An Investigation of Early Life Stress and Depression (ID: 2012P002593)

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Early Life Stress and Depression: Molecular and Functional Imaging Approaches
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I. BACKGROUND and SIGNIFICANCE

a. Historical background

The recent National Comorbidity Survey showed that severe childhood adversity explains nearly 32% of psychiatric disorders, and an even higher percentage (44%) of disorders with childhood onset (Green et al 2010). Strikingly, early adversities account for about 67% of suicide risk (Dube et al 2001). In a birth cohort of 1,000 children, early maltreatment was linked to an increased odds ratio of 4.6 for MDD (Fergusson et al 1996a,b; see also Kendler et al 2000; Wise et al 2001). In addition, CSA has been linked to higher rates of maladaptive coping behaviors, including 2.7-fold risk for alcohol abuse and dependence and 6.6-fold risk for abuse of other substances (Kendler et al 2000). Although maladaptive behaviors may serve an initial coping function to relieve CSA-related distress, these behaviors form a primary mediator between CSA and future sexual victimization (Messman-Moore et al 2009), which is consistently high (~30%; Messman-Moore et al 2010). Early adversity does not only increase the risk for psychopathology, but also medical illnesses (e.g., cardiovascular disease) and health-damaging behavior (e.g., smoking) (e.g., Anda et al 2006; Chapman et al 2004; Dong et al 2004b). In spite of these epidemiological data, the neurobiological underpinnings associated with adaptive (resilience) and maladaptive sequelae of CSA remain largely unknown. Furthermore, despite high prevalence rates and severe sequelae of CSA, little is known about underlying neurobiological mechanisms, particularly with respect to stress-mediated disturbances of mesocorticolimbic DA pathways. Progress in this area is currently hampered by a failure to consider the (1) specificity of the type of early adversity, (2) role of developmental trajectory of the brain at the timing of abuse, and (3) interplay of mesocortical vs. mesolimbic DA system.

b. <u>Previous research</u>

A large body of work has highlighted reduced affective, behavioral, and physiological responses to positive and reward-related cues in depression. Depressed and/or anhedonic participants: 1) fail to distinguish between options yielding large versus small rewards (Forbes et al, 2007); 2) experience diminished affective (Berenbaum & Oltmanns, 1992), behavioral (Sloan et al, 2001), and physiological (Piersonet al, 1987) responses to pleasant stimuli; 3) underestimate the frequency of correct feedback and positive reinforcement received in experimental tasks (Nelson & Craighead, 1977); and 4) require greater emotional intensity to correctly identify happy facial expressions (Joormann & Gotlib, 2006). In our work using a probabilistic reward task, we found that subjects with elevated depressive symptoms (Pizzagalli et al, 2005), unmedicated Major Depressive Disorder (MDD) patients (Pizzagalli et al, 2009), and euthymic bipolar patients (Pizzagalli et al, 2008) are characterized by reduced reward learning. Interestingly, in both clinical and non-clinical samples, reward responsiveness negatively correlated with anhedonic symptoms (Bogdan & Pizzagalli, 2006; Pizzagalli, et al., 2008; Pizzagalli et al., 2009; Pizzagalli et al., 2005), and predicted these symptoms one month later (Pizzagalli et al., 2005).

These behavioral findings have been complemented by neuroimaging data. Forbes and colleagues (Forbes et al., 2006) reported that, relative to controls, depressed children displayed reduced activation in the anterior cingulate cortex (ACC), caudate, and right orbitofrontal cortex (OFC) during the decision/anticipation and outcome phases of a reward-based decision making task (particularly for small rewards). Similarly, replicating and extending their finding of increased affective response to a dopamine (DA) agonist in MDD, Tremblay and colleagues (Tremblay et al., 2005) reported that,

relative to controls, MDD subjects showed increased affective responses to a DA agonist along with decreased BOLD signals in the OFC, caudate, and putamen. The decreased BOLD signal in these regions was interpreted as resulting from disinhibition of DA neurons. Thus, both the self-reported hyper-response to the drug and the fMRI results were interpreted as suggesting hypofunction in the brain's reward system in MDD. Further evidence of dysfunctional reward pathways in MDD arises from studies highlighting reduced responsiveness to positive stimuli in reward-related regions, including the ventral striatum (Epstein et al., 2006; Lawrence et al., 2004) and medial PFC/OFC (Elliott et al, 2002). Notably, a recent study using a gambling task reported that MDD subjects failed to show the behavioral (RT reduction) and neural (ventral striatum activation) patterns displayed by controls upon receiving positive feedback (Steele et al, 2007). Finally, anhedonic symptoms correlated negatively with activation in the ventral striatum and dorsolateral PFC in response to visual stimuli (Epstein et al., 2006; Harvey et al, 2007; Keedwell et al, 2005).

Preclinical data suggest that chronic stressors, including early adversity, exert long-lasting effects on mesocorticolimbic DA pathways (e.g., Anisman & Matheson 2005; Matthews & Robbins 2003). For example, chronic unavoidable stressors led to a 64% reduction in the number of spontaneously active DA neurons in the ventral tegmental area (VTA) (Moore et al 2001) and reduced DA output in the nucleus accumbens (NAc) up to 14 days post-stressor (Gambarana et al 1999). Reduction in mesolimbic DA was closely related to coping failures and maintenance of depression-like behaviors (Mangiavacchi et al 2001), and was normalized by antidepressants (Gambarana et al 1999). In adult monkeys (age 19-24), social deprivation in the first 9 months was linked to altered distribution and density of neurotransmitters in basal ganglia regions (globus pallidus, caudate, and putamen; Martin et al 1991). Similarly, early life stress reduced dopamine transporter (DAT) density and enhanced stress-induced DA release in the NAc and PFC (Brake et al 2004; Di Chiara et al 1999).

Notably, in Matthews and Robbins's (2003) maternal separation model, impaired reward anticipation emerged in spite of normal consummatory behavior, suggesting that specific phases of reward processing might be particularly affected by early adversity. Pryce et al (2004) extended these findings to non-human primates: adult monkeys subjected to early maternal separation displayed diminished motivation to obtain reward despite normative consummatory behavior. Similarly, maternal deprivation in rats led to an adult phenotype with decreased motivation to work to obtain a sucrose reinforcer (Ruedi-Bettschen et al 2005). While preclinical data point to reward system abnormalities following early adversity, little attention has been devoted to clarify the role of DA in CSA.
The role of stress in the development, expression, and exacerbation of depression, and particularly, anhedonia, is well established (Brown and Harris 1978; Kendler, Kessler et al. 1995; Hammen 2005; Monroe and Harkness 2005). Decades of animal research have shown that exposure to uncontrollable stressors down-regulates mesolimbic DA pathways and induces anhedonia-like behavior. Because phasic DA responses play a key role in the acquisition and expression of motivated behavior and reinforcement learning (Schultz 2007), these preclinical findings suggest that dysfunctions in DA mechanisms might underlie anhedonia in MDD. Of primary relevance to the proposed research, this preclinical work has also shown that these behavioral and physiological effects are dependent on the nature of the stressor (e.g., uncontrollable vs. controllable), prior encounters with chronic stressors, and genetics (Cabib and Puglisi-Allegra 1996). The ability of an organism to exert control over a stressful situation, in particular, has been found to have important effects (Anisman and Matheson 2005). Critically, elegant work by Maier and colleagues has shown that detection of control during a stressful situation activates the medial prefrontal cortex (mPFC), which in turn inhibits stress-induced activation of brainstem and limbic regions (Amat, Baratta et al. 2005; Amat, Paul et al. 2008). Intriguingly, experience of stress controllability modifies mPFC responses to future

uncontrollable stressors, boosting resilience. Evidence of increased stress sensitivity in MDD is complemented by reports of (1) Hypothalamic-Pituitary-Axis (HPA) hyperactivity, including chronically elevated cortisol and increased stress-related corticotrophin-releasing hormone (CRH) levels in MDD, which is seen in up to 70% of patients (Holsboer 2000); (2) HPA hyperactivity in both remitted MDD subjects (Vreeburg, Hoogendijk et al. 2009) as well as healthy first-degree relatives of MDD patients (Modell, Lauer et al. 1998); and (3) normalization of HPA axis function anteceding remission (Holsboer 2000).

In addition to stress-induced alterations in stress-hormone pathways, acute stressors have also been found to engage the inflammatory response system (IRS). IRS reactivity has been previously associated with anhedonic symptoms, directly impacts multiple aspects of reward-processing pathways such as DA turnover and basal ganglia function, and is dysfunctional in a substantial sub-group of patients meeting criteria for MDD (Capuron, Su et al. 2008; Raison and Miller 2011). Despite this robust evidence, the specific role of IRS function in relation to neurophysiological, neuroendocrine and anhedonic responses to stress is not well-characterized. Therefore, the current study will measure pro-inflammatory cytokines levels, specifically tumor-necrosis factor alpha (TNF-α) and interleukin-6 (IL-6). These markers were chosen as they have shown the most robust associations with both laboratory stress-paradigms as well as depressive symptoms in clinical populations (Steptoe, Hamer et al. 2007; Dowlati, Herrmann et al. 2010).

c. Study rationale

In summary, these data indicate that MDD is characterized by reduced hedonic responses and ability to modulate behavior as a function of positive reinforcers. Functional MRI studies suggest that these deficits may reflect ventral striatal, ACC, and OFC abnormalities. Because positive reinforcers increase the likelihood of behavior (Rescorla & Wagner, 1972), blunted reward responsiveness may lead to reduced motivational drive to pursue pleasurable activities, which may in turn contribute to depressive symptoms. Indeed, reduced hedonic capacity (1) characterizes both currently depressed and remitted depressed individuals (Kasch et al, 2002; Pinto-Meza, 2006); and (2) predicts depressive symptoms (Hundt et al, 2007), time to recovery (McFarland et al, 2006), and poor treatment outcome (Kasch et al., 2002; Spijker et al, 2001). The overarching goal of the proposed research is to investigate mesocorticolimbic dopaminergic pathways within young adult females with a history of CSA between the ages of 5-14 years with ("CSA/MDD group") and without ("CSA/RES group") a current diagnosis of major depressive disorder (MDD). To disentangle CSA- vs. MDD-related effects, these groups will be compared to not only healthy controls but also MDD females without a history of CSA. We hypothesize that, relative to healthy control and CSA/RES groups, the CSA/MDD participants will be characterized by (1) reduced reward responsiveness and ventromedial prefrontal cortex activation but cortisol hypersecretion when exposed to an acute psychosocial stress manipulation, (2) reduced dopaminergic transmission, and (3) reduced dopamine transporter binding. These hypotheses will be tested using a novel integration of behavioral, endocrinological, and functional/molecular imaging approaches. Improving our understanding of neurobiological mechanisms associated with different CSA outcomes (specifically MDD and resilience) is of paramount importance in order to (1) identify individuals at risk for psychopathology and maladaptive behavior, (2) prevent revictimization, and (3) develop more targeted therapeutic interventions.

II. SPECIFIC AIMS

a. Objectives and hypotheses

The findings outlined above demonstrate that MDD subjects display blunted reinforcement learning and reduced striatal activation (assessed by fMRI) in response to rewards. Based on the US Department of Health and Human Services (2011), over 63,000 children experienced childhood sexual abuse (CSA) in the US in 2010 alone. Early adversity accounts for 25-32% of adult psychiatric illnesses (Green et al 2010), with over 60% of adults meeting criteria for major depressive disorder (MDD) following CSA (Teicher et al 2009). An even larger risk of depression has been found in those who experienced CSA between the ages of 5-14 years. Preclinical research has shown that early adversities lead to a downregulation of mesolimbic dopaminergic (DA) pathways – including reduced DA transporter (DAT) levels – and a sensitization of the mesocorticolimbic system in response to later acute stressors. These data suggest that increased risk for psychopathology following CSA might be mediated by dysfunctions in mesocorticolimbic DA pathways leading to anhedonia and stress sensitization. Conversely, a well-functioning reward system and increased prefrontal cortex activity might be markers of resilience following adversity. The goals of the proposed research are to (1) test precise hypotheses about the role of DA mechanisms in the sequelae of CSA, and (2) identify biomarkers of resilience. Moreover, to gather a better understanding of the cellular mechanisms linked to these important outcomes, we will use in a small sub-set of the participants (n=20) directed conversion of participants' skin fibroblasts into functional DA neurons to investigate (1) DAT functions in human-induced neuronal cells, and (2) relationships between DAT function and fMRI, PET, and behavioral markers of anhedonia.

Specific Aim 1: To investigate that increased DA transmission normalizes reward responsiveness and optimism in females with histories of CSA. We will use reward paradigms from our pilot work in conjunction with a pharmacological challenge to test that decreased phasic DA transmission within mesolimbic regions plays a key role in CSA sequelae. We hypothesize that increased phasic DA release – achieved through presynaptic DA autoreceptor blocking by means of single low dose of the D_2/D_3 antagonist amisulpride – will improve positive reinforcement learning and boost activation in reward-related brain regions, particularly the ventral and dorsal striatum (**Hypothesis 1a**). Due to hypothesized downregulation of mesolimbic DA pathways, we expect that individuals with CSA and current MDD ("CSA/MDD") will show the largest improvements. Confirming that a well-functioning reward system is a marker of resilience, adults with CSA without lifetime psychopathology ("CSA/RES") will show normative activation and performance under placebo **(Hypothesis 1b)**. We also hypothesize that increased phasic DA release – achieved through amisulpride – will re-orient MDD individuals from a pessimistic outlook to an optimistic outlook as measured by the Optimism Bias Task (**Hypothesis 1c**). In addition, under placebo, MDD participants will show a pessimistic outlook as compared with healthy controls (**Hypothesis 1d**).

Specific Aim 2: To investigate dopamine transporter and its relation to reward processing in females with CSA history. We will utilize positron emission tomography (PET) and a highly selective radioactive ligand (^{11}C-altropane) to assess DAT binding in CSA adults. Animal studies have shown that early adversity leads to DAT downregulation within mesolimbic regions. In humans, we have observed links between altropane binding and depressive symptoms, the most common CSA sequelae). Based on our data, we expect that, relative to control and CSA/RES subjects, CSA/MDD adults will show reduced altropane binding (**Hypothesis 2a**). We further hypothesize that, among the CSA/MDD group, reduced DAT will correlate with blunted reinforcement learning and striatal activation (**Hypothesis 2b**).

