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**Take control**

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Summary and General Discussion



## SUMMARY AND GENERAL DISCUSSION

Over the past decades, several models have emerged that aim to explain (the development) of addiction, such as habit formation (Everitt & Robbins, 2005, 2016), incentive salience (Robinson & Berridge, 1993, 2003, 2008), and the role of negative affect and sensitivity to stress (Koob, 2015; Robinson & Berridge, 2003). These models are not necessarily mutually exclusive and can also be incorporated into one model to explain several aspects of addiction (e.g. Koob & Volkow, 2016). However, the studies in this dissertation were broadly based on Dual Process Models of Addiction, which state that (the development of) substance use disorders are associated with neuro-adaptations that yield hyper-reactive motivational and suboptimal reflective control processes (Bechara, 2005; Gladwin et al., 2011; Volkow et al., 2004; Wiers et al., 2007). These different types of neuroadaptations were the broad targets for the experimental treatments tested here: dampening of hyper-reactive motivational processes and improving cognitive control. New pharmaceutical compounds have been proposed to normalize the motivational processes, including N-acetylcysteine (NAC), aimed at normalizing the disrupted balance of glutamate in the dorsal anterior cingulate cortex (dACC), and thus potentially reducing cue-induced craving. In addition, novel training paradigms have been developed to improve cognitive control functions, including working memory training (WM-training). However, very little is known about the neurobiological bases of the changes involved in these new treatments and the relationship with clinical outcomes.

Therefore, the first aim of this dissertation was to investigate the neurocognitive and neurochemical mechanisms underlying substance use and substance use disorders, specifically with respect to the neurotransmitters glutamate and GABA and their association with impulsivity. The second aim was to investigate the effect of NAC on glutamate normalization and smoking cessation in the dACC. The third aim was to investigate the combined effect of NAC and WM-training on cocaine use cessation, craving, cognition, and brain activity associated with craving and working memory in regular cocaine users. Finally, we investigated the correspondence between data acquired under lab conditions and data collected using ecological momentary assessment (EMA).

## SUMMARY OF THE RESULTS

Chapter 2 provides a review on the effects of sustained abstinence after substance dependence on the recovery of neurocognitive functions. Based on the reviewed longitudinal studies, it appeared that many cognitive functions recover, at least partially, when substance use is discontinued for a long period. Moreover, there were indications that remaining impairment (partial recovery) may to some extent reflect pre-existing impaired cognitive functioning in verbal skills, impulsivity, IQ, and

working memory. In addition, some studies reported alterations in brain structure and functions associated with neurocognitive recovery. Such studies were predominantly performed in the field of alcohol dependence and virtually absent for other substances. Based on these findings, possibilities for treatment were suggested, such as training of impaired neurocognitive functions, pharmacotherapy, and neuromodulation.

In chapter 3, differences in glutamate and GABA concentrations in the dACC between substance users and non-using controls, and associations with impulsivity and substance use were investigated.  $^1\text{H-MRS}$  was used to obtain neurotransmitter concentrations in the dACC of cigarette smokers, smoking polysubstance users and non-using controls. In smokers and smoking polysubstance users, dACC glutamate concentrations were higher than in non-using controls and there were no glutamate differences between the substance using groups. There were no group differences in dACC GABA concentrations. Neurotransmitter concentrations were not associated with the amount of substance use in the past or the level of substance use disorder severity. Groups differed on impulsivity, with polysubstance users showing the highest levels of impulsivity, followed by smokers, and the lowest levels in non-substance using controls, but impulsivity was not associated with dACC neurotransmitter concentrations.

In chapter 4, neurotransmitter abnormalities in smokers versus non-smokers and the clinical and neurobiological effects of NAC in smokers were investigated. Smokers received 2400 mg/day NAC or placebo for two weeks. Glutamate and GABA concentrations in the dACC were obtained by means of  $^1\text{H-MRS}$ . Smokers showed higher baseline glutamate, but similar GABA concentrations compared to non-smokers. However, NAC did not affect dACC glutamate or GABA concentrations. Both groups showed similar reductions in smoking and craving, indicating no beneficial effects of NAC compared to placebo.

