The M1 Muscarinic Positive Allosteric Modulator PQCA Improves Performance on Translatable Tests of Memory and Attention in Rhesus Monkeys

Henry S. Lange, Christopher E. Cannon, Jason Drott, Scott D. Kuduk, Jason M. Uslaner

Merck Research Laboratories, West Point, Pennsylvania
a) Running Title: PQCA Improves Cognition in Rhesus Monkeys

b) Corresponding Author:

Jason Uslaner
Merck & Co., Inc.
WP45-1114
770 Sumneytown Pike, PO Box 4
West Point PA 19486
Phone: (215) 652-6617
Fax: (215) 652-7758
Email: Jason_Uslaner@merck.com
c) Page Count - 14
Table Count - 3
Figure Count - 7
Reference Count - 68
Word Count

Abstract - 198

Introduction - 581
d) Nonstandard Abbreviations – AchEI, AD, CD, CPT, GI, NHP, PAL, PAM, PQCA, Tmax

e) Recommended Section - Behavioral Pharmacology
Abstract

Improved treatment for Alzheimer’s disease is a significant unmet medical need which is becoming even more critical given the rise in the number of patients and the substantial economic burden. The current standards of care, acetylcholinesterase inhibitors (AChEIs), are hindered by GI side effects due to their nonselective activation of muscarinic and nicotinic receptors. Recently, the highly selective M1 positive allosteric modulator PQCA has been demonstrated to improve cognition in a variety of rodent and non-human primate cognition models without producing significant GI side effects. Here we describe the effect of PQCA and the AChEI donepezil on two clinically relevant and highly translatable touchscreen cognition tasks in NHPs, paired-associates learning (PAL) and the continuous performance task (CPT). Blockade of muscarinic signaling by scopolamine produced significant impairments in both PAL and CPT. PQCA and donepezil attenuated the scopolamine deficits in both tasks, and the action of these two compounds was similar in magnitude. In addition, the combination of sub-effective doses of PQCA and donepezil enhanced PAL performance. These results further suggest that M1 positive allosteric modulators have potential to reduce the cognitive deficits associated with Alzheimer’s disease either as monotherapy or as an add-on to current standards of care.
**Introduction**

Alzheimer’s disease (AD) is a profoundly debilitating neurodegenerative disorder characterized by neuronal cell dysfunction and death, particularly in the cholinergic neurons of the basal forebrain (Bartus et al., 1982; Schliebs and Arendt, 2006). Most currently approved symptomatic treatments for AD promote cholinergic function; 4 of the 5 drugs approved for symptomatic AD treatment are acetylcholinesterase inhibitors (AChEIs). Unfortunately, AChEIs provide relatively modest cognitive improvement and tend to produce significant gastrointestinal (GI) side effects (Raina et al., 2008). Due to these limitations and the severity of the disease, novel treatments for AD are a significant unmet medical need.

The M1 muscarinic receptor has gained interest as a target for novel treatments due to its high level of expression in brain areas associated with cognitive processing and its limited expression in the periphery (Levey, 1993; Volpicelli and Levey, 2004). Furthermore, M1 knockout mice show a variety of cognitive deficits (Miyakawa et al., 2001; Anagnostaras et al., 2003), and compounds that activate the M1 receptor have demonstrated pro-cognitive effects pre-clinically (Sedman et al., 1995; Hatcher et al., 1998; Wienrich et al., 2001).

Orthosteric M1 agonists have been developed and, in some cases, tested clinically (Sedman et al., 1995; Hatcher et al., 1998; Wienrich et al., 2001). However, the development of these compounds has been unsuccessful due to issues including insufficient selectivity, GI side-effects, and modest efficacy potentially due to the use of animal models that lack predictive validity (Fisher et al., 2003; Schenk et al., 2005; Cummings, 2008; Lindner et al., 2008; Conn et al., 2009). It is unclear which of these factors has resulted in the lack of clinical success.

To overcome the issue of selectivity and GI tolerability, we have developed M1 positive allosteric modulators (PAMs) that are potent and highly selective (Ma et al., 2009). For example PQCA (1-((4-cyano-4-(pyridine-2-yl)piperidin-1-yl)methyl-4-oxo-4 H-quinolizine-3-carboxylic acid) has an $EC_{50}$ value
of 49 and 135 nM on rhesus and human M1 receptor, respectively, and is inactive up to 30 μM versus the other muscarinic receptors (Kuduk et al., 2011). Previously we have demonstrated that PQCA enhances cognition across preclinical species and cognitive domains while providing a significantly improved therapeutic window in terms of GI effects, relative to the AChEI donepezil and the non-selective M1 orthosteric agonists xanomeline (Uslaner et al., 2013; Vardigan et al., 2014).

Here we aimed to provide more convincing translatable cognition data by extending our characterization of PQCA to two rhesus macaque touchscreen cognition tests, paired associates learning (PAL) and the continuous performance task (CPT). These tests are particularly valuable pre-clinically because nearly identical tests can be administered to humans. Results from such efforts show a high degree of cross-species translatability (Sahakian and Owen, 1992; Robbins et al., 1994; Fray and Robbins, 1996; Fray et al., 1996; Taffe et al., 1999; de Jager et al., 2002; Taffe et al., 2002; Blackwell et al., 2004; Buccafusco, 2006; Egerhazi et al., 2007; Lindner et al., 2008).

