Effects of donepezil in healthy young adults

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Rationale: The cholinergic system is involved in the modulation of both bottom-up and top-down attentional control. Top-down attention engages multiple executive control processes, but few studies have investigated whether all or selective elements of executive functions are modulated by the cholinergic system.. Objective: To investigate the acute effects of the pro-cholinergic donepezil in young, healthy volunteers on distinct components of executive functions. Methods: We conducted a double-blind, placebo-controlled, independent groups design study including 42 young healthy male participants who were randomly assigned to one of three oral treatments: glucose (placebo), donepezil 5 mg or donepezil 7. 5 mg. The test battery included measures of different executive components (shifting, updating, inhibition, dual-task performance, planning, access to long-term memory), tasks that evaluated arousal/vigilance/visuomotor performance, as well as functioning of working memory subsidiary systems. Results: Donepezil improved sustained attention, reaction times, dual-task performance and the executive component of digit span. The positive effects in these executive tasks did not correlate with other attentional arousal/visuomotor/vigilance measures. Conclusions: Among the various executive domains investigated donepezil selectively increased dual-task

performance in a manner that could not be ascribed to improvement in arousal/vigilance/visuomotor performance nor working memory slave systems. Other executive tasks that rely heavily on visuospatial processing may also be modulated by the cholinergic system.

Cholinergic manipulations consistently alter sensory-driven, bottom-up attention but their effects on top-down, controlled processing have been less explored (e. g. Furey et al. 2008b; Hasselmo and Stern 2006; Sarter et al. 2001; Thomas et al. 2008), specially as pertains executive functioning.

Executive-type processing comprises a wide range of cognitive processes that have a role in the control of action and are considered a function of the central executive in the multiple component model of working memory (see Baddeley 2007, p. 11; Repovs and Baddeley 2006). In the latest version of this model the central executive is responsible for manipulating information contained in subsidiary slave components that store information of different modalities for short periods of time, as well as information activated from long-term memory (Baddeley 2007; Repovs and Baddeley 2006).

Today, executive processing is considered a multiple construct, consisting of different cognitive domains or components that, despite being correlated, are dissociable (Collette et al. 2006; Fisk and Sharp 2004; Friedman et al. 2006; Mantyla et al. 2007; Rabbitt 1997; Smith and Jonides 1999). Miyake (2000), in their influential paper on the diversity of executive functions, showed the dissociability of four postulated executive functions: updating, or modification of the content of working memory by deleting no longer relevant information and incorporating more relevant data (Miyake et al.

2000; Shimamura 2000); inhibition, the ability to inhibit distracting information when selecting relevant information, or to attend selectively to one stream of information while discarding others (Baddeley 1996a; Kane and Engle 2000); shifting, the ability to suppress response strategies when shifting between different tasks (Miyake et al. 2000; Monsell 2003); and dual-task performance, the ability to perform in parallel two tasks that rely on different cognitive systems (see Baddeley et al. 1997; Logie and Della Sala 2001).

Other types of executive processes that were not evaluated by Miyake (2000) have been suggested as separate cognitive entities. One of these is planning, the ability to organize behavior in relation to a specific goal (Owen 1997; Shallice 1982), and the other is the efficiency of access to long-term memory (Baddeley 1996b, 1998; Fisk and Sharp 2004).

Acute administration of anticholinergic drugs has been shown to impair executive functions by many authors (Curran et al. 1991; Green et al. 2005; Rusted and Eaton-Williams 1991; Rusted 1988; Rusted et al. 1991; Rusted and Warburton 1988) but in these publications usually only one executive test was used, mostly with unknown loading on the different executive components discussed previously. These finding are therefore not comprehensive in examining the executive domains that are affected by cholinergic manipulations. Aside from studying anticholinergic effects in different executive components, in order to demonstrate that the cholinergic system is in fact directly responsible for executive effects it would be important to show that drugs which increase the availability of acetylcholine, or pro-cholinergics, have the opposite effects.

