Beyond the Cholinergic Hypothesis: Do Current Drugs Work in Alzheimer's Disease?

Alessandro Martorana,^{1,2} Zaira Esposito¹ & Giacomo Koch²

¹ Clinica Neurologica, Dipartimento di Neuroscienze, Università di Roma "Tor Vergata", Rome, Italy
² IRCCS S. Lucia, Ospedale di Neuroriabilitazione, Via Ardeatina, Rome, Italy

Keywords

Acetylcholine; Alzheimer's disease; Amines; Circadian rhythm; Nicotinic receptor.

Correspondence

Alessandro Martorana, M.D., Clinica Neurologica, Dipartimento di Neuroscienze, Università di Roma "Tor Vergata", via Montpellier, 1-00133, Rome, Italy.
Tel.: +039-06-72596020;
Fax: +039-06-72596022;
E-mail: martorana@med.uniroma2.it

doi: 10.1111/j.1755-5949.2010.00175.x

SUMMARY

Alzheimer's disease (AD) is a neurodegenerative disease characterized by memory and cognitive loss, and represents the leading cause of dementia in elderly people. Besides the complex biochemical processes involved in the neuronal degeneration (formation of senile plaques containing A β peptides, and development of neurofibrillary tangles), other molecular and neurochemical alterations, like cholinergic deficit due to basal forebrain degeneration, also occur. Because acetylcholine has been demonstrated to be involved in cognitive processes, the idea to increase acetylcholine levels to restore cognitive deficits has gained interest (the so-called cholinergic hypothesis). This has led to the development of drugs able to prevent acetylcholine hydrolysis (acetylcholinesterase inhibitors). However, the analysis of clinical efficacy of these drugs in alleviating symptoms of dementia showed unsatisfactory results. Despite such critical opinions on the efficacy of these drugs, it should be said that acetylcholinesterase inhibitors, and for some aspects memantine also, improve memory and other cognitive functions throughout most of the duration of the disease. The pharmacological activity of these drugs suggests an effect beyond the mere increase of acetylcholine levels. These considerations are in agreement with the idea that cognitive decline is the result of a complex and not fully elucidated interplay among different neurotransmitters. The role of each of the neurotransmitters implicated has to be related to a cognitive process and as a consequence to its decline. The current review aims to highlight the positive role of cholinergic drugs in alleviating cognitive deficits during wake as well as sleep. Moreover, we suggest that future therapeutic approaches have to be developed to restore the complex interplay between acetylcholine and other neurotransmitters systems, such as dopamine, serotonin, noradrenaline, or glutamate, that are likely involved in the progressive deterioration of several cognitive functions such as attention, memory, and learning.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder representing the leading cause of dementia in elderly people worldwide. The brain areas characteristically involved in the degenerative process are the hippocampus and the neocortex. In AD, the pathological hallmark is classically represented by the presence of extracellular deposits of β -amyloid, which is derived from

an abnormal cleavage of the amyloid protein precursor (APP) in the senile plaques, and by the intracellular formation of neurofibrillary tangles, containing abnormally phosphorylated forms of a microtubule-associated protein (tau). These pathological features have been associated to the loss of neuronal synapses and pyramidal neurons [1]. Such pathological changes result in the development of typical symptoms of AD, which are characterized by progressive impairment of memory and

cognitive functions associated to behavioral disturbances as disease progresses. Despite recent advances in the understanding of AD pathogenesis, the therapeutic rationale is still based on early biochemical and pathological investigations made on AD brains. The results of these studies showed an alteration of acetylcholine (ACh) synthesis (i.e., decrease of ACh release and up-take) associated to a marked loss of cholinergic cells from the nucleus basalis of Meynert, involving a presynaptic cholinergic deficit. These findings, together with the demonstration of the role played by ACh in cognitive functions, has provided a rationale for therapeutic intervention in AD, similarly to L-dopa treatment for Parkinson's disease. This suggests that the deterioration of memory and cognitive functions seen in AD patients is a consequence of the progressive degeneration of cholinergic neurons of the basal forebrain and the loss of cholinergic transmission in the hippocampus and cerebral cortex (cholinergic hypothesis of AD) [2]. Thus, increase of the cholinergic transmission would improve cognitive and memory functions, and perhaps behavioral symptoms in AD patients (Figure 1).

The pharmacological approach currently adopted in AD patients contemplates the use of drugs that increase the concentration of ACh, inhibiting its degrading enzyme acetylcholine-esterase, namely physostigmine, tacrine, and more recently donepezil, rivastigmine, and galantamine. Moreover, dysfunction of glutamatergic transmission, which was previously underestimated due to the majority of research focusing on amyloid cascade hypothesis, has instead been identified as a precocious and typical process responsible for degeneration in AD [3,4]. For such reasons, a drug that targets glutamate (uncompetitive NMDA receptor antagonist) rather than ACh, namely memantine, was introduced for the treatment of dementia.

However, although theoretical and experimental evidence encourage the use of AChEIs and memantine for the treatment of AD, the efficacy of these drugs is still a matter of contrasting opinions, as emerges from clinical studies on the efficacy of these drugs, especially in terms of costs and effectiveness [5–18]. Despite such opinions, AChEIs and memantine are still currently in use for treatment of dementia.

The use of AChEIs in AD patients increases ACh levels inducing improvement in cognitive functions and functional autonomy. This occurs because ACh itself is involved in learning and memory processes during wake, and also because it is essential in consolidation of memory during sleep. The latter aspect is fundamental when treating patients with probable AD, principally because AD patients with a preserved wake–sleep cycle have a reduced probability of manifesting behavioral disorders

Figure 1 Summary of the synthetic pathway of acetylcholine. Principle of Achels functioning. Presynaptic cholinergic neuron synthesise acetylcholine (ACh) from choline and acetyl-CoA. Choline-acetyl transferase (ChAT) is the limiting rate enzyme for synthesis of ACh. It is stored in vesicles, and then released in the synaptic cleft. ACh acts on postsynaptic neurons on M1 subtype of muscarinic receptors and also on nicotinic receptors; in particular, the $\alpha 7$ nicotinic receptor is involved in most of the ACh effects on cognitive functions. ACh is then hydrolyzed through the enzyme acetylcholine-esterase (AChE), allowing for its reuptake through the choline transporter and the high-affinity choline transporter (HACU). To replace cholinergic transmission in patients with a diagnosis of AD, drugs able to inhibit AChE (AChEI) were developed, to increase the level and action duration of the neurotransmitter ACh. ("cholinergic hypothesis").

and thus there is a decrease in the use of inappropriate therapies (i.e., neuroleptics). In this view, it must also be said that the pharmacological actions of AChEIs (and for some aspects memantine as well) go beyond the mere cholinesterase inhibition, as these are able to interfere with other neurotransmitter systems, such as the aminergic or glutamatergic systems [19–21]. Therefore, it is worth discussing the efficacy of the drugs used for the treatment of AD, in light of recent findings on the effects of ACh, and its relationship with other neurotransmitter systems, as well their relevance to the development of cognitive deterioration. Furthermore, as recently proven, treatment with AChEIs may also have neuroprotective effects, by interfering with the pathological process responsible for death of cortical neurons, and therefore slowing down the progression of the disease [22,23].

