PROBING MESOCORTICOLIMBIC DOPAMINE FUNCTION IN ALCOHOL
DEPENDENCE USING DEXTROAMPHETAMINE:
BEHAVIOURAL AND FMRI STUDIES

by

Xavier Laurent Balducci

A thesis submitted in conformity with the requirements for the degree of

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Graduate Department of Pharmacology and Toxicology, University of Toronto

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Background: A dysfunctional mesocorticolimbic dopamine system has been reported in alcohol dependence and major depressive disorder. Probing mesocorticolimbic dopamine function in severe depression using dextroamphetamine revealed an altered behavioural response and a disrupted mesocorticolimbic circuitry in behavioural and functional magnetic resonance imaging (fMRI) studies. The purpose of this study was to use a similar approach in alcohol dependence. Behavioural Study: to assess dextroamphetamine subjective effects in alcohol-dependent and depressed alcohol-dependent participants. FMRI Study: to assess how the mesocorticolimbic circuitry would respond to a dextroamphetamine challenge in alcohol-dependent participants exposed to alcohol cues. Methods: In both studies, a single oral 30 mg dose of dextroamphetamine was the pharmacological intervention. Behavioural Study: randomized, double-blind, placebo-controlled, between-subject study. Eighteen alcohol-dependent and 22 depressed alcohol-dependent participants were compared using validated self-report drug effect tools (e.g. Addiction Research Center Inventory). FMRI Study: single-blind, between-subject study. FMRI blood oxygen level–dependent (BOLD) activation was measured in 14 alcohol-dependent and 9 healthy control participants during an alcohol-cue exposure task pre- and post-drug. Results: Behavioural Study: DRUG ($F_{1,40}=18.6; p<0.001$)
and GROUP (F_{1,40}=16.6; \ p<0.001) main effects but no GROUP\times DRUG interaction effects (F_{1,40}=0.02; \ p=0.88) were detected, even when only severely depressed alcohol-dependent individuals were included (F_{1,30}=0.04; \ p=0.84). FMRI Study: Alcohol-dependent participants exhibited greater ventral striatal activation compared to controls pre-drug and post-drug effect (F_{1,40}=20.1; \ z=3.8; \ p<0.001; \ k>10; \ (x=10;y=-2;z=-14)). A GROUP\times DRUG interaction effect was detected in the medial orbitofrontal cortex (mOFC) (F_{1,40}=21.5; \ z=4.0; \ p<0.001; \ k>10; \ (x=-12;y=28;z=-20). The alcohol-dependent group exhibited a negligible mOFC response across both pre- and post-drug scanning sessions. In contrast, controls exhibited attenuation of mOFC response post-drug. Conclusion: The lack of significant GROUP\times DRUG interaction effects in the Behavioural Study may suggest different neurobiological mechanisms underlying alcohol dependence and depression mesocorticolimbic dysfunction. Alcohol dependence appeared to mitigate the impact of depression severity on participants’ behavioural responses to dextroamphetamine. The FMRI Study data suggest there may be ventral striatal and mOFC disruption in alcohol-dependent participants. We suggest the mOFC may be involved in the reported loss of prefrontal modulation of dopamine cell activity in alcohol dependence. This supports a key role for the mOFC in mesocorticolimbic dysfunction in alcohol dependence.
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Section 1 INTRODUCTION

Statement of Problem

Alcohol dependence (AD) is a clinically defined psychiatric disorder that is characterized by excessive and uncontrollable alcohol consumption accompanied by significant interference in psychosocial functioning. It is the second most prevalent substance use disorder after tobacco dependence; up to 6% of individuals in Ontario will develop alcohol dependence in their lifetime [1]. Unlike many abused psychoactive substances, alcohol has no known specific receptor system within the central nervous system. However, many neurotransmitter systems are modulated by alcohol. In particular, alcohol increases dopamine levels, like most drugs of abuse, in the nucleus accumbens (Nac); in the case of alcohol by increasing the firing rate of dopamine cell bodies in the ventral tegmental area (VTA) [2-10]. Major depressive disorder (MDD) is the most common psychiatric illness in individuals with alcohol dependence [11]. Indeed, 80% of individuals with alcoholism (abuse and dependence) report depressive symptoms [12] and between 15 to 50% of them have a lifetime diagnosis of major depressive disorder [13]. Poorer clinical outcomes and a more severe course of illness are found in patients with co-morbid alcohol dependence and major depressive disorder (AD/MDD) [14]. It was hypothesized that the use of alcohol represents an attempt at self-medication in people in alcohol withdrawal [15] and with depression [16]. The monoamine theory of depression is based on reduced monoamine levels – including dopamine - that underlie the pathophysiology and symptomatology of depression [16, 17]. An explanation for the monoamine imbalance of depression was recently suggested involving monoamine oxidase A, a major dopamine metabolism enzyme [18]. Based on the role of mesocorticolimbic dopamine in reward and motivation, it was hypothesized that a
dysfunctional mesocorticolimbic system underlies some of the symptoms of depression [16, 19]. Accordingly, participants with severe major depressive disorder showed evidence of mesocorticolimbic dopamine system dysfunction when challenged with a dopaminergic probe (an oral 30 mg dose of dextroamphetamine) in both a behavioural study [20] as well as in a functional magnetic resonance imaging (fMRI) study [21]. A dysfunctional mesocorticolimbic dopamine system has also been reported in alcohol dependence [22-31]. Therefore, using a similar paradigm to probe mesocorticolimbic dopamine system function in alcohol dependence could enhance our understanding of the neurobiological mechanisms underlying alcohol dependence.

**Purpose of Study and Objective**

To probe mesocorticolimbic dopamine function in alcohol dependence with dextroamphetamine using measures of behavioural rewarding effects (i.e. Addiction Research Center Inventory) and brain activation (i.e. fMRI cue-induced blood oxygenation level-dependent (BOLD) response).

**Statement of Research Hypothesis**

A dysfunctional mesocorticolimbic dopamine system in alcohol dependence will be revealed by an altered behavioural response to dextroamphetamine rewarding effects and by a disrupted brain activity in areas of the mesocorticolimbic dopamine circuitry.
Review of the literature

1. Characteristics of Alcohol Dependence

Alcohol dependence is a psychiatric disorder clinically defined by excessive and uncontrollable alcohol consumption and an inability to stop its use despite negative consequences. It is accompanied by significant interference in psychosocial functioning. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [32], alcohol dependence, as any substance dependence, is present when an individual exhibits a maladaptive pattern of alcohol use, leading to clinically significant impairment or distress, as demonstrated by three or more of the following criteria occurring within the previous twelve months: tolerance (a need for increased amounts to achieve the same effect that was originally achieved with lower doses); withdrawal physiological effects (e.g. anxiety, tremor, insomnia, hallucinations, etc.) due to decreasing or stopping of alcohol use; ingestion of alcohol in larger amounts or for a longer period of time; having the desire yet unsuccessful in attempts to limit or cease alcohol intake; spending significant amounts of time in obtaining and using alcohol and in recovering from its effects; important social, occupational, or recreational activities are given up or reduced due to alcohol use; and, continued use despite knowledge of a persistent or recurrent physical or psychological problem due to alcohol use. Alcohol dependence is the second most prevalent substance use disorder after tobacco dependence; up to 6% of individuals in Ontario will develop alcohol dependence in their lifetime [1]. The National Institute for Alcohol Abuse and Alcoholism (NIAAA) indicates that alcohol dependence affects 7.9 million individuals in the U.S.A with major social, health and economic costs [33]. Alcohol-dependent individuals are quite heterogeneous which has led some researchers/clinicians to subclassify them in 2 broad categories. Type A [34], Type
I [35], or anxious [36] alcoholics define alcohol-dependent individuals who developed alcohol problems later in life, with less severe alcohol dependence, with fewer childhood risk factors (e.g. hyperactivity), less antisocial behaviour and fewer co-morbid psychiatric conditions. Conversely, Type B, Type II, or antisocial alcoholics define alcohol-dependent individuals with an earlier onset of alcohol problems, a more severe level of dependence, greater impulse-control deficits or violent behaviour, and more relapse to and more frequent episodes of craving for alcohol [37, 38]. They also have antisocial behaviour, polydrug use, familial alcoholism, more childhood risk factors and life stress.

2. Neurotransmitter Systems, Alcohol and Alcohol Dependence

Many neurotransmitter systems have been implicated in alcohol dependence: Gamma-aminobutyric acid (GABA) [39-43]; glutamate [44-51]; opioid [52-57]; cannabinoid [58-65]; stress-related neurotransmitters such as corticotropin-releasing hormone (CRH) [66-77] and substance P (SP) [78, 79]; serotonin (5-HT) [80-88]; and dopamine (DA) (see section 3, Dopamine in Alcohol Dependence). Although alcohol has no specific neurotransmitter system, many of them are modulated by alcohol. It is generally accepted that alcohol acts not only by enhancing inhibitory neurotransmission at GABA synapses but also by reducing excitatory neurotransmission at glutamate synapses. Recent molecular pharmacology studies demonstrated that alcohol has a few known primary targets such as the glutamate NMDA, GABA$_A$, glycine, serotonin 3 and nicotinic acetylcholine receptors as well as L-type Ca$^{2+}$ and G-protein-activated inwardly rectifying K$^+$ (GIRK) channels [41]. Alcohol’s reinforcing effects may be mediated by its effects specifically on mesolimbic circuitry. Indeed, like other commonly abused and seemingly unrelated substances of abuse (e.g. nicotine, cocaine, amphetamine, and opioids), alcohol has been shown to increase dopamine levels in the
nucleus accumbens (Nac) in animals by increasing the firing rate of dopamine cell bodies in the ventral tegmental area (VTA) [2-10]. In a human functional neuroimaging study, it was reported that alcohol ingestion increased striatal dopamine release [89]. Therefore, alcohol-induced dopamine release within the nucleus accumbens may produce the reinforcing effects of alcohol. Cannabinoid 1, nicotinic, mu opioid, and serotonin 3 receptors may alter the reinforcing effects of alcohol by facilitating dopamine release [41]. Nevertheless, none of the clinical treatments currently available in Canada to treat alcohol dependence (e.g. naltrexone and acamprosate) directly involve the dopamine system, suggesting that dopamine’s role in alcohol dependence remains to be fully clarified.

The overall approach in Dr. C.A. Naranjo’s Neuropsychopharmacology Research Program has been to investigate underlying neurobiological mechanisms of alcohol dependence and co-morbid alcohol dependence and major depressive disorder focusing on three major neurotransmitter systems: dopamine, serotonin, and opioid. My Ph.D. research projects focused on the dopamine system. Therefore, there will be an emphasis on the dopamine system in this literature review section.

3. Dopamine in Alcohol Dependence

As mentioned, several neurotransmitter systems, other than dopamine, are involved in alcohol dependence [41] but for the purpose of this thesis, we will focus on studies (animal and human) of dopamine in alcohol dependence.

3.1 Animal Studies

Several markers of decreased central dopamine function have been found using animal models of alcohol dependence. Exposure to alcohol in utero resulted in marked hypofunction of the dopamine system in the hypothalamus [90, 91], striatum and frontal cortex in rats [92-...
Morphological changes in dopamine neurons have also been found following perinatal alcohol exposure in rats [95], however, such findings were not always consistent [96, 97]. Alcohol-preferring (AP) rats showed genetic factors that determined greater sensitivity to the effects of ethanol [98], and reduced dopamine transporter (DAT) levels in the mesocorticolumbic terminal pathways in the cingulate cortex [99].

Increasing dopamine concentrations with dextroamphetamine in rats decreased measures of impulsivity, but D₂ antagonists increased them [100]. Increased impulsivity is a characteristic more often found in severe alcohol dependence, and is associated with rapid progression of the disease and poorer outcome [14, 34]. D₂ agonists selectively decreased ethanol consumption [101] and an overexpression of D₂ receptors in rats resulted in significantly reduced ethanol intake, suggesting that an upregulation of D₂ receptors might confer neuroprotective compensatory response in alcohol dependence [102]. Monoamine oxidase inhibitors (MAOI), anti-depressants that increase serotonin and dopamine levels, have been found to decrease ethanol intake in rats [103]. Dopamine antagonists showed attenuation of alcohol intake in rodents [104, 105] but other studies reported increased relapse in animals and human clinical trials [106, 107]. Dopamine agonists and antagonists may modulate oral ethanol self-administration depending on the route of administration. Indeed, in rodents, ethanol self-administration was increased by local infusion of dopamine agonists into the nucleus accumbens whereas it was reduced by systemic administration [108-112]. Conversely, ethanol self-administration was decreased by local infusion of dopamine antagonists into the nucleus accumbens but was enhanced by systemic administration [110, 112].
Other studies show a toxic effect of chronic alcohol exposure on dopamine neurons in the mesocorticolimbic system of animals. Decreased numbers of active cells with reduced dopamine neurotransmission [113], along with prolonged changes in the neurochemistry of dopamine neurons [114], were found in the ventral tegmental area of mice following chronic ingestion of alcohol.

**Key Points:**

- Functional, morphological, and genetic markers of mesocorticolimbic dopamine hypofunction in animal studies of alcohol dependence have been reported.

- Increasing or decreasing dopamine neurotransmission with various pharmacological agents has been shown to modulate impulsivity and alcohol intake in animals.

- Chronic alcohol ingestion has been reported to have toxic effects on mesocorticolimbic dopamine neurons in animals.

### 3.2 Human Studies

Similar to the evidence found in the animal research literature, support for the genetic predisposition to alcohol use has also been found in humans [115]. A greater prevalence of the A1 allele of the dopamine D₂ receptor gene was found in more severe versus less severe alcohol-dependent individuals, and in alcohol-dependent individuals compared to controls, which resulted in decreased D₂ receptors [116, 117]. This allele was linked to increased alcohol intake [118], and with greater level of alcohol dependence severity [119]. Moreover, there is evidence linking the alcohol dehydrogenase gene to alcohol dependence [120-123] and the D₄ receptor gene polymorphism to alcohol craving and problematic alcohol use [124-126]. Furthermore, Hallikaninen and colleagues (2000) reported an association between the low activity allele of catechol-O-methyl transferase (COMT) gene and Type I but not Type II
alcohol-dependent individuals [127]. Early-onset alcohol-dependent persons (Type II) display higher levels of impulsivity, which may contribute to alcohol dependence. Impulsivity is a characteristic commonly found in alcohol dependence.

Increasing dopamine brain concentrations with dextroamphetamine [128] showed a decrease in impulsivity. On the other hand, increased striatal dopamine transporter densities (i.e. reduced dopamine availability in the synaptic cleft) correlated with increased novelty seeking behaviour in alcohol-dependent individuals [129]. Nonetheless, decreasing dopamine neurotransmission with acute tyrosine depletion [130] or with a dopamine antagonist [131] reduced alcohol self-administration in social drinkers as well as in patients with a diagnosis of alcohol dependence or abuse [132].

Evidence of neurotoxicity in humans following chronic alcohol use has been reported [133], with a ∼35% decrease in DAT levels and ∼18% decrease in heterogeneity of dopamine neurons found in the striatum of individuals with alcohol-dependence post-mortem [134, 135]. Neuroimaging studies support the finding of neurotoxicity to the dopamine system following chronic alcohol intake, with reduced striatal D$_2$ and D$_3$ levels found in vivo [136-138]. More recent neuroimaging studies have confirmed lower D$_2$/D$_3$ receptor bioavailability in detoxified alcohol-dependent participants [25-27, 29, 139].

**Key Points:**

- Genetic predispositions to alcohol use in humans have been related to dopamine D$_2$, D$_4$ receptors, alcohol dehydrogenase, and COMT genes.
- Increasing or decreasing dopamine neurotransmission has been shown to modulate impulsivity in alcohol-dependent participants and alcohol intake in alcohol-dependent participants and social drinkers.
4. Dopamine in Major Depressive Disorder

The pathophysiology of major depressive disorder consists of functional changes in neurotransmitter and neuroendocrine systems, such as the monoamines and the hypothalamic-pituitary-adrenal axis [140-145]. For the purpose of this thesis, we will focus on studies (animal and human) of dopamine in major depressive disorder.

4.1 Animal Studies

Numerous animal models of depression are used in the research and development of antidepressant drugs [146, 147]. The wide range of symptoms that characterize depression are difficult to mimic in the laboratory, limiting the usefulness of animal models, however, these models do support the role of dopamine in depression[16, 19]. For example, in a mouse model of depression, evidence for genetic influences with reduced dopamine in the mesocorticolimbic system was found [148, 149].

Chronic antidepressant therapy with such diverse approaches as electroconvulsive shock therapy and antidepressant pharmacotherapy, increased both striatal dopamine [150-152] and dopamine system neurosensitivity in rats [153-157]. Psychostimulants such as cocaine and amphetamine, which increase dopamine in mesocorticolimbic regions, are readily self-administered by animals. These drugs are often abused by humans, and produce increases in mood and euphoria [20, 128]. Moreover, dopamine antagonists (e.g. haloperidol) antagonize the reward of these drugs in the mesocorticolimbic dopamine system of animals [158].
4.2 Human Studies

Several studies have linked alleles of dopaminergic genes (e.g. D$_2$/D$_3$ receptors, tyrosine hydroxylase, COMT) to reduced mesocorticolimbic function [159, 160]) and to depression [161, 162]. Dr. Naranjo’s research group has recently provided further support for reduced dopamine function in depressed patients. An exaggerated response to a dextroamphetamine challenge in non-medicated severely depressed participants was found compared to controls, suggesting a hypofunctional mesocorticolimbic dopamine system in severe depression [20]. Dopamine-releasing drugs such as dextroamphetamine and methylphenidate have shown to decrease depressive symptoms and/or produced euphoria upon administration [163, 164]. Hence, dopaminergic drugs have been used to manage depression when conventional antidepressant treatment has failed [165].

Dr. Naranjo’s group has further investigated mesocorticolimbic dopamine dysfunction in major depressive disorder by combining a dextroamphetamine challenge with f-MRI. When compared to controls, depressed participants showed reduced activity in important mesocorticolimbic dopamine system areas such as the ventrolateral prefrontal cortex, the orbitofrontal cortex and the caudate/putamen [21]. Other neuroimaging studies have demonstrated dopamine function abnormalities in major depressive disorder. A lower striatal dopamine transporter (DAT) binding potential was reported during major depressive episodes [166] as well as in medication-free depressed participants with seasonal affective disorder [167]. Several studies have shown a D$_2$ receptor density increase in depression [168-172]. Other neuroimaging studies, however, have reported an unchanged [173-177] or lower [178] striatal D$_2$ receptor density in depression compared to controls. More severe major depressive disorder with psychotic features has been linked to the dopamine beta-
hydroxylase enzyme gene [179, 180], whereas increased suicidality has been linked to reduced dopamine activity [181]. Dopamine function abnormalities in major depressive disorder also involve monoamine oxidase A (MAO-A), a major dopamine metabolism enzyme. The monoamine theory of depression is based on reduced monoamine levels – including dopamine - that underlie the pathophysiology and symptomatology of depression [16, 17]. An explanation for the monoamine imbalance of depression was recently suggested by Meyer and colleagues involving monoamine oxidase A. [18]. The authors demonstrated elevated monoamine oxidase A density during major depressive episodes and they concluded this phenomenon was the primary monoamine-lowering process during major depression. Moreover, they argued that the regional density of monoamine transporters (e.g. DAT) also has a selective influence on particular monoamines (e.g. dopamine) with a strong relationship with particular symptoms (e.g. motor retardation).

**Key Points:**

- Genetic predispositions to major depression have been related to alleles of dopaminergic genes (e.g. D2/D3 receptors, tyrosine hydroxylase, COMT)

- Animal and human studies have reported a hypofunctional mesocorticolimbic dopamine system in depression.

- Many studies have reported an increase in D2 receptor density in depression and lower dopamine transporter binding sites.

- It was recently suggested that the monoamine imbalance in major depressive disorder may be explained by elevated monoamine oxidase A levels during major depressive episodes.
5. Co-morbid Alcohol Dependence and Major Depressive Disorder

Research on co-morbid alcohol dependence and major depressive disorder is limited [159, 182, 183]. However, depression is the most common psychiatric illness in individuals with alcohol-dependence [11]. Indeed, 80% of individuals with alcoholism (abuse and dependence) report depressive symptoms [12] and more so in women than in men [184-186]. Moreover, it was estimated that between 15 to 50% of alcohol-dependent individuals have a lifetime diagnosis of major depressive disorder [13]. Furthermore, in the Canadian general population, the total prevalence of co-morbid alcohol dependence and major depressive disorder was estimated to be 10% to 12% [13]. These findings were based on the Canadian National Population Health Survey 1994-95 [187], which may have actually under-reported the true incidence of co-morbid disorders in severe alcohol-dependent individuals [13].

Treating co-morbid alcohol dependence and major depressive disorder is difficult and suggests the need to understand mechanisms of the co-morbidity of those two disorders [188, 189].

Poorer clinical outcomes and a more severe course of illness are found in alcohol-dependent individuals who also have depression than in those who do not [14]. Co-morbid patients have significantly greater impairment of function with respect to family, work, and adaptation, compared to either depressed or alcohol-dependent only patients [14]. A common etiology for alcohol dependence and major depressive disorder has been suggested based on epidemiological and familial studies [190], yet the familial vulnerability is complex and likely heterogenous [191-193]. In a large twin study in male Vietnam veterans in the U.S.A, heritability estimates for lifetime alcohol dependence or major depressive disorder were 56%
and 40% respectively, with significant genetic effects accounting for 50% and 38% of that risk [194].

