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Acute administration of roflumilast enhances immediate recall of verbal word memory in healthy young adults

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ABSTRACT

The need for new and effective treatments for dementia remains indisputably high. Phosphodiesterase inhibitors (PDE-Is) have proven efficacy as cognitive enhancers based on their positive effects in numerous preclinical studies. Especially the PDE4 subfamily is of interest due to its expression in the hippocampus, the key structure for memory formation. The current study investigates the memory enhancing effects of the clinically approved PDE4-I roflumilast in a test battery including the Verbal Learning Task (VLT) combined with electroencephalography (EEG) recording. This acute study was conducted according to a double-blind, randomized, placebo-controlled, 4-way crossover design. Three capsulated dosages of roflumilast HCl (Daxas) and a placebo were administered in four study periods. Administration occurred 1 h before testing to reach maximal plasma concentrations. Memory performance was assessed using a 30 word Verbal Learning Task. The number of words recalled both immediately and after 45 min and 24 h were included as outcome measures. EEG was recorded during the cognitive tasks on the first day. Different event-related potentials (ERPs) were considered with special emphasis on P600, as this peak has been related to word learning. Memory performance was significantly improved after acute administration of 100 μg roflumilast. Specifically, immediate recall performance on the VLT increased 2–3 words, accompanied by an enhanced P600 peak during word presentation at the third learning trial. No side effects typical for PDE4-Is were reported for the lowest and effective dose of 100 μg roflumilast. The current proof-of-concept study shows for the first time the potential of low-dose roflumilast administration as a memory enhancer in humans.

1. Introduction

Despite the awareness of the increasing impact of dementia on society in the upcoming ages, an unjustified discrepancy exists between the extent of investigations into its underlying pathological mechanisms and current treatment strategies. Part of this can be attributed to the multi-causal nature of dementia. Recently, the FDA has indicated to be open for considering treatments for dementia and in particular Alzheimer's Disease (AD), that focus on having an effect on cognitive impairment (Kozauer and Katz, 2013), which will positively affect the array of approved treatment options.

Phosphodiesterase inhibitors (PDE-Is) can be considered as cognitive enhancers based on their positive effect on cognitive processes in numerous animal studies (Reneerkens et al., 2009; Heckman et al., 2015a,b). PDE-Is exert their effects downstream via modulation of the cyclic nucleotides cGMP and cAMP. These second messengers transfer an extracellular signal, such as the binding of a neurotransmitter to its receptor, into nonstructural (e.g. increased neurotransmitter release, receptor mobilization) and structural (e.g. receptor generation and/or synapse formation) cellular responses (Wei et al., 1998; Lu and Hawkins, 2002). The former implicates the activation of protein kinases and the latter the additional activation of specific transcription factors. Both responses increase the efficacy of signal transduction and may underlie neuronal plasticity including long-term potentiation (LTP);

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the underlying physiological substrate of memory (Bliss and Collingridge, 1993).

PDE4 represents one of the eleven phosphodiesterase families (PDE1-PDE11), each of which shows a different distribution throughout the body. PDE4 is known to be widely distributed in the brain, both in rats and humans (Reneerkens et al., 2009; Lakics et al., 2010). More specifically, PDE4 is highly expressed in the frontal cortex and hippocampus, key structures in memory function (Mclachlan et al., 2007). PDE4-Is exert their actions by the selective inhibition of PDE4, an enzyme which degrades the second messenger cAMP (Bender and Beavo, 2006). cAMP activates protein kinase A (PKA), which can eventually result in the phosphorylation of the transcription factor cAMP response element binding protein (P-CREB). PKA as well as P-CREB, which induces expression of CREB responsive genes, are involved in synaptic plasticity, memory and cognition (Frey et al., 1993; Barad et al., 1998; Li et al., 2011). Improvements in LTP and memory performance in rodents after PDE4 inhibition can be attenuated by concomitant inhibition of hippocampal cAMP/PKA/CREB signaling (Bollen et al., 2014; Bernabeu et al., 1997). Taken together, animal studies show that central PDE4 inhibition and its effect on LTP underlies the memory enhancing effects of PDE4-Is.