The aim of this review is to emphasize the complex interplay among multiple transmitters' alterations

Table 1 Clinical status of drugs currently in use for AD treatment

Donepezil (Aricept, Pfizer; Memac, Bracco)	Approved for treatment of AD in 1997, most widely prescribed AD therapy. Piperidine-based, reversible cholinesterase inhibitor, with high selectivity for AChE and less for BuChE.
Rivastigmine (Exelon, Novartis)	Approved in 2000 for treatment of AD. Carbamate, pseudo-irreversible cholinesterase inhibitor, and inhibitor of BuChE.
Galantamine (Reminyl, Jansen-Cilag)	Approved in 2001 for AD treatment. Tertiary alkaloid is a reversible competitive cholinesterase inhibitor, and allosteric modulator of nicotinic ACh receptors.
Memantine (Ebixa, Lundbeck)	Voltage-dependent, moderate-affinity uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, indicated for the treatment of moderate to severe AD.

involved in the progressive deterioration of cognitive functions, based on the current evidence on the effects of cholinesterase inhibitors and memantine on cognitive functions, circadian rhythmicity, and neuroprotection.

Acetylcholinesterase Inhibitors for Treatment of Cognitive Deficits

The second generation of AChEIs, namely donepezil, rivastigmine, and galantamine, has largely supplanted the first approved drug in this class, that is, tacrine, and to date represents the only possible treatment able to alleviate symptoms of demented people (for their pharmacological profile, see Table 1). In general, the analysis of numerous reviews examined since 2000 demonstrate that these drugs have positive effects on cognition, function, behavior, and global change. In many patients, the long-term treatment with AChEIs showed improved cognitive functions even after 1 or 2 years of therapy [9,16]. Therefore, this treatment surely improves activities of daily living of these patients, reduces the emotional impact for the caregiver, and limits overall care costs, attributing a role of disease modifiers to AChEIs, as reported in recent literature. Despite such encouraging results, the efficacy of these drugs in the treatment of AD still remains a matter of contrasting opinions.

Although some studies on the efficacy of donepezil [18] concluded that the treatment is not cost-effective, showing benefits that are below a minimally relevant threshold, a recent trial, which investigated the efficacy of donepezil, rivastigmine, and galantamine, encouraged the use of AChEIs in mild to moderate AD, independently of their specific pharmacological profile (NICE, 2007) [24]. Thus, although the numerous studies performed showed a clear improvement of cognitive functions, the clinical results of these drugs remain unsatisfactory. Reasons for such discouraging conclusions may reside in the "great expectations" that were generated by the introduction of these drugs for the treatment of dementia. In fact, the cholinergic hypothesis focused much

on the necessity to restore ACh to treat cognitive impairment in AD, idea that was strengthened by the demonstration that ACh is involved in cognitive and attentive processes in healthy subjects [25,26].

However, cognitive processes are the result of more complicated and partially known interactions between different neurotransmitter systems. Anatomical and pharmacological evidence showed that there is a tight relationship between ACh and dopamine (DA), serotonin (5-HT) noradrenaline (NA), or glutamate (Glu) in the modulation of cognitive processes such as attention, memory, and learning [27–30]. In particular, the relationship between amines and glutamate systems with ACh occur with a certain degree of reciprocity, and is able to modulate the release of transmitters from axon terminals, as demonstrated in recent experimental studies [31–37]. The majority of ACh effects on these neuron subtypes are directly mediated by postsynaptic nicotinic ACh receptors, in most cases of $\alpha 7$ subtype. In recent years, such receptor has gained interest because of its suggested putative role in the modulation of *cognitive symptoms* as well as in neurodegenerative processes [38–40]. This has been revealed by experimental work on AD patients, in whom dopamine or serotonergic transmission modulates the central cholinergic transmission in different brain areas [41–47]. The administration of AChEIs not only increases the ACh levels, but can also induce a marked upregulation and sensitization of $\alpha 7$ nACh receptor in prefrontal neocortex [48,49] and hippocampus [50,51]. Moreover, it has been shown that the administration of AChEIs induces the release of other neurotransmitters like NA, DA, or Glu [52–55], by acting either on the abovementioned $\alpha 7$ nACh or on other nicotinic receptor subtypes.

Thus, cognitive deterioration occurring in AD can be considered more as the result of complex neurotransmitters dysfunction rather than the effect of a single transmitter deficit. In this view, several recent neuropathological studies showed that subcortical nuclei, like the locus coeruleus (synthesizing NA), the raphe nuclei

(synthesizing 5-HT), and the midbrain neurons (synthesizing DA), are severely and precociously altered in AD patients [56,57]. Taken together, these findings confirm that cognitive decline could be the result of multiple transmitters' alterations, due to the pathological accumulation of β -amyloid peptide at synaptic level.

In such a complicated frame, the relative success of cholinomimetics has to be reconsidered. In this view, the replacement of ACh may compensate only in part for the transmitters dysfunction, improving some cognitive functions. As the disease progresses, severe dysfunction of synaptic transmission and impairment of neurotransmitters release, make the cognitive decline more evident and less treatable.

Circadian Rhythmicity, Cholinergic Treatment, and Alzheimer's Disease

Circadian rhythmicity weakens with aging, when insomnia, difficulties in falling asleep and early waking appear. In early as well as moderate AD, patients' sleep is in general slightly affected. Insomnia is the most frequently reported, while sleep architecture is well maintained, except for REM sleep, which is shorter than in healthy subjects. With the progression of AD, non-REM and REM sleep deteriorate, in parallel with cognitive impairment.

Sleep is a complex phenomenon regulated by neurons located in the preoptic area. Neurons in this area promote sleep by inhibiting neurons of the arousal system, represented by terminals arising from the noradrenergic LC, and the pontine cholinergic neurons. Transmitters used by these systems, namely ACh and NA, reciprocally exert an inhibitory action on hypothalamic neurons. In normal conditions, ACh release is maximal during waking, motor activity and REM sleep, while it decreases during non-REM-sleep (Figure 2).

