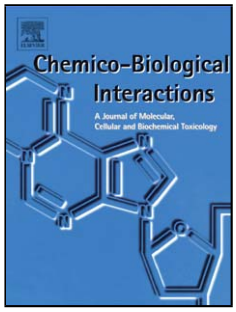
Accepted Manuscript



Title: Cholinesterase Inhibitors And Memory

Authors: Giancarlo Pepeu, Maria Grazia Giovannini

PII: S0009-2797(09)00489-X
DOI: doi:10.1016/j.cbi.2009.11.018
Reference: CBI 6061

To appear in: *Chemico-Biological Interactions*

Received date: 17-9-2009
Revised date: 17-11-2009
Accepted date: 18-11-2009

Please cite this article as: G. Pepeu, M.G. Giovannini, Cholinesterase Inhibitors And Memory, *Chemico-Biological Interactions* (2008), doi:10.1016/j.cbi.2009.11.018

CHOLINESTERASE INHIBITORS AND MEMORY

Giancarlo Pepeu and Maria Grazia Giovannini

Department of Pharmacology, University of Florence, Viale Pieraccini 6,

50139 Florence, Italy

Corresponding author:

Giancarlo Pepeu
Department of Pharmacology,
University of Florence,
Viale Pieraccini 6,
50139 Florence, Italy
Tel +39 0554271274
Fax +39 0554271280
GIANCARLO.PEPEU@UNIFI.IT

Abstract

A consensus exists that cholinesterase inhibitors (ChEIs) are efficacious for mild to moderate Alzheimer's Disease (AD). Unfortunately, the number of non-responders is large and the therapeutic effect is usually short-lasting. In experimental animals, ChEIs exert three main actions: inhibit cholinesterase (ChE), increase extracellular levels of brain acetylcholine (ACh), improve cognitive processes, particularly when disrupted in models of AD. In this overview we shall deal with the cognitive processes that are improved by ChEI treatment because they depend on the integrity of brain cholinergic pathways and their activation. The role of cholinergic system in cognition can be investigated using different approaches. Microdialysis experiments demonstrate the involvement of the cholinergic system in attention, working, spatial and explicit memory, information encoding, sensory-motor gating, skill learning. No involvement in long term memory has yet been demonstrated. Conversely, memory consolidation is facilitated by low cholinergic activity. Experiments on healthy human subjects, notwithstanding caveats concerning age, dose, and different memory tests, confirm the findings of animal experiments and demonstrate that stimulation of the cholinergic system facilitates attention, stimulus detection, perceptual processing and information encoding. It is not clear whether information retrieval may be improved but memory consolidation is reduced by cholinergic activation. ChEI effects in AD patients have been extensively investigated using rating scales that assess cognitive and behavioural responses. Few attempts have been made to identify which scale items respond better to ChEIs and therefore, presumably, depend on the activity of the cholinergic system. Improvement in attention and executive functions, communication, expressive language and mood stability have been reported. Memory consolidation and retrieval may be impaired by high ACh levels. Therefore, considering that in AD the degeneration of the cholinergic system is associated with alteration of other

neurotransmitter systems and a diffuse synaptic loss, a limited efficacy of ChEIs on memory processes should be expected.

1.*Introduction*

A consensus exists [1, 2], that the three cholinesterase inhibitors (ChEIs) donepezil, galantamine and rivastigmine are efficacious for mild to moderate Alzheimer's Disease (AD). Unfortunately, the number of non-responders is large [3, 4] and the therapeutic effect is usually short-lasting [5, 6]. The reasons of these limitations are not fully understood but it is assumed that they depend on the clinical conditions of the patients and the complexity of the cognitive processes in which the brain cholinergic system, which is the target of ChEIs [6], plays a crucial though partial role.

In this overview, we shall identify the cognitive processes subserved by the cholinergic system that may be impaired by the dysfunction of cholinergic neurons occurring in Alzheimer's Disease (AD) and Levy Bodies Dementia, and to some extent in normal aging. An improvement of these processes by treatment with ChEIs, aimed at restoring the cholinergic function, could therefore be expected.

The use of ChEIs was suggested as a tool to improve memory within the framework of the "Cholinergic hypothesis of geriatric memory dysfunction" proposed by Bartus et al 27 years ago [7]. This hypothesis was based on three groups of observations. i) The dysfunction of the forebrain cholinergic neurons in subjects affected by Alzheimer's disease [8] (see also [9]) and, to some extent, in normal aging [10], shown by the decrease in choline acetyltransferase (ChAT) activity. ii) The amnesic effects induced by antimuscarinic drugs, first among them scopolamine, mimicking the age-associated memory impairment [11]. iii) The improvement of cognitive deficits by physostigmine, the only ChEI that could be safely administered to man at the time of the hypothesis formulation [12]. The hypothesis postulated that, if the loss of memory occurring in AD as well as its decline in healthy older adults [13] depend on a cholinergic deficit, it should be possible to restore the memory impairment by compensating the cholinergic deficit. For this purpose tacrine, donepezil, rivastigmine and galantamine were developed and

marketed. These drugs inhibit acetylcholinesterase (AChE) and to some extent also butyrylcholinesterase (BuChE), with the exception of donepezil, which is selective for AChE [14], increase extracellular ACh levels in the brain of experimental animals [6] and restore the cognitive deficits observed in animal models of dementia (see ref in [15]). The increase in ACh extracellular levels, demonstrated by microdialysis studies in behaving rats after acute and chronic ChEI administration, is a direct evidence of enhanced cholinergic function [16, 17]. An activation of the cholinergic function also occurs in the periphery and is responsible for ChEI side effects, mostly gastrointestinal [3].

