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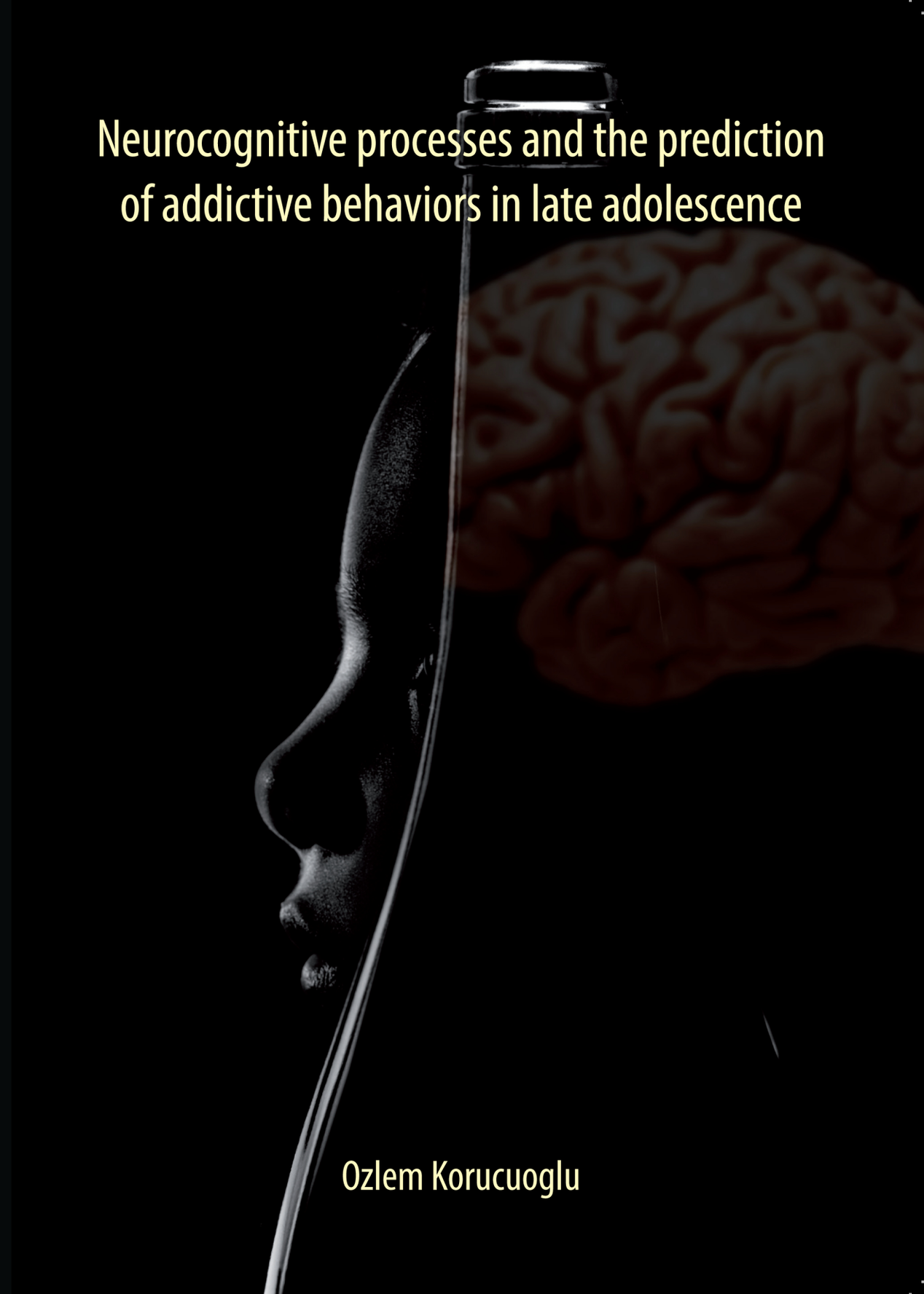

Neurocognitive processes and the prediction of addictive behaviors in late adolescence

Ozlem Korucuoglu

NEUROCOGNITIVE PROCESSES AND THE PREDICTION OF ADDICTIVE BEHAVIORS IN LATE ADOLESCENCE

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CHAPTER 1

GENERAL INTRODUCTION

ADOLESCENCE AS A VULNERABLE PERIOD

Adolescence, derived from the Latin word 'adolēscō' or 'adolēscere' refers to 'to grow up, mature' with a secondary meaning 'to burn'. These translations capture the turmoil of overlapping physical and psychological events that takes place in adolescence. Adolescence is a period of transition from childhood to adulthood that involves major physical, social, psychological, and physiological changes. Changes in hormone levels in adolescence contribute to social and affective development (Crone and Dahl, 2012; Forbes and Dahl, 2010) and play a role in the increased drive, thrill, sensation seeking, defensive and appetitive motivations (Quevedo *et al*, 2009). Whilst during childhood, parents provide a structure in the life of a child, with increasing age, adolescents develop their own identity, explore possible life directions for the future and need to gain necessary skills to become independent (Arnett, 2000). With the separation from the family and setting their own goals in life, social interactions become more important and adolescents are more sensitive to social influences (Petersen, 1988). For the development of an identity and the attainment of adult-like skills, exploration and risk taking behaviours increase during adolescence.

Many health risk behaviours, such as smoking or drinking, are initiated during this phase and this may affect later life. For example, many studies have reported that an early age of onset of substance use increases chances of later problems with and addiction to that substance (e.g., Grant and Dawson, 1997). Among the licit and illicit drugs, alcohol is often the first drug of choice in adolescence. A survey including 36 countries in Europa reported that among 15-16 year-olds on average 90% consume alcohol at least once in their lifetime and 57% in the last month. The quantity of alcohol use in the most recent drinking episode was on average 2-3 drinks of spirit, 40 centilitres of wine or one litre of beer (ERAB; Hibell *et al*, 2012). In another survey with 41 European participating counties on children and adolescents, 4% at age 11, 8% at age 13 and 21% at age 15 reported weekly drinking (HBSC; Currie *et al*, 2012). In the Netherlands, 60% of the 13 to 16 years olds had their first alcoholic drink and around 45% adolescents consume 5 or more drinks on a Friday or Saturday evening (Boekhoorn *et al*, 2007, cited in Hagemann, 2010). According to a recent report on alcohol and drug use in the Netherlands, alcohol use among 12 to 16 years olds in 2009 was less than alcohol use in 2003 (van Laar *et al*, 2011). These surveys demonstrate that underage drinking is common across European adolescents.

Early onset of alcohol use is one of the major risk factors both for the transition from occasional alcohol use to alcohol addiction in later life and for initiation of other health risk behaviours. Grant and Dawson (1997) reported that adolescents who start drinking before the age of 15 are four times more likely to become addicted in later life than those who started drinking at ages 20. The same study reported that alcohol dependence and abuse decreased 14

and 8% respectively, with each year alcohol use onset was delayed. While onset of alcohol use between 11-14 years old of age increased the risk of developing alcohol use disorder, this risk was greatly lower for onset at ages 19 and older. The risk profile for very early starters (before the age of 11) did not differ from the risk profile observed for late onset (19 and older) (Dewit *et al*, 2000). Similar to many other substances, early initiation of alcohol and cigarette use has also been found to be predictive of use of illicit drugs later in life (Agrawal *et al*, 2006). Moreover onset of smoking, alcohol, marihuana and cocaine use (besides other demographic characteristics) were predictive of other health risk behaviours (i.e. not wearing seatbelt, unsafe sexual behaviours, current substance use etc., Durant *et al*, 1999; Hanna *et al*, 2001). In short, early onset of alcohol (and other substances) increases the risk for transition to addiction and deteriorates adolescents' life also by increasing the likelihood of other unhealthy practices.

Dual Process Models: Adolescence and Addiction

Age-specific behavioural changes in adolescence are not limited to the increased prevalence rates of drug/alcohol use and unhealthy behaviours. Adolescence is a period accompanied with an increase in sensation seeking and risk taking broadly (Forbes and Dahl, 2010). During this period, many higher-order cognitive functions are under development, such as decision making, problem solving, attention and inhibitory control (Luna *et al*, 2010; Yurgelun-Todd, 2007). Moreover, social interaction and peers become a central driving motivation in the life of adolescents. For instance, peer interactions are shown to be more rewarding for adolescents than adults and children (Csikszentmihalyi *et al*, 1977). Therefore, behaviours like greater impulsivity and poor decision making are heightened especially under affective and social context (Blakemore and Robbins, 2012; Crone and Dahl, 2012). Such phenomena likely involve interactions between what the literature describes as "hot" versus "cold" cognition (*see* Casey and Jones, 2010; Gladwin *et al*, 2011). Changes during the maturation of the adolescent brain provide a biological basis for the changes in behaviour, advancements in cognitive functioning and emotional processing. A neurobiological dual-systems model has been proposed stating that a temporal difference in the maturation of two interacting systems, namely the prefrontal and the limbic system, accounts for increased incentive motivations and decreased regulatory processes in adolescence (Steinberg, 2005). The adolescent brain is characterized by a quickly maturing hyperactive limbic system, including the ventral striatum and amygdale, and an underdeveloped prefrontal system, including the inferior frontal cortex and anterior cingulate cortex (Casey *et al*, 2008; Jentsch and Taylor, 1999; Somerville *et al*, 2010; for an alternative three-system approach of adolescent brain, *see* Ernst *et al*, 2006). Other sources of evidence for the dual-systems model have been provided by the neuroimaging studies on structural and functional remodelling of the adolescent brain. From childhood

through adolescence, increase in functional connectivity, which has been linked with an increase in white matter (Giedd *et al*, 1999; Lebel and Beaulieu, 2011; Uddin *et al*, 2011), is necessary to promote acquisition of complex cognitive functions (Paus, 2005). Moreover, nonlinear changes (early peaks and later declines) in gray matter structures, especially in the associative areas, play a role in the development of higher cognitive functions (Giedd *et al*, 1999; Gogtay *et al*, 2004). Dopamine receptors in the striatal and nucleus accumbens show a peak during adolescence (Tarazi *et al*, 1998; Teicher *et al*, 1995). Adolescents are more sensitive to large rewards and show greater striatal activation to reward receipt due to an increased activity of the striatal and limbic system (Doremus-Fitzwater *et al*, 2010; Galvan, 2010), but diminished activity to rewards with low value (Galvan *et al*, 2006), suggesting that tendency to seek for rewards with higher values might play a role in adolescent high risk-taking behaviour. Increased striatal activity also plays a role in the functioning of frontal cortex. In adolescence, higher midbrain dopamine levels, which also increase reward-related signals to the prefrontal cortex, have been associated with increased frontal activity and reduced functioning (Dreher *et al*, 2008). During performance of a higher-level executive task, namely during the preparation phase of an inhibition task, increased frontal activity was observed in reward-trials compared to neutral ones in adolescents, suggesting that the behaviour was guided by the reward (Geier *et al*, 2010). As a result of these neuroadaptations, adolescence is a period marked by heightened drug motivations (especially after initiation) and limited cognitive capacity to control them. It has also been shown that alcohol-exposed adolescent rats learn better-than-expected outcomes faster than worse-than-expected outcomes and this biased learning may promote risk-based decision making in later life (Clark *et al*, 2012).

The rewarding effects of drugs and alcohol for which the adolescent is more responsive coupled with decreased inhibitory capacity to regulate behaviour leads to higher drug and alcohol use prevalence among adolescents. A review of the development of addictive behaviours in adolescence proposes that with repeated alcohol use during this period an approach oriented system becomes more sensitized while the regulatory system is compromised by (excessive) alcohol use (Wiers *et al*, 2007). These dual system models are able to account for the behavioural changes that takes place in adolescents, adolescent vulnerabilities for psychiatric disorders, and also several clinical disorders related to impulse regulation (Pfeifer and Allen, 2012). For instance, neurocognitive changes in chronic drug and alcohol users have been proposed to be a result of a dysfunction in the impulsive (or appetitive) system that promotes automatic approach tendencies towards alcohol and a deficit in executive control processes, which fail to inhibit these automatic approach tendencies. An additional theoretical concept called incentive-sensitization, which we will elaborate on later, describes the sensitization of the impulsive system with drug and alcohol use, and has been related to increased implicit cognitions in addictive individuals (i.e. drug-related cues capturing early

selective attention). In short, this model states that, with repetitive drug and alcohol use, drug-related environmental stimuli gain incentive salience (Berridge and Robinson, 2003; Robinson and Berridge, 1993; 2008). To sum, addicts and adolescents seem to be both characterized by an oversensitive impulsive system and a compromised cognitive control system.

Although dual process models are widely used, it is important to note some criticism for the application of these models. Regarding adolescent development, dual process models have been criticized for being overly simple and neglecting the role of social-affective changes on adolescent vulnerabilities (Crone and Dahl, 2012). Moreover, age-related increases and decreases in the frontal activity have been observed in neuroimaging studies of adolescent samples and it is argued that these findings cannot be fully explained by an immature prefrontal cortex. Other critics stated that neurocognitive functions of the two systems are not anatomically separable (e.g., Keren and Schul, 2009). Although the essence of dual-process models has partly been forged based on neural evidence for their existence, up to now this evidence has been based on studies mapping brain functions to neural structures, instead of looking at connections across networks, and are inadequate to explain the complexities of human brain and behaviour (Pfeifer and Allen, 2012).

From this introduction, it can be concluded that future investigation and experimentation is needed for a better understanding of adolescent vulnerabilities for the development of addiction. While alcohol and drug use tend to peak in early adolescence and subsequently declines in most individuals, a minority maintains an excessive and hazardous drinking pattern (Chen and Kandel, 1995; Johnston *et al*, 2012; Schulenberg *et al*, 2006). When entering adulthood, with the change in set of priorities and responsibilities, many individuals decrease their level of alcohol consumption (Kandel and Yamaguchi, 1985). One of the challenges in the addiction field is to identify vulnerability factors that can predict why some adolescents become addicted while others not.

SENSITIVITY TO ALCOHOL AS A RISK FACTOR FOR THE DEVELOPMENT OF SUBSTANCE USE DISORDER

There are indications that adolescents might be affected differently by alcohol consumption in comparison with adults. Altered sensitivity to alcohol during this period might play a role in the continuation and escalation of alcohol use. One way to study alcohol sensitivity is to test individuals after administration of a single dose of alcohol and compare the results with sensitivity to a placebo dose, referred as *alcohol challenge studies*. Alcohol consumption induces distinct and measurable stimulant and sedative effects based on the dosage and the limb of the blood alcohol curve. At low doses and during the rising limb of the blood alcohol curve, drinkers typically experience stimulating, positive and reinforcing effects. At high doses and

during the falling limb of the curve, drinkers typically report sedative and aversive effects from alcohol. Individual differences in subjective responses to alcohol is an important topic, as it is a well-established risk factor for the development of addiction. However, would high or low sensitivity to the rewarding and stimulating effects of alcohol promote drinking exclusively due to their pharmacological effects or would these sensitivities play a role in addiction through other mechanisms as well? For instance, although in earlier studies a great deal of attention has been paid to performance differences across high and low sensitive individuals in response to administration of alcohol and placebos, recent evidence suggests that individuals with low and high subjective response to alcohol demonstrate differences in brain function in sober conditions as well. The alcohol sensitivities discussed in this section have two main foci: 1) Individual differences in subjective experiences to pharmacological effects of alcohol (reinforcing/stimulating and aversive/sedative effects of alcohol); 2) Behavioural and neurobiological processes that are typically more sensitive to alcohol and may show variability across age groups or individuals. The current thesis investigated individual differences in responses to alcohol in human participants. Given the lack of pharmacological studies in human adolescents, for the second part an overview of findings in animal studies will be reviewed, followed by effects observed in studies with human adults. Moreover, genetic factors may play a role individual differences in response to alcohol. Next, a single nucleotide polymorphism, which plays a role in sensitivity to the rewarding effects of alcohol will be introduced.

Level of response as a risk factor: subjective experiences

Initial evidence for the involvement of subjective response to alcohol as a risk factor in addiction has been established in studies where Family History Positive (FHP) subjects demonstrated less intense reactions to alcohol, suggesting heritability of LR response (Schuckit *et al*, 1988; Schuckit *et al*, 1991). Over the years, the role of individual differences in subjective response to alcohol has been studied with measures on body sway, heart rate (Ray *et al*, 2006), cortisol, skin conductance (Newlin and Thomson, 1999) and brain responses (Schuckit *et al*, 1988). Based on a critical review on the effects of alcohol on FHP and FHN individuals (Newlin and Thomson, 1990) proposed a differentiator model (DM) stating that FHP individuals (and other individuals at risk for alcoholism) experience both an increased sensitivity to the rewarding effects of alcohol during the rising limb of blood alcohol concentration (acute sensitization) and a decreased sensitivity to the sedative effects of alcohol during the falling limb (acute tolerance). Contrary to the DM, which focuses on biphasic effects of alcohol, the Low Level of Response Model (LLR) studies alcohol sensitivity after a large dose of alcohol and a relatively long time-frame (high acute tolerance). Self-report measures of alcohol effects questionnaires demonstrate that people with low level of response (low LR) require higher

quantities of alcoholic beverages to feel the same pharmacological effect compared with high LR individuals (Schuckit *et al*, 1997). LLR model states that individuals with low LR may have a faulty feedback mechanism regarding their level of alcohol intoxication, resulting in a lack of warning signals to regulate drinking which promotes excessive drinking and development of tolerance (Schuckit, 1994; also for a review, *see* Morean and Corbin, 2010).

Alcohol effects in adolescents: animal studies

Animal models of addiction show that adolescents react differently to the sedative high dose and stimulative low dose effects of acute alcohol. These age-specific differences in response to alcohol may promote increased vulnerabilities during adolescence. To begin with, compared to adult animals, adolescent animals are less sensitive to the alcohol-induced motor impairment and alcohol-induced sedation (Little *et al*, 1996; White *et al*, 2002b). Research comparing adult rats with adolescents revealed that motor impairment was greater in adults than adolescents at higher doses of alcohol (White *et al*, 2002b). Moreover, while a low dose of alcohol decreased locomotor activity related with the sedative effects of alcohol in adult rats, this measure was unchanged in adolescent rats (Little *et al*, 1996). Sedative effects of alcohol and alcohol-induced impairment in motor coordination are important factors in deciding the maximum amount of alcohol that an individual can consume. A lack of alcohol-induced impairments might affect the limit of alcohol consumption per occasion and therefore this limit might be higher for adolescents that are relatively insensitive to its sedative effects. Moreover, a study comparing adolescent and adult rats treated with ethanol or saline demonstrated that only adolescent rats which were exposed to alcohol repeatedly were less sensitive to the motor impairment effects of alcohol during adulthood (White *et al*, 2002a), suggesting that the lack of an alcohol effect on motor impairment might be due to excessive drinking in adolescence, which in turn decreases adult sensitivity to the negative sedating effects of alcohol.

In contrast, adolescents appear to be more sensitive to the alcohol-induced impairment on cognitive functioning (see White and Swartzwelder, 2005, for a review), which has implications for inhibiting or regulating maladaptive behaviours. Adolescent rats are more sensitive to the deteriorating effects of alcohol on memory and learning compared to adults (Blitzer *et al*, 1990; Markwiese *et al*, 1998). In adults, the interference of alcohol with performance requires much higher alcohol levels. Another effect of alcohol is the increase in locomotor activity, which is associated with the stimulating effect of low dose of alcohol, and manifests itself differently in adult and adolescent samples. Contrary to adult mice, adolescent mice exhibited locomotor tolerance rather than locomotor sensitization when drinking is paired with environmental cues, but they exhibited an increase in context-independent locomotor sensitivity after a low dose stimulating effects of alcohol (Faria *et al*, 2008). Moreover low-

dose alcohol stimulation of locomotor activity has been associated with high alcohol consumption in adolescent rats, which was present at the age of onset of alcohol drinking (White *et al*, 2002a).

Acute alcohol effects in adults: human studies

Alcohol challenge studies in human adult samples have shown that acute alcohol impairs processes related to the executive functions (such as inhibition) and enhances processes related to the appetitive system in a dose dependent manner (for a review, *see* Field *et al*, 2010). Previous studies revealed that following alcohol consumption, alcohol-related cues become highly salient, as reflected in increased appetitive processes and cognitive biases towards alcohol-related stimuli (Adams *et al*, 2012; de Wit and Chutuape, 1993; Duka and Townshend, 2004; Hodgson *et al*, 1979; Kirk and de Wit, 2000; Schoenmakers *et al*, 2008). Interestingly, it has been shown that acute alcohol heightens the motivational system not only towards alcohol-related stimuli but also towards smoking cues suggesting that acute priming has a general facilitative effect on appetitive approach tendencies (Field *et al*, 2005). It is important to note that the priming effects of alcohol on impulsive (or reflective) processes are not linear. Generally speaking, although a low dose of alcohol is sufficent to prime the processes related to the appetitive system, in order to observe the detrimental effects of alcohol on the reflective processes at least a moderate dose of alcohol is required (Field *et al*, 2010). There are some indications that the priming effects on the appetitive processes is greater at low doses compared to higher dosages and placebos (Duka and Townshend, 2004; Schoenmakers and Wiers, 2010), which has not been reported for impairing effects on reflective processes (Field *et al*, 2010). As pointed out in the previous section when discussing dual process models of addiction, the chronically induced increase in appetitive processes and the decrease in cognitive functioning mimic the acute effects of alcohol on these processes.

Synopsis of alcohol studies in adults and adolescents

To sum up, acute alcohol studies show that while the stimulating effects of a low dose alcohol lead to an increase in the motivational reaction toward drug-related stimuli, sedative effects of moderate to high dose of alcohol lead to a decrease in cognitive functions (Field *et al*, 2010; Hernández and Vogel-Sprott, 2010; Ridderinkhof *et al*, 2002), and that both are outcomes of long-term repetitive use. Based on animal studies it seems that the process of sensitization and the low level of response to alcohol's detrimental effects on cognitive processes are magnified in adolescents. These age-specific effects of alcohol might promote the development of alcohol use disorders in adolescence. The finding of an association between the stimulating effects of

locomotor activity in adolescent and drinking behaviour is in line with this notion. Moreover, alcohol use during adolescence interferes with the development of sensitivity in adults (White *et al*, 2000), consistent with the notion that alcohol consumption during this period affects sensitization in later life. Empirical evidence also supports the view that both the acute and the chronic use of alcohol leads to similar neuroadaptations in the brain. In a review on the development of addictive behaviours in adolescence, it was proposed that acute alcohol mimics the effect of long term use and could be a unique predictor of vulnerability to alcohol abuse in later life (Wiers *et al*, 2007). This was tested (for a short time-period) in the present research project.

Limitations of existing studies

Most of our knowledge on the age-specific effects of acute alcohol comes from animal studies. However, certain differences across species likely limit the generalizability of results. Compared to mice or rats, the human cortex is a more complex structure, making a direct comparison of functional interactions between brain regions across species difficult. Many processes associated with normal human development are more complicated than in animal models (Concha *et al*, 2010; Schmierer *et al*, 2007). There are also developmental differences in the brain of primates and humans. For instance, contrary to simultaneous gray matter development in non-primates in all regions, human gray matter development and synapse elimination follows a temporal difference with some regions completing earlier than others (Giedd *et al*, 1999). These differences makes it difficult to compare findings of animal and human studies, especially for the period when the brain is still in the process of development. Moreover, in humans effects of continued heavy drinking have been examined by cross-sectional studies comparing brains of adolescents with substance use disorder (SUD) with brains of adolescents who have no or limited experience with drinking (for examples, see, de Bellis *et al*, 2000; Tapert *et al*, 2001; 2003; Thomas *et al*, 2005). It is unclear whether the deficits observed in individuals with SUD predate the development of addiction or were induced by repeated administration of alcohol.

Genetic Vulnerability to the Rewarding Effects of Alcohol

Genetic factors can account for variance in responses to drug cues and they pose a predisposition for the development of excessive incentive sensitization. The endogenous opioid system has been implicated in the pathophysiology of some aspects of alcoholism as it modulates some of the reinforcing effects of alcohol via activation of opioid receptors in the ventral tegmental area and nucleus accumbens, which enhances extracellular concentrations of

dopamine (DA) in the mesolimbic pathway (Gianoulakis, 2009; Koob and Kreek, 2007; Ramchandani *et al*, 2011). This has raised an interest in research to genes encoding for endogenous opioid receptors, with a particular focus on a single-nucleotide polymorphism (SNP) located in the *OPRM1* gene of mu opioid receptor (A118G).

Acute administration of alcohol releases B-endorphin, which is part of the system involved in reward and reinforcement (Merrer, 2009). In the G-allele carriers of this SNP, the receptor binding affinity for β-endorphin is thought to be 3-fold higher, therefore carriers of the G-allele experience greater reinforcement from acute administration of drugs and alcohol compared to A-carriers (Bond *et al*, 1998). Moreover, these individuals have over-reactive appetitive system and demonstrate greater cognitive biases towards alcohol-related cues; such as approach and attentional bias (Pieters *et al*, 2011; Wiers *et al*, 2009). In line with their higher subjective response to alcohol, alcohol cues and alcohol priming elicit a higher biological response in G-carriers; both in neurochemical and functional level. G-allele carriers show higher striatal dopamine level and increased striatal neural activity toward alcohol administration (Filbey *et al*, 2008b; Ramchandani *et al*, 2011). Treatment studies also provide evidence for the involvement of opioid receptors in sensitivity to rewarding effects of alcohol. In a treatment study with non-treatment seeking heavy drinkers who had more relatives with alcohol problems, administration of opiate receptor antagonist Naltrexone; which reduces the stimulating effects of alcohol; increased the time between drinks (Tidey *et al*, 2008). Also in adolescent problem drinkers, administration of naltrexone decreased craving and reduced drinking (Miranda *et al*, 2013). These studies support the view that the OPRM1 polymorphism moderate responses to drug-related cues.

Despite an abundance of research focusing on the OPRM1 genotype, the existing accounts fail to unravel the exact mechanism through which the OPRM1 genotype affects the alcohol dependence. The accumulating evidence from the association and the clinical studies are inconclusive so far. While some studies report that the prevalence rates of alcohol addiction is higher in G-allele carriers, including adult (Bart *et al*, 2005; Koller *et al*, 2012; Kranzler *et al*, 1998; Schinka *et al*, 2002) and adolescent samples (Miranda *et al*, 2010), others fail to replicate (Bergen *et al*, 1997; Franke *et al*, 2001; Gelernter *et al*, 1999; Loh *et al*, 2004). Critical reviews on the topic suggest that observed inconsistencies in the literature may be due to factors that differ across studies; such as heterogeneity of study sample, selection of control groups, clinical heterogeneity (van der Zwaluw *et al*, 2007). Moreover, vulnerabilities to drug addiction are likely to be the result of an interaction between genes and environment. Environmental events, by their influence on mechanisms that alter the function of genes, may result in the development of complex phenotypes. For instance, it has been shown that binge-like drinking during adolescence can induce alterations in the mesolimbic dopaminergic and glutamatergic systems and can trigger changes in gene expression, which are involved in drug-related

behavioural sensitization (Pascual *et al*, 2009). Epigenetic mechanisms involved in the regulation of the saliency of environmental stimuli may promote alcohol intake in adulthood (Alfonso-Loeches and Guerri, 2011; Guerri and Pascual, 2010; Renthal and Nestler, 2008). These studies support that genetic predisposition and early exposure to alcohol contribute to the development of addiction and moderate responses to drug-related cues. Note that studies on the OPRM1 genotype up to date have been conducted in heavy or treatment seeking adults and adolescents, making it difficult to know whether observed effects are a consequence of their predisposition or their heavy drinking.

COGNITIVE AND AFFECTIVE PREDICTORS OF ALCOHOL ESCALATION

During adolescence, the brain undergoes a series of functional and anatomical changes linked to advancements in cognitive and emotional processing. There is a large volume of cross-sectional studies describing the detrimental effects of alcohol use during this period on cognitive and emotional development. Few studies addressed questions like how drinking-related abnormalities in brain functioning contribute to escalation in alcohol use or whether individual differences in neurocognitive functioning prior to the progression of drinking behaviour have an influence on drinking-induced changes. In an effort to identify the cognitive risk pathways, in recent years there has been an increasing interest in longitudinal neuroimaging studies. Emerging findings demonstrate that atypical brain responses pre-existing before the initiation of alcohol use pose neural vulnerabilities. But also, alcohol use during this period intervenes with typical neural maturation of the brain and leads to further alterations in brain functioning. A number of studies have found that adolescents who transitioned to a heavy drinking pattern demonstrated less activity during a response inhibition paradigm before the onset of alcohol use (Norman *et al*, 2011; Squeglia *et al*, 2012; Wetherill *et al*, 2013), however after transition to heavy drinking they exhibited increased activity (Squeglia *et al*, 2012; Wetherill *et al*, 2013). This increased baseline activity has been associated with poor performance (Squeglia *et al*, 2011). However for adolescents with limited alcohol exposure (four to five years of heavy drinking after initiation) a different pattern was observed; these individuals demonstrated a decrease in brain function together with poorer performance, suggesting that at the initial phase of drinking adolescents' brain was able to compensate for drinking-related neural deficiencies, however, further continuation with drinking damaged this compensation mechanism (Squeglia *et al*, 2009; Squeglia *et al*, 2012).

The majority of these longitudinal neuroimaging studies focused on brain functioning during response inhibition paradigms. Response inhibition is important for behavioural control. Poor response inhibition and related brain abnormalities have been associated with risk for alcohol abuse and also with consequences of acute and chronic alcohol use (Easdon and Vogel-

Sprott, 2000; Field *et al*, 2010; Ivanov *et al*, 2008; Lawrence *et al*, 2009; Nigg *et al*, 2006, but also *see* Goudriaan *et al*, 2011). Several studies focusing on age-related changes in cognitive control mechanisms revealed that action-monitoring processes necessary for behavioural adjustment (monitoring response conflict, error detection and response inhibition) undergo developmental changes in adolescence (Davies *et al*, 2004; Hogan *et al*, 2005; Ladouceur *et al*, 2004; 2007). In adolescents, poor response inhibition predicted alcohol-related problems, drug use, comorbid alcohol and drug use; independent of IQ, parental risk or personality (Nigg *et al*, 2006). These studies suggest that brain functioning associated with response inhibition represents a neural vulnerability that both predate and precede alcohol use.

To the contrary, regarding the predictive value of abnormal affective processing and underlying neural mechanism in the development and maintenance of alcohol use, there is still insufficient data from prospective studies. Prospective behavioural studies have shown that alcohol-related associations and cognitive biases predict alcohol use in at-risk and healthy adolescents (Pieters *et al*, 2012; Thush and Wiers, 2007; Thush *et al*, 2007; Thush *et al*, 2008). Moreover increased brain activation towards alcohol-related pictures differs across groups of young individuals who transition to heavy drinking and maintain same levels of alcohol use (Dager *et al*, 2013a). In another study looking at the prospective predictive value of reward-related brain responses, personality and behaviour demonstrated that personality predicted initiation of alcohol use better than behavioural measures and brain responses, with brain responses being a moderate predictor (Nees *et al*, 2012). As explained by the authors and in line with the observed findings of Dager and colleagues' study, reward-related brain responses might be an important factor for the development of alcohol abuse rather than initiation of alcohol use. Complementary evidence supporting the involvement of cognitive biases and affective processes in the progression of drug use comes from studies on tobacco and cannabis. In heavy cannabis users, behavioural approach tendencies for cannabis cues and related brain activity predicted cannabis use and problems after six months (Cousijn *et al*, 2011; 2012). Further, smokers with greater attentional bias for tobacco cues were more likely to relapse after cessation (Waters *et al*, 2003). These studies show that in addition to deficiencies in cognitive processes, altered behavioural output and brain functioning of the appetitive system are reliable predictors of alcohol and drug escalation. Further research regarding the role of alcohol and other drug-related cognitive biases would be of great help in understanding trajectories of drug and alcohol escalation.

THE ROLE OF CONDITIONED CUES

Sensitization to Environmental Cues

Anticipation to biologically relevant environmental cues may also play a vital role in determining control of motivated behaviour. Over decades, many studies focused on the reactions to alcohol cues and their' clinical relevance in samples with a diagnosis of SUD and/or in individuals with a history of moderate to heavy drug/alcohol use (in hazardous or social drinkers) compared to controls. Heavy drinking was associated with positive ratings of alcohol pictures and this effect was consumption related (personal drinking experience) rather than environmental (family, peers etc.) (Pulido *et al*, 2009). Moreover, the degree of pleasurable effects were higher for pictures depicting pre-drinking or preparatory scenes (i.e. alcohol being poured) compared with post-consumption scenes (Lee *et al*, 2006). Functional imaging studies have revealed that substance cues can stimulate brain regions associated with the reward system (referred as *cue-reactivity response*) and can elicit craving (Myrick and Anton, 2004). Therefore, appetitive or drug-related cues are likely to influence the behaviour of substance dependent individuals. In the addiction literature, the process of sensitization towards drug-related stimuli has been very influential due to the Intensive-sensitization theory by Robinson and Berridge (1993). According to this model, repeated exposure to an addictive substance induces neural sensitization towards drugs and conditioned drug-related environmental cues, leading to the excessive attribution of incentive salience and approach inclinations toward those cues (Flagel *et al*, 2009; Robinson and Berridge, 1993; 2008). Yet it remains unclear at which stage in the development of addictive behaviors these neuroadaptations emerge, especially in humans. Suboptimal choices or maladaptive behaviours can also promote development of such conditioned responses. For instance, it is important for the cognitive control system to effectively inhibit impulsive drug-related behaviours in face of negative consequences; a process which might be compromised in adolescence due to underdeveloped frontal cognitive functions. Thus recently the focus in cue reactivity and craving shifted to younger samples in order to understand the time course and the nature of these neuroadaptations (for an early example, *see* Tapert *et al*, 2003).

Interaction between Alcohol Cues and Alcohol Administration

A second line of research focuses on the specific biases in the processing of alcohol-related stimuli in individuals with excessive drinking profiles and/or with SUD. A variety of these biases represent the significance of alcohol-related stimuli, including spatial and non-spatial attentional biases, implicit memory associations and approach tendencies (Field *et al*, 2004;

Field and Cox, 2008; Wiers and Stacy, 2006). However, only recently has the performance on executive functions in the context of alcohol cues received attention. Studies revealed that in the presence of alcohol-related stimuli, heavy drinkers demonstrated difficulty in inhibiting response, decreased accuracy and response speed in interference inhibition task (Field *et al*, 2007; Petit *et al*, 2012; Rose and Duka, 2008). These findings are consistent with earlier observations of increased attentional processes and approach tendencies towards alcohol-related cues. Likewise, as presented in an earlier section, while a prime dose of alcohol increased attentional bias towards alcohol-related cues, a high dose of alcohol decreased accuracy for alcohol-related cues in an interference inhibition task (Duka and Townshend, 2004). However, some controversy remains. Literature has emerged that offers findings supporting the notion that conditioned alcohol-related cues might elicit compensatory responses to counter alcohol effects (Birak *et al*, 2010; 2011). In these studies after alcohol administration performance was less impaired during an affective response inhibition paradigm.

AIM OF THIS DISSERTATION

The primary aim of this dissertation was to investigate the effect of acute alcohol on neurocognitive systems involved in the development of addictive behaviours in adolescents. A secondary aim of the project was to investigate whether alcohol-induced changes in cognitive and affective processes would be predictive of alcohol escalation in young people. While addressing the above research questions, the methodological approach taken in this dissertation and the secondary aims of each individual study discussed in the following chapters provide new perspectives to the existing literature. First, contrary to earlier studies where functional differences across individuals with different levels of response to alcohol were studied at group level (i.e. low vs. high), we took an individual differences approach, where variance in brain functions in response to alcohol administration were tested as predictors. Second, many studies provide findings supporting the similarities between chronic and acute effects of alcohol on behaviour and brain function, however no studies attempted to make a more direct association between an individual's response to acute alcohol and his propensity for a chronic alcohol abuse disorder. Therefore this dissertation provides a first step in bridging this gap in the field by focusing on the predictive value of functional changes after a single dose administration in later alcohol escalation. Third, based on earlier studies of developmental psychology focusing on affective processing and social interactions, adolescent cognitive performance is expected to vary depending on the context of the task at hand. In this regard, by comparing performance in a cognitive control task across two versions; one with an affective context and the other with a neutral context; the current thesis also contributes to our understanding of motivational influences on cognition in adolescence. Also extending on earlier behavioural prospective

studies of alcohol approach biases, in the current thesis we tested the predictive value of alcohol-induced changes on brain activation. Moreover, with an additional experiment, we focused on how a genetic vulnerability for alcohol's rewarding effects observed in adult samples would manifest itself in the adolescent brain with a limited prior exposure to alcohol.

To answer our research questions, we conducted a longitudinal study, where adolescents between ages 16 to 20 were tested in different phases. Until now, there have been a limited number of neuroimaging studies on implicit alcohol cognitions, and these were done exclusively in adults. In the first phase of the study, we aimed to develop an EEG version of an approach-avoidance task focusing on motor-related processes after alcohol administration. This study included graduate and undergraduate students. In the second phase, we turned our focus to the adolescent sample and conducted an EEG experiment where we looked at how cognitive processes and alcohol-related biases were influenced by alcohol administration in late adolescence. In this project 145 adolescents between 16-20 years old were tested once after alcohol and once after placebo administration. The aim of the acute alcohol administration in the EEG project was twofold: first we investigated the effects of acute alcohol on performance and brain activation in this sample. Second, we tested the predictive power of alcohol-induced changes on neurocognitive processes on alcohol escalation. In order to test the effects of acute alcohol as a predictor in the development of addiction, first we needed to demonstrate which specific behavioural and neurocognitive processes were influenced by acute alcohol. Possible changes in subject's drinking habits were followed-up with online surveys after six months preceding their participation to the EEG session. Subjects' saliva samples were collected for a following genotype-based fMRI experiment which took place at the last phase of the study. The aim of this fMRI study was to investigate differences in neural responses across genetic groups of individuals with increased sensitivity towards alcohol.

Chapter 2 describes the study of alcohol-induced changes on response preparation for the tendency to approach alcohol (approach bias). To study response preparation, a typical approach avoidance paradigm was modified according to earlier examples of response preparation in the EEG literature. Neural correlates of advance response preparation were tested for approach alcohol tendencies after placebo and alcohol administration.

Chapter 3 investigates the effect of acute alcohol administration on response preparation for approach tendencies in a sample of heavy and light drinking adolescents. Using a more implicit version of the alcohol approach bias task in Chapter 2, acute alcohol effects on response preparation were studied by looking at motor-related lateralization index after placebo and alcohol administration. Relationship between neural processes underlying response preparation for approach alcohol tendencies, drinking-related problems and motives were investigated. In

addition, alcohol-induced-changes on the lateralization index were used for the prediction of alcohol escalation over six-months.

Chapter 4 describes a study of alcohol effects on neurocognitive processes of conflict monitoring and error detection processes in the context of motivationally relevant alcohol cues in an adolescent sample. Using an affective Go-NoGo task, the N2 and the ERN event-related components for alcohol and soft drink cues that signal the inhibition of a prepotent response were studied after alcohol and placebo administration. In addition, the predictive value of alcohol-induced changes on ERP components for alcohol and soft drink cues on alcohol escalation over six-months was tested.

Chapter 5 focuses on the neural circuitry involved in alcohol taste-cue reactivity in a selected adolescent sample (from the larger study) with genetic vulnerability to the acute reinforcing effects of alcohol and at early stages of alcohol use. Using functional magnetic resonance imaging (fMRI), brain activity and frontostriatal functional connectivity after delivery of alcohol-taste were analysed across G- and A-alleles of the OPRM1 gene in an adolescent sample at early stages of alcohol use.

Chapter 6 provides an overview and a general conclusion of the studies together with limitations, suggestions for future research and possible implications of our findings.

CHAPTER 2

Preparing to approach or avoid alcohol: EEG correlates, and acute alcohol effects

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ABSTRACT

Recently an approach-bias for alcohol has been described as an important cognitive motivational process in the etiology of alcohol use problems. In the approach-bias, perception and action are inextricably linked and stimulus response associations are central to this bias: performance improves when task instructions are congruent with a pre-existing stimulus-response association. These pre- existing response associations could potentially allow advance response preparation and execution. The present study aimed at investigating the effect of the alcohol approach bias on response preparation by means of event-related desynchronization in the beta band (beta-ERD) of the EEG signal and the effect of acute alcohol in the approach bias in response to alcohol cues. Subjects (18 social drinkers) performed an adapted alcohol-Approach Avoidance Task, in which a preparatory period was provided between alcohol/soft drink cues and approach/avoid responses. Subjects were tested both in a placebo and in an alcohol condition (counterbalanced). Posterior beta-ERD was found to increase during preparation for alcohol-approach trials. The beta-ERD in the congruent block increased following alcohol administration. These results suggest that advance response preparation may play a role in the alcohol approach bias and that acute alcohol facilitates response preparatory processes for approach alcohol trials. Future EEG studies using the adapted AAT may help understanding approach biases in addiction.

INTRODUCTION

A large number of neuroadaptations are known to develop over time in response to repeated experience with drugs and the significance of drug-related stimuli is reflected in a variety of cognitive biases, including attentional biases (Field and Cox, 2008; Field *et al*, 2004), implicit associations (Ostafin and Palfai, 2006; Palfai and Ostafin, 2003) and approach tendencies (Field *et al*, 2008; Wiers and Stacy, 2006). These processes may play an important role in drug seeking and relapse as those motivationally relevant stimuli will elicit conditioned approach responses (i.e. approach bias toward drug related stimuli measured by approach avoidance tasks). Not only dependent patients but also heavy and social drinkers show an approach bias toward alcohol-related stimuli, yet in various degrees (Field *et al*, 2008). Moreover, approach tendencies can be retrained which helps patients to stay abstinent for longer periods (Eberl *et al*, 2013; Wiers *et al*, 2011). Although the approach bias has such clinical relevance, there are as-yet few studies aimed at unraveling neurocognitive processes underlying this approach bias.

In a typical alcohol-Approach Avoidance Task (alcohol-AAT), reaction times are measured while subjects are instructed to approach or avoid alcohol-related or non-alcohol-related pictures with a joystick movement (Wiers *et al*, 2009). In a relevant-feature version of the task (Rinck and Becker, 2007), congruent and incongruent arm movements are required in separate blocked conditions and the alcohol approach bias is measured as facilitations in response times when the valence of the task-related response is congruent with the valence of the stimulus (i.e. approaching pleasant stimuli and avoiding aversive stimuli) compared to incongruent situations (i.e. approaching aversive or avoiding pleasant stimuli). The alcohol approach bias is measured as the reaction time (RT) differences between congruent and incongruent block trials, note that this controls for general response bias due to a specific action (approach/avoid) or due to a specific stimulus category (alcohol/control cues). Recent reviews on approach bias state the importance of learning through which appetitive response outcomes reinforce stimulus-response associations and over time conditioned cues start to evoke an anticipatory response (Watson *et al*, 2012). Approach bias for a certain stimulus type is unique compared to other motivational processes (i.e. attentional bias) in a way that in the approach bias, perception and the production of actions are inextricably linked via stimulus-response associations. It follows that performance improves when task instructions are congruent with the pre-existing stimulus-response associations and these stimulus response associations could potentially influence advance response preparation and execution. In the current study we wanted to study response preparation in approach bias with the use of EEG.

