Supporting Online Material for

Changes in Cortical Dopamine D1 Receptor Binding Associated with Cognitive Training

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This PDF file includes:

Methods
Table S1
References
Supporting Online Material

Methods

Participants
Thirteen healthy male volunteers (ages 20-28, right handed) gave informed consent to participate in the study, which was approved by the local ethics committee of the Karolinska Hospital (Forskningetikprövningsnämnden).

Training
Prior to training the participants were briefed by a psychologist, either by telephone or in person. Each participant installed the training software on their home computer. Each training session included 100-150 trials, (30 to 45 minutes in total). Participants completed on average 23.9 (s.d. 1.8) training sessions during a 5 week period, with no more than one training session per day.

There were 10 different training tasks. An adaptive algorithm was used to adjust the WM load in line with performance, ensuring that participants trained at a level that matched their performance. The tasks were as follows: i) to remember a sequence of dots appearing on a stationary grid, and repeat the sequence in the same order; ii) to remember a sequence of positions appearing on the sides of a cube, and repeat the sequence in the same order; iii) to remember a sequence of dots appearing on a circular rotating grid, and repeat the sequence in the same order; iv) to remember a sequence of dots appearing on a rotating rectangular grid, and repeat the sequence in the same order; v) to remember a sequence of cubes which moved around the screen, and repeat the sequence in the same order; vi) to remember a sequence of dots which appeared at different positions on a circular grid, and a sequence of letters which they heard at the same time as the dots were displayed, and recall both in the same order; vii) to remember a sequence of heard letters, and repeat the sequence in the same order; viii) to remember a sequence of heard syllables, and repeat the sequence in the same order; ix) to remember a sequence of heard numbers, and repeat the sequence in the same order; x) to remember a sequence of visually presented numbers, and repeat the sequence in the reverse order.

Following each training session the results were automatically sent to the psychologist, who monitored progress, and gave feedback during training.

Behavioural testing
Before and after training participants completed a series of behavioural tests (approximately one hour). Five computerised WM tasks were administered. Three of the tests were designed to measure visual-spatial WM capacity. “Forward Span Board” required participants to view an array of cubes, a subset of which were sequentially illuminated, and then repeat the sequence by clicking on the same subset of cubes, in the same order. In “Backwards Span Board” the participants were required to repeat the sequence in the reverse order. In a third visual-spatial WM test the stimuli were circles presented on a 2D grid and participants were required to click the sequence of grid positions in the same order as the dots had appeared. The two remaining tests were tests of verbal WM. These involved repeating a sequence of syllables (auditory stimuli) in the
same order as they had been presented, and repeating a sequence of numbers (visual stimuli) in the reverse order. The number of items to be remembered in each of these tests (the level) increased in line with performance. Two sequences were given at each level, until the participant responded incorrectly to both sequences, at which point the task finished. A measure of WM capacity was determined according to performance on the highest level reached. For example, two correct responses at level three, and two incorrect responses at level four, would give a WM score of 3. Two correct responses at level two, one correct response at level three, and two incorrect responses at level four would give a WM score of 2.5. One correct response at level two, one correct response at level three and two incorrect responses at level four would also give a WM score of 2.5. For each participant, the mean WM score across all tasks was computed, and used in subsequent analysis.

Two additional pencil and paper tests were completed (a subset of Raven’s Advanced Progressive Matrices and the Paced Auditory Serial Addition Task), the results of which are not included here.

fMRI procedure
During fMRI scanning, stimuli were presented using E-prime software. A 4 x 4 grid was displayed (white lines on a black background). There were two conditions; a WM condition (50% of trials) and a control condition (50% of trials). In WM trials, five yellow circles were presented sequentially in different grid positions, but not in corner spaces. Each circle was displayed for 500 ms and was followed by presentation of the empty grid for 500 ms. 1000 ms after the last circle disappeared, a cue was presented within one of the grid spaces. This took the form of a number between 1 and 5, referring to the serial position in the previous stimulus sequence, and a question mark. The participant was asked to indicate with a yes/no response whether the number and the grid position matched, for example a “2?” in a certain grid position would prompt the participant to indicate whether the second circle had appeared in that particular grid position. In the control condition red circles were presented in the same sequence of corner grid positions, starting in the top left of the grid and progressing clockwise. The cue in the control condition always consisted of the number 8 presented in a non-corner space, and always required a “yes” response.

Trials were pseudo-randomly presented (with the trial type randomised), in an event-related design. There were four sessions of approximately 10 minutes, with 40 trials in each session. The order of the sessions was counterbalanced across participants. Yes/no responses were made with a button press using the index or middle finger of the right hand. The data from two participants were excluded from the fMRI analysis due to movement of >4 mm during fMRI recording. Before training, seven of the remaining participants completed four sessions and four completed three sessions. After training, nine of the remaining participants completed four sessions and two completed three sessions.

MRI acquisition
Imaging data were collected using a 1.5 T scanner. T2*-weighted, gradient echo, spiral echo-planar images were acquired with TR = 2100 ms, TE = 40 ms, flip angle = 76°, 22
axial slices, 5.0 mm slice thickness, 220 x 220 mm FOV, 64 x 64 grid, resulting in voxels that were 3.4 x 3.4 x 5.0 mm. Each session lasted 364.2s and included acquisition of 174 volumes. T1-weighted spin echo images (FOV = 220 x 220 mm, 256 x 256 grid) were acquired in the same position as the functional images.

