Challenging the cholinergic system : ageing, cognition & inflammation

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REVERSAL OF MECAMYLAMINE-INDUCED EFFECTS IN HEALTHY SUBJECTS BY NICOTINE RECEPTOR AGONISTS: COGNITIVE AND (ELECTRO)PHYSIOLOGICAL RESPONSES

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ABSTRACT

Establishing a pharmacologic challenge model could be an important tool to understand the complex nicotinic cholinergic system role in cognition and to develop novel compounds acting on the nicotine acetylcholine receptor. We examined not only the effects of the nicotinic antagonist mecamylamine on a battery of cognitive and neurophysiologic test, but also the effect of nicotine or galantamine co-administration in reversing the cognitive impairment caused by mecamylamine. We conducted a randomized, double-blind, double-dummy, placebo-controlled, four way cross-over study in 33 healthy subjects receiving a single oral dose of 30 mg of mecamylamine (or placebo) in combination with either 16 mg of oral galantamine or 21 mg of transdermal nicotine (or its double-dummy). Mecamylamine 30 mg induced significant disturbances of cognitive functions. Attention and execution of visual - (fine) motor tasks was decreased, short- and long-term memory was impaired and the reaction velocity during the test was slower when compared to placebo. Mecamylamine 30 mg produced a decrease in posterior α and β power in the surface EEG, effects that were reversed by nicotine co-administration. Memory and motor coordination tests could be partially reversed by the co-administration of nicotine. Mecamylamine administration induced slowing of the EEG and produced decrease in performance of tests evaluating motor coordination and short and long-term memory. These effects could be partially reversed by the co-administration of nicotine, and to a lesser extent by galantamine.

INTRODUCTION

The nicotinic cholinergic system plays an important role in key cognitive processes such as attention and working- and associative-memory, and is therefore essential for learning (Jones *et al*, 1999; Levin *et al*, 2006). Cholinergic dysfunction is recognized to be involved in the pathophysiology of neurodegenerative diseases (e.g. dementia) and psychiatric conditions (e.g. schizophrenia) and is, therefore, considered a promising therapeutic target (Court *et al*, 2000; Parri *et al*, 2011).

Scopolamine is the most frequently used challenge drug to induce temporary, reversible, cognitive disturbances resembling those of Alzheimer's disease (AD) in healthy subjects (Ebert and Kirch, 1998). Scopolamine is a selective and competitive muscarinic acetylcholine receptor antagonist, binding to all muscarinic receptor types (Ali-Melkkilä *et al*, 1993). With several nicotinic receptor agonists in the clinical phase of drug development (Beinat *et al*, 2015; Vallés *et al*, 2014), the interest in nicotinic acetylcholine receptor (nAChR) pharmacology is rising. The use of muscarinic receptor antagonist scopolamine to investigate the pharmacology of nicotinic receptor agonists would seem less direct and therefore we aimed to develop a pharmacological challenge model targeting the nicotinic cholinergic system.

Mecamylamine is a selective non-competitive nAChR antagonist (Webster *et al*, 1999). Mecamylamine 20 mg produced impairments in learning and retrieval (Newhouse *et al*, 1994), acquisition, increased reaction time and errors (Newhouse *et al*, 1992) and an increased inspection time during a visual discrimination test (Thompson *et al*, 2000). In order to be able to use mecamylamine as a challenge model to prove pharmacological effects of nicotinic compounds, it is necessary to demonstrate reversal of its temporary negative effects on cognition. In animals, successful reversal of mecamylamine-induced disturbances was demonstrated with nicotine co-administration (Brucato *et al*, 1994; Woodruff-Pak, 2003). To our knowledge, only one study in humans described partial reversal of increased inspection time induced by 20 mg of mecamylamine, when 5 mg of donepezil, an acetylcholinesterase inhibitor, was co-administered (Thompson *et al*, 2000).

In an previous exploratory study we confirmed that administration of 10 and 20 mg of mecamylamine in healthy subjects led to a temporary, dose-dependent

disturbance of several cognitive functions including fine motor coordination and fluency, short- and long-term memory, attention and concentration (Baakman *et al*, 2016). In this confirmatory study we further investigated the dose-effect relationship of mecamylamine with a higher dose of 30 mg. Furthermore, we aimed to further validate mecamylamine as a nicotinic anticholinergic challenge model by investigating the potential reversal of the observed effects of mecamylamine on cognition by co-administering galantamine (a cholinesterase inhibitor) and nicotine (a nAChR agonist).

MATERIALS AND METHODS

STUDY DESIGN

This was a randomized, double-blind, double-dummy, placebo-controlled, four-way cross-over study of a single oral dose of mecamylamine (or placebo) in combination with either galantamine or nicotine. The treatment arms were: mecamylamine plus placebo, mecamylamine plus nicotine, mecamylamine plus galantamine and (double)placebo. A minimal wash-out period of one week was utilized.

Oral medication was administered with water at time point zero of every visit. Five minutes thereafter, a nicotine or placebo patch was placed on the skin at the shoulder blade region. Subjects were discharged 32 hours post-dose after monitoring of vital signs was performed and if subjects were asymptomatic.

SUBJECT SELECTION

A medical ethics committee approved the study protocol. After giving written informed consent, all subjects were medically screened prior to study participation. Healthy male incidental smokers (age between 18 and 45 years and Body Mass Index (BMI) between 18 and 32 $kg \cdot m^{-2}$; both inclusive) were included in the study. Incidental smokers, defined as subjects smoking at least once a month, but no more than 5 cigarettes per day within the past 3 months, were included in the study due to the fact that non-smokers might have experienced more severe side effects derived from the nicotine and galantamine administration. Main exclusion criteria included any relevant medical abnormalities including conditions causing cognitive impairment, orthostatic hypotension (Kaufmann, 1996) or hypertension (>140/90 mmHg). Use of agents or drugs known to influence CNS performance were not allowed during study participation.

MEDICINAL PRODUCTS AND DOSING RATIONAL

Drug accountability of all medicinal products was managed by the Leiden University Medical Centre Clinical Trials pharmacy.

Mecamylamine 30 mg capsules (Euticals SpA, Milan, Italy) containing 36.6 mg mecamylamine HCl and microcrystalline cellulose as filling agent (used also in the placebo capsules) were administered orally. Based on an interim PK-PD modeling of the concentration-effect relationship of mecamylamine 10 and 20 mg on the blood pressure investigated in the exploratory study (data not presented), a single oral dose of 30 mg was considered safe. Moreover, the dose was expected not to exceed E_{max} (still allowing reversal) and not to cause functionally limiting hypotension.