To this end, we studied the modulatory role of the cholinergic system on the 6 different types of executive processes outlined above by investigating dose-dependent effects of acute oral doses (5 and 7. 5 mg) of donepezil, a potent, specific, non-competitive inhibitor of acetylcholinesterase (Jann et al. 2002; Shigeta and Homma 2001) that increases the availability of acethylcholine. We administered acute doses to young healthy volunteers because neurologic/psychiatric disorders and aging (Gron et al. 2005), as well as chronic use (Poirier 2002; Tsukada et al. 2004), alter the status of the cholinergic system.

To assess executive functioning we employed tests that have been shown to reflect each of these 6 separable processes. To evaluate updating, inhibition and switching we used tasks described by Miyake (2000) that showed high loading in the confirmatory factor analysis performed by these authors in each of these executive components. For dual-task performance we employed a standardized paradigm (Baddeley et al. 1997; Della Sala et al. 1995; Greene et al. 1995). For evaluating access to long-term memory we used word generation tasks (see Fisk et al. 2004), and for planning we selected the ecological Zoo Map Test (Wilson et al. 1996). We also evaluated arousal and sustained attention/vigilance changes that could interfere with executive measurements, in addition to performance on other working memory subsidiary components (see Baddeley 2007; Repovs and Baddeley 2006) that store visuospatial data (visuospatial sketchpad), phonological information (phonological loop), and integrated information from different modalities, including activated long-term memory (episodic buffer).

MATERIAL AND METHODS

Participants: participants were 42 healthy native Portuguese speaking volunteers (aged 18 to 35) with body mass index between 20 and 25, with at least 12 years of schooling. They were non-smokers, in good physical and mental health as determined by medical history, scored within normal ranges in the Beck Depression Inventory and the State-Trait Anxiety Inventory (Gorenstein and Andrade 1996), and were on no psychotropic medication at the time of the study.

Procedure: this was a randomized, double-blind, placebo-controlled, independent group-design study in which participants were randomly allocated to three acute oral treatments formulated in identical capsules (14 subjects each): placebo (glucose), donepezil 5 mg and donepezil 7. 5 mg. The Ethics Committee of the institution (Universidade Federal de São Paulo – UNIFESP) approved the study protocol (project no. 0335/07) which was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent and their IQ was estimated using the Raven’s Progressive Matrices (Raven et al. 1988). On the day of the experiment participants were required to have a light breakfast after which they receive treatments. They were submitted to a test battery (see below) at 210 min. after treatments (close to peak-plasma concentration of donepezil: Jann et al. 2002) that lasted 1. 5 h. with no prior training to insure that executive processing was involved (see Rabbitt 1997). Tests were presented in 4 randomly assigned orders, balanced between treatments.

Test Battery

Executive tasks

– Plus-minus task (Miyake et al. 2000): a measure of shifting that consisted of three lists of 30 two-digit numbers (the numbers 10-99 pre-randomized without replacement) on a single sheet of paper. On the first list, the participants were instructed to add 3 to each number and write down their answers. On the second list, they were instructed to subtract 3 from each number. Finally, on the third list, the participants were required to alternate between adding and subtracting 3 (i. e., add 3 to the first number, subtract 3 from the second number, and so on). List completion times, omission and comission errors were determined. The cost of shifting between the operations of addition and subtraction was then calculated as the difference between the time to complete the alternating list and the average times to complete the addition and subtraction lists. All the lists were performed under articulatory suppression (uttering the letter “ T”) to prevent the use o phonological stratergies while the task was performed.

– Letter memory task (Miyake et al. 2000): a measure of updating in which several letters from a list were presented serially for 2000 ms per letter. The task was to recall the last 4 letters presented in the list. To ensure that the task involved continuous updating, the instructions required that participants rehearse out loud the last 4 letters by mentally adding the most recent letter and dropping the 5th letter back. For example, if the letters presented were ” T, H, G, B, S” the participants should say, ” T . . . TH . . . THG . . . THGB . . . HGBS and answer “ HGBS” at the end of the trial. The number of letters presented (5, 7, 9, or 11) varied randomly across trials to ensure that participants would continuously update their working memory

representations until the end of each trial. After practicing on 2 trials with 5 and 7 letters, participants performed 12 trials for a total of 48 letters recalled, which took approximately 12 minutes. The dependent measure was the proportion of letters recalled correctly in the right serial order.

