PHARMACOKINETICS

An anti-nicotinic cognitive challenge model using mecamlamine in comparison with the anti-muscarinic cognitive challenge using scopolamine

Correspondence Anne Catrien Baakman, Zernikedreef 8, 2333 CL Leiden, The Netherlands. Tel.: +31 71 5246 400; Fax: +31 71 5246 499; E-mail: abaakman@chdr.nl

Received 15 June 2016; **Revised** 13 February 2017; **Accepted** 15 February 2017

Anne Catrien Baakman^{1,*}, Ricardo Alvarez-Jimenez^{1,*} , Robert Rissmann¹, Erica S. Klaassen¹, Jasper Stevens¹, Sebastiaan C. Gouloze¹ , Jeroen C. G. den Burger², Eleonora L. Swart², Joop M. A. van Gerven¹ and Geert Jan Groeneveld¹

¹Centre for Human Drug Research, Leiden, The Netherlands and ²Department of Clinical Pharmacology and Pharmacy, VU University Medical Center, Amsterdam, The Netherlands

*Both authors contributed equally.

Keywords clinical trials, drug development, neuropharmacology, pharmacotherapy

AIMS

The muscarinic acetylcholine receptor antagonist scopolamine is often used for proof-of-pharmacology studies with pro-cognitive compounds. From a pharmacological point of view, it would seem more rational to use a nicotinic rather than a muscarinic anticholinergic challenge to prove pharmacology of a nicotinic acetylcholine receptor agonist. This study aims to characterize a nicotinic anticholinergic challenge model using mecamlamine and to compare it to the scopolamine model.

METHODS

In this double-blind, placebo-controlled, four-way cross-over trial, 12 healthy male subjects received oral mecamlamine 10 and 20 mg, intravenous scopolamine 0.5 mg and placebo. Pharmacokinetics were analysed using non-compartmental analysis. Pharmacodynamic effects were measured with a multidimensional test battery that includes neurophysiological, subjective, (visuo)motor and cognitive measurements.

RESULTS

All treatments were safe and well tolerated. Mecamlamine had a $t_{\max}$ of 2.5 h and a $C_{\max}$ of 64.5 ng ml⁻¹ for the 20 mg dose. Mecamlamine had a dose-dependent effect decreasing the adaptive tracking performance and VAS alertness, and increasing the finger tapping and visual verbal learning task performance time and errors. Scopolamine significantly affected almost all pharmacodynamic tests.

CONCLUSION

This study demonstrated that mecamlamine causes nicotinic receptor specific temporary decline in cognitive functioning. Compared with the scopolamine model, pharmacodynamic effects were less pronounced at the dose levels tested; however, mecamlamine caused less sedation. The cognitive effects of scopolamine might at least partly be caused by sedation. Whether the mecamlamine model can be used for proof-of-pharmacology of nicotinic acetylcholine receptor agonists remains to be established.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The muscarinic acetylcholine receptor antagonist scopolamine is often used as pharmacological challenge in proof-of-pharmacology studies with pro-cognitive compounds.
- Nicotinic acetylcholine receptor agonists are currently in development to improve cognition or prevent decline of cognition in, for example, Alzheimer's disease.
- Mecamlamine is an antagonist specific for nicotinic acetylcholine receptors and also causes a temporary reduction in memory and other cognitive functions.

WHAT THIS STUDY ADDS

- Pharmacodynamic effects of mecamlamine were determined using a CNS test battery that incorporates a wide range of tests, reflecting different CNS domains, which yielded a much more complete picture of the pharmacodynamic effects of mecamlamine than previously known.
- Through frequent repetition of the pharmacodynamic test battery over the 10 h after drug administration, in parallel with pharmacokinetic measurements, a more complete time profile could be established, which is essential in assessing the effects of a nicotinic acetylcholine receptor agonist in future studies.
- A clearly different pharmacodynamic profile of mecamlamine and scopolamine could be shown and an important difference in level of sedation was observed.

Tables of Links

TARGETS
Ligand gated ion channels [2]
nAChR
G protein-coupled receptors [3]
mAChR

LIGANDS
mecamlamine
scopolamine

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2, 3].

Introduction

Alzheimer's disease (AD) is the most common form of dementia, with a prevalence of 3–7% in the Western European population [4]. AD causes significant burden for the patients and their caregivers and high health care costs for society. Even though many research groups are trying to unravel the pathophysiology and many pharmaceutical companies are searching for pharmacological targets for a curative treatment, no new drugs have been registered for this indication since 2003. The only approved therapy for mild to moderate AD is symptomatic treatment with cholinesterase inhibitors (CEIs), increasing the acetylcholine level in the synaptic cleft of cholinergic neurons. The cholinergic system is hypothesized to play an important role in several cognitive processes such as attention and memory [5]. Also, pathology studies have shown decreased levels of acetylcholine in the brains of patients with AD. Nevertheless, treatment with CEIs is only effective in about 14–36% of AD patients and the dose is limited by peripheral side effects such as nausea, vomiting and diarrhoea [6–10]. CEIs inhibit esterases peripherally and in the central nervous system (CNS), so they not only enhance functioning of the cholinergic neuronal system, but also induce peripheral cholinergic side effects, mainly *via* autonomic parasympathetic neurons. These peripheral

side effects could be avoided with agonists that are more selective for AChRs with a higher presence in the CNS than peripherally, such as the $\alpha 7$ and $\alpha 4\beta 2$ nicotinic acetylcholine receptors (nAChRs). nAChRs are mainly located in the hippocampus, thalamus, amygdala, striatum, entorhinal, frontal and pre-frontal cortex. Based on the localization of nAChRs in the human brain, nicotinic blockade could be expected to result in an impairment of cognitive functions such as acquisition, processing and recall of information [11]. Accumulating evidence suggests that $\alpha 7$ nAChRs play an important role in the pathophysiology of neuropsychiatric diseases, including schizophrenia and AD. Hence, a number of pharmaceutical industries have developed selective and high affinity $\alpha 7$ nAChR agonists as therapeutic drugs for these neuropsychiatric diseases [12]. Therefore, specific agonists targeting nAChR are currently being developed.

Proof-of-pharmacology studies with cholinergic compounds are often performed in healthy subjects after administration of scopolamine [13–20]. Scopolamine is a competitive muscarinic acetylcholine receptor (mAChR) antagonist with similar binding to all five known muscarinic receptor subtypes. From a pharmacological point of view, it seems more rational to use a nicotinic rather than a muscarinic anticholinergic challenge in a proof-of-pharmacology study of a nicotinic acetylcholine receptor agonist.

Mecamylamine is a nAChR antagonist that has been used for the treatment of severe hypertension since the 1950s. In 2009 it was withdrawn from the market because of its unfavourable risk–benefit profile compared with many other available antihypertensives. The antihypertensive effects of mecamylamine are mediated through nAChRs in peripheral autonomic ganglia. However, it also binds to nAChR present in the CNS [21]. Previous studies have confirmed that mecamylamine, temporarily and reversibly, perturbs the above-mentioned cognitive processes in healthy volunteers [22–26].

