Addiction and Lifestyles in Contemporary Europe: Reframing Addictions Project (ALICE RAP)

Marketing and Brain Activity

Deliverable 11.3, Work Package 11 (Task 4)

Jurriaan Witteman
Elizabeth de Sousa Fernandes Perna
Kim Kuypers
Eef Theunissen
Wim van Dalen
Johannes Ramaekers

October 2014

Table of Contents

GLOSSARY

- Acute (or immediate-) effects of alcohol advertisement exposure: the effect of a single session of exposure to alcohol advertisement on alcohol related cognitive processing immediately following exposure
- Chronic (or long-term) effect of alcohol advertisement exposure: the effects of repeated exposure to alcohol advertisement on alcohol related cognitive processing and drinking behavior
- Cue-elicited reactivity: an excessive cognitive, behavioral or physiological response to drug-related cues as compared to non-drug cues
- Physiological reactivity: an excessive physiological response to drug-cues as compared to non-drug cues
- Deliberative (or reflective-) alcohol related processing: cognitive processing of alcohol related cues that is relatively slow, intentional and open to introspection
- Automatic alcohol related processing: cognitive processing of alcohol related cues that is relatively fast, unintentional and not open to introspection
- Neuropathology of addiction: Drug addictions are chronic brain disorders that affect neural circuits that regulate reward, motivation, memory, and decision-making. Drug-induced pathological changes in these brain regions are associated with addictive behaviors.
- Dopamine: In the brain, dopamine acts as a neurotransmitter—a chemical released by nerve cells to send signals to other nerve cells. Dopamine has been implicated to play key roles in motor control, motivation, arousal, cognition, and reward
- Stressor: an event that induces a stress response in an organism
- fMRI BOLD activation: the blood-oxygen-level dependent (BOLD) contrast or signal is the primary outcome measure in functional magnetic resonance imaging (fMRI). It maps neural activity in the brain or spinal cord by imaging the change in blood flow (hemodynamic response) related to energy use by brain cells during task performance. Active neurons receive more blood released oxygen than inactive neurons. This causes a difference in magnetic susceptibility between oxygenated or deoxygenated blood.
- Valence: whether a stimulus (or emotion) is regarded as 'positive' or 'negative'
- Appetitive response: a response of the dopaminergic reward system that is associated with approach behavior
- Phasic response: an immediate and temporary response to an event

EXECUTIVE SUMMARY

In the present deliverable (D11.3) three multidisciplinary studies are reported about effects of marketing exposure on brain activity and drug-related cognitive processing in users of alcohol and cannabis and in controls. As was described in the Description of Work of the ALICE RAP project, the work consists of two parts. In Part I, a neuroimaging study about the effects of alcohol and cannabis marketing on brain activity is reported. Part II consists of two studies. In study 1 of part II, the immediate effect of alcohol advertisement exposure in light and heavy (but not clinically dependent) drinkers of alcohol is reported. In study 2 of Part II, immediate and long-term effects of alcohol advertisement in alcohol dependent patients are reported.

PART I: Effects of alcohol and cannabis marketing on brain activity: A functional Magnetic Resonance Imaging (fMRI) study

Recreational drug use is a pervasive phenomenon in our society. An important characteristic of recreational drug use is its social context. Drugs like alcohol and cannabis elicit reward and pleasure, which are common motives for people to use them in social settings. Recreational drug use is most popular among young people.

Motivations to use alcohol or cannabis may increase due to alcohol marketing or exposure to drug cues. Earlier studies on soft drink brands have shown that brand knowledge influences behavioral preferences and measured brain responses. Likewise, cue-elicited reactivity to cannabis has been shown to activate reward pathways in the brain associated with the neuropathology of addiction.

Most previous research on drug cue-reactivity has been conducted in abstinent users. The present study aimed to assess brain reactivity to cannabis and alcohol cues during abstinence as well as intoxication, and compare brain network activations during both states. Reinforcing stimuli have previously been shown to cause burst firing of midbrain dopamine neurons that leads to a temporary, phasic release of dopamine in the striatum. The striatal response or reward sensitivity to such phasic dopaminergic innervations however may decrease in the presence of elevated tonic dopamine levels induced by alcohol and cannabis. It was therefore predicted that brain networks that are activated after alcohol/cannabis cue exposure are identical during abstinence and intoxication, but that marketing reinforcement of these brain areas will decrease during intoxication

Heavy users of alcohol (n = 20) and regular cannabis users (n = 21) participated in a double-blind, placebo-controlled, within-subject study involving two experimental conditions consisting of placebo and alcohol (0.8 g/kg) treatment in the alcohol group and placebo and cannabis (300 µg THC/ kg) treatment in the cannabis group. A group of non-drug users (N=20) was included as reference. Treatment conditions were separated by a minimum washout period of 7 days to avoid carry-over effects. The control group received no treatment but the testing day was similar on all other aspects. The study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008) and approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University.

Functional magnetic resonance imaging (fMRI) was conducted between 20-60 minutes after treatment administration. Subsequently, implicit cognition was measured by means of implicit association tests, i.e. the single category implicit association task (SC-IAT) and the approach

avoidance task (AAT), between 0.15 – 1 h after receiving booster dose of the active treatment or placebo.

Brain activity was assessed by functional magnetic resonance imaging (fMRI) during a cue-exposure task using a block design. In this task, marketing clips were randomly presented on a computer screen in blocks of 30s. The clips consisted of three categories, i.e. alcohol marketing clips, cannabis-related clips and neutral clips.

The SC-IAT is a modification of the Implicit Association Test (IAT) which measures the strength of subjects´ affective evaluative associations with a single attitude object (drug marketing stimuli). In this task, two target concepts (positive and negative words) were coupled with a single attribute (cannabis or alcohol marketing pictures). Target concepts and attribute were presented one by one in the middle of a computer screen.

The AAT measures implicit or automatic action tendency towards target stimuli (e.g. drug-related or neutral stimuli). In this task, alcohol, cannabis, soda and neutral cues that are rotated 3° left or right were presented on a screen. Depending on the instruction, subjects had to pull or push a joystick in response to a feature that is unrelated to the contents of the presented stimuli, which was the orientation of the image.

Alcohol and cannabis marketing significantly increased blood-oxygen-level-dependent BOLD activation in parietal, temporal and frontal networks across all groups. Region of interest (ROI) analysis likewise revealed that alcohol and marketing movies increased striatal activation during abstinence. Striatal activation, however, decreased during alcohol and cannabis intoxication. There was a significant difference in performance between groups during exposure to cannabis marketing cues in the SC-IAT. It revealed a positive bias towards cannabis marketing cues in both the alcohol and cannabis group, relative to the controls. The alcohol group displayed a positive bias towards alcohol marketing cues that approached significance. Treatment did not affect performance during the SC-IAT. AAT performance did not differ between groups and treatment conditions.

These findings suggest that alcohol and drug marketing can trigger similar brain responses to those that occur during drug use and drug craving. Activation of the brain reward system through drug and alcohol advertisement may therefore directly increase the motivation to use drugs or alcohol. Moreover, the present study also demonstrates that the actual use of alcohol and cannabis in turn reduces the reinforcing strength of drug and alcohol marketing cues by responding to and saturating the need for cannabis and alcohol use. This notion of heightened and blunted reward sensitivity during abstinence and drug/alcohol intoxication fits well with current notions of the role of dopamine in reward processing. Reinforcing stimuli have been shown to cause burst firing of midbrain dopamine neurons that leads to a temporary, phasic release of dopamine in the striatum. The striatal response or reward sensitivity to such phasic dopaminergic innervations varies with the availability of tonic dopamine in the same area. Reward sensitivity is high when tonic dopamine is low and vice versa. In the present study, marketing exposure produced an increase in reward sensitivity as evinced by increased striatal BOLD activation. The phasic response to marketing, however, decreased in the presence of alcohol and cannabis induced elevation of tonic dopamine levels. In other words, [the effect of marketing / the influence of drug-cues] seem to be stronger when one has not consumed any drugs, but appear to be less strong when one has consumed drugs / is intoxicated with drugs. The brain includes several distinct dopamine systems, one of which plays a major role in reward-motivated behavior. This is consists out of several brain regions and pathways, which includes regions such as the striatum and pallidum, which are collective terms for the caudate

nucleus and putamen and for the dorsal pallidum (globus pallidus) and the ventral pallidum, respectively. The level of dopamine release is modulated by two interacting mechanisms in the brain: tonic and phasic dopamine release. Tonic dopamine release occurs when small amounts of dopamine are released independently of neuronal activity and is regulated by the activity of other nerve cells in the brain. Phasic dopamine release results from the activity of the dopamine-containing cells themselves. Most types of rewarding stimuli increase dopamine levels in the brain, and a variety of addictive drugs, such as alcohol and cannabis, increase dopamine neuronal activity. However the phasic dopamine release depends on the tonic dopamine level within a particular brain area. Alcohol and cannabis stimuli caused a phasic release of dopamine in the reward system and thereby increasing [striatal response to or reward sensitivity of] drug-cues. Intoxication with alcohol and cannabis in turn, caused an elevation of tonic dopamine release in the same brain region, [influencing / modulating / reducing] the level of phasic dopamine release and thereby decreasing [striatal response to or reward sensitivity of] drug-cues.

PART II: Study 1. Acute effects of alcohol marketing exposure on automatic and deliberative cognitive processes in heavy and light drinkers

Part II studied whether alcohol advertisement exposure has immediate effects in light drinkers and heavy, but not clinically dependent, drinkers (study 1) and whether it has immediate and long-term effects in clinically alcohol dependent drinkers (study 2). Specifically, it was studied through what psychological and physiological mechanisms exposure to alcohol advertisement may affect drinking behaviour in these populations. Note that, as can be read in the glossary provided on page 4 of this deliverable, with 'acute effects' or 'immediate effects' of alcohol advertisement exposure, we refer to the effect of a single session of alcohol advertisement exposure on alcohol related cognitive processes and drinking behavior immediately following the exposure. With 'chronic' or ' long-term' effects on the other hand, we refer to the effect of repeated exposure to alcohol advertisement over time on alcohol related cognitive processing and drinking behavior.

Previous studies had suggested two kinds of processes that might mediate effects of alcohol advertisement exposure on drinking behaviour. On the one hand, it had been proposed that alcohol advertisement exposure might engage 'automatic' processes in the brain that could promote drinking even without subjects being aware of such effects. Furthermore, it had been suggested that such automatic effects of alcohol advertisement exposure might be particularly evident in heavy users of alcohol, as repeated heavy alcohol consumption may in itself promote such automatic processes. On the other hand, it had been proposed that alcohol advertisement exposure might influence behaviour through more reflective processing of the (alcohol favourable) information presented in advertisement. Thus, in study 1 and 2 of part II, it was examined whether exposure to alcohol advertisement in light drinkers, heavy but not clinically dependent drinkers, and clinically dependent drinkers primarily affects automatic or reflective alcohol related psychological processes, and whether alcohol advertisement might affect drinking behaviour through these processes.

In study 1 of part II, the immediate effect of alcohol advertisement exposure on automatic and reflective psychological processes was studied, and whether such effects might differ between light and heavy (but not clinically dependent) drinkers of alcohol. This was achieved by exposing light and heavy drinkers of alcohol to a block of alcohol advertisements and, as a control, to soft-drink advertisements. Immediately after each block of advertisement, participants completed two tasks that measured automatic alcohol related processing. Furthermore, participants completed questionnaires tapping reflective alcohol related

psychological processes after each block. In addition, drinking behaviour was assessed one month following the experiment, allowing for a test of whether automatic and reflective alcohol related processing can predict actual drinking behaviour.

The results of study 1 of part II indicated that there were no acute effects of alcohol advertisement on automatic psychological processes in light or heavy drinkers of alcohol. For male light drinkers of alcohol, however, acute alcohol advertisement exposure increased the tendency to drink at the reflective level. Furthermore, reflective level tendency to drink predicted future drinking behaviour. In other words, the results of this experiment suggests that a single session of alcohol advertisement exposure seems to primarily affect those alcohol related cognitive processes that are relatively slow, intentional and that people are themselves aware of (mental processes that are introspectively accessible) and not alcohol related processes that are fast, unintentional and that can operate independently of subjects' awareness of these processes (i.e., automatic alcohol-related processes)

PART II: Study 2. Acute and chronic effects of alcohol advertisement exposure in alcohol dependent inpatients

In study 2 of part II, acute and chronic (long-term) effects of alcohol advertisement exposure were investigated in clinically dependent users of alcohol. Acute effects were measured by exposing alcohol dependent inpatients, who were receiving detoxification treatment, to alcohol advertisement and, as a control, soft-drink advertisement. After each block of advertisement patients indicated their level of alcohol craving to test for craving effects of alcohol advertisement exposure. Furthermore, the automatic response of the nervous system to the presence of alcohol cues in the advertisements was measured indirectly based on heart rate variability. Following discharge from the addiction center, patients monitored the level of exposure to alcohol advertisement in daily life for five consecutive weeks using a diary. Five weeks and three months after discharge, the drinking status of the participants in the study was assessed testing whether chronic alcohol advertisement exposure in patients' daily lives affects the course of their disease.

The results of study 2 indicated that acute alcohol advertisement exposure causes a robust increase in alcohol craving among alcohol-dependent patients. Furthermore, this craving response was associated with an automatic response of the nervous system to alcohol cues present in the advertisements (such as a glass of beer or the portrayal of drinking behaviour). Furthermore, patients reported being chronically exposed to a mean of five alcohol advertisements per day. Acute or chronic alcohol advertisement exposure, however, did not influence subsequent drinking behaviour. Although it is always difficult to explain negative results, there are three main possible explanations for the absence of chronic alcohol advertisement exposure effects on drinking behavior in alcohol dependence. On the one hand, although the best measure available to date, our measure of chronic alcohol advertisement exposure (an alcohol diary) may have been too imprecise in measuring true alcohol advertisement exposure, preventing us from detecting an effect of chronic alcohol advertisement exposure on drinking behavior in alcohol dependent patients. A different explanation is that society is so saturated with alcohol advertisement, that there is not much variation in exposure between individuals, reducing the opportunity to detect the effect statistically through a statistical phenomenon called 'restriction of range'. As a last explanation, the patients that participated in the study suffered from relatively severe alcohol dependence. It has been suggested that in such an advanced stage of alcohol dependence, drinking behavior is more governed by internal motivation than external stimulation such as alcohol advertisement exposure. Future studies with a large sample should clarify this issue.

Together the two studies reported in part II of this deliverable suggest that acute alcohol advertisement exposure affects reflective level tendency to drink in male light drinkers but not heavy drinkers, Because in light (male) drinkers strong reflective level alcohol-related processes has not developed yet (in contrast to heavy drinkers, who have already developed strong positive attitudes about alcohol) alcohol advertisement can more easily mould these processes in light drinkers as compared to heavy drinkers. Furthermore, both reflective (craving) and automatic cognitive processes in alcohol-dependent patients were affected by acute alcohol advertisement. An effect of chronic alcohol advertisement exposure on drinking behaviour in alcohol-dependent patients could not be demonstrated, but this result should be seen as preliminary due to methodological limitations of the work.

Policy recommendations
Based on these results the following recommendations for policy can be formulated:

(1) Alcohol and cannabis marketing increases reward sensitivity for these substances and increases motivation for actual use. A reduction of alcohol and drug marketing would diminish its impact, particularly in regular alcohol and cannabis users, by reducing brain exposure to reward cues that motivate and prepare for alcohol or drug use.

(2) Reducing exposure to alcohol advertising could reduce the reflective tendency to drink in light drinkers, and hence theoretically reduce actual drinking behaviour.

(3) Removing alcohol cues from alcohol advertisement may reduce craving and automatic nervous system reactivity in alcohol dependent patients, and hence theoretically affect the course of dependence positively. For instance, removing images of beer glasses being filled with beer or imagery of people drinking beer may reduce craving and cue-reactivity.

(4) Reducing levels of alcohol advertisement exposure altogether might be a more feasible and effective way to reduce these potentially drinking promoting, and hence public health damaging, effects.

PART I

Effects of alcohol and cannabis marketing on brain activity: A functional Magnetic Resonance Imaging (fMRI) study

EB de Sousa Fernandes Perna[1], EL Theunissen[1], KPC Kuypers[1], EA Evers[1], P Stiers [1], SW Toennes[2], J Witteman[3], W van Dalen[3] & JG Ramaekers[1]

[1] Department Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, The Netherlands
[2] Department of Forensic Toxicology, Institute of Legal Medicine, Goethe University of Frankfurt, Frankfurt, Germany,
[3] Dutch institute for alcohol policy (STAP), Utrecht, The Netherlands.

Abstract

Drugs stimulate dopamine (DA) release in mesolimbic reward pathways. Exposure to drug cues has also been shown to stimulate DA release and activate reward pathways of abstinent drug users. It can therefore be predicted that marketing of alcohol and drug use will also stimulate the reward pathways. This was the first study that examined the impact of alcohol and cannabis marketing cues on brain reward neurocircuitry in alcohol and cannabis users during abstinence and intoxication.

Heavy alcohol (n = 20) and regular cannabis users (n = 21) participated in a double-blind, placebo-controlled, within-subject study involving two experimental conditions consisting of placebo and alcohol (0.8 g/kg) /cannabis (300 µg THC/ kg) treatment. A group of non-drug users (N=20) was included for between group comparisons. Using functional magnetic resonance imaging (fMRI), brain activation after exposure to alcohol and cannabis marketing movies was compared between the three groups. Implicit cognition was assessed by means of a single-category implicit association test (SC-IAT) and approach avoidance task (AAT).

Alcohol and cannabis marketing significantly increased BOLD activation in parietal, temporal and frontal networks across all groups. Region of interest (ROI) analysis likewise revealed that alcohol and marketing movies increased striatal activation during abstinence. Striatal activation however decreased during intoxication with alcohol and cannabis. There was a significant difference in performance between groups during exposure to cannabis marketing cues in the SC-IAT. It revealed a positive bias towards cannabis marketing cues in both the alcohol and cannabis group. The alcohol group displayed a positive bias towards alcohol marketing cues that approached significance. Treatment did not affect performance during the SC-IAT. Performance in the AAT did not differ between groups and was not affected by treatment.

These findings suggest that alcohol and drug marketing can trigger similar brain responses observed during drug use and drug craving, but that associations between drug marketing and brain reward appear to be less strong during drug and alcohol intoxication. Activation of the brain reward system through drug and alcohol advertisement may therefore directly increase the motivation for actual use of drugs or alcohol. Actual use subsequently reduces the reinforcing strength of drug and alcohol marketing cues by responding to and saturating the desire to use. It is suggested that a striatal increase in tonic dopamine levels following alcohol and cannabis use underlies the reduction in sensitivity to phasic dopamine release that is commonly observed during exposure to alcohol and drug cues.

1. Introduction

Alcohol and cannabis are the most widely used drugs in the western world. It is estimated that around 2 billion individuals consume alcohol worldwide (World Health Organization, 2004), with the highest consumption levels found in Europe and America (World Health Organization, 2014). Around 200 million individuals have used cannabis at least once in their lifetime (United Nations Office on Drugs, 2010). The highest levels of recorded use were in North America, Western Europe, and Oceania (Degenhardt & Hall, 2012; United Nations Office on Drugs, 2010). Drugs facilitate the tonic release of dopamine in reward and motivation circuits in the brain via dopaminergic projections from the ventral tegmental area (VTA) through the ventral striatum extending further to limbic structures including the amygdala, hippocampus, anterior cingulate and (pre)frontal cortex areas (Anton, 1999; Heinz et al., 2005; Yacubian & Büchel, 2009).

Dopamine neurotransmission in mesolimbic reward pathways accounts for the rewarding (pleasurable) effects of drugs, which involves reward-related learning of both predictive cues and efficient action sequences directed toward obtaining the rewarding stimuli (Hyman, Malenka, & Nestler, 2006). These hedonic responses are often a motive for people to repeat drug use (Franken, Booij, & van den Brink, 2005). In addition, drug-associated cues have also been shown to stimulate phasic dopamine release (Berger et al., 1996; Koob & Volkow, 2010) and activate the reward circuit of abstinent drug users (Cousijn et al., 2013; Filbey, Schacht, Myers, Chavez, & Hutchison, 2009; Goudriaan, de Ruiter, van den Brink, Oosterlaan, & Veltman, 2010; Vollstädt-Klein et al., 2010). This suggests that drug-related cues may trigger the reward system to a similar extent as do drugs.

Consequently, motivations to use alcohol or drugs may increase due to marketing exposure to drug-related cues such as alcohol and drug advertisements. Motivations to use alcohol or cannabis may increase due to alcohol marketing or exposure to drug cues. Earlier studies on soft drink brands have shown that brand knowledge influences expressed behavioural preferences and measured brain responses (McClure et al., 2004). Likewise, cue-elicited reactivity to alcohol and marijuana has been shown to activate reward pathways in the brain associated with the neuropathy of addiction (Filbey et al., 2009; Tapert et al., 2003). Research on alcohol and tobacco marketing has shown that marketing can significantly increase consumption patterns (Anderson, de Bruijn, Angus, Gordon, & Hastings, 2009; Cassisi, Delehant, Tsoutsouris, & Levin, 1998; L. a Smith & Foxcroft, 2009; Tye, Warner, & Glantz, 1987). When exposed to drug-related versus neutral stimuli, drug using individuals, report robust increases in craving and exhibit modest changes in autonomic responses. These include increases in heart rate and skin conductance and decreases in skin temperature, reflecting excessive attentional bias for drugs, especially in drug-dependent individuals (Carter & Tiffany, 1999; Gray, K. M., LaRowe, S. D., Upadhyaya, 2009). Yet, reinforcing properties of drug and alcohol cues may actually diminish during drug and alcohol intoxication. Reinforcing stimuli have previously been shown to cause burst firing of midbrain dopamine neurons that leads to a temporary, phasic release of dopamine in the striatum (Schultz, 2007). The striatal response or reward sensitivity to such phasic dopaminergic innervations varies with the availability of tonic dopamine in the same area (Cools & D´Esposito, 2011). Reward sensitivity is high when tonic dopamine is low and vice versa. This implies that a phasic response to marketing may decrease in the presence of elevated tonic dopamine levels induced by alcohol (Gilman, Ramchandani, Davis, Bjork, & Hommer, 2008) and cannabis (Bossong et al., 2009).

Repeated exposure to drug or drug cues may increase implicit cognitions and attentional bias toward drug use and as such contribute to the aetiology and maintenance of drug abuse (Field, Eastwood, Bradley, & Mogg, 2006; Field, Mogg, & Bradley, 2004; Marhe, R., Waters, A. J., van de Wetering, B. J., & Franken, 2014; Newcomb, 1988; Wiers, Van Woerden, Smulders, & De Jong, 2002). Expectancies and implicit attitudes towards drug use are often reported motivations for drug use (Newcomb, 1988; Wiers et al., 2002). Expectancies include individuals´ beliefs about the effects of drugs on behaviour, moods and emotions. Implicit cognitions represent spontaneous or impulsive decisions that may surpass higher order cognitive control that is normally activated during critical decision points (Greenwald, Nosek, & Banaji, 1995; Kahneman, 2003). According to the implicit cognition theory, memory associations are formed and strengthened through repetitive experiences with alcohol or drugs (Schacter, 1992), resulting in strengthened attentional processing of drug-related stimuli (Franken, 2003) and an increased motivation to use drugs or alcohol (Stacy, Ames, & Knowlton, 2004; Wiers, R. W., Houben, K., Smulders, F. T., Conrod, P. J., & Jones, 2005).

While longitudinal studies consistently show that alcohol and tobacco marketing negatively affects adolescents' drinking and smoking behaviour (Anderson et al., 2009; Lovato, C., Linn, G., Stead, L. F., & Best, 2003), no research has examined the impact of marketing on brain activity and implicit cognition in heavy drug users during abstinence as well as during intoxication. This research will fill this gap by exposing heavy alcohol and cannabis users to alcohol and cannabis marketing, investigating the hypothesis that marketing cues elicit drug craving as indicated by an increased activation of the reward neurocircuitry in the brain, particularly at the level of the striatum. It is hypothesized that drug-marketing cues elicit activations in brain structures that are identical to those affected by cannabis and alcohol during intoxication. Implicit cognitions will furthermore be assessed, during abstinence and intoxication. It is predicted that brain networks that are activated after alcohol/cannabis cue exposure are identical during abstinence and intoxication, but that reinforcement of the striatum after cue exposure will be stronger during abstinence as compared to intoxication. In addition it is also predicted that implicit cognitions will be stronger in the sober state compared to the intoxicated state.

2. Methods

2.1 Participants

The present study included a group of heavy alcohol users, a group of regular cannabis users and a control group. Heavy alcohol use was defined as using on average 21 to 50 alcoholic drinks a week for males or 15 to 35 alcoholic drinks a week for females during the last year (Cassisi et al., 1998). Experimental use of cannabis in the alcohol group was allowed only if it occurred more than a year ago. Regular cannabis use was defined as having used cannabis at least 3 times a week but no more than 10 times a week, during the previous year (Ramaekers, Kauert, Theunissen, Toennes, & Moeller, 2009). Alcohol use between 1-14 units/week was allowed in the cannabis group. Controls were defined as not currently using cannabis or other drugs; experimental use of cannabis was allowed if it occurred more than a year ago and incidental alcohol use was permitted (1-7 units/week for women and 1-14 units of alcohol/week for men).