In the current set of studies, we first characterized donepezil in PAL and CPT in scopolamine-impaired rhesus monkeys. As far as we know, this is the first time an AChEi has been tested in these assays in non-human primates and so represents the first pharmacological validation of these potentially translatable assays. Secondly, the effects of PQCA were characterized in both PAL and CPT, representing the first M1 PAM characterized in these assays. Finally, we examined whether sub-effective doses of PQCA and donepezil could be combined to produce effects in PAL, which could suggest the potential for additivity in the clinic.
Materials and Methods

Animals

18 adult male rhesus monkeys (*Macaca mulatta*) were trained on either the PAL (n=10) or CPT (n=8) tasks. Sample size was determined using a power analysis based off of performance during training and an expected effect of ≥ 20% on PAL accuracy and CPT hit rate. In order to achieve >80% power, the sample size was determined to be ≥ 7 animals. Many of these animals had received test compounds in the past. However, all animals received a two week washout prior to the experiments described here which is many times longer than the half-lives of the compounds previously tested.

Animals were fed their full allotment of dry food (Purina High Protein Monkey Diet no. 5045), a multivitamin, and fresh fruits and vegetables after cognition testing at ~1500 hrs. The amount of dry food for each animal was manipulated in order to maintain engagement in cognitive testing studies. Dietary restrictions were approved by the veterinary staff, and body condition assessments were made every other week. Body scores were maintained at or above a 2 on the Clingerman & Summers (2005) scale. Water was available ad libitum. Temperature and relative humidity were maintained at 21–24 °C and 50–55 %, respectively. All experimental protocols described in this study were approved by the Merck and Co., Inc. Institutional Animal Care and Use Committee and conducted in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to use alternatives to in vivo methods where possible.

Apparatus

Cognitive testing for each animal took place inside a sound attenuated testing chamber (35”x35”x49.5”) in a room outside of the housing colony. Each chamber was equipped with a touch-screen computer system running the Cambridge Neuropsychological Test Automated Battery (CANTAB) software (Cambridge Cognition, Cambridge, UK) designed for use with nonhuman primates. A pellet dispenser
provided a food reward (100 mg flavored pellets; Bio-Serv, Flemington, NJ) to reinforce participation and correct responses. Testing chambers were ventilated and also included a stimulus light, house light, and speakers for white noise and auditory stimuli. Monkeys were unrestrained and free to move around the test chamber during testing. All animals were monitored via live video feeds streamed from cameras mounted inside the test chambers.

Paired-Associate Learning (PAL)

Ten single- or pair-housed male rhesus monkeys, ages 6-10 years old (5.2-10.5 kg), were tested on the PAL task, a task dependent on both frontal cortical and temporal-hippocampal systems (Gould et al., 2003; Meltzer and Constable, 2005; Gould et al., 2006). The PAL task required the subject to learn to associate specific visual stimuli with specific locations on a trial by trial basis, as previously described (Taffe et al., 2002). The stimuli for the PAL task consisted of ~70 distinct colored shapes or patterns. In each test session, animals were presented with 75 trials which were made up of 3 different trial types: 2-2, 2 samples in 2 locations; 3-3, 3 samples in 3 locations; and 4-4, 4 samples in 4 locations. At the beginning of a trial, one sample stimulus was presented in one of the four corners of the touchscreen. The animal had to acknowledge the stimulus by touching it, and no additional stimuli were presented until the animal responded. Once the animal responded, the second sample was presented in its own unique location until the animal responded. Depending on the trial type, 2, 3, or 4 sample stimuli were presented sequentially in unique locations. Sample presentations were separated by a 0.5 sec blank screen. After all of the sample stimuli were presented, and followed by a 1 sec blank screen, the choice phase began. In the choice phase, one of the sample stimuli was presented simultaneously in each sample phase location. The animal was then required to touch the correct sample location for that particular stimulus. Following a 0.5 sec blank screen, the next stimulus was presented in each of the sample locations, and the animal was again required to choose the correct location for that particular stimulus. The animal had to successfully complete a trial, by responding correctly to all choices, in order to obtain a food reward. Food reward was accompanied by a 1000 Hz, 85 dB tone and illumination of the food magazine. If the animal made an
error, the trial ended, accompanied by a 40 Hz, 85 dB tone, and the animal received a short timeout (5s) before the next trial began. Animals were trained on PAL until performance on the three trial types was stable at >80% correct on 2-2, 40-60% correct on 3-3, and <40% correct on 4-4. The percent correct measure was calculated for each trial type as the number of successfully completed trials divided by the number of trials presented. Figure 1 further illustrates the task.

