

The effects of Roflumilast, a phosphodiesterase type-4 (PDE4) inhibitor, on EEG biomarkers in schizophrenia: a randomised controlled trial.

Running head: PDE4 drug improves EEG markers in schizophrenia

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Abstract

Background

Patients with schizophrenia have significant cognitive deficits, which may profoundly impair quality of life. These deficits are also evident at the neurophysiological level with patients demonstrating altered Event Related Potentials (ERPs) in several stages of cognitive processing compared to healthy controls; within the auditory domain, for example, there are replicated alterations in Mismatch Negativity (MMN), P300, and Auditory Steady State Response (ASSR). However, currently there are no approved pharmacological treatments for cognitive deficits in schizophrenia.

Aims

Here we examine whether the PDE4 (phosphodiesterase-4) inhibitor, roflumilast, can improve neurophysiological deficits in schizophrenia.

Methods

Using a randomised, double-blind, placebo-controlled, crossover design study in 18 patients with schizophrenia, the effect of the PDE4 inhibitor, roflumilast (100µg and 250µg) on ASSR (early stage), MMN and theta (intermediate stage) and P300 (late stage) was examined using EEG. A total of 18 subjects were randomised and included in the analysis.

Results

Roflumilast 250µg significantly enhanced the amplitude of both the MMN ($p=0.04$) and working memory-related theta oscillations ($p=0.02$) compared to placebo but not in the other (early or late stage) cognitive markers.

Conclusions

The results suggest that PDE4 inhibition, with roflumilast, can improve EEG cognitive markers, which are impaired in schizophrenia, and that PDE4 inhibition acts at an intermediate rather than early or late cognitive processing stage. This study also underlines the use of neurophysiological measures as cognitive biomarkers in experimental medicine.

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Clinical Trials Registration

This trial was pre-registered at clinicaltrials.gov. Study Title: “Roflumilast Plus Antipsychotics Proof of Mechanism Study in Schizophrenia”. Identifier: NCT02079844. URL: <https://clinicaltrials.gov/ct2/show/NCT02079844>

Introduction

Patients with schizophrenia show significant cognitive deficits in multiple domains of cognitive functioning (Kern et al., 2011) which have a major negative impact on patients' day to day lives (Narvaez et al., 2008; Sensky et al., 2000). These deficits impair function and do so even more than positive symptoms (Green, 1966). Despite this and evidence that a number of compounds may improve cognition, there are still currently no approved medications to improve cognition in schizophrenia (Gilleen, 2017; Keefe, 2019). Current 'antipsychotic' medications which principally act via dopamine D2 receptors, also fail to improve cognition (Percudani et al., 2004). Thus, there is an urgent need to develop treatment strategies that improve cognitive functioning in this patient group.

PDE4 inhibitors offer a potential new line of treatment for these deficits. PDE4 inhibitors target one of eleven members of a family of intracellular enzymes that hydrolyse the cyclic nucleotide cyclic adenosine monophosphate (cAMP), thus raising cAMP levels. cAMP plays an important role as a second messenger molecule controlling multiple cellular processes (Pérez-Torres et al., 2000). Modulators of cAMP are promising therapeutic targets for schizophrenia as studies have shown impairments in levels of cAMP and cAMP signalling in the brains of people with schizophrenia (Garver et al., 1982; Muly, 2002; Tardito et al., 2000).

PDE4 may be particularly relevant for the treatment of schizophrenia, since it regulates cAMP levels in the axon terminal afferents to the striatum (Heckman

et al., 2016). Evidence has been accumulating to indicate that PDE4 inhibitors can remediate cognitive deficits, particularly memory deficits in animals (Barad et al., 1998; Hosseini-Sharifabad et al., 2012; Vanmierlo et al., 2016) including in primates (Rutten et al., 2008) in a rodent model of psychosis (Wiescholleck and Manahan-Vaughan, 2012) and most recently in patients with schizophrenia (Gilleen et al., 2018).

Improved task performance can be the result of changes at any point in task process from sensory detection to response selection and motor output and it is unclear whether PDE4 leads to improvements across the processing chain or at specific processing stages. One way to explore this issue is through the measurement EEG biomarkers, increasingly used in psychiatric and pharmacological studies (Light et al., 2012; Wilson and Danjou, 2015) and used previously to examine PDE4 inhibition in a preclinical hyperdopaminergic model of schizophrenia (Halene and Siegel, 2008; Maxwell et al., 2004). This approach offers valuable utility in identifying early indicators for prediction of potential efficacy (18).

In schizophrenia three EEG measures have been shown to be consistently reduced, which, based on the temporal profile and component frequencies, correspond approximately to: i) early sensory processing - 40 Hz Auditory Steady State Response (ASSR) (Kwon et al., 1999; O'Donnell et al., 2013; Thuné et al., 2016); ii) intermediate processing - Mis-Match Negativity (Erickson et al., 2016; Kasai et al., 1999) and iii) late processing – posterior P300 (or P3b) (Hirayasu et al., 1998; Winterer et al., 2003). The 40 Hz ASSR arises from the

resonant response of neural circuits to auditory clicks presented at a frequency in the gamma range and assesses the integrity of sensory pathways. MMN is a fronto-centrally distributed negative potential evoked by a regularity violation in a repeating series of stimuli. The auditory MMN ERP is independent of attention and smaller MMN amplitudes are associated with poorer cognitive function in schizophrenia (Baldeweg et al., 2004; Light et al., 2015). The P300 is a later ERP component evoked in similar tasks to the MMN that require a response. It is thought to relate to the updating of contextual memory (Donchin and Coles, 1988) and inhibition of extraneous brain activation, amongst other processing functions (Polich, 2007).

