OPINION

The probability of neurotransmitter release: variability and feedback control at single synapses

Tiago Branco and Kevin Staras

Abstract | Information transfer at chemical synapses occurs when vesicles fuse with the plasma membrane and release neurotransmitter. This process is stochastic and its likelihood of occurrence is a crucial factor in the regulation of signal propagation in neuronal networks. The reliability of neurotransmitter release can be highly variable: experimental data from electrophysiological, molecular and imaging studies have demonstrated that synaptic terminals can individually set their neurotransmitter release probability dynamically through local feedback regulation. This local tuning of transmission has important implications for current models of single-neuron computation.

Revealing the fundamental principles of how neural circuits are organized and operate is a key challenge in neuroscience. In the past 60 years, intensive effort has been invested in characterizing the properties of neuronal connections — the basic substrates of information transfer in neuronal networks. Electrophysiological studies, as well as light, fluorescence and electron microscopy approaches, have advanced our understanding of the functional and morphological characteristics of synaptic connections between many neuron types. In particular, two important principles have emerged from these efforts. First, in many cases, individual connections between two neurons are comprised of multiple synaptic contacts, the number varying with the connection type. Second, the synapses that mediate these connections can be functionally very different, even when they belong to the same axon and contact the same postsynaptic target. What is the basis for this multiplicity and variability in the composition of a connection and what function does it serve?

For technical reasons early investigations were limited to studies of connections as a whole (that is, the compound output of all participating synaptic contacts) and to the use of indirect analytical methods for extracting the average unitary properties of the synapses composing them. These studies, pioneered by Katz and colleagues, established that the strength of a connection is fundamentally dependent on three main factors: the number of synaptic contacts, the size of the postsynaptic depolarization caused by neurotransmitter release from a single synaptic vesicle (termed quantal size) and the probability of neurotransmitter release at each synapse[1]. This third parameter, which is the main focus of this Perspective, is termed release probability (p_r) and is a consequence of the inherently stochastic nature of the molecular and cellular processes that drive vesicle exocytosis[2]. Thus, for each action potential, neurotransmitter release has a certain likelihood of occurrence that defines the reliability of a synapse for transmitting the action potential signal and determines the average synaptic strength[1].

Recently, developments in molecular and imaging techniques have enabled studies to go beyond the level of whole connections and analyse functional details of the individual synaptic contacts. Using this approach, evidence has accumulated which shows that single terminals contributing to a connection can have release probabilities that are diverse and that can change over time.

Box 1 | **Defining the synaptic composition of a connection**

Establishing the number of active neurotransmitter release sites in CNS neurons is a major technical challenge. Why is this the case? The central problem is that unequivocally defining a synaptic specialization between two neurons requires ultrastructural confirmation. Ideally, a complete morphological reconstruction of the two target cells of interest and their contact points should be carried out in ultrastructural detail, coupled with some form of physiological measurement that establishes active neurotransmission. In reality, a full reconstruction is hard to achieve, particularly because dendrites and axons are often long and branched. Thus, researchers have generally relied on approaches that are less exhaustive but can still offer valuable information. One classical experimental strategy is based on stimulating a single putative axon and recording intracellularly from target neurons. Subsequent analysis and fitting of statistical models to the excitatory postsynaptic potential fluctuations allows estimations of the connection parameters, including the number of release sites — a method commonly known as quantal analysis[133]. This method, when used in combination with horseradish peroxidase (HRP) injections to the stimulated axon, can identify putative contact points. A second approach relies on paired recordings and quantal analysis, combined with HRP or biocytin filling of the recorded cells to identify contact points. This is sometimes followed by ultrastructural analysis to confirm the presence of a synapse (FIG. 1). Until recently, this second approach has represented the gold standard for mapping brain connectivity. Other approaches to estimate how many synapses constitute a given connection include the use of synaptic markers, such as antibodies directed at presynaptic proteins, in combination with some form of cell morphology visualization — for example, expression of cytoplasmic green fluorescent protein or filling the cells with fluorescent dyes. These methods have been especially useful in cultured neurons, where the low synaptic density facilitates identification of individual contacts. New technical advances, such as block-face electron microscopy[134], circuit tracing with engineered viruses and various genetic approaches[135], already offer major new strategies to characterize neuronal connections and will certainly revolutionize our understanding of the details of the brain wiring diagram.

Moreover, p_r seems to be regulated with high spatial precision. Defining the factors that contribute to setting the efficacy of the synaptic vesicle release process and how they interact is crucial for understanding both the nature and the functional value of neurotransmitter release regulation. Here we consider how individual synapses are organized in neuronal connections and examine the experimental evidence for heterogeneity in neurotransmitter release between different synaptic terminals. We focus on local feedback control as an important mechanism for p_r regulation and heterogeneity and, from a theoretical standpoint, consider its possible implications for information processing.

Composition of neuronal connections

As mentioned above, the number of synaptic contacts that contribute to a connection is one of the three main parameters that define the function and regulation of that connection. Nonetheless, estimating this value for a given connection is not a trivial problem (BOX 1). The first studies to provide definitive evidence that neuron–neuron connections in the CNS could be composed of multiple synaptic contacts were made in the 1980s using electrophysiological recordings combined with light and sometimes electron microscopy. Researchers demonstrated that, in the cat spinal cord, single axons could make almost 20 synapses onto individual neurons of the dorsal spinocerebellar tract[3]. Similarly, goldfish Mauthner cells were shown to receive up to 25 synapses from a single interneuron[4,5]. Information on connection properties has now been accumulated for many neuron classes (FIG. 1; TABLE 1), revealing striking variability in the composition of connections for different cell types. In the cerebellum, parallel fibres from granule cells usually make only one synapse with Purkinje cell dendritic trees[6]. In the cortex, connections between pyramidal neurons make on average five synapses[7,8], whereas interneurons usually make more than ten synaptic contacts[9,10]. More extreme examples include the climbing fibres in the cerebellum, which establish more than 500 synapses with a single Purkinje cell[11], and the Calyx of Held nerve terminals, where ~600 neurotransmitter release sites are concentrated in one giant synapse[12,13]. Multiple and variable numbers of synapses are also features of cultured neurons. In primary cultures of dissociated hippocampal cells, for example, connections are typically comprised of 5 to 20 synaptic contacts, the number being in part determined by culture geometry and cell density[14].