Cholinergic inactivity during non-REM sleep has been suggested to play a critical role in the consolidation of declarative memory [58–60]. ACh exerts its function by acting on muscarinic receptors located at postsynaptic level in the hypothalamus, and on nicotinic receptors located at presynaptic level on noradrenergic terminals [61] as well as at postsynaptic level on hypothalamic neurons [62].

Dys-regulation of the noradrenergic and/or cholinergic neurotransmission systems may be responsible for arousal and/or cognitive dysfunction associated to a variety of psychiatric disorders, including attention deficit, hyperactivity, sleep–arousal disorder [63,64]. Sleep and behavioral disorders are reported in about one-third of patients with mild to moderate AD, and worsen as the disease progresses [65]. Common symptoms include night-time sleep fragmentation, increased sleep latency,

decreased slow-wave sleep (SWS), and increased daytime napping. Moreover, “sundowning” is another common phenomenon that occurs as AD progresses and which consists of increased confusion, wandering, and anxiety that often occurs in the late afternoon and evening, with

Figure 2 ACh inhibits sleep-promoting neurons in the SCN. Hypothalamic supra-chiasmatic nucleus (SCN) neurons are considered sleep-promoting neurons. SCN neurones send projections to the histaminergic (H) tuberomammillary nucleus (TMN), a hypothalamic nucleus involved in arousal and cortical activation. Moreover, SCN neurones are also reciprocally connected with the activating systems, namely locus coeruleus (LC) and the cholinergic pontine tegmental system (PPT). NA as well as ACh are able to inhibit SCN neurons, maintaining wakefulness. NA, released from LC terminals, exert an inhibitory effect through the activation of postsynaptic α_2 -adrenergic receptors. At the same time, LC projects to the PPT, exerting an excitatory effect on cholinergic neurons acting on postsynaptic α_1 -adrenergic receptors set on these neurons. This effect is made possible also due to the inhibition of GABA neurons of the tegmental region: inhibition mediated through α_2 -adrenergic receptors. PPT releases ACh, which acts inhibiting SCN neurons directly via the postsynaptic muscarinic receptors and indirectly modulating the nicotinic receptors, localized on presynaptic terminals from LC. The effect of ACh is present during waking, although aminergic tone is prevalent, and during REM sleep, where it plays a fundamental role in memory consolidation. In the control of circadian rhythm are involved other transmitters playing function not fully known. Among others, orexin and adenosine are involved in the control of wake–sleep cycle, with opposite effect. Orexin, released from hypothalamic neurones acts through two different types of receptor differently expressed in LC (OX1), or TMN (OX2), where OX1 seem to be related more with vigilance. Adenosine (Ado) coreleased with neurotransmitters, exerts excitatory effects on PPT neurones modulating ACh release, and also on SCN neurones having a modulatory role during sleep induction.

peaks of improvement observed in day-light hours. As shown in neuropathological studies, most of these symptoms may be due to the degeneration of hypothalamic as well as adrenergic and cholinergic neurons observed in the course of AD [66,67]. The use of AChEIs showed positive effects not only on cognitive functions but also on the architecture of sleep in both elderly demented and nondemented people [25,26,68–70]. However, most of these positive effects were observed in diurnal hours, during wake activity of patients, while in nocturnal hours, due to the increased levels of ACh, there was an exacerbation of sleep disorders. This was particularly true for donepezil. In fact, due to the pharmacokinetic profile, donepezil induced a stable increase of ACh during the whole day, contrasting with the normal physiology of ACh release. Although this could potentially create adverse sleep-related events (insomnia, nightmares), as confirmed by some clinical trials [71,72], the continued administration of low doses of donepezil seemed to attenuate the effects on REM and SWS. Such evidence suggests the existence of counter-regulatory adaptative mechanisms that may reduce the adverse effects of donepezil [73]. However, such mechanisms raise questions regarding the possible development of tolerance to the drugs, which could potentially reduce their therapeutic effects. In this view, low doses administered during the day might represent a therapeutic choice; alternatively, shifting to galantamine could be a good option to reduce adverse effects during nighttime.

The abovementioned symptoms are less common for galantamine [74] and rivastigmine [73,75]. Galantamine has a pharmacokinetic profile that closely resembles the natural circadian rhythmicity of ACh, with decreased drug plasma levels during the evening hours. This induced positive effects on sleep, memory consolidation and possibly in delaying behavioral disorders in AD patients. Thus, AChEIs improve cognitive deficits and also circadian rhythm in AD patients. Available data suggest that the type of drug chosen and time of administration are critical clues for optimizing cholinergic treatment during AD.

Acetylcholinesterase Inhibitors as Disease Modifiers

Experimental evidence indicates that AChEIs are able to induce long-lasting effects well beyond the replacement of ACh. These drugs are thought to play a neuroprotective role, because they can interfere with β -amyloid synthesis and cell death mechanisms like glutamate excitotoxicity, mitochondrial dysfunction and free radical production, and oxidative stress [22,23,76]. Recent studies show that the neuro-protection induced by

AChEIs has to be connected to the stimulation of nicotinic receptors, which have been shown to protect against toxicity induced by $A\beta$ peptides and toxins, in both *in vitro* and *in vivo* systems [77,78]. In particular, the role played by the activation of $\alpha 7$ nACh in neuro-protection has been recently described. Such mechanism involves the modulation of glutamatergic activity as well as the activation of the antiapoptotic pathway Bcl-2 and Bcl-xL [39] (Figure 3). Furthermore, the activation of $\alpha 7$ nAChR is also able to influence the β -amyloid pathway, by either reducing the formation of fibrillar β -sheets from $A\beta$ monomers [79,80], or by reducing the production of insoluble $A\beta$ 1–42 and $A\beta$ 1–40 peptides [81]. Interestingly, some researchers found that $A\beta$ peptides were also found to act as agonists of $\alpha 7$ nAChR, inducing the activation of

Figure 3 “Neuroprotective effects of ChEIs. Role of $\alpha 7$ nicotinic receptor.” Under normal conditions ChEIs (in particular, Donepezil or Galantamine) stimulate nAChR, likely through an allosteric site distinct from the acetylcholinesterase binding site (which is known for galantamine while only suggested for donepezil). Upon stimulation of these drugs $\alpha 7$ nAChR activates phosphatidylinositol 3-kinase (PI3K), through the activation and association of Janus-activated kinase 2 (JAK2) and with nonreceptor type tyrosine kinase Fyn. PI3K activated in turn leads to the activation of Akt by phosphorylation (Akt-p). The level of Akt-p increases upon nicotine treatment. Its activation increases the expression level of Bcl-2 transcript, protecting neurons from cell death. Hypoactivated $\alpha 7$ nAChR decreases the activation of Jak2 and PI3K, which in turn increases the activity of the GSK-3 β enzyme, leading to increased phosphorylation of tau proteins, until cell death.

