A joint approach: brain structure & function in heavy cannabis users & their relationship with future use
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severity of cannabis-related problems, not to levels of cannabis use: most core regions associated with cue-reactivity were only active in heavy users scoring high for problem-severity, independently of frequency and history of cannabis use. These findings are consistent with previous findings and support a role of cue-reactivity in assessing problem severity of cannabis use.

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ABSTRACT

Cognitive biases, including implicit memory associations, are thought to play an important role in the development of addictive behaviors. The aim of the present study was to investigate implicit memory associations in heavy cannabis users. Implicit positive-arousal, sedation, and negative associations towards cannabis were measured with three Single Category Implicit Association Tests (SC-IAT's) and compared between 59 heavy cannabis users and 89 controls. Moreover, we investigated the relationship between these implicit affective associations and explicit affective expectancies, subjective craving, cannabis use, and cannabis related problems. Results showed stronger implicit positive-arousal associations but weaker implicit negative associations towards cannabis in heavy cannabis users had compared to controls. Weekly cannabis use was associated with stronger implicit negative associations in heavy cannabis users, decreasing the difference between cannabis users and controls. No other associations were observed between implicit associations and measures of cannabis use, cannabis use related problems, and subjective craving. These findings indicate that, in contrast to other substances of abuse like alcohol and tobacco, the relationship between implicit associations and cannabis use appears to be weak in heavy cannabis users.
INTRODUCTION

Cannabis is the most widely used illegal substance in most countries and treatment demands for cannabis dependence directly follow demands for alcohol and opiates (UNDOC World Drug Report, 2009; Degenhardt et al., 2008). However, relatively little is known about the processes underlying continued cannabis use and the eventual progression towards dependence. Theoretical models of addiction suggest that motivational processes play an important role in the development of addictive behaviors (Koob and Volkow, 2010; Wiers et al., 2007). A better understanding of these motivational processes could gain deeper insight into the processes underlying cannabis abuse and dependence.

In regular substance users, substance use is repeatedly paired with certain cues, such as specific contexts or emotional states. This is thought to result in a sensitization of the motivational system towards the substance of abuse. Subsequent exposure to these substance-related cues may then bias motivation towards substance use. These so-called ‘cognitive biases’ are thought to be relatively automatically triggered but may reach awareness (for reviews see, McCusker, 2001; Wiers et al., 2007).

Indirect or implicit measures of drug-related motivations are considered promising in the field of addiction since they appear less prone to self-awareness (De Houwer et al., 2009). This is especially important as substance users may lack insight into the cognitive processes underlying their own behavior (Goldstein et al., 2009a).

Prior studies showed that substance-related cues indeed automatically capture attention (e.g., attentional bias; i.e., Field, 2005; Mogg et al., 2005), elicit approach tendencies (e.g., approach bias; i.e., Field, 2005; Mogg et al., 2005; Wiers et al., 2009b), and activate implicit memory associations in heavy substance users (i.e., Field et al., 2004; Wiers et al., 2002a) Furthermore, cognitive biases tend to correlate with subjective craving (i.e., Field et al., 2004; Franken et al., 2000; Mogg et al., 2003), although not consistently over studies (Field et al., 2009). Additionally, cognitive biases have been found to predict relapse in heroine dependent individuals (Marissen et al., 2006), cigarette smokers (Kahler et al., 2007; Waters et al., 2003), and alcohol dependent individuals (Cox et al., 2002).

A test that has often been used to assess implicit memory associations is the Implicit Association Test (IAT; Greenwald et al., 1998). The underlying idea of this task is that the categorization of associated stimuli (i.e., flowers and positive) is easier compared to the categorization of non-associated stimuli (i.e., flowers and negative). The IAT has extensively been used to assess implicit memory associations towards substances of abuse, such as alcohol and cigarettes (for meta-analyses see; Reich et al., 2010; Rook et al., 2008). Previous IAT studies demonstrated implicit negative associations towards alcohol for both light drinkers and heavy drinkers (De Houwer et al., 2004; Wiers et al., 2002a). Interestingly, it was found that heavy drinkers had stronger alcohol-arousal compared to alcohol–sedation associations, but this effect
was absent in light drinkers (Wiers et al., 2002a). Furthermore, implicit associations were found to be related to craving (Waters et al., 2007) and to predict alcohol use (Ostafin et al., 2008).

