**Supplementary Material**

**Article Title:** A Neurofunctional Domains Approach to Evaluate D1/D5 Dopamine Receptor Partial Agonism on Cognition and Motivation in Healthy Volunteers With Low Working Memory Capacity

**Article Category:** Regular research article

**Authors:** Rita Balice-Gordon, Garry D. Honey, Christopher Chatham, Estibaliz Arce, Sridhar Duvvuri, Melissa Graham Naylor, Wenlei Liu, Zhiyong Xie, Nicholas DeMartinis, Brian Harel, Gabriel H. Braley, Rouba Kozak, Lovingly Park, David L. Gray

**Supplementary Methods**

***Neuroimaging/fMRI Battery***

MRI images were acquired with a Siemens 3T Tim Trio scanner using a 32-channel head coil. A T1-weighted high-resolution MPRAGE image was collected (TR=2300 ms; TE=2.48 ms; TI=900 ms; FA=8; voxel size = 1x1x1 mm3; matrix size=256x256). Fieldmaps were also acquired for distortion correction of functional images. A multi-band, accelerated, echo planar-imaging pulse sequence (common mode rejection ratio release R012; MB=4; TR=750 ms; TE=30 ms; FA=52; voxel size = 3.0x3.0x3.2 mm3; matrix size=70x70, slices=44) was used to collect functional images.

FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl)) was used to conduct functional image analysis. Motion correction was performed by aligning all volumes of the series to the middle volume via rigid body transformation using MCFLIRT. FSL-BET was used on the middle volume of each series, and then the resulting mask was applied to all other volumes of each series, to remove non-brain structures. Following B0-field distortion correction, spatial smoothing with 6 mm full width at half maximum, and high-pass temporal filtering (sigma=500s, filter cut-off 0.005 Hz), the mean functional volume was registered to the high-resolution T1 anatomical image using affine transformation. T1 anatomical images were transformed to the Montreal Neurological Institute (MNI) 152 standard space using affine transformation and then non-linear transformation. Finally, all regions of interest (ROIs) were defined in MNI space using the Harvard-Oxford Atlas (2015).

For the MID Task results (reported below), task-induced changes in the blood-oxygenation-level dependent (BOLD) response were assessed at the voxel level using the general linear model (GLM). Specifically, 18 event regressors were used to model the combined influence of 3 conditions (cue, hit, miss) and 6 levels of reward (-$0, +$0, -$1, +$1, -$5, +$5). Six motion regressors were also included to account for head motion. Contrast images were derived by comparing BOLD response to gain cues (+$1, +$5) with the response to no gain cues (+$0). The T-value at each voxel was converted to a Z-statistic via standard statistical transformation. The 90th percentile of the Z-stats of voxels inside each ROI was reported as the summary measurement of the ROI, and then subjected to further statistical analysis (see also “Statistical Analysis” below).

An analogous GLM- and ROI-based approach was used for assessing task-related BOLD changes for the other assessments in the fMRI battery, but is not described here for lack of space. For resting-state analyses, a seed-based approach was used (additional information on MRI methods is available upon request).

***Evoked-Response Potential Battery***

For the Contralateral Delay Activity Task, EEG data were processed according to the analysis method of prior work (Vogel and Machizawa, 2004; Vogel et al., 2005). Briefly, EEG data were segmented and any segments in which the voltage on the EOG channels exceeded 400 μV for vertical EOG or 120 μV horizontal EOG were excluded from ERP averages, resulting in an average of 3.7% of trials rejected. Average amplitudes of N2pc and contralateral delay activity from the ERP difference waveforms were then extracted from the intervals 250–350 ms and 300–900 ms relative to stimulus onset, respectively. Behavioral data were also extracted from the task in the form of both an estimate of working memory capacity (Cowan’s K) and “filtering efficiency”, as quantified by prior work (Vogel and Machizawa, 2004; Vogel et al., 2005).

For the Implicit Reward-Biasing Task, electroencephalogram (EEG) data were first visually inspected; electromyogram (EMG) artifacts and gross artifacts with absolute voltage >75 µV were rejected. Noisy EEG channels were then detected by visual inspection and noted. Independent Component Analysis (ICA) was next used to correct horizontal and vertical electrooculogram (EOG) artifacts, as well as small ECG and EMG artifacts. Following ICA correction, EEG data were again visually inspected and previously missed artifacts or segments with inadequate EOG correction were rejected. Noisy EEG channels (if any remained) were then interpolated, followed by re-referencing of the EEG data to an average reference and band-pass filtering to 1–30 Hz. These pre-processed EEG data were next segmented using markers for trials with feedback given and correct responses into -250 to 1000 ms segments relative to feedback onset. Each such segment was averaged for every electrode independently; average ERPs at the electrodes Fz, FCz, Cz, and Pz were further analyzed. Using the semiautomatic peak detection function of Brain Vision Analyzer 2 (BVA2), the feedback-related negativity was identified as the most negative peak at 200–400 ms latency. Amplitudes from the defined peaks were then exported from BVA2 for analysis. Behavioral data were also extracted from the task in the form of the Response Bias, as in prior work (Santesso et al., 2009).

