Chapter 7

General Discussion

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A lcohol dependence (AD) is a chronic, relapsing disease associated with impaired functionalities of the brain (Leshner 1997). These impairments may represent a pre-existing vulnerability to develop AD and/or the detrimental consequences of long-term heavy alcohol use. The main objective of this thesis was to gain a better insight into the neurobiological processes associated with a vulnerability to develop alcohol dependence and those resulting from chronic excessive alcohol use. We therefore studied the neurobiological correlates of a family history of alcohol dependence in non-alcoholic adults (Section II) and the neurobiological processes that contribute to a time-dependent progression towards habitual or compulsive alcohol use in established alcohol dependent patients (Section III).

In this General Discussion we discuss the results from chapters 2 to 6 in a broader context. In addition, we discuss some methodological considerations and directions for future research. We conclude this discussion with the clinical implications and provide a new vision on therapeutic targets.

SUMMARY AND DISCUSSION

Following the general framework for the studies presented in this thesis in the General Introduction in Section I, we report on the studies about the role of a family history of alcohol dependence (FH) in Section II. In this section two studies are described that focus on this important vulnerability factor to develop alcohol dependence and related psychopathology.

A family history of alcohol dependence: a fingerprint in the brain

An important vulnerability factor possibly underlying premorbid impairments in function or structure of the brain is the presence of a family history of alcohol dependence (Bjork et al. 2008; Heitzeg et al. 2008; Hill et al. 2001; Hill et al. 2009). In our family history studies, in chapters 2 and 3, we showed that the presence of a first degree family member (father/mother/brother/sister) with alcohol dependence influences the structural as well as functional neurobiological profile in non-alcoholic adults (healthy individuals or individuals with depression/anxiety) (Sjoerds et al. 2012a; Sjoerds et al. 2012b).

Although volumetric abnormalities in local brain structures have repeatedly been shown to be present in alcohol dependent patients (Agartz et al. 1999; Mechtcheriakov et al. 2007), studies in young non-alcoholic individuals with a family history of AD have indicated that certain volumetric reductions are present even before the development of problematic alcohol use (Benegal et al. 2007; Hill et al. 2001; Hill et al. 2007b; Venkatasubramanian et al. 2007). However, the brains of children and adolescents have not reached full maturation yet (Jernigan et al. 1991), and abnormalities in local brain structures and associated functions could be just a transient effect of slow maturation related to a family history of alcohol dependence. In this line, electroencephalographic (EEG) studies have found that differences in P300 amplitude disappear over time, indicating a developmental delay in brain maturation in children at high risk for alcohol-related disorders (Bauer and Hesselbrock 1999; Polich et al. 1994). Nevertheless, it is possible that some (other) neural correlates of familial AD are more persistent and remain observable in the adult brain. We therefore looked at persistent neurobiological abnormalities in the fully matured, adult brain of non-alcoholic individuals with a family history of alcohol dependence.

In chapter 2 we studied the influence of a family history of alcohol dependence on regional brain volumes using voxel-based morphometry (VBM) in a group of 36 non-alcoholic adults with reported first-degree familial AD (FH+). We compared these VBM data with those of a group of 107 non-alco-

holic adults without familial AD (FH–). The findings showed a smaller right parahippocampal gyrus (PHG) in our FH+ group compared with the FH– group; a brain area that has previously been shown to be smaller in children and adolescents with a positive family history of alcohol dependence (Benegal et al. 2007). Moreover, a very recent study found reduced hippocampal volumes in adult alcohol dependent patients with adolescent-onset alcohol use disorders (compared with late-onset alcohol use disorders) (Ozsoy et al. 2013), which could be associated with the presence of a specific premorbid vulnerability factor such as a family history of alcohol dependence, known to lead to early-onset alcohol use problems (Prescott and Kendler 1999). The smaller PHG volume in the adult FH+ sample of our study implies that the presence of familial AD is associated with a persistent biological factor, possibly associated with an increased risk for the development of AD or other related psychiatric disorders. By including solely non-alcoholic individuals, we could assure that the reported volumetric differences were not a consequence of long-term excessive drinking.