The development of PDE4-Is as cognition-enhancing drugs has been hampered by the dose-limiting emetic side effects in humans, particularly nausea and even vomiting (Hebenstreit et al., 1989; Puhan, 2011), as was evident with rolipram. Currently, PDE4-Is are being developed with a strongly improved therapeutical window by reducing the emetic side effects. Roflumilast (Daliresp or Daxas) is such an example which was approved by the FDA for the treatment of Chronic Obstructive Pulmonary Disease (COPD) in 2011 (Izquierdo and Aparicio, 2010; Chong et al., 2011). Recently, we (Vanmierlo et al., 2016) and others (Jabaris et al., 2015) have shown that roflumilast is brain penetrant and improves short-term and long-term memory in rodents. Importantly, a PET study with the ligand [18F]B9302-107 for roflumilast confirmed that the currently marketed dose for COPD is also brain penetrant in humans (Ji, 2009). On basis of these data, and the low emetic effects of roflumilast, this offered an excellent opportunity to investigate the cognitive effects of a PDE4-I in humans in a neuropsychological test battery.

In order to find the optimal acute dose for cognition enhancement in human subjects, we estimated the dose on basis of animal data (Vanmierlo et al., 2016). In addition, the dose should not induce emetic effects in humans. For COPD treatment, a daily dose of 500 μg roflumilast is prescribed which causes mild to moderate nausea in approximately 5% of the COPD patients (Rabe, 2011).

The performance on the verbal word learning task was the primary outcome measure. In addition, electroencephalography (EEG) recordings were included to examine whether the drug affected information processing in the brain. For the current study, we specifically expected potential memory improvements to be reflected in altered P600 amplitude since this Event-Related Potential (ERP) peak has been related to word learning (Balass et al., 2010).

2. Methods

2.1. Subjects

Forty-four healthy young university students were recruited through advertisements. The age-range for inclusion was 18–35 years of age. Informed consent was obtained from all volunteers and they received financial compensation. Exclusion criteria included current or history of cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, or hematological illness. In addition, volunteers with a first-degree relative with a psychiatric disorder including history of depressive disorder with or without suicidal risk were excluded as well. Other exclusion criteria were excessive drinking (>20 glasses of alcohol containing beverages a week), pregnancy or lactation, use of chronic medication other than oral contraceptives, use of recreational drugs from 2 weeks before until the end of the experiment, smoking, orthostatic hypotension, lactose intolerance, and sensory or motor deficits which could reasonably be expected to affect test performance. Subjects with current or a history of psychiatric illness were excluded as well based on the outcomes of a semi-structured neuropsychiatric interview (Apa, 1994). Two subjects were excluded because of psychopathology. The physical health of the remaining subjects was evaluated by a physician by means of a medical questionnaire and medical examination, including ECG, and blood and urine screening.

Out of this group, twenty-two subjects were selected for participation, based on their performance in the verbal learning task (VLT; Van Der Elst et al., 2005). Subjects performing in the lower and upper quartile were excluded in order to minimize floor- or ceiling effects (cf. Reneerkens et al., 2013). All procedures were approved by the local Medical Ethical Committee and in accordance with the declaration of Helsinki.

2.2. Design

This acute study was conducted according to a double-blind, randomized, placebo-controlled, 4-way crossover design. Order of treatments was balanced over the test days using orthogonal Latin square design. Test days were separated by a washout period of at least 10 days. Beforehand, subjects were familiarized with the setting and the cognitive test battery. After each test day, participants returned 24 h later to perform two cognitive tasks. EEG was recorded during the first day of testing only.

2.3. Treatment

Roflumilast HCl (Daxas) 500 μg tablets were grinded, and the appropriate quantities (i.e., 100, 300, 1000 μg) were distributed over capsules with lactose monohydrate as the principle constituent. The placebo capsules contained lactose monohydrate in an equivalent amount and the appearance was identical to the roflumilast capsules. The capsules were manufactured, blinded, and labelled by Basic Pharma Technologies BV (Geleen, the Netherlands) according to GMP regulations. Roflumilast was administered orally 1 h before testing based on maximum plasma concentrations (EMA, 2010).

