Title: Dopaminergic Mechanisms Underlying Human Social Behavior

NCT Number: NCT04205994

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STUDY PROTOCOL

Interested subjects will contact the study via the contact information provided in the study advertisement. Dr. Hsu, Dr. Kayser, or their research assistant(s) will then contact each potential subject by phone (or by each subject's preferred method of communication) in order to set up a brief phone interview, during which the requirements for the study will be reviewed. Dr. Hsu, Dr. Kayser, or their research assistant(s) will then perform the initial phone screening to verify that subjects meet the criteria specified in the advertisement. The Recruitment Script Phone will be used for note taking during the call, but the sheet is then shredded after the call and the information is recollected when the subject arrives for her/his screening visit (if eligible), and after they provide signed informed consent. If a prospective subject is a staff under the supervision of either Dr. Hsu or Dr. Kayser, the supervising individual will not participate in the recruitment process in order to avoid potential coercion or undue influence. In addition, UCB students, in general, will be routinely screened (initial phone screening) by research assistants, rather than by faculty.

For subjects who state that they are potentially eligible for enrollment, we will arrange an initial visit, at the subject's convenience, to perform further screening and to gather initial behavioral data. The Subject Health screening sheets will be filled out immediately (and only) after obtaining written informed consent. The Tolcapone package insert will be shared with subjects to inform them of possible side effects. Consent will be obtained only at approved sites for this study, including either the subject suite in the UC Berkeley Imaging Center (BIC) or the clinic and testing rooms at the UCSF Research Clinic. Subjects will read through the consent form prior to signing the document; if for some reason a subject is unable to read the form, the consenting study investigator will read the forms to the subject. Subjects will be provided as much time as required for them to familiarize themselves with the contents of the consent form. Any questions the subject has concerning the contents of the consent form, or the consent process itself, will be answered prior to signing. Only after informed consent is obtained will the remainder of the initial visit, described below, be completed.

We will administer an extensive questionnaire listing contraindications to MRI scanning. Because the MRI scanner attracts certain metals, subjects who may have metallic objects in their bodies will be excluded. (As an additional measure of protection, we will use a hand-held metal detector to screen subjects before entering the scanner.)

Subjects will provide a saliva sample in order to determine catechol-O-methyltransferase (COMT) and dopamine receptor subtype 2 (DRD2) genotypes. DNA extraction and analysis will be conducted using standard methods to assess the Val158Met polymorphism of the COMT gene and the A1+ polymorphism in the DRD2 gene. Examiners and researchers who interact with study subjects or study data will remain blinded to genotype until the end of the study. Furthermore, genes affecting dopamine metabolism in addition to COMT and DRD2 will be examined (including the dopamine transporter variable number tandem repeat (DAT VNTR) and dopamine receptor subtype 4 single nucleotide polymorphism 521 (DRD4 SNP-521)). In order to further assess potential contraindications to tolcapone, a blood sample will be tested for routine chemistries including white cell count, red cell count, platelet count, hemoglobin, hematocrit, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Additionally, because of potential hepatic effects of tolcapone, a hepatic screen will assay total protein, albumin, globulin, A/G ratio, bilirubin (total, direct, and indirect), alkaline phosphatase, aspartate aminotransferase

(AST, aka serum glutamic oxaloacetic transaminase (SGOT)), and alanine aminotransferase (ALT, aka serum glutamic pyruvic transaminase (SGPT)). Elevation of plasma bilirubin, AST (SGOT), ALT (SGPT), or alkaline phosphatase greater than 3 times normal values will be grounds for subject exclusion. (Note that ongoing monitoring of liver enzymes will not be necessary, as only a single, counterbalanced dose of tolcapone will be administered to each subject.) The blood sample may also be used for genetic testing if the saliva sample is non-diagnostic, compromised, or otherwise unavailable. Tests for blood chemistries following phlebotomy are currently performed at Quest Diagnostics in Berkeley, CA or the main laboratory at UCSF.