As part of the hippocampal formation, the PHG is highly involved in learning and memory functions (Eichenbaum et al. 1992; Squire et al. 1989; van Strien et al. 2009). Underlying its role in memory functions, the PHG and surrounding areas in the medial temporal lobe are relatively more dependent on glutamate signaling than other neocortical tissues (Amaral and Witter 1989). The observed volume reduction of the PHG in FH+ people may therefore be indicative of alterations of the glutamate signaling in this brain area, although direct evidence is not provided in this study. Interestingly, the N-methyl-d-aspartate (NMDA) subtype of glutamate receptors is associated with the intoxicating effects of alcohol through direct blockage by ethanol (Grant and Lovinger 1995; Krystal et al. 2003b), especially in the hippocampal area (Möykkynen and Korpi 2012). Moreover, the NMDA receptor has repeatedly been associated with a genetically controlled risk factor and involved in the pharmacological treatment of alcohol dependence (Krystal et al. 2003a; Spanagel and Vengeliene 2013). Antagonism of the NMDA-receptor by alcohol consumption conveys an important component of the objective and subjectively experienced alcohol-induced intoxication signal. A reduced sensitivity, however, to the direct intoxicating effects of alcohol (low alcohol response) is shown to be related to an increased risk for alcohol use problems and is seen in individuals with a family history of alcohol dependence (Quinn and Fromme 2011; Schuckit 1985; Schuckit et al. 1996). Alterations in NMDA-receptor function, associated with a reduced sensitivity to the actions of NMDA-receptor antagonists, could be associated with this decreased sensitivity to alcohol intoxication in individuals with an alcoholic family history, possibly by genetic effects on the brain (Schuckit et al. 2004). Memantine, an NMDA-receptor antagonist has been shown to differentially affect cognitive functions and related BOLD-responses in individuals with and without a family history of alcohol dependence (Jamadar et al. 2012). Thus, volumetric abnormalities in the hippocampal area, as found in our study in chapter 2, could in this way contribute to the decreased sensitivity to the antagonistic effects of alcohol on NMDA receptors, and the increased risk of developing alcohol dependence. However, no direct evidence on the association between smaller GM volumes and NMDA-receptor sensitivity are provided, and therefore our conclusion is rather speculative and more research is needed.

Additionally, the PHG has also been considered to play a key role in behavioral inhibition (Gray and McNaughton 2000), possibly indirectly through the memory system, and impulsivity has repeatedly been shown to be associated with an increased risk to develop substance use disorders (e.g. Verdejo-Garcia et al. 2008). Thus, alterations in PHG may contribute to increased impulsivity, and this could be an additional mechanism through which FH+ may contribute to an increased risk to develop AD.

These volumetric changes under influence of a family history of alcohol dependence can be either a reflection of genes and their effects on the brain (Devor and Cloninger 1989) or the result of environmental factors associated with alcoholic family member, such as role models, stress or

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prenatal alcohol exposure (Campbell et al. 2009; Gilman et al. 2007; Latendresse et al. 2008). A decrease in (para)hippocampal GM volume has been observed previously in various neuropsychiatric disorders associated with (high) stress (Sapolsky 2000), suggesting that negative environments associated with familial alcoholism may affect volumes of the hippocampal area through a similar mechanism (Lindauer et al. 2006; Pruessner et al. 2007). In our study we post-hoc controlled for environmental factors such as childhood trauma and maternal alcoholism (for the case of prenatal alcohol exposure), however these did not change the result. This could indicate that reduced parahippocampal volume in subjects with familial risk of AD is probably not mediated by these environmental factors but by pre-existing, possibly genetic factors. However, future studies should be performed taking genetics and additional environmental factors into account, in order to make a valid statement about the reflection of genes or environments in the neural correlates of an alcoholic family history.

In conclusion, whilst FH studies in non-alcoholic children and adolescents show a neural fingerprint associated with an increased vulnerability to develop AD, we were able to show a non-neurotoxic permanent brain abnormality in non-alcoholic FH+ adults. FH+ individuals may have a persistent neurobiological vulnerability for the development of AD or AD-related neuropsychiatric disorders.

Depression/anxiety and family history of AD

Decreased PHG volumes have been found in several studies on anxiety disorders (Liao et al. 2011; Massana et al. 2003) and in studies on other neuropsychiatric disorders associated with stress (Sapolsky 2000). Reciprocal connections between the medial temporal lobe, the orbitofrontal gyrus and the amygdala suggest that the PHG is involved in the processing of negative information (Iidaka et al. 2001). This may suggest an additional role for the PHG in emotion regulation, and next to a possible mediating effect in the risk to develop AD; a smaller PHG may thus play a role in the development of mood- or anxiety disorders. Interestingly, although objective depression measurements (MADRS) did not differ between groups, MDD was more prevalent in our non-alcoholic FH+ group than in the FH– group in chapter 2. Therefore it appears that the prevalence of other types of psychiatric pathology, often associated with alcohol use disorders, may increase under the familial load of AD. Similar observations were reported by Araujo and Monteiro (1995) and Dawson and Grant (1998) with an increased presence of depression and anxiety disorders in people with a family history of alcohol dependence.

In addition to an increased prevalence of depressive / anxiety disorders under the influence of an alcoholic family history, it is also possible that a positive family history of alcohol dependence influences the symptom profile or underlying neurobiology of established mood disorders. Indeed, the neural substrate of mood/anxiety disorders has been shown to be specifically influenced by the presence of an alcoholic family history. And more importantly, it is actually at the level of NMDA-receptors where these modifications are reported: depressed patients with an alcoholic family history have demonstrated a significantly better anti-depressant response to the NMDA-receptor antagonist ketamine compared with patients without such a family history (Phelps et al. 2009).

