Research Report

Dissociating cholinergic influence on alertness and temporal attention in primates in a simple reaction-time paradigm

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Abstract

The ability to promptly respond to behaviourally relevant events depends on both general alertness and phasic changes in attentional state driven by temporal expectations. Using a variable foreperiod simple reaction time (RT) task in four adult male rhesus macaques, we investigated the role of the cholinergic system in alertness and temporal expectation. Foreperiod-effects on RT reflect temporal expectation, while alertness is quantified as overall response speed. We measured these RT parameters under vehicle treatment and systemic administration of the muscarinic receptor antagonist scopolamine. We also investigated whether and to what extent the effects of scopolamine were reversed by donepezil, a cholinesterase inhibitor widely used for the treatment of dementia. In the control condition, RT showed a continuous decrease as the foreperiod duration increased, which clearly indicated the effect of temporal expectation on RT. This foreperiod effect was detectable only on the faster tail of the RT distribution and was completely eliminated by scopolamine. Furthermore, scopolamine treatment slowed down the average RT. Donepezil treatment was efficient on the slower tail of the RT distribution and only improved the scopolamine induced impairments on average RT reflecting a beneficial effect on alertness without any improvement in temporal expectation. The present results highlight the role of the cholinergic system in temporal expectation and alertness in primates, and help delineate the efficacy and scope of donepezil and other cholinomimetic agents as cognitive enhancers in present and future clinical practice.

1. Introduction

In a simple reaction time (RT) task, subjects have to detect and give speeded response to target stimuli that are either preceded by a previous target stimulus, or by one or more warning signals or cues. Varieties of the RT task can be used to measure factors that influence general alertness and vigilant attention in animal models of cognitive performance in rats (Robbins 2002), macaque monkeys (Baxter and Voytko 1996), and also in humans ranging from healthy subjects to Alzheimer's disease (AD) patients (McGuinness et al. 2010). In these tasks RT – as a reliable measure of alertness – is known to be influenced by sleep deprivation (Dinges and Powell 1985), pharmacological interventions (Killgore et al. 2008; Unrug-Neervoort, van Luijtelaar, and Coenen 1992), age (Blatter et al. 2006) or neurocognitive disorders such as AD.

Besides alertness, implicit (learned from the statistics of the environment) or explicit (informed by cues) temporal contingencies also reportedly influence RT through inducing temporal expectation and temporal attentional orienting, respectively (Nobre and Van Ede 2018). Specifically, in a simple RT task, the time elapsed between two target stimuli (Drazin 1961) or between a warning stimulus and the target (also known as the foreperiod) is thought to modulate RT through probabilistic target expectation (Weinbach and Henik 2012): If there is uncertainty in the occurrence of an expected event (e.g. random foreperiods from the 1-10 s duration range), the average RT is slower and attentional lapses occur more frequently for relatively short foreperiods (Basner and Dinges 2011; Matthews et al. 2017; Tsunoda and Kakei 2008), whereas for long foreperiods, RT becomes faster (Niemi and Näätänen 1981). In human subjects, these foreperiod effects remain stable after repetitive testing (5-day practice, unpublished observations) or even after sleep deprivation (Tucker et al. 2009) and is independent from the time of day, the prior duration of wakefulness and the relative fatigue occurring during task performance (time-on-task) (Matthews et al. 2017).

In patients with cognitive decline, responses are generally slower and RT shows higher variability both within and between individuals compared to cognitively healthy elderly control subjects (Gorus et al. 2008). Also, in contrast to young or healthy elderly subjects, AD patients show no RT improvement in the presence of alerting cues which predictably precede the target, indicating impairments in their ability to quickly and adaptively modulate phasic alertness (Tales et al. 2002). Importantly, patients with AD express slower RT for early targets in a sequential auditory oddball paradigm compared to age-matched controls (Golob and Starr 2000). Thus, besides the deficits of phasic alertness, deteriorated temporal expectation might also be a significant hallmark of pathological cognitive aging, and assessing foreperiod-related effects in a simple RT task could prove to be a valuable method to dissect the impairments of temporal expectation within the framework of a highly translatable experimental paradigm.

In the present study, therefore, to probe the influence of the cholinergic system in temporal expectation and alertness, we assessed the foreperiod-induced effects on RT and task performance in macaque monkeys. To fully exploit the information contained in RT data, we applied both parametric models and analyses that characterize the influence of treatment on the whole RT distribution (shift functions). Our first aim was to demonstrate the effects of foreperiod on RT and validate our paradigm for testing temporal expectation. Second, we investigated the effects of systemic muscarinic acetylcholine receptor suppression using scopolamine, a frequently used cholinolytic agent to induce transient amnesia in animal models of dementia (Ebert and Kirch 1998). We also hypothesized that cholinergic inhibition, besides causing increases in RT and impairments in performance accuracy, would decrease the beneficial effect of foreperiod on RT, indicating a parallel impairment of temporal expectation. Finally, we looked at whether and to what extent would scopolamine-induced impairment of attentional functions be alleviated or reversed by the cholinesterase enzyme inhibitor donepezil,

a cholinomimetic agent most commonly prescribed to alleviate symptoms in mild to moderate stages of AD.

2. Methods

2.1. Subjects

Five male 7 to 10 years old rhesus macaques (*Macaca mulatta*) were included in the study weighing 8 to 9 kg at the beginning of the experiments. One subject was excluded because of not displaying the foreperiod-dependent RTs we aimed to investigate (see details in the next section), therefore the final sample size was N=4. In order to ensure motivation during task performance, we applied a mild fluid restriction schedule on weekdays, where daily water intake, if not already consumed, was supplemented to 200 ml/day in the home cages. On weekends, water was available ad libitum. Animals were fed once per day, in the afternoons, following the daily testing session. Diet was standard nutritionally complete lab chow especially designed for non-human-primates (Altromin Spezialfutter GmbH, Lage, Germany) and was daily supplemented with fresh fruit and vegetable. In the home cage and testing rooms, temperature and humidity were maintained at 24 ± 1 °C and 55 ± 5 RH%, respectively.