With this study we aimed to better characterize the pharmacodynamic and pharmacokinetic effects of mecamylamine compared to scopolamine in order to improve the knowledge about an nAChR-specific anti-cholinergic challenge and to develop a challenge model that may be suitable for proof-of-pharmacology studies with nAChR agonists.

Methods

Trial design and subjects

This double-blind, double-dummy, placebo-controlled, four-way cross-over study was performed in young, healthy, non-smoker male subjects. Concomitant medication or abuse drugs were not allowed during the clinical trial. On four different occasions with a wash-out of 7 days in between, all subjects received an oral dose of mecamylamine 10 mg with intravenous placebo, an oral dose of mecamylamine 20 mg with intravenous placebo, an intravenous dose of scopolamine hydrobromide 0.5 mg with oral placebo and both oral and intravenous placebo. The expected $t_{\max}$ of scopolamine was 15 min after the start of the infusion, while the expected $t_{\max}$ of mecamylamine was 3 h after oral administration [27, 28]. Therefore, the intravenous dose of scopolamine or placebo was given 2.45 h after administration of mecamylamine or placebo with an infusion duration of 15 min in order to have a $t_{\max}$ of both drugs at approximately the same time point. All subjects gave written informed consent for participation in the study. The ethics committee of the Leiden University Medical Center (The Netherlands) approved the study.

Dosing rationale

For the treatment of hypertension, the approved starting dose of mecamylamine was 25 mg per day and in various cognitive studies, a maximum of 20 mg orally produced few adverse effects, other than mild hypotension [22–26, 28–36]. Cognitive impairments are observed at dose levels of 15 mg and higher [22–25]. For the pharmacological challenge in this study a lower (10 mg) and higher (20 mg) dose were chosen in order to better determine concentration–effect relationships. Mecamylamine uptake is characterized by complete absorption from the gastrointestinal tract [28].

Scopolamine has been validated and frequently used as a pharmacological challenge in previously published studies with minimal adverse effects and demonstrable cognitive impairments at 0.5 mg scopolamine intravenously dosed [27].

Pharmacokinetics

Venous blood samples were obtained via an indwelling catheter before administration of mecamylamine or placebo and at 0.5, 1.0, 2.0, 3.0, 3.25, 4.0, 6.0, 8.0, 10.0 and 22.0 h after drug administration. Plasma concentrations of mecamylamine and scopolamine were determined at the Department of Clinical Pharmacology and Pharmacy at VU University Medical Centre (Amsterdam, The Netherlands) by a validated method using high performance liquid chromatography coupled to tandem-mass spectrometry (LC/MS–MS).

The LC–MS/MS consisted of a Waters Alliance 2795 separation module and a Quattro Micro tandem mass spectrometer from Waters (Watford, UK). System control, data acquisition and data processing were performed using MassLynx v4.1. Chromatography was performed on a Kinetex C18 analytical column from Phenomenex. The particle size was 2.6 μM , column length was 150 mm and column diameter was 3.0 mm. The mobile phase ratio of 70% mobile phase A and 30% mobile phase B was run with a flow of 0.5 ml min^{-1} . Both mobile phases contained 0.05% (v/v) trifluoroacetic acid and 5 mM ammonium formate, whereas mobile phase A was prepared in purified water and mobile phase B was prepared in methanol. Ionization of the drugs was achieved in the positive electrospray modus. The respective multiple reaction monitoring (MRM) transitions were 168.1 > 137.1 m/z for mecamylamine, 304.2 > 138.1 m/z for scopolamine, 171.2 > 137.1 m/z for mecamylamine-D3 and 307.1 > 141.1 m/z for scopolamine-D3. For sample preparation, 100 μL of an aqueous solution containing 1 M zinc sulphate was added to 40 μL plasma and short vortexed. Thereafter 100 μL of the internal standard was added containing 100 $\mu\text{g l}^{-1}$ of mecamylamine-D3 and scopolamine-D3 in methanol. After vortexing for 3 min the samples were centrifuged at 10 900 g for 3 min. The clear supernatant was transferred to vials and 25 μL was injected on the LC–MS/MS.

Pharmacodynamic assessments

To determine the pharmacodynamic effects of mecamylamine, a battery of tests (NeuroCart®) with a previously shown sensitivity to drug effects on a wide range of CNS domains was used [27, 37–39]. All tests were performed twice at baseline, and repeated at 1.0, 2.0, 3.25, 4.0, 6.0, 8.0 and 10.0 h after administration of mecamylamine or placebo. The only exception was the visual verbal learning test, which was performed 3.5 h after dosing (immediate recall) and 5 h after dosing (delayed recall and recognition). Measurements were performed in a quiet room with ambient illumination with only one subject per session in the same room. A more detailed description of the tests performed in this study has recently been published by our group [40].

Finger tapping. This test evaluates motor activation and fluency and has been adapted from the Halstead Reitan Test Battery [41]. The volunteer was instructed to tap as quickly as possible with the index finger of the dominant hand. Each session contained five performances of 10 s. Feedback on performance was given by a counter in the centre of the screen, while the amount of taps of each 10 s trial was

shown on the screen in between the trials. The mean tapping rate of five trials per time point was used for statistical analysis.

N-back. This test evaluates the working memory and requires buffering and updating consonants, matching, encoding and responding. The N-back test consists of three conditions, with increased working memory load. Letters were presented consecutively on the screen with a speed of 30 letters per minute. In the first condition subjects had to indicate whether the letter on the screen was an 'X'. In the second condition, subjects indicated whether the letter seen was identical to the previous letter. In the third condition, subjects were asked to indicate whether the letter was identical to two letters before the letter seen [42–44].

Adaptive tracking. Adaptive tracking is a pursuit-tracking task, measuring attention and eye-hand coordination. A circle moves pseudo-randomly about a screen. The subject must try to keep a dot inside the moving circle by operating a joystick. If this effort is successful, the speed of the moving circle increases. Conversely, the velocity is reduced if the test subject cannot maintain the dot inside the circle. The average performance scores over a 3-min period was used for analysis. Before study participation, subjects performed three training sessions and on each occasion two baseline measurements were taken [38, 39, 45, 46].

Saccadic peak velocity. Saccadic peak velocity (SPV) is one of the most sensitive parameters for sedation. The use of a computer for measurement of saccadic eye movements has been described elsewhere [38, 39, 47]. Average values of latency (reaction time), saccadic peak velocity of all correct saccades and inaccuracy of all saccades were used as parameters. Saccadic inaccuracy was calculated as the absolute value of the difference between the stimulus angle and the corresponding saccade, expressed as a percentage of the stimulus angle.