Transdermal patches containing 21 mg nicotine (NiQuitin®, Glaxo-Smithkline, Bolton, UK), with blinding covering were administered to reverse mecamylamine effects. Blinded vaseline patches were used as placebo. Nicotine 21 mg patches are the highest commercially available dose that is well tolerated without significant adverse events in smokers (DeVeaugh-Geiss *et al*, 2010).

Galantamine hydrobromide 4 mg over encapsulated capsules (Reminyl®, Janssen-Cilag SpA, Latina, Italy) or matching placebo capsules were orally administered. Four Galantamine 4 mg capsules or placebo were administered for a total dose of 16 mg. Doses up to 15 mg without titration have been safely administered in healthy subjects (Riemann *et al*, 1994) and in our center, galantamine 16 mg was previously administered in healthy elderly subjects (unpublished data). Galantamine was chosen as it exerts an allosteric nicotinic modulatory activity that donepezil lacks (Coyle and Kershaw, 2001; Maelicke *et al*, 2001; Maelicke and Albuquerque, 2000).

SAMPLE SIZE DETERMINATION

Sample size calculations were performed using the data obtained from the Visual Verbal Learning test, performed in the previous study with

mecamylamine 10 and 20 mg. The sample size was calculated using 80% power in a paired t-test with a two-sided 0.05 significance level.

COGNITIVE AND NEUROPHYSIOLOGY MEASUREMENTS

The NeuroCart® is a computerized test-battery of sensitive tests used to evaluate a wide range of central nervous system (CNS) effects of neuro- and psychoactive drugs. A practice session of all tests was performed at screening. At each study visit, baseline training was performed twice to ensure stable performance and minimize learning effects. The NeuroCart® test battery was subsequently performed at time points 30, 80, 130, 180, 230, 280, 360 and 480 minutes post-dose, except for the Visual Verbal Learning Test (VVLT) which was only performed once per occasion and the Milner Maze test (MMT) which was not performed at 130 and 230 minutes.

N-BACK TEST ✳ Subjects were asked to remember and correlate a sequence of letters presented in a random order (Lim *et al*, 2008) thereby allowing evaluation of (short-term) working memory. Performance is expressed as the ratio of correct and incorrect answers ([correct – incorrect]·total^{-1}) and reaction time on the 0-, 1- and 2-back conditions.

ADAPTIVE TRACKING TEST ✳ The test was performed as previously described (Borland and Nicholson, 1984; van Steveninck *et al*, 1991). The test mainly evaluates vigilance and arousal and visuo-motor coordination.

ELECTRO-ENCEPHALOGRAM (EEG) ✳ Resting state eyes-closed EEG recordings were obtained for 64 seconds per time point using four cranial superficial gold electrodes (Fz, Cz, Pz, Oz), placed following the 10-20 system and fixed with EC2 paste with the same common ground and eye movement registration. Electrode resistance was kept below 5 kΩ. Grass 15LT series Amplifier Systems was used for signal amplification with a time constant of 0.3 seconds and a low pass filter at 100 Hz. The signal was AD-converted using CED 1401 Power (Cambridge Electronics Design, Cambridge, UK). Fast Fourier transformed absolute power (μV) was calculated from the raw measurements in the α [7.5 – 13.5 Hz], β [13.5 – 35 Hz], δ [2 – 4 Hz], ϑ [4 – 7.5 Hz] and γ [>35 Hz] frequency ranges in two bipolar leads: Fz-Cz and Pz-Oz.

FINGER TAPPING ✳ Dominant hand finger tapping test was performed to evaluate motor activation and fluency (Andrew, 1977; Liem-Moolenaar *et al*, 2010).

SIMPLE REACTION TIME TEST (SRT) ✳ Subjects were instructed to react as soon as possible after a visual stimulus was presented.

VISUAL VERBAL LEARNING TEST (VVLT) ✳ This test evaluates the different aspects of learning (i.e. acquisition, consolidation, storage, retrieval) and was performed as previously described (Liem-Moolenaar *et al*, 2010; Schmitt *et al*, 2006; Zuurman *et al*, 2010).

MILNER MAZE TEST (MMT) ✳ The MMT is a visuo-spatial working memory test (Milner, 1965). The computerized version has an immediate, a delayed and a reverse trial where the same maze has to be completed in the reverse order. Outcome measures are time to complete (milliseconds) and accuracy (number correct and incorrect steps).

VISUAL ANALOGUE SCALES (VAS) ✳ The VAS is a frequently used scale to measure subjective feelings of drug effects, as previously described (Bond and Lader, 1974). From these measurements, three main factors are the calculated as described by the authors: alertness (from nine scores), contentedness (often called mood; from five scores), and calmness (from two scores). A VAS was added evaluating nausea.

PUPIL DIAMETER MEASUREMENTS ✳ The pupil/iris ratio was measured as previously described (Liem-Moolenaar *et al*, 2010; Twa *et al*, 2004).

PHYSIOLOGIC MEASURES

Safety assessments, including registration of adverse events, electrocardiogram (ECG), body temperature, blood pressure and heart rate were performed at predefined times throughout the study. Hematology, biochemistry, urinalysis, alcohol and drugs test were performed at medical screening, pre-dose per visit and at follow-up.

STATISTICAL ANALYSIS

All variables were summarized by treatment and time. Repeated measured data were analyzed with a mixed model analysis of variance with fixed factors treatment, period, time and treatment-by-time and as random factors subject, subject-by-treatment and subject-by-time and the average pre-dose values as covariate. Single measured pharmacodynamic data were compared with a mixed model analysis of variance with fixed factors treatment, period, random factors subject and the average pre-dose values as covariate. The analysis was performed by an independent statistician using SAS software for windows v9.4 (SAS Institute, Inc., Cary, NC, USA). Graphs were created using R v2.14.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

SUBJECT DEMOGRAPHICS

Fifty-one healthy male subjects underwent medical screening and thirty-three subjects were included in the study. The mean age was 23.3 years (range 19–35), average body weight was 74.5 ± 8.3 kg (range 60.25–91.25) and BMI was 22.6 ± 2.4 $kg \cdot m^{-2}$ (range 19.4–27.7). Twenty-six subjects completed all four-study visits. Five subjects cancelled their participation after the first visit due to the side effects. One subject was withdrawn from the study because it was not possible to place an intravenous catheter and one subject stopped his participation for personal reasons.

COGNITIVE AND NEUROPHYSIOLOGICAL MEASUREMENTS

The SRT and pupil diameter test were not significantly influenced by mecamylamine, nicotine or galantamine.