All procedures were conducted in the Grastyán Translational Research Center of the University of Pécs. The study was approved by the Department of Animal Health and Food Control of the County Government Offices of the Ministry of Agriculture (BA02/2000-11/2012). Measures were taken to minimize pain and discomfort of the animals in accordance with the Directive 40/2013. (II.14.): 'On animal experiments' issued by the Government of Hungary, and the Directive 2010/63/EU 'On the protection of animals used for scientific purposes' issued by the European Parliament and the European Council.

2.2. Reaction time paradigm

Animals performed a simple RT task (Figure 1A) in one session per day. Each experimental session consisted of the total of 405 trials and lasted for approximately 60 min. During task performance, animals were seated in a primate chair in front of a computer screen (~50 cm distance). A response knob with an electric touch sensor was placed at a comfortable

reaching distance (~20 cm) from the primate chair. At the beginning of each trial, a short tone was played to indicate that the subjects had to touch the response knob and prepare for the key release response. If the animal did not touch the knob within 2 s, the trial was not initiated and an intertrial interval of 2±0.5 s followed. If the sensor knob was touched, a warning stimulus (a black disc with a radius of 2.5 mm/ 0.57 diameter in degrees of visual angle in the centre of screen) appeared within 0.3 s indicating the start of the foreperiod and remained displayed for the entire duration of the foreperiod. The duration of the foreperiod was set between 1.1 and 9.9 s (randomly drawn from 9 local probability bins: 1.1-1.9 s; 2.1-2.9 s; 3.1-3.9 s; 4.1-4.9 s; 5.1-5.9 s; 6.1-6.9 s; 7.1-7.9 s; 8.1-8.9 s; 9.1-9.9 s, incl. 45 trials per bin). When the foreperiod elapsed, the black disc turned white, which served as the target stimulus. The task required the animals to react as quickly as possible to the target stimulus by releasing the knob. In the absence of a response the target stimulus disappeared after 1000 ms the trial was considered unsuccessful and was terminated with no reward delivered. If the subject responded while the target was still displayed, the trial was completed correctly (completed trial). Upon a correct response the target stimulus disappeared, and subjects received a drop of liquid reward immediately after the response. If the trial was correct, then following a 2±0.05 s inter-trial interval the next trial started, if it was incorrect the inter-trial interval was 2±0.5 s. Stimulus presentation, response and reward delivery events were controlled by a script written by the authors in MATLAB programming environment (MathWorks, Natick, MA) using the Psychophysics Toolbox (Brainard 1997; Pelli 1997).

Animals performed the task at the same time of the day on every weekday. All animals had been previously trained to a criterion of at least 70% performance accuracy measured as performance rate (PR: number of correct (responded in time) trials divided by the number of initiated (touched the knob in time) trials and had been above criterion for at least five days consecutively prior to the start of the experiments.

In each subject, we modelled reaction time as a linear or quadratic function of the foreperiod, selecting the most appropriate model using the Akaike Information Criterion. In four out of five subjects, RT displayed a significant linear and/or quadratic dependence on foreperiod, indicating the presence of the temporal expectation that we aimed to investigate. One of the five subjects who were initially trained did not show temporal expectation in RT, therefore that subject was excluded from the study.

2.3. Procedures and drug administration

In the present placebo-controlled crossover and repeated measures experimental design all subjects underwent 8 recording sessions with at least 72 hours of washout interval between the sessions. All experimental treatments were repeated twice. The first four sessions covered all the pharmacological treatment conditions, which were administered again for the next four treatment sessions; otherwise treatment order was randomized and counterbalanced. To achieve stable and high plasma levels at the time of task performance, intramuscular injections of donepezil (Gedeon Richter Plc., Budapest, Hungary) were administered 40 min prior to behavioural testing, followed by scopolamine (Tocris Bioscience, Bristol, UK) or the corresponding vehicle treatment (saline) at 30 min before the behavioural testing session (Figure 1B). Donepezil and scopolamine were both dissolved in saline (saline, 0.9% NaCl). Saline was also used for vehicle (sham) treatments. Injection volume was set to 0.05 ml per kg bodyweight. There were four types of treatments (vehicle + vehicle (VEH); vehicle + 10.5 μg/kg dose of scopolamine (SCOP); 100 μg/kg dose of donepezil + 10.5 μg/kg scopolamine (DON00); 200 μg/kg dose of donepezil + 10.5 μg/kg dose of scopolamine (DON200)). Each solution was freshly prepared before each recording session and was stored for less than two hours.

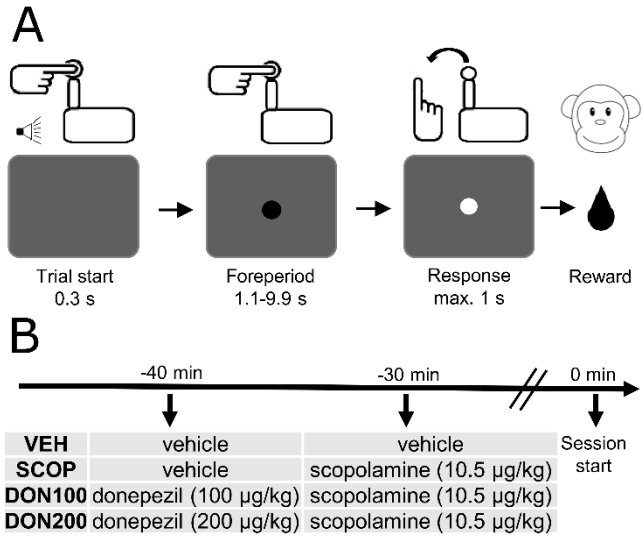

Figure 1. (A) Schematic illustration of a single trial in the variable foreperiod simple RT paradigm. On 'Trial start', animals had to touch a response knob after a short tone. Then, in the centre of the display, a warning stimulus (black disc) appeared and stayed on screen for a duration between 1.1 and 9.9 seconds, chosen randomly in each trial (Foreperiod). After the foreperiod the target stimulus (white disc) appeared. The task was to respond as quickly as possible to the target stimulus by releasing the knob (Response). If the subject responded in time the stimulus disappeared and the subject received the liquid reward. **(B) Schematic illustration of the timeline of treatments.** In each experimental session animals were administered two pharmacological agents (or vehicles). We administered the first injection at 40 and the second injection at 30 min before the task. We applied four types of treatment combinations, shown in the table under the timeline.

