Nonsynaptic Chemical Transmission Through Nicotinic Acetylcholine Receptors

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I. INTRODUCTION

It is generally accepted that chemical transmission at the synapse is the primary method of conveying a message from one neuron to another. In addition to synaptic transmission, neurochemical (175–177) and morphological (25) evidence has revealed the significance of nonsynaptic interactions between neurons: transmitters are released from axon terminals without conventional synaptic contacts that do not appose a postsynaptic density. In this case, the transmitter is released into the extracellular space (12–18% of brain volume) (116), diffuses, and influences the activity of other neurons through stimulation of extrasynaptically located receptors. These receptors are...
mainly high-affinity metabotropic receptors that are sensitive to monoamines (norepinephrine, dopamine, serotonin) (177). However, it has been shown that synaptically released glutamate not only acts locally at intrasynaptic receptors, but it also escapes from the synaptic cleft to exert remote effects on nonsynaptic and channel-operated NR2B N-methyl-D-aspartate (NMDA) receptors (19, 144). In addition, a tonic current was described in cerebellar granule cells (154) that reflects the action of high-affinity GABA$_A$ receptors (119) by ambient concentrations of GABA present in extrasynaptic space.

Recently, strong evidence was obtained demonstrating that a high proportion of cholinergic boutons (86–93%) in the central nervous system (CNS) do not make synaptic contacts (26, 74) but are still able to release acetylcholine (ACh) into the extracellular space (80). ACh, released in this way, reaches only low concentrations (0.1–2 μM) and may only have an effect on high-affinity receptors. Surprisingly, the ion-channel operated nAChRs of the CNS are mainly found in nonsynaptic localization (70). nAChRs, involved in the presynaptic modulation of transmitter release, are not postsynaptic receptors (180, 189).

Today, it is well established that nAChRs are highly permeable to Na$^+$, K$^+$, and Ca$^{2+}$. The relatively large Ca$^{2+}$ flux through the receptor (145, 173) provides extra power for nAChRs to efficiently modulate subcellular signaling cascades. Experiments on α4$^*$ knock-in mice, which have been sensitized to nicotine, revealed that nicotine can interact with nAChRs even at very low concentrations. Although the resolution of current optical methods may be insufficient to reveal small changes in intracellular Ca$^{2+}$ under normal conditions (159), the nAChR-induced Ca$^{2+}$ accumulation helps these “fast” receptors create significant responses at the level of intracellular Ca$^{2+}$. In addition to nAChRs, brain metabotropic muscarinic acetylcholine receptors (mAChRs) are also important receptors of ACh signaling. The operation of the muscarinic subsystem fits well with the nonsynaptic nature of ACh, producing tonic and much slower responses following cholinergic activity, compared with nicotinic responses. ACh released into the extracellular space is able to diffuse far away from release sites. The role of acetylcholinesterase (AChE) is to keep extrasynaptic, ambient level of ACh within physiological limits. Nonsynaptic transmission operates at a slower time-scale compared with synaptic transmission and is responsible for tonic changes in brain activity (176, 177). However, the ion-channel-type nAChRs produce considerably faster responses than metabotropic receptors, which is an observation that is inconsistent with the nonsynaptic theory. nAChR-mediated effects seem to be slow because their activation occurs after a rather long-lasting diffusion of the endogenous ligand to the receptor, but at the same time, they are fairly fast because of rapid ion flux through their ion channels following activation.

What experimental approach can be applied for studying nonsynaptic transmission via nAChRs? If one defines synaptic transmission as an event that occurs at the postsynaptic membrane, then presynaptic receptors are predominantly nonsynaptic because of the lack of axo-axonic synapses in most cell-to-cell contacts in many regions of the brain. Therefore, the majority of actions mediated by presynaptic nAChRs are nonsynaptic (182). Another possible way of investigation on nonsynaptic transmission is to study the effect of agonist ejection in small volume. Nonsynaptic and high-affinity excitatory nAChRs are activated under this condition because the fast dilution eliminates the agonist before it could enter the synapse at appropriate concentrations. Today, the challenge is to interpret how nAChRs use their ion channels to generate responses at the relatively slow time-scale of nonsynaptic transmission. This review attempts to address this issue and to partly resolve the contradictions. Details of the subtype composition (16, 49, 87) that shape central nicotinic functions, molecular biology (16), as well as structure and channel properties (114, 158) of nAChRs have been comprehensively reviewed recently (18, 107) and are beyond the focus of this paper.

II. A NEW AVENUE IN NICOTINIC FUNCTION: UNIQUE ROLE OF NONSYNAPTIC NICOTINIC ACETYLCHOLINE RECEPTORS IN CHEMICAL TRANSMISSION

A. Morphological Evidence of Cholinergic Boutons Without Synaptic Contacts and Release of ACh Into the Extracellular Space

Ultrastructural morphometric studies have revealed that the cholinergic axons of the adult rat parietal cortex (169) and hippocampus (168) rarely make synapses on other neurons. In layer 5 of the parietal cortex of young rats, the majority (66%) of the cholinergic boutons can be found in synaptic contact, but most of these synapses are symmetric, indicating muscarinic transmission (166). In older rats, cholinergic boutons establish significantly fewer synapses than in young animals (166). In the developing brain (postnatal day 16–32), the synaptic incidence of varicosities is 6% in the cortex (11) and 17% in the hippocampus, respectively (112). These numbers are quite close to the adult values of 7 and 14% (169, 168). These observations support the idea that ACh participates primarily in nonsynaptic interactions reaching its receptor via nonsynaptic routes (27). The position of cholinergic axons can be identified by staining for vesicular ACh transporter immunoreactivity, which is strongest in the
stratum oriens of the CA1 region within the hippocampus (164), indicating that the cholinergic input may predominantly target the basilar dendrites of pyramidal neurons and local interneurons. Although less evident, ACh synthesis [shown by choline acetyltransferase (ChAT)-immunostained varicosities] is also more pronounced in the strata pyramidale and oriens than in the radiatum or lacunosum moleculare (11). Another important piece of evidence for the nonsynaptic nature of the cholinergic system is the localization of AChE. The fact that AChE can be found at areas distinct from cholinergic axon terminals and nAChRs (57, 122, 178) suggests that the enzyme must sense and degrade ACh molecules that have already traveled far in the extracellular space. This assumption is consistent with the nonsynaptic nature of most cholinergic boutons. We can assume that ACh released from terminals without synaptic contact diffuses in the extracellular space to reach remote receptors, and this action is terminated by extracellular AChE. Thus it is not surprising that neostigmine, an AChE inhibitor, enhances the extracellular level of norepinephrine (NE) measured by microdialysis (80). Before its degradation by G4 form of AChE (26), the released ACh stimulates nAChRs of noradrenergic terminals causing NE release. Therefore, the slower degradation of ACh leads to an expansion of the volume transmission of NE.