– Stroop task (Stroop 1935): a test of inhibition that consists of a Word Colored Page, with common words printed in colors, and a Color-Word Page with names of colors printed in incongruent colors. The examinee must name the ink colors as quickly as possible. For each list the test yields two scores, the number of errors and the time necessary to complete the task. In addition, scores from the word colored page (which measures naming speed) are subtracted from those of the color-word page (naming with inhibition) to yield a score of the extra time needed for overriding the inconcruency of word name versus ink-color name.

– Dual-task paradigm (Baddeley 1997; Della Sala et al. 1995): evaluates dual-task performance. This is a paper and pencil test which involves a visuospatial tacking task (circle crossing) and a phonological/verbal one (digit span). The digit span task consists of the 90 sec. long repetition of digit sequences presented orally which the subject had to repeat in the proper order. Lists of increasing number of digits are read aloud at the rate of one digit per second and the participants are asked to repeat them in their order of presentation (forward digit span, which measures phonological loop functioning). Participants' digit span was taken to be the maximum length at which subjects repeated correctly 5 of 6 sequences of digits. Spans or scores were the number of digits contained in the last sequence repeated correctly. The circle crossing task consists of traversing with an " X" a chain of 240

circles linked with arrows to form a path laid out on an A3-sized sheet of paper, which was practiced with a 240 circles path. Subjects are required to cross-out the circles as rapidly as possible for a period of 90 sec. The dual-task condition consists of the simultaneous execution of both tasks within a 90 sec period. To quantify participant's performance we used the measure proposed by Baddeley et al. (1997), the mu index which expressed the overall percentage loss in the dual-tasks in relation to single tasks considering the contributions of both tasks to be of equal weight: mu = [1-(pm + pt)/2] x 100, where pm and pt were, respectively, the proportional phonological loss and visuospatial loss in the performance in the dual-task condition in relation to the single-task condition; pm equaled the number of correctly repeated digit sequences for the single-task (ps), and for the dual-task (pd), both divided by the total o sequences remembered (pm = ps – pd); pt equalled the number of traversed circles for the single-task (ts) minus those traversed in the dual-task (td), divided by ts [pt = (ts – td)/ts].

– Zoo Map Test (Wilson et al. 1996): a task that measures planning abilities from the ecological Behavioural Assessment of the Dysexecutive Syndrome test battery (BADS). Participants are given a map of a zoo and a set of instructions relating to places they have to visit (e. g. elephant house, lion cage) and rules they must stick to (e. g. starting at the entrance and finishing at a designated picnic area, using designated paths in the zoo just once). There are two trials with identical aims that involve a visit to six out of the 12 possible locations. The first trial consists of a " high demand" version in which the planning abilities of the participants are rigorously tested. In the second, or " low demand" version, the participant is simply required to follow

some instructions to reach specific locations. Scoring was based on the total number of errors in the high and low demand tasks, as well as the difference in time to conduct the high and low demand tasks [i. e. planning/thinking time and execution (drawing time) of the route in the high demand trials minus the drawing time in the low demand task (Allain et al. 2005)].

– Word and letter fluency (Lezak 2004): to test access to long-term memory participants were told to orally generate as many words as possible that belonged to a given category and that began with a given letter in 2 minutes each. The participants were instructed not to use proper nouns or morphological variations of words and to void repetitions. Scores were the total number of words generated and errors.

– “ Executive” digit span (modified from Della Sala et al. 1995): this task was the same as the digit span described above in the dual-task performance except that participants had to repeat the sequences backwards (backwards digit span). Spans or scores were the number of digits contained in the last sequence repeated correctly. A delta score (backward minus forward digit span) was also calculated because participant’s capacity to recall items backwards depends on their forward span.

Other working memory test

– Corsi block test [computerized version based on Miyake et al. (2001)]: participants were shown a set of blocks (drawn as white boxes) and asked to remember the order in which they were “ tapped” (shown as changing color). One box at a time turns black for 650 ms each, a duration short enough to discourage the use of idiosyncratic coding strategies. Five similar

but different configurations of blocks were used in each trial to discourage participants from using numerical coding of box locations. Immediately after a sequence of “ taps,” participants repeated the order (Corsi Block task direct, a measure of the visuospatial sketchpad) by clicking on the boxes with the mouse. Once the sequence of flashing boxes was completed, they had unlimited time to respond. There was a practice trial with two taps each, after which the sequences progresses in length from three to 10 taps or until the participants made two mistakes with a sequence of the same number of taps. Scores were the largest number of blocks recalled in the right sequence. The same procedure was conducted at the end of this task, except that subjects were asked to remember the taps in the inverse order (Backwards Corsi Block task, a general measure of executive functioning). Delta scores as calculated for digit span were also computed.

