Selected references


Hippocampal synapses: do they talk to their neighbours?

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Recent experimental findings show that fast synaptic transmission can extend its actions beyond the immediate synaptic cleft. Whether this phenomenon results in significant crosstalk between typical neighbouring synapses remains unclear. This article considers two areas of the hippocampus, the CA1 and dentate gyrus, where important neural processing occurs. The results discussed do not provide a simple answer to the question of whether synapses can ‘talk’ to their neighbours, but they do reveal crucial physiological constraints that determine the significance of synaptic crosstalk, thus adding considerably to our understanding of chemical synaptic transmission.

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Much evidence has emerged recently to suggest that basal transmission at conventional ‘fast’ CNS synapses exerts actions that extend beyond the synaptic cleft. On the one hand, synaptically released GABA and glutamate appear to bind to receptors that are at a considerable distance from the activated synapse. On the other hand, synaptic activity might cause transient depletion of extracellular Ca2+ in the synaptic vicinity and thus affect synaptic communication. This article aims to examine the evidence concerning the spatial separation of synapses in the hippocampus and to consider the consequences for possible interactions between neighboring synapses. Some of the experimental observations and theoretical implications regarding extrasynaptic neurotransmitter spillover have recently been discussed. An alternative insight into the extrasynaptic neurotransmitter concentration profiles that follow exocytosis comes from an examination of the currents elicited in astrocytes by electrophoretic glutamate uptake. Transporter currents in hippocampal slices are considerably slower than those observed in outside-out membrane patches of astrocytes following rapid application of glutamate. This observation implies that an elevated glutamate concentration persists for a long time (possibly as long as 10 millisecond) in the vicinity of the astrocyte transporters, which could be a considerable distance from the release site. Relatively little is known about the kinetics of local Ca2+ depletion. At a calyxal central synapse, approximately 2.5 × 107 Ca2+ ions have been estimated to flow into the presynaptic terminal during an action potential. If Ca2+ enters the cell exclusively from the synaptic...
Box 1. Distances between nearest neighbours in a synaptic scatter

The mean nearest-neighbour distance (NND), \( r_p \), between synapses in the neuropil is determined not only by the numerical density of synapses, \( N_s \), but also by their spatial arrangement. This is illustrated in Fig. IA–C where three characteristic point patterns are shown. These patterns exhibit the same numerical density of synapses, \( N_s \), but different distributions of NNDs and consequently different values of \( r_p \). In the case of a regular square lattice (Fig. IA) in 2D, geometry gives \( r_p = \sqrt{N_s} \), whereas a uniformly random or Poisson scatter (Fig. IB) in 3D gives \( r_p \approx 0.54 N_s^{-1/3} \) (Ref. a). However, the synaptic centroids (which we define here as the geometric centres of the synaptic cleft) cannot be closer to each other than is allowed by the 3D sizes of the synapses. A common stereological measure of the synaptic size is the mean projected height of the synapses, which in fact represents the mean ‘calliper’ diameter (in 3D) of synaptic elements that are immediately associated with the synaptic cleft. Setting the mean size of synapses, \( d \) (illustrated as shadowed circular areas in Fig. IC), results in an additional correction for \( r_p \), which can be estimated using a Monte-Carlo experiment as follows. First, a Poisson point process (uniformly random scatter) is generated with a numerical density that is slightly higher than \( N_s \). Second, points located at distances that are smaller than \( d \) from their nearest neighbours are deleted. Third, the numerical density of the remaining points (\( N_s^* \)) is calculated. Fourth, the entire procedure is repeated with a higher initial density until \( N_s^* \approx N_s \). This algorithm is also known as the Matern process of rigid spheres, because it is equivalent to throwing a known number of weightless solid spheres into a box of known dimensions. This methodology was adopted in our previous work to study populations of dendritic spines (Ref. b). By avoiding points located near the box edges (these will give biased measurements and must not be sampled), the NND is found for each point within the generated scatter.