In addition to these measures we also explored the effects of roflumilast on prefrontal theta (3.5-7 Hz) spectral power, which reflects coordination of brain networks and cognitive control (Cavanagh and Frank, 2014) and has been shown to underpin memory in both humans and experimental animals (for reviews see (Hsieh and Ranganath, 2014; Johnson and Knight, 2015)). Theta is linked to anterior cingulate function and sustained attention (Gevins et al., 1997); and increases parametrically with working memory load (Jensen and Tesche, 2002; Raghavachari et al., 2001) and is reduced in schizophrenia (Schmiedt et al., 2005).

Here we report the EEG findings of a study testing PDE4 enhancement of cognition in schizophrenia using roflumilast. This study also included a cognitive test battery and MRI assessments, which have been reported separately (Gilleen et al., 2018). The specific predictions were that roflumilast would

increase the magnitude of these EEG measures known to be dysfunctional in schizophrenia, and that the pattern of enhancement would help determine the processing stage modulated by roflumilast.

Methods and Materials

Study Design

This was a randomized, double-blind, placebo-controlled, single-site, 3-period crossover study to evaluate the effect of roflumilast as an add-on to second generation antipsychotics. The study consisted of three counter-balanced 8-day treatment periods separated by washout intervals of at least 14 days (beginning immediately after dosing on Day 8; see figure 1 for study schedule of activities). Randomisation codes, assigned sequentially to participants at the randomisation stage, dictated treatment order of low and high dose roflumilast and placebo. For information on drug treatment sequences, compliance, plasma, exposure and safety see Supplementary Materials. EEG recordings were obtained prior to dosing on Day 1 and approximately 5 hours (approximately mid-afternoon) after dose on Day 8 for each of the three dosing periods.

Participants

Participants were men or women aged 18 to 60 years, inclusive, who met Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5 Association, 2013)) criteria for schizophrenia, were cognitively impaired, and were on a stable dose of second-generation antipsychotic medication for at least 2 months prior to screening. Participants continued taking second

generation antipsychotic medication at a stable dose and regimen throughout the study and were clinically stable with mean PANSS scores [changing by fewer than 6 points](#) across the study assessment points. Exclusion criteria are provided in Supplementary Materials. Consent was conducted in accordance with Good Clinical Practice, and specifically, for this vulnerable patient group, a psychiatrist conducted a Capacity To Consent assessment before consent was requested. Briefly, this involves a participant being able to understand and explain back what they are being asked to do in the study.

This research was conducted at the Institute of Psychiatry, Psychology and Neuroscience at Kings College London, UK and in accordance with the protocol procedures complied with the Declaration of Helsinki, the International Conference on Harmonisation (ICH) E6 GCP guidance, and all applicable local or regional regulatory requirements. The study was pre-registered with clinicaltrials.gov (identifier: NCT02079844) and ethics approvals were given by NRES Committee South Central-Berkshire (reference: 12/SC/0443).

Sample

31 participants were screened, 21 of whom were randomized, and 15 completed all the EEG sessions (see Supplementary Materials for drop-out information). Three more participants completed at least one period (including both Day 1 and Day 8). All 18 participants were included in the statistical analysis (see table 1 for sample baseline demographic and clinical information).

EEG Acquisition

EEG was acquired using a Compumedics Neuroscan SYNAMPS 2 amplifier, with 64 channels. A linked ear recording reference was used, and additional electrodes were added on the left and right Mastoids as well as horizontal and vertically around the eyes, in order to capture eye movement artefacts. A sampling rate of 5kHz was used, and the low pass and high pass filters were set at 1kHz and 0.05 Hz respectively. Impedances were kept below 10 k Ω . EEGs were recorded in the early afternoon, and the study day also included fMRI, cognitive assessment and questionnaires, reported elsewhere (Gilleen et al., 2018).

Paradigms

ASSR

Click trains at frequencies of 40 Hz, 30 Hz and 20 Hz were played through earphones. Each click train was 800ms long and inter-train interval was between 1050ms and 1150ms. For each frequency, there were 55 click trains. Participants were asked to keep their eyes closed during this task.

MMN

Unlike the P300, the MMN paradigm does not require a response to the deviant tone. The standard stimuli was a tone of duration frequency 1000 KHz and duration 150 ms, whilst the deviant had the same frequency, but a duration of 100 ms. The fixed interstimulus interval was 500 ms. There were 150 deviants, with a probability of occurrence of 0.2. The participants were asked to pay

attention to a silent video, and if they became drowsy and/or closed their eyes, they were prompted to wake up.

P300

The P300 is a positive event related potential, which occurs around 300 ms after a target tone to which the participant is required to make a response. The target tone was at a frequency of 1500 Hz, whereas the standard tone was at a frequency of 1000Hz. Both tones had durations of 200ms. The probability of a target tone was 16%, with 39 targets. The time between tones was varied randomly between 1 and 1.5 seconds. The participants were required to maintain gaze on a fixation cross during this paradigm.

