Innovative cholinergic compounds for the treatment of cognitive dysfunction

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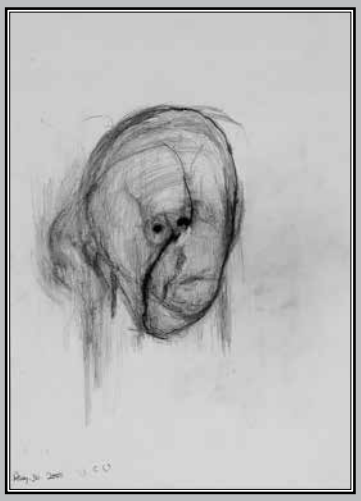
CHAPTER VIII



BIOMARKERS FOR THE EFFECTS OF CHOLINERGIC DRUGS IN THE CENTRAL NERVOUS SYSTEM IN HEALTHY SUBJECTS

Submitted to British Journal of Clinical Pharmacology

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ABSTRACT

Novel therapeutic agents targeting the central cholinergic system are under development. In early phase development studies in healthy volunteers biomarkers are used to proof pharmacology and determine the optimal dose level for further development. There is no consensus, however, on which biomarkers are most useful. This review provided an overview of biomarkers used to investigate effects of pro- and anticholinergic drugs in healthy subjects and their ability to detect drug effects was evaluated. In total 132 useful articles were included, comprising 223 individual tests. The most prominent effects were found for muscarinic receptor antagonists, which produced consistent deteriorations in learning and memory tests in 69% to 79% of the cases, in general dose related, and less consistent reductions in alertness (56% of the cases). Fewer tests were able to demonstrate effects of nicotinic receptor antagonists on learning and memory (36% to 50% of the cases). Nicotinic receptor agonist produced moderate improvements (up to 32% of the cases). By themselves, cholinesterase inhibitors did not produce reliable effects on any test in healthy volunteers. However, the well measurable temporary effects of anti-cholinergic drugs could be used as pharmacological challenge in healthy subjects, in order to demonstrate pharmacological activity of pro-cholinergic drugs.

INTRODUCTION

The cholinergic system is involved in a wide range of central nervous system (CNS) activities. It comprises neurons that are activated by or contain and release the neurotransmitter acetylcholine. Acetylcholine is produced by neurons in the synaptic bud and released from vesicles into the synaptic cleft where it binds to acetylcholine receptors. These receptors can be divided into two classes: the nicotinic acetylcholine receptors and the muscarinic acetylcholine receptors. The nicotinic receptor consists of 5 subunits that can be classified as α (α_2-α_7, α_9 and α_{10}) or β (β_2-β_4), which can be combined in a heteromeric and homomeric way. The nicotinic receptors that are most present in the brain are $\alpha_4\beta_2$ and the α_7 subunit combinations[1]. The $\alpha_4\beta_2$ receptors are widely distributed throughout the brain, however the highest density is in the thalamus, intermediate density in the basal ganglia and brain stem, and are slightly lower in the cortical regions. Also the α_7 receptor subunits are widely distributed in all brain areas, although a higher concentration is found in the cerebral cortex and putamen and a lower concentration in the caudate and cerebellum[1]. The muscarinic receptors can be divided in five subtypes, M_1-M_5. The M_1 receptor is the predominant muscarinic receptor in the brain with a high density in the hippocampus and cortex[2,3]. These brain structures are involved in memory and learning[4,5]. M_2 receptors are mainly expressed in the occipital cortex, dorsal side of the caudate nucleus, putamen and brain stem[2,3]. The expression of the M_3 receptors in the brain is low, this subtype is mainly present in the peripheral autonomic nervous system[3]. The M_4 receptor is highly expressed in neocortex and in the striatum where it modulates dopaminergic neurotransmission and to a lower extent in the occipital region of the cortex [2, 3, 6, 7]. M_5 receptors are present at a low level in the outermost layer of the cortex, hippocampus, striatum and superior and inferior colliculi. Their presence on the dopaminergic neurons of the ventral tegmental area mediates a key role in the mesolimbic reward pathway[8].

Acetylcholine is removed from the synaptic cleft in less than a millisecond through diffusion and degradation by the enzyme acetylcholinesterase[9]. Inhibition of cholinesterase increases the availability of the neurotransmitter in the synaptic cleft and consequently the duration of transmitter action.

Disturbance of the cholinergic system have been found in a.o. Alzheimer's disease, Lewy body disease (including Parkinson's disease, dementia with Lewy bodies and Parkinson's disease dementia), and schizophrenia. In these diseases, cognitive dysfunction due to cholinergic deficits is an important symptom starting either in early or later phase of the disease. The current treatment to improve the cholinergic balance is only symptomatic. In Alzheimers disease, dementia with Lewy bodies

and Parkinson's disease dementia cholinesterase inhibitors galantamine, rivistigmine and donepezil are prescribed. The efficacy of these drugs is limited and therefore there is room for improvement. Multiple new pro-cholinergic compounds are under development, targeting acetylcholinesterase, muscarinic receptors (mainly selective for the M_1 and/or M_4 subtypes) and nicotinic receptors (mainly selective for the $\alpha 7$ and $\alpha 4\beta 2$ subtypes)[10-12]. In patients with schizophrenia, treatment with cholinesterase inhibitors donepezil and rivastigmine showed no significant improvement in cognition[13,14] and galantamine treatment resulted only in temporary improvement of social memory[15]. Therefore treatment affecting the cholinergic system does not belong to the standard of care for schizophrenics, however, development of new therapeutics for this disease targeting the cholinergic system is ongoing[16].

