Proof-of-Mechanism Study of the PDE10 Inhibitor RG7203 in Patients with Schizophrenia and Negative Symptoms

Supplemental Information

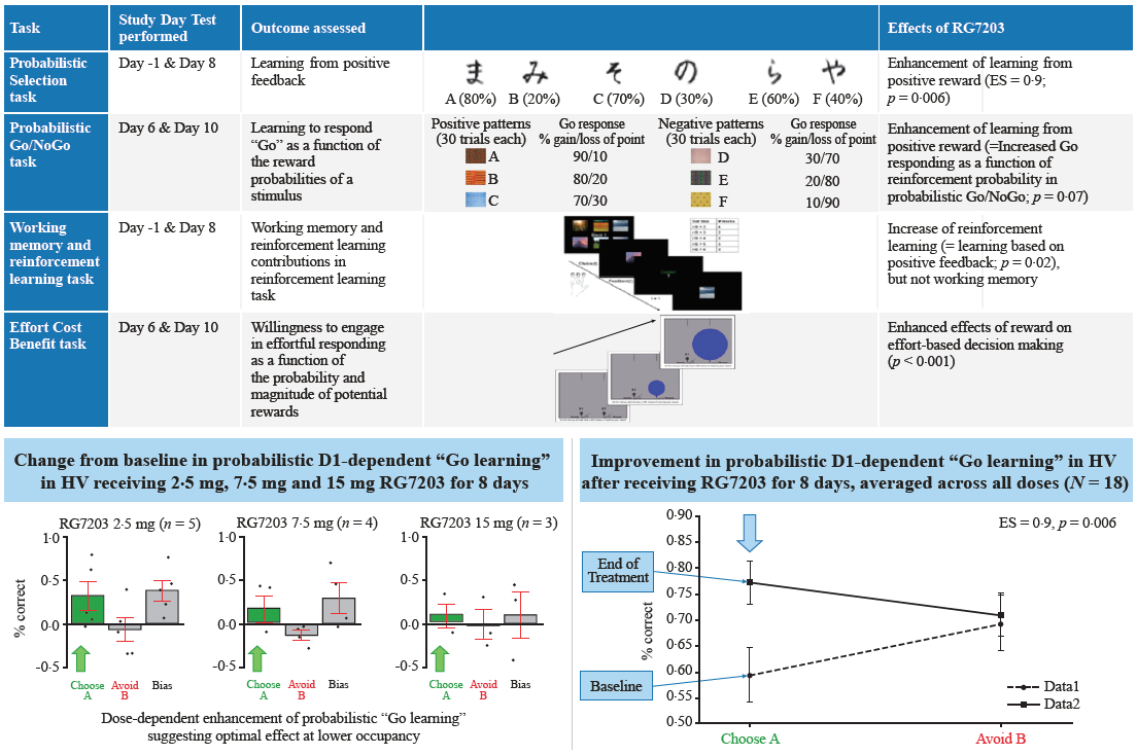

Figure S1. Behavioral assessments and outcomes in healthy volunteer multiple ascending dose study (1). D1, dopamine type 1; ES, effect size; HV, healthy volunteer; MAD, multiple ascending dose.

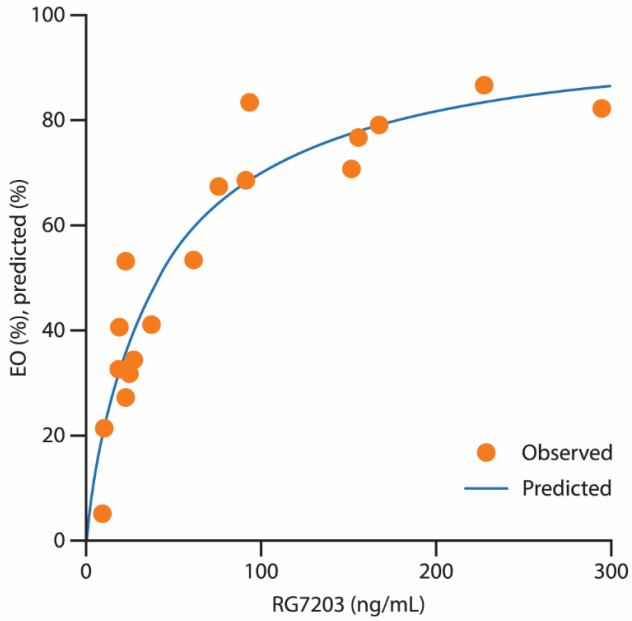

Figure S2. Relationship between plasma concentration and PDE10 enzyme occupancy (EO) following a single oral dose of RG7203 (also referred to as RO5545965). The E_{max} and EC_{50} parameters were estimated to range between 92.1–100% and 37.1–52.8 ng/mL for the 2-tissue compartmental modeling method, and between 89.3–100% and 32.2–50.2 ng/mL for the simplified reference tissue model method, respectively, across regions of interest. E_{max}, the maximal effect at high drug concentrations when all receptors are occupied by the drug; EC_{50}, the drug concentration to give the half-maximal effect.