To date, studies investigating implicit memory associations towards cannabis are sparse. In a study of Field et al. (2004), positive and negative implicit associations for cannabis related words were examined in regular cannabis users and controls. It was found that cannabis users showed weaker implicit negative associations for cannabis compared to controls, whereas the implicit positive associations did not differ between groups. Furthermore, no correlations were found between implicit associations and craving or other measures of cannabis use. In a second study, positive-arousal, sedation, and negative implicit associations were examined in adolescent cannabis users (Ames et al., 2007). In this study it was found that implicit positive-arousal associations towards cannabis predicted cannabis use, over and beyond explicit measures of affective expectancies. Finally, a study comparing implicit positive-arousal, sedation, and negative associations towards cannabis between male patients with schizophrenia and healthy controls found no differences in implicit associations between groups (Dekker et al., 2010). However, the patients showed stronger explicit negative expectancies towards cannabis compared to controls. Overall, inconsistent findings and methodological differences between studies preclude drawing strong conclusions about the relationship between implicit memory associations and cannabis use.

Therefore, the present study examined a large sample of non-treatment seeking heavy cannabis users. The primary aim was to investigate implicit affective memory associations in a large sample of heavy cannabis users compared to controls. Moreover, within the group of heavy cannabis users we assessed the relationship between these implicit affective associations and explicit affective expectancies, craving, quantitative cannabis use, and cannabis use related problem severity. Participants performed three Single Category Implicit Association Tests (SC-IAT’s; Karpinski and Steinman, 2006) to measure implicit associations in three dimensions: positive arousal (excitement), negative (negative affect), and sedation (negative reinforcement) associations towards cannabis use. We hypothesized that cannabis users would show stronger implicit sedation cannabis associations, whereas controls would have stronger implicit negative associations. Furthermore, we expected to find a relationship between implicit associations and craving, cannabis use, and cannabis related problems.
MATERIALS AND METHODS

Participants
For the current study, data from two separate studies investigating neurocognitive processes related to cannabis use were combined (see also Cousijn et al., 2012a; Cousijn et al., 2011; Cousijn et al., 2012b). From these studies participants that completed the IATs were included, resulting in a sample of 59 heavy cannabis users and 89 controls aged 18–25. Participants were recruited through advertisements on the Internet and in cannabis outlets (coffee-shops). Groups were matched for age and estimated intelligence (Schmand et al., 1991, Table 1). Heavy cannabis use was defined as using cannabis at least on 10 days in the previous month, 240 or more days in the last 2 years, and not seeking treatment or having a history of treatment for cannabis abuse. Participants in the control group used cannabis on fewer than 50 life-time occasions and did not use the previous year. To control for other illicit substance and alcohol use, participants with an Alcohol Use Disorder Identification Test (AUDIT; Saunders et al., 1993) score higher than 10, smoking more than 20 cigarettes per day, or using any noncannabinoid drugs on more than 100 occasions were excluded (no participant > 25 occasions). Further exclusion criteria were a history of major medical, physical or psychiatric disorders, assessed with the Mini-International Neuropsychiatric Interview (M.I.N.I., Sheehan et al., 1998; Dutch version 5.0.0). The study was approved by the medical ethics committee of the Academic Medical Center and the ethics committee of the University of Amsterdam All participants signed informed consent before participation.

Questionnaires
The Cannabis Use Disorder Identification Test (CUDIT; Adamson and Sellman, 2003) was used to assess cannabis use and related problems. The CUDIT is a screening instrument for at-risk cannabis use and consists of 10 items on cannabis use frequencies, symptoms of dependence and use-related problems (Adamson et al., 2010; Adamson and Sellman, 2003). Furthermore, detailed information about past and present cannabis use was obtained, such as duration of use, weekly use (days, grams), and lifetime use. Tobacco use and dependence was measured with the Fagerström Test for Nicotine Dependence (FTND; Heatherton et al., 1991).

