(\frac{B_{\max} - B}{B_{\max}}\right)\left(\frac{B}{B_{\max}}\right) + \left(\frac{B_{\max} - B}{B_{\max}}\right)^2$$

This is of interest, because of suggestions that singly-liganded AMPA receptors may desensitize (see text). The dashed line represents the crosstalk limit for the fraction of AMPA receptors in the model with at least one transmitter molecule bound.

In conclusion, our simple model for diffusion of glutamate in brain tissue suggests that crosstalk between separate spine synapses will not activate AMPA receptors significantly, and is also unlikely to be important for NMDA receptors, unless many

neighbouring synapses are active simultaneously. Uptake, and KBP in certain species, could reduce crosstalk. Similar principles should apply to crosstalk for other transmitters, such as GABA. It should be pointed out that the model is intended to represent diffusion between synapses made by separate boutons onto separate spines; synapses where separate postsynaptic densities share the same presynaptic terminal will experience greater crosstalk.

The apparent contradiction between the prediction of the model that crosstalk induces minimal activation of AMPA receptors and the experimental indications of crosstalk at AMPA receptors during parallel fibre–Purkinje cell transmission[12] remains to be accounted for. The model is approximate (see Boxes 1 and 2). Several of the assumptions of the model would have to be modified in any more detailed simulation. For example, synapses are not distributed uniformly and the density of synapses can certainly, at least locally, exceed the mean value of our model. In particular, a significant fraction of parallel fibre varicosities make two synaptic contacts[53], so these contacts will have at least one very close neighbour. Also, more than one vesicle might be released per synapse. The geometry of the extracellular space around Purkinje cell spines may also cause significant deviations from the equations based upon the average properties of the tissue (see Box 2). More accurate estimates of the importance of crosstalk at particular synapses will require better knowledge of the distribution and properties of receptors and uptake transporters (in particular their association rate constants), the number of vesicles released per release site per action potential, and will ultimately depend upon detailed reconstructions and explicit models of the synaptic and intersynaptic geometry.

Benefits of crosstalk

Crosstalk can be considered to be disadvantageous, reducing synaptic independence and thus the memory capacity of the brain. In some situations, however, crosstalk may be beneficial. One example is where multiple, closely-spaced synaptic contacts are formed by the same presynaptic fibre. Here, synaptic independence may be less important and reliability of transmission may be the goal. Examples include the climbing fibre–Purkinje cell connection[9–12,20], synapses formed by hippocampal[33] and cerebellar mossy fibres[21,37], and some inhibitory connections[1,17], for each of which data consistent with crosstalk have been reported. In these cases crosstalk could provide an amplification mechanism (more current per given amount of transmitter) as well as reducing variability by saturating receptors[21]. Since crosstalk preferentially activates high-affinity receptors which often produce long-lasting responses (e.g. NMDA or metabotropic receptors), it might also represent a 'background signal' which is not synapse- or cell-specific and is related to the level of activity in the network as a whole.

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