**Statistical Analyses**

For CBS, EVO, and PANAS-X endpoints where there was a baseline measure and multiple post-baseline measurements, treatment effect of PF-06412562 was tested using a mixed model for repeated measures (MMRM) with a restricted maximum likelihood method for the estimation of the covariance parameters. The dependent variable was the change from baseline measures and the model included treatment, time, and treatment-by-time interaction as fixed categorical factors, as well as the baseline scores and age as fixed-effect continuous covariates. Subject was included in the model as a random effect. An unstructured covariance matrix was used to model the within-subject errors. The Kenward–Roger approximation was used to estimate denominator degrees of freedom. Model-based contrasts/confidence intervals (2-sided 95% CIs for cognition endpoints and 92% CIs for reward endpoints) and 2-sided *P*-values of pairwise treatment effect were reported.

For other neurofunctional domain endpoints where there was a baseline measure and a single post-baseline measurement, the treatment effect of PF-06412562 was tested using an analysis of covariance model. The dependent variable was the change from baseline measure, and the model included treatment as a fixed categorical factor as well as the baseline scores and age as fixed-effect continuous covariates. The difference between each PF-06412562 dose and the placebo group was compared using appropriate contrasts of leastsquares (LS) means. Model-based contrasts/CIs (2-sided 95% CIs for cognition endpoints and 92% CIs for reward endpoints) and 2-sided *P*-values of pairwise treatment effect were reported.

**Supplementary Table S1.** Functional MRI session

**Supplementary Table S2.** ERP Battery

**Supplementary Table S3.** Plasma PF-06412562 concentration versus time summary on day 6

**Supplementary Table S4.** Statistical summary (ANCOVA) of change from baseline in Digit Symbol Coding: total number of correct symbols with *P*<0.05

**Supplementary Table S5.** Statistical summary (RM-ANCOVA) of change from baseline in Risk-Based Decision-Making Task endpoints with *P*<0.08

**Supplementary Table S6.** Statistical summary (ANCOVA) of change from baseline in MID endpoints with *P*<0.08

**Supplementary Table S7.** Statistical summary (MMRM) of change from baseline in CBS Battery endpoints with *P*<0.05 at day 5 hour 2

**Supplementary Table S8.** Statistical summary(MMRM) of change from baseline in EVO testing endpoints with *P*<0.05 on day 7

**References**

Cole DM, Oei NY, Soeter RP, Both S, van Gerven JM, Rombouts SA, Beckmann CF (2013) Dopamine-dependent architecture of cortico-subcortical network connectivity. Cereb Cortex 23:1509-1516.

Paxton JL, Barch DM, Racine CA, Braver TS (2008) Cognitive control, goal maintenance, and prefrontal function in healthy aging. Cereb Cortex 18:1010-1028.

Santesso DL, Evins AE, Frank MJ, Schetter EC, Bogdan R, Pizzagalli DA (2009) Single dose of a dopamine agonist impairs reinforcement learning in humans: evidence from event-related potentials and computational modeling of striatal-cortical function. Hum Brain Mapp 30:1963-1976.

Vogel EK, Machizawa MG (2004) Neural activity predicts individual differences in visual working memory capacity. Nature 428:748-751.

Vogel EK, McCollough AW, Machizawa MG (2005) Neural measures reveal individual differences in controlling access to working memory. Nature 438:500-503.

Vytlacil J, Kayser A, Miyakawa A, D'Esposito M (2014) An approach for identifying brainstem dopaminergic pathways using resting state functional MRI. PLoS One 9:e87109.

**Supplementary Table S1.** Functional MRI session

|  |  |
| --- | --- |
| **Endpoint** | **Details** |
| Monetary Incentive Delay (MID) | fMRI parameter estimates |
| Reaction time across trial type (valence and magnitude) |
| N-back | fMRI parameter estimates |
| Accuracy, d-prime, and reaction time for 0-back and 2-back trials |
| AX-Continuous Performance Task (AX-CPT) (Paxton et al., 2008) | Accuracy and standardized reaction time across trial type |
| MRI parameter estimates |
| Resting-state fMRI (Cole et al., 2013; Vytlacil et al., 2014) | Connectivity between dopamine-rich midbrain areas (substantia nigra and ventral tegmental area) and 2 regions in the dorsal striatal region (caudate and putamen) |

fMRI, functional magnetic resonance imaging.