– Counting span [Conway et al. (2005) in the version designed for adults by Engle et al. (1999)]: a task of working memory capacity that evaluates storage in the episodic buffer component of working memory (see Baddeley 2007). Participants were presented with displays on screen which consisted of a random arrangement of three to nine dark blue circles, one to nine dark blue squares, and one to five light blue circles. The participants’ task was to count and remember, in the right serial order, the total number of dark blue circles presented in consecutive displays which varied randomly in number from 2 to 6 (3 sequences each). Scores were the number of correct sequences retrieved.

Arousal/vigilance/visuomotor performance measures

– Psychomotor Vigilance Test (Dinges and Powell 1985): a portable device (Model PVT-192, CWE, Inc, Ardmore, PA) was used. The task consists of responding by button press to a small, bright red-light stimulus (light-emitting diode digital counter) as soon as it appears. Consecutive stimuli appear randomly in the range of 2 to 10 s for 5 minutes, resulting in 30-45 reaction time (RT) measures, depending on RT latency (Roach et al. 2006). Participants are instructed to press the button as soon as they see the stimulus, but not to press the button too soon (which yields false-start warnings on the display). Each subject was allowed a single 1-minute acclimation practice before the task commenced. Scores were mean total reaction time (RT), mean 10% fastest and slowest reaction times (Mean F RT and Mean S RT), all of which indicate arousal/psychomotor performance (Lim and Dinges 2008) and measures that indicate better sustained attention/vigilance (Lim and Dinges 2008), the percent change in RT throughout the test (% change) and slope reaction time (negative numbers indicate slowing from the beginning to the end of the test).

Statistical analysis

To compare treatment groups we employed one-way analyses of variance (ANOVAs) with treatment as factor (3 levels: placebo, donepezil 5 mg and donepezil 7. 5 mg) followed by post hoc Tukey HSD tests when appropriate. The level of significance adopted was pâ‰¤0. 05. Only measures that elicited significant drug effects are reported below. Magnitude of effects on the executive measures was determined through effect size calculations (Cohen d, Cohen 1988) as proposed by Snyder et al. (2005) and Fredrickson et al. (2008). In addition we calculated the Pearson Product Moment

correlation between changes in arousal/vigilance measured by the PVT and the variables that showed significant effects.

RESULTS

Comparability of treatment groups

The ANOVAs showed the comparability of participants in the three treatment groups in terms of age, body mass index and estimated intelligence measured by Raven’s Progressive Matrices (ps> 0. 27), so performance differences between treatments could not be accounted for by these characteristics.

Treatment effects (Table 1)

Data on PVT task of one subject in the placebo group and data on fluency test of one subject in the placebo group and of two subjects in donepezil 7. 5 mg group were lost due to technical problems with the equipments.

The ANOVAs showed PVT treatment effects for the minimum reaction time (F2, 38= 4. 42, p <0. 02), a measure of visuomotor performance and arousal, and also for the measure of sustained attention, the percentual change throughout the test (F2, 38= 4. 44, p <0. 02). In these tasks the 7. 5 mg dose of donepezil led to better performance when compared to placebo (ps <0. 05), the performance in the 5 mg dose having been intermediate, but non-significantly different from the other groups. Treatment effects were also observed for the dual-task mu index (F2, 39= 3. 34, p <0. 05) (Figure 1), as well as on the delta score in the digit span task (backward minus the forward span) (F2, 39= 3. 48 , p <0. 05). Performance in none of the

subsidiary working memory tasks showed significant treatment effects. For the zoo map test we found a marginal effect (F2, 39= 3. 09, p <0. 057) (mean±SD placebo: 167. 64±176. 71; donepezil 5 mg 61. 43±37. 89; donepezil 7. 5 mg: 92. 71±88. 46). However, in the placebo group there were two outliers which had delta zoo scores higher than mean plus 4 SD. When these subject were excluded from the analysis, the treatment effect was no longer significant (F2, 37= 1. 22, p <0. 31).