The short 12-item version of the Marijuana Craving Questionnaire (MCQ; Heishman et al., 2009) was used to assess subjective craving after the test-session. The MCQ is reliable for assessing craving in cannabis users not seeking treatment (Heishman et al., 2009; Heishman et al., 2001). The MCQ distinguishes four three-item craving factors: compulsivity (inability to control use), emotionality (relief from withdrawal and negative affect), expectancy (anticipation of positive outcomes) and purposefulness (planning/intention to use for positive outcomes). Items were rated on a Likert response scale ranging from 1 (strongly disagree) to 7 (strongly agree).
 Implicit Association Test

Participants completed three SC-IATs in a row to assess implicit affective associations towards cannabis. The SC-IAT’s were presented on a computer screen with E-prime software (version 2.0, Psychology Software Tools, Inc.). Each SC-IAT measured one of the three different affective associations towards the use of cannabis: positive-arousal, sedation, and negative. Each affective category was compared to a neutral category. The SC-IAT’s were performed in random order, counterbalanced over participants and groups. Each SC-IAT contained four categories: two target categories (cannabis or other) and two attribute categories (i.e., excited or neutral). Stimuli in the target categories were five cannabis related pictures (i.e., joints) and five neutral pictures (stationeries), matched on color and image composition. Stimuli in the attribute categories were five affective and five neutral words. These words were matched on the number of letters, syllables, familiarity, valence and arousal.

Each SC-IAT consisted of five blocks. The first block was a target discrimination practice block (e.g., left = cannabis and right = other), consisting of 20 trials, in which each image was presented twice. Participants were asked to categorize the images to one of the target categories by pressing a corresponding button (e-key = left or i-key = right). The second block was an attribute discrimination practice block consisting of 20 trials in which participants had to categorize words to one of the attribute categories. The third block was a combination block consisting of 20 practice and 20 test trials, in which target and attribute categories were presented together (left = cannabis + negative and right = other + neutral). Participants were now required to categorize stimuli to a target category combined with an attribute category. After this combination block, the target categories were reversed and practiced in another target discrimination practice block (e.g., left = other and right = cannabis). The final block was a second combined categorization block of 20 practice and 20 test trials (e.g., left = cannabis + neutral and right = other + negative). The IAT effect was considered the difference in RT between the two combined categorization blocks. Thus, participants with an implicit negative cannabis association were faster in responding to the cannabis-negative combined blocks compared to the cannabis-neutral combined blocks.

Each trial started with a word or image presented in the centre of the screen. Target and attribute words were presented on the left or right top of the screen in order to remind participants of the categories. Participants were instructed to categorize stimuli as quickly as possible by pressing a left or right response button with their index fingers. If an incorrect response was made, participants saw a red ‘X’ on the screen and were asked to correct there response before the next trial started. If participants did not response within 2500 ms, they received the feedback ‘too slow’. 
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Participants of the categories. Participants were instructed to categorize stimuli as quickly as attribute words were presented on the left or right top of the screen in order to remind five affective and five neutral words. These words were matched on the number of letters, (stationeries), matched on color and image composition. Stimuli in the attribute categories were presented twice. Participants were asked to categorize the images to one of the target categories block (e.g., left = cannabis and right = other), consisting of 20 trials, in which each image was presented twice. Participants completed three SC-IATs in a row to assess implicit affective associations towards the use of cannabis: positive-arousal, sedation, and negative. Each affective association was assessed with twenty-nine cannabis expectancy statements on cannabis use and positive-arousal (i.e., ‘if I smoke cannabis I feel excited’), negative (i.e., ‘I feel sick’), and sedation (i.e., ‘I feel relaxed’) expectancies had to be rated. The words used in the IATs were integrated in these statements. Participants were instructed to indicate the extent to which they disagreed or agreed with the statements on a Likert response scale ranging from 1 (strongly disagree) to 6 (strongly agree). Internal reliability of the scales in the presents sample was good: positive-arousal expectancies Cronbach’s α = .86, negative expectancies Cronbach’s α = .93, and sedation expectancies Cronbach’s α = .95.