Development of new medicines targeting the central nervous system is a long and expensive trajectory with high failure rates, of which 30% is caused by a lack of efficacy[17]. To reduce attrition rates, there is need to carry out proof-of-concept clinical trials in early phase of development. In these trials, biomarkers are used to demonstrate acute drug effects and dose/concentration-effect relationships that can support the proof of pharmacology. Considering the widespread distribution of nicotinic and cholinergic targets in different CNS-networks, a large variety of functional test can be used to demonstrate effects of cholinergic agonists or antagonists. This large choice complicates the selection of useful tests in early development studies.

The current review aims to identify the most useful types of tests, by providing an overview and an evaluation of the extensive literature that described the effects of biomarkers for CNS-active pro- and anti-cholinergic drugs in healthy subjects.

METHODS

STRUCTURED LITERATURE EVALUATION An overview of registered drugs affecting the cholinergic system was found on drugbank.ca. Only compounds approved by the regulatory agencies that are able to pass the blood brain barrier and thus can affect the cholinergic system in the central nervous system were selected. As it has to be certain that the compound is effective in order to be able to assess the effectiveness of a biomarker, no experimental compounds were included in this review. An overview is shown in Table 1. The compounds were grouped based on target receptor or enzyme.

To date, there are no approved drugs that selectively stimulate or positively modulate muscarinic receptors. To our best knowledge, the results of seven muscarinic agonists/positive allosteric modulators (PAMS) investigating trials in healthy humans have been published in full text or abstract form. No PD effects were investigated or observed, or no PD effects have been published in three of these compounds (NGX267, VU319, TAK-071). PD effects of GSK1034702 were only demonstrated in a challenge model. The remaining three compounds (xanomeline, MK-7622, HTL0018318) showed PD effects in healthy subjects, however, in addition to our requirement that a drug has to be approved, not enough data were available to draw a conclusion on the effect of muscarinic agonists on biomarkers. Therefore these were not included in this formal review.

The literature search was performed in PubMed up to 15 January 2020 using the following keywords: '[name of cholinergic drug] healthy' All searches were limited to humans, and in case of more than 1000 results also limited to clinical studies (article type). The results were manually scanned for:

- Administration of compounds in healthy subjects
- Administration of a known dose
- Being an original investigation
- Measurement of pharmacodynamics effects

Both studies investigating single doses and multiple doses were included. Specific interactions of compounds, in particular with age, personality features, challenge models, other drugs or nicotine addiction were not considered in this review, and MRI-studies or studies in animals were excluded.

The study characteristics and each individual test result were put into a database (Microsoft Excel). The following items were recorded: number of subjects exposed to the compound and included in the analyses of acute effects, sex (male; female), age, blinding (double blind; single blind; open; unknown), design (crossover; parallel; unknown), drug name, dose, route of administration and test name, as well as test cluster and functional domain as explained below. The subdivision of tests and effect scores were initially performed by one author and 10% of the manuscripts was checked by another author. The total number of evaluated tests (cases) was a product of the number of articles, drugs, doses and tests.

INDIVIDUAL TEST RESULTS The actual results of tests could not be recorded quantitatively, due to large the diversity of methods, outcome variables and treatments. Therefore, the results were scored as + (significant improvement/increase),=(no significant effect) or – (significant impairment/decrease) per outcome variable, compared with placebo or baseline. Although statistical significance is dependent on several factors such as test variability and group size, this approach at least allows an evaluation of the applicability of a test as a biomarker. No efforts were made to further quantify the overall level of statistical significance. The

different outcome variables of a single test were grouped together, if they provided information on the same cluster. When multiple dose levels were tested within a single study, and the test outcome of the dose levels showed conflicting but statistically significant responses, the items were separately scored for each dose level. When a certain outcome variable in a task from one cluster improved, while another outcome variable within the same task deteriorated, both items were scored separately within the different clusters. If studies described tests in the methods sections, but the results were not presented without a clear reason (*eg* publication elsewhere), we included these tests and assumed that they had shown no significant effects.

CLUSTERING OF INDIVIDUAL TEST RESULTS Since this review intended to identify generally applicable biomarkers, results from tests that were used only once or by one research group were not individually analysed. Such tests were grouped ('clustered') with other comparable tests. The first step in this process included grouping of tests that could be regarded as variants from a basic form into a single cluster, using compendiums of neuropsychological tests (ref). Single tests could include different outcome variables that measure various functions (e.g. memory, executive function) and can therefore provide information on different clusters. Subsequently, tests and clusters were grouped into domains.

TEST CRITERIA Ideally, a good biomarker for activity of a drug class should meet the following criteria:

- a clear, consistent response across studies and drugs from the same class;
- a clear response of the biomarker to therapeutic doses;
- a dose (concentration)–response relationship; and
- a plausible relationship between the function addressed with the biomarker, the pharmacological activity of the drug class and the pathogenesis of the therapeutic area.