The primary focus of the current study was the neural activity during this preparation period in response to approach toward and avoidance from alcohol-related stimuli before the actual motor response is given. Therefore, we converted the relevant-feature version of alcohol-

AAT to a cued reaction time paradigm suitable for electroencephalogram (EEG) analyses. Preparatory activity can be studied with cued reaction time paradigms, in which a warning or a preparatory stimulus (S1) is followed by an imperative stimulus (S2) to which the subject has to give a response (i.e. approach or avoid). Informative cues allow preparatory processes to be disentangled from movement execution. In studies using cued reaction time paradigms, the oscillatory activity associated with processes involved in response preparation shows a characteristic modulation. At the level of oscillations, preparation and execution of movements are preceded by a decrease of spectral amplitude (event-related desynchronization, ERD) in the beta frequencies (13–30 Hz). The topography of this deactivation varies: while frontal and centro-parietal beta-ERD is observed during preparation and execution of hand and finger movements (Gladwin *et al*, 2006; Stancák and Pfurtscheller, 1995; Wheaton *et al*, 2005), visually guided responses that demand sensory motor integration, such as object and tool manipulation, show a centro-parietal and occipital distribution (Kranczioch *et al*, 2008; Labyt *et al*, 2003).

A second goal of this study was to determine acute alcohol effects on approach bias-related components. Acute alcohol enhances processes related to the cognitive biases in a dose dependent manner (for a review see Field *et al*, 2010). A low dose of alcohol has been found to enhance cognitive biases in addiction (Field *et al*, 2010), sometimes referred to as an alcohol-priming effect. Previous studies revealed that following alcohol consumption alcohol-related cues become highly salient, as reflected in increased motivational processes and cognitive biases toward alcohol-related stimuli (Adams *et al*, 2012; Duka and Townshend, 2004; Hodgson *et al*, 1979; Schoenmakers *et al*, 2008). However, the effect of a prime dose of alcohol on EEG indices involved in the appetitive processes have not yet been studied, to the best of our knowledge. Thus, in this study, subjects performed an AAT, adapted for use with EEG measurements, under a low dose of alcohol and placebo conditions. We hypothesized that approach-alcohol trials would be associated with stronger response preparation. Thus, we expected congruent trials to be accompanied by higher beta-ERD. Priming approach tendencies with alcohol administration was expected to lead to an enhanced response preparation for congruent trials, and hence an increase in beta-ERD.

METHOD

Subjects

Twenty-three undergraduate students (10 males, mean age = 21.9 years, range = 18–27 years) were recruited. Participants had a minimum weight of 50 kg and had consumed at least one full drink in their lifetime. None of the subjects reported current or past neurologic or psychiatric illness. None of the female participants reported any risk for pregnancy. Prior to the

appointment, subjects abstained from any alcohol for at least 24 h, from any legal or illegal drugs for at least 1 week, and from all food and caffeine for at least 4 h (for alcohol-placebo designs, see Marlatt and Rohsenow, 1980). Four subjects' data were excluded due to misinterpretation of task instructions, equipment failure, or severe movement artifacts. One subject's data were excluded due to an extreme AUDIT score (AUDIT = 20, z = 2.55). The analysis was conducted with the remaining 18 subjects. All participants had normal or corrected- to-normal visual acuity and two were left-handed.

Alcohol procedure

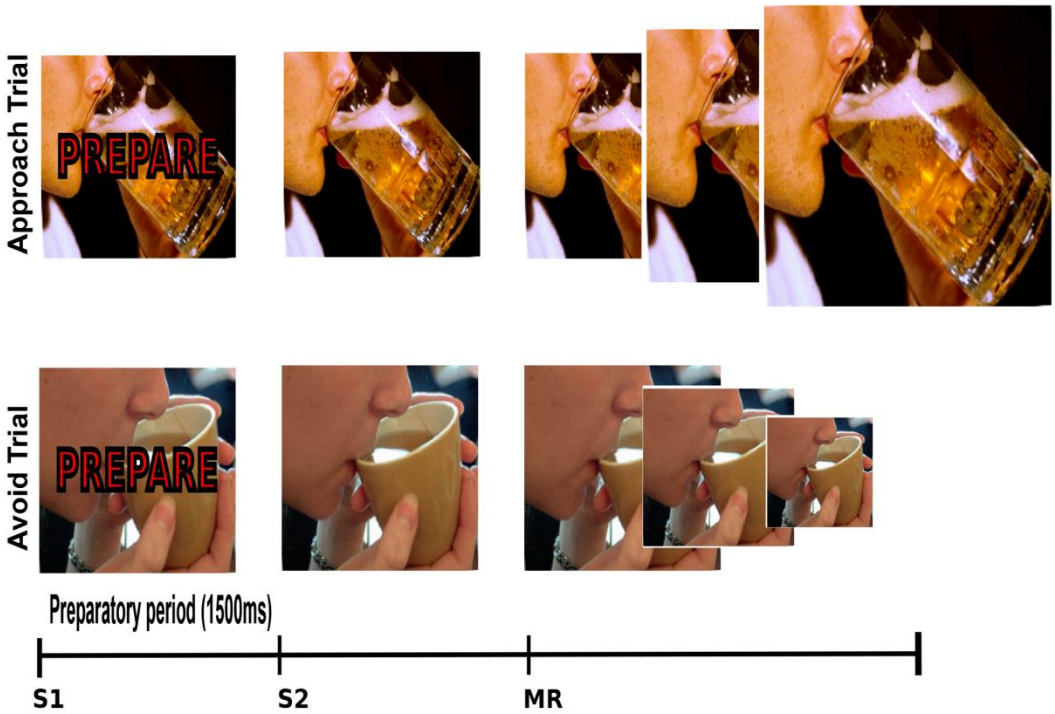
All subjects participated once in an alcohol and once in a placebo session in counterbalanced order. Participants were led to expect to receive either a high or a low dose of alcohol in each session, instead of the actual alcohol dose versus placebo dose. This was done in order to evoke expectancy effects in both conditions. A double blind procedure was used. The placebo dose was achieved by using tonic (300 ml) in a 40 proof vodka bottle. The alcohol dosage was calculated for each participant by using formulas from (Watson *et al*, 1981) to reach a level of 50 mg/100 ml. The dose of alcohol was filled until 300 ml with tonic and equally divided into 3 portions. Two of the drinks were served with 5 min apart, prior to commencing the tasks. The last drink was served as booster drink in the middle of the testing period to reduce noise due to measuring during the ascending versus descending flanks of the blood alcohol curve. On arrival at the laboratory, an initial Breath Alcohol Concentration (BrAC) of 0.00% was confirmed. Participants then completed demographic information and questionnaires among which the AUDIT (Saunders *et al*, 1993) was discussed in the current study. Subjects also performed three unrelated tasks (not reported here). The sequence of the tasks was counterbalanced. BrAC was collected 5 min after the first two drinks, after every task, and at the end of the experiment by using the Lion alcolmeter® SD-400 (Lion Laboratories Limited, South Glamorgan, Wales).

Approach-avoidance task

In this experiment we used the relevant-feature version of the task, in which the instructions explicitly involved the expected motivational classification of the stimuli (e.g., pull alcohol and push soft drink pictures). The trial started with a fixation (500 ms), followed by the presentation of word "PREPARE" on the screen together with the stimuli (1500 ms). During this preparation period, subjects were instructed to prepare their response depending on the block instructions, but to withhold their response until the word "PREPARE" disappeared. The task consists of two blocks with 2 practice and 80 experimental trials each. In the congruent block subjects were instructed to pull in response to alcohol-related and push in response to soft drink pictures using the joystick. In the incongruent block, stimulus response contingency was reversed (i.e. pull soft drinks and push alcohol-related drinks). The order of block types was randomized. As

subjects responded, pulled pictures became bigger and pushed pictures became smaller along with the joystick movement (Rinck and Becker, 2007). Subjects received feedback only if the response was incorrect (i.e. to initiate an avoid response for alcohol cues and an approach response for soft drink cues in the congruent block). Soft drink (4 stimuli) and alcohol-related pictures (4 stimuli) were presented equally often for the approach and the avoid action. Subjects were allowed to practice the task and the joystick movements prior to the testing to ensure that instructions were understood and followed. Error trials were excluded from the behavioral data for RT analysis. RT was calculated from the presentation of S2 until the time the subject fully completed the pull/push movement. Due to the preparation period, responses were fast and no trials were excluded based on RT. Median RTs were analyzed using repeated measures ANOVA as in previous AAT studies (e.g., Cousijn *et al*, 2011; Wiers *et al*, 2009). For the analysis of accuracy and RT, a repeated measure ANOVA with Condition (placebo, alcohol), Action (approach, avoid) and Stimulus Category (alcohol-related, soft drink pictures) as within subject variables was conducted. Note that the effect of congruency is tested by the interaction of Action by Stimulus Category.

Figure 1 Schematic representation of the congruent block type in the alcohol-AAT. S1 represents the warning stimulus and S2 represents the imperative stimulus to which motor response (MR) should be given. Following the MR, stimuli becomes bigger or smaller during approach and avoid action, respectively.



EEG/ERP data collection and analysis

Electrophysiological data were recorded at 512 Hz from the scalp using an Active-Two amplifier (Biosemi, Amsterdam, the Netherlands) from 32 scalp sites. Electrodes were placed according to the 10–20 international system. Two electrodes were placed at the outer canthi of the eyes and two below and above the left eye to measure horizontal and vertical eye movements. Error trials were excluded from analysis. All electrodes were re-referenced off-line to the average of the mastoids. For the time-frequency analyses, the data were low-pass filtered at 40 Hz and high-pass filtered at 0.01 Hz. Vertical and horizontal eye movements were detected by ICA analysis using the method of (Joyce *et al*, 2004). The time course of instantaneous amplitude (IA) around a given frequency was calculated by convolving the EEG signal by a Morlet wavelet: IA(t, f) = |w(t, f)s(t)| where w(t, f) is a Morlet wavelet:

$$w(t, f) = \frac{2}{\sigma_t sqrt(2\pi)} \exp(-0.5(\frac{t}{\sigma_t})^2) \exp(i2\pi ft)$$

where f is the center frequency with σ_t the standard deviation of the Gaussian envelope. Calculation of the IA was followed by segmenting the IA data and averaging IA across trials. The beta-band IA was calculated for the center frequency of 22 Hz with 3 Hz standard deviation. The IA was baselined to the mean of 500 ms period before cue onset. The average IA over the preparation period was then calculated for four successive time points by taking a moving average with overlapping intervals of 0.25 s at midline electrodes (Fz, Cz, Pz and Oz) (intervals: T1: 0–0.5 s, T2: 0.25–0.75 s, T3: 0.5–1 s, T4: 0.75–1.25 s). As a compromise between statistical power and type- I error, an FDR correction was applied for the total number of time points and channels, with a 5% desired false discovery rate (Benjamini *et al*, 2006). IA per interval was analyzed using repeated measure ANOVA with factors Condition (placebo, alcohol), Action (approach, avoid) and Stimulus Category (alcohol-related, soft drink pictures) as within subject variables.

RESULTS

Behavioural Results

The mean AUDIT score was 6.72 (*SD* = 4.09). No significant differences between males and females were found on the AUDIT questionnaire (p = 0.5).

On average, subjects made 2.11 (SD = 1.57) and 1.72 (SD = 1.07) mistakes in the placebo and alcohol condition, respectively. The accuracy data showed a trend towards a main effect of Action type, $F(1, 17) = 3.76$, $p = .07$, $\eta^2_p = .18$, due to subjects making more mistakes

during the avoid trials. None of the other main or interaction effects were significantly different (all $p > .1$).

Average reaction times for the placebo condition were 284, 261.28, 269.5, 261.22 ms, and for the alcohol condition were 272.86, 238.38, 249.83, 266.67 ms, for the approach soft drink, avoid soft drink, approach alcohol and avoid alcohol conditions, respectively. The repeated measures ANOVA of *RT* revealed a significant main effect of Action, $F(1, 17) = 10.82$, $p = .004$, $\eta^2_p = .39$; response times for avoid action were faster compared to approach action. A statistical trend towards an interaction effect of Action by Stimulus Category was observed, $F(1, 17) = 3.59$, $p = .07$, $\eta^2_p = .17$; subjects were faster to avoid compared to approach soft drink trials, $t(16) = 3.53$, $p = .003$, and faster to approach alcohol compared to approach soft drink trials, $t(16) = 2.12$, $p = .05$.

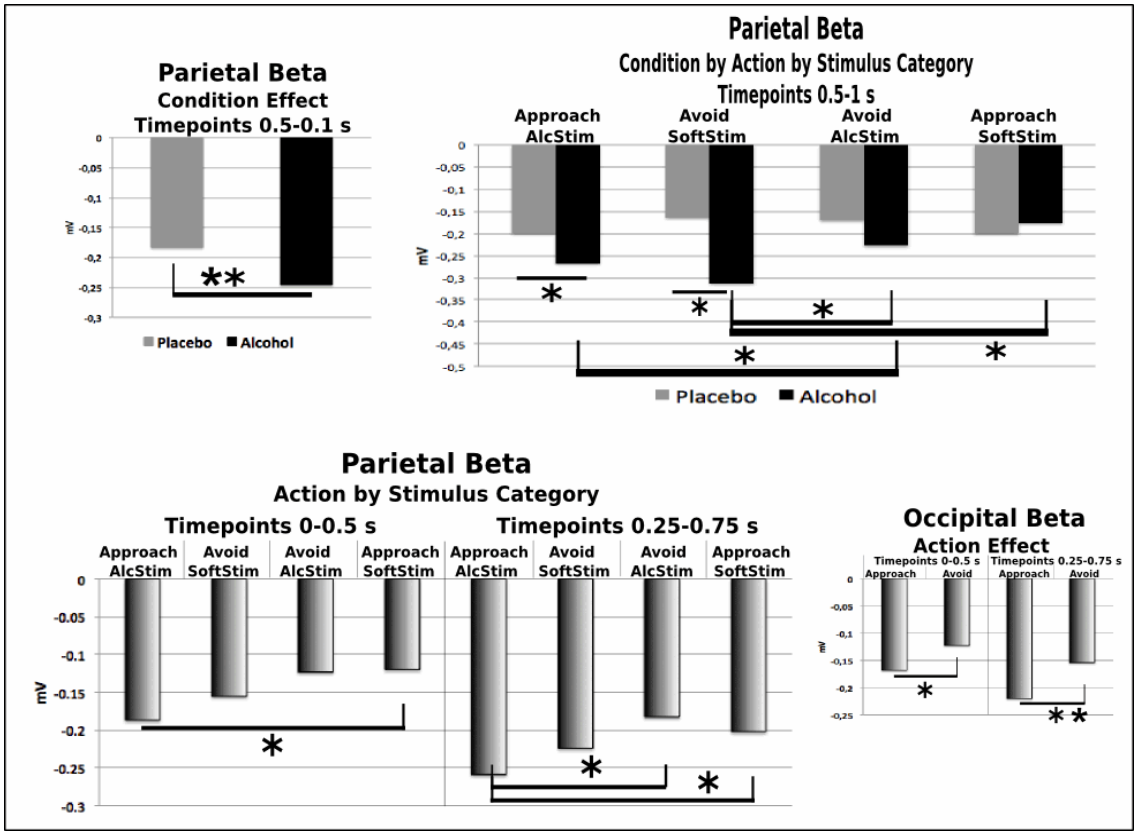
Time-Frequency Results

Parietal beta showed a two-way interaction of Action by Stimulus Category, *T1*: $F(1, 17) = 4.92$, $p = 0.04$, $\eta^2_p = .22$; *T2*: $F(1, 17) = 6.31$, $p = 0.02$, $\eta^2_p = .27$. On approach alcohol trials beta-ERD was stronger compared to approach soft drink trials during the time period of 0-0.75 s, *T1*: $t(17) = 1.96$, $p = .03$; *T2*: $t(17) = 1.94$, $p = .03$, and compared to the avoid alcohol condition during the time period of 0.25-0.5 s, $t(17) = 2.06$, $p = .03$.

Moreover, during the time period 0.5-1 s. beta amplitude at the parietal site showed a main effect of Condition, $F(1, 17) = 9.64$, $p = 0.006$, $\eta^2_p = .36$, and a three-way interaction of Condition by Action by Stimulus Category, $F(1, 17) = 5.56$, $p = 0.03$, $\eta^2_p = .25$. Compared to placebo, after alcohol a stronger parietal beta-ERD was observed. Post-hoc comparisons of the three-way interaction revealed, first that, compared to placebo, the congruent block trial types (approach alcohol, $t(17) = -1.84$, $p = .04$; and avoid soft drink trials, $t(17) =-3.62$, $p = .001$) showed higher beta-ERD in the alcohol condition. Second, in the alcohol condition, approach alcohol trials showed higher beta-ERD compared to the avoid alcohol trials ($t(17) = 1.94$, $p = .03$), but this effect was absent in the placebo condition. Moreover, the beta-ERD for the avoid soft drink trials was higher relative to approach soft drink trials ($t(17) = -2.06$, $p = .03$) and avoid alcohol trials ($t(17) = 1.94$, $p = .03$) in the alcohol condition only.

Finally, occipital beta-ERD showed a main effect of Action, *T1:* $F(1, 17) = 6.48$, $p = 0.02$, $\eta^2_p = .28$; *T2*: $F(1, 17) = 10.1$, $p = 0.005$, $\eta^2_p = .37$. Occipital beta-ERD was higher for approach trials than for avoid trials during the time period from 0 s until 0.75 s (*See* Fig. 2).

Figure 2 Beta-band IA. A bar plot with negative values represent desynchronization. * p < .05, ** p < .005.



DISCUSSION

In the current EEG study, we investigated the preparatory beta-ERD response for approach and avoidance behaviors in the context of alcohol cues and the effects of a low dose of alcohol on this preparatory activity. The results of the behavioral data were in line with previous studies of alcohol approach bias in various samples (Barkby *et al*, 2012; Field *et al*, 2008, 2011a; Schoenmakers *et al*, 2008; Wiers *et al*, 2009). In a previous acute alcohol study (Schoenmakers *et al*, 2008), alcohol approach bias and attentional bias were examined with a different task under the effect of a low dose of alcohol. An approach and an attentional bias toward alcohol-related stimuli were found, of which only the attentional bias was significantly increased after alcohol administration as compared with placebo administration. In the current study a tendency to approach faster toward alcohol- related cues as compared to soft drink cues was present; however alcohol administration did not facilitate this tendency. Moreover overall faster responses for avoid compared to the approach movement were observed, which indicates that our participants in the present experimental setup seem to have a general response time advantage for avoidance. The presence of a marginally significant Action by Stimulus category

interaction might suggest that this avoidance RT advantage was more prominent for soft drink than for the alcohol cues. Therefore these results suggest that although our participants showed an RT advantage for general avoidance responses, when relative RT differences between cues with and without alcohol contents were inspected, participants demonstrated a relative approach bias for alcohol compared with soft drinks.

As expected, analyses involving oscillatory activity revealed a beta-ERD during the preparatory period. The level of beta-ERD was modulated both by congruency and alcohol administration, and by their interaction. Higher desynchronization for approach alcohol cues (compared to the approach soft drink and to avoid alcohol trials in the incongruent block) is in accordance with our expectations of better preparation in the congruent block. Studies have shown that parietal and premotor areas play a role in the preparation of performance of complex hand movements. For instance, one study showed greater involvement of parietal beta during the planning of a targeting movement (requires visual-motor control such as hand eye coordination) compared with simple finger/arm movements (Labyt *et al*, 2003). Authors concluded that the parietal cortex is involved in the integration of visual–spatial information to specify the movement parameters (i.e. direction and extend). Another study observed a beta-ERD over the centro-parietal electrode sites during preparation of visually guided power-grip task, which requires monitoring the visual feedback to adjust the applied force (Kranczioch *et al*, 2008). In the context of the current task, the parietal distribution of the beta-ERD might be related to the expectation of the visual feed- back (zoom in/out) for the prepared movement. With respect to the acute alcohol effect, parietal beta-ERD was enhanced following alcohol administration specifically at the middle of the preparatory period (500 and 1000 ms), although the congruency effect was present in early preparatory period. As can be seen in Fig. 2, acute alcohol increased the beta-ERD (left upper plot), yet inspection of the three-way interaction revealed that this effect was specific to the congruent block (approach alcohol cues and avoid soft drink cues, right upper plot). This result suggests a possible role of acute alcohol on enhancing response preparation for a certain stimulus- response rule set (i.e. approach alcohol and avoid soft drink cues) when stimulus-response mapping is congruent with the subjects' active stimulus response representations (c.f. Schoenmakers *et al*, 2008). Enhancing the effect of acute alcohol on beta-ERD in the congruent block might emphasize the importance of stimulus-action representations in the AAT task. This could potentially explain effects of acute alcohol on alcohol-related behavior and biases.

The results provide clues on the mechanisms underlying approach tendencies, and the approach of ERP/EEG analyses of the adapted AAT appears to be a promising direction for further study. However, we note a number of limitations of the current study. First, the EEG version of the alcohol-AAT involved a long preparatory period and this might have reduced the effectiveness of the task in measuring behavioral effects. The reaction time data is reported in

the present study only for the sake of completeness. However, even with the adapted version of the alcohol-AAT, we observed a trend for an approach bias for alcohol. Second, two different versions of the AAT task have been proposed in the literature so far, each of them involving a different experimental design. Different from the relevant-feature version used in this paper, in an irrelevant-feature version of AAT, participants are instructed to react to another feature of the stimulus (unrelated to the contents), such as the format of the pictures (Cousijn *et al*, 2011; Wiers *et al*, 2009). The explicit nature of the instructions for the incongruence manipulation in the relevant version of the task might prompt the blocked design for the AAT task more susceptible to the manipulation of congruency. Third, the current sample was relatively small, and consisted of healthy subjects. Subjects with relatively low drinking patterns generally show weaker approach tendencies toward alcohol stimuli (Field *et al*, 2008; Wiers *et al*, 2009), which might have affected the results here. The current study should ide- ally be replicated in a larger sample and with clinical groups.

In summary, increased beta-ERD was observed for congruent trials, suggesting that response preparation may play a role in the alcohol approach bias. Further, a prime dose of alcohol facilitated preparatory processes for approach alcohol trials. Such results are of theoretical interest, and may also have clinical implications. Studies aimed at disentangling the processes underlying alcohol approach biases and their relationship to drinking behavior may help to further increase the efficacy of such interventions.

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CHAPTER 3

The effect of acute alcohol on motor-related EEG asymmetries during preparation of approach or avoid alcohol responses

This chapter is submitted as:

Korucuoglu O, Gladwin TE, Wiers RW. The effect of acute alcohol on motor-related EEG asymmetries during preparation of approach or avoid alcohol responses. Submitted.

ABSTRACT

Alcohol approach tendencies have been associated with heavy drinking and are hypothesized to play a role in the transition from initial drug use to drug abuse. The process of preparing an action (approach/avoid) for conditioned cues requires mapping a motor response to a category of stimuli. The present study investigated adolescents' (16-20 year olds) motor-related amplitude asymmetries (MRAA) during preparation for approach or avoidance responses in relation to cues (alcohol/non-alcohol) both after a small dose of alcohol and placebo. The predictive value of alcohol-induced changes on approach-avoidance bias and bias-related cortical asymmetries in change in alcohol use over a six months period was also tested. In heavy drinkers, for approach vs avoidance responses faster reaction times were observed for alcohol cues and greater asymmetries were observed for soft-drink cues. Moreover, the magnitude of the MRAA was related to problems with the self-control of alcohol intake: Individuals with more difficulty in regulating their drinking, had greater approach-related lateralization for soft-drinks and individuals with less difficulty had greater approach-related lateralization for alcohol. Regarding prospective predictions, we found that a relatively strong approach soft-drink and weak approach alcohol reaction-time bias after alcohol predicted decreasing drinking. To conclude, the beta-lateralization measured in this study may represent a compensatory effort for the weaker S-R mapping in heavy and light drinkers. The extent of alcohol-induced changes on the bias was related with changes in alcohol use, suggesting that the capacity to control over the bias under alcohol could be a protective factor.

INTRODUCTION

In recent years, researchers have shown an increasing interest in drug-related cognitive biases due to their value in predicting drug-related behaviours and clinical outcomes. Cognitive biases have been found in adolescents and young adults in attentional processes (e.g. Field *et al*, 2007), action tendencies (approach biases, Field *et al*, 2008; Wiers *et al*, 2009) and implicit memory associations (e.g. Thush *et al*, 2007). In adolescents these biases have been found to be predictive of drinking (memory bias: Thush and Wiers, 2007; Thush *et al*, 2008; approach bias: Peeters *et al*, 2013). Note that some of these studies involved high-risk groups, either defined by education (special education for adolescents with externalizing problems, Peeters *et al*, 2013) or by genotype (e.g., Wiers *et al*, 2009). Training varieties of these tasks have been found to change the bias and reduce relapse rates (Eberl *et al*, 2013; Schoenmakers and Wiers, 2010; Wiers *et al*, 2011). Such results have clinical implications but are also of theoretical interest. Studies in young samples may provide important insights for our understanding of the role of automatic motivational processes in the continuation of drug use later in life (i.e. Curtin et al., 2005).

The approach avoidance task (AAT) assesses automatically activated action tendencies to approach or avoid a category of stimuli (Rinck and Becker, 2007; Wiers *et al*, 2009). The approach bias is measured as the relative difference in reaction time when the valence of the task-related response is congruent with the valence of the stimulus (approaching alcohol and avoiding control cues) compared to when it is incongruent (approaching control and avoiding alcohol cues). These stimulus-response compatibility effects are thought to emerge when implicit action tendencies are in line with the instructed responses during congruent blocks and/or it is difficult to maintain a stimulus-response association during incongruent blocks. If indeed the motivational value of the alcohol cues drives the bias in the alcohol AAT, facilitation in approach alcohol responses might be related to subjects' drinking profile. This was exemplified by the finding of a stronger approach bias in heavier drinkers (Field *et al*, 2008; especially in those with a g-allele in the OPRM1 gene, Wiers *et al*, 2009).

Stimulus-response compatibility effects on motor programs can be studied through the hand-related response preparation. Regarding hand-related neural activity, both during movement preparation and execution, the beta (14–30 Hz) and mu (8-12 Hz) amplitude, decrease in amplitude (event-related desynchronization, ERD) over the motor cortex contralateral to the movement limb (Doyle *et al*, 2005; Gladwin et al., 2006; 2008; Pfurtscheller *et al*, 2000; Poljac and Yeung, 2014; Stancák and Pfurtscheller, 1995). These movement-related amplitude asymmetries (MRAA) can be quantified by using a formula similar to the calculation of the Lateralized Readiness Potentials in the time domain (LRP; Colebatch, 2007) as follows; Left-right hemisphere activity during preparation of left-hand response minus Left-Right

hemisphere activity during preparation of right-hand response (Gladwin *et al*, 2006). Given that the calculation of the MRAA eliminates motor-unrelated hemispheric lateralization, the remaining activity reflects motor-related preparatory lateralized activity. The first aim of the present study was to investigate the motor preparation in alcohol approach –avoidance bias by means of motor-related asymmetries as a function of drinking profile (light and heavy drinkers). Thus, we used a modified version of the AAT task that resembles the one used in our previous study (Korucuoglu *et al*, 2014), extending it by focusing on lateralized spectral analysis. In our previous study, preparatory activity was measured by presenting a warning (or a preparatory) stimulus before the presentation of an imperative stimulus (S2) to which the subject had to give a motor response. Contrary to our previous study where right-hand joystick movement was required for response, the task used in this study required both left and right hand responses to approach/avoid alcohol-related/control cues to allow the study of motor-related lateralization.

In an earlier study, we showed that a low dose of alcohol administration increased the parietal beta-ERD during preparation for the alcohol-compatible trials ('approach-alcohol/avoid-control picture trials') following alcohol administration (Korucuoglu *et al*, 2014), similar to facilitating effects of alcohol on appetitive processes (Duka and Townshend, 2004; Hodgson *et al*, 1979). A second aim of the current study was to assess whether acute alcohol would enhance asymmetries associated with drug-related approach/avoidance motivations. Finally, we tested whether alcohol-induced effects on lateralized power spectra would be related to alcohol consumption, problems, and motivations; and would predict alcohol escalation in a young sample.

METHOD

Participants

Forty adolescents (age range = 16-20 years) were recruited from local high schools in Amsterdam. Seven participants were excluded from data analysis (see Supplementary materials), analysis was conducted with the remaining 33 participants. In this study we examined participants with light and heavy drinking patterns, drinking groups were formed by using an inventory on alcohol use and problems (Alcohol Use Disorder Identification Test, AUDIT) using a median-split (heavy drinking: AUDIT > 8). All participants had normal or corrected-to-normal visual acuity. Prior to the experiment, written informed consent was obtained from all participants and from parents of participants under 18. The study was approved by the Ethical Committee of University of Amsterdam Psychology Department. Participants received financial compensation.

Procedure

All participants participated in a placebo (0 ml/kg) and alcohol (0.45 ml/kg) session administered on two different days, between 2 to 7 days apart (for the alcohol administration procedure, see Supplementary materials). Upon arrival in the lab, participants filled out demographics, questionnaires related to personality and drinking habits. At the start of each session, participants completed the *Desire for Alcohol Questionnaire* (DAQ; Love *et al*, 1998) and the *Positive and Negative Affect Scale* (PANAS; Watson *et al*, 1988) to measure differences in current mood and craving across sessions. Current alcohol use and problems were assessed with the AUDIT (Saunders *et al*, 1993); we used both the standard past year version, and a version about the past three months. Motives to drink alcohol and drinking restraint were assessed with the *Drinking Motives Questionnaire-Revised* (DMQR-R; Cooper, 1994) and *Temptation and Restraint Inventory* (TRI; Collins and Lapp, 1992), respectively. Before and after alcohol administration, participants also performed other unrelated tasks. Order of the tasks was counterbalanced across participants, but was kept same across sessions for each subject. The data of the other tasks are not reported in this paper.

Each session took approximately two and a half hours, including breaks and the application of electrodes, during one afternoon. Six months after these two assessments, participants were contacted with e-mail for an online assessment on recent alcohol and drug use. If no response was received within a week, participants were contacted by phone. During follow-up assessment, participants filled out the same alcohol-related scales as during pre-test.

Alcohol Approach Avoidance Task (A-AAT)

In the original A-AAT (Wiers *et al*, 2009) participants were instructed to pull (approach) or push (avoid) alcohol-related and control pictures by using a joystick. The EEG version of the AAT used in the current study was developed to compare the neural activity during preparation of alcohol approach and avoidance responses. Compared to the relevant-feature version used in our previous study (Korucuoglu *et al*, 2014), in this experiment the irrelevant-feature version of the task was used, where participants were presented with alcohol-related or soft-drink pictures tilted 3^0 to the left and right with participants being instructed to approach or avoid pictures depending on the orientation of the picture (cf. Cousijn *et al*, 2011).

The sequence of events in the trial was as follows (Figure 1): The trial started with a fixation period (500 ms or 700 ms), followed by a preparation period. During the preparation period, the word “Voorbereiden” (“prepare”) was presented on top of the stimuli. Participants were instructed to prepare their response depending on the orientation of the picture (left- or right-tilted) and instructions (pull or push by pressing the left or right button, assigned per block; see below for more details), and to withhold their response until the word disappeared. The word “Voorbereiden” was displayed centrally for a *randomly selected* amount of time

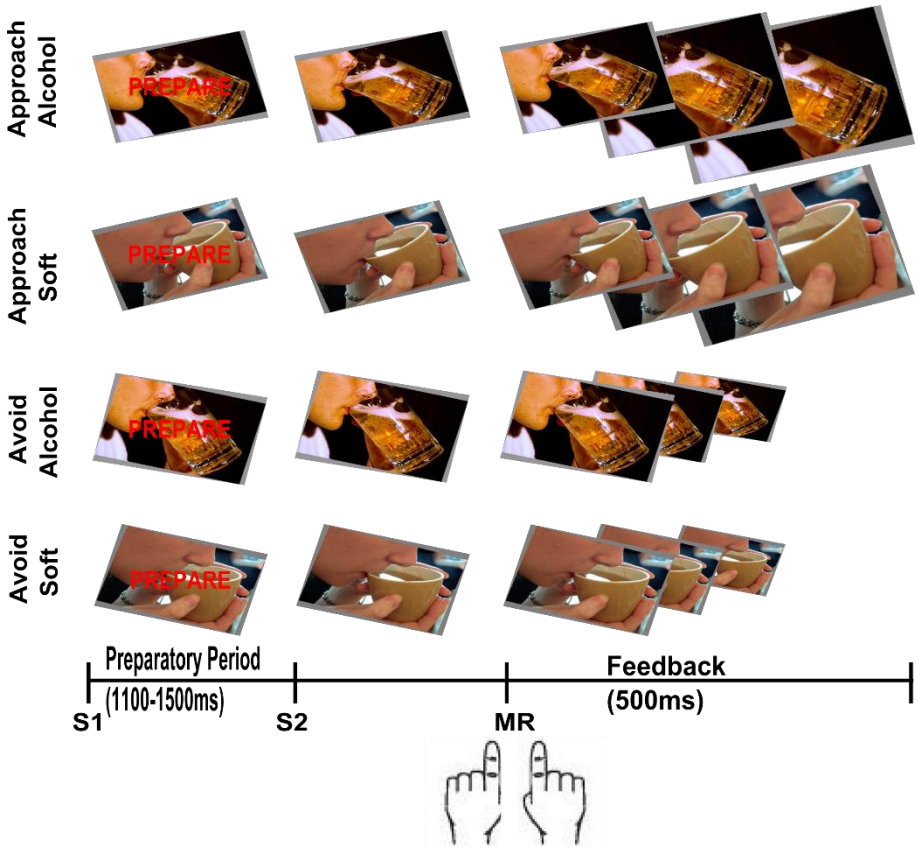
between 1000 ms and *1500 ms* with *100-ms increments*. The stimuli remained on the screen until the response was given. After the response there was a zoom effect with a fixed duration of 500 ms. During this zoom effect, pulled pictures became bigger and pushed pictures became smaller. Participants received feedback only if the response was incorrect. Each picture was presented equally often in the left- and right-tilted orientations. Since each picture (alcohol-related or soft-drink pictures) was presented in both orientations (left and right-tilted), the task consisted of *four experimental conditions*: 1) approach alcohol-related pictures, 2) avoid soft-drink pictures, 3) approach soft-drink pictures and 4) avoid alcohol-related pictures.

The task contained four blocks in total. In order to disentangle left/right hand and push/pull responses and allow motor-related asymmetry analyses, the assignment of the buttons (left or right hand side) to each action type (approach or avoid) alternated across blocks. For this reason, the sequence of block type during 4 experimental blocks followed either ABBA for half of the participants or BAAB design for the other half. During the block type A, the left button was assigned to the approach action and the right button was assigned to the avoid action. During the block type B, the mapping of left-right response buttons on action type was reversed. The contingencies of orientation (left or right-tilted) and the target action (pull or push) were randomized across participants in such a way that half of the participants were instructed to pull the left-tilted pictures and push right-tilted pictures and the other half received opposite instructions.

Each block started with 16 practice trials and was followed by 48 experimental trials. Non-beverage images (grey rectangles) were used during practice trials. During the first 6 practice trials in each block, the correct response was presented on top of the rectangles. Participants repeated a trial during practice block if the response was incorrect. 12 alcohol and 12 soft-drink pictures were used as stimuli, each presented half of the time.

Figure 1. Task illustration

Schematic representation of the Approach Avoidance Task. S1 represents the warning stimulus and S2 represents the imperative stimulus to which motor response (MR) should be given. Following the motor response, stimuli becomes bigger or smaller during approach and avoid action (feedback period), respectively.



Behavioural Data Analysis

All analyses were conducted using a RM-ANOVA in SPSS. Median RTs were analysed as in previous AAT studies (e.g., Cousijn *et al*, 2011; Wiers *et al*, 2009). For the analysis of accuracy, RM-ANOVA was conducted with *Dose* (placebo, alcohol), *Action* (approach, avoid) and *Stimulus Category* (alcohol-related, soft-drink pictures) as within-subject factors and *Group* (Light, Heavy drinkers) as between-subjects factor. Moreover, bias-scores for alcohol-related and soft-drink pictures were calculated separately by subtraction the median RT in pull trials from the median RT in push trials. Positive scores represent an approach bias and negative scores represent an avoidance bias. Bias scores were analysed with *Dose* (placebo, alcohol) and *Stimulus Category* (alcohol-bias, soft-drink bias) as within-subject factors and *Group* (Light, Heavy drinkers) as between-subjects factor.

EEG Analysis

The MRAA index was calculated for the alpha, mu and beta band (See Supplementary materials). Similar to the calculation of bias scores, MRAA bias-scores were calculated separately for alcohol and soft-drink cues by subtracting the MRAA in push trials from the MRAA in pull trials (i.e. MRAA alcohol bias-scores in placebo= $MRAA_{pull}$–$MRAA_{push\ alcohol\text{-}stimuli}$ at T1 after placebo). Given that MRAA is calculated based on ERDs (decrease in activity), negative MRAA bias-scores represent relatively higher ERDs for the approach compared to avoid responses, and positive MRAA bias-scores represent relatively higher ERDs for the avoid compared to approach responses. Statistical analysis was conducted with *Dose* (Placebo, Alcohol), and *MRAA Bias-scores* (Alcohol-bias, Soft-drink bias) as within-subject variables and *Group* (Light, Heavy drinkers) as between-subjects variable, and for each time interval (T1:0-350ms, T2:350-700ms, T3:700-1000ms) separately. Post-hoc comparisons were conducted by using paired sample t-tests and independent sample t-tests. For brevity, here we reported the results of post-hoc comparisons with t-test statistics, the results of the RM-ANOVA are provided in the Supplementary materials).

Relationships between MRAA and Individual Differences

For the correlation analysis, first a contrast score was calculated by taking the difference between the MRAA bias-score for the soft-drink and for the alcohol-related cues separately in the placebo and alcohol conditions (i.e. $(MRAA_{pull}–MRAA_{push})_{alcohol\text{-}stimuli}$ - $(MRAA_{pull}–MRAA_{push})_{control\text{-}stimuli}$ in the placebo dose). Positive MRAA contrast scores represent relatively higher ERDs for the soft-drink bias and negative MRAA contrast scores represent relatively higher ERDs for the alcohol bias. Correlations between MRAA contrast score and alcohol-related problems/drinking motives (TRI/DMQR) were assessed with Pearson correlations.

Prediction of future drinking

In order to assess whether differences in RT bias-scores and MRAA bias-scores across sessions predicted unique variance in the change in alcohol use during the six months after the experiment, a hierarchical multiple regression analysis was conducted. The difference between the contrast scores in the alcohol and placebo condition were calculated both for the RT and the MRAA data and used as predictors. First, behavioural measures (AUDIT score for recent use at baseline from the version about the past 90 days -sum of scores of items on frequency of drinking, typical quantity and frequency of heavy drinking-) were entered to the regression model, followed by the alcohol-induced changes in the RT and the MRAA contrast scores.

RESULTS

Accuracy

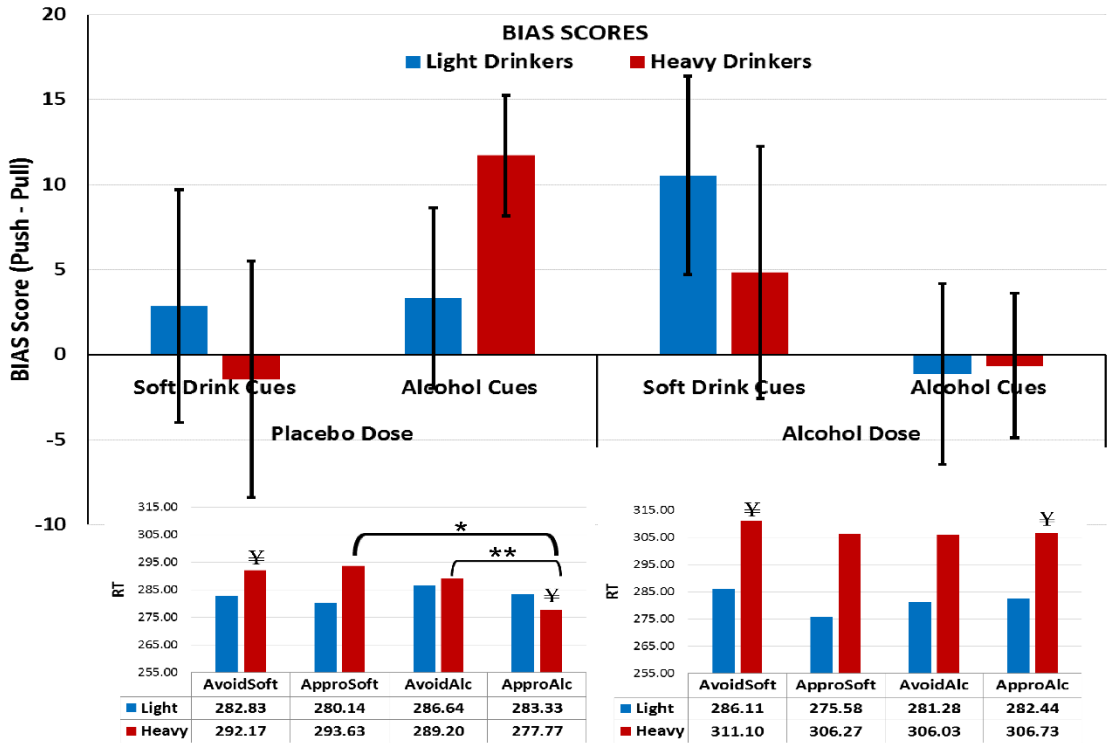
Compared to placebo, after alcohol administration participants made more errors in trials in which they had to avoid alcohol-related stimuli (t(32) = -2.292, *p* = .029).

Bias Scores on Reaction Time

Participants demonstrated a non-significant positive bias for alcohol pictures after placebo and a positive bias for soft-drink pictures after alcohol. Analysis revealed that after alcohol administration, bias scores for neutral pictures tended to be higher compared to bias scores for alcohol, which did not reach significance (t(32) = 1.816, *p* = .079) (See Figure 2).

Figure 2. Behavioral results

Upper panel: Bias scores for the light and heavy drinkers after placebo and alcohol dose. Positive scores represent an approach bias and negative scores represent an avoidance bias. Lower panel: Mean reaction times for the light and heavy drinkers after placebo (left) and alcohol dose (right). ¥: indicates differences across placebo and alcohol conditions. *p ≤ .05, **p ≤ .005. AvoidSoft: Avoid soft-drink cue trials, ApproSoft: Approach alcohol cue trials, AvoidAlc: Avoid alcohol cue trials, ApproAlc: Approach alcohol cue trials.



Mu-MRAA Bias

Task effect per Group: No significant differences were observed across task conditions.
Heavy vs. Light Drinkers: After the alcohol dose, light drinkers' negative MRAA bias-scores for the alcohol cues were different than heavy drinkers' positive MRAA bias-scores at 0-350ms ($t(31) = -2.332$, $p = .026$) and 350-700ms ($t(31) = -2.08$, $p = .046$). At 700-1000ms, after alcohol, light drinkers' negative MRAA soft-drink bias-scores was different than the heavy drinkers' MRAA positive bias-scores ($t(31) = -2.178$, $p = .037$) (See Figure 3).