Specific Aim 3: To investigate stress sensitization of the mesocortical system following CSA. We will use high-density, source localized electrophysiology (EEG), and cortisol assessments to probe cortical activation and stress reactivity during a reward learning paradigm performed under acute stress. Relative to control and CSA/RES subjects, the CSA/MDD group will show decreased ventromedial prefrontal cortex (vmPFC) activation and increased cortisol reactivity following acute stress accompanied by impaired reward learning **(Hypothesis 3a)**. Relative to healthy controls, the CSA/RES

group will show increased vmPFC activation but similar reward sensitivity and cortisol reactivity **(Hypothesis 3b)**.

Specific Aim 4a: To investigate DAT function from DA neurons derived from directed conversion of skin fibroblasts. We will collect a skin biopsy from a subset of the study sample (20 participants) to analyze human-induced neuronal DA cells. We hypothesize that relative to the healthy group (n = 8), individuals with MDD and a history of CSA (n=8) will show altered/downregulated DA-related phenotype (e.g., DAT downregulation; **Hypothesis 4a). Similarly, we hypothesize that the CSA/RES subjects (n=4) will show similar DA-related phenotype as the healthy group.**

Specific Aim 4b: To Investigate relations between DAT function and fMRI, PET, ERP, endocrinological and behavioral markers of anhedonic behavior and stress responsiveness. Across all groups, downregulated DAT function will be associated with decreased ^{11}C altropane binding in striatal regions (PET), lower reward-related striatal responsiveness (fMRI), potentiated stress-induced cortisol responses, and stress-induced anhedonia (**Hypoyhesis 4b)**.

Specific Aim 4c: To provide more conclusive tests of the potential role of DAT and NOC dysfunction in the pathophysiology of depression. Post-mortem tissue will be obtained from the Douglas Bell Canada Brain Bank in Montreal, QC, Canada through our collaborator Gustavo Turecki, Professor and Chair, Department of Psychiatry, McGill University. Please see Appendix 2 for more details.

III. SUBJECT SELECTION

Inclusion Criteria:

- Females of all ethnic origins, age between 20 and 45; right-handed (Chapman & Chapman 1987);
- Absence of any psychotropic medications for at least 2 weeks (6 weeks for fluoxetine; 6 months for neuroleptics; 2 weeks for benzodiazepines; 2 weeks for any other antidepressants);

Childhood Sexual Abuse/MDD (CSA/MDD):

- At least one incident of contact sexual abuse between the ages 5-14 years;
- Current DSM-IV diagnostic criteria for MDD (as diagnosed with the use of the SCID);

Childhood Sexual Abuse/Resilient (CSA/RES):

- At least one incident of contact sexual abuse between the ages 5-14 years;
- Absence of past or current DSM diagnosis, including MDD or alcohol/substance abuse;

Non-traumatized, MDD (MDD):

- No incidents of childhood sexual, verbal, or physical abuse (ascertained using the Traumatic Antecedents Questionnaire);
- Current DSM-IV diagnostic criteria for MDD (as diagnosed with the use of SCID);

Non-traumatized, healthy controls (controls):

- No incidents of childhood sexual, verbal, or physical abuse (ascertained using the Traumatic Antecedents Questionnaire);
- Absence of any medical, neurological, and psychiatric illness (including alcohol/substance abuse)

Exclusion Criteria:

- Participants with suicidal ideation where study participation is deemed unsafe by the study clinician;
- Women who are not fully fluent (reading, speaking, & writing) in English;
- Pregnant women or women of childbearing potential who are not compliant with the requirements of a urine and blood pregnancy test. Pregnancy is determined by serum pregnancy tests conducted during sessions 1 and 4 and by a urine pregnancy test conducted during session 3.

- Women of childbearing potential not using adequate form of contraception. Adequate contraception includes birth control pills, birth control patch, birth control sponge, birth control vaginal ring, birth control shots, a non-metallic, MRI-compatible intrauterine device (IUD), condom, diaphragm, female condom, spermicide, partner with vasectomy, and abstinence. Those not of childbearing potential include post-menopausal for more than 6 months or surgically sterilized
- Failure to meet MRI or PET safety requirements.
- Serious or unstable medical illness, including cardiovascular, hepatic, renal, respiratory, endocrine (hypothyroidism), neurologic or hematologic disease;
- Past/current DSM diagnosis of: OCD, ADHD, schizophrenia, schizoaffective disorder, delusional disorder, bipolar disorder, mood congruent/incongruent psychotic features, substance dependence, substance abuse within the last 12 months (with the exception of cocaine or stimulant abuse, which will lead to automatic exclusion); Bulimia and Binge Eating Disorder within the last 2 years; Anorexia
- PTSD*, simple phobia, social anxiety disorder and generalized anxiety disorder will be allowed only if secondary to MDD and only in the CSA/MDD and MDD groups (which will be matched for comorbidities);

* Eligibility has been revised to include participants with PTSD secondary to MDD in either the CSA/MDD or MDD groups. History of seizure disorder;

- Renal insufficiency, defined as eGFR of 60 or less, as determined by blood test conducted at session 1
- Abnormal result on differential, comprehensive metabolic panel (CMP) as determined by blood test conducted at session 1. This includes Urea Nitrogen (BUN) outside of 7-25 mg/dL; Creatinine outside of 0.6-1.35 mg/dL, BUN/Creatinine Ratio outside of 6-22; Sodium outside of 135-146 mmol/L; Potassium outside of 3.5-5.3 mmol/L; Chloride outside of 98-110 mmol/L; Carbon Dioxide outside of 21-33 mmol/L; Calcium outside of 8.6-10.3 mg/dL; Total Protein outside of 6.2-8.3 g/dL, Albumin outside of 3.6-5.1 g/dL, Globulin outside of 2.1-3.7 g/dL, Albumin/Globulin Ratio outside of 1.0-2.1, Total Bilirubin outside of 0.2-1.2 mg/dL, Alkaline Phosphatase outside of 40/115 U/L, AST/SGOT outside of 10-40 U/L, ALT outside of 9-60 U/L, and Total Creatine Kinase outside of 44-196 U/L. If a lab result is outside the reference range, but is determined by a M.D. to be of minimal clinical significance and have no impact on their safety in participating in our research, a subject may not be excluded.
- Abornmal levels of Thyroid-Stimulating Hormone (TSH), defined as outside the rage of 0.4-4.50 mIU/L, as determined by blood test conduted at session 1.
- Abnormal result on complete blood count (CBC) as determined by blood test conducted at session 1. This includes White Blood Cell Count outside of 3.8-10.8 Thousand/uL; Red Blood Cell Count outside of 4.2-5.8 Million/uL; Hemoglobin outside of 13.2-17.1 g/dL; Hematrocrit outside of 38.5-50.0%; Mean Corpuscular Volume (MCV) outside of 80-100 fL; Mean Corpuscular Hemoglobin (MCH) outside of 27-33 pg; Mean Corpuscular Hemoglobin Concentration (MCHC) outside of 32-36 g/dL; Red Blood Cell Distribution Width (RDW) outside of 11-15%; Platelet Count outside of 140-400 Thousand/uL; Mean Platelet Volume (MPV) outside of 7.5-11.5 fL; Absolute Neutrophils outside of 1500-7800 cells/uL; Absolute Lymphocytes outside of 850-3900 cells/uL; Absolute Monocytes outside of 200-950 cells/uL; Absolute Eosinophils outside of 15-500 cells/uL; and Absolute Basophils outside of 0-200 cells/uL. If a lab result is outside the reference range, but is determined by a M.D. to be of minimal clinical significance and have no impact on their safety in participating in our research, a subject may not be excluded.
- History of adverse reactions to amisulpride;
- History of cocaine, stimulant, and other DA drug use [e.g., (meth)amphetamine), methylphenidate].
- Current use of cocaine, amphetamines, methamphetamines, opiates, PCP, barbiturates, benzodiazepines, methadone, oxycodone, MDMA, or tricyclic antidepressants, as

determined by a urine drug test which is conducted at each session. Current marijuana use exceeding two occasions in two weeks prior to each session will be exclusionary.

- Abnormal electrocardiogram (EKG) as determined by M.D. Family history* of sudden early death due to sudden cardiac arrest
- QT abnormalities
- Family history* of QT abnormalities

*Family history is operationalized to include first degree relatives, including parents, siblings, and children.

- Exclusionary for the skin biopsy session: History of keloids, bleeding disorder, lidocaine allergy, or skin adhesive (such as Dermabond) allergy.
- Reynaud's Disease (exclusionary for EEG session due to ice water task)

b. Source of subjects and recruitment methods

The subjects for this research will be 184 females (20-45 years) recruited from the greater Boston area by McLean Hospital's Center for Depression, Anxiety and Stress Research. Only females will be recruited due to expected sex-specific differences in (i) abuse-related (e.g., child-perpetrator gender) and unrelated variables (e.g., brain development; Pechtel & Pizzagalli 2010), and (ii) HPA axis reactivity (e.g., Young & Korszun 2010). No employees of McLean Hospital, or students or employees of Harvard Medical School will be enrolled.

IV. SUBJECT ENROLLMENT

a. Methods of enrollment

Recruitment for participants will be conducted through the Center of Depression, Anxiety, and Stress Research at McLean Hospital (Director: Diego Pizzagalli, Ph.D.). Participants will be recruited through flyer postings, advertisements in Craigslist, postings on student job sites at local universities and colleges , postcards, outreach letters sent to local education and mental health centers, advertisements on the Partners Clinical Trials website (http://www.clinicaltrials.partners.org), and online advertisement campaign via TrialSpark, a Partners-approved recruiting system that helps investigators recruit patients/participants for clinical trials more efficiently using social media, software, and machine learning. Participants will also be recruited using google adwords (brief advertisements that appear in google search results and direct potential participants to the study's recruitment website which features information on eligibility criteria, information on study procedures and reimbursement). Participants responding to ads may either complete an online screening tool on REDCap, a Partners secure website, be screened over the phone by study coordinators, or both, Additionally participants will be recruited in collaboration with McLean's Developmental Biopsychiatry Program (DBP, Director: Martin Teicher, MD, Ph.D.). Individuals who are potentially eligible for the present study who participated in studies conducted by DBP may be contacted and invited to complete phone screening conducted by members of the Center for Depression, Anxiety and Stress Research. To facilitate this collaboration and ensure confidentiality of data, DBP staff members will use secure file transfer to convey participant information to the Center for Depression, Anxiety and Stress Research. Note that personal identifying information and eligibility information will be stored in distinct files linked only by an ID number. All information received from DBP will be destroyed at the time of study completion as it is used solely for purposes of identifying potential participants for initial contact. Prospective candidates will be screened over the phone by study coordinators or via the online screening survey on RedCap (a Partners secure website). They will be informed that only subjects currently on no medications are eligible for the study. Those meeting criteria based on the phone interview or screening survey will be scheduled for a screening visit with one of the study physicians, At the screening visit, all subjects will undergo a number of laboratory tests to ensure safe participation, including complete blood count with differential, comprehensive metabolic panel (CMP), serum

concentrations of electrolytes, BUN, SGOT, SGPT, CPK, alkaline phosphatase, total bilirubin, albumin, total protein, blood pregnancy test, TSH, eGFR, and electrocardiogram (EKG), and a urine drug test. These tests must be performed within four weeks prior to amisulpride administration.

Preliminary screening for sex, handedness, neurological disorders, exclusionary physical conditions, medication status, weight, history of childhood trauma and history of depression, and MRI and PET safety will take place at the time of the initial telephone contact or interview or via the online screening survey on RedCap (a Partners secure website). Subjects will be re-screened for MRI safety at the beginning of the fMRI session.

To avoid the confounding effects of medication, only subjects off medication will be considered**.** Importantly, no subject will be requested to discontinue treatment for the purpose of participating in the studies proposed here. Further, if any subject becomes emergently ill or suicidal, access to emergency clinical care will be provided according to established procedures at McLean Hospital. If at any point between signing of consent and the conclusion of the second session, a subject becomes suicidal, homicidal, manic, or psychotic, (s)he will be withdrawn from the study and appropriate care and resources will be provided. Depending on the outcome of the first session, eligible participants will be assigned to one of four groups: (1) CSA between the ages of 5-14 with current diagnosis of MDD (*CSA/MDD)*, (2) CSA between the ages of 5-14 without current or past diagnosis of Axis I or II disorders (CSA/RES), (3) current diagnosis of MDD without a history of CSA (*MDD*), and (4) healthy controls. Each group will contain 23 subjects who complete at least two imaging sessions (n=23/group).

A subset of subjects (20 participants) will be invited to participate in an additional study component involving a skin biopsy (see details below) after they have completed the ERP session. A subset of participants from the healthy group (n=8), CSA/MDD group (n=8), and the CSA/RES group (n=4) will participate. Invitations to participate will be extended to all subjects in the healthy group, CSA/MDD group, and CSA/RES group until recruitment for this additional component is complete. Participation in the additional session will require signing of a separate consent form prior to participation. In order to minimize phenotypic heterogeneity and maximize our ability to detect group differences, we will prioritize the cell conversion for participants based on their performance in a laboratory-based measure of anhedonia, the Probabilistic Reward Task (PRT), administered during the ERP session. Specifically, we will prioritize the subjects in the CSA/MDD group showing the greatest impairments in reward learning (as assessed by the PRT) when tested under stress and the healthy control subjects showing the greatest resistance against stress-induced reduction in reward learning (as assessed by the PRT). For all other subjects that do not meet this criteria (including all subjects in the CSA/RES group), skin samples will be frozen until additional funding is acquired. If any of these subjects refuse to participate, investigators will continue prioritizing subjects showing the next greatest level of impairment or resistance (as assessed by the PRT) until they find 3 subjects in each group. A normative database collected by Dr. Pizzagalli's laboratory will be used to identify participants meeting pre-specified cutoffs with respect to their performance in the PRT during stress.

b. <u>Procedures for obtaining informed consent</u>

After screening and approval for participation, subjects will read and sign an IRB-approved informed consent detailing the general purposes and procedures of the experiment, and any questions they may `have will be answered. The subjects of this study must be judged capable of understanding the nature of this study as well as the discomforts and potential benefits. This determination will be made by a

clinician or masters-level clinical interviewer (e.g., Nancy Hall Brooks, M.Ed., LMHC) after a comprehensive psychiatric evaluation. The informed consent will clearly state that the subjects may quit participation at any time without penalty. Potential risks and benefits will be explained in full by the project staff, and the subjects will be asked to sign a consent form. The informed consent will be signed by the subjects and a licensed study clinician.