In chapter 5, NAC was combined with WM-training to investigate their effects on cocaine use, craving, and cognition. For 25 consecutive days, regular cocaine users received 2400mg/day NAC or placebo and performed online WM-training. Cocaine use, craving and cognition were assessed before and after treatment. During treatment, cocaine use, craving and cognition were assessed with questionnaires, urine specimens, and with EMA. There were no differences between groups at baseline. Although there were no effects of NAC on self-reported cocaine use, objective measures (urinalysis) of cocaine use indicated a greater decrease in cocaine use in the NAC compared to the placebo group. In addition, experienced problems associated with cocaine use (as measured with the DUDIT) also decreased more strongly in the NAC group. There were no effects of NAC on craving, but reduced impulsivity in terms of improved inhibition on the Stop Signal task was

found. The results from the assessments in the lab generally corresponded to the EMA assessments, except for decreased performance on the classical Stroop task and no effect on the cocaine Stroop task. There were no effects of WM-training on any of the outcomes on either type of assessment.

In the study described in chapter 5, participants also performed an n-back task and a cue reactivity task during fMRI scanning at baseline and follow up. In chapter 6, therefore, the effect of NAC and WM-training on neural mechanisms of WM and cue reactivity in regular cocaine users was investigated. There were no differences between groups in baseline WM performance or in cue reactivity. There were no effects of NAC (compared to placebo) or the number of WM-training sessions on changes in cue reactivity, self-reported craving or WM performance. However, NAC did reduce WM-associated activity in the ventrolateral prefrontal cortex (VLPFC) at every memory load condition except 0-back. There was no effect of NAC on activity in the dorsolateral prefrontal cortex (DLPFC) or the dACC. In addition, there was a significant effect of the number of WM-training sessions on VLPFC activity in the NAC group but not in the placebo group. For the NAC group, more WM-training sessions resulted in a greater decrease of VLPFC activity during the 1-back and 2-back memory load condition. There were no effects of WM-training on other outcomes measures.

Taken together, the following conclusions arose from the aims: 1) Based on the literature review in chapter 2, recovery of several neurocognitive functions and recovery in brain structure and function appears to be present after abstinence. The functions for which partial or no recovery are present could represent pre-existing risk factors for the development of SUDs. Furthermore, differences in glutamate, but not GABA, concentrations in the dACC between substance users and non-users were found in chapter 3 and chapter 4. Although there also were differences in impulsivity between groups, impulsivity was not correlated with neurotransmitter concentrations. 2) NAC did not have an effect on glutamate concentrations in smokers, but no firm conclusions can be drawn with respect to the effect of NAC on smoking cessation and craving. 3) No effect was found of NAC and/or WM-training on cocaine use cessation, craving or neural cue reactivity. However, a decrease in objective measures of cocaine use (urinalysis) was found, as well as a decrease in cocaine use related problems. NAC also resulted in a decrease in WM associated activity in the VLPFC. The effects of NAC on cognition were ambiguous, with findings of increased, decreased and unaffected performance. There was, however, an effect of WM-training on WM associated activity in the VLPFC in the NAC group. 4) Except for the cognitive measures, there was correspondence between the lab assessments and the EMA assessments with respect to measures of substance use and craving.

## GENERAL DISCUSSION

### UNDERLYING MECHANISMS OF SUBSTANCE DEPENDENCE

The literature review in chapter 2 indicated that many neurocognitive functions differ between chronic and/or excessive substance users compared with controls, but also that most of these affected functions recover after a period of abstinence. Some changes in brain structures and functions associated with neurocognitive recovery were reported. However, some cognitive functions and associated neurobiological changes did not (fully) recover. It can be argued that these functions that do not (fully) recover either represent a pre-existing risk factor for the development of SUDs, or substance-induced neurocognitive adaptations or damage. This latter argument is in line with the brain disease model of addiction (BDMA) perspective on addiction, stating that addiction is a brain disease due to its association with changes in brain structure and function (e.g. Leshner, 1997; Volkow, Koob, & McLellan, 2016), although there are also arguments against this perspective (e.g. Heyman, 2009; Lewis, 2017).