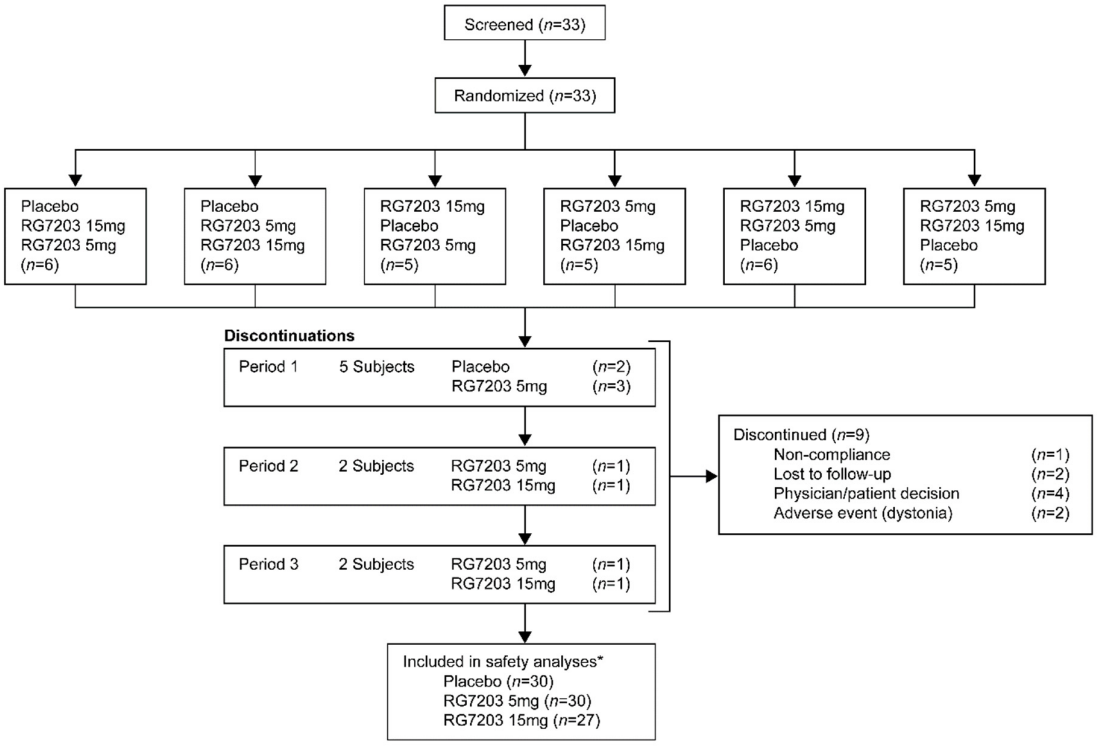

Figure S3. Patient flow through the study (CONSORT diagram). *Patients used for the safety analyses were also included in the analysis of the primary endpoint if they contributed data from at least 2 periods. AE, adverse event.

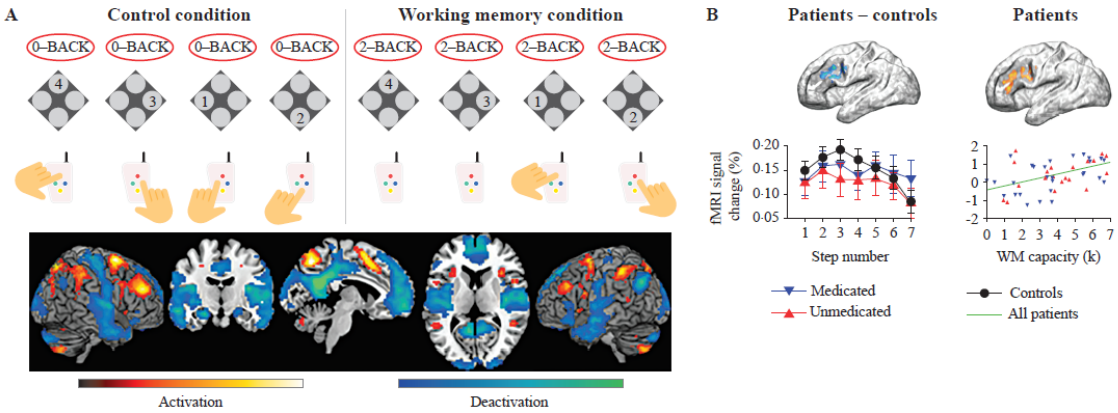

Figure S4. N-back working memory task. **(A)** Examples illustrating reduced activation in schizophrenia patients during the performance of the N-back working memory task. **(B)** Reprinted from: Van Snellenberg JX, Girgis RR, Horga G, van de Giessen E, Slifstein M, Ojeil N, *et al.* (2016): Mechanisms of Working Memory Impairment in Schizophrenia. *Biol Psychiatry*. 80: 617–626 (2). Copyright 2021, with permission from Elsevier.