Beta-MRAA Bias

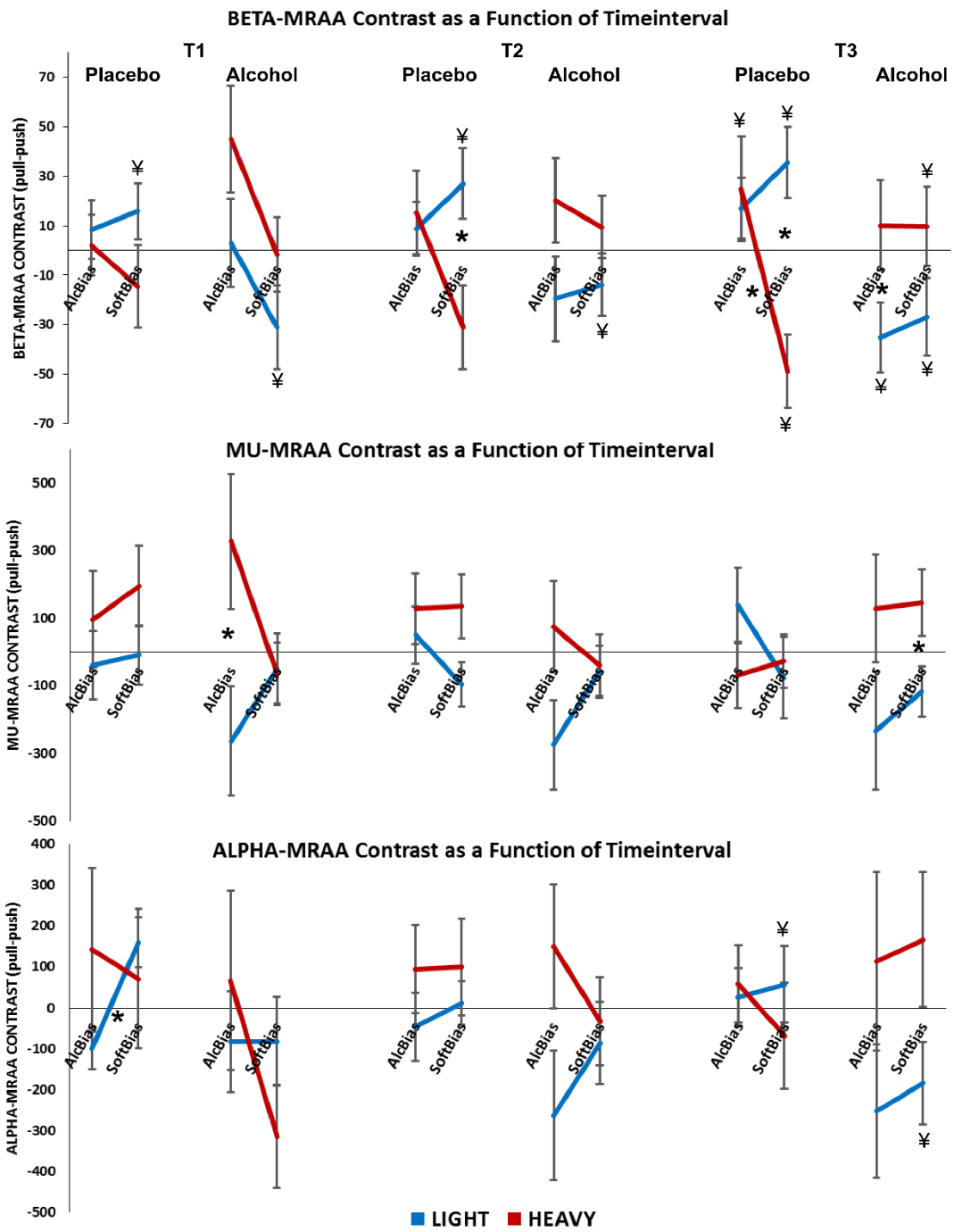
Task effect per Group: At 700-1000ms, heavy drinkers' negative MRAA soft-drink bias-scores were different than the positive MRAA alcohol bias-scores after placebo ($t(14) = 3.143$, $p = .007$). Also, for heavy drinkers at 700-1000ms, negative MRAA soft-drink bias-scores after placebo were different than positive MRAA bias-scores after alcohol ($t(14) = -2.641$, $p = .019$). Light drinkers had negative MRAA soft-drink bias-scores after alcohol which was different than the positive MRAA bias-scores after placebo at 0-350ms ($t(17) = 2.742$, $p = .014$), at 350-700ms ($t(17) = 2.447$, $p = .026$), and at 700-1000ms ($t(17) = 2.608$, $p = .022$). Moreover, at 700-1000ms, light drinkers had negative MRAA alcohol bias-scores after alcohol which was different than the positive MRAA bias-scores after placebo ($t(17) = 2.527$, $p = .018$).
Heavy vs. Light Drinkers: At 0-350ms, no differences were observed. At 350-700ms and 700-1000ms, after placebo dose, heavy drinkers' negative MRAA contrast for the soft-drink bias in placebo condition was different than light drinkers' positive MRAA scores, (350-700ms: $t(31) = 2.644$, $p = .013$; 700-1000ms: $t(31) = 4.055$, $p < .001$). Light drinkers' negative MRAA alcohol bias-scores in alcohol condition was different than heavy drinkers' positive MRAA bias-scores ($t(31) = -1.987$, $p = .056$) at 700-1000ms.

Parietal alpha-MRAA Bias

Task effect per Group: At 0-350ms, after placebo, light drinkers' negative MRAA alcohol bias-scores was different than the positive MRAA soft-drink bias-scores ($t(17) = -3.008$, $p = .008$). At 700-1000ms, positive MRAA soft-drink bias-scores after placebo and the negative MRAA soft-drink bias-scores after alcohol were significantly different ($t(17) = 2.31$, $p = .034$).
Heavy vs. Light Drinkers: No differences were observed across groups.

Figure 3. MRAA

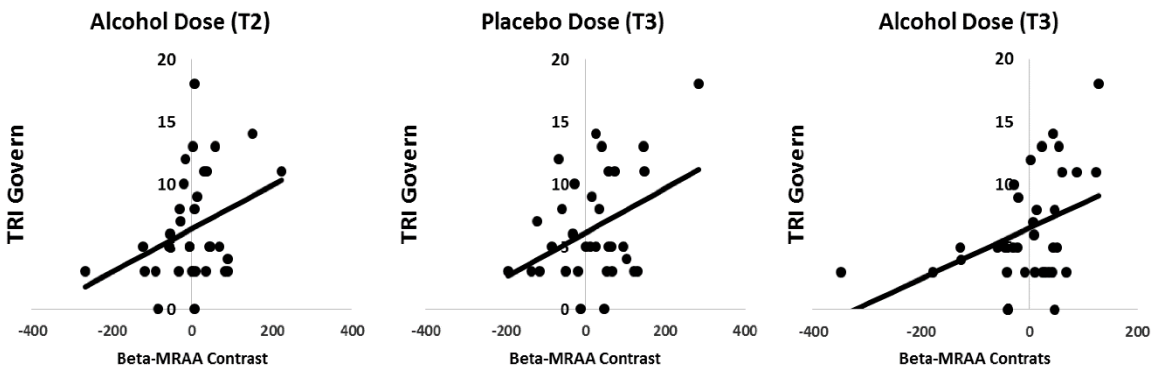
Beta-, mu- and alpha-MRAA bias scores for three successive time points (T1: 0-350ms, T2: 350-700ms, T3: 700-1000ms) following the presentation of the cue. ¥: indicates differences across placebo and alcohol conditions. Negative MRAA bias-scores represent relatively higher ERDs for the approach compared to avoid responses (similar to approach bias based on RT), and positive MRAA bias-scores represent relatively higher ERDs for avoid compared to approach responses (similar to avoid bias based on RT). Heavy: heavy drinkers, Light: Light drinkers.



Correlations

The Govern subscale of the TRI questionnaire ('difficulty controlling alcohol intake') positively correlated with the central beta-MRAA contrast scores in the alcohol condition at 350-700ms (r = .34, p = .05) and 700-1000ms (r = .43, p = .012) and with the MRAA contrast scores in the placebo condition at 700-1000ms (r = .37, p = .032) (See Figure 4). Individuals with higher TRI scores had more positive contrast scores, and individuals with lower TRI scores had more negative MRAA contrast scores.

Figure 4. Scatterplots for the TRI Govern sub-scale and the beta-MRAA contrast scores after alcohol at T2 (350-700ms) and T3 (700-1000ms) and after placebo at T3 (700-1000ms). Positive MRAA contrast scores represent relatively higher ERDs for the soft-drink bias (and greater lateralization for approach soft-drink bias relative to approach alcohol bias) and negative MRAA contrast scores represent relatively higher ERDs for the alcohol bias (and greater lateralization for approach alcohol bias relative to approach soft-drink bias).



Neural Predictors of Alcohol Use After Six Months

Six months after the baseline assessment, 82.5% follow-up response rate was achieved in the full sample of 40 participants. Alcohol-induced changes on the bias scores and the parietal alpha-MRAA at 350-700ms predicted future alcohol use beyond the variance explained by baseline AUDIT scores. The total variance explained by the full model was 81.5% (F-change$_{1,24}$ = 5.903, p = .023). The baseline AUDIT scores explained 70.5% of the variance (F-change$_{1,26}$ = 62.103, p < .001). Alcohol effects on the behavioural alcohol/soft-drink bias scores (alcohol minus placebo) and the parietal alpha-MRAA contrast scores explained an additional 6.4 and 4.6% of the variance (F-change$_{1,25}$ = 6.931, p = .014; F-change$_{1,24}$ = 5.903, p = .023) (See Figure

5). To follow up, a correlation analysis between change in AUDIT scores ($AUDIT_{follow\text{-}up}$ – $AUDIT_{baseline}$) and the predictors of the change in alcohol use was conducted (bias scores for the RT and the parietal alpha-MRAA). Individuals who had relatively more negative bias scores for the RT after alcohol administration at baseline (due to a stronger approach soft-drink and weaker approach alcohol bias, as depicted in Figure5b), had lower Audit scores, 6 months later (r = .384, p = .044). Follow-up correlations for the parietal-MRAA contrast scores did not reveal significant effects.

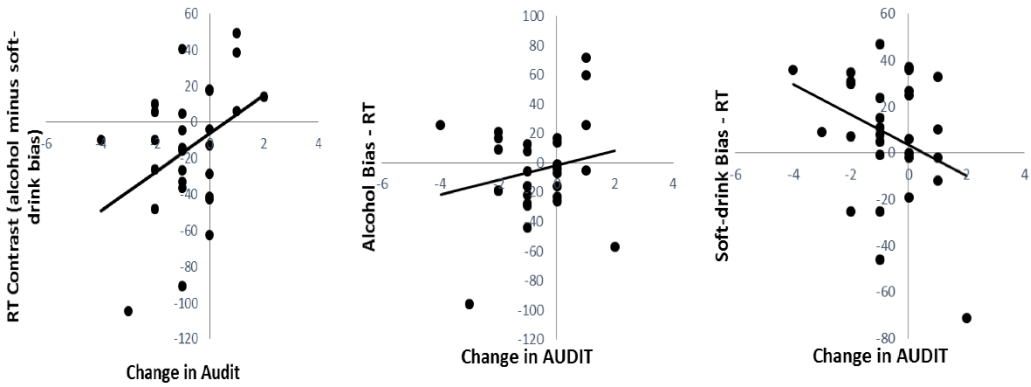
Figure 5. a) Hierarchical multiple regression analysis for variables predicting AUDIT at 6-months follow-up (n=28). **b)** Scatterplots between change in AUDIT scores ($AUDIT_{follow\text{-}up}$, last 90 days – $AUDIT_{baseline}$, last 90 days) and (from left to right) alcohol-induced changes on the contrast score (alcohol minus control bias) and alcohol-induced changes on alcohol and the control (soft-drink) bias, separately.

a)

	B	SE B	Beta	P – values
Step1: change R^2: .705, p<.001				
AUDIT baseline	.676	.086	.84	<.001
Step2: change R^2: .064, p=.014				
AUDIT baseline	.679	.077	.844	<.001
Alcohol Bias minus Control Bias – RT	.013	.005	.253	.014
Step3: change R^2: .046, p=.023				
AUDIT baseline	.643	.072	.798	<.001
Alcohol Bias minus Control Bias – RT	.016	.005	.305	.003
Parietal alpha-MRAA contrast –(alcohol minus control bias)	-.001	.00026	.225	.023

Alcohol Bias minus Control Bias – RT: (i.e. Approach Alcohol bias RT – Approach Control bias RT) in alcohol dose – (Approach Alcohol bias RT – Approach Control bias RT) in placebo dose. Parietal alpha-MRAA contrast: (i.e. Approach Alcohol bias MRAA – Approach Control bias MRAA) in alcohol dose – (Approach Alcohol bias MRAA – Approach Control bias MRAA) in placebo dose.

b)



DISCUSSION

In the current EEG study, we investigated motor-related lateralization during preparation for approach and avoidance behaviours in the context of alcohol cues and the effects of a prime dose of alcohol on these neurophysiological measures in heavy and light drinking adolescents. Preparation of a left/right hand response during the alcohol approach-avoidance task led to an ERD following the presentation of the imperative stimulus (Supplementary materials). A further analysis on motor-related asymmetries was conducted to identify the condition across drinking groups in which the increase in ERD was greater. In earlier studies, the mu- and beta-MRAA indices have been studied with switch task, pre-cueing RT paradigm, and motor imagery task (de Jong *et al*, 2006; Deiber *et al*, 2012; Doyle *et al*, 2005; Gladwin *et al*, 2006, 2008; Nam *et al*, 2011; Poljac and Yeung, 2014). During task switching paradigms (subjects need to switch their response hand when the current task switches), a reversal of lateralization of the mu and beta-MRAA from previous to current task set has been observed (de Jong *et al*, 2006; Poljac and Yeung, 2014), suggesting that MRAA reflects selection of motor goal and advance task preparation. This interpretation is strengthened by the finding of higher beta-band MRAA in 100% informative cues compared to 50% informative one (Doyle *et al*, 2005). In this study visuospatial attention to the imperative cues was also measured and it was found to be unrelated to the magnitude of the MRAA index. However, in another pre-cueing RT task, a cento-parietal alpha-MRAA was found to be reflecting visuospatial attention (Deiber *et al*, 2012). This study revealed a spectral pattern for weak lateralizers suggesting the recruitment of more visuospatial attentional resources (alpha ERD) and for high lateralizers suppression of irrelevant visual activity (alpha event-related synchronization, ERS).

Based on earlier findings, we expected that heavier drinkers would show an increased (more negative) mu- and beta-MRAA index for the approach versus avoidance alcohol-related cues compared to soft-drink cues, representing advance response preparation for these trial types. In heavy drinkers, greater approach-related lateralization was observed for approach soft-drink cues especially during the late preparation period, suggesting an increased asymmetry index for the bias in the direction opposite to the one hypothesized. The effects for the mu- and alpha-MRAA bias scores were found to be in the same direction, higher lateralization of the ERD for soft-drink bias in heavy drinkers. For the alpha and the mu, differences across conditions were moderate and did not lead to significant results. Given that heavy drinkers showed an approach bias for alcohol cues and also greater lateralization for approaching soft drink; the findings of the current study suggest that the asymmetry index measured with the AAT is likely to reflect an effortful response preparation process rather than an advance response preparation. This could be due to two reasons: first, a lack of lateralization for approach alcohol response might represent presence of an automatic response bias. However,

another likely scenario is a possible relationship between behaviour and lateralization which resembles the "speed-accuracy" trade-off for perceptual tasks. In the present case, our heavy drinking participants showed an approach alcohol bias in behaviour (failed to overcome this bias) when they lacked a lateralization in the brain. In line with this, for the soft-drink bias an increased lateralization was observed for more effortful approach behaviour. In sum, rather than a lack of lateralization possibly meaning an automatized process, the presence of lateralization could reflect effortful processing to overcome pre-existing biases. Also our correlational analysis revealed that individuals with greater difficulty in regulating their drinking (note that heavy drinkers had greater difficulty), had greater approach-related lateralization for soft-drink cues and individuals with less problem with control over drinking had greater approach-related lateralization for alcohol cues. Using an alcohol implicit association test (IAT), it has been shown that young heavy drinkers hold both positive and negative alcohol associations (Houben and Wiers, 2006), most likely reflecting ambiguity towards alcohol. Therefore, a likely explanation for the MRAA pattern in heavy drinkers is that problems in controlling alcohol intake may have caused ambivalence in these individuals, and subjects may have compensated for this ambiguity by putting more effort in preparing their response for trials incongruent with their state of drinking profile (approaching soft-drink cues).

Based on earlier findings of alcohol's priming effects on cognitive biases in adult samples (Field *et al*, 2011b), one may expect acute alcohol to increase this bias. However, earlier studies failed to show such an effect on RT with a relevant-feature version of the task with explicit instructions to approach/avoid alcohol-related cues (Korucuoglu *et al*, 2014; Schoenmakers *et al*, 2008). Results of the current study employing an irrelevant-feature version of the task demonstrated that after alcohol, the bias for the alcohol cues decreased especially in heavy drinkers. With alcohol administration, while heavy drinkers slowed down their responding for congruent trials (approach alcohol and avoid soft-drink cues) (this could be due to a decrease in inhibition or an increase in distraction), light drinkers showed a non-significant decrease in response time during incongruent trial types. Moreover, regression analysis revealed that individuals who had relatively strong avoid alcohol bias after alcohol administration at baseline (due to a stronger approach soft-drink and a weaker approach alcohol bias), had lower Audit scores, six months later. The evidence in this study suggests that the ability to respond adaptively under the influence of alcohol can be a protective factor for the development of addictive behaviours. Earlier studies showed that if alcohol is consumed in the presence of conditioned cues (drug-related environmental cues), individuals are able to counter the effects of alcohol on cognitive function (Birak *et al*, 2010; 2011), suggesting a cognitive tolerance to drugs in the presence of drug cues. It is important to note that these results might be specific to irrelevant version of the task used here, given that the implicit nature of the

instructions probably give more room for the top-down influence of task instructions on performance.

To conclude, results revealed greater preparatory approach-related lateralized activity for approach soft-drink cues in heavier drinkers in comparison to light drinkers and also in comparison to lateralization for the alcohol cues. The beta-lateralization measured in this study may represent a compensatory effort for the weaker S-R mapping in heavy drinkers. Moreover, alcohol administration decreased approach alcohol bias in heavy drinkers. The extent of alcohol-induced changes on the bias were related with changes in alcohol use, suggesting that the capacity to control over the bias under alcohol could be a protective factor. It is important to note here, heavier drinkers in the present study also reported greater problems with controlling their drinking behaviour. Studies with preselected samples can be considered to compare lateralization index in heavy drinkers with and without problems to control their drinking levels. Also with a larger sample future studies can focus on asymmetry differences between heavy drinking individuals who can and cannot overcome their approach alcohol bias.

Acknowledgements

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SUPPLEMENTARY MATERIALS

MATERIALS AND METHODS

Participants: Exclusion criteria

Exclusion criteria were psychiatric disorders, diagnosed cases of drug use disorder, head trauma, seizures, severe physical illness, cardiovascular disease, chronic obstructive pulmonary disease, the presence of major medical conditions, and use of medication. Further exclusion criteria for female participants were pregnancy and breast-feeding; which were assessed with self-report.

Four participants were excluded from further data analysis; two due to a positive drug test for THC and two due to missing data in one session. One subject was left-handed and excluded from the analysis. One subject's data was excluded due to broken electrode. In this study we examined participants with light and heavy drinking patterns, drinking groups were formed by using an inventory on alcohol use (Alcohol Use Disorder Identification Test) based on a median-split approach (for heavy drinkers AUDIT > 8, note that one subject's AUDIT score was missing, this subject was excluded). Data analysis was conducted with the remaining 33 participants.

Session restrictions

Prior to the testing sessions, participants were informed about the study restrictions by email. Participants were required to be minimally 16 years-old (minimum drinking age in Netherlands at the time of the study), with a minimum weight of 50 kg and to have had at least one full drink in their lifetime. Participants were requested not to drink any alcohol 24 hours before testing and eat a meal or drink caffeine 4 hours prior to testing. Participants' compliance with these restrictions was confirmed with self-report. Moreover, participants were instructed to abstain from any legal and illegal drugs for at least 1 week; their compliance with this restriction was confirmed with a urine test.

Alcohol Administration

All subjects participated in two sessions administered on two different days, between 2 to 7 days apart. Sessions started between 12:00 and 18:00 PM. Alcohol was administered in one session and placebo in the other. Dose order was counterbalanced across subjects. Participants were told that they would receive a different dose of alcohol during both sessions, to keep expectancy effects similar across sessions.

To keep the participants as well as the experimenter oblivious to the condition, a double blind procedure was used. Over-age subjects (18 year-olds and above) received a mix of vodka

and orange juice. Under-age subjects (16 and 17 year-olds) received a vodka-orange premixed drink (Eristoff & Orange Can, commercial ready-to-drink alcoholic beverages with a 7 % Vol). The alcohol content and the total volume of the liquid delivered to the participants under and over the age of 18 were the same (0.45g/kg with a maximum cut-off of 100 ml vodka). The mix was divided into three equal portions. Two of the drinks were served with 5 minutes apart, prior to commencing the task, and after electrode placement. Up to 3 minutes was allowed for drinking followed by 2 minutes of mouthwash to remove the residual alcohol in the mouth. In between the tasks 1/3 of the mix was administered as a booster drink in order to eliminate measurement during the descending limb of the BrAC. To enhance the alcohol taste, all the drinks had a lemon soaked in vodka and the glass in which drinks were served was sprayed with vodka beforehand. To mask the alcohol taste all drinks had three drops of tabasco sauce (McIlhenny Co., USA). The procedure was identical in each session, except alcohol was replaced with orange juice in the placebo condition.

Breath alcohol concentration (BrAC) was collected 5 minutes after the first two drinks, before and after the booster drink, and at the end of the experiment by using the Lion alcolmeter® SD-400 (Lion Laboratories Limited, South Glamorgan, Wales). Participants filled out the *Brief Biphasic Alcohol Effects Scale* (B-BAES, Rueger *et al*, 2009) each time a breath sample was taken, except before the booster drink. Throughout the experiment the BrAC was measured three times during which subjects also filled the B-BAES questionnaire: after alcohol administration, before the booster drink and at the end of the experiment. Moreover, an additional BrAC measurement was collected after the booster drink in order to monitor alcohol level following the top-up dose.

After completion of both sessions, a short manipulation check interview was conducted to determine whether the participants were aware of the alcohol contents of the drinks. Deception was not successful for one of the participants. Participants were debriefed about the true nature of the study and remained at the research site until their breath sample was 25mg/100ml or less.

2.4. Questionnaires

Desire for Alcohol Questionnaire (DAQ; Love *et al*, 1998): The desire for alcohol questionnaire (*DAQ*) is a 14-item instrument with a 7-point likert scale, measuring 4 dimensions of craving: Desires and intentions to drink, negative reinforcement, control over drinking, and mild desires to drink. Subjects were required to rate the items from “strongly disagree” to “strongly agree”.
Positive and Negative Affect Scale (PANAS; Watson *et al*, 1988): PANAS is a 20 item scale that measures subjects’ positive (such as enthusiasm, active, alert) and aversive mood states (such as subjective distress and unpleasurable engagement) during a specific time frame. This questionnaire consist of 20 descriptors such as ‘distressed’, ‘upset’, ‘excited’ etc. The subjects

are asked to rate each descriptor on a 5-point scale ranging from 1 (*very slightly*) to 5 (*very much*).

Alcohol Use Disorders Identification Test (AUDIT; Saunders *et al*, 1993): AUDIT is a 10-item questionnaire developed to screen for excessive drinking. The questionnaire includes three domains to measure subjects' current drinking habits as follow: recent alcohol use (Items 1-3), alcohol dependence symptoms (Items 4-6), and alcohol related problems (Items 7-10). In the baseline assessment, subjects filled out this questionnaire once for the last 3 months and once for the lifetime. In the online follow-up, subjects filled out this questionnaire only for the last 3 months. In the current study, the total score of items on recent alcohol use (Items1-3) at baseline (last 3 months) and six-months follow-up assessment were used in a hierarchical multiple regression analysis in order to identify factors that predicted changes in alcohol use.

Brief Biphasic Alcohol Effects Scale (B-BAES; Rueger *et al*, 2009): Participants' subjective stimulant and sedative effects of alcohol were assessed by the brief version of the BAES. The B-BAES is a 6-item adjective rating scale that measures the stimulant and sedative effects of alcohol as distinct constructs at ascending and descending limbs of the blood alcohol curve. The brief Stimulation subscale is the summation of the adjectives *energized*, *excited*, and *up*, and the brief Sedation subscale is the summation of the adjectives *sedated*, *slow thoughts*, and *sluggish*. Participants asked to rate the extent to which they were feeling each adjective at the present time on an 11-point scale ranging from 0 (*not at all*) to 10 (*extremely*).

Drinking Motives Questionnaire-Revised (DMQR; Cooper, 1994): DMQR-R is a 20 item questionnaire on a 5-point scale (1: never, 5: always) measuring motives to drink alcoholic beverages. The questionnaire has 4 subscales: social (social motives for alcohol use), coping (coping motives for alcohol use), enhancement (enhancement motives for alcohol use), conformity (external motives to engage in drinking behaviours).

Temptation and Restraint Inventory (TRI; Collins and Lapp, 1992): TRI is a 15 items inventory with a 9-point scale (1 reflects a lack of preoccupation and 9 reflects a high degree of preoccupation) measuring preoccupation to restraint drinking behaviour. The inventory consisted of three factors are: Govern (difficulty controlling alcohol intake), Restrict (attempts to limit drinking), and Emotion (negative affect as a reason for drinking).

Behavioural Data Analysis

The PANAS and DAQ scores were analysed with *Dose* (Placebo, Alcohol) as a within subject factor. The Stimulation and Sedation subscales of B-BAES scores were separately analysed with *Dose* (Placebo, Alcohol), and *Time* (pre-task and post-task) as within-subjects factors. The BrAC were subjected to a RM-ANOVA, with *Time* (BrAC pre-task and post-task) as within-subject variable. Two participants' B-BAES data and five participants' BrAC scores were lost; the analysis was conducted with the remaining participants.

Practice trials and trials with incorrect response (i.e a pull response in a push trial) were excluded from the behavioural data for RT analysis. RT was calculated from the end of the preparation period until the motor response. Due to the preparation period, responses were fast and no trials were excluded based on RT. Median RTs were analysed using RM-ANOVA as in previous AAT studies (e.g., Cousijn *et al*, 2011; Wiers *et al*, 2009).

Electroencephalogram (EEG) recording and statistical analysis

Electrophysiological data were recorded from the scalp using an Active-Two amplifier (Biosemi, Amsterdam, the Netherlands) from 32-scalp sites. Electrodes were placed at the standard positions of the 10-20 international system. Two electrodes were placed at the outer canthi of the eyes to measure horizontal eye movements. Two electrodes were placed at below and above the left eye to measure vertical eye movements. EEG was recorded at 2048 Hz sampling rate. The distance between the screen and the subject was kept at 75 cm.

EEG preprocessing was conducted using Brain Vision Analyzer (version 2.0, Brain Products GmbH, Munich, Germany). Data were down-sampled to 250Hz, re-referenced offline to the average of left and right mastoids, low pass filtered at 50Hz, and high pass filtered at 0.1 Hz. Ocular correction was applied using the algorithm of (Gratton *et al*, 1983). EEG data were segmented into 3 sec epochs starting 1 sec. before the cue presentation to 2 sec. afterwards. Trials were considered artefacts when the difference between consecutive data points was larger than 75 mV and the difference between the lowest and the highest voltage within a segment was higher than 200 mV. Epochs with an amplitude exceeding ±100 mV were excluded.

The Fieldtrip toolbox for EEG/MEG analysis was used for the time-frequency analysis (Oostenveld *et al*, 2011) running under Matlab 2010b. Because of their sensitivity to muscle activity, the (most) peripheral electrodes from left to right earlobes (Fp1, FP2, F7, F8, T7, and T8) were excluded from further data analysis. Time-frequency was performed by convolving the time series with a family of Morlet Wavelets with a family ratio of ($f0/\sigma f=7$), where f0 represent the frequency of interest. Frequencies of interest were alpha (8-12 Hz with 1 Hz frequency steps) and beta (13-30 Hz with 2 Hz frequency steps) frequency ranges. An absolute baseline correction was applied to the power spectrum by using the time period of -600 to -200 ms preceding the presentation of the cue.

For the calculation of motor-related amplitude asymmetries (MRAA), condition specific grand averages were calculated separately for each response hand. The power estimates were averaged for three successive time points (T1: 0-350ms, T2: 350-700ms, T3: 700-1000ms) following the presentation of the cue. Based on previous reports MRAA was calculated for the central beta and mu (de Jong *et al*, 2006; Gladwin *et al*, 2006; Poljac and Yeung, 2014) and for the parietal alpha (Deiber *et al*, 2012; note that for the parietal alpha we used P3-P4 channels in the same line with C3-C4, instead of CP3-CP4 used by the authors). To estimate lateralization, first the difference in power between two equal measuring points in the left and right hemispheres (C3-C4, P3-P4) was calculated for the left and right hand responses separately. Subsequently, a difference score was calculated between the left and the right hand responses (example for the central electrodes: $[(C3\text{-}C4)_{\text{Right hand response}} - (C3\text{-}C4)_{\text{Left hand response}})]$). Given that a decrease in power is expected for the hemisphere contralateral to the movement, more negative MRAA values would indicate greater motor-related lateralization due to increased ERD contralateral to the movement. Lateralization was calculated for the alpha, mu and beta band, separately. Statistical analysis was conducted by using a RM-ANOVA with *Dose* (Placebo, Alcohol), *Bias* (Alcohol bias, Soft-drink bias) as within-subject variables and *Group* (Light, Heavy drinkers) as between-subjects variable in SPSS and for each time interval (T1: 0-350ms, T2: 350-700ms, T3: 700-1000ms) separately.

RESULTS

Control Questionnaires

The overall DAQ scores were higher for the placebo dose compared to the alcohol dose ($F(1, 32) = 4.559$, $p = .058$, $\eta^2_p = .108$). With an additional ANOVA it was confirmed that higher scores on the DAQ was not different across heavy and light drinkers ($p = .725$). The same RM-ANOVA on PANAS scores revealed no significant differences between placebo and alcohol dose (*p*s >.2).

Manipulation Checks

For the Stimulation and Sedation subscales of B-BAES scores, results revealed a significant main effect of *Dose* for the sedation subscale ($F(1, 32) = 5.016$, $p = .032$, $\eta^2_p = .15$). Sedation scores were higher for the alcohol dose compared to the placebo dose. All other main and interaction effects were not significant (*p*s > .15).

Three subjects' post-task BrAC data were lost, the analysis was completed with the remaining participants. Similarly, the estimated blood alcohol levels (BAL) were subjected to repeated measures ANOVA, with time (BAL pre-AAT and BAL post-AAT) as within subject

variable. Results revealed that subjects performed the task during the steady state of alcohol level ($p > .198$) (See Table S2).

Accuracy

Accuracy data revealed a two way interaction effect of *Dose* by *Action Type* ($F(1, 31) = 5.874$, $p = .021$).

Reaction Times

Reaction time data comparing all task conditions revealed a three-way interaction of *Dose* by *Action Type* and *Stimulus Category* ($F(1, 31) = 6.579$, $p = .015$, $\eta^2_p = .18$) and *Stimulus Category* by *Group* ($F(1, 31) = 4.48$, $p = .042$, $\eta^2_p = .13$) interaction effect. To follow-up, we performed analysis separately for light and heavy drinkers. Results revealed that only heavy drinkers showed a three-way interaction of *Dose* by *Action Type* by *Stimulus Category* ($F(1, 14) = 5.99$, $p = .028$, $\eta^2_p = .3$), and main effects for *Dose* ($F(1, 14) = 6.62$, $p = .021$, $\eta^2_p = .326$) and *Stimulus Category* ($F(1, 14) = 8.287$, $p = .012$, $\eta^2_p = .3$). Analysis revealed that heavy drinkers were faster to approach than avoid alcohol ($t(14) = -3.318$, $p = .005$) and faster to approach alcohol compared to soft-drinks ($t(14) = 2.624$, $p = .02$). Remarkably, after drinking alcohol compared to placebo, heavy drinkers were slower in the approach alcohol and avoid soft-drink cue trials; all $t(14) > -2$, all $p < .032$) and had a tendency to respond slower in avoid alcohol-related cue trials ($t(14) = -1.984$, $p = .067$).

Bias Scores

The analysis of bias scores revealed a two way interaction of *Dose* and *Bias Type* ($F(1, 31) = 6.602$, $p = .015$, $\eta^2_p = .176$).

<u>Mu-MRAA</u>: Asymmetry indices over the time period of 0 to 1 sec. are presented in supplementary Figure1. After placebo dose MRAA for the avoid alcohol trials at T3 were significantly lower than baseline ($t(32) = -2.284$, $p = .029$). After alcohol dose, there was a significant decrease from baseline for the approach alcohol condition at T2 ($t(32) = -3.276$, $p = .003$), and in all conditions at T3 expect avoid alcohol condition (all $t(32) < -2$, all $p < .05$).

During the early preparation period (T1), analysis of the mu-MRAA for the bias revealed an interaction effect of *Dose* by *Bias* by *Group* ($F(1, 31) = 4.12$, $p = .051$, $\eta^2_p = .117$). During the middle preparation period (T2), only *Dose* by *Bias* by *Group* was marginally significant ($F(1, 31) = 3.846$, $p = .059$, $\eta^2_p = .11$). During the late preparation period (T3) a marginally significant *Dose* by *Group* interaction effect was observed ($F(1, 31) = 3.958$, $p = .056$, $\eta^2_p = .113$).

Beta-MRAA: In placebo, avoid alcohol at T2 (t (32) = -2.583, p = .015), and all conditions at T3 (all t (32) < -3, all p < .05) were different than baseline. After alcohol dose, avoid soft at T1 (t (32) = 3.171, p = .003), avoid alcohol at T3 (t (32) = -2.797, p = .009) and approach alcohol condition at T3 (t (32) =-2.956, p = .006) were different than baseline.

Analysis of the beta-MRAA revealed a *Dose* by *Group* interaction effect at T1 (F(1, 31) = 7.927, p = .008, η^2_p = .204), T2 (F(1, 31) = 9.158, p = .005, η^2_p = .113) and T3 (F(1, 31) = 8.958, p = .005, η^2_p = .224). At T3, a significant *Dose* by *Bias* by *Group* interaction effect (F(1, 31) = 6.988, p = .013, η^2_p = .184) and marginally significant *Dose* by *Bias* (F(1, 31) = 3.987, p = .055, η^2_p = .114) interaction effect were observed.

Parietal alpha-MRAA: The parietal MRAA was lower than baseline in the avoid alcohol condition after placebo dose (t(32) = -2.243, p = .032), in the approach alcohol condition after placebo (t(32) = -2.126, p = .041) and in the approach soft-drink condition after alcohol dose (t(32) = -2.038, p = .05) at T3.

The parietal alpha revealed an interaction effect of Bias by Group at T1 (t(31) = 6.284, p = .018) and an interaction effect of Dose by Group at T3 (t(31) = 4.374, p =.045).

Correlation with DMQR

The parietal alpha-MRAA contrast scores in the alcohol condition, positively correlated with the coping (r = .35, p =.044) and enhancement (r =.34, p = .05) subscales of the DMQR questionnaire and also with the total scores (r =.39, p = .024) at T2. For all subscales of the DMQR questionnaire, individuals with higher scores had more positive MRAA contrast scores, and individuals with lower scores had more negative MRAA contrast. In the placebo condition, no correlations with the MRAA contrast scores were observed.

Table S1. Demographic information for the light and heavy drinking groups.

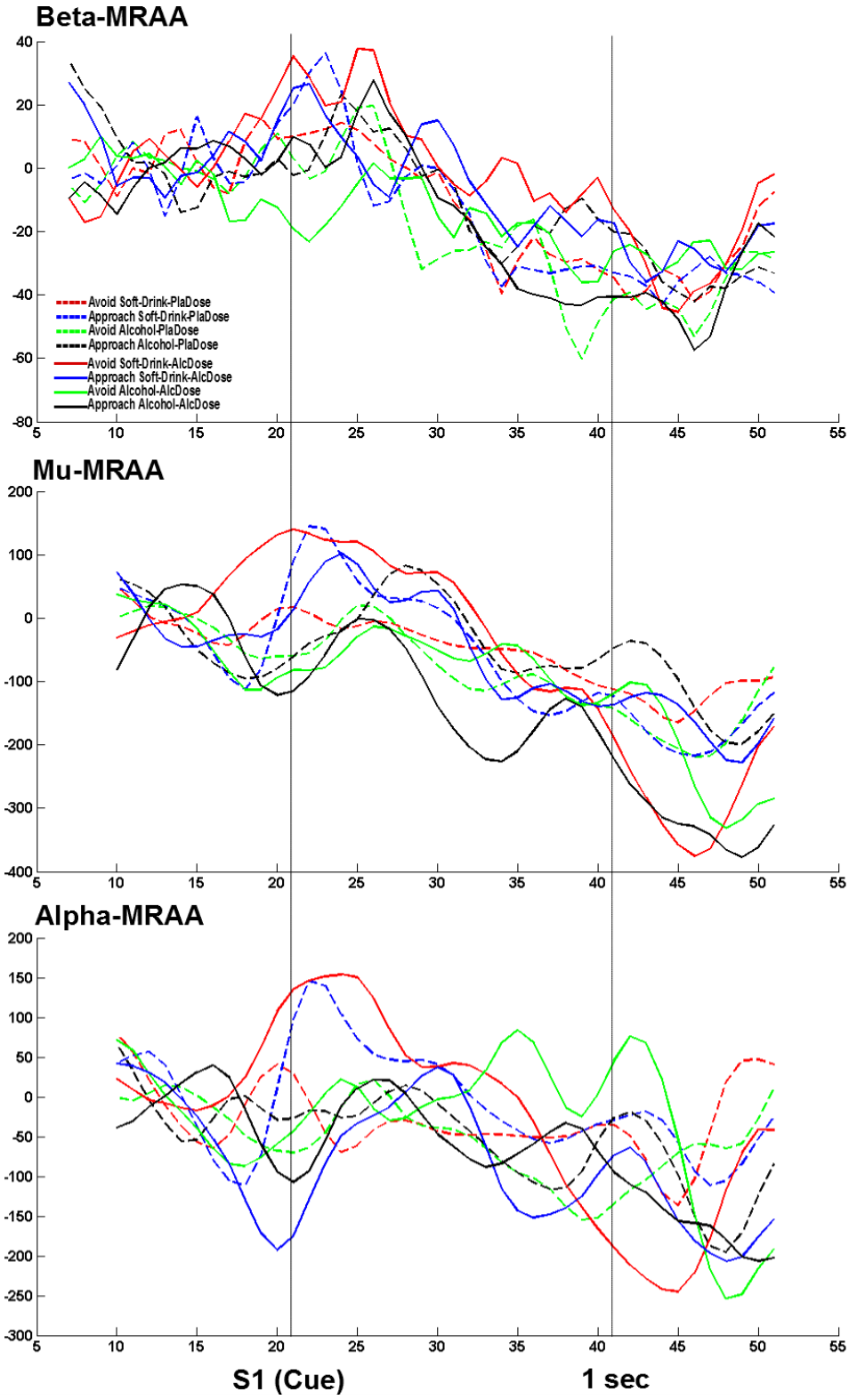
Variable	Light (n=18)	Heavy (n=15)	Light vs. Heavy (p-values)
Age (mean, SD)	18(1.19)	17.4(1.24)	.167
Sex (M/F)	6/12	7/8	-
AUDIT(mean, SD)	5.33(2)	13.87(3.14)	<.001
Smoking? (lifetime) (Yes/No, frequency)*	10/7, 31-40 times	14/1, 61-70 times	-
Drug Use (lifetime)*			
Marijuana (Yes/No, frequency)	10/7, 11-20 times	13/2, 31-40 times	-
Ecstasy (Yes/No, frequency)	0/17	4/11, 1-10 times	-
Hallucinogens (Yes/No, frequency)	1/16, 1-10 times	2/13, 1-10 times	-
Stimulants (Yes/No, frequency)	0/17	3/12, 1-10 times	-
Volatile Substances (Yes/No, frequency)	1/16, 1-10 times	4/11, 1-10 times	-
RAPI (last 3 months)	1.72(1.52)	5(4.07)	.003
RAPI (lifetime)	6.17(6.01)	14.67(6.02)	<.001
TRI			
Govern	4.11(2.63)	9.53(4.19)	<.001
Restrict	8.83(4.96)	13.8(5.43)	.01
Emotion	4.78(3.3)	9.6(5.05)	.002
Concern	6.33(4.65)	6.73(4.43)	.803
Cognitive	3.39(1.65)	5.53(2.72)	.009
Total	27.44(14.08)	45.2(14.62)	.001
DMQR			
Social	15.44(3.96)	17.33(2.87)	.134
Coping	6.83(1.51)	10(4.49)	.001
Enhancement	12.44(4.38)	14.13(4.88)	.303
Conformity	6.56(2.09)	6.33(1.84)	.751
Total	41.28(9.18)	47.8(8.98)	.049

* One light drinkers smoking and drug use information was missing.

Table S2. Mean scores and standard deviations for the BrAC and the Brief Biphasic Alcohol Effects Scale (B-BAES) before (pre-task) and after (post-task) participants completed the alcohol-Approach-Avoidance Task in the placebo and in the alcohol condition (n=33).

	Pre-AAT	Post-AAT
BAL (g/l, [Mean (SD)])	.55(.4)	.46(.15)
B-BAES Stimulation subscale		
Placebo [Mean (SD)]	18.15(5.72)	17.09(5.8)
Alcohol [Mean (SD)]	17.39(4.87)	16.12(5.7)
B-BAES Sedation subscale		
Placebo [Mean (SD)]	11.64(5.32)	12.(4.43)
Alcohol [Mean (SD)]	13.24(5.6)	14.24(5.49)

Figure S1. Asymmetry indices over the time period of 0 to 1 sec. for the beta-, mu- and alpha-MRAA.



CHAPTER 4

Alcohol-induced changes in conflict monitoring and error detection as predictors of alcohol use in late adolescence

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ABSTRACT

Adolescence is a vulnerable period for the development of substance use and related problems. Understanding how exposure to drugs influences the adolescent brain could reveal mechanisms underlying risk for addiction later in life. In the current study 87 adolescents (16-20 year-olds; the local legal drinking age was16, allowing the inclusion of younger subjects than usually possible) underwent EEG measurements during a Go/No-Go task with and without alcohol cues; after placebo and a low dose of alcohol (.45g/kg). Conflict monitoring and error detection processes were investigated with the N2 and the ERN (Error-Related Negativity) ERP-components. Participants were followed-up after six months to assess changes in alcohol use. The NoGo-N2 was larger for alcohol cues and acute alcohol decreased the amplitude of the NoGo-N2 for alcohol cues. ERN amplitude was blunted for alcohol cues. Acute alcohol decreased the amplitude of the ERN, specifically for control cues. Furthermore, the differences in ERN for alcohol cues between the placebo and alcohol conditions predicted alcohol use six months later: subjects who showed stronger blunting of the ERN after acute alcohol were more likely to return more moderate drinking patterns. These results suggest that cues signalling reward opportunities might activate a go-response mode and larger N2 (detection of increased conflict) for these cues might be necessary for inhibition. The ERN results suggest a deficiency in the monitoring system for alcohol cues. Finally, a lack of alcohol-induced deterioration of error monitoring for cues with high salience might be a vulnerability factor for alcohol abuse in adolescents.

INTRODUCTION

Dual process models explain addiction as the result of an imbalance between an appetitive and a regulatory system (Deutsch *et al*, 2006; Stacy *et al*, 2004; Wiers *et al*, 2007; but see Gladwin *et al*, 2011). Accordingly, poor response inhibition predicts drinking problems in high-risk children (Nigg *et al*, 2004; 2006) and a transition to problem drinking in adolescents (Norman *et al*, 2011). This may be related to the more general finding that adolescent cognitive performance is relatively weak in "hot" (emotionally or motivationally salient) versus "cold" contexts (Crone and Dahl, 2012; Gladwin and Figner, 2014; Grose-Fifer *et al*, 2013). Relatively weak performance of adolescents in an affective context has been tentatively related to a delay in the development of neural system needed for behavioural regulation, relative to the development of emotional-motivational system (Casey and Jones, 2010; Jentsch and Taylor, 1999). The Anterior Cingulate Cortex (ACC) is a key structure involved in response inhibition and monitoring of response conflicts (co-activation of competing actions) (Bekker *et al*, 2005; Yeung *et al*, 2004). Given its rich connections to the Prefrontal Cortex (PFC) and limbic structures, ACC regulated processes are likely to be affected by the interplay between control and motivation. Neural activity associated with conflict monitoring has been associated with alcohol use severity (Claus *et al*, 2013) and density of family history of alcoholism (Fein and Chang, 2008). Thus, inhibition and conflict monitoring in an affective context are likely to play a role in the vulnerability for addiction in adolescents.

