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An EEG nicotinic acetylcholine index to assess the efficacy of pro-cognitive compounds

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HIGHLIGHTS

- We developed an EEG index sensitive to mecamylamine, for monitoring nicotinic cholinergic effects.
- We demonstrate reversal of mecamylamine-induced EEG disturbances both with nicotine and galantamine.
- The nicotinic acetylcholine receptor index could enable quantifying EEG effects of pro-cognitive cholinergic compounds.

ABSTRACT

Objectives: Cognitive impairment models are used in clinical studies aimed at proving pharmacology of drugs being developed for Alzheimer's disease and other cognitive disorders. Due to rising interest in nicotinic agonists, we aimed to establish a method to monitor neurophysiological effects of modulating the nicotinic cholinergic system.
Methods: In a four-way cross-over study, eyes-closed rest EEG was recorded in 28 healthy subjects receiving mecamylamine—a nicotinic acetylcholine receptor (nAChR) antagonist, which induces temporary cognitive dysfunction in healthy subjects—with co-administration of placebo, nicotine or galantamine.
Results: Using machine learning to optimally contrast the effects of 30 mg of mecamylamine and placebo on the brain, we developed a nAChR index that consists of 10 EEG biomarkers and shows high classification accuracy (~95% non-cross-validated, ~70% cross-validated). Importantly, using the nAChR index, we demonstrate reversal of mecamylamine-induced neurophysiological effects due to 16 mg of galantamine as well as administering 21 mg of nicotine transdermally.
Conclusions: Our findings indicate that the mecamylamine challenge model jointly with the nAChR index—a measure of the nicotinic EEG profile—could aid future proof-of-pharmacology studies to demonstrate effects of nicotinic cholinergic compounds.
Significance: This novel measure for quantifying nicotinic cholinergic effects on the EEG could serve as a useful tool in drug development of pro-cognitive compounds.

1. Introduction

The cholinergic system is involved in cognitive processes such as attention, memory and learning (Jones et al., 1999; Terry and Buccafusco, 2003; Levin et al., 2006; Nees, 2015) and it holds promise as a therapeutic target due to its role in the pathophysiology of neurodegenerative and psychiatric disorders (Court et al., 2000; Sacco et al., 2004; Parri et al., 2011). Hence, anti-cholinergic pharmacological challenges have been used to induce temporary cognitive disturbances mimicking Alzheimer's disease (AD), scopolamine being the most frequently used challenge drug. Scopolamine is a selective competitive muscarinic acetylcholine receptor antagonist, with a high affinity for all muscarinic receptor subtypes (Ebert and Kirch, 1998). Recently, there is increased

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interest in the nicotinic acetylcholine receptor (nAChR) as a pharmacological target, as reflected in various clinical trials with nAChR agonists (Hurst et al., 2013; Valles et al., 2014; Beinat et al., 2015). Nicotinic receptor agonists are being developed as symptomatic treatment for cognitive dysfunction in AD and schizophrenia. However, the use of muscarinic receptor antagonists to prove the pharmacology of nicotinic receptor agonists is less direct, therefore a pharmacological challenge model targeting nicotinic cholinergic systems is needed.

Recently, mecamylamine has regained attention as a potential anti-nicotinergic challenge model to prove pharmacology of nicotinic agonists (Alvarez-Jimenez et al., 2017). Mecamylamine is a selective non-competitive nAChR antagonist, active in peripheral autonomic ganglia, but also binding to nAChRs in the brain. In healthy subjects, mecamylamine leads to temporary, reversible perturbations of cognitive processes such as attention, reaction time and memory. Mecamylamine produced errors in learning, liberalization of response bias and increased reaction times (Newhouse et al., 1992, 1994) and slowing of inspection time during a visual discrimination test (Thompson et al., 2000). The effects of mecamylamine are smaller compared to scopolamine (Baakman et al., 2017). However, scopolamine also exhibits sedative effects, which interferes with cognitive functions (Curran et al., 1991; Robbins et al., 1997). To use mecamylamine as a credible anti-cholinergic challenge, it is necessary to demonstrate reversibility of its effects on cognition and physiology.

In humans, co-administration of donepezil partially reverses mecamylamine-induced cognitive effects (Thompson et al., 2000). To the best of our knowledge, no study investigated reversal of mecamylamine effects using EEG. Here, we aimed to further validate mecamylamine as a nicotinic anti-cholinergic challenge model by identifying effects of mecamylamine on EEG and investigating their reversal by co-administration of galantamine or nicotine. Galantamine is a cholinesterase inhibitor, which also exerts allosteric nicotinic modulatory activity and is used to treat patients with AD. Nicotine is a nAChR agonist, which acts on all nicotinic receptors that are believed to play a role in cognitive function (Levin et al., 2006; Jasinska et al., 2014). Using EEG for proof-of-pharmacology in early-phase clinical trials supports cognitive tests in shedding light on physiological effects; however, it remains an important challenge to select and validate reliable biomarkers from the complex EEG signals.