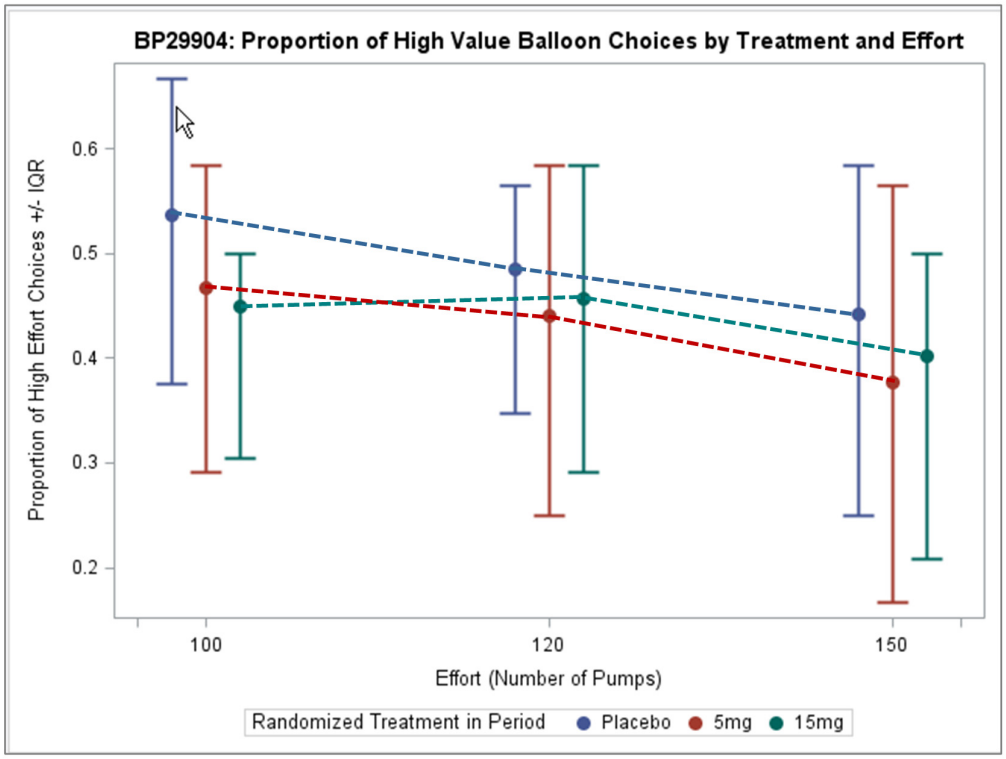

Figure S5. ECBT results: Proportion of high effort choices by effort required across all reward levels at 100% certainty of receiving the reward.