These findings indicate that depressed (and probably also anxious) patients may have a different neurobiological profile depending on the presence of a FH of AD, including possible differences in neurotransmitter and receptor profile, which in turn may be associated with performance differences during cognitive or emotional tasks. Taking the presence or absence of a family history of alcohol dependence into account when studying cognitive and emotional functions and their neural correlates could therefore explain some of the heterogeneous neuroimaging findings repeatedly reported in studies on depression and anxiety disorders (Fitzgerald et al. 2008).

Therefore in chapter 3 we focused on the functional correlates of a family history of alcohol dependence in a group of patients with depression/anxiety disorders and without an alcohol use disorder. This study aimed to examine the association between a family history of alcohol dependence and the neurophysiological manifestations of psychopathology other than AD, e.g., mood/anxiety disorders. We combined a cognitive and emotional paradigm in one fMRI study comparing 31 non-alcoholic FH+ patients with depression/anxiety disorders and 77 FH– patients with depression/anxiety disorders. To facilitate the interpretation of our findings, differences between the FH– and the FH+ depression/anxiety groups in performance levels and brain activation patterns were compared to those of 31 FH– healthy controls (HC).

During the planning test (Tower of London) there were no group differences in performance, but we found increased dorsal prefrontal activity in FH+ patients compared with both FH– patients and FH– HCs, suggesting the need for compensatory activity in the FH+ group, possibly associated with declined cognitive capacities.

During an emotional appraisal/memory task however, we only found decreased right insular activation in response to positive (mood-incongruent) emotional stimuli in the FH– patient group, whereas the FH+ patient group showed brain activation patterns comparable to the FH– HC group. Hypoactivation in the insula (as seen here in FH– patients) may be related to a blunted response to positive stimuli, which would be in concordance with the mood-incongruence bias hypothesis, i.e. attentional and memory bias away from positive information (Burt et al. 1995; van Tol et al. 2012; van Wingen et al. 2010), which is associated with a decreased ability to enjoy positive situations, possibly reflective of anhedonia (Bower 1981). When considering two subtypes of depression, atypical and melancholic depression, atypical depression is normally characterized by a disturbing sense of disconnectedness and emptiness (Gold and Chrousos 1999), which can be associated with anhedonia. The IDS questionnaire, as also assessed during the current studies, differentiates between atypical and melancholic items (Novick et al. 2005), however the FH+ and FH– groups did not differ on either melancholic or atypical items, nor on total IDS score, providing no direct clinical evidence for higher anhedonia in the FH– group.

Together with the required compensation during a cognitive task, these results suggest that the presence of a FH of AD is associated with impaired cognitive functions in mood/anxiety disorder patients, but not with abnormal processing of positive stimuli. The presence of depression / anxiety symptoms/disorders in FH+ patients therefore seems to be driven mainly by a reduced ability to regulate negative emotional states due to diminished cognitive control capacity, rather than by reduced reactivity to positive stimuli. This suggests a different pathway through which depression and anxiety symptoms are maintained in patients with depressive / anxiety disorders with a family history of AD compared to patients with similar symptoms without a family history of AD. These results could not be explained by differences in depression and anxiety severity or medication use, and therefore confirm our hypothesis that a FH of AD affects the neurophysiological profile related to emotional processing.

Similar to a vulnerability to develop AD, the underlying neurobiology of this increased risk related to FH+ can be transferred in families through genes as well as environmental factors. For example, the hypothesis that negative environmental factors associated with familial alcoholism may affect hippocampal volumes is supported by the presence of decreased PHG volumes in patients with a posttraumatic stress disorder (PTSD) (Nardo et al. 2010; Wignall et al. 2004), which are correlated with trauma load (Woodward et al. 2009). In this context, the volume decrease could be mediated through a mechanism of increased cortisol levels (Lindauer et al. 2006; Pruessner et al. 2007) or stress-related decreased levels of brain derived neurotrophic factor (BDNF), reducing neurogenesis in the hippocampal area (Hajek et al. 2012; Montag et al. 2009). However, in contrast to studies sug-