Previously, these criteria were used to evaluate the usefulness of biomarkers for the effects of antipsychotic drugs, benzodiazepines, selective serotonin reuptake inhibitors, and 3,4-methylene-dioxy-methamphetamine (ecstasy)[18-21]. These criteria are also applied in the current review to evaluate the biomarkers.

DOSE-EFFECT RELATIONSHIPS A clear increase of an effect with dose provides strong support for the usefulness of a test as a biomarker of pharmacological activity. To investigate this , for the most frequently used tests and drug dosages it was determined whether the number of statistically significant results increased with dose. To this end, drug doses were pooled into 'lower', 'medium' and higher' dosages (Table 2). The 'medium' dose was determined as the range between the lowest recommended therapeutic starting dose and halfway the highest recommended clinical maintenance dose[22]. The 'lower' and 'higher' doses were all dosages below or above this level.

STATISTICAL EVALUATION All data processing steps and calculations were performed using R software for Statistical Computing (version R 4.0.3). In order to calculate the average responses with confidence intervals for binomial proportions, responses were coded as follows: Impairment/decrease was coded as 0, no change was coded as 0.5 and improvement/increase was coded as 1. A cumulated response code was calculated by multiplying the number of occurrences for each response by the coding and adding this over the three responses. A proportion was calculated by dividing the cumulated response code by the total number of responses. This resulted in an average response between 0 (impairment/decrease) and 1 (improvement/increase) for which two-sided 95% exact (Clopper-Pearson) confidence intervals for binomial proportions were calculated.

RESULTS

LITERATURE In total 132 studies were included; 38 trials investigated cholinesterase inhibitors[23-60], 41 trials studied nicotinic receptor agonists[61-101], 13 studies used nicotinic receptor antagonists[88,102-113] and 54 trials investigated muscarinic receptor antagonists[23-25,56,102-105,108,110,111,113-155]. In 13 studies more than one drug class was investigated and in 13 studies more than one dose was administered. Characteristics of these studies are provided in Table 3. Across all studies 16 different study designs were used.

In total 223 tests were used, which were grouped into 69 clusters. Subsequently the tests and clusters were grouped into 9 domains (Table 4). An overview of the effects on each individual test in each study is shown in Suppl table S1a-d.

TESTS In the 38 studies investigating cholinesterase inhibitors, 99 unique tests were used. Of these, 11 tests were used more than 5 times (Table S2). Only the verbal learning task was used more than 10 times and showed an improvement in 2 cases, no significant effect in 7 cases and an impairment in 1 case (Table S2).

Nicotinic receptor agonists were investigated using 77 individual measurements of which the saccadic and anti-saccadic eye movements were used the most (both n=5). The anti-saccadic eye movements were improved in 3 cases, the saccadic eye movements was improved in 1 case Impairments were not observed.

In the 13 studies investigating nicotinic receptor antagonists 50 individual tests were used. The n-back test (n=6) and pupil size (n=5) were used most frequently. In the majority of the cases no effect on these test could be demonstrated. The n-back tests was impaired once and the pupil size increased once.

Also in the 54 papers studying muscarinic receptor antagonists many different tests were used (n=117). However, there seemed to be less variety as 18 tests were used more than 5 times and of these 6 tests were used more than 10 times (Table S3). These 6 tests (Simple reaction time, Digit span, N-back, Critical flicker fusion test, Verbal learning task and visual analogue scale (VAS) according to Bond and Lader) were able to show an impairment in 18% (Digit span) to 90% (Verbal learning task) of the cases. An improvement was only observed once (n-back test) and in the other cases, no effect was observed.

CLUSTERS Although many different tests were used to evaluate the effect of each drug class, most tests were not used frequently enough for further analysis. Therefore, the tests were grouped into clusters. In table 5, 14 clusters are presented which were used most frequently across all drug classes.

- **Cholinesterase inhibitors**

In the majority of the clusters, (71-100% of the cases) no effect was observed. The improvements and impairments that were shown occurred in a maximum of 18% of the cases and were inconsistent within almost each cluster (Tabel 5, Figure S4). A higher percentages of improvements (27%) and deteriorations (18%) were observed within the cluster Focused/selective attention, however, these were inconsistent.

- **Nicotinic receptor agonists**

Inhibition was improved in 32% of the cases, Sustained attention showed an improved in 30% of the cases and Scale alertness was impaired in 33% of the cases. In the other clusters an effect of this drug class was demonstrated in a maximum of 18% of the cases (Table 5, Figure S5). The high percentages showing an effect on Focused/selective attention (33%) and delayed recall of the auditory/verbal memory (50%) can be attributed to the low frequency of these clusters.

- **Nicotinic receptor antagonists**

In the domain Memory, an impairment of the Learning (50%), Auditory/verbal memory: immediate recall (38%) and delayed recall (36%) clusters was demonstrated (Table 5, Figure S6). The high percentages showing an effect on Inhibition and Motor control can be attributed to the low frequency of these clusters. In the remaining clusters, there was no clear effect of nicotinic antagonists.

- **Muscarinic receptor antagonist**

Impairments were demonstrated repeatedly in many clusters (table 5). In most of these clusters, there was still a lack of effect in at least 50% of the cases. Only in the clusters Learning, Auditory/verbal memory: immediate recall, delayed recall, delayed recognition and Scale alertness an impairment was observed more often than a lack of effect. Visualizing the data in a forest plot (Figure S7) shows a fairly consistent impairment within the clusters Working memory, Auditory/verbal memory: immediate recall and delayed recall and Scale alertness.