In recent years, glutamate and GABA have received more attention in relation to SUDs. The findings of increased glutamate concentrations in the included substance-using groups (chapter 3 and chapter 4) are in line with previous research showing elevated dACC glutamate concentrations in SUD patients (Bauer et al., 2013; Lee et al., 2007; Schmaal et al., 2012b; Thoma et al., 2011). However, no differences between smokers and smoking polysubstance users were found in chapter 3, which is in contrast with previous literature suggesting that the use of multiple substances is associated with larger group differences in neurotransmitter concentrations (Abé et al., 2013; Ke et al., 2004; Mason et al., 2006). More research on the effect of polysubstance use on the glutamate homeostasis is warranted to investigate if there is a limit to the extent with which the glutamate homeostasis can get disbalanced, and if it is unaffected by the intensity or extent of substance use once a specific threshold has been reached.

Even though lower GABA concentrations have been reported (Abé et al., 2013; Durazzo et al., 2016; Ke et al., 2004; Prescott et al., 2013; Silveri et al., 2014), no differences in dACC GABA concentrations in the substance using groups were found compared to non-substance using groups in chapter 3 and chapter 4. This discrepancy could be explained by the fact that previous studies mainly recruited non-smokers (Ke et al., 2004; Prescott et al., 2013; Silveri et al., 2014), whereas the substance using groups in chapter 3 and chapter 4 were smokers. Smoking could have a differential influence on the GABA systems through stimulation of nicotinic acetylcholine receptors (nAChRs). Nicotine modulates the release of certain neurotransmitters in the brain (e.g. increased GABA release) by binding to nAChRs on GABA-ergic neurons in the nucleus accumbens (Corrigall, Coen, Adamson, Chow, & Zhang, 2000;

Mansvelder, Keath, & McGehee, 2002). Therefore, it could be speculated that GABA in the dACC of the substance using groups was indeed decreased due to the use of other substances (the smokers were also recreational drinkers), but that smoking led to an increase of GABA and thereby leveled out the effects of other substances.

Although Mon et al. (2012) also found no differences in GABA concentrations despite a period of alcohol abstinence, another explanation could be the effect of abstinence at the moment of testing on the GABA system. All the aforementioned studies tested GABA concentrations after at least one week of abstinence, whereas in our studies substance users were allowed to smoke ad libitum until the session started. This could have had an effect on the results since the GABA system has been associated with abstinence and withdrawal. For instance, there is an increase in the enzyme glutamic acid decarboxylase (GAD) during the development of alcohol use disorder, which is involved in the synthesis of GABA (Sherif et al., 1997). From animal studies it appeared that the increased GAD resolves during withdrawal, possibly leading to a decrease in GABA. In addition, alcohol dependence is associated with a shift from benzodiazepine-sensitive to benzodiazepine-insensitive GABA<sub>A</sub> receptors. During withdrawal, there is an upregulation of benzodiazepine-sensitive GABA<sub>A</sub> receptors in early sobriety, which has been shown to depress GABA levels in humans (Staley et al., 2005). However, since the relationship between abstinence and the GABA system appears to be ambiguous, future research should further elucidate the mechanism underlying the effect of abstinence on the GABA system.

The literature on the associations of neurotransmitter deviations with intensity and severity of SUDs is ambiguous. For example, glutamate concentrations have been negatively associated with the amount of alcohol intake and alcohol use disorder severity (Ende et al., 2013; Thoma et al., 2011), but the absence of such associations with alcohol use and alcohol use disorder severity have also been reported (Mon et al., 2012; Silveri et al., 2014). These associations have also been explored in chapter 3 in smokers and smoking polysubstance users versus non-substance using controls. Similarly, although glutamate concentrations were higher in both substance-using groups, glutamate and GABA concentrations were not significantly correlated with the amount of substance use or with substance use disorder severity. Future research should aim to clarify the current discrepancies. However, similarly to why the use of multiple substances does not lead to greater glutamate deviations, it could be argued that, once a specific threshold has been reached, glutamate deviations are also not affected by the amount of substance use or by the severity of the SUD. On the other hand, it could be speculated that deviations in glutamate concentrations are more likely to be associated with specific cognitive functions that are a pre-existing risk factor or affected during SUD (e.g. impulsivity

(Verdejo-García, Lawrence, & Clark, 2008), rather than the toxic effect of substance use.