2.4. Data analysis

In the analyses not involving foreperiod length as a factor or detailed analysis of RT distributions (see below), all sessions (8 per animal) of all animals (N=4) were analysed, leading to 32 sessions altogether. For the analyses of foreperiod dependence using a linear mixed model and RT distribution shift, the number of completed trials was insufficient in one scopolamine session of one animal, therefore that session was excluded from further RT analyses, leading to a dataset of 31 sessions from the 4 animals.

We analysed the ratio of completed trials (performance rate, PR), the number of omission and commission errors and RTs. Performance rate was determined by the number of correct (responded in time) trials divided by the number of initiated (touched the knob in time) trials. The number of trials with specific types of errors were also counted: A commission error occurred when the subject released the knob before of appearance of target stimulus, and an omission error occurred if an initiated trial ended without a response or with a late response (after the target disappeared). We also examined the effect of foreperiod on RT as the effect of temporal expectation. In the case of average PR, the effects of treatments and foreperiod were analysed with repeated measures of analysis of variance (ANOVA). To test sphericity we used Mauchly's test, and found that the sphericity assumption was not violated. In this analysis, Fisher's Least Significant Difference (LSD) tests were used for post hoc comparisons. To test the number of commission and omission errors we used non-parametric related samples Friedman's analysis of variance by ranks test. Here, Dunn's test with Bonferroni correction was used for pairwise comparisons. These analyses were conducted using MATLAB (Mathworks, Natick, MA) and IBM SPSS (IBM Corp., Armonk, NY).

To test the interaction between the effect of treatment and foreperiod on RT, we used a linear mixed model on single-trial RT data. Foreperiod lengths were standardized for model fitting. Fixed effects were fitted for intercept, treatment, foreperiod and the

treatment×foreperiod interaction, with treatment coded using Helmert contrasts (all non-VEH vs. VEH, DON100 and DON200 vs. SCOP, DON200 vs. DON100). Random intercepts for subjects were fitted, and sessions were nested within subjects with random terms for intercept and foreperiod. The model was fitted with the restricted maximum likelihood criterion using the lme4 (Bates et al. 2015) package in R 3.6.0 (R Core Team 2019) using the following model formula: `RT ~ 1 + treatment * foreperiod.z + (1 | subject.id) + (1 + foreperiod.z | subject.session.id)`, where `treatment` was a categorical factor with the 4 levels corresponding to pharmacological treatment conditions, `foreperiod.z` was the standardized foreperiod as a continuous variable, `subject.id` is the subject clustering variable for the 4 subjects and `subject.session.id` was the sessions-within-subjects clustering variable for the 31 sessions. P values were calculated using the Kenward-Roger approximation of degrees of freedom using the lmerTest package (Kuznetsova, Brockhoff, and Christensen 2017).

Reaction time distributions are known to be non-normal and are theorized to contain more than one response component. To complement the parametric analysis and also capitalize on the rich information contained in the shape RT distributions, RT data was analysed by shift function analysis (Rousselet, Pernet, and Wilcox 2017) in MATLAB. The shift function describes the difference between the deciles of two distributions as a function of the deciles of one of the distributions. Deciles and medians are estimated using the Harrel-Davis quantile estimator in the shift function analyses and throughout the whole manuscript (including Figure 2). For statistical inference, confidence intervals of decile differences were estimated using the percentile bootstrap technique with n=10000 resamples (Wilcox et al. 2014). First, we tested the differences of distribution of RT data between the SCOP and VEH, DON100 and SCOP, DON200 and SCOP, DON200 and DON100 sessions. Treatment sessions within the first four sessions and the four repetition sessions were compared separately; for example, data from the

first SCOP session was compared to the first VEH session, and a separate shift function was used to compare the second SCOP session to the second VEH session. As an exception, for the animal with an omitted SCOP session, the remaining SCOP session was used in all relevant comparisons. Within each shift function analysis, bootstrap confidence intervals are adjusted for multiple testing across the 9 deciles. This method was used because a validated shift function estimation method encompassing the hierarchical structure of the present experimental design is currently not available – this aspect of inference is encompassed by the former linear mixed model analysis. Next, we analysed the difference between short (1.1-3.9 s) and long (7.1-9.9 s) foreperiod length categories within all the 31 treatment sessions included in the analysis (separately for both first and repeated sessions of VEH, SCOP, DON100, DON200).

3. Results

Animals showed reliable and high performance rates and fast response latencies in the vehicle control sessions. Specifically, the average PR was 95.8 ± 2.8% (Mean ± s.e.m., see Figure 2A), the number of omission errors was 5 ± 1.2, and the number of commission (early response) errors was 13 ± 11.7. The group average of median RT was 361 ± 12 ms (see also Figure 2B). Reaction times were significantly shorter for longer foreperiods in vehicle sessions (linear mixed model, simple effect of foreperiod in VEH: $t_{34.7}$=-3.31, p=0.0022; see Figure 2B; note that the presence of this pattern was an inclusion criterion for the study). However, foreperiod did not have a significant effect on PR ($F_{8,24}$=1.59, p=0.18, η_p^2=0.35). Thus, we first analysed treatment effects PR without regard to foreperiod (Section 3.1), followed by analysis of RTs taking foreperiod-dependence into account as well (Section 3.2). Finally, using shift functions, we investigated how the effects of treatments (Section 3.3) and foreperiod (Section 3.4) varied across the whole RT distribution.