PET acquisition

PET studies were performed before and after WM training, in resting conditions, using the ECAT EXACT HR System run in 3D mode (S1). The second round of PET measurements was conducted several days after completion of the WM training. Prior to each emission scan a transmission scan of 10 min was performed using three rotating $^{68}$Ge-$^{68}$Ga sources. The transaxial and axial resolution of the system is 3.8 and 4 mm full width at half-maximum in the centre of the field of view. [$^{11}$C]Raclopride and [$^{11}$C]SCH23390 were prepared as described previously (S2, S3). The radioligands were administered intravenously as a rapid bolus and the cannula was immediately flushed with saline. A total of 52 PET measurements were conducted for 51 min with a series of frames of increasing duration (3x1 min, 4x3 min, 6x6 min). After correction for attenuation, random and scatter events, images were reconstructed with filtered-back-projection using a Hanning filter of 2.0 mm, with a reconstructed volume of 47 slices, a centre-to-centre distance of 3.125 mm and a pixel size of 2.02 mm x 2.02 mm.

Data analysis

Preprocessing and statistical analysis of the fMRI data were carried out with SPM5 (Welcome Department of Cognitive Neurology, http://www.fil.ion.ucl.ac.uk/spm/software/spm5). Preprocessing included slice-time correction, motion correction, normalisation to the template EPI (interpolating to 2 mm cubic voxels) and spatial smoothing with an 8 mm Gaussian kernel. The data were modelled using a canonical hemodynamic response and its temporal derivative. The model used to identify WM activity included separate regressors for WM and control conditions (epoch analysis beginning at the onset of the first stimulus, with a duration of 8.5 s, corresponding to the trial duration). Only trials that received a correct response were included in the model.

The WM versus control condition contrast was implemented as a linear contrast. The analysis was carried out individually, and contrast images for each participant were used in a second level analysis, treating each participant as a random effect. The statistical map was thresholded with a false discovery rate of $p < 0.05$, and differences were considered significant if they fulfilled the criteria of an extent threshold of 600 voxels. From this statistical map, three clusters were identified.

ROI generation

Five ROIs were generated from the three clusters. A left frontal ROI corresponds exactly to cluster 2. A right ventral frontal ROI corresponds exactly to cluster 3. As cluster 1 was so large (21023 voxels) it was used to generate three ROIs. We subdivided cluster 1 into right and left hemispheres (dividing at x = 0), and also into anterior and posterior regions.
(dividing at y = -22). In this way three ROIs were generated (right posterior, left posterior and right dorsal frontal ROIs).

**PET analysis**

PET images were co-registered to T1-weighted MR images using SPM2 (Welcome Department of Cognitive Neurology). The outcome measure for D1- and D2-receptor quantification was $BP_{ND} (k_3/k_4)$. $BP_{ND}$ refers to the ratio at equilibrium of specifically bound radioligand to that of nondisplaceable radioligand in tissue and equals the ratio of receptor density ($B_{max}$) and apparent affinity ($K_D$), times the free fraction of the ligand in the nondisplaceable tissue ($f_{ND}$) ($S4$).

Parametric images of $BP_{ND}$ were generated using a wavelet-transform aided parametric imaging approach ($S5$). Briefly, the MR-coregistered PET images were transformed frame-by-frame to the wavelet space using a 3D stationary wavelet transform ($S6$). The depth of decomposition was set at 2 and the kernel length was 16. The resulting coefficients were analyzed quantitatively using the non-invasive Logan graphical approach ($S7$) using the cerebellum as a reference region. The end product of the calculation was a 3D parametric map of distribution volume ratio (DVR). $BP_{ND}$ images were calculated from the DVR maps by subtracting one from the DVR values.

$BP_{ND}$ images were spatially normalized to the MNI space using SPM2. First, each individual T1-weighted MRI scan was normalized to the MNI template, and then the transformation matrix was applied to the corresponding co-registered $BP_{ND}$ image. The functionally defined ROIs were applied to the normalized $BP_{ND}$ images to derive individual $BP_{ND}$ values for each fMRI cluster.

For simplicity, $BP_{ND}$ is referred to as binding potential (BP) in the article.
References


Table S1. Working memory capacity measured with each of the computerised tests before and after training.

<table>
<thead>
<tr>
<th></th>
<th>Forward Span Board</th>
<th>Backwards Span Board</th>
<th>Circles Forwards</th>
<th>Syllables Forwards</th>
<th>Numbers Backwards</th>
<th>Mean of all tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before training Mean ± SD</td>
<td>5.81 ± 0.75</td>
<td>5.79 ± 1.08</td>
<td>6.46 ± 1.42</td>
<td>6.00 ± 0.94</td>
<td>5.04 ± 1.41</td>
<td>5.81 ± 0.72</td>
</tr>
<tr>
<td>After training Mean ± SD</td>
<td>7.12 ± 0.92</td>
<td>7.08 ± 1.22</td>
<td>7.38 ± 1.33</td>
<td>6.81 ± 1.13</td>
<td>8.38 ± 1.88</td>
<td>7.40 ± 0.87</td>
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</tbody>
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