N-Back Working Memory task

Strings of letters were sequentially presented on the screen every 1 second with a 2 second inter-stimulus interval. There were four different conditions indicated by instruction on the screen: "Is it an X?" indicated that the participants were required to press a key when they saw the letter X; "1-back" indicated that a response was required if the letter was the same as the previous letter, whereas in the "2-back" and "3-back" conditions the participants responded if the letter was the same as the one two letters or three letters previously, respectively.

EEG analysis

Preprocessing

Using Neuroscan 4.5 analysis software, the EEG was re-referenced to the average of Mastoid channels. For the MMN, P300 and theta amplitude

measures, blink artefacts were corrected using the regression method (Croft and Barry, 2000). For the ASSR, power line noise was corrected using noise-cancellation and muscular artefacts were corrected using mathematical modelling and subtraction of muscle spikes (Nottage et al., 2013). Occasionally, the signal from a faulty channel, in a particular EEG recording for a participant, needed to be replaced by a signal reconstructed from nearby channels. In such a case exactly the same reconstructed channel was used for analysis of all EEG recordings from that participant.

ASSR

Epochs of length 819.2ms were cut, from 25 to 844ms after the start of each click train, and the linear trend was removed. Epochs with greater than ± 70 μV amplitude at Cz were rejected. The epochs were averaged to give an ERP at 40 Hz. A Fast Fourier transform was carried out on each ERP, using a 20% Hanning window and the evoked amplitude in the 39.06-40.28Hz frequency bin was obtained.

MMN

The MMN was calculated at Fz with an average mastoid reference. A low pass 20 Hz filter and a 1 Hz high pass filter were used, following published recommendations (Duncan et al., 2009), both being zero phase shift filters with 12dB roll-offs. These filters will not distort the MMN, as the power of this ERP is almost entirely in the theta band (Lee et al., 2017). The baseline was set as -75 ms to +5 ms. Epochs with amplitude greater than ± 70 μV at Fz were rejected. An ERP was calculated from all standard tones except the tone

following each deviant; a separate ERP was calculated for the deviant tones. The ERPs were exported to MATLAB for the remaining calculations. The standard average was subtracted from the deviant average, to give a difference wave. As the MMN is part of the MMN/P3a complex (Hermens et al., 2010), which has both positive and negative components, it is not suited to the mean area under the curve method of quantification. We therefore used an automated peak detection algorithm in MATLAB.

An unbiased estimate of each individual participant's ERP was estimated by averaging the ERPs from all 6 sessions for each participant and finding the highest amplitude negative peak in the difference wave. Since a 100ms duration deviant was used, whereas the standard tone was 150ms, the deviance begins at 100ms after the start of the tone. Therefore, a late time window is used for the MMN (Umbricht et al., 2000) of 200ms to 340ms after the start of the tone. For each session, the largest negative peak within 70 ms of the latency of the individual's mean MMN, but within the 200-340 ms window was taken as the MMN value for that session.

P300

A 45 Hz, a 12dB zero phase-shift low pass filter was applied, after which epochs were cut from -200 before each oddball tone to 1000 ms after. The epochs were baseline corrected using -150 to 0ms as the baseline. Epochs with greater than +/- 100 μ V in PZ were rejected. An average ERP was created, which was exported into MATLAB. For each session the mean amplitude in the +280ms to +420ms time window was calculated.

Working Memory

The working memory analysis was restricted to 2-back condition, which is the more commonly-used condition analysed. This provides sufficient WM challenge while data in the 3-back condition indicated that a number of participants were unable to engage sufficiently at such a high working memory level. The EEG was cut into 3276.8 ms segments, from 638.2 ms prior to the presentation of each letter in the N-back task, to 2638.4 ms after it, and the linear trend was removed. Epochs with amplitude greater than $\pm 70 \mu\text{V}$ at FZ were rejected. Fast Fourier Transforms were carried out using a 10% Hanning window, and the mean theta (3.5–7 Hz) amplitude at FZ was calculated in the 2-back condition. Percentage errors in the 2-back condition were calculated as the number of false alarms plus the number of missed targets, divided by the total number of possible targets and multiplied by 100.

Statistical Analysis

In the main statistical analysis a Mixed Model approach was used, with Subject as a Random Effect. This means that the differences between theta amplitudes across participants is taken into account by the model. However, if we used raw mean theta amplitudes and error rates in the regression, the differences between participants would confound the analysis. In order not to reduce the signal to noise ratio (Kenward and Roger, 2010) baseline (Day 1) values were not subtracted from Day 8 values for that period. Instead, as recommended (Kenward and Roger, 2010), baseline data were used to increase the precision of the estimate of each participants EEG measures. *The baselines are also*

treated as outcome variables but without associated treatment effects. Pairwise comparisons between active treatment groups and placebo were generated within the framework of analysis of variance (ANOVA), with Day (1 or 8), and Dose (0, 100 μ g, 250 μ g) as categorical fixed effects. Subject, nested within sequence to control for period effects, was included as a random effect. The least-squares means, p-values, and 2-sided 95% confidence intervals for the pairwise comparisons will be presented.