ADAPTIVE TRACKING TEST ✳ The mean performance on the Adaptive Tracking test was significantly influenced by mecamylamine administration (overall treatment effect $p<0.0001$), as shown in Table 4.1. As expected, mecamylamine alone produced a significant impairment in the mean performance of -3.3 % (-4.6 – -2.0, $p<0.0001$) in the Adaptive Tracking. Nicotine co-administration caused a significant improvement of 1.5 % (0.2 – 2. 8, $p<0.05$) in comparison to mecamylamine alone. Galantamine co-administration did not significantly reverse the effects of mecamylamine (mean group difference 0.2 %; Figure 4.1).

N-BACK TEST ✳ Examination of the mean correct - incorrect ratio by time in the 0-back condition showed a significant overall treatment effect, producing in average a decrease of -0.023 (-0.044 – -0.003, $p<0.05$) in the ratio after administration of mecamylamine (Figure 4.2, Table 4.1). Mecamylamine administration also produced a non-significant reduction in the ratio of correct-incorrect answers 1-back (-0.015) and 2-back (-0.018) condition. Nicotine co-administration non-significantly reversed mecamylamine effects during the 0-back (group mean 0.007), 1-back (0.015) and 2-back (0.018) conditions. Co-administration of galantamine produced a non-significant worsening of mecamylamine effects during the 0- (group mean -0.006), 1- (-0.013) and 2-back (-0.016) conditions, when compared to the mecamylamine group.

Regarding the reaction time (RT) during the N-back test, the only paradigm where a significant overall treatment effect ($p=0.0432$) was observed was the 2-back condition, the most difficult one. Mecamylamine administration produced a mean increase of 28.3 ms (2.0 – 54.6, $p<0.05$) in the 2-back RT (Figure 4.1). The increase in the RT due to mecamylamine administration was significantly reversed by the co-administration of both nicotine (mean difference -36.0 ms (-62.2 – -9.7, $p<0.01$)) and galantamine (mean difference -27.2 ms (-53.3 – -0.8, $p<0.05$). Mecamylamine administration increased non-significantly the RT in the 0-back (8.0 ms) and 1-back (6.2 ms). Nicotine non-significantly reversed mecamylamine effects during the 0-back (-9.6 ms) and 1-back (-4.5 ms) conditions. Galantamine reversed non-significantly mecamylamine effects during the 0-back (-4.0 ms) and further increased the 1-back (0.9 ms) condition.

ELECTRO-ENCEPHALOGRAM ✳ As shown in Figure 4.3, the mean *a* power over Pz-Oz by time showed a significant overall treatment effect ($p=0.0132$), however, the only significant contrast was an increase of 14.9% (6.0 – 24.6, $p<0.005$) when nicotine was co-administrated compared to

mecamylamine alone (Table 4.1). Administration of mecamylamine decreased non-significantly the *α* power over the Pz-Oz by -6.2% when compared to placebo and galantamine non-significantly reversed this effect (6.7%) when compared to placebo. Mecamylamine also decreased to a lesser extent and non-significantly the mean *α* power over Fz-Cz compared to placebo (-0.6%), effect that was non-significantly reversed by the co-administration of nicotine (3.0%) and galantamine (3.9%).

Mecamylamine showed a significant overall treatment effect on *β* power in the Pz-Oz lead. Mecamylamine administration reduced the *β* power by -7.1% (-13.7 - -0.1%, $p<0.05$) when compared to placebo. Nicotine co-administration reversed mecamylamine effects by 10.7% (2,9 – 19.1, $p<0.01$). Galantamine co-administration also appeared to reverse mecamylamine effects (4,5%), but the difference was not significant (Figure 4.3). Mecamylamine administration reduced also the *β* power Fz-Cz lead non-significantly (-2.6%).

No significant effects of mecamylamine were detected on the EEG in the γ, ϑ and δ frequency power at the Pz-Oz and Fz-Cz leads.

FINGER TAPPING ✳ Mecamylamine significantly decreased the mean number of taps recorded during the Finger Tapping tests by -5.3 taps (-6.8 – -3.8, $p<0.0001$). Mecamylamine plus nicotine or galantamine caused small non-significant decreases in the mean number of taps (-0.158 and -0.586, respectively).

VISUAL VERBAL LEARNING TEST ✳ The only parameter from the VVLT conditions where mecamylamine had a significant overall treatment effect was on the number of correct answers during the delayed word recognition ($p=0.0284$). Mecamylamine administration caused more mistakes than placebo (-1.87 correct answers; -3.46 – -0.28; $p=0.02$). Treatment with nicotine, appeared to reverse mecamylamine effects by 0.29 words, but this effect was not significant (Figure 4.2, Table 4.1).

MILNER MAZE TEST ✳ Mecamylamine administration produced a non-significant mean increase of 2195.1 ms in the exploration time during the Immediate condition (p=0.0167). Unexpectedly, mecamylamine caused a decrease of -1108 ms in the Delayed condition of the MMT when compared to placebo (p=0.0388). Contrary to what was observed in all other tests, galantamine co-administration produced a significant slowing (increase) in the mean exploratory time when compared to mecamylamine; the mean Exploratory Time in the group with galantamine co-administration was 5604.0 ms (429.1 – 10779, p<0.05) during the Immediate condition and 1740.3 ms (304.1 – 3176.6, p<0.05) during the Delayed condition. Nicotine co-administration slowed the mean Exploratory Time in the Delayed condition by 1976.8 ms (505.9 – 3447.6, p<0.01) when compared to the mecamylamine group.

VISUAL ANALOGUE SCALES ✳ Mecamylamine induced no significant differences compared to placebo on the mean VAS evaluating calmness and mood. A significant overall treatment effect was detected on the mean VAS alertness (overall treatment effect $p<0.05$) and nausea ($p<0.0001$). Mecamylamine administration produced a significant decrease in the mean subjective feeling of alertness by -1.82 mm (-3.61 – -0.02, $p<0.05$) on the VAS scale (Figure 4.1). This was not significantly reversed by either galantamine or nicotine.

Mecamylamine plus galantamine increased the mean VAS nausea measurement 90% (47% – 146%, $p<0.0001$; back-transformed), and the combination with nicotine caused an increase of 53% (19% – 98%, $p<0.005$; back-transformed) compared to mecamylamine alone.