Not only are connections made up of variable numbers of synapses, but their spatial organization can also vary greatly. At some connections synapses are distributed across the whole dendritic tree, but in others they target specific regions of the postsynaptic cell. For example, synapses between cortical pyramidal cells in the same layer tend to be restricted to the basal and apical oblique dendrites[8], whereas if the target is an interneuron, such as a basket cell, synapses

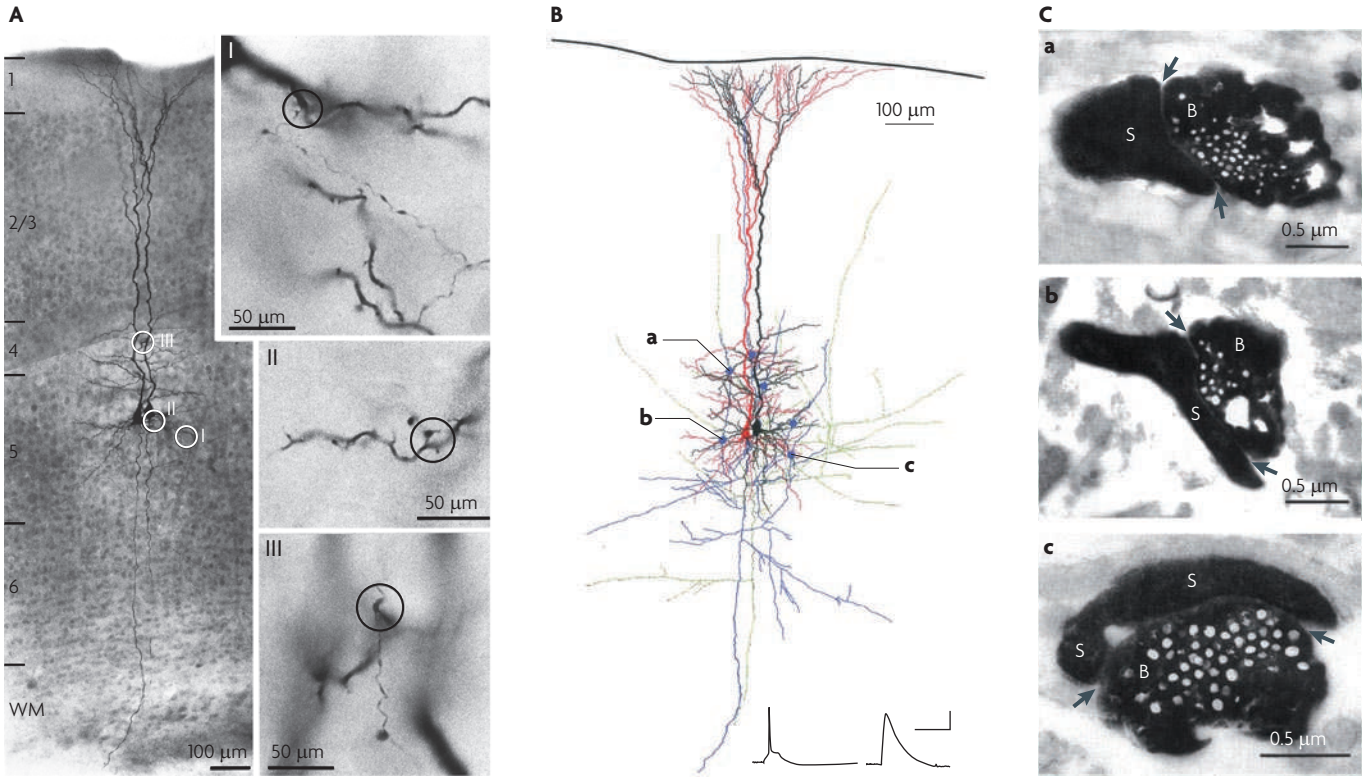

Figure 1 | **Characterizing neuronal connections with paired recordings and morphological reconstructions. A** | A light microscopy image of a connected pair of thick-tufted layer 5 pyramidal neurons filled with biocytin. The open circles indicate three contacts (I and II are synapses; III is an autapse) established by the left-hand neuron. The smaller panels show higher magnification views of these contacts. **B** | The same pair of neurons after camera lucida reconstruction. The dendritic arbor of the projecting neuron is in red and its axonal arborization is in blue. The dendritic arbor of the target neuron is in black and its axonal arborization is in green. The filled blue circles indicate putative synaptic contacts established by the red and blue neuron on the black and green neuron. The inset traces show an example of a presynaptic action potential and the corresponding excitatory postsynaptic potential, illustrating the presence of an excitatory connection. Scale bar: 50 ms (both traces), 40 mV (left trace) and 800 µV (right trace). **C** | Electron micrographs showing the ultrastructure of the synaptic contacts indicated in part **B**. The arrows point to the synaptic cleft. B, synaptic bouton; S, spine; WM, white matter. Figure is modified, with permission, from REF. 138 © (1997) The Physiological Society.

are typically made all over the dendritic tree and the cell body[15] (see also REF. 16). Other connections have a variety of different organizations and degrees of clustering. Differences in synaptic spatial organization will influence dendritic integration[17,18] and will have an impact not only on spike output but also on the way that single synapses are regulated; the functional consequences for this are considered later in this Perspective.

Synapses are functionally heterogeneous

Connections are typically comprised of multiple synaptic contacts, but do individual synapses behave similarly? Here, we address this question by considering one of the

Table 1 | **Summary of number of contacts and release probability (p_r) in different connections**