ChE inhibition was compared in rat and human neocortical tissue incubated *in vitro* with donepezil, rivastigmine and galantamine and similar IC50 were obtained in both species [18]. ChE inhibition has been measured in the CSF of subjects treated with ChEIs [19], and in the brain by PET, using as the substrate a radiolabeled ACh analogue [20, 21]. Although no direct evidence has yet been presented, we may assume, by analogy with animal experiments, that also in humans ChE inhibition is associated with an increase in ACh extracellular levels.

2. The role of the forebrain cholinergic system in cognition: animal studies.

Several experimental methods can be used for investigating the role of the cholinergic system in cognitive processes. They include the use of cholinergic antagonists, selective lesions of the cholinergic neurons by immunotoxins, nicotinic and muscarinic receptors knockout mice, stimulation of the cholinergic nuclei, and detection of the changes in extracellular ACh levels by microdialysis in specific brain areas during the performance of tasks involving cognitive processes [22]. In this section we shall confine ourselves to describe the relationships between cholinergic activity and cognitive processes, as studied using *in vivo* microdialysis. The extracellular ACh levels are an indication of the amount of ACh released by the firing of the cholinergic nerve terminals innervating specific brain areas during spontaneous behaviours or during acquisition and

performance of learned responses. It is therefore possible to identify, to some extent, which cognitive functions are accompanied and presumably subserved by modifications of the activity of the cholinergic neuronal systems [23]. Suppression of the behaviour or response by the administration of cholinergic receptor antagonists confirms that they depend on cholinergic activity [24].

When a rat is placed in a novel environment a large increase in extracellular ACh levels in the cerebral cortex and hippocampus, associated with active exploratory activity, is detected [25]. Not only a novel environment, but all novel situations activate the forebrain cholinergic system leading to increased ACh release both from the cerebral cortex and hippocampus, as shown in Table 1, irrespective whether they are accompanied by increased exploration or by fear-induced freezing.

Here TABLE 1

Tactile stimulation induces a larger increase of ACh release from the cortex than olfactory or auditory stimuli. Conversely, auditory, visual and tactile stimulations induce similar cholinergic activation in the hippocampus which, on the contrary, does not respond to olfactory stimuli [26]. A novel taste strongly stimulates the cholinergic neurons impinging to the insular cortex but has no effect on the cholinergic neurons of the parietal cortex [29]. These findings are consistent with the concept of functional specificity within the forebrain cholinergic neurons [30]. From these experiments it appears that the forebrain cholinergic system participates in arousal and attention induced by novelty and is involved in the analysis of the advantages and risks associated with the novel situation. Prolonged or repeated exposition to the environment or sensory stimulation leads to habituation, which is accompanied by small or no changes in brain ACh extracellular levels [27-29], because the novel information has been acquired and thus activation of the cholinergic system is no longer needed.

Attention and the associated activation of the cholinergic systems innervating the cerebral cortex and hippocampus are essential steps for the acquisition of new behaviours. As shown by Orsetti et al [31], acquisition of an operant behaviour, i.e. learning to obtain food by pressing a lever, is associated to a large increase in extracellular ACh levels in the frontal cortex and hippocampus, whereas similar cholinergic activation does not occur in rats already trained to press the lever to obtain food. Evidence of the role of cholinergic activation in information acquisition comes also from the observation that the acquisition of a passive avoidance response in the rat is accompanied by an increase in extracellular ACh levels associated with the activation of ERK (extracellular regulated protein kinase) in the prefrontal cortex and hippocampus of the rat. The finding that the acquisition can be blocked by systemic administration of scopolamine or intracerebral administration of ERK inhibitors confirms that the process is triggered by ACh release [32].

The involvement of the cortical cholinergic system in attention has been extensively investigated. By increasing the difficulty of an operant task a direct relationship between cortical ACh release and attentional demand was demonstrated in the rat [33]. In a further experiment [34] rats performing an operant task requiring sustained attention to obtain food reward showed a marked increase in cortical ACh release. If their ability to detect signals paired with the reward was impaired by infusing an NMDA receptor antagonist in the nucleus basalis, a further increase in ACh release was observed. The augmentation of the activation of the cholinergic nerve endings in the medial prefrontal cortex appears to be driven by “motivation” to maintain the performance under challenging conditions. “Motivation” may seem too much an anthropomorphic term for a rat behaviour but it has been shown that “anticipation” of a palatable reward is associated with a large increase in cortical ACh release [35]. The increase in cholinergic activity in the prefrontal cortex associated with attention, lasting minutes, seems to underlie and sustain a more rapid

cholinergic activation associated with cue detection. By the use of choline sensitive microelectrodes to measure cortical ACh release at a sub-second resolution, Parikh and collaborators [36] observed a transient increase in cholinergic activity (at the scale of seconds) occurring in the medial prefrontal cortex, but not in the motor cortex, when a rat detects a brief visual cue rewarded by food. When the cue was missed, the cholinergic transient was not observed. A low pre-cue cholinergic activity predicted a successful cue detection, a higher level predicted a miss. The cue-evoked cholinergic transients were superimposed over slower (at time-scale of minutes) changes in cholinergic activity. According to the authors, this finding is consistent with a model that assumes multiple cholinergic modules and a regulation of cholinergic activity in modality- and cortical area-specific manner. The results suggest that performance-related cholinergic activity manifests on multiple time scales.