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gesting smaller hippocampal volume as a consequence of stress, Gilbertson and colleagues (2002) provided evidence in their PTSD twin study that a smaller hippocampal volume is a pre-existing condition rendering the brain more vulnerable to the development of pathological stress responses. In our sample, there were significant differences in childhood trauma between the two patient groups, indicating that the FH+ group had experienced more childhood adversities before the age of 16 years than the FH– group; however the presence of these childhood adversities was not related to our main results. Our data could thus be consistent with a genetic predisposition for a smaller hippocampal area volume in FH+ and a distinguished neurofunctional profile in depression/anxiety patients with a family history of AD. A third possible explanation for the presence of small parahippocampal volumes in people with FH+ is that they are more likely to have a mother with AD and thus more likely to be exposed to alcohol prenatally, a situation known to negatively influence the development of the fetal brain, even leading to fetal alcohol syndrome (Coles and Li 2011; Lebel et al. 2011; Riley et al. 2011). Studies on the effects of prenatal alcohol exposure on the neurobiology later in life show that impaired cognitive functions are still discernible, and these are coupled with compromised functions of prefrontal areas, even compared with FH+ individuals without prenatal alcohol exposure (Norman et al. 2013). In our FH+ sample, only a few individuals (6 in the structural study and 4 in the functional study) reported to have had a mother with alcohol dependence. Removing these individuals from our main analyses did not change the results, indicating that the effects of a family history of AD on local brain structure and function in our study were not likely associated with prenatal alcohol exposure.

In conclusion, our family history studies show that a family history of alcohol dependence is associated with a neural fingerprint on both structure and function of the brain, either through genetic or environmental influences, or through gene-environment interactions. These impairments may contribute to an increased vulnerability to develop AD or related psychopathology through an effect on glutamatergic functions associated with the insensitivity to the intoxicating effects of alcohol, and to an increased anti-depressive response to NMDA-receptor antagonists like ketamine in depressed patients. This latter effect may be associated with a specific neurobiological and symptom profile characterized by decreased control over negative emotional states rather than insensitivity to positive stimuli, under the influence of a family history of AD.

Following Section II on the role of familial alcohol dependence in the neurobiological vulnerability to develop alcohol dependence and some neurobiological aspects of depression and anxiety disorders, in Section III we studied the neurobiological profile of patients that have actually developed AD, focusing on the progression of AD while considering several constructs thought to contribute to the relapsing nature of AD.

Habit formation and its neural correlates

When initial alcohol use becomes problematic and transits into an alcohol use disorder, additional changes in the brain are thought to contribute to the relapsing nature of alcohol dependence. However, substance use disorders are not seen as static disorders, but are progressive brain diseases, hypothesized to change over time under the influence of repeated drug exposure, even after the manifestation of dependence. Mainly based on animal studies, recent models hypothesize a shift towards habitual drug use (Everitt and Robbins 2005). In addicted rodents, this habit formation as a shift away from reward-driven or goal-directed behavior towards stimulus-driven or habitual drug seeking has been extensively studied (Everitt et al. 2008; Everitt and Robbins 2005). Devaluation of rewards in these experimental animal studies does not influence behavior once it has become to be more habit-driven. This behavioral shift is associated with a shift away from medial prefrontal

areas of the cortex towards the striatum, and within the striatum from ventral towards more dorsal striatal involvement (Everitt et al. 2008; Everitt and Robbins 2005).

To the best of our knowledge, this instrumental shift from reward-driven or goal-directed behavior towards stimulus-driven habits has not experimentally been studied in human drug abusers yet. Therefore we performed two studies focusing on the balance between goal-directed instrumental behavior and habit-guided behavior and its neural correlates in alcohol dependent patients, and the related shift from involvement of ventral striatum to the dorsal striatum in the underlying Pavlovian brain activation in response to visual alcohol stimuli.

In chapter 4 we examined brain reactivity and craving following visual alcohol cues in AD patients in an attempt to study Pavlovian alcohol cue-reactivation, which underlies instrumental actions, and which is hypothesized to also involve more habit-like pathways with longer lasting AD duration. We associated both imaging and clinical variables with duration and severity of AD. To further explore whether comorbid D/A disorders influence cue-reactivity and craving in subjects with AD, the association between visual cue-reactivity and craving with the severity of comorbid depression/anxiety symptoms was examined within the AD group, and compared with those in a group of 15 non-alcoholic D/A subjects and a group of 15 healthy controls. Exploratory assessments of neural cue-reactivity differences between the AD group and the two comparison groups showed higher involvement of motivational pathways in the AD group mainly in the orbitofrontal cortex, medial prefrontal cortex and a ventral part of the striatum (nucleus accumbens). These findings are consistent with those in numerous other studies in heavy alcohol users (Heinz et al. 2009), showing that the presentation of visual stimuli, which have been coupled to the rewarding effects of drugs by repeated drug exposure, can activate motivational pathways in drug users. We also reported an expected higher subjective baseline and cue-induced alcohol craving in AD patients compared with the two comparison groups; however these (subjective) craving scores were not significantly related to the (objective) cue-reactivity activation patterns in the AD group. In the past, cue-related brain activity has been shown to be related to craving scores (Newlin 1992). The mismatch between craving and cue-reactivity in our study could be due to the denial of craving by some detoxified alcohol-dependent patients, despite high relapse rates (Tiffany and Carter 1998), rendering it difficult, or even less valid, to measure craving in human subjects with self-report questionnaires (Sayette et al. 2000). Another explanation could be that drug cues motivate drug intake even in the absence of conscious craving, because habitual drug use has replaced reward/goal driven drug taking behavior in the maintenance of drug dependence (Tiffany and Carter 1998).