Five subjects from the alcohol group and 2 subjects from the cannabis group dropped out due to personal circumstances and 1 subject from the cannabis group failed to complete the fMRI session during placebo, but otherwise completed both behavioural sessions. The dropouts were replaced but the behavioural data of the subject with incomplete fMRI was also added to the final data set. The final dataset therefore consisted of 61 subjects spread among the alcohol and control group (N=20 each) and the cannabis group (N=21). Participants (35 male, 26 female) were aged between 18 and 28 (mean (SD) 22.5 (2.3) years). Subjects in the control group were matched across groups for age and educational level. Subjects' demographics and history of drug use in all three groups are shown in Table 1.

Participants were recruited through advertisements placed on a university website, university newspapers and by word of mouth. Subjects underwent a general medical examination including routine laboratory tests, provided a written informed consent and filled out a questionnaire on history of substance use. Inclusion criteria included: (i) age 18–40 years (ii) free from psychotropic medication; (iii) good physical health and, (iv) body mass index within 18.5 –28 kg/m^2. Exclusion criteria included: (i) addiction according to DSM-IV criteria, (ii) presence or history of psychiatric or neurological disorder as assessed by a medical questionnaire (iii) pregnancy (iv) cardiovascular abnormalities, (v) excessive smoking (>15 cigarettes per day) and (vi) hypertension.

This study was conducted according to the code of ethics on human experimentation established by the declaration of Helsinki (1964) and amended in Seoul (2008) and approved by the Medical Ethics Committee of the Academic Hospital of Maastricht and Maastricht University. A permit from the Dutch drug enforcement administration was acquired for obtaining, storing, and administering cannabis. Subjects received monetary compensation for their participation in the study.

Table 1. Subject demographics and history of alcohol and drug use

	Mean	SD	Min.	Max.
Age (years)	22.5	2.3	18.0	28.0
Weight (kg)	67.9	10.7	50.0	92.0
Alcohol group (N=20)				
Frequency of alcohol use (previous month)	107.6	47.8	28.0	197.1
Frequency of alcohol use (previous 3 months)	322.7	143.3	84.0	591.3
Frequency of alcohol use (previous year)	1290.8	573.4	336.0	2365.2
Cannabis group (N=21)				
Frequency of cannabis use (previous month	17.8	7.9	4.0	28.0
Frequency of cannabis use (previous 3 months)	53.4	23.9	12.0	84.0
Frequency of cannabis use (previous year)	240.5	104.6	52.0	364.0
Control group (N=20)				
Frequency of alcohol use (previous month)	32.4	20.9	2.0	58.8
Frequency of alcohol use (previous 3 months)	97.2	62.6	6.0	176.4
Frequency of alcohol use (previous year)	388.9	250.4	24.0	705.6

Occasional use of other drugs	**Total**	**Alcohol Group**	**Cannabis Group**	**Control Group**
Ecstasy	20	8	10	2
Amphetamine	8	2	5	1
Cocaine	6	1	5	0
LSD	3	0	3	0
Mushrooms	13	2	11	0
Other	11	3	8	0

2.2 Design & Treatments

Subjects in the alcohol and cannabis group participated in a double-blind, placebo-controlled, within-subject study involving two experimental conditions consisting of placebo and cannabis/alcohol treatment. Conditions were separated by a minimum washout period of 7 days to avoid carry-over effects. The control group received no treatment but the testing day was similar on all other aspects. A schematic representation of a test day is given in Table 2.
Alcohol was mixed with orange juice to a total volume of 250 ml. Alcohol doses were individually calibrated using the formula of Watson et al. (1981) to achieve a blood alcohol concentration (BAC) of 0.8 g/L. Subjects' BAC was monitored frequently (every 15-20 min approximately) with a breathalyser and was kept constant by administering maintenance drinks. Maintenance (booster) doses were administered during the break before behavioural testing. Each subject received 1-2 booster doses on average depending on their measured BAC level at the end of the scanning session.

The cannabis group received in total 300 µg THC/ kg bodyweight, divided over two successive doses of 200 µg and 100 µg THC /kg bodyweight (booster dose) with an interval of approximately 1 hour. THC was administered using a Volcano vaporizer produced by Storz-Bickel, Germany (http://www.storz-bickel.com). Hot air would pass through the filling chamber holding the cannabis (containing 12% THC), which caused the THC or placebo to vaporize and blend with the air. The THC molecules or the placebo (vapor) was trapped in a valve balloon. For inhalation, the valve of the balloon was put to subjects´ lips and they were instructed to inhale deeply. They would hold their breath for 10 s and then exhale. The volume of the balloon was inhaled in 7 to 10 subsequent breaths and the balloon had to be emptied within 5 to 10 min. As soon as subjects removed their lips from the mouthpiece, the valve closed automatically. All subjects were trained in using the Volcano vaporizer.

2.3 Procedures

Subjects were asked to refrain from drug use at least a week prior to the start and during the study. Subjects were not allowed to use alcohol on the day before an experimental session and were requested to arrive at experimental sessions well rested. Drug and alcohol screens were carried out upon arrival at our testing facilities. Urine drug screens assessed for the presence of benzodiazepines, opiates, cocaine, marijuana, MDMA and (meth)amphetamine. Women were also tested for pregnancy. Study treatments were only administered after negative drug screens, except for marijuana in the cannabis group, and negative pregnancy tests.

Brain activity was measured by means of functional magnetic resonance imaging (fMRI) at 20 to 30 minutes after cannabis and alcohol treatment (Tmax). Subjective measures were assessed throughout the test day and implicit cognition was measured by means of implicit association tests between 0.15 – 1 h after booster treatment (see table 2).
All subjects received a training session before onset of the experimental sessions in order to familiarize them with tests and procedures.

Table 2. Schematic representation of a testing day, specifying procedures which applied for all participants, and specific procedures which applied for the alcohol and cannabis group.

	Procedures for all participants	**Procedures for alcohol group**	**Procedures for cannabis group**
09:00-10:00	Arrival Drug screening Sleep scale POMS 1	BAC 1 VAS 1	Placement of venal catheter Blood sample 1 VAS 1
10:00-11:00	POMS 2	10:00-10:30 Placebo or alcohol 0.8 g/kg BAC 2 VAS 2	10:30-10:45 placebo or 200 µg THC /kg Blood sample 2 VAS 2
11:00-12:00	fMRI session		
12:00-12:45	Lunch and break POMS 3	12:15-12:30 placebo or alcohol booster (average: 0.3 g/kg) BAC 3 VAS 3	12:30-12:45 placebo or 100 µg THC /kg Blood sample 3 VAS 3
12:45-14:00	Implicit cognition tasks POMS 4	BAC 4 VAS 4	Blood sample 4 VAS 4

Note: POMS = Profile of Mood States; VAS = visual analogue scale; BAC = Blood Alcohol Concentration

2.3.1 Cue-Exposure Task / Pharmaco-fMRI

Brain activity was assessed by functional magnetic resonance imaging (fMRI) during a cue-exposure task using a block design. In this task, marketing clips were randomly presented on a computer screen in blocks of 30s. The clips consisted of three categories, i.e. alcohol marketing clips (10x), cannabis-related clips (10x) and neutral clips (10x). Alcohol clips were mainly non-local advertisement of beers, wines and other alcoholic beverages that were not readily available in the Netherlands and were spoken in foreign languages (e.g. Polish, Spanish or English) that did not correspond to the subjects´ native language (Dutch). This was done to ensure that subjects were not reacting to the specific alcohol brand, but to the alcohol itself. Cannabis clips were a selection of short film fragments where portrayal of cannabis use and marketing practices at cannabis selling points were displayed. The neutral clips consisted of local and non-local advertisement of non-drug-related stimuli (e.g. advertisement for cameras, water, hearing aid etc). The presentation order of the clips was kept constant across conditions. After the presentation of each clip, subjects were asked to rate their level of alcohol or cannabis craving (e.g. ¨How much do you feel like having alcohol/cannabis after seeing this clip?¨) with a joystick using a visual analogue scale (0-10cm). Total task time was approximately 33 min.

Image acquisition

fMRI images were acquired with a Siemens 3T head-only scanner (MAGNETOM Allegra, Siemens Medical Systems, Erlangen, Germany). During the cue exposure task whole brain functional volumes were acquired using gradient-echo echo-planar imaging (GE-EPI, TR= 2000 ms, TE= 30 ms; FA= 90°; FOV 224mm; matrix size= 64 x 64; voxel size= 3.5 x 3.5 x 3.5 mm). The T1-weighted anatomical scan was acquired using a 3D MPRAGE (magnetization-prepared rapid gradient echo; TR= 9,7 ms; TE= 4 ms; flip angle=12°; matrix=256×256; voxel size=1×1×1 mm^3).

Image preprocessing

Data preprocessing and analysis were conducted using SPM8 (Welcome Trust Center for Neuroimaging, London, UK). The first two volumes were removed from each fMRI data set to allow for magnetic equilibration. Firstly, framewise displacement (FD) calculations were carried out to quantify head displacement within and across runs (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012). The measured fMRI signal can be distorted by head motion within one scan as a result of spatial misallocation. Therefore, scans were identified during which head motion exceeded a particular threshold, i.e. absolute motion difference between successive scans in z direction greater than 0.35 mm (1/10th of the voxel size), and rotation around the x dimension greater than 0.26 degrees (angle corresponding to 0.35 mm displacement in the z direction of frontopolar voxels, assuming the rotation point in middle of brain is 88 mm from the anterior end of the brain frontal pole (Talairach & Tournoux, 1988)). Following this procedure, 2 subjects in the alcohol group, 1 subject in the cannabis group and 1 subject in the control group were excluded from further processing due to excessive movement (in >20% of the volumes). In addition, motion parameters in the alcohol and cannabis group were then compared to check for motion differences between placebo and active drug/alcohol conditions. These analyses indicated no difference between sessions for the most susceptible motion parameters (Mayer, Franco, Ling, & Cañive, 2007; Yoo, Choi, Juh, Pae, & Lee, 2005), namely translation in the z direction (Tz alcohol group; $t_{(1, 19)} = -.24$; $p = .82$, Tz cannabis group; $t_{(1, 19)} = 1.02$; $p = .32$) and rotation in the x direction (Rx alcohol group; $t(1, 19) = .53$; $p= .60$, Rx cannabis group; $t_{(1, 19)} = .95$; $p = .36$).

Thereafter the following preprocessing steps were carried out: (1) realignment, (2) slice time correction, (3) individual anatomical data sets were normalized to standard 3-D MNI space (voxel size was resampled to 2×2×2 mm), and (4) spatial smoothing was applied with a FWHM 6 mm Gaussian kernel.

2.3.2 Cognitive Assessment

Single Category Implicit Association Test

The Single Category Implicit Association Test (SC-IAT) is a modification of the Implicit Association Test (IAT) which measures the strength of subjects´ affective evaluative associations with a single attitude object (drug marketing stimuli). In this task, two target concepts (positive and negative words) are coupled with a single attribute (cannabis or alcohol marketing pictures). Target concepts and attribute were presented one by one in the middle of a computer screen.

During the 1st block of 24 trials (target discrimination), only the target concepts were presented and subjects had to respond using the corresponding keys (i.e. press left button for positive words, and the right button for negative words). In the 2nd block (compatible block) of 72 trials, positive words and drug marketing cues were categorized on the left key, and negative words were categorized on the right key. In the 3rd block (incompatible block) of 72 trials, negative words and drug marketing cues were categorized on the right key, and positive words were categorized on the left key. Subjects were instructed to respond to targets and attributes as quickly and accurately as possible. The rationale behind this task is that if subjects have a positive evaluation for alcohol or cannabis rather than a negative evaluation, they should be quicker to respond when alcohol/cannabis marketing + positive words (compatible block) share the same response key compared to the incompatible block, where alcohol/marketing clips + negative words share the same response key.

Data from the 1st block (practice block) was discarded. Non responses and responses faster than 350 ms were eliminated and error responses were replaced with the block mean plus an error penalty of 400 ms. Subjects who exceeded an error rate of 20% were excluded. The dependent variable was the D-score (originally derived from IAT D-scoring algorithm by Greenwald, Nosek and Banaji (2003) and adapted to SC-IAT D-scoring algorithm by Karpinski and Steinman (2006)), which was calculated by subtracting the mean reaction time (RT) of correct responses in the compatible block from the mean RT of correct responses in the incompatible block, divided by the standard deviation (SD) of all correct responses within the compatible and incompatible block. D-scores were log transformed (ln(D-score+1)) before entering statistical analysis.

Two versions of the SC-IAT were presented to all subjects in each group. In the first version, alcohol-marketing cues were presented as attributes. The second version presented cannabis-marketing pictures as attributes.

The Approach Avoidance Task

The Approach Avoidance Task (AAT) measures implicit or automatic action tendency towards target stimuli (e.g. drug-related or neutral stimuli). In this task, alcohol, cannabis, soda and neutral cues that are rotated 3° left or right were presented on a screen. Depending on the instruction, subjects had to pull or push a joystick in response to a feature that is unrelated to the contents of the presented stimuli, which was the orientation of the image. Half of the subjects were instructed to push images that were rotated left and to pull images that were

rotated right, while the other half received opposite instructions. Pushing or pulling the joystick respectively, gradually decreased image size upon a push movement and increased image size upon a pull movement. The combination of this ´zooming´ feature with actual arm flexion (pull) and extension (push) movements enhances the experience of a more realistic avoidance or approach reaction to drug cues. According to previous studies (Cousijn, Goudriaan, & Wiers, 2011; Stacy, A. W., & Wiers, 2012), attention is automatically guided to drug-related cues in drug-dependent individuals compared to non-dependent individuals. The drug cues elicit approach tendencies that are evaluated as more positive and arousing relative to neutral cues, which are manifested in faster pulling rather than pushing of the joystick (approach bias).

The raw data was corrected for outliers before calculating the bias scores; error trials were removed and RTs faster than 200 ms and slower than 2000 ms or more than 3 SDs above and below the mean were removed for each participant. The dependent variable was the bias score, which is the difference score of mean avoid RTs minus mean approach RTs. The subtractions resulted in bias scores for each stimuli category for each subject.

2.3.3 Subjective Assessment

The Groningen sleep scale (GSS) consists of 15 dichotomous questions about sleep complaints and an open question concerning the duration of sleep in order to assess respectively sleep quality and sleep quantity (hours of sleep) (Mulder-Hajonides van der Meulen, Weinberg, Hollanders, DeDiana, & Hoofdakker, 1980). The sum sleep quality score ranges from 0 (best quality of sleep) to 14 (worst quality of sleep) and is based on subjects' experienced sleep complaints during the night preceding the test day. Subjects completed the sleep questionnaire at the beginning of the test day.

The Profile of Mood States (POMS) is a self-assessment mood questionnaire with 72 five point-Likert scale items, representing eight mood states; i.e. Anxiety, Depression, Anger, Vigor, Fatigue, Confusion, Friendliness and Elation. Two extra scales are derived, i.e. Arousal ((Anxiety + Vigor) – (Fatigue + Confusion)) and Positive mood (Elation – Depression) (de Wit, Enggasser, & Richards, 2002).

Subjective intoxication was measured using a 100 mm visual analogue scale (VAS) with "not influenced by alcohol/cannabis at all" at one end and "very influenced by alcohol/cannabis" at the other end of the line. Subjects rated their mood and subjective intoxication at the beginning of a test day, after treatment, before and after cognitive assessment (table 2).

2.3.4 Pharmacokinetic Measures

In the cannabis group, blood samples (8mL) to determine cannabinoid concentrations (THC and metabolites OH-THC and THC-COOH) were collected at 4 successive times during each test day, i.e. at baseline and 0.5 h (Tmax), 2.75 h and 4 h after treatment. These blood samples were centrifuged immediately; serum was transferred into a tube and was stored at -20°C. Cannabinoid concentrations were determined by the Institute of Forensic Toxicology, University of Frankfurt, using solid phase extraction and gas chromatography with mass spectrometric detection with a limit of quantification of 1.0 ng/ml. In the alcohol group, BAC levels were measured throughout the test day (table 2).

2.4 Statistics

fMRI data

Two generalized linear model (GLM) full factorial models were built to calculate marketing cue-related BOLD activations during abstinence (i.e. placebo/no treatment) and how these were affected by cannabis or alcohol intoxication. For both models, contrast images from the individual GLM analysis (1st-level) were used as input for the 2nd level GLMs. Individual analysis consisted of contrast images of cannabis marketing movies vs neutral marketing movies (cannabis marketing; contrast [1 -1]) and alcohol marketing movies vs neutral marketing movies (alcohol marketing; contrast [1 -1]). All individual GLM models included the 6 realignment parameters as regressors.

The 1st GLM full factorial model focused on BOLD activation during cannabis marketing and alcohol marketing. The model included the factors Group (3 levels: cannabis group on placebo, alcohol group on placebo and controls) and Marketing Cue (cannabis marketing and alcohol marketing).

The 2nd GLM full factorial model was designed to assess the influence of cannabis and alcohol intoxication on brain activation during marketing exposure. The model consisted of the following factors: Group (2 levels: cannabis group and alcohol group); Treatment (2 levels: placebo and cannabis/alcohol) and Marketing Cue (2 levels: cannabis marketing and alcohol marketing).

For these two models, firstly, whole brain analyses were performed. Secondly, region of interest (ROI) analyses were used to assess marketing and drug/alcohol related BOLD activation in the striatum. Results were considered significant when P_{FWE}-corrected at cluster level < 0.05. ROI analyses were conducted in order to specifically test our hypotheses that marketing cues as well as drug intoxication would affect striatal activations within the brain reward network. A striatal ROI was built with the WFU PickAtlas (Maldjian, Laurienti, & Burdette, 2004; Maldjian, Laurienti, Kraft, & Burdette, 2003; Tzourio-Mazoyer et al., 2002) by combining the bilateral putamen, caudate and globus pallidus.

Finally, we quantified mean percent (%) BOLD signal change in striatal areas that showed significant brain activation following marketing exposure during abstinence and intoxication. Functional striatal masks were created with Marsbar to identify marketing and cannabis/alcohol related activations. Montreal Neurological Institute and Hospital (MNI) coordinates of the identified ROIs were 8 4 -4 (nucleus accumbens) for GLM1 and 8 18 8 (right caudate) for GLM2. Mean % BOLD signal change was quantified for all marketing clips (cannabis, alcohol and neutral) in each group and in each treatment condition using the SPM toolbox rfxplot (Gläscher, 2009). % BOLD signal change during abstinence was analyzed in SPSS following the same outline as previous GLM models. GLM1 consisted of 2 by means of a GLM univariate ANOVA with a main factors Group (3 levels; alcohol group on placebo, cannabis group on placebo and control) and Marketing cue (3 levels; alcohol, cannabis and neutral cues). GLM2 by means of repeated measures GLMs consisted of the factors Treatment (2 levels, alcohol/THC and placebo) Marketing cue (3 levels; alcohol, cannabis and neutral cues) with Group (2 levels; cannabis and alcohol group) as between subject factor.

Implicit cognition and subjective measures

Dependent parameters of the SC-IAT, AAT and subjective measures during abstinence were analyzed by means of a GLM univariate ANOVA with a main factor Group (3 levels; alcohol

group on placebo, cannabis group on placebo and control) and Stimulus Category (4 levels: alcohol, cannabis, soda and neutral) additionally for the AAT. These were followed by simple group contrast relative to the controls. The effects of the factors Alcohol treatment (2 levels, alcohol and placebo) and Cannabis treatment (2 levels; cannabis and placebo) cues were assessed in repeated measures GLMs in the alcohol and cannabis group respectively.

If the sphericity assumption was violated, the Greenhouse-Geisser correction was used. The alpha criterion significance level was set at p = 0.05. All statistical tests were conducted with SPSS version 20.0.

3. Results

3.1 Missing data

A total of 18 datasets for the alcohol and a total of 19 datasets for the cannabis and control group each entered the fMRI analyses. For analyses of the behavioural data, 20 complete datasets for the alcohol and control group and 21 for the cannabis group entered the analyses, except for the VAS where data from 2 subjects were missing and for the POMS where data from 3 subjects were missing.

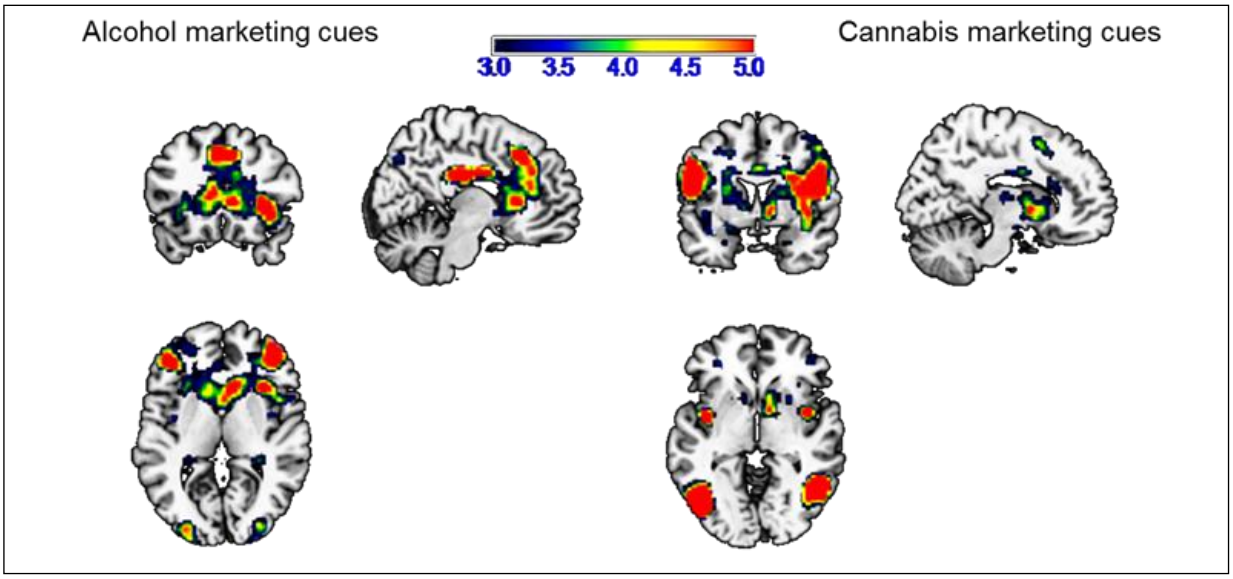
3.2 fMRI Analyses

Whole brain analyses

GLM1 analyses revealed a main effect of Group on BOLD response (red) in the left hippocampus and right precuneus and a main effect of Marketing Cue in parietal, temporal and frontal brain regions. Overall, exposure to marketing cues increased BOLD activations across these brain regions. Marketing related brain activities were equally present in all group as shown by the lack of a Group x Marketing cue interaction. Significant brain clusters associated with main effects of Group and Marketing cue are given in Table 3. Mean changes in BOLD activation following exposure to cannabis and alcohol marketing cues collapsed over the three groups are shown in Figure 1.

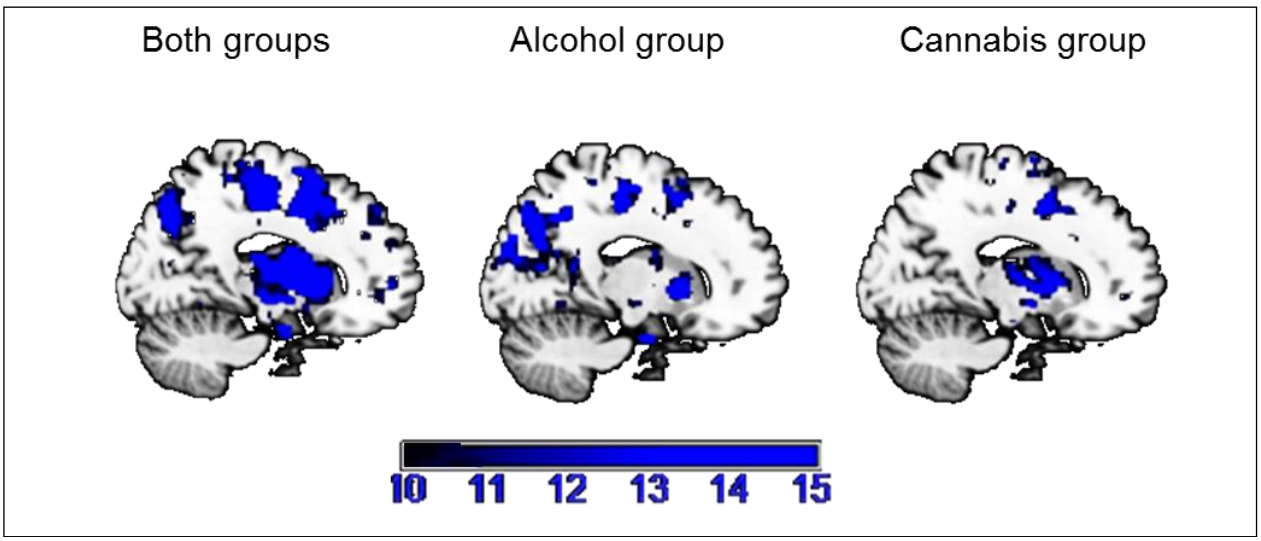
GLM2 analyses revealed a main effect of Group on BOLD response in the cuneus, rolandic operculum, brainstem, insula, amygdala, cerebellum and temporal and frontal clusters. A main effect of Treatment on BOLD response in the right supplementary motor area was observed. A main effect of Marketing Cue on BOLD response was found in the postcentral cluster, cingulum, temporal, parietal, frontal and occipital cortex. Significant brain clusters associated with main effects of Group, Treatment and Marketing cue are given in Table 4. Mean changes in BOLD activation (blue) following exposure to cannabis and alcohol marketing cues collapsed over the three groups are shown in Figure 2.