Smooth pursuit eye movements. The same system as used for saccadic eye movements was also used for measurement of smooth pursuit. For smooth pursuit eye movements, the target moves at a frequency ranging from 0.3 to 1.1 Hz, by steps of 0.1 Hz. The amplitude of target displacement corresponds to 22.5 degrees of eyeball rotation to both sides. Four cycles are recorded for each stimulus frequency. The time in which the eyes were in smooth pursuit of the target was calculated for each frequency and expressed as a percentage of stimulus duration. The average percentage of smooth pursuit for all stimulus frequencies was used as a parameter [47, 48].

Pharmaco-electroencephalography. Pharmaco-electroencephalography (p-EEG) was used to monitor any drug effects, which can be interpreted as evidence of penetration and activity in the brain [49, 50]. EEG recordings were made using gold electrodes, fixed with EC2 paste (Astromed) at Fz, Cz, Pz and Oz, with the same common ground electrode as for the eye movement registration (international 10/20 system). The electrode resistances were kept below 5 k Ω . 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 System with a time constant of 0.3 s and a low pass filter at 100 Hz. Data collection and analysis were performed using customized CED and Spike2 for Windows software (Cambridge Electronics Design, Cambridge, UK). In each session eight consecutive blocks of 8 s were recorded. The signal was AD-converted using a CED 1401 Power (Cambridge Electronics Design, Cambridge, UK). Data blocks containing artefacts were identified and these were excluded from analysis. For each lead, fast Fourier transform analysis was performed to obtain the sum of amplitudes in the very low (0.5–2 Hz), delta (2–4 Hz), theta (4–7.5 Hz), alpha (7.5–13.5 Hz), beta (13.5–35 Hz), and gamma (35–48.9 Hz) frequency ranges. The duration of EEG measurements was 64 s per session.

Pupil size. Pupil diameter was determined using a digital camera (Canon PowerShot A620) and a flash. The subject was instructed to look into the lens, and a sharp picture of the eyes was taken using a camera with flash. All pictures were stored digitally. The diameters of the pupil and the iris were determined using the number of horizontal pixels. For each eye, these values were recorded on data collection forms, and the pupil/iris ratio was subsequently calculated as a measure of pupil size.

Body sway. The body sway meter allows measurement of body movements in a single plane, providing a measure of postural stability. Body sway was measured with a pot string meter (Celesco) based on the Wright ataxia meter [51]. This method has been used to demonstrate effects of sleep deprivation [39], alcohol [46] and benzodiazepines [46, 52]. With a string attached to the waist, all body movements over a period of time were integrated and expressed as mm sway. The total period of body-sway measurement was 2 min.

Stroop. The Stroop test mainly investigates inhibition, interference and controlled vs. automatic processing. A two trial version of the colour-word Stroop task was presented to the subjects. In the first trial, six coloured items in green, red or blue were presented at random and subjects indicated which colour they saw. In the second trial, 34 colour and word pairs were presented randomly to the subject, forming either congruent or incongruent matches. The subjects were asked to indicate the colour of the word (for example: if the word blue was written in red, the correct answer was 'red') [53].

Simple Reaction Time Task. The Simple Reaction Time Task (SRTT) measures the attention and speed of information processing of the participant. In this task, participants view a black computer screen. At random intervals (0.5–1.5 s), a white circle appears in the centre of the computer screen. Participants were instructed to press the space bar with the index finger of their dominant hand each time the circle appears. They were instructed to respond as quickly as possible after the appearance of the circle. A total of 40 circles were presented, and the duration of the task was approximately 1 min. The outcome of the task is the time

between stimulus display and response. It has been shown to respond to several classes of sedative drugs [54].

Visual analogue scale according to Bond and Lader. Changes in subjective conditions are important aspects of drug effects, and a visual analogue scale (VAS) is one of the most common ways to assess subjective states. It is a psychometric response scale, which is particularly suited to repeatedly quantify present subjective states. In the VAS according to Bond and Lader, the 'directions' of different scales on a form were alternated, to avoid 'habitual scoring' by subjects. Composite scores were derived for alertness, mood and calmness [55].

Visual Verbal Learning Test. The Visual Verbal Learning Test (VVLTL) contains three different subtests that cover almost the whole scope of learning behaviour (i.e., acquisition, consolidation, storage and retrieval) [56]. Subjects were presented 30 words in three consecutive word trials. Each trial ended with a free recall of the presented words (immediate recall). Approximately 30 min after the start of the first trial, the volunteers were asked to recall as many words as possible (delayed recall). Immediately thereafter, the volunteers underwent memory recognition test, which consisted of 15 presented words and 15 'distractors' (recognition).

Safety assessments

All subjects underwent medical screening, including medical history, physical examination, vital signs measurement in supine and standing position, 12-lead electrocardiogram (ECG), urinalysis, drug screen and safety chemistry and haematology blood sampling. During study periods, safety was assessed using monitoring of adverse events, vital signs, ECG and safety chemistry and haematology blood sampling.

Pharmacokinetic and statistical analysis

The graphs and the pharmacokinetic parameters for mecamlamine were calculated by non-compartmental analysis in R [57]. Primary pharmacokinetic endpoints were: maximum plasma concentration ($C_{\max}$), time of maximum plasma concentration ($t_{\max}$), area under the plasma concentration vs. time curve ($AUC_{0-\text{last}}$), area under the plasma concentration vs. time curve extrapolated to infinity ($AUC_{0-\infty}$), apparent terminal half-life, apparent clearance (Cl/F) and apparent volume of distribution (Vd/F).

A mixed model analysis of covariance using SAS 9.1.3 for Windows (SAS Institute Inc., Cary, NC, USA) was used for analyses of pharmacodynamic effects, with subject, subject by treatment and subject by time as random effects; treatment, study period and treatment by time as fixed effects; and the average baseline value as covariate. VVLTL was analysed using a mixed model analysis of variance with fixed factors treatment and period, random factor subject and, if available, the (average) baseline. As this was an exploratory study, no formal adjustment for multiple testing was used. A P -value below 0.05 was considered statistically significant. In order to properly compare scopolamine and mecamlamine effects, two time points before scopolamine administration (1 and 2 h after mecamlamine administration) were not included in the LSM graphs.

Results

A total of 15 healthy male subjects participated in the trial. During execution of the study, three subjects stopped prematurely, due to personal circumstances (1), difficulties in blood sampling (1) and because of adverse events (nausea; 1). A total of 14 subjects completed at least one study period with treatment of mecamlamine and 12 subjects completed all study occasions. Subjects had a mean age of 25.9 (range 19–36) years, weight of 80.9 (range 59.9–90.0) kg and BMI of 24.4 (range 18.6–30.3) kg m^{-2} .

Safety

All subjects reported at least one treatment emergent adverse event. Most frequent occurring adverse events were somnolence, dizziness, fatigue, nausea, dry mouth and headache (Table 1). Adverse effects were mild and occasionally moderate and all disappeared spontaneously within a few hours. Three of 14 subjects reported postural dizziness at the 20 mg mecamlamine dose. This coincided in all cases with measurable orthostatic hypotension.

