Title of Study: A Study of Neural Circuit Responses to Catechol-O-methyl Transferase (COMT) Inhibitors

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Dopamine, Corticostriatal Connectivity, and Intertemporal Choice
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Materials and Methods

Subject training and task performance

We screened 45 subjects, of whom 27 healthy subjects (i.e. without a history of neurological or psychiatric illnesses) were eligible to participate. All subjects gave written informed consent in accordance with the Committee for the Protection of Human Subjects at the University of California, San Francisco and University of California, Berkeley. Subjects first underwent a history and physical exam, as well as blood testing for liver function, to ensure that there were no medical contraindications to tolcapone use or MRI scanning. They then completed a number of screening questionnaires, including the Barratt Impulsivity Scale (BIS). One subject was excluded prior to behavioral analysis for use of an albuterol inhaler immediately before MRI scanning. Three subsequent subjects were excluded after task performance: one reported an explicit change in discounting strategy prior to the second session, one was noted to be significantly sleep-deprived after completing final exams the day of the second session, and a third was a non-native English speaker who may have misunderstood task instructions. The differential impulsive choice ratio (ICR; see below) of the first of these excluded subjects favored tolcapone (-0.59) and of the other two favored placebo (0.45, 0.92); all values were identified as significant outliers ($p < 0.005$) by Rosner's generalized extreme studentized deviate (ESD) procedure (Rosner, 1983). Of the 23 subjects who remained, ages ranged from 19-41 years old; 13 were female. All subjects had normal neuroanatomy as reviewed by a neurologist (A.S.K.), were right-handed, and had normal or corrected-to-normal vision. Before scan sessions, subjects were trained on the delay discounting task to familiarize them with task procedures. Subjects then underwent two 2.25-hour fMRI sessions consisting of 8 runs of 32 task trials, for a

total of 256 trials, and two resting state runs. All subjects were compensated for their participation.

Experimental paradigm

Subjects were randomized in double-blind, counterbalanced, placebo-controlled fashion to either placebo or a single 200mg dose of tolcapone on their first visit and the alternative treatment on their second visit. After receiving task instructions and undergoing a brief practice session of 10-20 trials, subjects performed the delay discounting task (Figure 1) within the MRI scanner while blood-oxygen level dependent (BOLD) images were obtained. Subjects entered the MRI scanner 90 minutes after drug ingestion to ensure that the delay discounting task was performed while drug levels were at peak (approximately 120 minutes: tolcapone package insert, Valeant Pharmaceuticals). The 256 total trials were presented in pseudorandom order. At the start of each trial, subjects were cued to one of four trial types: Want (W), Don't Want (DW), Sooner (S), and Larger (L) (see below). For each of these trial types, subjects were then presented with two hypothetical alternatives: a smaller amount of money available today ("Now") and a larger amount available later ("Later"). We have previously shown that this paradigm with hypothetical rewards effectively engages subjects (Boettiger et al., 2007; Boettiger et al., 2009), consistent with reports that hypothetical rewards activate common brain regions involved in value computations (Kang et al., 2011). As in our previous work (Boettiger et al., 2007), the 'Later' option consisted of six amounts ($1, $2, $5, $10, $20, or $100) at one of five future delays (1 week, 2 weeks, 1 month, 3 months, or 6 months). The percentage difference between the Now and Later options was selected from one of four different values (30%, 15%, 10%, and 5%). Subjects then made a button press to select one of the two options, randomly assigned to

the left and right sides of the screen.

Each of the four trial types allowed us to investigate different functions. In the W condition, the primary analytic focus of this study, subjects chose the option they preferred. In the DW condition, subjects also chose the option they preferred, but then made a button-press to select the opposite choice. This condition permitted us to evaluate motor impulsivity by comparing the proportion of responses in the W and DW conditions. In the S and L conditions, which we combined to form a control (CON) condition, subjects simply selected the sooner or larger options, respectively. These trial types allowed us to ensure that subjects were appropriately following instructions, and to introduce a condition in which the decision about monetary options was not a motivated choice. The W condition comprised half of all trials; the CON conditions comprised one-third; and the DW condition comprised one-sixth. As expected, subjects performed very well in the CON condition (accuracy = 0.97 ± 0.008 (sem)) and therefore we do not further report results of the CON condition in this paper.

The primary behavioral outcome was the impulsive choice ratio (ICR), which represents the ratio of the number of sooner choices to the number of total choices in the W condition. As in our previous work, we calculated a number of related measures of impulsive choice, including a measure of the hyperbolic discounting rate (k) and the interest rate differentiating the two choices. Because values such as the hyperbolic discounting rate were highly correlated with ICR (e.g. r (ICR, k) = 0.78, $p < 1 \times 10^{-5}$) and consequently gave qualitatively similar results, we elected to study the simplest, most intuitive quantity, as in our previous work (Boettiger et al.,

2007; Boettiger et al., 2009). ICR values underwent an arcsine-square root transform – i.e. were variance-stabilized – to permit the application of parametric statistical tests.

Both before drug administration and after the scanner run, subjects completed a speeded responding task to assess potential changes in motor function on and off tolcapone. Subjects were required to make a button press response as soon as possible after the presentation of either a brief visual or auditory stimulus. Reaction times were compared both within each session and across the tolcapone and placebo conditions. Due to computer failures, these data were available for only 19 of our 23 subjects.