A subset of subjects (*n*=20) will be invited to participate in an additional component involving a skin biopsy immediately after they have completed the ERP session and will sign a separate informed consent form that will detail the risks of a small skin punch biopsy of their buttocks. The consent form will clearly state that participation is entirely voluntary and subjects may stop participation at any time, without a penalty or loss of benefits to which they are otherwise entitled. The procedure will be described in detail including a description of possible risks and benefits as well as steps taken to minimize the pain. Additionally, participants will be screened for ineligibility due to lidocaine allergy, skin adhesive (Dermabond) allergy, or history of or bleeding disorder. Consent will be acquired by Dr. Arthur Siegel, a physician board-certified in Internal Medicine, and the informed consent form will be signed by both Dr. Siegel and the subjects.

Confidentiality of all subjects will be honored. Individual records will be made anonymous immediately after recording using numeric codes only accessible to the experimenters. Only the experimenters will have access to identifiable subject information, which will be stored in locked cabinets.

V. STUDY PROCEDURES

a. Study visits and parameters to be measured

This study will include four sessions (for a subset of participants (n=20; see details below), the ERP session will include a skin biopsy.)

Session 1 (SCID Session)

The first session will take place at the Center for Depression, Anxiety, and Stress Research or McLean Imaging Center at McLean Hospital (Director: Diego Pizzagalli, Ph.D). The session will involve consenting, a clinical evaluation, and a medical assessment. The first session may be absolved in two separate visits if preferred by the participant (e.g. due to scheduling constraints). This will not affect data collection or reimbursement (participants will still be reimbursed at $15/hour).

Consenting

Subjects will read and sign an IRB-approved informed consent detailing the general purposes and procedures of the experiment, and any questions they may have will be answered. The subjects of this study must be judged capable of understanding the nature of this study as well as the discomforts and potential benefits. This determination will be made by a clinician (e.g., Dr. David Olson, MD, Ph.D.) or masters-level clinical interviewer (e.g., Nancy Hall Brooks, M.Ed., LMHC) after a comprehensive psychiatric evaluation. The informed consent will clearly state that the subjects may quit participation at any time without penalty. Potential risks and benefits will be explained in full by the project staff, and the subjects will be asked to sign a consent form. The informed consent will be signed by the subjects and a study doctor.

Clinical Evaluation

The Clinical evaluation involves the Structured Clinical Interview for DSM-IV (First et al 2002; the SCID is a diagnostic tool that assesses the presence of Axis I disorders); the Traumatic Antecedents

Questionnaire (Herman et al 1990; the TAQ evaluates the duration and severity of sexual abuse in childhood and confirms the absence of exposure to other types of childhood trauma) and the Columbia Suicide Severity Rating Scale (C-SSRS; to assess the emergence of suicidality).

Once eligibility is confirmed, participants move on to the medical assessment.

Medical Assessment

All subjects who meet criteria based upon the clinical evaluation will undergo a number of laboratory tests to ensure safe participation, including a blood draw with the following analyses: complete blood count with differential, urinalysis, comprehensive metabolic panel (CMP), serum concentrations of electrolytes, BUN, SGOT, SGPT, CPK, alkaline phosphatase, total bilirubin, albumin, total protein, serum pregnancy test, and TSH. An electrocardiogram (EKG) will be performed as a measure of heart health. Any subjects with signs of possible heart disease will be excluded from the study. A urine drug test will be performed to measure any current drug use. Participants who test positive for a substance or test positive for pregnancy will be excluded from the remainder of the study.
We will also collect a plasma sample (will be used for analysis of inflammatory signaling molecules). An additional 6ml of blood will be drawn for the plasma sample.

The Abnormal Involuntary Movement Scale (Guy, 1976) is a 12-item scale designed to record the occurrence of dyskinetic, involuntary movements. Participants will complete the AIMS task during the screening visit, prior to dismissal from the study and, in the case of a dystonic reaction during the scanning visit, during a final exit interview one to two weeks after the scanning visit.
Patients presenting with acute dystonic reaction, who are not in extreme distress, would be first treated with 50mg to 100mg of diphenhydramine orally. This is a safe and effective treatment, and widely available as an over-the-counter preparation. This is routinely used, (in oral or IV form), for example, for dystonic occulogyric crises in patients taking regular antipsychotic medication.

Patients with an acute dystonic reaction, who are in extreme distress would be directed to go to the nearest emergency room for treatment unless they were at McLean in which case IM cogentin or diphenhydramine would be given - after a brief assessment.

Benztropine (1mg - 4mg orally or 1mg - 2mg IM/IV) (available only by prescription, or in a physician's office or the ER) is also safe and effective for resolving acute dystonic reactions. IM/IV cogentin or diphenhydramine tends to work faster than oral treatment - but both strategies are effective.

The AIMS task will be administered and scored by one of the study staff physicians (e.g., David Olson, M.D., Ph.D.). During the AIMS task, participants are observed in stationary positions as well as during activated movements and any involuntary movements are recorded. This scale provides a quantitative measure of symptoms of dyskinesia and will help identify possible adverse reactions to the study drug, which are deemed unlikely due to the low and single dose administration.

Sessions 2-4 will include procedures for assessment of brain and cognitive functioning, specifically: a functional magnetic resonance imaging (fMRI) session, a positron emission tomography (PET) session, and an event related potential (ERP) session. The order in which these sessions are scheduled will depend on facility availability and convenience for participants.

ERP Session

This session will take place at the Center for Depression, Anxiety, and Stress Research at McLean Hospital (Director: Diego Pizzagalli, Ph.D) and will be scheduled between 1 and 5pm to control for

diurnal cortisol fluctuations. This session will involve an EEG recording while participants perform a probabilistic reward task. This session also involves a stress manipulation, the Maastricht Acute Stress Test (MAST; Smeets et al. 2012), and saliva samples for cortisol analysis.

EEG Recording

The probabilistic reward task used in our prior work (see below) will be administered in conjunction with 128-channel ERP recording; subjects will perform the task twice, once before exposure to an acute stressor and once immediately following the stressor. The 128-channel EEG will be recoded using the Geodesic EGI system in an electrically shielded room.

Probabilistic Reward Task

The probabilistic reward task (PRT) has been successfully used by the PI to assess reward responsiveness (e.g., Pizzagalli et al 2005, 2007, 2009b). In each trial, subjects choose which of two difficult-to-differentiate stimuli was presented. Stimuli consist of simple cartoon faces (diameter: 25 mm; eyes: 7 mm) presented in the center of the monitor. At the beginning of the trial, the face has no mouth. After a given delay, either a straight mouth of 11.5 mm ("short mouth") or 13 mm ("long mouth") is presented for 100 ms. Subjects are instructed to press an appropriate button to decide whether a long or small mouth had been presented. Unbeknownst to subjects, correct identification of one stimulus ("rich stimulus") is rewarded three times more frequently (*"Correct! You won 20 cents"*) than the other ("lean") stimulus. In healthy controls, this reinforcement schedule leads to a response bias (i.e., a preference for the more frequently rewarded stimulus). The degree of response bias toward the more frequently reinforced alternative will be used for operationalizing sensitivity to reward.

To avoid carry-over effects, participants complete the PRT twice in which only the stimuli type will vary. In the second version, instead of determining the length of the mouth, participants will need to decide whether a long or short 'nose' had been presented on the cartoon face by pressing the appropriate button. All other task parameters remain identical.

One version of the PRT is completed prior to the 'stress manipulation' (see below) and the other is completed after the 'stress manipulation'. The order of the two versions (mouth vs. nose) will be counterbalanced across subjects.

Maastricht Acute Stress Test (MAST)

The MAST is a physically and psychologically challenging laboratory stress test. It combines the most stressful feature of the Trier Social Stress Test (TSST; i.e., psychosocial evaluative threat, uncontrollability, and unpredictability; Foley and Kirschbaum, 2010) and the Cold Pressure Test (CPT; i.e., the painful aspect; e.g., Mitchell, 2004). The test starts with a short instruction and preparation phase (5 min). After that, over a 10 min time span, participants will be instructed to perform 5 socially evaluated cold pressor trials that vary in duration (ranging from 60 s to 90 s), with the water temperature held between 0° and 2 °C. In between the cold pressure trials (intertrial intervals also vary in duration), participants will be asked to do a mental arithmetic task as fast and accurate as possible and receive negative feedback on their performance. Specifically, starting at 2043, participants will be asked to count backwards in intervals of 17. If participants make a mistake, they will need to start again at 2043. During the procedure, participants are told that they are being videotaped and thus will need to look into the camera and are monitored by the experimenter. In reality, the camera is not saving data. Following the final immersion, participants will be told that their performance was poor and that they will need to repeat the task at a later stage of the session. Immediately after this announcement, participants will complete the post-stress set of tasks, including the PRT. Following the tasks, participants will be informed that they will not need to complete the water immersion and calculation task again.

Saliva samples for cortisol analysis

Saliva samples will be acquired for cortisol assessment 20 min after arrival (baseline saliva sample), immediately following the stress manipulation (acute saliva sample), following block 1 of the second PRT (post-stressor saliva sample #1), following block 2 of the second PRT (post-stressor saliva sample #2), and 68 minutes following the stress manipulation (recovery saliva sample). Saliva samples will be collected using passive-drooling cryovials (Salimetrics, State College, PA, USA). Subjects will be instructed to not eat, drink, smoke, brush their teeth or engage in physical activity for at least 30 min before the session. To avoid contamination from food particles, water will be handed to rinse oral cavities prior to data collection. In our prior work, intra- and inter-assay coefficients of variance were <10% (Koslov et al 2011). The protocol is in line with large cortisol studies (e.g., Lederbogen et al 2010) and adheres to the policies on tissue storage and transfer of McLean Hospital.

fMRI Session

This session will take place at the McLean Hospital Imaging Center. Participants will complete the Monetary Incentive Delay (MID) task during fMRI (collected using the 3T MR scanner). Because the pharmacokinetics of amisulpride are linear and a first peak plasma concentration is seen 1-1.5 hr after oral administration (Curran & Perry 2001), fMRI scanning will start 1 hr after administration of amisulpride (or placebo). The dose (50 mg) was selected based on prior studies (e.g., Hamon-Vilcot et al 1998; Samuels et al 2006), and the observation that all prior studies in depressive disorders have used 50 mg/day doses. Using a double-blind design, half of the participants in each group will be administered amisulpride (or placebo).

Amisulpride is a substituted benzamide neuroleptic with high affinity for D_2/D_3 DA receptors and low affinity for other receptors, and higher binding to DA receptors in limbic (e.g., NAc) than nigrostriatal regions. Animal studies have shown that at low doses, amisulpride preferentially blocks presynaptic DA autoreceptors, leading to increased DA synthesis and release and prohedonic effects (Coukell et al 1996; Schoemaker et al 1997).

The Monetary Incentive Trial (MID) task used in our published work with maltreated samples will be used with fMRI (Dillon et al, 2009). The Probabilistic Stimulus Selection Task (PSST) we have used in our pilot data in women with CSA will be administered after fMRI scanning (Pechtel & Pizzagalli, 2013). The subject will also complete the Implicit Learning Serial Reaction Time Task (SRTT) before the administration of amisulpride.

Implicit Learning Serial Reaction Time Task (SRTT)

To exclude the possibility of global learning deficits in MDD, an implicit learning serial reaction time task (Nissen & Bullemer, 1987) will be administered before fMRI scanning. The SRTT will be used to probe procedural learning, a form of implicit memory used to acquire, for example, simple stimulus-response associations. The task involves five blocks of 100 stimuli (an asterisk appearing in one of four boxes aligned horizontally). Each box, designated 1–4, corresponds to a button on a keyboard. When a cue appears, at the start of each trial, a participant selects the appropriate response button, which ends the trial. At the end of each trial, there is a short fixed delay before another cue is presented. In blocks 1-4, the visual cues play out a repeating sequence of positions (i.e., each location can be predicted by the preceding sequence of two locations), and subjects show systematic RT reduction in spite of no declarative knowledge of the sequence rule. In block 5, the sequence is fully random, and the visual cue no longer plays out a repeating pattern of positions. Performance on the SRTT is interpreted by measuring the gradual reduction in response time that takes place across the sequential trials. This provides a measure of participants' growing expertise in performing the

sequence and learning the visuomotor association, or mapping, between the position of the visual cute and the required response.

Self-Concept Attention (SCATT) Task. In this task, participants view a series of words, each superimposed on an image of either themself, a gender-matched other individual, or a scrambled image of another person. For attention-interference trials, participants must judge each word based on how self-descriptive is the word or based on the valence of the word, while ignoring the image. For memory trials, participants must manipulate word-image pairs in working memory (e.g. replace old word-image pairs with new word-image pairs). This task will take approximately 30 minutes. If there is not sufficient time for this task at the fMRI study visit, the task may instead be administered at the beginning of the EEG session. This will not affect data collection or change the reimbursement to participants.

Monetary Incentive Delay (MID) Task

During fMRI data collection, participants will complete an MID task featuring balanced incentive delivery and analytic strategies designed to identify activity specific to anticipation or consumption of incentives. The structure of the MID paradigm will be identical to our prior studies (Dillon et al 2008, 2009). Briefly, individual trials begin with presentation of one of three visual cues (1.5 s) signaling potential trial outcomes (reward: +$; loss: -$; no-incentive: 0$). Following a jittered ISI (3- 7.5 s), a red square target stimulus will be presented; participants are instructed to respond to the target with a button press. Following a second ISI (4.4-8.9 s), visual feedback (1.5 s) will be provided. In the reward condition, successful trials are associated with monetary gains ($1.96 to $2.34) whereas unsuccessful trials lead to no change. In the loss condition, successful trials are associated with no change whereas unsuccessful trials are associated with monetary penalties (-$1.81 to -$2.19). No feedback about cumulative earnings will be provided. Intertrial intervals (ITIs) will range from 3 to 12 s. The task will include five blocks of 24 trials (8 reward, 8 loss, 8 no-incentive). Thus, 40 trials/condition will be available for the analyses of anticipatory activation, whereas 20 trials/condition will be used for post-feedback responses. Participants will be told that faster reaction times (RT) will increase the likelihood of obtaining rewards and avoiding losses. RT data in three independent control samples confirmed that the task induces motivated responding: mean RT was fastest on reward trials, intermediate on loss trials, and slowest on no-incentive trials (all ps<.001; (Dillon et al., 2008, 2009).