References

Box 2. The mean nearest-neighbour distance between synapses: Monte-Carlo estimates and 3D reconstruction from serial sections

Loosen that the distribution of synapses can be reasonably approximated by a hard-core Poisson or Matern scatter, only two experimental measures are needed to determine the mean nearest-neighbour distance (NND): the mean synaptic density, $N_s$, and the mean size of synapses, $d$. Alternatively, a small sample of direct measurements of NNDs can be obtained using 3D reconstruction of a tissue fragment from serial sections. In our experiments, 14 ultra-thin (60–75 nm) sections were taken from area CA1 in the rat hippocampus. In larger samples of serial sections, it was difficult to control the accumulated alignment bias that is typical for the large sampling windows we used (Ref. 13). The sections were collected on 0.1-mm-thick grids and analysed using an electron microscope. Fields of interest in the neuropil (14 μm wide, 10 μm high) were imaged and aligned in consecutive sections.

In each individual section, the 2D coordinates of the centres of all synaptic boutons were measured. To obtain quantitative values of synaptic density, $N_s$, and synaptic size, $d$, spheres generated using the Monte-Carlo algorithm and the above values of $d$ and $N_s$. These estimates of mean synaptic density, $N_s$ (1.25 × 10$^{-5}$), and mean synaptic size ($d = 220$ nm), were obtained. Figure IA shows a Matern scatter of spheres generated using the Monte-Carlo algorithm and the above values of $d$ and $N_s$. Figure IB illustrates the synaptic scatter reconstructed directly from the stack of serial sections. Thus, data in Fig. 1 allow not only a numerical comparison but also a visual comparison of Monte-Carlo experiment results and 3D-reconstruction data within a similar slab of tissue.

References

Although the methods underlying the Monte-Carlo assessment are based on the use of representative samples of data (sections are sampled throughout the region of interest in several animals), it is useful to have a direct verification of the estimated mean NND. This can be achieved by using 3D reconstruction of a tissue fragment from serial ultra-thin sections, as described and illustrated in Box 2. Our 3D-reconstruction experiment yielded the NND histogram shown in Fig. 2, which gives a mean NND of 0.48 μm for dentate gyrus and of 0.49 μm for area CA1 (including the correction for tissue shrinkage).

Distances between synapses: direct measurements from 3D reconstruction

It seems reasonable to approximate the local distribution of synapses with a simple model of a hard-core Poisson process, or to sometimes describe the Matern process for rigid spheres because it is analogous to spheres scattered randomly in space. In terms of stochastic geometry, this type of scatter is termed a hard-core Poisson process, or is sometimes described as the Matern process for rigid spheres because it is analogous to spheres scattered randomly in space. It seems reasonable to approximate the local distribution of synapses with such a pattern. In this case, the mean NND can be estimated from a Monte-Carlo sampling experiment that uses two experimental measures: the numerical synaptic density, $N_s$, and the mean synaptic size (or projected height), $d$, as explained in Box 2. The values of $N_s$ and $d$ in area CA1 and dentate gyrus have been obtained from our previous work. These estimates are consistent with earlier reports by Geminian and colleagues, and also included a measured tissue-shrinkage factor for the electron-microscopy embedding protocol we used. An example of the synaptic scatter generated in our Monte-Carlo simulations, the mean NND is estimated by generating large samples (>1000) of synaptic coordinates scattered in a cube so that their numerical density matches $N_s$, as described in more detail in Box 1. Once the appropriate scatter is generated, measuring distances between nearest neighbours is straightforward. Figure 1 shows histograms of inter-synaptic distances measured in such samples and gives a mean NND of 0.46 μm for dentate gyrus and of 0.49 μm for area CA1 (including the correction for tissue shrinkage).