Recently, we developed a muscarinic acetylcholine receptor index which performed with superior accuracy to classify scopolamine-induced EEG changes compared to any single biomarker (Simpraga et al.; 2017). Here, we used similar machine-learning procedures to develop a nicotinic cholinergic index using data from a clinical trial with healthy subjects receiving mecamylamine. The index consists of complementary EEG biomarkers that, when taken together, outperform any single-biomarker classification and, therefore, provides a superior characterization of the anti-nicotinergic challenge. We further show reversal of mecamylamine-induced effects on the nAChR index by co-administration of nicotine or galantamine. The high sensitivity of the nAChR index could be beneficial in early clinical studies, for evaluating the efficacy of drugs that aim to induce effects opposite to mecamylamine, such as those for treatment of AD.

2. Methods

2.1. Study design

Data were obtained from a clinical trial conducted at the Centre for Human Drug Research (Leiden, the Netherlands) and approved by the Medical Ethics Review Committee of the Leiden University Medical Center. All subjects gave written informed consent prior to study participation and were medically screened.

The trial (EudraCT number 2014-001358-41) was a four-way cross-over study of a single oral dose of mecamylamine in combination with either a cholinesterase inhibitor or a nAChR agonist and matching placebos, as described previously (Alvarez-Jimenez et al., 2017). The study aimed to demonstrate whether the impairment of cognitive function caused by mecamylamine administration could be diminished by administration of galantamine or nicotine. A detailed description of neurophysiologic tests performed in this study has been reported previously (Liem-Moolenaar et al., 2010b, a; Alvarez-Jimenez et al., 2017). A total of 33 healthy-smoker male subjects aged 18–45 years were recruited. However, some subjects had missing measurements, so the remaining number of subjects available for analysis in the placebo and mecamylamine condition was 28. No subjects or recordings were excluded from the analysis. Healthy status is defined by the absence of evidence of any active or chronic disease following a detailed medical and surgical history, a complete physical examination including vital signs, 12-lead electrocardiogram (ECG), haematology, blood chemistry, and urinalysis. Exclusion criteria included the use of agents or drugs known to influence cognitive performance and evidence of relevant medical abnormalities including conditions that could cause any kind of cognitive impairment. We did not use additional structured assessment tools to ascertain healthy status. Incidental smokers, defined as smoking tobacco at least once a month and no more than 5 cigarettes per day in the last 3 months, were included because non-smokers might have experienced more severe side effects derived from the nicotine and galantamine administration. The treatment arms were: mecamylamine plus placebo, mecamylamine plus nicotine, mecamylamine plus galantamine and (double)placebo. Investigational drugs were administered in the following way: mecamylamine (30 mg) and galantamine hydrobromide (16 mg) orally, nicotine (21 mg) as a transdermal patch. Placebo was administered as matching placebo capsules, in appearance identical to active compounds (mecamylamine and galantamine), orally and as placebo (vaseline) patch with blinded covering.

Study periods were separated by a washout period of at least one week. Eyes-closed rest EEG measurements were performed at 10 time points from baseline (pre-dose) to 8 h after the drug administration. Measurements would start in the morning, with two baseline recordings and then 0.5 h, 1.2 h, 2.1 h, 3 h, 3.5 h, 4.4 h, 6 h and 8 h afterwards. Oral medication (mecamylamine/galantamine/placebo) was administered with water at time point zero of every visit. Five minutes afterwards, a nicotine or placebo patch was placed on the skin of the shoulder. Apart from the EEG recordings, a study period day consisted of performing tests such as the aforementioned neurophysiological tests, vital signs measurements and ECG at several time points. Subjects were discharged 32 h post-dose after monitoring of vital signs was performed and if subjects were asymptomatic.

2.2. EEG recordings and pre-processing

EEG recordings were made using gold electrodes fixed at Fz, Cz, Pz, and Oz positions (international 10/20 system), with the same common ground electrode as for the eye movement registration. The impedance was kept below 5 kΩ. EEG signals were obtained from leads Fz-Cz and Pz-Oz and a separate channel to record eye movements (for artefacts). The signals were amplified by use of a Grass 15LT series Amplifier Systems with a time constant of 0.3 s and a low-pass filter at 100 Hz. The duration of the recordings was 64 s. Sampling frequency was 64768 Hz, afterwards down-sampled to 1012 Hz for the analysis. The ongoing EEG was visually inspected in windows of 10 s and sharp transient artefacts were

cut out, as well as eye movement and muscle artefacts. Noisy channels were excluded from the subsequent analysis. After artefact removal, the average length of recordings was 61 s. In 2.5% of the recordings, one noisy channel was removed.

2.3. EEG analysis

For the EEG analysis, we used the Neurophysiological Biomarker Toolbox (NBT) (http://www.nbtwiki.net/) (Hardstone et al., 2012) to calculate biomarkers and custom-made scripts were integrated with the NBT analysis pipeline for advanced statistics, employing data mining algorithms to combine information from multiple biomarkers. We employed biomarker algorithms to extract both temporal and spectral information from EEG signals in frequency bands: delta (1–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–30 Hz). The power in these frequency bands was computed using the Welch method with an 8192-point Hamming window and a frequency resolution of 0.12 Hz. Relative power was calculated by dividing absolute power in each frequency band with integrated power over 1–45 Hz. Central frequency, f_c, and bandwidth, f_δ were computed according to these formulas:

$$f_c = \frac{\sum_{f=f_L}^{f_H} fP(f)}{\sum_{f=f_L}^{f_H} P(f)} \quad (1)$$

$$f_\delta = \sqrt{\frac{\sum_{f=f_L}^{f_H} (f - f_c)^2 P(f)}{\sum_{f=f_L}^{f_H} P(f)}}, \quad (2)$$

where f_L and f_H represent the lowest and highest frequency that defines a given frequency band (Vural and Yildiz, 2010), and $P(f)$ denotes power at frequency f. Thus, the central frequency biomarker indicates where the power is concentrated within a frequency band, whereas the bandwidth indicates how much the power is spread out around the central frequency.