2.4. Cognitive assessment

2.4.1. verbal learning task

The VLT is an adapted version of the original 15 word Rey auditory verbal learning test (Lezak, 1995), which assesses short- and long-term memory function for verbal information. The current task was developed to maximize the possibility of measuring enhancement rather than only impairment, by means of prolonging the list to 30 words (Riedel et al., 1999). The test consists of a list of 30 Dutch monosyllabic words (18 nouns and 12 adjectives). For each test day, a different validated version was used in each period. The use of the different versions was counterbalanced over the four periods. Words were shown on a computer screen for 1 s which was followed by a 2 s inter-trial interval. Each trial ended with a free recall of the words (immediate recall). Forty-five minutes after the first exposure, the participants were asked to recall as many words as possible (delayed recall). EEG was recorded during the learning

trials on the test day.

2.4.2. Spatial memory task

The Spatial Memory Task (SMT) assesses spatial memory and is based on the object relocation task by Kessels and colleagues (Kessels et al., 1999; Gazzaniga et al., 2002). It consists of 1 immediate and 2 delayed conditions. In the immediate condition, a set of 10 pictures was presented 1 by 1 on different locations within a white square on a computer screen. All pictures were every day, easy-to-name objects, presented in grayscale (about 3.5 × 5 cm). Each picture was presented for 2 s with an inter-stimulus interval of 1 s. This was followed by a 'relocation' part, which consists of the presentation of a picture in the middle of the screen, followed by a '1' and a '2' being presented on 2 different locations. The participants' task is to decide where the picture was originally presented, in location '1' or location '2'. The '1' and '2' remain on the screen until the participant responds. After relocation, which is accomplished by a button press, the next picture is presented followed by the '1/2' choice option. This continues until all 10 pictures are relocated. Thereafter, the next set of 10 pictures was presented. A total of 6 sets of 10 pictures were displayed. Forty-five minutes later, participants performed the first delayed relocation version. The original locations were not presented again.

Twenty-four hours after the immediate condition, participants returned to the lab to perform the task again. Once more, they had to decide where the picture was previously presented in location '1' or location '2'. The '1' and '2' remained on the screen until the participant responded. The number of correctly localized items was collected during the immediate and two delayed periods. As with the other tests, the EEG was recorded during this task on the test day.

2.4.3. Stroop task

The Stroop task is well known for its ability to induce interference, and assesses response inhibition and focused attention. In this task, color names (in Dutch) are printed in colored ink; in the congruent category, the color name and the color of the ink are the same, in the incongruent category they are not. The subjects have to name the color of the ink, not the words themselves. However, because of the urge to read the printed words (even if one is asked to ignore them) interference occurs. Since the printed words and ink color differ in the incongruent category, interference is larger in this category than in the congruent category; this is called the 'Stroop effect' and is known to remain even after extended practices (Gazzaniga et al., 2002). The colors used in this task are blue, red, green and yellow. The color of the ink has to be named by pressing one out of four buttons, which each represent one of the colors. In total, 144 stimuli are presented; 72 congruent and 72 incongruent items. The main performance measures are the RT and the correct responses. EEG was recorded during the task and it was analyzed similarly to the EEG recorded during the memory tasks.

2.5. Plasma levels and PK modelling

Blood samples were collected for measurement of roflumilast and roflumilast N-oxide concentrations at approximately the same time as the cognitive battery measurements (i.e., 2.25 and 24.5 h following administration). Plasma concentrations of roflumilast and roflumilast N-oxide were determined using a validated assay using high-performance liquid chromatography with tandem mass spectrometry method (Knebel et al., 2012).

Pharmacokinetics of roflumilast and roflumilast N-oxide were previously described using a 2- compartment model with linear absorption and elimination and a 1-compartment model with zero-order absorption and linear elimination (Lahu et al., 2010). The model parameters, except residual error, were initially fixed to those estimated from the published models to avoid model over-parameterization. Model evaluation was based on reduction in objective function value (OFV), improvement in goodness-of-fit diagnostics, reduction in inter-subject and residual variability, and improvement in parameter precision. Changes to the published model included increased oral bioavailability for roflumilast, estimation of a separate absorption rate constant (KA) for roflumilast and formation rate (D1) for roflumilast N-oxide for the 1000 μg cohort, and estimation of inter-subject variability for KA and D1 to eliminate bias due to changes in the administered formulation as a results of reformulation of the commercial formulation and the limited amount of PK samples.

Given the sparse nature of the PK sample collection, model-based simulations were performed to project the individual time course of roflumilast and roflumilast N-oxide concentrations over the treatment period using the empirical Bayesian estimates derived from the model. The simulated data were subsequently summarized using non-compartmental methods (i.e., C_{max}, AUC).