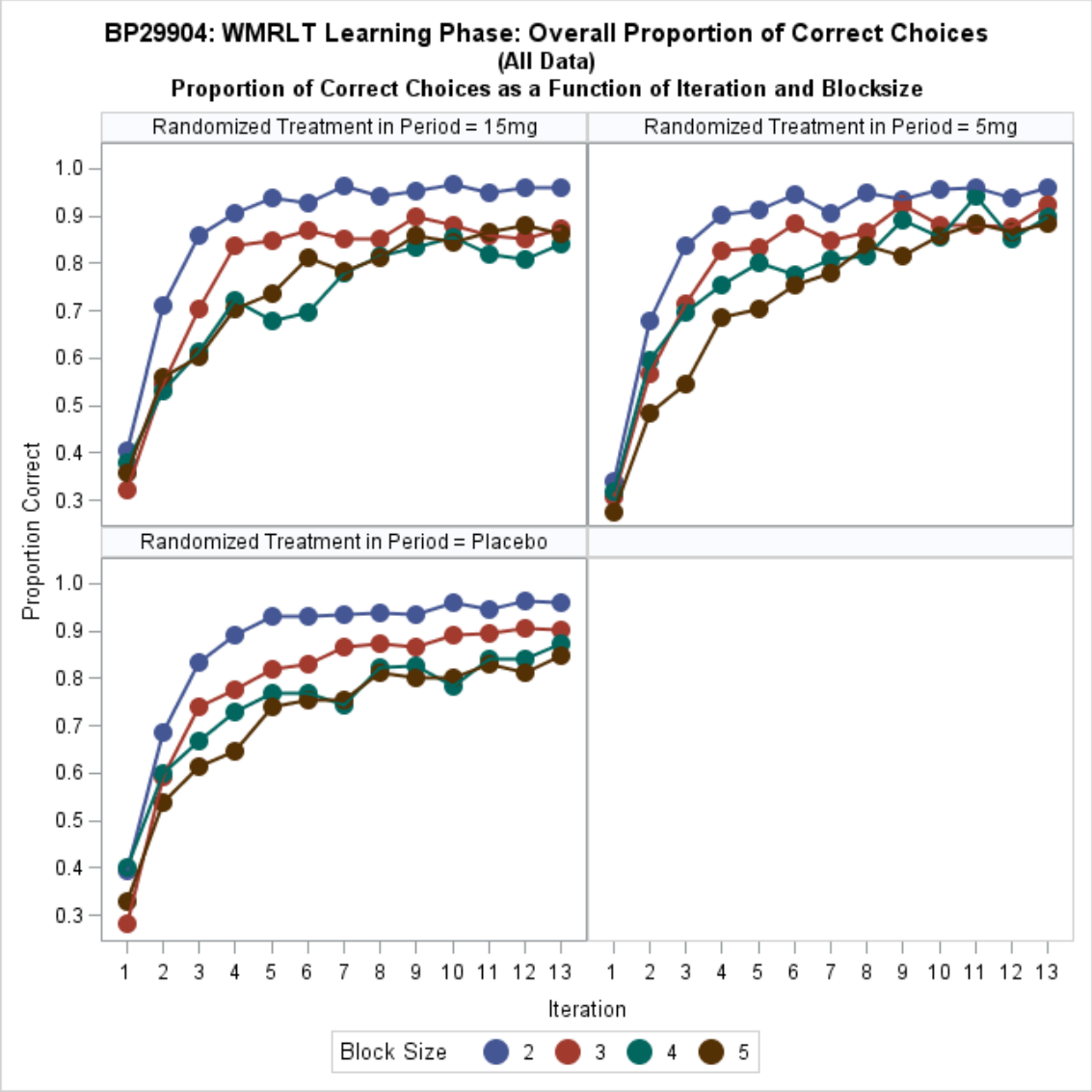

Figure S6. WMRLT Learning phase: Proportion of correct choices as a function of iteration and block size. During treatment with 5 mg patients showed a slight increase in correct choices at the end of the trial.

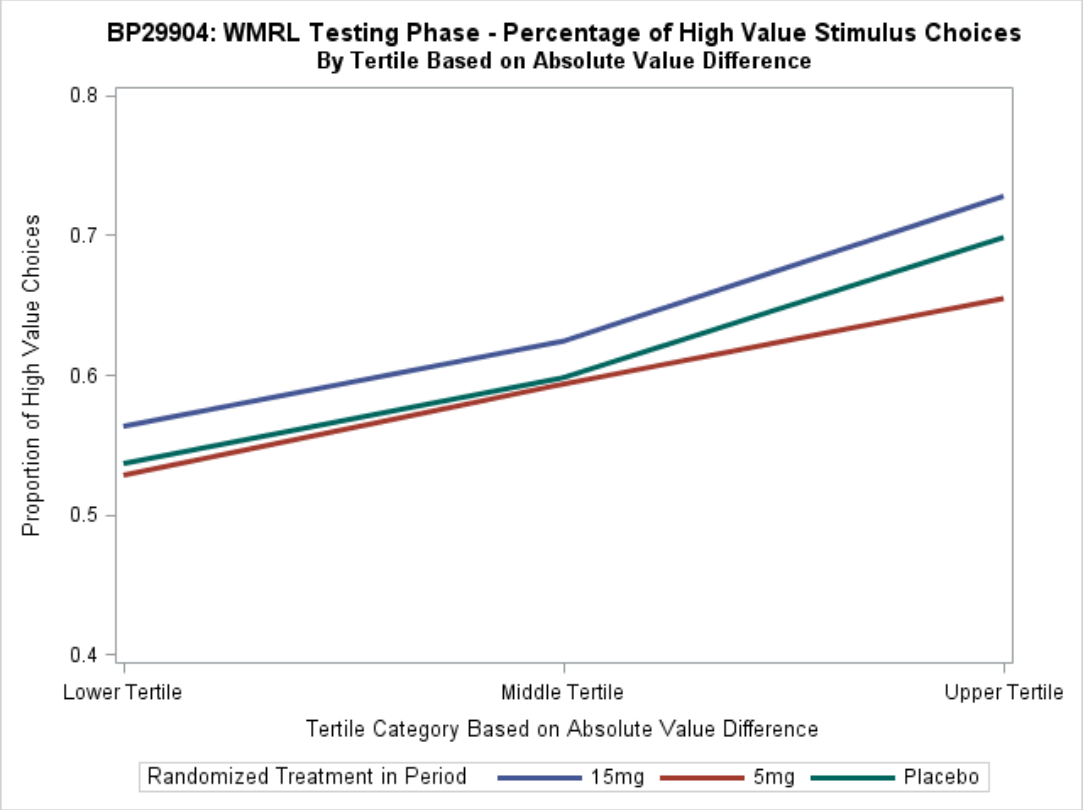

Figure S7. WMRLT Test phase: trials were binned by the absolute value difference between the two test stimuli into three tertile categories. With increasing value difference, the proportion of choosing the high value stimulus is expected to increase. This effect is observed. However, in both periods in which patients were treated with RG7203 the proportion of high value choices decreased.