The difference between standing and supine blood pressure increased significantly on the 20 mg mecamlamine dose, compared to placebo, while heart rate was significantly higher (Table 2). Also, the difference in blood pressure between supine and standing position was significantly higher on the 20 mg mecamlamine dose, compared to placebo. On the 10 mg dose of mecamlamine, only the increase in supine and standing heart rate was statistically significant compared to placebo. There were no other consistent changes in ECG or laboratory safety parameters.

Pharmacokinetics

The mean $t_{\max}$ of mecamlamine was 2.1 h (range 1–3.3) with a $C_{\max}$ of 33.9 ng ml^{-1} (range 23.4–44.1) for the 10 mg dose and 2.5 h (range 0.5–6) with a $C_{\max}$ of 64.5 ng ml^{-1} (range 45.9–80.1) for the 20 mg dose (Table 3). When analysing the individual plots (see Figure 1), the terminal half-life was estimated to be 8.5 h for 10 mg and 11.7 h for 20 mg mecamlamine. This difference was not statistically significant. Other pharmacokinetic parameters were estimated as follows: $Cl/F = 17.9 \text{ l h}^{-1}$ (range 15.1–20.7 l h^{-1}) and $Vd/F = 283 \text{ l}$ (range 260–307 l).

Scopolamine pharmacokinetics could not be described in detail due to the low sample frequency after administration of scopolamine. The mean $C_{\max}$ of scopolamine was 2549 pg ml^{-1} (range 1349–4835 pg ml^{-1}) measured 15 min after the start of scopolamine infusion in all subjects. This is consistent with a previously published PK model of scopolamine [27].

Pharmacodynamics

The main outcome parameters of the pharmacodynamic effects are summarized in Table 4 and Figure 2; more detailed information is reported in the Supporting information Table S1. Both administration of scopolamine and the 20 mg dose of mecamlamine led to a significant decrease in performance compared to placebo on adaptive tracking, the second and third trial of the immediate recall and the delayed recall of the visual verbal learning test (Figure 3), finger

Table 1

Most frequent treatment emergent adverse events. Number of adverse events and percentage of the subjects experiencing the adverse events

	Placebo <i>n</i> = 14	Mecamylamine 10 mg <i>n</i> = 12	Mecamylamine 20 mg <i>n</i> = 14	Scopolamine 0.5 mg <i>n</i> = 13
Subjects with at least one AE	7 (50.0%)	8 (66.7%)	12 (85.7%)	13 (100%)
Number of different AEs	8	9	33	19
Somnolence	2 (14.3%)	6 (50.0%)	9 (64.3%)	7 (53.8%)
Dizziness	–	2 (16.7%)	4 (28.6%)	10 (76.9%)
Fatigue	2 (14.3%)	2 (16.7%)	5 (35.7%)	4 (30.8%)
Nausea	2 (14.3%)	1 (8.3%)	5 (35.7%)	3 (23.1%)
Dry mouth	1 (7.1%)	–	1 (7.1%)	5 (38.5%)
Headache	2 (14.3%)	2 (16.7%)	1 (7.1%)	2 (15.4%)
Disturbance in attention	–	1 (8.3%)	2 (14.3%)	1 (7.7%)
Dysgeusia	1 (7.1%)	–	2 (14.3%)	1 (7.7%)
Diplopia	–	–	1 (7.1%)	2 (15.4%)
Dizziness postural	–	–	3 (21.4%)	–

Table 2

Effect on haemodynamic parameters. Estimates of difference, 95% confidence intervals and *P*-values

	Treatment <i>F</i> -ratio <i>P</i> -value	Mecamylamine 10 mg <i>n</i> = 12	Mecamylamine 20 mg <i>n</i> = 14	Scopolamine 0.5 mg <i>n</i> = 13
Diastolic BP (supine) (mmHg)	<i>F</i> = (3,33) 1.97 <i>P</i> = 0.1372	1.5 (–1.2, 4.2) <i>P</i> = 0.2674	–0.6 (–3.1, 2.0) <i>P</i> = 0.6652	–1.7 (–4.3, 1.0) <i>P</i> = 0.2067
Diastolic BP (standing) (mmHg)	<i>F</i> = (3,31) 6.13 <i>P</i> = 0.0021	0.1 (–3.4, 3.5) <i>P</i> = 0.9682	–6.2 (–9.5, –2.8) <i>P</i> = 0.0007	–2.2 (–5.7, 1.2) <i>P</i> = 0.1995
Diastolic BP (standing-supine) (mmHg)	<i>F</i> = (3,33) 5.72 <i>P</i> = 0.0028	–1.0 (–4.3, 2.3) <i>P</i> = 0.5428	–5.5 (–8.6, –2.5) <i>P</i> = 0.0009	–0.3 (–3.4, 2.9) <i>P</i> = 0.8698
Systolic BP (supine) (mmHg)	<i>F</i> = (3,32) 3.16 <i>P</i> = 0.0379	–0.4 (–4.0, 3.3) <i>P</i> = 0.8436	–4.5 (–8.0, –0.9) <i>P</i> = 0.0149	–3.4 (–7.0, 0.2) <i>P</i> = 0.0632
Systolic BP (standing) (mmHg)	<i>F</i> = (3,31) 5.77 <i>P</i> = 0.0030	–1.7 (–6.0, 2.6) <i>P</i> = 0.4277	–7.8 (–12.0, –3.7) <i>P</i> = 0.0005	–1.6 (–5.9, 2.7) <i>P</i> = 0.4507
Systolic BP (standing-supine) (mmHg)	<i>F</i> = (3,33) 4.18 <i>P</i> = 0.0129	–1.7 (–5.3, 1.9) <i>P</i> = 0.3445	–4.9 (–8.4, –1.3) <i>P</i> = 0.0090	0.8 (–2.8, 4.5) <i>P</i> = 0.6441
Heart rate (supine) (bpm)	<i>F</i> = (3,32) 31.00 <i>P</i> < 0.0001	6.9 (3.4, 10.3) <i>P</i> = 0.0003	9.4 (6.3, 12.6) <i>P</i> < 0.0001	–4.5 (–7.8, –1.2) <i>P</i> = 0.0099
Heart rate (standing) (bpm)	<i>F</i> = (3,34) 21.50 <i>P</i> < 0.0001	8.7 (2.9, 14.5) <i>P</i> = 0.0042	16.0 (10.4, 21.5) <i>P</i> < 0.0001	–4.4 (–10.3, 1.5) <i>P</i> = 0.1390
Heart rate (standing-supine) (bpm)	<i>F</i> = (3,33) 3.26 <i>P</i> = 0.0335	2.4 (–2.7, 7.5) <i>P</i> = 0.3495	6.8 (2.0, 11.7) <i>P</i> = 0.0074	0.9 (–4.4, 6.2) <i>P</i> = 0.7279

tapping, body sway and VAS alertness. The effects of scopolamine were significantly stronger than those of mecamlamine on all these parameters, except for finger tapping and body sway. In contrast to mecamlamine, scopolamine administration resulted in an increase in reaction time and an increased score on the VAS for calmness

compared to placebo. Scopolamine also induced a decrease in performance on all N-back parameters, a decrease in alpha and beta power on the p-EEG, and a decreased performance on the first immediate recall and the delayed recognition of the VVLT, the SRT and saccadic peak velocity and accuracy and smooth pursuit eye movements, while mecamlamine