The acute disinhibiting effects of alcohol may lead to escalation of alcohol use (review: Field *et al*, 2010). This may be due to an increase in appetitive motivation towards drug cues and/or a decrease in regulatory cognitive control (Adams *et al*, 2013; Duka and Townshend, 2004; Hernández and Vogel-Sprott, 2010; Ridderinkhof *et al*, 2002). Acute alcohol effects may mimic long-term effects and could thus predict escalation (Wiers *et al*, 2007). Note that both relatively strong direct appetitive effects and relatively weak later responses to alcohol in terms of negative effects (e.g. on balance) have been related to individual differences in the risk for later addiction (Newlin and Thomson, 1990; Schuckit *et al*; 2000).

The electroencephalogram (EEG) can be used to further study conflict monitoring, response inhibition and error detection. According to the conflict monitoring theory, the ACC monitors conflict that arises due to co-activation of competing actions in order to deploy additional cognitive resources. The ACC generates the N2 event-related potential (ERP) component, when it detects *pre-response conflict* on correctly inhibited trials (Van Veen and Carter, 2002). Another ERP component, the Error-Related Negativity (ERN), is thought to be related to *error detection* and generated by the ACC when a correct response is activated after an error, resulting in *post-response conflict*. Evidence supports the involvement of both conflict monitoring and response inhibition in N2 generation: the N2 was enhanced for low-frequency

stimuli regardless whether a response must be generated or suppressed (Nieuwenhuis *et al*, 2003) and for NoGo stimuli when the frequency of required Go/NoGo responses was equal, also the response conflict (Lavric *et al*, 2004).

In adults, effects of alcohol on the ERP suggest impaired error detection but intact conflict monitoring (Bartholow *et al*, 2012; Easdon *et al*, 2005; Ridderinkhof *et al*, 2002). In a simulation study, Yeung and Cohen (2006) showed that the ERN and the N2 could be sensitive to relevant and irrelevant stimulus information, respectively. Further, the ERN is modulated by affective cues (Larson *et al*, 2006), which may be related to the disruption of inhibition in an affective context (Grose-Fifer *et al*, 2013; Noël *et al*, 2007). To our knowledge, acute alcohol effects on conflict monitoring in the context of motivationally relevant alcohol cues has not been investigated yet in drinking adolescents.

The current study focused on two questions: 1) Are response inhibition and conflict monitoring processes influenced by acute alcohol in adolescents and is this moderated by the motivational relevance of the cues? 2) Do brain potentials, moderated by alcohol, predict future alcohol use in adolescents? To this aim, a Go/NoGo task including both alcohol and soft drink stimuli was used. We expected the ERN and the N2 to be dampened after acute alcohol and for alcohol versus soft drink cues. Participants' change in alcohol use was assessed after six months. Differences across dose conditions were expected to predict short-term prospective escalation of alcohol use.

MATERIALS AND METHODS

Participants

Ninety-seven adolescents were recruited from local high schools via advertisements. Participants were required to be minimally 16 years-old (minimum drinking age in Netherlands at the time of the study), with a minimum weight of 50 kg and to have had at least one full drink in their lifetime (see Supplementary Materials for exclusion criteria). Prior to the experiment, a written informed consent was obtained from all participants and from parents of participants under the age of 18. Ten subjects' data were excluded for the following reasons: three due to positive drug test for THC, one due to a drop-out in the second session, four due to equipment failure, one due to incorrect beverage administration, and one due to an extreme number of omission trials. The analysis was conducted with the remaining 87 subjects (33 males, mean age = 17.6 years, range= 16-20 years).

Alcohol Administration and Procedure

The study consisted of two sessions on two different days. On each session, either a placebo or an alcoholic drink (.45ml/kg) was administered. Beverages were divided into three equal portions. Two of the drinks were served prior to commencing the tasks and the last drink was administered as a booster drink halfway through the session (for details on the alcohol administration see Supplementary Materials). Upon arrival in the lab, subjects filled out demographics, questionnaires related to personality and drinking habits. At the start of each session, subjects completed the *Desire for Alcohol Questionnaire* (DAQ, Love *et al*, 1998) and the *Positive and Negative Affect Scale* (PANAS, Watson *et al*, 1988) to control for current mood and craving. Current alcohol use and problems were assessed with the *Alcohol Use Disorder Identification Test* (AUDIT, Saunders *et al*, 1993; we used both the standard past year version, and a version about the past 3 months), the Ruthers Alcohol Problem Index (RAPI, White and Labouvie, 1989) and an adjusted version of the Timeline Followback method developed by Sobell and Sobell, (1992) as reported in Wiers *et al* (1997). Drug use behaviour was assessed with an 11-item rating scale (Graham *et al*, 1984). In order to assess drinking frequency separately for weekdays and weekends subjects filled out three additional questions. This additional set included questions on the frequency and the quantity of drinking in the last 3 months and lifetime binge drinking frequency (see Supplementary Materials). The session started with an unrelated task, followed by beverage administration. Approximately 10 minutes after beverage administration, subjects also performed three unrelated tasks (see Supplementary Materials). Order of the tasks was counterbalanced across subjects, but was kept the same across sessions for each subject. Breath alcohol concentration (BrAC) was collected 5 minutes after the first two drinks, before and after the booster drink, and at the end of the experiment by using the Lion alcolmeter® SD-400 (Lion Laboratories Limited, South Glamorgan, Wales). Participants filled out the *Brief Biphasic Alcohol Effects Scale* (B-BAES, Rueger *et al*, 2009) each time a breath sample was taken, except before the booster drink.

The sessions were carried out at least 48 h and maximally 1 week apart. Sessions started between 12:00 and 18:00 PM. Each session took approximately two and a half hours, including breaks and the application of electrodes. Six months after the baseline assessment, participants were contacted via email for an online assessment on recent alcohol and drug use. During the follow-up assessment, subjects filled out the same alcohol-related scales as during pre-test. The study was approved by the local ethical committee.

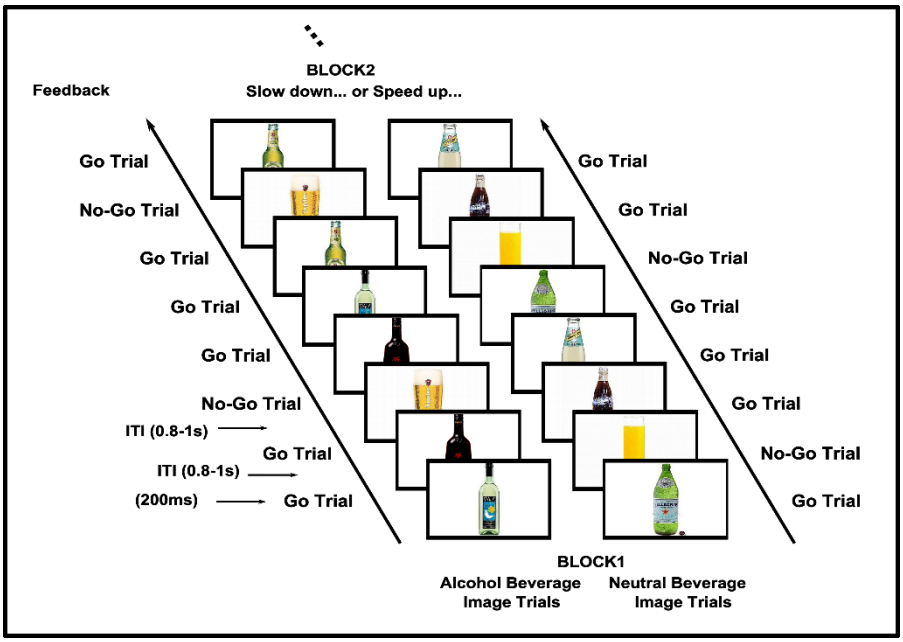
Go/NoGo Task

Subjects were presented with pictures of beverages in a bottle or in a glass. The task consisted of blocks with sets of either alcohol or soft drink pictures. In the alcohol blocks the stimuli were alcohol-related pictures (e.g. beer, wine), and in the neutral blocks the stimuli were pictures of

soft drinks (e.g. cola, sprite). In the Go trials, a right button response was required for the pictures of a beverage in a bottle (as quick as possible while maintaining accurate). In the NoGo trials, when the picture of a beverage in a glass was presented, subjects were required to withhold their responses. Four pictures were used in each block type, each consisting of three go stimuli and one no-go stimulus (e.g. beer, Figure 1). Each stimulus was presented with equal frequency, leading to 25 % no-go and 75 % go trials (90 no-go and 270 go trials per block). Pictures with and without alcohol contents were matched for perceptual characteristics (i.e., colour, shape etc.).

The task started either with the alcohol or the neutral block, counterbalanced across participants. After12 practice trials, the task consisted of 10 assessment blocks, with neutral and alcohol blocks alternating. Each stimulus was presented for 200 ms, followed by 800ms and 1000 ms of ITI for go and no-go trials, respectively. In order to study error-related EEG activity, an adequate amount of commission errors were required, therefore an adaptive procedure was used. After each block, subjects' overall commission errors and correct responses in the no-go trials were calculated. When the ratio between commission errors/correct no-go responses was smaller or larger than 50/50, the next block started with a feedback encouraging, respectively, to "speed up" or "slow down" the response. If the ratio was equal, subjects received no feedback. In order to control for the effect of picture familiarity across sessions, two sets of pictures with and without alcohol contents were matched, and each stimulus set was randomly assigned to a session.

Figure 1.Schematic representation of the alcohol-related Go/NoGo Task. Subjects were presented with pictures of beverages in a bottle or in a glass. The task consisted of blocks with sets of either alcohol or soft drink pictures. In the Go trials, a right button response was required for the pictures of a beverage in a bottle. In the NoGo trials, when the picture of a beverage in a glass was presented, subjects were required to withhold their responses. The task consisted of 10 blocks, with neutral and alcohol blocks alternating. Each stimulus was presented for 200 ms, followed by 800ms and 1000 ms of ITI for go and no-go trials, respectively.



Electroencephalogram (EEG) recording and data analysis

Electrophysiological data were recorded from the scalp using an Active-Two amplifier (Biosemi, Amsterdam, the Netherlands) from 32-scalp sites. Electrodes were placed at the standard positions of the 10-20 international system. Two electrodes were placed at the outer canthi of the eyes to measure horizontal eye movements. Two electrodes were placed at below and above the left eye to measure vertical eye movements. EEG was recorded at 2048 Hz sampling rate. The distance between the screen and the subject was kept 75 cm.

EEG analysis was conducted using Brain Vision Analyzer (version 2.0, Brain Products GmbH, Munich, Germany). Data were down-sampled to 250Hz, re-referenced offline to the average of scalp electrodes, low pass filtered at 20Hz, and high pass filtered at 0.1 Hz. Ocular correction was applied using the algorithm of Gratton *et al*, (1983). Stimulus and response-locked epochs ranged from -200 to 1000ms and from -300 to 800ms, respectively. Trials were considered artefacts when the difference between consecutive data points was larger than 50 mV and the difference between the lowest and the highest voltage within a segment was higher

than 150 mV. Epochs with an amplitude exceeding ±100 mV were excluded. The mean 200 ms pre-stimulus and pre-response period was used as baseline. After baseline correction, average stimulus-locked ERPs were calculated for artefact-free trials at each scalp location for trials with correct go, correct no-go and commission responses separately. Average response-locked ERPs were created for trials with commission error responses only (for details on ERP quantification and subject/trial exclusion procedure, see Supplementary Materials).

Data Preparation and Statistical Analysis

For behavioural performance, mean RTs for correct go and commission error responses, average hit rates (trials with correct go response/trials with correct go plus omission responses) and false alarm rates (trials with commission response/trials with commission plus correct no-go responses) were calculated separately for the blocks with neutral and alcohol stimulus set in each condition.

All analyses were conducted using a repeated measures analysis of variance (RM-ANOVA). The PANAS and DAQ scores were analysed with *Dose* (Placebo, Alcohol) as a within subject factor. The Stimulation and Sedation subscales of B-BAES scores were separately analysed with *Dose* (Placebo, Alcohol), and *Time* (pre-task and post-task) as within-subjects factors. The BrAC were subjected to a RM-ANOVA, with *Time* (BrAC pre-task and post-task) as within-subject variable. Two subjects' B-BAES data and five subjects' BrAC scores were lost; the analysis was conducted with the remaining subjects. Behavioural data were analysed with *Dose* (Placebo, Alcohol) and *Beverage Image Class* (Neutral, Alcohol Beverage Images) as within-subject factors. ERP data were analysed with *Dose* (Placebo, Alcohol) and *Beverage Image Class* (Neutral, Alcohol Beverage Images) as within subjects' variables. Further analysis for each ERP component focused on the channel locations where the amplitude was maximal. When appropriate, Greenhouse-Geisser corrected values were reported.

In order to assess whether ERP differences across sessions predicted unique variance in the change in alcohol use during the six months after the experiment, a hierarchical multiple regression analysis was conducted. First, subjects' demographic characteristics (age, gender and education) were entered to the regression model, followed by the AUDIT score for recent use (sum of scores of items on frequency of drinking, typical quantity and frequency of heavy drinking) at baseline from the version about the past 90 days. In the last step, the contrast scores (alcohol minus placebo) for the alcohol and the neutral stimulus sets were entered. This way the predictive value of acute alcohol effect on ERP indices was tested beyond the predictive value of subjects' demographics and AUDIT scores at baseline.

RESULTS

Questionnaires

Subjects' craving scores and their positive and negative mood scores at the start of the experiment were the same in the placebo and in the alcohol condition (*p*-values>.2).

Manipulation Checks

The differences in the BAES stimulation subscale before and after the task performance revealed that subjects felt less stimulated as the session proceeded ($F(1, 84) = 14.01$, $p < .001$, $\eta^2_p = .14$). Moreover, subjects felt more sedated after alcohol than after placebo ($F(1, 84) = 29.84$, $p < .001$, $\eta^2_p = .26$). BAL levels were lower post-task compared to pre-task ($F(1, 81) = 4.519$, $p = .037$, $\eta^2_p = .05$; See Table 1).

Table1. Mean scores and standard deviations for the BrAC and the Brief Biphasic Alcohol Effects Scale (B-BAES) before (pre-task) and after (post-task) subjects completed the Go/NoGo task in the placebo and in the alcohol condition.

	Pre-task	**Post-task**
BAL (g/l, [Mean (SD)])	.53(.31)	.43(.19)
B-BAES Stimulation subscale		
Placebo [Mean (SD)]	16.75 (5.93)	14.76 (5.79)
Alcohol [Mean (SD)]	16.14 (5.98)	14.75 (6.09)
B-BAES Sedation subscale		
Placebo [Mean (SD)]	11.39 (6.12)****[a]	11.99 (5.64)****[b]
Alcohol [Mean (SD)]	14.87 (6.96)****[a]	15.92 (6.85)****[b]

Abbreviation: BAL, blood alcohol level.
* p < .05, ** p < .01, *** p < .005, **** p < .001.
Significant differences for the sedation subscale are not across time points (pre vs post-task), but across conditions (placebo vs. alcohol). [a] and [b] indicates significant differences across conditions at pre- and post-task, respectively.

Behavioural Measures

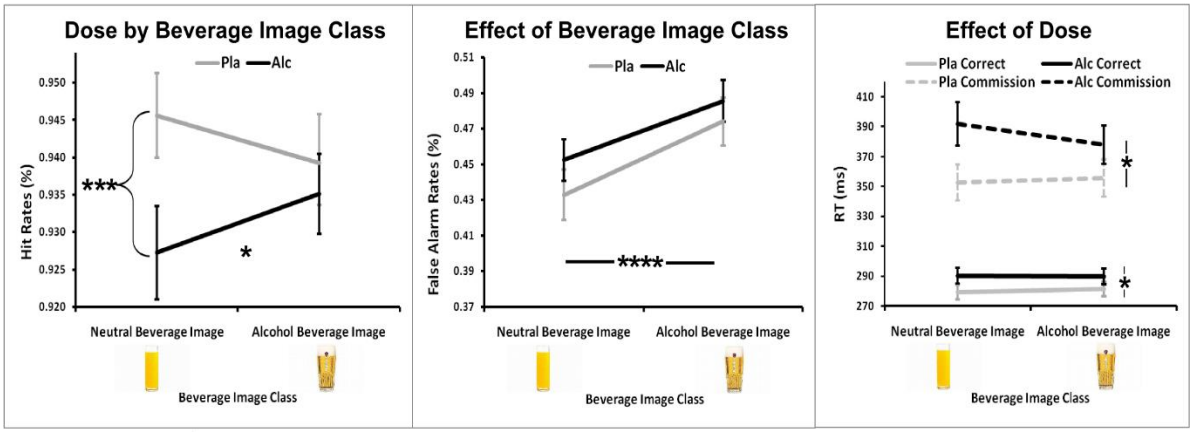
Dose by Beverage Image Class Effect on Hit Rates. Hit rates trended toward a main effect of *Dose* ($F(1, 86) = 3.89$, $p = .052$, $\eta^2_p = .04$), an effect superseded by a significant interaction effect of *Dose* by *Beverage Image Class* ($F(1, 86) = 7.85$, $p = .006$, $\eta^2_p = .08$). On the average, hit-rates tended to be higher in the placebo condition. Post-hoc analysis of the two-way interaction revealed that in the alcohol condition, hit-rates were higher for the *Alcohol Beverage Images* compared with the *Neutral Beverage Images* ($t(85) = -2.39$, $p = 0.02$), in the absence of

such an effect in the placebo condition ($p > 0.15$). Moreover, hit-rates were higher for the *Neutral Beverage Images* in the placebo condition compared with the alcohol condition ($t(85) = 3.09$, $p = 0.003$), with no differences observed between conditions for the *Alcohol Beverage Images* ($p > 0.55$) (See Figure 2, left panel).

Effect of Beverage Image Class on False Alarms. False alarm rates revealed a main effect of *Beverage Image Type* ($F(1, 86) = 32$, $p < .001$, $\eta^2_p = .27$), subjects made more commission errors for the *Alcohol* than the *Neutral Beverage Images* (See Figure 2, middle panel).

Effect of Dose on RT. In the trials with correct-go and commission responses, subjects tended to respond faster in the placebo than the alcohol condition (Correct-Go: $F(1, 86) = 3.56$ $p = .063$, $\eta^2_p = .04$; Commission: $F(1, 86) = 5.91$, $p = .017$, $\eta^2_p = .06$; *See* Figure 2, right panel).

Figure 2. Behavioural results for hit rates (left side), false alarm rates (middle) and RT (right side). Hit rates were lower in alcohol condition for neutral beverage images. False alarm rates were lower for neutral beverage images. RTs for trials with commission errors and correct Go responses were shorter in the placebo condition. Pla = placebo; Alc = alcohol; Pla Correct: Correct go trials in the placebo condition; * p < .05, ** p < .01, *** p < .005, **** p < .001.



N2

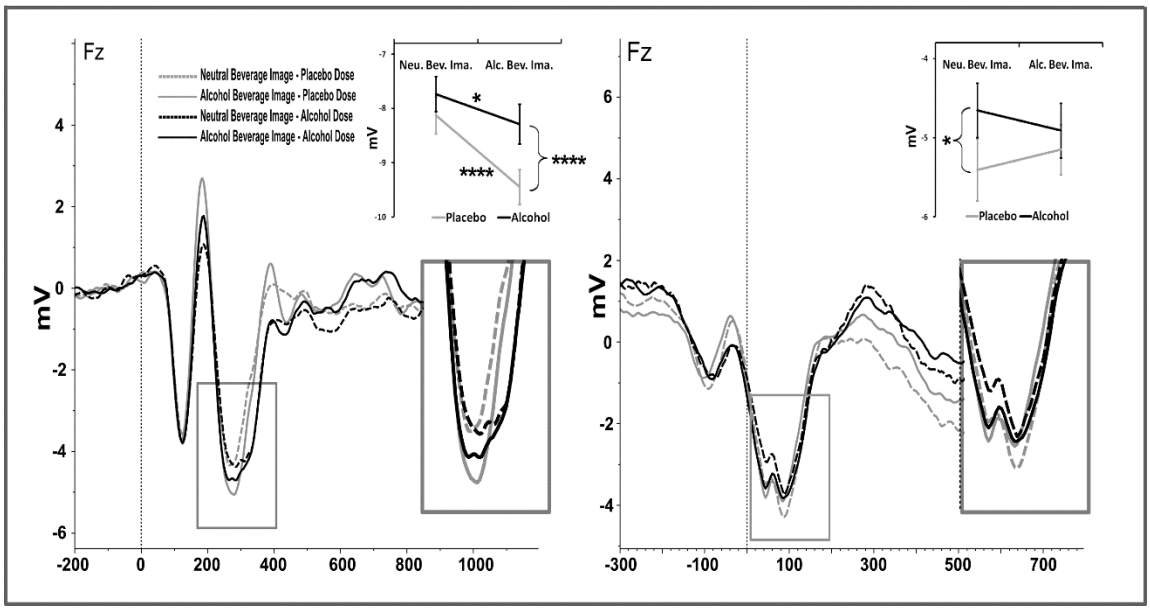
Dose by Beverage Image Class Effect on N2. The NoGo-N2 for the correct responses revealed a main effect of Dose ($F(1, 77) = 10.103$, $p = .002$, $\eta^2_p = .12$) and a main effect of Beverage Image Class ($F(1, 77) = 24.888$, $p < .001$, $\eta^2_p = .24$). An interaction effect of Block Type by Beverage Image Class qualified these main effects ($F(1, 77) = 6.021$, $p = .016$, $\eta^2_p = .073$). Inspection of the two-way interaction effect revealed that: 1) The NoGo-N2 for the *Alcohol*

Beverage Images was larger than for the *Neutral Beverage Images*, both in the placebo and in the alcohol conditions (placebo: $t(77) = 5.47$, $p < 0.001$, alcohol: $t(77) = 2.02$, $p = 0.047$); 2) For the *Neutral Beverage Images*, the NoGo-N2 had comparable amplitudes ($p > .2$) after alcohol and placebo, however, for the *Alcohol Beverage Images*, acute alcohol decreased the amplitude of NoGo-N2, ($t(77) = -4.136$, $p < 0.001$) (See Figure 3, left). NoGo-N2 for the incorrect trials did not reveal any main or interaction effects (p-values > .07).

ERN

Dose by Beverage Image Class Effect on ERN. The ERN was smaller in the alcohol than the placebo condition ($F(1, 68) = 4.073$, $p = .048$, $\eta^2_p = .057$) (See Figure 3, right panel). Given the predictive effects of the ERN (see below), additional exploratory pair-wise comparisons were conducted. These results revealed that acute alcohol decreased the ERN for the *Neutral Beverage Images* ($t(68) = -2.22$, $p = 0.03$), but not for the *Alcohol Beverage Images* ($p > .3$).

Figure 3. Stimulus–locked N2 (left side) for trials with correct responses and response-locked ERN for trials with error responses (right side) at Fz. Stimulus and response onset occurred at 0 ms. The NoGo-N2 for the *Alcohol Beverage Images* was larger than the *Neutral Beverage Images* in both placebo and alcohol conditions and acute alcohol decreased the amplitude of NoGo-N2 only for the *Alcohol Beverage Images (left side)*. The ERN was smaller in the alcohol than the placebo condition. Acute alcohol decreased the ERN only for the *Neutral Beverage Images (right side).* Alc. Bev. Ima: alcohol beverage images; Neu. Bev. Ima: neutral beverage images; * p < .05, ** p < .01, *** p < .005, **** p < .001.

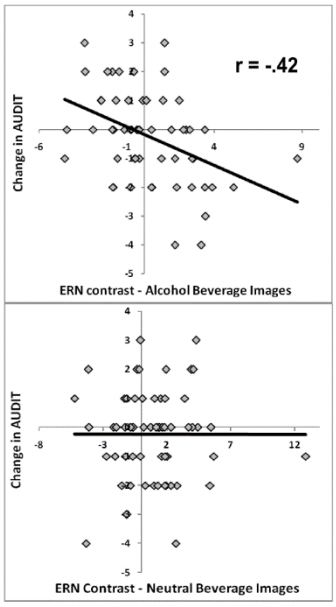


Neural Predictors of Alcohol Use After Six Months

Six months after the baseline assessment, 82.5% follow-up response rate was achieved in the full sample. On average `completers` and `drop-outs` were similar on demographic characteristics, yet drop-outs scored higher on drinking-related problems (RAPI), contained more smokers and reported higher drug use frequency (see Table S2). In the hierarchical multiple regression model, one subject's Cook's distance was .7 (mean Cook's Distance=.01, SD Cooks Distance=.03, before exclusion) and this subject was excluded from the analysis. The frontal ERN in the *Alcohol Beverage Images* significantly predicted future alcohol use beyond the variance explained by demographics and baseline AUDIT scores. The total variance explained by the full model was 69% (F change$_{2,51}$ = 5.886, p = .005). The demographics and the baseline AUDIT scores explained 19.3% (F change$_{4,54}$ = 3.237, p = .019) and 42.5 % (F change$_{1,53}$ = 58.875, p < .001) of the variance in alcohol use six months later, respectively. The frontal ERN in the *Alcohol Beverage Images* explained an additional 7.2% of the variance. In order to interpret the contribution of the ERP contrast in the *Alcohol Beverage Images*, we conducted a correlation analysis between change in AUDIT scores (AUDIT follow-up – AUDIT baseline), the ERN contrast in the alcohol and neutral blocks. The results revealed a negative correlation between change in AUDIT and the ERN contrast in the *Alcohol Beverage Images* (r = -.42, p = .001) (*See* Figure 4). Subjects, who showed a relatively strong ERN decrease after acute alcohol in the alcohol blocks, had lower AUDIT scores, relative to baseline, 6 months later.

Figure 4. Hierarchical multiple regression analysis for variables predicting AUDIT at 6-month follow-up (n = 59) (left side). The correlation between change in AUDIT scores (AUDIT follow-up, last 90 days – AUDIT baseline, last 90 days), the ERN contrast (Alcohol - Placebo) for the Alcohol (upper, right) and Neutral Beverage Images (lower, right). SE: Standard errors. AlcBevIma: alcohol beverage images; NeuBevIma: neutral beverage images.

	B	SE B	Beta	P - values
Step1: change R^2: .193, p=.019				
Gender	1.432	.539	.333	.01
Age	.317	.205	.194	.127
Middle level education	.149	.819	.036	.856
High level education	1.271	.797	.316	.117
Step2: change R^2: .425, p<.001				
Gender	.837	.382	.195	.033
Age	.318	.142	.194	.029
Middle level education	.564	.572	.136	.328
High level education	.675	.559	.168	.233
Audit baseline	.636	.083	.701	<.001
Step3: change R^2: .072, p=.005				
Gender	.86	.361	.2	.021
Age	.362	.131	.221	.008
Middle level education	.795	.53	.191	.139
High level education	.762	.515	.19	.145
Audit baseline	.714	.079	.787	<.001
ERN Contrast-AlcBevIma	-.241	.071	-.288	.001
ERN Contrast-NeuBevIma	.022	.057	.033	.694



DISCUSSION

We examined whether acute alcohol and alcohol cues affect conflict monitoring and error detection processes in drinking older adolescents. Moreover, we tested whether alcohol-induced changes on these cognitive processes predict future alcohol use. Behavioural data revealed that RT for commission and correct-go responses were slower after alcohol administration, suggesting a psychomotor slowing in order to maintain accuracy. In line with this interpretation, false alarm rates did not vary across the alcohol and placebo conditions. Similar to previous findings (Adams *et al*, 2013; Kreusch *et al*, 2013), subjects gave more go-responses for alcohol cues both in Go and NoGo trials, suggesting that alcohol cues may be more associated with an approach or a go response. Moreover, hit rates for neutral cues were more sensitive to acute alcohol effects, subjects made more omissions for neutral cues after acute alcohol.

The ERP data showed that the NoGo-N2 for alcohol cues was higher than for soft drink cues; suggesting a relatively strong simultaneous activation of Go (stimulus-induced) and NoGo (task-induced) responses towards alcohol cues. In line with previous simulation research

(Yeung and Cohen, 2006), acute alcohol decreased the N2 specifically for task-irrelevant alcohol cues. The ERN was not influenced by the motivationally salient alcohol cues. Exploratory analyses revealed lower ERN amplitudes after acute alcohol, specifically for neutral cues. Finally, alcohol-induced changes in the ERN for alcohol cues predicted changes in alcohol use six months later.

In young adult drinkers, many studies have shown increased salience of alcohol-related stimuli (Bartholow *et al*, 2007; 2010; Herrmann *et al*, 2000), engagement of attentional resources and automatic approach tendencies towards alcohol cues (Johnsen *et al*, 1994; Sharma *et al*, 2001; Field *et al*, 2008). Thus the results of the Nogo-N2 associated with inhibition and conflict monitoring might indicate that alcohol cues pre-activate a go-response due to increased attention allocation and approach tendencies. In correct NoGo trials, the greater N2 for alcohol cues might suggest that when increased conflict between stimulus-induced and task-relevant responses is detected, inhibition of this pre-potent response has been successful. In incorrect NoGo trials, the lack of this additional process of conflict detection might have resulted in comparable N2 amplitudes for alcohol and non-alcohol cues. Moreover, the ERN associated with error detection was smaller for alcohol cues during commission errors, a result in line with the idea that detecting the conflict between competing responses might be important for giving correct responses. Moreover, relatively small ERN amplitudes for alcohol cues might suggest a relative dysfunction involving error detection in the presence of alcohol cues.

To the best of our knowledge, only two previous studies investigated the influence of drug-related context on the N2, one in the context of smoking cues (Luijten *et al*, 2011) and the other in the context of alcohol cues (Petit *et al*, 2012). These studies did not reveal any effects of drug-related cues on the N2. Both studies implemented alcohol-related contexts as backgrounds; the feature that signalled the correct response was not itself drug-related. This may have allowed the drug-related stimuli to be more effectively suppressed. The study by Luijten *et al* (2011) implemented intermittent presentation of drug-related and control cues, unlike our continuous presentation of drug cues in a blocked design. Rapid attention alterations required by the task might have reduced the effect of task irrelevant stimulus information on the N2. Differences across studies could also be due to studying different samples. The current study tested such effects with a younger sample, likely to have heightened reward sensitivity.

A second aim of the current study was to examine whether alcohol-induced changes on ERPs associated with action monitoring would predict changes in alcohol use. The effect of acute alcohol in the ERN for alcohol cues predicted changes in alcohol use in adolescents. Subjects for whom alcohol disrupted the error detection processes for alcohol cues, as indexed by the ERN, were more likely to show a decrease in their drinking at the six months follow-up. A tangible deleterious effect of acute alcohol on the ERN for alcohol cues might indicate a ‘protective sensitivity’, comparable to the protective value of negative alcohol effects on body

sway (cf., Schuckit *et al*, 2000). An earlier study showed that expectancy of cognitive and motor impairment due to alcohol is associated with non-drinking in adolescents and young adults (Wiers *et al*, 1997). Moreover, individuals with high sensitivity to negative alcohol effects are more likely to show adaptive strategy adjustments (Bartholow *et al*, 2003). Taken together, alcohol-induced changes in the monitoring system might be a protective factor for alcohol abuse.

In summary, the results of the current study are in line with previous studies showing decreased performance in the presence of motivational cues. We showed that the conflict monitoring system is sensitive to alcohol cues. This could be because cues signalling reward opportunities might activate a go-response mode. Future research is needed to replicate and extend the current findings in adults with substance use disorders. Moreover low and high doses of alcohol affect different processes therefore future studies in adult samples could study the acute effects of higher dosages of alcohol and relate them to future alcohol use. Responses of the error detection system towards drugs and drug-related stimuli appear to be related to changes in drug-related behaviours. An interesting route for future studies would be to understand how sensitivity to positive and negative response outcomes (i.e. feedback-based learning) could affect processes such as error detection and conflict monitoring in adolescents and how these learning processes could contribute to addictive behaviours in later life. Lastly, we would like to note that until now our knowledge of acute alcohol effects on neurocognitive processes in younger samples have exclusively been based on either relatively old adolescents due to legal limitations or animal studies, hence the current study uniquely contributes to the literature by providing initial findings of acute alcohol effects on human adolescent sample.

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SUPPLEMENTARY MATERIALS

MATERIALS AND METHODS

Participants: Exclusion criteria

Full exclusion criteria were psychiatric disorders, drug use disorder, head trauma, seizures, severe physical illness, cardiovascular disease, chronic obstructive pulmonary disease, the presence of major medical conditions, and use of medication. Further exclusion criteria's for female participants was pregnancy and breast-feeding; this was confirmed with self-report.
For participants under the age of 18, parental consent was mandatory to take part in the study. A written informed consent was obtained from all participants prior to the experiment. Participants received financial compensation (€25) for their participation.

Session restrictions

Prior to the appointment, participants were instructed to abstain from any alcohol for at least 24 hr, and any meal or caffeine for at least 4 hr. Subjects' compliance with these restrictions was confirmed with self-report. Moreover, participants were instructed to abstain from any legal and illegal drugs for at least 1 week; their compliance with this restriction was confirmed with a urine test.

Questionnaires

Desire for Alcohol Questionnaire (DAQ, Love *et al*, 1998): The desire for alcohol questionnaire (*DAQ*) is a 14-item instrument with a 7-point likert scale, measuring 4 dimensions of craving: Desires and intentions to drink, negative reinforcement, control over drinking, and mild desires to drink. Subjects were required to rate the items from "strongly disagree" to "strongly agree".

Positive and Negative Affect Scale (PANAS, Watson *et al*, 1988): PANAS is a 20 item scale that measures subjects' positive (such as enthusiasm, active, alert) and aversive mood states (such as subjective distress and unpleasurable engagement) during a specific time frame. This questionnaire consist of 20 descriptors such as 'distressed', 'upset', 'excited' etc. The subjects are asked to rate each descriptor on a 5-point scale ranging from 1 (*very slightly*) to 5 (*very much*).

Alcohol Use Disorders Identification Test (AUDIT, Saunders *et al*, 1993): AUDIT is a 10-item questionnaire developed to screen for excessive drinking. The questionnaire includes three domains to measure subjects' current drinking habits as follow: recent alcohol use (Items 1-3),

alcohol dependence symptoms (Items 4-6), and alcohol related problems (Items 7-10). In the baseline assessment, subjects filled out this questionnaire once for the last 3 months and once for the lifetime. In the online follow-up, subjects filled out this questionnaire only for the last 3 months. In the current study, the total score of items on recent alcohol use (Items1-3) at baseline (last 3 months) and six-months follow-up assessment were used in a hierarchical multiple regression analysis in order to identify factors that predicted changes in alcohol use.

Brief Biphasic Alcohol Effects Scale (B-BAES, Rueger *et al*, 2009): Participants' subjective stimulant and sedative effects of alcohol were assessed by the brief version of the BAES. The B-BAES is a 6-item adjective rating scale that measures the stimulant and sedative effects of alcohol as distinct constructs at ascending and descending limbs of the blood alcohol curve. The brief Stimulation subscale is the summation of the adjectives *energized*, *excited*, and *up*, and the brief Sedation subscale is the summation of the adjectives *sedated*, *slow thoughts*, and *sluggish*. Participants asked to rate the extent to which they were feeling each adjective at the present time on an 11-point scale ranging from 0 (*not at all*) to 10 (*extremely*).

Rutgers Alcohol Problem Index (RAPI, White and Labouvie, 1989): RAPI is a 23- item survey developed to measure alcohol related problems. Subject were asked to report how many times they experienced each statement on a 5-point scale (0=never, 1 = 1 to 2 times, 2 = 3 to 5 times, 3 = 6 to 10 times, 4 = more than 10 times).

Self-Rating of the Effects of Alcohol (SRE, Schuckit *et al*, 1997): SRE is a 12-question survey used to determine an individual's level of response to alcohol. Subjects were asked the number of standard drinks required to produce four possible type of effects at three different time points (first 5 times they ever drank, 3 months drinking of once a month, and period heaviest drinking). Subjects received information regarding the amount of alcohol present in a standard drink of different sort (beer, wine, spirits etc.)

Alcohol Questions on frequency of alcohol use and binges:

Question1: During the last 3 months, how often did you usually have any kind of drink containing alcohol? (response options for weekends: once per month or less, two or three times per month, once per weekend, twice per weekend; response options for weekdays: once per month or less, two or three times per month, once per weekday, twice per weekday, three or four times per weekday, every weekday). Question2: During the last 3 months, how many alcoholic drinks did you have on a typical day when you drank alcohol? (response options: less than 1 drink, 1 drink, 2 drinks, 3 drinks, 4 drinks, 5 drinks, 6 drinks, 7-9 drinks, 10-12 drinks, more than 12 drinks). Question3: How often did you have 5 or more drinks containing any kind

of alcohol on one occasion? (response options: never, 1-3 times, 3-5 times, 5-6 times, 7-9 times, 9-12 times, 12-15 times, 15-17 times, 17-20 times, more than 20 times).

Alcohol preparation/administration procedure

Dose order was counterbalanced across participants such that half of the participants were tested under the alcohol dose first and the other half was tested under the placebo dose first. Participants were given the high and low dose expectancy in order to assure the presence of expectancy effects in both sessions. To keep the participants as well as the experimenter unaware of the experimental conditions, a double blind procedure was used. Participants under the age of 18 received a vodka-orange pre-mixed spirit (Eristoff & Orange Can, commercial ready-to-drink alcoholic beverages with a 7 % Vol) and participants over the age of 18 received a mix of vodka and orange juice. The alcohol content and the total volume of the liquid delivered to the participants under and over the age of 18 were the same (.45 ml/kg with a maximum cut-off of 100 ml vodka in the alcohol condition). On average alcoholic beverages contained 28.54 grams of alcohol. This amount equals to two standard drinks in the USA (A standard drink contains 10 grams of alcohol in the Netherlands; 14 grams of alcohol in the USA). It is important to note that in the Netherlands, the legal age to consume pre-mixed drinks is 16 years of age or over. The procedure was identical in both sessions, except alcohol was replaced with orange juice in the placebo session. Beverages were divided equally into three portions. Two of the drinks were served with 5 minutes apart, prior to commencing the tasks, and after electrode placement. Up to 3 minutes were allowed for drinking followed by 2 minutes of mouthwash to remove the residual alcohol in the mouth. 1/3 of the mix was administered as a booster drink halfway through the session. To enhance the alcohol taste, all drinks had a lemon soaked in vodka in it and the glass in which drinks were served was sprayed with vodka beforehand (cf., Marlatt & Rohsenow, 1980). In order to mask the vodka taste, all drinks were mixed with three drops of tabasco sauce (McIlhenny Co., USA). The session started with a working memory task, followed by beverage administration. Following the beverage administration, subjects also performed an alcohol-approach avoidance task, a Dot probe task and a task switching paradigm. Order of the tasks was counterbalanced across subjects, but was kept same across sessions. After completion of both sessions, a short manipulation check interview was conducted to determine whether the participants were aware of the alcohol contents of the drinks. Five of the subjects reported that they received no alcohol in the placebo condition. Participants were debriefed about the true nature of the study and remained at the research site until their breath sample was 25mg/100ml or less.

Electroencephalogram (EEG) recording and data analysis – ERP quantification and subject/trial exclusion procedure

Three subjects' EEG data in one session were lost. A participant had braids and completed the study without EEG measurement. Trials in which the RTs were slower than 100 ms or faster than 1000 ms were excluded from the behavioural data and EEG epochs. Due to RT exclusion, on average approximately 10% and 12% of the Go trials and 6% and 10% of the no-go trials were excluded in the placebo and alcohol conditions, respectively.

The quantification of ERPs were based on the previous literature (Bartholow *et al*, 2012; Easdon *et al*, 2005; Nieuwenhuis *et al*, 2001; 2003). The N2 was defined as the difference between the most negative value within 200-380 ms time interval after stimulus presentation minus the immediately preceding positive peak. The ERN was defined as the most negative value within 0-150 ms following a commission response. Pe was defined as the most positive value within 150-350 ms following a commission response. Go- and NoGo-N2's were measured for trials with correct responses at fronto-central electrode (Fz). Response locked ERPs; the ERN and Pe; were measured at frontal and central electrodes (Fz and Cz), respectively. The minimum number of artefact-free trials for each subject and condition was kept at six for the analyses of ERN and Pe (Hajcak and Simons, 2008; Olvet and Hajcak, 2009). Due to a lack of adequate artefact-free trials, one subject's data were excluded from the response-locked No-go epochs and three subjects' data were excluded from the stimulus-locked No-go epochs. Following peak detection, averaged segments were visually inspected. Due to noise or due to a lack of signal from a channel, two and three subjects' data were excluded from the stimulus-locked Go and No-go epochs, respectively. From the response-locked ERN and Pe epochs, thirteen and eleven subjects' data were excluded. After the exclusion of artefacts and noisy data, in the placebo condition an average of 480.68, 105.69/72.52 (correct/commission), 73.57 and 72.52 trials remained for subsequent analysis for the Go-N2, NoGo-N2, ERN and Pe, respectively. In the alcohol condition the numbers of remaining trials were 466.01, 105.23/69.65, 70.85 and 69.65, respectively for the Go-N2, NoGo-N2 (correct/commission), ERN and Pe epochs.

RESULTS

Go-N2 and Baseline Alcohol Use

The Go-N2 was smaller for the *Alcohol Beverage Images* compared with the *Neutral Beverage Images* ($F(1, 79) = 4.83$, $p = .031$, $\eta^2_p = .06$) and it was smaller after alcohol administration ($F(1, 79) = 14.06$, $p < .001$, $\eta^2_p = .15$).

The relationships between the effect of acute alcohol on ERP measures and subjects' alcohol use at baseline were explored by correlating AUDIT scores with planned contrasts.

Contrast scores were calculated representing the difference between the placebo and alcohol conditions and between the *Neutral* and *Alcohol Beverage Images* for the ERP measures of interest (i.e. N2 Contrast for Alcohol Task Set = Alcohol minus Placebo for the Alcohol Beverage Images; N2 Contrast for Neutral Beverage Images = Alcohol minus Placebo for the Neutral Beverage Images).

A correlation between AUDIT scores and Beverage Image Class contrast for the Go-N2 was present in the placebo condition (r = .3, p = .007)[4], and this correlation was absent in the alcohol condition. The data suggests that in the placebo condition, subjects with high AUDIT scores had larger Go-N2 for the *Alcohol Beverage Images* and subjects with low AUDIT scores had larger Go-N2 for the *Neutral Beverage Images*. However the relationship between AUDIT scores and the Go-N2 contrast scores disappeared after acute alcohol administration.

Pe

The results revealed that the Pe was smaller in the alcohol condition than the placebo condition ($F(1, 70) = 10.019$, $p = .002$, $\eta^2_p = .125$).