We also investigated whether neurotransmitter concentrations were associated with self-reported impulsivity. In line with previous studies showing increased impulsivity in relation to substance use (e.g. Moeller et al., 2001; Schmaal et al., 2012a), group differences in impulsivity were found with the highest scores in smoking polysubstance users followed by smokers and the lowest impulsivity scores for non-using controls. However, there were no significant correlations of dACC glutamate or GABA concentrations with impulsivity. The absence of a between-group difference in GABA concentrations could explain why GABA was not associated with impulsivity. The absence of an association between impulsivity and glutamate concentrations is in contrast with previous studies (Pattij & Vanderschuren, 2008; Schmaal et al., 2012b). This discrepancy might be explained by methodological differences. For example, Pattij and Vanderschuren (2008) used different indicators for impulsivity in an animal study. Another explanation might be that abstinence has a differential effect on the association between glutamate and the construct of impulsivity, mediating an association between glutamate and trait impulsivity (as measured with the BIS-11). Abstinence has been found to affect impulsivity and this might be dependent on the type of impulsivity measure (de Wit, 2009). During active substance use there may be an association between glutamate and state impulsivity, which was not measured.

Finally, it should be mentioned that MRS measurements primarily reflect redundant intracellular neurotransmitters, and that it is not possible to distinguish between intracellular and extracellular neurotransmitter concentrations. In addition, we were unable to differentiate between glutamate, glutamine and glutathione due their substantial overlap in spectral alignment. Therefore, Glx was used as a proxy measure for glutamate, but some contribution of glutamate and glutathione cannot be ruled out. This could have contributed to the discrepancies between the current findings and previous literature.

## N-ACETYLCYSTEINE

As argued in chapter 2, new treatment opportunities arise from neurobiological alterations associated with substance use disorder, among which is pharmacological treatment using NAC. Some of the results of chapter 3 and chapter 4 point to the possibility of restoring deviating glutamate concentrations with NAC, which may also mediate effects on cognitive functions and ultimately on clinical outcomes. However, we found no normalizing effect of NAC on the increased dACC glutamate concentrations in smokers (chapter 4). This is in contrast to the study by Schmaal et al. (2012b), who found a normalization of dACC glutamate concentrations in cocaine



dependent participants. This could be explained by the fact that the participants in the study of Schmaal et al. (2012b) were recruited from addiction treatment centers and were already abstinent when they entered the study, whereas the smokers recruited in the study reported in chapter 4 were allowed to smoke ad libitum when they entered the study. It could be that continued substance use maintains the unbalanced glutamate homeostasis and due to the poor bioavailability of NAC (only up to 10% e.g. Olsson, Johansson, Gabrielsson, & Bolme, 1988), the current dose might not have been sufficient to achieve glutamate normalization during active substance use. When substance use cessation is achieved by means of other (e.g. pharmacological) treatment strategies, NAC could be applied to promote abstinence by conserving the rebalanced glutamate homeostasis. This was also argued in a recent review by McClure et al. (2014).

The clinical effects of NAC were explored in chapters 4 to 6, describing generally negative results. For the effect of NAC on smoking cessation (chapter 4), we were unable to draw any conclusion on the beneficial effects of NAC given that both groups showed a reduction in smoking and craving. In addition, we found no effect of NAC on cocaine use cessation and craving. The results regarding cocaine use and craving measured in the lab were similar to the results of the EMA measurements. The lack of an effect of NAC on substance use and craving is in contrast with the growing body of research reporting beneficial effects of NAC with respect to reduced substance use (Froeliger et al., 2015; Gray et al., 2012, 2010; Knackstedt et al., 2009; LaRowe et al., 2013; Mardikian et al., 2007), reduced craving (Amen et al., 2011; Back et al., 2016; Froeliger et al., 2015; Gray et al., 2010; LaRowe et al., 2006, 2007, 2013; Mousavi et al., 2015), reduced rewarding effects of cigarette smoking (Schmaal et al., 2011) and reduced levels of nicotine dependence (Grant et al., 2014). This discrepancy could have been caused by the fact that some of these studies used NAC as an augmentation to other treatment strategies (e.g. Froeliger et al., 2015; Grant et al., 2014). It could be argued that positive clinical effects were the result from the treatment NAC was augmented to. And as argued before, abstinence from substance use could have led to an initial normalization of the glutamate homeostasis. The addition of NAC could in turn have led to conservation of this normalization, mediating the clinical effects. This is to some extent supported by the fact that NAC did not show an effect on glutamate concentrations in smokers (chapter 4). If this is also the case in cocaine users remains to be analyzed, since these data were not included in this dissertation and remain to be analyzed.