The cholinergic system is also involved in spatial memory formation. A large cortical ACh increase is detected when rats alternate spontaneously in an Y maze [28] and an increase in hippocampal ACh release occurs during spontaneous alternation in a cross maze [37]. The increase in ACh is presumably induced by the novel environment represented by the maze. However, the cholinergic activation is crucial for the rat to determine its location in space [38] and alternate correctly, as demonstrated by the finding that spontaneous alternation is disrupted by cholinergic receptor blockade [39]. According to Anzalone et al [40], spontaneous alternation is accompanied not only by activation of the hippocampal cholinergic system but also by an intense and persistent increase in ACh release in retrospenial cortical area 29ab that integrates the spatial cues encoded by the hippocampus. Actually, it appears that the involvement of the cortical and hippocampal cholinergic systems depends on the complexity of the task. In an alternation task for food reward in an operant chamber, Hironaka et al [41] observed that during the acquisition of a delayed alternation there was an increase of ACh release in the prefrontal cortex, similar

to that occurring in an attentional task. Conversely, when the task was made easier using a cued alternation and thus involving only reference memory, the increase of ACh release occurred in the hippocampus. Fadda et al [42] showed that hippocampal ACh release progressively increased from 139% to 245% during the 12 days of radial-maze learning and the magnitude of change in ACh output was positively correlated with spatial memory performance, thus suggesting that changes in the functioning of these neurons are involved in spatial learning. The cholinergic system of dorsomedial striatum is not involved in place acquisition in a maze rewarded with food but ACh efflux selectively increases in the dorsomedial striatum, but not dorsolateral or ventromedial striatum during place reversal learning. The results reveal an essential role of the striatal cholinergic interneurons in cognitive flexibility [43].

Finally, the performance of spatial and operant tasks may require the activation of the cholinergic neurons in some brain regions and their inactivation in others. McIntyre et al [44] observed an increase in ACh release during a spontaneous alternation and an amygdala-dependent conditioned place preference tasks. However, the magnitude of hippocampal ACh release is negatively correlated with good performance in the conditioned place preference task, indicating a competition between the two cholinergic structures in this type of memory, and that the activation of the cholinergic system adversely affects the expression of an amygdala-dependent type of memory.

So far we described evidence showing the importance of the activation of the cholinergic system for attention, stimulus detection and analysis and information encoding. The question arises whether the cholinergic system plays also a role in memory consolidation and recall.

According to Hasselmo and McGaughy [45], low cortical levels of ACh, like those accompanying slow-wave sleep [46], are needed to set the appropriate dynamic for memory consolidation by reducing the afferent input and facilitating feedback connections.

This model of the cholinergic function was confirmed by Gais and Born in man [47]. These authors were able to block the consolidation of declarative memory for word pairs by physostigmine infusion (0.75 mg) during slow-wave rich sleep. In rats the retrieval of spatial information evaluated in a maze was disrupted by physostigmine administration. However, it cannot be excluded that the cholinesterase inhibitor, physostigmine, had boosted ACh action during a time when cholinergic levels need to decline for proper consolidation [48].

In conclusion, the animal experiments demonstrate that activation of the cholinergic system plays a role in maintaining the level of attention toward the environment and in detecting cues and creating the condition for a response. At the same time it makes possible the encoding of new information of various types, including spatial information. Conversely, memory consolidation is facilitated by low cholinergic activity. The role of the cholinergic system in information retrieval has not yet been defined.

3. ChEI effects on cognitive functions in human healthy subjects.

ChEIs have been administered to healthy subjects for two purposes, to investigate the role of brain cholinergic system in man, and to test their use as potential cognition enhancers [49]. The first demonstration that physostigmine infusion (1 mg in 1 hr) significantly enhanced information storage into long-term memory, evaluated as number of words remembered 80 min after infusion, was reported by Davis et al [50]. Short term memory was not improved. In a study by Wetherell [51] physostigmine (0.5 mg) induced a 25 – 30% ChE inhibition accompanied by an improvement of perception as well as, or instead of, memory, evaluated by a memory-scanning test. Furey et al [52], using functional magnetic resonance imaging (fMRI), investigated the mechanisms by which cholinergic enhancement improves working memory. They demonstrated that cholinergic enhancement, obtained by physostigmine infusion, improves memory performance,

assessed by a face recognition task, by augmenting the selectivity of perceptual processing during encoding, thereby simplifying processing demand during memory maintenance and reducing the need for prefrontal participation. According to an event-related fMRI investigation by Bentley et al [53], physostigmine administered to healthy adults appears to modulate both selective attention and emotional processes through independent, region-specific effects within the extrastriate cortex. Furthermore, cholinergic inputs to the frontoparietal cortex may influence the allocation of attention to emotional information. In agreement with animal studies [45], and with the work of Gais and Born [47], Kukolja et al [54] confirmed, using event-related fMRI, that activation of the cholinergic system by physostigmine infusion (1 mg/h) enhances neural activity associated with encoding but reduces neural activity associated with retrieval.