The effects of all four drug classes on clusters are presented in a spider plot (Figure 1). Impairments were clearer following muscarinic receptor antagonist than after nicotinic receptor antagonist.

DOSE-RESPONSE RELATIONSHIPS The potential relationships between the dose levels of each drug class and the effects on the 14 clusters were investigated (Table S8, Figure S7)). There were no clear dose related effects after administration of cholinesterase inhibitors, nicotinic receptor agonists and nicotinic receptor antagonists. From the studies investigating muscarinic receptor antagonists there appeared to be a relationship between the effects on clusters Scale alertness and Auditory/verbal memory: immediate recall and delayed recall (Figure S9). Following a low dose, an impairment on Scale alertness was observed in 33% of the cases which is less frequently than after a medium dose (42%) and a high dose (80%). The cluster Auditory/verbal memory immediate recall showed no effect after a low dose (only one case present) and deterioration after a medium dose in 77% of the cases and after a high dose in 83% of the cases. Impairment increased with dose for the cluster Auditory/verbal memory delayed recall from 50% in the lowest dose group (2 cases present at this dose level) to 85% in the highest.

DISCUSSION

In this review we aimed to provide an overview and evaluation of biomarkers that were used to detect acute drug effects of cholinergic drugs acting on the central nervous system in healthy subjects. The biomarkers were evaluated for the drug classes cholinesterase inhibitors, nicotinic receptor agonist, nicotinic receptor antagonists and muscarinic receptor antagonists separately. No studies with (subtype) selective muscarinic receptor agonists were included, these drugs are not (yet) used in clinical practice, and experimental compounds were excluded. A large number of 223 tests were described in 132 publications, the majority of which were used infrequently. This huge variability is comparable to the results of similar reviews of biomarkers used to investigate CNS-active drugs in healthy subjects [156, 20, 21, 19, 18, 157]. In

each of the reviews, a call has been made for a harmonisation and standardisation of tests in drug development, in order to facilitate selection of methods, comparisons of compounds and functional interpretations of effects. Although some tests seem to be sensitive to drug effects such as the anti-saccadic eye movements after nicotinic receptor agonists (improved in 3/5 cases), and digit span (improved in 2/6 cases) and EEG alpha (decreased in 2/5 cases) after cholinesterase inhibitors, no conclusions about individual test used to evaluated the effect of cholinesterase inhibitors, nicotinic receptor agonists and antagonists can be drawn due to this low frequency. The tests used for muscarinic receptor antagonists show a more consistent effect (mainly impairment), but also in this drug class, there was a lack of effect in more than 50% of the cases. Because of the wide variety of tests and their low frequency, we have grouped these test in clusters of tests that measure similar CNS-functions. Grouping these tests in clusters might obscure information: the 'perfect' biomarker could be masked by nonresponsive tests in the same cluster. Additionally test variants and differences among research groups were bypassed. However, excluding tests based on their limited application could have resulted in missing possibly valuable information.

Analysis of the clusters showed moderate effects of nicotinic receptor agonists (improvement in up to 30% of the cases on inhibition and sustained attention) and a lack of clear effects after cholinesterase inhibitors. As most of the clusters represent a cognitive function, these lack of effects and moderate cholinergic-induced improvements could reflect the challenge of investigating cognitive improvement in healthy subjects: most tests in this review have ceiling effects in healthy optimally functioning subjects.

Ceiling effects are also suggested by the contrast between the limited results of the pro-cholinergic drugs, with the clearer impairments observed with anticholinergic compounds. Muscarinic receptor antagonists, for instance, showed deteriorations in 58-79% of memory tests.

The effects of nicotinic receptor antagonists were more limited, but this seems to be at least partly related to the low numbers of studies (n=13) included in this review. In several specifically designed human pharmacological studies, evident dose- and concentration-response relationships found on a number of sensitive tests [108, 158, 107]. However, these methods were all from the same centre, and not used often enough by other groups to be analysed in this review. The same investigators showed a different pharmacodynamic profile of a nicotinic receptor antagonist (mecamylamine) compared with the (more pronounced) effects of a muscarinic receptor antagonist (scopolamine)[108].

A consistent impaired effect on multiple clusters was shown after muscarinic receptor antagonist. Data of muscarinic receptor agonists were not included in this review, as these drugs are not approved (yet), The few clinical studies investigating the experimental muscarinic receptor agonists/PAMS in healthy subjects that were published showed a reduction in 2nd REM latency on a sleep EEG after xanomeline[159], an increase in pupil size after single doses of HTL0018318[160], and increases in sigma, delta and theta EEG frequency bands after multiple doses of MK-7622[161]. EEG delta and theta were also increased after muscarinic receptor antagonists thus no opposite effects were observed. Sleep EEG, pupil size, and EEG sigma were not used frequently enough after muscarinic receptor antagonists to compare with agonists/PAMS.

Analysing a dose-response relationship of the clusters revealed a relationship between the muscarinic receptor antagonists and the clusters Scale alertness and Auditory/verbal memory: immediate recall and delayed recall. These three relationships can be explained by the pharmacology of the drug, as the muscarinic receptors are highly prevalent in the hippocampus, a brain structure involved in memory[4,5] and in the brain stem and thalamus[3] which are involved in alertness[162]. In the remaining clusters, the low number of cases per dose level could have masked potential dose-effect relationships easily.