ERK2-MAPK signaling cascade [82–85]; whereas, other research groups clearly found a greater inhibitory action of A β peptides on α 7nAChR. Further studies showed that the blockage of α 7nAChR with antagonists can attenuate A β -mediated toxic effects [86,87]. These conflicting data shed confusion on the role of α 7nAChR in ameliorating cognitive functions. For this purpose it was suggested that such receptor could be rapidly desensitized following activation, making the distinction between agonistic and antagonistic activity difficult to understand. Thus, given the possibility that the enhancing effects of AChEIs on cognition may be mediated through nAChRs, the role of agonistic and desensitizing actions of these receptors deserves further investigation [4,88]. Moreover, recent experimental studies showed that the stimulation of 5-HT receptors helps to protect neurons of the hippocampus and neocortex. This seems to be the case with five HT4 subtype receptor, which is localized in limbic structures, particularly amygdala and hippocampus, and involved in cognitive processes such as memory and learning [89–91]. In experimental models of AD, the 5-HT4 stimulation has been demonstrated to reduce the amyloid burden in murine models of AD, and also to promote the nonamyloidogenic pathway of the amyloid-precursor protein (APP) [92–95]. Interestingly, the association between donepezil and 5-HT4 agonist was beneficial to memory function in laboratory models [96]. Moreover, Ach effects are also mediated by a different class of receptors, namely muscarinic Ach receptors. In AD brains, *muscarinic receptor expression* remains unaltered, although alteration of the coupling between receptors, G-protein and second messenger are reported [97]. On the other hand the M2 mAChR subtypes, which are located preferentially on presynaptic cholinergic terminals, are reduced in AD brains [98]. mAChR has long remained a high-profile target for the treatment of cognitive dysfunction. In particular, many efforts have been dedicated to the development of either M1 mAChR agonists or M2 mAChR antagonists. In fact, besides their ability to restore cholinergic deficits and memory function, these compounds have shown to be capable of modifying the course of the disease. In particular, mAChR M1 agonists are able to interfere with the nonamyloidogenic pathway of APP processing, resulting in increased soluble APP α secretion and, as a consequence, reduced A β toxicity [99–102]. Furthermore, attenuation of the apoptotic effects induced by A β peptides was also shown [103].

Unfortunately, the prevalence of adverse effects has limited the development and introduction of these drugs for the treatment of AD. Even the antagonism of the M2 mAChR could represent a key to restore cholinergic tone and to improve cognitive functions, but the risk of serious adverse effects (due to the presence of M2 re-

ceptors in the heart) has limited the use of these drugs [102].

Therefore, despite the great amount of data elucidating the possible neuroprotective role of AChEIs, no *in vivo* studies are available at the moment. The recent experimental findings described above may certainly represent a valid pharmacological tool for future treatments. Again, it is evident that like cholinergic drugs, aminergic drugs can also enhance cognitive processes and play a neuroprotective role; more importantly, when the two are combined [44,104,105]. Thus, poly-therapy may represent a valid option to improve cognitive functions and at the same time to delay the disease progression.

Memantine for Treatment of Cognitive Deficits

Memantine is a moderate affinity, uncompetitive glutamate antagonist, acting on ionotropic NMDA receptors, with strong voltage dependency and fast kinetics. Glutamate (Glu) is one of the major excitatory neurotransmitters in the brain and spinal cord, and is of critical importance for learning and cognitive processes. The idea of treating AD patients with memantine is due to the concept that overstimulation of NMDA receptors leads to excitotoxicity and cell death, a mechanism that is non-specific for AD, but diffusely recognized for supporting neurodegenerative processes. In AD, due to the effects of A β peptides, glutamatergic neurons are however overactive, releasing glutamate continuously and in greater amounts compared to non-AD subjects, and the NMDA receptor is more sensitive in the course of AD. Also, in accordance with these results there has been neurophysiological evidence of cortical hyper-excitability in AD patients, interpreted as the result of glutamatergic dysfunction [45,106–108]. Interestingly, recent findings show that levels of vesicular transporters for glutamate are altered in different brain areas of AD patients, well before cell loss and disease onset [109,110], indicating that in early phases of the disease glutamatergic transmission might be hypoactive rather than hyperactive. Reasons for such discrepancies might reside in the effect of A β peptides: A β is in fact able to inhibit vesicular glutamate transporters as well as increase glutamate release. Thus, as a result of this altered glutamatergic homeostasis it is assumed that increased levels of Glu can tonically activate glutamate receptors, namely NMDA, leading to continuous activation until cell damage/death is reached [3,4]. Alternatively (or in addition), memantine was supposed to act on extrasynaptic NMDARs, leading to receptor overactivation [111,112]. Indeed, as previously demonstrated, these receptors preferentially

mediate toxic effects of glutamate [113,114]. Thus, although the excitatory dysfunction is not unique and typical of AD pathological process, such dysfunction makes the use of glutamate antagonists particularly indicated. In particular, Parsons et al. [3] hypothesized that memantine, due to its fast kinetics and relative selectivity, could act by restoring the glutamate homeostasis, when glutamate is inappropriately activated.

However, despite its pharmacological profile, memantine has been recommended only for treatment of severe cases of AD. In this view, data on the efficacy of memantine are not encouraging due to the limited benefits produced by this drug, which have been often restricted to behavioral symptoms of AD [13,15,115].

Recent studies show a higher efficacy when memantine is administered with AChEIs such as donepezil or galantamine [116]. Experimental evidence supports this idea, showing how glutamate and ACh cooperate in the modulation of learning processes. Anatomical and pharmacological data, in particular, showed that glutamatergic axon terminals of rat frontal cortex expresses $\alpha 7$ subtype as well as non- $\alpha 7$ subtype nACh receptors [34,36]. Considering the growing evidence on the role of the nicotinic receptor subtype $\alpha 7$, the relationship afore mentioned strengthens the cooperation between transmitter systems.

Further neurophysiological studies [45,106,107] show that the altered glutamatergic transmission observed in AD patients is modulated by the administration of either AChEIs or L-dopa, supporting the hypothesis of a neurotransmitters' interplay at the basis of cognitive dysfunction seen in AD patients. Moreover, *in vitro* studies show how modulation of nicotinic cholinergic receptors are also able to dampen glutamate overactivity, protecting neurons from cell death [115,116]. Thus, the association between AChEIs and Memantine seems fruitful, and may take the treatment of symptoms of dementia a step further.

Limits to the evaluation of efficacy are likely due to the fact that it is restricted to advanced cases of dementia. This could in turn limit the role of ACh and Glu to behavioral disorders rather than to more proper cognitive deficits. However, numerous studies to further evaluate the real efficacy of memantine are still in course (DOMINO-AD 2009 Study protocol) [117], and the results premature for discussion.