PHYSIOLOGIC MEASURES

VITAL SIGNS ✳ Examination of the mean standing systolic blood pressure (SBP) by time showed a significant overall treatment effect ($p<0.005$). While mecamylamine non-significantly decreased the mean standing SBP by -5.3 mmHg, nicotine co-administration produced an additional decrease of -8.8 mmHg (-16.1 – -1.6, $p<0.05$) when compared to mecamylamine alone (Table 4.1). A significant overall treatment effect in standing and supine position ($p<0.0001$) was observed in both position measurement of the heart rate. Mecamylamine administration produced an increase in heart rate in supine (mean 12.3 bpm [9.7 – 14.9], $p<0.0001$) and standing (mean 26.7 bpm [19.7 – 33.8], $p<0.0001$) positions. Co-administration of nicotine and galantamine did not influence the heart rate significantly. There were no changes in the body temperature in any of the groups compared to placebo.

There were no clinically significant changes in values for hematology, chemistry and urinalysis parameters.

ADVERSE EVENTS (AE) ✳ AE were less frequently reported in the placebo group (46.4%), followed by the galantamine (89.3%), nicotine (89.7%) and finally the mecamylamine (93.1%) group had the highest incidence of AEs in the trial. Table 4.2 displays the most incident AEs per treatment group. No severe or serious AEs were reported.

DISCUSSION

A consistent pattern was observed after mecamylamine was administered: healthy subjects performed worse compared to placebo across cognitive and neurophysiological tests evaluating attention, motor fluency, visual (fine) motor coordination, short- and long-term memory and reaction time. Mecamylamine *in vitro* non-competitively antagonizes the most important central nicotinic receptors, $\alpha_3\beta_4$, α_7 and $\alpha_4\beta_2$, related to cognitive functions (Papke *et al*, 2001). These receptors are situated principally in the prefrontal, motor and entorhinal cortex, and with lower density, in the cingular and temporal cortex, in the thalamus (principally the dorsomedial and ventrolateral nuclei) and basal ganglia in the human brain (Paterson and Nordberg, 2000). The afore-mentioned structures are associated with visuo-spatial and declarative memory, decision-making processes, integration of acquired stimuli, fine motor skills and learning, correlating with the measured mecamylamine induced effects observed as a result of nAChR blockade in this study.

Reversal of mecamylamine effects by a nAChR agonist has not been previously demonstrated in humans, probably because lower doses were used in previous experiments. In this study we provided evidence that nicotine partially reversed the effects produced after mecamylamine administration. Nicotine 21 mg administered transdermally over a period of 8 hours, significantly but not completely, reversed mecamylamine effects on the tests evaluating visual (fine) motor coordination, short- and long-term memory and reaction time. Co-administration of nicotine also appeared to reverse mecamylamine effects in tests evaluating alertness and visuo-spatial memory, but these effects were not significant. *In vivo* reversal by nicotine of the cognitive effects resulting from mecamylamine administration indicates that both drugs affect the same system, namely the nicotinic cholinergic central neuronal system. Mecamylamine is a nicotinic non-competitive antagonist that *in vitro* completely blocks the effect of nicotine on several nAChRs (Albuquerque *et al*, 2009). In order to determine the competitive effect-concentration relationship between nicotine and mecamylamine, a range of nicotine doses should be explored to better elucidate this relationship *in vivo* and determine if the partial nature of the reversal can be complete. Co-administration of a nicotinic agonist with different activity, i.e. selective $\alpha_3\beta_4$, α_7 and $\alpha_4\beta_2$ agonist, should produce different profiles in the different cognitive areas and may help better characterize the drug *in vivo,* a reason for nicotine to reverse almost all test where mecamylamine had an effect, except for tests evaluating motor fluency and verbal short- and long-term memory. Different cognitive profiles with different nicotinic agonist might provide a functional challenge model with an interesting proof-of-pharmacology profile.

While galantamine appeared to reverse mecamylamine induced cognitive effects, the differences with placebo were not significant in any of the mecamylamine-induced cognitive or neuro-physiological effects except for the reaction time during the 2-back test. Galantamine has been reported to reverse electroencephalographic and sedative disturbances produced by scopolamine. One possible explanation might be that in the scopolamine study in which partial reversal by galantamine was shown, a galantamine dose of 0.5 mg · kg^{-1} was used (Baraka and Harik, 1977), while in the current study the dose was on average 0.21 mg · kg^{-1}. We expected that the 'direct' reversal of a nicotinic antagonist by a nicotinic would require a lower concentration range than 'indirect' reversal of a muscarinic antagonist. Still, we cannot exclude that higher galantamine doses would have produced a more extensive reversal of mecamylamine-induced cognitive effects. Even though a higher galantamine dose in this study was considered, the expected side effects (severe nausea and vomiting) in healthy subjects after an acute administration of galantamine was an important argument not to administer higher doses of galantamine. In

retrospect, this was the right decision, as in this study there was already a high incidence of adverse events related to the mechanism of action of the drug (see Table 4.2).

Mecamylamine produced in the EEG a decrease in β frequency power in the posterior bipolar leads of the surface EEG, and also led to a non-significant decrease in α power and an increase in ϑ power, which corresponds to reports from previous studies with mecamylamine (Pickworth *et al*, 1997). A decrease in posterior α power and an increase in frontal and posterior θ power has also been observed in patients with Alzheimer's disease (van Straaten *et al*, 2014). Nicotine significantly diminished the decrease in α and β power induced by mecamylamine in the posterior leads of the EEG, mainly at the last time points (>300 minutes), producing an even greater increase when compared to placebo. The T_{max} during transdermal nicotine patch administration is reported at 6 hours (360 minutes), consistent with the time where the maximum effect was observed in the EEG (DeVeaugh-Geiss *et al*, 2010). The increase of the β power at the end of the trial observed in the EEG could be explained by a difference in the T_{max}, of mecamylamine and nicotine.

Administration of a single dose of 30mg of mecamylamine was safe, and generally tolerated well enough for a challenge model involving cognitive testing. The most common AEs in the active groups were known symptoms related to gastrointestinal and central nervous system AChR agonists administration. Nausea and vomiting were the most frequently reported adverse events on occasions where nicotine and galantamine were co-administered. It could be postulated that the mechanism for the nausea and vomiting is related to the high density of α_3, α_4, β_2 and to a less extent α_5 and β_4 nAChRs in the area postrema (Léna and Changeux, 1997). Although we deliberately enrolled sporadic smokers in the study to avoid nausea due to administration of nicotine 21 mg (the approved starting dose for patients willing to abstain from smoking) a high incidence of nausea and vomiting was still observed. Mecamylamine decreased the BP in supine and standing positions, only significantly different compared to the placebo group in standing position. Blockage of the sympathetic system by mecamylamine and its effects on the BP has been extensively studied and described before in patients with hypertension, however not in healthy subjects (Ford *et al*, 1956). Mecamylamine effect on BP in healthy subjects mainly impaired the compensatory mechanisms of orthostatic hypotension.

