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Modulatory compartments in cortex and local regulation of cholinergic tone

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1. Introduction

Chemical signaling between neurons is often viewed as a relatively simple relationship in which an action potential in one neuron leads to signal transduction in another neuron. In classical synaptic transmission, action potentials travel to the axon terminal, where signaling molecules are released into the synapse, across which they will diffuse and subsequently bind to postsynaptic receptors, thereby transmitting a neural signal. This type of “point-to-point” transmission generally results in a signal that is both temporally and spatially precise.

Synapses open signaling to local modification. Here we argue that, in the case of diffuse communication by long-range neuromodulatory systems, there are many more opportunities to violate an assumption of a tightly coupled relationship between signal sent (by presynaptic neurons) and signal received (at postsynaptic neurons). In neuromodulatory systems signaling via non-synaptic transmission, mechanisms exist that can alter the coupling between action potentials (spikes) at the soma and the resulting response in local cortical circuits. Features such as patterns of axonal innervation, tissue tortuosity and molecular diffusion, effectiveness of degradation and reuptake pathways, subcellular receptor localization, and patterns of receptor expression across the local receiving circuit can offer the capacity to locally modify long-range communication between neurons.

Signaling by neuromodulators is generally considered broad in its impact, creating a neuromodulatory “tone” across large regions of the cortex. In much of the literature exploring the spatial and temporal scale of modulatory signaling, there is an implicit assumption that the number of neurons in the innervating modulatory structure defines a lower limit on the size of a uniquely modulated compartment in cortex. Here, we argue that

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local characteristics of cortical circuits can loosen spike-response coupling and introduce locally specific responses to broadcast modulatory signals, thereby creating small modulatory compartments in cortex without increasing neuron numbers in subcortical structures. We further argue that the signaling between these modulatory compartments can vary considerably and that these local compartments have the ability to regulate their own modulatory input leading to a two-way, interactive communication. As such, the circuit itself can influence the modulatory signals that regulate its activity.

Acetylcholine (ACh) acts a neuromodulator in cortical circuits and is believed to support cognition. Its role in the central nervous system is still poorly understood, although it has been implicated in many processes including learning, memory consolidation (reviewed by Hasselmo & McGaughy 2004), arousal (Jasper & Tessier 1971), and attention (reviewed by Everitt & Robbins 1997; reviewed by Sarter et al. 2005). We argue that anatomical differences across cortex that constrain and shape the way ACh interacts with local cortical circuitry result in the capacity for these circuits to regulate their own cholinergic modulatory tone, resulting in distinct “cholinergic compartments” across cortex. Furthermore, the existence of these compartments calls for thinking about cholinergic modulation in terms of finer-grained control of local cortical circuits than is implied by the traditional view of this system as a diffuse and loosely topographic modulator. These compartments are not limited to traditionally defined cortical areas, or even anatomically or functionally defined subregions within cortical areas, but comprise any region of cortex over which modulatory conditions are relatively uniform. Additionally, these compartments provide a substrate for an interactive relationship between cholinergic inputs and task-relevant cortical circuitry. This configuration allows for task relevant circuitry to “demand” its own modulation, which prompts a cholinergic “response.” The following review emphasizes evidence from studies of the macaque monkey. However, when data regarding primates are not available, or when notable species differences exist, we include information from other model systems.

2. Volume transmission

Classical synaptic transmission provides one mechanism for chemical signaling between neurons. Non-synaptic transmission, often termed “volume” transmission is another method by which cells communicate. This term, introduced by Fuxe and Agnati (1991), describes the diffusion of signaling molecules throughout the extracellular space, beyond the confines of a synapse (Figure 1). Many neuromodulatory molecules are thought to signal, at least in part, through volume transmission. This type of communication differs from classical synaptic (point-to-point) transmission (Figure 1A) in that molecules are released from varicosities that are not apposed to a specialized receptive surface (Figure 1B). Under these circumstances, it is often thought that the resulting signal will be both slow, and homogeneous over large regions of cortex. This is in contrast to classical synaptic transmission, in which signals are generally assumed to be fast and precise. Here, we challenge this simple dichotomy. We offer a new interpretation of volume transmission in which modulatory signals have the capacity for higher spatial and temporal precision—conferred by features of local cortical compartments—than has traditionally been assumed. This likely results in the capacity for a spectrum of signaling precision that varies across time and differs over the space of the cortical mantle, possibly on a very fine scale.