Figure 1. BOLD activation (red) following cannabis and alcohol marketing exposure collapsed over all three groups.



Note: Images are shown in neurological convention (left=left, right=right) and T-values are presented on a standard MNI T1 brain. The scale represents the T-values associated with the statistical analyses, which depicts the strength of activation in the positive range of a particular brain region compared to other brain regions, from least active (blue) to very active (red).

Figure 2. BOLD deactivation (blue) following marketing cue exposure during alcohol and cannabis intoxication, collapsed over the cannabis and alcohol group and for each group separately.



Note: Images are shown in neurological convention (left=left, right=right) and F-values are presented on a standard MNI T1 brain. The scale represents the F-values associated with the statistical analyses, which depicts the strength of activation in the negative range of a particular brain region compared to other brain regions, from least inactive (dark blue) to very inactive (lighter blue).

ROI analyses

GLM1 revealed a main effect of Group on BOLD response in the left pallidum during marketing exposure across all groups. The factor Marketing cue did not differentially affect BOLD

response in the striatum. The GLM2 analysis revealed a main effect of Group on BOLD response in the right caudate. The factor treatment caused an overall decrease in the BOLD response in the bilateral pallidum and thalamus. The factor Marketing cue did not differentially affect BOLD response in the striatum. Significant brain clusters associated with main effects of Group, Treatment and Marketing cues for the 2 ROI analyses are given in Table 3 and 4.

Table 3. Brain areas activated after marketing cue exposure during placebo (no drug) conditions across 3 groups

	BA	Number of voxels	Peak MNI coordinates	F-value	p-value FWE cluster corrected <.05
WHOLE BRAIN ANALYSES					
Group					
Left hippocampus	30	185	20, 20, 26	17.68	0.007
Right precuneus	7,31	915	0, 50, 20	14.71	0.050
Marketing cue					
Right superior parietal cluster	7	2027	32, 48, 66	38.81	0.000
Left middle temporal cluster	37	1253	54, 68, 4	35.61	0.001
Right inferior temporal cluster	37	1190	50, 64, 6	33.90	0.002
Left interior parietal cluster	40	1202	36, 38, 52	30.46	0.006
Right inferior frontal cluster	44	397	54, 10, 26	28.21	0.014
TEMPLATE-BASED REGION OF INTEREST ANALYSES					
Group					
Left pallidum		22	24, 8, 6	13.22	0.006

Note: BA= Brodmann area; FWE=Familywise error

% Signal change

GLM 1 revealed a main effect of Marketing cue ($F_{2,52}$ = 12,8; p<.001). Simple contrast indicated that cannabis marketing cues (p<.001) and alcohol marketing cues (p<.001) increased BOLD activation in the striatum, relative to neutral marketing cues. The factors Group and Group x Marketing cue did not reach significance.

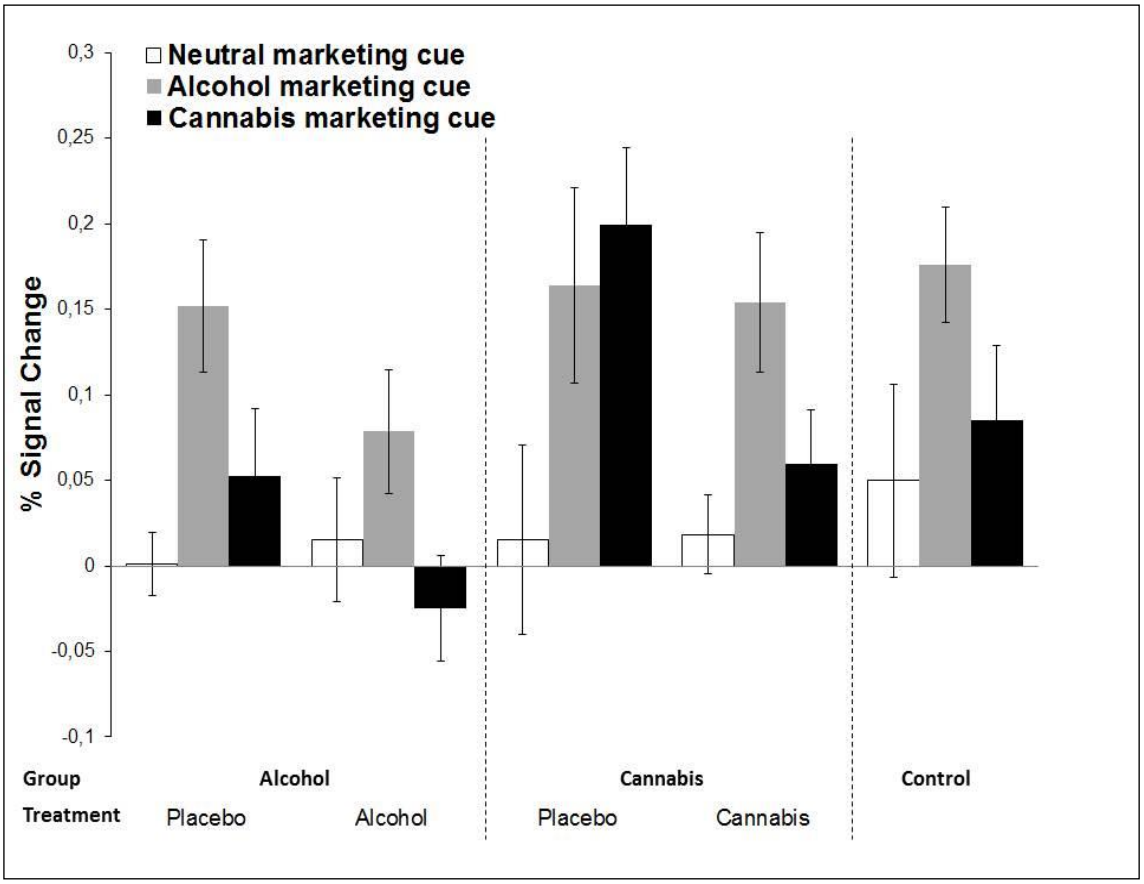
GLM 2 revealed main effects of Treatment ($F_{1,35}$ = 4,18; p=.048) and Marketing cue ($F_{2,34}$ = 14,6; p<.001). Treatment with alcohol and cannabis generally reduced BOLD activation in the striatum relative to placebo (p=.048) whereas cannabis (p=0.014) and alcohol (p<.001) marketing cues generally increased BOLD activation, relative to neutral cues. The interactions between Treatment, Group and Marketing cue did not reach significance.

Table 4. Brain areas activated after marketing cue exposure during placebo and drug conditions in the alcohol and cannabis group

	BA	Number of voxels	Peak MNI coordinates	F value	P value FWE corrected
WHOLE BRAIN ANALYSES					
Group					
Left cuneus		5658	10, 72, 26	46.57	0.000
Right superior temporal cluster	42	432	62, 44, 20	33.87	0.001
Right rolandic operculum	48	898	50, 24, 20	27.83	0.012
Brainstem		130	0, 12, 26	27.09	0.015
Right insula	48	317	36, 20, 30	26.38	0.020
Left middle temporal cluster	21	245	56, 32, 12	25.55	0.027
Left amygdala		38	16, 4, 14	24.76	0.037
Left medial superior frontal cluster	10	230	2, 60, 34	24.72	0.038
Left cerebellum	30	109	16, 42, 14	24.02	0.049
Treatment					
Right supplementary motor area	6	39977	14, 8, 54	41.61	0.000
Marketing cue					
Right inferior temporal cluster	37	1703	56, 64, 4	47.25	0.000
Left middle occipital cluster	19	1336	46, 76, 4	46.87	0.000
Right superior parietal cluster	2	1607	36, 44, 62	39.08	0.000
Left middle temporal cluster	22	417	60, 20, 2	30.44	0.004
Left postcentral cluster	40	976	38, 34, 44	30.14	0.005
Right superior temporal cluster	21	372	60, 2, 6	28.69	0.008
Right inferior frontal cluster	44	458	54, 12, 24	28.00	0.011
Right middle cingulum	24	117	2, 26, 36	24.80	0.036
TEMPLATE-BASED REGION OF INTEREST ANALYSES					
Group					
Right caudate		32	18, 8, 22	16.87	0.035
Treatment					
Right pallidum		1672	16, 6, 2	39.45	0.000
Left pallidum		1533	26, 6, 2	33.25	0.000
Thalamus		6	20, 24, 20	16.76	0.037

Note: BA= Brodmann area; FWE=Familywise error

Figure 3. Mean (SE) percent signal change for the striatal activation clusters, in each group, treatment and stimulus type.



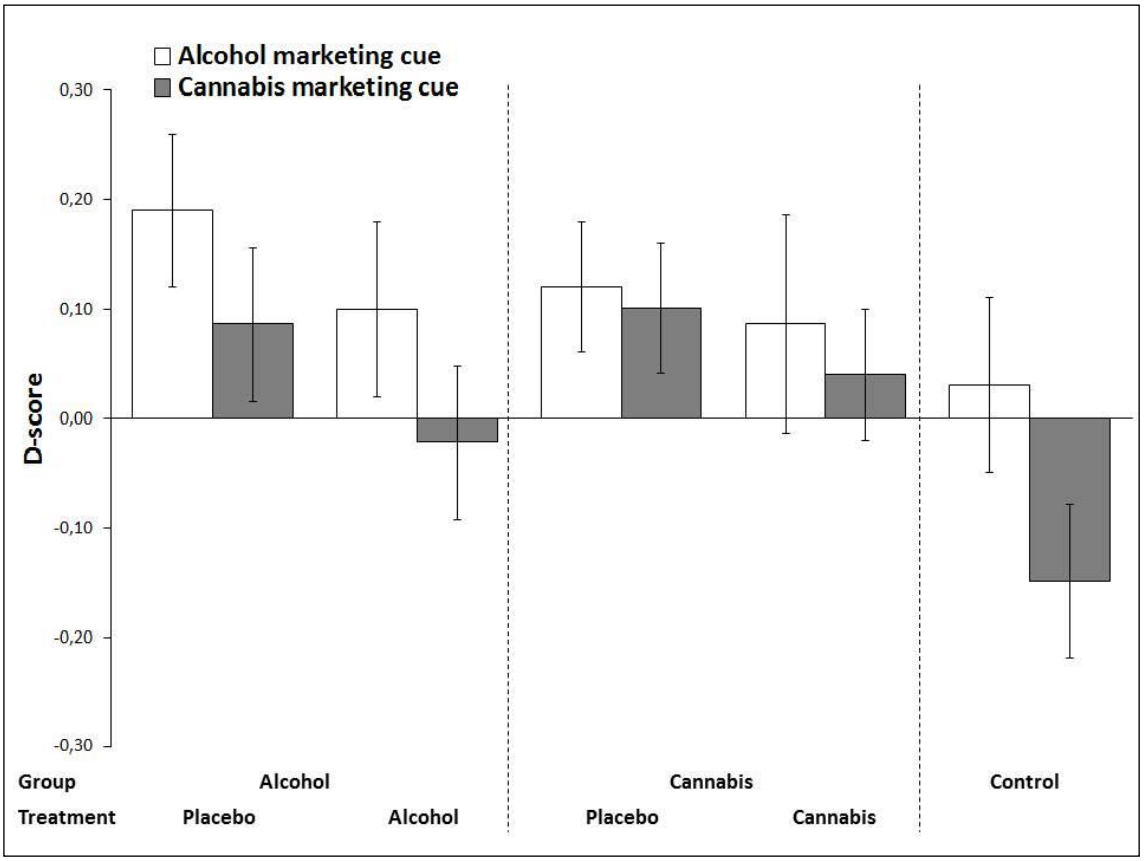
3.3 Implicit cognition

Overall, implicit associations (SC-IAT) following exposure to cannabis cues significantly differed between groups during abstinence ($F_{2,58}$ = 4.16; *p* = .021). Simple groups contrast revealed that D-scores following cannabis cues were more positive in the cannabis (p = .012) and alcohol group (p = .020) relative to controls. Overall, implicit associations with alcohol cues did not differ between groups. Simple contrasts revealed that associations with alcohol cues tended to be higher in the alcohol group as compared to the group of controls (p = .058).

During intoxication with alcohol and cannabis, mean D-scores were less relative to placebo but failed to reach statistical significance. Mean D-scores obtained in the cannabis group, alcohol group and controls are shown in Figure 4.

Bias scores in the AAT did not differ between Groups and were also not affected by alcohol and cannabis intoxication. Stimulus category did also not affect bias. Mean bias scores are presented in Table 5.

Figure 4. Mean (SE) D-Scores from the SC-IAT, following alcohol and cannabis marketing cues in each group and each treatment condition.



3.4 Subjective Assessment

A summary of mean (SE) subjective ratings in all groups during all treatment conditions is given in Table 5. There were no significant differences in sleep quality and quantity between the two conditions as measured by the GSS.

POMS ratings did not differ between Groups during abstinence. GLM analysis revealed an effect of Alcohol and Cannabis treatment on POMS factor scores. Subjects under the influence of alcohol experienced more anxiety ($F_{1,19}$ = 7.19; p = .015) and more confusion ($F_{1,18}$ = 6.14; p = .037). Subjects under the influence of cannabis experienced more fatigue ($F_{1,20}$ = 6.50; p = .019) and confusion ($F_{1,20}$ = 10.64; p = .004) compared to placebo. Other POMS scales were not affected by alcohol or cannabis treatment.

Subjective intoxication did not differ between Groups. There was a significant effect of Alcohol and Cannabis treatment on subjective intoxication ratings. Subjective intoxication ($F_{1,17}$ = 89.3; p = .000) was significantly increased after alcohol consumption in the alcohol group. In the cannabis group, subjective intoxication ($F_{1,20}$ = 136.5; p = .000) was significantly increased after cannabis administration.

GLM 1 revealed that subjective craving for alcohol significantly increased after presentation of Marketing movies across all groups during abstinence ($F_{2,54}$= 39,5; p<.001). Both alcohol movies (p<.001) and cannabis movies (p=.008) increased alcohol craving relative to neutral movies. The Factor Group and Group x Marketing cue did not affect subjective craving during

abstinence. GLM 2 revealed that subjective craving for alcohol was not affected by the factor Treatment. Marketing cues ($F_{2,35}$= 20,9; p<.001) increased subjective craving, both following alcohol (p<.001) and cannabis (p=.003) marketing movies. GLM 1 also indicated that the factor Marketing cue significantly increased subjective cannabis craving ($F_{2,54}$= 17,7; p<.001). Both alcohol (p=.043) and cannabis (p<.001) marketing movies increased cannabis craving relative to neutral movies. Overall, subjective craving differed between Groups ($F_{2,55}$= 28,7; p<.001) and was higher in the cannabis group as compared to controls (p<.001). GLM 2 further revealed that the Factor Treatment slightly increased subjective cannabis craving ($F_{1,36}$= 4,1; p=.05). GLM2 also revealed main effects of Marketing ($F_{2,35}$= 17,9; p<.001, Group ($F_{1,36}$= 55,1; p<.001) and Marketing cue x Group ($F_{2,35}$= 9,4; p<.001). The latter indicated that subjective cannabis craving following marketing cues was most pronounced in the cannabis group as compared to the alcohol group.

Table 5. Mean bias scores in the AAT and subjective ratings for groups and treatments separately.

	Alcohol group		**Cannabis group**		**Control group**
Treatment	**Placebo treatment**	**Alcohol treatment**	**Placebo treatment**	**Cannabis treatment**	
AAT					
Neutral cues	44.48 (12.76)	54.20 (14.76)	16.50 (18.64)	32.24 (11.65)	24.40 (15.47)
Soda cues	36.28 (9.06)	33.45 (11.88)	28.79 (9.47)	22.14 (16.61)	16.85 (20.12)
Alcohol cues	42.13 (10.33)	39.15(14.07)	13.57 (17.57)	18.31 (10.06)	22.08 (11.13)
Cannabis cues	18.65 (8.65)	34.68 (10.67)	11.69 (28.32)	−7.24 (19.03)	33.23 (15.09)
POMS					
Anxiety	2.61 (0.43)	3.84(0.72)	3.34 (0.40)	3.84 (0.42)	4.31 (0.55)
Depression	1.42 (0.77)	1.29 (0.52)	0.46 (0.19)	0.45 (0.17)	0.92 (0.57)
Anger	1.95 (0.44)	2.26 (0.68)	1.49 (0.41)	0.99 (0.25)	1.96 (0.49)
Vigor	13.33 (1.01)	13.86 (0.92)	11.38 (1.19)	10.77 (0.99)	12.68 (1.14)
Fatigue	2.72 (0.61)	3.64 (0.72)	2.27 (0.64)	3.19 (0.61)	2.28 (0.68)
Confusion	4.12 (0.41)	5.08 (0.49)	3.94 (0.41)	5.41 (0.51)	3.99 (0.51)
Positive mood	10.37 (1.02)	11.33 (0.93)	9.91 (1.06)	11.11 (0.96)	10.22 (1.07)
Friendliness	20.55 (0.98)	20.98 (1.03)	19.55 (1.24)	20.54 (1.10)	18.61 (1.15)
Elation	11.79 (0.79)	12.62 (0.82)	10.37 (1.02)	11.56 (0.90)	11.14 (0.92)
Arousal	9.14 (1.51)	8.99 (1.38)	8.51 (1.69)	6.02 (1.49)	11.03 (1.63)
VAS					
Subjective high	6.51 (1.69)	34.4 (3.05)	4.51 (1.68)	41.11 (3.23)	N/A
Craving					
Alcohol Craving					
Alcohol cues	27.95 (5.95)	28.91 (5.65)	21.46 (4.56)	23.83 (4.09)	15.49 (4.76)
Cannabis cues	12.01 (3.49)	15.62 (4.06)	12.09 (3.09)	11.53 (3.27)	5.33 (2.83)
Neutral cues	12.05 (3.93)	14.03 (4.04)	9.40 (3.01)	10.04 (2.75)	4.51 (3.03)
Cannabis Craving					
Alcohol cues	3.46 (0.95)	4.73 (1.53)	19.98 (4.65)	24.51 (5.19)	2.12 (2.73)
Cannabis cues	8.49 (2.67)	8.55 (2.87)	48.51 (6.12)	51.77 (6.16)	3.12 (3.85)
Neutral cues	3.43 (1.13)	3.85 (1.29)	17.63 (4.93)	21.29 (5.06)	1.75 (2.90)

3.5 Pharmacokinetics

Mean alcohol concentrations in breath and cannabinoid concentrations in serum for the alcohol and cannabis treatment conditions are shown in Table 6.

Table 6. Mean (SE) concentrations of THC and metabolites in serum in the cannabis group and blood alcohol concentrations levels in the alcohol group, at the different time points.

	THC [µg/L]	THC-OH [µg/L]	THC-COOH [µg/L]	BAC [g/L]
Baseline	1.24 (.45)	.44 (.28)	15.89 (1.36)	.00 (.00)
Before scanning (11:00)	46.48 (1.59)	3.93 (.26)	27.66 (0.84)	.76 (.03)
Before implicit cognition (12:45)	24.17 (1.46)	3.16 (.28)	27.34 (1.02)	.79 (.02)

Note: THC = Tetrahydrocannabinol; THC-OH = 11-Hydroxy-THC; THC-COOH = 11-nor-9-Carboxy-THC; BAC = Blood Alcohol Concentration

4. Discussion

The goal of this study was to assess brain reactivity to drug marketing cues in heavy alcohol and cannabis users during abstinence as well as intoxication. Brain activity of drug-users was compared to a non-drug using control group. All three groups were exposed to both alcohol and cannabis marketing cues during which functional imaging and a series of implicit cognition tests were performed.

The influence of marketing cues on brain activity were specifically addressed in a GLM model that included the imaging data obtained during the placebo condition in the alcohol and cannabis group and in controls. Overall, cannabis and alcohol marketing cues significantly increased BOLD activation in the hippocampus and precuneus across all groups during non-drug use (placebo). Specific analyses of the striatal region of interest furthermore indicate a strong increase in BOLD activation in the pallidum. In addition, exposure to marketing cues specifically stimulated BOLD activations in a wide range of parietal, temporal and frontal networks. These results are consistent with those from studies that reported wide-spread brain activations in reward, motivation and memory circuits in drug users compared to non-drug users after exposure to drug-cues (e.g. Cousijn et al., 2013; Goldstein et al., 2009; Janes et al., 2010; McClernon, Hiott, Huettel, & Rose, 2005; Myrick et al., 2004; Smolka et al., 2006; Zijlstra, Veltman, Booij, van den Brink, & Franken, 2009).

Activation of striatal and cortical networks following exposure to marketing movies of cannabis and alcohol use strongly suggests that such marketing can trigger similar brain responses that have also been observed during drug use or drug craving (Martín-Santos et al., 2010; Volkow et al., 2006; Wong et al., 2006). The ventral pallidum is involved in reward, motivation and drug reinforcement. It integrates reward signals from the limbic system and is considered to be an essential convergent point for motivation and reward signaling pathways in the brain as it is responsible for coding and causing enhancements of reward learning, motivation and hedonics (Everitt & Robbins, 2005; K. S. Smith, Tindell, Aldridge, & Berridge, 2010). Hence, cue-triggered activation of the ventral pallidum such as in the present study might promote motivational reactions in drug users to engage in drug taking and could trigger relapse in abstinent users.

Activation in the precuneus and frontal cortex areas along with the striatum was higher in the alcohol and cannabis using groups. The precuneus and (pre)frontal cortex are involved in cognitive processes responsible for the regeneration of rich episodic contextual associations of environmental stimuli (Lundstrom et al., 2003; Lundstrom, Ingvar, & Petersson, 2005). Therefore, cue-triggered activation of these areas in the cannabis but not control group might reflect neuro-cognitive processes resulting from memory recollections of drug-related activities during cue exposure. The cognitive bias toward drug-related stimuli during abstinence reflected as increased activity in the striatum, is a potential result of neural sensitization induced by repeated drug exposure (Frankel, Alburges, Bush, Hanson, & Kish, 2008; Koob & Volkow, 2010) and has been shown to be stronger in heavy users as compared to occasional users (Vollstädt-Klein et al., 2010).

A second GLM analysis was conducted to specifically assess treatment effects in the alcohol and cannabis group. Both alcohol and cannabis treatments significantly decreased BOLD activation in the right supplementary area and the striatum across the alcohol and cannabis groups. In addition, a range of parietal, temporal and frontal activations was reflected differential activations across the alcohol and cannabis groups as well as across the alcohol and cannabis marketing cues, independent of drug treatment. As expected, brain areas associated with marketing cue exposure showed a strong overlap with those areas identified during first GLM across non-drug conditions all three groups.

The finding that active treatment with alcohol and cannabis caused a reduction in BOLD activation in the striatum was further explored in separate analyses of 2 striatal ROIs that showed the largest % signal change. Striatal ROIs were determined for GLM1 (Marketing activation) and GLM2 (Treatment activation) separately. ROI was located in the right nucleus accumbens and the right caudate nucleus for GLM1 and GLM2 respectively. Analyses of mean % signal change confirmed the overall findings reported above. Overall, cannabis and alcohol marketing movies significantly increased % signal changes in both striatal ROIs. Treatment with alcohol and cannabis significantly decreased % signal change in the pallidum. Together, these results indicate that alcohol and cannabis marketing movies can stimulate striatal parts of the human reward system when drug users are not under the influence of drug or alcohol. The impact of marketing however was less pronounced when under the influence of alcohol or cannabis as shown to be a decrease in striatal brain activation. This suggests that the reinforcing effects of marketing movies are reduced during alcohol or cannabis intoxication.

Performance during the cannabis SC-IAT differed significantly between the alcohol and cannabis users and controls. The control group had negative bias scores, which contrasted with positive bias scores of the alcohol and cannabis group indicating positive implicit association for cannabis-related stimuli in cannabis and alcohol users. This result is in line with previous reviews indicating that a positive, implicit attitude towards drug-related cues is a characteristic of alcohol and substance users (Field & Cox, 2008; Field, Wiers, Christiansen, Fillmore, & Verster, 2010). The observation that alcohol users also showed positive bias scores in response to cannabis cues also indicates that this bias towards drugs might not only be limited to their drug of choice (alcohol) exclusively. Negative bias scores of controls during this task imply negative implicit association for cannabis-related stimuli further indicating that non-drug using individuals do not show implicit bias towards drug cues.

Performance during the alcohol SC-IAT was not affected by alcohol or cannabis treatment nor was there a difference in performance between the 3 groups during abstinence. Although alcohol and cannabis users did display higher alcohol bias scores during placebo as compared to controls, these differences only tended to reach significance in the alcohol group ($p=0.058$).