Probabilistic Stimulus Selection Task (PSST)

The PSST task (Frank et al, 2004) will allow us to examine whether participants exhibit a bias for choosing frequently reinforced stimuli or avoiding frequently punished stimuli, and thus to assess positive and negative reinforcement learning. Of note, this task has been found to be sensitive to DA modulations, suggesting that hypodopaminergic states affect phasic DA signals required for learning from positive outcomes. Thus, MDD subjects are expected to be better at learning from punishment than reward in the PSST. The task has two phases: a learning and testing phase. In the learning phase, subjects are randomly presented with three different stimuli pairs (AB, CD, EF), and are instructed to choose one of the two stimuli by pressing a key. After the response, feedback is given to indicate whether the choice was correct or incorrect. Importantly, the feedback is probabilistic: for AB trials, a choice of stimulus A leads to positive feedback in 80% of the trials, while a choice of stimulus B leads to negative feedback in 80% of the trials. The stimulus pair CD is less reliable, with stimulus C being correct in 70% of the trials, and the stimulus pair EF is the least reliable, with stimulus E being correct in 60% of the trials. Subjects learn to choose stimuli A, C, and E more frequently than B, D, or F, during the learning phase. Of note, learning to choose A over B can be achieved either by learning that

A usually leads to positive feedback or that B usually leads to negative feedback, or both. The learning phase ends after participants reach performance criteria (65% accuracy for A, 60% for C, and 50% for E) or after 6 blocks. The performance criteria are set so that all subjects are at the same level before starting the testing phase. In the testing phase, the same three pairs as well as all novel combinations of stimuli pairs are presented, and no feedback is provided. To examine whether subjects learned more about positive or negative outcomes of their decisions, the stimuli pairs of interest are those involving an A or B stimulus paired with a novel stimulus (e.g., AC, AD, BC, BD), referred to as transfer pairs. These transfer pairs will allow us to assess whether subjects learn from prior positive feedback to choose the most reinforced stimulus ("Choose A") or learn from prior negative feedback to avoid the most punished stimulus ("Avoid B").

MRI data will be acquired on a 3T Siemens Tim Trio system using a 32-channel brain array coil. As in our prior work (Dillon et al 2009), data will be acquired using tilted slice acquisition (30^0 to the AC-PC line). To minimize susceptibility dephasing of spins and loss of magnetization, we will use a short echo time (30 ms), a GRAPPA acceleration factor of 2 to reduce the length of the EPI readout, and thin slices. During functional scans, gradient echo T2*-weighted echoplanar images will be acquired with: TR/TE: 3000/30ms; FOV: 224 mm; matrix: 64x64; 42 slices; in-plane resolution: 3.5 mm (2.5 mm slices, 1.0-mm gap); voxels 3.5 x 3.5 x 3.5 mm). The EPI acquisition will use prospective motion correction to minimize the effects of subject motion.

Self-report questionnaires will also be administered during the course of this session as well as the Columbia Suicide Severity Rating Scale (C- SSRS) to assess the emergence of suicidality. The appropriate procedures in the event of emergence of suicidality are outlined in 'Psychosocial (non-medical) risks' in section VII (Risks and Discomforts). In addition, urine drug and pregnancy tests will also be administered prior to the MRI scan.

In case that a subject is observed or determined to be in an altered state, (s)he will be evaluated by one of the study physicians (e.g., David Olson, M.D., Ph.D.) and will be given appropriate care. Subjects deemed not to be at risk by one of the study physicians will be offered transportation by cab and reimbursed through a voucher. In the rare case that a subject exhibits dystonic symptoms, as assessed by AIMS, the subject will be invited back for a final exit interview one to two weeks after the scan at which time AIMS will be repeated.

Asymptomatic subjects will be dismissed 3 hours after administration of amisulpride, following a final evaluation by one of the study physicians (e.g., David Olson, M.D., Ph.D.).

PET Session

This session will take place at the Massachusetts General Hospital PET facility. After re-screening for compatibility with the PET environment, any remaining questions will be answered. Subjects will then be positioned in the gantry of a PET camera. Head alignment will be made, relative to the canthomeatal line, using projected laser lines. A Civco vac-lok cushion with velcro head strap will be used to prevent movement. A peripheral venous catheter will be inserted for radiopharmaceutical injection. 9 mCi of [^{11}C] altropane will be injected either by a physician or a trained nuclear medicine technicians and PET scanning will begin. The minimum specific activity that we use for C-11 altropane is 300 mCi / micromole at time of administration. PET data will be acquired using an ECAT EXACT HR + PET camera.

Subjects will be instructed that they are being continuously monitored and can be heard by the investigators if there is a problem. They will also be reminded that it is best for them to remain still

and refrain from any head, face or jaw movements. After conclusion of the PET scan, participants will be removed from the scanner.

Compared to other DAT tracers, altropane offers the following advantages (Fischman et al 2001; Madras et al 1998a,b,c): (1) rapid (max. accumulation 15-30 min post-injection) and specific striatal binding; (2) high selectivity for DA sites; (3) reversible binding kinetics; and (4) a relatively low level of nonspecific binding.

Prior to the PET scan, a blood serum pregnancy test and a urine drug will be administered, and participants will complete two tasks on cognitive functioning: the Optimism Bias Task, and the Self-Concept Attention Task.

Optimism Bias Task
This task is a belief updating task. On each trial participants are presented with a description of 40 different adverse life events (e.g. Alzheimer's disease, robbery). They are asked to provide estimates of their likelihood of experiencing these events in their lifetime. After each trial, participants are presented with the average probability of that event occurring to a person from the same sociocultural environment. We then ask participants to estimate their likelihoods for the same 40 events again in a second session to examine how they incorporate the information into their estimates. To control for potential confounds participants complete a memory test for the information presented and rate all stimuli on emotional arousal, negativity and past experience. The task takes approximately 30 minutes to complete.

Skin Fibroblast Component

A subset of subjects (20 participants) will be invited to participate in an additional component involving a skin biopsy immediately after they have completed the ERP session. As aforementioned (see section IV a. "Methods of Enrollment" for details), this subset will not be selected based on a specific criteria, but rather based on willingness to participate. Cell conversion, however, will be prioritized based on performance on the Probabilistic Reward Task (PRT) administered during the ERP session. For this component, participants will be accompanied by a staff to the Internal Medicine unit in the Admission Building (located 2 min walking distance from CDASR). Next Dr. Arthur Siegel, a physician board-certified in Internal Medicine, will acquire informed consent using a separate consent form for this component of the study; see section IV b. "Procedures for obtaining informed consent". Dr. Siegel will then ask the participant some questions to determine eligibility (exclusion criteria include history of keloids or bleeding disorder). If eligible, Dr. Siegel will take a 4mm (0.157 inch) skin sample from the subject's buttocks via a new, sterile disposable punch biopsy for each subject. The area will first be cleaned, and then a very small injection of local anesthesia will be used, 0.6 to 1.0 ml of 1% xylocaine with 1/200,000 epinephrine intracutaneously, so that the pain associated with the procedure will be minimal; the subject should not feel any pain from the procedure other than the needle stick. Finally, 25% aluminum chloride will be applied for hemostasis, if needed, after which a skin adhesive (DERMABOND by ETHICON, REF DNX6, Advanced topical skin adhesive, which comes in a single-dose vial [0.7 ml] and requires no dressing) will be applied. This procedure is identical to that used by the IRB approved study "Sensitive Periods, Brain Development and Depression", P 2010P001100, led by Dr. Martin Teicher. This study session will take approximately 30 minutes.

A series of self-report questionnaires will also be administered during the course of the experiment.

Postmortem Analysis

To provide more conclusive tests of the potential role of DAT and NOC dysfunction in the pathophysiology of depression, we are collaborating with Dr. Berretta and her group, at McLean Hospital. Together we would like to perform analyses on post-mortem tissues from individuals with MDD and demographically matched healthy controls. Post-mortem tissue will be obtained from the Douglas Bell Canada Brain Bank in Montreal, QC, Canada through our collaborator Gustavo Turecki, Professor and Chair, Department of Psychiatry, McGill University. The Douglas Bell Canada Brain Bank collected these samples under their IRB protocol, which covers informed consent process for postmortem tissue donation, communication with donor families, tissue processing and information de-identification and secure storage.

Information provided by the Douglas Bell Canada Brain Bank to group at McLean Hospital will be strictly anonymized, so that no personal identifiable information will be included. Information provided will include demographic and clinical data strictly needed for meaningful interpretations of potential findings, such distributive diagnosis, available clinical assessment, cause of death, post-mortem interval and tissue characteristics, exposure to pharmacological and non-pharmacological treatment, and neuropathology report. This information will be kept at McLean in hard copy form in locked cabinets within locked offices and, electronically, using access-restricted encrypted computers, backed up using Syncplicity (FIPS 140-2 compliant). Tissue samples will be coded and stored in locked freezers within the Mailman Research Center at McLean Hospital. Under no circumstances, they will be shared with investigators outside this collaboration without prior permission from the Douglas Bell Canada Brain Bank. Please see Appendix 2 for more details.

Self-report measures

During the course of the study, participants will complete a number of standardized instruments on paper, on the computer and through the secure, online survey collection system, REDCap Survey. The instruments will include the following:

1) Positive and Negative Affect Schedule – State (PANAS) (Watson et al, 1988)
    - Measures positive and negative affect
2) State-Trait Anxiety Inventory (STAI) (Spielberger et al, 1970)
    - Measures and differentiates between temporary or emotional state anxiety versus long standing personality trait anxiety in adults.
3) Visual Analogue Mood Scales (VAMS) (Aitken, 1969)
    - Measures current affective state
4) Beck Depression Inventory-II (BDI-11) (Beck et al., 1996)
    - Measures the presence and severity of depressive symptoms
5) Mood and Anxiety Symptom Questionnaire (MASQ) (Watson et al., 1995)
    - Measures anxiety and depressive symptoms, including Anhedonic Depression, General Distress (Depression), General Distress (Anxiety), and Anxious Arousal subscales.
6) Snaith Hamilton Pleasure Scale (SHPS) (Snaith et al., 1995)
    - Assesses hedonic tone

7) Apathy Evaluation Scale (Marin et al, 1991)
    - Assesses apathy, defined as a state characterized by simultaneous reduction in the overt behavioral, cognitive, and emotional concomitants of goal-directed behavior.
8) Menstrual Cycle Questionnaire
    - Record information on last menstruation and use of hormonal contraceptives (oral, implant, hormone-secreting IUD).
9) Race & Ethnicity Demographics
    - Background Information Questionnaire
10) General Habit Questionnaires
    - Record information on alcohol, nicotine use
    - Two versions: one assesses typical alcohol and nicotine consumption and one probes alcohol and nicotine consumption in the past 24 hours
11) Caffeine Assessment Questionnaires
    - Record information on caffeine and stimulant/depressant use
    - Two versions: one assesses typical caffeine consumption and one probes caffeine consumption in the past 24 hours
12) Traumatic Antecedents Questionnaire (Herman et al 1990)
    - Evaluates the duration and severity of sexual abuse in childhood and confirms the absence of exposure to other types of childhood trauma
13) Modified Adverse Childhood Experience Scale
    - Measures the severity of life stress in the first 18 years of life
14) Quick Inventory of Depressive Symptomatology – Clinican Rated(Rush et al 2003)
    - Measures depressive symptoms experienced in the past seven days
15) Coping in Stressful Situations (Endler & Parker 1990)
    - Assesses three coping styles (emotion-oriented, task-oriented, avoidant) to deal with difficult and stressful situations)
16) Perceived Stress Scale (Cohen et al 1983)
    - Probes the extent of stress experienced during the past months)
17) Limbic System Function Questionnaire (Teicher et al 1993)
    - Records symptoms related to limbic system dysfunction
18) Post MID Questionnaire
    - Probes participants' thoughts regarding their performance in the MID task
19) Post-PRT Questionnaire
    - Probes participants' thoughts regarding their performance in the PRT task
20) Post-EEG Questionnaire
    - Probes whether participants were uncomfortable, anxious, sleepy, or inattentive during the EEG recording
21) Youth Risk Behavior Survey-Adult Version
    - Probes participants' level of engagement in behaviors related to safety, violence-related behaviors, self-harm behavior and ideation, tobacco use, alcohol use, marijuana use, other drug use, sexual behavior, body weight
22) Trait Ratings Survey
    - Assesses participants' self-concept by asking participants to rate a list of personality trait words on how self-descriptive, positive or negative, or important, those traits are.
23) Ruminative Responses Scale
    - Assesses participant's use of ruminative response patterns when they feel down or sad.
24) Experiences Questionnaire

- Assesses levels of decentering and rumination by asking how frequently participant's engage in various thoughts and experiences.

25) Hamilton Rating Scale for Depression
   - 17-item scale assessing depressive symptoms (Hamilton, 1960)

26) ADHD module from the Mini International Neuropsychiatric Interview
   - Short assessment of lifetime and current ADHD symptoms.

27) Childhood Trauma Questionnaire (CTQ; Bernstein et al., 2003)

28) Threat-Challenge Questionnaire (Mendes, Gray et al. 2007)

Debriefing

At the conclusion of each session, subjects will be fully debriefed by a member of the study staff. (S)he will outline the purpose of the study and explain the purposes of each procedure (e.g. the scans, tasks, and the stressor). The experimenter will also explain how results of the project might inform our understanding of the interactions between dopamine, mood, reward responsiveness, and early life stress.

Material Inducements

Subjects will be paid $15/hour for the SCID session (session 1), up to $70 for the ERP session including $38 for completing the EEG and up to $32 on the probabilistic reward task, up to $150 for the fMRI session including $100 for completing the session and up to $50 on the MID task, and $200 for the PET session. In addition, participants who complete all four sessions of the study will earn an additional $75. Lastly, if participants complete only part of a study session, they will be compensated in a prorated manner, such that if they complete half of a study session, they will be compensated half of the total amount (e.g., participant completes half of the PET scan session, he/she will be compensated $100). If the participant completes all of the sessions, they can earn up to $555. The subset of subjects who participate in the additional skin fibroblast study (n=20) will be compensated $100.