The amplitude envelope was extracted using the Hilbert transform and analyzed for long-range temporal correlations using detrended fluctuation analysis (DFA) (Peng et al., 1995, Linkenkaer-Hansen et al., 2001, Hardstone et al., 2012). DFA quantifies how slowly auto-correlations of amplitude modulation decay with the DFA power-law exponent ranging from 0.5 (uncorrelated) to 1.0 (strong auto-correlations). Signals were filtered using a FIR filter with a Hamming window with a length corresponding to two f_1 Hz cycles for frequency band $[f_1, f_2]$. To minimize temporal correlations introduced by the FIR filter, DFA was fitted in the interval from 4 to 20 s for delta and theta band, from 2 to 20 s for alpha and 1 to 20 s for the beta band (Hardstone et al., 2012). The oscillation-burst lifetime was used to quantify differences in amplitude dynamics of oscillations on short to intermediate time scales (< 1 s) (Montez et al., 2009, Poil et al., 2011). We used a threshold at the median of the amplitude envelope and defined beginning and end of an oscillation burst as time points of crossing this threshold. The duration of oscillation bursts was calculated by taking the 95th percentile of all durations measured within each channel, which we refer to as oscillation-burst "lifetime" biomarker (Montez et al., 2009). In total, 20 biomarkers were extracted from each EEG signal. Each biomarker was computed over two bipolar channels (Fz-Cz and Pz-Oz), therefore resulting in 40 features for classification analysis.

2.4. Statistical analysis

Machine learning techniques were employed to determine biomarkers that best distinguished mecamylamine from placebo. From time-dependent curves of EEG biomarkers, we identified the peak effect of mecamylamine on most EEG biomarkers to be 3 h after administration—in agreement with the peak drug effect time point according to cognitive measurements (Baakman et al., 2017); therefore, we performed classification on the EEG biomarkers from the recording 3 h after administration. To eliminate variation between days, we subtracted the biomarkers of the baseline recording from biomarkers at 3 h. A feature matrix was built from EEG biomarkers—in the form #features × #samples—with the aim of identifying sets of biomarkers that were more discriminative between the two groups than each individual biomarker. The list of features was as follows: relative power, central frequency, bandwidth, DFA and oscillation burst lifetime in the delta, theta, alpha and beta band, for two bipolar channels. Per subject, there were two samples: the placebo EEG recording and the mecamylamine recording. In total, this gave 40 features and 55 samples (28 placebo and 27 mecamylamine recordings). Feature selection and classification were performed simultaneously using elastic net logistic regression.

The nAChR index was identified by applying the classification algorithm to the whole dataset; however, cross-validation was used to evaluate the stability of the result, i.e., classification with 100 different splits of the data into training and test sets were performed to obtain the median and median absolute deviation of the classification performance, which provides an estimate of the classification performance on a "novel" sample (Witten et al., 2011). In the training phase, the index was developed by applying the feature-selection algorithm to training data and in the test phase, the index was applied to predict the class membership on the test data. The features used for machine learning were z-scored EEG biomarker values. To avoid introducing test data information into the classifier, we normalized both the training and the test data by subtracting the mean and dividing by the standard deviation of biomarker values from the training data only. We performed Monte-Carlo cross-validation with 70/30% random splitting, i.e., from a random permutation of the samples (i.e. recordings), 70% were used for training and 30% for testing. The number of samples is twice the number of subjects: per subject, the placebo EEG recording was used as the first sample and the mecamylamine recording as the second sample. Therefore, the total number of samples was 55. Correspondingly, the training set consisted of 39 EEG recordings and the test set 16 recordings.

2.5. Elastic net logistic regression

Because of correlation between some of the features and an interest in reducing the number of features, we chose to use the elastic net (Zou and Hastie, 2005), which has sparsity and grouping of correlated features as properties. Additionally, elastic net is an embedded method, which performs both feature selection and classification. It is a regularized logistic regression that bridges the gap between lasso (Tibshirani, 2011) and ridge regression (Hoerl and Kennard, 1970) by combining their penalties and optimizing the number of features included in the integrated index through minimizing the function:

$$L(\lambda_1, \lambda_2, \beta) = |y - X\beta|^2 + \lambda_1 ||\beta||_1 + \lambda_2 ||\beta||_2^2, \quad (3)$$

where X is the feature matrix, y is the response vector (the class labels), β the weights, and λ_1 and λ_2 coefficients determining the influence of the L_1 and L_2 norm penalties, respectively. The first term is similar to logistic regression while the second and third terms form the elastic net penalty function. If we denote:

$$\alpha = \lambda_2/(\lambda_1 + \lambda_2)$$

then the elastic net penalty can be rewritten as

$$(1 - \alpha)||\beta||_1 + \alpha||\beta||_2^2$$

where α acts as the balancing term between the L_1 and L_2 norm penalties. We optimized α in a 5-fold cross-validation procedure and found the best classification performance with $\alpha = 1$, which is the lasso regression.