3.1. Analysis of task performance

The analysis of treatment effects showed a marginal main effect on PR ($F_{3,9}$=3.28, p=0.073, η_p^2=0.52; see Figure 2A). In particular, scopolamine (SCOP) treatment significantly decreased PR compared to vehicle (SCOP vs. VEH post hoc p=0.017). Application of 100 µg/kg dose of donepezil (DON100) significantly reversed the scopolamine-induced impairments on PR (SCOP vs. DON100 post hoc p=0.037; see Figure 2A). 200 µg/kg dose of donepezil (DON200) had only a marginally significant effect on scopolamine induced-impairment on PR (SCOP vs. D200 post hoc p=0.085), however, there was no significant difference between the effects of the 100 and 200 µg/kg dose of donepezil (DON100 vs. DON200 post hoc p=0.623). These effects on PR were mainly driven by changes in the number of omission errors (failing to respond in time) rather than commission (false start) errors (Friedman's ANOVA along the 4 treatments, omission: $\chi^2(3)$=10.8; p=0.013; commission:

$\chi^2(3)=3{,}9$; $p=0.272$), with higher number of omissions under scopolamine treatment (157.3±71.8) compared to vehicle control (5±1.2; Dunn's test for SCOP vs. VEH: $p<0.01$). The effect of donepezil on omissions relative to Scopolamine was not significant (DON100: 42.8 ± 9.5; DON200: 89.3 ± 61.2; Dunn's test for DON100 vs. SCOP: $p=0.6$; Dunn's test for DON200 vs. SCOP: $p=0.6$).

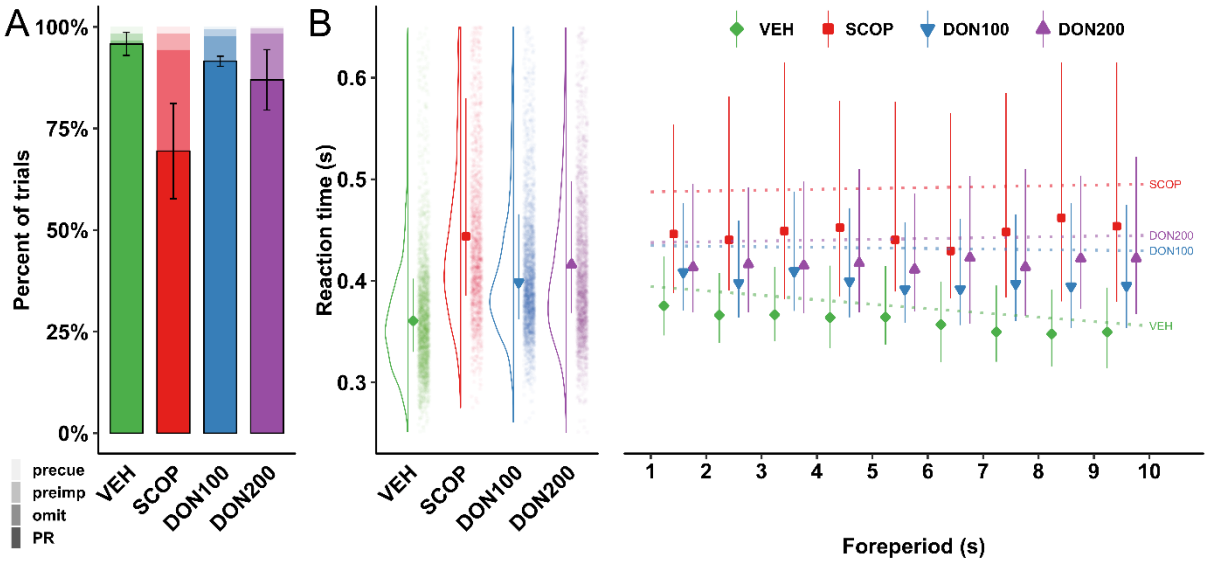

Figure 2. The effects of scopolamine and donepezil on mean performance rate (PR) and reaction time (RT). (A) Scopolamine (SCOP) significantly decreased PR compared to vehicle control (VEH), and this effect was partially reversed by donepezil (DON100, DON200). Bars with black contours filled with opaque colours show group mean PR, error bars here show s.e.m. Over the PR bar, areas shaded with decreasing saturation (legend in bottom right corner) show the proportion (among initiated trials) of omission errors ('omit'), commission errors ('preimp') and responses preceding the warning stimulus ('precue'). It is clearly seen here that the scopolamine-related impairment in PR is largely caused by omission errors. **(B)** The effect of scopolamine and donepezil on RTs and the foreperiod dependence of RTs for each of the four treatment combinations. On the left, RT distributions for each treatment are shown. The marker in the middle indicates the group mean of individual medians, the error bars cover the

range from the 2nd to the 9th deciles (also group averages). The distributions of RTs pooled across subjects and sessions are also visualised with kernel smoothing (to the left) and as jittered dotplots (to the right, each dot is one trial). The right part of the plot shows RT for each 1-sec bin of foreperiod length, with markers and error bars using the conventions described above (group averaged 2nd, 5th and 8th deciles). Dotted lines correspond to the linear relationship between foreperiod as a continuous variable and RT as modelled by the linear mixed model in Section 3.2. Note that the distance between the marginal means of the linear model and the medians is commensurate with the skewness of the RT distribution. Reaction times decrease as foreperiod increases in the VEH treatment condition. Scopolamine treatment slowed down RTs and eliminated the effect of foreperiod on RT, and donepezil, while mitigating the overall RT increase, did not restore the foreperiod dependence in any of the doses applied.