Brain microdialysis, which samples from the extracellular space, further supports the nonsynaptic mechanism of action for nAChRs by showing changes in extracellular ACh levels during different treatments. It seems that the effect of ACh released from cholinergic boutons is tonic in several brain areas: neostriatal cholinergic interneurons produce spontaneous tonic firing in the absence of synaptic input and evoke a tonic release of ACh (12). Similarly, the released ACh keeps the striatal dopaminergic terminal under tonic control (195). The tonic mode of cholinergic transmission also favors the involvement of nAChRs in nonsynaptic transmission. A continuous, low level of transmitters is characteristic of extracellular information exchange, as the removal of the message from a large volume can never be as efficient as it is at the synapse. In contrast, there is evidence for the participation of cholinergic boutons in synaptic transmission in the cerebral cortex where two-thirds of the cholinergic axon terminals form synaptic specializations. However, the large majority of these cholinergic synapses are symmetric, indicating the inhibitory nature of transmission, most likely mediated by mAChRs (152). Indeed, electrophysiological measurements of synaptic currents revealed mAChR-mediated cholinergic transmission in associational-commissural synapses of the hippocampal CA3 region (184).

**B. Nonsynaptic Localization of nAChRs**

There have been a number of observations of nAChRs at nonsynaptic sites (Fig. 1) (31, 58–59, 62–64, 167, 186). In chicken ciliary ganglion neurons, nAChRs contribute to both synaptic (nicotinic) and nonsynaptic transmission: α3/α5 nAChR mediate synaptic responses, while α7 nAChRs appear at perisynaptic locations (59, 186). The existence of α7 nAChRs in the chick ciliary ganglion has been shown earlier by α-bungarotoxin labeling (66). Ultrastructural studies using labeled α-bungarotoxin have revealed that most α7 nAChRs are located predominantly in nonsynaptic (at peri- and extrasynaptic loci) regions of axon terminals, with only 12% of labeled terminals showing synaptic α7 nAChRs (70). Low-versus high-affinity human α4β2 nAChRs can be separated by the different conductance levels of nicotinic currents (15). The high-affinity α4β2 nAChRs correspond most likely to nonsynaptic nAChRs receptors, which normally sense very low concentrations of the endogenous agonist ACh.

There is evidence that α7- and β2-containing nAChRs exist in both synaptic and nonsynaptic locations in hippocampal neurons (1, 31, 34, 51, 58, 75). However, most synapses containing nAChRs are not cholinergic, but rather glutamatergic or GABAergic (31). For these receptors, ACh must diffuse from the extracellular space into the synaptic cleft. Therefore, these unique ‘postsynaptic heteroreceptors,’ which may modify the synaptic transmission by glutamate or GABA, participate in nonsynaptic transmission rather than in synaptic transmission and

**Fig. 1. Possible locations of nonsynaptic nicotinic acetylcholine receptors (nAChRs).** A: presynaptic axon terminals without synaptic contacts and containing various transmitters can also be equipped with nAChRs whose stimulation results in transmitter release. B: dendritic nAChRs are located extrasynaptically at most neurons receiving cholinergic messages via nAChRs. C: nAChRs may occur inside the synapse. In glutamatergic synapses, their location is postsynaptic but the activation requires nonsynaptic routes, i.e., release of ACh from a nonsynaptic varicosity. In this case, the diffusion of ACh into the synapse is even more difficult.
their existence further strengthens the idea of nonsynaptic nicotinic transmission (Fig. 1). The finding, that GABAergic synapses contain postsynaptic α7 nAChRs in activity-dependent clusters together with an overlapping distribution with GABA<sub>A</sub> receptors (75), also confirms the hypothesis of nicotinic heteroreceptors, which are supposed to receive nonsynaptic messages (while GABA<sub>A</sub> receptors would convey synaptic GABA messages at these synapses). What could be the function of nAChRs in this unusual localization? In the hippocampus, GABAergic inhibitory potentials could be postsynaptically inhibited by activation of α7 nAChRs, indicating that one potential function of the postsynaptic heteroreceptor is to suppress synaptic transmission among interneurons during the activity of septohippocampal fibers (172). Further evidence for the existence of nicotinic heteroreceptors is that cholinergic boutons do not colocalize with α7 nAChRs (70). In the hippocampus, pyramidal neurons show less staining for nAChRs than interneurons (34, 58), indicating a smaller but existing nicotinic input in these cells. α4 or α7 null mutant mice show labeling nearly equivalent to the control (71, 56); however, it should be noted that data obtained with α4 or α7 antibodies have to be used with caution until the specificities of these antibodies have been ascertained. Although nearly all of the GABAergic interneurons of the hippocampus express nAChR subunits (155), and exhibit a dense α7 nAChR labeling (1, 34, 37), synaptic transmission by nAChRs occurs in ~20% of the interneurons (6). In some cases, nicotinic synaptic responses can be difficult to resolve (111). This may be partly attributable to the relatively sparse cholinergic innervation compared with the glutamatergic inputs. It seems likely that most nAChRs are located at extrasynaptic membrane surfaces and do not mediate synaptic transmission.