CNS area	Presynaptic	Postsynaptic	N	p_r	Methods
Cat spinal cord	Group 1a axons	Motor neurons	2–5 (REFS 22,24,146)	0–1 (CB) (REFS 21,22,24)	R[21,22,24], LM[22,146], QA[21,22,24]
Cat spinal cord	Group 1a and 1b axons	DSCT neurons	1–18 (REF. 3)	0.07–1 (CB) (REF. 23) 0.06–0.85 (CB) (REF. 3)	R[3,23], LM[3], QA[3,23]
Frog spinal cord	Primary afferent fibres	Motor neurons	21–72	0.15–0.69	R, LM, QA[147]
Goldfish brainstem	Interneurons	Mauthner cells	3–28 (REFS 4,5)	0.17–0.62 (REFS 4,5)	PR[4,5], LM[4,5], EM[4,5], QA[4,5]
Cat and rat L2/3	Pyramidal cells	Interneurons	1–7 (REF. 15)	0–0.84 (CB) (REF. 15) 0.13–0.64 (REF. 49)	PR[15,49], LM[15], EM[15], QA[15], CaI[49]
Cat L2/3	Interneurons	Pyramidal and spiny stellate cells	3–17 (REF. 10)	ND	PR, LM, EM[10]
Cat and rat L2/3	Pyramidal cells	Pyramidal cells	3.9±0.8 (REF. 148) 2–4 (REF. 8) 7.6±4.7 (REF. 149)	0.5±0.05 (REF. 148) 0.46±0.26 (REF. 49) 0.65±0.18 (REF. 149)	PR[25,35,42,148], LM[8], QA[148,149], CaI[49]
Rat L2/3	L4 spiny cells	Pyramidal cells	4–5 (REF. 150) 4–6 (REF. 151)	0.79±0.04 (REF. 151)	PR[150,151], LM[150,151], EM[151], QA[151]
Cat and rat L4	L4 pyramidal and spiny stellate cells	L4 pyramidal and spiny stellate cells	2–5 (REF. 152) 8±4.2 (REF. 149)	0.69–0.98 (REF. 153) 0.86±1.09 (REF. 149)	PR[152], R[153], LM[22,153], QA[153]
Rat L5/6	Pyramidal cells	Pyramidal cells	2–8 (REF. 7) 4–8 (REF. 138) 8.1±4.2 (REF. 149)	0.16–0.9 (REF. 138) 0.53±0.22 (REF. 149)	PR[7,138,149], LM[7,138], EM[138], QA[138,149]
Rat L5/6	Interneurons	Pyramidal cells	1–5 (REF. 154)	ND	PR, LM, EM[154]
Rat L5/6	Pyramidal cells	Interneurons	6–12 (REF. 155)	<0.1 (REF. 155)	PR, LM, EM[155]
Rat CA1	Interneurons	Pyramidal cells	6–12 (REF. 9)	ND	PR, LM, EM[9]
Rat CA1	Pyramidal cells	Pyramidal cells	2 (REF. 156)	ND	PR, LM, EM[156]
Rat CA1	Stratum radiatum	Pyramidal cells	3–18 (REF. 157)	0.14–0.81 (REF. 157) 0.06–0.37 (REF. 26)	R[26,157] QA[157], MK[26]
Rat CA3	Interneurons	Pyramidal cells	2–13 (REF. 158)	ND	PR, LM, EM, QA[158]
Guinea pig CA3	Pyramidal cells	Interneurons	1–3 (REF. 159)	0.75±0.19 (REF. 159)	PR, LM, EM, QA[159]
Rat hippocampal cultures	Excitatory cells	(Autapse)	ND	0.09–0.54 (REF. 27) 0.05–0.9 (REF. 28)	R[27,28] MK[27] FM[28]
Rat hippocampal cultures	Excitatory cells	Excitatory and inhibitory cells	3–19 (REF. 14)	0.03–0.9 (REF. 14)	PR, FM, EM, QA[14]
Rat cerebellum	Climbing fibres	Purkinje cells	510±50 (REF. 25) 221–392 (REF. 11)	0.9±0.03 (REF. 25)	R[25], QA[25], LM[11], EM[11]
Rat cerebellum	Parallel fibres	Purkinje cells	1–2 (REF. 6)	0.05 (REF. 160)	R[160], M[160], LM[6], EM[6]
Rat cerebellum	Interneurons	Stellate and basket cells	ND	0.1–0.54 (REF. 161)	R, MS[161]
Striatum	L4/5 afferents	Medium spiny neurons	ND	0.42 (REF. 162)	R, QA[162]
Rat auditory brainstem	Calyx of Held	Principal cells in MNTB	637±113 (REF. 13)	0.25–0.4 (REF. 13)	PR, QA[13,163,164]
Striatum	Thalamic afferents	Medium spiny neurons	ND	0.72 (REF. 162)	R, QA[162]
Olfactory bulb	Olfactory receptor neurons	Principal mitral and tufted cells and periglomerular interneurons	ND	0.92±0.03 (REF. 6)	R, QA[6]
Olfactory bulb	Interneurons	Juxtaglomerular cell	ND	0.21–0.32 (REF. 165)	R, MS[165]

Summary of the number of release sites and p_r for some connections in the CNS, illustrating the diversity of these parameters across different connections. CA1, hippocampal area CA1; CA3, hippocampal area CA3; CaI, Ca^{2+} imaging; CB, compound binomial; DSCT, dorsal spinocerebellar tract; EM, electron microscopy; FM, FM-dye based method; L, layer; LM, light microscopy; M, modelling; MK, MK-801 method; MNTB, medial nucleus of the trapezoid body; MS, minimal stimulation; N, number of contacts; ND, not determined (no absolute value was estimated); PR, paired electrophysiological recording; QA, quantal analysis; R, electrophysiological recording.

crucial determinants of synaptic performance, p_r. This variable not only defines the reliability of synaptic transmission, but also changes with the short-term activity history of the synapse, thus shaping the way in which a connection dynamically adapts to input[19,20]. Although p_r is a fundamental synaptic parameter, it is difficult to measure directly and many investigations rely on methods that can provide only estimates of its value (BOX 2).

In most studies of synaptic function, p_r is considered to be the same for all terminals in a connection between two neurons and the measured p_r therefore represents the average of all contributing synapses. As such, this value provides no specific information about the properties of individual terminals, which could in fact be highly variable, as del Castillo and Katz insightfully noted when they first formulated their theory of synaptic transmission[1]. Indeed, early experiments using classic quantal analysis of postsynaptic responses in a single cell suggested that there is considerable variability across terminals of the same axon. For example, response amplitude histograms from recordings of cat spinal cord neurons were shown to be better fit by a compound binomial model, in which each release site has a different p_r, than by a simple binomial model[21–23]. Similar conclusions were drawn from variance mean analysis applied to both spinal cord neurons[24] and cerebellar climbing fibres[25]. Also, in CA1 pyramidal cells, NMDA (*N*-methyl-D-aspartate) current block curves (BOX 2) were better fit with a bi-exponential curve than with a simple exponential curve, implying that the connections had at least two groups of synapses with very different p_rs[26]. Even in autaptic cell cultures, in which a cell makes synaptic contacts with itself, p_r seems to be extremely non-uniform across different boutons, as demonstrated both by the NMDA current block approach[27] and by fluorescence-based measurements of p_r at individual synapses[28]. Based on the latter methodology, Murthy *et al.* reported a wide and continuous distribution of p_rs across different boutons, skewed to larger values and with a coefficient of variation larger than 0.5 (REF. 28), a finding that was recently confirmed by other groups[14,29,30] (FIG. 2a,b). Moreover, morphological correlates of p_r at synapses, such as the active zone size and the number of docked vesicles, have been shown to exhibit a similar distribution[31].