The aim was to determine if there was sufficient evidence of drug signal on EEG measures. Given this desire to avoid Type II errors, adjustment was not made for multiple comparisons - results do not survive multiple-comparison correction. Effect sizes were calculated to compare active treatment groups to placebo using Cohen's d (Cohen, 1988).

Association between reductions in working memory theta and error performance in the 2-back condition were assessed by Pearson's product moment correlation coefficient. To reduce the effect of inter-subject variability baseline corrected values from participants who had completed all sessions were used. The baseline error rate and theta amplitudes for each participant were calculated as the mean of all the Day 1 sessions for that participant. The mean baseline percentage error for each person was subtracted from each session value. However, as the magnitude of EEG amplitudes can vary substantially between subjects due to non-neural factors such as skull thickness and as the magnitude of differences in theta amplitude, from baseline, will also

be affected by such factors the oscillation magnitudes were divided by baseline magnitudes.

Results

Auditory Steady-State Response

There were no statistically significant differences for the 40Hz ASSR amplitude. While 40Hz ASSR with 100 µg roflumilast ($0.316 \pm 0.038 \mu\text{V}$) tended to be larger than with placebo ($0.270 \pm 0.025 \mu\text{V}$), with a moderate effect size (Cohen's $d=0.344$) this difference was not significant ($F=1.24$, $p=0.270$, $df=60.9$). The 40Hz ASSR amplitude was also unchanged by roflumilast 250 µg ($0.280 \pm 0.046 \mu\text{V}$), (Cohen's $d=0.067$, $F=0.064$, $p=0.801$, $df=57.1$).

Mismatch Negativity

MMN amplitudes at Fz were significantly larger with roflumilast 250 µg ($-2.007 \pm 0.245 \mu\text{V}$) than with placebo ($-1.492 \pm 0.168 \mu\text{V}$), ($F=4.40$, $p=0.040$, $df=61.2$) and the effect size was moderate to large (Cohen's $d=0.614$). Even with the lower 100 µg dose of roflumilast the MMN amplitudes were larger and effect size was moderate (Cohen's $d=0.438$, Mean= $-1.846 \pm 0.231 \mu\text{V}$) although this effect was not significant ($F=1.916$, $p=0.171$, $df=64.2$).

P300

There was no significant difference in P300 amplitudes at Pz between roflumilast 250 µg ($4.56 \pm 1.06 \mu\text{V}$) and placebo ($4.11 \pm 1.75 \mu\text{V}$, $F=0.30$, $p=0.587$, $df=58.4$) and the effect size was small (Cohen's $d=0.06$). The P300 amplitude

was also not changed by roflumilast 100 µg (Mean= 4.33±1.0µV, Cohen's d=0.05, F=0.06, p=0.804, df =60.6).

Theta amplitude in the working memory task

Prefrontal theta amplitude in the 2-back condition of the working memory task was significantly increased by roflumilast 250 µg (0.94±0.13 µV) compared with placebo (0.83 ±0.12 µV, F=5.46, p=0.023, df=59.7) and the effect size was small to moderate (Cohen's d=0.25). The 100 µg dose of roflumilast had no effect on the prefrontal theta amplitude (0.83±0.12 µV) compared with placebo (Cohen's d=0.00, F<0.01, p=0.998, df=61.8).

Working memory task performance

The post-drug baseline corrected theta amplitude was found to be negatively correlated with the baseline corrected 2-back percentage errors on Day 8 (Correlation Coefficient = -0.316, p=0.037) but not on Day 1 (Correlation Coefficient =-0.064, p=0.682). Although the trend for both doses was towards fewer errors, compared to placebo (mean percentage errors = 62.8± 10.5 %), neither 250 µg roflumilast (mean percentage errors = 41.4 ± 15.0 %, F=2.18, p=0.145, df=57.3) nor 100 µg roflumilast (mean percentage errors = 40.3 ± 9.7 % (F=2.10, p=0.153, df=62.8) had a statistically significant effect on the percentage errors. The effect sizes for accuracy were moderate for both doses (roflumilast 250 µg: Cohen's d= -0.420, roflumilast 100 µg: Cohen's d=-0.405).

Discussion

The present study examined the potential for roflumilast to improve EEG biomarkers of cognitive impairment in schizophrenia. Mis-match negativity, shown to be reduced in schizophrenia (Erickson et al., 2016), was significantly improved with 250 µg roflumilast and with a medium-large effect size (0.61). This dose of roflumilast also significantly enhanced frontal theta associated with working memory functioning. No effect of roflumilast, at either dose, was seen on P300 or ASSR ERPs, nor on working memory performance itself.

MMN reflects sensory memory processing and occurs when violations in environmental regularity happen regardless of attention. Hence, MMN may index the capacity for short-term sensory memory traces (Giard et al., 1990). Recently, EEG was used to show that roflumilast improves a neural signal linked to attentional sensory gating in healthy volunteers (Heckman et al., 2018) and improves the P600 associated with improved verbal memory in young healthy adults (Van Duinen et al., 2018). Our study demonstrated increased MMN ERPs, which may indicate that PDE4 inhibition increases neural signals to represent currently-relevant environmental information at low-level attentional states. Heckman et al. (Heckman et al., 2018) propose that neuronal tuning to environmental stimuli is improved by PDE4's capacity to modulate the reciprocity of GABA-ergic inhibitory interneurons and pyramidal neurons as they respond to environmental stimuli, and alter ('gate') their firing response to subsequent stimuli. Elevated dopamine neurotransmission (as in

schizophrenia) negatively affects such processes and so dopamine-related disturbances in low-level processing could be modulated by PDE4 inhibition.