Table 3

Summary of mecamlamine PK parameters

Characteristic	Mecamlamine 10 mg (<i>n</i> = 12)				Mecamlamine 20 mg (<i>n</i> = 14)			
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
C_{max} (ng ml⁻¹)	33.9	5.96	23.4	44.1	64.5	10.9	45.9	80.1
t_{max} (h)	2.05	0.92	1	3.28	2.57	1.61	0.5	6
Terminal half life (h)	8.48	1.47	5.44	11.22	11.66	5.41	6.16	23.9
AUC_{0-inf}	503.8	126.3	332.9	746.1	1346.1	564.7	672.3	2621.8
AUC_{0-last}	410.1	90.0	277.7	607.0	913.8	187.3	603.5	1260.6

Figure 1

Mecamlamine plasma concentrations vs. time per dose group. Dots represent the measured mecamlamine concentrations. The red dotted line represents the mean and the shaded polygon the lower and upper 95% confidence intervals

Table 4Pharmacodynamic effects on cognition. Estimates of difference, 95% confidence intervals and *P*-values. *F* = (NumDF, DenDF) *F*-ratio

	Treatment <i>F</i> -ratio <i>P</i> -value	Mecamlamine 10 mg <i>n</i> = 12	Mecamlamine 20 mg <i>n</i> = 14	Scopolamine 0.5 mg <i>n</i> = 13
Adaptive tracking (%)	<i>F</i> = (3,33) 43.25 <i>P</i> < 0.0001	-1.89 (-3.90, 0.12) <i>P</i> = 0.0647	-2.06 (-3.97, -0.15) <i>P</i> = 0.0355	-10.4 (-12.4, -8.39) <i>P</i> < 0.0001
VAS alertness (mm)	<i>F</i> = (3,33) 7.07 <i>P</i> = 0.0009	-1.3 (-3.7, 1.2) <i>P</i> = 0.2962	-2.5 (-4.8, -0.2) <i>P</i> = 0.0342	-5.3 (-7.7, -2.9) <i>P</i> < 0.0001
Finger tapping (taps/10 s)	<i>F</i> = (3,31) 5.93 <i>P</i> = 0.0025	-2.87 (-4.75, -0.99) <i>P</i> = 0.0040	-3.25 (-5.05, -1.46) <i>P</i> = 0.0008	-3.04 (-4.89, -1.18) <i>P</i> = 0.0022
VVLT 3rd recall (number of words)	<i>F</i> = (3,33) 15.17 <i>P</i> < 0.0001	-2.7 (-5.1, -0.3) <i>P</i> = 0.0286	-3.6 (-5.9, -1.4) <i>P</i> = 0.0025	-7.7 (-10.1, -5.4) <i>P</i> < 0.0001
VVLT delayed recall (number of words)	<i>F</i> = (3,34) 9.98 <i>P</i> < 0.0001	-3.1 (-5.8, -0.4) <i>P</i> = 0.0259	-3.8 (-6.4, -1.2) <i>P</i> = 0.0051	-7.1 (-9.8, -4.5) <i>P</i> < 0.0001
Simple reaction time task (% change)	<i>F</i> = (3,32) 15.61 <i>P</i> < 0.0001	7.0% (-0.8%, 15.5%) <i>P</i> = 0.0786	3.8% (-3.5%, 11.7%) <i>P</i> = 0.3080	26.8% (17.6%, 36.8%) <i>P</i> < 0.0001
Saccadic peak velocity (deg/sec)	<i>F</i> = (3,28) 2.56 <i>P</i> = 0.0745	-14.3 (-33.5, 4.8) <i>P</i> = 0.1367	-10.9 (-29.0, 7.1) <i>P</i> = 0.2232	-25.4 (-44.2, -6.6) <i>P</i> = 0.0098

Figure 2

Mecamlamine effects on cognitive tests. Figures represent the estimates of the change from baseline and the shaded area represents the 95% confidence interval. P -value vs. placebo

Figure 3

Mecamlamine effects on the Visual Verbal Learning Test. Boxplot graphs of the performance on the VVL per treatment group. The mean is represented by the black middle line with the first and third quartile in the central boxplot. 'M' represents the mean. P -value vs. placebo

administration did not affect these tests. On the Stroop test, mecamlamine administration led to a decrease in reaction time compared to placebo, while scopolamine led to an increase in performance. Saccadic reaction time only increased after administration of mecamlamine. No consistent differences between mecamlamine and placebo could be observed for N-back, SRT, p-EEG, saccadic inaccuracy, saccadic peak velocity, smooth pursuit eye movements and VAS calmness. Reaction time on the VVLT recognition, pupil size and VAS mood were not affected by either scopolamine or mecamlamine compared to placebo.

Discussion

In this study, we investigated the pharmacodynamic and pharmacokinetic profile over time of mecamlamine using an extensive CNS test battery that included cognitive as well as visuomotor and neurophysiological measures. Two oral doses of mecamlamine were compared to intravenously administered scopolamine and placebo in order to determine the profile of an nAChR-specific anti-cholinergic pharmacological challenge model. All treatments administered were considered safe and well tolerated, as all adverse events were transient and mild to moderate in severity. Pharmacokinetics of scopolamine are in line with previously described results [27]. The plasma concentrations of mecamlamine almost doubled with the doubling of the dose, which suggests dose proportionality, as has been described before [28].

Mecamlamine showed a dose-dependent decrease in performance on several tests that represent different cognitive domains. The decline in performance on adaptive tracking and reduced VAS alertness reflected a deficiency in sustained attention. The decrease on the third trial of the immediate and the delayed recall of the VVLT represents a reduction in learning ability and memory retrieval. This mecamlamine induced impairment in acquisition and recall of information was expected, based on the localization of nAChRs in the brain [11]. These effects last up to 10 h after drug administration. Mecamlamine did not have any significant effects on measures for sedation (SRTT and saccadic peak velocity).

The cognitive effects of mecamlamine found in this study are consistent with previous research, where mecamlamine was administered at doses of 5, 10 and 20 mg to healthy young and elderly volunteers [23, 24]. In these studies, the effects on cognition were studied 1 and 2 h after dosing. A dose-dependent decrease in learning ability and reaction time was reported, which was more pronounced in elderly volunteers. There was no effect on subjective scales for drowsiness. Another study reported significant decrease in learning ability and semantic memory after administration of 15 mg mecamlamine [22] and also a decrease in inspection time after administration of 20 mg of mecamlamine was reported [25]. Cognitive testing was done at one [22, 25] or two [23, 24] time points after dosing, and tests for sustained attention were not performed in these studies. In none of the previously mentioned studies were plasma mecamlamine concentrations measured.