Additional support for the idea that synapses from a single axon can have different p_rs comes from work that investigated the synaptic properties along one axon that contacted different cell types. The first evidence for heterogeneity among synapses that share an axon but have different targets came from studies on the neuromuscular junction (NMJ). Direct focal recordings from different regions of an endplate belonging to one motor neuron axon, in the crayfish opener muscle, showed that boutons at different locations have different p_rs[32–35]. It was also demonstrated that an axon that innervates two different types of muscle can exhibit facilitation at one target and depression at the other[36]. Similar findings were reported from focal recordings at the frog NMJ[37–39] and from double recordings from two muscles contacted by the same axon in the lobster stomatogastric system[40]. This type of experiment, in which the short-term plasticity of synapses belonging to one axon is measured in two or more postsynaptic targets, has also been widely used to reveal p_r variability in the CNS. In the leech nociceptive system two different motor cells can be innervated by the same sensory cell, and the two synapses display opposite forms of short-term plasticity[41]; the same phenomenon has been observed in the cat spinal cord[42], in the giant reticulospinal axon synapses onto spinal neurons in the lamprey[43], in hippocampal cultures[44] and in cortical layer 2/3 and 5 circuits[45,46]. For some multiple-target recordings in cricket[47] and locust interneurons[48], quantal analysis confirmed that different p_rs underlie the differences in short-term plasticity. More recently, in a remarkable *tour de force*, Koester *et al.* performed optical quantal analysis using single-synapse Ca^{2+} imaging in layer 2/3 pyramidal cells and interneurons, and directly showed that p_r varies with the postsynaptic cell type[49] (FIG. 2c).

In most studies that demonstrated p_r variability between synapses, p_r was measured in a 'resting' ('basal') state, in response to a single action potential. However, as mentioned above, p_r is dynamic and varies on a very short timescale[50]. Therefore, although heterogeneity of basal p_rs is evident, during continuous activity differences between synapses will also be strongly influenced by the size and replenishment rate of vesicle pools[51]. As such, the basal p_r values might be the most influential neurotransmitter release

Box 2 | **Estimating release probability**

Release probability (p_r) can be estimated in many different ways. Some methods allow only relative comparisons whereas others provide absolute p_r estimates for either connections as a whole or individual synapses.

Quantal analysis
Analysis and binomial-model fitting of synaptic-response amplitude fluctuations is the classic method for extracting quantal parameters, including p_r. Many techniques for quantal analysis are available, and all of them require long and stable electrophysiological recordings[133].

Paired-pulse ratio
The degree of facilitation or depression of a synaptic connection depends on p_r and can be quantified by the paired-pulse ratio (PPR), which is defined as the amplitude ratio of the second to the first postsynaptic response after stimulating the connection with two action potentials[50]. An important caveat of this method is that the relationship between p_r and PPR is not known in most cases[28,136].

Failure rate
The frequency of failures of the synaptic connection can be used as an indication of p_r[137,138]. A major problem is that the failure rate depends on both p_r and the number of release sites, and so differences in the number of failures can be due to either of these parameters. An alternative is to stimulate single synapses, by careful placement of an electrode directly adjacent to a fluorescently labelled synapse[139,140].

Progressive block of NMDA synaptic current
When NMDAR (*N*-methyl-D-aspartate receptor)-mediated synaptic currents are recorded in the presence of an irreversible open-channel blocker (MK-801), the response amplitude is progressively reduced owing to the increasing number of receptors that become blocked after use. The rate of the block depends on how often glutamate is released, and a kinetic model can be fitted to the block curve and used to convert it into p_r[26,27].

Optical methods
FM dyes[14,28,141] and vesicle proteins tagged with pHluorins (recombinant fluorescent pH indicators)[30,142] allow p_r measurements at single synapses by imaging and quantifying vesicle exocytosis. When the signal/noise ratio is high enough to allow detection of single fusion events[142,143], p_r can be directly measured. Otherwise, estimations can be obtained from the average release in response to a series of action potentials. A different approach, termed optical quantal analysis, detects release events by imaging postsynaptic NMDAR-mediated Ca^{2+} accumulation in single contacts[49,140,144,145].

parameter in cells that fire infrequently, but other factors should be taken into consideration for neurons with high output rates. For example, although marked heterogeneity in release is apparent during low-frequency stimulation in hippocampal neurons in culture, after 60 action potentials at 10 Hz most terminals release at similar rates[51].

Feedback control of p_r

The marked heterogeneity of p_r for synapses along an axon raises the question of what actually determines p_r at a single synapse. Neurotransmitter release is the final result of a complex series of cellular and molecular steps, in which an action potential increases the intracellular Ca^{2+} concentration and triggers full or transient fusion of synaptic vesicles with the plasma membrane[2,52]. The success of this whole process fundamentally depends on three variables: the number of release-ready vesicles, the Ca^{2+} concentration in the presynaptic terminal and the molecular coupling between Ca^{2+} and vesicle fusion. A large number of factors can modify these variables, including the regulation of Ca^{2+} channel function[53], the modulation of release machinery proteins[2], the regulation of Ca^{2+} entry by the action potential waveform at the presynaptic terminal[54] and by different Ca^{2+} buffering capabilities[55], and the different architectures of Ca^{2+} channels and sensors[56,57] (FIG. 3). These and many other examples, the detailed description of which is beyond the scope of this article, clearly show that p_r regulation is a complex process, and that the p_r of a synapse depends on the balance of all these factors. But what engages these p_r regulators? For example, different synapses from the same axon in hippocampal cultures have different distributions of Ca^{2+} channel subtypes[58,59]. So what determines the types of Ca^{2+} channels expressed at the terminal or the phosphorylation state of a particular release machinery protein? The answer is not entirely clear, but some p_r regulators are determined by the nature of the cell itself and its developmental programme, whereas others depend on the cell's environment and network activity. In recent years, feedback from the postsynaptic site has emerged as a major influence on the variables and regulators that determine p_r. In this section we discuss examples that illustrate how the postsynaptic site can contribute to the determination of p_r at individual synaptic contacts.

Postsynaptic cell identity. As previously mentioned, there are many examples of one axon that contacts different targets exhibiting different release properties, suggesting that the identity of the postsynaptic cell is an important determinant of p_r. This postsynaptic influence can in principle be exerted either during synaptogenesis or through retrograde regulation after synapse formation. Furthermore, the fact that synapses next to each other in a single axon can contact different cells[60] suggests that this type of p_r regulation can be restricted to single boutons. One notable example of such compartmentalized differentiation of