Subjects received four test sessions weekly (Mon, Tues, Thurs, and Fri). Mondays and Thursdays were typically baseline sessions. Tuesdays and Fridays were possible drug treatment days, which allowed at least three days for compound washout. Drug administration only occurred if each subject’s performance on vehicle (IM saline in the case of Scopolamine, p.o. saline for donepezil, and p.o. 20% vitamin E TPGS for PQCA) was stable. Animals were considered stable if their standard error for % correct trials was less than 7.5 for each trial type over the previous three vehicle sessions. Once stability was attained, animals began scopolamine (Sigma Aldrich, 5-30 ug/kg; i.m.) titration. Scopolamine dosing was individualized for each subject, as behavioral sensitivity to impairment agents in monkeys varies greatly from subject to subject (Cannon et al., 2013). Each subjects’ scopolamine dose was titrated to identify a dose that produced a significant decrease in trial success while still completing at least 80% of trials. The scopolamine impairment was also assessed for stability over multiple sessions using the criteria described above. Once stable performance for both vehicle and scopolamine was achieved, donepezil (Sequoia Research Products, 0.3-3 mg/kg; p.o.), validation began. Utilizing a Latin square study design, scopolamine (or vehicle) and donepezil (or vehicle) were administered 30 min and 4-5 h prior to testing, respectively. Finally, following donepezil validation and after at least a one week washout period, characterization of PQCA (3, 10, or 30 mg/kg; p.o.) began. Doses of PQCA were based on Uslaner et al. (2013), which demonstrated that doses within this range reliably attenuated a scopolamine-induced deficit in other NHP cognition tasks. Utilizing a Latin square study design, scopolamine (or vehicle) and PQCA (or vehicle) were administered 30 min and 4 h prior to testing, respectively. Finally, a combination study was performed using subeffective doses of donepezil and PQCA to assess possible additivity or synergy.
between the compounds. Vehicle performance was monitored and remained stable throughout the drug studies.

Continuous Performance Task (CPT)

Eight single- or pair-housed male rhesus monkeys, ages 6-10 years old (4.9-6.8 kg), were tested on the CPT task; a test of sustained attention and impulsivity. This task was a modified version of the continuous performance task previously described by Zeamer et al. (2011). Each CPT session consisted of rapidly presented identically shaped stimuli, which only varied by color (white (RGB: 255 255 255), light yellow (RGB: 255 255 20-100), and yellow (RGB: 255 255 0)). Yellow was set as the target stimulus. Touching the target was rewarded with a food pellet, accompanied by a 1000 Hz tone. These responses were recorded as hits. Failing to touch the target stimulus was recorded as a miss. The white stimulus was used as an easy distractor to confirm that each subject was responding selectively to the target stimulus. This allowed for exclusion of animals who responded indiscriminately to all stimuli. The light yellow stimulus was used as a difficult distractor. The RGB value of the light yellow color was titrated for each animal to closely resemble the target and to establish a baseline level of false alarms. Because drug treatments have the potential to impact general levels of responding, these incorrect responses provided a way to dissociate a decrease in accuracy from a general decrease in responsivity. Touching either distractor stimulus resulted in a 10s time-out during which the stimulus light was extinguished and a 40Hz tone was presented. These responses were recorded as false alarms. Correctly rejecting either type of distractor did not result in food reward but was accompanied by a 1000 Hz tone. Animals received 100 trials per session with 60% of the stimuli presented as targets and the other 40% split evenly between easy and difficult distractors. Stimuli were presented for 1s with a 2s ITI. The measures recorded for each testing session were, 1) hits - the number of correct touches to the target, 2) misses - the number of missed targets, 3) false alarms - the number of incorrect touches to distractors, 4) correct rejections – the number of correctly avoided distractors, and 5) reaction time – the time between appearance and response (hit or
false alarm). Animals were trained until they reached an asymptotic performance level of ~80% correct responses to hits and ~30% false alarms to the difficult distractor.

In addition to CPT, a color discrimination (CD) task was given to control for potential drug effects on color vision. The CD task used the same stimuli as CPT. Impairment in both CD and CPT may reflect an impairment in color vision while an impairment in only CPT performance with a sparing of CD accuracy suggests an effect on attention. In this task the target yellow stimulus was presented concurrently with either the easy or difficult distractor. Animals received 40 randomly presented trials immediately after the CPT task completed, thus all compound treatments were identical to the CPT task described above.

Animals completed CPT/CD testing in 15-20 mins. Considering the relatively long half-lives of the drugs described in these studies, we expect plasma drug concentrations to be similar between the CPT and CD tasks. The CD trials used the same timing parameters as CPT except that each discrimination trial was presented for up to 5s in order to reduce the attentional strain. The measures recorded for each testing session were, 1) correct responses and 2) omissions. Figure 2 further illustrates CPT and the CD task.