Although we found motivational pathways to be more involved during cue-reactivity in AD compared with non-AD individuals, we expected that drug cues would induce brain activity more in habit pathways after prolonged drug use. Therefore we examined the relation between the cue-reactivity related BOLD-response (and subjective craving scores) with duration and severity of AD within our AD group of 30 patients using regression analyses. As hypothesized, we found increased cue-reactivity BOLD-response in the posterior putamen with longer AD duration, indicating a stronger activation of habit pathways at the sight of alcohol cues after more prolonged excessive alcohol use. In addition, higher AUDIT scores were associated with less activation in the anterior putamen, suggesting that patients with more severe AD show less alcohol cue-related involvement of brain areas underlying goal-directed actions. These results indicate that a hypothesized shift in instrumental drug-seeking behavior from goal-directed towards habitual behavior is associated with an increased involvement of habit pathways during Pavlovian cue-reactivity. This higher involvement of habit pathways could contribute to stimulus-driven habitual drug seeking and drug taking behavior. To study the balance between goal-directed and habitual actions therefore the next logical step was to study instrumental habit learning in human alcohol dependent individuals.

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In chapter 5 we tested the hypothesized increased involvement of habit pathways following longer AD duration using an instrumental learning paradigm. In an fMRI study, we compared 31 AD patients with 19 healthy controls (HC) during an instrumental learning task, designed to distinguish between general goal-directed and habit learning (de Wit et al. 2009). Behaviorally, AD patients showed an imbalance between goal-directed and habit learning compared with HC, with reduced goal-directed learning and an increased reliance on S-R habit learning in the AD group. This behavioral imbalance in the AD group was associated with increased recruitment of brain areas implicated in habit learning (posterior putamen), and decreased engagement of brain areas implicated in goal-directed action (ventromedial prefrontal cortex and anterior putamen), while activity in the ventromedial prefrontal cortex marginally further decreased with longer AD duration.

Since the imbalance in AD patients between the goal-directed and habit system was not merely based on specific alcohol-use habits, but was initiated in a more general context, it seems that chronic alcohol dependence is associated with a general imbalance in goal-directed versus habit learning possibly underlying alcohol-specific habit formation. This general imbalance could be a pre-existing phenomenon, increasing the risk of drug abuse and dependence after initial drug intake. Impulsivity, another trait shown to precede the development of substance use disorders (Verdejo-Garcia et al. 2008), was shown to be associated with a higher reliance on the habit system (Hogarth et al. 2012). Of course, increased reliance on general S-R habits could also be an effect of repeated drug exposure (Nelson and Killcross 2006) possibly further exacerbating a habitual trait already present, rendering vulnerable drug users more prone to develop habitual drug-seeking behaviors. This way, a vicious cycle is entered, longitudinally spiraling into clinical dependence (Everitt et al. 2008; Hogarth and Chase 2011). In this case chronic drug dependents would rely on much stronger on compulsive S-R habits than individuals who recently started to develop substance dependence. Even though we lacked to find behavioral associations with AD duration during instrumental learning, we do show a progression of neurobiological functions with longer AD during Pavlovian (chapter 4) and instrumental (chapter 5) experiments, possibly indicating a process that further continues with more chronic compulsive drug use.

The habit construct can be deduced from historical models of behavioral conditioning such as Skinner's (1938) behaviorism, which stated that this behavior is not primarily driven by cognitive and motivational mediators, but rather turned into an automatic drive directly guided by stimuli in the environment. However, critiques to this view, which can be traced back to the 1960s (Mowrer 1960), emphasize the inadequacy of reducing such complex human behavior to these 'simple' behavioral perspectives. In the context of addiction, however, such a 'simple' point of view does make sense in the way that addicted patients lose control over their behavior, expressed by decreased cognitive control functions (see below), spiraling into chronic relapsing drug abuse, despite decreasing drug-related rewards and increasing negative consequence. The motivational and cognitive mediators that are thought to make (healthy) human behavior complex, have decreased in the addicted brain, making way for the automatic drives guided by direct stimuli in the environment as seen when habits drive behavior. In other words, these chronic cases have been said to have a 'hijacked' brain by drugs stimuli. Animal literature generally describes this stimulus-guided habitual behavior as 'compulsive'. In the context of obsessive-compulsive and impulse control disorders such as trichotillomania, compulsions are rooted in a need to reduce tension or anxieties (Grant and Potenza 2004). The current definition of S-R habits however does not directly describe this tension releasing factor, thereby disconnecting habitual drug use from compulsive disorders. However interestingly, it has been shown that high levels of stress induce or accelerate the formation of habits (Schwabe and Wolf 2009), indicating that tension or stress at least to some extent do seem to mediate habit