Nonsynaptic nAChRs do not restrict to extrasynaptic membranes of neurons. nAChRs are also expressed in nonneuronal cells in the nervous system (149). For example, microglial cells and hippocampal astrocytes contain nAChRs (43, 151, 160). Because of the lack of synapses, ACh can only reach these cells by diffusion. Functionally, the α4, α7, β2, and β3 subunits and related Ca<sup>2+</sup> channel responses have been found in primary rat astrocytes (191). The presence of nAChRs on these cells also implies a broader scope for the actions of nicotine that needs to be considered from a clinical viewpoint (149). Clinically, an increase in the proportion of astrocytes expressing α7 immunoreactivity was observed in Alzheimer’s disease (160). This may contribute to alterations in Ca<sup>2+</sup> homeostasis and could interfere with β-amyloid-mediated inflammatory processes (191). The activation of microglia, brain mononuclear phagocyte cells, establishes an endogenous “cholinergic anti-inflammatory pathway” via α7 nAChR in the peripheral nervous system. The most likely function of these nAChRs is to inhibit lipopolysaccharide (LPS)-induced tumor necrosis factor (TNF)-α release (151). These data indicate that nAChRs on nonexcitable cells may also represent an additional mechanism underlying the neuroprotective properties of nicotine.

C. Mismatch Between AChE and ChAT Immunoreactivity

The above findings support the hypothesis that the main function of nAChRs is to mediate nonsynaptic transmission. However, the widespread distribution of AChE, which degrade the released ACh, also raises further questions. It can be speculated that the large number of degrading enzymes could stop the action of extracellular ACh. However, AChE and the ChAT distributions do not overlap perfectly. The mismatch between AChE and ChAT immunoreactivity (57) has been clearly shown in the retina (22) and in the rat interpeduncular nucleus (76). In the rodent cerebral cortex, ChAT-immunoreactive neurons do not contain detectable AChE activity, indicating that the released ACh is not degraded immediately after release. Thus ACh must diffuse a certain distance to reach its receptors while the surrounding AChEs create a “nonsynaptic tunnel” by degrading ACh at particular positions (92). Supporting the permissive role of AChE for ACh diffusion, central cholinergic pathways can be established in AChE knockout animals by butyrylcholinesterase substitution (113). It has already been proposed that the high extracellular level of AChE aims primarily to keep the concentration of ACh within limits, in time and in space, rather than to totally eliminate the released ACh (26). Furthermore, the degradation product of ACh, choline, can activate α7 nAChRs. In this regard, AChE-rich patches in brain tissue may relate to areas controlled by α7 nAChRs. The other uncertainty regarding the nonsynaptic nature of nicotinic transmission derives from the question of how a fast ion-channel-type receptor, like the nAChR, is capable of sensing the slowly building ACh levels around the receptor. Below, we made an attempt to provide an answer to this question.

D. Operation of Nicotinic Transmission in the Nonsynaptic Mode

With the assumption that cholinergic varicosities are in close proximity to remote elements, the released ACh should diffuse a relatively short distance to reach its receptors (as compared, for example, with the monoamine system; see Fig. 2). In this regard, the nature of this form of nonsynaptic transmission is different from monoamine transmission (176–177). A large and fluctuating monoamine pool in the extracellular matrix of the brain continuously modulates cell excitability and synaptic transmission. In contrast, nicotinic communication must
represent a faster form of nonsynaptic transmission operating at shorter distances. Nevertheless, this form of interneuronal communication might still be much slower than synaptic transmission. The shorter distance allows a short diffusion time of ACh so that the fast response on the target dendrite may appear within the physiological time limits of neural integration. Membrane nicotinic ion channels mediate fast Na$^+$ and Ca$^{2+}$ influx, indeed, but the subsequent Ca$^{2+}$ accumulation below the receiver membrane takes longer. Slow Ca$^{2+}$ accumulation is corroborated by membrane voltage-sensitive Ca$^{2+}$ channels and by Ca$^{2+}$ release from intracellular stores (13, 29, 78, 142, 148, 153, 157, 174, 181). The Ca$^{2+}$ influx through the nAChR can add to the Ca$^{2+}$ response induced by synaptic activity or action potential back-propagation, which may lead to supralinear addition of inputs. Overall, nicotinic transmission can be taken as “fast nonsynaptic transmission.” How can different time-scale transmission types work together? Based on the structural data regarding cholinergic boutons and remote neurons, one can assume a neuronal assembly of relatively close elements. Very distant pairs of elements (boutons and dendrites) are unlikely because AChE activity would then turn off most of the cholinergic message. Of course, these neuronal elements do not (or very rarely) form a synapse, but the diffusion barrier may be much smaller than that of the monoaminergic system. In one cell of the network, the cholinergic message may come shortly after a glutamatergic excitation as a second wave of excitation. Changes in monoamine levels can only slowly modulate the response of the cell. An ACh pulse from a nearby cholinergic bouton can increase the concentration of ACh around the remote cell and activate local nAChRs, which have just recovered from desensitization. The assumption of close, but not synaptic, appositions of cholinergic boutons and their targets is in line with the mismatch localization of ChAT (at the release site) and AChE (at the target neuron). Following a short diffusion, ACh can activate remote receptors, and this effect is promptly terminated by the closely located AChE (Fig. 2). In this regard, the effect on the target neurons appears secondary to synaptic activity, but still faster than the effect of monoamine neuromodulators (Fig. 2). Therefore, the activated receptors are able to evoke a large Ca$^{2+}$ influx through the receptor (especially in the case of the α7 nAChR), leading to a local excitation of the host cell. Overall, ACh