synapses in area CA1 and in the dentate gyrus of the hippocampus we found no regular pattern. The minimum distance between the centres of the synaptic profiles is apparently restricted by the size of the synapses themselves (this size can be thought of as twice the mean distance between the synaptic-cleft centre and the closest nonsynaptic-element of the neuropil in 3D). In terms of stochastic geometry, this type of scatter is termed a hard-core Poisson process, or is sometimes described as the Matern process for rigid spheres because it is analogous to spheres scattered randomly in space. It seems reasonable to approximate the local distribution of synapses with such a pattern. In this case, the mean NND can be estimated from a Monte-Carlo sampling experiment that uses two experimental measures: the numerical synaptic density, $N_s$, and the mean synaptic size (or projected height), $d$, as explained in Box 2. The values of $N_s$ and $d$ in area CA1 and dentate gyrus have been obtained from our previous work. These estimates are consistent with earlier reports by Geminian and colleagues, and also included a measured tissue-shrinkage factor for the electron-microscopy embedding protocol we used. An example of the synaptic scatter generated in our Monte-Carlo simulations, the mean NND is estimated by generating large samples (>1000) of synaptic coordinates scattered in a cube so that their numerical density matches $N_s$, as described in more detail in Box 1. Once the appropriate scatter is generated, measuring distances between nearest neighbours is straightforward. Figure 1 shows histograms of inter-synaptic distances measured in such samples and gives a mean NND of 0.46 μm for dentate gyrus and of 0.49 μm for area CA1 (including the correction for tissue shrinkage).
volume of tissue in one animal, the consistency be-
tween the two 3D-reconstruction measures and the
Monte-Carlo estimate of NND is reasonable.
These data, therefore, indicate that the mean NND
between centres of synaptic clefts in dentate gyrus or
area CA1 is around 0.5 μm rather than 1.0 μm, the value
often deduced from observing 2D-electron micrographs
(see discussion in Ref. 20). What are the implications of
this structural constraint for inter-synaptic crosstalk?

**Diffusion of neurotransmitter following exocytosis**

Following exocytosis, neurotransmitter diffuses
rapidly within the synaptic cleft and further into the
extracellular space. Does this diffusion activate synaptic
receptors at the neighbouring synapse? Experimental
detection of neurotransmitter levels with this temporal
and spatial resolution is not yet feasible so the alterna-
tive is to try to answer a simple question: what do the
laws of physics predict regarding extrasympatic diffu-
sion? A common criticism of any analytical approach is
that we simply do not know many constraints that are
built into a particular model (for example, the amount
of glutamate released or the density of its binding sites).
However, a simple test for robustness of the conclusions
drawn from modelling is to explore the unknown
parameters over the known physiological range. This
approach has been applied successfully in several stud-
ies22–24, which include Monte-Carlo simulations where
movements of individual molecules of neurotransmitter
have been traced explicitly22–24. One constraint, however,
remains to be clarified in such simulations: how to rep-
resent the geometry of the synaptic micro-environment
for the synaptic population of interest.

Box 3 illustrates an experimental approach that we
used recently to address this issue. We analysed a statis-
tical sample of electron micrographs of synapses in order
to establish the occurrence of the perisynaptic extra-
cellular space around synaptic profiles in area CA1 of
the hippocampus31. We used this average occurrence
profile to build a 3D model of the typical synaptic
environment in area CA1 with the available
parameters over the known physiological range. This
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remains to be clarified in such simulations: how to rep-
represent the geometry of the synaptic micro-environment
for the synaptic population of interest.

Although synaptic activity is likely to elicit substan-
tial Ca2+ depletion in the synaptic vicinity, extracellular
Ca2+ transients have only been measured relatively indi-
rectly, with low spatial and temporal resolution relative
to inter-synaptic distances and time courses of release,
respectively25–30. The extent to which Ca2+ depletion might
affect neighbouring synapses remains unclear.

In this study, we combined our geometric model of the
synaptic environment in area CA1 with the available

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**Fig. 1.** Histograms of the nearest-neighbour distances between excitatory synapses obtained from Monte-Carlo experiments that model synaptic scatter in hippocampal neuropil. The arrows indicate the mean value (also denoted as m). The values of numeric synaptic density, Nv, account for tissue shrinkage and the mean synaptic size parameter, d, is 0.23 μm. The experimental values of Nv and d were calculated in accordance with the Disector technique (using adjacent ultra-thin (~65 nm) sections taken throughout the regions of interest from four (CA1) and six (dentate gyrus) animals31–33.