In volume transmission, signaling molecules are thought to diffuse away from a release site and bind to receptors over some volume of tissue. Volume transmission can also occur when molecules released into a synapse spillover, outside of the synapse, and subsequently bind to nearby non-synaptic receptors. All chemical communication between cells provides an opportunity for a signal to be modified. In volume transmission, however, there is a greater opportunity for signal modification as a result of the time and distance over which the modulatory molecule is able to diffuse. That is, local features of the receiving circuit have the ability to influence cortical modulation, both across and within regions. For example, ACh is synthesized by neurons in the basal forebrain, which provides the only source of cortical ACh in adult primates (Mesulam et al. 1983). It is released from axon terminals to bind to receptors that are usually not associated with synaptic specializations (Umbriaco et al. 1994, Mrzljak et al. 1995; but see Turrini et al. 2001). However, despite all of neocortex receiving ACh from the same subcortical nuclei, differing patterns of axonal innervation may provide varying levels of ACh to each area. The cholinergic innervation of cortex in macaques (Mesulam et al., 1983) and humans (Mesulam, 2004) exhibits a broad topography based upon the projections from subdivisions of the basal forebrain. Raghanti et al. (2008) have shown that the density of this cholinergic innervation differs on a still finer scale between Brodmann areas 4, 9, and 32 in the frontal lobe of the macaque. Dense networks of cholinergic axons are likely to yield a higher density of release sites and thus more ACh being delivered to an area.

Axonal innervation of the cortical mantle by cholinergic neurons exhibits still finer grained laminar differences within a single cortical area. In macaque frontal area 46, the supragranular layers I, II, and superficial III show denser patterns of cholinergic axons than the infragranular layers (Mrzljak et al. 1995), again suggesting the supragranular layers will be exposed to high levels of extracellular ACh. These studies provide evidence that while all ACh release across cortex is provided by a relatively small number of neurons in the basal forebrain, the signal received in a patch of cortex may vary, as a result of differences in the density of cholinergic innervation.

In addition to differences in the density of cholinergic innervation, local regulation of ACh synthesis can influence the capacity for cholinergic signaling to follow demands placed on it by the cortical circuits it serves. Choline, which is necessary for the synthesis of ACh, is taken up by cholinergic neurons via high affinity choline transporters (ChTs). These transporters are the rate-limiting factor in ACh synthesis (reviewed by Okuda & Hugo 2003). Therefore, ChT expression density at release sites may influence the dynamics of local ACh release. For example, when comparing across cortical regions, those areas with a higher density of ChTs may have a greater capacity for ACh production, and therefore for sustained or high intensity cholinergic signaling. Similarly, cortical areas with a lower density of ChTs may not be able to follow faithfully high and/or prolonged axonal firing. ChT expression density (which determines the ability to synthesize ACh in response to demand), combined with patterns of cholinergic innervation (which influence the local extracellular ACh concentration, and therefore the “load” placed on local ChTs), ultimately provides an opportunity for more finely tuned local regulation of ACh release. This interplay between release and reuptake is very likely dynamic, potentially offering greater temporal precision under conditions of high demand. It has been shown, for example, that ChTs can

be moved from cytosolic pools to the cell membrane in response to local demands on ACh signaling that result from varying activity-relevance of local cortical circuits (Ferguson et al. 2003). It is not known the extent to which the ready pool of ChTs varies across time—or differs between cortical cell types, layers, or areas—but such variation could lead to highly local constraints on dynamic ACh signaling.