By minimizing the L-function, we obtain the set of n selected features corresponding to the ones with highest β values. If p is the probability that an EEG recording belongs to the mecamylamine condition, then the odds ratio is $p/(1-p)$, which is the ratio of the probability of mecamylamine to the probability of baseline recording. Logistic regression models the log odds ratio as a linear combination of the independent variables, via this equation:

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 f_1 + \cdots + \beta_n f_n, \quad (4)$$

where f_i are the features and β_i the associated weights. The log odds can be transformed back to probabilities as:

$$p(t) = \frac{1}{1+\exp(-t)}, t = \beta_0 + \beta_1 f_1 + \cdots + \beta_n f_n. \quad (5)$$

2.6. Classification outcome evaluation

To evaluate the classification performance of the nAChR index we used four different measures, defined as:

Accuracy (AC): (number of correctly classified mecamylamine and placebo recordings)/(total number of recordings).

Sensitivity (SE): (number of correctly classified mecamylamine recordings)/(number of mecamylamine recordings).

Specificity (SP): (number of correctly classified placebo recordings)/(number of placebo recordings).

Area Under Curve (AUC): area under the Receiver Operating Characteristic (ROC) curve, which plots the true positive rate (SE) against the true negative rate (1-SP) as the discrimination threshold of the classifier is varied. A higher AUC means better classification performance.

3. Results

3.1. Enhanced classification with integrated nAChR index

To gain a comprehensive understanding of the effects of mecamylamine on the EEG, we employed biomarker algorithms characterizing spectral content as well as temporal dynamics of neuronal oscillations. A significant mecamylamine effect compared to placebo was observed for several biomarkers. Power spectrum analysis of broadband EEG signals revealed a reduction in relative power in alpha and beta bands, as well as an increment in delta and theta relative power, most notably in parieto-occipital regions. Central frequency was decreased in alpha band, while increased in the delta band. The short-time scale temporal structure of narrowband oscillations was quantified using oscillation-burst lifetime analysis and revealed a tendency towards longer alpha bursts and shorter beta bursts after mecamylamine administration. Long-range temporal correlations were quantified using detrended fluctuation analysis and were generally stronger in the mecamylamine condition across frequency bands and regions, except for the delta band. Considering that mecamylamine administration affected both spectral and temporal dynamics of the EEG and that many of these biomarkers carry complementary information about EEG effects of mecamylamine, combining this information may result in a more sensitive measure of the nicotinic anticholinergic effects compared to any of the individual ones.

We used machine learning techniques to find biomarkers that best distinguish placebo from mecamylamine conditions, at the peak effect time point. Previous studies have reported mecamylamine-induced "slowing" of the EEG, reflected in power and frequency shifts mostly in the delta, theta and alpha frequency band (Pickworth et al., 1988, Knott et al., 1997, Pickworth et al., 1997). Therefore, to identify the peak effect of mecamylamine, we inspected time-dependent curves of those biomarkers where we expected to find the strongest effects, known from the literature. Biomarkers curves were noisy due to many time points, missing data points, and few subjects but they revealed the peak effect occurring around 3 h after mecamylamine administration (Fig. 1A). Therefore, the classification was performed on EEG recorded 3 h after administration of mecamylamine subtracted by the respective baseline recording, for placebo vs mecamylamine (see Methods, Statistical analysis). An integrated index was developed using elastic net on data from healthy subjects ($n = 28$ males, see Methods) that received mecamylamine, which allows a fraction—determined by the algorithm—of the 40 available biomarkers to be included. Accuracy, sensitivity, specificity, and area under curve increased with the number of features included in the index increased to 10 (Fig. 1B). Adding more features did not improve classification performance further. Thus, the integrated nAChR index consists of 10 biomarkers and their associated weights as visualized in Fig. 1C. Descriptive statistics of the nAChR biomarkers are provided in Table 1.

The nAChR index had excellent performance when training and testing on the same data (accuracy 95%, sensitivity 93%, specificity 96% and area under curve 0.98), and much higher than relative theta power, which was the single-best biomarker (accuracy 72%, sensitivity 70%, specificity 74% and area under curve 0.78) (Fig. 1D). Accordingly, differences between the placebo predicted group and the mecamylamine predicted group (Fig. 1D) was much more pronounced for the nAChR index ($p < 10^{-9}$, Wilcoxon rank sum test) than for relative delta ($p < 10^{-4}$). The single-best biomarker was determined by performing elastic net classification using each of the features alone and then ranking them by the average of the different classification outcome measures from cross-validation. To obtain a more accurate estimate of the classification performance, we used cross-validation with 100 iterations. The difference in performance per cross-validation is due to different subsets of subjects used for training and testing in each iteration, and results in slightly different biomarker selections and weights. Cross-validation resulted in an accuracy of 69 ± 6%, sensitivity of 75 ± 13%, specificity of 75 ± 13% and area under curve 79 ± 9%, which is higher than using just relative theta power: accuracy of 69 ± 6%, sensitivity of 63 ± 13%, specificity of 75 ± 13% and area under curve 77 ± 9%.

3.2. The nAChR index consists of complementary biomarkers

To better understand why the integrated index offers more accurate monitoring of changes in brain dynamics following mecamylamine administration, we cross-correlated all biomarkers in the nAChR index, using Pearson correlation coefficient (Fig. 2). Correlations among selected biomarkers were relatively low, with the average absolute correlation of 0.25, suggesting a high degree of complementarity and reinforcing the idea that specific manipulation of receptor functioning leads to a multitude of changes in neuronal population activity and the associated EEG signals.