Randomization

For protocol 2010-P-002593, all subjects in study are administered a single low dose of amisulpride (50 mg) in order to test the role of dopaminergic mechanisms in the pathophysiology of MDD. This proof-of mechanism study uses a double-blind, placebo-controlled between-subject design (for each group in study, 50% of the subjects in each group will receive placebo, with the remaining 50% receiving a single dose of amisulpride), involves a single MRI session, and does not include a treatment component. Though data collection has not yet been completed, the blind was broken as of May 2016 to conduct preliminary analyses. Accordingly, the medication blind will be broken as of November, 2016 in order to allow further analyses. In order to preserve the scientific integrity of the study, we propose the following precaution:

The Research Assistant responsible for running participants (Ms. Clegg), the study doctors (Dr. Olson, Dr. Vitaliano), and any other member of the Pizzagalli Lab who directly interacts with participants will remain blind to drug group assignment. Accordingly, if we discover during statistical analyses that a few participants yielded unusable data (e.g., due to excessive motion artifacts), we will be able to enroll and test a few final participants in a fully unbiased way.

b. Drugs to be used

Amisulpride

Rationale: Many studies have shown that low doses of amisulpride (50 mg/day) are effective in relieving depressive and negative symptoms across a variety of disorders. Numerous studies in MDD, dysthymia, and double depression have shown that administration of low doses of amisulpride (50

mg/day) has antidepressant effects comparable to other antidepressants (e.g., Amore & Jori, 2001; Boyer et al, 1999; Cassano & Jori, 2002). Antidepressant efficacy has been attributed to increased DA release due to presynaptic D_2/D_3 autoreceptor blocking. Similarly, in an outpatient sample with dysthymia and/or MDD, amisulpride was superior to imipramine in ameliorating anhedonic symptoms (Lecrubier et al, 1997). Adverse events and drop-off rates in amisulpride-treated patients with depressive disorders or schizophrenia were similar or lower than those of other antidepressant treatments (Amore & Jori, 2001; Boyer et al., 1999) or conventional antipsychotics (Leucht et al, 2002), respectively. These findings fit animal data showing that low doses of amisulpride potentiate DA release in the nucleus accumbens and other limbic regions, have strong prohedonic effects, and increase the incentive value of environmental cues. Because increased DA transmission and potentiation of D_2/D_3 receptor function in the nucleus accumbens might represent a final common pathway of antidepressant effects (D'Aquila et al, 2000; Gershon et al, 2007), the strong affinity of amisulpride for D_2/D_3 receptors in limbic regions makes it an ideal drug for testing mechanisms underlying reduced hedonic capacity in MDD. An Investigational New Drug (IND) application to use amisulpride in the proposed research has been approved by the FDA on 01/19/2010 (see attached IND approval letter).

Procedure: Because the pharmacokinetics of amisulpride are linear and similar across gender, and a first peak plasma concentration is seen 1-1.5 hr after oral administration (Ascalone et al, 1996), fMRI scanning will start 1 hr after administration of amisulpride (or placebo). During the one-hour wait between the administration of amisulpride (or placebo), participants may read or view a neutral film. During this time, experimenters will carefully monitor participants to assess the presence/absence of side effects. A designated study physician will be present at the neuroimaging facility throughout the session with a response time of 2-3 minutes in case study participants experience adverse side effects during the fMRI scanning. After one hour, participants will be asked to enter the scanner, and data collection will begin. The dose (50 mg) was selected based on prior studies (Hamon-Vilcot et al., 1998; Samuels et al, 2006), and the observation that all prior studies using amisulpride in depressive disorders have used 50 mg/day doses (see above). Using a double-blind design, half of the participants in each group will be administered amisulpride, whereas the other half will receive placebo.

c. Devices to be used

Not applicable

d. Surgical interventions

Not applicable

e. Data to be collected

Please see below for details on data collected and analyses performed.

VI. BIOSTATISTICAL ANALYSIS

a. Specific data variables being collected

Probabilistic Reward Task (PRT)

The main variable is response bias, which assesses participants' preference for the stimulus paired with more frequent reward, and will be calculated using a published formula (Pizzagalli et al 2005). To test the specificity of findings, control analyses will be run on discriminability, which provides a measure of the ability to discriminate the two stimuli. To test **Hypothesis 3a** and **3b**, G*roup* (CSA/MDD, CSA/RES, MDD, controls) x *Block* (1,2,3) ANOVAs will be run for response bias and discriminability.

Implicit Learning Serial Reaction Time Task (SRTT)
SRTT data will be analyzed using established procedures (median reaction times for blocks presenting random vs. the predetermined sequence will be calculated; (Kathmann et al, 2005). If group differences emerge in procedural learning, SRTT data will be used as covariates/regressors in all analyses.

Optimism Bias Task
For each trial we compare the extent to which participants altered their estimates in response to the information presented (update score = second estimate- first estimate). We then compare these scores for trials in which participants receive good news (trials where the actuarial probability of a negative event is less than participants' estimate of their likelihood) with trials in which they receive bad news trials (where the actuarial probability of a negative event is greater than participants estimate of their likelihood).

Self-Concept Attention Task
Reaction time (RT) and responses to trait words are compared between the accompanying image types (self, other person, or scrambled).

Scalp ERP and Source Analyses
ERP analyses will follow prior work (Pizzagalli et al 2002, 2003). ERPs will be collected during the Probabilistic Reward Task. Briefly, ERPs will be computed covering 1024 ms and time-locked to the onset of the stimulus (Rich, Lean) and feedback using a 100-ms pre-stimulus baseline. Analyses will focus on the feedback-related negativity (FRN). The FRN (measured as the most negative deflection 200-400ms after reward feedback over fronto-central electrodes) is hypothesized to reflect phasic DA reward prediction signals projecting from the basal ganglia to the ACC and used to guide behavior according to reinforcements (Holroyd & Coles 2002). Studies in the PI's lab have shown that reduced reinforcement learning is linked to a larger (i.e., more negative) FRN, likely due to a reliance on external feedback to guide behavior (Bogdan et al 2011; Santesso et al 2008, 2009). Thus, relative to controls, the CSA/MDD – but not CSA/RES group – will show larger FRN and reduced OFC and vmPFC activation to reward feedback. *Group* x *Electrode* (AFz, Fz, FCz, Cz) ANOVAs will be conducted on FRN amplitude and latency. As in prior work (Bogdan et al 2011; Santesso et al 2008), intracortical current density will be computed at the time of the FRN using LORETA (Pascual-Marqui et al 1999). Whole-brain voxelwise tests will test differences between groups or conditions. To control for Type I error, permutation procedures will be used to calculate the false-positive rate under the Null Hypothesis (Nichols & Holmes 2002).

Amisulpride Behavioral Analyses for PSST and MID
A *Group* (CSA/MDD, CSA/RES, MDD, controls) x *Drug* (amisulpride, placebo) x *Trial Type* (PSST: Choose A, Avoid B or MID: Reward, loss, no-incentive) ANOVA will be run. Based on prior findings, we expect a significant *Drug* x *Trial Type* interaction, due to larger positive reinforcement learning but lower negative reinforcement learning in the amisulpride relative to the placebo group. Moreover, although amisulpride is expected to affect reinforcement learning in all groups, we hypothesize that CSA/MDD subjects will show the largest gains, leading to a significant *Group* x *Drug* x *Trial Type* interaction. Planned contrast will show no difference in positive reinforcement learning between controls receiving placebo and CSA/MDD subjects receiving amisulpride. To test Hypothesis 1b, a *Group* x *Drug* x *Trial* ANOVA is expected to yield no group differences between CSA/RES and controls and significant differences to the CSA/MDD in the placebo condition and CSA/MDD subjects receiving amisulpride.

fMRI Analyses

Analyses will be conducted using FreeSurfer (Fischl et al 2002, 2004). Volumetric analysis will be conducted and, if applicable, fMRI data will be adjusted for volumetric group differences. Pre-processing will include motion/slice-time correction, removal of slow linear trends, intensity normalization, and spatial smoothing (6-mm). The hemodynamic response will be modeled as a gamma function (2.5 s delay to rise, 1.25 s dispersion time) and convolved with stimulus onsets. Data will be analyzed using two approaches: an ROI approach to evaluate *a priori* hypotheses and a whole-brain approach to test finding specificity. In the first, regression coefficients ("beta weights") will be extracted from ROIs obtained from FreeSurfer's parcellation and selected based on prior studies (Knutson & Cooper 2005). ROIs will include basal ganglia regions (globus pallidus, NAc, caudate, putamen), dorsal/ rostral anterior cingulate, vmPFC, and OFC. For each subject and ROI, beta weights will be entered into *Group* x *Condition* (amisulpride, placebo) x *Hemisphere* x *Trial* (reward, loss, no-incentive) ANOVAs. We expect that the main effects of *Group* (controls ≈ CSA/RES > MDD > CSA/MDD) and *Condition* (amisulpride > placebo) will be significant. Moreover, although amisulpride will increase basal ganglia responses to reward – but not punishment or no-incentive – cues in all groups, we expect that the CSA/MDD group will show the greatest improvements (significant *Group* x *Condition* x *Cue* interaction). Planned contrast will show no difference between controls and CSA/RES receiving placebo, and CSA/MDD subjects receiving amisulpride will show similar activation as controls under placebo. In the second approach, whole-brain analyses will be run. Each subject's data will first be re-sampled into Talairach space and random effects analyses will be run. Significance levels in the fMRI data will be set to yield a corrected mapwise significance level of $p<.05$.

PET Analyses

Images will be acquired in 3-D mode and reconstructed using a filtered back projection method while modeling the point spread function of the HR+ in the projector to ensure recovery of in-plane spatial resolution of ~4.5 mm without increasing noise levels. All projection data will be corrected for nonuniformity of detector response, dead time, random coincidences, and scattered radiation. After realignment, motion-corrected PET frames will be summed, registered to the respective MRI (Woods et al 1993), and transformed into stereotaxic space (Talairach & Tournoux 1988) based on an automated feature-matching algorithm (Collins et al 1994). ROIs will be manually drawn on high-resolution MRI images for dorsal (caudate, putamen) and ventral (NAc) striatal regions. The cerebellum will be used as reference tissue. A simplified reference tissue method (Gunn et al 2001; Lammertsma & Hume 1996) will be used to calculate binding potential. To increase statistical power, a Bayesian method recently developed by the MGH team will be used (Alpert et al 2009; Fang et al 2010). Briefly, this method utilizes prior normative data in a cohort of 51 healthy subjects who underwent 60-min ^{11}C-altropane scan. Bayesian estimates yielded a substantial variance reduction in SRTM parameters, making it possible to reduce the sample size needed to achieve a given power level by $\sqrt{2}$. ANOVAs contrasting age-adjusted altropane binding (covariates: smoking & phase of menstrual cycle) across the groups will be performed. Correlational analyses between binding and (1) response bias in the probabilistic reward task, and (2) beta weights in striatal regions during the reward task, will be run.

Skin Fibroblast Analyses

Procedures to Obtain Skin Fibroblasts: After the skin biopsy has been collected, it will be frozen at -80^{o}C in the laboratory of Dr. Kwang-Soo Kim, Ph.D. The biopsy will be transferred to a petri dish and washed in balanced salt solution with antibiotics. Large chunks of tissue will be diced smaller with a scalpel. The epidermis will be separated from the dermal layer using dispase incubation. The growth surface of tissue culture flasks will be coated with fetal bovine serum (FBS) to lay down a matrix of fibronectin to allow for more rapid fibroblast growth. Dispase will be washed with fresh 1x phosphate buffered saline and the tissue minced to smaller chunks < 4mm using a sterile blade. The minced tissue will be placed evenly around the growth surface of flasks with minimal Dulbecco's Modified Eagle

Medium (DMEM) containing 20% FBS, so the tissue can adhere easily. The medium will be changed every 4-5 days, increasing the volume gradually without disturbing the adhered tissue. Large patches of fibroblasts will appear to grow from the chunks in 2 weeks. Dermal fibroblasts will be passaged by trypsinization, redistributed evenly expanded for 2-3 weeks, and cryopreserved for further use.

Direct Conversion of Skin Fibroblasts into DA Neurons: To efficiently convert human fibroblasts into mDA neurons, Dr. Kim will combine lentiviral vectors expressing (i) neurogenesis-promoting TFs (e.g., BAM), (ii) mDA-promoting TFs (e.g., Nurr1, Pitx3, FoxA2) and/or (iii) miRNAs (e.g., miR-9* and miR-124). Biopsied adult fibroblasts will be maintained in DMEM fibroblast media with 10% FBS as previously described[41]. Lentiviral vectors expressing the above factors or miRNAs will be prepared in Lenti-X 293T cells and used for transducing fibroblasts overnight. The transduced fibroblasts will be maintained in fibroblast media for 2-3 days, followed by a change to Neuronal Media (ScienceCell) supplemented with bFGF and BDNF and other survival-promoting factors. Media will be changed every 3-4 days until differentiated cells are used for functional and phenotype analyses. The optimal conditions will be determined by testing combinations of the above factors, as well as small molecules that may promote mDA differentiation and/or inhibit alternative differentiation.

Comparison of Molecular and Cellular Properties of Converted DA Neurons from Fibroblasts of MDD/CSA (n=8), CSA/RES (n=4), and Psychiatrically Healthy Subjects (n=8): Once we optimize conversion of fibroblasts to mDA neurons, we will use these biopsied fibroblasts to convert them to mDA neurons using our optimized protocol. To evaluate whether converted DA neurons from each group have distinct properties, we will test their ability to synthesize and release DA in response to membrane depolarization. We will treat *in vitro* differentiated cells with 50 mM KCl for 30 minutes and assess DA release by HPLC. Media will be collected after 30 min then concentrated perchloric acid (PCA) will be added to a final concentration of 0.1 M PCA/0.1mM EDTA. The DA content of the supernatants will be measured by reverse-phase HPLC. We will also compare mRNA expression patterns for dopamine-related genes such as synthesizing (TH and AADC), uptake (DAT), degrading (MAO and COMT), and storage function (VMAT2) at mRNA and protein levels in converted mDA neurons of each group.