3.2. Reaction times and foreperiod effects

Linear mixed effects modelling confirmed a main effect of Treatment on RT (main effect of Treatment: $F_{3,24.1}$=15.2, p=10^{-5}; see Figure 2A). Responses were significantly slowed down by scopolamine relative to control (contrast for all vs. VEH: $t_{24.0}$=5.8, p=5×10^{-6}), while donepezil partly reversed this impairment (contrast for DON100 and DON200 vs. SCOP: $t_{24.4}$=-3.6, p=0.0015), apparently in a dose-independent manner (contrast for DON200 vs. DON100: $t_{23.8}$=0.55, p=0.59). Importantly, RT showed a clear and continuous dependence on foreperiod length in the VEH condition: RTs decreased as foreperiod length increased (simple effect of foreperiod in VEH: $t_{34.7}$=-3.31, p=0.0022; see Figure 2B). Treatments clearly modulated this foreperiod dependence of RT (Treatment×Foreperiod: $F_{3,26.6}$=3.29, p=0.036, Figure 2B): in contrast to control sessions, no such effect was observed in either of the treatment conditions (simple effects of foreperiod in all treatment conditions: $|t|\leq0.56$, $p\geq0.58$). This pattern is also reflected in an interaction contrast, wherein vehicle control sessions differed with respect to the foreperiod effect from all the treatment sessions (contrast for [all treatments vs. VEH]×Foreperiod: $t_{26.0}$=3.06, p=0.0052). Treatment conditions did not differ from each other with regard to foreperiod effects (contrast for [DON100 and DON200 vs. SCOP] × Foreperiod: $t_{29.5}$=-0.42, p=0.68, [DON200 vs. DON100] × Foreperiod: $t_{24.9}$=0.74, p=0.47), and the main effect of delay was not significant ($F_{1,26.9}$=1.47, p=0.24).

3.3. Effects of treatments on reaction time distributions

The effect of treatments on RT distributions are depicted on Figure 3A, where reaction times were pooled across sessions and animals, followed by kernel smoothing for visualisation purposes. To statistically characterize distribution effects, we analysed the treatment effects on RT in all deciles (1st-9th) using shift functions (Rousselet et al. 2017) for pairs of sessions for each animal separately. Shift function analysis is a versatile tool for visualisation and inference, which entails estimating the deciles of the two distributions to compare and plotting their differences (see Methods and Figure 3B, C for details).

Scopolamine treatment increased RT relative to vehicle across the whole distribution (Figure 3A), but particularly in the case of slower RT, creating a strong tail of slow responses. This slow response component probably reflects attentional lapses. Shift analysis confirmed this, showing significant differences in all deciles between RTs in the vehicle and scopolamine treatment conditions for all sessions of all animals (Figure 3B, C, D). Application of donepezil significantly reversed the decreased RT (Figure 3A, blue curves, Figure 3E, Supplementary Figure S1 A). In the case of the 100 µg/kg donepezil dose, the shift was significant across the whole distribution, except for one session of two animals (Figure 3E). Mirroring the effect of scopolamine, donepezil was more effective in the slower RT deciles, that is, it reduced the number of lapses. Reaction time distributions under donepezil treatment in the higher 200µg/kg dose were not significantly different from those of the lower dose (Supplementary Figure S1).