Subjects received four test sessions weekly (Mon, Tues, Thurs, and Fri) with drug administration typically occurring on Tuesdays and Fridays. Drug administration only occurred if each subject’s vehicle performance (i.m. saline for all compounds) was stable, as described above. For CPT, subjects’ scopolamine doses were titrated to identify a dose that reliably decreased hits (>20%) with minimal effect on false alarms. Once a dose that met these parameters was identified, it was repeated at least once. After stable performance for both vehicle and scopolamine was achieved, donepezil (0.1-0.25 mg/kg; i.m.) validation and PQCA (0.3-1.0 mg/kg; i.m.) characterization began. The CPT animals were dosed IM due to scheduling issues that prevented us from testing CPT at pre-treatment times required for PO dosing with these compounds (Tmax ~4 hr when given PO). As shown in Table 1, we chose IM doses to match plasma exposures from our previous studies (Uslaner et al., 2013; Vardigan et al., 2014) where compounds were given PO. Utilizing a Latin square study design, scopolamine (or saline) and donepezil (or saline) were administered 60 min and 30 min prior to testing, respectively. In the PQCA study,
scopolamine (or saline) and PQCA (or saline) were administered 60 min and 30 min prior to testing, respectively.

Data analysis

Each dependent measure was analyzed via one-way repeated measures analysis of variance (ANOVA). Within-subjects t-tests were performed to compare differences between scopolamine and all other treatments. In addition, in order to characterize sustained attention, CPT data was divided into the first and second half of each session and analyzed using a two-way repeated measures ANOVA, with treatment and testing phase as within subject factors. Finally, a two-way repeated measures ANOVA was used to assess color discrimination with treatment and distractor type as within subject factors.
Results

PAL

The effect of scopolamine and donepezil on paired associate learning performance is presented in Fig 3. A one-way repeated measures ANOVA revealed a main effect of treatment on 2-2 \( (F(4, 36) = 4.136, p = 0.007) \), 3-3 \( (F(4, 36) = 15.937, p < 0.001) \), and 4-4 \( (F(4, 36) = 5.506, p = 0.001) \) trial types. Post-hoc analysis demonstrated that scopolamine impaired performance relative to vehicle on all three trial types. For 3-3 trials, 1.0 and 3.0 mg/kg donepezil significantly attenuated the scopolamine effect \( (p=0.047 \text{ and } p=0.001, \text{ respectively}) \). 3.0mg/kg donepezil showed a trend to reduce the scopolamine effect \( (p=0.067) \) on 4-4 trials.

Figure 4 shows the effect of PQCA on PAL. One-way repeated measures ANOVA revealed a main effect of treatment for 2-2 \( (F(4, 32) = 11.012, p < 0.001) \), 3-3 \( (F(4, 32) = 12.165, p < 0.001) \), and 4-4 \( (F(4, 32) = 4.607, p = 0.005) \) trial types. Similarly to the donepezil study, scopolamine significantly impaired performance for all three trial types. 10 and 30 mg/kg PQCA attenuated the scopolamine effect on 2-2 \( (p=0.003 \text{ and } p=0.05, \text{ respectively}) \) and 3-3 trials \( (p=0.009 \text{ and } p=0.015, \text{ respectively}) \).

The results from a combination study characterizing ineffective doses of donepezil (0.3 mg/kg) and PQCA (3.0 mg/kg) is shown in Figure 5. A repeated measures ANOVA revealed a main effect of treatment for 2-2 \( (F(2, 14) = 10.171, p = 0.002) \), 3-3 \( (F(2, 14) = 16.355, p < 0.001) \), and 4-4 \( (F(2, 14) = 8.046, p = 0.005) \) trial types. Again, scopolamine produced a robust and significant effect for all trial types. The combination of donepezil and PQCA significantly attenuated the scopolamine effect for 2-2 trials \( (p=0.05) \) and trended toward reducing the scopolamine effect for both 3-3 \( (p=0.094) \) and 4-4 \( (p=0.073) \) trial types. When given alone, neither of these doses of donepezil or PQCA attenuated the scopolamine impairment.

CPT
Table 2 and Figure 6 show the effect of scopolamine and donepezil on the continuous performance task (CPT). A one-way repeated measures ANOVA revealed a main effect of treatment on hit rate \((F_{(3,21)} = 7.843, p = 0.001)\) (Figure 6). Scopolamine treatment significantly impaired hit rate \((p=0.001)\), and the high dose of donepezil \((0.25 \text{ mg/kg})\) attenuated the scopolamine effect \((p=0.018)\). Difficult distractors produced significantly more false alarms than easy distractors \((F_{(1,7)} = 41.924, p < 0.001)\), but neither type of false alarm was impacted by compound treatment \((\text{difficult: } F_{(3,21)} = 1.645, p = 0.209; \text{easy: } F_{(3,21)} = 0.982, p = 0.420)\). Reaction time for targets was also affected by compound treatment \((F_{(3,21)} = 6.559, p = 0.003)\). Scopolamine significantly increased reaction time \((p=0.021)\) and 0.25 mg/kg donepezil reduced the scopolamine effect \((p=0.042)\). A two-way repeated measures ANOVA revealed main effects of testing phase \((F_{(1,7)} = 21.595, p = 0.002)\) and treatment \((F_{(3,21)} = 8.433, p = 0.001)\) but not a phase x treatment interaction \((F_{(3,21)} = 0.888, p = 0.463)\) for the number of missed targets. The number of missed targets significantly increased during the second half of testing \((p=0.002)\) suggesting difficulty sustaining attention. A one-way ANOVA on the first half of testing revealed a main effect of treatment on missed targets \((F_{(3,21)} = 4.799, p = 0.011)\). Scopolamine significantly increased missed targets during the first phase of testing \((p=0.004)\), and the high dose of donepezil failed to significantly impact the effect of scopolamine, though there was a trend \((p=0.095)\). A one-way ANOVA on the second half of testing revealed a main effect of treatment on missed targets \((F_{(3,21)} = 7.840, p = 0.001)\). Scopolamine significantly increased missed targets during the second half of testing \((p=0.002)\), and the high dose of donepezil significantly reduced the scopolamine effect \((p=0.018)\).