Plasma Samples

Concentrations of pro- an anti-inflammatory cytokines and related receptors will be assessed using enzyme-linked immunosorbent assays (ELISA, R&D systems, Minneapolis, MN). All assays will be performed at the CLIA-certified Harvard Catalyst Central Laboratory (HCCL) in accordance with manufacturer specifications, and will be run in duplicate.

b. Study endpoints

Not applicable

c. Statistical methods

Please see above.

d. Power analysis

Sample size and power calculations

Sample size was estimated after considering effect sizes obtained in preliminary studies. Effect sizes were calculated using $p = 0.05$ for detecting differences between (1) controls vs. CSA/MDD, and (2) CSA/MDD receiving a DA challenge vs. placebo. Based on these estimates and allowing for 5% data loss, the sample size is 92 (23 subjects/group). In our study using the PSST task proposed, relative to controls, women with histories of CSA and MDD showed lower accuracy in trials in which they needed to rely on reward (Cohen's $d = -0.85$). In a study using the probabilistic reward task, we found that unmedicated MDD subjects had significantly reduced response bias compared to controls ($d = -0.70$; Pizzagalli et al 2009b). In another study using the same task, we found that healthy subjects receiving

a single dose of pramipexole – hypothesized to reduce phasic DA transmission due to inhibition of presynaptic autoreceptors – had significantly lower reward learning than those receiving placebo (d = -1.45; Pizzagalli et al 2008b). In an fMRI study using the MID task, relative to controls, a maltreated sample showed weaker activation to reward-predicting cues in the left putamen (d = -0.54) and left pallidus (d = -1.02), yielding a mean effect size of -0.78. In a separate study using the MID task, unmedicated MDD subjects showed weaker activation than controls in response to gains in the caudate (d = -0.98), left NAc (d = -0.73) and left putamen (d = -0.78) during reward anticipation (Pizzagalli et al 2009a), yielding a mean effect size of -0.87. In a pharmacological fMRI study in unmedicated MDD subjects we found that a single 150 mg dose of Bupropion – assumed to partially block DAT – increased reward-related responses in components of the brain's reward circuit, including the midbrain (d = -1.88), subcallosal gyrus (d = -1.64), and SLEA (d = -1.89), leading to an mean effect size of -1.80 (Pizzagalli et al 2008a). In sum, based on the effect sizes we have observed for the behavioral (mean d = -1.00) and fMRI (mean d = -1.15) components, a total of 92 subjects leads to a power >0.95 of detecting group differences.

For the PET study, secondary analyses of altropane data collected in ADHD and control samples (Spencer et al 2007) indicated that a history of depression and/or current subthreshold depressive symptoms were linked to decreased DAT binding (d = -0.58). Because this sample was carefully screened to exclude current, clinically significant depressive symptoms and because analyses did not involve the recently developed Bayesian method, we expect that effect size between control and CSA/MDD groups will be larger (expected d > 0.80). Thus, 92 subjects leads to a power >0.85 of detecting group differences.

VII. RISKS AND DISCOMFORTS

a. Complications of surgical and non-surgical procedures

Maastricht Acute Stress Test (MAST)

The risks associated with the stress manipulation are minimal. This manipulation has been implemented in numerous labs due to its efficiency and safety. First of all, it is a non-intrusive manipulation that causes only very minor pain, if any. Secondly, at all times participants are allowed to remove their hand from the cold water and thus immediately stop the manipulation.

EEG

We foresee only minimal risks from the EEG recording, a commonly and widespread procedure used to non-invasively measure electrical brain activity. The EEG will be recorded with the Geodesics Sensors Net, which requires no scalp abrasion. On rare occasions, individuals with very sensitive skin may experience a slight irritation at the site of sensor application due to the use of mildly salinated water.

fMRI

Implants/Prostheses: The magnetic field of the scanner exerts a force on ferromagnetic objects within the field. This force can cause a ferromagnetic implant, such as some brands of aneurysm clips, surgical clips, or prostheses, to move or be displaced and cause injury or death. If the implant is both large and ferromagnetic, sufficient currents can be induced in the metal by the magnetic field to cause heating of the implant. *Individuals with any such devices will be excluded from enrolling in the study.*

Pregnancy: The safety of the 3.0T MRI scanner for imaging embryos/fetuses has not been clearly established. *Therefore, women who are pregnant or who are of unknown pregnancy status will be excluded.*

Collision Hazard: The magnetic field near the scanner is strong enough to attract ferromagnetic objects with great force. Near the magnet this force can be strong enough to pull objects in and cause them to fly down the axis of the magnet. Such objects become projectiles that can cause injury or death. A security zone has been established to prevent ferromagnetic objects from coming into proximity of the magnet. The likelihood of a collision in the context of the present experiment is thought to be low, much lower than the likelihood in clinical practice.

RF and Magnetic Field Interference: Implants electronic devices, such as cardiac pacemakers and cochlear implants, may be susceptible to interference from the magnetic and RF fields produced by the scanner. This interference may destroy or adversely affect operation of these devices. Since interference to cardiac pacemakers is observed in magnetic fields as low as 13 gauss, means have been provided to prevent persons with cardiac pacemakers or other implanted electronic devices from entering a zone where the magnetic field exceeds 5 gauss. *Individuals with such devices will be excluded from the study.*

Biomagnetic Hazards: It is possible that subtle genetic or molecular changes could be caused by the magnetic fields produced by the MR system. To date, however, no harmful biological effects have been demonstrated at the magnetic field strengths and exposure times utilized by the scanner. At the present time, the likelihood of any significant adverse biomagnetic effect is considered to be very low.

Neurostimulation: Some subjects undergoing functional MRI have experienced minor neurostimulation effects, such as muscle twitches and "tingling" sensations, due to the rapidly oscillating magnetic field gradients used in these examinations. There are no known risks associated with these effects. Specifically, the potential for cardiac stimulation has been examined and judged not to be a problem. The devices used in our research create field gradients that are within the limits specified by the FDA. Likewise, the head resonator for the McLean 3.0T scanner operates within FDA guidelines.

Clinical Hazards: The confining conditions of the MR system can precipitate claustrophobia in a subject. *Subjects will be screened for possible claustrophobia before they are enrolled in the study.*

Access to MR Area: Access to all areas exceeding the 5 gauss level will be controlled by warning signs, barriers, staffed entry locations, and adequate interrogation to assure avoidance of incidents. Access to the magnet room by any personnel will be closely controlled for safety of persons, in particular to prevent accidental introduction of ferromagnetic objects that could be attracted by the magnetic field generated by the MR system.

PET

The primary risk associated with the study is exposure to ionizing radiation. However, the exposure will be small, and there is no evidence that it causes major health risks. Subjects will be exposed to approximately 2 milliSieverts (2 mSv). This amount of radiation is equal to about 55% of the annual background radiation everyone is exposed to each year from the earth and the sky. Careful screening for contraindications will be conducted via written questionnaire both at the time of recruitment and just prior to the experiment. No side effects from the radiopharmaceutical [11C] altropane are expected. [11C] Altropane is similar to cocaine, which is known to cause physical dependence and addiction after repeated use at high doses. The dose of [11C] Altropane being administered in this study is below that at which we would expect any effect, including physical dependence and addiction. However, since this drug is investigational, unexpected adverse effects could occur.

An IV injection is made either by a physician or trained nuclear medicine technician. The risks associated with injections include: bruising, local discomfort, or infection at the site of the needle puncture. The placement of an intravenous line will be necessary and local infection swelling, and redness could occur at this site and temporary loss of pulse at the wrist could occur. The area may have a bruise or feel uncomfortable for 2 - 3 days after the tube is removed.

In addition, subjects will be required to lie still on the imaging table with their heads in the scanner, which may be uncomfortable.

The safety of the PET scanner for imaging embryos/fetuses has not been clearly established. Therefore, women who are pregnant or who are of unknown pregnancy status will be excluded. In addition, the safety of the PET scanner for participants with a history of cancer or exposure to radiation has not been established. Participants with a history of cancer will be excluded. In cases where participants have a history of radiation exposure, the MGH Radiation Safety Office will be consulted to determine if participation in the study is safe.

The proposed PET procedures are identical to those previously approved by both the McLean (2010-P-001447) and Partners (2009-P-001360) IRB board ("*Depression and Dopamine Transporter Function: A Positron Emission Tomography Study Using C-11 Altropane*").

Fibroblast Biopsy

Risks to study subjects are modest. Skin punch biopsies are simple medical procedures performed by an experienced physician. As stated in the informed consent form, the biopsy procedure may cause a small scar (generally smaller than the size of the biopsy itself 4mm or 0.157 inch in diameter). The site (buttocks) is chosen to minimize discomfort and so that the minimal scarring that may occur will have no adverse cosmetic effects. The biopsy site will be cleaned. Local anesthesia will be used, 0.6 to 1.0 ml of 1% xylocaine with 1/200,000 epinephrine intracutaneously, so that the pain associated with the procedure will be minimal. There is a slight risk that the biopsy site might become infected. However, new, disposable, sterile punch biopsy kits will be used for each subject, and antibiotic ointment and a band -aid will be applied to minimize this risk. Dr. Siegel will assess eligibility before the procedure to ensure that no participants undergoing the skin biopsy procedure have a previous history of keloids (a type of scar which results in an overgrowth of tissue at the site of a healed skin injury) or bleeding disorders.

No subject will have more than one biopsy. The subject will be told that the biopsy site should be kept clean. The site should not be submerged in water (i.e. no swimming, hot tubs, baths, etc) for a few days. This information is also specified in the informed consent form, a copy of which will be given to the subject.

We believe that these risks are minimized by having an experienced physician perform these procedures.

b. Drug side effects and toxicities

Amisulpride

The risks posed to the subjects due to a single 50 mg dose of amisulpride, are minimal. At the low dose proposed for the current study (50 mg), amisulpride caused an incidence of extrapyramidal symptoms similar to placebo and lower than that of haloperidol. At low doses, neuroendocrinological adverse events, gastrointestinal intolerance, anxiety, and excitation were reported rarely. Moreover, in healthy

controls, single administrations of 50-mg doses of amisulpride had no observable effects on mood or a variety of cognitive functions, including memory, attention, vigilance, sensory-motor coordination, and information processing. No potentiation of the effects of alcohol was seen after co-administration of 50-mg amisulpride. However, because amisulpride undergoes substantial renal elimination, subjects with moderate or severe renal insufficiency will be excluded. In sum, the likelihood of discomforts arising from a single, low dose administration of amisulpride is deemed to be very low; based on available studies, we expect that if discomfort were to occur, it would be transient and benign, and resolve quickly.

The proposed pharmacological challenge and fMRI procedures are identical to those previously approved by the McLean IRB board (2010-P-001568) ("*The Effects of Dopamine on Reward Processing*")

c. Device complications/malfunctions

Not applicable.

d. Psychosocial (non-medical) risks

Actively Suicidal Participants

SCID Session: If a diagnosis of current severe major depressive disorder is made when the SCID is administered at the first session, the interviewer will make a further assessment of current suicidal intent and planning. If the interviewer, in consultation with the PI and McLean Study Physicians, determines that that subject is at imminent risk, our protocol will be to call hospital security to arrange a walk-over to the McLean Clinical Evaluation Center or Massachusetts General Hospital Acute Psychiatric Services (depending upon subject location at the time). If risk is judged to be serious but not imminent, the project protocol calls for the interviewer, in consultation with the PI and the study physicians, to provide crisis phone numbers as well as relevant referrals for therapy, and to verify that the subject understands how best to access these resources.

EEG, fMRI, PET, and fibroblast biopsy sessions:

If an individual appears to be at imminent risk at the EEG, fMRI, PET or biopsy sessions, we will consult a physician, who will be available for these sessions. If the physician determines that the subject is at imminent risk, our protocol will be to call the McLean Clinical Evaluation Center or the Boston Emergency Services Team.

SCID and Questionnaires

Possible discomfort may occur while answering personal questions during the psychiatric (SCID) interview, and while completing the questionnaires.

MID, PSST, PRTT, PRT, and Optimism Bias Tasks

Minimal discomfort may occur due to the time and effort related to completing these tasks.

Maastricht Acute Stress Test (MAST)

This paradigm is designed to induce physiological and psychological stress and thus most participants will find this task discomforting. However, participants are told that they can take their hand out of the ice water at any time if it is too uncomfortable. All subjects will be de-briefed at the end of the experiment regarding the stress manipulation.

e. Radiation risks

Exposure from the PET scan is considered small, and there is no evidence that it causes major health risks. Subjects will be exposed to approximately 2 milliSieverts (2 mSv). This amount of radiation is equal to about 55% of the annual background radiation everyone is exposed to each year from the earth and the sky. Subjects who have a history of cancer or a history of exposure to mutagens or radiation will be excluded from the study.

VIII. POTENTIAL BENEFITS

a. Potential benefits to participants

There will be no direct benefits to the subjects themselves for participating in this research.

b. Potential benefits to society

From a scientific perspective, the study may benefit people with depression and early life stress by furthering our understanding of the role of dopamine in the risk for and manifestation of depression and also the effects of early life stress on the dopaminergic system.

IX. MONITORING AND QUALITY ASSURANCE

a. Independent monitoring of source data

Not applicable.

b. Safety monitoring (e.g., Data Safety Monitoring Board, etc.)

As there is no clinical intervention, an external Data and Safety Monitoring Board will not be required. However, formal procedures for managing potential safety concerns will be implemented prior to study initiation.

Subjects are required to have a structural MRI scan of clinical quality at least once per year at McLean Hospital that is reviewed by a board certified radiologist or neurologist and a clinical report is generated usually within 2-4 days. If the subject has not received a structural clinical scan in the past year, one will be obtained for the present study during session 3 (fMRI session). Subjects will not receive more than one structural clinical MRI within a given 12-month period unless a follow-up scan is recommended by the radiologist who reads the scan.

Abnormal reports are reviewed by the Clinical Director of the McLean Imaging Center (MIC), Dr. David Olson and forwarded to the Principal Investigator, in this case, Dr. Pizzagalli, and the designated study physician. If an abnormality is present, the study physician and PI will coordinate the process of contacting the subject so that an appointment can be made to discuss the findings. The MIC Clinical Director may also assist with this process when requested and appropriate.

c. Outcomes monitoring

The principal investigator and co-investigators will hold regular meetings to review data integrity and safety concerns. Confidentiality will be maintained throughout the project period. Names will not be kept on data records, and a standardized code will be used for each subject's data. Subjects will be identified only by a code number. Any records with subject-specific information will be kept in a locked file.

For the skin biopsy samples, confidentiality will be protected by labeling information and samples with a study subject number, not the subject's name. The coded skin sample will be processed in Dr. Kim's McLean Hospital laboratory on grounds at McLean Hospital. Dr. Pizzagalli will maintain any information that identifies you in a locked file, accessible only to clinical research staff directly involved in the study. As stated in the informed consent form for the addendum, individual genetic results will not be given to anyone (including the subject and the subject's family, doctor, insurance

company and employer) unless required by law. The consent form will emphasize that results will not be shared with the subject and that even given these protective measures we cannot guarantee that the subject's individual genetic results could never be linked to them. Furthermore, it is stated in the informed consent that subjects have the right to have their skin sample destroyed if they request so before testing has been done and the sample has been anonymized.

d. Adverse event reporting guidelines

In the unlikely event of serious adverse events, these events will be documented and reported immediately to the McLean IRB, as well as to the safety board and to the FDA (as appropriate). An event that is serious must be recorded on the case record and requires expeditious handling to comply with regulatory requirements. In the event that a subject becomes ill or injured as a direct result of participation in the research study, necessary medical care will be made available. The NIMH Project Officer will be informed of any actions taken by the IRBs as a result of such adverse events.