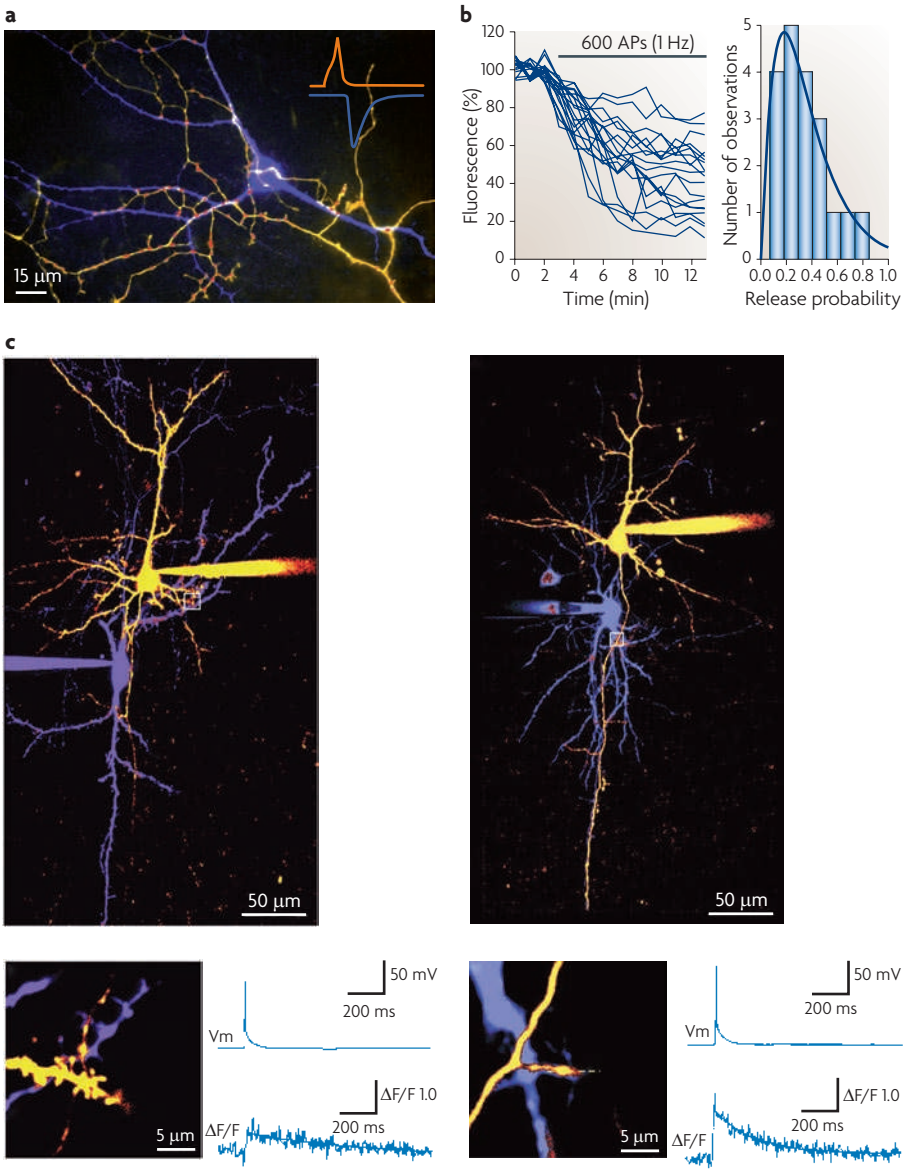

Figure 2 | **Variability of release probability measured at single synapses. a** | An epifluorescence image of a connected pair of hippocampal neurons in culture, with presynaptic terminals labelled with FM4-64 (red). The presynaptic cell is yellow and the target cell is blue. The inset plot shows the recorded action potential (AP) and the corresponding excitatory postsynaptic current. **b** | FM dye destaining curves of the synapses between the two cells (left plot) show considerable heterogeneity, reflecting the broad distribution of release probabilities for this connection (right plot). **c** | Two-photon images of pyramidal cells (yellow), filled with a Ca^{2+} indicator, connected to (left panel) a bitufted interneuron (blue) and (right panel) a multipolar interneuron (blue). The presynaptic Ca^{2+} signal in response to an action potential was measured at single synaptic contact points between the cell pairs (boxed in the upper panels and magnified in the lower panels). Ca^{2+} transients are bigger when the pyramidal cell target is a multipolar neuron, indicating that there are target-specific differences in release probability. ΔF/F, relative Ca^{2+} fluorescence change; Vm, membrane voltage. Parts **a** and **b** are modified, with permission, from REF. 14 © (2008) Cell Press. Part **c** is reproduced, with permission, from REF. 49 © (2005) American Association for the Advancement of Science.

presynaptic properties is the demonstration that terminals of hippocampal CA3 pyramidal cells that contact metabotropic glutamate receptor 1α (mGluR1α)-positive interneurons have ten times as much mGluR7 as terminals that contact mGluR1α-negative cells[61]. This specificity is so high that even active zones that belong to the same terminal but contact different targets exhibit these differences[61]. This biochemical divergence translates to a functional one, as the activation of presynaptic mGluR7 has been shown to depress neurotransmitter release[62].

Long-term Hebbian plasticity. Release probability is modulated by long-term synaptic plasticity. In the hippocampus, for example, high-frequency stimulation of mossy fibres causes long-term potentiation in pyramidal cells, owing to increased vesicle fusion efficiency following activation of the cyclic AMP–protein kinase A cascade and RIM1α (also known as RIMS1) phosphorylation in the presynaptic cell[63]. Interestingly, p_r

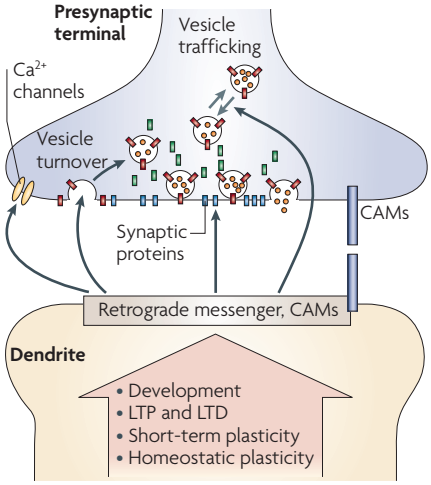

Figure 3 | **Postsynaptic influences on release probability.** Several processes, such as developmental changes and synaptic plasticity, can be initiated in the postsynaptic terminal and retrogradely modulate release probability through various targets in the presynaptic terminal. These targets include factors that change the number of vesicles available for release (by affecting vesicle trafficking or turnover), factors that change the amount of Ca^{2+} that enters the presynaptic terminal (for example, by influencing Ca^{2+} channels), factors that change the nature and properties of the synaptic proteins that form the release machinery, and factors that change the way in which these and other variables interact. Feedback communication between the two sides of the synapse can be mediated by a secreted factor or can operate directly through, for example, cell adhesion molecules (CAMs). LTD, long-term depression; LTP, long-term potentiation.

modulation in this context is also exquisitely dependent on the nature of the postsynaptic cell: at mossy fibre–interneuron synapses the same protocol causes a long-term decrease in p_r as a result of presynaptic mGluR7 activation and protein kinase C-dependent inhibition of voltage-gated Ca^{2+} channels at the terminal[64,65]. This process requires elevation of Ca^{2+} in the postsynaptic cell, again indicating retrograde regulation of p_r[66]. Although in this example the nature of the retrograde messenger is not clear, in several brain areas[67–72] long-term p_r depression results from endocannabinoid release from the dendrite. Endocannabinoids activate presynaptic cannabinoid receptors, leading to inhibition of voltage-gated Ca^{2+} channels and activation of K^+ channels (resulting in terminal hyperpolarization), which ultimately decreases p_r[73]. Although endocannabinoids are diffusible messengers, studies in the hippocampus suggest that their effects are locally restricted[73]. This would make them highly appropriate for implementing p_r control in a local and dynamic manner.