3.3. Reversal of mecamylamine effects with nicotine and galantamine successfully demonstrated by the nAChR index

To verify that the mecamylamine model can be used for proof-of-pharmacology, we tested the ability of nicotine and galantamine to reverse mecamylamine-induced effects. Administration of these compounds together with mecamylamine resulted in a strong reversal of effects observed when mecamylamine was adminis-

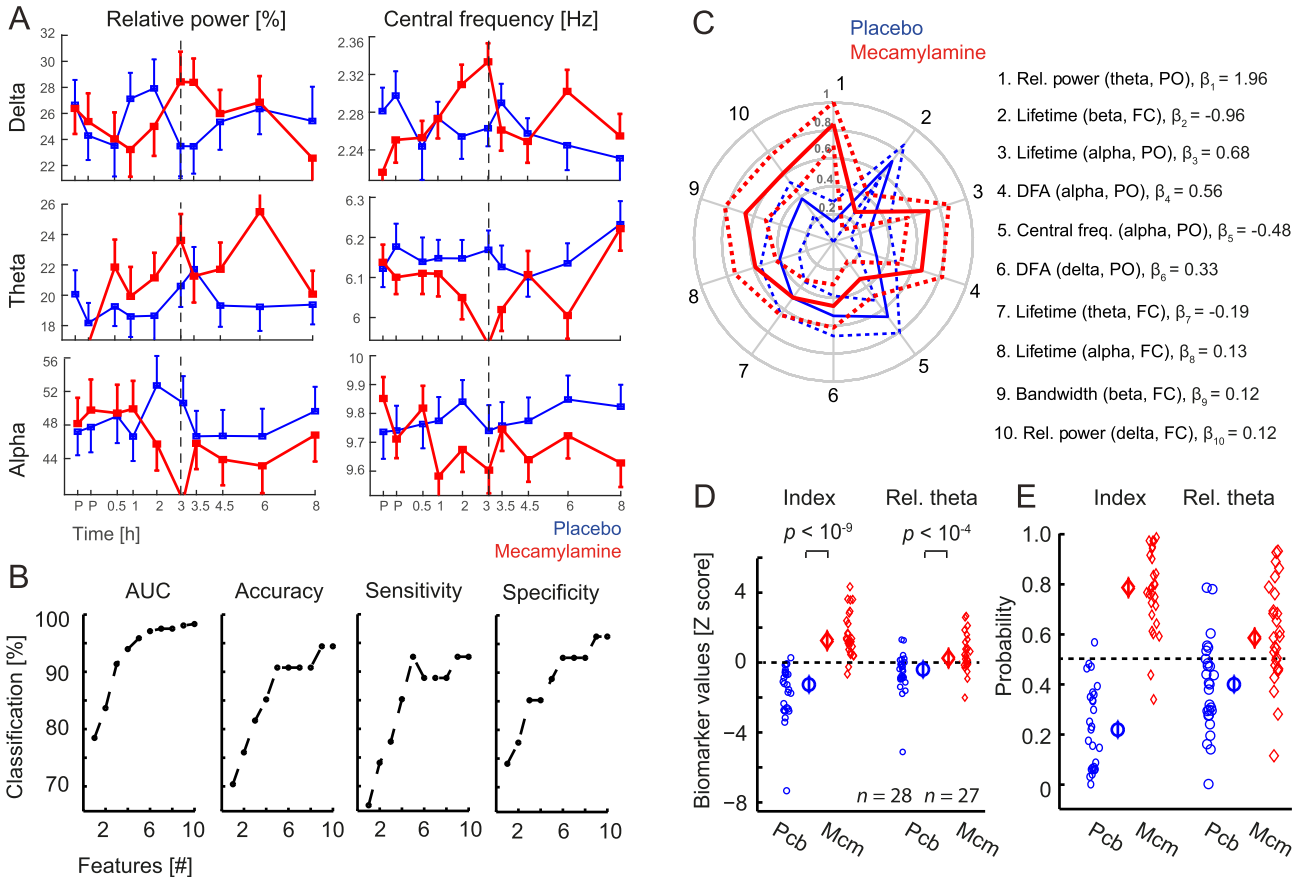

Fig. 1. Machine learning enhances the ability to detect mecamylamine-induced changes in EEG. (A) Mecamylamine affects several EEG characteristics, with the effect peaking around 3 h for most biomarkers. Time dependence curves of relative power and central frequency (columns) for delta, theta and alpha frequency bands (rows) are shown for placebo (*blue*) and mecamylamine (*red*). All biomarkers are shown as averages over the 2 channels (Fz-Cz and Pz-Oz). Time denotes time elapsed from administration of mecamylamine/placebo and P stands for pre-dose (before administration). Vertical dashed line points to the peak effect time point (3 h). (B) Classification performance increased with the number of features included in the integrated index. (C) Nearly all of the biomarkers selected by elastic net logistic regression for inclusion in the integrated nAChR index differed significantly between placebo and mecamylamine. Biomarkers are ordered by their absolute weights, decreasing clockwise from the top. Weights (β) are listed next to each biomarker in the legend (PO denotes Pz-Oz and FC denotes Fz-Cz). The values plotted on the spider plot are the z-score group means and standard error of the mean, normalized to [0, 1] by subtracting the minimum across all biomarkers and dividing with the largest range present (i.e., the difference between the minimum and maximum value found for the biomarkers with the largest difference). (D) The nAChR index was more sensitive to the mecamylamine intervention than relative theta power ($p < 10^{-8}$, paired-samples t-test, performed on the differences between placebo and mecamylamine with matched subjects). The plot shows z-scored biomarker values per subject recording. Singled-out symbols represent median values per group with standard error bars. The dashed line indicates the threshold of the classifier to predict the recordings as a placebo (below) or a mecamylamine (above) recording. Pcb denotes placebo and mcm mecamylamine. (E) Same as (D) but instead of z-scored biomarker values, predictive probabilities obtained from the classifier are shown. Significance levels for this figure are based on the Wilcoxon signed rank test.