Based on alcohol’s effect in the nucleus accumbens, it has been hypothesized that use of alcohol represents an attempt at self-medication in those with depression [16] or in alcohol withdrawal [195]. The monoamine theory of depression is based on reduced monoamine levels – including dopamine - that underlie the pathophysiology and symptomatology of depression [16, 18]. Based on the ample evidence for a role of mesocorticolimbic dopamine in reward (see section 6. Pharmacological Probing of the Mesocorticolimbic Dopaminergic System), a dysfunctional mesocorticolimbic dopamine system, mediating a core major depressive disorder symptom (i.e. anhedonia), has been postulated [16, 19]. A behavioural study done in Dr. Naranjo’s laboratory recently showed that severely depressed participants demonstrated evidence of dysfunctional mesocorticolimbic dopamine system as revealed by a dextroamphetamine challenge using a single oral dose (30 mg) of dextroamphetamine. These results support the hypothesis of an involvement of a dysfunctional mesocorticolimbic dopamine system in major depressive disorder [20]. A subsequent fMRI study was performed and revealed a disruption in mesocorticolimbic dopamine system circuitry (in striatal and prefrontal cortical areas) in major depressive disorder using dextroamphetamine (30 mg, p.o.) as a dopaminergic probe [21].

Reduced mesocorticolimbic dopamine system function in alcohol dependence has also been reported, with lower dopamine activities associated with decreased rates of abstinence in alcohol-dependent individuals [196, 197], alcohol ingestion in social drinkers [130], and alcohol preference in animals [99, 198]. Moreover, recent human neuroimaging
studies have reported a dysfunctional mesocorticolimbic dopamine system in alcohol dependence in vivo [25-27, 29, 139] and post-mortem [199, 200].

Therefore, using similar approaches as in Tremblay and colleagues’ behavioural and fMRI studies [20, 21] may allow probing of mesocorticolimbic dopamine system (dys)function in alcohol dependence with or without co-morbid depression.

Evidence of pre-morbid involvement of dopamine neurotransmitter system in both alcohol dependence [115, 159] and major depressive disorder [161, 162] has been reported. Reduced dopamine function was postulated to predispose individuals to mood disorders [159, 160] and affect impulse control [128, 201] via the mesocorticolimbic system. Several findings indicate a possible common dopaminergic etiology in alcohol dependence and depression. Lower DAT density in Type I alcohol-dependent individuals [134, 135] as well as a correlation between DAT levels and depressive scores in alcohol-dependent participants during withdrawal [202] have been demonstrated. Similarly, a reduced DAT binding potential has been reported during depression [166]. Associations between various dopaminergic genes (e.g. D2/D3 receptors, tyrosine hydroxylase, COMT) and reduced dopamine function in the mesocorticolimbic system [159, 160] have been linked to both depression [161, 162] and alcohol dependence [116, 118-120]). Thus, it has been suggested the mesocorticolimbic dopamine system may be a common substrate between the two disorders [159, 203].

Traditional therapy for co-morbid alcohol dependence and major depressive disorder has required the classification of one condition as preceding the other, thus designating one of them the primary and the other the secondary condition. However, the designation of “primary” or “secondary” was suggested not to be possible or correct in depressed alcohol-
dependent individuals [204]. Several studies show that up to 60% of alcohol-dependent people continue to drink during treatment and that the relapse rate is generally between 40 to 50% [205-207]. Psychosocial treatment programs remain the standard of care for alcohol-dependent patients who have encountered difficulty in achieving or maintaining sobriety and the commonly prescribed medications for co-morbid alcohol dependence and depression are fluoxetine and citalopram [208]; 2 serotonin reuptake inhibitors (SSRIs) that are not specifically aimed at the dopamine system.

Targeting the dopamine system in alcohol dependence (with or without depression) would appear to be a therapeutic option. Indeed, increasing dopamine activity with dextroamphetamine increased mood scores in severely depressed participants [20], decreased measures of impulsivity in rats [100] and in humans [128]. This is particularly compelling as increased impulsivity is found in the most severe alcohol-dependent individuals [34], and is associated with poorer disease outcome [35]. Increasing dopamine activity using the D2 agonist quinpirole produced dose-dependent decreases in ethanol intake in rats [101]. Similar findings were found by reducing dopamine metabolism using monoamine oxidase inhibitors [103] and by overexpressing D2 receptors in rats [102]. Although early research showed positive results in humans using bromocriptine [209, 210], more recent human clinical trials with D2 agonists have not shown any benefit for improving abstinence rates in alcohol-dependent patients [196, 211]. On the other hand, olanzapine, a D2/D4 antagonist reduced alcohol taste cue-induced craving and alcohol consumption in alcohol-dependent individuals with the longer D4 receptor gene alleles [212]. Other studies have also shown benefits of increasing dopamine activity in the mesocorticolimbic system. Both desipramine and imipramine – 2 tricyclic antidepressants (TCAs) – were reported to increase mood scores and
to decrease alcohol intake in depressed alcohol-dependent participants in a series of studies [213-216].

Treating both alcohol dependence and major depressive disorder concurrently appears logical, since the two conditions have a complex interaction, with a relapse in either triggering a relapse in the other [213]. Moreover, the treatment of both disorders concurrently may improve success rates, since symptoms of depression may interfere with treatment requirements of alcohol dependence [217]. Although depression is best addressed concurrently, it has been suggested to await for a prolonged period of sobriety in order to make a definitive major depressive disorder diagnosis [208].

6. Pharmacological Probing of the Mesocorticolimbic Dopamine System

6.1 Neurobiology of the Mesocorticolimbic Dopamine System

There are several dopaminergic pathways in the central nervous system [218]: the nigrostriatal pathway projects from the substantia nigra to the dorsal striatum (caudate and putamen) and is primarily involved in motor function; the tuberoinfundibular pathway arising and projecting in various areas of the hypothalamus is involved in neuroendocrine (prolactin and growth hormone) regulation in the anterior pituitary; the mesocortical pathway projects from the ventral tegmental area to frontal and temporal cortices, particularly the anterior cingulate, entorhinal and prefrontal cortices; and the mesolimbic pathway which also projects from the ventral tegmental area to the ventral striatum (including the nucleus accumbens), hippocampus, and amygdala. The mesocorticolimbic pathway/system is involved in reward behaviour including pleasure and motivation. Indeed it has been shown to play important roles in natural rewards and in drug addiction [219-224]. Nevertheless, the precise contribution of mesocorticolimbic dopamine to reward still offers debate as several
hypotheses have emerged. Reward-learning hypotheses have suggested that dopamine systems are involved in associative learning about rewards and that addiction results from neural learning in mesocorticolimbic circuits which may cause excessive drug-taking habits and exaggerated reward predictions [219, 225-230]. The hedonia hypothesis has suggested that dopamine mediates the sensory pleasure of food, drugs, and other rewards (“liking”) as well as that addiction results from withdrawal-induced anhedonia caused by a hypodopamine function [164, 222, 231-233]. Several neuroimaging studies have reported a correlation between mesocorticolimbic dopamine neurotransmission and measures of drug “liking” [137, 163, 164, 234-241]. However, drug-induced pleasure effects have not consistently been reduced by treatments that attenuate mesocorticolimbic dopamine neurotransmission (amphetamine [242-244]; cocaine [245-249]; alcohol [130, 131]; and tobacco [250]). The incentive salience hypothesis has suggested that dopamine systems modulate the perceived incentive value of reward stimuli (“wanting”) and that addiction results from sensitization of the mesocorticolimbic system, causing excessive “wanting” to consume drugs [220, 223, 251-257]. Recent PET \([^{11}C]\)raclopride studies have demonstrated mesocorticolimbic dopamine implication in drug “wanting”, novelty-seeking, and reward prediction in healthy males [258-260]. Moreover, acute dopamine precursor depletion studies have shown that decreased mesocorticolimbic dopamine transmission decreased the salience of reward-related cues and the ability to respond to them preferentially [244, 261-263]; as well as reduced motivation to obtain alcohol in male drinkers not fulfilling alcohol abuse or dependence criteria [264]. Therefore, a new model on mesocorticolimbic dopamine neurotransmission contribution to the acquisition of drug taking and the development of addiction has recently been suggested [265] based on animal and human studies demonstrating that drug-induced
activation of the mesocorticolimbic dopamine system increased the incentive salience of drug-related stimuli. Further research will be needed in order to thoroughly define mesocorticolimbic dopamine involvement in reward.

6.2 Dextroamphetamine: Mesocorticolimbic Dopamine System Probe

The mesocorticolimbic dopamine system probe used in the studies presented in this thesis was a single oral dose of dextroamphetamine (30 mg). It was chosen because of its well known dopamine releasing properties, its robust and consistent rewarding subjective effects, its use in neuroimaging studies and because the dose needed is safe and well within therapeutic doses (5 to 60 mg per day) [266]. The "dextro" isomer has been found to demonstrate greater potency than the "levo" isomer [267]. Previous testing in Dr. Naranjo’s research group with 10 mg and 30 mg doses in healthy human volunteers found that the latter dose produced more robust and consistent subjective effects (i.e. euphoria) than the lower dose.

Amphetamine has the ability to reliably stimulate the release of dopamine in the human mesocorticolimbic system by increasing dopamine concentrations at the synapse through an impulse-independent presynaptic dopamine release and dopamine re-uptake blocking action [268-273]. Dextroamphetamine has a higher inhibition of dopamine reuptake ($IC_{50} = 41 \text{ nM}$) and norepinephrine reuptake ($IC_{50} = 23.2 \text{ nM}$) compared to serotonin reuptake ($IC_{50} = 11,000 \text{ nM}$) [274]. A 30 mg dose of dextroamphetamine produces strong subjective positive (e.g. stimulation, elation) and minimal or no negative (e.g. confusion, dysphoria) effects compared to placebo. Peak behavioural effects occur between 1 to 2 hours. Behavioural and fMRI studies in Dr. Naranjo’s research group using dextroamphetamine (30 mg p.o.) reported peak effects at approximately 90 minutes post-ingestion [20, 21]. Plasma
concentration levels peak at 3 to 4 hours after administration [275]. Ten to twelve hours is the expected elimination half-life with metabolism occurring in part via enzyme CYP 2D6 [276]. Dextroamphetamine has dose-dependent effects [277-280]. In humans, at higher doses, dextroamphetamine possesses a high abuse potential due to its well known reinforcing properties [281, 282]. Moreover, sensitization to dextroamphetamine, a phenomenon that may confer vulnerability to drug addiction, was reported in healthy volunteers and was associated with dopamine release in the ventral striatum [258]. Clinically, at low doses, the drug produces euphoria and elation in some individuals; and as the dose is increased these effects are intensified but in addition, individuals begin to show signs of motor activity, mental alertness, diminished drowsiness, and decreased fatigue. Additionally, glucose metabolism is increased in the attentional (e.g. frontal cortex, cerebellum) as well as in the motivational and emotional (e.g. limbic/paralimbic system) pathways resulting in the behavioural effects typically seen (e.g. good mood, fast thinking) after dextroamphetamine administration [283, 284].

Clinically, dextroamphetamine is prescribed for narcolepsy and Attention Deficit-Hyperactivity Disorder (ADHD) under the name Dexedrine®. According to the Compendium of Pharmaceuticals and Specialties [266], therapeutic doses fall within the range of 5 to 60 mg per day for treating narcolepsy and 2.5 to 40 mg per day for treatment of ADHD. Dextroamphetamine effectively crosses the blood-brain barrier and, therefore, is able to exert its effects centrally through the stimulated release of dopamine. The related norepinephrine release results in increased heart rate and blood pressure. The most common side effects of dextroamphetamine (nervousness, agitation, decreased sleep) are related to its sympathomimetic pharmacology. With respect to the anorectic effects of amphetamines, the
role of hypothalamic neuropeptide Y (NPY), a potent stimulant of appetite, is being considered as a possible mechanism [285, 286].

Various animal studies have confirmed the reinforcing effects of dextroamphetamine. Self-administration, place preference, and decreased brain stimulation thresholds all reported positive reinforcing effects of the drug [287, 288]. Blocking dopamine activity has been shown to attenuate psychomotor stimulant effect [289]. Moreover, the effects of amphetamine as a discriminative stimulus in animals (i.e. choosing amphetamine over placebo) was mimicked by dopamine D1 and D2 receptor agonists and blocked by D1 and D2 antagonists. The use of D2/5-HT2A antagonists (e.g. risperidone) in intracranial self-stimulation studies has shown that only the D2 activity of these drugs has significant anti-amphetamine effects [290]. Furthermore, it appears D1 receptors are mostly involved in the stimulus effects of the drug but there may be an interaction involving the D2 receptors as well. More specifically, it has been reported that dextroamphetamine-induced dopamine release may primarily act on D1 receptors at low doses and on D2 receptors at higher doses [291]. Recent studies have revealed the ability of D1 and D2 receptors to heterooligomerize resulting in different signaling complexes than those in each separate receptor [292-294]. The latter studies suggest there may be diverse potential mechanisms modulating dextroamphetamine effects that need further investigation.

The importance of D2 receptors in the reinforcing effects of psychostimulants such as amphetamine has been verified in humans through the use of different brain imaging techniques [164, 236]. It was found that dextroamphetamine-induced dopamine release showed binding to D2 receptors located in mesocorticolimbic dopamine system areas such as the caudate nucleus, putamen, and nucleus accumbens [236]. Moreover, studies have shown
a correlation between the intensity of dextroamphetamine-induced behavioural effects (e.g. euphoria) and the extent of binding of dopamine to D<sub>2</sub> receptors [163, 236], specifically in the nucleus accumbens and ventral medial caudate; both major mesocorticolimbic brain areas. Furthermore, a recent human neuroimaging study demonstrated that oral administration of a single 30 mg dose of dextroamphetamine (the same dose and route of administration used in the studies in this thesis) produced a significant decrease in D<sub>2</sub> receptor availability measured by PET [<sup>11</sup>C]raclopride [295]. It also was reported that doubling the dextroamphetamine dose produced a doubling in dopamine binding to D2 receptors in primates [296]. The two latter studies are consistent with the literature involving dopamine in cocaine-induced euphoria [164, 239, 297].

Based on dextroamphetamine’s ability to exert its effects on the dopamine system, several recent PET studies have used this dopamine releasing compound in order to investigate dopamine function in humans with either an intravenous [27] or an oral [258, 260, 298] administration. Moreover, several fMRI studies using dextroamphetamine demonstrated that the drug did not affect the BOLD signal [21, 299, 300].

Dextroamphetamine has been reported to cause norepinephrine and serotonin release in the nucleus accumbens, but to a lower degree compared to dopamine [301-303]. However, drugs that block norepinephrine receptors failed to produce changes in the rewarding effects of the drug whereas blocking dopamine activity attenuated psychostimulant-induced reward [291, 304-306]. Moreover, it has recently been reported that dopamine, serotonin and norepinephrine have functional reciprocal interactions that may enhance or attenuate one or several other monoamine neurotransmitter activities [307, 308]. Acetylcholine also was reported to be
released by dextroamphetamine [301] but the effect of this neurotransmitter in amphetamine reward (e.g. “high”) remain unclear and deserves further investigation.

Therefore, despite its ability to release neurotransmitters other than dopamine, dextroamphetamine may probe the mesocorticolimbic system through a relatively specific mechanism (i.e. dopamine release). Hence, we will focus on dextroamphetamine-induced dopamine release in the two studies of this thesis, while recognizing its effects on other neurotransmitters.

7. Functional Magnetic Resonance Imaging (fMRI)

Magnetic Resonance Imaging (MRI) is used ubiquitously in medical applications that require depiction of the soft tissues of the body, especially the central nervous system. Compared to other forms of neuroimaging (e.g. Positron Emission Tomography [PET], Single Photon Emission Computerized Tomography [SPECT]), fMRI has excellent time and spatial resolution, costs less, requires no radioactive substrates, and is non-invasive. This imaging technique employs cognitive tests performed during the scan to identify the spatial location of elevated brain activity associated with a particular task. Indeed, fMRI is used for mapping neuronal activation in a safe and non-invasive manner [309-311]. It uses radio frequency pulses, called echo planar pulse sequences, in a strong static magnetic field to detect changes in brain activity. The basis for fMRI lies in the changes in blood oxygenation, blood volume and blood flow caused by metabolic demands of activated neurons. These neurons produce localized changes in magnetic resonance signals in the brain known as the blood-oxygenation level-dependent (BOLD) effect or signal. The BOLD technique takes advantage of the fact that the change from diamagnetic oxyhemoglobin to paramagnetic deoxyhemoglobin that takes place with brain activation results in decreased signal intensity on MRI. The blood
itself therefore acts as an endogenous contrast agent which produces BOLD signal changes of 2% to 4% in grey matter, using magnetic field strengths ranging from 1.5 to over 7 Tesla.

Cognitive tests performed during the scan may be used to identify related structural correlates with a spatial resolution of approximately 1-3 millimetre and a temporal resolution of approximately 3-5 seconds [310]. Activation is usually quantified in fMRI studies such that the temporal on-off sequence of the task is correlated with the BOLD signal, with the task often repeated several times to increase statistical power. Cognitive tasks can therefore be used to engage a certain known region of the brain that is related to the question of interest. More recently, fMRI has been combined with various pharmacological agents to explore their sites and mechanisms of action within the brain. Pharmacological MRI has shown potential usefulness in studies of neurotransmitter systems (e.g. dopamine [312, 313]), psychiatric [314, 315] and neurological conditions [316, 317], and drugs of abuse (e.g. alcohol [318-321]) (reviewed in [322]). Indeed, fMRI can provide specific information relating to the neural substrates of the probe’s effects. For example, as we previously mentioned, Tremblay and colleagues have successfully combined the administration of a dextroamphetamine challenge with fMRI in 12 depressed participants to identify brain regions associated with altered behavioural response to dextroamphetamine [21]. Severely depressed participants reported higher dextroamphetamine rewarding effects than the controls with corresponding differences in a network of well-localized brain regions including prefrontal, cingulate, and striatal regions. Moreover, pharmacological MRI has shown utility in predicting treatment response and supporting the development of novel compounds in neuropsychiatry [322] including in alcohol dependence [318]. Other studies reported the involvement of specific brain regions in major depressive disorder and alcohol-
dependent participants. Exposure to pleasant visual stimuli using the International Affective Picture System (IAPS) [323] revealed decreased activation in the medial frontal cortex, but increased activation in inferior frontal cortex, anterior cingulate, thalamus, putamen, and insula in anhedonic depressed patients compared to controls [324]. Similarly, depressed participants demonstrated elevated responses in rostral anterior cingulate, medial prefrontal cortex, and lateral orbitofrontal cortex but attenuated neural responses in the ventral cingulate and posterior orbitofrontal cortex during an emotional go/no-go task [325]. The Stroop Interference Task also revealed increased brain activation in the anterior cingulate and dorsolateral prefrontal cortex [326]. Alcohol-dependent participants displayed increased craving and brain activity in the left dorsolateral prefrontal cortex and anterior thalamus compared to controls after a sip of alcohol while watching alcoholic beverage cues [327]. Olfactory alcohol cue-induced BOLD response revealed activation in subcortical-limbic regions of the right amygdala/hippocampus and cerebellum in currently drinking alcohol-dependent participants [321], which was no longer present after abstinence and behavioural therapy. An alcohol cue exposure paradigm elicited activation of mesocorticolimbic brain regions [22, 30] in alcohol-dependent participants compared to controls. Thus, such a paradigm is relevant to neuroimaging studies aimed at investigating mesocorticolimbic circuitry in alcohol-dependence.

8. FMRI Cue-Induced BOLD Response and Dextroamphetamine to Reveal Mesocorticolimbic Dopamine System Dysfunction in Alcohol Dependence

In the fMRI study by Tremblay and colleagues [21] investigating the neural correlates of the altered behavioural response to dextroamphetamine rewarding effects in depressed participants, the International Affective Picture System (IAPS) [323] was used as a cognitive task. However, in this thesis FMRI Study, an alcohol cue exposure task would be more
appropriate in order to engage certain known brain regions that are related to the question of interest in alcohol dependence.

As was previously mentioned, a dysfunctional mesocorticolimbic dopamine system has been reported in alcohol dependence. Specifically, lower dopamine D2/D3 receptor availability has been reported in alcohol dependence in functional neuroimaging [25-27, 29, 139] and post-mortem [199, 200] studies. Blunted ventral striatal dopamine release has also been documented [27] as well as a low level of striatal dopamine synthesis [25] in alcohol dependence.

Alcohol cue exposure paradigms have been used in several fMRI studies of alcohol dependence using validated alcohol cues [22, 26, 30, 31, 318, 327-329]. An increase in BOLD response/activation in the ventral striatum (VS) was shown in response to alcohol-associated cues compared to affectively neutral cues using fMRI in abstinent alcohol-dependent individuals [31]. These standardized pictures of alcoholic beverages have been shown to activate important areas of the mesocorticolimbic dopamine system such as the ventral striatum and the orbitofrontal cortex (OFC) and therefore they allow assessing brain circuits involved in the processing and evaluation of alcohol cues [22, 30, 31]. The mesocorticolimbic circuitry has been suggested to be a substrate for incentive salience attribution to drug-related cues [255] and to be implicated in the pathophysiology of addiction [330, 331]. The orbitofrontal cortex is an area of the mesocorticolimbic circuitry that is involved in incentive salience attribution [332]. Its disruption has been suggested to be central to the addiction process [333]. Animal studies have shown a prefrontal regulation of dopamine release in the nucleus accumbens and dopamine cells in the ventral tegmental area [334, 335] and more recently, Volkow and colleagues documented a possible orbitofrontal
cortex involvement in striatal dopamine release decreases in detoxified alcohol-dependent individuals using $[^{11}C]$(raclopride PET and methylphenidate [29]. The latter study results are consistent with the hypothesis of a loss of prefrontal modulation of dopamine cell activity in alcohol dependence, speculating on an orbitofrontal cortex involvement [29, 336]. As was previously mentioned, other dopaminergic compounds have been used in functional neuroimaging studies of alcohol dependence, e.g., amisulpride was used in order to block cue-induced brain activation using fMRI [319]. However, none of these studies specifically stimulated dopamine activity in relation to alcohol cues in alcohol-dependent individuals. As previously mentioned, dextroamphetamine has been used in combination with fMRI to reveal neural substrates of altered reward processing in major depressive disorder [21]. Indeed, combining functional neuroimaging with a dopaminergic drug challenge makes it possible to study dopaminergic system function in psychiatric disorders [337].