All adverse effects will be immediately reported upon their discovery. Potentially serious adverse events (SAEs) should be followed to resolution or stabilization and reported as SAEs if they become serious.

In the case that a subject is observed or determined to be in an altered state at any point during the study, (s)he will be evaluated by one of the study physicians (e.g., David Olson, M.D., Ph.D.) and will be given appropriate care.

In addition, in order to assess any dystonic symptoms caused by amisulpride administration, a study physician will now utilize the Abnormal Involuntary Movement Scale (AIMS) to rate subject involuntary movements during the screening visit and prior to dismissal on the scanning visit.

Asymptomatic subjects will be dismissed 3 hours after administration of amisulpride, following a final evaluation by a study physician. If a participant does report an adverse event or symptom following the administration of the Amisulpride, they will remain with the researcher until they are feeling well, and will be assessed by the study physician before dismissal. Participants who report an adverse response to the Amisulpride dose will be contacted 24 hours following dismissal from the study, and will also be contacted for an exit interview one to two weeks after the drug administration session where the Abnormal Involuntary Movement Scale (AIMS) will be repeated.

All subjects will be provided with taxi transportation to and from McLean Hospital for the fMRI session. Participants will be given the contact information of the study staff contact and the principal investigator, as well as the McLean Clinical Evaluation Center and/or the Boston Emergency Services Team and relevant referrals for therapy as needed.

For a detailed description of the published literature supporting the Amisulpride specific precautions summarized above, please refer to "Appendix 1"

X. REFERENCES

Aitken, R. C. (1969). Measurement of feelings using visual analogue scales. *Proc R Soc Med, 62*(10), 989-993.

al'Absi, M., K. L. Petersen and L. E. Wittmers (2002). "Adrenocortical and hemodynamic predictors of pain perception in men and women." Pain **96**(1-2): 197-204.

Amat, J., M. V. Baratta, et al. (2005). "Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus." Nat Neurosci **8**(3): 365-371.

Amat, J., E. Paul, et al. (2008). "Activation of the ventral medial prefrontal cortex during an uncontrollable stressor reproduces both the immediate and long-term protective effects of behavioral control." Neuroscience **154**(4): 1178-1186.

Amore, M., & Jori, M. C. (2001). Faster response on amisulpride 50 mg versus sertraline 50-100 mg in patients with dysthymia or double depression: a randomized, double-blind, parallel group study. *Int Clin Psychopharmacol, 16*(6), 317-324.

Ascalone, V., Ripamonti, M., & Malavasi, B. (1996). Stereospecific determination of amisulpride, a new benzamide derivative, in human plasma and urine by automated solid-phase extraction and liquid chromatography on a chiral column. application to pharmacokinetics. *J Chromatogr B Biomed Appl, 676*(1), 95-105.

Beck, A. T., Steer, R. A., Ball, R., & Ranieri, W. (1996). Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J Pers Assess, 67*(3), 588-597.

Berenbaum, H., & Oltmanns, T. F. (1992). Emotional experience and expression in schizophrenia and depression. *J. Abnorm. Psychol., 101*(1), 37-44.

Bernstein DP, Stein JA, Newcomb MD,Walker E, Pogge D, Ahluvalia T, et al. (2003). Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child Abuse & Neglect 27(2), 169-190.

Bogdan, R., & Pizzagalli, D. A. (2006). Acute stress reduces reward responsiveness: implications for depression. *Biol. Psychiatry, 60*(10), 1147-1154.

Boyer, P., Lecrubier, Y., Stalla-Bourdillon, A., & Fleurot, O. (1999). Amisulpride versus amineptine and placebo for the treatment of dysthymia. *Neuropsychobiology, 39*(1), 25-32.

Brown, G.W. and Harris, T, (1978). "Social origins of depression: A study of psychiatric disorder in women." London: Tavistock.

Buchanan, T. W., D. Tranel and R. Adolphs (2006). "Impaired memory retrieval correlates with individual differences in cortisol response but not autonomic response." Learn Mem **13**(3): 382-387.

Cabib, S. and S. Puglisi-Allegra (1996). "Stress, depression and the mesolimbic dopamine system." Psychopharmacology (Berl) **128**(4): 331-342.

Capuron, L., S. Su, et al. (2008). "Depressive symptoms and metabolic syndrome: is inflammation the underlying link?" Biol Psychiatry **64**(10): 896-900.

Cassano, G. B., & Jori, M. C. (2002). Efficacy and safety of amisulpride 50 mg versus paroxetine 20 mg in major depression: a randomized, double-blind, parallel group study. *Int Clin Psychopharmacol, 17*(1), 27-32.

Chapman, L. J., & Chapman, J. P. (1987). The measurement of handedness. *Brain Cogn, 6*(2), 175-183.

Chapman L.J., Chapman J.P., Kwapil T.R., Eckblad M., Zinser M.C. (1994). Putatively psychosis-prone subjects 10 years later. *J. Abnorm. Psychol.* 103(2), 171-183.

Cloninger, C. R. (1994). Temperament and personality. *Curr Opin Neurobiol, 4*(2), 266-273.

Cox, R. W. (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res, 29*(3), 162-173.

D'Aquila, P. S., Collu, M., Gessa, G. L., & Serra, G. (2000). The role of dopamine in the mechanism of action of antidepressant drugs. *Eur J Pharmacol, 405*(1-3), 365-373.

Dillon, D.G., Bogdan, R., Fagerness, J., Holmes, A.J., Perlis, R.H., Pizzagalli, D.A. (in press). Variation in TREK1 gene linked to depression-resistant phenotype is associated with potentiated neural responses to rewards in humans. Human Brain Mapping

Dillon, D. G., Holmes, A. J., Jahn, A. L., Bogdan, R., Wald, L. L., & Pizzagalli, D. A. (2008). Dissociation of neural regions associated with anticipatory versus consummatory phases of incentive processing. *Psychophysiology, 45*(1), 36-49.

Dillon, D.G., Holmes, A.J., Birk, J.L., Brooks, N., Lyons-Ruth, K., Pizzagalli, D.A. (2009). Childhood adversity is associated with left basal ganglia dysfunction during reward anticipation in adulthood. *Biological Psychiatry*, 66, 206-213.

Elliott, R., Rubinsztein, J. S., Sahakian, B. J., & Dolan, R. J. (2002). The neural basis of mood-congruent processing biases in depression. *Arch Gen Psychiatry, 59*(7), 597-604.

Epstein, J., Pan, H., Kocsis, J. H., Yang, Y., Butler, T., Chusid, J., et al. (2006). Lack of ventral striatal response to positive stimuli in depressed versus normal subjects. *Am. J. Psychiatry, 163*(10), 1784-1790.

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1994). *Structured Clinical Interview for axis 1 DSM-IV disorders.* New York: Biometric Research Department, New York State Psychiatric Institute.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res, 12*(3), 189-198.

Forbes, E. E., Christopher May, J., Siegle, G. J., Ladouceur, C. D., Ryan, N. D., Carter, C. S., et al. (2006). Reward-related decision-making in pediatric major depressive disorder: an fMRI study. *J. Child Psychol. Psychiatry, 47*(10), 1031-1040.

Forbes, E. E., Shaw, D. S., & Dahl, R. E. (2007). Alterations in reward-related decision making in boys with recent and future depression. *Biol. Psychiatry, 61*(5), 633-639.

Frank, M. J., Seeberger, L. C., & O'Reilly R, C. (2004). By carrot or by stick: cognitive reinforcement learning in parkinsonism. *Science, 306*(5703), 1940-1943.

Friston, K. J., Tononi, G., Reeke, G. N., Jr., Sporns, O., & Edelman, G. M. (1994). Value-dependent selection in the brain: simulation in a synthetic neural model. *Neuroscience, 59*(2), 229-243.

Gershon, A. A., Vishne, T., & Grunhaus, L. (2007). Dopamine D2-like receptors and the antidepressant response. *Biol Psychiatry, 61*(2), 145-153.

Gluck, M. E., A. Geliebter, J. Hung and E. Yahav (2004). "Cortisol, hunger, and desire to binge eat following a cold stress test in obese women with binge eating disorder." Psychosom Med **66**(6): 876-881.

Greden, J.F. (2001). The burden of disease for treatment-resistant depression. *J Clin Psychiatry* 62 Suppl 16, 26-31.

Guy, W. (1976). ECDEU assessment manual for psychopharmacology, revised ed. Washington, D.C., U.S. Department of Health, Education, and Welfare.

Hammen, C. (2005). "Stress and depression." Annu Rev Clin Psychol **1**: 293-319.

Hamilton, M. (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery & Psychiatry, 23*, 56-62.

Hamon-Vilcot, B., Chaufour, S., Deschamps, C., Canal, M., Zieleniuk, I., Ahtoy, P., et al. (1998). Safety and pharmacokinetics of a single oral dose of amisulpride in healthy elderly volunteers. *Eur J Clin Pharmacol, 54*(5), 405-409.

Harvey, P. O., Pruessner, J., Czechowska, Y., & Lepage, M. (2007). Individual differences in trait anhedonia: a structural and functional magnetic resonance imaging study in non-clinical subjects. *Mol Psychiatry, 12*(8), 703, 767-775.

Hasler, G., Drevets, W.C., Manji, H.K., Charney, D.S. (2004). Discovering endophenotypes for major depression. *Neuropsychopharmacology,* 29(10), 1765-1781.

Holsboer, F. (2000). "The corticosteroid receptor hypothesis of depression." Neuropsychopharmacology **23**(5): 477-501.

Hundt, N. E., Nelson-Gray, R. O., Kimbrela, N. A., Mitchella, J. T., & Kwapila, T. R. (2007). The interaction of reinforcement sensitivity and life events in the prediction of anhedonic depression and mixed anxiety-depression symptoms *Personality and Individual Differences, 43*(5), 1001-1012.

Joormann, J., & Gotlib, I. H. (2006). Is this happiness I see? Biases in the identification of emotional facial expressions in depression and social phobia. *J. Abnorm. Psychol., 115*(4), 705-714.

Kasch, K. L., Rottenberg, J., Arnow, B. A., & Gotlib, I. H. (2002). Behavioral activation and inhibition systems and the severity and course of depression. *J. Abnorm. Psychol., 111*(4), 589-597.

Kathmann, N., Rupertseder, C., Hauke, W., & Zaudig, M. (2005). Implicit sequence learning in obsessive-compulsive disorder: further support for the fronto-striatal dysfunction model. *Biol Psychiatry, 58*(3), 239-244.

Keedwell, P. A., Andrew, C., Williams, S. C., Brammer, M. J., & Phillips, M. L. (2005). The neural correlates of anhedonia in major depressive disorder. *Biol. Psychiatry, 58*(11), 843-853.

Kendler, K. S., R. C. Kessler, et al. (1995). "Stressful life events, genetic liability, and onset of an episode of major depression in women." Am J Psychiatry **152**(6): 833-842.

Lawrence, N. S., Williams, A. M., Surguladze, S., Giampietro, V., Brammer, M. J., Andrew, C., et al. (2004). Subcortical and ventral prefrontal cortical neural responses to facial expressions distinguish patients with bipolar disorder and major depression. *Biol Psychiatry, 55*(6), 578-587.

Lecrubier, Y., Boyer, P., Turjanski, S., & Rein, W. (1997). Amisulpride versus imipramine and placebo in dysthymia and major depression. Amisulpride Study Group. *J Affect Disord, 43*(2), 95-103.

Leucht, S., Pitschel-Walz, G., Engel, R. R., & Kissling, W. (2002). Amisulpride, an unusual "atypical" antipsychotic: a meta-analysis of randomized controlled trials. *Am J Psychiatry, 159*(2), 180-190.

Marin, R. S., Biedrzycki, R. C., & Firinciogullari, S. (1991). Reliability and validity of the Apathy Evaluation Scale. *Psychiatry Res, 38*(2), 143-162.

McFarland, B. R., Shankman, S. A., Tenke, C. E., Bruder, G. E., & Klein, D. N. (2006). Behavioral activation system deficits predict the six-month course of depression. *J. Affect. Disord., 91*(2-3), 229-234.

Meehl, P.E. (1975). Hedonic capacity: some conjectures. *Bull. Menninger Clin.,* 39(4), 295-307.

Mendes, W. B., Gray, H., Mendoza-Denton, R., Major, B. & Epel, E. (2007). Why egalitarianism might be good for your health: Physiological thriving during stressful intergroup encounters. Psychological Science, 18, 991-998.

Modell, S., C. J. Lauer, et al. (1998). "Hormonal response pattern in the combined DEX-CRH test is stable over time in subjects at high familial risk for affective disorders." Neuropsychopharmacology **18**(4): 253-262.

Monroe, S. M. and K. L. Harkness (2005). "Life stress, the "kindling" hypothesis, and the recurrence of depression: considerations from a life stress perspective." Psychol Rev **112**(2): 417-445.

Nelson, R. E., & Craighead, W. E. (1977). Selective recall of positive and negative feedback, self-control behaviors, and depression. *J. Abnorm. Psychol., 86*(4), 379-388.

Nissen, M. J., & Bullemer, P. (1987). Attentional requirements of learning: evidence from performance measures. *Cognitive Psychology, 19*, 1-32.

Patton, J. H., Stanford, M. S., & Barratt, E. S. (1995). Factor structure of the Barratt impulsiveness scale. *J Clin Psychol, 51*(6), 768-774.

Pechtel, P., Pizzagalli, D.A. (2013). Disrupted reinforcement learning and maladaptive behavior in women with a history of childhood sexual abuse: A high-density event-related potential study. JAMA Psychiatry, 70, 499-507.

Pierson, A., Ragot, R., Ripoche, A., & Lesevre, N. (1987). Electrophysiological changes elicited by auditory stimuli given a positive or negative value: a study comparing anhedonic with hedonic subjects. *Int J Psychophysiol, 5*(2), 107-123.

Pinto-Meza, A., Caseras X, Soler, J., Puigdemont, D., Perez, V., Torrubia, R. (2006). Behavioural inhibition and behavioural activation systems in current and recovered major depression participants. *Personality and Individual Differences, 40*, 215-226.

Pizzagalli, D. A., Evins, A. E., Schetter, E. C., Frank, M. J., Pajtas, P. E., Santesso, D. L., et al. (2008). Single dose of a dopamine agonist impairs reinforcement learning in humans: behavioral evidence from a laboratory-based measure of reward responsiveness. *Psychopharmacology (Berl), 196*(2), 221-232.