3. The extracellular space: diffusion and tortuosity

The region of tissue over which a neuromodulatory molecule, once released, can exert its influence is limited in part by features of the extracellular space (ECS). The ECS is composed of those regions of brain tissue that separate one cell from its neighbors. Functionally, the ECS forms a conduit for the movement of molecules, including neuromodulators. In the case of neuromodulators such as ACh, which exhibit non-synaptic volume transmission, it is important to understand how these molecules move through the ECS. The ECS is estimated to compose between 20 and 25% of the normal adult brain volume in vivo (Syková & Vargová 2008). ECS morphology likely varies within and across brain regions imparting region-specific signaling potential upon molecules that communicate in a diffuse manner (McBain et al. 1990, Kinney et al. 2013). Characteristics of the ECS, therefore, partially define neuromodulatory compartments.

Once released into the ECS, the volume of tissue within which a neuromodulator can exert its effects is determined by a number of factors. The first is that movement of these neuromodulators through the ECS is by diffusion. This diffusion is influenced by the presence of structures such as neuronal and glial cell membranes that serve as physical barriers to molecular movement. The degree to which physical features of the tissue impede diffusion of the neuromodulator is referred to as the “tissue tortuosity.”

Local cellular topology influences characteristics of the ECS. For example, ECS domains can be classified as being sheet-like or tunnel-like (Figure 2A; Kinney et al. 2013). These ECS domains are believed to differently influence molecular diffusion, with molecules diffusing more slowly through sheets than tunnels. Such sheet and tunnel characteristics are non-uniformly distributed throughout the ECS, and therefore may also create unique neuromodulatory compartments.

Neural tissue also requires the presence of biochemical components in the ECS that, among other functions, form a matrix that serves as a cellular scaffold. This matrix contains long-chain macromolecules that can either be tethered to the cell surface or float freely in the ECS (Syková & Nicholson 2008, Bignami & Asher 1992). In either case, the presence of these macromolecules can further hinder diffusion (Figure 2B). Altogether, variations in extracellular matrix composition and structure will contribute to differences in the ability of neuromodulators to diffuse throughout certain microenvironments, thereby altering local neuromodulation.

Given that the brain exhibits structural plasticity, it is unsurprising that ECS characteristics do not remain static across time. The extracellular volume fraction in the hippocampus decreases during early postnatal development until it reaches a value similar to that seen in

adults (Fiala et al. 1998). Alterations of the brain due to pathology are also known to affect ECS parameters (Nicholson & Syková 1998). ECS volume decreases in response to nerve stimulation (Svoboda & Syková 1991) and the magnitude of this reduction in volume is related to the frequency and duration of stimulation. Thus, the tendency of a region of tissue toward activity may further influence the dynamics of neuromodulator diffusion.

4. Signal termination

The effective range (across the tissue) and the temporal dynamics of a neuromodulatory signal are further delimited by mechanisms that terminate the signal. In the context of cholinergic signaling, termination is accomplished by the enzyme acetylcholinesterase (AChE), which catalyzes the conversion of ACh into choline and acetate. AChE is a fast and efficient enzyme that has been extensively characterized at the neuromuscular junction, where it terminates, on a millisecond timescale, the action of ACh on nicotinic receptors found in muscle (Descarries et al. 1997, Gašperšič et al. 1999). Within the central nervous system, however, less is known about the expression, regulation, and function of AChE's membrane-bound and soluble forms (Sarter et al. 2009, Fass and Hamill 1984). Likewise, the extent to which cholinergic signaling is fully terminated by AChE is difficult to characterize. Following ACh degradation, as described above, choline is transported by ChTs back across the cell membrane into neurons where it can be used to synthesize more ACh or to support the turnover of phosphatidylcholine. However, it has been proposed that some portion of the extracellular choline may act as a ligand at some nicotinic receptors (Alkondon & Albuquerque 2006), although it is not clear that choline is an effective agonist at these receptors at the concentrations likely to be found in vivo (Mike et al. 2000)