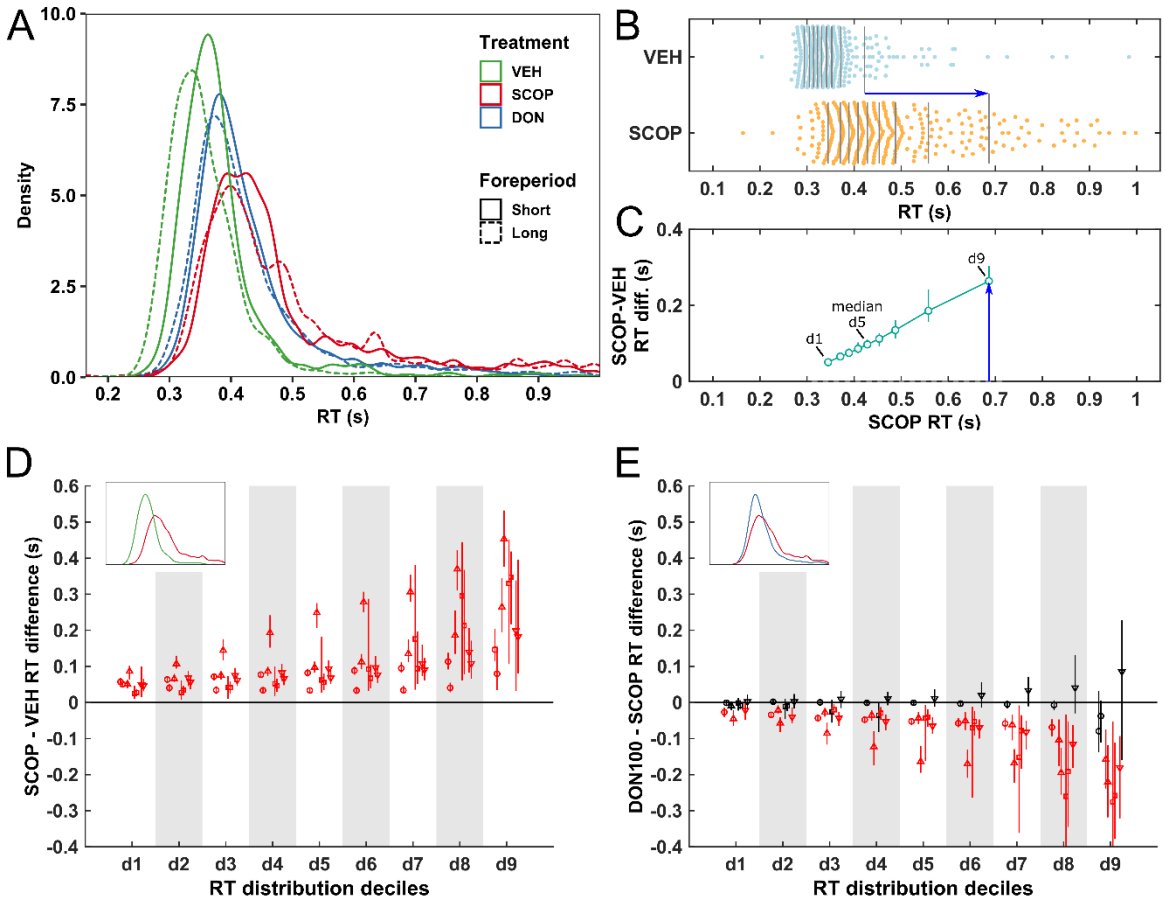

Figure 3. The effect of scopolamine and donepezil on RT distributions. (A) Distribution of reaction times in each treatment condition and foreperiod category (Short – continuous line: 1.1-3.9 s; Long – dashed line: 7.1-9.9 s), pooled across subjects and sessions. Data of the two donepezil doses was also pooled here as DON for visualisation to avoid clutter. Scopolamine treatment (SCOP, red curves) shifted the RT distribution rightwards (towards slower responses) compared to vehicle (VEH, green curves). This effect was partially compensated by donepezil treatment (DON, blue curves). Responses were slower for the short (continuous curves) compared the long foreperiods (dashed curves) in the vehicle control session (green curves), but this pattern is absent in all of the pharmacological treatment conditions. **(B) RT distributions from a VEH and a SCOP session** of one animal that illustrates the typical Scopolamine effect. Vertical grey lines indicate the deciles of the distributions. The blue arrow marks the shift of the 9th decile in the SCOP session relative to its value in the VEH session.

(C) Shift function for the difference of RT distributions from the VEH and SCOP session shown above in *B*. In the shift function, the difference between SCOP and VEH at each decile is plotted against the decile values in the SCOP session. For example, the blue arrow denotes the same shift as in *B*, i.e. the large RT difference between SCOP and VEH in the 9th decile. Vertical lines indicate the 95% simultaneous bootstrap confidence intervals. On the following panels, plots are composed of altogether 8 shift functions, 2 for each subject (see Methods). **(D) Effects of scopolamine treatment** relative to vehicle compared using shift functions for the two pairs of sessions in each animal separately. Here, instead of plotting against actual decile values as on *C*, deciles (labelled d1 to d9) are distributed evenly on the x axes. The insets illustrate which distributions are compared using the colour key of Figure 3A. Markers indicate individual animals. Red colour indicates significant differences. Scopolamine slowed down responses, especially at slower deciles. **(E) Shift functions comparing 100 µg/kg dose of donepezil** (DON100) with scopolamine treatment sessions, with the notation described above. Donepezil (DON100) decreases RTs relative to scopolamine monotreatment (SCOP), more effectively in slow deciles.

3.4. The effects of foreperiod on reaction time distributions

To test the effect of foreperiod on RT distributions, we pooled foreperiods in two categories, short (1.1-3.9 s) and long (7.1-9.9 s), and compared them within each treatment using shift functions. In the VEH treatment longer foreperiods induced shorter RTs, especially in faster deciles (Figure 4A). Scopolamine treatment abolished the foreperiod dependence across the whole RT distribution (Figure 4B). Donepezil was not effective in any of the applied doses to alleviate the scopolamine-induced impairments on temporal expectation (Figure 4C, D).