Given the effects on the tests, clusters and the dose-relationship in this review, there are only a limited number of clusters that meet the criteria of a good biomarker as defined in the method section. This does not exclude the existence of other good biomarkers. The success of a biomarker depends on multiple factors such as sample size and characteristics of the study population, study design and timing of the application, which were not taken in account in our analysis. Additionally, as mentioned before, grouping the tests into clusters could have masked good biomarkers. It was also mentioned that studies that are specifically designed to detect concentration-effect relationships (by employing different doses and frequent measurements of concomitant drug concentrations and effects) can provide unequivocal evidence for the suitability of a test as a pharmacological biomarker, even in a single study. An example of a good biomarker included in this review is the adaptive tracking test, a measure for attention[163-165]. This test was used to measure effects of cholinesterase inhibitors donepezil and an experimental CNS-penetrating prodrug of galantamine[54,52]. This example is encouraging to further evaluate and validate the existing biomarkers, because the reliability of biomarkers can be more carefully assessed when more data is available. Because of this example and the effects of cholinesterase inhibitors on individual tests such as digit span and EEG alpha we also strongly recommend to keep using biomarkers in experimental studies in healthy subjects for

the investigation of pro-cholinergic drugs, as is recommended by the guideline of the EMA[166]. If test improvements or impairments are observed in early phase clinical trials, these can be further investigated by analysing the concentration-response relationship in order to avoid a type I error. Additionally, to avoid the ceiling effects of biomarkers, challenge situations can be applied such as the scopolamine, mecamylamine or biperiden challenge models, sleep deprivation challenge or inclusion of elderly subjects. Scopolamine, mecamylamine and biperiden temporary induce cognitive deficits and neurophysiological effects [108, 158, 167], which create the possibility to improve cognition in healthy subjects. Co-administration of the pro-cholinergic compound can then (partially) reverse these effects, and elucidate drug effects which cannot be demonstrated in unchallenged optimally functioning individuals[107]. Cholinesterase inhibitors have been investigated in scopolamine challenge models. These ameliorated the magnitude of the scopolamine-induced effects on learning efficiency of the Groton maze learning test[56] and power and continuity of attention and quality of working memory, measured as a combination of multiple tests[168]. As these tests are sensitive to the effects of cholinesterase inhibitors, they it be considered to also use them in early phase drug clinical studies.

In conclusion, an excessive number of tests has been used to evaluate the effects of pro-cholinergic and anti-cholinergic drugs in healthy subjects. This huge variability is detrimental to the proper use of biomarkers in early drug development. From this review, no single test could be identified that was able to demonstrate pro-cholinergic effects consistently, although there are tests that are able to detect dose dependent effects of pro-cholinergic drugs in healthy subjects, such as the adaptive tracking test. Therefore further evaluation and validation of the the potential pro-cholinergic functional biomarkers is recommended. Effects of nicotinic and muscarinic receptor antagonists could be demonstrated more consistently. These well measurable temporary anti-cholinergic effects can be used ins pharmacological challenge experiments in healthy subjects, in order to allow detection of the effects of pro-cholinergic drugs.

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TABLE 1 **Cholinergic drugs included in this review.**

Drug class	Drugs
Cholinesterase inhibitor	Galantamine, rivastigmine, donepezil, physostigmine, tacrine
Nicotinic receptor agonists	Nicotine, varenicline
Nicotinic receptor antagonist	Mecamylamine
Muscarinic receptor antagonist	Scopolamine, biperiden, atropine, procyclidine

TABLE 2 **Classification of dose levels per drug.**

	Low dose level	Medium dose level	High dose level
Nicotine			
Chewing gum	<2 mg	2 mg	>2 mg
Plaster (transdermal)	<14 mg/24h	14 -20 mg/24h	>20 mg/24h
Tablet	<2 mg	2-4 mg	>4 mg
Intranasal		1 mg	
Mouth spray	<1 mg	1-2 mg	>2 mg
Subcutaneous		6 ug/kg	12ug/kg, 1 mg
Donepezil (oral)	<5mg	5-7.5mg	>7.5mg
Galantamine (oral)	<8 mg	8 mg	16 mg
Rivastigmine			
Capsule	<3 mg single dose	3-5mg single dose	>5 mg single dose
Plaster (transdermal)	4.6 mg/24h	9.5 mg/24h	
Mecamylamine (oral)	<5 mg/day	5-20 mg/day	> 20 mg/day
Biperiden			
Oral	<1 mg single dose	1-3 mg single dose	>3 mg single dose
Intraveneous	<2.5 mg	2.5 mg-4 mg	>4 mg
Scopolamine			
Transdermal	<1.0 mg	1-1.5 mg	>1.5 mg
Intramuscular	<0.3 mg single dose	0.3-0.5 mg single dose	>0.6 mg single dose
Intravenous	<0.3 mg single dose	0.3-0.5 mg single dose	>0.6 mg single dose
Oral	<0.4 mg	0.4-08 mg	>0.8
Procyclidine (oral)	2.5 mg single dose	0.5 mg-5 mg single dose	>5 mg single
Varenicline (oral)	<0.5 mg single dose	0.5-1 mg single dose	2 mg single dose
Tacrine (oral)	low <20mg per dose	medium 20-60mg per dose	>60mg per dose
Physostigmine (IM, IV)		0.5 to 2 mg	