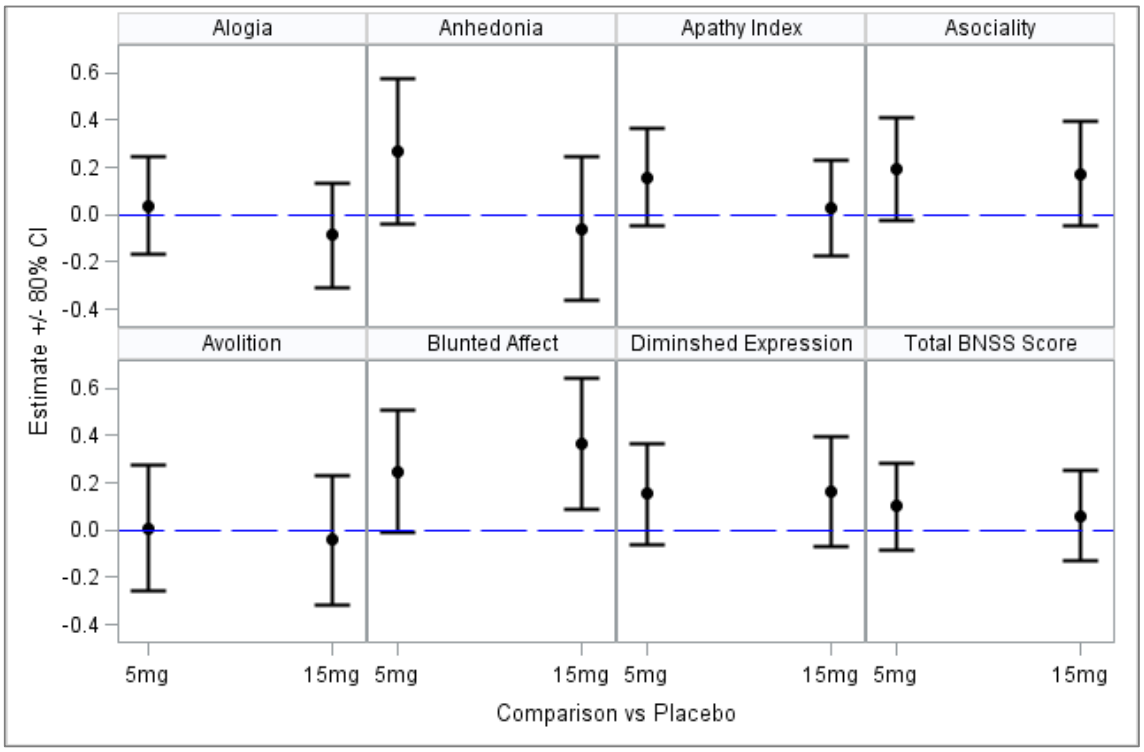

Figure S8. BNSS: Treatment group comparisons – total and subscales. A change of > 0 indicates worsening versus placebo; a change of < 0 indicates improvement versus placebo. Figure shows estimated mean change per item of each subscale. Apathy Index = sum of Anhedonia, Asociality and Avolition; Diminished Expression = sum of Alogia and Blunted Affect.

Table S1. BNSS Total and subscores at the beginning and end of each treatment period.

			Placebo	RG7203 5 mg	RG7203 15 mg
BNSS Total score	Day 1	n	30	30	27
		Mean (SD)	33.7 (10.9)	35.8 (10.4)	32.0 (13.3)
	Day 22	n	28	26	25
		Mean (SD)	30.6 (11.2)	32.0 (11.4)	30.6 (11.9)
Blunted affect	Day 1	Mean (SD)	8.4 (3.5)	8.3 (3.3)	8.0 (3.6)
	Day 22	Mean (SD)	7.3 (3.1)	7.6 (3.4)	7.6 (3.8)
Alogia	Day 1	Mean (SD)	4.1 (2.6)	4.5 (2.8)	4.0 (2.6)
	Day 22	Mean (SD)	4.0 (2.5)	4.0 (2.7)	3.7 (2.8)
Anhedonia	Day 1	Mean (SD)	7.9 (3.5)	8.6 (3.3)	7.6 (4.4)
	Day 22	Mean (SD)	7.5 (3.5)	8.2 (3.9)	7.0 (4.1)
Asociality	Day 1	Mean (SD)	5.7 (2.3)	5.9 (2.3)	5.5 (2.9)
	Day 22	Mean (SD)	4.9 (2.6)	5.3 (2.6)	5.2 (2.7)
Avolition	Day 1	Mean (SD)	5.5 (2.2)	6.0 (2.1)	5.1 (3.0)
	Day 22	Mean (SD)	5.2 (2.2)	5.1 (2.6)	5.0 (2.5)
Distress	Day 1	Mean (SD)	2.1 (1.5)	2.4 (1.4)	1.8 (1.5)
	Day 22	Mean (SD)	1.8 (1.6)	1.8 (1.5)	2.1 (1.7)

Table S2. PANSS Total and Negative and Positive Symptom Factor scores at the beginning and end of each treatment period.