Conversely, several other studies found no effects of mecamlamine on various cognitive tests [26, 30, 32, 33,

35, 36]. However, these studies all used a dose of 15 mg and investigated the cognitive effects at only one time point after dosing. With measurements at only one time point, modest effects may have been missed. This is supported by the finding that the attentional network measured with fMRI was downregulated after administration of the same dose of mecamlamine, while cognitive tests were not influenced [31, 36]. The slightly higher dose of mecamlamine and the frequency and sensitivity of our test may have attributed to the positive results of our study.

The second aim of this study was to compare the mecamlamine model with the anti-muscarinic scopolamine model. Several previous studies attempted to do this, but none of these studies found significant cognitive effects of mecamlamine to compare with, probably due to low doses and few measurements [22, 26, 30, 32, 33, 35]. In this study, scopolamine had a significant effect on all cognitive domains measured, including inhibition and working memory, as has been described before [22, 27, 32, 35, 58]. The increase in reaction time and decrease in saccadic peak velocity, which was not observed after mecamlamine administration, and the larger reduction of VAS alertness, suggest that scopolamine has a strong sedative effect. These sedative effects of scopolamine have been reported previously [59–61]. It is unlikely that this is related to relative dose differences between the doses of mecamlamine and scopolamine given in this study, as sedation is also reported after lower doses of scopolamine [60] and mecamlamine has been given as an antihypertensive in doses up to 80 mg in the past without any relevant sedation. The brainstem and basal brain areas controlling arousal and wakefulness contain more mAChR than nAChR [62], which is a likely explanation for the difference in sedative effects between mecamlamine and scopolamine. The scopolamine-induced sedation may contribute to the cognitive effects of scopolamine in this study which are more pronounced than those of mecamlamine [34, 63]. The larger magnitude of the effects of scopolamine may seem attractive, but smaller, though still relevant effects of a new compound might get lost in the margins of variability or get overshadowed by the sedation caused by scopolamine. Due to the absence of sedation, the mecamlamine challenge may not only be more suitable for proof-of-pharmacology studies with an nAChR agonist, but also for other pro-cognitive compounds. Low doses of mecamlamine (<5 mg) have been described to enhance cognition in several animal studies [64, 65]; however, the neurophysiological mechanism is unclear. This phenomenon has not been described in healthy subjects, although something similar was observed in a single study in patients with Attention Deficit Hyperactivity Disorder [66]. As we did not observe any improvement in cognitive functioning due to mecamlamine in the current study, we did not take this factor into account here.

As previously mentioned, mecamlamine has been used as an effective anti-hypertensive in the past [34]; however, haemodynamic changes of mecamlamine and scopolamine were poorly studied in healthy volunteers. Mecamlamine decreased both systolic and diastolic blood pressure in supine position; nevertheless, these changes were more subtle in the diastolic parameters. Scopolamine, on the other hand,

produced negligible changes in blood pressure. As previously described, scopolamine marginally (on average four beats per minute) reduced the heart rate when compared to placebo. Decrease of heart rate after acute scopolamine administration has been described previously after a paradoxical short increase in the heart rate [67].

We can conclude from this study that the nicotinic anticholinergic pharmacological challenge with mecamlamine results in measurable cognitive deficits with an nAChR-specific profile, which is clearly distinguishable from the profile of the mAChR antagonist scopolamine. The mecamlamine challenge could therefore be suitable for proof-of-pharmacology studies with nAChR agonists. Furthermore, the relevant lack of sedation is an advantage of the mecamlamine challenge, compared with the scopolamine challenge.

Mecamlamine plasma concentrations correlated dose-dependently to the measured temporary cognitive deficits measured with the battery of tests. A PK/PD model of mecamlamine would be helpful in designing studies with the mecamlamine challenge. With the results of this study (i.e. doses up to 20 mg), PK/PD modelling of the neurophysiological endpoints was not possible due to the narrow range of difference in pharmacodynamic effects between the mecamlamine lower and higher dose. In order to better characterize the pharmacokinetic and pharmacodynamic relationships a follow-up study was conducted with higher doses of mecamlamine (up to 30 mg). Using the data of this study and the current study, we successfully developed a PK/PD model that can be used to simulate probable outcomes in future clinical trials [40].

In conclusion, this study demonstrated that mecamlamine causes nicotinic receptor-specific temporary decline in cognitive functioning and affects differently other CNS domains. Compared with the scopolamine model, pharmacodynamic effects were less pronounced at the dose levels tested and caused less sedation. Whether the mecamlamine model can be used for proof-of-pharmacology of nicotinic acetylcholine receptor agonists remains to be established.

Competing Interests

There are no competing interests to declare.