Therefore, dextroamphetamine and fMRI cue-induced BOLD response in combination would appear to be an appropriate strategy to assess how brain areas of the mesocorticolimbic circuitry would respond to a dopamine challenge in alcohol-dependent participants, and specifically in order to reveal a disruption in areas of this circuitry in alcohol dependence.
As mentioned in the literature review, the mesocorticolimbic dopamine system may mediate the rewarding effects of drugs of abuse and it has been reported to be dysfunctional in major depressive disorder and alcohol dependence. Probing mesocorticolimbic dopamine function in major depressive disorder with a single oral dose of dextroamphetamine (30 mg) showed an altered behavioural response (i.e. hypersensitive) to dextroamphetamine rewarding effects in participants with severe major depressive disorder compared to controls [20]. The purpose of the Behavioural Study was to use a similar protocol in order to probe mesocorticolimbic dopamine function in alcohol dependence. Because major depressive disorder is the most common psychiatric disorder in those suffering with alcohol dependence and because of the dysfunctional mesocorticolimbic system reported in both disorders, the primary objective was to determine if alcohol-dependent participants with co-morbid major depressive disorder would have a significantly greater behavioural response to dextroamphetamine rewarding effects compared to non-depressed alcohol-dependent participants. Indeed, we hypothesized the presence of the 2 disorders would potentiate the behavioural response to dextroamphetamine rewarding effects compared to the presence of alcohol dependence alone. Secondly, as a post-hoc analysis, the objective was to compare alcohol-dependent and depressed alcohol-dependent participants with control and only-depressed (MDD) participants whose data were previously collected by L. Tremblay using similar experimental conditions [20].
BEHAVIOURAL STUDY OBJECTIVES

Primary: To compare the subjective effects induced by dextroamphetamine (one single oral dose) between alcohol-dependent and depressed alcohol-dependent participants.

Secondary: To compare these results with control and only-depressed participants (data previously collected by L. Tremblay using a similar protocol).

BEHAVIOURAL STUDY HYPOTHESIS

A hypofunctional mesocorticolimbic dopamine system, present in major depressive disorder, may also be present in alcohol dependence; therefore the administration of dextroamphetamine, which causes dopamine release, may elicit a similar (i.e., hypersensitive) response in those suffering from alcohol dependence, and more so in those with co-morbid depression and alcohol dependence.
Section 2A  MATERIALS AND METHODS

2A.1 Study Design

This study was a randomized, double-blind, placebo-controlled, between-subject study. An assessment session was conducted to determine the participants’ eligibility. Each subject participated in one study day session. On the study day, a series of symptom severity assessments were measured at baseline, and then a battery of subjective measurements as well as physiological measurements were performed at baseline and repeated at 30, 60, 120, 180, and 240 min after dextroamphetamine or placebo administration.

The subjective effects of dextroamphetamine 30 mg as a single oral dose were compared to placebo in participants with alcohol dependence and participants with co-morbid depression and alcohol dependence.

As a post-hoc analysis, comparisons were also made to control and depressed participants (without alcohol dependence) using data collected in a previous study in our laboratory using the same study design [20].

2A.2 Participant selection

Participants were of either sex; aged 19-65 years; met Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [32] criteria for alcohol dependence or co-morbid major depressive disorder and alcohol dependence (confirmed using the Structured Clinical Interview for DSM-IV for Axis I disorders (SCID-IV)); and were not receiving pharmacological treatment for either disorder (e.g. acamprosate, antidepressants for at least 2 weeks (5 weeks for fluoxetine)). Exclusion criteria included the following: Pregnancy/lactation; current or past history of a cardiovascular disorder; medical condition requiring immediate investigation or treatment; positive urine screen for psychoactive drugs.
(other than nicotine); recent use (within last three months) of any illicit drugs such as cocaine, amphetamine, heroin; recent (≤ 1 yr)/current history of drug abuse or dependence on a substance (other than alcohol, caffeine, or nicotine); current use of any medication known to interact with the study drug (e.g. antihypertensives, sedative hypnotics, antipsychotics; see Appendix A for list of medications known to interact with dextroamphetamine); current suicidal ideation posing immediate threat to the participant's life; co-morbid DSM-IV Axis I or Axis II mental illness (other than alcohol dependence and major depressive disorder); psychotic depression. All participants were recruited through city newspaper advertisements.

As a post-hoc analysis, alcohol-dependent and depressed alcohol-dependent participants were compared to control and depressed-only participants recruited for a similar study in our laboratory using the same protocol [20]. Participants in the depressed group fulfilled DSM-IV criteria for major depressive disorder. Control participants could not have a personal history of mood disorders or other psychiatric disorders. Some participants in the control and depressed groups were referred by outpatient psychiatrists.

The protocol was approved by the research ethics board of Sunnybrook Health Sciences Centre. Signed informed consent was obtained from all participants prior to participating in the study (see Behavioural Study consent form in Appendix B).

2A.3 Study Session

Participants arrived between 8 and 10 AM for their study session. They were instructed not to consume alcohol, nicotine or caffeine for 12 hours prior to arriving. A breathalyzer (Alert, Alcohol Countermeasure Systems, Canada) was used to verify participants’ sobriety. A urine sample was collected for toxicology testing (see Appendix C for substances that can be detected by the urine toxicology screen). After a light standardized breakfast, symptom
severity assessment, subjective and physiological measurements were performed before drug or placebo ingestion (i.e. at baseline). The subjective measurements as well as physiological measurements were repeated at 30, 60, 120, 180, and 240 min after dextroamphetamine or placebo administration. The overall Behavioural Study session procedures are outlined in Table 1.

Table 1: Summary of Behavioural Study Session Procedures

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0</th>
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<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
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</tr>
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</tr>
<tr>
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<td>x</td>
<td>x</td>
<td>x</td>
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<td>x</td>
</tr>
</tbody>
</table>
2A.4 Symptom Severity Assessments

Participants were evaluated for alcohol withdrawal using the Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar) [338]. Those reporting significant withdrawal symptoms would be excluded. The Alcohol Dependence Scale (ADS) [339, 340], the Fagerström Test for Nicotine Dependence (FTND) [341], the Beck Depression Inventory (BDI) [342], the Snaith-Hamilton Pleasure Scale (SHAPS) [343] and a modified version of the Sunnybrook Psychomotor Agitation and Retardation Questionnaire (SPARQ) [344] were administered to evaluate alcohol dependence, tobacco dependence, depression, anhedonia and psychomotor symptom severity respectively. Severity of depressive episodes experienced by participants during the 2 to 3 weeks before the study session was evaluated using the Hamilton Rating Scale for Depression (21-item HAM-D) [345]. Details of these symptom severity assessment measures are described below:

1) Clinical Institute Withdrawal Assessment for Alcohol (revised) (CIWA-Ar): This 10-item scale was used to measure the severity of alcohol withdrawal symptoms. The maximum score is 67. It is important for our results that participants were not undergoing significant withdrawal while being tested so only scores \(<10\) were permitted. Those reporting a score \(\geq 10\) would be excluded as such a level of withdrawal is generally considered significant (e.g. could warrant a dose of medication in a diazepam loading protocol for detoxification).

2) Alcohol Dependence Scale (ADS): The ADS provides a quantitative measure of the severity of alcohol dependence consistent with the concept of the alcohol dependence syndrome. Its 25 items cover alcohol withdrawal symptoms, impaired
control over drinking, awareness of a compulsion to drink, increased tolerance to alcohol, and salience of drink-seeking behaviour.

3) Fagerström Test for Nicotine Dependence (FTND): This 6-item questionnaire was used to determine the level of nicotine dependence is current smokers. The maximum score is 10, which denotes a high level of nicotine dependence. Scores greater than 5 generally define heavy smokers. The FTND has a high level of internal consistency and determinations of dependence severity using this measure closely match biochemical indices of heaviness of smoking.

4) Beck Depression Inventory (BDI): This self-assessment scale measures depression severity. It is composed of 21 questions. Scoring is as follows: <10 considered non-depressed; 10-15 dysphoric; >16 depressed.

5) Snaith-Hamilton Pleasure Scale (SHAPS): A validated self-assessment scale estimating the degree to which a person is able to experience pleasure or the anticipation of a pleasurable event (i.e. hedonic tone). A score of 2 or more "disagree/definitely disagree" is considered to be indicative of an anhedonic state.

6) Sunnybrook Psychomotor Agitation and Retardation Questionnaire (SPARQ): The modified version of this self-assessment scale was administered solely to evaluate psychomotor symptom severity.

7) Hamilton Depression Scale (HAM-D): This scale contains 17 questions aimed at such variables as depressed mood, suicide, somatic symptoms, and lost of interest. Four additional variables are included (i.e. diurnal variation, derealization, paranoid symptoms and obsessional symptoms), making the total questionnaire 21 questions in length. The first of these latter questions is included more as a way of classifying the
depression rather than contributing to the rating while the other three were originally not included because of their infrequency. A score of 18 and higher suggests the presence of major depressive disorder, whereas a score of 7 or lower is not considered to represent significant depressive symptoms.

2A.5 Study Probe

Dextroamphetamine sulfate (Dexedrine, GlaxoSmithKline Inc., Mississauga, Ontario, Canada) and placebo doses were prepared in identical 10-mg capsules filled with drug or dextrose powder. A research pharmacist dispensed the medication and kept the randomization code. Dextroamphetamine (30 mg) was administered orally and used as a pharmacological probe. This dose was selected based on its ability to produce reliable and measurable positive subjective effects (euphoria, “liking”) [295]. The dose is also safe and is within the therapeutic daily dose range (5 to 60 mg). [266].

2A.6 Dextroamphetamine Effects Measurement Tools

2A.6.a Subjective Effects

Instruments used to measure behavioural subjective drug effects were computerized versions of the Addiction Research Center Inventory (ARCI) [346-348] and Visual Analog Scales (VAS) [349-352]. The ARCI is the main outcome measure used for dextroamphetamine rewarding effects. The POMS and VAS are additional, less specific self-report measures administered to assess acute mood changes. Below are details of each measurement tool:

1) Addiction Research Center Inventory (ARCI): This 77-item questionnaire measures subjective effects of drugs both positive and reinforcing (e.g. euphoria, stimulation) and negative or dysphoric (e.g. sedation, confusion). This inventory
allows the quantification of subjective drug effects with scales sensitive to the effects of specific drugs and drug classes (e.g. amphetamine).

2) Profile of Mood States (POMS): This scale consists of a series of 72 adjectives and it is commonly used for assessing drug-induced changes in mood. With respect to each adjective, participants indicate how they feel using a five-point scale ranging from "extremely" to "not at all". Tension-Anxiety, Anger-Hostility, Depression-Dejection, Friendliness, Fatigue, Confusion, Vigor, Elation, Arousal, and Positive Mood are the 10 scales covered in the POMS.

3) Visual Analog Scale (VAS): The Visual Analog Scales are often used in the assessment of momentary changes in affect. They consist of a selection of visual analog rating scales (100mm lines) anchored at each end by opposing adjectives. Participants were instructed to rate how they felt by making a mark anywhere along the line. The VAS scales including the word anchors at each end of the 100mm scale were as follows: “I feel high from the drug” (absolutely normal‡ very high), “I feel anxious” (certainly not‡ certainly), “I feel irritable” (certainly not‡ certainly), “I feel alert” (certainly not‡ certainly), “I feel restless” (certainly not‡ certainly), “I feel an increase of energy” (certainly not‡ certainly), “I feel an increase in my speed of thinking” (certainly not‡ certainly), “I feel a drug effect” (certainly not‡ certainly), “I like the drug” (dislike intensely‡ like intensely), “I feel the drug’s good effects” (undetectable‡ detectable), “I feel the drug’s bad effects” (undetectable‡ detectable).
2A.6.b Objective Effects

Physiological measurements (heart rate and blood pressure) were recorded using a stethoscope and sphygmomanometer at baseline and at 30, 60, 120, 180, and 240 min after 30 mg of dextroamphetamine or placebo administration.

2A.7 Data Analysis

Demographics between the 2 groups (alcohol-dependent and depressed alcohol-dependent) and amongst the 4 groups (control, depressed, alcohol-dependent and depressed alcohol-dependent), in the post-hoc study, were compared using t-tests and analysis of variances (ANOVAs), respectively. Those that were significantly different were used as covariates in the analyses.

The dextroamphetamine behavioural effect was defined as the maximum change from baseline score at any time point following 30-, 60-, 120-, 180-, and 240-minute recordings (the highest magnitude value after dextroamphetamine administration irrespective of whether or not it was a higher or lower value than the corresponding baseline value). The main dependent outcome variable, termed ARCI Rewarding Effects Composite, consisted of a composite of change scores from scales that measure positive reinforcing/rewarding effects: Abuse-Potential, Amphetamine, Benzedrine Group, Morphone-Benzedrine Group and Stimulation-Euphoria. A similar rewarding effects composite was calculated with the POMS using the Elation, Vigor and Friendliness scales. The ARCI Negative Effects Composite was also calculated, using the LSD, Pentobarbital-Chlorpromazine-Alcohol Group, Sedation-Mental, Sedation-Motor, Unpleasantness-Dysphoria, and Unpleasantness-Physical scales in order to evaluate increases in negative (i.e. unpleasant) drug effects. Because of the different
score ranges within the various scales, the maximum change from baseline scores were converted to a score on a 100% scale before being added into the composite score. The Cronbach \( \alpha \) coefficient was obtained for each composite measure to evaluate internal consistency. For that purpose, the Cronbach \( \alpha \) coefficient should at least be 0.8. The ARCI, POMS and VAS scales mean scores represented the maximum change from baseline.

The objective drug effect measures (systolic (SBP) and diastolic (DBP) blood pressures; heart rate (HR)) were also defined as the maximum change from baseline score among the 30-, 60-, 120-, 180-, and 240-minute recordings.

The control (\( n=20 \)) and depressed (\( n=22 \)) participants’ data included in the post-hoc exploratory study were collected in a previous study in our laboratory by Tremblay and colleagues with the same experimental conditions [20]. In order to be included in the post-hoc analysis, the control and depressed participants’ raw data were computed in the same way as the alcohol-dependent and depressed alcohol-dependent participants’ data.

In the study by Tremblay and colleagues [20] - comparing the behavioural response to dextroamphetamine rewarding effects between controls and depressed participants - the rewarding effects of the 30 mg oral dose of dextroamphetamine were found to be highly correlated with the severity of depression (as measured by the HAM-D) in the depressed group (\( r^2=0.88 \)). Furthermore, it was found that those with mild to moderate depression reacted similarly to controls but those categorized with severe depression showed a 64% larger response to the rewarding effects of dextroamphetamine. Therefore, we extracted the severely depressed alcohol-dependent participants from the whole depressed alcohol-
dependent group. The mean and median HAM-D scores in depressed alcohol-dependent participants (as well as depressed-only participants in the study by Tremblay) were consistently found to be 23; therefore this was used as the cut-off for selecting the more severely depressed participants. Only our main outcome measure (ARCI Rewarding Effects Composite) was compared between alcohol-dependent and severely depressed alcohol-dependent participants.

All data were analyzed using a statistical software program (SPSS version 15.0; SPSS Inc, Chicago, Ill). Pearson’s Chi-Square test was used to determine if the female/male ratio was significantly different among the groups. Independent t-tests were used to compare other demographic and baseline characteristics between the alcohol-dependent and depressed alcohol-dependent participants as well as to compare between the dextroamphetamine and the placebo arms within each group. An ANOVA on the same clinical data was used to compare amongst the 4 groups in the post-hoc study. Subjective data (ARCI, POMS and VAS) as well as physiological data (SBP, DBP, HR) were analyzed by 2-factor ANOVAs (GROUP (with 2 levels) x DRUG (with 2 levels)) on the main outcome dependent variable: ARCI Rewarding Effects Composite, on the ARCI Negative Effects Composite, on the POMS Rewarding Effects Composite, on all of the ARCI, POMS, and VAS scales as well as on the physiological scores (SBP, DBP and HR). The binary factors were as follows: GROUP (alcohol-dependent (AD), depressed alcohol-dependent (AD/MDD)) and DRUG (placebo, dextroamphetamine). In the post-hoc study, ARCI Rewarding Effects Composite data were similarly analyzed with 4 levels in the GROUP factor (control (CON), depressed (MDD), alcohol-dependent (AD), depressed alcohol-dependent
Pearson’s correlation coefficient tests were used for bivariate correlations in order to determine if the ARCI Rewarding Effects Composite scores correlated with baseline measures of anhedonia (i.e. SHAPS scores) or psychomotor retardation (i.e. psychomotor retardation sub-scores of the SPARQ).

Section 3A RESULTS

3A.1 AD vs. AD/MDD

3A.1.a Participants

In recruiting the alcohol-dependent and co-morbid depressed alcohol dependent groups, 132 individuals underwent initial screening on the telephone: 38 were eligible to come for an assessment session and the remaining potential participants were excluded because: 1) they did not fulfill DSM-IV criteria for alcohol dependence (n=25); 2) they were taking medications (e.g. antidepressants) (n=19), reported another DSM-IV Axis I Illness (e.g. bipolar disorder) (n=9) or were using illicit drugs (e.g. cocaine) (n=13). Twenty-eight individuals were not interested in the study after receiving all the information by phone. Of the 38 individuals who were eligible to come for an assessment session after the telephone screening, 12 did not show up and 26 completed assessment sessions; 3 of these were excluded after the assessment session because of the presence of other Axis I disorders. Twenty-three participants completed the study (11 alcohol-dependent and 12 depressed alcohol-dependent). These numbers were supplemented in the analyses by data (7 alcohol-dependent and 10 depressed alcohol-dependent) collected by Laura Abbott, a Master of Science student in our laboratory using the same protocol. Therefore, the total number of participants in the alcohol-dependent and depressed alcohol-dependent groups was 18 and 22
respectively. This resulted in the analysis of data from 4 study arms: alcohol dependence-placebo (n=10), alcohol dependence-dextroamphetamine (n=8), alcohol dependence/major depressive disorder-placebo (n=10), and alcohol dependence/major depressive disorder-dextroamphetamine (n=12).

Three participants were positive for THC the day of the study but were included in the data set. According to the information given on the assessment and study session days, these 3 participants did not consume marijuana on a regular basis and their last day of consumption was at least 1 week prior to the study day. Moreover, the statistical analysis on our main outcome measure was repeated excluding the 3 THC positive participants and no difference was observed. Therefore, the results presented in the results section include the 3 individuals.

3A.1.b Demographic and baseline characteristics data
No alcohol-dependent or depressed alcohol-dependent participant reported significant withdrawal symptoms on a study session day (CIWA-Ar \( \geq 10 \)) and none was intoxicated (as measured by breathalyzer). Participants’ characteristics are summarized in Table 2. As expected, the HAM-D scores were significantly different between the alcohol-dependent and depressed alcohol-dependent participants. The baseline SHAPS and psychomotor agitation or retardation sub-scores of the SPARQ mean scores in the depressed alcohol-dependent group were significantly different from the scores of the alcohol-dependent group. Anhedonia, according to the SHAPS (score \( \geq 2 \)), was not present in every participant within the alcohol dependent and depressed alcohol-dependent groups. Six alcohol-dependent and 16 depressed alcohol-dependent participants scored at least 2 on the SHAPS. Three alcohol-dependent participants reported a family history of a psychiatric disorder compared with 12 in the
depressed alcohol-dependent group. Furthermore, 9 alcohol-dependent and 15 depressed alcohol-dependent participants reported a family history of alcoholism (abuse or dependence). There was no significant difference between the alcohol-dependent and the depressed alcohol-dependent groups in terms of average number of alcoholic drinks per day and time since the last alcoholic drink.

None of the demographic variables were significantly different between the alcohol-dependent and the depressed alcohol-dependent groups; therefore no covariates were included in our analyses.

There was no difference in the demographic and baseline characteristics between placebo and dextroamphetamine arms within the co-morbid depressed alcohol dependent group. The alcohol dependent group had a statistically significant difference in the HAM-D scores at baseline between placebo and dextroamphetamine sub-groups (2.9±1.8 versus 1.1±1.4, respectively, p=0.03); however, HAM-D scores < 7 are not considered to represent clinically relevant levels of depressive symptoms. All other demographic and baseline characteristics between placebo and the dextroamphetamine arms in the alcohol-dependent group were not different. There were no differences in baseline drug effect scores between placebo and dextroamphetamine arms within the alcohol-dependent group, as well as those within the depressed alcohol-dependent group.
Table 2: Participant demographic and baseline characteristics (AD vs. AD/MDD)

<table>
<thead>
<tr>
<th></th>
<th>AD (n=18)</th>
<th>AD/M DD (n=22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.44 ± 11.00</td>
<td>38.82 ± 11.38</td>
<td>*0.47</td>
</tr>
<tr>
<td>Sex (female/male ratio)</td>
<td>4/14</td>
<td>8/14</td>
<td>†0.33</td>
</tr>
<tr>
<td>Education (years after high school)</td>
<td>2.78 ± 1.56</td>
<td>3.09 ± 1.77</td>
<td>*0.56</td>
</tr>
<tr>
<td>Alcohol Dependence Scale (ADS)</td>
<td>17.56 ± 6.67</td>
<td>21.27 ± 5.43</td>
<td>*0.06</td>
</tr>
<tr>
<td>Fagerström Test for Nicotine Dependence (FTND)</td>
<td>1.50 ± 2.66</td>
<td>3.09 ± 3.13</td>
<td>*0.10</td>
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<td>Hamilton Rating Scale for Depression (HAM-D)</td>
<td>2.11 ± 1.81</td>
<td>22.27 ± 5.90</td>
<td>*&lt;0.001</td>
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<td>Beck Depression Inventory (BDI)</td>
<td>7.22 ± 5.59</td>
<td>25.36 ± 8.02</td>
<td>*&lt;0.001</td>
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<td>Snaith-Hamilton Pleasure Scale (SHAPS)</td>
<td>1.28 ± 1.81</td>
<td>4.45 ± 4.00</td>
<td>*0.002</td>
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<tr>
<td>Psychomotor Agitation sub-score of the SPARQ</td>
<td>7.56 ± 6.69</td>
<td>29.05 ± 16.43</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Psychomotor Retardation sub-score of the SPARQ</td>
<td>3.44 ± 3.54</td>
<td>16.73 ± 9.35</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Number of alcoholic drinks per day</td>
<td>7.11 ± 2.83</td>
<td>7.00 ± 2.94</td>
<td>*0.90</td>
</tr>
<tr>
<td>Time since last alcoholic drink (hours)</td>
<td>29.78 ± 15.12</td>
<td>22.91 ± 13.22</td>
<td>*0.13</td>
</tr>
</tbody>
</table>

Group differences analyzed with independent t-tests (*) or with Pearson’s Chi Square tests (†)
3A.1.c Objective Drug Effect Measures: Blood Pressure and Heart Rate

The 2-factor ANOVA (GROUP (alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, dextroamphetamine)) on the maximum change from baseline systolic blood pressure (SBP) confirmed that the 2 groups experienced a dextroamphetamine effect compared to placebo. Indeed, main effects of DRUG were detected ($F_{1,40}=15.8; p<0.001$). No GROUP ($F_{1,40}=0.7; p=0.41$) or interaction ($F_{1,40}=1.1; p=0.31$) effects were detected. The 2-factor ANOVAs on the maximum change from baseline diastolic blood pressure (DBP) and heart rate (HR) detected no main effects of DRUG (DBP: $F_{1,40}=2.6; p=0.12$; HR: $F_{1,40}=3.4; p=0.07$) or GROUP (DBP: $F_{1,40}=0.1; p=0.76$; HR: $F_{1,40}=0.07; p=0.79$) and no interaction effect (DBP: $F_{1,40}=0.04; p=0.84$; HR: $F_{1,40}=0.2; p=0.69$). All mean SBP, DBP and HR maximum change from baseline scores with the GROUP, DRUG and interaction $p$-values are summarized in Table 3.