**FIG. 2.** Schematic drawing of the contribution of fast nonsynaptic transmission mediated by nAChRs relative to fast synaptic and slow monoaminergic nonsynaptic transmission. In this example, we assume simultaneous activation of glutamate-, monoamine-, and ACh-containing boutons, which converge to one target dendrite (gray). Note that the distributions of acetylcholinesterase (AChE) and choline-acetyltransferase (ChAT) enzymes do not overlap. Diffusion of ACh is limited by the high density of AChE, providing relatively shorter delay in action on target neurons, while the monoamines, such as noradrenaline/norepinephrine (NA), serotonin (5-HT), and dopamine (DA) diffuse over larger distances because they do not make synaptic contacts. **Left panel** (synaptic): target receptors are first activated by the released glutamate because of the high speed of synaptic transmission. **Middle panel** (channel-operated nonsynaptic): ACh, released from a nearby bouton, can reach the target after traveling a short distance between the cholinergic boutons and the receptive dendrite. **Right panel** (metabotropic nonsynaptic): the released monoamines have to pass a much larger distance. Thus they arrive at the target dendrite later, when the glutamatergic and cholinergic activation are over.

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molecules arriving nonsynaptically can activate extrasynaptic nAChRs, or synaptic hetero-nAChRs in glutamatergic or GABAergic synapses, resulting in fast nonsynaptic transmission.

III. PRESYNAPTIC AND/OR PRETERMINAL NICOTINIC ACETYLCHOLINE RECEPTORS: RELEASE BY RECEPTOR STIMULATION

A. Location of nAChRs

nAChRs located in the axon have been described in various brain regions. Because of the low incidence of axo-axonic synapses in most areas of the brain, ACh must diffuse to these receptors from nearby release sites. Thus axonal (in a wider sense “presynaptic”) nAChRs may represent one target of nonsynaptic transmission. Activation of these receptors may induce regenerative spikes, which ultimately lead to release of transmitters in a tetradotoxin (TTX)-dependent manner. These receptors, residing on axon terminals and/or preterminal axon branches typically far from the release site, have been termed preterminal receptors (88, 89, 187–189). In some cases, the term presynaptic receptors is limited to receptors located very close to the release site (89). Because of the close proximity to the release site, these receptors can influence release directly while avoiding the use of voltage-sensitive Na⁺ channels. The closer the receptor to the release site, the less sensitive it is to TTX. Nevertheless, both preterminal and presynaptic receptors should be at nonsynaptic sites of the axon because of the scarcity of axo-axonic synapses. Why are presynaptic/preterminal nAChRs so important for understanding cholinergic transmission? First of all, activation of presynaptic nAChRs that precedes or coincides with the arrival of an action potential into the terminal region of the neuron can increase the probability of release via the integrative capability of nAChR-induced Ca²⁺ influx. Indeed, catecholamine release could be elicited by the application of nicotinic agonists through mobilization of intracellular Ca²⁺ in chromaffin cells (42). The enhancement of miniature excitatory postsynaptic currents (mEPSCs) by nicotinic agonists in hippocampal pyramidal neurons is sufficient to drive the postsynaptic cell above the threshold for firing action potentials through synchronization of the release process (148). Nicotinic action on transmitter release evoked by electrical field stimulation is a useful indicator of presynaptic receptors, as the field stimulation opens all channels in the field, including Na⁺ and Ca²⁺ channels of the axon terminal. Thus the evoked release can be modulated only by local presynaptic receptors. Upstream (somatic) activity, either excitatory or inhibitory, exerts less or no influence on the evoked release. In addition, nicotine can induce release independent of action potentials (67).

There has been accumulating neurochemical evidence for the transmitter releasing action of presynaptic/preterminal nAChRs (180, 181). Activation of nAChRs in the hippocampus leads to the release of NE (139, 146, 179), GABA (83, 101), and serotonin (90, 156). In the striatum, nicotinic stimulation evokes the release of ACh (140), glutamate (162), dopamine (DA) (20, 24, 30, 50, 105, 153, 165), and serotonin (132). Brief pulses of low (3 μM) concentrations of nicotine, which do not induce desensitization of nAChRs, can cause a large enhancement of DA release evoked by high K⁺ application in striatal synaptosomes by altering the size of the readily releasable pool of vesicles (165). Evidence has been provided for presynaptic nAChRs on GABAergic axons in the rat interpeduncular nucleus (89). One mechanism by which GABA release could be enhanced is the insertion of α7 nAChRs into axon terminals (192). Nicotine, epibatidine, and anatoxin-a can evoke the release of [³H]aspartate in a Ca²⁺-dependent manner in frontal cortex slices (135). Nicotine enhances glutamate release via α7 nAChRs from synaptosomes isolated from rat prefrontal cortex (185). In accordance with the neurochemical evidence for nAChRs modulating glutamate release, α7 nAChRs are present in approximately one-third of the glutamatergic axon terminals in the ventral tegmental area (VTA) (70). Stimulation of nAChRs in this region also causes DA release in the nucleus accumbens through the indirect activation of midbrain NMDA receptors (143). In cultured hippocampal neurons, fluorescent α7 nAChRs highly colocalize with synaptotagmin, which labels active axon terminals (193), further confirming the presynaptic/preterminal function of the α7 subtype. The presynaptic α7 nAChR seems cell-type specific; for example, cholinergic boutons apparently lack nAChRs (70).