			Placebo	**RG7203 5 mg**	**RG7203 15 mg**
PANSS Total score	Day 1	n	30	30	27
		Mean (SD)	65.5 (10.9)	62.2 (11.2)	64.0 (12.0)
	Day 22	n	28	26	25
		Mean (SD)	60.8 (11.8)	59.4 (11.7)	60.7 (11.8)
PANSS Negative Symptom Factor score	Day 1	Mean (SD)	20.9 (3.7)	21.3 (4.0)	20.4 (4.8)
	Day 22	Mean (SD)	18.9 (4.1)	19.2 (5.3)	19.0 (4.4)
PANSS Positive Symptom Factor score	Day 1	Mean (SD)	18.6 (5.4)	17.8 (5.6)	19.4 (5.5)
	Day 22	Mean (SD)	17.5 (5.7)	17.3 (5.2)	18.1 (5.6)

Inclusion Criteria

Patients must meet the following criteria for study entry:

1. A Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 diagnosis of schizophrenia as established by the Structured Clinical Interview for DSM Clinical Trials version (SCID-5-CT) at screening.
2. Age 18–50 years (inclusive).
3. Outpatient with no hospitalization for worsening of schizophrenia within 3 months prior to screening (hospitalization for social management within this time is acceptable).
4. Medically stable over 1 month and psychiatrically stable without symptom exacerbation over the previous 3 months prior to screening.
5. Males and females with no childbearing capacity; females must be either surgically sterile (by means of hysterectomy, bilateral oophorectomy or tubal ligation) or post-menopausal for at least 1 year (confirmed by follicle stimulating hormone and estradiol, if not on hormone replacement).
6. Has a caregiver or other identified responsible person considered reliable by the investigator to provide support to the patient to help ensure compliance with study treatment, study visits and protocol procedures, and who preferably is also able to provide input helpful for completing study rating scales.
7. Body mass index > 18.5 kg/m^2 and < 35 kg/m^2.
8. Fluent in English, even if English is not the primary language.
9. Able and willing to provide written informed consent according to International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and local regulations and to comply with the study protocol. (If the patient has a legal representative, the informed consent must be signed by this person as well.)
10. Able to complete study procedures.

Symptom severity at screening:

11. The patient has a Clinical Global Impression–Severity scale (CGI-S) score ≥ 3 (mildly ill).
12. The patient has a score of < 4 (moderate) on the following Positive and Negative Syndrome Scale (PANSS) items:
    a) P7 (hostility)
    b) G8 (uncooperativeness)
13. Total score of ≥ 18 on the PANSS negative symptom factor score (items scored 1–7 for a maximum possible score of 49).
14. PANSS item G6, depression score of ≤ 4 (moderate).
15. Depressive symptoms, defined as a score of ≤ 8 on the Calgary Depression Rating Scale for schizophrenia.

Antipsychotic treatment:

16. On stable treatment, that is 6 weeks without change, with no more than 2 antipsychotics (oral and long-acting injectable formulations of the same medication are considered to be two different antipsychotics) prior to screening. Antipsychotic regimen:
    c) Patients must be on a “primary” antipsychotic and may be on a secondary antipsychotic. The amount of the secondary antipsychotic has to be equal or less than the equivalent dose of the primary antipsychotic and the sum of the primary and secondary antipsychotics has to be ≤ 6 mg of risperidone equivalents.
    d) The allowed “primary” antipsychotics are quetiapine, paliperidone, risperidone, aripiprazole, lurasidone and ziprasidone. Antipsychotics have to be used in the dose range according to the prescribing information approved in the US. In the case that the primary antipsychotic is a first-generation antipsychotic (oral or injectable) an exception may be granted if discussed and clearly documented

between the investigator and the Sponsor (Translational Medicine Leader or delegate) and approved by the Sponsor.

Exclusion Criteria

Patients who meet any of the following criteria will be excluded from study entry:

1. Moderate to severe substance use disorder within 6 months (excluding nicotine or caffeine) as defined by DSM-5.
2. Positive urine drug screen for amphetamines, methamphetamines, opiates, buprenorphine, methadone, cannabinoids, cocaine and barbiturates.
3. Other current Axis I diagnosis requiring exclusion (e.g., bipolar disorder, schizoaffective disorder, major depressive disorder) based on DSM-5.
4. The patient is at significant risk of suicide or harming him- or herself or others according to the Investigator's judgment.
5. History of neuroleptic malignant syndrome.
6. A prior or current general medical condition that might be impairing cognition or other psychiatric functioning (e.g., migraine headaches requiring prophylaxis treatment, head trauma, dementia, seizure disorder, stroke, neurodegenerative, inflammatory, infectious, neoplastic, toxic, metabolic, endocrine etc.).
7. A movement disorder due to antipsychotic treatment not currently controlled with anti-EPS (extrapyramidal symptoms) treatment or another movement disorder which might affect the ratings on the EPS scales (e.g., Parkinson's disease).
8. The patient has a score > 2 (mild) in any of the four CGI-S items of the Extrapyramidal Symptom Rating Scale – Abbreviated; Parkinsonism, akathisia, dystonia, and tardive dyskinesia at screening or on Day -1 and is requiring anti-Parkinson medication including anticholinergic drugs.
9. History of HIV infection, or history of Hepatitis B infection within the previous year, or history of Hepatitis C infection which had not been adequately treated.