As PDE4 inhibition increases intracellular cAMP levels in striatum and frontal cortex it was expected that roflumilast would improve WM, which relies, in part, on the fronto-striatal circuit (McNab and Klingberg, 2008). Theta is a distributed network rhythm (Başar et al., 2001) thought to coordinate and control activity in different brain areas (Cavanagh and Frank, 2014), and is related to WM efficiency (Raghavachari et al., 2001; Tóth et al., 2014). Roflumilast (at 250ug) significantly improved prefrontal theta; and while higher theta predicted fewer errors, WM error rates were not significantly better (vs placebo). Earlier data from our group (Gilleen et al., 2018) provided preliminary evidence that roflumilast may reduce prefrontal cortical demands during spatial working memory as assessed with fMRI, in a dose-dependent manner. Taken together, while WM performance was not significantly improved (nor a different spatial span task reported previously (Gilleen et al., 2018)), the changes seen in prefrontal activity (with fMRI; Gilleen et al., 2018), and elevated prefrontal theta during the Nback task (with EEG) indicate roflumilast has the potential to improve working memory ability.

The potential to improve working memory performance aligns with evidence from pre-clinical pharmacological challenge (Egawa et al., 1997; Rutten *et al.*, 2008; Vanmierlo et al., 2016) which could be consistent with frontal theta effects. The effects may reflect increase dopamine synthesis and turnover in striatum which may enhance the dopamine D1 receptor/PKA/DARPP-32

signalling cascade in frontal cortex. In animals, rolipram increases dopamine D1 receptor signaling in frontal cortical pyramidal neurons and dopamine D1 receptor signalling and improves working memory performance (Castner and Goldman-Rakic, 2004. (Kuroiwa et al., 2012). The correlation between theta magnitude and performance suggests that theta oscillations may be involved in the remediation of working memory impairments by roflumilast. Future work should aim to further investigate these effects in a larger sample size as behavioural improvements would necessarily be harder to detect than drug effects on brain activity per se.

PDE4 inhibition has been shown to impact on hippocampal activity in CA1 (Barad et al., 1998; Navakkode et al., 2004; Wiescholleck and Manahan-Vaughan, 2012) while hippocampal theta activity has been shown to increase with WM demands (Tesche and Karhu, 2000). Theta oscillations may be indicative of encoding of information directly and rapidly into long-term memory via synaptic modification (Greenstein et al., 1988; Huerta and Lisman, 1993; Raghavachari et al., 2001) and play an important integrative role in working memory organization and cognitive coordination (See (Sauseng et al., 2010)). WM-related theta activity, evident during retention periods, may index a gating mechanism controlling WM task relevance (signal to noise ratio) (Raghavachari et al., 2001) reminiscent of the improvements in lower level 'gating' reported by Heckman *et al.* (Heckman et al., 2018).

These results add to the growing evidence of the potential of PDE4 inhibitors as pro-cognitive agents and are consistent with positive effects on attention and

memory observed in preclinical (e.g. (Maxwell et al., 2004), and healthy human studies (Heckman et al., 2018; Van Duinen et al., 2018), including the significant improvements in verbal memory in schizophrenia that we recently reported (Gilleen et al., 2018). However, Roflumilast showed no effect on P300 (specifically P3b) or ASSR suggesting PDE4 does not have an effect on this lower-level sensory response. Together therefore, roflumilast appears to have an effect on brain physiology, but its precise capacity to alter the individual's brain function has yet to be fully determined.^[1]_[SEP]

Limitations

As this was a proof of concept study, the numbers of patients included were modest, and correction for multiple comparisons was not used, though directional predictions were made and pre-registered. Nevertheless, it is noteworthy that any numerical trends in the data for either drug dose, across all EEG measures, were all in the expected direction. The time-course of effects of roflumilast on the EEG measures could not be assessed as there was only one EEG recording after dosing for each period. Future studies could improve on these limitations by increasing the number of included patients and adding additional recordings.

Conclusions

This study demonstrates that 250 µg roflumilast increases the magnitude of MMN and of theta oscillations during working memory. Together with the cognitive and fMRI results from Gilleen et al. (Gilleen et al., 2018), roflumilast appears to ameliorate multiple types of sustained mental representation of

external, task-related, and environmental stimuli – but not contextual memory (as indexed by P300), nor in the entraining of neural firing to auditory frequency and phase (ASSR). In this study, the effects of high dose Roflumilast were specific to preattentive sensorial memory and working memory biomarkers. This study was a proof of concept study and should now be followed up by more research using a larger sample size. The fact that significant changes in EEG measures with a small number of subjects has been observed reinforces the usefulness of EEG in pharmacological studies as a marker of potential for efficacy (Wilson and Danjou, 2015). More importantly, these results add support to the proposition that PDE4 inhibitors have therapeutic potential in patients with schizophrenia.