Synaptic homeostasis. Other examples of p_r regulation come from studies of synaptic homeostasis. Despite initial controversy, it has now been shown that p_r can also be modified by this form of plasticity. This was first convincingly demonstrated in the *Drosophila melanogaster* NMJ, in elegant experiments carried out by Davis and colleagues. Their studies showed that when a motor neuron is biased to differentially innervate two adjacent muscle targets, both targets nevertheless develop normal levels of depolarization, in part owing to homeostatic adaptations in presynaptic neurotransmitter release and active zone density[74,75]. Also, in mutants in which postsynaptic excitability was decreased[74,76–78], p_r increased to restore normal levels of activity; this process was in part mediated by changes in voltage-gated Ca^{2+} channels[79,80] and active zone structure[81].

Homeostatic plasticity can also change p_r in hippocampal cell cultures. Blocking glutamate receptors or preventing action potential generation leads to an increase in the frequency of miniature excitatory postsynaptic currents and overall p_r[82–84]. This is associated with increases in the sizes of the total and the recycling vesicle pools[85], suggesting that scaling of vesicle pools — for example, through modulation of vesicle trafficking[86,87] — could be an efficient means of regulating p_r. Homeostatic regulation of p_r can also result from changes in synaptic vesicle recycling, active zone size and the number of docked vesicles[85]. Similar presynaptic adaptations are seen in hippocampal organotypic slice cultures[88] and cortical cultures[89]. Recently, it was shown that in dissociated hippocampal cultures these changes can be synapse-specific and are triggered by dendritic depolarization[14]. Furthermore, the same study showed that p_r homeostatically adapts to the synaptic density of each dendritic branch: the more synapses one axon makes on a dendritic branch, the lower the p_r of each synapse. Interestingly, quantal analysis of paired recordings of L2/3 pyramidal cells showed an inverse relationship between p_r and the number of contacts in the connection[90]. Again, this argues for a feedback control of p_r.

Although the nature of the retrograde messenger in homeostatic p_r changes is mostly not known, work in the *D. melanogaster* NMJ suggests that growth factor signalling pathways might be involved[81,91]. In principle, however, a number of different mechanisms could have important roles, including modulation of the release machinery by cell adhesion molecules such as postsynaptic density 95 (PSD95)–neuroligin[92] (by *trans*-synaptic activation of signalling cascades) or any other messengers that are involved in long-term potentiation and depression.

Short-term activity. Release probaility also changes with the short-term history of synaptic activity. For example, prolonged stimulation or depolarization of the postsynaptic target can suppress neurotransmitter release[93] through an endocannabinoid-dependent feedback loop, which also shows target cell heterogeneity. Once again, this emphasizes p_r control by the postsynaptic cell[94,95]. The most classic forms of short-term plasticity, however, occur over a shorter timescale, and their dynamics depend on a number of properties of the terminal[50]; these include the vesicle pool size, the vesicle recycling rate, the level of Ca^{2+} buffering and the expression of different kinds of metabotropic and ionotropic receptors. Interestingly, many of these properties have been shown to vary from synapse to synapse, indicating that not only the resting p_r but also the dynamic p_r response to activity can be regulated at the level of single synapses[96–101] (reviewed in REFS 102,103).

In summary, p_r is influenced by a large range of factors acting through various mechanisms and targets. Although some p_r adjustments can be induced by the presynaptic terminal itself[104], most rely on a feedback loop from the dendritic target, suggesting that p_r is highly controlled by the identity and activity of the postsynaptic cell. Although

p_r is also likely to be affected by cell-wide adjustments of synaptic function[105], a substantial body of evidence suggests that p_r changes can be tailored to the specific needs of individual synapses. At any given point in time, therefore, the p_r of a synapse will be the result of all of these influences, each of which has a different weight, timescale and duration of action. In view of this, it perhaps is less than surprising that any two terminals belonging to the same axon can exhibit strikingly different p_rs.

Functional implications

It is evident that many neuronal connections are composed of multiple unreliable synaptic contact points, and that the probability of successful neurotransmitter release is variable and adjustable at the single-synapse level. Why is this so? Is there any functional advantage to this design?

The consequences of synaptic unreliability for information transfer have been widely debated[106–111]. There is a general consensus that one of the most important outcomes of having synaptic p_rs <1 is the flexibility it provides. The short-term activity dependence of p_r means that synaptic strength is dynamic, and that synapses can act as filters of the input pattern of the presynaptic action potential[20,112–114]. An adjustable p_r therefore gives synapses a broad dynamic range that is sensitive to the activity pattern, and can function as a gain control mechanism. Furthermore, setting the basal p_r to <1 also permits longer-term plasticity-driven changes in synaptic weight — an effective means of changing the strength and dynamics of a connection[115]. Given that sometimes stimulation does not result in synaptic transmission at a contact point (that is, there is synaptic failure), it seems intuitively desirable that connections be composed of more than one contact point to ensure that information transfer occurs every time[116]. Also, a high number of release sites per connection with p_r <1 would seem to permit high fidelity of transmission during prolonged high-frequency stimulation. At steady state, when the readily releasable pool of vesicles at a synapse has been depleted and the maximum rate of release depends on the kinetics of vesicle pool replenishment, having multiple synapses may offer a further advantage: while presynaptic terminals that have recently undergone vesicle fusion are in recovery and therefore not available for transmission, other synapses in the connection will be operational. Thus, a more constant overall release rate can be achieved. Nevertheless, other specializations such as large vesicle pools, fast vesicle recycling rates[117,118] and neurotransmitter release without full vesicle collapse[119] are necessary for synapses that operate at high frequencies.