Improvement of perception and perceptual processing was also observed in healthy subjects treated with a single donepezil administration (5 mg) performing a task evaluating inspection time [55]. Improvement of performance was detected in pilots tested in a flight simulator after the administration of donepezil (5 mg) daily for 30 days. Donepezil, similarly to nicotine, showed the largest effects on flight tasks requiring sustained visual attention [56]. Apparently at variance with the previous studies, Gron [57] did not observe in healthy young subjects, receiving donepezil 5 mg daily for 30 days, an improvement in attentional, executive, as well as various memory functions—comprising short-term and working memory, semantic memory (priming) performances— but a selective enhancement of episodic memory performance. The same dose of donepezil given for 6 weeks to older adults (mean age 71.5 ± 5.2) improved immediate or delayed recall performance of semantically encoded words. Attention was not evaluated. More recently [58], in a double-blind, placebo controlled, parallel group design of cognitive effects of an acute oral dose of donepezil (5 mg), it has been demonstrated that positive effects of donepezil can be observed in various cognitive domains including mood, but its full nootropic potential,

including an improvement of performance in the central executive measure (backward digit span) occurred only at times close to its theoretical peak-plasma concentration (around 210 min after oral administration).

In conclusion, the relatively small number of trials on the effect of ChEIs on cognitive processes in normal humans described in this review are summarized in Table 2

Here TABLE 2

Differences in age of the subjects, memory functions tested, experimental conditions make it difficult to compare their results. However, they mostly confirm the

findings of animal experiments and demonstrate that stimulation of the cholinergic system facilitates attention, stimulus detection, perceptual processing and information encoding. In some experiments long-term memory also appears improved by ChEI administration. The effects can be observed also after repeated administrations and no non-responders are reported. It is not clear whether information retrieval may be improved but memory consolidation is definitely reduced by cholinergic activation. Whether the magnitude of the effects observed justifies ChEI use as memory enhancers in working activity, still needs to be convincingly demonstrated.

4. ChEI treatment on cognitive functions in AD patients

ChEI effects on cognitive deficits in AD patients have been extensively investigated using rating scales that assess cognitive and behavioural responses . Few attempts have been made to identify which items of the scales respond better to the treatment with ChEIs and therefore, presumably, depend on the activity of the cholinergic system. Lemstra et al [59] observed that the AD patients who responded better to 6 month treatment with rivastigmine were characterized by attentional deficits, combined with a cluster of behavioural symptoms including hallucinations, apathy, anxiety and psychomotor disturbances that the authors assumed to be the clinical profile of cholinergic deficiency.

According to Persson et al [5], using the Mini-Mental State Examination (MMSE) and the Alzheimer's Disease Assessment Scale – Cognitive subscale (ADAS-Cog), it is possible to evaluate three different cognitive domains. They can be defined as: 1) a general domain, including, among other, comprehension, concentration, naming; 2) a memory domain, including orientation, word recall, word recognition; 3) a spatial domain including attention, writing ability, constructional praxis. In a group of AD patients treated with donepezil for 36 months, only the general and spatial domains showed some improvement during the first 6 months of treatment whereas the memory domain score tended to drop more rapidly in the first 18 months of treatment, after which all three domains worsened equally. Bohnen et al, [60], measured by positron emission tomography the AChE inhibition in the cerebral cortex after 12 weeks donepezil (5 and then 10 mg) administration in subjects with mild AD, and assessed the cognitive performance with a battery of tests. AChE inhibition was rather low, on average 16 – 24%. Only the Stroop test, which evaluates attention and executive functions, showed an improvement correlated with AChE inhibition. Improvements in communication, expressive language, attention, and mood stability were noted by Hanyu et al [61] in all patients in the responder group after treatment with donepezil (5 mg) daily for 12- 16 weeks. The improvement, quantified by MMSE, inversely correlated with the thickness of the substantia innominata. In humans, this area is the site of a large part of the forebrain cholinergic neurons and its atrophy reflects their degeneration. This observation was recently confirmed [62] and supports the viewpoint of Venneri et al [20] that prolonged cholinergic enhancement appears beneficial only in states of reduced or altered cholinergic neurotransmission. Nevertheless, it remains unclear which cognitive processes are actually improved, besides those depending on attention and stimulus perception.