**Fig. 2.** Histogram of nearest-neighbour distances between synapses measured in a fragment of neuropil reconstructed in 3D. Nearest-neighbour distances (NNDs) were measured between synaptic cleft centres in a 15 μm × 9 μm × 0.95 μm block of tissue from area CA1 of rat hippocampus, which was reconstructed in 3D using a serial section technique. The arrow indicates the mean NND (m). N represents the number of measured distances and Nv represents numeric synaptic density.
We attempted to quantify the typical geometry of the synaptic microenvironment by analysing samples of electron micrographs. Profile fragments of the extracellular space surrounding 88 axo-spinous synapses were aligned with respect to the synaptic cleft and then superimposed as described by Rusakov and Kullmann. The outcome of this procedure is shown in Fig. 1A, where the grey level indicates the probability of encountering the extracellular space at different positions relative to the synaptic cleft (darker grey represents higher probability). These results allow one to build a realistic 3D model of the typical synaptic environment, of which a 2D section is shown in Fig. 1B. In this model, the neurotransmitter is released into a ~220 nm wide, 20 nm thick cleft between two hemispherical obstacles to diffusion, which represent the presynaptic and postsynaptic elements. Beyond the cleft and obstacles, the neurotransmitter moves within a homogeneous porous medium, a robust approximation that reduces the effect of geometric obstacles to two parameters: the extracellular volume fraction, $\alpha$, and the geometric tortuosity, $\lambda$ (Ref. b,c). Morphometric analysis of neuropil in CA1 performed in our earlier study gave $\alpha = 0.12$ and $\lambda = 1.36$ (Ref. a). The value $\lambda \approx 1.4$ was subsequently confirmed to be unique for an isotropic porous medium with no preferred orientation of planar 2D pores. The space in this model was divided into small compartments (thin concentric shells) to allow direct computation of the mass transfer of glutamate (or Ca$^{2+}$) in accordance with Fick’s first law. Given sufficiently small time steps and a suitable shell width, this procedure has no limitations regarding the complexity of the reactions involved. Glutamate release from the synaptic vesicle (which contains 1000 molecules) was represented by the function $\delta(t) = 1\exp(-t/\tau_0)$ with $\tau_0 = 39 \text{ ms}^{-1}$ (Ref. a). The extracellular diffusion coefficient for glutamate was variably estimated between 0.2 $\mu\text{m}^2\text{ms}^{-1}$ and 0.75 $\mu\text{m}^2\text{ms}^{-1}$ in order to reflect retarded diffusion caused by geometric or viscous components of $\alpha$, or both (Ref. d). Glutamate transporters present in cell membranes had binding, unhinding and translocation rates of $1\text{ms}^{-1}$, $1\text{ms}^{-1}$ and $0.03 \text{ms}^{-1}$, respectively (Ref. e). The activation of AMPA and NMDA receptors at different distances from the release site was computed in accordance with kinetic schemes proposed by Lester and Jahr (1998) and Kocho (1995), respectively.

The same geometric model was used to calculate extracellular Ca$^{2+}$ transport following synaptic activation. The presynaptic Ca$^{2+}$ influx that triggers excitosynaptic activity has been studied with sufficient accuracy to justify the modelled parameters (Ref. i). In this case, synaptic activation (through action potential) opened $\approx 10$ presynaptic voltage-gated channels with an $\alpha$-function opening time course: $\alpha(t) = \alpha_0 [1 - \exp(-t/\tau_0)]$, where $Q_0 = 10\text{mC}$, $\tau_0 = 0.5 \text{ ms}$ (an individual peak channel current, $Z = 2$ for Ca$^{2+}$, $e = 1.9 	imes 10^{19}$ is the elementary charge, $N_e = 6 	imes 10^{23} \text{mol}^{-1}$ (Avogadro’s number), and $\sigma = 20 \text{ mC}$ (Ref. j,m)). During Ca$^{2+}$ depletion, this influx current was attenuated with a proportionality factor of $\sigma$ (extracellular Ca$^{2+}$ concentration $\approx$ intracellular Ca$^{2+}$ concentration), in accordance with the Nernst equation. Active Ca$^{2+}$ extrusion was represented by a first-order process, with a rate of $0.5 \text{ms}^{-1}$ (Ref. f), which also incorporates an estimate of the cell-membrane surface area per unit volume. Less is known about the permeability of barriers extending 0.25 nnm from the synaptic cleft cleft. The crucial unknown parameter explored in these simulations was the total peak Ca$^{2+}$-current density through channels in the postsynaptic membrane.