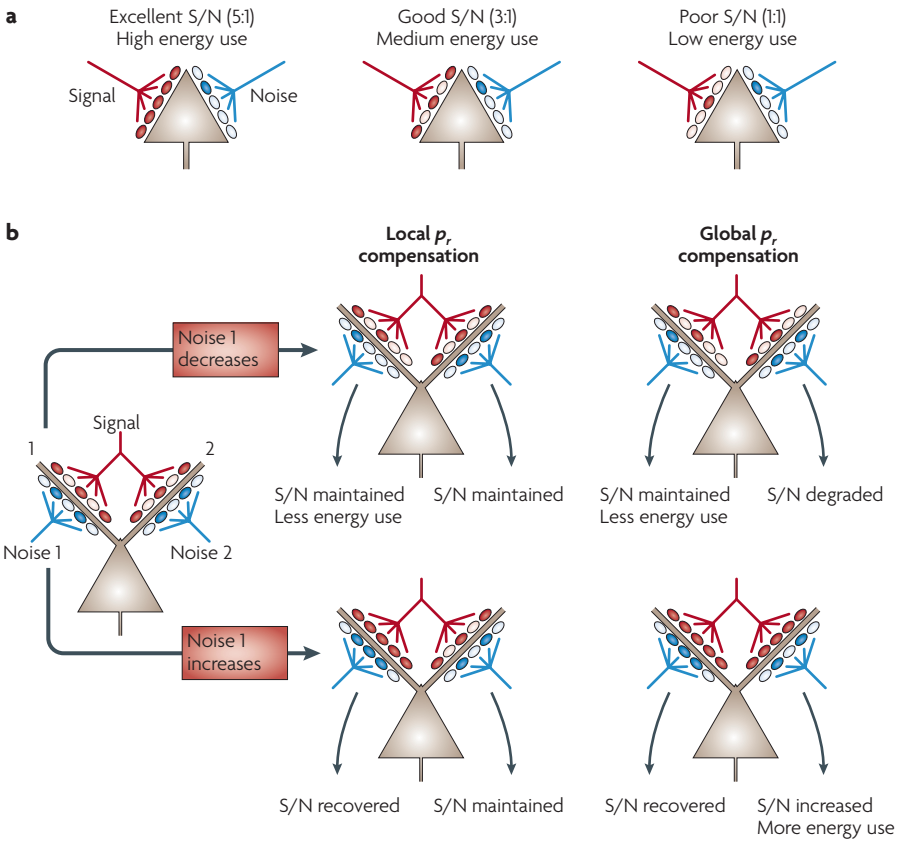

Figure 4 | **Consequences of p_r adjustments for signal/noise ratio and energy usage. a** | A neuron receiving an input of interest (signal) and noise. Filled circles at the end of each input are active synapses and open circles are inactive synapses — a representation of release probability (p_r). In the three examples shown the p_r of the noise is constant and the p_r of the signal varies, leading to different signal/noise (S/N) ratios and energy expenditures. The optimal arrangement is the middle one, in which an adequate S/N ratio is achieved with a minimum number of active synapses. **b** | The same schematic representation as in part **a**, but in this case two independent sources of noise arrive at two different dendrites. The response to changes in the noise of dendrite 1 is illustrated using two possible modes of p_r regulation — local, in which p_r adapts only in synapses on dendrite 1, and global, in which p_r changes are made for all synapses of the signal. Local p_r changes lead to energy-efficient S/N ratio maintenance, whereas global p_r regulation may result in S/N ratio degradation or in unnecessarily high, energetically expensive S/N ratios.

It also makes sense from an energy-efficiency standpoint to ensure that information about action potential firing is transmitted every time, given that action potential generation is a highly energy-consuming process[120,121]. On the other hand, recovery from the postsynaptic actions of neurotransmission is also very energetically costly[120,121], and so for maximum efficiency the number of active synapses per connection should be kept to the minimum that ensures a signal above noise[122–124]. An adjustable probability of neurotransmitter release under feedback control seems a sensible mechanism by which to constantly and quickly adapt to the postsynaptic noise level while ensuring minimal energy consumption (FIG. 4a).

If one assumes that the goal of synaptic transmission between two neurons is for the presynaptic cell to influence the firing of the postsynaptic cell, in principle feedback control of p_r as a means of maintaining efficient signal transfer should be implemented in a cell-wide manner, so that it acts on all synapses that compose a connection. If synaptic signals were linearly transmitted from the dendrites to the soma this would be an efficient means of control because only voltage fluctuations near to the action potential initiation site would influence the signal/noise ratio. However, dendritic branches are very independent electrotonic compartments[125–127] that can be highly nonlinear owing to the presence of several

voltage-gated conductances[128] and to shunting[129]. Thus, local voltage fluctuations have an impact on the degree of nonlinearity (by changing the recruitment of voltage-gated channels and the degree of shunting) and change the magnitude of signals that reach the action potential initiation site. One can therefore propose that feedback adjustments of p_r that maintain an adequate signal/noise ratio are implemented by the cell at the compartment level to account for the local noise (FIG. 4b). The observation that synapses from one axon onto a single dendritic branch have more similar p_rs than synapses onto different branches is consistent with this hypothesis[14]. Interestingly, in most connections that have been documented to date, synaptic terminals in a single connection tend to contact different dendritic branches. The reason for this is unclear, but it could be a strategy to reduce shunting and to provide a more complete 'sampling' of the dendritic tree and the overall activity of the cell.

Another potential reason for the local regulation of p_r is that neuronal compartments might perform regional integration operations, acting as semi-independent computation units[18,130,131]. In this scenario, in which a neuron can be thought of as a multiple-unit network, it makes sense that signal/noise adjustments are performed separately for each unit rather than for the cell as a whole. Also, having synapses with different p_rs in different dendritic branches means that information from a single axon can be dynamically filtered in a different way at each dendritic compartment. Another argument for neurons to use single-synapse p_r adjustments is that a more local regulation of synaptic weights might be easier to implement than a global one. Although a globally coordinated adjustment of p_r could be achieved by a glia-secreted factor such as tumour necrosis factor-α[132], such a uniform adjustment of synapses in all dendritic branches would require coordinated release from multiple glial cells, adding extra complexity to the regulation process. By contrast, local retrograde messengers or cell adhesion molecules can efficiently reach their target with high precision and specificity.

Local feedback regulation of p_r provides an explanation for p_r heterogeneity in a connection. However, it is important to note that it does not imply that each synapse should necessarily be different. Terminals belonging to the same axon generate similar levels of postsynaptic activity because they have the same presynaptic firing history. If the local dendritic environment of each terminal is also similar — for example, owing to functional spatial segregation of inputs on the dendritic tree — then p_r should be essentially uniform among synaptic contacts in a given connection, as has been found for some cortical connections[49,90].

We think that the model of local p_r regulation that we discuss here should be taken only as a general principle that particular cells and circuits tailor to suit their functional needs. For example, a single climbing fibre makes more than 500 synapses with a p_r>0.9 onto a Purkinje cell, whereas axons from the Schaffer collaterals in the hippocampus make a small number of very unreliable synapses onto CA1 pyramidal cells. The reason for this diversity most likely resides in the type and importance of the information carried by these fibres, as well as in the degree of circuit redundancy and plasticity. A variable probability of neurotransmitter release that is independently adjustable for each single synapse seems to be an excellent means by which to ensure optimal functional specialization for different connections.