We did, however, find a beneficial effect of NAC on an objective measure of cocaine use (urinalysis) and on cocaine-use related problems (DUDIT) in chapter 5. It must be pointed out that the results of chapter 5 were not corrected for multiple testing, and that these results would no longer reach significance when this correction

was applied. Therefore, the positive results of chapter 5 could be the result of a Type 1 error, and should consequently be considered exploratory and hypothesis-generating. Future research on the effect of NAC on cocaine use cessation, craving and cognition should aim to include larger samples. However, the current studies indicated that this can be difficult, emphasizing the need to prevent attrition.

NAC has also been found to have an effect on a variety of cognitive functions (e.g. executive functioning, processing speed and memory) in a variety of populations, such as patients suffering from Alzheimer's or schizophrenia and even healthy participants (Skvarc et al., 2017). However, there are no reports yet with respect to patients with SUDs. The results in this dissertation on the effect of NAC on cognitive functions are ambiguous and are inconsistent between lab and EMA data. In chapter 5, we found improved performance on the Stop Signal task, whereas we found decreased performance on the classical Stroop task. However, no effects of NAC on performance on the cocaine Stroop task (also chapter 5) or WM performance (chapter 6) were found. The improved performance on the Stop Signal task indicates reduced impulsivity in terms of improved inhibition. Since increased impulsivity has been associated with increased glutamate concentrations (Schmaal et al., 2012b), it could be argued that the effect of NAC on inhibition is mediated by a normalization of glutamate. However, this remains speculation pending analyses on the effect of NAC on glutamate. The classical Stroop task measures a different construct compared to the Stop Signal task. Whereas the Stop Signal task is considered a reliable measure to assess inhibition (Congdon et al., 2012), many theoretical explanations have been offered to explain the classical Stroop effect, among which are attentional processes and or processing bias (see Cox, Fadardi, & Pothos, 2006). It might be that these different processes are affected differentially by NAC, deviations in glutamate, or the stage of dependence. However, these results should be interpreted with caution since they would no longer be significant if a correction for multiple comparisons would be applied. In addition, due to the lack of a non-using control group, no inferences can be made about the baseline deviations in these cognitive functions.

There are several reasons that could explain why NAC did not have an effect on working memory or performance on the cocaine Stroop task, including low statistical power due to small sample size ( $N=9$ ). In addition, since there was no non-using control group included, it could not be verified if working memory performance was impaired at baseline. There are indications from preclinical and clinical studies that NAC only has an effect on glutamate when the homeostasis is unbalanced. For instance, Schmaal et al (2012b) only found an effect of NAC in cocaine dependent patients with deviating glutamate levels, but not in healthy controls with normal glutamate levels. Future research should further investigate the effect of NAC on

(different types of) cognitive processes more elaborately, preferably with larger sample sizes, different types and severities of SUDs and a non-using control group, before definitive conclusions can be drawn.