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Declaration of Conflicting Interests Statement

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References

- Association AP (2013) *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*. American Psychiatric Pub.
- Baldeweg T, Klugman A, Gruzelier J, et al. (2004) Mismatch negativity potentials and cognitive impairment in schizophrenia. *Schizophrenia Research* 69(2–3): 203–217.
- Barad M, Bourtchouladze R, Winder DG, et al. (1998) Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. *Proceedings of the National Academy of Sciences of the United States of America* 95(25): 15020–15025.
- Başar E, Schürmann M and Sakowitz O (2001) The selectively distributed theta system: functions. *International Journal of Psychophysiology* 39(2–3): 197–212. DOI: 10.1016/S0167-8760(00)00141-0.
- Castner SA and Goldman-Rakic PS (2004) Enhancement of working memory in aged monkeys by a sensitizing regimen of dopamine D1 receptor stimulation. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 24(6): 1446–1450. DOI: 10.1523/JNEUROSCI.3987-03.2004.
- Cavanagh JF and Frank MJ (2014) Frontal theta as a mechanism for cognitive control. *Trends in Cognitive Sciences* 18(8): 414–421. DOI: 10.1016/j.tics.2014.04.012.
- Cohen J (1988) *Statistical Power Analysis for the Behavioral Sciences (2nd Edition)*. Routledge.
- Croft RJ and Barry RJ (2000) Removal of ocular artifact from the EEG: a review. *Neurophysiologie Clinique/Clinical Neurophysiology* 30(1): 5–19. DOI: 10.1016/S0987-7053(00)00055-1.
- Donchin E and Coles MGH (1988) Is the P300 component a manifestation of context updating? *Behavioral and Brain Sciences* 11(3): 357–374. DOI: 10.1017/S0140525X00058027.
- Duncan CC, Barry RJ, Connolly JF, et al. (2009) Event-related potentials in clinical research: guidelines for eliciting, recording, and quantifying mismatch negativity, P300, and N400. *Clinical Neurophysiology* 120(11): 1883–1908.
- Egawa T, Mishima K, Matsumoto Y, et al. (1997) Rolipram and its optical isomers, phosphodiesterase 4 inhibitors, attenuated the scopolamine-induced impairments of learning and memory in rats. *Japanese Journal of Pharmacology* 75(3): 275–281.

- Erickson MA, Ruffle A and Gold JM (2016) A Meta-Analysis of Mismatch Negativity in Schizophrenia: From Clinical Risk to Disease Specificity and Progression. *Biological Psychiatry* 79(12): 980–987. DOI: 10.1016/j.biopsych.2015.08.025.
- Garrido MI, Kilner JM, Stephan KE, et al. (2009) The mismatch negativity: a review of underlying mechanisms. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology* 120(3): 453–463. DOI: 10.1016/j.clinph.2008.11.029.
- Garver DL, Johnson C and Kanter DR (1982) Schizophrenia and reduced cyclic AMP production: evidence for the role of receptor-linked events. *Life Sciences* 31(18): 1987–1992.
- Gevins A, Smith ME, McEvoy L, et al. (1997) High-resolution EEG mapping of cortical activation related to working memory: effects of task difficulty, type of processing, and practice. *Cerebral Cortex* 7(4): 374–385. DOI: 10.1093/cercor/7.4.374.
- Giard MH, Perrin F, Pernier J, et al. (1990) Brain generators implicated in the processing of auditory stimulus deviance: a topographic event-related potential study. *Psychophysiology* 27(6): 627–640.
- Gilleen J (2017) Cognitive enhancement in schizophrenia. In: Keefe RSE, Reichenberg AA, and Cummings J (eds) *Cognitive Enhancement in CNS Disorders and Beyond*. Oxford University Press.
- Gilleen J, Farah Y, Davison C, et al. (2018) An experimental medicine study of the phosphodiesterase-4 inhibitor, roflumilast, on working memory-related brain activity and episodic memory in schizophrenia patients. *Psychopharmacology*.
- Green MR (1966) Common problems in the treatment of schizophrenia in adolescents. *The Psychiatric Quarterly* 40(2): 294–307.
- Greenstein YJ, Pavlides C and Winson J (1988) Long-term potentiation in the dentate gyrus is preferentially induced at theta rhythm periodicity. *Brain Research* 438(1–2): 331–334.
- Halene TB and Siegel SJ (2008) Antipsychotic-like properties of phosphodiesterase 4 inhibitors: evaluation of 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (RO-20-1724) with auditory event-related potentials and prepulse inhibition of startle. *The Journal of Pharmacology and Experimental Therapeutics* 326(1): 230–239. DOI: 10.1124/jpet.108.138586.
- Heckman PRA, van Duinen MA, Bollen EPP, et al. (2016) Phosphodiesterase Inhibition and Regulation of Dopaminergic Frontal and Striatal Functioning: Clinical Implications. *The International Journal of Neuropsychopharmacology*.