B. Subtypes of nAChRs Involved in the Release of Transmitters

Different subtypes of nAChRs can be involved in the presynaptic regulation of transmitter release. The α3β2 nAChR has been suggested to induce NE release from acute hippocampal slices (146, 179) and striatal DA release (86). The α3β2-like nAChR-induced release of NE was TTX dependent in hippocampal slices (146). In contrast, using hippocampal synaptosomes, α3β4-like nAChRs were found to mediate the release of NE because the subtype-selective α-conotoxin AuIB blocked the nicotine-evoked release (98). In addition, the α3β2 selective α-conotoxin MII was ineffective in preventing nicotine-evoked NE release from synaptosomes (98). The TTX sensitivity of the α3β2 responses in the slice preparation suggests a preterminal localization of this receptor subtype for the hippocampal NE release. Taken together, we can conclude that the most likely structure for the nico-
nicotine modulation of the noradrenergic innervation of the hippocampus consists of αδβ4 nAChRs on the terminals and αβ2 receptors in upper segments of the axon. This can be explained by assuming that stimulation of αβ2 receptors may produce local regenerative potentials, which further increase the release by the additional Na⁺ influx. β2-Containing nAChRs are involved in the regulation of GABA release in the thalamus (88). In the superior cervical ganglion, activation of nAChRs enhanced the electrical field stimulation-induced release of ACh, most likely via α7 nAChRs (96). This kind of modulation represents a positive feedback for the ACh release. The role of α7 subtype is supported by the finding that α7 nAChR-mediated currents can also be measured in cultured superior cervical ganglion neurons (97). In addition, two different splice variants of α7 nAChRs have been detected in the superior cervical ganglion with distinct desensitization properties (23, 147). In the case NE release, αδβ4 nAChRs were assumed for both TTX-sensitive and TTX-insensitive release forms in cultured sympathetic neurons (85). Later, the participation of αδ subunits was also linked to the presynaptic facilitation of NE release (32). Looking at the binding of radioligands, αδβ4 was shown to be the dominant subtype, but axonal receptors for modulating the release were not identified separately from those mediating synaptic transmission on the postsynaptic side (121). The observation, that not only the α7 transcript, but also αδ, α5, β2, and β4 transcripts are present in the superior cervical ganglion with distinct desensitization properties (23, 147). In the case NE release, αδβ4 nAChRs were assumed for both TTX-sensitive and TTX-insensitive release forms in cultured sympathetic neurons (85).

A. Receptors Mediating Synaptic Transmission

There is only scattered experimental evidence for functional nAChRs, which mediate synaptic transmission. Some of these studies have been designed using the “remaining current principle,” i.e., the nicotinic antagonist-sensitive current following the block of all other synaptic currents. With the use of this approach, nicotinic synapses have been detected in stratum radiatum and oriens interneurons (6, 33), in hippocampal CA1 pyramidal cells (54), in pyramidial cells and interneurons of the developing visual cortex (134), in cells of the supraoptic nucleus (53), and in chick ciliary ganglion neurons (150). However, it is somewhat surprising that only 20% of stratum radiatum interneurons exhibit fast synaptic transmission mediated by nAChRs in acute hippocampal slices (6). Despite the fact that most interneurons (~90%) express currents in response to application of nicotinic agonists (33–34), α7-mediated synaptic responses can be detected in only 14% of the cells, strongly suggesting that nonsynaptic transmission, and not synaptic transmission, may be the principal method of nicotinic transmission in interneurons. McQuiston and Madison (111) did not find any fast synaptic response mediated by nAChRs in interneurons, most likely because of the different methods of tissue preparation. With the assumption of a sparse distribution of nicotinic synapses over the dendrites of interneurons, many cells in the slice preparation may lack intact nicotinic synapses because of the angle of the cut or the selective degradation of superficial cells and dendrites. Nevertheless, the impact of the nicotinic synapses on interneuron dendrites is much less than the weight of glutamatergic inputs, which can be activated in 100% of the cells. These data favor the hypothesis that most nicotinic inputs of interneurons are not synaptic.

IV. NONSYNAPTIC MODULATION OF SYNAPTIC TRANSMISSION BY NICOTINIC ACETYLCHOLINE RECEPTORS

To estimate the impact of nonsynaptic nAChRs in neural circuit, we need to understand how nAChRs influence synaptic functions. In particular, the major aim of this discussion is to review how hard-wired connections between cell types combine with the slower time-scale modulatory effects of nonsynaptic nAChRs. To demonstrate the nonsynaptic effects of nAChRs, we first summarize the limited number of cases where nAChRs mediate synaptic transmission in the brain. Then, the highly effective and widespread nicotinic modulation of fast synaptic transmission will be reviewed.
This is even more characteristic for hippocampal pyramidal neurons where synaptic currents mediated by nAChRs can only be dissected from a synaptic current of at least 1 nA amplitude (54). Taking this observation in conjunction with an earlier morphological study showing cholinergic synapses on pyramidal neurons (41), it seems that the contribution of nicotinic synapses to the synaptic innervation of pyramidal neurons is small. Most of the cholinergic synaptic innervation may conduct muscarinic-type responses including Ca\(^{2+}\) waves (126).

In the supraoptic nucleus, nicotinic excitatory postsynaptic potentials (EPSPs) are also mediated via α7 nAChRs similar to hippocampal interneurons (53). Non-α7 nAChRs (150) can also contribute to synaptic transmission. In chick ciliary ganglion neurons, which innervate the iris and the choroid body, persynaptic α7 nAChRs cooperate with fast synaptic currents mediated by non-α7 nAChRs (17, 194). nAChRs located on somatic spines of chick ciliary ganglion neurons mediate fast synaptic transmission confined to spines (150). During high-frequency synaptic stimulation (50 Hz), non-α7 nAChRs appear to mediate the Ca\(^{2+}\) transients confined to spines, and the sustained Ca\(^{2+}\) signal shows a decrement as an indication of receptor desensitization (150). Synaptic transmission through α3 subunit-containing nAChRs has been identified in the sympathetic ganglia (109, 129). Both α7 and non-α7 nAChRs contribute to the remaining synaptic currents following the elimination of GABA and glutamatergic transmission, suggesting a synaptic role for nAChRs in dopaminergic neurons of the substantia nigra (108).