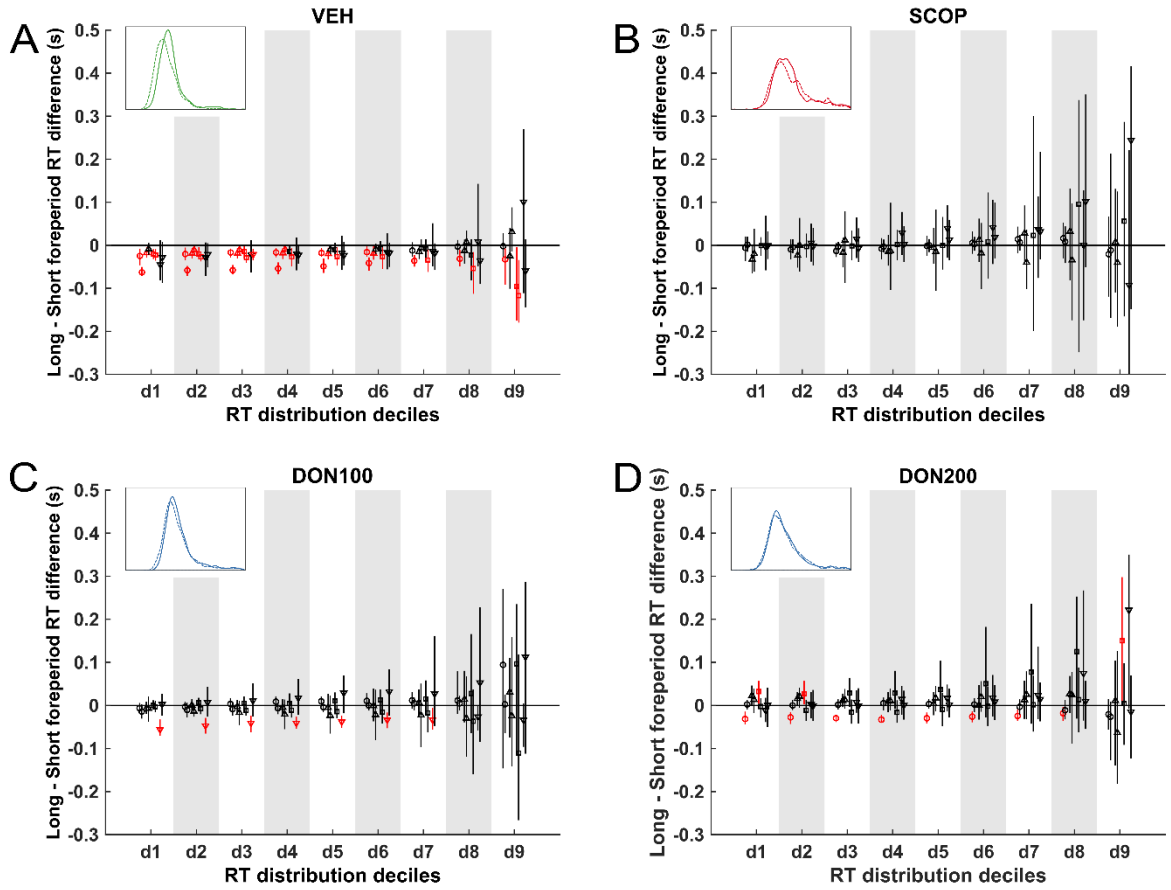

Figure 4. Shift functions showing the effect of foreperiod length (RTs from long minus short foreperiods) on reaction time distributions. Eight shift functions are plotted on each panel (except where noted), one for both sessions for each of the 4 animals. Insets illustrate which distributions are compared using the colour key of Figure 3A. Markers indicate individual animals and red colour indicates a significant shift. **(A)** For vehicle (VEH) treatment, longer foreperiods induced faster RTs, especially in the faster deciles. **(B)** Scopolamine (SCOP) treatment abolished the foreperiod effect. **(C, D)** During the donepezil treatments neither of the doses applied (DON100, DON200) were able to reverse the effects of scopolamine.

4. Discussion

In the present study, we applied a variable foreperiod simple RT paradigm to investigate the effects of foreperiod on RT and task performance accuracy. First, the effect of foreperiod on RT, an indicator of temporal expectation, was detectable in rhesus macaques in our RT paradigm. The observed foreperiod effect primarily manifested in the faster deciles of the RT distribution, implying the presence of a fast response component (Noorani and Carpenter 2016). Second, we went on to test how alertness and temporal expectation are modulated by the muscarinic acetylcholine receptor antagonist scopolamine as monotreatment or as co-administered with the cholinesterase inhibitor donepezil. Under scopolamine treatment, responses were generally slower, and also more lapses occurred, manifesting as a strong right tail component in the RT distribution (i.e., a shift of the RT distribution in the slow direction). In addition, the influence of foreperiod on RT was eliminated by the scopolamine treatment. Slower responses and lapses imply reduced alertness, whereas the lack of a foreperiod effect is a sign of impaired temporal attention. When donepezil was administered to alleviate scopolamine-induced impairments, task performance greatly improved and, though not reaching control levels, RTs became faster. Donepezil, mirroring the effects of scopolamine, was particularly effective at the slower deciles of the RT distribution, so it could largely alleviate the lapses introduced by scopolamine. In contrast to the alertness deficit, donepezil failed to mitigate impairments in temporal expectation, since the foreperiod effect was not reinstated under any of the donepezil treatment doses.

We investigated temporal expectation in a variable foreperiod simple RT task and confirmed that RT decreased (became faster) as the foreperiod increased. Faster responses are expected in increased states of arousal or alertness, or as a result of anticipatory attentional processes due to explicit or implicit knowledge about the timing of the expected event. It is known that rapid increases of phasic alertness can be induced by pre-target warning cues that

precede the target by a relatively small and constant time interval, leading to reduction in RTs (Lawrence and Klein 2013). However, in our experiment, when the imperative stimulus arrived at the earliest time point (1100 ms) from the range of possible foreperiod lengths (up to 9900 ms), RTs were not fast, but the slowest of all. This indicates that in our case, uncertainty in the timing of the imperative stimulus precluded the rapid burst of phasic alertness for early target arrivals. The way such uncertainty modulates RTs has been investigated using temporal attentional cueing experiments (Coull et al. 2000; Miniussi et al. 1999), where cues of varying validity predicted whether the target would arrive after a short or a long foreperiod. In the case of short elapsed time between the cue and target stimuli, the valid cue resulted in shorter RT, and the invalid cue slowed down RT. In the case of long foreperiods, the validity of a cue usually had smaller or no effects on RT. These reaction time changes reflect the attentional costs and benefits of temporal orienting (Correa, Lupiáñez, and Tudela 2006; Coull et al. 2000; Lawrence and Klein 2013; Miniussi et al. 1999). In our paradigm, which lacked explicit temporal cues, the mere passing of time during the foreperiod induces continuously increasing subjective probability that the target would finally appear. The conditional probability of appearance of the target given that it has not appeared yet – also known as the hazard rate – increases parallel with the foreperiod length, and this can induce probabilistic temporal expectations, leading to changes in RT that are analogous to those observed in the case of explicit probabilistic temporal attentional cueing. In such paradigms, RT is characteristically longest after the shortest foreperiod length (Niemi and Näätänen 1981), corresponding to an attentional cost of the target appearing at a low-probability time point. Therefore, temporal expectation can be examined by manipulating the time interval between the warning cue and the target and measuring concomitant changes in RT (Milliken et al. 2003; Weinbach and Henik 2012).

Preparing for an anticipated event for which a response is required involves the coordinated activity of sensorimotor and attentional networks in the brain. Related cortical processes have been observed in humans in the form of slow cortical potentials over sensory and motor areas (stimulus-preceding negativity and contingent negative variation, respectively, see Brunia 2003; Brunia, van Boxtel, and Böcker 2011), and activation in premotor and parietal areas in fMRI experiments (Coull and Nobre 1998). Anticipatory spiking activity of neurons in the lateral intraparietal area of macaques has been found to reflect both elapsed time and the probability of the target appearance (Janssen and Shadlen 2005). In interval timing tasks, the dopaminergic system appears to be important in the operation of an internal clock, while memory for temporal intervals and attentional mechanisms are thought to be linked to the cholinergic system. A fronto-striatal circuit is theorized to connect these two systems, and their coordinated operation underlies time perception and duration discrimination (Meck 1996). Parikh et al (2007) established the role of cue-evoked prefrontal cholinergic transients in cue detection in a rodent model (Hasselmo and Sarter 2011; Parikh and Sarter 2008). Importantly, they have shown that the time course (but not the amplitude) of the cholinergic transients depend on the length of the cue-reward interval (or foreperiod). Also, before successfully detected cues, cholinergic activity in the mPFC shows a negative trend, conversely, missed trials are preceded by gradually increasing cholinergic activity. Thus, the temporal dynamics of cholinergic activity play a crucial role both before and during cue-evoked shifts of attention.