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formation. Therefore, habitual behaviors, as hypothesized in addictions such as AD, can be seen as compulsive behavior to reduce stress. Possibly in line with this, in our study in chapter 4 we also looked at the influence of depression / anxiety on the hypothesized involvement of habit pathways during Pavlovian cue-reactivity. We showed that brain activation in reaction to visual alcohol cues as found in the striatum was not associated with depression/anxiety severity, indicating that this effect was solely based on alcohol use characteristics, and not on the comorbid, possibly stress-related, mood problems. However, subjective craving was positively correlated with depression scores in AD, indicating that the subjective experience of drug-taking urges is mediated, and maybe even enhanced, by the presence of depressive symptoms, whereas the more objective measurement of BOLD response in reactivation to alcohol cues is probably not.

Together, the studies on the shift of conditioned processes towards habits presented in chapters 4 and 5 confirm the hypothesis that also in human addicts an increased reliance on S-R habit learning and its neural pathways is seen in chronic alcohol dependent patients. As such, this is the first clinical study supporting the conceptualization of addiction as a condition of pathological habit-based learning.

Cognitive control of drug-use drives
Together with increased (reward- and/or habit-guided) drives leading to increased and repeated drug seeking and drug use behavior, most addiction models also mention the important role of decreased control functions (e.g. Impaired Response Inhibition and Salience Attribution (I-RISA) model; (Goldstein and Volkow 2002). Therefore we also studied the neurobiological correlates of cognitive control functioning in AD patients in chapter 6. We aimed to distinguish AD-related impairments in response inhibition from response inhibition effects associated with comorbid depression/anxiety. In addition, we intended to explore the relationship of AD-specific response inhibition impairments with the severity and duration of AD. In order to test our hypotheses, we compared AD patients with depression/anxiety patients and healthy controls on a Stop Signal Task during fMRI scanning. Our results showed that overall, the AD group performed similar to D/A patients and HCs. However, alcohol problem severity was significantly associated with impulsivity measurements in AD patients, indicating that more severe alcohol dependence was associated with more severe inhibition impairments, whereas severity of depression and anxiety was not. Importantly, the AD group showed a stronger activation during response inhibition in subcortical areas such as thalamus and putamen than D/A patients and HCs, whereas AD patients showed less activation in the supplementary motor area than HCs, indicating a shift from cortical to subcortical engagement during response inhibition in AD patients. In the context of similar behavioral performance in all groups, the larger subcortical involvement by AD patients can be interpreted as a compensation mechanism, which may decrease with longer disease duration in the thalamus. This implies that AD patients with a longer drinking history were less able to invoke this compensatory or alternative strategy during inhibition. Therefore we suggest that a longer drinking history, associated with more severe alcohol use problems, underlies an increasing disability to compensate for inhibition impairments by recruitment of the thalamus, leading to higher impulsivity. However, because we did not find a direct association between behavioral inhibition measurements and AD duration, we can only speculate about this indirect association. Future studies are needed, to further assess the association between AD duration and behavioral cognitive control measurements.

These findings indicate an AD-specific endophenotype as the neurobiological basis for cognitive control impairments in AD patients, which is subject to alcohol use characteristics like severity and disease duration. Compensation mechanisms, which allow AD patients to perform at 'normal' levels

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during a response inhibition task, decline with longer lasting AD. This could lead to a progressively decreasing ability to control drug urges, which in turn increases with the formation of inflexible drug-use habits.

METHODOLOGICAL CONSIDERATIONS

The findings of the studies presented in this dissertation need to be viewed in light of some methodological considerations.

For our first aim, in Section II of this dissertation, we identified the presence of an alcoholic family history as a vulnerability factor to develop alcohol dependence. To study the permanent neural impairments associated with an increased vulnerability to develop AD-related psychopathology, we studied adults with and without a family history of AD. The mean age of our non-alcoholic adult sample, however, was around 38 years, and although late onset AD could still occur, this average age could be considered relatively old to still develop AD. Thus, instead of being highly vulnerable for AD, a proportion of the older individuals could be actually protected to develop AD. This fact could have rendered our results weaker than expected, or actually could explain a part of the current findings. A known protective factor in the brain against developing AD in unaffected family members of alcoholics is the increase of D2-receptor levels (Volkow et al. 2006b). Even though our current results cannot be directly linked to dopamine receptor profiles, it is possible that for example decreased parahippocampal gyrus volume could contribute to another protective factor in the brain to develop AD. However, at this point we cannot make any statement about the risk to eventually develop AD in the studied individuals. A solution to this would be to follow up on the studied individuals, to check if they eventually did develop AD or related psychopathology.