**Supplementary Table S2.** ERP Battery

|  |  |
| --- | --- |
| **Endpoint** | **Details** |
| Contralateral Delay Activity (CDA) (Vogel and Machizawa, 2004; Vogel et al., 2005) | Amplitude of the CDA |
| Amplitude of the N2pc |
| Working memory capacity, Cowan’s K |
| Filtering efficiency |
| Implicit Reward-Biasing Task (Santesso et al., 2009) | Feedback-related negativity (FRN) at midline electrodes (Fz and FCz) during late Blocks 2 and 3 compared with early (Block 1) learning phases |
| Response Bias (Block 3) – Response Bias (Block 1) |

**Supplementary Table S3.** Plasma PF-06412562 concentration versus time summary on day 6

|  |  |  |
| --- | --- | --- |
| **Treatment** | **Time post-dose (hr)** | **Concentration (ng/mL)** |
| PF-06412562 3 mg BID (N=27)  | 0 | 32.88 (11.98) |
|  | 5 | 43.51 (12.00) |
|  | 12 | 26.73 (10.45) |
| PF-06412562 15 mg BID (N=27) | 0 | 151.4 (73.02) |
|  | 5 | 224.7 (117.6) |
|  | 12 | 123.8 (60.65) |

Summary statistics were calculated by setting concentration values below the LLOQ to 0. The LLOQ is 0.500 ng/mL.

Mean (SD) are presented.

BID, twice daily; hr, hour(s); LLOQ, lower limit of quantification; N, number of observations (non-missing concentrations); SD, standard deviation.

**Supplementary Table S4.** Statistical summary (ANCOVA) of change from baseline in Digit Symbol Coding: total number of correct symbols with *P*<0.05

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  |  | **Contrast of PF-06412562 versus placebo** |
| **Treatment** | **n** | **LS means** | **SE** | **LS means** | **SE** | **95% CI** | ***P*-value** |
| PF-06412562 3 mg BIDPF-06412562 15 mg BIDPlacebo | 272722 | 6.646.2211.63 | 1.471.461.63 | -4.98-5.41 | 2.212.19 | (-9.39, -0.57)(-9.78, -1.04) | 0.02740.0159 |

ANCOVA, analysis of covariance; BID, twice daily; CI, confidence interval; LS, least squares; n, number of subjects; SE, standard error.

**Supplementary Table S5.** Statistical summary (RM-ANCOVA) of change from baseline in Risk-Based Decision-Making Task endpoints with *P*<0.08

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  | **Contrast of PF-06412562 versus placebo** |
| **Endpoint** | **Treatment** | **n** | **LS means** | **SE** | **LS means** | **SE** | **92% CI** | ***P*-value** |
| Proportion of experimental gambles when magnitude of possible gains was 70–30 | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 272722 | 0.06630.05010.1681 | 0.02750.02730.0305 | -0.1018-0.1180 | 0.04010.0402 | (-0.1730, -0.0306)(-0.1892, -0.0467) | 0.01320.0043 |
| Median RT when magnitude of possible losses was 30 | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 272722 | -379.16-558.23-204.92 | 97.5497.41108.10 | -174.24-353.30 | 145.84145.55 | (-433.90, 85.43)(-612.45, -94.16) | 0.23680.0182 |
| Median RT when magnitude of possible gains was 30 | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 272722 | -417.79-566.46-289.42 | 96.2396.11106.69 | -128.38-277.04 | 143.98143.68 | (-384.93, 128.18)(-533.04,-21.04) | 0.37630.0587 |
| Median RT when magnitude of possible gains was 70 | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 272722 | -400.77-547.22-212.55 | 96.2696.09106.65 | -188.21-334.67 | 143.87143.60 | (-444.59, 68.16)(-590.53, -78.81) | 0.19600.0233 |
| Median RT when probability of winning was low | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 272722 | -417.18-541.89-216.71 | 100.0999.94110.96 | -200.47-325.18 | 149.68149.35 | (-466.60, 65.67)(-590.71, -59.64) | 0.18510.0330 |
| Median RT when probability of winning was high | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 272722 | -401.38-571.79-285.26 | 100.09100.07110.95 | -116.12-286.54 | 149.66149.51 | (-382.23, 149.99)(-552.35, -20.72) | 0.44060.0596 |

BID, twice daily; CI, confidence interval; LS, least squares; n, number of subjects; RM-ANCOVA, repeated measure analysis of covariance; RT, reaction time; SE, standard error.