Scopolamine 0.5 mg induced in previous studies in healthy subjects a higher incidence of somnolence (ranging from 24.0 to 58.3%; unpublished data) and dizziness (ranging from 48.0 to 76.9%; unpublished data) when compared to mecamylamine 30 mg (dizziness 17.2 and somnolence 34.5%) as shown in this study in Table 4.2. The decrease in attention after mecamylamine administration might suggest that this is not due to sedation (as with muscarinic antagonists) but to impairment of attention/concentration due to mecamylamine, suggesting that mecamylamine as challenge drug might be preferred to induce cognitive impairment with fewer sedative effects. Donepezil 5 mg has been reported as the only drug that partially reversed the effects induced by mecamylamine 20 mg in healthy subjects, which consisted of slowing of the inspection time during visual discrimination (Thompson *et al*, 2000). Similar to our study in humans, mecamylamine-induced cognitive effects were significantly reversed by nicotine in mice. In this animal study, however, nicotine did not reverse scopolamine induced effects (Levin *et al*, 1997). While numerous groups have been able to demonstrate reversal of scopolamine effects by co-administration of compounds with nAChR agonist activity in animal models, none of these results were ever reproduced in humans with the mecamylamine challenge model. The proposed mecamylamine model therefore seems superior to the scopolamine challenge model to use in translational and early phase clinical drug studies investigating novel nicotinic agonists.

In conclusion, we have confirmed in humans that a single dose of mecamylamine 30 mg induces a significant disturbance in cognitive functions such as visual (fine) motor coordination, short- and long-term memory, reaction time and changes in the EEG (decrease in α and increase in ϑ power), and that these effects could be partially reversed by the co-administration of nicotine. This suggests that the mecamylamine challenge model can be used for proof-of-pharmacology studies nAChR agonists in humans, providing a useful tool in drug development of cognition enhancing compounds currently being developed to treat Alzheimer's disease and schizophrenia, between other diseases.

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TABLE 4.1 Mean differences (contrasts) and Least Squared (LS) means per treatment group on the neuro- and physiological parameters.