Table 3: Results summary of 2-factor ANOVAs (GROUP (AD, AD/MDD) x DRUG (placebo, d-amph)) on SBP, DBP and HR

<table>
<thead>
<tr>
<th>Maximum change from baseline</th>
<th>AD (Mean±SD)</th>
<th>AD/MDD (Mean±SD)</th>
<th>GRP effect $p$-value</th>
<th>DRUG effect $p$-value</th>
<th>Inter. effect $p$-value</th>
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<tr>
<td></td>
<td>placebo n=10</td>
<td>d-amph n=8</td>
<td>placebo n=10</td>
<td>d-amph n=12</td>
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<tr>
<td>SBP (mmHg)</td>
<td>0.0±19.2</td>
<td>31.0±11.5</td>
<td>1.2±20.1</td>
<td>19.4±22.6</td>
<td>0.41 &lt;0.001 0.31</td>
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<tr>
<td>DBP (mmHg)</td>
<td>2.4±17.9</td>
<td>12.5±12.8</td>
<td>5.3±16.6</td>
<td>13.1±19.7</td>
<td>0.76 0.12 0.84</td>
</tr>
<tr>
<td>HR (beats/min.)</td>
<td>-11.4±13.2</td>
<td>1.3±17.9</td>
<td>-7.6±21.5</td>
<td>0.5±17.1</td>
<td>0.79 0.07 0.69</td>
</tr>
</tbody>
</table>
3A.1.d.1 ARCI Scales

The 2-factor ANOVA (GROUP (alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, dextroamphetamine)) on the maximum change from baseline score on all ARCI scales detected DRUG effects on all rewarding ARCI scales (Stimulation Motor, Stimulation Euphoria, Abuse Potential, Amphetamine, Morphine Benzedrine Group and Benzedrine Group) (p<0.005). Main effects of GROUP were detected on the following ARCI scales: Unpleasantness-Dysphoria, Stimulation-Euphoria, Abuse Potential, Amphetamine, Pentobarbital Chlorpromazine Alcohol Group, Morphine Benzedrine Group and Benzedrine Group (p<0.05). No interaction effect was detected on any of the ARCI scales. All mean ARCI scales scores with the GROUP, DRUG and interaction p-values are summarized in Table 4.

3A.1.d.2 ARCI Rewarding Effects Composite

The Cronbach α coefficient for the ARCI Rewarding Effects Composite was 0.95. The 2-factor ANOVA (GROUP (alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, dextroamphetamine)) on the ARCI Rewarding Effects Composite dependent variable detected main effects for DRUG (F_{1,40}=18.6; p<0.001) and GROUP (F_{1,40}=16.6; p<0.001) factors but no interaction effect was detected (F_{1,40}=0.02; p=0.88) (Figure 1). Results of the 2-factor ANOVA on ARCI Rewarding Effects Composite are summarized in Table 4.

Pearson Correlation tests revealed that the ARCI Rewarding Effects Composite scores of the alcohol-dependent and depressed alcohol-dependent participants who received
dextroamphetamine did not correlate with the baseline SHAPS ($r=0.21; \ p=0.37$) or the psychomotor retardation sub-score of the SPARQ ($r=0.30; \ p=0.20$) scores.

3A.1.d.3 ARCI Negative Effects Composite

The Cronbach $\alpha$ coefficient for the ARCI Negative Effects Composite was 0.91. The 2-factor ANOVA (GROUP (alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, dextroamphetamine)) on the ARCI Negative Effects Composite dependent variable detected no main effects of DRUG ($F_{1,40}=0.9; \ p=0.35$) or GROUP ($F_{1,40}=3.4; \ p=0.07$) as well as no interaction effect ($F_{1,40}=0.3; \ p=0.58$) (Table 4).
Table 4: Results summary of 2-factor ANOVAs (GROUP (AD, AD/MDD) x DRUG (placebo, d-amph)) on ARCI scales

<table>
<thead>
<tr>
<th>ARCI maximum change from baseline</th>
<th>AD (Mean±SD)</th>
<th>AD/MDD (Mean±SD)</th>
<th>GRP effect p-value</th>
<th>DRUG effect p-value</th>
<th>Inter. effect p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEDMOT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>placebo n=10</td>
<td>1.2±4.8</td>
<td>1.9±4.4</td>
<td>0.42</td>
<td>0.99</td>
<td>0.25</td>
</tr>
<tr>
<td>d-amph n=8</td>
<td>3.5±5.1</td>
<td>-0.4±8.4</td>
<td>0.06</td>
<td>0.30</td>
<td>0.81</td>
</tr>
<tr>
<td>SEDMENT</td>
<td>2.8±10.1</td>
<td>-2.8±9.0</td>
<td>0.51</td>
<td>0.30</td>
<td>0.93</td>
</tr>
<tr>
<td>placebo n=10</td>
<td>0.1±8.6</td>
<td>-7.1±12.3</td>
<td>0.04</td>
<td>0.58</td>
<td>0.41</td>
</tr>
<tr>
<td>UNPHYS</td>
<td>2.4±4.1</td>
<td>1.3±5.6</td>
<td>0.06</td>
<td>0.005</td>
<td>0.81</td>
</tr>
<tr>
<td>placebo n=10</td>
<td>0.8±2.8</td>
<td>4.9±3.8</td>
<td>0.06</td>
<td>0.005</td>
<td>0.805</td>
</tr>
<tr>
<td>UNDYS</td>
<td>0.7±2.7</td>
<td>-1.4±5.1</td>
<td>0.008</td>
<td>0.001</td>
<td>0.805</td>
</tr>
<tr>
<td>placebo n=10</td>
<td>1.1±3.8</td>
<td>-3.6±6.4</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td>0.75</td>
</tr>
<tr>
<td>STIMOT</td>
<td>0.3±2.4</td>
<td>1.9±2.5</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.88</td>
</tr>
<tr>
<td>placebo n=10</td>
<td>2.9±2.4</td>
<td>4.9±3.8</td>
<td>0.02</td>
<td>0.17</td>
<td>0.85</td>
</tr>
<tr>
<td>placebo n=10</td>
<td>9.3±9.3</td>
<td>17.3±10.8</td>
<td>0.002</td>
<td>0.001</td>
<td>0.55</td>
</tr>
<tr>
<td>STIEUPH</td>
<td>-1.6±5.4</td>
<td>7.5±10.3</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.58</td>
</tr>
<tr>
<td>placebo n=10</td>
<td>9.3±9.3</td>
<td>17.3±10.8</td>
<td>0.002</td>
<td>0.001</td>
<td>0.58</td>
</tr>
<tr>
<td>ABPOT</td>
<td>-2.7±3.6</td>
<td>4.2±4.5</td>
<td>0.79</td>
<td>0.67</td>
<td>0.25</td>
</tr>
<tr>
<td>placebo n=10</td>
<td>2.8±6.9</td>
<td>10.2±6.2</td>
<td>0.04</td>
<td>0.35</td>
<td>0.58</td>
</tr>
<tr>
<td>AMPH</td>
<td>-3.3±6.1</td>
<td>4.7±8.1</td>
<td>0.07</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>placebo n=10</td>
<td>7.9±9.0</td>
<td>14.3±7.3</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>PCAG</td>
<td>4.1±8.9</td>
<td>-6.7±8.4</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>placebo n=10</td>
<td>-2.1±9.9</td>
<td>-11.4±17.7</td>
<td>0.001</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>MBG</td>
<td>-4.7±8.1</td>
<td>21.0±10.0</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>placebo n=10</td>
<td>4.5±12.9</td>
<td>20.1±10.0</td>
<td>0.001</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>BG</td>
<td>-2.4±6.4</td>
<td>14.8±8.0</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>placebo n=10</td>
<td>5.4±7.1</td>
<td>0.2±8.7</td>
<td>0.002</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>1.8±6.5</td>
<td>0.2±8.7</td>
<td>0.79</td>
<td>0.67</td>
<td>0.25</td>
</tr>
<tr>
<td>placebo n=10</td>
<td>3.5±5.6</td>
<td>0.2±8.7</td>
<td>0.07</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>*ARCI Rewarding</td>
<td>43.5±66.9</td>
<td>275.1±89.8</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.88</td>
</tr>
<tr>
<td>placebo n=10</td>
<td>158.3±102.7</td>
<td>151.8±85.5</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>*ARCI Negative</td>
<td>123.2±103.0</td>
<td>15.3±157.7</td>
<td>0.07</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>placebo n=10</td>
<td>108.5±90.1</td>
<td>73.3±105.3</td>
<td>0.07</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

SEDMOT: Sedation-Motor; SEDMENT: Sedation-Mental; UNPHYS: Unpleasantness-Physical; UNDYS: Unpleasantness-Dysphoria; STIMOT: Stimulation-Motor; STIEUPH: Stimulation-Euphoria; ABPOT: Abuse Potential; AMPH: Amphetamine; PCAG: Pentobarbital Chlorpromazine Alcohol Group; MBG: Morphine Benzedrine Group; BG: Benzedrine Group; *ARCI Rewarding: ARCI Rewarding Effects Composite (STIEUPH+ABPOT+AMPH+MBG+BG at the % scale); *ARCI Negative: ARCI Negative Effects Composite (SEDMOT+SEDMENT+UNPHYS+UNDYS+PCAG+LSD at the % scale)
Figure 1: Degree of rewarding effects experienced by participants as measured by the maximum change from baseline Addiction Research Center Inventory (ARCI) Rewarding Effects Composite score vs. participants group (alcohol-dependent or co-morbid depressed alcohol-dependent). In the GROUP (alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, d-amph) ANOVA, main effects were detected for GROUP and DRUG (***p<0.001) factors but no interaction effects were detected. Error bars represent SD.
3A.1.e Profile of Mood States

3A.1.e.1 POMS Scales

The 2-factor ANOVA (GROUP (alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, dextroamphetamine)) on the maximum change from baseline score on all POMS scales detected DRUG effects only on Vigor, Elation and Arousal scales (p<0.01). Main effects of GROUP were detected on all POMS scales (p<0.05) except on Tension-Anxiety, Friendliness and Confusion scales. No interaction effect was detected on any of the POMS scales. All mean POMS scales scores with the GROUP, DRUG and interaction p-values are summarized in Table 5.

3A.1.e.2 POMS Rewarding Effects Composite

The Cronbach α coefficient for the POMS Rewarding Effects Composite was 0.86. Similar to the ARCI Rewarding Effects Composite results, the 2-factor ANOVA (GROUP (alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, dextroamphetamine)) on the POMS Rewarding Effects Composite dependent variable detected main effects for DRUG (F_{1,40}=10.0; p=0.003) and GROUP (F_{1,40}=15.3; p<0.001) factors but no interaction effect was detected (F_{1,40}=2.2; p=0.14) (Table 5).
Table 5: Results summary of 2-factor ANOVAs (GROUP (AD, AD/MDD) x DRUG (placebo, d-amph)) on POMS scales

<table>
<thead>
<tr>
<th>POMS maximum change from baseline</th>
<th>AD (Mean±SD)</th>
<th>AD/MDD (Mean±SD)</th>
<th>GRP effect p-value</th>
<th>DRUG effect p-value</th>
<th>Inter. effect p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>placebo n=10</td>
<td>d-amph n=8</td>
<td>placebo n=10</td>
<td>d-amph n=12</td>
<td></td>
</tr>
<tr>
<td>TENANX</td>
<td>-1.9±4.2</td>
<td>-0.3±3.2</td>
<td>-3.3±2.9</td>
<td>-2.8±6.5</td>
<td>0.19</td>
</tr>
<tr>
<td>ANGHOS</td>
<td>1.1±2.2</td>
<td>-0.3±1.6</td>
<td>-3.4±6.7</td>
<td>-6.4±8.4</td>
<td>0.007</td>
</tr>
<tr>
<td>DEPDEJ</td>
<td>-1.8±3.3</td>
<td>-1.9±5.5</td>
<td>-9.5±8.8</td>
<td>-14.3±12.5</td>
<td>0.001</td>
</tr>
<tr>
<td>FRIEND</td>
<td>-4.6±6.2</td>
<td>-3.0±9.7</td>
<td>-2.1±7.0</td>
<td>7.8±5.5</td>
<td>0.20</td>
</tr>
<tr>
<td>FATIGUE</td>
<td>1.4±7.3</td>
<td>-1.0±2.0</td>
<td>-2.0±8.3</td>
<td>-8.3±8.0</td>
<td>0.03</td>
</tr>
<tr>
<td>CONF</td>
<td>-0.8±1.8</td>
<td>-2.1±2.0</td>
<td>-0.6±4.2</td>
<td>-2.5±7</td>
<td>0.95</td>
</tr>
<tr>
<td>VIGOR</td>
<td>-4.9±3.4</td>
<td>-0.6±9.3</td>
<td>2.4±6.1</td>
<td>10.8±7.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ELATION</td>
<td>-0.4±3.2</td>
<td>2.0±4.8</td>
<td>2.1±4.5</td>
<td>7.4±4.6</td>
<td>0.007</td>
</tr>
<tr>
<td>*AROUSAL</td>
<td>-3.0±9.8</td>
<td>2.4±10.0</td>
<td>2.6±13.9</td>
<td>21.8±17.7</td>
<td>0.007</td>
</tr>
<tr>
<td>*POSMOOD</td>
<td>1.7±4.0</td>
<td>3.0±8.8</td>
<td>9.8±12.3</td>
<td>20.2±15.8</td>
<td>0.002</td>
</tr>
<tr>
<td>*POMS Rewarding</td>
<td>-31.4±35.0</td>
<td>-3.0±74.8</td>
<td>9.7±56.1</td>
<td>89.0±46.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

TENS-ANX: Tension-Anxiety; ANGHOS: Anger-Hostility; DE-DEJ: Depression-Dejection; FRIEND: Friendliness; CONF: Confusion; *AROUSAL: TENANX+VIGOR+DEPDEJ+CONF; *POSMOOD: Positive Mood (ELATION-DEPDEJ); *POMS Rewarding: POMS Rewarding Effects Composite (FRIEND+VIGOR+ELATION).
3A.1.f Visual Analog Scales

The 2-factor ANOVA (GROUP (alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, dextroamphetamine)) on the maximum change from baseline score on all VAS scales detected DRUG effects on “I feel an increase of energy”, “I feel a drug effect” and “I feel the drug’s good effects” scales (p<0.05). Main effects of GROUP were detected on “I feel anxious”, “I feel irritable” and “I feel an increase of energy” scales (p<0.05). No interaction effect was detected on any of the VAS scales. All mean VAS scales scores with the GROUP, DRUG and interaction p-values are summarized in Table 6.

Table 6: Results summary of 2-factor ANOVAs (GROUP (AD, AD/MDD) x DRUG (placebo, d-amph)) on VAS scales

<table>
<thead>
<tr>
<th>VAS scale maximum change from baseline</th>
<th>AD (Mean±SD)</th>
<th>AD/M DD (Mean±SD)</th>
<th>GRP effect p-value</th>
<th>DRUG effect p-value</th>
<th>Inter. effect p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>placebo n=10</td>
<td>d-amph n=8</td>
<td>placebo n=10</td>
<td>d-amph n=12</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>15.7±28.2</td>
<td>27.5±22.1</td>
<td>16.3±19.6</td>
<td>38.4±44.9</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxious</td>
<td>1±7.4</td>
<td>9.5±12.4</td>
<td>-33.4±35.5</td>
<td>-7.3±46.6</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritable</td>
<td>13.2±20.2</td>
<td>0.1±6.9</td>
<td>-15.8±31.6</td>
<td>-20.0±43.1</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.38</td>
<td>0.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alert</td>
<td>-23.6±52.2</td>
<td>-17.8±60.7</td>
<td>-16.0±30.4</td>
<td>33.1±50.8</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>0.09</td>
<td>0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restless</td>
<td>21.8±25.1</td>
<td>5.5±28.2</td>
<td>-9.3±36.5</td>
<td>1.9±50.1</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>0.83</td>
<td>0.26</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>↑Energy</td>
<td>-3.0±35.9</td>
<td>51.9±31.9</td>
<td>36.0±25.3</td>
<td>58.5±37.7</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>↑Thinking</td>
<td>30.7±31.6</td>
<td>26.5±43.3</td>
<td>18.3±36.4</td>
<td>46.7±49.5</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>0.37</td>
<td>0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug effect</td>
<td>31.0±33.7</td>
<td>58.6±36.4</td>
<td>41.7±37.9</td>
<td>69.8±35.7</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug liking</td>
<td>20.3±23.7</td>
<td>24.1±38.0</td>
<td>27.8±38.5</td>
<td>51.2±25.9</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>0.19</td>
<td>0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good effects</td>
<td>22.3±37.6</td>
<td>45.0±42.5</td>
<td>38.9±37.1</td>
<td>65.3±34.4</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>0.048</td>
<td>0.88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bad effects</td>
<td>2.7±33.1</td>
<td>15.0±26.8</td>
<td>16.7±23.2</td>
<td>29.9±29.9</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>0.17</td>
<td>0.96</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3A.2 Post-hoc Study: CON vs. MDD vs. AD vs. AD/MDD

Data from 22 participants with major depressive disorder and 20 controls were collected under the same experimental conditions [20] and computed in the same way as data from the alcohol-dependent and depressed alcohol-dependent groups. This allowed comparison among the four groups. This resulted in the analysis of data from 8 study arms: control-placebo (n=10), control-dextroamphetamine (n=10), major depressive disorder-placebo (n=11), major depressive disorder-dextroamphetamine (n=11), alcohol dependence-placebo (n=10), alcohol dependence-dextroamphetamine (n=8), alcohol dependence/major depressive disorder-placebo (n=10), and alcohol dependence/major depressive disorder-dextroamphetamine (n=12).

There were no differences in baseline characteristics between the placebo and dextroamphetamine arms in the only-depressed or the control groups. Baseline ARCI Rewarding Effects Composite scores between the placebo and dextroamphetamine arms within the 4 groups were not different.

The number of females in the depressed group was higher than in the other groups. There were significant sex and Fagerström Test for Nicotine Dependence scores (FTND) differences among the 4 groups. There also were significant age and education (years after high school) differences between the control group and the 3 others (depressed, alcohol-dependent, co-morbid depressed alcohol-dependent). Since sex, level of tobacco dependence, age, and education could have influences on our study results, they were included as covariates in the 2-factor ANOVA (GROUP (control, depressed, alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, dextroamphetamine)) on our main outcome measure: ARCI Rewarding Effects Composite. The covariate analysis indicated that sex,
Fagerström Test for Nicotine Dependence scores, age, and education did not alter the ARCI Rewarding Effects Composite measure.

### 3A.2.a ARCI Rewarding Effects Composite

The Cronbach $\alpha$ coefficient for the ARCI Rewarding Effects Composite was 0.94. The 2-factor ANOVA (GROUP (control, depressed, alcohol-dependent, depressed alcohol-dependent) $\times$ DRUG (placebo, dextroamphetamine)) on the ARCI Rewarding Effects Composite dependent variable detected main effects for DRUG ($F_{1,82}=30.0; p<0.001$) and GROUP ($F_{3,82}=7.2; p<0.001$) factors. No interaction effect was detected ($F_{3,82}=0.4; p=0.79$).

Although there was a similar increase in dextroamphetamine response compared to placebo in the four groups (DRUG effect but no interaction effect), depressed and co-morbid depressed alcohol-dependent groups exhibited similar responses to dextroamphetamine as well as to placebo while the control and alcohol-dependent groups appeared to respond similarly (GROUP effect) (Figure 2).
Figure 2: Degree of rewarding effects experienced by participants as measured by the maximum change from baseline Addiction Research Center Inventory (ARCI) Rewarding Effects Composite score vs. participants group (control, depressed, alcohol-dependent or co-morbid depressed alcohol-dependent). In the GROUP (control, depressed, alcohol-dependent, depressed alcohol-dependent) x DRUG (placebo, d-amph) ANOVA, main effects were detected for GROUP (***p<0.001) and DRUG (***p<0.001) factors but no interaction effects were detected. Error bars represent SD. Non-filled bars represent participants in the control and depressed groups from a previous study in our research group using the same protocol.
3A.3 Influence of Depression Severity: AD vs. AD/MDD severe

In the following ANOVA, only participants with HAM-D scores ≥23 (severe depression) were included in the depressed alcohol-dependent group (n=12 instead of 22). The Cronbach α coefficient for the ARCI Rewarding Effects Composite was 0.92. The 2-factor ANOVA (GROUP (alcohol-dependent, severely depressed alcohol-dependent) x DRUG (placebo, dextroamphetamine)) on the ARCI Rewarding Effects Composite dependent variable detected main effects for DRUG (F_{1,30}=11.2; \ p=0.003) and GROUP (F_{1,30}=8.8; \ p=0.006) factors but no interaction effect was detected (F_{1,30}=0.04; \ p=0.84).