Conclusions

Our considerations are not in contrast with the results of the numerous reviews in recent literature. Instead, they may help to ease ongoing conflicts on the use and effec-

tiveness of AChEIs in the treatment of patients suffering from AD and other forms of dementia.

In conclusion, these drugs remain a good option for the treatment of progressive cognitive deterioration. However, limits in their effectiveness are related to the lack of effects on the multiple transmitters impairment, and also to the progression of pathological processes leading to severe and diffused neuronal loss in hippocampus and neocortex. Further therapeutic approaches may be developed to restore the complex interplay among multiple transmitters alterations, such as dopamine, serotonin, noradrenaline, or glutamate, that are likely involved in the progressive worsening of several cognitive functions such as attention, memory, and learning.

Conflict of Interests

The authors have no conflict of interest.

References

1. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* 2002;**297**:353–356.
2. Bartus RT, Dean RL III, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. *Science* 1982;**217**:408–414.
3. Parsons CG, Stöfler A, Danysz W. Memantine: A NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system—too little activation is bad, too much is even worse. *Neuropharmacology* 2007;**53**:699–723.
4. Ondrejcek T, Klyubin I, Hu NW, Barry AE, Cullen WK, Rowan MJ. Alzheimer's disease amyloid beta-protein and synaptic function. *Neuromolecular Med* 2010;**12**:13–26.
5. Geldmacher DS. Long-term cholinesterase inhibitor therapy for Alzheimer's disease: Practical considerations for the primary care physician. *Prim Care Companion J Clin Psychiatry* 2003;**5**:251–259.
6. Geldmacher DS, Frolich L, Doody RS, et al. Realistic expectations for treatment success in Alzheimer's disease. *J Nutr Health Aging* 2006;**10**:417–429.
7. Ritchie CW, Ames D, Clayton T, Lai R. Metaanalysis of randomized trials of the efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer disease. *Am J Geriatr Psychiatry* 2004;**12**:358–369.
8. Forchetti CM. Treating patients with moderate to severe Alzheimer's disease: Implications of recent pharmacologic studies. *Prim Care Companion J Clin Psychiatry* 2005;**7**:155–161.
9. Birks J. Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst Rev* 2006;CD005593.

10. Loveman E, Green C, Kirby J, Takeda A, Picot J, Payne E, Clegg A. The clinical and cost-effectiveness of donepezil, rivastigmine, galantamine and memantine for Alzheimer's disease. *Health Technol Assess* 2006;**10**:iii–iv, ix–xi, 1–160.
11. Schmitt FA, Wichems CH. A systematic review of assessment and treatment of moderate to severe Alzheimer's disease. *Prim Care Companion J Clin Psychiatry* 2006;**8**:158–159.
12. Takeda A, Loveman E, Clegg A, Kirby J, Picot J, Payne E, Green C. A systematic review of the clinical effectiveness of donepezil, rivastigmine and galantamine on cognition, quality of life and adverse events in Alzheimer's disease. *Int J Geriatr Psychiatry* 2006;**21**:17–28.
13. Raina P, Santaguida P, Ismaila A, et al. Effectiveness of cholinesterase inhibitors and memantine for treating dementia: Evidence review for a clinical practice guideline. *Ann Intern Med* 2008;**148**:379–397.
14. Hansen RA, Gartlehner G, Lohr KN, Kaufer DI. Functional outcomes of drug treatment in Alzheimer's disease: A systematic review and meta-analysis. *Drugs Aging* 2007;**24**:155–167.
15. Hansen RA, Gartlehner G, Webb AP, Morgan LC, Moore CG, Jonas DE. Efficacy and safety of donepezil, galantamine, and rivastigmine for the treatment of Alzheimer's disease: A systematic review and meta-analysis. *Clin Interv Aging* 2008;**3**:211–225.
16. Rountree SD, Chan W, Pavlik VN, Darby EJ, Siddiqui S, Doody RS. Persistent treatment with cholinesterase inhibitors and/or memantine slows clinical progression of Alzheimer disease. *Alzheimers Res Ther* 2009;**1**:1–7.
17. Robles A. Pharmacological treatment of Alzheimer's disease: Is it progressing adequately? *Open Neurol J* 2009;**3**:27–44.
18. Birks JS, Melzer D, Beppu H. Donepezil for mild and moderate Alzheimer's disease. *Cochrane Database Syst Rev* 2000;CD001190.
19. Zhu XD, Giacobini E. Second generation cholinesterase inhibitors: Effect of (L)-huperzine-A on cortical biogenic amines. *J Neurosci Res* 1995;**41**:828–835.
20. Trabace L, Coluccia A, Gaetani S, et al. In vivo neurochemical effects of the acetylcholinesterase inhibitor ENA713 in rat hippocampus. *Brain Res* 2000;**865**:268–271.
21. Giacobini E, Zhu XD, Williams E, Sherman KA. The effect of the selective reversible acetylcholinesterase inhibitor E2020 on extracellular acetylcholine and biogenic amine levels in rat cortex. *Neuropharmacology* 1996;**35**:205–211.
22. Francis PT, Nordberg A, Arnold SE. A preclinical view of cholinesterase inhibitors in neuroprotection: Do they provide more than symptomatic benefits in Alzheimer's disease? *Trends Pharmacol Sci* 2005;**26**:104–111.
23. Pepeu G, Giovannini MG. Cholinesterase inhibitors and beyond. *Curr Alzheimer Res* 2009;**6**:86–96.
24. Iliffe S. The National Institute for Health and Clinical Excellence (NICE) and drug treatment for Alzheimer's disease. *CNS Drugs* 2007;**21**:177–184.
25. Schredl M, Weber B, Leins ML, Heuser I. Donepezil-induced REM sleep augmentation enhances memory performance in elderly, healthy persons. *Exp Gerontol* 2001;**36**:353–361.
26. Hornung OP, Regen F, Danker-Hopfe H, Schredl M, Heuser I. The relationship between REM sleep and memory consolidation in old age and effects of cholinergic medication. *Biol Psychiatry* 2007;**61**:750–757.
27. Blokland A. Acetylcholine: A neurotransmitter for learning and memory? *Brain Res Brain Res Rev* 1995;**21**:285–300.
28. Wisman LA, Sahin G, Maingay M, Leanza G, Kirik D. Functional convergence of dopaminergic and cholinergic input is critical for hippocampus-dependent working memory. *J Neurosci* 2008;**28**:7797–7807.
29. Millan MJ, Di Cara B, Dekeyne A, et al. Selective blockade of dopamine D(3) versus D(2) receptors enhances frontocortical cholinergic transmission and social memory in rats: A parallel neurochemical and behavioural analysis. *J Neurochem* 2007;**100**:1047–1061.
30. Brooks DJ. Imaging the role of dopamine in health and disease Parkinson's disease as a lesion model. *Wien Klin Wochenschr* 2006;**118**:570–572.
31. Cassel JC, Jeltsch H, Neufang B, Lauth D, Szabo B, Jackisch R. Downregulation of muscarinic- and 5-HT1B-mediated modulation of [3H]acetylcholine release in hippocampal slices of rats with fimbria-fornix lesions and intrahippocampal grafts of septal origin. *Brain Res* 1995;**704**:153–166.
32. Clarke PB, Reuben M. Release of [3H]-noradrenaline from rat hippocampal synaptosomes by nicotine: Mediation by different nicotinic receptor subtypes from striatal [3H]-dopamine release. *Br J Pharmacol* 1996;**117**:595–606.
33. Kenny PJ, File SE, Neal MJ. Evidence for a complex influence of nicotinic acetylcholine receptors on hippocampal serotonin release. *J Neurochem* 2000;**75**:2409–2414.
34. Marchi M, Risso F, Viola C, Cavazzani P, Raiteri M. Direct evidence that release-stimulating alpha7* nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. *J Neurochem* 2002;**80**:1071–1078.
35. Woolley ML, Marsden CA, Sleight AJ, Fone KC. Reversal of a cholinergic-induced deficit in a rodent model of recognition memory by the selective 5-HT6 receptor antagonist, Ro 04-6790. *Psychopharmacology* 2003;**170**:358–367.
36. Barik J, Wonnacott S. Indirect modulation by alpha7 nicotinic acetylcholine receptors of noradrenaline release in rat hippocampal slices: Interaction with glutamate and GABA systems and effect of nicotine withdrawal. *Mol Pharmacol* 2006;**69**:618–628.