In the color discrimination control task, total accuracy \((F_{(3,21)} = 0.609, p = 0.616)\) and total omissions \((F_{(3,21)} = 2.215, p = 0.116)\) were not affected by scopolamine or donepezil dosing. Furthermore, a two-way repeated measures ANOVA for accuracy revealed a main effect of distractor type \((F_{(1,7)} = 15.257, p = 0.006)\), with difficult distractors producing more errors, but no main effect of treatment \((F_{(3,21)} = 0.747, p = 0.536)\). The treatment x distractor type interaction trended toward significance \((F_{(3,21)} = 2.810, p = 0.064)\). In addition, the two-way repeated measures ANOVA for omissions was not significant for
distractor type ($F_{(1, 7)} = 0.089, p = 0.775$), treatment ($F_{(3, 21)} = 1.997, p = 0.145$), or the interaction ($F_{(3, 21)} = 0.529, p = 0.667$).

Table 3 and Figure 7 show the effect of PQCA on scopolamine-impaired CPT. A repeated measures ANOVA revealed a main effect of treatment on hit rate ($F_{(3, 21)} = 17.157, p < 0.001$). Scopolamine significantly impaired hit rate ($p < 0.001$), and 0.3 and 1.0 mg/kg PQCA significantly attenuated the scopolamine effect ($p = 0.021$ and $p = 0.041$, respectively) (Figure 7). False alarm rate was not affected by treatment for difficult ($F_{(3, 21)} = 0.983, p = 0.420$) or easy ($F_{(3, 21)} = 0.011, p = 0.998$) distractors. Difficult distractors produced significantly more false alarms than easy distractors ($F_{(1, 7)} = 98.882, p < 0.001$).

Reaction time for targets was also influenced by treatment ($F_{(3, 21)} = 9.917, p < 0.001$). Scopolamine significantly increased reaction time ($p = 0.012$) but neither dose of PQCA mitigated that effect. A two-way repeated measures ANOVA revealed a significant main effect of testing phase ($F_{(1, 7)} = 12.976, p = 0.009$) and treatment ($F_{(3, 21)} = 17.100, p < 0.001$), and a phase x treatment interaction ($F_{(3, 21)} = 6.446, p = 0.003$) for the number of missed targets. The number of missed targets significantly increased during the second half of testing ($p = 0.009$). A one-way ANOVA on the first phase of testing revealed a main effect of treatment on missed targets ($F_{(3, 21)} = 7.577, p = 0.001$). Scopolamine significantly increased missed targets during the first phase of testing ($p = 0.002$) and 0.3 mg/kg PQCA significantly reduced that effect ($p = 0.009$). In addition, 1.0 mg/kg PQCA showed a trend of attenuation ($p = 0.074$). A one-way ANOVA on the second phase of testing revealed a main effect of treatment on missed targets ($F_{(3, 21)} = 19.866, p < 0.001$). Scopolamine significantly increased missed targets during the first phase of testing ($p < 0.001$) and the high dose of PQCA significantly reduced the scopolamine effect ($p = 0.05$).

In the color discrimination control task, total accuracy ($F_{(3, 18)} = 0.680, p = 0.576$) was not affected by scopolamine or PQCA. However, the two-way repeated measures ANOVA for omissions was significant for treatment ($F_{(3, 18)} = 5.233, p = 0.009$); scopolamine alone ($p = 0.038$), and scopolamine + 0.3 ($p = 0.047$), and scopolamine + 1.0 mg/kg PQCA ($p = 0.023$) produced more omissions than vehicle. Neither distractor type ($F_{(1, 6)} = 0.051, p = 0.829$) nor the distractor type x treatment interaction ($F_{(3, 18)} = 0.205, p = 0.892$)
significantly affected omissions. Furthermore, a two-way repeated measures ANOVA for accuracy revealed a main effect of distractor type ($F_{(1,6)} = 19.364, p = 0.005$), with difficult distractors producing more errors, but no main effect of treatment ($F_{(3,18)} = 0.302, p = 0.823$) or treatment x distractor type interaction ($F_{(3,18)} = 1.633, p = 0.217$).
Discussion

The present studies demonstrate the pro-cognitive effects of donepezil and the novel M1 PAM PCQA in two different, highly translatable cognition assays in rhesus monkeys. In addition, the combination of sub-effective doses of PQCA and donepezil showed additivity/synergy in attenuating the scopolamine deficit in PAL. To our knowledge this is the first report of an improvement in scopolamine-impaired NHP PAL and CPT by either an AChEI or an M1 PAM. These results further support the therapeutic potential of highly selective M1 PAMs for Alzheimer’s disease and other diseases characterized by cognitive dysfunction.