Post-error slowing

In order to test the effect of acute alcohol on post-error adjustment, the mean reaction times for correct responses following errors and following correct trials were calculated and subjected to an RM-ANOVA with *Dose* (Placebo, Alcohol), *Beverage Image Class* (Neutral, Alcohol Beverage Images) and *Response Type* (post-error, post-correct) as within subjects factors. Overall, the response time in trials following an error were slower compared to response times in trials following a correct response ($F(1, 68) = 27.698$, $p < .001$, $\eta^2_p = .29$). Moreover the two-way interaction of effect *Dose* by *Response Type* was significant ($F(1, 68) = 6.7$, $p = .012$, $\eta^2_p = .09$). Pairwise comparisons of RTs revealed that this interaction term was present because response times for the post-error trials were higher in the alcohol condition compared to the placebo condition ($t(68) = -2.2$, $p = 0.03$), however the RTs for the post-correct trials did not reveal any differences across conditions ($p = .57$). An exploratory analysis was conducted to test possible distinct effects of alcohol on *Beverage Image Class*. This analysis revealed that post-error slowing increased after alcohol administration, only for N*eutral Beverage Images* ($t(68) = -2.6$, $p = 0.01$).

Table S1. Demographic information

Variable	
Age (mean, SD)	17.6 (1.27)
Sex (M/F)	30/57
Education Level †	
High school (Level1/Level2/Level3)	1/15/29
Tertiary Education (Level1/Level2/Level3)	12/14/16
Academic Achievement (CITO scores*)	
High school (Level1/Level2/Level3)	541/541.77/547.52
Tertiary Education (Level1/Level2/Level3)	531.5/543.08/546.42
Parents' SES †	
(Below/about/above average)	10/60/17
Favorite alcoholic drink †	
(Beer/wine/mix drink/strong drink/other)	38/17/17/7
AUDIT (mean, SD, range)	8.82, 4.82, 2-24
Drinking Behavior (last 90 days)	
Drinks per drinking day (Wkdy/wknd)	2 drinks/5 drinks
Alcohol use frequency (Wkdy/wknd)	2-3 times per month/once per wknd
Binge drinking (>5 drinks) (Yes/No, frequency) †	78/9, 5-7 times
Drinking Problems (last 90 days)	
RAPI (mean, SD)	3.94(5.08)
Smoking (Yes/No) †	32/55
Smoking Frequency †	
(Occasional/once or twice a day/regular/ex-smoker)	6/15/11/3
Drug Use (last 90 days) †	
Marijuana (Yes/No, frequency)	35/51, btw. 21-30 times
Ecstasy (Yes/No, frequency)	10/77, < 10 times
Hallucinogenic (Yes/No, frequency)	1/86, < 10 times
Stimulants or amphetamine (Yes/No, frequency)	6/86, < 10 times

High School Level1: VMBO, Level2: HAVO, Level3: VWO, Tertiary Education Level1: MBO, Level2: HBO, Level3: WO. † Units of measurement: total number of subjects. * General academic achievement was measured using the Dutch CITO scores. The CITO test is a national test of educational achievement used to determine high-school entrance level. CITO scores range from 501 to 550. 73 subjects CITO scores were available. The mean CITO score for the general Dutch sample is 535 (www.cito.nl). Wkdy: Weekdays; wknd: weekend.

Table S2. Demographic Information for Completers and Drop-Outs in the follow-up assessment.

Variable	Completers (n=82)	Drop-Outs (n=15)
Age (mean, SD)	17.67(1.24)	17.2(1.26)
Sex (M/F)	32/50	5/10
Current Education Level †		
High school (Level1/Level2/Level3)	1/14/29	1/4/2
Tertiary Education (Level1/Level2/Level3)	8/15/15	5/2/1
Academic Achievement (CITO scores*)		
High school (Level1/Level2/Level3)	541/541.5/547.34	-/542.67/550
Tertiary Education (Level1/Level2/Level3)	530.2/542.5/546.91	538/546/541
Parents' SES †		
(Below/about/above average)	10/55/17	3/9/13
Favorite alcoholic drink †		
(Beer/wine/mix drink/strong drink/other)	31/15/16/6	8/2/2/2
AUDIT (mean, SD)	8.48(4.83)	11.57(4.42)
Drinking Behavior (last 90 days)		
Drinks per drinking day (Wkdy/wknd)	2 drinks/5 drinks	1 drink/6 drinks
Alcohol use frequency (Wkdy/wknd)	2-3 times per month /once per wknd	2-3 times per month /once per wknd
Binge drinking (>5 drinks) (Yes/No†, frequency)	9/67, 7-9 times	1/14, 9-12 times
Drinking Problems (last 90 days)		
RAPI (mean, SD)	3.26 (4.1)	6.87 (7.86)
Smoking (Yes/No) †	25/57	11/4
Smoking Frequency †		
(Occasional/once or twice a day/regular/ex-smoker)	6/11/8/3	1/4/5/0
Drug Use (last 90 days) †		
Marijuana (Yes/No, frequency)	27/48, < 10 times	10/5, 11-20 times
Ecstasy (Yes/No, frequency)	7/68, < 10 times	3/12, < 10 times
Hallucinogenic (Yes/No, frequency)	1/74, < 10 times	0/15
Stimulants or amphetamine (Yes/No, frequency)	4/71, < 10 times	2/13, < 10 times

High School Level1: VMBO, Level2: HAVO, Level3: VWO, Tertiary Education Level1: MBO, Level2: HBO, Level3: WO. † Units of measurement: total number of subjects. * 73 subjects CITO scores were available. The mean CITO score for the general Dutch sample is 535 (www.cito.nl). Wkdy: Weekdays; wknd: weekend.

Table S3. Accuracies and mean reaction times as a function of Beverage Image Class in the placebo and in the alcohol condition.

		Beverage Image Class [Mean(SD)]	
		Neutral Beverage Image	**Alcohol Beverage Image**
Hit Rates	**Placebo**	.95(.05) ***	.94(06)
	Alcohol	.92(06)	.93(05) *
False Alarm Rates	**Placebo**	.43(.13)	.47(.13) ****
	Alcohol	.45(.11)	.49(.11)
RT CorrectGo	**Placebo**	279.29(45.26)	281.43(45.22)
	Alcohol	290.20(49.56)	289.76(47.83)
RT Commission	**Placebo**	352.57(112.95) **	355.71(115.93)
	Alcohol	391.86(136.35)	377.93(120.29)

Table S4. Correlations between ERP indices and drinking behaviour. (n= 59).

	1	2	3	4	5	6	7
1.N2 Contrast Neu. Bev. Ima.							
2.N2 Contrast Alc. Bev. Ima.	.349**						
3.ERN Contrast Neu. Bev. Ima.	-.233	-.081					
4.ERN Contrast Alc. Bev. Ima.	.113	-.07	.216				
5.AUDIT lifetime	.003	-.14	.014	.092			
6.AUDIT lifetime (recent use)	.068	-.197	.024	.046	.773**		
7.AUDIT (last 90 days)	.016	.086	.09	.2	.67**	.346**	
8.AUDIT (last 90 days, recent use)	.05	.121	.041	.27*	.503**	.375**	.855**

Neu. Bev. Ima: Neutral beverage images, N2 Contrast: N2 in the alcohol dose minus in the placebo dose, For 6 and 8, the total score of items on recent alcohol use (AUDIT Items1-3) are reported, *< .05, ** < .01

Table S5. Correlations between ERP indices and measures of subjective response to alcohol. (n= 59).

	1	2	3	4
1.N2 Contrast Neu. Bev. Ima.				
2.N2 Contrast Alc. Bev. Ima.				
3.ERN Contrast Neu. Bev. Ima.				
4.ERN Contrast Alc. Bev. Ima.				
5.BAES at peak BAL - Stimulation	-.188	-.2	.11	.074
6.BAES at peak BAL - Sedation	.031	-.027	.036	.121
7.SRE	.15	.127	-.085	.115

Note: 5 and 6, BAES scores at the time of peak intoxication (BAL), time of peak = T4 (See Figure S1) (Schuckit *et al*, 1997). Neu. Bev. Ima: Neutral beverage images, N2 Contrast: N2 in the alcohol dose minus in the placebo dose, *< .05, ** < .01

Table S6. Correlations between AUDIT scores and measures of subjective response to alcohol (n=59)

	1	2	3	4	5	6	7	8
1.SRE								
2.AUDIT lifetime	.421**							
3.AUDIT lifetime, recent use	.447**	.773**						
4.AUDIT, 90 days	.189	.67**	.346**					
5.AUDIT, 90 days, recent use	.226	.503**	.375**	.855**				
6.AUDIT, 90 days, T2	.355**	.672**	.363**	.711**	.631**			
					.32* [a]			
7.AUDIT, 90 days, recent use, T2	.344**	.543**	.37**	.692**	.741**	.871**		
						.269* [b]		
8.BAES at peak BAL - stimulation	-.046	.032	.086	.06	.091	-.13	.038	
9.BAES at peak BAL - sedation	-.078	-.01	-.091	.01	-.054	-.017	-.068	.155

[a] partial correlation, corrected for variable 4
[b] partial correlation, corrected for variable 5.

Figure S1. Mean scores and standard errors for the blood alcohol levels (BAL, the line graph), for the stimulation and sedation subscales of the Brief Biphasic Alcohol Effects Scale in the placebo and alcohol dose conditions (B-BAES, bar graph) and the timeline of events during experimental session (bottom part). B-BAES = brief biphasic alcohol effects scale; BAL = blood alcohol levels; Stim = stimulation subscale; Sed = sedation subscale; Pla = placebo dose condition; Alc = alcohol dose condition (n=87).

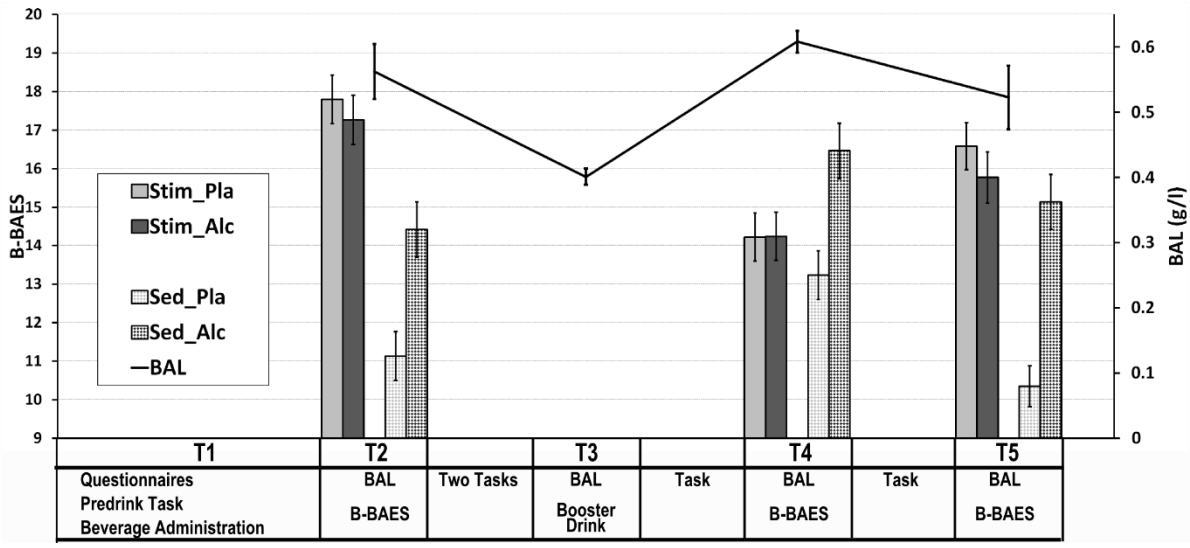
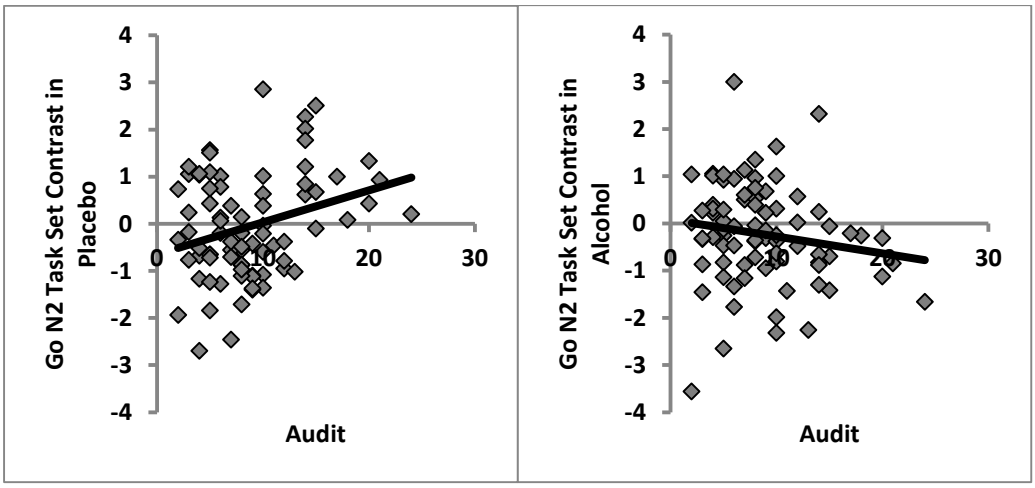


Figure S2. Scatterplots depicting associations between the Audit scores in baseline and the contrast scores for the Go-N2.



CHAPTER 5

Neural response to alcohol taste cues in youth with high alcohol sensitivity: effects of the *OPRM1* gene

This chapter is in preparation as:

Korucuoglu O, Gladwin TE, Baas F, Mocking RJT, Ruhe HG, Groot PFC, Wiers RW. Neural response to alcohol taste cues in youth with high alcohol sensitivity: effects of the *OPRM1* gene.

ABSTRACT

Genetic variations in the mu-opioid receptor (*OPRM1*) gene have been related to high sensitivity to rewarding effects of alcohol. The current study focuses on the neural circuitry underlying this phenomenon using an alcohol vs. water taste-cue reactivity paradigm in a sample with limited exposure to alcohol, thus avoiding the confound of variations in duration of alcohol use. Drinkers (17-21 years-old) were selected on genotype carrying the AA- (n=20) or the AG- (n=16) variant of the A118G single nucleotide polymorphism (SNP) of the *OPRM1* gene (rs1799971), and underwent functional magnetic resonance imaging (fMRI). Magnitude of the neural activity and frontostriatal functional connectivity in response to alcohol vs. water were investigated. The AG-group demonstrated reduced activation in prefrontal and parietal regions, including the inferior and middle frontal gyrus, superior and inferior parietal lobule, compared with the AA-group. No activation differences were observed in the mesolimbic pathway. Connectivity from the ventral-striatum to frontal regions for alcohol vs. water trials was higher in the AG than the AA group. For the dorsal-striatum seed region, the AG group showed increased connectivity to non-PFC regions. These results indicate that adolescents carrying the G-allele may be more vulnerable for the alcohol to hijack the reward system in the absence of frontal control to regulate craving. This implies that findings of hyperactivation in the mesolimbic structures of G-allele carriers in earlier studies might result from both genetic susceptibility and heavy drinking.

INTRODUCTION

Incentive sensitization towards drugs and drug-related stimuli develop during the development of dependence due to neuroadaptations in the mesolimbic dopaminergic system controlling the incentive values assigned to drug stimuli (Berridge & Robinson, 2003; Berridge *et al.*, 2009). At early stages, drug use is goal-directed, and drug-taking behaviour is promoted by the hedonic properties of drugs (associated with `liking`) in order to obtain pleasurable outcomes. In susceptible individuals, long-term drug use can produce changes in the brain, leading to incentive salience ('wanting'). In animal studies, it has been shown that individual differences in the tendency to attribute incentive salience to drug-related stimuli is associated with vulnerability for the transition to compulsive drug seeking behaviour (Flagel *et al.*, 2009). In humans, genetic variants which play a role in the brain reward circuitry have been proposed as one factor contributing to the extent of incentive salience attribution (Blum *et al.*, 2011).

A single-nucleotide polymorphism (SNP) located in the *OPRM1* gene of the mu opioid receptor (A118G) has been found to contribute to individual differences in sensitivity to the rewarding effects of alcohol. The A118G SNP results in an amino acid shift from asparagine to aspartate and is thought to increase receptor binding affinity for β-endorphin by 3-fold (Bond *et al.*, 1998). Interestingly, mu opioid receptors are expressed heavily in the ventral tegmental area (VTA), nucleus accumbens (NAc), thalamus, and with less consistency in the amygdala (Merrer, 2009). Moreover, alcohol consumption induces opioid release (primarily β-endorphin) binding to the mu opioid receptors and leads to heightened dopamine levels in brain reward circuitry (Merrer, 2009). Thus carriers of the G-variant with higher binding affinity experience higher reinforcement from acute administration of alcohol. In experimental studies, heavy drinkers with a G-allele of the *OPRM1* gene demonstrated relatively strong automatic approach action-tendencies (Wiers *et al.*, 2009), attentional bias towards alcohol-related stimuli (Pieters *et al.*, 2011), alcohol craving (van den Wildenberg *et al.*, 2007) and stronger subjective feelings of intoxication, stimulation, sedation after alcohol as compared with participants homozygous for the A-allele (Ray & Hutchison, 2004). Imaging studies have revealed that G-allele carriers demonstrated a relatively potent striatal dopamine response to alcohol (Ramchandani *et al.*, 2011) and showed relatively strong neural activity in the mesocorticolimbic pathway (i.e., ventral striatum, ventromedial prefrontal cortex, and orbitofrontal cortex) to alcohol taste cues before and after alcohol priming (Filbey *et al.*, 2008*b*). Furthermore, activations in these regions were correlated with state measures of alcohol craving and with measures of drinking behaviour and problems. Although in adults the OPRM1 g-allele has not been robustly associated with risk for alcoholism (Van der Zwaluw et al., 2009), a recent study associated the *OPRM1* gene with increased risk for alcoholism in adolescents (Miranda *et al.*, 2010). Moreover, G-allele carrying adolescents reported higher levels of enhancing positive affect compared to A carriers

(Miranda *et al.*, 2010). Therefore, these studies suggest that this A118G polymorphism may be associated with increased sensitivity towards the rewarding effects of alcohol, which in return is consistent with the role of the opioidergic system in the hedonic properties of alcohol as well as natural rewards (Robinson & Berridge, 1993).

While incentive sensitization to alcohol-related cues strengthens due to acute rewarding properties of drugs on mesolimbic structures, control over drug use could also fail as a result of a weak frontal regulatory mechanism, either pre-existing prior to drug use and/or as a consequence of chronic use (Gladwin *et al.*, 2011; Volkow *et al.*, 2004; Wiers *et al.*, 2007). Current literature suggests the involvement of two interacting systems (limbic and frontal) in addiction and craving. Therefore, the role of dysregulation of frontostriatal circuitry in sustained drug-seeking behaviour is a topic of interest (e.g., Feil *et al.*, 2010). An additional mechanism involves the shift from ventral to dorsal striatal (VS/DS) activation to drug cues, which co-occurs with increasing habitual responses to alcohol (Everitt *et al.*, 2008). For instance, in an alcohol dependent sample, disrupted frontostriatal connectivity predicted maladaptive drug-related behaviours and impairments in learning (Park *et al.*, 2010). Another study showed higher frontal activation in light versus heavy drinkers after cue exposure, which was associated with better cortical control over alcohol-related cues (Vollstädt-Klein *et al.*, 2010). Lastly, individuals with substance use disorder showed lower connectivity between VS to frontal regions, but comparable connectivity between DS (eg. caudate) and cortex (Motzkin *et al.*, 2014). These studies show that frontal regulation of striatal activation towards rewarding effects of drugs and alcohol could play an important role in addiction.

In adult samples (dependent or non-dependent), alcohol use history (duration) and patterns (frequency, dose) typically covary strongly, potentially confounding the results and making it difficult to distinguish preexisting neural predispositions from neural dysregulations induced by chronic use (Fernandez-Serrano *et al.*, 2011). Therefore, a benefit of studying young people is that it enables the study of responses towards alcohol cues at early stages of alcohol use without the confound of duration of use. Yet most of the cue reactivity studies looking at the *OPRM1* gene have been conducted in adult samples or heavy-drinking/dependent adolescents with a substantial amount of drinking history. For instance, Filbey and colleagues (2008b) focused on adult samples (mean age ~23) with a score of ~12 on self-report questionnaire of alcohol use disorder (AUDIT, Alcohol Use Disorder Identification Test). Studies conducted by Courtney and Ray (2014) and Ray *et al.* (2014) focused on cue reactivity in adult samples (ages 21-51) who met DSM criteria for an Alcohol Use Disorder (AUD). The heavy drinking young men in Wiers *et al.* (2009) and van den Wildenberg *et al.* (2007) studies had a mean AUDIT score of 14. Unfortunately neither of these studies reported the age of onset of alcohol use. To our knowledge, studies in younger samples without extensive drinking histories are largely lacking (for an example in adolescents with alcohol use disorder, see Tapert

et al. 2003), while they are essential to answer whether heightened cue-reactivity in clinical samples with genetic vulnerability is already present in a sample without long-term neuroadaptations as a result from chronic use. Only one study showed that adolescents with heightened neural response to alcohol pictures transitioned to heavy drinking (Dager *et al*. 2014). Therefore, the current study specifically targeted a younger sample while comparing two groups with different genetic vulnerability for the acute reinforcing effects of alcohol at *early* stages of alcohol use. We focus on the neural circuitry during an alcohol cue-taste reactivity paradigm; a phenomenon established to measure neural reactions provoked by cues with reinforcing properties.

To overcome this knowledge gap, we here studied the neural circuitry involved in the processing of alcohol-taste-cues in a young sample with limited exposure to alcohol and alcohol-related cues. We expected that G-allele carriers would be more sensitive to alcohol taste-cues than non-carriers. We studies both activation and connectivity measures. First, we studied regional activations in the reward circuitry, expecting increased responses in G-allele carriers. Second, we studied frontostriatal functional connectivity in processing alcohol-related cues in both groups. Functional connectivity analysis focused on *a priori* selected seed regions of NAc and dorsal caudate (VS/DS). We expected that the mu-opioid system would be uniquely involved in the brain circuitry associated with hedonic responses to drugs (NAc), thus hypothesizing that G-alleles would show an increased ventral-to-frontal connectivity.

MATERIALS AND METHODS

Participants

Thirty-six participants were selected from a larger group of adolescents (n = 145), who participated in a study in which they were genotyped. In the larger sample, only one participant was GG carrier and not included in this study. Groups were created in such a way to have AA and AG groups well-matched on demographics and drinking patterns so that the observed differences could be attributed to genetic variance alone. Due to technical problems with liquid administration, cue-reactivity task failed with 5 participants, these participants were replaced based on their demographics from the same pool. In the final sample, 20 participants were homozygous for the A-allele of the A118G SNP of the *OPRM1* gene (rs1799971), while 16 participants had the AG genotype. At the time of the fMRI study, our participants had 3-4 years of experience with alcohol, were in secondary education, scored an average of 7.5 on the AUDIT and had fairly stable drinking pattern for the last two years (see Table1), therefore they could be considered as being at an early stage of alcohol use. Participants were instructed to abstain from any alcohol for at least 24 hr and any legal or illegal drugs for at least 1 week (for exclusion criteria, see supplementary materials). The study was approved by the Ethics

Committee of the Faculty of Social and Behavioral Sciences of the University of Amsterdam. For participants under the age of 18, parental consent was mandatory to take part in the study. A written informed consent was obtained from all participants prior to the experiment. Participants received financial compensation (€35) for their participation.

Genotyping

Saliva samples were collected using Oragene saliva collection kit (DNA Genotek, Inc., Ottawa, Ontario, Canada) for DNA analysis. Genotyping was performed at the Academic Medical Center, the Netherlands. Genotyping was performed with a Taqman assay (Life Technologies) on a LC480 lightcycler (Roche) at the Genetics core facility of the Academic Medical Center, the Netherlands. Sanger sequencing of 5 samples with the different genotypes was perform to confirm the genotypes of the Taqman assays. Duplicate genotyping was done for five samples as a quality control, which showed 100% consistency. The allele frequencies did not violate Hardy-Weinberg Equilibrium ($X^2(1)_{HW}$= .233, P = .63).

Procedure

Upon arrival, participants filled out questionnaires (see supplementary information). Participants first completed a behavioural testing session, where they completed an Electromyogram (EMG) measurement and performed two unrelated tasks, followed by an fMRI session. A minority of participants performed (part/all of -6/2 participants-) their behavioural session last due to scheduling related problems. In the scanner participants performed two tasks, of which the second one was the cue-reactivity task. Before and after the scanning session, participants rated the pleasantness of the tastes (alcohol and water) on a 10-point scale.

Cue Reactivity Task with Tastes

A blocked-design taste-cue paradigm was adapted from Filbey and colleagues (Filbey *et al.*, 2008a; 2008*b*). The task consisted of 16 mini blocks during which either an alcohol-containing beverage or a control taste was delivered (8 alcohol and 8 control blocks). Each block comprised of two taste-delivery periods of 10 sec, in which 1 ml liquid was administered, each followed by a swallowing period of 2 sec. During the taste and swallowing periods, participants were presented with visual instructions of “Taste” and “Swallow” (See Fig. 1). Vodka-apple pre-mixed spirit (Smirnoff, commercial ready-to-drink alcohol beverage with a 6.4 % Vol) was used as alcoholic taste and distilled water was used as control taste. Taste stimuli were delivered via a plastic tube attached to an electronic syringe pump positioned in the scanner control room, using a computer-controlled delivery system running under E-prime2 (Psychology Software Tools, Inc., Sharpsburg, PA). Each taste was equally presented across blocks and randomized

with the restriction that two consecutive blocks would be of the same type. The block was completed with a rest period of 16 sec followed by taste ratings for *pleasantness* and *urge* in that order (with a maximum duration of 5 sec). Participants rated the tastes on a 1-10 Likert scale via an MRI compatible optic response device (fORP) with a four-button paddle. The start of the next block was informed via a "Ready?" warning on the screen (2 sec).

Figure 1. Schematic representation of the alcohol-taste cue reactivity task.

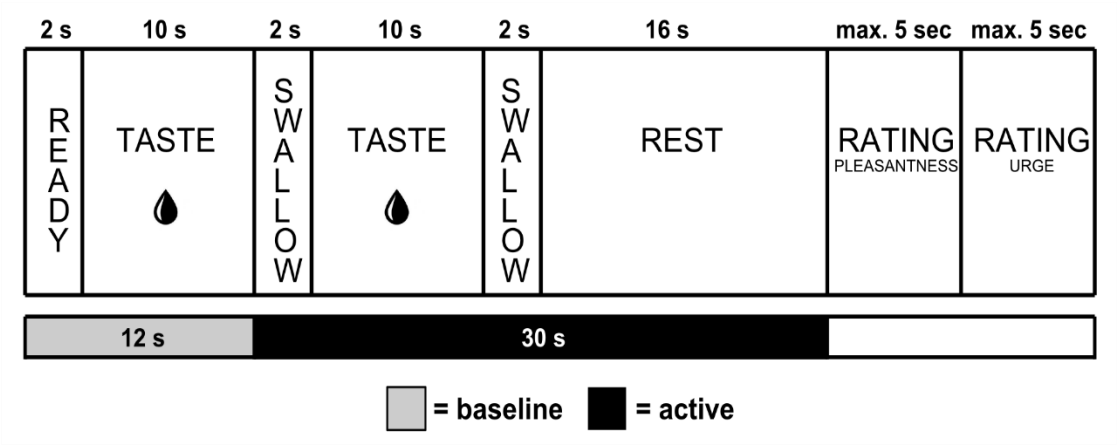


Image Acquisition

Functional and anatomical images were acquired on a Philips 3 Tesla Achieva TX MRI scanner with a 32-channel SENSE head coil, at the Spinoza Center, Amsterdam, the Netherlands. A structural T1-weighted echo planar image was acquired with the following parameters: voxel size of 1 × 1 × 1 mm, FOV = 240 × 188, TR = 8.17ms, TE = 3.8 ms, flip angle = 8°, slice thickness = 1mm, 0 mm gap, matrix = 240 × 240, 220 slices per volume, with a total scan duration of ~6 min. Functional T2*-weighted images were acquired with a single-shot gradient echo EPI sequence. The following parameters were used for the functional scans: FOV = 240 × 240, voxel size of 3 × 3 × 3 mm, 420 volumes, TR= 2000 ms, TE = 27.63 ms, matrix size = 80 × 80, flip angle of 76.1°, 37 slices per volume, slice gap 0.3 mm, slice thickness = 3 mm, sensitivity encoding factor of 2. Stimuli were projected on a projection screen, which the participants viewed through a tilted mirror attached to the head coil.

Image Processing and Statistical Analyses

MRI data were analyzed using statistical parametric mapping (SPM8, Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 7.11. Preprocessing steps included motion correction using rigid body transformations, coregistration to the anatomical scans, spatial normalization to a T1 template based on Montreal Neurological Institute (MNI)

stereotaxic space, spatial smoothing (8 mm full width – half maximum) and high pass filtering with a cutoff period of 128 s.

For the first-level analysis, hemodynamic response function was convolved with the time course of the blocked design. Realignment parameters were used as model regressors. The analysis focused on the contrast of Alcohol versus Water taste delivery, depicted as active period in Fig.1. Specifics of the fMRI analysis (the events modelled, the contrast selected etc.) were based on previous studies (Filbey *et al.*, 2008a; Ray *et al*., 2014). Fixation, first taste delivery, urge and pleasantness rating periods were not modelled (Filbey *et al*. 2008a). To verify main effects of the task, a whole brain analysis was conducted for all participants with a threshold of $p = .05$ (FWE), 10 voxels. Given that the influence of a single SNP on brain responses is usually modest, the statistical threshold for group comparison contrasts were set to $p < 0.005$, with a minimum cluster size of 20. This threshold produces a desirable balance between Type-I and Type-II errors (Lieberman & Cunningham, 2009).

Functional connectivity was assessed using psychophysiological interactions (PPI) analysis (Friston *et al.*, 1997). The aim of a PPI analysis is to detect regions whose activity is coupled with the activity of a seed region over the time course of the alcohol taste blocks, but not during the water blocks. The regions of interest for the PPI analysis were based on previous research (Ray *et al.*, 2014) and included the following regions: a) the right NAc and b) the right dorsal caudate, to investigate connectivity between the ventral/dorsal striatum (VS/DS) and the PFC. A mask image for the right NAc and caudate were acquired from the IBASPM 71 anatomical atlas toolbox (Alemán-Gómez *et al.*, 2006). The tail of the caudate mask (ventral part) was excluded using an in-house package programmed in Matlab (for masks, see Fig. S1, supplementary materials). The mean deconvolved time courses in these seed regions were extracted from the preprocessed individual images. Regressors were created by multiplying extracted time courses of ROIs with condition specific regressors. The PPI analysis was conducted for each individual separately and then entered into a random-effects analysis using a one sample t-test. Between-group analysis was conducted using a two-sample t-test with the thresholds described above. Anatomical labelling was based on the AAL atlas (Tzourio-Mazoyer *et al.*, 2002) with the SPM probabilistic toolbox (Eickhoff *et al.* (2005) and the Hiro software (Gladwin & Vink, 2008). When the effect of task condition on the activity of the seed region increases, increases and decreases in activity in the other regions represent the positive and negative connectivity, respectively.

RESULTS

Participant Characteristics

Allele groups were not different in any of the demographical or substance use characteristics ($p>.1$, see Table1).

Table1. Demographic information, drug and alcohol use, and urge-pleasantness ratings for the AA and AG groups of the OPRM1 genotype.

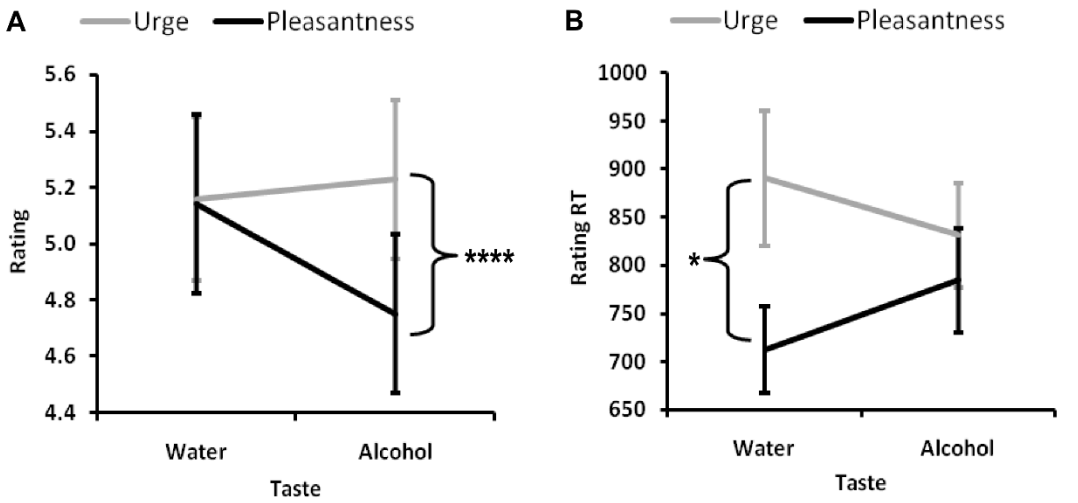
Variable	AA (n=20)	AG (n=16)	AA vs AG
Age (mean, SD)	19.2(1.82)	18.81(1.72)	ns.
Sex (M/F)	10/10	13/3	-
Ethnicity (Caucasian/other)	20/0	13/3	-
DAQ (mean, SD)	36.5(11.58)	38.38(8.46)	ns.
PANAS – Positive affect(mean, SD)	28.2(7.35)	26.7(5.02)	ns.
PANAS – Negative affect(mean, SD)	12.5(2.21)	13.31(2.24)	ns.
AUDIT T1(last 90 days) (mean, SD)* (n=18,14)	7.06(4.24)	7.5 (5.52)	ns.
AUDIT T2(last 90 days) (mean, SD)* (n=19,14)	7.37(4.98)	6.93 (4.43)	ns.
AUDIT T3(last 90 days) (mean, SD)* (n=19,14)	7.42(4.34)	6.62 (4.01)	ns.
AUDIT T4(last 90 days)– fMRI session(mean, SD)* (n=20,16)	7.65(4.85)	7.56(4.11)	ns.
Age of first drink (mean, SD)	15.1(1.62)	14.94(1.34)	ns.
Smoking? (Yes/No, frequency)	9/11, 11-20 times	9/7, 21-30 times	-
Drug Use (last 90 days)			-
Marijuana (Yes/No, frequency)	6/14, < 10 times	9/7, 11-20 times	
Ecstasy (Yes/No, frequency)	2/18, < 10 times	4/12, < 10 times	
Volatile Substances (Yes/No, frequency)	2/18, < 10 times	0/16	
Real Time Urge and Pleasantness Ratings			
Alcohol Taste Pleasantness	4.58(1.8)	4.97(1.55)	ns.
Water Taste Pleasantness	5.34(1.92)	4.9(1.94)	ns.
Alcohol Taste Urge	5(1.68)	5.5(1.69)	ns.
Water Taste Urge	5.36(1.78)	4.91(1.7)	ns.

***** In this study participants were selected from a pool of subjects (n = 145), who took part in a larger study in which they were genotyped (Time 1, T1). Participants filled out AUDIT questionnaire once again, 3 and 6-months after the inclusion to the study (T2 and T3, respectively). The fMRI session (T4) took place approximately 1 to 2 years after T1. SD: standard error; M: male; F: female; DAQ: Desire for alcohol Questionnaire; PANAS: Positive and Negative Affect Scale; AUDIT: Alcohol Use Disorder Identification Test.

Urge and Pleasantness Rating During Scanning

Real time urge and pleasantness ratings and response times are shown in Fig. 2. Urge and pleasantness rating scores and reaction times during fMRI scanning were subjected to a repeated-measures ANOVA (RM-ANOVA) with *Scale* (pleasantness, urge) and *Taste* (alcohol, water) as within-subjects factors and *Group* (AA, AG) as between-subjects factor. No main or interaction effects were observed for Group. Analysis revealed a significant main effect of *Scale* ($F(1, 34) = 14.432$, $p = .001$, $\eta^2_p = .3$) and an interaction effect of *Scale* by *Taste* ($F(1, 34) = 8.544$, $p = .006$, $\eta^2_p = .2$). In post-hoc tests, for the alcohol taste, urge rating was higher than pleasantness rating ($F(1, 34) = 4.076$, $p < .001$, $\eta^2_p = .37$). However post-hoc analysis revealed that neither the pleasantness rating for alcohol and water nor the urge ratings for alcohol and water significantly differed from each other. RT data revealed a main effect of Scale ($F(1, 34) = 4.92$, $p < .033$, $\eta^2_p = .58$), post-hoc analysis revealed that only for water, participants were slower to rate urge than pleasantness ($F(1, 34) = 5.7$, $p < .023$, $\eta^2_p = .64$).

Figure 2. Mean scores for the real-time pleasantness and urge ratings (A) and reaction times (RTs) (B) for the alcohol and control tastes. Behavioural results for ratings revealed that for alcohol taste urge rating was higher than the pleasantness rating (A) and subjects were slower to rate water urge than water pleasantness (B); * p < .05, ** p < .01, *** p < .005, **** p < .001.



Pleasantness Rating Pre- and Post-scanning

Pleasantness ratings before/after the scanning session were analyzed with a RM-ANOVA, with *Time* (pre- and post-scanning) and *Taste* (alcohol, water) as within-subject variables and *Group* (AA, AG) as between-subject variable. No group differences were observed. Overall, participants liked alcohol more than water ($F(1, 34) = 5.733$, $p = .022$, $\eta^2_p = .14$). An interaction effect of *Time* by *Taste* was observed ($F(1, 34) = 5.49$, $p = .025$, $\eta^2_p = .14$). This two-way interaction was inspected by separately examining the effect of *Time* on each *Taste*. Results revealed that compared to pre-scanning, participants rated alcohol less pleasant during post-scanning ($F(1, 34) = 5.31$, $p = .027$, $\eta^2_p = .14$). Pre- and post-scanning ratings for water were the same. Lastly, during pre-scans, participants rated alcohol as more pleasurable than water ($F(1, 34) = 12.41$, $p = .001$, $\eta^2_p = .27$).

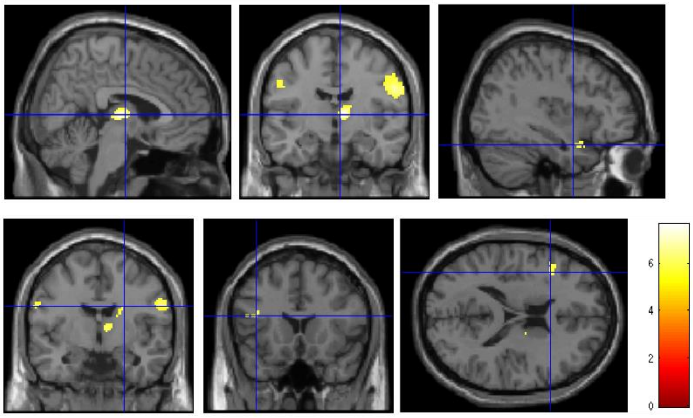
Whole-Brain Analysis Alcohol vs. Water Contrast

The whole brain analysis in the full sample revealed activation in several regions over the frontal, parietal, temporal and limbic regions. The alcohol-taste cues elicited activation in the thalamus, inferior frontal gyrus, superior temporal gyrus and a region close to caudate (See Fig. 3a and Table 2). No deactivations were observed. Analysis across genotypes revealed that AA-carriers showed higher activation over the frontal and parietal areas; including middle and inferior frontal gyrus, angular gyrus, superior and inferior parietal gyrus; compared to G-allele

carriers (See Fig. 3b and Table 3). The G-allele carriers revealed higher activation in the hippocampus.

Figure 3. A) Significant areas of activation for the Alcohol > Control Taste contrast (FWE, $p < .05$, $k \geq 10$ voxels); top-row left to right; thalamus (sagittal and coronal view), and temporal pole; bottom-row left to right; caudate and inferior frontal gyrus (coronal and transverse). B) Regions showing greater activation for the G allele carriers of the OPRM1 genotype compared to AA carriers ($p < .005$, uncorrected, $k \geq 20$ voxels); top-row; middle frontal gyrus (transverse, sagittal and coronal view); bottom-row; inferior frontal gyrus (transverse, sagittal and coronal view).

A



B

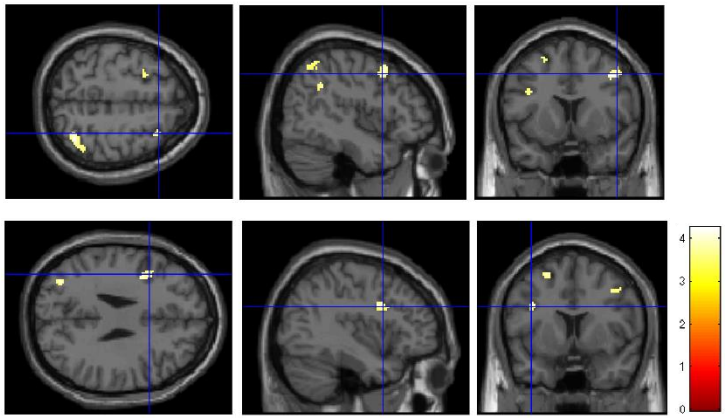


Table 2: Significant areas of activation for the Alcohol > Control Taste contrast (Whole-brain analysis, FWE, $p < .05$, $k \geq 10$ voxels).

Region	Hemisphere	Cluster size (in voxels)	Peak Value	MNI coordinates x,y,z
Thalamus	R	298	7.63	8, -10, 6*
			6.64	14, -20, 4*
Superior temporal gyrus	L	60	6.46	-48, -26, 8*
Inferior frontal gyrus	L	26	6.13	-40, 18, 18*
Caudate	R	16	6.08	22, -4, 24^
Temporal pole	R	23	5.99	38, 12, -22*
Postcentral gyrus	R	407	6.75	60, -8, 30*
			6.45	66, -14, 34*
			6.22	48, -16, 38*
	L	85	6.07	-44, -16, 34*
			5.9	-52, -12, 36*
			5.53	-44, -20, 42*
	L	17	5.84	-58, -4, 28*

L = left; R = right; MNI: Montreal Neurological Institute. Anatomical labelling was based on the AAL atlas (Tzourio-Mazoyer *et al.*, 2002) with the SPM probabilistic toolbox* (Eickhoff *et al.* (2005) and the Hiro software^ (Gladwin & Vink, 2008).

Table 3: Whole-Brain Group Comparison by *OPRM1* polymorphism genotype ($p < .005$, uncorrected, $k \geq 20$ voxels).

Region	Hemisphere	Cluster size (in voxels)	Peak Value	MNI coordinates x,y,z
AG vs AA				
Hippocampus/Heschl	R	36	3.08	28 -40 16^*
AA vs AG				
Middle occipital gyrus	L	67	3.49	-32, -74, 32*
Middle frontal gyrus	R	132	3.46	44, 10, 44*
	L	35	3.32	-24, 8, 60*
	R	35	3.26	30, 20, 56*
Superior parietal lobule	R	260	3.47	36, -60, 56*
Inferior parietal lobule	R		3.04	44, -54, 52*
Angular gyrus	R		3.46	38, -64, 48*
Angular gyrus	R	23	3.14	46, -48, 32*
Precentral gyrus	L	45	3.35	-38, 4, 30*
Inferior frontal gyrus	L		2.99	-40, 12, 28*

L = left, R = right; MNI: Montreal Neurological Institute. Anatomical labelling was based on the AAL atlas (Tzourio-Mazoyer *et al.*, 2002) with the SPM probabilistic toolbox* (Eickhoff *et al.* (2005) and the Hiro software^ (Gladwin & Vink, 2008).

Functional Connectivity

For the VS seed region, relative to the AA group G-allele carriers exhibited stronger alcohol-taste cue related connectivity with middle and superior frontal gyrus, parahippocampal, and motor cortex, as well as with voxels in or near the caudate and insula (See Fig.4a and Table 4). For the DS seed region, the G-allele carriers revealed stronger connectivity with hippocampal, thalamic, precuneus and occipital regions, however, no connectivity was observed with frontal regions (See Fig.4b and Table 4). There was no significant increased connectivity across the brain in the AA group vs. the AG group.