This model does not consider the role of Ca$^{2+}$ ions that accumulate near cell membranes in form part of the electrical charge-screening layer, which apparently includes two components. The first component arises from the surface excess charge density, $\sigma$, induced by the depolarized membrane. The value of $\sigma$ can be assessed from a simple formula for the planar capacitor: $\sigma = \frac{eN_e}{d}$, where $e$ is the membrane dielectric constant ($\approx 2$), $\lambda$ is the permittivity of vacuum, $d$ is the membrane thickness (~5 nm), and $V_e$ is the membrane potential (~70 mV). This formula gives $\sigma = 2.5 \times 10^{-4} \text{C} \text{m}^{-2}$ implying an excess density of divalent ions of $10^{-5} \text{m}^{-2}$. Given an intercellular gap width of ~20 nm, this corresponds to $V_e$-dependent changes of local extracellular Ca$^{2+}$ concentration at a level of less than ~0.1 m, which is unlikely to affect our calculations. The second component is due to acidic phospholipids of cell membranes, which induce an electrostatic potential ($\phi$) that ranges from ~10 to ~100 mV in the extracellular space (Ref. g). This predicts a 100- to 1000-fold increase in Ca$^{2+}$ levels near the membrane surface, according to the classical Debye–Hückel theory (or its Gouy–Chapman modification). However, it remains to be ascertained whether the classical theory is readily applicable in this case: for example, sub-membrane ion densities that exceed ~0.25 mm$^{-3}$ would be inconsistent with Debye’s radius of ~1 nm, which is characteristic for the extracellular medium. Furthermore, the thermal energy of sub-membrane Ca$^{2+}$ ions, $k$T, reduces $\phi$ in the Boltzmann term, $\exp(-\phi/kT)$, and their electric energy, $Z\phi$ (where $Z = 2$, and $\phi$ is the elementary charge), appear to be of the same order of magnitude (~10$^{-5}$ mV), in which case the ion distribution might not follow the classical theory. Finally, it is unlikely that any release of these Ca$^{2+}$ ions ‘bound’ by the fixed $p$ potential is as rapid as Ca$^{2+}$ diffusion in the free extra- cellular medium. The issue of the sub-membrane Ca$^{2+}$ store, however, requires more experimental tests and detailed theoretical consideration.

References
physiological data concerning properties of Ca\(^{2+}\) channels, in order to simulate extracellular Ca\(^{2+}\) transients, as explained in Box 3. Figure 4 shows the simulated time course of the extracellular Ca\(^{2+}\) level within and at different distances from the centre of the synaptic cleft. The results demonstrate that the action-potential-driven presynaptic Ca\(^{2+}\) influx that triggers exocytosis can deplete the level of the free ion in the cleft very rapidly (whereas outside the cleft Ca\(^{2+}\) levels remain almost unaffected). The level inside the cleft, however, returns to its resting value rapidly (within 1 ms) because of Ca\(^{2+}\) diffusion from the perisynaptic space. A more prolonged decrease in extracellular Ca\(^{2+}\) levels can occur following the opening of various postsynaptic Ca\(^{2+}\) channels. Their opening probability, and therefore the magnitude of the postsynaptic Ca\(^{2+}\) influx, depends on the membrane depolarization, which in turn depends on the synaptic activation of AMPA and NMDA receptors. Given that synaptic activation is likely to induce Ca\(^{2+}\) influx only through the spine-head membrane, changes in the extracellular Ca\(^{2+}\) concentration around neighbouring synapses are small (Fig. 4A). Only when the postsynaptic current is an order of magnitude larger than is thought to be a strong synaptic signal does the Ca\(^{2+}\) depletion begin to affect neighbouring synapses, as illustrated in Fig. 4B. Although these data were computed for the resting extracellular Ca\(^{2+}\) concentration of 1 mM, other values for the resting levels produce similar profiles, provided that the Ca\(^{2+}\) influx is proportionately adjusted.