- Heckman PRA, Van Duinen MA, Blokland A, et al. (2018) Acute administration of roflumilast enhances sensory gating in healthy young humans in a randomized trial. *Psychopharmacology* 235(1): 301–308.
- Hermens DF, Ward PB, Hodge MAR, et al. (2010) Impaired MMN/P3a complex in first-episode psychosis: cognitive and psychosocial associations. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 34(6): 822–829.
- Hirayasu Y, Asato N, Ohta H, et al. (1998) Abnormalities of auditory event-related potentials in schizophrenia prior to treatment. *Biological Psychiatry* 43(4): 244–253. DOI: 10.1016/S0006-3223(97)00275-8.
- Hosseini-Sharifabad A, Ghahremani MH, Sabzevari O, et al. (2012) Effects of protein kinase A and G inhibitors on hippocampal cholinergic markers expressions in rolipram- and sildenafil-induced spatial memory improvement. *Pharmacology, Biochemistry, and Behavior* 101(3): 311–319. DOI: 10.1016/j.pbb.2012.01.017.
- Hsieh L-T and Ranganath C (2014) Frontal midline theta oscillations during working memory maintenance and episodic encoding and retrieval. *NeuroImage* 85 Pt 2: 721–729. DOI: 10.1016/j.neuroimage.2013.08.003.
- Huerta PT and Lisman JE (1993) Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. *Nature* 364(6439): 723–725. DOI: 10.1038/364723a0.
- Javitt DC, Lee M, Kantrowitz JT, et al. (2018) Mismatch negativity as a biomarker of theta band oscillatory dysfunction in schizophrenia. *Schizophrenia Research* 191: 51–60. DOI: 10.1016/j.schres.2017.06.023.
- Jensen O and Tesche CD (2002) Frontal theta activity in humans increases with memory load in a working memory task. *The European Journal of Neuroscience* 15(8): 1395–1399.
- Johnson EL and Knight RT (2015) Intracranial recordings and human memory. *Current Opinion in Neurobiology* 31: 18–25. DOI: 10.1016/j.conb.2014.07.021.
- Kasai K, Okazawa K, Nakagome K, et al. (1999) Mismatch negativity and N2b attenuation as an indicator for dysfunction of the preattentive and controlled processing for deviance detection in schizophrenia: a topographic event-related potential study. *Schizophrenia Research* 35(2): 141–156.
- Keefe RS (2019). Why are there no approved treatments for cognitive impairment in schizophrenia?. *World Psychiatry*, 18(2), 167.
- Kenward MG and Roger JH (2010) The use of baseline covariates in crossover studies. *Biostatistics (Oxford, England)* 11(1): 1–17. DOI: 10.1093/biostatistics/kxp046.

- Kern RS, Gold JM, Dickinson D, et al. (2011) The MCCB impairment profile for schizophrenia outpatients: results from the MATRICS psychometric and standardization study. *Schizophrenia Research* 126(1–3): 124–131. DOI: 10.1016/j.schres.2010.11.008.
- Kuroiwa M, Snyder GL, Shuto T, et al. (2012) Phosphodiesterase 4 inhibition enhances the dopamine D1 receptor/PKA/DARPP-32 signaling cascade in frontal cortex. *Psychopharmacology* 219(4): 1065–1079. DOI: 10.1007/s00213-011-2436-8.
- Kwon JS, O'Donnell BF, Wallenstein GV, et al. (1999) Gamma frequency-range abnormalities to auditory stimulation in schizophrenia. *Archives of General Psychiatry* 56(11): 1001–1005.
- Lee M, Sehatpour P, Hoptman MJ, et al. (2017) Neural mechanisms of mismatch negativity dysfunction in schizophrenia. *Molecular psychiatry* 22(11): 1585.
- Light GA, Swerdlow NR, Rissling AJ, et al. (2012) Characterization of neurophysiologic and neurocognitive biomarkers for use in genomic and clinical outcome studies of schizophrenia. *PLoS One* 7(7): e39434. DOI: 10.1371/journal.pone.0039434.
- Light GA, Swerdlow NR, Thomas ML, et al. (2015) Validation of mismatch negativity and P3a for use in multi-site studies of schizophrenia: characterization of demographic, clinical, cognitive, and functional correlates in COGS-2. *Schizophrenia Research* 163(1–3): 63–72. DOI: 10.1016/j.schres.2014.09.042.
- Maxwell CR, Kanos SJ, Abel T, et al. (2004) Phosphodiesterase inhibitors: a novel mechanism for receptor-independent antipsychotic medications. *Neuroscience* 129(1): 101–107. DOI: 10.1016/j.neuroscience.2004.07.038.
- McNab F and Klingberg T (2008) Prefrontal cortex and basal ganglia control access to working memory. *Nature Neuroscience* 11(1): 103–107. DOI: 10.1038/nn2024.
- Muly C (2002) Signal transduction abnormalities in schizophrenia: the cAMP system. *Psychopharmacology Bulletin* 36(4): 92–105.
- Narvaez JM, Twamley EW, McKibbin CL, et al. (2008) Subjective and objective quality of life in schizophrenia. *Schizophrenia Research* 98(1–3): 201–208. DOI: 10.1016/j.schres.2007.09.001.
- Navakkode S, Sajikumar S and Frey JU (2004) The Type IV-Specific Phosphodiesterase Inhibitor Rolipram and Its Effect on Hippocampal Long-Term Potentiation and Synaptic Tagging. *Journal of Neuroscience* 24(35): 7740–7744. DOI: 10.1523/JNEUROSCI.1796-04.2004.