In general, these findings seem in line with previous research. Duka and Townshend (2004) investigated the acute effects of alcohol (BAC 0.08%) on implicit bias in heavy social drinkers and reported no differences between placebo and alcohol in attentional bias towards alcohol-cues. Other researchers only found a decrease in implicit bias above certain alcohol dose thresholds in binge-drinkers, as expressed by a negative correlation between the amount of units consumed and the magnitude of attentional bias (Schoenmakers & Wiers, 2010). Other studies on the other hand did show an increase in bias in heavy drinkers following a small dose (0.3 g/kg, BAC 0.03%) of alcohol (Schoenmakers, Wiers, & Field, 2008), but no effect was found on alcohol-approach associations, suggesting a dissociation in dose-dependent increases of attentional bias after alcohol intoxication.

There appears to be a parallel between imaging data and behavioural data. BOLD activation during fMRI sessions as well as alcohol and cannabis bias during the implicit association tasks were increased during exposure to alcohol and cannabis marketing. Yet, BOLD activation during marketing exposure significantly decreased during intoxication with alcohol and cannabis. Likewise, mean alcohol and cannabis bias scores in the SC-IAT were lower during alcohol and cannabis intoxication as compared to placebo, albeit non-significantly. According to some models of addictive behaviours, attentional bias is thought to develop partly due to classical conditioning causing substance-related stimuli to elicit the expectancy of drug availability in absence of the drug itself. This expectation causes an experience in subjective craving and enhances the attention-grabbing properties of substance-related cues that they are exposed to (Field & Cox, 2008). The present data indeed confirm that alcohol and cannabis marketing movies increase explicit liking for alcohol and cannabis as assessed with subjective questionnaires, both during abstinence and during actual drug use. This notion along with the current results further confirms that subjective craving and implicit drug bias following alcohol and cannabis marketing is particularly evident in alcohol and drug users. However, in contrast to subjective craving, brain reactivity to marketing cues is prominent during abstinence but very minimal during alcohol and cannabis intoxication. This strongly suggests that the actual reinforcing properties of marketing decrease when striatal networks involved in reward interact with actual drug or alcohol use. Reinforcing stimuli have previously been shown to cause burst firing of midbrain dopamine neurons that leads to a temporary, phasic release of dopamine in the striatum (Schultz, 2007). The striatal response or reward sensitivity to such phasic dopaminergic innervations varies with the availability of tonic dopamine in the same area (Cools & D´Esposito, 2011). Reward sensitivity is high when tonic dopamine is low and vice versa. The present data fits this notion very well. Marketing exposure produced an increase in reward sensitivity as evinced by increased striatal BOLD activation. The phasic response to marketing however decreased in the presence of elevated tonic dopamine known to be caused by alcohol (Gilman, Ramchandani, Davis, Bjork, & Hommer, 2008) and cannabis (Bossong et al., 2009) administration.

Alcohol and cannabis both significantly increased ratings of subjective intoxication and increased negative mood as measured by the POMS questionnaire. Alcohol made subjects feel more anxious and confused and cannabis made subjects feel more confused and fatigued. Subjective increases in levels of intoxication and measures of confusion have been previously reported after cannabis smoking (Mathew & Wilson, 1993). Results of studies that investigated the anxiolytic effects of alcohol on the other hand are mixed, some report an increase while others report a reduction in anxiety (for a review see Eckardt et al., 1998). These results confirm that alcohol and cannabis have a significant effect on mood, whether these are positive or negative.

5. Conclusion and recommendations for policy

This was the first study to examine the impact of marketing cues on brain activity/reward neurocircuitry in alcohol and cannabis users during abstinence and during intoxication. Alcohol and cannabis marketing significantly increased BOLD activation in the striatum across all groups. The impact of marketing on striatal activation, however, decreased during alcohol and cannabis intoxication. It is concluded that alcohol and cannabis marketing activate the brain reward circuit, which may increase craving for alcohol and cannabis. The reward circuitry response to drug marketing was blunted during actual drug and alcohol intoxication.

Alcohol and cannabis marketing increase reward sensitivity for these substances and increases motivation for actual use. A reduction of alcohol and drug marketing volume would lessen its impact, particularly in regular alcohol and cannabis users, by reducing brain exposure to reward cues that motivate and prepare for alcohol or drug use.

6. Acknowledgements

The authors thank Anita van Oers, Emina Helena van Veen, Fabian van den Berg and Laura Steenbergen for their contribution to this study.

The research leading to these results or outcomes has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013), under Grant Agreement nº 266813-Addictions and Lifestyle in Contemporary Europe – Reframing Addictions Project (ALICE RAP). Participant organisations in ALICE RAP can be seen at http://www.alicerap.eu/about-alice-rap/partner-institutions.html.

The views expressed here reflect those of the authors only and the European Union is not liable for any use that may be made of the information contained therein.

7. References

Anderson, P., de Bruijn, A., Angus, K., Gordon, R., & Hastings, G. (2009). Impact of alcohol advertising and media exposure on adolescent alcohol use: a systematic review of longitudinal studies. *Alcohol and Alcoholism (Oxford, Oxfordshire)*, *44*(3), 229–43. doi:10.1093/alcalc/agn115

Anton, R. F. (1999). What is craving? Models and implications for treatment. *Alcohol Research & Health : The Journal of the National Institute on Alcohol Abuse and Alcoholism*, *23*(3), 165–73. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10890811

Berger, S. P., Hall, S., Mickalian, J. D., Reid, M. S., Crawford, C. a, Delucchi, K., & Carr, K. (1996). Haloperidol antagonism of cue-elicited cocaine craving. *Lancet*, *347*(9000), 504–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8596268

Bossong, M. G., van Berckel, B. N. M., Boellaard, R., Zuurman, L., Schuit, R. C., Windhorst, A. D., … Kahn, R. S. (2009). Delta 9-tetrahydrocannabinol induces dopamine release in the human striatum. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *34*(3), 759–66. doi:10.1038/npp.2008.138

Carter, B. L., & Tiffany, S. E. N. T. (1999). Meta-analysis of cue-reactivity in addiction research. *Addiction*, *94*(April 1998).

Cassisi, J. E., Delehant, M., Tsoutsouris, J. S., & Levin, J. (1998). Psychophysiological reactivity to alcohol advertising in light and moderate social drinkers. *Addictive Behaviors*, *23*(2), 267–274.

Cools, R., & D´Esposito, M. (2011). Inverted-U shaped dopamine actions on human working memory and cognitive control. *Biological Psychiatry*, *69*(12), 113–125. doi:10.1016/j.biopsych.2011.03.028.Inverted-U

Cousijn, J., Goudriaan, A. E., Ridderinkhof, K. R., van den Brink, W., Veltman, D. J., & Wiers, R. W. (2013). Neural responses associated with cue-reactivity in frequent cannabis users. *Addiction Biology*, *18*(3), 570–80. doi:10.1111/j.1369-1600.2011.00417.x

Cousijn, J., Goudriaan, A. E., & Wiers, R. W. (2011). Reaching out towards cannabis: approach-bias in heavy cannabis users predicts changes in cannabis use. *Addiction (Abingdon, England)*, *106*(9), 1667–74. doi:10.1111/j.1360-0443.2011.03475.x

De Wit, H., Enggasser, J. L., & Richards, J. B. (2002). Acute administration of d-amphetamine decreases impulsivity in healthy volunteers. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *27*(5), 813–25. doi:10.1016/S0893-133X(02)00343-3

Degenhardt, L., & Hall, W. (2012). Extent of illicit drug use and dependence, and their contribution to the global burden of disease. *Lancet*, *379*(9810), 55–70. doi:10.1016/S0140-6736(11)61138-0

Duka, T., & Townshend, J. M. (2004). The priming effect of alcohol pre-load on attentional bias to alcohol-related stimuli. *Psychopharmacology*, *176*(3-4), 353–61. doi:10.1007/s00213-004-1906-7

Eckardt, M. J., File, S. E., Gessa, G. L., Grant, K. a, Guerri, C., Hoffman, P. L., … Tabakoff, B. (1998). Effects of moderate alcohol consumption on the central nervous system. *Alcoholism, Clinical and Experimental Research*, *22*(5), 998–1040. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9726269

Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature Neuroscience*, *8*(11), 1481–9. doi:10.1038/nn1579

Field, M., & Cox, W. M. (2008). Attentional bias in addictive behaviors: a review of its development, causes, and consequences. *Drug and Alcohol Dependence*, *97*(1-2), 1–20. doi:10.1016/j.drugalcdep.2008.03.030

Field, M., Eastwood, B., Bradley, B. P., & Mogg, K. (2006). Selective processing of cannabis cues in regular cannabis users. *Drug and Alcohol Dependence*, *85*(1), 75–82. doi:10.1016/j.drugalcdep.2006.03.018

Field, M., Mogg, K., & Bradley, B. P. (2004). Cognitive bias and drug craving in recreational cannabis users. *Drug and Alcohol Dependence*, *74*(1), 105–11. doi:10.1016/j.drugalcdep.2003.12.005

Field, M., Wiers, R. W., Christiansen, P., Fillmore, M. T., & Verster, J. C. (2010). Europe PMC Funders Group Acute Alcohol Effects on Inhibitory Control and Implicit Cognition : Implications for Loss of Control Over Drinking. *Alcohol Clin Exp Res*, *34*(8), 1346–1352. doi:10.1111/j.1530-0277.2010.01218.x.Acute

Filbey, F. M., Schacht, J. P., Myers, U. S., Chavez, R. S., & Hutchison, K. E. (2009). Marijuana craving in the brain. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(31), 13016–21. doi:10.1073/pnas.0903863106

Frankel, P. S., Alburges, M. E., Bush, L., Hanson, G. R., & Kish, S. J. (2008). Striatal and ventral pallidum dynorphin concentrations are markedly increased in human chronic cocaine users. *Neuropharmacology*, *55*(1), 41–6. doi:10.1016/j.neuropharm.2008.04.019

Franken, I. H. a. (2003). Drug craving and addiction: integrating psychological and neuropsychopharmacological approaches. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *27*(4), 563–79. doi:10.1016/S0278-5846(03)00081-2

Franken, I. H. a, Booij, J., & van den Brink, W. (2005). The role of dopamine in human addiction: from reward to motivated attention. *European Journal of Pharmacology*, *526*(1-3), 199–206. doi:10.1016/j.ejphar.2005.09.025

Gilman, J. M., Ramchandani, V. a, Davis, M. B., Bjork, J. M., & Hommer, D. W. (2008). Why we like to drink: a functional magnetic resonance imaging study of the rewarding and anxiolytic effects of alcohol. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *28*(18), 4583–91. doi:10.1523/JNEUROSCI.0086-08.2008

Goldstein, R. Z., Alia-Klein, N., Tomasi, D., Carrillo, J. H., Maloney, T., Woicik, P. a, … Volkow, N. D. (2009). Anterior cingulate cortex hypoactivations to an emotionally salient task in cocaine addiction. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(23), 9453–8. doi:10.1073/pnas.0900491106

Goudriaan, A. E., de Ruiter, M. B., van den Brink, W., Oosterlaan, J., & Veltman, D. J. (2010). Brain activation patterns associated with cue reactivity and craving in abstinent problem gamblers, heavy smokers and healthy controls: an fMRI study. *Addiction Biology*, *15*(4), 491–503. doi:10.1111/j.1369-1600.2010.00242.x

Gray, K. M., LaRowe, S. D., Upadhyaya, H. P. (2009). Cue reactivity in young marijuana smokers: a preliminary investigation. *Psychology of Addictive Behaviors*, *22*(4), 582–586. doi:10.1037/a0012985.Cue

Greenwald, A. G., Nosek, B. a., & Banaji, M. R. (1995). Greenwald & Banaji, 1995.pdf. *Psychological Review*, *102*(1), 4–27.

Greenwald, A. G., Nosek, B. a., & Banaji, M. R. (2003). Understanding and using the Implicit Association Test: I. An improved scoring algorithm. *Journal of Personality and Social Psychology*, *85*(2), 197–216. doi:10.1037/0022-3514.85.2.197

Heinz, A., Braus, D. F., Smolka, M. N., Wrase, J., Puls, I., Hermann, D., … Büchel, C. (2005). Amygdala-prefrontal coupling depends on a genetic variation of the serotonin transporter. *Nature Neuroscience*, *8*(1), 20–1. doi:10.1038/nn1366

Hyman, S. E., Malenka, R. C., & Nestler, E. J. (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annual Review of Neuroscience*, *29*, 565–98. doi:10.1146/annurev.neuro.29.051605.113009

Janes, A. C., Pizzagalli, D. a, Richardt, S., Frederick, B. D. B., Holmes, A. J., Sousa, J., … Kaufman, M. J. (2010). Neural substrates of attentional bias for smoking-related cues: an FMRI study. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *35*(12), 2339–45. doi:10.1038/npp.2010.103

Kahneman, D. (2003). A perspective on judgment and choice: mapping bounded rationality. *The American Psychologist*, *58*(9), 697–720. doi:10.1037/0003-066X.58.9.697

Karpinski, A., & Steinman, R. B. (2006). The single category implicit association test as a measure of implicit social cognition. *Journal of Personality and Social Psychology*, *91*(1), 16–32. doi:10.1037/0022-3514.91.1.16

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *35*(1), 217–38. doi:10.1038/npp.2009.110

Lovato, C., Linn, G., Stead, L. F., & Best, A. (2003). Impact of tobacco advertising and promotion on increasing adolescent smoking behaviours (Review). *Cochrane Database of Systematic Reviews*, (3).

Lundstrom, B., Ingvar, M., & Petersson, K. (2005). The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. *NeuroImage*, *27*(4), 824–34. doi:10.1016/j.neuroimage.2005.05.008

Lundstrom, B., Petersson, K., Andersson, J., Johansson, M., Fransson, P., & Ingvar, M. (2003). Isolating the retrieval of imagined pictures during episodic memory: activation of the left precuneus and left prefrontal cortex. *NeuroImage*, *20*(4), 1934–1943. doi:10.1016/j.neuroimage.2003.07.017

Maldjian, J. a, Laurienti, P. J., & Burdette, J. H. (2004). Precentral gyrus discrepancy in electronic versions of the Talairach atlas. *NeuroImage*, *21*(1), 450–455. doi:10.1016/j.neuroimage.2003.09.032

Maldjian, J. a., Laurienti, P. J., Kraft, R. a., & Burdette, J. H. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *NeuroImage*, *19*(3), 1233–1239. doi:10.1016/S1053-8119(03)00169-1

Marhe, R., Waters, A. J., van de Wetering, B. J., & Franken, I. H. (2014). NIH Public Access. *Consult Clin Psychol.*, *81*(1), 1–12. doi:10.1037/a0030754.Implicit

Martín-Santos, R., Fagundo, a B., Crippa, J. a, Atakan, Z., Bhattacharyya, S., Allen, P., … McGuire, P. (2010). Neuroimaging in cannabis use: a systematic review of the literature. *Psychological Medicine*, *40*(3), 383–98. doi:10.1017/S0033291709990729

Mathew, R. J., & Wilson, W. H. (1993). Acute changes in cerebral blood flow after smoking marijuana. *Life Sciences*, *52*, 757–767.

Mayer, A. R., Franco, A. R., Ling, J., & Cañive, J. M. (2007). Assessment and quantification of head motion in neuropsychiatric functional imaging research as applied to schizophrenia. *Journal of the International Neuropsychological Society : JINS*, *13*(5), 839–45. doi:10.1017/S1355617707071081

McClernon, F. J., Hiott, F. B., Huettel, S. a, & Rose, J. E. (2005). Abstinence-induced changes in self-report craving correlate with event-related FMRI responses to smoking cues. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *30*(10), 1940–7. doi:10.1038/sj.npp.1300780

McClure, S. M., Li, J., Tomlin, D., Cypert, K. S., Montague, L. M., & Montague, P. R. (2004). Neural correlates of behavioral preference for culturally familiar drinks. *Neuron*, *44*(2), 379–87. doi:10.1016/j.neuron.2004.09.019

Mulder-Hajonides van der Meulen, W. R. E. H., Weinberg, J. R., Hollanders, J. J., DeDiana, I., & Hoofdakker, R. (1980). Measurements of subjective sleep quality. In Elsevier (Ed.), *Fifth European Congress on Sleep Research, Amsterdam*.

Myrick, H., Anton, R. F., Li, X., Henderson, S., Drobes, D., Voronin, K., & George, M. S. (2004). Differential brain activity in alcoholics and social drinkers to alcohol cues: relationship to craving. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *29*(2), 393–402. doi:10.1038/sj.npp.1300295

Newcomb, M. D. (1988). Cognitive Motivations for Drug Use Among Adolescents : Longitudinal Tests of Gender Differences and Predictors of Change in Drug Use, *35*(4), 426–438.

Power, J. D., Barnes, K. a, Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *NeuroImage*, *59*(3), 2142–54. doi:10.1016/j.neuroimage.2011.10.018

Ramaekers, J. G., Kauert, G., Theunissen, E. L., Toennes, S. W., & Moeller, M. R. (2009). Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. *Journal of Psychopharmacology (Oxford, England)*, *23*(3), 266–77. doi:10.1177/0269881108092393

Schacter, D. L. (1992). Understanding implicit memory. A cognitive neuroscience approach. *The American Psychologist*, *47*(4), 559–69. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1595984

Schoenmakers, T., & Wiers, R. (2010). Craving and attentional bias respond differently to alcohol priming: a field study in the pub. *European Addiction Research*, *16*(1), 9–16. doi:10.1159/000253859

Schoenmakers, T., Wiers, R., & Field, M. (2008). Effects of a low dose of alcohol on cognitive biases and craving in heavy drinkers. *Psychopharmacology*, *197*(1), 169–78. doi:10.1007/s00213-007-1023-5

Schultz, W. (2007). Multiple dopamine functions at different time courses. *Annual Review of Neuroscience*, *30*, 259–88. doi:10.1146/annurev.neuro.28.061604.135722

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2010). NIH Public Access, *196*(2), 155–167. doi:10.1016/j.bbr.2008.09.038.Ventral

Smith, L. a, & Foxcroft, D. R. (2009). The effect of alcohol advertising, marketing and portrayal on drinking behaviour in young people: systematic review of prospective cohort studies. *BMC Public Health*, *9*, 51. doi:10.1186/1471-2458-9-51

Smolka, M. N., Bühler, M., Klein, S., Zimmermann, U., Mann, K., Heinz, A., & Braus, D. F. (2006). Severity of nicotine dependence modulates cue-induced brain activity in regions involved in motor preparation and imagery. *Psychopharmacology*, *184*(3-4), 577–88. doi:10.1007/s00213-005-0080-x

Stacy, A. W., Ames, S. L., & Knowlton, B. J. (2004). Neurologically Plausible Distinctions in Cognition Relevant to Drug Use Etiology and Prevention. *Substance Use & Misuse*, *39*(10-12), 1571–1623. doi:10.1081/LSUM-200033204

Stacy, A. W., & Wiers, R. W. (2012). Implicit cognition and addiction: a tool for explaining paradoxical behavior. *Annual Review of Clinical Psychology*, *6*, 551–575. doi:10.1146/annurev.clinpsy.121208.131444.Implicit

Talairach, J., & Tournoux, P. (1988). Co-planar stereotaxic atlas of the human brain. 3-Dimensional proportional system: an approach to cerebral imaging.

Tapert, S. F., Cheung, E. H., Brown, G. G., Frank, L. R., Paulus, M. P., Schweinsburg, A. D., … Brown, S. a. (2003). Neural response to alcohol stimuli in adolescents with alcohol use disorder. *Archives of General Psychiatry*, *60*(7), 727–35. doi:10.1001/archpsyc.60.7.727

Tye, J. B., Warner, K. E., & Glantz, S. a. (1987). Tobacco advertising and consumption: evidence of a causal relationship. *Journal of Public Health Policy*, *8*(4), 492–508. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3323236

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., … Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *NeuroImage*, *15*(1), 273–89. doi:10.1006/nimg.2001.0978

United Nations Office on Drugs. (2010). *World drug report 2010*.

Volkow, N. D., Wang, G.-J., Telang, F., Fowler, J. S., Logan, J., Childress, A.-R., … Wong, C. (2006). Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(24), 6583–8. doi:10.1523/JNEUROSCI.1544-06.2006

Vollstädt-Klein, S., Wichert, S., Rabinstein, J., Bühler, M., Klein, O., Ende, G., … Mann, K. (2010). Initial, habitual and compulsive alcohol use is characterized by a shift of cue processing from ventral to dorsal striatum. *Addiction (Abingdon, England)*, *105*(10), 1741–9. doi:10.1111/j.1360-0443.2010.03022.x

Wiers, R. W., Van Woerden, N., Smulders, F. T. Y., & De Jong, P. J. (2002). Implicit and explicit alcohol-related cognitions in heavy and light drinkers. *Journal of Abnormal Psychology*, *111*(4), 648–658. doi:10.1037//0021-843X.111.4.648

Wiers, R. W., Houben, K., Smulders, F. T., Conrod, P. J., & Jones, B. T. (2005). To drink or not to drink: the role of automatic and controlled cognitive processes in the etiology of alcohol-related problems. In *Handbook of implicit cognition and addiction* (pp. 339–361).

Wong, D. F., Kuwabara, H., Schretlen, D. J., Bonson, K. R., Zhou, Y., Nandi, A., … London, E. D. (2006). Increased occupancy of dopamine receptors in human striatum during cue-elicited cocaine craving. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *31*(12), 2716–27. doi:10.1038/sj.npp.1301194

World Health Organization. (2004). *Global Status Report on Alcohol 2004*.

World Health Organization. (2014). Global status report on alcohol and health 2014.

Yacubian, J., & Büchel, C. (2009). The genetic basis of individual differences in reward processing and the link to addictive behavior and social cognition. *Neuroscience*, *164*(1), 55–71. doi:10.1016/j.neuroscience.2009.05.015

Yoo, S.-S., Choi, B.-G., Juh, R., Pae, C.-U., & Lee, C.-U. (2005). Head motion analysis during cognitive fMRI examination: application in patients with schizophrenia. *Neuroscience Research*, *53*(1), 84–90. doi:10.1016/j.neures.2005.06.004

Zijlstra, F., Veltman, D. J., Booij, J., van den Brink, W., & Franken, I. H. a. (2009). Neurobiological substrates of cue-elicited craving and anhedonia in recently abstinent opioid-dependent males. *Drug and Alcohol Dependence*, *99*(1-3), 183–92. doi:10.1016/j.drugalcdep.2008.07.012

PART II

STUDY 1: The malleability of automatic and deliberative alcohol related cognitive processes by alcohol advertisement among light and heavy drinkers: Relationship with prospective drinking

J Witteman[1], EB de Sousa Fernandes Perna[2], J Cousijn[3], A De Bruijn[1], Wiers R[3], W van Dalen[1], EL Theunissen[2], KPC Kuypers[2], & JG Ramaekers[2]

[1] Dutch institute for alcohol policy (STAP), Utrecht, The Netherlands
[2] Department of Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, The Netherlands
[3] Department of Developmental Psychology, University of Amsterdam, The Netherlands

Abstract

Dual-process theories of addiction have proposed two different levels of alcohol related cognitive processing to be involved in addiction. On the one hand, addiction behavior is driven by 'automatic' cognitive processes. These processes are fast and efficient, unintentional and not open to introspection. Deliberative processes have been suggested to be involved in drinking behavior too. These processes, in contrast, are relatively slow, intentional and can be introspectively assessed. The present study investigated whether exposure to alcohol advertisement might affect drinking behaviour through automatic or deliberative cognitive processing, or both. Furthermore, it was examined whether the nature of alcohol advertisement effects differ between light and heavy drinkers of alcohol. Last, it was studied to what extent automatic and deliberative alcohol related processing could predict future drinking behaviour. Therefore, automatic and deliberative alcohol related processing was measured in light and heavy drinkers of alcohol following exposure to a block of alcohol and soft-drink advertisements. Additionally, prospective drinking behaviour was assessed one month later. The results indicated that acute (i.e., a single sessions of-) alcohol advertisement exposure does not affect the automatic level of alcohol related processing, but does affect deliberative level processing in male light drinkers. Furthermore, deliberative alcohol related processing predicted prospective drinking behaviour while automatic processing did not. It is concluded that acute alcohol advertisement exposure influences alcohol related processing through the deliberative re-evaluation of validity tags in male light drinkers. Further, deliberative alcohol related processing might play a role in the development of addiction and hence modulation of deliberative processing may be a suitable target for prevention.

1. Introduction

Alcohol advertisement is a pervasive phenomenon in society. A systematic review of longitudinal studies concluded that exposure to alcohol advertisement increases the probability that youngsters initiate drinking and increases drinking among those who have already started drinking (Anderson, De Bruijn, Angus, Gordon, & Hastings, 2009). Thus theoretically, alcohol advertisement exposure may contribute to the onset and progression of alcohol dependence by initiating and intensifying drinking behavior. However, at present it is unknown how (through what psychological mechanisms) alcohol advertisement influences behaviour. Furthermore, it is unknown whether the same or different mechanisms may explain the influence of marketing on drinking behaviour in populations that vary in drinking history, such as light versus heavy (but not clinically dependent) drinkers. To shed light on these issues, the present investigation exposed light and heavy drinkers to alcohol and control advertisement, tested what psychological processes might be affected by acute alcohol advertisement exposure (i.e., a single session of alcohol advertisement exposure) and to what extent these processes predict future drinking.