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Effects of mecamylamine intervention on the nAChR index biomarkers, at the peak effect time point (3 h after administration of mecamylamine/placebo) subtracted by the baseline recording. Mean, standard deviation, difference and a *p* value are given per biomarker, using the paired-samples t-test. Pcb denotes placebo and mcm mecamylamine.

Variable	Mean			Standard deviation		*P*-value
	Placebo	Mecamylamine	Mcm-Pcb	Placebo	Mecamylamine	
Rel. power (theta, PO)	−1.55	5.2	6.75	7.33	6.54	0.0008
Lifetime (beta, FC)	5.99	−14.8	−20.79	39.91	30.62	0.04
Lifetime (alpha, PO)	−455.06	67.97	523.03	1012.4	815.66	0.04
DFA (alpha, PO)	−0.08	0.01	0.09	0.21	0.22	0.12
Central freq. (alpha, PO)	−0.04	−0.17	−0.13	0.26	0.31	0.12
DFA (delta, PO)	0.01	−0.01	−0.02	0.16	0.18	0.75
Lifetime (theta, FC)	2.74	1.47	−1.27	283.42	448.2	0.99
Lifetime (alpha, FC)	−81.42	18.49	99.91	396.1	477.44	0.4
Bandwidth (beta, FC)	0.02	0.13	0.11	0.24	0.28	0.12
Rel. power (delta, FC)	−1.82	0.74	2.56	8.81	9.2	0.3

tered alone. As expected, nicotine—a nicotinic agonist—achieved a stronger reversal compared to galantamine. Mecamylamine intervention produced a significant increase in the nAChR index compared to placebo, with all except one subject displaying this effect (Fig. 3A). Co-administration of galantamine greatly reduced the nAChR index scores, and nicotine co-administration even more so (Fig. 3B, C). Importantly, both galantamine and nicotine show a consistent reversal pattern towards placebo, with most of the

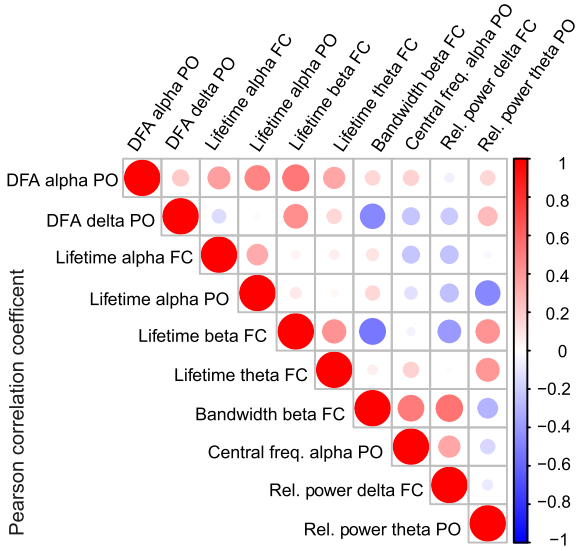

Fig. 2. The EEG biomarkers in the nAChR index exhibit a high degree of complementarity. The correlation matrix visualizes the relationship between pairs of nAChR biomarkers. Low correlations among the selected biomarkers indicate high complementarity and low redundancy, confirming the added value gained by integrating them into a combined index. The correlations are double-coded with the color and size of the disk.