**Explicit expectancies**

Similarly to Wiers et al. (2002b, 2005), Ames et al. (2007) and Dekker et al. (2010), explicit cannabis expectancies were assessed with twenty-nine cannabis expectancy statements on cannabis use and positive-arousal (i.e., ‘if I smoke cannabis I feel excited’), negative (i.e., ‘I feel sick’), and sedation (i.e., ‘I feel relaxed’) expectancies had to be rated. The words used in the IATs were integrated in these statements. Participants were instructed to indicate the extent to which they disagreed or agreed with the statements on a Likert response scale ranging from 1 (strongly disagree) to 6 (strongly agree). Internal reliability of the scales in the presents sample was good: positive-arousal expectancies Cronbach’s α = .86, negative expectancies Cronbach’s α = .93, and sedation expectancies Cronbach’s α = .95.

**Procedure**

Test-sessions took place during the late afternoon and at the beginning of the evening. All participants were asked to refrain from alcohol and drug use 24 hours prior to testing. Each session started with signing the informed consent form. After completing questionnaires and the
diagnostic interview, participants performed the three SC-IAT’s. Participants were then asked to rate the statements on cannabis use expectancies. Craving was assessed at the end of the test session.

**Statistical analysis**

Participants who made more than 35 % errors during the IATs and with an average RT more than 3 standard deviations (SD) above or below the group mean were considered as outliers. For each of the three affective dimensions a D-2SD measure was calculated based on the scoring algorithm of Greenwald (Greenwald et al., 2003). To investigate main effects of study and gender, a multivariate analysis of variance (MANOVA) was performed. To further investigate the relative contribution of the different variables, discriminant analyses were conducted. In order to examine differences between heavy cannabis users and controls, independent t-tests were performed. One-sample t-tests were used to test if IAT scores differed significantly from zero within each group. In order to investigate associations between implicit associations and explicit expectancies, craving, cannabis use and cannabis use related problems Pearson’s correlations were calculated. To investigate potential confounding effects of nicotine use, within the group of heavy cannabis users IAT scores were correlated with scores for nicotine dependence and smokers and non-smokers were compared.

**Figure 1** Mean implicit associations (IAT D2SD score + standard error) per group for each IAT dimension.

**Figure 2** Mean explicit expectancies (likert scale + standard error) per group for each IAT dimension.
RESULTS

Sample characteristics
The IAT scores of 3 controls were excluded from analysis because their errors exceeded 35 % (40-49 %). Furthermore, one control was discarded as outlier because the IAT score was above 4 SD from the group mean (results were similar when this participant was included in the analyses). The groups did not differ in age (t_{148} = .57, p = .57), IQ (t_{148} = .79, p = .43), and alcohol use (t_{148} = -1.03, p = .31). However there were more men (χ^2 = 7.41, p = .006) and more cigarette smokers (χ^2 = 20.62, p < .001) in the heavy cannabis users group. CUDIT scores (t_{148} = -17.73, p < .001) and craving were higher (compulsivity: t_{146} = 8.81, p < .001, emotionality t_{146} = 8.37, p < .001, expectancy t_{146} = 10.84, p < .001, and purposefulness t_{146} = 11.07, p < .001) in the heavy cannabis users compared to controls (see Table 1).

Group comparison implicit associations and explicit expectancies
A MANOVA was performed to investigate overall effects of group, study and gender on implicit memory associations. There was a significant main effect of group (F_{3,135} = 4.76, p = .003, η^2 = .10), but not for study (F_{3,135} = .89, p = .45) or gender (F_{3,135} = 2.13, p = .10). No significant interactions between group and study (F_{3,135} = .71, p = .55) or group and gender (F_{3,135} = .45, p = .72) were found.