While further characterization of central expression and function of AChE will be important, it has long been used to distinguish areal boundaries across cortex. It is important to recognize that AChE histochemistry can describe aspects of the local function of cholinergic modulation within those areas as well. The density of AChE expression provides at least two pieces of information. First, this density serves as an indirect indicator of local cholinergic tone. Though it seems intuitive to expect areas with dense cholinergic innervation to exhibit high levels of AChE, and areas with sparse or no cholinergic innervation to exhibit low levels or an absence of AChE, this is not always the case (Kawaja et al. 1990, Dani & Bertrand 2007). Second, AChE density provides a direct indicator of the efficacy of ACh degradation within a given compartment. Differential expression of AChE between regions of tissue will affect the spread of ACh molecules in both space and time (Figure 3A). When comparing between two regions of tissue, ACh will be able to move farther through tissue over a longer period of time in a region that expresses a lower level of AChE. In a region with higher levels, the movement of ACh molecules is expected to be more restricted in both space and time, as they will more readily be degraded. This means that spatiotemporal precision of the cholinergic signal is likely to be influenced by AChE level.

Density of AChE within human and nonhuman primate auditory cortex, for example, differs sharply across the border between the core and belt cortical areas (Kaas & Hackett 2000). Furthermore, within these regions, AChE expression is highest in layers IIIc and IV and thus exhibits both laminar and inter-regional expression differences. In mice, clear differences in

AChE expression exist across layers of both the visual and somatosensory cortices. In contrast, auditory cortex exhibits diffuse labeling without notable laminar differences (Anderson et al. 2009). Combined, these studies indicate that AChE expression differences exist not only within regions of a cortical area, but across cortical areas as well.

5. Receptor expression

Critical to understanding the circuitry receptive to a diffusible molecule is an understanding of receptor expression. ACh binds to a diverse array of receptors. These receptors are categorized into two distinct families, muscarinic (mAChR) and nicotinic (nAChR), named according to the agonists to which they are particularly responsive: muscarine and nicotine, respectively. nAChRs are ligand-gated cation channels, which mediate fast depolarization of the receiving cell and can be characterized based on their relative affinity for nicotine. Homomeric $\alpha 7$ subunit-containing receptors have a low affinity for nicotine and desensitize rapidly, while heteromeric receptors that contain the $\beta 2$ -subunit have a high nicotine affinity and desensitize slowly. mAChRs receptors, on the other hand, are metabotropic; they can act via direct channel coupling or intracellular second messenger systems. Five mAChR subtypes have been identified and are characterized based on the type of G protein to which they are coupled. The m2 and m4 mAChRs are preferentially coupled to the $G_{i/o}$ signaling pathway, and correspond to the M2 (pirenzepine-insensitive) pharmacological class. The m1, m3, and m5 mAChRs are preferentially coupled to the G_q signaling pathway and correspond to the M1 pharmacological class, although pirenzepine sensitivity varies within the class.

These ACh receptor types are expressed differently across the receiving circuits of the cortex. Receptor autoradiography in human indicates that, within a cortical area, both mAChRs and nAChRs exhibit differences in receptor density when examined across layers (Zilles et al. 2004). Across cortical areas, mAChR autoradiography reveals differences in mean binding site concentration and laminar receptor distribution (Eickhoff et al. 2007). For example, when comparing between visual areas, binding site densities for the m2 mAChR are highest in primary visual cortex V1 and decrease with the progression to visual area V2 and to visual area V3 (Eickhoff et al. 2008).

Even within a cortical area, modulatory compartments can be defined based on receptor expression. For example, Zilles et al. (2004) reported differences in regional distribution of cholinergic receptors within human secondary somatosensory cortex. Likewise, when comparing between ventral and dorsal regions of both V2 and V3, binding site densities for m1 and m3 mAChRs exhibit significant differences (Eickhoff et al. 2008). Receptor expression can also vary across elements of the circuit; when comparing m1 mAChR-expressing neurons in areas V1 and V2 of the macaque, the percentage of those neurons that are also inhibitory is higher in V1 than in V2 (Disney et al. 2006).