[Table 1 and Figure 1 near here]

Magnitude of effects

Effect sizes (see Table 1) comparing placebo and 7. 5 mg of donepezil were large (pâ‰¥0. 8, see Cohen 1988; Sloan et al. 2005) for most of the PVT measures, as well as the delta score of the digit span. The remaining comparisons between these groups yielded medium effect sizes (between 0. 5 and 0. 8), that together with large effect sizes are considered meaningful differences (Cohen 1988; Sloan et al. 2005), and included the dual-task measure.

Correlations between executive and other general attentional measures

In order to determine whether arousal/vigilance/visuomotor changes were responsible for the observed executive effects, we calculated Pearson Product Moment correlations between the PVT measures and the executive measures that showed significant effects of donepezil (mu dual-task index and delta scores of the digit span). Correlation values were small and non-significant (all ps> 0. 05 and rs between -0. 22 and 0. 17).

5. Sample sizes

In order to show that the sample size was adequate for this set of data we calculated the number of individuals necessary to show significant differences between placebo and the donepezil dose that showed significant differences in relation to placebo (7. 5 mg). To do so we used the calculations proposed by Rosner (1999) [with an Î± of 0. 05 and 80% power]. This takes into account the mean values of the groups under comparison and their common standard deviation. We carried out these calculations considering one-sided differences since our hypothesis was that donepezil would increase performance (see table 1).

DISCUSSION

In the present study we completed a comprehensive examination of the potential capacity of a pro-cholinergic drug to improve executive functions in healthy young adults exploring diverse processes associated with executive tasks. Our findings extend previous reports on the acute nootropic potential of this drug in young, healthy volunteers (Hutchison et al. 2001; Thompson et al. 2000; Zaninotto et al. 2009). More specifically, an acute 7. 5 mg dose of donepezil improved arousal/vigilance/visuomotor measures in addition to increasing performance especifically in the executive dual-task domain. An increment in delta digit span was also observed, a task that has unknown loading on the 6 executive components studies here.

A role for acetylcholine in modulating executive function is consistent with earlier work reporting impairment after acute doses of the antimuscarinic scopolamine (Ellis et al. 2006; Green et al. 2005; Rusted et al. 1991a; Rusted

1988; Rusted and Warburton 1988; Thomas et al. 2008). However, in the present study we found this effect to be highly selective within the broad battery of executive domains. Only the dual-task domain measure was sensitive to the effects of donepezil while this drug left the remaining 5 tested executive domains unchanged.

These evidences suggests cholinergic enhancement in the coordination of two tasks that rely on different cognitive systems, possibly due to activation of cortical cholinergic inputs which facilitate cognitive processes by increasing filtering of noise and distractors, which are necessary under taxing attentional conditions (Sarter et al. 2001). The magnitude of these positive changes reflected medium effect sizes which are treated as clinically meaningful (e. g. see Sloan et al. 2005) and that should be considered in light of the fact that the participants had optimum baseline performance having been young, highly educated, physically and mentally healthy, not deprived of sleep, food or otherwise compromised.

The present result was not mediated by increases in speed of information processing, improvement in performance that relies in subsidiary working memory systems, nor task demands, as discussed below. This (see Logie and Della Sala 2001) lends support to the idea that the cholinergic system is involved in the executive process that coordinates different specialized functions when considered together with previous reports of scopolamine induced impairment of dual-task performance (Rusted and Warburton 1988). It is also noteworthy that patients with Alzheimer´s disease, which is in part characterized by cholinergic deficiency (Everitt and Robbins 1997), display particular problems in dual-task in comparison with single-task performance

using the same (Baddeley et al. 1991; Greene et al. 1995; Kaschel et al. 2009) and different (MacPherson et al. 2007, see also Logie and Della Sala 2001) dual-task paradigms. Hence, we here obtained a pharmacological dissociation that confirms behavioural data suggesting the separability of dual-task coordination from other executive domains (e. g., Baddeley 1996b; Baddeley and Della Sala 1996; Bourke et al. 1996; Bourke 1997; Miyake et al. 2000; see also de Ribaupierre and Ludwig 2003).