Recent insights from psychology into the nature of attitudes suggest that attitudes about an object (such as alcohol) may be determined by both automatic and deliberative levels of processing (Petty, Briñol, & DeMarree, 2007; Wiers et al. 2007). Automatic processing is characterized by several features such as efficiency, goal-independence, and uncontrollability, while deliberative processing on the other hand is slower, goal-directed and controllable (Moors and de Houwer, 2006). The 'Meta-Cognitive Model" (MCM) of attitudes (Petty et al., 2007) integrates these two processing levels into a model of attitudes and proposes that at each level of processing there may be both positive and negative attitudes about an object. The net sign (positive versus negative) of an attitude towards an object is then determined by

the interaction of both within level balance of positive and negative attitudes and the interaction between both levels of processing. Additionally, the net attitude towards an object is determined by 'meta-cognitive processes', such as how valid or useful one considers an attitude. This implies that the net sign of attitudes towards an object (such as alcohol) as measured with a test of (primarily) automatic attitudes such as the Implicit Association Task (Greenwald, Nosek, & Banaji, 2003) could be different from the net sign as obtained from a self report measure (which would be sensitive to the combination of automatic, deliberative and meta-cognitive processes). Also, the model implies that to obtain a complete understanding of the effects of advertisement it would be advantageous to combine measures of automatic and deliberative processes (Comello & Slater, 2011). Thus, in the present investigation effects of alcohol advertisement on automatic- and deliberative alcohol related processing were systematically investigated.

At what level of processing then, might alcohol advertisement influence attitudes towards alcohol? To the best of the authors' knowledge, this has not been investigated directly and only a handful of experimental studies outside of the alcohol field have examined the issue. These studies have found that exposure to (anti-) advertisement affects both automatic processes as measured with automatic priming paradigms and the IAT (Czyzewska & Ginsburg, 2007; Grande, Frosch, Perkins, & Kahn, 2009; Strick, Van Baaren, Holland, & Van Knippenberg, 2009; Goodall & Slater, 2010; but see Gibson, 2008) and deliberative processes that are additionally captured by self-report measures such as liking and favourableness of self reported attitudes (Martin et al., 2002; Czyzewska & Ginsburg, 2007; but see Gibson, 2009; Grande et al., 2009; Goodall & Slater, 2010). Thus, the previous literature suggests that both automatic and deliberative processing of a marketed object can be influenced by marketing.

Several mechanisms have been proposed to account for advertisement effects on automatic and deliberative processes. First, evaluative conditioning (Brunel, Tietje, & Greenwald, 2004; Goodall & Slater, 2010) could influence automatic processing by repeatedly paring the object of advertisement with a pleasant stimulus and through repeated exposure increasing the favourableness of automatic attitudes towards the marketed object in an associative manner. Additionally, the mere exposure effect, i.e., the phenomenon that familiar stimuli are perceived as relatively pleasant, has been proposed to be mediated by automatic processes and hence could account for automatic effects of advertisement exposure (Brunel et al., 2004; Grimes & Kitchen, 2007). Last, applying the MCM model to advertisement, meta-cognitive evaluation of attitudes may be an additional deliberative mechanism through which the net attitude towards the marketed object may be influenced (i.e., through the deliberative consideration of the validity of attitude favourableness towards the marketed object) and that will be apparent on self-report measures (Petty, Briñol, & DeMarree, 2007).

Both automatic and deliberative processes then, might account for the (mixed) evidence that has been presented in favour of acute alcohol advertisement exposure effects on drinking behaviour (for negative results see Koordeman et al. 2011; Koordeman et al. 2012; for a positive result, see Engels et al, 2009). Acute effects of context on automatic processes have been criticized however, both from a methodological perspective (regarding context effect on the IAT, see Han, Czellar, Olson, & Fazio, 2010) and because it seems unlikely that automatic processes could be easily sculpted by relatively low levels and/or low intensity of exposure (Han et al., 2010). Indeed, by definition, automatic processes have been proposed to be hard to influence by external factors (Moors & De Houwer, 2006). Thus, only chronic (such as practice) and/or profound (such as repeated direct pharmacological action by addictive substances, Robbinson & Berride, 2008) exposure to an external or internal stimulus has been

proposed to be able to (de-)automate neurocognitive processes, rendering it unlikely that a single session of exposure to advertisement would be able to mould automatic processes.

For habitual heavy drinkers of alcohol, an additional mechanism may mediate effects of alcohol advertisement. Incentive sensitization theory (Robbinson & Berridge, 2008) proposes that with repeated heavy alcohol use, alcohol cues will be associated with the primary reinforcing effect of alcohol through classical conditioning and gain 'incentive salience'. In turn, increased incentive salience of alcohol cues would promote the development of automatic attitudes about and automatic approach behaviour towards alcohol, ultimately increasing further drinking (Wiers et al, 2007). Indeed, using the Approach Avoidance Task (AAT), automatic approach behaviour towards alcohol cues has been demonstrated in heavy drinkers (Schoenmakers, Wiers, & Field, 2008; Field, Kieran, Eastwood, & Child, 2008; Wiers, Rinck, Dictus, & Van den Wildenberg, 2009; Field, Caren, Fernie, & De Houwer, 2011). Further, using the Implicit Association Task (IAT), negatively valenced automatic attitudes (Wiers, Van Woerden, Smulder, & De Jong 2002; De Houwer, Crombez, Koster, & Beul, 2004; Houben, Rothermund, & Wiers, 2009) and positive automatic arousal attitudes (De Houwer et al., 2004; Wiers et al., 2002) have been demonstrated in heavy drinkers, which has been interpreted as simultaneously disliking (due to negative experiences with the drug) but at the same time (pathological) wanting of the drug (Wiers et al., 2002) as predicted by incentive sensitization theory (Robbinson & Berridge, 2008). Exposure to alcohol cues such as presented in alcohol advertisement may then engage the sensitized reward system (Tapert et al., 2003) and engage automatic processes (Cox, Brown & Rowlands, 2003) to approach alcohol and ultimately promote drinking in heavy drinkers (Koordeman, Anschutz, & Engels, 2012). If true, we would expect a more pronounced response of automatic processes in heavy than light drinkers in response to alcohol cues as presented during alcohol advertisement.

To shed light on these issues, in the present investigation light and heavy drinkers of alcohol were exposed to alcohol- and control advertisement and purported effects on automatic and deliberative processes were measured. To maximize comparability of the effect on automatic and deliberative processes, we adopted the approach by Wiers, Van Woerden, Smulders, and De Jong (2002) and maximized the comparability of both levels of processing by maximizing the similarity of stimulus material of indirect (automatic) and self-report measures. It was expected that acute alcohol marketing exposure would influence automatic processes, particularly in heavy drinkers, or deliberative processes, or both. Last, as previous work has suggested that automatic and deliberative alcohol related processes predict unique variance in future drinking behaviour (Wiers et al., 2002; Houben, Rothermund, & Wiers, 2009), we additionally tested the predictive power automatic and deliberative processes in predicting prospective drinking behaviour.

2. Methods

2.1 Procedure

Approximately one week before the first experimental session, all participants were familiarized with the automatic measures of attitudes and approach behaviour (see materials section for more details) and all participants filled in the retrospective alcohol calendar (the Time Line Follow Back, see materials section for more details) probing drinking behaviour in the previous month.

During the first experimental session, either a block of alcohol or soda advertisement (see materials section for more details) was shown (the order of advertisement content over the

first versus second experimental session was counterbalanced across subjects) after which subjects indicated their level of enjoyment of the commercials. Subsequently, subjects performed the automatic measures of automatic attitudes and approach behaviour (task order counterbalanced across subjects). After completion of the automatic measures, subjects completed the self-report scales probing attitudes about alcohol and approach behaviour towards alcohol (see materials section for more details).

During the second experimental session approximately one week later, participants watched the other category of (soda versus alcohol) advertisements. All the other experimental procedures we exactly the same as for the first experimental session except that subjects additionally filled in a questionnaire probing alcohol problems (see materials section for more details) at the end of the second session. Subsequently, participants rated all the stimulus material used in the measure of automatic approach behaviour on a 9-point Manakin scale for arousal and valence. Finally, participants were debriefed. Both experimental sessions took approximately 30 minutes to complete. One month after the second experimental session participants were interviewed by telephone to assess frequency and intensity of alcohol use.

The present experiments were part of a larger scale (neuroimaging) study that was evaluated by the ethics committee of Maastricht University and was conducted in accordance with the Declaration of Helsinki. Participants in the control group received a total of € 70 and participants in the cannabis and the alcohol group € 175 for completion of the study.

2.2 Participants

Sixty two young adults participated in the study: (i) Heavy Drinkers (HD) (N = 20) of alcohol, defined as using 21 to 50 standard units (10 grams of pure alcohol) per week for males and 15 to 35 standard units per week for females (total number standard units in the previous 30 days: M = 107 (SD = 47); age: M = 22.65 (SD = 2.43); 10 males); (ii) Light Drinkers (LD) of alcohol (N =20), defined as using 1 to 14 standard units of alcohol per week, but that do not use cannabis (total number standard units in the previous 30 days: M = 32.41 (SD = 21.41); age: M = 22.85 (SD = 2.27); 10 males) and (iii) Light Drinkers of alcohol (1 to 14 standard units of alcohol per week) that use cannabis regularly (N = 22), defined as using cannabis at least 3 times a week but not more than 10 times a week (total number standard units in the previous 30 days: M = 32.11 (SD = 23.57); age: M = 21.82 (SD = 2.20); 16 males). For the present investigation, the light drinkers of alcohol that did and the group that did not use cannabis were collapsed resulting in a group of 42 light drinkers (alcohol in standard units: M = 32.26 (SD = 22.22); age: M = 22.31 (SD = 2.27); males) as the groups did not significantly differ in alcohol consumption (independent T-Test: p = 0.96). Exclusion criteria were (1) history of drug abuse other than alcohol or cannabis (2) DSMV-IV diagnosis substance dependence and (3) current or history of psychiatric disorder

2.3 Materials

Drug use

Alcohol use in the previous month was assessed using the timeline follow-back method (Sobell & Sobell, 1990). This method uses a calendar on which participants indicate how many standard units (using a conversion table) were consumed for every day of the previous month. Mean frequency of cannabis use in the previous month was assessed over the previous 3 months was assessed one week before the first experimental session using a self report questionnaire.

Prospective drinking behaviour was assessed by telephone interview based on the Quick Drinking Screen (QDS), a 'quantity-frequency' method for assessing alcohol use that correlates highly with the Time Line Follow Back Method (Sobell et al., 2003). This method assesses the mean number of drinking days per week and the mean number of standard units consumed on a drinking day to estimate the total number of standard units consumed in the previous month. When the drinking pattern was variable (e.g., a large difference in number of drinks consumed during the week versus during the weekend) mean number of drinks consumed on each of these days was assessed separately to derive the total number of standard units consumed. Additionally, the maximum number of standard units consumed in the previous month and the number of binge drinking days (50 and 60 grams of pure alcohol or more for females and males respectively within one drinking session) was assessed.

Alcohol related problems

The Rutgers Alcohol Problem Index (RAPI: White & Labouvie, 1989) was used to assess the severity of alcohol related problems. This questionnaire uses a 5-point Likert scale to assess how frequently a participant experiences alcohol problems (e.g., unable to study for exams due to alcohol use). The RAPI has adequate reliability (> .80) and validity (discriminates between clinical and non-clinical populations (White & Labouviee, 1989). In the present investigation the mean RAPI score for HD was 20.36 (7.18), and for LD 9.67 (6.63) indicating that the mean alcohol problem severity was just below the clinical range.

Advertisement

Advertisement blocks consisted of a three-minute series of five soda or alcohol commercials. The commercials were pre-existing commercials from brands not available in the Netherlands in order to exclude brand specific effects. The alcohol commercials contained images of mainly beer but also of wine and liquor (shooters and vodka), footage of these beverages being poured into a glass and of people consuming these beverages. The soda commercials were matched in content to the alcohol commercials.

Automatic measures

Two automatic measures were used. First, the Approach and Avoidance Task (AAT) developed by Cousijn, Goudriaan, & Wiers (2011) was adapted to measure automatic action tendencies towards alcohol. Participants viewed 15 alcohol related, 15 soda related and 15 neutral pictures. Alcohol and soda related images depicted common brands of alcohol beer, wine and liquor and soda in the Netherlands and close ups of individuals drinking these beverages. Neutral images consisted of (people using) office appliances. The three image categories were matched on colour and composition. At the end of the study, all images were rated on a 9-point Manakin scale for arousal and valence (in other words, to what extent a picture is considered to be positive or negative) by the subjects (for two subjects, one from the control group and one from the alcohol group, the data was not available). Concerning arousal, the analyses indicated that for Heavy Drinkers (HD), alcohol was rated as more arousing than soda and soda was rated more arousing than office supplies. For Light Drinkers (LD), both soda and alcohol images were rated as more arousing than office supplies, but there was no difference between soda and alcohol images. Concerning valence, analyses indicated that HD rated the valence of alcohol images higher than valence of soda images, and the valence of soda images higher than the valence of office supplies. LD rated the valence of soda images higher than of alcohol images and office supplies, while valence of office supplies and alcohol was not significantly different. The full analyses can be found in the Supplementary material. Arousal and valence ratings for each category and each group can be found in Table 1.

All images were presented twice rotated 3º to the left and twice rotated 3º to the right (resulting in a total of 60 trials per image category). Subjects were instructed to either pull or push a joystick for pictures rotated to the left or right. Push or pull instruction for left versus right orientated pictures was counterbalanced across participants. Note that, as the (alcohol versus non-alcohol-) content of the images was task irrelevant, the current adaptation of the AAT can be argued to be a more valid index of *automatic* action tendency towards alcohol as compared to versions of the AAT in which the content of the images is task relevant as in the present version of the task any observed action tendency towards alcohol pictures would exist despite low attention to the alcohol content (Moors & De Houwer, 2006). The push or pull action resulted in a zooming out or zooming in of the picture on the computer screen respectively which, combined with the flexion or extension action on the joystick, simulates avoid and approach behaviour (Wiers, Rinck, Dictus, & Van den Wildenberg, 2009). Two stimulus orders were used to eliminate stimulus order effects (counterbalanced across participants). The task started with a short practice session with grey rotated squares to familiarize subjects with the test procedures.

Table 1. Mean (SD) arousal and valence ratings for the stimulus categories used in the AAT for each experimental group.

Dimension	Category	Light drinkers	Heavy drinkers
Arousal	Alcohol	3.17 (1.66)	4.19 (1.61)
	Soda	3.24 (1.85	3.37 (1.36)
	Neutral	2.47 (1.73)	2.05 (1.05)
Valence	Alcohol	5.01 (0.86)	5.99 (1.11)
	Soda	5.45 (0.88)	5.54 (0.88)
	Neutral	4.97 (0.37)	4.78 (0.61)

Second, the Single Category bipolar Implicit Association Task (SC-IAT) was used to assess automatic attitudes towards alcohol. In the SC-IAT, positive (e.g., 'HAPPY') and negative (e.g., 'SAD') words are combined with alcohol pictures and presented in blocks. In the first block, only affective words were presented and participants had to affectively categorize each word by pressing the appropriate response key (positive or negative) as fast as possible. In the second block, the alcohol pictures were coupled with one of the affective (e.g. the negative) words by placing the word 'ALCOHOL' in one of the upper corners of the computer screen with one of the affective words (e.g., 'NEGATIVE'). Participants had to press the appropriate response key (e.g., 'NEGATIVE') as fast as possible for alcohol pictures and the respective affective category. In the final block, the alcohol pictures were combined with the other affective (e.g. positive) category and participants had to press the other response key (see Table 2 for a schematic presentation of the task). The last two (critical) blocks consisted of 72 trials, each comprising 36 critical trials (alcohol pictures). The positive and negative words used can be found in Appendix A. Alcohol pictures consisted of alcohol commercial prints for wine, beer and alcoholpops with the respective products (i.e. bottles of beer, wine and alcoholpop) clearly visible. There were 6 target pictures that were repeated 5 times. The target stimuli were randomly presented (with the restriction that a particular target picture could not be presented in subsequent trials) in the center of the screen until the participant responded, with an inter stimulus interval of 250 msec. Subjects were instructed to respond as quickly and accurately as possible. If an error was made, a red cross appeared on the screen. Assignment of the response keys (left versus right) to the affective categories (positive versus negative) and order of blocks (press positive for alcohol vs. press negative for alcohol) was counterbalanced across subjects.

Table 2. Schematic presentation of the SC-IAT

Block	Task	Left key	Right key
1	Target discrimination	Positive words	Negative words
2	Initial combination	Positive words	Negative words, Alcohol
3	Reversed combination	Positive words, Alcohol	Negative words

Self-report measures

As a manipulation check of whether the alcohol advertisements appealed to the participants, participants indicated how enjoyable they found alcohol and soda advertisement by indicating their level of enjoyment on an unmarked 11 cm VAS-scale.

Self-reported attitudes about alcohol were matched as much as possible to the automatic measure (the SC-IAT) to maximize comparability between automatic and self-reported effects. Thus, with respect to deliberative attitudes towards alcohol, we adopted the approach by Wiers, Van Woerden, Smulders, and De Jong (2002) and used exactly the same affective words as used in the SC-IAT and asked participants to indicate on an 11 cm unmarked VAS-scale to what extent they would agree with statements associating each affective word with alcohol (e.g., '*After drinking alcohol I am happy',* where 'happy' was also used as an affective target word in the SC-IAT).

Similarly, we used the Approach and Avoidance of Alcohol Questionnaire (AAAQ) as a deliberative analogue of the AAT. The AAAQ is a 14-item questionnaire to measure self-reported approach and avoidance tendency towards alcohol. It has been found to have a two-factor structure among alcohol dependent patients (Klein, Stasiewicz, Koutsy, Bradizza, & Coffey, 2007) and a three-factor structure among non-dependent adults (McEvoy, Stritzke, French, Lang, & Ketterman, 2004). Further, adequate reliability and initial support for convergent validity has been presented (McEvoy et al., 2004; Klein et al. 2007).

2.4 Pre-processing

For the IAT, the algorithm recommended by Greenwald, Nosek, and Banaji (2003) was used to obtain the 'D-score'. This score can be seen as a standardized difference in correct reaction time for positive versus negative associations with alcohol. A significant departure of this measure from zero indicates a significant (positive versus negative) implicit attitude towards alcohol.

Regarding self-reported attitudes towards alcohol, responses on the six items denoting positive aspects of alcohol (such as 'Happy') were summed into a 'positive attitudes' towards alcohol scale, and the six items denoting negative aspects of alcohol (such as 'Nauseous') into a 'negative attitudes towards alcohol' scale.

Regarding the AAT, a very similar procedure was adopted to obtain a standardized difference score between approach and avoidance reaction time. This 'D measure' has been recommended based on various methodological grounds (Greenwald, Nosek, & Banaji, 2003; Siriam, Greenwald, & Nosek, 2010) in stead of taking the mean difference between the two conditions without taking into account the variance. Therefore, first all latencies for correct

responses smaller than 150 ms and larger than 1500 were removed from the analyses (2% of the data). Further, subjects with latencies smaller than 150 ms on more than 10% of the trials were excluded from the analysis (N = 0). Subsequently, the mean latency for correct responses was computed for approach trials and for avoid trials. The mean difference between the two conditions was then divided by the standard deviation for all correct trials of both blocks to obtain the standardized difference between approach and avoidance trials. Thus, a significant deviation of this measure from zero indicates a significant automatic approach/avoidance tendency.

Regarding deliberative approach and avoidance of alcohol using the AAAQ, items were scored into three scales in correspondence with the three-factor structure found in the previous literature among non-dependent drinkers (McEvoy, Stritzke, French, Lang, & Ketterman, 2004), i.e., the dimensions 'Inclined' (mild approach behaviour), 'Obsessed' (strong approach behaviour) and 'Resolved' (avoidance behaviour).

2.5 Statistical analysis

In the case of the IAT and the AAT, an Analysis of Variance (ANOVA) was performed with Advertisement (Alcohol; Soda) as the within-subject factor and Gender and Group (Light Drinker, Heavy Drinker) as between-subject variables and the D-score as dependent variable. For the self-report measures, Multivariate Analyses of Variance (MANOVA) were performed for each set of subscales with identical within and between subject factors. One-sample T-tests were performed to test whether the mean D-scores deviated significantly from zero across all conditions and groups.

Multiple linear regression analysis was performed to analyse whether the automatic measures (IAT, AAT) and self-report measures (deliberative attitudes towards alcohol, deliberative approach behaviour towards alcohol) could predict future drinking behaviour beyond pre-experimental drinking levels. Thus, the predictor variables were entered in three steps: (1) baseline drinking as reported with the Timeline Follow Back (TLFB); (2) self-reported attitudes and approach behaviour; (3) automatic attitudes (IAT) and approach behaviour (AAT).

3. Results

Internal consistency, test-retest reliability of the measures used and their associations can be found in Table 3. It should be noted that internal consistency and test-retest reliability of the SCIAT was relatively low. Furthermore, only positive self-reported attitudes towards alcohol and strong self-reported approach tendency towards alcohol (Obsesses subscale of the AAAQ) correlated with retrospectively assessed alcohol use in the month preceding the first experimental session in the expected positive direction.

Table 3. Alpha and Pearson correlations for internal consistency and test-retest reliability for the measures used and Spearman correlations between the measures and retrospective drinking.

	No. of items	Mean α	r^2	1	2	3	4	5	6	7	8
1. SCIAT	72	.17	.27	-	.						
2. AAT	60	.40	.33	.11	-						
3. Positive attitudes	5	.87	.75	-.03	.02	-					
4. Negative attitudes	5	.84	.52	.07	.12	-.23	-				
5. AAAQ inclined	5	.81	.73	-.13	-.05	.46**	-.08	-			
6. AAAQ obsessed	4	.68	.79	-.07	.04	.29*	.17	.65**	-		
7. AAAQ resolved	5	.59	.62	.01	.26*	.16	.20	.35**	.43**	-	
8. Alcohol use (TLFB)	-	-	-	-.19	.02	.38**	-.20	.23	.32*	.08	-

Note: Alpha's denote split-half reliability of each measure (mean of both sessions), test-retest reliability the Pearson correlation between scores of the two sessions. Mean (over both sessions) Spearman correlations among measures indicate associations between the measures. Spearman correlations were used because multiple measures did not adhere to a normal distribution.

Manipulation check

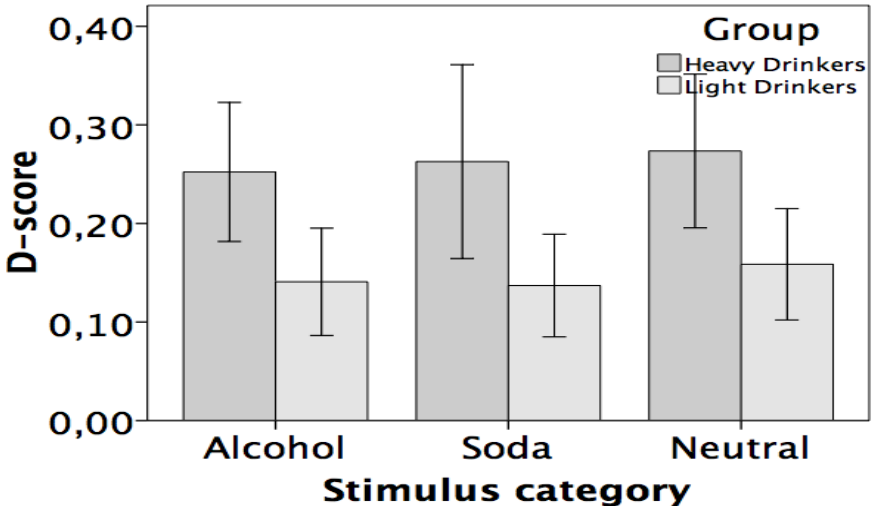
As a manipulation check of whether advertisements were positively evaluated, a repeated measures (RM-) ANOVA performed with Advertisement as within- and Gender and Group as between-subjects factors and the VAS-score evaluating the enjoyableness of the commercials as dependent variable (with 0 indicating extremely unenjoyable and 100 extremely enjoyable) revealed no main effects or interactions. For both sessions however, the mean enjoyableness rating (95% CI in brackets) was significantly higher than the mid-point (50) of the scale (Alcohol: M = 70.58 [65.28 – 75.88]; Soda: M = 74.96 [69.72 – 80.20]), indicating that both advertisement blocks were evaluated as enjoyable.

Automatic and deliberative attitudes about alcohol

Repeated-measures ANOVA, with Advertisement as within-subject factor, Gender and Group as between-subjects factor and D-score for the SCIAT as dependent, revealed no main effects or interactions. D-scores did not deviate significantly from zero across groups and conditions.

A repeated measures MANOVA with identical factors but positive and negative self-reported attitudes towards alcohol as dependents, only showed a significant multivariate main effect of Group ($F(2,57) = 5.16$, $p = .009$). Follow-up univariate tests revealed that HD scored significantly higher on positive attitudes towards alcohol than LD ($F(1,58) = 10.46$, $p = .002$) while there was no difference between the two groups on negative attitudes (See Table 4).

Figure 1. AAT D-scores for alcohol, soft-drink (soda) and neutral pictures for the heavy and light drinkers.