Functionally, differences in cholinergic receptor expression can affect the receipt of ACh. For example, a compartment more predominantly expressing the low affinity, $\alpha 7$ -containing nAChRs will become desensitized to ACh more quickly, resulting in a decreased capacity for continuous cholinergic modulation. The high affinity nAChRs, however, desensitize more slowly, likely resulting in prolonged modulation by ACh. Therefore, based on the

specific expression of receptor subtypes, modulatory signaling can range from more temporally precise to more prolonged.

In addition to previously mentioned factors that affect receptor binding via molecular diffusion, modulatory signaling will also depend upon the distance that separates a release site and a receptor (Muñoz & Rudy 2014). Because signaling by ACh is not always synaptic, increasing the distance between varicosities and receptors is expected to decrease the likelihood of a molecule binding to a receptor (Figure 3B). Therefore, variability in receptor expression as it relates to distance from nearest release site can affect subsequent binding of ACh. Additionally, affinity for ACh differs across receptor subtypes, meaning that the instantaneous local ACh concentration may activate only certain subtypes of receptors. In the case of mAChRs, m2 and m4 receptors exhibit higher affinity for ACh than do m1, m3, and m5 receptors (Kuczewski et al. 2005). As ACh diffuses away from its site of release, the concentration of that released ACh will decrease with distance. At distances farther from a release site, it can therefore be hypothesized that m2 and m4 mAChRs will more likely be activated than will m1, m3, and m5 mAChRs. Altogether, distance between ACh release sites and receptors will affect the likelihood of receptor binding as well as the subtypes to which ACh will bind.

This variability in receptor expression across and within cortical areas highlights the ability of the receiving circuit itself to respond to signals in a locally-specific fashion. Receipt of a signaling molecule may result in widely different signals depending on the local receptor expression.

6. Subcellular receptor localization

When a signaling molecule is bound by a receptor, the localization of that receptor can significantly determine the resulting impact on the receiving neuron. In addition to expression by traditionally understood postsynaptic circuit elements (dendrites, spines, and cell bodies), receptors are also expressed by neuromodulatory axons. These presynaptic receptors can be broadly classified as autoreceptors or as heteroreceptors. Autoreceptors respond to the molecules released by the same axons that express them and act as an inhibitory feedback mechanism, reducing further release (Figure 4A–B). Conversely, heteroreceptors are activated by ligands released by neighboring cells and thereby allow one signaling molecule to modulate the release of another (Figure 4C–D; Gilsbach & Hein 2008). Heteroreceptor activation thus depends on action potentials in other neurons and regulates local release by the neurons upon which the heteroreceptors are expressed. Currently, most of the pharmacological evidence for the function of heteroreceptors comes from rodent model systems. For example, noradrenaline release in the rat cortex, while dependent upon firing in the locus coeruleus, is also locally facilitated by axonally-expressed receptors for γ -aminobutyric acid (GABA; Bonanno & Raiteri 1987). Studies such as these provide evidence that while autoreceptors are considered inhibitory upon release, heteroreceptors have the ability to bi-directionally regulate the release of neuromodulators within local cortical circuits.

There are well-documented examples of cholinergic receptors implicated as heteroreceptors. The m2 mAChR is expressed by non-cholinergic terminals, allowing ACh to regulate release of other transmitters (Mrzljak et al. 1993). Furthermore, activation of nAChRs can enhance noradrenaline release in hippocampal synaptosomes (Wonnacott 1997). Activation of cholinergic heteroreceptors has in fact been shown to elicit neurotransmitter release *independent of axonal action potentials* in the receiving neuron's axon. For example, Cachepe et al. (2012) report that stimulation of nAChRs is sufficient to elicit dopamine release in the nucleus accumbens of mice, without the occurrence of a spike in the dopaminergic axon. Similarly, glutamate release from hippocampal mossy fiber synapses has been shown to increase, again independent of a somatic action potential, through activation of axonal nAChRs (Sharma et al. 2008).