There was little effect of NAC on brain activity associated with cue reactivity and WM. With respect to cue reactivity, there was no effect of NAC on activity in the dACC, rostral ACC (rACC), dorsal striatum (DS) or ventral striatum (VS). The lack of reduced activity in the DS and VS can be explained by the absence of baseline cue reactivity in these regions, despite the fact that the stimuli were able to increase craving. In addition to the ongoing substance use as described in chapter 5, small sample size could have contributed to the inability to find NAC-induced reductions in cue reactivity in the dACC and rACC. In addition, since there was no non-using control group included, it could also not be verified if the participants had deviating brain activity. However, NAC led to decreased WM associated activity in the VLPFC (in the absence of changes in performance), but not in the DLPFC or dACC. These regions are all involved in cognitive control functions, but their activity is not univocally affected. This could be because cognitive control encompasses several more specific cognitive functions (Miyake et al., 2000) and there is no consensus on which brain areas are associated with which cognitive functions (Owen et al., 2005). Therefore, it is possible that the decrease in activity in the VLPFC was associated with change in a different cognitive function related to cognitive control, which was not assessed in the study.

Generally, we found little effects of NAC on neurobiological, clinical or cognitive outcomes. One general explanation has to do with the sample sizes of the two studies reported on in chapters 4 to 6. For example, both studies suffered from substantial dropout due to various reasons, and for chapter 4 several MRS spectra had to be excluded due to technical errors. However, for the study reported in chapter 4 and the study reported in chapter 5 and chapter 6, we had 80% power to mostly detect medium effect sizes for a time by group interaction effect with  $\alpha=0.05$ . Despite numerous reports of clinical effects, it is also argued that the efficacy of NAC may depend on the specific circumstances under which it is provided. For instance, the type of SUD seems to be of importance for the clinical efficacy of NAC, as was argued in a review by Deepmala et al. (2015). These authors concluded there was little support to recommend NAC for the treatment of tobacco dependence (Deepmala et al., 2015). In addition, as noted above, NAC seems to be more effective as an adjunct treatment, supporting the maintenance of abstinence and prevention of relapse in currently abstinent SUD patients than to induce abstinence in currently substance using SUD patients, as argued in a recent review by McClure and colleagues (2014).

## WORKING MEMORY TRAINING

WM-training was performed in an attempt to improve cognitive control functions, of which the results were presented in chapters 5 and 6. Several studies have used WM-training to strengthen cognitive control in various SUDs (for review, see Bickel et al., 2014). However, the studies in this dissertation failed to replicate these generally beneficial effects of WM-training. WM training did not have an effect on WM performance or measures of impulsivity and WM-training also had no effect on cocaine use. The latter is in contrast to previous studies which did find reductions in alcohol consumption (Houben et al., 2011) and opioid use (Rass et al., 2015). However, the lack of effect on substance use can be explained by the fact that training did not affect WM itself and therefore there is no transfer to other cognitive functions or clinical outcomes. Finally, there was no effect of WM-training on cue reactivity in the dACC, rACC, DS or VS. The lack of effect of WM-training can be explained by the lack of baseline cue reactivity for the DS and VS, the high drop-out rate and the low treatment adherence.

Although there was no effect of WM-training on WM-associated activity in the dACC or DLPFC, there was an effect of WM-training on activity in the VLPFC in the NAC group but not in the placebo group. Why there was only an effect in the VLPFC can be explained, as mentioned earlier, by the possibility of specific cognitive control functions being associated with specific brain areas, a debate on which there is no consensus yet (Owen et al., 2005). Future studies should further investigate whether training-induced improvements in cognitive functions are associated with changes in activity in specific brain regions, and whether these changes in cortical activity are associated with clinical effects. For instance, WM-training has been found to affect underlying neural mechanisms, such as the middle frontal gyrus and parts of the parietal cortices (Olesen et al., 2004).

Except for the additional effect of WM-training to the effect of NAC on activity in the VLPFC, there were no effects of training on cognitive functions or clinical outcomes. The lack of transfer effects to other cognitive functions and clinical effects can be explained by the fact that training did not improve the cognitive function it aimed to improve directly, that is working memory. Other possible explanations include the high drop-out rate and the low adherence to working memory training. Due to the extensiveness of the training, 3 tasks consisting of 30 trials for 25 consecutive days, a high level of motivation is required to complete all tasks every day. Future studies should aim to improve motivation to train, for example by making the training more engaging by using gaming elements (Boendermaker et al., 2015).