It has been demonstrated that the impairment of the cortical cholinergic inputs affects information processing and attentional function worsening neuropsychiatric

disorders [63]. The question whether ChEIs are also effective in the management of the behavioural and psychological symptoms of dementia (BPSD) in AD remains open. In a recent review, based on 14 studies with treatment lasting from 12 to 170 weeks, Rodda et al [64] conclude that in the absence of alternative safe and effective management options, the use of ChEIs is an appropriate pharmacological strategy for the management of BPSD. Pre-pulse inhibition (PPI) is considered a valid operational measure of information processing and attentional deficits in man, namely in schizophrenic subjects, and animals [65]. The finding that rivastigmine restores ACh levels and PPI in rats with a lesion of the cholinergic pathways [66] supports the hypothesis that the cholinergic deficits in AD may be also responsible of the BDSD and offer a rational to their treatment with ChEIs.

5. Conclusions

A recent editorial on ChEIs concluded that “few drugs with such a modest clinical effect have elicited such controversial and emotional responses” [67] and, we may add, have caused so much investigation in order to understand and possibly overcome the limits of their effects. Aim of this overview was to define which cognitive processes are subserved by the cholinergic system and therefore may respond to ChEI treatment. The complex functions of the cholinergic system have clearly emerged. The activation of the cholinergic neurons induced by novel environment and stimuli occurs at different time scales, seconds and minutes, [36] with differences between cortical areas and balances between brain regions such as hippocampus and amygdala [30, 44]. Memory consolidation seems to require low cortical cholinergic activity [45]. Under these conditions, blocking the hydrolysis of ACh with ChEIs and, and thus uniformly and constantly elevating extracellular ACh levels, may improve or restore only some cognitive processes and therefore has a limited clinical usefulness . Most investigations in animal and humans demonstrate, in agreement with Frankfort et al [68], that attention responds significantly and favourably to ChEIs and sharpening the attention may play a mediating

role in other cognitive domains. Animal studies and, to some extent, work on normal subjects demonstrate a facilitation of information encoding [55-57]. Whether ChEIs improve memory formation and recall has not been convincingly proved. If we consider that in AD the degeneration of the cholinergic system is associated with alteration of other neurotransmitter systems [69] and a diffuse loss of synapses [70], a limited efficacy of ChEIs on memory processes should be expected.

References

[1] Giacobini E, Cholinesterase in human brain: the effect of cholinesterase inhibitors on Alzheimer's disease and related disorders in: Giacobini E, Pepeu G (Eds.), The Brain Cholinergic System in Health and Disease, Informa Healthcare, Abingdon, 2006, pp. 235-265,

[2] J. Birks, Cholinesterase inhibitors for Alzheimer's disease, Cochrane. Database. Syst. Rev.2006) CD005593.

[3] K. L. Lanctot, N. Herrmann, K. K. Yau, L. R. Khan, B. A. Liu, M. M. LouLou and T. R. Einarson, Efficacy and safety of cholinesterase inhibitors in Alzheimer's disease: a meta-analysis, CMAJ. 169 (2003) 557-564.

[4] R. Raschetti, M. Maggini, G. C. Sorrentino, N. Martini, B. Caffari and N. Vanacore, A cohort study of effectiveness of acetylcholinesterase inhibitors in Alzheimer's disease, Eur. J. Clin. Pharmacol. 61 (2005) 361-368.

[5] C. M. Persson, A. K. Wallin, S. Levander and L. Minthon, Changes in cognitive domains during three years in patients with Alzheimer's disease treated with donepezil, BMC. Neurol. 9 (2009) 7.

[6] G. Pepeu and M. G. Giovannini, Cholinesterase inhibitors and beyond, Curr. Alzheimer Res. 6 (2009) 86-96.

[7] R. T. Bartus, R. L. Dean, B. Beer and A. S. Lippa, The cholinergic hypothesis of geriatric memory dysfunction, Science 217 (1982) 408-417.

[8] P. Davies and A. J. R. Maloney, Selective loss of central cholinergic neurons in Alzheimer's disease, The Lancet 2 (1976) 1403-1405.

[9] P. T. Francis, A. M. Palmer, M. Snape and G. K. Wilcock, The cholinergic hypothesis of Alzheimer's disease: a review of progress, J Neurol. Neurosurg. Psychiatry 66 (1999) 137-147.

[10] E. K. Perry, M. Johnson, J. M. Kerwin, M. A. Piggott, Court JA, P. J. Shaw, P. G. Ince, A. Brown and R. H. Perry, Convergent cholinergic activities in aging and Alzheimer's disease, Neurobiol. Aging 13 (1992) 393-400.

[11] D. A. Drachman and J. Leavitt, Human memory and the cholinergic system: A relationship to aging?, Arch. Neurol. 30 (1974) 113-121.

[12] K. L. Davis, L. E. Hollister, J. Overall, A. Johnson and K. Train, Physostigmine: effects on cognition and affect in normal subjects, Psychopharmacology (Berl) 51 (1976) 23-27.

[13] P. A. Balota, P. O. Dolan and J. M. Duchek, Memory changes in healthy older adult in: E. Tulving, G. I. M. Craik (Eds.), The Oxford Handbook of Memory, Oxford University Press, Oxford, 2000, pp. 395-409,

[14] M. Weinstock, Selectivity of Cholinesterase Inhibition. Clinical Implications for the Treatment of Alzheimer's Disease, CNS Drugs 12 (1999) 307-323.