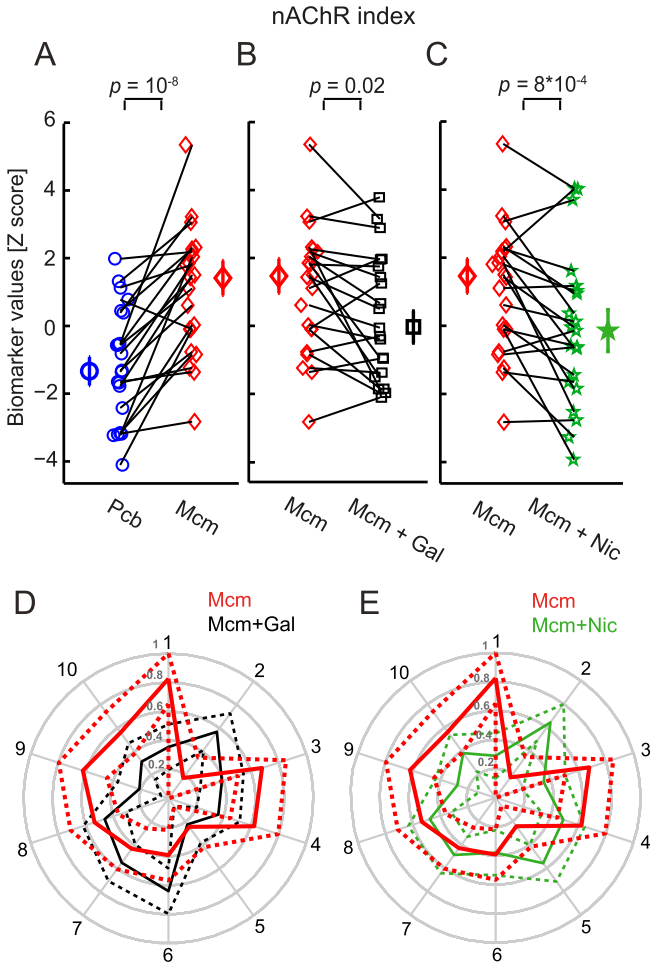

Fig. 3. nAChR index demonstrates successful reversal of mecamylamine-induced effects by nicotine and galantamine. Values of nAChR index are shown for individual subjects and paired between different conditions. Pcb stands for placebo, mcm for mecamylamine, gal for galantamine and nic for nicotine. (A) Individual nAChR index values differ significantly between the placebo and mecamylamine administration. A remarkably consistent effect is visible, with all but one subject showing higher nAChR values in mecamylamine condition compared to placebo. (B) Paired individual nAChR values for subjects receiving mecamylamine and mecamylamine co-administered with galantamine. Galantamine administration rescued mecamylamine effects as reflected in a reduction of the nAChR index values for most subjects, albeit not reaching the low mean index score of the placebo in (A). (C) Nicotine co-administration reversed mecamylamine effects, reducing the nAChR index value for almost all subjects. Significance levels are based on repeated-measures ANOVA ($p = 1.75 * 10^{-7}$) and Fisher's least significant difference as a post-hoc test. (D, E) Galantamine and nicotine reverse all the nAChR biomarkers towards placebo (Fig. 1C). Details on construction of the plots as well as the index biomarker list can be found in Fig. 1C and its legend.

nAChR-sensitive biomarkers participating in the reversal (Fig. 3 D, E).

We quantified the reversal effects by performing repeated-measures ANOVA, which identified a statistically significant effect of drug condition on the nAChR index ($F(3,60) = 15.24$, $p = 1.75 * 10^{-7}$). Post-hoc analysis using Fisher's least significant difference revealed a reversal of mecamylamine effects both for nicotine and galantamine co-administration ($p = 8 * 10^{-4}$ for nicotine, $p = 0.02$ for galantamine), with the nicotinic effect being stronger (Fig. 3B, C). When using the more conservative Bonferroni correction, the reversal only reached significance for nicotine ($p = 4 * 10^{-3}$ for nicotine, $p = 0.14$ for galantamine). Of note, whereas drug condition had a significant effect on relative theta power (ANOVA: $F(3,60) = 6.6$, $p = 6.3 * 10^{-4}$), both post-hoc tests indicated that neither nicotine nor galantamine reversed relative theta power—the single best biomarker (Fisher's least significant difference: $p = 0.15$ for nicotine, $p = 0.4$ for galantamine, Bonferroni: $p = 0.88$ for nicotine, $p = 1$ for galantamine). We take this as proof-of-principle that using the nAChR index is better than a single-biomarker approach for proof-of-pharmacology studies aiming to demonstrate effects of nicotinic cholinergic compounds.

4. Discussion

In this study, we aimed to develop a nicotinic cholinergic EEG index, based on data from a study testing mecamylamine as a proof-of-pharmacology challenge model. Mecamylamine administration induced widespread EEG changes, affecting both the spectral content and temporal dynamics of neuronal oscillations. Using machine learning, we integrated the multitude of effects into a nAChR index, which had a high accuracy for distinguishing the mecamylamine condition from placebo. Importantly, the nAChR index had a higher performance than any single EEG biomarker. We also established a low degree of cross-correlations between the biomarkers composing the nAChR index, thus indicating complementarity in the information they carry. Finally, we demonstrated that both nicotine and galantamine reverse mecamylamine-induced effects on the EEG using the nAChR index. We hope that in the near future it will be possible to validate or improve the index using a better spatial coverage as well as a larger sample size.

Using EEG and spectral analysis to monitor the effects of mecamylamine, previous studies have reported a so-called "slowing" of the EEG (Pickworth et al., 1988, Knott et al., 1997, Pickworth et al., 1997). We also observed this, with mecamylamine being related to a decrease in relative alpha and beta power, while increasing the relative power in the delta and theta frequency bands. The frequency within these bands was correspondingly affected as reflected in a decrease in the central frequency of the alpha band, and an increase in central frequency in the delta band. Such EEG changes are reminiscent of those observed in patients with Alzheimer's disease, e.g., a decrease in posterior alpha power and an increase in frontal and posterior theta power (van Straaten