PAL

Deficits in PAL, a test of visuo-spatial memory dependent on the hippocampus, are among the earliest impairments observed in AD (Fowler et al., 2002; O’Connell et al., 2004; de Jager and Budge, 2005; Egerhazi et al., 2007; Junkkila et al., 2012). In healthy subjects, pharmacological impairments and improvements in cholinergic function can produce deficits and rescue PAL performance, respectively (Harel et al., 2013).

In the present set of studies, scopolamine treatment significantly and reliably impaired PAL performance across all trial types. These results are consistent with Taffe, et al. (2002) who showed an impairment of all trial types at the highest scopolamine doses tested. Although the doses of scopolamine tested here were individually titrated to produce a reliable impairment, it is worth noting that the mean dose tested (~17 µg/kg) is the same as the high dose used by Taffe et al. (2002). Donepezil treatment significantly improved PAL performance for the moderately difficult 3-3 trials, but not the easiest (2-2) or the most difficult (4-4) trials. This pattern is similar to effects of donepezil seen in the NHP delayed match to sample task (Buccafusco et al., 2008) in which donepezil only improved scopolamine deficits at the small to moderate delays but not at the longer most demanding delays. We believe that the lack of significant effect on the demanding (4-4) trials might simply be due to the smaller dynamic range between the
scopolamine and vehicle treated animals for those trials. Indeed, both donepezil and donepezil + PQCA
trended towards significantly reversing the effects in the more demanding trials. Of course, the lack of
donepezil’s effect on easy and difficult trial types might also be due to subject variability or a small
sample size. Importantly, scopolamine-impaired PAL performance in humans is also attenuated by acute
donepezil treatment, showing that effects in non-human primates appear to translate well to humans
(Harel et al., 2013).

PQCA improved PAL performance for both easy and moderate trial types. It is unclear whether
the difference in effect compared to donepezil on easy trials is due to a true difference in the mechanism
of action or due to between study variability in the scopolamine deficit. Regardless, it is clear that PCQA
produces pro-cognitive effects at least equal in magnitude to donepezil in this task. The effective doses
here are consistent with our group’s previous findings in the object retrieval detour task (ORD) and self-
ordered spatial search (SOSS) (Uslaner et al., 2013). These data further suggest that compounds which
selectively increase M1 activity will improve cognitive function.

The combination of sub-effective doses of PQCA and donepezil enhanced PAL performance.
Interestingly, these effects were achieved with the same doses, which given in combination, improved
performance in ORD (Vardigan et al., 2014). Considering the lack of side effects when combining low
doses of PQCA and donepezil (Vardigan et al., 2014), a combination therapy may allow for improvement
in cognition and a reduction in GI side effects compared to donepezil alone.

CPT

Attentional impairments arise shortly after the memory deficits in AD (Parasuraman and Haxby, 1993;
Parasuraman and Martin, 1994; Parasuraman et al., 1995; Perry and Hodges, 1999; Rizzo et al., 2000;
Berardi et al., 2005; Bohnen et al., 2005). Similar to memory, activation of the cholinergic system
improves attention in humans (Furey et al., 2006; Furey et al., 2008; Ricciardi et al., 2013) and NHPs
(Callahan, 1999; O’Neill et al., 1999; Schwarz et al., 1999; Terry et al., 2002; O’Neill et al., 2003; Katner
et al., 2004) while cholinergic blockade produces attentional impairments in both species (Callahan et al., 1993; Callahan, 1999; Taffe et al., 1999; Thienel et al., 2009a; Thienel et al., 2009b).

In this novel version of CPT, scopolamine treatment significantly and reliably impaired accuracy to the target stimulus while maintaining baseline false alarm rates for both easy and difficult distractors. Scopolamine treatment also increased reaction time in both studies, which theoretically could result from a reduction in attention or a decrease in motivation/motor function. However, the stability of false alarm rates during scopolamine treatment suggests a sparing of motivation/motor function. False alarms to the difficult distractor did increase with scopolamine treatment although this effect did not reach statistical significance. This increase in false alarms may signify an increase in impulsivity (Zeamer et al., 2011).

Finally, scopolamine significantly impacted the number of misses during both the early and late phases of testing, findings that are consistent with the scopolamine-impaired mouse model of CPT described by Young et al. (2013). Importantly, in the current study, the magnitude of this effect increased in the late phase. This late stage increase in misses suggests a greater impact of scopolamine on sustained attention, which has been shown to be impaired in AD patients (Sahakian et al., 1989; Mendez et al., 1997; Levin et al., 1998; White and Levin, 1999).