Subjects will then be urine-screened for illicit drug use (Biotechnostix) and screened for intoxication via breathalyzer (Lifeloc Technologies). To confirm that subjects do not have potential drugs of abuse in their system, an on-site rapid urine-screening and a breathalyzer test will be done before administering tolcapone or placebo. Urine-screening will be done via an integrated test cup that includes a temperature sensor. The drug classes detected include cocaine, amphetamine, methamphetamine, tetrahydrocannabinol (THC), and opiates. Additionally, two opiates (methadone and oxycodone) may not be reliably detected by the 5-Test Integrated cup, so urine dipstick screening (Innovacon, Inc.) will also be performed on each subject's urine sample for these two

compounds. These drugs have been chosen due to their possible interaction with tolcapone and their possible cognitive and cardiovascular effects. No identifiers will be put on the test cup and it will be read immediately and discarded by the researcher. Similarly, the results of the breathalyzer will be read and then the test will be discarded. Moreover, due to the potentially sensitive nature of this information, the results will not be written down. Subjects who test positive for any of these substances will be excluded from further participation in the study. Subjects will also be excluded if they regularly use medications that affect dopamine levels, or will have used these medications within two weeks of tolcapone administration (including tolcapone, except as part of our study, or any of the following: levodopa/carbidopa, entacapone, amantadine, pergolide, pramipexole, ropinirole, selegeline, isocarboxazid, phenelzine, tranylcypromine, clozapine, olanzapine, quetiapine, risperidone, ziprasidone, aripiprazole, fluphenazine, haloperidol, perphenazine, pimozide, thiothixene, trifluoperazine, loxapine, molindone, chlorpromazine, mesoridazine, thioridazine, dextroamphetamine, dexmethylphenidate, or methylphenidate).

A licensed health care provider listed in the protocol will also conduct a brief physical exam. This exam will search for signs of medical illness, including jaundice or abdominal distension associated with liver disease, that would exclude subjects from participating in the study. (See exclusion criteria.) For the purposes of the exam, female subjects will not be limited to a female provider, and male subjects will not be limited to a male provider. However, our practice has been, and will continue to be, that a research subject may always request a male or female provider. If for some reason the requested provider is not available, a member of the research team of the requested gender can also be present for the exam, if desired.

Female subjects will also be screened for pregnancy, as the effects of the study drug during pregnancy are not adequately known and the drug may appear in breast milk. Since subjects may not know they are pregnant, all female subjects recruited to participate in the study will be required to have a urine pregnancy test prior to each session of the study. They will self-administer the test after coming into the research lab. These pregnancy tests have been in

reliable use at the UC Berkeley Brain Imaging Center for approximately 10 years without any reported failures. Subjects will be provided with printed instructions in the use of the test.

Active use of substances other than alcohol or tobacco, use of alcohol on the day of the meeting as assessed by breathalyzer testing, and/or a positive pregnancy test, will be grounds for exclusion.

STATISTICAL ANALYSIS PLAN

For each of the three arms of the study we plan to recruit a minimum of 35 subjects. The total number of subjects is consistent with both our initial behavioral results in a group of 35 patients and seminal neuroimaging papers that suggest numbers of at least 20 subjects per group are necessary for adequate reliability of fMRI results (Murphy and Garavan, 2004; Thirion et al., 2007) – assuming certain standard estimates of signal-to-noise, spatial smoothing, and BOLD time series duration that are well-established in the fMRI literature across studies including perceptual, attentional, language-related, and executive tasks. Based on our previous data, for a behavioral effect size of 0.5 at a significance level of $p = 0.05$, our sample size will provide at least 88% power to detect a behavioral difference in a 2x2 ANOVA with factors of group and drug. For neuroimaging, our sample size will provide at least 80% power to detect a difference in the BOLD signal of 0.5% in a 2x2 (group x drug) ANOVA at a two-tailed significance of $p = 0.05$, corrected (Desmond and Glover, 2002). For PET imaging, our sample will provide at least 80% power to detect correlation of 0.3, which is on the lower end of effects observed in related studies at a two-tailed significance of $p = 0.05$ (Berry et al., 2017; Jonasson et al. 2017; Kodaka et al. 2013).

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