	LS Means					Contrasts		
Parameter	Placebo	Mecamylamine	Mecamylamine + Galantamine	Mecamylamine + Nicotine	Treatment effect p-value	Mecamylamine vs. placebo	Mecamylamine + Galantamine vs Mecamylamine	Mecamylamine + Nicotine vs. Mecamylamine
Adaptive tracking (%)	31.05	27.78	28.00	29.24	p <0.0001	-3.27 (-4.58--1.97) p<0.0001	0.223 (-1.09-1.537) p=0.7355	1.467 (0.153-2.780) p=0.0292
Taps: Mean of 5 trials (n/10 sec)	64.30	59.00	58.41	58.84	p <0.0001	-5.30 (-6.80--3.80) p<0.0001	-.586 (-2.08-0.909) p=0.4367	-.158 (-1.66-1.341) p=0.8343
Simple reaction time task (sec)	288.13	293.41	294.92	298.54	p =0.4640	1.8% (-2.6%-6.4%) p=0.4166	0.5% (-3.9%-5.1%) p=0.8182	1.7% (-2.7%-6.4%) p=0.4429
N-back corr-incorr/total 0	0.94	0.92	0.92	0.93	p =0.0410	-.023 (-.044--.003) p=0.0270	-.006 (-.027-0.015) p=0.5784	0.007 (-.013-0.028) p=0.4839
N-back mean RT 0 back (msec)	447	455	451	445	p =0.7043	8.0 (-9.8-25.8) p=0.3721	-4.0 (-21.8-13.9) p=0.6588	-9.6 (-27.4-8.2) p=0.2836
N-back corr-incorr/total 1	0.90	0.89	0.87	0.90	p =0.0602	-.015 (-.038-0.008) p=0.2021	-.013 (-.036-0.010) p=0.2634	0.015 (-.009-0.038) p=0.2184
N-back mean RT 1 back (msec)	497	503	504	498	p =0.7850	6.2 (-10.0-22.4) p=0.4485	0.9 (-15.3-17.2) p=0.9076	-4.5 (-20.8-11.9) p=0.5873
N-back corr-incorr/total 2	0.84	0.82	0.80	0.84	p =0.1079	-.018 (-.049-0.014) p=0.2664	-.016 (-.048-0.015) p=0.3043	0.018 (-.014-0.049) p=0.2689
N-back mean RT 2 back (msec)	575	603	576	567	p =0.0432	28.3 (2.0-54.6) p=0.0356	-27.2 (-53.5--0.8) p=0.0437	-36.0 (-62.2--9.7) p=0.0079
Word recall correct 1	9.4	8.4	8.2	8.1	p =0.2031	-1.00 (-2.32-0.32) p=0.1344	-0.19 (-1.52-1.15) p=0.7806	-0.30 (-1.64-1.05) p=0.6601
Word recall correct 2	14.3	12.5	12.4	12.9	p =0.0790	-1.79 (-3.39--0.20) p=0.0281	-0.10 (-1.71-1.52) p=0.9040	0.37 (-1.26-2.00) p=0.6534
Word recall correct 3	16.9	15.6	15.0	15.5	p =0.1835	-1.29 (-2.97-0.39) p=0.1314	-0.53 (-2.23-1.17) p=0.5365	-0.04 (-1.76-1.68) p=0.9664
Delayed word recall correct	12.1	10.9	9.7	11.0	p =0.0470	-1.22 (-2.89-0.45) p=0.1502	-1.24 (-2.93-0.45) p=0.1484	0.08 (-1.63-1.79) p=0.9275
Delayed word recognition correct	25.1	23.2	22.7	23.5	p =0.0284	-1.87 (-3.46--0.28) p=0.0220	-0.49 (-2.10-1.12) p=0.5440	0.29 (-1.33-1.91) p=0.7200
Delayed word recog RT corr (msec)	903.1	925.1	912.3	941.1	p =0.4593	21.98 (-26.4-70.34) p=0.3681	-12.8 (-61.7-36.12) p=0.6039	15.99 (-33.4-65.40) p=0.5213
EEG α Fz-Cz (uV)	3.07	3.05	3.17	3.14	p =0.7186	-0.6% (-7.9%-7.3%) p=0.8760	3.9% (-3.8%-12.2%) p=0.3262	3.0% (-4.7%-11.2%) p=0.4518
EEG α Pz-Oz (uV)	5.48	5.14	5.48	5.91	p =0.0132	-6.2% (-13.4%-1.6%) p=0.1149	6.7% (-1.6%-15.6%) p=0.1124	14.9% (6.0%-24.6%) p=0.0012
EEG β Fz-Cz (uV)	2.08	2.03	2.00	2.13	p =0.1292	-2.6% (-7.9%-3.1%) p=0.3628	-1.4% (-6.8%-4.5%) p=0.6362	5.3% (-0.6%-11.5%) p=0.0778
EEG β Pz-Oz (uV)	2.42	2.25	2.35	2.49	p =0.0439	-7.1% (-13.7%--0.1%) p=0.0474	4.5% (-2.8%-12.5%) p=0.2315	10.7% (2.9%-19.1%) p=0.0068
EEG δ Fz-Cz (uV)	2.06	2.04	1.99	1.99	p =0.4487	-1.0% (-6.3%-4.6%) p=0.7281	-2.7% (-8.0%-2.9%) p=0.3282	-2.6% (-7.8%-3.0%)p=0.3563
EEG δ Pz-Oz (uV)	2.04	2.08	2.05	2.02	p =0.9445	1.8% (-6.8%-11.3%) p=0.6822	-1.1% (-9.5%-8.1%) p=0.8062	-2.6% (-11.0%-6.5%) p=0.5571
EEG γ Fz-Cz (uV)	0.66	0.65	0.66	0.68	p =0.3345	-0.6% (-6.1%-5.3%) p=0.8386	0.8% (-4.9%-6.8%) p=0.7872	5.0% (-0.9%-11.2%) p=0.0987
EEG γ Pz-Oz (uV)	0.64	0.63	0.61	0.67	p =0.3225	-1.4% (-10.8%-9.1%) p=0.7825	-2.2% (-11.6%-8.2%) p=0.6580	7.3% (-3.0%-18.7%) p=0.1708
EEG ϑ Fz-Cz (uV)	2.37	2.51	2.51	2.48	p =0.2088	5.7% (-0.5%-12.3%) p=0.0735	0.2% (-5.8%-6.6%) p=0.9437	-0.9% (-6.8%-5.4%) p=0.7755
EEG ϑ Pz-Oz (uV)	2.55	2.77	2.79	2.66	p =0.1770	8.8% (-0.8%-19.2%) p=0.0716	0.9% (-7.9%-10.6%) p=0.8385	-3.8% (-12.1%-5.4%) p=0.4066
VAS Alertness (mm)	48.9	47.1	45.8	47.2	p =0.0124	-1.82 (-3.61--0.02) p=0.0470	-1.30 (-3.13-0.53) p=0.1610	0.17 (-1.63-1.97) p=0.8539
VAS Calmness (mm)	52.9	53.8	53.7	53.2	p =0.6272	0.85 (-0.66-2.35) p=0.2641	-0.04 (-1.55-1.47) p=0.9533	-0.54 (-2.10-1.02) p=0.4906
VAS Mood (mm)	52.1	52.5	51.8	51.8	p =0.5487	0.44 (-0.73-1.60) p=0.4567	-0.74 (-1.91-0.42) p=0.2060	-0.73 (-1.91-0.45) p=0.2199
VAS Nausea log(mm)	0.331	0.365	0.644	0.551	p <0.0001	.0337 (-.079-.1462) p=0.5526	.2787 (.1670-.3903) p<0.0001	.1860 (.0746-.2974) p=0.0013
Left Pupil/Iris ratio	0.4807	0.5003	0.5060	0.4879	p =0.1729	0.01960 (-0.00506-0.04426) p=0.1177	0.00574 (-0.01896-0.03044) p=0.6449	-0.01235 (-0.03702-0.01231) p=0.3220
Right Pupil/Iris ratio	0.4944	0.5066	0.5101	0.4886	p =0.1946	0.01217 (-0.01007-0.03441) p=0.2795	0.00353 (-0.01870-0.02576) p=0.7529	-0.01797 (-0.04023-0.00430) p=0.1123
MMT_Imm: Exploratory Moves	192	192	194	194	p =0.6157	-0.3 (-4.4-3.7) p=0.8725	2.0 (-2.0-6.1) p=0.3250	2.2 (-1.9-6.3) p=0.2930
MMT_Imm: Exploratory Errors	23	23	24	24	p =0.6191	-0.2 (-2.2-1.8) p=0.8226	1.0 (-1.0-3.0) p=0.3366	1.1 (-0.9-3.2) p=0.2688
MMT_Imm: Exploratory Time (msec)	74337	76533	82137	79947	p =0.0167	2195 (-3024-7414) p=0.4042	5604 (429.1-10779) p=0.0342	3415 (-1731-8560) p=0.1899
MMT_Rev: Exploratory Moves	38	38	38	37	p =0.3082	0.3 (-0.9-1.5) p=0.6378	-0.3 (-1.5-1.0) p=0.6823	-1.1 (-2.4-0.1) p=0.0749
MMT_Rev: Exploratory Errors	4	4	4	4	p =0.4044	0.1 (-0.5-0.7) p=0.7542	-0.1 (-0.7-0.5) p=0.6960	-0.5 (-1.1-0.1) p=0.1128
MMT_Rev: Exploratory Time (msec)	16826	17439	17016	17286	p =0.9342	612.6 (-1422-2648) p=0.5501	-423 (-2488-1643) p=0.6845	-153 (-2196-1889) p=0.8815
MMT_Del: Exploratory Moves	37	37	36	36	p =0.5768	-0.1 (-1.3-1.0) p=0.7952	-0.6 (-1.7-0.5) p=0.2993	-0.3 (-1.4-0.9) p=0.6292
MMT_Del: Exploratory Errors	4	4	3	4	p =0.7074	-0.1 (-0.6-0.5) p=0.8285	-0.2 (-0.8-0.3) p=0.3747	-0.1 (-0.6-0.5) p=0.7600
MMT_Del: Exploratory Time (msec)	15770	14662	16402	16639	p =0.0388	-1108 (-2541-325.2) p=0.1277	1740 (304.1-3177) p=0.0182	1977 (505.9-3448) p=0.0091
Diastolic BP standing (mmHg)	70	66	65	61	p =0.0088	-4.3 (-9.3-0.7) p=0.0880	-0.9 (-5.6-3.8) p=0.7078	-4.3 (-9.2-0.5) p=0.0769
Diastolic BP supine (mmHg)	62	63	63	62	p =0.6500	0.6 (-1.3-2.6) p=0.5105	0.3 (-1.6-2.2) p=0.7535	-0.8 (-2.7-1.2) p=0.4372
Systolic BP standing (mmHg)	121	116	112	107	p =0.0027	-5.3 (-12.8-2.2) p=0.1623	-3.4 (-10.5-3.7) p=0.3487	-8.8 (-16.1--1.6) p=0.0177
Systolic BP supine (mmHg)	116	114	115	115	p =0.3579	-1.7 (-3.8-0.4) p=0.1099	0.2 (-1.9-2.3) p=0.8358	0.3 (-1.8-2.4) p=0.7886
Heart rate standing (bpm)	65	92	89	90	p <0.0001	26.7 (19.7-33.8) p<0.0001	-3.3 (-10.2-3.6) p=0.3383	-2.1 (-8.9-4.8) p=0.5555
Heart rate supine (bpm)	57	69	69	71	p <0.0001	12.3 (9.7-14.9) p<0.0001	-0.5 (-3.1-2.2) p=0.7224	1.3 (-1.3-3.9) p=0.3227