**Supplementary Table S6.** Statistical summary (ANCOVA) of change from baseline in MID endpoints with *P*<0.08

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  | **Contrast of PF-06412562 versus placebo** |
| **Endpoint** | **Treatment** | **n** | **LS means** | **SE** | **LS means** | **SE** | **92% CI** | ***P*-value** |
| Contrast of hit loss > miss loss in left caudate | PF-06412562 3 mg BIDPF-0641256215 mg BIDPlacebo | 262521 | -0.335-0.1950.144 | 0.1550.1530.177 | -0.480-0.339 | 0.2330.232 | (-0.888, -0.071)(-0.746, 0.068) | 0.03980.1446 |
| Contrast of cue gain > cue no gain in left nucleus accumbens | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 262521 | 0.1690.035-0.464 | 0.2130.2150.245 | 0.6320.499 | 0.3210.323 | (0.069, 1.196)(-0.067, 1.065) | 0.04920.1229 |
| Contrast of cue gain > cue no gain in leftputamen | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 262521 | 0.1170.009-0.552 | 0.2390.2380.263 | 0.6680.561 | 0.3450.356 | (0.064, 1.273)(-0.063, 1.184) | 0.05290.1154 |
| Contrast of hit loss > miss loss in right caudate | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 262521 | -0.360-0.2130.109 | 0.1650.1730.190 | -0.469-0.322 | 0.2540.248 | (-0.913, -0.024)(-0.757, 0.113) | 0.06500.1952 |
| Contrast of cue gain > cue no gain in rightputamen | PF-064125623 mg BIDPF-0641256215 mg BIDPlacebo | 262521 | 0.056-0.061-0.561 | 0.2250.2270.259 | 0.6170.500 | 0.3410.344 | (0.019, 1.215)(-0.102, 1.103) | 0.07070.1460 |

Baseline is defined as the day 0 assessment. Subjects with <40% accuracy at baseline were excluded.

*P*-values were obtained from an ANCOVA model on change from baseline with baseline, treatment, and age as fixed effects.

ANCOVA, analysis of covariance; BID, twice daily; CI, confidence interval; LS, least squares; MID, monetary incentive delay; n, number of subjects; SE, standard error.

**Supplementary Table S7.** Statistical summary (MMRM) of change from baseline in CBS Battery endpoints with *P*<0.05 at day 5 hour 2

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  | **Contrast of PF-06412562 versus placebo** |
| **Endpoint** | **Treatment** | **n** | **LS means** | **SE** | **LS means** | **SE** | **95% CI** | ***P*-value** |
| Composite Short-Term Memory Score | PF-064125623 mg BID | 27 | 2.20 | 0.27 | -1.14 | 0.40 | (-1.94, -0.35) | 0.0046 |
| PF-0641256215 mg BIDPlacebo | 2722 | 1.923.35 | 0.270.30 | -1.43 | 0.40 | (-2.22, -0.64) | 0.0004 |
| CompositeReasoning Score | PF-064125623 mg BID | 27 | 3.13 | 0.27 | -1.14 | 0.41 | (-1.94, -0.34) | 0.0051 |
| PF-0641256215 mg BIDPlacebo | 2722 | 2.974.27 | 0.270.30 | -1.30 | 0.41 | (-2.10, -0.51) | 0.0013 |

*P*-values were obtained from a MMRM model of change from baseline with baseline, age, treatment, time, and treatment-by-time interaction as fixed effects and subject as a random effect.

BID, twice daily; CBS, Cambridge Brain Sciences; CI, confidence interval; LS, least squares; MMRM, mixed model repeated measures; n, number of subjects; SE, standard error.

**Supplementary Table S8.** Statistical summary(MMRM) of change from baseline in EVO testing endpoints with *P*<0.05 on day 7

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  |  |  |  | **Contrast of PF-06412562 versus placebo** |
| **Endpoint** | **Treatment** | **n** | **LS means** | **SE** | **LS means** | **SE** | **95% CI** | ***P*-value** |
| Mean Threshold Single Task | PF-064125623 mg BID | 27 | 0.749 | 0.276 | -0.421 | 0.413 | (-1.243, 0.402) | 0.311 |
| PF-0641256215 mg BIDPlacebo | 2722 | 0.0911.170 | 0.2760.306 | -1.079 | 0.412 | (-1.900, -0.258) | 0.011 |
| Mean ThresholdAveraged Across Single Task and Multi-task | PF-064125623 mg BID | 27 | 0.854 | 0.230 | -0.283 | 0.343 | (-0.968, 0.401) | 0.412 |
| PF-0641256215 mg BIDPlacebo | 2722 | 0.2761.137 | 0.2300.255 | -0.861 | 0.343 | (-1.545, -0.178) | 0.014 |

*P*-values were obtained from a MMRM model of change from baseline with baseline, age, treatment, time, and treatment-by-time interaction as fixed effects.

BID, twice daily; CI, confidence interval; LS, least squares; MMRM, mixed model repeated measures; n, number of subjects; SE, standard error.