37. Rousseau SJ, Jones IW, Pullar IA, Wonnacott S. Presynaptic alpha7 and non-alpha7 nicotinic acetylcholine receptors modulate [3H]d-aspartate release from rat frontal cortex in vitro. *Neuropharmacology* 2005;**49**:59–72.
38. Ikonomic MD, Wecker L, Abrahamson EE, et al. Cortical alpha7 nicotinic acetylcholine receptor and beta-amyloid levels in early Alzheimer disease. *Arch Neurol* 2009;**66**:646–651.
39. Bencherif M, Lippiello PM. Alpha7 neuronal nicotinic receptors: The missing link to understanding Alzheimer's etiopathology? *Med Hypotheses* 2010;**74**:281–285.
40. Counts SE, He B, Che S, Ikonomic MD, DeKosky ST, Ginsberg SD, Mufson EJ. Alpha7 nicotinic receptor up-regulation in cholinergic basal forebrain neurons in Alzheimer disease. *Arch Neurol* 2007;**64**:1771–1776.
41. Galindo-Charles L, Hernandez-Lopez S, Galarraga E, et al. Serotonergic dorsal raphe neurons possess functional postsynaptic nicotinic acetylcholine receptors. *Synapse* 2008;**62**:601–615.
42. Fone KC. An update on the role of the 5-hydroxytryptamine6 receptor in cognitive function. *Neuropharmacology* 2008;**55**:1015–1022.
43. Garcia-Alloza M, Gil-Bea FJ, Diez-Ariza M, Chen CP, Francis PT, Lasheras B, Ramirez MJ. Cholinergic-serotonergic imbalance contributes to cognitive and behavioral symptoms in Alzheimer's disease. *Neuropsychologia* 2005;**43**:442–449.
44. Smith GS, Kramer E, Ma Y, Hermann CR, Dhawan V, Chaly T, Eidelberg D. Cholinergic modulation of the cerebral metabolic response to citalopram in Alzheimer's disease. *Brain* 2009;**132**:392–401.
45. Martorana A, Stefani A, Palmieri MG, Esposito Z, Bernardi G, Sancesario G, Pierantozzi M. L-dopa modulates motor cortex excitability in Alzheimer's disease patients. *J Neural Transm* 2008;**115**:1313–1319.
46. Martorana A, Mori F, Esposito Z, et al. Dopamine modulates cholinergic cortical excitability in Alzheimer's disease patients. *Neuropsychopharmacology* 2009;**34**:2323–2328.
47. Preda S, Govoni S, Lanni C, Racchi M, Mura E, Grilli M, Marchi M. Acute beta-amyloid administration disrupts the cholinergic control of dopamine release in the nucleus accumbens. *Neuropsychopharmacology* 2008;**33**:1062–1070.
48. Reid RT, Sabbagh MN. Effects of donepezil treatment on rat nicotinic acetylcholine receptor levels in vivo and in vitro. *J Alzheimers Dis* 2003;**5**:429–436.
49. Reid RT, Sabbagh MN. Effects of cholinesterase inhibitors on rat nicotinic receptor levels in vivo and in vitro? *J Neural Transm* 2008;**115**:1437–1444.
50. Kenney JW, Gould TJ. Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. *Mol Neurobiol* 2008;**38**:101–121.
51. Placzek AN, Zhang TA, Dani JA. Nicotinic mechanisms influencing synaptic plasticity in the hippocampus. *Acta Pharmacol Sin* 2009;**30**:752–760.
52. Zhang L, Zhou FM, Dani JA. Cholinergic drugs for Alzheimer's disease enhance in vitro dopamine release. *Mol Pharmacol* 2004;**66**:538–544.
53. Sharp BM, Yatsula M, Fu Y. Effects of galantamine, a nicotinic allosteric potentiating ligand, on nicotine-induced catecholamine release in hippocampus and nucleus accumbens of rats. *J Pharmacol Exp Ther* 2004;**309**:1116–1123.
54. Shearman E, Rossi S, Szasz B, et al. Changes in cerebral neurotransmitters and metabolites induced by acute donepezil and memantine administrations: A microdialysis study. *Brain Res Bull* 2006;**69**:204–213.
55. Schilström B, Ivanov VB, Wiker C, Svensson TH. Galantamine enhances dopaminergic neurotransmission in vivo via allosteric potentiation of nicotinic acetylcholine receptors. *Neuropsychopharmacology* 2007;**32**:43–53.
56. Berlanga ML, Simpson TK, Alcantara AA. Dopamine D5 receptor localization on cholinergic neurons of the rat forebrain and diencephalon: A potential neuroanatomical substrate involved in mediating dopaminergic influences on acetylcholine release. *J Comp Neurol* 2005;**492**:34–49.
57. Kumar U, Patel SC. Immunohistochemical localization of dopamine receptor subtypes (D1R–D5R) in Alzheimer's disease brain. *Brain Res* 2007;**1131**:187–196.
58. Power AE. Slow-wave sleep, acetylcholine, and memory consolidation. *Proc Natl Acad Sci USA* 2004;**101**:1795–1796.
59. Gais S, Born J. Declarative memory consolidation: Mechanisms acting during human sleep. *Learn Mem* 2004;**11**:679–685.
60. Rasch BH, Born J, Gais S. Combined blockade of cholinergic receptors shifts the brain from stimulus encoding to memory consolidation. *J Cogn Neurosci* 2006;**18**:793–802.
61. Saint-Mieux B, Eggermann E, Bisetti A, et al. Nicotinic enhancement of the noradrenergic inhibition of sleep-promoting neurons in the ventrolateral preoptic area. *J Neurosci* 2004;**24**:63–67.
62. Hatton GI, Yang QZ. Synaptic potentials mediated by alpha 7 nicotinic acetylcholine receptors in supraoptic nucleus. *J Neurosci* 2002;**22**:29–37.
63. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev* 2003;**42**:33–84.
64. Berridge CW. Noradrenergic modulation of arousal. *Brain Res Rev* 2008;**58**:1–17.
65. Deschennes CL, McCurry SM. Current treatments for sleep disturbances in individuals with dementia. *Curr Psychiatry Rep* 2009;**11**:20–26.