The mean response to dextroamphetamine rewarding effects did not increase in the severely depressed alcohol-dependent group (n=12) compared to the whole group (n=22) (Figure 3B).
Figure 3: Degree of rewarding effects experienced by participants as measured by the maximum change from baseline Addiction Research Center Inventory (ARCI) Rewarding Effects Composite score vs. participants group (A: control and severely depressed; B: alcohol-dependent and severely depressed alcohol-dependent). In Figure 3A, the MDD severe group is a sub-group of the MDD group (Figure 2) including only those with severe depression. In Figure 3B, the AD/MDD severe group is a sub-group of the AD/MDD group (Figure 2) including only those with severe depression. Severe depression: HAM-D score ≥23. Error bars represent SD. Non-filled bars represent participants in the control and depressed groups from a previous study in our research group using the same protocol [20]. The previous study showed a significant differential response to dextroamphetamine rewarding effects compared to placebo between the severely depressed (MDD severe) and control participants (* = GROUP x DRUG interaction, p<0.05). In the GROUP (alcohol-dependent, severely depressed alcohol-dependent) x DRUG (placebo, d-amph) ANOVA with only severely depressed individuals, main effects were detected for GROUP (***p<0.001) and DRUG (***p<0.001) factors but no interaction effects were detected.
FMRI STUDY: Probing Mesocorticolimbic Dopamine Function in Alcohol Dependence Using Dextroamphetamine and FMRI Cue-Induced Brain Activation.

The primary purpose of the FMRI Study was to determine the neural correlates of the altered behavioural response to dextroamphetamine in alcohol-dependent and depressed alcohol-dependent participants. However, there was no altered behavioural response detected in these two groups (no GROUP x DRUG interaction), which made it less compelling to investigate these groups.

Therefore, the FMRI Study was conceptualized and designed as an exploratory study focusing on how brain areas of the mesocorticolimbic circuitry would respond to a dextroamphetamine challenge in alcohol dependence (with or without major depressive disorder) when exposed to alcohol visual cues. As mentioned in the literature review section, validated alcoholic beverage pictures have been shown to activate important areas of the mesocorticolimbic dopamine system such as the ventral striatum and the orbitofrontal cortex and therefore they allow assessing brain circuits involved in the processing and evaluation of alcohol cues [22, 30, 31]. Using an alcohol cue exposure paradigm would allow us to probe mesocorticolimbic dopamine function in alcohol dependence by investigating the modulatory effects of dextroamphetamine on mesocorticolimbic circuitry activation induced by alcohol visual cues. We included a control group in the FMRI Study, composed of social drinkers (<14 drinks/week), in order to compare the effect of a single oral dose of dextroamphetamine on cue-induced mesocorticolimbic circuitry activation with the alcohol-dependent group, hypothesizing a disruption in this circuitry would be revealed in alcohol dependence.
FMRI STUDY OBJECTIVE:
To assess how brain areas of the mesocorticolimbic dopamine system would respond to a dextroamphetamine challenge in alcohol-dependent participants compared to control participants exposed to alcohol visual cues.

FMRI STUDY HYPOTHESIS:
Cue-induced BOLD response and dextroamphetamine would reveal a disruption in mesocorticolimbic circuitry in alcohol-dependent participants compared to controls.
Section 2B MATERIALS AND METHODS

2B.1 Study design

This study was a single-blind, between-subject study. After an assessment session determining eligibility, subjects participated in a study session. On the study day, outside the scanner at baseline, pre-scan procedures included a series of symptom severity assessments as well as dextroamphetamine subjective effects measures. Some measures were repeated post-dextroamphetamine effects post-scan.

In the scanner, fMRI data were acquired while control and alcohol-dependent participants were presented with a validated alcohol visual cue exposure task before and after dextroamphetamine peak effect (~ 90min).

2B.2 Participant selection

Participants were all males; aged 19-65 years. All participants in the control and alcohol-dependent groups were right-handed. Alcohol-dependent participants met DSM-IV criteria for alcohol dependence [32], which was confirmed using the Structured Clinical Interview for DSM-IV for Axis I disorders (SCID-IV) [353], and were not receiving pharmacological treatment for alcohol dependence (e.g. acamprosate). Control participants were social drinkers (<14 drinks/week) and they did not have a personal history of substance abuse/dependence (except for tobacco in smokers) or other psychiatric disorders. Exclusion criteria included the following: current or past history of a cardiovascular disorder; medical conditions requiring immediate investigation or treatment; positive urine screen for psychoactive drugs (other than nicotine); recent use (within last three months) of any illicit drugs such as cocaine, amphetamine, heroin; recent (< 1 yr)/current history of drug abuse or dependence on a substance (other than alcohol in the alcohol-dependent group, caffeine, or
tobacco); current use of any medication known to interact with the study drug (e.g. antihypertensives, sedative hypnotics, antipsychotics) (see Appendix A for list of medications known to interact with dextroamphetamine); current suicidal ideation posing immediate threat to the participant's life; co-morbid DSM-IV Axis I (except for major depressive disorder) or Axis II mental illness; psychotic depression; and MRI exclusion criteria (e.g. metal in body, tattoos on the neck and/or face). All participants were recruited through city newspaper advertisements.

The experimental protocol was approved by the research ethics board of Sunnybrook Health Sciences Centre. Signed informed consent was obtained from all participants prior to participating in the study (see FMRI Study consent form in Appendix D).

2B.3 Study Session

Participants arrived between 8 and 10 AM on the study session day. They were instructed not to consume alcohol, nicotine or caffeine for 12 hours prior to arriving. A breathalyzer (Alert, Alcohol Countermeasure Systems, Canada) was used in the alcohol-dependent group to verify participants’ sobriety on study day. A urine sample was collected for toxicology screening (see Appendix C for substances that can be detected by the urine toxicology screen). Participants were evaluated for alcohol withdrawal using the Clinical Institute Withdrawal Assessment for Alcohol-Revised (CIWA-Ar) [338]. Those reporting significant withdrawal symptoms would be excluded. After a light standardized breakfast, self-report instruments were administered at baseline. The Alcohol Dependence Scale (ADS) [339, 340], the Beck Depression Inventory (BDI) [342], the Fagerström Test for Nicotine Dependence (FTND) [341], and the Alcohol Craving Questionnaire-NOW (ACQ-NOW) [354] were administered to evaluate alcohol dependence, depression, tobacco dependence and alcohol
craving severities respectively. Details about the CIWA-Ar, the ADS, the BDI, and the FTND can be found in section 2A.4 (Symptom Severity Assessments). The ACQ-NOW is a 47-item self-administered, multidimensional state measure of acute alcohol craving. It assesses the multidimensional aspects of alcohol craving among current users and is a measure of acute alcohol craving [355-357]. These tests were conducted outside of the scanner at baseline.

After this testing, control and alcohol-dependent participants were administered a single oral 30 mg dose of dextroamphetamine. Dextroamphetamine sulfate (Dexedrine, GlaxoSmithKline Inc., Mississauga, Ontario, Canada) doses were over-encapsulated to establish single-blind conditions. A research pharmacist dispensed the medication. The dose is within the therapeutic daily dose range (5 to 60 mg) [266].

The scanning procedure lasted approximately 2 hours during which participants performed a battery of tasks at baseline and at presumed peak drug effect as was previously demonstrated by Tremblay and colleagues [20, 21] (90 minutes after drug administration) while fMRI data were acquired. At baseline, participants performed an alcohol cue exposure task (detailed in section 2B.3.a fMRI task). This task was repeated at 90 minutes after drug administration (peak dextroamphetamine effect).

Subjective ratings for drug effect (Visual Analog Scales (VAS) and Addiction Research Center Inventory (ARCI)) were recorded at baseline and at approximately 140 min post-drug outside of the scanner. Details about the VAS [349-352] and ARCI [346-348] drug effects measurement tools can be found in section 2A.6 (Dextroamphetamine Effects Measurement Tools). The ACQ also was administered post-dextroamphetamine effect, out of the scanner, at around 140 min post-drug. Baseline systolic and diastolic blood pressures
were recorded in the scanner and were repeated at the end of the scanning procedure – in the scanner - at approximately 120 min post-drug. For a condensed form of the study procedure, see Figure 4: FMRI Study session summary.
Figure 4: FMRI Study session summary. The study day protocol involved both in and out of scanner procedures. The pre-scan procedures included a breathalyzer measurement to confirm participants’ sobriety, a urine toxicology screen, a blood pressure (BP) measure as well as several measures to characterize the study participants such as the Alcohol Dependence Scale (ADS), the Beck Depression Inventory (BDI), the Fagerström Test for Nicotine Dependence (FTND), the Alcohol Craving Questionnaire (ACQ), the Addiction Research Center Inventory (ARCI) and Visual Analog Scales (VAS). Some post-scan measures were repeated outside the scanner (BP, ACQ, ARCI, and VAS). Inside the triangle are the in-scanner procedures. FMRI data were acquired while participants were presented with validated alcohol cues and neutral cues before and after dextroamphetamine peak effect (~ 90 min). An anatomical scan was performed between the baseline and the peak dextroamphetamine scans. During the anatomical scan, participants were performing on the ARCI (~ 60 min).
2B.3.a FMRI task

The alcohol cue exposure task was presented using a block design. It consisted of rating a set of validated images containing affectively neutral, abstract, and alcohol-associated images, which delineate the three rating conditions. The standardized alcohol-associated images [30, 31] were generously provided by Dr. Jana Wrase and Dr. Andreas Heinz from Charité University Medical Center in Berlin, Germany. The selected alcohol pictures have previously been shown to elicit significant activation in mesocorticolimbic and other regions in alcohol-dependent participants [30, 31]. The alcohol cue exposure task block-design consisted of 15 stimulation blocks alternating with fixation blocks (fixation cross). Hence, there were 5 blocks for each of the 3 conditions. Condition order was pseudo-randomized across the 15 stimulation-fixation cycles. In each block, 3 images from one of the 3 categories (affectively neutral, abstract, and alcohol-associated) were presented sequentially for 4 seconds, resulting in a total duration of 12 seconds per block. The total duration of the alcohol cue exposure task was 7 minutes and 50 seconds. An International Affective Picture System task [323], a counting and emotional Stroop task [358, 359] as well as finger tapping and visual pattern tasks were included as “filler” tasks in order to reach dextroamphetamine peak effect while keeping participants’ attention and avoid states of inattention (resting states) that can affect spontaneous BOLD oscillations [360]. Accordingly, the IAPS, Stroop, finger-tapping and visual pattern tasks data were not analyzed for the purpose of this thesis.

2B.3.b FMRI setup

The alcohol cue exposure task was programmed using E-Prime (version 1.1; Psychology Software Tools, Inc, Pittsburgh, Pennsylvania) to control timing of stimulus presentation and to measure accuracy, picture ratings, and reaction times. Participants were positioned supine
on the scanner bed and outfitted with the MR-compatible bi-manual button boxes and LCD viewing goggles. Participant responses were recorded using the button boxes, which contained 2 buttons for each hand (Lumitouch; Light Wave Technology Inc, Surrey, British Columbia) and provided measurements of accuracy and reaction times. Participants were instructed to interpret and capture the images and to rate how much craving for alcohol each image elicited by pressing 1 of the 4 available buttons, i.e. not at all, a little, somewhat, or very much. The LCD goggles were connected to an LCD projector (Silent Vision, model SV022; Avotec, Inc, Jensen Beach, Florida) and were adjustable for visual acuity.

2B.3.c Imaging parameters

Imaging data were collected using a 3.0 T scanner (General Electric Medical Systems, Waukesha, Wisconsin). High resolution T₁-weighted anatomical volumes were obtained for each participant using a three dimensional T₁-weighted spoiled gradient recall echo sequence with the following parameters: TI/TR/TE/FA = 300 ms/7.0 ms/3.1 ms/15°, voxel dimensions of 0.86 mm x 1.15 mm x 1.4 mm, field-of-view (FOV) = 220-mm, matrix size = 256 x 192 x 128). Next, a T₂*-weighted gradient-echo spiral in-out pulse sequence was prescribed and higher order shimmed for the functional trials [361]. Acquisition parameters were as follows: TR/TE/FA = 2000 ms/30 ms/70°, in-plane voxel resolution = 3.1 mm x 3.1 mm x 5 (no skip), FOV = 200 mm, slices/TR = 26. As described by Glover and Thomason [361], spiral IO data with signal-weighted averaging increases the signal-to-noise ratio significantly in high-susceptibility areas such as the ethmoid sinuses compared to conventional spiral out sequences, without sacrificing temporal resolution. In the 7 minute 50 sec scanning session comprising the alcohol cue exposure task, 232 volumes were acquired, of which the first 15 volumes were discarded (for a total of 217 reconstructed time-points).
2B.4 Data analysis

2B.4.a Subjective and physiological data

Statistical analysis of demographics, alcohol cue exposure task cue rating data, reaction time, subjective data (VAS, ARCI and ACQ) and physiological data were conducted using SPSS (version 15.0; SPSS Inc, Chicago, Illinois).

Independent t-tests were used to compare the two groups in terms of age, education, and Fagerström Test for Nicotine Dependence. Repeated-measures ANOVAs (((GROUP (control, alcohol-dependent) x DRUG (pre-drug, post-drug)) on alcohol cue exposure task subjective measures (cue rating), reaction times, subjective data (VAS and ACQ total scores) and on systolic and diastolic blood pressures were performed. An ARCI Rewarding Effects Composite measure was defined the same way as detailed in section 2A.7 Data Analysis, in the Behavioural Study. A repeated-measures ANOVA (((GROUP (control, alcohol-dependent) x DRUG (pre-drug, 60 min post-drug, 140 min post-drug) on ARCI Rewarding Effects Composite scores also was performed.

2B.4.b FMRI data

Functional activation was determined from the blood oxygenation level-dependent (BOLD) signal using the software Statistical Parametric Mapping (SPM5, University College London, UK; http://www.fil.ion.ucl.ac.uk). Following image reconstruction, the time series data for each participant were motion-corrected (translational motion parameters were less than one voxel for all included participants) and co-registered with their T1-weighted structural image. The T1 image was then normalized into standard atlas (Montreal Neurological Institute (MNI)) space. Warping parameters obtained from the T1 normalization process
were subsequently applied to the time-series data (resampling to 2 mm$^3$ voxels). The time-series data were spatially smoothed to an 8 mm$^3$ full-width half maximum Gaussian kernel.

We constructed a custom brain template from the alcohol-dependent participants’ T$_1$ volumes. The T$_1$ template was segmented into separate gray and white matter tissue maps. To ensure that functional data originating from each group were estimated from a common volumetric space, the gray matter map was then applied as an explicit mask to all participants’ first level statistical parametric maps.

Single subject time series data were submitted to general linear statistical models [362] examining the alcohol cue exposure. The task-specific boxcar stimulus functions were convolved with the canonical hemodynamic response function (HRF). Each model included within-session global scaling (default), high-pass filtering to remove low-frequency signal drift (period = 128 s), and the AR1 method of estimating temporal autocorrelation. For each participant, 2 individual t-contrasts were specified for the alcohol cue exposure task statistical model: [alcohol cues > neutral cues] and [alcohol cues > abstract cues]. The [alcohol cues > neutral cues] contrast was our primary contrast of interest as it is the most widely used in cue-induced reactivity studies in alcohol-dependent participants [22, 30, 31].

To compare group-dependent effects of dextroamphetamine administration, the above first-level contrasts were subsequently entered into separate GROUP (control, alcohol-dependent) x DRUG (pre-drug effect, post-drug effect) factorial ANOVAs. Inference of statistical significance for the interaction F-tests effects was assessed using an uncorrected p value < 0.001 and voxel extent of 10 as base thresholds. All group level t-contrasts used a $p_{uncorrected}$ < 0.001 and voxel extent of 10 as base thresholds.
Control and alcohol-dependent groups differed at baseline in measures of depression (indexed using the Beck Depression Inventory) and level of nicotine dependence (indexed using the Fagerström Test for Nicotine Dependence). Accordingly, these measures were included as covariates in the ANOVA model to ensure that differential effects in BOLD signal were not attributable to depressive symptomatology or level of nicotine dependence. The BDI and the Fagerström Test for Nicotine Dependence scores were also included as covariates in all subjective and physiological data analyses.

Section 3B RESULTS

3B.1 Participants

There were 14 male alcohol-dependent and 9 male healthy control participants; matched by age and smoking status. Eight alcohol-dependent participants fulfilled DSM-IV criteria for major depressive disorder, which is consistent with the population of Ontario, Canada where approximately more than 55% of alcohol-dependent individuals have a co-morbid mood disorder [1]. In recruiting the alcohol-dependent group, 58 individuals underwent initial screening on the telephone: 17 were eligible and the remaining potential participants were excluded, primarily because they were taking medications (e.g. antidepressants), reported another DSM-IV Axis I Illness, other than major depressive disorder (e.g. bipolar disorder) or were using illicit drugs (e.g. cocaine). Of the 17 individuals who were eligible after the telephone screening, 17 completed assessment sessions; one of them was excluded because of the presence of another Axis I disorder (bipolar disorder) and one was excluded due to hypertension. Fifteen participants completed the study but only data from 14 sessions could
be used for analysis. The data from one participant were excluded due to equipment failure on that scanning day.

3B.1.a Including AD and AD/MDD participants in one group

In the FMRI Study, the alcohol-dependent group (n=14) includes 8 participants fulfilling DSM-IV criteria for major depressive disorder on the assessment day. A decision was made to include alcohol-dependent participants with or without a co-morbid diagnosis of depression in 1 group for the reasons stated below:

1- There was no significant difference in the behavioural response to dextroamphetamine rewarding effects compared to placebo between alcohol-dependent and depressed alcohol-dependent participants (no GROUP x DRUG interaction) in the Behavioural Study.

2- Separating alcohol-dependent participants with or without a co-morbid diagnosis of depression often is questioned as most individuals with alcohol dependence have experienced major depressive episodes in their lifetime and a large proportion has depressive symptoms yet not fully fulfilling DSM-IV criteria for major depressive disorder [12, 13, 363, 364]. As such, it is suggested that a period of prolonged sobriety be awaited for a definitive diagnosis of major depressive disorder [208].

3- The alcohol-dependent and depressed alcohol-dependent participants in the FMRI Study were similar in mood rating scales scores – specifically in terms of Beck Depression Inventory (BDI) scores - as well as all other baseline characteristics (Table 7).
Table 7: Participant baseline characteristics and mood rating scales scores (AD vs. AD/MDD)

<table>
<thead>
<tr>
<th></th>
<th>AD (n=6)</th>
<th>AD/M DD (n=8)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SD</strong></td>
<td><strong>Mean ± SD</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (in years)</td>
<td>47.2 ± 8.7</td>
<td>38.9 ± 11.2</td>
<td>*0.16</td>
</tr>
<tr>
<td>Fagerström Test for Nicotine Dependence (FTND) scores</td>
<td>4.2 ± 3.4</td>
<td>3.4 ± 3.2</td>
<td>*0.66</td>
</tr>
<tr>
<td>Alcohol Dependence Scale (ADS) scores</td>
<td>21.5 ± 6.5</td>
<td>23.8 ± 7.9</td>
<td>*0.58</td>
</tr>
<tr>
<td>Beck Depression Inventory (BDI) scores</td>
<td>13.5 ± 7.1</td>
<td>18.5 ± 4.3</td>
<td>*0.13</td>
</tr>
<tr>
<td>Presence of past major depressive episode</td>
<td>4/6</td>
<td>4/8</td>
<td>†0.53</td>
</tr>
<tr>
<td>Number of past major depressive episodes</td>
<td>1.8 ± 2.1</td>
<td>3.5 ± 4.0</td>
<td>*0.33</td>
</tr>
<tr>
<td>Presence of family history of depression</td>
<td>5/6</td>
<td>6/8</td>
<td>†0.71</td>
</tr>
<tr>
<td>Presence of family history of alcoholism</td>
<td>4/6</td>
<td>7/8</td>
<td>†0.35</td>
</tr>
<tr>
<td>Number of alcoholic drinks per day</td>
<td>11.33 ± 7.03</td>
<td>7.00 ± 2.56</td>
<td>*0.20</td>
</tr>
<tr>
<td>Time since last alcoholic drink (hours)</td>
<td>32.33 ± 19.61</td>
<td>37.13 ± 16.95</td>
<td>*0.64</td>
</tr>
</tbody>
</table>

Group differences analyzed with independent t-tests (*) or with Pearson’s Chi Square tests (†). Presence of family history: first or second degree relative (Ψ).

4- In a GROUP (alcohol-dependent, depressed alcohol-dependent) x DRUG (pre-, post-drug effect) ANOVA, the interaction F-contrast showed that the [alcohol cues > neutral cues] contrast did not identify an interaction effect in any brain region (p<0.001; k>10). This suggests there was no differential BOLD response to dextroamphetamine between the alcohol-dependent and depressed alcohol-dependent participants. Additionally, no main
effect of DRUG was detected \((p<0.001; k>10)\). The main effect of GROUP F-contrast only detected greater activation in the left lingual gyrus in alcohol-dependent participants compared to depressed alcohol-dependent participants at both pre-drug and post-drug effect \((F_{1,23}=19.5; z=3.5; p<0.001; (x \ y \ z) = (-8 \ -68 \ -4)) \ (k>10)\). The lingual gyrus is part of the occipital lobe and its main function is to process visual information. It is not involved in the mesocorticolimbic dopamine system.