Conclusion and future directions

Heterogeneity in the synaptic weights of a connection seems to result, in part, from individualized regulation of p_r, and as we discussed above it has important functional implications. But what is the role of this heterogeneity? Does it merely relate to noise in the system and is it simply a consequence of a non-optimized design? Or does it reflect the particular history and environment of each synapse and influence synaptic function in ways that can change the output of neuronal networks? The answers to these questions are unclear at present and will probably emerge only from detailed theoretical models of connections between neurons in combination with experiments designed to better understand synaptic integration in complex dendritic trees. Additionally, many aspects of p_r regulation need to be explored before definitive answers can be reached. For example, the nature of the regulators and effectors must be identified through experiments that manipulate candidate proteins and pathways, so that we can begin to understand how different influences such as long-term potentiation and depression and homeostatic plasticity interact to shape p_r. Furthermore, the exact spatial precision of p_r regulation needs to be characterized and the 'monitoring' variables that trigger p_r changes need to be identified. Are adjustments in p_r driven by the local Ca^{2+} level in the dendrite, for example? If so, what is it that determines whether a cell triggers a Hebbian-like change or a homeostatic one? Finally, although much information can be gathered from experiments in cell culture and brain slices, links between the details of synaptic physiology and the functional parameters that are relevant to the intact system will come only from studying single-synapse physiology *in vivo*, which at the present time is still a formidable challenge.

Glossary

Active zone
A specialized area of the presynaptic membrane where synaptic vesicle exocytosis occurs.

Dendritic integration
The process through which synaptic inputs interact with each other and with the electrical properties of the dendritic tree to generate patterns of action potential output.

Gain control
Regulation of the relationship between synaptic input and neuronal output.

Miniature excitatory postsynaptic currents
The postsynaptic signals that are produced in response to spontaneous release of a single quantum of transmitter (usually a single vesicle).

Quantal analysis
A statistical approach for decomposing the synaptic response into the underlying 'quanta', usually involving estimation of quantal size, release probability and number of release sites.

Shunting
A decrease in the size of synaptic responses that results from an increase in the membrane conductance (for example, through neurotransmitter-activated ion channels).

Synaptic homeostasis
A regulatory process that stabilizes synaptic weights around a set point.

Tiago Branco is at the Wolfson Institute for Biomedical Research and Department of Neuroscience, Physiology and Pharmacology, University College London, WC1E 6BT, UK.

Kevin Staras is at the School of Life Sciences, University of Sussex, BN1 9QG, UK.

e-mails: t.branco@ucl.ac.uk; k.staras@sussex.ac.uk
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OPINION

Phasic acetylcholine release and the volume transmission hypothesis: time to move on

Martin Sarter, Vinay Parikh and W. Matthew Howe

Abstract | Traditional descriptions of the cortical cholinergic input system focused on the diffuse organization of cholinergic projections and the hypothesis that slowly changing levels of extracellular acetylcholine (ACh) mediate different arousal states. The ability of ACh to reach the extrasynaptic space (volume neurotransmission), as opposed to remaining confined to the synaptic cleft (wired neurotransmission), has been considered an integral component of this conceptualization. Recent studies demonstrated that phasic release of ACh, at the scale of seconds, mediates precisely defined cognitive operations. This characteristic of cholinergic neurotransmission is proposed to be of primary importance for understanding cholinergic function and developing treatments for cognitive disorders that result from abnormal cholinergic neurotransmission.

The entire cortical mantle is innervated by cholinergic neurons that originate in the nucleus basalis of Meynert, the substantia innominata and the horizontal limb of the diagonal band — all structures of the basal forebrain (BF) (FIG. 1). Traditionally, the cortical cholinergic input system has been categorized as the rostral component of the brain's ascending arousal systems, complementing the modulatory roles of, and interacting with, noradrenergic, serotonergic and other projection systems that broadly influence the readiness of the forebrain for input processing, wakefulness and somnolence[1]. However, more recent evidence has supported the more specific hypothesis that cortical cholinergic inputs mediate essential aspects of attentional information processing[2–9]. As a result, efforts to develop treatments for a wide range of cognitive disorders have focused on cholinomimetic approaches, particularly acetylcholinesterase (ACHE) inhibitors and agonists at muscarinic (m) and nicotinic (n) acetylcholine (ACh) receptors (AChRs)[10–12].

The anatomical organization of the cortical cholinergic input system seems to be largely consistent with the notion of a diffuse pathway (this article does not address the hippocampal cholinergic projection system or cholinergic projections to the amygdala). Tracing studies revealed a roughly ventrolateral, dorsomedial and rostrocaudal topographical organization of cholinergic BF projections but did not suggest a more precise topography that would indicate, for example, that adjacent neurons in the BF innervate adjacent regions in the cortex[13–16] (FIG. 1b,c). Nearly all cortical layers and regions are innervated by BF cholinergic neurons[17], although the distribution of choline acetyltransferase (CHAT)- or ACHE-positive fibres in the cortex indicates differences in the density of the cholinergic innervation of specific layers[18–21] (FIG. 2). This seemingly diffuse organization of the cortical cholinergic input system has supported descriptions that it exerts general, uniform effects across the cortical hemispheres[20].

In contrast to other diffusely organized ascending systems, such as the ascending reticular systems of the brainstem, the axons of corticopetal cholinergic neurons (subcortical afferents that project to both cerebral hemispheres) do not seem to be extensively collateralized: individual neurons innervate a relatively small cortical field[22–24]. Thus, separate cortical regions, such as frontal and parietal regions, are not innervated by the same cholinergic neurons, suggesting that these regions may be differentially modulated by the cholinergic input system.

It has recently been proposed[14,15,25] that the corticopetal cholinergic system is less diffusely organized than was traditionally assumed (FIG. 1b,c). In support of this hypothesis, it has been demonstrated that there are clusters of cholinergic cells in the BF[15,25,26] and that the BF receives modality-specific projections[27]. The morphological heterogeneity of BF cholinergic neurons (see REFS 28,29) and of their efferent and afferent projection systems, including the degree to which they exhibit a topographical organization, remains insufficiently understood[13]. For example, the finding that manipulations of the excitability of the nucleus accumbens affect prefrontal ACh release but not the release of ACh in parietal regions[30,31] does not correspond with traditional descriptions of the organization of this system: it is more consistent with views suggesting a refined anatomical or functional topographical organization of the BF corticopetal projection system.