### B. Modulation of Synaptic Transmission by Nicotine

Bath application of nicotinic drugs in low concentrations is a frequently used experimental approach to study nicotinic modulatory actions. This method of drug delivery simulates the action of low extracellular ACh concentrations, which occur during normal neural activity, and even more importantly, it corresponds well with the effects of low-dose nicotine during cigarette smoking. Low concentrations of the agonist are not expected to interfere with synaptic transmission because the receptors in the synapse accommodate high (millimolar) concentrations of the synaptic transmitter (177, 182). In this regard, the dominant mode of the nicotinic effect is to influence, rather than mediate, synaptic transmission through nAChRs. Especially in the case of presynaptic nicotinic actions, the nonsynaptic form of interneuronal communication predominates.

In fact, the activation of presynaptic α7 nAChRs by extracellular ACh or choline facilitates glutamatergic synaptic currents in cultured hippocampal neurons (127), in CA1 and CA3 pyramidal neurons of the hippocampus (69, 100, 148), in olfactory bulb-amygdala preparations (48), and in pyramidal neurons of the rat auditory cortex (10).

Non-α7 nAChRs may also contribute to the facilitation of glutamatergic synaptic events in the medial habenula-interpeduncular nucleus (48). Non-α7 nAChRs, presumably of the α3β4 subtype, control the glutamatergic input to CA1 stratum radiatum interneurons (2). Additional evidence for the role of non-α7 nAChRs in the modulation of synaptic transmission comes from studies on thalamocortical synapses showing that bath application of 1 μM nicotine facilitates synaptic responses (46). Bath application of 1 μM nicotine increases the AMPA-mediated excitatory postsynaptic current amplitude via α7 nAChRs in VTA dopaminergic neurons through a presynaptic/preterminal mechanism (103). Activation of nAChRs can release aspartate from the frontal cortex using neurochemical methods, supporting the nicotinic enhancement of glutamatergic transmission (135). Corroborating the functional studies, α7 nAChRs have been identified in VTA glutamatergic axon terminals (70). There is experimental evidence for functional nAChRs on GABAergic axon terminals as well. Bath-applied nicotine enhances the frequency of giant depolarizing potentials (depolarizing mEPSCs) rather than mediate, synaptic transmission through nAChRs.

The mechanism of synaptic enhancement most likely involves the activation of presynaptic nAChRs that causes Ca\(^{2+}\) influx into the axon terminal and induces transmitter release. Low-dose nicotine can increase mEPSC frequency and presynaptic Ca\(^{2+}\) influx (47, 52, 100, 110). This effect is TTX insensitive. Therefore, fast depolarization by voltage-sensitive Na\(^+\) channels can be excluded in these cases. In addition to the presynaptic enhancement of synaptic potentials, which appears as an increased frequency of EPSCs, there could be postsynaptic amplification by nAChRs as well. For example, low-dose nicotine can enhance synaptic transmission by increasing the amplitude of evoked glutamatergic EPSCs via postsynaptic nAChRs in the interpeduncular nucleus and in the hippocampus (110, 148). It should be noted that large-amplitude mEPSCs may appear in the absence of postsynaptic effects by preferential release of large vesicles, selective activation of synapses with larger synaptic potentials, or through the synchronization of release from more active zones (148).

### V. NICOTINIC FUNCTIONS BEYOND SYNAPTIC TRANSMISSION

There is a large amount of experimental data regarding nicotinic transmission that is hard to explain on the
basis of synaptic transmission. As it was discussed above, ACh is released into the extracellular space and, therefore, cholinergic transmission is primarily associated with a nonsynaptic form of communication in the CNS. “Postsynaptic” nAChRs mostly receive messages forwarded to extrasynaptic membranes. Thus it is more precise to define them as somatodendritic receptors. Although only a few studies discuss nAChRs directly as extrasynaptic receptors, there are many indications of nonsynaptic operations during nicotinic stimulation.

In the early study of Rovira et al. (136) using in vivo field recordings, ACh and the classical nicotinic agonist DMPP, applied to the somata of pyramidal neurons, enhanced population spikes (many coordinated action potentials), while application of ACh directly onto the dendrite increased dendritic inhibition. The enhancement of population spikes evoked by a very high concentration (800 μM) of nicotine, applied in the bath, was still prevented by mecamylamine despite the high concentration of the agonist (38). Nicotine was proposed to have a preferential net inhibitory effect on hippocampal basket cells and an excitatory effect on oriens/alveus interneurons (130, 131). In the following sections, several examples are provided of how nAChRs can interact with various neural functions at the cellular level, most likely through a nonsynaptic mechanism of action. Because central nAChRs have been extensively studied in the hippocampus, we provide a short overview of nAChR function in this region.

A. Hippocampal Interneurons

While it is known that the GABAergic portion of the septohippocampal innervation produces disinhibition in the hippocampus (163), much less is understood about the function of the cholinergic part of the septohippocampal projection. The most likely targets of the cholinergic septohippocampal pathway are the GABAergic interneurons. Although a population of hippocampal interneurons inhibits other interneurons, raising the possibility of a nicotine-induced disinhibition (39), this indirect excitation by the GABAergic loop does not appear in pyramidal neurons of the hippocampus under normal circumstances (14). However, in the case of high GABAergic activity, disinhibition can be observed in pyramidal neurons as a reduction of spontaneous GABA-α-mediated currents (68). In the dentate gyrus, nicotinic stimulation of hiliar and subgranular interneurons consistently produces inhibition in granule cells via α7 nAChRs (35). Hippocampal inhibitory interneurons are markedly excited by activation of nAChRs (7, 34, 72, 111). Large currents can be evoked by application of nicotinic agonist onto stratum radiatum interneurons that persist even when voltage-sensitive Na⁺ and Ca²⁺ channels or synaptic glutamate and GABA receptors are blocked (3, 34, 72), indicating a postsynaptic/dendritic localization of these nAChRs. This also highlights that nAChRs, which receive the message of the septohippocampal cholinergic neurons, are largely located outside of the synapse. Selective nicotinic ligands induce firing of action potentials in a concentration-dependent manner (3, 4). At concentrations above 30 μM, nicotine is able to evoke action potentials in all interneurons. In contrast, at 10–15 μM nicotine concentrations, only a fraction (~50%) of the neurons can respond (4). It seems unlikely that nicotinic drugs at the applied concentration (10–30 μM) could activate low-affinity synaptic nAChRs and induce a synaptic current. It is much more likely that the involved nAChRs are of high affinity and located at extrasynaptic membranes, indicating the role of nonsynaptic transmission. Stimulation of nAChRs also induces fast Ca²⁺ responses in stratum radiatum interneurons (183). There is a subtype heterogeneity among hippocampal interneurons at different localizations. α7 nAChRs have been found to “sense” the nicotine message on stratum radiatum interneurons while stimulation of α4β2 nAChRs in stratum lacunosum moleculare interneurons induces a current with very little desensitization (4, 9, 111). nAChRs, expressed in interneurons of the hippocampus, can be excited by nicotine at concentrations found in smokers (4). Again, this finding implies that these receptors are of high affinity and suggest a role of nonsynaptic nicotinic transmission for hippocampal interneurons. All interneurons in the stratum radiatum and stratum lacunosum moleculare can be excited by nicotinic ligands, but many interneurons in the pyramidal cell layer do not respond (111).