It could be argued that this was solely due to the lack of power of the study. Sample size calculations taking into account data from the placebo and donepezil 7. 5 mg groups showed that the number of participants necessary for the obtention of statistical effects in the measures that were statistically significant here were close to that used in this study. However, the number of individuals in each group had to be larger than 66 to show significant effects in the remaining executive domains (Table 1). To our knowledge no study in this field of research has ever used such a large sample size. Hence, we believe that dual-task performance, among the executive domains investigated here, is particularly sensitive to improvement by increases in acetylcholine levels.

On measures of general attention, donepezil improved (significantly with large effect sizes) sustained attention, arousal and visuomotor performance in the PVT, cognitive functioning measures that have been previously shown to be affected by cholinergic manipulations (Furey et al. 2000, 2008a; Meinke et al. 2006). These changes could in themselves have led to better executive performance, but this seems unlikely in the present case because better overall attention would not have benefited only this single executive

component. In addition, no significant correlation was found between these general attentional scores and those of the executive tasks that were enhanced by donepezil, and r values were small.

The changes in executive functioning found here could also not be ascribed to improvement in the subsidiary working memory systems which were unchanged by donepezil, in accordance with previous lack of effects with other acute cholinergic manipulations of the articulatory loop and visuospatial sketchpad (see Mintzer and Griffiths 2007; Rusted 1988; Zaninotto et al. 2009), as well as the episodic buffer (see Zaninotto et al. 2009). These changes could also not be attributed to task difficulty, as the letter memory task was at least as demanding as the dual-task. This latter task involved continuous updating of information of letter sequences, some of which extended way beyond subjects' spans, for approximately 12 minutes, and showed no treatment effect. Performance in this task was unchanged by donepezil administration, but a similar n-back updating task has been shown to be impaired by acute doses of scopolamine (Green et al. 2005). In the latter case, though, the n-back task relied heavily on visuospatial perception and processing, which seem particularly sensitive to cholinergic manipulations (Ellis and Nathan 2001; Thomas et al. 2008; Zaninotto et al. 2009). In retrospect we noted that none of the executive tasks used here made specific demands on this type of processing, neither did the executive inhibition task employed by Mintzer and Griffiths (2007), which was unaltered by acute scopolamine administration. In effect, Thomas (2008) suggested that visuomotor and working memory processes that subserve visuospatial executive function are specifically dependent on

cholinergic neurotransmission. Hence, enhancement of cholinergic activity could cause specific top-down optimization of visuospatial input processing which could lead to improved executive visuospatial performance, especially if the extensive involvement of executive functions with visuospatial short-term memory is taken into account (see Miyake et al. 2001).

Based on this suggestion it may be hypothesized that the improvement in the delta digit span measure obtained here (high effect size) and in a recent donepezil study (Zaninotto et al. 2009), as well as impairment after anticholinegic drugs (Guthrie et al. 2000) reflect effects of cholinergic manipulations because backward digit span seems to involves activation of occipital visual cortical areas (more so than the forward version of this test) in addition to prefrontal ones (see Sun et al. 2005). Therefore, a conjunction of executive attention and facilitated visual processing by donepezil may have led to the increase in performance in this task.

Although we found cholinergic effects it is not possible to determine whether the present finding are due to the activation of nicotinic or muscarinic receptors because donepezil increases the amount of acetylcholine that can activate all acetylcholine receptors. Both types of receptors have been found to interact functionally, having synergistic effects particularly on visuospatial attention (Greenwood et al. 2009), working memory, and vigilance tasks (see Ellis et al. 2006; Erskine et al. 2004) so our data may reflect the effects of their combined activation.

In sum, acute oral administration of 7. 5 mg of donepezil to young, healthy volunteers had a selective positive effect in executive dual-task performance

that was seemingly independent of the donepezil-induced improvement on broad attentional processes (arousal/visuomotor/vigilance) and working memory slave systems, corroborating the proposal that this type of executive processing constitutes a separable cognitive construct. In addition, improvement in the digit span delta scores points to the role of cholinergic modulation on other central executive measures, possibly those that rely more heavily in visuospatial processing.