Pizzagalli, D. A., Goetz, E., Ostacher, M., Iosifescu, D. V., & Perlis, R. H. (2008). Euthymic patients with bipolar disorder show decreased reward learning in a probabilistic reward task. *Biol. Psychiatry, 64*(2), 162-168.

Pizzagalli, D. A., Holmes, A. J., Dillon, D. G., Goetz, E. L., Birk, J. L., Bogdan, R., et al. (2009). Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. *Am J Psychiatry, 166*(6), 702-710.

Pizzagalli, D. A., Iosifescu, D., Hallett, L. A., Ratner, K. G., & Fava, M. (2009). Reduced hedonic capacity in major depressive disorder: evidence from a probabilistic reward task. *J Psychiatr Res, 43*(1), 76-87.

Pizzagalli, D. A., Jahn, A. L., & O'Shea, J. P. (2005). Toward an objective characterization of an anhedonic phenotype: a signal-detection approach. *Biol. Psychiatry, 57*(4), 319-327.

Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning and the
effectiveness of reinforcement and non-reinforcement. . In A. H. Black & W. F. Prokasy (Eds.), *Classical Conditioning. 2. Current Research and Theory.* (pp. 64–69). New York: Appleton-Century-Crofts.

Raison, C. L. and A. H. Miller (2011). "Is depression an inflammatory disorder?" Curr Psychiatry Rep **13**(6): 467-475.

Samuels, E. R., Hou, R. H., Langley, R. W., Szabadi, E., & Bradshaw, C. M. (2006). Comparison of pramipexole and amisulpride on alertness, autonomic and endocrine functions in healthy volunteers. *Psychopharmacology (Berl), 187*(4), 498-510.

Sartorius, N. (2001). The economic and social burden of depression. *J. Clin. Psychiatry,* 62 (Suppl 15), 8-11.

Schultz, W. (2007). "Behavioral dopamine signals." Trends Neurosci **30**(5): 203-210.

Schwabe, L., L. Haddad, et al. (2008). "HPA axis activation by a socially evaluated cold-pressor test." Psychoneuroendocrinology **33**(6): 890-895.

Schwabe, L., L. Haddad and H. Schachinger (2008). "HPA axis activation by a socially evaluated cold-pressor test." Psychoneuroendocrinology **33**(6): 890-895.

Sloan, D. M., Strauss, M. E., & Wisner, K. L. (2001). Diminished response to pleasant stimuli by depressed women. *J. Abnorm. Psychol., 110*(3), 488-493.

Smeets, T., Cornelisse, S., Quaedflieg, C. W. E. M., Meyer, T., Jelicic, M., & Merckelbach, H. (2012). Introducing the Maastricht Acute Stress Test (MAST): a quick and non-invasive approach to elicit robust autonomic and glucocorticoid stress responses. *Psychoneuroendocrinology*, *37*(12), 1998–2008. doi:10.1016/j.psyneuen.2012.04.012

Snaith, R. P., Hamilton, M., Morley, S., Humayan, A., Hargreaves, D., & Trigwell, P. (1995). A scale for the assessment of hedonic tone the Snaith-Hamilton Pleasure Scale. *Br J Psychiatry, 167*(1), 99-103.

Spielberger, C. D., Goruch, R. L., & Lushene, R. E. (1970). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Spijker, J., Bijl, R. V., de Graaf, R., & Nolen, W. A. (2001). Determinants of poor 1-year outcome of DSM-III-R major depression in the general population: results of the Netherlands Mental Health Survey and Incidence Study (NEMESIS). *Acta Psychiatr Scand, 103*(2), 122-130.

Steele, J. D., Kumar, P., & Ebmeier, K. P. (2007). Blunted response to feedback information in depressive illness. *Brain, 130*(Pt 9), 2367-2374.

Steptoe, A., M. Hamer, et al. (2007). "The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis." Brain Behav Immun **21**(7): 901-912.

Tremblay, L. K., Naranjo, C. A., Graham, S. J., Herrmann, N., Mayberg, H. S., Hevenor, S., et al. (2005). Functional neuroanatomical substrates of altered reward processing in major depressive disorder revealed by a dopaminergic probe. *Arch. Gen. Psychiatry, 62*(11), 1228-1236.

Vreeburg, S. A., W. J. Hoogendijk, et al. (2009). "Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study." Arch Gen Psychiatry **66**(6): 617-626.

Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol, 54*(6), 1063-1070.

Watson, D., Weber, K., Assenheimer, J. S., Clark, L. A., Strauss, M. E., & McCormick, R. A. (1995). Testing a tripartite model: I. Evaluating the convergent and discriminant validity of anxiety and depression symptom scales. *J. Abnorm. Psychol., 104*(1), 3-14.

Appendix 1- Amisulpride Research

For the study 2012-P-002119 "Early Life Stress and Depression: Molecular and Functional Imaging Approaches", we are using a 50 mg oral dose of Amisulpride to study the role of dopamine in reward processing. In this study, female subjects with and without a history of childhood sexual abuse and with and without current MDD will receive either 50 mg of Amisulpride or placebo and will participate in an fMRI scan. Asymptomatic subjects will be dismissed from the study session after an assessment by the study physician (using the Abnormal Involuntary Movement Scale (AIMS)) 3 hours following the drug administration In a study by Jocham and colleagues (2011) entitled "Dopamine-Mediated Reinforcement Learning Signals in the Striatum and Ventromedial Prefrontal Cortex Underlie Value-Based Choices", investigators administered a 200 mg dose of Amisulpride to healthy participants. Subjects' heart rate and blood pressure were measured before dismissal and if they felt well, they were dismissed. In consulting with Dr. Jocham, he clarified that subjects were dismissed immediately after the study if they did not feel anything unusual (roughly 4 hours after the 200 mg drug administration). Dr. Jocham reports that none of the participants reported any unusual symptoms, and therefore all were dismissed following the final heart rate and blood pressure assessment. Furthermore, in another recent study by Dr. Jocham, a 400 mg dose of Amisulpride was administered to study participants, and no adverse reactions were observed or reported. Participants in this study were often unable to distinguish between placebo and drug administration, despite the dose being eight times higher than the 50 mg we will be using in our study (Jocham et al., unpublished observation).

In a study by Ersche and colleagues (2010) entitled "Influence of Compulsivity of Drug Abuse on Dopaminergic Modulation of Attentional Bias in Stimulant Dependence", investigators administered a single oral 400 mg dose of Amisulpride to stimulant dependant and healthy controls. Participants were released following the completion of the session, which was approximately six hours after the Amisulpride administration. The participants were also contacted by telephone the following day by researchers to ensure no adverse events occurred following dismissal. They reported that the 400 mg dose was well tolerated, and no serious adverse events were reported in either of the populations.

In a separate study in patients with OCD, the same group (Ersche et al., 2011) recently reported that a 400-mg Amisulpride dose evoked unpleasant side effects in a subgroup of patients with OCD. Specifically, the study investigated the effect of the single 400 mg dose of Amisulpride on 22 OCD patients, 20 were being successfully treated with SSRIs, and the remaining 2 were not being treated with medication. The following side effects were reported:

- 8 of the 20 SSRI-treated OCD patients experienced the subjective effects of akathisia (inner restlessness, agitation, feelings of anxiety, irritability, panic attacks or aggression) (36.3% of total OCD patients)
- 2 of the 8 patients who reported akathisia also reported aggravation of their OCD symptoms (9% of total OCD patients)

Critically, a diagnosis of OCD is an exclusionary criterion in our IRB protocol. Furthermore, use of SSRI's within the previous two weeks is also an exclusionary criterion in our IRB protocol. Based on these recent findings by Ersche et al., both past and current OCD diagnosis will lead to exclusion to minimize the likelihood of drug-related side effects. In addition, all of the akathisia reports and increased OCD symptoms reported by Ersche et al. were from participants who were currently being treated by SSRI's. Our exclusion of participants who have taken SSRI's

in the past two weeks will minimize the likelihood of the drug-related side effects reported by Ersche et al (2011) occurring in our study.

After a systematic review of studies investigating Amisulpride use in healthy participants, Rosenzweig and colleagues (2002) concluded that "[Amisulpride] exhibits no significant detrimental effects in psychometric or memory tests up to the dose of 400 mg a day, inducing only mild impairment at high doses, whereas EEG data suggest an alertness enhancing effect at low doses (< 50 mg)" (p. 1). Fifty mg doses have been shown to be without clinically significant detrimental effects in a wide range of psychometric tests intended to explore attention, vigilance, sensory motor coordination and information processing. Single, 100 mg and 200mg doses were also free of deleterious effects on psychomotor performance and body sway. Finally, a single administration of a high dose (400mg) did not cause any alteration in selective and divided attention, motor activity, sensory-motor coordination or vigilance in healthy volunteers. Based on their review, the authors concluded that "In most studies, Amisulpride did not show any sedative effects at dosages up to 400 mg/day in young subjects, or after a single 200 mg dose in elderly subjects" (p. 9).

Hamon-Vilcot and colleagues (1998) administered a single dose of 50 mg Amisulpride to healthy volunteers aged 65-79. Despite the fact that older people are especially sensitive to antipsychotics and many will experience extrapyramidal and anticholinergic effects, "no serious adverse events were recorded" (p. 407). One subject vomited 9 h after dosing. A further subject complained of mild somnolence lasting 12 h, which occurred 4 hrs after dosing. No extrapyramidal symptoms were observed. Neither clinically significant hemodynamic variations nor ECG abnormalities were observed during the study. Overall, these findings indicate that a single dose of 50 mg Amisulpride was not associated with any clinically significant side effects. Importantly, to minimize age-related fluctuations in dopamine levels, our protocol proposes to include only participants between the age of 20 and 45. Accordingly, the likelihood that sedation or nausea will occur will be further diminished.

Samuels and colleagues (2006) administered to 16 healthy males a 50-mg Amisulpride dose, and its functional effects were assessed at peak plasma levels. The following side effects were recorded.

- Sleepiness (3 mild, 1 moderate, 2 severe= 6 total) 37.50%
- Nausea (2 mild) 12.50%
- Vomiting 0 0%
- Headache (5 mild, 1 moderate= 6 total) 37.50%
- Dizziness (5 mild) 31.25%
- Irritated throat (1) 6.25%
- General tiredness (1) 6.25%

References:

Ersche KD, Bullmore ET, Craig KJ, Shabbir SS, Abbott S, Müller U, Ooi C, Suckling J, Barnes A, Sahakian BJ, Merlo-Pich EV, Robbins TW. Influence of compulsivity of drug abuse on dopaminergic modulation of attentional bias in stimulant dependence. Arch Gen Psychiatry. 2010 Jun;67(6):632-44.

Hamon-Vilcot B, Chaufour S, Deschamps C, Canal M, Zieleniuk I, Ahtoy P, Chretien P, Rosenzweig P, Nasr A, Piette F. Safety and pharmacokinetics of a single oral dose of Amisulpride in healthy elderly volunteers. Eur J Clin Pharmacol. 1998 Jul;54(5):405-9.

Jocham G, Klein TA, Ullsperger M. Dopamine-mediated reinforcement learning signals in the striatum and ventromedial prefrontal cortex underlie value-based choices. J Neurosci. 2011 Feb 2;31(5):1606-13.

Rosenzweig P, Canal M, Patat A, Bergougnan L, Zieleniuk I, Bianchetti G. A review of the pharmacokinetics, tolerability and pharmacodynamics of Amisulpride in healthy volunteers. Hum Psychopharmacol. 2002 Jan;17(1):1-13.

Samuels ER, Hou RH, Langley RW, Szabadi E, Bradshaw CM. Comparison of pramipexole and Amisulpride on alertness, autonomic and endocrine functions in healthy volunteers. Psychopharmacology (Berl). 2006 Sep;187(4):498-510.

Appendix 2 – Postmortem Analysis

For protocol 2012-P-002593, interim analyses indicate that, relative to healthy controls, individuals with major depressive disorder (MDD) show reduced dopamine transporter (DAT) binding in both the striatum and ventral tegmental area, as assessed by Positron Emission Tomography (PET). Prior studies assessing DAT density in MDD have yielded inconsistent findings, with some studies describing down-regulation of DAT densities (e.g., Sarchiapone et al. 2006), and others describing opposite results (e.g., Brunswick et al. 2003). In contrast, post-mortem studies have provided more consistent evidence of reduced DAT levels in striatal regions (e.g., caudate, putamen) of depressed subjects (Bowden et al. 1997; Klimek et al. 2002).

In addition, growing evidence highlights the potential role of the Nociceptin (NOC)/ Orphanin FQ Receptor in stress-related, depression-like phenotypes. For example, blockade of NOC receptors has been found to reverse depression-like phenotypes induced by chronic mild stress (Gavioli and Calo, 2013). In addition, NOC antagonists have been found to block the behavioral and neurobiological effects of acute and chronic stressors (Gavioli and Calo, 2013; Witkin et al., 2014). In parallel, human PET (Lohith et al., 2014) and post-mortem autoradiography (Berthele et al., 2003) studies have described the highest distribution of NOC in the prefrontal cortex, anterior cingulate cortex and striatum.

To provide more conclusive tests of the potential role of DAT and NOC dysfunction in the pathophysiology of depression, we are collaborating with Dr. Berretta and her group, at McLean Hospital. Together we would like to perform analyses on post-mortem tissues from individuals with MDD and demographically matched healthy controls. Post-mortem tissue will be obtained from the Douglas Bell Canada Brain Bank in Montreal, QC, Canada through our collaborator Gustavo Turecki, Professor and Chair, Department of Psychiatry, McGill University. The Douglas Bell Canada Brain Bank collected these samples under their IRB protocol, which covers informed consent process for postmortem tissue donation, communication with donor families, tissue processing and information de-identification and secure storage.
Information provided by the Douglas Bell Canada Brain Bank to group at McLean Hospital will be strictly anonymized, so that no personal identifiable information will be included. Information provided will include demographic and clinical data strictly needed for meaningful interpretations of potential findings, such distributive diagnosis, available clinical assessment, cause of death, post-mortem interval and tissue characteristics, exposure to pharmacological and non-pharmacological treatment, and neuropathology report. This information will be kept at McLean in hard copy form in locked cabinets within locked offices and, electronically, using access-restricted encrypted computers, backed up using Syncplicity (FIPS 140-2 compliant). Tissue samples will be coded and stored in locked freezers within the Mailman Research Center at McLean Hospital. Under no circumstances, they will be shared with investigators outside this collaboration without prior permission from the Douglas Bell Canada Brain Bank.