[15] Pepeu G, Preclinical pharmacology of cholinesterase inhibitors in: Giacobini E (Eds.), *Cholinesterases and Cholinesterase Inhibitors*, Martin Dunitz, London, 2000, pp. 145-155,

[16] C. Scali, M. G. Giovannini, L. Bartolini, C. Prosperi, V. Hinz, B. H. Schmidt and G. Pepeu, Effect of metrifonate on extracellular brain acetylcholine levels and object recognition in aged rats, Eur J Pharmacol 325 (1997) 173-180.

[17] M. G. Giovannini, C. Scali, L. Bartolini, B. H. Schmidt and G. Pepeu, Effect of subchronic treatment with metrifonate and tacrine on brain cholinergic function in aged F334 rats, European Journal of Pharmacology 354 (1998) 17-24.

[18] R. Jakisch, S. Forster, M. Kemmerer, A. K. Rothmaler, A. Ehret, J. Zenter and T. J. Feuerstein, Inhibitory potency of choline esterase inhibitors on acetylcholine release and choline esterase activity in fresh specimens of human and rat neocortex, J. Alzheimers. Dis. 16 (2009) 635-647.

[19] A. Nordberg, T. Darreh-Shori, E. Peskind, H. Soininen, M. Mousavi, G. Eagle and R. Lane, Different cholinesterase inhibitor effects on CSF cholinesterases in Alzheimer patients, Curr. Alzheimer Res. 6 (2009) 4-14.

[20] A. Venneri, Imaging treatment effects in Alzheimer's disease, Magn Reson. Imaging 25 (2007) 953-968.

[21] V. Kaasinen, K. Nagren, T. Jarvenpaa, A. Roivainen, M. Yu, V. Oikonen, T. Kurki and J. O. Rinne, Regional effects of donepezil and rivastigmine on cortical acetylcholinesterase activity in Alzheimer's disease, J. Clin. Psychopharmacol. 22 (2002) 615-620.

[22] G. Pepeu and M. G. Giovannini, The role of cholinergic system in cognitive processes in: Giacobini E, Pepeu G (Eds.), Brain Cholinergic Mechanisms, Tailor & Francis, Oxford, 2006, pp. 221-233,

[23] G. Pepeu and M. G. Giovannini, Changes in acetylcholine extracellular levels during cognitive processes, Learn. Mem. 11 (2004) 21-27.

[24] Pepeu G. and Giovannini M.G, Acetylcholine: I Muscarinic receptors in: Riedel G, Platter D (Eds.), From Messengers to Molecules: Memories Are Made of These , Plenum Press, New York, 2004, pp. 90-112,

[25] M. G. Giovannini, A. Rakovska, R. S. Benton, M. Pazzagli, L. Bianchi and G. Pepeu, Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats, Neuroscience 106 (2001) 43-53.

[26] F. M. Inglis and H. C. Fibiger, Increases in hippocampal and frontal cortical acetylcholine release associated with presentation of sensory stimuli, Neuroscience 66 (1995) 81-86.

[27] E. Acquas, C. Wilson and H. C. Fibiger, Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: effects of novelty, habituation, and fear, J Neurosci. 16 (1996) 3089-3096.

[28] M. G. Giovannini, L. Bartolini, S. R. Kopf and G. Pepeu, Acetylcholine release from the frontal cortex during exploratory activity, Brain Res 784 (1998) 218-227.

[29] M. I. Miranda, L. Ramirez-Lugo and F. Bermudez-Rattoni, Cortical cholinergic activity is related to the novelty of the stimulus, Brain Res. 882 (2000) 230-235.

[30] G. N. Fournier, K. Semba and D. D. Rasmusson, Modality- and region-specific acetylcholine release in the rat neocortex, Neuroscience 126 (2004) 257-262.

[31] M. Orsetti, F. Casamenti and G. Pepeu, Enhanced acetylcholine release in the hippocampus and cortex during acquisition of an operant behavior, Brain Res 724 (1996) 89-96.

[32] M. G. Giovannini, M. Pazzagli, P. Malmberg-Aiello, C. L. Della, A. D. Rakovska, F. Cerbai, F. Casamenti and G. Pepeu, Inhibition of acetylcholine-induced activation of extracellular regulated protein kinase prevents the encoding of an inhibitory avoidance response in the rat, Neuroscience 136 (2005) 15-32.

[33] A. M. Himmelheber, M. Sarter and J. P. Bruno, Increases in cortical acetylcholine release during sustained attention performance in rats, Brain Res Cogn Brain Res 9 (2000) 313-325.

[34] R. Kozak, J. P. Bruno and M. Sarter, Augmented prefrontal acetylcholine release during challenged attentional performance, Cereb. Cortex 16 (2006) 9-17.

[35] F. M. Inglis, J. C. Day and H. C. Fibiger, Enhanced acetylcholine release in hippocampus and cortex during the anticipation and consumption of a palatable meal, Neuroscience 62 (1994) 1049-1056.

[36] V. Parikh, R. Kozak, V. Martinez and M. Sarter, Prefrontal acetylcholine release controls cue detection on multiple timescales, Neuron 56 (2007) 141-154.