In order to ensure that the differential effects of BOLD signal were not attributable to participants’ depressive symptomatology in our FMRI Study analyses (ANOVA model), BDI scores were used as covariates. Indeed, clinical diagnosis of depression does not necessarily indicate biological brain differences. However, participants’ self-report mood states at the time of the scan were captured by the BDI as participants were appraising affective stimuli. Moreover, because BDI scores are a continuous measure of symptomatology (as opposed to the categorical nature of the major depressive disorder diagnosis), they are more widely used in neuroimaging studies.

### 3B.2 Demographic and Subjective data

#### 3B.2.a Demographic and Baseline Characteristics Data

No alcohol-dependent participant reported significant withdrawal symptoms on a study session day \((\text{CIWA-Ar} \geq 10)\) and none was intoxicated (as measured by breathalyzer). No significant group difference was observed in age \((t=-0.4; df=21; p=0.67)\) and education \((t=1.9; df=21; p=0.08)\) between control and alcohol-dependent participants whereas Fagerström Test for Nicotine Dependence scores were significantly different \((t=-2.9; df=19.5; p=0.009)\). As expected, the BDI scores were significantly different between control and alcohol-dependent groups \((t=-6.5; df=21; p<0.001)\) (Table 8).
<table>
<thead>
<tr>
<th></th>
<th>Control (n=9)</th>
<th>AD (n=14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (in years)</strong></td>
<td>40.3 ± 12.0</td>
<td>42.4 ± 10.7</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Years of Education</strong></td>
<td>4.6 ± 2.5</td>
<td>2.9 ± 1.9</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>(after high school)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol Dependence</strong></td>
<td>0.7 ± 0.9</td>
<td>22.8 ± 7.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Scale scores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Beck Depression</strong></td>
<td>2.1 ± 3.3</td>
<td>16.4 ± 6.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Inventory scores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fagerström Test for Nicotine Dependence</strong></td>
<td>0.9 ± 1.5</td>
<td>3.7 ± 3.2</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>scores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Number of alcoholic</strong></td>
<td>N/A</td>
<td>8.86 ± 5.25</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>drinks per day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Time since last alcoholic</strong></td>
<td>N/A</td>
<td>35.07 ± 17.57</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>drink (hours)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol cues rating</strong></td>
<td>1.4 ± 0.4</td>
<td>2.9 ± 0.9</td>
<td>* GROUP: &lt;0.001</td>
</tr>
<tr>
<td><strong>(pre-drug)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol cues rating</strong></td>
<td>1.5 ± 0.4</td>
<td>3.1 ± 0.8</td>
<td>* DRUG: 0.09</td>
</tr>
<tr>
<td><strong>(post-drug)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reaction time (alcohol</strong></td>
<td>1614.4 ± 360.8</td>
<td>1732.6 ± 462.9</td>
<td>* GROUP: 0.57</td>
</tr>
<tr>
<td><strong>cues) in ms (pre-drug)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reaction time (alcohol</strong></td>
<td>1390.6 ± 408.7</td>
<td>1581.3 ± 511.0</td>
<td>* DRUG: 0.052</td>
</tr>
<tr>
<td><strong>cues) in ms (post-drug)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Visual Analog Scale</strong></td>
<td>0.6 ± 1.7</td>
<td>0.1 ± 0.3</td>
<td>* GROUP: 0.29</td>
</tr>
<tr>
<td><strong>feel high - (pre-drug)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Visual Analog Scale</strong></td>
<td>25.8 ± 32.6</td>
<td>62.6 ± 25.2</td>
<td>* DRUG: 0.009</td>
</tr>
<tr>
<td><strong>feel high - (post-drug)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Repeated measures ANOVA with GROUP and DRUG effects p-values. Analyses include Beck Depression Inventory and Fagerström Test for Nicotine Dependence scores as covariates.
3B.2.b VAS and ARCI Data

Several of the VAS scales detected a dextroamphetamine effect. Indeed, there were main effects of DRUG detected for: “I feel high from the drug” (Table 8), “I feel anxious”, “I feel irritable”, “I feel an increase of energy”, “I feel an increase in my speed of thinking”, “I feel a drug effect” and “I feel the drug’s good effects”. There were significant GROUP main effects as well as GROUP x DRUG interaction effects in the “I feel anxious” and “I feel a drug effect” VAS scales. No main effects of DRUG, GROUP or interaction effects were detected on ARCI Rewarding Effects Composite scores.

3B.2.c Alcohol Craving Data

Performing the alcohol cue exposure task, the alcohol-dependent group reported stronger alcohol craving than the control group (alcohol cue exposure task subjective data – cue rating -) indicative of groups’ difference (Table 8): there were main effects of GROUP (F_{1,19}=22.7; p<0.001) but no DRUG (F_{1,19}=3.1; p=0.09) or GROUP x DRUG interaction effects (F_{1,19}=2.2; p=0.16) detected. Moreover, there was no significant difference in the mean reaction time between the two groups when they were presented with alcohol cues (F_{1,19}=0.3; p=0.57) but an almost significant DRUG effect (F_{1,19}=4.3; p=0.052) was detected with an increase in reaction time in all participants. However, no GROUP x DRUG interaction effect (F_{1,19}=0.4; p=0.55) was detected (Table 8). Thus, reaction time may not be an important factor in any neural differences detected. With regards to the Alcohol Craving Questionnaire (ACQ) total scores, there were main effects of GROUP (F_{1,19}=13.1; p=0.002) and DRUG (F_{1,19}=4.9; p=0.04), and GROUP x DRUG interaction effects (F_{1,19}=4.6; p=0.04) detected in which alcohol-dependent participants reported stronger alcohol craving than controls with a
significant increase in craving from pre- to post-dextroamphetamine effect in those with alcohol dependence only (Figure 5).

![Graph showing alcohol craving questionnaire scores](image)

**Figure 5:** Mean Alcohol Craving Questionnaire (ACQ) total scores vs. participants’ groups (control, alcohol-dependent) pre- and post-dextroamphetamine effect. The repeated-measures ANOVA (GROUP (control, alcohol-dependent) x DRUG (pre-, post-dextroamphetamine effect) on ACQ scores detected GROUP and DRUG main effects as well as interaction effects. *p<0.05; **p<0.005. Analysis includes Beck Depression Inventory and Fagerström Test for Nicotine Dependence scores as covariates.
3B.3 Physiological data

The repeated-measures ANOVAs ((GROUP (control, alcohol-dependent) x DRUG (pre-drug, post-drug effect) on systolic (SBP) (Figure 6) and diastolic (DBP) blood pressures detected the following effects: main effects of DRUG were detected in both systolic ($F_{1,19}=11.1; p=0.004$) and diastolic ($F_{1,19}=7.4; p=0.01$) blood pressures in which an increase in blood pressures in both groups was detected. Alcohol-dependent participants had a higher systolic blood pressure overall compared to controls ($F_{1,19}=17.2; p=0.02$) but this GROUP effect was not detected in diastolic blood pressure ($F_{1,19}=0.02; p=0.88$). No GROUP x DRUG interaction effect was detected in systolic ($F_{1,19}=0.1; p=0.74$) and diastolic ($F_{1,19}=2.2; p=0.15$) blood pressures. All SBP and DBP means with the GROUP, DRUG and interaction p-values are summarized in Table 9.

Table 9: Results summary of repeated-measures ANOVAs ((GROUP (CON, AD) x DRUG (pre-drug, post-drug) on SBP and DBP

<table>
<thead>
<tr>
<th></th>
<th>CON (n=9) (Mean±SD)</th>
<th>AD (n=14) (Mean±SD)</th>
<th>GRP effect p-value</th>
<th>DRUG effect p-value</th>
<th>Inter. effect p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-drug</td>
<td>Post-drug</td>
<td>Pre-drug</td>
<td>Post-drug</td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>110.8±15.8</td>
<td>141.2±13.2</td>
<td>115.9±14.7</td>
<td>164.2±11.1</td>
<td>0.015</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>65.9±9.4</td>
<td>84.8±10.1</td>
<td>67.5±9.8</td>
<td>93.1±8.8</td>
<td>0.88</td>
</tr>
</tbody>
</table>


Figure 6: Mean systolic blood pressure (SBP) in mmHg vs. participants’ groups (control, alcohol-dependent) pre- and post-dextroamphetamine effect. The repeated-measures ANOVA (GROUP (control, alcohol-dependent) x DRUG (pre-, post-dextroamphetamine effect) on SBP detected GROUP and DRUG main effects but no interaction effects. *p<0.05; **p<0.005. Analysis includes Beck Depression Inventory and Fagerström Test for Nicotine Dependence scores as covariates.
3B.4 FMRI Data

3B.4.a Main effect of GROUP: alcohol cues compared to neutral cues

The main effect of GROUP F-contrast detected greater ventral striatal activation in the right ventral striatum, contiguous with the anterior perforated substance in alcohol-dependent participants compared to control participants at both pre-drug and post-drug effect ($F_{1,40} = 20.1; \ z = 3.8; \ p < 0.001; \ (x \ y \ z) = (10 -2 -14))$ with an extent threshold of 10 voxels (Figure 7). Other brain regions came out significant in the main effect of GROUP F-contrast (Table 10), including superior frontal gyrus, postcentral gyrus, parahippocampal gyrus and cerebellum. The latter brain areas all showed greater activation in alcohol-dependent participants relative to controls pre- and post-drug effect.
Figure 7: Brain activation elicited by alcohol cues > neutral cues. Main effect of GROUP from a GROUP (control, alcohol-dependent) x DRUG (pre-, post-dextroamphetamine effect) ANOVA: Identified BOLD signatures in the ventral striatum that differentiated control and alcohol-dependent groups. The main effect activation is overlayed on a canonical T₁ template (Colin27) as well as on a custom brain template from the alcohol-dependent participants’ T₁ volumes in MNI space (coronal reference slice: y-coordinate = -2). To better elucidate how signal in this region differentiated groups, parameter estimates (error bar, 90% C.I.) of the peak activation voxel in this locus is plotted across GROUP and DRUG factors; (**p<0.001). Y-axis: Parameter estimates (beta values) in arbitrary units at the VS. X-axis labels: pre=pre-dextroamphetamine effect, post=post-dextroamphetamine effect.
Table 10: Brain activation elicited by alcohol cues > neutral cues. Main effect of GROUP F-contrast. Threshold $p < 0.001$. Cluster size $k > 10$ voxels.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Region</th>
<th>MNI coordinates</th>
<th>$F$ value</th>
<th>$p_{unc} &lt; 0.001$</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.0000008</td>
</tr>
<tr>
<td></td>
<td>l. sup. parietal lob.</td>
<td>-34 -50 64</td>
<td>32.70</td>
<td>0.000001</td>
</tr>
<tr>
<td></td>
<td>r. cerebellum</td>
<td>20 -50 -32</td>
<td>22.09</td>
<td>0.00003</td>
</tr>
<tr>
<td></td>
<td>r. parahippo. gyr.</td>
<td>24 -14 -22</td>
<td>21.81</td>
<td>0.00003</td>
</tr>
<tr>
<td></td>
<td>l. post. orbital gyr.</td>
<td>-26 12 -18</td>
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<td>0.00005</td>
</tr>
<tr>
<td></td>
<td>r. ventral striatum</td>
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<td>20.06</td>
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<tr>
<td></td>
<td>l. sup. frontal gyr.</td>
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</tr>
<tr>
<td></td>
<td>r. postcentral gyr.</td>
<td>24 -16 46</td>
<td>16.01</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>r. cerebellum</td>
<td>4 -54 -12</td>
<td>15.79</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>r. supramarg. gyr.</td>
<td>64 -28 24</td>
<td>15.50</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>r. postcentral gyr.</td>
<td>64 -4 14</td>
<td>14.62</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

l.=left; gyr.=gyrus; sup.=superior; lob.=lobule; r.=right; parahippo.=parahippocampal; supramarg.=supramarginal
3B.4.b Main effect of DRUG: alcohol cues compared to neutral cues

The main effect of DRUG $F$-contrast detected left medial orbitofrontal cortex (mOFC) activation ($F_{1,40}=16.1; z=3.5; p<0.001; (x \ y \ z) = (-8 \ 46 \ -4)$. The omnibus $F$ was plotted for signal extracted from the mOFC cluster in order to examine the direction of the drug effects at each cell of the ANOVA (using a cluster-based small volume correction, SVC) (Figure 8). The effect of drug appeared to be solely driven by an attenuation of mOFC activity from pre- to post-drug effect in the control group, whereas mOFC activity appeared negligible at both drug conditions in the alcohol-dependent group. This was further interrogated by a GROUP $\times$ DRUG interaction (see section 3B.4.c GROUP $\times$ DRUG interaction analysis: alcohol cues compared to neutral cues below). The main effect of DRUG $F$-contrast also detected right subgenual anterior cingulate cortex (sACC) activation ($F_{1,40}=15.3; z=3.4; p<0.001; (x \ y \ z) = (2 \ 22 \ -4)$) with similar patterns of response as in the mOFC activation (Figure 8). The inferior frontal gyrus also was sensitive to dextroamphetamine effect as its activity decreased from pre- to post-drug effect in both groups. All areas where main effects of DRUG were detected are summarized in Table 11.
Figure 8: Brain activation elicited by alcohol cues > neutral cues. Main effect of DRUG from a GROUP (control, alcohol-dependent) x DRUG (pre-, post-dextroamphetamine effect) ANOVA: Identified BOLD signatures in the medial orbitofrontal cortex (mOFC) and the subgenual anterior cingulate cortex (sACC) that differentiated pre- and post-dextroamphetamine effect conditions. The main effect activation is overlayed on a canonical $T_1$ template (Colin27) as well as on a custom brain template from the alcohol-dependent participants’ $T_1$ volumes in MNI space (coronal reference slice: $z$-coordinate = -4). To better elucidate how signal in the mOFC and sACC differentiated pre- and post-dextroamphetamine effect conditions, parameter estimates (error bar, 90% C.I.) of the peak activation voxel in those loci is plotted across GROUP and DRUG factors; (**p < 0.001). Y-axis: Parameter estimates (beta values) in arbitrary units at the mOFC and sACC. X-axis labels: pre=pre-dextroamphetamine effect, post=post-dextroamphetamine effect.
Table 11: Brain activation elicited by alcohol cues > neutral cues. Main effect of DRUG F-contrast. Threshold \( p < 0.001 \). Cluster size \( k > 10 \) voxels.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Region</th>
<th>MNI coordinates</th>
<th>( F ) value</th>
<th>( p_{\text{unc}} &lt; 0.001 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol – Neutral</td>
<td>l. cuneus</td>
<td>-4 -90 24</td>
<td>16.66</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td>l. medial OFC</td>
<td>-8 46 -4</td>
<td>16.07</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>r. sACC</td>
<td>2 22 -4</td>
<td>15.32</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>l. inf. frontal gyr.</td>
<td>-48 14 8</td>
<td>14.92</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

l.=left; OFC=orbitofrontal cortex; r.=right; sACC=subgenual anterior cingulate cortex; inf.=inferior; gyr.=gyrus
3B.4.c GROUP x DRUG interaction analysis: alcohol cues compared to neutral cues

The GROUP x DRUG interaction F-contrast showed that the [alcohol cues > neutral cues] contrast identified an interaction effect in the left mOFC ($F_{1,40}=21.5; z=4.0; p<0.001; (x \ y \ z) = (-12 \ 28 \ -20)$) (Figure 9). The [alcohol cues > neutral cues] contrast in the GROUP x DRUG interaction analysis also yielded the bilateral superior temporal sulcus with 5 peak activations all with an $F_{1,40}>15.9$ (Table 12). Given our a priori hypotheses regarding cue-induced mesocorticolimbic system activation (i.e. OFC), we plotted the omnibus F for signal extracted from the mOFC cluster to further examine the interaction effects in the [alcohol cues > neutral cues] analysis (SCV centered on MNI coordinates: $x = -12, y = 28, z = -20$) in order to examine the direction of the drug effects at each cell of the ANOVA. The interaction was driven by a decrease in signal from pre- to post-drug effect in controls. In contrast, alcohol-dependent participants exhibited a stable and modest elevation of mOFC signal across both pre- and post-drug effect time-points (Figure 9). We confirmed the direction and significance of these interaction effects with post-hoc t-contrasts. We also tested with 2 t-contrasts the differential effects of group at each time point (i.e. control>alcohol-dependent pre- and post-drug effect). The left mOFC showed greater signal activity in control than in alcohol-dependent participants only pre-drug effect ($t_{1,40}=4.1; z=3.7; p<0.001; (x \ y \ z) = (-8 \ 56 \ -18)$) with an extent threshold of 10 voxels. The right precentral gyrus ($t_{1,40}=4.0; z=3.6; p<0.001; (x \ y \ z) = (24 \ -16 \ 46)$) also came out significant in the control>alcohol-dependent pre-drug effect t-contrast. No brain area came out significant in the control>alcohol-dependent post-drug effect t-contrast. A t-contrast looking at the
alcohol-dependent group pre- vs. post-drug effect in both directions showed no significant
different activity in the mOFC.
Figure 9: Brain activation elicited by alcohol cues > neutral cues. GROUP x DRUG interaction effect from a GROUP (control, alcohol-dependent) x DRUG (pre-, post-dextroamphetamine effect) ANOVA: Identified BOLD signatures in the medial orbitofrontal cortex (mOFC). The interaction effect activation is overlayed on a canonical T\textsubscript{1} template (Colin27) as well as on a custom brain template from the alcohol-dependent participants’ T\textsubscript{1} volumes in MNI space (coronal reference slice: z-coordinate = -20). To better elucidate the differential response to dextroamphetamine between the groups at the mOFC signal, parameter estimates (error bar, 90% C.I.) of the peak activation voxel in this locus is plotted across GROUP and DRUG factors; (***p<0.001). Y-axis: Parameter estimates (beta values) in arbitrary units at the mOFC. X-axis labels: pre=pre-dextroamphetamine effect, post=post-dextroamphetamine effect.
Table 12: Brain activation elicited by alcohol cues > neutral cues. GROUP x DRUG interaction F-contrast. Threshold \( p < 0.001 \). Cluster size \( k > 10 \) voxels.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Region</th>
<th>MNI coordinates</th>
<th>( F ) value</th>
<th>( p_{unc} &lt; 0.001 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( X )</td>
<td>( Y )</td>
</tr>
<tr>
<td>Alcohol – Neutral</td>
<td>l. medial OFC.</td>
<td>-12</td>
<td>28</td>
<td>-20</td>
</tr>
<tr>
<td></td>
<td>r. sup. temp. sulc.</td>
<td>30</td>
<td>-54</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>l. sup. temp. sulc.</td>
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<td>-24</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>r. sup. temp. sulc.</td>
<td>44</td>
<td>-22</td>
<td>-12</td>
</tr>
<tr>
<td></td>
<td>l. sup. temp. sulc.</td>
<td>-44</td>
<td>-32</td>
<td>-10</td>
</tr>
<tr>
<td></td>
<td>r. sup. temp. sulc.</td>
<td>62</td>
<td>-28</td>
<td>6</td>
</tr>
</tbody>
</table>

l.=left; OFC=orbitofrontal cortex; r.=right; sup.=superior; temp.=temporal; sulc.=sulcus
3B.4.d GROUP x DRUG interaction analysis: alcohol cues compared to abstract cues

The GROUP x DRUG interaction F-contrast showed that the [alcohol cues > abstract cues] contrast also identified an interaction effect in the left mOFC ($F_{1,40}=21.7; z=4.0; p<0.001; (x\ y\ z) = (-12\ 30\ -20)$. As in the [alcohol cues > neutral cues] contrast, the interaction was driven by a decrease in signal from pre- to post-drug effect in controls whereas alcohol-dependent participants exhibited a stable and modest elevation of mOFC signal across both pre- and post-drug effect time-points. The [alcohol cues > abstract cues] contrast in the GROUP x DRUG interaction analysis also yielded the bilateral superior temporal sulcus with 2 peak activations ($F_{1,40}=20.1; z=3.8; p<0.001; (x\ y\ z) = (-44\ -10\ -18); (F_{1,40}=15.8; z=3.4; p<0.001; (x\ y\ z) = (42\ -22\ -4)$).