Donepezil treatment attenuated the scopolamine impairments in accuracy, reaction time, and misses over time. Similarly, PQCA treatment attenuated the scopolamine impairments in accuracy and misses over time but not reaction time. The reason for the lack of effect of PQCA on reaction time compared to donepezil is unclear. There is some evidence suggesting that reaction time is influenced more by nicotinic receptors than muscarinic receptors (Mirza and Stolerman, 2000). Thus, although donepezil’s wide-spread effect on muscarinic and nicotinic receptors might be responsible for many of its adverse effects, donepezil’s activation of nicotinic receptors might be beneficial for reaction time improvements. Additional research is needed to test this hypothesis.
It is important to acknowledge that the scopolamine model is limited and is not meant to model the neuronal loss, plaques, and tangles observed in AD. Furthermore, the behavioral disturbances of AD do not manifest with this model. However, we do believe that the scopolamine model is useful for preclinical translation for a number of reasons. First, many of the symptoms observed in AD are thought to be due to a loss of cholinergic function, which is meant to be modelled by the muscarinic receptor antagonist scopolamine. Indeed, many of the same cognitive domains affected by scopolamine are impacted in AD (Ebert and Kirch, 1998), and donepezil, the standard of care for AD, produces its effects by increasing cholinergic neurotransmission. Second, AD patients perform an average of 2-4 standard deviations below normal age-matched controls (Pillon et al., 1991). In both of the tasks described here, the scopolamine deficit represents a 2-3 standard deviation reduction from baseline performance, which suggests a similar level of impairment to AD patients. Third, donepezil is able to attenuate a scopolamine deficit in healthy volunteers in the PAL task, and AD patients show a modest but significant improvement with donepezil treatment. Thus, there is some pharmacological validation of the scopolamine model in humans, and the present findings demonstrate that similar responses are seen preclinically.

Here we have demonstrated the ability of the highly specific M1 PAM, PQCA to attenuate a scopolamine deficit in two very different and highly translatable NHP cognition assays. The expression and magnitude of PQCA’s effect was similar to the clinical standard, donepezil for both visuo-spatial memory (PAL) and attention (CPT) and the effects of donepezil observed here are similar to those observed in the human scopolamine model. Thus, the use of NHP models like these should improve the probability of success in the clinic by accurately predicting which cognitive tests and domains will be impacted by novel medicines, such as M1 PAMs.
Acknowledgments: The authors would like to thank the Merck Chemistry group for preparing the compounds for these studies; the Merck SALAR group for dosing assistance and exceptional animal care, Spencer Tye for helpful discussions regarding assay design, and Joshua Vardigan for comments on this manuscript.
Authorship Contributions:

Participated in research design: Lange, Cannon, Drott, and Uslaner

Conducted experiments: Lange, Cannon, and Drott

Contributed new reagents or analytic tools: Kuduk

Performed data analysis: Lange, Cannon, and Drott

Wrote or contributed to the writing of the manuscript: Lange, Cannon, Drott, and Uslaner
References


Young JW, Geyer MA, Rissling AJ, Sharp RF, Eyler LT, Asgaard GL and Light GA (2013) Reverse translation of the rodent 5C-CPT reveals that the impaired attention of people with schizophrenia is similar to scopolamine-induced deficits in mice. *Transl Psychiatry* **3**:e324.

Figure Legends

Fig. 1. Diagram of paired-associates learning task trials. Two to four sample stimuli are presented sequentially during the sample phase of each trial. After the animal touches each sample stimulus, the stimuli are re-displayed in all of the locations used during the sample phase. During this choice phase, the animal must remember the stimulus-location pairing for each stimulus. The order of the stimuli in the choice phase is randomized. A trial is successfully completed if the animal correctly identifies the sample location of each stimulus.

Fig. 2. Diagram of trials for the continuous performance task (CPT) and the color discrimination task (CD). In CPT (A), identically shaped stimuli which varied by color were presented rapidly in the center of the screen. Animals were trained to respond to yellow stimuli and ignore white and light yellow distractors. Touches to the yellow stimulus were recoded as hits while touches to the distractors were recorded as false alarms. The RGB value of the light yellow color was titrated for each animal to establish a baseline level of false alarms which provided a way to separate a decrease in accuracy from a general decrease in responsivity. Animals received 100 trials per session with 60% of the stimuli presented as targets and the other 40% split evenly between easy and difficult distractors. Stimuli were presented for 1s with a 2s ITI. In CD (B), one target stimulus and one type of distractor were presented concurrently. Animals were trained to respond to the yellow stimuli and ignore white and light yellow distractors. Animals received 40 trials immediately following completion of each CPT session. Stimuli were presented for 5s with a 2s ITI.

Fig. 3. Donepezil improves accuracy on scopolamine impaired paired-associates learning in rhesus monkeys. Data shown are percent successful trials for each trial type. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to scopolamine treatment ($n=10$).

Fig. 4. PQCA improves accuracy on scopolamine impaired paired-associates learning in rhesus monkeys. Data shown are percent successful trials for each trial type. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared to scopolamine treatment ($n=10$).
Fig. 5. The combination of sub-effective doses of donepezil and PQCA improves accuracy on scopolamine impaired paired-associates learning in rhesus monkeys. Data shown are percent successful trials for each trial type. *$P < 0.05$ and **$P < 0.01$ compared to scopolamine treatment (n=10).