Figure 4. Regions showing greater positive functional connectivity for the AG vs AA carriers of the OPRM1 polymorphism with seed regions (A) the ventral striatum (NAc) (top-row: superior frontal gyrus, caudate, and insula; bottom-row: middle frontal gyrus) and (B) the dorsal striatum (caudate); (top row: middle cingulate, precuneus; bottom-row: thalamus/hippocampus) ($p < .005$, uncorrected, $k \geq 20$ voxels).

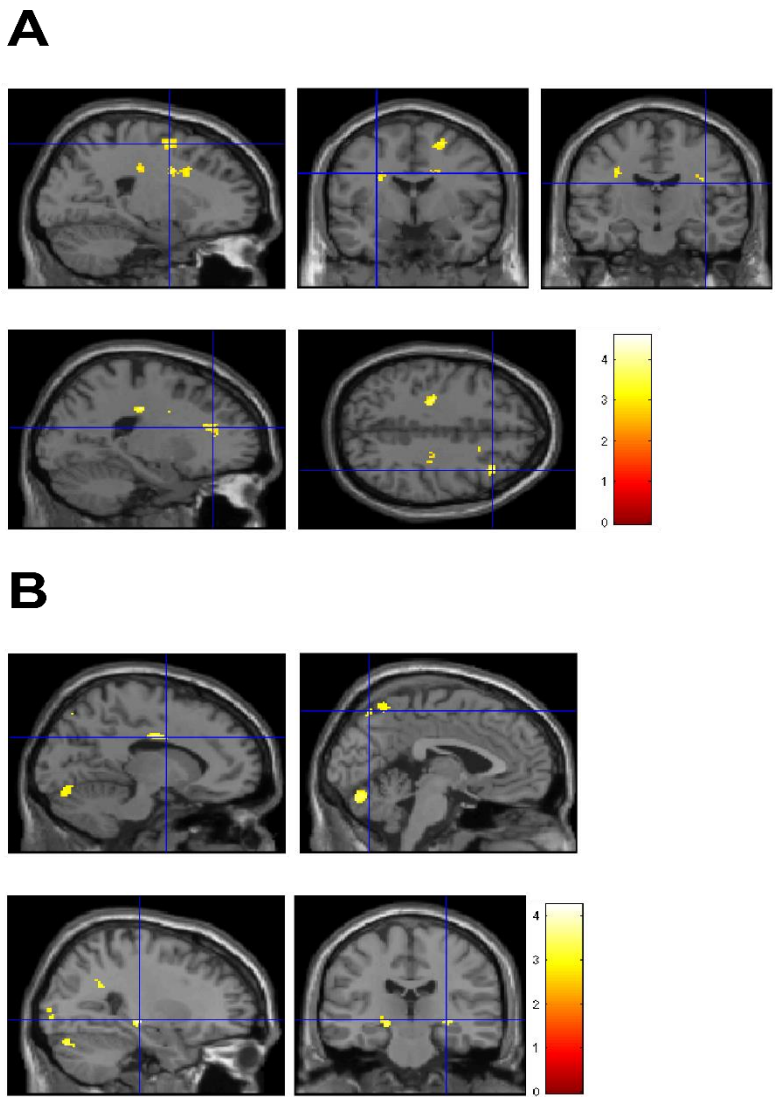


Table 4: Regions showing greater positive functional connectivity with the ventral and dorsal striatum for the AG vs AA carriers of the *OPRM1* polymorphism genotype ($p < .005$, uncorrected, $k \geq 20$ voxels).

Region	Hemisphere	Cluster size (in voxels)	Peak Value	MNI coordinates x,y,z
Ventral Striatum seed region (NAc)				
Precentral	L	166	4.59	-28, -20, 34^
			3.5	-32, -12, 34^
	R	128	3.8	30, -22, 34^
Precentral/Insula	R		3.22	34, -14, 28^
Postcentral	R	21	3.29	46, -34, 64*
	L	25	3.14	-40, -42, 66*
Middle frontal gyrus(BA9)	R	44	4.1	38, 26, 40*
	L	78	3.6	-20, 30, 22^
			3.24	-20, 38, 14^
			2.82	-28, 38, 20^
Superior frontal gyrus	R	77	3.54	22, 0, 58*
Mid cingulum	R	133	3.51	20, 12, 32^
			3.46	22, 4, 36^
			2.94	18, 16, 40^
Caudate	L	28	3.48	-24, 0, 26^
Parahippocampal	L	25	3.44	-34, -46, -2^
Dorsal Striatum seed region (Dorsal Caudate)				
Hippocampus	R	39	4.26	28, -24, -6^
Hippocampus/Thalamus	R		2.98	22, -24, 0*
Hippocampus/Thalamus	L	51	3.49	-18, -24, -6*
Cerebellum	R	972	3.97	34, -78, -26*
			3.85	22, -80, -22*
			3.69	12, -82, -16*
Inferior orbital gyrus	R	36	3.73	38, 20, -20^
Mid cingulate cortex	L	79	3. 69	-12, -12, 34^
	L		2.92	-18, -16, 38^
	L		2.75	-8, -2, 30^
	R	29	3.47	18, -4, 36^
Inferior frontal tri	R	30	3.65	38, 22, 20^
Sup parietal lobule	R	201	3.56	24, -72, 54*
Precuneus	R		3.37	14, -74, 48*
	L		3.14	-2, -72, 56*
	R	163	3.42	4, -48, 64*
	R		3.1	2, -58, 60*
	R	25	3.14	28, -52, 26^
Angular gyrus/Precuneus	R		2.95	38, -58, 26*^
Inferior occipital gyrus	L	74	3.29	-44, -76, -10*
	L		3.16	-42, -84, -8*
Fusiform Gyrus	L		2.89	-42, -64, -16*
Fusiform gyrus	L	29	3.15	-42, -44, -22*
Middle occipital gyrus	R	29	3.21	34, -88, 12*
	R		2.99	40, -84, 8*

L = left, R = right, NAc: Nucleus Accumbens; MNI: Montreal Neurological Institute. Anatomical labelling was based on the AAL atlas (Tzourio-Mazoyer *et al.*, 2002) with the SPM probabilistic toolbox* (Eickhoff *et al.* (2005) and the Hiro software^ (Gladwin & Vink, 2008).

DISCUSSION

The main aim of this study was to assess differences in neural activity and frontostriatal functional connectivity during an alcohol-taste paradigm between the *OPRM1* AG- and AA-genotypes in a sample of young individuals (17- to 21-year-olds) at the early stage of their drinking career. Main findings can be summarized as follows: concerning brain activations across genetic groups, G-allele carriers of the *OPRM1* gene demonstrated reduced activation by alcohol in the prefrontal and parietal regions, including the inferior and middle frontal gyrus, superior and inferior parietal lobule, compared with A-allele homozygotes. Contrary to our expectations, no activation differences were observed in the mesolimbic reward pathway between the A-allele homozygotes and G-allele carriers. Concerning connectivity, we observed that the coupling from the VS seed region to the frontal regions (middle –including dlPFC- and superior frontal gyrus) after alcohol tasting (compared to water) was higher in G-allele carriers than in AA carriers. For the DS seed region, the AG group showed increased connectivity to non-PFC regions.

Both increases and decreases in the PFC activation have been implicated in the literature, albeit with distinct functional roles. Higher activation in OFC and DLPFC to alcohol cues has been observed in non-treatment seeking drug users but was lacking in treatment seeking drug users, which has been associated with context-dependent processing, e.g., related to the actual availability of drugs (Wilson *et al.*, 2004). Prefrontal activation could reflect the cue-evoked activation of expectancy of drug-related reinforcement and planning to acquire drugs (Wilson *et al.*, 2004). Moreover, higher medial PFC activity towards alcohol cues has been reported in patients with subsequent relapse compared to non-relapsers and control subjects (Beck *et al.*, 2012). Increased cue-induced activations in frontal regions have previously been found in emotion regulation areas (e.g. dorsolateral prefrontal cortex) and has been associated with the regulation of craving and decreases in craving (Kober *et al.*, 2010). Inferior and middle frontal cortex are part of the emotion network that has been documented in earlier reviews of emotion regulation (Quirk & Beer, 2006). Thus, OFC and DLPFC may be associated with processes that are involved with problematic responses to drug cues as well as more healthy regulatory control, depending on other psychological factors. Given that G-allele carriers are more vulnerable to hazardous drinking, their reduced frontal activation appears to reflect a lack of regulatory responding. In the absence of this cortical readiness to regulate craving, in the long run, G-allele carriers may be more vulnerable for the alcohol and drugs to hijack the reward system. G-allele carriers may be more prone to rapidly acquire incentive salience of alcohol cues with increasing alcohol use, also due to a decreased regulatory behaviours to monitor or to control alcohol use.

Vulnerabilities to or protective factors against addiction cannot be explained by genetics alone and are likely to be the result of an interaction between genes and environment. Epigenetic mechanisms involved in the regulation of the saliency of environmental stimuli may promote alcohol intake in adulthood. For instance, it has been shown that repeated alcohol administration in adolescent rats induce alterations in the mesolimbic dopaminergic and glutamatergic systems and can trigger changes in gene expression (Pascual *et al.*, 2009), which are involved in drug-related behavioural sensitization (Renthal & Nestler, 2008). To date there has been little agreement whether epigenetic alterations increase vulnerabilities for addiction or chronic drug use induces epigenetic responses to substance exposure (Nielsen *et al.*, 2012). However, the studies support that genetic predisposition and early exposure to alcohol can both contribute to the development of addiction and moderate responses to drug-related cues.

Studies in human samples also provide evidence for the notion that genetic factors and heavy drinking may have distinct contributions to the development of addiction and cue reactivity towards drugs and drug-related stimuli. Previous research comparing family history positive (FHP; a global hereditary risk factor) and family history negative (FHN) groups with heavy and light drinking patterns suggested that heavy drinking and risk for alcoholism influences different neural circuitry involved in cue reactivity (Dager *et al.*, 2013b). Moreover, only heavy drinking young adults carrying a G-variant of the *OPRM1* gene showed a relatively strong approach bias towards alcohol-related stimuli and more craving for alcohol compared to heavy drinking A-homozygotes (van den Wildenberg *et al.*, 2007; Wiers *et al.*, 2009). This might suggest that findings of hyperactivation in mesolimbic structures of G-allele carriers reported in earlier studies could be the composite outcome of genetic vulnerability to attribute incentive sensitization to reward cues, reduced regulation of emotional-motivational responses to drug cues, and excessive drinking history.

Genes bias behaviours and risk for psychiatric disorders by partly through neural systems mechanisms (endophenotype). Although some earlier studies associated certain genotypes with functionally specialized regions (i.e. 5HTTLPR gene and amygdala activity), perhaps a more plausible hypothesis is that genes affect the brain at the network level (Viding, Williamson, & Hariri, 2006). This would be expected especially during development, given that even in individuals carrying the risk allele, social and environmental adversity would play a role in the transition from normal to pathological behaviours (Viding *et al.*, 2006). It is relevant to mention that association studies failed to support a consistent relationship between *OPRM1* gene and alcohol dependence (for a review, *see* van der Zwaluw et al., 2007), therefore it could be argued that other factors that interact with the presence of the G-allele of this polymorphism increase the risk for alcohol addiction and functional connectivity may be the endophenotype that relates to the behavioural outcome. In earlier studies with late adolescents at risk for alcohol addiction, differential reward network functional connectivity has been

reported between vulnerable and resilient individuals (Heitzeg & Nigg, 2008; Weiland *et al.*, 2013). For instance, in FHP adolescents between 16-20 years old, during processing of affective stimuli, a problem drinking group showed greater frontal and lesser VS activation than a group with no problem drinking (Heitzeg & Nigg, 2008). Moreover, in another study with FHP and FHN young adults, increased coupling of the NAc with attention and motor structures was associated with personality characteristics and drinking profile (Weiland *et al.*, 2013). These studies suggest that heavy or problem drinking in youth at risk for alcohol dependence is related to changes in functional networks. Lastly, associations have been reported between neural response to alcohol taste cues and factors like years of alcohol exposure and severity of alcohol use, especially for the DLPFC, NAc and OFC activity (Claus *et al.*, 2011). In this regard, the G-allele carriers of the *OPRM1* gene included in earlier imaging genetics studies with heavy drinking profile or alcohol dependence could potentially be composed of a sub-sample with low resilience or high risk.

A recent study in heavy drinking adults reported *OPRM1* genotype involvement in the regulation of frontostriatal functional connectivity during an alcohol-taste paradigm (Ray *et al.*, 2014). Specifically, the study in heavy drinkers revealed negative frontostriatal connectivity in G-allele carriers both for the ventral and the dorsal part of the striatum (Ray *et al.*, 2014). Negative directionality of this connectivity suggests that heavy drinking G-allele carriers required inhibitory frontal control over both ventral and dorsal striatum during processing of alcohol cues. Contrary to earlier findings, in the current study with late adolescents, allele differences were specific to frontostriatal connectivity from the VS seed region only in G-allele carriers, but a greater connectivity of the DS with frontal structures was absent. The positive connectivity of the PPI analysis in the present study could be due to the dominance of bottom-up feedback system in late adolescents in general (Gladwin *et al.*, 2011). Previous findings of increased frontal to dorsal connectivity in heavy drinking adult samples might indicate increased need for frontal control of reward-related striatal signals due to neuroadaptations or cognitive impairments that took place in long term users (Ray *et al.*, 2014). Alternatively, the negative connectivity observed in the previous study with an adult sample may be related to the recruitment procedure: individuals reporting alcohol problems, which might result in the context-dependent processing discussed above (Wilson *et al*, 2004).

Some limitations of the current study need mentioning. It is important to note that although at pre-scanning participants rated the alcohol taste more pleasant than water, throughout the experimental session pleasantness rating for the alcohol taste decreased (yet not significantly different from the rating of water), although urge ratings were stable. Consequently, post-scanning pleasantness ratings of alcohol tastes were comparable with ratings for water. Although higher pleasantness ratings at the beginning of the experiment only for the alcohol taste suggests that alcohol taste cues were more rewarding than water, decrease

in the pleasantness rating of alcohol with repeated administration might have had an effect on the activation pattern. Moreover, the present study consists of relatively a small sample size. However we focused on a priori hypotheses based on earlier findings of imaging genetic studies and tested this in a sample with limited age range, which may (partly) compensate for this limitation. Another consideration is that the risk group in our study included only AG carriers which might have limited our power to detect small effects; stronger effects might have been observed with the inclusion of GG carriers. Despite these limitations, however, this is the first study testing the neural responses to real-time alcohol administration in a genetically selected young group without excessive drinking histories.

In this imaging genetics study, we found that young individuals carrying the *OPRM1* G-allele genotype revealed lower activation in frontal regions compared to AA carriers in a taste-paradigm. Functional connectivity analysis revealed that G-allele carriers had more dominant input from VS to frontal regions compared to A-allele homozygotes, which could be related to the observed lower PFC activity. Thereby, the present study provides various findings that may provide novel insights and new directions for the future studies. The role of *OPRM1* gene on the acquisition of alcohol addiction could be studied from a broader perspective, in different age groups and as a function of drinking profiles. In a recent review it has been emphasized that besides its role in rewarding effects of alcohol, mu opioid receptors play a role in many other mechanisms; such as social reward, response inhibition and decision making processes (Lutz & Kieffer, 2013). As a dysfunction in these processes contribute to the development of addiction, it may also be the case that such dysregulations might be attenuated in the G-carriers (Mitchell *et al*., 2007). If such causal links can be established, cognitive enhancers can be used in early stages of alcohol use for the vulnerable groups. Moreover, earlier studies showed that young adult carriers of the *OPRM1* G-allele have stronger approach tendencies towards alcohol-related cues (Wiers *et al.*, 2009). Given that this approach bias for alcohol appears to be reversible through training (Wiers *et al.*, 2011), the *OPRM1* gene carriers could be a target group.

In conclusion, these results indicate that previous findings of hyperactivity in mesocorticolimbic structures observed in G-allele carriers of the *OPRM1* gene may result not only from genetic susceptibility but also from excessive alcohol use. In G-allele carrying adolescents without extensive alcohol use, the present study observed reduced prefrontal and parietal activations to alcohol taste-cues, together with increased VS to frontal coupling, which may constitute a mechanism of vulnerability that could be targeted in treatment.

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SUPPLEMENTARY MATERIALS

Exclusion Criteria

Exclusion criteria were psychiatric disorders, drug use disorder, head trauma, seizures, severe physical illness, cardiovascular disease, the presence of major medical conditions, and use of medication. Further exclusion criteria for the fMRI were metal implants, claustrophobia, pregnancy and breast-feeding.

Questionnaires

At the start of the session, subjects completed the *Desire for Alcohol Questionnaire* (DAQ; Love *et al.*, 1998) and the *Positive and Negative Affect Scale* (PANAS; Watson *et al.*, 1988) to compare groups on current mood and craving. Current alcohol use was assessed with the *Alcohol Use Disorder Identification Test* (AUDIT; Saunders *et al.*, 1993). Frequency of drug use behavior was assessed with a 10-item rating scale (marijuana, cocaine, ecstasy, hallucinogenic, stimulators, sedatives, opiates, volatile substances, other club/party drugs) on a 11-point scale (Graham *et al.*, 1984), ranging from 1 (never used) to 11 (91+ times), with intermediate points referring to frequency of use in increments of 10 (2 = 1-10 times, 3 = 11-20 times, etc.).

Figure S1: Seed region of interest masks, for the right Nucleus Accumbens (NAc) and dorsal caudate, used to extract time course data in the PPI analysis. L = left, R = right.

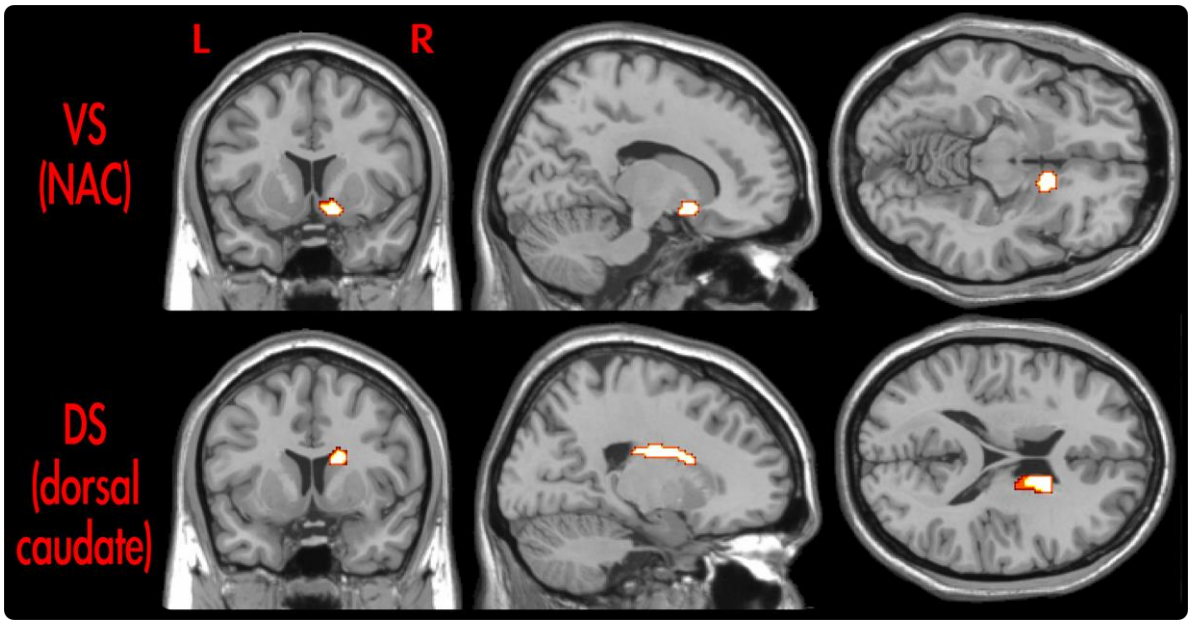

Table S1: Regions showing greater negative functional connectivity with the ventral and dorsal striatum for the full sample ($p < .005$, uncorrected, $k \geq 20$ voxels).

Region	Hemisphere	Cluster size (in voxels)	Peak Value	MNI coordinates x,y,z
Ventral Striatum seed region (NAC)				
Precuneus	L	413	4.45	-6, -82, 44*
	L		4.28	-4, -76, 52*
	R		3.32	4,-66,60*
Thalamus/Parahippocampus	R	53	3.95	12, -20, -8^
Hippocampus	R	38	3.86	10, 0, -14^
Thalamus/Pallidum	R		3.04	10, -6, -6^
Olfactory	L		2.98	0, 4, -10^
Cerebellum	R	129	3.68	38, -66, -22*
Fusiform gyrus	R		3.59	32,-70,-18*
Temporal lobe	R	90	3.68	44,20,-24*
Inferior frontal gyrus	R		3.15	34,20,-22*
Temporal pole	R		3.1	48,22,-16*
Frontal Inferior Operculum	L	48	3.53	-34,8,22^
Dorsal Striatum seed region (Dorsal Caudate)				
Inferior parietal gyrus	L	58	3.63	-60, -42, 50^
Supramarginal gyrus	R	73	3.38	58, -40, 34*
			2.81	60, -38, 26*
Insula	R	36	3.21	38, 8, -2^
Superior frontal gyrus	R	25	3.12	20, 40, 34*
Postcentral gyrus	R	23	2.97	58, -4, 32*
Precentral gyrus	L	29	2.96	-50, -8, 28*
			2.88	-56, 0, 26*

L = left, R = right, NAc: Nucleus Accumbens, MNI: Montreal Neurological Institute. Anatomical labelling was based on the AAL atlas (Tzourio-Mazoyer *et al.*, 2002) with the SPM probabilistic toolbox* (Eickhoff *et al.* (2005) and the Hiro software^ (Gladwin & Vink, 2008).

Neural Correlates of Pleasantness and Urge Ratings

Here we report the relationship between real time "pleasantness/urge" ratings (reflecting 'liking/wanting' aspects of drug use) in the scanner with neural responses during the alcohol taste-cue exposure in relation to the OPRM1 gene.

Separate regression analyses were conducted to investigate genotype effects on the relationship between the *pleasantness* and *urge* ratings in real time during fMRI scanning within the limbic clusters identified with a whole-brain analysis on the full sample. Similar to the contrast of interest as in the fMRI analysis, a contrast score was also calculated for in-scanner pleasantness and urge ratings, separately, by subtracting the mean rating for the water from the mean rating for the alcohol taste (i.e. Contrast score for urge rating = Urge rating alcohol – Urge rating water). Following that, contrast scores for *pleasantness* and *urge* ratings were centered by subtracting the overall mean score from each participant's rating score.

Inspection of contrast scores for *urge* and *pleasantness* ratings revealed a strong correlation ($r = .95$, $p < .001$). Note that there was a significant positive correlation between *pleasantness* and *urge* ratings for each liquid type as well ($r_{\text{urge-pleasantness for water}} = .95$, $p < .001$ and $r_{\text{urge-pleasantness for alcohol}} = .93$, $p < .001$). Given that the contrast scores for the *pleasantness* and *urge* ratings were highly correlated, first a principle component analysis (PCA) method was applied in order to reduce two correlated variables into one factor. Following, the PCA factor was used in the regression model to predict neural pattern of activation commonly relating to both scales.

Given that the difference scores for the *urge* and *pleasantness* ratings correlated significantly, we reported the neural activity across genotypes during the alcohol>water contrast that has been predicted by the PCA factor, which has been identified via combining urge and pleasantness variables into one. The strength of the connectivity from the VS to the frontal regions (inferior and superior frontal regions) positively correlated with the PCA factor of urge/pleasantness ratings in G- compared with A-carriers. Moreover, G- than the A-carriers also demonstrated a positive correlation with the level of DS-to-frontal connectivity (to inferior frontal cortex) and the PCA factor (see Table S2 for the full list).

Regarding real time urge and pleasantness ratings, two points are particularly worth noticing here. Earlier reviews stated that in the initial phases of drug use, wanting and liking are closely linked to each other. With repetitive use, liking behaviour can either be stable or decrease, while “wanting” increases with progression of alcohol and drug use (Berridge & Robinson, 2003). In the current sample, real time pleasantness and urge ratings were highly correlated, and therefore we looked at brain regions showing correlation with the variable accounting for the variance common to both rating scales. Interestingly, correlations of real time pleasantness and urge ratings with connectivity from the striatum highlighted two frontal regions: inferior and superior frontal gyrus. Changes in coupling from the VS and DS to the IFG were correlated with both urge and pleasantness ratings. The IFG has been involved in successful inhibition and regulation of emotions (Shafritz *et al.*, 2006). The correlation of the urge and pleasantness with the striatum connectivity to the superior frontal gyrus was specific to the VS seed region. The superior prefrontal cortex has been associated with modulating craving reactivity in tobacco addiction (Rose *et al.*, 2011). In sum, observed correlations between pleasure and urge ratings and frontostriatal connectivity patterns are in line with the idea of a conceptual and neural overlap between liking and wanting behaviour in initial phases of drug use.

Table S2: Regions correlated with the real-time pleasantness and urge ratings for the contrast AG vs AA carriers of the *OPRM1* polymorphism genotype ($p < .005$, uncorrected, $k \geq 20$ voxels).

Region	Hemisphere	Cluster size (in voxels)	Peak Value	MNI coordinates x,y,z
PPI analysis – Ventral striatum seed region (NAc)				
Negative Correlations for Pleasantness and Urge Ratings (Principle component)– AG vs AA				
Superior Occipital G	L	374	4.21	-8,-96,10*
Cuneus	L		4	-6,-92,20*
Superior Occipital G	L		3.59	-20,-90,22*
Inferior Frontal G. (BA47)	R	43	3.72	48,44,-10*
Inferior Frontal G.	R		3.25	48,36,-12*
Superior Orbital G.	R	33	3.52	34,60,-4*
Superior Frontal G. (BA10)	R		2.92	30,66,2*
Insula	L	26	3.48	-26,-6,20^
Superior Occipital G	R	153	3.40	22,-92,8*
Calcarine gyrus/Cuneus	R		3.16	16,-88,12*^
Middle Occipital G.	R		3.05	32,-84,8*
ACC/Caudate	L	62	3.3	-8,14,18^
ACC	L		2.99	-10,22,20^
Frontal Superior Medial G. (BA8)	L	35	3.18	-2,28,46^
Positive Correlations for Pleasantness and Urge Ratings (Principle component)– AG vs AA				
Amygdala	R	23	3.32	22,2,-18*
Postcentral Gyrus	R	36	3.29	30,-44,64*
	R		2.85	38,-42,64*
PPI analysis – Dorsal striatum seed region (Dorsal Caudate)				
Negative Correlations for Pleasantness and Urge Ratings (Principle component) - AG vs AA				
Inferior Frontal G.	L	33	3.36	-48,24,16*
Positive Correlations for Pleasantness and Urge Ratings (Principle component) - AG vs AA				
Amygdala	R	118	3.76	26, 0, -16*
Hippocampus	R		3.67	32, -8, -18*
Temporal Pole	R		2.87	32, 8, -22*
Hippocampus	L	33	3.55	-12, -16, -12^
Putamen	R	26	3.3	20, 10, 6*

L = left, R = right, NAc: Nucleus Accumbens, ACC: Anterior cingulate cortex, G: gyrus, MNI: Montreal Neurological Institute. Anatomical labelling was based on the AAL atlas (Tzourio-Mazoyer *et al.*, 2002) with the SPM probabilistic toolbox* (Eickhoff *et al.* (2005) and the Hiro software^ (Gladwin & Vink, 2008).

CHAPTER 6

GENERAL DISCUSSION

The studies discussed in this dissertation had two main aims. One first main aim was to assess the effect of a moderate dose of alcohol on the neurocognitive processes involved in the aetiology of problem drinking in an adolescent sample. Alcohol-induced effects on brain responses were measured for processes associated with executive functions and appetitive processes. Second, we aimed to identify specific neurocognitive processes associated with executive functions and appetitive processes that would predict escalation in alcohol use. Over and above these main goals, each study pursued different but related secondary objectives, together providing a novel and integrative perspectives on acute alcohol effects. For instance, the first and second study exemplified how neurocognitive studies can provide insights into the mechanisms involved in implicit alcohol-related processes and how they were affected by acute alcohol. These studies described protocols that included the development of approach-avoidance tasks compatible with the measurement of EEG. The third study integrated the manipulation of a hot vs. cold context in an executive function task by using an affective Go/NoGo task and described context-dependent alcohol-induced performance changes in adolescents. Moreover, while the first three studies tested specific processes and their sensitivity to acute alcohol, and how variability across individuals related to these alcohol-induced changes could contribute to escalation of alcohol use, the last study described whether a trait-like (genetic) individual difference in sensitivity to rewarding effects of alcohol affected neural responses towards alcohol-taste cues in adolescents. These research questions were studied with a prospective neurocognitive study, involving adolescents between the ages of 16 to 20 years old (n=145). Note that until now our understanding of acute alcohol effects on behaviour and brain functions in adolescents was exclusively based on animal research. In this final chapter we will provide an overview of our findings, discuss our limitations and give suggestions for future research.

Specific behavioural and neurocognitive processes sensitive to acute alcohol in late adolescence

In this thesis, we tested the acute effects of alcohol on processes associated with executive functions and on appetitive processes thought to be involved in addictive behaviours. Specifically, we studied alcohol-induced changes first during an approach-avoidance task in young adults and late adolescents, and during an inhibition task in adolescents. Finally, we looked at real-time administration of alcohol tastes on brain function across individuals with genetic individual differences in sensitivity to acute alcohol.

Until now, studies on approach tendencies for alcohol-related stimuli have been conducted with behavioural measures (Barkby *et al*, 2012; Christiansen *et al*, 2012a; 2012b; Farris and Ostafin, 2008; Field *et al*, 2008; 2011a; Fleming and Bartholow, 2014; Pieters *et al*,

2012; Schoenmakers *et al*, 2008; Sharbanee *et al*, 2012; 2014; van Hemel-Ruiter *et al*, 2011; R.W. Wiers *et al*, 2009; 2010; 2011) and neuroimaging studies focusing on this bias are limited (Ernst *et al*, 2014; C.E. Wiers *et al*, 2014a). The main findings in these studies can be summarized as 1) different levels of approach bias towards alcohol-related stimuli have been observed in samples with different drinking profiles (dependent patients, social and heavy drinkers; Christiansen *et al*, 2012b; Field *et al*, 2008; Fleming and Bartholow, 2014; C.E. Wiers *et al*, 2014a), 2) the alcohol approach bias measured in the lab has been associated with alcohol-related behaviours and problems in real life (Barkby *et al*, 2012; Field *et al*, 2008), 3) evidence suggests that regulatory processes are partially involved in approach bias (Sharbanee *et al*, 2012), 4) approach biases can be retrained which helps people to stay abstinence (Eberl *et al*, 2013; R.W. Wiers *et al*, 2010; 2011). However, an understanding of the mechanisms underlying the approach bias or how the approach alcohol bias contributes to addictive behaviours is largely lacking (but see Field *et al*, 2011a; C.E. Wiers *et al*, 2014b). Only recently, a review addressed this issue by discussing the empirical findings in the literature under the theoretical framework of associative learning (Watson *et al*, 2012). The review of the existing findings suggested that the involvement of both Pavlovian (stimulus outcome contingencies) and instrumental learning processes (response outcome contingencies) in approach tendencies is in line with the observed findings in the literature (Watson *et al*, 2012).

In the current thesis, we aimed to study the nature of this biased action tendency and the effect of acute alcohol by looking at response preparatory processes for approach and avoid responses. Using this approach, we tested the effect of acute alcohol on two different versions of the approach avoidance task (AAT): relevant and irrelevant-feature versions, each involving different experimental manipulations (De Houwer, 2003). In a relevant-feature version, participants are instructed in one block to approach alcohol and to avoid soft-drinks, and in the other block to avoid alcohol and to approach soft-drinks (De Houwer, 2001; Schoenmakers *et al*, 2008). In an irrelevant-feature version, participants are instructed to react to another feature of the stimulus unrelated to the contents (Cousijn *et al*, 2011; Huijding and de Jong, 2005; R.W. Wiers *et al*, 2009). The irrelevant-feature version of this task may be considered to be more implicit given that subjects do not need to make an explicit judgment about the stimuli in order to generate an accurate behavioural response. The majority of the studies on approach tendencies measure the alcohol approach bias as the reaction time difference between push and pull responses, therefore controlling for general response bias due to a specific action (approach/avoid).

Concerning alcohol effects on approach bias, earlier studies revealed conflicting results. Farris and Ostafin tested the effect of acute alcohol on the strength of associations between ‘approach/avoidance’ and alcohol-related stimuli with an implicit word association task. The results revealed that ‘approach’ and ‘alcohol’ associations increased after alcohol

administration (Farris and Ostafin, 2008). Fernie and colleagues reported that no effects of alcohol (compared to placebo) were observed on the bias with an irrelevant-feature version of the AAT (Fernie *et al*, 2012). With a relevant version of the task, Schoenmakers and colleagues found no increase in approach bias after a low dose of alcohol as compared with placebo administration. However, correlation of the approach bias with another cognitive bias (attentional bias) increased after alcohol administration suggesting that alcohol increased the association between different measures of cognitive biases for alcohol (Schoenmakers *et al*, 2008). Interestingly, a recent study comparing alcohol approach bias after administration of alcohol, placebo or control beverages, reported increased approach tendencies after placebo and alcohol compared to the control condition with a relevant-feature version of the task. This suggests that this bias might be more sensitive to the expectancy or anticipation effects of alcohol than the pharmacological effects (Christiansen *et al*, 2012b).

In Chapter 2 and Chapter 3, we studied the neural activity during advance response preparation and hand-related response preparation for approach and avoid alcohol responses. In both versions a preparatory period was provided between the presentation of the stimulus and the motor response. The behavioural results with the revised EEG-versions of the approach avoidance task suggested an approach bias for alcohol. Alcohol did not affect the approach bias in social drinkers mainly composed of young adults (Chapter 2) but it had an influence on the bias as a function of drinking profile in adolescents (Chapter 3). Heavy drinking adolescents slowed down their response after alcohol and this effect was more pronounced in approach alcohol and avoid soft drink trials, probably due to fast responses during these trial types in the placebo condition.

Neural activity related to advance response preparation was studied by comparing decrease in spectral activity (event-related desychronization) in the mu (Chapter 3) and beta band (Chapter 2 and 3). In Chapter 3, hand-related motor preparation was studied by focusing on motor-related asymmetry index. Results suggested that the neural response during response preparation measured as central ERD (Chapter 2) and lateralized ERD (Chapter 3) showed a characteristic modulation that could be explained by specific requirements of the task version employed. Regarding the oscillations in Chapter 2, approach alcohol and avoid soft drink responses were preceded by a decrease in beta power over parietal region. The parietal cortex plays an important role in visuomotor transformations involved in response preparation tasks (Toni *et al*, 1999). Therefore, increased parietal beta-ERD observed during congruent trials of the AAT may suggest the contribution of visual input in movement preparation. In this study, beta-ERD was measured with a relevant version of the AAT where subjects needed to categorize the stimuli as alcohol-related or not and to map the stimuli to the correct response direction (approach/avoid) in order to produce the correct response. The stimulus categorization step required in the relevant-feature version of the task may have influenced the spatial

distribution of the beta-ERD. It could also be argued that observed effects were partially modulated due to certain stimulus-response associations being overlearned. However, earlier studies on response preparation and execution conducted with EEG found that learning in visuomotor tasks is associated with an increase in activity during preparation and a decrease in activity during execution (Kranczioch *et al*, 2008). Therefore, observed EEG effects on the congruent trials cannot be explained by automatic motor reactions. Moreover, alcohol administration increased the beta-ERD for congruent trials which is in line with the attentional account of congruent trials, given that earlier research showed increased attention towards alcohol cues after alcohol administration (Duka and Townshend, 2004; Fernie *et al*, 2012; Nikolaou *et al*, 2013; Townshend and Duka, 2001).

In our second study (Chapter 3), we focused on response preparation for approach tendencies by testing a motor-related asymmetry index. In previous studies, an increased asymmetry index has been associated with advance task preparation (Gladwin *et al*, 2006; Deiber *et al*, 2012; Doyle *et al*, 2005; Nam *et al*, 2011; Poljac and Yeung, 2014). With the irrelevant-feature version of the AAT, where both left and right hand responses were required for approach/avoidance responses and motor unrelated EEG components were removed, we observed an increased approach-related asymmetry for soft-drink cues in heavy drinkers. This finding was in contrast with our expectation of an increased asymmetry index for alcohol bias in heavier drinkers indicating an advance task preparation due to greater automatic approach tendencies. This result suggests that the observed asymmetry index in the current paradigm may have reflected a different psychological process. This interpretation was strengthened by the observation that the asymmetry index was not associated with the behavioural measure of the bias (no significant correlation between brain and behavioural measures). However, the asymmetry index was associated with difficulties to regulate drinking, assessed with a self-report measure (Collins and Lapp, 1992). Individuals who reported more difficulties in regulating their drinking, had greater approach-related lateralization for soft-drink cues and individuals who reported less difficulties, had greater approach-related lateralization for alcohol cues. This result paralleled what was observed when asymmetry was compared across groups: heavy drinkers had more difficulty in controlling alcohol intake compared to light drinkers and they also showed approach-related lateralization for soft-drink cues. These results suggest that the asymmetry index may not represent an automatic but perhaps more controlled processes, which may be intentional or implicit in nature (for a review on unconscious/automatic influences on cognitive control, see Suhler and Churchland, 2009; also see Lau and Passingham, 2007). For instance, during incompatible avoid alcohol and approach soft drink cue trials, heavy drinkers may have invested more efforts in order to overcome their automatic reactions of approaching alcohol and avoiding soft drink cues. Moreover, intentional processes, such as regulating behaviour due to negative attitudes towards one's own drinking habits, may

also play a role in the direction and the magnitude of the asymmetry index given the observed association between problem drinking and the approach lateralization for the S-R mapping that is incompatible with a pre-existing stimulus-response association.

Alcohol administration reversed the asymmetry index in such a way that after alcohol the greater approach-related lateralization for soft drink cues in heavy drinkers and avoid-related lateralization for both cue types in light drinkers shifted to an avoid-related lateralization in heavy and approach-related lateralization in light drinkers, which was independent of cue type. Put differently, after alcohol administration, lateralization was higher for approach behaviours in heavy drinkers and for avoidance behaviours in light drinkers, but these effects were not related to a specific stimulus type. In line with this finding, after alcohol, the RT-bias for alcohol cues compared to soft-drink cues disappeared. Until now, only three studies tested the effect of acute alcohol on lateralization (Marinkovic *et al*, 1994; Rhodes *et al*, 1975; Tsujii *et al*, 2011). The study of Marinkovic and colleagues investigated the motor-related asymmetry index in the time domain (Lateralized readiness potential, LRP; Colebatch, 2007) by having subjects inhibit responses when presented with novel stimuli rather than previously presented items. The authors found lateralization only in trials that required a motor response (Go-trials). In trials where subjects were required to inhibit a motor response (correct inhibition trials), acute alcohol induced lateralization compared to the placebo condition and this lateralization terminated around the time when a decision for the correct response could be achieved (~500ms). This study concluded that increased lateralization induced by alcohol could reflect increased impulsive behaviour. Tsujii and colleagues examined alcohol-induced changes on lateralization of the inferior frontal cortex (IFG) between blocks that required inhibitory responses and blocks that contained only Go-responses. Alcohol decreased the right lateralization of the IFG and increased the false alarm rates (Tsujii *et al*, 2011). In Rhodes and colleagues' study alcohol attenuated asymmetry of visually evoked potentials (VEP), especially for late components. The findings from all three studies are in line with the idea that alcohol may modulate asymmetry of the EEG components via its deleterious effects on controlled/effortful processes. Moreover, observed increased and decreased asymmetry indices in all studies are consistent with the idea that lateralization might be a process aimed at optimizing performance and alcohol's detrimental effects on lateralization and performance observed in the previous and the current study supports this interpretation.

In a second task (Chapter 4), we measured alcohol effects on an executive control task during which subjects were required to inhibit prepotent motor responses. According to an influential model by Miyake and colleagues (Miyake *et al*, 2000), inhibitory control is one of the core executive functions and is an important factor for the regulation of behaviour. A deficiency in inhibitory control has been associated with risk for drug and alcohol addiction (Ivanov *et al*, 2008; Nigg *et al*, 2004; 2006; Norman *et al*, 2011). Both acute and chronic use

of alcohol decreases inhibitory control capacity (Lawrence *et al*, 2009; Loeber and Duka, 2009). In the current thesis, we studied the effect of acute alcohol on response inhibition and associated neurocognitive mechanisms in an adolescent sample. In behaviour, our adolescent sample demonstrated a psychomotor slowing after alcohol administration, which could be a compensatory motor-slowing effect to maintain behavioural performance after alcohol. Similarly, post-error response times, which represent behavioural adjustment after an error, were higher after alcohol administration compared with placebo. Note that such compensatory motor-slowing effects were also observed after alcohol administration for heavy drinkers in the AAT task. In addition to the increase in response times, hit rates for neutral cues were also found to be lower following alcohol, this decrease in hit rates represents an increase in omission responses following alcohol. Increased omission responses could also be the by-product of the compensatory psychomotor slowing after alcohol, given that in response inhibition tasks, fast response deadlines and infrequent NoGo trials are utilized to enhance pre-potent response tendencies. In an earlier study with adults comparing performance before and after different beverage administrations (placebo, low alcohol, and moderate alcohol) faster responses were reported after beverage administration compared to before (irrespective of beverage type), but post-error slowing was not affected (Easdon *et al*, 2005). Two other studies, which focused on alcohol effects on conflict monitoring by using a flanker task in adult samples, reported that alcohol did not affect reaction times (Bartholow *et al*, 2012; Ridderinkhof *et al*, 2002). In sum, up till now in adult samples no evidence was found in favour of a psychomotor slowing following acute alcohol, therefore this might be an effect specific to adolescents.

Regarding effects of acute alcohol on neurocognitive mechanisms, contrary to the findings in adults, the NoGo-N2 ERP component associated with conflict was higher for alcohol cues, and these enhanced NoGo-N2 component for alcohol cues decreased after alcohol administration. These results suggest that alcohol-related cues might have induced a ‘go’ or an ‘approach’ response and conflict may have increased due to a mismatch between stimulus induced Go response and task induced NoGo response during alcohol cue trials. As discussed in the general introduction, acute alcohol has been found to prime appetitive processes such as approach tendencies and impairs cognitive control functions. Both in the AAT and the inhibition task, acute alcohol resulted in a general decrease in response speed in adolescents rather than a decrease in reaction times towards alcohol cues, as mentioned earlier that could be due to a compensatory reaction to maintain a stable level of performance. These general slowing effects observed after alcohol administration might have unexpectedly resulted in better control over automatic reactions towards alcohol. Automatic activation of Go responses in the Go/NoGo task requires fast responding and a general psychomotor slowing following alcohol might have decreased the conflict due to NoGo responses towards alcohol cues, similar to the increase in omission responses. In a simulation study it has been shown that factors that

impair target processing affect the amplitude of the ERN, but factors that increase the processing of the irrelevant stimuli affect the N2 amplitude (Yeung and Cohen, 2006). Acute alcohol effects on the N2 amplitude for alcohol cues could be due to alcohol promoting processing of irrelevant stimulus information. It has been shown that capacity to attend relevant and to ignore irrelevant stimulus information increases during development (Berman and Friedman, 1995). Therefore the influence of alcohol on processing of relevant/irrelevant information could be more pronounced in adolescents.