2.6. Questionnaires

Potential side effects and subjects' wellbeing was monitored with questionnaires. At baseline and during cognitive testing at 100 min after drug intake, a questionnaire was administered to assess changes in mood using the profile of mood states (POMS). Subjective alertness was assessed using a visual analogue rating scale consisting of in total 16 items, 9 of which comprise the subjective alertness subscale (Bond and Lader, 1974). Also physical complaints were measured by a general questionnaire consisting of 31 items with a 4 point scale ranging from 0: 'not at all' to 3: 'strongly'. During the 24 h measurement, subjects completed both questionnaires twice: first to assess their well-being at the time of arrival and second to assess the occurrence of symptoms during the past 24 h.

2.7. EEG acquisition

EEG was recorded using the NeuroScan SynAmps system, with sample rate set at 1000 Hz. Data were filtered between 0.05 and 100 Hz. Electrophysiological activity was recorded from 32 electrodes, but for the memory related tasks only included the five midline electrodes in our analyses. Electrodes were positioned according to the international 10–20 system (Jasper, 1958) using an elastic cap (Easycap, MedCaT Neurosupplies). Eye movements were detected by horizontal and vertical electro-oculogram recordings. Reference and ground electrodes were placed on the mastoids and the forehead, respectively. Brain Vision Analyzer (Brain Products GmbH) was used as the software package to process data.

2.8. Statistical analyses

Statistical analyses were performed using PASW17 for Windows. General Linear Model for repeated measures was applied with the placebo condition included as contrast for all primary and secondary outcomes. For the analysis of EEG data, the five midline electrodes (Fz, FCz, Cz, CPz, Pz) were included as within subject variables.

3. Results

Before randomization and after the first test day, in total, two male subjects dropped-out. The remaining 20 subjects (age: 20.9 ± 2.3 yrs; 16 females) completed the study protocol.

3.1. Verbal learning task (VLT) - behavior

Roflumilast had no effect on the number of words recalled in the first two immediate recall trials. However, the number of recalled words was increased immediately after the third learning trial ($F(3,57) = 2.868$, $p = .04$. See Fig. 1). The within-subject contrast analysis showed that only treatment with 100 μg significantly increased the number of words recalled ($F(1,19) = 10.514$, $p < .004$; Cohen's d: 0.68). The higher doses did not affect the number of words in the third immediate recall trial (300 μg, $p > .62$; 1000 μg $p > .14$). In the delayed recall trials (45 min and 24 h) no general treatment effects were found ($F(3,57) = 0.827$, $p = .48$; $F(3,57) = 0.308$, $p = .74$, respectively).

3.2. Verbal learning task (VLT) - EEG

GLM tests of within subjects effects did not reveal an effect of roflumilast dose on ERP amplitude ($F(3, 54) = 2.066$, $p > .12$). A-priori defined test of within-subjects contrasts was included in the analysis and revealed a significantly increased P600 amplitude in the roflumilast 100 μg condition ($F(1,39) = 4.493$, $p = .048$; Cohen's d: 0.62) as compared to the placebo condition during the third learning trial of the VLT only (Fig. 2). The other two doses did not affect the P600 peak, or any other pre-selected peak (all p values > 0.10). No other treatment effects on peak amplitudes or peak latencies were observed (p values > 0.10).

3.3. Spatial memory task

No roflumilast treatment effects were found for the immediate and both delayed measurements ($F(3,57) = 0.341$, $p = .80$; delayed 45 min $F(3,57) = 0.866$, $p = .46$; delayed 24 h $F(3,57) = 1.399$, $p = .25$, respectively).

The location of on average 52 out of 60 pictures was correctly recalled in the immediate phase, with no difference between treatment conditions. Neither were any differences observed for the delayed phases at 45 min and 24 h with on average 46 items and 35 items (near chance level), respectively. With regard to EEG, no overall effects for roflumilast treatment were found, but incidentally statistically significant effects appeared from within subject contrasts tests. In the immediate phase, recorded when the picture was presented in the middle of the screen, immediately before the location had to be chosen, a-priori defined test of within-subjects contrasts revealed a significantly decreased P3b amplitude in the roflumilast 100 μg condition only ($F(1,39) = 6.679$, $p = .019$) as compared to the placebo ( ). For the N400, a-priori defined test of within-subjects contrasts revealed increased negativity in the roflumilast 100 μg condition only ($F(1,39) = 6.239$, $p = .023$; supplementary materials). Without any behavioral effects, interpretation of the meaning of these findings would be highly speculative. With regard to the P600, no treatment effects were found ($F(3,51) = 0.695$, $p = .56$).