References

- 1 Southan C, Sharman JL, Benson HE, Faccenda E, Pawson AJ, Alexander SP, *et al.* The IUPHAR/BPS Guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. *Nucl Acids Res* 2016; 44: D1054–D1068.
- 2 Alexander SPH, Peters JA, Kelly E, Marrion N, Benson HE, Faccenda E, *et al.* The Concise Guide to PHARMACOLOGY 2015/16: Ligand-gated ion channels. *Br J Pharmacol* 2015; 172: 5870–903.
- 3 Alexander SPH, Davenport AP, Kelly E, Marrion N, Peters JA, Benson HE, *et al.* The Concise Guide to PHARMACOLOGY 2015/16: G protein coupled receptors. *Br J Pharmacol* 2015; 172: 5744–869.
- 4 Takizawa C, Thompson PL, van Walsem A, Faure C, Maier WC. Epidemiological and economic burden of Alzheimer's disease: a systematic literature review of data across Europe and the United States of America. *J Alzheimers Dis* 2015; 43: 1271–84.
- 5 Drachman DA, Leavitt J. Human memory and the cholinergic system: a relationship to aging? *Arch Neurol* 1974; 30: 113–21.
- 6 Birks J. Cholinesterase inhibitors for Alzheimer's disease. *Cochrane Database Syst Rev* 2006; CD005593.
- 7 Birks J, Grimley Evans J, Iakovidou V, Tsolaki M, Holt FE. Rivastigmine for Alzheimer's disease. *Cochrane Database Syst Rev* 2009; CD001191.
- 8 Olin J, Schneider L. Galantamine for Alzheimer's disease. *Cochrane Database Syst Rev* 2002; 1: CD001747.
- 9 Rösler M, Anand R, Cicin-Sain A, Gauthier S, Agid Y, Dal-Bianco P, *et al.* Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. *BMJ* 1999; 318: 633–8.
- 10 Tariot PN, Solomon PR, Morris JC, Kershaw P, Lilienfeld S, Ding C. A 5-month, randomized, placebo-controlled trial of galantamine in AD. The Galantamine USA-10 Study Group. *Neurology* 2000; 54: 2269–76.
- 11 Paterson D, Nordberg A. Neuronal nicotinic receptors in the human brain. *Prog Neurobiol* 2000; 61: 75–111.
- 12 Toyohara J, Hashimoto K. $\alpha 7$ Nicotinic receptor agonists: potential therapeutic drugs for treatment of cognitive impairments in schizophrenia and Alzheimer's disease. *Open Med Chem J* 2010; 4: 37–56.
- 13 Blin O, Audebert C, Pitel S, Kaladjian A, Casse-Perrot C, Zaim M, *et al.* Effects of dimethylaminoethanol pyroglutamate (DMAE p-Glu) against memory deficits induced by scopolamine: evidence from preclinical and clinical studies. *Psychopharmacology (Berl)* 2009; 207: 201–12.
- 14 Buccafusco JJ. The revival of scopolamine reversal for the assessment of cognition-enhancing drugs. In: *Methods of Behavior Analysis in Neuroscience*, ed Buccafusco JJ. Boca Raton, FL: CRC Press, 2009; 329–43.
- 15 Cho W, Maruff P, Connell J, Gargano C, Calder N, Doran S, *et al.* Additive effects of a cholinesterase inhibitor and a histamine inverse agonist on scopolamine deficits in humans. *Psychopharmacology (Berl)* 2011; 218: 513–24.
- 16 Deiana S, Harrington CR, Wischik CM, Riedel G. Methylthioninium chloride reverses cognitive deficits induced by scopolamine: comparison with rivastigmine. *Psychopharmacology (Berl)* 2009; 202: 53–65.
- 17 Lee J-Y, Kim KY, Shin KY, Won BY, Jung HY, Suh Y-H. Effects of BT-11 on memory in healthy humans. *Neurosci Lett* 2009; 454: 111–4.
- 18 Liem-Moolenaar M, Zoethout RWM, de Boer P, Schmidt M, de Kam ML, Cohen AF, *et al.* The effects of a glycine reuptake inhibitor R231857 on the central nervous system and on scopolamine-induced impairments in cognitive and psychomotor function in healthy subjects. *J Psychopharmacol* 2010; 24: 1681–7.
- 19 Snyder PJ, Bednar MM, Cromer JR, Maruff P. Reversal of scopolamine-induced deficits with a single dose of donepezil, an acetylcholinesterase inhibitor. *Alzheimers Dement* 2005; 1: 126–35.

- 20 van Ruitenbeek P, Vermeeren A, Riedel W. Histamine H1-receptor blockade in humans affects psychomotor performance but not memory. *J Psychopharmacol* 2008; 22: 663–72.
- 21 Stone CA, Torchiana ML, Navarro A, Beyer KH. Ganglionic blocking properties of 3-methylaminoisocamphane hydrochloride (mecamylamine): a secondary amine. *J Pharmacol Exp Ther* 1956; 117: 169–83.
- 22 Little JT, Johnson DN, Minichiello M, Weingartner H, Sunderland T. Combined nicotinic and muscarinic blockade in elderly normal volunteers: cognitive, behavioral, and physiologic responses. *Neuropsychopharmacology* 1998; 19: 60–9.
- 23 Newhouse PA, Potter A, Corwin J, Lenox R. Acute nicotinic blockade produces cognitive impairment in normal humans. *Psychopharmacology (Berl)* 1992; 108: 480–4.
- 24 Newhouse PA, Potter A, Corwin J, Lenox R. Age-related effects of the nicotinic antagonist mecamylamine on cognition and behavior. *Neuropsychopharmacology* 1994; 10: 93–107.
- 25 Thompson JC, Stough C, Ames D, Ritchie C, Nathan PJ. Effects of the nicotinic antagonist mecamylamine on inspection time. *Psychopharmacology (Berl)* 2000; 150: 117–9.
- 26 Voss B, Thienel R, Reske M, Habel U, Kircher T. Cognitive performance and cholinergic transmission: influence of muscarinic and nicotinic receptor blockade. *Eur Arch Psychiatry Clin Neurosci* 2010; 260 (Suppl): S106–S110.
- 27 Liem-Moolenaar M, de Boer P, Timmers M, Schoemaker RC, van Hasselt JGC, Schmidt S, *et al.* Pharmacokinetic-pharmacodynamic relationships of central nervous system effects of scopolamine in healthy subjects. *Br J Clin Pharmacol* 2011; 71: 886–98.
- 28 Young JM, Shytle RD, Sanberg PR, George TP. Mecamylamine: new therapeutic uses and toxicity/risk profile. *Clin Ther* 2001; 23: 532–65.
- 29 Dumas J, Hancur-Bucci C, Naylor M, Sites C, Newhouse P. Estrogen treatment effects on anticholinergic-induced cognitive dysfunction in normal postmenopausal women. *Neuropsychopharmacology* 2006; 31: 2065–78.
- 30 Dumas JA, Saykin AJ, McDonald BC, McAllister TW, Hynes ML, Newhouse PA. Nicotinic versus muscarinic blockade alters verbal working memory-related brain activity in older women. *Am J Geriatr Psychiatry* 2008; 16: 272–82.
- 31 Dumas JA, McDonald BC, Saykin AJ, McAllister TW, Hynes ML, West JD, *et al.* Cholinergic modulation of hippocampal activity during episodic memory encoding in postmenopausal women: a pilot study. *Menopause* 2010; 17: 852–9.
- 32 Ellis JR, Ellis KA, Bartholomeusz CF, Harrison BJ, Wesnes KA, Erskine FF, *et al.* Muscarinic and nicotinic receptors synergistically modulate working memory and attention in humans. *Int J Neuropsychopharmacol* 2006; 9: 175–89.
- 33 Erskine FF, Ellis JR, Ellis KA, Stuber E, Hogan K, Miller V, *et al.* Evidence for synergistic modulation of early information processing by nicotinic and muscarinic receptors in humans. *Hum Psychopharmacol* 2004; 19: 503–9.
- 34 Ford RV, Madison JC, Moyer JH. Pharmacology of mecamylamine. *Am J Med Sci* 1956; 232: 129–43.
- 35 Green A, Ellis KA, Ellis J, Bartholomeusz CF, Illic S, Croft RJ, *et al.* Muscarinic and nicotinic receptor modulation of object and spatial n-back working memory in humans. *Pharmacol Biochem Behav* 2005; 81: 575–84.
- 36 Thienel R, Voss B, Kellermann T, Reske M, Halfter S, Sheldrick AJ, *et al.* Nicotinic antagonist effects on functional attention networks. *Int J Neuropsychopharmacol* 2009; 12: 1295–305.
- 37 de Visser SJ, van der Post JP, de Waal PP, Cornet F, Cohen AF, van Gerven JMA. Biomarkers for the effects of benzodiazepines in healthy volunteers. *Br J Clin Pharmacol* 2003; 55: 39–50.
- 38 van Steveninck AL, Schoemaker HC, Pieters MSM, Kroon R, Breimer DD, Cohen AF. A comparison of the sensitivities of adaptive tracking, eye movement analysis, and visual analog lines to the effects of incremental doses of temazepam in healthy volunteers. *Clin Pharmacol Ther* 1991; 50: 172–80.
- 39 van Steveninck AL, van Berckel BN, Schoemaker RC, Breimer DD, van Gerven JM, Cohen AF. The sensitivity of pharmacodynamic tests for the central nervous system effects of drugs on the effects of sleep deprivation. *J Psychopharmacol* 1999; 13: 10–7.
- 40 Alvarez-Jimenez R, Baakman AC, Stevens J, Gouloze SC, Hart EP, Rissmann R, *et al.* Pharmacokinetics and pharmacodynamics of oral mecamylamine – development of a nicotinic acetylcholine receptor antagonist cognitive challenge test using modelling and simulation. *J Psychopharmacol* 2017; 31: 192–203.
- 41 Andrew JM. Delinquents and the tapping test. *J Clin Psychol* 1977; 33: 786–91.
- 42 Lim H-K, Juh R, Pae C-U, Lee B-T, Yoo S-S, Ryu S-H, *et al.* Altered verbal working memory process in patients with Alzheimer's disease: an fMRI investigation. *Neuropsychobiology* 2008; 57: 181–7.
- 43 Rombouts SARB, Barkhof F, Van Meel CS, Scheltens P. Alterations in brain activation during cholinergic enhancement with rivastigmine in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2002; 73: 665–71.
- 44 Sweet LH, Rao SM, Primeau M, Durgerian S, Cohen RA. Functional magnetic resonance imaging response to increased verbal working memory demands among patients with multiple sclerosis. *Hum Brain Mapp* 2006; 27: 28–36.
- 45 Gijsman HJ, Van Gerven JM, Tieleman MC, Schoemaker RC, Pieters MS, Ferrari MD, *et al.* Pharmacokinetic and pharmacodynamic profile of oral and intravenous meta-chlorophenylpiperazine in healthy volunteers. *J Clin Psychopharmacol* 1998; 18: 289–95.
- 46 van Steveninck AL, Gieschke R, Schoemaker HC, Pieters MS, Kroon JM, Breimer DD, *et al.* Pharmacodynamic interactions of diazepam and intravenous alcohol at pseudo steady state. *Psychopharmacology (Berl)* 1993; 110: 471–8.
- 47 Baloh RW, Sills AW, Kumley WE, Honrubia V. Quantitative measurement of saccade amplitude, duration, and velocity. *Neurology* 1975; 25: 1065–70.
- 48 Bittencourt PR, Wade P, Smith AT, Richens A. Benzodiazepines impair smooth pursuit eye movements. *Br J Clin Pharmacol* 1983; 15: 259–62.
- 49 Cohen AF, Ashby L, Crowley D, Land G, Peck AW, Miller AA. Lamotrigine (BW430C), a potential anticonvulsant. Effects on the central nervous system in comparison with phenytoin and diazepam. *Br J Clin Pharmacol* 1985; 20: 619–29.
- 50 Van Gteveninck AL, Mandema JW, Tuk B, Van Dijk JG, Schoemaker HC, Danhof M, *et al.* A comparison of the concentration-effect relationships of midazolam for EEG-derived parameters and saccadic peak velocity. *Br J Clin Pharmacol* 1993; 36: 109–15.
- 51 Wright BM. A simple mechanical ataxia-meter. *J Physiol* 1971; 218: 27P–28P.Suppl