Foreperiod effects have already been demonstrated in a primate model (Sharma et al. 2015), but the effects of cholinergic agents on temporal expectation in primates has not yet been investigated. In our experiment, the foreperiod effect on RT was eliminated by the scopolamine treatment. This finding confirms that neuromodulation via muscarinic receptors may play an important role in temporal expectation. A possible explanation of this finding is that scopolamine may have inhibited prefrontal evoked cholinergic activity that is known to have a

key role in cue detection (Parikh et al. 2007). Indeed, in macaques performing a working memory task, systemically administered scopolamine have been shown to affect delay activity in the prefrontal cortex (Zhou et al. 2011). Scopolamine also caused a global slowing of RTs in our experiments, in line with several studies that found deteriorated performance in simple RT tasks in humans (Ebert and Kirch 1998) and NHPs (Taffe, Weed, and Gold 1999).

We also investigated the effect of the acetylcholinesterase inhibitor donepezil on the scopolamine-induced deficit of temporal expectation and alertness. Donepezil is an agent widely used for symptomatic treatment of AD (Martorana, Esposito, and Koch 2010), moderately improving the quality of life (Gauthier et al. 2010) and several neuropsychological test results (Sugimoto et al. 2002). Furthermore, cholinesterase inhibitors are known to be moderately effective in reversing scopolamine-induced cognitive and/or psychomotor impairments in humans (Snyder et al. 2005; Wesnes et al. 1991). Here we confirmed that donepezil partially reverses scopolamine-induced slowing in a simple reaction time task; however, it failed to attenuate the scopolamine-induced impairment on temporal expectation, since the foreperiod effect was not restored. The present results are in line with previous observations showing that donepezil was partially able to compensate the effect of scopolamine in rhesus monkeys in a delayed matching to sample task (Buccafusco et al. 2008).

Reaction time distributions are known to be non-normal, but are rather composed of multiple response components, therefore conventional parametric models do not fully capture potential treatment effects. To this end, we analysed treatment effects across the whole RT distribution using a nonparametric quantile-based method. Based on our results, the effect of donepezil was stronger on the slower tail of the RT distribution, where, otherwise, the effect of scopolamine was also more pronounced. In contrast, the foreperiod effect was more expressed in the fast RT deciles, and this effect was completely eliminated by scopolamine. This suggests that temporal expectation is chiefly manifested in a fast response component that might require

attentional mechanisms involving cholinergic mechanisms on muscarinic receptors. While donepezil mitigated the response slowing caused by scopolamine to some degree, the fast response component for long foreperiods did not reappear under any of the donepezil treatment doses – that is, the impaired foreperiod dependence of RT was not restored. We further hypothesize that this is because donepezil may improve general alertness but not temporal expectation, which only together may serve as the functional basis of optimized task performance.

A possible neuropharmacological mechanism that could underlie the present results is that muscarinic receptors might play a role in either the production of cholinergic transients or the readout (Howe et al. 2017) of their temporal dynamics, and these mechanisms could be required for adaptive behavioural responses to temporal contingencies in the environment (Hasselmo and Sarter 2011; Parikh et al. 2007; Parikh and Sarter 2008). Further, it can be conceived that the increased availability of ACh under donepezil treatment can partly restore the cholinergic tone and thus tonic behavioural alertness, but at the same time, acetylcholinesterase activity would be required for the temporal specificity of phasic prefrontal cholinergic activity, for example by facilitating the decay phase of cholinergic transients. Under high tonic ACh levels resulting from inhibited acetylcholinesterase activity, cholinergic transients might be smeared, leading to lower signal-to-noise ratio of the temporal attentional processes they normally support. Also, research on rats suggests that the temporal specificity of cholinergic transients during sustained attention, and concomitant better task performance can be achieved by targeting specific receptor subtypes (Howe et al. 2010) – pharmacological modulation of tonic ACh levels probably cannot achieve this (Trocme et al. 2010). This raises the possibility that pro-cholinergic drugs that increase the sensitivity of particular subtypes of ACh receptors, i.e. agonists, and especially positive allosteric modulators, might be potentially

more successful in supporting higher level cognitive functions that require the adaptive dynamics of phasic cholinergic neuromodulation.

To detect the effect of temporal expectation on RT, here we used a simple RT paradigm with different foreperiods, creating probabilistic expectations for the time of target appearance. Measuring the reduction in RT with increasing foreperiod length may provide a simple method for behavioural pharmacological testing of temporal expectation in primates. Scopolamine is suggested to impair cue detection evoked cholinergic activity that is known to optimize attentional performance, and we also show that increasing the availability of ACh by donepezil is not sufficient on its own to reverse the observed deficits in temporal expectation. The present results also help delineate the efficacy and scope of donepezil and other cholinomimetic agents as cognitive enhancers in present and future clinical practice.

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Conflict of Interest Statement

B.L. is employee of Gedeon Richter Plc. This does not alter our adherence to EJN policies on sharing data and materials. The remaining authors (V.O., B.K., A.T., I.H.) declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

V.O., A.T., B.L. and I.H. designed the research. V.O. conducted the experiments, V.O., A.T. and B.K. performed data analysis. V.O., B.K., B.L. and I.H. wrote and reviewed the manuscript.

Data Accessibility Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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