3.4. Stroop task

No roflumilast treatment effects were found for correct responses in the Stroop for both congruent and incongruent stimuli (resp. $F(3,57) = 0.886$, $p = .45$; $F(3,57) = 1.11$, $p = .35$); neither for reaction time at the congruent items ($F(3,57) = 1.661$, $p = .19$). Tests of Within-Subjects Contrast did not show a significant difference between any of the roflumilast conditions as compared to placebo ($p > .1$).

With regard to EEG, different Event Related Potentials were evaluated for both congruent and incongruent items. A priori defined analyses included P300. For congruent items, no difference in peak amplitude was found ($F(3,57) = 0.712$, $p = .46$). For incongruent items, no difference in P300 peak amplitude was found either ($F(3,57) = 0.296$, $p = .72$). Post-hoc analyses were performed for N200 peak amplitude. For congruent items, no difference was found ($F(3,57) = 1.539$, $p = .23$); neither for incongruent items ($F(3,57) = 1.239$, $p = .30$ (supplementary materials)).

3.5. Plasma levels and pharmacokinetics

The observed and simulated roflumilast and roflumilast N-oxide concentration data grouped by dose are presented in Fig. 3.

The distribution of individual C_{max} and AUC values for roflumilast and roflumilast N-oxide are presented in Table 1.

3.6. Questionnaires

Delta values (post-treatment − baseline) for individual items on the physical complaints list were compared between roflumilast treatment conditions and placebo. Symptoms related to gastrointestinal functioning did not show increased prevalence at 100 min

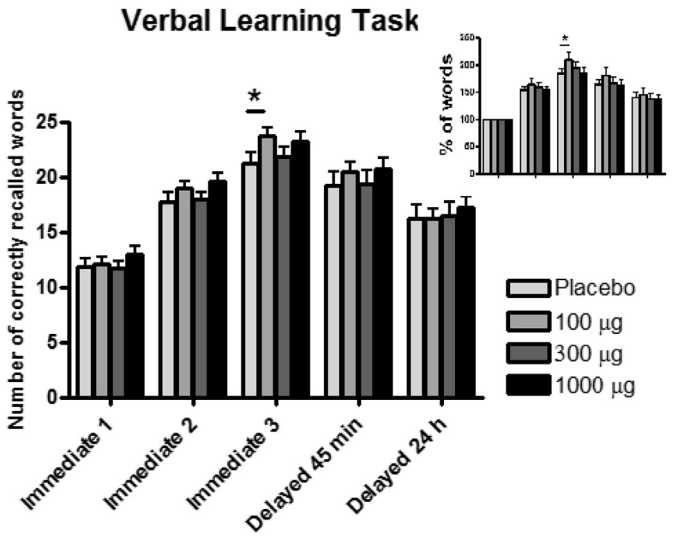

Fig. 1. Number of words recalled immediately after the learning trials (immediate 1–3) and during delayed recall at 45 min and 24 h. Values represent mean ± SEM (n = 20). The insert represents the percentage of words recalled relative to the first immediate recall for each treatment condition set at 100%.

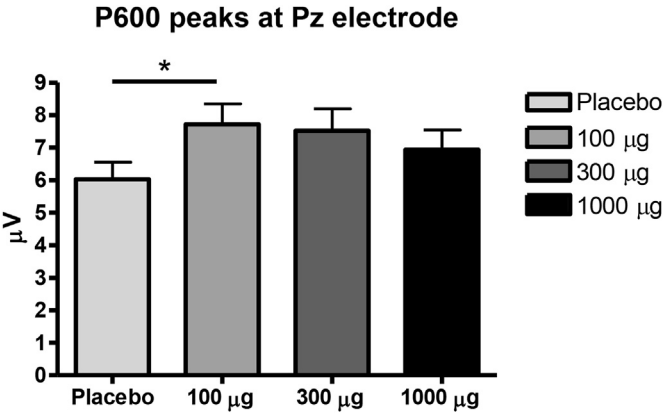

Fig. 2. Mean values of the P600 peak at Pz electrode under different roflumilast treatment conditions. *p < .05.