Section 4 DISCUSSION

4A Behavioural Study Discussion

To our knowledge, the Behavioural Study is the first study exploring mesocorticolimbic dopamine function in alcohol dependent and co-morbid depressed alcohol-dependent individuals using a single oral dose of dextroamphetamine (30 mg) as a probe. The main findings of our study indicated there was no GROUP x DRUG interaction effects detected on any of our dextroamphetamine behavioural effects measures between the alcohol-dependent and the depressed alcohol-dependent groups as measured by the ARCI, POMS or VAS scales. Specifically, no differential response to dextroamphetamine rewarding effects compared to placebo was detected between the alcohol-dependent and the depressed alcohol-dependent groups as evidenced by our main outcome measure: ARCI Rewarding Effects.
Composite. Nevertheless, a dextroamphetamine effect was objectively confirmed by the physiological data analysis that detected DRUG main effects in both the alcohol-dependent and the depressed alcohol-dependent participants. Moreover, dextroamphetamine effects were distinguishable from placebo in the alcohol-dependent and depressed alcohol-dependent groups in all of the subjective drug effect data analyses, such as in our main outcome measure: ARCI Rewarding Effects Composite. Indeed, participants experienced more rewarding effects with a single dose of dextroamphetamine (30 mg p.o.) in each group compared to placebo. Similar results were found among the 4 groups in the post-hoc study. Furthermore, GROUP main effects were detected demonstrating that alcohol-dependent and depressed alcohol-dependent participants responded differently to both placebo and dextroamphetamine in this study although the effect of dextroamphetamine compared to placebo was not significantly different between the 2 groups. Main effects of GROUP revealed greater placebo as well as dextroamphetamine responses in the depressed alcohol-dependent group compared to the alcohol-dependent group. Similar results were found in the post-hoc study in which groups with major depressive disorder present (depressed and depressed alcohol-dependent) experienced more placebo and dextroamphetamine rewarding effects than alcohol-dependent and control groups although no GROUP x DRUG interaction effects were detected. The increased placebo response in the depressed and depressed alcohol-dependent groups is consistent with previous findings of placebo effects in major depressive disorder [365, 366]. These results suggest high placebo response rates may also be present in depression co-morbid with alcohol dependence. In terms of the patterns of response to dextroamphetamine rewarding effects, groups with major depressive disorder present were similar which is consistent with results by Tremblay and colleagues [20],
whereas control and alcohol-dependent participants appeared to have similar response patterns (Figure 2). However, alcohol-dependent participants did not feel effects as rewarding as those with major depressive disorder. These results cannot be explained by the alcohol-dependent group being more sensitive to dextroamphetamine negative effects than other groups (as shown by the ARCI Negative Effects Composite results). Nonetheless, they may suggest different underlying neurobiological mechanisms responsible for the dysfunctional mesocorticolimbic dopaminergic state in alcohol dependence and major depressive disorder. In their study, Tremblay and colleagues suggested the altered behavioural response to dextroamphetamine rewarding effects in major depressive disorder was related to an underlying dopaminergic dysfunction [20]. Indeed, dextroamphetamine has the ability to stimulate the mesocorticolimbic dopamine system [236, 270, 272, 367] and its rewarding effects are linked to dopamine binding to D<sub>2</sub> receptors in the human ventral striatum [368]. Orally administered dextroamphetamine increases dopamine binding to D<sub>2</sub> receptors [295], thus strengthening the link between dextroamphetamine-induced dopamine release and rewarding effects in humans. Hence, the enhanced behavioural response to the dopaminergic probe’s rewarding effects in major depressive disorder may have reflected decreased dopamine output, where compensatory mechanisms (such as increased dopamine D<sub>2</sub> receptors sensitivity or secondary up-regulation of D<sub>2</sub> receptors) were unmasked by an exogenous source (e.g. dextroamphetamine) which generated a hypersensitive response. Indeed, depression generally is characterized by a relative hypo-dopaminergic state and more specifically reduced mesocorticolimbic dopamine levels [369-371] that could be attributed to abnormalities at the presynaptic level [18, 369, 372]. Similarly, in detoxified alcohol-dependent participants, Martinez and colleagues showed a blunted dextroamphetamine-
induced dopamine release in limbic striatum [27] compared to controls. The latter study strengthens the similarity in presynaptic dopamine dysfunction in alcohol dependence and major depressive disorder. Nevertheless, Parsey and colleagues did not find an association between major depressive disorder and alterations in dextroamphetamine (0.3 mg/kg, i.v.)-induced dopamine release in a small sample of non-psychotic depressed patients, using single photon emission computed tomography (SPECT) [174]. Moreover, various studies have shown evidence of a link between mesocorticolimbic dopamine hypofunction and presence of specific major depression symptoms such as psychomotor retardation [172, 372, 373] or impulsivity [373] in depressed participants. For example, Meyer and colleagues recently reported elevated D2 receptor binding potential in depressed individuals with motor retardation in a [11C]raclopride PET study [172]. The link between mesocorticolimbic dopamine dysfunction and specific depressive symptoms needs to be more thoroughly investigated. Although there seems to be a dopamine dysfunction commonality at the presynaptic level in both major depressive disorder and alcohol dependence, there is ample evidence suggesting differences at the postsynaptic level, which could likely explain the lack of altered response to dextroamphetamine rewarding effects in alcohol dependence. Indeed, several studies have shown a D2 receptor density increase in depression that could be a result of presynaptic dopamine hypofunction in major depressive disorder [168-172]. Other neuroimaging studies, however, have reported an unchanged [173-177] or lower [178] striatal D2 receptor density in depression compared to controls. In alcohol dependence, several studies have recently shown a postsynaptic D2 receptors down-regulation in central areas described as a result of either chronic alcohol-related dopamine release or alcohol dependence-related neurotoxicity. Indeed, Volkow and colleagues reported reduced D2
receptor availability in the caudate and putamen of detoxified alcohol-dependent participants using $[^{11}C]$raclopride and a high resolution PET camera [28]. A decrease in D$_2$ receptor availability in the ventral striatum in alcohol dependence also was reported by Heinz and colleagues using $[^{18}F]$desmethoxyfallypride [25, 26]. The latter studies results were supported by another neuroimaging study reporting reduction in D$_2$ receptor availability in alcohol dependence in the caudate, putamen, and ventral/limbic striata [27].

Interestingly, the presence of severe depression did not increase the response to dextroamphetamine rewarding effects in the depressed alcohol-dependent group whereas it did in the depressed-only group. These results may suggest that the presence of alcohol dependence had a mitigating effect on the impact of depression severity on participants’ behavioural responses to dextroamphetamine rewarding effects. Although our participants in the depressed alcohol-dependent group were not stratified in terms of primary or secondary alcohol dependence sub-groups, the results suggest the presence of alcohol dependence was a central influence in our study sample of depressed alcohol-dependent participants. Separating alcohol-dependent participants with or without a co-morbid diagnosis of depression often is questioned as most individuals with alcohol-dependence have experienced major depressive episodes in their lifetime and many have depressive symptoms but do not fully fulfill DSM-IV criteria for major depressive disorder [12, 13, 363, 364]. Moreover, depressive symptomatology can rapidly change in alcohol-dependent individuals should they experience alcohol intoxication, withdrawal, craving or sobriety [374-380]. As such, many clinicians primarily focus on alcohol dependence and see it as the central disorder to treat; indeed it is suggested that a period of prolonged sobriety occur in order to obtain a definitive diagnosis of major depressive disorder [208]. Hence, a DSM-IV diagnosis of major depressive disorder
in non-abstinent, non-detoxified alcohol-dependent participants may be difficult to interpret. Our study results may suggest clinical and neurobiological similarities between alcohol-dependent individuals with or without co-morbid major depressive disorder.

Overall, the Behavioural Study results did not support the hypothesis that a single oral dose of dextroamphetamine (30 mg) elicits a similar hypersensitive response to its rewarding effects as in major depressive disorder in those suffering from alcohol dependence, and more so in those with co-morbid depression and alcohol dependence. Nonetheless, as discussed in the literature background section, there is plenty evidence for a dysfunctional mesocorticolimbic system in alcohol dependence. Therefore, these results suggest that different neurobiological mechanisms (occurring at the dopamine synapse level) could be responsible for the mesocorticolimbic dopamine system dysfunction in alcohol dependence and in major depressive disorder, which may not solely be revealed by dextroamphetamine subjective effects.

4A.1 Limitations
Several limitations of the Behavioural study need to be recognized. 1) Participants with alcohol dependence present were not detoxified as in most other clinical studies cited on alcohol dependence, but neither were they under the influence of alcohol during the testing sessions, nor experiencing withdrawal. 2) Only alcohol-dependent participants fulfilling DSM-IV criteria for major depressive disorder were included in the co-morbid depressed alcohol-dependent group, leaving participants with sub-threshold depressive symptoms in the alcohol-dependent group. 3) There was a significant difference in the female/male ratio and the age amongst the 4 groups. Although sex and age were taken into account in our analysis, this is particularly relevant as sex differences in striatal dopamine release in healthy adults
have been demonstrated [381] as well as age-related changes in dopaminergic neurotransmission in healthy volunteers [382-385]. This should therefore lead to similar studies in alcohol-dependent and depressed participants as well as studies with age-matched groups. 4) In the post-hoc study, there was an uneven distribution of smokers amongst the 4 groups. However, the level of nicotine dependence was controlled for in the analysis and did not influence the results. Furthermore, Cardenas and colleagues demonstrated that smoking did not modify the response to dextroamphetamine in depressed and control participants [386]. 5) Although there is strong evidence both in animals and humans that dextroamphetamine-induced rewarding effects are mediated by dopamine, one should acknowledge dextroamphetamine’s ability to release other neurotransmitters than dopamine such as norepinephrine and serotonin to a lesser extent [301, 303, 306, 387]. This is particularly relevant since it was recently shown that dopamine, serotonin and norepinephrine have physiologically functional reciprocal interactions [308] such that both norepinephrine and serotonin may have an inhibitory action on dopamine function. Therefore, these interactions could have had an effect on the lack of altered response in alcohol-dependent participants with or without depression. 6) Statistical power could have been increased if a within-subject design had been used rather than a between-subject design. However, it would have been very difficult to sample our study population of alcohol-dependent individuals on two occasions (placebo day and dextroamphetamine day). Moreover, it was desirable to follow the same study design as in Tremblay and colleagues [20] in order to compare our study data in the post-hoc analysis.
4B FMRI Study Discussion

To our knowledge, this is the first study assessing how areas of the mesocorticolimbic circuitry respond to a dopamine challenge in alcohol dependence using cue-induced BOLD response with dextroamphetamine as a pharmacological probe. The strengths of this study design include the use of a relatively dopamine-specific probe, the use of well-validated alcohol cues and participants that represent non-abstinent and non-treatment seeking alcohol-dependent individuals. Our study paradigm included neural measures that implicate brain areas of the mesocorticolimbic circuitry, such as the ventral striatum and the medial orbitofrontal cortex that appear to be primarily dysfunctional in alcohol dependence.

4B.1 Subjective and Physiological Data

Subjective and objective data results provided evidence of a dextroamphetamine effect in our participants. Subjective ratings of drug effect (i.e. VAS) and blood pressures significantly increased in both groups after dextroamphetamine administration. Although VAS feel high scores appeared different between control and alcohol-dependent participants post-drug, there was no significant GROUP or GROUP x DRUG interaction when the Beck Depression Inventory and Fagerström Test for Nicotine Dependence scores were used as covariates. Systolic blood pressure was significantly higher in alcohol-dependent participants, this difference is characteristic of a direct alcohol pressor effect [388]. Nevertheless, a dextroamphetamine effect was not detected using the ARCI Rewarding Effects Composite, our main outcome measure of dextroamphetamine behavioural effects, in the Behavioural Study. However, the ARCI Rewarding Effects Composite data presented in the FMRI Study were not similarly analyzed. Indeed, only 3 ARCI measures were taken in the FMRI Study (baseline, 60 min post-drug and 140 min post-drug), this did not allow us to look at the
maximum change from baseline at various time points, including at 240 min post-drug as in the Behavioural Study. Moreover, the 60 min post-drug ARCI measure was administered to participants while confined in a scanner and lying on the scanner bed with all the visual equipment in place whereas the first and last ARCI measures were acquired outside the scanner, sitting in front of a computer. Psychoactive drug effects, such as in the case of amphetamine, are highly dependent on environmental settings [389] and the ARCI Rewarding Effects Composite was analyzed differently between the 2 studies, therefore this may explain the lack of consistency between the 2 studies and the lack of detected dextroamphetamine effect by the ARCI Rewarding Effects Composite, in the FMRI Study.

Dextroamphetamine Effect on Alcohol Craving

This is the first study showing an increase in craving for alcohol in alcohol-dependent participants after administration of an oral dose of dextroamphetamine (30 mg). Although Lingford-Hughes and colleagues showed no significant change in craving scores in response to alcohol cues alone in abstinent alcohol-dependent individuals [363], one could cautiously attribute the detected increase in alcohol craving in our study to the direct influence of dextroamphetamine. Indeed, although systemic amphetamine, at a range of doses, consistently primed psychostimulant drug seeking in animals familiar with cocaine [390-392], amphetamine did not reliably prime alcohol seeking in animals familiar with alcohol [393-395]. However, in human studies, dextroamphetamine-induced drug wanting and novelty seeking increases were reported in healthy men [260] and in an acute phenylalanine/tyrosine depletion study, decreased catecholamine, and hence dopamine neurotransmission reduced alcohol self-administration in healthy female social drinkers
Acute phenylalanine/tyrosine depletion also induced an effect on alcohol progressive ratio breakpoints in heavier social drinkers [264]. Similarly, nicotine increased progressive ratio breakpoints for alcohol in occasional smokers [396]. One could speculate that an increase in dopamine neurotransmission (e.g. induced by dextroamphetamine) may stimulate alcohol wanting/seeking in alcohol-dependence. Nonetheless, in another human study assessing priming effects of oral dextroamphetamine (30 mg) in problem gamblers, drinkers and gambler-drinkers, there was little evidence that dextroamphetamine directly primed desire for alcohol in problem drinkers [397]. The question remains whether those meeting criteria for alcohol dependence would respond differently. The craving increase detected from pre- to post-drug effect in the alcohol-dependent group in our study might be due to the long motionless period inside the scanner during the study procedure which may have exacerbated alcohol-dependent participants’ craving for alcohol. Furthermore, the visual craving task subjective scores did not markedly change after dextroamphetamine administration. However this task is not a measure of acute alcohol craving and habituation to the alcohol cues might have occurred since the same pictures were presented pre- and post-drug effect. Nevertheless, it is significant to note that some commonalities in alcohol and dextroamphetamine subjective effects at low doses were found in non-problem drinkers [398].

4B.2 Imaging Data

The imaging data showed that the frontal gyrus was sensitive to our dopaminergic drug in both groups with a similar pattern (reduction) as that reported in a recent functional imaging study using $[^{15}O]H_2O$ PET and methylphenidate in healthy volunteers [337]. Moreover, the fMRI data indicated ventral striatal dysfunction during presentation of alcohol cues at
baseline in alcohol-dependent participants, which is consistent with previous literature [22, 399]. But to extend this literature, we showed that this increased striatal activity also is present in non-abstinent alcohol-dependent individuals and persists after dextroamphetamine administration. It should be noted that our results with non-abstinent alcohol-dependent participants are similar to other studies with abstinent alcohol-dependent participants in whom subsequent neuroadaptation may have presumably occurred after detoxification [31]. Greater ventral striatal activation in alcohol-dependent participants relative to controls during presentation of alcohol cues pre- and post-dextroamphetamine effect may suggest that this brain area is dysfunctional with or without the presence of a dopamine releasing compound in alcohol-dependent individuals. We did not show greater ventral striatal activation post-dextroamphetamine relative to pre-dextroamphetamine effect in either of the two groups, which is consistent with a functional neuroimaging study reporting no methylphenidate-induced activation of the striatum in healthy participants [337]. Although fMRI does not provide a direct measure of dextroamphetamine-induced dopamine release, PET studies using dopaminergic probes (amphetamine [27] or methylphenidate [29]) have demonstrated an increase in dopamine release in controls and detoxified alcohol-dependent participants and the increase was significantly higher in controls than in alcohol-dependent participants. Although the ventral striatum appeared hyperfunctional in alcohol-dependent participants compared to controls when exposed to alcohol cues, there was a similar response to dextroamphetamine between the two groups at this brain area (i.e. no change in ventral striatal signal magnitude from pre- to post-drug effect in both groups). This suggests that non-abstinent alcohol-dependent individuals relied more on ventral striatal activity when processing and evaluating alcohol cues irrespective of drug conditions.
There was a differential response to dextroamphetamine between the control and the alcohol-dependent groups at the medial orbitofrontal cortex and the bilateral superior temporal sulcus. The superior temporal sulcus primarily has been implicated in social brain functions [400-402] as well as speech perception [402] whereas the orbitofrontal cortex is part of the mesocorticolimbic circuitry. It has been shown to be involved in reward processing [332] and to be crucial in relation to drug addiction in animals and humans [403]. Specifically, the orbitofrontal cortex has been suggested to link reward to hedonic experience in humans [332]. It also has been suggested there is a functional dissociation of the orbitofrontal cortex such that the lateral part would be related to the evaluation of punishers whereas the medial part would have an activity related to monitoring, learning and memory of the reward value of many different reinforcers [404]. Moreover, it has been suggested that the medial orbitofrontal cortex implements monitoring processing of the incentive salience of a stimulus [332, 404, 405]. Our fMRI data determined that a decrease in medial orbitofrontal cortex activity from pre- to post-dextroamphetamine effect may represent the normal response when participants are evaluating and processing alcohol cues. One could speculate that dextroamphetamine-induced dopamine release in the ventral striatum interferes with orbitofrontal cortex modulation of ventral striatal dopamine activity. The fMRI data also determined medial orbitofrontal cortex activity was negligible with or without dextroamphetamine effect in the alcohol-dependent group, which may demonstrate a dysfunctional medial orbitofrontal cortex in alcohol dependence. There was no differential response to dextroamphetamine between the control and the alcohol-dependent groups detected at the subgenual anterior cingulate cortex (as evidenced by the interaction analysis). Interestingly, this brain region (involved in neuropsychiatric disorders [406]) was detected in
the main effect of DRUG analysis with similar response patterns as in the medial orbitofrontal cortex. Indeed, tracer studies in non-human primates have shown that the subgenual anterior cingulate cortex has connections with the orbitofrontal cortex [407, 408] and the ventral striatum [409-411], strengthening the modulatory role of this mesocorticolimbic region between limbic and frontal cortex areas. Moreover, a diffusion tractography study recently demonstrated strong connections between the subgenual anterior cingulate cortex and the medial orbitofrontal cortex as well as the ventral striatum in healthy participants [412].

Consistent with the hypothesis speculating on an orbitofrontal cortex involvement in the loss of prefrontal modulation of dopamine cell activity in alcohol dependence [29, 336], our imaging study results provide further support of a dysfunctional orbitofrontal cortex in alcohol dependence. Our results may support the hypothesis that the orbitofrontal cortex modulates the value of rewards by regulating dopamine activity in the ventral striatum through a corticolimbic gateway, the subgenual anterior cingulate cortex. Therefore, based on our results and the strong connections linking the subgenual anterior cingulate cortex to the medial orbitofrontal cortex and to the ventral striatum, the medial part of the orbitofrontal cortex appears to be a more precise region responsible for the prefrontal dysregulation in alcohol dependence.

Overall, the FMRI Study data support our hypothesis in that dextroamphetamine - a dopaminergic probe - in combination with fMRI (cue-induced BOLD response) revealed a disruption in important areas of the mesocorticolimbic dopamine system (e.g. orbitofrontal cortex and ventral striatum) in alcohol-dependent participants compared to controls.
4B.3 Limitations

Several limitations of the FMRI Study need to be recognized. 1) The main findings of the FMRI Study suggest that the medial orbitofrontal cortex may participate in the loss of prefrontal modulation of ventral striatal dopamine cell activity; however no connectivity analyses were performed to demonstrate the two brain areas were causally linked. 2) Although our group is representative of male alcohol-dependent individuals, it does not include female alcohol-dependent individuals. This may be important because it has been shown that ovarian steroids modulate reward-evoked neural activity in humans in areas such as the orbitofrontal cortex and the striatum [413]. 3) We did not have the statistical power to stratify study participants in terms of alcohol subtypes (i.e. Type I/II) or in terms of primary/secondary alcohol dependence in those who also fulfilled major depression criteria. 4) The single dose of dextroamphetamine was administered a couple of minutes before the pre-drug effect scanning session (i.e. baseline). Although it was previously demonstrated in our laboratory that dextroamphetamine peak effect occurred approximately 90 minutes after drug administration [20, 21], we cannot entirely rule out any dextroamphetamine effect in some participants during the baseline scanning session. Although pharmacodynamic variability among participants is mitigated by the repeated-measure within subject nature of the design, its effect on the study results could be better controlled with measures of dextroamphetamine or dopamine in participants. Moreover, the oral dose of dextroamphetamine was identical in all participants (30 mg); therefore, differences in drug-metabolizing abilities could also have influenced our results. 5) As mentioned in the Behavioural Study limitations section, dextroamphetamine has the ability to release dopamine as well as other neurotransmitters [301, 303, 306, 387] which all have functional
reciprocal interactions [308]. Thus, this could have influenced brain activity post-dextroamphetamine effect. 6) Although we used the Fagerström Test for Nicotine Dependence scores as covariates in all of our FMRI Study analyses in order to control for cigarette smoking, using time since last cigarette consumption could have been another option to ensure that the differential BOLD signal was not attributable to cigarette smoking.; however these data were not collected.

4C Summary

4C.1 Behavioural Study

Participants experienced more rewarding effects with a single dose of dextroamphetamine (30 mg p.o.) compared to placebo in the alcohol-dependent and depressed alcohol-dependent groups or in the 4 groups in the post-hoc study. There were greater placebo and dextroamphetamine responses in the depressed alcohol-dependent group compared to the alcohol-dependent group. Similar results were found amongst the control, depressed, alcohol-dependent, and depressed alcohol-dependent groups in the post-hoc study in which greater placebo and dextroamphetamine responses were found in the 2 depressed groups (depressed and depressed alcohol-dependent) whereas controls and alcohol-dependent participants had similar patterns of response. Nevertheless, we showed no differential response to dextroamphetamine rewarding effects compared to placebo between alcohol-dependent participants with or without depression as well as amongst control, depressed, alcohol-dependent and depressed alcohol-dependent participants (no GROUP x DRUG interaction effects). This lack of interaction effects may be originating from differences in availability of post-synaptic D₂ receptors (reduced in alcohol dependence and increased in depression) whereas both disorders seem to feature a common presynaptic dysfunction, creating a hypo-
dopaminergic state. Moreover, the presence of alcohol dependence had a mitigating effect on the impact of depression severity on participants’ behavioural responses to dextroamphetamine rewarding effects. Therefore, the data suggest the presence of alcohol dependence was a central influence in our study sample of co-morbid depressed alcohol-dependent participants.

4C.2 FMRI Study

We demonstrated greater ventral striatal activation in non-abstinent alcohol-dependent participants when presented with alcohol cues and we also showed this effect remained post-dextroamphetamine. Finally, our major finding indicated that there was a differential response to dextroamphetamine – a dopaminergic releaser – between the control and alcohol-dependent groups, when presented with alcohol cues, at the medial orbitofrontal cortex, an area involved in incentive salience attribution [332], and that we suggest is involved in the reported loss of prefrontal modulation of dopamine cell activity in alcohol dependence. This supports a key role of the medial orbitofrontal cortex in mesocorticolimbic dysfunction in alcohol dependence.