Fig. 6. Donepezil improves accuracy on the scopolamine impaired continuous performance task in rhesus monkeys. Scopolamine significantly impaired hit rate and donepezil (0.25 mg/kg) attenuated this impairment. *$P < 0.05$ and ***$P < 0.001$ compared to scopolamine treatment (n=8).

Fig. 7. PQCA improves accuracy on the scopolamine impaired continuous performance task in rhesus monkeys. Scopolamine significantly impaired hit rate and PQCA (0.3-1.0 mg/kg) attenuated this impairment. *$P < 0.05$ and ***$P < 0.001$ compared to scopolamine treatment (n=8).
Tables

Table 1

Comparison of drug plasma concentrations between dosing routes.

Data are mean ± S.D., n = 3/group

<table>
<thead>
<tr>
<th>Compound</th>
<th>Donepezil</th>
<th></th>
<th></th>
<th>PQCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route</td>
<td>PO (4hr)</td>
<td>IM (0.5hr)</td>
<td>PO (4hr)</td>
<td>IM (0.5hr)</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>0.3</td>
<td>1.0</td>
<td>3.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Plasma Level (µM)</td>
<td>0.003 ±</td>
<td>0.010 ±</td>
<td>0.065 ±</td>
<td>0.015 ±</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.004</td>
<td>0.027</td>
<td>0.007</td>
</tr>
</tbody>
</table>
Table 2

Effect of donepezil on the scopolamine impaired continuous performance task in rhesus monkeys

Data are mean ± S.E.M, n=8

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle</th>
<th>0</th>
<th>0.1</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>False Alarm Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Easy</td>
<td>0.07 ± 0.04</td>
<td>0.09 ± 0.04</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.03</td>
</tr>
<tr>
<td>Difficult</td>
<td>0.32 ± 0.03</td>
<td>0.42 ± 0.06</td>
<td>0.33 ± 0.07</td>
<td>0.36 ± 0.05</td>
</tr>
<tr>
<td>Reaction Time (ms)</td>
<td>499.0 ± 24.89*</td>
<td>566.9 ± 26.85</td>
<td>555.6 ± 24.81</td>
<td>531.7 ± 28.21*</td>
</tr>
<tr>
<td>Early Phase</td>
<td>3.38 ± 1.02**</td>
<td>11.25 ± 1.46</td>
<td>10.50 ± 2.43</td>
<td>7.25 ± 1.25</td>
</tr>
<tr>
<td>Late Phase</td>
<td>4.50 ± 0.98**</td>
<td>16.13 ± 1.90</td>
<td>12.63 ± 2.81</td>
<td>9.00 ± 1.70*</td>
</tr>
</tbody>
</table>

*P < 0.05 and **P < 0.01 compared to scopolamine treatment.
Table 3

Effect of PQCA on the scopolamine impaired continuous performance task in rhesus monkeys

Data are mean ± S.E.M, n=8

<table>
<thead>
<tr>
<th>Variable</th>
<th>PQCA (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle 0 0.3 1</td>
</tr>
<tr>
<td>False Alarm Rate</td>
<td></td>
</tr>
<tr>
<td>Easy</td>
<td>0.05 ± 0.04 0.06 ± 0.03 0.05 ± 0.03 0.05 ± 0.03</td>
</tr>
<tr>
<td>Difficult</td>
<td>0.24 ± 0.04 0.31 ± 0.06 0.33 ± 0.06 0.35 ± 0.03</td>
</tr>
<tr>
<td>Reaction Time (ms)</td>
<td>504.4 ± 20.43* 580.4 ± 23.97 580.4 ± 26.53 591.5 ± 21.15</td>
</tr>
<tr>
<td># of Misses</td>
<td></td>
</tr>
<tr>
<td>Early Phase</td>
<td>4.63 ± 1.63** 13.75 ± 1.92 9.00 ± 1.82** 10.25 ± 1.76</td>
</tr>
<tr>
<td>Late Phase</td>
<td>3.63 ± 0.75*** 19.00 ± 1.67 16.75 ± 2.22 13.13 ± 2.99*</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, and ***P < 0.001 compared to scopolamine treatment.
Figure 1
Figure 2
Figure 3

The bar chart shows the percentage of correct trials across different trial types for various treatments:

- **Vehicle**
- **Scopolamine**
- **0.3 Don+Scop**
- **1.0 Don+Scop**
- **3.0 Don+Scop**

The y-axis represents the percentage of correct trials, ranging from 0 to 100. The x-axis represents different trial types: 2-2, 3-3, and 4-4.

Significance levels are indicated as follows:

- **(*)** p < 0.05
- **(**) p < 0.01
- **(***) p < 0.001

The graph illustrates a clear trend of decreasing correct trials with increasing doses of the combination treatment.
Figure 6

Hit Rate

Vehicle | 0 | 0.1 | 0.25

Scopolamine

* * *

Donepezil (mg/kg, i.m.)
Figure 7

![Graph showing hit rate against PQCA (mg/kg, i.m.)](image)