TABLE 3 **Characteristics of the studies included in this review. One study consisted of two differently designed study parts resulting in n=133 for the design related columns.**

Randomization (total n=133)	Blinding (total n=133)	Design (total n=133)	Control (total n=133)	Age (total n=132)	Number of subjects included (total n=132)	Sex of subjects (total n=132)
Randomized n= 112 (84%)	Double-blind n= 111 (83%)	Cross-over n= 97 (73%)	Placebo-controlled n= 122 (92%)	Mean (range) age 29.2 (21-73.10)	Mean (range) 23.5 (6-116)	Only males n=40 (30%)
Pseudo-randomized n= 3 (2%)	Single-blind n= 9 (7%)	Parallel n= 31 (23%)	Not placebo-controlled n= 11 (8%)			Only females n=3 (3%)
Non-randomized n= 12 (9%)	Open label n= 12 (9%)	Unknown n= 5 (4%)				Both males and females n=86 (n=64%)
Unknown n= 6 (5%)	Unknown N=1 (1%)					Unknown n=3 (3%)

n=number of studies

TABLE 4 **Overview of all the tests included in this review and the grouping in clusters and domains.**

Test	Cluster	Domain
Divided attention, Paced Auditory Serial Addition Test	Divided attention	Attention
Digit symbol substitution test, Symbol digit substitution test	DSST-like	
Attention Network Test	Executive control	
Critical flicker fusion task	Flicker discrimination	
Attention Network Test	Orienting	
5-choice reaction time, Choice Reaction Time task, Detection task, Faces Dot Probe Task, Go no go paradigm, Incongruent choice reaction time task, Multiple choice reaction time test, Psychomotor Vigilance Task, Rapid visual information processing, Self paced and externally triggered reaction times, Simple reaction time test, Spatial attentional resource allocation task, Sustained attention to response task, Visual choice reaction oddball task	Reaction time	
3-D multiple object tracking, Adaptive tracking, Attention Network Test, Choice Reaction Time task, Continuous performance test, Continuous performance test – identical pairs version, Digit vigilance, Identify task, Multiple choice reaction time test, Paced Auditory Serial Addition Test, Psychomotor Vigilance Task, Rapid visual information processing, Span of apprehension test, Sustained attention to response task, Unstable tracking, Visual choice reaction oddball task, Wilkins counting test	Sustained attention (vigilance)	
Attentional blink test, Posner cueing task, D2 concentration test, Digit span, Inspection time task, Shape matching task, Simple two-choice visual discrimination task, Spatial attentional resource allocation task, Spatial span, Visual pop out search, Visual scanning test, Visual selective attention, Visuospatial cueing task	Focused/selective attention	
ECG	ECG	Autonomic
Pupil size	Pupil size	
Visual accommodation and acuity	Visual acuity	
Blood pressure, Oral temperature, Pulse rate	Vital signs	
Emotion-potentiated startle task, Flanker task, Go no go paradigm, Magnetoencephalography (pre pulse inhibition), Prepulse inhibition of the acoustic startle reflex, Simon task, Stop signal task, Stroop Test, Three card stroop task	Inhibition	Executive
Inspection time task	Judgement	
Controlled Oral Word Association Test, Oral language, Phonemic letter fluency, Reading, Regensburger Wortfluessigkeitstes, Spelling	Language	
Tower of London task, Zoo map test	Planning	
Emotion recognition task, Emotion recognition/matching, Facial expression recognition task, Mathematical processing, Emotional Categorisation Task, Word categorization and memory task	Reasoning/association	
Balloon Analogue Risk Task, Signal detection task	Reward	
Dual task paradigm, Intra/extradimensional shift, Plus-minus task, Trail Making Test, Wisconsin card sorting test	Shifting	
Little man test, Manikin task, Stockings of Cambrigde	Spatial orientation	
Time wall	Time estimation	
Speed anticipation test	Time-distance estimation	
Corsi block test, Counting span, Digit span, Immediate memory task, Letter-number sequencing, Letter memory task, Match to sample, Maze learning task, N-back, Non-spatial working memory, Paced Auditory Serial Addition Test, Short Blessed Test, Spatial information processing, Spatial recognition memory, Spatial span, Spatial working memory, Sternberg working memory task, Symbol digit recall test, The arena, Visual spatial working memory test	Working memory	
Buschke Selective Reminding Test , California verbal learning test, Emotional Recall Task, Levels of processing, Logical memory test, Word categorization and memory task, Paired associate learning, Rey Auditory Verbal Learning Test, Selective reminding task, Verbal learning task	Auditory/verbal memory: immediate recall	Memory
Buschke Selective Reminding Test, California verbal learning test, Cued recall task, Free recall test, Hi-lo imaginary test, Hopkins Verbal Learning Test-Revised, Levels of processing, Logical memory test, Memory task of Jacoby (adjusted version), Paired associate learning, Repeated Acquisition Task, Rey Auditory Verbal Learning Test, Selective reminding task, Verbal learning task	Auditory/verbal memory: delayed recall	
California verbal learning test, Emotional Recognition Memory Task, Word categorization and memory task, Rey Auditory Verbal Learning Test, Verbal learning task, Verbal recognition task, Word completion task	Auditory/verbal memory: delayed recognition	
Continuous recognition memory test, Episodic memory paradigm, Running word recognition, Hi-lo imaginary test	Auditory/verbal memory: immediate recognition	
Continuous Visual Recognition, Object relocation task, Rivermead Behavioral Memory Task, Spatial free recall: selective reminding, Spatial memory task, Visual episodic memory, Visual reproduction	Visual/spatial memory: immediate recall	
Maze learning task, Memory task of Jacoby (adjusted version), Object relocation task, Rivermead Behavioral Memory Task, Self-paced subsequent recognition memory task, Spatial memory task, Visual episodic memory, Visual memory task, Visual reproduction	Visual/spatial memory: delayed recall	
Benton Visual Retention Test, Delayed picture recognition, Face recognition, Novelty test, Object recognition, Pattern recognition memory, Processing depth, Spatial memory task, Spatial recognition memory, Sternberg working memory task	Visual/spatial memory: delayed recognition	
Change detection task, Continuous recognition memory test, Pattern recognition memory, Picture memory test, Running picture recognition, Spatial memory task, Spatial recognition memory, Visual working memory task, Word categorization and memory task	Visual/spatial memory: immediate recognition	
10-response sequences task, Buschke Selective Reminding Test, California verbal learning test, Continuous paired associate learning task, Face encoding recognition task, Hi-lo imaginary test, Learned irrelevance task, Levels of processing, Motion direction discrimination task, One Card learning test, Paired associate learning, Repeated Acquisition Task, Rey Auditory Verbal Learning Test, Selective reminding task, Spatial memory task, Symbol digit recall test	Learning	
Automatic task, Controlled task, Priming task	Priming	
Prospective memory task	Prospective memory	
Circular lights task, Finger tapping task, Finger tapping task (auditory paced), Hand cooperation test, Pointing task	Motor control	Motor
Detect task	Reaction time	
Adaptive tracking, Compensatory tracking task, Handwriting test, Line copying task, Maze learning task, Tangle, Tangle task, The grooved pegboard, Trail Making Test	Visuo-motor control	
Brain-derived neurotrophic factor	Brain-derived neurotrophic factor	(Neuro) endocrine
Acetylcholinesterase activity, Butyrylcholinesterase activity	Cholinesterase	
Cortisol	Cortisol	
Follicle-stimulating hormone	FSH	
Ghrelin	Ghrelin	
Luteinizing Hormone	LH	
Prolactin	Prolactin	