A discriminant analysis, that focused on the structure coefficients, was performed to determine the relative contribution of the different variables to the main effect of group (Huberty and Morris, 1989). The relative contributions to the difference between heavy cannabis users and controls were: Implicit negative associations (-.93), positive-arousal associations (.64), and sedation associations (.30). Groups differed significantly in negative (t_{144} = 3.04, p < .01) and positive-arousal associations (t_{144} = -2.01, p = .046), with heavy cannabis users showing stronger positive-arousal associations towards cannabis (M = .99, SD = .52) compared to controls (M = .82, SD = .50) and weaker negative associations (M = .79, SD = .53) compared to controls (M = 1.05, SD = .49). Post hoc one-sample t-tests indicated that both groups had a significant negative, arousal, and sedation association towards cannabis.

With regard to the explicit affective expectancies, a MANOVA revealed overall effects for group (F_{3,138} = 21.25, p < .001, η^2 = .32) and study (F_{3,138} = 4.66, p = .004, η^2 = .09) but not for gender (F_{3,138} = 1.76, p = .16). A discriminant analysis showed the following relative contributions to the difference between groups: Negative expectancies (.81), relaxed expectancies (-.36), and positive-arousal expectancies (.22).

Differences between groups were found in negative (t_{146} = 9.10, p < .001) and sedation expectancies (t_{146} = -6.78, p < .001), with controls showing stronger negative expectancies (M = 3.01, SD = .91) compared to cannabis users (M = 1.82, SD = .50) and heavy cannabis users showing stronger relaxed expectancies (M = 4.53, SD = .62) compared to controls (M = 3.54, SD = 1.01), see
Figure 2. No significant differences between groups were found in positive-arousal expectancies ($t_{146} = -1.56, p = .11$). Participants differed between studies concerning positive-arousal expectancies ($t_{146} = 4.00, p < .001$).

**Correlations**

In the heavy cannabis users group, no significant correlations between implicit and explicit associations were found. In the control group, implicit and explicit positive-arousal associations ($R = .23, p = .033$) and implicit and explicit relaxed associations ($R = .26, p = .012$) correlated positively. There was no significant correlation between implicit and explicit negative associations ($R = -.01, p = .958$).

Within the group of heavy cannabis users, implicit negative associations correlated positively with weekly cannabis use (grams, $R = .28, p = .038$). No other significant correlations were found between implicit measures and measures of cannabis use, cannabis use related problems, and craving.

Within the group of heavy cannabis users, a positive correlation between the CUDIT and explicit negative expectancies was found ($R = .29, p = .026$), indicating that cannabis users with more cannabis related problems have stronger explicit negative expectancies towards cannabis.

**DISCUSSION**

The present study aimed at investigating affective implicit memory associations in heavy cannabis users. Significant group differences in the IATs measuring implicit negative and positive-arousal associations were found: Heavy cannabis users had stronger positive-arousal but weaker negative associations towards cannabis compared to controls. Positive-sedation associations did not differ between groups. Within the group of heavy cannabis users, weekly cannabis use (grams) was associated with stronger implicit negative associations, decreasing the difference between heavy cannabis users and controls. We did not observe any other association between implicit memory associations and measures of cannabis use, problems, or craving.

Although heavy cannabis users had weaker negative associations, both cannabis users and controls had a significant negative association towards cannabis. This significant negative association towards cannabis in heavy cannabis users is in accordance with earlier studies examining implicit associations towards alcohol (De Houwer, et al., 2004; Wiers, et al., 2002) and cannabis (Dekker, et al., 2010; Field, et al., 2004). However, in the study of Field et al. (2004) negative associations towards cannabis were only observed in controls, not in heavy cannabis users. The latter finding may be explained by the use of a bipolar IAT, in which negative associations were directly compared with positive associations (Karpinski & Steinman, 2006). We observed a similar pattern regarding the explicit negative expectancies as both groups showed
significantly explicit negative expectancies, but in heavy cannabis users they were less strong compared to controls. This finding could imply that cannabis users generally have less negative attitudes towards cannabis.