[37] M. E. Ragozzino, S. N. Pal, K. Unick, M. R. Stefani and P. E. Gold, Modulation of hippocampal acetylcholine release and spontaneous alternation scores by intrahippocampal glucose injections, J. Neurosci. 18 (1998) 1595-1601.

[38] E. S. Brazhnik, R. U. Muller and S. E. Fox, Muscarinic blockade slows and degrades the location-specific firing of hippocampal pyramidal cells, J. Neurosci. 23 (2003) 611-621.

[39] L. Bartolini, R. Risaliti and G. Pepeu, Effect of scopolamine and nootropic drugs on rewarded alternation in a T-maze, Pharmacol Biochem Behav 43 (1992) 1161-1164.

[40] S. Anzalone, J. Roland, B. Vogt and L. Savage, Acetylcholine efflux from retrosplenial areas and hippocampal sectors during maze exploration, Behav. Brain Res. 201 (2009) 272-278.

[41] N. Hironaka, K. Tanaka, Y. Izaki, K. Hori and M. Nomura, Memory-related acetylcholine efflux from rat prefrontal cortex and hippocampus: a microdialysis study, Brain Res. 901 (2001) 143-150.

[42] F. Fadda, S. Cocco and R. Stancampiano, Hippocampal acetylcholine release correlates with spatial learning performance in freely moving rats, NeuroReport 11 (2000) 2265-2269.

[43] M. E. Ragozzino, E. G. Mohler, M. Prior, C. A. Palencia and S. Rozman, Acetylcholine activity in selective striatal regions supports behavioral flexibility, Neurobiol. Learn. Mem. 91 (2009) 13-22.

[44] C. K. McIntyre, S. N. Pal, L. K. Marriott and P. E. Gold, Competition between memory systems: acetylcholine release in the hippocampus correlates negatively with good performance on an amygdala-dependent task, J. Neurosci. 22 (2002) 1171-1176.

[45] M. E. Hasselmo and J. McGaughy, High acetylcholine levels set circuit dynamics for attention and encoding and low acetylcholine levels set dynamics for consolidation, Prog. Brain Res. 145 (2004) 207-231.

[46] G. G. Celesia and H. H. Jasper, Acetylcholine released from cerebral cortex in relation to state of activation, Neurology 16 (1966) 1053-1063.

[47] S. Gais and J. Born, Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation, Proc. Natl. Acad. Sci. U. S. A 101 (2004) 2140-2144.

[48] J. L. Rogers and R. P. Kesner, Cholinergic modulation of the hippocampus during encoding and retrieval, Neurobiol. Learn. Mem. 80 (2003) 332-342.

[49] C. Lanni, S. C. Lenzken, A. Pascale, V. Del, I, M. Racchi, F. Pistoia and S. Govoni, Cognition enhancers between treating and doping the mind, Pharmacol. Res. 57 (2008) 196-213.

[50] K. L. Davis, R. C. Mohs, J. R. Tinklenberg, A. Pfefferbaum, L. E. Hollister and B. S. Kopell, Physostigmine: improvement of long-term memory process in normal humans, Science 201 (1978) 272-274.

[51] A. Wetherell, Effects of physostigmine on stimulus encoding in a memory-scanning task, Psychopharmacology (Berl) 109 (1992) 198-202.

[52] M. L. Furey, P. Pietrini and J. V. Haxby, Cholinergic enhancement and increased selectivity of perceptual processing during working memory, Science 290 (2000) 2315-2319.

[53] P. Bentley, P. Vuilleumier, C. M. Thiel, J. Driver and R. J. Dolan, Cholinergic enhancement modulates neural correlates of selective attention and emotional processing, Neuroimage. 20 (2003) 58-70.

[54] J. Kukolja, C. M. Thiel and G. R. Fink, Cholinergic stimulation enhances neural activity associated with encoding but reduces neural activity associated with retrieval in humans, J. Neurosci. 29 (2009) 8119-8128.

[55] C. W. Hutchison, P. J. Nathan, L. Mrazek and C. Stough, Cholinergic modulation of speed of early information processing: the effect of donepezil on inspection time, Psychopharmacology (Berl) 155 (2001) 440-442.

[56] M. S. Mumenthaler, J. A. Yesavage, J. L. Taylor, R. O'Hara, L. Friedman, H. Lee and H. C. Kraemer, Psychoactive drugs and pilot performance: a comparison of nicotine, donepezil, and alcohol effects, Neuropsychopharmacology 28 (2003) 1366-1373.

[57] G. Gron, M. Kirstein, A. Thielscher, M. W. Riepe and M. Spitzer, Cholinergic enhancement of episodic memory in healthy young adults, Psychopharmacology (Berl) 182 (2005) 170-179.

[58] A. L. Zaninotto, O. F. Bueno, M. Pradella-Hallinan, S. Tufik, J. Rusted, C. Stough and S. Pompeia, Acute cognitive effects of donepezil in young, healthy volunteers, Hum. Psychopharmacol. 24 (2009) 453-464.

[59] A. W. Lemstra, K. J. Kalisvaart, R. Vreeswijk, W. A. van Gool and P. Eikelenboom, Pre-operative inflammatory markers and the risk of postoperative delirium in elderly patients, Int. J. Geriatr. Psychiatry 23 (2008) 943-948.