10. QTcF interval > 450 msec (470 msec for females) or other clinically significant abnormality on screening electrocardiogram based on centralized reading.
11. Clinically significant abnormalities in laboratory safety test results (including hepatic and renal panels, complete blood count, chemistry panel and urinalysis). In case of uncertain or questionable results, tests performed during screening may be repeated before randomization to confirm eligibility or may be accepted if they are, in the opinion of the Investigator, clinically insignificant.
10. Significant or unstable physical condition which in the Investigator's judgment might require a change in medication or hospitalization within the next 3 months.
11. Receipt of an investigational drug within 90 days or 5 times the half-life of the investigational drug, whatever is longer, prior to screening.
12. Previously received RO5545965.
13. Electroconvulsive treatment within 6 months prior to screening.
14. Currently on treatment with olanzapine or clozapine.
15. Having received olanzapine or clozapine within 3 months of screening.
16. On more than one antidepressant, or if on one antidepressant, a change in dose within 4 weeks prior to screening.
17. Change in benzodiazepine or sleep medication regimen within 2 weeks (regimen could be as needed or continuous treatment) prior to screening.
18. Change in anti-EPS medication within two weeks prior to screening.
19. Use of prohibited medications taken within 14 days or within 5 times the elimination half-life of the medication before the first study drug administration (whichever is longer), except for allowed medication:
    - Concomitant therapy includes any medication, e.g., prescription drugs, over the counter drugs, approved dietary and herbal supplements, nutritional supplements and any nonmedication interventions (e.g., individual psychotherapy, cognitive behavioral therapy, smoking cessation

therapy, and rehabilitative therapy) used by a patient from 6 weeks prior to screening until the follow-up visit.

20. Use of any strong or moderate inhibitor of CYP3A or CYP2C8 and any inducer of CYP3A within 14 days or within 5 times the elimination half-life of the medication (whichever is longer) before the first study drug administration, including but not limited to the following: rifampicin, carbamazepine, St. John's wort, ketoconazole, itraconazole, fluconazole, erythromycin, cimetidine, fluoxetine, gemfibrozil and phenytoin.
21. Use of any other nutrients known to modulate CYP3A activity (e.g., grapefruit containing products) within 1 week before the first study drug administration.
22. The patient is a member of the study personnel or of their immediate families, or is a subordinate (or immediate family member of a subordinate) to any of the study personnel.

Behavioral Tasks

Working Memory Reinforcement Learning Task (WMRLT)

Learning phase (LP): The WMRLT was modified from a classic conditional associative learning paradigm (3, 4). In the LP, performed on a laptop in a quiet room, patients learned to select 1 correct out of 3 button presses for each stimulus (1 stimulus presented at a time). For each stimulus a single button corresponded to the “correct” choice. The stimuli were presented in 12 blocks, with 4 blocks of size $n_s = 2$ (i.e., 4 blocks containing 2 stimuli each), 3 blocks of size $n_s = 3$, 2 blocks of size $n_s = 4$, and 3 blocks of size $n_s = 5$. The stimuli in each block corresponded to a different category of images (e.g., sports, fruits, places, etc.) leading to a total of $(4 \times 2) + (3 \times 3) + (2 \times 4) + (3 \times 5) = 40$ different stimuli. Within each block, the stimuli were presented in a pseudo-random intermixed order. Patients selecting the incorrect action for a stimulus received feedback indicating they had won 0 points, while patients selecting the correct action obtained a reward of 1 or 2 points. Each image had a fixed probability q of resulting in 2 (vs. 1) points of reward for correct actions (q is 0.25, 0.50, and 0.75); the

probabilities were pre-assigned to counterbalance across set sizes and other factors. The correct action was always correct and the incorrect one was always incorrect. In recent research using this task, patients with schizophrenia displayed an impaired reinforcement learning performance with increased working memory load (4) For this reason, the proportion of correct choices (where a choice has been made) in late trials (trials 11, 12, and 13) for blocks of size 4 and 5 (high working memory load) was used for further group level analyses described below.