## METHODOLOGICAL CONSIDERATIONS

Some methodological considerations of this dissertation should be taken into account when interpreting the results. Perhaps the most important limitations are related to the participant groups included in the studies. First, the experimental groups in the studies investigating treatment efficacy (chapters 3 to 5) were rather small. For example in chapters 4 and 5, only 9 of the 17 participants in the group allocated to receive NAC returned for the second lab assessment. Although high drop-out rates are a typical feature of this population, especially in internet-based self-help eHealth interventions (Eysenbach, 2005), this resulted in a lower than expected statistical power to detect a treatment effect. Second, although participants were included based on their smoking or cocaine use behavior, the confounding effect of the use of multiple substances cannot be ruled out, especially in the groups of regular cocaine users, where frequent polysubstance use was not an exclusion criterion. Although concurrent alcohol and/or tobacco dependence is very common in cocaine users in the community and in treatment settings (EMCDDA, 2009; Connor et al., 2014; Tang et al., 2007), it could be that different substances have a different or even opposite effect on glutamate concentrations. Since this differential effect could influence treatment efficacy (Connor et al., 2014), the potentially different effect of multiple substances should be taken into account in the treatment of polysubstance use. Third, although the inclusion of only male participants led to more homogeneous groups, the generalizability of the results to the general population of smokers and regular cocaine users is limited. Future research should therefore consider including larger samples of both male and female (poly)substance users, to further elucidate the neurobiological effects of polysubstance use and its implications for treatment, that may or may not be gender specific.

Further methodological considerations concern the design of the studies included in this dissertation. The original protocol of the studies described in Chapter 5 and 6, had a 2 (NAC/placebo) by 2 (WM-training/placebo training) design, resulting in the possibility to independently examine the effect of NAC and WM-training as well as their combined effect. However, due to slow enrollment of regular cocaine using men with the desire to quit, the placebo WM-training condition was dropped, resulting in only two randomized treatment conditions (NAC/placebo) with all participant receiving active WM-training. As a consequence, there was no placebo condition for the WM-training, and only a continuous measure of the number of completed WM-training sessions to assess the potential effect of WM-training, which was necessarily confounded by motivation to train, was included. In addition, there was no non-using control group to establish the presence of baseline differences with unaffected neurocognitive functions and associated neurobiological mechanisms.

The final methodological considerations are related to the assessments used in this dissertation. First, with respect to MRS acquisition, the sequence that was used to assess glutamate and GABA concentrations in chapter 3 and chapter 4 was not optimized to differentiate between glutamate, glutamine and glutathione due to their largely overlapping range in chemical shift (Ramadan et al., 2013). Therefore, Glx was used as a proxy measure for glutamate, but some contributions of glutamine and glutathione to glutamate cannot be ruled out. In addition, many MRS spectra had to be excluded for GABA and Glx due to unreliable spectra caused by noisy data, which could have been caused by head movements. It should also be noted that it is not possible to distinguish between intracellular and extracellular neurotransmitter concentrations with MRS, and measures primarily reflects a surplus of intracellular neurotransmitters. Furthermore, substance use does not only affect brain function but also brain structure, which in turn can have an influence on neurotransmitter concentrations. Future research should take these factors into account when investigating neurotransmitter concentrations, and glutamate in particular.

Second, region of interest (ROI) analyses were used to explore treatment effects in *a priori* specified brain regions associated with cue reactivity and WM. ROI analyses have the advantage that less power, and thus a smaller sample size, is needed to detect an effect compared to conventional (whole brain) fMRI analyses strategies. However, the disadvantage is that, inherent to the analysis, only these particular regions are investigated and potential activity in other brain regions is disregarded.