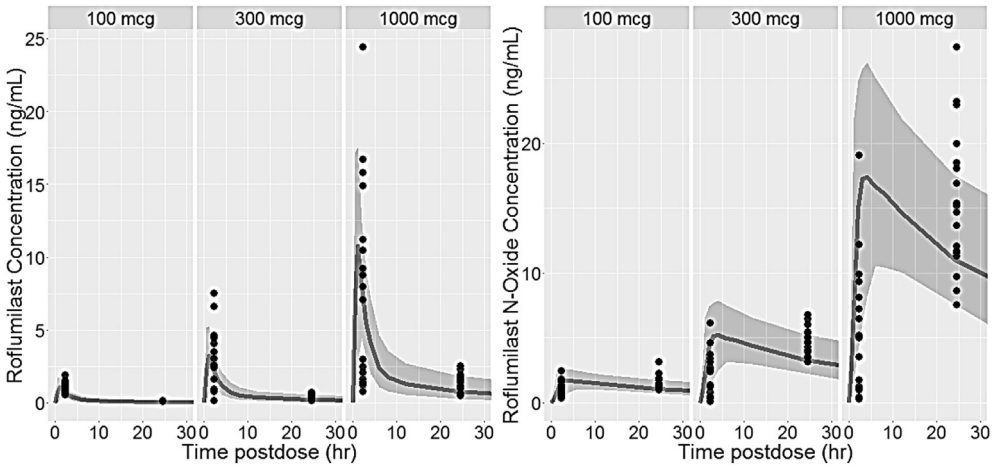

Fig. 3. Observed (black dots) and simulated historical data (dark gray areas; median and 95% prediction interval) of roflumilast and roflumilast-N-oxide for different doses 2.25 h and 24.5 h after treatment. Simulated data were based on a population PK model (Lahu et al., 2010).

after drug intake. Tiredness was reported most strongly in the roflumilast conditions as compared to the placebo condition with increased tiredness delta values in 4 subjects in the placebo and 100 μg condition, 8 subjects in the in the 300mcg condition and 7 in the 1000 μg condition. Measurements obtained at 24 h after drug intake, questioning symptoms over 24 h, showed no differences between the placebo and 100 μg condition. Increased frequencies for some physical complaints were reported. Most notably; headache, insomnia, dizziness, nausea, and restlessness were reported more frequently after 300 and 1000 μg roflumilast (supplementary materials). No significant differences were found between any roflumilast condition as compared to placebo on the five POMS subscales (depression, anger, fatigue, vigor, tension; all p-values > 0.1) or the three subscales of the Bond & Lader (alertness, contentedness, calmness; all p-values > 0.1).

4. Discussion

This study shows for the first time memory improvement after acute administration of the PDE4-I roflumilast in young, healthy subjects. The lowest dose (100 μg) improved immediate recall performance on the VLT and was accompanied by changes in the EEG. More specifically, the subjects performed well and the average number of words recalled in the placebo condition was about 21 out of 30 words after 3 learning trials. Treatment with 100 μg roflumilast increased the immediate recall performance with 2–3 words.

The P600 during the presentation of the words in the third trial was enhanced after the 100 μg dose. These findings together indicate that roflumilast affected information processing during the encoding of the words, which was associated with an improved performance on the immediate recall scores. However, this effect on memory performance was not observed during the delayed recall trials.

Table 1
Mean (SD) values for exposure metrics.

Dose (μg)	Roflumilast		Roflumilast N-oxide	
	C_{max} (ng/mL)	AUC (ng·h/mL)	C_{max} (ng/mL)	AUC (ng·h/mL)
100	2.09 (1.19)	13.06 (2.84)	1.91 (0.37)	98.97 (9.70)
300	6.27 (3.56)	39.19 (8.51)	5.74 (1.10)	296.91 (29.08)
1000	8.19 (5.19)	130.52 (28.47)	17.02 (3.51)	980.42 (98.29)

On basis of animal data with positive effects on long-term memory (Vanmierlo et al., 2016), improved delayed recall performance was expected as well. However, the current study did not find an improved memory performance after 45 min or 24 h. It should be noted that the performance in the placebo condition was better as compared to previous studies from our lab (Sambeth et al., 2007; Linssen et al., 2012; Kuypers et al., 2016). Moreover, the amount of words forgotten during the period in between learning and delayed recall was smaller than normal. Thus, it could be that potential treatment effects on the delayed recall scores were masked by relative high scores in the placebo condition.