EEG delta	EEG delta	Neuro-physio-logical
EEG theta	EEG theta	
EEG alpha	EEG alpha	
EEG beta	EEG beta	
EEG gamma	EEG gamma	
EEG sigma	EEG sigma	
EEG (novelty oddball paradigm), ERP (3 types of auditory stimuli paradigm), ERP (attentional blink test), ERP (during rapid visual information processing, ERP (learned irrelevance paradigm), ERP (match to sample probe matching cue), ERP (MMN paradigm), ERP (Novelty oddball paradigm), ERP (P50 paradigm), ERP (Paired-click paradigm), ERP (Verbal learning test), ERP (visual memory task), High frequency oscillations, Magnetoencephalography (relevance modulation task), Magnetoencephalography (somatosensory stimuli (electrical pulses)), Magnetoencephalography (standard and deviate auditory stimulus), Magnetoencephalography (visuomotor task), Somatosensory evoked potentials	Evoked potential	
Smooth pursuit eye movements	Eye movements – pursuit	
Anti-saccadic eye movement, Saccadic eye movements	Eye movements – saccadic	
Body sway	Postural stability	
TMS	TMS	
Object relocation task	Visual perception	Perception
Profile of Mood States, VAS mood scale	Scale aggression	Subjective experience
Eigenschaftswörterliste, Profile of Mood States, Subjective well-being, VAS Bond and Lader, VAS Norris, Visual analogue scale	Scale alertness	
Brief Psychiatric Rating Scale, Positive and negative affective schedule, Profile of Mood States, State trait anxiety inventory, Visual analogue scale	Scale anxiety	
Profile of Mood States, Subjective well-being, VAS Bond and Lader	Scale calmness	
Addiction Research Center Inventory, Brief Questionnaire of Smoking Urges, Drug Effects Questionnaire	Scale craving	
Side effects, Visual analogue scale	Scale dizziness	
Addiction Research Center Inventory, Drug Effects Questionnaire	Scale drug effect	
Positive and negative affective schedule, Profile of Mood States, Side effects	Scale fatigue	
Addiction Research Center Inventory, VAS Bowdle	Scale high	
Addiction Research Center Inventory, Beck depression inventory, Befindlichkeits scale, Brief Psychiatric Rating Scale, Positive and negative affective schedule, Profile of Mood States, Subjective well-being, VAS Bond and Lader, VAS mood scale, Visual analogue scale	Scale mood	
Subjective well-being, VAS Bond and Lader	Scale performance	
Brief Psychiatric Rating Scale, Positive and Negative Symptoms Scale, VAS Bowdle, VAS Norris	Scale psychotomimetic	
Visual analogue scale	Scale satiety	
Biphasic Alcohol Effects Questionnaire, Bodily symptoms, Neurovegetative effects, Side effects, VAS nausea, Visual analogue scale	Scale symptoms	