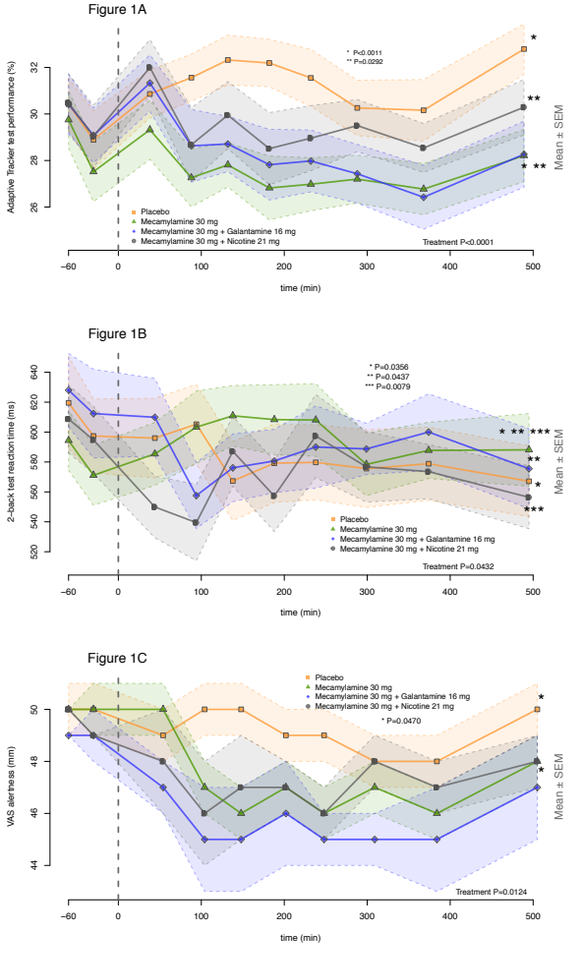
TABLE 4.2 Summary of number of subjects with an adverse event and number of adverse events with the highest incidence in descending order of incidence.

	Mecamylamine (n=29)		Mecamylamine + Galantamine (n=28)		Mecamylamine + Nicotine (n=29)		Placebo (n=28)	
Adverse event	**Nr of events**	**Nr of subjects (%)**	**Nr of events**	**Nr of subjects (%)**	**Nr of events**	**Nr of subjects (%)**	**Nr of events**	**Nr of subjects (%)**
All Events	76	27 (93.1)	101	25 (89.3)	108	26 (89.7)	26	13 (46.4)
Nausea	3	3 (10.3)	15	14 (50.0)	12	12 (41.4)	-	-
Somnolence	14	10 (34.5)	12	12 (42.9)	11	10 (34.5)	1	1 (3.6)
Dizziness	5	5 (17.2)	13	11 (39.3)	13	11 (37.9)	1	1 (3.6)
Fatigue	8	7 (24.1)	8	8 (28.6)	6	6 (20.7)	4	4 (14.3)
Orthostatic hypotension	8	8 (27.6)	4	4 (14.3)	5	5 (17.2)	6	4 (14.3)
Application site pruritus	1	1 (3.4)	-	-	7	6 (20.7)	1	1 (3.6)
Ocular hyperemia	3	2 (6.9)	2	2 (7.1)	6	6 (20.7)	-	-
Vision blurred	6	5 (17.2)	1	1 (3.6)	4	4 (13.8)	-	-
Constipation	5	4 (13.8)	2	2 (7.1)	5	5 (17.2)	-	-
Vomiting	1	1 (3.4)	3	3 (10.7)	4	4 (13.8)	-	-
Headache	3	3 (10.3)	3	3 (10.7)	6	6 (20.7)	4	2 (7.1)
Dizziness postural	2	1 (3.4)	1	1 (3.6)	3	3 (10.3)	-	-
Abdominal pain	3	3 (10.3)	3	3 (10.7)	2	2 (6.9)	-	-
Feeling abnormal*	1	1 (3.4)	3	3 (10.7)	-	-	2	2 (7.1)
Abdominal distension	1	1 (3.4)	3	3 (10.7)	-	-	-	-

** Feeling abnormal was used by the research physician when no other symptom could describe the feeling the subject was experiencing.*

FIGURE 4.1 Effect on Tests Evaluating Fine Coordination, Reaction Time, Attention and Alertness.

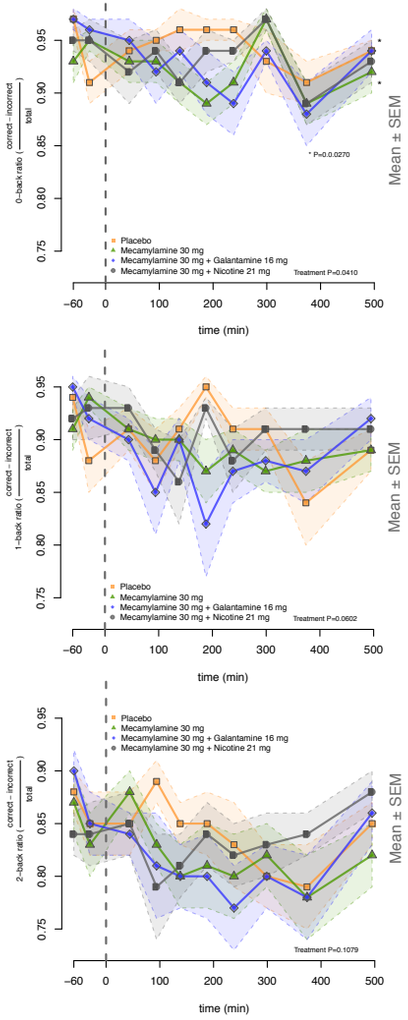
Mecamylamine, nicotine and galantamine effect versus time during the Adaptive Tracking test, Reaction Time during the 2-back condition and Visual Analogue Scale evaluating Alertness.