- 52 Van Steveninck AL, Walln AE, Schoemaker RC, Pieters MSM, Danhof M, Van Gerven JMA, *et al*. A study of the effects of long-term use on individual sensitivity to temazepam and lorazepam in a clinical population. *Br J Clin Pharmacol* 1997; 44: 267–75.
- 53 Laeng B, Lg T, Brennen T. Reduced Stroop interference for opponent colors may be due to input factors: evidence from individual differences and a neural network simulation. *J Exp Psychol Hum Percept Perform* 2005; 31: 438–52.
- 54 Wezenberg E, Sabbe BGC, Hulstijn W, Ruigt GSF, Verkes RJ. The role of sedation tests in identifying sedative drug effects in healthy volunteers and their power to dissociate sedative-related impairments from memory dysfunctions. *J Psychopharmacol* 2007; 21: 579–87.
- 55 Norris H. The action of sedatives on brain stem oculomotor systems in man. *Neuropharmacology* 1971; 10: 181–91.
- 56 de Haas SL, Franson KL, Schmitt JA, Cohen AF, Fau JB, Dubruc C, *et al*. The pharmacokinetic and pharmacodynamic effects of SL65.1498, a GABA-A α 2,3 selective agonist, in comparison with lorazepam in healthy volunteers. *J Psychopharmacol* 2009; 23: 625–32.
- 57 R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, 2013.
- 58 Broks P, Preston GC, Traub M, Poppleton P, Ward C, Stahl SM. Modelling dementia: effects of scopolamine on memory and attention. *Neuropsychologia* 1988; 26: 685–700.
- 59 Kamboj SK, Curran HV. Scopolamine induces impairments in the recognition of human facial expressions of anger and disgust. *Psychopharmacology (Berl)* 2006; 185: 529–35.
- 60 Koller G, Satzger W, Adam M, Wagner M, Kathmann N, Soyka M, *et al*. Effects of scopolamine on matching to sample paradigm and related tests in human subjects. *Neuropsychobiology* 2003; 48: 87–94.
- 61 Pergolizzi JV, Philip BK, Leslie JB, Taylor R, Raffa RB. Perspectives on transdermal scopolamine for the treatment of postoperative nausea and vomiting. *J Clin Anesth* 2012; 24: 334–45.
- 62 Brown RE, Basheer R, McKenna JT, Strecker RE, McCarley RW. Control of sleep and wakefulness. *Physiol Rev* 2012; 92: 1087–187.
- 63 McQueen EG, Smirk FH. Use of mecamlamine in the management of hypertension. *Br Med J* 1957; 1: 422–5.
- 64 Driscoll P. Nicotine-like behavioral effect after small dose of mecamlamine in Roman high-avoidance rats. *Psychopharmacologia* 1976; 46: 119–21.
- 65 Terry AV, Buccafusco JJ, Prendergast MA. Dose-specific improvements in memory-related task performance by rats and aged monkeys administered the nicotinic-cholinergic antagonist mecamlamine. *Drug Dev Res* 1999; 47: 127–36.
- 66 Potter AS, Ryan KK, Newhouse PA. Effects of acute ultra-low dose mecamlamine on cognition in adult attention-deficit/hyperactivity disorder (ADHD). *Hum Psychopharmacol* 2009; 24: 309–17.
- 67 Gravenstein JS, Thornby JI. Scopolamine on heart rates in man. *Clin Pharmacol Ther* 10: 395–400.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

<http://onlinelibrary.wiley.com/doi/10.1111/bcp.13268/supinfo>

Table S1 Pharmacodynamic effects on all measured outcome parameters