To recap, during the relevant-feature version of the AAT, where stimulus categorization is central for response preparation, alcohol enhanced brain processes during approach alcohol trials, without affecting behavioural performance. During an irrelevant-feature version of the task, where response preparation does not require processing of stimuli, acute alcohol disrupted lateralization, which was greater for incongruent trial types in heavier drinkers. In the Go/NoGo task, alcohol affected the EEG component associated with conflict monitoring specifically for task-irrelevant alcohol cues. Moreover, in adolescents, acute alcohol increased response times both during approach-avoidance and inhibition tasks. Based on the literature discussed in Chapter 1, suggesting less sensitivity to the alcohol-induced motor impairment and sedation in adolescents, it could be the case that adolescents maintain their level of motor performance by adjusting their reaction time. This could be because due to their younger age and limited experience with alcohol, adolescents might be more concerned with their performance. Such successful compensatory mechanisms could become a protective or a risk factor in the long-term, depending on how they shape the expectancies of adolescents about alcohol.

Interaction between cognition and affect

The general framework provided by dual process models states that repetitive drug and alcohol use changes processes related to two interacting systems. Although dual process models propose that two interrelated neural systems (appetitive and regulatory) play a central role in addiction, until now the majority of research addressed the effects of alcohol and drugs on these processes in isolation. However, the few studies that did focus on this interaction, produced interesting results. For example, it has been shown that selectively inhibiting responses to alcohol-cues, makes implicit alcohol attitudes more negative and reduces alcohol intake in the short run (Houben *et al*, 2012), and reduces approach motivation towards alcohol (Bowley *et al*, 2013). Therefore, greater regulatory capacity over drug-related cues, innate or acquired, might contribute to lower drug or alcohol use in real life. Likewise, an inept control may result in failure to resist temptations especially in the face of appetitive cues. These interactions might be even more important for the assessment of adolescent cognitive capacity in an affective

context, given that the temporal gap in the maturation of the adolescent brain tips the balance towards enhanced appetitive processes. Also, whether chronic or acute alcohol use affects cognitive capacity and context-dependent regulatory processes in the same way is still unknown. We may be able to disentangle the compound effects of these two interacting processes by different approaches, for instance by using mathematical modelling, integrating appetitive stimuli in cognitive control tasks and systematically varying the cognitive load in these paradigms, or by studying connectivity between neural structures that tap into both systems.

The present thesis provides some evidence for the interaction between these two systems. In Chapter 4, we tested adolescent inhibitory capacity with two versions of the same task, using either soft drink cues or alcohol cues as stimuli. The results of this study demonstrated that in adolescents, commission errors and neural activity associated with conflict were higher for alcohol cues compared to control cues. These findings suggest that alcohol cues induced a 'go' or an 'approach' tendency, leading to higher commission errors and greater conflict when subjects needed to inhibit a motor response. In Chapter 5, we studied the connectivity between two neural substrates (prefrontal cortex and striatum) during a cue reactivity paradigm, for adolescents carrying different alleles of the OPRM1 genotype. This study demonstrated that adolescents with limited drinking experience carrying the G-allele of the OPRM1 gene did not reveal a higher striatal reactivity to alcohol cues, as it has been shown in adult heavy drinkers, but they did demonstrate a lack of frontal regulation of striatal activity. The results of this study are in line with the notion that excessive drinking in the long-term may change the balance between these two systems in susceptible individuals. As mentioned previously, there is some indication that executive control processes may moderate the approach bias. For instance, Sharbanee and colleagues (2012) showed that responses during avoid alcohol trials -which require greater regulatory control-, explained group differences across problem and social drinkers, rather than approach alcohol trials. In the study by Cousijn *et al* (2012), greater DLPF/ACC activity, brain areas involved in the regulatory and evaluative processes, were associated with decreased cannabis use. In a young adolescent sample, the study by Pieters *et al*, (2012) showed that association between alcohol approach tendencies and alcohol use was moderated by parental rule setting, so that a relatively strong approach bias only predicted heavy drinking in adolescents with parent who did not impose restrictions on the drinking of their children. In those who did set strict rules regarding their children's drinking, the approach bias was not predictive. Therefore, weak regulatory capacity combined with excessive drinking in the long run may contribute to the excessive incentive salience attribution and development of implicit and explicit cognitive biases towards drug-related stimuli. Recently it has been found that alcohol-dependent patients trained to give avoid responses for alcohol cues demonstrated decreased alcohol cue reactivity (C.E. Wiers *et al*, 2014a).

Moreover, we found increased asymmetry for incongruent trials (approaching soft drink trials) in heavy drinkers, suggesting that the asymmetry may represent an effortful compensatory process. This finding in Chapter 3 is consistent with the notion that approach bias might be moderated by executive control processes. This could partially be: 1) because an irrelevant-feature version of the task may give more room for top-down influence of task instructions, and/or 2) presenting a preparatory period may allow for regulatory processes to have an influence (as it has been stated by earlier researchers that tasks with informative cues and preparatory period allow advance planning, which is more of a controlled process).

Interaction between alcohol cues and alcohol administration

The presence or absence of conditioned drug-related stimuli has an influence on brain responses associated with motivational state following drug challenges (Leyton and Vezina, 2012, 2013) and on tolerance to drug-induced negative influences on executive functions (Birak *et al*, 2010, 2011). The findings from previous studies on the effects of acute alcohol in the presence of alcohol cues can be summarized as follows: First, it has been shown that acute alcohol administration increases attention towards alcohol cues which probably plays a role in heightened cognitive biases following acute alcohol (Duka and Townshend, 2004; Fernie *et al*, 2012; Nikolaou *et al*, 2013; Townshend and Duka, 2001). Second, it has also been shown that when conditioned cues are present, deleterious effects of alcohol on executive functions are alleviated (Birak *et al*, 2010). Lastly literature focusing on striatal reactions states that blunted striatal responses can be observed when drugs and alcohol are administered in the absence of drug cues (Leyton and Vezina, 2012). In the current thesis, acute alcohol administration interacted with performance and brain responses during the affective inhibition task. In trials requiring a motor response, performance was unaffected by the administration of alcohol when alcohol cues were present, while in neutral cue trials, performance decreased after alcohol administration. Moreover, a measure of behavioural adjustment following errors was influenced by acute alcohol but not when alcohol cues were present. In sum, in the presence of alcohol cues, acute alcohol did not deteriorate performance in tasks requiring cognitive control. During a cognitive bias task, to the contrary, acute alcohol did not increase approach tendencies towards alcohol cues. Therefore, a possible increase in attention towards alcohol cues cannot fully account for the observed behavioural effects. A lack of deterioration in performance specific to alcohol cue trials is consistent with the findings of Birak and colleagues, where the presentation of conditioned drug cues has been found to counteract the effects of acute alcohol.

Regarding brain responses, distinct findings were found following acute alcohol. During the inhibition task, acute alcohol specifically decreased the neural activity associated with conflict when alcohol cues were present. To the contrary, acute alcohol increased the

neural response associated with advance response preparation for alcohol approach tendencies. These results demonstrate that whereas during a cognitive control task, acute alcohol disrupts the neural response for alcohol cues, this response is augmented during an appetitive task. In a separate study we tested whether this increased response to alcohol cues was moderated by the individual differences in the OPRM1 gene, previously associated with individual differences towards alcohol-related stimuli in adults. Our study revealed no activation differences in the mesolimbic pathway across G and A alleles in adolescent drinkers.

Predictors of alcohol escalation

As discussed in Chapter 1, increased sensitivity to sedative effects and decreased sensitivity to stimulating effects of alcohol are risk factors for the development of addictive behaviours. Most of the empirical evidence in support of this hypothesis focused on comparing the physiological reactions to alcohol of high vs. low risk individuals (family history positive vs. family history negative). The effects of acute alcohol, however, is broader than its effects on physiology, since it induces long-term changes in brain and behaviour. Variability in brain responses to alcohol may explain some of the variability in risk propensity for alcohol addiction. However, until now, no studies bridged the gap between individual differences in cognitive or neural sensitivity in response to acute alcohol and the development of drinking problems with prospective studies. Moreover, individual differences in neurocognitive functioning prior to the progression of drinking behaviour have been used as successful predictors of escalation in alcohol use. Assessing these neurocognitive processes under a low dose of alcohol may provide better prediction.

In the current project, the predictive value of alcohol-induced changes in brain and behaviour were tested with two different paradigms. In an affective inhibition paradigm, alcohol-induced changes in an event-related component associated with error detection were used as predictors. In this study, we found that the subjects for whom alcohol disrupted the error detection processes for alcohol cues, as indexed by the ERN, were more likely to show a decrease in their drinking at the six months follow-up. In a second study, the predictive power of alcohol effects on approach tendencies and on a motor-related asymmetry index were tested regarding their prediction of subsequent escalation of drinking. Although both behavioural and brain responses predicted changes in alcohol use in the full model, follow-up analysis revealed an association between the variability in alcohol-induced effects on behavioural measure and future drinking. We found a relatively stronger approach soft-drink and weaker approach alcohol bias after acute alcohol with decreasing drinking.

We believe that this is the first study where variability in alcohol-induced changes on neurocognitive processes was under investigation as a risk factor. The results of both studies

demonstrated that acute alcohol effects on neurocognitive processes in an affective context were successful predictors of alcohol escalation in adolescents. To the contrary, acute alcohol effects on cognitive processes with a neutral context did not add unique variance to the prospective prediction of alcohol use. As discussed earlier, generally speaking, acute alcohol administration decreases cognitive capacity and increases processes related with impulsive (appetitive) system. In the current thesis, empirical evidence revealed that adolescents who are less prone to alcohol's deleterious effects on the monitoring system were more likely to escalate drinking and adolescents who are more prone to show stronger avoid alcohol bias after alcohol administration were more likely to reduce their drinking. The former pattern of results may be indicative of a lack of a signal to limit or stop drinking during a drinking episode, the latter may increase resistance to the sensitization-related changes that are the result of long-term neuroadaptations. However, these interpretations should be taken with caution as this line of research has limited empirical data to support firm conclusions about the mechanisms underlying the role of alcohol-induced changes as a risk or protective factor. Alcohol-induced effects on specific processes and their neural correlates that are better predictors of alcohol escalation are to be uncovered in the future.

Concluding Remarks and Limitations

In his interesting review, Arnett focuses on a period that he calls *emerging adulthood* (Arnett, 2000). This phase is characterized by a prolonged period of adolescence during which young people gain independence from their families, however, due to altered expectations in modern societies, adult commitments (volitional or marital) are delayed to later ages. Many individuals decrease their alcohol and drug use with the transition to adulthood when they take more adult-like responsibilities. However, with the changes in social life, cessation of heavy drinking may shift to later ages leading to some young adults being exposed to excessive drinking for longer periods of time. Differentiating vulnerable individuals from resilient ones might gain more importance in modern societies due to altered expectations. Moreover, understanding what makes these individuals vulnerable is important for the development of prevention programs. The results of the current study are the first steps in identifying the factors to be targeted in behavioural interventions. The alcohol-induced changes on executive function measures can be used for the development of training paradigms (for a review, see R.W. Wiers *et al*, 2013). One of the limitations in this study is the administration of only a low dose of alcohol instead of comparing responses under low and high dose of alcohol. As mentioned earlier, the ascending and descending limbs of the blood alcohol curve capture sensitivities to alcohol's simulative and sedative effects (Newlin and Thomson, 1990).

Processes with an affective context were the best predictors of alcohol escalation in the adolescent sample tested in this project. In both tasks the predictive value of alcohol-induced changes was tested with alcohol-related pictorial cues used as appetitive stimuli. Some studies report significant task effects with pictorial cues and some others with verbal cues, therefore the effects seem to depend on the type of cues employed. Moreover, a recent review suggests that the neural reactions provoked by the multisensory drug cues, which depict real life drug exposure, are more consistently associated with clinical outcomes (Yalachkov *et al*, 2012). Lastly, the results observed in this thesis may differ in groups with various degrees of alcohol exposure. Similar findings should be tested in adult studies and future studies should also test the interplay between cognition and impulse by looking at the moderating effect of one process on the other one. With a similar approach, it has been shown in adolescents that implicit cognitions are better predictors of alcohol (Grenard *et al*, 2008; Peeters *et al*, 2013; Thush *et al*, 2008) and cigarette use (Grenard *et al*, 2008) in individuals with poor cognitive control capacity.

To this end, the research presented in this thesis suggests that inclusion of appetitive cues in cognitive tasks can contribute to our understanding of adolescent performance in motivational situations. Adolescents' cognitive performance and associated neural processes differ in an affective and neutral context. However, our findings revealed no evidence for a genetic modulation of neural responses underlying motivation in adolescents. Further research needs to be done to establish whether these genetic influences manifests as drinking progresses, also possibly in combination with a lack of frontal regulatory system. At the behavioural level, alcohol administration did not lead to greater impulsive behaviours, to the contrary, the findings suggested that adolescents might adjust their responses adaptively to counteract the anticipated effects of alcohol on motor responses. During an implicit cognitive bias task, this compensatory effort was also evident in the neural level in heavy drinking adolescents who had greater problems to control their drinking in real life. Moreover, alcohol-induced changes both on task performance and neural activity seem to contribute to the prediction of changes in alcohol use. Future studies should focus on determining which specific processes influenced by acute alcohol are better predictors of escalations in future drinking, which in turn may provide clues about ways to curb this development.

Reference List

Adams S, Ataya AF, Attwood AS, Munafò MR (2012). Effects of acute alcohol consumption on alcohol-related cognitive biases in light and heavy drinkers are task-dependent. *J Psychopharmacol* **26**: 245–253.

Adams S, Ataya AF, Attwood AS, Munafo MR (2013). Effects of alcohol on disinhibition towards alcohol-related cues. *Drug Alcohol Depend* **127**: 137-142.

Agrawal A, Grant JD, Waldron M, Duncan AE, Scherrer JF, Lynskey MT, *et al* (2006). Risk for initiation of substance use as a function of age of onset of cigarette, alcohol and cannabis use: findings in a Midwestern female twin cohort. *Prev Med (Baltim)* **43**: 125–128.

Alemán-Gómez Y, Melie-García L, Valdes-Hernández P (2006). IBASPM: Toolbox for automatic parcellation of brain structures. *Present 12th Annu Meet Organ Hum Brain Mapping, June 11– 15, 2006, Florence, Italy Available CD-Rom NeuroImage*.

Alfonso-Loeches S, Guerri C (2011). Molecular and behavioral aspects of the actions of alcohol on the adult and developing brain. *Crit Rev Clin Lab Sci* **48**: 19–47.

Arnett JJ (2000). Emerging adulthood: A theory of development from the late teens through the twenties. *Am Psychol* **55**: 469–480.

Barkby H, Dickson JM, Roper L, Field M (2012). To approach or avoid alcohol? Automatic and self-reported motivational tendencies in alcohol dependence. *Alcohol Clin Exp Res* **36**: 361–368.

Bart G, Kreek MJ, Ott J, LaForge KS, Proudnikov D, Pollak L, *et al* (2005). Increased attributable risk related to a functional mu-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. *Neuropsychopharmacology* **30**: 417–422.

Bartholow BD, Henry EA, Lust SA (2007). Effects of alcohol sensitivity on P3 event-related potential reactivity to alcohol cues. *Psychol Addict Behav* **21**: 555–63.

Bartholow BD, Henry EA, Lust SA, Saults JS, Wood PK (2012). Alcohol effects on performance monitoring and adjustment: affect modulation and impairment of evaluative cognitive control. *J Abnorm Psychol* **121**: 173–186.

Bartholow BD, Lust SA, Tragesser SL (2010). Specificity of P3 event-related potential reactivity to alcohol cues in individuals low in alcohol sensitivity. *Psychol Addict Behav* **24**: 220–228.

Bartholow BD, Pearson M, Sher KJ, Wieman LC, Fabiani M, Gratton G (2003). Effects of alcohol consumption and alcohol susceptibility on cognition: a psychophysiological examination. *Biol Psychol* **64**: 167-190.

Beck A, Wüstenberg T, Genauck A, Wrase J, Schlagenhauf F, Smolka MN, *et al* (2012). Effect of brain structure, brain function, and brain connectivity on relapse in alcohol-dependent patients. *Arch Gen Psychiatry* **69**: 842–852.

Bekker EM, Kenemans JL, Verbaten MN (2005). Source analysis of the N2 in a cued Go/NoGo task. *Brain Res Cogn Brain Res* **22**: 221-231.

Benjamini Y, Krieger A, Yekutieli D (2006). Adaptive linear step-up procedures that control the false discovery rate. *Biometrika* **93**: 491–507.

Bergen AW, Kokoszka J, Peterson R, LongJC, Virkkunen M, Linnoila M, *et al* (1997). Mu opioid receptor gene variants: lack of association with alcohol dependence. *Mol Psychiatry* **2(6)**: 490-494.

Berman S, Friedman D (1995). The development of selective attention as reflected by event-related brain potentials. *J Exp Child Psychol* **59**: 1–31.

Berridge KC, Robinson TE (2003). Parsing reward. *Trends Neurosci* **26**: 507–513.

Berridge KC, Robinson TE, Aldridge JW (2009). Dissecting components of reward: "liking", "wanting", and learning. *Curr Opin Pharmacol* **9**: 65-73.

Birak KS, Higgs S, Terry P (2011). Conditioned tolerance to the effects of alcohol on inhibitory control in humans. *Alcohol Alcohol* **46**: 686–693.

Birak KS, Terry P, Higgs S (2010). Effect of cues associated with an alcoholic beverage on executive function. *J Stud Alcohol Drugs* **71**: 562–569.

Blakemore S-J, Robbins TW (2012). Decision-making in the adolescent brain. *Nat Neurosci* **15**: 1184–91.

Blitzer R, Gil O, Landau E (1990). Long-term potentiation in rat hippocampus is inhibited by low concentrations of ethanol. *Brain Res* **537**: 203–208.

Blum K, Gardner E, Oscar-Berman M, Gold M (2011) "Liking" and "Wanting" linked to reward deficiency syndrome (RDS): Hypothesizing differential responsivity in brain reward circuitry. *Curr Pharm Des* **18**: 113-118.

Bond C, LaForge KS, Mingting T, Melia D, Zwang S, Borg L, *et al* (1998). Single-nucleotide polymorphism in the human mu opioid receptor gene alters B-endorphin binding and activity : Possible implications for opiate addiction. *Proc Natl Acad Sci USA* **95**: 9608–9613.

Bowley C, Faricy C, Hegarty B, J Johnstone S, L Smith J, J Kelly P, *et al* (2013). The effects of inhibitory control training on alcohol consumption, implicit alcohol-related cognitions and brain electrical activity. *Int J Psychophysiol* **89**: 342–348.

Casey BJ, Jones RM (2010). Neurobiology of the adolescent brain and behavior: implications for substance use disorders. *J Am Acad Child Adolesc Psychiatry* **49**: 1189–1201.

Casey BJ, Jones RM, Hare TA (2008). The adolescent brain. *Ann N Y Acad Sci* **1124**: 111–126.

Chen K, Kandel DB (1995). The natural history of drug use from adolescence to the mid-thirties in a general population sample. *Am J Public Health* **85**: 41–47.

Christiansen P, Cole JC, Goudie AJ, Field M (2012a). Components of behavioural impulsivity and automatic cue approach predict unique variance in hazardous drinking. *Psychopharmacology (Berl)* **219**: 501–510.

Christiansen P, Rose AK, Cole JC, Field M (2012b). A comparison of the anticipated and pharmacological effects of alcohol on cognitive bias, executive function, craving and ad-lib drinking. *J Psychopharmacol* **27**: 84–92.

Clark JJ, Nasrallah NA, Hart AS, Collins AL, Bernstein IL, Phillips PEM (2012). Altered risk-based decision making following adolescent alcohol use results from an imbalance in reinforcement learning in rats. *PLoS One* **7**: e37357.

Claus E, Feldstein Ewing SW, Filbey FM, Hutchison KE (2013). Behavioral control in alcohol use disorders: relationships with severity. *J Stud Alcohol Drugs* **74**: 141–151.

Claus ED, Ewing SWF, Filbey FM, Sabbineni A & Hutchison KE (2011) Identifying neurobiological phenotypes associated with alcohol use disorder severity. *Neuropsychopharmacology* **36**: 2086–2096.

Colebatch JG (2007). Bereitschaftspotential and movement-related potentials: origin, significance, and application in disorders of human movement. *Mov Disord* **22**: 601–610.

Collins RL, Lapp WM (1992). The temptation and restraint inventory for measuring drinking restraint. *Br J Addict* **87**: 625–633.

Concha L, Livy DJ, Beaulieu C, Wheatley BM, Gross DW (2010). In vivo diffusion tensor imaging and histopathology of the fimbria-fornix in temporal lobe epilepsy. *J Neurosci* **30**: 996–1002.

Cooper M (1994). Motivations for alcohol use among adolescents: Development and validation of a four-factor model. *Psychol Assess* **6**: 117–128.

Courtney KE, Ray LA (2014) Subjective responses to alcohol in the lab predict neural responses to alcohol cues. *J Stud Alcohol Drugs* **75**: 124–135.

Cousijn J, Goudriaan AE, Ridderinkhof KR, van den Brink W, Veltman DJ, Wiers RW (2012). Approach-bias predicts development of cannabis problem severity in heavy cannabis users: results from a prospective FMRI study. *PLoS One* **7**: e42394.

Cousijn J, Goudriaan AE, Wiers RW (2011). Reaching out towards cannabis: approach-bias in heavy cannabis users predicts changes in cannabis use. *Addiction* **106**: 1667–74.

Crone EA, Dahl RE (2012). Understanding adolescence as a period of social-affective engagement and goal flexibility. *Nat Rev Neurosci* **13**: 636–50.

Csikszentmihalyi M, Larson R, Prescott S (1977). The ecology of adolescent activity and experience. *J Youth Adolesc* **6**: 281–94.

Currie C, Zanotti C, Morgan A, Currie D, de Looze M, Roberts C, *et al* (2012). *Social determinants of health and well-being among young people. Health Behaviour in School-aged Children (HBSC) study: international report from the 2009/2010 survey. Copenhagen, WHO Reg Off Eur 2012 (Health Policy Child Adolesc No 6)*.

Curtin JJ, Barnett NP, Colby SM, Rohsenow DJ, Monti PM (2005). Cue reactivity in adolescents: measurement of separate approach and avoidance reactions. *J Stud Alcohol* **66**: 332–343.

Dager AD, Anderson BM, Rosen R, Khadka S, Sawyer B, Jiantonio-Kelly RE, *et al* (2013a). Functional magnetic resonance imaging (fMRI) response to alcohol pictures predicts subsequent transition to heavy drinking in college students. *Addiction* **109**: 585–595.

Dager AD, Anderson BM, Rosen R, Khadka S, Sawyer B, Jiantonio-Kelly RE, *et al* (2014). Functional magnetic imaging (fMRI) response to alcohol pictures predicts subsequent transition to heavy drinking in college students. *Neuropsychopharmacology* **109**: 585-595.

Dager AD, Anderson BM, Stevens MC, Pulido C, Rosen R, Jiantonio-Kelly RE, *et al* (2013b). Influence of alcohol use and family history of alcoholism on neural response to alcohol cues in college drinkers. *Alcohol Clin Exp Res* **37** Suppl 1:E161–71.

Davies PL, Segalowitz SJ, Gavin WJ (2004). Development of error-monitoring event-related potentials in adolescents. *Ann New York Acad Sci* **1**: 7–11.

De Bellis MD, Clark DB, Beers SR, Soloff PH, Boring AM, Hall J, *et al* (2000). Hippocampal volume in adolescent-onset alcohol use disorders. *Am J Psychiatry* **157**: 737–744.

De Houwer J (2001). A structural and process analysis of the implicit association test. *J Exp Soc Psychol* **37**: 443–451.

De Houwer J (2003). A structural analysis of indirect measures of attitudes. In: Musch, J (eds), Klauer C (eds). *The psychology of evaluation; Affective processing in cognition and emotion*. Mahwah, NJ, US: Lawrence Erlbaum Associates Publishers, pp 219–244.

De Jong R, Gladwin TE, ’t Hart BM (2006). Movement-related EEG indices of preparation in task switching and motor control. *Brain Res* **1105**: 73–82.

De Wit H, Chutuape M (1993). Increased ethanol choice in social drinkers following ethanol preload. *Behav Pharmacol* **4**: 29–36.

Deiber M-P, Sallard E, Ludwig C, Ghezzi C, Barral J, Ibañez V (2012). EEG alpha activity reflects motor preparation rather than the mode of action selection. *Front Integr Neurosci* **6**: 59.

Deutsch R, Gawronski B, Strack F (2006). At the boundaries of automaticity: negation as reflective operation. *J Pers Soc Psychol* **91** (3), 385–405.

Dewit DJ, Adlaf EM, Offord DR, Ogborne AC (2000). Age at first alcohol use: A risk factor for the development of alcohol disorders. *Am J Psychiatry* **157**: 745–750.

Doremus-Fitzwater TL, Varlinskaya EI, Spear LP (2010). Motivational systems in adolescence: possible implications for age differences in substance abuse and other risk-taking behaviors. *Brain Cogn* **72**: 114–123.

Doyle LMF, Yarrow K, Brown P (2005). Lateralization of event-related beta desynchronization in the EEG during pre-cued reaction time tasks. *Clin Neurophysiol* **116**: 1879–1888.

Dreher J-C, Meyer-Lindenberg A, Kohn P, Berman KF (2008). Age-related changes in midbrain dopaminergic regulation of the human reward system. *Proc Natl Acad Sci USA* **105**: 15106–15111.

Duka T, Townshend JM (2004). The priming effect of alcohol pre-load on attentional bias to alcohol-related stimuli. *Psychopharmacology (Berl)* **176**: 353–361.

Durant RH, Smith JA, Kreiter SR, Krowchuk DP (1999). The relationship between early age of onset of initial substance use and engaging in multiple health risk behaviors among young adolescents. *Arch pediatryic Adolesc Med* **153**: 286–291.

Easdon C, Izenberg A, Armilio ML, Yu H, Alain C (2005). Alcohol consumption impairs stimulus- and error-related processing during a Go/No-Go Task. *Brain Res Cogn Brain Res* **25**: 873–883.

Easdon CM, Vogel-Sprott M (2000). Alcohol and behavioral control: Impaired response inhibition and flexibility in social drinkers. *Exp Clin Psychopharmacol* **8**: 387–394.

Eberl C, Wiers RW, Pawelczack S, Rinck M, Becker ES, Lindenmeyer J (2013). Approach bias modification in alcohol dependence: do clinical effects replicate and for whom does it work best? *Dev Cogn Neurosci* **4**: 38–51.

Eickhoff SB, Stephan KE, Mohlberg H, Grefkes C, Fink GR, Amunts K, *et al* (2005) A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* **25**: 1325–1335.

Ernst LH, Plichta MM, Dresler T, Zesewitz AK, Tupak S V, Haeussinger FB, *et al* (2014). Prefrontal correlates of approach preferences for alcohol stimuli in alcohol dependence. *Addict Biol* **19(3)**: 497-508.

Ernst M, Pine DS, Hardin M (2006). Triadic model of the neurobiology of motivated behavior in adolescence. *Psychol Med* **36**: 299–312.

Everitt BJ, Belin D, Economidou D, Pelloux Y, Dalley JW, Robbins TW (2008). Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos Trans R Soc Lond B Biol Sci* **363**: 3125–3135.

Faria RR, Lima Rueda AV, Sayuri C, Soares SL, Malta MB, Carrara-Nascimento PF, *et al* (2008). Environmental modulation of ethanol-induced locomotor activity: Correlation with neuronal activity in distinct brain regions of adolescent and adult Swiss mice. *Brain Res* **1239**: 127–140.

Farris SR, Ostafin BD (2008). Alcohol consumption primes automatic alcohol-approach associations. *Am J Drug Alcohol Abuse* **34**: 703–711.

Feil J, Sheppard D, Fitzgerald PB, Yücel M, Lubman DI, Bradshaw JL (2010). Addiction, compulsive drug seeking, and the role of frontostriatal mechanisms in regulating inhibitory control. *Neurosci Biobehav Rev* **35**: 248–275.

Fein G, Chang M (2008). Smaller feedback ERN amplitudes during the BART are associated with a greater family history density of alcohol problems in treatment-naïve alcoholics. *Drug Alcohol Depend* **92**: 141–148.

Fernandez- Serrano MJ, Perez-Garcia M, Verdejo-Garcia A (2011). What are the specific. vs. generalized effects of drugs of abuse on neuropsychological performance? *Neurosci Biobehav Rev* **35**: 377-406.

Fernie G, Christiansen P, Cole JC, Rose AK, Field M (2012). Effects of 0.4g/kg alcohol on attentional bias and alcohol-seeking behaviour in heavy and moderate social drinkers. *J Psychopharmacol* **26**: 1017–1025.

Field M, Caren R, Fernie G, De Houwer J (2011). Alcohol approach tendencies in heavy drinkers: comparison of effects in a Relevant Stimulus-Response Compatibility task and an approach/avoidance Simon task. *Psychol Addict Behav* **25**: 697–701.

Field M, Christiansen P, Cole J, Goudie A (2007). Delay discounting and the alcohol Stroop in heavy drinking adolescents. *Addiction* **102**: 579–586.

Field M, Cox WM (2008). Attentional bias in addictive behaviors: a review of its development, causes, and consequences. *Drug Alcohol Depend* **97**: 1–20.

Field M, Kierman A, Eastwood B, Child R (2008). Rapid approach responses to alcohol cues in heavy drinkers. *J Behav Ther Exp Psy* **39**: 209-218.

Field M, Mogg K, Bradley BP (2005). Craving and cognitive biases for alcohol cues in social drinkers. *Alcohol Alcohol* **40**: 504–510.

Field M, Mogg K, Zetteler J, Bradley BP (2004). Attentional biases for alcohol cues in heavy and light social drinkers: the roles of initial orienting and maintained attention. *Psychopharmacology (Berl)* **176**: 88–93.

Field M, Schoenmakers T, Wiers R (2011). Cognitive Processes in Alcohol Binges: A Review and Research Agenda. *Addict Res* **1**: 263–279.

Field M, Wiers RW, Christiansen P, Fillmore MT, Verster JC (2010). Acute alcohol effects on inhibitory control and implicit cognition: Implications for loss of control over drinking. *Alcohol* **34**: 1346–1352.

Filbey FM, Claus E, Audette AR, Niculescu M, Banich MT, Tanabe J, *et al* (2008*a*). Exposure to the taste of alcohol elicits activation of the mesocorticolimbic neurocircuitry. *Neuropsychopharmacology* **33**: 1391–1401.

Filbey FM, Ray L, Smolen A, Claus ED, Audette A, Hutchison KE (2008*b*). Differential neural response to alcohol priming and alcohol taste cues is associated with DRD4 VNTR and *OPRM1* genotypes. *Alcohol Clin Exp Res* **32**: 1113–1123.

Flagel SB, Akil H, Robinson TE (2009). Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. *Neuropharmacology* **56 Suppl 1**: 139–148.

Fleming KA, Bartholow BD (2014). Alcohol cues, approach bias, and inhibitory control: applying a dual process model of addiction to alcohol sensitivity. *Psychol Addict Behav* **28**: 85–96.

Forbes EE, Dahl RE (2010). Pubertal development and behavior: hormonal activation of social and motivational tendencies. *Brain Cogn* **72**: 66–72.

Franke P, Wang T, Nöthen MM, Knapp M, Neidt H, Albrecht S, *et al* (2001). Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *Am J Med Genet* **105**: 114–119.

Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ (1997). Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage* **6**: 218–229.

Galvan A (2010). Adolescent development of the reward system. *Front Hum Neurosci* **4**: 1–9.

Galvan A, Hare TA, Parra CE, Penn J, Voss H, Glover G, *et al* (2006). Earlier development of the accumbens relative to orbitofrontal cortex might underlie risk-taking behavior in adolescents. *J Neurosci* **26**: 6885–6892.

Geier CF, Terwilliger R, Teslovich T, Velanova K, Luna B (2010). Immaturities in reward processing and its influence on inhibitory control in adolescence. *Cereb Cortex* **20**: 1613–1629.

Gelernter J, Kranzler HR, Cubells J (1999). Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. *Mol Psychiatry* **4**: 476–483.

Gianoulakis C (2009). Endogenous opioids and addiction to alcohol and other drugs of abuse. *Curr Top Med Chem* **9(11)**: 999-1015.

Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, *et al* (1999). Brain development during childhood and adolescence: a longitudinal MRI study. *Nat Neurosci* **2**: 861–863.

Gladwin TE, Vink M (2008). hiro3 [Computer Software]. Available from tegladwin.com.

Gladwin TE, 't Hart BM, de Jong R (2008). Dissociations between motor-related EEG measures in a cued movement sequence task. *Cortex* **44**: 521–536.

Gladwin TE, Figner B, Crone EA, Wiers RW (2011). Addiction, adolescence, and the integration of control and motivation. *Dev Cogn Neurosci* **1**: 364–376.

Gladwin TE, Figner B. (2014) "Hot" cognition and dual systems: Introduction, criticisms, and ways forward. In: Wilhelms E, Reyna VF (eds.) *Frontiers of Cognitive Psychology Series: Neuroeconomics, Judgment and Decision Making*. Psychological Press: New York, pp 157–180.

Gladwin TE, Lindsen JP, de Jong R (2006). Pre-stimulus EEG effects related to response speed, task switching and upcoming response hand. *Biol Psychol* **72**: 15–34.

Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, *et al* (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Natl Acad Sci U S A* **101**: 8174–8179.

Goudriaan AE, Grekin ER, Sher KJ (2011). Decision making and response inhibition as predictors of heavy alcohol use: a prospective study. *Alcohol Clin Exp Res* **35**: 1050–1057.

Graham JW, Flay BR, Johnson CA, Hansen WB, Grossman L, Sobel JL (1984). Reliability of self-report measures of drug use in prevention research: Evaluation of the Project SMART questionnaire via the test–retest reliability matrix. *J Drug Edu* **14**: 175–193.

Grant BF, Dawson DA (1997). Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: Results from the national longitudinal alcohol epidemiologic survey national institute on alcohol abuse and alcoholism. *J Subst Abuse* **9**: 103–110.

Gratton G, Coles MGH, Donchin E (1983). A new method for off-line removal of ocular artifact. *Eletroencephalography Clin Neurophysiol* **55**: 468–484.

Grenard JL, Ames SL, Wiers R, Thush C, Sussman S, Stacy AW (2008). Working memory capacity moderates the predictive effects of drug-related associations on substance use. *Psychol Addict Behav* **22**: 426–432.

Grose-Fifer J, Rodrigues A, Hoover S, Zottoli T (2013). Attentional capture by emotional faces in adolescence. *Adv Cogn Psychol* **9**: 81–91.

Guerri C, Pascual M (2010). Mechanisms involved in the neurotoxic, cognitive, and neurobehavioral effects of alcohol consumption during adolescence. *Alcohol* **44**: 15–26.

Hagemann T (2010). Alcohol en het Adolescente Brein. *Trimbos Inst* .

Hajcak G, Simons RF (2008). Oops!.. I did it again: an ERP and behavioral study of double-errors. *Brain Cogn* **68**: 15–21.

Hanna EZ, Yi H, Dufour MC, Whitmore CC (2001). The relationship of early-onset regular smoking to alcohol use, depression, illicit drug use, and other risky behaviors during early adolescence: Results from the youth supplement to the Third National Health and Nutrition Examination Survey. *J Subst Abuse* **13**: 265–282.

Heitzeg M, Nigg J (2008). Affective circuitry and risk for alcoholism in late adolescence: differences in frontostriatal responses between vulnerable and resilient children of alcoholic parents. *Alcohol Clin Exp Res* **32**: 414–426.

Hernández OH, Vogel-Sprott M (2010). Alcohol slows the brain potential associated with cognitive reaction time to an omitted stimulus. *J Stud Alcohol Drugs* **71**: 268–277.

Herrmann MJ, Weijers HG, Wiesbeck GA, Aranda D, Böning J, Fallgatter AJ (2000). Event-related potentials and cue-reactivity in alcoholism. *Alcohol Clin Exp Res* **24**: 1724–1729.

Hibell B, Guttormsson U, Balakireva O, Bjarnason T, Kokkevi A, Kraus L (2012). The 2011 ESPAD Report: Substance use among students in 36 European countries. *Swedish Counc Inf Alcohol Other Drugs*.

Hodgson R, Rankin H, Stockwell T (1979). Alcohol dependence and the priming effect. *Behav Res Ther* **17**: 379–387.

Hogan AM, Vargha-Khadem F, Kirkham FJ, Baldeweg T (2005). Maturation of action monitoring from adolescence to adulthood: an ERP study. *Dev Sci* **8**: 525–534.

Houben K, Havermans RC, Nederkoorn C, Jansen A (2012). Beer à no-go: learning to stop responding to alcohol cues reduces alcohol intake via reduced affective associations rather than increased response inhibition. *Addiction* **107**: 1280–1287.

Houben K, Wiers RW (2006). Assessing implicit alcohol associations with the Implicit Association Test: fact or artifact? *Addict Behav* **31**: 1346–1362.

Huijding J, de Jong PJ (2005). A pictorial version of the extrinsic affective simon task. *Exp Psychol* **52**: 289–295.

Ivanov I, Schulz KP, London ED, Newcorn JH (2008). Inhibitory control deficits in childhood and risk for substance use disorders: a review. *Am J Drug Alcohol Abuse* **34**: 239–258.

Jentsch JD, Taylor JR (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl)* **146**: 373–390.

Johnsen BH, Laberg JC, Cox WM, Vaksdal A, Hugdahl K (1994). Alcoholic subjects ' attentional bias in the processing of alcohol-related words. *Psychol Addict Behav* **8**: 111–115.

Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE (2012). *Monitoring the future. National results on adolescence drug use: Overview of key findings, 2011*. Ann Arbor: Inst Soc Res Univ Michigan.

Joyce CA, Gorodnitsky IF, Kutas M (2004). Automatic removal of eye movement and blink artifacts from EEG data using blind component separation. **41**: 313–325.

Kandel DB, Yamaguchi K (1985). Developmental patterns of the use of legal, illegal, and medically prescribed psychotropic drugs from adolescence to young adulthood. *NIDA RES Monogr* **56**: 193-235.

Keren G, Schul Y (2009). Two is not always better than one: A critical evaluation of two-system theories. *Perspect Psychol Sci* **4**: 533–550.

Kirk JM, de Wit H (2000). Individual differences in the priming effect of ethanol in social drinkers. *J Stud Alcohol* **61**: 64–71.

Kober H, Mende-Siedlecki P, Kross EF, Weber J, Mischel W, Hart CL, *et al* (2010). Prefrontal-striatal regulation of craving pathway underlies cognitive regulation of craving. *Proc Natl Acad Sci USA* **107**: 14811–14816.

Koller G, Zill P, Rujescu D, Ridinger M, Pogarell O, Fehr C, *et al* (2012). Possible association between OPRM1 genetic variance at the 118 locus and alcohol dependence in a large treatment sample: relationship to alcohol dependence symptoms. *Alcohol Clin Exp Res* **36**: 1–7.

Koob G, Kreek MJ (2007). Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am J Psychiatry* **164**: 1149–1159.

Korucuoglu O, Gladwin TE, Wiers RW (2014). Preparing to approach or avoid alcohol: EEG correlates, and acute alcohol effects. *Neurosci Lett* **559**: 199–204.

Kranczioch C, Athanassiou S, Shen S, Gao G, Sterr A (2008). Short-term learning of a visually guided power-grip task is associated with dynamic changes in EEG oscillatory activity. *Clin Neurophysiol* **119**: 1419–1430.

Kranzler HR, Gelernter J, Malley SO, Hernandez-avila CA, Kaufrnan D (1998). Association of alcohol or other drug dependence with alleles of the M opioid receptor gene (OPRM1). *Alcohol Clin Exp Res* **22**: 1359–1362.

Kreusch F, Vilenne A, Quertemont E (2013). Response inhibition towards alcohol-related cues using an alcohol go/no-go task in problem and non-problem drinkers. *Addict Behav* **38**: 2520-2528.

Labyt E, Szurhaj W, Bourriez J-L, Cassim F, Defebvre L, Destée A, *et al* (2003). Changes in oscillatory cortical activity related to a visuomotor task in young and elderly healthy subjects. *Clin Neurophysiol* **114**: 1153–1166.

Ladouceur CD, Dahl RE, Carter CS (2004). ERP correlates of action monitoring in adolescence. *Ann New york Acad Sci* **1021**: 329–336.

Ladouceur CD, Dahl RE, Carter CS (2007). Development of action monitoring through adolescence into adulthood: ERP and source localization. *Dev Sci* **10**: 874–891.

Larson MJ, Perlstein WM, Stigge-Kaufman D, Kelly KG, Dotson VM (2006). Affective context-induced modulation of the error-related negativity. *Neuroreport* **17**: 329–333.

Lau HC, Passingham RE (2007). Unconscious activation of the cognitive control system in the human prefrontal cortex. *J Neurosci* **27**: 5805–5811.

Lavric A, Pizzagalli DA, Forstmeier S (2004). When “go” and “nogo” are equally frequent: ERP components and cortical tomography. *Eur J Neurosci* **20**: 2483-2488.

Lawrence AJ, Luty J, Bogdan N a, Sahakian BJ, Clark L (2009). Impulsivity and response inhibition in alcohol dependence and problem gambling. *Psychopharmacology (Berl)* **207**: 163–172.

Lebel C, Beaulieu C (2011). Longitudinal development of human brain wiring continues from childhood into adulthood. *J Neurosci* **31**: 10937–10947.

Lee E, Namkoong K, Lee CH, An SK, Lee BO (2006). Differences of photographs inducing craving between alcoholics and non-alcoholics. *Yonsei Med J* **47**: 491–497.

Leyton M, Vezina P (2012). On cue: striatal ups and downs in addictions. *Biol Psychiatry* **72**: e21–e22.

Leyton M, Vezina P (2013). Striatal ups and downs: their roles in vulnerablity to addictions in human. *Neurosci Biobehav Rev* **37**: 1999-2014.

Lieberman MD, Cunningham WA (2009). Type I and Type II error concerns in fMRI research: re-balancing the scale. *Soc Cogn Affect Neurosci* **4**: 423–428.

Little PJ, Kuhn CM, Wilson WA, Swartzwelder HS (1996). Differential effects of ethanol in adolescents and adult rats. *ACER* **20(8)**: 1346-1351.

Loeber S, Duka T (2009). Acute alcohol impairs conditioning of a behavioural reward-seeking response and inhibitory control processes--implications for addictive disorders. *Addiction* **104**: 2013–2022.

Loh EW, Fann CSJ, Chang YT, Chang CJ, Cheng ATA (2004). Endogenous opioid receptor genes and alcohol dependence among Taiwanese Han. *Alcohol Clin Exp Res* **28**: 15–19.

Love A, Es DJAM, Willner P (1998). A comparison of two alcohol craving questionnaires. *Addiction* **93**: 1091–1102.

Luijten M, Littel M, Franken IHA (2011). Deficits in inhibitory control in smokers during a Go/NoGo task: an investigation using event-related brain potentials. *PLoS One* **6**: e18898.

Luna B, Padmanabhan A, O'Hearn K (2010). What has fMRI told us about the development of cognitive control through adolescence? *Brain Cogn* **72**: 101–113.

Lutz P-E, Kieffer BL (2013). The multiple facets of opioid receptor function: implications for addiction. *Curr Opin Neurobiol* **23**: 473–479.

Marinkovic K, Halgren E, Klopp J, Maltzman I (1994). Alcohol effects on Movement-related Potentials : A Measure of Impulsivity?*. *J Stud Alcohol* **61**: 24–31.

Markwiese BJ, Acheson SK, Levin ED, Wilson WA, Swartzwelder HS (1998). Differential effects of ethanol on memory in adolescent and adult rats. *Alcohol Clin Exp Res* **22**: 416–421.