Test phase (TP): After LP, patients underwent a TP, in which they were presented with pairs of images they previously encountered. Patients were asked to choose the image that they perceived to have given them most points in LP. They received no reward for the choices being made. TP includes 115 trials, i.e., 115 pairs of images. These 115 pairs were selected among all possible pairs. The selection was based on the actual responses of the patient in LP and done to ensure that sufficient pairs with a range of value differences but also value means were included. For TP, a general linear model was fitted for each patient and session. The model included: 1) the value difference between the right and left image based on the number of points the patient gained in the LP; 2) an interaction term between the value difference and the value mean; 3) an interaction term between the value difference and the set size average of the respective images; 4) the set size difference; 5) the difference between learning blocks in which the images were learned; and 6) the side of the previously chosen image. As suggested in previous research using this task in schizophrenia patients, the beta coefficients (5) describing how the value difference is modulated by the value mean was used for subsequent evaluation of treatment effects (6). This coefficient is considered to capture the deficit observed in schizophrenia patients with negative symptoms that is related to the “choose A / avoid B” paradigm in which patients learn to choose a highly rewarded stimulus and avoid a less valuable stimulus.

Effort Cost Benefit Task (ECBT)

In the ECBT, adapted from Gold *et al.* (7), patients choose between a low effort task and a high effort task. When making the choice, the patients were informed about the number of pumps ('effort') required for the low (20 pumps) and the high effort task (100, 120, or 150 pumps). The patients were also informed about the maximum possible reward (1 point for the low effort task and 3, 5, or 7 points for the high effort task). Each set of cumulated 20 points converted to a $1 bonus. If they selected the low effort balloon, patients needed to complete all 20 pumps to obtain their reward. If they selected the high effort balloon, they obtained the reward after 25 seconds, no matter how many times they pumped, and in proportion to the amount of effort exerted. For example, if they selected the balloon for 5 points and 120 pumps, and end up pumping 90 times, they received a reward of 5*(90/120) points. Similarly, they could earn more than 5 points if they pumped more than 120 times. The patients were also informed about the certainty with which the reward will be paid (100% or 50%), with this certainty applying equally to the low and high effort task. Furthermore, the high effort balloon could appear on the left or the right side of the screen. In total this led to 3*3*2*2 = 36 different combinations of 'number of pumps for high effort balloon', 'reward of high effort balloon', 'reward certainty', and 'side of high effort balloon'. Each combination was presented twice, leading to a total of 72 experiments per session. The overall percentage of high effort choices under deterministic reward condition (100% reward) for reward magnitudes 5 and 7 was used for subsequent evaluation comparing drug and placebo conditions. At the end of each session, patients received the actual amount of money earned.

Sample Size Considerations

The sample size was based on the change in ventral striatal activation in the monetary incentive delay task, a medium effect size of 0.5 was considered as evidence of relevant pharmacodynamic effect; a sample size of 27 patients was considered sufficient to detect this magnitude of effect at an uncorrected 2-sided significance level of 0.10 and power of 80%. To ensure a fully counter-balanced 3-period crossover design with 6 sequences, this was

increased to 33 patients**.** In alignment with this, 2-sided *p*-values are reported throughout and will be considered statistically significant if less than 0.1. No corrections for multiplicity have been performed in this hypothesis generating study.

Supplementary References

1. Umbricht D, Dukart J, Abt M, Tamburri P, Chatham C, Frank M*, et al.* (2017): T187. A proof-of-mechanism study of the PDE10A inhibitor RG7203 in patients with schizophrenia and negative symptoms probing reward functions with imaging and behavioral approaches. *ACNP 56th Annual Meeting*, pp S415.
2. Van Snellenberg JX, Girgis RR, Horga G, van de Giessen E, Slifstein M, Ojeil N*, et al.* (2016): Mechanisms of Working Memory Impairment in Schizophrenia. *Biol Psychiatry*. 80: 617–626.
3. Petrides M (1985): Deficits on conditional associative-learning tasks after frontal- and temporal-lobe lesions in man. *Neuropsychologia*. 23: 601–614.
4. Collins AG, Brown JK, Gold JM, Waltz JA, Frank MJ (2014): Working memory contributions to reinforcement learning impairments in schizophrenia. *J Neurosci*. 34: 13747–13756.
5. Bobes J, Arango C, Garcia-Garcia M, Rejas J, Group CSC (2010): Prevalence of negative symptoms in outpatients with schizophrenia spectrum disorders treated with antipsychotics in routine clinical practice: findings from the CLAMORS study. *J Clin Psychiatry*. 71: 280–286.
6. Collins AGE, Albrecht MA, Waltz JA, Gold JM, Frank MJ (2017): Interactions among working memory, reinforcement learning, and effort in value-based choice: A new paradigm and selective deficits in schizophrenia. *Biol Psychiatry*. 82: 431–439.
7. Gold JM, Strauss GP, Waltz JA, Robinson BM, Brown JK, Frank MJ (2013): Negative symptoms of schizophrenia are associated with abnormal effort-cost computations. *Biol Psychiatry*. 74: 130–136.