Automatic and deliberative approach/avoidance of alcohol

A repeated measures MANOVA with Advertisement as within-subject factor and Gender and Group as between-subjects factor and D-scores for the AAT (for alcohol, soda and neutral pictures) as dependents, revealed no main effects or interactions. As can be seen in Figure 1 however, mean AAT D-scores across both sessions for alcohol pictures deviated significantly from zero for both heavy and light drinkers. The AAT effect was not specific for alcohol pictures however, as both groups also showed a significant positive mean D-score (95% CI in brackets) indicating approach behaviour for HD (Soda M = .26 [.14 - .38]; Neutral M = .27 [.11 - .43]) and LD (Soda M= .14 [.03 - .24]; Neutral M = .16 [.06 - .26]).

A repeated measures MANOVA with identical factors but the three self-reported approach/avoidance scales as dependents, revealed a significant multivariate effect of Group (F(3,56) = 2.93, p = .04) and a multivariate Advertisement × Group × Gender (F(3,56) = 3.58, p = .02) interaction. Follow-up univariate tests, showed a significant group effect for the Obsessed subscale only (F(1,58) = 8.28, p = .006) with the heavy drinkers scoring significantly higher than the light drinkers (see Table 4). Follow-up univariate tests revealed that the multivariate Advertisement × Group × Gender effect was significant for the Inclined subscale only ((F(1,58) = 5.52, p = .02). Following up univariate repeated measures ANOVAs for males and females separately, revealed a significant Advertisement × Group interaction for males only ((F(1,58) = 4.25, p = .047). Finally, following up the two-way interaction with separate repeated measures ANOVAs for the LD and HD group among male participants, with Advertisement as the within subjects factor, revealed a highly significant effect of Advertisement for the LD-group only ((F(1,25) = 10.17, p = .004) with male light drinkers showing a higher Inclined subscale score after alcohol advertisement (M = 14.81, SD = 6.82) than after soda advertisement (M =10.89, SD = 7.92).

Table 4. Means (SD's) of the self-report measures for light drinkers and heavy drinkers.

	Light drinkers	Heavy drinkers
Positive attitudes	65.72 (16.15)**	78.08 (15.22)
Negative attitudes	24.83 (15.67)	25.61 (16.12)
AAAQ inclined	11.26 (6.59)	13.70 (7.37)
AAAQ obsessed	1.76 (2.65)**	4.15 (3.57)
AAAQ resolved	7.30 (5.07)	9.50 (5.26)

*Note: **p<0.01 Significant group difference*

Prospective drinking behaviour

Prospective alcohol use was obtained for 81% of the sample. Hierarchical multiple linear regression was performed to predict total units drunk in the month following the second experimental session based on pre-experimental total drinking (step 1), self-reported attitudes and approach/avoidance behaviour towards alcohol (step 2) and automatic attitudes (IAT) and approach/avoidance behaviour (AAT) towards alcohol (step 3). Because total units drunk was not normally distributed (Shapiro-Wilk; < 0.9, p < .001), it was log-transformed. Multicolinnearity, normality of residuals and outlier diagnostics were uneventful (minimum tolerance = .39, maximum Cook's distance = 0.18)

The final model explained 63.6% of prospective drinking which was significant ((*F*(1,48) = 12.22, p < .001). The first step (retrospective drinking) explained 47.5% of future drinking, which was significant ((*F*(1,48) = 42.46, p < .001). The second step (self-reported attitudes about alcohol and approach/avoidance towards alcohol) explained a significant additional amount of variance (R^2 change = 16%, p = .007), with in addition to baseline drinking (β = .55, p < .001) the Inclined subscale of the AAAQ as the sole significant predictor (β = .45, p = 0.005), showing a positive relationship with prospective drinking beyond predicted by baseline drinking. The final third step (automatic attitudes about alcohol and automatic approach/avoidance behaviour towards alcohol) did not explain a significant amount of additional variance in prospective drinking behaviour (R^2 change = 1.4%, NS).

4. Discussion

This study investigated whether alcohol advertisement exposure has acute effects on automatic and deliberative levels of alcohol-related processing (in other words, whether a single session of alcohol advertisement exposure has effects on alcohol-related cognitive processing immediately following exposure), and if so, whether effects differ between light and heavy drinkers of alcohol.

An acute effect of alcohol marketing was found on the deliberative- but not automatic level of processing for light drinkers of alcohol, although this effect was restricted to males. More specifically, for male light drinkers, self-reported weak approach behaviour towards alcohol (as measured with the Inclined subscale of the AAAQ) was increased following alcohol advertisement as compared to soda advertisement. This effect can be understood within the framework of the Meta-Cognitive Model (MCM) developed by Petty, Briñol, and DeMarree (2007). As these authors point out, a change in a deliberative measure (in the present study, the Inclined subscale of the AAAQ) without a change in a corresponding automatic measure (in the present study, the approach-avoidance task) may indicate the re-evaluation of 'validity-tags' concerning an already existing attitude. Thus, it can be argued that in light drinkers pre-existing approach attitudes towards alcohol were re-evaluated as more valid or more reliable after exposure to alcohol-favourable messages embedded in alcohol marketing, which resulted

in an increase on the Inclined AAAQ scale for light drinkers. For (male) heavy drinkers, approach behaviour towards alcohol was likely already regarded as valid or reliable, resulting in less room for change of validity tags and hence exposure did not result in a similar increase on the Inclined subscale among heavy drinkers. Because the alcohol advertisement primarily consisted of beer clips, which may have been particularly appealing to males (who are typically beer drinkers, e.g., see Koordeman, Anschutz, & Engels, 2011) the marketing exposure may have only translated into an effect on deliberative processing in male light drinkers.

The absence of acute advertising effects on automatic alcohol related processing seems to be at odds with previous studies that did find acute marketing effects on automatic measures. There are, however, both theoretical and empirical grounds to question the reality of acute marketing effects on automatic processing. First, by definition (Moors & De Houwer, 2006) automatic processes have been suggested to be hard to control and are therefore unlikely to be influenced by relatively weak phasic external stimulation such as one session of alcohol marketing exposure as employed in the present study (for a similar argument, see Han, Czellar, Olson, & Fazio (2010). Furthermore, it should be noted that two out of three mechanisms that have been proposed to be responsible for automatic level effects of marketing exposure, the mere exposure effect (Brunel, Tietje, & Greenwald, 2004; Grimes & Kitchen, 2007) and evaluative conditioning (Brunel et al., 2004; Goodall & Slater, 2010) assume repeated exposure and repeated paring of the marketed object with an unconditioned stimulus respectively, in order to reach sizeable automatic effects. Thus, these two mechanisms would not predict strong acute marketing effects. The third mechanism, incentive salience sensitization for alcohol cues in heavy drinkers due to repeated heavy drinking (Robinson & Berridge, 2008), does seem to allow for acute alcohol marketing exposure effects for heavy drinkers but no such effect was found in the present study. Although acute effects of alcohol marketing exposure on cue reactivity have been found previously in young heavy drinkers (Tapert et al., 2003), these effects may not generalize to automatic approach tendency. Based on the theoretical mechanisms of automatic processing effects discussed above, it is plausible however that *chronic* exposure (i.e., repeated exposure to alcohol advertisement over time) to marketing (as is the case in society) is able to mould automatic level of processing of a marketed object (such as alcohol). Indeed, the automatic approach bias found towards alcohol (and other stimulus categories) in the present study among both groups of drinkers may, although admittedly speculatively, partly have been caused by chronic alcohol marketing exposure. Thus, future studies could investigate effects of chronic alcohol marketing exposure on automatic alcohol related processing to shed light on this issue.

Furthermore empirically, it should be noted that two out of four previous studies that have found automatic-level effects of marketing have used the Implicit Association Task (Czyzewska & Ginsburg, 2007; Grande, Frosch, Perkins, & Kahn, 2009; but for different paradigms see Strick, Van Baaren, Holland, & Van Knippenberg, 2009; Goodall & Slater, 2010). In the present study, the IAT scores were limited by low reliability, as is often the case for implicit measures (Atatya et al., 2012), precluding a formal comparison. As Han, Czellar, Olson, & Fazio (2010) have pointed out, however, because there is ambiguity in the interpretation of verbal labels in the traditional IAT, a change in IAT scores due to influence of context may be caused by a change in responding with respect to the frame of (personal versus extrapersonal) reference, without a true change in automatic attitudes. In the present study the AAT had reasonable reliability (e.g, see Cousijn, Luijten, & Wiers, 2014) but did not show a corresponding change as a function of alcohol marketing as the Inclined subscale did for male light drinkers. Because the different stimulus categories were not task-relevant in the version of the AAT employed in the present study, such label ambiguity effects are less likely to occur with the AAT. Therefore, we suggest that acute marketing effects on automatic action tendency are small at best and that,

at least for the IAT, previous results may have at least partially been caused by other factors than a true change in automatic attitudes (but for acute effects of marketing on automatic measures that cannot be explained by label ambiguity effects, see Strick et al., 2009; Goodall & Slater, 2010). Thus, our results concur with the conclusion reached by previous work, that automatic processes seem to be relatively stable and hard to influence by a single session of exposure (Han et al., 2010) as employed in the present study.

Independently of marketing exposure, automatic approach tendency towards alcohol and other stimulus categories was found for both light and heavy drinkers. This result replicates a recent study by Cousijn, Luijten & Wiers (2014), which used a very similar version of the AAT (where stimulus content was task irrelevant and a joystick was used as the mode of responding with a zooming feature) and similarly found automatic approach bias towards alcohol and other stimulus categories in heavy and light drinkers. Further, non-alcohol specific approach bias in heavy drinkers with a similar version of the AAT has been found in an earlier study as well (Wiers, Rinck, Dictus, & Van den Wildenberg, 2009). Thus, although automatic approach bias for alcohol cues in heavy drinkers indeed has been observed previously (Schoenmakers, Wiers, & Field, 2008; Field, Kieran, Eastwood, & Child, 2008; Wiers, Rinck, Dictus, & Van den Wildenberg, 2009; Field, Caren, Fernie, & De Houwer, 2011; Cousijn et al., 2014) of the two studies with the necessary design to test for group-specificity (Field et al., 2011; Cousijn et al., 2014) only one study (Field et al., 2011) found a specific approach bias for heavy drinkers while the other did not (Cousijn et al., 2014). Similarly, of all the studies that reported approach bias statistics for an additional stimulus category than alcohol (Field et al., 2008; Wiers et al., 2009; Cousijn et al., 2014) only Field and colleagues (2008) reported an effect specific for alcohol-cues. Thus, the present results similarly raise the question to what extent AAT effects are indeed specific for heavy drinkers and alcohol cues, as would be predicted by incentive salience theory (Robinson & Berridge, 2008). Future studies could systematically vary both the responding procedure and task-relevance of the stimulus material with multiple groups and stimulus categories to investigate the role of methodological factors in the specificity of AAT effects.

Independently of marketing exposure, stronger positive (but not negative) self-reported attitudes and stronger self-reported strong approach tendency (as measured with the Obsessed subscale of the AAAQ) was found for heavy drinkers as compared to light drinkers. Regarding self-reported attitudes, positive attitudes have been reported for heavy drinkers previously (Wiers, Van Woerden, Smulders, & De jong, 2002; Houben, Rothermund, & Wiers, 2009). Further, the only previous study that had the necessary design to compare self reported positive and negative attitudes between light and heavy drinkers, similarly found that heavy drinkers report significantly more positive attitudes than light drinkers while no difference in negative attitudes was found (Wiers et al., 2002). Interestingly, in clinical alcohol dependence both stronger positive *and* negative self-reported attitudes about alcohol have been observed (Dickson, Gately, & Field, 2013). Thus, heavy drinking without clinical dependence may represent a stage of drinking where the many negative consequences of drinking as typically experienced by clinically dependent patients have not yet been encountered. Therefore, positive deliberative attitudes about alcohol might dominate in heavy drinking without clinical dependence in contrast to the conflict between strong deliberative positive and negative attitudes that has been observed in clinical dependence.

Similarly, regarding self-reported approach-avoidance behaviour towards alcohol, significantly stronger strong-approach behaviour (as measured with the Obsessed subscale of the AAAQ) was found for heavy drinkers as compared to light drinkers but no difference was found in avoidance behaviour. In clinically dependent patients, both stronger self-reported approach

and avoidance tendency has been observed as compared to light drinkers (Klein, Stasiewicz, Koutsky, Bradizza, & Coffey, 2007; Barky, Dickson, Roper, & Field, 2012). Thus, analogously to deliberative positive and negative attitudes about alcohol, deliberative approach tendency among non-clinically dependent heavy drinkers may represent a stage of drinking where due to the relatively low frequency of negative experiences with heavy drinking as compared to clinically dependent patients, deliberative approach tendency dominates in stead of the conflict between strong approach and avoidance behaviour as observed in clinical dependence.

Finally, weak self-reported approach tendency towards alcohol but not automatic measures predicted prospective drinking behaviour beyond pre-experimental drinking. This result seems at odds with previous studies that have found complementary predictive power of automatic and deliberative measures in predicting future drinking (Wiers, Van Woerden, Smulder, & De Jong, 2002; Houben, Rothermund, & Wiers, 2009). However, in these previous studies, the IAT was used as the automatic measure, which was limited by low reliability in the present study. Thus, the lack of predicative power of the automatic measure used in the present study, the Approach Avoidance Task (AAT), may be specific to automatic approach tendency. Future studies could employ multiple automatic measures to investigate the specificity of the predictive value of automatic measures. Last, the present study showed for the first time that deliberative weak approach tendency towards alcohol, but not deliberative positive and negative attitudes about alcohol predicted future drinking beyond baseline levels. However, it should be noted that there is significant conceptual overlap between positive and negative self reported attitudes and self-reported approach and avoidance behaviour. Future studies could examine the specificity deliberative measures in predicting prospective drinking.

In conclusion, the present study finds evidence for the malleability of deliberative approach tendency in male light drinkers by acute alcohol marketing exposure but no corresponding effect on automatic alcohol related processing, which can be interpreted as marketing induced re-evaluation of the validity of pre-existing attitudes. Further, deliberative approach tendency was successful in predicting future drinking and hence may be important for the development of addictive behaviour. If future studies replicate these results, deliberative processing might be a suitable target for the prevention of progression of drinking.

5. Recommendations for policy

The present results suggest that acute alcohol advertisement exposure promotes the tendency to drink in male light drinkers, by acting on deliberate level alcohol-related processing. Therefore, reducing alcohol advertisement exposure in male light drinkers may reduce tendency to drink and theoretically may also reduce actual drinking behaviour.

6. Acknowledgements

The research leading to these results or outcomes has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013), under Grant Agreement nº 266813-Addictions and Lifestyle in Contemporary Europe – Reframing Addictions Project (ALICE RAP). Participant organisations in ALICE RAP can be seen at http://www.alicerap.eu/about-alice-rap/partner-institutions.html.

The views expressed here reflect those of the authors only and the European Union is not liable for any use that may be made of the information contained therein.

7. References

Anderson, P., de Bruijn, A., Angus, K., Gordon, R., & Hastings, G. (2009). Impact of Alcohol Advertising and Media Exposure on Adolescent Alcohol Use: A Systematic Review of Longitudinal Studies. *Alcohol and Alcoholism, 44*, 229-243.

Ataya, A. F., Adams, S., Mullings, E., Cooper, R. M., Attwood, A. S., & Munafò, M. R. (2012). Internal reliability of measures of substance-related cognitive bias. *Drug and Alcohol Dependence, 121*, 148-151.

Brunel, F. F., Tietje, B. C., & Greenwald, A. G. (2004). Is the Implicit Association Test a Valid and Valuable Measure of Implicit Consumer Social Cognition? *Journal of Consumer Psychology, 14*, 385-404.

Comello, M. L. G., & Slater, M. D. (2011). Effects of adverts from a drug and alcohol prevention campaign on willingness to engage in alcohol-related risky behaviors. *Journal of Health Psychology, 16*, 1268-1276.

Cousijn, J., Goudriaan, A. E., & Wiers, R. W. (2011). Reaching out towards cannabis: approach-bias in heavy cannabis users predicts changes in cannabis use. *Addiction, 106*, 1667-1674.

Cousijn, J., Luijten, M., & Wiers, R. (2014). Mechanisms underlying alcohol approach action tendencies: the role of emotional primes and drinking motives. *Frontiers in Psychiatry, 5,* 44.

Cox, W. M., Brown, M. A., & Rowlands, L. J. (2003). The effects of alcohol cue exposure on non-dependent drinkers' attentional bias for alcohol-related stimuli. *Alcohol and Alcoholism, 38*, 45-49.

Czyzewska, M., & Ginsburg, H. J. (2007). Explicit and implicit effects of anti-marijuana and anti-tobacco TV advertisements. *Addictive Behaviors, 32*, 114-127.

De Houwer, J., Crombez, G., Koster, E. H. W., & Beul, N. D. (2004). Implicit alcohol-related cognitions in a clinical sample of heavy drinkers. *Journal of Behavior Therapy and Experimental Psychiatry, 35, 275-286.*

Dickson, J., Gately, C., & Field, M. (2013). Alcohol dependent patients have weak negative rather than strong positive implicit alcohol associations. *Psychopharmacology*, 228, 603-610.

Engels, R. C. M. E., Hermans, R., van Baaren, R. B., Hollenstein, T., & Bot, S. M. (2009). Alcohol portrayal on television affects actual drinking behaviour. *Alcohol and Alcoholism, 44*, 244-249.

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. *Psychology of Addictive Behaviors, 25*, 697-701.

Field, M., Kiernan, A., Eastwood, B., & Child, R. (2008). Rapid approach responses to alcohol cues in heavy drinkers. *Journal of Behavior Therapy and Experimental Psychiatry, 39*, 209-218.

Gibson, B. (2008). Can evaluative conditioning change attitudes toward mature brands? new evidence from the implicit association test. *Journal of Consumer Research, 35*, 178-188.

Goodall, C. E., & Slater, M. D. (2010). Automatically activated attitudes as mechanisms for message effects: the case of alcohol advertisements. *Communication Research*, 37, 620-643.

Grande, D., Frosch, D. L., Perkins, A. W., & Kahn, B. E. (2009). Effect of exposure to small pharmaceutical promotional items on treatment preferences. *Archives of Internal Medicine, 169*, 887-893.

Grimes, A., & Kitchen, P. J. (2007). Researching mere exposure effects to advertising. *International Journal of Market Research, 49*, 191-219.

Han, H. A., Czellar, S., Olson, M. A., & Fazio, R. H. (2010). Malleability of attitudes or malleability of the IAT? *Journal of Experimental Social Psychology, 46*, 286-298.

Houben, K., Rothermund, K., & Wiers, R. W. (2009). Predicting alcohol use with a recoding-free variant of the Implicit Association Test. *Addictive Behaviors, 34*, 487-489.

Klein, A., Stasiewicz, P., Koutry, J., Bradizza, C., & Coffery, S. (2007). A psychometric evaluation of the approach and avoidance of alcohol questionnaire (AAAQ) in alcohol dependent outpatients. *Journal of Psychopathology and Behavioral Assessment*, *29,* 241-240.

Koordeman, R., Anschutz, D. J., & Engels, R. C. (2011). Exposure to alcohol commercials in movie theaters affects actual alcohol consumption in young adult high weekly drinkers: an experimental study. *American Journal of Addiction, 20*, 285-291.

Koordeman, R., Anschutz, D. J., & Engels, R. C. M. E. (2012). The effect of alcohol advertising on immediate alcohol consumption in college students: an Experimental Study. *Alcoholism: Clinical and Experimental Research, 36*, 874-880.

Martin, S. E., Snyder, L. B., Hamilton, M., Fleming-Milici, F., Slater, M. D., Stacy, A., . . . Grube, J. W. (2002). Alcohol advertising and youth. *Alcoholism: Clinical and Experimental Research, 26*, 900-906.

McEvoy, P. M., Stritzke, W. G. K., French, D. J., Lang, A. R., & Ketterman, R. (2004). Comparison of three models of alcohol craving in young adults: a cross-validation. *Addiction, 99*, 482-497.

Moors, A., & De Houwer, J. (2006). Automaticity: A theoretical and conceptual analysis. *Psychological Bulletin, 132*, 297-326.

Petty, R. E., Briñol, P., & DeMarree, K. G. (2007). The meta–cognitive model (MCM) of attitudes: Implications for attitude measurement, change, and strength. *Social Cognition, 25*, 657-686.

Robinson, T. E., & Berridge, K. C. (2008). The incentive sensitization theory of addiction: Some current issues. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363*, 3137-3146.

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

Sobell, L. C., Agrawal, S., Sobell, M. B., Leo, G. I., Young, L. J., Cunningham, J. A., & Simco, E. R. (2003). Comparison of a quick drinking screen with the timeline followback for individuals with alcohol problems. *Journal of Studies on Alcohol, 64*, 858-861.

Sriram, N., Greenwald, A. G., & Nosek, B. A. (2010). Correlational biases in mean response latency differences. *Statistical Methodology, 7*, 277-291.

Strick, M., van Baaren, R. B., Holland, R. W., & van Knippenberg, A. (2009). Humor in advertisements enhances product liking by mere association. *Journal of Experimental Psychology: Applied, 15*, 35-45.

Tapert, S. F., Cheung, E. H., Brown, G. G., Frank, L. R., Paulus, M. P., Schweinsburg, A. D., . . . Brown, S. A. (2003). Neural Response to Alcohol Stimuli in Adolescents With Alcohol Use Disorder. *Archives of General Psychiatry, 60*, 727-735.

White, H. R., & Labouvie, E. W. (1989). Towards the assessment of adolescent problem drinking. *Journal of Studies on Alcohol, 50*, 30-37.

Wiers, R. W., Bartholow, B. D., van den Wildenberg, E., Thush, C., Engels, R. C. M. E., Sher, K. J., . . . Stacy, A. W. (2007). Automatic and controlled processes and the development of addictive behaviors in adolescents: A review and a model. *Pharmacology, Biochemistry and Behavior, 86*, 263-283.

Wiers, R. W., 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 and Behavior, 8*, 101-106.

Wiers, R. W., van Woerden, N., Smulders, F. T., & de Jong, P. J. (2002). Implicit and explicit alcohol-related cognitions in heavy and light drinkers. *Journal of Abnormal Psychology, 111*, 648-658.

8. Supplementary material

Analysis of arousal and valence ratings for the stimulus materials used in the AAT
A RM-MANOVA with Category (alcohol, soda, neutral) as within subject factor, Group (Heavy drinkers, light Drinkers) as between subject factor and Arousal and Valence as dependents revealed a main effect of Category that was qualified by a Category × Group interaction. Univariate tests showed that the Category × Group interaction was significant for both Arousal ($F(4,110)=11.85$, $p < 0.001$) and Valence ($F(4,110)=11.13$, $p < 0.001$). Following up the interaction with separate tests for each group for arousal ratings, showed a main effect of Category for both the Heavy Drinkers (($F(2,36)=14.16$, $p < 0.001$) and Light Drinkers (($F(2,36)=31.04$, $p < 0.001$). Bonferoni corrected pairwise comparisons indicated that for the Heavy Drinkers (HD), alcohol was rated as more arousing than soda and soda was rated more arousing than office supplies. For Light Drinkers (LD), both soda and alcohol images were rates as more arousing than office supplies, but there was no difference between soda and alcohol images. Following up the interaction with separate tests for each group for valence ratings showed a significant effect of Category for HD (($F(2,36)=13.76$, $p < 0.001$) and LD (($F(2,36)= 7.38$, $p < 0.01$), Bonferroni corrected pairwise comparisons indicated that HD rated the valence of alcohol images higher than soda images, and valence of soda images higher than of office supplies. LD rated the valence of soda images higher than of alcohol images and office supplies, while valence of office supplies and alcohol was not significantly different.

9. Appendix A.

Affective words used and their English translation in the Implicit Association Task:

Positive

Gezellig	-	Cosy
Gelukkig	-	Happy
Opgewekt	-	Light hearted
Vrolijk	-	Cheerful
Grappig	-	Funny
Energiek	-	Energetic

Negative

Misselijk	-	Nauseous
Lusteloos	-	Lifeless
Ellendig	-	Miserable
Beroerd	-	Lousy
Somber	-	Sad
Vervelend	-	Annoying

STUDY 2: Cue-reactivity and its relation to craving and relapse in alcohol dependence: A combined laboratory and field study.

J Witteman[1] , H Post[5], M Tervainen[4], A De Bruijn[1], EB de Sousa Fernandes Perna [2], JG Ramaekers[2], W van Dalen[1], Wiers R[3].

[1]Dutch institute for alcohol policy (STAP), Utrecht, The Netherlands.

[2]Department Neuropsychology and Psychopharmacology, Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, The Netherlands,

[3]Department of Developmental Psychology, University of Amsterdam, The Netherlands

[4] Department of Applied Physics, Faculty of Science and Foresty, University of Eastern Finland, Finland.

[5] VICTAS addiction centre, Utrecht, The Netherlands.