et al., 2014). Other biomarkers affected by mecamylamine included the oscillation burst lifetime duration biomarker, which decreased in the beta band and increased in the alpha band. DFA increased most notably in the alpha band, indicating stronger alpha long-range temporal correlations, which was included in the nAChR index. In early-stage AD, the findings for DFA alpha and lifetime alpha were opposite to mecamylamine effects that we report (Montez et al., 2009). However, the mecamylamine effects are comparable to scopolamine for DFA, with both anticholinergic compounds inducing a global increase in DFA across multiple frequency bands (Simpraga et al., 2017). It is possible that these seemingly conflicting observations relate to differences in the dosage which can exert divergent responses due to u-shaped dose-response in pharmacology (Poil et al., 2011). For a better insight into the effect of cholinergic functioning and the stability of neuronal oscillations, more studies are needed. The effects of mecamylamine administration on bandwidth were most pronounced in the beta band and included in the nAChR index. The bandwidth was increased in beta band, which has been associated with less frequency stability of the beta oscillations and potentially to a less efficient working memory (Kopell et al., 2011). It is remarkable that lifetime and DFA biomarkers play such a prominent role in the composition of the nAChR index considering the short recordings available to our study and the intrinsic need for considerable recording durations for these time-series analyses. We speculate that recordings of 3–5 min would have resulted in better classification results; however, we also note that the selection of biomarkers quantifying the temporal structure of the amplitude modulation of neuronal oscillations even from relatively short 1-min recordings is in line with our previous study using similar recording durations to develop a mAChR index (Simpraga et al., 2017).

Compared to scopolamine, the magnitude of the EEG effects following mecamylamine administration were overall lower, but the directionality was in agreement for most of the biomarkers, with the exceptions being oscillation-burst lifetime duration in the alpha and beta bands and central frequency alpha, which exhibited the opposite changes for mecamylamine than scopolamine intervention (Simpraga et al., 2017).

To our knowledge, this is the first study using machine learning for classifying mecamylamine effects on the EEG. Past studies have focused on developing a muscarinic index (Snaedal et al., 2010, Johannsson et al., 2015, Simpraga et al., 2017). The nicotinic index provides a physiological means to non-invasively monitor the nicotinic cholinergic activity in the human brain. The greater performance of the nAChR index compared to any single biomarker can be attributed to the feature selection and classification with lasso, which is suitable for correlated variables and aims at selecting sparse models. To verify this, we cross-correlated the biomarkers composing the nAChR index and found these correlations to be low, thus displaying a high degree of complementarity among the selected biomarkers.

Reversal of mecamylamine-induced effects by nAChR agonists has not been previously demonstrated in humans with EEG. So far, the only drug reported to partially reverse mecamylamine-induced effects in healthy subjects was donepezil, using a visual discrimination test (Thompson et al., 2000). Here, we provided evidence for the nicotinic reversal of effects produced by mecamylamine administration using the nAChR index. The reversal of the EEG effects resulting from mecamylamine administration by nicotine indicates that both drugs affect the nicotinic cholinergic central neuronal system. We also showed the reversal effect of galantamine, albeit less pronounced compared to nicotine. The reason could be that the dose used was too low. Namely, galantamine has been reported to reverse electroencephalographic and sedative disturbances produced by scopolamine (Baraka and Harik, 1977), but in that study 0.5 mg kg^{-1} of galantamine was used, while in the current study the dose was 0.21 mg kg^{-1}.

The present study recruited incidental smokers in order to diminish the risk of severe side effects from nicotine and galantamine administration. This should not be a confounding factor, as previous studies have found mecamylamine to produce similar EEG effects in smokers and non-smokers (Pickworth et al., 1997). However, this has not been established for nicotine and galantamine using EEG; therefore, it would be of interest for future studies to investigate this.

Scopolamine has been used as the standard drug for inducing cognitive impairment in healthy volunteers; however, it also induces undesirable sedative effects, as well as non-specific behavioral effects and peripheral side-effects (Klinkenberg and Blokland, 2010). Therefore, more selective muscarinic antagonists have been proposed. Furthermore, some studies suggest the use of muscarinic and nicotinic cholinergic blockade combined as a model of memory impairment, as both muscarinic and nicotinic receptors synergistically modulate cognitive function (Erskine et al., 2004; Green et al., 2005; Ellis et al., 2006). However, an EEG study on this topic found that the co-administration of scopolamine and mecamylamine induced similar changes to those observed with mecamylamine alone in the spectral content of the EEG (Knott et al., 1997). In the growing field of drug development focusing on nicotinic agonists (Hurst et al., 2013; Valles et al., 2014; Beinat et al., 2015), the most adequate cognitive impairment model would be one targeting the nicotinic receptor such as mecamylamine. Moreover, in order for a drug to be used as a credible model for dementia, its temporary cognitive disturbance needs to be reversible, and for mecamylamine we demonstrate this with a standard cholinesterase inhibitor drug as well as a nicotinic agonist.

In conclusion, we have presented a nAChR index, which serves as an EEG signature of the nicotinic anticholinergic challenge. It combines the most prominent, complementary biomarkers from both the spectral and temporal EEG domain. The index is a highly accurate measure of the nicotinic anticholinergic intervention and can successfully detect reversal of mecamylamine-induced EEG disturbances by co-administration of either nicotine or galantamine. Therefore, our findings indicate that the mecamylamine challenge model in combination with modern EEG assessments could play an important part in understanding the complex role of the nicotinic cholinergic system in cognition and for evaluating novel pro-cognitive compounds acting on the nicotinic acetylcholine receptor.

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Conflict of interest

K.L.-H. and S.-S.P. are shareholders of NBT Analytics BV, which provides EEG analysis services based on index analysis for clinical trials.

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