Interneurons play an important role in the neural circuit of the hippocampus. Stratum radiatum interneurons primarily mediate feed-forward inhibition because they receive inputs from fibers entering the CA1 that mostly inhibit dendrites of pyramidal neurons. Therefore, nicotinic stimulation can shift the balance of inhibition/excitation in a slower time-scale, likely integrating a few consecutive synaptic events, as would be expected from a nonsynaptic message with a relatively large Ca²⁺ accumulation on the second scale. Nicotinic currents through functional nAChRs of hippocampal interneurons have been confirmed in carbachol uncaging experiments, revealing a decremental scaling of the nicotinic responses along the dendrite (77). Nicotinic excitation in interneurons can interact with network activity: simultaneous activation of AMPA and NMDA receptors boosts the postsynaptic nicotinic current in interneurons of the hippocampus (5).

B. Interneurons of the Cortex and Striatum

Certain types of cortical layer 5 interneurons can be excited by puff application of nicotinic agonists, provid-
C. Pyramidal Neurons

In addition to nAChRs on hippocampal interneurons, there are functional dendritic nAChRs in pyramidal neurons of the hippocampus (44, 69). Earlier studies have shown nicotinic action only in a fraction of pyramidal neurons, which respond with small depolarizations corresponding to inward currents of 10–20 pA amplitude mediated by α7 nAChRs (111). Interestingly, other studies failed to resolve the function of these nAChRs (34, 72, 77). In cultured hippocampal neurons, roughly two-thirds of the cells can respond to nicotinic agonists with an α7 nAChR-mediated current (193). Activation of somatodendritic nAChRs of pyramidal neurons by puffs of nicotinic agonists evokes excitatory events and Ca\(^{2+}\) accumulation in acute slices (44, 69, 91) (Fig. 3).

Functional data are supported by structural findings: pyramidal neurons of the hippocampus show immunoreactivity for different nAChR subunits including the α7 and β2 subunits (34, 51, 58). The level of radiolabeled α-bungarotoxin binding has been found to be particularly high in the hippocampus and in the pyramidal cells of the CA1 region (137). In addition, hippocampal pyramidal neurons express α4, α5, β2, β3, and β4 nAChR subunits (155). Immunostaining of α7 nAChRs in cell cultures revealed that only 17% of the immunopositive neurons are GABAergic, while the remaining 83% are presumed to be glutamatergic pyramidal neurons (75), further supporting the view of pyramidal neurons as targets of nicotine. Most of these nAChRs on pyramidal neurons are believed to be located at extrasynaptic sites. Although septal cholinergic afferents also synapse onto hippocampal pyramidal neurons, and not only onto interneurons (40), it has been demonstrated that trains of stimulations can activate slow EPSPs through nAChRs rather than nAChRs in CA1 pyramidal neurons (21, 99). These findings suggest that even the existing cholinergic synapses can transmit muscarinic messages. These data strongly support the existence of nonsynaptic nAChRs in pyramidal neurons. A functional role in cellular memory formation has also been assigned to dendritic nAChR, since their activation boosts synaptic plasticity in hippocampal CA1 pyramidal neurons (44, 69), exemplifying how nAChRs can interfere with network dynamics. Nevertheless, the low expression level of nAChRs in pyramidal neurons favors a modulatory role in various cellular functions. The modulation of wired transmission occurs on a rapid time-scale; therefore, synaptic plasticity becomes even more flexible and shows adaptation to the actual level of nicotinic transmission.