Symbols represent the mean per treatment group and the polygon (shaded area around the mean) the standard error. Asterisks represent significance between groups (p value is mentioned per overall treatment effect and per group, when applicable). Vertical discontinuous line represents time point zero.

FIGURE 4.2 Effect on Tests Evaluating Short and Long Term Retrieval.

Mecamylamine, nicotine and galantamine effect versus time during the o-back condition Ratio of Correct-Incorrect answers. Symbols represent the mean per treatment group and the polygon (shaded area around the mean) the standard error. Asterisks represent significance between groups (*p* value is mentioned per overall treatment effect and per group, when applicable). Vertical discontinuous line represents time point zero.



Asterisks represent significance between groups (p value is mentioned per treatment and per group, when applicable). Mecamylamine, nicotine and galantamine effect versus time during the Delayed Word Recognition condition of the Verbal Visual Learning Test number of correct answers. The box plots represent the first and third quartile, the middle line the group mean and the vertical lines the confidence interval. Individual observations are plotted as well.

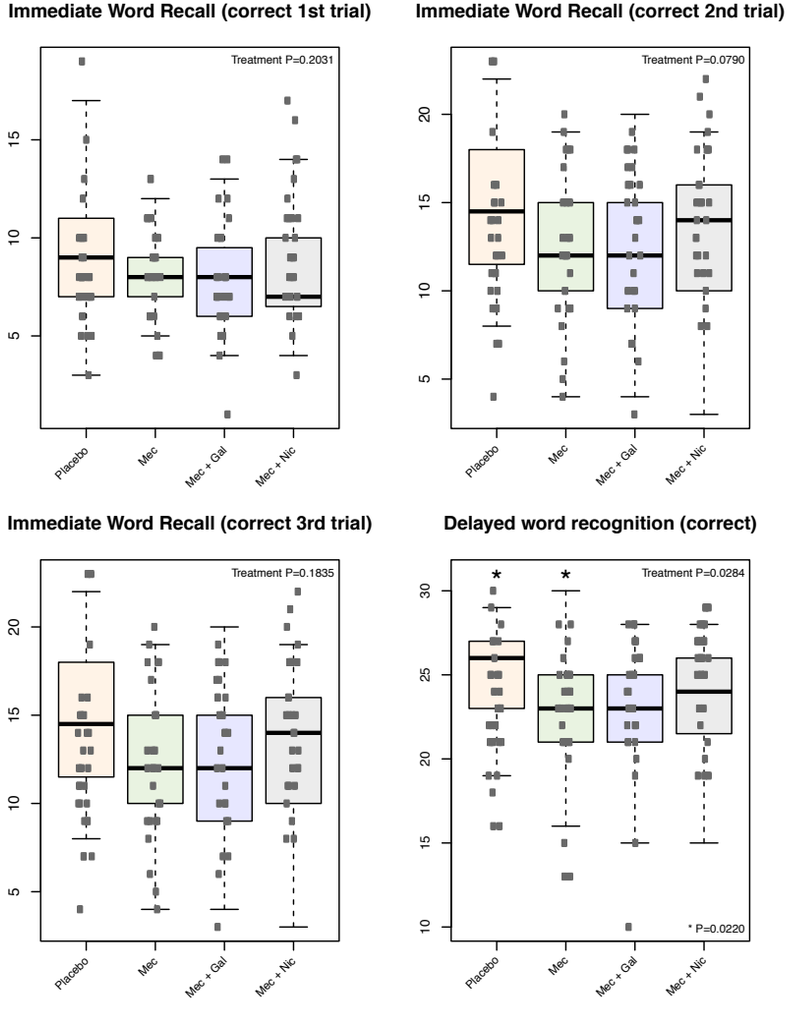
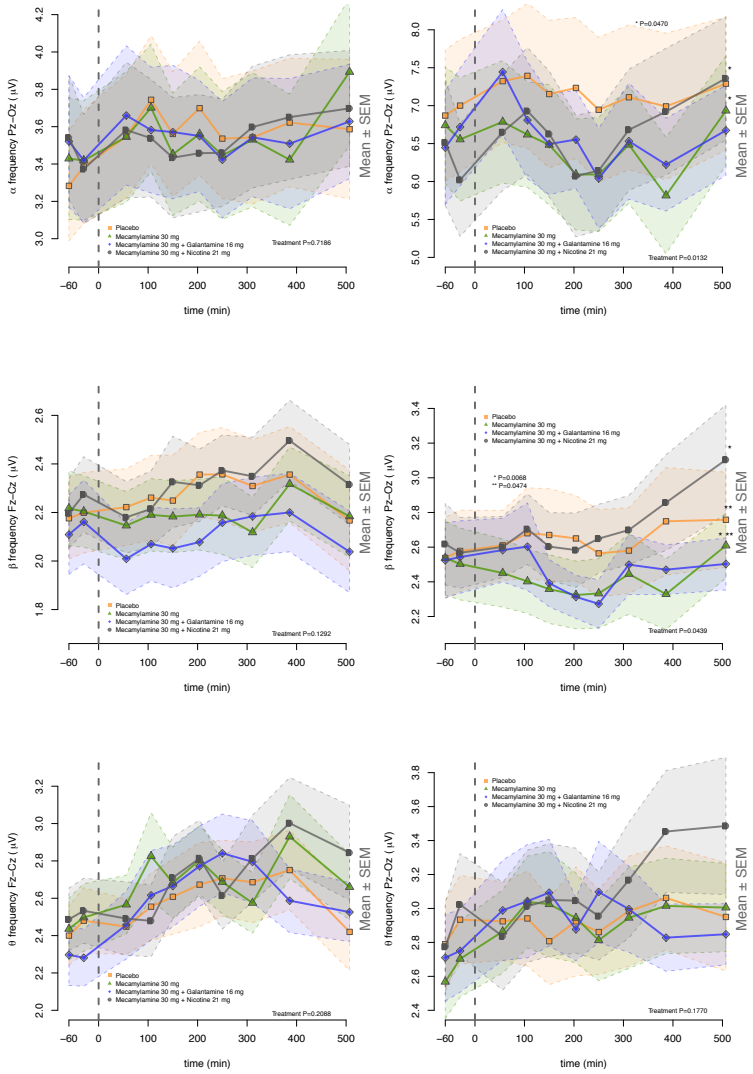


FIGURE 4.3 Effect on the Electro-Encephalogram.

Mecamylamine, nicotine and galantamine effect versus time for the EEG *α*, *β* and *θ* frequency.



Symbols represent the mean per treatment group and the polygon the standard error around the mean. Asterisks represent significance between groups (p value is mentioned per treatment and per group, when applicable). The vertical discontinuous line represents time point zero