66. Stopa EG, Volicer L, Kuo-Leblanc V, Harper D, Lathi D, Tate B, Satlin A. Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. *J Neuropathol Exp Neurol* 1999;**58**:29–39.
67. Weinshenker D. Functional consequences of locus coeruleus degeneration in Alzheimer's disease. *Curr Alzheimer Res* 2008;**5**:342–345.
68. Moraes Wdos S, Poyares DR, Guilleminault C, Ramos LR, Bertolucci PH, Tufik S. The effect of donepezil on sleep and REM sleep EEG in patients with Alzheimer disease: A double-blind placebo-controlled study. *Sleep* 2006;**29**:199–205.
69. Cooke JR, Loreda JS, Liu L, et al. Acetylcholinesterase inhibitors and sleep architecture in patients with Alzheimer's disease. *Drugs Aging* 2006;**23**:503–511.
70. Mizuno S, Kameda A, Inagaki T, Horiguchi J. Effects of donepezil on Alzheimer's disease: The relationship between cognitive function and rapid eye movement sleep. *Psychiatry Clin Neurosci* 2004;**58**:660–665.
71. Burns A, Rossor M, Hecker J, et al. The effects of donepezil in Alzheimer's disease – Results from a multinational trial. *Dement Geriatr Cogn Disord* 1999;**10**:237–244.
72. Rogers SL. Perspectives in the management of Alzheimer's disease: Clinical profile of donepezil. *Dement Geriatr Cogn Disord* 1998;**9**(Suppl 3):29–42.
73. Davis B, Sadik K. Circadian cholinergic rhythms: Implications for cholinesterase inhibitor therapy. *Dement Geriatr Cogn Disord* 2006;**21**:120–129.
74. Grossberg GT, Sadowsky C, Olin JT. Rivastigmine transdermal system for the treatment of mild to moderate Alzheimer's disease. *Int J Clin Pract* 2010;**64**:651–660.
75. Nieoullon A, Bentué-Ferrer D, Bordet R, Tsolaki M, Förstl H. Importance of circadian rhythmicity in the cholinergic treatment of Alzheimer's disease: Focus on galantamine. *Curr Med Res Opin* 2008;**24**:3357–3367.
76. Giacobini E. Cholinesterases: New roles in brain function and in Alzheimer's disease. *Neurochem Res* 2003;**28**:515–522.
77. Shimohama S, Kihara T. Nicotinic receptor-mediated protection against beta-amyloid neurotoxicity. *Biol Psychiatry* 2001;**49**:233–239.
78. Zeng H, Zhang Y, Peng L, et al. Nicotine and amyloid formation. *Biol Psychiatry* 2001;**49**:248–257.
79. Salomon AR, Marciniowski KJ, Friedland RP, Zagorski MG. Nicotine inhibits amyloid formation by the beta-peptide. *Biochemistry* 1996;**35**:13568–13578.
80. Hellström-Lindahl E, Court J, Keverne J, et al. Nicotine reduces A beta in the brain and cerebral vessels of APPsw mice. *Eur J Neurosci* 2004;**19**:2703–2710.
81. Nordberg A, Hellström-Lindahl E, Lee M, et al. Chronic nicotine treatment reduces beta-amyloidosis in the brain of a mouse model of Alzheimer's disease (APPsw). *J Neurochem* 2002;**81**:655–658.
82. Takada-Takatori Y, Kume T, Sugimoto M, Katsuki H, Sugimoto H, Akaike A. Acetylcholinesterase inhibitors used in treatment of Alzheimer's disease prevent glutamate neurotoxicity via nicotinic acetylcholine receptors and phosphatidylinositol 3-kinase cascade. *Neuropharmacology* 2006;**51**:474–486.
83. Takada-Takatori Y, Kume T, Izumi Y, et al. Roles of nicotinic receptors in acetylcholinesterase inhibitor-induced neuroprotection and nicotinic receptor up-regulation. *Biol Pharm Bull* 2009;**32**:318–324.
84. Dineley KT, Westerman M, Bui D, Bell K, Ashe KH, Sweatt JD. Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal alpha7 nicotinic acetylcholine receptors: In vitro and in vivo mechanisms related to Alzheimer's disease. *J Neurosci* 2001;**21**:4125–4133.
85. Dineley KT, Xia X, Bui D, Sweatt JD, Zheng H. Accelerated plaque accumulation, associative learning deficits, and up-regulation of alpha 7 nicotinic receptor protein in transgenic mice co-expressing mutant human presenilin 1 and amyloid precursor proteins. *J Biol Chem* 2002;**277**:22768–22780.
86. Martin SE, de Fiebre NE, de Fiebre CM. The alpha7 nicotinic acetylcholine receptor-selective antagonist, methyllycaconitine, partially protects against beta-amyloid1-42 toxicity in primary neuron-enriched cultures. *Brain Res* 2004;**1022**:254–256.
87. Mousavi M, Hellström-Lindahl E. Nicotinic receptor agonists and antagonists increase sAPPalpha secretion and decrease Abeta levels in vitro. *Neurochem Int* 2009;**54**:237–244.
88. Gay EA, Giniatullin R, Skorinkin A, Yakel JL. Aromatic residues at position 55 of rat alpha7 nicotinic acetylcholine receptors are critical for maintaining rapid desensitization. *J Physiol* 2008;**586**:1105–1115.
89. Barthet G, Framery B, Gaven F, et al. 5-Hydroxytryptamine 4 receptor activation of the extracellular signal-regulated kinase pathway depends on Src activation but not on G protein or beta-arrestin signaling. *Mol Biol Cell* 2007;**18**:1979–1991.
90. Mlinar B, Mascalchi S, Morini R, Giachi F, Corradetti R. MDMA induces EPSP-Spike potentiation in rat ventral hippocampus in vitro via serotonin and noradrenaline release and coactivation of 5-HT4 and beta1 receptors. *Neuropsychopharmacology* 2008;**33**:1464–1475.
91. Perez-Garcia G, Meneses A. Memory formation, amnesia, improved memory and reversed amnesia: 5-HT role. *Behav Brain Res* 2008;**195**:17–29.
92. Maillet M, Robert SJ, Lezoualc'h F. New insights into serotonin 5-HT4 receptors: A novel therapeutic target for Alzheimer's disease? *Curr Alzheimer Res* 2004;**1**:79–85.