Marlatt GA, Rohsenow DJ (1980). Cognitive processes in alcohol use: expectancy and the balanced placebo design. In: Mello NK (eds.) *Advances in substance abuse: behavioral and biological research.* Greenwich: JAI Press, pp 159-199.

Merrer JLE (2009). Reward processing by the opioid system in the brain. *Physiol Rev* **89**: 1379–1412.

Miranda R, Ray L, Blanchard A, Reynolds EK, Monti PM, Chun T, *et al* (2013). Effects of naltrexone on adolescent alcohol cue reactivity and sensitivity: an initial randomized trial. *Addict Biol* **18**: 193–201.

Miranda R, Ray L, Justus A, Meyerson LA, Knopik VS, McGeary J, *et al* (2010). Initial evidence of an association between OPRM1 and adolescent alcohol misuse. *Alcohol Clin Exp Res* **34**: 112–122.

Mitchell JM, Tavares VC, Fields HL, D'Esposito M, Boettiger CA (2007). Endogenous opioid blockade and impulsive responding in alcoholics and healthy controls. *Neuropsychopharmacology* **32**: 439–449.

Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A, Wager TD (2000). The unity and diversity of executive functions and their contributions to complex "Frontal Lobe" tasks: a latent variable analysis. *Cogn Psychol* **41**: 49–100.

Morean ME, Corbin WR (2010). Subjective response to alcohol: a critical review of the literature. *Alcohol Clin Exp Res* **34**: 385–395.

Motzkin JC, Baskin-Sommers A, Newman JP, Kiehl KA, Koenigs M (2014). Neural correlates of substance abuse: Reduced functional connectivity between areas underlying reward and cognitive control. *Hum Brain Mapp* **35**: 4282-4292.

Myrick H, Anton R (2004). Recent advances in the pharmacotherapy of alcoholism. *Curr Psychiatry Rep* **6**: 332–338.

Nam CS, Jeon Y, Kim Y-J, Lee I, Park K (2011). Movement imagery-related lateralization of event-related (de)synchronization (ERD/ERS): motor-imagery duration effects. *Clin Neurophysiol* **122**: 567–577.

Nees F, Tzschoppe J, Patrick CJ, Vollstädt-Klein S, Steiner S, Poustka L, *et al* (2012). Determinants of early alcohol use in healthy adolescents: the differential contribution of neuroimaging and psychological factors. *Neuropsychopharmacology* **37**: 986–995.

Newlin D, Thomson J (1999). Chronic tolerance and sensitization to alcohol in sons of alcoholics: II. Replication and reanalysis. *Exp Clin Psychopharmacol* **7**: 234–243.

Newlin DB, Thomson JB (1990). Alcohol challenge with sons of alcoholics: a critical review and analysis. *Psychol Bull* **108**: 383–402.

Nielsen D, Utrankar A, Reyes J, Simons D, Kosten T (2012). Epigenetics of drug abuse: predisposition or response. *Pharmacogenomics* **13**: 1149–1160.

Nieuwenhuis S, Ridderinkhof KR, Blom J, Band GP, Kok A (2001). Error-related brain potentials are differentially related to awareness of response errors: evidence from an antisaccade task. *Psychophysiology* **38**: 752–760.

Nieuwenhuis S, Yeung N, van den Wildenberg W, Ridderinkhof KR (2003). Electrophysiological correlates of anterior cingulate function in a go/nogo task: effects of response conflict and trial type frequency. *Cogn Affect Behav Neurosci* **3:** 17-26.

Nigg JT, Glass JM, Wong MM, Poon E, Jester JM, Fitzgerald HE, *et al* (2004). Neuropsychological executive functioning in children at elevated risk for alcoholism: findings in early adolescence. *J Abnorm Psychol* **113**: 302–314.

Nigg JT, Wong MM, Martel MM, Jester JM, Puttler LI, Glass JM, *et al* (2006). Poor response inhibition as a predictor of problem drinking and illicit drug use in adolescents at risk for alcoholism and other substance use disorders. *J Am Acad Child Adolesc Psychiatry* **45**: 468–475.

Nikolaou K, Field M, Critchley H, Duka T (2013). Acute alcohol effects on attentional bias are mediated by subcortical areas associated with arousal and salience attribution. *Neuropsychopharmacology* **38**: 1365–1373.

Noël X, van der Linden M, d'Acremont M, Bechara A, Dan B, Hanak C, *et al* (2007). Alcohol cues increase cognitive impulsivity in individuals with alcoholism. *Psychopharmacology (Berl)* **192**: 291–298.

Norman AL, Pulido C, Squeglia LM, Spadoni AD, Paulus MP, Tapert SF (2011). Neural activation during inhibition predicts initiation of substance use in adolescence. *Drug Alcohol Depend* **119**: 216–223.

Olvet DM, Hajcak G (2009). The stability of error-related brain activity with increasing trials. *Psychophysiology* **46**: 957–961.

Oostenveld R, Fries P, Maris E, Schoffelen J-M (2011). FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput Intell Neurosci* **2011**: 156869.

Ostafin BD, Palfai TP (2006). Compelled to consume: the implicit association test and automatic alcohol motivation. *Psychol Addict Behav* **20**(3): 322-327.

Palfai TP, Ostafin BD (2003). Alcohol-related motivational tendencies in hazardous drinkers: assessing implicit response tendencies using the modified-IAT. *Behav Res Ther* **41**: 1149–1162.

Park SQ, Kahnt T, Beck A, Cohen MX, Dolan RJ, Wrase J, *et al* (2010). Prefrontal cortex fails to learn from reward prediction errors in alcohol dependence. *J Neurosci* **30**: 7749–7753.

Pascual M, Boix J, Felipo V, Guerri C (2009). Repeated alcohol administration during adolescence causes changes in the mesolimbic dopaminergic and glutamatergic systems and promotes alcohol intake in the adult rat. *J Neurochem* **108**: 920–931.

Paus T (2005). Mapping brain maturation and cognitive development during adolescence. *Trends Cogn Sci* **9**: 60–68.

Peeters M, Monshouwer K, van de Schoot RAGJ, Janssen T, Vollebergh WAM, Wiers RW (2013). Automatic processes and the drinking behavior in early adolescence: a prospective study. *Alcohol Clin Exp Res* **37**: 1737–1744.

Petersen AC (1988). Adolescent development. *Annu Rev Psychol* **39**: 583–607.

Petit G, Kornreich C, Noël X, Verbanck P, Campanella S (2012). Alcohol-related context modulates performance of social drinkers in a visual Go/No-Go task: a preliminary assessment of event-related potentials. *PLoS One* **7**: e37466.

Pfeifer JH, Allen NB (2012). Arrested development? Reconsidering dual-systems models of brain function in adolescence and disorders. *Trends Cogn Sci* **16**: 322–329.

Pfurtscheller G, Neuper C, Pichler-Zalaudek K, Edlinger G, Lopes da Silva FH (2000). Do brain oscillations of different frequencies indicate interaction between cortical areas in humans? *Neurosci Lett* **286**: 66–68.

Pieters S, Burk WJ, van der Vorst H, Wiers RW, Engels RCME (2012). The moderating role of working memory capacity and alcohol-specific rule-setting on the relation between approach tendencies and alcohol use in young adolescents. *Alcohol Clin Exp Res* **36**: 915–922.

Pieters S, van der Vorst H, Burk WJ, Schoenmakers TM, van den Wildenberg E, Smeets HJ, *et al* (2011). The effect of the *OPRM1* and DRD4 polymorphisms on the relation between attentional bias and alcohol use in adolescence and young adulthood. *Dev Cogn Neurosci* **1**: 591–599.

Poljac E, Yeung N (2014). Dissociable neural correlates of intention and action preparation in voluntary task switching. *Cereb Cortex* **24**: 465–478.

Pulido C, Mok A, Brown SA, Tapert SF (2009). Heavy drinking relates to positive valence ratings of alcohol cues. *Addict Biol* **14**: 65–72.

Quevedo KM, Benning SD, Gunnar MR, Dahl RE (2009). The onset of puberty: effects on the psychophysiology of defensive and appetitive motivation. *Dev Psychopathol* **21**: 27–45.

Quirk GJ, Beer JS (2006). Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Curr Opin Neurobiol* **16**: 723–727.

Ramchandani VA, Umhau J, Pavon FJ, Ruiz-Velasco V, Margas W, Sun H, *et al* (2011). A genetic determinant of the striatal dopamine response to alcohol in men. *Mol Psychiatry* **16**: 809–817.

Ray LA, Hutchison KE (2004). A polymorphism of the mu-*OPRM1* receptor gene (*OPRM1*) and sensitivity to the effects of alcohol in humans. *Alcohol Clin Exp Res* **28**: 1789-1795.

Ray LA, Courtney KE, Hutchison KE, Mackillop J, Galvan A, Ghahremani DG (2014). Initial evidence that *OPRM1* genotype moderates ventral and dorsal striatum functional connectivity during alcohol cues. *Alcohol Clin Exp Res* **38**: 78-89.

Ray LA, McGeary J, Marshall E, Hutchison KE (2006). Risk factors for alcohol misuse: examining heart rate reactivity to alcohol, alcohol sensitivity, and personality constructs. *Addict Behav* **31**: 1959–1973.

Renthal W, Nestler EJ (2008). Epigenetic mechanisms in drug addiction. *Trends Mol Med* **14**: 341–350.

Rhodes LE, Obitz FW, Creel D (1975). Effect of alcohol and task on hemispheric asymmetry of visually evoked potentials in man. *Electroencephalogr Clin Neurophysiol* **38**: 561–568.

Ridderinkhof KR, de Vlugt Y, Bramlage A, Spaan M, Snel J, Band GPH (2002). Alcohol consumption impairs detection of performance errors in mediofrontal cortex. *Science* **298**: 2209–2211.

Rinck M, Becker ES (2007). Approach and avoidance in fear of spiders. *J Behav Ther Exp Psychiatry* **38**: 105–120.

Robinson TE, Berridge K (1993). The neural basis of drug craving: an incentive - sensitization theory of addiction. *Brain Res Rev* **18**: 247–291.

Robinson TE, Berridge KC (2008). The incentive sensitization theory of addiction: some current issues. *Philos Trans R Soc Lond B Biol Sci* **363**: 3137–3146.

Rose AK, Duka T (2008). Effects of alcohol on inhibitory processes. *Behav Pharmacol* **19**: 284–291.

Rose JE, McClernon FJ, Froeliger B, Behm FM, Preud'homme X, Krystal AD (2011). Repetitive transcranial magnetic stimulation of the superior frontal gyrus modulates craving for cigarettes. *Biol Psychiatry* **70**: 794–799.

Rueger SY, McNamara PJ, King AC (2009). Expanding the utility of the Biphasic Alcohol Effects Scale (BAES) and initial psychometric support for the Brief-BAES (B-BAES). *Alcohol Clin Exp Res* **33**: 916–924.

Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M (1993). Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption--II. *Addiction* **88**: 791–804.

Schinka JA, Town T, Abdullah L, Crawford FC, Ordorica PI, Francis E, *et al* (2002). A functional polymorphism within the m-opioid receptor gene and risk for abuse of alcohol and other substances. *Mol Psychiatry* **7**: 224–228.

Schmierer K, Wheeler-Kingshott CAM, Boulby PA, Scaravilli F, Altmann DR, Barker GJ, *et al* (2007). Diffusion tensor imaging of post mortem multiple sclerosis brain. *Neuroimage* **35**: 467–477.

Schoenmakers T, Wiers RW, Field M (2008). Effects of a low dose of alcohol on cognitive biases and craving in heavy drinkers. *Psychopharmacology (Berl)* **197**: 169–178.

Schoenmakers TM, Wiers RW (2010). Craving and attentional bias respond differently to alcohol priming: a field study in the pub. *Eur Addict Res* **16**: 9–16.

Schuckit MA (1994). Low level of response to alcohol as a predictor of future alcoholism. *Am J Psychiatry*, **151**: 184-189.

Schuckit MA, Duthie LA, Mahler HI, Irwin M, Monteiro MG (1991). Subjective feelings and changes in body sway following diazepam in sons of alcoholics and control subjects. *J Stud Alcohol* **52**: 601–608.

Schuckit MA, Gold EO, Croot K, Finn P, Polich J (1988). P300 latency after ethanol ingestion in sons of alcoholics and in controls. *Biol Psychiatry* **24**: 310–315.

Schuckit MA, Smith TL, Kalmijn J, Tsuang J, Hesselbrock V, Bucholz K (2000). Response to alcohol in daughters of alcoholics: a pilot study and a comparison with sons of alcoholics. *Alcohol Alcohol* **35**: 242-248.

Schuckit MA, Smith TL, Tipp JE (1997). The Self- Rating of the Effects of alcohol (SRE) from as a retrospective measure of the risk for alcoholism. *Addiction* **92**: 979-988.

Schulenberg JE, Merline AC, Johnston LD, Malley PMO, Bachman JG, Virginia B (2006). Trajectories of marijuana use during the transition to adulthood: the big picture based on national panel data. *J Drug Issues* **35**: 255–279.

Shafritz KM, Collins SH, Blumberg HP (2006). The interaction of emotional and cognitive neural systems in emotionally guided response inhibition. *Neuroimage* **31**: 468–475.

Sharbanee JM, Hu L, Stritzke WGK, Wiers RW, Rinck M, MacLeod C (2014). The effect of approach/avoidance training on alcohol consumption is mediated by change in alcohol action tendency. *PLoS One* **9**: e85855.

Sharbanee JM, Stritzke WGK, Wiers RW, Young P, Rinck M, Macleod C (2012). The interaction of approach-alcohol action tendencies, working memory capacity, and current task goals predicts the inability to regulate drinking behavior. *Psychol Addict Behav* **27**: 649–661.

Sharma D, Albery IP, Cook C (2001). Selective attentional bias to alcohol related stimuli in problem drinkers and non-problem drinkers. *Addiction* **96**: 285–295.

Sobell, LC, Sobell MB (1992). Timeline followback: A technique for assessing self-reported alcohol consumption. In: J. Allen & R. Z. Litten (eds.) *Measuring alcohol consumption: Psychosocial and biological methods*. Totowa NJ: Humana, pp 41-72.

Somerville LH, Jones RM, Casey BJ (2010). A time of change: behavioral and neural correlates of adolescent sensitivity to appetitive and aversive environmental cues. *Brain Cogn* **72**: 124–133.

Squeglia LM, Jacobus J, Tapert SF (2009). Development, the influence of substance use on adolescent brain. *Clin EEG Neurosci* **40**: 31–38.

Squeglia LM, Pulido C, Wetherill RR (2012). Brain response to working memory over three years of adolescence: influence of initiating heavy drinking. *J Stud Alcohol Drugs* **73**: 749–760.

Squeglia LM, Schweinsburg AD, Pulido C, Tapert SF (2011). Adolescent binge drinking linked to abnormal spatial working memory brain activation: differential gender effects. *Alcohol Clin Exp Res* **35**: 1831–1841.

Stacy AW, Ames SL, Knowlton BJ (2004). Neurologically plausible distinctions in cognition relevant to drug use etiology and prevention. *Subst Use Misuse* **39** (10–12), 1571–1623.

Stancák A, Pfurtscheller G (1995). Desynchronization and recovery of β rhythms during brisk and slow self-paced finger movements in man. *Neurosci Lett* **196**: 21–24.

Steinberg L (2005). Cognitive and affective development in adolescence. *Trends Cogn Sci* **9**: 69–74.

Suhler CL, Churchland PS (2009). Control: conscious and otherwise. *Trends Cogn Sci* **13**: 341–347.

Tapert SF, Brown GG, Kindermann SS, Cheung EH, Frank LR, Brown SA (2001). fMRI measurement of brain dysfunction in alcohol-dependent young women. *Alcohol Clin Exp Res* **25**: 236–245.

Tapert SF, Cheung EH, Brown GG, Frank LR, Paulus MP, Schweinsburg AD, *et al* (2003). Neural response to alcohol stimuli in adolescents with alcohol use disorder. *Arch Gen Psychiatry* **60**: 727–735.

Tarazi FI, Tomasini EC, Baldessarini RJ (1998). Postnatal development of dopamine D4 -like receptors in rat forebrain regions: comparison with D2 -like receptors. *Dev Brain Res* **110**: 227–233.

Teicher MH, Andersen SL, Jr JCH (1995). Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. *Dev Brain Res* **89**: 167–172.

Thomas SE, Drobes DJ, Deas D (2005). Alcohol cue reactivity in alcohol-dependent adolescents. *J Stud Alcohol* **66**: 354–360.

Thush C, Wiers R (2007). Explicit and implicit alcohol-related cognitions and the prediction of future drinking in adolescents. *Addict Behav* **32**: 1367–1383.

Thush C, Wiers RW, Ames SL, Grenard JL, Sussman S, Stacy AW (2007). Apples and oranges? Comparing indirect measures of alcohol-related cognition predicting alcohol use in at-risk adolescents. *Psychol Addict Behav* **21**: 587–591.

Thush C, Wiers RW, Ames SL, Grenard JL, Sussman S, Stacy AW (2008). Interactions between implicit and explicit cognition and working memory capacity in the prediction of alcohol use in at-risk adolescents. *Drug Alcohol Depend* **94**: 116–124.

Tidey JW, Monti PM, Rohsenow DJ, Gwaltney CJ, Miranda R, McGeary JE, *et al* (2008). Moderators of naltrexone's effects on drinking, urge, and alcohol effects in non-treatment-seeking heavy drinkers in the natural environment. *Alcohol Clin Exp Res* **32**: 58–66.

Toni I, Schluter ND, Josephs O, Friston K, Passingham RE (1999). Activity in the Human Brain: An Event-related fMRI Study. *Cereb Cortex* **9**: 35–49.

Townshend JM, Duka T (2001). Attentional bias associated with alcohol cues: differences between heavy and occasional social drinkers. *Psychopharmacology (Berl)* **157**: 67–74.

Tsujii T, Sakatani K, Nakashima E, Igarashi T, Katayama Y (2011). Characterization of the acute effects of alcohol on asymmetry of inferior frontal cortex activity during a Go/No-Go task using functional near-infrared spectroscopy. *Psychopharmacology (Berl)* **217**: 595–603.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, *et al* (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* **15**: 273–289.

Uddin LQ, Supekar KS, Ryali S, Menon V (2011). Dynamic reconfiguration of structural and functional connectivity across core neurocognitive brain networks with development. *J Neurosci* **31**: 18578–18589.

Van den Wildenberg E, Wiers RW, Dessers J, Janssen RGJH, Lambrichs EH, Smeets HJM, , *et al* (2007) A functional polymorphism of the mu-opioid receptor gene (*OPRM1*) influences cue-induced craving for alcohol in male heavy drinkers. *Alcohol Clin Exp Res* **31**: 1–10.

Van der Zwaluw CS, van den Wildenberg E, Wiers RW, Franke B, Buitelaar J, Scholte RHJ, *et al* (2007). Polymorphisms in the mu-opioid receptor gene (OPRM1) and the implications for alcohol dependence in humans. *Pharmacogenomics* **8**: 1427–1436.

Van Hemel-Ruiter ME, de Jong PJ, Wiers RW (2011). Appetitive and regulatory processes in young adolescent drinkers. *Addict Behav* **36**: 18–26.

Van Laar MW, Cruts AAN, Ooyen-Houben MMJ, Meijer RF, Croes EA, Brunt T, *et al* (2011). Netherlands National Drug Monitor: NDM Annual Report 2010. Utrecht, the Netherlands: Trimbos Institute.

Van Veen V, Carter C (2002). The anterior cingulate as a conflict monitor: fMRI and ERP studies. *Psychol Behav* **77** (4-5): 477-482.

Viding E, Williamson DE, Hariri AR (2006). Developmental imaging genetics: Challenges and promises for translational research. *Dev Psychopathol* **18**: 877–892.

Volkow ND, Fowler JS, Wang G-J, Swanson JM (2004) Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Mol Psychiatry* **9**: 557–569.

Vollstädt-Klein S, Wichert S, Rabinstein J, Bühler M, Klein O, Ende G, *et al* (2010). Initial, habitual and compulsive alcohol use is characterized by a shift of cue processing from ventral to dorsal striatum. *Addiction* **105**: 1741–1749.

Waters AJ, Shiffman S, Sayette MA, Paty JA, Gwaltney CJ, Balabanis MH (2003). Attentional bias predicts outcome in smoking cessation. *Heal Psychol* **22**: 378–387.

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

Watson P, de Wit S, Hommel B, Wiers RW (2012). Motivational mechanisms and outcome expectancies underlying the approach bias toward addictive substances. *Front Psychol* **3**: 440.

Watson PE, Watson ID, Batt RD, Phil D (1981). Prediction of blood alcohol concentrations in human subjects. *J Stud Alcohol* **42**: 547-556.

Weiland BJ, Welsh RC, Yau W-YW, Zucker RA, Zubieta J-K, Heitzeg MM (2013). Accumbens functional connectivity during reward mediates sensation-seeking and alcohol use in high-risk youth. *Drug Alcohol Depend* **128**: 130–139.

Wetherill RR, Squeglia LM, Yang TT, Tapert SF (2013). A longitudinal examination of adolescent response inhibition: neural differences before and after the initiation of heavy drinking. *Psychopharmacology (Berl)* **230**: 663–671.

Wheaton L a, Nolte G, Bohlhalter S, Fridman E, Hallett M (2005). Synchronization of parietal and premotor areas during preparation and execution of praxis hand movements. *Clin Neurophysiol* **116**: 1382–1390.

White AM, Bae JG, Truesdale MC, Ahmad S, Wilson WA, Swartzwelder HS (2002a). Chronic-intermittent ethanol exposure during adolescence prevents normal developmental changes in sensitivity to ethanol-induced motor impairments. *Alcohol Clin Exp Res* **26**: 960–968.

White AM, Ghia AJ, Levin ED, Swartzwelder HS (2000). Binge pattern ethanol exposure in adolescent and adult rats: Differential impact on subsequent responsiveness to ethanol. *Alcohol Clin Exp Res* **24**: 1251–1256.

White AM, Swartzwelder HS (2005). Age-related effects of alcohol on memory and memory-related brain function in adolescents and adults. *Recent Dev Alcohol* **17**: 161-176.

White AM, Truesdale MC, Bae JG, Ahmad S, Wilson WA, Best PJ, *et al* (2002b). Differential effects of ethanol on motor coordination in adolescent and adult rats. *Pharmacol Biochem Behav* **73**: 673–677.

White HR, Labouvie EW (1989). Towards the assessment of adolescent drinking problems. *J Stud Alcohol* **50**: 30–37.

Wiers CE, Stelzel C, Gladwin TE, Park SQ, Pawelczack S, Gawron CK, *et al* (2014a). Effects of Cognitive Bias Modification Training on Neural Alcohol Cue Reactivity in Alcohol Dependence. *Am J Psychiatry*, doi:10.1176/appi.ajp.2014.13111495.

Wiers CE, Stelzel C, Park SQ, Gawron CK, Ludwig VU, Gutwinski S, *et al* (2014b). Neural correlates of alcohol-approach bias in alcohol addiction: the spirit is willing but the flesh is weak for spirits. *Neuropsychopharmacology* **39**: 688–697.

Wiers RW, Bartholow BD, van den Wildenberg E, Thush C, Engels RCME, Sher KJ, *et al* (2007). Automatic and controlled processes and the development of addictive behaviors in adolescents: a review and a model. *Pharmacol Biochem Behav* **86**: 263–283.

Wiers RW, Eberl C, Rinck M, Becker ES, Lindenmeyer J (2011). Retraining automatic action tendencies changes alcoholic patients' approach bias for alcohol and improves treatment outcome. *Psychol Sci* **22**: 490–497.

Wiers RW, Gladwin TE, Hofmann W, Salemink E, Ridderinkhof KR (2013). Cognitive bias modification and cognitive control training in addiction and related psychopathology: mechanisms, clinical perspectives, and ways forward. *Clin Psychol Sci* **1**: 192–212.

Wiers RW, Hoogeveen K, Sergeant JA, Gunning WB (1997). High- and low-dose alcohol-related expectancies and the differential associations with drinking in male and female adolescents and young adults. *Addiction* **92**(7): 871-888.

Wiers RW, Rinck M, Dictus M, van den Wildenberg E (2009). Relatively strong automatic appetitive action-tendencies in male carriers of the OPRM1 G-allele. *Genes Brain Behav* **8**: 101–106.

Wiers RW, Rinck M, Kordts R, Houben K, Strack F (2010). Retraining automatic action-tendencies to approach alcohol in hazardous drinkers. *Addiction* **105**: 279–287.

Wiers RW, Stacy AW (2006). Implicit cognition and addiciton. *Curr Dir Psychol Sci* **15**: 292–296.

Wilson S, Sayette M, Fiez J (2004). Prefrontal responses to drug cues: a neurocognitive analysis. *Nat Neurosci*. **7**: 211-214.

Yalachkov Y, Kaiser J, Naumer MJ (2012). Functional neuroimaging studies in addiction: multisensory drug stimuli and neural cue reactivity. *Neurosci Biobehav Rev* **36**: 825–835.

Yeung N, Botvinick MM, Cohen JD (2004). The neural basis of error detection: Conflict monitoring and the error-related negativity. *Psychol Rev* **111**: 931-959.

Yeung N, Cohen JD (2006). The impact of cognitive deficits on conflict monitoring. *Psychol Sci* **17**: 164–171.

Yurgelun-Todd D (2007). Emotional and cognitive changes during adolescence. *Curr Opin Neurobiol* **17**: 251–257.

English Summary

Adolescence is a period accompanied with an increase in sensation seeking, exploration and risk taking behaviours. Many health risk behaviours, such as smoking or drinking, are initiated during this phase and deteriorates adolescents' health in later life. Dual process models are employed by many researchers in explaining behavioural changes that take place both in adolescents and in addiction. According to these models, both adolescents and individuals suffering from addictive behaviours, are characterized by an oversensitive impulsive system and a compromised cognitive control system, in the case of adolescents due to a maturational gap and in the case of addiction due to chronic drug use. Moreover, both acute and chronic use, affect similar processes, suggesting that we can gain knowledge on the long-term effects of alcohol by studying the effect of acute alcohol administration. Although many adolescents initiate alcohol and drug use during this period, only a minority develop substance use problems later in life. An important challenge in the field is to identify adolescent vulnerabilities for the development of addiction. Sensitivity to acute alcohol administration is one of the well-established risk factors for the development of addiction. Moreover, neurocognitive functioning prior to the progression of drinking behaviour has also been successfully used to identify cognitive risk pathways. However until now, no studies have assessed the predictive value of individual differences in neurocognitive processes under the effect of acute alcohol. The primary aim of this dissertation was to investigate the effect of acute alcohol on neurocognitive systems involved in the development of addictive behaviours in adolescents. A secondary aim of the project was to investigate whether alcohol-induced changes in cognitive and affective processes would be predictive of alcohol escalation in young people.

Relatively fast responses to approach compared to avoid alcohol cues (referred as approach bias) has been described as an important cognitive motivational process in the aetiology of alcohol use problems. Stimulus-response associations are central to the approach tendencies: For drinkers approaching compared to avoiding an alcohol cue is more compatible with their dominant action tendencies. Such stimulus-response compatibility effects can be investigated by comparing brain activity during response preparation for compatible and incompatible responses. In the first and second study, we investigated the effects of acute alcohol on response preparation for approach and avoid responses by studying brain signals in the beta frequency in young adults (Chapter 2) and adolescents (Chapter 3). When a preparatory period is provided between an informative cue and motor response, the beta frequency decreases over time (event-related desynchronization in the beta band, beta-ERD). The behavioural finding of these two studies suggested a tendency to respond faster when approaching alcohol pictures compared with avoiding them. Alcohol administration did not affect the approach bias in social drinkers mainly composed of young adults (Chapter 2) but it had an influence on the approach bias as a function of drinking profile in adolescents (Chapter 3). In a version of this task with explicit instructions to approach/avoid alcohol-related cues

(Chapter 2), posterior beta-ERD was found to increase during preparation for alcohol-approach trials, suggesting relatively strong advance response preparation for approaching alcohol. The posterior beta-ERD was further attenuated after alcohol administration. In a version of the task with implicit instructions (Chapter 3), we studied response preparation by comparing the strength of motor-related asymmetry of the beta-ERD. In earlier studies it was shown that when a task required more preparation, an increased asymmetry was observed. In heavy drinking adolescents, greater approach-related asymmetry in the beta-band was observed for soft-drink cues compared to alcohol cues and this increase was associated with increase in difficulty to regulate alcohol intake. Individuals who reported more difficulties in regulating their drinking (mostly heavy drinkers), had greater approach-related lateralization for soft-drink cues and individuals who reported less difficulties, had greater approach-related lateralization for alcohol cues. These findings suggest that greater beta-lateralization for soft-drink cues measured in heavy drinkers may represent a compensatory effort for the weaker S-R mapping. Earlier findings demonstrated that young heavy drinkers hold both positive and negative alcohol associations, reflecting an ambiguity towards alcohol. The MRAA findings in this study may highlight a mechanism related to overcompensation of ambivalent attitudes about drinking in our heavy drinking sample who had greater problems to limit their alcohol intake compared to light drinkers.

In a third study (Chapter 4), we tested alcohol-induced changes on inhibitory capacity in adolescents with an affective version of a Go/NoGo task. In this task subjects were instructed to give a motor response for frequent Go stimuli and stop responding for infrequent NoGo stimuli. Subjects performed two versions of the same task, one with alcohol pictures and the other one with soft-drink pictures as stimuli. Conflict monitoring and error detection processes were investigated with the N2 and the error-related negativity (ERN) ERP components. The main findings in this study were as follows: Both commission errors and the NoGo-N2 associated with conflict were greater for alcohol cues, suggesting that cues signalling reward opportunities might activate a go-response mode. The ERN results suggested a deficiency in the monitoring system for alcohol cues. Acute alcohol deteriorated error detection for control cues and conflict monitoring for alcohol cues. In a separate study (Chapter 5), we focused on how a genetic vulnerability for alcohol's rewarding effects observed in adult samples would affect adolescent brain response to alcohol taste-cues with a limited prior exposure to alcohol. This study demonstrated that adolescents with limited drinking experience carrying the G-allele of the OPRM1 gene did not reveal a higher striatal activity as it has been shown in adult heavy drinkers, but they demonstrated a lack of frontal regulation of striatal activity.

In the two EEG studies with adolescents (Chapter 3 and 4) the power of alcohol-induced changes in brain and behaviours in predicting subsequent escalation of drinking were also tested. In the study with Go/NoGo paradigm, alcohol-induced changes in an event-related

component associated with error detection were used as predictors. In this study, we found that those participants for whom alcohol disrupted the error detection processes for alcohol cues, were more likely to show a decrease in their drinking at the six months follow-up. In the approach-avoidance paradigm, behavioural approach tendencies and motor-related asymmetry index associated with response preparation were used as predictors. An association between alcohol-induced effects on a behavioural measure and future drinking was found. Individuals with relatively strong approach soft-drink and weak approach alcohol bias after acute alcohol, decreased their drinking six-months later.

In this project, tasks that tap into cognitive control, implicit and explicit action tendencies were used to assess late adolescents' brain responses after alcohol administration. In all studies, motivational influences were under investigation by either using contextual cues in cognitive tasks or directly assessing alcohol-related cognitions. Increased motivation towards alcohol-related cues was evident in all studies despite our adolescent sample having no substance use disorder. However, this increased motivation towards alcohol cues was not affected by a gene previously associated with appetitive motivation, suggesting that this genetic factor might only become important after repeated use. In adolescents neural activity associated with executive control processes was greater for control cues and alcohol administration dampened this activity. At the group level, alcohol administration did not decrease brain activity associated with error detection for alcohol cues. However, at the individual level, adolescents who showed a decrease in error detection for alcohol cues after alcohol, also decreased drinking six months later. In sum, the evidence in this study suggests that alcohol-induced changes in the monitoring system and the ability to regulate alcohol-related cognitions under the influence of alcohol can be a protective factor for the development of addictive behaviours. Alcohol-induced changes both on task performance and neural activity are shown to contribute to the prediction of changes in alcohol use. These neurocognitive predictors of alcohol escalation could be used in differentiating risk from resilient individuals or targeted in prevention research.

Nederlandse Samenvatting

De adolescentie is levensfase waarin de neiging om nieuwe ervaringen op te doen (sensatiezucht) en risicovol gedrag toenemen. Veel gedragingen die een risico voor de gezondheid vormen, zoals roken en drinken worden in deze fase van het leven geïnitieerd en zijn gerelateerd aan een slechtere gezondheid op latere leeftijd. Op basis van de resultaten uit verschillende neuroimaging studies wordt verondersteld dat zowel in de adolescentie als bij het ontstaan van verslaving, de balans tussen verschillende processen (tijdelijk) verdwenen is. Volgens dit model worden verslaafden en adolescenten beide gekarakteriseerd door een overgevoelig impulsief systeem en een relatief zwak cognitief controlesysteem. Bij adolescenten komt dit door een discrepantie in de ontwikkeling van deze systemen en bij verslaafden komt dit door het chronisch gebruiken van alcohol en drugs. Zowel acuut als chronisch gebruik van drugs heeft invloed op deze processen. Mogelijk kunnen we dus de langere termijn effecten van alcohol beter leren begrijpen door het acute effect alcohol goed te bestuderen. Hoewel veel adolescenten beginnen met het gebruik van alcohol en drugs in deze periode, blijft slechts een kleine groep ook veel drinken op latere leeftijd. Een belangrijke uitdaging in dit onderzoeksveld is het om te identificeren welke adolescenten een grotere kans hebben op het ontwikkelen van een verslaving. De gevoeligheid voor de farmacologische effecten van alcohol is een goed onderzochte voorspeller voor het ontwikkelen van een verslaving. Daarnaast kan ook het neurocognitief functioneren voor aanvang van het drinkgedrag gebruikt worden om het risico op alcoholisme te voorspellen. Tot nu toe zijn er geen studies geweest die hebben onderzocht of het neurocognitief functioneren van adolescenten terwijl ze onder invloed van alcohol zijn, een goede voorspeller is voor alcoholisme. Het primaire doel van deze dissertatie was om te onderzoeken wat het effect is van acute alcohol inname op de neurocognitieve systemen die betrokken zijn bij het ontwikkelen van verslavingsgedrag in adolescenten. Het tweede doel was om te onderzoeken of deze door alcohol geïnduceerde veranderingen in cognitieve en affectieve processen voorspellend zijn voor escalatie in het drink gedrag van jongeren.

Bij herhaald gebruik van alcohol, zullen stimuli die geassocieerd zijn met alcohol een sterke motiverende waarde krijgen die interfereert met cognitief functioneren. Deze sterke motivaties voor alcohol-gerelateerde stimuli zullen ook de snelheid van bepaalde motorische reacties veranderen. De toenaderings-vermijdingstaak, is een taak waarin mensen sommige stimuli als het ware naar zich" toe moeten trekken" (toenaderen) met behulp van een beweging van de joystick of het keyboard, andere plaatjes moeten ze juist van zich "afduwen" (vermijden). Als mensen sneller reageren wanneer ze een met alcohol geassocieerde stimuli benaderen, vergeleken met het vermijden van dezelfde stimuli, wordt van een toenaderingsneiging (*bias*) gesproken. Deze toenaderingsneiging is beschreven als een belangrijk cognitief-motivationeel proces in het ontstaan van alcohol problematiek.

De associaties tussen afbeeldingen en reacties daarop kunnen onderzocht worden door hersenactiviteit te onderzoeken in de periode waarin mensen een reactie voorbereiden die in lijn is of juist niet in lijn is met hun dominante gedragspatronen. Hersensignalen kunnen we bestuderen in verschillende frequenties uit het EEG, die elk hun eigen rol spelen in het functioneren van de hersenen. In de eerste en de tweede studie hebben we de effecten van alcohol inname onderzocht op toenadering- en vermijdende reacties op afbeeldingen van alcohol en frisdranken, door te kijken naar hersensignalen in de beta frequentie band in jong volwassenen (hoofdstuk 2) en adolescenten (hoofdstuk 3). Wanneer mensen een korte periode hebben waarin ze zich kunnen voorbereiden op een motorische reactie nadat ze taak instructies hebben gekregen, neemt de beta frequentie af over tijd. Dit wordt "event gerelateerde desynchonisatie" genoemd (beta-ERD). De gedragsresultaten in deze twee studies lieten zien dat de deelnemers de neiging hadden om sneller te reageren wanneer ze alcohol-gerelateerde afbeeldingen benaderden dan wanneer ze deze vermeden. De toediening van alcohol in deze deelnemers had geen invloed op de toenaderingsbias in sociale drinkers die vooral bestonden uit jong volwassenen (hoofdstuk 2). Maar in adolescenten had alcohol toediening wel een invloed op de toenaderingsbias die afhankelijk was van de drink-gewoontes (hoofdstuk 3).

Beta-ERD die gemeten werd achter op het hoofd nam toe gedurende een periode waarin mensen zich voorbereidden op het benaderen van alcohol stimuli in de hierboven beschreven taak. Dit geeft aan dat deelnemers deze reactie al aan het voorbereiden waren (hoofdstuk 2). De beta-ERD nam af na de toediening van alcohol aan de deelnemers. In een andere studie (hoofdstuk 3) hebben we het voorbereiden van de reactie bekeken door te kijken naar de sterkte van de lateralisatie (de hoeveelheid hersenactiviteit van de rechter hersenhelft ten opzichte van de linker hersenhelft) van de beta-ERD. In eerdere studies is aangetoond dat de lateralisatie in het brein toeneemt als een taak meer voorbereiding vereist. In deze studie vonden we dat zware drinkers een sterkere toenaderings- gerelateerde lateralisatie lieten zien bij afbeeldingen van frisdranken vergeleken met afbeeldingen van alcohol. Individuen die meer problemen hadden met het reguleren van hun drinkgedrag, vooral zware drinkers, hadden een grotere toenaderings-gerelateerde lateralisatie voor frisdrank afbeeldingen. Individuen die minder alcohol gerelateerde problemen rapporteerden hadden een grotere toenaderings-gerelateerde lateralisatie voor alcohol afbeeldingen. Dit zou kunnen betekenen dat het voor zware drinkers meer moeite kost om een reactie voor te bereiden waarin ze de frisdrank toenaderen, omdat ze een sterkere neiging hebben om een toenadering tot alcohol te maken

In de derde studie (hoofdstuk 4) gebruiktem we de zogenoemde Go/NoGo taak om de bekijken wat de invloed van alcohol inname was op het vermogen om reacties te onderdrukken. In deze taak werden deelnemers geïnstrueerd om snel te reageren op sommige afbeeldingen die die vaak werden gepresenteerd. Bij andere afbeeldingen die niet vaak voorkwamen moesten ze juist niet reageren. Doordat deze laatste categorie veel minder vaak voorkomt, is het moeilijk

om in deze situatie geen reactie te geven. Wanneer deelnemers per ongeluk toch reageren, wordt dit een omissie fout genoemd. Deelnemers deden twee versies van deze taak. Een waarin afbeeldingen van alcohol gepresenteerd werden en een waarin alleen afbeeldingen van frisdrank gepresenteerd werden. Hersenprocessen die geassocieerd zijn met het detecteren van conflict en het detecteren van fouten werden vergeleken tussen deze twee versies om te zien of adolescenten slechter presteerden wanneer de afbeeldingen een motiverende waarde hadden. Deelnemers die normaal gesproken meer alcohol drinken maakten meer omissie fouten bij alcohol afbeeldingen en hadden grotere neurale reacties die geassocieerd zijn met conflict. Dit geeft aan dat afbeeldingen die een beloning signaleren mogelijk een reactie activeren. Daarnaast vonden we dat de neurale activiteit geassocieerd met de detectie van fouten lager was voor alcohol afbeeldingen. De inname van alcohol leidde tot een afgenomen detectie van fouten voor frisdrank afbeeldingen en een afname in conflict monitoren voor alcohol afbeeldingen.

In een andere studie (hoofdstuk 5), hebben we gekeken naar een mogelijke genetische kwetsbaarheid voor de belonende effecten van alcohol die gevonden is in volwassenen, in G-allel van het OPRM1 gen. Wij hebben onderzocht of deze kwetsbaarheid in adolescenten de reactie van het brein op alcoholische smaken zou beïnvloeden. In volwassenen die veel drinken is dit gen geassocieerd met een toename in activiteit van het striatum, een hersengebied dat te maken heeft met beloning en motivatie. Resultaten van onze studie lieten zien dat er in adolescenten met deze kwetsbaarheid niet een vergelijkbare toename is van activiteit in het striatum. Wel vonden we dat er een afname was in de regulatie van de activiteit in het striatum door frontale hersengebieden.

In de twee EEG studies met adolescenten (hoofdstuk 3 en 4) onderzochten we of we op basis van alcohol-geïnduceerde veranderingen in de hersenen en het gedrag, toekomstige escalaties in drinkgedrag konden voorspellen. In de Go/NoGo taak, gebruikten we de alcohol geïnduceerde veranderingen in neurale processen die geassocieerd zijn met het detecteren van fouten als voorspeller. In deze studie vonden we dat de deelnemers bij wie alcohol inname het fout-detectie proces voor alcohol afbeeldingen verminderde, vaker een afname in het consumeren van alcohol lieten zien bij de her-test na zes maanden. In het toenaderings-vermijdingsparadigma, gebruikten we twee maten als voorspellers; de gedragsmaten van toenadering tot alcohol afbeeldingen en de lateralisatie geassocieerd met het voorbereiden van de reactie. Daar vonden we dat individuen die na alcohol toediening een relatief sterkere toenadering voor frisdranken en een zwakkere toenadering voor alcohol hadden, minder alcohol dronken na zes maanden.

In dit project, hebben we taken gebruikt die cognitieve controle en de toenadering tot alcohol afbeeldingen meten om de hersenreacties van adolescenten na alcohol toediening te bestuderen. In alle studies hebben we verschillende motiverende invloeden bekeken door

contextuele afbeeldingen in een cognitieve taak te manipuleren (frisdrank versus alcohol afbeeldingen in de Go/NoGo taak) of door directe reacties op alcohol-gerelateerde afbeeldingen te bekijken. Ondanks het feit dat onze adolescente deelnemers geen verslaving hadden, lieten de neurale patronen zien dat ze een hogere motivatie hadden voor alcohol afbeeldingen in vergelijking met frisdrank afbeeldingen. Deze hogere motivatie voor alcohol afbeeldingen werd niet beïnvloed door het onderzochte gen, wat suggereert dat deze genetische factor mogelijk pas een rol gaat spelen na herhaald gebruik.

In adolescenten vonden we dat neurale activiteit geassocieerd met controlerende processen hoger was voor de controle afbeeldingen van frisdrank, en dat alcohol inname leidde tot aan afname van deze activiteit. Gemiddeld genomen over alle deelnemers, leidde alcohol inname niet tot een afname van de fout-detectie bij alcohol afbeeldingen. Echter, op het niveau van het individu vonden we dat alcohol leidde tot een afname in de hersenactiviteit gerelateerd aan fout-detectie bij alcohol afbeeldingen. Dit was geassocieerd was met een afgenomen alcohol consumptie na 6 maanden. Individuen die na inname van alcohol in staat waren hun alcohol toenaderings-neigingen te controleren hadden een lagere alcohol consumptie na zes maanden.

Met deze studies hebben we laten zien dat alcohol-geïnduceerde veranderingen in zowel prestatie als neurale activiteit bijdragen aan de voorspelling van toekomstige veranderingen in alcohol gebruik. Deze neurocognitieve voorspellers van alcohol escalatie kunnen gebruikt worden om individuen met een hoog en laag risico voor verslaving van elkaar te onderscheiden, en kunnen helpen bij het ontwikkelen van preventieve interventies.

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Ozlem