[60] N. I. Bohnen, D. I. Kaufer, R. Hendrickson, L. S. Ivanco, B. J. Lopresti, R. A. Koeppe, C. C. Meltzer, G. Constantine, J. G. Davis, C. A. Mathis, S. T. DeKosky and R. Y. Moore, Degree of inhibition of cortical acetylcholinesterase activity and cognitive effects by donepezil treatment in Alzheimer's disease, J. Neurol. Neurosurg. Psychiatry 76 (2005) 315-319.

[61] H. Hanyu, T. Asano, H. Sakurai, Y. Tanaka, M. Takasaki and K. Abe, MR analysis of the substantia innominata in normal aging, Alzheimer disease, and other types of dementia, AJNR Am. J. Neuroradiol. 23 (2002) 27-32.

[62] H. Kanetaka, H. Hanyu, K. Hirao, S. Shimizu, T. Sato, T. Akai, T. Iwamoto and K. Koizumi, Prediction of response to donepezil in Alzheimer's disease: combined MRI analysis of the substantia innominata and SPECT measurement of cerebral perfusion, Nucl. Med. Commun. 29 (2008) 568-573.

[63] M. Sarter, J. P. Bruno and J. Turchi, Basal forebrain afferent projections modulating cortical acetylcholine, attention, and implications for neuropsychiatric disorders, Ann. N. Y. Acad. Sci. 877 (1999) 368-382.

[64] J. Rodda, S. Morgan and Z. Walker, Are cholinesterase inhibitors effective in the management of the behavioral and psychological symptoms of dementia in Alzheimer's disease? A systematic review of randomized, placebo-controlled trials of donepezil, rivastigmine and galantamine, Int. Psychogeriatr. 21 (2009) 813-824.

[65] M. A. Geyer and D. L. Braff, Startle habituation and sensorimotor gating in schizophrenia and related animal models, Schizophr. Bull. 13 (1987) 643-668.

[66] M. Ballmaier, F. Casamenti, C. Scali, R. Mazzoncini, M. Zoli, G. Pepeu and P. F. Spano, Rivastigmine antagonizes deficits in prepulse inhibition induced by selective immunolesioning of cholinergic neurons in nucleus basalis magnocellularis, Neuroscience 114 (2002) 91-98.

[67] J. Rodda and Z. Walker, Ten years of cholinesterase inhibitors, Int. J. Geriatr. Psychiatry 24 (2009) 437-442.

[68] S. V. Frankfort, B. A. Appels, A. de Boer, L. R. Tulner, J. P. van Campen, C. H. Koks, J. H. Beijnen and B. A. Schmand, Identification of responders and reactive domains to rivastigmine in Alzheimer's disease, Pharmacoepidemiol. Drug Saf 16 (2007) 545-551.

[69] J. Hardy, J. Adolfsson, I. Alafuzoff, J. Marcusson, P. Nyberg, E. Perdahl, P. Wester and B. Winblad, Transmitter deficits in Alzheimer's disease, Neurochemistry International 7 (1985) 345-363.

[70] T. Arendt, Synaptic degeneration in Alzheimer's disease, Acta Neuropathol. 118 (2009) 167-179.

TABLE 1. NOVELTY ACTIVATES THE FOREBRAIN CHOLINERGIC SYSTEM AS SHOWN BY THE INCREASE IN EXTRACELLULAR ACh IN THE RAT.

Novel Stimulus	Brain Area	% ACh increase	Ref
Auditory, olfactory, tactile	Cortex	200 -250 (tactile > olfactory = auditory)	[26]
Auditory, visual, tactile	Hippocampus	160 - 200 (auditory = visual = tactile)	[26]
Paired tone and light	Cortex Hippocampus	250 200	[27]
Novel environment	Cortex	150 - 200	[28]
Novel taste	Insular cortex	500	[29]

TABLE 2. ChEIs EFFECTS ON COGNITIVE FUNCTIONS IN HUMAN HEALTHY SUBJECTS.

Drug	Dose mg iv	Improved cognitive function	Test used	REF
Physostigmine	1.0	Long term memory	Verbal learning tasks	50
Physostigmine	0.5 + 0.6	Perceptual processes	Memory scanning test	51
Physostigmine Glycopyrrolate	1.0/h 0.2	Perceptual processing during encoding	Visual working memory for faces task + fMRI	52
Physostigmine Glycopyrrolate	1.0/h 0.2	Selective attention and emotional processes	Matching task: reaction time and accuracy + fMRI	53
Physostigmine Glycopyrrolate	1.0/h 0.2	Spatial encoding	Spatial source memory task + fMRI	54
Donepezil	5.0 po	Inspection time	Visual discrimination	55
Donepezil	5.0 po 30 d	Sustained visual attention	Flight-performance tasks	56
Donepezil	5.0 po 30 d	Verbal and visual episodic memory, long-term visual episodic recall	Extensive neuropsycho-logical test battery	57
Donepezil	5.0 po	Long term recall of prose, object, spatial location, mood	Visual spatial tasks, object relocation task, memory test, digit span, mood rating scale	58