These data suggest that, unlike neurotransmitter spillover, the occurrence of synaptic crosstalk directly through the tissue volume, owing to extracellular Ca\(^{2+}\) depletion, is unlikely. An alternative mechanism, however, which could contribute to extracellular Ca\(^{2+}\) depletion, is the depolarization of the postsynaptic membrane that contains voltage-dependent Ca\(^{2+}\) channels. Our previous simulation study explored a vertically built compartmental-cell model with dendritic spines that contained a mixed population of Ca\(^{2+}\) channels. We used the time course of regenerative postsynaptic Ca\(^{2+}\) currents obtained in that study to drive Ca\(^{2+}\) influx in our present diffusion model. The results gave a profile of postsynaptic Ca\(^{2+}\) depletion, which was only 10–15 ms long but otherwise qualitatively similar to that in Fig. 4. In these simulations, dendritic ‘spiking’ and, therefore, significant Ca\(^{2+}\) depletion were observed when the peak conductance density of postsynaptic Ca\(^{2+}\) channels exceeded the range of ~10–20 pA/μm\(^2\) (Ref. 36). This value is several times lower than the somatic membrane conductance (surface area of ~500–1000 μm\(^2\)) predicted by the peak Ca\(^{2+}\) influx of 1–2 nA following an action potential generated at a giant axo–somatic synapse in the brainstem. One factor that could, in principle, attenuate any long-term Ca\(^{2+}\) depletion, is the release of ‘bound’ Ca\(^{2+}\) ions that constitute the submembrane electrical double layer (see Box 3). However, it is less likely to occur following the release of neurotransmitter spillover.
On the role of NO in the thalamus

The article by Cudeiro and Rivadulla is an important and timely review that attempts to provide an overview of the possible effects of NO in the visual system, and, as such, represents a novel way of interpreting the subject matter. In particular, the authors appear to suggest that in the lateral geniculate nucleus (LGN) of the thalamus, NO affects visual responses via a specific modulation of NMDA receptors that do not involve the guanylate-cyclic-GMP system. This interpretation rests on a significant extent to the finding made by Cudeiro and co-workers that l-arginine application of N-bromo-GMP onto lateral LGN neurones in vivo does not mimic the effects of NO donors.

We feel that in their endeavour the authors have not taken into consideration all of the available data concerning NO-related modulation of neurotransmission in the thalamus and, thus, provide an incomplete view of the function(s) of this modulator in the LGN and in other thalamic areas. There is a need in the physiological evidence to suggest that NO is released in the thalamus during arousal, most probably because of the activity of the cholinergic fibres arising from the brainstem. In addition, biochemical evidence suggests that these effects are mediated via the guanylate-cyclic-GMP pathway. This suggestion is further supported by in vitro electrophysiological data, showing that NO donors cause a shift in the voltage dependence of the hyperpolarization-activated cation current I, in thalamo-cortical neurones of the cat and guinea-pig lateral and medial geniculate nuclei in vivo, and in hippocampal neurones. Inhibitors of NO generation associated with a decrease in input resistance and interruption of rhythmic burst activity in these neurones. The effects of NO donors are blocked by GMP analogues, indicating a common pathway of action. Taken together, these data suggest that this phenomenon has any effect on fast extracellular Ca2+-transients, and more empirical data are required to ascertain its spatial and temporal characteristics.

Concluding remarks

The mean inter-synaptic distance is an important constraint for physiological hypotheses that address neurotransmission between synapses in the CNS. Building this constraint and other morphometric information into a realistic model of the synaptic environment reveals two mechanisms that could mediate inter-synaptic communication. First, NMDA-receptor-mediated cross-talk is likely to occur between conventional excitatory synapses. However, the modulator of NMDA receptors that does not involve the guanylate-cyclic-GMP system is likely to be especially important at the 30–50% of synapses that are separated by less than the mean NND (371.13,21) and could explain the observation that some synaptic release events are mediated exclusively by NMDA receptors. Whether crossstalk occurs appreciably in vivo depends on the relatively poorly understood role of glial transporters. Transporters could either rapidly reduce the initial level of released neurotransmitter or, after one or two milliseconds, retard its diffusion, or both, in a temperature-dependent manner. Second, transient depletion of extracellular Ca2+-ions caused by the fast presynaptic influx that follows synaptic activation is unlikely to extend far beyond the synaptic cleft in CA1 (although it might do so at some calyceal synapses). Ca2+-depletion-mediated cross-talk could potentially occur, however, if the elicited post synaptic Ca2+-influx is five to ten times higher than can be produced by the estimated average individual synaptic signal that activates AMPA and NMDA receptors. Alternatively, the opening of extracellular voltage-dependent Ca2+-channels in the postsynaptic membrane could extend Ca2+-depletion to the extracellular space adjacent to the postsynaptic cell membrane outside the immediate synaptic cleft. How often the properties of individual synapses meet these crucial requirements for synaptic cross-talk remains to be ascertained experimentally. The conclusions of this study are related to young adult animals, and additional data would be required to assess the plausibility of extrasympathetic communication in animals of different ages.

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