- Nottage JF, Morrison PD, Williams SCR, et al. (2013) A novel method for reducing the effect of tonic muscle activity on the gamma band of the scalp EEG. *Brain Topography* 26(1): 50–61. DOI: 10.1007/s10548-012-0255-9.
- O'Donnell BF, Vohs JL, Krishnan GP, et al. (2013) The auditory steady-state response (ASSR): a translational biomarker for schizophrenia. *Supplements to Clinical Neurophysiology* 62: 101–112.
- Percudani M, Barbui C and Tansella M (2004) Effect of second-generation antipsychotics on employment and productivity in individuals with schizophrenia: an economic perspective. *Pharmacoeconomics* 22(11): 701–718. DOI: 10.2165/00019053-200422110-00002.
- Pérez-Torres S, Miró X, Palacios JM, et al. (2000) Phosphodiesterase type 4 isozymes expression in human brain examined by in situ hybridization histochemistry and [3H]rolipram binding autoradiography. Comparison with monkey and rat brain. *Journal of Chemical Neuroanatomy* 20(3–4): 349–374.
- Polich J (2007) Updating P300: an integrative theory of P3a and P3b. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology* 118(10): 2128–2148. DOI: 10.1016/j.clinph.2007.04.019.
- Raghavachari S, Kahana MJ, Rizzuto DS, et al. (2001) Gating of human theta oscillations by a working memory task. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 21(9): 3175–3183.
- Rutten K, Basile JL, Prickaerts J, et al. (2008) Selective PDE inhibitors rolipram and sildenafil improve object retrieval performance in adult cynomolgus macaques. *Psychopharmacology* 196(4): 643–648. DOI: 10.1007/s00213-007-0999-1.
- Sauseng P, Griesmayr B, Freunberger R, et al. (2010) Control mechanisms in working memory: a possible function of EEG theta oscillations. *Neuroscience and Biobehavioral Reviews* 34(7): 1015–1022. DOI: 10.1016/j.neubiorev.2009.12.006.
- Schmiedt C, Brand A, Hildebrandt H, et al. (2005) Event-related theta oscillations during working memory tasks in patients with schizophrenia and healthy controls. *Brain Research. Cognitive Brain Research* 25(3): 936–947. DOI: 10.1016/j.cogbrainres.2005.09.015.
- Sensky T, Turkington D, Kingdon D, et al. (2000) A randomized controlled trial of cognitive-behavioral therapy for persistent symptoms in schizophrenia resistant to medication. *Archives of General Psychiatry* 57(2): 165–172.

- Tardito D, Tura GB, Bocchio L, et al. (2000) Abnormal levels of cAMP-dependent protein kinase regulatory subunits in platelets from schizophrenic patients. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* 23(2): 216–219. DOI: 10.1016/S0893-133X(99)00161-X.
- Tesche CD and Karhu J (2000) Theta oscillations index human hippocampal activation during a working memory task. *Proceedings of the National Academy of Sciences of the United States of America* 97(2): 919–924.
- Thuné H, Recasens M and Uhlhaas PJ (2016) The 40-Hz Auditory Steady-State Response in Patients With Schizophrenia: A Meta-analysis. *JAMA psychiatry* 73(11): 1145–1153. DOI: 10.1001/jamapsychiatry.2016.2619.
- Tóth B, Kardos Z, File B, et al. (2014) Frontal midline theta connectivity is related to efficiency of WM maintenance and is affected by aging. *Neurobiology of Learning and Memory* 114: 58–69. DOI: 10.1016/j.nlm.2014.04.009.
- Umbricht D, Schmid L, Koller R, et al. (2000) Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: implications for models of cognitive deficits in schizophrenia. *Archives of General Psychiatry* 57(12): 1139–1147.
- Van Duinen MA, Sambeth A, Heckman PRA, et al. (2018) Acute administration of roflumilast enhances immediate recall of verbal word memory in healthy young adults. *Neuropharmacology* 131: 31–38. DOI: 10.1016/j.neuropharm.2017.12.019.
- Vanmierlo T, Creemers P, Akkerman S, et al. (2016) The PDE4 inhibitor roflumilast improves memory in rodents at non-emetic doses. *Behavioural Brain Research* 303: 26–33. DOI: 10.1016/j.bbr.2016.01.031.
- Wiescholleck V and Manahan-Vaughan D (2012) PDE4 inhibition enhances hippocampal synaptic plasticity in vivo and rescues MK801-induced impairment of long-term potentiation and object recognition memory in an animal model of psychosis. *Translational Psychiatry* 2: e89. DOI: 10.1038/tp.2012.17.
- Wilson FJ and Danjou P (2015) Early Decision-Making in Drug Development: The Potential Role of Pharmaco-EEG and Pharmaco-Sleep. *Neuropsychobiology* 72(3–4): 188–194. DOI: 10.1159/000382022.
- Winterer G, Egan MF, Raedler T, et al. (2003) P300 and genetic risk for schizophrenia. *Archives of General Psychiatry* 60(11): 1158–1167. DOI: 10.1001/archpsyc.60.11.1158.