MRI image acquisition

MRI scanning was conducted on a Siemens MAGNETOM Trio 3T MR Scanner at the Henry H. Wheeler, Jr. Brain Imaging Center at the University of California, Berkeley. Anatomical images consisted of 160 slices acquired using a T1-weighted MP-RAGE protocol (TR = 2300 ms, TE = 2.98 ms, FOV = 256 mm, matrix size = 256 x 256, voxel size = 1 mm^3). Functional images consisted of 24 slices acquired with a gradient echoplanar imaging protocol (TR = 1370 ms, TE = 27 ms, FOV = 225 mm, matrix size = 96 x 96, voxel size = 2.3 x 2.3 x 3.5 mm). A projector (Avotec SV-6011, http://www.avotec.org) was used to display the image on a translucent screen placed within the scanner bore behind the head coil. A mirror was used to allow the subject to see the display. Subjects made their responses via an MRI-safe fiber optic response pad (Inline Model HH-1x4-L, http://www.crsltd.com).

fMRI preprocessing

fMRI preprocessing was performed using both the AFNI (http://afni.nimh.nih.gov) and FSL (http://www.fmrib.ox.ac.uk/fsl/) software packages. Functional images were converted to 4D NIfTI format and corrected for slice-timing offsets. Motion correction was carried out using the AFNI program *3dvolreg*, with the reference volume set to the mean image of the first run in the series. Images were then smoothed with a 5mm FWHM Gaussian kernel. Co-registration was performed with the AFNI program *3dAllineate* using the local Pearson correlation cost function optimized for fMRI-to-MRI structural alignment. The subsequent inverse transformation was used to warp the anatomical image to the functional image space. Anatomical images were normalized to a standard volume (MNI_N27) using the FSL program *fnirt* available from the Montreal Neurological Institute (MNI; http://www.bic.mni.mcgill.ca). The same normalization parameters were later applied to native-space statistical maps as necessary for the generation of group statistical maps (see below).

Univariate analysis

To address a series of hypotheses, we carried out a number of voxel-wise fMRI statistical analyses for each subject using the general linear model (GLM) framework implemented in the AFNI program 3*dDeconvolve*. The BOLD correlates of different decisions were assessed by modeling the cue and decision phases of the task for the four different conditions (Want, Don't Want, Sooner, Larger) with separate regressors, each of which was derived by convolving a gamma probability density function (peaking at 6 seconds) with a vector of stimulus onsets for each condition. Map-wise significance ($p < 0.05$, corrected for multiple comparisons) was determined by applying a cluster-size correction derived from the AFNI programs *3dFWHMx* and *3dClustSim* on data initially thresholded at a value of $p < 0.005$ (uncorrected). For the main

effect of task (figure 3), a whole-brain correction was applied. This contrast compared event onsets from all conditions – including both cue and outcome – with baseline fMRI activity on tolcapone versus placebo to illustrate that there are widespread changes in BOLD signal across the brain resulting from the use of tolcapone. Because cortical dopamine projections are frontally predominant (Cools, 2008), subsequent univariate analyses designed to address more specific task-related hypotheses about changes in frontostriatal regions used the AAL template brain (Tzourio-Mazoyer et al., 2002) to generate a frontostriatal mask (AAL areas 3-32 and 71-76; analyses illustrated in figures 4 and 5a).

Multivariate analysis

Resting state data were used to evaluate the connectivity between brain regions in both the tolcapone and placebo conditions in all subjects who completed both resting state sessions (N = 22; due to technical failures, complete resting state data from one of our 23 subjects were not obtained). This approach allowed us to identify any drug-related changes in connectivity independent of task performance. These data were smoothed by a 2.5mm FWHM Gaussian kernel prior to temporal bandpass filtering between 0.009 Hz and 0.08 Hz to reduce the influence of cardiac and respiratory artifact, per the protocol of Fox and colleagues (Fox et al., 2005). Movement parameters and the white matter and ventricular time series, but not the global mean signal, were included as regressors of no interest. A striatal region of interest in the left ventral putamen was chosen, based on the results of our univariate analyses (see Results, Figure 4a). A time course defined by averaging across voxels in this region was then correlated with every other voxel in the brain, and correlation coefficients were Fisher-transformed to allow for the application of parametric statistical tests. Images were then normalized to the MNI template

prior to the application of group-level statistics. Because of our hypotheses about changes in frontostriatal connectivity, data were masked with a prefrontal mask (AAL regions 3-32) before the appropriate cluster-size correction was calculated.

For the evaluation of connectivity during task performance, we used the seed region (left ventral putamen) and significantly-connected region (left pregenual anterior cingulate cortex) identified in the resting state connectivity analysis shown in Figure 5b. For each of these two ROIs, the time series for each of the eight task-state runs was windowed with a 4-point split-cosine bell and concatenated with the other segments to produce a subject-specific time series. Time series within each ROI were then averaged across voxels to generate a single time series for each ROI. Coherency values were obtained by applying a fast Fourier transform (Matlab, http://www.mathworks.com) to the data for each pair of ROIs, implemented via Welch's periodogram averaging method using a 64-point discrete Fourier transform, Hanning window, and overlap of 32 points (Kayser et al., 2009). Coherence values for each ROI were then computed using the band-averaged coherence. To compute correlations between coherence results and other values, we first Fisher-transformed the coherence values to generate an approximately normal distribution (Rosenberg et al., 1989) that permitted us to apply parametric statistical tests.

Statistical Analysis

For analysis of behavioral data, paired T-tests and Pearson's correlation coefficients were used to calculate statistical significance. For univariate and multivariate analyses of BOLD data, signficance was calculated using statistical techniques and corrections implemented in the Afni

software package, including the functions *3dDeconvolve*, *3dFWHMx*, *3dClustSim*, and *3dttest++*.