4C.3 Overall Summary

The Behavioural Study results did not support the overall research hypothesis in that a single oral dose of dextroamphetamine (30 mg) did not reveal a dysfunctional mesocorticolimbic dopamine system in alcohol dependence, even when major depressive disorder was co-morbid, in terms of our main behavioural response outcome measure (i.e. ARCI Rewarding Effects Composite). Although there is evidence of a dysfunctional mesocorticolimbic dopamine system in alcohol dependence, an altered behavioural response to dextroamphetamine rewarding effects may not have been revealed because of a low
availability of dopamine D2 post-synaptic receptors that has been reported in alcohol-dependence. On the other hand, the FMRI Study did support the overall research hypothesis in that a single oral dose of dextroamphetamine (30 mg) did reveal a dysfunctional mesocorticolimbic dopamine system in alcohol-dependent participants compared to controls in terms of alcohol cue-induced BOLD response. The data suggest that there may be ventral striatal and medial orbitofrontal cortex disruption in alcohol-dependent participants, 2 areas of the mesocorticolimbic circuitry.

4D Recommendations

4D.1 Behavioural Study

The Behavioural Study results suggest that different neurobiological mechanisms could be responsible for the mesocorticolimbic dopamine system dysfunction in alcohol dependence and major depressive disorder. Future research should be carried out to determine whether these different underlying mechanisms 1) occur at different levels of the dopamine synapse in alcohol dependence and major depressive disorder using PET [11C]raclopride in order to thoroughly compare presynaptic dopamine release as well as post-synaptic dopamine receptor function in both disorders, 2) involve other neurotransmitter systems, 3) are dependent on participant populations (e.g. primary vs. secondary alcohol dependence), alcohol dependence (e.g. Type I or II) and major depressive disorder (e.g. with melancholic features) sub-types, or 4) are dependent on the presence of specific symptoms (e.g. psychomotor retardation).

Overall, further research is needed in order to understand mesocorticolimbic dopamine function and therefore, partly elucidate the neurobiological mechanisms underlying alcohol dependence and how it mitigates the impact of major depressive disorder
severity on behavioural responses to dextroamphetamine rewarding effects when they are co-
morbid.

Using pharmacological fMRI with the same dopaminergic probe could help reveal the neural substrates of dysfunctional mesocorticolimbic dopamine system in alcohol dependence as we showed in the FMRI Study.

4D.2 FMRI Study

In terms of dextroamphetamine effect on alcohol craving, future research should investigate whether dextroamphetamine alone - without alcohol visual cues presented in a scanner setting – can increase craving for alcohol in alcohol-dependent individuals or not. This would add to the body of knowledge on the role of dopamine in the motivational aspect of reward as well as in alcohol craving.

The imaging data suggest a dysfunctional medial orbitofrontal cortex in alcohol dependence. Therefore, future studies focusing on the medial orbitofrontal cortex may help in determining the underlying neurobiology of alcohol dependence, and may lead to future treatment targets. Sex differences were reported with regards to striatal dopamine release [381] and reward-evoked neural activity in the orbitofrontal cortex and striatum [413]; therefore a similar fMRI study in female alcohol-dependent participants is warranted.

Combining fMRI, PET and a dextroamphetamine challenge would be useful in revealing neural substrates of the dysfunctional mesocorticolimbic system in alcohol dependence as well as in vivo neurotransmission, because it would provide a direct measure of dextroamphetamine-induced dopamine release. This would lend support for the main findings of the FMRI Study based on the involvement of the medial orbitofrontal cortex in the modulation of ventral striatal dopamine cell activity suggested in this study.
Psychophysiologic interactions and/or dynamic causal modeling analyses would allow to demonstrate that the medial orbitofrontal cortex and the ventral striatum were causally linked, hence supporting the main suggested findings. Additionally, pharmacodynamic variability effects among participants would be better controlled. Moreover, as most neuroimaging studies on alcohol dependence include abstinent, detoxified alcohol-dependent participants, a study combining fMRI, PET and a dextroamphetamine challenge in detoxified and non-detoxified alcohol-dependent participants would reveal if dopamine receptors bioavailability is different in those 2 groups. Furthermore, it would confirm if similar results in the medial orbitofrontal cortex exist in detoxified and non-detoxified alcohol-dependent groups thus enhancing cross-study consistency.

Some alcohol cue-exposure paradigms also use olfactory [414] and gustatory [327, 329] in addition to visual cues. Alcohol taste cues (a sip of the preferred alcoholic beverage) have recently shown to activate mesocorticolimbic circuitry in heavy alcohol drinkers [415] and they also generally induce craving for alcohol in alcohol-dependent individuals [327, 329]. Since the mesocorticolimbic dopamine system also is involved in craving [25, 26, 31, 416], fMRI studies using an alcohol taste paradigm in combination with dextroamphetamine as a dopaminergic probe may help elucidate the neurobiological mechanisms underlying alcohol craving.

Overall, combining a dextroamphetamine challenge with fMRI may be a useful tool to study the functional integrity of the mesocorticolimbic dopamine system in many psychiatric disorders, particularly in substance use disorders.
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Papers Under Internal Review

Balducci, XL, Sproule, BA, Herrmann, N, Busto, UE, Naranjo, CA, Probing dopamine function in alcohol-dependent and co-morbid depressed alcohol-dependent individuals.

Balducci, XL, Schmitz, TW, Sproule, BA, De Rosa, E, Herrmann, N, Graham, SJ, Busto, UE, Naranjo, CA, Medial orbitofrontal cortex dysfunction in alcohol dependence assessed by using a dextroamphetamine probe and functional magnetic resonance imaging.

Abstracts


Balducci, XL, Sproule, BA, Herrmann, N, Busto, UE, Naranjo, CA, Oral d-amphetamine effect on alcohol craving in alcohol-dependent and co-morbid alcohol-dependent/major depressive disorder participants. 69th Annual Scientific Meeting of the College on Problems of Drug Dependence, Quebec City, Quebec. June 16-21, 2007. Poster presentation.


Balducci, XL, Schmitz, TW, Sproule, BA, De Rosa, E, Herrmann, N, Busto, UE, Naranjo, CA, Dysfunction of reward processing in alcohol dependence assessed by fMRI and dextroamphetamine. 70th Annual Scientific Meeting of the College on Problems of Drug Dependence, San Juan, Puerto Rico, June 14-19, 2008. Oral communication.
APPENDICES

APPENDIX A  Medications known to interact with dextroamphetamine
APPENDIX B  Behavioural Study Consent Form
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APPENDIX D  FMRI Study Consent Form
APPENDIX A

Medications known to interact with dextroamphetamine

ascorbic acid
anesthetics, inhalation
antacids with calcium and magnesium
anticonvulsants
antihypertensives or diuretics used as antihypertensives
antipsychotics
beta-adrenergic blocking agents
carbonic anhydrase inhibitors
citrates
CNS depressants (e.g. alcohol)
CNS stimulants
digitalis glycosides (digoxin)
glutamic acid hydrochloride
levodopa
loxapine
molidone
phenothiazines
pimozide
sodium bicarbonate
thioxanthenes
meperidine
metrizamide
monoamine oxidase inhibitors
propoxyphene
thyroid hormones
tricyclic antidepressants
sedatives-hypnotics (e.g. diazepam)
APPENDIX B

Behavioural Study Consent Form

The Role of the Brain Reward System in Depressed Alcoholics

Principal Investigators: Dr. Claudio A. Naranjo
Dr. Usoa Busto

VOLUNTEER INFORMATION SHEET AND CONSENT FORM

You are being asked to participate in a study to understand how depression and alcohol dependence occur. The main procedure involves giving a single dose of a widely prescribed medication called dextro-amphetamine. This study will be conducted at the Sunnybrook and Women's College Health Sciences Centre-Sunnybrook Campus, 2075 Bayview Ave, under the supervision of Dr. Claudio Naranjo. Your participation is voluntary and will involve the following:

Background
Close to 6% of Canadians aged 18 years and over have experienced depression at least once in their lives. The risk of depression among alcoholics is even higher than in the general population. For example, it has been estimated that up to 80% of alcoholics complain of depressive symptoms. In addition, individuals who have both of these disorders are more likely to have problems related to low self-esteem and greater impairment in day to day functioning. A specific area of the brain is associated with the perception of pleasure. This area of the brain can be stimulated either by certain kinds of drugs or meaningful life events. A malfunction in this particular brain region could be associated with symptoms of depression and alcohol dependence.

Purpose
The purpose of this study is to look at the effects of a single oral dose (a capsule swallowed by mouth) of dextro-amphetamine on mood and feelings. We will compare these effects in a group of depressed alcohol-dependent individuals and a group of non-depressed alcohol dependent individuals. D-amphetamine (Dexedrine ®) is prescribed in children or adults with attention deficit disorder (ADD) or narcolepsy (a sleep disorder). Results from this study will give us a better understanding of the brain systems involved in the symptoms of depression and alcohol dependence.
Location
The study will take place in the Neuropsychopharmacology Research Laboratory, Sunnybrook and Women's College Health Sciences Centre-Sunnybrook Campus, 2075 Bayview Ave., Toronto. The room is located in the F-wing, on the third floor, room # F3-13.

Duration
If you agree to participate in this study, you will be asked to come to the Sunnybrook and Women's College Health Sciences Centre-Sunnybrook Campus for one study session that will take approximately six hours (half a day).

Procedures
In order to participate in this study, you must be diagnosed with Alcohol Dependence. One half of the participants included will also be diagnosed with Depression. You must be between the ages of 19 and 65 years of age. If you agree to participate in this study, you will undergo an initial assessment, which will consist of several questionnaires, a urine sample for drug screening and a blood sample for routine blood work. You may not participate in the study if you have used any illicit drugs such as cocaine, amphetamine, heroin or marijuana within the last 3 months, or, if you have a recent/current history of drug abuse or dependence on a substance other than alcohol, nicotine or caffeine. Our intention isn't to discriminate against users. Instead, our basis for this exclusion is scientific, that is, it would gravely influence our results and we would not be able to use them. Females who are pregnant or breast-feeding are excluded from this study due to the medication given in this study.

Once you have been determined eligible for this study, you will not be able to consume any foods or drinks containing caffeine (chocolate, coffee, tea, soft drinks/pop such as cola) 24 hours before the scheduled session. Other restrictions are as follows: No food 12 hours before the test session, until we provide you with a light breakfast, drinking nothing but water after midnight the night before the session, and no use of any recreational drugs (e.g. alcohol) or over the counter medication 24 hours prior to the session. For those who were prescribed an anti-depressant medication, you may take your first dosage only after the test session.

You will be asked to come to the laboratory by 8:00 or 9: 00 a.m. on at least one occasion. The procedures for each session are as follows: At 9:30 a.m. you will swallow a dose of either the drug (dextro-amphetamine) or placebo (an inactive substance). Neither you nor the person testing you will be aware of which one you are receiving. The dose of dextro-amphetamine will be 30 mg. A urine sample will be collected at baseline. You will be asked to answer some paper and computerized questionnaires evaluating your mood and feelings before and a few times after you take the medication. You will also be given a task called the Stroop Attentional task that basically involves reading the colour of words as quickly as you can. There will also be one psychomotor task to measure your hand coordination. Your blood pressure and heart rate will also be measured. The entire session will take approximately 6 hours.

Risks
Some effects that you may experience after the administration of a single dose of 30 mg of dextro-amphetamine include mild euphoria (feelings of pleasure), irritability, nervousness, and restlessness. Other effects may include difficulty sleeping and agitation. It is important to note that the dose of dextro-amphetamine administered in this study is safe
and has been used in many studies before. If you develop any other side effects, they are usually mild and last for a few hours. The symptoms are all reversible and pose no danger to you.

Benefits
You will have no direct medical benefit from participating in this study, but the findings of this study may help future patients with alcoholism and depression.

Compensation
Your participation in this study is voluntary and you can withdraw from the study at any time. If you are diagnosed with depression, you should strictly follow the advice of your psychiatrist. If you decide not to participate or to withdraw from the study, this will not in any way affect your medical care or any benefits to which you may be entitled. The investigator may terminate your involvement in the study at any time (for example, due to medical reasons or for non-compliance with the study protocol). If you withdraw from the study, your compensation will be prorated to reflect the time you participated in the study.

Confidentiality
Your identity and the information obtained in this study will be kept strictly confidential and secure, available only to the researchers in the study. The data will be identified by your initials only, and not by your name. Published reports and presentations at scientific meetings will refer to grouped data and no person will be identifiable.

Contact Persons
Dr. Claudio A. Naranjo (416) 480-6761
Dr. Usoa E. Busto (416) 535-8501 ext. 6812
Xavier Balducci, M.Sc. (416) 480-6100 ext. 3557
I, (please print your name on line) ___________________________________ have read
the above text and fully understand the nature and the purpose of the study entitled "The Role
of the Brain Reward System in Depressed Alcoholics" in which I have been asked to
participate. The explanations that I have been given mentioned both the possible risks and
benefits of the study. I understand that I will be free to withdraw from the study at any time
without affecting my medical care in any way. I voluntarily consent to participate in this
study.

________________________________________   _________________________
Signature of the Volunteer      Date

____________________________________
Name of the Volunteer (typed or printed)

________________________________________  _________________________
Signature of the Witness      Date

____________________________________
Name of the Witness (typed or printed)

________________________________________  _________________________
Signature of the Principal Investigator    Date

____________________________________
Name of the Principal Investigator (typed or printed)
APPENDIX C

The following substances can be detected by the urine toxicology screen

• By chromatography:
  Methadone
  Methadone metabolite
  Morphine
  6-AM (heroin metabolite)
  Codeine
  Oxycodone
  Hydromorphone
  Hydrocodone
  Meperidine
  Amphetamine
  Methamphetamine
  Diphenhydramine/Dimenhydramine
  Phenylpropanolamine
  Ephedrine/Pseudoephedrine
  Cocaine
  Nortryptyline
  Desipramine
  Doxepin
  Ranitidine
  Cotinine

• By immunoassay:
  Amphetamines
  Barbiturates
  Benzodiazepines
  Cocaine Metabolite
  Ethanol
  Methadone
  Opiates
  THC
APPENDIX D

FMRI Study Consent Form

The Role of the Brain Reward System in Comorbid Major Depressive Disorder and Alcohol Dependence: an f-MRI Study

Principal Investigators: Dr. Claudio A. Naranjo
Dr. Usoa E. Busto

VOLUNTEER INFORMATION SHEET AND CONSENT FORM

You are being asked to participate in a study to help us understand how depression occurs. The main procedures involve giving you a single dose of a commonly prescribed medication called dextro-amphetamine (Dexedrine®) and to use a brain scanning technique called functional magnetic resonance imaging (f-MRI) which involves taking live “pictures” of your brain. This study will be conducted at the Sunnybrook and Women’s College Health Sciences Centre, 2075 Bayview Ave., Toronto, Ontario M4N 3M5, under the supervision of Dr. C.A. Naranjo and Dr. Usoa Busto. Your participation is completely voluntary and will involve the following:

Background
Close to 6% of Canadians aged 18 years and over have experienced depression at least once in their lives. The risk of depression among alcoholics is even higher than in the general population. For example, it has been estimated that up to 80% of alcoholics complain of depressive symptoms. In addition, individuals who have both of these disorders are more likely to have problems related to low self-esteem and greater impairment in day to day functioning. A specific area of the brain is associated with the perception of pleasure. This area of the brain can be stimulated either by certain kinds of drugs or meaningful life events. A malfunction in this particular brain region could be associated with symptoms of depression. Therefore, we have decided to test the functioning of this brain region in non-depressed alcoholics and depressed alcoholics. This study may give us a better understanding of how symptoms of depression and alcohol dependence occur.

Purpose
The purpose of this study is to look at the effects of a single oral dose (capsules swallowed by mouth) of 30 mg dextro-amphetamine on your mood and feelings. We will compare these effects in non-depressed alcoholics vs. depressed alcoholics.

Location
The study will take place at the Human Psychopharmacology Laboratory, Department of Psychiatry, room EG-04 at the Sunnybrook Health Science Centre, 2075 Bayview Ave, Toronto,
Ontario. The room is located in the E-wing, on the ground floor in room number EG-04. The f-MRI procedure will take place in the S-wing.

**Duration**

If you agree to participate in this study, you will be asked to come to Sunnybrook Health Sciences Centre once for an interview session (2 hours) and once for a study session (4 hours). If you have been diagnosed with depression and will be receiving treatment, you will be able to start antidepressant treatment after completing the study session.

**Procedures**

In order to participate in this study, you must be diagnosed with Alcohol Dependence. One half of the subjects included will also be diagnosed with Depression.

**Interview Session**

If you agree to participate in this study, you will undergo an initial assessment in which you will be asked a series of standard questions. You will also be required to provide us with a urine sample for drug screening. You may not be able to participate in the study if you have used any illicit drugs such as cocaine, amphetamine, or heroin within the last 3 months, or, if you have a recent/current history of drug abuse or dependence on a substance other than alcohol, nicotine or caffeine. Our intention isn’t to discriminate users. Instead, our basis for this exclusion is scientific, that is, it would gravely influence our results and we would not be able to use them. For those volunteers experiencing depressive symptoms, you may not be able to participate if your episode is significantly different from those who experience Major Depressive Disorder. Females who are pregnant or lactating are not eligible for this study.

**Study Session**

For the study session, you will be asked to come to the Human Psychopharmacology Laboratory in room EG-04 at the Sunnybrook Health Sciences Centre at a set time. The procedures for the session are as follows: After a “practice run” of computerized questionnaires about your mood and thinking patterns, you and the researcher will go to the lab (f-MRI lab) where the brain scanning machine is located. You will be asked to answer a short questionnaire to make sure you’re suitable for the scanning procedure called f-MRI or functional magnetic resonance imaging. You will be given capsules containing the dose of either the drug (dextro-amphetamine) or placebo (an inactive substance). Neither you nor the person testing you will be aware of which one you are receiving. The exact dose of dextro-amphetamine will be 30 mg or 0 mg (placebo). You will be asked to lie down and your head will be oriented within a cylindrical scanner (machine that takes the “pictures”) for up to 2 hours, so that we can measure the activity in the brain before and after the drug is in your system. During that time, your head will be rested on top of a pillow so that you don’t move your head significantly. We will be taking pictures of your brain while you answer questionnaires on the computer by tapping your finger. These questionnaires will ask you about your mood, feelings, and thinking patterns. The researcher and staff members will be close to you at all times to help you understand the procedure and to make you as comfortable as possible. The details of how they will take pictures of changes in the brain will be described to you by a staff member in that area and the researcher. One blood sample will be drawn near 2 hours after administration of the drug/placebo. After the scanning, we will return to EG-04 where you will sit comfortably in a chair completing further questionnaires at set times. Your blood pressure and heart rate will also be measured throughout the day.
Risks

Risks with d-amphetamine

D-amphetamine (Dexedrine ®) is commonly prescribed in children or adults with attention deficit hyperactivity disorder (ADHD) or narcolepsy (a sleep disorder). Some effects that you may experience after the administration of a single dose of 30 mg of dextro-amphetamine include mild euphoria (feelings of pleasure), irritability, nervousness, and restlessness. Other effects may include difficulty sleeping and agitation. We advise that you not drive on the day of the study session (i.e. the day that you receive the drug). Moreover, if you have difficulty sleeping the night after the study session, we advise that you not drive your vehicle the next day due to the potential dangers induced by fatigue while operating a vehicle. It is important to note that the dose of dextro-amphetamine administered in this study is safe and has been used in many studies before. In the case that you develop any side effects, they will be minor and last only for a few hours. All symptoms are reversible and pose no danger to you.

Risks with scanning (f-MRI)

The MRI scanning technique is commonly used in medicine to diagnose and examine abnormalities in different areas of the body including the brain as well as research (i.e. functional MRI or f-MRI). The known hazards associated with functional-magnetic resonance imaging (f-MRI) are minimal. There are no painful or deleterious effects of MRI on your body. Some people may become nervous while being scanned, because the scanner is confining and makes some knocking sounds. To make you as comfortable as possible, the f-MRI staff will introduce you to the technique so you can be more familiar and they will give you ear plugs to minimize the sound from the machine. The scanning procedure can cause some people to feel knocking, tapping, or buzzing sensations in their arms and legs. These feelings or sensations are completely harmless. During your time in the scanner, you can speak to the operator at any time by speaking through the intercom system.

Benefits

You will have no direct medical benefit from participating in this study, but the findings of this study may help future patients with depression and alcohol dependence by knowing more about how these illnesses occur.

Compensation

You will receive $150.00 for your participation after completing the test session as compensation for the incidental expenses (e.g. transportation) and the time commitment incurred by you while participating in this study.

Participation/termination

Your participation in this study is voluntary and you can withdraw from the study at any time. If you are diagnosed with depression, you should strictly follow the advice of your psychiatrist. If you decide not to participate or to withdraw from the study, this will not in any way affect your medical care or any benefits to which you may be entitled. The investigator may terminate your involvement in the study at any time (for example, due to medical reasons or for non-compliance with the study protocol). If you withdraw from the study, your compensation will be prorated to reflect the time you participated in the study.
Confidentiality

Your identity and the information obtained in this study will be kept strictly confidential and secure, available only to the researchers in the study. The data will be identified by your initials only, and not by your name. Published reports and presentations at scientific meetings will refer to grouped data and no person will be identifiable.

If you have any questions, feedback, concerns, suggestions, or complaints about the study, please don’t hesitate to contact us:

Dr. Claudio A. Naranjo (416) 480-6761
Dr. Usoa E. Busto (416) 535-8501 ext. 6812
Xavier Balducci, M.Sc., Ph.D. Candidate (416) 480-6100 ext. 3557
e-mail: xavier.balducci@sw.ca
I, (write your name on the following line) ______________________________ have read the above text and fully understand the nature and the purpose of the study entitled “The Role of the Brain Reward System in Comorbid Major Depressive Disorder and Alcohol Dependence: an f-MRI Study” in which I have been asked to participate. The explanations that I have been given mentioned both the possible risks and benefits of the study. I understand that I will be free to withdraw from the study at any time without affecting my medical care in any way. I also understand that I’m not waiving any legal rights by signing this form. I voluntarily consent to participate in this study.

________________________________           ______________________
Signature of the Volunteer             Date

___________________________________
Name of the Volunteer (typed or printed)

________________________________
Signature of the Witness             Date

__________________________________
Name of the Witness (typed or printed)

__________________________________            _________________________
Signature of the Principal Investigator              Date

__________________________________
Name of the Principal Investigator (typed or printed)