A major challenge to our studies focusing on habit formation in human AD, in Section III, was the fact that models and hypotheses which we used for our rationale and interpretation have been mainly derived from pre-clinical studies in rodents. The translation from the rodent towards the human brain can be tricky. Brain anatomy is very different and consequently different terminology is being used in animal research compared with human research. In general this translational problem should receive more attention, since most fundamental knowledge used in human studies is based on rodent studies, and we do not want to get 'lost in translation'. Additional studies on habit formation in healthy humans as well as other addictions need to be performed in order to replicate and confirm the findings of the current pioneer study in human AD.

A note should be made about chapter 5 of Section III. The observed imbalance towards habit learning in AD patients seems to be general, since the effect is seen with non-alcohol related stimuli. A stronger effect could probably be seen with alcohol-specific cues, and longitudinal studies should further clarify the shift towards habits over time, shedding more light onto the habit formation occurring before, during, and/or after repeated and compulsive drug intake.

A more general methodological topic covers the fact that all current studies were set up in the context of the Netherlands Study of Depression and Anxiety (NESDA). NESDA is a longitudinal cohort study, providing a large amount of demographic and clinical information on patients who were primarily seeking or receiving therapy for depression/anxiety disorders, and on healthy controls. For the studies in established AD, we were able to select individuals for recruitment who appeared to

have an established DSM-IV diagnosis of alcohol dependence during the diagnostic interviews. However, since the main focus of the NESDA study is depression/anxiety, the presence of depression/anxiety symptoms was high in our selected samples for the family history as well as for the alcohol dependent studies. Nonetheless, since AD is highly comorbid with depression and/or anxiety disorders in the general population (Boschloo et al. 2011; de Graaf et al. 2002), the selected sample of our studies should have an expected high empirical validity, and thus is representative for the general population. Additionally, it should be noted that in the NESDA study, treatment seeking for disorders other than depression/anxiety (such as AD) was an exclusion criterion, resulting in inclusion of AD patients who were non-treatment seeking at baseline and therefore less severe and less chronic. We additionally recruited patients from local treatment clinics, in order to have a wider range of AD severity and duration for regression analyses, and to be able to study patients with more severe and longer lasting AD. For matching purposes, and to avoid severe physical and psychiatric comorbidity (including personality disorders, but except for depression/anxiety) in the treatment-seeking AD group, we recruited these patients from treatment groups focusing on lifestyle changes, resulting in turn in a less severe subgroup of treatment-seeking AD patients. Therefore, our overall AD sample was relatively light with regard to AD severity, possibly explaining the only subtle behavioral results. Additionally, although the two AD samples (NESDA-recruited and treatment-clinic recruited) were matched regarding age, education, gender and physical and psychiatric comorbidity, the two groups evidently still differed on therapy-related characteristics such as treatment-seeking status, abstinence period and AD severity / duration. This prevented us from comparing the two recruited AD groups with each other. Nonetheless, we were able to scan a –for neuroimaging standards– relatively large sample of AD patients (N = 42), with a wide range of AD severity and duration, in order to perform valid regression analyses.

The definition of AD duration, one of the main independent variables of our studies within AD patients, should however be considered with caution. To calculate the duration of AD, we subtracted the age of AD onset from the age of recent AD symptoms (by definition in the past six months). We obtained information on the onset of AD only retrospectively by self-report. However, studies on the consistency of retrospectively reported age at first drink show patterns of inconsistent reporting (Bailey et al. 1992; Engels et al. 1997; Johnson and Mott 2001; Prause et al. 2007; Shillington and Clapp 2000). Intuitively, age at onset of alcohol dependence (instead of simply alcohol use), e.g. the first period of twelve months when at least three AD symptoms according to the DSM-IV occurred, should be even more difficult to estimate retrospectively, and therefore the age of onset of AD could be biased. This could lead to an underestimation of the association between AD duration and clinical, behavioral or neuroimaging outcome measurements, and could be the reason that the found associations with AD duration in the current study are mostly only moderate. Ideally, longitudinal studies, following patients from, or even before, the onset of AD towards compulsive drug use, monitoring time-dependent changes in behavior and neurophysiology should be performed. The need for longitudinal cohorts also holds for the family history studies, following children of alcoholics from childhood or adolescence into adulthood, when the brain has been fully matured.