## **FUTURE DIRECTIONS AND CLINICAL IMPLICATIONS**

Several recommendations for future research and clinical implications arise from this dissertation. First, it should be further investigated which cognitive functions are affected by the use of a particular addictive substance or multiple addictive substances, and how these altered cognitive functions are associated with neurobiological alterations. In addition, it needs to be further investigated if and how deviations in glutamate and GABA are associated with different stages of dependence and by particular features of SUD, such as the type of substance(s) used, the quantity of use and the severity of the dependence. This can be achieved by assessing different groups of substance users with different combinations of substance use (for an example of such an approach see Fernández-Serrano et al., 2011). However, since polysubstance use is often a more valid representation of reality (e.g. EMCDDA, 2009; Connor et al., 2014; Tang et al., 2007), researchers should also study the cognitive and neurobiological effects of polysubstance use. This could also be more beneficial for clinical purposes. More knowledge on the specific effects of combinations of particular substances could lead to the development of treatment strategies that are more tailored to the profile of specific patient groups, thereby

possibly improving treatment outcome. In addition, the assessment of neurocognitive effects should become more standardized and larger samples should be used. This can be achieved by the collaboration between research institutes and treatment centers. Standardized assessment of neurocognitive functions leads to greater generalizability of study results in different psychiatric disorders and may foster development of treatment strategies in clinical practice. Eventually this may result in a transdiagnostic approach, in which neurocognitive functions are targeted irrespective of the diagnosed psychiatric disorder (Krueger & Eaton, 2015).

One of the aims of this thesis was to investigate the effect of NAC and WM-training on clinical outcomes. Although NAC and/or WM-training generally did not have a significant effect on any of the clinical outcomes, this still provides relevant information for future studies and it does have clinical implications. In the current studies NAC and WM-training were applied simultaneously in an attempt to achieve substance cessation. Even though we were unable to find beneficial effects of this approach, it could be argued that improving the approach could lead to more efficacious treatment. For example, since it is argued that improving self-control before trying to quit is more effective (Muraven, 2010), therapists could first aim to improve cognitive control, for instance by means of working memory training, before starting pharmacotherapy such as NAC. However, research should first elucidate on how the efficacy of and adherence to such training paradigms can be improved.

Furthermore, as argued in a previous review by Deepmala et al. (2015), and in line with the findings in this dissertation, there is little support to recommend NAC for the treatment of smoking dependence. The primary substance of abuse should therefore be taken into account when tailoring a treatment strategy to the patient. In addition, NAC seems more effective to maintain abstinence and prevent relapse (see McClure et al., 2014) than to induce abstinence. Therapists should first aim to achieve substance cessation by other means, and start to prescribe NAC only after the patient has been successful in quitting use. Further studies are needed to elucidate how the glutamate homeostasis is affected by abstinence in order to determine the appropriate moment to subscribe NAC during treatment. In addition, future research should investigate for which type of substance use dependence NAC is most effective.

Ecological momentary assessment (EMA) minimizes recall bias and permits a more intensive assessment of personal experiences in the context of environmental changes. The utility of EMA has been illustrated in chapter 5, and EMA has already been shown to be beneficial in clinical trials (Kowalczyk 2015, Moran, 2016). EMA could be an interesting tool to incorporate in future clinical trials in order to obtain more detailed information of the effects of treatment during the trial. Additionally, since it is important that a therapist receives accurate information on a patient's progress, something that can be influenced by the possible memory bias of the



patient, EMA could be used to more accurately monitor treatment effect. This also gives the opportunity to prevent relapse by intervening when a patient indicates high levels of temptations and subsequent craving. However, future research should aim to develop EMA strategies that are more reliable and more interactive during treatment.

High dropout rates are a typical feature of interventions, especially when it concerns internet-based self-help eHealth interventions (Eysenbach, 2005). Although often viewed as negative, attrition potentially provides researchers with information about risk factors for drop-out. To this end researchers should compare usage metrics, such as age of onset and severity of substance use, and other descriptive measures between the drop-outs and those who complete the trial, as we did in chapter 5 and chapter 6. However, attrition also results in a reduction of power to detect any effects, and it is therefore advisable to keep attrition levels to a minimum. One way to achieve this is by increasing the motivation to adhere to the treatment. This can be achieved by increasing the intrinsic motivation to change behavior using motivational interviewing (MI; Miller & Rollnick, 2012), which in turn mediates the motivation to perform the training. Another possibility is to increase the extrinsic motivation, for example with contingency management (e.g. Gray et al., 2012) or by making the training more engaging by using gaming elements (Boendermaker et al., 2015).