Abstract

The present study investigated the nature of physiological cue-reactivity and craving in response to alcohol cues among alcohol-dependent patients (N=80) who were enrolled in detoxification treatment. Further, the predictive value with regard to future drinking of both the magnitude of the physiological and craving response to alcohol cues while in treatment and the degree of alcohol cue exposure in patients' natural environment was assessed. Physiological reactivity and craving in response to experimental exposure to alcohol and soda advertisements were measured during detoxification treatment using heart rate variability and subjective rating of craving. Following discharge, patients monitored exposure to alcohol advertisement for five consecutive weeks with a diary and were followed up with an assessment of relapse at 5 weeks and 3 months post-discharge. The results indicated that alcohol cues such as the portrayal of the drug and drinking behaviour induced physiological cue-reactivity and craving. Additionally, cue-reactivity and craving were positively correlated, and cue-reactivity was larger for patients with shorter histories of alcohol dependence. Further, patients reported a substantial daily exposure to alcohol cues. The magnitude of physiological cue-reactivity and the craving response to alcohol cues at baseline and degree of exposure to alcohol cues in patients' natural environment did not predict relapse. It is concluded that portrayal of cues such as alcoholic beverages and drinking behaviour induce cue-reactivity and craving through a conditioned appetitive response.

1. Introduction

Alcohol dependence can be regarded as a chronic condition (Koob & Volkow, 2009), characterized by high rates of relapse into problematic drinking soon after initial successful treatment (Witkiewitz & Marlat, 2007). Insight into what factors promote relapse could provide a starting point for developing treatments that reduce relapse. The present study aimed to test the influence of one such factor, exposure to alcohol related cues, by measuring the physiological and craving response to alcohol cues in the laboratory and the influence of naturally occurring alcohol cues (i.e., alcohol advertisement) in the daily environment of alcohol dependent patients on relapse.

A large evidence base suggests that an exaggerated central nervous system response to alcohol related cues is a key phenomenon in alcohol dependence (Bechara, 2005; Koob & Volkow, 2009; Wiers et al., 2007). A prominent hypothesis about the mechanism behind reward circuitry hyperactivity for alcohol related cues in alcohol dependence is the 'incentive salience' hypothesis (Robinson & Berridge, 2008), which proposes that initially neutral cues (such as the sight of a beer bottle) by repeated paring with the direct pharmacological effect of alcohol on the reward circuitry gain 'incentive salience' through classical conditioning and can ultimately also evoke a response of the reward circuitry in the absence of the direct pharmacological effect (in other words, the direct biochemical effect of alcohol in the brain). The magnitude of this conditioned reward circuitry response has subsequently been hypothesized to be associated with craving (Litt & Cooney, 1999; Volkow, Wang, Fowler & Tomasi, 2012), ultimately promoting relapse (Niaura et al., 1988; Volkow et al., 2010).

Indeed, recent meta-analyses of the neuroimaging literature of alcohol cue exposure have indicated that in alcohol dependence there is hyperactivity of the reward system for alcohol related cues as compared to controls (Kühn & Gallinat, 2011; Schacht, Anton & Myrick, 2013). Further, studies have indeed found a relationship between the magnitude of physiological reactivity to alcohol cues during treatment and subsequent probability of relapse after

discharge (Rosenhow et al., 1994; Grüsser et al., 2004; Beck et al., 2012; Garland, Franken & Howard, 2012; but see Heinz et al., 2007). For baseline cue elicited craving during treatment however, results have been mixed with two studies showing a relationship between the magnitude of the craving response during treatment and subsequent relapse (Cooney, Litt, Morse, Bauer & Gaupp, 1997; Papachristou, Nederkoorn, Giesen & Jansen, 2014) while another study (Rosenhow et al., 1994) did not find such a relationship. Interestingly, Rosenhow et al. (1994) measured both craving and physiological (salivation) response to alcohol-cues, allowing for a direct comparison of predictive validity, and found that physiological cue-reactivity did but craving did not predict future drinking behaviour.

This apparent differential predictive value of physiological responses to alcohol related cues on the one hand and the craving response on the other hand suggests that these two phenomena may be caused by partially overlapping (but also distinct) mechanisms. Indeed, previous studies have shown that physiological cue reactivity does not (Erblich, Bovbjerg & Sloan, 2011) or does only moderately correlate (Myrick et al., 2003; Wrase et al., 2007; Mason, Light, Escher & Drobes, 2008) with subjective craving, which would be expected if these two phenomena were only partially overlapping. One explanation for this moderate relationship is that craving reflects cue reactivity of the reward system but also additional processes, such as the (verbal) interpretation of the physiological response (Rosenhow et al.,1994; Carter & Tiffany, 1999; Drummond, 2000). Subjective craving is likely to be influenced by factors such as demand characteristics, resulting in moderate correlations between cue-reactivity and craving and a more direct relationship between cue reactivity and relapse (versus craving and relapse). Thus, in the present study both physiological cue-reactivity and cue elicited craving to alcohol cues was measured in order to test the purported differential predictive power of these two phenomena with regard to future drinking behaviour.

An abundant and salient source of alcohol cues in society is alcohol advertisement. Thus, alcohol advertisement may act as a conditioned stimulus and engage the sensitized reward system (Tapert et al., 2003) and subsequently induce craving and motivation to drink among alcohol dependent patients. Indeed, one study showed hyperactivity of the prefrontal cortex and thalamus and higher craving for alcohol dependent patients versus controls after exposure to printed alcohol advertisement (George et al., 2001). Similarly, alcohol dependent adolescents have been found to show hyperactivity in (inter alia) the reward circuitry after exposure to printed alcohol advertisements as compared to controls (Tapert et al., 2003). Therefore, in the present study both physiological reactivity and craving in response to alcohol advertisement was tested when patients were still in treatment and its relation with subsequent drinking behavior.

What cues in alcohol advertisement then, might engage the reward circuitry and induce craving and motivation to drink? Staiger & White (1991) suggested that particularly the sight and smell of an alcohol dependent patient's favourite drink induces cue reactivity. Thus, this study suggests that cue-reactivity due to alcohol advertisement exposure may be specific to the favourite brand of alcohol dependent patients. A more recent study by Mucha, Geier, Stuhlinger and Mundle (2000) using the startle response, however, suggests that specifically observing the preparation of drug use may be particularly potent in evoking a response. However, both of these studies did not use alcohol advertisements as alcohol cues. Thus, in the present study it was tested whether observing drug related cues such as the preparation and actual use of the drug (i.e. people preparing a drink and actual drinking behaviour) in alcohol advertisement may be particularly potent in eliciting cue-reactivity among alcohol dependent patients.

The conditioned reward circuitry response to drug related cues is accompanied by activity of the autonomous nervous system (Bechara, 2005). Parasympathetic nervous system activity in response to external stimulation can be measured using the High Frequency (HF) Heart Rate Variability (HRV) component (Thayer & Lane, 2000). However, only two previous studies have investigated the parasympathetic nervous system response to drug cues. Erblich, Bovbjer and Sloan (2011) found an increase in HF HRV when smokers imagined a smoking script versus a control script. Similarly, Garland (2011) found an increase in the HF HRV component during stress primed alcohol cue exposure among alcohol dependent patients. It has been suggested that this HF HRV increase either reflects a homeostatic response to an aversive stimulus (Erblich et al., 2011) or the regulation of an appetitive response to the drug cues (Garland, 2011). Only one recent study has examined whether the HF HRV response to stress primed alcohol cues also shows a relation with subsequent drinking behaviour. A larger HF HRV response to stress primed alcohol cues was associated with an increased probability of relapse (Garland, Franken & Howard, 2012). Thus, HF HRV might be a cost effective and valid psychophysiological marker of relapse vulnerability.

Not only the *magnitude* of the physiological and craving response to alcohol cues while in treatment (Niaura et al., 1988), but also the *degree* of actual alcohol cue exposure in daily life could contribute to probability of relapse in alcohol dependence. Thus, there is a need to extend lab measurements of cue-elicited craving and physiological cue-reactivity with more ecologically valid measures (Litt & Cooney, 1999). However, in the previous literature so far only lab measurement of craving and cue-reactivity magnitude have been examined while the degree of alcohol cue exposure in the natural environment of patients remains unexplored. Therefore, the present study attempted to extend previous work by not only measuring the HF HRV and craving response to alcohol cues experimentally while patients were still in treatment, but additionally the degree of (self-reported) exposure to alcohol cues (more specifically, alcohol advertisement) in patients' daily life. It was expected that exposure to alcohol cues would induce physiological cue-reactivity and craving. Further, it was predicted that the magnitude of the cue-reactivity and craving response to alcohol cues or the degree of actual alcohol cue exposure in daily life, or both, would predict future drinking behaviour.

2. Methods

2.1 Study design and procedure

All patients who fulfilled the inclusion criteria (see below) participated in a one hour baseline session. During this session it was first assessed whether the patient fulfilled the inclusion criteria. Subsequently, patients performed two tasks, the results of which will be reported elsewhere (as these tasks were not relevant to the research questions raised in the description of work). The tasks were the Approach Avoidance Task (AAT), to measure automatic action tendency towards alcohol described in the previous chapter, and a cognitive control task (in other words, a task measuring impulsivity). In between the two tasks retrospective measures drinking quantity (and other drug use) were administered (see Measures section for more details) and clinical background variables assessed. Further, all participants watched a five minute series of alcohol and a five minute series of soda advertisements (order of soda versus alcohol advertisement counterbalanced between participants) while HR data was recorded. Patients were instructed to attentively watch the commercials. After each film, patients indicated their current level of craving for alcohol on the VAS-scale. Last, patients eligible for the longitudinal part of the study (i.e. all patients in the short detoxification program) were asked to participate. For patients enrolled in the longitudinal part of the study, the advertisement diary was handed out at the end of the baseline session and relapse was

assessed 5 weeks and 3 months post-discharge by a telephone interview that lasted 5 minutes. The research protocol was evaluated by the ethics committee of Maastricht University and the study was conducted in accordance with the Declaration of Helsinki. Patients received a 10 € reward voucher for participation in the baseline session which took one hour to complete and an additional 50€ worth of vouchers for 5 weeks of diary monitoring.

2.2 Subjects

A total of 80 alcohol dependent inpatients who were enrolled into detoxification treatment from Victas addiction center (Utrecht, The Netherlands) participated in the study. The mean duration of stay in the addiction center at the time of testing was 9.32 (SD = 4.32) days and the mean interval between testing and discharge was 4.6 (SD= 2.5) days, resulting in a mean total stay duration of 14.1 (SD = 4.3) days. The detoxification program was typically followed by an ambulatory cognitive behavioural therapy program. Cue-exposure therapy was not part of the detoxification program.

Inclusion criterion were (1) a Diagnostic and Statistical Manual of Mental Disorders – fourth edition (DSM-IV) diagnosis of alcohol dependence for the 12 months leading up to admission to the addiction centre based on the M.I.N.I.-plus International Neuropsychiatric Interview (Sheehan et al., 1998; Van Vliet and De Beurs, 2007) and (2) between 18 and 70 years old and (3) currently stable condition as indicated by the cessation benzodiazepine administration as prescribed by the addiction physician for the treatment of withdrawal. Exclusion criteria were (1) the presence of a severe psychiatric (severe depression, psychotic disorder), neurological (e.g., severe amnesia or tremor) or other somatic disease or (2) very low intelligence (based on clinical impression), as these factors would significantly complicate adherence to the study procedures. Alcohol abuse was required to be the main substance use problem but other substance use than alcohol did not serve as an exclusion criterion in order to increase ecological validity

For a small subset (N=8) of patients, it was decided that they would be enrolled in a longer additional treatment program after participation in the baseline session. These participants were excluded form the longitudinal part of the study (as including them would induce variation in the interval between testing and discharge) but retained for the analyses of baseline measures. Additionally, one patient was excluded because severe neuropsychiatric disorder was suspected based on baseline testing, leaving us with 79 patients (age M = 45.9, SD= 11.21; 70% male) for the baseline measurement. For the longitudinal part of the study 68 patients (age M = 46.5, SD = 10.8; 69% male) were enrolled, of whom 65 agreed to additionally to take part in the diary part of the study. For the total sample of 79 patients, alcohol dependence was relatively severe, as indicated by a mean endorsement of 6.1 (SD = .93) out of 7 DMS-IV alcohol dependence symptoms, a mean intake of 16.6 standard (10 g of pure alcohol) units per day (SD = 7.5) in the year before treatment and a mean AUDIT score of 25.5 (SD = 4.7). Mean duration of problematic alcohol use was 14 years with significant heterogeneity (SD = 9.9). A significant proportion of patients used other recreational drugs in addition to alcohol and tobacco: 21% could be classified as a polysubstance user (defined as the use of two or more recreational drugs in addition to alcohol and tobacco at least twice in the previous year). All patients were abstinent from psychoactive recreational drugs during detoxification as measured with routine urinalysis.

2.3 Measures

Substance use and medication

Several indices of the quantity and nature of substance use were obtained. Mean alcohol use in the 12 months before admission was assessed using the Quick Drinking Screen (QDS), a 'quantity-frequency' method for assessing alcohol use that correlates highly with the Time Line Follow Back Method (Sobell et al., 2003). The Alcohol Use Disorders Identification Test (AUDIT) was used to assess the severity of alcohol abuse in the last 12 months (Saunders, Aasland, Babor, De La Fuente, & Gramt, 1993). Similarly to Joos et al. (2012), age of problematic drinking onset was assessed by asking: "At what age did you start drinking problematically, according to yourself and/or your environment?" and used to compute the duration of problematic drinking. Additionally, frequency of other substance use than alcohol in the 12 months preceding admission was assessed. Finally, it was assessed whether relapse prevention medication (naltrexone, disulfiram, acamprosate) had been prescribed by the addiction physician and whether the patient used a psychopharmacologically active agent (antidepressant, antipsychotic, stimulant or anticonvulsant).

Cue-elicited Heart Rate Variability (HRV) and craving

All participants watched two five minute films, containing a series of eight soft-drink or alcohol commercials. The commercials were pre-existing commercials from brands not available in the Netherlands in order to remove preferred brand specific effects. The alcohol marketing collage contained five beer commercials, one wine commercial, and two liquor (shooters and vodka) commercials and contained images of beer, wine and liquor, footage of the beverage being poured into a glass and of people consuming these beverages. The soft-drink commercials were matched in content to the alcohol commercials.

Cue-elicited HRV was measured using a Polar RS800 CX (Polar Electro Oy, Kempele, Finland) heart rate monitor (HRM) at 1000 Hz. The device collects HR data through a two-lead chest band which wirelessly transmits the data to a wristwatch. Although there has been some discussion concerning the validity of Polar HRM in measuring HRV (Wallen et al., 2012; Quintana et al., 2013), in subjects without heart disease the measures obtained with Polar HRM in the time domain and for normalised power in the frequency domain show high correspondence with the gold standard, traditional electrocardiography (Weippert et al., 2010). Cue elicited subjective craving for alcohol was assessed by asking patients to indicate their current craving for alcohol on a 100 mm visual analogue (VAS) scale. Although the use of multi-item instruments to assess craving has gained popularity, it has been shown that VAS scales are reliable in assessing craving (Kozlowski et al., 1996; Papachristou et al., 2013). In the present investigation, it was decided to use a VAS scale because we were interested in acute changes (i.e., changes in craving occurring immediately following exposure to alcohol advertisement) in craving level in response to alcohol cues, which demanded a rapid assessment procedure.

Advertisement diary

Patients used prospective diaries to estimate actual exposure to alcohol marketing following their discharge from the clinic. Among self-reported measures available, retrospective reports and retrospective diaries suffer from recall bias effects more than a prospective diary (Patrick & Lee, 2010). Recall effects can be expected to be prominent in the case of alcohol advertisement exposure monitoring, as the relevant events are relatively frequent and brief. Therefore, prospective monitoring of alcohol advertisement using a diary was employed. Using the diary, patients monitored alcohol advertisement by briefly marking in a table every time an advert was noticed. All relevant advertisement channels (television, film, outdoor advertisement, radio, in shop advertisement) were covered. All patients monitored advertisement for two days a week, one weekend day and a weekday (particular weekday and

weekend day counterbalanced across patients) during five weeks following discharge. As a control (and to reduce attention for alcohol cues) patients monitored soft-drink advertisement as well.

Assessment of relapse

There is no consensus on a definition of the term relapse (Witkiewitz & Marlat, 2007). In the interest of comparability, various measures of relapse were therefore collected through telephone interviews. First, it was assessed whether the patient had consumed any alcohol at all (abstinence), and if so, how many days after discharge the first alcoholic drink had been consumed ('time to first drink'). Further, the number of days since discharge on which the patient had consumed any alcohol was indexed ('number of drinking days'). Similarly, we asked every patient whether six or more standard units had been consumed on any occasion (i.e., binge drinking) and if so, what the time to first binge drink was and how many binge drinking days had occurred. Lastly, we asked patients whether they evaluated the current drinking behaviour as problematic, and if so, whether they evaluated the current problem drinking behaviour as less severe, equally severe, or more severe than pre-detoxification.

2.4 Data analysis

HRV pre-processing

Concerning cue-elicited HRV, the raw RR data for each experimental condition were extracted from the HRM and visually inspected for abnormalities. The data were then imported into Kubios HRV software (version 2.0, 2008, Biosignal Analysis and Medical Imaging Group, University of Kupio, Finland, MATLAB). For each series of commercials (alcohol, soft-drink) an epoch of exactly 5 minutes was first automatically checked for artefacts (i.e., impossible values), using the medium artefact correction setting. After a Fast Fourier Transform (FFT), normalised power of the high frequency (HF) component (0.04 – 0.15 Hz) was extracted and used as the HRV measure from the frequency domain. It has been shown that the HF component of HRV at least partially reflects parasympathetic control over the heart, particularly with a within subject analysis as in the present study (Bernston et al., 1997). Patients who reported heart disease (N=3) or who showed abnormalities upon visual inspection of the RR data (N= 2) were excluded from the statistical analyses.

In the 5 minute series of alcohol advertisements, alcohol cues were only sporadically presented. Therefore, we were interested in whether the presentation of alcohol cues (i.e., the presentation of objects associated with drinking such as a bottle, the pouring of an alcoholic beverage in a glass and people displaying drinking behaviour) in alcohol advertisement might specifically elicit an HRV response, as predicted by cue-reactivity theory. To test this hypothesis, an 'Event Related Heart Variability' (EVHRV) analysis was additionally performed, for which absolute HF power was extracted from five segments with a duration of eight seconds during which alcohol cues were presented (including people consuming an alcoholic beverage) and compared to the immediately preceding eight second interval that did not contain any alcohol cues (pre-cue baseline). Due to practical limitation of the requirement that all alcohol cue segments had to be preceded by an alcohol cue free baseline, all alcohol cue segments were extracted from beer commercials (because there were no segments in the non-beer commercials containing alcohol cues that were preceded by a baseline without alcohol cues).

Diary data pre-processing

Total exposure to alcohol and soft-drink advertisement was computed as the sum of all individual alcohol and soft-drink exposures over the five week interval (comprising a total of 10 monitoring days). The amount of exposure prior to relapse was additionally computed as the mean number of alcohol/soft-drink ads reported in the period prior to relapse. Last, weekly total exposure to alcohol/soft-drink ads was computed as the sum of all alcohol/soft-drink ads for each of the five monitoring weeks.

Statistical analysis

Because both craving VAS-scores and HRV-HF power (except normalised HF for soft-drink advertisement) were not normally distributed (for all, Shapiro-Wilk < .9, p <.001), Wilcoxon Signed-ranks tests were used to test for the effect of alcohol advertisement on craving and HF HRV power (as compared to soft-drink advertisement) and the presence of alcohol cues (as compared to the immediately preceding alcohol cue free baseline) on EVHRV HF power. The relationship between craving and cue-reactivity was examined using non-parametric (Spearman) correlation coefficients.

Hierarchical regression analyses were performed to predict relapse at 5 weeks and 3 months post-discharge, with addiction severity (AUDIT) and addiction duration (number of problem drinking years), cue-reactivity (HRV) and cue-elicited craving at baseline, and self reported exposure to alcohol advertisement in the field as predictors. More specifically, for all regression analyses, baseline difference scores between the alcohol and soft-drink commercials for craving and HRV (Llabre et al., 1991; Garland et al., 2011) also known as 'delta' (Δ), were used as predictors. Similarly to Garland et al. (2011) these three classes of variables (addiction severity and duration; baseline measures; field exposure) were entered into the regression analyses in three blocks.

For binary relapse variables (abstinence and binge drinking), logistic multiple regression analyses were performed with mean self-reported exposure to alcohol ads and soft-drink ads (as a control) per day *before* the day of relapse (i.e., drinking or binge drinking) as predictor (since exposure after relapse cannot have contributed to relapse). For continuous relapse variables (number of drinking and number of binge drinking days), self reported exposure to alcohol and soft-drink ads over the whole five week monitoring period was used as the measure of field exposure, as in this case both alcohol advertisement exposure before and after the first (binge) drink day could theoretically have contributed to the number of drinking days. Because number of (binge) drinking days was not normally distributed (for all, Shapiro-Wilk < .9, p <.001), these variables were first natural log transformed. Last, Cox-regression with the baseline measures as predictors and weekly exposure to alcohol advertisement as time-varying covariates was performed to predict time to first (binge) drink.

3. Results

Baseline session

HRV measurements for alcohol and soft-drink advertisement were available for 67 patients. Wilcoxon Signed-ranks test revealed no significant difference in normalised HF power between exposure to alcohol versus soft-drink advertisement (Z= -1.56, N.S). HF absolute power measurements during exposure to alcohol advertisement were available for 71 patients. As can be observed in Figure 1, EVHRV analysis of HF absolute power during the presentation of alcohol cues as compared to pre-cue baseline using a Wilcoxon Signed-ranks test revealed a highly significant increase in HF power during the presentation of alcohol cues (*Mdn* = 100.54)

as compared to the pre-cue (*Mdn* = 89.05) baseline (*Z* = 2.74, p = .006). Although soft-drink cues also increased HF power as compared to baseline, this increase was not significant.

Craving VAS-scores after soft-drink and alcohol advertisement exposure were available for 79 patients. Wilcoxon Signed-ranks test indicated a highly significant increase in craving after exposure to alcohol (*Mdn* = 14) advertisement as compared to soft-drink (*Mdn* = 5) advertisement (*Z* = 5.54, p < .001). However, in absolute terms, craving after alcohol advertisement exposure was relatively low (see Figure 2). Further, 38.8% of patients did not show an increase in craving after alcohol advertisement.

Exploratory Spearman correlations indicated no significant correlation between the increase in craving during alcohol ads as compared to soft-drink ads and increase of HF normalised power (p = .6, N.S). However, the increase in absolute HF power during the presentation of alcohol cues (as compared to pre-cue baseline) showed a significant positive correlation with absolute craving after alcohol advertisement exposure (*r*(71) = .33, p = .004), as can be seen in Figure 3. Further, duration of problem drinking (number of problem drinking years) correlated with increase in HF power during the presentation of alcohol cues (*r*(71) = -.26, p = .03). Because age significantly correlated with the number of problem drinking years, we performed a multiple linear regression analysis with age and number of problem drinking years as predictors and HF-HRV cue reactivity as dependent variable, revealing that only the number of problem drinking years was significantly negatively associated with cue-reactivity. As can be seen in Figure 4, the increase in HF power was larger for patients with *shorter* histories of problematic drinking. Severity of alcohol dependence in the previous year (AUDIT) did not correlate with HF power during presentation of alcohol cues.

Figure 1. Mean High Frequency (HF) power of heart rate variability during pre-alcohol cue baseline and alcohol cue exposure.

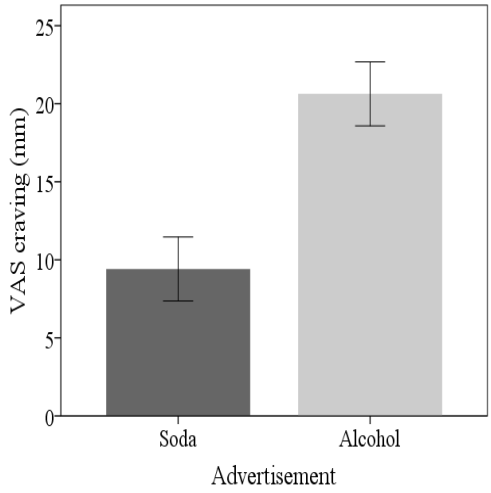


Figure 2. Mean craving score after alcohol and soft-drink advertisement.

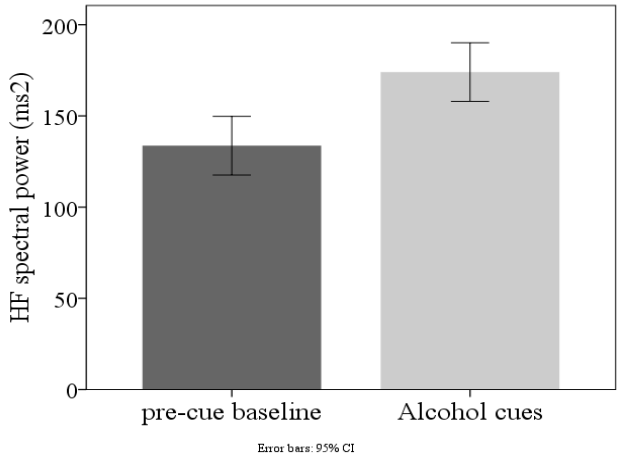


Figure 3. Scatterplot of the increase in HF power during alcohol-cue exposure in alcohol advertisement (y-axis) against the craving score after exposure to alcohol advertisement (x-axis).