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CLINICAL IMPLICATIONS AND TREATMENT TARGETS

The identification of a neural endophenotype associated with a family history of AD may help to develop prevention targets for the development of AD in highly vulnerable individuals. Smaller

parahippocampal volumes as found in our current family history study on brain structure (chapter 2) could contribute to an increased vulnerability to develop alcohol dependence through several system, such as the stress or the memory system, or through decreased inhibition, or a combination of all these. An interesting, but so far rather speculative connection could also be made with the glutamate system, underlying the important memory functions of the hippocampal area (Amaral and Witter 1989; van Strien et al. 2009). In this context a smaller PHG may be directly or indirectly related with the family history-related decreased NMDA-receptor sensitivity to its agonists, such as the intoxicating effects of ethanol in alcoholic and non-alcoholic FH+ individuals (Krystal et al. 2003a; Spanagel and Vengeliene 2013). Additionally, in our family history study on functional correlates within depressive and anxious patients (chapter 3), we show a differentiating functional profile in FH+ patients compared with FH– patients, which indicates a subgroup within the population of depression / anxiety patients. A subgroup of depression/anxiety patients based on a family history of alcohol dependence has previously been identified, actually at the level of glutamate receptors, showing differentiating anti-depressant effects following administration of the NMDA-receptor antagonist ketamine (Phelps et al. 2009). Such a differentiating receptor profile could underlie differential functionality of the brain, leading to a distinct symptom profile in FH+ patients compared with FH– patients. Our study suggests that negative symptoms in our FH+ patient group are mainly based on cognitive regulatory difficulties, possibly leading to a disability to regulate negative mood, instead of a lack of blunting reactivation to positive stimuli. These differentiating effects between depression/anxiety patients by familial alcohol dependence imply the importance to monitor alcoholic family histories in patients with depression/anxiety in order to guide (personalized) therapy, but also in non-alcoholic healthy individuals, to assess vulnerability factors for prevention. Additionally, this obtained knowledge on functional subtypes should be taken into account in the study of neurobiological correlates of both depression and anxiety disorders. In general, the presence of a family history of alcohol dependence should be monitored better in healthy controls and psychiatric patients, for both prevention and research purposes.

Our findings within established AD (chapters 4 and 5) contribute to the conceptualization of addiction as an overreliance on stimulus-driven habits at the expense of flexible, goal-directed action (O'Brien and McLellan 1996) leading to frequent and persistent substance use despite serious negative consequences. This should have consequences for treatment programs, since to date, treatment of AD has mainly been focused on the reduction of the positive reinforcing properties of drugs ("blocking the buzz" i.e. with the opioid antagonist naltrexone), or the negative reinforcing aspects of chronic alcohol use represented by relief craving ("curing the blues" i.e. with the glutamatergic NMDA-receptor antagonist acamprosate) (Heilig et al. 2010). The most researched reported interventions to reduce craving and relapse are disulfiram, naltrexone, acamprosate and nalmefene (Spanagel and Vengeliene 2013). In the context of the glutamate theory of alcoholism, targeting the NMDA-receptor is a main therapeutic action in the treatment of alcohol dependence, using antagonists such as acamprosate, topiramate, n-acetylcysteine and memantine by substitution drugs for alcohol dependent patients (Spanagel and Vengeliene 2013). This way the negative effects of withdrawal, often inducing new alcohol intake, can be reduced. It should be noted that the NMDA-receptor can also be targeted for cognitive enhancement in the treatment of alcohol dependence (Collingridge et al. 2013), since it is involved in long-term potentiation, underlying learning and memory functions (Collingridge et al. 1983; Morris et al. 1986).

Moreover, naltrexone, an opioid receptor antagonist with a high affinity for μ-opioid receptors (Littleton and Zieglgansberger 2003) has repeatedly been proven to be effective in alcohol dependence (Ray et al. 2010; Rosner et al. 2010), and with the presence of an alcoholic family history (Krish-

nan-Sarin et al. 2007). Next to the opioid mechanism, naltrexone has additionally been shown to decrease NAcc alcohol-induced dopamine levels through the antagonism of midbrain dopaminergic activity (Benjamin et al. 1993). Putative mechanisms of action of naltrexone include the reduction of alcohol cravings/urges to drink, blunting of the stimulatory effects (Middaugh and Bandy 2000) and the potentiation of sedative and unpleasant effects of alcohol, and the increase of cognitive control (Ray et al. 2010). However, preclinical research shows that naltrexone only reduced alcohol-intake in heavy drinking mice, and not in mice that showed compulsive addicted behavior, as characterized by continued alcohol intake despite aversive consequences (Fachin-Scheit et al. 2006). This suggests a therapeutic efficiency only in early stages of the addiction.

While the abovementioned therapeutic interventions for the reevaluation of drug outcomes are of crucial importance to modify the initial goal status of addictive substances, recent research has provided evidence that addictive stimulus-driven behavior may still persist (Hogarth 2012). The findings of the current studies in AD patients could explain why blockage of the reinforcing effects by abovementioned pharmacological options have been shown to be effective only in non-dependent heavy alcohol users or alcohol-dependent patients who have only recently developed the dependence (Heilig and Koob 2007). In this stadium reinforcing effects and rewarding goals in ventral parts of the prefrontal cortex and striatum still play an important role for the use of alcohol or other substances. More chronic and longer lasting AD is more likely driven by S-R habits, and therefore these habitual drug-taking behaviors should be differently targeted using new pharmacological, psychotherapeutic or neuromodulatory interventions to disrupt inflexible drug habits.

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