TABLE 5 **Effect of drug classes on clusters.**

		Pro-cholinergic drugs								Anti-cholinergic drugs							
Domain		**Cholinesterase inhibitors**				**Nicotinic receptor agonists**				**Nicotinic receptors antagonists**				**Muscarinic receptor antagonists**			
	Cluster	n	–	=	+	n	–	=	+	n	–	=	+	n	–	=	+
Executive	Working memory	35	1 (2.9%)	30 (85.7%)	4 (11.4%)	11	1 (9.1%)	9 (81.8%)	1 (9.1%)	11	1 (9.1%)	10 (90.9%)	0 (0.0%)	41	18 (43.9%)	22 (53.7%)	1 (2.4%)
	Inhibition	9	1 (11.1%)	8 (88.9%)	0 (0.0%)	22	1 (4.5%)	14 (63.6%)	7 (31.8%)	3	2 (66.7%)	1 (33.3%)	0 (0.0%)	6	3 (50.0%)	3 (50.0%)	0 (0.0%
Motor	Motor control	2	0 (0.0%)	2 (100.0%)	0 (0.0%)	3	0 (0.0%)	3 (100.0%)	0 (0.0%)	3	2 (66.7%)	1 (33.3%)	0 (0.0%)	13	5 (38.5%)	8 (61.5%)	0 (0.0%)
	Visuo-motor control	7	0 (0.0%)	6 (85.7%)	1 (14.3%)	7	0 (0.0%)	6 (85.7%)	1 (14.3%)	1	0 (0.0%)	1 (100.0%)	0 (0.0%)	12	6 (50.0%)	6 (50.0%)	0 (0.0%)
Memory	Learning	12	2 (16.7%)	9 (75.0%)	1 (8.3%)	1	0 (0.0%)	1 (100.0%)	0 (0.0%)	6	3 (50.0%)	3 (50.0%)	0 (0.0%)	19	13 (68.4%)	6 (31.6%)	0 (0.0%)
	Auditory/ verbal memory: immediate recall	18	1 (5.6%)	17 (94.4%)	0 (0.0%)	3	0 (0.0%)	3 (100.0%)	0 (0.0%)	8	3 (37.5%)	5 (62.5%)	0 (0.0%)	28	20 (71.4%)	6 (21.4%)	2 (7.1%)
	Auditory/verbal memory: delayed recall	13	1 (7.7%)	10 (76.9%)	2 (15.4%)	2	0 (0.0%)	1 (50.0%)	1 (50.0%)	11	4 (36.4%)	7 (63.6%)	0 (0.0%)	29	23 (79.3%)	6 (20.7%)	0 (0.0%)
	Auditory/verbal memory:delayed recognition	7	1 (14.3%)	5 (71.4%)	1 (14.3%)	3	0 (0.0%)	3 (100.0%)	0 (0.0%)	5	0 (0.0%)	5 (100.0%)	0 (0.0%)	12	7 (58.3%)	5 (41.7%)	0 (0.0%)
Attention	Sustained attention (vigilance)	21	0 (0.0%)	20 (95.2%)	1 (4.8%)	10	0 (0.0%)	7 (70.0%)	3 (30.0%)	13	2 (15.4%)	11 (84.6%)	0 (0.0%)	29	11 (37.9%)	18 (62.1%)	0 (0.0%)
	Focused/selective attention	11	3 (27.3%)	6 (54.5%)	2 (18.2%)	3	1 (33.3%)	2 (66.7%)	0 (0.0%)	5	0 (0.0%)	5 (100.0%)	0 (0.0%)	23	2 (8.7%)	21 (91.3%)	0 (0.0%)
	Reaction time	17	0 (0.0%)	17 (100.0%)	0 (0.0%)	6	0 (0.0%)	6 (100.0%)	0 (0.0%)	14	3 (21.4%)	11 (78.6%)	0 (0.0%)	32	10 (31.2%)	22 (68.8%)	0 (0.0%)
Subjective experience	Scale mood	12	0 (0.0%)	10 (83.3%)	2 (16.7%)	9	1 (11.1%)	8 (88.9%)	0 (0.0%)	10	0 (0.0%)	10 (100.0%)	0 (0.0%)	17	2 (11.8%)	14 (82.4%)	1 (5.9%)
	Scale alertness	9	1 (11.1%)	7 (77.8%)	1 (11.1%)	6	2 (33.3%)	4 (66.7%)	0 (0.0%)	6	1 (16.7%)	5 (83.3%)	0 (0.0%)	25	14 (56.0%)	11 (44.0%)	0 (0.0%)
	Scale calmness	7	0 (0.0%)	7 (100.0%	0 (0.0%)	3	0 (0.0%)	3 (100.0%)	0 (0.0%)	3	0 (0.0%)	3 (100.0%)	0 (0.0%)	15	3 (20.0%)	10 (66.7%)	2 (13.3%)

n= The frequency of the cluster used (cases) as a product of the number of articles, drugs, doses and tests; '–' is impairment; '=' is no effect; '+' is improvement

FIGURE 1 Effect of all drug classes on the 14 most investigated clusters. The line moving towards the centre of the spider plot represents an impairment. The line moving towards the edge of the spider plot represents an improvement.