The P300 and N400 peaks during the VLT word presentation, have been related to attentional and language related processes, respectively (Kutas and Federmeier, 2011; Huang et al., 2015). On the other hand, the P600 peak has been specifically related to memory-related processes (Balass et al., 2010). Roflumilast did not affect the P300 and N400 deflections, but −as mentioned above-the lowest dose of roflumilast increased the P600 peak amplitude. The same low dose was also found to increase the concurrent immediate recall performance in the VLT. Therefore, these ERP effects support early, centrally-mediated improved memory performance by roflumilast.

Roflumilast might improve memory performance via a release in neurotransmitters including glutamate as the latter is directly related to an increase in cAMP (Schoffelmeer et al., 1985; Imanishi et al., 1997; Rodriguez-Moreno and Sihra, 2013). In a study using a very mild induction of LTP accompanied with increased release of glutamate in rat hippocampal slices, it was found that stimulation of a spine resulted already after 2 min in an enlargement of predominantly small spines (Matsuzaki et al., 2004). This effect was persistent for at least 100 min. The described spine enlargement was associated with an increase in AMPA-receptor-mediated currents (Matsuzaki et al., 2001). Based on this it has been suggested that these large spines could be the physical traces for memory formation (Matsuzaki et al., 2004). If stimulation would be strong enough, even new synapses might be generated as has been observed to occur within 1 h after stimulation (Engert and Bonhoeffer, 1999). However, in the present study the memory improvement is not present anymore 45 min after learning. Possibly roflumilast resulted in spine enlargement, yet without any new spine generation. Another explanation could be that the effect of roflumilast on memory can be explained with a transient increase in glutamate release sufficient for transient memory improvement via AMPA receptor stimulation without any structural changes.

A second memory task was included to study spatial memory performance. No signs of memory improvement were found on the SMT. In contrast to the VLT, during the immediate recall of the SMT, participants performed all at a very high level (upper quartile) maximum ceiling effects. This may have prevented any effects to be exhibited. Conversely, during the 24 h delayed version, the lack of effect might be explained task difficulty in remembering, i.e. performing at chance level.

The Stroop task was included as it is well known for its ability to induce interference, and assesses response inhibition and focused attention. As no effects of roflumilast were detected on any of the outcome measures of the Stroop task after drug intake, it favors the memory effects not being explained through improvements in attention. However based on this one task we cannot fully exclude the possibility of drug effects on attention as non-human primate studies have shown positive effects of PDE4 inhibition on in the object retrieval task (Rutten et al., 2008; Sutcliffe et al., 2014). Of note, the object retrieval task is also a task that not only measures attention but also other cognitive abilities (e.g., response inhibition and executive control (Heckman et al., 2015a)). Further studies are indicated to more specifically address the effects of roflumilast on attention.

Based on our previous rodent pharmacokinetic data (Vanmierlo et al., 2016) and others (Jabaris et al., 2015), roflumilast is clearly brain penetrant (brain/plasma ratio of about 1). The IC50 of roflumilast for inhibition of isoforms of PDE4D, which is the most important isoform for memory (Li et al., 2011; Gurney et al., 2015), is maximally 0.4 nM (Hatzelmann et al., 2010). Data obtained with mice suggests that the free brain concentration of roflumilast is close to its IC50 for PDE4D (0.93 times) when improving memory consolidation processes (Vanmierlo et al., 2016). The N-oxide metabolite of roflumilast is considered to be the major contributor to PDE4 inhibition (>90% of the overall pharmacological effect (Hermann, 2006)). However, the contribution of the metabolite to memory processes may be less important based on its lower potency for PDE4D (IC50 is maximally 0.8 nM; see Hatzelmann et al., 2010) and lower brain penetration (brain/plasma ratio of 0.08; see Vanmierlo et al., 2016).