Finally, cholinergic tone can be regulated by other modulatory systems as well. Altering local levels of serotonin (Hirano et al., 1995) or glutamate receptor activation (Parikh et al., 2008) modulates local ACh release in rodent prefrontal cortex.

These studies indicate the importance of receptor expression in receiving cortical circuits. Appropriately localized receptors can modify feedforward signaling via postsynaptic receptors, inhibit further release via an autoreceptor, or regulate (bi-directionally) axonal release via an axonal heteroreceptor. As such, receptor localization is an important aspect of signal receipt and response by the local circuitry.

7. Visual Cortex

Further research on the structure and function of the cholinergic modulatory compartments in the visual system is needed, but the data already available clearly show that—structurally—these compartments exist and thus that cholinergic modulation is likely to differ functionally across the visual pathway. As mentioned above, the proportion of m1 mAChR-expressing neurons that is also inhibitory differs between areas V1, V2, and the middle temporal area (MT) of the macaque (Disney et al. 2006; and unpublished data). Similarly, levels of AChE vary, both within and across V1 and V2 of the macaque (Balaram et al. 2015). Finally, ChAT immunolabeling in both cat and rat V1 reveals laminar distinctions in the innervation by cholinergic axons (Stichel & Singer 1987, Eichenstein et al. 1988).

Given the potential for cholinergic signaling within V1 to be spatially inhomogeneous, ACh's functional role in visual attention may not be limited to a general “vigilance” or “alertness” signal as might be suggested by the assumed slow and diffuse nature of cholinergic signaling. Instead, ACh, given the precision allowed by cortical compartments, may have the capacity to support more specific processes. If the variation in structural features of cholinergic signaling we describe has functional correlates (as it likely would) then ACh may well have the capacity to modulate smaller regions of tissue than would be suggested based on the number of cholinergic neurons in the basal forebrain. In this case, a role for ACh in processes that occur on small spatial or fine temporal scales cannot be ruled out. Important support for the idea that ACh supports temporally precise signaling of cognitive variables important to cortical circuits is the recent finding that basal forebrain cholinergic projection neurons in rodents respond to reward and punishment with exquisite

temporal precision (Hangya et al. 2015). Therefore, ACh, in the context of cortical compartments, may have the capacity to modulate cortical processes such as the fine scale modification of receptive fields that supports perceptual learning, and the cortical mechanisms for spatial and feature based attention.

8. Conclusion

The studies described above suggest a complexity to modulatory signaling throughout cortex. In diffuse communication, in which there is no point-to-point connection, the relationship between signal sent and signal received can be readily weakened. Each stage of diffuse signal transmission offers the opportunity to modify the modulatory signal; from differences in axonal innervation, through control of release and diffusion, to the response by the local circuit. Neuromodulatory compartments encompass a multidimensional space in which signaling can vary considerably based on features of the local circuit.

This structural inhomogeneity makes it essential to understand specific signaling conditions in any local cortical circuit of interest; without such data it becomes difficult to infer mechanisms of cholinergic modulation in cortex. For example, in order understand the role of ACh release in a task that is known to be dependent upon cholinergic innervation (such as a sustained vigilance task in a rodent; Himmelheber et al. 2000), recording electrophysiological activity in the basal forebrain will provide valuable insight. Further, because the signal sent from the basal forebrain does not necessarily equate to signal received in the cortex, additionally measuring local levels of released ACh across visual areas (V1, V2, etc.) will provide a second layer of valuable information regarding ACh's role in task performance. Beyond this, describing features of the local circuit and the local response to ACh release will help to characterize the transformation from signal sent to signal received (Figure 5). First, understanding the tissue tortuosity will describe the movement of molecules through the tissue once released. Second, some limits on the capacity for spatial and temporal precision of the signal can be determined by quantifying the local levels of AChE. Next, understanding the types of neurons that express ACh receptors (i.e. inhibitory, excitatory), as well as the subcellular localization of those receptors (e.g. autoreceptor, heteroreceptor; somatic versus dendritic) will help to describe the effects of ACh binding in a given area. Finally, measuring the resulting neuronal responses will reveal the effect of the locally transformed ACh signal on the local computation and spiking output of the cortical area of interest. Altogether, these data will help us to understand the transformation from signal sent in a diffuse modulatory system to signal received in cortex. Not considered in this review, but also important are the changes in compartments across development and adult plasticity. An understanding of modulatory compartments as dynamic features of cortical circuits will allow us to better characterize the many points along the circuit where signal modification is possible. This offers opportunities to understand, and perhaps correct, signal modifications that lead to pathological states—a critical step given that the majority of medications prescribed for neuropathological states target modulatory systems that signal diffusely in cortex.