Interestingly, higher weekly cannabis use was associated with stronger implicit negative associations. This suggests that more heavy (problematic) users have implicit negative association towards cannabis, similarly to the controls. A similar relationship was observed regarding explicit negative expectancies: The higher cannabis-related problems (CUDIT) the stronger the explicit negative expectancies towards cannabis. This finding is in accordance with studies showing that stronger negative expectancies were related to relapse in heavy drinkers (Jones & McMahon, 1994, 1996). In contrast to our hypothesis, we did not observe any other association between implicit or explicit associations towards cannabis and measures of cannabis use, cannabis use-related problems, or craving. In a subsample of the heavy cannabis users we previously showed that the cannabis approach bias, as measured with a joystick approach avoidance task, could predict escalation of cannabis use 6 months later (Cousijn, et al., 2011). A post-hoc analysis with the participants included in the Cousijn et al. (2011) study indicated that implicit memory associations did not significantly predict future cannabis use (associations IAT measures with change in cannabis use and cannabis use related problems over six months was $R^2 < .078 \ p > .11$). All together, these findings suggest that, in contrast to e.g., alcohol and nicotine (Wiers, et al., 2007), the relationship between implicit affective associations and cannabis use is rather weak. Alternatively, implicit associations towards cannabis may only play an important role in earlier stages of cannabis use, stimulating onset and repeated cannabis use rather than chronic cannabis use. This should then result in strong associations between implicit associations and cannabis use in a less heavy or regular group of cannabis users. These strong associations between implicit associations and cannabis use were found in the study of Ames et al. (2007) with a sample of adolescent cannabis users. Future research is needed to clarify these issues. Therefore, different groups of heavy cannabis users with various levels of cannabis use should be investigated.

The stronger positive-arousal associations observed in heavy cannabis users are in line with an earlier study from Wiers et al. (2002), which found stronger implicit and explicit arousal associations in heavy drinkers compared to light drinkers. The findings in our study suggest similar motivational mechanisms underlying heavy cannabis and alcohol use.

In the Netherlands cannabis is decriminalized, which means that use and possession of cannabis are to a certain extent legal. Furthermore, cannabis can be purchased in so called ‘coffee-shops’. One might expect that this decriminalization would lead to less negative attitudes towards cannabis. However, in the present study both heavy cannabis users and controls had implicit and explicit negative associations towards cannabis, which is similar to results from studies conducted in countries with opposite cannabis policies or studies investigating legal substances of abuse like alcohol (Field, et al., 2004; Wiers, Van Woerden, Smulders, & de Jong, 2002). This leads to the
assumption that associations towards cannabis are not dependent on drug policy, which is in line with studies that failed to find that frequency and quantity or prevalence of cannabis use is influenced by drug policy (MacCoun & Reuter, 1997; Reinarman, Cohen, & Kaal, 2004). For further research it would be interesting to test this hypothesis across countries.

Some limitations have to be taken into account. Among the heavy cannabis users more participants also smoked tobacco compared to controls. Since tobacco and cannabis cigarettes show resemblance, it is possible that the implicit associations we observed towards cannabis partly reflect tobacco associations activated in tobacco smokers. However, IAT scores did not differ between smokers and non-smokers and scores for nicotine dependence did not correlate with any of the IAT scores. Nevertheless, since almost all cannabis users smoked cannabis combined with tobacco, we cannot entirely discriminate between cannabis and tobacco effects. Concerning methodological issues, it has previously been argued that the IAT effect could be caused by salience of stimuli rather than by implicit attitudes, that is a ‘figure ground effect’ (Rothermund & Wentura, 2004). However, with regard to alcohol, alcohol-arousal associations could not be explained by figure ground effects (Houben & Wiers, 2006). Finally, it has been suggested that extra-personal associations (i.e., cultural norms) could (at least partly) contaminate the IAT effect (Houben & Wiers, 2007). These extra-personal associations are suggested to be irrelevant to our personal behavior, which may explain why we observed only a weak association between implicit associations and measures of cannabis use and cannabis use related problems.

In summary, the present study demonstrates that both, controls and heavy cannabis users have implicit negative associations and explicit negative expectancies towards cannabis; however, these associations were stronger in controls. Moreover, heavy cannabis users showed stronger implicit arousal associations compared to controls. In contrast to other substances of abuse, implicit and explicit associations towards cannabis appear to be only weakly associated with cannabis use, suggesting only a limited role of implicit associations in cannabis abuse.

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