Also in humans, brain penetration has been demonstrated with a PET ligand (Ji, 2009). As expected, in the current study the highest dose of 1000 μg roflumilast resulted in plasma levels which were similar to the ones of roflumilast or slightly lower compared to the ones of roflumilast N-oxide reported in the literature for repeated daily administration of 500 μg roflumilast (Lahu et al., 2010). When a PK model was fit to the study data (Lahu et al., 2010), there was some bias observed from the goodness-of-fit plots (mainly the metabolite at 24.5 h after treatment). This might be due to the reformulation of the commercial formulation and the limited amount of PK samples. Further, based on our effects on both memory performance and EEG, it is clear that a dose of 100 μg roflumilast is centrally active. To have an estimation of its related biological activity, i.e. PDE4D inhibition, values for total brain penetration, i.e. brain/plasma ratio, as well as the free brain fraction are needed. However, these values for human populations are not known to our knowledge. One could assume a similar brain/plasma ratio in humans as in rodents which is about 1 (Vanmierlo et al., 2016) and a similar free fraction in the brain of 1.1% as in the plasma of humans (Lahu et al., 2008). Based on our plasma values, C_{max} for the effective 100 μg dose is 2.09 ng/ml. Of note, t_{max} for roflumilast is 1 h after administration (Lahu et al., 2011), which is the time point at which the VLT task was assessed. Based on these measures, the hypothetical concentration of the free brain fraction is estimated to be 0.057 nM. This would be about 7 times lower as the PDE4D IC50. An explanation may be that a very low dose and accompanying low brain concentration of roflumilast to inhibit PDE4 isoforms related to memory function is already be sufficient to improve memory, as downstream molecular signaling cascades can be amplified after PDE4 inhibition (Mackenzie et al., 2011).

The 300 μg dose was estimated to result in hypothetical brain concentrations of about 2 times the PDE4D IC50, i.e. 100% enzyme inhibition. Apparently, stronger enzyme inhibition is not effective to improve brain function (VLT performance and N600 peak). How this translates to central enzyme inhibition due to the N-oxide metabolite of roflumilast is not clear when taking into consideration that although in humans the plasma free fraction of the metabolite is higher than that of roflumilast itself (3.5% vs. 1.1%; Lahu et al., 2008), its brain penetration is supposedly less (brain/plasma ratio in mice about 0.08; Vanmierlo et al., 2016) and its IC50 higher than for roflumilast (PDE4D IC50 is maximally 0.8 nM; Hatzelmann et al., 2010). Nevertheless, this suggest that the 300 and 1000 μg dosages of roflumilast may be at the right side of the inverted U-curve and that it would be interesting to test lower doses of roflumilast in future studies.

The finding that the 100 μg was effective in improving memory performance in young healthy volunteers is very interesting when considering further clinical development of roflumilast. In COPD a daily dose of 500 μg is used and is associated with diarrhea and nausea in respectively about 10% and 5% of patients (Martinez et al., 2015, 2016). These are typical side effects of PDE4-Is (Richter et al., 2013). Our cognition enhancing effects were observed at a 5-fold lower dose and no side effects were reported at the 100 μg dose, whereas some subjects reported nausea (4 out of 20) at the 300 μg. This supports the notion that roflumilast may have a favorable therapeutic index for cognition enhancement when compared to COPD (Vanmierlo et al., 2016).

Currently, there are not many pharmacological treatments capable of improving verbal memory performance in healthy young subjects (Riedel and Blokland, 2015). Methylphenidate has shown to improve memory performance in the same version of the VLT as used in the current study (Linssen et al., 2012). However, methylphenidate had no effect on the immediate recall but improved the 30 min delayed recall performance. Disadvantage of methylphenidate as a drug treatment is its persisting side-effects (Storebo et al., 2015). The current study shows that a dose of 100 μg roflumilast improves VLT performance after acute treatment without obvious side effects. This puts roflumilast in a unique position as a possible cognition enhancer.

In summary, this is a first double-blind human study in which memory enhancing effects of a PDE4-I were found. The effects were found on immediate recall and were accompanied with a higher P600 amplitude. The effects were specifically found at a 100 μg dose, which did not induce any adverse effects typical for PDE4-Is in this single-dose trial. Further studies are indicated to examine whether lower doses can be effective and whether the effects can be found after steady state plasma levels. Moreover, the effects reported here warrant studies in older subjects with memory complaints to investigate the clinical potential of roflumilast.

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Statement of interest

MT, GL and TU are employees of Takeda Development Center Americas. MAvD, AB, JP and AS have a proprietary interest in the PDE4 inhibitor roflumilast.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuropharm.2017.12.019.

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