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Highlights

- Acetylcholine signals partly by non-synaptic volume transmission
- In volume transmission, molecular diffusion is implicated as a signaling variable
- Diffusion and factors affecting it can alter neuromodulatory signals in cortex
- Here we consider local circuit features and their capacity to affect diffuse inputs

Figure 1. Synaptic and non-synaptic “volume” transmission

Example of an axonal varicosity releasing signaling molecules. *A* demonstrates classical synaptic transmission, in which molecules (shown in light green) released from the varicosity cross the synapse and bind to a receptor (shown in dark green). *B* demonstrates the release of molecules into a volume of tissue that diffuse away to bind to nearby receptors. Dashed lines indicate the extent to which molecules will diffuse in each type of transmission. Arrows indicate paths of molecular diffusion through the extracellular space toward receptors.

Figure 2. Physical characteristics of the extracellular space

A is a representation of neural tissue reconstructed from electron micrographs. Intracellular space is depicted in gray; extracellular space (ECS) is shown in white. Morphological features of the ECS are indicated by sheet-like features (shown in red) and tunnel-like features (shown in green). Molecular diffusion through sheet-like domains is predicted to occur more slowly than through tunnel-like domains. Figure adapted from Kinney et al. 2013. B shows the path of diffusion (indicated by dashed line) of a neuromodulator (indicated by black circle) through the ECS. Diffusion along this path can be hindered by the presence of macromolecules attached to the cellular membrane or floating freely through the ECS. Figure is adapted from Syková & Nicholson 2008.

Figure 3. Acetylcholine diffusion, binding, and degradation

Examples of axonal varicosities releasing ACh into a volume of tissue. *A* illustrates the effect of AChE levels on molecular diffusion. In regions of low AChE concentration (light brown), ACh diffuses over a greater area whereas in regions of high AChE concentration (dark brown), ACh has a more limited area of diffusion. Blue clouds indicate the distance over which ACh can diffuse in each region. *B* shows the distance (indicated by dashed lines) between varicosities and receptors (shown in green). Molecules are more likely to bind to nearby receptors than to distant receptors.

Figure 4. Autoreceptor- and heteroreceptor-mediated release of Acetylcholine

(A–B) Example of a cholinergic autoreceptor expressed on a cholinergic axon. A depicts ACh (shown in light green) release in the absence of activation of the cholinergic autoreceptor (shown in dark green). In B, released ACh binds to the autoreceptor and inhibits further acetylcholine release. (C–D) Example of a serotonergic heteroreceptor expressed on a cholinergic axon. C demonstrates the release of acetylcholine from a varicosity in the absence of serotonin (shown in yellow) binding to the serotonergic heteroreceptor (shown in orange). In D, serotonin binds to the heteroreceptor, which mediates a greater output of acetylcholine release from the varicosity.

Figure 5. Features of the local circuit in cortex

Cholinergic projections originating in the basal forebrain innervate different cortical compartments. *A* illustrates differences in receptor expression among neurons in the receiving circuit. *B* shows the presence of receptors along cholinergic axons that may influence local output of acetylcholine. *C* depicts regions with low and high concentrations of acetylcholinesterase. *D* demonstrates that together, these features create a multidimensional space in which modulatory signals can be modified at many points.