VI. NONSYNAPTIC NICOTINIC ACETYLCHOLINE RECEPTORS AND HIGHER BRAIN FUNCTIONS

A. Role of Nonsynaptic nAChR in Smoking

Immediately after nicotine enters the brain during smoking, it augments the cholinergic route of reward; the blood level of nicotine in smokers reaches the firing threshold of VTA neurons leading to DA release in the nucleus accumbens (NAc) via nAChRs, which later desensitize leading to addiction (73). Nicotine in the brain most likely acts on high-affinity receptors because of the low concentration of the drug. Nicotine concentrations in the smoker’s blood can vary between 250 and 500 nM for ~10 min just after smoking a cigarette (55). During the first minute of smoking a cigarette, the blood level of nicotine goes up to 250 nM (55). Nicotine can remain at a low level (~200 nM) for hours. Just before smoking a cigarette, the concentration of nicotine in the smoker’s blood is ~40 nM (55). Although nicotine accumulates in the brain with a greater concentration than in the blood, reaching a nearly fourfold increase (45), the final concentration is still too low to activate any synaptic receptor, because synaptic activation may require millimolar concentration of the
endogenous agonist. Thus the conclusion must be drawn that nonsynaptic transmission is the dominant mode of action for the exogenous (smoked) nicotine. Nicotine reaches a maximal concentration around cells that is far below the concentration for activation of the sparse synaptic nAChRs. At the cellular level, nicotine can indeed activate nAChRs at concentrations that likely occur in the brain during smoking (4). There are experimental data showing that nicotine is able to activate receptors in spite of the rapid desensitization. Pretreatment with 40 nM nicotine fails to alter GABAergic responses to 1 μM nicotine, suggesting that nAChRs may have already recovered from desensitization by the time the blood level of nicotine drops down to 40 nM and are ready to be activated during the next cigarette (104). However, pretreatment with 250 nM nicotine could desensitize nAChR and abolish the GABAergic response to nicotine administration (104). Exposure to 0.5 μM nicotine for longer than 5 min can cause a deeper state of desensitization (123). The long half-life of nicotine and the slow recovery from desensitization may explain why smokers report that the first cigarette is the most pleasurable of the day (138). It has been shown that nicotine in nanomolar concentrations can interact with nAChRs in an α4-sensitized preparation (159) and may produce local Ca2+ responses in the nicotinic microdomain under normal circumstances. Thus even very low, nanomolar concentrations of nicotine are enough to produce small changes, which could be significant for local neural functions, most likely via high-affinity (extrasynaptic) receptors. It can be assumed that in contrast to the nicotine application during experiments, the synchronization of openings by low-dose nicotine does not all nAChRs become desensitized, with a few receptors escaping desensitization and remaining ready to open.

Nicotine at 1 μM causes a robust increase in the frequency of spontaneous GABAergic inhibitory postsynaptic currents in the VTA, indicating a role for presynaptic/preterminal nAChRs at GABAergic innervation (104). Presynaptic/preterminal α7 nAChRs, which are necessarily nonsynaptic receptors, contribute to long-term potentiation (LTP) induction in the VTA. The pairing of nicotine application with postsynaptic depolarization of VTA dopaminergic neurons potentiates the evoked EPSCs to form LTP (103). The glutamatergic transmission is still enhanced when the enhancement of GABAergic transmis-

![Fig. 3](image-url)
sion by nicotine has already desensitized (104). Although nAChRs on VTA dopaminergic neurons desensitize rapidly, the sustained dopaminergic input to the NAc after nicotinic stimulation still exits because of the differential desensitization of the GABAergic and glutamatergic innervation: nAChRs on GABAergic neurons have already desensitized while nicotine is still able to enhance glutamatergic transmission, shifting the balance of synaptic inputs to prolonged excitation (104).

B. Therapeutic Potential of nAChR Stimulation on Cognition and Nonsynaptic Transmission

Further evidence for the nonsynaptic nature of nicotinic transmission comes from the effect of nicotine in human therapy. Nonsynaptic receptors located on extrasynaptic surfaces are activated by a low concentration of the agonist and are, therefore, characterized by high-affinity binding (177). High-affinity receptors and transporters are the major targets of medicines because drugs can reach only low concentration in the brain during clinical therapy (177). In the light of these considerations, it is not surprising that nicotine has therapeutic power in humans. The effect of nAChR stimulation in Alzheimer’s disease (AD) is based on the experimental evidence that nicotine improves memory in animals, healthy subjects, and AD patients (118). Transdermal nicotine patches improve performance on a nonverbal learning task and increase attention performance in AD patients (133). The nicotinic agonist ABT-418 improves verbal learning and memory in AD patients (125), and administration of the nicotinic antagonist mecamylamine to elderly subjects and AD patients produces cognitive impairment (118). In addition, levels of different nAChRs decrease with age, which is even more pronounced in AD (118). The cortical nAChR deficits significantly correlate with cognitive impairment in AD patients (118). Positron emission tomography studies revealed reduced cortical AChE activity in AD patients (65). Cholinesterase inhibitors including tacrine, rivastigmine, and galantamine have been shown to slow the progression of AD in clinical studies (171). It is possible that these inhibitors, at least in part, act through direct activation of nAChRs (118). Nicotine skin patches attenuate the haloperidol-induced deficit in working memory tests (133). Animal models further support the human importance of nicotine therapy in AD by showing that nicotine can reduce β-amyloidosis in mice (117).

Supporting the observations of positive nicotinic effects in AD, there are numerous experimental data available showing that nicotine improves cognitive performance in deprived smokers and in patients with impaired cognition (115). The clearest effect of transdermal nicotine patches is an improvement of cognition in humans, which is reflected by the reduction in the number of errors of omission on the continuous performance task showing primarily the effect on attentional processes (133). It has been shown that long-term nicotine treatment can be important for improving cognitive function (170). In animal tests, both acute and chronic nicotine treatments have been shown to improve working memory (93, 133). Choice of accuracy significantly increased following nicotine administration in behavioral studies on rats, indicating the nicotinic effect on the working memory (133). Block of the nicotinic system shows the opposite: local infusion of mecamylamine into the hippocampus impairs working memory performance in rats (120). Mecamylamine and dihydro-β-erythroidine (DHβE) impair short- and long-term memory retrieval in behavioral tests on rats, while nicotine enhances it (106). The in vivo effects of DHβE or the α7 nAChR-specific methyllycaconitine (MLA) suggest a role for both α7 and non-α7 receptors in memory formation in rats (94). In support of the clinical importance and effectiveness of nicotinic therapy, it has been shown that people with schizophrenia smoke cigarettes at a very high rate, ~80–90% compared with the 45–70% of patients with other psychiatric disorders and 30% of the general population (60). Schizophrenics have a deficient number of nAChRs in the hippocampus (36).

These data indicate that clinical pharmacotherapy, which mostly targets nonsynaptic receptors because of their high affinity (177), utilizes the beneficial effects of drugs on nAChRs, which highlights the importance of nonsynaptic communication in this context. Similarly, nicotine inhaled from smoking can reach only a low concentration in the brain; therefore, it can preferentially activate nonsynaptic nAChRs of high affinity. The low-affinity nAChRs situated in the nicotinic synapses of the brain are most likely silent during drug treatment or smoking.

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