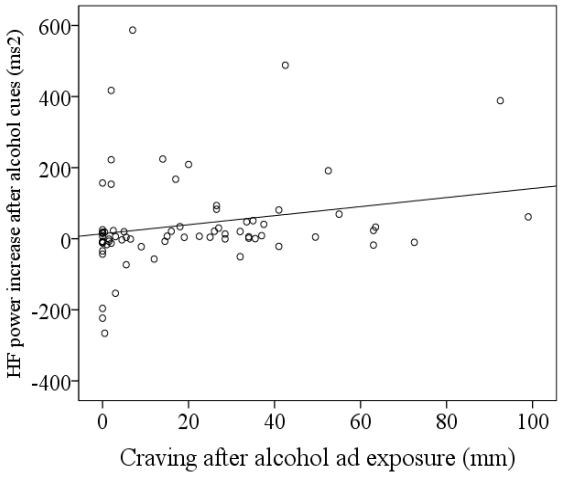
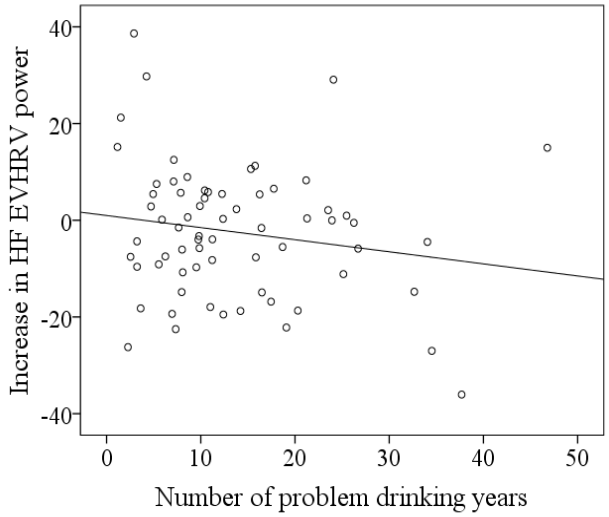


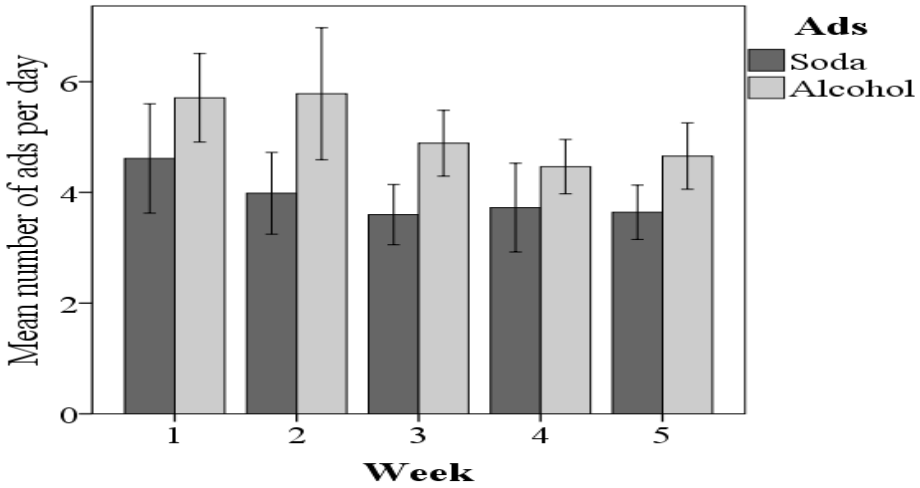
Figure 4. Scatterplot of the increase in HF power during alcohol-cue exposure in alcohol advertisement (y-axis) against the number of problem drinking years (x-axis).



Field exposure to alcohol and soft-drink advertisement

Mean exposure to alcohol and soft-drink advertisement as reported in the diary for each of the five weeks in the interval between discharge and the first follow-up can be found in Figure 5. Week to week reliability of total alcohol advertisement exposure was high (Pearson correlations ranging from .65 to .87). Mean number of advertisement exposures was 5.38 per day for alcohol (SD = 4.14) and 4.05 per day for soft-drink (SD = 4.14) over the whole five week monitoring period.

Figure 5. Mean number of self-reported exposures to soda and alcohol advertisement per day for each of the five monitoring weeks.



Follow-up

Of the 68 patients that were enrolled in the longitudinal part of the study, 91% could be retained at 5 week follow-up and 74% at three months follow-up. Of the 58 patients that took

part in the diary monitoring however, only 37 (63.8%) returned the diary at the end of the monitoring period.

At five weeks and three months post-discharge, non-abstinence rates were 47.1% and 66.2%, respectively. For binge drinking, relapse rates at five weeks and three months post-discharge were 25% and 39.7 % respectively. Mean time to relapse was 27.12 (SD = 22.65) and 30.11 (SD = 23.19) days for non-abstinence and binge drinking respectively. However, the mode was 21 and 14 days respectively, showing that that a substantial number of patients relapses within the first 3 weeks after discharge. Last, at five weeks and three months post-discharge, 29% and 42.9% of patients respectively evaluated their current drinking behaviour as problematic.

Chi-square tests indicated that patients who did not return the diary had a significantly higher non-abstinence rate at five weeks ($X^2(1, N = 54) = 5.17$, $p = .02$) and three months $X^2(1, N = 53) = 4.14$, $p = .04$) follow-up than patients that returned the diary. Further, independent T-tests revealed that patients who did not return the diary had a significantly higher number of drinking days at five weeks ($T(51) = 2.57$, $p = .02$) and three months ($T(52) = 2.25$, $p = .04$) follow-up.

The omnibus-tests for the multiple regression analyses did not reveal a significant amount of variance explained by the predictors (AUDIT, problem drinking years; cue-reactivity, cue-elicited craving; self-reported exposure to alcohol and soft-drink advertisement) for any of the dependent variables (abstinence, binge drinking status, number of drinking days, number of binge drinking days, time to relapse) for neither the five-weeks nor the 3-months follow-up.

4. Discussion

The present study investigated the nature of psychophysiological cue-reactivity and craving in response to alcohol cues in alcohol dependence and its relation to subsequent drinking behaviour using a combined laboratory and field approach.

While patients were still in treatment no significant HF HRV response to alcohol advertisements was observed as compared to a block of soft-drink advertisements. However, there was a robust increase in craving after alcohol advertisement exposure as compared to soft-drink advertisement. Further, during the presentation of alcohol cues (individuals drinking or preparing to do so) in alcohol advertisements, a significant increase in EV HFHRV power was observed as compared to an interval of identical length immediately preceding the alcohol cues. As has been hypothesized previously, it is possible that the presentation of soft-drink cues might have also elicited a HF HRV response, either through a primary appetitive response to soft-drink cues (Schacht, Raymond, & Myrick, 2013) or because soft-drink cues could be interpreted as alcohol cues (Vollstädt-Klein et al., 2010), resulting in a non-significant increase between the block of soft-drink and alcohol advertisements. Indeed, in the present investigation HF power after the presentation of soft-drink cues was increased as compared to baseline, although not significantly.

A significant *in*crease in event-related HF HRV spectral power in response to the presentation of drug cues as found during alcohol advertisement in the present investigation has been found previously (Erblich et al., 2011; Garland et al. 2011). There has been a debate on whether the increase in vagal tone (as reflected in HRV HF power) represents an appetitive response (Garland et al., 2011) or a regulatory homeostatic response to an aversive stimulus (Erblich et al., 2011) or both (Niaura et al. 1988; Wiers et al., 2007). Interestingly, a recent meta-analysis of studies examining cue-reactivity to stress found a *de*crease in HF HRV in

response to stress (Brindle, Ginty, Philips, & Carroll, 2014). Further, a recent study also found an *in*crease in HF HRV spectral power when confronting an obese population with high-caloric food (Udo et al., 2014). Therefore, EV HFHRV cue-reactivity may represent a conditioned appetitive response to conditioned drug cues as has been proposed for cue-reactivity as measured with fMRI (Tapert et al., 2003; Kuhn & Gallinat, 2011; Schacht, Anton, & Myrick, 2013). Additionally, the present HR-data are in line with studies that have shown significant cue-reactivity in response to drug cues as presented in tobacco (Vollstad-Klein et al., 2011) and alcohol (George et al., 2001; Tapert et al., 2003) advertisement among nicotine and alcohol dependent patients respectively. Further, our results are in keeping with the suggestion that the presentation of drug cues and scenes depicting (preparation of) drug use may be driving such conditioned physiological cue-reactivity (Mucha, Geier, Stuhlinger, & Mundle, 2000).

A significant but moderate association was observed between the EV HFHRV power increase during alcohol cue exposure and absolute craving after alcohol advertisement exposure. Such modest associations between physiological cue-reactivity and craving have been observed previously (Myrick et al., 2003; Wrase et al., 2007; Mason, Light, Escher, & Drobes, 2008). Therefore, craving and physiological cue-reactivity may represent partial overlapping phenomena. It has been suggested that cue-reactivity is a primary response of the nervous system to conditioned drug cues, as predicted by incentive salience theory (Robinson & Berrigde, 2008) and that craving represents additional processes such as the interpretation of this response or the will to resist drinking (Rosenhow et al., 1994; Drummond, 2000) which may be influenced by various organismic and contextual factors, resulting in an association of small magnitude between the two phenomena. Together, the baseline measures suggest that alcohol advertisement has generic (non brand-specific) cue-reactivity and craving effects in alcohol dependent patients. The results furthermore suggest that physiological cue-reactivity and craving effects of alcohol advertisement are driven by portrayal of drug-cues such as presentation of the drug (i.e., alcohol), individuals preparing to drink and actual drinking behaviour.

During the five week follow-up period after discharge, patients reported being exposed to a mean of five alcohol advertisements per day. Week to week reliability of the diary was high, suggesting that a diary may be a sensitive method to assess exposure to alcohol advertisement in the field. However, there was a substantial drop-out in the diary measure. Therefore, future investigations could use an electronic version of the diary (allowing for instant data collection) and reduce the number of monitoring days to increase data retention and reduce drop-out rates. Taking the baseline laboratory and follow-up field results together then, although tentative, our results suggest that alcohol dependent patients may experience cue reactivity and craving as a result of alcohol advertisement exposure on a daily basis.

Even when using a relatively conservative definition of relapse, i.e., the occurrence of at least one binge drinking episode, relapse rates were high in the present study, with two thirds of patients reporting non-abstinence at the three month follow-up, in line with previous work (Witkiewitz & Marlatt, 2007). However, baseline physiological cue-reactivity and cue-elicited craving did not predict relapse in the present study. Regarding HF HRV cue reactivity, one previous study did find a relationship with relapse, with patients showing a greater increase in HF spectral power of HRV after alcohol cue exposure having a higher probability of relapse (Garland et al., 2012). However, in this study patients were first exposed to a stressor (exposure to pictures containing unpleasant scenes) before being exposed to alcohol cues. Thus, it might specifically be the HF HRV response to stress primed alcohol cues that is predictive of relapse. Regarding the relationship between baseline cue-elicited craving and subsequent relapse, previous results have been mixed, with three studies finding a positive

relationship (Cooney, Litt, Morse, Bauer, & Gaupp, 1997; Seo et al., 2013; Papachristou, Nederkoorn, Giesen, & Jansen, 2014) but a different study failing to do so (Rosenhow et al., 1994). Interestingly, the magnitude of alcohol craving in response to stress might be particularly predictive of future drinking as well, as two of the three studies above that did find a relationship used a (negative) mood induction procedure before measuring alcohol craving (Cooney, Litt, Morse, Bauer, & Gaupp, 1997; Seo et al., 2013). A final explanation that has been suggested previously is that in severe drug dependence (as was the case in the present study) external cues play a less important role in determining craving and drug use, as behaviour in this advanced stage of dependence is governed by internal (withdrawal) cues (Vollstädt-Klein et al., 2011) or habit (Everitt & Robins, 2005) rather than appetitive cues. In (indirect) support of this interpretation, we found a reduced HF HRV response to alcohol cues in the present investigation, with longer problematic drinking histories.

In addition to the *magnitude* of the cue-reactivity and the craving response to drug cues at baseline, we were interested whether the *degree of actual exposure* to alcohol cues in the natural environment may show a relationship with relapse. Although patients reported a substantial daily exposure to alcohol advertisement, no robust relationship between the degree of exposure to alcohol advertisement and drinking behaviour was found. However, several factors could have obscured a true relationship between alcohol advertisement exposure and drinking behaviour. First, there was a relatively large and selective drop-out from the diary monitoring part of the study, which may have reduced power to detect the relationship and may have distorted the observed relationship. Second, society is saturated with alcohol advertisement, resulting in low variation in the dose of advertisement among individuals and therefore reducing power to detect the relationship through restriction of range. Alternatively, as suggested above, external cues may play a minor role in severe alcohol dependence (Vollstädt-Klein et al., 2011). All in all then, concerning the relationship between alcohol advertisement and relapse, our results should be taken as preliminary.

5. Recommendations for policy

Alcohol advertisement exposure causes a robust craving response in alcohol-dependent patients. Further, display of the drug (i.e., an alcoholic beverage), individuals preparing to drink and actual drinking behaviour seem to drive physiological cue-reactivity and craving in response to such alcohol advertisement. Therefore, reducing alcohol cues in alcohol advertisement might reduce physiological cue-reactivity and craving in alcohol-dependent patients, which could theoretically reduce probability of relapse. For instance, images of beer being poured into a beer glass or imagery of people consuming alcohol could be removed from alcohol advertisement to reduce these aversive effects. Reducing the volume of alcohol advertisement exposure altogether would be expected to have a similar effect.

6. Acknowledgements

We thank Loes Balai and Maaike Vintges for their logistic support.

The research leading to these results or outcomes has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013), under Grant Agreement nº 266813-Addictions and Lifestyle in Contemporary Europe – Reframing Addictions Project (ALICE RAP). Participant organisations in ALICE RAP can be seen at http://www.alicerap.eu/about-alice-rap/partner-institutions.html.

7. References

Bechara, A. (2005). Decision making, impulse control and loss of willpower to resist drugs: A neurocognitive perspective. *Nature Neuroscience, 8*, 1458-1463.

Beck, A., Wüstenburg, T., Genauck, A., Wrase, J., Schlagenhauf, F., Smolka, M. N., & Heinz, A. (2012). Effect of brain structure, brain function, and brain connectivity on relapse in alcohol-dependent patients. *Archives of General Psychiatry, 69*, 842-852.

Ernston, G.G., Bigger, J. T., Eckberg, D., Grossman, P., Kaufmann, P. G. Malik, M., Nagaraja, H. N., …Van der Molen, M. W. (1997). Heart rate variability: Origins, methods, and interpraive caveats. *Psyhcophysiology, 34,* 623-648.

Brindle, R. C., Ginty, A. T., Phillips, A. C., & Carroll, D. (2014). A tale of two mechanisms: A meta-analytic approach toward understanding the autonomic basis of cardiovascular reactivity to acute psychological stress. *Psychophysiology*, [e-pub ahead of print]

Carter, B. L., & Tiffany, S. T. (1999). Meta-analysis of cue-reactivity in addiction research. *Addiction, 94*, 327-340.

Cooney, N. L., Litt, M. D., Morse, P. A., Bauer, L. O., & Gaupp, L. (1997). Alcohol cue reactivity, negative-mood reactivity, and relapse in treated alcoholic men. *Journal of abnormal psychology, 106*, 243-250.

Drummond, D. C. (2000). What does cue-reactivity have to offer clinical research? *Addiction, 95*, 129-144.

Erblich, J., Bovbjerg, D. H., & Sloan, R. P. (2011). Exposure to smoking cues: Cardiovascular and autonomic effects. *Addictive Behaviors, 36*, 737-742.

Everitt, B. J., & Robbins, T.W. (2005). Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. *Nature Neuroscience, 8*, 1481-1489.

Garland, E., Franken, I., & Howard, M. (2012). Cue-elicited heart rate variability and attentional bias predict alcohol relapse following treatment. *Psychopharmacology, 222,* 17-26.

Garland, E. L. (2011). Trait mindfulness predicts attentional and autonomic regulation of alcohol cue-reactivity. *Journal of Psychophysiology, 25*, 180-189.

George, M. S., Anton, R. F., Bloomer, C., Teneback, C., Drobes, D. J., Lorberbaum, J. P., et al. (2001). Activation of prefrontal cortex and anterior thalamus in alcoholic subjects on exposure to alcohol-specific cues. *Archives of General Psychiatry, 58*, 345-352.

Gossop, M., Keaney, F. et al. (2002). A Short Alcohol Withdrawal Scale (SAWS): development and psychometric properties. *Addiction Biology, 7,* 37-43.

Grüsser, S. M, Wrase, J., Klein, S., Hermann, D., Smolka, M. N., Ruf, M., Weber-Fahr, W., …Heinz, A. (2004). Cue-induced activation of the striatum and medial prefrontal cortex is associated with subsequent relapse in abstinent alcoholics. *Psychopharmacology, 175*, 296-302.

Joos, L., Schmaal, L., Goudriaan, A. E., Fransen, E., Van den Brink, W., Sabbe, B. G.., et al. (2012). Age of onset and neuropsychological functioning in alcohol dependent inpatients. *Alcoholism: Clinical and Experimental Research*, *37,* 407-416.

Koob, G. F., & Volkow, N. D. (2009). Neurocircuitry of Addiction. *Neuropsychopharmacology, 35*, 217-238.

Kozlowski, L. T., Pillitteri, J. L., Sweeney, C. T., Whitfield, K. E., & Graham, J. W. (1996). Asking questions about urges or cravings for cigarettes. *Psychology of Addictive Behaviors, 10*, 248-260.

Kühn, S. J. (2011). Common biology of craving across legal and illegal drugs - a quantitative meta-analysis of cue-reactivity brain response. *European Journal of Neuroscience, 33*, 1318-1326.

Lit, M. D., & Cooney, N. L. (1999). Inducing craving for alcohol in the laboratory. *Alcohol Research & Health, 23*, 174-178.

Llabre, M. M., Spitzer, S. B., Saab, P. G., Ironson, G. H., & Schneiderman, N. (1991). The reliability and specificity of delta versus residualized change as measures of cardiovascular reactivity to behavioral challenges. *Psychophysiology, 28*, 701-711.

Mason, B., Light, J., Escher, T., & Drobes, D. (2008). Effect of positive and negative affective stimuli and beverage cues on measures of craving in non treatment-seeking alcoholics. *Psychopharmacology, 200*, 141-150.

Mucha, R. F., Geier, A., Stuhlinger, M., & Mundle, G. (2000). Appetitive effects of drug cues modelled by pictures of the intake ritual: Generality of cue-modulated startle examined with inpatient alcoholics. *Psychopharmacology, 151*, 428-432.

Myrick, H., Anton, R. F., Li, X., Henderson, S., Drobes, D., Voronin, K., et al. (2003). Differential brain activity in alcoholics and social drinkers to alcohol cues: relationship to craving. *Neuropsychopharmacology, 29*, 393-402.

Niaura, R. S., Rohsenow, D. J., Binkoff, J. A., Monti, P. M., Pedraza, M., & Abrams, D. B. (1988). Relevance of cue reactivity to understanding alcohol and smoking relapse. *Journal of Abnormal Psychology, 97*, 133-152.

Papachristou, H., Nederkoorn, C., Giesen, J. C. A. H., & Jansen, A. (2014). Cue reactivity during treatment, and not impulsivity, predicts an initial lapse after treatment in alcohol use disorders. *Addictive Behaviors, 39*, 737-739.

Papachristou, H., Nederkoorn, C., Havermans, R., Bongers, P., Beunen, S., & Jansen, A. (2013). Higher levels of trait impulsiveness and a less effective response inhibition are linked to more intense cue-elicited craving for alcohol in alcohol-dependent patients. *Psychopharmacology*, *228,* 641-649.

Patrick, M. E., & Lee, C. M. (2010). Comparing numbers of drinks: College students' reports from retrospective summary, followback, and prospective daily diary measures. *Journal of Studies on Alcohol and Drugs, 71*, 554-561.

Quintana, D. S., McGregor, I. S., Guastella, A. J., Malhi, G. S., & Kemp, A. H. (2012). A meta-analysis on the impact of alcohol dependence on short-term resting-state heart rate variability: Implications for cardiovascular risk. *Alcoholism: Clinical and Experimental Research*, 37, E23-E29

Robinson, T. E. & Berridge, K. C. (2008). The incentive sensitization theory of addiction: some current issues. *Philosophical Transactions of the Royal Society B: Biological Sciences, 363,* 3137-3146.

Rohsenow, D. J., Monti, P. M., Rubonis, A. V., Sirota, A. D., Niaura, R. S., Colby, S. M., et al. (1994). Cue reactivity as a predictor of drinking among male alcoholics. *Journal of Consulting and Clinical Psychology, 62*, 620-626.

Schacht, J. P., Anton, R. F., & Myrick, H. (2013). Functional neuroimaging studies of alcohol cue reactivity: a quantitative meta-analysis and systematic review. *Addiction Biology, 18*, 121-133.

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Weller, E., Hergueta, T… Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of Clinical Psychiatry, 59,* 22-33.

Seo D, L. Lacadie, C. M., Tuit, K., Hong, K. L., Constable, R. T., & Sinha, R. (2013). Disrupted ventromedial prefrontal function, alcohol craving, and subsequent relapse risk. *JAMA Psychiatry*, 70, 727-739..

Sobell, L. C., Agrawal, S., Sobell, M. B., Leo, G. I., Young, L. J., Cunningham, J. A., et al. (2003). Comparison of a quick drinking screen with the timeline followback for individuals with alcohol problems. *Journal of Studies on Alcohol and Drugs, 64*, 858-861.

Staiger, P. K., & White, J. M. (1991). Cue reactivity in alcohol abusers: Stimulus specificity and extinction of the responses. *Addictive Behaviors, 16*, 211-221.

Saunders, J. B., Aasland, O. G., Babor, T. F., De La Fuente, J. R., & 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.

Tapert, S. F., Cheung, E. H., Brown, G. G., Frank, L. R., Paulus, M. P., Schweinsburg, A. D., et al. (2003). Neural response to alcohol stimuli in adolescents with alcohol use disorder. *Archives of General Psychiatry, 60*, 727-735.

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders, 61*, 201-216.

Udo, T., Weinberger, A. H., Grilo, C. M., Brownell, K. D., DiLeone, R. J., Lampert, R., et al. (2014). Heightened vagal activity during high-calorie food presentation in obese compared with non-obese individuals—Results of a pilot study. *Obesity Research & Clinical Practice, 8*, e258-e265.

van Vliet, I. M., & de Beurs, E. (2007). The MINI-International Neuropsychiatric Interview. A brief structured diagnostic psychiatric interview for DSM-IV en ICD-10 psychiatric disorders. *Tijdschrift voor de Psychiatrie, 49*, 393-397.

Volkow, N. D., Wang, G.-J., Fowler, J. S., & Tomasi, D. (2012). Addiction circuitry in the human brain. *Annual Review of Pharmacology and Toxicology, 52*, 321-336.

Volkow, N. D., Wang, G.-J., Fowler, J. S., Tomasi, D., Telang, F., & Baler, R. (2010). Addiction: Decreased reward sensitivity and increased expectation sensitivity conspire to overwhelm the brain's control circuit. *BioEssays, 32*, 748-755.

Vollstädt-Klein, S., Kobiella, A., Bühler, M., Graf, C., Fehr, C., Mann, K., & Smolka, M N. (2011). Severity of dependence modulates smokers' neuronal cue reactivity and cigarette craving elicited by tobacco advertisement. *Addiction Biology, 16*, 166-175.

Vollstädt-Klein, S., Wichert, S., Rabinstein, J., Bühler, M., Klein, O., HErmann, D., & Mann, K. (2010). Initial, habitual and compulsive alcohol use is characterized by a shift of cue processing from ventral to dorsal striatum. *Addiction, 105*, 1741-1749.

Wallén, M., Hasson, D., Theorell, T., Canlon, B., & Osika, W. (2012). Possibilities and limitations of the polar RS800 in measuring heart rate variability at rest. *European Journal of Applied Physiology, 112*, 1153-1165.

Weippert, M., Kumar, M., Kreuzfeld, S., Arndt, D., Rieger, A., & Stoll, R. (2010). Comparison of three mobile devices for measuring R–R intervals and heart rate variability: Polar S810i, Suunto t6 and an ambulatory ECG system. *European Journal of Applied Physiology, 109*, 779-786.

Wiers, R. W., Bartholow, B. D., van den Wildenberg, E., Thush, C., Engels, R. C., Sher, K. J., Grenard, J.,…Stacy, A.W. (2007). Automatic and controlled processes and the development of addictive behaviors in adolescents: A review and a model. *Pharmacology Biochemistry and Behavior, 86*, 263-283.

Witkiewitz, K., & Marlatt, G. A. (2007). Modeling the complexity of post-treatment drinking: It's a rocky road to relapse. *Clinical Psychology Review, 27*, 724-738.

Wrase, J., Schlagenhauf, F., Kienast, T., Wüstenberg, T., Bermpohl, F., Kahnt, T., et al. (2007). Dysfunction of reward processing correlates with alcohol craving in detoxified alcoholics. *NeuroImage, 35*, 787-794.