93. Cho S, Hu Y. Activation of 5-HT₄ receptors inhibits secretion of beta-amyloid Peptides and increases neuronal survival. *Exp Neurol* 2007;**203**:274–278.
94. Cachard-Chastel M, Lezoualc'h F, Dewachter I, et al. 5-HT₄ receptor agonists increase sAPP alpha levels in the cortex and hippocampus of male C57BL/6j mice. *Br J Pharmacol* 2007;**150**:883–892.
95. Russo O, Berthouze M, Giner M, et al. Synthesis of specific bivalent probes that functionally interact with 5-HT(4) receptor dimers. *J Med Chem* 2007;**50**:4482–4492.
96. Cachard-Chastel M, Devers S, Sicsic S, Langlois M, Lezoualc'h F, Gardier AM, Belzung C. Prucalopride and donepezil act synergistically to reverse scopolamine-induced memory deficit in C57BL/6j mice. *Behav Brain Res* 2008;**187**:455–461.
97. Warpman U, Alafuzoff I, Nordberg A. Coupling of muscarinic receptors to GTP proteins in postmortem human brain – Alterations in Alzheimer's disease. *Neurosci Lett* 1993;**150**:39–43.
98. Nordberg A, Alafuzoff I, Winblad B. Nicotinic and muscarinic subtypes in the human brain: Changes with aging and dementia. *J Neurosci Res* 1992;**31**:103–111.
99. Müller DM, Mendla K, Farber SA, Nitsch RM. Muscarinic M1 receptor agonists increase the secretion of the amyloid precursor protein ectodomain. *Life Sci* 1997;**60**:985–991.
100. DeLapp N, Wu S, Belagaje R, et al. Effects of the M1 agonist xanomeline on processing of human beta-amyloid precursor protein (FAD, Swedish mutant) transfected into Chinese hamster ovary-m1 cells. *Biochem Biophys Res Commun* 1998;**244**:156–160.
101. Caccamo A, Oddo S, Billings LM, Green KN, Martinez-Coria H, Fisher A, LaFerla FM. M1 receptors play a central role in modulating AD-like pathology in transgenic mice. *Neuron* 2006;**49**:671–682.
102. Langmead CJ, Watson J, Reavill C. Muscarinic acetylcholine receptors as CNS drug targets. *Pharmacol Ther* 2008;**117**:232–243.
103. Fuentealba RA, Farias G, Scheu J, Bronfman M, Marzolo MP, Inestrosa NC. Signal transduction during amyloid-beta-peptide neurotoxicity: Role in Alzheimer disease. *Brain Res Brain Res Rev* 2004;**47**:275–289.
104. Bragin V, Chemodanova M, Dzhafarova N, Bragin I, Czerniawski JL, Aliev G. An integrated treatment approach improves cognitive function in demented and clinically depressed patients. *J Alzheimers Dis Other Dement* 2005;**20**:21–26.
105. Rozzini L, Chilovi BV, Conti M, Bertolotti E, Zanetti M, Trabucchi M, Padovani A. Efficacy of SSRIs on cognition of Alzheimer's disease patients treated with cholinesterase inhibitors. *Int Psychogeriatr* 2010;**22**:114–119.
106. Di Lazzaro V, Oliviero A, Tonali PA, et al. Noninvasive in vivo assessment of cholinergic cortical circuits in AD using transcranial magnetic stimulation. *Neurology* 2002;**13**:392–397.
107. Di Lazzaro V, Oliviero A, Pilato F, et al. Motor cortex hyperexcitability to transcranial magnetic stimulation in Alzheimer's disease. *J Neurol Neurosurg Psych* 2004;**75**:555–559.
108. Pierantozzi M, Panella M, Palmieri MG, et al. Different TMS patterns of intracortical inhibition in early onset Alzheimer dementia and frontotemporal dementia. *Clin Neurophysiol* 2004;**115**:2410–2418.
109. Kirvell SL, Esiri M, Francis PT. Down-regulation of vesicular glutamate transporters precedes cell loss and pathology in Alzheimer's disease. *J Neurochem* 2006;**98**:939–950.
110. Kashani A, Lepicard E, Poirel O, et al. Loss of VGLUT1 and VGLUT2 in the prefrontal cortex is correlated with cognitive decline in Alzheimer disease. *Neurobiol Aging* 2008;**29**:1619–1630.
111. Lipton SA. Pathologically-activated therapeutics for neuroprotection: Mechanism of NMDA receptor block by memantine and S-nitrosylation. *Curr Drug Targets* 2007;**8**:621–632.
112. Léveillé F, El Gaamouch F, Goux E, Lecocq M, Lobner D, Nicole O, Buisson A. Neuronal viability is controlled by a functional relation between synaptic and extrasynaptic NMDA receptors. *FASEB J* 2008;**22**:4258–4271.
113. Lau CG, Zukin RS. NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nat Rev Neurosci* 2007;**8**:413–426.
114. Zhang SJ, Steijaert MN, Lau D, Schütz G, Delucinge-Vivier C, Descombes P, Bading H. Decoding NMDA receptor signaling: Identification of genomic programs specifying neuronal survival and death. *Neuron* 2007;**53**:549–562.
115. van Marum RJ. Update on the use of memantine in Alzheimer's disease. *Neuropsychiatr Dis Treat* 2009;**5**:237–247.
116. Geerts H. Indicators of neuroprotection with galantamine. *Brain Res Bull* 2005;**64**:519–524.
117. Jones R, Sheehan B, Phillips P, et al.; DOMINO-AD team. DOMINO-AD protocol: Donepezil and memantine in moderate to severe Alzheimer's disease – A multicentre RCT. *Trials* 2009;**10**:57:1–11.